PART I

A CIRCULAR DICHROISM STUDY OF KETAL FORMATION OF SOME STEROIDAL KETONES

PART II

STUDIES IN THE SYNTHESIS OF CAMPTOTHECIN AND ANALOGS

A THESIS

Presented to

The Faculty of the Division of Graduate Studies

By

Kenneth Alfred French

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in the School of Chemistry

Georgia Institute of Technology

March, 1975

PART I

A CIRCULAR DICHROISM STUDY OF KETAL FORMATION OF SOME STEROIDAL KETONES

PART II

STUDIES IN THE SYNTHESIS OF CAMPTOTHECIN AND ANALOGS

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THIS WORK IS DEDICATED TO THE CHRIST

THE SON OF THE LIVING GOD

ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. Leon H. Zalkow for providing the environment for this work to take place and for his patience with me.

I would like to thank Dr. James B. Nabors, Jr., for his interest in the work and especially for ideas and encouragement he provided. Similarly, I would like to thank Dr. Robert H. Hill, Jr.

I also express thanks to Dr. C. L. Liotta and to Dr. Drury S. Caine, III, for serving as members of the Reading Committee. I also thank them and Dr. E. C. Ashby for help and encouragement.

Thanks are due to the people of the United States of America for providing funds for a National Defense Education Act fellowship (1967-1970). Support from the National Cancer Institute, National Institutes of Health (PH-43-68-1288) is also gratefully acknowledged.

I would also like to thank Mr. D. E. Lilly and Mr. James Kinlock for preparing and repairing numerous glass items. I thank Mr. Gerald O'Brien and Mr. Charles Taaffe for repairs on electronic instruments. Thanks are also due to Mr. George Turner and Mr. Lawrence Abbey for obtaining mass spectral data.

I would also like to thank Mrs. J. B. McLaughlin for

her interest in this work and moral support. I also wish to thank my mother, Anna Mary French, and all my family and friends who prayed for me to succeed in this work.

The National Science Foundation is acknowledged for providing funds for the purchase of a Hitachi (Perkin-Elmer) RMU-TL High Resolution Mass Spectrometer which was used in the latter part of this study.

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GLOSSARY OF ABBREVIATIONS

Ac	Acetyl (CH_3CO-)
Anal.	elemental analysis
CD	circular dichroism
cm	centimeter
d	doublet (NMR)
EMD	exact mass determination
g	gram
GLC	gas-liquid chromatography
hr	hour
IR	infrared spectroscopy
J	coupling constant (NMR)
ℓ	liter
m	multiplet, a complex pattern of signals (NMR)
M^+	molecular ion in mass spectrum
m/e	mass to charge ratio
mg	milligram
mm	millimeter
mmole	millimole
min	minute
ml	milliliter
nm	nanometer (millimicron)
NMR	nuclear magnetic resonance spectroscopy
ORD	optical rotatory dispersion

q	quartet (NMR)
R_f	distance relative to solvent front (TLC)
R_t	retention time GLC (1.0 unit equals ~0.2 min)
rel	relative
RT	room temperature (25°±3°)
s	singlet (NMR)
s	strong (IR)
soln	solution
t	triplet (NMR)
TLC	thin layer chromatography
TMS	tetramethylsilane (NMR standard)
USDA	United States Department of Agriculture
UV	ultraviolet spectroscopy
μl	microliter

SUMMARY

Part I

Treatment of selected 2-keto and 3-keto steroids with hydrochloric acid in methanol, in ethanol and in 2-propanol gave the expected ketals in equilibrium with their ketones. The circular dichroism (CD) spectrum was observed before and after addition of acid. The percentage of change (decrease) is a measure of the extent of ketal formation.

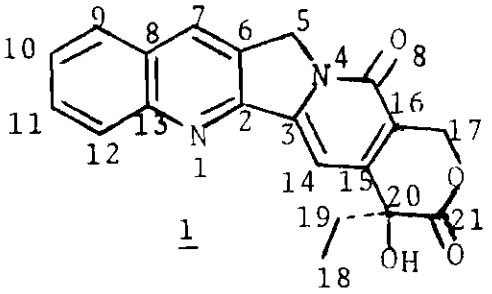
The dimethyl ketal of 5α-cholestan-3-one was isolated in crystalline form after treatment of the ketone in methanol with just a trace of hydrochloric acid. Thus it is not the hemi-ketal which is formed as had been proposed.

The circular dichroism study of ketal formation in steroidal ketones, originally developed by Djerassi, *et al.*[12,13], is able to provide valuable structural and stereochemical information, such as locating the site of the carbonyl functionality and the position of alkyl groups. Moisture must be rigorously excluded, however, if meaningful results are to be derived.

SUMMARY

Part II

Our approach to a synthesis of camptothecin (1), the well-known alkaloid tumor-inhibitor and antileukemic agent, revolved primarily around intermediate 25, the product of the dihydropyrroloquinoline 10 with the highly-oxygenated intermediate 46.



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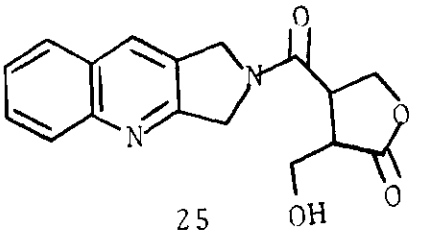

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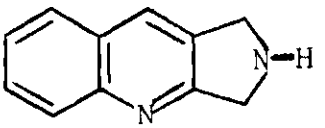

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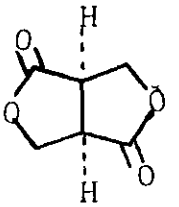

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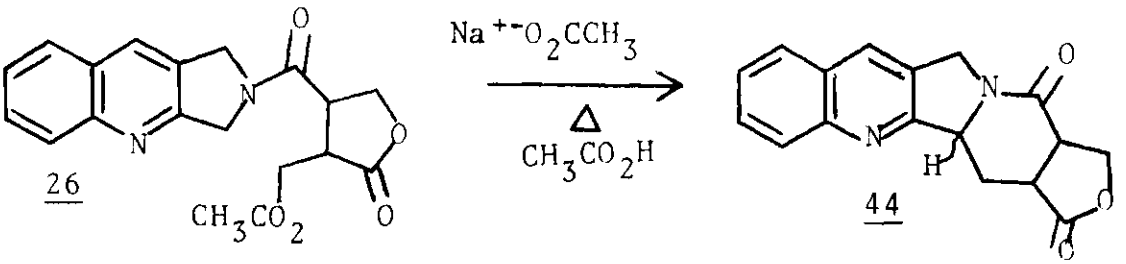
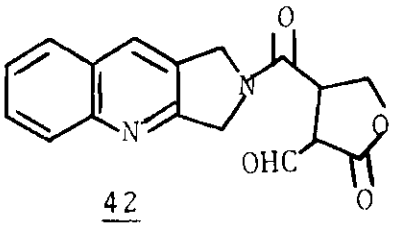
Amide 25 was prepared by Nabors.[20] Efforts to convert the hydroxyl function to a better leaving group were successful only in providing the acetate 26. Attempts to cyclize 26 to 44 were not successful.



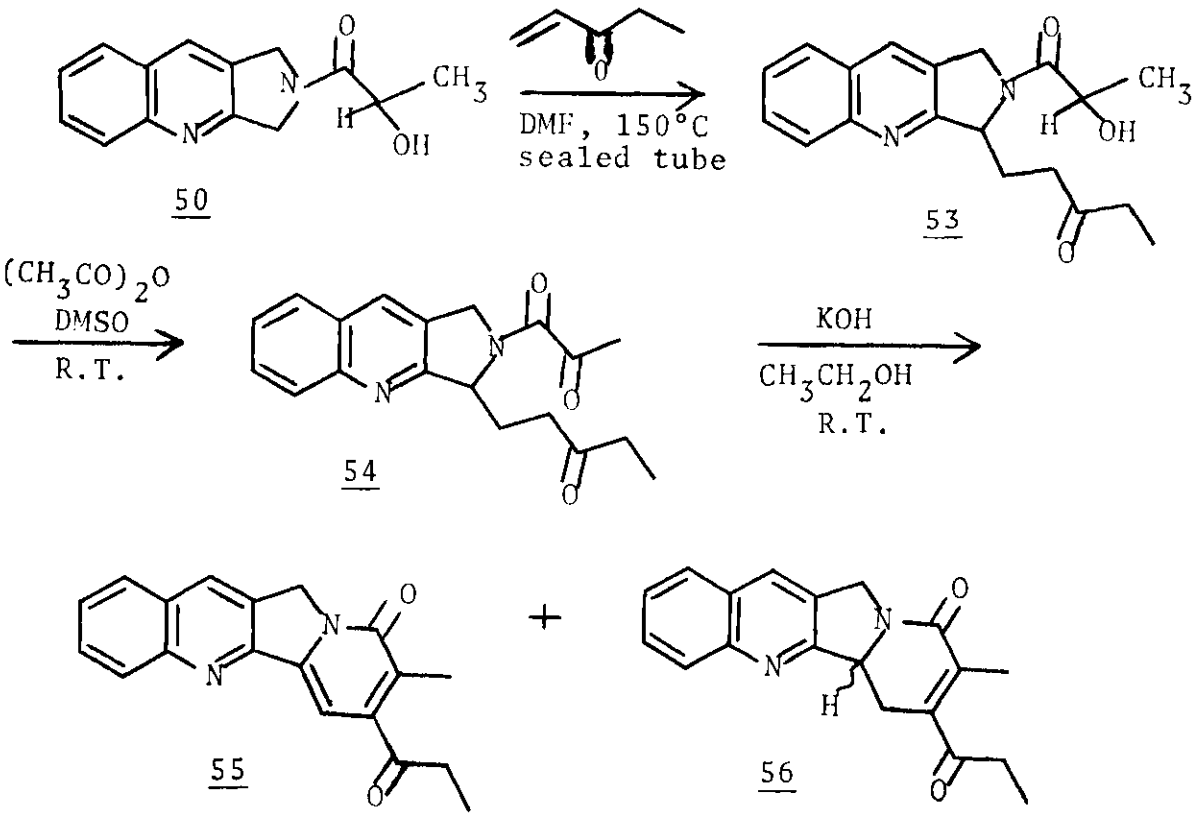
26 44

Chiefly because compound 25 failed to be oxidizable to the useful intermediate 42 and since no facile leaving group, such as the mesylate or tosylate could be introduced, this intermediate was abandoned.

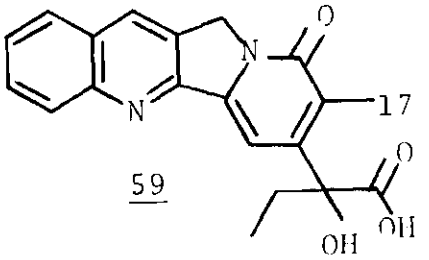


42

Attention was then turned toward a synthesis involving the lactamide 50. This intermediate was carried through the sequence shown below.



The tetracyclic intermediate 55 was then reacted unsuccessfully with hydrogen cyanide in the hope of forming, upon hydrolysis, the camptothecin analog 59, 17-desoxy-camptothecin, as a racemic mixture.



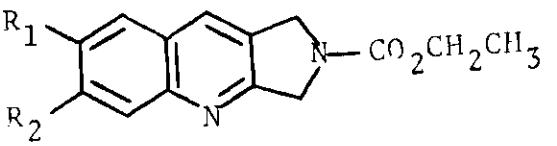
59

With a sufficient supply of 59 it was hoped the C-17 methyl group could be functionalized to yield racemic camptothecin. The antitumor activity of 59 is of interest in further establishing the structure-activity relationship of the camptothecin molecule.

Compounds of the type shown below (11, 13, 15, 17, 19, and 21) were prepared with the intention of carrying them through to the camptothecin analogs.

Each of these compounds was tested against cancer in mouse along with more than 46 other intermediates. Anti-malarial studies in mouse were conducted on 28 intermediates. No significant activity was observed with any of the compounds.

11 $R_1 = R_2 = H$
13 $R_1 = Cl$, $R_2 = H$
15 $R_1 = OCH_3$, $R_2 = H$
17 $R_1 = H$, $R_2 = OCH_3$
19 $R_1 = R_2 = OCH_3$
21 $R_1, R_2 = -OCH_2O-$



Part I

CHAPTER I

INTRODUCTION

The reaction of a carbonyl group in a ketone or aldehyde with one or two moles of alcohol to give a hemiketal (hemiacetal) or a ketal (acetal), respectively, is well known.[1-3] More recently the reaction of certain aliphatic and monocyclic ketones with methanol in the presence of acid has been studied quantitatively by several authors.[4-7]

McCoy *et al.* detected cyclohexanone dimethylketal by mass spectral examination of a reaction mixture, as well as by the disappearance of the C=O absorption in IR and by the appearance of OCH_3 groups. Suter and Guedin[8] reported that acid solutions of simple aliphatic ketones in methanol introduced into a mass spectrometer indicate that some ketal is formed. The criterion used by Wheeler[4] was the lowering of the UV extinction coefficient of the carbonyl chromophore at 290 nm.

Such techniques encounter some technical drawbacks. On the one hand, IR measurements are not always convenient for equilibrium studies, since they require a substantial amount of material. Moreover, polar solvents (such as methanol) cannot be used routinely. This is important for chloroform insoluble substances. On the other hand, UV

absorption measurements of saturated ketones and aldehydes are often unsatisfactory because of unsharp maxima and low extinction coefficients. In addition, the UV method is not easily applicable to substances which contain strongly absorbing chromophores (e.g. conjugated acids or esters and aromatic rings).

If the substance is optically active, the conversion of a carbonyl compound into its hemiketal or its ketal should result in a decrease of the Cotton effect observed by either CD or ORD.[9] This is illustrated by the plain ORD curves of various carbohydrates in which the carbonyl function is masked by acetal formation[10] and the contrasting Cotton effect ORD curves of polyacetylated aldehydo-sugars and fructose in which acetal or ketal formation is hampered.[11]

These conditions led Djerassi[12] to investigate solvent effects and the influence of structure and stereochemistry in (hemi) ketal formation. For this he used ORD. By measuring the ORD of optically active ketones in methanol compared with the ORD of the same solution after a drop of hydrochloric acid has been added (and after a suitable time interval), Djerassi *et al.*[12,13] have shown that hemiketal or ketal formation is translated into a proportional decrease in the Cotton effect.

In spite of these studies, some doubt still existed about the hemiketal *versus* ketal nature of the species devoid of UV absorption and Cotton effect. Although several

authors[5-7] agreed that linear ketones and aldehydes, as well as monocyclic saturated ketones form mainly ketals (acetals) in acidic methanol solution, there was neither chemical nor spectroscopic evidence to support either hemiketal or ketal formation in polycyclic ketones. Furthermore, the reports mentioning the amount of ketal formed with such simple ketones as cyclohexanone in acidic methanol solution are conflicting.[4-7]

In the text which follows[16] it will be shown that the ketal, and not the hemiketal, is formed in steroidal system. The influence of solvent and stereochemistry on the formation of ketals from some steroidal ketones is reported as well.

CHAPTER II

INSTRUMENTATION AND EQUIPMENT

The microanalysis is due to Alfred Bernhardt, Mikroanalytisches Laboratorium, West Germany. The ORD and CD curves were obtained with a JASCO model ORD/UV-5 Optical Rotatory Dispersion Recorder equipped with a CD attachment. The NMR spectrum was recorded at 60 MHz in $CDCl_3$ containing TMS as internal standard. We are indebted to Syntex, S. A., Mexico for a generous gift of steroids.

Drying of Solvents

Methanol[15]

To Fisher spectroanalyzed methanol (1.5 ℓ) was added magnesium turnings (Grignard grade, 10 g). The reaction was allowed to proceed, and the mixture was let stand overnight. Then the methanol was heated at reflux for two hours. Distillation through a heated, two-foot Vigreux column was begun, and the first 500 ml of distillate was discarded. (A silica gel drying tube was used.) The next 500 ml of distillate was collected in a dry, 500 ml round-bottom flask for use in our studies.

Ethanol

A mixture of 95% ethanol (1.5 ℓ) and lump calcium oxide was heated at reflux for an additional two hours.

The first 250 ml of ethanol distilled was discarded. The next 750 ml of ethanol (99.5% pure) was collected for further drying.

A mixture of 100 ml of dried ethanol, 5 g of magnesium and a few drops of ethyl bromide was heated at reflux for 24 hours under a drying tube. Then 650 ml of dried ethanol (99.5% pure) was added. The mixture was heated at reflux for one hour. The first 100 ml of distillate was discarded. Then 350 ml of absolute ethanol was collected in a dry flask for use in our studies.

2-Propanol

Fisher spectroanalyzed solvent was distilled over magnesium.

Standard Alcoholic Acid Solutions

Dry hydrogen chloride gas was passed through the dry alcohol solution for about one to two minutes (from a lecture bottle) with gas being introduced through a stainless steel 17 gauge needle. The acid solutions were titrated with 0.010 N sodium hydroxide solution using phenolphthalein indicator. The following normalities were found (the average of three titrations): 0.117 N HCl in methanol; 0.104 N HCl in ethanol; 0.167 N HCl in 2-propanol.

Transferring Solutions

All transferring of alcohol and acidic alcohol solutions was done with dry syringes and dry, ten inch, stainless steel needles. Each solution was kept in a dry

vessel which had a standard taper ground glass joint fitted with a stop-cock having a T-tube for flushing with nitrogen whenever the stop-cock is opened. See Figure 1 below. The jacketed quartz CD cuvette (10 mm length was also fitted with the piece shown above to keep any traces of moisture out. (Unfortunately, the cuvette could never be "flamed-out" with a Bunser burner under a nitrogen flush.)

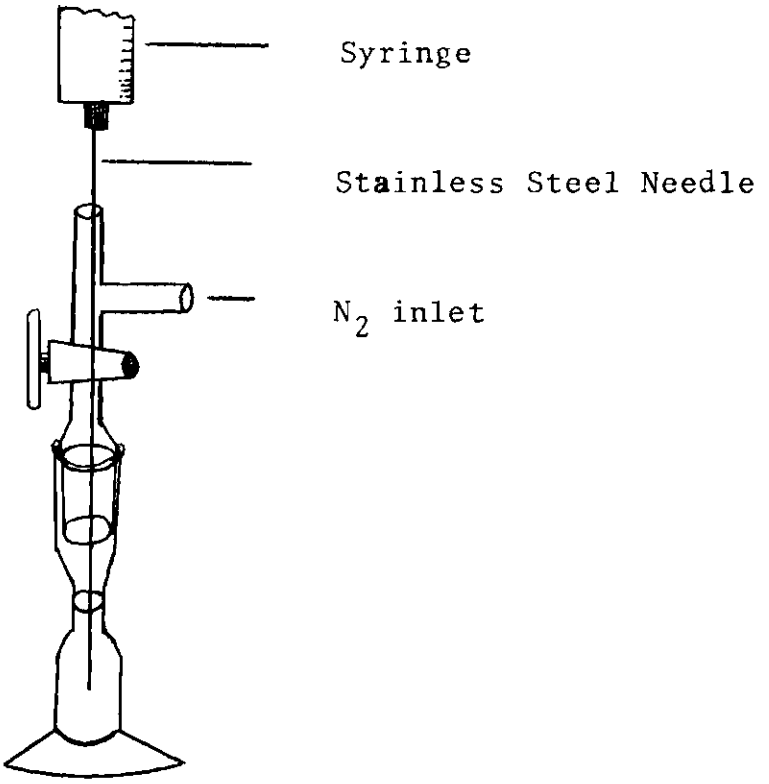


Figure 1. Apparatus for Anhydrous Transfer of Solutions

CHAPTER III

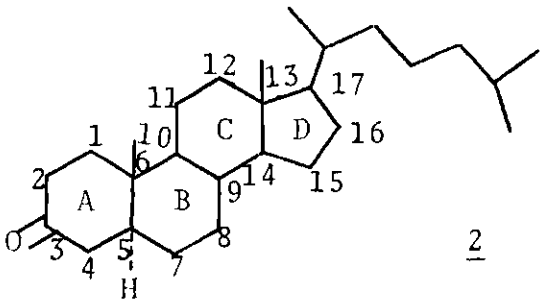
EXPERIMENTAL

General Procedure

Every precaution was taken to exclude moisture from our solutions. After each spectrum of the pure compound was run in dry alcohol, approximately 25 microliters of standardized, alcoholic HCl solution (in the same alcohol) was added using a syringe with a 20 cm needle. When the curve amplitude stabilized at a minimum value (usually within about 45 minutes), the spectrum was run again on the same chart paper (with the same baseline). Finally a baseline was determined using pure solvent. All spectra were recorded at 20° ± 1°C in a water-jacketed 10 mm quartz cell maintained at this temperature with an Ultra-Kryomat TK-30 cooling unit.

ORD and CD Spectra of 5α-Cholestan-3-one (2) and the Dimethyl Ketal (7)

A solution was prepared by dissolving 151.2 mg of 2 in methanol (Fisher spectro-analyzed) and diluting to 50 ml. Approximately 3 ml of this solution was used in a 10 mm cell to obtain the ORD curve: $[\phi]_{700}$ + 134°; $[\phi]_{589}$ + 153°; $[\phi]_{305.5}$ + 3121°; $[\phi]_{287}$ 0°; $[\phi]_{268}$ - 2240°; $[\phi]_{230}$ - 1614°; $[\phi]_{215}$ - 1532°; a = +54. A minute trace of concentrated hydrochloric acid was added by dipping a 10 microliter



2

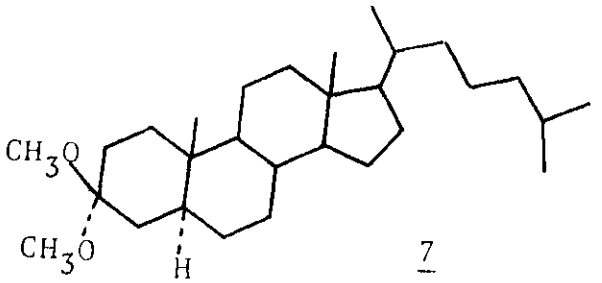
syringe needle into the acid, wiping lightly with tissue and momentarily inserting the needle into the sample. After 15 minutes were allowed for equilibration, the ORD spectrum was recorded again. The Cotton effect was almost imperceptible on the plain positive curve obtained.

Another 3 ml of the original solution (151.2 mg/50 ml) was used to determine the CD spectrum of 2. The molecular ellipticities [θ] and the width at half height (Γ) were: $[\theta]_{233}$ 0; $[\theta]_{289}$ + 4084; $[\theta]_{239}$ 0; Γ = 33 nm. The addition of a trace of acid as before gave $[\theta]_{289}$ + 168, a 96% reduction. Adding one microliter of water shifted the equilibrium to give $[\theta]_{289}$ + 211. Further aliquots of water had the expected effect as shown by Table 1 below.

Table 1. The Effect of Traces of Water on the CD Spectrum of a Ketone-Ketal Equilibrium Mixture

μl of water	$[\theta]_{289}$	μl of water	$[\theta]_{289}$
0	168	50	1600
1	211	60	1810
5	337	80	2126
10	674	100	2379
20	926	150	2910
30	1179	200	3221
40	1410	250	3452

Preparation of 7



7

The remainder of the original solution (approximately 44 ml) was treated with a trace of concentrated hydrochloric acid and after 30 minutes, was treated with solid sodium bicarbonate (0.5 g, dried one hour at 110°). After shaking, the material was filtered. The filtrate was evaporated at reduced pressure on the rotary evaporator to give 150 mg of a gum, which slowly crystallized on cooling. Recrystallization from ether gave 7 as a white solid: m.p. 83.5-84°; $[\alpha]_D$ + 23°; ν_{max}^{KBr} 1110, 1060 cm^{-1}; NMR ($CDCl_3$) CH_3 singlets at δ0.65 (3H), 0.79 (6H), 0.91 (6H), 3.14 (3H) and 3.19 (3H). Mol. wt.: 432.397, mass spectrum: M^+ at m/e 432.390. Anal. Calc. for $C_{29}H_{52}O_2$: C, 80.49; H, 12.11. Found: C, 80.28; H, 12.08.

CHAPTER IV

RESULTS AND DISCUSSION

The addition of hydrochloric acid to a methanol solution of 5α-cholestan-3-one 2 followed by addition of anhydrous sodium bicarbonate, allowed the isolation of the dimethyl ketal. The formation of this crystalline compound 7, characterized by the absence of both a CO band and an -OH absorption band in its IR spectrum, two $-OCH_3$ signals appearing in the NMR spectrum at δ 3.14 and δ 3.19, respectively, and the appearance of the molecular ion M^+ 432.39 in the mass spectrum, strongly indicates that the ketal, and not the hemiketal, is formed under these conditions. The table lists the steriodal ketones which were investigated. The CD molecular ellipticity [θ] was obtained at 20°, in anhydrous methanol, ethanol and 2-propanol solution, since ketal formation is known to be very responsive to the size of the alcohol.[12-14] Table 2 also reports the molecular ellipticity values after addition of a standardized amount of acid as [θ]', and the percent reduction in magnitude of the Cotton effect as %Δ.

When 17β-hydroxyandrostan-2-one (1a) in methanol solution was treated with acid, the Cotton effect was reduced by 54%. The corresponding 17-acetate (1b) showed 73%

Table 2. The CD Data for Compounds 1a-6

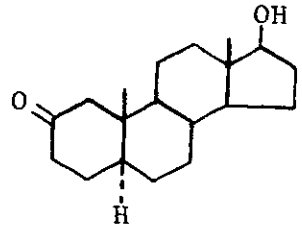
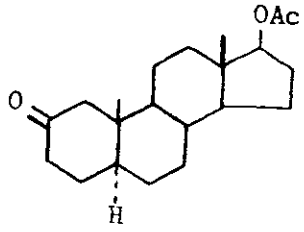
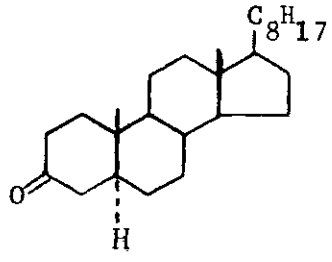
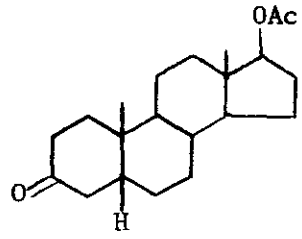
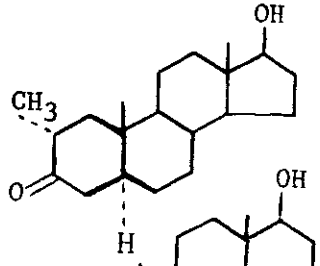
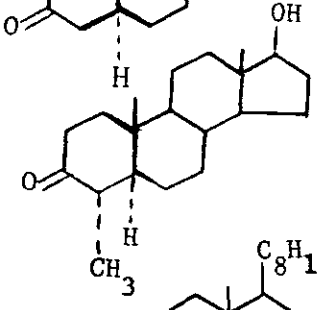
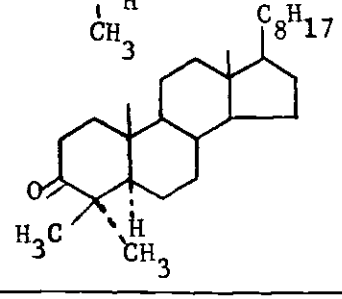
Compound	Structure	Methanol	Ethanol	2-Propanol
1a		$[\theta]_{291}$ = 6293 $[\theta]'$ = 2876 %Δ = 54	$[\theta]_{292}$ = 6138 $[\theta]'$ = 5206 %Δ = 15	$[\theta]_{292}$ = 6742 $[\theta]'$ = 6376 %Δ = 5
1b		$[\theta]_{292}$ = 6525 $[\theta]'$ = 1775 %Δ = 73	$[\theta]_{292}$ = 6138 $[\theta]'$ = 5206 %Δ = 15	$[\theta]_{293}$ = 6199 $[\theta]'$ = 5818 %Δ = 6
2		$[\theta]_{289}$ = 4185 $[\theta]'$ = 168 %Δ = 96	$[\theta]_{290}$ = 4339 $[\theta]'$ = 696 %Δ = 84	$[\theta]_{290}$ = 3682 $[\theta]'$ = 2762 %Δ = 25

Table 2 (concluded)

Compound	Structure	Methanol	Ethanol	2-Propanol
3		$[\theta]_{289}$ = -1339 $[\theta]'$ = 0 %Δ = 100	$[\theta]_{291}$ = -1411 $[\theta]'$ = -92 %Δ = 94	$[\theta]_{290}$ = -1471 $[\theta]'$ = 844 %Δ = 43
4		$[\theta]_{290}$ = 3901 $[\theta]'$ = 2900 %Δ = 26	$[\theta]_{291}$ = 3805 $[\theta]'$ = 3601 %Δ = 5	$[\theta]_{292}$ = 3642 $[\theta]'$ = 3509 %Δ = 4
5		$[\theta]_{289}$ = 3509 $[\theta]'$ = 2986 %Δ = 15	$[\theta]_{288}$ = 3448 $[\theta]'$ = 3270 %Δ = 5	$[\theta]_{289}$ = 3607 $[\theta]'$ = 3456 %Δ = 4
6		$[\theta]_{307}$ = -864 $[\theta]'$ = -709 %Δ = 18	-- -- --	$[\theta]_{309}$ = -701 $[\theta]'$ = -701 %Δ = 0

formation of the ketal. As indicated by Djerassi,[12] the fact that the 2-keto-steriod 1b could not be converted entirely to its ketal is due to steric factors, *in extenso* the 1,3-diaxial interactions between the 2β-OCH_3 and the 10β-CH_3 groups. However, the amount of ketal formed (up to 73% *viz* 12% reported earlier) indicates that the 1,3-diaxial interactions are not as strong as previously indicated, so that other factors may be involved in the ketone ⇄ ketal equilibrium. In agreement with previous observations,[13] only a small proportion of ketal is formed in ethanol (15%) and in 2-propanol solution (5%). This is attributed to steric factors inherent to the nature of the ketal.

The 3-keto-steroid 5α-cholestan-3-one (2) gave 96% of the dimethyl ketal (7), 84% of the diethyl ketal, and 25% of the corresponding diisopropyl ketal. Still more striking is the quantitative dimethyl ketal formation in the case of the 3-keto-5β-steriod 3. Moreover, the diethyl ketal was formed in 94%, and the diisopropyl ketal in 43% yield. These results emphasize the influence of the stereochemistry at C-5 on the amount of ketal formed and seem to indicate that the 3-keto chromophore is less hindered in the 5β H-series than in the 5α H-series and/or there are less steric interactions in the ketals derived from the 3-keto-5β-steroid 3 than in the 5α-compound 2. (Note that 2 shows a positive Cotton effect, while 3 shows a negative Cotton effect.) Each of the 3-keto-steroids 4, 5, and 6 have alkyl

substituents either at C-2 or at C-4. Provided that the precautions mentioned in the experimental section are taken, the formation of ketal in acidic methanol is not fully inhibited in these three cases. It is substantially reduced, however. Even the presence of gem-dimethyl groups adjacent to the carbonyl in the cyclohexanone ring, such as in 4,4-dimethyl-cholestanone (6), does not completely inhibit conversion to the ketal. The reduced ketal formation, ranging from 15 to 26%, can be used as an indication of the presence of a methyl substituent vicinal to the keto-group[12,13].

The difference in ketal formation observed between compounds 4 and 5 in methanol solution (see Table 2) may mean that an equatorial $-CH_3$ group at C-4 causes more steric hindrance than an equatorial $-CH_3$ at C-2. It is noteworthy that the value of %Δ of the gem-dimethyl-cholestanone (6) in methanol solution is higher (18%) than that of 5 (15%), in extenso two $-CH_3$ groups at C-4 are no worse sterically than the equatorial $-CH_3$ in 5. In both 5 and 6, the really severe interaction comes from the equatorial $-CH_3$ group at C-4, which, in the ketal, is gauche to both $-OCH_3$ groups at C-3. The slight difference in %Δ values between 5 and 6, may be due to changes of conformation occurring in ring A of these polycyclic compounds.

The difference noted in the ketal formation in the case of the alcohol 1a and its acetate 1b could indicate that the time required to reach a constant equilibrium may

vary from one substance to another. Indeed, it was observed that addition of hydrochloric acid to cholestanone (2) in 2-propanol is not accompanied by an immediate decrease of the magnitude of the Cotton effect. The formation of the ketal is not instantaneous, but takes some time to level off.

It should be noted that in the cases of compounds 1b and 3 the cleavage of the acetate by alcohol, particularly by methanol, probably occurs rapidly in the presence of a trace of hydrochloric acid.

CHAPTER V

CONCLUSIONS

In the case of 2-keto-steroids, it was found that the previously reported[13a] value 12% for ketal formation in methanol of 1b was low. With the precautions indicated in the experimental section, the ketone ⇄ ketal equilibrium can be displaced to the right. This indicates the importance of keeping the solvent absolutely anhydrous in order to get the maximum of ketal formation which is possible for the system. This has been demonstrated in the case of a 3-keto-steroid. The addition of a "trace" (see experimental section) of concentrated hydrochloric acid to a dry methanol solution of 2 gave 96% of the ketal 7. The introduction of 1 microliter of water to this solution shifted the ketone ⇄ ketal equilibrium to the left (perceptible in the CD spectrum), and the further addition of microliter quantities of water regenerated increasing amounts of ketone 2 (see Table 1 in experimental section). This observation emphasizes that the amount of ketal formed is not only a function of the structure and stereochemistry of the keto-compound and of the nature of the solvent, but the solution must also be strictly anhydrous, or the results become meaningless.

In summary, ORD or CD ketal study, initially developed

by Djerassi *et al.*[12,13] can provide valuable information. However, before drawing structural and stereochemical conclusions from such an experiment, one must be sure that all precautions have been taken to exclude moisture from the system.

CHAPTER VI

RECOMMENDATIONS

It would be wise to carefully check each solution, prior to running its spectrum, for traces of water. This can be done by the rather elaborate and expensive Karl Fisher analysis.

Careful studies should be done on other standard carbonyl systems which show a Cotton effect. The results should be tabulated eventually as a handy reference for all researchers who might use CD or ORD information to help determine the location of carbonyl groups and alkyl substituents in the A-ring of steroidal ketones.

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PART II

CHAPTER I

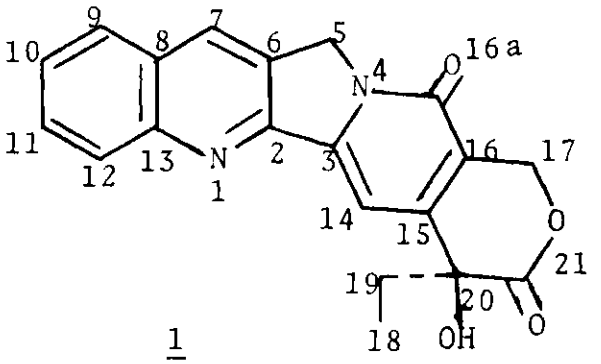
INTRODUCTION

Camptothecin (1), a novel pentacyclic alkaloid with potent antileukemia and antitumor activities in animals, was isolated from the stemwood of a tree which grows abundantly only in mainland China.[1] The tree, *Camptotheca acuminata*, belongs to the family Nyssaceae. No other species are found in this family. The tree in its native China reaches a height of 60 feet growing along riverbanks and other wet areas.

Camptothecin has high activity in the P-388 and P-1534 leukemia systems. (The latter was used to screen the Vinca alkaloids.) It is also very active in inhibiting the growth of solid tumors including Walker Carcinoma 256 (intramuscular). Camptotheca acuminata extracts gave excellent inhibition in the CA-755 tumor, and this is one of the few plants to show strong activity in the L-1210 (leukemia) system with crude extracts.[2]

The isolation of camptothecin was carried out using bioassay.[3] The dry plant material was extracted thoroughly with hot heptane. Bioassay showed no activity in this extract. The plant material was then extracted with 95% ethanol. The ethanol concentrate was partitioned between

chloroform and water. The chloroform solution proved to be highly active. The chloroform extract was subjected to an 11-stage preparative Craig Countercurrent Distribution System (CCl_4, $CHCl_3$, CH_3OH, H_2O; ratio 7:3:8:2). This concentrated the activity in a few fractions containing 1-2% camptothecin. Silica gel chromatography, followed by crystallization gave further purification. In addition to X-ray analysis, the structure was confirmed by UV, IR, NMR, mass spectrum and a limited amount of chemical data.[1]



1

Camptothecin showed a remarkable ability in the inhibition of macromolecular synthesis (DNA and RNA).[4,5]

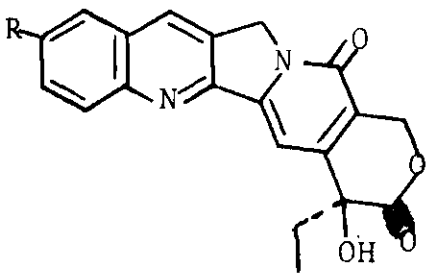
Camptothecin survived even advanced pharmacological screening in dogs and monkeys. Finally, trials were made on human patients who had failed to respond to conventional treatment.[6] The alkaloid has been reported to be effective in the treatment of cancer of the colon, as well as other types of cancer in human patients.[7] Each year cancer of the colon strikes 75,000 Americans and causes more deaths than

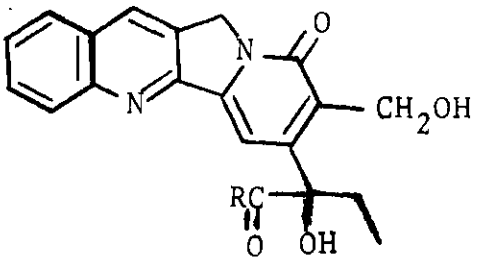
any other type of cancer except lung cancer. Tests on patients at the National Cancer Institute's Baltimore Research Center produced the surprising and encouraging discovery that small doses of camptothecin remain in the body from two to eight days. By comparison, methotrexate, another cancer drug, is often administered by continuous infusion and is excreted from the body in a little more than two hours. Full scale human treatments at the Mayo Clinic tested camptothecin as a single agent and in combination with radiation therapy and with other drugs. Parallel programs would have been underway to study the effect of the agent against breast cancer, leukemia and lung cancer, but camptothecin was too scarce.

In late 1963, Dr. Robert E. Perdue, Jr. of USDA's Agricultural Research Service began an intensive search for new sources of camptothecin. From a single tree in southern California that produces viable seeds and from two trees at Chico, California, the Agricultural Research Service began cultivating seedlings. In 1968 the first major crop was harvested. By the fall of 1969 the planting at Chico included 6,000 seedlings 2 to 5 years old and 4 to 14 feet tall. At that time 2,000 *Camptotheca acuminata* trees were harvested at a cost of about $150,000 to yield 1-2 pounds (less than one kilogram) of camptothecin.

In contrast to the situation with many other anti-tumor agents a great deal is known about structure activity

relationships in the camptothecin series.[2] Hydroxy- and methoxy-camptothecin, 2 and 3, respectively, have been isolated from *Camptotheca acuminata*.[8] These compounds are at least as active as camptothecin. When treated with meta-chloroperbenzoic acid, camptothecin gives the moderately active 1-N-oxide. The sodium salt 4 and the amide 5 both have appreciable activity. Both are converted to camptothecin at pH 6.0 or less.





1 R = H

2 R = OH

3 R = OCH_3

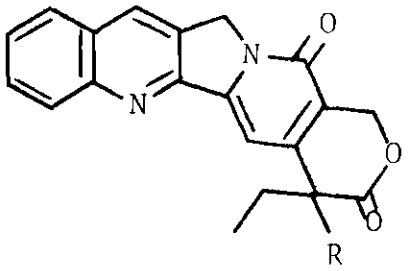
4 R = ONa

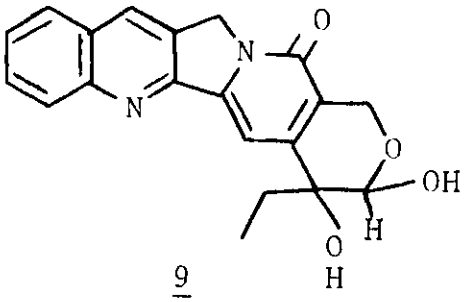
5 R = $NHCH_3$

Changes in the C-20-hydroxyl function lead to complete inactivation. Thus the acetate 6 is essentially inactive, and the C-20-chloro and C-20-desoxy analogs 7 and 8, respectively, are totally inactive. Reduction of camptothecin under mild conditions with sodium borohydride gives the lactol 9, also inactive.

Table 1. Historical Sketch of Camptothecin

1954	The first chemical studies were done on Camptotheca acuminata extracts. The plant gave no significant tests for saponins, alkaloids or flavonoids.
1959	Camptothecin's anticancer activity in animals was established.
1963	The search for seeds and the first planting of seedlings occurred.
1966	The structure of camptothecin was established.
1970	Human patients were given camptothecin.
1970	Bosmann reported that camptothecin inhibits macromolecular synthesis (DNA and RNA) in human cervical carcinoma cell culture (Hela) and mouse lymphoma cell culture (L5178Y).
1971	The first total synthesis of racemic camptothecin was published.





6 R = O-$COCH_3$

7 R = Cl

8 R = H

9

It can be rationalized that the mode of action of camptothecin might involve nucleophilic attack on the lactone carbonyl by R-NH_2 or S-SH groups in enzyme systems or substrates essential to tumor growth. An α-hydroxy lactone is more reactive than a simple lactone. Hydrogen bonding of the hydroxyl group to the carbonyl increases the positive character of the carbonyl carbon atom, permitting a facile, reversible reaction as shown in Figure 1. This also accounts for the fact that camptothecin reacts quantitatively with base, amine and borohydride under mild conditions.

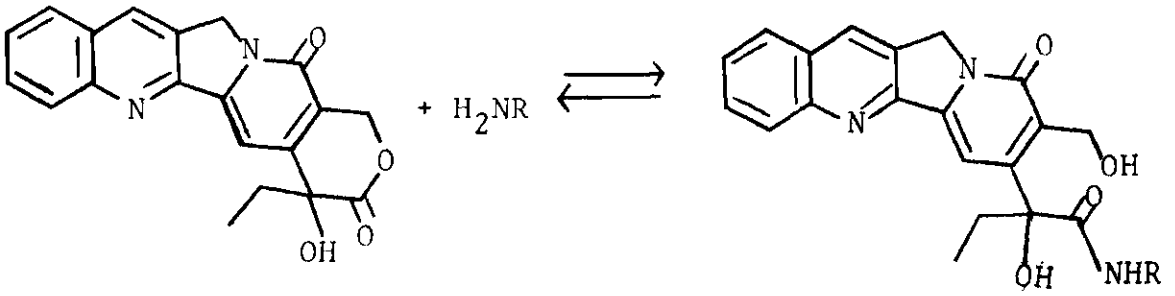


Figure 1. Possible Mode of Action of Camptothecin on a Biological Substrate.

Camptothecin has been the object of much attention as a challenge to organic synthesis chemists.[8-36] During the course of our work Stork and Schultz reported the first total synthesis of dl-camptothecin.[26] A key step involved the use of a new reaction between an α-hydroxy ester and an α,β-unsaturated lactam, as shown in Figure 2.

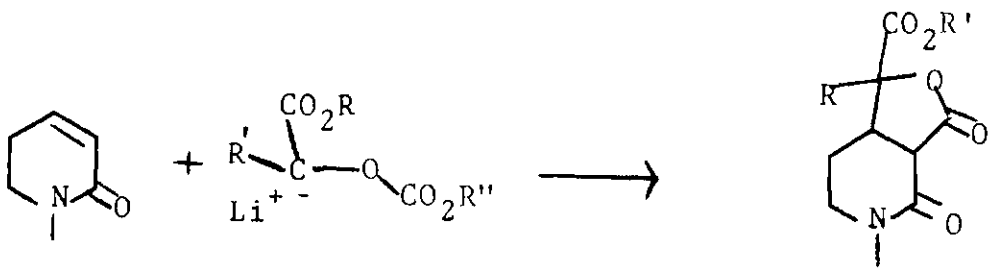


Figure 2. Stork's Key Step, A New Reaction

Since then a number of quite various approaches have borne fruit.[27-33] The highest yield, 11%, (and one of the most interesting syntheses) was published by Rapoport.[32] He had the pyridone D ring intrinsically built into the starting material, pyridone-2,5-dicarboxylic acid. One of the most ingenious steps involved the use of the "methylene lactam rearrangement," shown in Figure 3.

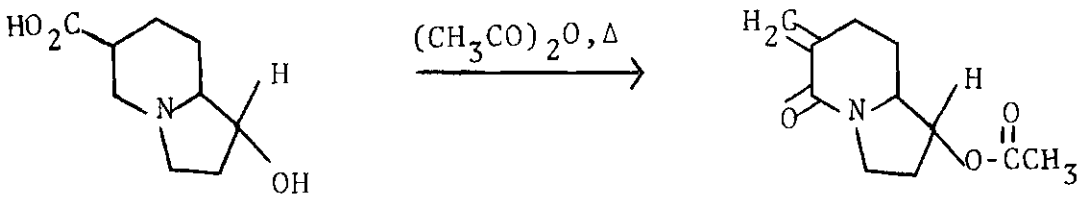


Figure 3. One of Rapoport's Key Steps

Rapoport has also prepared some DE and CDE ring analogs of camptothecin. Danishefsky and coworkers observed that desoxycamptothecin 8 exposed to air is transformed to camptothecin (Figure 4).

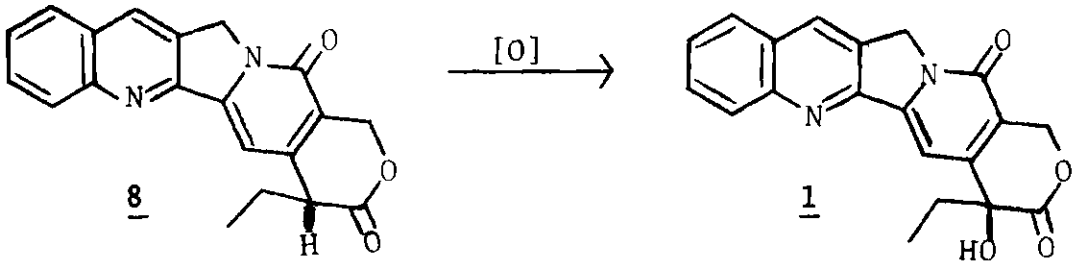
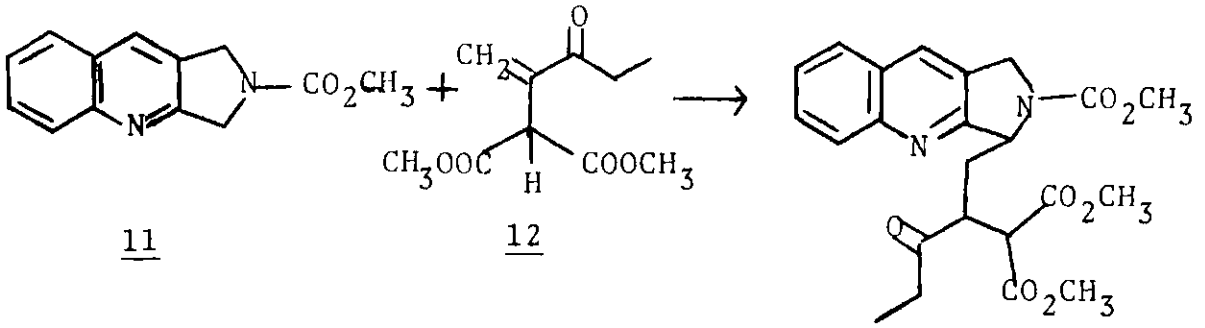


Figure 4. Danishefsky's Oxidative Method

They obtained a 20% yield using potassium tert-butoxide in dimethyl sulfoxide (DMSO)-butanol with one equivalent of aqueous hydrogen peroxide.

Wall, Wani and coworkers[29] developed a synthesis based on the pyrroloquinoline 11 and the highly oxygenated moiety 12.



Wall and Wani also prepared a DE ring analog which has weak cytotoxic activity.[25] Winterfeldt and coworkers[31]

succeeded in a biogenetically oriented synthesis suggested by Wenkert.[24] The key step made use of an indole-camptothecin rearrangement, shown in Figure 5.

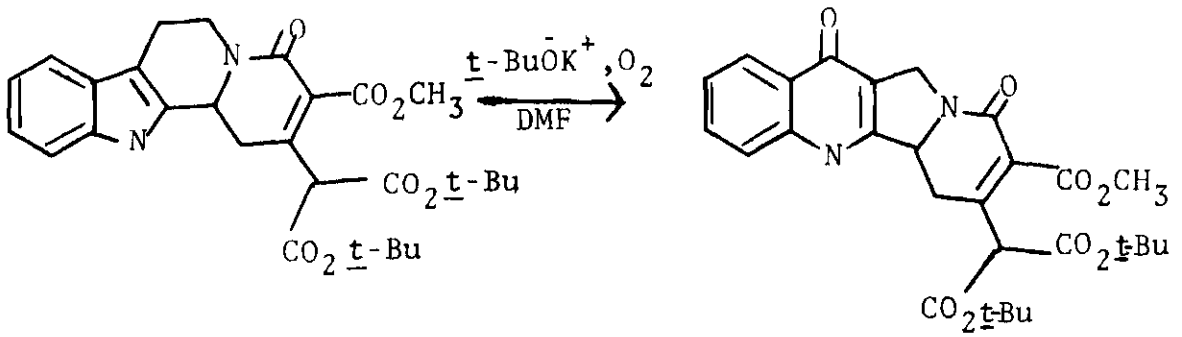


Figure 5. Winterfeldt's Indole-Camptothecin Rearrangement

Winterfeldt also used Danishefsky's discovery that desoxycamptothecin is readily converted camptothecin. He reports "high yield" using oxygen, dimethylformamide (DMF) and copper (II) chloride.

An excellent review by Schultz covers the camptothecin literature through July, 1972.[37] He notes that due to its extreme toxicity camptothecin is no longer of prime interest in clinical testing.

The text which follows describes our efforts toward the synthesis of camptothecin and its analogs, especially 17-desoxycamptothecin. This particular analog is expected to show activity comparable to that of camptothecin.

CHAPTER II

INSTRUMENTATION AND EQUIPMENT

Melting points are uncorrected and were determined on a Thomas-Hoover capillary melting point apparatus. The boiling points are also uncorrected. Infrared spectra were recorded using a Perkin-Elmer 237B spectrophotometer with solids in the form of a potassium bromide pellet and liquids as a thin film between sodium chloride plates or in chloroform solution. The band at 1601 cm^{-1} of a polystyrene film was used as a reference point. Nuclear magnetic resonance (NMR) spectra were recorded with a Varian Associates Model A-60D spectrometer using solutions containing tetramethylsilane as an internal standard. Mass spectra were obtained in the vast majority of cases using a Varian Associates Model M-66 mass spectrometer. A few, which are specified, were run on the Hitachi (Perkin-Elmer) RMU-72 high resolution, mass spectrometer. The ultraviolet (UV) spectra were recorded with a Jasco Model ORD/UV-5 spectrophotometer. Gas-liquid chromatography (GLC) was performed using an F&M Model 400 biomedical gas chromatograph with a hydrogen flame ionization detector and helium as the carrier gas. Helium flow rate was ~90 ml/min. Glass columns (6 ft. x 1/8 in. inside diameter) bent in a U shape were used. The relative peak

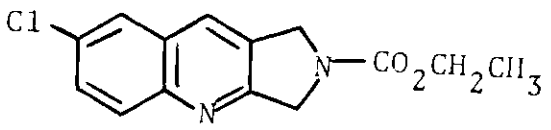
areas were measured by cutting and weighing on an analytical balance.

Gas chromatographs were run isothermally (200-250°C) on columns packed with Chromosorb W impregnated with either 3% SE-30 or 3% XE-60, injection temperature 300°C, detector temperature 250°C. Microanalyses were performed by Alfred Bernhardt Microanalytisches Laboratorium, Mulheim, West Germany, or by Atlantic Microlab, Inc., Atlanta, Georgia.

CHAPTER III

EXPERIMENTAL

Preparation of Ethyl 7-Chloro-2, 3-dihydro-1H-pyrrolo [3,4-b] quinoline-2-carboxylate[18] (13) and Ethyl 7-Chloro-2,3-dihydro-1H-pyrrolo [2,3-b] quinoline-1-carboxylate (14)



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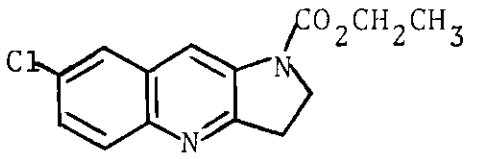


14

Ethyl 3-oxopyrrolidine-1-carboxylate[38] (1.4 g, 8.9 mmole) was mixed with 5-chloro-2-aminobenzaldehyde[39] (1.4 g, 9.0 mmole) and p-toluenesulfonic acid monohydrate (0.40 g, 2.1 mmoles) in a 250 ml round bottomed flask. A distilling head with a vacuum adapter and a 50 ml round bottomed flask as receiving vessel was attached. The oily mixture was then put under a slow stream of nitrogen and immersed in an oil bath at 190°C for about five minutes (until no further vapors came off). The crude black mixture was allowed to cool, and chloroform was added to the warm mass. Gas chromatography on 3% XE-60 at 225°C showed two major peaks in a 46:54 ratio (ret. time 30 and 34 units,

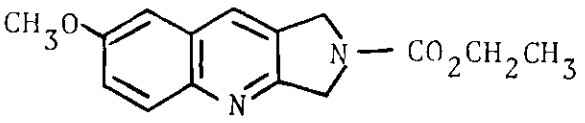
respectively). These were 14 and 13, respectively.

It was convenient to isolate 13 by selective hydrolysis[18] of 14. The reaction mixture was dissolved in 95% ethanol (40 ml). Then 5 ml of 85% aqueous potassium hydroxide was added. The mixture was stirred in a closed vessel for 18 hours at RT. Solvent was removed at reduced pressure on the rotary evaporator, and the residue was then partitioned between water (70 ml) and chloroform (70 ml). The organic layer was separated and dried. Evaporation gave a solid. Two 400 ml extractions with boiling cyclohexane gave, upon evaporation, 0.66 g (2.4 mmoles, 26% yield) of crude 13. Chromatography on silica gel gave pure 13 in the chloroform-benzene (1:1) eluent, m.p. 173-175°C. ν_{max}^{KBr} 1685, 1600 cm^{-1}. NMR $\delta(CDCl_3)$ 1.32 (3H,t,J = 6 Hz), 4.29 (2H,q, J = 6 Hz), 4.82 (4H,s), 7.47-8.10 (4H, complex). UV λ_{max}^{MEOH} 310,318 and 325 nm (log ε 3.74, 3.68 and 3.89). Mol. wt. by mass spectrum 276. Anal. Calculated for $C_{14}H_{13}N_2O_2Cl$: C, 60.76; H, 4.73; N, 10.13; Cl, 12.81. Mol. wt. 276. Found: C, 60.89; H, 4.90; N, 9.97; Cl, 12.73.

The isomer 14 was isolated in another run using 5.0 g (32 mmoles) of 5-chloro-2-aminobenzaldehyde[39] and 5.1 g (32.5 mmoles) of ethyl 3-oxopyrrolidine-1-carboxylate[38] with 1.5 g (7.9 mmoles) of PTSA. The crude product (applied in 1,2-dichloroethane) was chromatographed on a neutral alumina column (100 g) using 1:1 benzene-cyclohexane (600 ml), benzene (300 ml), 1% $CHCl_3$ in benzene (300 ml), then

gradient with $CHCl_3$ in benzene up to 1:1 (2ℓ). The isomer 14 eluted first in the benzene and 1% $CHCl_3$ in benzene fractions. There was obtained 1.33 g (4.8 mmole, 15%) of 14. Recrystallization from EtOH: H_2O gave 1.14 g of pure 14, m.p. 118-121°C. ν_{max}^{KBr} 1315, 1620 (m) and 1720. NMR $\delta(CDCl_3)$ 1.38 (3H,t, J = 7Hz), 3.34 (2H,t, J = 8 Hz), 4.12 (2H, t, J = 8 Hz), 4.39 (2H,q, J = 7 Hz), 7.34-8.16 (4H, complex). UV λ_{max}^{MeOH} 328 and 343 nm (log ε 4.12 and 4.25). Anal. Calculated for $C_{14}H_{13}N_2O_2Cl$: (60.76;H, 4.73; N, 10.13; Cl, 12.81. Found: C, 60.98; H, 4.89; N, 4.96. Note: methylene chloride selectively dissolved 14 away from 13. Acetone also dissolved 14 and impurities from 13. Ether dissolved 13 and a high ret. time (GLC) impurity away from 14. Absolute ethanol was a good crystallization solvent for 13.

Preparation of Ethyl 2,3-Dihydro-7-methoxy-1H-pyrrolo [3,4-b] quinoline-2-carboxylate[18] (15) and Ethyl 2,3-Dihydro-7-methoxy-1H-pyrrolo-[2,3-b] quinoline-1-carboxylate[18] (16)



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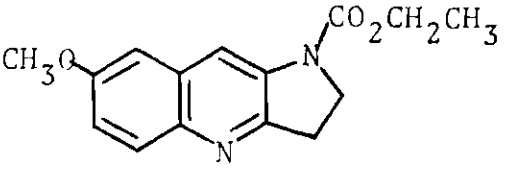


16

To 3.2g (21.2 mmoles) of 2-amino-5-methoxybenzaldehyde[25] was added 3.2g (20.4 mmoles) of ethyl 3-oxopyrrolidine-1-carboxylate[38] in a 500 ml round bottomed flask equipped with distilling head and condenser. Then 1.2g (6.3 mmoles) of p-toluenesulfonic acid hydrate was added, and the reaction vessel was immersed in an oil bath at 200°C while dry nitrogen passed through the flask. Magnetic stirring was attempted. After five minutes, the heat was removed and chloroform was carefully added to the warm mixture. Gas chromatography on 3% XE-60 at 225-230°C showed a 2:1 ratio of 15 to 16. The chloroform solution was diluted to 150 ml and extracted with 175 ml and 100 ml of water in turn. The organic solution was dried over sodium sulfate, decolorized with 0.6g of carbon and filtered through Celite.[R] The solvent was removed by rotary evaporation, and the oily residue was extracted with four 100 ml portions of boiling cyclohexane. Concentration of the combined (filtered) extracts gave 0.35g of orange solid. Gas chromatography showed this solid to be a 42:58 mixture of 15 and 16, respectively. From 0.25g of this solid, 0.0921g of 15 (>95% pure by GLC) and 0.1176g of 16 (>95% pure by GLC) were obtained by trituration with acetone.

Further concentration from 75 ml to 35 ml gave 0.1213g of

[R] A Johns-Manville registered trademark for a diatomaceous silica product.

reddish solid. Washing with 0.8 ml of benzene gave 0.030g of yellow solid. Thin layer chromatography on alumina using 16% chloroform in benzene gave an analytical sample of 16 (0.017g), m.p. 142-144° (benzene). M^+ at m/e 272 (calc. 272). ν_{max}^{KBr} 1700, 1615, 1115 and 1040 cm^{-1}. NMRδ(C_6D_6) 1.25 (3H, t, J = 7 Hz), 3.0 (2H, t, J = 8 Hz), 3.7 (3H,s), 3.75 (2H, t, J = 8 Hz), 4.2 (2H, q, J = 7 Hz), 7.1 (2H, m) and 8.0 (2H, m). UV λ_{max}^{MeOH} 250 and 329 nm (logε 4.81 and 4.18). Anal. Calculated for $C_{15}H_{16}N_2O_3$: C, 66.18; H, 5.88, N, 10.29. Found: C, 66.06; H, 5.99; N, 10.14.

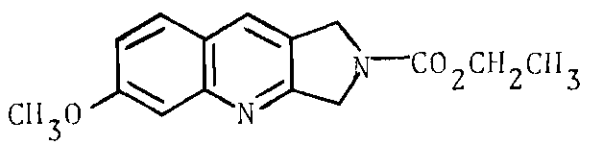
Again 0.0797g of solid was obtained from the filtrate and washings. Nearly quantitative recovery of pale yellow solid (~95% pure by GLC) was obtained by column chromatography on 12.5g of alumina (activity I, neutral) using cyclohexane and benzene. Similarly, chromatography of the remaining filtrate and trituration of the recovered solid with ethanol gave 0.070g of 16 (>95% pure by GLC).

The tarry, dark residue left after boiling cyclohexane extraction was triturated with chloroform to give 0.400g of yellow solid. Recrystallization from chloroform: acetone (20 ml: 70 ml) gave 0.182g of analytically pure 15 (and 0.1259g in a second crop), m.p. 218-220°C (acetone: chloroform). M+ at m/e 272 (calc. 272). ν_{max}^{KBr} 1680, 1625, 1105 and 1025 cm^{-1}. NMRδ($CDCl_3$) 1.5 (3H, t, J = 7 Hz), 3.9 (3H,s), 4.25 (2H, q, J = 7 Hz), 4.8 (4H,s), 7.5 (4H,m). UV λ_{max}^{MeOH} 335 nm (logε 3.94). Anal. Calculated for $C_{15}H_{16}N_2O_3$:

C, 66.18; H, 5.88; N, 10.29. Found: C, 66.01; H, 5.97; N, 10.23.

Evaporation of chloroform and trituration of the residue with acetone gave an additional 0.3090g of yellow solid (single peak on GLC). Total yield of 15 was 0.837g (15%), and 0.3439g (5%) of 16.

Preparation of Ethyl 2,3-Dihydro-6-methoxy-1H-pyrrolo [3,4-b] quinoline-2-carboxylate[18] (17) and Ethyl 2,3-Dihydro-6-methoxy-1H-pyrrolo [2,3-b] quinoline-1-carboxylate[18] (18)



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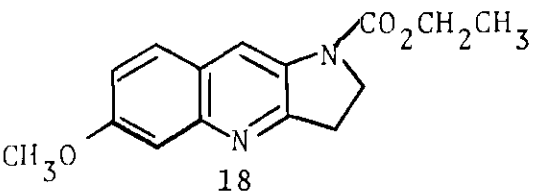
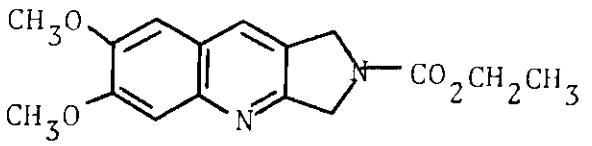


18

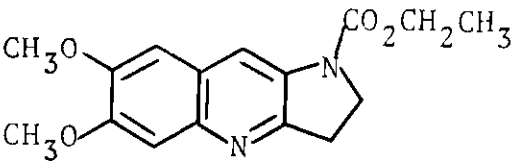
4-Methoxyanthranilaldehyde (2.58g, 17.1 mmoles), prepared from 4-methoxy-2-nitrobenzaldehyde[40] by a procedure similar to that used for anthranilaldehyde,[41] was mixed with ethyl 3-oxopyrrolidine-1-carboxylate[38] (2.68g, 17.1 mmoles). After p-toluenesulfonic acid hydrate (0.80g, 4.2 mmoles) was added with swirling, the mixture was heated at 200°C for five minutes (under nitrogen gas). Gas chromatography (3% XE-60 at 225°C) showed two products,

17 and 18 in a 58:42 ratio, respectively. The crude reaction mixture was dissolved in chloroform (100 ml) and extracted twice with water (100 ml portions). The chloroform solution was dried over sodium sulfate, and the solvent was removed under reduced pressure on a rotary evaporator. The tarry residue was triturated with ethyl acetate to give crude 17 (0.77g, 2.8 mmoles, 17%). The ethyl acetate was evaporated, and the residue was chromatographed on silica gel (50g) using 1:1 benzene-chloroform to give 18 (0.42g, 1.5 mmole, 9%), m.p. 153-155°C. ν_{max}^{KBr} 1695, 1620, and 1040 cm^{-1}. NMR δ 1.38 (3H, t, J = 7 Hz), 3.28 (2H, t, J = 8 Hz, 3.88 (3H, s), 4.05 (2H, t, J = 8 Hz), 4.32 (2H, q, J = 7 Hz) and 7.50-8.10 (4H, complex). UV λ_{max}^{MeOH} 270, 346 and 355 nm (log ε 4.08, 3.98 and 4.00). Anal. Calculated for $C_{15}H_{16}N_2O_3$: C, 66.18; H, 5.88; N,10.29. Mol. wt. 272. Found: C, 66.05; H, 6.15; N, 10.14. Mol. wt. by mass spectrum 272. The same chromatography yielded an additional amount of pure 17 (0.65g, 2.4 mmoles, 14%), m.p. 174-176° (transforms at 164-166°C). ν_{max}^{KBr} 2850, 1700, 1615, 1120, 1025 and 1010 cm^{-1}. NMR δ 1.35 (3H, t, J = 7 Hz), 4.27 (2H, q, J = 7 Hz), 4.75 (4H,s) and 7.00-7.90 (4H, complex). UV λ_{max}^{MeOH} 330 nm (log ε 4.07). Anal. calculated for $C_{15}H_{16}N_2O_3$: C, 66.18; H, 5.88; N, 10.29. Mol. wt. 272. Found: C, 66.07; H, 6.02; N, 10.33. Mol. wt. by mass spectrum 272. Note: The undesired isomer 18 is practically insoluble in methanol.

Preparation of Ethyl 2,3-Dihydro-6,7-dimethoxy-1H-pyrrolo [3,4-b] quinoline-2-carboxylate (19) and Ethyl 2,3-Dihydro-6,7-dimethoxy-1H-pyrrolo [3,2-b] quinoline-1-carboxylate (20)



19

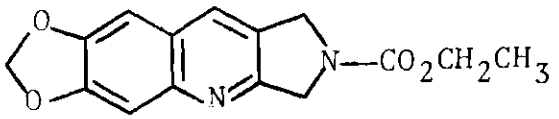


20

6-Aminoveratraldehyde[42] (8.5g, 45 mmoles) and ethyl 3-oxopyrrolidine-1-carboxylate[38] (3.62g, 23 mmoles) along with crystals of p-toluenesulfonic acid hydrate (1.5g, 7.9 mmoles) were mixed in a 250 ml round-bottom flask. The mixture was heated under nitrogen at 190-200°C for five minutes. Gas chromatography (3% XE-60) showed two products at 22.5 and 27.0 units in a 38:62 ratio, respectively. Chloroform (250 ml) was added to give a dark green solution. The solution was extracted with water twice (275 and 150 ml). Evaporation of the chloroform, followed by azeotropic drying with benzene, gave a crude, black tar. Chromatography on silica gel (55g) with benzene-chloroform gave a dark solid upon evaporation of the solvent. Trituration with acetone removed the undesired isomer 20 and impurities leaving GLC pure 19 (0.66g, 2.18 mmole, 10% yield). Recrystallization from acetone gave an analytical sample of 19, m.p. 203-204.5°. ν_{max}^{KBr} 1010, 1115, 1245, 1435, 1620,

1685, 2935 and 2980 cm^{-1}. NMR $\delta(CDCl_3)$ 1.37 (3H, t, J = 7 Hz), 4.00 (6H, s), 4.27 (2H, q, J = 7 Hz), 4.77 (3H (calc. 4H), s), 7.02 (1H, s), 7.35 (1H, s) and 7.80 (1H, broad s). UV λ_{max}^{MeOH} 323 and 335 nm (log ε 4.05 and 4.15). Mass spectrum m/e (rel intensity) 302 (21), 273 (100) and 229 (30). Anal. Calculated for $C_{16}H_{18}N_2O_4$: C, 63.58; H, 5.96; N, 9.27. Found: C, 63.76; H, 5.92; N, 9.36. The acetone containing crude 20 and impurities was evaporated to give 1.47g of crude residue. This material was chromatographed on neutral alumina (10g) with ether to give 20, (10.48g, 7% yield), m.p. 125-129°C (ether). ν_{max}^{KBr} 1030, 1140, 1235, 1445, 1615, 1695 and 2965 cm^{-1}. NMR $\delta(CDCl_3)$ 1.37 (3H, t, J = 7 Hz), 2.12 (2H, s), 2.66-3.90 (2H, complex), 3.96 (6H, s), 4.32 (3H, (calc. 2H), q, J = 7 Hz), 6.95 (1H, s), 7.25 (1H, s) and 8.04 (1H, broad s). UV λ_{max}^{MeOH} 341 and 353 nm (log ε 4.18 and 4.28). Mass spectrum m/e (rel intensity) 302 (100), 274 (5) and 229 (12). Anal. Calculated for $C_{16}H_{18}N_2O_4$: C, 63.58; H, 5.96; N, 9.27. Found: C, 63.48; H, 6.10; N, 9.33. Note: In general the undesired isomer (e.g. 20) has the shorter R_t in both GLC and column chromatography.

Preparation of Ethyl 2,3-Dihydro-6,7-methylenedioxy-1H-pyrrolo [3,4-b] quinoline-2-carboxylate[18] (21)

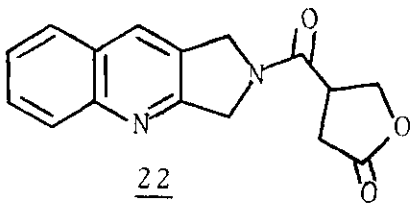


21

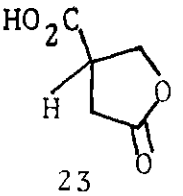
Ethyl 3-oxopyrrolidine-1-carboxylate[38] (3.52g, 22.4 mmoles) was mixed with 6-aminopiperonal[43] (7.39g, 44.7 mmoles) in a 250 ml round bottom flask. After addition of solid p-toluenesulfonic acid hydrate (2.26g, 11.9 mmole), the mixture was swirled and lowered into an oil bath at 200°C while the flask was being flushed with a slow stream of nitrogen gas. The heating was maintained for five minutes. Gas chromatography (3% XE-60 at 250°C) indicated two products in a 1:1 ratio. The crude reaction mixture was dissolved in ethanol (100 ml), and potassium hydroxide solution (85%, aqueous, 18 ml) was added. The mixture was stirred in a closed flask at RT for 18 hr, concentrated and the residue was partitioned between water (160 ml) and chloroform (160 ml). The organic layer was dried over sodium sulfate, concentrated and chromatographed on silica gel (35g) to give in the 1:1 benzene-chloroform eluent 0.78g (2.7 mmoles, 12.1% yield) of 21, m.p. 257-259°. ν_{max}^{KBr} 1700, 1630, 1030 and 1110 cm^{-1}. NMR δ ($CDCl_3$) 1.30 (3H, t, J = 7 Hz), 4.20 (2H, q, J = 7 Hz), 4.80 (4H, s), 6.10 (2H, s), 7.10-8.00 (4H, complex). UV λ_{max}^{MeOH} 324 and 347 nm (log ε 4.18 and 4.40). Anal. Calculated for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Mol. wt. 286. Found: C, 62.87; H, 5.09; N, 9.74. Mol. wt. by mass spectrum 286. Note: The very poor solubility of this set of compounds hindered isolation of the undesired isomer. The free amine from the undesired isomer had m.p. 150-155 and m/e 214 (100%).

Reaction of 2(2-Hydroxymethyl)Succinoyl Lactone (23) with 2,3-Dihydro-1H-pyrrolo [3,4-b] quinoline[18] (10)

A. Preparation of Paraconic Acid[47] (23)



22



23

Ethyl malonate (377g, 2.36 moles) and then freshly distilled ethyl chloroacetate (291g, 2.37 moles) were added, with stirring and cooling, to an ethanolic solution of sodium ethoxide prepared by slowly adding 54g (2.34 moles) of sodium, in small pieces, to 900 ml of dry ethanol. The reaction mixture was stirred at RT for 6 hr, and then the usual workup gave 600g of a viscous oil which was distilled through a 50 cm vigreux column. The fraction (300g) boiling at 120-140°/1.5 mm was shown by NMR to be ~95% pure triethyl 1,1,2-tricarboxyethane. Redistillation gave pure triester (60%), b.p. 114-115°/1.3 mm (lit[44] b.p. 156-158°/16 mm). NMR $\delta(CDCl_3)$ ~1.33 (9H, t, J = 7 Hz, 2 sets of overlapping triplets) 2.87 (2H, d, J = 7.5 Hz), 3.76 (1H, t, J = 7 Hz), 4.24 (4H, q, J = 7.5 Hz), 4.19 (2H, q, J = 7.5 Hz). Further fractionation of the pot residue gave 30g of tetraethyl 1,2,2,3-tetracarboxypropane, b.p. 142-148°/1 mm. NMR $\delta(CDCl_3)$ 1.24 (12H, t, J = 7 Hz), 3.05 (4H, s), 4.08 (4H, q, J = 7 Hz), 4.14 (4H, q, J = 7 Hz). Triethyl

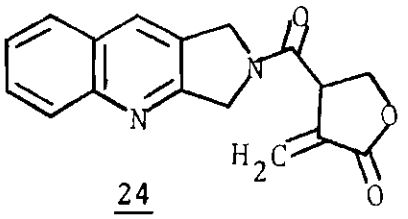
1,1,2-tricarboxyethane was converted into β,β-dicarboethoxybutyrolactone as previously described[47] in about 80% yield. NMR δ($CDCl_3$) 1.28 (6H, t, J = 7 Hz), 3.08 (2H,s), 4.28 (4H, q, J = 7 Hz), 4.55 (2H,s). Distillation through a 50 cm Vigreux column gave the desired lactone ester, b.p. 144-145°/2.0 mm (lit[47] b.p. 128-132°/2.0 mm). The usual saponification gave 23.

B. Preparation of 22

From treating 2.7g (11 mmoles) of ethyl 2,3-dihydro-1H-pyrrolo [3,4-b] quinoline-2-carboxylate[18] with 100 ml of 48% hydrobromic acid (distilled from stannous chloride, b.p. 125-126°C, 760 mm) for 14 hr on the steam bath and then basifying the residue from rotary evaporation with triethylamine in water there was obtained 1.4g (8.2 mmoles) of solid 10 upon methylene chloride extraction, drying, and evaporation of the solvent. The freshly prepared amine 10 was then added to 50 ml of dry dioxane containing 2.1g (10 mmoles) of dicyclohexyl carbodiimide. The solution was cooled in an ice bath, and paraconic acid[47](1.3g, 10 mmoles) 23 was added. This solution was stirred at RT for 5 hr. The reaction mixture was then poured into 800 ml of hot acetone and allowed to crystallize. This gave 1.8g (8 mmoles) of dicycohexyl urea. Concentrating the solution to 150 ml and allowing it to cool gave another 0.2g (0.9 mmole) of the urea. Evaporation in vacuum and recrystallization of the residue from ethyl acetate gave 0.7g of tan solid 22,

(ν_{max}^{KBr} 1780 and 1620 cm^{-1}). The mother liquor was evaporated, and a chloroform solution of the residue was poured through an alumina column (100g, neutral, activity II) to give 0.4g of pure 22. The total yield was 47.5%, m.p. 220-227°C (dec, CH_2Cl_2). NMR $\delta(CD_3CO_2D)$ 1.40 (1H, broad), 2.08 (1H, broad), 3.07 (1H,s), 2.93 (1H,s), 3.65-4.35 (1H, complex), 4.60 (1H,s), 4.73 (1H, d, J = 2 Hz), 4.97 (1H,s), 5.13 (1H, broad s) and 7.45-8.60 (5H, complex). UV λ_{max}^{MeOH} 319, 311, 304, 298, 292, 286 and 281 nm (log ε 3.93, 3.61, 3.79, 3.66, 3.65, 3.60 and 3.59). Mass spectrum m/e (rel intensity): M^+ 282 (73), 254 (7), 253 (11), 223 (15), 197 (10), 169 (100) and 168 (67). Anal. Calculated for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Mol. wt. 282. Found: C, 67.86; H, 4.85; N, 10.09.

Reaction of 2-(2-Hydroxymethyl)-3-methylenesuccinoyl Lactone with 2,3-Dihydro-1H-pyrrolo [3,4-b] quinoline

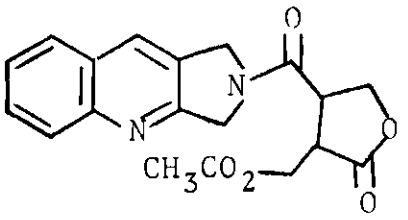


24

In 10 ml of methylene chloride, 2.0g (11.8 mmoles) of the free amine 2,3-dihydro-1H-pyrrolo [3,4-b] quinoline[18] (10) was added. To this solution was added first 2.6g (12.6 mmoles) of dicyclohexyl carbodiimide, and then 1.7g

(12.0 mmoles) of 2-(2-hydroxymethyl)-3-methylene succinoyl lactone[45] (α-methylene paraconic acid) was added in three portions at RT. The mixture began to reflux spontaneously. This lasted only about one minute. The mixture was allowed to cool to RT. After 0.5 hr, the precipitated dicyclohexyl urea was filtered off and washed with 15 ml methylene chloride. Evaporation on the rotary evaporator at reduced pressure gave 3.2g of gummy material. Chromatography on silica gel (3.5 x 35 cm) using 1ℓ of benzene, 1ℓ of 1:1 benzene-chloroform, 800 ml of chloroform and 600 ml of 9:1 chloroform-methanol gave 24 (1.1g, 3.74 mmoles, 31.6% yield), in the 29th-35th 100 ml fractions (chloroform-methanol), m.p. 193-195°C. ν_{max}^{KBr} 1773, 1655, 1637, 1500, 1450, 1410 and 760 cm^{-1}. NMR $\delta(CDCl_3)$ 3.40-5.25 (6H,expect 7H, s), 6.37 (1H, d, J = 2.5 Hz), 6.70 (1H, d, J = 2.5 Hz) and 7.25-8.20 (5H, complex). A single spot was observed with silica gel TLC using 20% acetone in benzene. Mass spectrum m/e (rel intensity): 295 (17.5), M^+ 294 (77), 169 (100), 168 (23), 140 (21), 125 (31) and 115 (18). Anal. Calculated for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.76; N, 9.52. Mol. wt. 294. Found: C, 69.33; H, 4.90; N, 9.33.

Reaction of Nabors' Alcohol[20] 25 with Acetic Anhydride in Pyridine: Brabson's Acetate*(26)



26

Nabors' alcohol[20] (25) was dissolved with vigorous shaking and swirling in 4:1 acetic anhydride-pyridine (3.0g, 9.9 mmoles, in 120 ml). The solution was then allowed to stand overnight at RT. After 18 hr crystals had formed. The crystalline substance was collected by filtration and washed with ether. After vacuum drying there remained 2.94g (8.3 mmoles, 87% yield), m.p. 190-192°C. ν_{max}^{KBr} 1745, 1730 and 1650 cm^{-1}. UV $\lambda_{max}^{CHCl_3}$ 291, 297, 304, 311 and 319 (log ε 3.73, 3.75, 3.88, 3.83 and 4.00) NMR δ(trifluoroacetic acid) 1.3 (3H,s), 2.6-3.1 (1H, complex), 3.1-3.6 (1H, complex), 3.7-4.1 (4H, complex), 4.4-5.0 (4H, complex) 7.1-7.7 (4H, complex) and 8.3 ppm (1H,s). Anal. Calculated for $C_{19}H_{18}N_2O_5$: C, 64.39; H, 5.13; N, 7.91. Mol. wt. 354. Found: C, 64.44; H, 5.08; N, 8.08.

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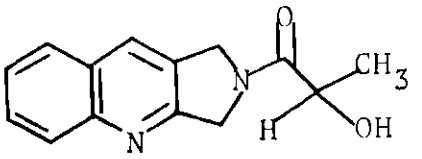
*First prepared by John S. Brabson in Dr. Zalkow's laboratory.

Preparation of 2,3-Dihydro-2-lactoyl-1H-pyrrolo [3,4-b] quinoline 50



50

An aqueous solution (1ℓ) of 2,3-dihydro-1H-pyrrolo [3,4-b] quinoline dihydrobromide* (20.5g, 61.7 mmoles) was treated with excess triethylamine (50 ml) and then extracted with methylene chloride (3 x 50 ml). Rotary evaporation of the undried methylene chloride gave 10.[18] Freshly distilled ethyl lactate (15.4g, 130 mmoles) was added to the free amine 10, and the neat mixture was heated under nitrogen at 80-100° for 12 hr. Chloroform (40 ml) was then added to the mixture which had solidified. The mass was broken up, filtered and dried to give 10.55g (43.6 mmoles, 71% yield), m.p. 222-226°, which showed a single peak on GLC (3%SE-30 at 230°). ν_{max}^{KBr} 3375, 3250, 1660, 1625 cm^{-1}. NMR δ(d-6 DMSO), 1.43 (3H, d, J = 6.5 Hz), 3.42 (1H, complex, disappears on addition of deuterium oxide), 4.60 (1H, complex), 5.10 (4H, two overlapping d of d's, J = 5 Hz, Δν = 17.5 Hz). 7.50-8.28 (5H, complex). Mass spectrum m/e (rel intensity)

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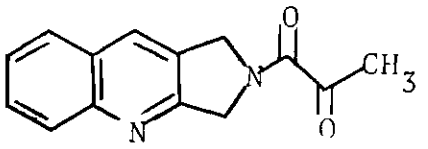
*We are indebted to Dr. R. E. Engle of Drug Research and Development, National Cancer Institute, National Institutes of Health, for supplying us with a large sample of this dihydrobromide, prepared by Stark Laboratories according to our procedure.[18]

M^+ 242 (83), 197 (100) and 169 (70). Anal. Calculated for $C_{14}H_{14}N_2O_2$: C, 69.42; H, 5.78; N, 11.57. Mol. wt. 242. Found: C, 69.29; H, 5.67; N, 11.73.

Preparation of 2,3-Dihydro-2-pyruvoyl-1H-pyrrolo [3,4-b] quinoline (51)

A. Sarrett's Oxidation

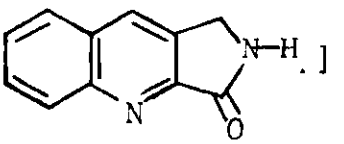


51

In 300 ml of pyridine (ACS certified) 7.0g (28.5 mmoles) of lactamide 50 was dissolved by heating and stirring. Then 7.0g (70 mmoles) of chromium trioxide was added to 72 ml of fresh pyridine at 15°C in an ice-water bath. The additions were made portion-wise with stirring. While still warm, the pyridine solution of lactamide 50 was added to the chromium trioxide-pyridine complex in the ice-water bath. The addition of the first 200 ml of reactant solution was gradual (as a steady stream from a buret), while the last 100 ml was added in 25 ml portions. The reaction vessel was flushed with nitrogen, stoppered and allowed to stand at RT for 22 hr. Then the solution was poured into one liter of water. The aqueous pyridine solution was extracted with 1.5ℓ of 1:1 benzene-ether solution. The combined organic extracts (upper layer) were

divided into two equal portions. Each was extracted with ten 100 ml portions of water. The water was discarded.

[The aqueous pyridine solution above was further extracted with chloroform. The chloroform contained variable amounts of impure starting material. The major impurity (a few %) was isolated by TLC on silica gel-GF developed twice in ethyl acetate, R_f = 0.71, m.p. 262-266° (dec), single peak on GLC (3% SE-30 at 190-195°), R_t = 13 units (compare with R_t = 19 units for 51 and R_t = 25 units for 50). ν_{max}^{KBr} 1635, 1700, 2890, 3080 and 3200 cm^{-1}. Mass spectrum m/e (rel intensity) M^+ 184 (100), 183 (65), 155 (13) and 140 (58). EMD (Hitachi instrument) Mol. wt. calculated for $C_{11}H_8N_2O$: 184.06366; rel intensity M+1, 12.82; M+2, 0.95. Found: Mol. wt. 184.06305 $\pm$ .0031; rel intensity M^+, 100; M+1, 13.02; M+2, 1.75. The above data are in accord with the following structure or the isomeric lactam:



]

The benzene-ether solution was dried over sodium sulfate, filtered and concentrated to 5-10 ml on the rotary evaporator at reduced pressure. The concentrate was treated with acetone (5-10 ml) while still warm. Upon cooling the solution precipitated crystals of 51. In all, there was obtained 1.4g (20%) of product, m.p. 192.5-197° (dec.). ν_{max}^{KBr} 3400, 1700, 1630, 1570, 1500, 1400, 1415, and 1355 cm^{-1}.

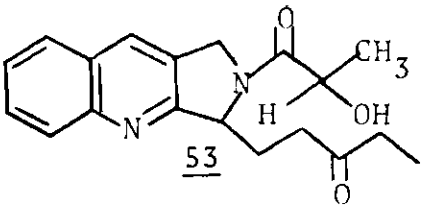
NMR $\delta(CDCl_3)$ 2.53 (3H,s), 4.97 (2H, broad s), 5.17 (1H, s, overlaps with δ5.22 signal), 5.22 (1H, d, J = 1.5 Hz), 7.47-8.17 (5H, complex). Mass spectrum m/e (rel intensity) M^+ 240 (10), 213 (5), 197 (100), 184 (7) and 169 (37). Anal. Calculated for $C_{14}H_{12}N_2O_2$: C, 70.00; H, 5.00; N, 11.67. Mol. wt. 240. Found: C, 70.17; H, 4.90; N, 11.70.

B. Dimethyl Sulfoxide--Acetic Anhydride Oxidation

The lactamide 50 (0.485g, 2.0 mmoles) was added to a 50 ml round-bottom flask, and dimethyl sulfoxide (DMSO, 4.9 ml, dried by vacuum distilling over sodium hydride) was added. Finally acetic anhydride (3.4 ml distilled) was added. The reaction was followed by GLC (3% SE-30 at 200°). The optimum time was found to be 3.5 hr. At this point 11 ml of absolute ethanol was added. After another 4 hr, the solution was cooled in ice-water, and 2.7 ml of water was added. Then 7.3 ml of concentrated aqueous ammonia solution was added in portions, while the temperature was kept at 15-20°. Finally, another 11 ml of water was added to the cold solution with stirring being maintained throughout the entire reaction and work-up. Filtration gave a solid, which was washed well with water and dried for 3 hr at 65° under vacuum. The gray product weighed 0.310g (1.29 mmole). The aqueous solution above was saturated with sodium chloride and extracted with two 100 ml portions of 1:1 benzene-ether. The organic phase was washed with four 50 ml portions of water, then with 50 ml of saturated sodium chloride solution

and dried over sodium sulfate. Evaporation and drying as before gave 0.54g (0.64 mmole, total yield 96%). The product is identical to 51 by IR and by GLC (very pure).

Preparation of 2,3-Dihydro-2-lactoyl-3-(3-oxopentyl)-1H-pyrrolo [3,4-b] quinoline (53)

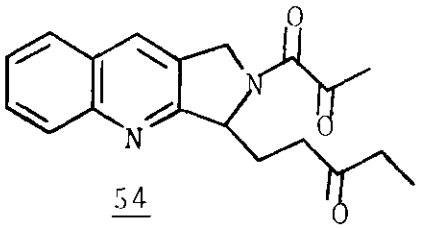


53

The lactamide 50 (0.75g, 3.1 mmoles) and ethyl vinyl ketone (0.47g, 5.5 mmoles, lachrymator) in 6.0 ml of dimethylformamide (DMF) were cooled in dry-ice in a combustion tube, and the mixture was sealed by means of a flame (after the atmosphere above the mixture had been purged with nitrogen. The tube (1 x 20 cm) was heated at 148° for 48 hr. Four such tubes were heated together in a combustion tube oven behind a safety shield. Upon cooling to RT, the tubes were put in dry-ice for 20 minutes and carefully opened behind a shield. Unreacted 50 was recovered (total 0.16g, 0.6 mmole, 4.8%) by filtration of the dimethyl formamide. Benzene (200 ml) was added to the filtrate, and the benzene solution was extracted with water, washed with saturated sodium chloride solution and dried over sodium sulfate. Evaporation of the benzene gave a viscous oil, which on trituration with 1:1 ether-acetone deposited an additional 0.11g, (0.45 mmole, 3.6% total recovered 8.4%) of 50. The filtrate was

chromatographed on silica gel (150g) with 1:4:4 chloroform-methylene chloride-ether. The product 53 was found in the 23rd-40th fractions of 100 ml each to the extent of 2.39g (62% yield) as a viscous oil, which showed a single peak by GLC (3% SE-30 at 235°). $\nu_{max}^{CHCl_3}$ 3460, 1705, 1635, 1355, 1110 cm^{-1}. NMR $\delta(CDCl_3)$ 0.95 (3H, s, J = 6.5 Hz), 1.47 (3H, d, J = 6.5 Hz), 2.08-2.83 (6H, complex), 3.50-4.17 (1H, broad), 4.33-4.83 (1H, broad), 4.99 (2H, d of d), 5.46 (1H, complex), 7.38-8.22 (5H, complex). Mass spectrum m/e (rel intensity) M^+ 326 (25), 253 (62) and 169 (100). The picrate was prepared by treating an ethanolic solution of 53 with a saturated solution of picric acid in 95% ethanol. Recrystallization of this product gave dark green crystals, m.p. 143.5-144°. ν_{max}^{KBr} 3400, 1700, 1650 (doublet) and 1605 cm^{-1}. Mass spectrum m/e (rel intensity) M^+ less picric acid 326 (25), 253 (56) and 169 (100). Anal. Calculated for $C_{25}H_{25}N_5O_{10}$: C, 54.05; H, 4.54; N, 12.61. Mol. wt. less picric acid 326. Found: C, 54.38; H, 4.63; N, 12.41. Note: GLC (3% SE-30 at 235°) of the crude DMF filtrate showed a few per cent of a high R_t impurity, probably the product of addition of a second molecule of ethyl vinyl ketone to 53. It came off the silica gel column in the two fractions just prior to 53.

Preparation of 2,3-Dihydro-3-(3-oxopentyl)-2-pyruvoyl-1H-pyrrolo [3,4-b] quinoline (54)

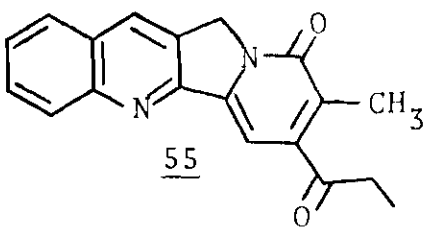


54

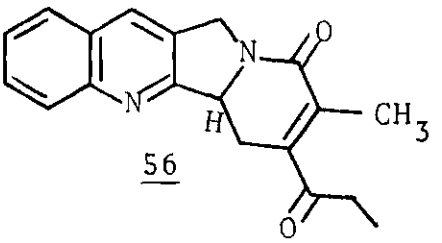
Compound 53 (0.940g, 2.5 mmoles) was dissolved in 9 ml of dimethyl sulfoxide, and 6.8 ml of freshly distilled acetic anhydride was added. After stirring at RT for 20 hr, 20 ml of absolute ethanol was added, and after an additional 1 hr of stirring, 5 ml of water was added. The solution was cooled in an ice-water bath, and 133 ml of concentrated aqueous ammonia solution was added dropwise over a period of 1 hr. Water (21.6 ml) was added to the cooled solution. After standing in an ice-water bath for 2 hr, the solution was filtered. The filtrate was extracted with 1:1 benzene-ether. The organic extract was washed with water and saturated sodium chloride solution and then dried over sodium sulfate. Evaporation of the solvent at reduced pressure gave 0.711g (76%) of a red oil. GLC (3% SE-30 at 230°) showed this oil to contain ~80% 11. The analytical sample was obtained by chromatography on silica gel and was eluted with 1:1 methylene chloride-ether, m.p. 88-91°. ν_{max}^{KBr} 1707, 1645, 1625 (sh), 1570 cm^{-1}. NMR $\delta(CDCl_3)$ 0.97 (3H, t, J = 7 Hz), 2.00-2.66 (9H, complex), 4.50-5.75 (3H, complex), 7.34-8.16 (5H, complex). UV λ_{max}^{MeOH} 320 (log ε 4.43), 239, 208 nm.

Mass spectrum m/e (rel intensity) M^+ 324 (83), 281 (63), 253 (100), 239 (22), 169 (96). Picrate m.p. 98-103°. Anal. Calculated for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Mol. wt. 324. Found: C, 70.24; H, 6.28; N, 8.62.

Isolation of Tetracyclics 55 and 56



55



56

An oily sample of 54 (1.81g, 5.8 mmoles) was dissolved in 100 ml of absolute ethanol in a 250 round-bottom flask. The solution was purged of oxygen by bubbling dry nitrogen through it for five minutes. Then 4 ml of ethanolic potassium hydroxide solution (3M, 1.68g in 10 ml of ethanol) was added. The mixture was stirred at RT for five hours. During this time a deep purple color developed. The solution was poured into 300 ml of water. A mildly exothermic reaction took place. Vigorous shaking with 1:1 benzene-ether (4 x 125 ml) caused the purple color to fade, leaving a yellow solution. The combined organic layers were treated with 75 ml of saturated sodium chloride solution, dried over sodium sulfate and evaporated to a gummy, brown solid. Treatment with 5-10 ml of ethanol and filtering gave yellow crystals, which were washed with water and with

methanol (~15 ml). This gave 0.201g (12%) of 55, m.p. 233-235°, showed a single peak by GLC (3% SE-30). ν_{max}^{KBr} 1400, 1450, 1500, 1625 (sh), 1650, 1700, 2875, 2925, 2970 and 3400 cm^{-1}. NMR $\delta(CDCl_3)$ 1.25 (3H, t, J = 7.5 Hz), 2.18 (4H,s), 2.92 (2H, q, J = 7.5 Hz), 5.22 (2H,s), 7.20 (1H,s), 7.57-8.37 (5H, complex). UV λ_{max}^{MeOH} 219, 245, 253, 2.90 and 3.70 nm (log ε 4.60, 4.39, 4.40, 3.77 and 4.30). Mass spectrum m/e (rel intensity) M^+ 304 (100), 303 (18), 289 (67), 276 (37), 275 (43), 261 (9), 248 (92), 247 (83), 219 (62), 218 (38) and 169 (9). Anal. Calculated for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.21. Mol. wt. 304. Found: C, 74.77; H, 5.44; N, 9.03. The ethanol filtrate was concentrated and applied in spots to a silica gel preparative (1 mm) TLC plate (20 cm x 20 cm). The plate was developed in methylene chloride and dried; the process was repeated two more times. The plate was divided into three equal sections. GLC (3% SE-30 at 235°) showed 55 and 56 to be present only in the middle section (0.6g, 36% of oil), in approximately a 1:1 ratio with R_t 70 and 37 units, respectively. The top third contained a small amount of 56 which was isolated by crystallization from acetone, m.p. 146.5-149.5°. ν_{max}^{KBr} 1600, 1655, 1680, 2935, 2975 and 3055. UV λ_{max}^{MeOH} 304, 318 and 350 nm (log ε 4.20, 4.25 and 3.77). Mass spectrum m/e (rel intensity) M^+ 306 (90), 305 (18), 304 (10), 277 (11), 249 (13), 219 (9), 169 (85) and 138 (100).

Attempted Reaction of Tetracyclic 55 with Hydrogen Cyanide

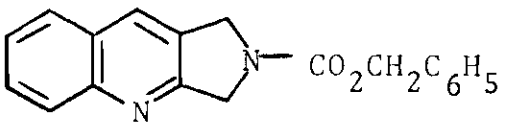
Crystalline 55 (0.200g, 0.66 mmole) in 5.5 ml of dimethyl sulfoxide (ACS certified) was cooled in dry-ice for one hour with stirring in a 50 ml 3-necked round-bottom flask. One neck was connected to a mercury-filled bubbler and then to a solution of concentrated sodium hydroxide. Another neck was fitted with a glass coil, surrounded by a water-ethanol mixture at -10° and coming from a gaseous hydrogen cyanide (in the hood) generator. A trace of saturated aqueous potassium cyanide solution was used as catalyst. Hydrogen cyanide was generated by the slow addition of 7.0g (140 mmoles) of sodium cyanide in 15 ml of water to a stirred 50% aqueous sulfuric acid solution 150 ml. After 10 hr the system was flushed with nitrogen for five minutes. Then 40 ml of water was added, and the white precipitate was filtered washed with water and with methanol and dried, wt. 0.124g (62% recovery), m.p. 230-231.5°. Mass spectrometry showed this substance to be 55. In an attempt to recover the remaining material, the aqueous dimethyl sulfoxide solution was extracted with 60 ml of methylene chloride and 60 ml of chloroform. These were combined and extracted with water (4 x 50 ml) and saturated sodium chloride solution, and dried over magnesium sulfate. The filtered solution was evaporated to give only 0.001g of additional material.

Attempted Reaction of Tetracyclic 55 with Hydrogen Cyanide Generated *in situ*

To oily 55 (1.01g, 3.3 mmoles) in 7 ml of acetic acid at 0° was added excess potassium cyanide (0.24g, 3.7 mmoles). The mixture was shaken vigorously for 2 minutes in a 100 ml round-bottom flask. The cooled solution was allowed to stand overnight, then added dropwise to stirred, ice-cooled concentrated hydrochloric acid (1.5 ml, 18 mmoles). After standing overnight again, the solution was stirred and saturated with hydrogen chloride gas. Next, 50% sodium hydroxide solution was added to the ice-cooled, stirred solution until it was basic to litmus. After three hours the solution was put on the steam bath and heated overnight. Then solid sodium hydroxide (0.26g, 61 mmoles) was added, and the heating was continued another 2 hours. The filtered solution was adjusted to pH 7.0 and extracted with chloroform (nothing was extracted). Slow evaporation gave crystalline material which was mainly inorganic (non-flammable, water-soluble, broad bands in the IR spectrum), probably sodium chloride. An ethanol wash of the crystals gave a greenish, gummy solid. The bulk of the material could not be extracted out of the water layer. No conclusive data were obtained.

Preparation of Benzyl 2,3-Dihydro-1H-pyrrolo [3,4-b] quinoline-2-carboxylate (27)

A. With Sodium Hydroxide



27

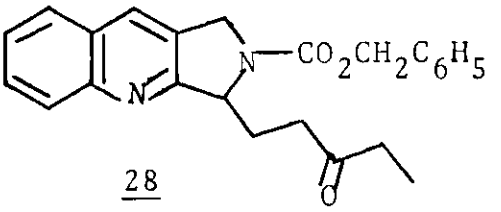
An aqueous solution (50 ml) of the dihydrobromide salt of 10 (4.0g, 12 mmoles) was treated with sodium hydroxide solution (50 ml, 0.13M). The mixture was cooled in ice to 6-10° with stirring under a slow stream of nitrogen. Gradually another 150 ml of sodium hydroxide solution (0.13M) was added. Then five 20 ml portions of sodium hydroxide solution (0.13M) were added alternately with neat portions (~0.44g each) of carbobenzoxy chloride (2.15g, 13 mmoles total) with shaking during about 20 min. The solution was stirred another five min and filtered. The solid obtained was air dried for 30 min, then at 50° in the vacuum oven for 1 hr. The yield was 1.9g (43%), m.p. 160-161.5° (methylene chloride-methanol). ν_{max}^{KBr} 700, 1100, 1400, 1695, 2850, 2940 and 3020 cm^{-1}. NMR $\delta(CDCl_3)$ 4.80 (4H, broad s), 5.20 (2H,s), and 7.35-8.10 (10H, complex, with a singlet at 7.40). Mass spectrum m/e (rel intensity) M^+ 304 (39), 91 (100), 169 (41) and 168 (33). Anal. Calculated for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.21. Mol. wt. 304. Found: C, 74.80; H, 5.32; N, 9.16. Extraction of the

aqueous filtrate with chloroform gave 1.0g (49%) of crude 10 (m.p. 115-116°, lit[18] m.p. 139-141° from benzene and lit[36] m.p. 101-103° from ether; identical by IR to an authentic sample).

B. With Sodium Carbonate and Sodium Bicarbonate

The dihydrobromide of 10 (4.0g, 12 mmoles) was added to 20 ml of water in an 80 ml beaker with magnetic stirring. Then 1.27g (12 mmoles) of sodium carbonate in 10 ml of water was added. Next, solid sodium bicarbonate (2.6g, 31 mmoles) was added. Then carbobenzoxy chloride (2.24g, 15 mmoles) was added dropwise with stirring during 20 min. Sufficient water was added to bring the volume to 60 ml. Then 20 ml of ethanol was added. Although precipitation began immediately, the mixture was stirred for 3.5 hrs before it was filtered. The water-washed, dry product weighed 2.1g (58% yield). This product was identical by m.p. and IR spectrum to the above product 27.

Preparation of Benzyl 2,3-Dihydro-3-(3-oxopentyl)-1H-pyrrolo [3,4-b] quinoline-2-carboxylate (28)



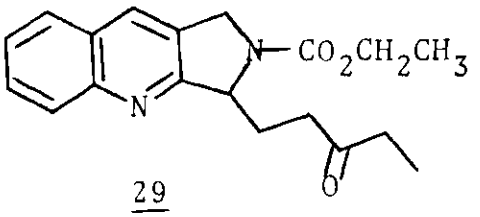
28

Compound 27 (3.75g, 12 mmoles) was divided into four equal portions, which were added to combustion tubes

(8 x 10 x 200 mm), along with 4 ml of dimethylformamide per tube and ~0.5g (2.1g, 25 mmole total) of ethyl vinyl ketone. The tubes were cooled in dry-ice for 15-30 min and then sealed. After being heated at 147° (±5°) for 37.5 hr, the tubes were allowed to cool to RT, then further cooled in dry-ice and were opened behind a safety shield. The dimethylformamide solution was filtered to give a light gray solid, 0.64g (17%) of recovered 27, as found by GLC (3% SE-30 at 250°). The filtrate showed a single peak (~90% of the total) on GLC at a significantly higher R_t than 27. The filtrate was diluted to 60 ml with methylene chloride and extracted with water (3 x 100 ml) and with saturated sodium chloride solution (100 ml). The organic phase was dried over sodium sulfate and evaporated at reduced pressure. The oily residue was heated at 60° in the vacuum oven (1-5 mm) to a weight of 5.83g. This residue was dissolved in warm benzene, and chromatography on silica gel (80g) was begun. First, 2ℓ of benzene-hexane (gradient from 1:9 to pure benzene) gave no product, only polymers of ethyl vinyl ketone. Then 3.6ℓ of ether-benzene (gradient from 1:45 to 1:15) was used to elute the desired product 28. m.p. 124-125.5° (methanol), a single peak on GLC (3% SE-30 at 230°). ν_{max}^{KBr} 700, 1100, 1360, 1400, 1620 and 1685 cm^{-1}. NMR $\delta(CDCl_3)$ 0.93 (3H, t, J = 7 Hz), 2.42 (6H, complex), 4.78 (2H, s), 5.25 (3H, broad s) and 7.40-8.14 (10H, complex, with a large singlet at 7.40). Mass spectrum m/e (rel

intensity) M^+ 388 (57), 317 (5), 303 (12), 259 (41), 253 (100), 169 (24), 168 (18) and 91 (98). Anal. Calculated for $C_{24}H_{24}N_2O_3$: C, 74.20; H, 6.23; N, 7.21. Mol. wt. 388. Found: C, 74.33; H, 6.37; N, 7.12.

Preparation of Ethyl 2,3-Dihydro-3-(3-oxopentyl)-1H-pyrrolo [3,4-b] quinoline-2-carboxylate (29)

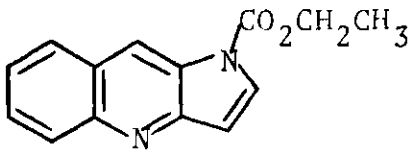


29

Ethyl 2,3-Dihydro-1H-pyrrolo [3,4-b] quinoline-2-carboxylate[18] 11 (0.508g, 2.1 mmoles) was put in a combustion tube (1 x 4 cm), along with ethyl vinyl ketone (0.779g, 9.3 mmoles) and 2 ml of dimethylformamide. The tube was cooled in dry-ice and sealed under nitrogen. Then it was heated at 150° (±5°) for 48 hr. Once the tube had cooled to RT, it was again cooled in dry-ice and opened behind a shield. The crude mixture on GLC (3% SE-30 at 182°), in addition to low R_t ethyl vinyl ketone polymers, contained the starting pyrroloquinoline 11 (27%, R_t 11 units), and new product 29 (68%, 42 units), and a higher R_t product (5%, 90 units) which probably represents the addition on a second molecule of ethyl vinyl ketone to the starting pyrroloquinoline. The crude mixture was diluted with 50 ml of benzene and extracted with two 150 ml portions of water (to remove

dimethylformamide). Evaporation of the benzene gave 0.757g of red-brown oil. Chromatography on silica gel (10g, in benzene), using pure ether gave 0.409g (1.3 mmoles, 62%) of a clear orange oil. (Hot-box molecular distillation failed to give pure product due to decomposition.) IR ν_{max}^{film} 1115, 1410, 1690-1715, 2925 and 2965 cm^{-1}. NMR $\delta(CDCl_3)$ 0.90 (4H, t, J = 7 Hz), 1.32 (3H, t, J = 7 Hz), 2.1-2.8 (8H, complex), 3.20 (0.44H, s, impurity), 4.25 (2H, q, J = 7 Hz), 4.75 (2H, s), 4.93 (1H, broad) and 7.30-8.20 (5H, complex). EMD (Hitachi instrument) Mol. wt. calculated for $C_{19}H_{22}N_2O_3$: 326.163; rel intensity M+1, 21.76; M+2, 2.86. Found: 326.169 ± 0.007; rel intensity M^+, 5.0; M+1, 22.66; M+2, 3.03; m/e 57 (100%). Semicarbazone m.p. 189-190° (acetone). Anal. Calculated for $C_{20}H_{25}N_5O_3$ (the semicarbazone): C, 62.48; H, 6.82; N, 18.22. Found: C, 61.93; H, 6.52; N, 18.03.

Preparation of Ethyl 1H-Pyrrolo [3,2-b] quinoline-1-carboxylate (61)

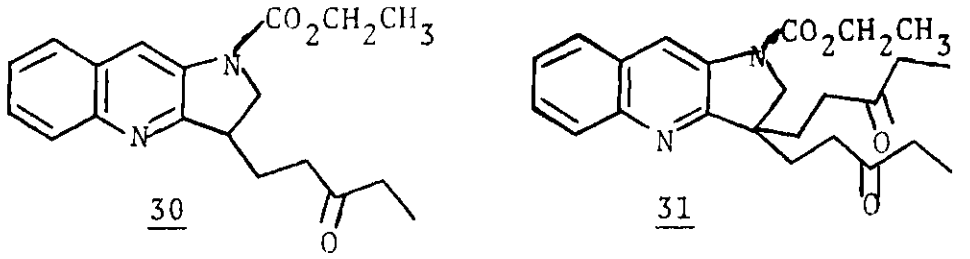


61

Ethyl 2,3-dihydro-1H-pyrrolo [3,2-b] quinoline-1-carboxylate[18] (m.p. 80-81°; ν_{max}^{KBr} 1705, 1615 and 1575 cm^{-1}; 0.50g, 2.1 mmoles) was dissolved in 125 ml of toluene. The

solution was stirred under nitrogen and chloranil (tetrachloro-1,2-benzoquinone, 0.53g, 2.15 mmoles) was added. The mixture was heated at reflux for 18 hr. Upon cooling to RT the mixture was extracted twice with 100 ml, of 3% sodium hydroxide solution (tends to form an emulsion), and then with 100 ml of 1% hydrochloric acid solution (to neutralize the base and prevent cleavage of the N-carboethoxy group) and with 100 ml of water. The toluene solution was evaporated at reduced pressure and azeotropically dried with several portions of absolute ethanol to give 0.32g of dark solid. Chromatography on basic alumina (17g) with 1:1 benzene-chloroform gave 61 as a dark purple solid (0.18g, 36% yield), m.p. 102-105°, m.p. from carbon tetrachloride 93-97° (pink crystals). ν_{max}^{KBr} 1620, 1730 (s), 2910, 2965, 3035 and 3095 cm^{-1}. UV λ_{max}^{MeOH} 324 and 330 mm (log ε 3.18 and 3.16). EMD (Hitachi instrument) Mol. wt. calculated for $C_{14}H_{12}N_2O_2$: 240.090; rel intensity M+1, 16.16; M+2, 1.62. Found: M^+ 240.088 ± 0.004; rel intensity M^+, 97; M+1, 16.88; M+2, 1.79; m/e 168 (100%).

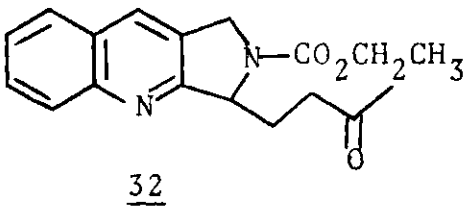
Preparation of Ethyl 2,3-Dihydro-3-(3-oxopentyl)-1H-pyrrolo [3,2-b] quinoline-1-carboxylate (30) and Ethyl 2,3-Dihydro-3,3-bis(3-oxopentyl)-1H-pyrrolo [3,2-b] quinoline-1-carboxylate (31)



Ethyl 2,3-dihydro-1H-pyrrolo [3,2-b] quinoline-1-carboxylate[18] (12) (1.01g, 4.2 mmoles) in 13 ml of dry dimethylformamide was mixed with ethyl vinyl ketone (1.01g, 12 mmoles). The mixture was sealed under nitrogen in a combustion tube which had been cooled with dry-ice. The mixture was then heated at 148° (±5°) for about 18 hr. The tube was again cooled in dry-ice (after it had reached RT) and opened behind a safety shield. The dimethylformamide solution was poured into benzene (50 ml). After three 75 ml water extractions, the benzene solution was dried over sodium sulfate. GLC (3% SE-30 at 230°) of the crude mixture showed three major peaks at R_t 5.5, 19.2 and 46.0 units in the ratio 9:10:6, respectively. These represent the starting pyrroloquinoline 12 (R_t 5.5), 30 (R_t 19.2) and 31 (R_t 46.0). Concentration of the benzene solution was followed by chromatography on silica gel (150g). Low boiling petroleum ether (30-60°, 1ℓ) gave only low R_t ethyl

vinyl ketone polymers by GLC (see above). The product 30 representing monoaddition of ethyl vinyl ketone to the starting pyrroloquinoline was eluted in 9:1 petroleum ether-ethyl ether (1ℓ). The diaddition product 31 and starting pyrroloquinoline were eluted in the next 1ℓ of 9:1 petroleum ether-ethyl ether. The monoaddition product 30 had m.p. 97-99°. ν_{max}^{KBr} 1045, 1150, 1310, 1445, 1620, 1710, 2980 and 3030 cm^{-1}. NMR $\delta(CDCl_3)$ 1.05 (3H, t, J = 7 Hz), 1.40 (3H, t, J = 7 Hz), 1.82-2.98 (7H, complex), 3.82 (2H, d of d, J = 12 Hz, $\Delta\nu$ = 18 Hz), 4.35 (3H, 2H is calculated, q, J = 7 Hz), 7.15-8.40 (5H, complex). EMD Mol. wt. calculated for $C_{19}H_{22}N_2O_3$: 326.1630. Found: 326.1647. Anal. Calculated: C, 69.92; H, 6.79; N, 8.58. Found C, 70.03; H, 6.86; N, 8.49. For the diaddition product 31 the only physical evidence is an exact mass; however, the chemistry of alkyl quinolines points to the structure shown. 31 EMD (Hitachi instrument) Mol. wt. calculated for $C_{24}H_{30}N_2O_4$: 410.221; rel intensity M+1, 27.33; M+2, 4.39. Found: 410.218 ± 0.007; rel intensity M^+, 10; M+1, 27.47; M+2, 4.58; m/e 255 (100%). Of the seven formulas that may be written for this mass (410.218), all can be eliminated for various reasons (e.g. an odd number of nitrogen atoms with an even molecular weight) except $C_{24}H_{30}N_2O_4$.

Preparation of Ethyl 2,3-Dihydro-3-(3-oxobutyl)-1H-pyrrolo [3,4-b] quinoline-2-carboxylate (32) and Ethyl 2,3-Dihydro-3,3-bis(3-oxobutyl)-1H-pyrrolo [3,4-b] quinoline-2-carboxylate (33)



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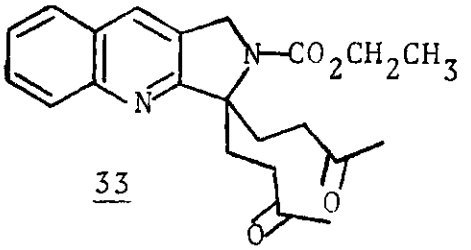


33

Ethyl 2,3-dihydro-1H-pyrrolo [3,4-b] quinoline-2-carboxylate[18] 11 (1.0g, 4.1 mmoles) in 20 ml of acetic anhydride was treated with methyl vinyl ketone (10 ml, 8.6g, 123 mmoles, Aldrich, lachrymator) in a 250 ml round-bottom flask. The mixture, under nitrogen, was heated for 24 hr at reflux. GLC (3% SE-30 at 205°) showed a new peak at higher R_t then the starting pyrroloquinoline. These were present in a 68:32 ratio, respectively. Then another 5 ml (4.3g, 62 mmoles) of methyl vinyl ketone was added. After another 44 hr, the crude mixture was reinjected on GLC. Apart from low R_t methyl vinyl ketone polymers, the GLC showed only a few percent starting pyrroloquinoline, mainly the desired product 32 and a few percent higher R_t product 33. The volatile materials were removed on the rotary evaporator at reduced pressure. The residue (7.65g) was chromatographed on silica gel (35g). Carbon tetrachloride (1.2ℓ) was used to elute methyl vinyl ketone

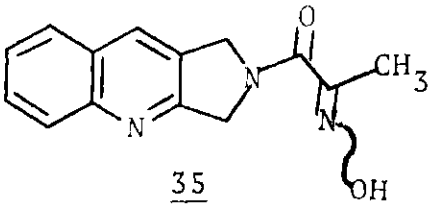
polymers (3.4g). Then 1:1 benzene-chloroform (200 ml) was used. Finally pure chloroform (300 ml) gave very crude 32. Another 600 ml of chloroform gave 33. Crystallization from acetone gave pure 33, m.p. 149-152°. ν_{max}^{KBr} 1690 and 1715 cm^{-1}. NMR showed a singlet ~δ5.0 (2H) which indicated both methyl vinyl ketone additions occurred at the same site. EMD (Hitachi instrument) Mol. wt. calculated for $C_{22}H_{26}N_2O_4$: 382.189; rel intensity M+1, 25.11; M+2, 3.82. Found: 382.190 ± .007; rel intensity M^+, 5; M+1, 26.59; M+2, 4.3; m/e 43 (100%). The crude sample of 32 (2.5g) was chromatographed on silica gel (25g). Benzene (200 ml) eluted polymeric impurities. Then benzene-chloroform gradient elution gave ~1.0g of 32 as an oil. A gummy, reddish solid formed on standing. This substance was triturated with carbon tetrachloride. ν_{max}^{KBr} 1415, 1700, 2900 and 2950 cm^{-1}. NMR δ($CDCl_3$) 1.35 (4H, calc. 3H, t, J = 7 Hz), 1.95-2.58 (19H, calc. 7H, complex), 4.28 (2.6H, calc. 2H, q, J = 7 Hz), 4.87 (2H, d of d, J = 1 Hz, Δν = 5 Hz), 5.22 (1H, t, J = 4 Hz) and 7.34-8.22 (5H, complex). Mol. wt. calculated for $C_{18}H_{20}N_2O_3$: 312. Mass spectrum m/e (rel intensity) M^+ 312 (43), 255 (20), 241 (74) and 169 (100).

Preparation of Ethyl Pyruvate Oxime (34)

A mixture of ethyl pyruvate (1.0g, 8.6 mmoles), hydroxylamine hydrochloride (1.0g, 14.5 mmoles), pyridine (1 ml), ethanol (10 ml) and water (10 ml) was heated on a

steam bath for 1.5 hr. The next day there were long needles present. Filtration gave 0.10g (9%) of 34, m.p. 94-95° (lit[46] m.p. 93.5-94°). ν_{max}^{KBr} 1020, 1185, 1315, 1725, 2970 and 3225 cm^{-1}. No peak was seen by GLC (3% SE-30 at 215°). Solid sodium chloride was added, and further precipitation of 34 occurred until another 0.52g (45%) was obtained. Finally the mixture was extracted with ether (75 ml). The ether was dried over sodium sulfate and evaporated to give a yellow oil. Needle crystals formed in the oil and were separated (0.14g, 13%, total yield 67%). These needles had the same IR as above.

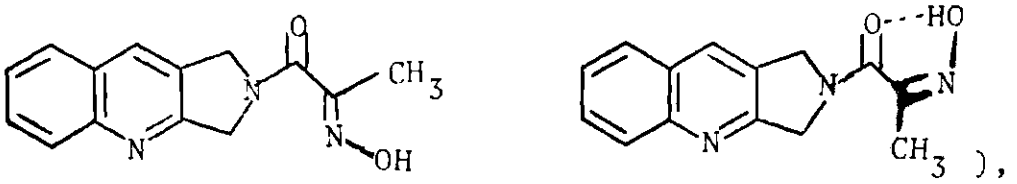
Reaction of Ethyl Pyruvate Oxime (34) with 2,3-Dihydro-1H-pyrrolo [3,4-b] quinoline 10



35

A benzene solution (75 ml) containing freshly prepared 10[18] (0.77g, 4.5 mmoles) was treated with ethyl pyruvate oxime (0.51g, 4.6 mmoles) in a 150 ml round-bottom flask. The mixture was refluxed under nitrogen with a Dean-Stark water separator attached during 36 hr on a steam bath. About 30 ml of partly cloudy distillate was collected in the water separator. The solvent was evaporated and the oily mixture was heated on the steam bath for another 24 hr

(since only 10 was observed by GLC with 3% SE-30 at 23°). The IR spectrum showed that some amide (1625 cm^{-1}) had been formed in the crude reaction mixture. Treatment of the oily mixture with ether gave a black solid (0.40g), which was washed with acetone and methanol. Chromatography on silica gel (1.7g) with 10:1 chloroform-methanol gave 0.16g (14%) of gray-white solid, m.p. 263-268° (dec). ν_{max}^{KBr} 1580, 1625, 2820, 3050 and 3150 cm^{-1}. δ(d6-DMSO) 2.09 and 2.17 (3H, two s in a ratio of 1:3, respectively; probably representing A and B:



A B

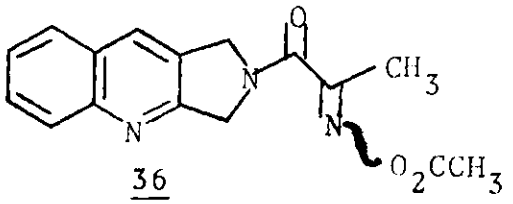
5.35 (4H, d of d, J = 5 Hz, Δν = 15 Hz) and 7.1-8.4 (5H, complex). Mass spectrum m/e (rel intensity) M^+ 255 (0), 238 (34), 197 (4) and 169 (100). EMD (Hitachi instrument) fragment (M^+-OH) wt. calculated for $C_{14}H_{14}N_3O$: 238.098. Found: 238.097 ± 0.004.

Alternate Preparation of 35 from 51

2,3-Dihydro-2-pyruvoyl-1H-pyrrolo [3,4-b] quinoline 51 (0.29g, 1.2 mmole) was added to a solution of 4.4 ml of water containing 0.78g (1.1 mmole) of hydroxylamine hydrochloride and 3.0 ml of 10% (w/w) aqueous sodium hydroxide solution. Then 12 ml of ethanol was added. The solution

was heated on the steam bath (with a boiling stick for 12 min. GLC (3% SE-30 at 230°) showed no evidence of 51. (It was found that pure 35 does not show a GLC peak under these conditions.) On stirring and cooling in ice-water a precipitate formed. Filtration gave a gray solid. Acetone washing removed some of the color, leaving 0.11g (36% yield) of 35, m.p. 258-262°C.

Reaction of 35 with Acetic Anhydride-Pyridine

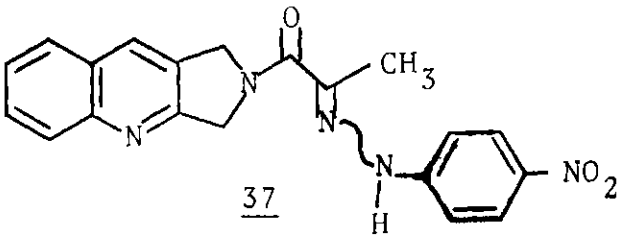


36

The oxime 55 (0.15g, 0.6 mmole) was treated with 20 ml of dry pyridine and 19 ml of distilled acetic anhydride. After stirring for 2 hr, the oxime had completely dissolved. The solution was stirred another 26 hr at RT. Then the excess acetic anhydride and pyridine were removed on the rotary evaporator at 35° and 5-10 mm. The solid residue was further dried for 25 min at 25-60° and 1-5 mm to give 0.178g (98% yield) of 36, m.p. 185-187° (methylene chloride). ν_{max}^{KBr} 1185, 1410, 1450, 1612, 1635, 1650, 1765 and 1780 cm^{-1}. NMR $\delta(CDCl_3)$ 2.23 (6H,s; with addition of Europium shift reagent this singlet becomes three closely spaced sharp signals), 4.98 (2H, s), 5.20 (1H, s), 5.27 (1H, d, J = 1 Hz) and 7.37-8.17 (5H, complex). Anal. Calculated for $C_{16}H_{15}N_3O_3$:

C, 64.64; H, 5.09; N, 14.13. Found: C, 64.25; H, 5.27; N, 13.92.

Reaction of Pyruvoyl Chloride (p-Nitrophenyl)-hydrazone with 2,3-Dihydro-1H-pyrrolo [3,4-b] quinoline (10)

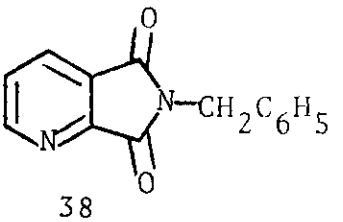


37

Freshly prepared 10 (6 mmoles from 2.0g of dihydrobromide salt) in 120 ml of methylene chloride was added dropwise to a solution of pyruvoyl chloride (p-nitrophenyl) hydrazone (Fisher, 1.42g, 5.9 mmoles) and 2 ml of triethylamine in 100 ml of methylene chloride with stirring under a nitrogen atmosphere. Only after about 80 ml of solution had been added did the yellow solid (the starting hydrazone) dissolve. Then after 4 hr, a precipitate began to form. When the mixture had been stirred for 23 hr at RT, it was filtered to give 0.50g (22%) of 37, m.p. 230-235°. ν_{max}^{KBr} 1260, 1325, 1590 (s), 1625 and 3400 cm^{-1}. The filtrate was treated with seven 100 ml portions of saturated sodium bicarbonate solution. Evaporation of the methylene chloride gave an oil (0.32g), which was triturated with ethyl acetate to give 0.02g (0.9%, total yield 23%) of 37, m.p. 230-235°. ν_{max}^{KBr} 1260, 1325, 1590 (s), 1625 and 3400 cm^{-1}. The sample

was too insoluble in dimethyl sulfoxide for an NMR spectrum to be obtained. Mass spectrum m/e (rel intensity) M^+ 375 (29) and 169 (100). EMD (Hitachi instrument) Mol. wt. calculated for $C_{20}H_{17}N_5O_3$: 375.133; rel intensity M+1, 23.91; M+2, 3.34. Found: 375.135 ± 0.006; rel intensity M+1, 25.03; M+2, 3.67.

Reaction of 2,3-Pyridinedicarboxylic Anhydride with Benzylamine

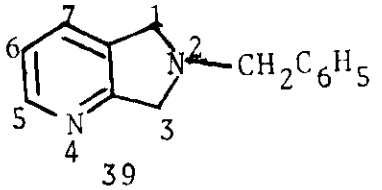


38

2,3-Pyridinedicarboxylic anhydride (Aldrich, quinolinic anhydride, 1.91g, 12.8 mmoles), benzylamine hydrochloride (2.0g, 14 mmoles) and anhydrous sodium acetate (4.0g, mmoles) were added to glacial acetic acid (30 ml) in a 50 ml round-bottom flask. The solution was stirred by a magnetic stirring bar under a nitrogen atmosphere and heated by a heating mantle. The solution was warmed to reflux and held at moderate reflux for 45 min. Upon slow cooling (after 60 hr), white crystals formed in the yellow solution. The acetic acid solution was evaporated to dryness. The residue and the white crystals were combined and dissolved in 50 ml of 4:1 methanol-chloroform. Then 1:1 benzene-chloroform (120 ml) was added to precipitate any inorganic salts. The

solution was filtered, extracted with 25 ml of water and dried over sodium sulfate. Evaporation of the organic solvents gave an oil which crystallized on standing. Trituration with acetone gave a fine white solid (2.37g, 78% yield), m.p. 159-161°, m.p. of the analytical sample 162-163°(chloroform-acetone). ν_{max}^{KBr} 1380, 1590, 1723 (s) and 1775 cm^{-1}. NMR δ(trifluoracetic anhydride) 4.25 (2H,s) and 6.44-8.44 (8H, complex). Mass spectrum (Hitachi instrument) m/e (rel intensity) M^+ 238 (100), 201 (31), 200 (25), 182 (18), 181 (69), 155 (20), 91 (27) and 79 (99). Anal. Calculated for $C_{14}H_{10}N_2O_2$: C, 70.58; H, 4.23; N, 11.76. Mol. wt. 238. Found: C, 70.77; H, 4.28; N, 11.61.

Reaction of 38 with Lithium Aluminum Hydride: 2-Benzyl-2,3-dihydro-1H-pyrrolo [3,4-b] pyridine (39)



39

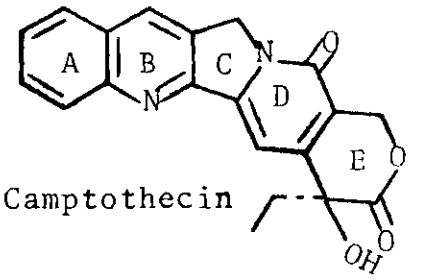
To 0.80g (21 mmoles) of lithium aluminum hydride in 150 ml of dry tetrahydrofuran was added 1.93g (8.1 mmoles) of N-benzyl-2,3-pyridinedicarboximide (38) in 50 ml of dry tetrahydrofuran. (Solubility is poor.) The mixture was refluxed for five hours after slow addition of 38. Magnetic stirring was employed. Excess lithium aluminum hydride

was destroyed by the addition of 5 ml of ethyl acetate, and then 3-5 ml of 2.5% sodium sulfate solution. The mixture was allowed to stir at 0-5°C for 30 minutes and then filtered. The precipitate was washed with three 10 ml portions of ether. The solvent was removed at reduced pressure (20 mm) on the rotary evaporator. The oily residue was dried by azeotropic distillation of benzene (three times). Finally the residue was distilled ($bp_{0.3}$ 130-170°C) to give 0.45g (26% yield) of crude 39. ν_{max}^{film} 1425, 1580, 1590, 1665 (impurity), 1765 (impurity), 2775, 2920 and 3040 cm^{-1}. Mass spectrum m/e (rel intensity) M^+ 210 (30), 209 (53), 135 (15), 133 (18), 119 (63), 107 (25), 106 (38), 92 (37), and 91 (100). This compound turned dark brown on standing and appeared to be unstable.

CHAPTER IV

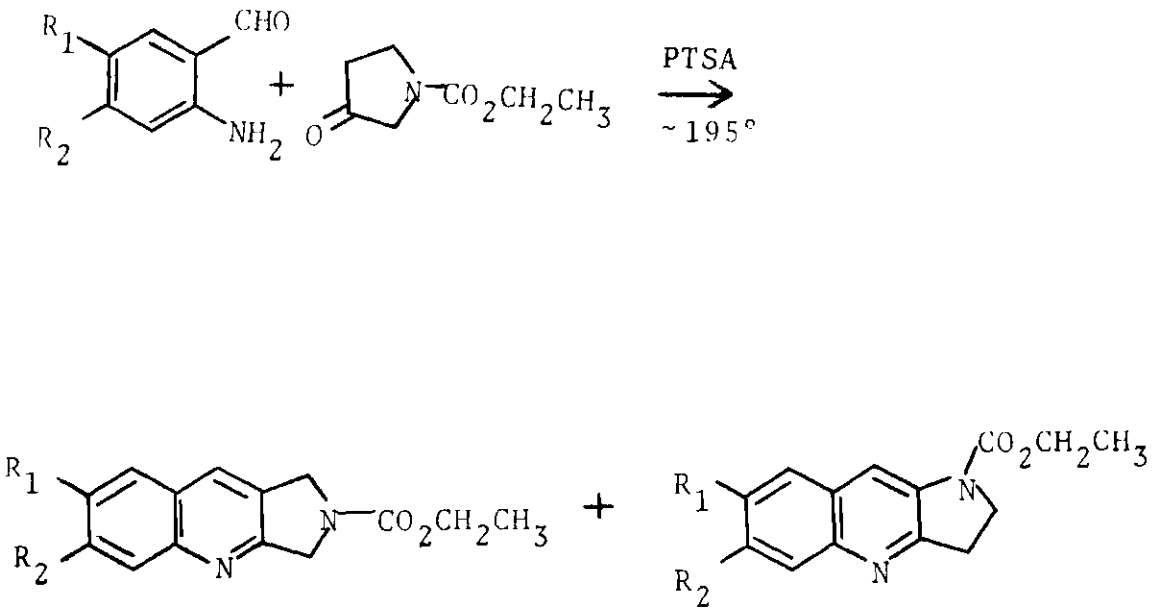
DISCUSSION OF RESULTS

The purpose of this work was to prepare racemic camptothecin and some of its analogs. Several promising intermediates have been prepared including one tetracyclic compound. All of our efforts revolved around reacting the dihydropyrroloquinoline 10[18] with a highly oxygenated compound which could be elaborated into the D and E rings of camptothecin. Compound 10 was prepared from the N-carbethoxy derivative 11 either by treatment with refluxing, aqueous hydrobromic acid or with aqueous ethanolic potassium hydroxide solution at room temperature. Various A-ring substituted derivatives of this dihydropyrroloquinoline type were prepared along with their isomers.



Camptothecin

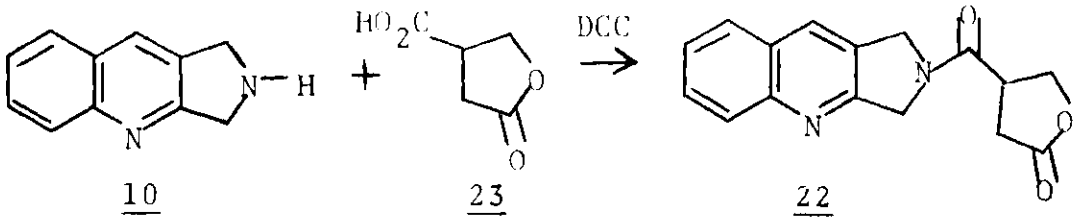
A Friedlander synthesis was employed using para-toluenesulfonic acid as catalyst. The appropriately substituted ortho-aminobenzaldehyde was mixed with ethyl 3-oxopyrrolidine-1-carboxylate[38] in a 2:1 molar ratio and heated for 3-5 minutes under nitrogen without solvent.



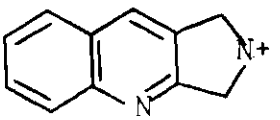
11	R_1, R_2 = H	12	R_1, R_2 = H
13	R_1 = Cl, R_2 = H	14	R_1 = Cl, R_2 = H
15	R_1 = OCH_3, R_2 = H	16	R_1 = OCH_3, R_2 = H
17	R_1 = H, R_2 = OCH_3	18	R_1 = H, R_2 = OCH_3
19	R_1 = OCH_3, R_2 = OCH_3	20	R_1 = OCH_3, R_2 = OCH_3
21	R_1, R_2 = $-OCH_2O-$		

It was found that these conditions favored formation of isomer 11 over isomer 12. The compounds of the isomer 11 type were prepared as possible intermediates in a synthesis of camptothecin analogs.

Various approaches toward a camptothecin synthesis were tried starting with compound 10. One of the oxygenated intermediates selected for reaction with 10 was paraconic acid (23)[47].

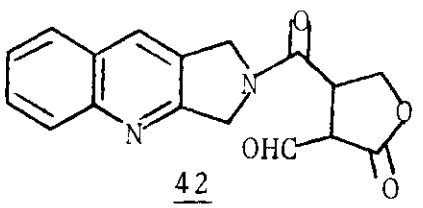


The product of this reaction, which employed dicyclohexyl carbodimide (DCC) to effect the condensation, was the expected amide 22. The infrared spectrum of the product showed bands at 1780 (lactone) and 1620 (amide) cm^{-1}. The NMR spectrum represented spectral characteristics similar to the two starting compounds. The mass spectrum of the product showed the anticipated molecular ion at m/e 282 with a base peak at m/e 169 representing the ion 41. This fragment was characteristically present in the mass spectrum of all our N-substituted, uncyclized dihydro-pyrroloquinoline intermediates.

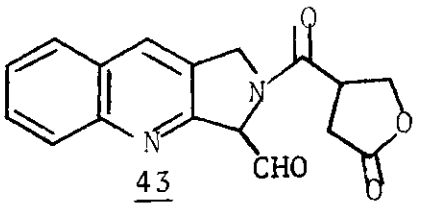


41

Formylation of 22 was attempted in expectation that one of the active methylene positions would react to provide a useful intermediate such as 42 or 43.



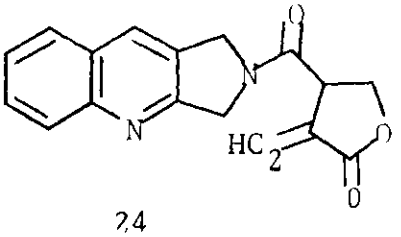
42



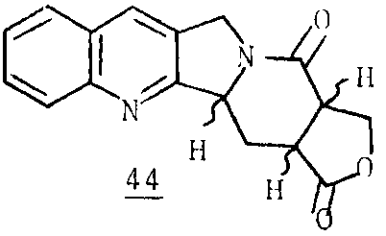
43

Under the alkaline reaction conditions employed for formylation, it was thought that ring closure might even occur and yield a pentacyclic intermediate through condensation at the other active methylene position. Various attempts at formylation using ethyl orthoformate and ethyl formate gave no indication of reaction.

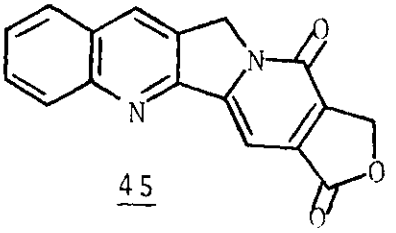
Attention was then focused on a synthesis based on 24. It was anticipated that cyclization of 24, which was prepared analogously to 22, would afford 44. Further oxidation was expected to lead ultimately to 45.



24



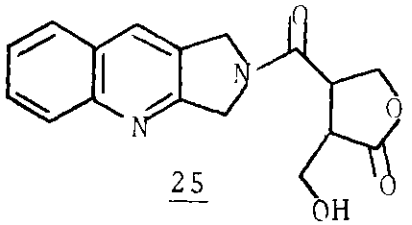
44



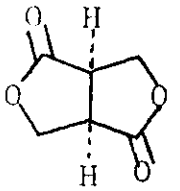
45

Compound 24 gave the appropriate combustion analysis data. The infrared spectrum showed bands at 1773 (lactone) and 1655 (amide) cm^{-1}. The NMR spectrum showed the expected quinoline protons at δ7.25-8.20 (5H) as well as exocyclic methylene protons at δ6.37 (1H) and δ6.70 (1H) as doublets with J = 2.5 Hz. A single spot was observed by thin layer chromatography using 20% acetone in benzene on silica gel. The expected ion at m/e 294 (77%) was observed in the mass spectrum. Efforts to cyclize 24 to 44 were unfruitful.

Considerable effort was spent on alcohol 25 prepared by Nabors,[20] who reacted the dihydropyrroloquinoline 10 with the symmetrical dilactone 46.[47]



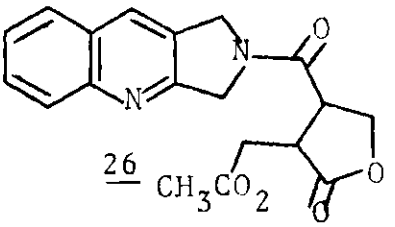
25



46

Attempts to cyclize 25 via the mesylate or the tosylate prepared *in situ* failed to provide 44. The acetate 26 was prepared in 87% yield, however, by employing refluxing acetic anhydride-pyridine (4:1, v/v). The crystalline solid produced melted at 190-192°, gave the expected combustion analysis, and showed infrared bands at 1745 (lactone), 1730 (acetate) and 1650 (amide) cm^{-1}. The NMR spectrum showed a singlet at δ1.3 (3H) for the acetate methyl group, two complex signals at δ2.9 (1H) and δ3.4 (1H), lactone protons at δ3.7-4.1 (4H), pyrrole-ring protons at δ4.4-5.0 (4H) and quinoline protons at δ7.1-7.7 and δ8.3 (5H).

Attempts were made to cyclize 25 to 44 in refluxing acetic acid-acetic anhydride.

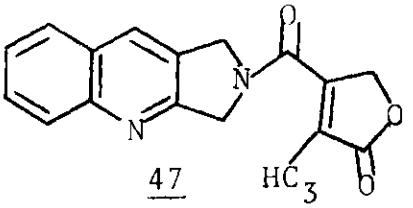
25 → 

26 → 44

Analogous reactions have been observed for 2-alkyl quinolines.[48] The resulting products were found to be 46

and the N-acetyl derivative of 10.

Acetate 26 was heated in acetic acid with sodium acetate to yield a mixture containing exocyclic lactone 24, endocyclic lactone 47 and a product having the same R_f value by thin layer chromatography as an authentic sample[16] of 44. It was concluded that the complexity of the mixture precluded the use of this reaction in a synthetic scheme.

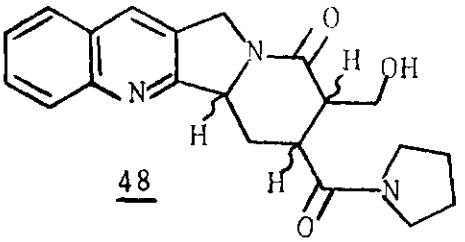


47

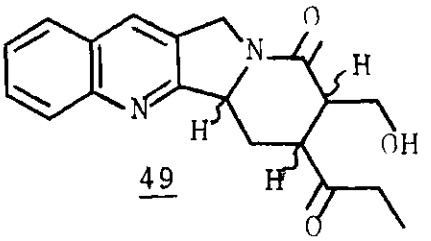
An authentic sample of 44 was converted to 45 in fair yield by heating to 250° with palladium on carbon. Compound 45 had m.p. 275-278° and displayed an ultraviolet spectrum (360 nm) characteristic of the pyridone ring system fused to the pyrroloquinoline system. This absorption is not observed in less fully aromatized compounds. See Table 2 on page 91.

Solvolysis of 26 in dimethyl sulfoxide containing sodium acetate gave a product melting at 275-278° in about 50% yield. This product did not give proper combustion analysis results. Heating with palladium on carbon gave only a very low yield of 45 as determined by UV. The dimethyl sulfoxide solvolysis product was treated with pyrrolidine in ethanol to yield a white, crystalline solid with infrared absorption bands at 1600-1625 and 3400 cm^{-1}.

The analysis found was in agreement with structure 48 as expected. Attempted dehydrogenation of 48, again gave unpromising results. Attempts to convert 48 to the ethyl ketone 49 via Grignard reagent were hindered by solubility problems. Reaction of 48 with ethyl magnesium chloride gave indications that diaddition had occurred to give the tertiary alcohol (by mass spectrometry). No indication of ketone 49 was found by infrared spectroscopy. Persistent efforts along these lines proved fruitless.

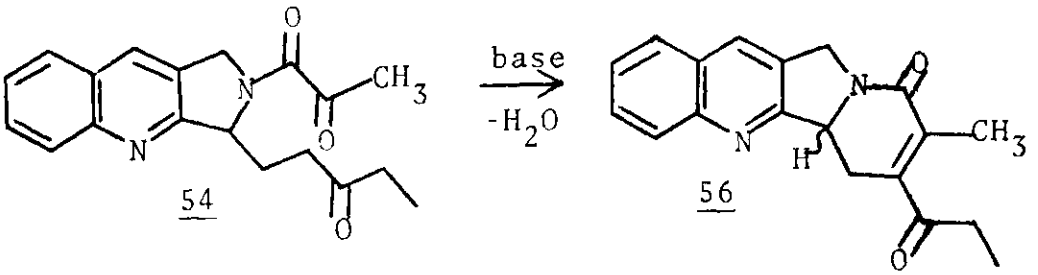


48



49

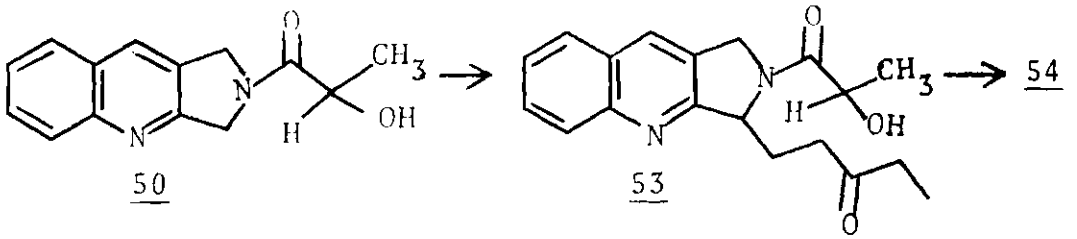
Attention was then given to the idea of constructing the D-ring of camptothecin by attaching two side chains to the crucial intermediate 10 and then condensing them together with base, as with 54 and 56.



54 56

The dihydropyrroloquinoline 10 was reacted with ethyl lactate to give 50, followed by a Michael reaction at C-3 using ethyl vinyl ketone to yield 53, which upon

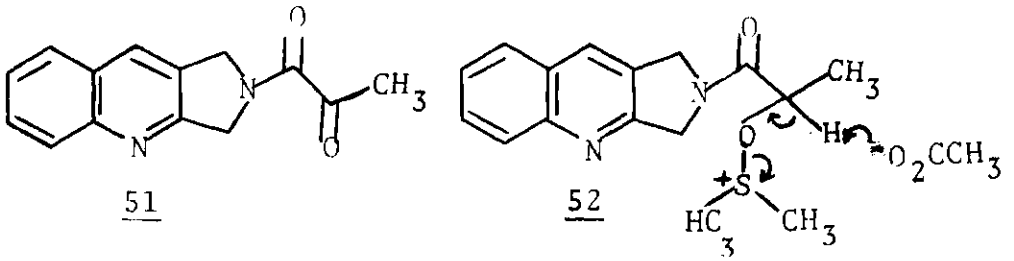
oxidation gave 54.



When 10 was heated with ethyl lactate at 80-100° for 12 hours under nitrogen, 50 was produced in 71% yield as an off-white solid, m.p. 222-226°, which showed a single peak by gas liquid chromatography on 3% SE-30 at 230°.

The infrared spectrum showed bands at 1625 (C=C, aromatic), 1660 (amide), 3250 and 3375 (alcohol) cm^{-1}. The NMR spectrum showed a doublet at δ1.43 (3H) for the terminal methyl group, a one-proton signal at δ3.42 which disappeared upon addition of deuterium oxide, a complex signal at δ4.60 (1H) and the usual pyrroloquinoline proton signals. Combustion analysis data were in agreement with structure 50. Oxidation of 50 with Sarrett's reagent gave 51 in low yield (~30%). The white crystalline product melted with decomposition at 192.5-197° and had bands in its infrared spectrum at 1700 (ketone) and 1630 (amide) cm^{-1}. The NMR spectrum of 51 contained a singlet at δ2.53 (3H), a complex pattern of signals at δ4.97-5.22 (4H), and the usual quinoline protons at δ7.42-8.17 (5H) in the characteristic pattern. The parent ion (M^+, 240) for 51 amounted to 10% of the base peak (m/e, 197) in the mass spectrum. The combustion

analysis was in agreement with structure 51.



Various attempts were made to improve the yield of 51 from 50. The reagents tried were: Jones reagent, ferric chloride in 0.1 N hydrochloric acid at 100° for 10 minutes, cupric acetate in refluxing aqueous acetic acid for 1-2 minutes, cupric sulfate and air with aqueous pyridine at 100° for 30 minutes, and bismuth trioxide in glacial acetic acid at 60° for 30 minutes.

These reagents were used since they are effective in converting α-hydroxy ketone compounds to α-diketo compounds. The reaction mixtures were observed by gas liquid chromatography (3% SE-30 at 230°). No significant amount of 51 was observed with any of these reagents. Freshly prepared, "active" manganese dioxide was also used without success, probably due to the difficulty in preparing the truly active reagent.[49] Sulfur trioxide-pyridine complex in dimethyl sulfoxide was used along with triethylamine. Gas liquid chromatography showed a 3:1 ratio of 51 to 50. Use of dimethyl sulfoxide-acetic anhydride[50] gave the best results, however. Initially a 73% yield of 51 was obtained. Using the work-up described by Clement, Dangieri and Tuman[51], a

quantitative yield was obtained. A good explanation of why compounds which give readily cleavable products (such as α-dicarbonyl compounds) are difficult to oxidize has been suggested by Clement et al. When the oxidation process involves a discrete electron transfer, cleavage is favored, hence a poor yield. A concerted process such as the one shown for 52[52] does not have this drawback, and the smooth conversion results in higher yields.

Preliminary studies on the model system 11 indicated that methyl vinyl ketone in refluxing acetic anhydride gave efficient conversion to a new, higher retention time product (on 3% SE-30 at 230°). A 40-fold excess of methyl vinyl ketone was heated with 11 for 48 hours to give a 15:85 ratio of 11 to product.

The Michael reaction was then attempted using ethyl vinyl ketone on pyruvamide 51. The excess ethyl vinyl ketone formed polymeric products which interfered with the isolation of the expected product and the gas liquid chromatography results were much less clear cut with 51, the "real" system. Other conditions were eventually tried after many failures with this set of conditions.

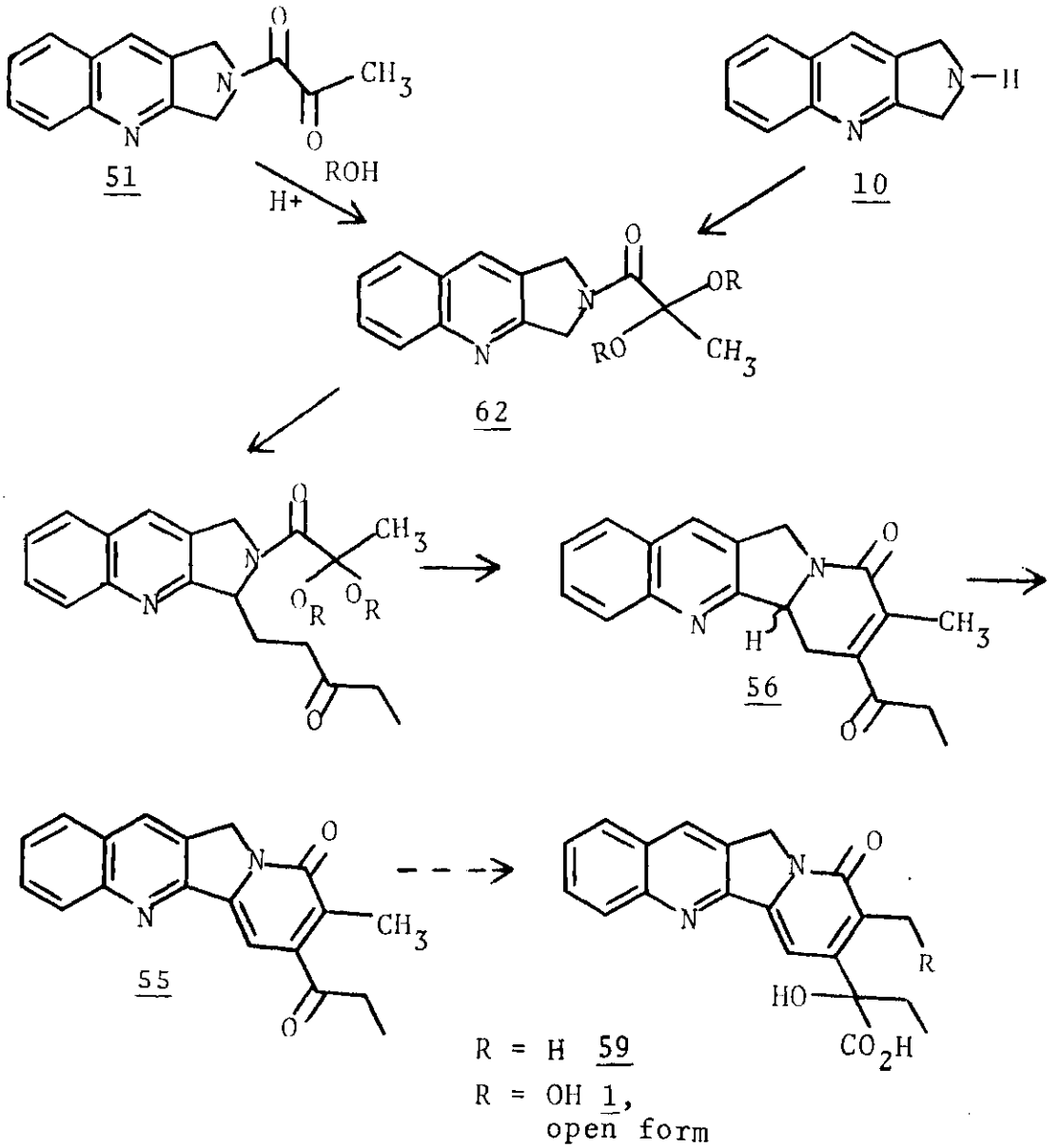
Compound 11 was dissolved in N,N-dimethylformamide and mixed with a three-fold excess of ethyl vinyl ketone. The mixture was sealed in a pyrex combustion tube and heated at 148° for periods of 24 and 48 hours. The better results were observed with longer reaction time. The starting

compound 11 was almost completely consumed in the reaction giving mainly a single higher retention time product plus a few percent of a much higher retention time product, probably due to addition of a second molecule of ethyl vinyl ketone. When pyruvamide 51 was treated similarly a much more complex mixture resulted. It was concluded that the pyruvamide side chain was competing in the Michael reaction. To overcome this problem it was decided to form the ketal of 51 in order to break up the resonance stabilization of the terminal enolate anion and to sterically restrict reaction at the terminal methyl group.

Pyruvamide 51 gave no reaction with ethylene glycol when heated at reflux in benzene under a water-separator for 20 hours with *para*-toluenesulfonic acid. Wall[8c] reported the failure of a simple ketone to give the ketal with ethylene glycol under standard conditions; with boron trifluoride, however, the product formed in 90% yield.

Attempts to prepare a ketal of 51 using the dimethyl,[53] diethyl[54] and ethylene glycol[55] ketals of pyruvate esters with 10 were unsuccessful, even using hindered amide synthesis techniques. No product could be detected by gas liquid chromatography or infrared spectroscopy (amide).

Thus the following sequence of reactions was proposed, but was never carried out.



Only after all these failures was it resolved to treat compound 50 with a threefold excess of ethyl vinyl ketone in N,N-dimethylformamide without catalyst in a sealed tube at 148° for 48 hours. About five percent of unreacted 50 was recovered from the work-up of the reaction. A 62% yield of 53 was produced, as indicated by infrared bands appearing at 3460 (hydroxyl), 1705 (ketone) and 1635

(amide) cm^{-1}; the NMR spectrum contained the expected quinoline protons at δ7.38-8.22 (5H), with the terminal methyl groups being observed at δ0.95 (3H) and δ1.47 (3H), with the methylene protons of the ketone side-chain appearing at δ2.08-2.83 (6H), the hydroxyl proton appeared at δ3.50-4.17 (1H), the C-2 proton on the lactoyl side chain at δ4.33-4.83 (1H), and the dihydropyrrole protons appeared as two signals centered at δ4.99 (2H), and a complex signal instead of the expected triplet appeared at δ5.46 (1H). Combustion analysis on the picrate (m.p. 143.5-144°) was very near the expected value (C, 54.38; calculated C, 54.05).

The oxidation with dimethyl sulfoxide-acetic anhydride was applied successfully to 53 to give 54. Thus the pyruvamide with ketone side-chain was obtained as a crystalline solid (m.p. 88-91°). The infrared spectrum showed no significant hydroxyl band (in practice all the pyrroloquinolines showed a trace of moisture), while bands were observed at 1707 and 1645 cm^{-1} for ketone and amide carbonyls, respectively. The NMR spectrum of 54 showed a terminal methyl at δ0.97 (3H, triplet), a complex pattern at δ2.00-2.66 (9H) representing the pyruvoyl methyl group and the methylene groups of the ketone side chain, a complex pattern at δ4.50-5.75 (3H) for the dihydropyrrole protons and quinoline protons in a complex pattern at δ7.34-8.16 (5H). Combustion analysis was in excellent agreement with the calculated values. The mass spectrum showed the expected

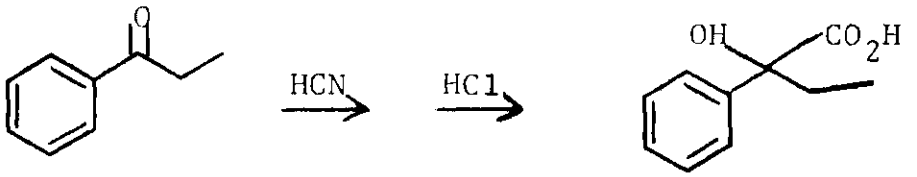
molecular ion at m/e 324. The strongest absorption in the UV spectrum was at 320 nm (log ε 4.43), which is characteristic of these 2,3-dihydro-1H-pyrrolo [3,4-b] quinolines containing the amide functionality.[18]

Treatment of 54 with ethanolic potassium hydroxide at room temperature for five hours gave not only the desired condensation product 56, but in addition afforded the fully aromatized pyrrolidone system in 55. A comparison of the UV spectra of pure 55 and 56 with that reported for natural camptothecin is shown in Table 2.

Table 2. A Comparison of Selected UV Absorptions for Compounds 55 and 56 with Natural Camptothecin

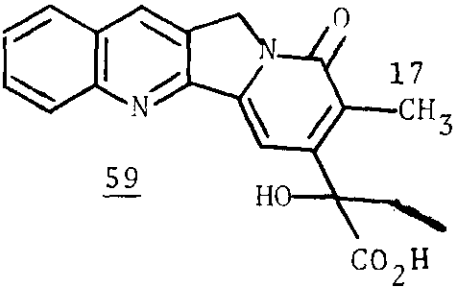
Compound	Wavelength (nm) (CH_3OH)	log ε
56	350	3.77
55	370	4.30
Camptothecin	370	4.30

A model system was examined to determine conditions for the addition of hydrogen cyanide to an ethyl aryl ketone, such as 55 or 56, followed by hydrolysis to the hydroxy acid.



57 58

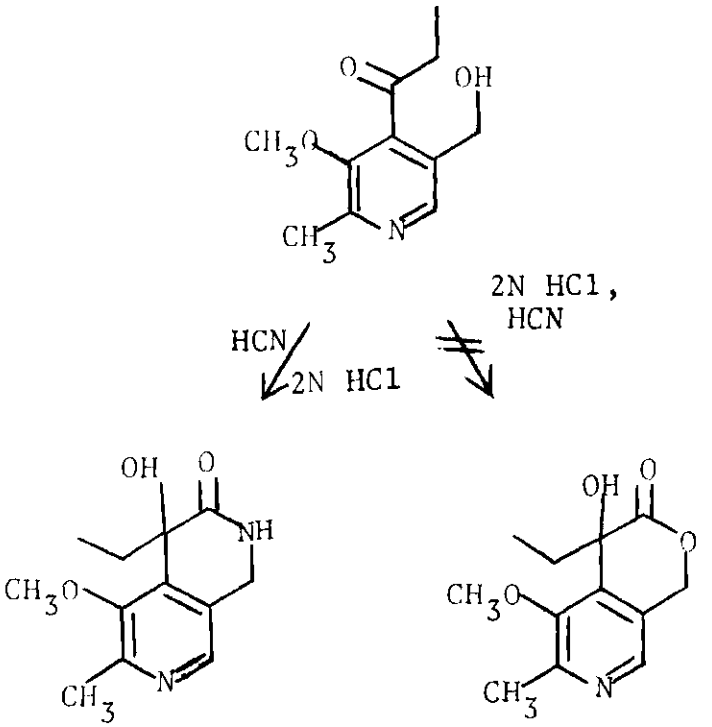
Propiophenene (57) was used as the model compound. It was converted to the corresponding hydroxy acid (58) in about 8% yield using an Organic Syntheses procedure.[56] When a similar reaction was applied to 55, no hydroxy acid was isolated. The use of freshly generated hydrogen cyanide at dry-ice temperature with a trace of potassium cyanide as catalyst in dimethyl sulfoxide solution also failed to afford the desired hydroxy acid 59 upon hydrolytic work-up.



59

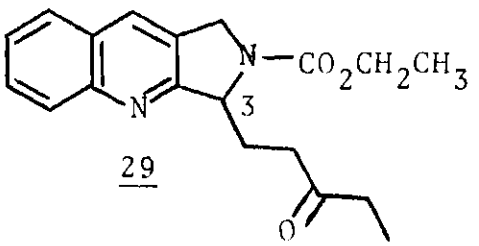
Compound 59 represents the racemic mixture of 17-desoxy camptothecin.

It is interesting to note the results of Kametani[36] and coworkers who reacted an aryl ethyl ketone (below) with hydrogen cyanide in hydrochloric acid. He obtained the lactam instead of the desired lactone.



Miscellaneous Reactions

Compound 11 previously prepared was treated with ethyl vinyl ketone in N,N-dimethylformamide without catalyst. The mixture was heated to 150° in a sealed tube for 48 hours to give 29.

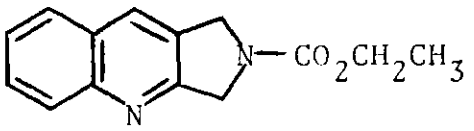


29

The oily product recovered in 62% yield was not fully characterized with elemental analysis, but an exact mass determination gave a molecular ion at 326.169 ± 0.007, compared with the theoretical value of 326.163 calculated for $C_{19}H_{22}N_2O_3$. The expected NMR spectrum was observed with poor integration for the alkyl protons on the C-3 side-chain. The C-3 proton appeared at δ5.15 (1H) as a broad triplet. It was concluded that compound 29 had been prepared, though not in pure form. Treatment of 29 with refluxing constant-boiling hydrobromic acid gave only a very complex, dark green mixture. Preparative thin layer chromatography on silica gel in 1:1 ethanol-chloroform with 1% methanol showed the crude reaction mixture to be inordinately complex. A 20 cm plate showed contiguous bands of green, grey, brown and black. Probably conditions of reaction were too harsh for the expected amino-ketone

product to survive. Wall[8c] found that even forming a simple amino-ketone from catalytic reduction of the nitro-ketone resulted in self condensation to the Δ^1-pyrroline N-oxide.

It was interesting to compare the reactivities of the isomers 11 and 12 with ethyl vinyl ketone in the uncatalyzed Michael reaction. During 18 hours in a sealed tube heated at 148°, each compound was reacted separately with three equivalents of ethyl vinyl ketone. The total amount of unreacted 11 was 75%. The mono addition product 29 was formed to the extent of 23% (GLC yield). Only a trace (<2%) of diaddition product was formed.

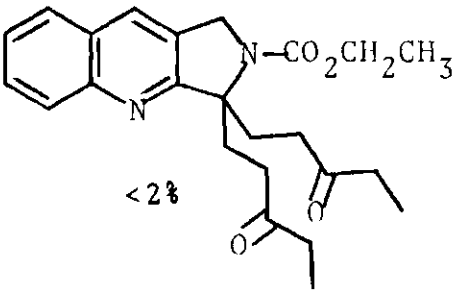


11

75% (unreacted)

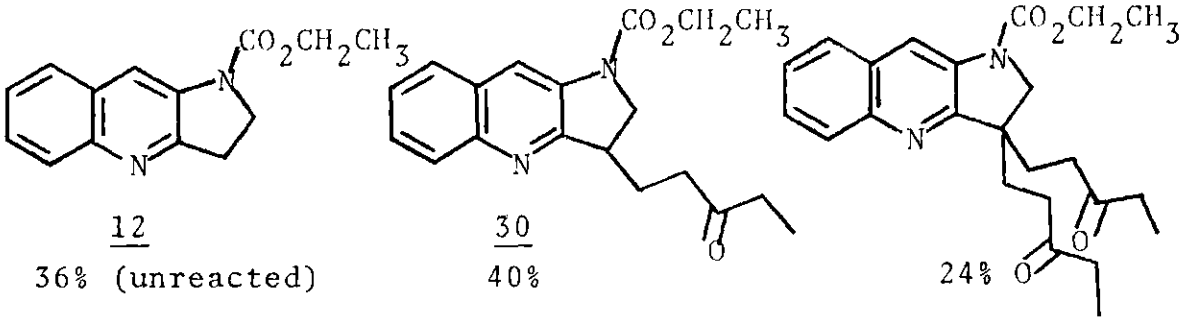
29

23%

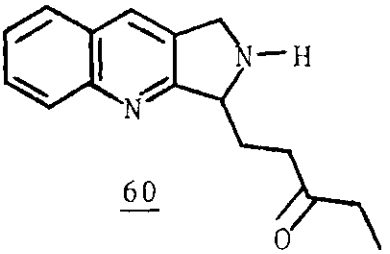


<2%

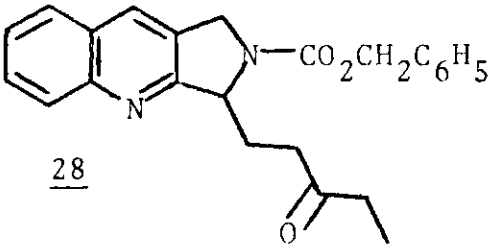
Compound 12, on the other hand, showed the greater reactivity in that only 36% of the starting compound was left unreacted under these same conditions. A single peak corresponding to monoaddition of ethyl vinyl ketone, 30, was present to the extent of 40%, while diaddition of ethyl vinyl ketone, 31, was represented by a higher retention time peak (24% of the total mixture).



When the hydrolysis of 29 proved to be impractical as a source of the amino ketone 60, a new N-protecting group



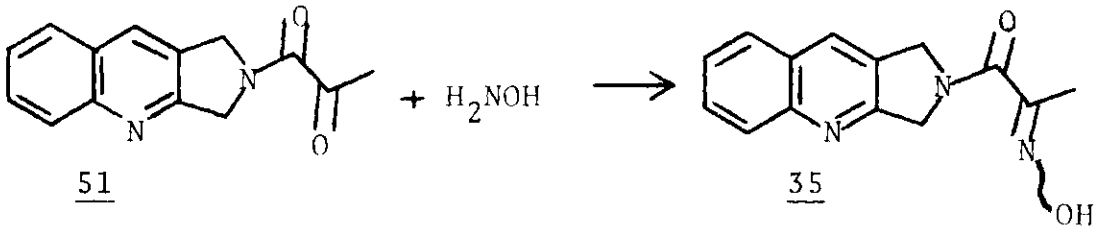
was considered. A simple hydrogenolysis was expected to improve the chances of obtaining compound 60 (before Wall's experience with a similar system was considered). Compound 27 was prepared from the free amine 10. Addition of ethyl vinyl ketone to 27 went smoothly to yield crystalline 28, m.p. 124-125.5° (methanol). The combustion analysis data closely agreed with the theoretical values. Unfortunately,



the hydrogenolysis of 28 with palladium on carbon at 60 psig

gave only what seemed to be a non-volatile, polymeric mixture. Therefore, this route (via 60) to 53 was abandoned.

Another approach which was considered is the sequence shown in Figure 6 (page 98). Compound 36 was prepared in two steps from 10 as shown. Combustion data and NMR spectra confirmed that 36 was indeed formed. The second step proved to be quantitative. Compound 36 was also prepared from 51 by the reaction shown below.



51 35

The scheme was abandoned, however, when another route proved more promising, namely, the route which ultimately gave 56, but could not be extended to yield 59. Furthermore, the transformation of 65 to 66 was not expected to exceed 48% efficiency based on an analogous system described by House.[57] Our case is also complicated because of salt formation at the quinoline nitrogen site.

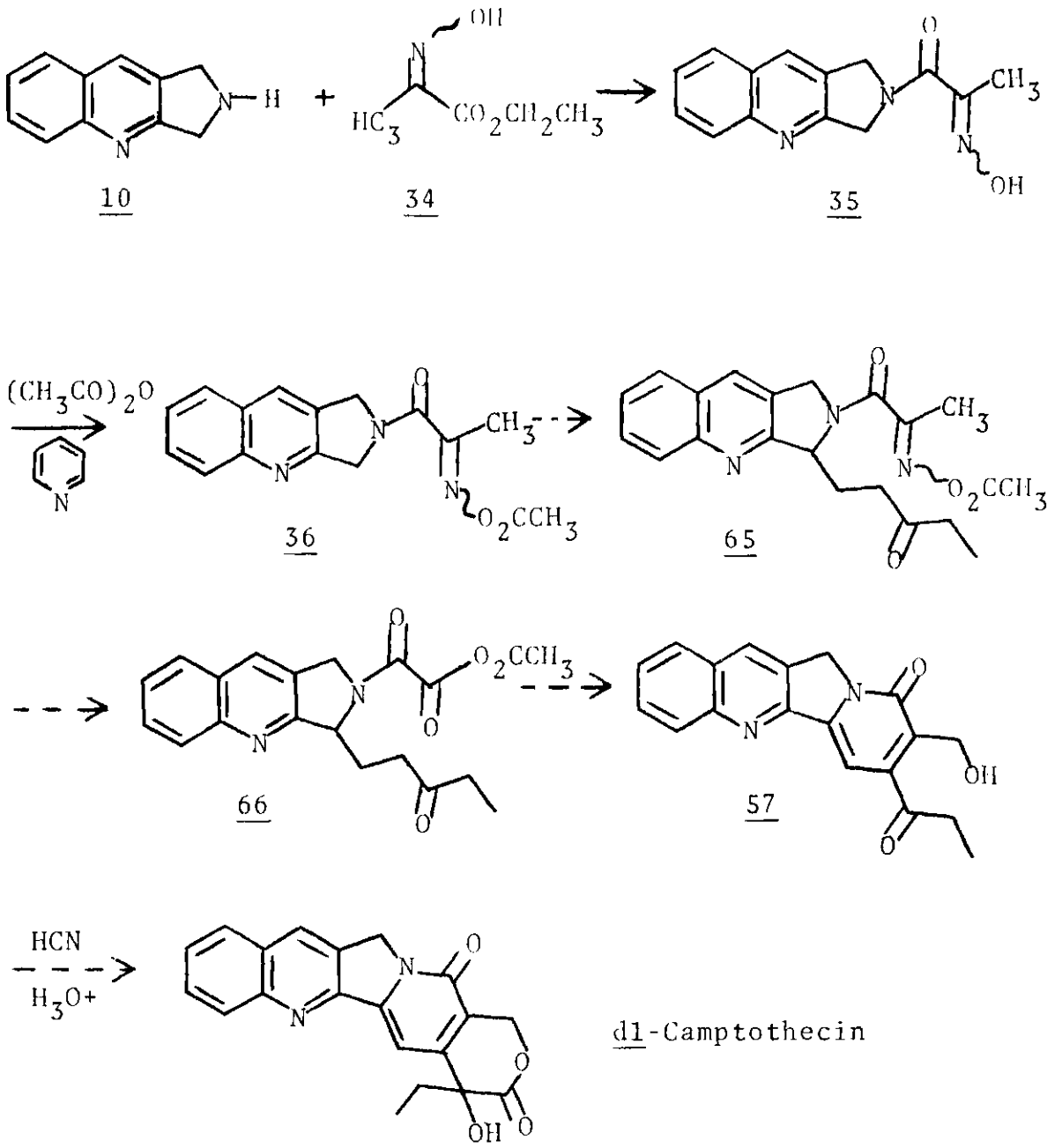


Figure 6. A Proposed Route to dl-Camptothecin

CHAPTER V

CONCLUSIONS

Several intermediates in the synthesis of camptothecin, an unusual pentacyclic alkaloidal tumor inhibitor, have been prepared. A number of approaches to the synthesis have also been explored with the intention of preparing analogous compounds for testing. Many intermediates (more than 52) were submitted to the National Cancer Institute for screening as potential anti-cancer drugs (Table 3, Appendix). As many as 28 compounds were also submitted for testing against malaria. No significant activity was noted, however, with any of these intermediates. As could be expected, none proved useful. Some did have high toxicity though.

The tetracyclic intermediate 55 was prepared in four steps from the pyrroloquinoline 10. Attempts to convert 55 to 17-desoxycamptothecin (59) were not successful. Aromatization of 56 to 55 was observed under alkaline condensation conditions.

CHAPTER VI

RECOMMENDATIONS

In order to be of practical value the synthesis of tetracyclic 55 should have the yields optimized to afford a workable supply of this intermediate.

Further attempts to transform compound 55 into racemic camptothecin or at least its 17-desoxy analog 59 might be worthwhile. Biological testing of compound 59 would shed light on the structure-activity relationship of camptothecin.

APPENDIX

Molecular ellipticity, $[\theta]$, is given by

$$[\theta] = \frac{4500}{\pi dc} \ln 10 \text{ (instrument scale)x(chart measurement)}$$

$$[\theta] = 3300 \left(\frac{1}{dc}\right) \text{ (instrument scale)x(chart measurement)}$$

where d is the cell path in cm and c is the concentration in moles per liter.

The data recorded for ORD measurements are given below, where α_{589} is the observed rotation at 589 nm, $[\alpha]_D$ is the specific rotation at 589 nm and $[\Phi]_{589}$ is the molecular rotation at 589 nm.

$$\alpha = \text{(instrument scale in degrees)x(chart measurement)}$$

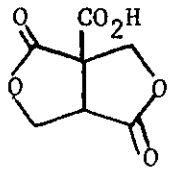
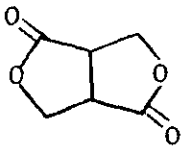
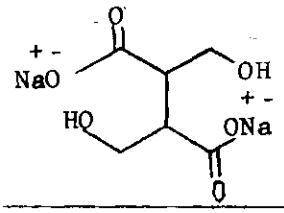
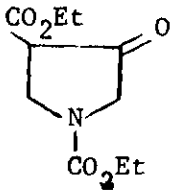
$$[\alpha]_D = \frac{100 \times \alpha_{589}}{\text{(conc. in g/100 ml)x(pathlength in decimeters)}}$$

$$[\Phi]_{589} = \frac{[\alpha]_{589} \times \text{(Mol. wt.)}}{100}$$

$$a = \frac{[\Phi]_1 - [\Phi]_2}{100}$$

The molecular amplitude, a, is defined as the difference between the molecular rotation at the extremum (peak or trough) of the longer wavelength $[\Phi]_1$, and the molecular rotation at the extremum of the shorter wavelength $[\Phi]_2$, divided by 100.

Table 3. National Cancer Institute Screen Data on Some Camptothecin Intermediates

Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	**SG-C-13-A[21,41]	124006	LE	400	6/6	101
			LE	200	6/6	106
			LE	100	6/6	106
			LE	50	5/5	97
	**SG-C-13-B[21,41]	124007	LE	400	6/6	109
			LE	200	6/6	107
			LE	100	6/6	101
			LE	50	6/6	107
	**SKG-26-1[58]	124008	LE	400	6/6	106
			LE	200	6/6	101
			LE	100	6/6	121
			LE	50	6/6	107
	JN-C-21-1[20,34]	124009	LE	400	6/6	109
			LE	200	6/6	107
			LE	100	6/6	106
			LE	50	6/6	103

*Refer to "Instruction 14, Screening Data Summary Interpretation," Drug Research and Development, Division of Cancer Treatment, NCI, Bethesda, Maryland 20014.

**The above compounds were tested for antimalarial activity at Walter Reed Army Institute of Research. A comparison of responses to test compounds by Plasmodium berghei KBG 173 malaria in mouse as expressed in mean survival times versus mean survival times of untreated controls indicated that no cures were effected.

†The T/C parameters were either mean or median survival time, except for host KB (Cell culture). The host in all other cases was mouse.

Table 3 (continued)

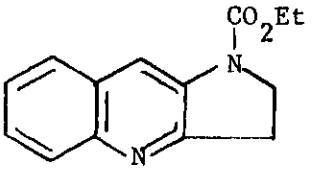
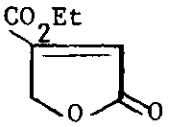
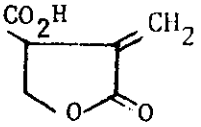
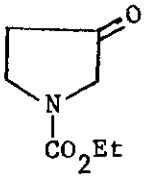
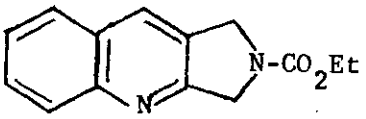
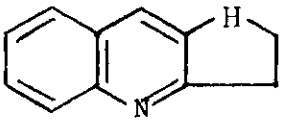
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	**JN-C-25-1[18,20]	124010	LE	400	3/6	---
			LE	200	5/6	97
			LE	100	6/6	109
			LE	50	6/6	106
			LE	400	6/6	---
			LE	200	6/6	93
			LE	100	6/6	90
			LE	50	6/6	100
	JN-C-30-1[58]	124641	LE	400	6/6	102
	**SCB-28[42]	124901	LE	75	1/6	---
			LE	18	6/6	106
			LE	400	0/6	---
			LE	300	0/6	---
			LE	150	1/6	---
			LE	75	6/6	98
	JN-C-23-1[16,18,24]	126739	LE	360	6/6	100
			LE	400	6/6	94
	**JN-C-23-2[16,18,20]	126740	LE	400	3/6	---
			LE	300	4/6	95
			LE	150	6/6	104
			LE	75	6/6	98
			LE	300	2/6	---
			LE	150	5/6	101
			LE	75	6/6	100
	JN-C-71-1[18,20]	126870	LE	400	6/6	97
			LE	400	5/6	109
			LE	200	6/6	97
			LE	100	6/6	95

Table 3 (continued)

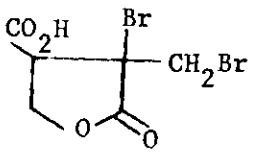
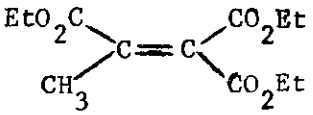
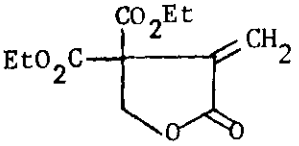
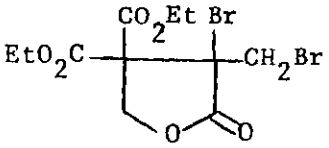
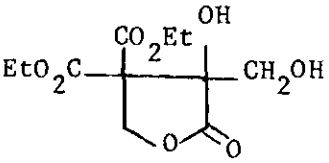
Structure	GTT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	**SCB-34[58]	126871	LE	400	0/6	---
			LE	300	4/6	101
			LE	150	6/6	98
			LE	75	5/6	101
	** 1-PTZ-5.2[58]	126872	LE	400	5/6	108
	**1-PTZ-5.3[42]	126873	LE	400	0/6	---
			LE	300	1/6	---
			LE	150	0/6	---
			LE	75	1/6	---
			LE	80	2/6	---
			LE	40	6/6	108
			LE	20	6/6	100
			LE	10	6/6	100
			LE	5	6/6	100
			LE	40	1/6	---
			LE	20	6/6	95
			LE	10	6/6	103
			LE	5	6/6	98
	1-PTZ-7.1[58]	126874	LE	400	6/6	97
	1-PTZ-9.1[58]	126878	LE	400	0/6	---
			LE	300	0/6	---
			LE	150	5/6	95
			LE	75	6/6	106

Table 3 (continued)

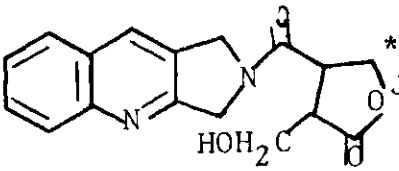
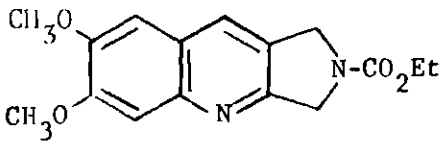
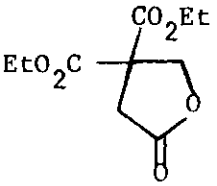
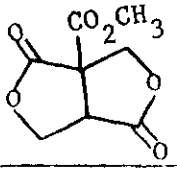
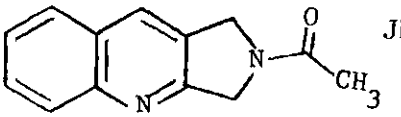
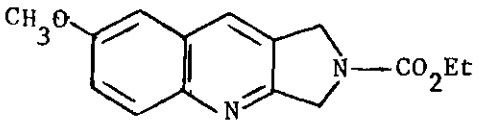
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	** JNC-73-1[20]	129229	LE	400	6/6	91
			LE	400	6/6	98
	**KF-88-A	146041	LE	400	6/6	108
			LE	200	6/6	94
			LE	100	6/6	109
			KB w slope -0.72 ED50 4.9 x 10			
			PS	400	6/6	105
			PS	200	6/6	90
			PS	100	6/6	105
	**GPN-4-A[40]	138323	LE	400	6/6	98
			LE	200	6/6	96
			LE	100	6/6	107
			LE	500	6/6	94
	** LZ-256[58]	138324	LE	400	6/6	109
			LE	200	6/6	98
			LE	100	6/6	100
	JN-C-89.1[18,20]	138325	LE	400	5/6	96
			LE	200	6/6	107
			LE	100	6/6	98
	**KF-66-B-1	138326	LE	400	6/6	105
			LE	200	6/6	89
			LE	100	6/6	94
			LE	200	6/6	95
			LE	100	6/6	95
			LE	500	6/6	98
			LE	300	6/6	91

Table 3 (continued)

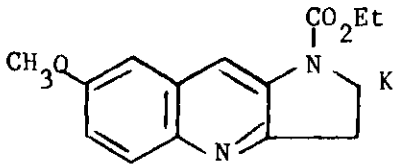
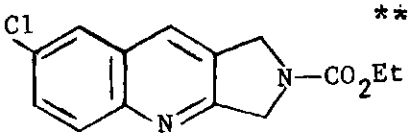
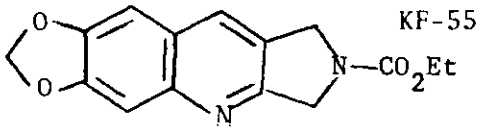
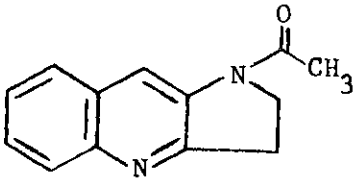
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	KF-66-B-2	138327	LE	400	5/6	100
	** KF-49-A	138328	LE	400	6/6	102
	KF-55-A	138329	LE	400	6/6	100
			LE	200	6/6	96
			LE	100	6/6	94
	JN-C-11-1[18,20]	138330	LE	400	0/6	---
			LE	300	0/6	---
			LE	150	6/6	101
			LE	75	6/6	95
			LE	37	6/6	104
			LE	340	0/6	---
			LE	225	0/6	---
			PS	150	5/6	---
			PS	75	6/6	125
			PS	37	6/6	130
			PS	56	6/6	104
			PS	37	6/6	104
			PS	24	6/6	123
			PS	56	6/6	110
			PS	37	5/6	105
			PS	25	6/6	105
			LE	150	5/6	88
			LE	75	6/6	94
			LE	37	6/6	96
			LE	150	6/6	106
			LE	75	6/6	104
			LE	37	6/6	104

Table 3 (continued)

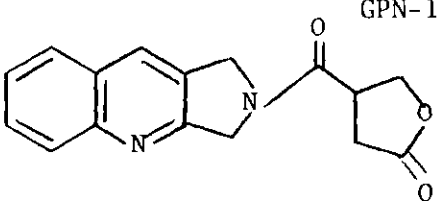
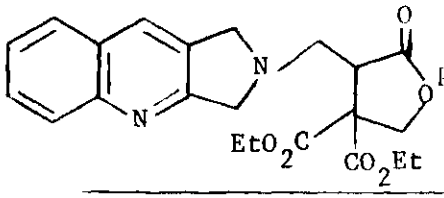
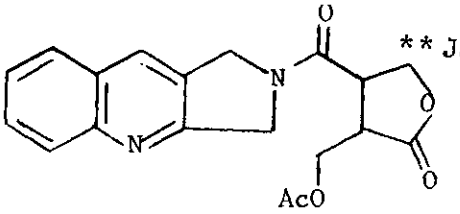
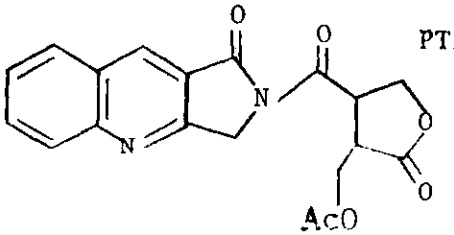
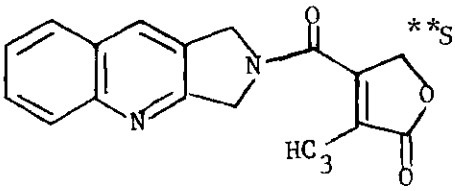
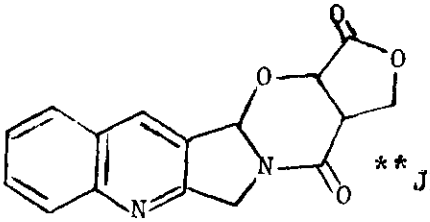
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	**GPN-1[58]	138331	LE	400	6/6	92
	PTZ-11.1[58]	138332	LE	400	6/6	96
	** JSB-C-1-2[58]	138333	LE	400	6/6	87
	PTZ-3.4[58]	138334	KB	with slope-0.69 ED50 1.2 with slope-1.37 ED50 3.6		
	**SKG-13-3[58]	138335	LE	400	6/6	96
	**JN-C-95-1[20]	138336	LE	400	6/6	97

Table 3 (continued)

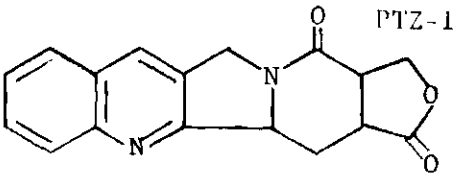
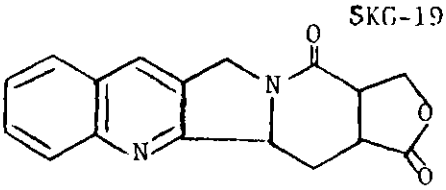
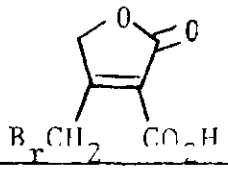
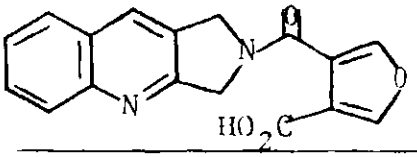
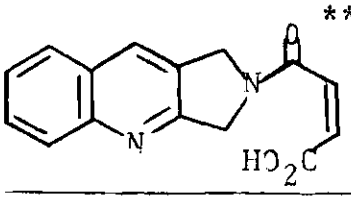
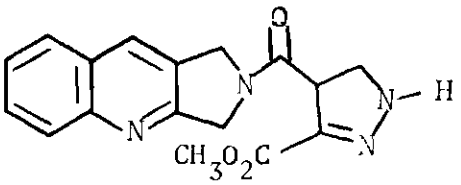
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	PTZ-134.1[58]	138337	LE	400	6/6	90
	SKG-19-2[58]	138338	LE	400	6/6	92
	1-PTZ-77.12[58]	138482	KB			
		with slope -0.97 ED50 25 x 10				
	DAM-55[14]	140898	LE	160	6/6	103
			LE	80	6/6	100
			LE	40	6/6	105
			LE	20	6/6	97
			LE	10	6/6	103
	**JN-C-275[58]	140899	LE	160	6/6	96
			LE	80	6/6	100
			LE	40	6/6	105
			LE	20	6/6	99
			LE	10	6/6	99
	JN-C-281[58]	140900	LE	160	6/6	97
			LE	80	6/6	92
			LE	40	6/6	103
			LE	20	6/6	99
			LE	10	6/6	103
			LE	160	6/6	99
			LE	80	6/6	97
			LE	40	6/6	91
			LE	20	6/6	97
			LE	10	6/6	94

Table 3 (continued)

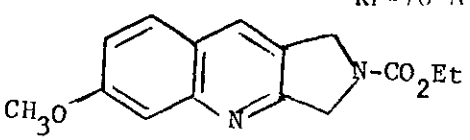
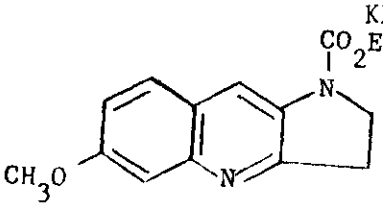
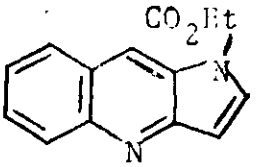
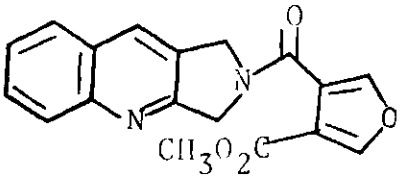
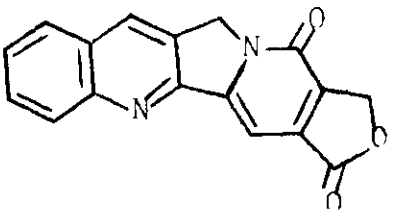
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	** KF-76-A	142989	LE	400	6/6	103
			LE	200	6/6	101
			LE	100	6/6	98
	KF-76-B	142990	LE	400	6/6	98
			LE	200	6/6	98
			LE	1--	6/6	103
			LE	400	6/6	90
			LE	200	6/6	94
			LE	100	6/6	92
	KF-111	145947	LE	400	6/6	90
			LE	200	6/6	105
			LE	100	6/6	100
			KB	-0.92 ED 50 1.9 x 10		
	JTB-3-6-71[58]	145948	LE	400	6/6	94
			LE	200	6/6	95
			LE	100	6/6	103
			PS	100	6/6	104
			PS	50	6/6	100
			PS	25	6/6	100
			KB			
		with slope -0.0 ED50 M 1.0 x 10^2				
	JN-C-348[16]	145951	LE	400	6/6	103
			LE	200	6/6	89
			LE	100	6/6	89
			KB			
			slope ED50 -0.0 1.0 x 10^2			

Table 3 (continued)

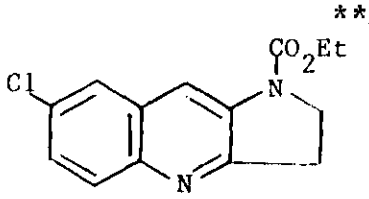
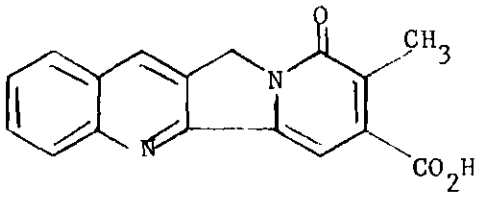
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	**KF-97	146042	LE	400	6/6	106(98)
			LE	200	6/6	110(93)
			LE	100	6/6	104(95)
			KB	with slope -0.56 ED50 1.0 x 10		
	JN-C-361[58]	147740	LE	400	6/6	98
			LE	200	6/6	106
			LE	100	6/6	95
			PS	200	6/6	96
			PS	100	6/6	100
			PS	50	6/6	92
			PS	200	4/6	100
			PS	100	6/6	113
			AK	200	10/10	94
			AK	100	10/10	100
			AK	50	9/10	102
			KB	slope -0.0 ED50 1.0×10^2		
			PS	200	4/6	100
			PS	100	6/6	113
			PS	50	6/6	95
			LE	400	6/6	94
			LE	200	6/6	95
			LE	100	6/6	103
			PS	100	6/6	104
			PS	50	6/6	100
			PS	25	6/6	100
			AK	200	10/10	---
			AK	100	10/10	100
			AK	50	10/10	93
			B1	400	3/10	---
			B1	200	10/10	---
			B1	100	10/10	100
			B1	100	10/10	89
				50	10/10	94
				25	10/10	102

Table 3 (continued)

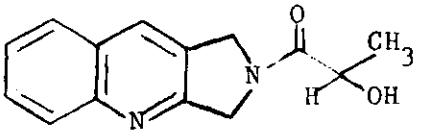
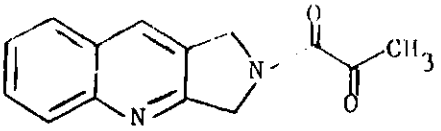
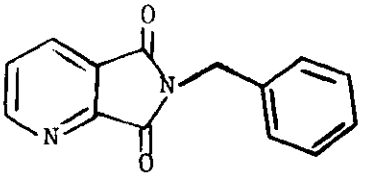
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	**KF-138	151662	LE	400	3/6	---
			LE	200	6/6	96
			LE	100	5/6	100
			PS	200	6/6	95
			PS	100	5/6	104
			PS	50	6/6	95
			KB with slope -0.0 ED50 1.0×10^2			
	**KF-139	151663	LE	400	5/6	102
			LE	200	5/6	102
			LE	100	6/6	115
			PS	200	6/6	100
			PS	100	6/6	100
			PS	50	6/6	100
			KB slope -0.0 ED50 1.0×10^2			
	**KF-82	151664	LE	400	6/6	102
			LE	200	6/6	103
			LE	100	6/6	107
			LE	400	6/6	102
			LE	200	5/6	96
			LE	100	6/6	103
			PS	400	6/6	90
			PS	200	6/6	95
			PS	100	6/6	95
			PS	37	6/6	100
			PS	18	6/6	95
			PS	9	6/6	109
			KB slope -0.0 ED50 1.0×10^2			

Table 3 (continued)

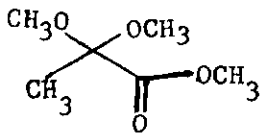
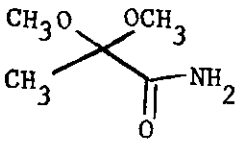
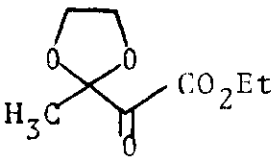
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	KF-8'-A[54,58]	159637	LE	400	6/6	96
			LE	200	6/6	100
			LE	100	6/6	96
			LE	400	5/6	105
			LE	200	6/6	112
			LE	100	6/6	104
			PS	400	5/6	100
			PS	200	5/6	100
			PS	100	6/6	100
			PS	400	6/6	100
			PS	200	6/6	109
			PS	100	6/6	100
			KB	slope -0.0 ED50 1.0×10^2		
	KF-12'-A[58]	159638	LE	400	2/6	---
			LE	200		
			LE	100	6/6	102
			LE	100	1/6	
			LE	50	5/6	88
			LE	25	6/6	94
			PS	50	6/6	103
			PS	25	6/6	96
			PS	12	6/6	100
			PS	50	4/6	104
			PS	25	6/6	122
			PS	12	6/6	118
			KB	slope -0.96 ED50 2.0 x 10		
	**KF-17'-A[55]	163401	LE	400	6/6	99
			LE	200	6/6	96
			LE	100	6/6	103
			KB	no detail lines		
			LE	400	6/6	95
			LE	200	6/6	100
			LE	100	6/6	101
			KB	Slope -0.27 ED50 1.0×10^2		

Table 3 (continued)

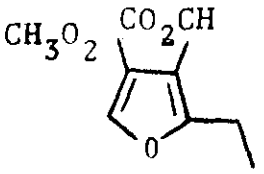
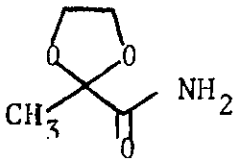
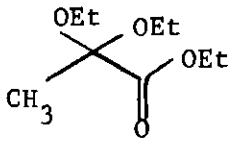
Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	KF-17'-A[55] continued		B1	400	10/10	104
			B1	200	10/10	104
			B1	100	10/10	95
			LE	400	6/6	99
			LE	200	6/6	96
			LE	100	6/6	103
			LE	400	6/6	95
			LE	200	6/6	100
			LE	100	6/6	101
	**KF-95[58]	164303	LE	400	6/6	104
			LE	200	6/6	94
			LE	100	6/6	95
			KB	slope -0.31 ED50 7.5 x 10		
			LE	400	5/6	100
			LE	200	6/6	96
			LE	100	6/6	96
			B1	400	10/10	95
			B1	200	10/10	97
			B1	100	10/10	102
			LE	400	6/6	104
			LE	200	6/6	94
			LE	100	6/6	95
			LE	400	5/6	100
			LE	200	6/6	96
			LE	100	6/6	96
	KF-27'[58]	163402	LE	400	6/6	100
			LE	200	6/6	96
			LE	100	6/6	103
			PS	400	6/6	95
			PS	200	6/6	95
			PS	100	6/6	100
			KB	slope -0.0 ED50 1.0×10^2		

Table 3 (concluded)

Structure	GIT No.	CCNSC No.	Tumor*	Dose (mg/kg)	Toxicity (survivors)	%T/C†
	**KF-163[53,58]	38952	LE	450	6/6	97
			LE	400	6/6	102
			LE	200	6/6	96
			LE	100	6/6	109
			LE	400	5/6	88
			LE	200	6/6	96
			LE	100	5/6	103
			PS	400	6/6	91
			PS	200	6/6	91
			PS	100	6/6	100
			PS	200	6/6	115
			PS	100	6/6	110
			PS	50	6/6	105

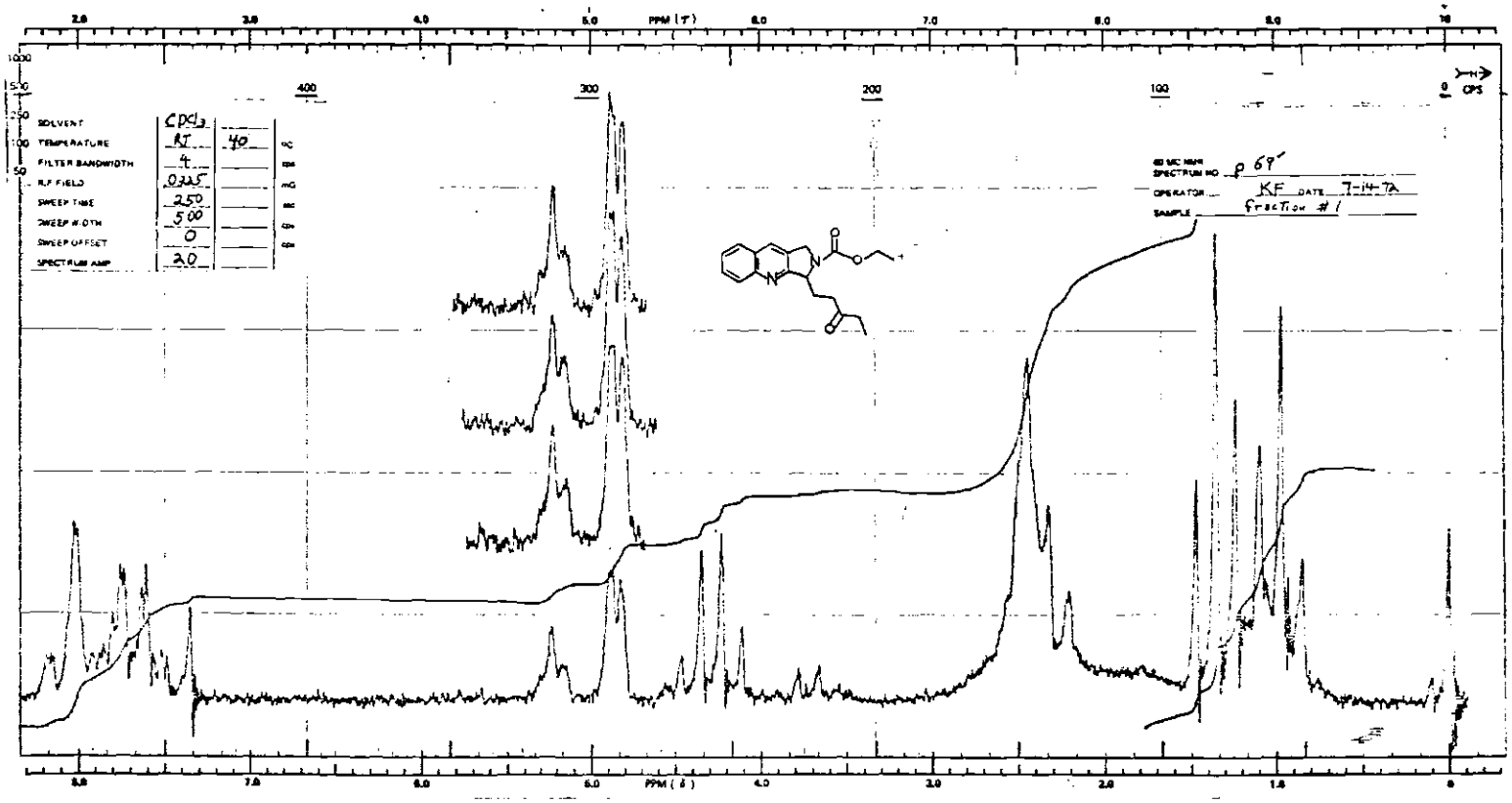


Plate 1. NMR Spectrum of 29

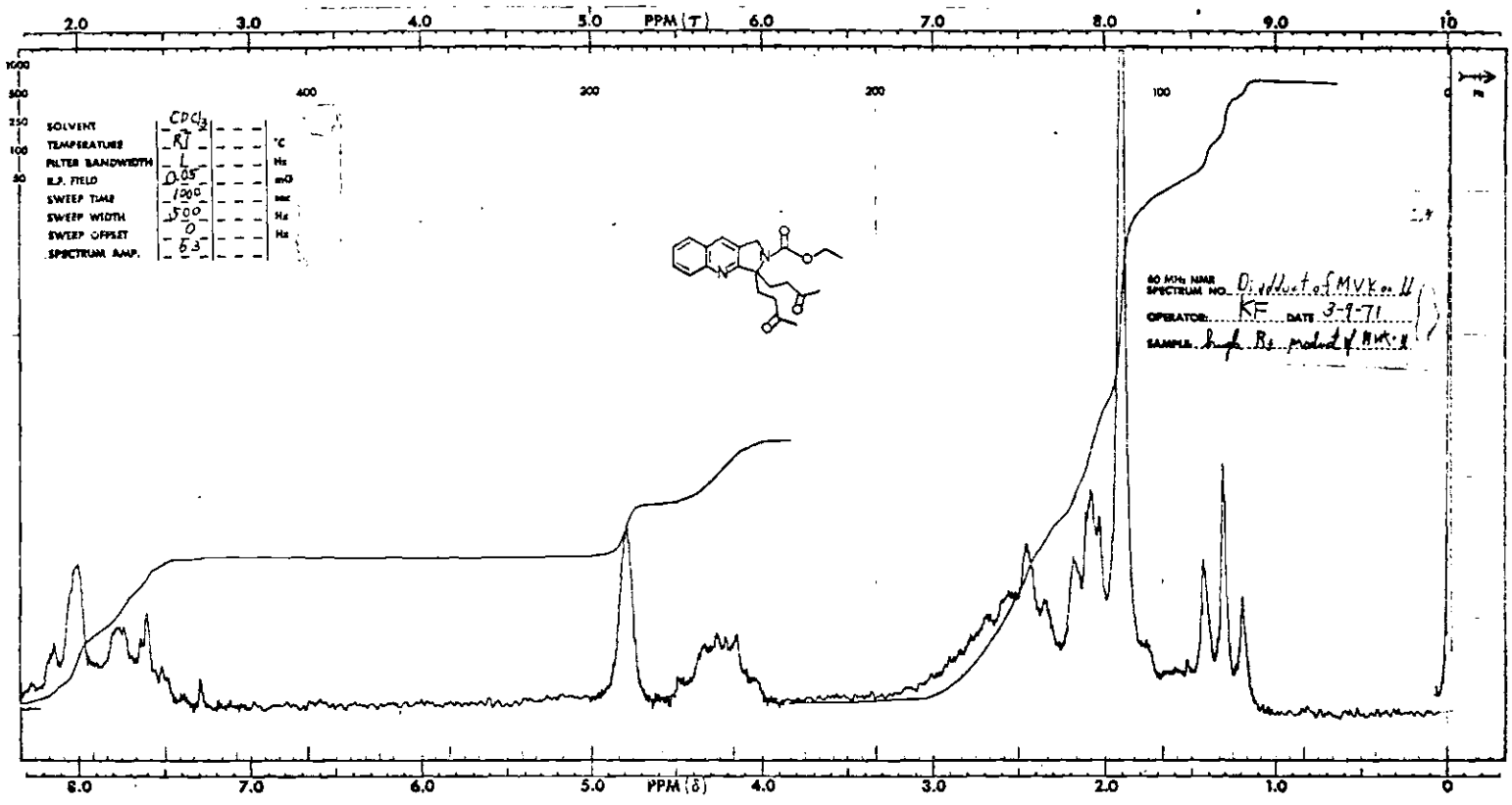


Plate 2. NMR Spectrum of Methyl Vinyl Ketone Diaddition Product from 11

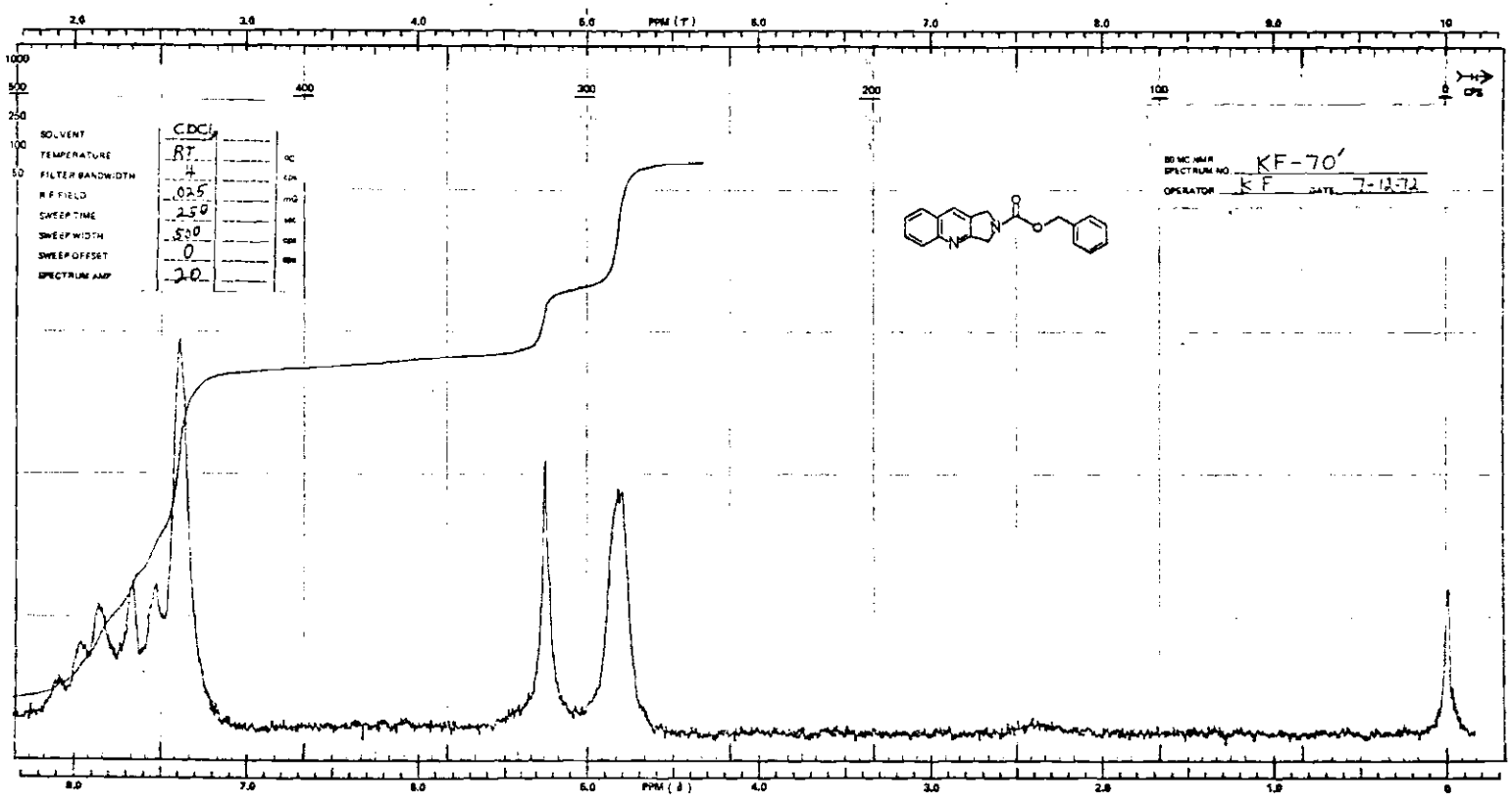


Plate 3. NMR Spectrum of 27

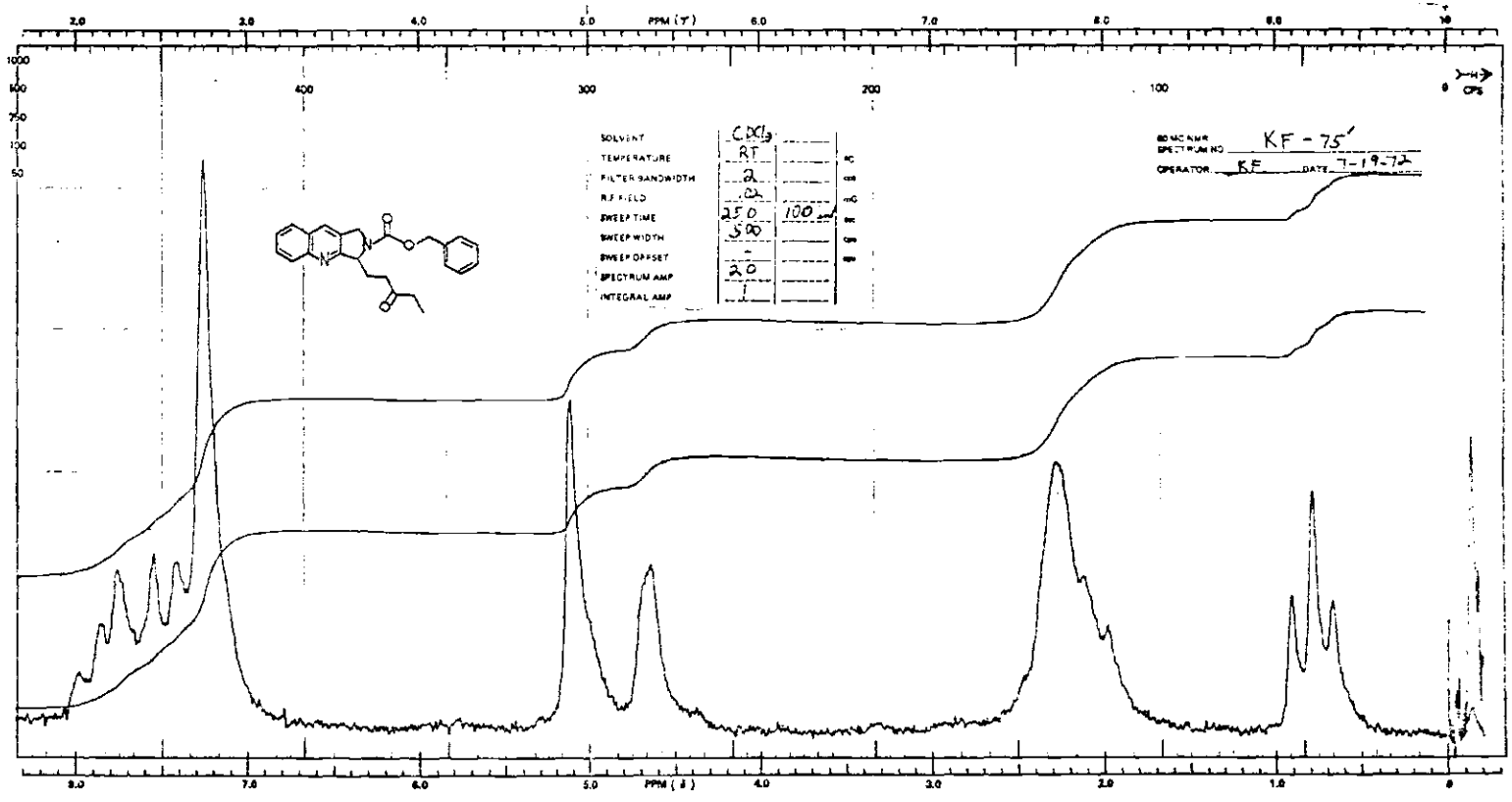


Plate 4. NMR Spectrum of 28

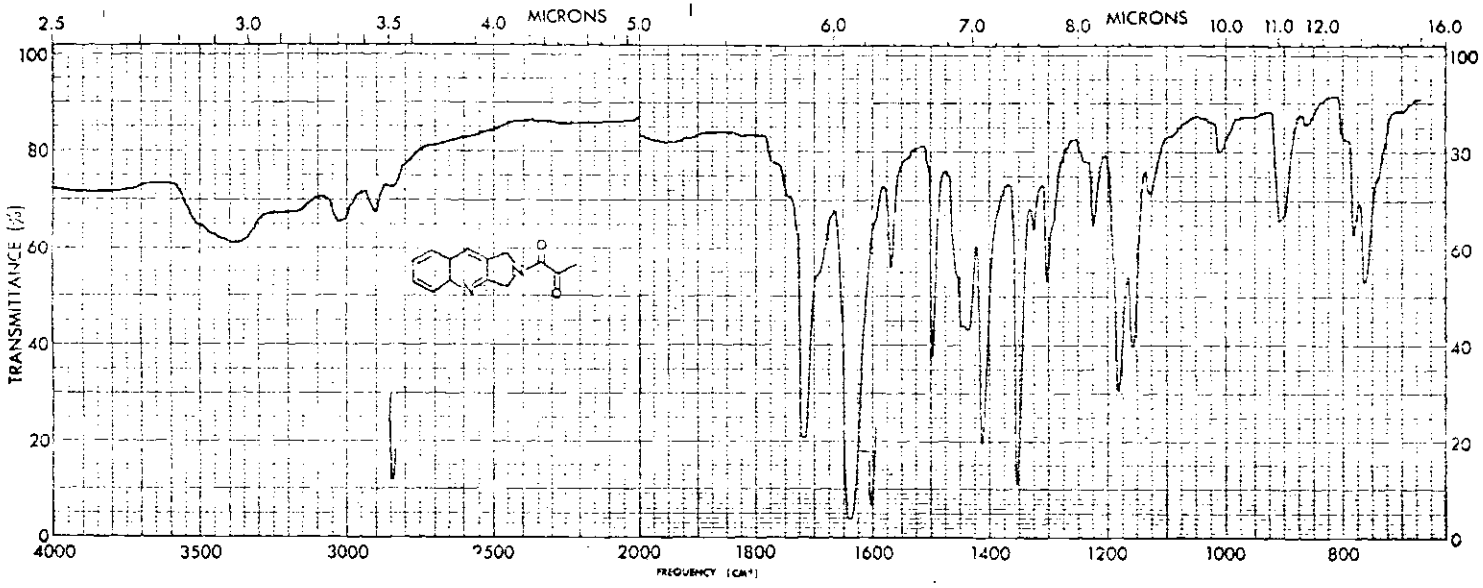


Plate 5. IR Spectrum of 51

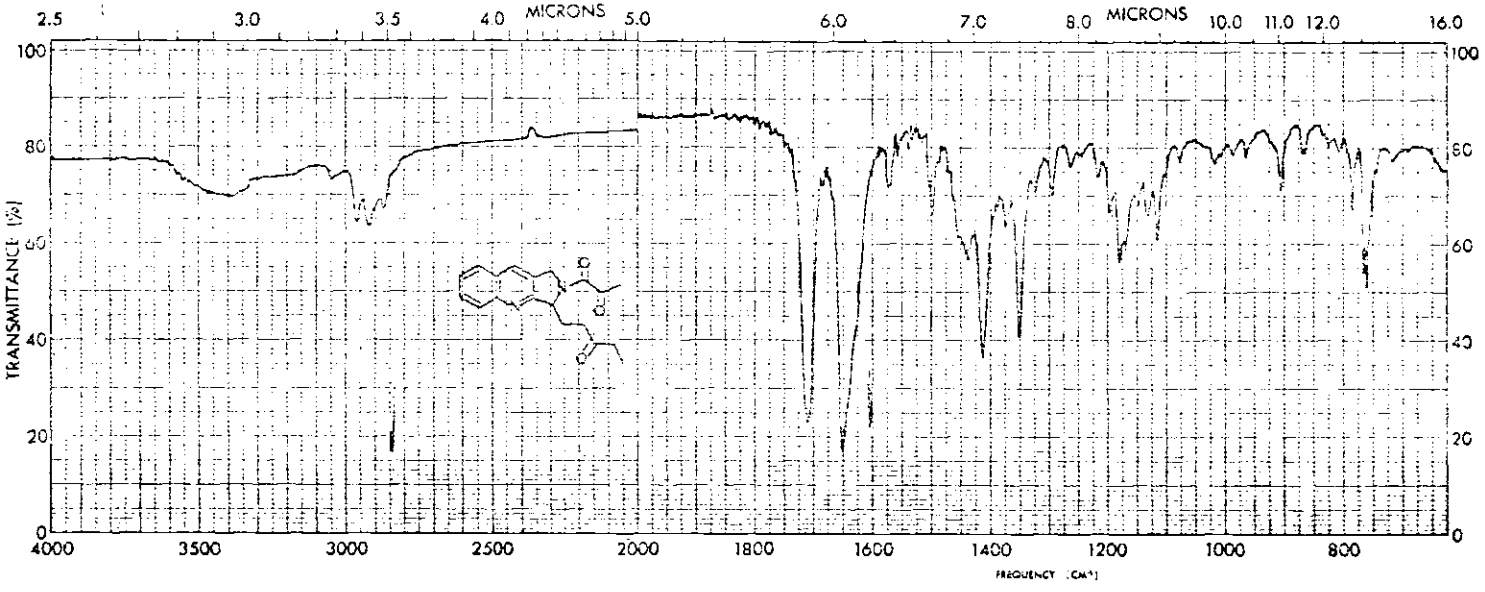


Plate 6. IR Spectrum of 54

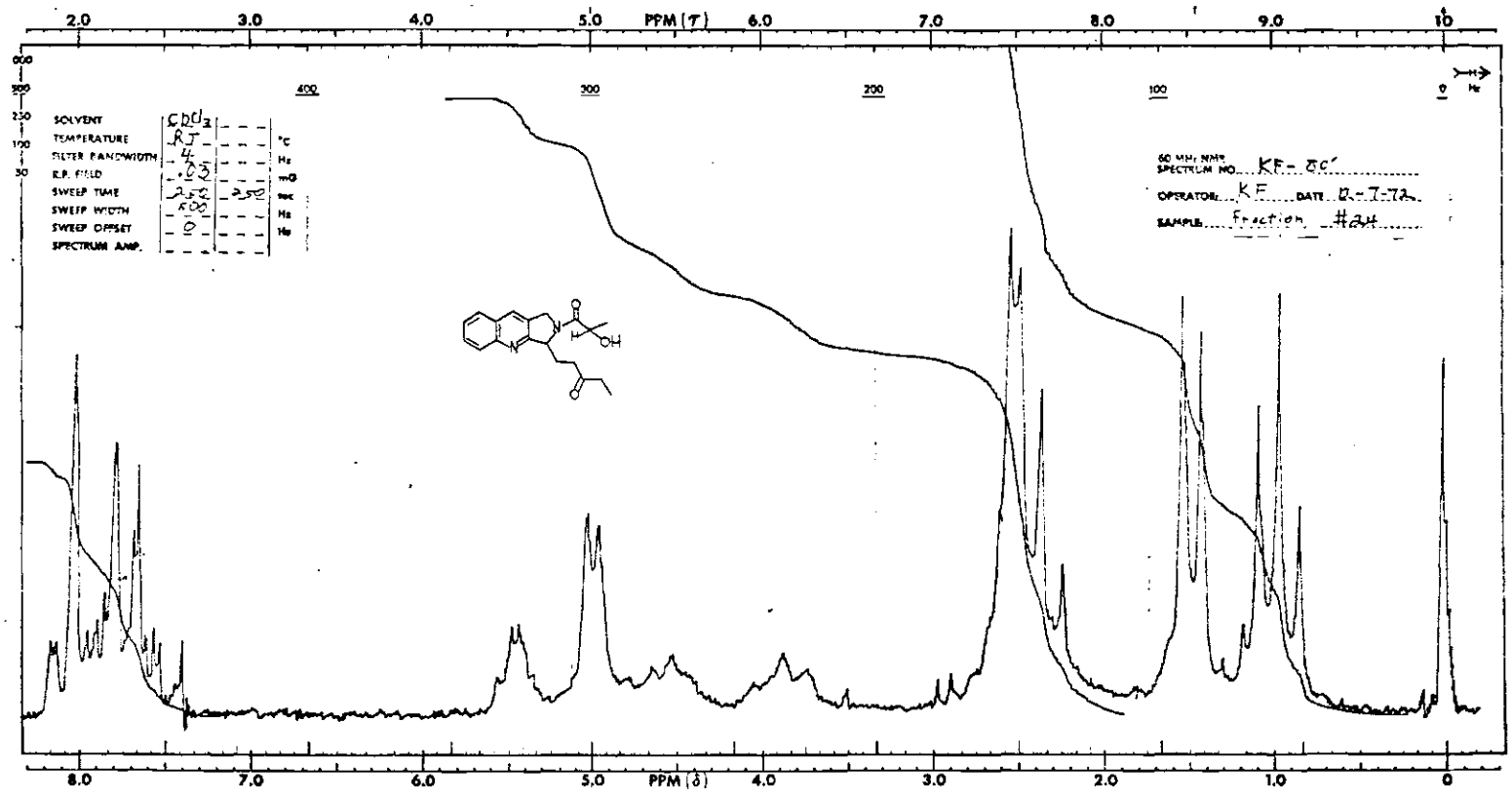


Plate 7. NMR Spectrum of 53

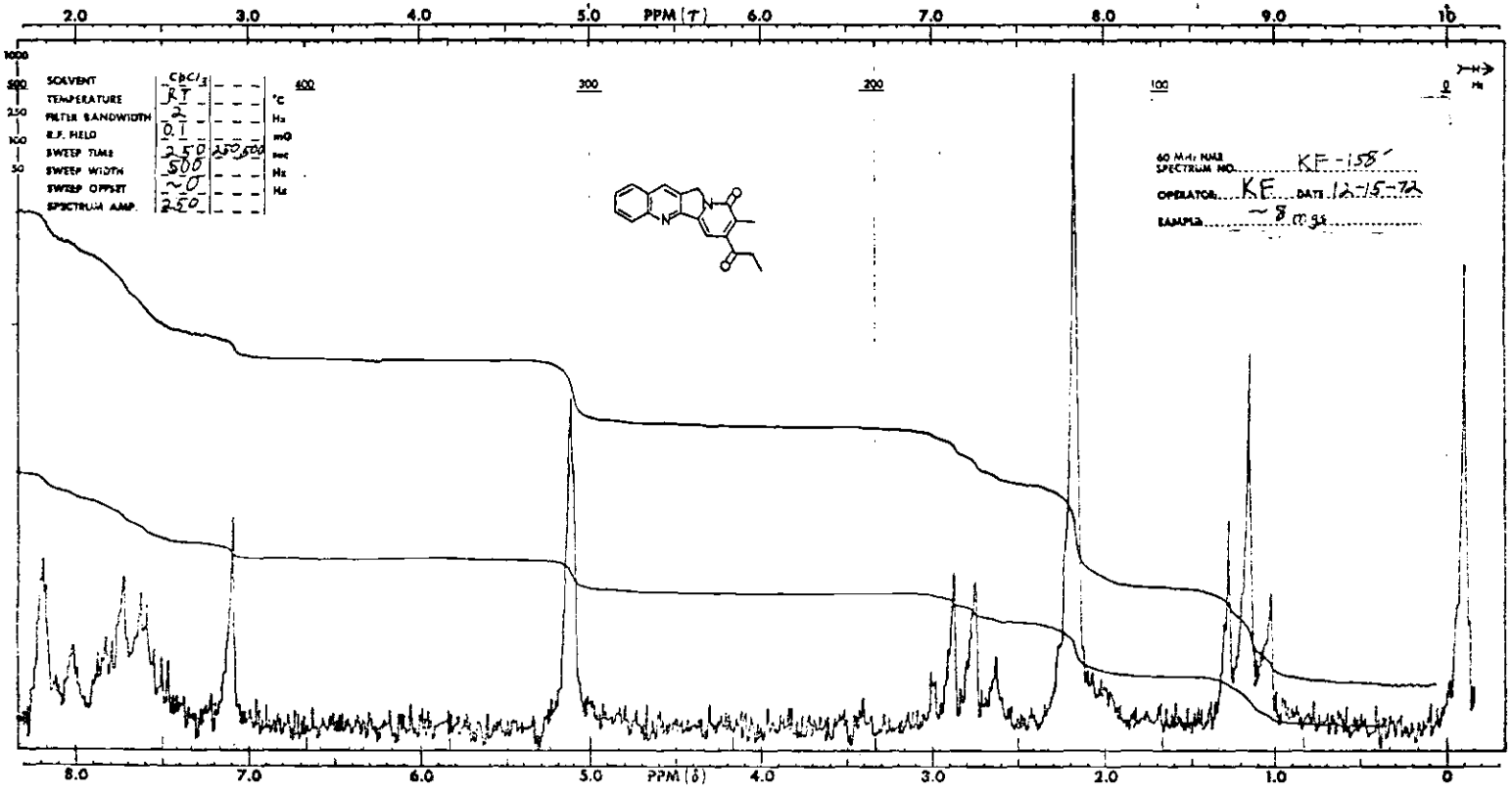


Plate 8. NMR Spectrum of 55

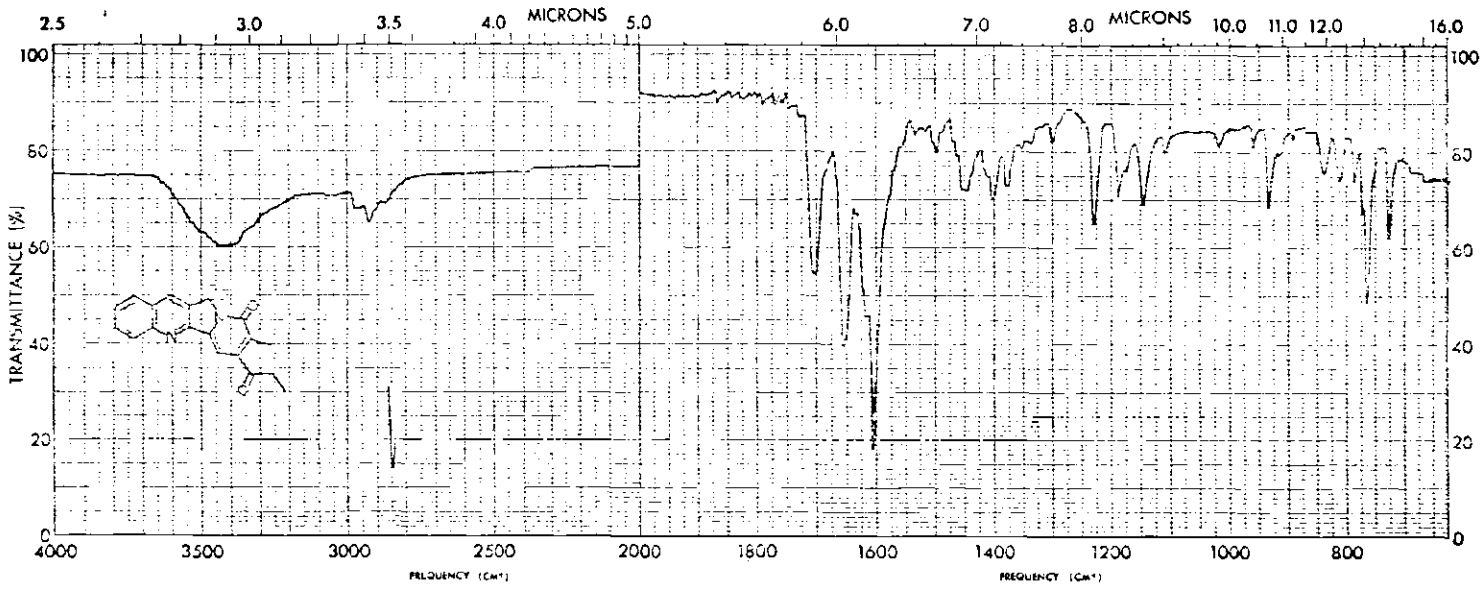


Plate 9. IR Spectrum of 55

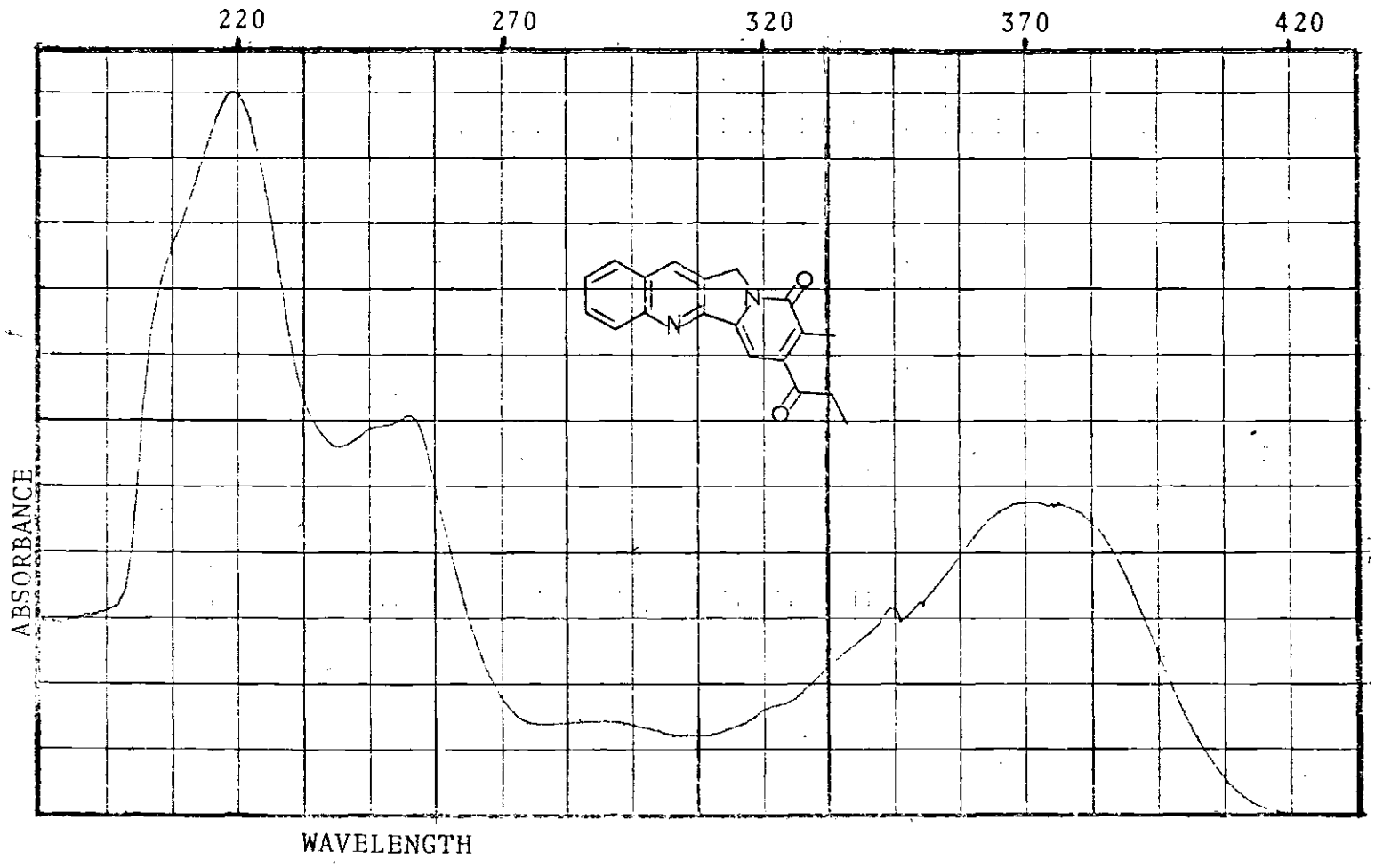


Plate 10. UV Spectrum of 55

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Kenneth Alfred French was born in Louisville, Kentucky, on May 24, 1945. He attended Flaherty (formerly St. Martin's) Elementary and High Schools in Meade County, Kentucky. Upon graduation from Flaherty High, he was admitted to Bellarmine College in Louisville on a partial scholarship (50 percent of tuition, based on need).

He was encouraged by the chemistry staff at Bellarmine, headed by Dr. John M. Daly, to apply to graduate school. Kenneth entered Georgia Institute of Technology in the fall of 1967 on an NDEA fellowship.

Kenneth entered the Army Reserve Officers Training Corps program at Georgia Tech in June, 1970, and was commissioned in June, 1972.