IDENTIFICATION OF MAJOR AND MINOR CLASSES OF

NATURAL ORGANIC SUBSTANCES FOUND IN DRINKING WATER

Final Progress Report

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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

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classification ranging from number average molecular weight $(\bar{M}_N) \approx 500$ to $\bar{M}_N \approx 3000$. 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 signi-
ficant 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 sur-
face 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 alco-
hol. 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).

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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 micro- meter 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). Ali- quots 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.

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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 $\underline{M}$ $Ca(NO_3)_2$; 0.100 $\underline{M}$ NaCl

[c] 0.100 $\underline{M}$ $Ca(CH_3COO)_2$; 0.100 $\underline{M}$ $Na(CH_3COO)$; 0.150 $\underline{M}$ 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

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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

reservoir containing 10 ppm model compound

funnel for concentrated chlorine flocculant addition

constant-head device overflow drain

to mixing chamber

mixing chamber

magnetic stirrer

perforated ring for exit flow

settling chamber

flocculation chamber

clamp

for sampling, draining

floc drain

carbon/sand filter

clear well storage prior to final pH, residual adjust

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Figure 2   Dimensions and Volumes of Mini-Pilot Facility.
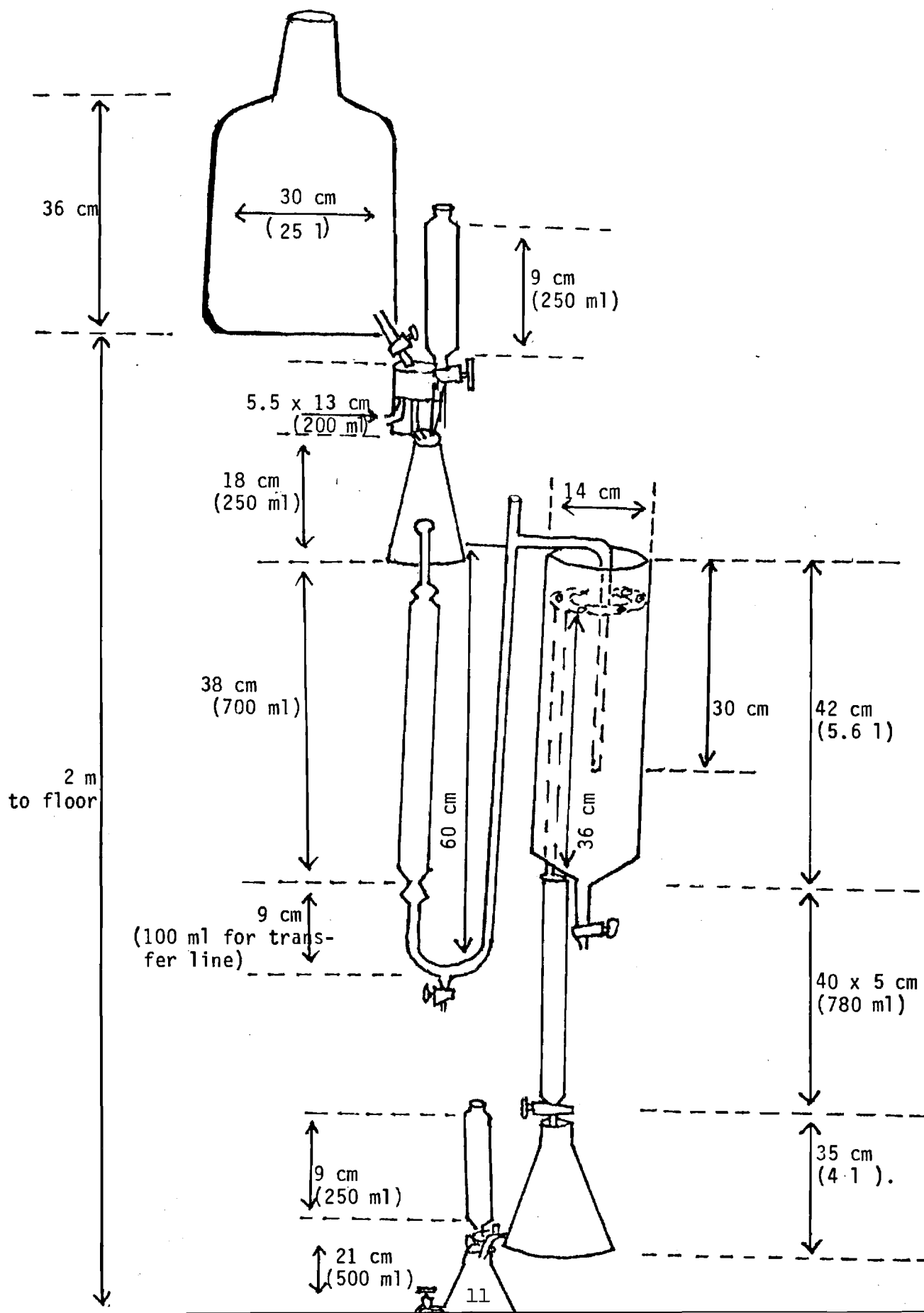
aquatic humic material together with whatever buffering agents were required

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 $65^\circ C$, 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.

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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 1) 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 $\underline{N}$ hydrochloric acid per bed, thus providing 2 1 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 puri-fied $6\underline{N}$ hydrochloric acid. A brown precipitate slowly developed in this solu-tion (2.8 1) 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 1 of water had passed through the resin. Once again a portion of the eluate was retained for TOC determination.

The remaining eluate (2.3 1) 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 $3\underline{N}$ 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 1, pH 1.2) was retained for TOC analysis. The remaining eluate was brought to pH 7.0 with $1\underline{N}$ 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 $1\underline{N}$ sodium hydroxide solution and washed with 200 ml of distilled water. The remaining 1.6 1 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 1, 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 0.1$\underline{N}$ sodium hydroxide and o.1$\underline{N}$ 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 environ-
ment is source selection. In the case of our research, source selection was
governed by the following requirements: (1) high DOC* content, (2) pre-
dominance of low molecular weight aquatic humic materials of high solubility
and (3) a pure "humic" character. DOC concentration shows a positive corre-
lation 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 ground-
water 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 solu-
bility 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 0.4μ m 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.
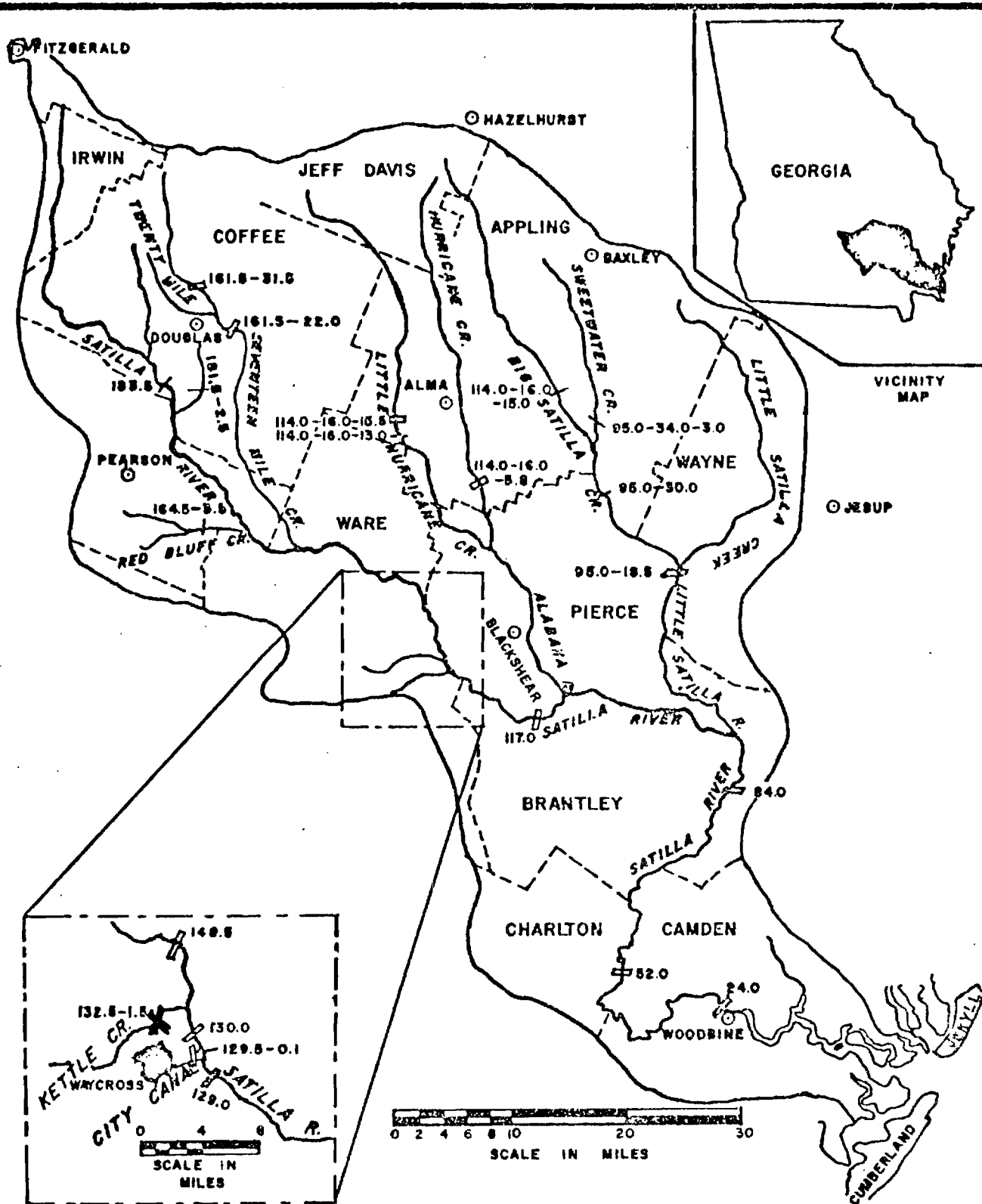
Figure 3.

MAP OF THE
SATILLA RIVER BASIN

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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.

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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):

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80.7-81.7   broad, aliphatic                                  5H
83.4        broad, CH or CH2 in electronegative environment   8H
83.8        broad, OCH3                                        2OH
87.35       sharp, aromatic                                    2H
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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 0.4μm 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 ($r \geqslant 0.999$) 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 ($r \geqslant .999$) 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

Direct Titrations:
Titration 1 in 0.66 N Ca $(NO_3)_2$
Titration 2 in 0.1 N NaCl

Indirect Titrations:
Titration 4 in 0.2 N Ca$(OAc)_2$
Titration 5 in 0.1 N NaOAc
(filtered on Amicon UM-2)

26
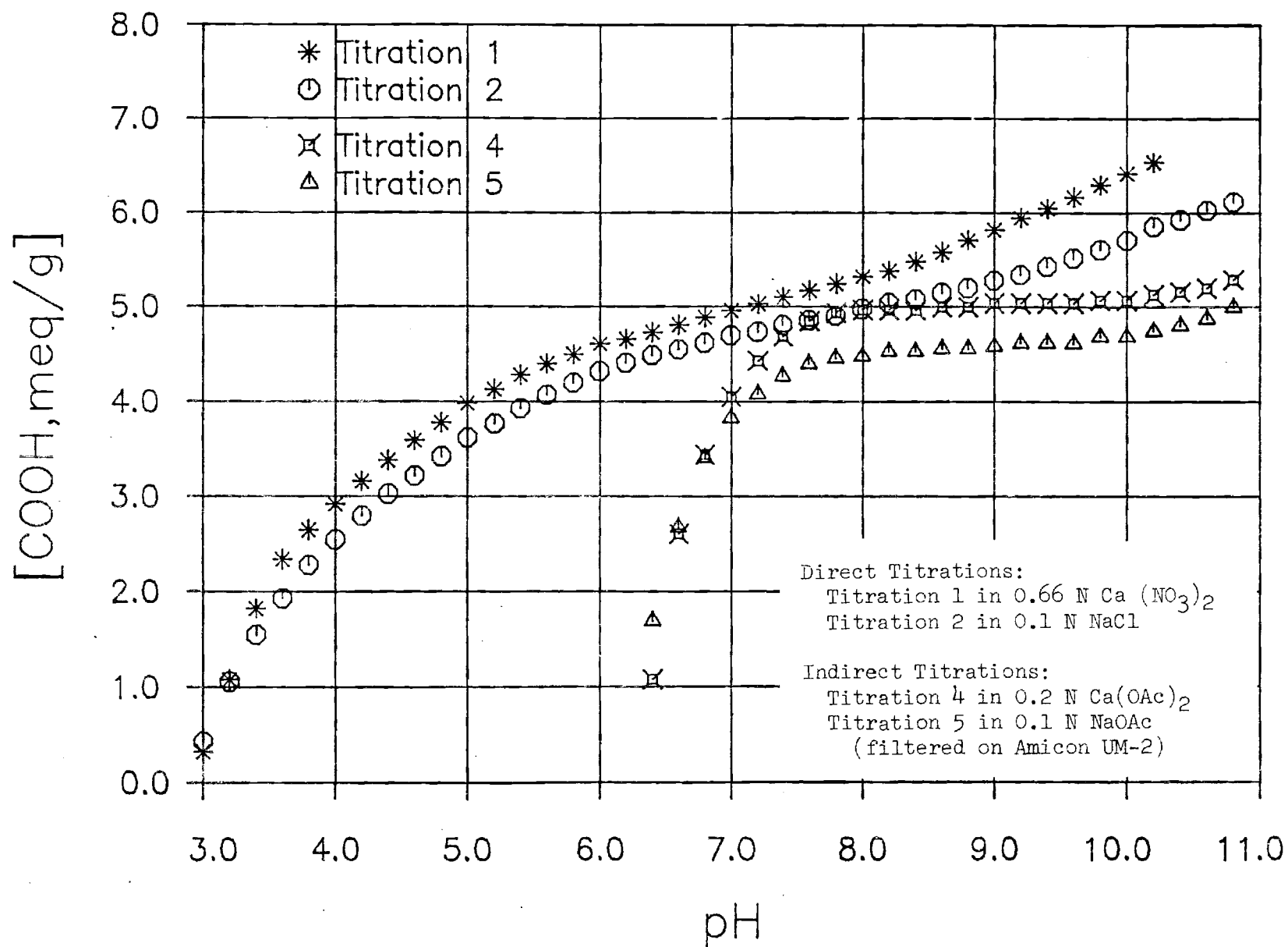
pH 10.2. At pH 9.8, the endpoint recommended for the calcium acetate method, 6.3 meq/g have been titrated.

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 (0.4μm). Only ultrafiltration through membranes of pore size 0.0001μm (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

28
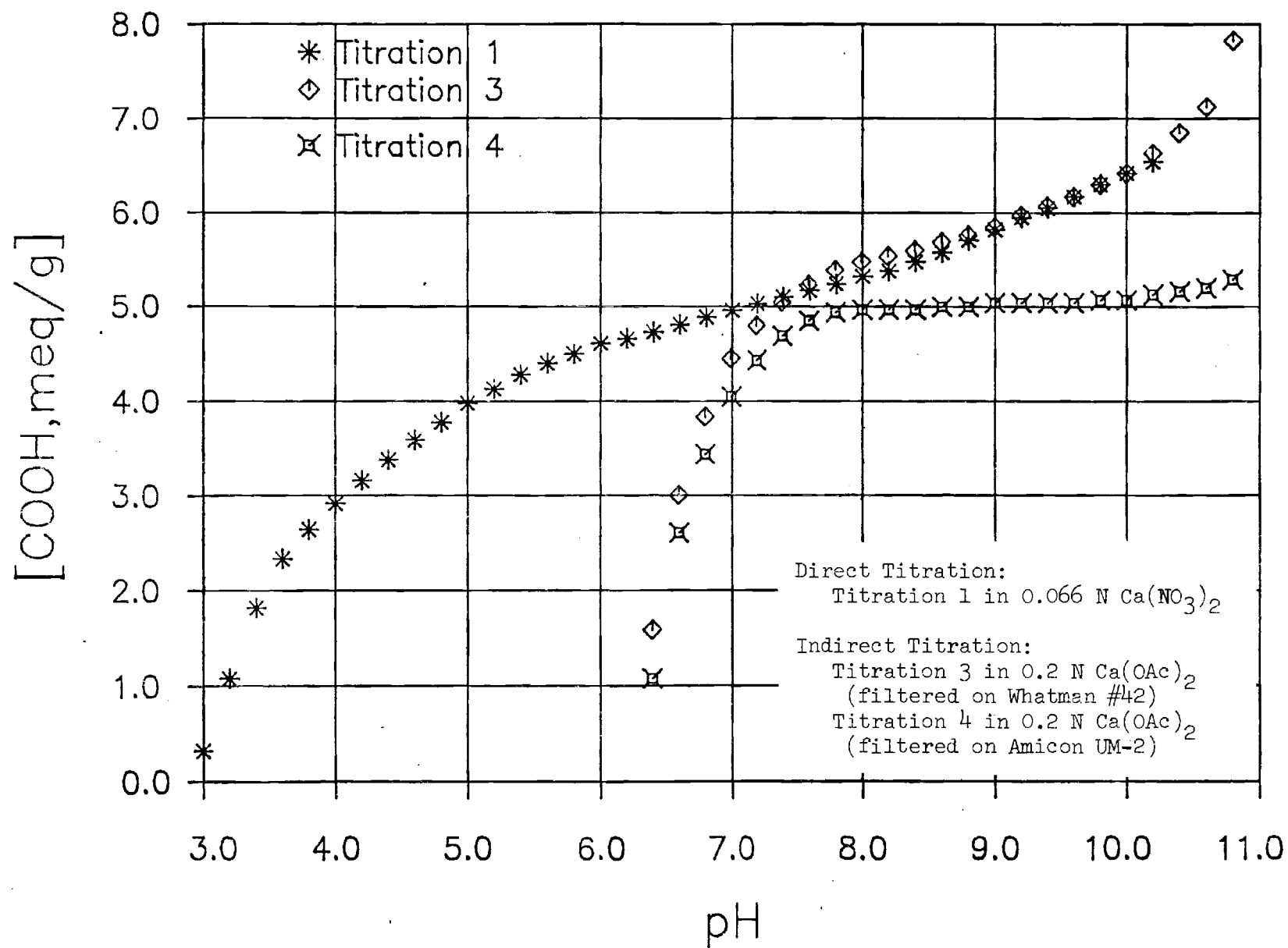
remove the colored humic material. In this case the sample after reaction of aquatic humic matter with calcium acetate, was filtered through Whatman #42 filter paper, which allowed passage of virtually all color. Compared with titration T4, the "net titration curve" of T3 continues to rise past the level 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, if colored humic substances are not removed quantitatively before titration. 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.

If partially neutralized humic substances have been removed quantitatively (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 (~ 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

Indirect Titrations:

Titration 5 in 0.1 N NaOAc
Titration 6 in 0.15 N pyridine -
NaCl (filtered on UM-2)

31
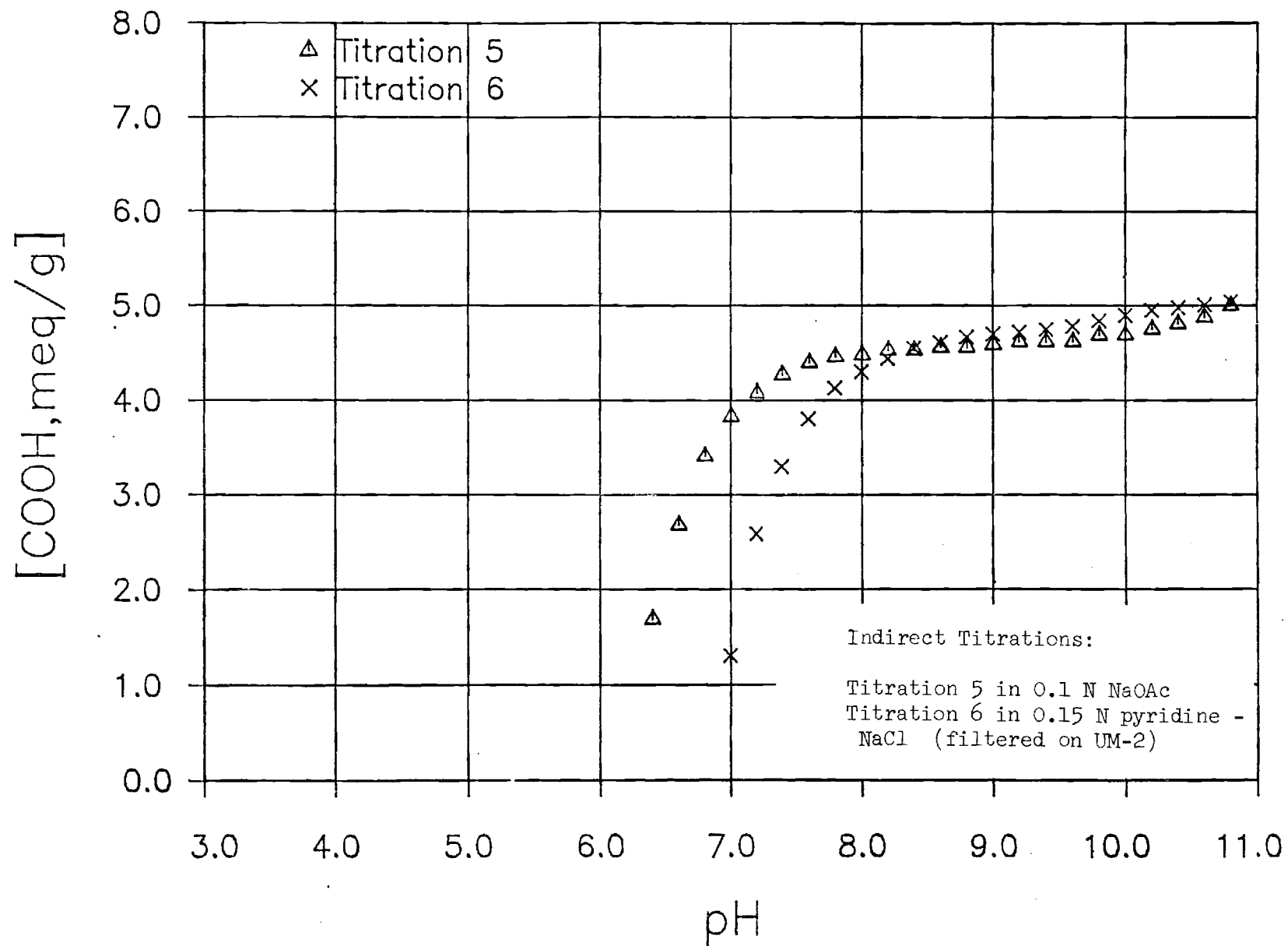
as measured by titrating the resulting pyridinium ion. Since pyridine
(pK_b = 8.83) is a slightly stronger base than acetate (pK_b = 9.25), a
higher yield in COOH can be expected on these grounds alone. This
differential yield illustrates the fact that pK dependent methods of
carboxyl analysis will always 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 reason-
able 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 determi-
nation 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 $1600-1640 cm^{-1}$ region) and the NMR (only 15% Ar-H).

33

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.

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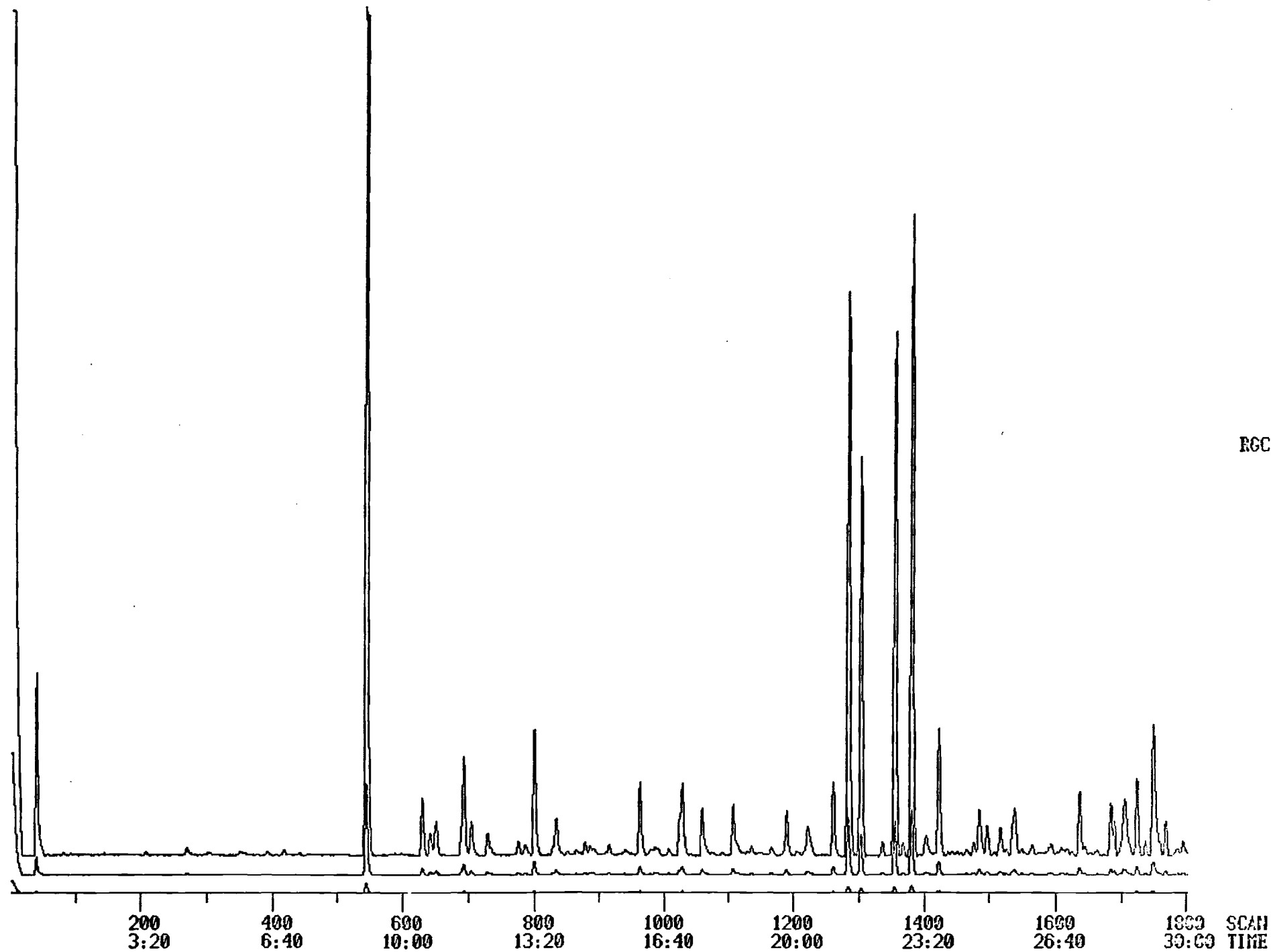
RGC

Figure 8. Total Ion Chromatogram, Methylated Oxidation Products From Humic Acid
Scans 1800-3507 (enhanced).
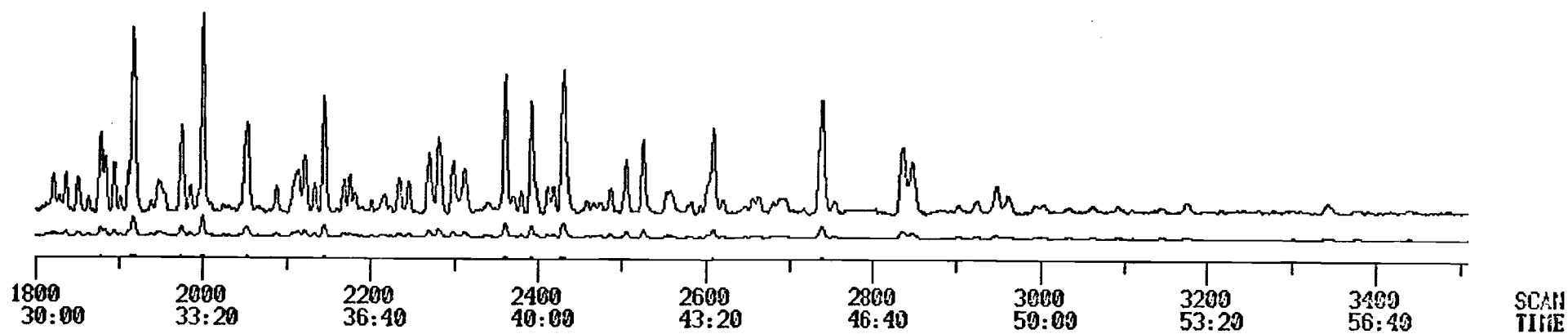
36

RGC

| 1800 | 2000 | 2200 | 2400 | 2600 | 2800 | 3000 | 3200 | 3400 | SCAN |
| 30:00 | 33:20 | 36:40 | 40:00 | 43:20 | 46:40 | 50:00 | 53:20 | 56:40 | TIME |

SAMPLE M/14

Figure 9. Comparison of Unknown Spectrum
With Library Spectrum for
Succinic Acid Dimethyl Ester
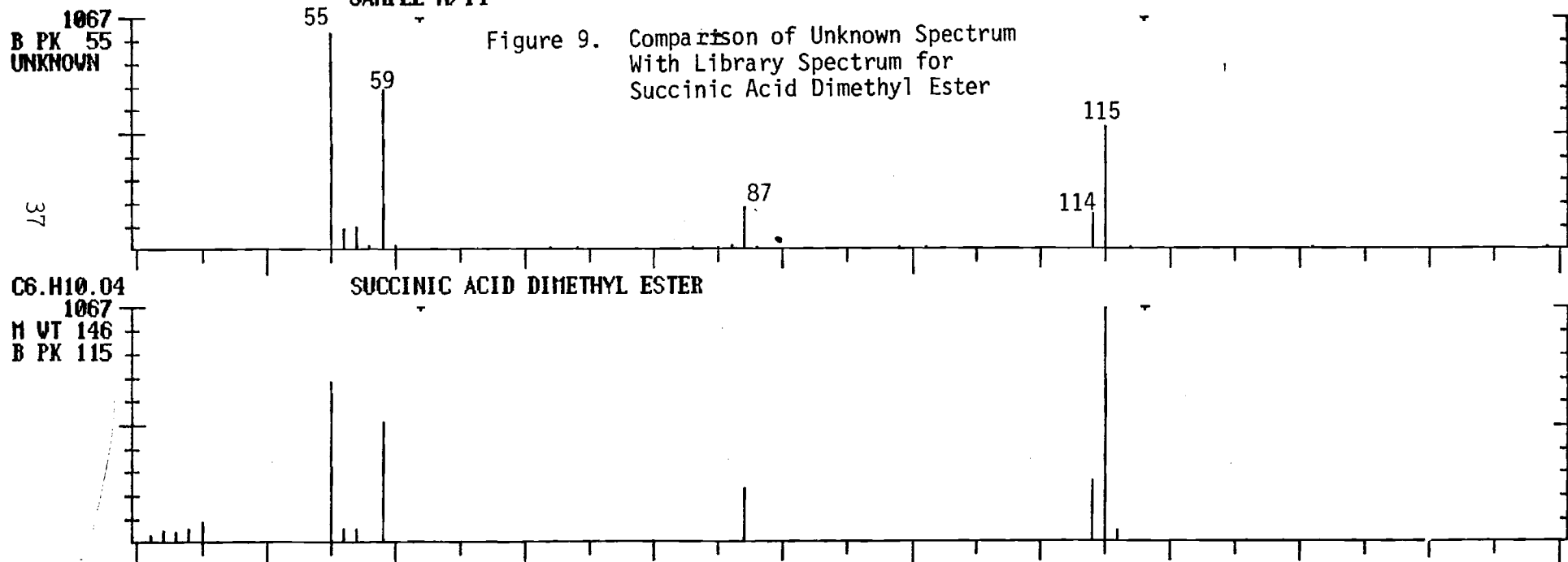
Figure 10. Fragmentation Sequence for Dimethyl Succinate.
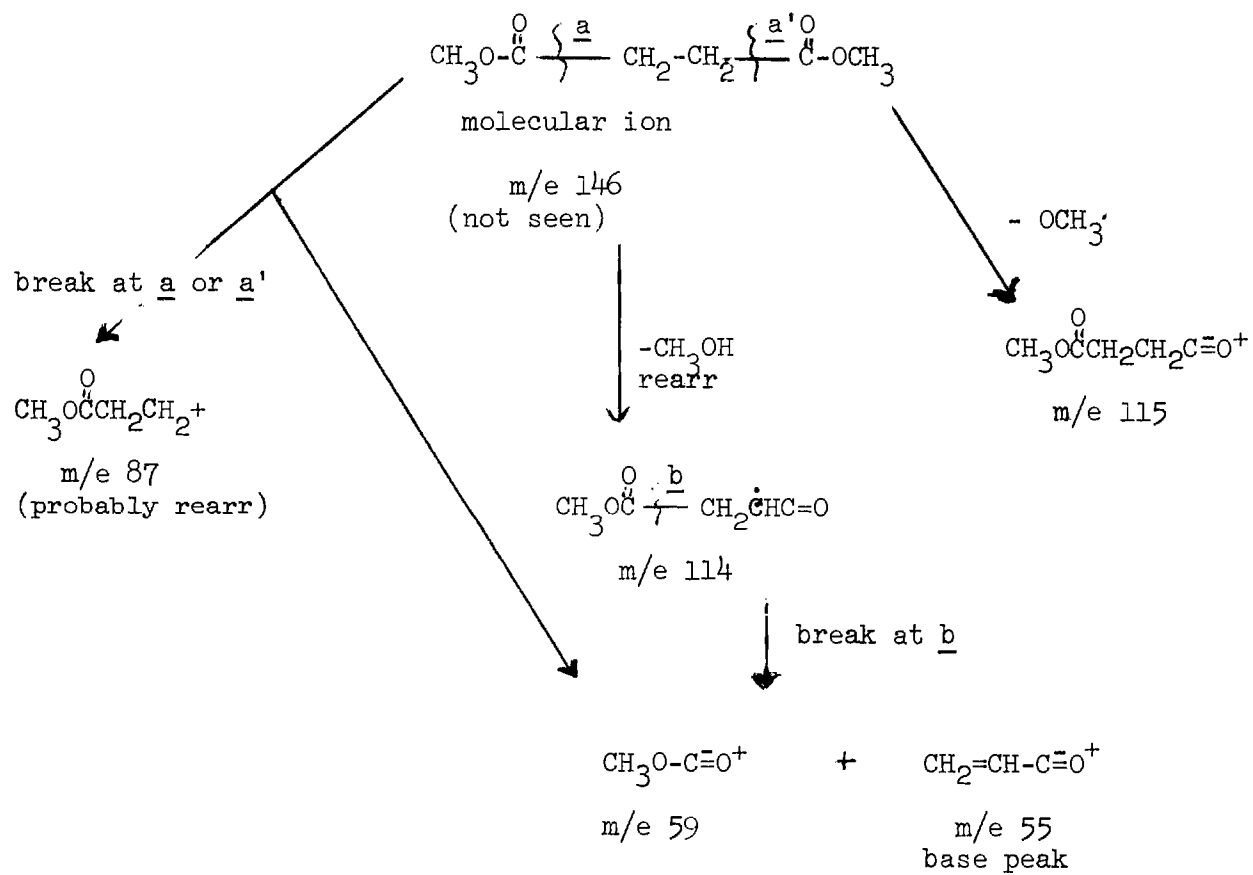
$$CH_3O-\overset{O}{\overset{\|}{C}} \overset{a}{)} CH_2-CH_2 \overset{a'}{(} \overset{O}{\overset{\|}{C}}-OCH_3$$

molecular ion

m/e 146
(not seen)

break at $\underline{a}$ or $\underline{a}'$

$$CH_3O\overset{O}{\overset{\|}{C}}CH_2CH_2{}^+$$

m/e 87
(probably rearr)

$-CH_3OH$
rearr

$$CH_3O\overset{O}{\overset{\|}{C}} \overset{b}{(} CH_2\overset{\cdot}{C}HC=O$$

m/e 114

break at $\underline{b}$

$- OCH_3\cdot$

$$CH_3O\overset{O}{\overset{\|}{C}}CH_2CH_2C\equiv O^+$$

m/e 115

$$CH_3O-C\equiv O^+ \qquad + \qquad CH_2=CH-C\equiv O^+$$

m/e 59   m/e 55
                     base peak

INTEN
10000.
1.

39

RIC

SCAN
TIME

500   1000   1500   2000
20:50   41:40   62:30   83:20
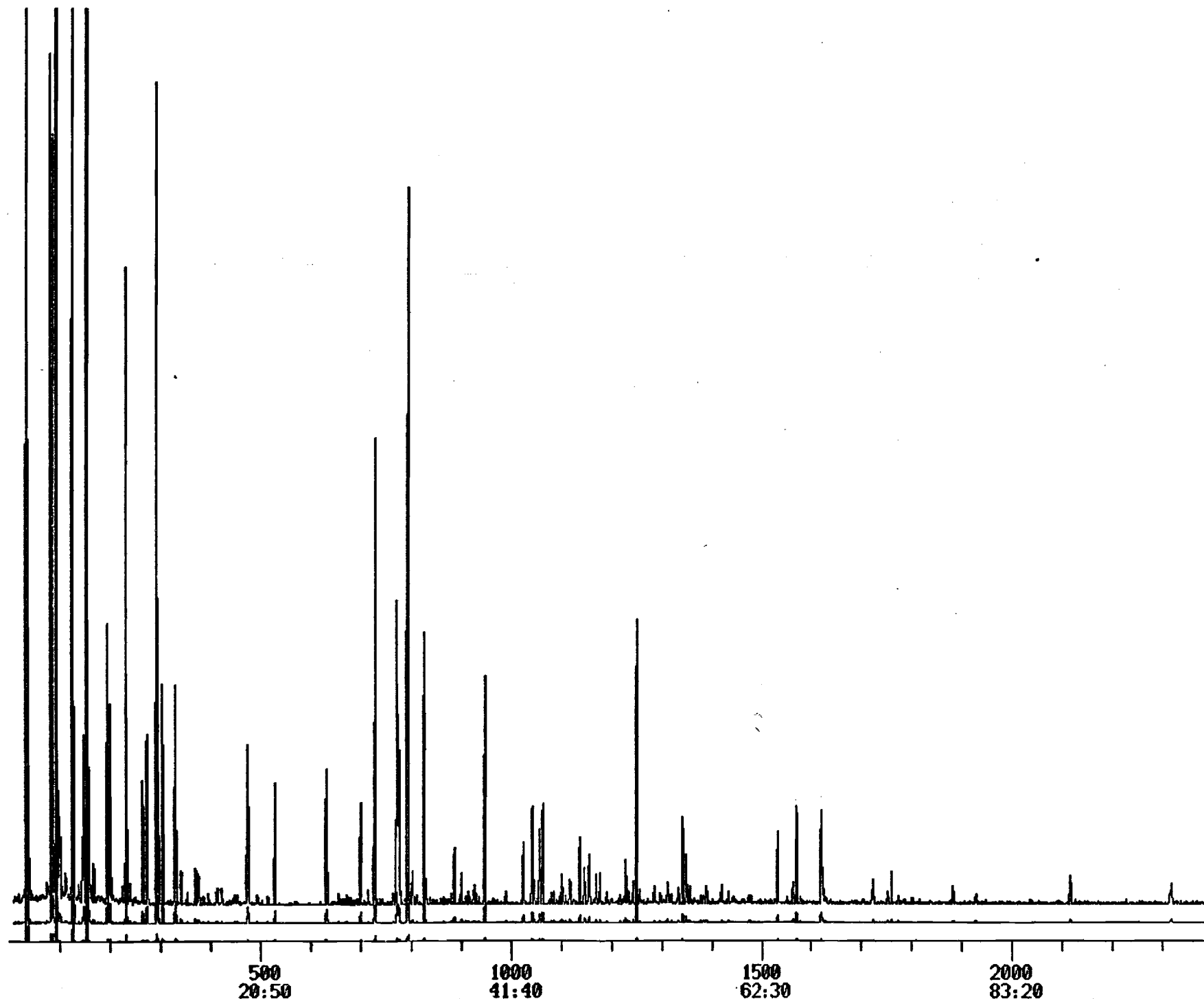
Figure 11. EI - Ion Chromatogram Methylated Humic Oxidation Products

RIC
03/16/78 16:23:00
SAMPLE: M/M/73 METHYLATED HUMIC OXIDATION

DATA: M73CI #1
CALI: 0316 #3

SCANS   1 TO 2400

INTEN
3000.
1.

RIC

40

500   1000   1500   2000   SCAN
20:50   41:40   62:30   83:20   TIME
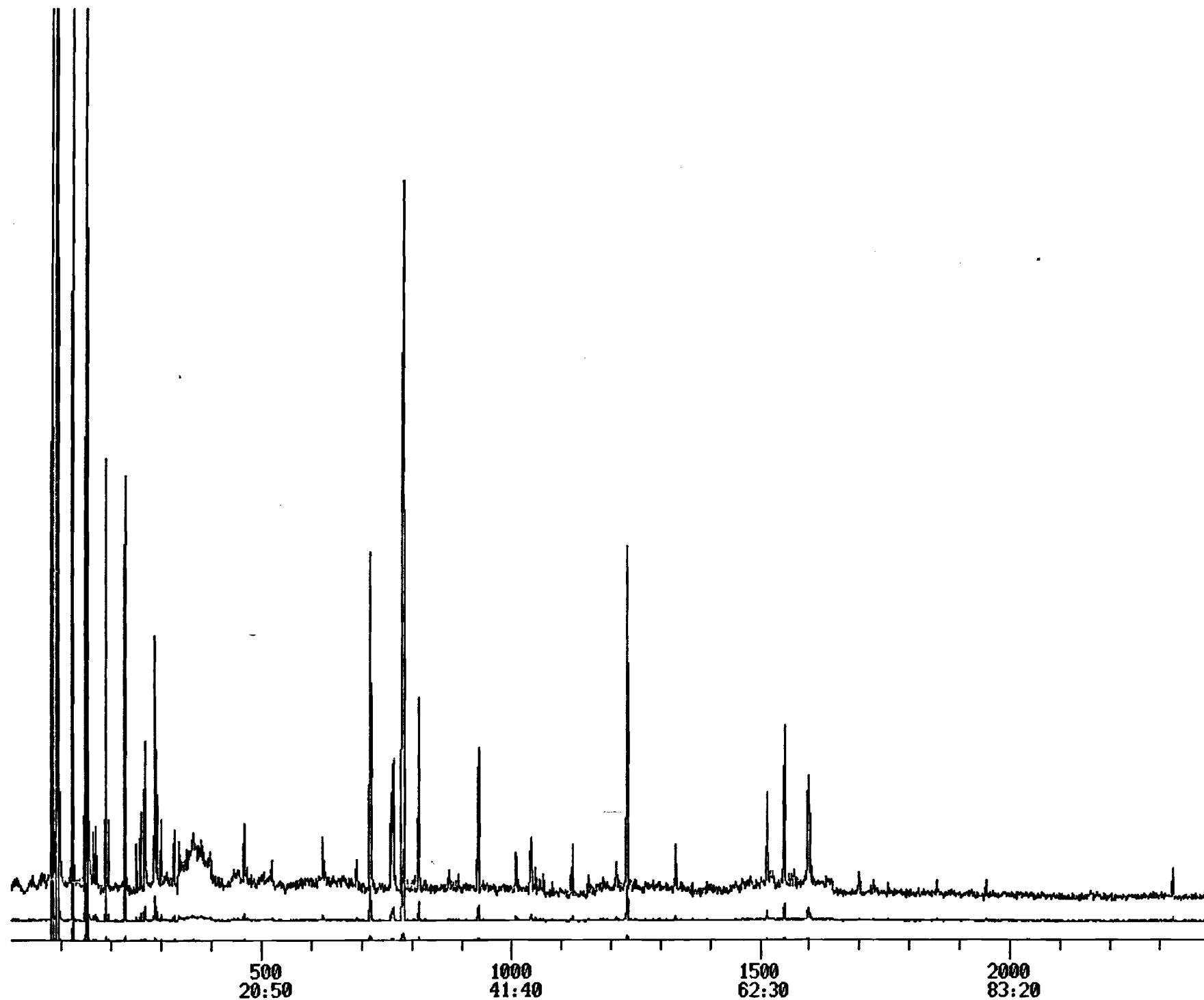
Figure 12   CI Ion Chromatogram Methylated Humic Oxidation Products

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.

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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 Dimethyloxaloace-tate	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
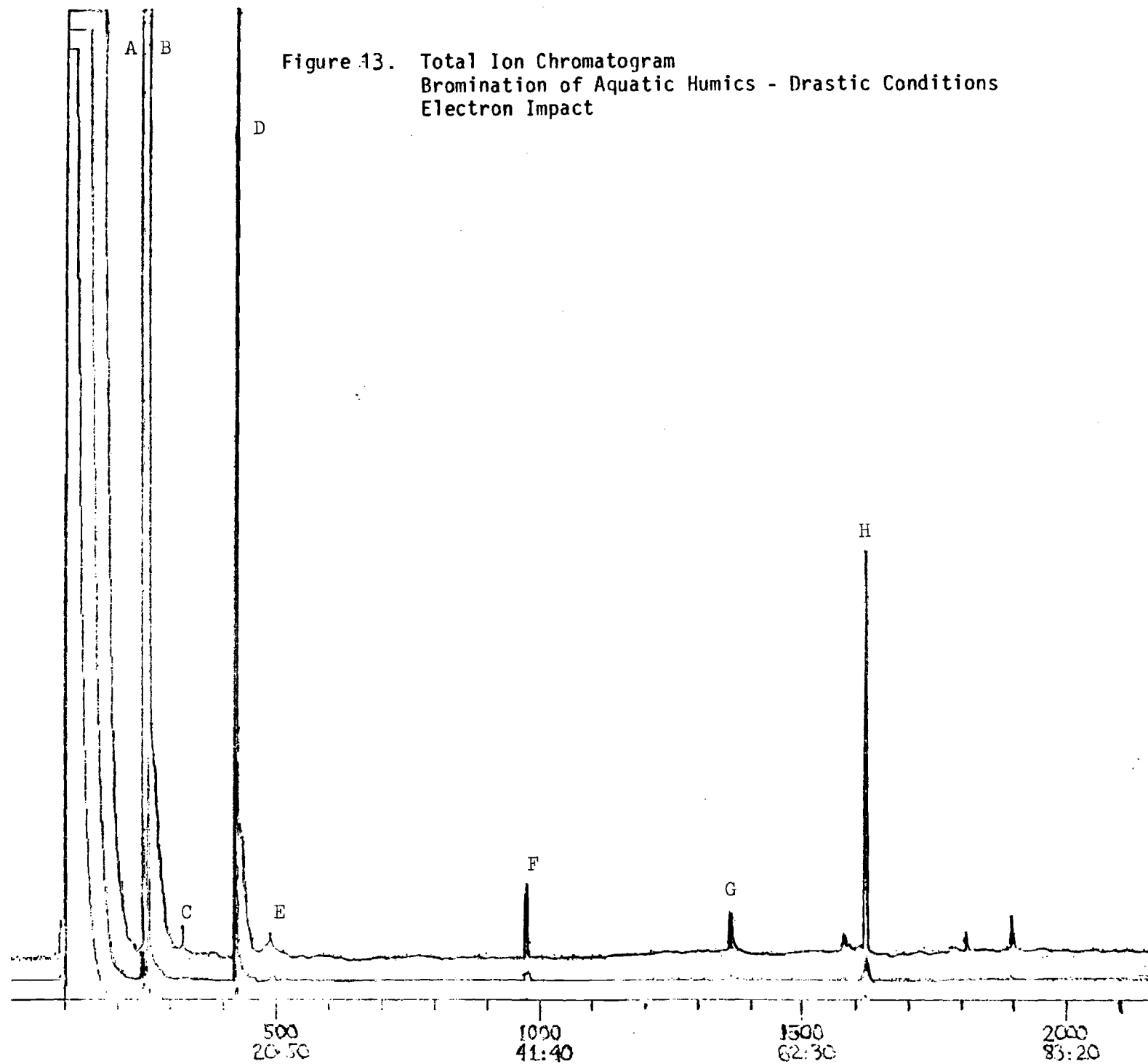
Figure 14. Total Ion Chromatogram
Bromination of Aquatic Humics - Drastic Conditions
Chemical Ionization
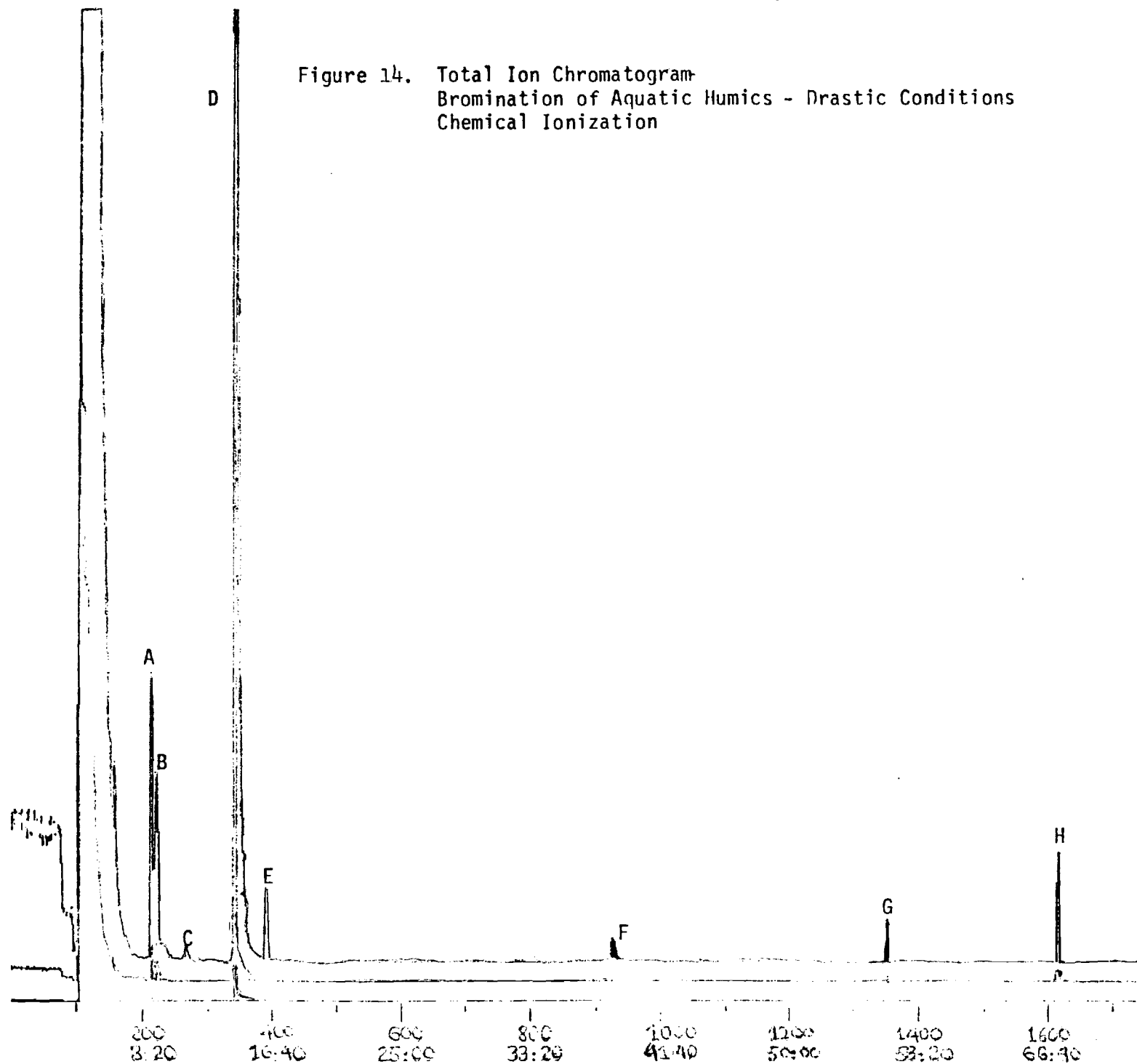
TABLE 7

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/1 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/1. 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

51

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

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

DATA: II11943D #1
CALI: 0507 #1

SCANS 200 TO 1000

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

INTEN
32309.
2.
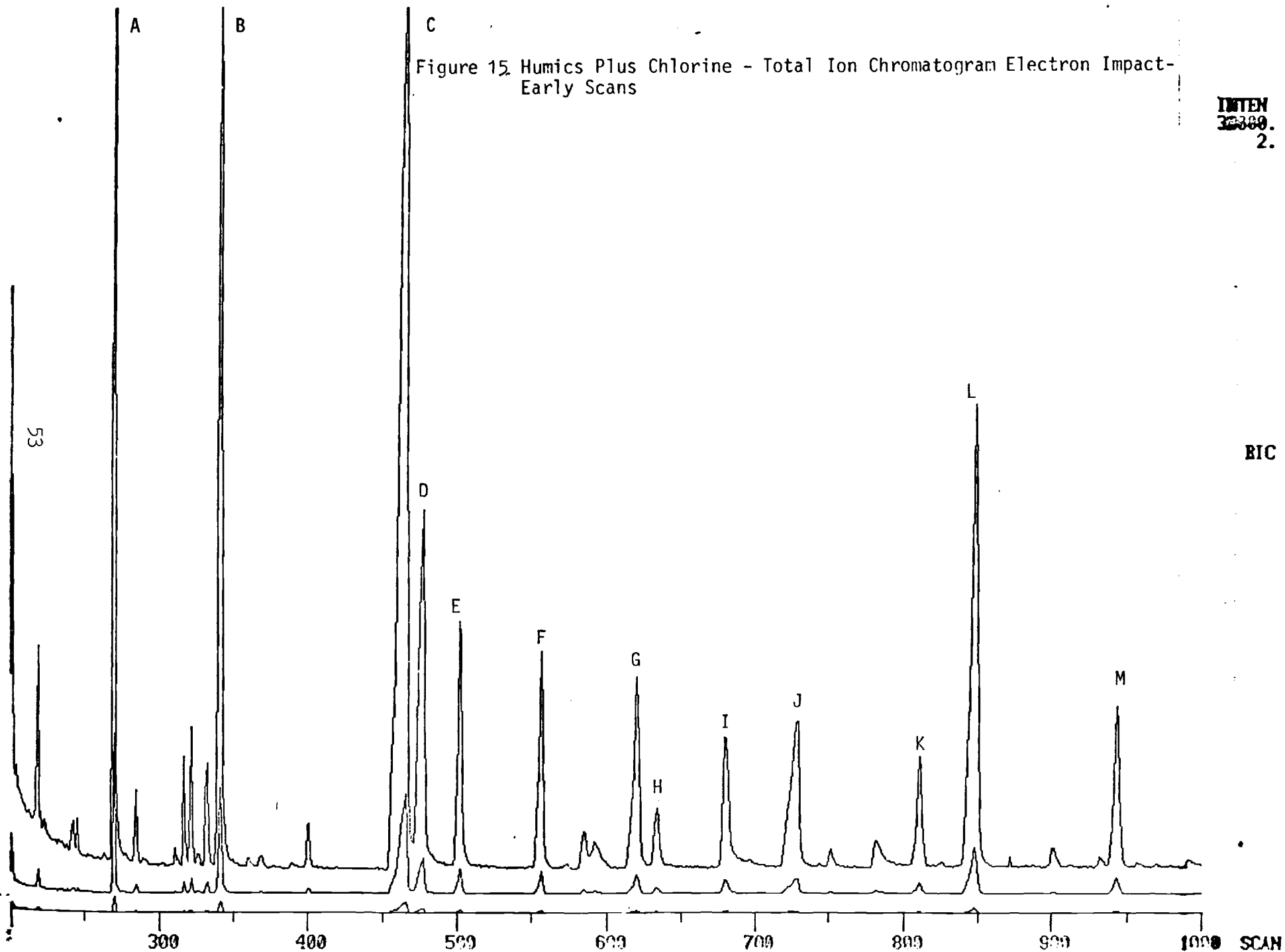
BIC

53

SCAN

Figure 16 Humics Plus Chlorine - Total Ion Chromatogram Chemical Ionization-Early Scans
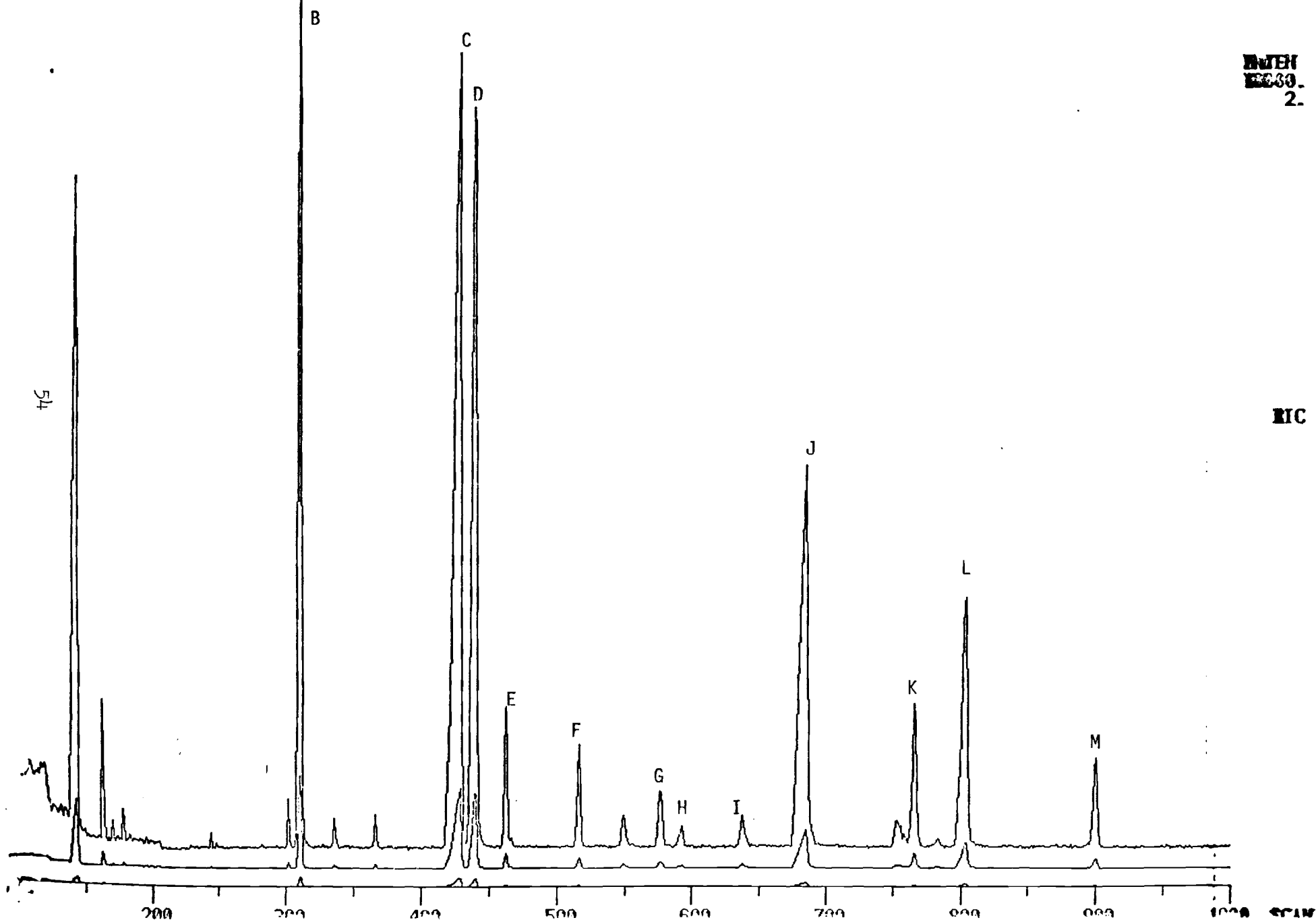
example, the monochloro alcohols (3-chloro-2-methyl-2-butanol, 1-chloro-2-methyl-2-butanol, 3-chloro-2-methyl-1-butanol and 4-chloro-3-methyl-1-butanol) all showed nearly identical chemical ionization (CI) spectra with major ions at m/e 87, m/e 105 and m/e 107 (see Figure 17 for a typical case) thus 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   ENHANCED (S 31B 2N 0T)   RIC: 599.
SAMPLE: HUMICS PLUS CHLORINE. CH4 CI
#517 TO #518 SUMMED - #516 X0.65

Figure 17
CHEMICAL IONIZATION SPECTRUM

1-CHLORO-2-METHYL-2-BUTANOL

Figure 18

ELECTRON IMPACT SPECTRUM

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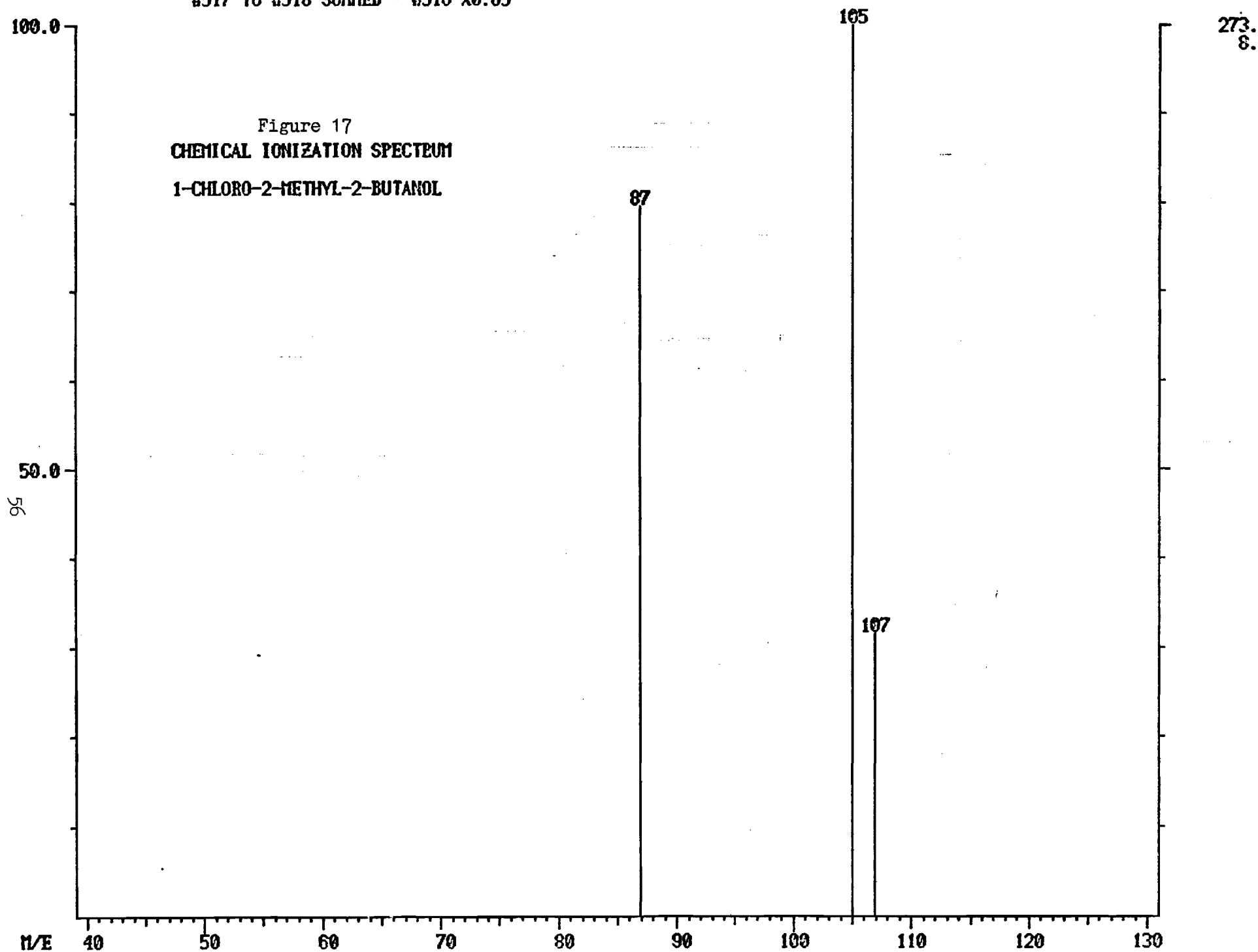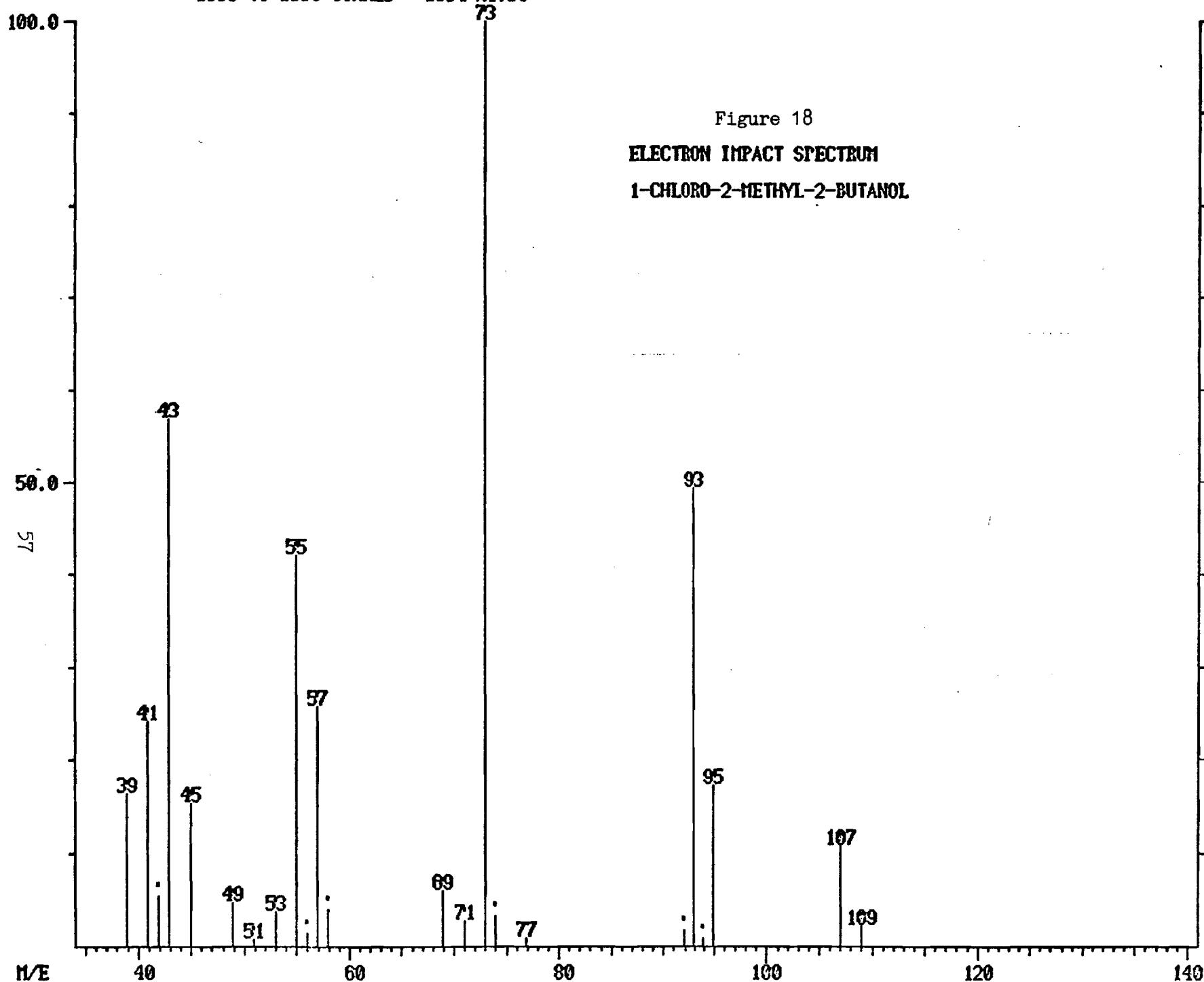
Figure 19. EI Fragmentation Sequence for 1-Chloro-2-methyl-2-butanol.

$$CH_3$$
$$C_2H_5-C \xrightarrow{a} CH_2Cl \qquad -C_2H_5 \qquad -CH_3$$
$$OH$$

molecular ion

m/e 122
(not seen)

break at a

$C_2H_5\overset{+}{\underset{OH}{C}}CH_3$ + $CH_2\overset{..}{-}Cl$

m/e 73 · · · · m/e 49,51

-HCl

$C_5H_{10}O^+$

m/e 86

/ - OH

$C_5H_9^+$

m/e 69

$$CH_3\overset{+}{-}C - CH_2Cl$$
$$OH$$

m/e 93,95

- HCl

$C_3H_5O^+$

m/e 57

- C_2H_4

$C_2H_3O^+$

m/e 43

- H_2O

$C_4H_7^+$

m/e 55

$C_3H_5^+$

m/e 41

$-C_2H_4$
rearr $C_2H_5O^+$

m/e 45

$$C_2H_5\overset{+}{-}C-CH_2Cl$$
$$OH$$

m/e 107,109

- HCl

$C_4H_7O^+$

m/e 71

- CO

$C_3H_7^+$

m/e 43
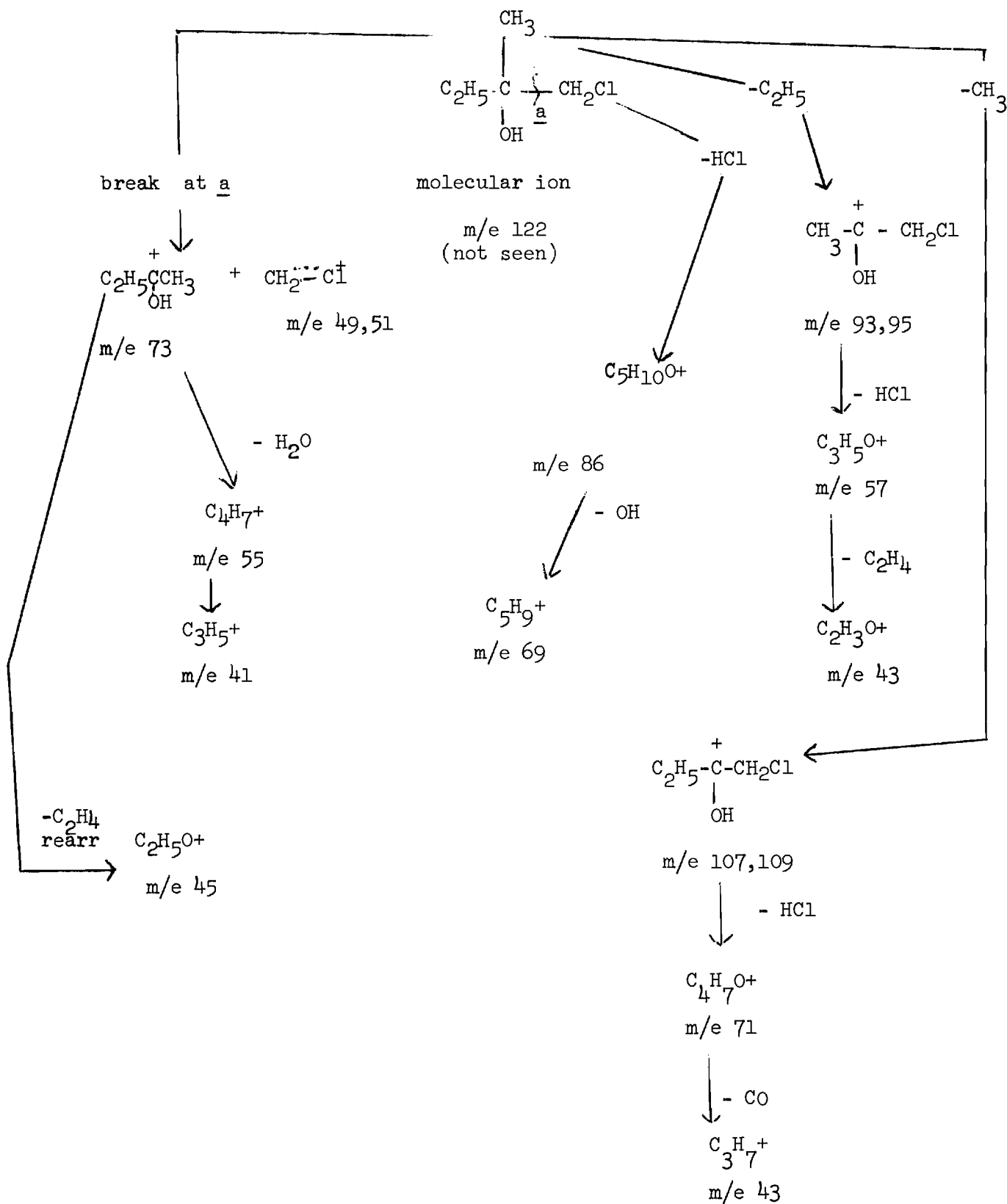
TABLE 8

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
06/09/79 18:46:00 + 15:52
SAMPLE: PENTAFLUOROBROMOBENZENE
309 TO # 310 SUMMED

DATA: BROM960979 # 309   BASE M/E: 117
CALI: Z063979 #   1   RIC: 240263.

1000

SAMPLE

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

06.BR.F5
BENZENE, BROMOPENTAFLUORO-
1000
H WT 246
B PK 117
LANK   1
IH 2030
PUR 925
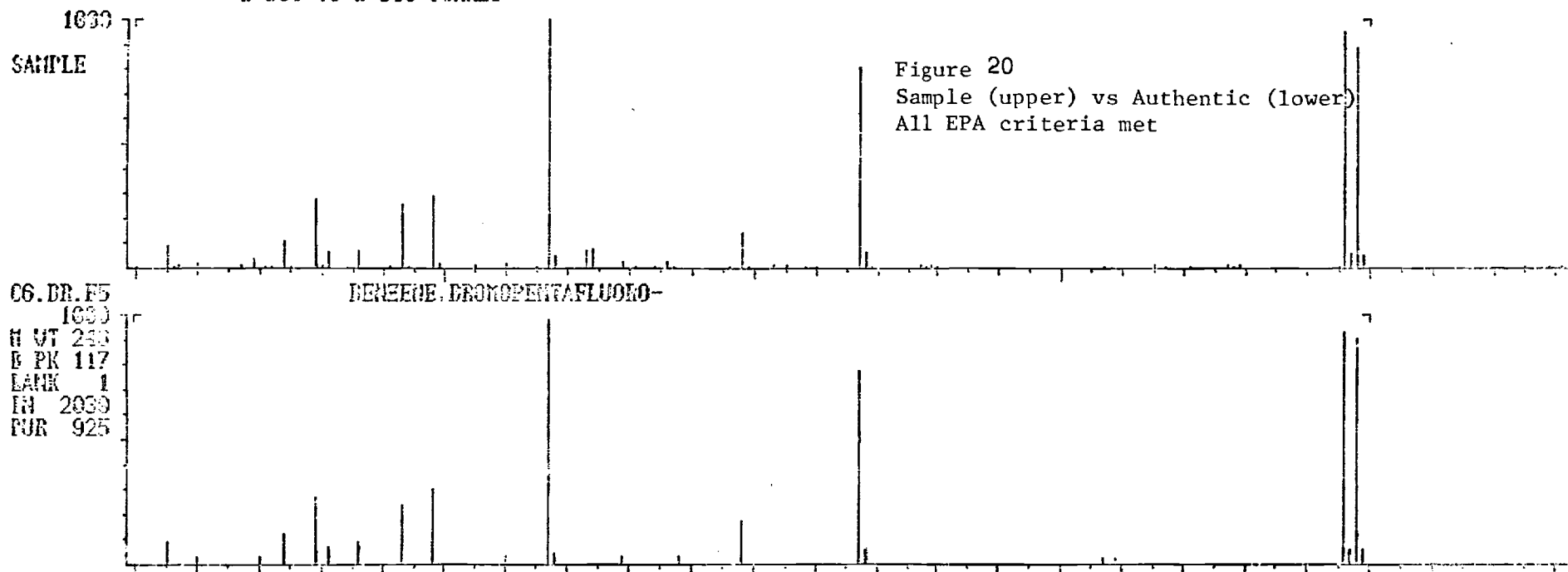
is compared with the EPA performance criteria in Table 9.

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

61

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: TTY1943DCTS #1137   BASE M/E: 121
ENHANCED (S 31B 2N 0T)   RIC: 1230.

Figure 21
CHEMICAL IONIZATION SPECTRUM
1,3-DICHLORO-2-METHYL-2-BUTANOL

63

5
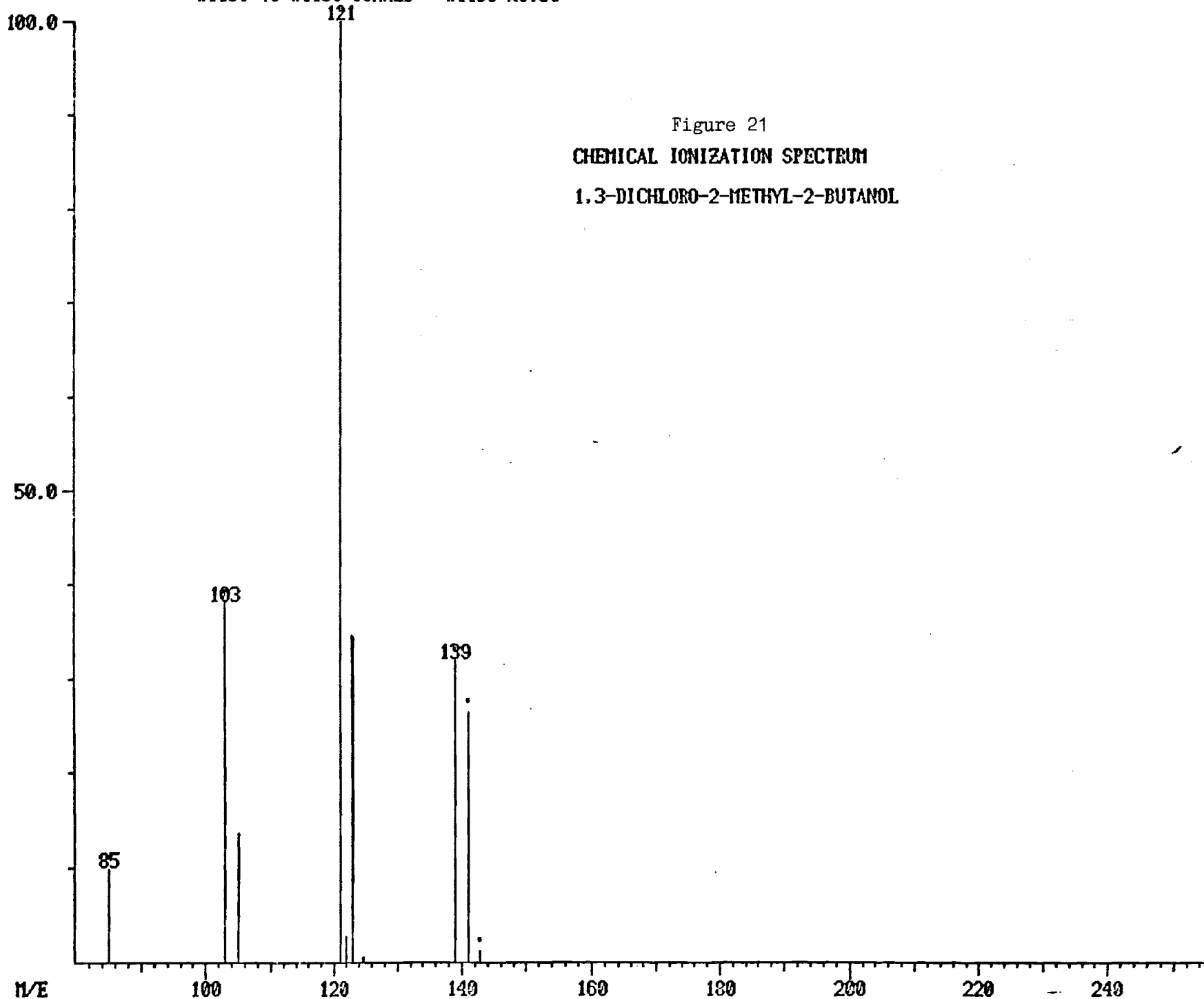
cluster at m/e 139, 141, 143. An analagous attack on the Cl function re-
sulting in the loss of HCl accounts for the base peak at m/e 121, 123. These
ions in turn can lose HCl and/or water to provide ions at m/e 103, 105 and
m/e 85. This information is summarized in the fragmentation sequence outlined
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 chloro-
methyl 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 rela-
tive 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)

quasi-molecular ion
(not seen)

m/e 121,123   m/e 157   m/e 139,141,143

−H₂O

−HCl

m/e 103,105

m/e 85
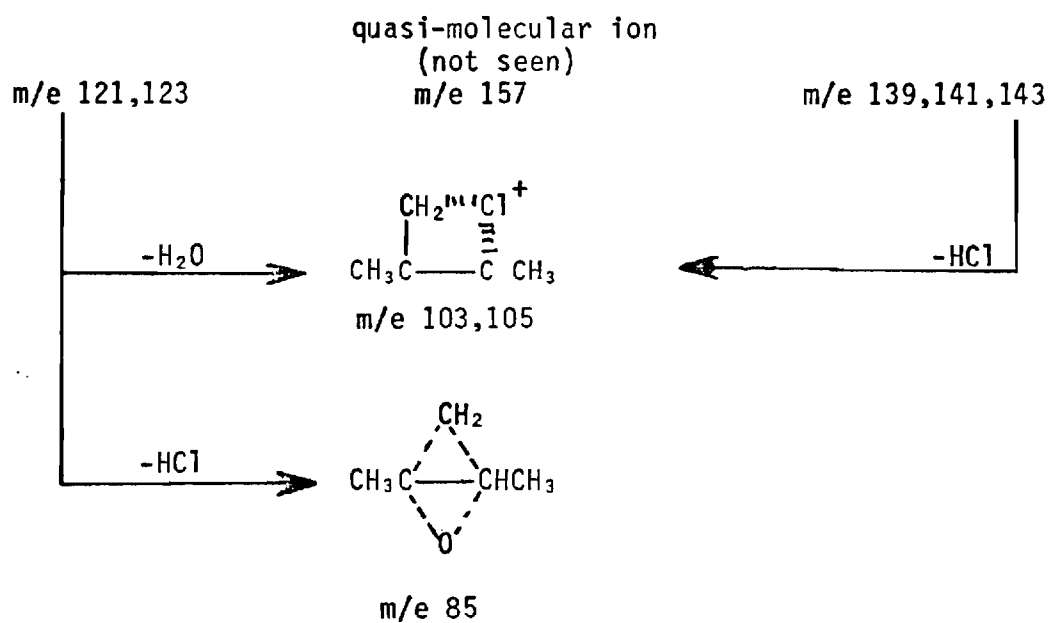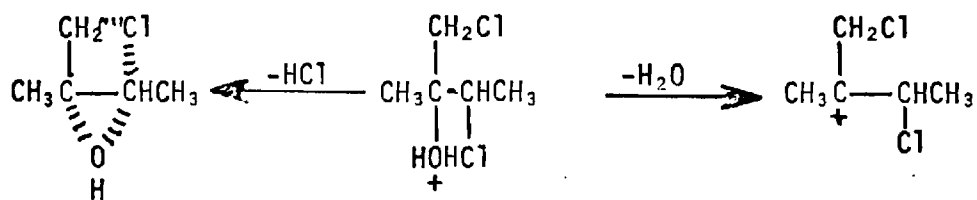
MASS SPECTRUM
05/08/78 15:29:00 + 29:30
SAMPLE: M/30 HUMICS PLUS CHLORINE, EI
#1180 - #1183 X1.00

DATA: II11943D #1180   BASE M/E: 43
CALI: 0507 #1   RIC: 5096.

50.0X

Figure 23

ELECTRON IMPACT SPECTRUM

COMPOUND Q

1.3-DICHLORO-2-METHYL-2-BUTANOL

Figure 23 — Electron impact spectrum of Compound Q, 1,3-Dichloro-2-methyl-2-butanol
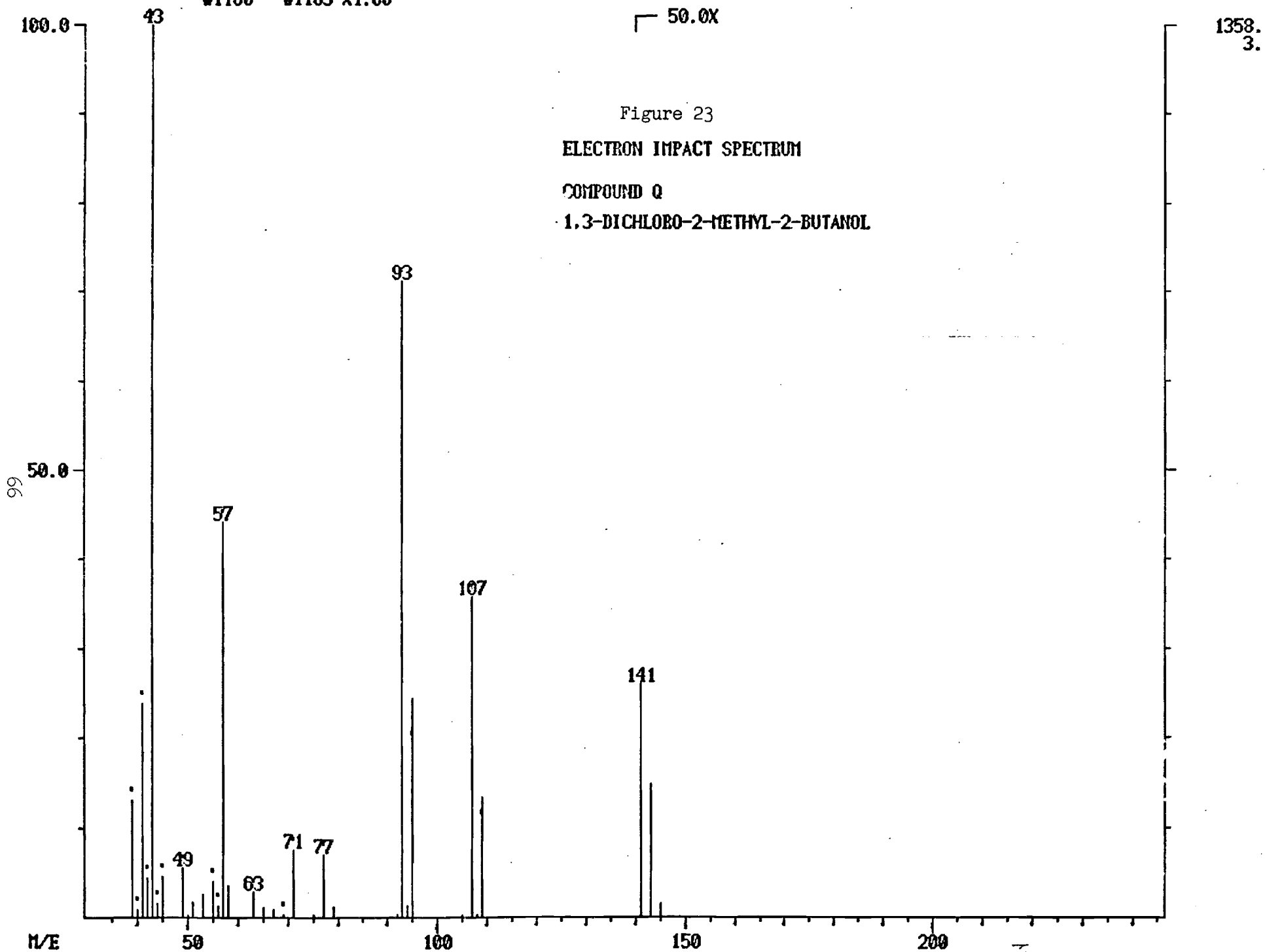
FIGURE 24

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

$$CH_2Cl-C(=\overset{+}{O}H)-CHCH_3(Cl) \xleftarrow{\text{break at a or a'}} CH_3-C(CH_2Cl)(HO)-CH(Cl)-CH_3 \xrightarrow{\text{break at b}} CH_3C(\overset{+}{H}O)-CH(Cl)-CH_3 + CH_2\overset{+}{Cl}$$
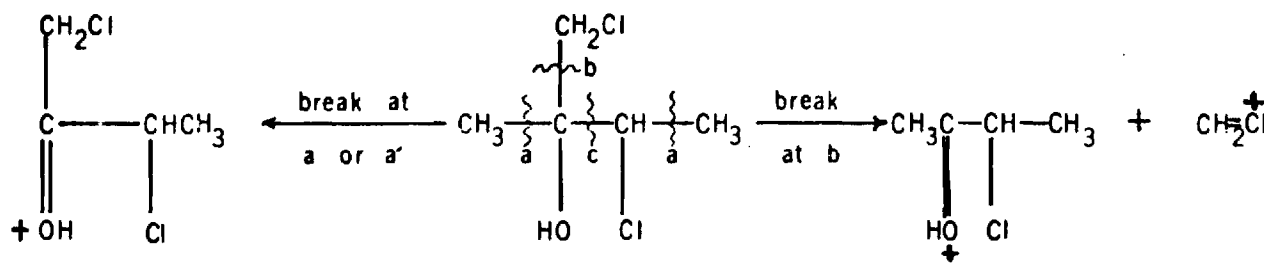
m/e 141, 143, 145

molecular ion

m/e   156, 158, 160

(not seen)

m/e 107, 109   m/e 49, 51

break | at c.

−HCl

$$\overset{+}{C}HClCH_3 \quad + \quad CH_3C(CH_2Cl)=\overset{+}{O}H \qquad CH_3C(=O,+H)-CH=CH_2$$
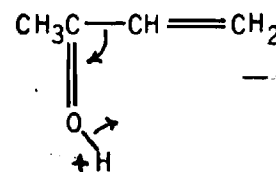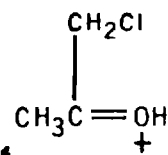
m/e 63, 65

m/e 93, 95

m/e 71

−HCl

−C₂H₄

$$C_3H_5\overset{+}{O} \longrightarrow CH_3\overset{+}{C}\equiv O$$

m/e 57   m/e 43

−H₂O

−H₂O

$$C_3H_3^+ \longleftarrow C_4H_5^+$$

m/e 39   m/e 53
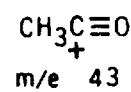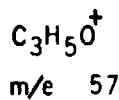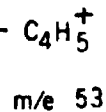
olefin or by means of some other common precursor. A possible sequence
of events is outlined in Figure 25. A more detailed discussion of the possible
overall sources for the chlorinated isoprenoids follows the discussion of
the products resulting from the chlorination of mature aquatic humics.

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 repre-
sent 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.

isoprene
(common in air)[14]

H^+

more stable, less reactive

more reactive, less stable

H_2O, $- H^+$

H_2O, H^+

Cl^- (from HCl)

HCl

2-methyl-3-butene-2-ol
(found in precipitation
and surface waters)

3-chloro-3-methyl-2-butanol
(dominant monochloro alcohol)

3-chloro-2-methyl-
1-butene
(dominant chlorinated
hydrocarbon, also
found by Glaze)[18]

Cl^+ (from Cl_2), H_2O

1,3-dichloro-2-methyl-2-butanol
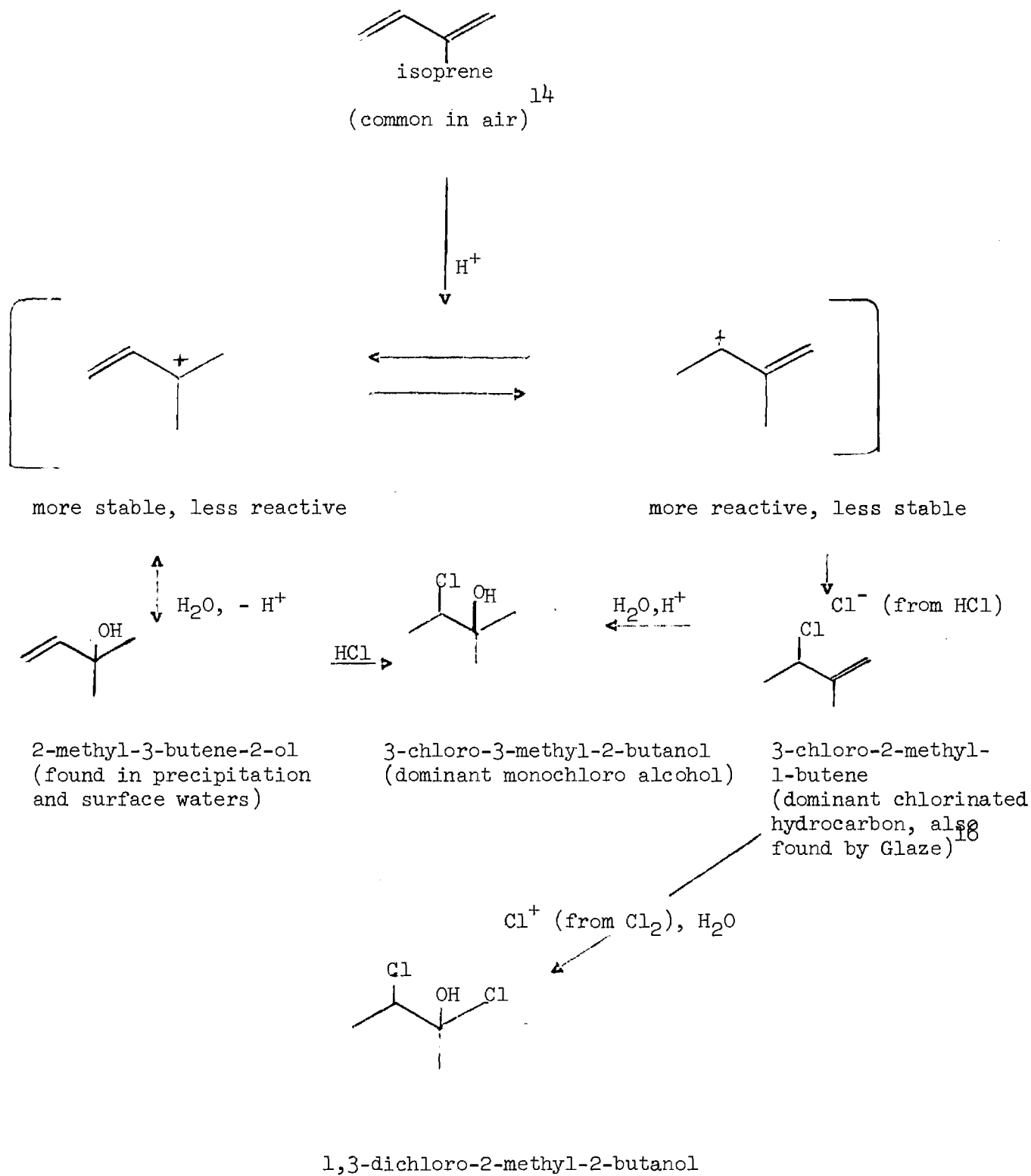
MASS SPECTRUM
05/04/78 14:57:00 + 6:19
SAMPLE: HUMICS PLUS CHLORINE. CH4 CI

DATA: II11943CCI #253
CALI: C594 #1

BASE M/E: 69
RIC: 4432.

100.0

68.9

3852.

Figure 26.   Humics Plus Chlorine: 2-methyl-3-chloro-1-butene.
Chemical Ionization (CH$_4$)

50.0

70

105.0

M/E   50   60   70   80   90   100   110   120   130   140   150

Figure 27   Humics Plus Chlorine:
2-methyl-3-chloro-1-butene.
Electron Impact
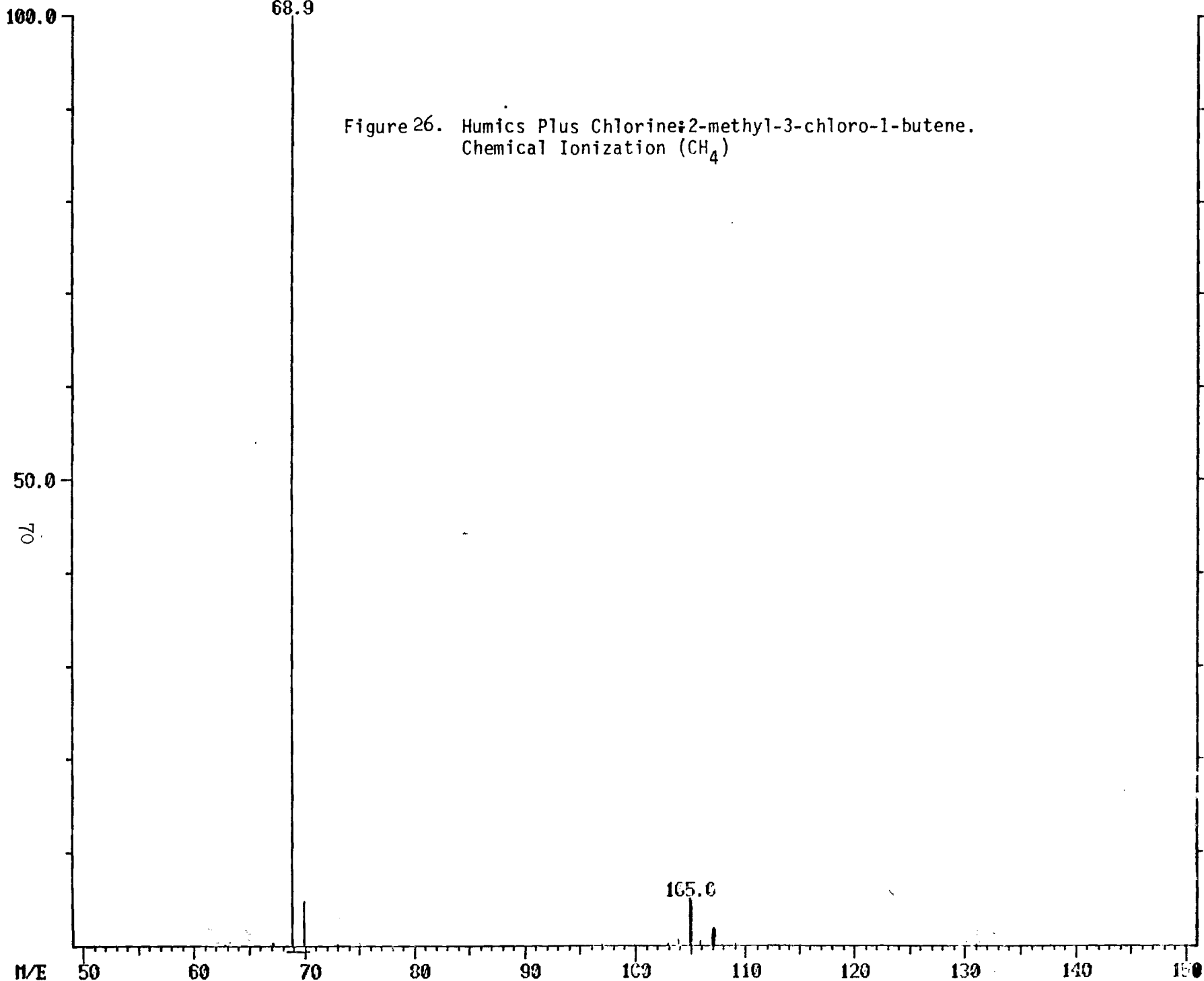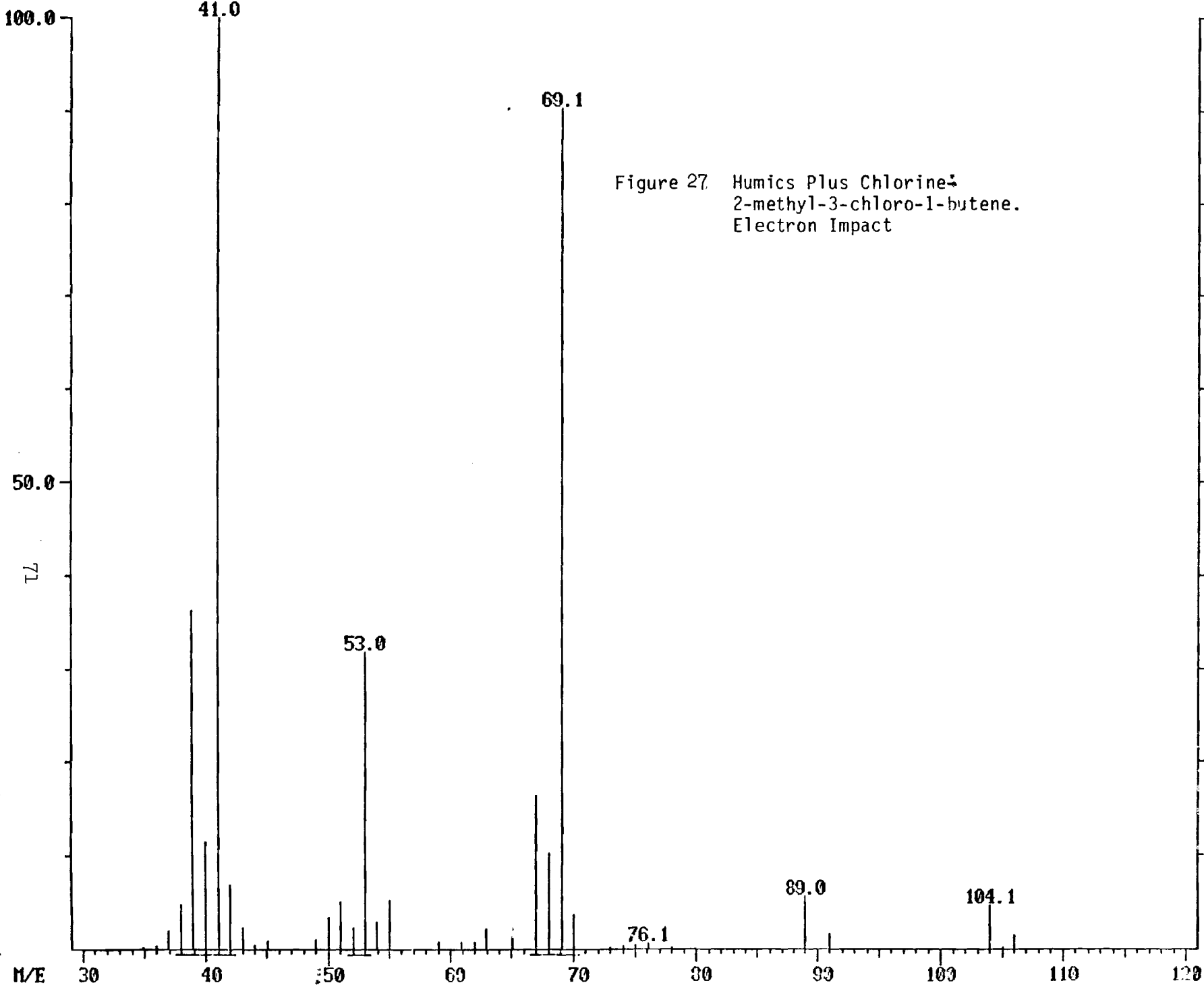
Figure 28

EI and CI Fragmentation Sequence for 3-Chloro-2-methyl-1-butene
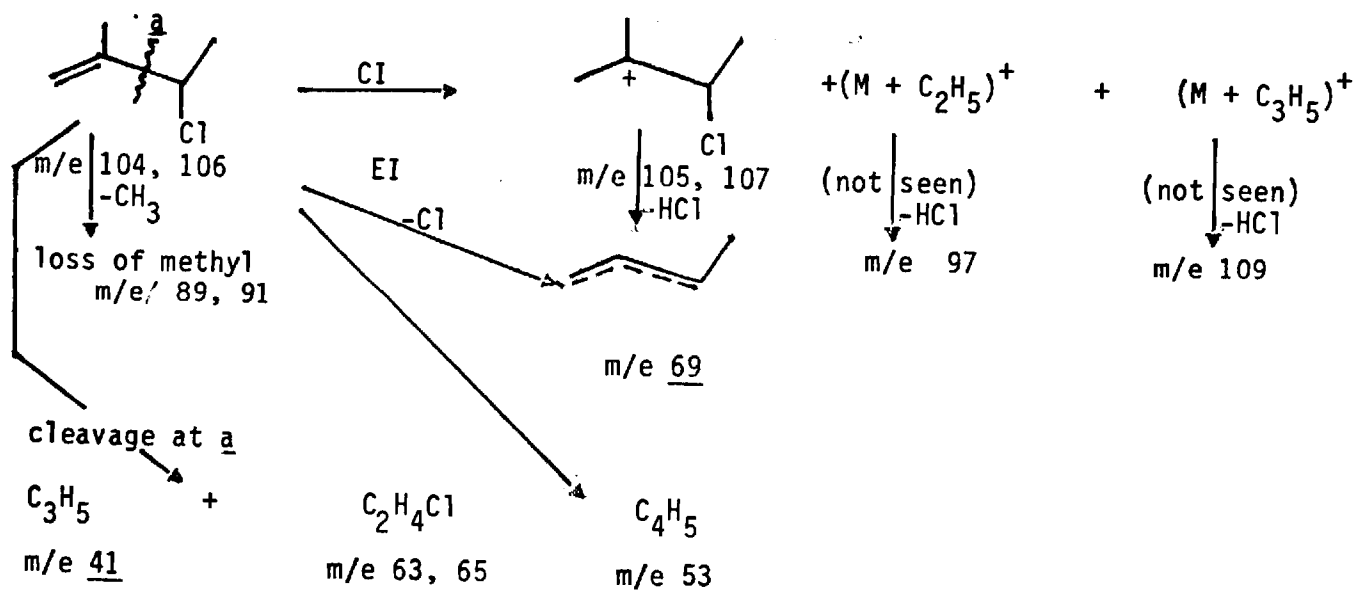
chlorine. The major chlorine-containing products are dichloracetic and trichloroacetic acids. Some aromatic compounds and several organic acids have been assigned. Some aromatic products were noted suggesting a closer link to the oxidative degradation work. A complete listing of the products identified 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.

74

RIC
05/14/79 21:16:00
DATA: SC051079 #1
CALI: Z051479 #16
SCANS   1 TO 2800
SAMPLE: PH 1.5 ET20 EXTRACT PLUS DIAZOMETHANE SETTLING CHAMBER HUMICS PL

Figure 29   Total Ion Chromatogram, Aquatic Humics Plus Chlorine,
1 Day, Experiment 2.
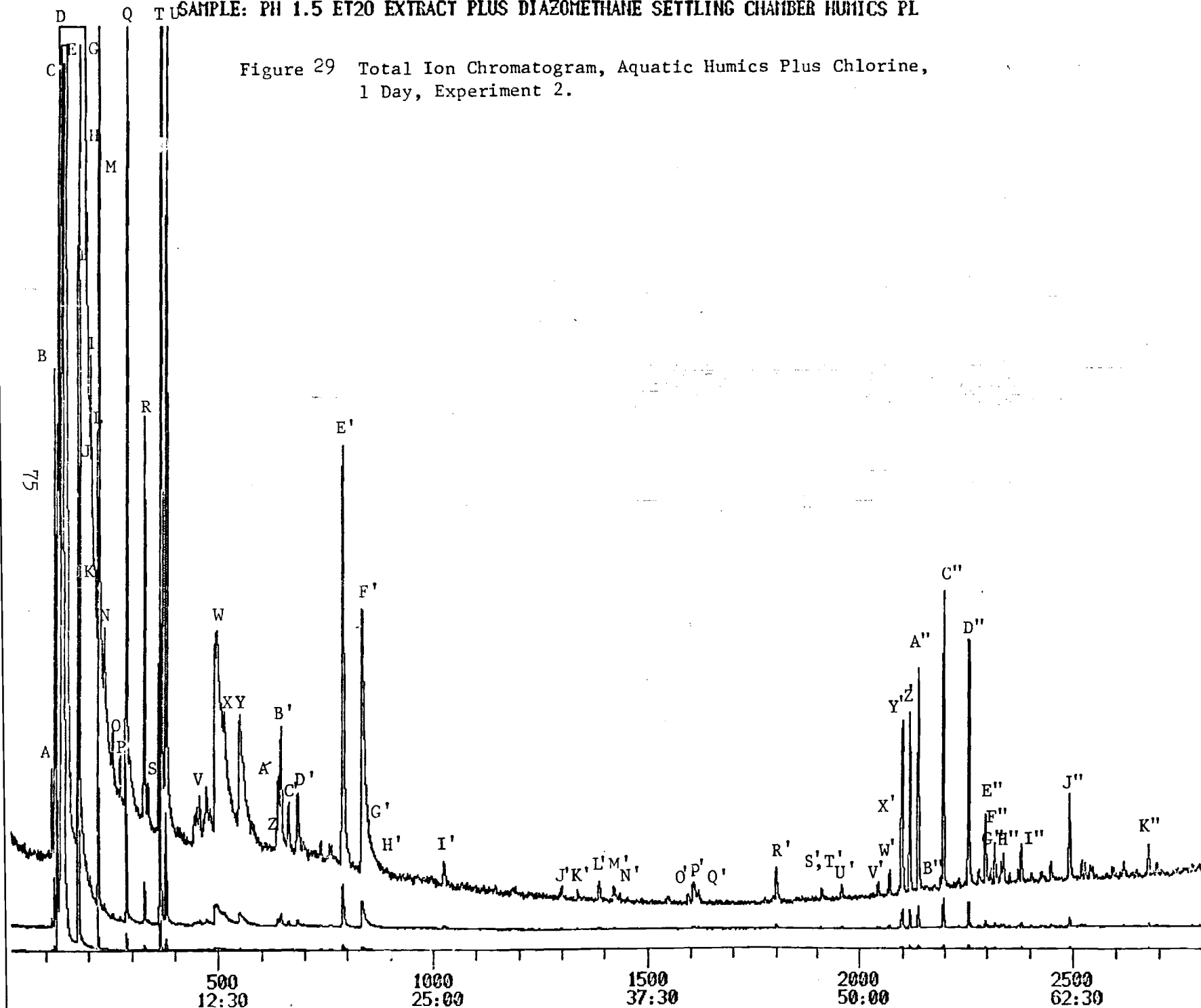
MASS SPECTRUM
05/13/79 2:10:00 + 11:07
SAMPLE: ET20 EXTRACT PH1.5 HUMICS PLUS CHLORINE
  #445 - #440 X1.00

DATA: EFF0508792 #445   BASE M/E: 59
CALI: Z051279 #25   RIC: 45952.

Figure 30

METHYL DICHLOROACETATE

FIT   981
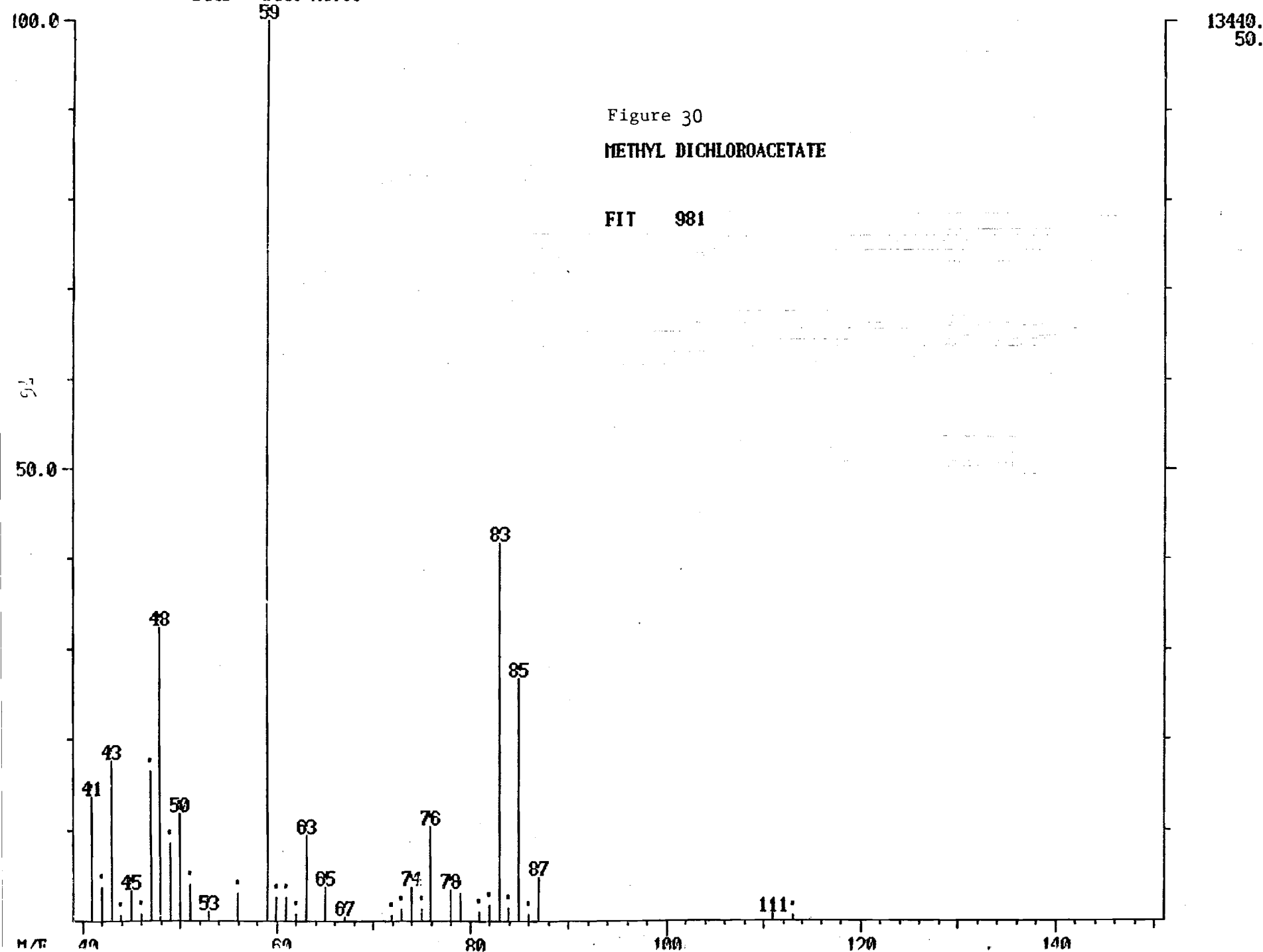
Figure 31
Fragmentation Sequence – Methyl Dichloroacetate

$$CHCl_2^+ \quad + \quad O{\equiv}C{-}OCH_3^+ \quad \xleftarrow{\hspace{2cm}} \quad CHCl_2\overset{\overset{O}{\|}}{C}OCH_3 \quad \xrightarrow{-OCH_3^{\bullet}\hspace{1cm}} \quad CHCl_2C{\equiv}\overset{+}{O}$$

m/e 83, 85, 87   m/e 59   Molecular ion   m/e 111, 113, 115*
                          m/e 142, 144, 146
                          (not seen)

$\downarrow$ -Cl· $\searrow$ -HCl $\searrow$ rearrange

$CHCl_2^+$ CCl^+ $ClC^+{\equiv}O$ + $\overset{Cl}{\underset{H}{>}}C{=}O - CH_3^+$

m/e 48,50   m/e 47,49   m/e 63,65   m/e 79 81
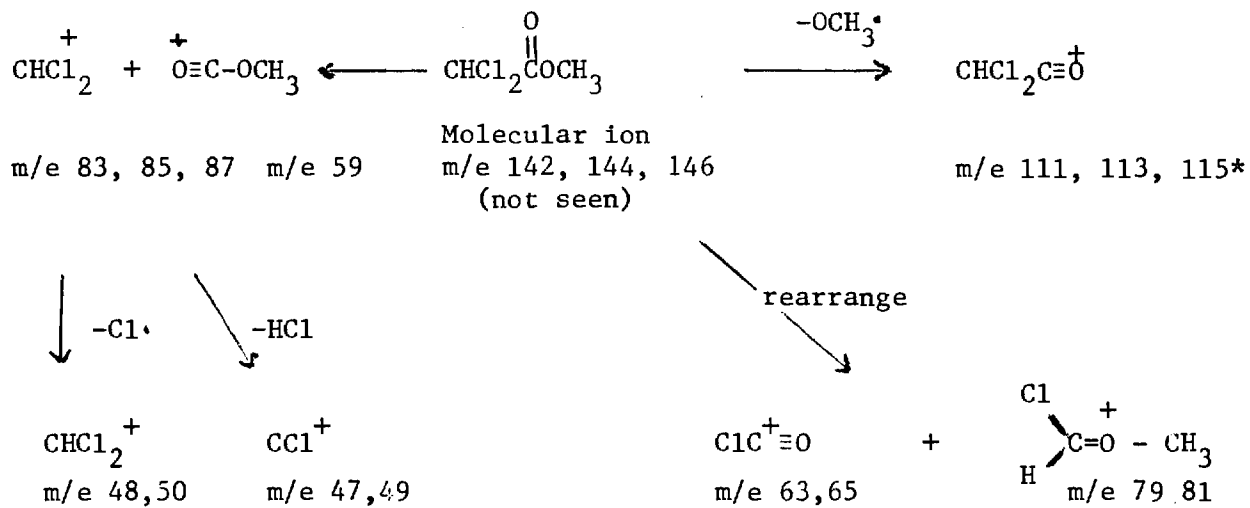
All of the ions shown with the exception of the molecular ion are present in the reference spectrum. A mechanism leading to the minor rearrangement ions at m/e 63, 65 and m/e 79,81 is difficult to postulate.

*Not seen because of low intensity

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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

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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 in-
crease 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

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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 chloro-alcohols 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 con-
centrations 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 per-
form 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

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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 $\mu g/L$	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

Percent Chlorine Uptake; Solid
TOCl; Dashed

$87\bar{M}_N$
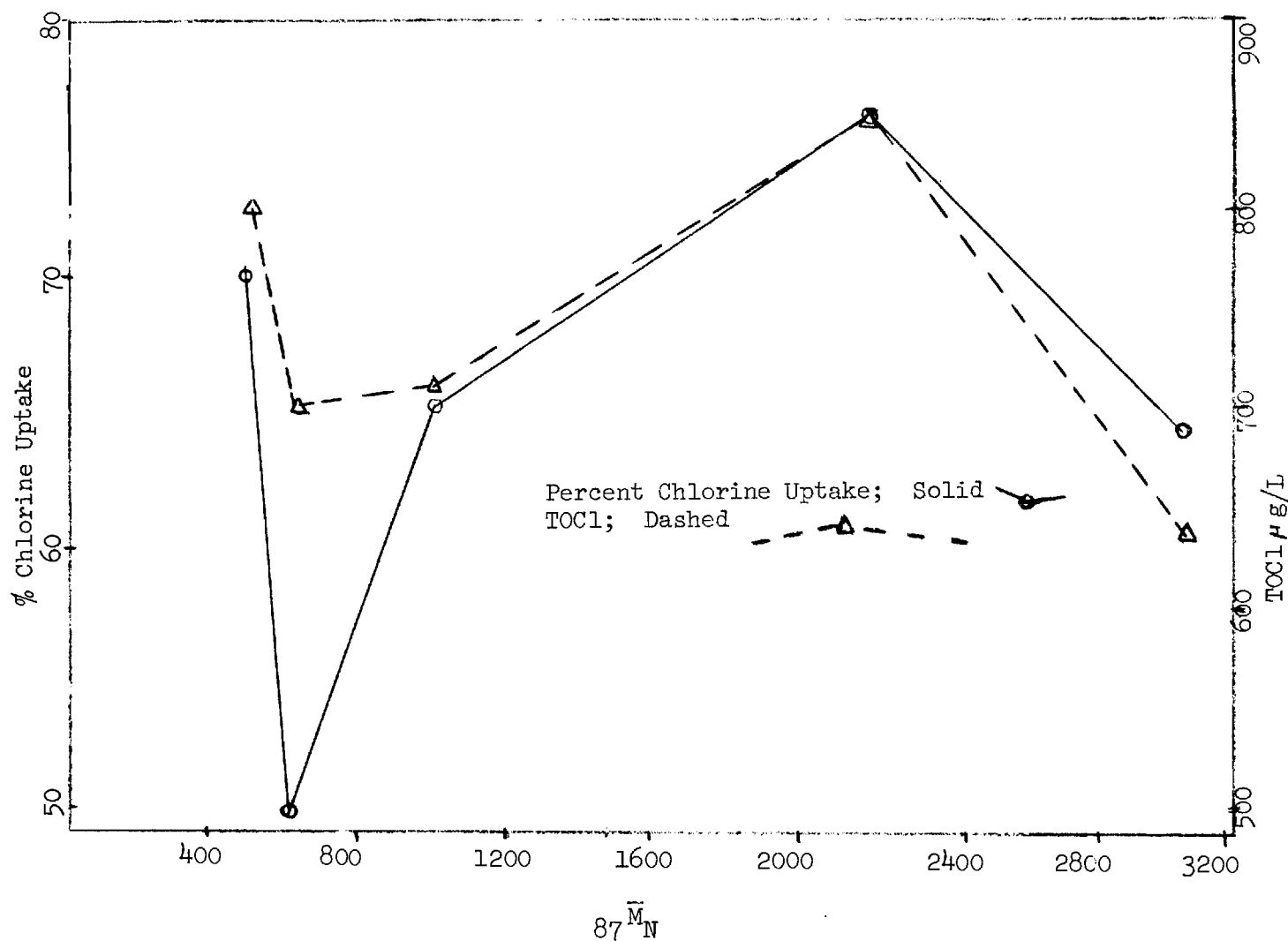
FIGURE 33.   SEPARATION-CONCENTRATON SCHEME

```
                          ┌──────────────────────┐
                          │     WATER SAMPLE      │
                          └──────────────────────┘
                             │
         Measure TOC         │    Filter TOC = 50.8
     ┌───────────────────────┴──────────────┐
┌─────────────────────┐            ┌─────────────────────┐
│ PARTICULATE ORGANIC │            │  DISSOLVED ORGANIC  │
│       CARBON        │            │       CARBON        │
└─────────────────────┘            └─────────────────────┘
                                         │
 Not part of this work        Measure TOC,    │  Closed Circuit Stripping TOC = 50.0
                                              │
    ┌─────────────────────────────────────────┴──────┐
┌─────────────────────┐            ┌──────────────────────────┐
│    VOLATILES AND    │            │  NON-VOLATILE ORGANIC    │   Direct
│    SEMI VOLATILES   │            │        CARBON            │   Lyophilization
└─────────────────────┘            └──────────────────────────┘
```

Quantify, separate by   Recyle ──────   Measure TOC, sequential   XAD-8 TOC= 51.1
appropriate means   SAC columns XAD-8 - XAD-2   XAD-2 TOC= 48.7
(Figue 4)

                                                              Backflush column
       Adjust to pH2   with o.1 N HCl
       (HCl), measure TOC

                                                ┌──────────────────────┐
       TOC = 44.2   │   HYDROPHOBIC BASE   │
                                                └──────────────────────┘
       pH 2
                                                   Quantify, separate by
                                                   appropriate means

                        Measure │ TOC   XAD-8 TOC 27.4
                                        XAD-2 TOC 22.0

                                                      Backflush column
                                                      with 0.1 N NaOH

                                                ┌──────────────────────┐
                                                │   HYDROPHOBIC ACID   │
                                                └──────────────────────┘

                                                   Quantify, separate by
                                                   appropriate means

                          ┌──────────────────────────────┐
                          │   HYDROPHILIC FRACTION   │
                          └──────────────────────────────┘

            Measure TOC   A G MP-50 (H$^+$ form)   TOC = 19.8

                                                      Backflush column
                                                      with 0.1 N NaOH
                                      ┌──────────────────────┐
                                      │   HYDROPHILIC BASE   │
                                      └──────────────────────┘

                                         Quantify, separate by
                                         appropriate means
                     A G MP-1 (OH$^-$ from)   TOC = 6.7
┌─────────────────────┐   Measure TOC,   Backflush column
│ HYDROPHILIC ACID   │   Lyophilize if is any   with 0.1 N HCl
└─────────────────────┘
Quantify, separate by
appropriate means
```

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 ⎫ | | 15 ⎫ | |
| Second Washing | 6+ ⎭ 26 | | 4.7 ⎭ 94 | |
| 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.

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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



ATMOSPHERIC MODIFICATIONS
OF ISOPRENE?
INTACT METHYL BUTANOLS

METHYL BUTANOLS
(found in precipitation)

+

ISOPRENE OR
MODIFIED ISOPRENE?

ISOPRENE (known in air)

+

METHYL BUTANOLS (known in plants)

RUN OFF

VOLATIZATION

ADSORPTION ON
SOIL HUMICS

SURFACE WATERS

DISINFECTION
DESORPTION
REACTION

CHLORINATED METHYL BUTANOLS
(found by authors)

VEGETATION

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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