GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 3/6/79

Project Title: Identification of Major and Minor Classes of Natural Organic Substances Found in Drinking Water

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Project Director: Dr. S. C. Havlicek

Sponsor: Environmental Protection Agency

Agreement Period: From 1/15/79 Until ~~6/15/79~~ 9/15/79

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Defense Priority Rating: n/a

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OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: 2/22/80

Project Title: Identification of Major and Minor Classes of Natural Organic Substances Found in Drinking Water

Project No: E-20-657

Project Director: Dr. Havlicek

Sponsor: Environmental Protection Agency

Effective Termination Date: 9/5/79

Clearance of Accounting Charges: 9/5/79

Grant/Contract Closeout Actions Remaining:

x Final Invoice and Closing Documents
_ Final Fiscal Report
x Final Report of Inventions
x Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
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CA-4 (1/79)

E-20-657
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IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

July 14, 1978

by

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EXECUTIVE SUMMARY

The purpose of the work described in this report and the overall objective of the research project is to identify major and minor classes of natural organic substances found in surface waters such as might be used as a source of potable water. A second major aim of the study is to evaluate the effect of a number of water treatment processes such as chlorine, ozone and chlorine dioxide on the transformations which these naturally occurring materials may undergo during the disinfection process.

A source water which is rich in the required organic materials but which is unusually low in such interfering materials as agricultural runoff, municipal wastewater effluents and industrial discharges is being used to provide a generous reserve of aquatic humic materials which are the dominant class (80%) of all natural organic substances found in drinking water sources. Although evidence gathered during this reporting period has indicated that pollutants were present in the raw water during the last sampling, this has been attributed to minor highway construction in the vicinity and is expected to be a temporary phenomenon.

Considerable progress continues to be made in the identification of products resulting from the chlorination of aquatic humics. Fragmentation sequences have been proposed for eight new compounds (five of which are chlorinated) isolated from the aforementioned product mixtures. The list of assigned structures has been expanded to include four new products and two new product mixtures.

Aquatic humics were brominated under rather drastic conditions to produce a product mixture which has been partially worked up to date. Four compounds have been identified in the product mixture thus far. Re-extraction of the product mixture after a waiting period of two days resulted in

the isolation of additional brominated material. This observation suggests that larger, water-soluble bromo-organics are initially produced which may subsequently break down into less hydrophilic substances which can then be removed by a later extraction.

Raw water from the Satilla River has been heavily dosed with chlorine both with and without extensive preliminary flocculation to reduce TOC. The product mixtures have been sampled at several points and are currently being worked up for analysis.

A sample of aquatic humic material was run through the mini-pilot facility in much the same manner as before except that the sand filter was replaced with a carbon bed filter. Samples were withdrawn at the reservoir (control), the settling chamber, and after six time intervals at the carbon bed. These samples are now being worked up for analysis.

Class separation of raw Satilla River water has been completed and a material balance established on a TOC basis. Each fraction has been removed from the resin for future analysis and further division into subclasses. The recovery of material from the resin was 99% on a TOC basis.

The analysis of products from the mild permanganate oxidation of aquatic humics is continuing. Four new structural assignments have been made and supported by reasonable fragmentation sequences. Interpretation of the extremely complex mass spectral data is continuing. New, even milder oxidation techniques will be employed during the next reporting period.

I. PERSONNEL

Dr. J. W. Ralls has informed us that he has taken a special assignment with a West Coast company as of September 1. Fortunately, he has given us sufficient notice so that we can find a successor and make an orderly transfer of his responsibilities to his replacement. We are presently soliciting candidates. No reduction in the quality or level of effort is anticipated. We are interviewing replacements for Dr. El-Barbary and are near a decision.

II. EQUIPMENT

The GC/MS continues to operate with downtime being limited only to routine maintenance such as source cleaning. New chemical ionization gases (ammonia and isobutane) have been connected to the system and preliminary experiments are underway.

The Finnigan Model 9500 stand-alone gas chromatograph has been successfully interfaced with a Motorola 6800 microcomputer system with 22K of core, a mini-floppy dual disc system (68K per disc), video display and teletype hardcopy. We are currently programming the system so that it will help us acquire, process and analyze our GC data in a more effective and precise manner. Within the next two months, we plan to add a RT/68 MX chip which will replace the Motorola 6800 chip. The use of the Microware Systems compiler will speed up the system 100X by converting BASIC to MACHINE language. Although none of this equipment has been purchased with project funds, it has been described in this report because any such general enhancement of capabilities is bound to be of value to the project.

III. ANALYSIS OF PRODUCTS-CHLORINATION OF AQUATIC HUMICS

It will be recalled that a systematic lettering scheme has been established in order to organize the interpretation and presentation of the data. The lettering of peaks is based on the total ion chromatograms of run II-110, sand filter eluate which are represented in Figures 1 and 2 and extended in Figures 3 and 4 for the reviewers convenience. A discussion of those new assignments shown in Table IV, page 34 of the previous report will be followed by other new assignments not in the table and finally by an expanded version of the table itself.

I. In spite of a very weak spectrum the chemical ionization fragmentation pattern with its striking similarity to that of compounds C (3-chloro-2-methyl-2-butanol), F (1-chloro-2methyl-2-butanol) and H (3-chloro-2-methyl-1-butanol) strongly suggests an isomeric structure. The peak at m/e 45 suggests a CH_2CH_2OH group. On this basis, a structural assignment of 4-chloro-3-methyl-1-butanol is postulated. A comparison of base peaks and structures as outlined in Figure 5 indicates that all four structural assignments are consistent thus far.

A complete description of the EI and CI fragmentation patterns is presented in Figure 6. The bar graphs themselves are presented in Figures 7 and 8 respectively.

K. In this case a good basis for preferring one isomeric dichloromethyl butene over another is not yet clear. Both 3,4-dichloro-2-methyl-1-butene and 3,4-dichloro-3-methyl-1-butene have a number of features which seem consistent with the observed electron impact fragmentation pattern (see Figure 9). The former structure is slightly preferred since it best explains m/e 67 as the base peak. This case, however, should not be considered closed. A dichlorocyclopentane structure was briefly

05/08/78 15:29:00
SAMPLE: M/30 HUMICS PLUS CHLORINE. EI

DATA: III1943D #1
CALI: 0507 #1

SCANS 200 TO 1000

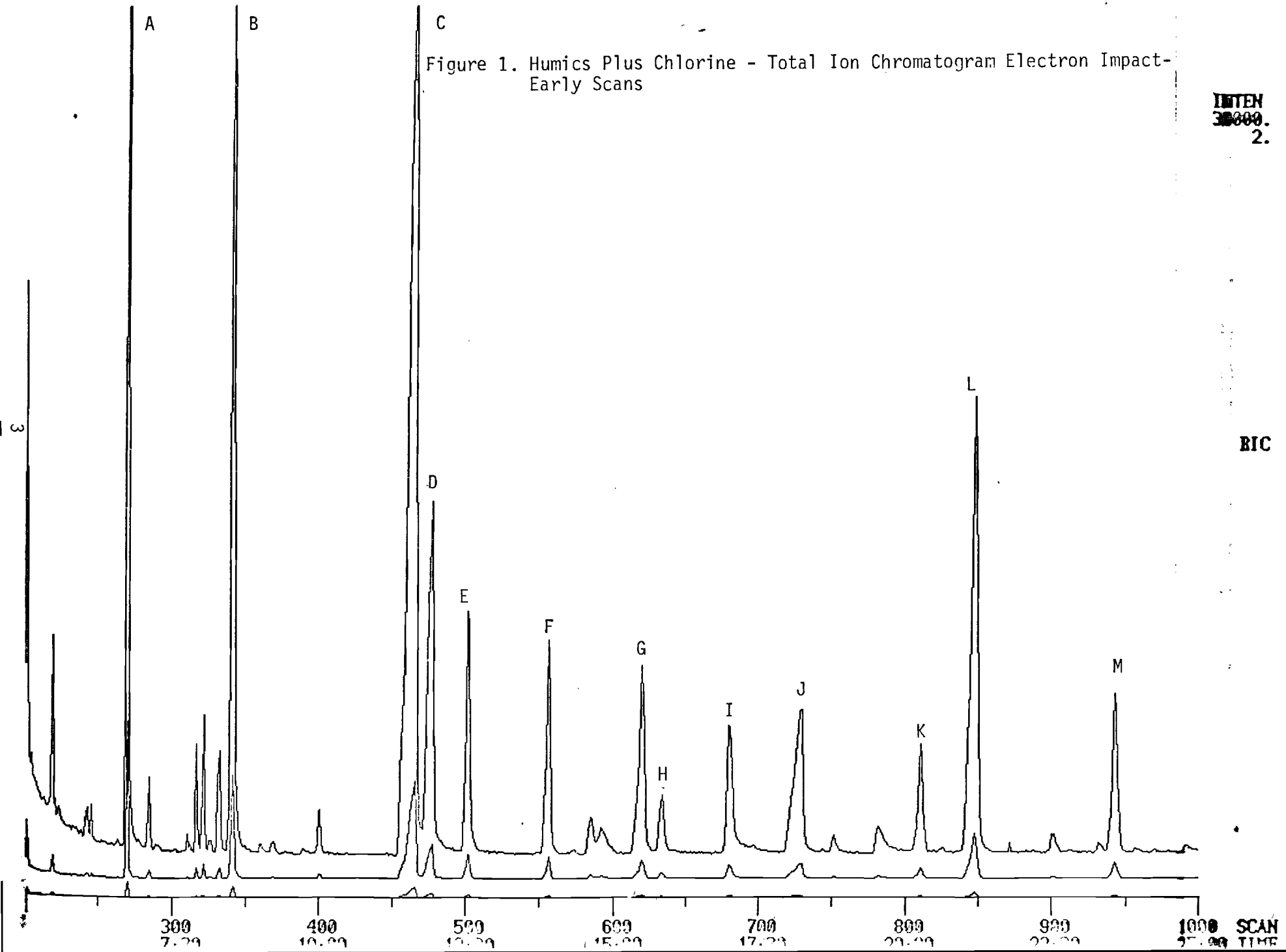


Figure 1. Humics Plus Chlorine - Total Ion Chromatogram Electron Impact-
Early Scans

RIC
05/09/78 9:56:00
SAMPLE: HUMICS PLUS CHLORINE, CH4 CI

DATA: 111943DCI #1
CALI: 0509 #3

SCANS 100 TO 1000

Figure 2. Humics Plus Chlorine - Total Ion Chromatogram Chemical Ionization-Early Scans

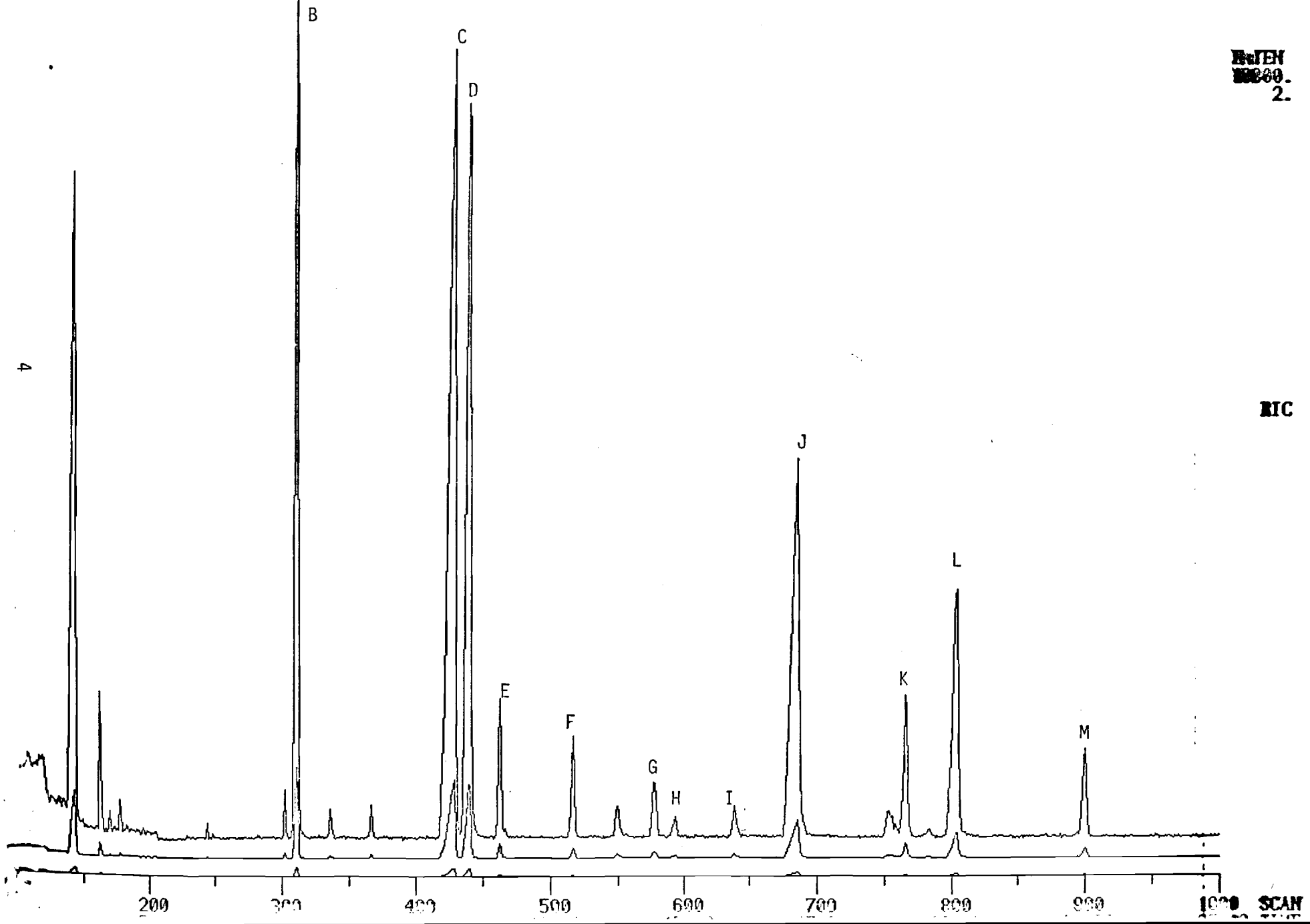


Figure 3. Aquatic Humics Plus Chlorine
Total Ion Chromatogram-Electron Impact
Early and Intermediate Scans

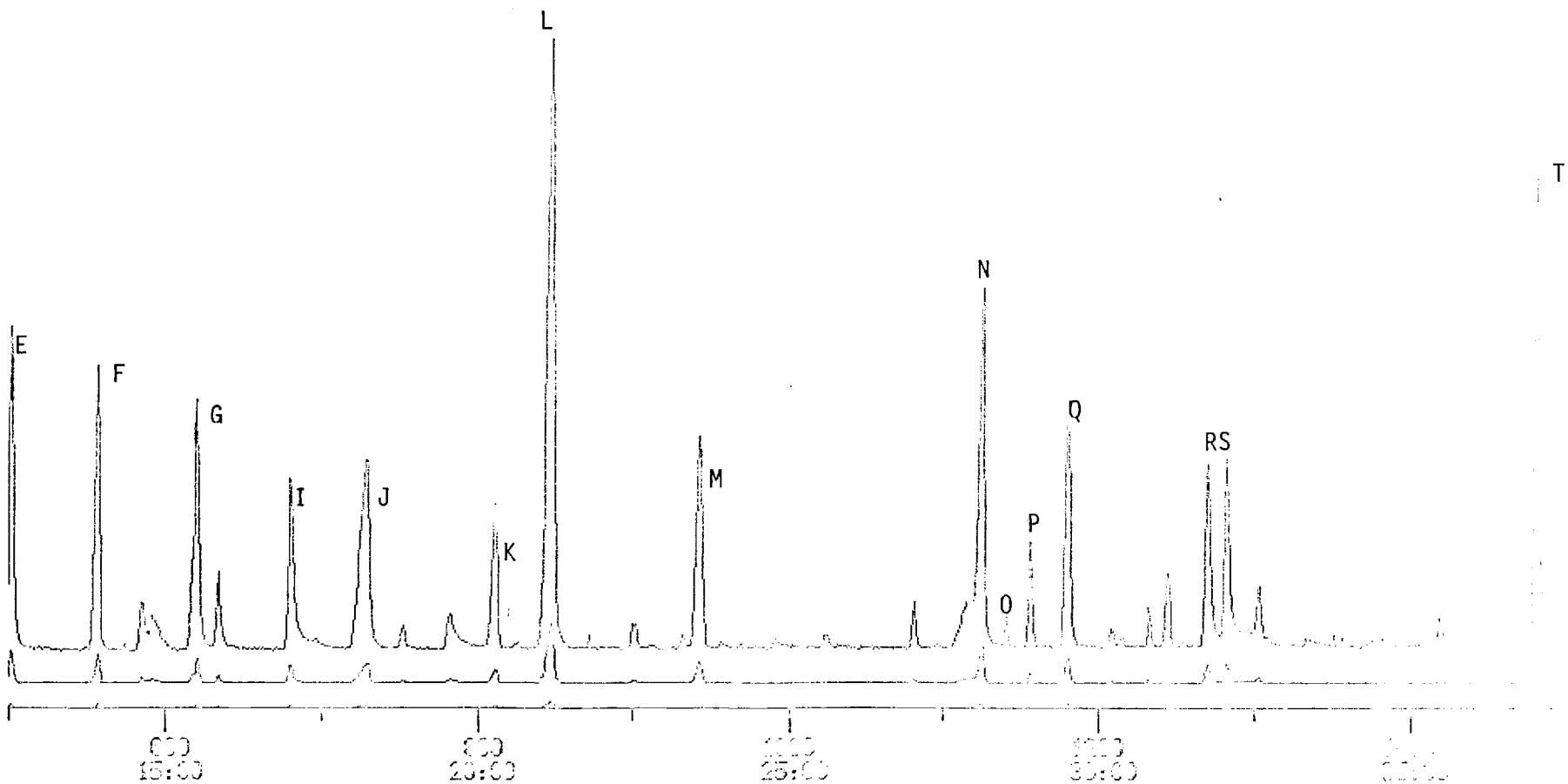
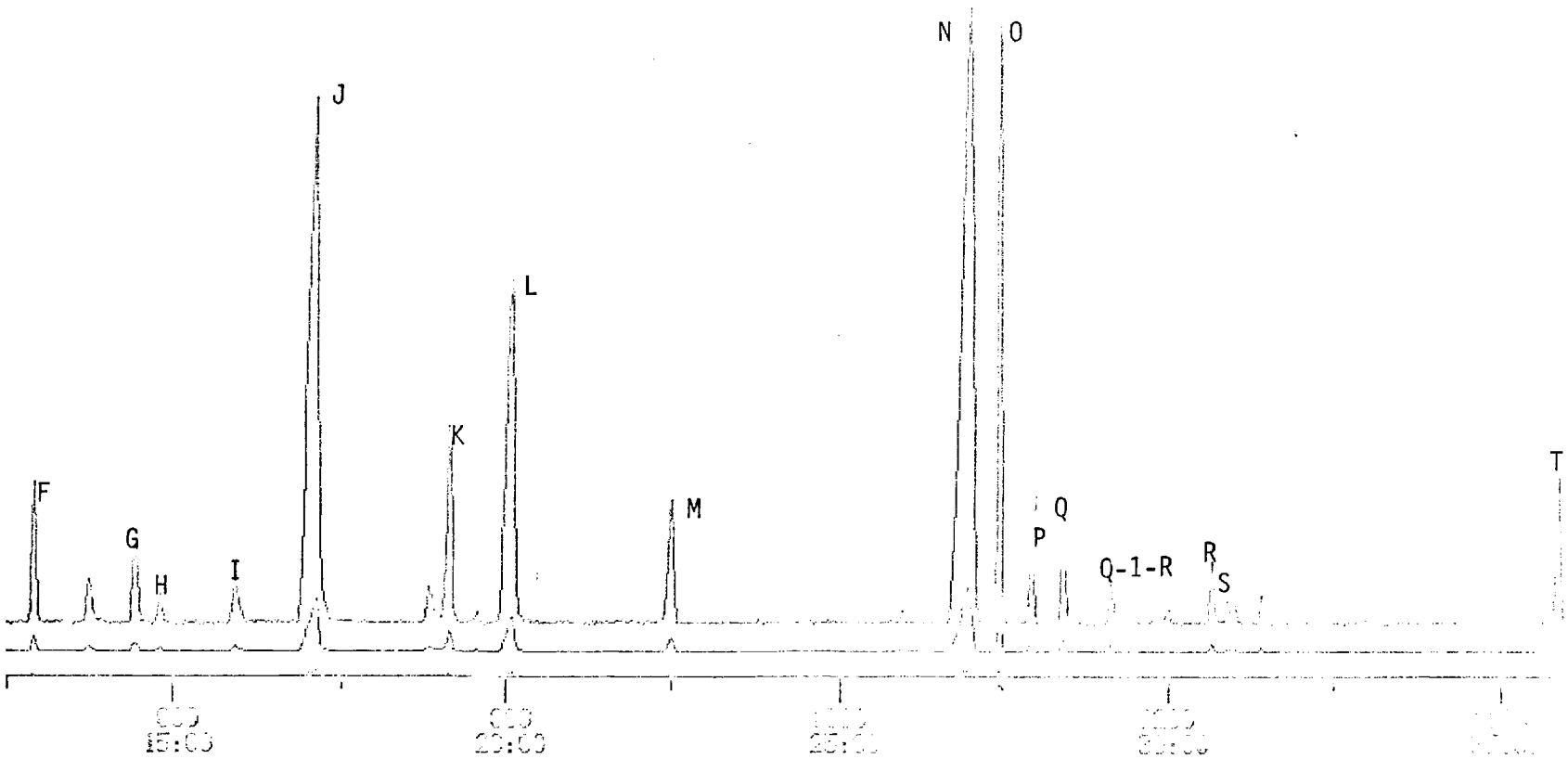
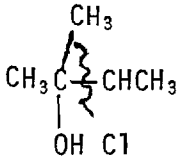
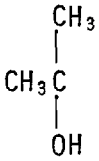


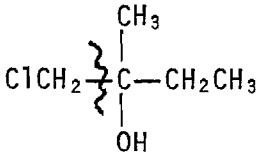
Figure 4. Aquatic Humics Plus Chlorine
Total Ion Chromatogram-Chemical Ionization
Early and Intermediate Scans

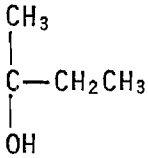




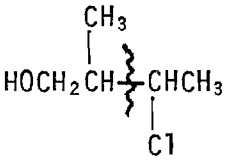


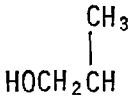
C m/e 59



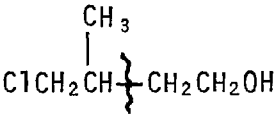


F m/e 73





H m/e 59



CH_2CH_2OH

I m/e 45

Figure 5.
Comparison of Base Peaks from Isomeric Chloro-Methyl-Butanols

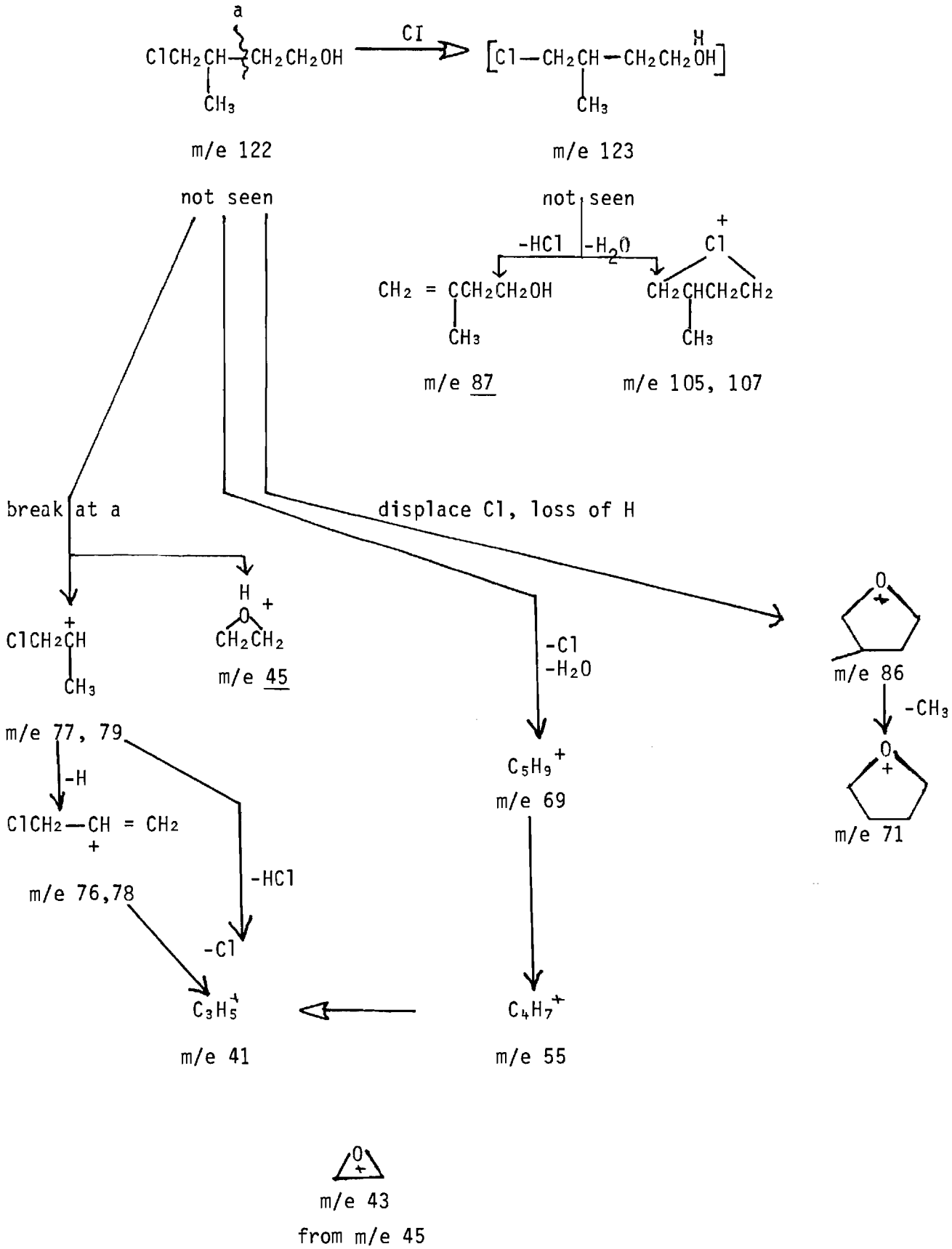


Figure 6. Compound I, EI and CI Fragmentation
Aquatic Humics Plus Chlorine
4-chloro-3-methyl-1-butanol

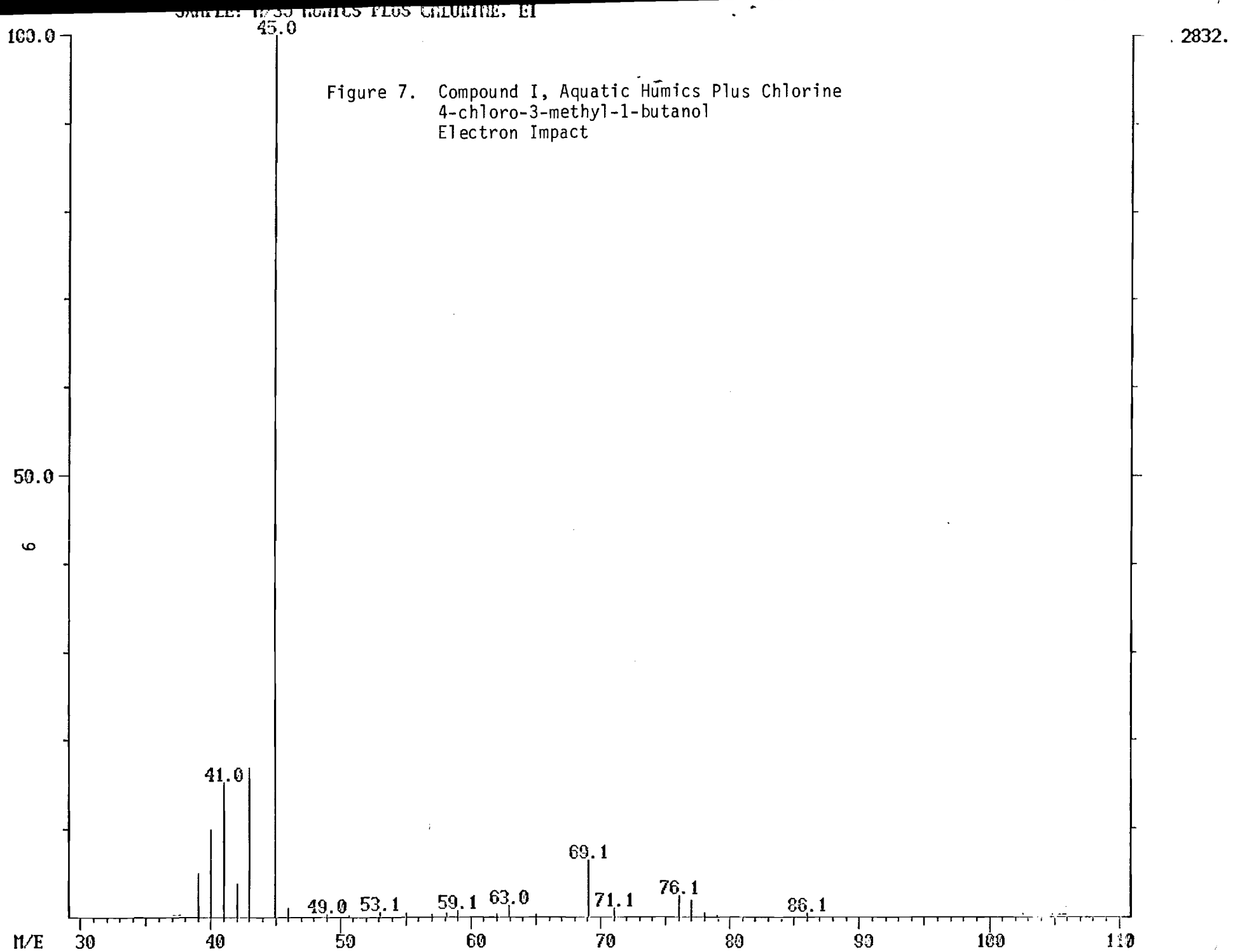


Figure 7. Compound I, Aquatic Humics Plus Chlorine
4-chloro-3-methyl-1-butanol
Electron Impact

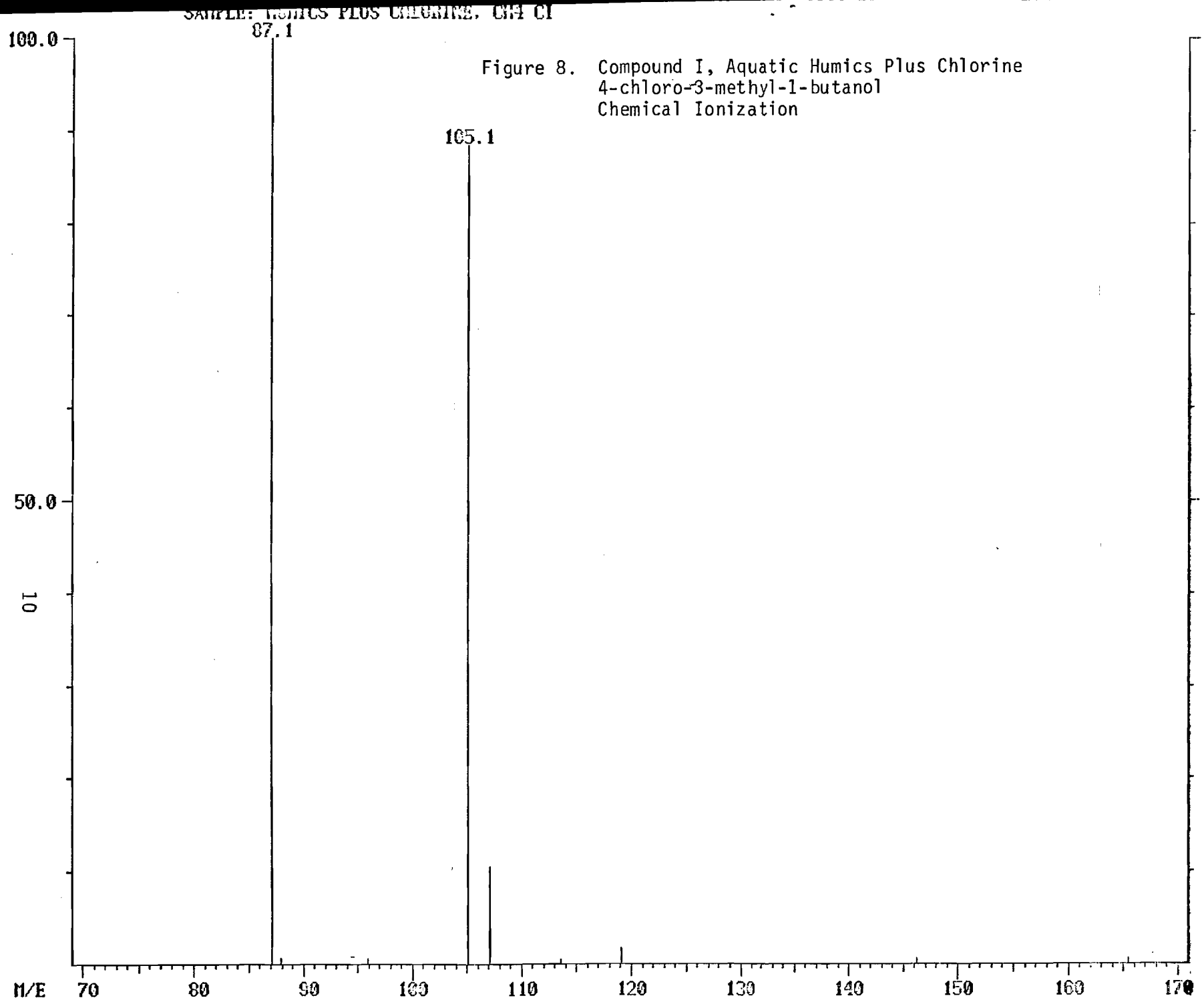


Figure 8. Compound I, Aquatic Humics Plus Chlorine
4-chloro-3-methyl-1-butanol
Chemical Ionization

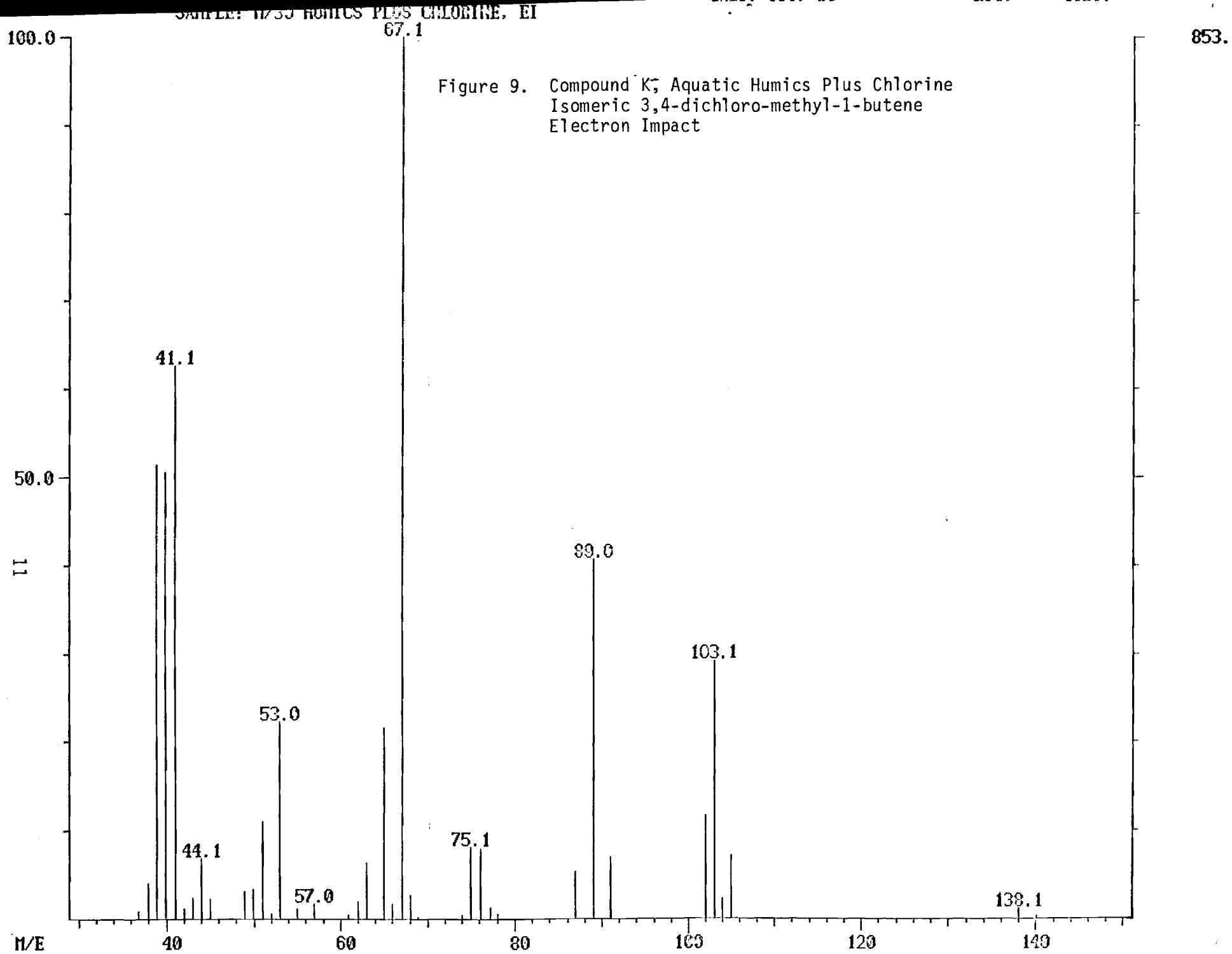
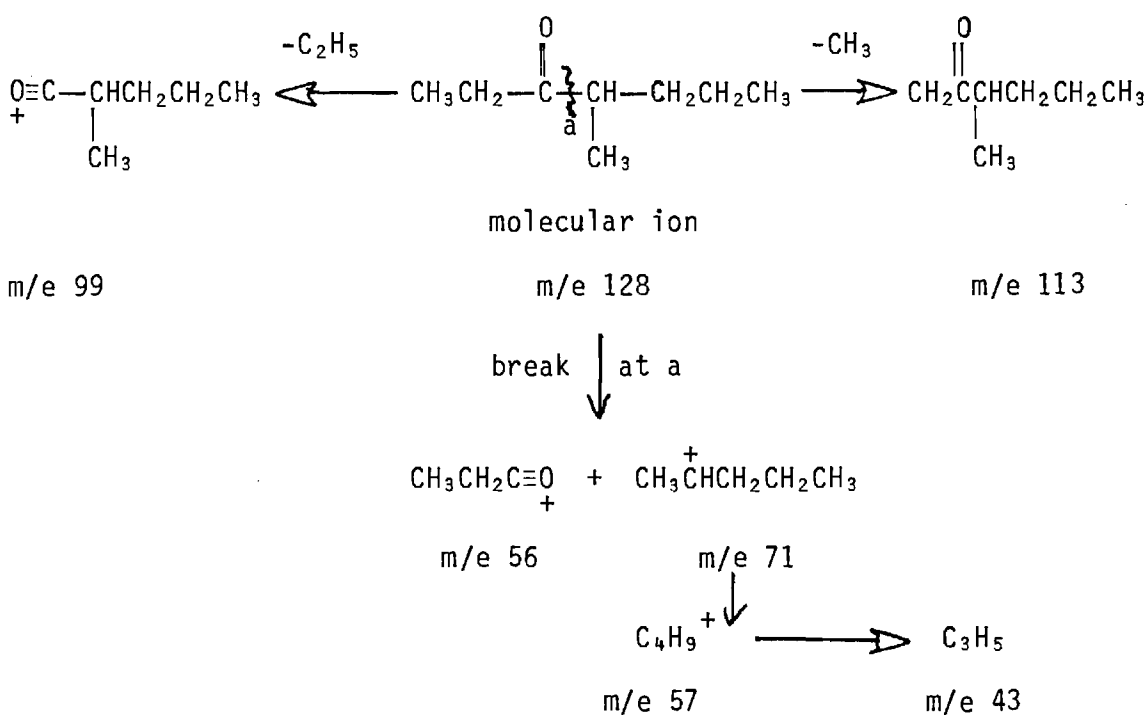


Figure 9. Compound K, Aquatic Humics Plus Chlorine
Isomeric 3,4-dichloro-methyl-1-butene
Electron Impact

considered since this assignment would explain the relative intensities of the m/e 67, m/e 41 and m/e 39 peaks. This assignment could not, however, explain the m/e 89,91 isotopic pair which strongly suggest the loss of a CH_2Cl group. Both assignments require a four-center rearrangement to explain the ions at m/e 76,78. The chemical ionization spectrum which is shown in Figure 10 provides little useful information beyond the base peak at m/e 103, 105 formed by loss of HCl from the quasi-molecular ion. The respective fragmentation patterns are outlined in Figures 11 and 12.

L. This component was identified by the data system as 4-methyl-3-heptanone on the basis of its electron impact fragmentation pattern which is shown in Figure 13. This structure was the first choice for this component in both the chlorination product mixture and in the aquatic humic control. A proposed fragmentation scheme is outlined below.



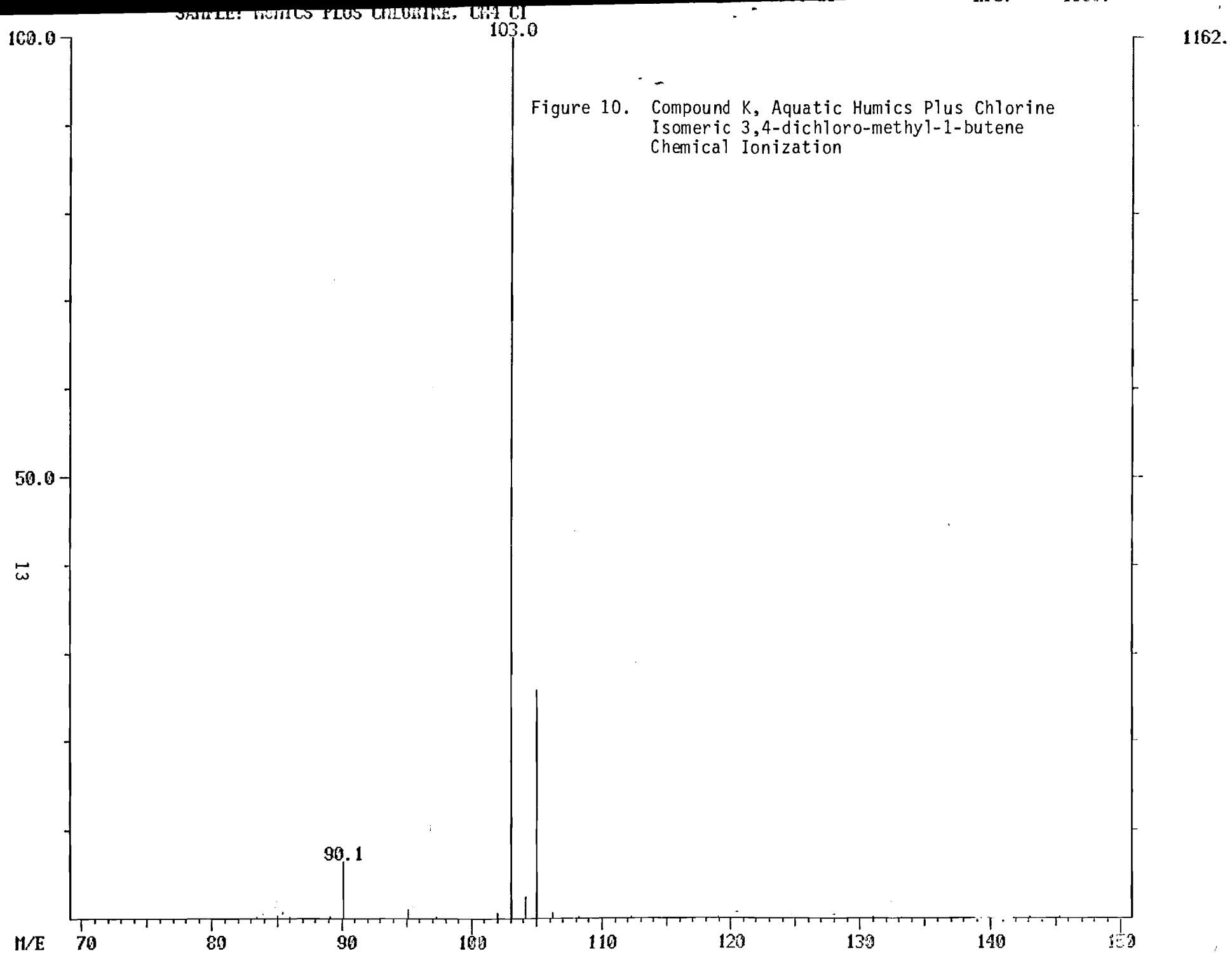
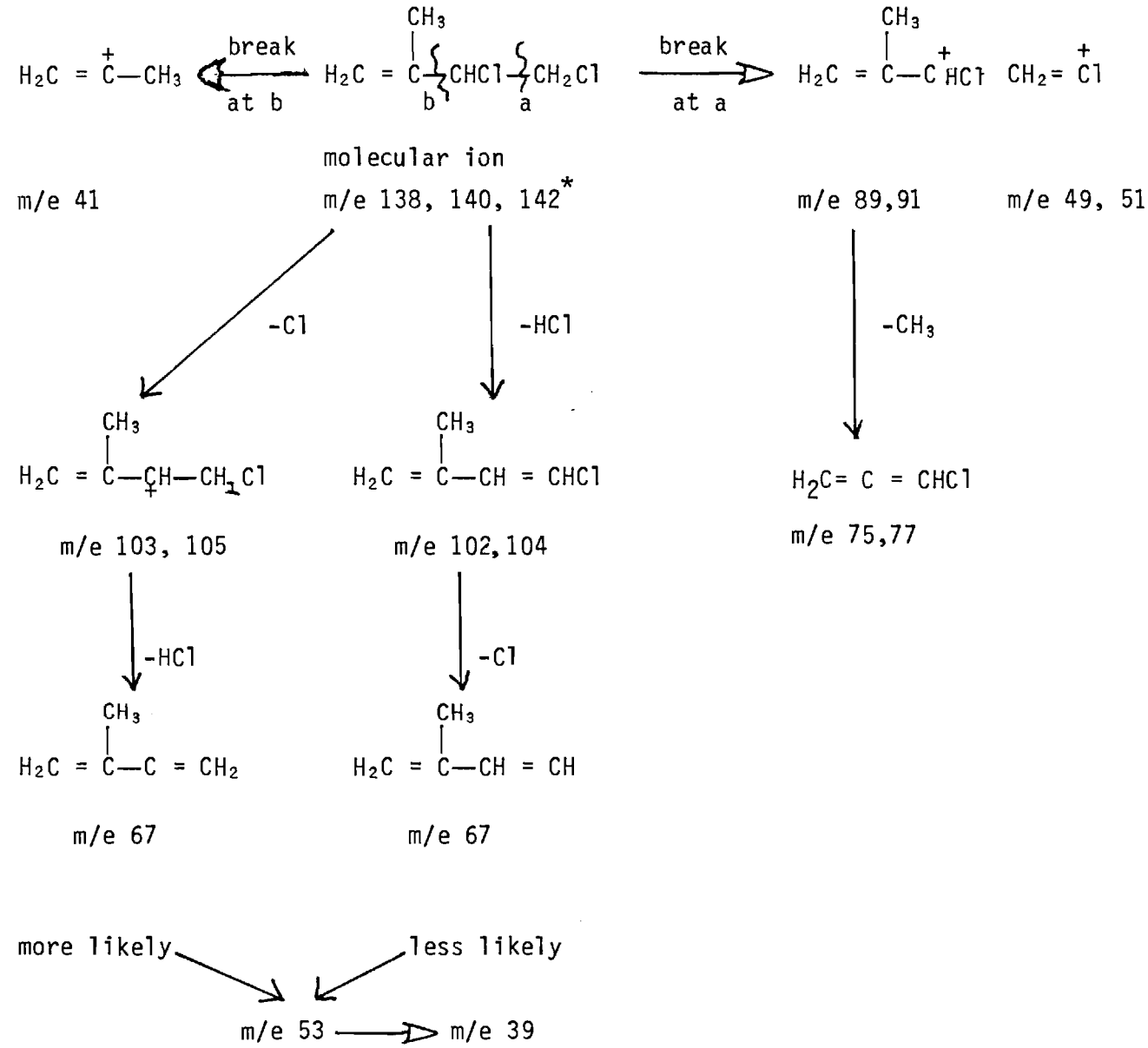


Figure 10. Compound K, Aquatic Humics Plus Chlorine
Isomeric 3,4-dichloro-methyl-1-butene
Chemical Ionization



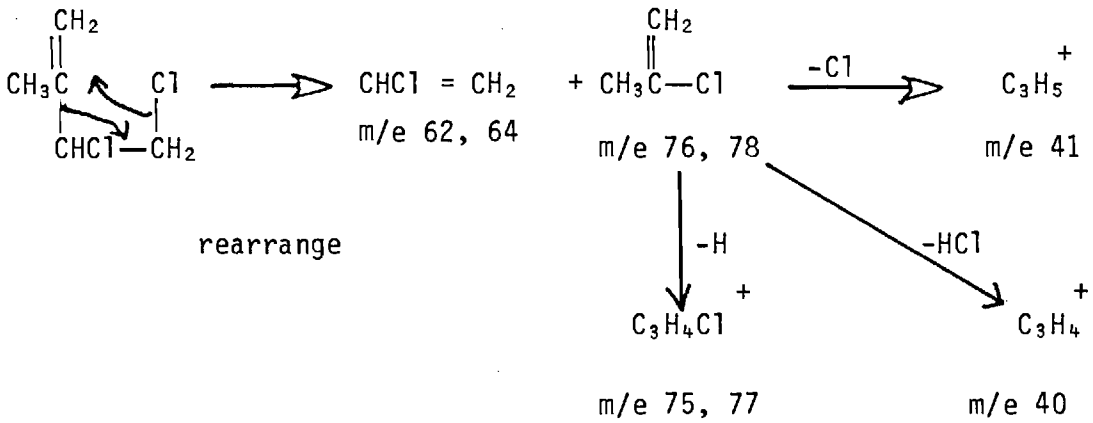


Figure 11. Compound K, Electron Impact Fragmentation
Aquatic Humics Plus Chlorine
3,4-Dichloro-2-methyl-1-butene

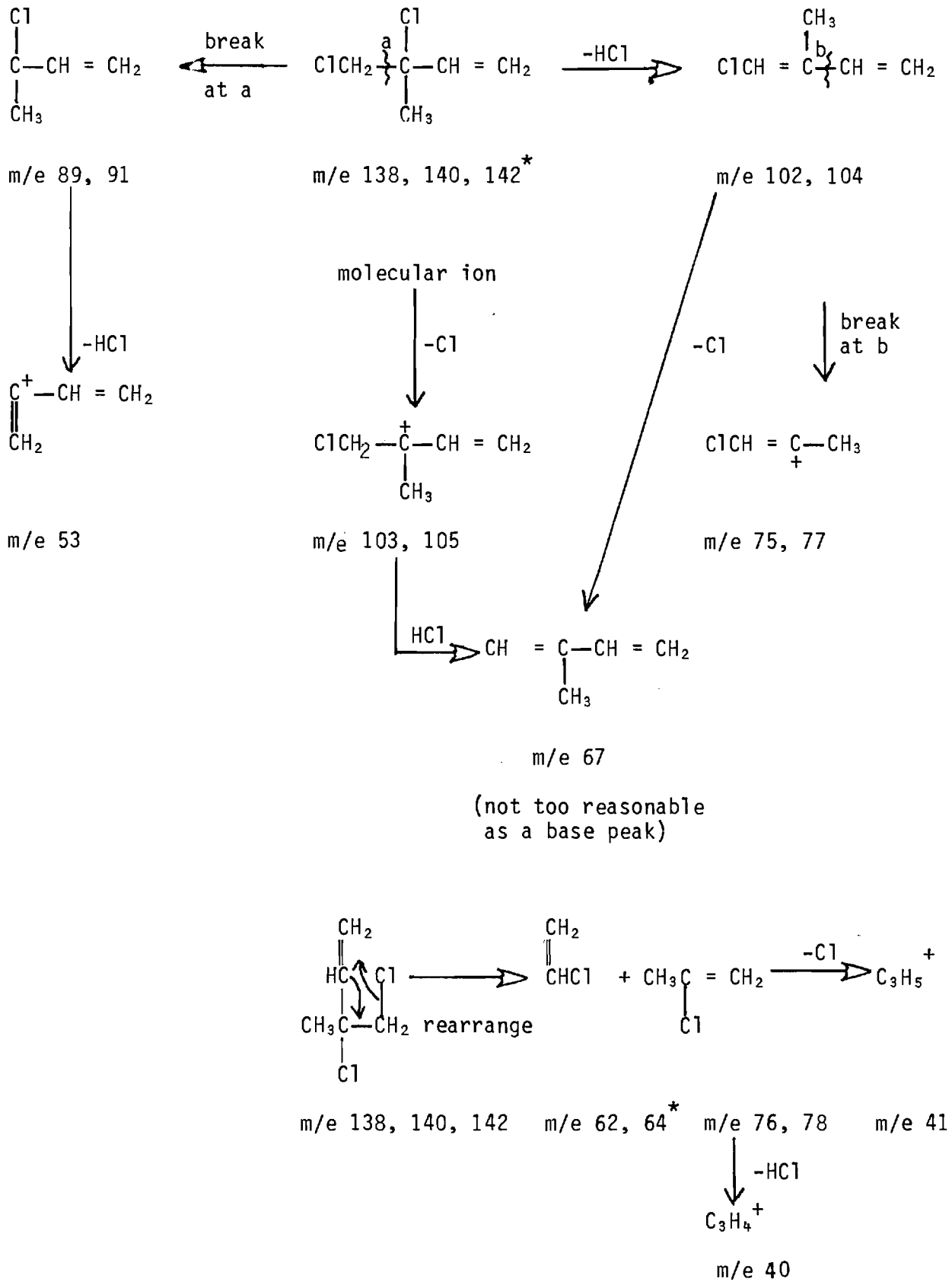


Figure 12. Compound K, Electron Impact Fragmentation
Aquatic Humics Plus Chlorine
3,4-Dichloro-3-methyl-1-butene

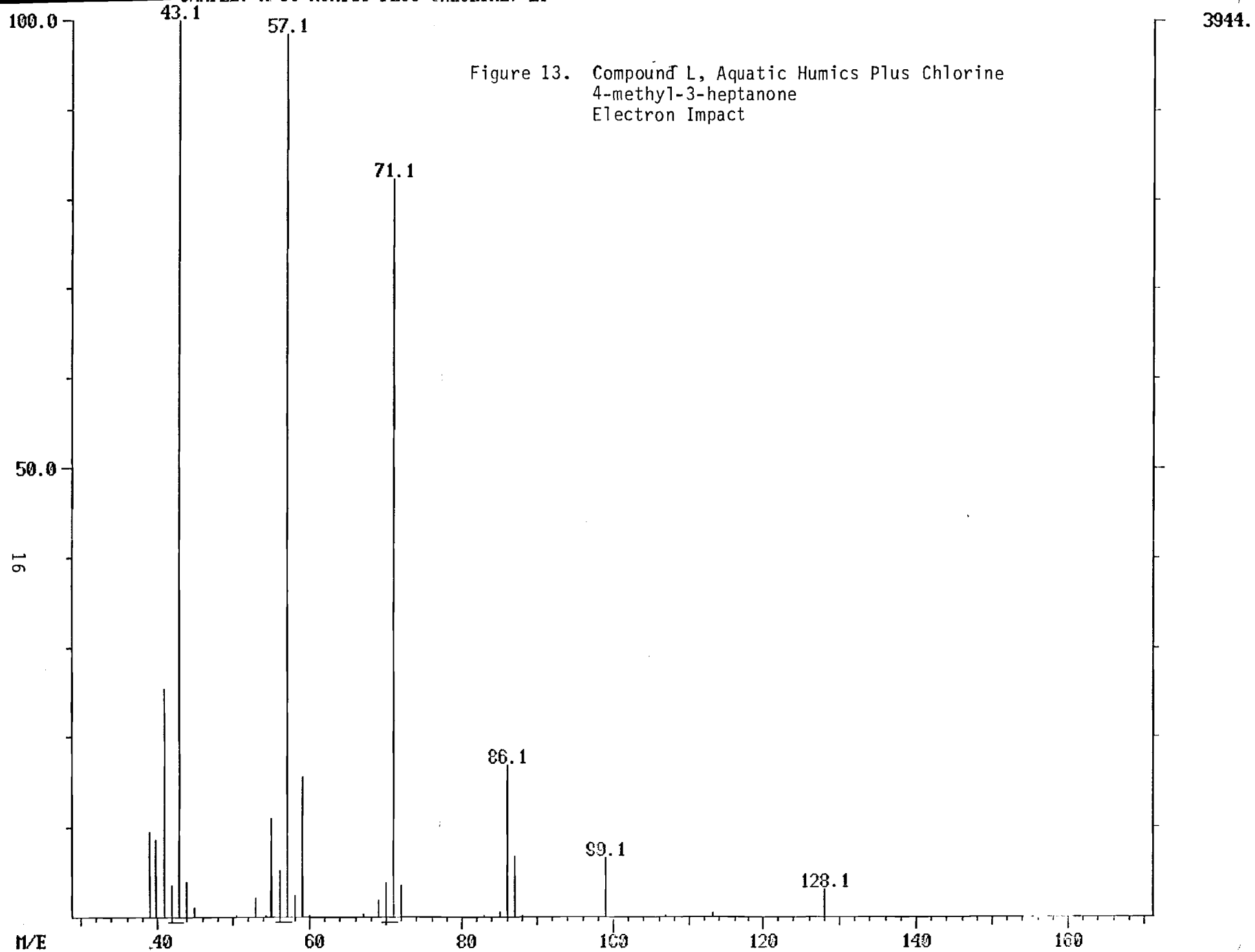
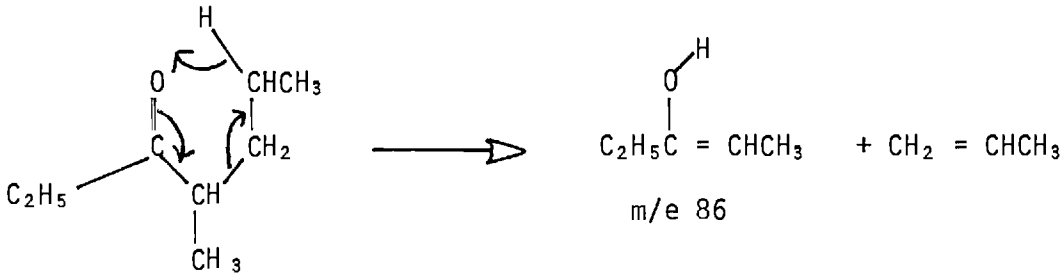


Figure 13. Compound L, Aquatic Humics Plus Chlorine
4-methyl-3-heptanone
Electron Impact

The m/e 86 peak is a classical case of the McLafferty rearrangement as shown below.



The chemical ionization spectrum supports a molecular weight of 128 with a quasi-molecular ion at m/e 129, an M + C_2H_5 ion at m/e 157 and an M + C_3H_5 ion at m/e 169. This pattern is presented in Figure 14.

M. A very tentative structural assignment has been made for compound M which again is found in the untreated aquatic humics as well as in the treated aquatic humics. The molecular weight was assigned to be 128 on the basis of a quasi-molecular ion at m/e 129 in the CI spectrum. This assignment is strengthened by the presence of strong peaks at m/e 58 and m/e 70 in the EI fragmentation pattern which presumably arise from the molecular ion by rearrangement. The loss of water from the quasi-molecular ion but not from the molecular ion suggests an alcohol which is not prone to dehydration under ordinary conditions. The suggested structure is 2-(3-OXO-) butyl cyclopropanol. The postulated fragmentation pattern is outlined in Figure 15. The EI and CI spectra are presented in Figures 16 and 17. The reviewer's comments would be particularly welcome in this case as strong peaks at m/e 41 and m/e 55 are not readily explained by this structure. Also an expected loss of methyl is not seen.

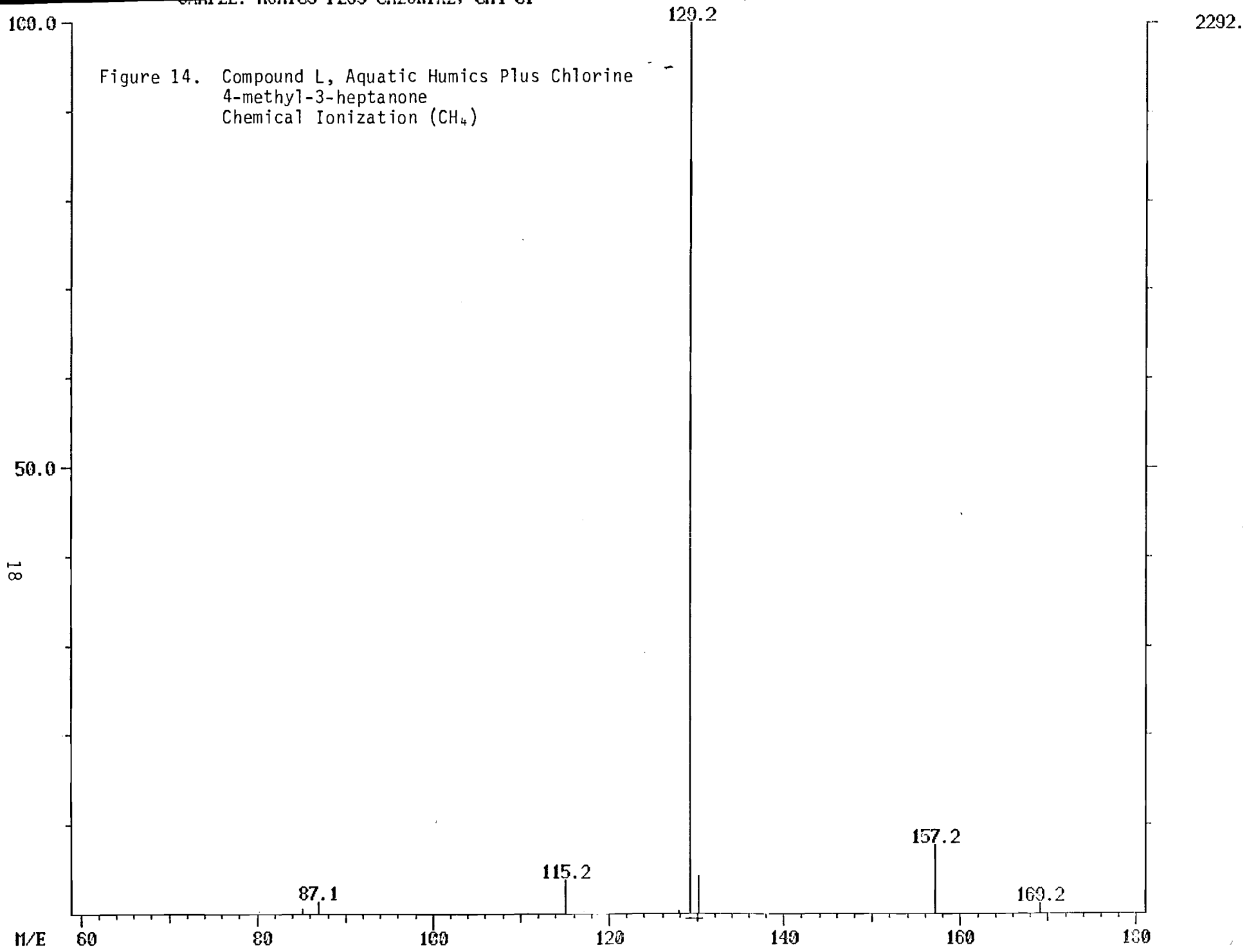
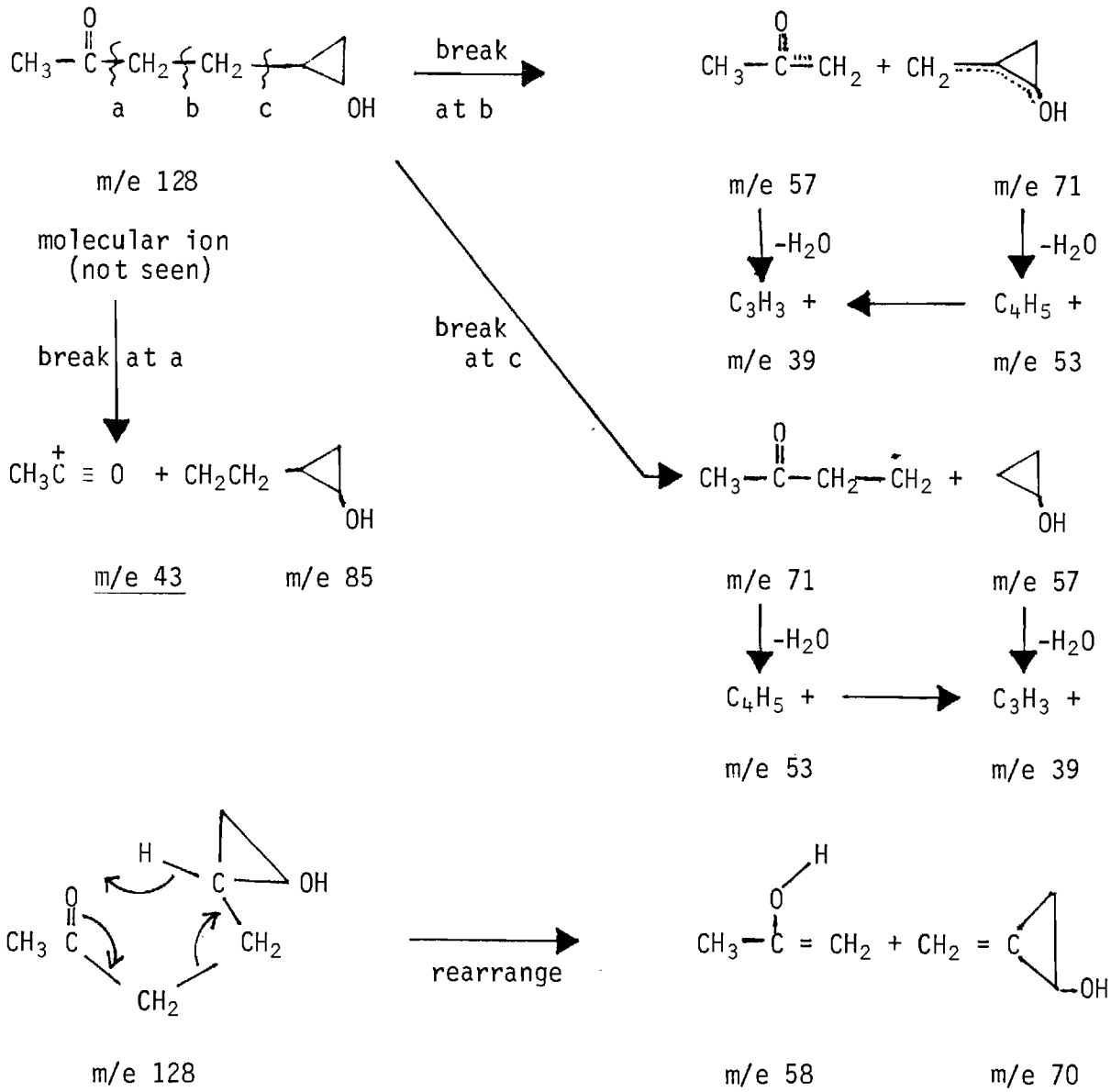


Figure 14. Compound L, Aquatic Humics Plus Chlorine
4-methyl-3-heptanone
Chemical Ionization (CH_4)

EI



CI

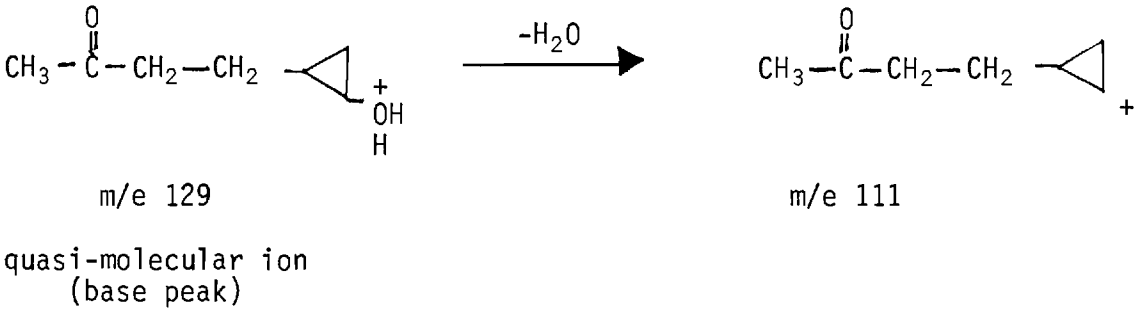


Figure 15. Compound M, Aquatic Humics Plus Chlorine
Fragmentation Patterns
2-(3-OXO-)butyl cyclopropanol

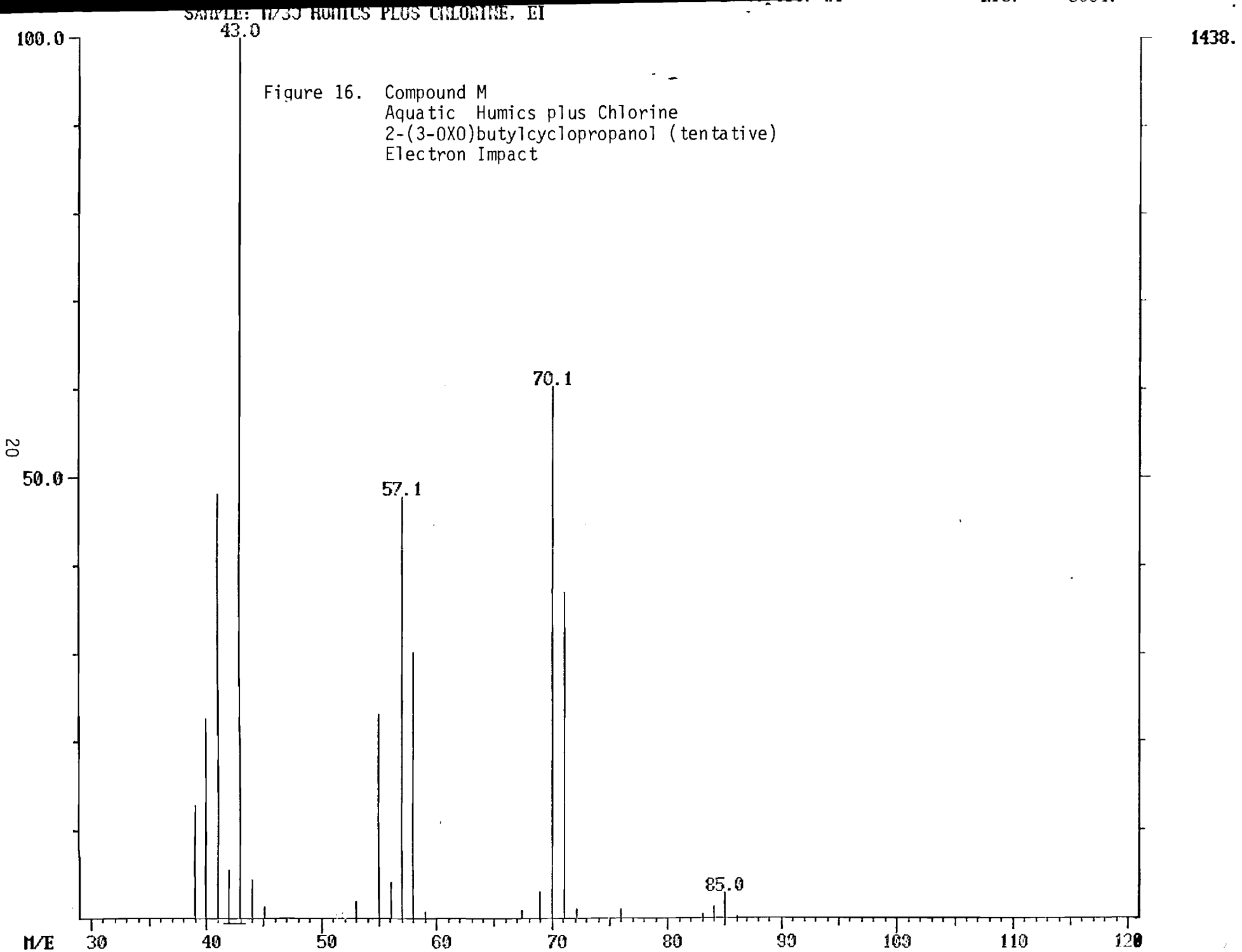


Figure 16. Compound M
Aquatic Humics plus Chlorine
2-(3-OXO)butylcyclopropanol (tentative)
Electron Impact

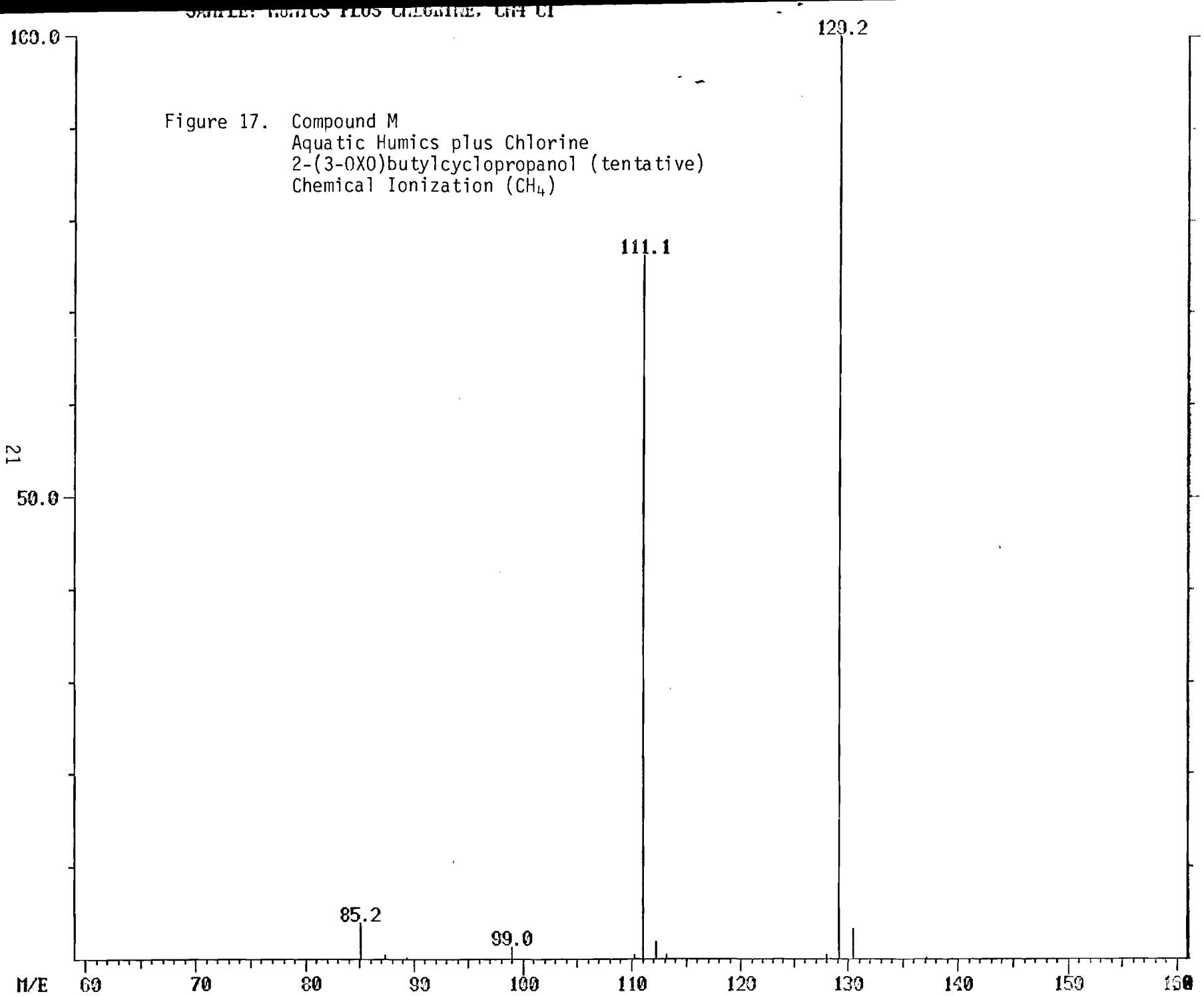
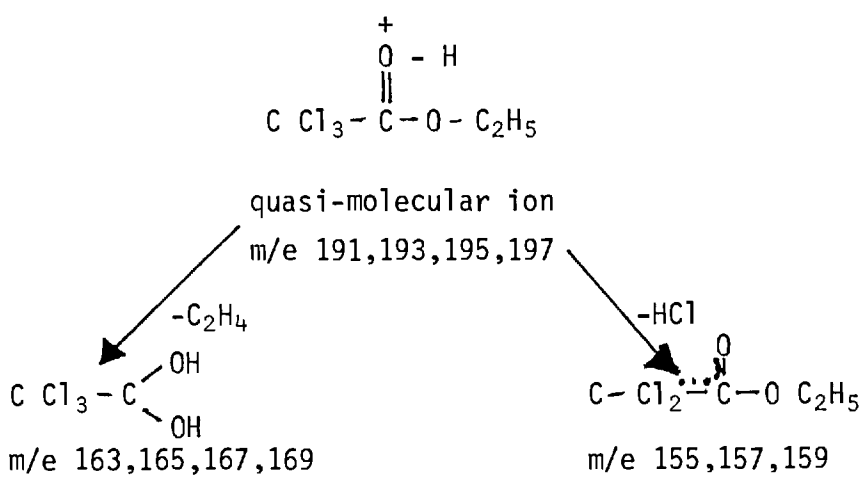


Figure 17. Compound M
Aquatic Humics plus Chlorine
2-(3-OXO)butylcyclopropanol (tentative)
Chemical Ionization (CH_4)

N. Although compound N was not completely resolved from a leading minor constituent, it has been possible to assign a structure on the basis of the EI and CI fragmentation patterns. While the CI isotope pattern at m/e 139, 141, 143 was the only major group suggesting the presence of more than one chlorine, the other major ions in the EI spectrum are more readily explained if one invokes two chlorines. The frequent loss of water seen in the CI spectrum, but not in the EI spectrum, suggests a tertiary alcohol or a similar function. It is our belief that the postulated structure 1,4-dichloro-2-methyl-2 butanol is best supported by the EI fragmentation pattern (Figure 18) and the CI spectrum (Figure 19). The postulated sequences are outlined in Figures 20 and 21, respectively.

O. The next compound to be identified was done so on the basis of its chemical ionization fragmentation pattern which is shown in Figure 22. In this case, the CI spectrum was much more intense than the EI spectrum which did not produce a good match with any of the library spectra although a trichloroacetate was one of the structures selected by the data system. A quasi-molecular ion corresponding to ethyl trichloroacetate was seen as the base peak at m/e 191, 193, 195, 197 all in the correct isotopic ratios. The protonated carboxylate was seen at m/e 163, 165, 167, 169 and a loss of HCl at m/e 155, 157, 159. This sequence is outlined below.



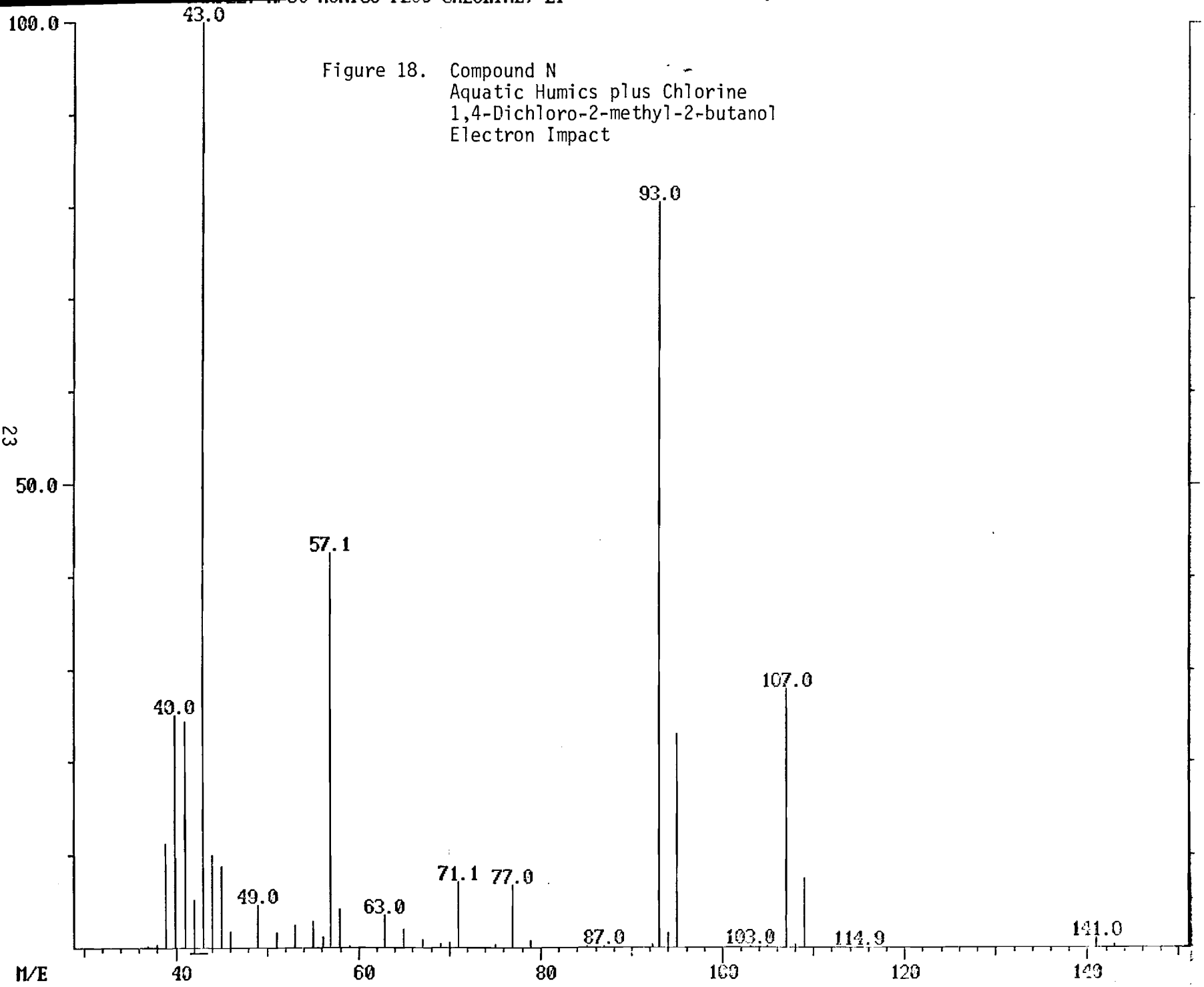


Figure 18. Compound N
Aquatic Humics plus Chlorine
1,4-Dichloro-2-methyl-2-butanol
Electron Impact

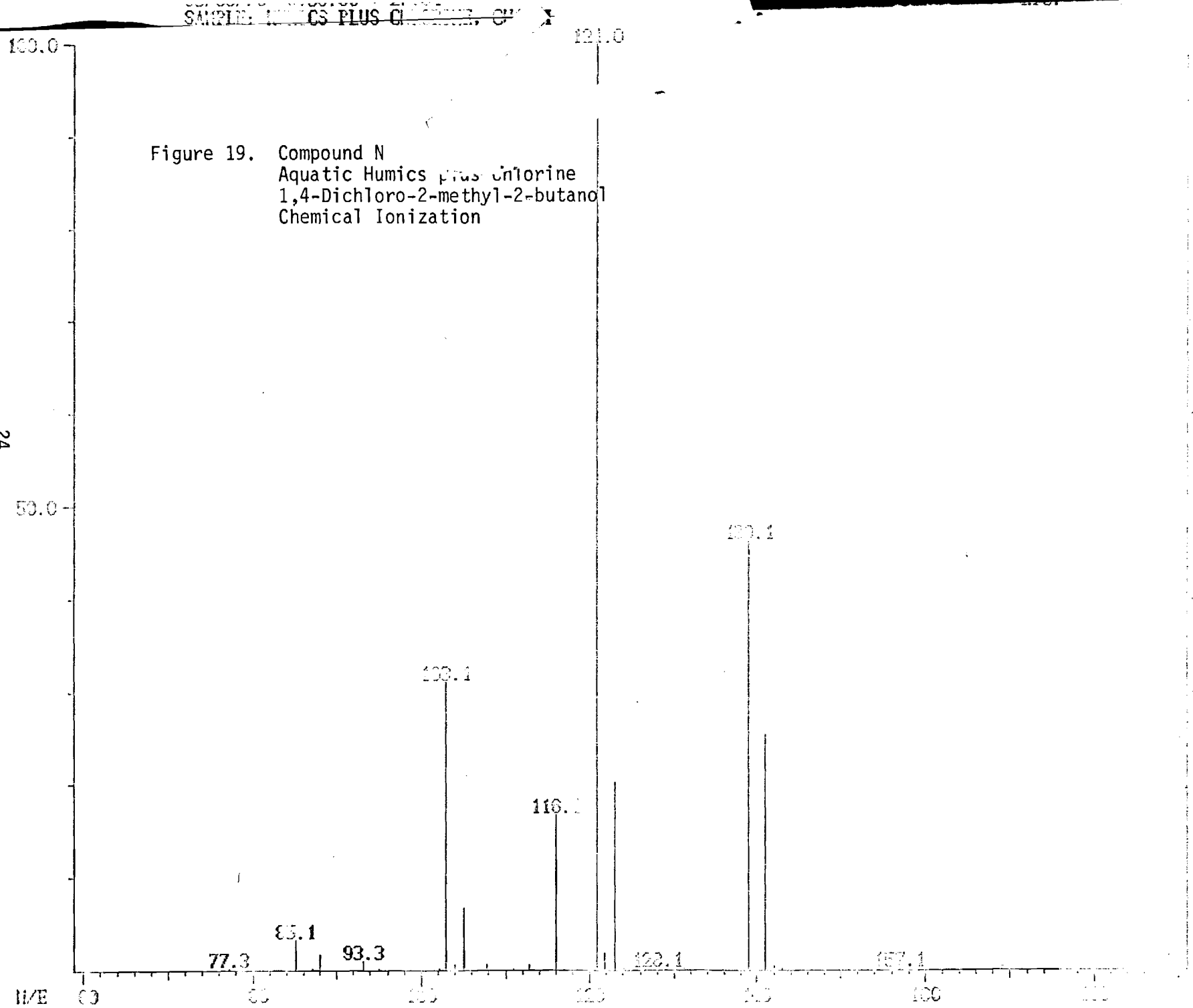


Figure 19. Compound N
Aquatic Humics plus chlorine
1,4-Dichloro-2-methyl-2-butanol
Chemical Ionization

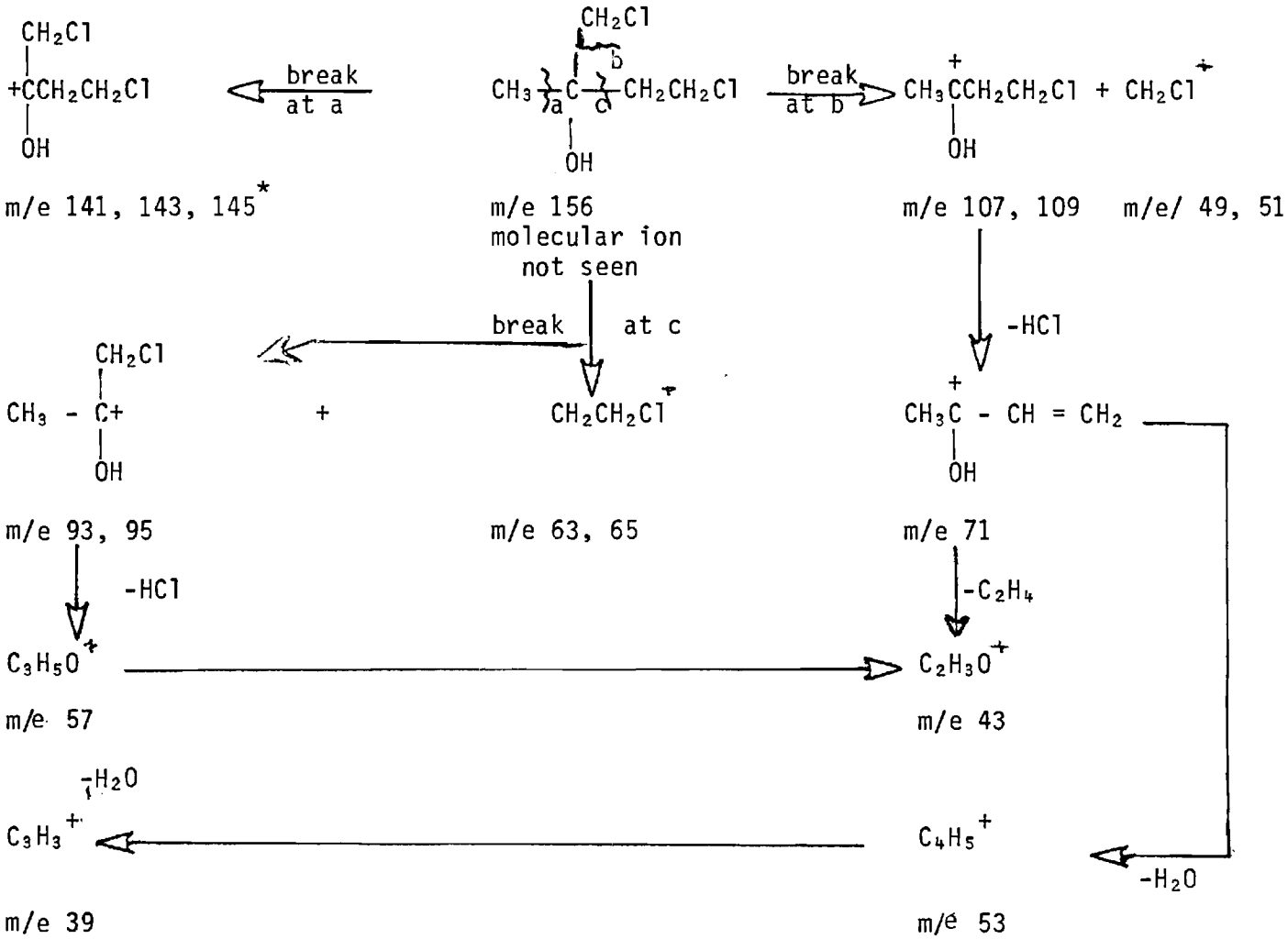
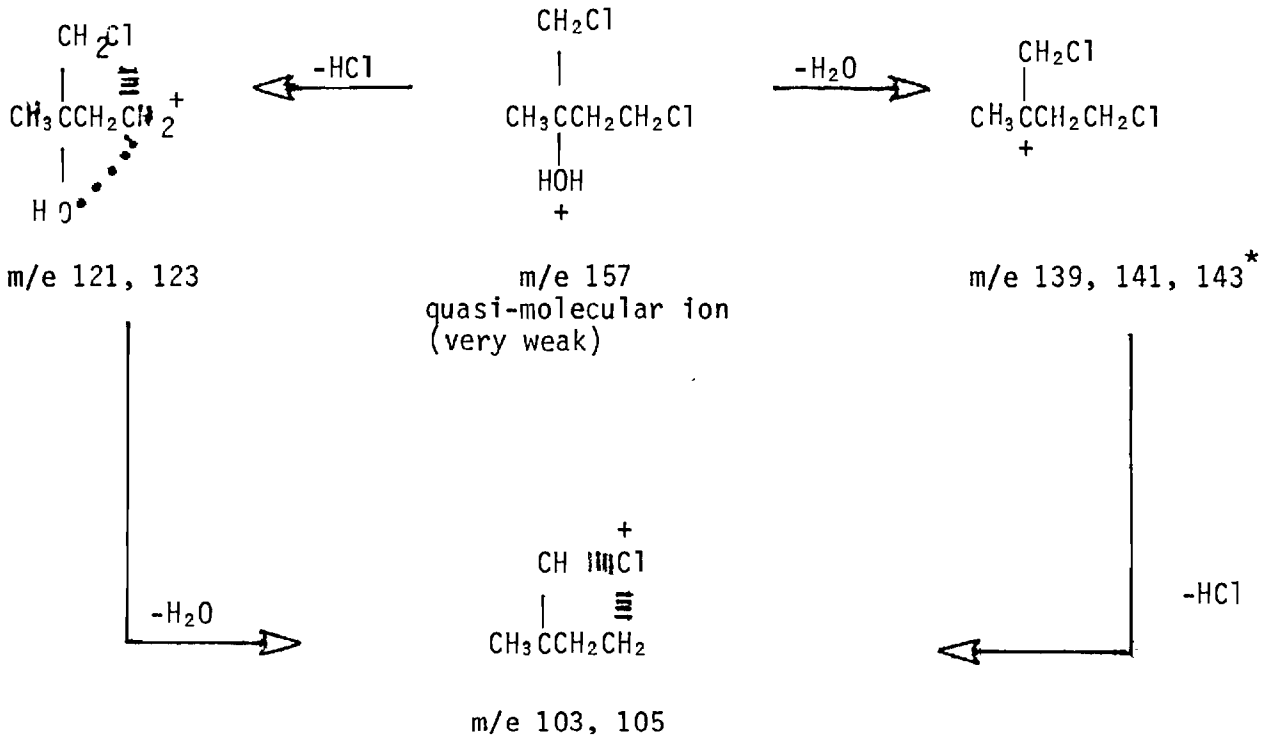


Figure 20. Electron Impact Ionization Fragmentation+
Aquatic Humics Plus Chlorine
1,4-Dichloro-2-methyl-2 butanol

*Not seen due to low intensity of the ion cluster.

+m/e 77 may be overlap from leading peak.



NOTE: m/e 116 is overlap from leading peak

Figure 21. Chemical Ionization Fragmentation
Aquatic Humics Plus Chlorine
1,4-Dichloro-2-methyl-2-butanol

*Not in proper ratio, possibly due to low intensity.

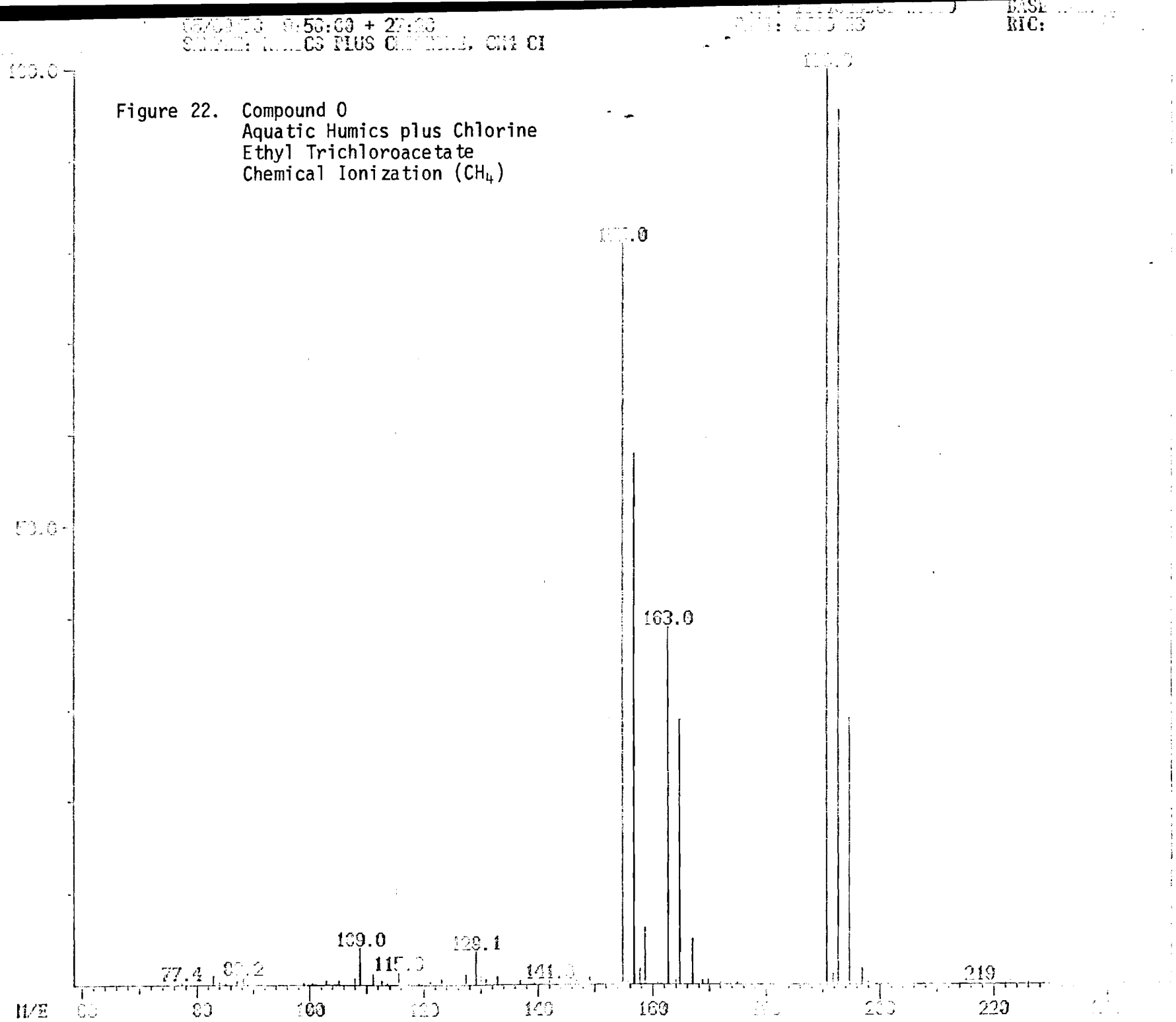
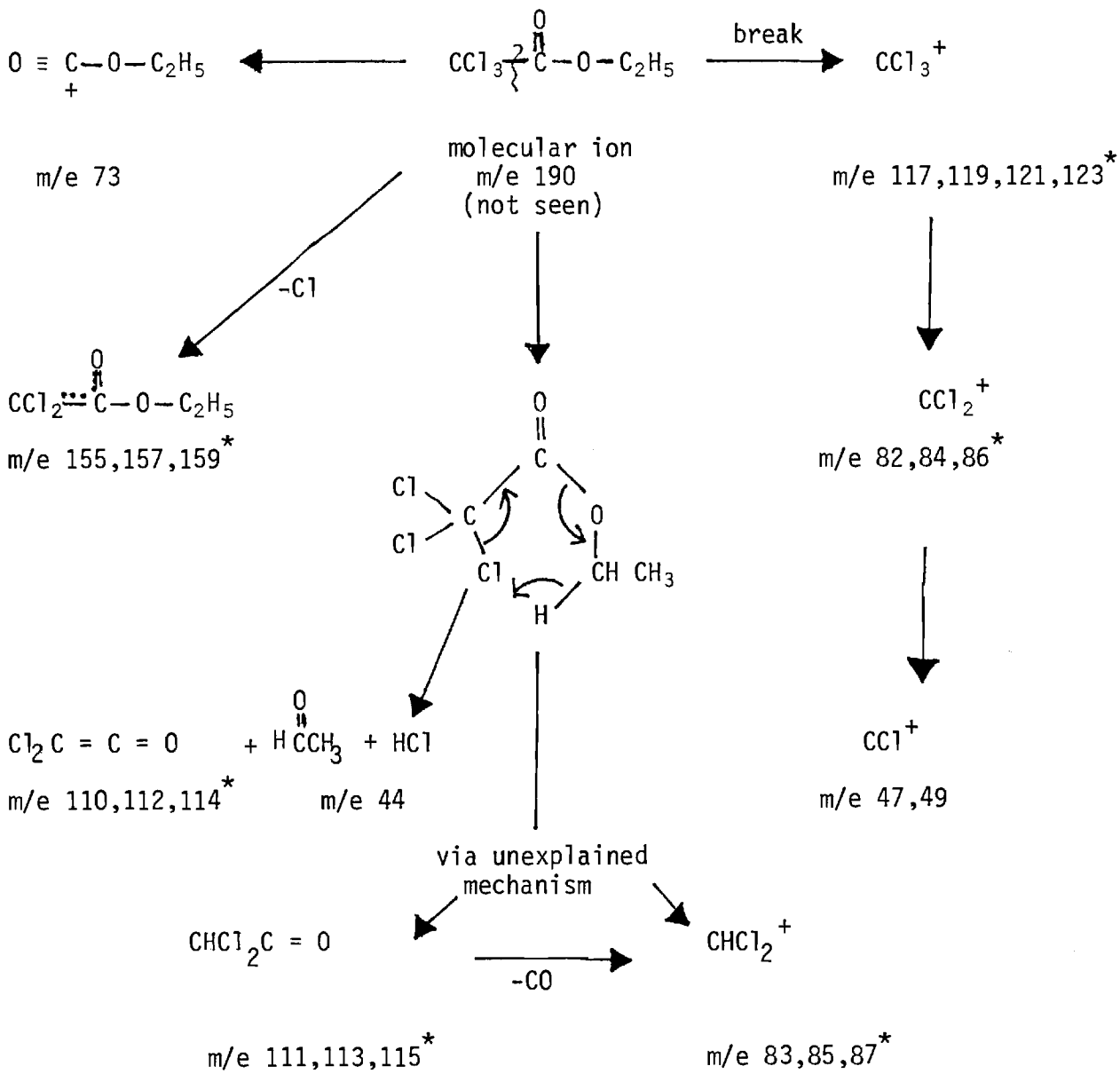


Figure 22. Compound O
Aquatic Humics plus Chlorine
Ethyl Trichloroacetate
Chemical Ionization (CH_4)

The electron impact fragmentation pattern which is shown in Figure 23 (an earlier run from the same sample is presented) is supportive of this structural assignment in that the isotopic ratios at m/e 117,119,121 are approximately correct for CCl_3^+ in spite of the fact that m/e 123 is missing. Other peaks can be explained as outlined below. The apparent presence of peaks at m/e 111,113 and m/e 83,85 require a rearrangement which is difficult to explain. The molecular ion is not seen.



03/28/78 17:18:00 + 13:52
SAMPLE: II-119-43. CL2/ETHYLATED M/30 BY EI

CALI: 0328 #1

BASE M/E: 117
RIC: 2064.

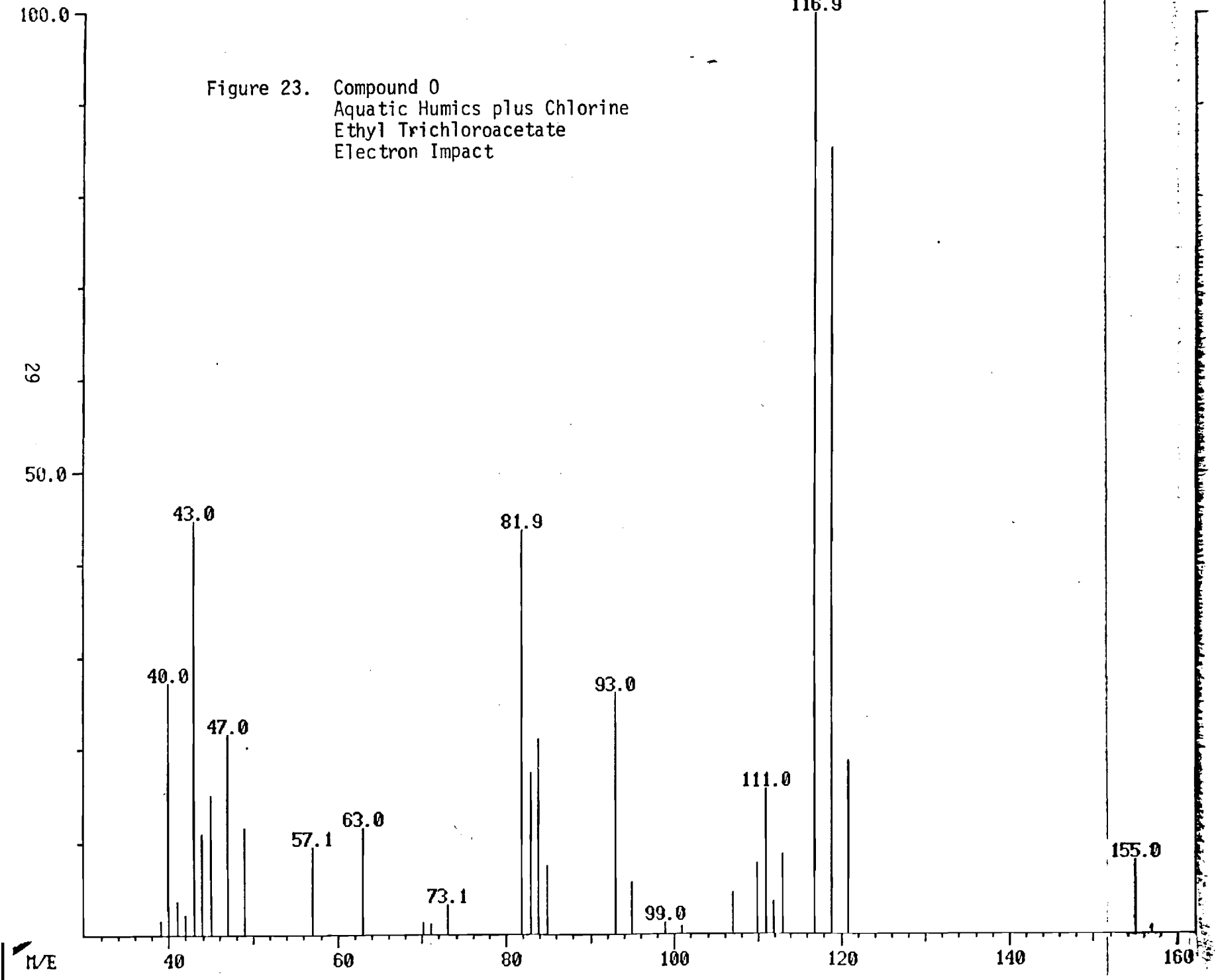


Figure 23. Compound O
Aquatic Humics plus Chlorine
Ethyl Trichloroacetate
Electron Impact

P. The next major peak eluting later than ethyl trichloroacetate in the reaction product mixture was also present in untreated aquatic humic material and exhibited a number of curious features; i.e., (1) the peak at highest mass in both the EI and CI fragmentation patterns was m/e 147, and (2) the EI fragmentation pattern showed a repetitive loss of 28 amu's. The data system reported a very good match with a compound having the formula $C_9H_{20}O_4$ and a name which made no sense at all. On the basis of the clue provided by the matched formula, we have been able to assign a structure which in retrospect, may be the very one matched in the data system's library. The proposed structure, tetra-ethoxy methane is the ortho ester of carbonic acid. It is not clear at this time whether this material is derived from aquatic humic matter or is an artifiact. The sequences outlined below completely explain the observed EI and CI fragmentation patterns which are shown in Figures 24 and 25.

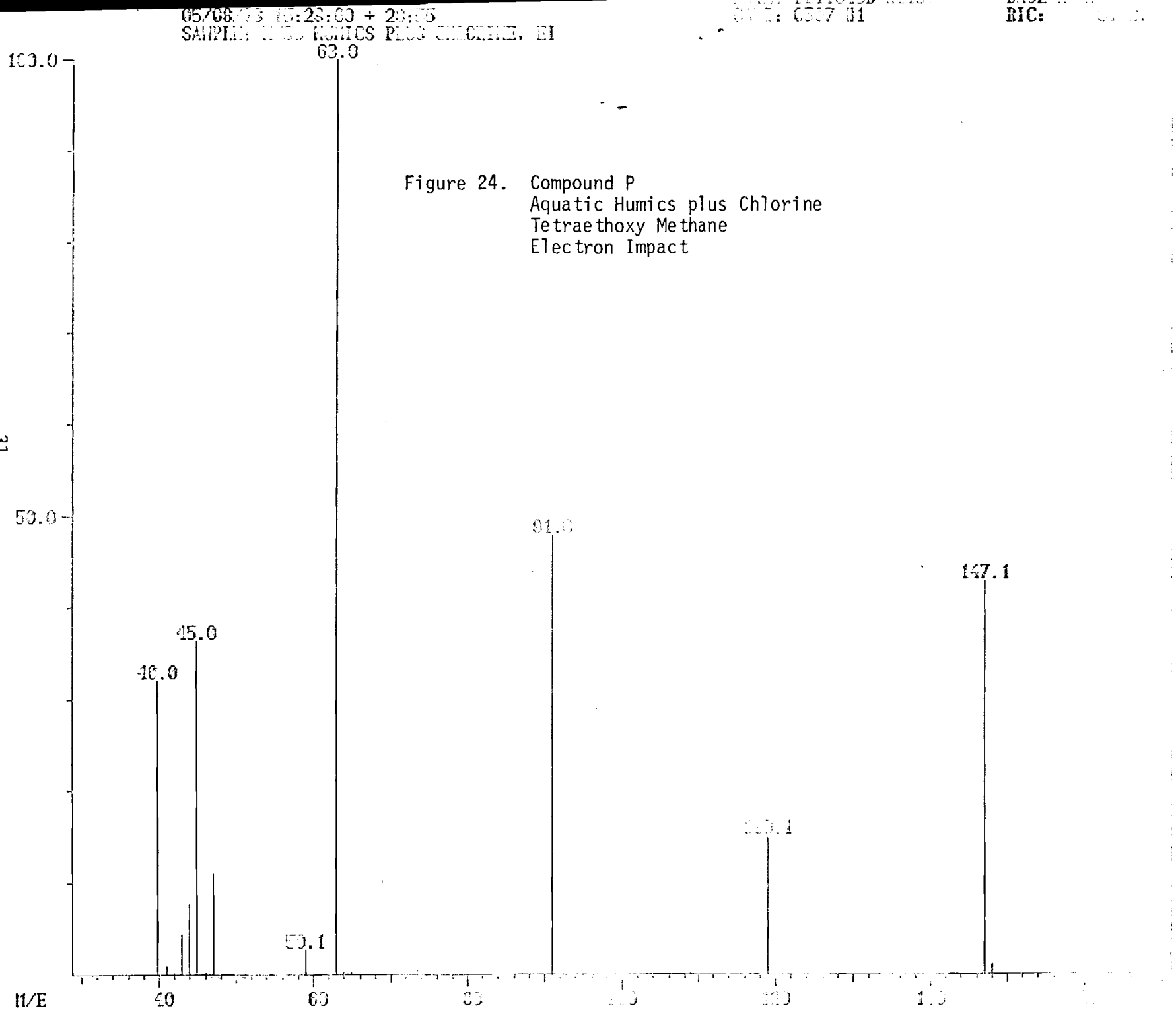


Figure 24. Compound P
Aquatic Humics plus Chlorine
Tetraethoxy Methane
Electron Impact

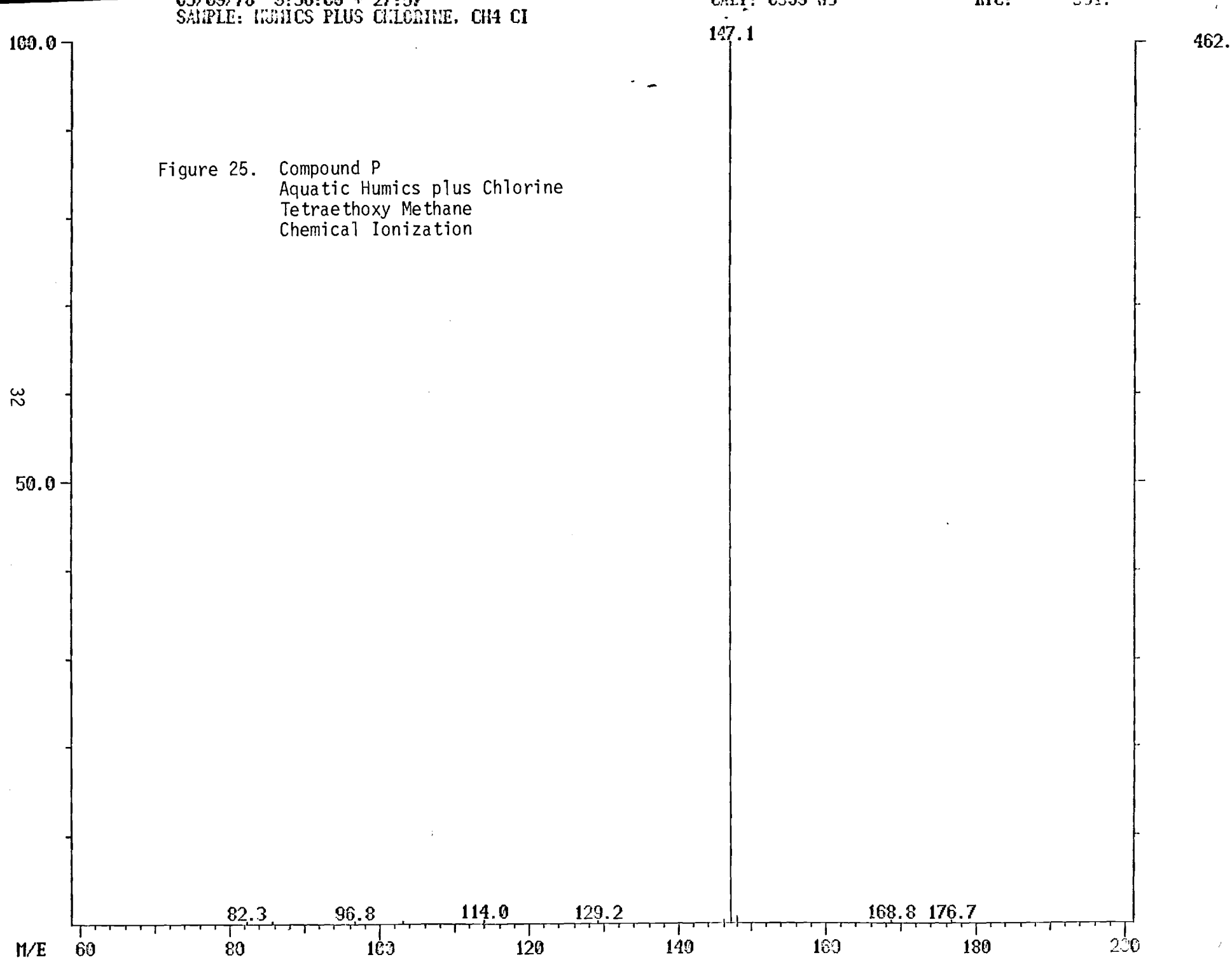
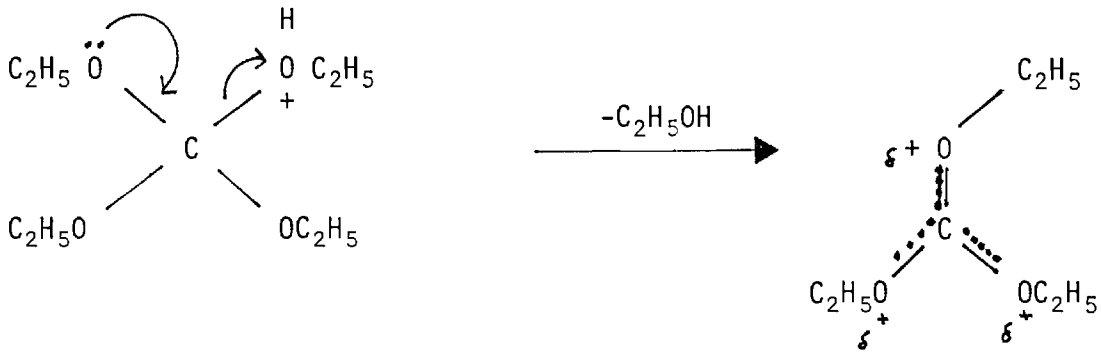


Figure 25. Compound P
Aquatic Humics plus Chlorine
Tetraethoxy Methane
Chemical Ionization



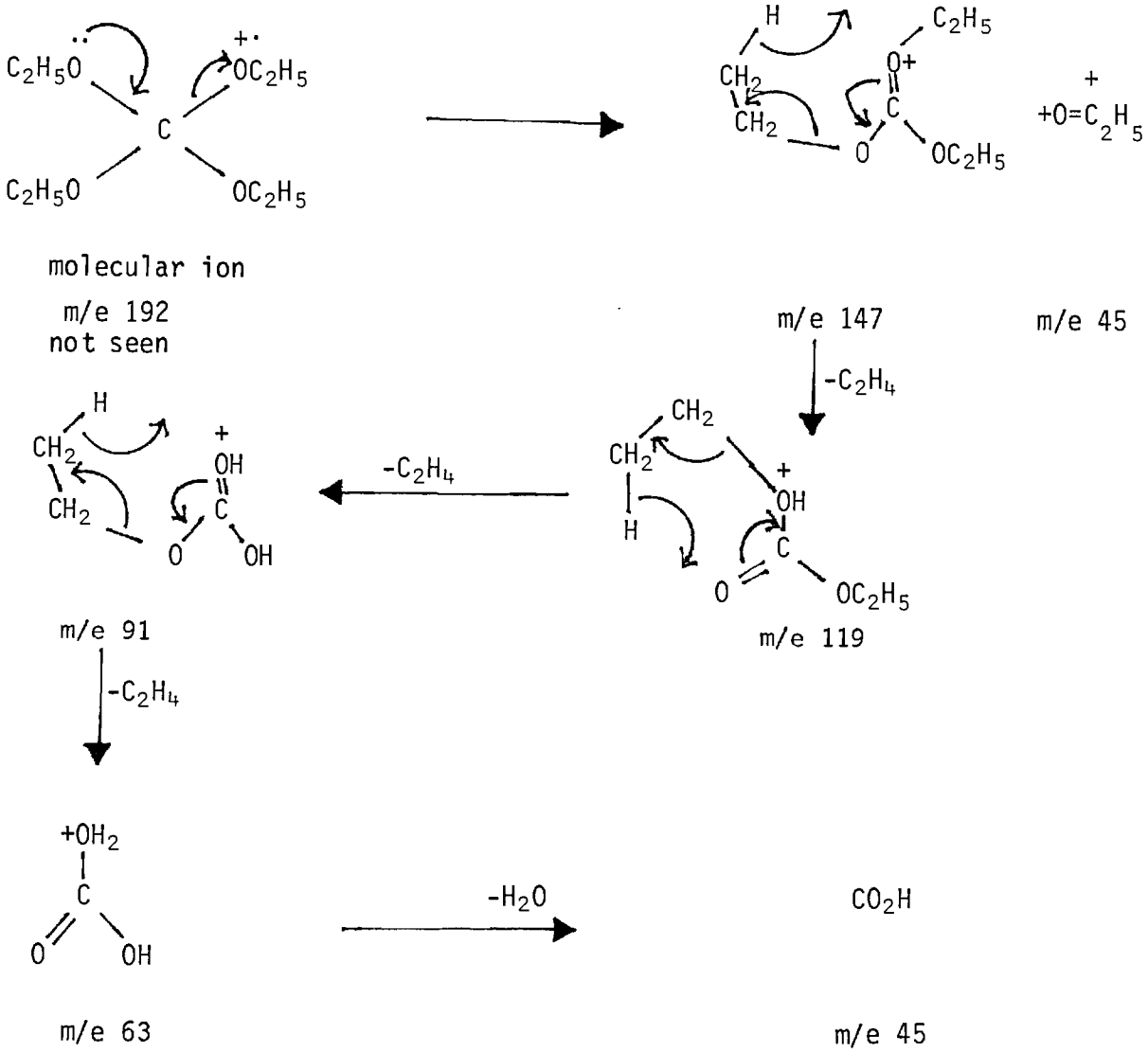
quasi-molecular ion

m/e 193
(not seen)

m/e 147
only peak

A-2-B. This component was found in run II-134 beyond heptane. It is evident from the EI spectrum (Figure 26) that this is an isomer of compound A. In fact, the two spectra are so similar that it is easy to mistake one for the other. The fact that both compounds occur in the same product mixture and elute at different times demands that different structural assignments be made. Accordingly, 2-chloro-3-methyl-2-butene was selected as being the best fit to the data. It may be that the assignments should be interchanged although the somewhat stronger molecular ion in A-2-B is supportive of the present structures. The CI spectrum (Figure 27) is presently available only from run II-134 and is partially obscured by an overlapping chromatographic peak. Thus the only ion peak which can be definitely assigned to compound A-2-B is m/e 69 representing loss of HCl from the quasi-molecular ion. A fragmentation sequence which explains the major ions in the spectra is outlined on page 37.

EI



Chemical ionization results in only one peak because the addition of a proton, $C_2H_5^+$ or $C_3H_5^+$ results in the creation of a species which can readily relieve the extreme crowding around the quaternary carbon by the expulsion of a stable neutral molecule. The common product ion is triply stabilized by participation of the remaining ethoxy oxygens as shown on the next page.

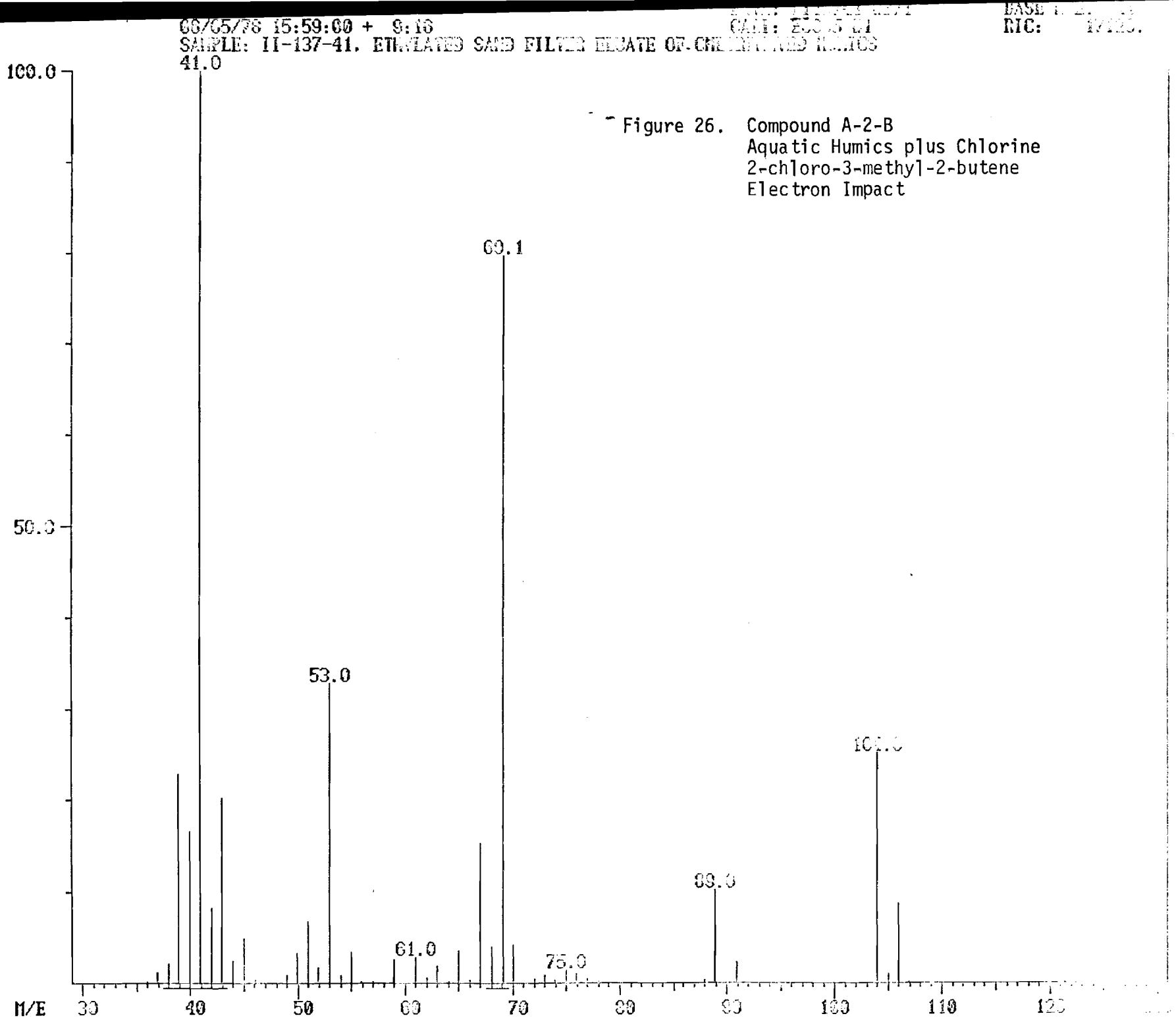


Figure 26. Compound A-2-B
Aquatic Humics plus Chlorine
2-chloro-3-methyl-2-butene
Electron Impact

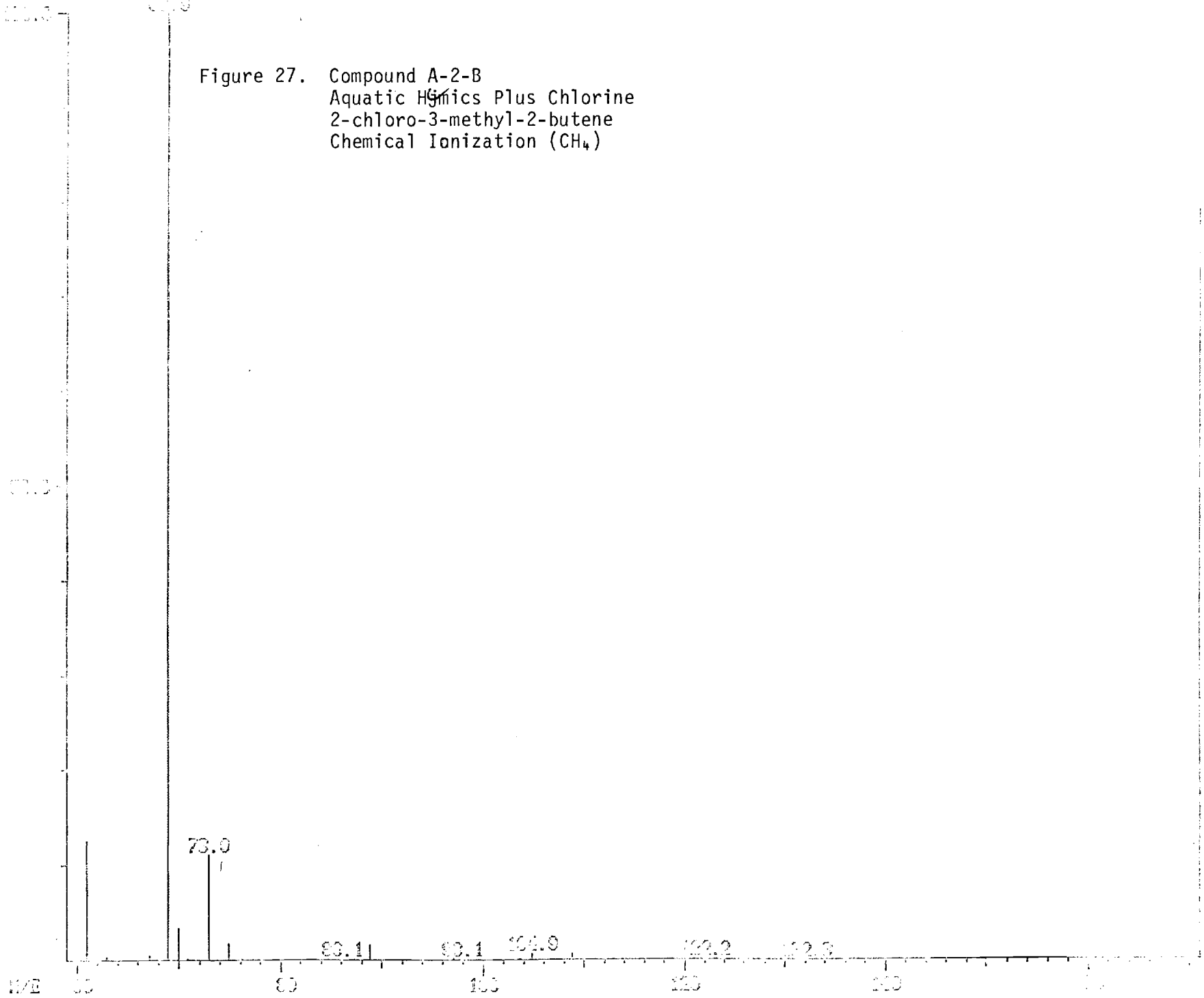
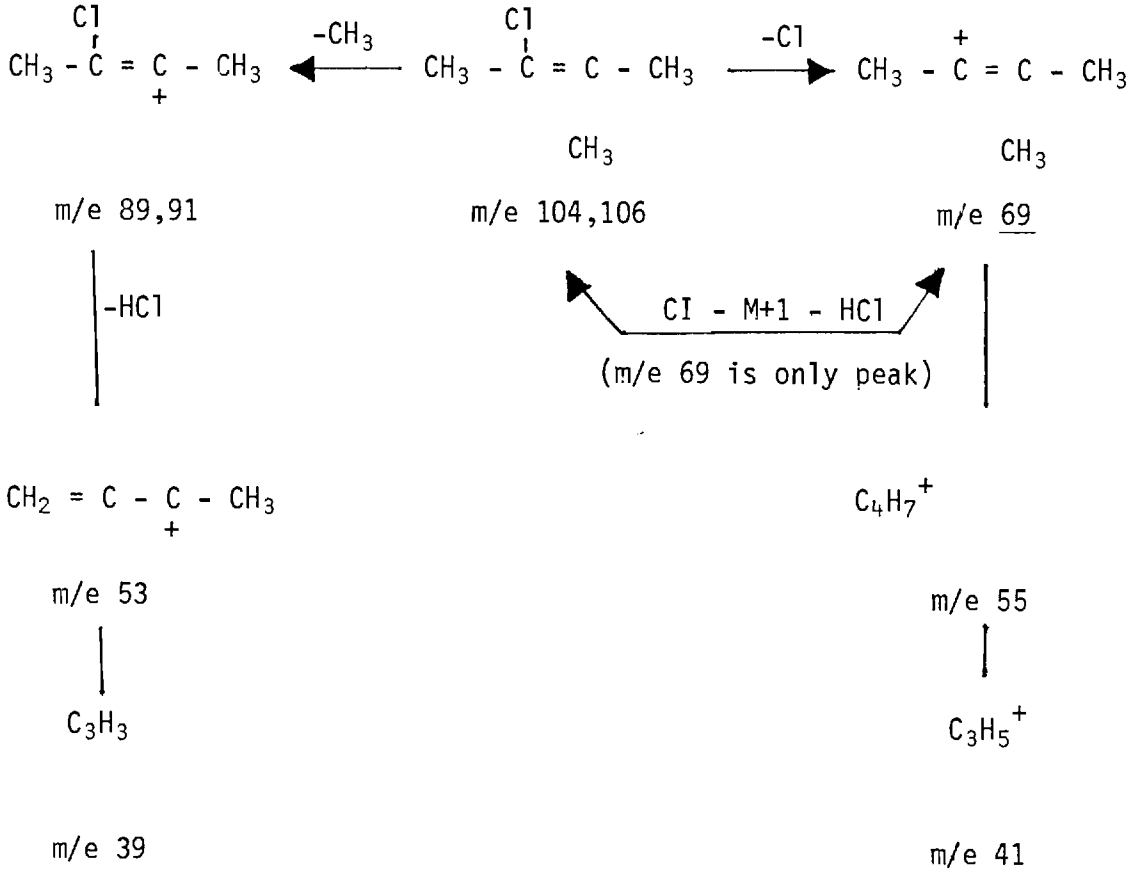


Figure 27. Compound A-2-B
Aquatic Humics Plus Chlorine
2-chloro-3-methyl-2-butene
Chemical Ionization (CH_4)



Fragmentation Sequence Compound A-2-B 2-Chloro-3-methyl-2-butene

IV. AQUATIC HUMICS PLUS CHLORINE - COMPARISON OF PRODUCT MIXTURES

In accordance with the systematic approach for interpreting and presenting the data gathered in connection with the mini-pilot experiments with aquatic humic substances, an expanded version of the product list (Table IV, p. 34, last report) is presented as Table I. Four new products were added to the list (compounds I, K, M and P). A number of other compounds have been tentatively identified in both the controls and product mixtures but have not been described in the previous section because both the EI and CI data have not been examined to date. In general, the writers prefer not to postulate a structure without matching EI and CI evidence. Product mixture II-145 is quite interesting in that it contains a large number of chlorinated products. Finding one's way through the data in this case was particularly difficult on account of the fact that a large number of related isomers, particularly of K and N are present. Thus chlorinated isopene derivatives continue to dominate the product mixtures. Mixture II-149, on the other hand, does not seem to contain any chlorinated products. In this case, the CI data have not yet been processed thus making it difficult to make positive assignments.

The relevant total ion chromatograms are presented in Figures 1-4 (run II-110, sand filter eluent), Figure 28 (run II-134, reservoir control), Figure 29 (run II-134, sand filter eluent), Figures 30 and 31 (run II-145, sand filter eluent), and Figure 32, (run II-149, sand filter eluent).

Table I

Chlorinated Aquatic Humics Product List

		Run Number				
		II-110 Sand Filter	II-134 Sand Filter	II-134 Reservoir Control	II-145 Sand Filter	II-149 Sand Filter
Pre A-1	3-Methyl-2-butanone	ND*	X***	X	ND	---
A	3-Chloro-2-methyl-1-butene	X	X	ND	X	ND
A-1-B	Heptane	ND	X	X	ND	---
A-2-B	2-Chloro-3-methyl-2-butene	ND	X	ND	X	ND
B	2-Methyl-3-pentanone	X	X	X	X	---
B-2-C	3-Methyl-2-butanol	ND	X	ND	---	---
C	3-Chloro-2-methyl-2-butanol	X	---**	ND	X	ND
D	4-Hydroxy-3-methylbutanal	X	---	X	X	---
E	2,3-Dichloro-2-methylbutane	X	---	ND	X	---
F	1-Chloro-2-methyl-2-butanol	X	---	ND	X	ND
G	1,3-Dichloro-2-methylbutane	X	---	ND	X	ND
H	3-Chloro-2-methyl-1-butanol	X	---	ND	X	ND
I	4-Chloro-3-methyl-1-butanol	X	---	ND	X	ND
J	N-Nitrosodiethylamine (artifact)	X	---	X	X	X
K	3,4-dichloro-methyl butene isomer	X	---	ND	X	X
L	4-Methyl-3-heptanone	X	---	X	ND	X
M	2-(3-oxo-)butylcyclopropanol	X	---	X	ND	---
N	1,4-Dichloro-2-methyl-2-butanol	X	---	ND	X	---
O	Ethyl Trichloroacetate	X	---	ND	X	---
P	Tetra-ethoxy methane	X	---	X	ND	---

*ND indicates looked for but not detected.
** indicates analysis incomplete.
*** indicates detected.

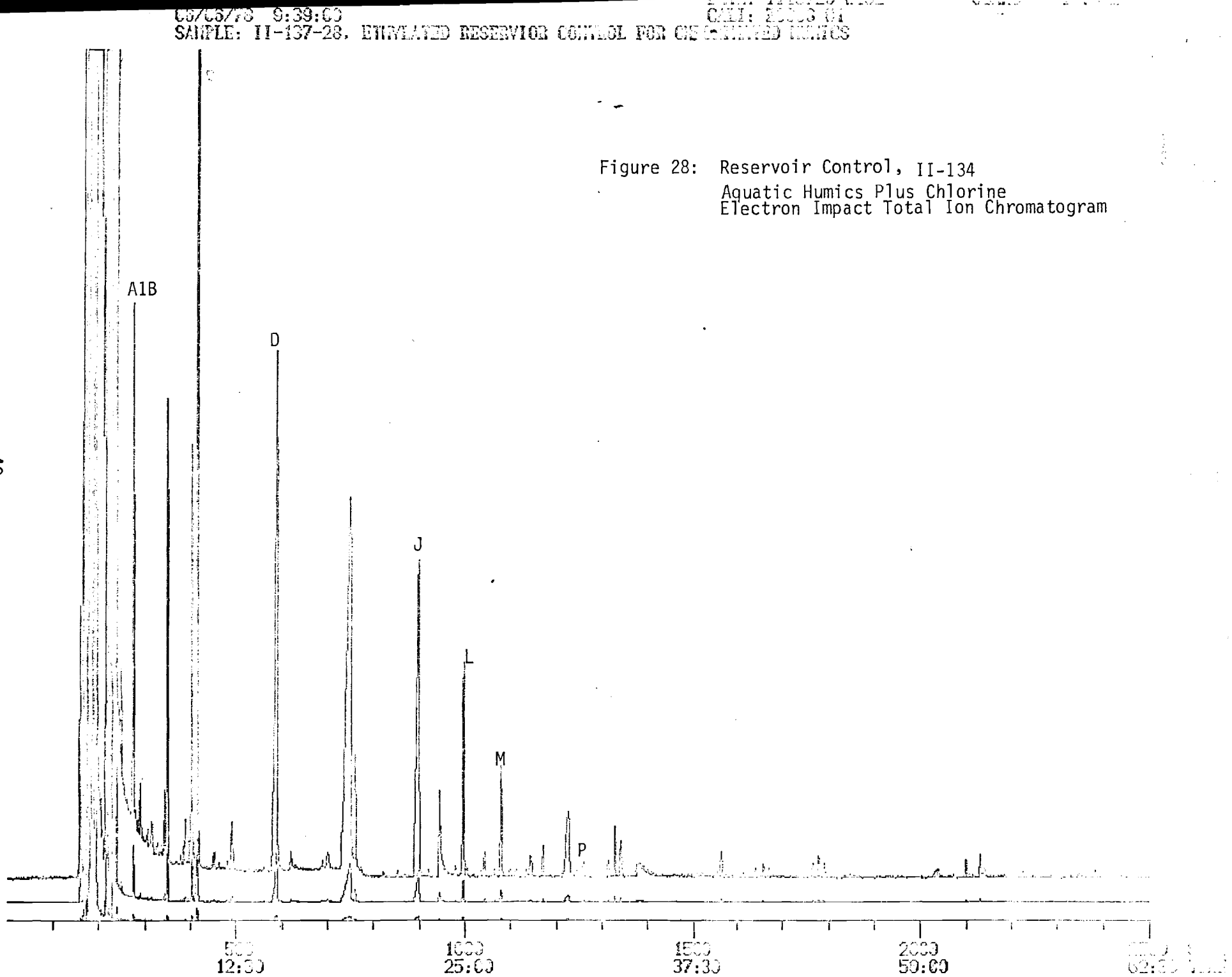


Figure 28: Reservoir Control, II-134
Aquatic Humics Plus Chlorine
Electron Impact Total Ion Chromatogram

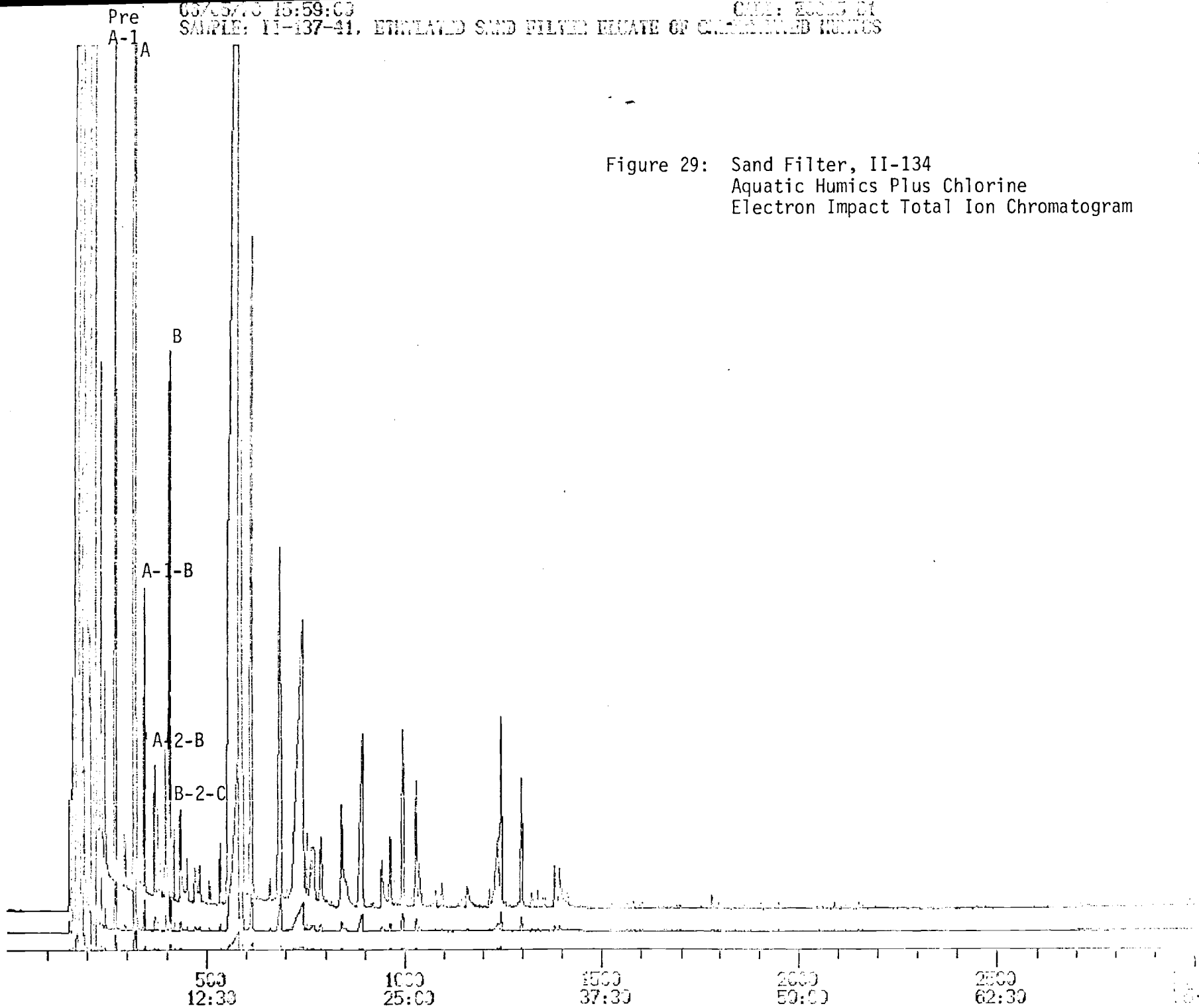


Figure 29: Sand Filter, II-134
Aquatic Humics Plus Chlorine
Electron Impact Total Ion Chromatogram

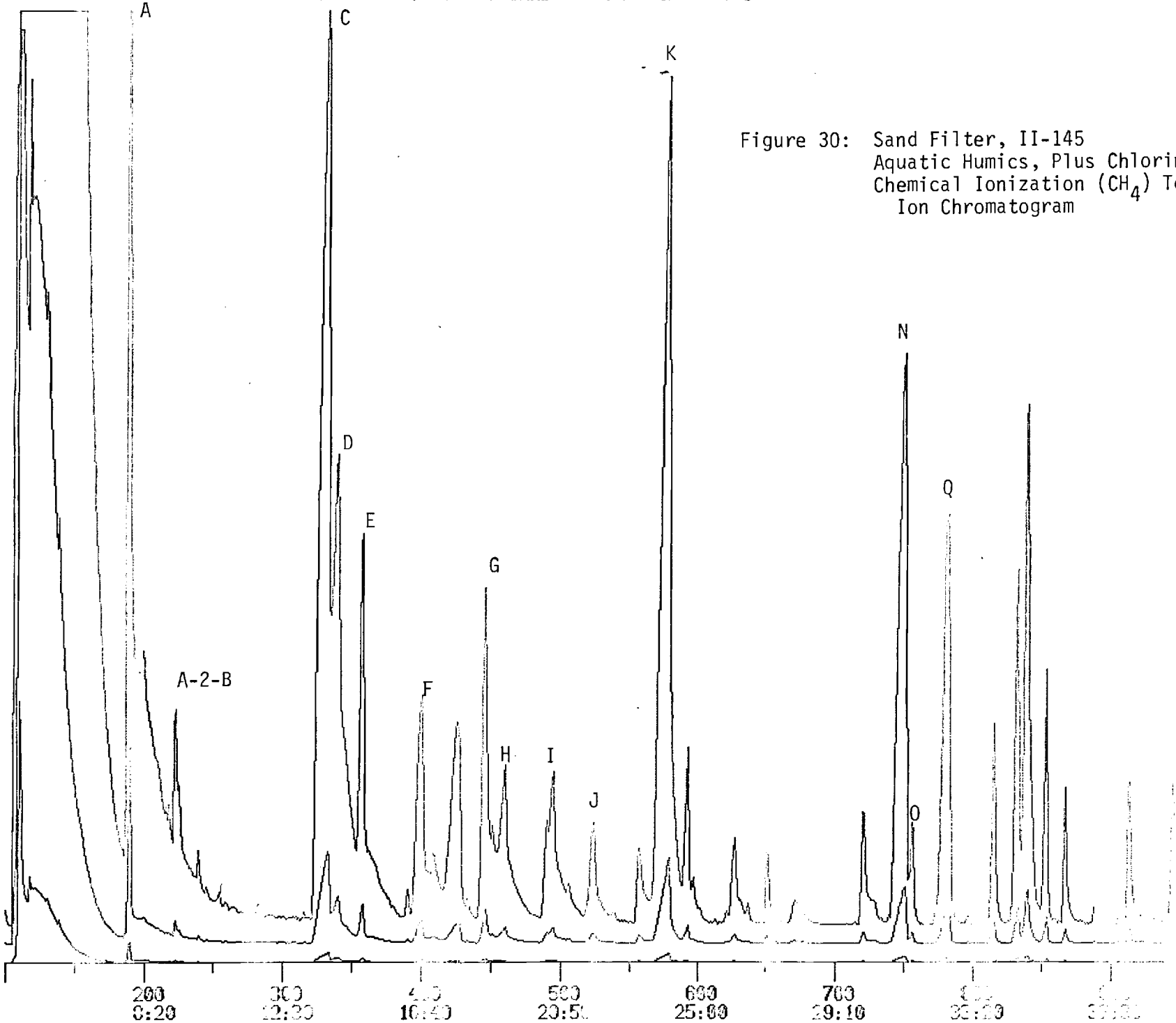


Figure 30: Sand Filter, II-145
Aquatic Humics, Plus Chlorine
Chemical Ionization (CH_4) Total
Ion Chromatogram

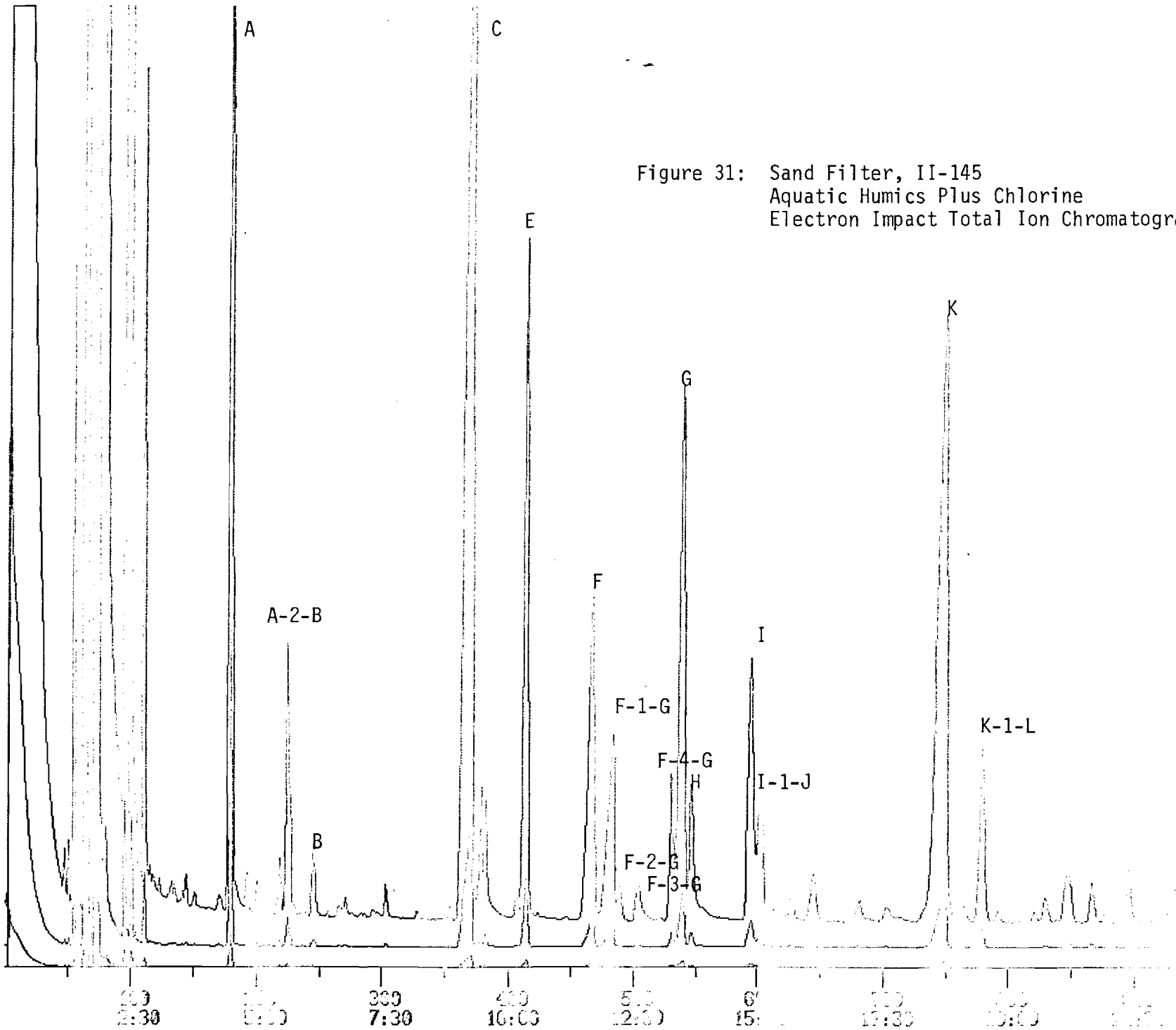


Figure 31: Sand Filter, II-145
Aquatic Humics Plus Chlorine
Electron Impact Total Ion Chromatogram

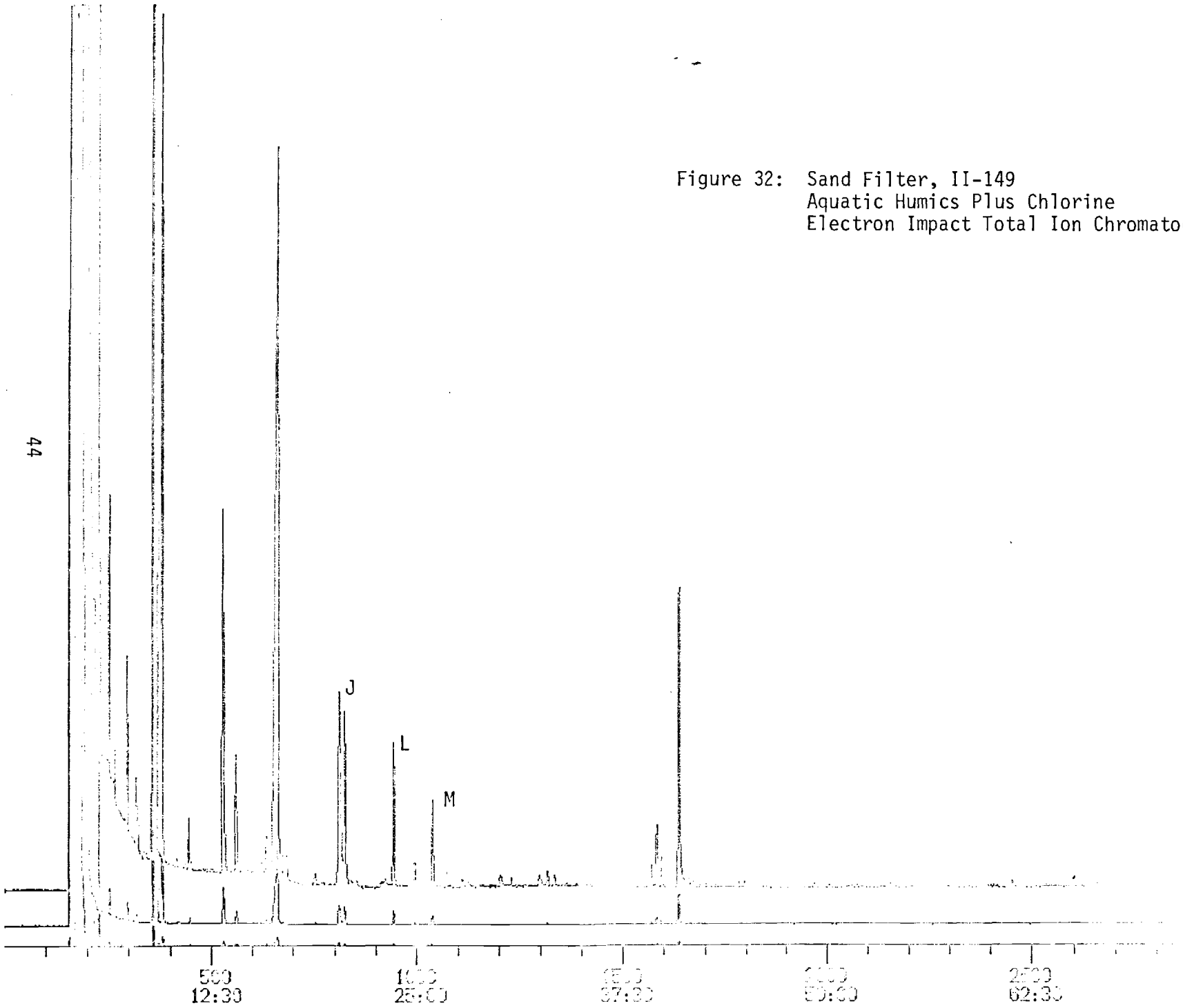


Figure 32: Sand Filter, II-149
Aquatic Humics Plus Chlorine
Electron Impact Total Ion Chromatogram

V. ISOLATION OF AQUATIC HUMICS

Isolation of aquatic humics from our most recent trip to the Satilla River has provided us with a yield of 24.1 g of material from 392 liters of river water. When sampling was undertaken, it was noted that the road crossing the tributary from which we sample was under construction. Although active work did not seem to be taking place at the bridge from which we sampled, this nearby activity may explain the increased levels of hydrocarbons found in the water.

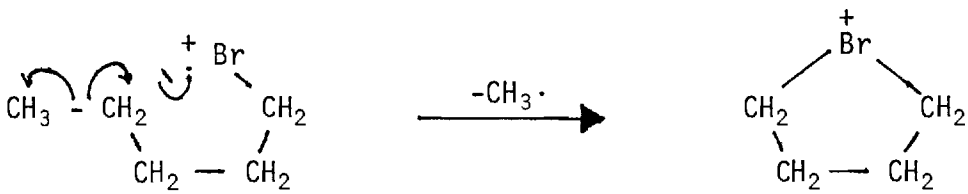
A few weeks later, another member of the staff who happened to be passing through the area noted that the wooden bridge was being replaced by a concrete structure. Upon closer inspection, a slight oil slick was noted on the tributary itself. Hopefully, this will clear up as the construction activity ceases. If not, a more suitable site will be found.

VI BROMINATION OF AQUATIC HUMICS

A sample (M/30, 1.0g) of aquatic humic material was dissolved in water (200 ml); brought to pH 7.1 by the addition of a few drops of concentrated sodium hydroxide solution and treated with excess bromine (2.5 ml). The reaction mixture was stirred overnight before being extracted, first with pentane and then with ethyl acetate. A second, delayed extraction performed two days later using ethyl acetate resulted in the isolation of additional brominated material. This observation suggests that larger, water-soluble bromoorganics are initially produced which may subsequently break down into less hydrophilic substances which are then removed by the second ethyl acetate extraction.

The EI and CI total ion chromatograms (Figures 33 and 34) of the pentane extracts show at least ten components in the mixture, four of which appear to be dominant. The peak labeling scheme is the same as that employed in earlier sections. Different structures, of course, will correspond to the letters. Thus it will be necessary in future correspondence to refer to the reaction first and the compound by letter second. A description of the structural assignments follows.

A. The electron impact spectrum of this compound (see Figure 35) appears to be quite straightforward and shows a good match with both 3-bromopentane and 2-bromopentane with all fit indices above 900. The lack of a strong peak at m/e 135,137 corresponding to the cyclic bromonium ion shown below argues that compound A is not 1-bromopentane.



m/e 135/137
(not seen)

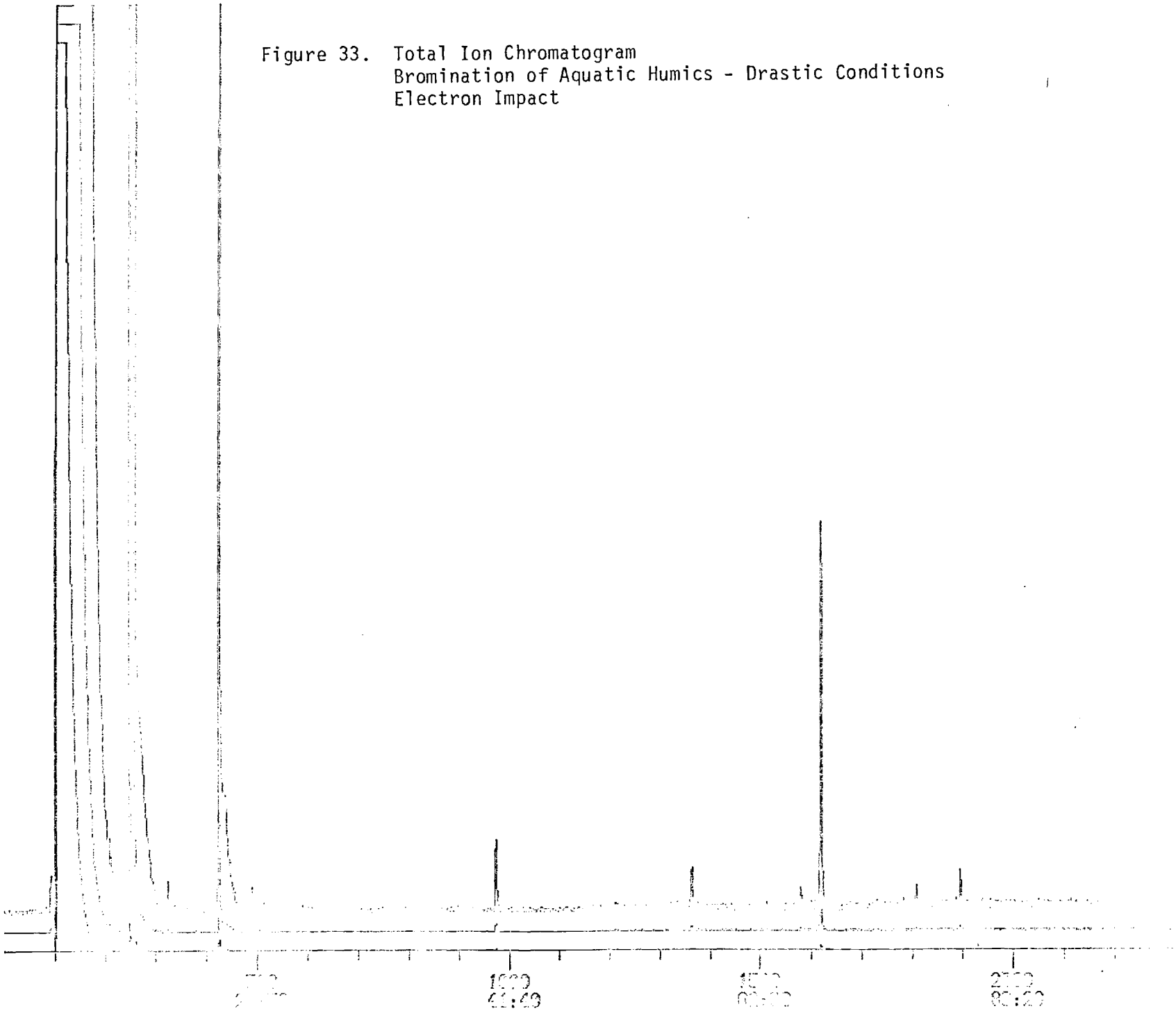


Figure 33. Total Ion Chromatogram
Bromination of Aquatic Humics - Drastic Conditions
Electron Impact

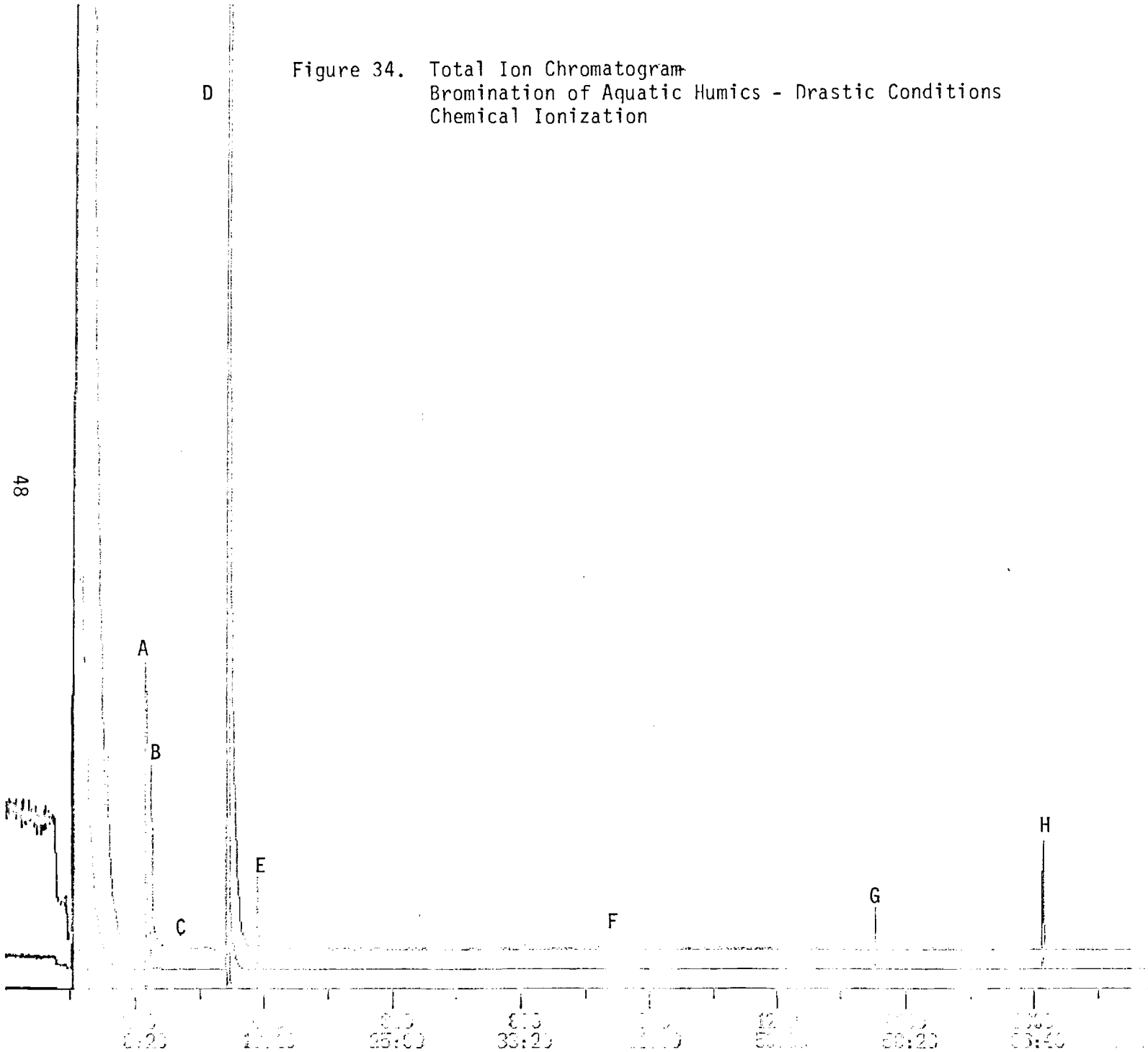


Figure 34. Total Ion Chromatogram
Bromination of Aquatic Humics - Drastic Conditions
Chemical Ionization

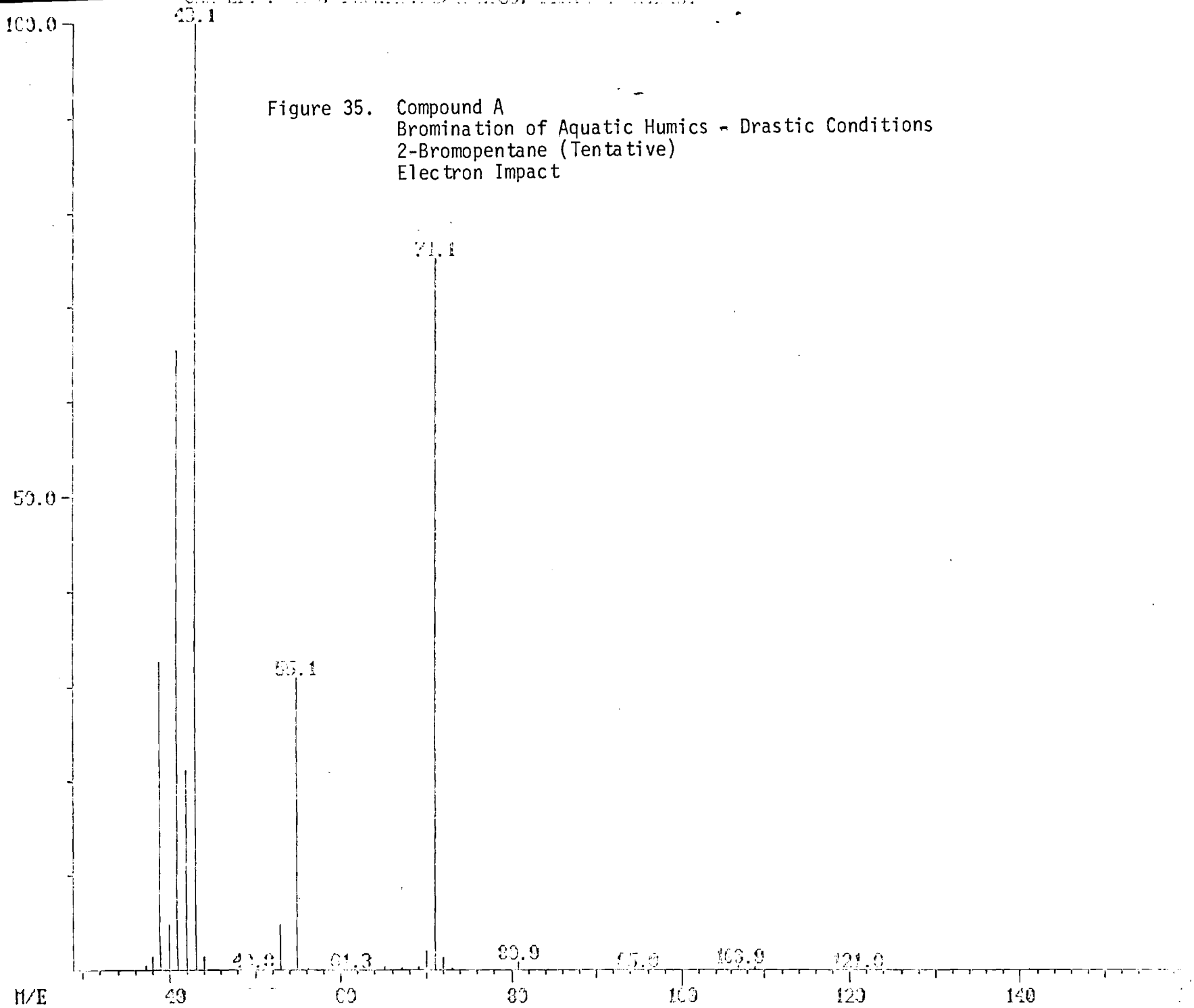
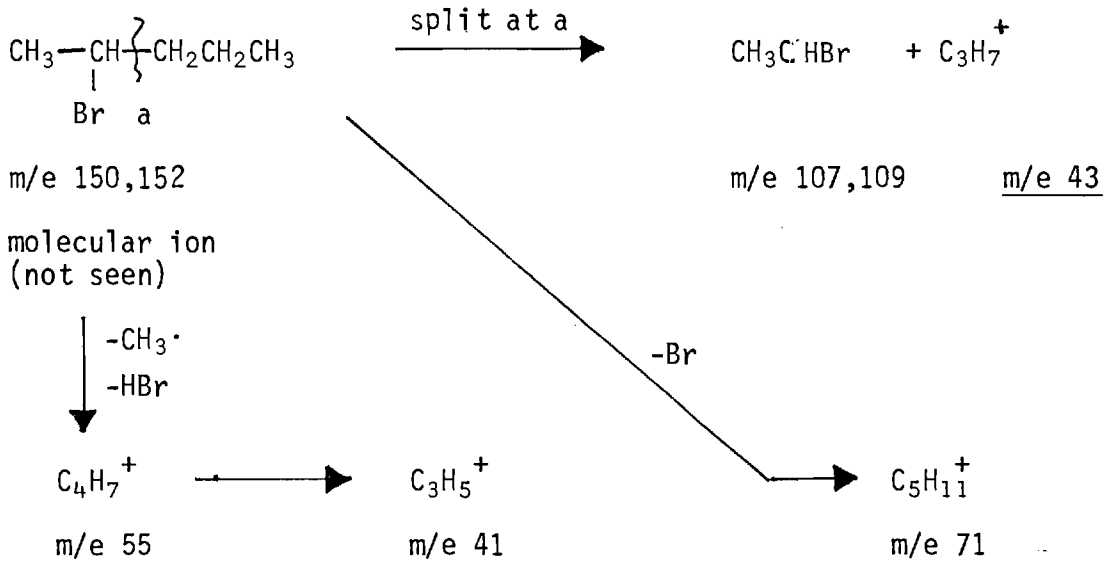


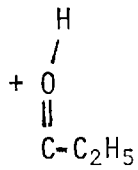
Figure 35. Compound A
Bromination of Aquatic Humics - Drastic Conditions
2-Bromopentane (Tentative)
Electron Impact

Since the ions which are seen are more easily rationalized by the 2-bromopentane structure, this is the structure which has been tentatively assigned. Unfortunately bromoalkanes are particularly prone to rearrangement. Therefore, the 3-bromopentane structure cannot be excluded. In fact, isoprene structures related to the chlorination products would seem to be more consistent with past results. Furthermore, until the control results are in, the possibility that the compound may be an artifact cannot be dismissed. A fragmentation sequence is outlined below. The spacing of 16 amu's between M+-Br and the next lower ion has been reported for other bromoalkanes.

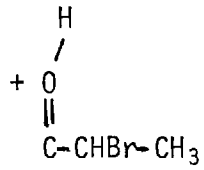


The chemical ionization spectrum which is presented in Figure 36 further confuses the structural assignments. The base peak, instead of being at m/e 71 resulting from the loss of HBr from the quasi-molecular ion is observed at m/e 72. This observation plus the fact that bromine-containing peaks are seen at m/e 136,138 and m/e 150,152 led us to consider structures related to bromoketones in which case the following ion structures might be assigned.

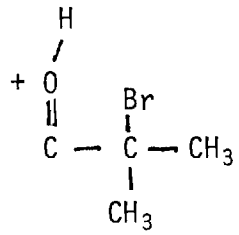
Figure 36. Compound A
Bromination of Aquatic Humics - Drastic Conditions
2-Bromopentane (Tentative)
Chemical Ionization (CH_4)



m/e 72



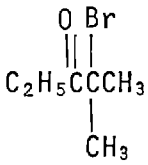
m/e 136,138



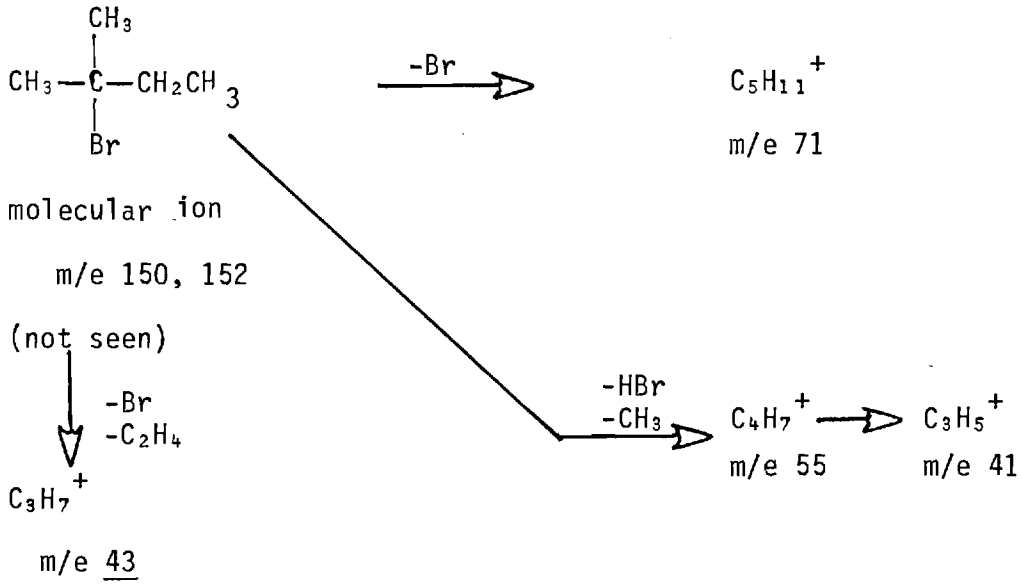
m/e 150,152

Unfortunately, we have not been able to fit all three ions into the same structure and therefore remain unable to explain this spectrum. Work with known bromoalkanes is underway in the hope that some of these compounds might also exhibit this unusual behavior.

B. Both the EI and CI spectra (see Figures 37, 38) recorded for compound B are virtual overlays with those of compound A. The CI peaks above m/e 72 are even weaker and no peak is seen at m/e 136, 138. From this standpoint, the ketonic structure shown below seems reasonable



However, one might expect to see a m/e 57, 43 series arising from the $C_2H_5C{\equiv}O$ ion at m/e 71 in the EI fragmentation. Instead, the 16 AMU gap characteristic of bromoalkanes is seen at m/e 71, 55. Thus, we are again inclined to agree with the data system's preferred choice of a bromopentane isomer. In this case an isoprene structure, 2-bromo-2-methyl butane is tentatively proposed



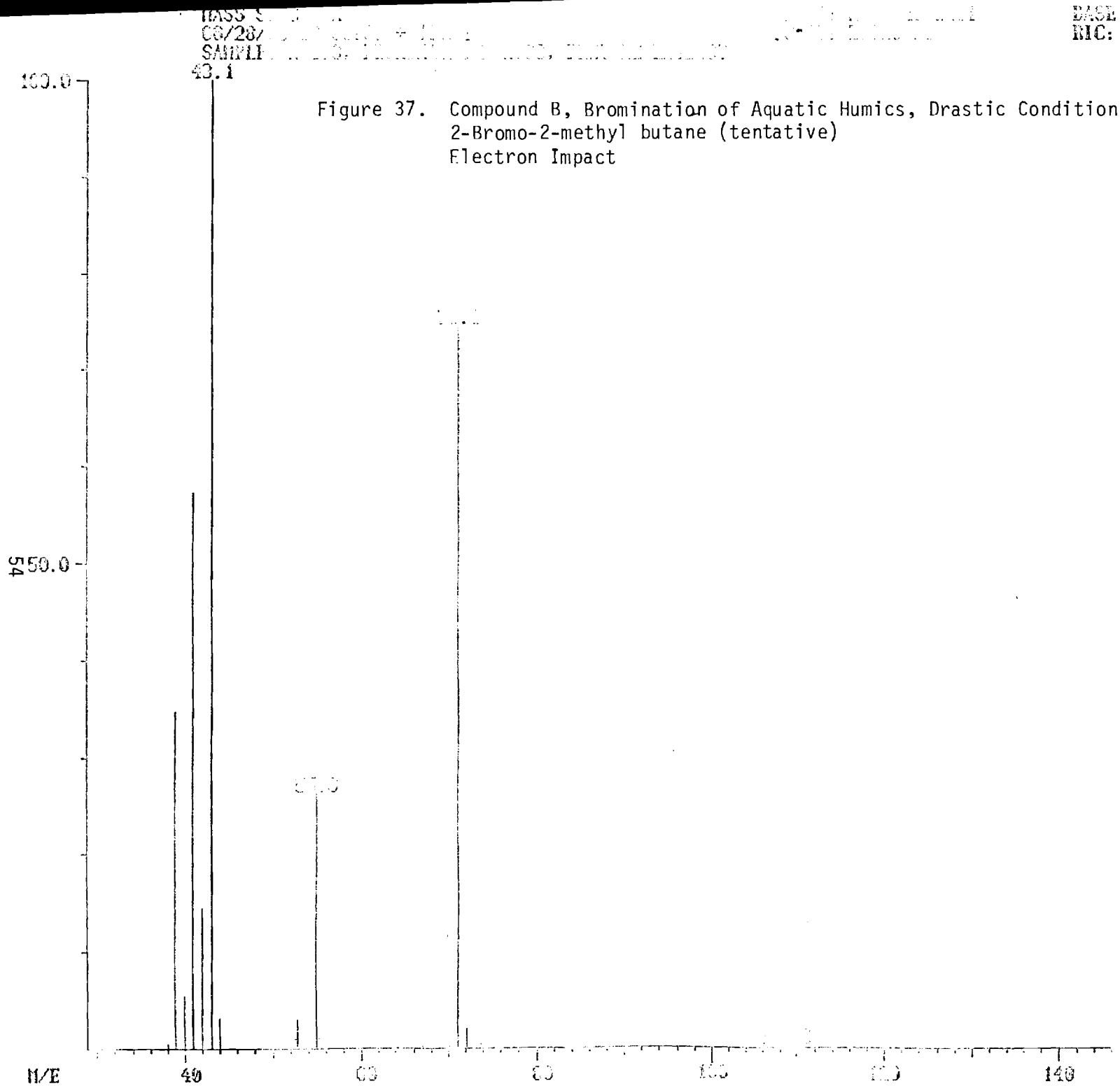
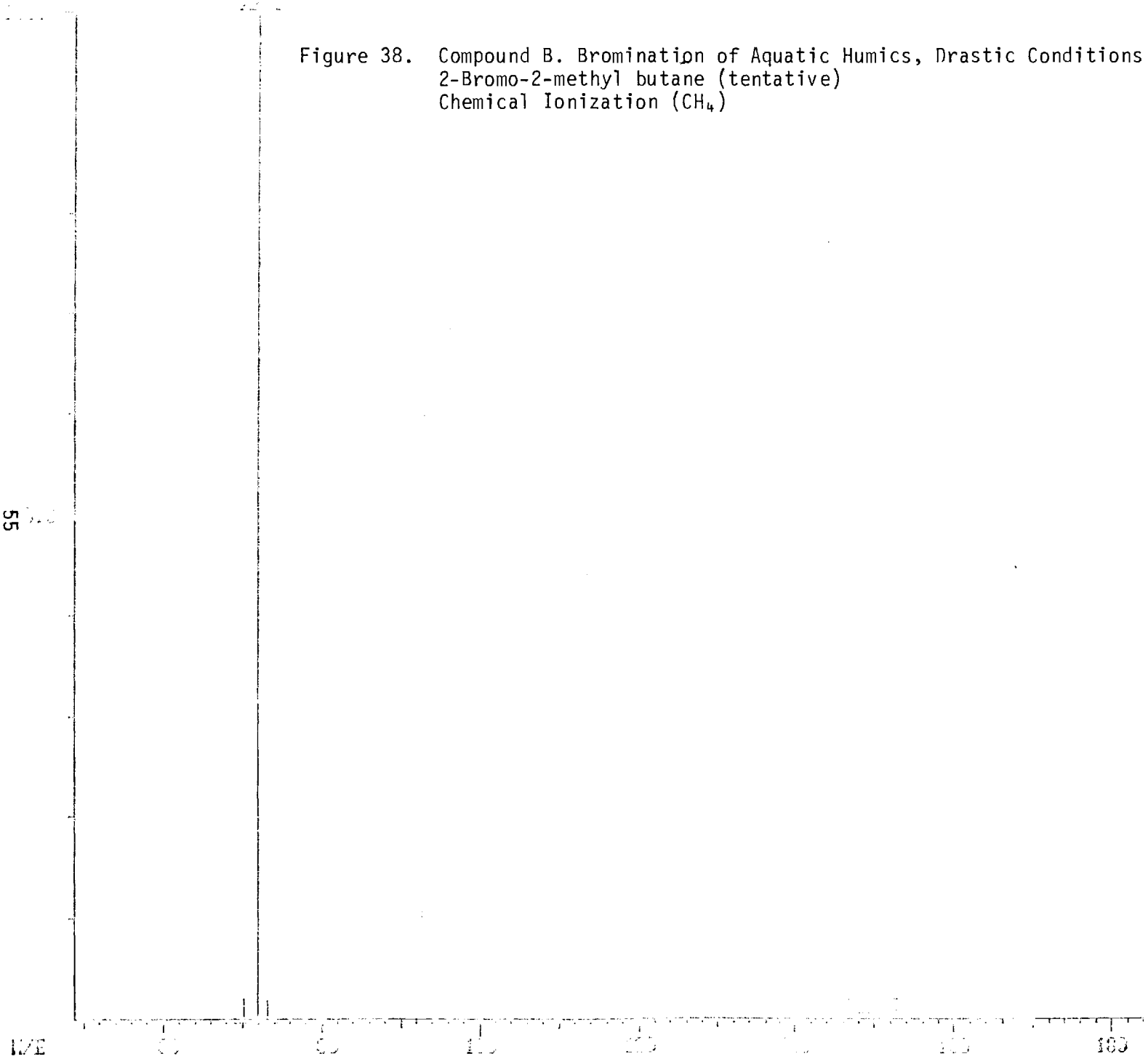
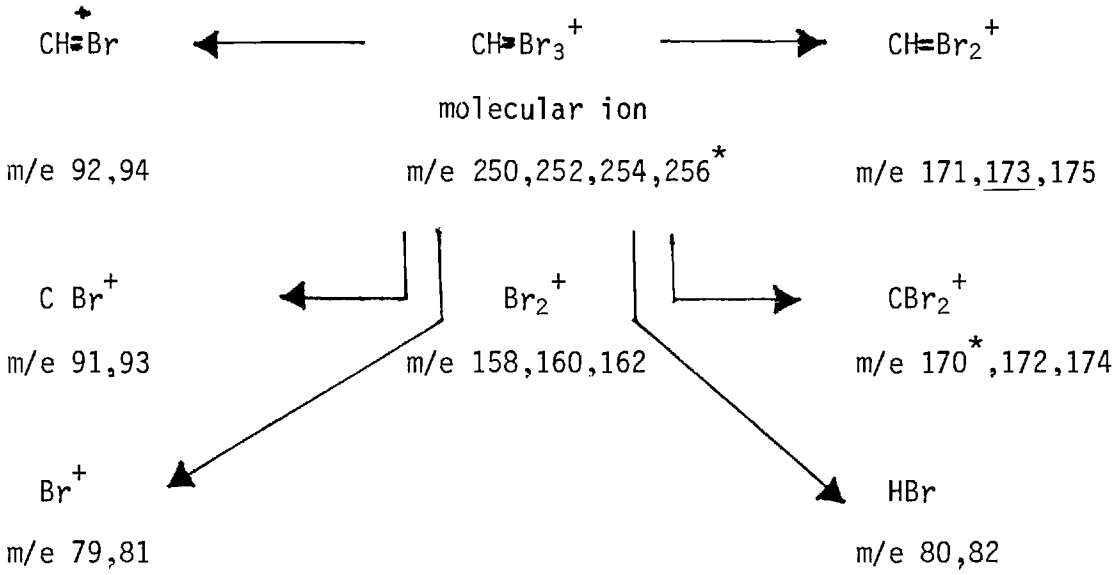


Figure 37. Compound B, Bromination of Aquatic Humics, Drastic Conditions
2-Bromo-2-methyl butane (tentative)
Electron Impact

Figure 38. Compound B. Bromination of Aquatic Humics, Drastic Conditions
2-Bromo-2-methyl butane (tentative)
Chemical Ionization (CH_4)



D. The electron impact spectrum of compound D which is shown in Figure 39 is identical to that of an authentic sample of bromoform. A probable fragmentation sequence is outlined below:



The chemical ionization fragmentation pattern is again somewhat difficult to understand (see Figure 40). A very weak molecular ion is seen rather than a quasi-molecular ion. The base peak is again the CH Br_2 group at m/e 171,173,175 with smaller CH_2Br_2 and CBr_2 peaks superimposed at the even masses. The ion cluster at m/e 189,191,193 which apparently contains two bromine atoms is particularly difficult to interpret. The fragmentation sequence is outlined on page 59.

*Not seen due to low intensity.

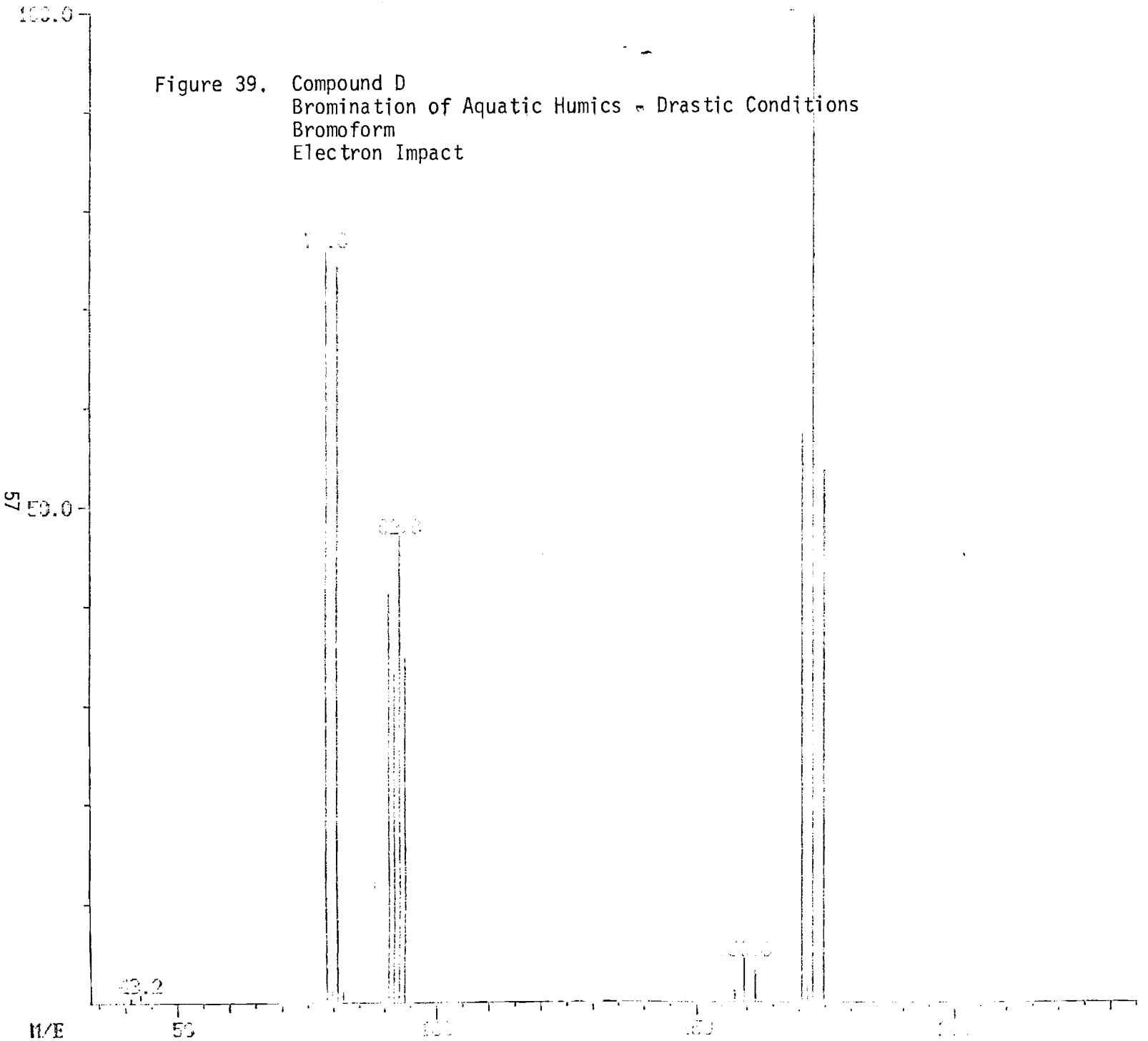
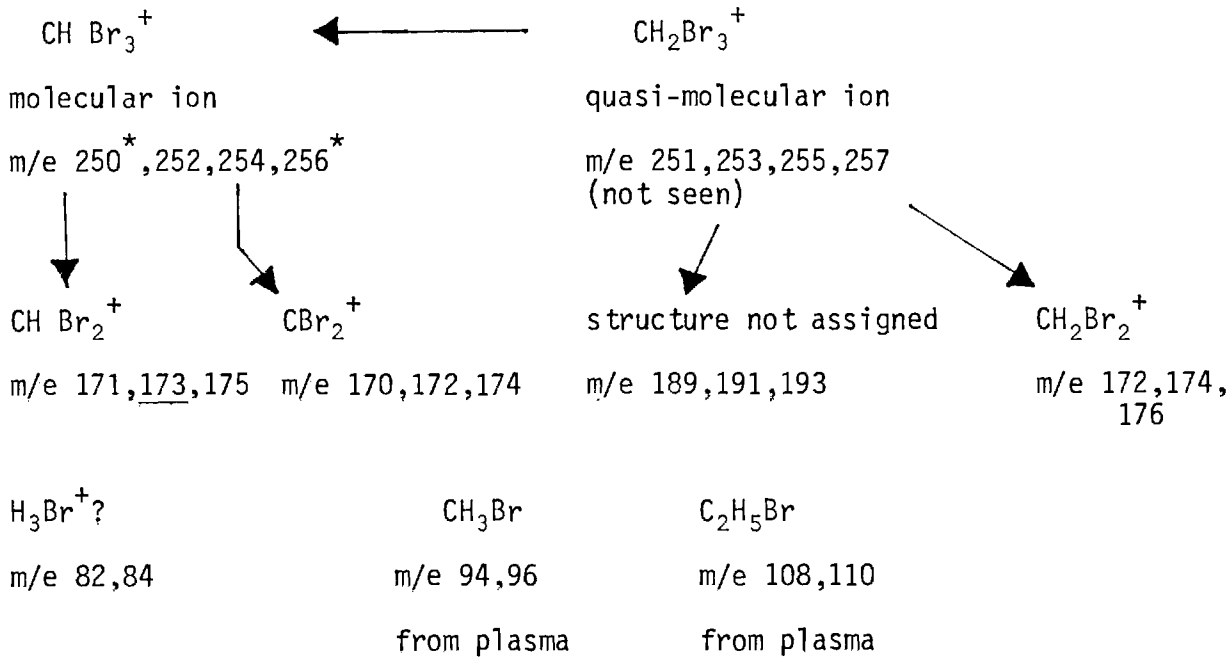


Figure 39. Compound D
Bromination of Aquatic Humics - Drastic Conditions
Bromoform
Electron Impact

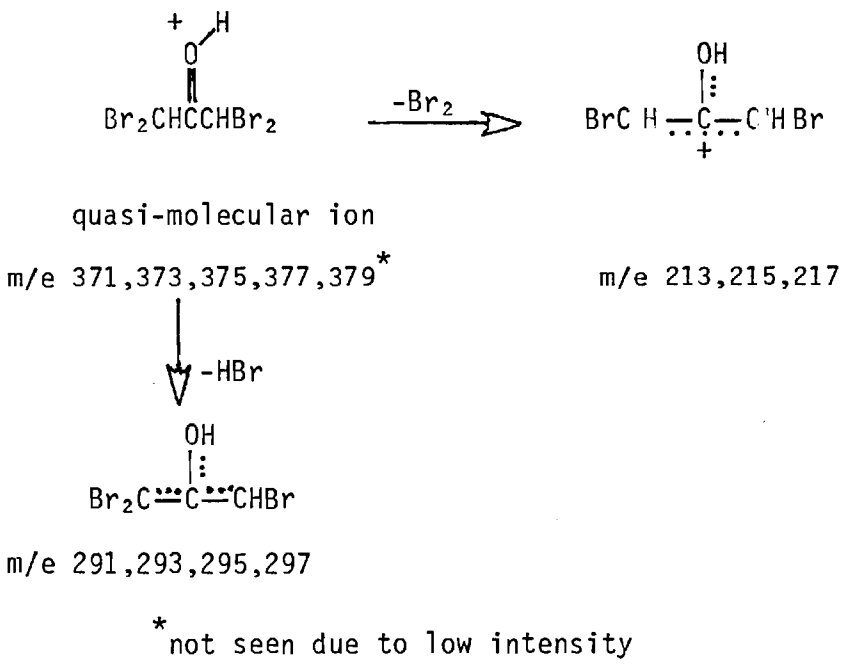
Figure 40. Compound D
Bromination of Aquatic Humics - Drastic Conditions
Bromoform
Chemical Ionization (CH_4)



Fragmentation Sequence Compound D Bromoform

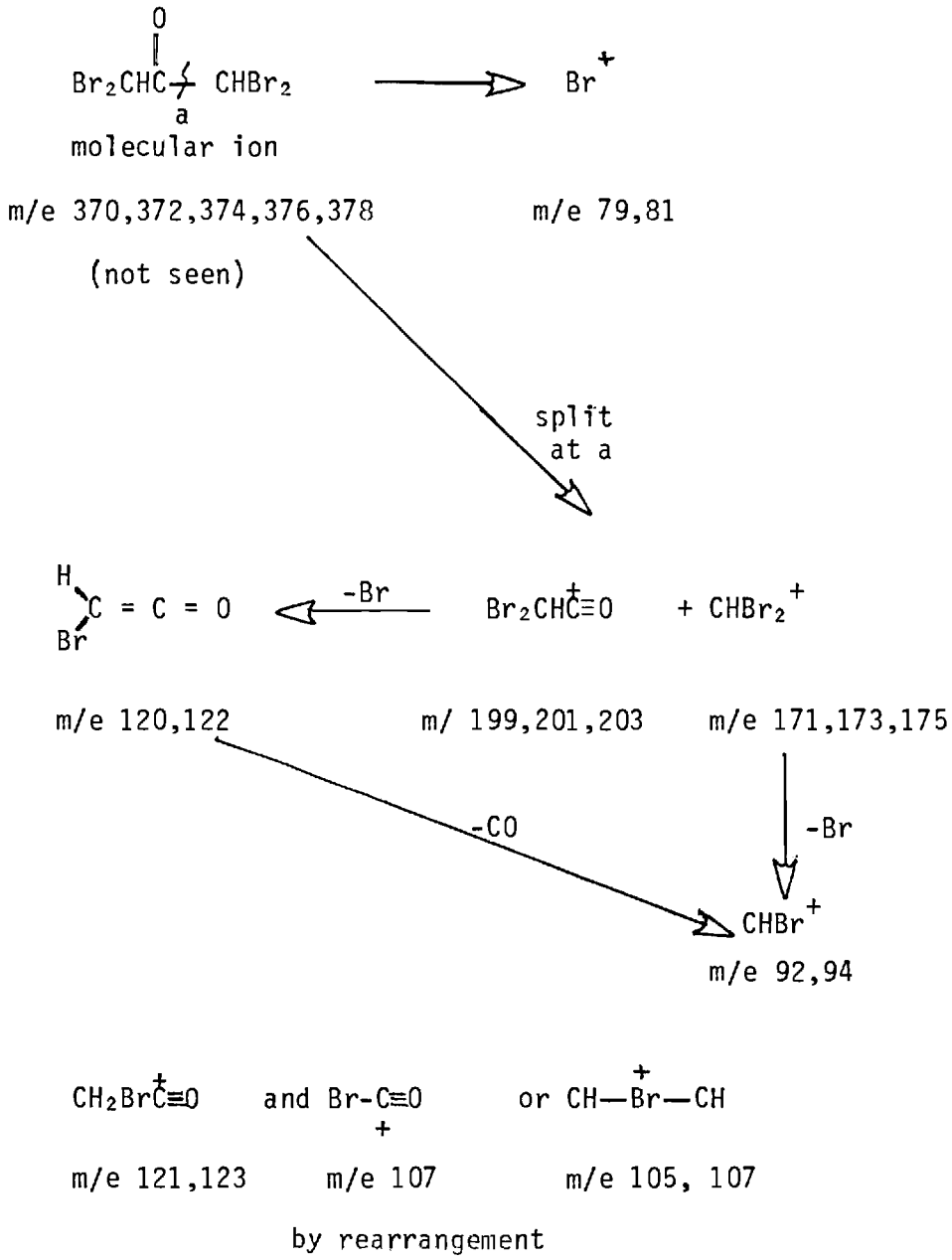
*Not seen due to low intensity.

G. Unlike compounds A and B in which the CI spectra merely added to the confusion, the CI fragmentation pattern of compound G which is shown in Figure 41 proved to be instrumental in making a structural assignment. A fairly strong quasi-molecular ion was seen at m/e 371, 373, 375, 377 and 379. While the low overall ion intenities gave poor isotope ratios, all ion clusters excepting the one marked at 137.5 are consistent with a tetrabromoacetone structure.



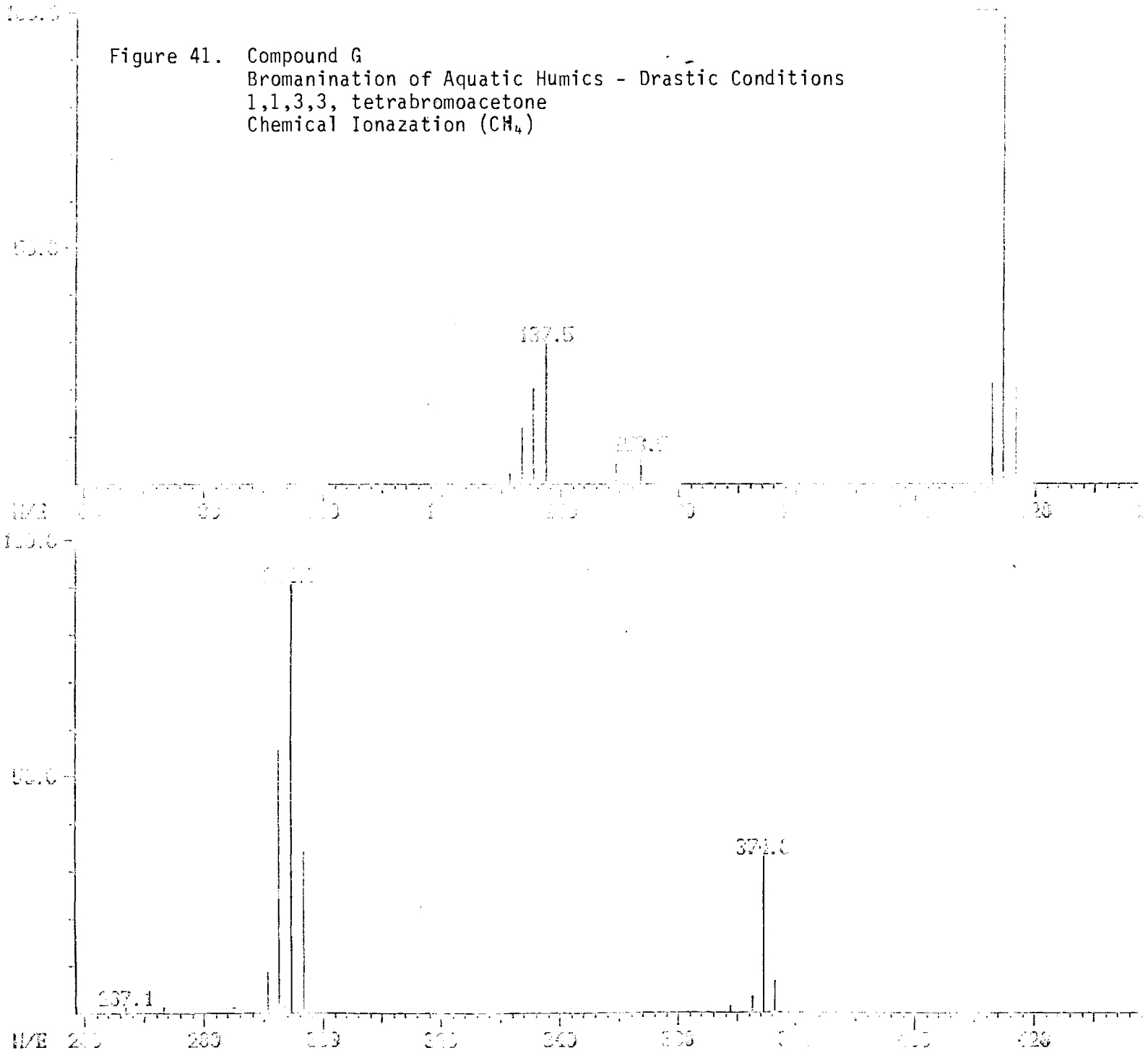
*not seen due to low intensity

The electron impact spectrum is fully consistent with this structure (see Figure 42) as outlined on the next page in the proposed fragmentation scheme. Once again, low overall ion intensities have unbalanced some of the isotope ratios.



EI Fragmentation Sequence Compound G 1,1,3,3 - Tetrabromoacetone

Figure 41. Compound G
Bromanination of Aquatic Humics - Drastic Conditions
1,1,3,3, tetrabromoacetone
Chemical Ionazation (CH_4)



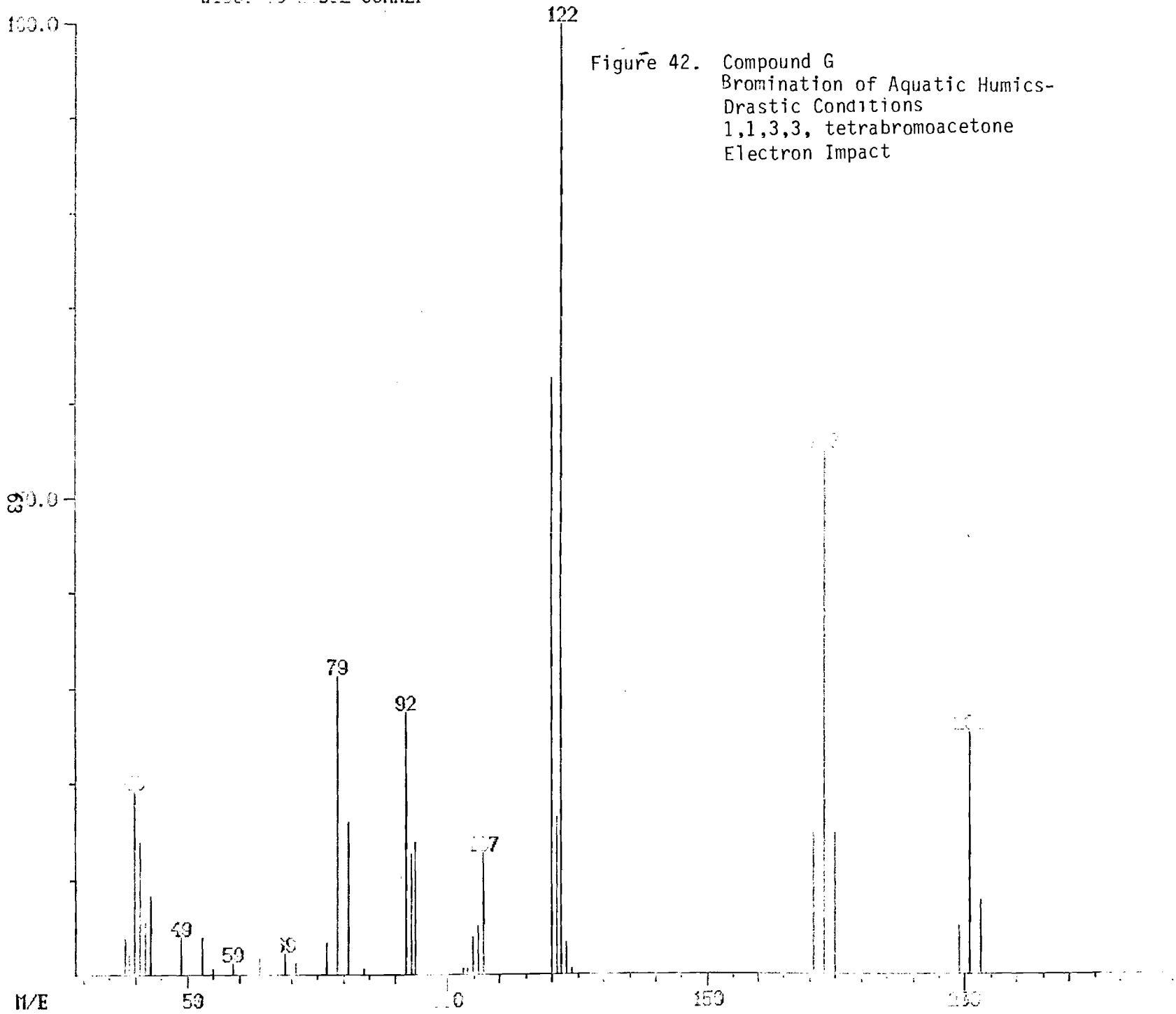


Figure 42. Compound G
Bromination of Aquatic Humics-
Drastic Conditions
1,1,3,3, tetrabromoacetone
Electron Impact

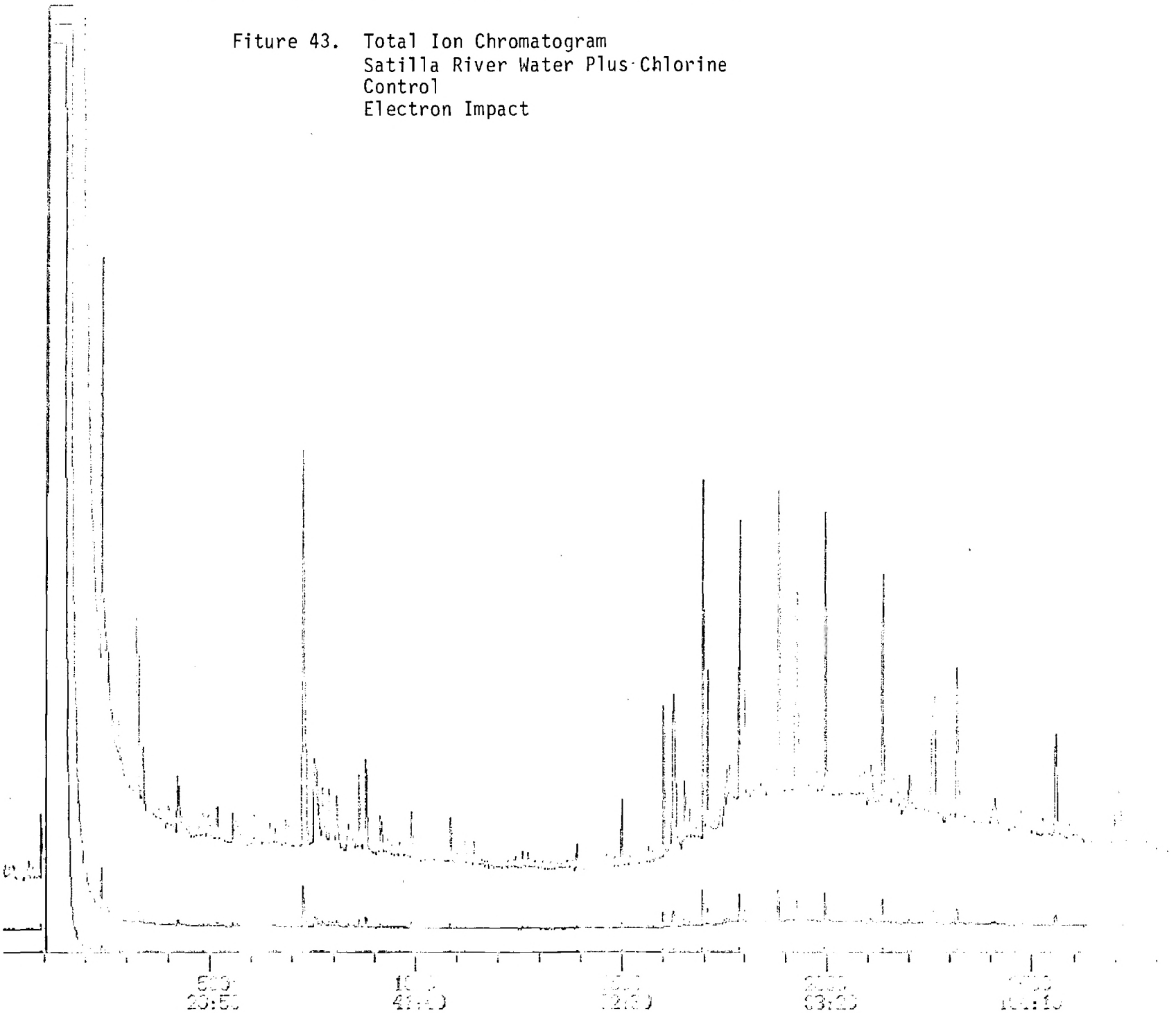
The remaining components in the pentane extracts occurred at levels which were too low to permit structural assignments to be made. It is evident, however, that they do contain bromine. Further work with this particular fraction beyond that mentioned in connection with compounds A and B is not contemplated.

VII. TREATMENT OF RAW WATER FROM THE SATILLA RIVER IN THE MINI-PILOT FACILITY

It will be recalled from Section IV, pages 9 and 10 of the previous monthly report that 10 ℓ of Satilla River water (TOC 51 mg/ℓ) was brought to pH 7.1 by adding 32 mg/ℓ calcium hydroxide and subsequently chlorinated (110 mg/ℓ) in the presence of aluminum sulfate (100 mg/ℓ). In spite of the relatively high chlorine dosage, no chlorine residual was observed either before or after the sand filter. This condition will be corrected in subsequent runs. Floc formation and improved color were noted. Nevertheless, a partial isolation and characterization of the organic material has been undertaken in realization of the fact that this run represents a good "worst case" test of what might happen when organic-rich waters are heavily dosed with chlorine. A volume of 1800 mℓ from each of the reservoir (control), settling chamber and sand filter eluate samples was treated with concentrated sulfuric acid until the pH was 1.6-1.8. The acidified aqueous solutions were extracted with (3x10 mℓ) Nanograde® pentane. Emulsion problems were cured by filtration of the upper layer through anhydrous sodium sulfate. The dried pentane extracts were then concentrated to a volume of 1-3 mℓ in a Kuderna-Danish apparatus and stored in a freezer prior to GC/MS analysis.

The concentrated extracts were not treated with diazoethane, nor were the GC/MS data extensively studied because the whole experiment was due to be repeated under conditions which would result in improved flocculation and in the maintenance of a chlorine residual. A comparison of the total ion chromatograms for the control (Figure 43) and the settled, unfiltered finished water (Figure 44) shows a great

Fiture 43. Total Ion Chromatogram
Satilla River Water Plus Chlorine
Control
Electron Impact



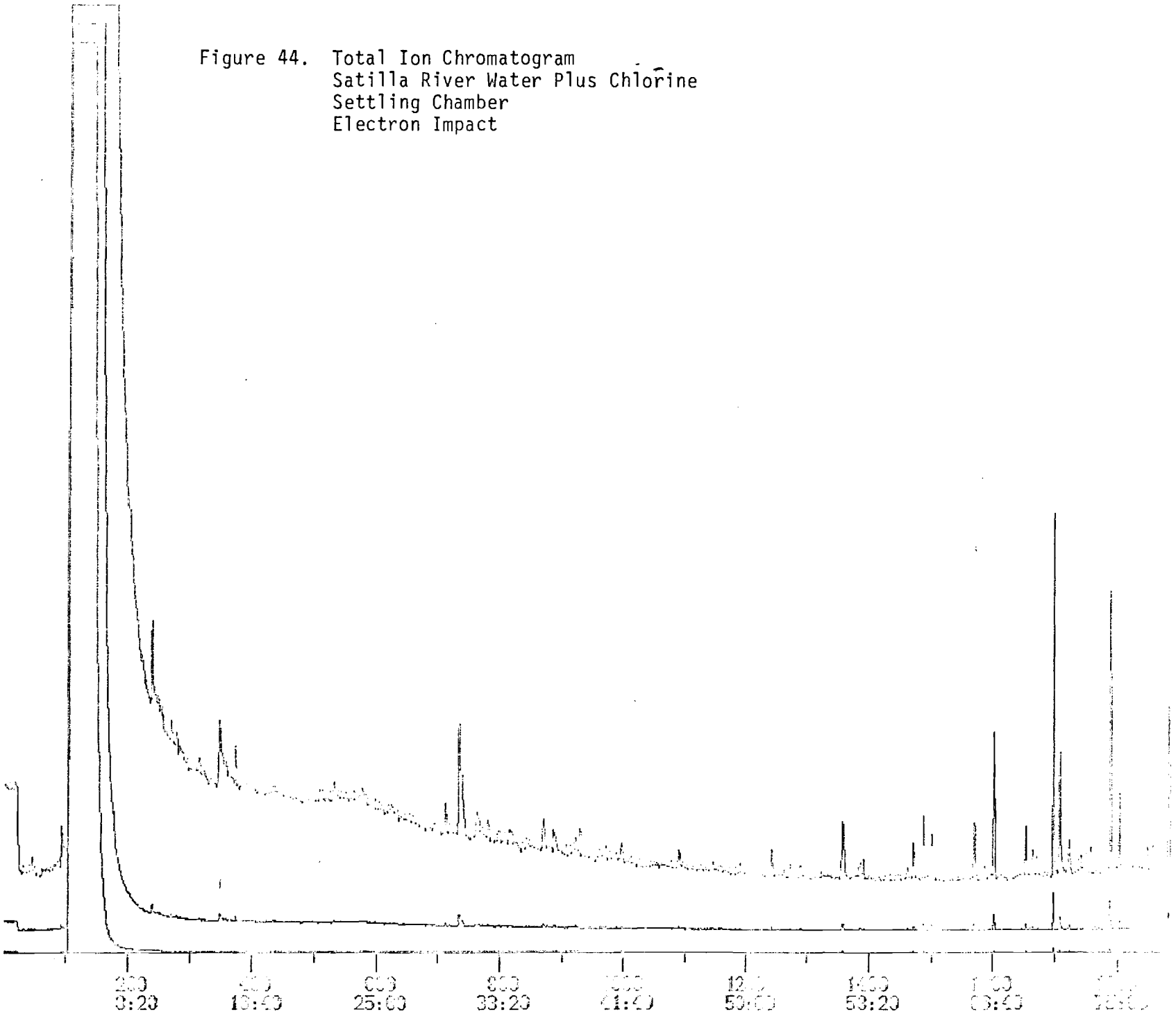


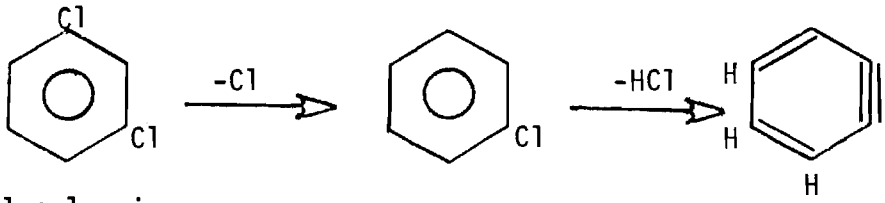
Figure 44. Total Ion Chromatogram
Satilla River Water Plus Chlorine
Settling Chamber
Electron Impact

deal of similarity, particularly in the region beyond scan 1600. The series of peaks in this area is suggestive of a hydrocarbon series. Preliminary examination of individual scans strongly supports this conclusion. It is interesting to note that this pattern was absent from the sand filter eluant (Figure 45) thus indicating that at least temporarily, the sand filter is capable of hydrocarbon removal.

The fact that the hydrocarbons are present at all is evidence that the river was being contaminated—possibly by the construction mentioned in Section V. For this reason, it was decided to postpone the sampling originally scheduled for this reporting period.

The only positive identification of a chlorinated compound (EI data only) was achieved in the case of scan 783 in the control run. Excellent matches were obtained for the three dichlorobenzenes. The presence of this material in all three samples again strongly suggests pollution. (A blank solvent concentrate showed no dichlorobenzene.) The fragmentation pattern (after background correction) is presented in Figure 46.

A proposed fragmentation sequence is presented below.



molecular ion
m/e 146,148,150 m/e 111, 113 m/e 75

Other assignments have not been made. Since a higher priority will be placed on the analysis of the data from a second run, it is unlikely that the identity of other components will be discussed in future reports.

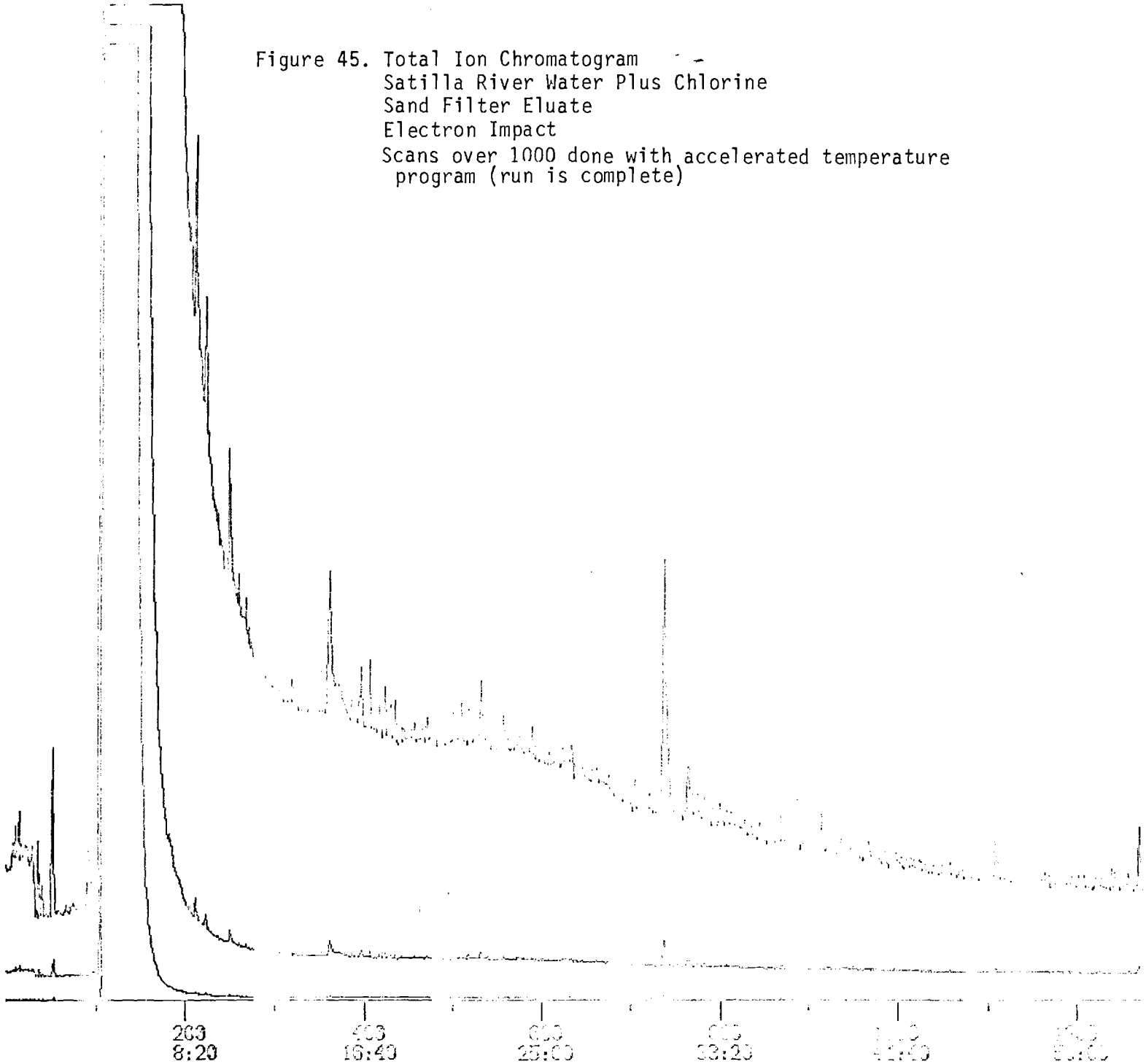


Figure 45. Total Ion Chromatogram
Satilla River Water Plus Chlorine
Sand Filter Eluate
Electron Impact
Scans over 1000 done with accelerated temperature program (run is complete)

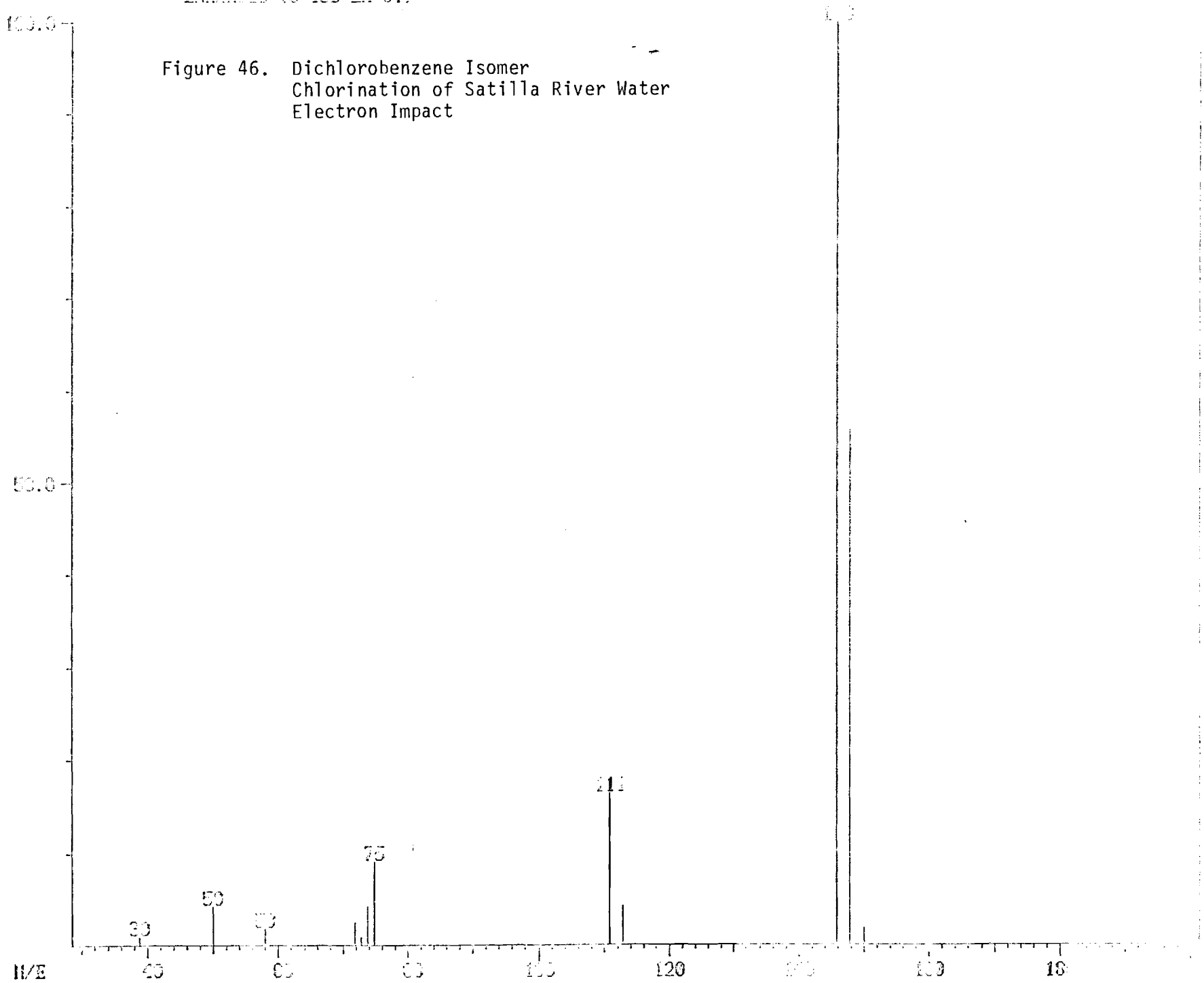


Figure 46. Dichlorobenzene Isomer
Chlorination of Satilla River Water
Electron Impact

VIII. TREATMENT OF PRE-FLOCCULATED SATILLA RIVER WATER IN THE MINI-PILOT FACILITY

The high TOC values of raw Satilla River water suggest that a pre-chlorination settling step should be employed to lower the chlorine demand prior to disinfection. Such a treatment would be more in keeping with recommended practices and in our last monthly progress report (p11) was shown to be effective in lowering the BOD from 51 to 17 mg/l.

Accordingly, an 8.8-liter sample of raw Satilla River water was dosed with 440 mg of ferric chloride, with stirring, to produce a heavy floc. The suspension was filtered through Whatman No. 1 filter paper to provide a light yellow filtrate (pH 3.45).

The treated water (7.4 l) was placed in the mini-pilot reservior along with 10g dipotassium hydrogen phosphate and 3g of potassium dihydrogen phosphate. The buffered water was added to the mixing chamber at a rate of one l/hour. A solution of 660 mg of chlorine and 650 mg of aluminum sulfate in 300 ml of purified water was added to the mixing chamber at a rate of 50 ml/hour.

After eight hours of contact time, the sand filter eluate was found to have a pH of 6.98 and a chlorine residual of 24.8 mg/l. The residual chlorine was quenched with 250 mg of anhydrous sodium sulfite.

After 20 hours, the pH in the settling chamber was 7.03 and the chlorine residual was 36 mg/l. The chlorine was quenched with 750 mg of sodium sulfite.* At the same time, 350 mg of sodium sulfite was added to the 1.4 l of buffered solution in the reservoir. Thus the control is more nearly an exact duplicate of the treated solutions.

A 1.4 l portion of each of the three samples described earlier were

---

*Settling chamber volume is 3.4 liters.

treated with concentrated sodium hydroxide solution to bring the pH to 10. The solutions were then extracted with Nanograde[R] pentane (3x100 ml). The combined pentane extracts were washed with 100 ml of purified water, dried over "roasted" anhydrous sodium sulfate and concentrated to a volume of 1-3 ml in a Kuderna-Danish apparatus. The pentane extracted solutions were subsequently adjusted to pH 1.8-1.9 with concentrated sulfuric acid. The acidified solutions were next extracted with (3x100 ml) ethyl acetate. The combined ethyl acetate extracts were washed with 100 ml of purified water, dried over "roasted" anhydrous sodium sulfate and concentrated to a volume of 3 ml in a Kuderna-Danish apparatus. Further workups are in progress.

IX. MINI-PILOT TREATMENT OF A QUATIC HUMICS - TERMINAL CARBON FILTRATION

A solution of 100mg of M/30 aquatic humic material in 10ℓ of deionized, distilled water containing 10ℓ of dipotassium hydrogen phosphate and 1 g potassium dihydrogen phosphate (ph 7.85) was purged as usual with a stream of nitrogen for one hour in order to remove volatiles which might have survived the water purification process. The solution in the reservoir bottle was added to the mixing chamber at a rate of 1ℓ/hour. A solution of 1428mg of chlorine and 860mg of aluminum sulphate in 457ml of purified water was added to the mixing chamber at a rate of 53ml/hour. A light floc formation was observed under these conditions. The overflow from the settling chamber was passed through a bed of prewashed Nuchar WV-H (6-16 mesh) (Westvaco Co.) 85mm deep and 40 mm in diameter. No sand filtration was used. The eluate from the carbon bed was collected in six-500ml portions. After collecting the first three 500ml portions of carbon bed eluate, a 500ml portion of the settling chamber liquid was taken (pH = 7.08; residual free chlorine = 50.6mg/ℓ). Another three samples of carbon bed eluate were then taken. The six carbon column eluates were found to have no free residual chlorine and a pH of 7.58, 7.54, 7.56, 7.43, 7.41, and 7.15, respectively. The 500ml portion of settling chamber eluate was quenched with 100mg of anhydrous sodium sulfate twenty minutes after collection

After 24 hours, the settling chamber liquid had a free residual chlorine content of 48.8mg/l which was quenched by the addition of 600mg of anhydrous sodium sulfite. A 150ml portion of each of the seven 500ml portions described above was extracted with three 40ml portions of Nanograde^{R} pentane. The combined pentane extracts were dried over "roasted" anhydrous sodium sulfate

and concentrated to a volume of 1ml in a Kuderna-Danish apparatus. A water-solvent control was prepared using 150ml of distilled water, 120ml of pentane followed by drying and concentration in the same way as for the seven-sample experimental set. The quenched reservoir sample is being reserved as a back-up sample for a possible tie-in with earlier runs.

X. CLASS SEPARATION OF SATILLA RIVER WATER

In the last progress report (Section III, page 5) the separation of a 4.4 liter sample of Satilla River water into a number of classes using macroroticular and ion-exchange resins was described. This scheme is represented in Figure 47 for the reviewer's convenience. A summary of the quantity of material recovered as mg/TOC is presented in Table II. The precision of the method was checked by submitting blind duplicates to the analyst along with our regular samples. Duplicates above 10 mg/ml differed by about 4% while those below 5 mg/l differed by as much as 50%. Since this represents an absolute difference of only 2 mg/l, we believe the procedure meets the needs of this project. Further subdivision and characterization of the fractions is in progress.

Figure 47. Separation-Concentration Scheme

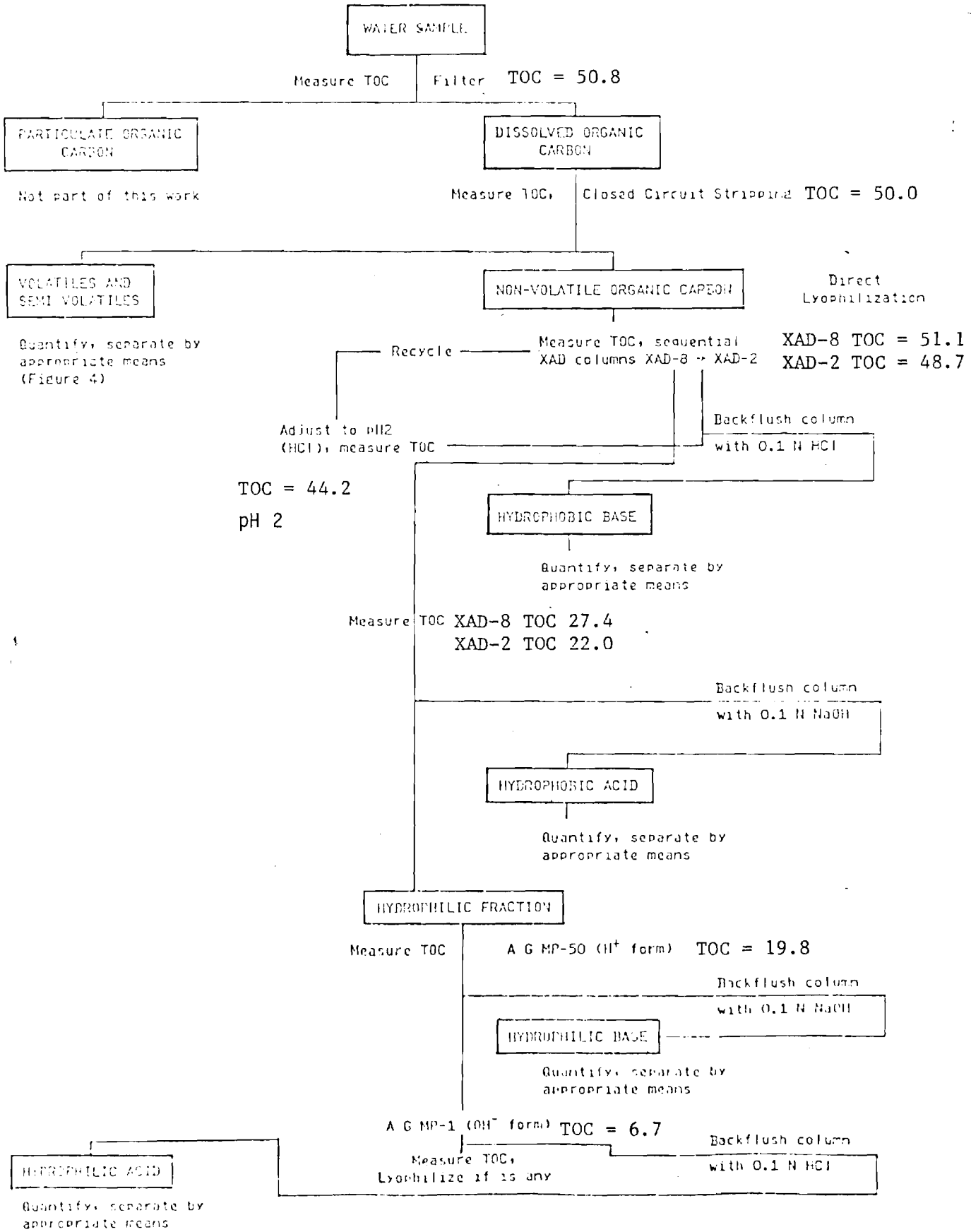


Table II

Recoveries of River Water Organics by Class

	Removed		Recovered	
	mg	%	mg	%
Hydrophobic Bases from XAD-8* and XAD-2	6.2	4	8.2	132
Hydrophobic Acids from XAD-8	49	35	63	140
Hydrophobic Acids from XAD-2	12	11	14	117
Hydrophilic Base from AGMP-50	4.2	3	2.5	60
Hydrophilic Acid from AGMP-1				
First Washing	21	26	15	94
Second Washing	6+		4.7	
Hydrophilic Neutrals	10	20		
SUM OR(OVERALL)	108	99	107	(99)

*Zero TOC removal on XAD-8. Extraction will be carried out nevertheless. At this time it seems inappropriate to present it as a separate figure.

+By difference.

XI. OXIDATION OF AQUATIC HUMICS WITH PERMANGANATE

The previous monthly progress report described the mild oxidation of methylated aquatic humic matter at 55°C for 6 hours (Section VII p. 17). The product mixture was methylated again following the destruction of unreacted permanganate to provide a complex mixture of methyl esters and ethers which was subsequently separated and analyzed by GC/MS. The EI and CI total ion chromatograms covering the early scans are shown again in Figures 48 and 49 for the convenience of the reviewers. A tabular summary of the structural assignments is presented in Table III. Interpretation of this data is still in progress.

H. Compound H was identified as dimethyl succinate on the basis of a good match of the EI data with library spectra. The CI spectrum was supportive with the loss of methanol from the quasi-molecular ion being the only ion of consequence. Since this compound has been discussed in earlier reports, the raw data and a discussion of the fragmentation pattern will not be included in this section.

K. Compound A exhibited an electron impact spectrum (see Figure 50) which is in excellent agreement with the library spectrum for dimethyl methyl succinate (all indicies greater than 940).As is common in methyl esters, the highest mass ion corresponds to M - 31. A fragmentation sequence which explains the observed EI spectrum is outlined on page 82.

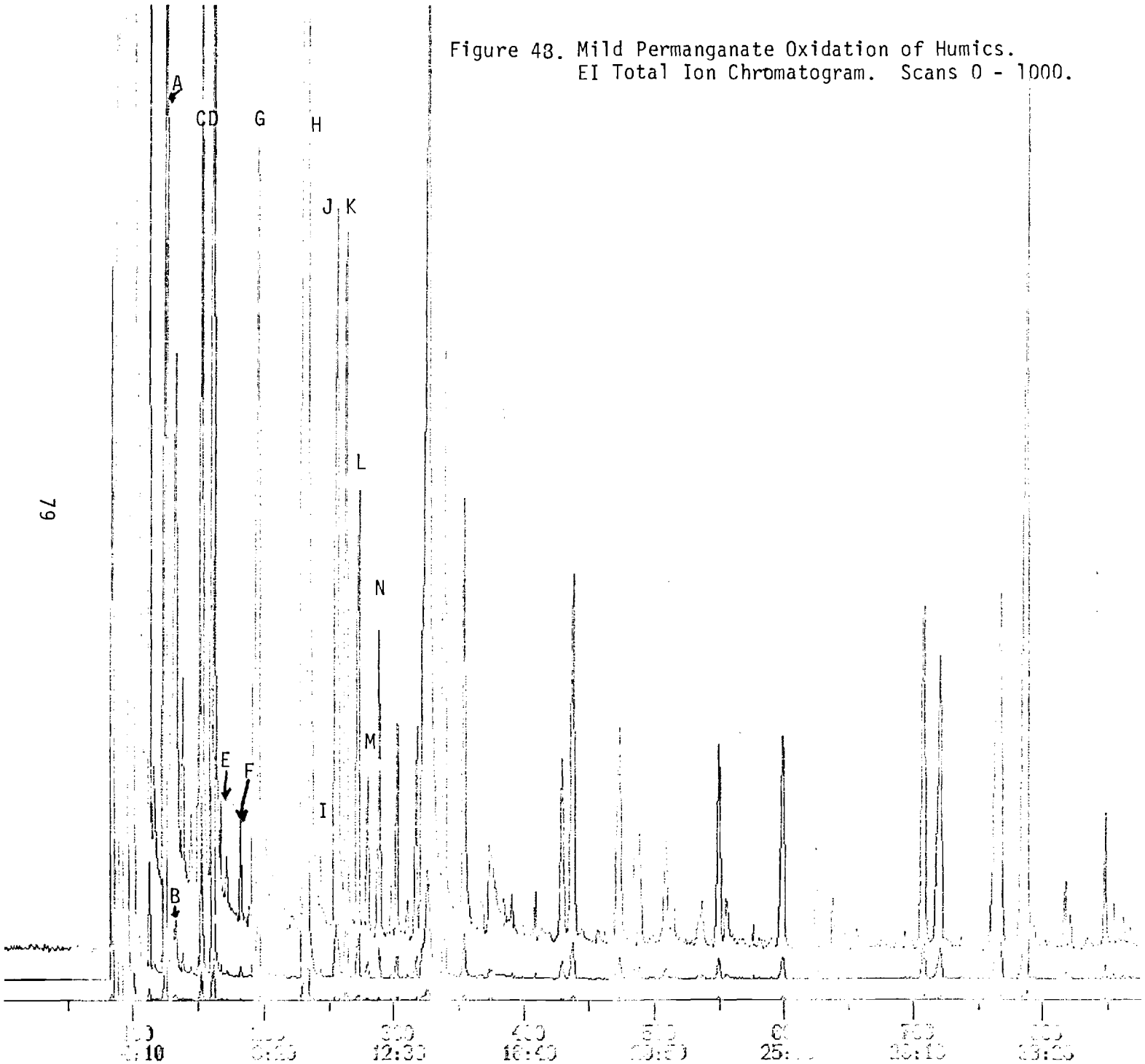


Figure 48. Mild Permanganate Oxidation of Humics.
EI Total Ion Chromatogram. Scans 0 - 1000.

Figure 49. Mild Permanganate Oxidation of Humics.
CI Total Ion Chromatogram. Scans 0 - 1000.

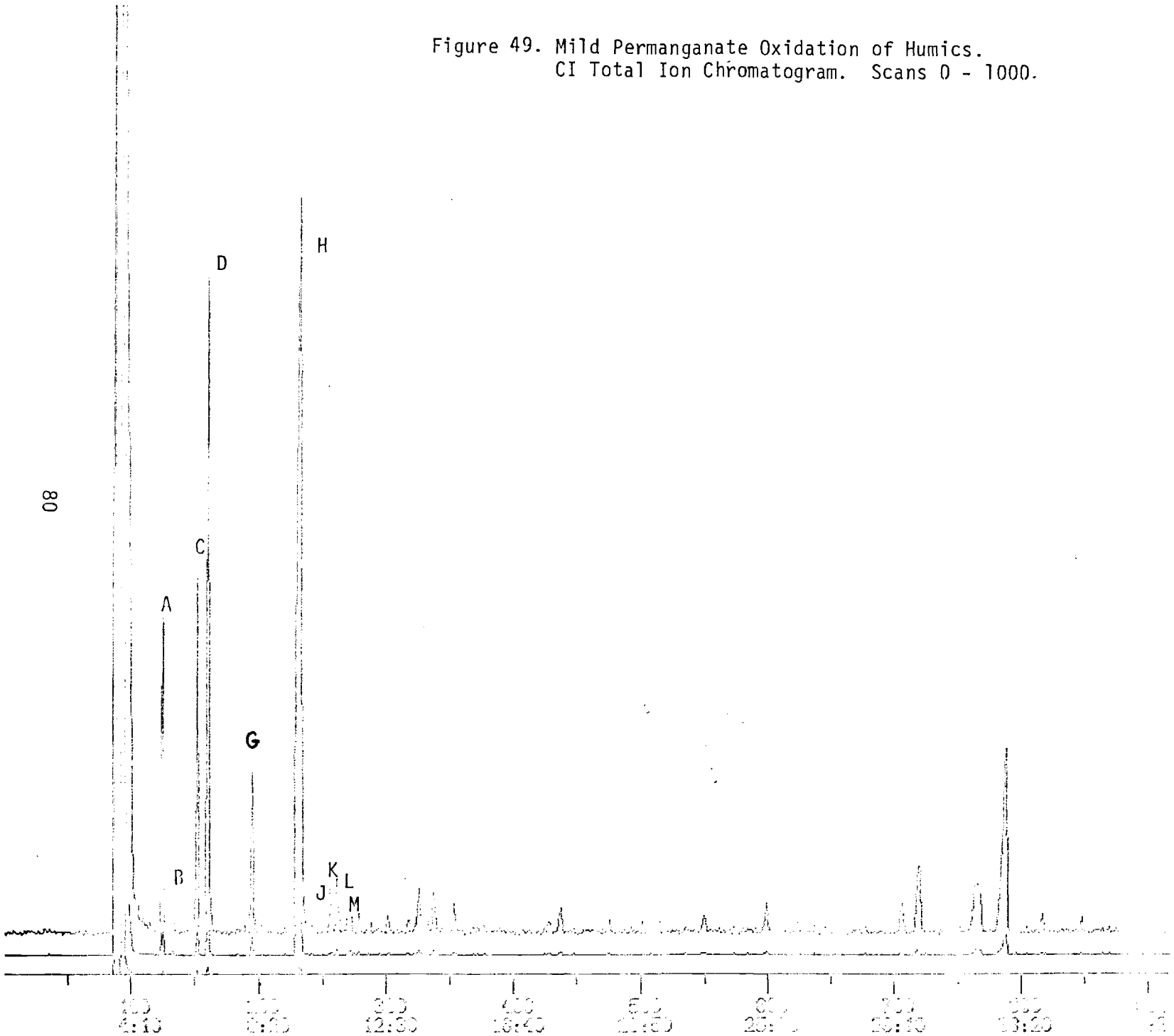
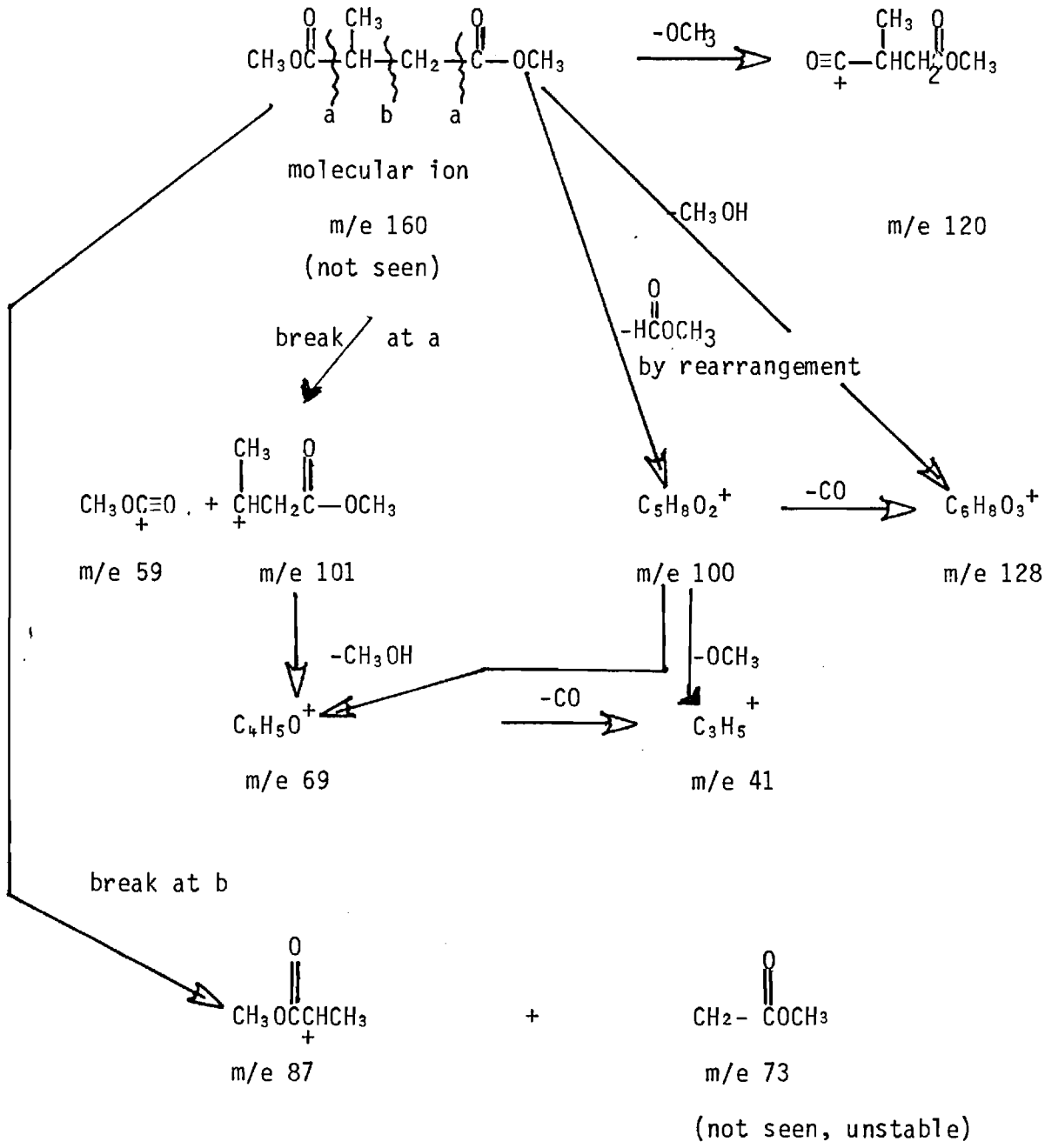


Table III

Mild Permanganate Oxidation of Humics

Compound	Identity	Comment
A	Dimethyl Oxalate	
D	Dimethyl Malonate	
F	Methyl Dimethyl Malonate	Weak CI
G	Methyl Levulinate	
H	Dimethyl Succinate	
K	Dimethyl Methyl Succinate	Weak CI
L	Methyl-5-Methyl Furan-2-Carboxylate	Weak CI
N	Dimethyl-2,2-Dimethyl Succinate	Weak CI



EI Fragmentation Sequence Compound K Dimethyl Methyl Succinate

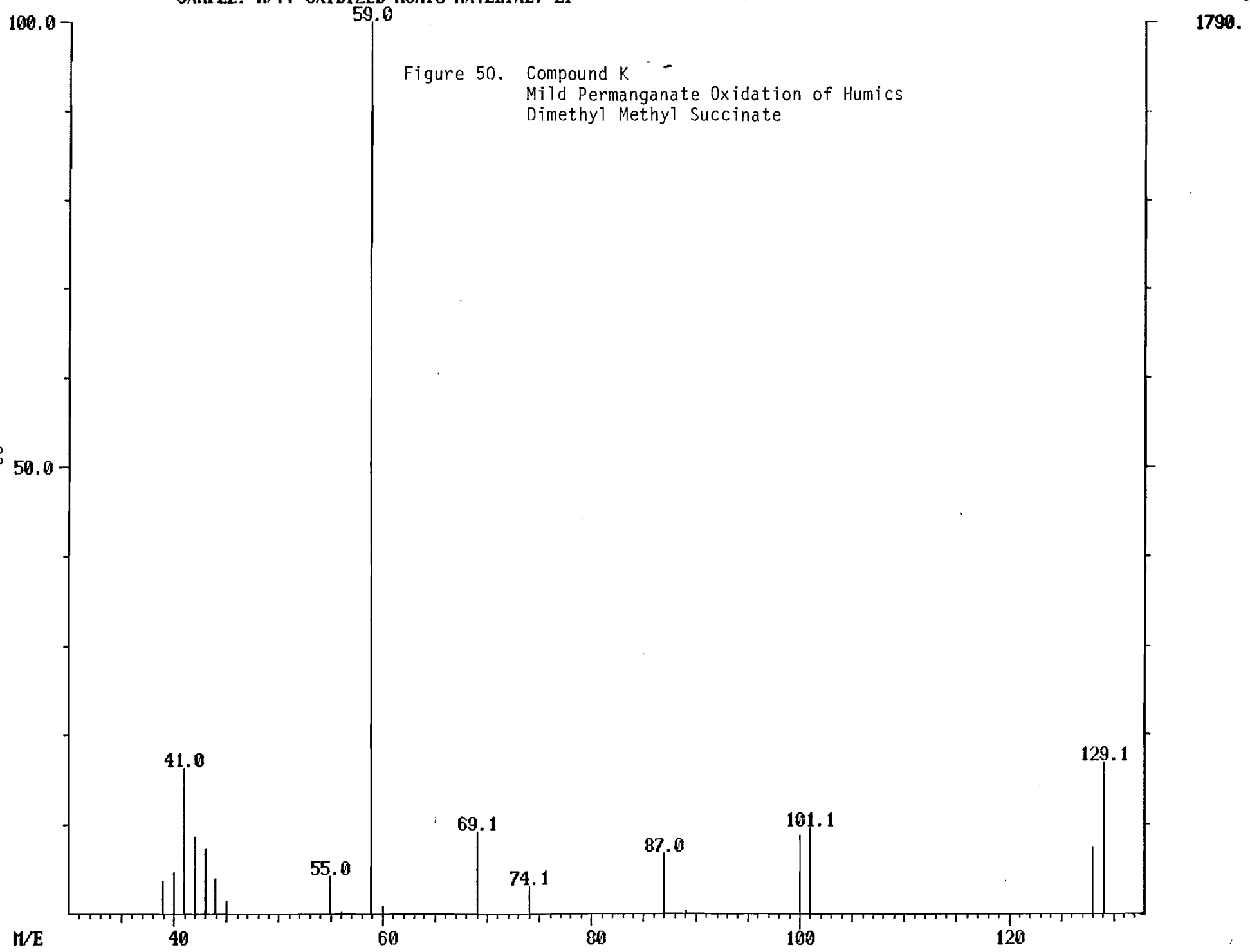
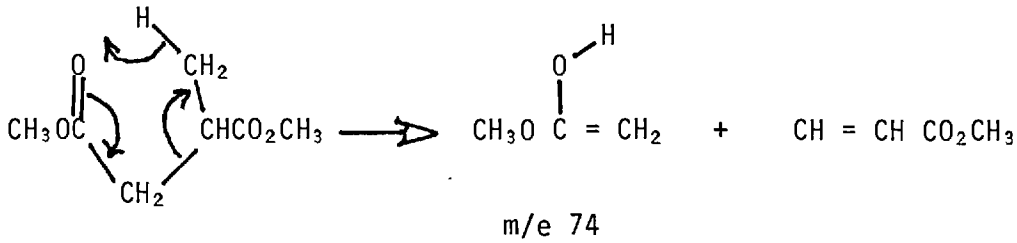


Figure 50. Compound K
Mild Permanganate Oxidation of Humics
Dimethyl Methyl Succinate

The peak at m/e 74 corresponds to a classical McLafferty rearrangement as shown below. (In this case, the charge is retained by the enol fragment)



Although the CI spectrum which is not shown was very weak, the base peak at m/e 129 corresponds to a loss of methyl alcohol from the quasi-molecular ion which is typical for methyl esters and is therefore supportive of the structural assignment.

L. Compound L was identified as methyl-5-methylfuran-2-carboxylate by comparison with earlier work. The parent ion is observed at m/e 140 and the base peak at m/e 109 corresponds to a loss of methoxy. The fragmentation pattern has been explained in earlier reports. The chemical ionization spectrum, although weak, is fully consistent with the assigned structure. The only two ions seen correspond to the quasi-molecular ion (base peak) and loss of methanol from the quasi-molecular ion.

N. Compound N showed a good match with the library spectrum for methyl-2,2-dimethyl succinate. The electron impact fragmentation pattern which is shown in Figure 51 shows the same kind of ion pair (this time at m/e 142, 143) corresponding to the loss of methanol and methoxyl as was noted for the dimethyl methyl succinate. The second curious pair of ions at m/e 114, 115 is also seen. Since the M-32 and M-60 ions are

04/12/78 11:57:00 + 12:02
SAMPLE: M/77 OXIDIZED HUMIC MATERIAL, EI

DATA: M77EI #289
CALI: 0412 #1

BASE M/E: 73
RIC: 1718.

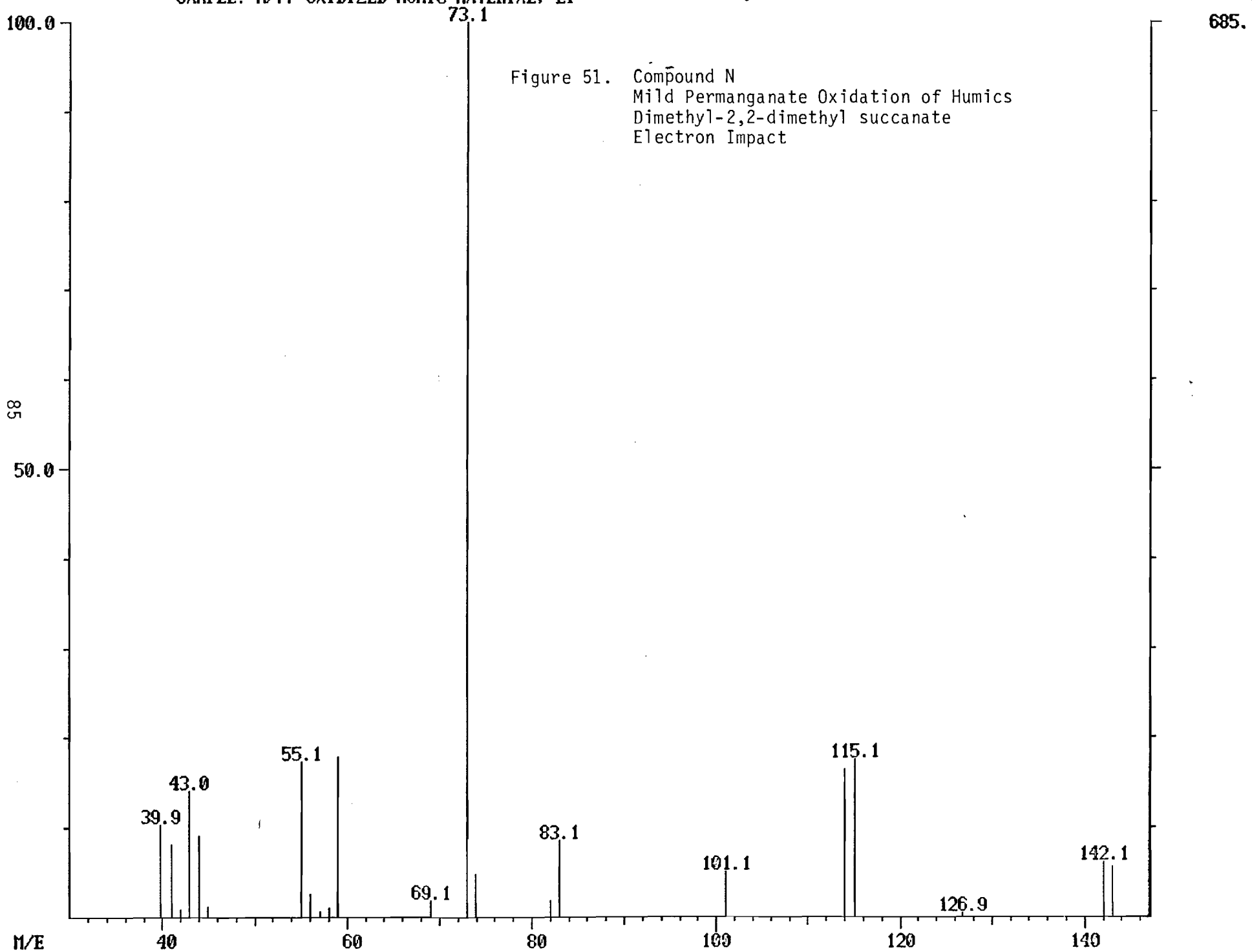
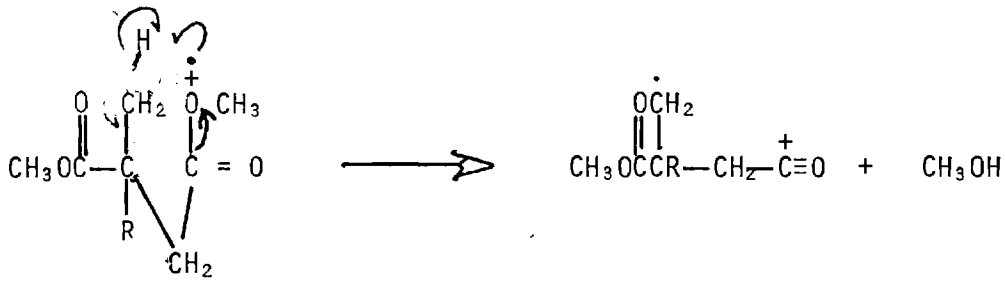


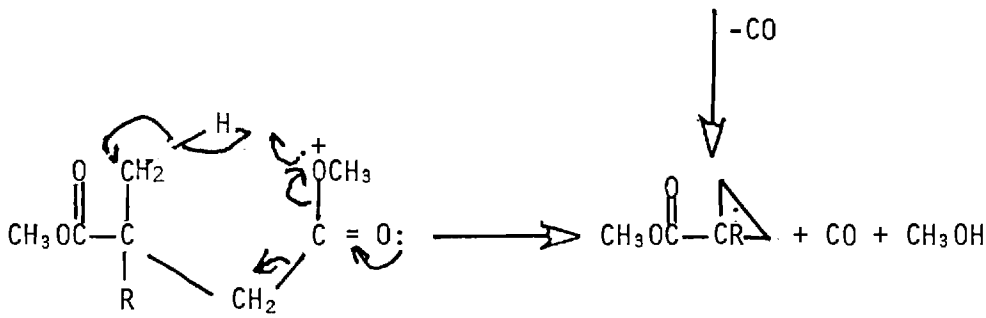
Figure 51. Compound N
Mild Permanganate Oxidation of Humics
Dimethyl-2,2-dimethyl succanate
Electron Impact

not present in dimethyl succinate itself, any explanation for their origin must involve the alkyl methyl group. We would suggest the following explanations. R = H for compound K and R = CH_3 for compound N.



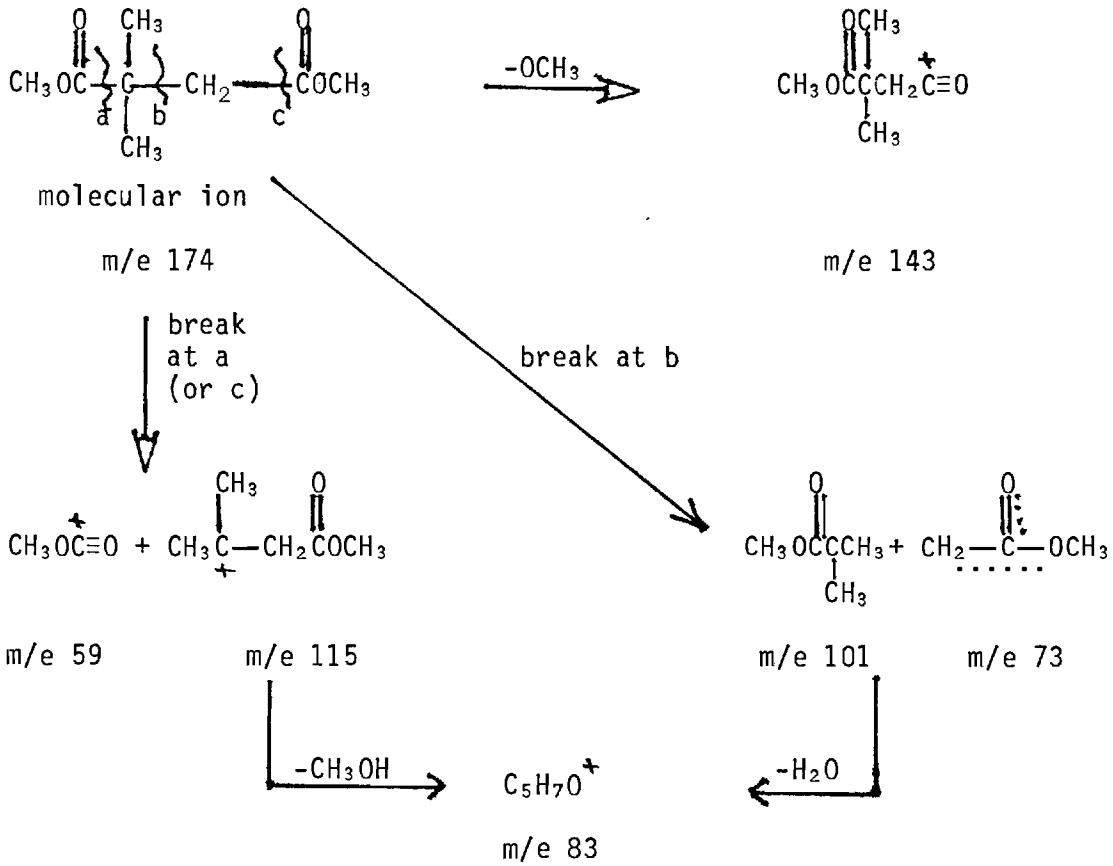
molecular ion

$M^{+\cdot}$ $[M-32\ ^{+\cdot}]$



$M^{+\cdot}$ $[M-60\ ^{+\cdot}]$

The remainder of the electron impact fragmentation pattern is explained below . The chemical ionization spectrum is so weak as to be nearly usless. A loss of methanol and possibly protonated methyl carboxylate from the quasi-molecular ion is noted, however.



Compound N Mild Permanganate Oxidation of Humics - EI Fragmentation

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

August 15, 1978

by

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EXECUTIVE SUMMARY

Access to a larger pool of personnel skilled in analytical chemistry has been assured by a reorganizational transfer of the project team from the Applied Sciences Laboratory to the Technology Development Laboratory. Further gains in access to an advanced laboratory equipment and a much improved laboratory facility will be realized as we move into our new 15,000 ft.^{2} research building the use of which will be totally devoted to chemical programs in the fields of energy, environmental analysis, polymers and materials science.

The data required by means of the GC/MS analysis of the complex product mixtures resulting from the reaction of aquatic humic substances with aqueous permanganate have been further studied. A number of structural assignments, many of which have not been mentioned in earlier reports, are described in detail. The following assignments have been fully described: methyl benzoate, dimethyl glutarate, the methyl ether of dimethyl oxaloacetate, dimethyl adipate, dimethyl tartrate, methyl-4-methoxy benzoate and trimethyl tricarballylate.

In order to achieve a homogeneous reaction system, methylated aquatic humics were dissolved in methylene chloride and treated with a solution of the potassium permanganate complex of 18-crown-6 (also in methylene chloride). The resulting highly complex mixture has been separated into fractions and analyzed by packed-column GC. Specific structural assignments will be made following capillary GC/MS analysis.

A comparison of the composition of the product mixture resulting from carrying out the oxidative degradation of aquatic humics under

progressively milder conditions shows a tenfold dominance of aliphatics over aromatics under the mildest conditions and only a twofold dominance under more severe conditions. A comprehensive product list including the previously mentioned structures plus other, less intensively studied assignments is presented.

Yet another method has been developed for the more accurate and convenient estimation of carboxyl groups in aquatic humics and similar polycarboxylic, polymeric molecules having a high degree of solutility in aqueous systems. The new method relies on the reaction of the carboxyl functions with pyridine to form the pyridinium ion, which is then titrated against standard base. Because there is no counter ion, the acidity of the initial substrate remains unchanged. Because only a single acid product is present (pyridinium) the end-point is sharp, thus improving accuracy. The new method is more convenient than the previsouly described calcium acetate/ultrafiltration method in that no ultrafiltration is required.

The reaction of aquatic humic material with iodine under conditions approximating those which would be employed for actual disinfection was carried out in the mini-pilot facility. Although only a treatment under conditions in which the disinfectant species is I_2 is described, a second run in which the active species is hypoidite (HOI) is in progress. Products are being worked up for GC/MS analysis.

Another drastic bromination of aquatic humics has been carried out. Hopefully this experiment will allow us to identify some of the minor products. Workup of the reaction mixture is in progress.

A systems blank using highly purified water in place of the aquatic humic solutions or raw river water was conducted in the mini-pilot facility. All other reagents were used in their usual proportions. Contact times, extractions, concentrations and sample storage have been carried out in the usual manner. Analysis of the various fractions is planned for the near future. Two new products have been identified in the complex reaction product mixture resulting from the treatment of dilute solutions of aquatic humics with chlorine in the mini-pilot facility. They are 1,4-dichloro-2-methyl-2-butanol and 3,4-dichloro-3-methyl-2-butanol.

Renewed activity is planned during the next working period on the characterization of nitrogeneous fractions and the liquid chromatographic characterization of non-volatile components.

I. PERSONNEL

As a result of an internal reorganization, our research group will be shifted from the Applied Sciences Laboratory to the Technology Development Laboratory effective September 2, 1978. This transfer is occurring as part of Georgia Tech's continuing effort to make better use of equipment and personnel. In our case, the transfer is particularly advantageous as we now are part of a larger group and can draw on a fourfold larger labor pool to fill the gaps left by departing personnel. Since Dr. El-Barbary is part of this larger group, it will be possible for him to rejoin the effort. Other organic chemists and experienced technicians can pitch in to keep the work going while we find a high-caliber replacement for Dr. Ralls.

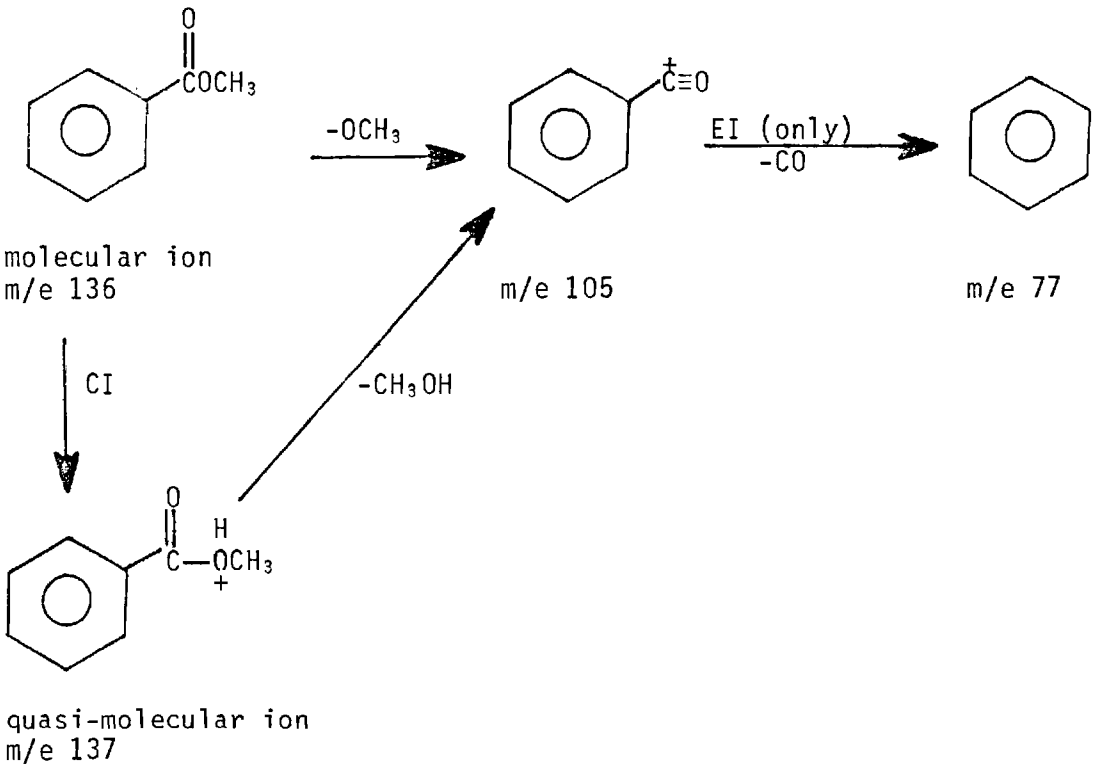
II. EQUIPMENT AND FACILITIES

Georgia Tech has recently acquired a modern,15,000 ft^2 chemical laboratory building with about 10,000 ft^2 of laboratory space and 5,000 ft^2 of office space. Much of this is far better equipped than the space we now occupy. The whole building has been given over to the Chemical and Materials Science Division of which we are now a part. In addition to providing us with greatly improved laboratory space and presenting us with a larger labor pool upon which to draw, the move which will take place on or about September 1, 1978 greatly expands the quality and quantity of research equipment co-located with our group. For example, we will now have access to five GC's and three LC's instead of just one of each. We are confident that this move will increase our ability to carry out the present research program in the best possible manner.

III. OXIDATION OF AQUATIC HUMICS WITH PERMANGANATE - MILD CONDITIONS

The previous monthly progress report (Section XI, p. 78) described the mild oxidation of methylated aquatic humic matter. It will be recalled that following the destruction of unreacted permanganate, the product was remethylated to provide a complex mixture of methyl ethers and methyl esters which was subsequently separated and analyzed by GC/MS. The electron impact total ion chromatogram was particularly intense. While the CI total ion chromatogram was considerably weaker, it is a good overlay and has been used to provide supportive evidence in many of the assignments. The early sections of these total ion chromatograms is presented in Figures 1 and 2. A tabular summary of the structural assignments is presented in Table I. Interpretation of the data is still in progress.

The identity of compound P was particularly difficult to assign because there is apparently single-scan overlap with a second component. A comparison of two adjacent scans across the top of an apparently flat-topped peak showed differences beyond what could be reasonably ascribed to ion statistics (see Figures 3 and 4). For example, scan 302 has m/e 105 as the base peak and m/e 115 is weaker at 30% RI while in scan 303, m/e 115 is the base peak and m/e 105 at 75% RI is the weaker of the two. Using this technique, it was possible to attribute m/e 136, 105, 77, 72 and 51 to compound P. All of these fit methyl benzoate with the exception of m/e 72. The CI spectrum, although very weak and also intermixed with the following compound was supportive with ions at m/e 137 and 105. A structure has not been postulated for the following compound. The fragmentation sequence is outlined on the next page.


O
COCH3
-OCH3
C≡O
EI (only)
-CO
molecular ion
m/e 136
m/e 105
m/e 77
CI
-CH3OH
O
H
C—OCH3
+
quasi-molecular ion
m/e 137

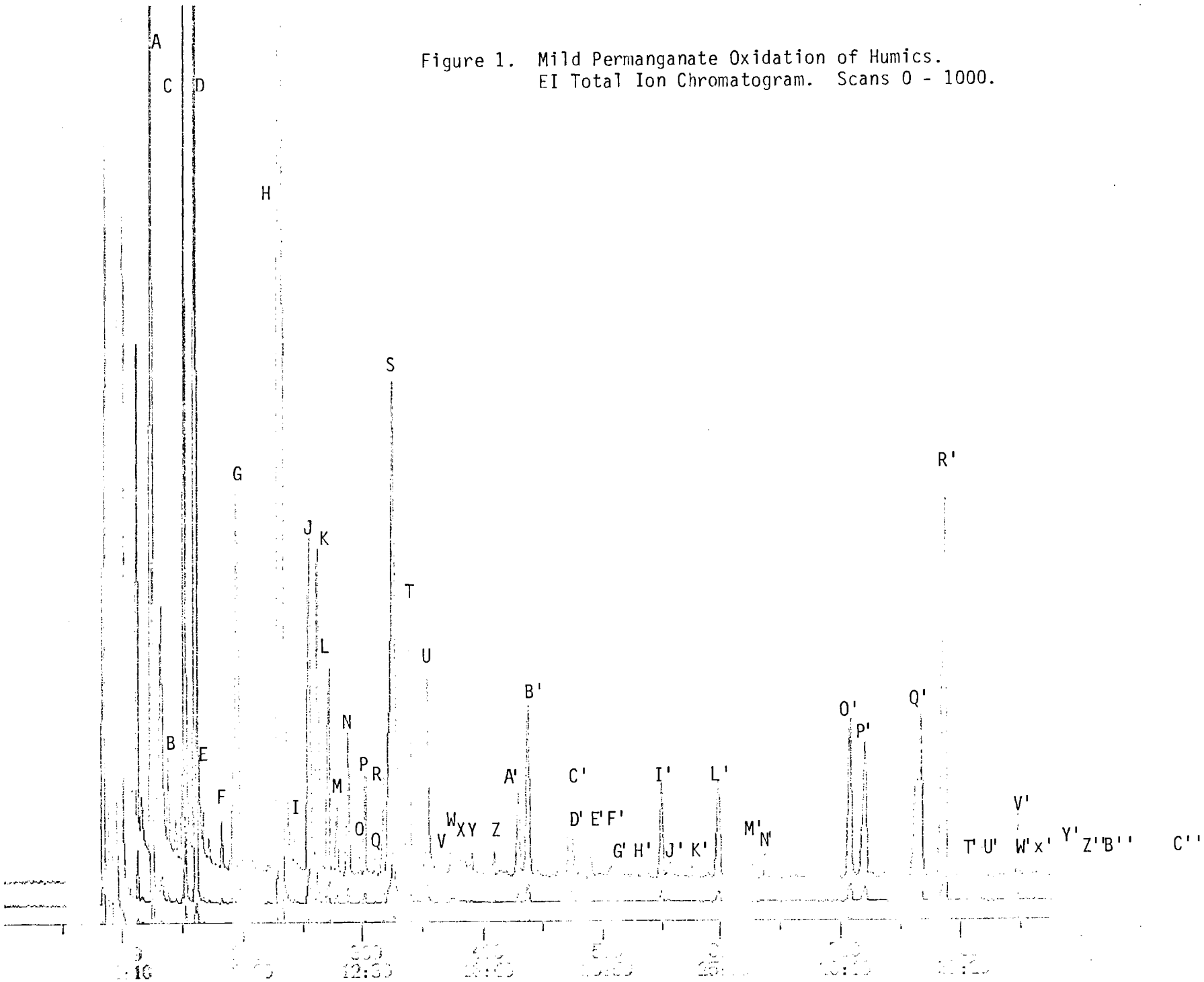


Figure 1. Mild Permanganate Oxidation of Humics.
EI Total Ion Chromatogram. Scans 0 - 1000.

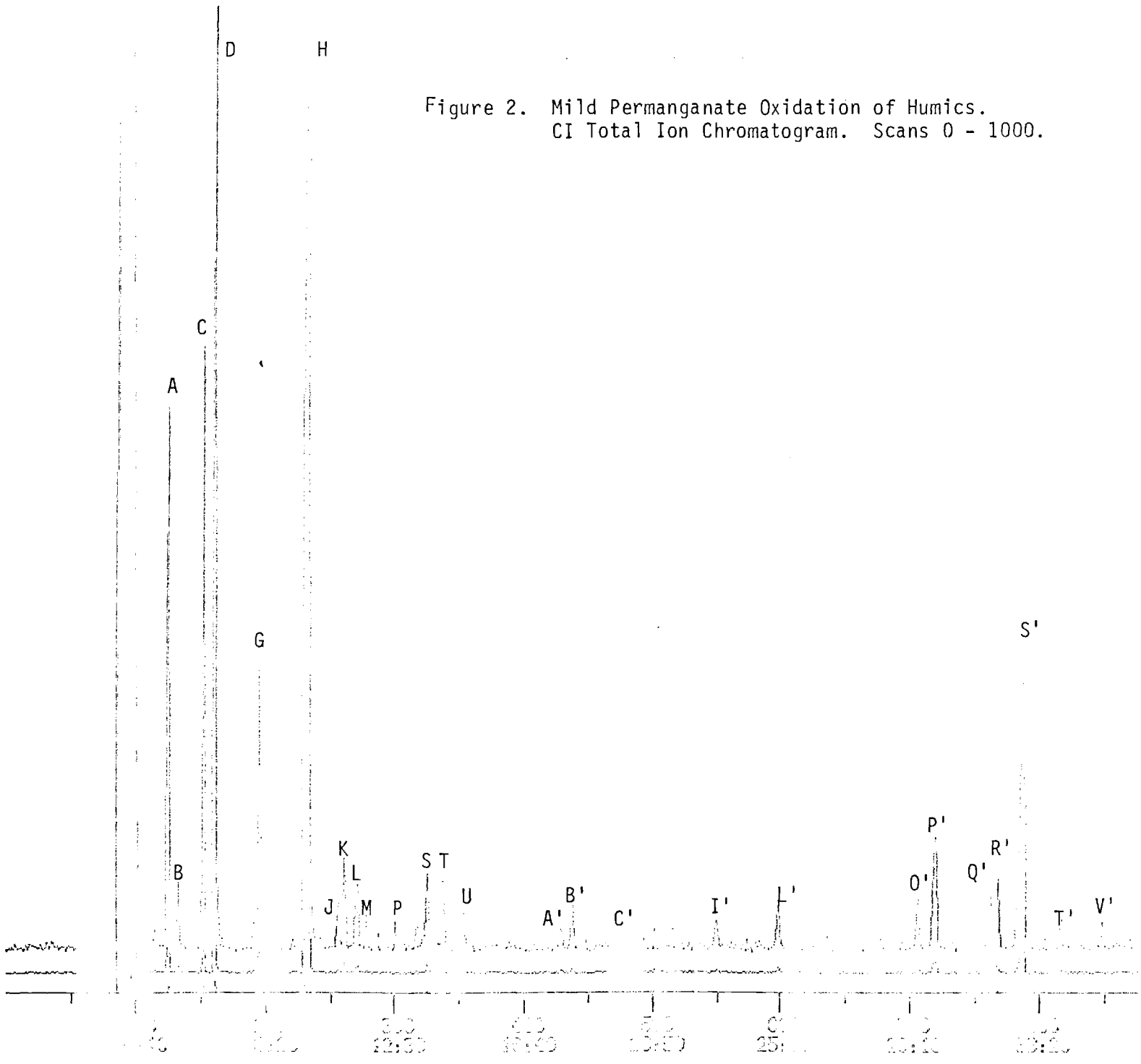


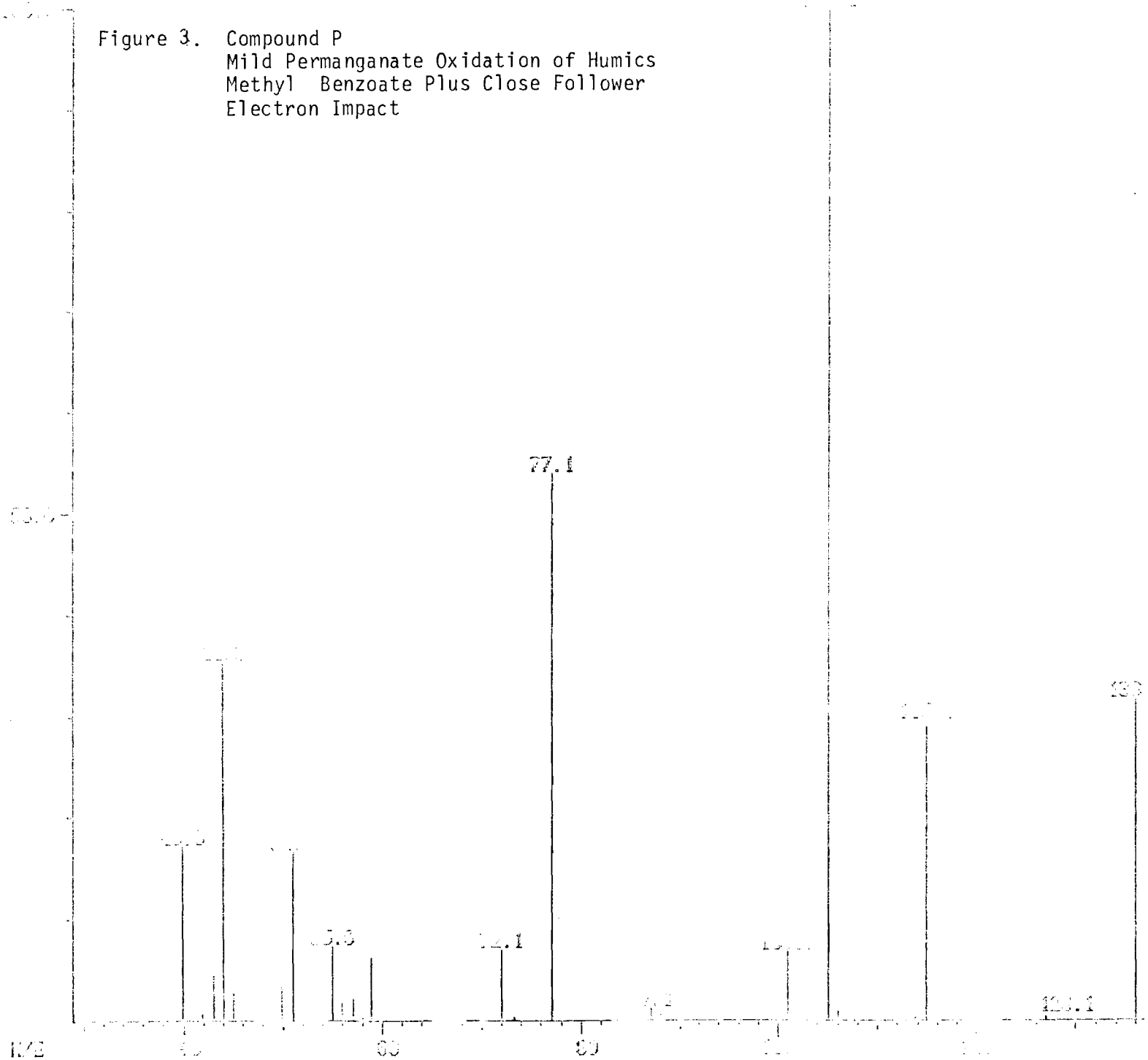
Figure 2. Mild Permanganate Oxidation of Humics.
CI Total Ion Chromatogram. Scans 0 - 1000.

Table I

Mild Permanganate Oxidation of Humics

Compound	Identity	Comment
A	Dimethyl Oxalate	
D	Dimethyl Malonate	
F	Methyl Dimethyl Malonate	Weak CI
G	Methyl Levulinate	
H	Dimethyl Succinate	
K	Dimethyl Methyl Succinate	Weak CI
L	Methyl-5-Methyl Furan-2-Carboxylate	Weak CI
N	Dimethyl-2, 2-Dimethyl Succinate	Weak CI
P	Methyl Benzoate	Incomplete Separation
T	Dimethyl Glutarate	Weak CI
B'	Methyl Ether of Dimethyl Oxaloacetate	Tentative
E'	Dimethyl Adipate	No CI
I'	Dimethyl Tartrate	Weak CI
O'	Methyl-4-Methoxybenzoate	
R'	Trimethyl Tricarballate	Incomplete Separation

Figure 3. Compound P
Mild Permanganate Oxidation of Humics
Methyl Benzoate Plus Close Follower
Electron Impact

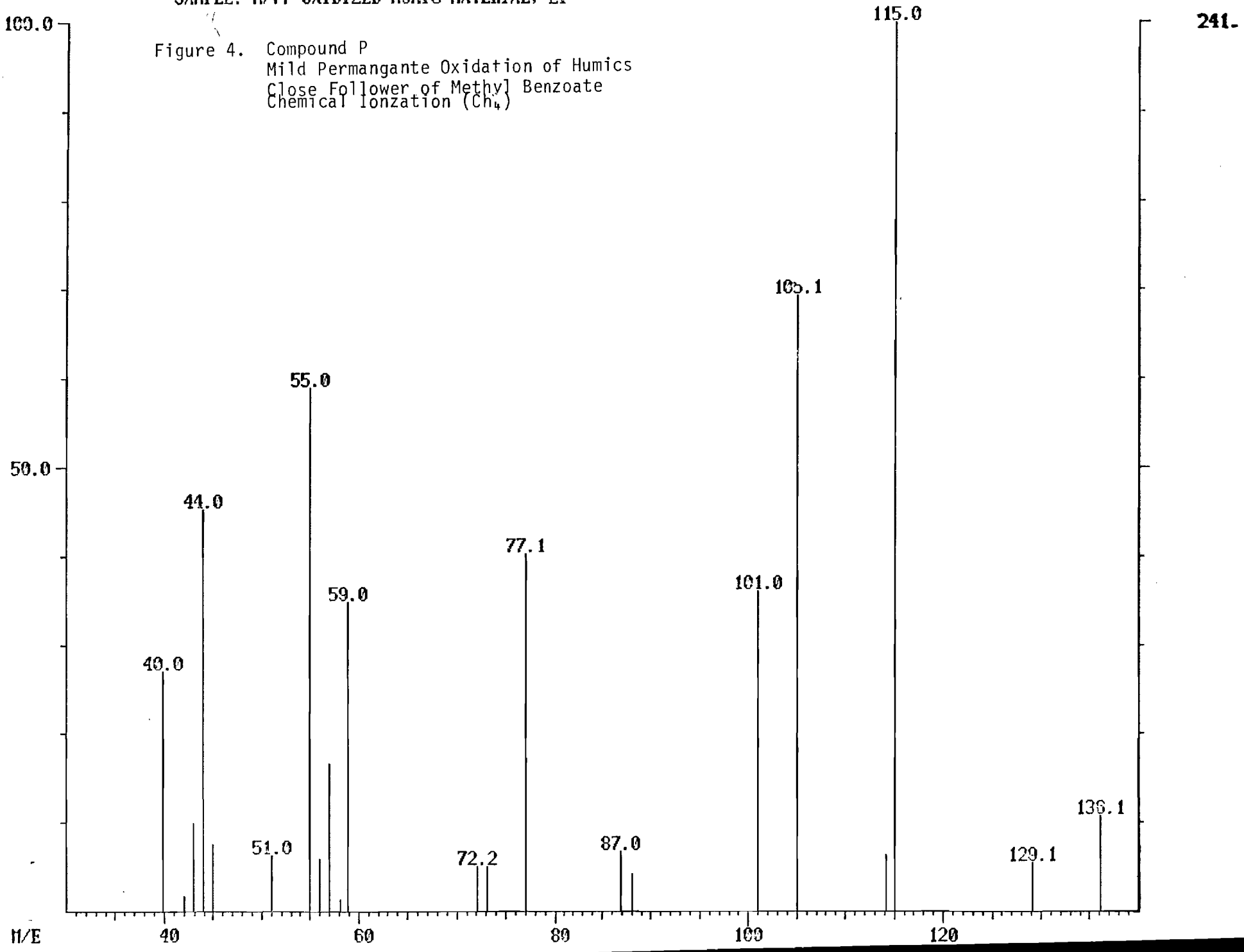


MASS SPECTRUM
04/12/78 11:57:00 + 12:37
SAMPLE: M/77 OXIDIZED HUMIC MATERIAL, EI

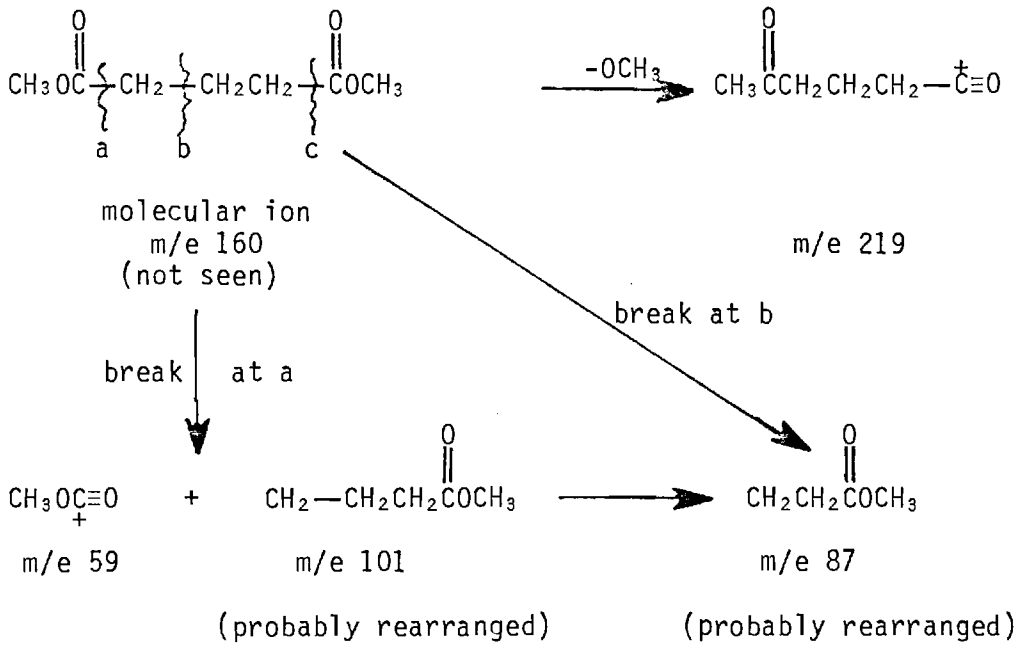
DATA: M77EI #303
CALI: 0412 #1

BASE M/E: 115
RIC: 1216.

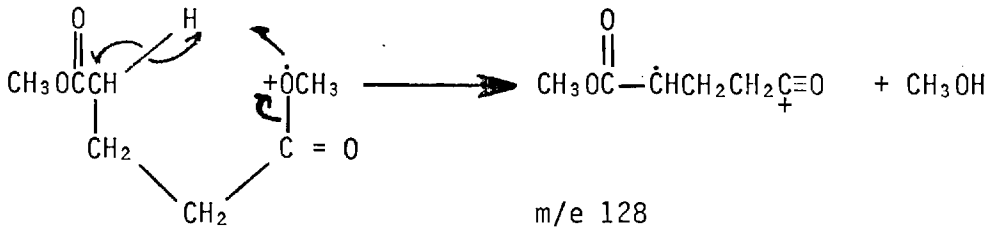
Figure 4. Compound P
Mild Permangante Oxidation of Humics
Close Follower of Methyl Benzoate
Chemical Ionzation (CH_4)



T. Compound T was identified by the data system as dimethyl glutarate with all indices above 850. The electron impact spectrum which is shown in Figure 5 produces a fragmentation pattern which is consistent with this assignment. An explanation is provided below.



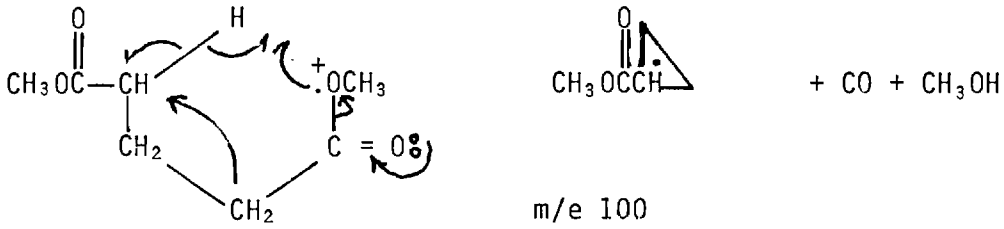
The same tendency to lose methanol or methoxy alone or in conjuction with a loss of carbon monoxide as had been reported for the esters of methyl-succinic acid and 2,2 dimethyl succinic acid is evident in compound T. A similar mechanism suffices to explain m/e 128, 129 and m/e 100, 101 as outlined on the next page.



$M^{+\cdot}$ (not seen)

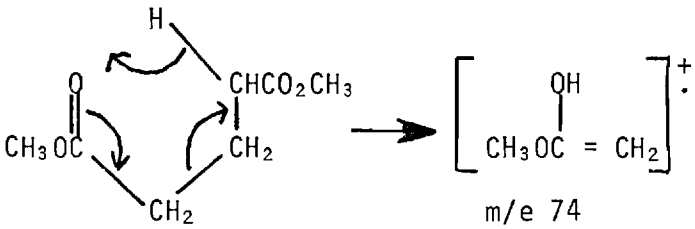
$[M - 32]^{+\cdot}$

-CO



m/e 100

The ion at m/e 74 arises from a classical McLafferty rearrangement as shown below. This rearrangement is commonly seen in methyl esters having a γ-hydrogen.



m/e 74

The chemical ionization spectrum (see Figure 6) is supportive with a base peak at m/e 129 due to the loss of methanol from the quasi-molecular ion.

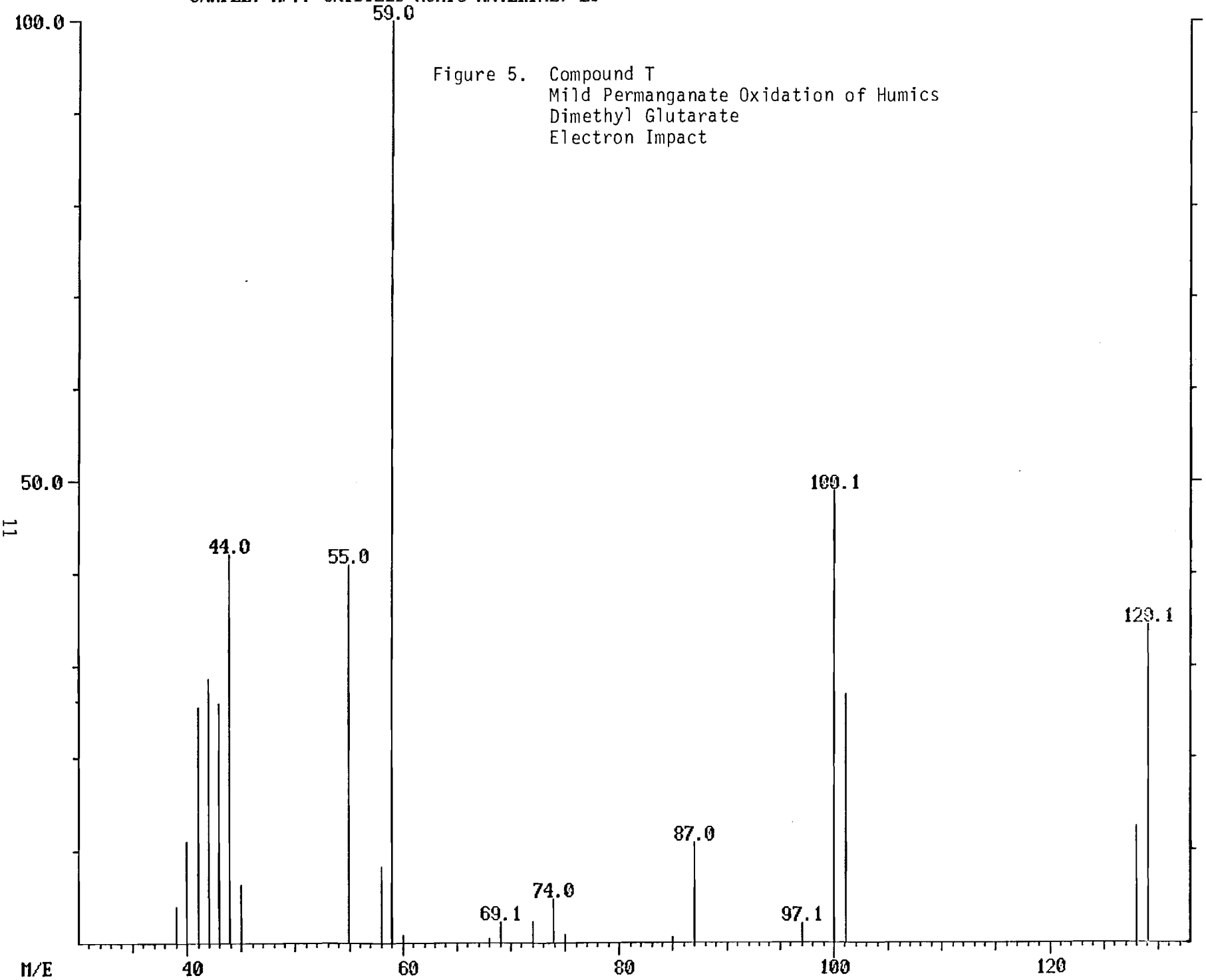
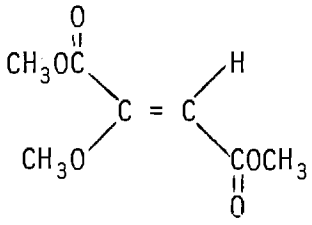


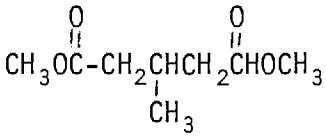
Figure 5. Compound T
Mild Permanganate Oxidation of Humics
Dimethyl Glutarate
Electron Impact

B'. Considerable effort was invested in the interpretation of the EI and CI spectra of compound B' which are shown in Figures 7 and 8 respectively. Although the library match is quite good for the dimethyl ester-methyl ether of oxaloacetic acid with all indices above 850, it has been quite difficult to rationalize the fragmentation pattern. Even the fragmentation pattern provided by the library is difficult to fully explain. The CI fragmentation is consistent with the structure only in that the major peaks represent the loss of neutral molecules (H_2O, CH_3OH, CH_3OH + CO) from the quasi-molecular ion. Unfortunately, it is not at all clear <u>how</u> they would arise.

At this moment, two structures are being considered - dimethyl-3-methyl glutarate (I) and the <u>trans</u>-dimethyl ester-methyl ether of oxaloacetic acid II. The two structures are shown below. Since there are elements in both structures which are difficult to rationalize with the observed fragmentation pattern, we are inclined to favor the choice of the data system on a tentative basis.



I



II

The EI fragmentations are outlined below:

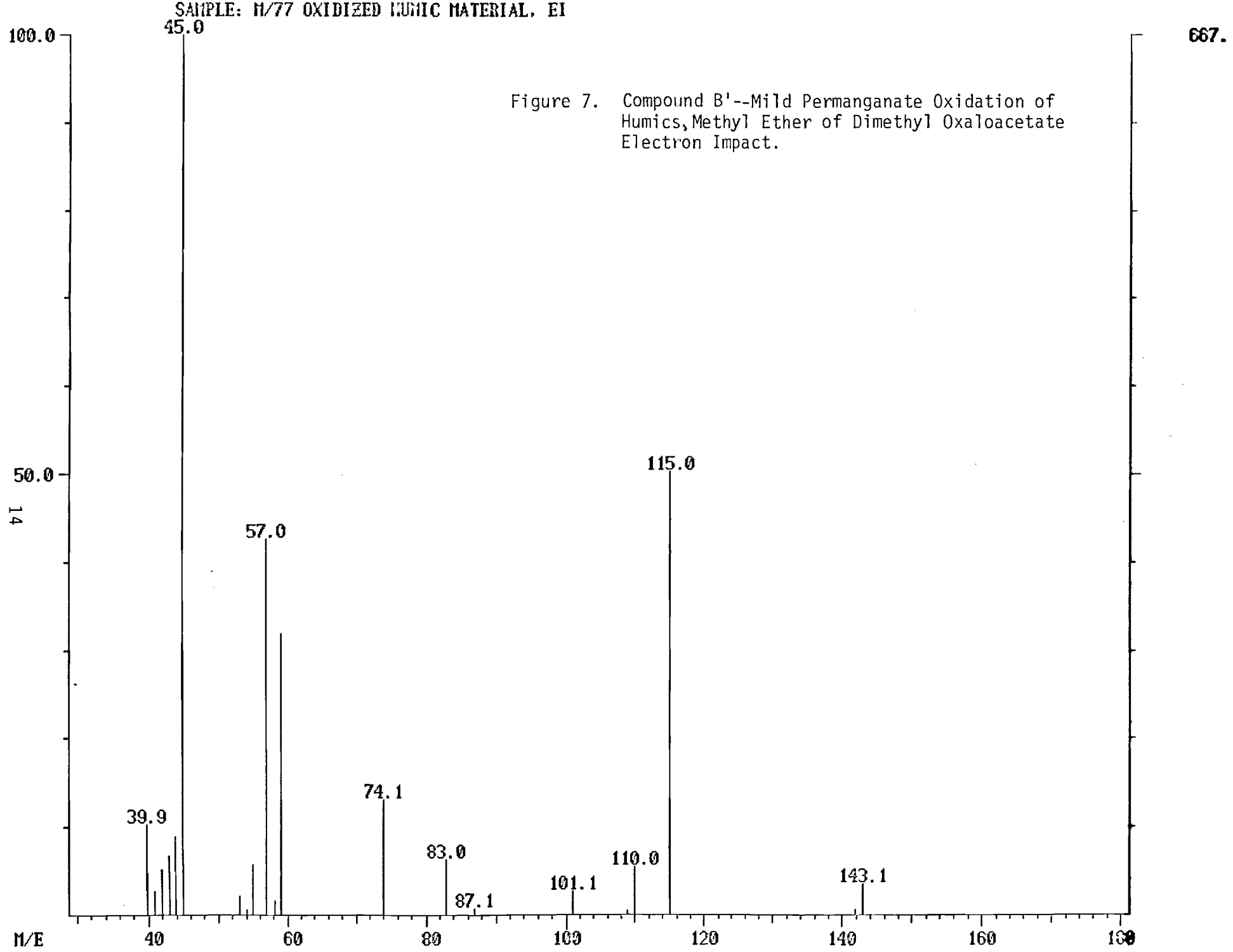


Figure 7. Compound B'--Mild Permanganate Oxidation of Humics, Methyl Ether of Dimethyl Oxaloacetate Electron Impact.

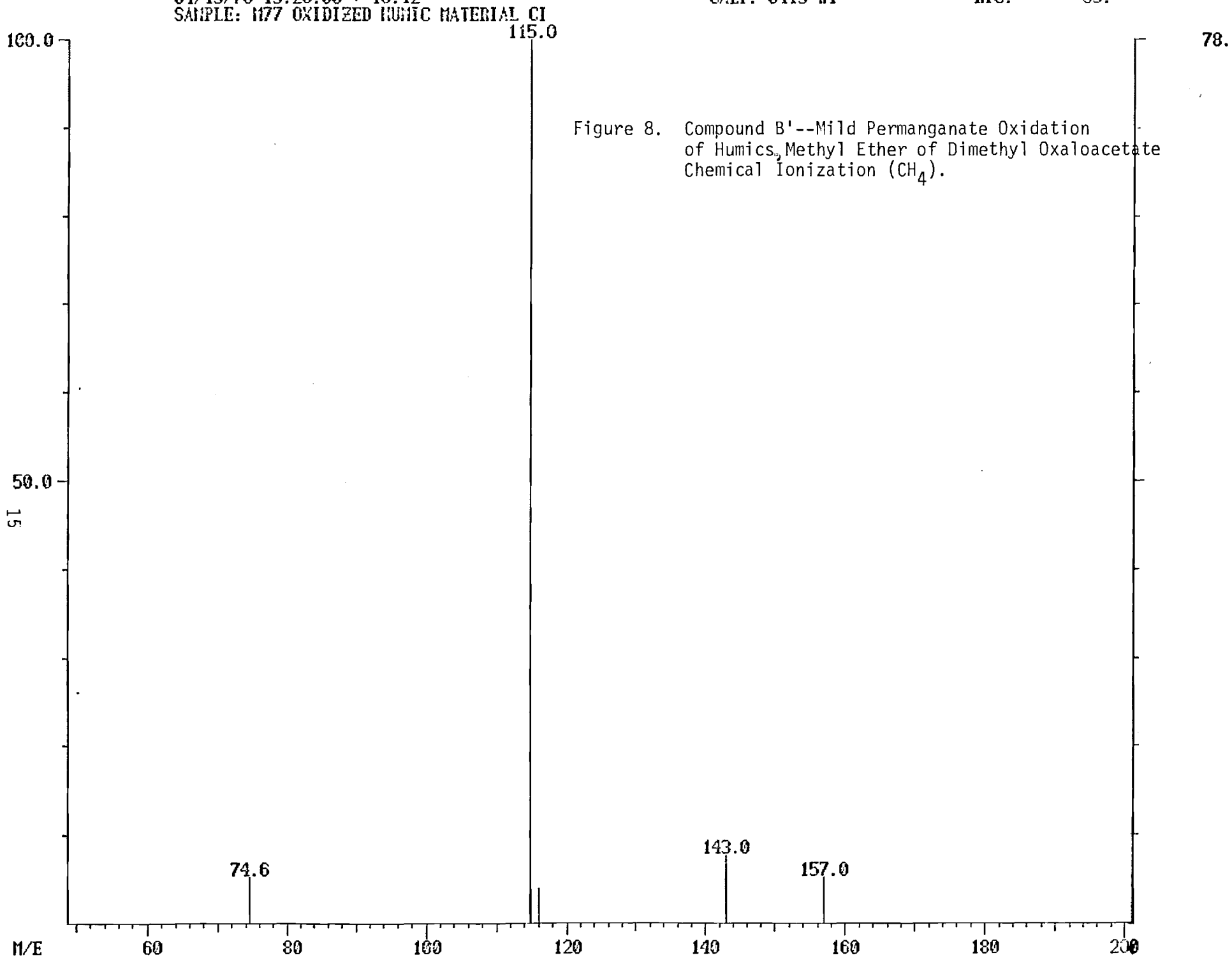
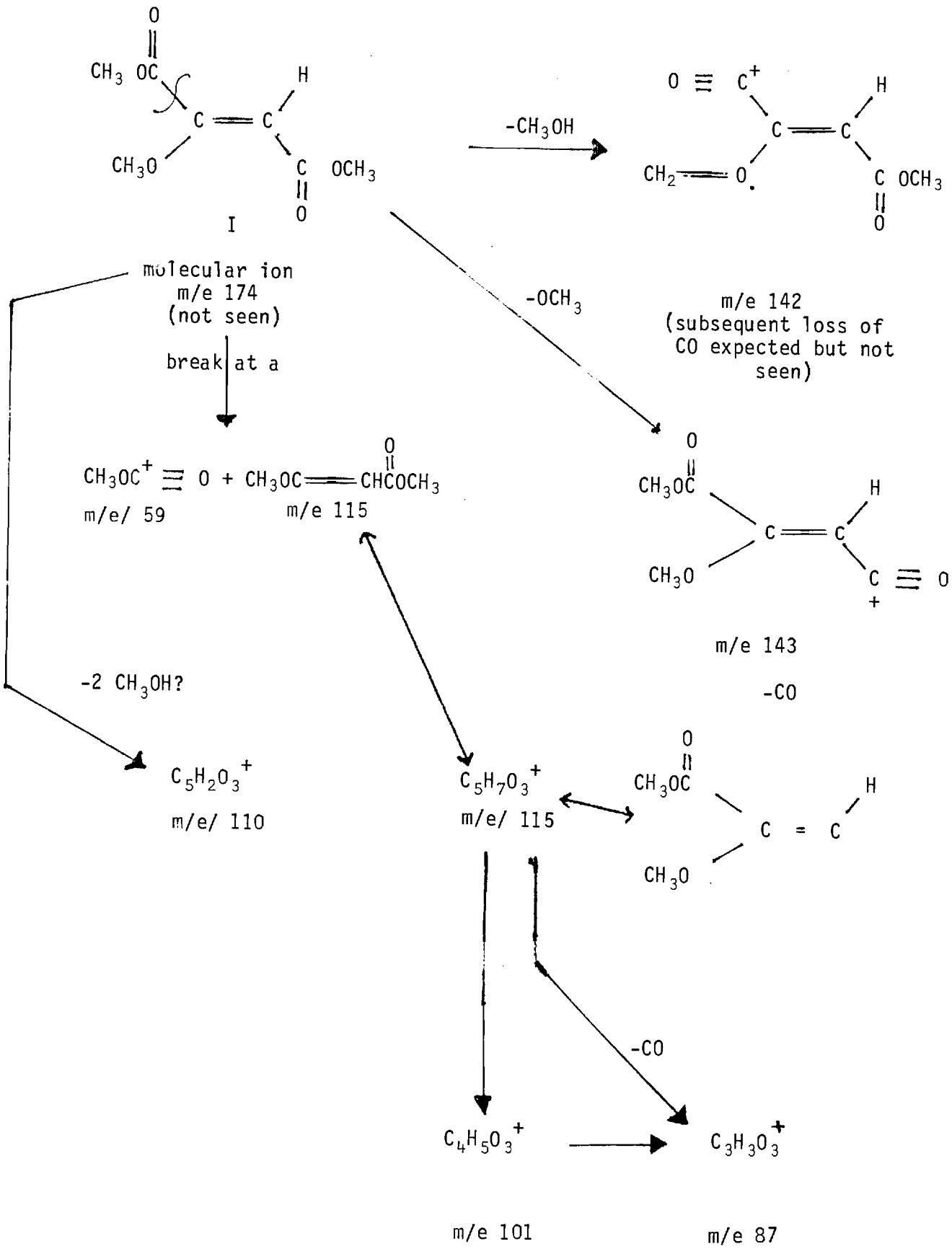
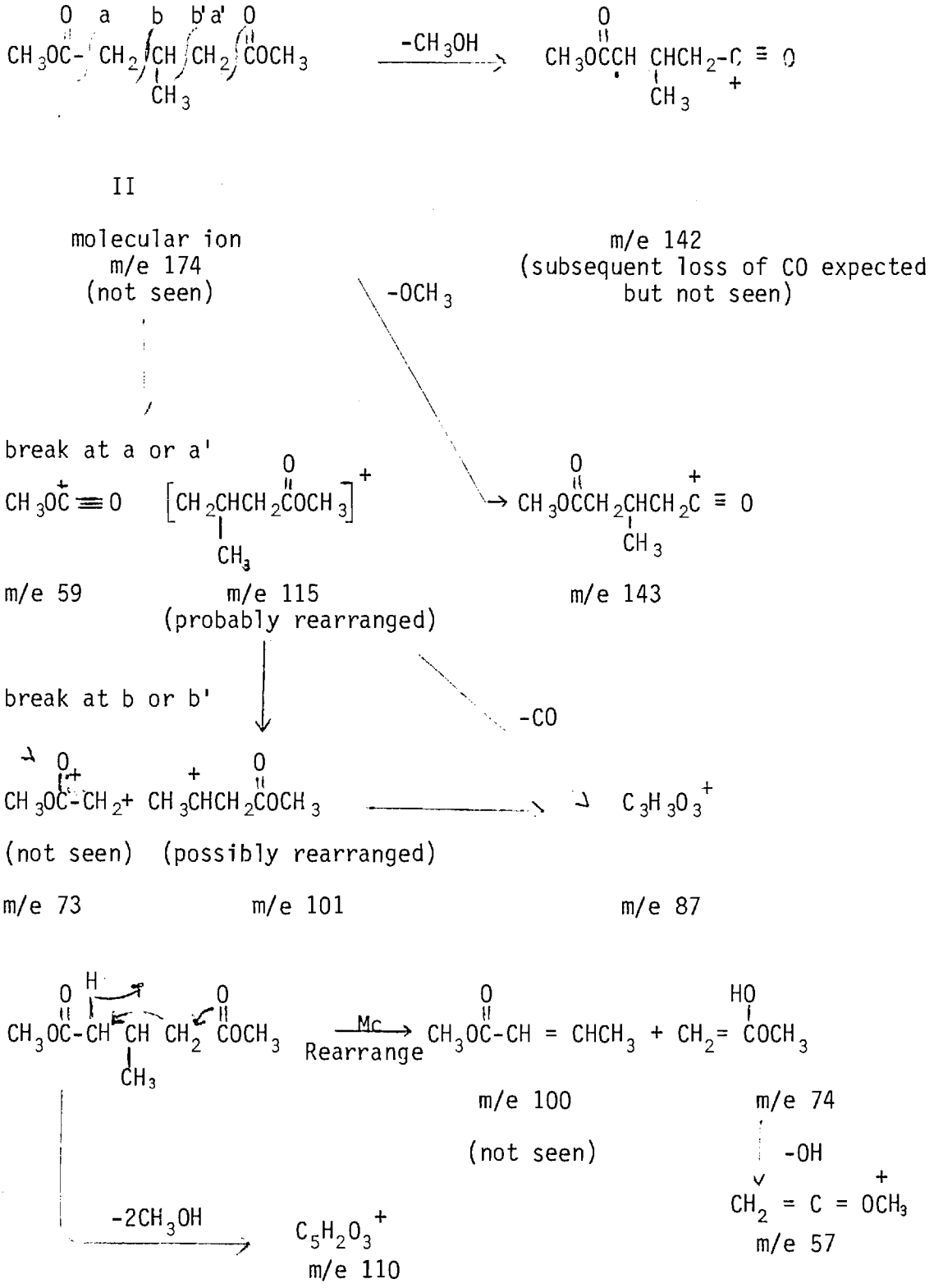
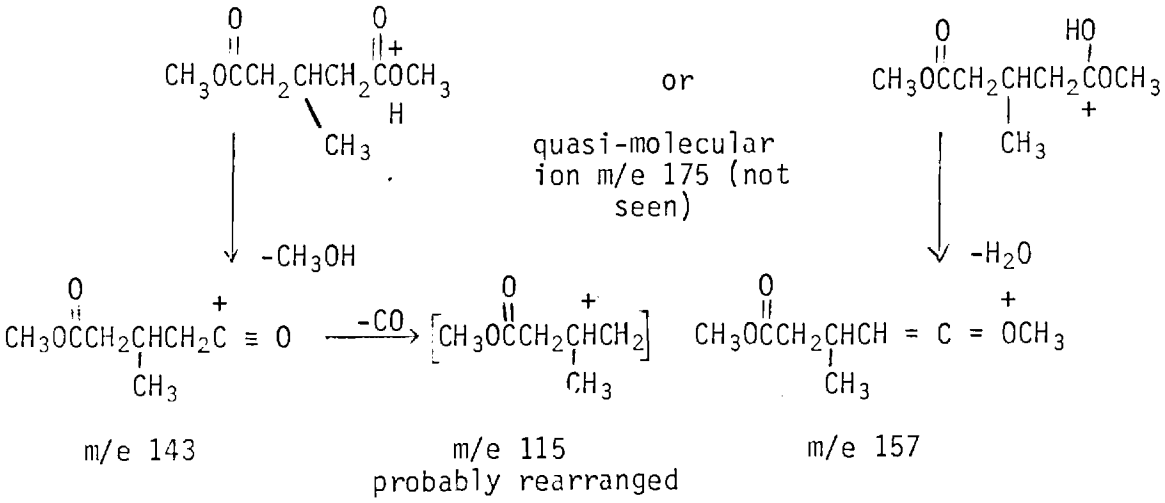


Figure 8. Compound B'--Mild Permanganate Oxidation of Humics, Methyl Ether of Dimethyl Oxaloacetate Chemical Ionization (CH_4).

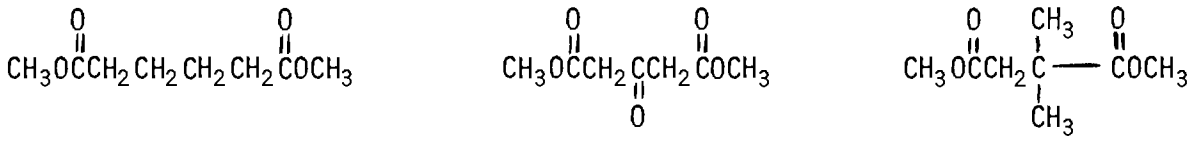

I
-CH3OH
-OCH3
molecular ion
m/e 174
(not seen)
break at a
m/e 142
(subsequent loss of
CO expected but not
seen)
CH3OC+ ≡ O + CH3OC═CHCOCH3
m/e/ 59
m/e 115
m/e 143
-CO
-2 CH3OH?
C5H2O3+
m/e/ 110
C5H7O3+
m/e/ 115
-CO
C4H5O3+
C3H3O3+
m/e 101
m/e 87


II
molecular ion
m/e 174
(not seen)
-OCH3
m/e 142
(subsequent loss of CO expected but not seen)
break at a or a'
m/e 59
m/e 115
(probably rearranged)
m/e 143
break at b or b'
-CO
(not seen)
(possibly rearranged)
m/e 73
m/e 101
m/e 87
Mc
Rearrange
m/e 100
(not seen)
m/e 74
-OH
m/e 57
-2CH3OH
m/e 110

In both cases, the base peak at m/e 45 is probably CO_2H^+ or $C_2H_5O^+$. Our inability to explain the peaks at m/e 110 and m/e 74 in the fragmentation of the methyl ether of dimethyl oxaloacetate (I) should not be taken as a reason for distrusting the assignment since these peaks are also present in the library spectrum. Neither of the postulated patterns explains the absence of a peak at m/e 114 which is thought to be derived from m/e 142 by loss of CO. In fact, this peak is recorded in the library spectrum of I. While the dimethyl-3-methyl glutarate (II) does make it easier to explain m/e 74 (McLafferty) rearrangement and m/e 110 (double rearrangement of the type previously described for dimethyl glutarate and dimethyl-2,2-dimethyl succinate), it is difficult to understand why a significant ion is not seen at m/e 73. Both structures are reasonable from the standpoint of sample history and pretreatment. Structure II is definitely more consistent with the CI fragmentation as shown below.



E'. The EI spectrum of compound E' which is shown in Figure 9 showed a fivefold match with that of dimethyl adipate III when compared with library spectra. Since 12 of the possible 15 fit indices were over 800, it was necessary to give this structure serious consideration. Alternate structures IV and V which are shown below were considered in an effort to explain the absence of peaks at m/e 87 and m/e 115 in the experimental spectrum. Neither of the alternate structures nor the computer matched structure explains the absence



III IV V

of the peak at m/e 142 (corresponding to the loss of water and methanol from the molecular ion) which has been seen in the other dicarboxylates in this series. Structure IV was rejected because it would be expected to be in equilibrium with the enol which would then be methylated during the diazomethane treatment. Structure V would be expected to give an even stronger ion at m/e 115 than III because a loss of CO_2CH_3 would relieve steric strain. Structure III, which at least has no extra ions of significance, therefore seems to be the most consistent with the fragmentation sequence outlined below. The missing ions at m/e 142, m/e 115, m/e 87 and m/e 82 are all weak in the library spectra and thus may be below the noise level in the experimental spectrum which itself is rather weak. The CI spectrum was too weak to be of use. The electron impact fragmentation sequence is outlined on page 21.

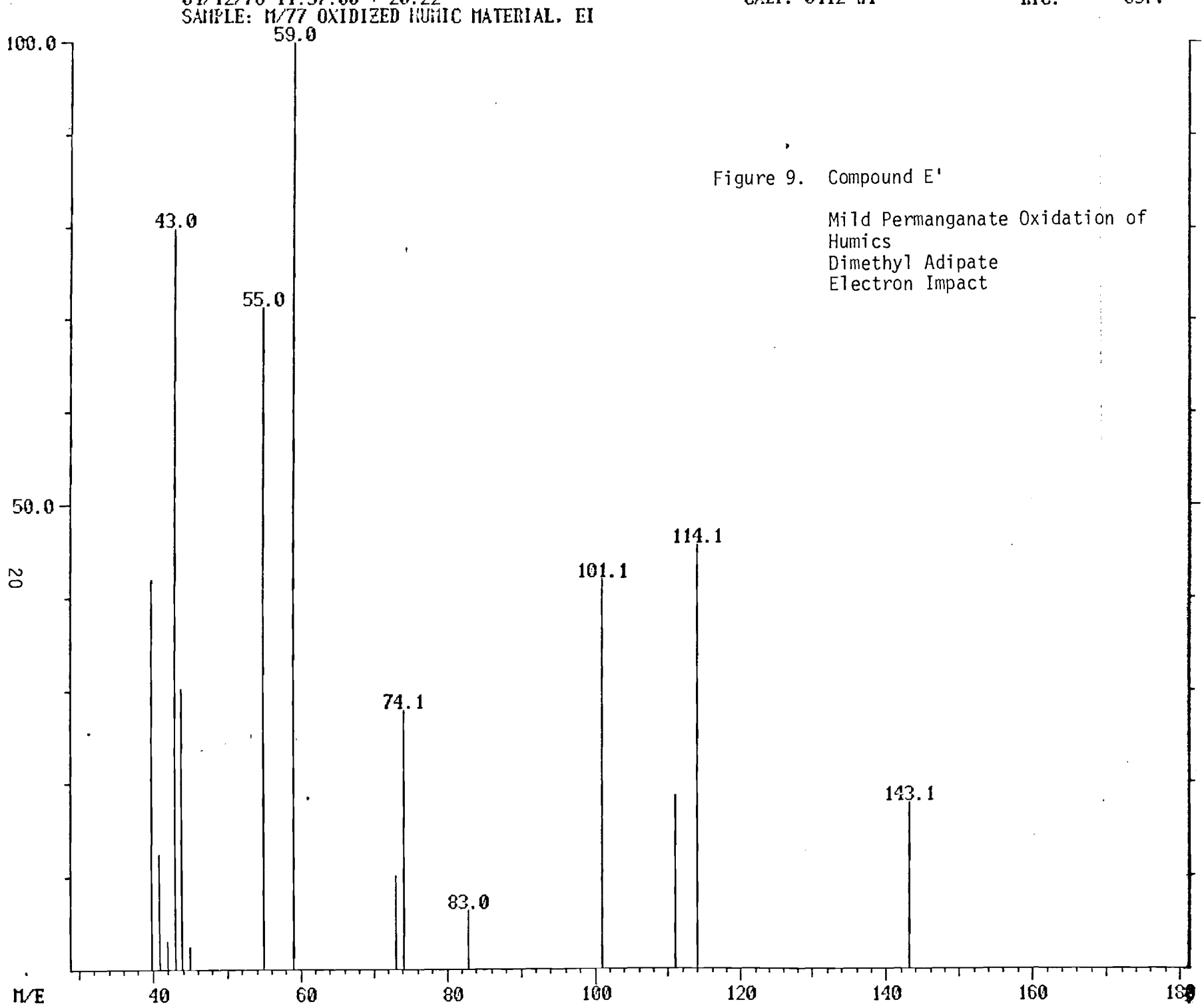
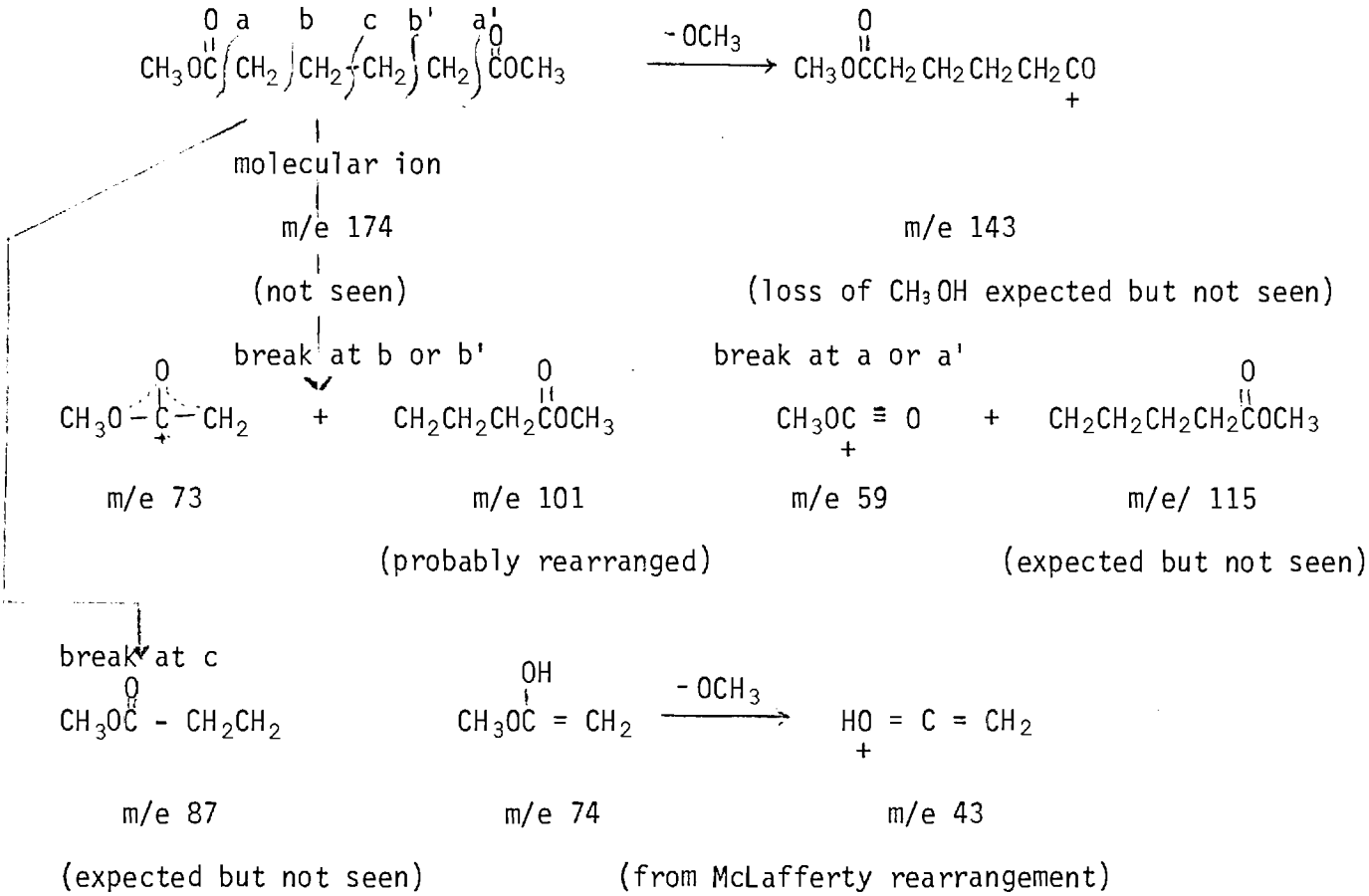
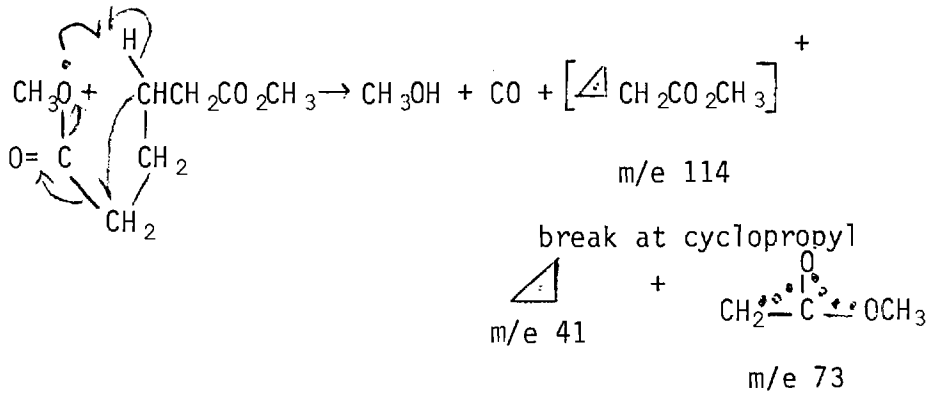


Figure 9. Compound E'

Mild Permanganate Oxidation of Humics
Dimethyl Adipate
Electron Impact

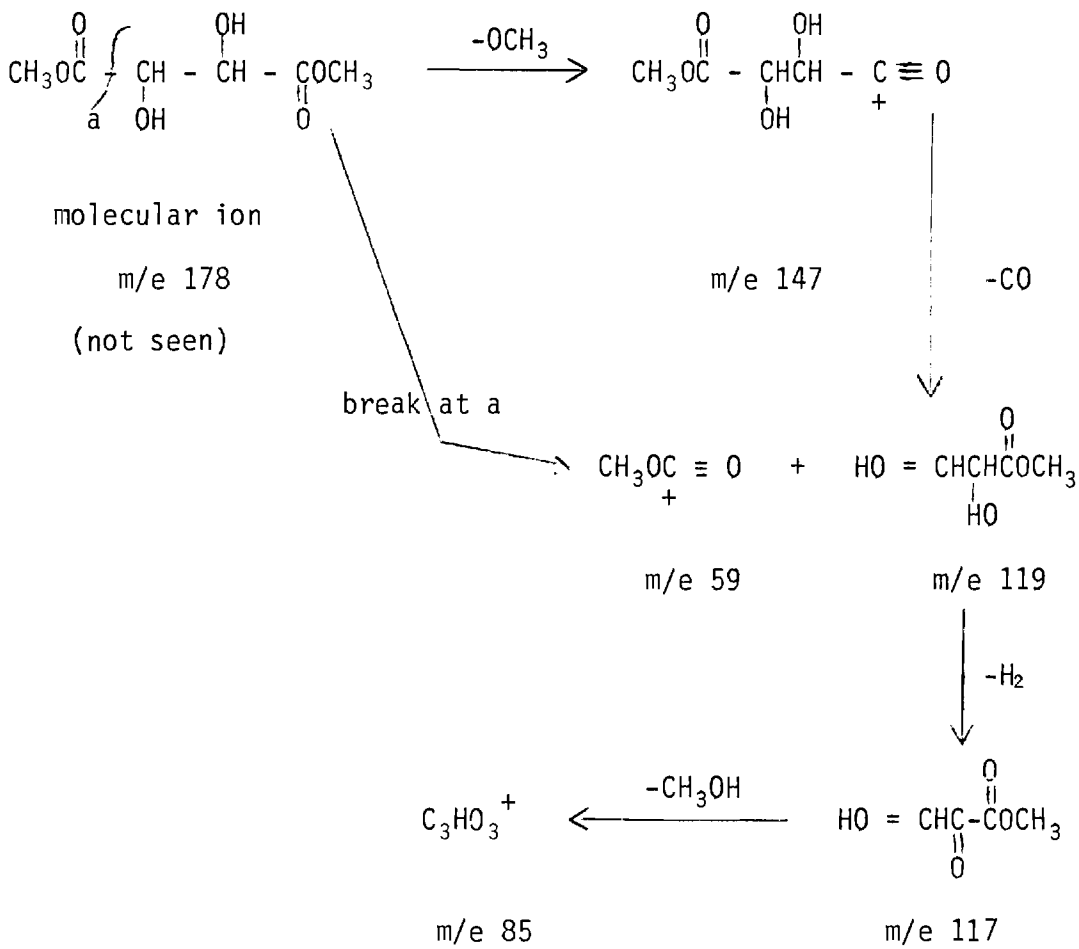


The production of m/e 43 from the McLafferty product at m/e 74 seems somewhat inconsistent with its high relative intensity. The rearrangement ion at me/ 114 is consistent with a loss of methanol plus carbon monoxide in a manner which agrees with earlier sequences. Further fragmentation could lead to m/e 73 and m/e 41 as shown below.



We are willing to consider other structures. The reviewers are invited to make comments.

I'. The EI spectrum of compound I' which is shown in Figure 10 showed a good fit (all indices above 880) with that of dimethyl tartrate. A fragmentation sequence which is consistent with the experimental spectrum is outlined below.



The close proximity and high proportion of functional groups in the molecule increases the opportunity for rearrangement. A number of such rearrangements have been postulated below in order to explain the observed fragmentation patterns. The first of these involves the simultaneous loss of

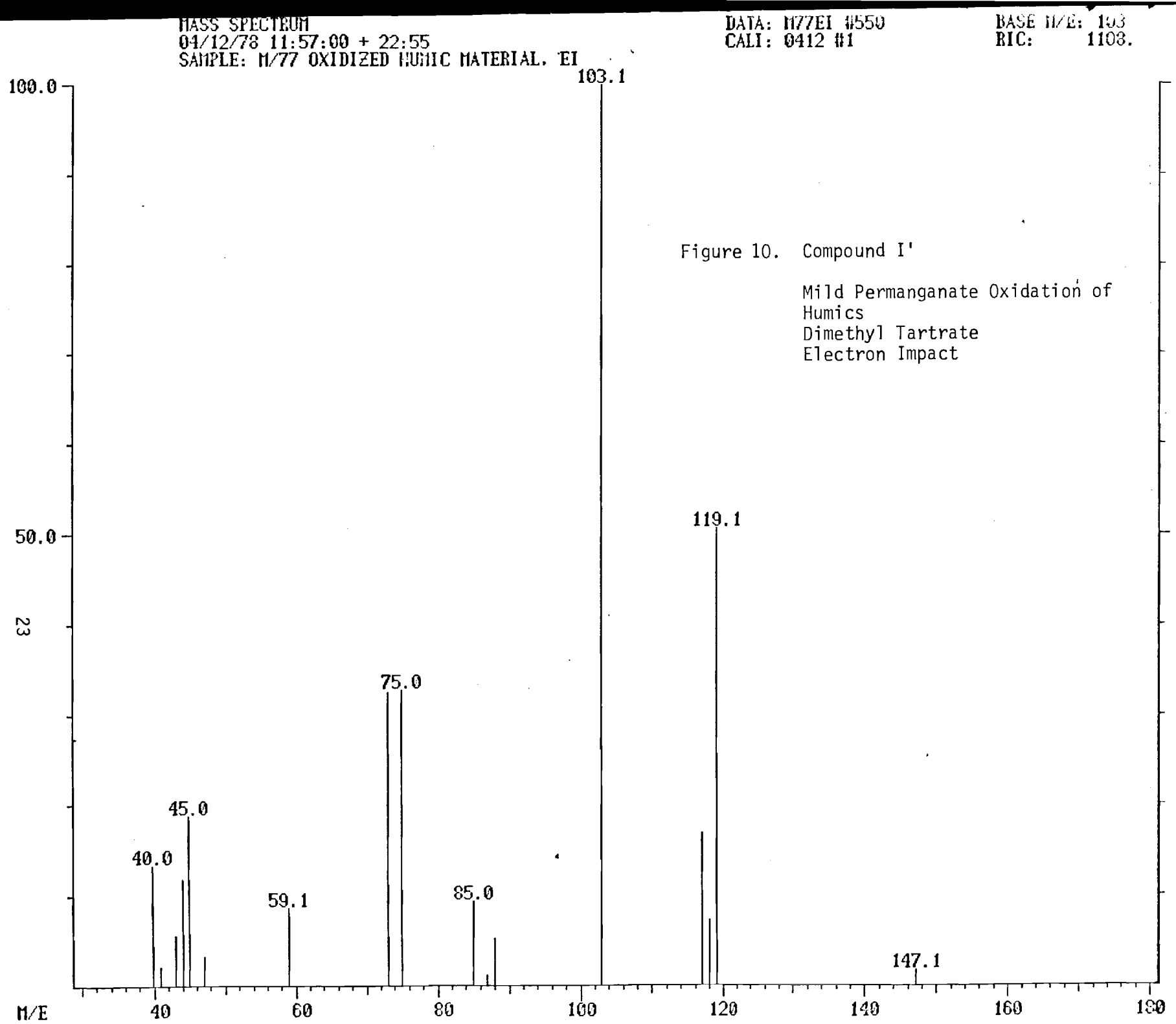
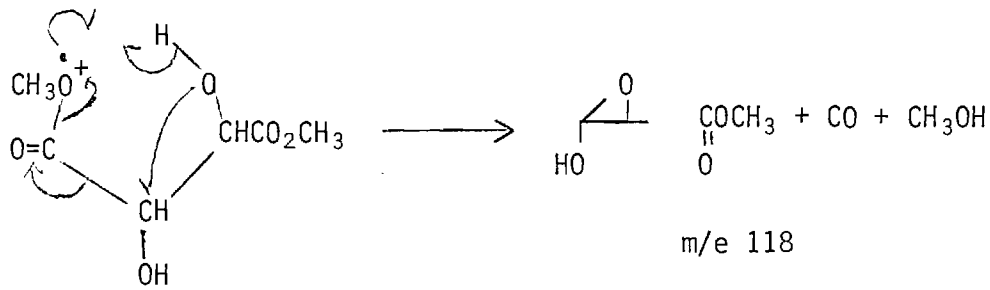


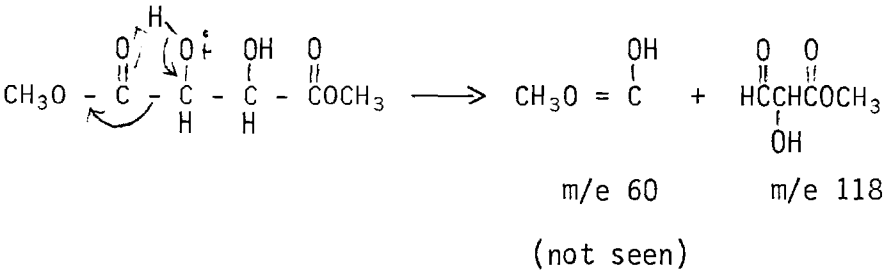
Figure 10. Compound I'

Mild Permanganate Oxidation of Humics
Dimethyl Tartrate
Electron Impact

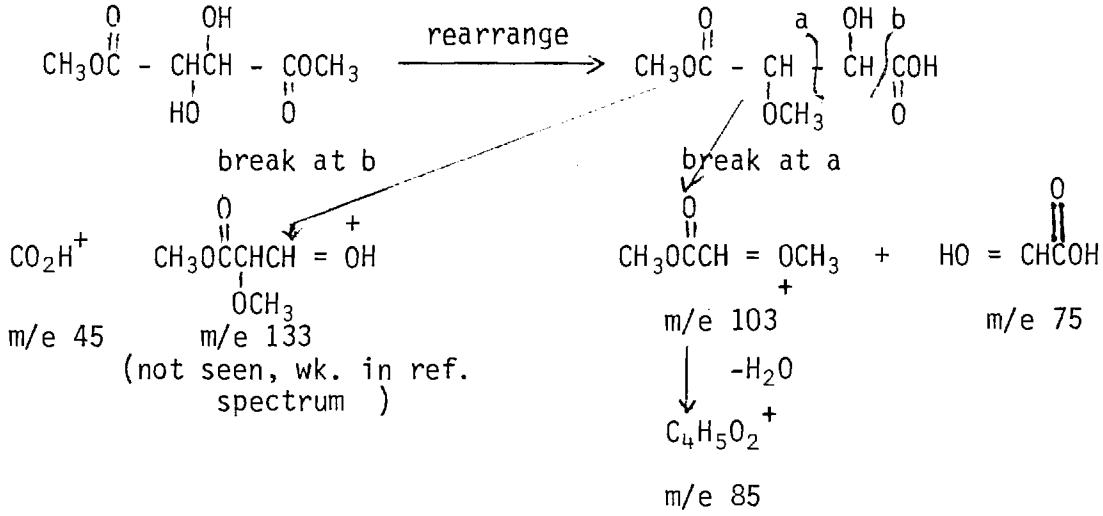
methanol and carbon monoxide. A mechanism which is similar to those described in earlier sections is suggested.



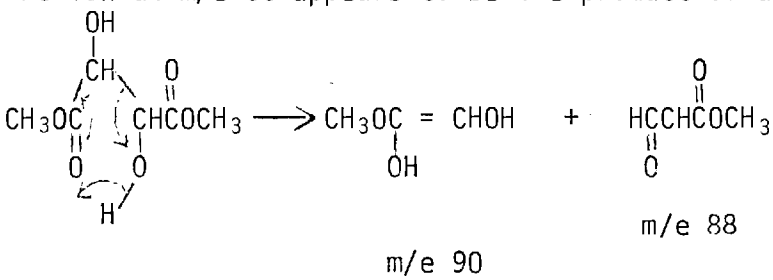
An alternate mechanism is also possible as shown below.



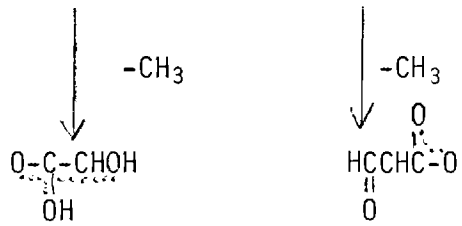
An exchange of methoxy and hydroxy (or hydrogen and methyl if six-membered process is preferred) involving a mechanism which we cannot postulate at this time is required to explain the ions at m/e 103 and m/e 75.



The ion at m/e 88 appears to be the product of a McLafferty rearrangement.



(not seen, not in ref. spectrum)

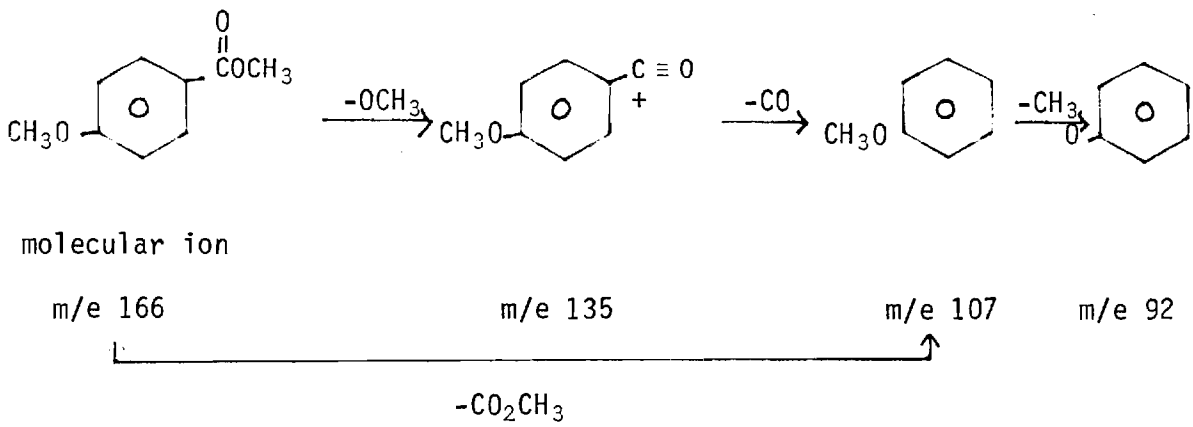


m/e 75 m/e 73

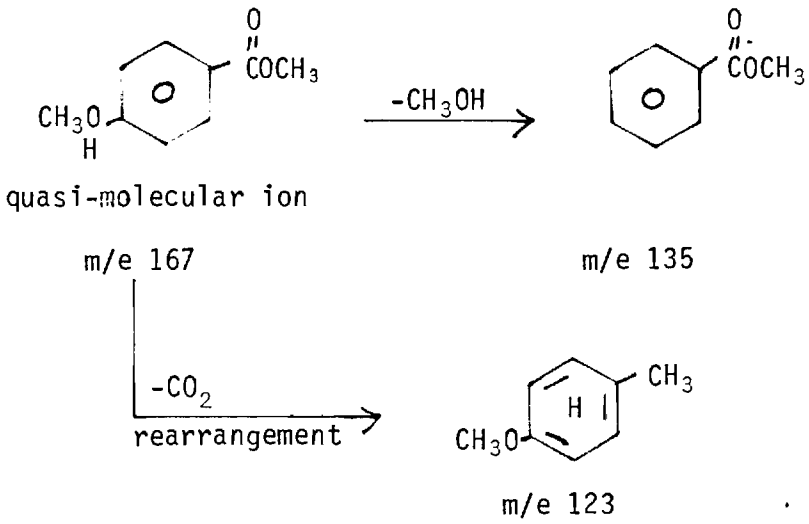
The chemical ionization spectrum is mildly supportive in that it shows m/e 119 as the only peak. This ion presumably arises from the quasi-molecular ion by loss of the protonated ester function.

O' Compound O' was readily identified as a methoxy benzoic ester by virtue of a strong molecular ion at m/e 166 and a methoxy benzoyl base peak at m/e 135. The lack of strong ions at m/e 133 and m/e 134 suggests that an <u>ortho</u> substituted structure should be ruled out. The choice between a 4-methoxybenzoate and a 3-methoxybenzoate is not so clear. The data system suggests a very slight preference for methyl p-methoxybenzo ate.. All fit indicies were above 960 for both isomers. The EI and CI spectra are presented in Figures 11 and 12. The respective fragmentation sequences which are fully consistent with the assigned structure are outlined below.

(EI)



(CI)



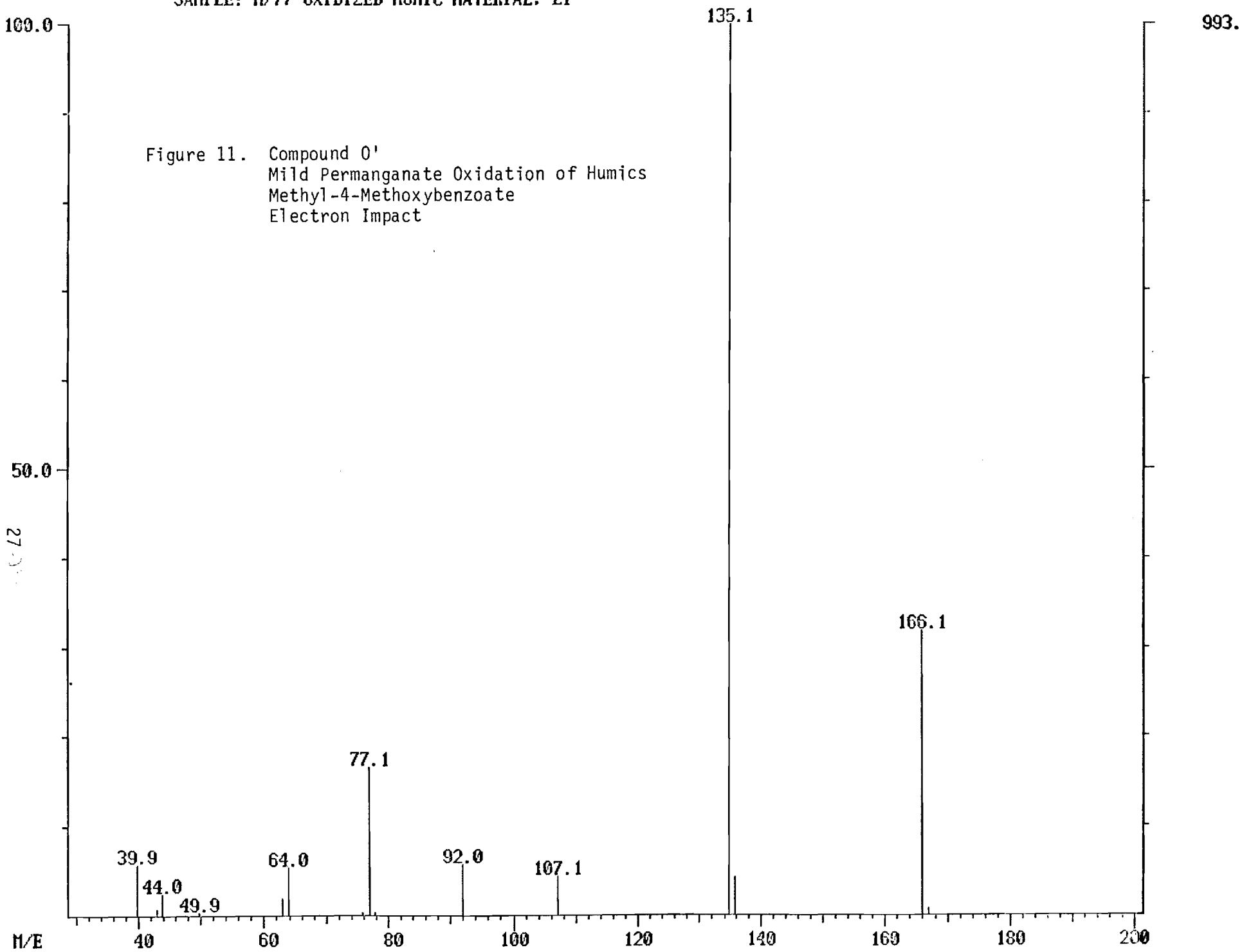


Figure 11. Compound O'
Mild Permanganate Oxidation of Humics
Methyl-4-Methoxybenzoate
Electron Impact

R' This compound can be given only a tentative structural assignment largely because the chromatographic peak is poorly resolved from the preceding component Q'. In fact, an enlargement of the total ion chromatogram in this region shows a third component in between Q' and R'. Nevertheless, the tricarballylic acid structure was found to be consistent with enough of the EI spectrum (see Figure 13) to warrant an assignment. The data system did not suggest a good match with any of the library spectra. A proposed fragmentation sequence is outlined on the next page. Enough branching is present among the proposed pathways so that a number of explanations may be found for the base peak and many of the lower mass ions. Anything not explained may also be attributed to incomplete resolution of the chromatographic peaks. The CI spectrum is supportive with the base peak at m/e 187 corresponding to loss of methanol and carbon monoxide or the protonated ester function from the quasi-molecular ion.

MASS SPECTRUM
04/12/78 11:57:00 + 32:00
SAMPLE: M/77 OXIDIZED HUMIC MATERIAL, EI

DATA: M77EI #768
CALI: 0412 #1

BASE M/E: 127
RIC: 1910.

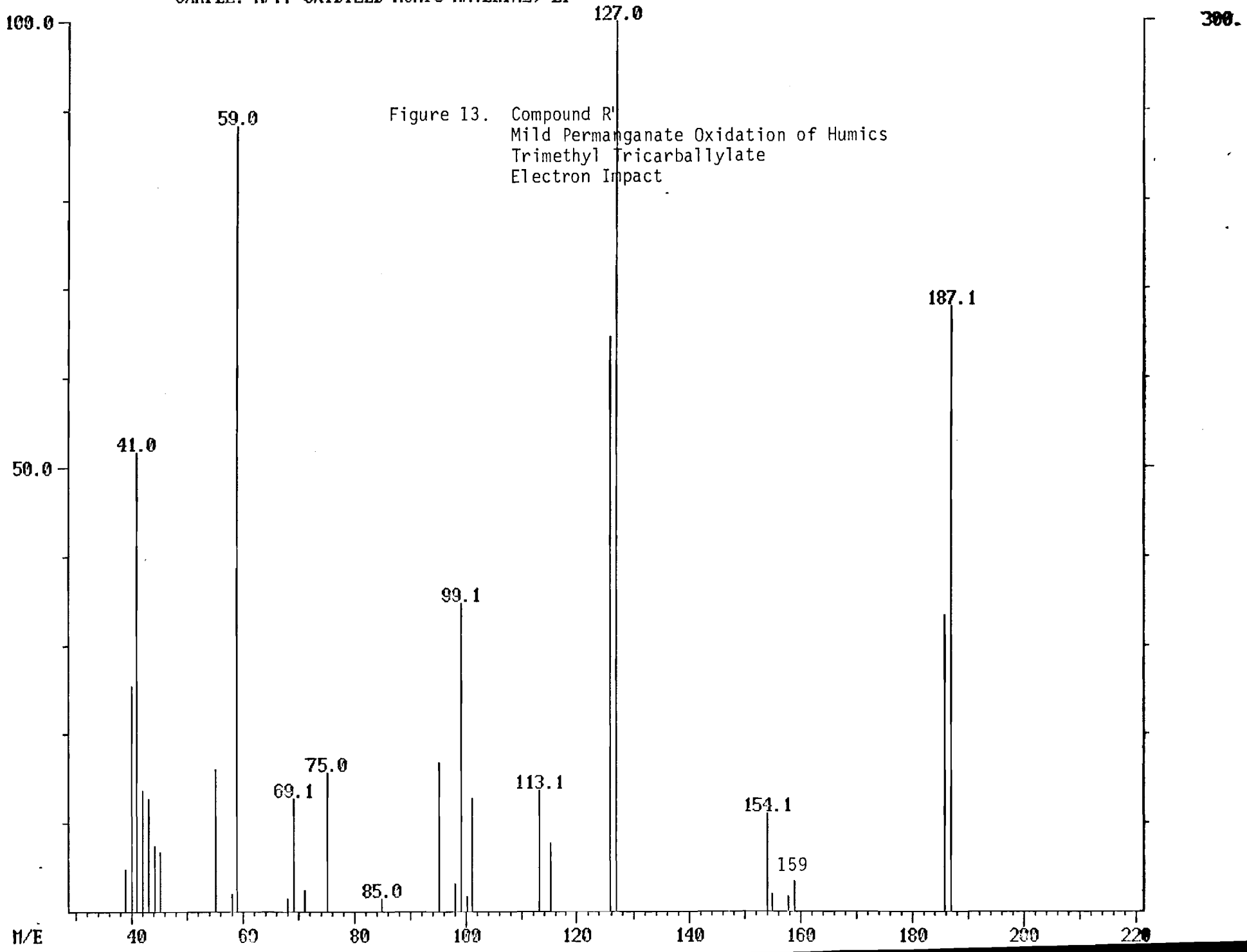
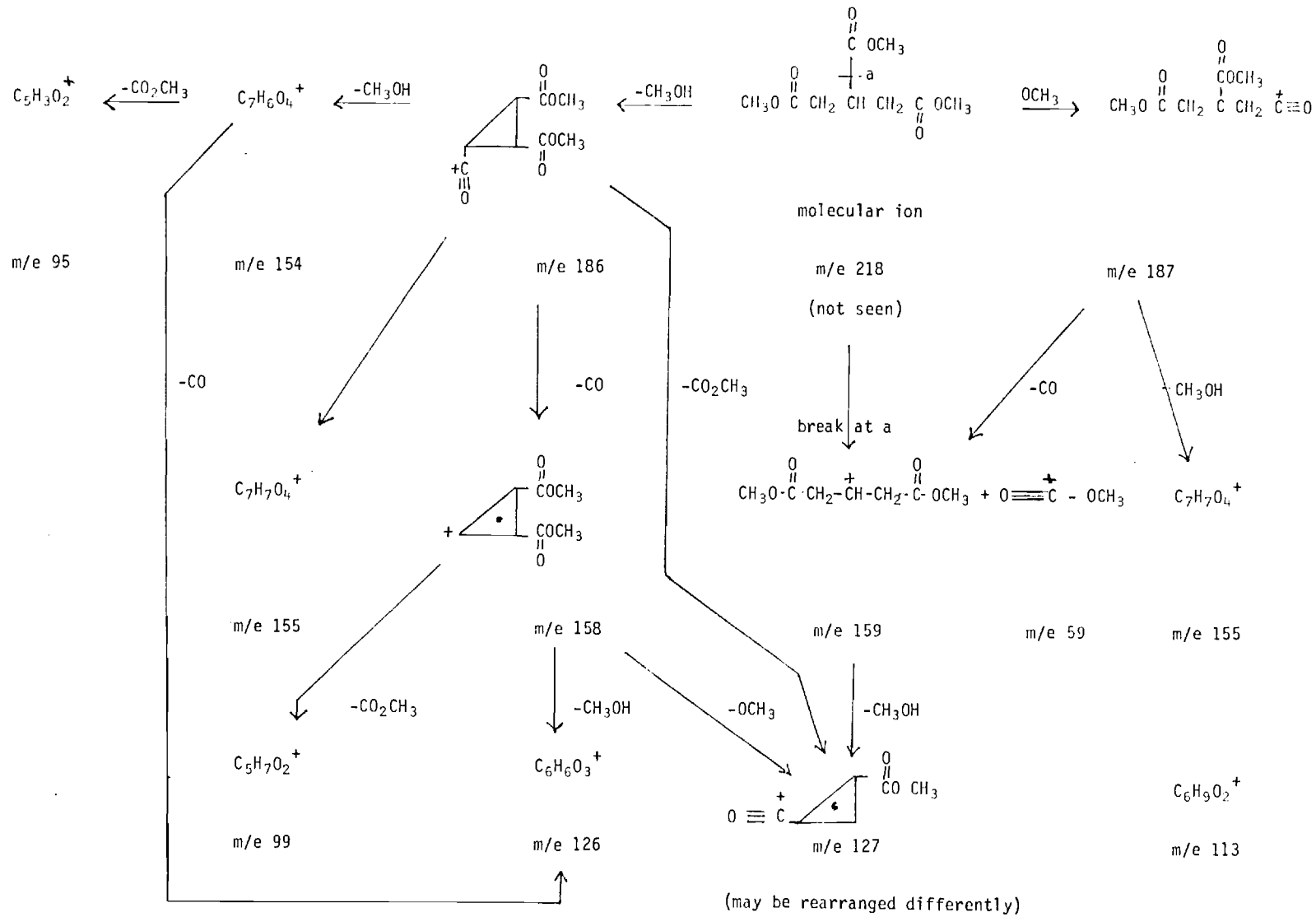


Figure 13. Compound R'
Mild Permanganate Oxidation of Humics
Trimethyl Tricarballylate
Electron Impact


$C_5H_3O_2^+$
$-CO_2CH_3$
$C_7H_6O_4^+$
$-CH_3OH$
$-CH_3OH$
$CH_3O\ C\ CH_2\ CH\ CH_2\ C\ OCH_3$
OCH_3
$CH_3O\ C\ CH_2\ C\ CH_2\ C\equiv O$
molecular ion
m/e 95
m/e 154
m/e 186
m/e 218
(not seen)
m/e 187
-CO
-CO
$-CO_2CH_3$
-CO
$-CH_3OH$
break at a
$CH_3O\text{-}C\text{-}CH_2\text{-}CH\text{-}CH_2\text{-}C\text{-}OCH_3 + O\equiv C - OCH_3$
$C_7H_7O_4^+$
$C_7H_7O_4^+$
m/e 155
m/e 158
m/e 159
m/e 59
m/e 155
$-CO_2CH_3$
$-CH_3OH$
$-OCH_3$
$-CH_3OH$
$C_5H_7O_2^+$
$C_6H_6O_3^+$
$C_6H_9O_2^+$
m/e 99
m/e 126
m/e 127
m/e 113
(may be rearranged differently)

IV. OXIDATION OF HUMICS WITH PERMANGANATE IN AN APROTIC SOLVENT USING AN 18-CROWN-6 COMPLEX

In the years since the discovery of the ability of crown polyethers to bring unusual amounts of inorganic salts into aprotic solvents and thus produce "naked anion" reagents, the unusual properties of such reagents have been the subject of much research. Since the 18-crown-6 ether forms an organic-soluble complex with potassium permanganate, we decided to attempt oxidation of methylated aquatic humics with this reagent. Two important reasons for taking this action can be brought forward: (1) Our previous reactions with $KMnO_4$ solutions were hampered by the fact that the reaction mixtures were inhomogeneous because the methylated humic substances were water insoluble. Both the 18-crown-6-$KMnO_4$ complex and the methylated humic substances are now dissolved in methylene chloride, thus creating a homogeneous system; and (2) In our previous oxidation reactions we had to raise the temperature initially to at least 55°C in order to get the reaction started. However, we have found that the distribution of degradation products depends on the conditions of the oxidation reaction, most importantly the temperature. The 18-crown-6-$KMnO_4$ complex in CH_2Cl_2 reacts with the methylated humics at room temperature, which could mean a much milder and more specific oxidative attack. Also, the potassium salts of the acid reaction products would be expected to be insoluble in methylene choride, which may protect them from further oxidation.

Our procedure is as follows:

A small amount of 18-crown-6-ether (2g) was dissolved in methylene choride (100 ml), and to this solution potassium permanganate (12 g) was added. The mixture was stirred vigorously to give a purple solution. A second solution of

methylated aquatic humics (starting from 1g M/30 sample) in 30 ml methylene chloride was added to the solution of oxidant complex. The reaction mixture was stirred vigorously for 24 hours at room temperature and then filtered. The black cake of MnO_2 remaining on the filter paper was washed with methylene chloride, and then stirred with water (200 ml) and sodium sulfite (4g) in order to destroy any unreacted permanganate. Fifty ml of 2N sodium hydroxide was added to the solution with continued stirring for 30 minutes. The colored alkaline aqueous solution thus obtained was extracted with ether after filtration in order to remove the crown ether. A gas chromatogram of the concentrated extract (Fraction A) showed two peaks, one of which is the crown ether.

The aqueous solution was next acidified with hydrochloric acid to pH 2 and continuously extracted with ethyl acetate. The residue obtained after evaporation of solvent was methylated with diazomethane to yield 0.194 g of product (Fraction B).GC/MS data of (A) and (B) are being acquired as of this writing.

A second extraction of the residual aqueous solution with ethyl acetate provided an additional 0.146 g of product (after methylation-Fraction C). The respective gas chromatograms which are shown in Figures 14 and 15 indicate a large number of components in each of the product mixtures. Capillary GC/MS analysis should provide information which is complementary to that provided in Section III.

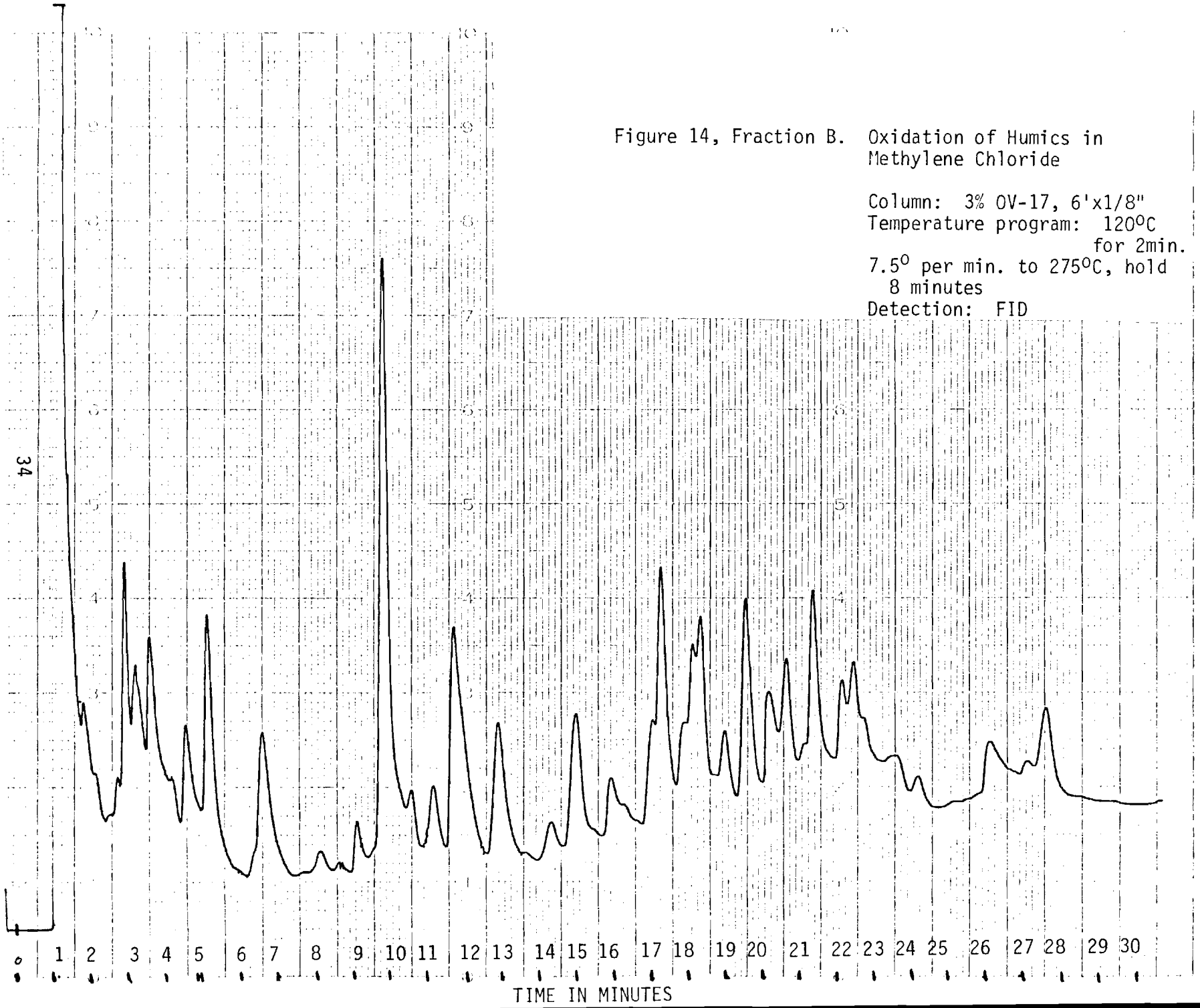


Figure 14, Fraction B. Oxidation of Humics in Methylene Chloride

Column: 3% OV-17, 6'x1/8"
Temperature program: 120°C for 2min.
7.5° per min. to 275°C, hold 8 minutes
Detection: FID

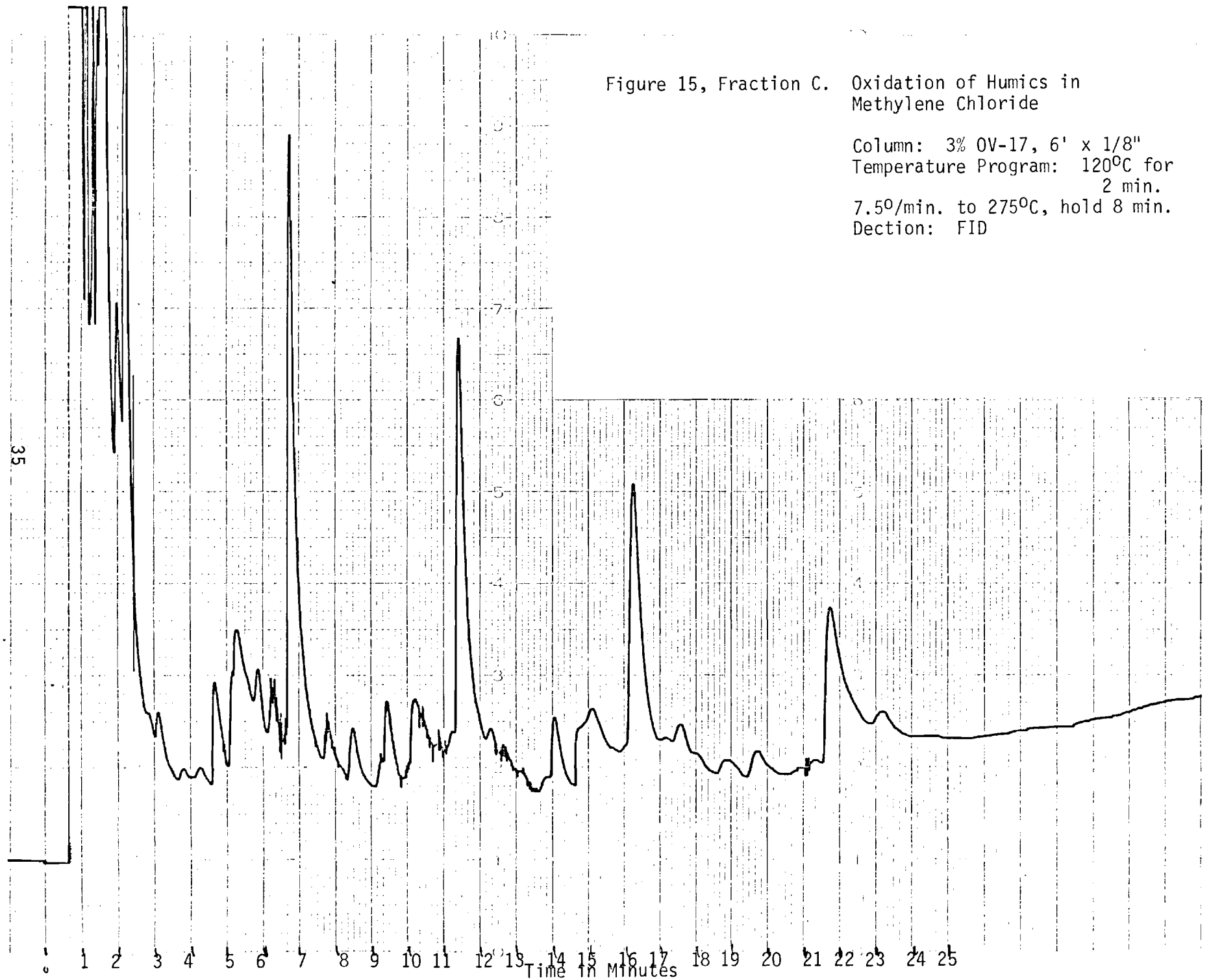


Figure 15, Fraction C. Oxidation of Humics in Methylene Chloride

Column: 3% OV-17, 6' x 1/8"
Temperature Program: 120°C for 2 min.
7.5°/min. to 275°C, hold 8 min.
Dection: FID

V . COMPARISON OF PRODUCT MIXTURES - PERMANGANATE OXIDATIONS

In addition to those assignments which have been discussed in detail in this and earlier reports (see Table I) a number of further assignments have been made. We have also been able to obtain peak area integrations on two of the more recent runs. This has enabled us to develop an estimate of the relative proportions of aromatic and aliphatic compounds derived from the oxidation procedures. The estimated ten-to-one dominance of aliphatics over aromatics in the milder aqueous oxidation is not expected to change dramatically as more components are identified. It is also important to note that this dominance drops to less than 2:1 in the oxidation carried out under more vigorous, but still not drastic conditions. Since much of the earlier work has been carried out using severe conditions of oxidation, it is not surprising that earlier workers have postulated a highly aromatic structure for humic substances. We feel that, at least as far as aquatic humics are concerned, a more aliphatic structure is now required. This is particularly true in view of the fact that much higher yields result when the milder conditions are employed. The results from three runs are summarized in Table II.

The reviewers should be reminded that the objects of Tables I and II are differenet. Table I provides elution orders and lists only those assignments which have been very thoroughly studied. Table II is meant to illustrate the dominance of aliphatic compounds and provide a total list of all compounds which have been identified on at least a "once-over" basis.

As a further general note it might be mentioned that a number of studied but unassigned components appear to be methyl esters of amino acids. Further manipulations of the data or the acquisition of additional data will be required

Table II

Oxidation Product Distributions- Aqueous Permanganate.

	Product	Mildest* Condition	Moderate* Condition	Somewhat Rigorous Condition
	Product	M/77	M/73	M/14
1.	Dimethyl Oxalate	12.47	11.76	---
2.	Dimethyl Malonate	10.18	---	---
3.	Dimethyl Methylmalonate	0.22	---	---
4.	Dimethyl Succinate	20.61	3.49	X
5.	Methyl-5-Methylfuran-2-Carboxylate	1.34	1.57	X
6.	Methyl Benzoate	0.51	1.34	X
7.	Dimethyl Glutarate	1.60	0.25	X
8.	Methyl Levulinate	2.76	---	---
9.	Dimethyl Methylsuccinate	X	?	---
10.	Dimethyl-2,2-Dimethylsuccinate	X	?	X
11.	Dimethyl Adipate	0.23	---	X
12.	Dimethyl Tartrate	0.55	---	X
13.	Methyl Ether of Dimethyl Oxaloacetate	X	---	---
14.	Methyl-4-Methoxybenzoate	1.14	0.10	X
15.	Methyl Tricarballylate	2.07	X	X
16.	Dimethyl Phthalate	0.14	1.95	X
17.	Dimethyl Terephthalate	---	0.41	X
18.	Dimethyl Isophthalate	---	0.23	X
19.	Trimethyl Citrate	0.33	---	---
20.	Trimethyl Benzene-1,3,5-Tricarboxylate	0.35	1.98	X
21.	Trimethyl Benzene-1-2-3-Tricarboxylate	0.07	0.33	X
22.	Tetramethyl Benzene Tetracarboxylates (3)	0.96	1.43	X
23.	Pentamethyl Benzene Pentacarboxylate	0.47	0.37	X
24.	Dimethyl-3-Methylglutarate	?	---	X
25.	Methyl Dimethoxy Benzoate Isomer	X	---	X
26.	Trimethyl Benzene-1,2,4-Tricarboxylate	---	X	X
27.	Methyl Pentadecanoate	---	X	X
28.	Trimethyl Methoxybenzene Tricarboxylates(5)	---	---	X

* Numbers refer to % of total integrated area; X indicates presence; --- indicates not found; ? indicates possible.

to firm up these assignments. Since our aquatic humics have been found to contain somewhat less than 1% total nitrogen of which roughly 3/4 is in the form of amino groups, we might expect to find as much as 4.5% of the total organic matter to be present in the form of amino acids. At least some of these should be liberated by the oxidative degradation.

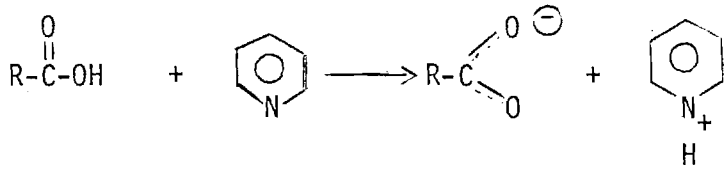
VI. REACTION OF M/30 AQUATIC HUMIC MATERIAL WITH DIAZOETHANE

In order to provide an unoxidized and unfractionated ethylated aquatic humic sample for comparative liquid chromatographic studies a sample of aquatic humic material was treated with excess diazoethane as follows. A solution of 12 g of potassium hydroxide pellets in 12 ml of distilled, purified water was cooled with stirring in running tap water and 200 ml of distilled-in-glass pentane added. The mixture was cooled in a freezer for 15 minutes. Solid N-nitroso-N-ethyl-N'-nitroguanidine (8.18 g) was added to the stirred pentane-KOH-H_2O over a period of five minutes. After an additional five minutes of stirring, the reddish orange solution of diazoethane in pentane was decanted from the aqueous sludge into a pre-chilled flask.

A suspension of 100 mg of solid aquatic humic material in 25 ml of ACS Certified Reagent ethyl acetate was treated with cold diazoethane in pentane. By the time 10-20 ml of the pentane had been added, most of the dark colored suspended humic material had gone into solution. After 50 ml of the diazoethane solution had been added, a light yellow solid began to precipitate. After the addition had been completed, the suspension was allowed to stand at room temperature for 2 hours. The unreacted diazoethane and most of the pentane was removed by gently boiling the suspension. This material is now ready for use as a control in our liquid chromatographic studies.

VII. ESTIMATION OF CARBOXYL GROUPS

In our June report we discussed briefly the non-specificity of the classical Ca-acetate method for the estimation of the carboxyl content of aquatic humic substances. We suspect that the presence of the Ca^{2+} spectator ion is responsible for an increase in acidity of phenolic hydroxyls. We have replaced the base acetate by pyridine for the reaction with the carboxyl groups of the humic substances. The corresponding acid formed is the pyridinium ion the pK_a of which



is 5.21 thus allowing the titration of this moiety with standard base. Since no spectator ion is introduced, the titration can be done in the presence of the humic substances without affecting the acidity of the phenolic groups. Our titration results are indeed very close to those obtained by direct titration, i.e., 5.10 meg/g vs. 5.02 meg/g (direct titration). The advantage of this indirect method is that instead of titrating carboxyl groups having a variety of pK_a values, one now titrates an acid with one defined pK_a, making the evaluation of the endpoint much more accurate. It should also be pointed out that the Ca-acetate method is still valid for those humic substances which form insoluble salts with Ca which then can be removed by filtration, thus leaving only acetic acid to be titrated. However, in the case of our aquatic humics, hardly any insoluble product is formed with Ca. Consequently, simple filtration does not remove anything. As we have shown previously, one would have to go to ultrafiltration to remove the Ca-humate from solution. Thus, the substitution

of pyridine for Ca-acetate offers a valuable and convenient alternative for the analysis of carboxyl groups in humic substances and other natural products having a high degree of solubility in water.

VIII. REACTION OF M/30 AQUATIC HUMIC MATERIAL WITH IODINE

A solution containing 100 mg of M/30 aquatic humic material, 10 g of dipotassium hydrogen phosphate and 3 g of potassium dihydrogen phosphate in 10 liters of deionized water was purged with a stream of nitrogen gas for thirty minutes in order to drive off any volatile materials which might have been introduced with the water.

A stock solution of disinfectant was prepared by dissolving 2.0 g of potassium iodide and 1.0 g of sublimed iodine in 100 ml of deionized distilled water. Aluminum sulfate (0.86 g) was added to 40 ml of the above stock solution and the volume brought to 500 ml. This solution was added to the mixing chamber at a rate of 50 ml/hr. The rate of addition of the buffered solution of aquatic humics was maintained at 1 liter/hr.

The strength of the disinfectant/flocculant solution was determined by diluting a 1 ml aliquot with 10 ml of purified water and titrating with sodium thiosulfate to a starch-iodine color endpoint. The titratable iodine content was 804 mg/l. Thus the concentration of iodine in the treated water would have been expected to be 38.3 mg/l. The actual concentration as measured in triplicate was found to be 7.1 mg/l. Thus most of the iodine evidently reacted with the aquatic humic matter in the course of the three-hour period between mixing and the second measurements. After 20 hours, the iodine concentration had dropped only to 5.1 mg/liter. Therefore, one would conclude that a rapid initial uptake is followed by a relatively slower reaction rate.

It may be of interest to note that no flocculation took place under these conditions. After 20 hours, the residual iodine in both the settling chamber and the sand filter eluate was quenced by the addition of 30 mg/l of anhydrous sodium sulfite.

Samples were set aside in septum-closed bottles for determination of volatile halogenated organics. Liquid-liquid extractions were made with ethyl acetate, concentrated and treated with diazoethane. Further fractionation and analysis of this product mixture is in progress.

IX. BROMINATION OF AQUATIC HUMICS

It will be recalled (Section VI, July 14 report, p. 46) that aquatic humics react with bromine under drastic conditions to produce a variety of brominated organics including bromoform, tetrabromoacetone, 2-bromopentane and 2-bromo-2-methylbutane. This room-temperature reaction was repeated under identical conditions with the exception that excess bromine was destroyed with sodium sulfite after 19 hours in order to eliminate all chances of reactions with solvents used in the subsequent extractions. The concentrated pentane extracts and ethyl acetate extracts await GC/MS analysis.

A slight delay in the acquisition of the GC/MS data was brought about by three back-to-back maintenance repairs 1) cleaning the rods, 2) replacing the electron multiplier which expired suddenly rather than slowly and 3) an unexpected power failure which caused oil backup in the source. While all three items plus an earlier source cleaning were handled by our staff in very short order, the instrument was down long enough to create a considerable sample backlog. Corrective measures are being taken to assure that future events of this kind will be of an even shorter duration.

X. REACTION OF DEIONIZED WATER WITH CHLORINE

In order to obtain a systems blank for our mini-plant studies, a solution of 20 g of dipotassium hydrogen phosphate and 6 g of potassium dihydrogen phosphate in 10 liters of deionized water was purged with a stream of nitrogen for 30 minutes. This corresponds to actual buffer levels used in earlier aquatic humic runs.

The buffered waste was added to the mixing chamber at a rate of 1 liter/hour. A solution of 49.5 mg of chlorine and 430 mg of aluminum sulfate was added to the mixing chamber at a rate of 50 ml/hour for four hours. A second portion of 94 mg of chlorine and 430 mg of aluminum sulfate in 200 ml of water was added over a second period of 4.6 hours. The liquid in the settling chamber and the sand filter eluate showed a strong chlorine residual after eight and twenty hours. After twenty hours contact time the chlorine residual was quenched in the settling chamber, the sand filter eluate and reservoir. In order to achieve correspondence with the aquatic humic runs, the amounts of sodium sulfite used were 370 mg, 300 mg and 140 mg, respectively. Two 125-ml samples of the solutions, after quenching, in the settling chamber, sand filter collection flask and the reservoir were prepared and stored in a refrigerator using septum closure bottles as had been done for the aquatic humic runs.

Portions of the reservoir solution (1.2 l), sand filter eluate and settling chamber contents were adjusted to pH 10 with concentrated sodium hydroxide solution and extracted with three 100 ml portions of distilled-in-glass pentane. The combined pentane extracts were washed with 100 ml of water, dried over "roasted" anhydrous sodium sulfate and

concentrated to a volume of 1-3 ml in a Kuderna-Danish apparatus.

The aqueous solutions after pentane extraction were treated with concentrated sulfuric acid until the pH was in the range of 1-2. The acidified solutions were then extracted with three 100 ml portions of ACS Certified ethyl acetate. The combined ethyl acetate extracts were washed with 100 ml of water, dried over "roasted" anhydrous sodium sulfate, and concentrated to a volume of 5 ml in a Kuderna-Danish apparatus.

The ethyl acetate concentrates were treated with a solution of diazoethane in pentane until a yellow color persisted for 15 minutes. After standing at room temperature for two hours, the excess diazoethane and some pentane was removed by fractional distillation.

XI. ANALYSIS OF PRODUCTS—CHLORINATION OF AQUATIC HUMICS

Although the greater portion of our interpretive efforts during this reporting period were directed towards assigning structures to the permanganate oxidation products, two new assignments were also made on the chorination product mixtures. Functionalized isoprenes continue to dominate the product mixtures. The EI and CI total ion chromatograms of the early and intermediate scans are presented once again in Figures 16 and 17 for the reviewer's convenience.

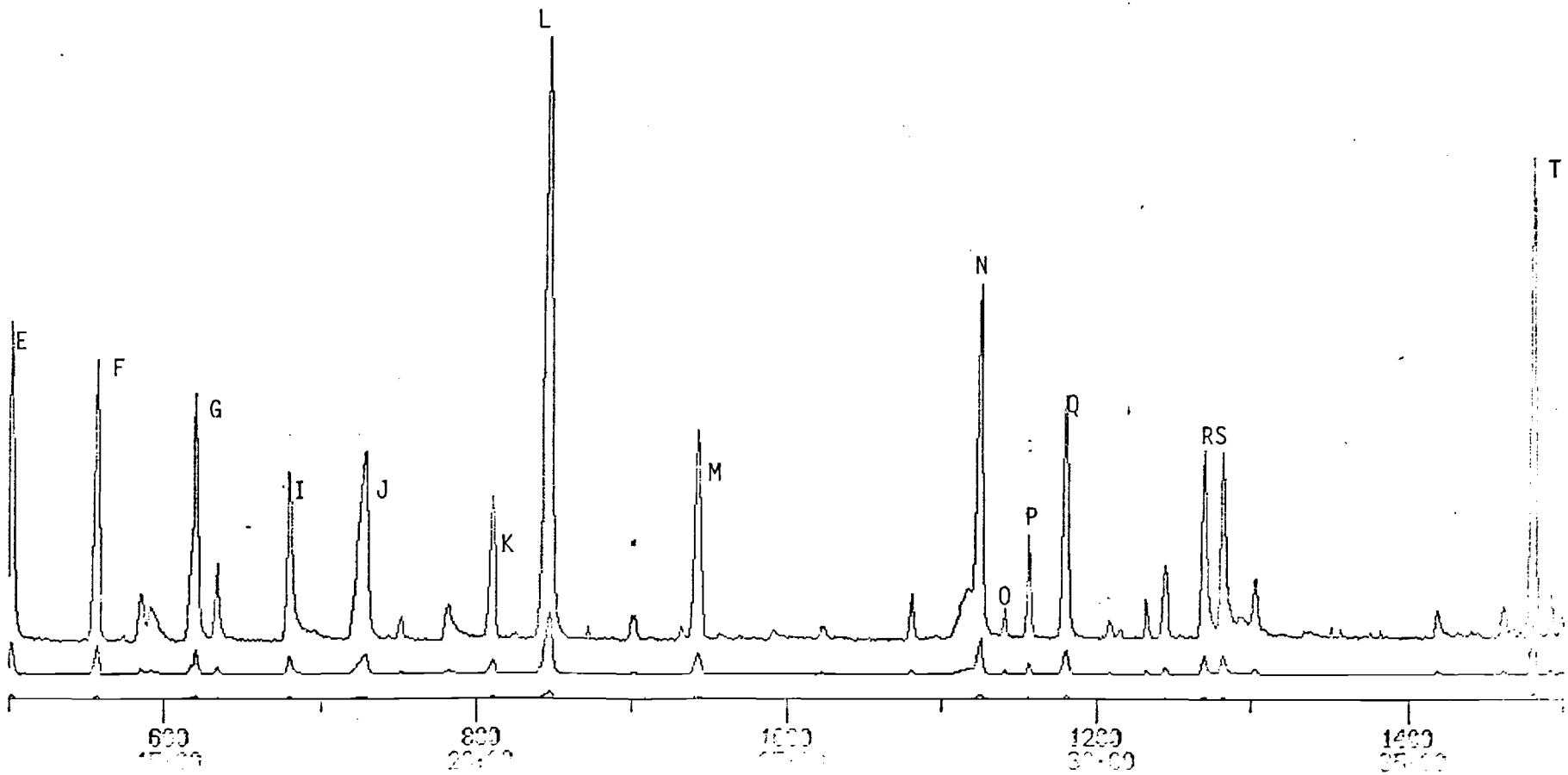
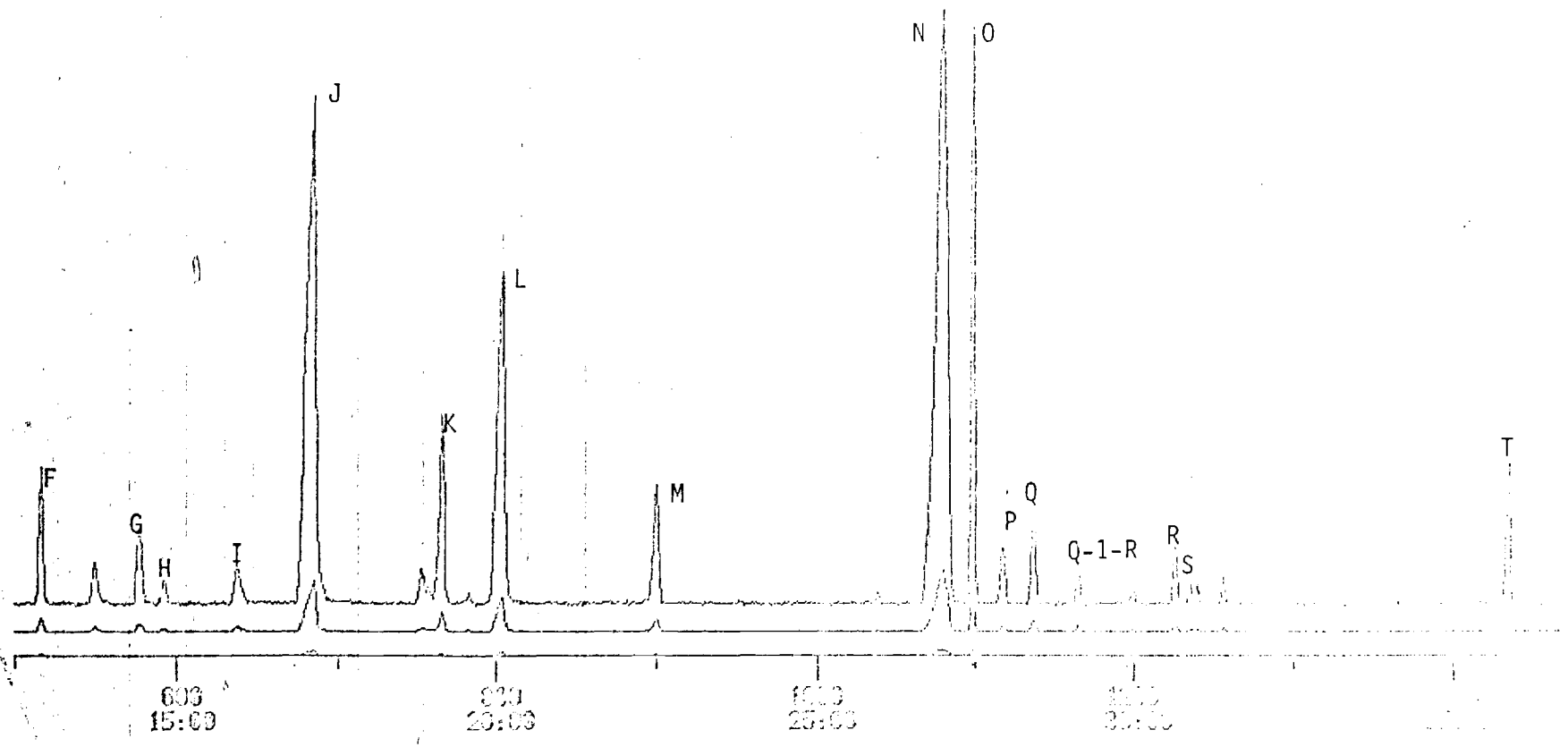
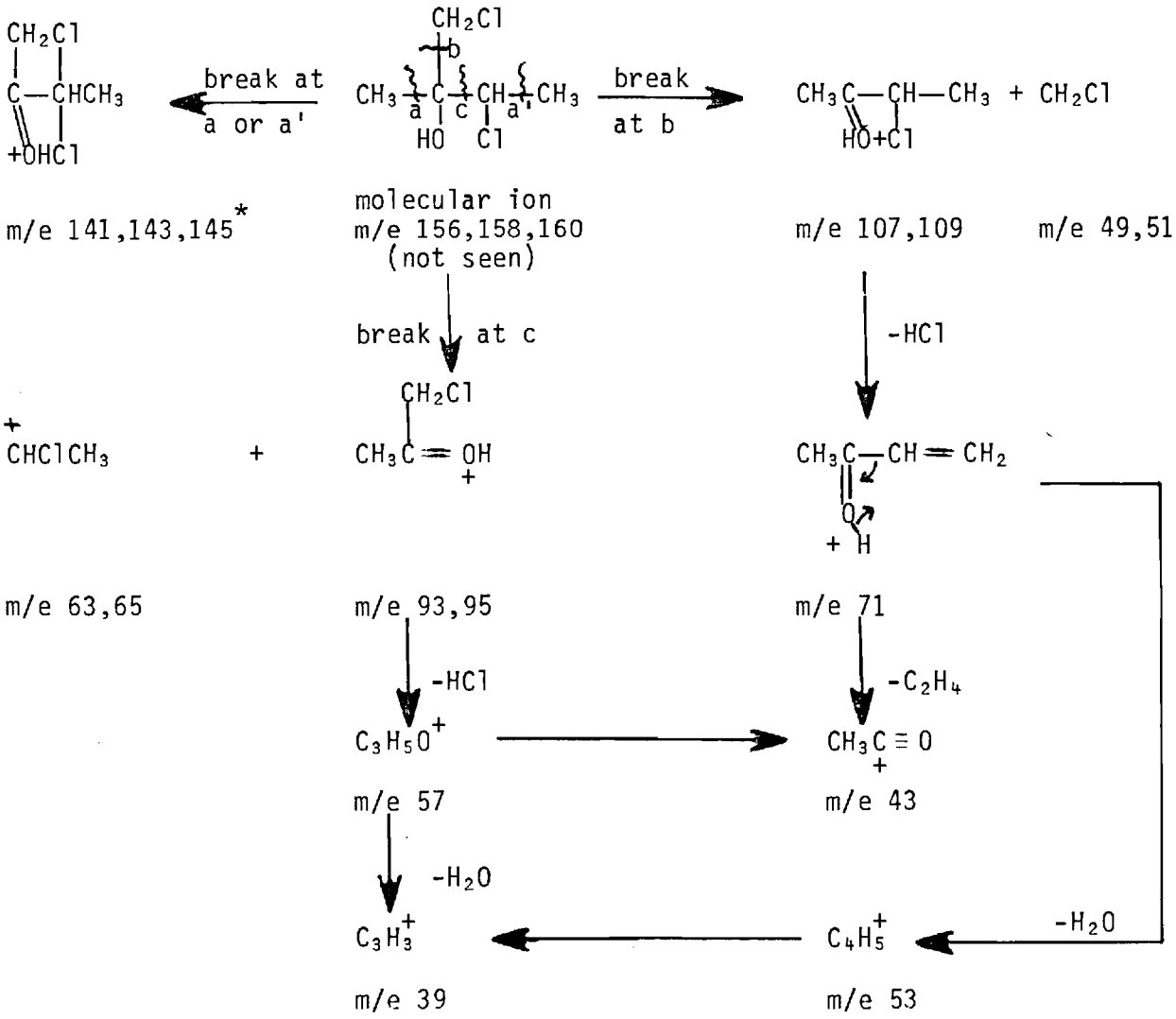


Figure 16. Aquatic Humics Plus Chlorine
Total Ion Chromatogram-Electron Impact
Early and Intermediate Scans

Figure 17. Aquatic Humics Plus Chlorine
Total Ion Chromatogram-Chemical Ionization
Early and Intermediate Scans



Q. Compound Q exhibits EI and CI fragmentation patterns (see Figures 18 and 19 which are virtual overlays for those shown by compound N. Therefore a second structural assignment must be proposed which is isomeric with 1,4-dichloro-2-methyl-2-butanol and which is also consistent with the fragmentation sequence. Accordingly, a 1,3-dichloro-2-methyl-2-butanol structure is proposed. It is also evident that the assignments for N and Q may be interchanged. An outline of the EI fragmentation sequence is presented below.



*Not seen due to low overall intensity.

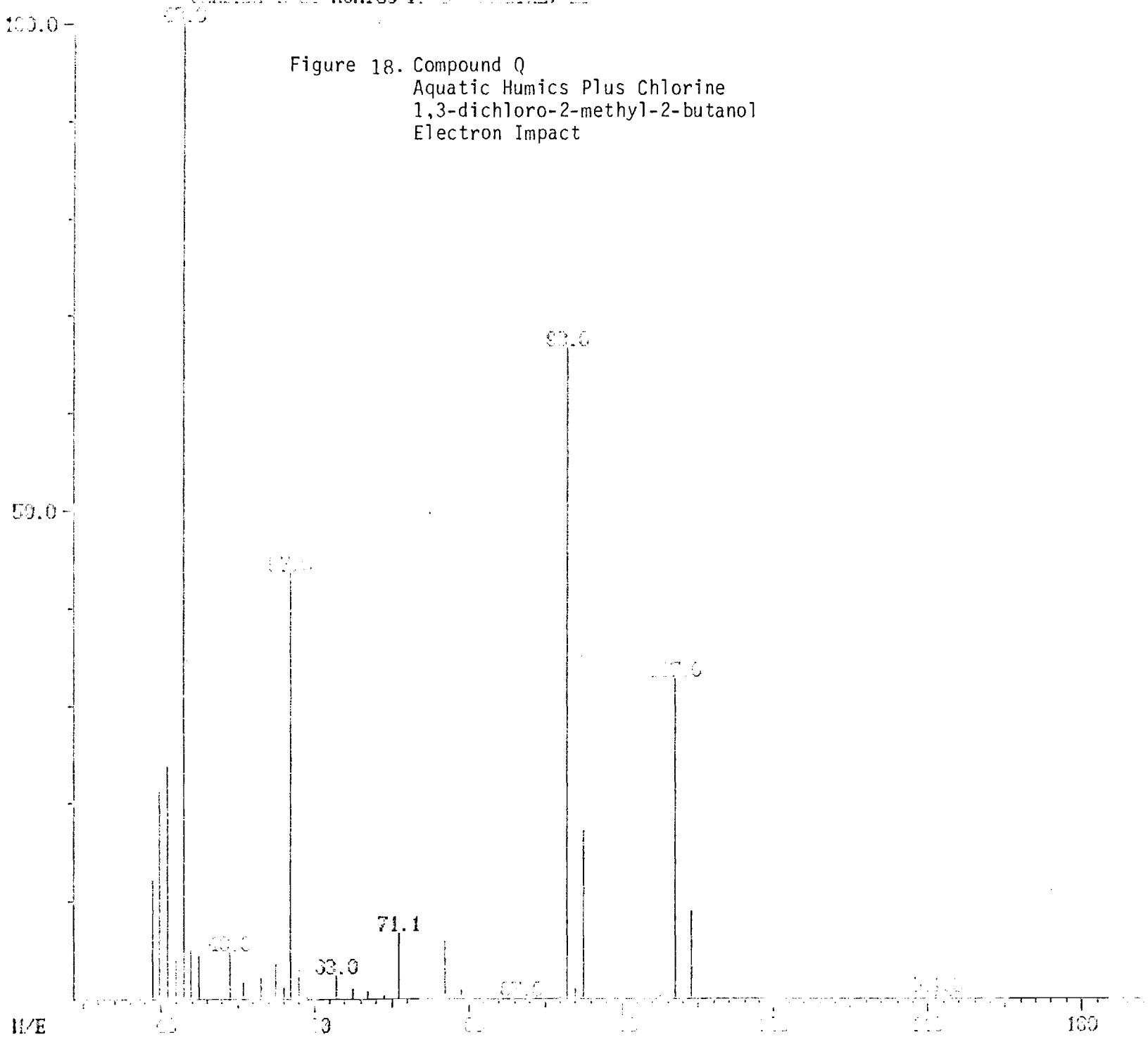


Figure 18. Compound Q
Aquatic Humics Plus Chlorine
1,3-dichloro-2-methyl-2-butanol
Electron Impact

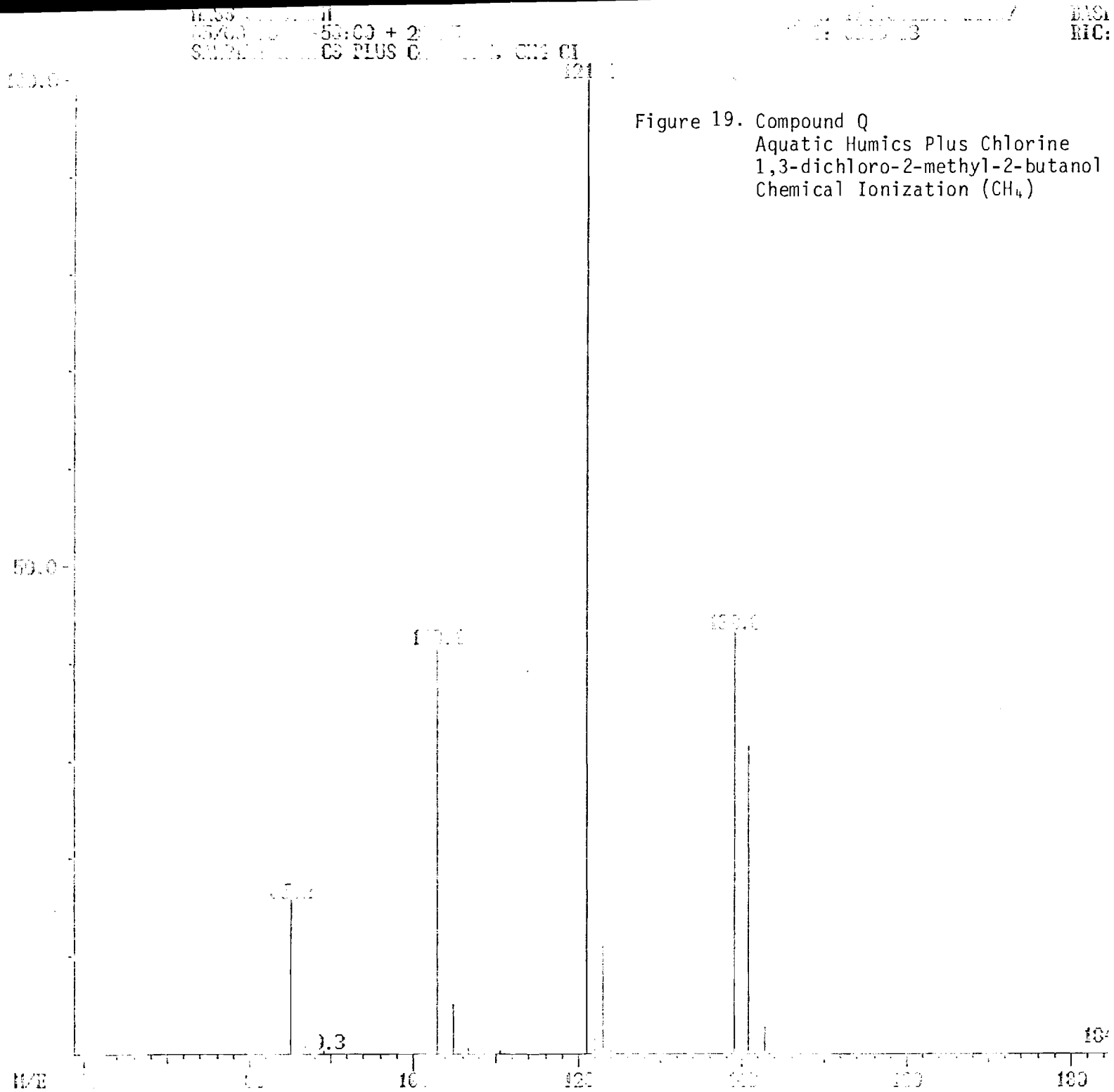
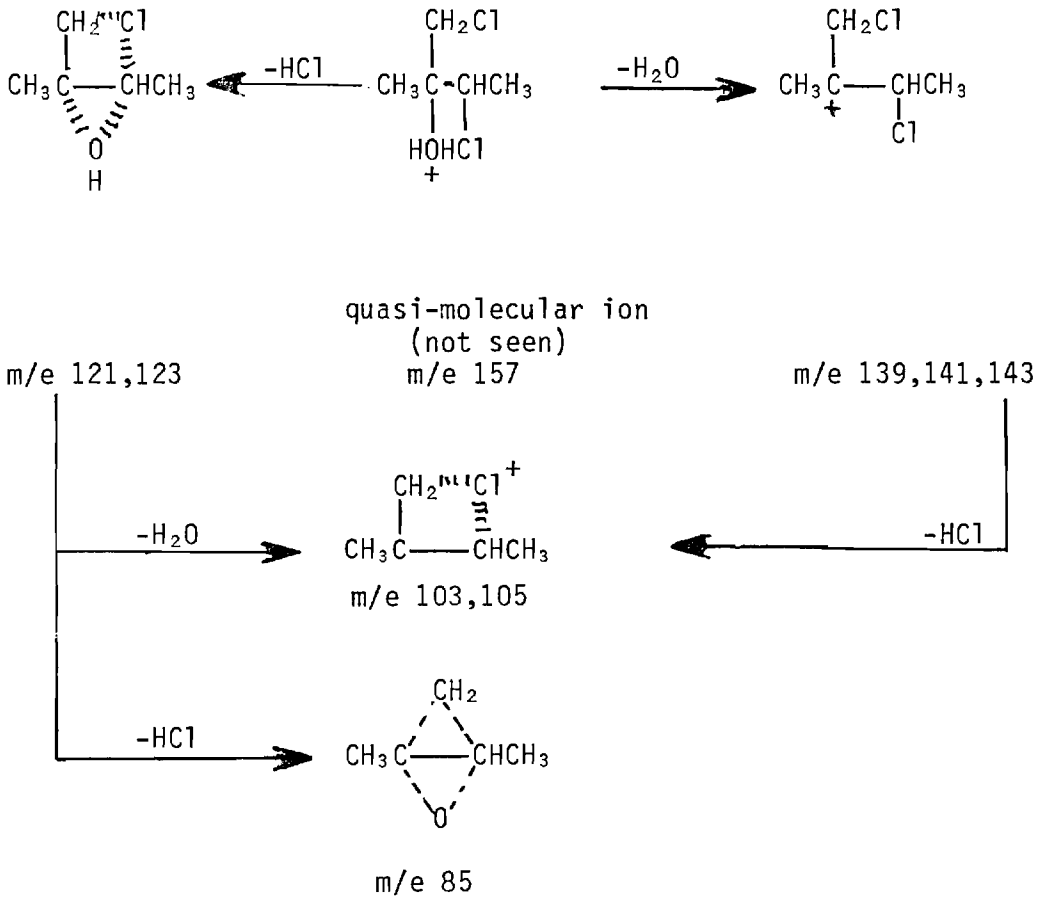
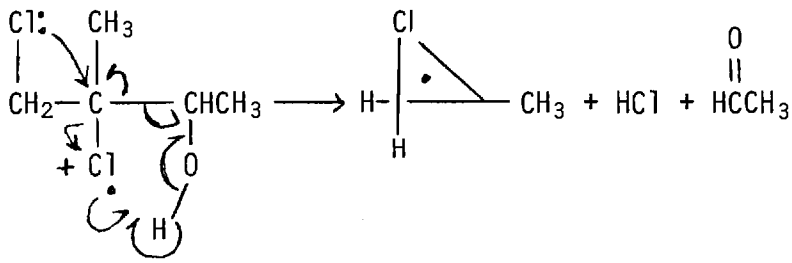


Figure 19. Compound Q
Aquatic Humics Plus Chlorine
1,3-dichloro-2-methyl-2-butanol
Chemical Ionization (CH_4)

The CI fragmentation pattern is supportive and can be explained as outlined below.



R. Compound R again showed a CI spectrum (see Figure 20) which indicated the compound was another dichlorohydroxyisoprene derivative. In this case, the electron impact spectrum which is shown in Figure 20 was quite different from those which had been observed for compounds N and Q. The fragmentation sequence which is outlined below is consistent with the 3,4-dichloro-3-methyl-2-butanol structure. The most unusual feature of the spectrum is the strong rearrangement ion at m/e 76,78. This is best explained by invoking a 5-membered cyclic mechanism (favored for halide). The resulting product ion can be backside-stabilized by the adjacent chlorine as shown below.

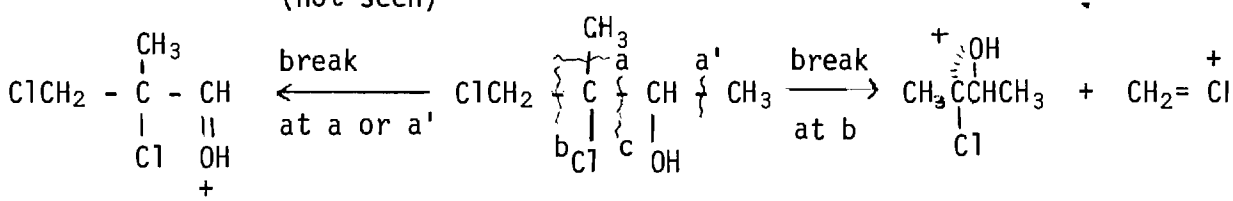


molecular ion

m/e 156, 158, 160

(not seen)

m/e 76, 78

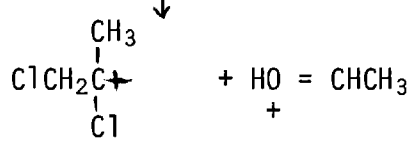


m/e 141,* 143, 145*

m/e 107, 109*

m/e 49, 51

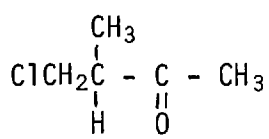
molecular ion
m/e 156, 158, 160
(not seen)



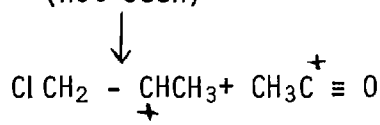
m/e 111, 113, 115
(not seen)

m/e 45
(base peak)

-HCl, with rearrangement



m/e 120, 122, 124
(not seen)



m/e 77, 79

m/e 43

* Not seen due to low overall intensity.

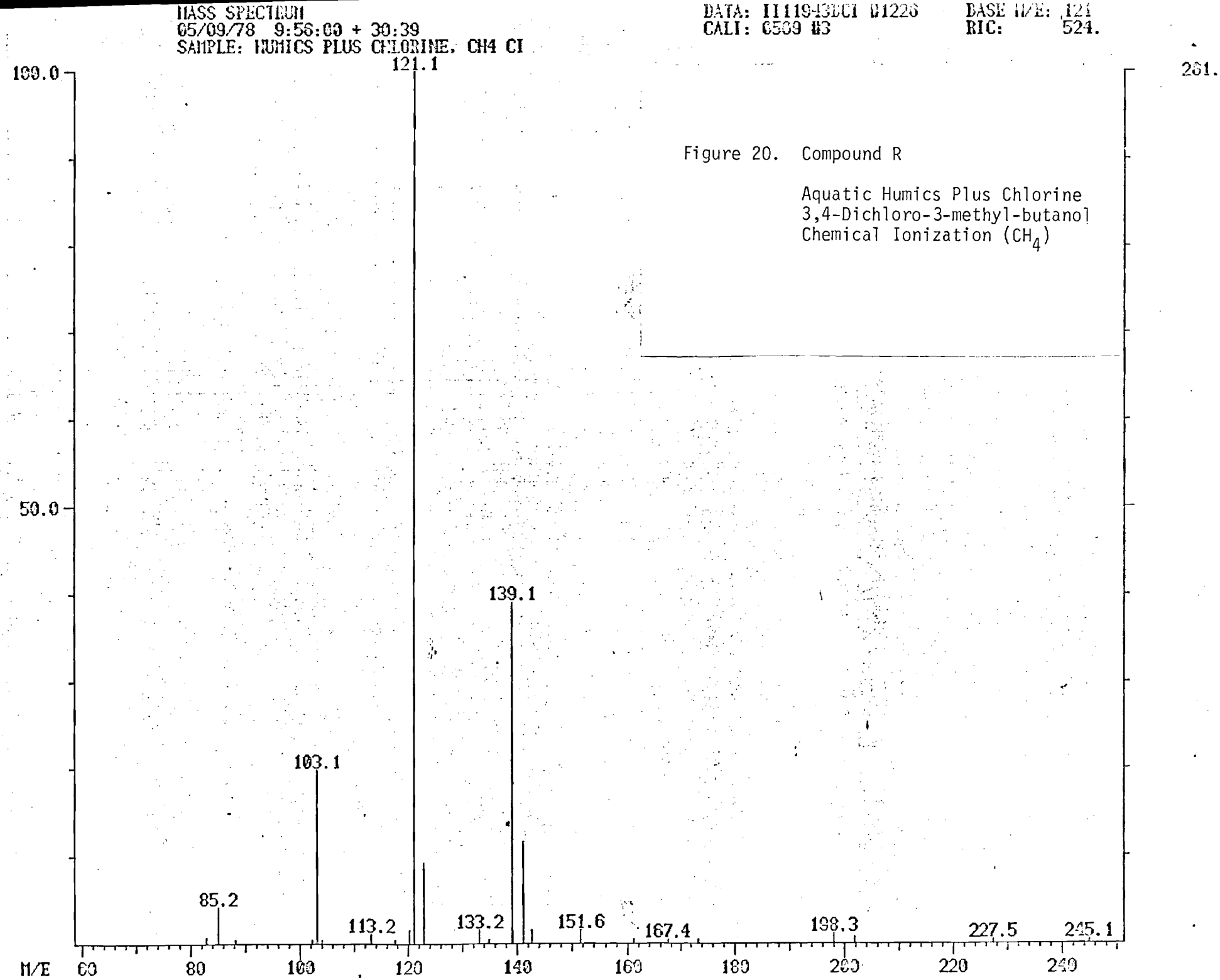


Figure 20. Compound R

Aquatic Humics Plus Chlorine
3,4-Dichloro-3-methyl-butanol
Chemical Ionization (CH_4)

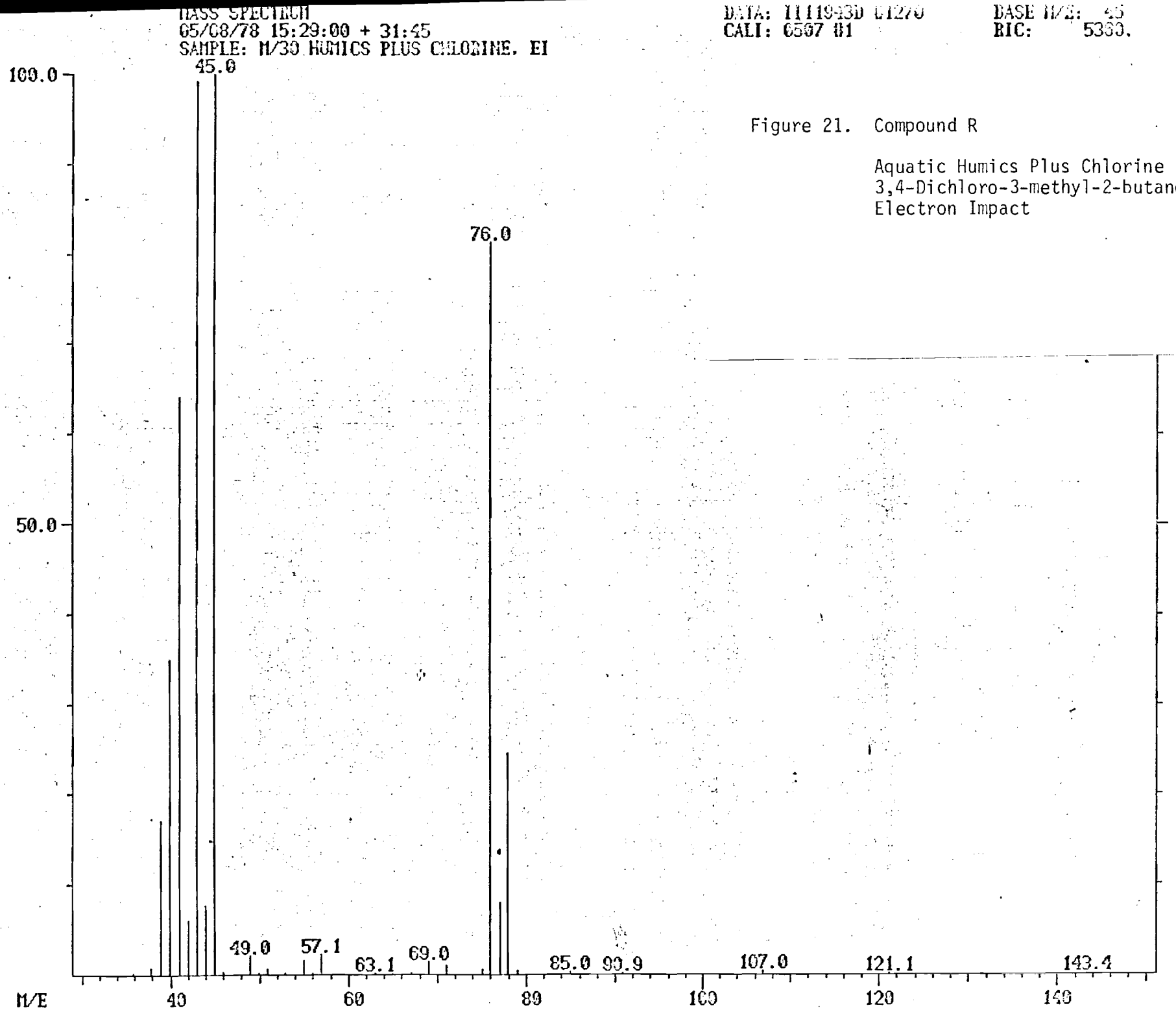


Figure 21. Compound R

Aquatic Humics Plus Chlorine
3,4-Dichloro-3-methyl-2-butanol
Electron Impact

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

September 15, 1978

by

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DISCLAIMER

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EXECUTIVE SUMMARY

The purpose of the work described in this report and the overall objective of the research project is to identify major and minor classes of natural organic substances found in surface waters such as might be used as a source of potable water. A second major aim of the study is to evaluate the effect of a number of water treatment processes such as chlorine, ozone and chlorine dioxide on the transformations which these naturally occurring materials may undergo during the disinfection process.

A source water which is rich in the required organic materials but which is unusually low in such interfering materials as agricultural runoff, municipal wastewater effluents and industrial discharges is being used to provide a generous reserve of aquatic humic materials which are the dominant class (80%) of all natural organic substances found in drinking water sources. Fractions representing the non-humic organic materials have been isolated, separated and worked up for further characterization.

A major advance made during this reporting period deals with the greatly improved yields (about 80%) of oxidation products resulting from the reaction of aquatic humics with a crown ether complex of potassium permanganate. These milder conditions have made it possible for the Georgia Tech research team to isolate and characterize many new products, mostly of an aliphatic nature. Even reactive products such as p-commaric acid have been identified on the basis of their electron impact mass spectra. Such products will be of fundamental importance in both defining the overall structure of a quatic humics and in understanding how they react with such disinfecting agents as chlorine, chlorine dioxide, ozone, bromine and iodine.

A second major advance is concerned with the development of a methods program for automatically processing GC/MS data to search reaction product

mixtures for preselected compounds. In this way it will be possible to study highly complex product distributions as a function of reaction conditions. The most obvious application will be documenting the presence/absence of key components in the reaction product mixture resulting from the chlorination of aquatic humics. To the extent that reproducibility can be established, the method is quantitative as well as qualitative and therefore may be capable of adding a whole new dimension to future studies. Work is continuing in these promising areas.

Future plans include the exploration of the use of still milder oxidizing agents, another sampling trip to the Satilla River, an increased pace of reactions and data processing and further work on the characterization of some of the minor classes of natural organics found in aqueous systems.

I. PERSONNEL

Dr. I. El Barbary will be returning to the project, at least for the duration of the period in which Mr. Lupton remains confined to bed with a bad case of infectious hepatitis. We believe that his familiarity with the project uniquely qualifies him to step in and guide the student labor in the performance of those tasks normally performed by Mr. Lupton. He will himself pick up some of the assignments formerly carried out by Dr. Ralls. Both Mr. Lupton and Dr. Havlicek have undergone an intensive training course at the Finnigan Institute on the operation of the INCOS Data System. Mr. Lupton also took the 4000 operators course. The courses were excellent. Some of the benefits of this extension of expertise will be apparent in later sections of this report. This extra training has been made necessary by the premature departure of Dr. Strattan who has been hired by EPA (Kansas City) effective October 1. While we shall sorely miss his talents, we congratulate him on a job well done and wish him well. Our loss is EPA's gain.

II. EQUIPMENT

The GC/MS and other major instrumentation continue to operate smoothly. We have learned to automate many of our GC/MS operations thus freeing the operator for interpretive tasks. Our planned merger with another division (Chemical and Materials Science Division) within EES has gained us access to more sophisticated GC and LC equipment. Since the equipment has not yet been colocated the full benefits of this reorganization have yet to be felt.

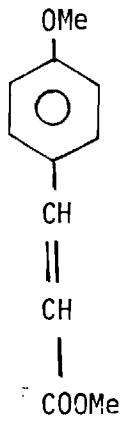
III. ESTIMATION OF CARBOXYL GROUPS

Preliminary studies are in progress in an effort to further differentiate between strongly acidic phenolic functions and carboxyl groups in such polyfunctional molecules as the aquatic humics. We have now acetylated a sample of M/30 and processed it in such a way that only the phenolic functions should be acetylated. Thus we should be able to accurately estimate the carboxyl groups remaining by means of the pyridine treatment and subsequent titration of the pyridinium ion with standard base. Unfortunately the results to date are inconclusive. Further work will be required in order to provide clear answers.

IV. OXIDATION OF HUMICS WITH PERMANGANATE IN AN APROTIC SOLVENT USING AN 18-CROWN-6 COMPLEX

Oxidation of aquatic humics with naked MnO_4^- in the presence of crown ether has been described in our previous report. Prolonged extraction of the aqueous reaction product with ethyl acetate produced three fractions which were methylated with diazomethane (128A, 128A1 and 128A2). The total yield of the oxidation products was 0.83 g, starting from 1 g of aquatic humics. This total represents a considerable improvement over literature values.

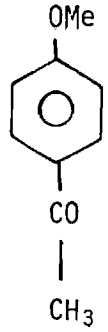
A list of compounds identified as a result of the GC/MS analysis of fraction 128A is presented in Table I. The occurrence of more long chain acid esters in comparison to the previous mild oxidation (M/77) is remarkable. Even more interesting is the occurrence of aromatic compounds with substitutions that ought to have been totally oxidized in our previous experiments.



(Scan 2076)

I

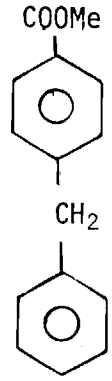
Methyl Ester-Methyl Ether of p-Hydroxy Cinnamic Acid (Dimethyl-p-Coumarate)



(1580)

II

p-Methoxy Acetophenone



(2218)

III

p-Benzyl Methyl Benzoate

Compounds I and II have not been previously reported among the oxidation products isolated from aquatic humic material. It is of interest to note that p-coumaric aldehyde is formed during the microbial degradation of coniferous lignin--presumably by demethoxylation of coniferyl residues.

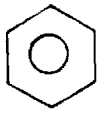
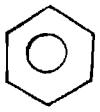
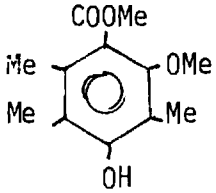
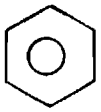
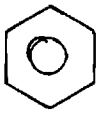
The data presented in Table I will be explained in greater detail in subsequent reports. The same type of reverse-search quantitation as will be described in subsequent sections can be applied to this data and will be included in the next report.

TABLE I

M/128A

Scan	Identity	
625	Malonic acid methyl ester	132,101
788	Levulinic acid methyl ester	130,115
903	Butenedioic acid di-methyl ester	144,113
924	Dimethyl succinate	144,115
1010	Methylsuccinic acid di-methyl ester	129,101
1015	Methyl 5-methyl-furan-2-carboxylate	140,109
1068	2,2-Dimethylsuccinic acid dimethyl ester	143,115
1074	Methylbenzoate	136,105
1166	Glutaric acid di-methyl ester	129
1249	2-Methyl glutaric acid di-methyl ester	143,115
1380	Dimethyl adipate	143,114
1461	Hexanedioic acid 3-Methyl dimethyl ester	157,
1556	Benzoic acid 3-methoxy-methyl ester	166,135
1563	Heptanedioic acid dimethyl ester	157,128
1580	Ethanone, 1-(4-methoxyphenyl)	150,135, 119,107
1620	Benzoic acid 4-methoxy-methyl ester	166,135
1683	Tricarballylic acid methyl ester	187
1706	Tetramethoxy benzene	198,167
1711	Benzene acetic acid, 4-methoxy, methyl ester	180,121
1747	Dimethyl phthalate	
1878	C_{12} acid ester	
1884	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	182,151, 123
1891	Nonanedioic acid dimethyl ester	185
1932	Mandelic acid, p-methoxy, methyl ester	196,137
1937	Benzoic acid, 2,6-dimethoxy, methyl ester	196,165
1954	Benzoic acid, 3,4-dimethoxy, methyl ester	196,165
2035	Benzenesulfonamide, N,N-4-trimethyl	
2040	Long chain fatty acid ester	

TABLE I (Continued)

Scan	Identity	
2045	 $(OMe)_4$	198,167
2076	2-Propenoic acid, 3-(4-methoxy-phenyl)-, methyl ester	192,161
2098 2130 2134 2177 2222	Isomers of  OMe $(COOMe)_2$ or  and isomers	
2136	Xanthenone	
2157	Benzenesulfonamide, N, 4-dimethyl	
2169	Tetradecanoic acid methyl ester	242,211
2218	Methyl p(benzyl)-benzoate	226,195
2303	Benzene 1,2,4-tricarboxyllic acid methyl ester	
2355 2363 2406 2414	 $(COOMe)_2$ $(OMe)_2$	254,223
2431	Hexadecanoic acid methyl ester	
2448	Dibutyl phthalate	
2455	 $(OMe)_3$ $(COOMe)_2$	

V. COMPARISON OF PRODUCT MIXTURES - CHLORINATION OF AQUATIC HUMICS

A computer program was developed jointly by Dr. Glenn Ouchi of the Finnigan Institute and the Project Manager which is designed to scan the GC/MS output gathered from all runs for the presence of products which have previously been identified in earlier work. At present, we have selected run II-110, sand filter eluate as the basis for the comparison and have not yet had the opportunity to add those products which have been identified in other runs.

The basic strategy of the program involves the creation of a library list LIBSH and a library SH in which we have previously placed the best available spectra of the identified compounds. Data from a complete GC/MS run are then "reverse searched" according to user specified parameters. The search proceeds one compound at a time. If a chromatographic peak falling within the specified scan window and meeting the "PURITY" and "FIT" criteria written into the program is found, the area of the peak is computed using pre-specified parameters and sent to a quantification list along with the retention time. The program then loops back to the second compound on the library list and repeats the search. If a compound is not found, this information is stored directly and the pointer moved to the next position on the library list. When the complete list has been searched, a quantitation report is printed according to the format specified in "QUAN." In this case, we have specified a printout of the names of each of the compounds involved in the search whether "found" or "not found." If found, the RIC, the absolute and relative retention times, the area of the chromatographic peak, the normalized amount and the percentage of the total area of the identified peaks contributed by the compound in question is sent to a quantitation list. In the absence of better information, we are currently

using response factoes of one for all compounds. In its present form, the program takes 10-15 minutes to run and accomplishes with less error what would otherwise take 1-2 days. The program runs automatically thus freeing the operator for other tasks. Upon completion, a song is played to remind the operator that another run can begin.

It should be pointed out that most of the work performed during this reporting period was concerned with developing, modifying and debugging the program, a listing of which is presented in Figure 1. The applications shown in Figures 2 and 3 represent test cases carried out on the sand filter eluates from run II-110, and the reservoir control from reaction II-134, respectively. We intend to apply the same program to the analysis of the oxidation products and such other work as involves the examination of the effect of changing reaction conditions upon product distributions. We believe this represents a major advance regarding our ability to process data and interpret results.

```
TRACE OF PROCEDURE STUFF
   * SET4 SH;SETL LIBSH;SETQ $;EDQL(-;W;E);CHRO(I;D;E);STUFF1;
   * QUAN $  (D;F;F1;H;E);SONG(@FF)
   *
   SET4 SH
   SETL LIBSH
   SETQ $
   EDQL  (-;W;E)
   CHRO  (I;D;E)
   STUFF1
      * GETL;SEAR (I;$;N1,500,900<;&;D-175,175;E)/V;CK1;LOOP
      *
      GETL
      SEAR  (I;$;N1,500,900<;&;D-175,175;E)/V
      CK1
         * CK2;EDQL (-;N;#;A;E);RETU
         *
         CK2
            * IF !14;SET1 !14;ERASE;CHRO (I;N-2,1.5;O;#;G-1,1;A>-1,1.5;D-10,10;E);RETU CK1
            *
            *
            IF !14
            SET1 !14
            ERASE
            CHRO  (I;N-2,1.5;O;#;G-1,1;A>-1,1.5;D-10,10;E)
            RETU CK1
         EDQL  (-;N;#;A;E)
         RETU
      LOOP
   QUAN $ (D;F;F1;H;E)
   SONG  (@FF)
```

Figure 1. Procedure for Product Composition/Distribution Analysis

QUANTITATION REPORT FILE: I111943D

AMOUNT=AREA * REF.AMNT/(REF.AREA* RESP.FACT)

NO	NAME
1	3-CHLORO-2-METHYL-1-BUTENE
2	2-CHLOROMETHYL-1-BUTENE
3	ETHYLENE GLYCOL,DIETHYL ETHER
4	2-METHYL-3-PENTANONE
5	DIETHYL CARBONATE
6	2-METHYLPENTANAL
7	3-CHLORO-2-METHYL-2-BUTANOL
8	4-HYDROXY-3-METHYLBUTANAL
9	2,3-DICHLORO-2-METHYLBUTANE
10	1-CHLORO-2-METHYL-2-BUTANOL
11	ETHYL ACETATE
12	1,3-DICHLORO-2-METHYLBUTANE
13	3-CHLORO-2-METHYL-1-BUTANOL
14	4-CHLORO-3-METHYL-1-BUTANOL
15	N-NITROSODIETHYLAMINE
16	3,4-DICHLORO-METHYL-1-BUTENE ISOMER
17	4-METHYL-3-HEPTANONE
18	2,2'-BIS-1,3-DIOXOLANE
19	2-(3-OXOBUTYL)-CYCLOPROPANOL
20	1,4-DICHLORO-2-METHYL-2-BUTANOL
21	ETHYL TRICHLOROACETATE
22	TETRAETHOXY METHANE
23	1,3-DICHLORO-2-METHYL-2-BUTANOL
24	1,3-DIOXOLANE
25	3,4DICHLORO-3-METHYL-2-BUTANOL
26	UNKNOWN CHLORO PRODUCT
27	ISOMER OF S
28	T-QUESTION-3,3,4,4-TETRAMETHYL-2-PENTANONE
29	U-UNASSIGNED

NO	M/E	SCAN	TIME	REF	RRT	METH	AREA	AMOUNT	%TOT
1	RIC	271	6:46	7	0.582	A BB	107331.	61.604	12.45
2	RIC	317	7:55	7	0.680	A BB	5667.	3.253	0.66
3	RIC	322	8:03	7	0.691	A VB	7747.	4.446	0.90
4	RIC	342	8:33	7	0.734	A BB	98065.	56.285	11.37
5	RIC	401	10:01	7	0.861	A BB	3845.	2.207	0.45
6	RIC	333	8:19	7	0.715	A BB	6983.	4.008	0.81
7	RIC	466	11:39	7	1.000	A BB	174228.	100.000	20.20
8	RIC	478	11:57	7	1.026	A BB	48981.	28.113	5.68
9	RIC	502	12:33	7	1.077	A BB	26948.	15.467	3.13
10	RIC	557	13:55	7	1.195	A BB	24489.	14.056	2.84
11	RIC	585	14:37	7	1.255	A BB	3370.	1.934	0.39
12	RIC	621	15:31	7	1.333	A BB	27724.	15.912	3.22
13	RIC	634	15:51	7	1.361	A BB	6706.	3.849	0.78
14	RIC	680	17:00	7	1.459	A BB	18360.	10.538	2.13
15	RIC	730	18:15	7	1.567	A BB	28542.	16.382	3.31
16	RIC	811	20:16	7	1.740	A BB	14716.	8.446	1.71
17	RIC	848	21:12	7	1.820	A BB	80155.	46.006	9.30
18	RIC	902	22:33	7	1.936	A BB	2505.	1.438	0.29
19	RIC	943	23:34	7	2.024	A BB	22415.	12.865	2.60
20	RIC	1126	28:09	7	2.416	A BB	37593.	21.577	4.36
21	RIC	1142	28:33	7	2.451	A BB	1961.	1.126	0.23
22	RIC	1157	28:55	7	2.483	A BB	8275.	4.750	0.96
23	RIC	1181	29:31	7	2.534	A BB	21445.	12.309	2.49

Figure 2. Product Distribution, Chlorination of Aquatic Humics, Run II-110 Sand Filter Eluate

NO	M/E	SCAN	TIME	REF	RRT	METH	AREA	AMOUNT	%TOT
24	RIC	1232	30:48	7	2.644	A BB	2773.	1.592	0.32
25	RIC	1270	31:45	7	2.725	A BB	16044.	9.209	1.86
26	RIC	1282	32:03	7	2.751	A BD	13921.	7.990	1.61
27	RIC	1303	32:34	7	2.796	A BB	3649.	2.094	0.42
28	RIC	1481	37:01	7	3.178	A BB	38527.	22.113	4.47
29	RIC	1530	38:15	7	3.283	A BB	9355.	5.369	1.08

Figure 2. (Continued)

QUANTITATION REPORT FILE: I113728

AMOUNT=AREA * REF.AMNT/(REF.AREA* RESP.FACT)

NO	NAME
1	3-CHLORO-2-METHYL-1-BUTENE
2	2-CHLOROMETHYL-1-BUTENE
3	ETHYLENE GLYCOL,DIETHYL ETHER
4	2-METHYL-3-PENTANONE
5	DIETHYL CARBONATE
6	2-METHYLPENTANAL
7	3-CHLORO-2-METHYL-2-BUTANOL
8	4-HYDROXY-3-METHYLBUTANAL
9	2,3-DICHLORO-2-METHYLBUTANE
10	1-CHLORO-2-METHYL-2-BUTANOL
11	ETHYL ACETATE
12	1,3-DICHLORO-2-METHYLBUTANE
13	3-CHLORO-2-METHYL-1-BUTANOL
14	4-CHLORO-3-METHYL-1-BUTANOL
15	N-NITROSODIETHYLAMINE
16	3,4-DICHLORO-METHYL-1-BUTENE ISOMER
17	4-METHYL-3-HEPTANONE
18	2,2'-BIS-1,3-DIOXOLANE
19	2-(3-OXOBUTYL)-CYCLOPROPANOL
20	1,4-DICHLORO-2-METHYL-2-BUTANOL
21	ETHYL TRICHLOROACETATE
22	TETRAETHOXY METHANE
23	1,3-DICHLORO-2-METHYL-2-BUTANOL
24	1,3-DIOXOLANE
25	3,4DICHLORO-3-METHYL-2-BUTANOL
26	UNKNOWN CHLORO PRODUCT
27	ISOMER OF S
28	T-QUESTION-3,3,4,4-TETRAMETHYL-2-PENTANONE
29	U-UNASSIGNED

NO	M/E	SCAN	TIME	REF	RRT	METH	AREA	AMOUNT	%TOT
1	NOT FOUND								
2	NOT FOUND								
3	RIC	391	9:46	4	0.940	A VB	3349.	2.537	0.74
4	RIC	416	10:24	4	1.000	A BB	132012.	100.000	29.22
5	RIC	492	12:18	4	1.183	A BB	4279.	3.241	0.95
6	RIC	405	10:07	4	0.974	A BB	38548.	29.200	8.53
7	NOT FOUND								
8	RIC	590	14:45	4	1.418	A BB	91072.	68.988	20.16
9	NOT FOUND								
10	NOT FOUND								
11	RIC	750	18:45	4	1.803	A BB	71642.	54.269	15.86
12	NOT FOUND								
13	NOT FOUND								
14	NOT FOUND								
15	RIC	899	22:28	4	2.161	A BB	63662.	48.224	14.09
16	NOT FOUND								
17	RIC	998	24:57	4	2.399	A BB	25775.	19.525	5.71
18	RIC	1043	26:04	4	2.507	A BB	2283.	1.729	0.51
19	RIC	1080	27:00	4	2.596	A BB	12861.	9.743	2.85
20	NOT FOUND								
21	NOT FOUND								
22	NOT FOUND								
23	NOT FOUND								

Figure 3. Product Distribution, Chlorination of Aquatic Humics, Run II-134, reservoir control

NO	M/E	SCAN	TIME	REF	RRT	METH	AREA	AMOUNT	%TOT
24	RIC	1329	33:13	4	3.195	A BB	4232.	3.206	0.94
25	NOT FOUND								
26	NOT FOUND								
27	NOT FOUND								
28	RIC	1567	39:10	4	3.767	A BB	2052.	1.554	0.45
29	NOT FOUND								

Figure 3. (Continued)

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Monthly Progress Reports for Oct., Nov., Dec. 1978

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

January 17, 1979

by

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EXECUTIVE SUMMARY

The purpose of the work described in this report and the overall objective of the research project is to identify major and minor classes of natural organic substances found in surface waters such as might be used as a source of potable water. A second major aim of the study is to evaluate the effect of a number of water treatment processes such as chlorine, ozone and chlorine dioxide on the transformations which these naturally occurring materials may undergo during the disinfection process.

A source water which is rich in the required organic materials but which is unusually low in such interfering materials as agricultural runoff, municipal wastewater effluents and industrial discharges is being used to provide a generous reserve of aquatic humic materials which are the dominant class (80%) of all natural organic substances found in drinking water sources.

During the reporting period a formal transfer of the project and staff to the department of Civil Engineering was arranged to take place in mid-January of 1979. This transfer completes the partnership arrangement between the Project Manager and Dr. Edward S. K. Chian in which the labor and equipment resources contributed by each can be used to strengthen the other's programs. The reaction series involving aquatic humics and chlorine dioxide has been completed as of this writing.

A series of preliminary experiments designed to test the ability of aquatic humics to remove selected organics from air and bind them in such a way as to resist extraction by LLE has been initiated. The finding of benzene and toluene among our oxidative degradation products lends further support to our hypothesis regarding the ability of aquatic humics to bind airborne organics for release upon oxidative alteration of the humic structure.

A tetrahydropranal (THP) derivitive of aquatic humics has been prepared and awaits further characterization. Infrared evidence indicates that THP groups have been added to the humic structure.

The last of the fractions resulting from the oxidative degradation of aquatic humic material with the crown ether complex of potassium permanganate has been examined by GC/MS. It appears to be composed of higher molecular weight material than the previously analyzed fractions.

A presentation entitled "Environmental Applications of GC/MS" was made before the meeting of the Atlanta Chromatography Discussion group in which the methods employed to obtain our results were discussed. A rough draft of another publication entitled "The Role of Aquatic Humics in the Transport of Organic Substances in the Environment" has been prepared and is currently being edited for submission.

PERSONNEL AND EQUIPMENT

In spite of a considerable investment of resources, our efforts to recruit seasoned talent for the mass spectrometry position associated with this project have not been successful. Our first two offers have been rejected and we are therefore, compelled to fall back on training our own operators from the present staff. As the sponsor is well aware, qualified workers in this area are in great demand and while we have been able to match salaries, we are unable to offer the added inducement of a tenure-track position. Dr. E.S.K. Chian of the Department of Civil Engineering, and myself, have decided to formalize our partnership and as a result my staff and I have joined the Department of Civil Engineering. As a result of this move we will have immediate access to four state-of-the-art microprocessor controlled GCs a sub-ppm TOC apparatus, a pair of state-of-the-art liquid chromatographs and a wide variety of custom-built "front-end" equipment. Dr. Chian, in turn will gain immediate access to our powerful GC/MS system. While we hope to eventually be housed under the same roof, we plan to make the move at such a time as to avoid a serious lapse in the work. The department has demonstrated its support for this move by ordering the 9-track tape attachment for the GC/MS system so that we can now transcribe raw data in the EPA format for retention in the EPA archives should this be desired.

In addition, Dr. Chian himself and the members of his highly qualified staff, particularly Dr. Gabbai and Dr. Meng, whose resumes are enclosed as an appendix, will be available for advice and actual "hands-on" assistance should the situation require it. Mr. Lupton continues to mend slowly and his doctor has suggested that he return to work at least on a part-time basis sometime in February.

REACTION OF AQUATIC HUMICS WITH CHLORINE DIOXIDE

Our mini-pilot runs with chlorine dioxide as the disinfectants are essentially complete. The samples await treatment with diazoethane prior to analysis by GC/MS. A slight delay has been brought about by the need to familiarize new personnel with the diazoethane treatment using test compounds prior to working with the real samples. A summary of the treatment conditions employed thus far is presented below.

Run 1 - Sand filter as a final step, pH 7.5. Dose 1.8 - 2.0 on a weight per weight basis. Concentration of humics 10/mg/l. (Samples II-19-1, II-19-11, II-19-19)

Run 2 - Sand filter as a final step. Aluminum sulfate added to the reservoir as a flocculating agent; pH 5.0. Dose 0.9:1 on a weight per weight basis concentration of humics 10 mg/l. (Samples II-24-1, II-21-20, II-25-1)

Run 3 - Sand filter as a final step. Same buffer system aluminum sulfate added with the disinfectant; pH 5.7. Dose 0.9:1 on a weight per weight basis. Concentration of humics 10 mg/l. Runs 1-3 made with humics taken from Satilla tributary. (Samples II-29-2, II-26-5, II-30-1)

Run 4 - Sand filter as a final step. All conditions as in Run 3 except humics drawn from main channel of the Satilla River and processed without the use of chloroform. All subsequent runs made with this material; pH 5.7. Dose 0.9:1. Concentration of humics 10 mg/l. (Samples II-34-2, II-34-4, II-35-1)

Run 5 - Carbon filter placed ahead of mixing chamber so that disinfectant was added after contact with carbon. No flocculating agent was used; pH 7.8. Dose 0.9:1. Concentration of humics 10 mg/l. Additional samples taken periodically during run to check for breakthrough effects. Carbon employed was Nuchar WV-H, 6x16 mesh. (Samples II-44-20, II-44-30, II-45-2, II-42-5, II-46-1)

Run 6 - Carbon filter positioned as in Run 5. No flocculating agent employed; pH 6.6. Dose 0.9:1. Concentration of humics 10 mg/l. Additional samples removed periodically. Carbon employed was Nuchar WV-H, 6 x 16 mesh. (Samples II-48-20, II-49-5, II-50-1, II-50-12, II-48-5).

Run 7 - Carbon filter placed at end of system so that disinfectant was added prior to contact with carbon. No flocculating agent employed; pH 6.3. Dose 1.4:1. Concentration of humics 10/mg/l. Samples taken periodically during the run. Carbon as above. (Samples II-54-20, II-56-3, II-56-12, II-57-3, II-57-19)

A final run buffered to keep the pH near 8 is in progress. This will balance Run 7. Further experiments are not envisioned unless, for some reason, the data are unacceptable or suggest a new avenue of research. The latter course of action would be persued only if time permitted. Further runs using the mini-pilot facility will employ ozone generated by a high intensity UV lamp. Following our relocation in the Civil Engineering building, we shall use a Welsbach generator if time permits. Otherwise, we shall continue or work with chlorine-possibly following some minor modifications in the design of mini-pilot facility. We have already honored the sponsor's desire to see more chlorination work and intend to present a more comprehensive summary of this work in the next report.

AQUATIC HUMICS AND ORGANICS IN AIR

The invocation of airborne isoprene as a possible adsorbed material in the aquatic humics which is ulitmately responsible for the production of the chlorinated isoprene alcohols has led us to undertake a series of experiments aimed at determining the ability of aquatic humics to solubilize airborne hydrocarbons. The experimental design is described in the following paragraphs.

Four 1000 ml volumetric flasks were charged with 50 ml of liquid toluene and warmed to 50°C in a constant temperature bath with the caps loosely sealed with aluminum foil. Two of the flasks were carefully loaded with 100 ml of high-purity water and fitted with glass stoppers. Three others were similarly loaded with 100 ml of a solution containing 20 ppm aquatic humics. The flasks

were returned to the water bath, shaken periodically, removed after one hour and allowed to stand overnight. A headspace analysis was performed on each of the flasks. One pair of the flasks was subjected to direct liquid-liquid extraction with high purity pentane (100 ml, 2 x 50 ml). The combined extracts were dried by passage over roasted sodium sulfate and concentrated to 0.5 ml in a Kuderna-Danish apparatus. The concentrates were brought to 1.0 ml and analyzed by gas chromatographic methods.

A second pair of flasks was purged prior to extraction and workup as before. The aqueous layers remaining from all four flasks were transferred to clean roundbottom flasks and gently refluxed with alkaline potassium permanganate for 1 hour after cooling the aqueous phase was extracted and worked up as before. It is hoped that this experiment will demonstrate an increased solubilization of the organic material by the aquatic humics, will show that that the aquatic humics interfere with the ability to remove the organic materials by liquid-liquid extraction or purging and that the destruction of the aquatic humic material by oxidative degradation restores the effectiveness of the liquid-liquid extraction. A preliminary run has been carried out as of this writing, but will have to be repeated in the near future as a malfunction of the gas chromatograph has placed some question on the interpretation of the results.

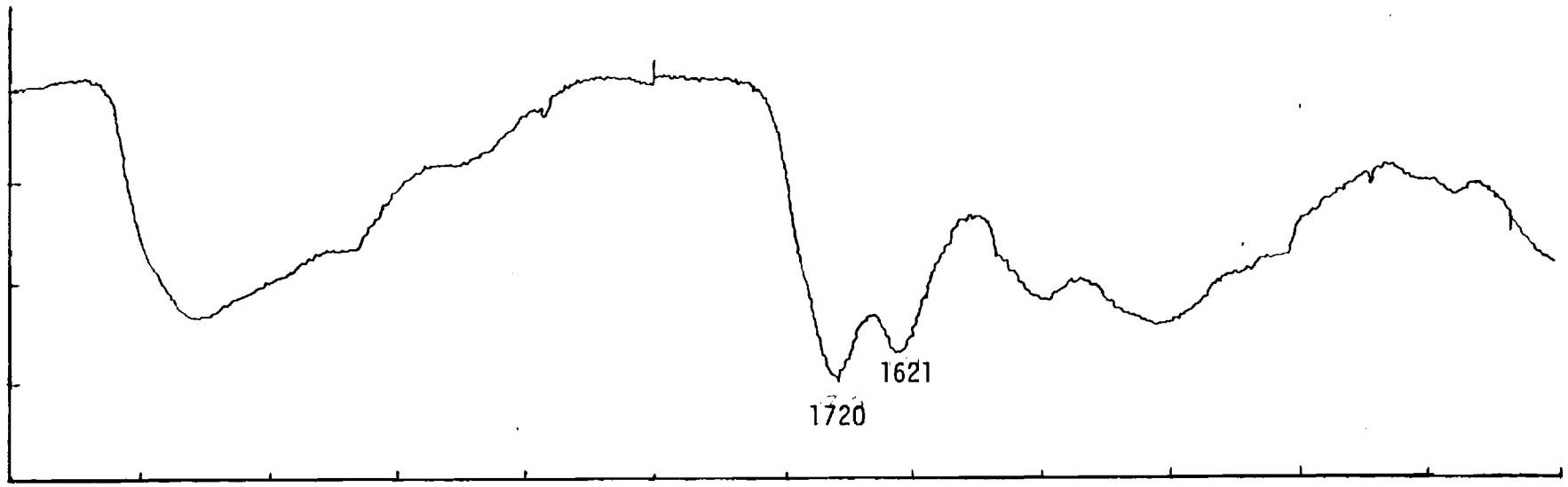
TETRAHYDROPYRANYL DERIVATIVE OF AQUATIC HUMICS

Aquatic humic material contains carboxyl groups as well as many hydroxyl groups, some of which may be strongly acidic. In order to estimate only the carboxyl groups without any interference from the acidic hydroxyl groups, the latter must be protected with a group that is stable to bases such as sodium hydroxide. The tetrahydropyranyl (THP) ether meets such requirements. Accordingly, we have attempted to prepare this derivative. The primary obstacle

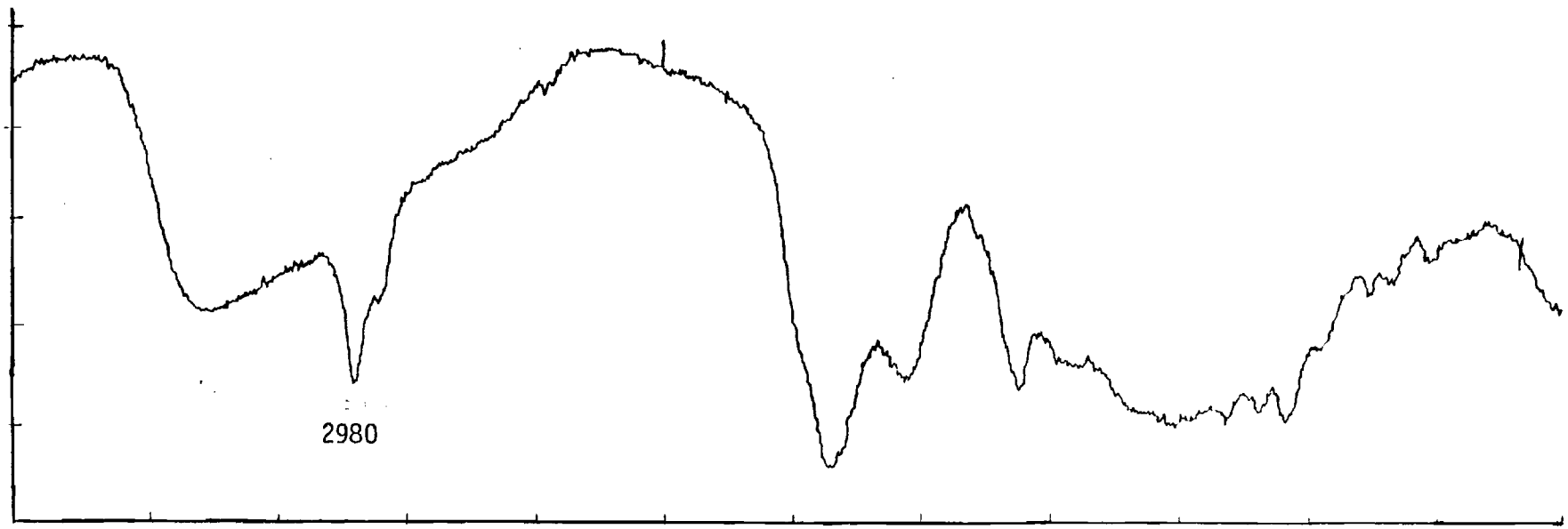
in preparing THP derivatives of aquatic humics is that this material is insoluble in organic solvents. However, it will be recalled that our sample (M/39) is soluble in anhydrous methanol as well as in water. Therefore, a solution of aquatic humic material was prepared in anhydrous methanol and treated with dihydropyran overnight at ambient temperature. It was first assumed that the acidity of the humic material would suffice to catalyze the reaction. This assumption proved to be incorrect. Only unchanged aquatic humic material was recovered. In a subsequent experiment, dry hydrogenchloride gas was bubbled through a solution of aquatic humic material (0.3g) in anhydrous methanol (8.0 ml) and treated with 2.0 ml DHP. The reaction mixture was left overnight at room temperature prior to removal of all volatile components under reduced pressure and subsequent drying of the residue in vacuum over sodium hydroxide. The infrared spectrum (see Figure 1) shows a dramatic increase in the strength of the C-H absorption bands just below 3000 cm^{-1} which must be taken as a strong indication that the desired reaction has taken place. Further investigation of the properties of this material is under way. Hopefully, the THP derivative will provide yet another means for distinguishing between true carboxylates and strongly acidic phenolic functions as subsequent reaction with strong base could not involve the "protected" functions.

OXIDATION OF AQUATIC HUMICS WITH CROWN ETHER COMPLEX OF POTASSIUM PERMANGANATE

The last major extract resulting from the oxidation of methylated aquatic humics with the permanganate/crown ether complex in methylene chloride has been analyzed on the GC/MS system. Several new components are present in this fraction which are generally characterized by longer GC-retention times. This property, coupled with the observed lower solubility in the extracting solvent, suggests that higher molecular weight materials may dominate this fraction.



Untreated Aquatic Humics



Awuatic Humics - THP Derivative

Figure 1 - IR Spectra of Aquatic Humics and Aquatic Humic THP Derivative

Unfortunately, it is apparent that we shall have to rely on our own interpretive skills for the assignment of structures as nearly all of the components of this product mixture fail to achieve an acceptable library match. Such efforts are time consuming and have therefore been reserved for a later date.

PUBLICATIONS AND PRESENTATIONS

A presentation entitled "Environmental Applications of GC/MS" was made by the project manager before the meeting of the Atlanta Chromatography Discussion Group held January 18, 1979 in Athens, Georgia. Upwards of 50 members were in attendance. THe presentation emphasized the techniques employed in getting the results described in our paper entitled "Reaction of Aquatic Humic Material with Chlorine - Isolation and Characterization of some New Chlorinated Organics" which is to be presented at the joint ACS/ Chemical Society of Japan meeting to be held this spring.

A rough draft of a publication entitled "The Role of Aquatic Humics in the Transport of Organic Substances in the Environment", by S. C. Havlicek, J. H. Reuter, J. A. Cotruvo and C. L. Trichilo has been completed and is currently being drastically shortened to include only the new evidence relating aquatic humics to airborne pollutant transport which has been developed in the course of this project. If the reviewers so desire, they may participate in this editorial process. Otherwise, their comments will be sought at a later stage.

COMPUTERIZED DATA PROCESSING

The correction of our minor problems with the disc drive mentioned in our last report has been carried out so that we have been able to run our reverse searching procedure STEVE against most of the data derived from our mini-pilot chlorination runs. Two fit criteria were employed. The loose criteria (L) (PURITY 500, FIT 500) were designed to catch everything which might be present which had been noted in earlier runs. The tight criteria (T) (PURITY 600, FIT 900) were designed to be more discriminating and thereby present a greater degree of assurance that the indicated components were really present. Since the program is still evolving, it is not surprising that some difficulties have been encountered in analyzing data gathered over a six-month's span. In some cases, corrective action can be taken in the way that the data are gathered so that the retention times for all components will be more nearly identical so that the dimensions of the searching "window" do not have to be changed from run to run. It has been found necessary to include -1 as a starting point in the search window even if all components lay behind their positions in the library from which the search is made. In such cases, a very large search window is created which lengthens the time required to complete the search and incrasees the opportunity for multiple "hits" or out-of-position hits. We intend to try creating a satellite library with altered retention times longer than those expected in any of our runs. Thus we will be able to assign a negative number to the window without making it too large. It has already been demonstrated that the program can successfully process data with both ends of the search window being assigned negative values. A change in the program might also be successful in circumventing this difficulty.

A compromise must always be made in setting up the peak area integration criteria, and the peak finding criteria within CHRO. For example, a peak might meet the search criteria but not project far enough above the noise level (6.0 standard deviations as marked in Figure 2) to be counted. It will not then be integrated. This ultimately results in a gap in the quantitation report in which case the compound is neither reported as a "not found" nor as a hit with a defined area. Such cases are reported as "NI" (for not integrated) in the comprehensive table which follows this section. Lowering this noise line increases the chances of picking up minor, closely related components instead of the component of interest. Tightening up the search criteria might permit a lowering of the noise level at this point without resulting in multiple hits providing nearby isomeric components were not too similar to the components being looked for. Improved reproducibility of conditions would, of course, be extremely helpful since the search window could then be made very narrow so that closely related compounds would not be mistaken for each other. Hopefully, the program itself could be adjusted to make the "not integrated" peaks readily recognizable.

The library list, itself was reordered so that the listing was more nearly in a direct relationship with retention time. This action was necessary in order to accomodate data from abbreviated runs. Otherwise the searches of such data would be prematurely terminated even though some early peaks had not been "looked for". Ideally, the sequence in the library list should be in direct correspondence with the elution order.

A complete presentation of the data from three of the mino-pilot runs involving aquatic humics and chlorine is presented in Table I. The key to the table is as follows:

```
TRACE OF PROCEDURE STEVE
   * SET4 SH;SETL LIBSH;SETQ TRASH;EDQL(-;W;E);CHRO(I;D;E);STEVE1;QUAN(D;F;F1;H;E);SONG(@FF)
   *
   SET4 SH
   SETL LIBSH
   SETQ TRASH
   EDQL  (-;W;E)
   CHRO  (I;D;E)
   STEVE1
      * GETL;SEAR(I;$;&;N1,500,500<;D-10,10;E)/V;CK1;LOOP;
      *
      GETL
      SEAR  (I;$;&;N1,500,500<;D-10,10;E)/V
      CK1
         * CK2;EDQL (-;N;*;A;E);RETU
         *
         CK2
            * IF !14;SET1 !14;ERASE;CHRO (I;N-1,6.0;O;*;G-1,1;A>-1,2.0;D-10,10;E);RETU CK1
            *
            *
            IF !14
            SET1 !14
            ERASE
            CHRO  (I;N-1,6.0;O;*;G-1,1;A>-1,2.0;D-10,10;E)
            RETU CK1
         EDQL  (-;N;*;A;E)
         RETU
      LOOP
   QUAN  (D;F;F1;H;E)
   SONG  (@FF)
```

Figure 2. Trace of procedure STEVE (Note height of quantitation line)

X indicates the component is found and quantitated.

NI indicates the component is found, but not quantitated.

NF indicates the component is not found.

NA indicates the search broke off prior to the scheduled appearance of the component.

TABLE I REVERSE SEARCH RESULTS

NO.	NAME	II 110 pH 7.3 No Floculant				II 134 pH 6.0, $Al_2(SO_4)_3$				II 145 pH 8.0 $Al_2(SO_4)_3$			
		Control		Treated		Control		Treated		Control		Treated	
		T	L	T	L	T	L	T	L	T	L	T	L
1	3-Chloro-2-methyl-1-butene	NF	NF	X	X	NF	X	X	X	NF	NF	X	X
2	2-Chloromethyl-1-butene	NF	NF	X	X	NF	X	X	X	NF	NF	X	X
3	Ethylene glycol, diethyl ether	NF	X	X	X	NF	X	X	X	X	X	NF	NI
4	2-Methyl-3-pentanone	X	X	X	X	X	X	X	X	X	X	X	X
5	Diethylcarbonate	NF	X	X	X	NF	X	X	X	NF	X	X	X
6	2-Methylpentanal	X	X	X	X	X	X	X	X	X	X	NF	NI
7	3-Chloro-2-methyl-2-butanol	NF	X	X	X	NF	X	X	X	NF	NF	X	X
8	4-Hydroxy-3-methylbutanal	X	X	X	X	NF	X	NI	NI	X	X	X	X
9	2,3-Dichloro-2-methylbutane	NF	NF	X	X	NF	NF	X	X	NF	NF	X	X
10	1-Chloro-2methyl-2-butanol	NF	NF	X	X	NF	NF	X	X	NF	NF	X	X
11	Ethyl acetate	NF	X	X	X	NF	X	NI	NI	X	X	NF	X
12	1,3-Dichloro-2-methylbutane	NF	NF	X	X	NF	NF	NF	NF	NF	NF	X	X
13	3-Chloro-2methyl-1-butanol	NF	NF	X	X	NF	X	NF	NF	NF	NF	X	X
14	4-Chloro-3-methyl-1-butanol	NF	NF	X	X	NF	NF	X	X	NF	NF	X	X
15	N-Nitrosodiethylamine (Artifact)	X	X	X	X	NF	X	NF	NF	X	X	X	X
16	3,4-Dichloro-methyl-1-butene isomer	NF	NF	X	X	NF	NF	NF	NF	NF	NF	X	X
17	4-Methyl-3-heptanone	NF	NF	X	X	NF	NF	NF	NF	X	X	NF	NF
18	2,2-Bis-1, 3-dioxolane (Tentative)	NF	NF	NF	X	NF	X	NF	NF	NF	NF	X	X
19	2-(3-Oxobutyl)-cyclopropanol	NF	NF	X	X	NF	X	X	X	X	X	NF	X
20	1,4-Dichloro-2-methyl-2-butanol	NF	NF	X	X	NF	NF	X	X	NF	NF	X	X
21	Ethyl trichloroacetate	NF	NF	NF	X	NF	NF	NF	NF	NF	NF	X	X
22	Tetraethoxymethane	NF	NF	X	X	NF	X	NF	NF	NF	NF	X	X
23	1,3-Dichloro-2-methyl-2-butanol	NF	NF	X	X	NF	NF	X	X	NF	NF	X	X
24	1,3-Dioxolane	NF	X	X	X	NF	X	X	X	NF	NF	X	X
25	3,4-Dichloro-3-methyl-2-butanol	NF	NF	X	X	NF	NF	X	X	NA	NA	X	X

TABLE I (continued)

NO.	NAME	II 110 pH 7.3 No Floculant				II 134 pH 6.0. $Al_2(SO_4)_3$				II 145 pH 8.0 $Al_2(SO_4)_3$			
		Control		Treated		Control		Treated		Control		Treated	
		T	L	T	L	T	L	T	L	T	L	T	L
26	Unknown chloro product	NF	NF	X	X	NF	NF	X	X	NA	NA	NF	X
27	Compound iso-S (a chloro compound)	NF	NF	NF	X	NF	NF	NF	X	NA	NA	X	X
28	3,3,4,4-Tetramethyl-2-pentanone (Tentative)	NF	NF	X	X	NF	X	NX	NF	NA	NA	NF	NF
29	Compound U unassigned	NA	NA	X	X	NF	X	NF	NF	NA	NA	NF	NF
30	2 Butanol (Tentative)	NF	NI	X	X	NF	NI	NI	NI	X	X	NF	X
31	Ethyl Ether (Tentative)	NF	NF	X	X	NF	NI	NF	NI	X	X	NF	X
32	Compound O-2-A (May contain Cl)	NF	NF	X	X	NF	NI	NF	X	NF	X	NF	X
33	Compound O-3-A (May contain Cl)	NF	NF	NF	X	NF	NI	NI	NI	NF	NI	NF	X
34	Oxirane (Tentative)	NF	NF	X	X	X	X	X	X	X	X	X	X
35	N-methyl Formamide	NF	NF	NF	NF	NI	NI	NF	NF	NF	NF	NF	NF
36	Methyl Formate (Tentative)	NF	NF	NF	NF	NI	NI	NF	NF	NF	NF	NF	NF
37	Ethyl Cyanate (Tentative)	NF	NF	NI	NF	NF	X	NF	NF	NF	NF	NF	NI
38	3-Butene-2-one (Tentative)	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
39	2-Butanone	NF	NF	X	X	NI	NI	NF	X	NF	NF	X	X
40	Chloroform	NF	NF	NI	NF	NF	NF	NF	NF	NF	NF	NF	X
41	Benzene	NF	NF	NF	NF	X	X	NF	NF	NF	NF	NI	NI
42	Post-Solvent Peak #7 (Possible Artifact)	NF	NI	NF	NF	NF	X	NF	NF	NF	NF	X	X
43	Cyclohexane (Artifact)	NF	NI	NF	X	NF	NF	NF	NF	NF	NF	NF	NF
44	2,2,3-Trimethyl oxetane (Tentative)	NF	NI	X	X	X	X	X	X	NF	NI	NF	X
45	2-Chloro-3-methyl-2-butene	NF	NF	NF	X	NF	X	X	X	NF	NF	X	X
46	Compound A-5-B	X	X	NF	X	X	X	NF	NI	NF	NF	NF	I
47	Compound B-1-C	X	X	NF	X	NF	X	X	X	NF	NF	X	X
48	Compound B-2-C	NF	NF	NF	X	NF	NI	NF	NF	NF	NF	NF	X
49	Compound B-3-C	X	X	NF	NF	NF	NI	X	X	NF	NF	X	X
50	Unassigned ester E-1-F	NF	X	NF	NF	NF	X	NF	NI	NF	NF	NF	X

TABLE I (continued)

NO.	NAME	II 110 pH 7.3 No Floculant				II 134 pH 6.0. $Al_2(SO_4)_3$				II 145 pH 8.0 $Al_2(SO_4)_3$			
		Control		Treated		Control		Treated		Control		Treated	
		T	L	T	L	T	L	T	L	T	L	T	L
51	Compound F-2-G	NF	NF	NF	NF	NF	X	NF	NF	NF	NF	X	X
52	Compound F-3-G	X	X	NF	X	NF	NI	NF	NF	X	X	NF	X
53	Compound F-4-G	X	X	NF	NF	NF	NI	NF	NF	X	X	NF	X
54	1,2 Propane diol (Tentative)	NF	X	NF	NI	NF	NI	NF	X	NF	NF	X	X
55	Compound I-1-J	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
56	Compound I-2-J	NF	NF	NF	NF	NF	X	NF	NF	NF	NF	X	X
57	Compound J-1-K	NF	NF	NF	X	NF	NF	NF	NF	NF	X	NF	NF
58	Compound J-2-K	X	X	X	X	NF	X	NF	NF	NF	NF	NF	NF
59	Compound 2-3-M (A dioxolane?)	NI	NI	NF	NF	NF	X	NF	NI	NA	NA	NI	NI
60	Compound M-1-N (Another isoprene?)	NF	NF	NF	NF	NF	X	NF	NF	NA	NA	X	X
61	Compound M-2-N	NI	NI	NF	X	NF	X	NF	X	NA	NA	NF	X
62	Compound M-3-N (May contain Cl)	NF	NF	X	X	NF	NF	NF	NF	NA	NA	X	X
63	Compound M-4-N	X	X	NI	NI	NF	X	X	X	NA	NA	NF	X
64	Compound Q-1-R (Another nitrosc artifact)	NF	NF	NF	X	NF	X	X	X	NA	NA	X	X
65	Compound Q-2-R	NF	NF	NF	NF	NF	NI	NF	NF	NA	NA	NF	X
66	Compound Q-4-R	NF	NF	X	X	NF	NF	X	X	NA	NA	NF	X
67	Compound S-3-T	NA	NA	NF	X	NF	NF	NF	NF	NA	NA	NF	X
68	Compound S-4-T (May contain Cl)	NA	NA	NF	X	NF	NF	NF	NF	NA	NA	NF	X
69	Compound T-1-U	NA	NA	X	X	X	X	NA	NA	NF	NF	NF	NF
70	Compound T-2-U (May contain Cl)	NA	NA	NF	X	NF	NF	NF	NF	NA	NA	X	X
71	Compound V	NA	NA	NF	NF	NF	X	NF	NF	NA	NA	NF	X
72	Compound W (May contain Cl)	NA	NA	NF	X	NF	X	NF	NF	NA	NA	NF	NF
73	Compound X	NA	NA	NF	NF	X	X	NF	NF	NA	NA	NF	X
74	Compound Y	NA	NA	NF	NF	X	X	NF	NF	NA	NA	X	X
75	Compound Z	NA	NA	NF	NF	X	X	NF	NF	NA	NA	X	X

TABLE I (continued)

NO.	NAME	II 110 pH 7.3 No Floculant				II 134 pH 6.0. $Al_2(SO_4)_3$				II 145 pH 8.0 $Al_2(SO_4)_3$			
		Control		Treated		Control		Treated		Control		Treated	
		T	L	T	L	T	L	T	L	T	L	T	L
76	Compound Z-1-A (Isoprenoid?)	NA	NA	NF	NF	NF	X	NF	NF	NA	NA	NF	NF
77	Compound A'	NA	NA	NF	NF	NF	X	NF	NF	NA	NA	NF	X
78	Compound B'	NA	NA	NF	NF	NF	NF	NF	NF	NA	NA	NF	NF
79	Compound E' (Another dioxolane?)	NA	NA	NF	NF	X	X	NF	X	NA	NA	X	X
80	Compound F'	NA	NA	NF	NF	NF	NF	NF	NF	NA	NA	NF	NF
81	Compound G' (Another dioxolane?)	NA	NA	X	X	X	X	NF	X	NA	NA	X	X
82	Compound H' (Another dioxolane?)	NA	NA	NF	X	X	X	NF	X	NA	NA	X	X
83	Compound I' (Another dioxolane?)	NA	NA	NF	X	X	X	NF	X	NA	NA	X	X
84	Compound J'	NA	NA	NF	NF	NF	NF	NF	NF	NA	NA	NF	NF
85	Compound K' (Possible dichloro compound)	NA	NA	NF	NF	NF	NF	NF	NF	NA	NA	X	X
86	Compound L' (Isopronoid?)	NA	NA	NF	NF	NF	NF	NF	NF	NA	NA	NF	NF
87	Compound M' (May contain Cl)	NA	NA	X	NF	NF	X	NF	NF	NA	NA	X	X
88	Compound N'	NA	NA	NF	X	NF	NF	NF	NF	NA	NA	NF	NF
89	Compound O'	NA	NA	NF	NF	NF	X	NF	NF	NA	NA	NF	X
90	Compound P'	NA	NA	NF	X	NF	X	NF	NF	NA	NA	NF	X
91	Compound Q'	NA	NA	NF	X	NF	X	NF	NF	NA	NA	NF	NF
92	Compound R'	NA	NA	NF	NF	NF	X	NF	NF	NA	NA	NF	NF
93	Compound S'	NA	NA	NF	NF	NF	NF	NF	NF	NA	NA	X	X
94	Compound T'	NA	NA	NF	NF	NF	NF	NF	NF	NA	NA	NF	X
95	Compound U' (May contain Cl)	NA	NA	NF	X	NF	NF	NF	NF	NA	NA	NF	X
96	Compound V'	NA	NA	NF	X	NI	NI	NF	NF	NA	NA	NF	NI
97	Compound W' (Another dioxolane?)	NA	NA	X	X	X	X	NF	X	NA	NA	X	X
98	Compound X'	NA	NA	NF	X	X	X	NF	X	NA	NA	X	X
99	Compound Y'	NA	NA	NF	X	X	X	NF	X	NA	NA	X	X
100	Compound Z'	NA	NA	NF	NF	NI	NI	NF	NF	NA	NA	X	X

TABLE I (continued)

NO.	NAME	II 110 pH 7.3 No Floculant				II 134 pH 6.0. $Al_2(SO_4)_3$				II 145 pH 8.0 $Al_2(SO_4)_3$			
		Control		Treated		Control		Treated		Control		Treated	
		T	L	T	L	T	L	T	L	T	L	T	L
101	Compound A"	NA	NA	NF	NF	NF	NI	NF	NF	NA	NA	NF	X
102	Compound B"	NA	NA	NF	NF	NF	X	NF	NF	NA	NA	NF	X
103	Compound C"	NA	NA	NF	NF	X	X	NF	NF	NA	NA	NF	X
104	Compound D"	NA	NA	NF	NF	NF	X	NF	NF	NA	NA	NF	NI
105	Compound D"-1-E"	NA	NA	NF	NF	NF	NI	NF	NF	NA	NA	NF	X
106	Compound E"	NA	NA	NF	NF	NF	X	NF	NF	NA	NA	X	X
107	Compound F" (A substituted oxirane?)	NA	NA	NF	NF	X	X	NF	NF	NA	NA	NF	NI
108	Compound G" (A substituted oxirane?)	NA	NA	NF	NI	X	X	NF	NF	NA	NA	NF	NF
109	Compound H"	NA	NA	NF	NF	X	X	NF	NF	NA	NA	NF	NF
110	Compound I"	NA	NA	NF	X	X	X	NF	NF	NA	NA	NF	NF
111	Compound J"	NA	NA	NF	X	X	X	NF	NF	NA	NA	NF	NI
112	Compound K" (Another dioxolane?)	NA	NA	NF	NF	NF	NI	NF	NF	NA	NA	X	X
113	Compound M" (A substituted oxitane?)	NA	NA	NF	NF	NF	X	NF	NF	NA	NA	NF	NF
114	Compound O"	NA	NA	NF	NF	NA	NA	NF	NF	NA	NA	NF	NF
115	Compound P"	NA	NA	NF	NF	NA	NA	NF	NF	NA	NA	NA	NA

It can be seen that the program is quite successful at finding the early components in the II-110 run, treated. This run provides a measure of the effectiveness of the program since it is the run from which the library was constructed. Even with the tight criteria, only three of the first 32 components are reported as not found. With the loose criteria, all of these are reported as found. As one progresses down the column, it is evident that fewer and fewer of the components are meeting the search criteria. This is not unexpected because these are all minor components for which a summation of adjacent scans was necessary in order to improve the statistics of the total ion counts. Since the search is conducted on individual scans, the statistics are often poorer and therefore do not meet the search criteria. Eventually even the loose criteria cannot be met. The "loose criteria" searches run into more "NI"s than the tight searches because they find more of these minor peaks which may not project sufficiently far above the noise level to qualify for integration. The high number of hits in the other two treated samples provides additional evidence that the program is doing its job. An examination of the data shows that none of the chlorinated products is found in the searches of the components of the controls[1] when the searches are conducted with the tight criteria.

An assessment of the relative importance of each of the components is presented in figure 4. A list of the compounds searched for is presented in figure 3. The quantitation report properly excludes the contribution of the solvent peak but, perhaps improperly, also excludes the areas of the peaks not meeting the search criteria. It would also exclude new peaks,

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[1]The control for run II-145 was processed a few days later than the product mixture. Otherwise the controls and treated samples were processed together.

QUANTITATION REPORT FILE: TRASH

AMOUNT=AREA * REF.AMNT/(REF.AREA* RESP.FACT)

NO	NAME
1	3-CHLORO-2-METHYL-1-BUTENE
2	2-CHLOROMETHYL-1-BUTENE
3	ETHYLENE GLYCOL,DIETHYL ETHER
4	2-METHYL-3-PENTANONE
5	DIETHYL CARBONATE
6	2-METHYLPENTANAL
7	3-CHLORO-2-METHYL-2-BUTANOL
8	4-HYDROXY-3-METHYLBUTANAL
9	2,3-DICHLORO-2-METHYLBUTANE
10	1-CHLORO-2-METHYL-2-BUTANOL
11	COMPOUND F-1-G ETHYL ACETATE
12	1,3-DICHLORO-2-METHYLBUTANE
13	3-CHLORO-2-METHYL-1-BUTANOL
14	4-CHLORO-3-METHYL-1-BUTANOL
15	N-NITROSODIETHYLAMINE
16	3,4-DICHLORO-METHYL-1-BUTENE ISOMER
17	4-METHYL-3-HEPTANONE
18	2,2'-BIS-1,3-DIOXOLANE
19	2-(3-OXOBUTYL)-CYCLOPROPANOL
20	1,4-DICHLORO-2-METHYL-2-BUTANOL
21	ETHYL TRICHLOROACETATE
22	TETRAETHOXY METHANE
23	1,3-DICHLORO-2-METHYL-2-BUTANOL
24	1,3-DIOXOLANE
25	3,4DICHLORO-3-METHYL-2-BUTANOL
26	UNKNOWN CHLORO PRODUCT
27	ISOMER OF S
28	T-QUESTION-3,3,4,4-TETRAMETHYL-2-PENTANONE
29	U-UNASSIGNED
30	COMPOUND 0-1-A 2-BUTANOL (TENTATIVE)(ALTERNATE)
31	ETHYL ETHER COMPOUND 0-1-A (ALTERNATE)
32	COMPOUND 0-2-A (UNASSIGNED-MAY CONTAIN CL)
33	COMPOUND 0-3-A (UNASSIGNED-MAY CONTAIN CL)
34	PRESOLVENT PEAK #1 OXIRANE?
35	PRE SOLVENT PEAK TAIL ON #1 N-METHYL FORMAMIDE
36	PRE SOLVENT PEAK #2 METHYL FORMATE
37	POST SOLVENT PEAK #1 ETHYL CYANATE?
38	LEADER ON POST SOLVENT PEAK#2 3-BUTENE-2-ONE(TENTATIVE)
39	2-BUTANONE(LEADER ON POST SOLVENT PEAK #3)
40	METHANE,TRICHLORO-
41	BENZENE-POST SOLVENT PEAK #6
42	POST SOLVENT PEAK #7 (UNASSIGNED)
43	CYCLOHEXANE (TENTATIVE)
44	COMPOUND A-1-B 2,2,3-TRIMETHYL OXETANE (TENTATIVE)
45	2-CHLORO-3-METHYL-2-BUTENE (COMPOUND A-2-B)
46	COMPOUND A-5-B (UNASSIGNED)
47	COMPOUND B-1-C (UNASSIGNED)
48	COMPOUND B-2-C (UNASSIGNED)
49	COMPOUND B-3-C (UNASSIGNED)
50	COMPOUND E-1-F (UNASSIGNED)
51	COMPOUND F-2-G (UNASSIGNED)
52	COMPOUND F-3-G (UNASSIGNED)
53	COMPOUND F-4-G (UNASSIGNED)
54	COMPOUND I-1-J (UNASSIGNED)

Figure 3. List of Products- Chlorination of Humics

NO	NAME
55	COMPOUND I-2-J(UNASSIGNED)
56	COMPOUND J-1-K (UNASSIGNED)
57	COMPOUND J-2-K (UNASSIGNED)
58	COMPOUND L-3-M (UNASSIGNED)
59	COMPOUND M-1-N (UNASSIGNED)
60	COMPOUND M-2-N (UNASSIGNED)
61	COMPOUND M-3-N (UNASSIGNED)
62	COMPOUND Q-1-R (UNASSIGNED)
63	COMPOUND Q-2-R (UNASSIGNED)
64	COMPOUND Q-4-R (UNASSIGNED)
65	COMPOUND S-3-T UNASSIGNED)
66	COMPOUND S-4-T (UNASSIGNED)
67	COMPOUND T-1-U (UNASSIGNED)
68	COMPOUND T-2-U (UNASSIGNED)
69	COMPOUND V (UNASSIGNED)
70	COMPOUND W UNASSIGNED
71	COMPOUND X (UNASSIGNED)
72	COMPOUND Y (UNASSIGNED)
73	COMPOUND Z (UNASSIGNED)
74	COMPOUND Z-1-A' (UNASSIGNED)
75	COMPOUND A' (UNASSIGNED)
76	COMPOUND B' (UNASSIGNED)
77	COMPOUND E' (UNASSIGNED)
78	COMPOUND F' (UNASSIGNED)
79	COMPOUND G' (UNASSIGNED)
80	COMPOUND H' (UNASSIGNED)

Figure 3. (continued) List of Products - Chlorination of Humics

NO	M/E	SCAN	TIME	REF	RRT	METH	AREA	AMOUNT	%TOT
1	RIC	271	6:46	7	0.582	A BB	106663.	62.363	10.23
2	RIC	317	7:55	7	0.680	A BB	5667.	3.313	0.54
3	RIC	322	8:03	7	0.691	A BB	7498.	4.384	0.72
4	RIC	342	8:33	7	0.734	A BB	97803.	57.183	9.38
5	RIC	401	10:01	7	0.861	A BB	3845.	2.248	0.37
6	RIC	333	8:19	7	0.715	A BB	6983.	4.083	0.67
7	RIC	466	11:39	7	1.000	A BB	171036.	100.000	16.40
8	RIC	478	11:57	7	1.026	A BB	48476.	28.343	4.65
9	RIC	502	12:33	7	1.077	A BB	26729.	15.628	2.56
10	RIC	557	13:55	7	1.195	A BB	24107.	14.095	2.31
11	RIC	585	14:37	7	1.255	A BB	3370.	1.970	0.32
12	RIC	621	15:31	7	1.333	A BB	27276.	15.948	2.62
13	RIC	634	15:51	7	1.361	A BB	6706.	3.921	0.64
14	RIC	680	17:00	7	1.459	A BB	18360.	10.735	1.76
15	RIC	730	18:15	7	1.567	A BB	28542.	16.688	2.74
16	RIC	811	20:16	7	1.740	A BB	14141.	8.268	1.36
17	RIC	848	21:12	7	1.820	A BB	79285.	46.356	7.60
18	RIC	902	22:33	7	1.936	A BB	2505.	1.465	0.24
19	RIC	943	23:34	7	2.024	A BB	22415.	13.105	2.15
20	RIC	1126	28:09	7	2.416	A BB	37593.	21.980	3.60
21	RIC	1142	28:33	7	2.451	A BB	1961.	1.147	0.19
22	RIC	1157	28:55	7	2.483	A BB	8275.	4.838	0.79
23	RIC	1181	29:31	7	2.534	A BB	21445.	12.538	2.06
24	RIC	1232	30:48	7	2.644	A BB	2523.	1.475	0.24
25	RIC	1270	31:45	7	2.725	A BB	15566.	9.101	1.49
26	RIC	1282	32:03	7	2.751	A BB	13921.	8.139	1.33
27	RIC	1303	32:34	7	2.796	A BB	3649.	2.133	0.35
28	RIC	1481	37:01	7	3.178	A BB	38527.	22.526	3.69

Figure 4. Quantitation Report - Chlorination of Humics

NO	M/E	SCAN	TIME	REF	RRT	METH	AREA	AMOUNT	%TOT
29	RIC	1530	38:15	7	3.283	A BB	9355.	5.470	0.90
30	RIC	198	4:57	7	0.425	A BB	1972.	1.153	0.19
31	RIC	198	4:57	7	0.425	A BB	1972.	1.153	0.19
32	RIC	201	5:01	7	0.431	A VV	23812.	13.922	2.28
33	RIC	198	4:57	7	0.425	A BB	1972.	1.153	0.19
34	RIC	139	3:28	7	0.298	A BB	41026.	23.987	3.93
35	NOT FOUND								
36	NOT FOUND								
37	NOT FOUND								
38	NOT FOUND								
39	RIC	190	4:45	7	0.408	A BB	63992.	37.414	6.14
40	NOT FOUND								
41	NOT FOUND								
42	NOT FOUND								
43	RIC	246	6:09	7	0.528	A VB	1355.	0.792	0.13
44	RIC	285	7:07	7	0.612	A BB	3275.	1.915	0.31
45	RIC	317	7:55	7	0.680	A B8	5667.	3.313	0.54
46	RIC	327	8:10	7	0.702	A B8	605.	0.354	0.06
47	RIC	361	9:01	7	0.775	A BB	588.	0.344	0.06
48	RIC	369	9:13	7	0.792	A BB	905.	0.529	0.09
49	NOT FOUND								
50	NOT FOUND								
51	NOT FOUND								
52	RIC	593	14:49	7	1.273	A VB	3182.	1.861	0.31
53	NOT FOUND								
54	NOT FOUND								
55	NOT FOUND								
56	RIC	752	18:48	7	1.614	A BB	1567.	0.916	0.15
57	RIC	782	19:33	7	1.678	A BB	4638.	2.712	0.44
58	NOT FOUND								
59	NOT FOUND								
60	RIC	1024	25:36	7	2.197	A BB	1044.	0.610	0.10
61	RIC	1082	27:03	7	2.322	A BB	3646.	2.132	0.35
62	RIC	1208	30:12	7	2.592	A BB	1046.	0.612	0.10
63	NOT FOUND								
64	RIC	1282	32:03	7	2.751	A BB	13921.	8.139	1.33
65	RIC	1419	35:28	7	3.045	A BB	2123.	1.242	0.20
66	RIC	1462	36:33	7	3.137	A BB	2268.	1.326	0.22
67	RIC	1493	37:19	7	3.204	A BB	2414.	1.411	0.23
68	RIC	1499	37:28	7	3.217	A BB	849.	0.496	0.08
69	NOT FOUND								
70	RIC	1570	39:15	7	3.369	A BB	1310.	0.766	0.13
71	NOT FOUND								
72	NOT FOUND								
73	NOT FOUND								
74	NOT FOUND								
75	NOT FOUND								
76	NOT FOUND								
77	RIC	1694	42:21	7	3.635	A BB	1021.	0.597	0.10
78	NOT FOUND								
79	RIC	1708	42:42	7	3.665	A BB	1294.	0.757	0.12
80	RIC	1708	42:42	7	3.665	A BB	1294.	0.757	0.12

Figure 4. (continued) Quantitation Report - Chlorination of Humics

not on the library list if such peaks were present. Since this search was conducted on the same run as was used to compile the library list, the last two considerations are of minor importance. The quantitation report (figure 4) accounts for 98.5% of the total "found" peak area, the remainder of which can be obtained from the quantitation list in a separate operation. This list has not been presented in the interests of brevity. Inspection of figure 4 shows that only three errors have been made in the form of multiple assignments even though loose search criteria were employed. In all three cases the percentages of total area involved were less than 0.2. The narrow search window employed is chiefly responsible for the relative freedom from this type of error. The dominant chlorinated products are 3-Chloro-2-methyl-1-butene (10%) and the corresponding alcohol (16%). It should be pointed out that it would be inappropriate to relate these proportions to the proportion of chloroform in the same fraction since chloroform is well buried in the solvent peak, can be found only with difficulty and therefore cannot be even crudely estimated. An independent estimation of the chloroform concentration performed on the finished water itself will be required to link the relative proportions of the chlorinated isoprenes to those of the trihalomethanes.

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

February 23 1979

by

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DISCLAIMER

This report has not been reviewed by the Criteria and Standards Division, Office of Water Supply, U.S. Environmental Protection Agency, and is intended for internal circulation only. The contents do not necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

EXECUTIVE SUMMARY

The purpose of the work described in this report and the overall objective of the research project is to identify major and minor classes of natural organic substances found in surface waters such as might be used as a source of potable water. A second major aim of the study is to evaluate the effect of a number of water treatment processes such as chlorine, ozone and chlorine dioxide on the transformations which these naturally occurring materials may undergo during the disinfection process.

A source water which is rich in the required organic materials but which is unusually low in such interfering materials as agricultural runoff, municipal wastewater effluents and industrial discharges is being used to provide a generous reserve of aquatic humic materials which are the dominant class (80%) of all natural organic substances found in drinking water sources.

During the reporting period, a compilation of our on-going quality control measures was completed and is included in this report. It is not clear whether it is appropriate to apply quality control standards developed for solids probe or packed column performance to capillary column work. Nevertheless,we have been able to meet over 90% of the standards and are hopeful of meeting them all in the very near future.

A series of experiments with diazoethane and diazomethane and a number of reference standards has been completed. The results of the diazoethane experiments indicate that, under the conditions employed for the conversion of our reaction products to their ethyl ethers and esters, a partial conversion of even a relatively hindered substrate such as syringic acid takes place. The employment of slightly more rigorous conditions in the future should assure a reasonable conversion of all of our products.

The chlorine dioxide products have been partially analyzed prior to conversion to their ethyl derivatives so that the samples can be split for mutagenic testing prior to contact with the carcinogenic derivitization reagents. The results of these analyses which we have not been able to include in the body of the text indicate a scarcity of volatile products of the type observed in the chlorination experiments. We believe it would

be in the best interests of the project to rerun the reaction on a vastly more concentrated scale so that we might have a better indication of the nature of the products in case the analytical scheme would have to be revised. In this way we would not be risking our valuable mini-piolet samples in a methods development scheme.

A number of our structural assignments have been confirmed by comparison with authentic materials. Unfortunately, we are not able to present this material at this time either. Also performed, but not described, are some further oxidative degradation work on the aquatic humic material, some preliminary ozone work in the mini-pilot facility and some air-aquatic humic interaction studies.

In view of the difficulties involved in preparing reports such as this and in getting all of the work done with a reduced labor pool, we have elected to adopt an assembly line approach in which experiments are performed in one period of time to be followed by a massive analytical effort in which all saples are to be analyzed in a brief time span.

QUALITY CONTROL MEASURES

As stated in the original proposal, the contractor has periodically checked instrument performance with decafluorotriphenylphosphine. We have found that it is extremely difficult to tune the instrument to meet these specifications when the sample is presented through the capillary gas chromatographic interface. This is true even when the instrument has been laboriously calibrated with perfluorotributylamine to the extent that all base peaks fit to within 1-3 millimass units and the ratios of the intensities at m/e 69, 219 and 131 are all correct to within 0.1. Part of the problem with DFTPP, if one wishes to call it a problem is that our instrument (Finnigan 4023) is more sensitive at high mass than the "standard" instruments by EPA to draw up the performance criteria. The 442 peak is always considerably stronger than the 40% minimum required by the criteria. Thus the m/e 275 criterion (10%-30% of m/e 198) is most often violated on the high side. The m/e 127 criterion (40-60% of m/e 198) on the other hand, being below the base peak in amu units would be expected to miss on the low side more often than on the high side. Such is indeed the case. Other problems have been noted which do not appear to be related to this bias such as the frequent absence of the m/e 365 peak which should be present at 1% or better. This may be either a consequence of tuning out noise or might also be due to a thermal effect which in turn could be caused by the fact that the DFTPP performance is supposed to be evaluated via introduction of the sample via the solids probe. Other occasional errors such as the failure to register sufficiently high isotope peaks have been infrequently observed and are more readily amenible to correction.

A second standard of instrument performance, bromopentafluorobenzene, which is intended to be presented through a packed column was introduced

through the capillary column and indicated that the instrument was properly in tune. While even the perfluorotributylamine ratios indicated a mid-range over sensitivity which caused a slight enhancement of the m/e 167 peak (65%-85%) relative to the m/e 246 and m/e 248 peaks (75%-98%) we are confident that this discrepancy can be tuned out. In fact, a summation of several scans produces a composite mass spectrum which meets the criteria. We shall use this material instead pf DFTPP for certification of instrument performance in future runs. The background material used to develop these comments was developed by examining past quality control data dating back to June of 1978. Over sixty spectra were examined immediately prior to drafting this section. Some typical results together with the criteria are presented in Table I.

TABLE I

Performance Criteria and Typical Results

Decafluorotriphenylphosphine (DFTPP)

	Criterion					
1	Is m/e 51 30-60% of m/e 198?	54	36	29	48	37
2	Is m/e69 less than 2% of m/3 69?	NP	NP	NP	NP	NP
3	Is m/e 70 less than 2% of m/e 69?	NP	NP	NP	NP	NP
4	Is m/e 127 40-60% of m/e 198?	48	40	38	48	42
5	Is m/e 197 less than 1% of m/e 198?	NP	NP	NP	NP	NP
6	Is m/e 198 the base peak?	yes	yes	yes	yes	yes
7	Is m/e 199 5-9% of m/e 198?	9	5	5	7	7
8	Is m/e 275 10-30% of m/e 198?	30	25	27	37	33
9	Is m/e 365 at least 1% of m/e 198?	0.4	1	1	1	NP
10	Is m/e 441 less than m/e 443?	yes	yes	yes	yes	yes
11	Is m/e 442 40% or more of m/e 198?	55	59	82	91	79
12	Is m/e 443 17-23% of m/e 442?	12	16	14	17	16

Bromopentafluorobenzene

1	Is m/e 78 less than 1% of m/e 117?	NP	NP
2	Is m/e 79 15-35% of m/e 117?	25	28
3	Is m/e 80 4-8% of m/e 79?	5	5
4	Is m/e 116 less than 1% of m/e 117?	NP	NP
5	Is m/e 117 the base peak?	yes	yes
6	Is m/e 118 4-8% of m/e 117?	8	8
7	Is m/e 166 less than 1% of m/e 167?	NP	NP
8	Is m/e 167 65-85% of m/e 117?	87	85
9	Is m/e 168 5-9% of m/e 167?	9	8

		Results	
10	Is m/e 245 less than 1% of m/e 246?	NP	NP
11	Is m/e 246 75-98% of m/e 117?	84	82
12	Is m/e 247 5-9% of m/e 246?	8	7
13	Is m/e 248 75-98% of m/e 117?	78	75
14	Is m/e 249 5-10% of m/e 248?	8	7
15	Is m/e 248 93-99% of m/e 246?	93	91

EXPERIMENTS WITH DIAZOETHANE

In response to a suggestion from the audience during one of our recent public presentations of this work, it was mentioned that diazoethane might not be quite as successful as diazomethane in protecting hindered or less reactive functions. The use of deuteriodiazomethane was suggested instead. In order to assess the desirability of acting on this suggestion prior to the commitment of our entire sample backlog to a possibly unnecessary treatment, a series of experiments was undertaken in which diazoethane was reacted with benzoic acid, phenol and syringic acid in an effort to gauge the relative success of the treatment on an easy case (benzoic acid), an unhindered phenol (phenol) and a difficult case (syringic acid). Previous yield studies had shown a nearly complete conversion of benzoic acid to ethyl benzoate under the conditions which we had subsequently employed to convert our reaction product mixtures to their more volatile ethyl ethers and ethyl esters. A comparative series of experiments with diazomethane has been carried out and worked up as of this writing. The GC/MS analysis of this material will be described in the next report.

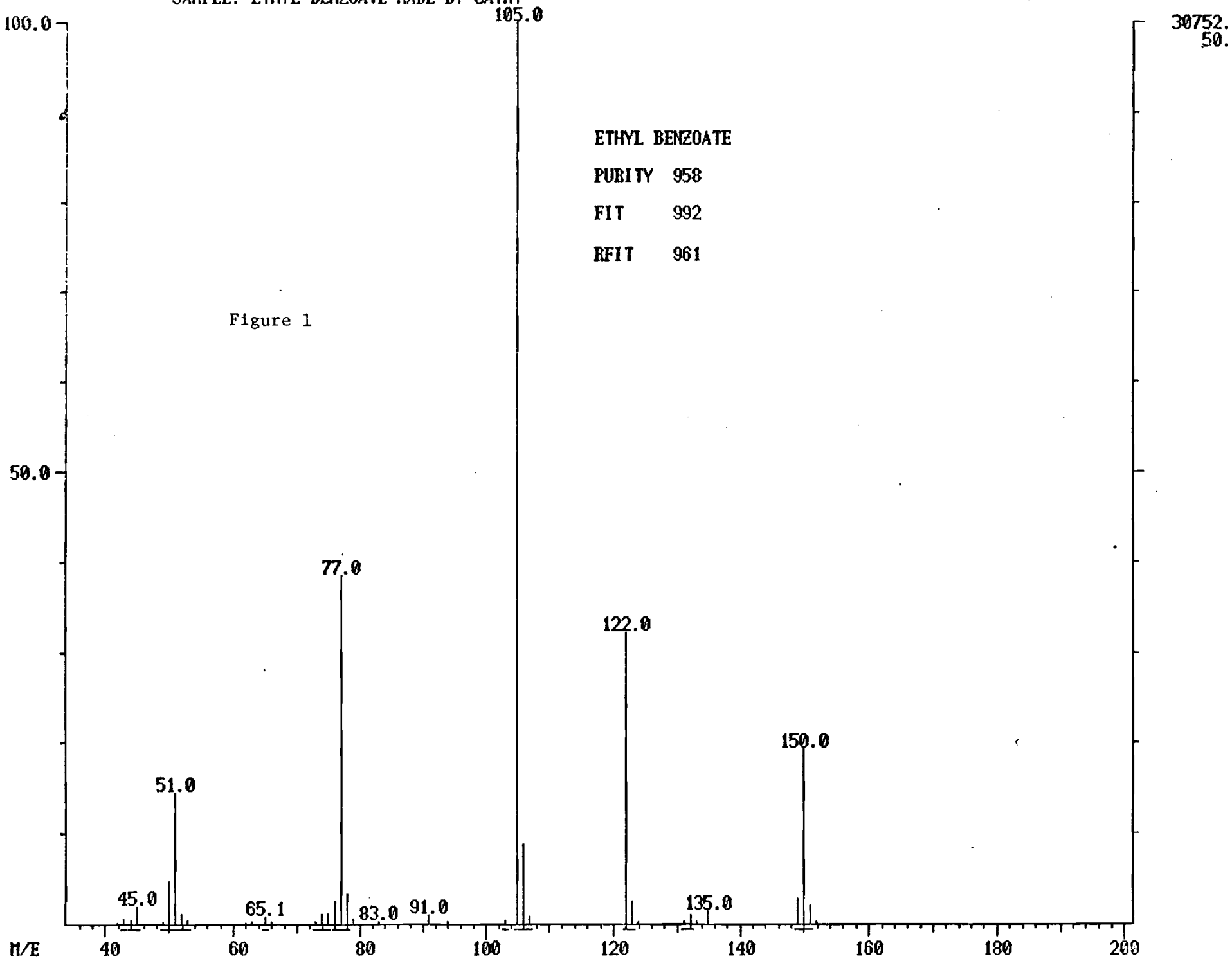
The reaction with benzoic acid resulted in a successful conversion of this substrate to ethyl benzoate, a spectrum of which is shown in Figure 1. The high fit indices are a further indication of the success of the quality control measures which have been discussed in the previous section. The fragmentation sequence is outlined below and is fully consistent with the assigned structure. The McLafferty rearrangement which cannot occur with the corresponding methyl ester is shown in the upper right hand corner. The acylium ion, with its added stability accounts for the base peak.

The fact that some benzoic acid was also found among the reaction

MASS SPECTRUM
02/07/79 15:41:00 + 4:50
SAMPLE: ETHYL BENZOATE MADE BY CATHY

DATA: ETBENZOATE #232
CALI: Z020779 #5

BASE M/E: 105
RIC: 76416.



ETHYL BENZOATE

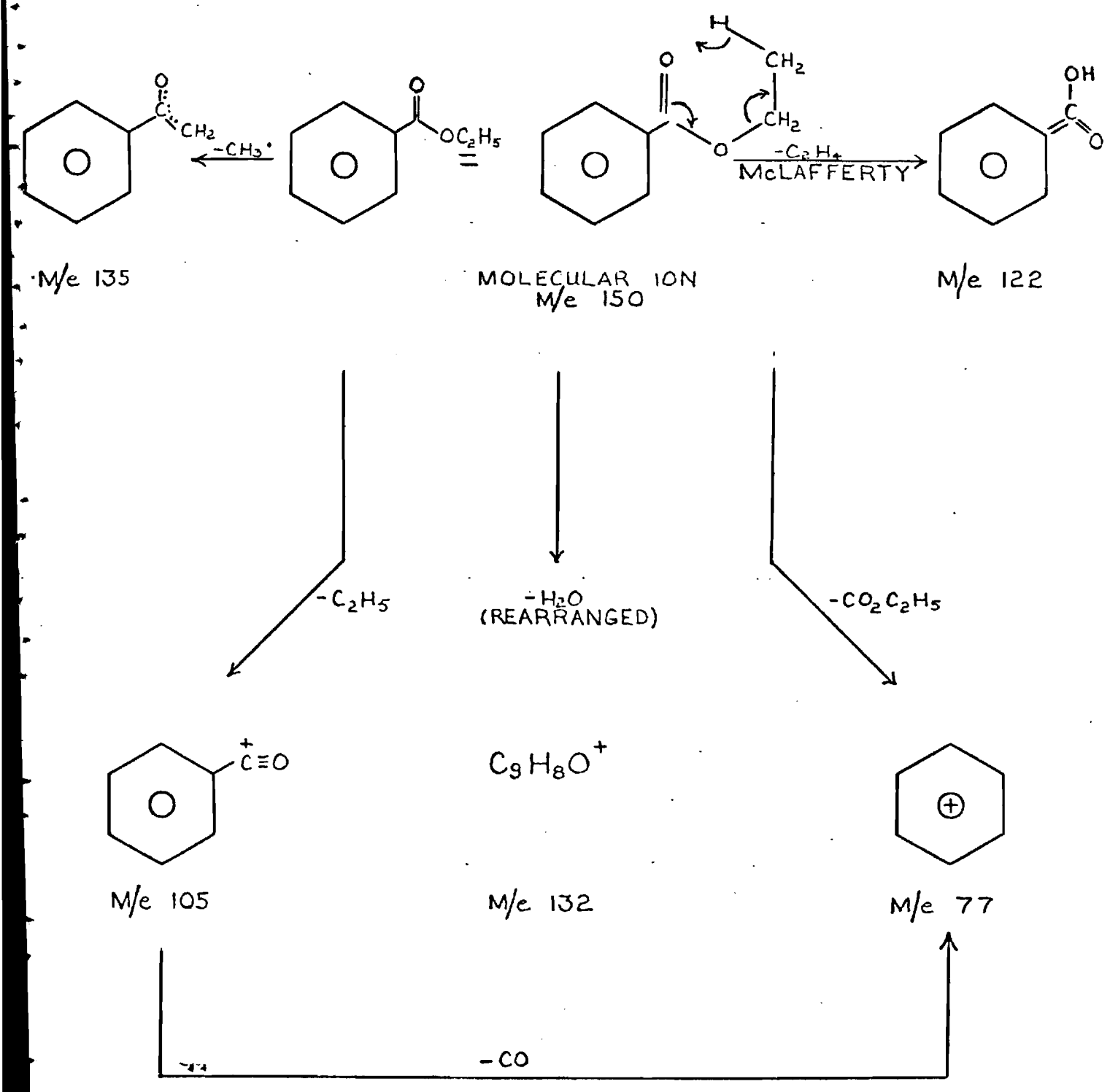
PURITY 958

FIT 992

RFIT 961

Figure 1

FRAGMENTATION SEQUENCE - ETHYL BENZOATE

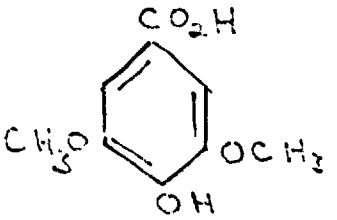


products indicates that the use of a larger excess of diazoethane and a longer reaction time may be helpful. Once again, the fit indices are very high (See Figure 2). The tendency to form the acylium ion at m/e 105 is so strong that even the usually unimportant loss of a hydroxyl radical from the molecular ion proceeds to such an extent that m/e 105 is still the base peak. The fragmentation sequence is straightforward and will not be described.

The reaction between phenol and diazoethane likewise did not proceed to completion under the conditions employed. This is evidenced by the presence of unreacted phenol in the reaction mixture (See Figure 3). Once again the fit indices are quite high. It is of interest to note the loss of CO from the molecular ion to provide a strong peak at m/e 66. This loss is typical of unprotected phenols. The desired ethyl-phenyl ether was also strongly present in the product mixture. In this case, a comparison spectrum is apparently not present in the NB library. The interpretation of the spectrum, which is shown in Figure 4 is straightforward however, and we are therefore quite confident regarding the accuracy of our assignments. The fragmentation sequence is outlined on the page following Figure 4. In this case, the McLafferty rearrangement (upper right) involves the aromatic ring because no carbonyl group is present.

The most significant experiment in this series was concerned with the reaction between syringic acid and diazoethane.

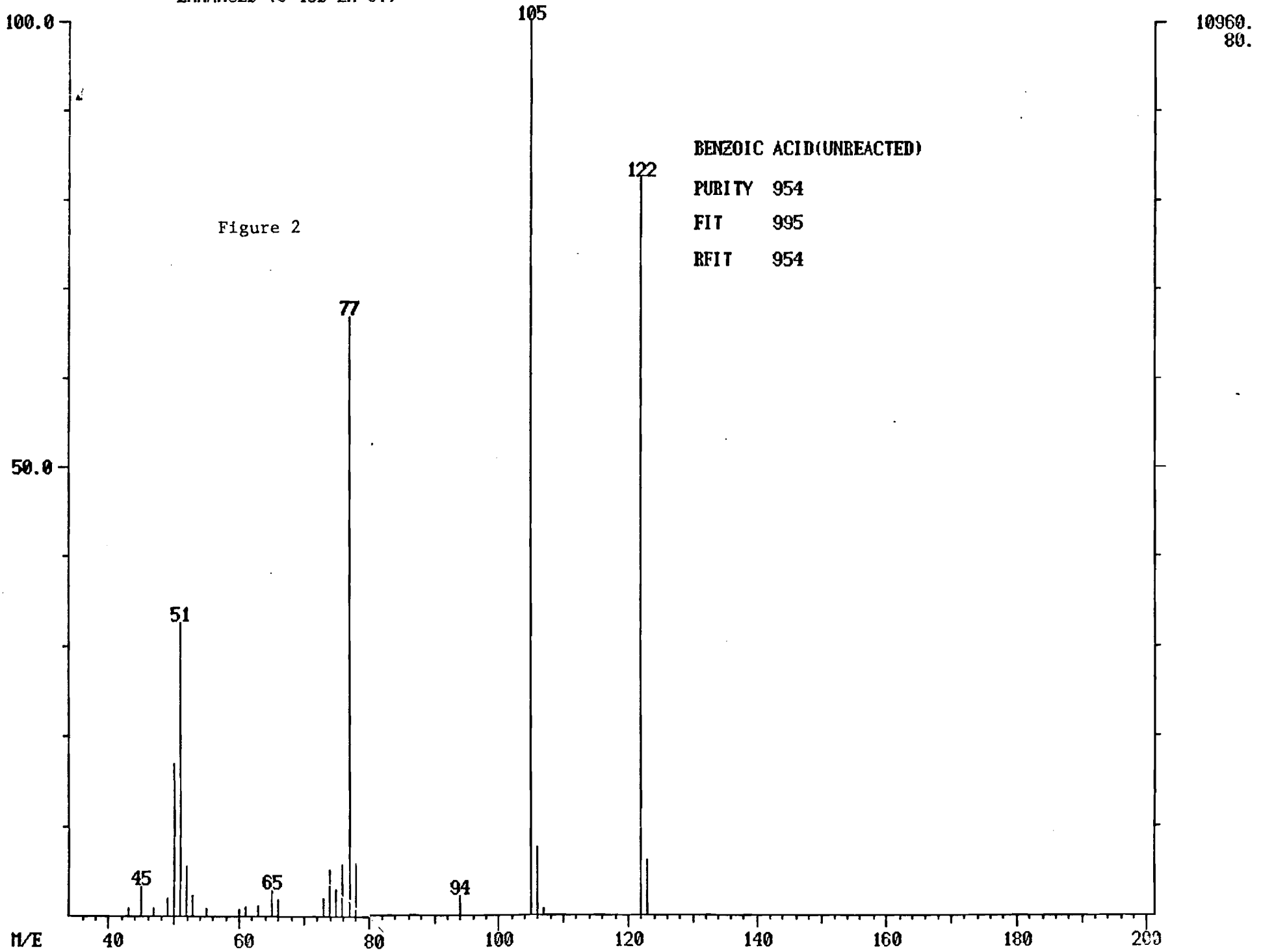
This substrate as shown below possesses two sites for reaction, the readily accessible carboxyl group and the moderately hindered hydroxyl group.



MASS SPECTRUM
02/07/79 15:41:00 + 4:55
SAMPLE: ETHYL BENZOATE MADE BY CATHY
ENHANCED (S 15B 2N 0T)

DATA: ETBENZOATE #236
CALI: Z020779 #5

BASE M/E: 105
BIC: 40512.



BENZOIC ACID(UNREACTED)

PURITY 954

FIT 995

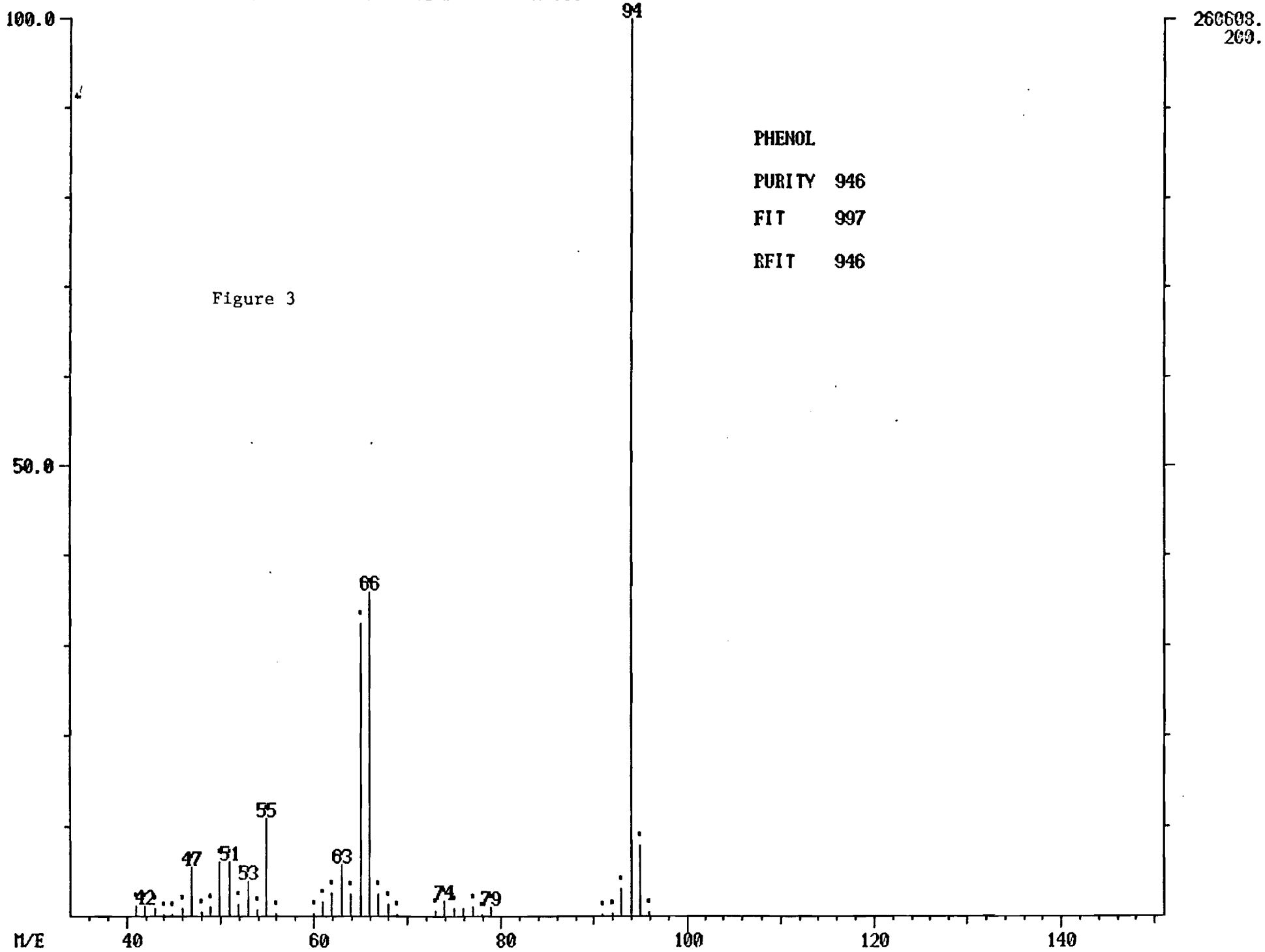
RFIT 954

Figure 2

MASS SPECTRUM
02/07/79 16:05:00 + 4:17
SAMPLE: PHENOL PLUS DIAZOETHANE
#206 TO #207 SUMMED - #203 - #219 X1.00

DATA: ETPHENOL #206
CALI: Z020779 #5

BASE M/E: 94
RIC: 631808.



PHENOL

PURITY 946

FIT 997

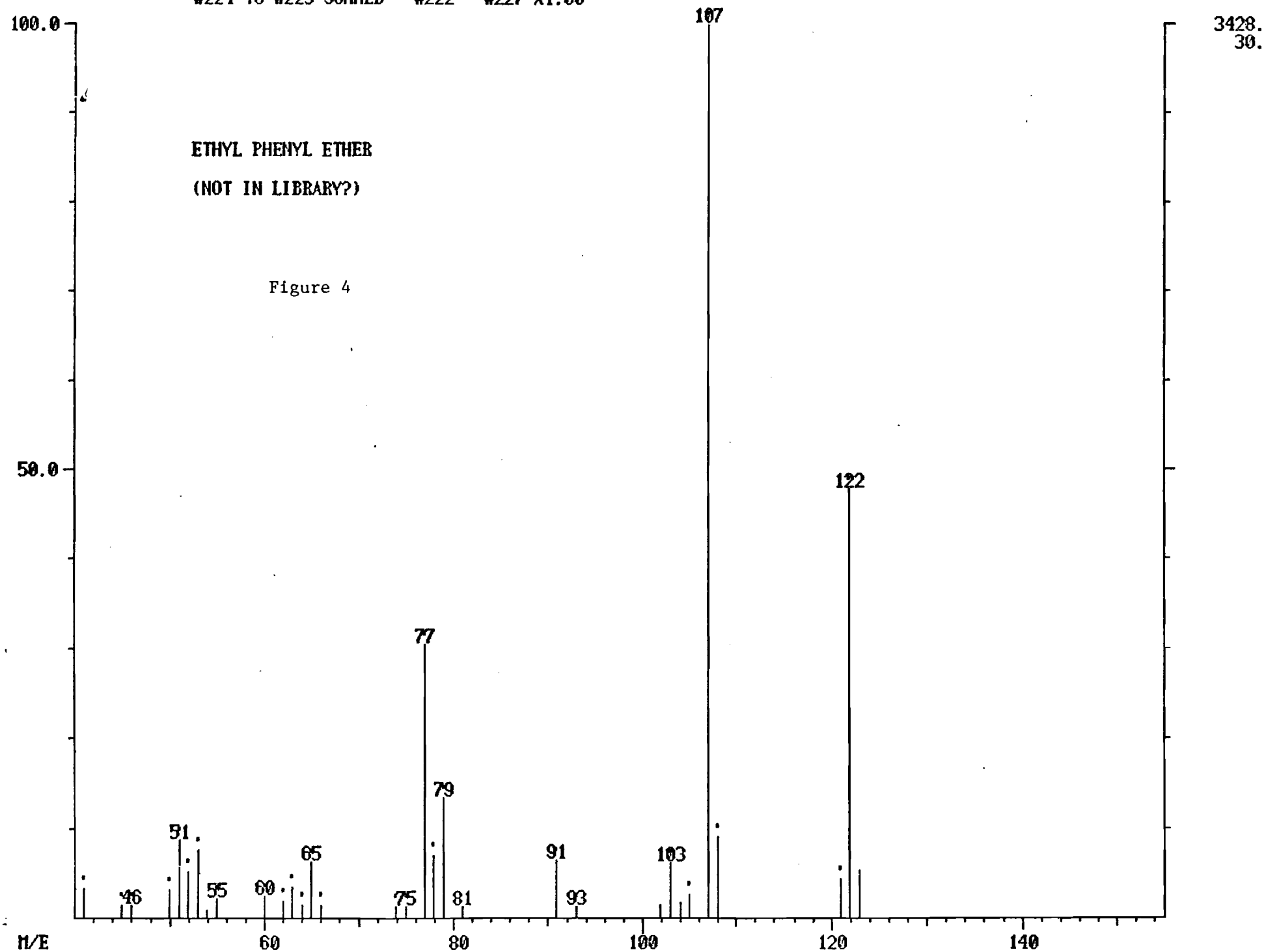
RFIT 946

Figure 3

MASS SPECTRUM
02/07/79 16:05:00 + 4:40
SAMPLE: PHENOL PLUS DIAZOETHANE
#224 TO #225 SUMMED - #222 - #227 X1.00

DATA: ETPHENOL #224
CALI: Z020779 #5

BASE M/E: 107
RIC: 10320.

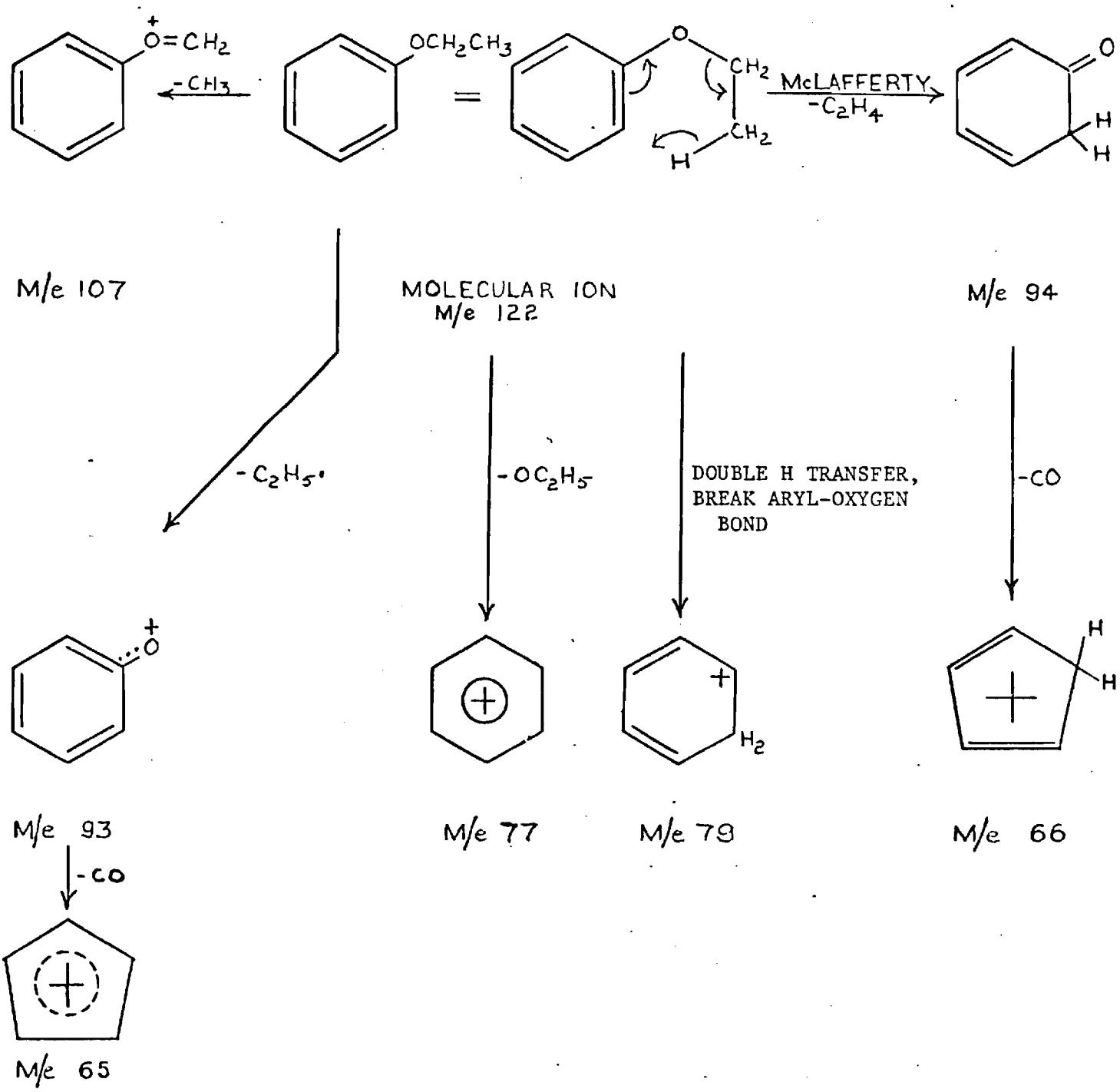


ETHYL PHENYL ETHER

(NOT IN LIBRARY?)

Figure 4

FRAGMENTATION SEQUENCE - ETHYL PHENYL ETHER



Production of the diethyl-derivative, even in modest yield, could be regarded as evidence that diazoethane is an effective reagent for the conversion of both phenols and acids to their more volatile ethyl derivatives. The total ion chromatogram which is presented in Figure 5 is encouraging in that three peaks are present - possibly the acid, the monoethyl derivative and the diethyl derivative. Application of the ion mapping technique described in earlier reports (see Figure 6) revealed that the first peak to be comprised of two components, the first and most important of which has been tentatively assigned a 3,4,5-trimethoxybenzoate structure on the basis of the high mass portion of the mass spectrum which is shown in Figure 7. A great deal of interpretive effort has not been placed on verifying the structure via a rigorous interpretation of the fragmentation sequence which is outlined on the page which follows Figure 7.

The curious ion clusters spaced 16 Amu's apart are also seen in the mono-and diethyl syringates and are most likely due to loss of CO_2 and CO or C_2H_4 in various combinations. Thus, this feature is generally supportive of the structural assignment. The tailing compound has not been assigned a structure.

The second chromatographic peak has been assigned the desired fully ethylated syringic acid structure on the basis of an interpretation of its electron impact spectrum which is presented in Figure 8. The polyfunctional nature of the structure makes the fragmentation sequence difficult to follow beyond the loss of two or three fragments.

The large number of branching points offers several possible origins for each of the lower ions. The sequence outlined on the page immediately after Figure 8 is therefore only one of several equally probable arrangements

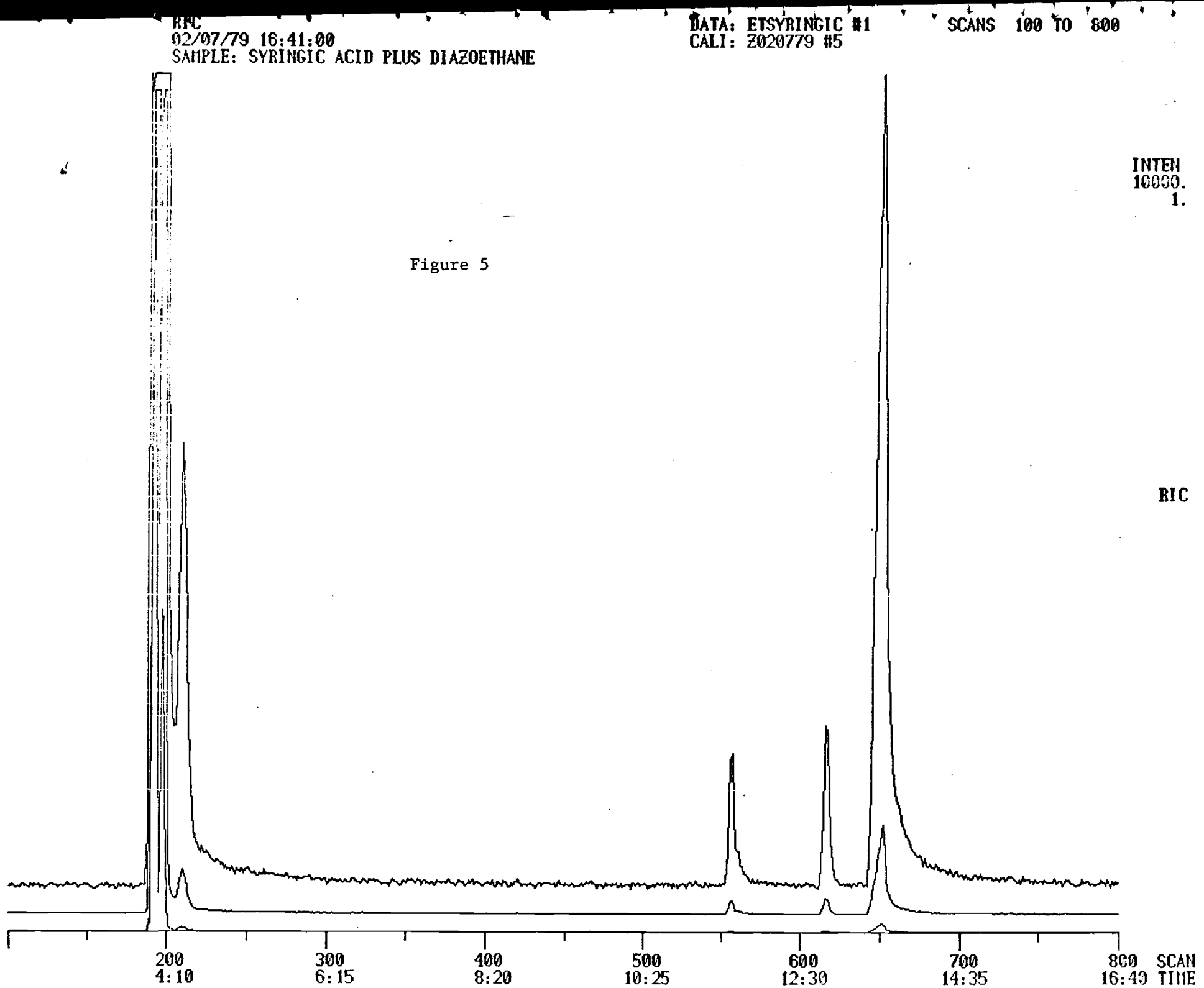

RFC
02/07/79 16:41:00
SAMPLE: SYRINGIC ACID PLUS DIAZOETHANE
DATA: ETSYRINGIC #1
CALI: Z020779 #5
SCANS 100 TO 800
INTEN
10000.
1.
RIC
200
4:10
300
6:15
400
8:20
500
10:25
600
12:30
700
14:35
800
16:40
SCAN
TIME

Figure 5

RIC + CHROMATOGRAM MAP
02/07/79 16:41:00
SAMPLE: SYRINGIC ACID PLUS DIAZOETHANE

DATA: ETSYRINGIC #1
CALI: Z020779 #5

SCANS 550 TO 570
MASS 150 TO 260

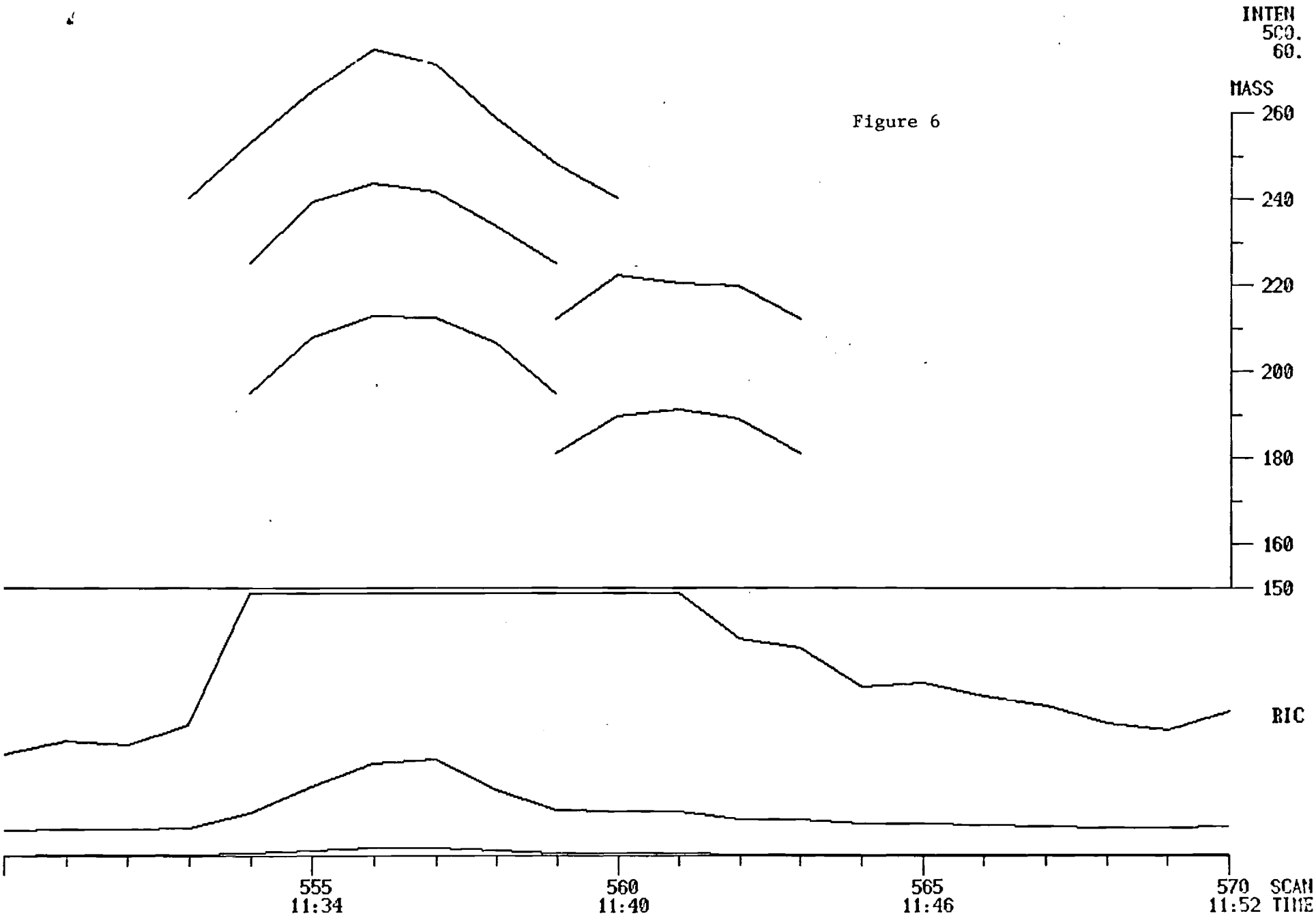
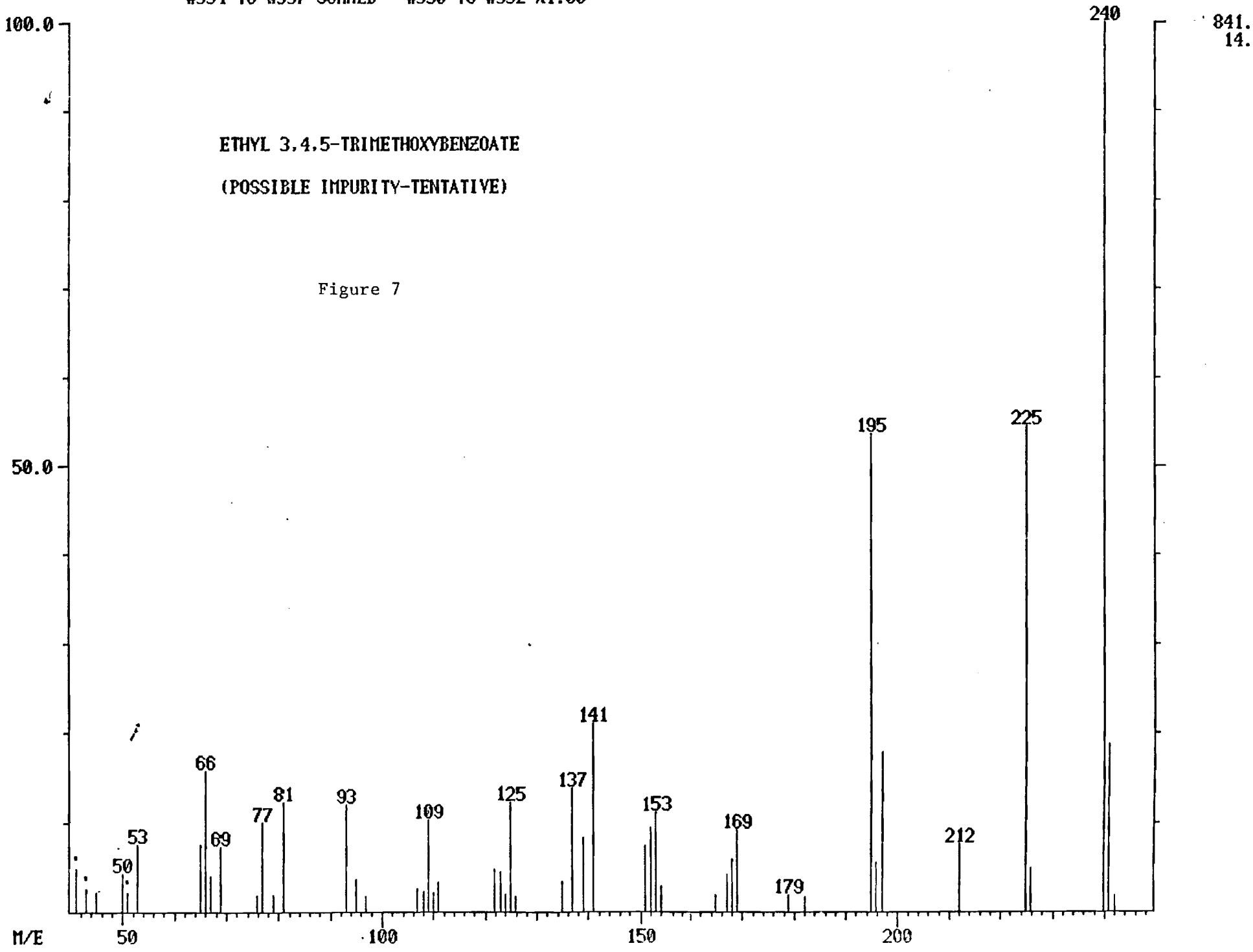


Figure 6

MASS SPECTRUM
02/07/79 16:41:00 + 11:34
SAMPLE: SYRINGIC ACID PLUS DIAZOETHANE
#554 TO #557 SUMMED - #550 TO #552 X1.00

DATA: ETSYRINGIC #555
CALI: Z020779 #5

BASE M/E: 240
RIC: 4504.

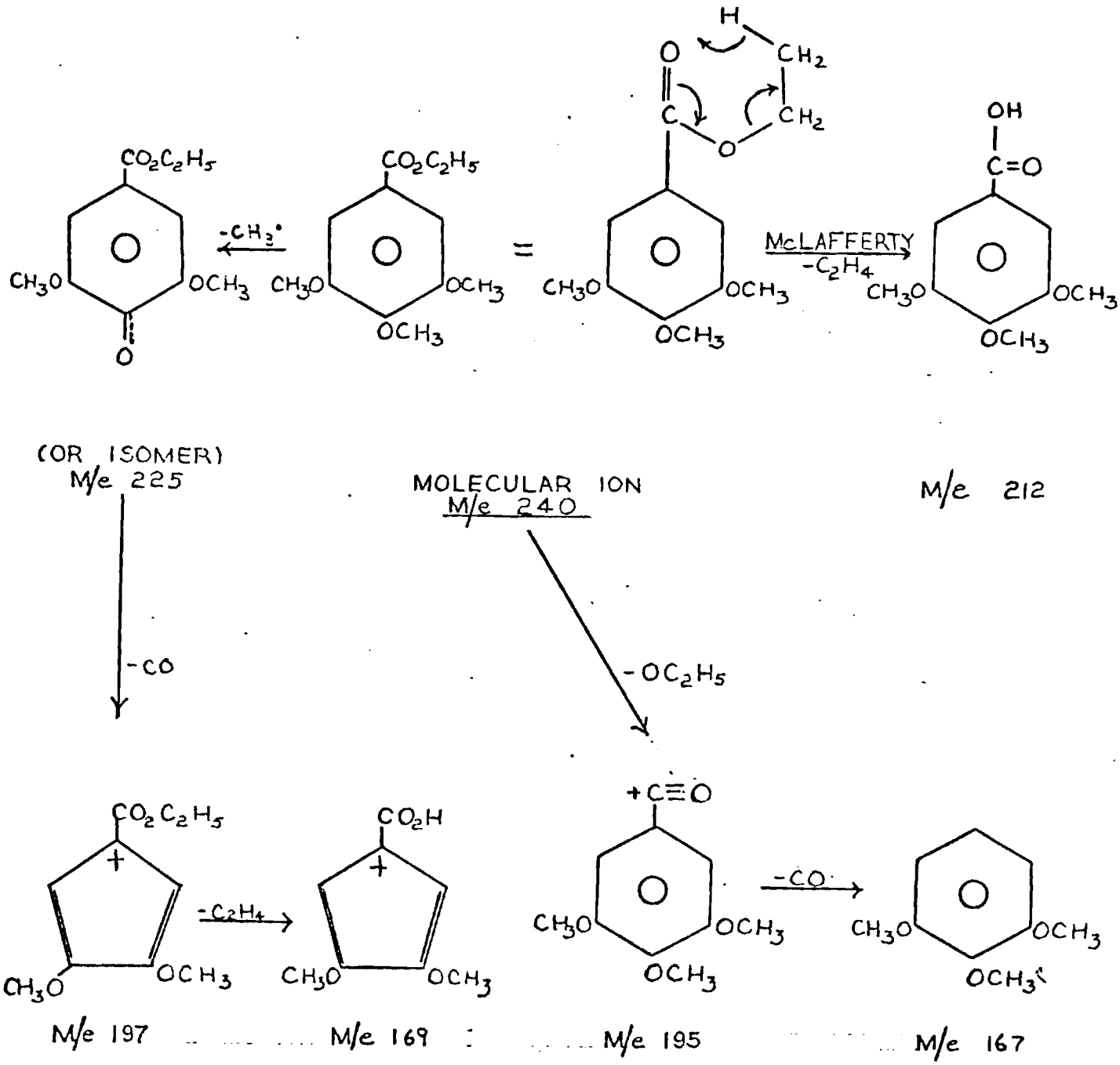


ETHYL 3,4,5-TRIMETHOXYBENZOATE

(POSSIBLE IMPURITY-TENTATIVE)

Figure 7

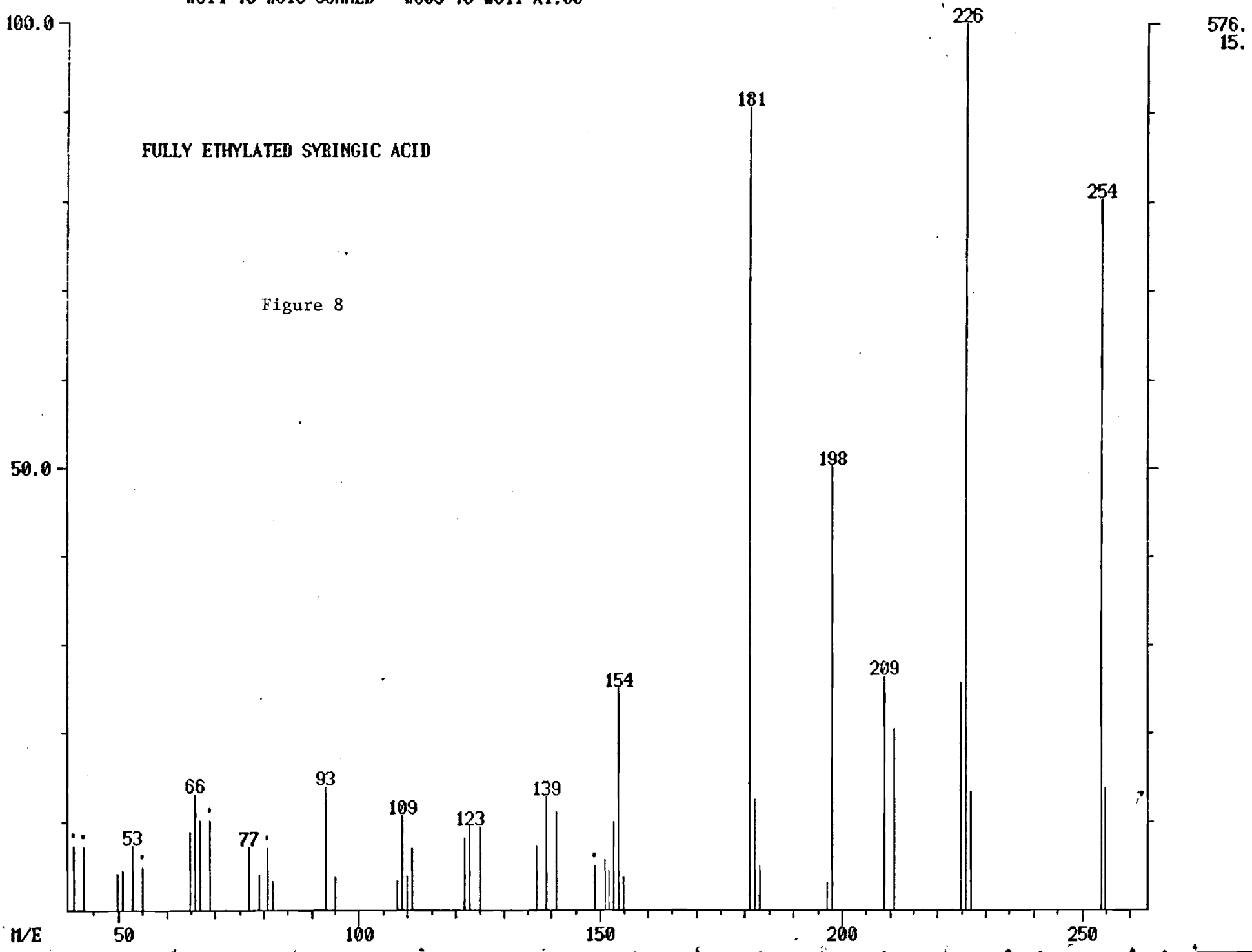
FRAGMENTATION SEQUENCE; 3,4,5-TRIMETHOXY BENZOATE



MASS SPECTRUM
02/07/79 16:41:00 + 12:49
SAMPLE: SYRINGIC ACID PLUS DIAZOETHANE
#614 TO #616 SUMMED - #609 TO #611 X1.00

DATA: ETSYRINGIC #615
CALI: Z020779 #5

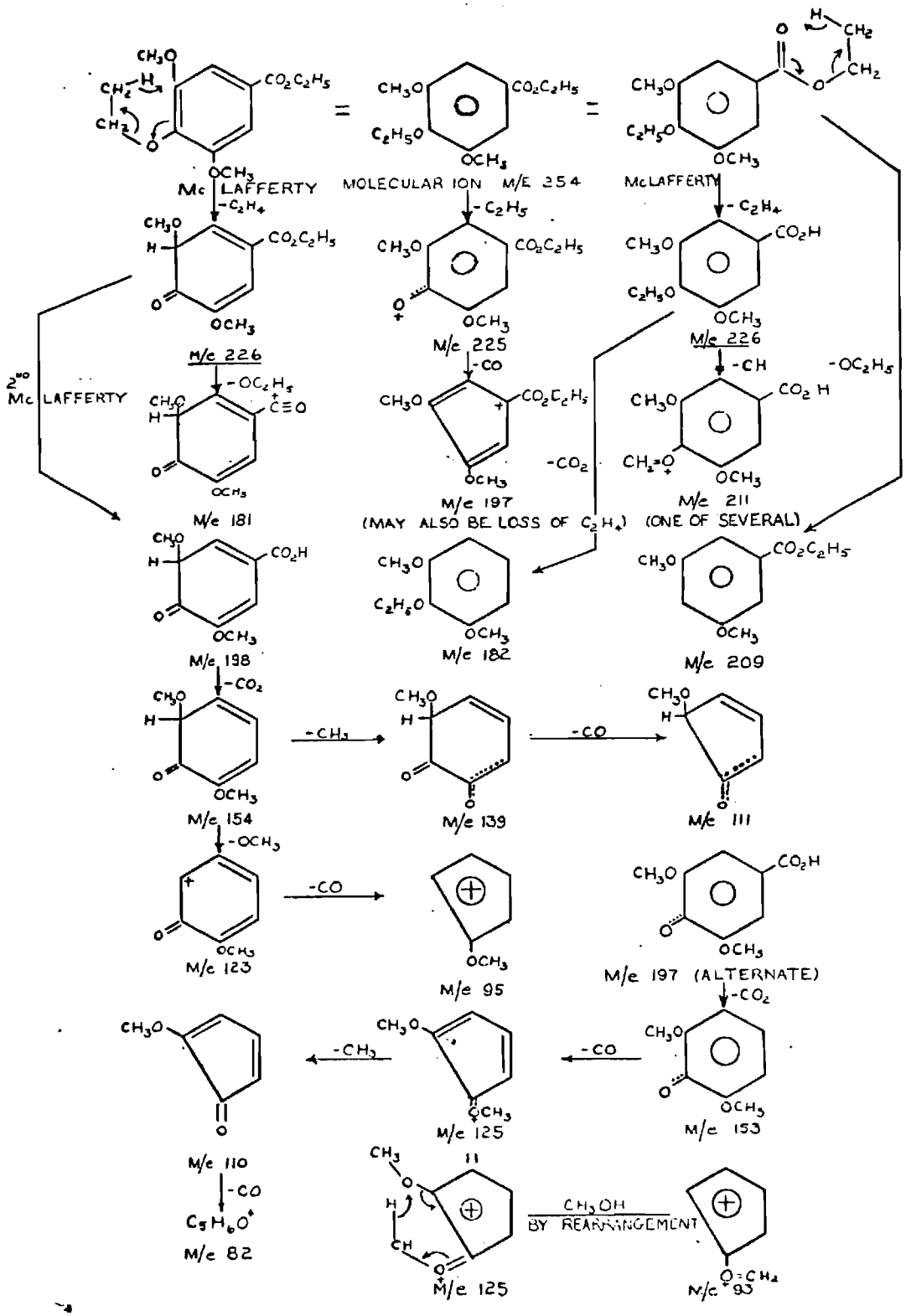
BASE M/E: 226
BIC: 4296.



FULLY ETHYLATED SYRINGIC ACID

Figure 8

FRAGMENTATION SEQUENCE - DIETHYL SYRINGATE



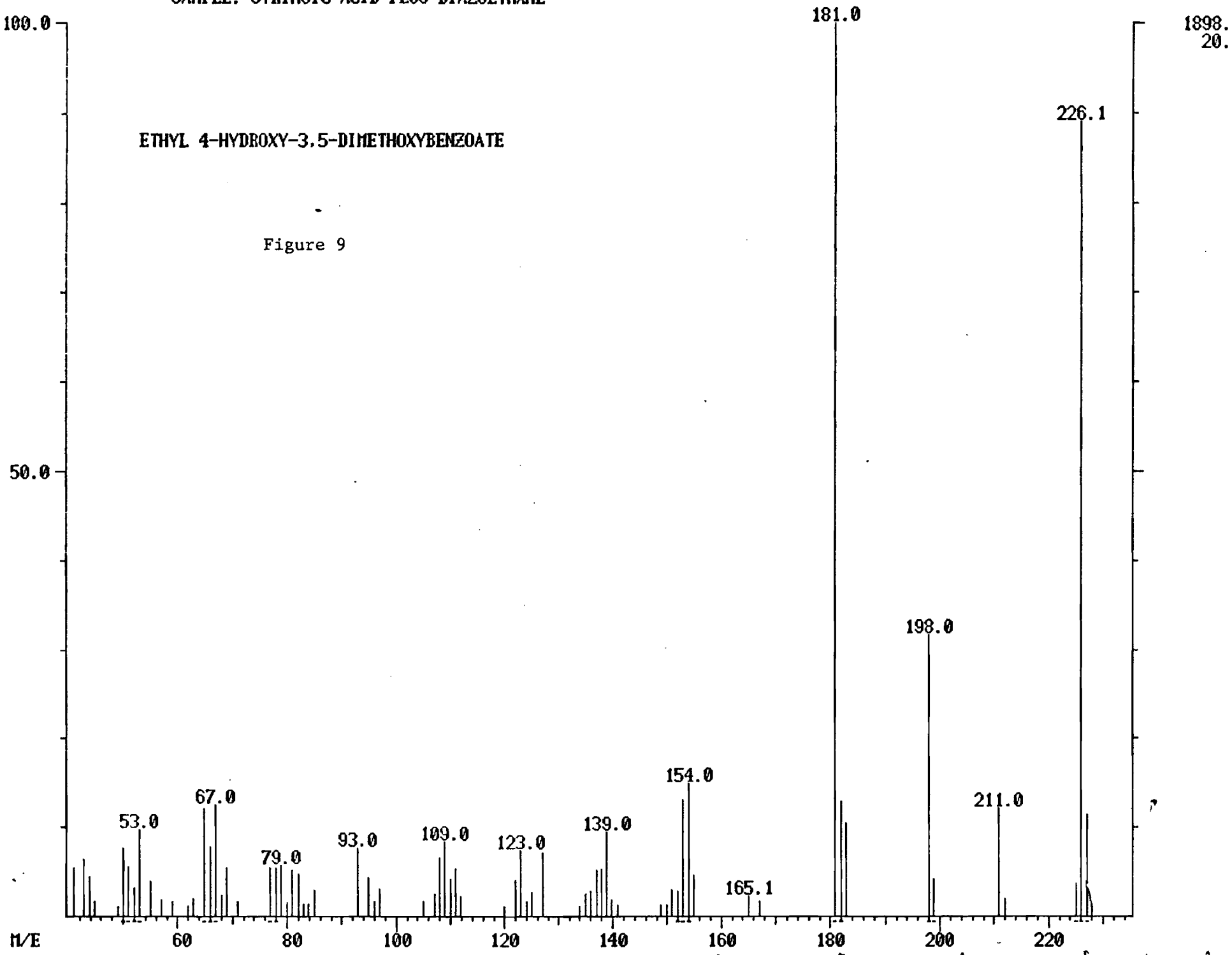
which could be drawn up in support of the structural assignment. Finding the hindered phenolic function in the form of its ethyl ether following treatment with diazoethane attests to the basic correctness of our approach. It is apparent however that the employment of more rigorous reaction conditions would be useful.

The major product derived from the reaction between syringic acid and diazoethane has been identified as ethyl 4-hydroxy-3,5-dimethoxybenzoate on the basis of its electron impact spectrum which is shown in Figure 9. It is not surprising that the fragmentation sequence which is outlined on the page following the figure is very nearly identical to that of the fully ethylated derivative to which it is closely related. The only peak missing is the m/e 209 peak which therefore must arise from the fully ethylated derivative by loss of the central ethoxy group from the molecular ion. The analagous experiments in which diazomethane has been substituted for diazoethane have been performed and await analysis.

MASS SPECTRUM
02/07/79 16:41:00 + 13:35
SAMPLE: SYRINGIC ACID PLUS DIAZOETHANE

DATA: ETSYRINGIC #652
CALI: Z020779 #5

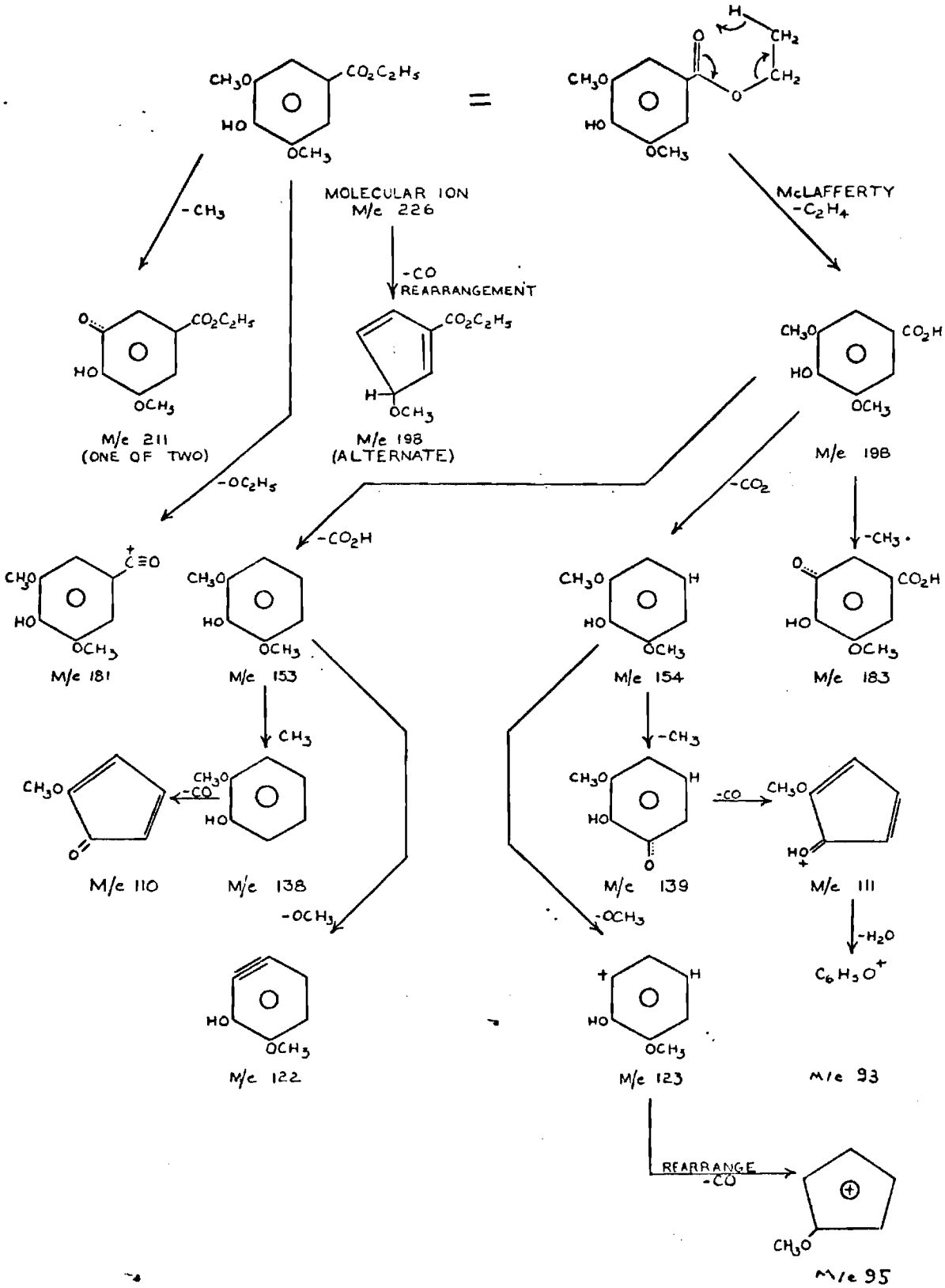
BASE M/E: 181
RIC: 10912.



ETHYL 4-HYDROXY-3,5-DIMETHOXYBENZOATE

Figure 9

FRAGMENTATION SEQUENCE - MONOETHYL SYRINGATE



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Monthly Progress Report for March 1979

E-20-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Monthly Progress Report

April 21, 1979

by

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The purpose of the work described in this report and the overall objective of the research project is to identify major and minor classes of natural organic substances found in surface waters such as might be used as a source of potable water. A second major aim of the study is to evaluate the effect of a number of water treatment processes such as chlorine, ozone and chlorine dioxide on the transformations which these naturally occurring materials may undergo during the disinfection process.

A source water which is rich in the required organic materials but which is unusually low in such interfering materials as agricultural runoff, municipal wastewater effluents and industrial discharges is being used to provide a generous reserve of aquatic humic materials which are the dominant class (80%) of all natural organic substances found in drinking water sources.

During the reporting period, a relocation of our facilities within the Department of Civil Engineering was accomplished. Four reactions were run on a concentrated scale with two samples of aquatic humic material using chlorine dioxide as the disinfectant and featuring both normal addition and inverse addition. The product mixtures were fractionated into base/neutrals, acids and polar compounds by serial liquid-liquid extraction. The concentrated extracts were divided with one portion being reserved for mutagenicity testing. The other portions were derivatized, if appropriate, for GC/MS. The GC/MS data has been acquired for all of the M/30 fractions and affordsa link with our earlier chlorination studies. The data for the normal addition fractions are fully presented in this report.

A similar experiment using photochemically generated ozone as the disinfectant has also been conducted. The GC/MS data for all of the fractions have been acquired. The data for the base/neutral fraction is fully discussed in this report.

The data from our most recent crown ether oxidation of aquatic humics has been acquired and partially processed during the reporting period but will be presented in a later report.

A paper entitled "Reaction of Aquatic Humic Material with Chlorine-Isolation of Some New Chlorinated Organics" by S. C. Havlicek, J. H. Reuter, J. W. Ralls, I. El Barbary, L. W. Strattan, J. A. Cotruvo and C. L. Trichilo was presented before the Division of Environmental Chemistry at the recent ACS/CSJ meeting. A summary is included in this report.

I. PERSONNEL AND EQUIPMENT

The mass spectrometer, the mini-pilot facility, and other supporting equipment used in connection with this project have all been relocated in the Daniel Laboratory, Department of Civil Engineering. While the moving has taken considerably longer than expected, the actual move was preceeded by a period of double-time effort in the laboratory and on the mass spectrometer so that our overall progress has not been significantly delayed.

A malfunction of the air conditioning system did cause a minor delay in restarting the GC/MS system so that it has not been possible to completely process all of the data acquired before the move. This problem has been corrected and we are once again operating normally. In order to compensate for any delays caused by moving, we plan to continue with some experimental work in April and May and put the final report together after the expiration date of the project.

We have begun to shift personnel to other projects in anticipation of a possible gap in funding while our application for renewal is being considered. As long as this gap is small, a complete loss of momentum will not occur.

II REACTION OF AQUATIC HUMICS WITH CHLORINE DIOXIDE

It has been reported in the literature (J. M. Symons, EPA publication "Ozone, Chlorine Dioxide and Chloramines as Alternatives to Chlorine for Disinfection of Drinking Water") that the ratio of chlorine dioxide to substrate can influence the degree of incorporation of chlorine into the product. It is also unclear to what extent the pH may influence the

uptake of chlorine. Early work by Sarkamen et al., TAPPI, 45, 24 (1962) reports uptake at strongly acid pH's such as would encourage the full utilization of the oxidizing power of chlorine dioxide and which would presumably involve chlorine in the plus one (hypochlorite) or zero oxidation states (Cl_2) - either of which could account for the observed production of chloroorganics. At pH's in the neutral range, the incorporation of chlorine was still reported even though the full oxidation capacity of chlorine dioxide or chlorite is supposedly not available. Obviously, more work will be required to fully resolve this question. It will be recalled, however, that the use of pH's far beyond neutrality was not a part of the original scope of this work. Therefore we have no plans at present to study chlorine dioxide at strongly acid pH's.

However, we have designed our experiments to complement the existing work regarding concentration effects by conducting paired studies. In one case, the chlorine dioxide was added to the humic material while in the other, the aquatic humic material was added to the chlorine dioxide. Thus, in one case the substrate was in large excess for most of the reaction period while in the other, the chlorine dioxide was in large excess for most of the reaction period. In both cases, the total amounts of all reactants employed were identical.

Experiments were carried out with two batches of aquatic humic material (M/39 and M/30) so that a tie-in with our earlier chlorination work could be established (M/30). The M/39 experiments provide a link with the mini-pilot studies in which it will be recalled very little volatile or semivolatile material has been observed. Thus these experiments also serve as a check on these studies in that much more material is available to be carried through the isolation and workup.

We will therefore be able to make statements comparing the relative volatility (and indirectly the molecular weights and polarities) of the products derived from the treatment of aquatic humic material with chlorine and with chlorine dioxides.

The M/39 experiments were run first due to the larger amount of this material which was available. However, once the conditions of reaction had been established, the product mixtures were given a lower analytical priority than the M/30 humics which afford a more direct link with earlier work. Accordingly, the experimental details and results derived from the M/30 samples will be presented first. Due to the time lost in moving coupled with a breakdown of the air conditioning equipment in our new facility, we are unable to present all of the results in the same report.

A solution of aquatic humic material (M/30, 100 mg in 10 ml high purity water) was mixed at ambient temperature with 20 ml of a chlorine dioxide solution containing 0.175 mg/ml ClO_2. A starch-iodide test was performed after 2 hours which indicated that all of the oxidant had been consumed. At this time another 20 ml of the chlorine dioxide solution was added to be followed at intervals of 1 hour by three separate additions of 10 ml each. After a total of seven hours the reaction was quenched with 5 ml of saturated sodium sulfite and brought to pH 10.

The resulting alkaline product mixture was extracted with pesticide grade pentane (3 x 70 ml). The combined extracts were dried over roasted sodium sulfate and concentrated at 60°C in a Kuderna-Danish apparatus. The concentrate was divided into two parts one for gc/ms analysis and the second for mutagenicity testing.

The aquous solution remaining after extraction was brought to pH 2 and extracted with pentane (3 x 70 ml). The combined extracts were dried, concentrated and divided as before. In this case, the portion destined for gc/ms analysis was treated with diazomethane prior to GC/MS analysis.

In the second experiment of this pair, a solution of aquatic humic material (M/30, 100 mg in 10 ml high purity water was added to 70 ml of a chlorine dioxide solution (0.175 mg/ml) over the course of two hours. The reaction mixture was then allowed to stand an additional five hours before being quenched with excess sodium sulfite. The product mixture was worked up, concentrated and subdivided as previously described. In this case, the aqueous solution remaining after the pH 2 pentane extraction was subjected to a second extraction with ethyl acetate, dried, concentrated and divided. The sample destined for GC/MS analysis was treated with diazoethane in pentane to avoid confusion created by possible transesterification. Reagent blanks accompanied both reactions. The pair of experiments run with the M/39 humic material were carried out under similar conditions.

The reagent blank for both the normal and the inverse additions exhibited a number of chromatographic peaks in the solvent region as can be seen in Figure 1. An examination of the mass spectral data provided the information summarized in Table I.

The high quality of the fit data are probably the result of our increased emphasis on instrument performance as described in earlier reports. The fact that all of the major chromatographic peaks with the possible exception of benzene and the late eluter are solvent components confirms that our reagents and equipment are contamination-free. Since the mass spectral data itself represents only solvent components, it will

RIC
04/06/79 21:15:00
SAMPLE: HUMICS PLUS CLO2 UNTREATED CONTROL

DATA: M30CLO2BLKS #1
ENHANCED (S 22B 2N 0T)

SCANS 50 TO 4000

Figure 1. Experiment 4 - Substrate (M/30) in Excess
Reagent Blank

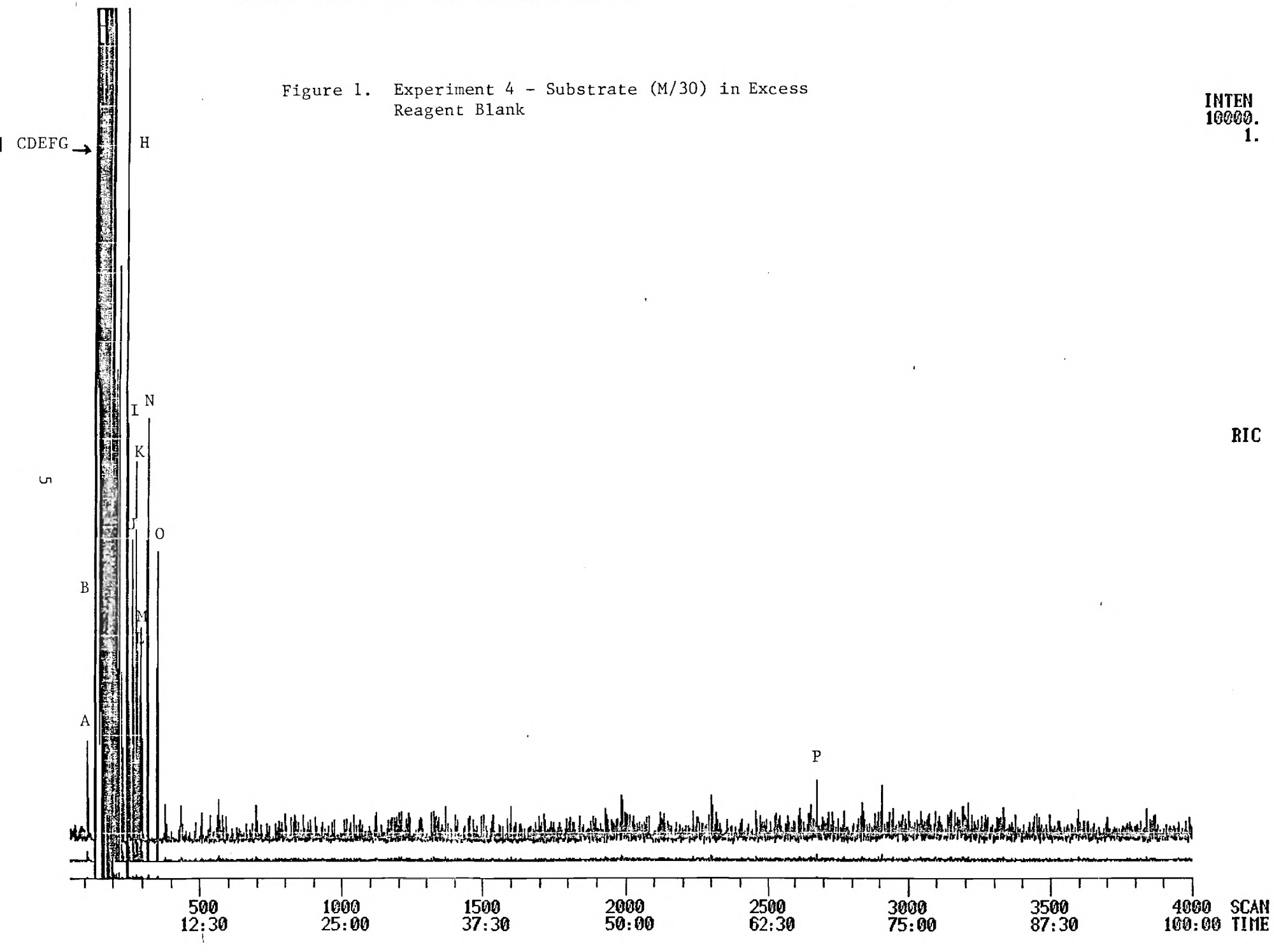


Table I
Identification of Chromatographic Peaks
Reagent Blank - Humics Plus Chlorine Dioxide

Component		Purity	FIT	RFIT	Comment
A	Carbon Dioxide	NA	NA	NA	Seen in all runs
B	2-Methylbutane	991	995	991	Solvent component
C	Pentane	976	982	976	Solvent
D	Cyclopentene	911	933	923	Minor solvent component
E	Unassigned	NA	NA	NA	Solvent component
F	3-Methylpentane	899	908	899	Solvent component
G	Hexane	987	995	987	Solvent component
H	Benzene	992	998	992	Solvent component
I	Dimethylpentane isomer	852	894	934	Solvent component
J	3-Methylhexane	947	976	953	Solvent component
K	1,3-Dimethylcyclopentane isomer	816	907	879	Solvent component
L	1,3-Dimethylcyclopentane isomer	809	870	909	Solvent component
M	Trans-1,2-Dimethylcyclopentane	834	915	872	Solvent component
N	Heptane	911	976	924	Solvent component
O	Methylcyclohexane	914	979	924	Solvent component
P	Unassigned late eluter	NA	NA	NA	Contaminant?

not be presented or further described in this report. The spectrum of the unassigned late eluter is presented in Figure 2. The dominance of higher mass ions suggests a polycyclic structure. If m/e 346 is the molecular ion, loss of a methyl to provide m/e 331 is reasonable. The loss of m/e 93 from m/e 346 or m/e 78 from m/e 331 is more difficult to explain. At present, we are unable to suggest a structure.

The pH 10 extract from the experiment in which the chlorine dioxide was added to the M/30 aquatic humics was the next to be examined. This fraction would be expected to contain all of the peaks found in the reagent blank (unless compound P is acidic) plus any base/neutral compounds which might have been generated during the disinfection. The total ion chromato gram which is presented in Figure 3 indicates that very little material has been extracted in this fraction. A summary of the results obtained by examining the spectra derived from each of the chromatographic peaks is presented in Table II.

Since this extract had been more highly concentrated than the others examined thus far, it is not surprising that additional solvent components have been identified. The spectrum of compound E was highly merged as indicated by the ion mapping technique. This prevented the clear identification of any of the merged components. The data system did suggest 3-methyl-2-butene-1-ol as a first choice, but the low fit indices and position in the chromatogram do not justify making an assignment at this time.

Since this product mixture is derived from the M/30 samples, it is not possible to rule out contamination as the source of the chloroform (compound G-1-H). Benzene and toluene were also found in this concentrate but, again, contamination cannot be ruled out as a source of these components.

MASS SPECTRUM
04/09/79 16:58:00 + 67:06
SAMPLE: HUMICS PLUS CHLORINE DIOXIDE EX4 PH10 EXTRACT
#2683 TO #2685 SUMMED - #2681 - #2686 X1.50

DATA: M30CLO2PH10 #2684
CALI: Z040979 #15

BASE M/E: 253
RIC: 3308.

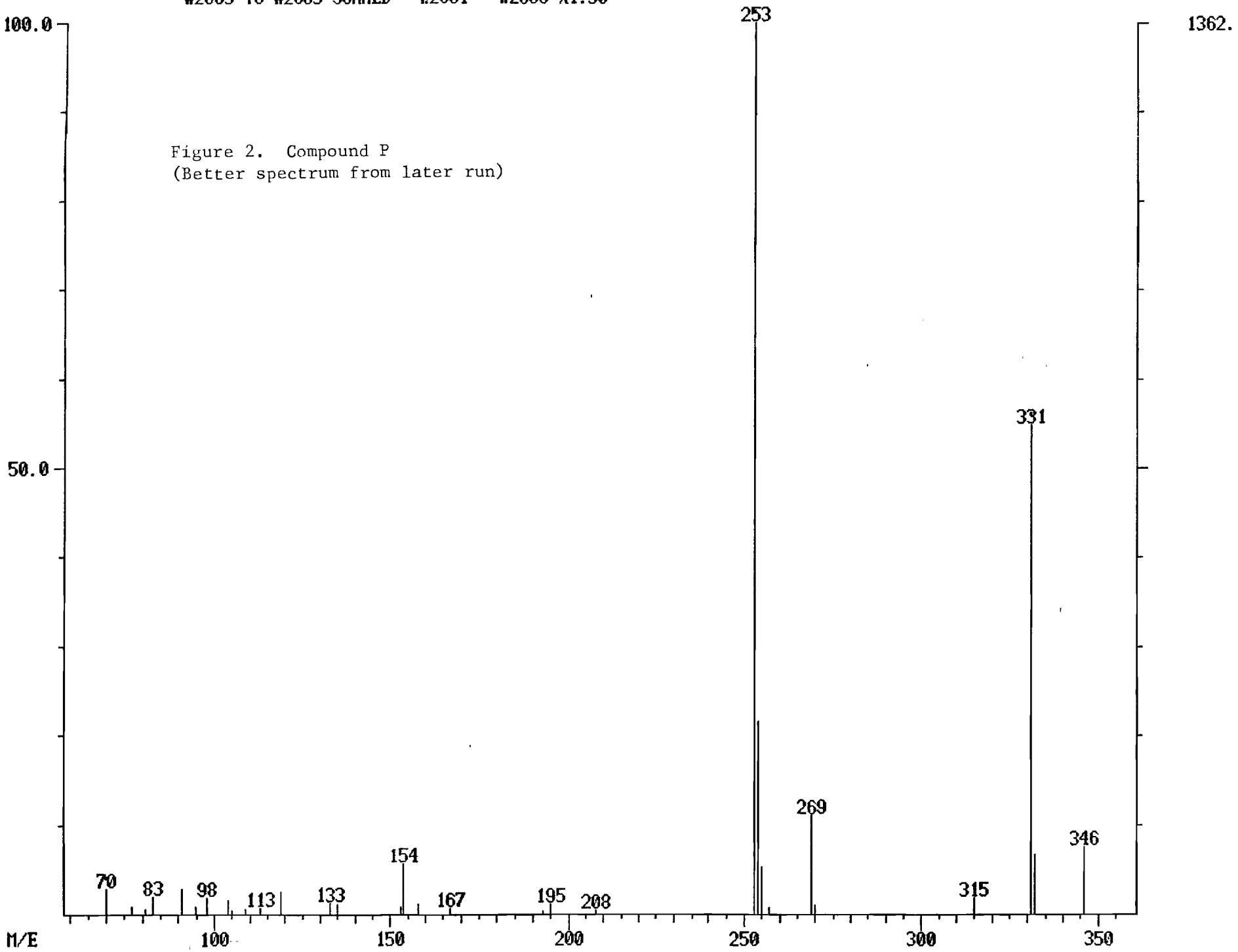


Figure 2. Compound P
(Better spectrum from later run)

RIC
04/09/79 16:58:00
SAMPLE: HUMICS PLUS CHLORINE DIOXIDE EX4 PH10 EXTRACT

DATA: M30CLO2PH10 #1
CALI: Z040979 #15

SCANS 100 TO 3000

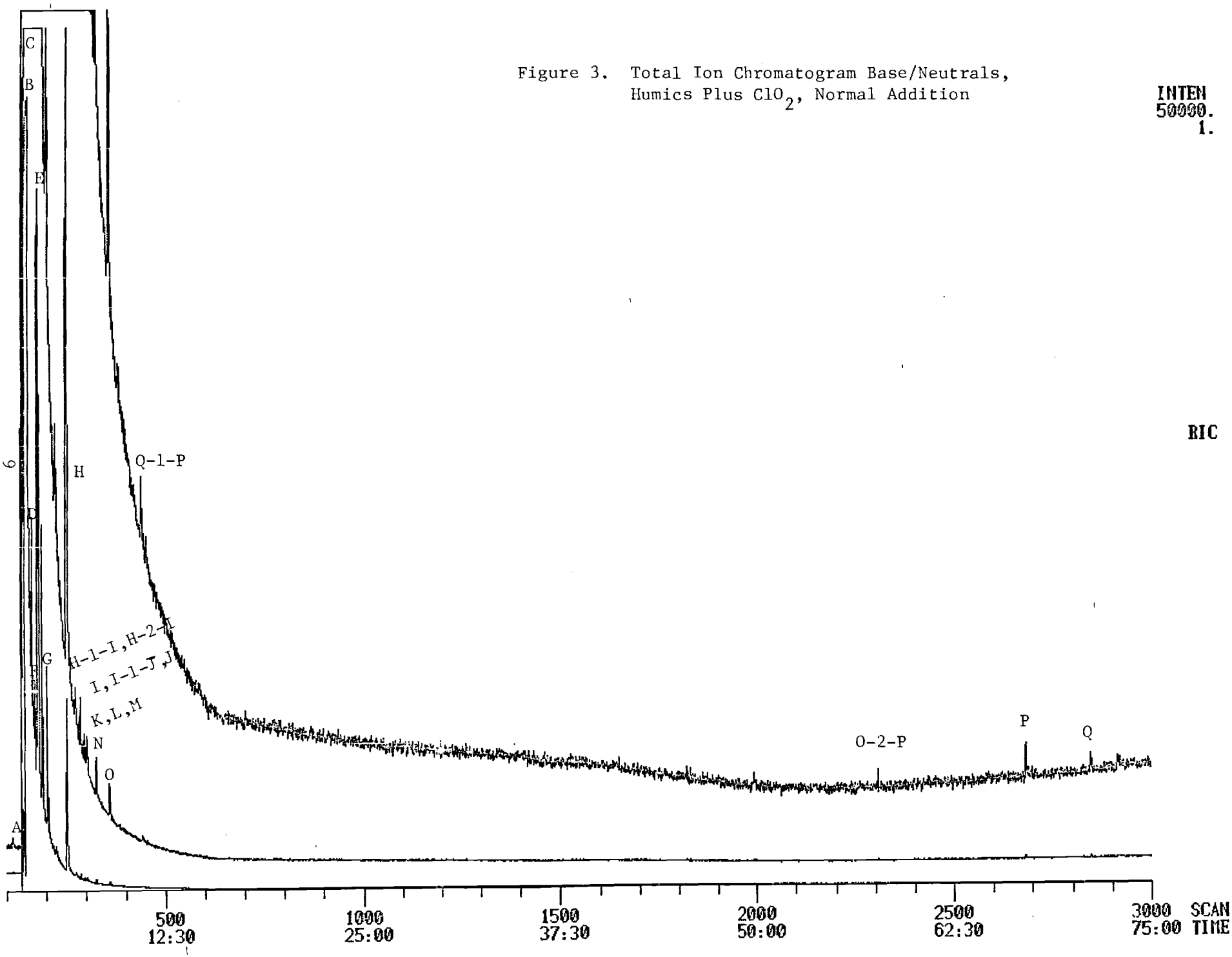


Figure 3. Total Ion Chromatogram Base/Neutrals, Humics Plus ClO_2, Normal Addition

Table II
Identification of Chromatographic Peaks - Base/Neutrals,
Humics Plus Chlorine Dioxide, Normal Addition

Component	Purity	FIT	RFIT	Comment
A Carbon Dioxide	NA	NA	NA	Present in most runs
B 2-Methylbutane	988	992	988	Solvent component
C Pentane	NA	NA	NA	Saturated spectra, no good fit
D Cyclopentene	899	902	983	Minor solvent component
E Unassigned	NA	NA	NA	Solvent component, 3-methyl-2-butene-1-ol is lo choice, but can't resolve chromatographically
F 3-Methylpentene	825	831	919	Again may be merged with an oxygenated compound
G Hexane	974	982	974	Solvent component
G-1-H Chloroform	958	972	979	Possible artifact
G-2-H Methylcyclopentane	916	929	967	Minor solvent component
H Benzene	993	997	993	Contaminant?
H-1-I Cyclohexane	915	962	933	Minor solvent component
H-2-I 2-Methylhexane	903	936	907	Minor solvent component
I 2,2-Dimethylpentane	873	933	878	Solvent component
I-1-J Unassigned	NA	NA	NA	Probable hydrocarbon
J 3-Methylhexane	945	958	983	Solvent component
K trans-1,3-Dimethylcyclopentane	864	953	864	Solvent component
L cis-1,3-Dimethylcyclopentane	902	925	949	Solvent component
M trans-1,2-Dimethylcyclopentane	930	968	938	Solvent component
N Heptane	963	984	970	Solvent component
O Methylcyclohexane	955	984	965	Solvent component
O-1-P Toluene	865	952	886	Very weak
O-3-P Unassigned	NA	NA	NA	Found in mini-pilot runs
P Unassigned	NA	NA	NA	Present in reagent blank
Q Unassigned	NA	NA	NA	Not found in mini-pilot runs
R Dibutyl Phthalate	796	858	906	Very weak, contaminant

Compound O-2-P has been found in our earlier mini-pilot runs. The spectrum has been presented and discussed in the previous report. Therefore, there is no need to review this item in this report. Compound P is present in the blank and has already been described. Compound Q was not observed in the blank nor in the previous mini-pilot runs. The electon impact spectrum which is presented in Figure 4 shows major ions only at m/e 405 and m/e 327. The gap of m/e 78 between these ions is unusual and may represent a clue. As of this writing, a structure has not been assigned. The weak chromatographic peak corresponding to component R has been identified as dibutylphthalate.

The derivatized concentrates from the pH 2 extracts derived from the same reaction mixture were the next to be examined by GC/MS. The total ion chromatogram is presented in Figure 5. Once again, the majority of the components appear to be solvent-related. The data derived from each of the chromatographic peaks is summarized in Table III.

Two new chromatographic peaks are observed between the carbon dioxide and the 2-methylbutane peaks which had been observed in the reagent blank and in the base/neutral extract. The first of these, a spectrum of which is presented in Figure 6 shows major ions at m/e 60 and m/e 45. A smaller ion at m/e 74 may be the molecular ion in which case it would be necessary to explain the loss of a fragment of 16 amu's. In order to be present only in this fraction, the compound must have some acidic properties. At present we are unable to suggest a structure. The second new compound has been identified as ethanol. Since ethanol was not observed in the reagent blank, it may arise via transesterification or hydrolysis of an ester or hydrolysis of a ketal within the aquatic humic structure.

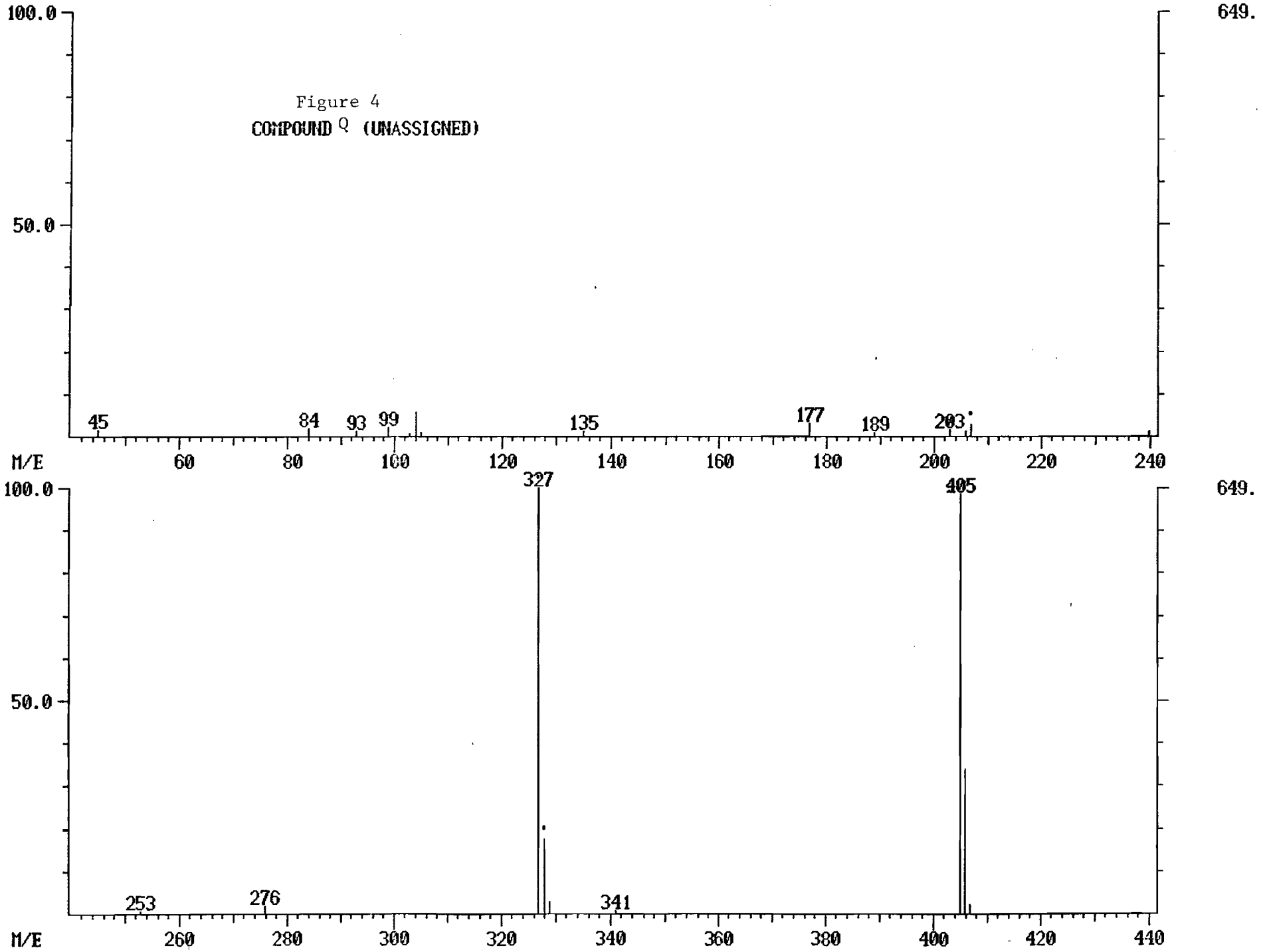
04/09/79 16:58:00 + 71:10
SAMPLE: HUMICS PLUS CHLORINE DIOXIDE EX4 PH10 EXTRACT
#2846 TO #2848 SUMMED - #2844 - #2849 X1.50

CALI: Z040979 #15

RIC: 1840.

Figure 4

COMPOUND Q (UNASSIGNED)



04/09/79 13:43:00
SAMPLE: HUMICS PLUS CLO2 PH2 EXTRACTS EX4

CALI: Z040979 #15

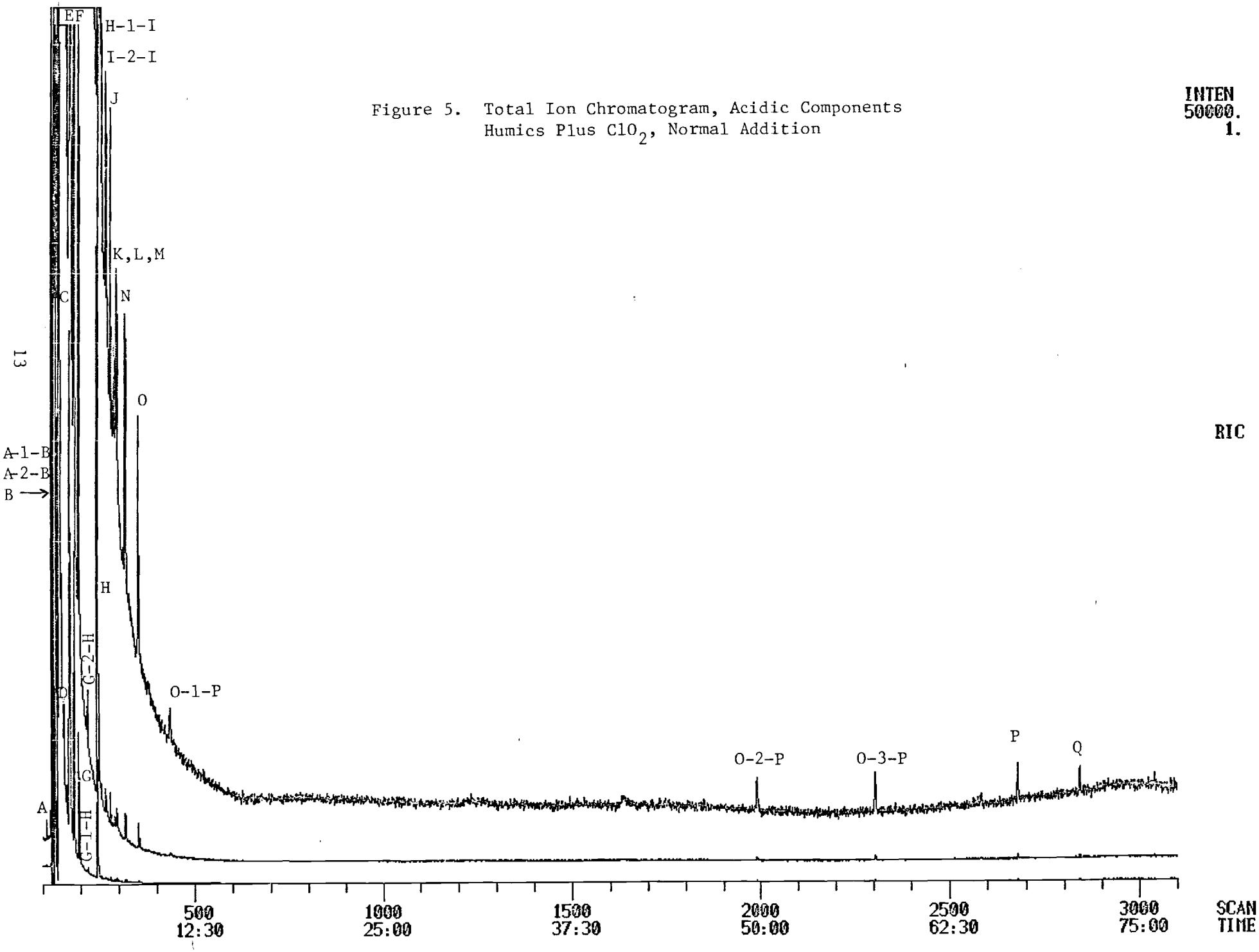


Figure 5. Total Ion Chromatogram, Acidic Components
Humics Plus ClO_2, Normal Addition

Table III
Identification of Chromatographic Peaks-Acids,
Humics Plus Chlorine Dioxide, Normal Addition

Component	Purity	FIT	RFIT	Comment
A Carbon Dioxide	NA	NA	NA	Present in most runs
A-1-B (Unassigned)	NA	NA	NA	New this fraction
A-2-B Ethanol	988	996	988	Origin unclear
B 2-Methylbutane	968	972	976	Solvent component
C Pentane	NA	NA	NA	Saturated spectrum
D Cyclopentene	948	963	973	Solvent component
E Unassigned	NA	NA	NA	As in Table II
F 3-Methylpentane	968	976	982	Solvent component
G Hexane	976	984	982	Solvent component
G-1-H Chloroform	876	951	917	Artifact
G-2-H Methylcyclopentane	953	965	953	Minor solvent component
H Benzene	990	997	990	Artifact in solvent
H-1-I Cyclohexane	891	926	943	Minor solvent component
H-2-I 2-Methylhexane	900	936	907	Minor solvent component
J 3-Methylhexane	934	964	966	Solvent component
K trans-1,3-Dimethylcyclopentane	917	961	929	Solvent component
L cis-1,3-Dimethylcyclopentane	880	930	904	Solvent component
M trans-1,2-Dimethylcyclopentane	914	965	941	Solvent component
N Heptane	969	990	977	Solvent component
O Methylcyclohexane	950	985	961	Solvent component
O-1-P Toluene	847	943	887	Artifact in solvent
O-2-P Unassigned	NA	NA	NA	Found in mini-pilot run
O-3-P Unassigned	NA	NA	NA	Present in pH 10 extract
P Unassigned	NA	NA	NA	Present in reagent blanks
Q Unassigned	NA	NA	NA	Present in pH 10 extract

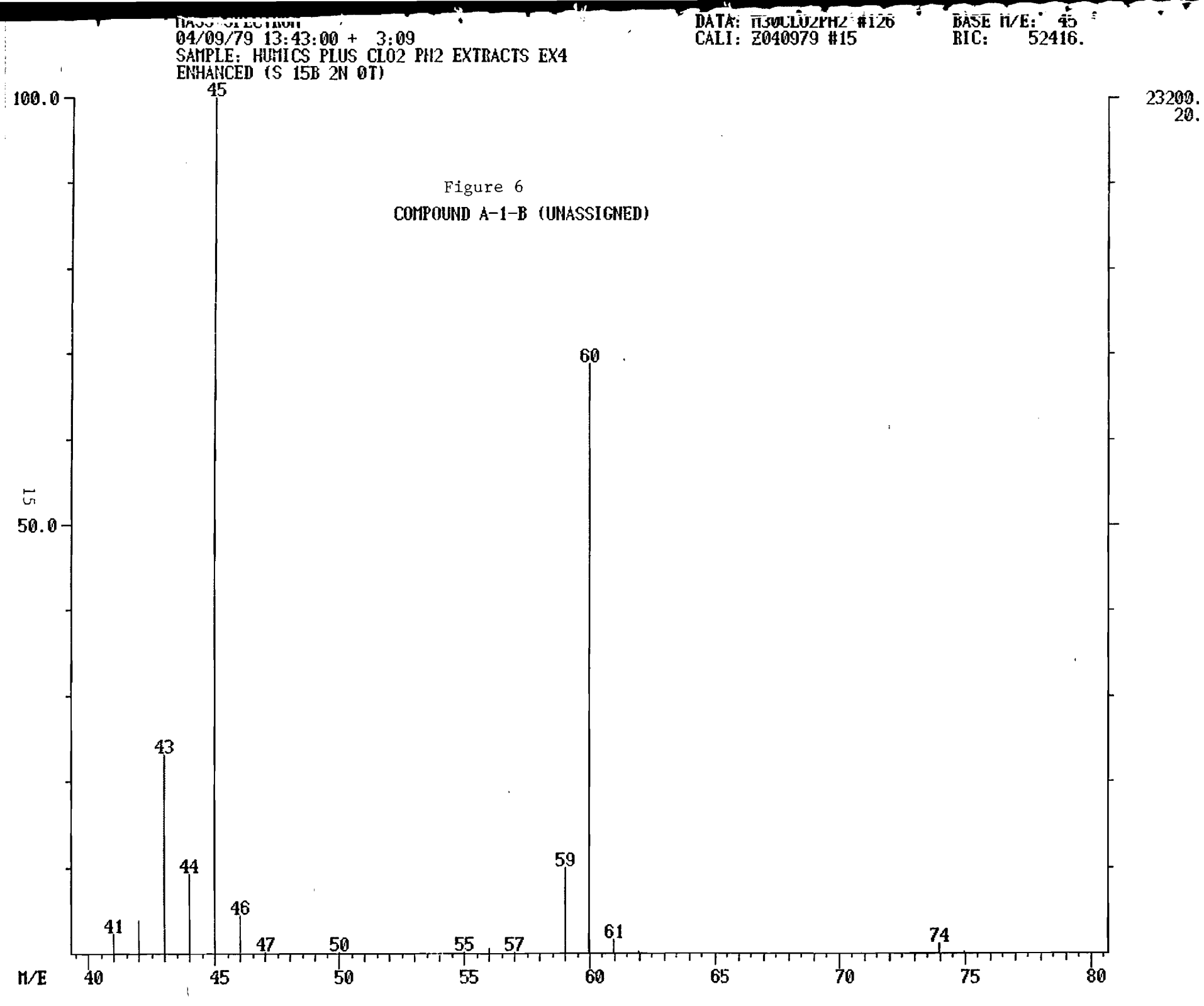


Figure 6

COMPOUND A-1-B (UNASSIGNED)

It was possible to detect a very tiny trace of chloroform in the product mixture only by developing selected ion chromatograms at m/e 83, m/e 85 and m/e 87. The amount present is clearly lower than that found in the base/neutral fraction by at least an order of magnitude. This evidence coupled with the absence of chloroform in the reagent blank argues that the chloroform is either released by the M/30 humics during oxidative degradation or else is created from them by the disinfectant. The results from the M/39 studies should clear up this question.

The benzene and toulene levels remain about the same, thus suggesting that they are derived from the solvent rather than from the substrate. Compound O-2-P has been found in the mini-pilot studies and has therefore been presented in the previous report (Figure 14). The solubility behavior suggests that the m/e 73 ion is derived from $CH_2\overset{O}{\overset{\|}{C}}OCH_3$ rather than from $\overset{O}{\overset{\|}{C}}OC_2H_5$ since an ethyl ester would have to preexist and would therefore be extracted in the base/neutral fraction unless some other acidic group were also present. The lack of a significant ion at m/e 74 argues that no γ hydrogen is present.

Compounds O-3-P and Q are present at higher levels in this fraction but were also found among the base neutrals thus suggesting only a weakly acidic nature. Compound P is present in both fractions and the blank and thus must be an artifact.

The GC/MS data for the inverse addition run was acquired during this reporting period. A casual inspection of the total ion chromatograms indicates a general similarity to those described in the pH 10 and pH 2 pentane extracts. An additional extract was taken at pH 2 with ethyl acetate in an effort to recover the more highly polar materials. It was hoped that by using a separatory funnel extraction instead of a con-

tinuous extraction some of the problems attributed to the transesterification and/or condensation reactions described in earlier reports could be avoided. The GC/MS analysis was run immediately after condensation. This extract was straw colored and as expected contained a large number of products. It will be recalled that this fraction was derivatized with diazoethane to further reduce possible confusion caused by ester interchange. The fractions for which the data have been collected are listed below. The total ion chromatograms are presented in the indicated figures. A full analysis will be presented in the next report.

Extract with pentane at pH 10	Figure 7
Extract with pentane at pH 2	Figure 8
Extract with ethyl acetate at pH 2	Figure 9

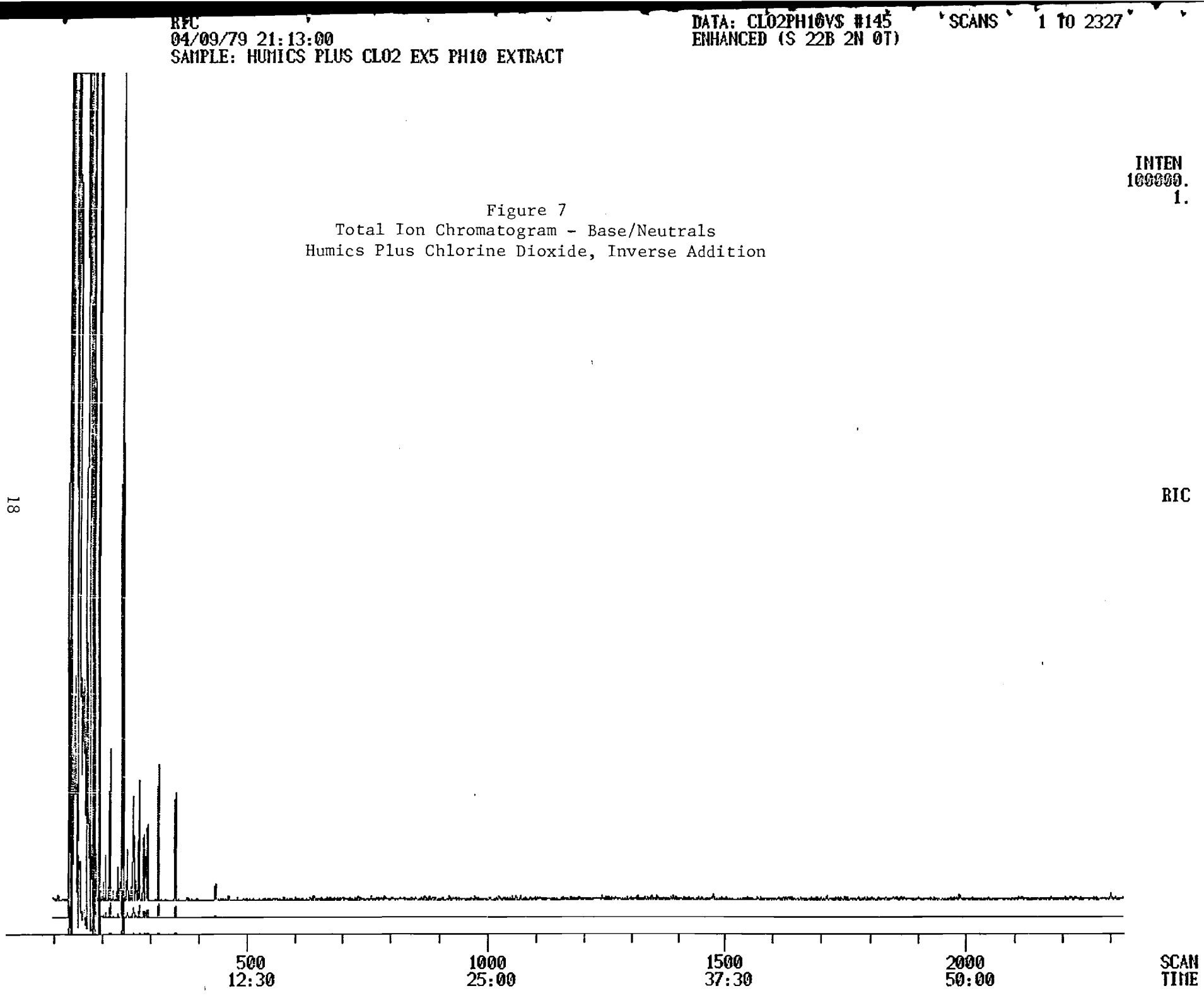


Figure 7
Total Ion Chromatogram - Base/Neutrals
Humics Plus Chlorine Dioxide, Inverse Addition

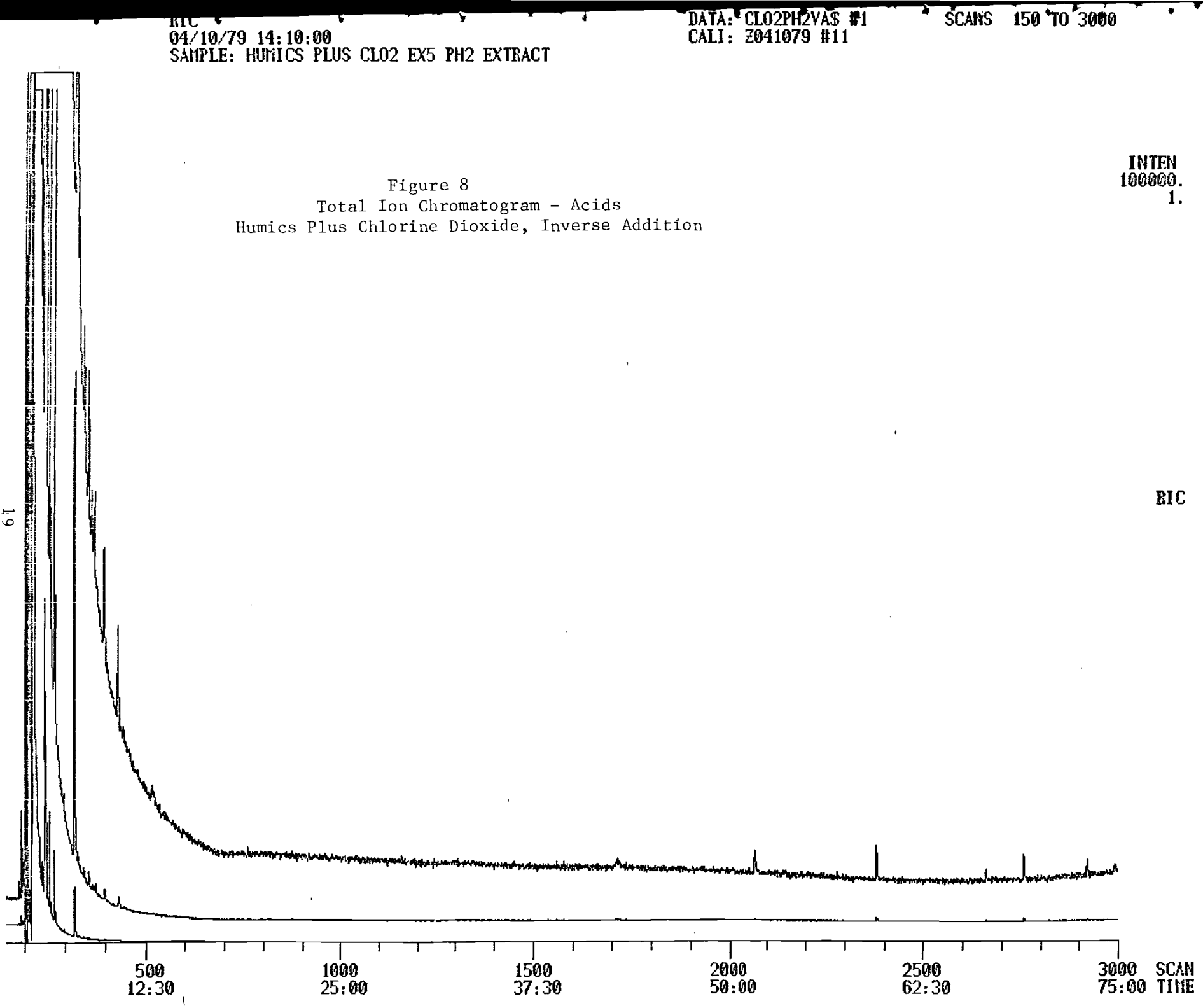


Figure 8
Total Ion Chromatogram - Acids
Humics Plus Chlorine Dioxide, Inverse Addition

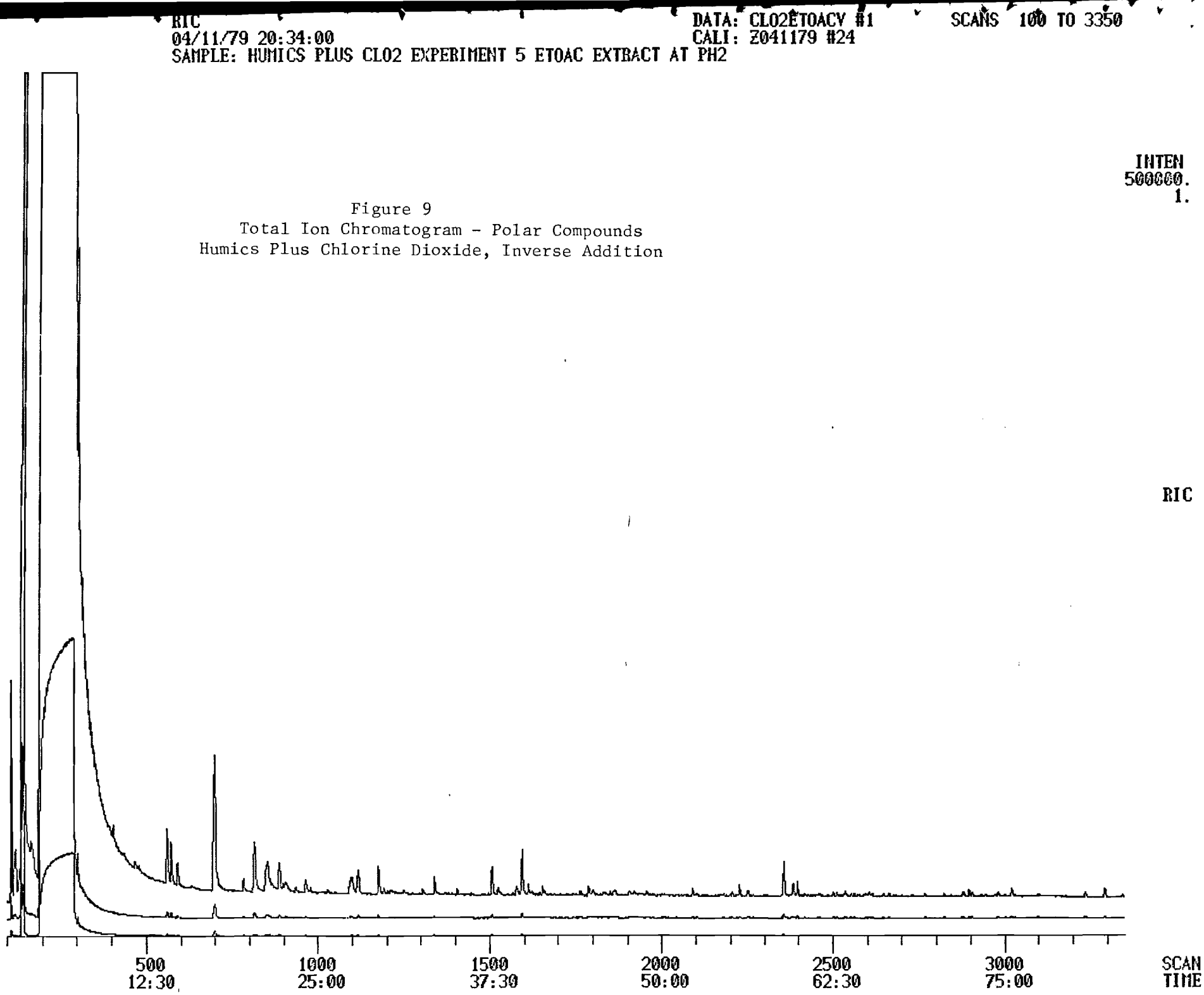


Figure 9
Total Ion Chromatogram - Polar Compounds
Humics Plus Chlorine Dioxide, Inverse Addition

III. REACTION OF AQUATIC HUMICS WITH OZONE

Aquatic humic material (M/39, 100 mg) was dissolved in high purity water (20 ml) and exposed to ozone from a short wavelength UV generator for 100 minutes. The rate of ozone generation was 240 mg/minute. Thus even at a low transfer efficiency, the ozone was in very large excess. At the end of the contact period, the reaction mixture was treated with a saturated solution of sodium sulfite (5 ml) to destroy any remaining oxidizing agent prior to extraction at pH 10 with pentane (3 x 50 ml). The combined extracts were dried over roasted anhydrous sodium sulfate and concentrated as previously described. The concentrate was divided into two equal parts - one for mutagenicity testing and the other for GC/MS analysis.

The aquous solution remaining after the first series of extractions was brought to pH 2 with hydrochloric acid and extracted again with pentane (3 x 50 ml). The combined extracts were dried, concentrated and divided as before. The portion destined for GC/MS analysis was derivatized with diazoethane. A second series of extractions was carried out with ethyl acetate (3 x 50 ml) on the aquous solution which remained after the pentane extraction had been completed. These extracts were combined, dried, concentrated and divided as before. A reagent blank was also performed. The data for each of the concentrates has been acquired. The total ion chromatograms are presented as indicated below:

Extracted with pentane at pH 10	Figure 10
Extracted with pentane at pH 2	Figure 11
Extracted with ethyl acetate at pH 2	Figure 12

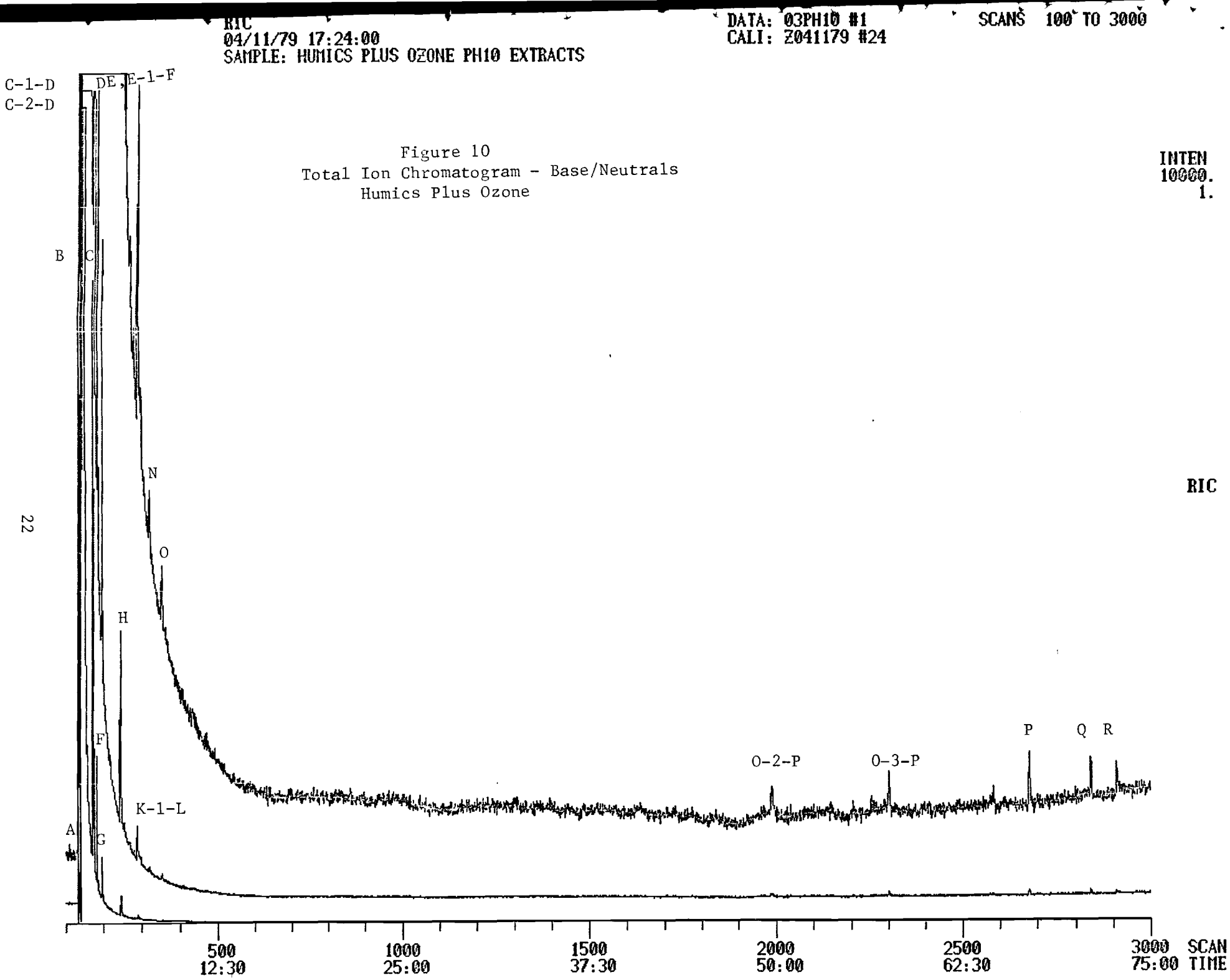


Figure 10
Total Ion Chromatogram - Base/Neutrals
Humics Plus Ozone

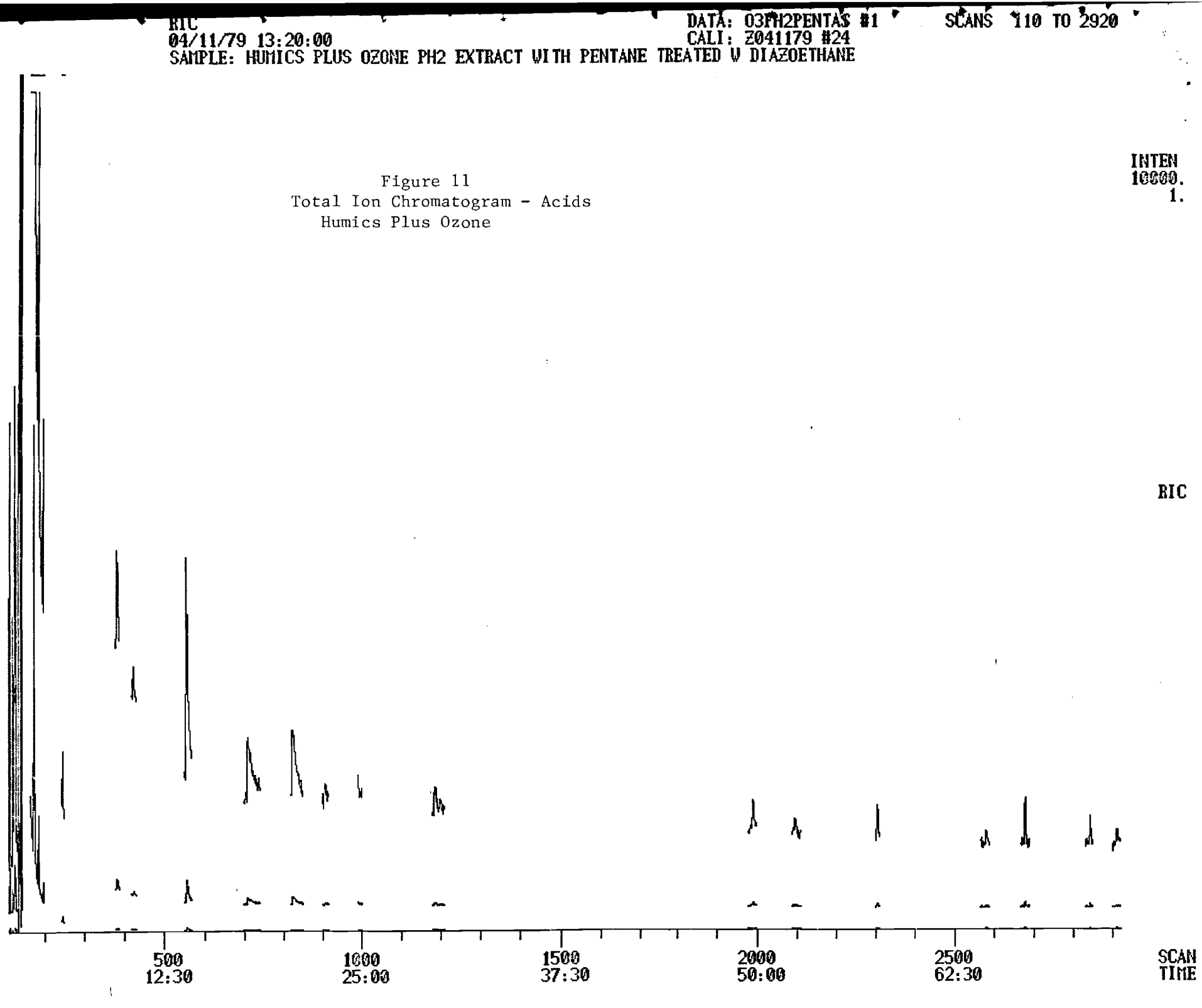


Figure 11
Total Ion Chromatogram - Acids
Humics Plus Ozone

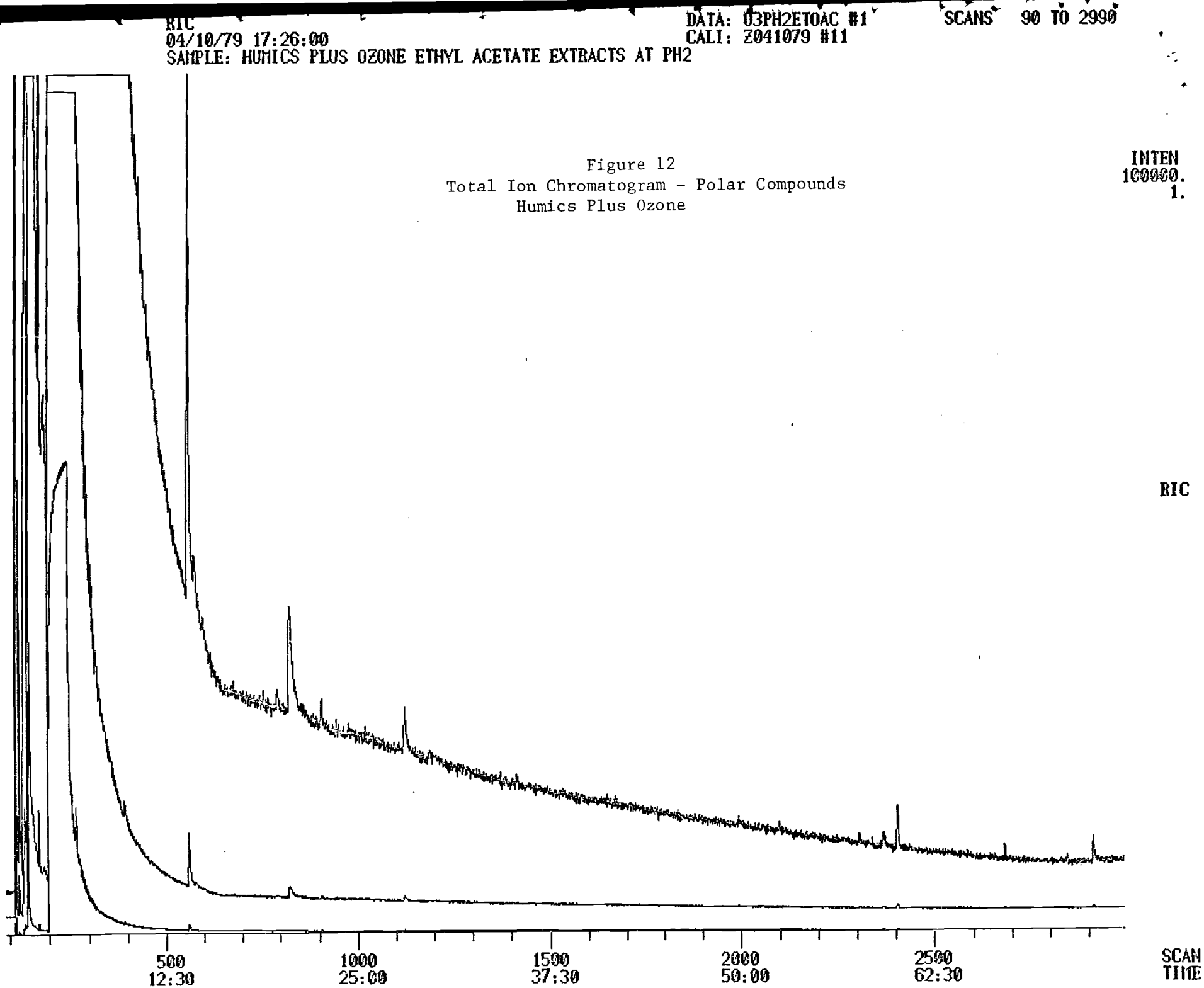


Figure 12
Total Ion Chromatogram - Polar Compounds
Humics Plus Ozone

The data from the pH 10 pentane concentrates have been subjected to interpretation. The results are outlined in Table IV.

Once again, the majority of the components in the mixture were found to be solvent-related. In this case, it was possible to identify compound E as 2-pentene due to an improved chromatographic separation. This finding indicates that the second component which has very nearly the same retention time was probably merged with compound E in earlier runs thus preventing a correct assignment from being made.

The apparent absence of chloroform in the pH 10 extracts may only be a consequence of file shortening rather than a reality. However, in order to be dropped during shortening, the concentration would have had to have been quite low thus indicating that by comparison with the M/30 ClO_2 runs there is less chloroform present. Whether this is a consequence of the fact that the M/39 humic material used for this study had never been contacted with chloroform while the M/30 humics had or whether the chlorine in the ClO_2 is somehow generating very small amounts of this trihalomethane is therefore unclear. We believe the former explanation to be correct. The data from the ClO_2 runs with M/39 should help resolve this question.

The presence of triethylamine in this sample may also be explained by the difference in treatments between the M/30 and the M/39 starting materials. Since triethylamine was used in the workup of both samples, its persistance in the M/39 sample may be due to the fact that a liquid-liquid extraction with chloroform was not performed on this material. Alternatively, the triethylamine may be present in both samples and is not released by the ClO_2 but is released by the ozone. Again, parallel experiments already performed should clear up this question.

The results obtained from the other fractions will be presented in the next report.

Table IV

Identification of Chromatographic Peaks-Base/Neutrals

Humics Plus Ozone

Compound	Purity	FIT	RFIT	Comment
A Carbon Dioxide	NA	NA	NA	Present in most runs
B 2-Methylbutane	977	998	977	Solvent component
C Pentane	986	997	986	Solvent
C-1-D 2,2-Dimethylcyclopropane	810	828	963	Tentative
C-2-D Unassigned Hydrocarbon	NA	NA	NA	Solvent component
D Cyclopentene	865	920	932	Solvent component
E 2-Pentene	928	941	928	Usually merged
E-1-F Unassigned	NA	NA	NA	
F 3-Methylpentane	983	995	983	Solvent component
G Hexane	975	995	975	Solvent component
G-2-H Methylcyclopentane	NA	NA	NA	Fit is weak, retention correct, is 1st choice
H Benzene	971	999	971	Contaminant in solvent
J 3-Methylhexane	NA	896	NA	Weak, correct retention
K-1-L Triethylamine	783	963	796	Merged with K and L
M Heptane	NA	936	NA	Weak, correct retention
O Methylcyclohexane	NA	972	NA	Weak, correct retention
O-2-P Unassigned	NA	NA	NA	Seen in ClO_2 runs
O-3-P Unassigned	NA	NA	NA	Seen in ClO_2 runs
P Unassigned	NA	NA	NA	Seen in ClO_2 runs
Q Unassigned	NA	NA	NA	Seen in ClO_2 runs
R Dibutyl Phthalate	804	835	902	Contaminant

IV. THE ACS/CSJ MEETING

The unfortunate occurrence of a major airline strike which cancelled more than half of the U.S. flights to Honolulu coupled with the extremely pleasant surroundings held down attendance at the environmental sessions to unusually low levels. Cancellations of talks also cut down on attendance by leaving some talks isolated between cancelled papers. Luckily, our presentation was scheduled right in the middle of a session with no cancellations and as a result was the best attended of the Monday or Tuesday papers in our symposium. The presentation itself went smoothly, was finished in the recommended amount of time and generated a lively question and answer session which continued until the chairman interrupted to get the next paper going.

While the presentation followed the abstract in general, the Project Manager was able to add a more rigorous definition of what the term "aquatic humic matter" means to us along with a few more details regarding the origin of this material. The spectral interpretations were discussed as presented in the extended abstract. The known ability of aquatic humics to adsorb small molecules was involved to explain the origin of the chlorinated isoprene alcohols. Supporting evidence was cited regarding the presence of isoprene and 2-methyl-2-butene-2-ol in air and the correlation of the latter with algal growth and die-off. The prediction was made that these chlorinated alcohols would be found, at least on an irregular basis, in public water supplies drawn from organic-rich surface waters.

The major lines of questioning dealt with the adsorption phenomenon with a gentleman from the Geological Survey being most interested in whether or not we had found benzene and toluene in our product mixtures. He was of the opinion that such entities are somehow derived directly from the

humic materials. As the reviewers are aware, we do regularly find these two compounds in our product mixtures. However, we are not yet completely satisfied that they are not artifacts and have therefore kept them off our "official" product lists.

A number of other interesting papers were presented at the symposium which had a link with some of our observations. At least two other papers discussed the presence of polyethylene oxides i.e. $H(CH_2CH_2O)CH_2CH_2\ O\ R$ in drinking waters. Thus it may be that similar products found in our crown ether runs are not entirely artifacts as we had originally supposed. Another paper described finding traces of toluene, ethylbenzene, xylenes and related alkylbenzenes by passing extremely large volumes of drinking water through macroreticular resins, extracting the resins and examining the products by GC/MS. The Project Manager suggested that such materials which we have seen from time to time might be coming from the resin itself. We believe that even the rigorous cleaning of such materials prior to use removes only that portion of the alkylbenzenes which is held near the surface. The remaining monomers can then slowly diffuse to the surface and can thus contribute to the extracts if the resin is stored prior to use. Thus the only way to obtain a satisfactory blank for a long-term experiment might be to hold a "resin blank" in contact with a fixed volume of ultra high purity water for the duration of the experiment. Short-term experiments can probably be successfully executed if the resin is cleaned just prior to use.

F-00-657

IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Progress Report

May 22, 1979

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DISCLAIMER

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SUMMARY

Approximately 80% of the organic matter present in unpolluted surface waters occurs in the form of aquatic humic substances. It was therefore deemed appropriate to investigate the properties and structure of this as yet uncharacterized polymeric material in the hope that inferences could be drawn which would be of use in the production of higher quality potable water from surface sources.

A large number of aliphatic compounds have been identified among the products resulting from the relatively mild oxidative degradation of aquatic humics. This result strongly suggests that aquatic humic materials which account for 80% of the TOC in typical surface waters are more aliphatic and less aromatic than previously supposed.

The isolation and identification of a series of chlorinated isoprenoid alcohols and hydrocarbons resulting from the treatment of aquatic humic matter with chlorine under conditions simulating the production of potable water have been accomplished. The fact that these compounds are structurally related to isoprene rather than to the typical oxidative degradation products of aquatic humic material itself suggests that the precursors are adsorbed within the aquatic humic matter. These compounds have been found to be produced at levels which are approximately one order of magnitude lower than those observed for the corresponding trihalomethanes.

The subdivision of aquatic humic material into five chromatographically homogeneous fractions using gel permeation methods has resulted in a size

classification ranging from number average molecular weight ($\bar{M}_N$)≈500 to $\bar{M}_N$≈3000. While these fractions do not appear distinct with respect to their gross properties such as IR, UV and elemental analysis, they do appear to differ significantly in their chlorine demands and in their ability to generate chloroform and total organic chlorine (TOCl) when treated with chlorine under conditions simulating the production of potable water.

The detection of adsorbed species in addition to the chloroalcohol precursors in aquatic humic material, which are released for detection only after oxidative disruption of the aquatic humic matter, supports the suggestions of other workers regarding the importance of aquatic humics in the transport of pollutant materials in the environment. Evidence developed in connection with this effort strongly suggests that air pollutants may also bind strongly to aquatic humic material.

INTRODUCTION

The use of chlorine for the disinfection of potable water has resulted in a general feeling of security regarding the freedom of public water supplies from objectionable materials. At least in the developed countries, this feeling is certainly justified from a microbiological standpoint. However, the National Organics Reconnaissance Survey conducted by the U.S. Environmental Protection Agency in 1974 and 1975[1] demonstrated the presence of trihalomethanes in the public water supplies of 80 major U.S. cities. These findings caused considerable concern because some of these compounds are known or suspected carcinogens.[2,3] Since it has also been demonstrated that these halogenated organics are formed during the disinfection process,[4,5] it is appropriate that EPA's Office of Drinking Water has awarded a significant research contract to the authors aimed at identifying the major and minor classes of natural organic substances in surface waters before and after disinfection. This report describes the findings obtained as a result of this investigation.

The work in question has had two major objectives, the first of which was to define the nature of the organic materials naturally present in surface waters such as might be used for the production of potable water. The second major objective of this work has been to isolate and identify those organic materials, particularly the chloroorganics which are produced from the aforementioned natural organics under conditions of disinfection currently practiced by the water industry.

It will be noted that the term "aquatic humic material" has been used in several places already within this document. It will be useful to define

this term in the sense in which it is used by the writers since not all of the workers in the area are necessarily in agreement regarding this matter. We shall preface our definition with a negative statement: whereas for soil humic substances a purely operational definition can be given to denote that fraction of soil organic matter which is extracted by alkali and can be separated into acid soluble (fulvic acid) and acid insoluble (humic acid) fractions by acidification of the extract, no equivalent definition can be given for aquatic humic material. These materials obviously have not been mobilized from soil by strong alkali, but rather by meteoric waters of low ionic strength and a pH range of 4 to 8. These mild extraction conditions in our sampling area lead to the solubilization of mostly low molecular weight humic fractions which are similar to soil fulvic acids. Secondary alterations, such as continued oxidative polymerization, e.g. in water bodies with longer residence times, can increase the relative abundance of high molecular weight humic fractions which may flocculate and precipitate at low pH values (pH <3.5). This material may then become more similar to soil humic acids. This "humic acid"-like fraction may be found in rivers in varying relative concentrations depending upon the soil chemistry of the drainage basin, the residence times of dissolved organic matter, pH and other as yet poorly understood factors. Additional work which is reserved for the future might be aimed at improving our understanding of the maturation process undergone by aquatic humics and at pinpointing the more subtle regional and seasonal differences which may exist in aquatic humic structures derived from different sources.

Considerable emphasis has been placed upon the aquatic humic fraction in connection with this project because this material accounts for such a large proportion of the organic matter typically found in surface waters. Some very recent work has demonstrated that 90% of the organics in river water were of molecular weight less than 3000 and furthermore, that these source materials account for 45% of the total trihalomethanes generated when treated with chlorine under conditions simulating the production of potable water.[6] This fraction corresponds exactly to what we have defined as aquatic humic matter.

Similarly, the major effort with disinfecting agents has been concentrated on chlorine since this is the agent having the most widespread use. Throughout this work, the empahsis has been on realism. Thus we have used aquatic humics at levels corresponding to 10mg/l or 5 mg/l TOC. This level is within the normal range of 2-10 mg/l TOC encountered in surface waters. Likewise the levels of chlorine dosed are in general agreement with current practice. Contact times, sequence of treatments, pH and selection of flocculant aids have similarly been chosen to be in general agreement with conditions employed for the production of potable water. In this way, the results described in this report have a special relevancy to the mission of the funding agency.

METHODS

Isolation of Aquatic Humics

River water was collected in batchwise fashion several times during the course of the project (see Table 3). Samples were collected in two 55-gallon stainless steel drums which had originally been used for USP alcohol. The drums were rinsed on site with river water, filled and brought to the laboratory for processing, which was begun within 24 hours of collection. The collected waters (470L in a typical case) were continuously clarified by centrifugation at 17,000 RPM with a residence time of one minute and a flow rate of 400 ml/min. This step prevents subsequent clogging of the resin columns used for concentration. The water was then acidified with hydrochloric acid to pH 1.8. Because the waters selected for study contained mostly low-molecular weight humics, no significant precipitation occurred. The protonation of the acidic functions on the aquatic humic material is necessary for the subsequent adsorption on the resin. Aqueous triethylamine (3.3%) is then employed to remove the adsorbed material from the macroreticular resin. Excess triethylamine is removed from the eluate under reduced pressure. An optional liquid-liquid extraction with an organic solvent can be performed at this time to remove triethylammonium hydrochloride. Such a step is not recommended if adsorbed organics in the isolated aquatic humic material are to be studied. (However, even continuous extraction does not completely remove such materials).

The triethylammonium salts of the aquatic humics are subsequently treated with an acidic cation exchange resin (Bio Rad AGW-50, H^+). This step also removes any inorganic cations still associated with the aquatic humic matter. The final product is obtained by lyophilization as an ash-free humic material which is completely soluble in both water and methanol.

Acid-Base Chemistry of Aquatic Humic Substances

Potentiometric titrations were carried out with a Fisher Accumet 144 pH meter and a Fisher combination pH electrode. One hundred milliliter aliquots containing 62.7 mg of Satilla River Humic Substances (SRHS) (3.135 g/L) in various ionic media (see Table 1) of varying ionic strength were titrated with 0.4997 N NaOH delivered from a Gilmont digital micrometer buret (2.5 ml capacity). Reactions of SRHS and acetylated derivatives with various bases were allowed to proceed for 24 hours with constant stirring under nitrogren. The reaction products were filtered using Amicon UM-2 filters, which removed all color (checked photmetrically at 420 nm). Aliquots ranging from 50 to 25 ml of filtrate were used for the titration. All blanks were prepared and titrated parallel to the samples. The use of the ultra-high precision micrometer buret allowed addition of very small volumes (0.001 ml) of more concentrated base (0.5 N NaOH), so that the final titration volume changed by less than one percent, eliminating the need to correct the evaluation of the titrations for dilution.

TABLE 1
General Parameters of Acid - Base Titrations of SRHS

Titration	Type[a]	Supporting Electrolyte[b]	Weak Base[c]	Filtration[d]
1	D	$Ca(NO_3)_2$	None	None
2	D	NaCl	None	None
3	I	None	$Ca(CH_3COO)_2$	W#42
4	I	None	$Ca(CH_3COO)_2$	UM-2
5	I	None	$Na(CH_3COO)$	UM-2
6	I	NaCl	C_5H_5N	UM-2

[a]D= direct titration; I - indirect titration.
[b]0.033 M $Ca(NO_3)_2$; 0.100 M NaCl
[c]0.100 M $Ca(CH_3COO)_2$; 0.100 M $Na(CH_3COO)$; 0.150 M C_5H_5N
[d]W#42 = Whatman #42 paper; UM-2 = Amicon UM-2 membrane

Reaction of Aquatic Humics with Permanganate

Aquatic humic material (1.0 g) isolated as previously described was dissolved in methanol (100 ml) and treated for 1-2 hours with diazomethane in ether at ambient temperature. The partially methylated product gradually became insoluble in methanol at which time the original solvent was removed and replaced with an equal volume of chloroform. After 1-2 hours reaction with additional etherial diazomethane, the reaction was presumed to be complete. Removal of solvent under reduced pressure provided 1.18g of methylated product. Unlike results reported recently in the literature,[7] this material is readily soluble in organic solvents. In earlier reactions, this material was suspended in aqueous potassium permanganate (4% w/v; 125 ml) and brought to reflux. Reaction conditions were gradually moderated to less vigorous conditions i.e., 60°C and no reflux and a non-aqueous system in which the crown-18-ether was employed to hold a low concentration of permanganate in solution at room temperature. Some typical conditions

are summarized in Table 2. The product mixtures were then treated with methanol, to destroy excess oxidant. Afterwards, the reaction mixtures were filtered, treated with cation exchange resin to strip out metallic salts, acidified to pH2 with HCl, extracted and treated once again with diazomethane. The weights of the final products were calculated and the mixtures analyzed by GC/MS.

TABLE 2

REACTION CONDITIONS OXIDATION OF AQUATIC HUMICS

	Temperature	Time	Percent Yield
Aqueous $KMnO_4$(5g/g)	Reflux	4 hrs	23
Aqueous $KMnO_4$(8g/g)	60°C	6 hrs	47
Aqueous $KMnO_4$(8g/g)	55°C	6 hrs	58
CH_2Cl_2,$KMnO_4$(12g/g) 18-crown-6-ether(2g)	room temp.	24 hrs	83

Chlorination of Aquatic Humic Material in the Mini-Pilot Facility

In order to duplicate conventional methods for the disinfection of potable water as accurately as possible, a mini-pilot facility was fabricated. This apparatus was constructed entirely of Teflon and glass and is shown schematically in Figure 1. The dimensions are presented in Figure 2. The modular design permitted the facility to be dismantled and baked out between runs.

In a typical run, the reservoir was loaded with 12 liters of high-purity (Continental Water Conditioning consisting of filtration, double ion exchange, carbon filtration and pre-purging) water containing 10 mg/L

Figure 1. Schematic - Mini-Pilot Facility

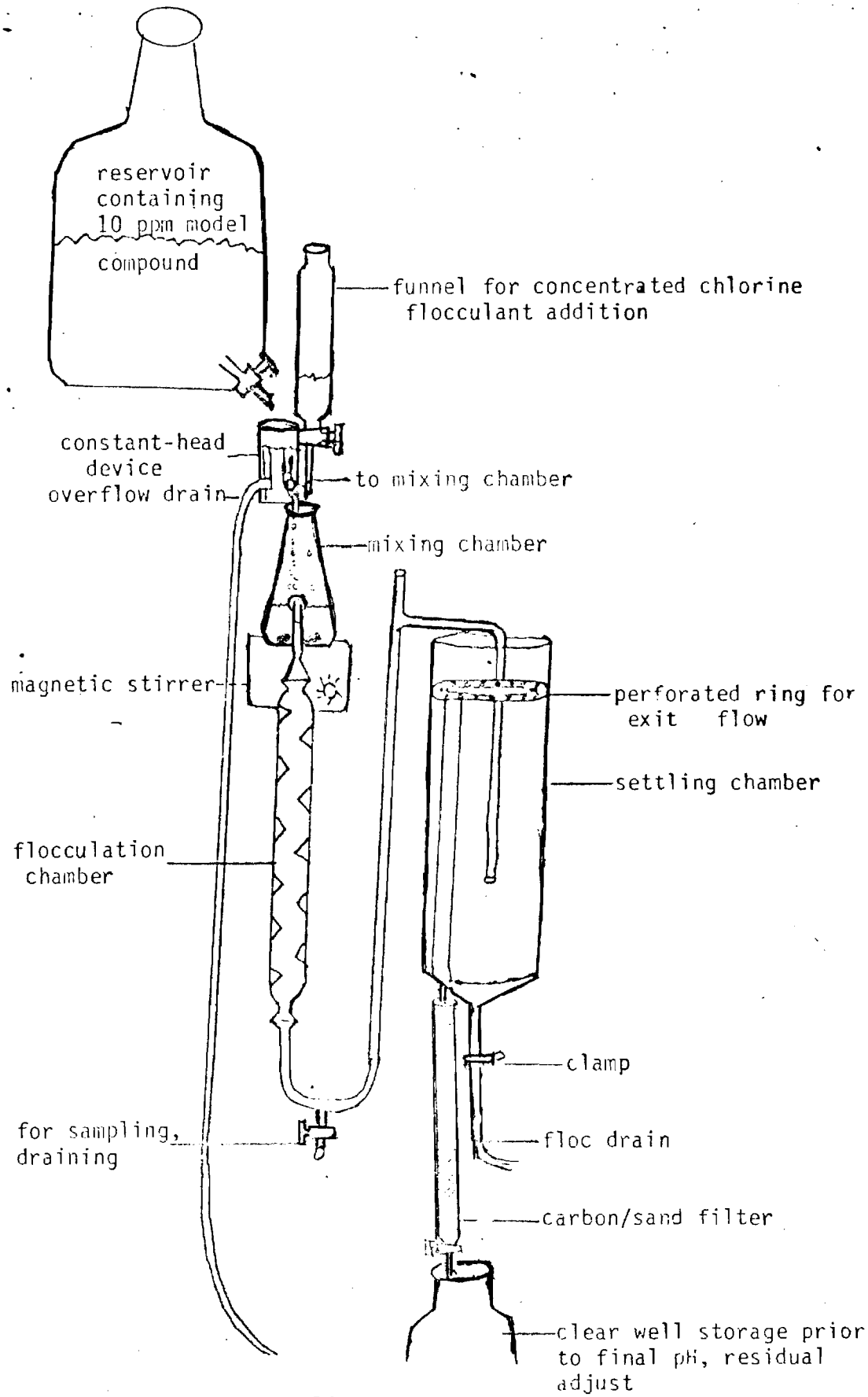
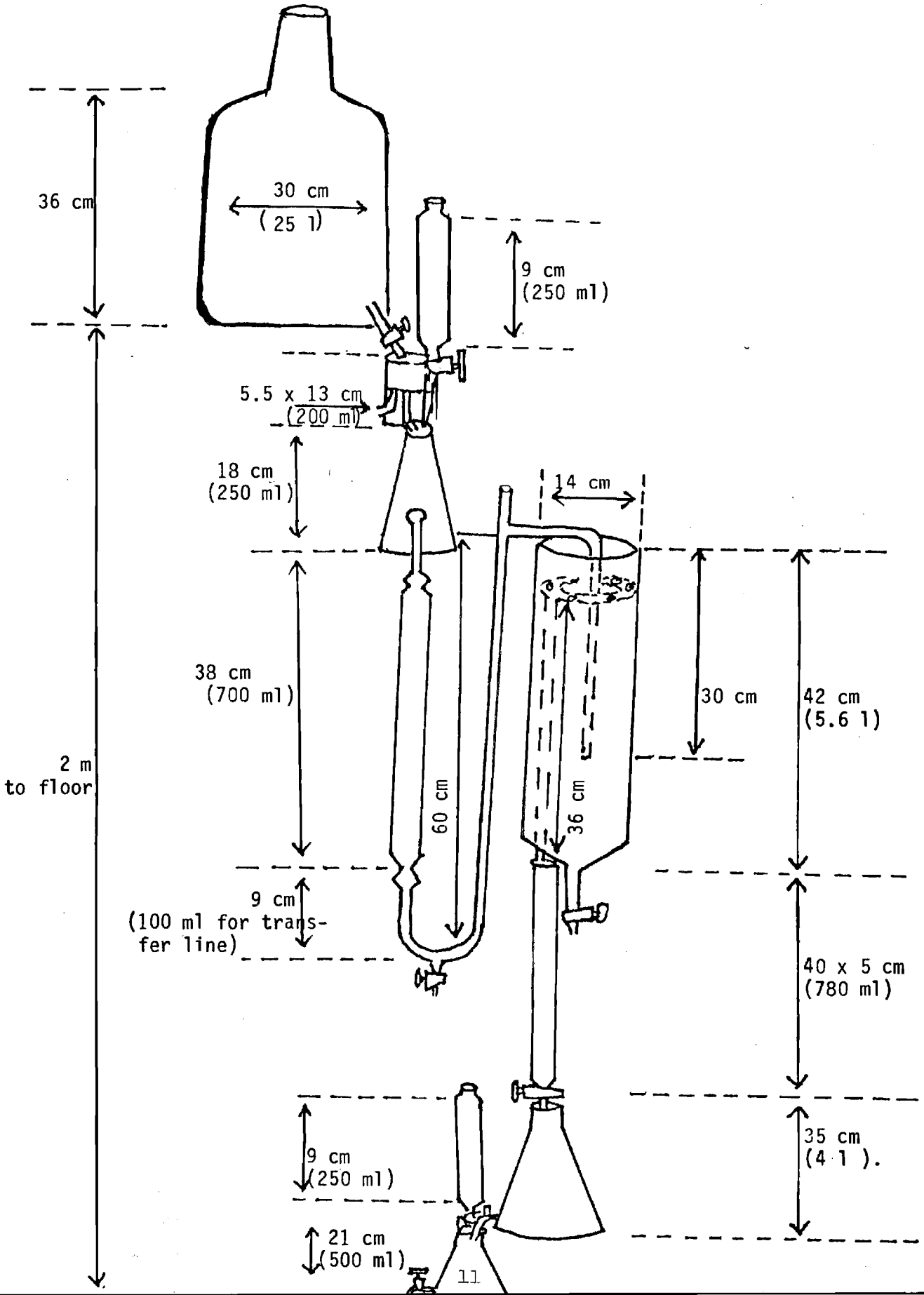


Figure 2 Dimensions and Volumes of Mini-Pilot Facility.



aquatic humic material together with whatever buffering agents were required to maintain the pH within the specified range for the experiment in question. The flow from the reservoir was adjusted to 1.0 L/hr and balanced with an appropriate flow of chlorine concentrate and flocculants. The desired levels of these chemicals were independently established ahead of time by jar tests and chlorine demand studies. The flocculants and buffering agents were all pretreated in a muffle furnace in order to remove all organic matter. The chlorine concentrate was prepared from gaseous chlorine generated in an all-glass system from prepurged hypochlorite and H_2SO_4. Incidental carryover of acid beyond that expected from the dissolution of chlorine in the water was not found to be a problem. Chlorine addition was adjusted to provide a 24 hour residual not to exceed 2 mg/L.

Samples (1 L or more) were withdrawn and immediately treated with pre-roasted sodium sulfite. Other samples were allowed to stand 24 hours prior to treatment with sodium sulfite. A sample withdrawn from the reservoir and similarly treated served as the control. All samples were acidified with HCl and extracted with high-purity ethyl ether.

The combined extracts (3x100 ml) were subsequently dried over pre-roasted sodium sulfate and concentrated to 1-2 ml using a Kuderna-Danish apparatus.

The concentrated extracts were then treated with diazoethane (generated from N-nitroso-N-ethyl-N'-nitroguanadine) at ambient temperature until the yellowish color of the reagent no longer faded. Independent tests with a number of standards confirmed that this reagent is about as effective as diazomethane if applied under the same conditions (i.e., 60-85% conversion).

The derivatized product mixtures were then reconcentrated and examined by capillary column GC/MS using the techniques of both electron impact (EI) and methane chemical ionization (CI). Spectra were compared with the EPA/NBS library using the INCOS software provided with the Finnigan Model 4023 GC/MS/DS. Structures were assigned on the basis of library comparisons, operator interpretation and user-developed automatic search routines.

Reactions were carried out at 3 pH values: 6.0, 7.3 and 8.2, in accordance with the sponsor's request to survey a rather narrow range of pH values. The pH values were adjusted using organic-free phosphate buffering systems. Ferric chloride was employed as the flocculant in some cases. The sand used in the filters was freed from organics by pretreatment in the muffle furnace. The granular activated carbon employed in some of the experiments was water treatment grade (Nuchar WV-H) donated by the Westvaco Corporation.

Subdivision of Aquatic Humic Materials According to Molecular Size

A sample of aquatic humic matter was fractionated by gel permeation chromatography. The partitioning was begun with Sephadex G-50 and continued consecutively with G-25, G-15 and G-10. Each excluded fraction was collected, concentrated and re-chromatographed on the same gel until it contained only excluded material. The retarded fractions likewise were collected, concentrated and chromatographed on the next lower grade of Sephadex. In this way, five fractions were obtained: (I) G-50 excluded; (II) G-25 excluded; (III) G-15 excluded; (IV) G-10 excluded and (V) retarded. Fraction II was found to contain almost half of the starting material. The amounts collected in each fraction were: (I) 3.6%; (II) 47%; (III) 14%; (IV) 15%; and, (V) 21%.

Chlorination of Aquatic Humics Following Molecular Weight Fractionation

A bench scale experiment was set up using a series of 100 ml glass-stoppered reaction flasks each containing 10 mg/L aquatic humics from the chromatographically resolved molecular size fractions described in the previous subsection. Duplicate runs were made for each of the five fractions. Each of the fractions was contacted with identical amounts of chlorine as required to maintain a residual throughout the experiment. After 24 hours, the excess chlorine was quenched with organic-free sodium sulfite and sealed in crimp-top bottles prior to being submitted for Total Organic Halogen (TOX) and chloroform analysis. A small portion of each reaction mixture was analyzed for residual chlorine immediately prior to the sodium sulfite treatment.

Oxidation of Aquatic Humics with Iodine in Base

A solution (25 ml) of aquatic humic material (500 mg), sodium hydroxide (2.5 g) and iodine (3.7 g) was heated to 65°C, held 30 min., and allowed to stand overnight at ambient temperatures. The resulting dark brown solution was directly extracted with ethyl ether (3x25 ml) and the combined extracts back extracted with water (2x25 ml). The ether layer was then dried over sodium sulfate and solvent and excess iodine removed under reduced pressure to provide 13.4 mg of yellow hexagonal prisms, mp 122° C. The product was tentatively identified as iodoform on the basis of these properties (lit mp 123) and subsequently confirmed by GC/MS.

The aqueous phase remaining after extraction was combined with the back extracts, acidified with HCl and reextracted with ethyl ether (3x25 ml). The combined extracts were dried over sodium sulfate, concentrated and examined by GC/MS.

Reaction of Aquatic Humics with Bromine in Acetic Acid

Aquatic humic material (sample M/30, 100 mg) was suspended in acetic acid (4 ml) to which bromine (0.3 ml) was subsequently added dropwise with swirling. The reaction mixture was then heated (60°) for 10 minutes during which time the suspension gradually dissolved. The solvent and unreacted bromine were removed overnight using a slow nitrogen sweep. Unfortunately, this procedure may have caused the loss of some of the more volatile products. The resulting deep red flakes were further dried and then examined using the infrared spectrophotometer. Compared to I.R. spectra of untreated humic material, a new band appeared in the product at 485-635 cm^{-1} which can be attributed to C-Br stretching. The relative intensity of the 1600-1650cm^{-1} region is somewhat decreased, possibly due to the bromination at enolizable carbonyl functions. A concentrated pentane extract of this material was examined by capillary column GC/MS.

Classification of Raw River Water

A 4.4-liter subsample of Satilla River water was filtered through Whatman No. 1 paper to remove the small amounts of suspended solids which had built up in the sample during transit. Portions of this filtered subsample were retained for TOC analysis. In this way, the TOC will provide a check on the material balance.

The remaining water sample (3.3 liters) was treated with 10 drops of concentrated potassium hydroxide in order to bring the pH to 9.0. This water (3.3 liters) was passed through four beds (11 x 50 mm) of precleaned XAD-8 macroreticular resin. Each bed contained about 2g of resin (four beds were used to speed the completion of the process without introducing rapid flow rate). In this way, the flow rate was maintained at 100 ml/hour/bed.

A sample of the XAD-8 resin column eluate was reserved for TOC measurement. The remaining eluate (3.1 l) was passed through four beds of XAD-2 resin in a similar way as that described for the XAD-8 treatment. A sample of the eluate (pH=7.5) from the XAD-2 resin was retained for TOC measurement. At this point in the separation-concentration sequence, any hydrophobic bases present in the sample are retained on the resin. The XAD-8 and XAD-2 resins were then back-washed with 500 ml 0.1 N hydrochloric acid per bed, thus providing 2 l of eluate containing hydrophobic bases from each type of resin.

The pH of the sample after passage over XAD-2 was adjusted to 2.0 with purified 6N hydrochloric acid. A brown precipitate slowly developed in this solution (2.8 l) as it was passed again over XAD-8 in the manner described above. As a result of the precipitate formation, the flow rate dropped to only a few ml/hr after about 2 l of water had passed through the resin. Once again a portion of the eluate was retained for TOC determination.

The remaining eluate (2.3 l) was subsequently passed over the XAD-2 resin. Very little additional colored material appeared on the resin. A portion of the eluate (pH 2.1) was also retained for TOC evaluation.

Two beds of pre-purified AG MP-50 ion exchange resin (H^+ form) equivalent to 4g of dry resin were prepared in water, following activation with 10 ml of 3N purified hydrochloric acid and washing with 100ml distilled water per bed, the columns were treated with the remaining XAD eluates at a flow rate of 125ml per hour per bed. A portion of this eluate (1.9 l, pH 1.2) was retained for TOC analysis. The remaining eluate was brought to pH 7.0 with 1N NaOH (65 ml).

Two columns of pre-purified AG MP-1 resin containing about 2g each of material on a dry weight basis were converted to the OH-form with 10 ml of 1N sodium hydroxide solution and washed with 200 ml of distilled water. The remaining 1.6 l of eluate was passed through the resin beds at a flow rate of 150ml/hr/bed. The color was completely removed by this treatment. The upper part of the resin became dark brown. A portion of this eluate (1.5 l, pH 7.0) was retained for the measurements. This eluate contains the hydrophilic neutrals.

Back-flushing of the AG MP-50 and AG MP-1 beds with a total of 200 ml of 0.1N sodium hydroxide and o.1N sulfuric acid provided the hydrophilic base and hydrophilic acid fractions.

TOC determinations were carried out using a Beckman 915 TOC Analyzer. The precision of the method for the 10-50 mg/l range has been established as ±0.5mg/l.

RESULTS

Isolation of Aquatic Humics

The first element in the isolation of aquatic humics from the environment is source selection. In the case of our research, source selection was governed by the following requirements: (1) high DOC* content, (2) predominance of low molecular weight aquatic humic materials of high solubility and (3) a pure "humic" character. DOC concentration shows a positive correlation with amount of runoff, which in turn indicates that DOC is allochthonous and therefore is derived from soil organic matter. Our source water is taken from a small stream originating in the flood plain swamps of the Satilla River in South Georgia. The humic material appears to be generated in the swampy subsurface soil and flushed out during periods of high groundwater levels. There is a linear correlation between TOC and absorbance at 425 nm. Since humic substances absorb strongly at this wavelength, they appear to be the overwhelming contributors to the TOC in the Satilla River with little additional contribution from biopolymers (e.g. peptides and polysaccharides).

The predominance of low molecular weight humic substances of high solubility is believed to be due to the proximity of the point of sampling to the point of origin of the material. As a result of our continuing research on the nature of organic matter in river systems, we have come to the conclusion that low molecular weight humic substances as they are "floated"

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*DOC = dissolved organic carbon

out of the subsurface soil layers by a rising water table continue to polymerize by acid-catalyzed and oxidative coupling. This progressive humification leads to higher molecular weight material which can subsequently and successively form colloidal, microparticulate and finally floccular humic aggregates. Thus aquatic humic substances in a river system occur in particle sizes ranging above and below 0.4μm which is the conventional DOC-POC (particulate organic carbon) boundary. However, for purposes of drinking water research it was considered to be more important to place emphasis on the low molecular weight fraction since this is the very material which is most likely to escape removal by such pre-treatment procedures as flocculation, settling and filtration.

The researchers were aware of the importance of selecting a source water for the gathering of the aquatic humic material which was nominally free from inputs of anthropogenic matter which might introduce unnecessary complications into subsequent studies performed on the isolated material. While the complete exclusion of such foreign material is not possible, particularly in the case of the dialkyl phthalates, it is desirable to hold it to a minimum. The location shown in Figure 3 was selected as the sampling site for this work. The Satilla River drains a swampy area and is relatively free from municipal, agricultural and industrial inputs of pollution. Furthermore, since the samples are collected from a region of highly leached sandy soil (kaolinite and gibbsite), the humic material thus obtained has an extremely low ash content which is easily removed leading to a highly soluble, ashfree material. A summary of the collection data is presented in Table 3.

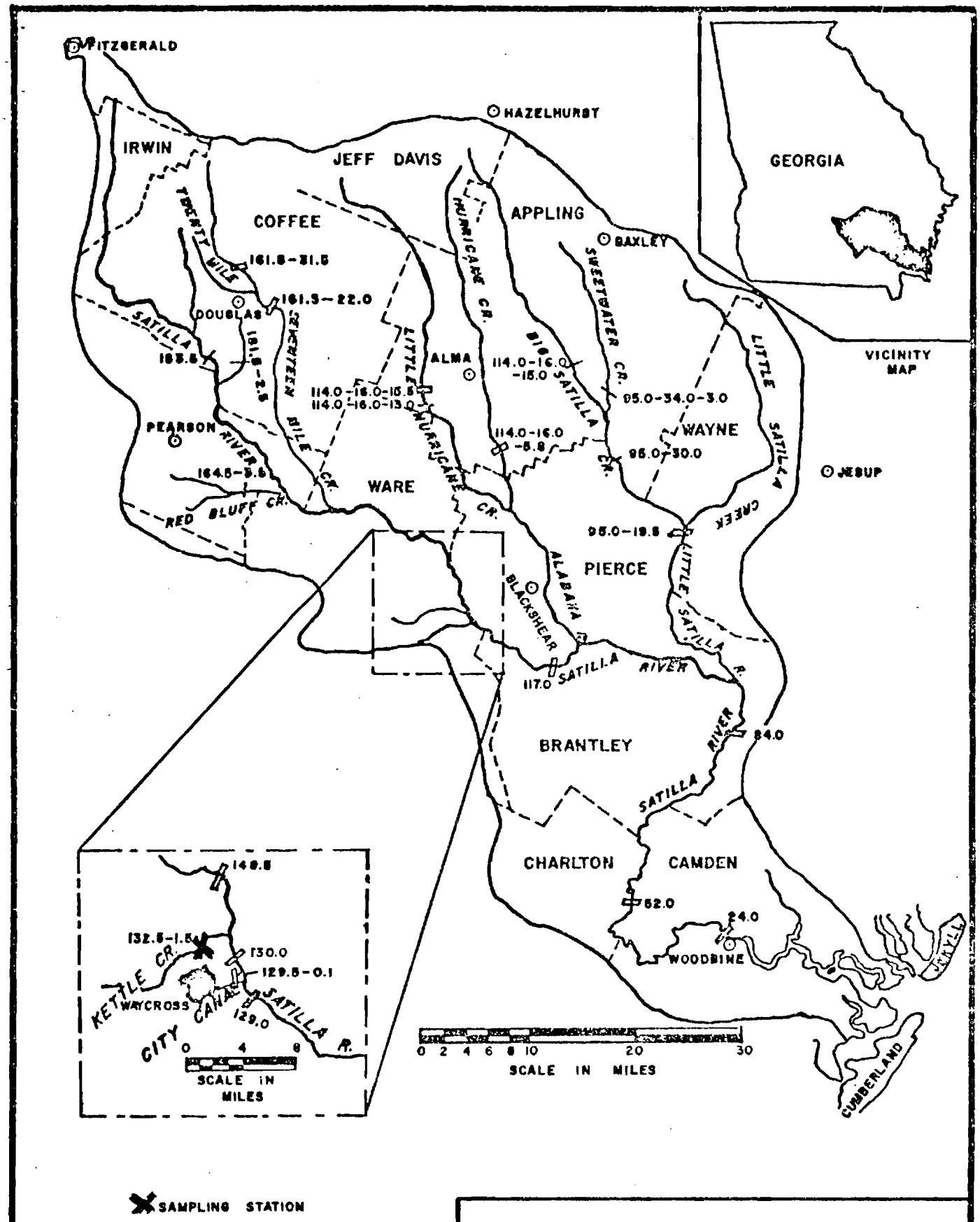


Figure 3.

MAP OF THE SATILLA RIVER BASIN

TABLE 3

COLLECTION DATA: AQUATIC HUMIC MATERIAL

Date	Level of Aquatic Humics in Source Water* (mg/L)	Liters Processed	Yield in Grams	Percent Recovery
October 13, 1977	58.5	294	39	73
December 27, 1977	45.5	411	23	60
May 24, 1978	51.5	390	24	60
October 5, 1978	7.0	395	3.3	61

*Based on UV absorbance at 425 nm

By avoiding the use of strong bases such as sodium hydroxide for the elution of the XAD resins, the oxidative coupling of phenolic groups and other base-catalyzed condensation reactions is minimized. In retrospect, it might also have been useful to exclude air from the system while it is at high pH although this precaution was not actually taken. The choice of a tertiary organic amine as the eluting base avoids the alteration of the concentrated aquatic humic substance via the inclusion of nitrogen as is known to occur with primary and secondary amines. Liquid-liquid extraction with chloroform is employed to eliminate trimethylammonium hydrochloride. It will be noted that this step is omitted if the isolated aquatic humics are destined for use in trihalomethane-generation studies. Since the acidic functions at this point are present as triethylammonium salts, it is desirable to replace these with protons by means of ion exchange treatment. This treatment also serves as a desalting step by removing other cations which might also be present. The final product is completely soluble in both water and methanol.

The convenience of the method permits the isolation of aquatic humic material in batches of 20-40 grams. It would not have been inconvenient to isolate the aquatic humic material in hundred-gram lots. The Project Manager is willing to provide EPA with large quantities of a representative aquatic humic material should EPA desire to conduct some in-house projects on aquatic humics. Enough material is on hand at both Georgia Tech and Bemidji State University to permit the immediate resumption of work should additional funding become available.

Physical and Chemical Properties

Some physical and chemical properties of the light brown amorphous solid are as follows:

Elemental Analysis:	C	50.13%
	H	3.50%
	N	0.80%
(by difference)	O	45.57%

Simplest formula: $C_{145}H_{123}N_2O_{100}$

IR (underivatized): 2500-3600cm^{-1} broad, strong; H-bonded OH
2950cm^{-1} strong; aliphatic CH
1700cm^{-1} broad, strong; acid carbonyl
1615cm^{-1} broad, strong; probable C=C

IR (after methylation):

3200-3600cm^{-1} broad, weak; non-acidic OH
3000cm^{-1} sh, weak; unsaturated CH
2950cm^{-1} strong,; aliphatic CH
1725cm^{-1} strong; ester carbonyl
1600cm^{-1} medium; probable C=C
1430cm^{-1} medium; possible C=C-H bend

NMR (exhaustively methylated):

80.7-81.7	broad, aliphatic	5H
83.4	broad, CH or CH_2 in electronegative environment	8H
83.8	broad, OCH_3	20H
87.35	sharp, aromatic	2H

Acid-Base Chemistry of Aquatic Humics

In the analysis of oxygen containing functional groups of water soluble humic substances no precise method has been devised in order to distinguish between carboxyl groups and phenolic hydroxyl groups. Usually one determines the amount of total exchangeable protons and considers these to be derived from the sum of both carboxyl and phenolic hydroxyl groups. Since the carboxyl functions on the humic polymer exhibit a considerable range of acidities, direct titrations are in many cases difficult to evaluate: the titration curves do rarely exhibit distinct inflection (or equivalence) points. In order to circumvent this problem, one usually reacts the humic substances with a weak base (e.g. calcium acetate) with the intention of titrating the resulting monoprotic acid (e.g. acetic acid), the titration curve of which should exhibit a sharp inflection point. Having thus arrived at a value for carboxyl content, one calculates phenolic hydroxyls by difference from the total exchangeable protons. It is obvious that the ratio of carboxyl to phenolic hydroxyl depends solely (if one considers the method for total acidity to be accurate) on the accuracy of the determination of carboxyl groups.

We have encountered a series of analytical difficulties and discrepancies in the analysis of carboxyl in aqueous humic substances which require a re-evaluation of the standard methods used for soil humic

substances (as given e.g. in Schnitzer and Khan, 1972).[8] For this reason we have investigated new analytical approaches to this problem.

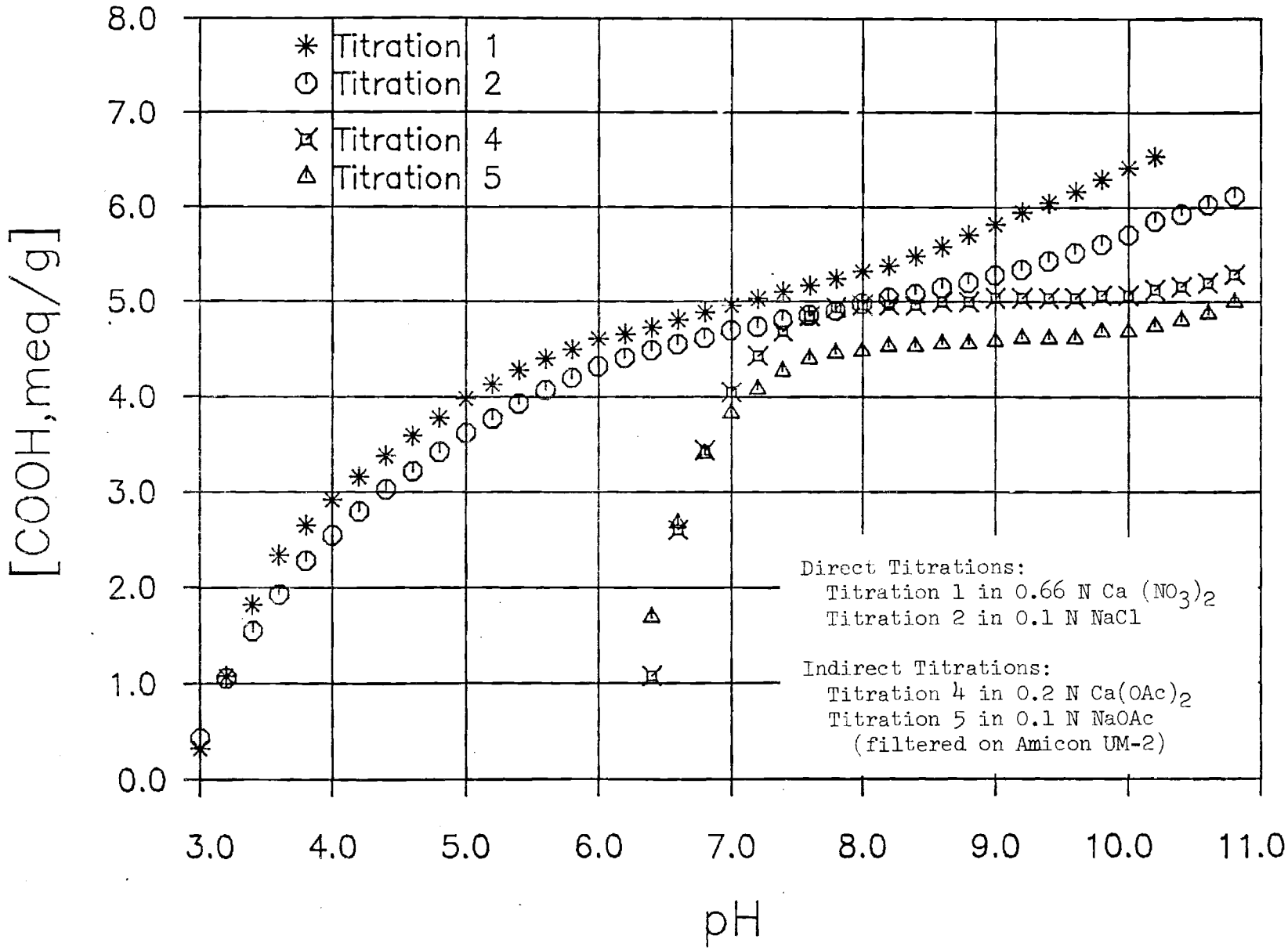
The reason for reacting humic substances with bases such as acetate or pyridine is, as mentioned above, to convert the polymer bound carboxyl function whose acid dissocation constants cover a range of values, to a monoprotic acid (e.g. acetic acid, pyridinium ion), and then to titrate this monoprotic acid after separation from the polymer. Originally the calcium-acetate method had been developed for humic acids which forms insoluble calcium salts which can then be separated from the solution containing the acetic acid by simple filtration through filter paper (e.g. Whatman #42). Water soluble humic substances, however, do not form appreciably insoluble salts. Therefore, filtration through filter papers or even 0.4 μm micropore filters does not impede passage of most of the dark colored polymer. Kwak et al. (1977)[9] have shown that acid soluble soil fulvic acids are retained by ultrafiltration through Amicon UM-2 membranes. We have likewise found a high degree of retention for aqueous humic substances on these filters, enabling us to separate acetic acid and pyridinium hydrochloride from the dark colored polymer.

The derivation of "net titration curves" (see Figures 4-6) is accomplished by subtracting the corresponding blank curves from the original titration curves. This method eliminates the effect of mere base dilution, so that only actual base consumption by titratable, i.e. acidic matter, becomes visible (shown on the ordinate in milliequivalents of base consumed per gram of humic material). A theoretical curve for the titration of a monoprotic acid with base demonstrates that as soon as the titration endpoint is reached, the slope of the curve becomes zero, indicating that all titratable substances

have been neutralized, so that no more base can be consumed. The titrations divide into two major types: 1) acidic polyprotic humic substances are directly titrated with a strong base, and 2) humic substances are reacted with a weak base (acetate, pyridine) to generate a monoprotic acid (acetic acid, pyridinium ion) which is subsequently separated from the colored humic material and titrated. In the following the problems encountered in determining the carboxyl group content of water soluble humic substances (e.g. fulvic acids, river water humic substances and the like) by potentiometric titrations will be discussed. From the shape of the "net titration curves" (T1, T2, see figure 4) resulting from the direct titration of aquatic humic matter with sodium hydroxide one recognizes the fact that acidic functions are continuously titrated even beyond pH 10. The curves have their flattest portion in the pH interval 6 to 8.6, but the slope never approaches zero. This titration behavior is characteristic for polyelectrolytes and illustrates the fact that carboxyl groups in fixed association with macromolecules exhibit a range of differential acidities. The fact that the slope of the curve never approaches zero, precludes a quantitative evaluation of the carboxyl content. Rather the flattest and almost perfectly linear ($r > 0.999$) portion of the "net titration curve" ranges from 4.6 to 5.2 milliequivalents of base consumed per gram of aquatic humic matter in the presence of 0.1 N NaCl, and from 4.6 to 5.2 meq/g in the presence of 0.666 N $Ca(NO_3)_2$. The "net titration curve" of aquatic humic matter in the presence of 0.666 N $Ca(NO_3)_2$ exhibits a second straight segment ($r > .999$) beyond 5.5 meq/g, intersecting the first one at 5.4 meq/g, indicating titratable material up to 6.5 meq/g at

Figure 4

TITRATION OF SATILLA RIVER HUMIC SUBSTANCES



pH 10.2. At pH 9.8, the endpoint recommended for the calcium acetate method, 6.3 meq/g have been titrated.

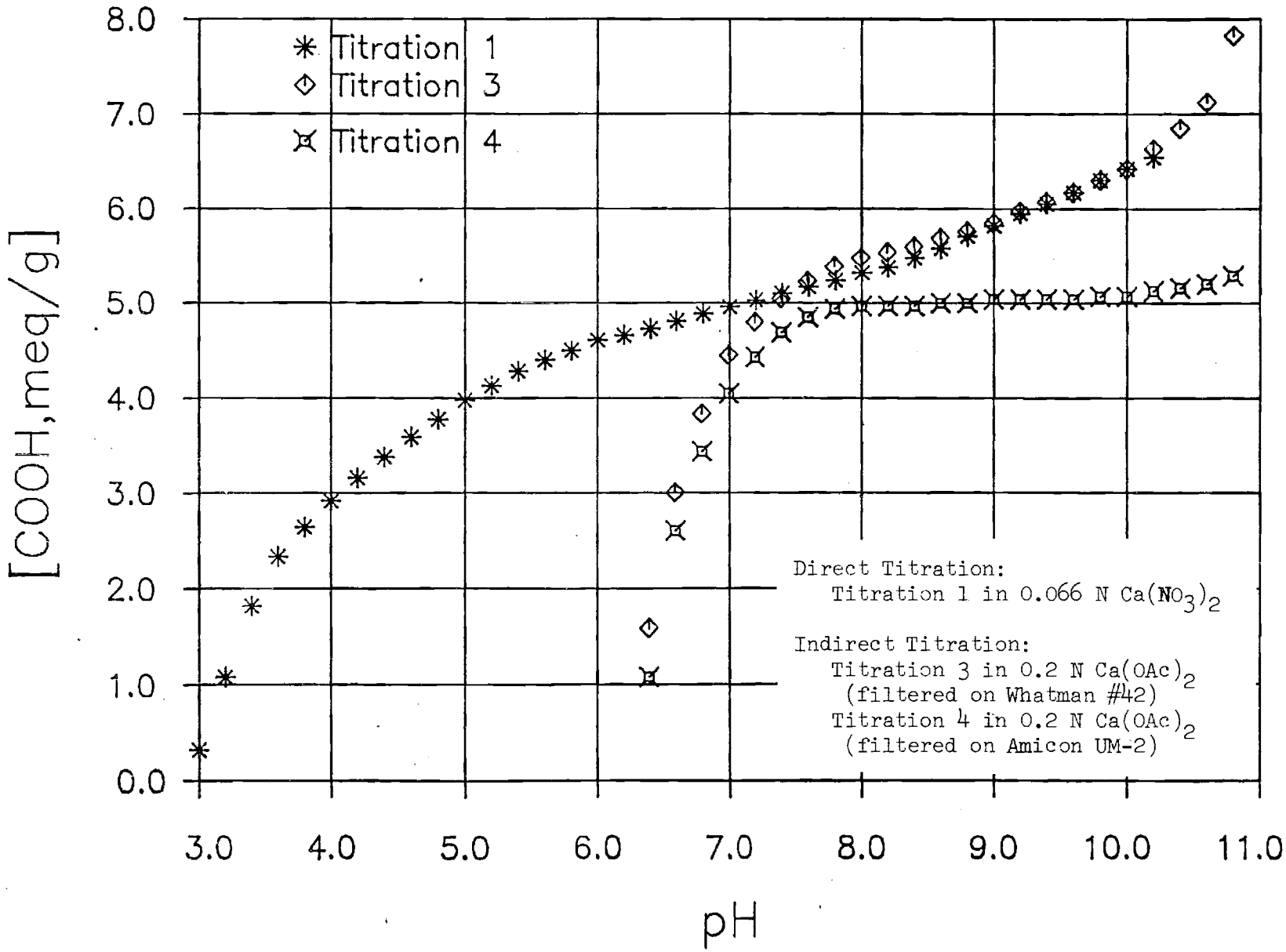
The first problem encountered in the indirect titrations is that of the separation of the monoprotic acid (e.g. acetic acid, pyridinium ion) generated by the reaction of the humic substances with the corresponding bases (acetate, pyridine). In the case of water soluble humic substances little or no solid reaction product is formed (e.g. insoluble calcium salts) which could be removed by filtration through either filter paper (e.g. Whatman #42) or micropore filters (0.4 μm). Only ultrafiltration through membranes of pore size 0.0001 μm (e.g. Amicon UM-2, 1000 MW cut-off) removes all color, as measured spectrophotometrically at 420 nm.

Typical "net titration curves" of aquatic humic matter after reaction with 0.1 M sodium acetate and 0.1 M calcium acetate and subsequent filtration through Amicon UM-2 are illustrated by T5 and T4 (see Fig. 4) respectively. The end of the titration of acetic acid is characterized by the almost horizontal region at 4.6 and 5.0 meq/g, for the titration after reaction above pH 8.4. Both titrations show an increase in slope starting at about pH 9.0, indicating the presence of small amounts of "excess" titratable material (0.3-0.4 meq/g at pH 10.8) with pKa's different from acetic acid. This may have been derived from the lower molecular weight fraction of fulvic acid which is able to pass the UM-2 filter, the nominal MW cut off of which is ^1000. Since number average molecular weights of aquatic humic substances and soil fulvic acids are on the order of 10^3, such leakage can be expected.

Titration T3 (Fig. 5) illustrates what happens if one fails to

Figure 5

TITRATION OF SATILLA RIVER HUMIC SUBSTANCES



remove the colored humic material. In this case the sample after reaction of aquatic humic matter with calcium acetate, was filtered through Whatman #42 filter paper, which allowed passage of virtually all color. Compared with titration T4, the "net titration curve" of T3 continues to rise past the level of 5 meq/g, which is equal to the amount of carboxyl groups reacted with acetate anions in the presence of Ca^{2+}. The curve never levels off. This means that after the initial neutralization of free acetic acid, additional acidic functional groups on the polymer are continuously being titrated. Indeed, from pH 8.85 (the theoretical equivalence pH for titration of acetic acid in calcium acetate) to about pH 10.2 the "net titration curve" for T3 follows exactly that for the direct titration in the presence of $Ca(NO_3)_2$ (see Fig. 5).

The analytical procedures recommended for the determination of COOH groups in fulvic acids (e.g. Schnitzer and Khan 1972, p. 40) require evaluation of the titration of fulvic acid--calcium acetate reaction products (after filtration - unspecified) at pH 9.8. According to our results, when analyzing water soluble humic substances (many filvic acids included), there is no difference between evaluating either direct or indirect (i.e. after reaction with calcium acetate) titrations at pH 9.8, <u>if colored humic substances are not removed quantitatively before titration</u>. Evaluation at pH 9.8 lies in a region of the titration curve in which in the presence of colored humic substances acidic groups of pK_a greater than that of acetic acid are being titrated.

<u>If partially neutralized humic substances have been removed quantitatively</u> (which for water soluble humics can be achieved by ultra-

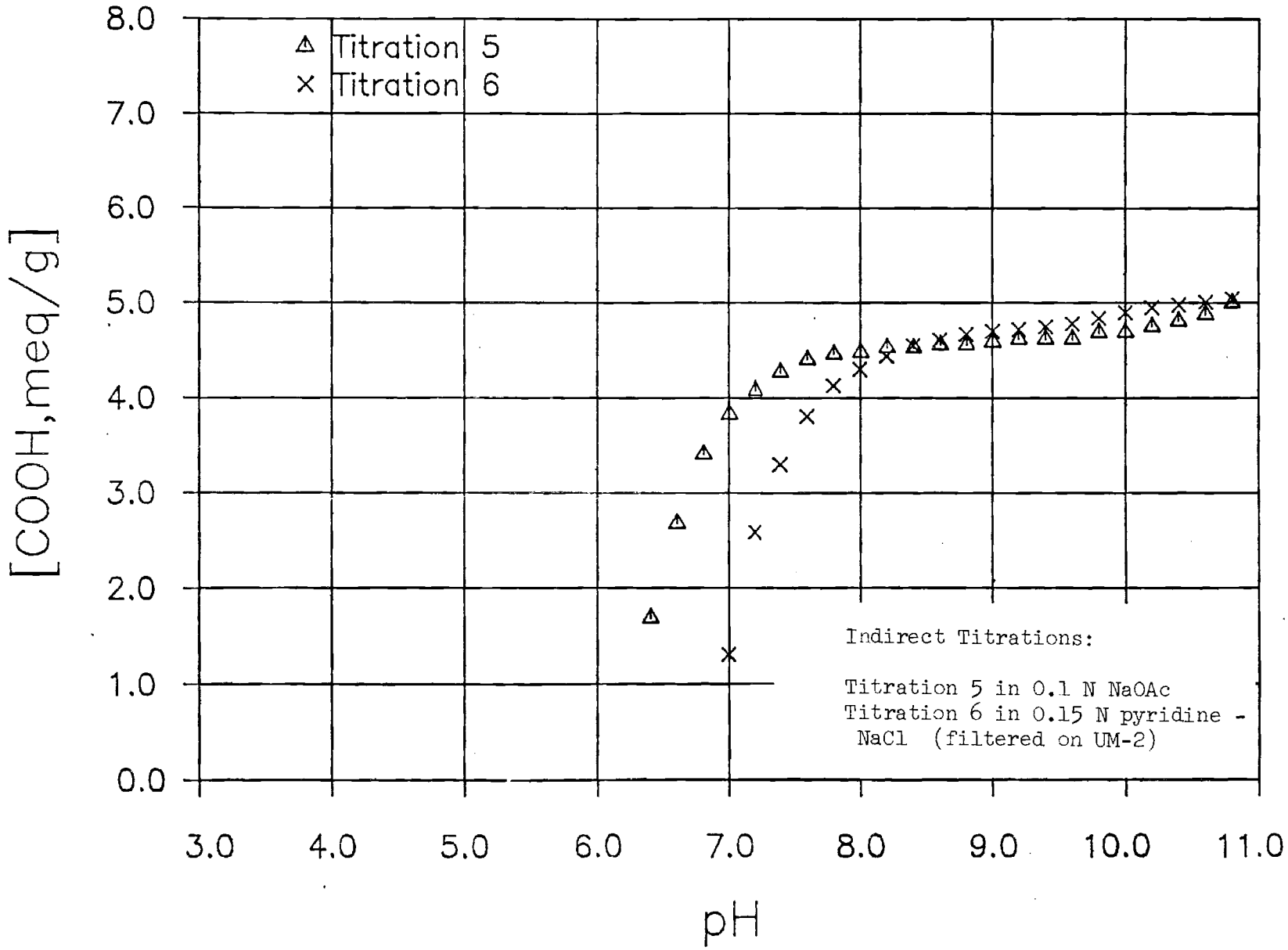
filtration), evaluation at pH 9.8 introduces an error commensurate with the efficiency of removal. Quantitative removal of colored humic material after reaction with acetate is therefore essential for the quantitative analysis of those carboxyl groups which are sufficiently acidic to protonate acetate ion.

Comparison of "net titration curves" T5 and T4, and T2 and T1 (see Fig. 4) reveals another important aspect of apparent carboxyl acidity. Titrations T2 and T5 were done in the presence of Na^+, T1 and T4 in the presence of Ca^{2+} as the counter ion. In the presence of Ca^{2+} the apparent COOH yield is increased. This increase, although small ($\sim$0.4 meq/g) is consistent and significant (in the case of aquatic humic matter $\sim$9%). Differences in ionic strength and base concentration can be neglected in producing this effect. We propose that calcium binding be considered the responsible factor for this increase. The positive charge associated with - $(COO^-Ca^{2+})^+$ can force an increase in acidity on other functional groups located in its vicinity on the polymer. However, this effect probably does not discriminate between carboxyl and phenolic hydroxyl functions. Thus the observed increased yield in apparent carboxyl content may be derived from either of the two types of acidic functional groups. For this reason it appears that a divalent counter ion, such as Ca^{2+}, should be avoided when reacting humic substances with acetate for carboxyl group analysis.

Titration T6 (Fig. 6) shows another aspect to be considered in carboxyl group analysis by means of pK-dependent methods. When reacted with pyridine, aquatic humic matter yields a slightly higher COOH content

Figure 6

TITRATION OF SATILLA RIVER HUMIC SUBSTANCES



as measured by titrating the resulting pyridinium ion. Since pyridine (pK_b = 8.83) is a slightly stronger base than acetate (pK_b = 9.25), a higher yield in COOH can be expected on these grounds alone. This differential yield illustrates the fact that pK dependent methods of carboxyl analysis will always give results dependent on the pK of the reacting base, since COOH groups on the polymer, as the direct titrations demonstrate, have a wide range of pK_a values. It appears therefore reasonable to redefine carboxyl content as the abundance of those acidic functional groups which are sufficiently acidic to protonate acetate ion (or pyridine) in the presence of a monovalent counter ion (e.g. Na^+), with acetate ion to be in sufficient excess (e.g. tenfold). Those acidic functional groups which are not protonated under these conditions may still in part be carboxyl groups but are excluded from the arbitrary definition since they are not acidic enough to react with acetate ion. It must be recognized therefore, that by using pK-dependent methods for the determination of carboxyl group content one has to compromise: directly titratable acidic functions in excess of those determined by reaction with acetate ion may still in part be carboxylic functions. However, so far none of the quantitative titration methods used can distinguish between carboxyl and phenolic hydroxyl functions in a sequence of progressively weaker acidic functional groups associated with humic polymers. This situation will prevail unless a method becomes available to block phenolic hydroxyl groups stably and selectively to allow reaction of the humic material with a series of increasingly stronger bases. Acetylation of humic material does not serve this purpose since the acetyl ethers are subject to alkaline hydrolysis.

Reaction of Aquatic Humics with Permanganate

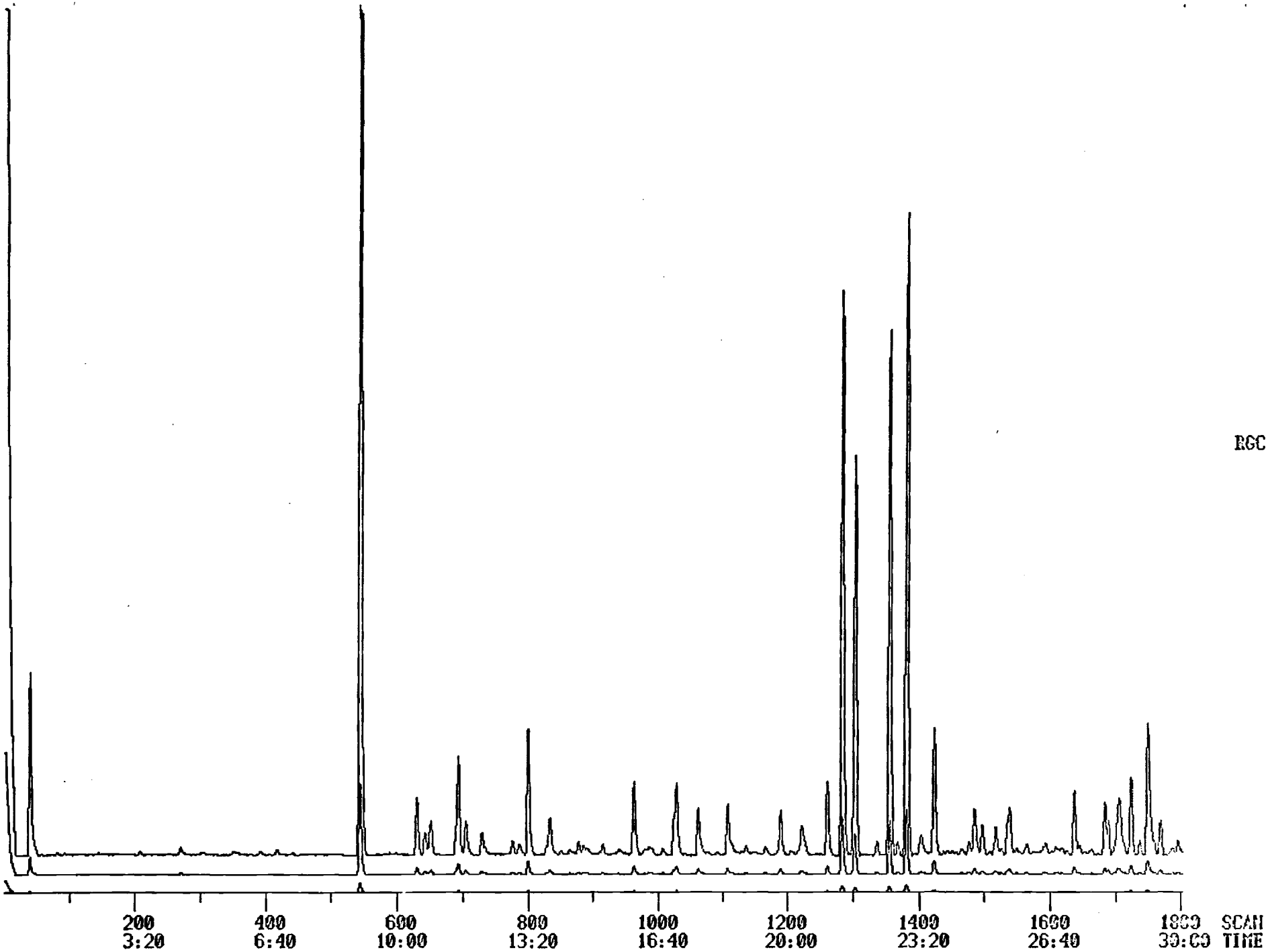
Humic substances have been considered by many workers in the field to consist of predominantly aromatic building blocks. Therefore benzene carboxylic and phenolic acids are expected to dominate the product mixtures upon oxidative degradation, particularly after prior protection of existing acidic functional groups on the aromatic rings in the form of their methyl ethers and esters. We have found a different picture to be the case for the immature aquatic humics isolated from the Satilla River. In the material isolated as previously described, aliphatic materials dominate the degradation product mixtures, particularly when mild conditions of oxidation are employed. Since the percentage yields rise significantly as the conditions of the oxidation reaction are moderated (see Table 2) while the absolute amount of aromatic products remains unchanged, it can be concluded that experiments of earlier workers were not designed to facilitate this observation. Thus it appears from our experimental evidence that aquatic humic matter has more aliphatic character than has generally been assumed.[10,11] On the other hand, one can argue that the natural maturation process whereby aquatic humic matter is transformed from a predominantly soil fulvic acid-type material to a mixture in which soil humic acid-type material becomes a significant fraction, selectively eliminates the less refractory, more aliphatic portions of the initial material. Thus different results could be expected if the aquatic humic matter had been collected from river water under low-flow conditions or from a body of water having a long residence time for the organic matter contained therein. Supportive evidence for this lack of aromatic dominance is offered by the IR (i.e., after methylation only a rather small absorbance remains in the 1600-1640cm^{-1} region) and the NMR (only 15% Ar-H).

The aforementioned product mixtures were analyzed by capillary column gas chromatography following a second treatment with diazomethane. The resolution of the capillary column is such that it is not unusual to resolve more than 100 peaks. A typical early result is shown in Figures 7 and 8. Structures are suggested by a computerized library searching routine an example of which is presented in Figure 9. In this case, the spectrum shown in the upper display corresponds to the major gas chromatographic peak seen in Figure 7. The lower display corresponds to that of the first choice suggested by the data system; i.e. dimethyl succinate. Since the structure is reasonable for the position in the chromatogram and the treatments given to the sample and since the major ions in the chromatogram can be rationalized in a fragmentation sequence (see Figure 10), the structure was accepted.

Other structures were assigned on the basis of a rigorous interpretation of the electron impact (EI) and chemical ionization (CI) data without the assistance of a good match with library spectra. The use of complementary EI/CI data was made possible even in the case of very complex mixtures by carefully matching conditions so that the EI and CI runs could be very nearly overlaid. An example of this matching is provided by Figures 11 and 12. Examples of an assignment made on an interpretive basis are provided in the section dealing with the chlorination of aquatic humics presented later in the text. A complete list of the oxidation products identified during the course of this project is presented in Table 4.

Computerized data processing clearly indicates the dominance of aliphatic acids with succinic and oxalic acids being the most abundant.

Figure 7. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Scans 1-1800 (enhanced).

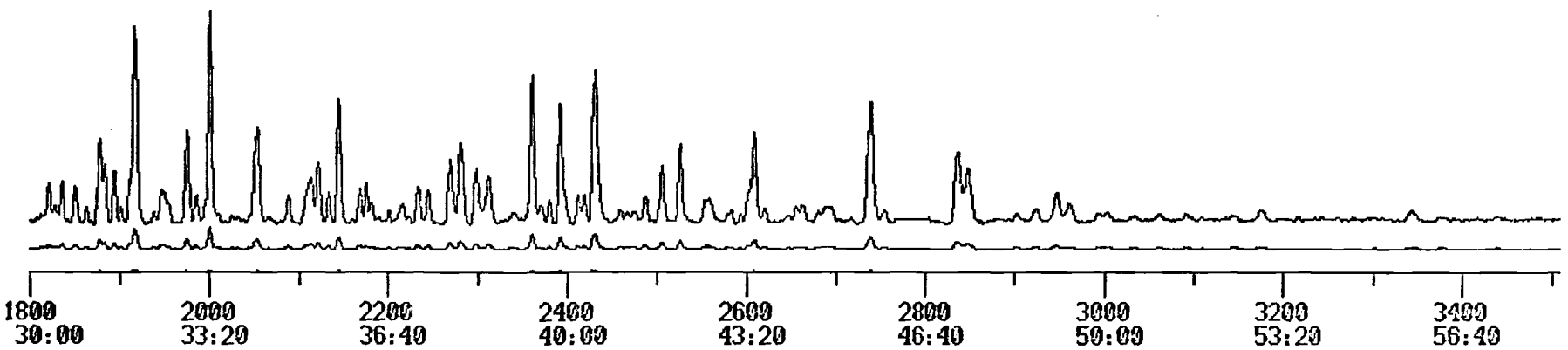


RGC
DATE: 08/23/77 TIME: 1030
SAMPLE: SAMPLE M/14

SAMPLE RUN: M14A$
CALIB. RUN: M14ACAL

SCANS 1800 TO 350.

Figure 8. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Scans 1800-3507 (enhanced).



RGC

LIBRARY SPECTRA
DATE: 08/23/77 TIME: 1030

SAMPLE RUN: M14A
CALIB. RUN: M14ACAL

SCAN 543
RET. TIME: 9:02

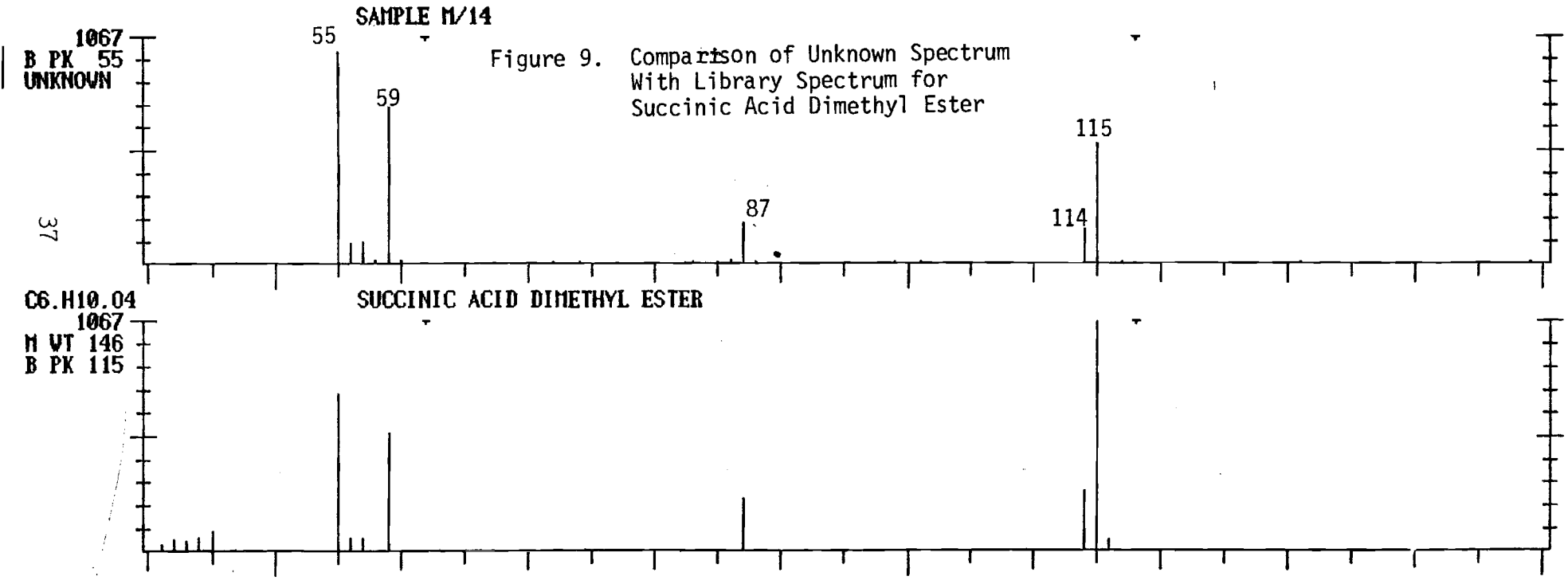
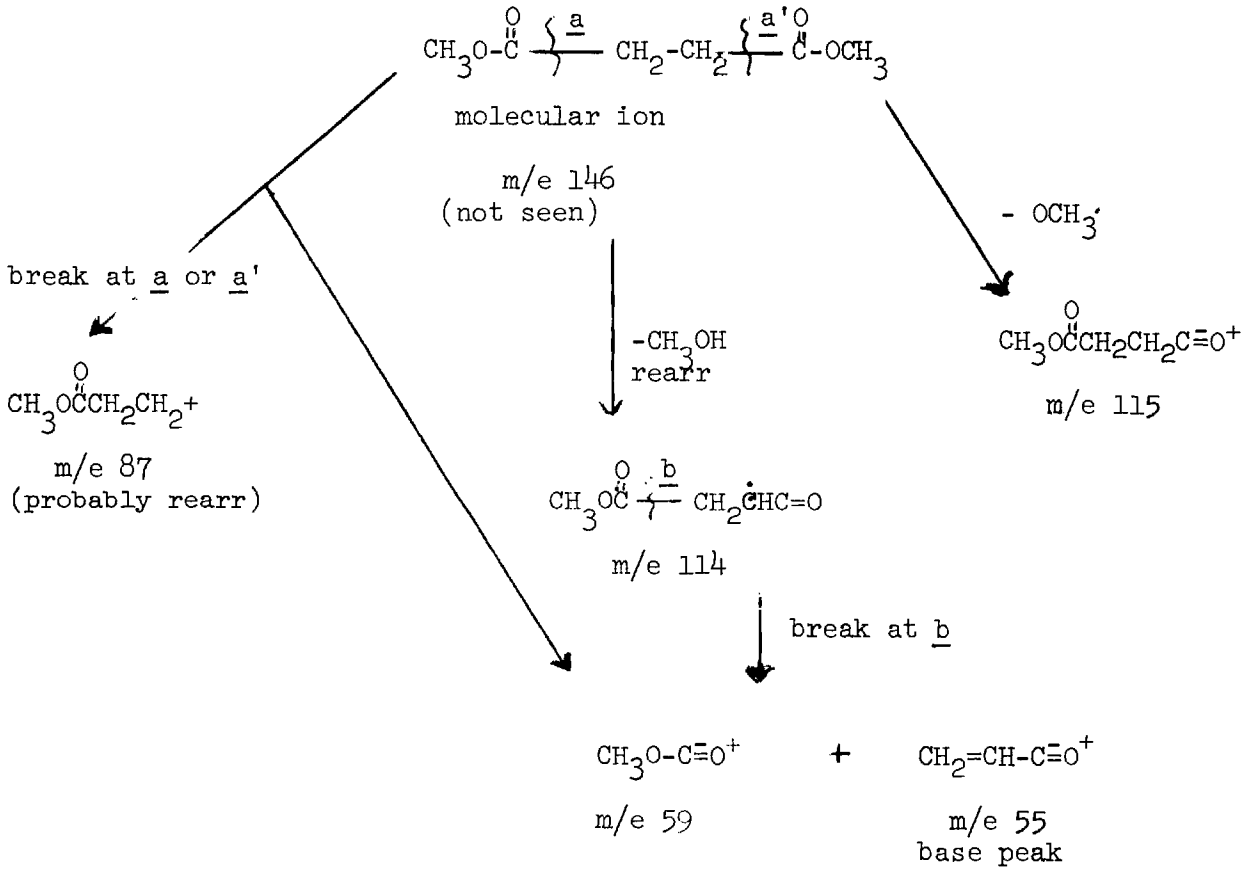


Figure 9. Comparison of Unknown Spectrum With Library Spectrum for Succinic Acid Dimethyl Ester

Figure 10. Fragmentation Sequence for Dimethyl Succinate.



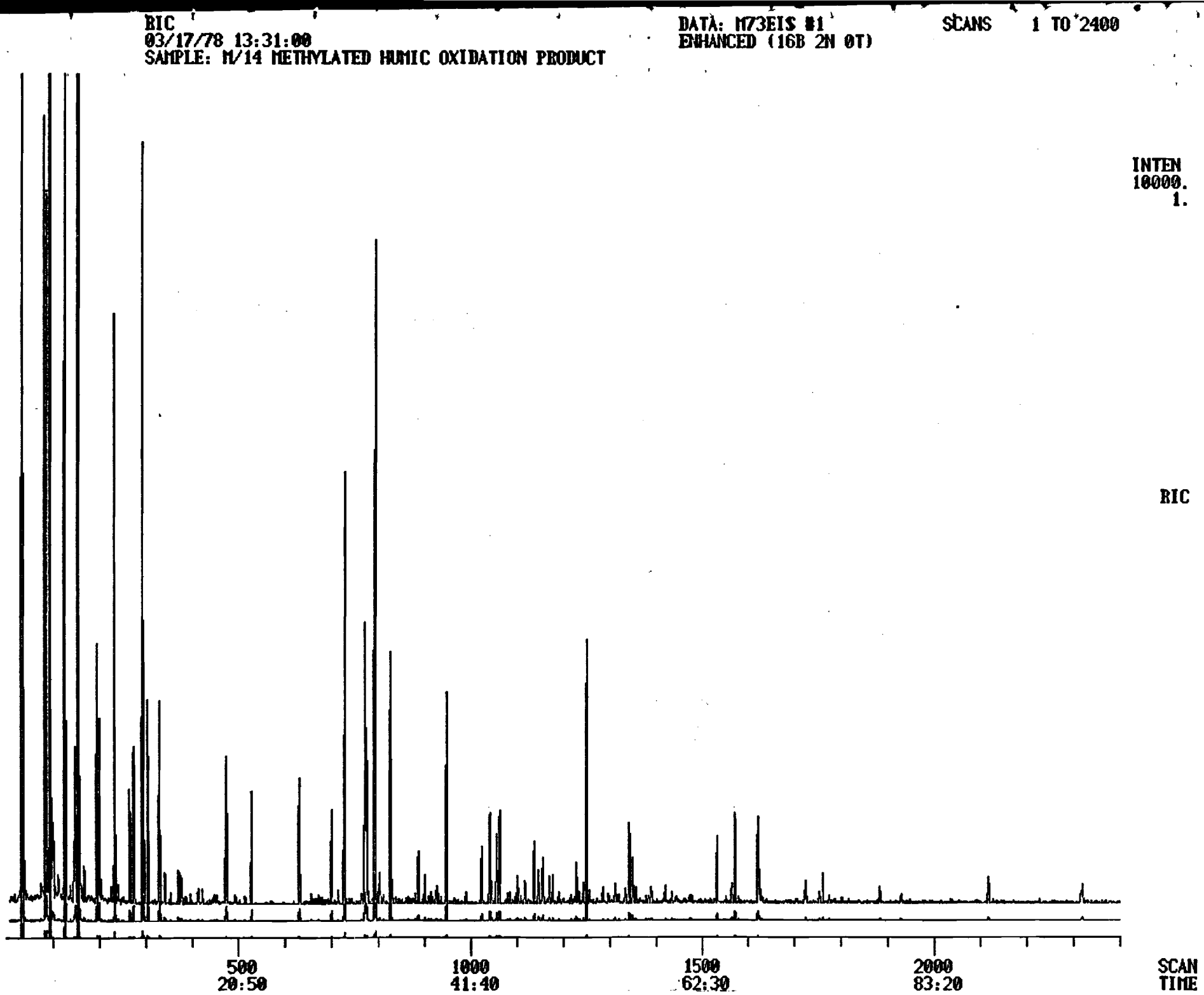


Figure 11 EI - Ion Chromatogram Methylated Humic Oxidation Products

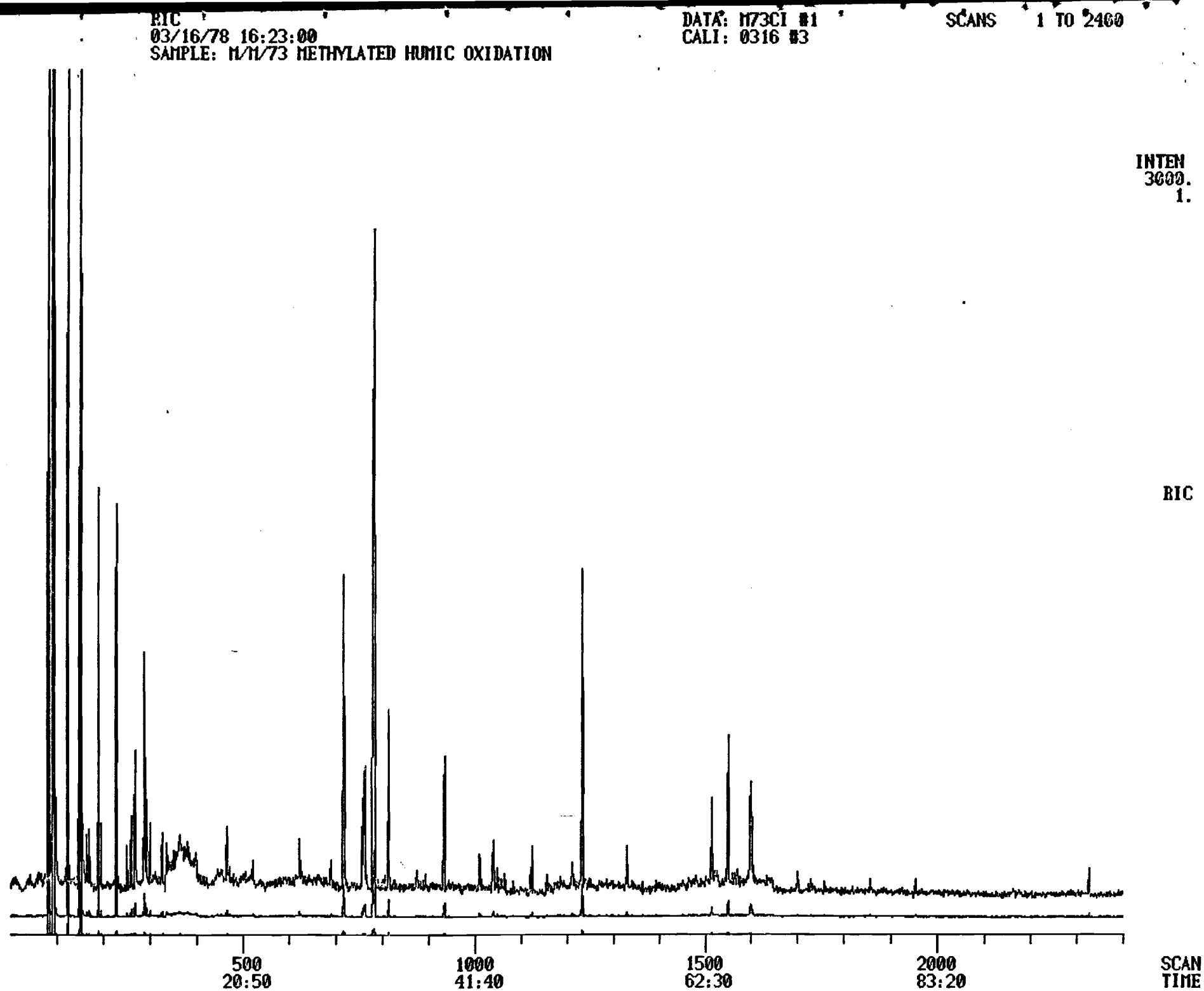


Figure 12 CI Ion Chromatogram Methylated Humic Oxidation Products

TABLE 4

OXIDATION PRODUCTS DERIVED FROM AQUATIC HUMIC MATERIAL

(acids and phenols chromatographed and identified after methylation)

1. Oxalic Acid
2. 3-Oxopropionic Acid
3. Malonic Acid
4. 2-Hydroxy-2-methylpropionic Acid
5. Levulinic Acid
6. Succinic Acid
7. Mandelic Acid
8. Benzoic Acid
9. Methylmalonic Acid
10. Methylsuccinic Acid
11. 2,2-Dimethylsuccinic Acid
12. Glutaric Acid
13. 5-Methylfuroic Acid
14. 4-Methoxybutanoic Acid
15. 2-Hydroxysuccinic Acid
16. 6,7-Dodecadione
17. Adipic Acid
18. Tartaric Acid
19. Oxaloacetic Acid
20. 3-Methoxybenzoic Acid
21. Tricarballylic Acid
22. Tetramethoxybenzene Isomer
23. Phthalic Acid
24. 3-Hydroxypentane-1,3,5-tricarboxylic Acid
25. 2-Hydroxypentane-1,3,5-tricarboxylic Acid
26. Dimethoxybenzoic Acid Isomers
27. Benzene-1,2,4-tricarboxylic Acid
28. Benzene Pentacarboxylic Acid
29. Octanoic Acid
30. 3-Methylglutaric Acid
31. Terephthalic Acid
32. Isophthalic Acid
33. Benzene Tricarboxylic Acid Isomers
34. Benzene Tetracarboxylic Acid Isomer
35. 3-Methoxybenzaldehyde
36. 4-Methoxyacetophenone
37. 3,4-Dimethoxybenzaldehyde
38. p-Coumaric Acid
39. 4-Benzylbenzoic Acid
40. 4-Methyladipic Acid
41. Pimelic Acid
42. 4-Methoxphenylacetic Acid
43. 4-Hydroxy-3-methoxybenzoic Acid
44. Azeliac Acid

TABLE 4, cont'd

45. Hexadecanoic Acid
46. Tetradecanoic Acid
47. Citric Acid
48. Fumaric Acid
49. 4-Methoxybenzoic Acid
50. Dodecanoic Acid
51. 4-Methoxymandelic
52. Methoxyphthalic Acid Isomers
53. Dimethoxyphthalic Acid Isomers
54. Trimethoxyphthalic Acid Isomers
55. Xanthenone
56. 5-Methylfuran-2,4-dicarboxylate

Using the least rigid search criteria and searching against the aqueous permanganate run with the highest yield, one obtains the percentage compositions outlined in Table 5. The list, of course, is incomplete in that many of the components remain unidentified. Only those products stable and volatile enough to travel through the gas chromatograph are counted. Nevertheless, the overall results remain useful. Differences from batch to batch of aquatic humics are evident but since reaction conditions were always being modified, it is not possible to attribute these differences to differences in the composition of the aquatic humic materials themselves.

It should be further emphasized that the results shown in Table 4 have been assembled from data gathered from five different runs. All of the dominant components have been found more than once.

Oxidation of Aquatic Humics with Iodine in Base

The treatment of aquatic humics in base with a large excess of iodine provided iodoform, 13.4 mg, mp 122° (lit. 123°) upon direct extraction with ethyl ether. The amount of material isolated corresponded to a 0.8% yield as chloroform on a weight/weight basis which is in excellent agreement with reported values.[4,12] This result suggests that working with iodine (and possibly bromine) in place of chlorine may provide new knowledge which directly relates to chlorination practices because the products of iodine or bromine incorporation will have higher molecular weights and can therefore be more conveniently isolated. A list of reaction products, some of which

TABLE 5

PERCENT COMPOSITION OF SEMIVOLATILE PRODUCTS OBTAINED FROM THE AQUEOUS PERMANGANATE OXIDATION OF AQUATIC HUMICS *

	Compounds	Area	% Total
1.	Dimethyl Oxalate	55201	12.5
2.	Methyl 3-Oxopropionate	7989	1.8
3.	C	31387	7.1
4.	Dimethyl Malonate	44099	10.0
5.	Methyl-2-hydroxy-2-methyl Propionate (tentative)	5586	1.3
6.	Chloroform	By Inspection	NA
7.	Methyl Levulinate	11924	2.7
8.	F-1-G	By Inspection	NA
9.	Dimethyl Succinate	90479	20.5
10.	H-1-I	Not Found	0.0
11.	I	Not Found	0.0
12.	K-1-L	By Inspection	NA
13.	M	1234	0.3
14.	S-Ethyl Pentanethioate (tentative)	Not Found	0.0
15.	Mandelic Acid (tentative)	309	0.1
16.	Methyl Benzoate	Not Found	0.0
17.	Methyl Dimethylmalonate	Not Found	0.0
18.	Methyl Dimethylsuccinate	7105	1.6
19.	Dimethyl 2,2-Dimethylsuccinate	3394	0.8
20.	Dimethyl Glutarate	6163	1.4
21.	Methyl 5-Methylfuran-2-carboxylate	5510	1.3
22.	J	11155	2.5
23.	Q	24649*	5.6
24.	Methyl-4-methoxybutanoate (tentative)	2021	0.5
25.	R-1-S	By Inspection	NA
26.	S	24649*	5.6
27.	Dimethyl 2-Hydroxysuccinate	Not Found	0.0
28.	U	4830	1.1
29.	V	Not Found	0.0
30.	W	By Inspection	NA
31.	Y	Not Found	0.0
32.	Z	347	0.1
33.	A'	1989	0.5
34.	B'-3-C'	Not Found	0.0
35.	B'-5-C'	Not Found	0.0
36.	C'	3618	0.8
37.	Dimethyl Adipate	1097	0.3
38.	F'	1163	0.3
39.	G'	313	0.1

*Acids and phenols chromatographed and estimated as their methyl esters and ethers.

TABLE 5, cont'd

40.	G'-1-H'	Not Found	0.0
41.	H'	641	0.2
42.	Dimethyl Tartrate	2472	0.6
43.	J'	536	0.1
44.	Methyl Ether of Dimethyloxaloacetate	4644	1.1
45.	X	Not Found	0.0
46.	L'	3113	0.7
47.	M'	1086	0.3
48.	N'	429	0.1
49.	N'-1-O'	100	0.1
50.	N'-2-O'	Not Found	0.0
51.	Methyl 3-Methoxybenzoate	5080	1.2
52.	O'-1-P'	By Inspection	NA
53.	P'	4590	1.0
54.	P'-1-A'	By Inspection	NA
55.	Q'	By Inspection	NA
56.	Trimethyl Tricarballylate	9260	2.1
57.	R'-2-S'	18172*	4.1
58.	S'	18172*	4.1
59.	T'	Not Found	0.0
60.	Dimethyl Phthalate	609	0.1
61.	V'	277	0.1
62.	Trimethyl 3-Hydroxypentane-1,3,5-tricarboxylate	1434	0.3
63.	Y'	109	0.1
64.	A'	Not Found	0.0
65.	A''	Not Found	0.0
66.	Trimethyl 2-Hydroxypentane-1,3,5-tricarboxylate	943	0.2
67.	C''	319	0.1
68.	D''	461	0.1
69.	D''	Not Found	0.0
70.	F''	349	0.1
71.	F''-1-G''	By Inspection	NA
72.	G''	Not Found	0.0
73.	H''	187	0.1
74.	I''	450	0.1
75.	J''	Not Found	0.0
76.	K''	5707	1.3
77.	M''	Not Found	0.0
78.	O''	1447	0.3
79.	N''	Not Found	0.0
80.	Q''	84	0.1
81.	R''	Not Found	0.0
82.	S''	299	0.1

TABLE 5, cont'd

83.	T''	333	0.1
84.	V''	By Inspection	NA
85.	Trimethyl Benzene-1,2,4-Tricarboxylate	1576	0.4
86.	X''	Not Found	0.0
87.	Z''	254	0.1
88.	A''-1-A''	By Inspection	NA
89.	A'''	208	0.1
90.	B'''	Not Found	0.0
91.	C'''	Not Found	0.0
92.	D'''	Not Found	0.0
93.	F'''	Not Found	0.0
94.	G'''	Not Found	0.0
95.	I'''	Not Found	0.0
96.	L'''-1-M'''	1676*	0.4
97.	M'''	1676*	0.4
98.	P'''	1131	0.1
99.	Q'''	Not Found	0.0
100.	S'''	1073	0.3
101.	U'''	Not Found	0.0
102.	V'''	382	0.1
103.	W'''	1163	0.3
104.	Y'''	Not Found	0.0
105.	Z'''	575	0.1
106.	Pentamethyl Benzene Pentacarboxylate	1134	0.3

*Error, duplicate hit.

lend further support to this idea is presented in Table 6.

TABLE 6

REACTION PRODUCTS-OXIDATION OF AQUATIC HUMICS WITH IODINE IN BASE

Compound	Ion	Comment
Methyl Iodide	142, 127	poorly resolved
Ethyl Iodide	156, 127	poorly resolved
3-Methyl-2-pentanone	100, 71, 57, 43	poorly resolved
Benzene	78, 63, 52, 91, 39	possible artifact+
Toluene	92, 91	possible artifact+
Methylene Iodide	268, 254, 141, 127	good fit
2,2,4-Trimethyl-3-hexanone	142, 100, 85, 57, 43, 41	minor
Iodoform		confirmed by mp

Reaction of Aquatic Humics with Bromine in Acetic Acid

The product mixture resulting from the treatment of non-methylated aquatic humic material with bromine in acetic acid was analyzed by GC/MS. Surprisingly, the number of products resulting from this relatively harsh treatment was not large. The complementary EI and CI ion chromatograms are presented in Figures 13 and 14 respectively. The structures which were assigned largely on the basis of interpretation are presented in Table 7.

Chlorination Studies - General

In keeping with the intent of the sponsoring agency as well as the stated goals of the original proposal, the research carried out in connection

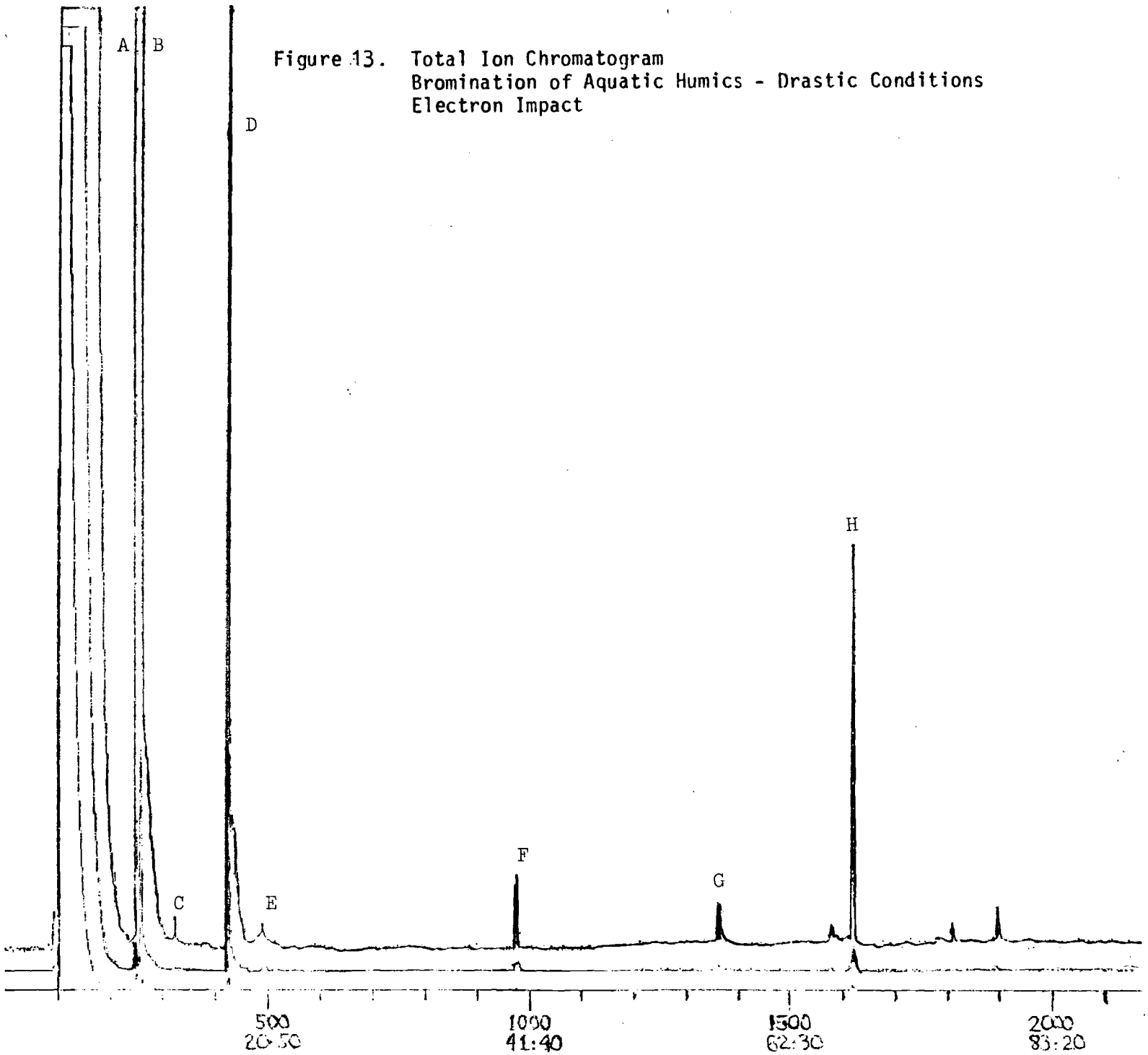


Figure 13. Total Ion Chromatogram
Bromination of Aquatic Humics - Drastic Conditions
Electron Impact

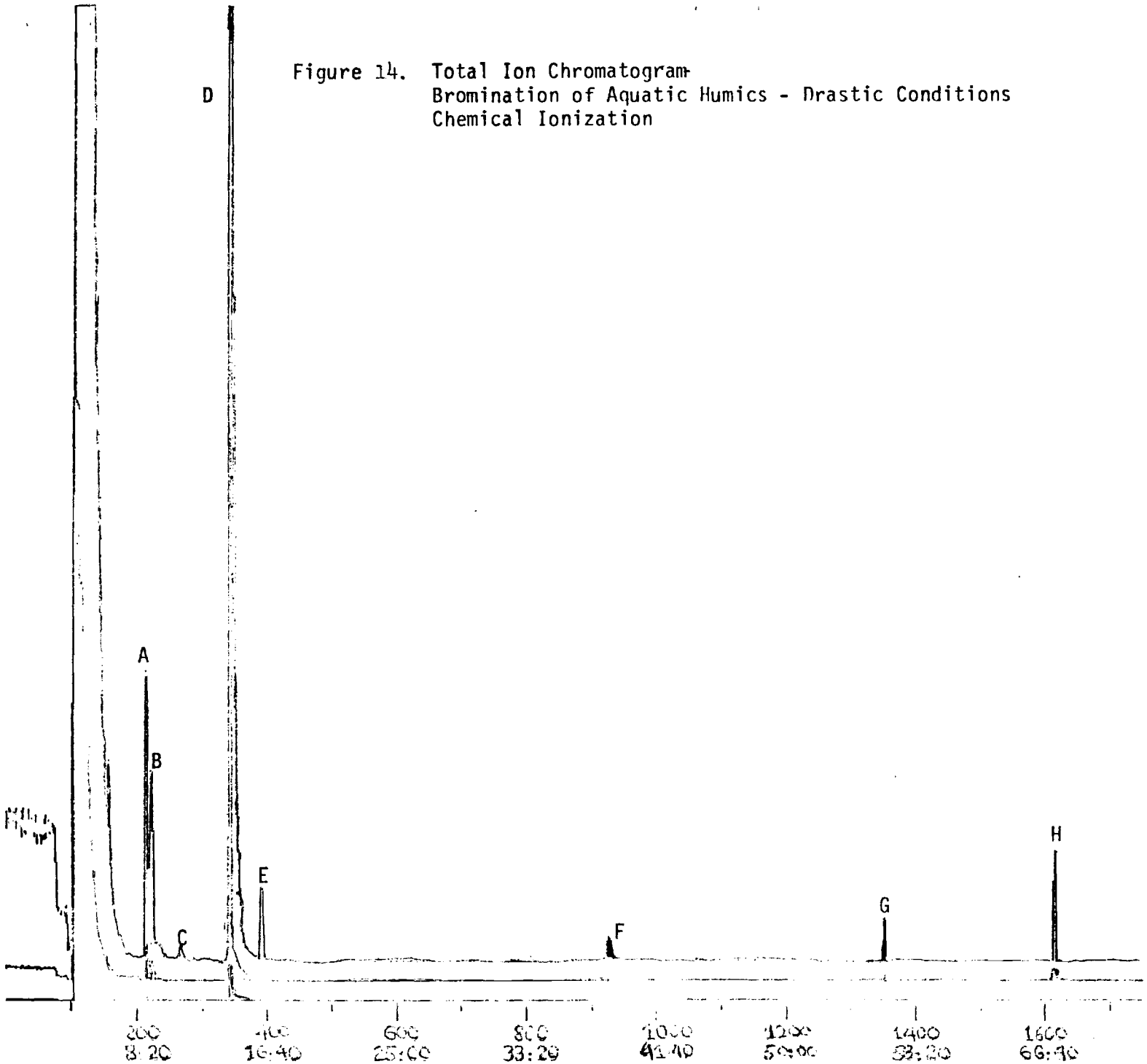


Figure 14. Total Ion Chromatogram
Bromination of Aquatic Humics - Drastic Conditions
Chemical Ionization

TABLE 7

DRASTIC BROMINATION OF AQUATIC HUMICS PRODUCT LIST

Product	Significant Ions	Comments
A. Contains Br	EI 107, 109; 71; 55; 43 CI 150, 152; 136, 138; 72	possible C-5 bromo-ketone medium component
B. Isomer of A	EI 71, 55, 43, 41 CI 150, 152; 72	possible C-5 bromo-ketone medium component
D. Bromoform	EI M^+ cluster, M-Br cluster, M-Br_2 cluster, M-HBr_2 cluster, M-HBr cluster, HBr, Br, Br_2 CI Again a misfit, with molecular ion instead of quasi molecular	matches authentic sample, major product
G. Tetrabromoacetone	EI m/e 199, 201, 203; 171, 173, 175; 120, 122; 92, 94; 121, 123; 105, 107 CI M+1 cluster; M+1-Br_2 cluster, M+1-HBr cluster	minor component

with this project has always aimed at the realistic. This has been especially true of our chlorination studies. Aquatic humics were reacted with chlorine at levels corresponding to only 5 ppm TOC (10 mg/l aquatic humics). Temperature and pH were maintained within a narrow range. Chlorine was dosed according to demand as required to maintain a residual of not more than 2 mg/l. Contact times, the use of inorganic flocculants etc. were all carried out at realistic levels. A special apparatus was constructed so that these studies might be carried out in a dynamic system simulating a typical water disinfection facility rather than in a static system as is usually employed for laboratory studies.

Although the investigators were unaware of the significance which would be later attached to it, the sample of aquatic humic material employed for the majority of the work described in this report was not entirely typical. A drought of considerable duration preceded the collection of the sample which was therefore collected immediately following the first significant rainfall which terminated the drought. Thus the aquatic humic material which was subsequently isolated was unusually rich in adsorbed organics. Furthermore, it will be recalled that the researchers sample from a small tributary rather than the main stream thus assuring a more immature isolate. The subsequent examination of the data obtained from those few runs conducted with other samples of more mature humic material showed considerable differences in the product mixtures. Accordingly the writers have decided to first present the data associated with the chlorination work in two additional subsections the first of which is concerned with

the immature humics. This discussion will be followed by a shorter presentation of information derived from reactions carried out with the more mature aquatic humics. In general, chlorination of the immature material resulted in a greater array of chlorine-containing products, presumably because this material was unusually enriched in adsorbed organics, the reactive nature of which was not as evident in mature aquatic humic matter.

Structural assignments were made chiefly on the basis of the mass spectral fragmentation patterns. Of course, supporting data such as solubility and gas chromatographic retention time were taken into account. Since the burden of structural proof rests so heavily on mass spectrometry and since authentic compounds are not generally available, the interpretive efforts practiced in connection with this project were considerably more rigorous than might otherwise be the case.

Chlorination of Immature Humics

To date, the most interesting products to be characterized among the reaction products resulting from the chlorination of immature aquatic humics are a series of chlorinated alcohols structurally related to isoprene. Their positions in a typical GC/MS total ion chromatogram are shown in Figure 15. The strucutural elucidation of such materials has been greatly enhanced by our total reliance on capillary-column gas chromatography to achieve the maximum resolution of components before they enter the source of the mass spectrometer. The complementary information provided by the matching electron-impact and chemical ionization data (see Figure 16) further increased our ability to make correct structural assignments. For

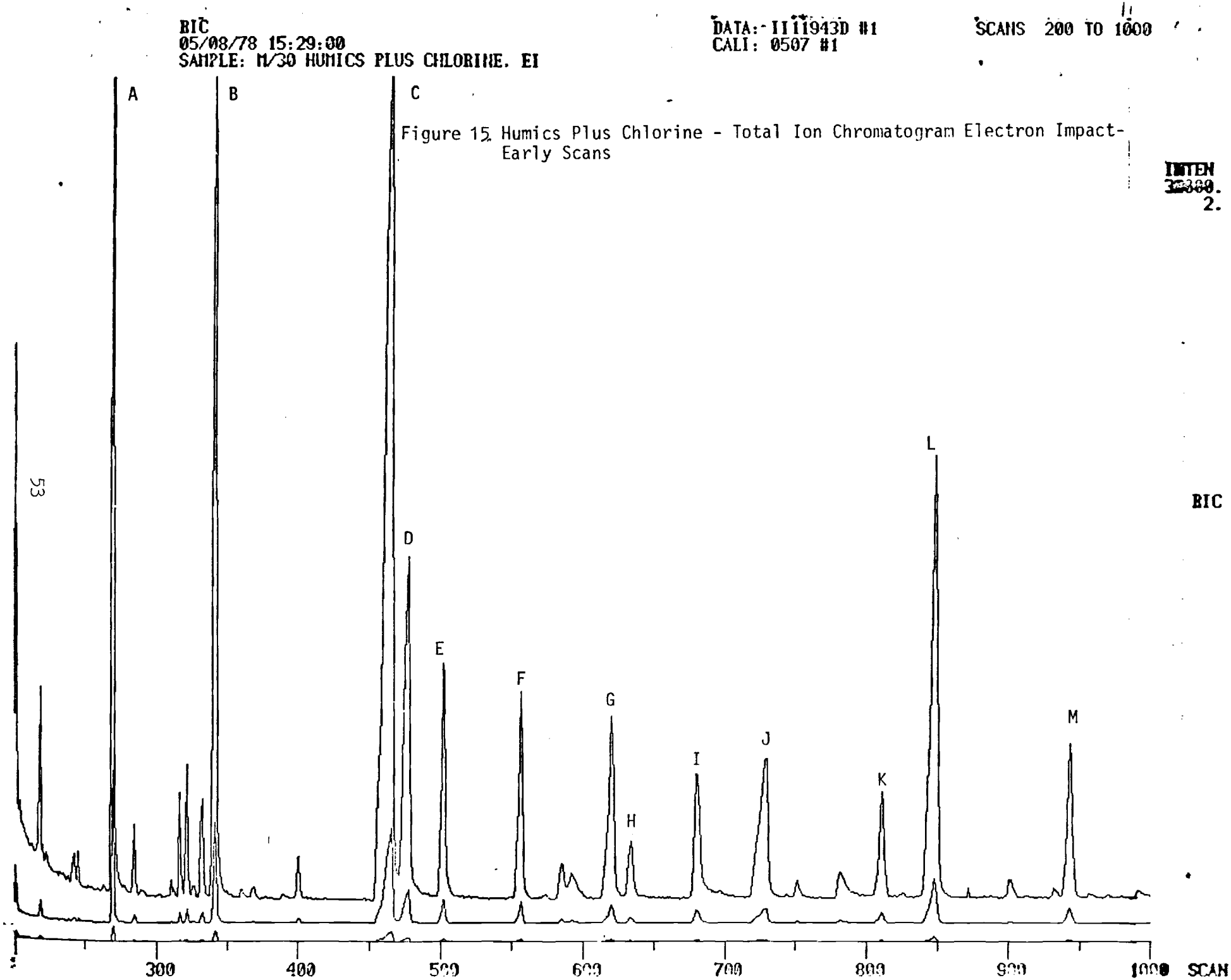


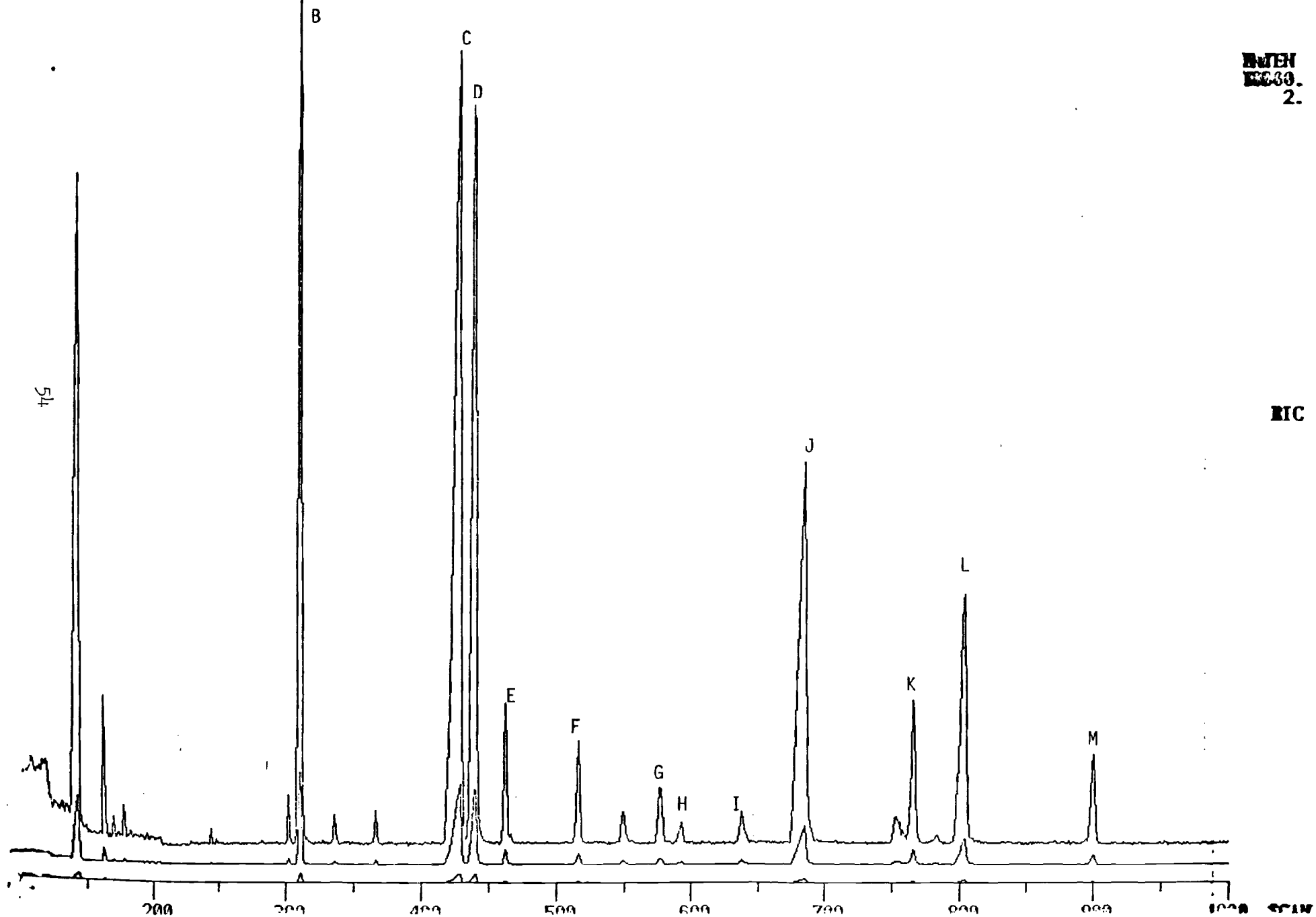
Figure 15. Humics Plus Chlorine - Total Ion Chromatogram Electron Impact-Early Scans

RIC
05/09/78 9:56:00
SAMPLE: HUMICS PLUS CHLORINE, CH4 CI

DATA: 111943DCI #1
CALI: 0509 #3

SCANS 100 TO 1000

Figure 16 Humics Plus Chlorine - Total Ion Chromatogram Chemical Ionization-Early Scans



RIC

54

example, the monochloro alcohols (3-chloro-2-methyl-2-butanol, 1-chloro-2-methyl-2-butanol, 3-chloro-2-methyl-1-butanol and 4-chloro-3-methyl-1-butanol) all showed nearly identical chemical ionization (CI) spectra with major ions at m/e 87, m/e 105 and m/e 107 (see Figure 17 for a typical case) thus indicating probable structural isomerism. The electron impact (EI) spectra were quite different, however. This fact made it possible to assign structures to each of the isomers on the basis of the differences in their EI base peaks and fragmentation patterns. A typical example is shown in Figure 18 which is compatible with the fragmentation sequence outlined in Figure 19. A more complete listing of the products identified thus far is presented in Table 8. Such advanced techniques as selected ion mapping, reversed-search quantitation and a number of other data processing methods were employed in order to obtain the maximum amount of information from each of the experiments.

A quality assurance program of considerable rigor was maintained throughout the course of this program. Sensitivity and spectral quality were monitored daily with pentafluorbromobenzene. The use of this compound is recommended by EPA as a measure of instrument performance. In the case of the work performed in connection with these studies, tuning was optimized for spectral quality at the sacrifice of some sensitivity. The Project Manager believes this approach to be best strategy for general screening purposes so that library matching and user interpretation will not be confused by random noise. A representative quality control spectrum together with the NBS/NIH library reference spectrum is presented in Figure 20. This result

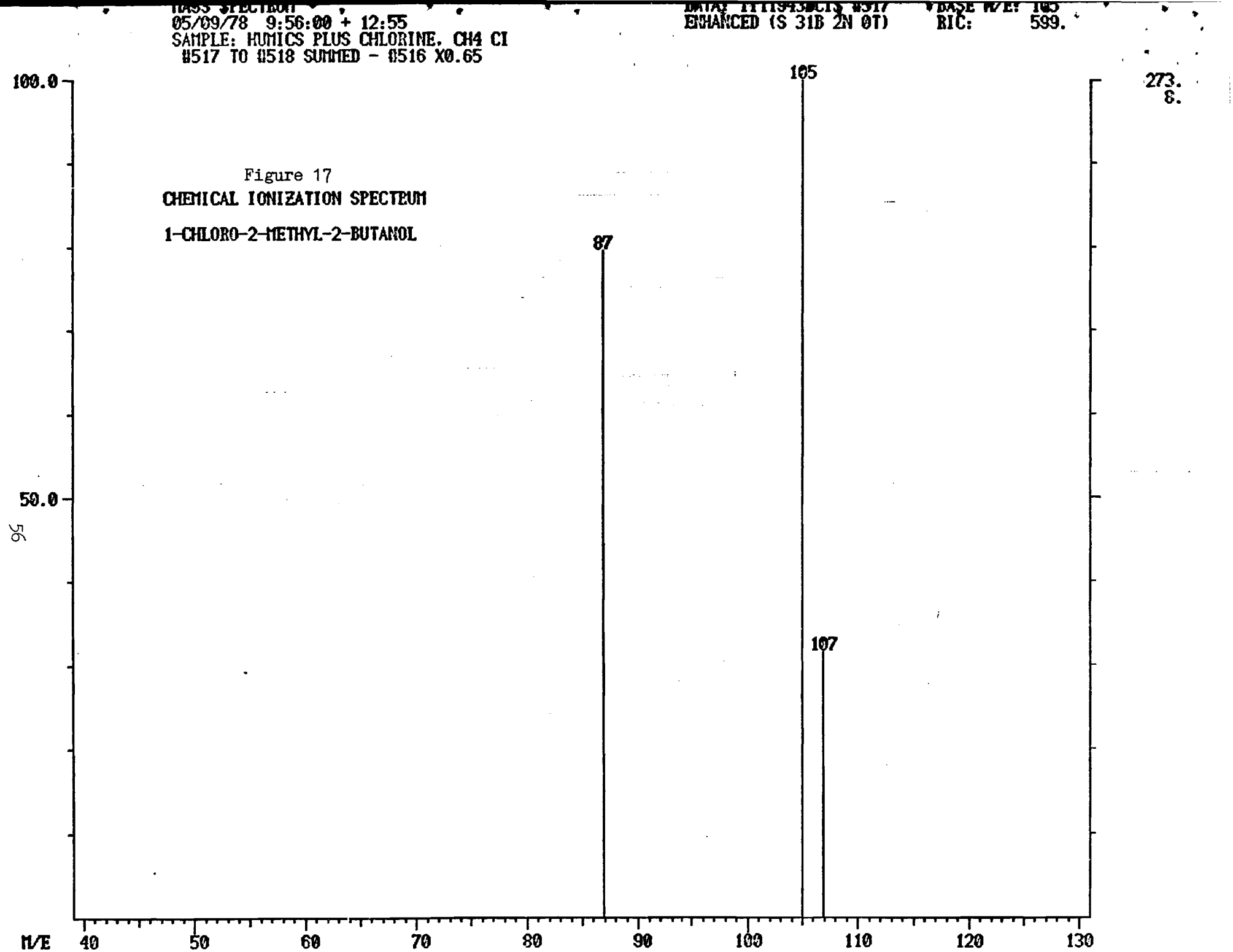


Figure 17
CHEMICAL IONIZATION SPECTRUM
1-CHLORO-2-METHYL-2-BUTANOL

05/08/78 13:29:00 + 13:54
SAMPLE: M/30 HUMICS PLUS CHLORINE. EI
#555 TO #558 SUMMED - #554 X1.50

CALI: 0507 #1

BASEPEAK: 73
RIC: 11568.

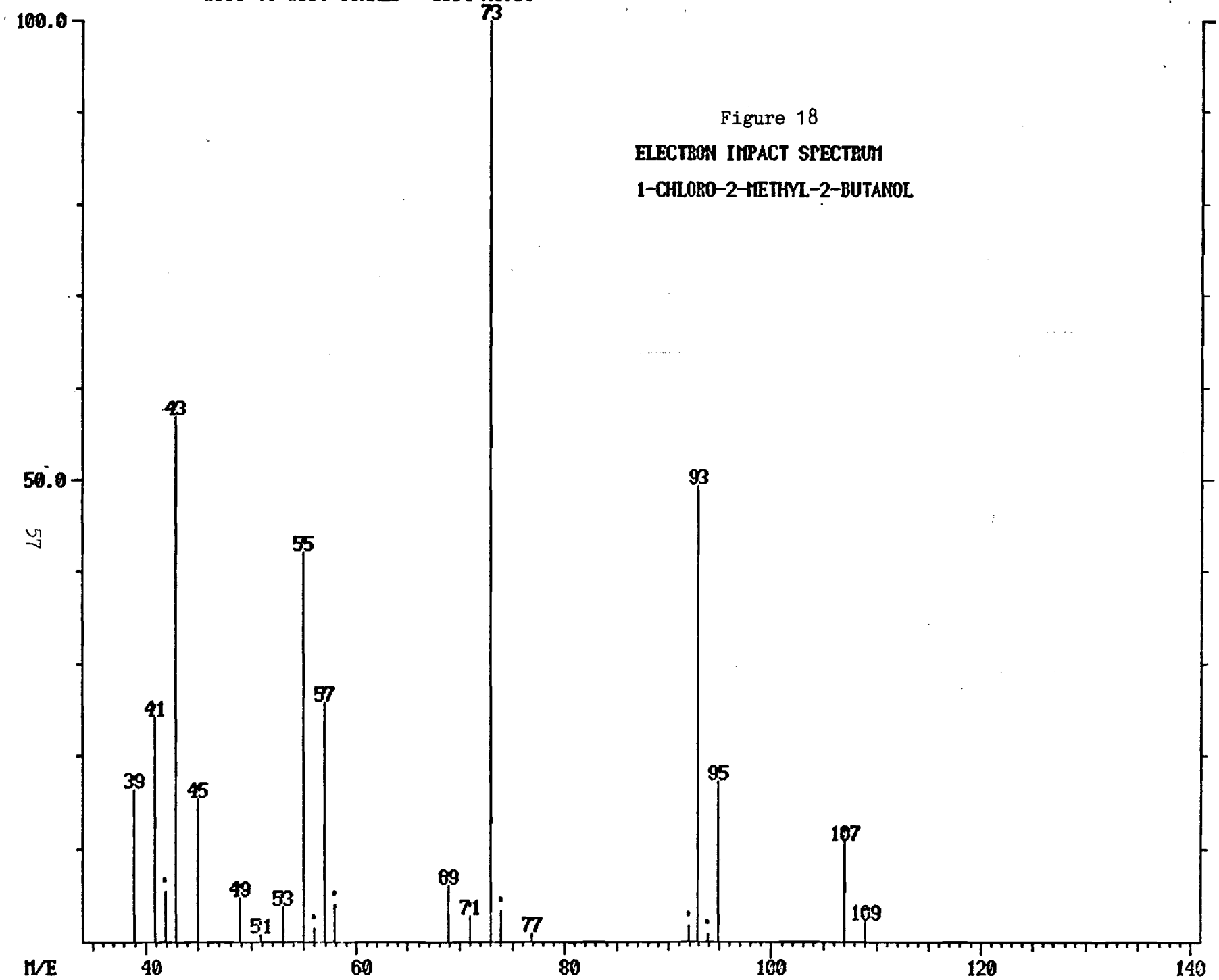


Figure 18

ELECTRON IMPACT SPECTRUM

1-CHLORO-2-METHYL-2-BUTANOL

Figure 19. EI Fragmentation Sequence for 1-Chloro-2-methyl-2-butanol.

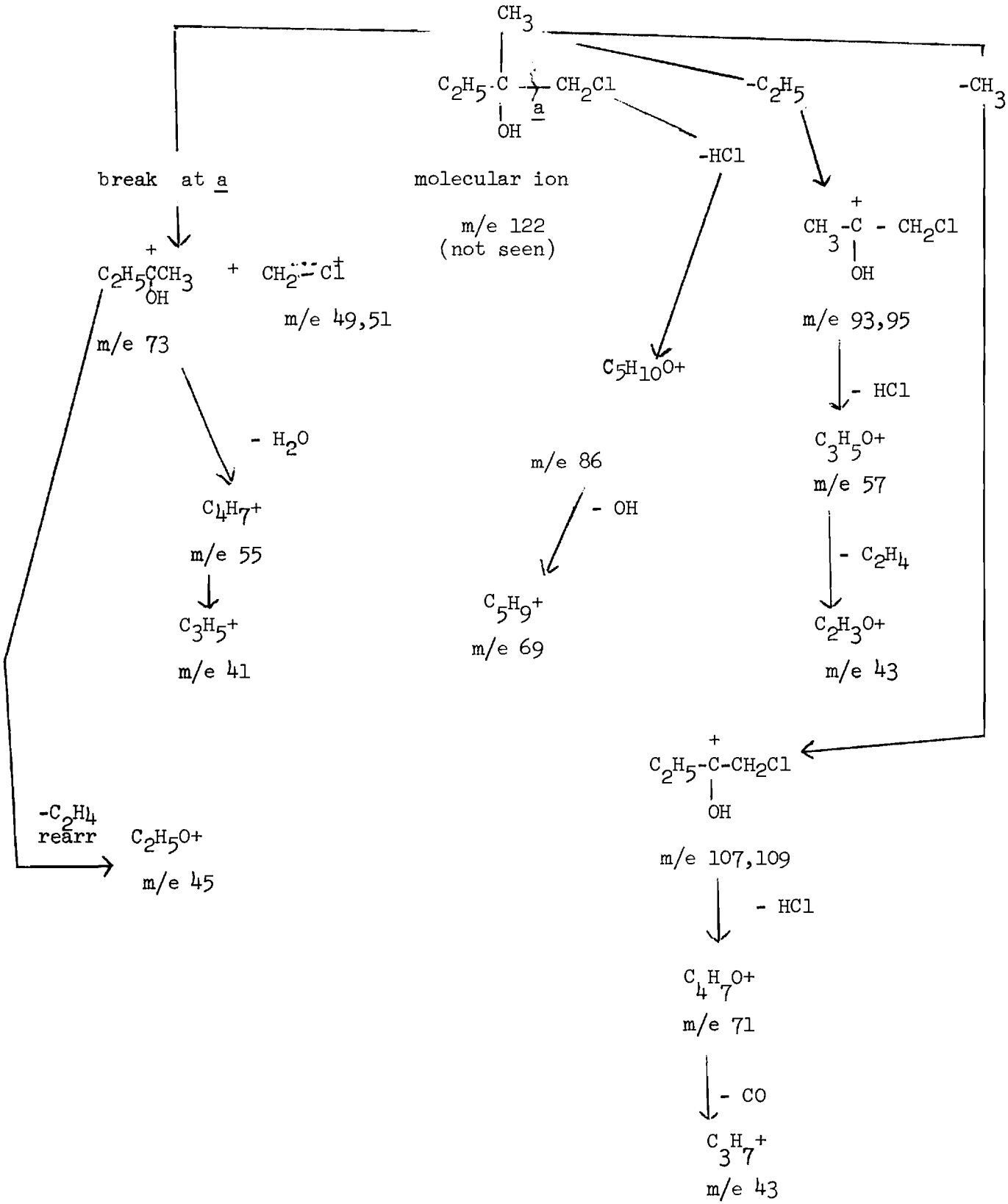


TABLE 8

CHLORINATION PRODUCTS DERIVED FROM AQUATIC HUMIC MATERIAL

1. 3-Chloro-2-methyl-1-butene*
2. 2-Chloromethyl-1-butene
3. 2-Methyl-3-pentanone+
4. 2-Methylpentanal+
5. 3-Chloro-2-methyl-2-butanol
6. 4-Hydroxy-3-methylbutanal+
7. 2,3-Dichloro-2-methylbutane
8. 1-Chloro-2-methyl-2-butanol
9. 1,3-Dichloro-2-methlbutane
10. 3-Chloro-2-methyl-1-butanol
11. 4-Chloro-3-methyl-1-butanol
12. 3,4-Dichloro-methyl-1-butene Isomer
13. 4-Methyl-3-heptanone
14. 2-(3-Oxobutyl)-cyclopropanol
15. 1,4-Dichloro-2-methyl-2-butanol
16. Trichloroacetic Acid
17. 1,3-Dichloro-2-methyl-2-butanol
18. 3,4-Dichloro-3-methyl-2-butanol
19. 2-Butanol+
20. Chloroform
21. 2-Chloro-3-methyl-2-butene
22. Dichloroacetic Acid
23. Toluene**
24. Benzene**

*Dominant product. It is of interest to point out that this is also the dominant product reported by Dr. W.H. Glaze in his paper on the superchlorination of secondary effluent - a material which is known to be rich in humic substances. (Reference 16)

+Found in controls, presumably adsorbed to the aquatic humics

**Unclear whether is an artifact or an adsorbed material originating in the atmosphere. Other workers have reported finding these two components (Note 19)

LIBRARY SEARCH
08/09/79 18:46:00 + 15:52
SAMPLE: PENTAFLUOROBROMOBENZENE
309 TO # 310 SUMMED

DATA: BROM060979 # 309
CALI: ZG60979 # 1

BASE M/E: 117
RIC: 248783.

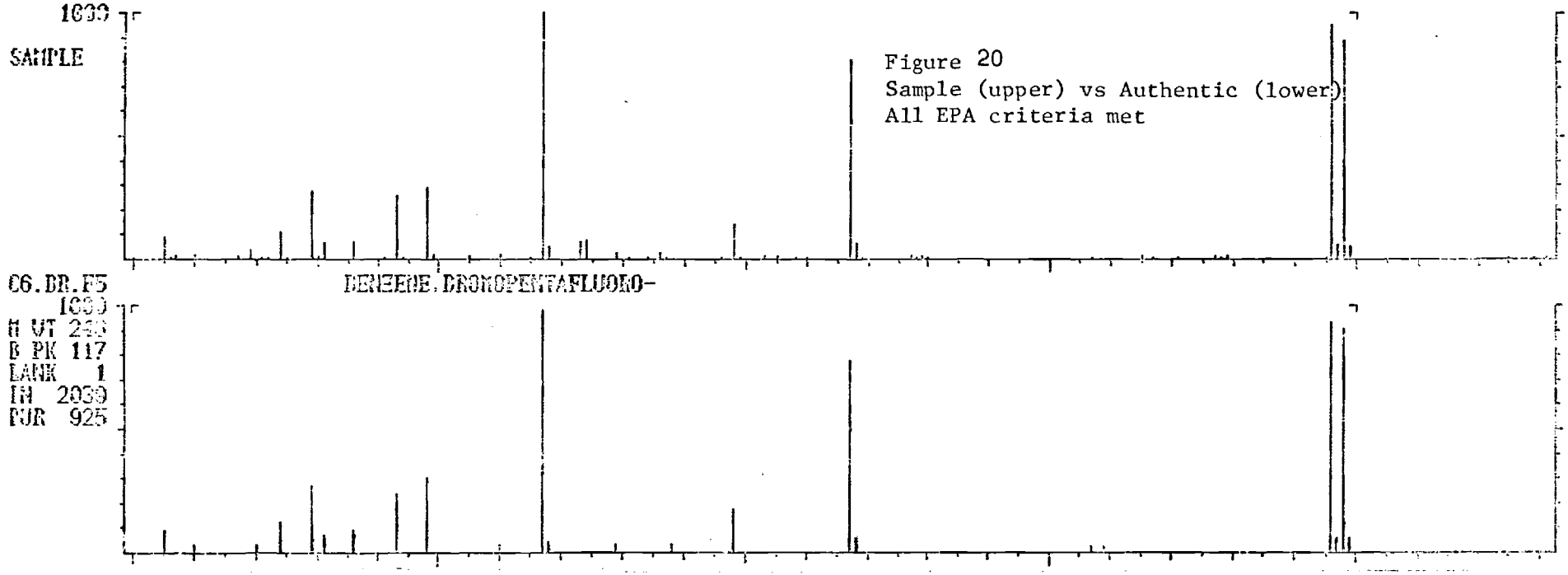


Figure 20
Sample (upper) vs Authentic (lower)
All EPA criteria met

is compared with the EPA performance criteria in Table 9.

Each chlorination run featured a companion blank run contacting all of the reagents with the exception of the chlorine, flocculant and filter packing. In addition to providing assurance that incidental contamination had not taken place, these control runs provided a body of information of their own regarding the nature of some of the more readily desorbed organics associated with the aquatic humics. Glassware and the mini-pilot facility were carefully cleaned between runs. Instrumental performance was assured by a program of routine maintenance. In this way, shutdown due to unscheduled maintenance has been held to only 5 days over the course of the entire project.

The studies with immature humics have resulted in the characterization of three general classes of chloroorganics all of which bear a structural relationship to isoprene. These are listed below:

1) Monochloroalcohols
2) Dichloroalcohols
3) Chlorinated hydrocarbons

The process by which the monochloroalcohols were structurally identified has already been illustrated by example in Figures 17, 18 and 19 together with the accompanying text. A similar strategy was employed to identify a series of dichloroalcohols. For example, all members of this series are characterized by CI spectrum such as that of 1,3-dichloro-2-methyl-2-butanol which is presented in Figure 21. Once again, the quasi-molecular ion (expected at m/e 157, 159, 161) is not recorded. Instead, a loss of water resulting from an attack on the protonated OH function is seen as an ion

TABLE 9

GC/MS PERFORMANCE CRITERIA PENTAFLUOROBROMOBENZENE

	Criterion Value	Georgia Tech Value
1.	Is m/e 78 less than 1% relative intensity (RI)?	Not present
2.	Is m/e 79 between 15% and 35% of RI?	28%
3.	Is m/e 80 etween 4% and 8% of m/e 79?	4%
4.	Is m/e 116 less than 1% RI?	Not present
5.	Is m/e 117 the base peak?	yes
6.	Is m/e 118 between 4% and 8% RI?	5%
7.	Is m/e 166 less than 1% RI?	Not present
8.	Is m/e 167 between 65% and 85% RI?	80%
9.	Is m/e 168 between 5% and 9% of m/e 167?	8%
10.	Is m/e 245 less than 1% RI?	0%
11.	Is m/e 246 between 75% and 98% RI?	95%
12.	Is m/e 247 between 5% and 9% of m/e 246?	6%
13.	Is m/e 248 between 75% and 98% RI?	89%
14.	Is m/e 249 between 5% and 10% of m/e 248?	6%
15.	Is m/e 248 between 93% and 99% of m/e 246?	94%

MASS SPECTRUM
05/09/78 9:56:00 + 28:25
SAMPLE: HUMICS PLUS CHLORINE, CH4 CI
#1136 TO #1138 SUMMED - #1138 X0.50

DATA: II M943DCI$ #1137
ENHANCED (S 31B 2N 0T)

BASE M/E: 121
RIC: 1230.

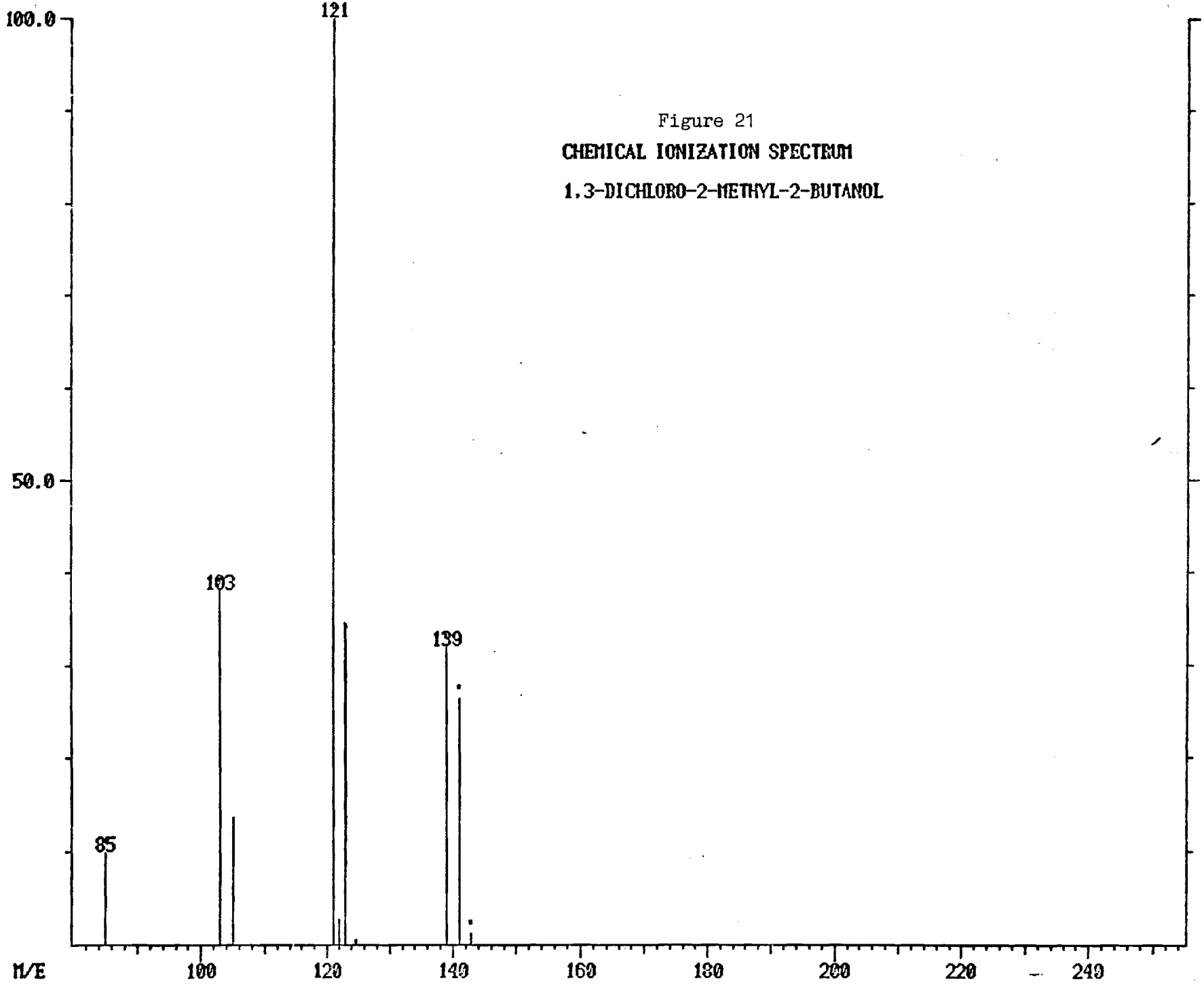


Figure 21

CHEMICAL IONIZATION SPECTRUM

1,3-DICHLORO-2-METHYL-2-BUTANOL

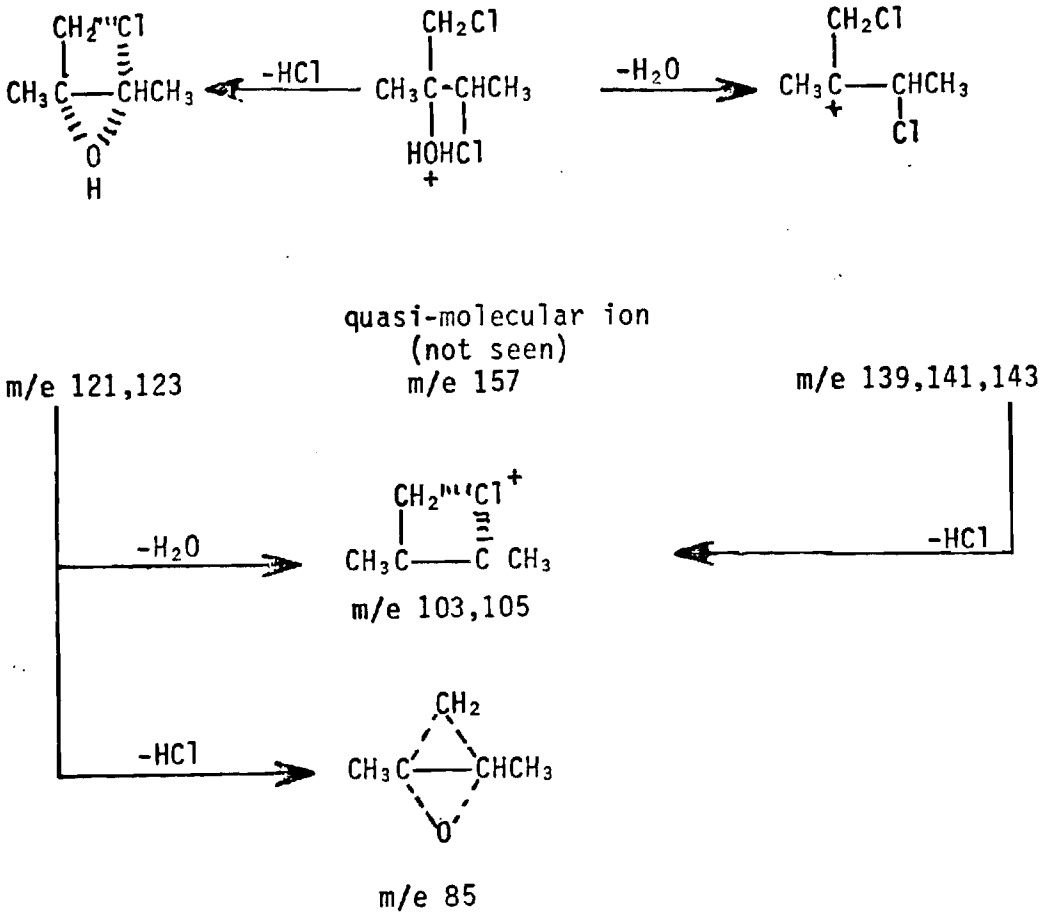
cluster at m/e 139, 141, 143. An analagous attack on the OH function resulting in the loss of HCl accounts for the base peak at m/e 121, 123. These ions in turn can lose HCl and/or water to provide ions at m/e 103, 105 and m/e 85. This information is summarized in the fragmentation sequence outlined in Figure 22.

As was the case with the monochloroalcohols, the EI spectra recorded for the dichloroalcohols were generally quite different from one another. The EI spectrum of 1,3-dichloro-2-methyl-2-butanol is presented in Figure 23 as an example. The molecular ion is not seen. Loss of a methyl radical generates the dichloro ion cluster at m/e 141, 143, 145. Loss of the chloromethyl group accounts for the ions at m/e 107, 109. A weak companion ion is seen at m/e 49, 51. Breakage between the functional groups generates the ion pair at m/e 93, 95. The complete fragmentation sequence is presented in Figure 24.

The final general group of chlorinated organics to be characterized among the products resulting from the simulated disinfection of immature aquatic humics are the chlorinated hydrocarbons. Once again, all of these products were found to be structurally related to isoprene. Furthermore, the dominant monochloroalcohol, 3-chloro-2-methyl-2-butanol is a close relative of the dominant isoprene hydrocarbon, 3-chloro-2-methyl-1-butene. At least one of the dichloroalcohols-i.e. 1,3-dichloro-2-methyl-2-butanol is also closely related. The co-principal investigators believe that this structural relationship is not coincidental and that the dominant products are either produced from each other via hydration/chlorination of a common definite precursor, or by dehydration of the monochloroalcohol to the chloro-

FIGURE 22

CHEMICAL IONIZATION SEQUENCE: 1,3-Dichloro-2-methyl-2-butanol(methane)



MASS SPECTRUM
05/08/78 15:29:00 + 29:30
SAMPLE: M/30 HUMICS PLUS CHLORINE. EI
#1180 - #1183 X1.00

DATA: I111943D #1180
CALI: 0507 #1

BASE M/E: 43
BIC: 5096.

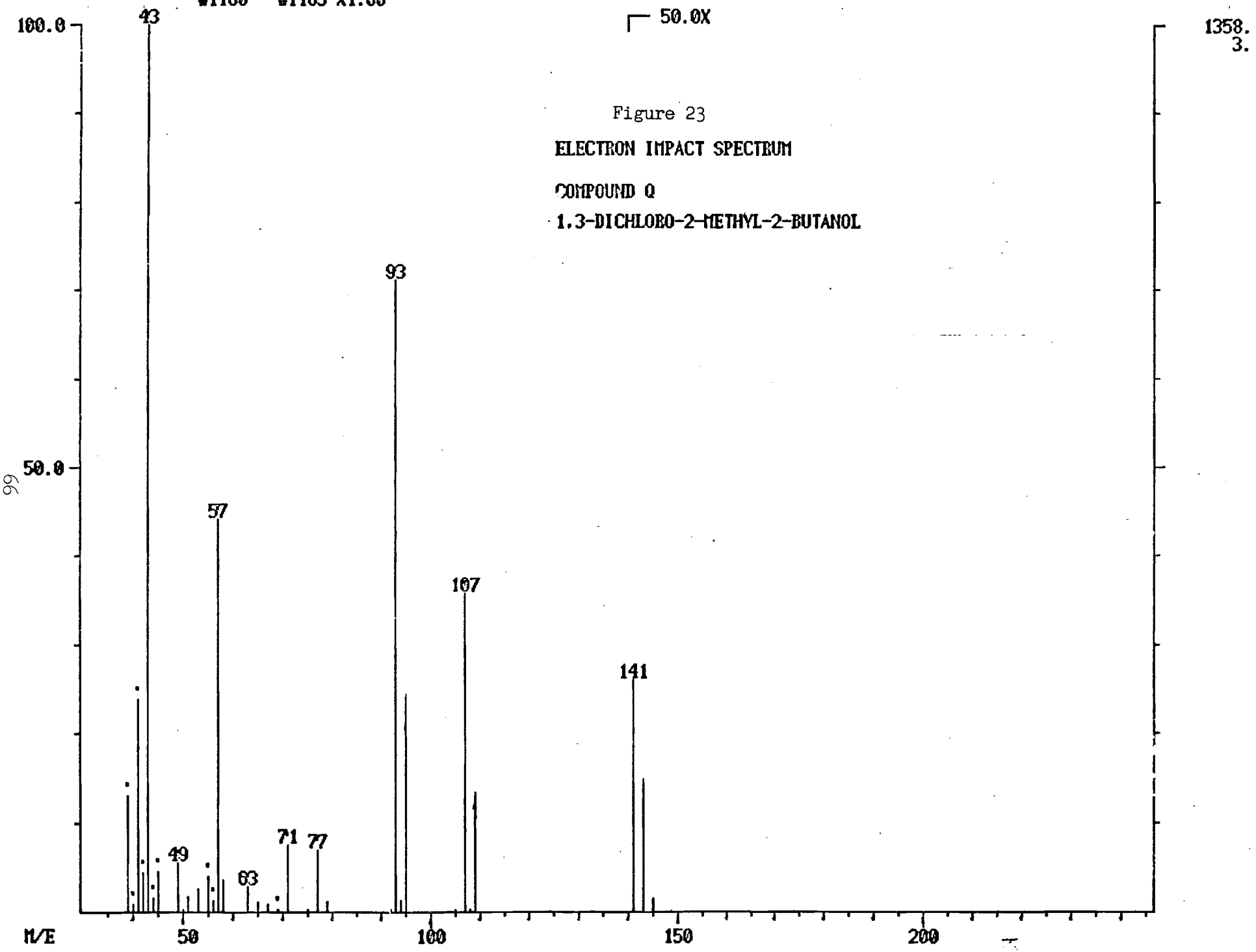


Figure 23

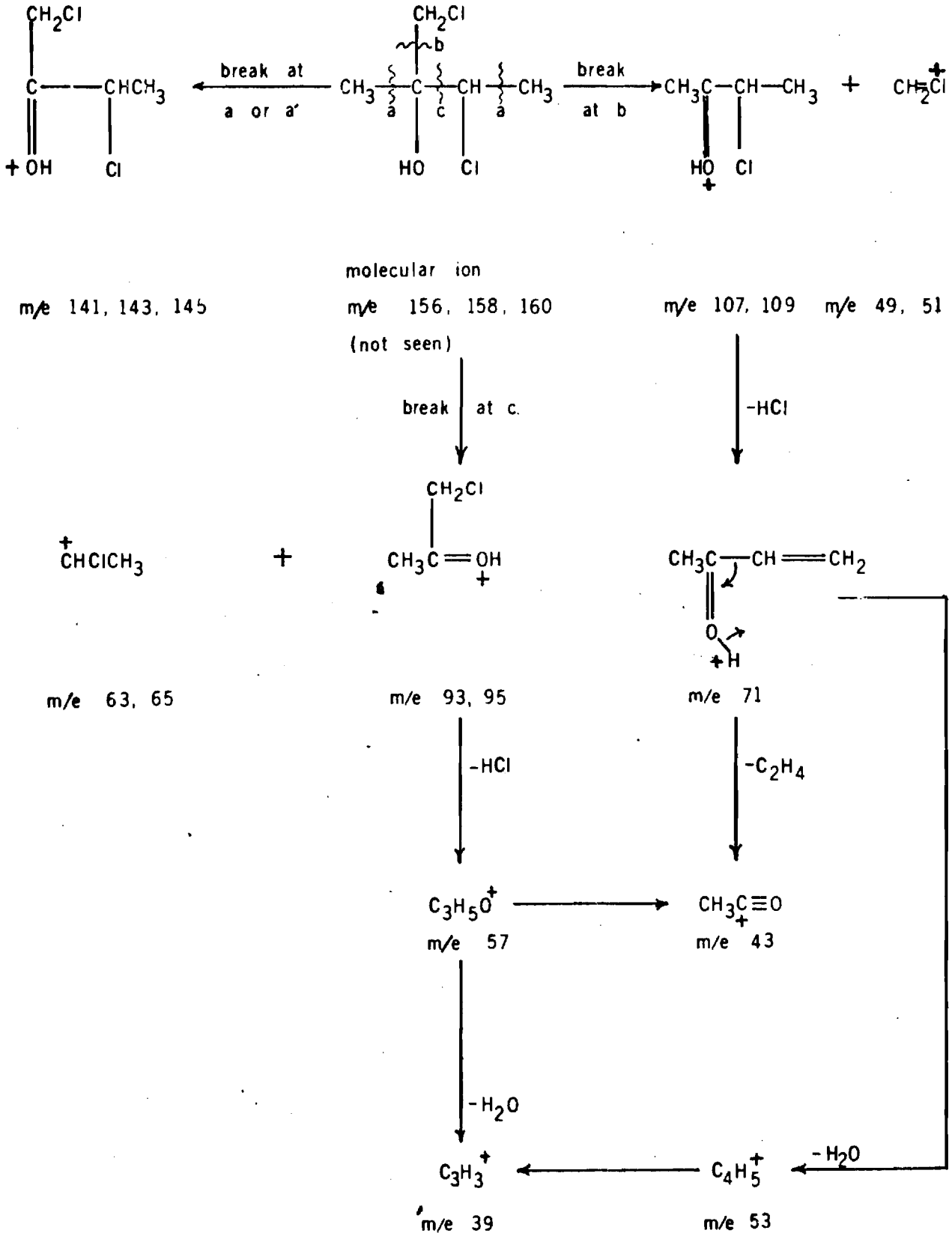
ELECTRON IMPACT SPECTRUM

COMPOUND Q

1,3-DICHLORO-2-METHYL-2-BUTANOL

FIGURE 24

ELECTRON IMPACT FRAGMENTATION: 1,3-Dichloro-2-methyl-2-butanol



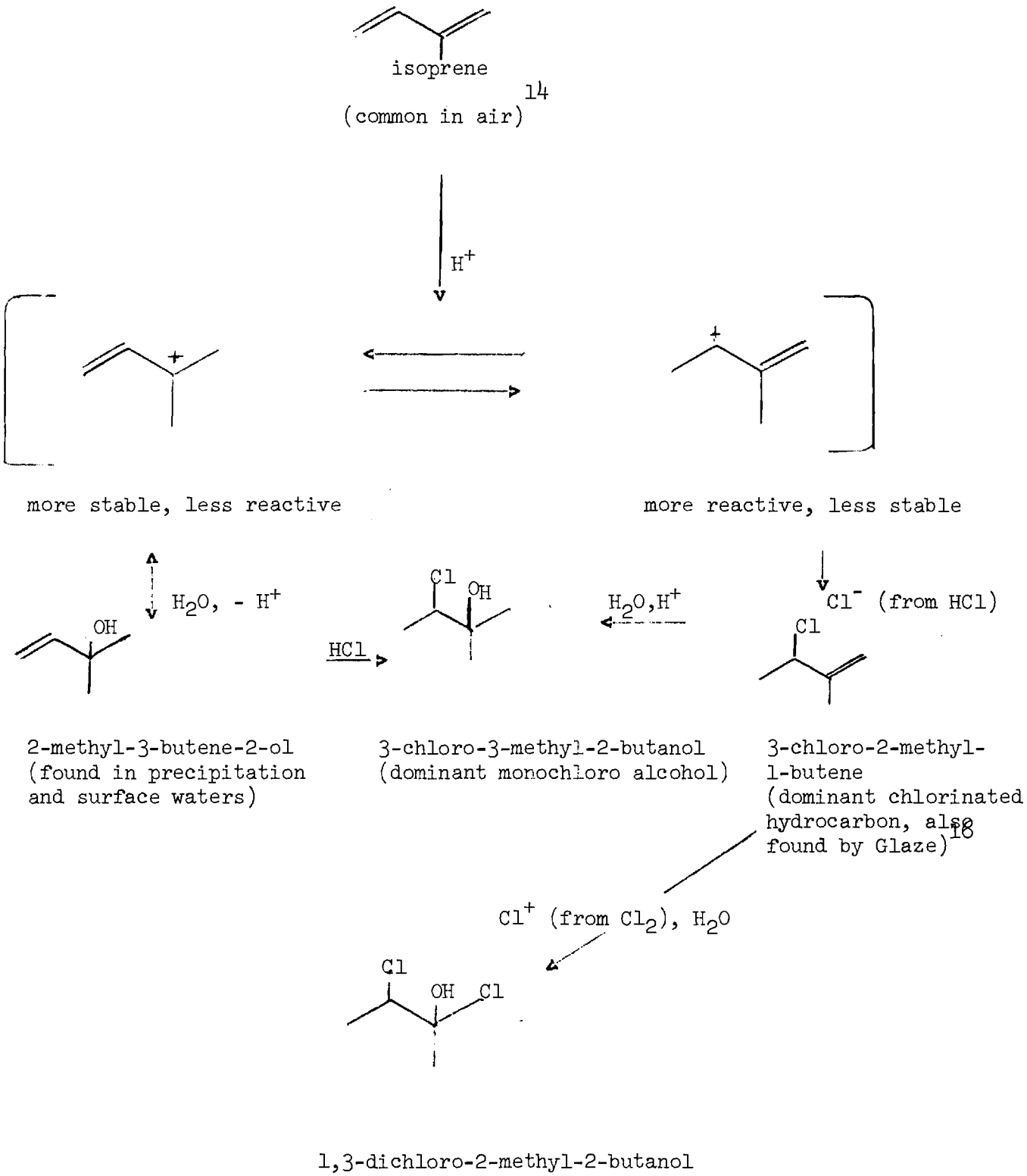
olefin or by means of some other common precursor. A possible sequence of events is outlined in Figure 25. A more detailed discussion of the possible overall sources for the chlorinated isoprenoids follows the discussion of the products resulting from the chlorination of mature aquatic humics.

Structural assignments were made according to the general considerations discussed earlier. In this case, however, a number of the compounds in question were represented in the data system library so that valid suggestions were made more often than not. The illustrative example selected to represent this class of compounds is 3-chloro-2-methyl-1-butene. While a weak quasi-molecular ion is seen in the CI spectrum (Figure 26) at m/e 105 and m/e 107, the base ion at m/3 69 is generated by loss of HCl from the quasi-molecular ion. The electron impact spectrum (see Figure 27) is more informative, particularly since a very good match was obtained by means of a computerized comparison with the NBS/EPA library. All fit indices exceeded 900 out of a possible 1000. In this case, the molecular ion is seen at m/e 104, 106. Loss of a methyl group provides another chlorine-containing ion at m/e 89 and m/e 91. Loss of the allylic chlorine accounts for the strong ion at m/e 69 while breakage between the functional groups generates m/e 41 and a weaker ion pair at m/e 63, 65. The full EI and CI fragmentation sequences are presented in Figure 28.

Chlorination of Mature Humics

While the chlorination of immature aquatic substances produces a product mixture comprised of a vast array of components about half of which may contain chlorine, the chlorination of mature aquatic humics is characterized by a reduced uptake of chlorine and a less complex product mixture. In the latter case, only about 25% of the product mixture was found to contain

Figure 25. Possible Interrelation of Major Chlorinated Isoprenoid Products.



MASS SPECTRUM
05/04/78 14:57:00 + 6:19
SAMPLE: HUMICS PLUS CHLORINE. CH4 CI

DATA: I111943CCI #253
CALI: 6594 #1

BASE M/E: 69
RIC: 4432.

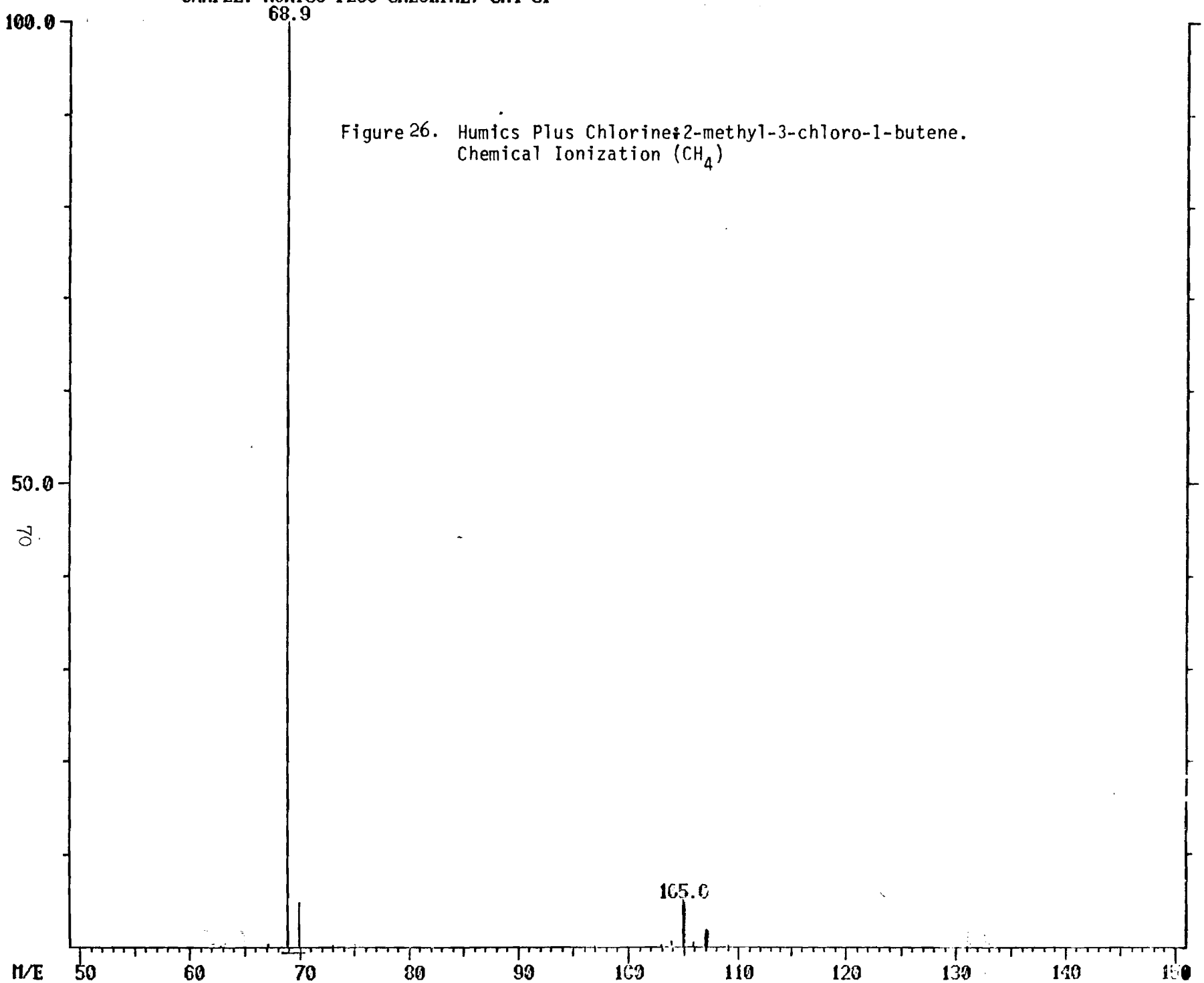


Figure 26. Humics Plus Chlorine: 2-methyl-3-chloro-1-butene. Chemical Ionization (CH_4)

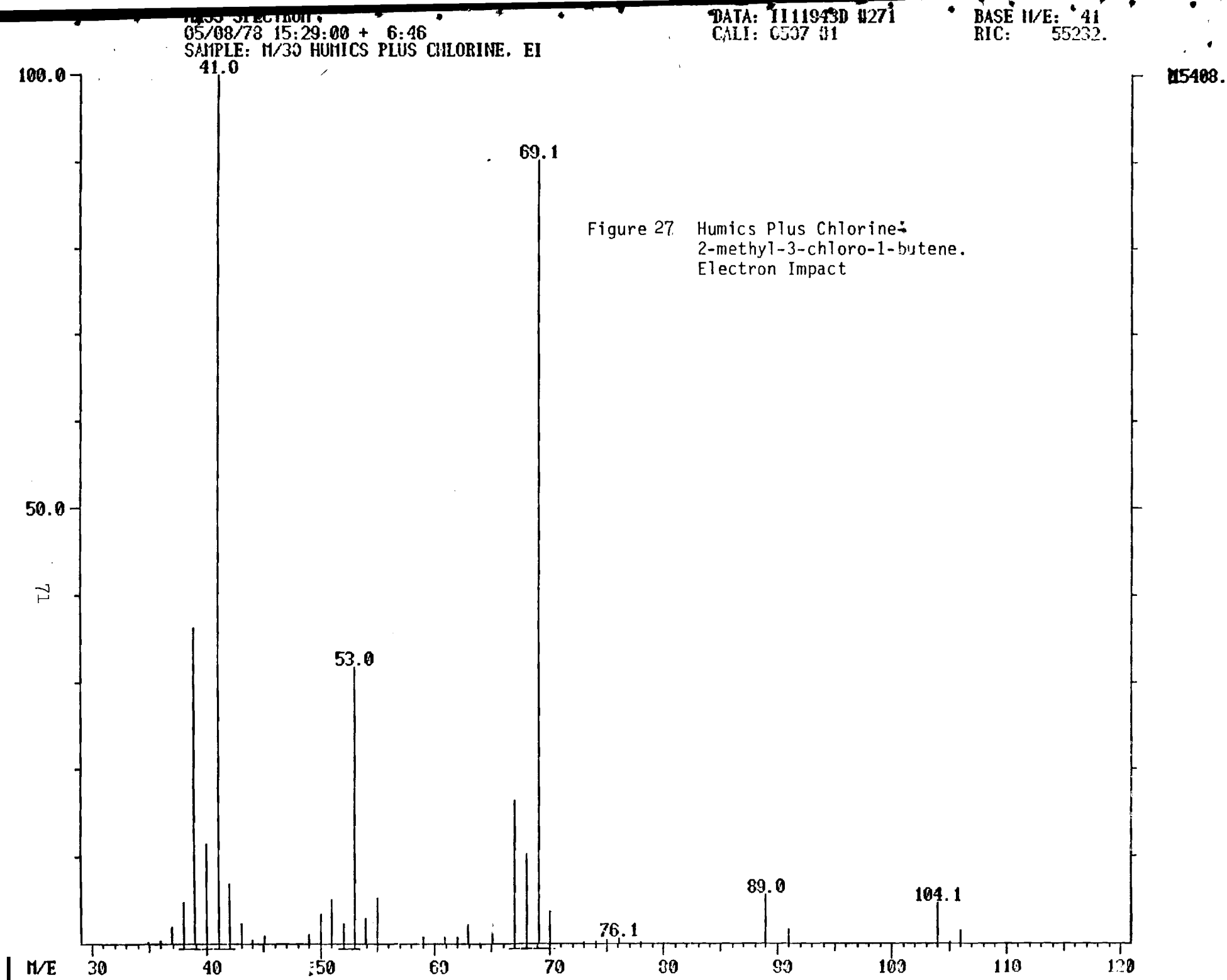
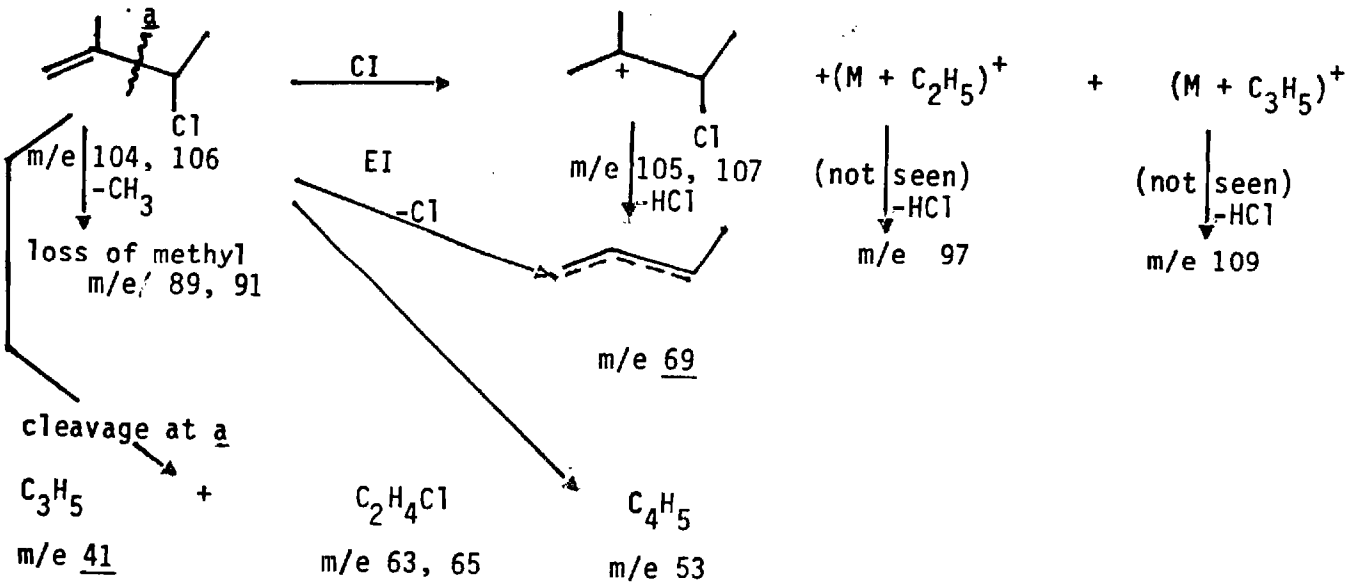


Figure 27 Humics Plus Chlorine: 2-methyl-3-chloro-1-butene. Electron Impact

Figure 28

EI and CI Fragmentation Sequence for 3-Chloro-2-methyl-1-butene



chlorine. The major chlorine-containing products are dichloracetic and trichloroacetic acids. Some aromatic compounds and several organic acids have been assigned. Some aromatic products were noted suggesting a closer link to the oxidative degradation work. A complete listing of the products identified during the support period is presented in Table 10. The EI total ion chromatogram is shown in Figure 29.

Structural assignments were made according to the procedures previously mentioned. In this case it will be noted that the methyl esters and ethers were prepared rather than the corresponding ethyl derivatives. A typical structural assignment is illustrated by the case of methyl dichloroacetate the EI spectrum of which is presented in Figure 30. The FIT index of 981 provided a very strong suggestion regarding the structure of this component. The molecular ion at m/e 142, 144, 146 is not seen. The weak ion cluster at m/e 111 and m/e 113 representing the loss of a methoxy radical does not include the last isotopic peak at m/e 115 because this signal is too weak to stand out above the background. The strong ion cluster at m/e 83, 85, 87 represents cleavage between the functional groups as does the base peak at m/e 59. A more complete fragmentation sequence is presented in Figure 31.

These results are especially significant when compared with the results described in the previous subsection. This is so because a definite contrast is noted between the two cases which in turn suggests that adsorbed or otherwise weakly bound, but highly reactive species are present when aquatic humics are initially flushed from the soil environment. The writers postulate that this material is only weakly shielded from degradation and

TABLE 10

PRODUCTS LIST: CHLORINATION OF MATURE AQUATIC HUMICS

1. 2-Methylpentanal
2. Propionic Acid*
3. 3-Methyl-2-butanone
4. Formaldehyde
5. Pentanoic Acid*
6. Toluene*
7. 2-Hydroxy-4-methylpentanoic Acid*
8. Dichloroacetic Acid
9. Trichloroacetic Acid
10. Benzoic Acid
11. Trimethyoxybenzene isomers**
12. 2-Methyl-2-butanol
13. 2-Methylpropionate Acid

*Acids were isolated after derivation to the methyl esters.

**Probably not completely methoxylated in the original product mixture.

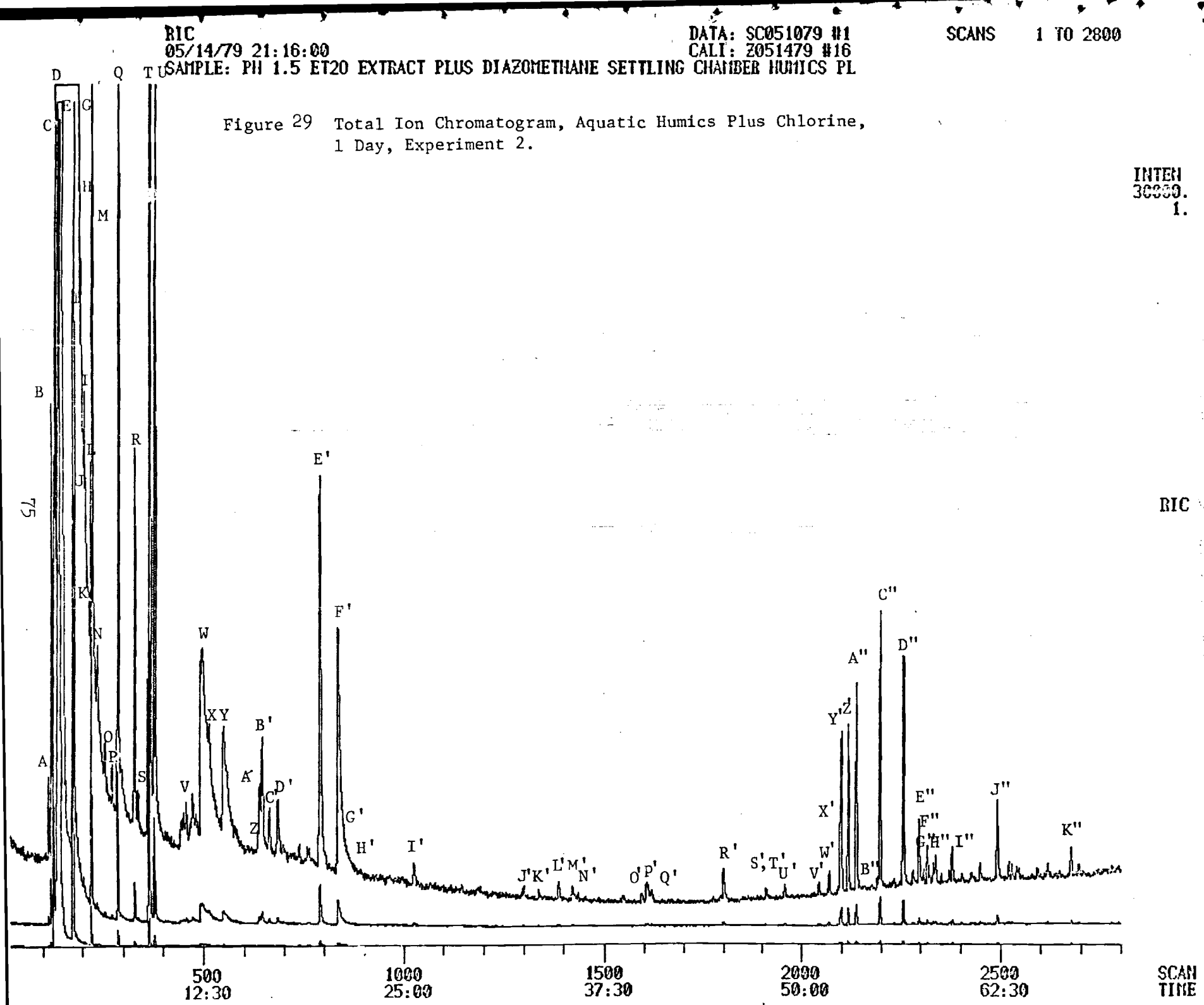


Figure 29 Total Ion Chromatogram, Aquatic Humics Plus Chlorine, 1 Day, Experiment 2.

MASS SPECTRUM
05/13/79 2:10:00 + 11:07
SAMPLE: ET20 EXTRACT PH1.5 HUMICS PLUS CHLORINE
#445 - #440 X1.00

DATA: EFF0508792 #445
CALI: Z051279 #25

BASE M/E: 59
RIC: 45952.

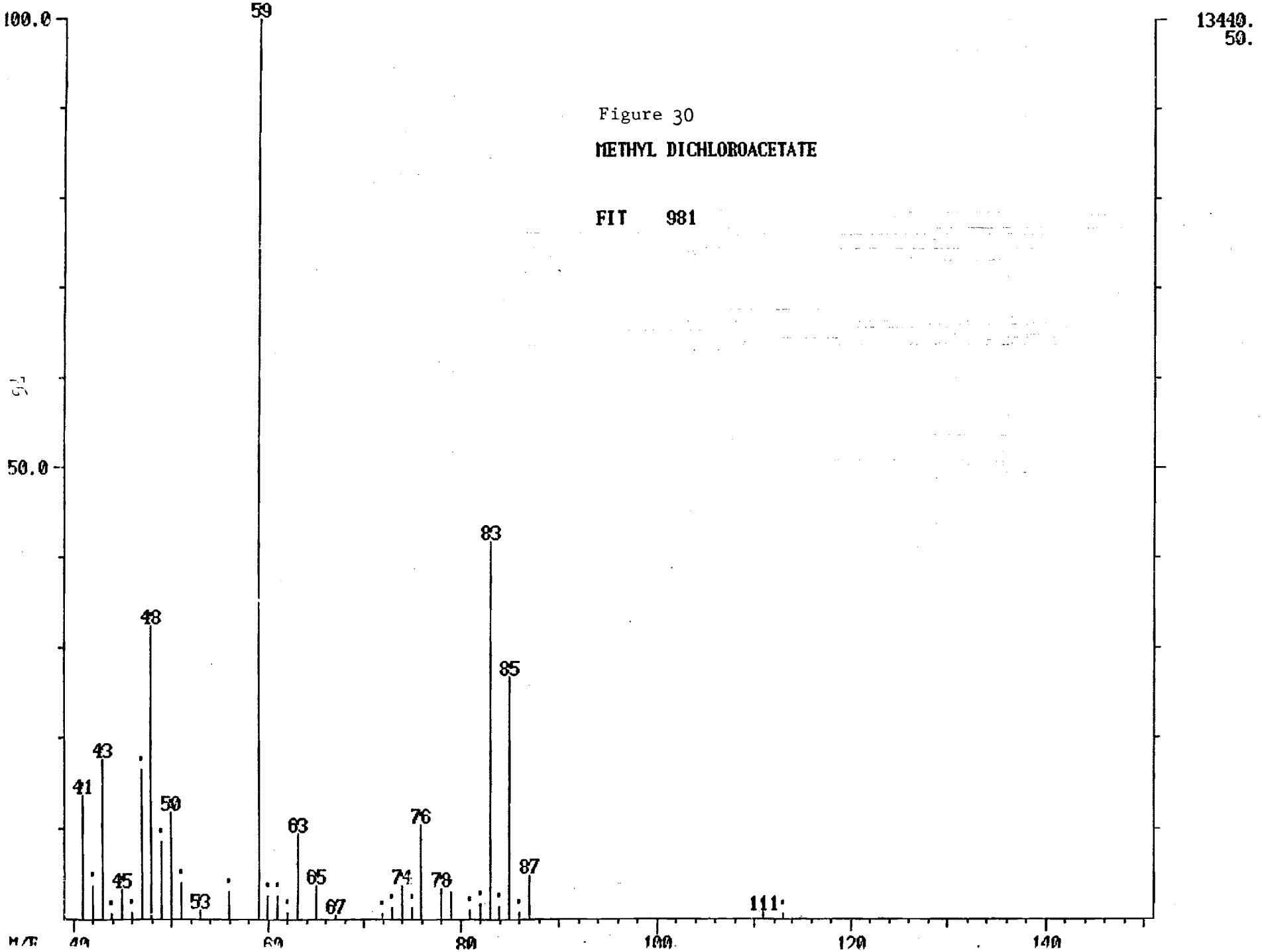
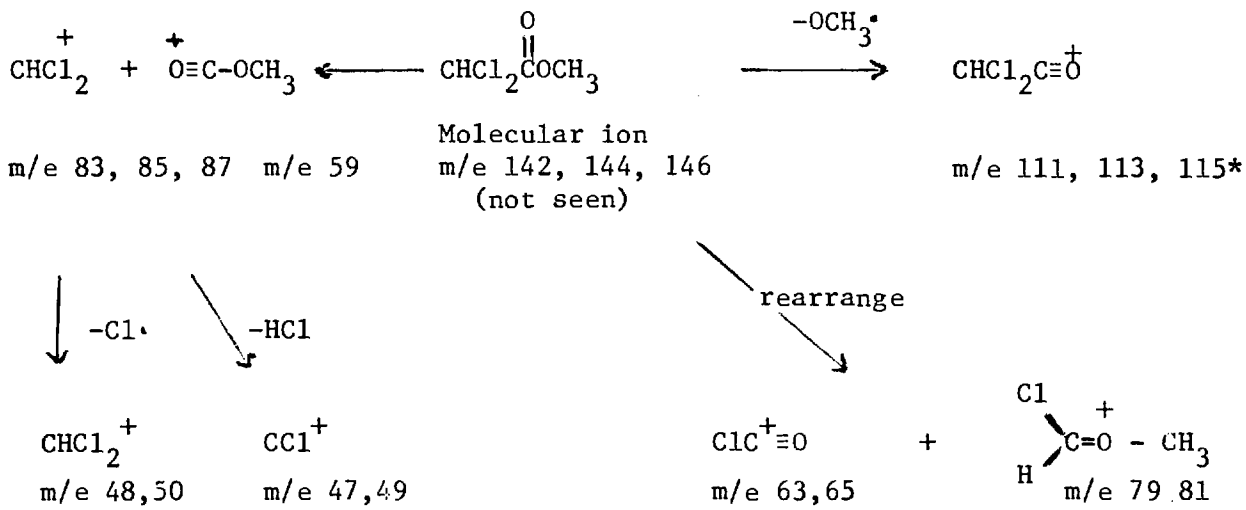


Figure 30

METHYL DICHLOROACETATE

FIT 981

Figure 31
Fragmentation Sequence - Methyl Dichloroacetate



All of the ions shown with the exception of the molecular ion are present in the reference spectrum. A mechanism leading to the minor rearrangement ions at m/e 63, 65 and m/e 79,81 is difficult to postulate.

*Not seen because of low intensity

in the presence of biological activity, sunlight, acid and dissolved oxygen it soon disappers. If the aquatic humics are isolated and stored in a more protected enviroment, these species can survive for a longer time period. Therefore, it is not surprising that we were able to repeat our initial findings with the immature humics over the course of several experiments. Eventually, even under the conditions of a more protected environment, the adsorbed isoprenoids are lost and/or altered so that the product mixture contains fewer chlorinated components and no chlorinated isoprenoids. Mature aquatic humics have already lost these precursors and therefore are less influenced by protracted storage.

These observations have implications for possible treatment protocols. Our contacts at the Atlanta water works inform us that unidentified volatile, halogenated compounds are noted among the components detected by volatile organics analysis (VOA) immediately following a period of heavy rainfall. We suggest that these compounds may be related to the chlorinated isoprenoids described in an earlier subsection. Removal might be effected by simply holding the water for a few days prior to treatment.

Quantitative Estimates of Chlorine-Containing Products Resulting from Disinfection of Aquatic Humics

Three experiments were carried out quite late in the course of the project in order to provide a connecting link between a property for which a maximum concentration limit has been set-i.e. the trihalomethanes (THM's)- and the experiments performed in connection with this project. Bromide was excluded thus making TOX equivalent to total organic chlorine (TOCl). In

order to successfully execute a TOCl analysis, one must guard against both the accidental loss of volatile, chlorine-containing entities such as the THM's while avoiding contamination with common chlorine-containing laboratory chemicals. Since the accepted methods depend on the adsorption of the organic chlorine on carbon, and its subsequent conversion to chloride, the carbon must be totally free from chloride to start with and furthermore must be sufficiently active to adsorb the chloroorganics when exposed to the test solutions. For these reasons, the samples collected for TOCl analysis were treated with excess sodium sulfite to destroy any remaining free chlorine, sealed in crimp top bottles and sent to two different commercial laboratories for subsequent analysis. Matching THM values were obtained in-house.

The results obtained from these experiments are presented in Table 11. It should be pointed out that different batches of aquatic humics were employed, four sets of results were obtained and two different laboratories were involved in to TOCl work. The analytical variability was found to exceed the differences between the two batches of aquatic humics in all sample categories with the exception of the reservoir controls. (A second pair of values, not shown in the table placed this level at 125 mg/l vs the 7.6 mg/l shown). TOCl values were significantly higher after 5 days than they were initially. This observation may be significant with regard to possible ongoing TOCl producing reactions in drinking water distribution systems. A recent publication, in fact, suggests that a significant increase in the mutagenicity of drinking waters does occur as it travels through the distribution system.[17] It is possible that this increase in mutagenicity is related to the increase in TOX.

THM levels were also found to exhibit the same upward trends as did the TOX values. Furthermore, the percent contribution of chloroform to

TABLE 11

TOCl AND CHLOROFORM RESULTS - CHLORINATION OF AQUATIC HUMICS

	Mean Level µg/L	Chlorofrom µg/L	Percent Contribution of $CHCl_3$ to TOCl
Blank	3.8	--	--
Reservoir Control	7.6	1.8	21
Finished Water (1 day)	1110	250	21
Finished Water (5 days)	1530	395	23

TOX is remarkably constant from the control through the 5-day samples even though the absolute values varied by more than two orders of magnitude. The Project Manager believes that these findings constitute evidence that the rise in chloroform levels in drinking waters reported after prolonged contact with chlorine does not occur at the expense of other TOCl. One might therefore conclude that the proportion of chloroform precursors generated from the aquatic humic fraction of typical source waters depends only on the reactivity of the humics and the conditions of disinfection and not upon the length of time in the distribution system. Since both values exhibit the same trend, either one is a valid indicator of the overall hazard potential posed by chlorinated organics in drinking water. Thus a regulation using total trihalomethanes as an indicator of TOX would seem to be justified in the light of this evidence. Other workers have monitored finding similar correlations between chloroform and THM.[18]

Using 3-methyl-3-butene-1-ol as a quantitation standard, the II11943D concentrate derived from immature aquatic humics and which had served as the master run for our past work was retrieved from frozen storage and rerun. A comparison of the proportions of each of the chloroalcohols in the new run to the same proportions in the original run provided strong evidence that losses of these materials had not occurred. The correlations are summarized in Table 12.

The standard compound was injected into the GC/MS system as a solution in methanol having a concentration of 50 mg/l. Since this level corresponds to a concentration of 25 μg/l in the finished water when adjusted for the concentration step performed on the real samples, it was hoped that it

TABLE 12

PROPORTIONS OF CHLOROALCOHOLS IN CHLORINATED AQUATIC HUMICS

EVIDENCE OF SAMPLE INTEGRITY

Compound	Original Proportion	Post-Storage Proportion
Monochloroalcohols		
3-Chloro-2-methyl-2-butanol	1.00	1.00
1-Chloro-2-methyl-2-butanol	0.14	0.12
4-chloro-3-methyl-1-butanol	0.04	0.03
Dichloroalcohols		
1,4-Dichloro-2-methyl-2-butanol	1.00	1.00
1,3-dichloro-2-methyl-2-butanol	0.57	0.56
3,4-Dichloro-3-methyl-2-butanol	0.42	0.37

would give a response of similar intensity to that recorded for the chloroalcohols with which it was to be compared. Duplicate injections showed a variability of only 10-15% based on total ion current across a chromatographic peak. This general tactic of making semiquantitative estimates on the basis of total ion currents has proven to be quite successful in some of our priority pollutant work in which case the average values found for six generally dissimilar standards was 105% of the real value. Individual results, however, did vary by up to a factor of four, thus the overall results are to be given greater credibility than any single result. In the case of the work performed in connection with the aquatic humics, the real sample showed peak areas which were bracketed by those exhibited by the standards. The concentrations of the monochloromethylbutanols and dichloromethylbutanols were calculated on the basis of total ion counts. While the chloromethylbutenes did not survive the storage period, the closeness of the data on the surviving materials encouraged us to extend the concentration estimates to include these compounds as well. These results are presented in Table 13.

In summary, it can be seen that the concentrations of chlorinated isoprene alcohols as a group in this product mixture is about 10% of the chloroform levels presented in Table 10. A similar percentage relationship was observed for the chlorinated C-5 hydrocarbons. It should be pointed out, however, that these estimates are only "ballpark" figures as they represent a comparison between one set of experiments performed with immature humics and another set performed with mature humics.

While chloroform accounts for about 20% of the total organic chlorine, the monochlorinated alcohols and hydrocarbons together account for only

Table 13
Concentrations of Chlorinated Products in
Mini-Pilot Studies with Aquatic Humics

Compound	Level in mg/l
3-Chloro-2-methyl-2-butanol	24
1-Chloro-2-methyl-2-butanol	2.9
3-Chloro-2-methyl-1-butanol	0.7
4-Chloro-3-methyl-1-butanol	2.4
Sum monochloromethylbutanols	30
1,4-Dichloro-2-methyl-2-butanol	4.4
1,3-Dichloro-2-methyl-2-butanol	2.3
3,4-Dichloro-3-methyl-2-butanol	1.6
Sum dichloromethylbutanols	8.3
3-Chloro-2-methyl-1-butene	15
2-Chlormethyl-1-butene	0.8
3,4-Dichloro-methyl-1-butene isomer	2.0
2,3-Dichloro-2-methylbutane	3.7
1,3-Dichloro-2-methylbutane	3.8
Sum chlorinated C-5 hydrocarbons	25.3

1% of the TOCl on account of their lower chlorine content. The dichloro-alcohols and hydrocarbons account for another 0.5% of the TOCl. The concentrations as shown in Table 13 are only approximate, partly because of underlying assumptions and partly due to experimental difficulties. Nevertheless, the authors believe they are correct within an order of magnitude. The elucidation of the nature of the remaining percentage of the TOCl remains as a task for the future.

Chlorination of Aquatic Humics by Molecular Weight Fraction

This series of experiments was performed on the molecular size fractions isolated according to the methods described earlier in this report. Because only a limited amount of material was available, it was necessary to perform these experiments in 100 ml glass-stoppered Erlenmeyer flasks rather than in the dynamic environment of the mini-pilot facility. Conditions were otherwise realistic, however (10 mg/L aquatic humics, chlorine residual to 2 mg/L or less). Fractions were run in duplicate to improve the quality of the data. Chlorine uptake, TOX (as TOCl) and chloroform concentrations were determined. Unfortunately, the chloroform runs produced erratic results so that statistical significance could not be established which would enable the authors to make statements regarding which of the fractions contributed the most to the chloroform production. This was so even though the high-TOCl and high-chlorine-demand fraction was also the high-$CHCl_3$ fraction. Overall levels of chloroform produced were higher than those found by Schnoor et.al.[6], but lower than those reported in the previous subsection or by Oliver's group at the Canada Center for Inland Waters.[12] We would refer the reader to the previous subsection for information regarding the trihalomethane-generating potential of unfractionated humics. The results

are presented in Table 14. The molecular size partitions studied represent a subdivision of those fractions already reported to be responsible for most of the THM production observed in natural surface waters.

A positive correlation between chlorine uptake and TOCl was observed. TOCl accounts for about 1/5 to 1/6 of the chlorine consumed. Fractions 2 ($\overline{M}_N$=2200, 47%) and 5 ($\overline{M}_N$=530, 21%) account for most of the chlorine uptake and TOCl. The distribution as presented graphically in Figure 32 is clearly bimodal.

Classification of Raw River Water

Thi experiment was concerned with the separation of river water organics by class by means of sequential adsorption on XAD resins. Using the scheme discussed earlier it was possible to isolate hydrophobic bases, hydrophobic acids, hydrophilic bases, hydrophilic acids and hydrophilic neutrals with a removal efficiency of 99% as measured by TOC. These results are presented in Figure 33. The TOC levels remaining after each step are noted on the flow chart together with the percent desorbed from each of the resins are listed in Table 15. Although there is considerable drift in individual values, the overall recovery for the whole scheme is 99%. The hydrophobic acid fractions (ca. 60%), hydrophilic acid fractions (26%) and the hydrophilic neutrals (20%) account for virtually all of the TOC removal.

TABLE 14

PRODUCTION AS A FUNCTION OF MOLECULAR SIZE

Fraction	Molecular Weight $\bar{M}_N$	Percent of Total Humics	TOCl μg/L	Percent Cl_2 Uptake
G-50 excluded	3100	3.6	639±5	64
G-25 excluded	2200	47	846±17	76
G-15 excluded	1100	14	713±02	65
G-10 excluded	660	15	709±11	50
G-10 excluded	530	21	803±03	70

FIGURE 32.

CHLORINE UPTAKE AND TOCl AS A FUNCTION OF MOLECULAR SIZE

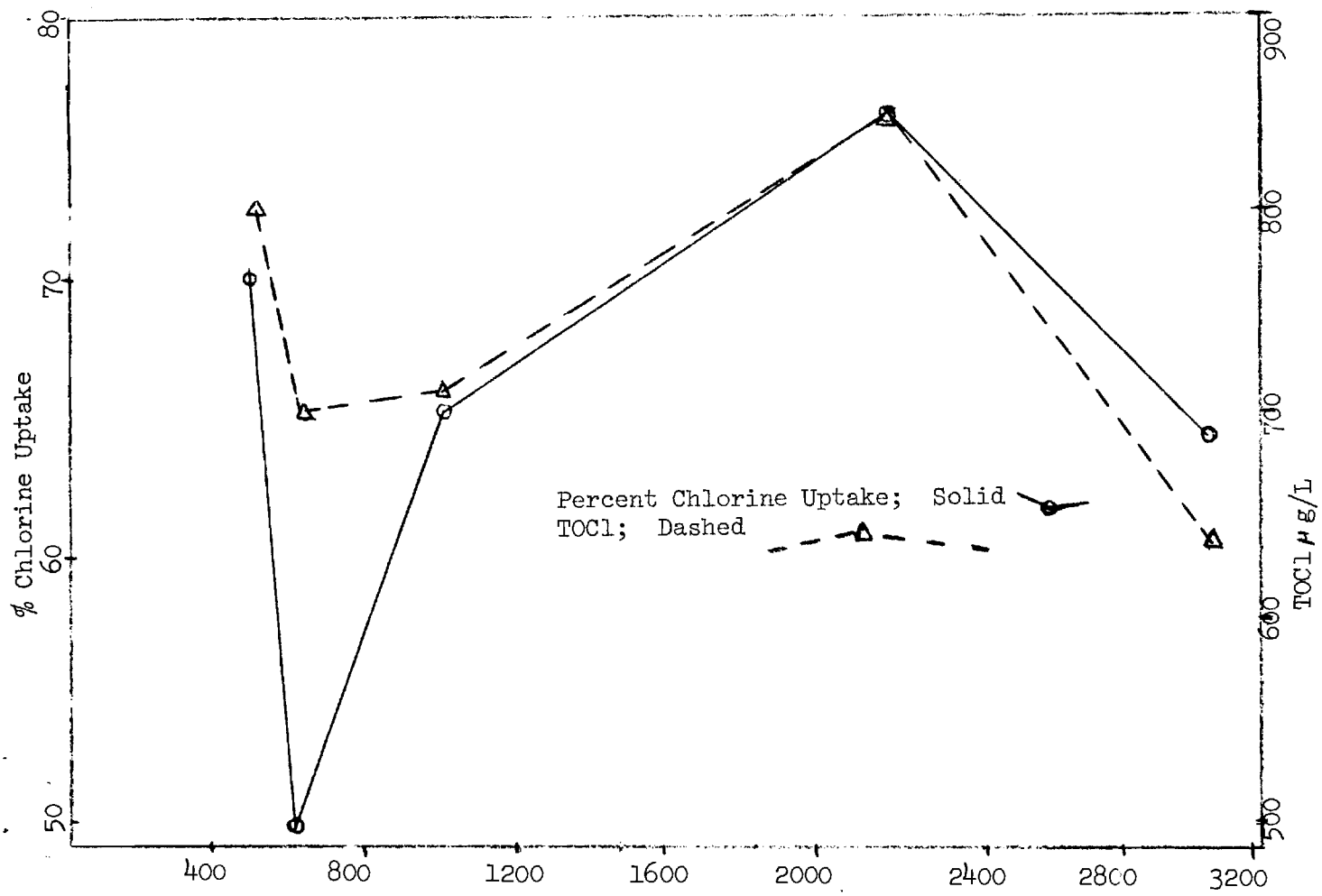


FIGURE 33. SEPARATION-CONCENTRATON SCHEME

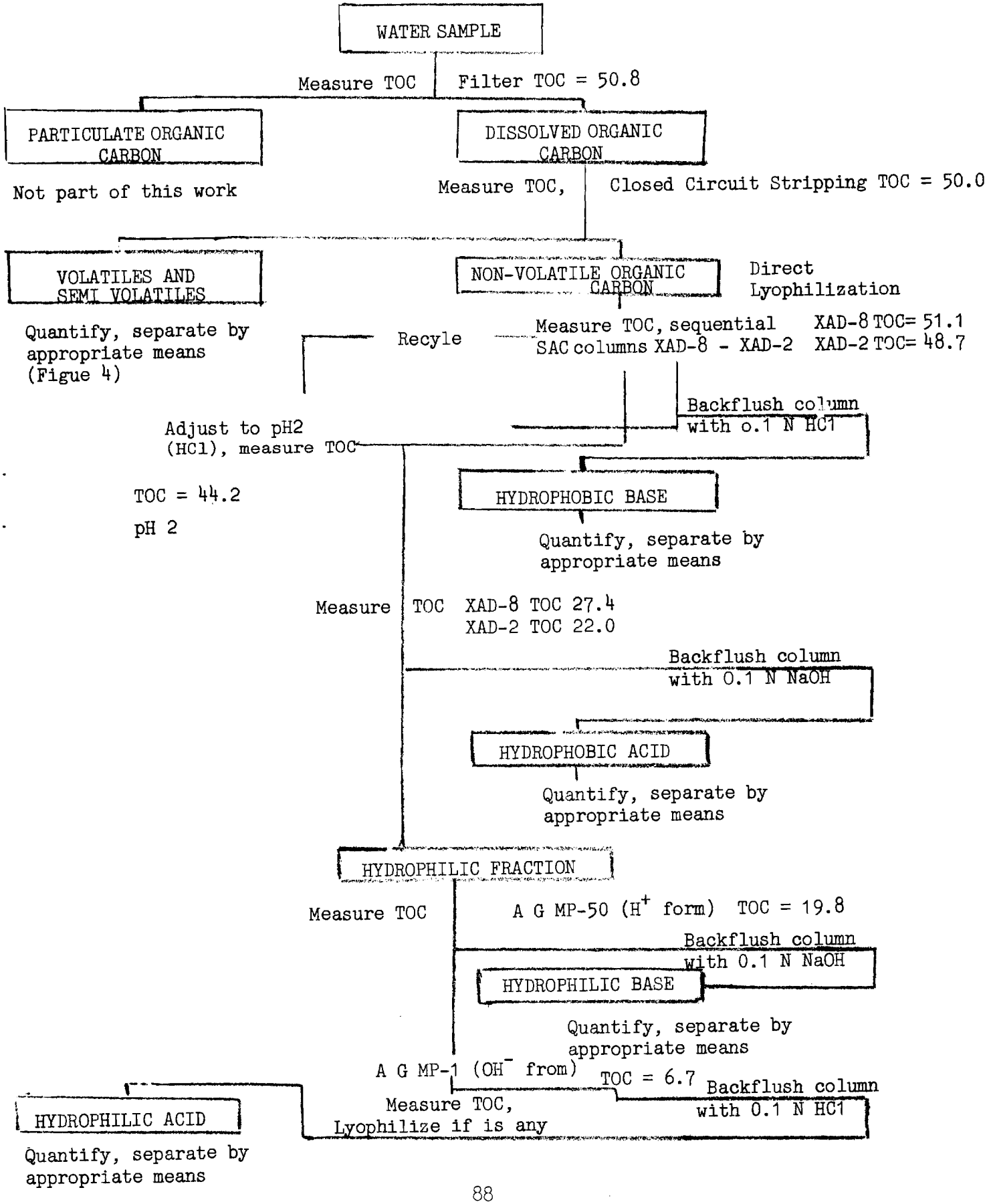


Table 15

Recoveries of River Water Organics by Class

	Removed		Recovered	
	mg	%	mg	%
Hydrophobic Bases from XAD-8* and XAD-2	6.2	4	8.2	132
Hydrophobic Acids from XAD-8	49	35	63	140
Hydrophobic Acids from XAD-2	12	11	14	117
Hydrophilic Base from AGMP-50	4.2	3	2.5	60
Hydrophilic Acid from AGMP-1				
First Washing	21	26	15	94
Second Washing	6+		4.7	
Hydrophilic Neutrals	10	20		
SUM OR(OVERALL)	108	99	107	(99)

*Zero TOC removal on XAD-8. Extraction will be carried out nevertheless. At this time it seems inappropriate to present it as a separate figure.

+By difference.

CONCLUSIONS

Isolation of Aquatic Humics

Aquatic humic matter can be isolated from acidified surface waters with recoveries of about 60% using a resin adsorption technique. Large-scale processing is possible so that pound-quantities of the material can be made available for future study should the sponsor so desire.

Acid-Base Chemistry of Aquatic Humic Substances

Improved analytical techniques developed during the course of this project have made it possible to arrive at more precise values for operationally defined carboxyl groups in aquatic humic substances.

Reaction of Aquatic Humics with Permanganate

The presence of oxalic and succinic acids as the dominant products resulting from the mild permanganate oxidation of methylated aquatic humics together with the general dominance of aliphatically-derived products provides strong evidence that aquatic humic materials are more aliphatic and less aromatic than previously supposed.

Oxidation of Aquatic Humics with Iodine in Base

The halogens exhibit similar behavior in a variety of chemical reactions including the ability to disinfect. The high atomic weight of iodine makes it relatively easy to isolate iodoorganics-even when produced in very low yields. For example, iodoform can be isolated as a crystalline solid although the yield is only 0.8%--a value which is incidentally quite typical of those recorded for chloroform in chlorination reactions of humic materials.[4,12] Thus the presence of methylene iodide among the products identified lends credence to the suggestion that dihalomethanes may be genuine products of disinfection. This observation is important since the near-ubiquity of methylene chloride as a laboratory contaminant makes it difficult to confirm, particularly at extremely low levels. The finding of methyl and ethyl iodide is also significant.

These findings fit in rather well with the detection of short-chain alkyl branched dicarboxylic acids among the permanganate oxidation products described in earlier sections. For example, malonic acid which is actually employed to remove chlorine from chlorine dioxide would be expected to produce methylene chloride upon decarboxylation. If only one of the reactive sites were occupied by a chlorine, methyl chloride would result. If methylmalonic acid or an intermediate with a similar substitution pattern were present, ethyl chloride would be the expected post-decarboxylation product. The Project Manager feels that this question needs further researching.

Reaction of Aquatic Humics with Bromine in Acetic Acid

The presence of tetrabromoacetone and the suggested presence of C-5

bromoketones among the products derived from the drastic treatment of aquatic humics with bromine offers tie-ins with both the oxidative degradations (short-chain oxygenated acids) and the chlorination studies (oxygenated isoprenes). Further work in this area might also prove rewarding.

Chlorination Studies

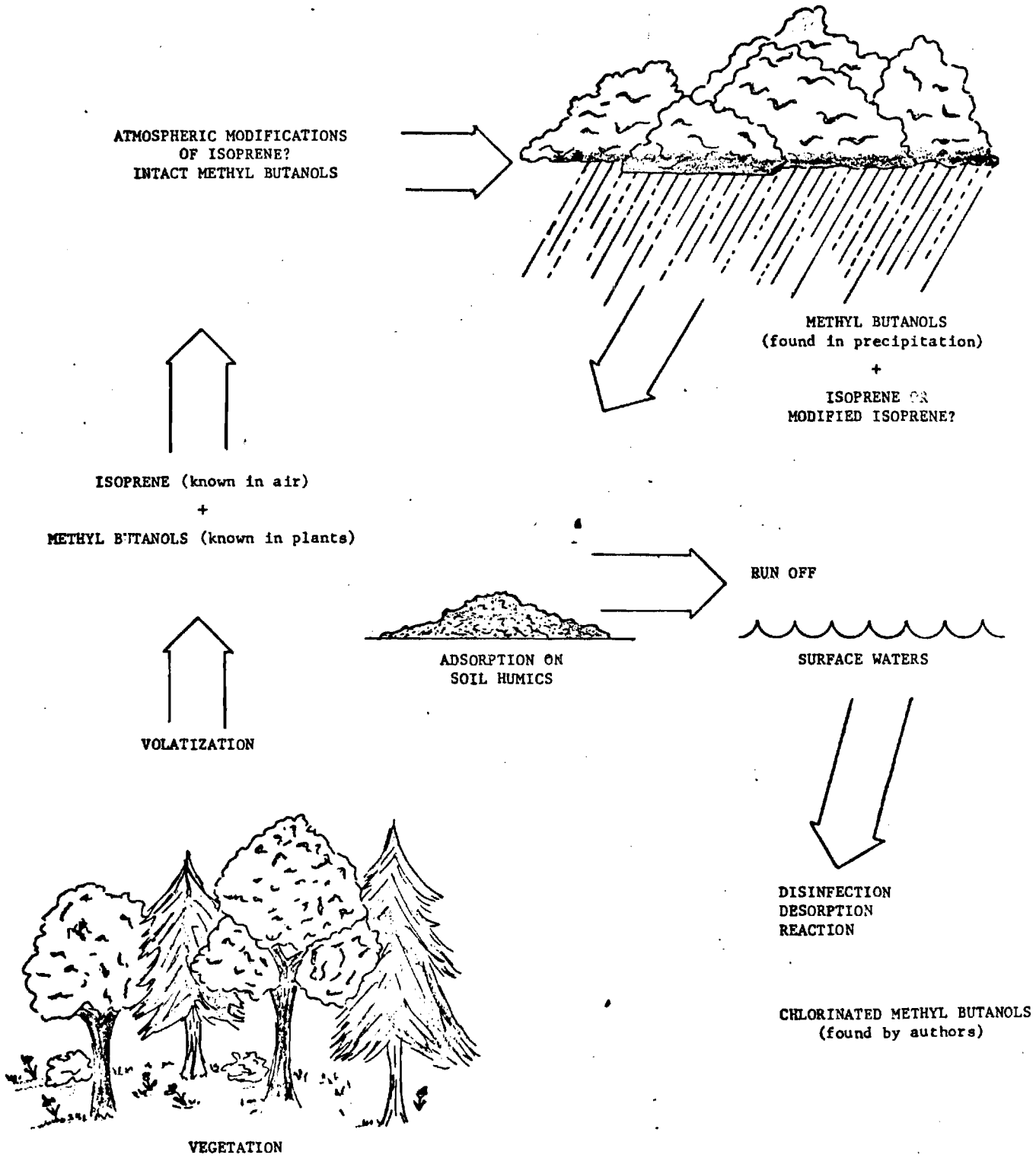
The isolation and identification of a series of chlorinated isoprenoid alcohols and hydrocarbons resulting from the treatment of immature aquatic humics with chlorine in a dynamic system simulating the disinfection of potable water is an observation of fundamental importance. We believe that the reported finding of the most dominant hydrocarbon in chlorinated secondary effluents by Glaze et.al.[18] is strong supportive and important evidence in the light of the fact that secondary effluent are rich in immature aquatic humics and are frequently chlorinated prior to discharge into surface waters. Other supportive evidence regarding the presence of isoprenoid precursors in the atmosphere, in precipitation and in surface waters makes it possible to suggest a mechanism for the origin of the chlorinated methylbutanols as shown in Figure 34.

The detection of other adsorbed organics in aquatic humic material such as toluene and benzene, which are released for detection only after oxidative disruption of the aquatic humic matter supports the suggestions of other workers regarding the importance of aquatic humics in the transport of pollutant materials in the environment.

Aquatic humic materials as isolated by the Co-principal Investigator have an overall number average molecular weight ($\bar{M}_N$) of 1270. When

Figure 34

ORIGIN OF CHLORINATED METHYL BUTANOLS



these are subdivided according to molecular size, almost half of the material is found to have $M_{\bar{N}}$ of 2200. This is also the fraction which was found to clearly generate the most TOCl and which showed the highest chlorine uptake. Fractions having $\bar{M}_N$ of 1100 or less accounted for very nearly all of the remaining material. It should therefore be noted that while these fractions do not appear distinct with respect to their gross properties such as IR, UV and elemental analysis, they do appear to differ significantly in their chlorine demands and in their ability to generate total organic chlorine (TOCl) when treated with chlorine under conditions simulating the production of potable water.

Classification of Raw River Water

Raw river water can be successfully classified according to the scheme presented earlier in Figure 33 with an overall recovery of 99% as measured by TOC. Over 70% of the components removed are acidic in nature thus reinforcing the conclusion that aquatic humics (which are acids) are the major organic component of surface waters. On the other hand, organic bases account for less than 10% of the components removed and are therefore of lesser importance. The hydrophilic neutrals however do account for the remaining 20% and represent an area in which more investigation is required.

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IDENTIFICATION OF MAJOR AND MINOR CLASSES OF NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Final Progress Report

January 15, 1980

by

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SUMMARY

Approximately 80% of the organic matter present in unpolluted surface waters occurs in the form of aquatic humic substances. It was therefore deemed appropriate to investigate the properties and structure of this as yet uncharacterized polymeric material in the hope that inferences could be drawn which would be of use in the production of higher quality potable water from surface sources.

A large number of aliphatic compounds have been identified among the products resulting from the relatively mild oxidative degradation of aquatic humics. This result strongly suggests that aquatic humic materials which account for 80% of the TOC in typical surface waters are more aliphatic and less aromatic than previously supposed.

The isolation and identification of a series of chlorinated isoprenoid alcohols and hydrocarbons resulting from the treatment of aquatic humic matter with chlorine under conditions simulating the production of potable water have been accomplished. The fact that these compounds are structurally related to isoprene rather than to the typical oxidative degradation products of aquatic humic material itself suggests that the precursors are adsorbed within the aquatic humic matter. These compounds have been found to be produced at levels which are approximately one order of magnitude lower than those observed for the corresponding trihalomethanes.

The subdivision of aquatic humic material into five chromatographically homogeneous fractions using gel permeation methods has resulted in a size

classification ranging from number average molecular weight ($\bar{M}_N$)≈500 to $\bar{M}_N$≈3000. While these fractions do not appear distinct with respect to their gross properties such as IR, UV and elemental analysis, they do appear to differ significantly in their chlorine demands and in their ability to generate chloroform and total organic chlorine (TOCl) when treated with chlorine under conditions simulating the production of potable water.

The detection of adsorbed species in addition to the chloroalcohol precursors in aquatic humic material, which are released for detection only after oxidative disruption of the aquatic humic matter, supports the suggestions of other workers regarding the importance of aquatic humics in the transport of pollutant materials in the environment. Evidence developed in connection with this effort strongly suggests that air pollutants may also bind strongly to aquatic humic material.

INTRODUCTION

The use of chlorine for the disinfection of potable water has resulted in a general feeling of security regarding the freedom of public water supplies from objectionable materials. At least in the developed countries, this feeling is certainly justified from a microbiological standpoint. However, the National Organics Reconnaissance Survey conducted by the U.S. Environmental Protection Agency in 1974 and 1975[1] demonstrated the presence of trihalomethanes in the public water supplies of 80 major U.S. cities. These findings caused considerable concern because some of these compounds are known or suspected carcinogens.[2,3] Since it has also been demonstrated that these halogenated organics are formed during the disinfection process,[4,5] it is appropriate that EPA's Office of Drinking Water has awarded a significant research contract to the authors aimed at identifying the major and minor classes of natural organic substances in surface waters before and after disinfection. This report describes the findings obtained as a result of this investigation.

The work in question has had two major objectives, the first of which was to define the nature of the organic materials naturally present in surface waters such as might be used for the production of potable water. The second major objective of this work has been to isolate and identify those organic materials, particularly the chloroorganics which are produced from the aforementioned natural organics under conditions of disinfection currently practiced by the water industry.

It will be noted that the term "aquatic humic material" has been used in several places already within this document. It will be useful to define

this term in the sense in which it is used by the writers since not all of the workers in the area are necessarily in agreement regarding this matter. We shall preface our definition with a negative statement: whereas for soil humic substances a purely operational definition can be given to denote that fraction of soil organic matter which is extracted by alkali and can be separated into acid soluble (fulvic acid) and acid insoluble (humic acid) fractions by acidification of the extract, no equivalent definition can be given for aquatic humic material. These materials obviously have not been mobilized from soil by strong alkali, but rather by meteoric waters of low ionic strength and a pH range of 4 to 8. These mild extraction conditions in our sampling area lead to the solubilization of mostly low molecular weight humic fractions which are similar to soil fulvic acids. Secondary alterations, such as continued oxidative polymerization, e.g. in water bodies with longer residence times, can increase the relative abundance of high molecular weight humic fractions which may flocculate and precipitate at low pH values (pH <3.5). This material may then become more similar to soil humic acids. This "humic acid"-like fraction may be found in rivers in varying relative concentrations depending upon the soil chemistry of the drainage basin, the residence times of dissolved organic matter, pH and other as yet poorly understood factors. Additional work which is reserved for the future might be aimed at improving our understanding of the maturation process undergone by aquatic humics and at pinpointing the more subtle regional and seasonal differences which may exist in aquatic humic structures derived from different sources.

Considerable emphasis has been placed upon the aquatic humic fraction in connection with this project because this material accounts for such a large proportion of the organic matter typically found in surface waters. Some very recent work has demonstrated that 90% of the organics in river water were of molecular weight less than 3000 and furthermore, that these source materials account for 45% of the total trihalomethanes generated when treated with chlorine under conditions simulating the production of potable water.[6] This fraction corresponds exactly to what we have defined as aquatic humic matter.

Similarly, the major effort with disinfecting agents has been concentrated on chlorine since this is the agent having the most widespread use. Throughout this work, the empahsis has been on realism. Thus we have used aquatic humics at levels corresponding to 10mg/l or 5 mg/l TOC. This level is within the normal range of 2-10 mg/l TOC encountered in surface waters. Likewise the levels of chlorine dosed are in general agreement with current practice. Contact times, sequence of treatments, pH and selection of flocculant aids have similarly been chosen to be in general agreement with conditions employed for the production of potable water. In this way, the results described in this report have a special relevancy to the mission of the funding agency.

METHODS

Isolation of Aquatic Humics

River water was collected in batchwise fashion several times during the course of the project (see Table 3). Samples were collected in two 55-gallon stainless steel drums which had originally been used for USP alcohol. The drums were rinsed on site with river water, filled and brought to the laboratory for processing, which was begun within 24 hours of collection. The collected waters (470L in a typical case) were continuously clarified by centrifugation at 17,000 RPM with a residence time of one minute and a flow rate of 400 ml/min. This step prevents subsequent clogging of the resin columns used for concentration. The water was then acidified with hydrochloric acid to pH 1.8. Because the waters selected for study contained mostly low-molecular weight humics, no significant precipitation occurred. The protonation of the acidic functions on the aquatic humic material is necessary for the subsequent adsorption on the resin. Aqueous triethylamine (3.3%) is then employed to remove the adsorbed material from the macroreticular resin. Excess triethylamine is removed from the eluate under reduced pressure. An optional liquid-liquid extraction with an organic solvent can be performed at this time to remove triethylammonium hydrochloride. Such a step is not recommended if adsorbed organics in the isolated aquatic humic material are to be studied. (However, even continuous extraction does not completely remove such materials).

The triethylammonium salts of the aquatic humics are subsequently treated with an acidic cation exchange resin (Bio Rad AGW-50, H^+). This step also removes any inorganic cations still associated with the aquatic humic matter. The final product is obtained by lyophilization as an ash-free humic material which is completely soluble in both water and methanol.

Acid-Base Chemistry of Aquatic Humic Substances

Potentiometric titrations were carried out with a Fisher Accumet 144 pH meter and a Fisher combination pH electrode. One hundred milliliter aliquots containing 62.7 mg of Satilla River Humic Substances (SRHS) (3.135 g/L) in various ionic media (see Table 1) of varying ionic strength were titrated with 0.4997 N NaOH delivered from a Gilmont digital micrometer buret (2.5 ml capacity). Reactions of SRHS and acetylated derivatives with various bases were allowed to proceed for 24 hours with constant stirring under nitrogren. The reaction products were filtered using Amicon UM-2 filters, which removed all color (checked photmetrically at 420 nm). Aliquots ranging from 50 to 25 ml of filtrate were used for the titration. All blanks were prepared and titrated parallel to the samples. The use of the ultra-high precision micrometer buret allowed addition of very small volumes (0.001 ml) of more concentrated base (0.5 N NaOH), so that the final titration volume changed by less than one percent, eliminating the need to correct the evaluation of the titrations for dilution.

TABLE 1
General Parameters of Acid - Base Titrations of SRHS

Titration	Type[a]	Supporting Electrolyte[b]	Weak Base[c]	Filtration[d]
1	D	$Ca(NO_3)_2$	None	None
2	D	NaCl	None	None
3	I	None	$Ca(CH_3COO)_2$	W#42
4	I	None	$Ca(CH_3COO)_2$	UM-2
5	I	None	$Na(CH_3COO)$	UM-2
6	I	NaCl	C_5H_5N	UM-2

[a]D= direct titration; I - indirect titration.
[b]0.033 M $Ca(NO_3)_2$; 0.100 M NaCl
[c]0.100 M $Ca(CH_3COO)_2$; 0.100 M $Na(CH_3COO)$; 0.150 M C_5H_5N
[d]W#42 = Whatman #42 paper; UM-2 = Amicon UM-2 membrane

Reaction of Aquatic Humics with Permanganate

Aquatic humic material (1.0 g) isolated as previously described was dissolved in methanol (100 ml) and treated for 1-2 hours with diazomethane in ether at ambient temperature. The partially methylated product gradually became insoluble in methanol at which time the original solvent was removed and replaced with an equal volume of chloroform. After 1-2 hours reaction with additional etherial diazomethane, the reaction was presumed to be complete. Removal of solvent under reduced pressure provided 1.18g of methylated product. Unlike results reported recently in the literature,[7] this material is readily soluble in organic solvents. In earlier reactions, this material was suspended in aqueous potassium permanganate (4% w/v; 125 ml) and brought to reflux. Reaction conditions were gradually moderated to less vigorous conditions i.e., 60°C and no reflux and a non-aqueous system in which the crown-18-ether was employed to hold a low concentration of permanganate in solution at room temperature. Some typical conditions

are summarized in Table 2. The product mixtures were then treated with methanol, to destroy excess oxidant. Afterwards, the reaction mixtures were filtered, treated with cation exchange resin to strip out metallic salts, acidified to pH2 with HCl, extracted and treated once again with diazomethane. The weights of the final products were calculated and the mixtures analyzed by GC/MS.

TABLE 2

REACTION CONDITIONS OXIDATION OF AQUATIC HUMICS

	Temperature	Time	Percent Yield
Aqueous $KMnO_4$(5g/g)	Reflux	4 hrs	23
Aqueous $KMnO_4$(8g/g)	60°C	6 hrs	47
Aqueous $KMnO_4$(8g/g)	55°C	6 hrs	58
CH_2Cl_2,$KMnO_4$(12g/g) 18-crown-6-ether(2g)	room temp.	24 hrs	83

Chlorination of Aquatic Humic Material in the Mini-Pilot Facility

In order to duplicate conventional methods for the disinfection of potable water as accurately as possible, a mini-pilot facility was fabricated. This apparatus was constructed entirely of Teflon and glass and is shown schematically in Figure 1. The dimensions are presented in Figure 2. The modular design permitted the facility to be dismantled and baked out between runs.

In a typical run, the reservoir was loaded with 12 liters of high-purity (Continental Water Conditioning consisting of filtration, double ion exchange, carbon filtration and pre-purging) water containing 10 mg/L

Figure 1. Schematic - Mini-Pilot Facility

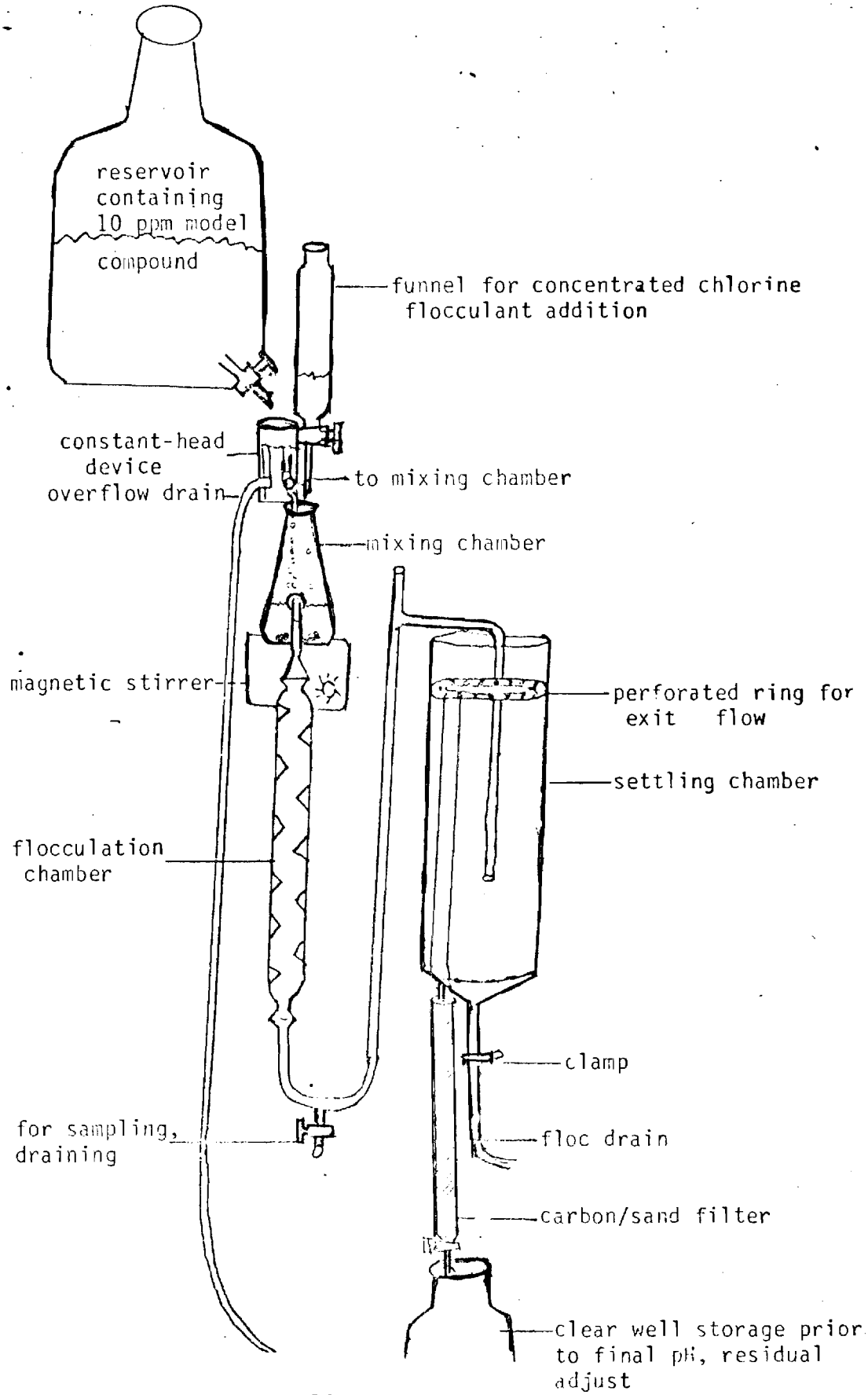
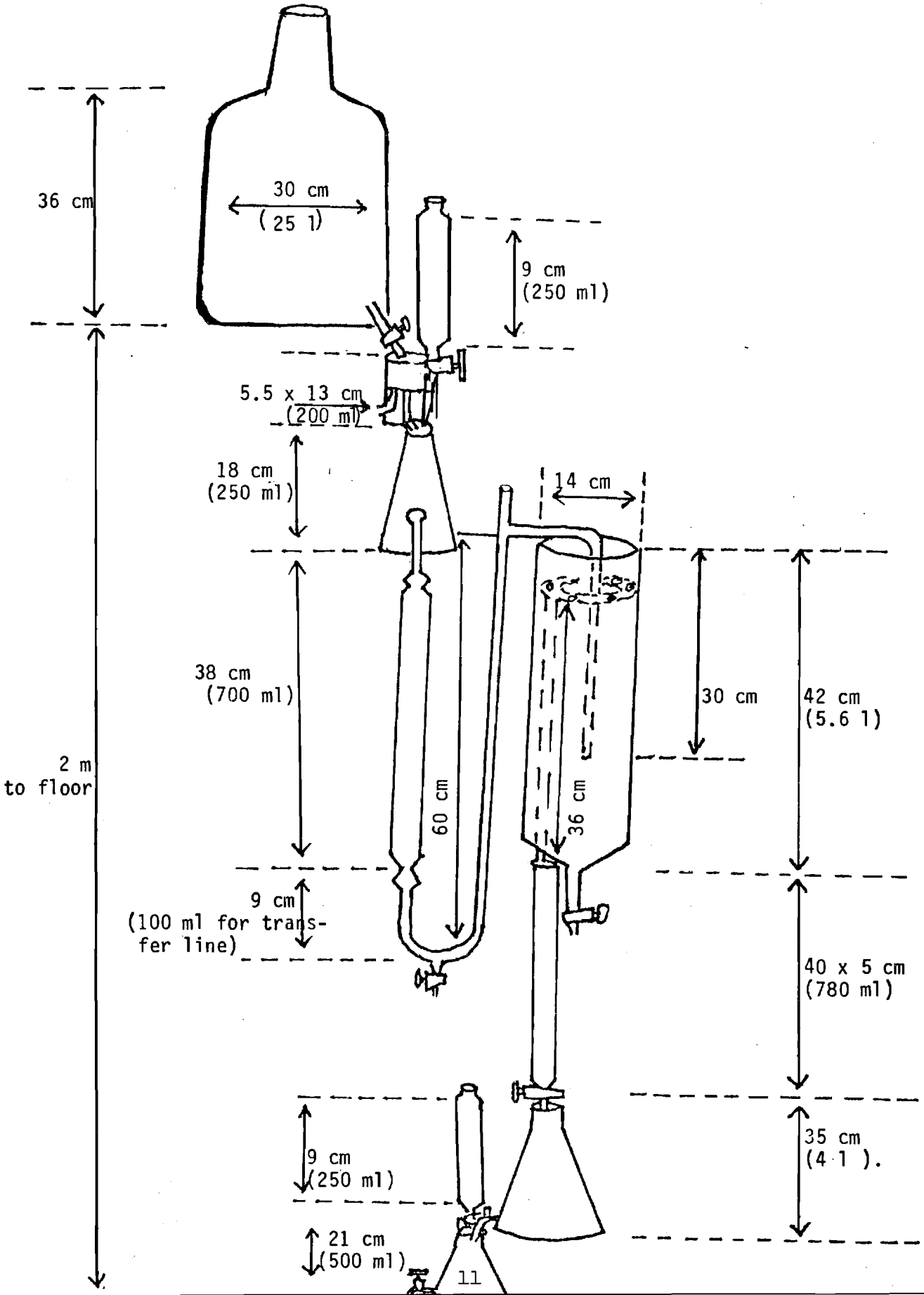


Figure 2 Dimensions and Volumes of Mini-Pilot Facility.



aquatic humic material together with whatever buffering agents were required to maintain the pH within the specified range for the experiment in question. The flow from the reservoir was adjusted to 1.0 L/hr and balanced with an appropriate flow of chlorine concentrate and flocculants. The desired levels of these chemicals were independently established ahead of time by jar tests and chlorine demand studies. The flocculants and buffering agents were all pretreated in a muffle furnace in order to remove all organic matter. The chlorine concentrate was prepared from gaseous chlorine generated in an all-glass system from prepurged hypochlorite and H_2SO_4. Incidental carryover of acid beyond that expected from the dissolution of chlorine in the water was not found to be a problem. Chlorine addition was adjusted to provide a 24 hour residual not to exceed 2 mg/L.

Samples (1L or more) were withdrawn and immediately treated with pre-roasted sodium sulfite. Other samples were allowed to stand 24 hours prior to treatment with sodium sulfite. A sample withdrawn from the reservoir and similarly treated served as the control. All samples were acidified with HCl and extracted with high-purity ethyl ether.

The combined extracts (3x100 ml) were subsequently dried over pre-roasted sodium sulfate and concentrated to 1-2 ml using a Kuderna-Danish apparatus.

The concentrated extracts were then treated with diazoethane (generated from N-nitroso-N-ethyl-N'-nitroguanadine) at ambient temperature until the yellowish color of the reagent no longer faded. Independent tests with a number of standards confirmed that this reagent is about as effective as diazomethane if applied under the same conditions (i.e., 60-85% conversion).

The derivatized product mixtures were then reconcentrated and examined by capillary column GC/MS using the techniques of both electron impact (EI) and methane chemical ionization (CI). Spectra were compared with the EPA/NBS library using the INCOS software provided with the Finnigan Model 4023 GC/MS/DS. Structures were assigned on the basis of library comparisons, operator interpretation and user-developed automatic search routines.

Reactions were carried out at 3 pH values: 6.0, 7.3 and 8.2, in accordance with the sponsor's request to survey a rather narrow range of pH values. The pH values were adjusted using organic-free phosphate buffering systems. Ferric chloride was employed as the flocculant in some cases. The sand used in the filters was freed from organics by pretreatment in the muffle furnace. The granular activated carbon employed in some of the experiments was water treatment grade (Nuchar WV-H) donated by the Westvaco Corporation.

Subdivision of Aquatic Humic Materials According to Molecular Size

A sample of aquatic humic matter was fractionated by gel permeation chromatography. The partitioning was begun with Sephadex G-50 and continued consecutively with G-25, G-15 and G-10. Each excluded fraction was collected, concentrated and re-chromatographed on the same gel until it contained only excluded material. The retarded fractions likewise were collected, concentrated and chromatographed on the next lower grade of Sephadex. In this way, five fractions were obtained: (I) G-50 excluded; (II) G-25 excluded; (III) G-15 excluded; (IV) G-10 excluded and (V) retarded. Fraction II was found to contain almost half of the starting material. The amounts collected in each fraction were: (I) 3.6%; (II) 47%; (III) 14%; (IV) 15%; and, (V) 21%.

Chlorination of Aquatic Humics Following Molecular Weight Fractionation

A bench scale experiment was set up using a series of 100 ml glass-stoppered reaction flasks each containing 10 mg/L aquatic humics from the chromatographically resolved molecular size fractions described in the previous subsection. Duplicate runs were made for each of the five fractions. Each of the fractions was contacted with identical amounts of chlorine as required to maintain a residual throughout the experiment. After 24 hours, the excess chlorine was quenched with organic-free sodium sulfite and sealed in crimp-top bottles prior to being submitted for Total Organic Halogen (TOX) and chloroform analysis. A small portion of each reaction mixture was analyzed for residual chlorine immediately prior to the sodium sulfite treatment.

Oxidation of Aquatic Humics with Iodine in Base

A solution (25 ml) of aquatic humic material (500 mg), sodium hydroxide (2.5 g) and iodine (3.7 g) was heated to 65°C, held 30 min., and allowed to stand overnight at ambient temperatures. The resulting dark brown solution was directly extracted with ethyl ether (3x25 ml) and the combined extracts back extracted with water (2x25 ml). The ether layer was then dried over sodium sulfate and solvent and excess iodine removed under reduced pressure to provide 13.4 mg of yellow hexagonal prisms, mp 122° C. The product was tentatively identified as iodoform on the basis of these properties (lit mp 123) and subsequently confirmed by GC/MS.

The aqueous phase remaining after extraction was combined with the back extracts, acidified with HCl and reextracted with ethyl ether (3x25 ml). The combined extracts were dried over sodium sulfate, concentrated and examined by GC/MS.

Reaction of Aquatic Humics with Bromine in Acetic Acid

Aquatic humic material (sample M/30, 100 mg) was suspended in acetic acid (4 ml) to which bromine (0.3 ml) was subsequently added dropwise with swirling. The reaction mixture was then heated (60°) for 10 minutes during which time the suspension gradually dissolved. The solvent and unreacted bromine were removed overnight using a slow nitrogen sweep. Unfortunately, this procedure may have caused the loss of some of the more volatile products. The resulting deep red flakes were further dried and then examined using the infrared spectrophotometer. Compared to I.R. spectra of untreated humic material, a new band appeared in the product at 485-635 cm^{-1} which can be attributed to C-Br stretching. The relative intensity of the 1600-1650cm^{-1} region is somewhat decreased, possibly due to the bromination at enolizable carbonyl functions. A concentrated pentane extract of this material was examined by capillary column GC/MS.

Classification of Raw River Water

A 4.4-liter subsample of Satilla River water was filtered through Whatman No. 1 paper to remove the small amounts of suspended solids which had built up in the sample during transit. Portions of this filtered subsample were retained for TOC analysis. In this way, the TOC will provide a check on the material balance.

The remaining water sample (3.3 liters) was treated with 10 drops of concentrated potassium hydroxide in order to bring the pH to 9.0. This water (3.3 liters) was passed through four beds (11 x 50 mm) of precleaned XAD-8 macroreticular resin. Each bed contained about 2g of resin (four beds were used to speed the completion of the process without introducing rapid flow rate). In this way, the flow rate was maintained at 100 ml/hour/bed.

A sample of the XAD-8 resin column eluate was reserved for TOC measurement. The remaining eluate (3.1 l) was passed through four beds of XAD-2 resin in a similar way as that described for the XAD-8 treatment. A sample of the eluate (pH=7.5) from the XAD-2 resin was retained for TOC measurement. At this point in the separation-concentration sequence, any hydrophobic bases present in the sample are retained on the resin. The XAD-8 and XAD-2 resins were then back-washed with 500 ml 0.1 N hydrochloric acid per bed, thus providing 2 l of eluate containing hydrophobic bases from each type of resin.

The pH of the sample after passage over XAD-2 was adjusted to 2.0 with purified 6N hydrochloric acid. A brown precipitate slowly developed in this solution (2.8 l) as it was passed again over XAD-8 in the manner described above. As a result of the precipitate formation, the flow rate dropped to only a few ml/hr after about 2 l of water had passed through the resin. Once again a portion of the eluate was retained for TOC determination.

The remaining eluate (2.3 l) was subsequently passed over the XAD-2 resin. Very little additional colored material appeared on the resin. A portion of the eluate (pH 2.1) was also retained for TOC evaluation.

Two beds of pre-purified AG MP-50 ion exchange resin (H^+ form) equivalent to 4g of dry resin were prepared in water, following activation with 10 ml of 3N purified hydrochloric acid and washing with 100ml distilled water per bed, the columns were treated with the remaining XAD eluates at a flow rate of 125ml per hour per bed. A portion of this eluate (1.9 l, pH 1.2) was retained for TOC analysis. The remaining eluate was brought to pH 7.0 with 1N NaOH (65 ml).

Two columns of pre-purified AG MP-1 resin containing about 2g each of material on a dry weight basis were converted to the OH-form with 10 ml of 1N sodium hydroxide solution and washed with 200 ml of distilled water. The remaining 1.6 l of eluate was passed through the resin beds at a flow rate of 150ml/hr/bed. The color was completely removed by this treatment. The upper part of the resin became dark brown. A portion of this eluate (1.5 l, pH 7.0) was retained for the measurements. This eluate contains the hydrophilic neutrals.

Back-flushing of the AG MP-50 and AG MP-1 beds with a total of 200 ml of 0.1N sodium hydroxide and o.1N sulfuric acid provided the hydrophilic base and hydrophilic acid fractions.

TOC determinations were carried out using a Beckman 915 TOC Analyzer. The precision of the method for the 10-50 mg/l range has been established as ±0.5mg/l.

RESULTS

Isolation of Aquatic Humics

The first element in the isolation of aquatic humics from the environment is source selection. In the case of our research, source selection was governed by the following requirements: (1) high DOC* content, (2) predominance of low molecular weight aquatic humic materials of high solubility and (3) a pure "humic" character. DOC concentration shows a positive correlation with amount of runoff, which in turn indicates that DOC is allochthonous and therefore is derived from soil organic matter. Our source water is taken from a small stream originating in the flood plain swamps of the Satilla River in South Georgia. The humic material appears to be generated in the swampy subsurface soil and flushed out during periods of high groundwater levels. There is a linear correlation between TOC and absorbance at 425 nm. Since humic substances absorb strongly at this wavelength, they appear to be the overwhelming contributors to the TOC in the Satilla River with little additional contribution from biopolymers (e.g. peptides and polysaccharides).

The predominance of low molecular weight humic substances of high solubility is believed to be due to the proximity of the point of sampling to the point of origin of the material. As a result of our continuing research on the nature of organic matter in river systems, we have come to the conclusion that low molecular weight humic substances as they are "floated"

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*DOC = dissolved organic carbon

out of the subsurface soil layers by a rising water table continue to polymerize by acid-catalyzed and oxidative coupling. This progressive humification leads to higher molecular weight material which can subsequently and successively form colloidal, microparticulate and finally floccular humic aggregates. Thus aquatic humic substances in a river system occur in particle sizes ranging above and below 0.4μm which is the conventional DOC-POC (particulate organic carbon) boundary. However, for purposes of drinking water research it was considered to be more important to place emphasis on the low molecular weight fraction since this is the very material which is most likely to escape removal by such pre-treatment procedures as flocculation, settling and filtration.

The researchers were aware of the importance of selecting a source water for the gathering of the aquatic humic material which was nominally free from inputs of anthropogenic matter which might introduce unnecessary complications into subsequent studies performed on the isolated material. While the complete exclusion of such foreign material is not possible, particularly in the case of the dialkyl phthalates, it is desirable to hold it to a minimum. The location shown in Figure 3 was selected as the sampling site for this work. The Satilla River drains a swampy area and is relatively free from municipal, agricultural and industrial inputs of pollution. Furthermore, since the samples are collected from a region of highly leached sandy soil (kaolinite and gibbsite), the humic material thus obtained has an extremely low ash content which is easily removed leading to a highly soluble, ashfree material. A summary of the collection data is presented in Table 3.

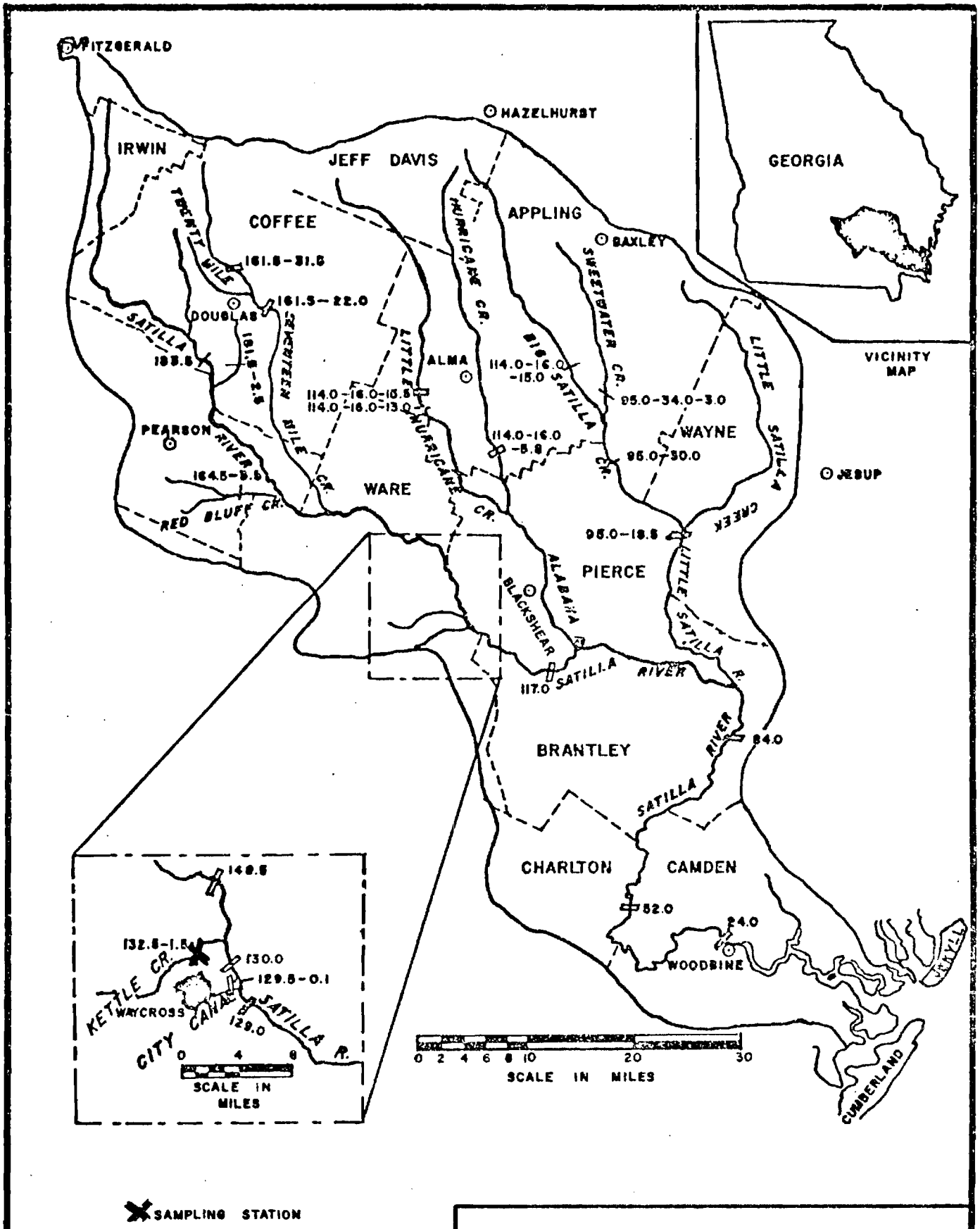


Figure 3.

MAP OF THE SATILLA RIVER BASIN

TABLE 3

COLLECTION DATA: AQUATIC HUMIC MATERIAL

Date	Level of Aquatic Humics in Source Water* (mg/L)	Liters Processed	Yield in Grams	Percent Recovery
October 13, 1977	58.5	294	39	73
December 27, 1977	45.5	411	23	60
May 24, 1978	51.5	390	24	60
October 5, 1978	7.0	395	3.3	61

*Based on UV absorbance at 425 nm

By avoiding the use of strong bases such as sodium hydroxide for the elution of the XAD resins, the oxidative coupling of phenolic groups and other base-catalyzed condensation reactions is minimized. In retrospect, it might also have been useful to exclude air from the system while it is at high pH although this precaution was not actually taken. The choice of a tertiary organic amine as the eluting base avoids the alteration of the concentrated aquatic humic substance via the inclusion of nitrogen as is known to occur with primary and secondary amines. Liquid-liquid extraction with chloroform is employed to eliminate trimethylammonium hydrochloride. It will be noted that this step is omitted if the isolated aquatic humics are destined for use in trihalomethane-generation studies. Since the acidic functions at this point are present as triethylammonium salts, it is desirable to replace these with protons by means of ion exchange treatment. This treatment also serves as a desalting step by removing other cations which might also be present. The final product is completely soluble in both water and methanol.

The convenience of the method permits the isolation of aquatic humic material in batches of 20-40 grams. It would not have been inconvenient to isolate the aquatic humic material in hundred-gram lots. The Project Manager is willing to provide EPA with large quantities of a representative aquatic humic material should EPA desire to conduct some in-house projects on aquatic humics. Enough material is on hand at both Georgia Tech and Bemidji State University to permit the immediate resumption of work should additional funding become available.

Physical and Chemical Properties

Some physical and chemical properties of the light brown amorphous solid are as follows:

Elemental Analysis:	C	50.13%
	H	3.50%
	N	0.80%
(by difference)	O	45.57%

Simplest formula: $C_{145}H_{123}N_2O_{100}$

IR (underivatized): 2500-3600cm^{-1} broad, strong; H-bonded OH
2950cm^{-1} strong; aliphatic CH
1700cm^{-1} broad, strong; acid carbonyl
1615cm^{-1} broad, strong; probable C=C

IR (after methylation):

3200-3600cm^{-1} broad, weak; non-acidic OH
3000cm^{-1} sh, weak; unsaturated CH
2950cm^{-1} strong,; aliphatic CH
1725cm^{-1} strong; ester carbonyl
1600cm^{-1} medium; probable C=C
1430cm^{-1} medium; possible C=C-H bend

NMR (exhaustively methylated):

80.7-81.7	broad, aliphatic	5H
83.4	broad, CH or CH_2 in electronegative environment	8H
83.8	broad, OCH_3	20H
87.35	sharp, aromatic	2H

Acid-Base Chemistry of Aquatic Humics

In the analysis of oxygen containing functional groups of water soluble humic substances no precise method has been devised in order to distinguish between carboxyl groups and phenolic hydroxyl groups. Usually one determines the amount of total exchangeable protons and considers these to be derived from the sum of both carboxyl and phenolic hydroxyl groups. Since the carboxyl functions on the humic polymer exhibit a considerable range of acidities, direct titrations are in many cases difficult to evaluate: the titration curves do rarely exhibit distinct inflection (or equivalence) points. In order to circumvent this problem, one usually reacts the humic substances with a weak base (e.g. calcium acetate) with the intention of titrating the resulting monoprotic acid (e.g. acetic acid), the titration curve of which should exhibit a sharp inflection point. Having thus arrived at a value for carboxyl content, one calculates phenolic hydroxyls by difference from the total exchangeable protons. It is obvious that the ratio of carboxyl to phenolic hydroxyl depends solely (if one considers the method for total acidity to be accurate) on the accuracy of the determination of carboxyl groups.

We have encountered a series of analytical difficulties and discrepancies in the analysis of carboxyl in aqueous humic substances which require a re-evaluation of the standard methods used for soil humic

substances (as given e.g. in Schnitzer and Khan, 1972).[8] For this reason we have investigated new analytical approaches to this problem.

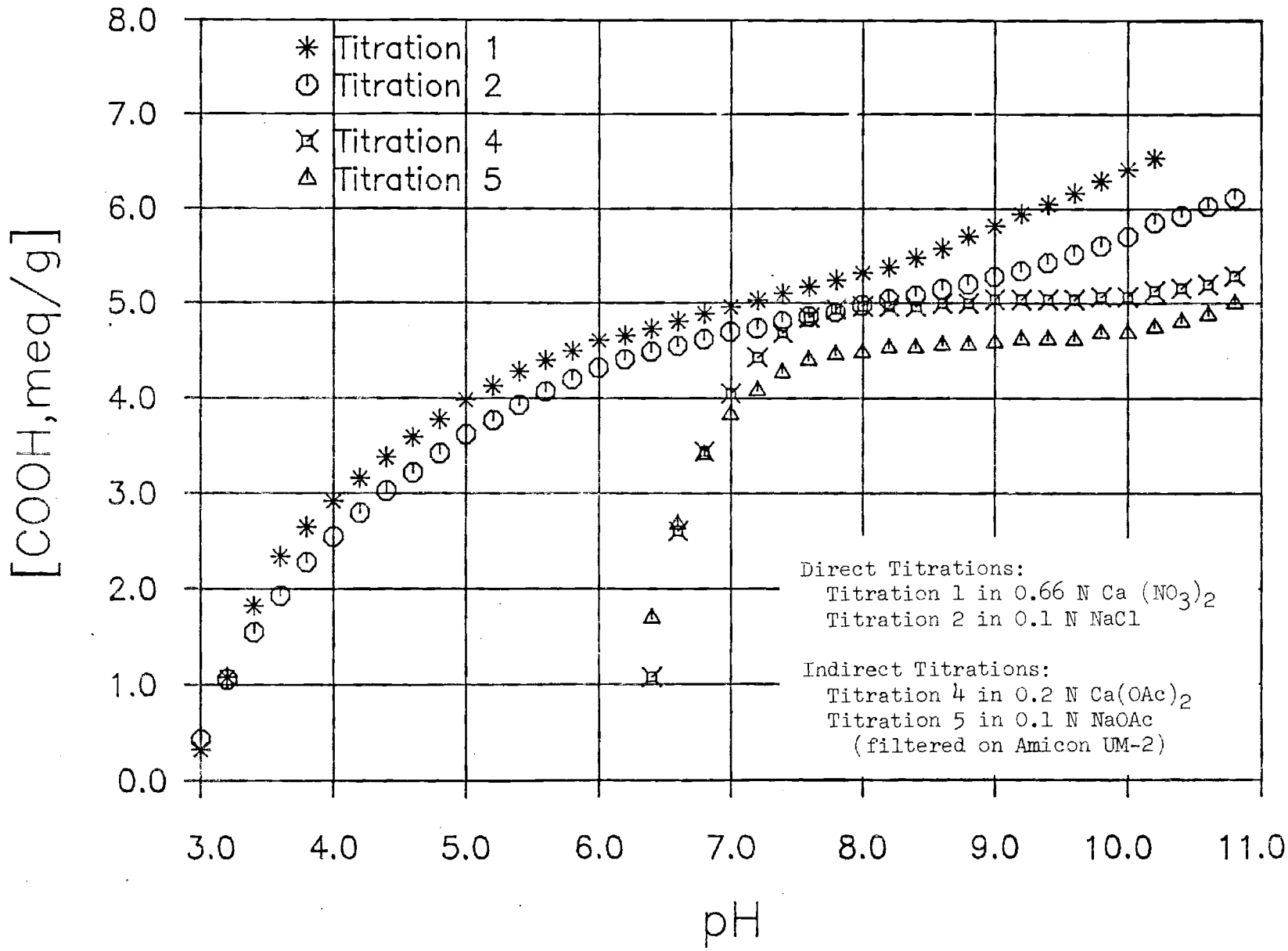
The reason for reacting humic substances with bases such as acetate or pyridine is, as mentioned above, to convert the polymer bound carboxyl function whose acid dissocation constants cover a range of values, to a monoprotic acid (e.g. acetic acid, pyridinium ion), and then to titrate this monoprotic acid after separation from the polymer. Originally the calcium-acetate method had been developed for humic acids which forms insoluble calcium salts which can then be separated from the solution containing the acetic acid by simple filtration through filter paper (e.g. Whatman #42). Water soluble humic substances, however, do not form appreciably insoluble salts. Therefore, filtration through filter papers or even 0.4 μm micropore filters does not impede passage of most of the dark colored polymer. Kwak et al. (1977)[9] have shown that acid soluble soil fulvic acids are retained by ultrafiltration through Amicon UM-2 membranes. We have likewise found a high degree of retention for aqueous humic substances on these filters, enabling us to separate acetic acid and pyridinium hydrochloride from the dark colored polymer.

The derivation of "net titration curves" (see Figures 4-6) is accomplished by subtracting the corresponding blank curves from the original titration curves. This method eliminates the effect of mere base dilution, so that only actual base consumption by titratable, i.e. acidic matter, becomes visible (shown on the ordinate in milliequivalents of base consumed per gram of humic material). A theoretical curve for the titration of a monoprotic acid with base demonstrates that as soon as the titration endpoint is reached, the slope of the curve becomes zero, indicating that all titratable substances

have been neutralized, so that no more base can be consumed. The titrations divide into two major types: 1) acidic polyprotic humic substances are directly titrated with a strong base, and 2) humic substances are reacted with a weak base (acetate, pyridine) to generate a monoprotic acid (acetic acid, pyridinium ion) which is subsequently separated from the colored humic material and titrated. In the following the problems encountered in determining the carboxyl group content of water soluble humic substances (e.g. fulvic acids, river water humic substances and the like) by potentiometric titrations will be discussed. From the shape of the "net titration curves" (T1, T2, see figure 4) resulting from the direct titration of aquatic humic matter with sodium hydroxide one recognizes the fact that acidic functions are continuously titrated even beyond pH 10. The curves have their flattest portion in the pH interval 6 to 8.6, but the slope never approaches zero. This titration behavior is characteristic for polyelectrolytes and illustrates the fact that carboxyl groups in fixed association with macromolecules exhibit a range of differential acidities. The fact that the slope of the curve never approaches zero, precludes a quantitative evaluation of the carboxyl content. Rather the flattest and almost perfectly linear ($r > 0.999$) portion of the "net titration curve" ranges from 4.6 to 5.2 milliequivalents of base consumed per gram of aquatic humic matter in the presence of 0.1 N NaCl, and from 4.6 to 5.2 meq/g in the presence of 0.666 N $Ca(NO_3)_2$. The "net titration curve" of aquatic humic matter in the presence of 0.666 N $Ca(NO_3)_2$ exhibits a second straight segment ($r > .999$) beyond 5.5 meq/g, intersecting the first one at 5.4 meq/g, indicating titratable material up to 6.5 meq/g at

Figure 4

TITRATION OF SATILLA RIVER HUMIC SUBSTANCES



pH 10.2. At pH 9.8, the endpoint recommended for the calcium acetate method, 6.3 meq/g have been titrated.

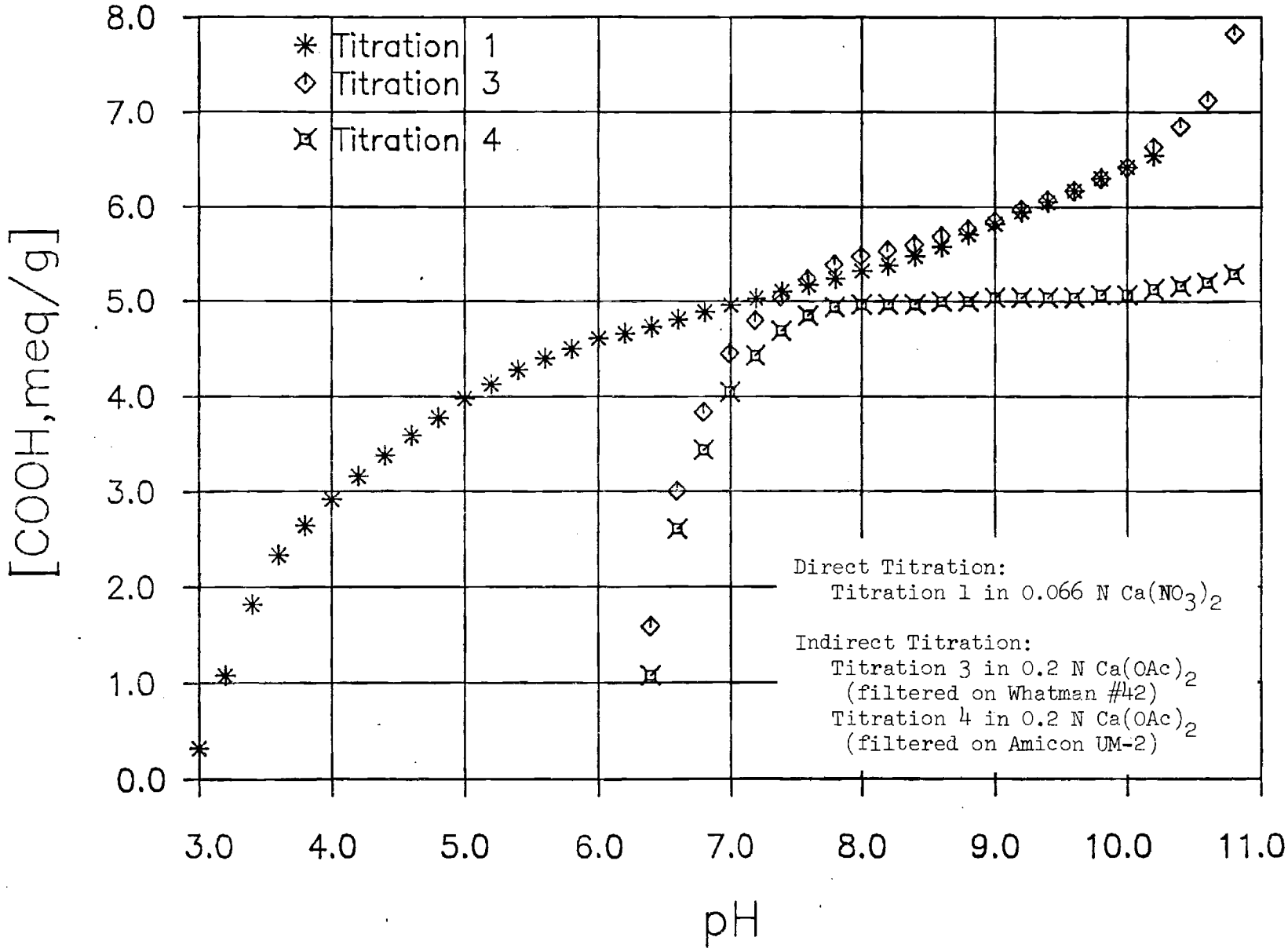
The first problem encountered in the indirect titrations is that of the separation of the monoprotic acid (e.g. acetic acid, pyridinium ion) generated by the reaction of the humic substances with the corresponding bases (acetate, pyridine). In the case of water soluble humic substances little or no solid reaction product is formed (e.g. insoluble calcium salts) which could be removed by filtration through either filter paper (e.g. Whatman #42) or micropore filters (0.4 μm). Only ultrafiltration through membranes of pore size 0.0001 μm (e.g. Amicon UM-2, 1000 MW cut-off) removes all color, as measured spectrophotometrically at 420 nm.

Typical "net titration curves" of aquatic humic matter after reaction with 0.1 M sodium acetate and 0.1 M calcium acetate and subsequent filtration through Amicon UM-2 are illustrated by T5 and T4 (see Fig. 4) respectively. The end of the titration of acetic acid is characterized by the almost horizontal region at 4.6 and 5.0 meq/g, for the titration after reaction above pH 8.4. Both titrations show an increase in slope starting at about pH 9.0, indicating the presence of small amounts of "excess" titratable material (0.3-0.4 meq/g at pH 10.8) with pKa's different from acetic acid. This may have been derived from the lower molecular weight fraction of fulvic acid which is able to pass the UM-2 filter, the nominal MW cut off of which is $\sim$1000. Since number average molecular weights of aquatic humic substances and soil fulvic acids are on the order of 10^3, such leakage can be expected.

Titration T3 (Fig. 5) illustrates what happens if one fails to

Figure 5

TITRATION OF SATILLA RIVER HUMIC SUBSTANCES



remove the colored humic material. In this case the sample after reaction of aquatic humic matter with calcium acetate, was filtered through Whatman #42 filter paper, which allowed passage of virtually all color. Compared with titration T4, the "net titration curve" of T3 continues to rise past the level of 5 meq/g, which is equal to the amount of carboxyl groups reacted with acetate anions in the presence of Ca^{2+}. The curve never levels off. This means that after the initial neutralization of free acetic acid, additional acidic functional groups on the polymer are continuously being titrated. Indeed, from pH 8.85 (the theoretical equivalence pH for titration of acetic acid in calcium acetate) to about pH 10.2 the "net titration curve" for T3 follows exactly that for the direct titration in the presence of $Ca(NO_3)_2$ (see Fig. 5).

The analytical procedures recommended for the determination of COOH groups in fulvic acids (e.g. Schnitzer and Khan 1972, p. 40) require evaluation of the titration of fulvic acid--calcium acetate reaction products (after filtration - unspecified) at pH 9.8. According to our results, when analyzing water soluble humic substances (many filvic acids included), there is no difference between evaluating either direct or indirect (i.e. after reaction with calcium acetate) titrations at pH 9.8, <u>if colored humic substances are not removed quantitatively before titration</u>. Evaluation at pH 9.8 lies in a region of the titration curve in which in the presence of colored humic substances acidic groups of pK_a greater than that of acetic acid are being titrated.

<u>If partially neutralized humic substances have been removed quantitatively</u> (which for water soluble humics can be achieved by ultra-

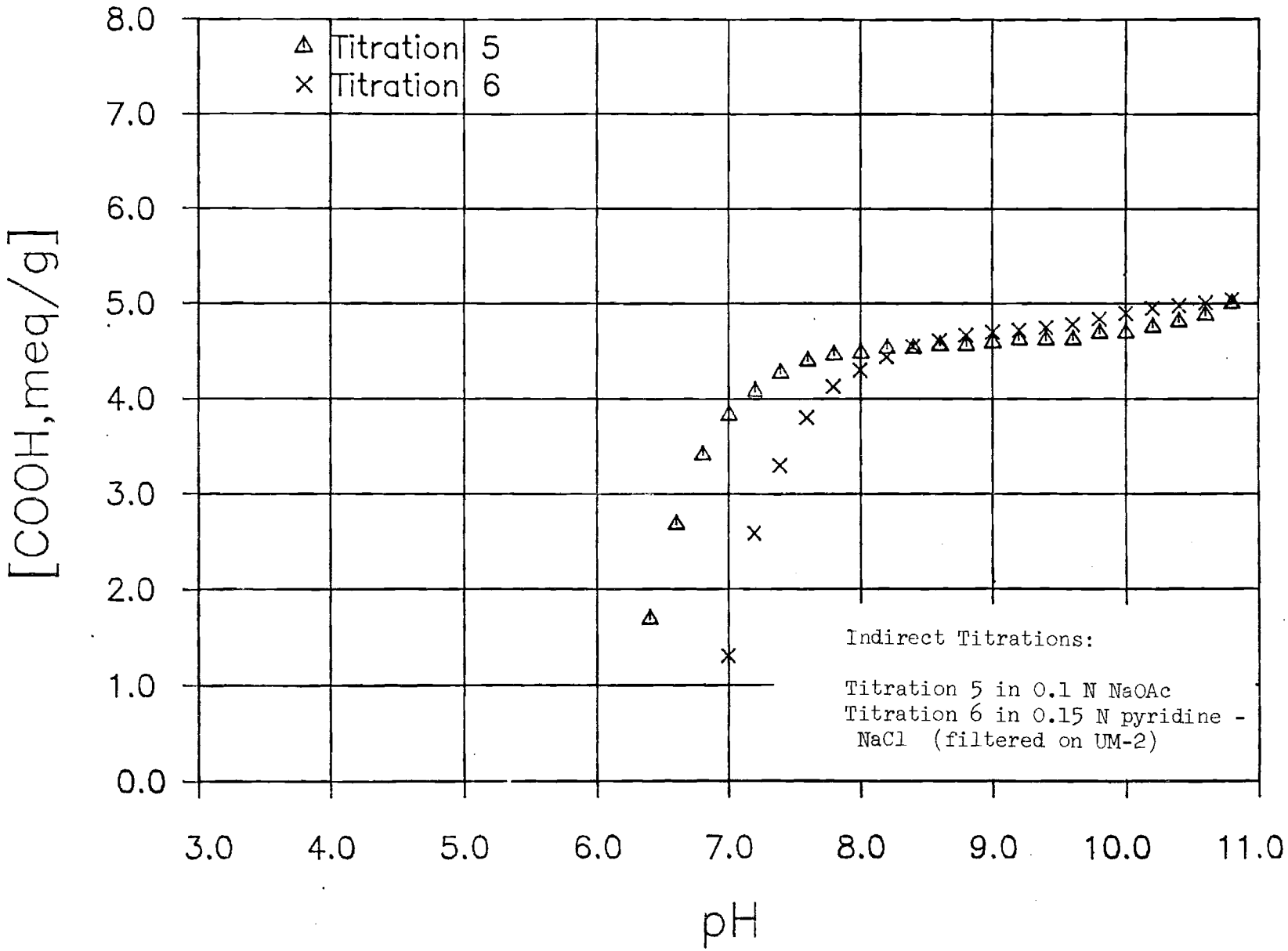
filtration), evaluation at pH 9.8 introduces an error commensurate with the efficiency of removal. Quantitative removal of colored humic material after reaction with acetate is therefore essential for the quantitative analysis of those carboxyl groups which are sufficiently acidic to protonate acetate ion.

Comparison of "net titration curves" T5 and T4, and T2 and T1 (see Fig. 4) reveals another important aspect of apparent carboxyl acidity. Titrations T2 and T5 were done in the presence of Na^+, T1 and T4 in the presence of Ca^{2+} as the counter ion. In the presence of Ca^{2+} the apparent COOH yield is increased. This increase, although small ($\sim$0.4 meq/g) is consistent and significant (in the case of aquatic humic matter $\sim$9%). Differences in ionic strength and base concentration can be neglected in producing this effect. We propose that calcium binding be considered the responsible factor for this increase. The positive charge associated with - $(COO^-Ca^{2+})^+$ can force an increase in acidity on other functional groups located in its vicinity on the polymer. However, this effect probably does not discriminate between carboxyl and phenolic hydroxyl functions. Thus the observed increased yield in apparent carboxyl content may be derived from either of the two types of acidic functional groups. For this reason it appears that a divalent counter ion, such as Ca^{2+}, should be avoided when reacting humic substances with acetate for carboxyl group analysis.

Titration T6 (Fig. 6) shows another aspect to be considered in carboxyl group analysis by means of pK-dependent methods. When reacted with pyridine, aquatic humic matter yields a slightly higher COOH content

Figure 6

TITRATION OF SATILLA RIVER HUMIC SUBSTANCES



as measured by titrating the resulting pyridinium ion. Since pyridine (pK_b = 8.83) is a slightly stronger base than acetate (pK_b = 9.25), a higher yield in COOH can be expected on these grounds alone. This differential yield illustrates the fact that pK dependent methods of carboxyl analysis will always give results dependent on the pK of the reacting base, since COOH groups on the polymer, as the direct titrations demonstrate, have a wide range of pK_a values. It appears therefore reasonable to redefine carboxyl content as the abundance of those acidic functional groups which are sufficiently acidic to protonate acetate ion (or pyridine) in the presence of a monovalent counter ion (e.g. Na^+), with acetate ion to be in sufficient excess (e.g. tenfold). Those acidic functional groups which are not protonated under these conditions may still in part be carboxyl groups but are excluded from the arbitrary definition since they are not acidic enough to react with acetate ion. It must be recognized therefore, that by using pK-dependent methods for the determination of carboxyl group content one has to compromise: directly titratable acidic functions in excess of those determined by reaction with acetate ion may still in part be carboxylic functions. However, so far none of the quantitative titration methods used can distinguish between carboxyl and phenolic hydroxyl functions in a sequence of progressively weaker acidic functional groups associated with humic polymers. This situation will prevail unless a method becomes available to block phenolic hydroxyl groups stably and selectively to allow reaction of the humic material with a series of increasingly stronger bases. Acetylation of humic material does not serve this purpose since the acetyl ethers are subject to alkaline hydrolysis.

Reaction of Aquatic Humics with Permanganate

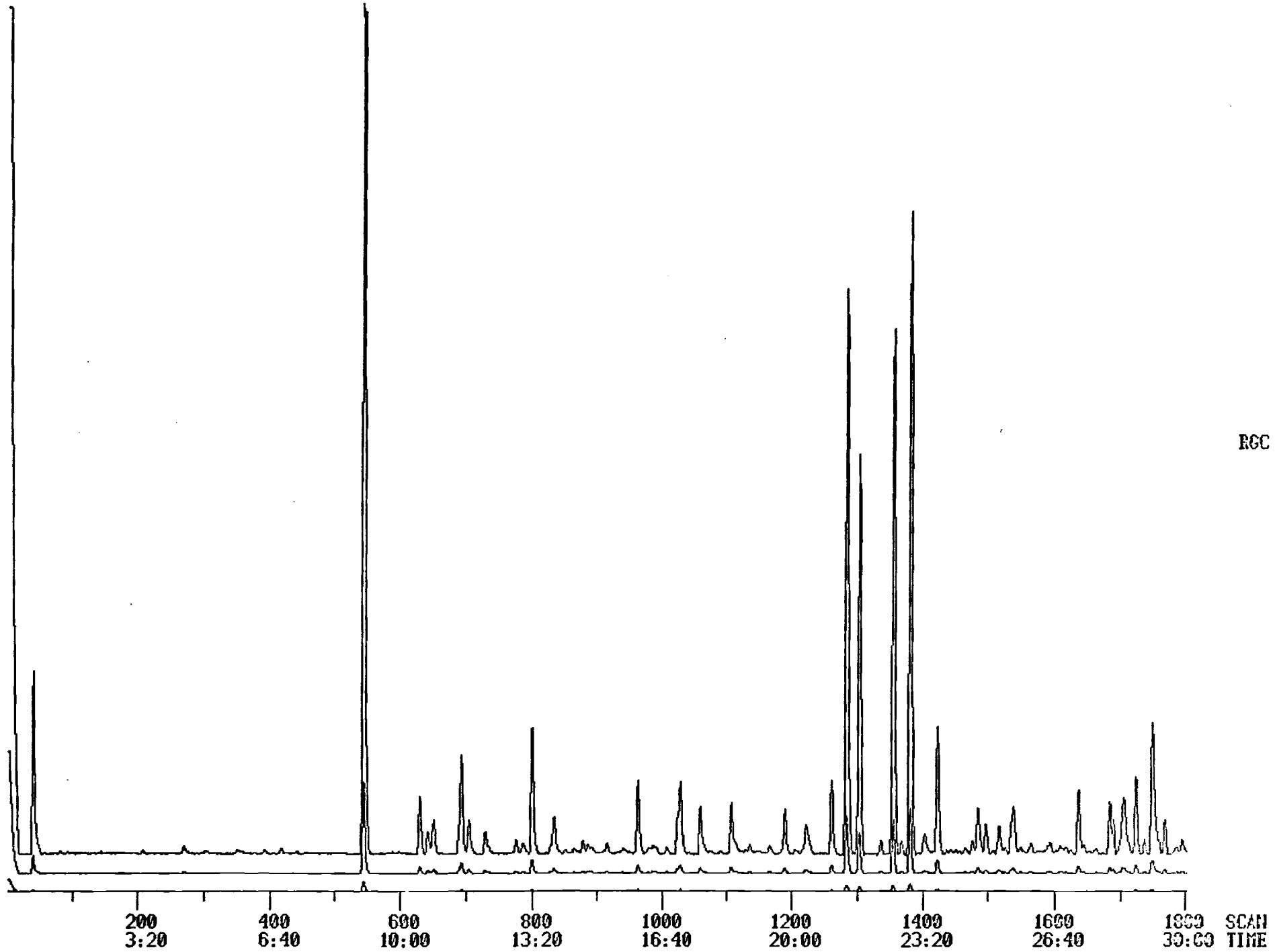
Humic substances have been considered by many workers in the field to consist of predominantly aromatic building blocks. Therefore benzene carboxylic and phenolic acids are expected to dominate the product mixtures upon oxidative degradation, particularly after prior protection of existing acidic functional groups on the aromatic rings in the form of their methyl ethers and esters. We have found a different picture to be the case for the immature aquatic humics isolated from the Satilla River. In the material isolated as previously described, aliphatic materials dominate the degradation product mixtures, particularly when mild conditions of oxidation are employed. Since the percentage yields rise significantly as the conditions of the oxidation reaction are moderated (see Table 2) while the absolute amount of aromatic products remains unchanged, it can be concluded that experiments of earlier workers were not designed to facilitate this observation. Thus it appears from our experimental evidence that aquatic humic matter has more aliphatic character than has generally been assumed.[10,11] On the other hand, one can argue that the natural maturation process whereby aquatic humic matter is transformed from a predominantly soil fulvic acid-type material to a mixture in which soil humic acid-type material becomes a significant fraction, selectively eliminates the less refractory, more aliphatic portions of the initial material. Thus different results could be expected if the aquatic humic matter had been collected from river water under low-flow conditions or from a body of water having a long residence time for the organic matter contained therein. Supportive evidence for this lack of aromatic dominance is offered by the IR (i.e., after methylation only a rather small absorbance remains in the 1600-1640cm^{-1} region) and the NMR (only 15% Ar-H).

The aforementioned product mixtures were analyzed by capillary column gas chromatography following a second treatment with diazomethane. The resolution of the capillary column is such that it is not unusual to resolve more than 100 peaks. A typical early result is shown in Figures 7 and 8. Structures are suggested by a computerized library searching routine an example of which is presented in Figure 9. In this case, the spectrum shown in the upper display corresponds to the major gas chromatographic peak seen in Figure 7. The lower display corresponds to that of the first choice suggested by the data system; i.e. dimethyl succinate. Since the structure is reasonable for the position in the chromatogram and the treatments given to the sample and since the major ions in the chromatogram can be rationalized in a fragmentation sequence (see Figure 10), the structure was accepted.

Other structures were assigned on the basis of a rigorous interpretation of the electron impact (EI) and chemical ionization (CI) data without the assistance of a good match with library spectra. The use of complementary EI/CI data was made possible even in the case of very complex mixtures by carefully matching conditions so that the EI and CI runs could be very nearly overlaid. An example of this matching is provided by Figures 11 and 12. Examples of an assignment made on an interpretive basis are provided in the section dealing with the chlorination of aquatic humics presented later in the text. A complete list of the oxidation products identified during the course of this project is presented in Table 4.

Computerized data processing clearly indicates the dominance of aliphatic acids with succinic and oxalic acids being the most abundant.

Figure 7. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Scans 1-1800 (enhanced).



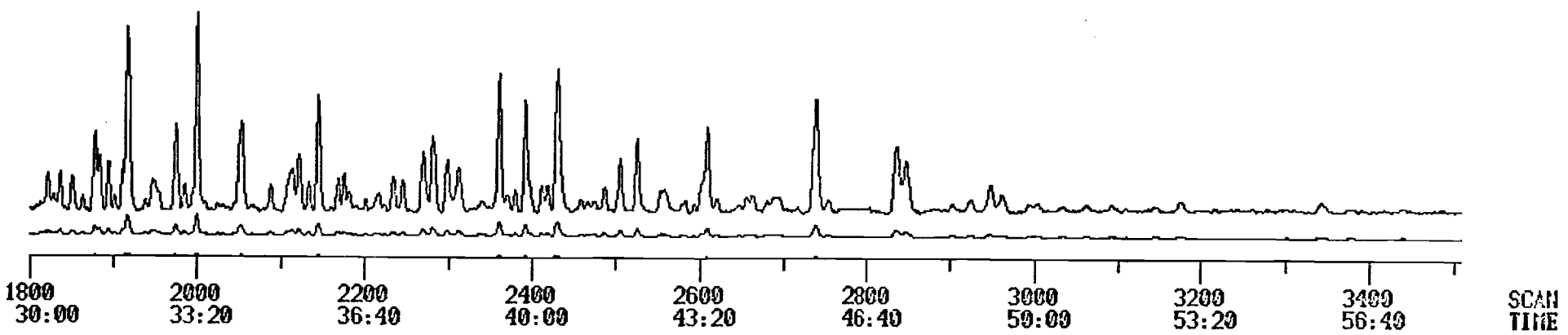
RGC

DATE: 08/23/77 TIME: 1030
SAMPLE: SAMPLE M/14

SAMPLE RUN: M14A$
CALIB. RUN: M14ACAL

SCANS 1800 TO 350.

Figure 8. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid Scans 1800-3507 (enhanced).



LIBRARY SPECTRA
DATE: 08/23/77 TIME: 1030

SAMPLE RUN: M14A
CALIB. RUN: M14ACAL

SCAN 543
RET. TIME: 9:02

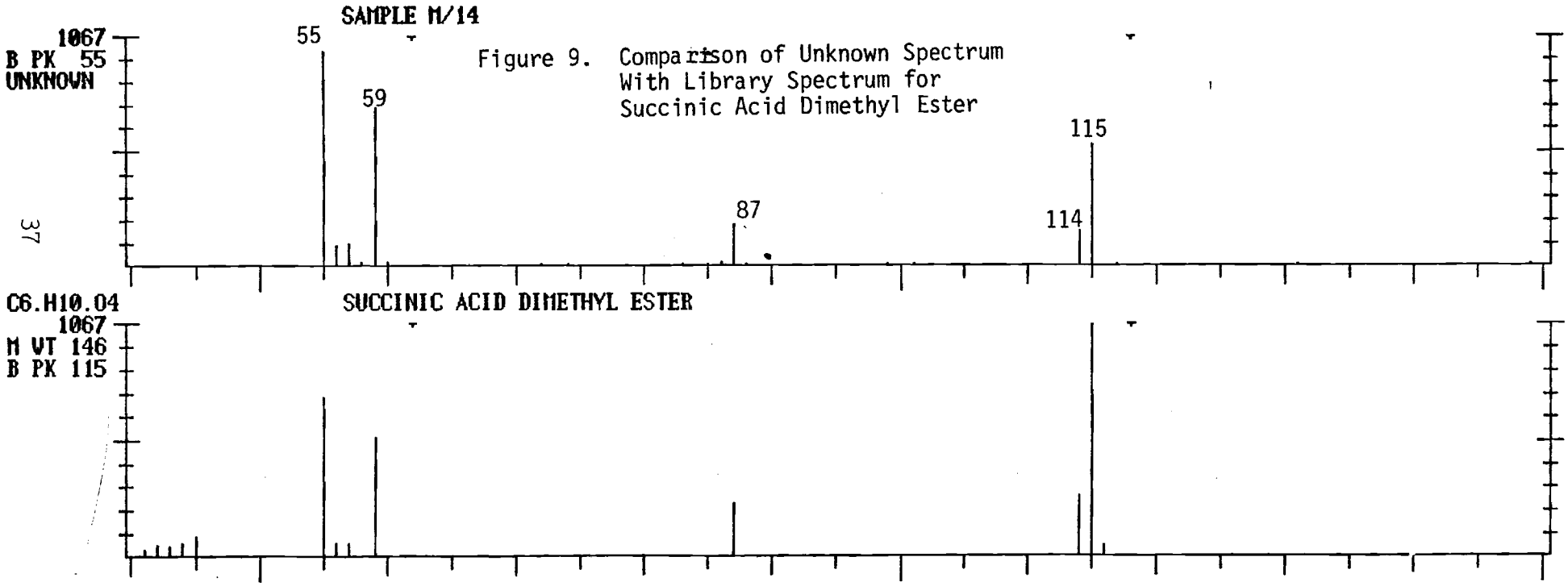
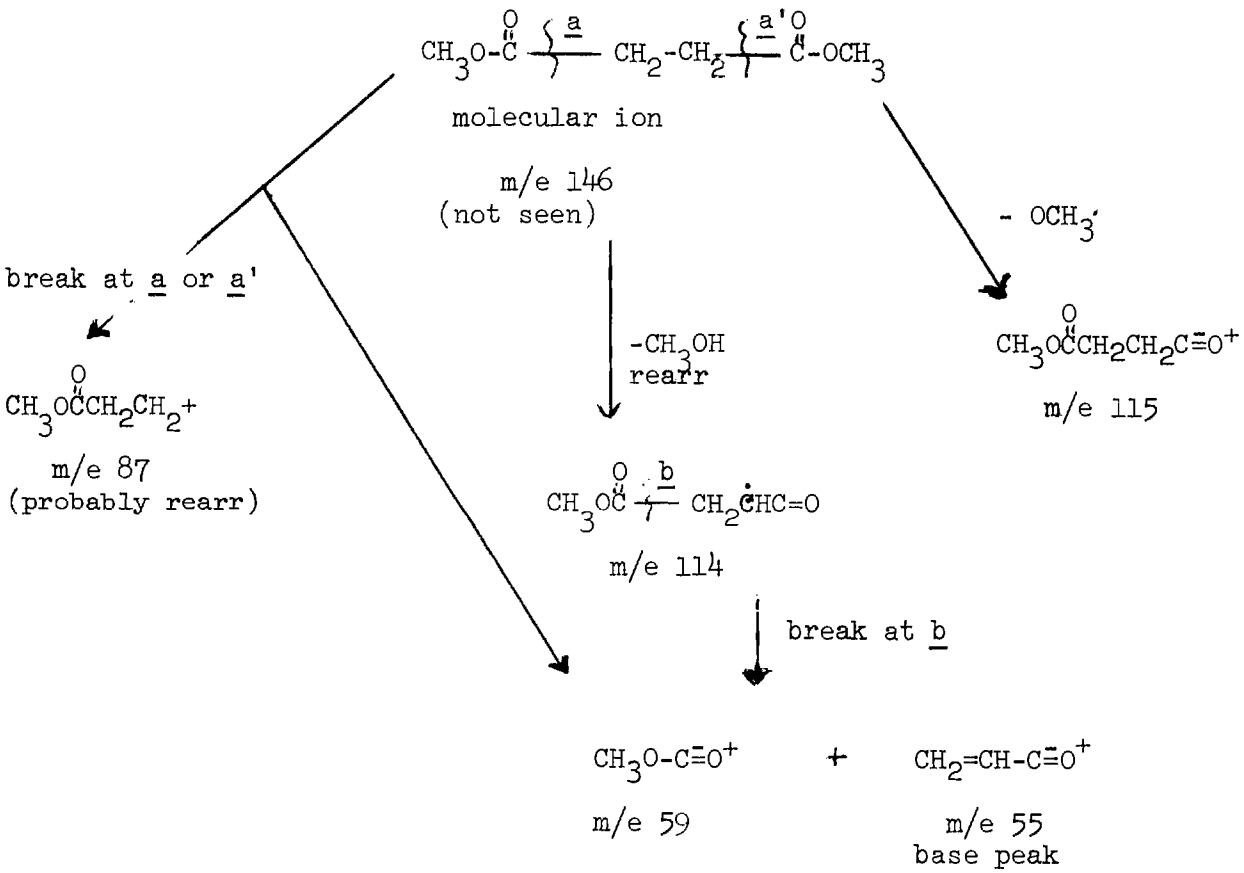


Figure 9. Comparison of Unknown Spectrum With Library Spectrum for Succinic Acid Dimethyl Ester

Figure 10. Fragmentation Sequence for Dimethyl Succinate.



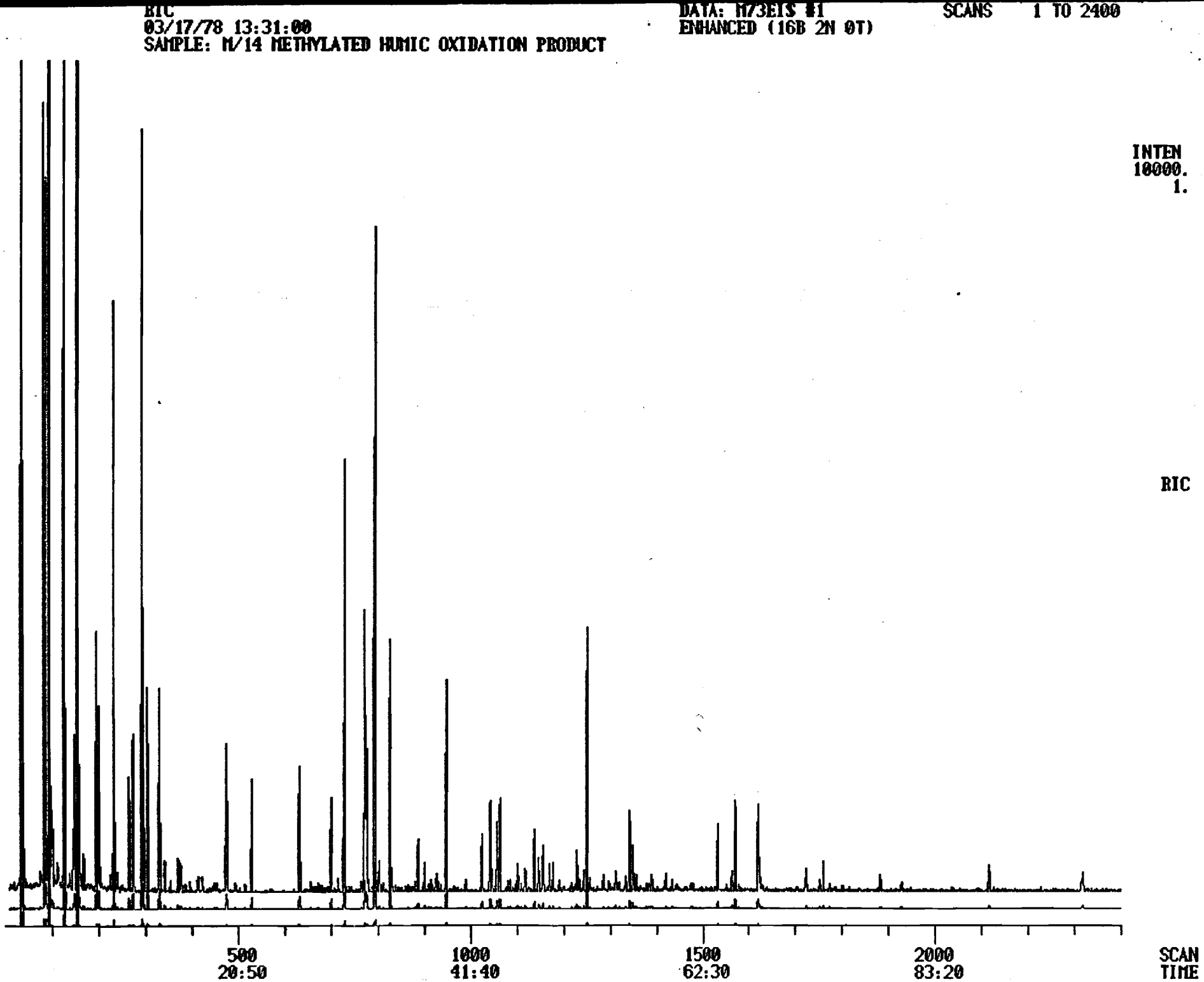


Figure 11 EI - Ion Chromatogram Methylated Humic Oxidation Products

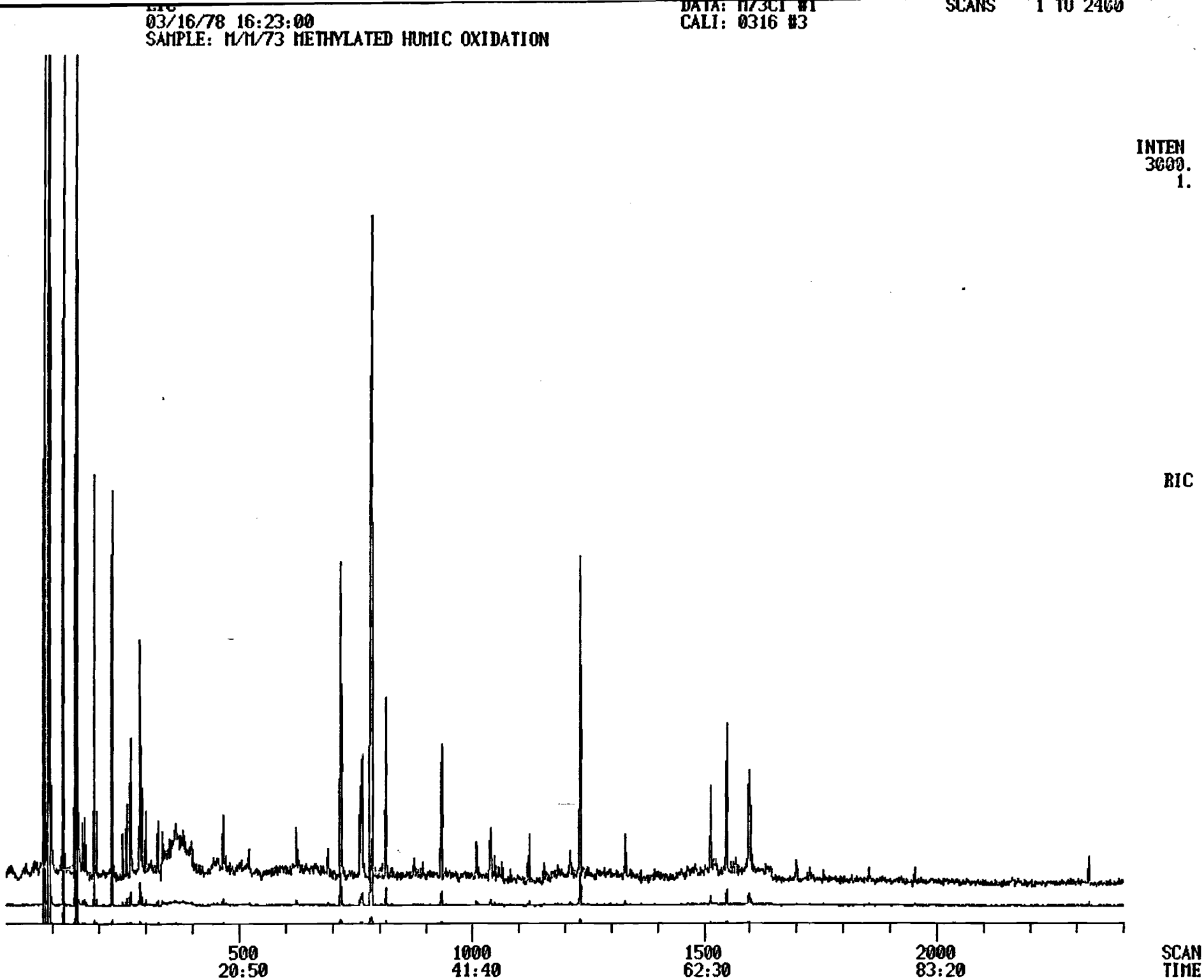


Figure 12 CI Ion Chromatogram Methylated Humic Oxidation Products

TABLE 4

OXIDATION PRODUCTS DERIVED FROM AQUATIC HUMIC MATERIAL

(acids and phenols chromatographed and identified after methylation)

1. Oxalic Acid
2. 3-Oxopropionic Acid
3. Malonic Acid
4. 2-Hydroxy-2-methylpropionic Acid
5. Levulinic Acid
6. Succinic Acid
7. Mandelic Acid
8. Benzoic Acid
9. Methylmalonic Acid
10. Methylsuccinic Acid
11. 2,2-Dimethylsuccinic Acid
12. Glutaric Acid
13. 5-Methylfuroic Acid
14. 4-Methoxybutanoic Acid
15. 2-Hydroxysuccinic Acid
16. 6,7-Dodecadione
17. Adipic Acid
18. Tartaric Acid
19. Oxaloacetic Acid
20. 3-Methoxybenzoic Acid
21. Tricarballylic Acid
22. Tetramethoxybenzene Isomer
23. Phthalic Acid
24. 3-Hydroxypentane-1,3,5-tricarboxylic Acid
25. 2-Hydroxypentane-1,3,5-tricarboxylic Acid
26. Dimethoxybenzoic Acid Isomers
27. Benzene-1,2,4-tricarboxylic Acid
28. Benzene Pentacarboxylic Acid
29. Octanoic Acid
30. 3-Methylglutaric Acid
31. Terephthalic Acid
32. Isophthalic Acid
33. Benzene Tricarboxylic Acid Isomers
34. Benzene Tetracarboxylic Acid Isomer
35. 3-Methoxybenzaldehyde
36. 4-Methoxyacetophenone
37. 3,4-Dimethoxybenzaldehyde
38. p-Coumaric Acid
39. 4-Benzylbenzoic Acid
40. 4-Methyladipic Acid
41. Pimelic Acid
42. 4-Methoxphenylacetic Acid
43. 4-Hydroxy-3-methoxybenzoic Acid
44. Azeliac Acid

TABLE 4, cont'd

45. Hexadecanoic Acid
46. Tetradecanoic Acid
47. Citric Acid
48. Fumaric Acid
49. 4-Methoxybenzoic Acid
50. Dodecanoic Acid
51. 4-Methoxymandelic
52. Methoxyphthalic Acid Isomers
53. Dimethoxyphthalic Acid Isomers
54. Trimethoxyphthalic Acid Isomers
55. Xanthenone
56. 5-Methylfuran-2,4-dicarboxylate

Using the least rigid search criteria and searching against the aqueous permanganate run with the highest yield, one obtains the percentage compositions outlined in Table 5. The list, of course, is incomplete in that many of the components remain unidentified. Only those products stable and volatile enough to travel through the gas chromatograph are counted. Nevertheless, the overall results remain useful. Differences from batch to batch of aquatic humics are evident but since reaction conditions were always being modified, it is not possible to attribute these differences to differences in the composition of the aquatic humic materials themselves.

It should be further emphasized that the results shown in Table 4 have been assembled from data gathered from five different runs. All of the dominant components have been found more than once.

Oxidation of Aquatic Humics with Iodine in Base

The treatment of aquatic humics in base with a large excess of iodine provided iodoform, 13.4 mg, mp 122° (lit. 123°) upon direct extraction with ethyl ether. The amount of material isolated corresponded to a 0.8% yield as chloroform on a weight/weight basis which is in excellent agreement with reported values.[4,12] This result suggests that working with iodine (and possibly bromine) in place of chlorine may provide new knowledge which directly relates to chlorination practices because the products of iodine or bromine incorporation will have higher molecular weights and can therefore be more conveniently isolated. A list of reaction products, some of which

TABLE 5

PERCENT COMPOSITION OF SEMIVOLATILE PRODUCTS OBTAINED FROM THE AQUEOUS PERMANGANATE OXIDATION OF AQUATIC HUMICS *

	Compounds	Area	% Total
1.	Dimethyl Oxalate	55201	12.5
2.	Methyl 3-Oxopropionate	7989	1.8
3.	C	31387	7.1
4.	Dimethyl Malonate	44099	10.0
5.	Methyl-2-hydroxy-2-methyl Propionate (tentative)	5586	1.3
6.	Chloroform	By Inspection	NA
7.	Methyl Levulinate	11924	2.7
8.	F-1-G	By Inspection	NA
9.	Dimethyl Succinate	90479	20.5
10.	H-1-I	Not Found	0.0
11.	I	Not Found	0.0
12.	K-1-L	By Inspection	NA
13.	M	1234	0.3
14.	S-Ethyl Pentanethioate (tentative)	Not Found	0.0
15.	Mandelic Acid (tentative)	309	0.1
16.	Methyl Benzoate	Not Found	0.0
17.	Methyl Dimethylmalonate	Not Found	0.0
18.	Methyl Dimethylsuccinate	7105	1.6
19.	Dimethyl 2,2-Dimethylsuccinate	3394	0.8
20.	Dimethyl Glutarate	6163	1.4
21.	Methyl 5-Methylfuran-2-carboxylate	5510	1.3
22.	J	11155	2.5
23.	Q	24649*	5.6
24.	Methyl-4-methoxybutanoate (tentative)	2021	0.5
25.	R-1-S	By Inspection	NA
26.	S	24649*	5.6
27.	Dimethyl 2-Hydroxysuccinate	Not Found	0.0
28.	U	4830	1.1
29.	V	Not Found	0.0
30.	W	By Inspection	NA
31.	Y	Not Found	0.0
32.	Z	347	0.1
33.	A'	1989	0.5
34.	B'-3-C'	Not Found	0.0
35.	B'-5-C'	Not Found	0.0
36.	C'	3618	0.8
37.	Dimethyl Adipate	1097	0.3
38.	F'	1163	0.3
39.	G'	313	0.1

*Acids and phenols chromatographed and estimated as their methyl esters and ethers.

TABLE 5, cont'd

40.	G'-1-H'	Not Found	0.0
41.	H'	641	0.2
42.	Dimethyl Tartrate	2472	0.6
43.	J'	536	0.1
44.	Methyl Ether of Dimethyloxaloacetate	4644	1.1
45.	X	Not Found	0.0
46.	L'	3113	0.7
47.	M'	1086	0.3
48.	N'	429	0.1
49.	N'-1-O'	100	0.1
50.	N'-2-O'	Not Found	0.0
51.	Methyl 3-Methoxybenzoate	5080	1.2
52.	O'-1-P'	By Inspection	NA
53.	P'	4590	1.0
54.	P'-1-A'	By Inspection	NA
55.	Q'	By Inspection	NA
56.	Trimethyl Tricarballylate	9260	2.1
57.	R'-2-S'	18172*	4.1
58.	S'	18172*	4.1
59.	T'	Not Found	0.0
60.	Dimethyl Phthalate	609	0.1
61.	V'	277	0.1
62.	Trimethyl 3-Hydroxypentane-1,3,5-tricarboxylate	1434	0.3
63.	Y'	109	0.1
64.	A'	Not Found	0.0
65.	A''	Not Found	0.0
66.	Trimethyl 2-Hydroxypentane-1,3,5-tricarboxylate	943	0.2
67.	C''	319	0.1
68.	D''	461	0.1
69.	D''	Not Found	0.0
70.	F''	349	0.1
71.	F''-1-G''	By Inspection	NA
72.	G''	Not Found	0.0
73.	H''	187	0.1
74.	I''	450	0.1
75.	J''	Not Found	0.0
76.	K''	5707	1.3
77.	M''	Not Found	0.0
78.	O''	1447	0.3
79.	N''	Not Found	0.0
80.	Q''	84	0.1
81.	R''	Not Found	0.0
82.	S''	299	0.1

TABLE 5, cont'd

83.	T''	333	0.1
84.	V''	By Inspection	NA
85.	Trimethyl Benzene-1,2,4-Tricarboxylate	1576	0.4
86.	X''	Not Found	0.0
87.	Z''	254	0.1
88.	A''-1-A''	By Inspection	NA
89.	A'''	208	0.1
90.	B'''	Not Found	0.0
91.	C'''	Not Found	0.0
92.	D'''	Not Found	0.0
93.	F'''	Not Found	0.0
94.	G'''	Not Found	0.0
95.	I'''	Not Found	0.0
96.	L'''-1-M'''	1676*	0.4
97.	M'''	1676*	0.4
98.	P'''	1131	0.1
99.	Q'''	Not Found	0.0
100.	S'''	1073	0.3
101.	U'''	Not Found	0.0
102.	V'''	382	0.1
103.	W'''	1163	0.3
104.	Y'''	Not Found	0.0
105.	Z'''	575	0.1
106.	Pentamethyl Benzene Pentacarboxylate	1134	0.3

*Error, duplicate hit.

lend further support to this idea is presented in Table 6.

TABLE 6

REACTION PRODUCTS-OXIDATION OF AQUATIC HUMICS WITH IODINE IN BASE

Compound	Ion	Comment
Methyl Iodide	142, 127	poorly resolved
Ethyl Iodide	156, 127	poorly resolved
3-Methyl-2-pentanone	100, 71, 57, 43	poorly resolved
Benzene	78, 63, 52, 91, 39	possible artifact+
Toluene	92, 91	possible artifact+
Methylene Iodide	268, 254, 141, 127	good fit
2,2,4-Trimethyl-3-hexanone	142, 100, 85, 57, 43, 41	minor
Iodoform		confirmed by mp

Reaction of Aquatic Humics with Bromine in Acetic Acid

The product mixture resulting from the treatment of non-methylated aquatic humic material with bromine in acetic acid was analyzed by GC/MS. Surprisingly, the number of products resulting from this relatively harsh treatment was not large. The complementary EI and CI ion chromatograms are presented in Figures 13 and 14 respectively. The structures which were assigned largely on the basis of interpretation are presented in Table 7.

Chlorination Studies - General

In keeping with the intent of the sponsoring agency as well as the stated goals of the original proposal, the research carried out in connection

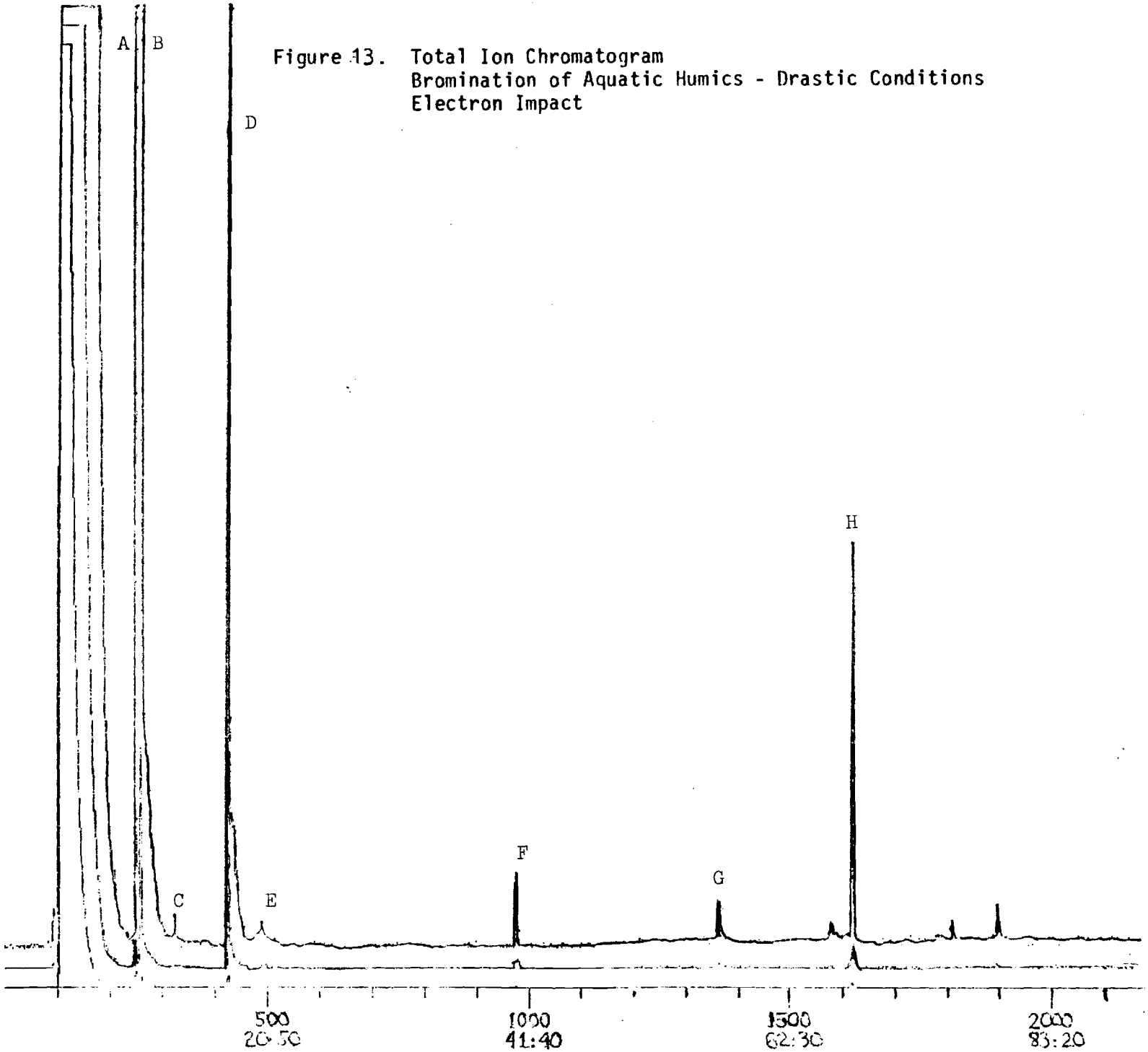


Figure 13. Total Ion Chromatogram
Bromination of Aquatic Humics - Drastic Conditions
Electron Impact

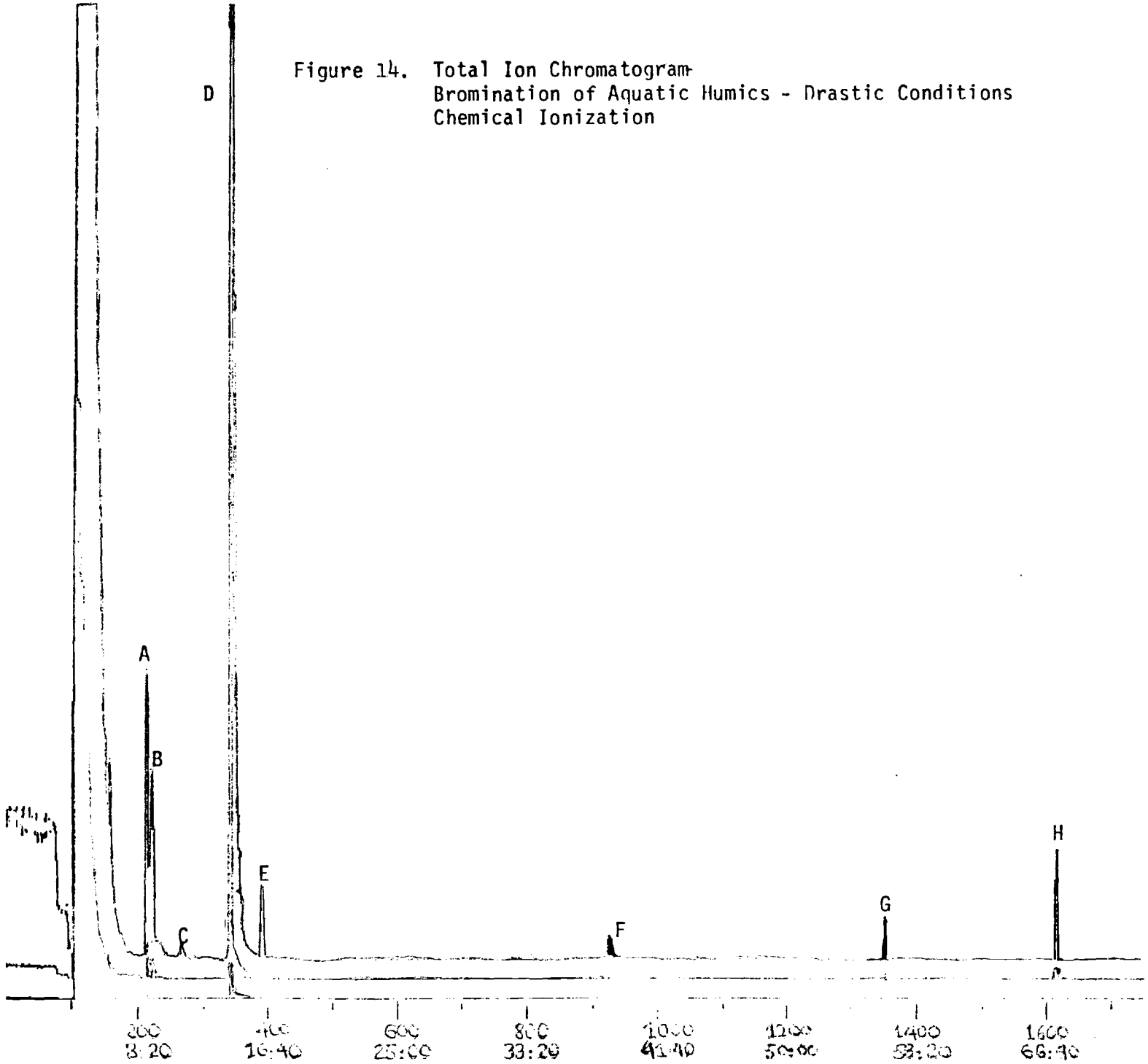


Figure 14. Total Ion Chromatogram
Bromination of Aquatic Humics - Drastic Conditions
Chemical Ionization

TABLE 7

DRASTIC BROMINATION OF AQUATIC HUMICS PRODUCT LIST

Product	Significant Ions	Comments
A. Contains Br	EI 107, 109; 71; 55; 43 CI 150, 152; 136, 138; 72	possible C-5 bromo-ketone medium component
B. Isomer of A	EI 71, 55, 43, 41 CI 150, 152; 72	possible C-5 bromo-ketone medium component
D. Bromoform	EI M^+ cluster, M-Br cluster, $M-Br_2$ cluster, $M-HBr_2$ cluster, M-HBr cluster, HBr, Br, Br_2 CI Again a misfit, with molecular ion instead of quasi molecular	matches authentic sample, major product
G. Tetrabromoacetone	EI m/e 199, 201, 203; 171, 173, 175; 120, 122; 92, 94; 121, 123; 105, 107 CI M+1 cluster; $M+1-Br_2$ cluster, M+1-HBr cluster	minor component

with this project has always aimed at the realistic. This has been especially true of our chlorination studies. Aquatic humics were reacted with chlorine at levels corresponding to only 5 ppm TOC (10 mg/l aquatic humics). Temperature and pH were maintained within a narrow range. Chlorine was dosed according to demand as required to maintain a residual of not more than 2 mg/l. Contact times, the use of inorganic flocculants etc. were all carried out at realistic levels. A special apparatus was constructed so that these studies might be carried out in a dynamic system simulating a typical water disinfection facility rather than in a static system as is usually employed for laboratory studies.

Although the investigators were unaware of the significance which would be later attached to it, the sample of aquatic humic material employed for the majority of the work described in this report was not entirely typical. A drought of considerable duration preceded the collection of the sample which was therefore collected immediately following the first significant rainfall which terminated the drought. Thus the aquatic humic material which was subsequently isolated was unusually rich in adsorbed organics. Furthermore, it will be recalled that the researchers sample from a small tributary rather than the main stream thus assuring a more immature isolate. The subsequent examination of the data obtained from those few runs conducted with other samples of more mature humic material showed considerable differences in the product mixtures. Accordingly the writers have decided to first present the data associated with the chlorination work in two additional subsections the first of which is concerned with

the immature humics. This discussion will be followed by a shorter presentation of information derived from reactions carried out with the more mature aquatic humics. In general, chlorination of the immature material resulted in a greater array of chlorine-containing products, presumably because this material was unusually enriched in adsorbed organics, the reactive nature of which was not as evident in mature aquatic humic matter.

Structural assignments were made chiefly on the basis of the mass spectral fragmentation patterns. Of course, supporting data such as solubility and gas chromatographic retention time were taken into account. Since the burden of structural proof rests so heavily on mass spectrometry and since authentic compounds are not generally available, the interpretive efforts practiced in connection with this project were considerably more rigorous than might otherwise be the case.

Chlorination of Immature Humics

To date, the most interesting products to be characterized among the reaction products resulting from the chlorination of immature aquatic humics are a series of chlorinated alcohols structurally related to isoprene. Their positions in a typical GC/MS total ion chromatogram are shown in Figure 15. The strucutural elucidation of such materials has been greatly enhanced by our total reliance on capillary-column gas chromatography to achieve the maximum resolution of components before they enter the source of the mass spectrometer. The complementary information provided by the matching electron-impact and chemical ionization data (see Figure 16) further increased our ability to make correct structural assignments. For

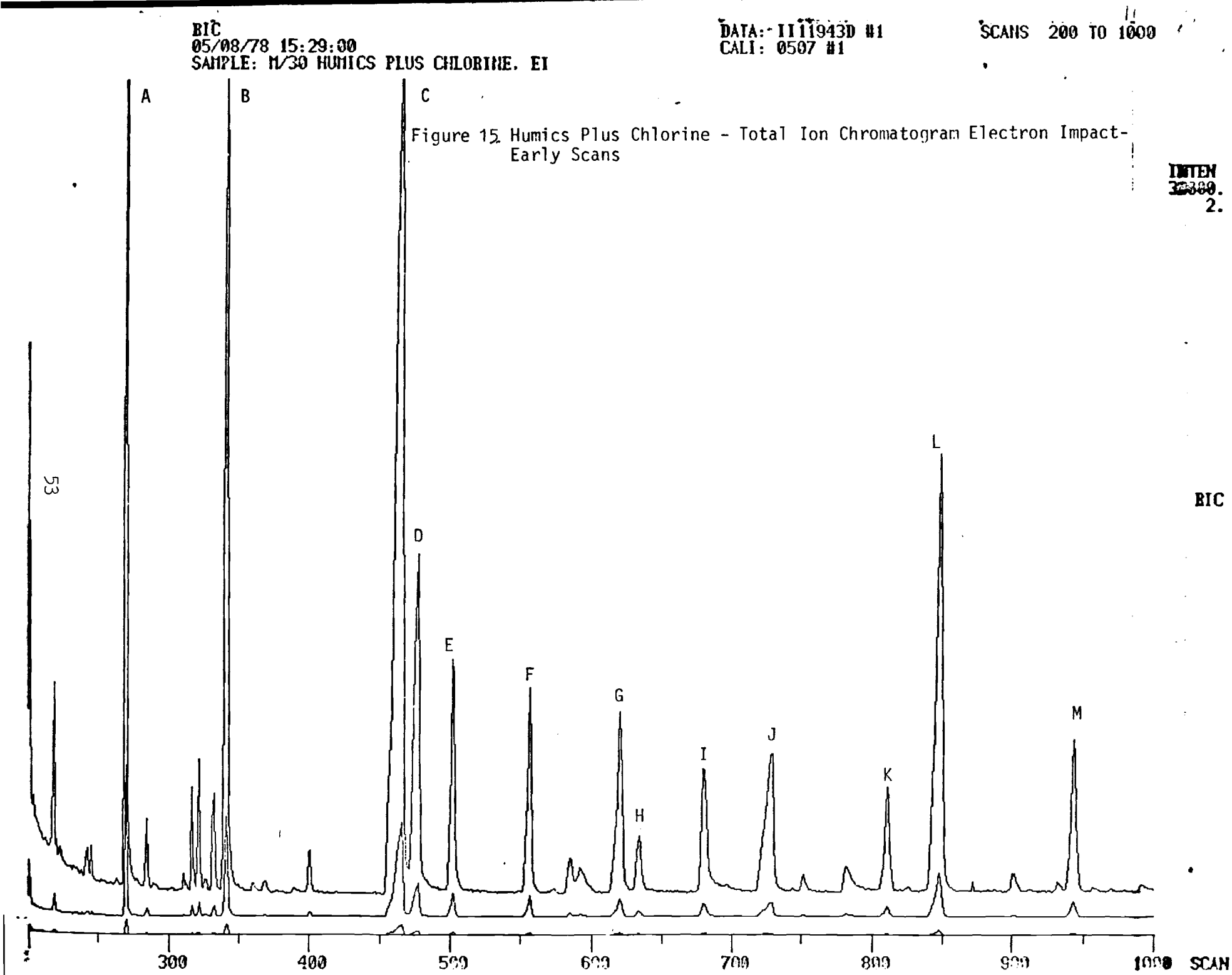


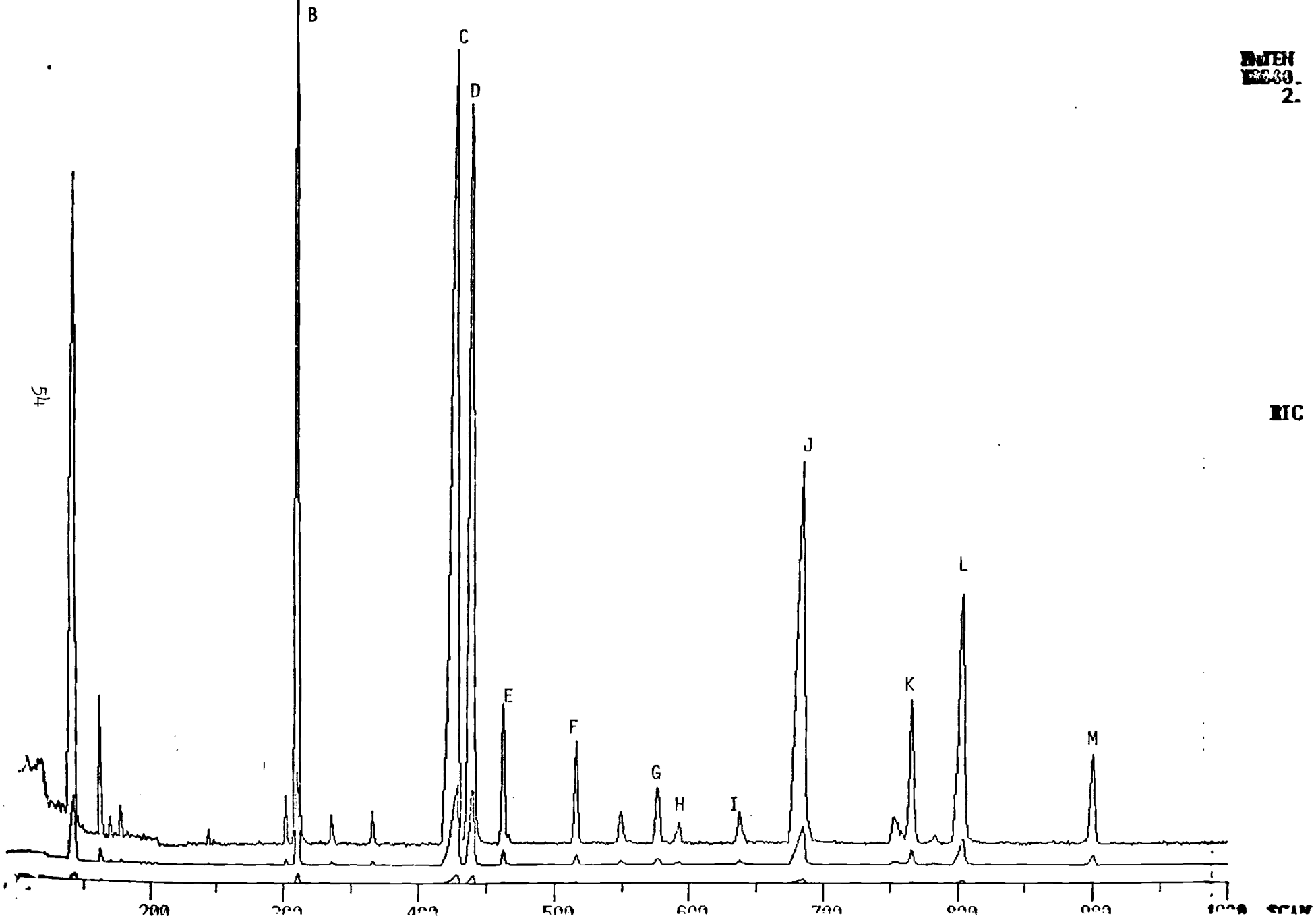
Figure 15 Humics Plus Chlorine - Total Ion Chromatogram Electron Impact-Early Scans

RIC
05/09/78 9:56:00
SAMPLE: HUMICS PLUS CHLORINE. CH4 CI

DATA: 111943DCI #1
CALI: 0509 #3

SCANS 100 TO 1000

Figure 16 Humics Plus Chlorine - Total Ion Chromatogram Chemical Ionization-Early Scans



RIC

example, the monochloro alcohols (3-chloro-2-methyl-2-butanol, 1-chloro-2-methyl-2-butanol, 3-chloro-2-methyl-1-butanol and 4-chloro-3-methyl-1-butanol) all showed nearly identical chemical ionization (CI) spectra with major ions at m/e 87, m/e 105 and m/e 107 (see Figure 17 for a typical case) thus indicating probable structural isomerism. The electron impact (EI) spectra were quite different, however. This fact made it possible to assign structures to each of the isomers on the basis of the differences in their EI base peaks and fragmentation patterns. A typical example is shown in Figure 18 which is compatible with the fragmentation sequence outlined in Figure 19. A more complete listing of the products identified thus far is presented in Table 8. Such advanced techniques as selected ion mapping, reversed-search quantitation and a number of other data processing methods were employed in order to obtain the maximum amount of information from each of the experiments.

A quality assurance program of considerable rigor was maintained throughout the course of this program. Sensitivity and spectral quality were monitored daily with pentafluorbromobenzene. The use of this compound is recommended by EPA as a measure of instrument performance. In the case of the work performed in connection with these studies, tuning was optimized for spectral quality at the sacrifice of some sensitivity. The Project Manager believes this approach to be best strategy for general screening purposes so that library matching and user interpretation will not be confused by random noise. A representative quality control spectrum together with the NBS/NIH library reference spectrum is presented in Figure 20. This result

05/09/78 9:56:00 + 12:55
SAMPLE: HUMICS PLUS CHLORINE. CH4 CI
#517 TO #518 SUMMED - #516 X0.65

ENHANCED (S 31B 2N 0T) RIC: 599.

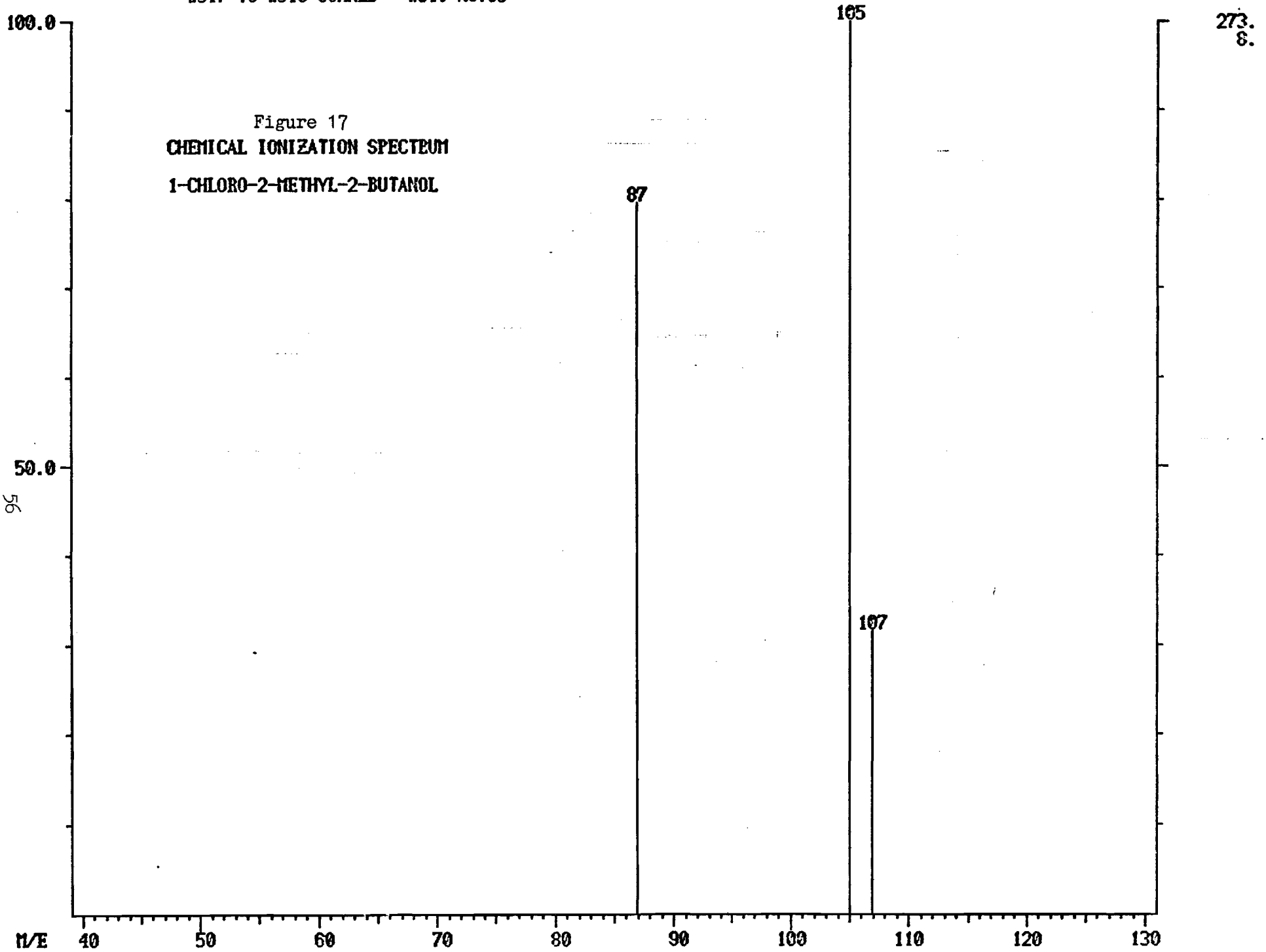


Figure 17
CHEMICAL IONIZATION SPECTRUM

1-CHLORO-2-METHYL-2-BUTANOL

SAMPLE: M/30 HUMICS PLUS CHLORINE. EI
#555 TO #558 SUMMED - #554 X1.50

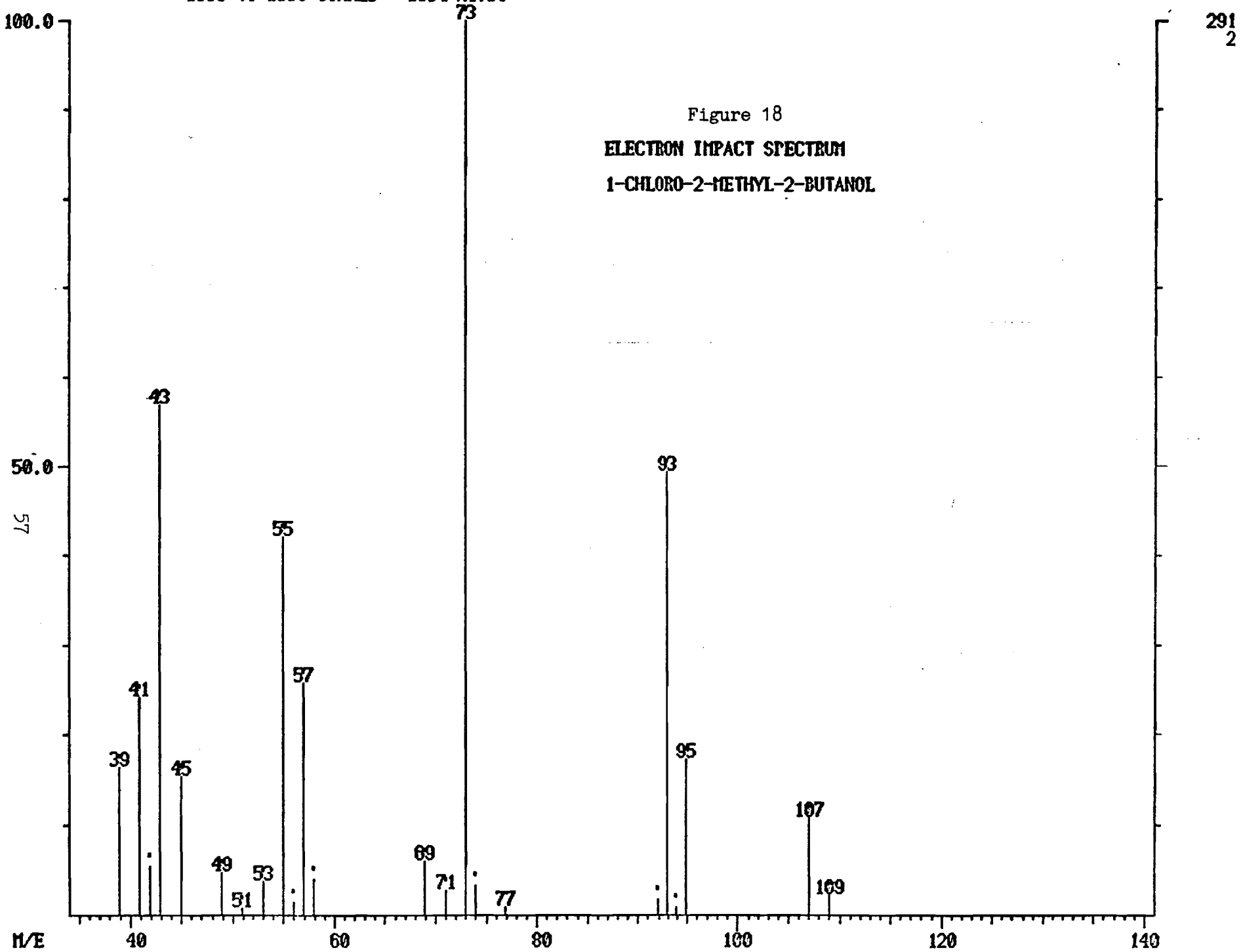


Figure 18

ELECTRON IMPACT SPECTRUM

1-CHLORO-2-METHYL-2-BUTANOL

Figure 19. EI Fragmentation Sequence for 1-Chloro-2-methyl-2-butanol.

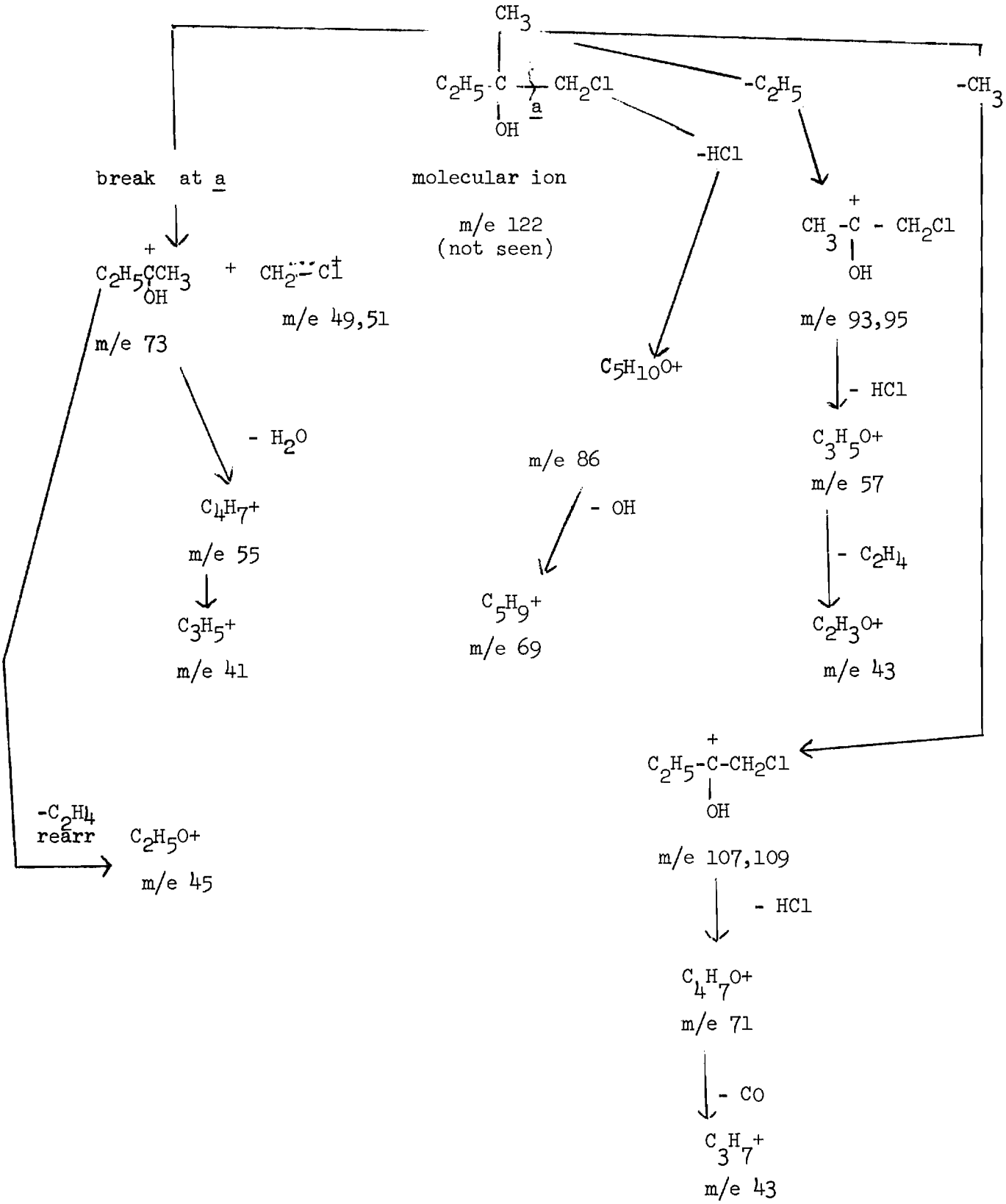


TABLE 8

CHLORINATION PRODUCTS DERIVED FROM AQUATIC HUMIC MATERIAL

1. 3-Chloro-2-methyl-1-butene*
2. 2-Chloromethyl-1-butene
3. 2-Methyl-3-pentanone+
4. 2-Methylpentanal+
5. 3-Chloro-2-methyl-2-butanol
6. 4-Hydroxy-3-methylbutanal+
7. 2,3-Dichloro-2-methylbutane
8. 1-Chloro-2-methyl-2-butanol
9. 1,3-Dichloro-2-methlbutane
10. 3-Chloro-2-methyl-1-butanol
11. 4-Chloro-3-methyl-1-butanol
12. 3,4-Dichloro-methyl-1-butene Isomer
13. 4-Methyl-3-heptanone
14. 2-(3-Oxobutyl)-cyclopropanol
15. 1,4-Dichloro-2-methyl-2-butanol
16. Trichloroacetic Acid
17. 1,3-Dichloro-2-methyl-2-butanol
18. 3,4-Dichloro-3-methyl-2-butanol
19. 2-Butanol+
20. Chloroform
21. 2-Chloro-3-methyl-2-butene
22. Dichloroacetic Acid
23. Toluene**
24. Benzene**

*Dominant product. It is of interest to point out that this is also the dominant product reported by Dr. W.H. Glaze in his paper on the superchlorination of secondary effluent - a material which is known to be rich in humic substances. (Reference 16)

+Found in controls, presumably adsorbed to the aquatic humics

**Unclear whether is an artifact or an adsorbed material originating in the atmosphere. Other workers have reported finding these two components (Note 19)

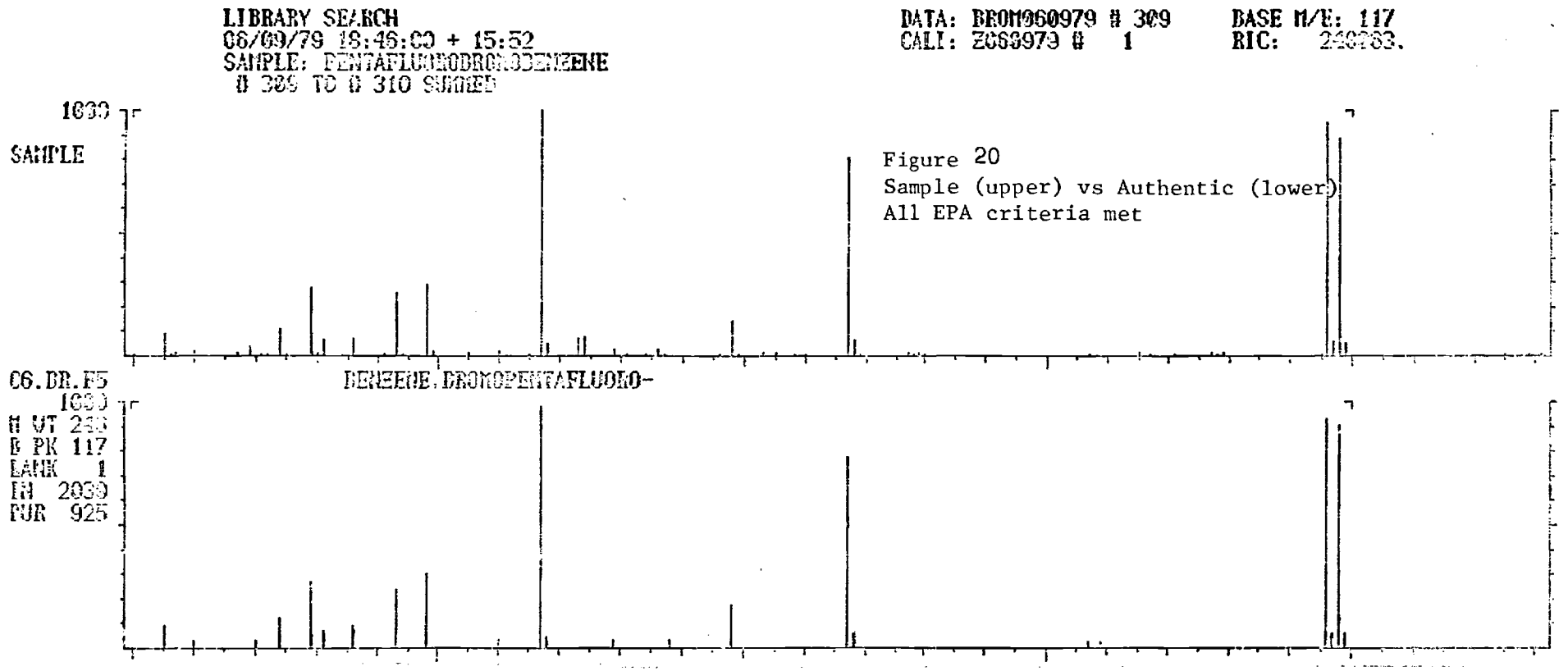


Figure 20
Sample (upper) vs Authentic (lower)
All EPA criteria met

is compared with the EPA performance criteria in Table 9.

Each chlorination run featured a companion blank run contacting all of the reagents with the exception of the chlorine, flocculant and filter packing. In addition to providing assurance that incidental contamination had not taken place, these control runs provided a body of information of their own regarding the nature of some of the more readily desorbed organics associated with the aquatic humics. Glassware and the mini-pilot facility were carefully cleaned between runs. Instrumental performance was assured by a program of routine maintenance. In this way, shutdown due to unscheduled maintenance has been held to only 5 days over the course of the entire project.

The studies with immature humics have resulted in the characterization of three general classes of chloroorganics all of which bear a structural relationship to isoprene. These are listed below:

1) Monochloroalcohols
2) Dichloroalcohols
3) Chlorinated hydrocarbons

The process by which the monochloroalcohols were structurally identified has already been illustrated by example in Figures 17, 18 and 19 together with the accompanying text. A similar strategy was employed to identify a series of dichloroalcohols. For example, all members of this series are characterized by CI spectrum such as that of 1,3-dichloro-2-methyl-2-butanol which is presented in Figure 21. Once again, the quasi-molecular ion (expected at m/e 157, 159, 161) is not recorded. Instead, a loss of water resulting from an attack on the protonated OH function is seen as an ion

TABLE 9

GC/MS PERFORMANCE CRITERIA PENTAFLUOROBROMOBENZENE

	Criterion Value	Georgia Tech Value
1.	Is m/e 78 less than 1% relative intensity (RI)?	Not present
2.	Is m/e 79 between 15% and 35% of RI?	28%
3.	Is m/e 80 etween 4% and 8% of m/e 79?	4%
4.	Is m/e 116 less than 1% RI?	Not present
5.	Is m/e 117 the base peak?	yes
6.	Is m/e 118 between 4% and 8% RI?	5%
7.	Is m/e 166 less than 1% RI?	Not present
8.	Is m/e 167 between 65% and 85% RI?	80%
9.	Is m/e 168 between 5% and 9% of m/e 167?	8%
10.	Is m/e 245 less than 1% RI?	0%
11.	Is m/e 246 between 75% and 98% RI?	95%
12.	Is m/e 247 between 5% and 9% of m/e 246?	6%
13.	Is m/e 248 between 75% and 98% RI?	89%
14.	Is m/e 249 between 5% and 10% of m/e 248?	6%
15.	Is m/e 248 between 93% and 99% of m/e 246?	94%

05/09/78 9:56:00 + 28:25
SAMPLE: HUMICS PLUS CHLORINE, CH4 CI
#1136 TO #1138 SUMMED - #1138 X0.50

DATA: TTT1943DCIS #1137
ENHANCED (S 31B 2N 0T)

BASE M/E: 121
RIC: 1230.

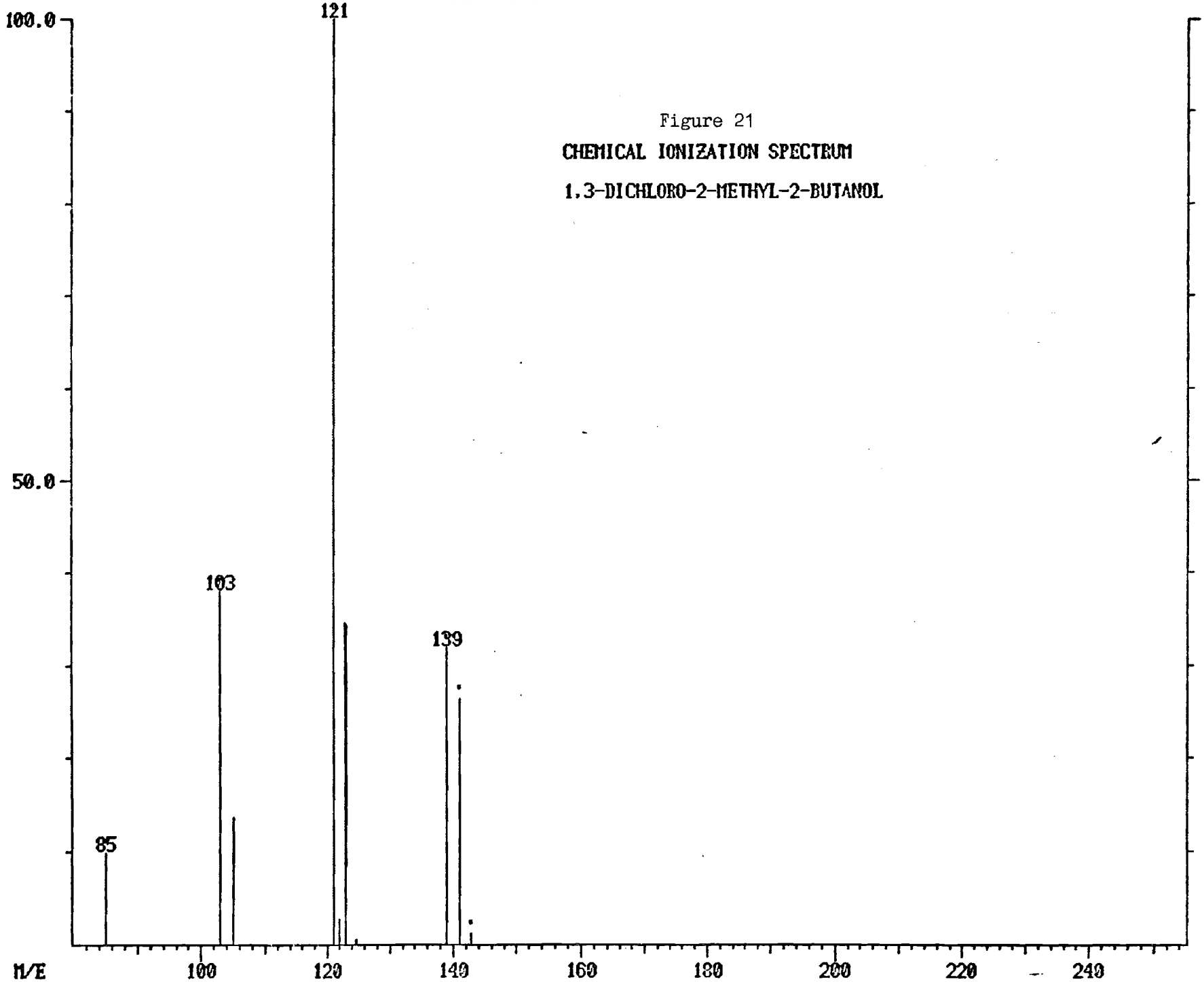


Figure 21

CHEMICAL IONIZATION SPECTRUM

1,3-DICHLORO-2-METHYL-2-BUTANOL

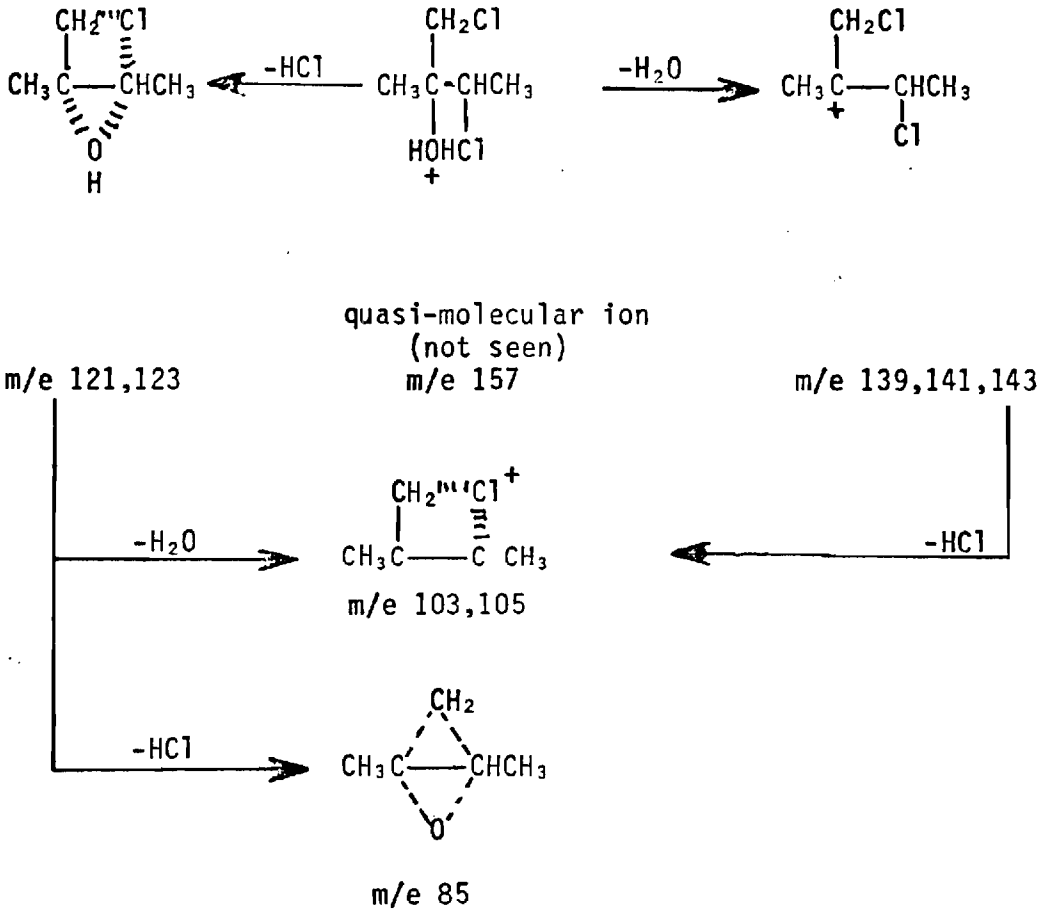
cluster at m/e 139, 141, 143. An analagous attack on the Cl function resulting in the loss of HCl accounts for the base peak at m/e 121, 123. These ions in turn can lose HCl and/or water to provide ions at m/e 103, 105 and m/e 85. This information is summarized in the fragmentation sequence outlined in Figure 22.

As was the case with the monochloroalcohols, the EI spectra recorded for the dichloroalcohols were generally quite different from one another. The EI spectrum of 1,3-dichloro-2-methyl-2-butanol is presented in Figure 23 as an example. The molecular ion is not seen. Loss of a methyl radical generates the dichloro ion cluster at m/e 141, 143, 145. Loss of the chloromethyl group accounts for the ions at m/e 107, 109. A weak companion ion is seen at m/e 49, 51. Breakage between the functional groups generates the ion pair at m/e 93, 95. The complete fragmentation sequence is presented in Figure 24.

The final general group of chlorinated organics to be characterized among the products resulting from the simulated disinfection of immature aquatic humics are the chlorinated hydrocarbons. Once again, all of these products were found to be structurally related to isoprene. Furthermore, the dominant monochloroalcohol, 3-chloro-2-methyl-2-butanol is a close relative of the dominant isoprene hydrocarbon, 3-chloro-2-methyl-1-butene. At least one of the dichloroalcohols-i.e. 1,3-dichloro-2-methyl-2-butanol is also closely related. The co-principal investigators believe that this structural relationship is not coincidental and that the dominant products are either produced from each other via hydration/chlorination of a common definite precursor, or by dehydration of the monochloroalcohol to the chloro-

FIGURE 22

CHEMICAL IONIZATION SEQUENCE: 1,3-Dichloro-2-methyl-2-butanol(methane)



MASS SPECTRUM
05/08/78 15:29:00 + 29:30
SAMPLE: M/30 HUMICS PLUS CHLORINE, EI
#1180 - #1183 X1.00

DATA: Y1119430 #1180
CALI: 0507 #1

BASE M/E: 43
RIC: 5096.

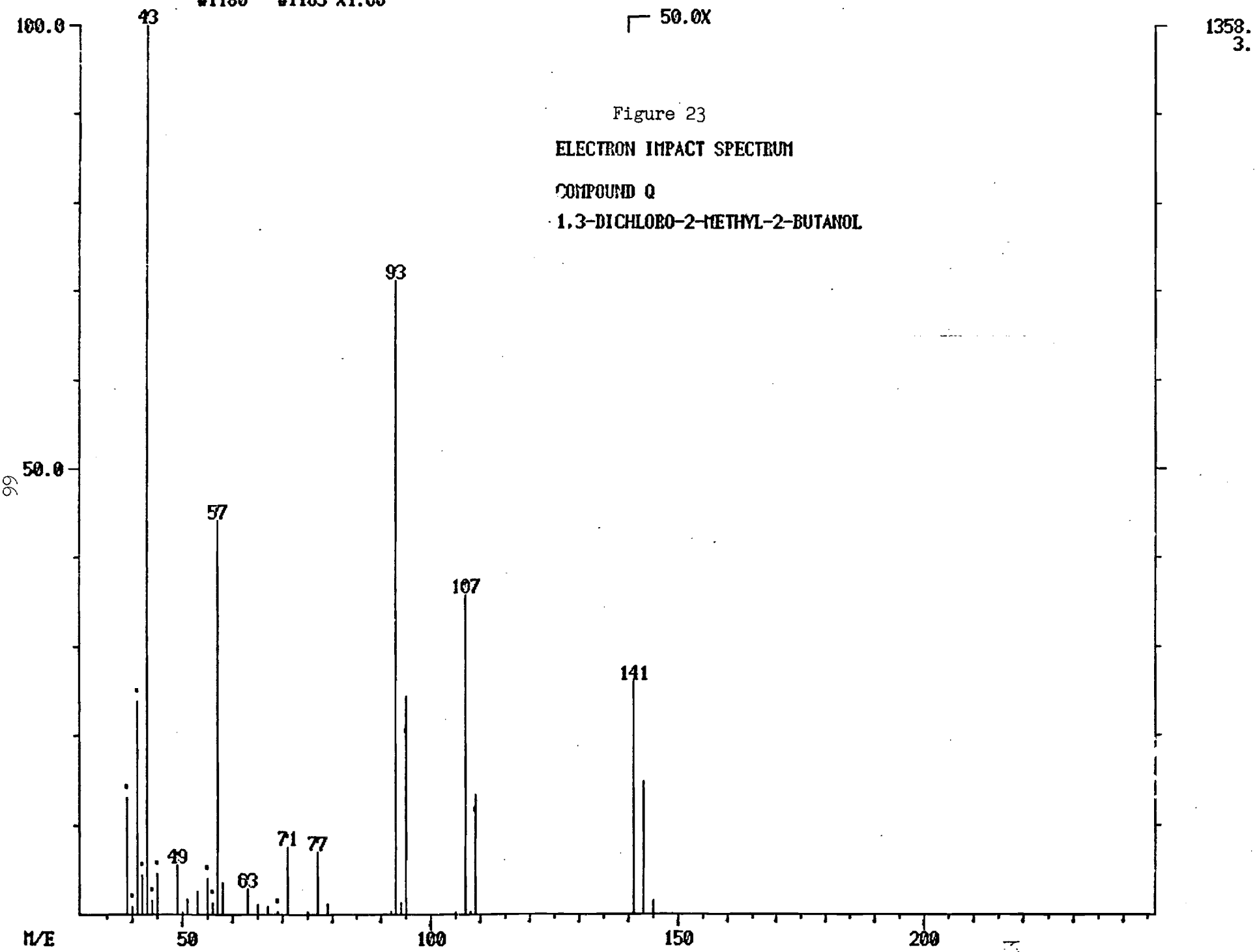


Figure 23

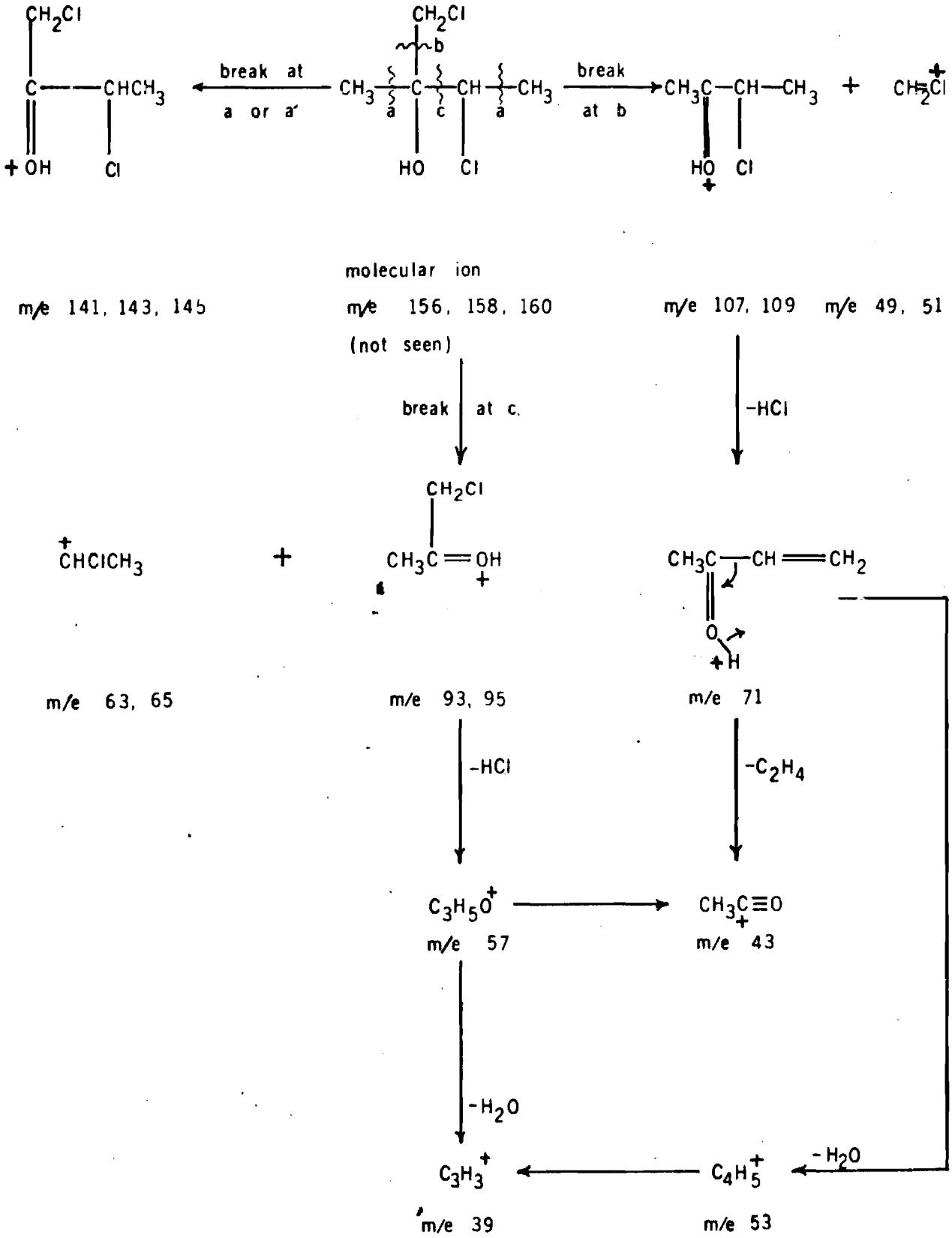
ELECTRON IMPACT SPECTRUM

COMPOUND Q

1,3-DICHLORO-2-METHYL-2-BUTANOL

FIGURE 24

ELECTRON IMPACT FRAGMENTATION: 1,3-Dichloro-2-methyl-2-butanol



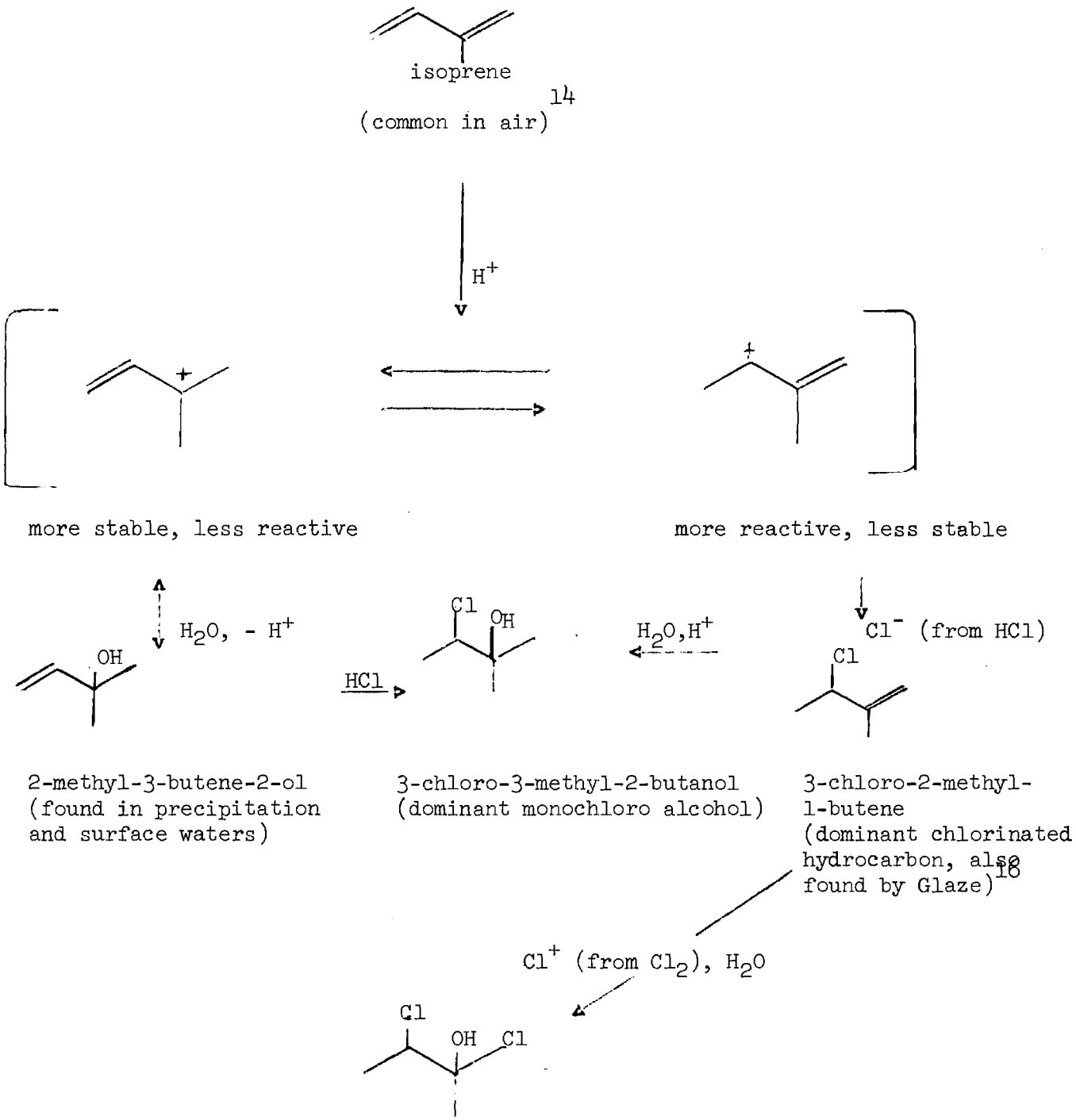
olefin or by means of some other common precursor. A possible sequence of events is outlined in Figure 25. A more detailed discussion of the possible overall sources for the chlorinated isoprenoids follows the discussion of the products resulting from the chlorination of mature aquatic humics.

Structural assignments were made according to the general considerations discussed earlier. In this case, however, a number of the compounds in question were represented in the data system library so that valid suggestions were made more often than not. The illustrative example selected to represent this class of compounds is 3-chloro-2-methyl-1-butene. While a weak quasi-molecular ion is seen in the CI spectrum (Figure 26) at m/e 105 and m/e 107, the base ion at m/3 69 is generated by loss of HCl from the quasi-molecular ion. The electron impact spectrum (see Figure 27) is more informative, particularly since a very good match was obtained by means of a computerized comparison with the NBS/EPA library. All fit indices exceeded 900 out of a possible 1000. In this case, the molecular ion is seen at m/e 104, 106. Loss of a methyl group provides another chlorine-containing ion at m/e 89 and m/e 91. Loss of the allylic chlorine accounts for the strong ion at m/e 69 while breakage between the functional groups generates m/e 41 and a weaker ion pair at m/e 63, 65. The full EI and CI fragmentation sequences are presented in Figure 28.

Chlorination of Mature Humics

While the chlorination of immature aquatic substances produces a product mixture comprised of a vast array of components about half of which may contain chlorine, the chlorination of mature aquatic humics is characterized by a reduced uptake of chlorine and a less complex product mixture. In the latter case, only about 25% of the product mixture was found to contain

Figure 25. Possible Interrelation of Major Chlorinated Isoprenoid Products.



MASS SPECTRUM
05/04/78 14:57:00 + 6:19
SAMPLE: HUMICS PLUS CHLORINE. CH4 CI

DATA: II11943CCI #253
CALI: 0584 #1

BASE M/E: 69
RIC: 4432.

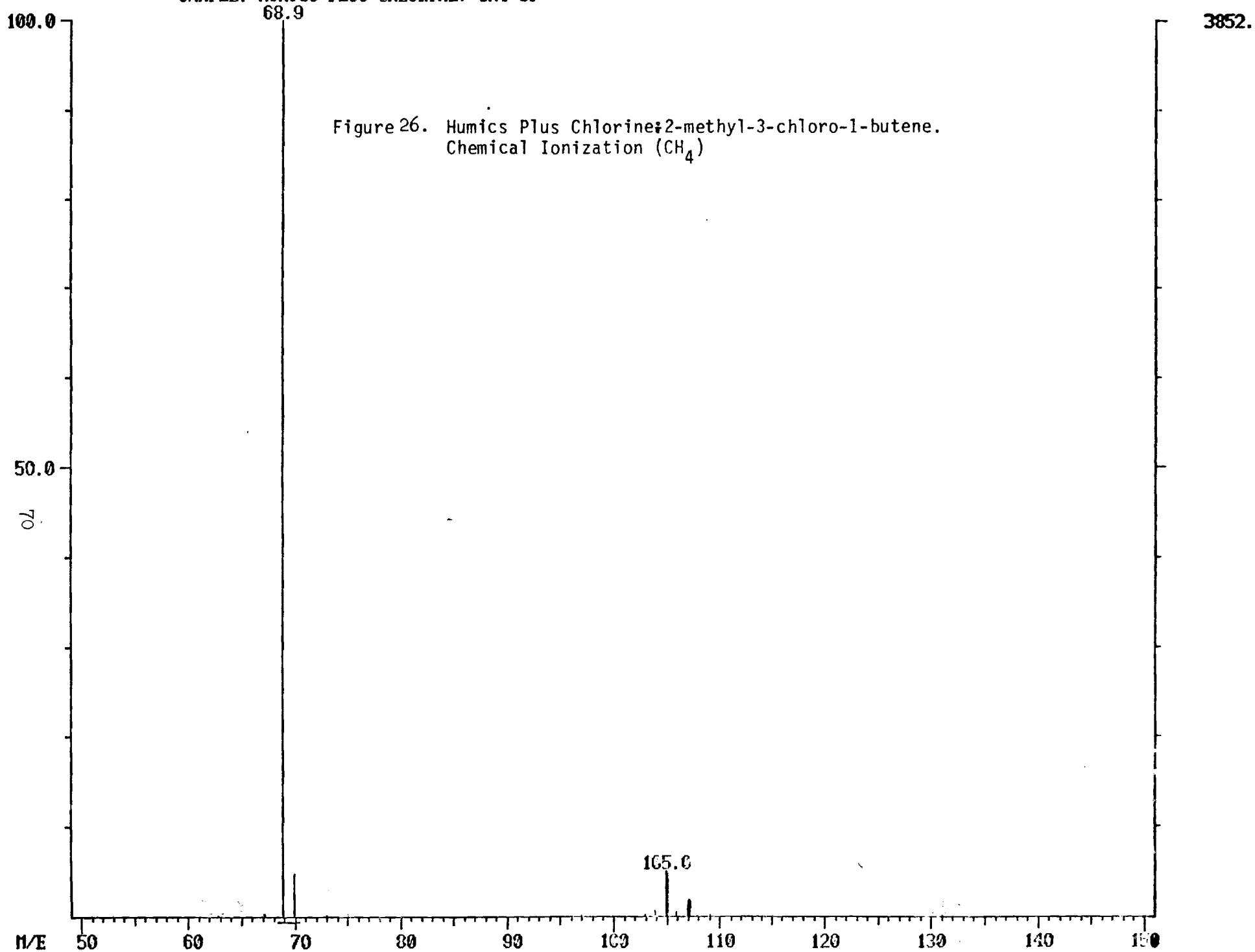


Figure 26. Humics Plus Chlorine: 2-methyl-3-chloro-1-butene. Chemical Ionization (CH_4)

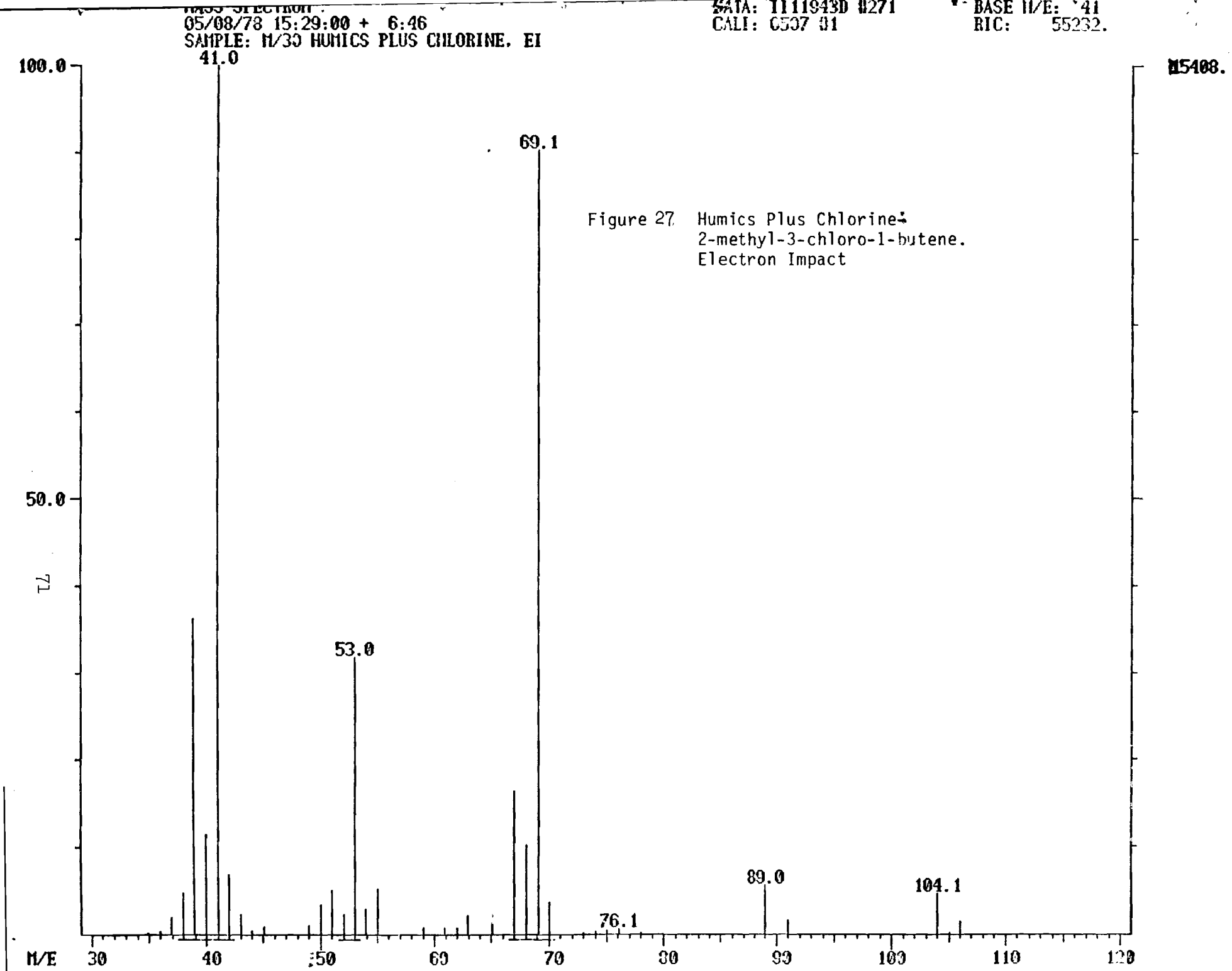
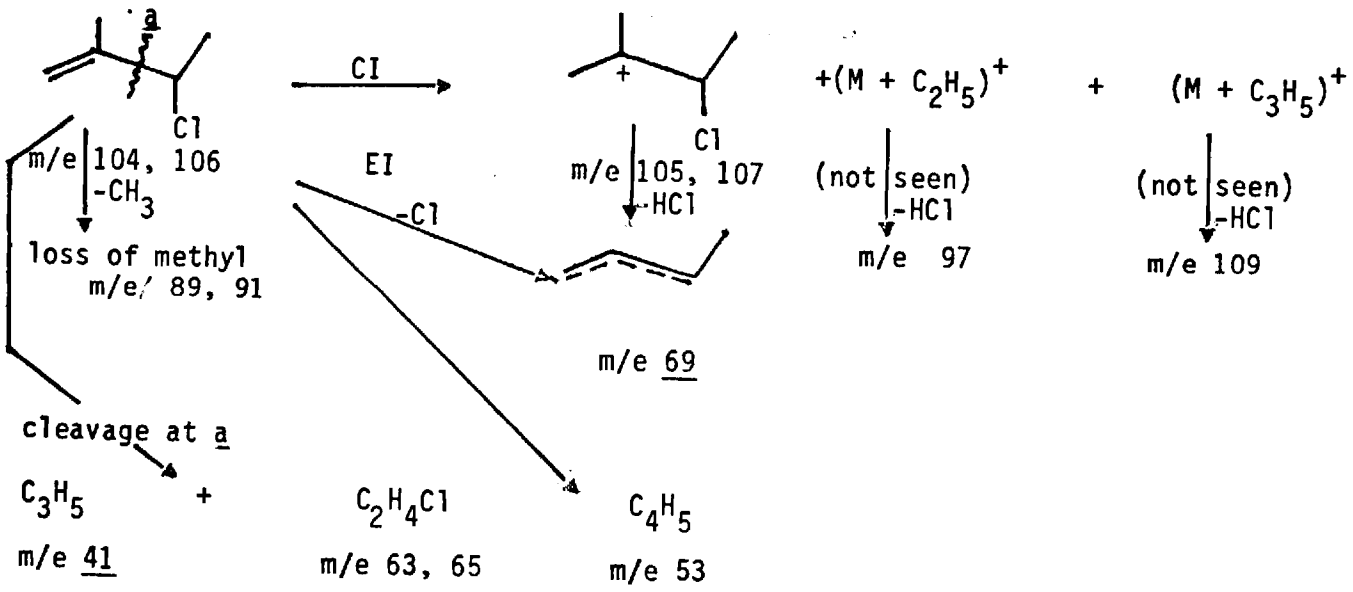


Figure 27 Humics Plus Chlorine: 2-methyl-3-chloro-1-butene. Electron Impact

Figure 28

EI and CI Fragmentation Sequence for 3-Chloro-2-methyl-1-butene



chlorine. The major chlorine-containing products are dichloracetic and trichloroacetic acids. Some aromatic compounds and several organic acids have been assigned. Some aromatic products were noted suggesting a closer link to the oxidative degradation work. A complete listing of the products identified during the support period is presented in Table 10. The EI total ion chromatogram is shown in Figure 29.

Structural assignments were made according to the procedures previously mentioned. In this case it will be noted that the methyl esters and ethers were prepared rather than the corresponding ethyl derivatives. A typical structural assignment is illustrated by the case of methyl dichloroacetate the EI spectrum of which is presented in Figure 30. The FIT index of 981 provided a very strong suggestion regarding the structure of this component. The molecular ion at m/e 142, 144, 146 is not seen. The weak ion cluster at m/e 111 and m/e 113 representing the loss of a methoxy radical does not include the last isotopic peak at m/e 115 because this signal is too weak to stand out above the background. The strong ion cluster at m/e 83, 85, 87 represents cleavage between the functional groups as does the base peak at m/e 59. A more complete fragmentation sequence is presented in Figure 31.

These results are especially significant when compared with the results described in the previous subsection. This is so because a definite contrast is noted between the two cases which in turn suggests that adsorbed or otherwise weakly bound, but highly reactive species are present when aquatic humics are initially flushed from the soil environment. The writers postulate that this material is only weakly shielded from degradation and

TABLE 10

PRODUCTS LIST: CHLORINATION OF MATURE AQUATIC HUMICS

1. 2-Methylpentanal
2. Propionic Acid*
3. 3-Methyl-2-butanone
4. Formaldehyde
5. Pentanoic Acid*
6. Toluene*
7. 2-Hydroxy-4-methylpentanoic Acid*
8. Dichloroacetic Acid
9. Trichloroacetic Acid
10. Benzoic Acid
11. Trimethyoxybenzene isomers**
12. 2-Methyl-2-butanol
13. 2-Methylpropionate Acid

*Acids were isolated after derivation to the methyl esters.

**Probably not completely methoxylated in the original product mixture.

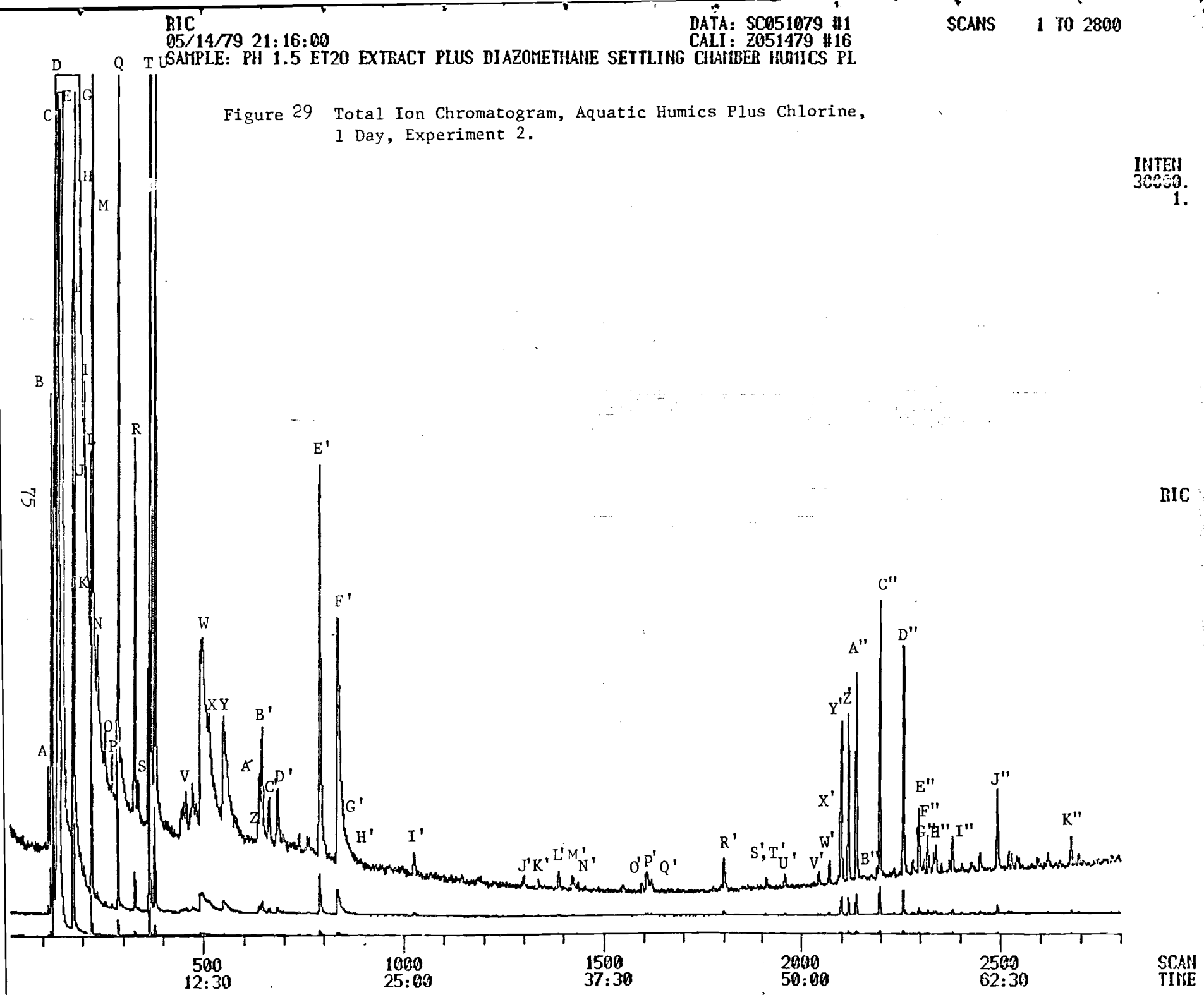


Figure 29 Total Ion Chromatogram, Aquatic Humics Plus Chlorine, 1 Day, Experiment 2.

MASS SPECTRUM
05/13/79 2:10:00 + 11:07
SAMPLE: ET20 EXTRACT PH1.5 HUMICS PLUS CHLORINE
#445 - #440 X1.00

DATA: EFF0508792 #445
CALI: Z051279 #25

BASE M/E: 59
RIC: 45952.

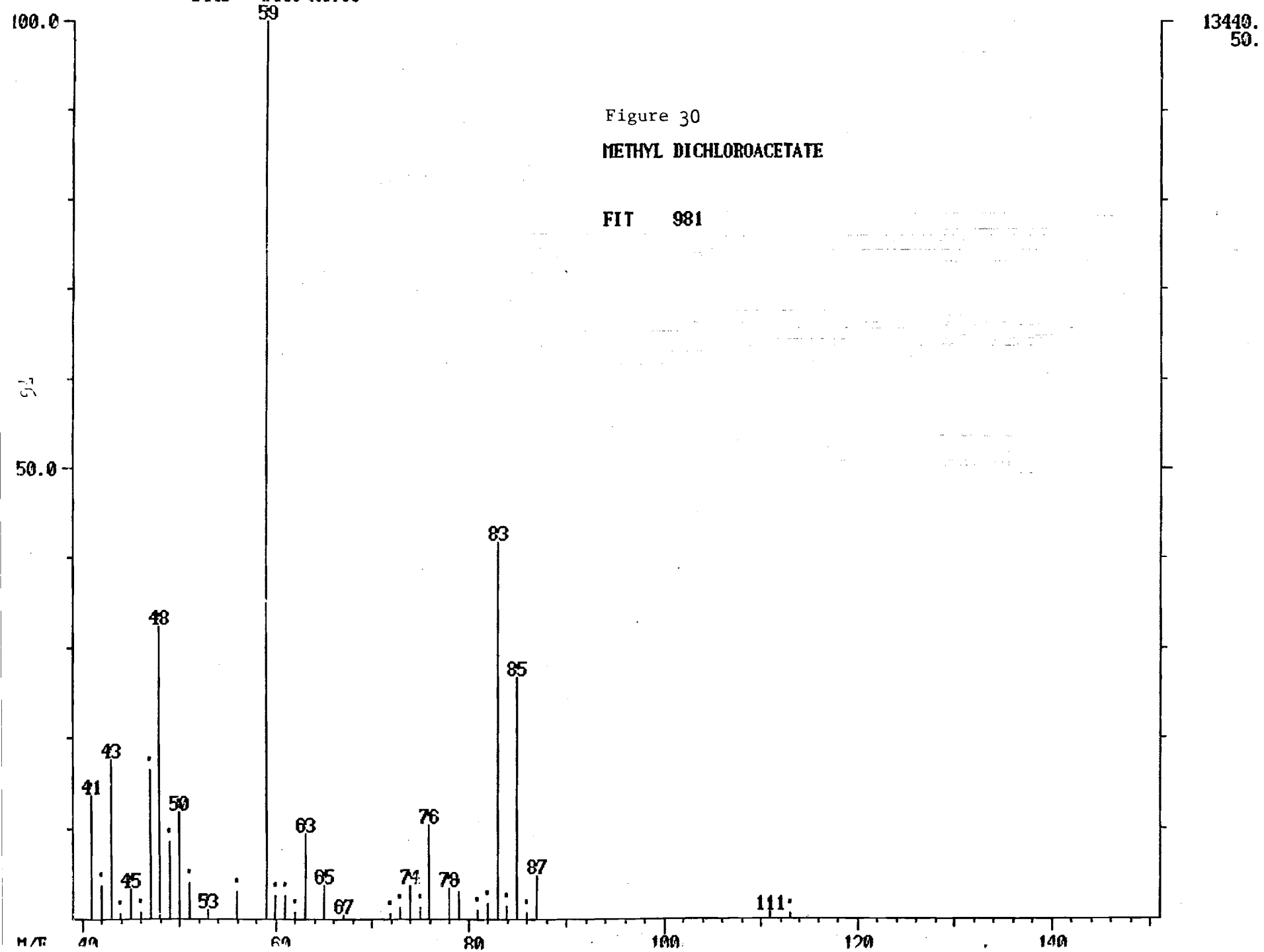
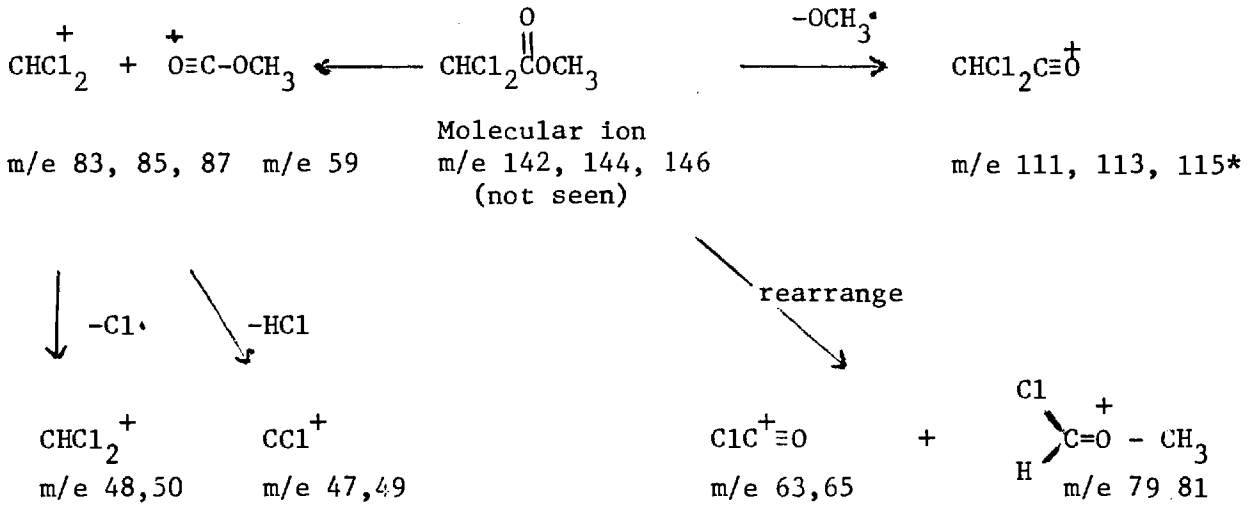


Figure 30

METHYL DICHLOROACETATE

FIT 981

Figure 31
Fragmentation Sequence - Methyl Dichloroacetate



All of the ions shown with the exception of the molecular ion are present in the reference spectrum. A mechanism leading to the minor rearrangement ions at m/e 63, 65 and m/e 79,81 is difficult to postulate.

*Not seen because of low intensity

in the presence of biological activity, sunlight, acid and dissolved oxygen it soon disappers. If the aquatic humics are isolated and stored in a more protected enviroment, these species can survive for a longer time period. Therefore, it is not surprising that we were able to repeat our initial findings with the immature humics over the course of several experiments. Eventually, even under the conditions of a more protected environment, the adsorbed isoprenoids are lost and/or altered so that the product mixture contains fewer chlorinated components and no chlorinated isoprenoids. Mature aquatic humics have already lost these precursors and therefore are less influenced by protracted storage.

These observations have implications for possible treatment protocols. Our contacts at the Atlanta water works inform us that unidentified volatile, halogenated compounds are noted among the components detected by volatile organics analysis (VOA) immediately following a period of heavy rainfall. We suggest that these compounds may be related to the chlorinated isoprenoids described in an earlier subsection. Removal might be effected by simply holding the water for a few days prior to treatment.

Quantitative Estimates of Chlorine-Containing Products Resulting from Disinfection of Aquatic Humics

Three experiments were carried out quite late in the course of the project in order to provide a connecting link between a property for which a maximum concentration limit has been set-i.e. the trihalomethanes (THM's)- and the experiments performed in connection with this project. Bromide was excluded thus making TOX equivalent to total organic chlorine (TOCl). In

order to successfully execute a TOCl analysis, one must guard against both the accidental loss of volatile, chlorine-containing entities such as the THM's while avoiding contamination with common chlorine-containing laboratory chemicals. Since the accepted methods depend on the adsorption of the organic chlorine on carbon, and its subsequent conversion to chloride, the carbon must be totally free from chloride to start with and furthermore must be sufficiently active to adsorb the chloroorganics when exposed to the test solutions. For these reasons, the samples collected for TOCl analysis were treated with excess sodium sulfite to destroy any remaining free chlorine, sealed in crimp top bottles and sent to two different commercial laboratories for subsequent analysis. Matching THM values were obtained in-house.

The results obtained from these experiments are presented in Table 11. It should be pointed out that different batches of aquatic humics were employed, four sets of results were obtained and two different laboratories were involved in to TOCl work. The analytical variability was found to exceed the differences between the two batches of aquatic humics in all sample categories with the exception of the reservoir controls. (A second pair of values, not shown in the table placed this level at 125 mg/l vs the 7.6 mg/l shown). TOCl values were significantly higher after 5 days than they were initially. This observation may be significant with regard to possible ongoing TOCl producing reactions in drinking water distribution systems. A recent publication, in fact, suggests that a significant increase in the mutagenicity of drinking waters does occur as it travels through the distribution system.[17] It is possible that this increase in mutagenicity is related to the increase in TOX.

THM levels were also found to exhibit the same upward trends as did the TOX values. Furthermore, the percent contribution of chloroform to

TABLE 11

TOCl AND CHLOROFORM RESULTS - CHLORINATION OF AQUATIC HUMICS

	Mean Level µg/L	Chlorofrom µg/L	Percent Contribution of $CHCl_3$ to TOCl
Blank	3.8	--	--
Reservoir Control	7.6	1.8	21
Finished Water (1 day)	1110	250	21
Finished Water (5 days)	1530	395	23

TOX is remarkably constant from the control through the 5-day samples even though the absolute values varied by more than two orders of magnitude. The Project Manager believes that these findings constitute evidence that the rise in chloroform levels in drinking waters reported after prolonged contact with chlorine does not occur at the expense of other TOCl. One might therefore conclude that the proportion of chloroform precursors generated from the aquatic humic fraction of typical source waters depends only on the reactivity of the humics and the conditions of disinfection and not upon the length of time in the distribution system. Since both values exhibit the same trend, either one is a valid indicator of the overall hazard potential posed by chlorinated organics in drinking water. Thus a regulation using total trihalomethanes as an indicator of TOX would seem to be justified in the light of this evidence. Other workers have monitored finding similar correlations between chloroform and THM.[18]

Using 3-methyl-3-butene-1-ol as a quantitation standard, the II11943D concentrate derived from immature aquatic humics and which had served as the master run for our past work was retrieved from frozen storage and rerun. A comparison of the proportions of each of the chloroalcohols in the new run to the same proportions in the original run provided strong evidence that losses of these materials had not occurred. The correlations are summarized in Table 12.

The standard compound was injected into the GC/MS system as a solution in methanol having a concentration of 50 mg/l. Since this level corresponds to a concentration of 25 μg/l in the finished water when adjusted for the concentration step performed on the real samples, it was hoped that it

TABLE 12

PROPORTIONS OF CHLOROALCOHOLS IN CHLORINATED AQUATIC HUMICS

EVIDENCE OF SAMPLE INTEGRITY

Compound	Original Proportion	Post-Storage Proportion
Monochloroalcohols		
3-Chloro-2-methyl-2-butanol	1.00	1.00
1-Chloro-2-methyl-2-butanol	0.14	0.12
4-chloro-3-methyl-1-butanol	0.04	0.03
Dichloroalcohols		
1,4-Dichloro-2-methyl-2-butanol	1.00	1.00
1,3-dichloro-2-methyl-2-butanol	0.57	0.56
3,4-Dichloro-3-methyl-2-butanol	0.42	0.37

would give a response of similar intensity to that recorded for the chloroalcohols with which it was to be compared. Duplicate injections showed a variability of only 10-15% based on total ion current across a chromatographic peak. This general tactic of making semiquantitative estimates on the basis of total ion currents has proven to be quite successful in some of our priority pollutant work in which case the average values found for six generally dissimilar standards was 105% of the real value. Individual results, however, did vary by up to a factor of four, thus the overall results are to be given greater credibility than any single result. In the case of the work performed in connection with the aquatic humics, the real sample showed peak areas which were bracketed by those exhibited by the standards. The concentrations of the monochloromethylbutanols and dichloromethylbutanols were calculated on the basis of total ion counts. While the chloromethylbutenes did not survive the storage period, the closeness of the data on the surviving materials encouraged us to extend the concentration estimates to include these compounds as well. These results are presented in Table 13.

In summary, it can be seen that the concentrations of chlorinated isoprene alcohols as a group in this product mixture is about 10% of the chloroform levels presented in Table 10. A similar percentage relationship was observed for the chlorinated C-5 hydrocarbons. It should be pointed out, however, that these estimates are only "ballpark" figures as they represent a comparison between one set of experiments performed with immature humics and another set performed with mature humics.

While chloroform accounts for about 20% of the total organic chlorine, the monochlorinated alcohols and hydrocarbons together account for only

Table 13
Concentrations of Chlorinated Products in
Mini-Pilot Studies with Aquatic Humics

Compound	Level in mg/l
3-Chloro-2-methyl-2-butanol	24
1-Chloro-2-methyl-2-butanol	2.9
3-Chloro-2-methyl-1-butanol	0.7
4-Chloro-3-methyl-1-butanol	2.4
Sum monochloromethylbutanols	30
1,4-Dichloro-2-methyl-2-butanol	4.4
1,3-Dichloro-2-methyl-2-butanol	2.3
3,4-Dichloro-3-methyl-2-butanol	1.6
Sum dichloromethylbutanols	8.3
3-Chloro-2-methyl-1-butene	15
2-Chlormethyl-1-butene	0.8
3,4-Dichloro-methyl-1-butene isomer	2.0
2,3-Dichloro-2-methylbutane	3.7
1,3-Dichloro-2-methylbutane	3.8
Sum chlorinated C-5 hydrocarbons	25.3

1% of the TOCl on account of their lower chlorine content. The dichloro-alcohols and hydrocarbons account for another 0.5% of the TOCl. The concentrations as shown in Table 13 are only approximate, partly because of underlying assumptions and partly due to experimental difficulties. Nevertheless, the authors believe they are correct within an order of magnitude. The elucidation of the nature of the remaining percentage of the TOCl remains as a task for the future.

Chlorination of Aquatic Humics by Molecular Weight Fraction

This series of experiments was performed on the molecular size fractions isolated according to the methods described earlier in this report. Because only a limited amount of material was available, it was necessary to perform these experiments in 100 ml glass-stoppered Erlenmeyer flasks rather than in the dynamic environment of the mini-pilot facility. Conditions were otherwise realistic, however (10 mg/L aquatic humics, chlorine residual to 2 mg/L or less). Fractions were run in duplicate to improve the quality of the data. Chlorine uptake, TOX (as TOCl) and chloroform concentrations were determined. Unfortunately, the chloroform runs produced erratic results so that statistical significance could not be established which would enable the authors to make statements regarding which of the fractions contributed the most to the chloroform production. This was so even though the high-TOCl and high-chlorine-demand fraction was also the high-$CHCl_3$ fraction. Overall levels of chloroform produced were higher than those found by Schnoor et.al.[6], but lower than those reported in the previous subsection or by Oliver's group at the Canada Center for Inland Waters.[12] We would refer the reader to the previous subsection for information regarding the trihalomethane-generating potential of unfractionated humics. The results

are presented in Table 14. The molecular size partitions studied represent a subdivision of those fractions already reported to be responsible for most of the THM production observed in natural surface waters.

A positive correlation between chlorine uptake and TOCl was observed. TOCl accounts for about 1/5 to 1/6 of the chlorine consumed. Fractions 2 ($\overline{M}_N$=2200, 47%) and 5 ($\overline{M}_N$=530, 21%) account for most of the chlorine uptake and TOCl. The distribution as presented graphically in Figure 32 is clearly bimodal.

Classification of Raw River Water

This experiment was concerned with the separation of river water organics by class by means of sequential adsorption on XAD resins. Using the scheme discussed earlier it was possible to isolate hydrophobic bases, hydrophobic acids, hydrophilic bases, hydrophilic acids and hydrophilic neutrals with a removal efficiency of 99% as measured by TOC. These results are presented in Figure 33. The TOC levels remaining after each step are noted on the flow chart together with the percent desorbed from each of the resins are listed in Table 15. Although there is considerable drift in individual values, the overall recovery for the whole scheme is 99%. The hydrophobic acid fractions (ca. 60%), hydrophilic acid fractions (26%) and the hydrophilic neutrals (20%) account for virtually all of the TOC removal.

TABLE 14

PRODUCTION AS A FUNCTION OF MOLECULAR SIZE

Fraction	Molecular Weight $\bar{M}_N$	Percent of Total Humics	TOCl μg/L	Percent Cl_2 Uptake
G-50 excluded	3100	3.6	639±5	64
G-25 excluded	2200	47	846±17	76
G-15 excluded	1100	14	713±02	65
G-10 excluded	660	15	709±11	50
G-10 excluded	530	21	803±03	70

FIGURE 32.

CHLORINE UPTAKE AND TOCl AS A FUNCTION OF MOLECULAR SIZE

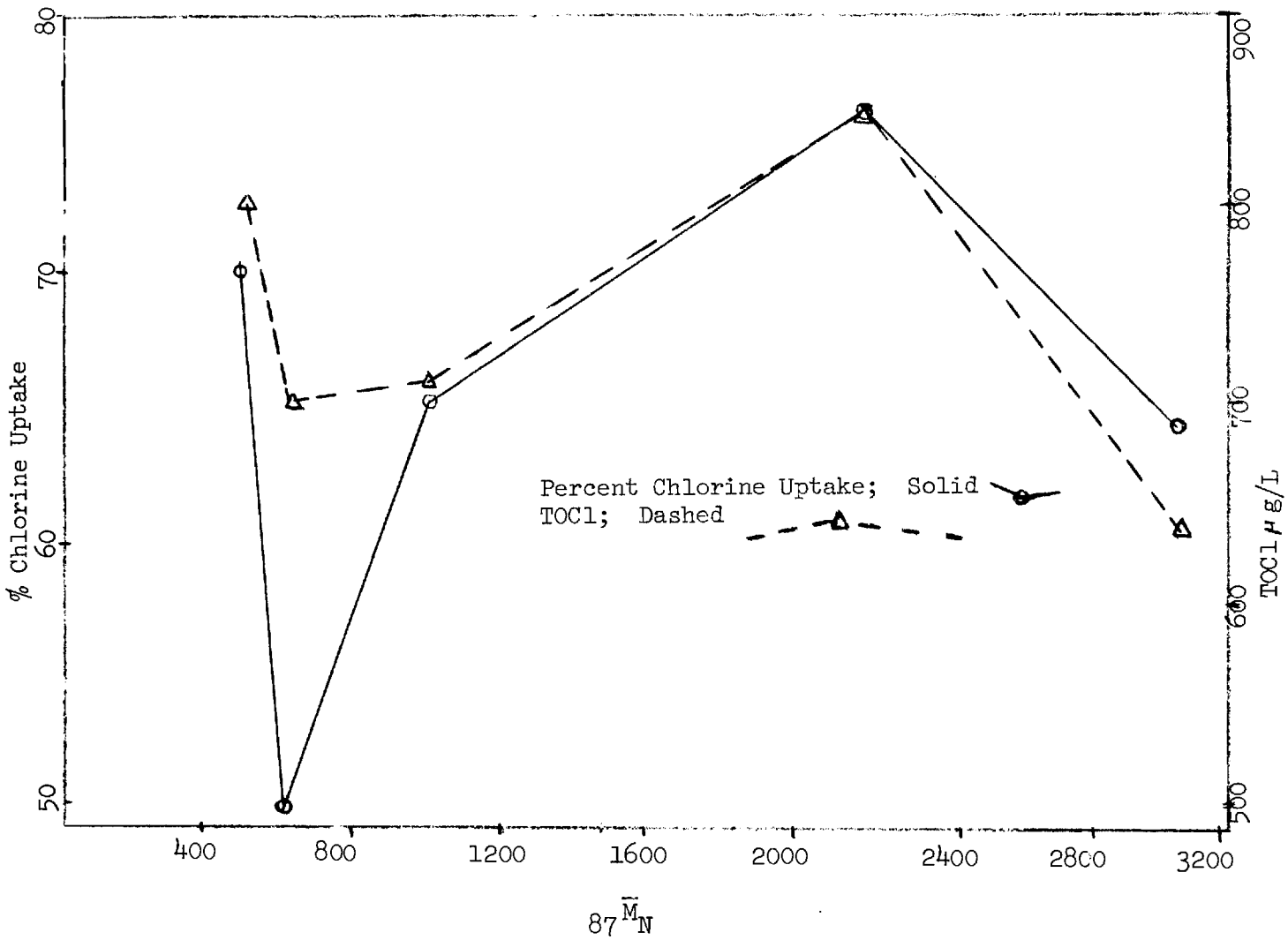


FIGURE 33. SEPARATION-CONCENTRATON SCHEME

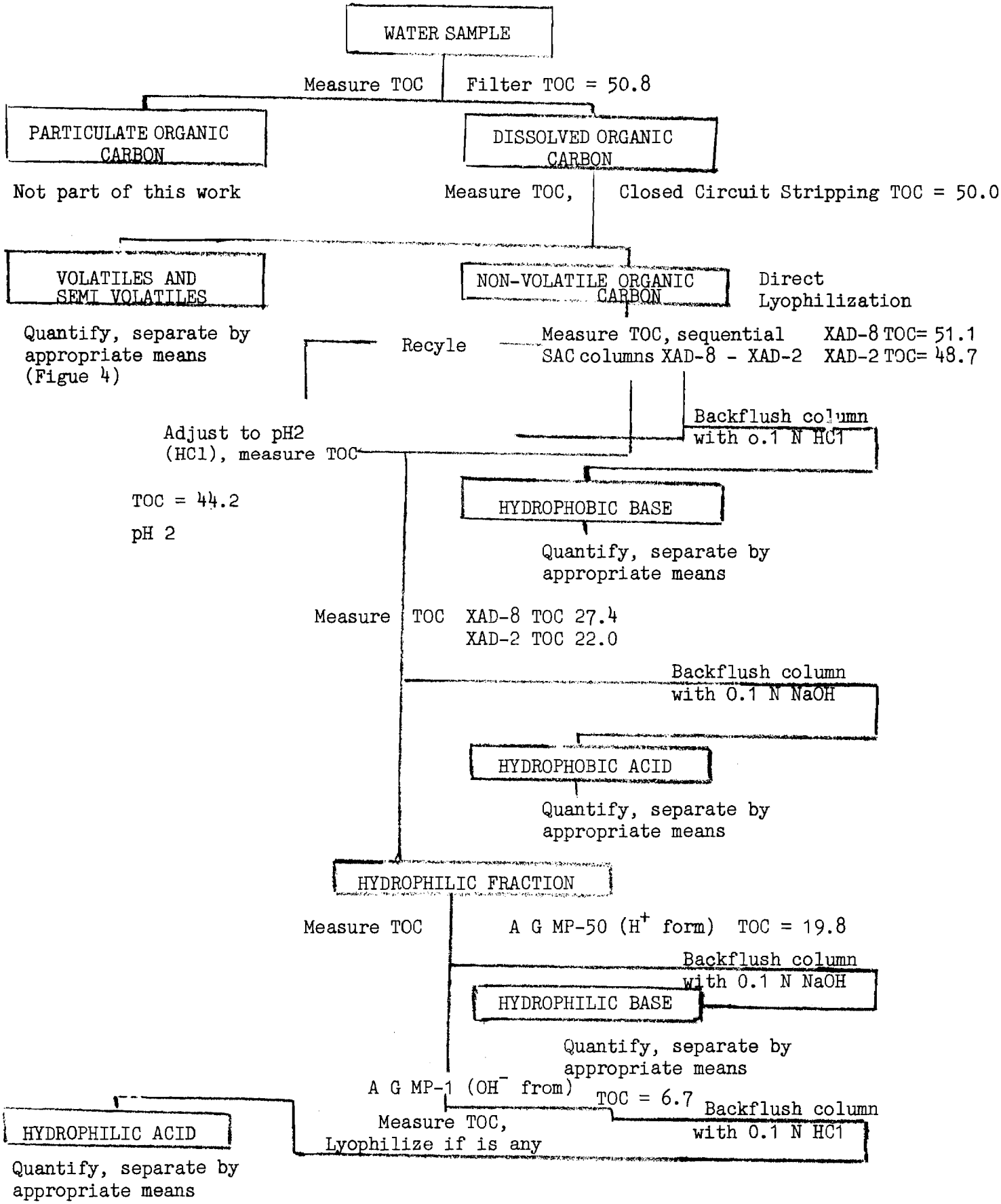


Table 15

Recoveries of River Water Organics by Class

	Removed		Recovered	
	mg	%	mg	%
Hydrophobic Bases from XAD-8* and XAD-2	6.2	4	8.2	132
Hydrophobic Acids from XAD-8	49	35	63	140
Hydrophobic Acids from XAD-2	12	11	14	117
Hydrophilic Base from AGMP-50	4.2	3	2.5	60
Hydrophilic Acid from AGMP-1				
First Washing	21	26	15	94
Second Washing	6+		4.7	
Hydrophilic Neutrals	10	20		
SUM OR(OVERALL)	108	99	107	(99)

*Zero TOC removal on XAD-8. Extraction will be carried out nevertheless. At this time it seems inappropriate to present it as a separate figure.

+By difference.

CONCLUSIONS

Isolation of Aquatic Humics

Aquatic humic matter can be isolated from acidified surface waters with recoveries of about 60% using a resin adsorption technique. Large-scale processing is possible so that pound-quantities of the material can be made available for future study should the sponsor so desire.

Acid-Base Chemistry of Aquatic Humic Substances

Improved analytical techniques developed during the course of this project have made it possible to arrive at more precise values for operationally defined carboxyl groups in aquatic humic substances.

Reaction of Aquatic Humics with Permanganate

The presence of oxalic and succinic acids as the dominant products resulting from the mild permanganate oxidation of methylated aquatic humics together with the general dominance of aliphatically-derived products provides strong evidence that aquatic humic materials are more aliphatic and less aromatic than previously supposed.

Oxidation of Aquatic Humics with Iodine in Base

The halogens exhibit similar behavior in a variety of chemical reactions including the ability to disinfect. The high atomic weight of iodine makes it relatively easy to isolate iodoorganics-even when produced in very low yields. For example, iodoform can be isolated as a crystalline solid although the yield is only 0.8%--a value which is incidentally quite typical of those recorded for chloroform in chlorination reactions of humic materials.[4,12] Thus the presence of methylene iodide among the products identified lends credence to the suggestion that dihalomethanes may be genuine products of disinfection. This observation is important since the near-ubiquity of methylene chloride as a laboratory contaminant makes it difficult to confirm, particularly at extremely low levels. The finding of methyl and ethyl iodide is also significant.

These findings fit in rather well with the detection of short-chain alkyl branched dicarboxylic acids among the permanganate oxidation products described in earlier sections. For example, malonic acid which is actually employed to remove chlorine from chlorine dioxide would be expected to produce methylene chloride upon decarboxylation. If only one of the reactive sites were occupied by a chlorine, methyl chloride would result. If methylmalonic acid or an intermediate with a similar substitution pattern were present, ethyl chloride would be the expected post-decarboxylation product. The Project Manager feels that this question needs further researching.

Reaction of Aquatic Humics with Bromine in Acetic Acid

The presence of tetrabromoacetone and the suggested presence of C-5

bromoketones among the products derived from the drastic treatment of aquatic humics with bromine offers tie-ins with both the oxidative degradations (short-chain oxygenated acids) and the chlorination studies (oxygenated isoprenes). Further work in this area might also prove rewarding.

Chlorination Studies

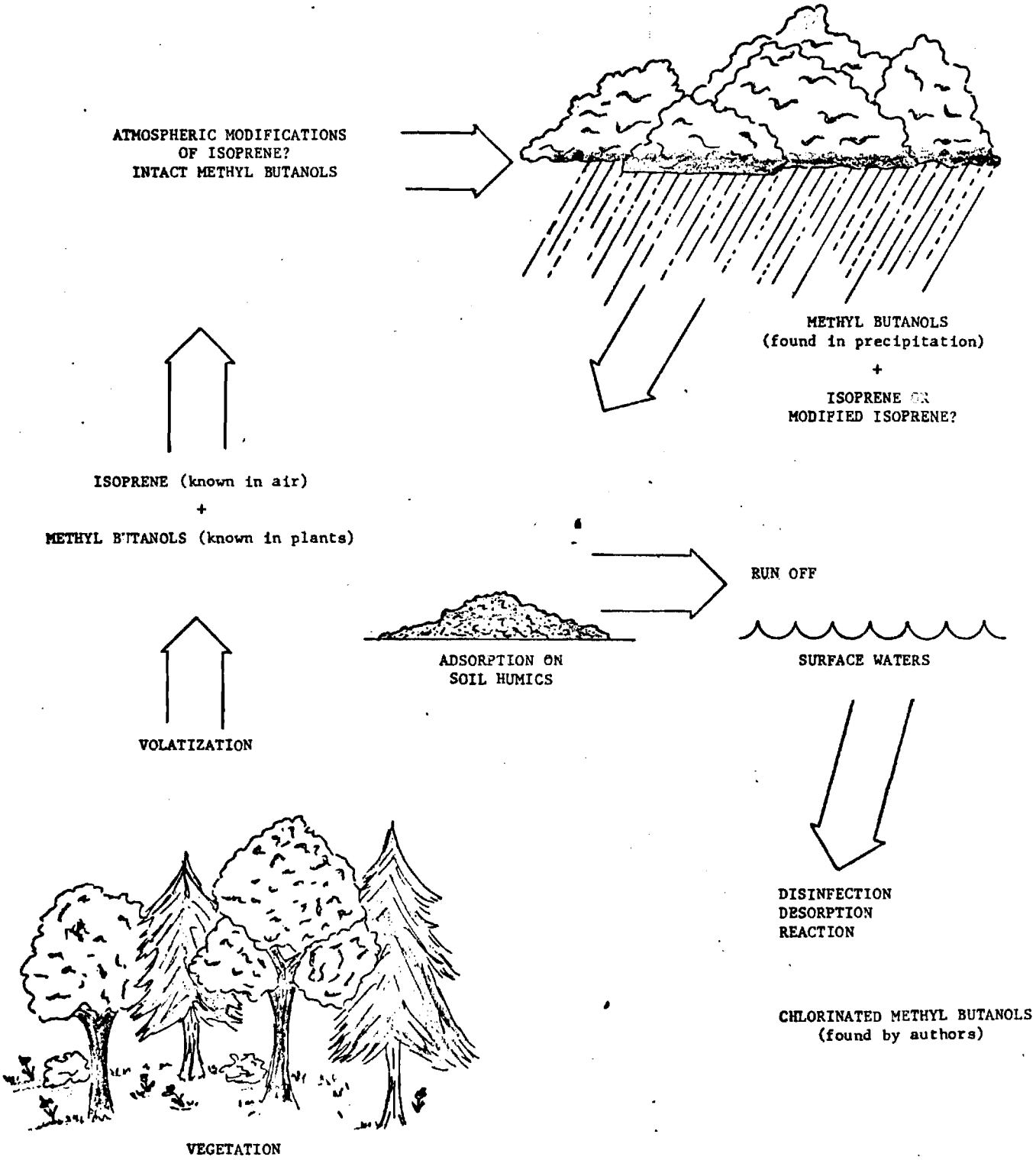
The isolation and identification of a series of chlorinated isoprenoid alcohols and hydrocarbons resulting from the treatment of immature aquatic humics with chlorine in a dynamic system simulating the disinfection of potable water is an observation of fundamental importance. We believe that the reported finding of the most dominant hydrocarbon in chlorinated secondary effluents by Glaze et.al.[18] is strong supportive and important evidence in the light of the fact that secondary effluent are rich in immature aquatic humics and are frequently chlorinated prior to discharge into surface waters. Other supportive evidence regarding the presence of isoprenoid precursors in the atmosphere, in precipitation and in surface waters makes it possible to suggest a mechanism for the origin of the chlorinated methylbutanols as shown in Figure 34.

The detection of other adsorbed organics in aquatic humic material such as toluene and benzene, which are released for detection only after oxidative disruption of the aquatic humic matter supports the suggestions of other workers regarding the importance of aquatic humics in the transport of pollutant materials in the environment.

Aquatic humic materials as isolated by the Co-principal Investigator have an overall number average molecular weight ($\bar{M}_N$) of 1270. When

Figure 34

ORIGIN OF CHLORINATED METHYL BUTANOLS



these are subdivided according to molecular size, almost half of the material is found to have $M_{\bar{N}}$ of 2200. This is also the fraction which was found to clearly generate the most TOCl and which showed the highest chlorine uptake. Fractions having $\bar{M}_N$ of 1100 or less accounted for very nearly all of the remaining material. It should therefore be noted that while these fractions do not appear distinct with respect to their gross properties such as IR, UV and elemental analysis, they do appear to differ significantly in their chlorine demands and in their ability to generate total organic chlorine (TOCl) when treated with chlorine under conditions simulating the production of potable water.

Classification of Raw River Water

Raw river water can be successfully classified according to the scheme presented earlier in Figure 33 with an overall recovery of 99% as measured by TOC. Over 70% of the components removed are acidic in nature thus reinforcing the conclusion that aquatic humics (which are acids) are the major organic component of surface waters. On the other hand, organic bases account for less than 10% of the components removed and are therefore of lesser importance. The hydrophilic neutrals however do account for the remaining 20% and represent an area in which more investigation is required.

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