THE STRUCTURE OF ACRIFOLINE

By

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SCOPE AND CONTENTS:

A detailed study of acrifoline ($C_{16}H_{23}O_2N$), a minor alkaloid of Lycopodium annotinum L., has shown its structure to be:

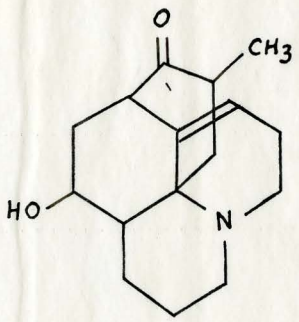

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE STRUCTURE OF ACRIFOLINE

GENERAL INTRODUCTION

The presence of alkaloids in Lycopodium, a member of the club moss family, was first reported in 1881 (1). Since that time, particularly in the last eighteen years, approximately sixty alkaloids have been isolated and characterized. Most of the alkaloids have been found to contain sixteen carbon atoms and a single nitrogen atom, and several have been shown to be simple derivatives of others (2, 3, 4). The majority of the alkaloids occur in only minor quantities, and their chemistry has been dealt with only in a cursory fashion or not at all. However, the chemistry of annotinine ($C_{16}H_{21}O_3N$) and lycopodine ($C_{16}H_{25}O_2N$), the two major Lycopodium alkaloids, has been extensively studied and the structures of these two alkaloids are now known (5, 6, 7).

The work reported in this thesis involves a structural investigation of acrifoline ($C_{16}H_{23}O_2N$), a minor alkaloid of Lycopodium annotinum. At the time the study began, little was known about the constitution of the alkaloid. Perry and MacLean (3) had established that the alkaloid contained a carbonyl group, a double bond, a hydroxyl

group and a tertiary nitrogen, and therefore that the molecule was tetracyclic. However, no degradations had been carried out.

During the course of the present investigation, sufficient evidence has been obtained to permit a complete structure for acrifoline to be formulated. The structure has the same skeleton as that proposed for lycopodine.

HISTORICAL INTRODUCTION

Isolation of the Alkaloids

Vascular plants are grouped into a single division, the Tracheophyta. The members of this phylum are distributed among four subphyla: Psilopsida, Sphenopsida, Lycopsida, and Pteropsida. The plants in the first three subphyla have received much less attention from chemists than have those in the subphylum Pteropsida (which includes flowering plants) since the former are of secondary economic importance and are frequently inconspicuous in appearance. The subphylum Lycopsida includes the Lycopodiaceae, the club moss family, and the Selaginellaceae, the small club moss family. The Lycopodiaceae comprise two living genera: Lycopodium with about 100 species and widely-spread over most of the earth, and Phylloglossum, a monotypic genus restricted to parts of Australia.

The presence of alkaloids in Lycopodiaceae was first reported in 1881 by Boedeker (1). From L. complanatum L. native to northern and central Europe he isolated a compound melting at 114-115° which he called lycopodine and formulated as $C_{32}H_{52}O_3N_2$. In 1892, Arata and Canzoneri (8) isolated a substance melting at 64-65° from the South American L. saurus Lam. The alkaloid was termed pillijanine and was formulated as $C_{15}H_{20}ON_2$. L. saurus Lam. was later

investigated by Deulofeu and de Langhe (9) but the presence of pillijanine was not detected. However, they did isolate and characterize two new bases, saururine ($C_{10}H_{19}N$) and sauroxine ($C_{17}H_{26}ON_2$). The presence of alkaloids in other Lycopodium species, including L. annotinum L., was confirmed by Orechov (10) and independently by Muszynski (11).

Lycopodine was again isolated (this time from L. clavatum L. of Polish origin) by Achmatowicz and Uzieblo (12) in 1938, and was assigned the correct formula of $C_{16}H_{25}ON$. In addition to the major alkaloid lycopodine, they were able to isolate clavatine ($C_{16}H_{25}O_2N$) and clavatoxine ($C_{16}H_{27}O_2N$).

The first comprehensive study of the Lycopodium alkaloids was carried out by Manske and Marion (13-22) using plant material mostly of North American origin. They investigated the species of L. flabelliforme Fernald., L. annotinum L., L. tristachyum Pursh., L. obscurum L., L. clavatum L., L. lucidulum Michx., L. sabinaefolium Willd., L. annotinum var. acrifolium Fern., L. cernuum L., and L. densum Labill. As a result of this investigation, approximately thirty-five new alkaloids were isolated and analyzed, of which only four were ascribed trivial names. For identification purposes, the alkaloids, including the above four, were numbered in the sequence in which they were isolated. Thus the first alkaloid to be isolated was identified as alkaloid Lycopodium 1, or, in short, L.1. The major alkaloid found in most of the species studied was lycopodine, occurring in all species except L., cernuum.

One of the most thoroughly investigated species of Lycopodium has been L. annotinum. In 1943, Manske and Marion (14) reported the isolation of eight alkaloids, including obscurine (L.6) and, in small amounts, lycopodine, from L. annotinum L. of Canadian origin. The main alkaloid, L.7 ($C_{16}H_{21}O_3N$), was named annotinine. The other five bases were obtained in a yield of less than 0.01% and included L.8 ($C_{16}H_{25}O_2N$), L.9a ($C_{20}H_{31}O_4N$) and L.9b ($C_{16}H_{23}ON$), L.10 ($C_{16}H_{27}ON$), L.11 ($C_{16}H_{21}O_3N$), and L.12 ($C_{18}H_{25}O_3N$). Obscurine, which had been previously isolated from L. flabelliforme, was later shown by Moore and Marion (23) to be a mixture of α- and β-obscurine.

A later investigation of L. annotinum var. acrifolium Fern. by Manske and Marion (20) led to the isolation of annotinine as the major alkaloid in addition to five new bases. The new bases included L.27 ($C_{16}H_{23}O_2N$), L.28 ($C_{17}H_{27}O_2N$), L.29 ($C_{16}H_{23}O_2N$), L.30 ($C_{16}H_{25}O_2N$), and L.31 ($C_{20}H_{29}O_4N$). Missing were the alkaloids L.8, L.9, L.11, L.12 and obscurine which had been previously isolated from L. annotinum L. Alkaloid L.27 was given the trivial name acrifoline. Because of the great difference in alkaloidal content of the two plants, Manske and Marion suggested that the acrifolium variety should be raised to the status of a new species. More recent work by Douglas, Lewis and Marion (2) has shown that L.8 and L.30 are identical.

In 1952, Bertho and Stoll (24) published the results of an investigation of L. annotinum L. of German origin. In addition to annotinine and lycopodine, they isolated the following compounds: acrifoline, annotoxine ($C_{31}H_{42}O_5N_2$), annotine ($C_{16}H_{23}O_4N$), and

two bases formulated as $C_{16}H_{25(23)}ON$ and $C_{10}H_{19(21)}ON$. They failed to find obscurine, or alkaloids L.8, L.9, L.10, L.11, and L.12.

In a detailed study of the alkaloidal content of L. annotinum L. of Polish origin, Achmatowicz and Rodewald (25) in 1955 showed that the differences between L. annotinum of Canadian origin and that of European origin were not as great as had appeared from the results of previous investigators. Eight alkaloids were isolated from the plant of Polish origin, five of which had been previously found by Manske and Marion (14) to occur in L. annotinum of Canadian origin. These five alkaloids were annotinine, α-obscurine, lycopodine, alkaloid L.8 and alkaloid L.11. Acrifoline (L.27) and annotoxine, previously reported as alkaloidal constituents of plant material of German origin, were also isolated in addition to a substance ($C_{16}H_{25}ON$) called isolycopodine that appeared to be new but was possibly identical with alkaloid L.13 originally isolated from L. tristachyum by Manske and Marion (15).

By a careful examination of annotoxine, the Polish workers established that the correct formula of the base was $C_{32}H_{44}O_5N_2$, and that the substance was an equimolecular combination of acrifoline and alkaloid L.11. The unusual chemical composition of annotoxine was proved in a number of ways. The hydriodide and methiodide of annotoxine were fractionally crystallized into two components which proved identical with the hydriodides and methiodides of acrifoline and alkaloid L.11 respectively. Furthermore, annotoxine was quantitatively formed when equimolar amounts of acrifoline and

alkaloid L.11 in acetone were mixed together. The thermal analysis of the acrifoline-alkaloid L.11 system also confirmed the chemical character of annotoxine.

A comparison of ultraviolet spectra, melting points and optical rotations of annotine and alkaloid L.11 by Achmatowicz and Rodewald showed that these two compounds were actually identical. Hence they were able to clarify the absence of annotine in the plant of Canadian and Polish origin on the one hand and the absence of alkaloid L.11 in the plant of German origin on the other. The absence of annotoxine in L. annotinum of Canadian origin was then ascribed to the method of isolation of the alkaloids. Since the Polish workers found that the crystallization of salts of annotoxine led to fractionation of acrifoline and annotine, it could be assumed that this fractionation process had occurred during the isolation procedure of Manske and Marion in which the alkaloids were crystallized as their perchlorates. Likewise, Bertho and Stoll failed to detect annotoxine in two out of three plant extracts because the bases in the two cases were fractionally crystallized in the form of their nitrates. In the third plant extract, the alkaloids were fractionally crystallized in the form of free bases and annotoxine was isolated. The alkaloidal content of European L. annotinum, and the character of annotoxine led Achmatowicz and Rodewald to suggest that acrifoline was probably present in fractions L.9a or L.9b of the Canadian L. annotinum investigated by Manske and Marion. Accordingly, Achmatowicz and Rodewald believed that little difference existed in the alkaloidal content of L. annotinum of Canadian and European origin.

An investigation by Perry and MacLean (3) has revealed that annotoxine can indeed be isolated from L. annotinum of Canadian origin. From several batches of L. annotinum of undetermined variety collected in Nova Scotia, New Brunswick, and Ontario, they isolated annotinine, lycopodine, α- and β-obscurine, acrifoline, L.8, L.11 (annotine) and L.12 as well as annotoxine. The complex nature of annotoxine was confirmed by the fact that the base was readily separated into acrifoline and annotine through fractional crystallization of the hydrobromide salts, and partially separated by chromatography on alumina. The isolation of acrifoline from plant material of several regions indicated that the alkaloid may be present in all varieties of L. annotinum, and not in the acrifolium variety alone as had been thought previously.

A further investigation of the alkaloidal content of L. annotinum of Polish origin by Achmatowicz and Rodewald (26) led to the isolation of several minor alkaloids, including five new ones. In 1958, they reported the isolation of nicotine and a base, $C_{16}H_{23}ON$, which was believed to be identical with the base, $C_{16}H_{25}ON$, isolated by Bertho and Stoll from plant material of German origin. Also found were three alkaloids believed to be identical with alkaloids L.28, L.29, and L.31 that had been isolated by Manske and Marion from L. annotinum var. acrifolium of Canadian origin. The five new alkaloids were of the following formulae: $C_{16}H_{21}O_3N$, $C_{17}H_{25}O_2N$, $C_{17}H_{25}O_3N$, $C_{18}H_{25}O_3N$, and $C_{18}H_{25}O_4N$. All together, Achmatowicz and Rodewald isolated eighteen alkaloids from L. annotinum, accounting for 93.3% of the total alkaloids.

Several of the alkaloids occurring in L. annotinum have also been found in L. selago L. of Polish origin. In 1955, Achmatowicz and Rodewald (27) reported the isolation of lycopodine, acrifoline and alkaloid L.8 from L. selago. Also isolated was pseudoselagine, $C_{16}H_{25}O_2N$, which may be identical with alkaloid L.23 previously isolated by Manske and Marion (18) from L. lucidulum Michx.

Five new alkaloids have been recently isolated from L. annotinum of Canadian origin. In 1958, Anet and Eves (28) reported the isolation of lycodine to which they assigned the formula $C_{17}H_{24}N_2$, while in 1959, Anet and Khan (29) reported the isolation of annofoline ($C_{16}H_{25}O_2N$), lycofoline ($C_{16}H_{25}O_2N$), α-lofoline ($C_{18}H_{29}O_3N$) and β-lofoline ($C_{18}H_{29}O_3N$).

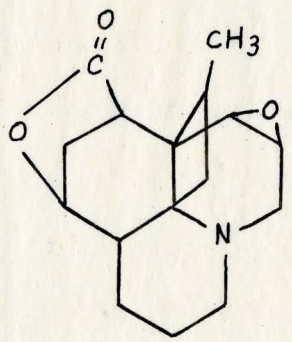
New alkaloids from other species of Lycopodium have recently been reported as well. In 1959, Burnell (30) reported the isolation of seven alkaloids from L. fawcettii Lloyd and Underwood which had been collected in the Blue Mountain Range of Jamaica. These alkaloids were: a base ($C_{16}H_{27}O_2N$), L.30, fawcettiine ($C_{18}H_{29}O_3N$), deacetylfawcettiine ($C_{16}H_{27}O_2N$), a base ($C_{17}H_{25}O_2N$), fawcettidine ($C_{16}H_{23}ON$), and a base ($C_{18}H_{27}O_3N$).

Chemical Investigation of the Alkaloids

Although many alkaloids have been isolated from several species of Lycopodium, comparatively few have received extensive structural investigation. One of the reasons for this is that most of the alkaloids are present in only trace quantities while another is the fact that the lack of reactive functional groups in many of the

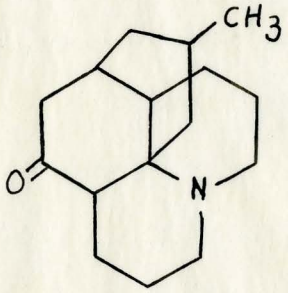
alkaloids renders their degradation to simpler compounds of constitutional value quite difficult. These factors are now partially compensated by the use of recently-developed instruments and techniques (e.g., infrared and nuclear magnetic resonance spectroscopy and vapor phase chromatography) that enable present investigators to gain structural information more readily and on less material than was the case with earlier workers.

The two major Lycopodium alkaloids, annotinine and lycopodine, have been extensively studied and their structures are now known. The structural elucidation of annotinine began in 1947 with the work of Manske and Marion (20) and terminated eleven years later. In 1958, Przybylska and Marion (5) published the complete structure obtained by X-ray diffraction studies while at the same time Wiesner and co-workers (6) put forward the same structure which they had elucidated chemically. The structure of annotinine is:

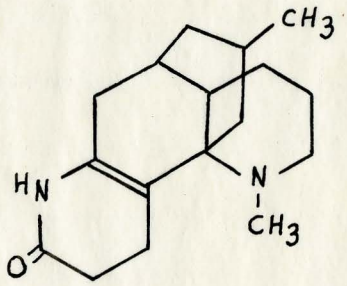


The structural investigation of lycopodine began with the work of Manske and Marion (31) in 1942 and the complete structure was

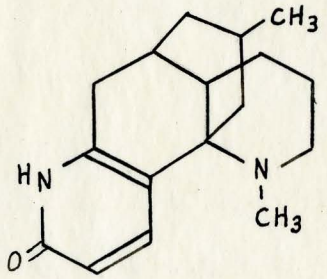
proposed in 1960 by Harrison and MacLean (7). The proposed structure of lycopodine is:



Very recently, the structures of four dinitrogen Lycopodium alkaloids have been published. Ayer and Iverach (32) have found the structures of α- and β-obscurine to be the following:

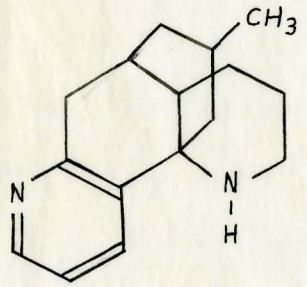


α-Obscurine

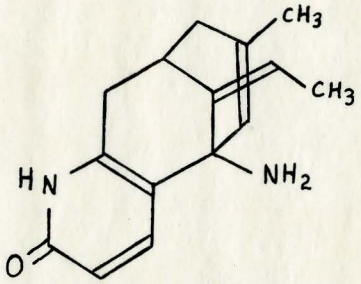


β-Obscurine

On the basis of published chemical evidence (28) and analogy to the above structures, Ayer and Iverach (32) have postulated that lycodine (the formula $C_{17}H_{24}N_2$ was originally assigned by Anet and Eves (28) but the formula $C_{16}H_{22}N_2$ equally well fits the analytical values) has the following structure:



Selagine ($C_{15}H_{18}ON_2$), which had been isolated from L. selago, was found by Valenta et al. (33) to have the following structure:



The chemical investigation of the remaining minor Lycopodium alkaloids has not been carried out in any great detail, and has been limited to only a few compounds. Bertho and Stoll (24) reported that annotine (L.11) had no NCH_3, OCH_3 or OH groups but possessed a carbonyl group. Perry and MacLean (3) later established the functional groups of annotine to be a carbonyl group, a double bond, a hydroxyl group and an inert oxygen probably present in an ether linkage.

Bertho and Stoll (24) reported that acrifoline did not have NCH_3, OCH_3 or OH groups but that it had a carbonyl function on the basis

of its ultraviolet spectrum and its reaction with phenyllithium. A fully saturated ring system was proposed since the alkaloid could not be hydrogenated. They reported that the base did not react with peracetic acid, selenium dioxide, 2,4-dinitrophenylhydrazine, or benzaldehyde and sodium ethoxide. Although acrifoline itself did not undergo Hofmann degradation on pyrolysis of its methyl quaternary ammonium hydroxide, Bertho and Stoll found that phenylacrifoline underwent partial degradation. The product, a base ($C_{23}H_{31}O_2N$) melting at 192-193°, was not investigated further.

Perry and MacLean (3) established the nature of the functional groups in acrifoline by chemical methods and by infrared spectroscopy. Reaction of acrifoline with acetic anhydride gave an O-acetyl derivative indicating the presence of a hydroxyl group. The O-acetyl derivative was identical with alkaloid L.12. Reduction of acrifoline with hydrogen and platinum gave dihydroacrifoline, $C_{16}H_{25}O_2N$, while reduction with lithium aluminum hydride yielded acrifolinol, $C_{16}H_{25}O_2N$. They reported that hydride reduction of the former compound gave α-dihydroacrifolinol, $C_{16}H_{27}O_2N$, m.p. 195°, while catalytic reduction of the latter compound gave β-dihydroacrifolinol, $C_{16}H_{27}O_2N$, m.p. 165-167°. Thus acrifoline was shown to have a double bond, a hydroxyl group, and a carbonyl group, and hence was tetracyclic.

An examination of the formulae of the Lycopodium alkaloids shows that many of the bases contain sixteen carbon atoms and one nitrogen atom. Furthermore, several of these alkaloids, including annotinine, lycopodine, annotine and acrifoline, have been shown to be

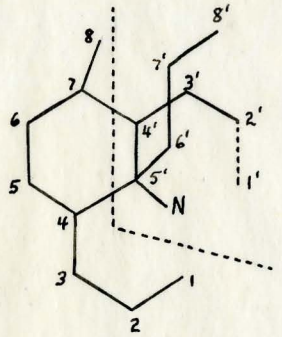
tetracyclic. However, there are at least two basic nuclei as exemplified by the structures of annotinine and lycopodine so that all the $C_{16}N$ alkaloids cannot bear a simple relationship to one another. It appears certain, though, that many of the alkaloids not yet investigated chemically will have either the annotinine nucleus or the lycopodine nucleus, or be otherwise closely related.

A second group of Lycopodium alkaloids are those containing two nitrogen atoms; e.g., α- and β-obscurine, pillijanine ($C_{15}H_{20}ON_2$), sauroxine ($C_{17}H_{26}ON_2$), alkaloid L.32 ($C_{16}H_{26}ON_2$), lycodine ($C_{16}H_{22}N_2$) and selagine ($C_{15}H_{18}ON_2$). As seen in the preceeding discussion, four of these compounds are known to have similar structures, and therefore it is probable that the others may be closely related.

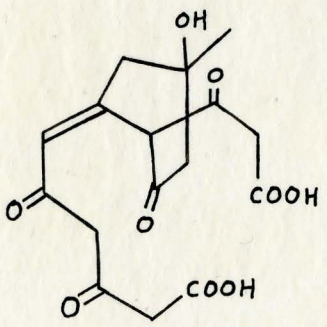
The remaining alkaloids, aside from those which may be acetyl derivatives of C_{16} compounds, do not appear to fall into any general category. A few alkaloids contain ten carbon atoms, e.g., nicotine ($C_{10}H_{14}N_2$), saurine ($C_{10}H_{19}N$), and a base ($C_{10}H_{19(21)}ON$). Others (e.g., L.8, $C_{11}H_{19}ON$; L.26, $C_{15}H_{25}ON$; L.34, $C_{14}H_{21}ON$) do not fit into the above categories and may be incorrectly formulated.

Although no relationship has been established between annotinine and other alkaloids, a relationship has been established between lycopodine and three other alkaloids. Thus, anhydrodihydrolycopodine ($>C=O \rightarrow >CHOH \longrightarrow \gg$) is identical with alkaloid L.14, $C_{16}H_{25}N$ (2), O-acetyldihydrolycopodine ($>C=O \rightarrow >CHOH \longrightarrow >CHOAc$) is identical with alkaloid L.2, $C_{18}H_{29}O_2N$ (2), and dihydrolycopodine is identical with alkaloid L.1 (4).

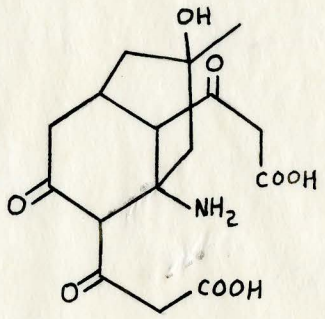
An examination of the known structures of the Lycopodium alkaloids shows that all have the following structural arrangements in common:



Very recently, Conroy (34) proposed a biogenetic pathway for the formation of Lycopodium alkaloids. A different pathway was proposed earlier by Leete (35), but the proposal was specific for the biogenesis of annotinine, the only Lycopodium alkaloid whose structure was known at that time. The scheme presented by Conroy is more flexible, and accounts for all Lycopodium alkaloid structures known to date. The scheme involves the initial aldol condensation of two moles of 3, 5, 7-triketooctanoic acid (a polyacetate unit) between C_7 and $C_{4'}$ followed by dehydration. A second aldol condensation between C_8 and $C_{7'}$ gives:



Reduction of the C_6-C_7 double bond followed by Mannich condensation with ammonia joins C_4 and $C_{5'}$ and gives:



Lactamization of the two carboxyl groups followed by reduction at the appropriate centers gives lycopodine. Other minor variations in the scheme yield annotinine, obscurine, and selagine.

DISCUSSION OF RESULTS

The Functional Groups of Acrifoline

This investigation was undertaken in order to elucidate the structure of acrifoline. When the work was initiated, it was known that acrifoline was tetracyclic, and had a double bond, a tertiary nitrogen atom, a hydroxyl group, and a carbonyl group. Spectroscopic studies were invaluable in extending the existing structural knowledge of acrifoline and its known derivatives, and were used liberally in elucidating the structures of the degradation products of acrifoline.

The infrared spectrum of acrifoline in nujol showed absorption at 3310 cm^{-1} in the hydroxyl region and weak bands at 1700 cm^{-1} and 1670 cm^{-1}, while in solution it exhibited strong absorption at 1700 cm^{-1} owing to a carbonyl group in a six-membered (or larger) ring and at 1675 cm^{-1} owing to unsaturation. Both spectra showed strong absorption near 1100 cm^{-1} that was attributed to an ether linkage. The absence of carbonyl absorption in the solid state implies that acrifoline crystallizes as a hemi-ketal, whereas in solution, a carbonyl absorption is observed because of the presence of a hydroxy-ketone in equilibrium with the hemi-ketal. Since only five- and six-membered cyclic hemi-ketals form spontaneously, the carbonyl group and the carbon atom bearing the hydroxyl group must be separated by two or three atoms. The above

phenomenon was also observed in many derivatives and degradation products of acrifoline in which the original carbonyl and hydroxyl groups were present.

Additional information was gained through an examination of the nuclear magnetic resonance spectrum of acrifoline. Chemical shifts are given in parts per million (ppm) from water. Absorption at a displacement of 0.85 ppm of an intensity of one proton was attributed to a >CHO-group, while a peak of equal area at a displacement of -0.46 ppm indicated the presence of a >C=C<^{H} group. The latter peak appeared to be in the form of a triplet so that a $-CH_2-$ group is probably adjacent to the =CH- fragment. A split peak occurring at a displacement of 3.70 ppm with an intensity of three protons established the presence of a $>CHCH_3$ unit. The presence of one C-methyl group was shown previously by Kuhn-Roth analysis of acrifoline (as its hydrobromide salt).

The above NMR results were confirmed by a study of acetylacrifoline. Its NMR spectrum showed absorptions attributed to >C=C<^{H}, >CHO-, and $>CHCH_3$ at displacements of -0.53, 0.14, and 3.80 ppm respectively. In addition, a sharp peak occurring at a displacement of 3.00 ppm was assigned to the $-COCH_3$ group. The occurrence of the absorption of the >CHO- group at a lower field strength in acetylacrifoline (0.14 ppm) than in acrifoline (0.85 ppm) is probably owing to the influence of the acetyl group in the former compound (36). The absence of absorption at low field expected of an aldehyde in both of the above NMR spectra indicated that the carbonyl group in acrifoline is ketonic. The hydroxyl group is secondary since the signal area of the >CHO- group corresponded to one proton.

The above chemical and spectroscopic data establish the following structural features in acrifoline: a ketone, a secondary hydroxyl group with the carbon atom bearing the hydroxyl group separated by two or three atoms from the carbonyl group, a trialkylated double bond, a $>CHCH_3$ group and a tertiary nitrogen atom. In order to interrelate the functional groups and to gain further knowledge of acrifoline, a number of degradations were investigated.

The Hofmann Degradation of Acrifoline

The first degradation of acrifoline to be studied in detail was the Hofmann reaction. Acrifoline readily formed a methiodide which, on treatment with potassium tertiary butoxide, underwent Hofmann elimination to give a crystalline base (I), $C_{17}H_{25}O_2N$, melting at 161°, in 70% yield. The presence of a terminal methylene group in the Hofmann product (I) was suggested by its infrared spectrum which contained a strong absorption at 900 cm^{-1} with an overtone at 1800 cm^{-1}. The carbonyl group showed normal absorption at 1700 cm^{-1}. Strong absorption in the ultraviolet spectrum with λ_{max} at 2400Å, ϵ =24,000, along with an enhanced double bond absorption at 1625 cm^{-1} in the infrared spectrum, indicated the presence of a conjugated diene system and that the double bond introduced during the Hofmann reaction must be in conjugation with the original double bond of acrifoline. The double bond in acrifoline would therefore be in a position γ, δ relative to the nitrogen atom. The presence of the $=CH_2$ group in the Hofmann product (I) was confirmed by ozonolysis and subsequent isolation and

identification of formaldehyde. Glyoxal, which would be expected from the ozonolysis of an unsubstituted conjugated diene, was not detected, inferring that a branch occurred in the carbon skeleton of acrifoline at the β or γ position relative to nitrogen. The four carbon atoms in the conjugated diene system in compound I are designated $\overset{\delta\ \gamma\ \beta\ \alpha}{>C=C-C=CH_2}$ in order to facilitate the present discussion. Acrifoline is designated $\overset{\delta\ \gamma\ \beta\ \alpha}{>C=C-C-CH_2-N<}$.

The Hofmann product (I) behaved abnormally in several ways. The base was a well-defined crystalline solid melting at 161°, with a pK_a value of 7.56, which did not form crystalline salts such as a hydrobromide or perchlorate. Furthermore, it did not react with methyl iodide when treated with this reagent in hot acetone, but gave a crystalline product when heated with methyl iodide in acetone in a sealed tube for several hours at 150°. However, further reactions with this product were unfruitful. Catalytic reduction of the diene system in compound I led to the formation of a non-crystalline product which was probably a mixture of partially reduced and fully reduced compounds. A quantitative microhydrogenation of the Hofmann product (I) showed the rapid uptake of one mole of hydrogen while the second mole was only partially consumed after several hours.

The diene system in the Hofmann product was satisfactorily reduced by chemical means. The carbonyl group was first reduced with sodium borohydride to give a diol (II), $C_{17}H_{27}O_2N$, melting at 252°. Compound II still retained the conjugated diene system since its ultraviolet spectrum had λ_{max} at 2400Å with ϵ=28,000. Treatment of the

diol (II) with sodium in alcohol gave a base (III), $C_{17}H_{29}O_2N$, melting at 195°, which no longer showed absorption in the ultraviolet spectrum. Conjugated dienes normally undergo 1, 4-reduction, but a 1, 2-reduction is not precluded since it has been reported that certain unsymmetrical conjugated dienes undergo the latter mode of reduction (37, 38). In either case, ozonolysis of compound III would give a volatile aldehyde (propionaldehyde or acetaldehyde) if the original diene system in the Hofmann product (I) were unsubstituted. However, ozonolysis of compound III did not yield any volatile carbonyl compound, and attempts to characterize the ozonized base were unsuccessful. In addition, oxidation of compound III with osmium tetroxide and periodate, or with permanganate-periodate also failed to yield a volatile cleavage product although oxidation appeared to have taken place. Therefore, these results again indicated that an alkyl branch occurred in the acrifoline skeleton at the carbon atom β or γ relative to nitrogen.

A determination of the alkyl groups present in compound III, and in the crude hydrogenated Hofmann product (I) showed that a branch did not exist on either the β or γ carbon atom in acrifoline. The nature of the alkyl groups was established by chromic acid oxidation and analysis of the volatile carboxylic acids produced. The oxidation procedure was a modified Kuhn-Roth (39, 40) with alterations (41) to permit the identification of the carboxylic acids as their methyl esters. Oxidation of compound III yielded propionic and acetic acids whereas acrifoline yielded only acetic acid, as expected. Therefore an ethyl group was present in compound III and hence a branch did not exist at the β carbon atom in acrifoline.

These results demonstrated that the conjugated diene had undergone a 1, 2-reduction on treatment with sodium and alcohol, and that the double bond remaining in compound III was in the same relative position as in acrifoline. However, acrifoline and compound III varied greatly in their ease of reduction with hydrogen and platinium. Whereas acrifoline underwent hydrogenation readily at 50 psig, but not at atmospheric pressure (3, 24), compound III was not reduced even at 750 psig and 100°. Acrifoline methiodide was similar in behaviour to compound III since it could not be hydrogenated at 1000 psig and 100°. Hence it would appear that the double bond in acrifoline is somewhat sterically hindered toward reduction, and that the presence of a methyl group on the nitrogen atom prevents hydrogenation from taking place.

The lack of an alkyl substituent on the γ carbon atom was established by modified Kuhn-Roth oxidation of the crude material obtained by hydrogenation of the Hofmann product (I). Oxidation of this mixture yielded butyric acid as well as propionic and acetic acids proving that a n-propyl group had been formed in the hydrogenation of compound I. Therefore the γ carbon atom in compound I and in acrifoline carried only a hydrogen substituent. Since the NMR data for acrifoline established the presence of one proton on the double bond, then the δ carbon atom must be joined to two other carbon atoms and represents a point of ring juncture. These results are summarized in Figure 1.

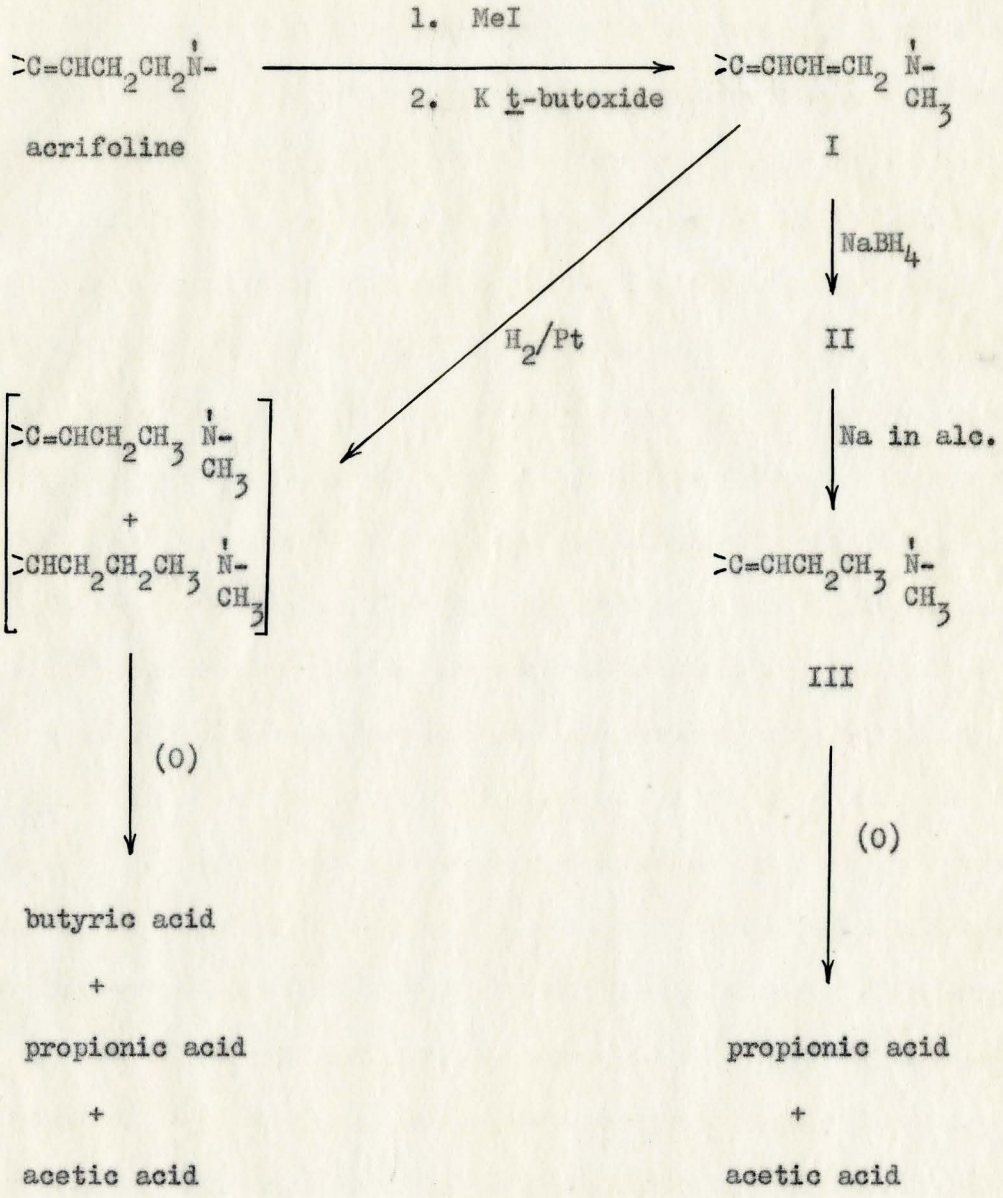


Figure 1. The Hofmann Reaction of Acrifoline

The NMR spectra of the Hofmann product (I) and compound III confirm the partial structures assigned above. In the NMR spectrum of the Hofmann product (I), a doublet at a displacement of 3.71 ppm with an intensity of 3 represented the $\geq CHCH_3$ group, a sharp peak at 2.61 ppm

represented the $>NCH_3$ group, and a peak at 0.89 ppm of intensity 1 represented the >CHO- group. Two peaks of equal size and intensity occurring at displacements of -0.40 and -0.11 ppm were attributed to the $=CH_2$ group while two peaks at displacements of -1.15 and -0.97 ppm, although having a signal area of less than 2, were apparently owing to the two protons on the β and γ carbon atoms. The NMR spectrum of compound III showed absorption characteristic of $>NCH_3$ and >CHO- groups at displacements of 2.62 and 0.87 ppm respectively. A complex peak at 3.70 ppm was ascribed to the superimposition of triplet and doublet signals created by the two methyl groups. A multi-split peak at a displacement of -0.36 ppm was attributed to the single proton on the double bond whose signal was split by protons on adjacent carbon atoms.

The Von Braun Reaction of Acrifoline

A study of the products of the von Braun reaction on acrifoline confirmed the results of the Hofmann degradation and in addition furnished information about the nature of three more carbon atoms. Reaction of acrifoline with cyanogen bromide yielded two isomeric cleavage products, α- and β-cyanobromoacrifoline, IV and V, respectively. β-Cyanobromoacrifoline (V), $C_{17}H_{23}O_2N_2Br$, was isolated in 30% yield as a crystalline product melting at 154.5°. α-Cyanobromoacrifoline (IV) was not isolated in crystalline form but was obtained in 22% yield as a crystalline quaternary ammonium bromide (VI), $C_{20}H_{32}O_2N_3Br$, on treatment of the filtrate from the isolation of β-cyanobromoacrifoline (V) with trimethylamine. The quaternary ammonium bromide (VI) readily

eliminated trimethylamine on treatment with potassium tertiary butoxide and formed a neutral product (VII), $C_{17}H_{22}O_2N_2$, which melted at 183.5°. The presence of a terminal methylene group in compound VII was suggested by infrared absorptions at 900 cm^{-1} with an overtone at 1800 cm^{-1}. The ultraviolet spectrum of compound VII showed an intense absorption with λ_{max} at 2400Å, ϵ =24,800, indicating a conjugated diene system. The position and intensity of the maximum in the ultraviolet spectrum of compound VII is almost identical with that of the Hofmann product (I) and it can be inferred that the same ring cleavage occurred in the formation of both the Hofmann product (I) and α-cyanobromoacrifoline (IV).

Reduction of the diene system in compound VII with hydrogen and platinum yielded products analogous to those formed by the hydrogenation of the Hofmann product (I). However, in the case of the cyano compound (VII), a crystalline product (VIII) melting at 154-155° was obtained in 50% yield. This material analyzed for $C_{17}H_{24}O_2N_2$, and yielded propionic acid in addition to the expected acetic acid on oxidation by the modified Kuhn-Roth procedure. Therefore compound VIII contained an ethyl group and had been formed by a 1, 2-reduction of the diene system in compound VII. The crude material obtained in the filtrate from the isolation of compound VIII yielded a small amount of butyric acid as well as propionic and acetic acids on oxidation by the modified Kuhn-Roth procedure. Hence, a small quantity of saturated material containing a n-propyl side-chain also was formed during the hydrogenation of the diene system in compound VII.

In contrast to the well-defined crystalline products (VI and VII) obtained from α-cyanobromoacrifoline (IV), the crystalline β-cyanobromoacrifoline (V) yielded non-crystalline products on similar treatment. Thus, reaction of compound V with trimethylamine gave a non-crystalline ether-insoluble quaternary ammonium bromide (IX). Other reactions of compound V, including reduction of the carbonyl group with sodium borohydride, and removal of the bromine atom with hydrogen and palladium at room temperature or dry ice temperature, also gave non-crystalline products. Treatment of the quaternary bromide (IX) with potassium tertiary butoxide gave a neutral product (X), obtained as an oil, that contained a terminal methylene group. The presence of the $=CH_2$ unit was indicated by infrared absorptions at 910 cm^{-1} with an overtone at 1820 cm^{-1}, and confirmed by ozonolysis and subsequent isolation and identification of formaldehyde. The terminal double bond was not in conjugation with the original double bond since there was no absorption in the ultraviolet spectrum. Reduction of the diolefin (X) with hydrogen and platinum gave an oil that partially crystallized from ether. This crystalline product (XI) analyzed correctly for $C_{17}H_{26}O_2N_2$ and was obtained in 24% overall yield from β-cyanobromoacrifoline (V).

A modified Kuhn-Roth oxidation of compound XI yielded butyric, propionic and acetic acids in an overall yield of 68% in a molar ratio of approximately 44:14:42. The presence of butyric acid among the oxidation products proved that a n-propyl side-chain was present in compound XI, and therefore that acrifoline had three $-CH_2-$ groups emanating from the nitrogen atom. The relatively high yield of butyric

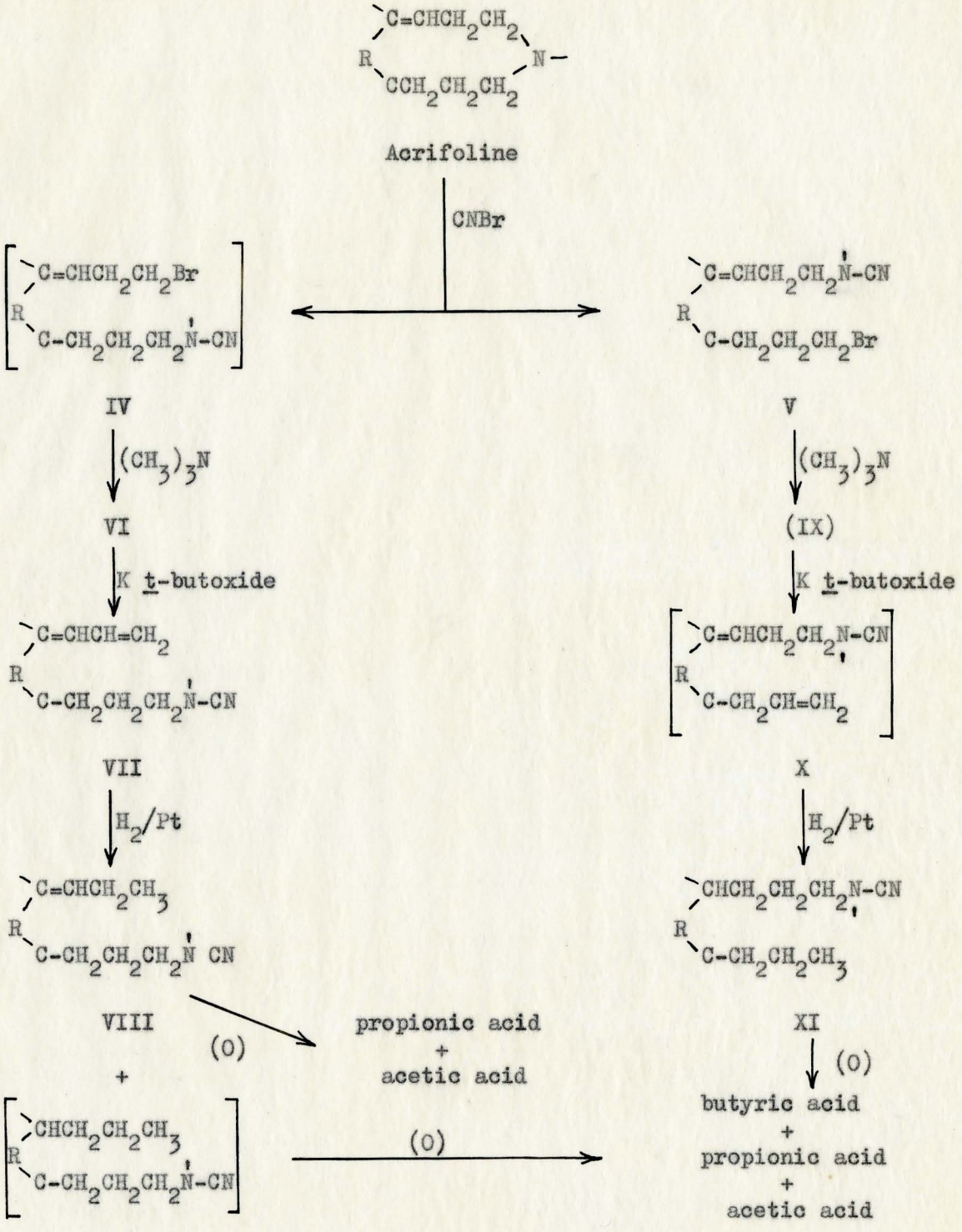


Figure 2. The von Braun Reaction of Acrifoline.

TABLE I

Results of the Modified Kuhn-Roth Oxidations

Compound oxidized	Percent yield of volatile acids	Volatile acids found	Percentage of individual acids in total product
Acrifoline	86	acetic	100
Reduced β-cyano compound (XI)	66	acetic propionic butyric	42 14 44
1, 2-Reduced Hofmann product (III)	85	acetic propionic	67 33
1, 2-Reduced α-cyano-acrifoline (VIII)	73	acetic propionic	69 31
Crude hydrogenated Hofmann product	(100)	acetic propionic butyric	77 19 4
Crude hydrogenated α-cyanoacrifoline	68	acetic propionic butyric	77 21 2

acid indicated that the n-propyl group in compound XI was not joined to a quaternary center. The reaction of acrifoline with cyanogen bromide is summarized in Figure 2, and the results of the modified Kuhn-Roth oxidations are given in Table I.

The Reactions of Phenylacrifoline

Evidence has been presented that the carbonyl group and the carbon atom bearing the hydroxyl group in acrifoline are separated by two or three atoms. A study of phenylacrifoline (XII) provided further support for the postulated relationship of these two groups. Phenylacrifoline, $C_{22}H_{29}O_2N$, was prepared by the reaction of acrifoline with phenyllithium. Its ultraviolet spectrum showed typical benzenoid absorption with λ_{max} at 2580Å, ϵ =228. Phenylacrifoline contains a tertiary hydroxyl located on a carbon atom alpha to the benzene ring, and might be expected to dehydrate with relative ease. However, dehydration proved to be fairly difficult. Phenylacrifoline was recovered unchanged after treatment with hot 37% hydrochloric acid but a reaction occurred in 85% phosphoric acid at 150°. The product (XIII), $C_{22}H_{27}ON$, apparently was an ether since it was formed from phenylacrifoline with the loss of one mole of water and did not have any hydroxyl absorption in its infrared spectrum. The ether linkage appeared to be quite stable since treatment of compound XIII with hot 48% hydrobromic acid caused no change. Therefore the ether oxygen may be located in a five- or six-membered ring formed by interaction of the two hydroxyl groups.

Dehydration of phenylacrifoline was achieved without ether formation by blocking one of the hydroxyl groups. Treatment of phenylacrifoline (XII) with acetic anhydride gave a monoacetate (XIV) that presumably was formed by the acetylation of the secondary hydroxyl group. Dehydration of compound XIV with wet phosphorus oxychloride (effectively phosphoric acid) gave a product (XV) whose ultraviolet spectrum showed styrenoid absorption with λ_{max} at 2420Å, $E^{1\%}_{1\ cm}$ =117 (ϵ ca 4,200). Compound XV was not obtained in a crystalline form, but the position and intensity of its ultraviolet absorption showed that the double bond introduced by dehydration was in conjugation with the benzene ring, and that the original double bond in phenylacrifoline was not part of the chromophoric system. Hydrolysis of the acetyl group in compound XV with aqueous potassium hydroxide gave a hydroxy compound (XVI) that formed a crystalline methiodide, $C_{22}H_{27}ON.CH_3I$.

Phenylacrifoline underwent Hofmann reaction in the same manner as acrifoline. Treatment of phenylacrifoline methiodide with potassium tertiary butoxide gave a crystalline base (XVII) melting at 194-195°. The analytical values for carbon and hydrogen were slightly lower than those calculated for $C_{23}H_{31}O_2N$ even though the product appeared to be pure. In fact, difficulty was experienced in obtaining good analytical results for most of the Hofmann products encountered in the present investigation. This may have been caused by traces of solvent and moisture not removed by conventional procedures. The ultraviolet spectrum of the Hofmann product (XVII) had λ_{max} at 2430Å with ϵ =27,000. Hence compound XVII contained a conjugated diene system whose absorption was not influenced by the presence of the phenyl group in the molecule.

Bertho and Stoll (24) had previously reported a Hofmann degradation of phenylacrifoline carried out by thermal decomposition of its methyl quaternary hydroxide. Their product melted at 192-193° and was obtained in 22% yield. Undoubtedly this compound is the same as compound XVII obtained above.

Selenium Dioxide Oxidation of Acrifoline

A study of the products of selenium dioxide oxidation of acrifoline revealed the nature of several carbon atoms near the carbonyl group, and their positions relative to the hydroxyl group. Two products were isolated from the reaction of acrifoline with selenium dioxide. The minor product (XVIII), obtained in 6.5% yield, analyzed for $C_{16}H_{21}O_2N$. The ultraviolet spectrum of this material (as the perchlorate) showed no strong absorption and its infrared spectrum showed carbonyl absorption at 1725 cm^{-1} but no hydroxyl absorption. The major product (XIX), obtained in 30% yield, had the same molecular formula - $C_{16}H_{21}O_2N$. The infrared spectrum of compound XIX (as its perchlorate) showed carbonyl absorption at 1690 cm^{-1}, enhanced double bond absorption at 1627 cm^{-1} and hydroxyl absorption at 3530 cm^{-1} while its ultraviolet spectrum showed absorption of medium intensity with λ_{max} at 2430Å, ϵ=5,330. The two spectra establish the presence of a $CO-\overset{\alpha}{C}=\overset{\beta}{C}$ unit in compound XIX and therefore the presence of a CO-CH-CH unit in acrifoline. The position of the ultraviolet absorption maximum at 2430Å in compound XIX suggested that the chromophore CO-C=C was at least trialkylated (42), or that the -CO-C=C- unit carried one alkyl substituent which might represent a point

of ring juncture. It also indicated that the -CO-C=C- chromophore was isolated from the double bond originally present in acrifoline. The possibility of rearrangement during the formation of compound XIX was precluded by hydrogenation of compound XIX to dihydroacrifoline.

The unsaturated carbonyl compound (XIX) was readily converted to the saturated carbonyl compound (XVIII) by base catalyzed addition of the hydroxyl group to the conjugated double bond system. Hence the formation of a small amount of compound XVIII during the reaction of acrifoline with selenium dioxide can be attributed to the intramolecular reaction of the primary product (compound XIX) in the reaction medium. The ease of addition of the hydroxyl group to the double bond in compound XIX implies that a five- or six-membered cyclic ether is formed, and that the carbon atom β to the carbonyl group and the carbon atom carrying the hydroxyl group are separated by two or three atoms.

The character of the carbon atoms α and β to the carbonyl group in acrifoline was established by an examination of the nuclear magnetic resonance spectra of compounds XVIII and XIX. The NMR spectrum of compound XVIII had a doublet at a displacement of 3.72 ppm representing the $>CHCH_3$ group, and a peak of area 1 at a displacement of -0.51 ppm representing the single proton on the double bond. Two peaks, each of area 1, occurring at displacements of 0.69 and 1.04 ppm were attributed to absorption by two >CHO- groups, one group at either end of the ether linkage. The presence of one proton on the carbon atom β to the carbonyl group in compound XVIII, and therefore in compound XIX as well, shows that this carbon atom is present as a $-CH_2-$ group in acrifoline.

The NMR spectrum of the unsaturated carbonyl compound (XIX) had two peaks of area 1 at displacements of -0.54 and 0.87 ppm representing the isolated double bond proton and the >CHO-group respectively. A single sharp peak at a displacement of 3.00 ppm was attributed to the methyl group. The singlet nature of this peak and its chemical shift of 3.00 ppm shows that the methyl group is located on the double bond α, β to the carbonyl group. The carbon atom β to the carbonyl group has already been shown to carry only a proton substituent so that the methyl group must be situated on the α-carbon atom. A peak of area 1 occurring at a displacement of -2.27 ppm was attributed to the proton on the β-carbon since protons on the β-carbon of α, β-unsaturated carbonyl compounds absorb at relatively low field strengths (e.g., tiglic and angelic acids (43)). The presence of a $-COCH(CH_3)CH_2-$ system in acrifoline with the $-CH_2-$ unit separated from the carbon atom bearing the hydroxyl group by two or three atoms is thus established. These reactions are summarized in Figure 3.

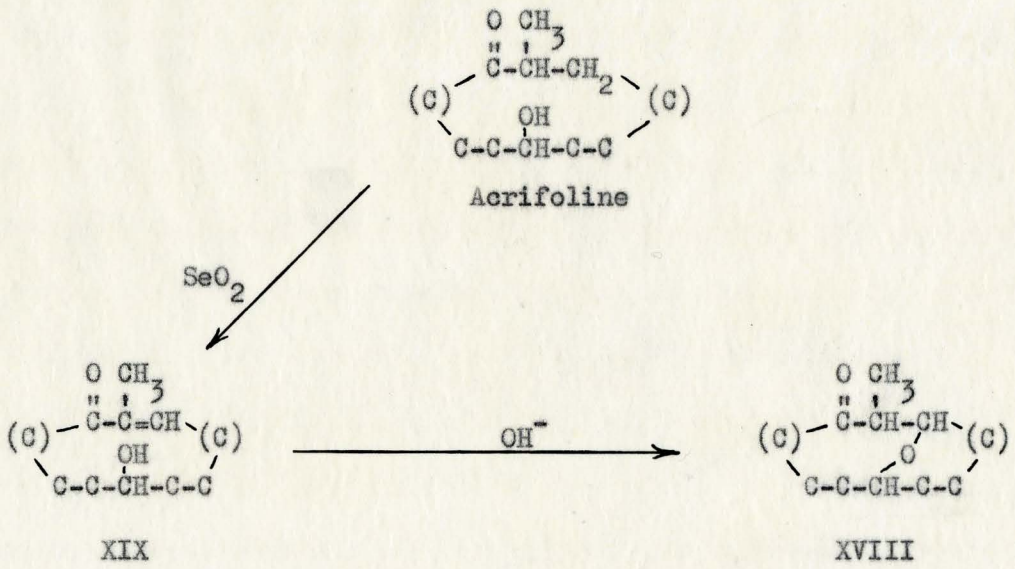


Figure 3. The Reaction of Acrifoline with Selenium Dioxide

Oppenauer Oxidations

Oppenauer oxidations have been carried out on acrifoline and several of its derivatives. In the early stages of the work, these oxidations were undertaken in an effort to establish the nature of the hydroxyl group since at that time the NMR data were not available. It was found that acrifoline and the Hofmann product (I) were not oxidized by the Oppenauer method using a variety of solvents and reaction conditions, which implied that the hydroxyl group was not secondary. However, acrifolinol underwent an Oppenauer oxidation on treatment with aluminum iso-propoxide and cyclohexanone in boiling toluene for 33 hours. The product (XX), $C_{16}H_{23}O_2N$, which was isolated in crystalline form as its methiodide, contained hydroxyl and carbonyl groups. The latter absorbed at 1700 cm^{-1} in the infrared and was located in a ring that was six-membered or larger. This result was of little value since the base XX could not be isolated in crystalline form and it was not established which of the hydroxyl groups in acrifolinol had been oxidized.

In contrast to acrifolinol and acrifoline, dihydroacrifolinol was readily oxidized by the Oppenauer method. Perry and MacLean (3) had reported two dihydroacrifolinols. α-Dihydroacrifolinol was reported to form by hydrogenation of acrifoline to dihydroacrifoline followed by reduction of the carbonyl group with lithium aluminum hydride. β-Dihydroacrifolinol was formed by initial reduction of the carbonyl group of acrifoline to give acrifolinol followed by hydrogenation. In the present investigation, the same product was formed by either of the above

procedures. The product, dihydroacrifolinol, melted at 192.5-193.0° and proved to be identical with the α-dihydroacrifolinol previously reported. Treatment of dihydroacrifolinol with aluminum tertiary butoxide and cyclohexanone in boiling toluene for seven hours gave a crude product that showed two strong absorptions of equal magnitude at 1705 and 1735 cm^{-1} in the carbonyl region of the infrared spectrum, and hydroxyl absorption at 3500 cm^{-1}. Chromatography of the crude product led to the isolation of only one compound (XXI), $C_{16}H_{23}O_2N$, in 45% yield, that was analyzed as its hydrobromide. The free base (XXI) melted at 128-129° but was difficult to obtain in crystalline form in good yield. The infrared spectrum of the base XXI showed a single strong carbonyl absorption at 1705 cm^{-1} and no hydroxyl absorption. Compound XXI is probably a diketone with both carbonyl groups located in rings that are six-membered or larger. Absorption at 1420 cm^{-1} in the spectra of both the diketone XXI and its hydrobromide and none in this region in the spectrum of acrifoline (nujol, chloroform of carbon tetrachloride) implies that a $-CH_2CO-$ group is present in compound XXI but is absent in acrifoline (44). Thus the $-CH_2CO-$ group in the diketone XXI must have arisen from a $-CH_2CHOH-$ group in acrifoline.

In Figure 4 an explanation for the occurrence of peaks at 1735 and 3500 cm^{-1} in the infrared spectrum of crude compound XXI is offered. It is proposed that the diketone XXI exists in equilibrium with the adduct XXII. Structure XXIII, an alternative formulation, is ruled out on steric grounds when one considers the total structure of the alkaloid.

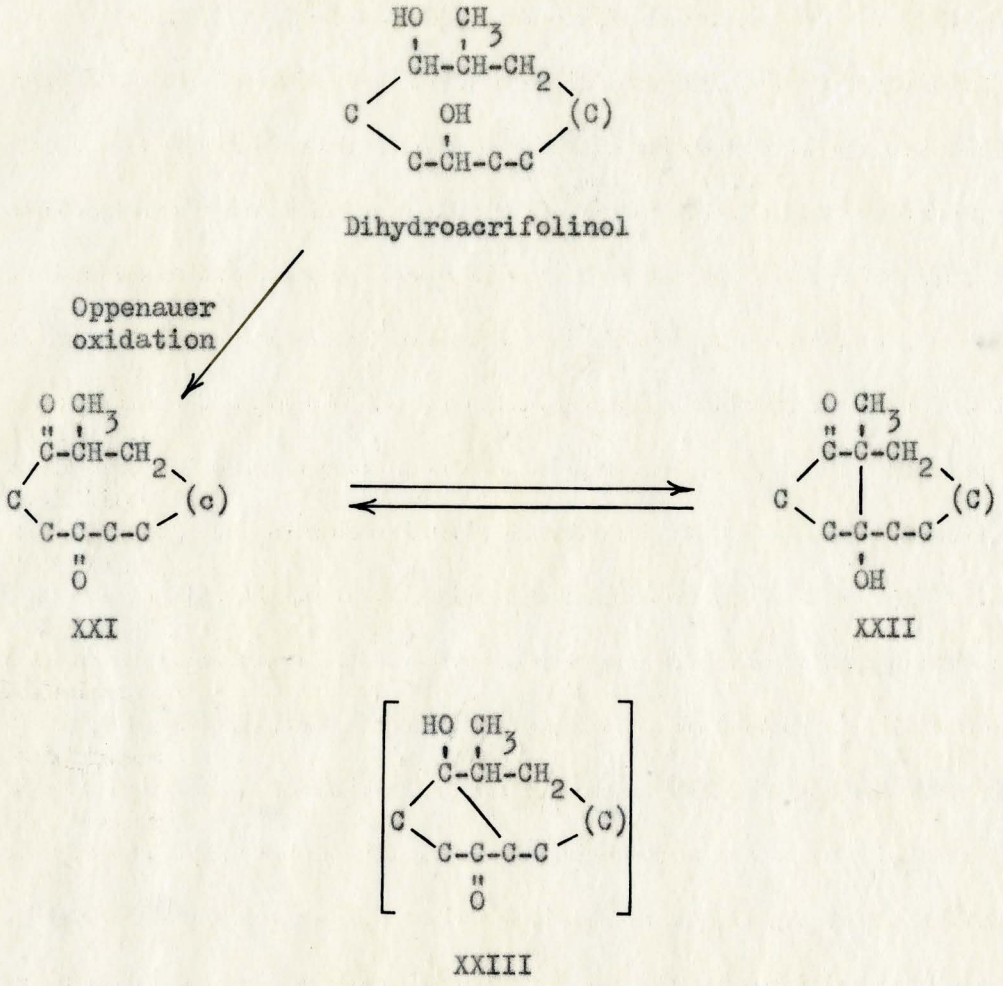
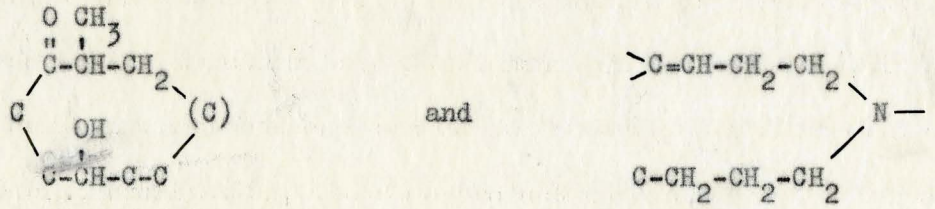


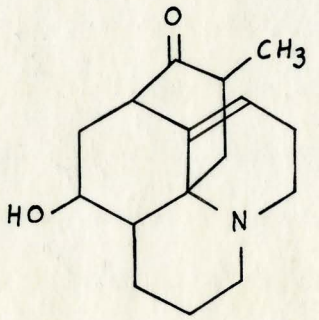
Figure 4. The Oppenauer Oxidation of Dihydroacrifolinol.

The Structure of Acrifoline

The evidence presented in the preceeding discussion has shown that acrifoline has the following structural features:



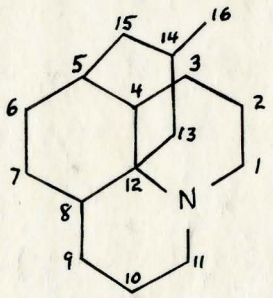
In addition, it has been shown that one of the carbon atoms adjacent to the carbon carrying the hydroxyl function may be present as a $-CH_2-$ group, and that the carbonyl group and the >CHOH group are both located in rings that are six-membered or larger. Structure A, which is formulated for acrifoline, is in accord with these facts



A

and is biogenetically feasible.

To facilitate the subsequent discussion, the numbering system in B will be used.



B

Other experimental evidence confirms structure A. The hydroxyl group does not appear to be allylic since attempts to replace it by halogen in acrifoline, acrifolinol, and the Hofmann product (I) were of no avail. Similarly, attempts to dehydrate these compounds were unsuccessful. The location of the bridgehead between the carbonyl

group and the double bond explains why an interaction was not observed between these two groups in acrifoline and its degradation products. The relatively good yield of butyric acid realized on oxidation of dihydro-β-cyanoacrifoline (XI) is consistent with a tertiary center at C_8.

The quaternary character of C_{12} is confirmed by NMR data. The NMR spectrum of dehydrobrominated α-cyanobromoacrifoline (compound VII) shows a single absorption at a displacement of 1.47 ppm characteristic of a $-CH_2-$ unit adjacent to the cyanamide group. There is no other absorption in this region which might be expected if a $\geq CH$ group were present as well. The NMR spectrum of the unsaturated selenium dioxide product (compound XIX) shows a single sharp absorption at -2.27 ppm owing to the proton on the -COC(Me)=CH- system. The sharpness of this peak precludes the presence of a proton on an adjacent carbon atom.

pK_a measurements on acrifoline and its derivatives are also in accord with structure A. The determinations were carried out in 50% aqueous methanol and the results are given in Table II. Perry and MacLean (3) previously reported pK_a values of 8.34 and 9.13 for acrifoline and dihydroacrifoline respectively, and proposed that the increase in basic strength on reduction might be due to the presence of a cyclic allylamine structure in acrifoline. A difference of pK_a values of these compounds is also found in the present case, but this difference (0.55 units) is somewhat less than previously reported (0.79 units), and is still less than values reported for other cyclic allylamines (45, 46) where differences are in the order of 1.3-1.5 units. Similarly, the

TABLE II

pK_a Values of Acrifoline and Derivatives

Compound	pK_a
Acrifoline	8.05
Dihydroacrifoline	8.60
Acrifolinol	8.36
Hofmann product (I)	7.55
Reduced Hofmann product (III)	7.40
Unsat'd SeO_2 product (XIX	7.40
Sat'd SeO_2 product (XVIII)	6.95
Copper sulphate product (XXIV)	7.70

difference between the pK_a values of acrifoline and the unsaturated selenium dioxide product (XIX), where the double bond is also in a β, γ-position relative to nitrogen, is of the same order of magnitude (0.65 units) as that between dihydroacrifoline and acrifoline (0.55 units).

The correlation of the pK_a values and structures is shown in Figure 5. The difference in pK_a values between the various structures is given beside the arrows in Figure 5. It is noted that the introduction of a double bond at a β, γ-position relative to nitrogen decreases the basicity by about 0.60 units (0.55 and 0.65), and the conversion of the hydroxyl group to an ether lowers the basicity about 1.0 unit (0.90 and 1.10).

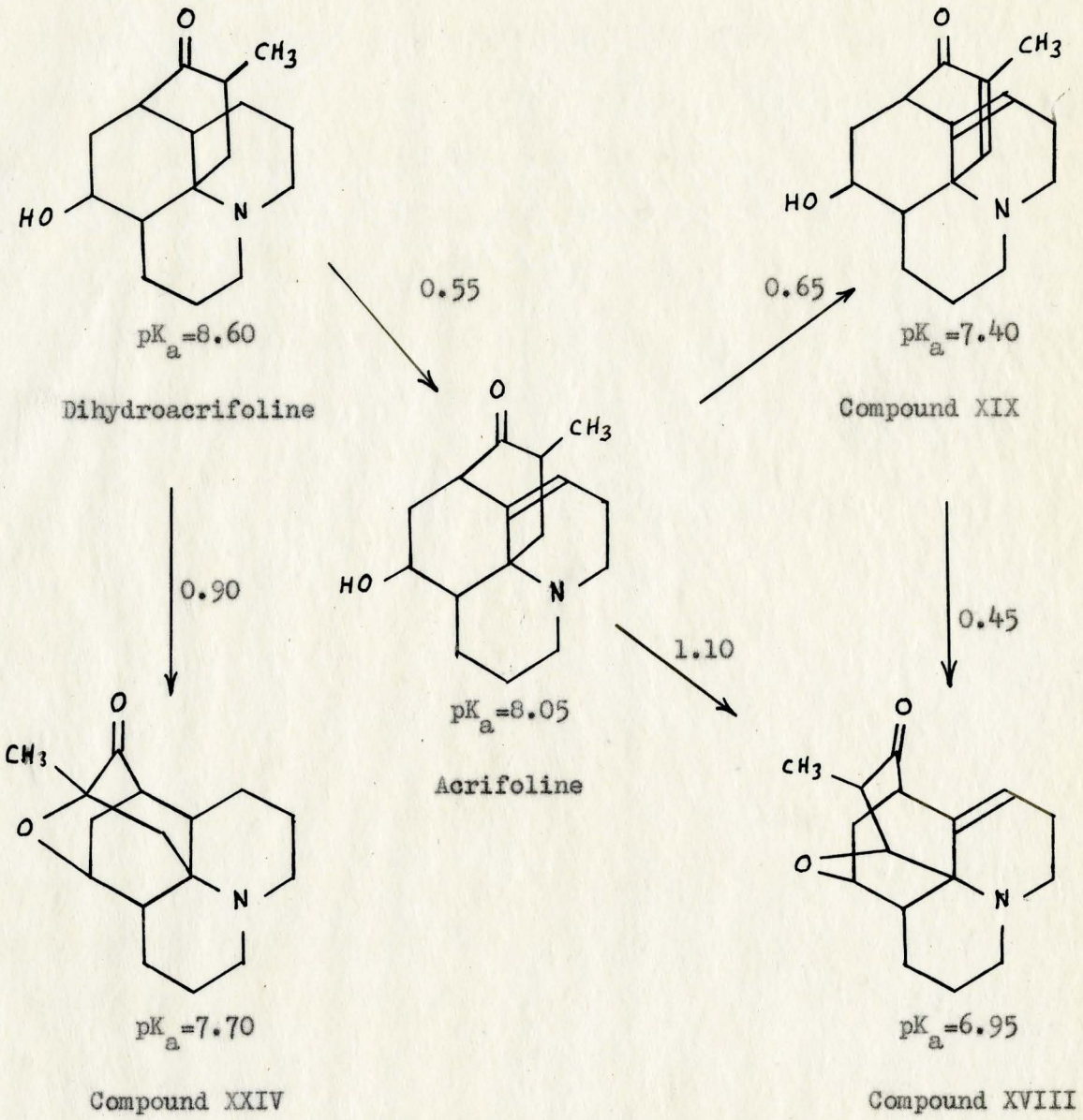
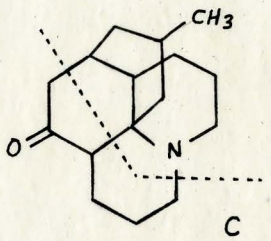


Figure 5. The Relationship of pK_a Values and Structures of Acrifoline and Some Derivatives.

An attempt was made to verify structure A through dehydrogenation studies. However, acrifoline failed to yield identifiable products in liquid phase or gas phase dehydrogenation experiments. Vapor phase chromatography of the products of these reactions gave peaks in the region

expected of quinoline and its derivatives but crystalline products or derivatives were not isolated in any case. This is in contrast to the behaviour of lycopodine which has been reported to yield 7-methylquinoline in good yield in liquid phase dehydrogenation (31). In the present study, a 15-20% yield of 7-methylquinoline was obtained from lycopodine by the gas phase dehydrogenation procedure of Linstead (47). The same procedure was used without success on acrifoline.

In lycopodine, cleavage of the ring system probably occurs as shown in structure C. In acrifoline, although



the skeletal structure is similar, the geometry of the molecule may be such that the compound cannot come into favorable contact with the catalyst for dehydrogenation to occur. The position of the carbonyl group on the bridge in acrifoline rather than in an activating position as in lycopodine may also hinder a dehydrogenation reaction.

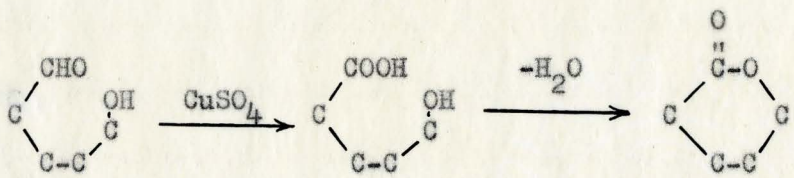
Other reactions of acrifoline which were studied are reported below.

The Reaction of Dihydroacrifoline with Copper Sulphate and with Peroxytrifluoroacetic Acid

In the early stages of the investigation (before the NMR data were obtained) it was not known whether the carbonyl group was present

in acrifoline as an aldehyde or ketone. However, acrifoline was sensitive to a variety of oxidizing agents and it appeared that the carbonyl group was present as an aldehyde. Tests with Tollen's and Fehling's reagents under ordinary conditions were negative, but a silver mirror was deposited when acrifoline was heated with silver acetate in aqueous pyridine. Acrifoline also reacted with copper sulphate in pyridine and gave a small amount of uncharacterized base that had carbonyl absorption at 1725 cm^{-1} in its infrared spectrum.

The reaction of dihydroacrifoline, $C_{16}H_{25}O_2N$, with copper sulphate in aqueous pyridine gave a new compound (XXIV), $C_{16}H_{23}O_2N$, that was isolated in 78% yield. The infrared spectrum of this material showed carbonyl absorption at 1725 cm^{-1} but no hydroxyl absorption. From the infrared and analytical data it appeared that compound XXIV might contain a six-membered lactone formed in the following manner:

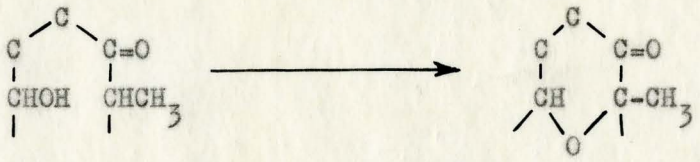


Compound XXIV was also obtained from an attempted Baeyer-Villiger oxidation of dihydroacrifoline. The reaction was carried out under standard conditions (48, 49) using peroxytrifluoroacetic acid in methylene chloride in the presence of disodium phosphate. The formation of compound XXIV under these conditions was considered to follow the same pathway as that proposed above for the oxidation of dihydroacrifoline with copper sulphate in aqueous pyridine. However, the reactions of compound XXIV were not compatible with a lactone structure.

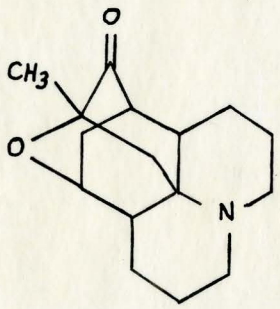
The carbonyl group in compound XXIV underwent reduction in excellent yields with several reducing agents. Thus, treatment of compound XXIV with sodium borohydride, lithium aluminum hydride, sodium methoxide, or barium hydroxide in aqueous methanol gave the same hydroxy compound (XXV), $C_{16}H_{25}O_2N$, in 90-95% yields. The latter two reductions were carried out in sealed tubes at 175° and represent rather uncommon types of Meerwein-Ponndorf-Verley reductions (50). The reduction product (XXV) contained only one hydroxyl group since it formed a monoacetate (XXVI), $C_{18}H_{27}O_3N$, on treatment with the mixed anhydride of trifluoroacetic and acetic acids. Chromic acid oxidation of compound XXV in aqueous acetic acid at -15° gave compound XXIV in 86% yield.

The carbonyl group in compound XXIV also reacted readily with phenyllithium and methyllithium. The reaction of compound XXIV with phenyllithium gave a monophenyl derivative (XXVII), $C_{22}H_{29}O_2N$, in 89% yield that had λ_{max} at 2580Å, ϵ=237 in its ultraviolet spectrum. Similarly, the reaction of compound XXIV with methyllithium afforded the corresponding methyl derivative (XXVIII), $C_{17}H_{27}O_2N$.

The structure of the copper sulphate product (XXIV) was established by NMR spectroscopy. Its NMR spectrum had absorption of area 1 at a displacement of 1.05 ppm owing to a >CHO- group, and a sharp single peak at a displacement of 3.66 ppm. The singlet nature of the latter peak established that the carbon atom bearing the methyl group no longer carried a proton, and its appearence at a displacement of 3.66 ppm indicated that this carbon atom might be joined to an oxygen atom. Therefore the reaction



must have occurred on treatment of dihydroacrifoline with either peroxytrifluoroacetic acid or copper sulphate in pyridine. The product (XXIV) was assigned structure D.



D

The overall reaction closely resembles that of the oxidation of other aldehydes and ketones by various reagents. For example, Waters et al (51, 52) have extensively studied the oxidations of carbonyl compounds and have found that oxidation occurs by a free radical process at the α-carbon atom after enolization has taken place. The oxidation of dihydroacrifoline by copper sulphate can be represented by a similar process, as in Figure 6.

The first step of the reaction involves the removal of a proton by a base to give the enolate ion (XXIX). Abstraction of an electron from XXIX by Cu^{++} gives the mesomeric radical XXX, and further electron loss from XXX yields the carbonium ion XXXI. The latter intermediate would readily yield the product E (compound XXIV) by loss of a proton. Step XXX → XXXI requires the removal of an electron from the resonance

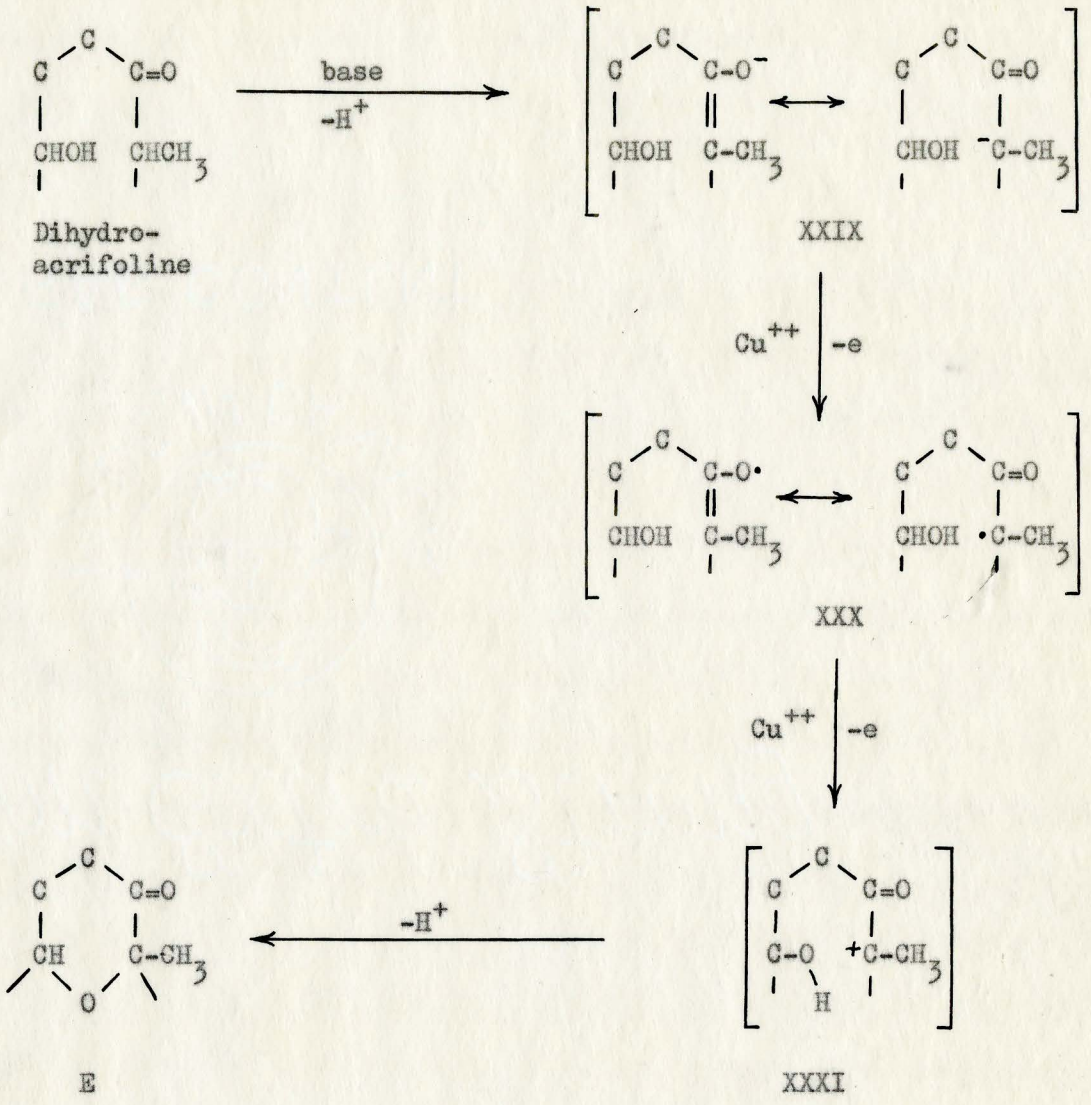


Figure 6. The Oxidation of Dihydroacrifoline

With Copper Sulphate

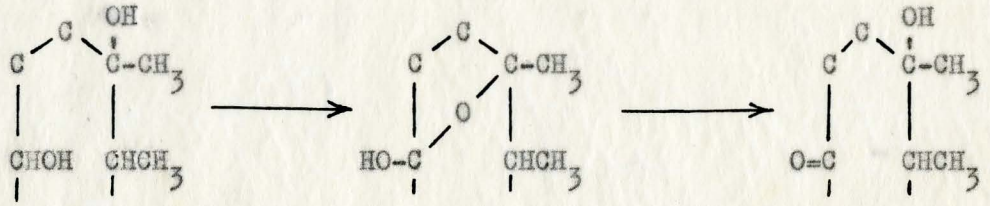
stabilized radical XXX. However, the reaction may proceed directly from the radical XXX to the product E by simultaneous removal of a proton and an electron, and not pass through the energetically unfavorable carbonium ion XXXI at all. It has been postulated (51) that such oxidations proceed through a number of organic-inorganic complexes that make the reaction

energetically favorable. In the case of cyclohexanone, a disproportionation of the free radical into an anion and a cation has been proposed (52).

The Reactions of Methylacrifoline and Methyldihydroacrifoline

Two derivatives of acrifoline whose reactions were studied in this investigation are methylacrifoline and methyldihydroacrifoline. Methylacrifoline (XXXII), $C_{17}H_{27}O_2N$, was prepared in 80% yield by the reaction of acrifoline with methyllithium. Oxidation of methylacrifoline either by chromic acid or by the Oppenauer procedure yielded a new base (XXXIII), $C_{17}H_{25}O_2N$, in yields of 40% and 31% respectively. This compound was formed by the loss of two hydrogen atoms but it had no carbonyl absorption in its infrared spectrum. Methyldihydroacrifoline (XXXIV), $C_{17}H_{29}O_2N$, behaved similarly. It was prepared in 80% yield by the reaction of dihydroacrifoline with methyllithium, and in 85% yield by the hydrogenation of methylacrifoline (XXXII). Oxidation of compound XXXIV by the Oppenauer procedure as well as by chromic acid gave a base (XXXV), $C_{17}H_{27}O_2N$, in yields of 70% and 54% respectively. Again, two hydrogens were lost in the oxidation process but no carbonyl absorption was present in the infrared spectrum of the product. However, on treatment with 37% hydrochloric acid in methanol, compound XXXV gave a new base (XXXVI) having the same formula - $C_{17}H_{27}O_2N$. This product had a strong absorption at 1720 cm^{-1} and a hydroxyl absorption at 3330 cm^{-1}. When compound XXXV was treated with sodium methoxide in methanol, starting material was recovered. To account for the observed behaviour of these

compounds it is proposed that the oxidation of compound XXXIV gave a hemi-ketal (XXXV), and that the hemi-ketal ring was opened on treatment with acid, as follows:

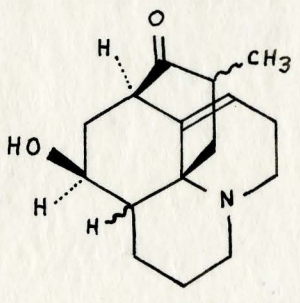


XXXIV XXXV XXXVI

The fact that the isomerization takes place in acid medium but not in basic medium may indicate that the tertiary alcoholic center is epimerized during cleavage of the hemi-ketal.

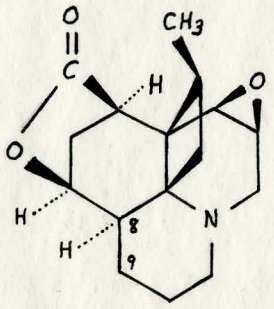
Stereochemistry of Acrifoline

An examination of the structure of acrifoline (A) shows that there are three asymmetric centers (C_7, C_8, and C_{14}) which are not fixed by the ring system. The hydroxyl group at C_7 must be cis to the C_{13}-C_{15} bridge because of the interaction of the hydroxyl group with these three carbon atoms. Center C_{14} adjacent to the carbonyl group will adopt the more stable configuration. The third asymmetric center (C_8) is not defined as readily. The stereochemistry of these centers is shown in structure F.

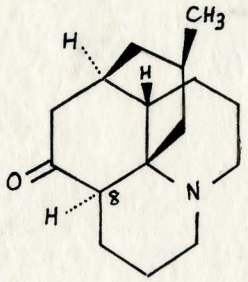


F

It is of interest to compare the geometry of structure F with that of annotinine (G) and that proposed for lycopodine (H). The configuration of C_8 in annotinine (G) is the less stable of the two possible forms, and epimerization occurs at this center whenever possible (6, 53). The instability at C_8 in G has been ascribed to the non-bonded



G



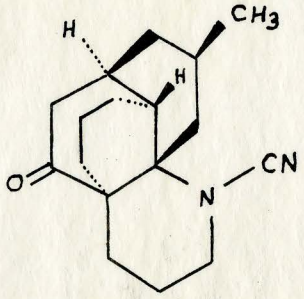
H

interaction between the hydrogens of the four-membered ring and a hydrogen on C_9 (6). The configuration at C_8 in lycopodine (H) may be the same as that in annotinine (G) and hence may represent the more stable of the two possible epimers of this skeletal system. This proposal is made because dihydrolycopodine, in which the hydroxyl group is probably oriented toward the C_{13}-C_{15} bridge, is readily dehydrated to anhydrodihydrolycopodine (2, 54) indicating that the hydroxyl group on C_7 and the hydrogen atom on C_8 in dihydrolycopodine are located trans and axial to each other.

In acrifoline, there is little or no evidence in favor of any specific configuration at the C_8 position. The fact that the hydroxyl group at C_7 could not be dehydrated may indicate that the proton on C_8 and the hydroxyl on C_7 are not trans and axial, and therefore that the C_8 configuration may be unlike that of annotinine or lycopodine. A study of molecular models of acrifoline does not allow a differentiation

between the two configurations since both appear to be equally stable. Therefore, a knowledge of the configuration at C_8 must await a more detailed chemical study of this region of the molecule. Such a study might lead to the conversion of acrifoline to lycopodine, and to other Lycopodium alkaloids as well.

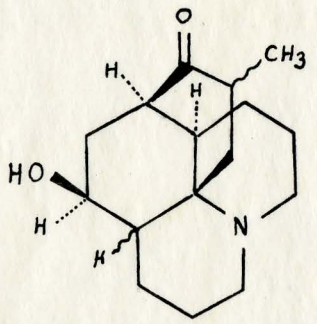
The greatest obstacle in the conversion of acrifoline to lycopodine would appear to be the attainment of the correct configuration at C_4. The configuration at C_4 in lycopodine is shown in H with the C_4-hydrogen cis to the C_{13}-C_{15} bridge. This assignment is made because α-cyanobromolycopodine forms a cyclic derivative with C_1 joined to C_8, as shown in structure J (55, 56).



J

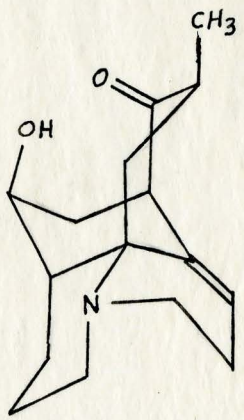
Examination of a molecular model of acrifoline reveals that the least hindered side of the double bond is that side opposite the C_{13}-C_{15} bridge. Since hydrogenation of a double bond occurs from the least hindered side, the product (dihydroacrifoline) from the hydrogenation of acrifoline would have a configuration at C_4 opposite to that in lycopodine. Chemical results appear to substantiate this proposed mode of reduction. Previously, it was shown that methyldihydroacrifoline (XXXIV) was formed in

good yield either by the hydrogenation of methylacrifoline or by the reaction of dihydroacrifoline with methyllithium. The formation of the same product demonstrates that the same mode of reduction of the double bond has occurred in each case. Methylacrifoline has an additional methyl group on the C_{13}-C_{15} bridge so that the double bond at C_3-C_4 would be even less prone to attack on the bridge side than in acrifoline. Therefore, reduction must occur on the side of the molecule opposite the C_{13}-C_{15} bridge, and hence dihydroacrifoline has structure K.

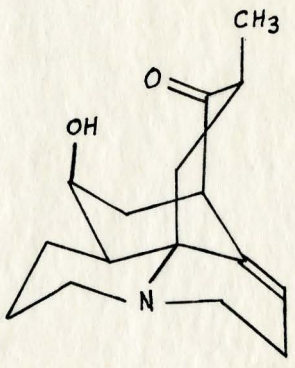


K

Two perspective structures of acrifoline differing only in configuration at C_8 are shown in M and N.

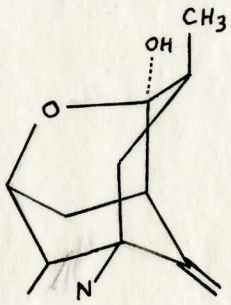


M

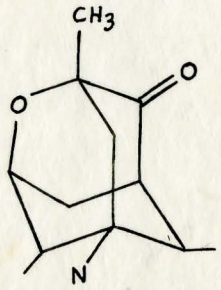


N

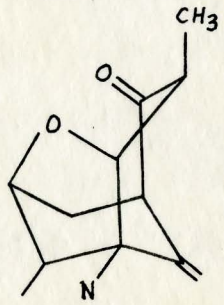
A study of molecular models indicates that the ring carrying the methyl group is probably in the boat form with the methyl group oriented as shown. In any case, the ring must be in this conformation for hemi-ketal and ether formation between C_7 and C_{15}. The geometry of the compounds formed by the interaction of the hydroxyl group with carbon atoms C_{13}, C_{14}, and C_{15} is shown in partial structures O, P, and Q. Structure O represents acrifoline in the hemi-ketal form, structure P



O



P



Q

represents the copper sulphate product (XXIV), and structure Q represents the saturated selenium dioxide product (XVIII).

EXPERIMENTAL

All melting points are corrected. Infrared spectra were measured in nujol mull unless otherwise specified and were determined on a Perkin-Elmer Model 21 recording spectrophotometer with sodium chloride optics. Ultraviolet spectra were measured in 95% ethanol on a Perkin-Elmer Model 4000 Spectracord. Nuclear magnetic resonance spectra were measured in concentrated chloroform solutions in spinning sample tubes using a Varion V-4300B NMR spectrometer equipped with field stabilizer, at a fixed frequency of 56.4 Mc/sec. Chemical shifts were measured by the side-band technique using chloroform as the reference material, and calculated to give the displacement (δ) in ppm from water.

The microanalyses were carried out by Drs. G. Weiler and F. B. Strauss of Oxford, England, by Mr. A. E. Ledingham of the Dominion Rubber Company, Guelph, Ontario, and by Mr. E. Thommen of Basel, Switzerland.

Source of Acrifoline

Acrifoline was available from the breakdown of annotoxine which had crystallized from the mother liquors remaining after the separation of annotinine from the mixed alkaloids of L. annotinum. The breakdown of annotoxine was accomplished by fractional crystallization of its hydrobromide salt. The hydrobromide salt, which melted above 315°, was analyzed for its C-methyl content.

Calc. for $C_{16}H_{23}O_2N.HBr$: one C-methyl, 4.39%.

Found: C-methyl, 4.15%.

Preparation of Acrifoline Methiodide

A solution of acrifoline (1.0 g) in 15 ml of acetone and 3 ml of methyl iodide was heated under reflux and within 2 minutes a crystalline methiodide began to separate. The mixture was heated under reflux for 15 minutes longer, cooled, and let stand at room temperature for 1 hour. Filtration gave 1.23 g (80%) of product melting at 278-279° (with decomposition). An additional 0.260 g (17%) of product was obtained from the filtrate on further treatment with methyl iodide in acetone. Recrystallization of the methiodide from methanol-acetone gave colorless needles melting at 280-281° (with darkening). Bertho and Stoll (24) report a melting point of 249-250° and Achmatowicz and Rodewald (25) a melting point of 267-268° for this compound.

Calc. for $C_{16}H_{23}O_2N.CH_3I$: C, 50.6; H, 6.49; N, 3.47%.

Found: C, 50.5; H, 6.47; N, 3.12%.

Treatment of Acrifoline Methiodide with Potassium Tertiary Butoxide

A mixture of acrifoline methiodide (1.10 g) and potassium tertiary butoxide (1.50 g) in 60 ml of tertiary butanol and 2 ml of benzene was heated under reflux for 4 hours. The mixture was cooled, water was added and the organic solvents removed under reduced pressure. The resulting alkaline aqueous mixture was extracted several times with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual oil was crystallized from petroleum ether to give 0.53 g (70%) of colorless needles melting

at 158-159°. Recrystallization from petroleum ether gave compound I melting sharply at 161°.

Calc. for $C_{17}H_{25}O_2N$: C, 74.1; H, 9.15%.

Found: C, 73.7; H, 9.02%.

The infrared spectrum of this material had hydroxyl absorption at 3500 cm^{-1}, carbonyl absorption at 1700 cm^{-1}, double bond absorption at 1650 cm^{-1} (weak) and 1625 cm^{-1} (strong), and $=CH_2$ absorption at 900 cm^{-1} with an overtone at 1800 cm^{-1}. The ultraviolet spectrum had λ_{max} at 2400Å, ϵ=24,000.

Ozonolysis of the Hofmann Product (I)

A stream of ozone enriched oxygen was passed through a solution of the Hofmann product (0.10 g) in 15 ml of glacial acetic acid until the uptake of ozone was complete (about 20 minutes), then zinc dust (0.50 g) was added and the mixture steam distilled. The steam distillate (50 ml) was divided into two portions. To one portion was added 5 ml of a 2, 4-dinitrophenylhydrazine solution (prepared by dissolving 2 g of 2, 4-dinitrophenylhydrazine in 20 ml of sulphuric acid, then diluting with 50 ml of ethanol and 25 ml of water). The resulting product (0.017 g; 50%) showed no depression of the melting point in admixture with authentic formaldehyde 2, 4-dinitrophenylhydrazone. The second portion of the steam distillate was neutralized with a 10% alcoholic sodium hydroxide solution and made slightly acidic with acetic acid. To this solution was added 5 ml of a dimedone solution (prepared by dissolving 1.5 g of dimedone in 25 ml of a 1:3 water:ethanol solution). The resulting product (0.030 g; 56%) had a sharp melting point of 189° on

purification. A mixture melting point with authentic formaldehyde dimedone derivative showed no depression.

Reduction of Compound I with Sodium Borohydride

A solution of compound I (0.20 g) in 15 ml of absolute ethanol was let stand at room temperature for 6 hours. The excess borohydride was destroyed with formaldehyde, water was added and the organic solvent removed under reduced pressure. The product was isolated by extraction of the aqueous alkaline mixture with chloroform, extraction of this chloroform solution with dilute aqueous acid, and extraction of the basified (ammonia) aqueous solution with chloroform. The latter chloroform solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure yielding 0.20 g of crystalline solid melting at 249-251°. An analytical sample of compound II, m.p. 251.5-252.0°, was obtained after two recrystallizations from methanol-acetone.

Calc. for $C_{17}H_{27}O_2N$: C, 73.6; H, 9.81; N, 5.05; NCH_3, 10.5%.

Found: C, 73.4; H, 9.83; N, 5.07; NCH_3, 10.8%.

The infrared spectrum had hydroxyl absorption in the 3000-3200 cm^{-1} region, no carbonyl absorption, double bond absorption at 1625 and 1590 cm^{-1}, and $=CH_2$ absorption at 900 cm^{-1} with an overtone at 1800 cm^{-1}. The ultraviolet spectrum had λ_{max} at 2400Å, ϵ=28,000.

Sodium in Alcohol Reduction of the Diol (II)

A solution of compound II (0.30 g) in 75 ml of absolute ethanol was heated to boiling, and sodium was added in small pieces over a period of 3.5 hours at such a rate that metallic sodium was always present in the medium. The solution was cooled, ethanol and water were added and

the organic solvent removed under reduced pressure. The resulting aqueous mixture was extracted several times chloroform. The combined pale yellow chloroform solution was extracted with dilute aqueous acid and the acid extract made alkaline with ammonia and extracted with chloroform. The latter chloroform solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual colorless oil was crystallized from acetone-ether yielding 0.264 g (88%) of compound III melting at 191.5-192.5°. Recrystallization from acetone-ether raised the melting point to 194.5-195.0°.

Calc. for $C_{17}H_{29}O_2N$: C, 73.0; H, 10.5%.

Found: C, 72.8; H, 10.5%.

Both the infrared and ultraviolet spectrum of this compound showed no absorption characteristic of a conjugated diene system.

Oxidative Determination of Alkyl Side-Chains

The oxidation was carried out in a boiling solution of 3 g of CrO_3 in 10 ml of water so that volatile components were removed by distillation as rapidly as formed. A slow stream of nitrogen passing through the solution served both as a bubbler and as an aid in sweeping out the volatile materials. When 5 ml of water had distilled, 5 ml more were added to the reaction flask from a dropping funnel, and this procedure repeated until 50 ml of distillate had been collected. The pale yellow distillate was redistilled to give a colorless solution which was neutralized with 0.2 N NaOH. Evaporation to dryness on a rotary evaporator yielded the sodium salts of the carboxylic acids. The free carboxylic acids were liberated from their sodium salts by means of

anhydrous hydrogen chloride, and their methyl esters prepared by treatment with ethereal diazomethane. The methyl esters were identified by vapor phase chromatography of the ether solution on a Perkin-Elmer Model 154 Vapor Fractometer using a 6.6 ft didecyl phthallate column (Perkin-Elmer Column A) at a temperature of 75° and helium flow rate of 140 ml/min. Under these conditions, the strong ether peak (taken as zero time) appeared about two minutes after injection of the sample, methyl acetate at 1.3 minutes after the ether peak, methyl propionate at 4.5 minutes and methyl butyrate at 10.9 minutes.

Modified Kuhn-Roth Oxidation of Acrifoline

Oxidation of 0.025 g (0.0957 millimole) of acrifoline yielded 0.082 milliequiv. (86%) of volatile carboxylic acid. Vapor phase chromatography of its methyl ester indicated only the presence of methyl acetate.

Modified Kuhn-Roth Oxidation of the 1, 2-Reduced Hofmann Product (III)

Oxidation of 0.030 g (0.1075 millimole) of compound III yielded 0.183 milliequiv. (85%) of volatile carboxylic acids. Vapor phase chromatography of their methyl esters revealed the presence of methyl acetate and methyl propionate in the molar ratio of 67:33 respectively.

Modified Kuhn-Roth Oxidation of Crude Hydrogenated Hofmann Product

The Hofmann product (I) (0.030 g, 0.109 millimole) was dissolved in methanol and treated with hydrogen (50 psig) and platinum (0.025 g PtO_2) for 15 hours. The catalyst was removed by filtration and the solvent evaporated under reduced pressure leaving a colorless oil. Chromic acid oxidation of this crude product yielded 0.218 milliequiv. of volatile

carboxylic acids. Vapor phase chromatography of their methyl esters revealed the presence of methyl acetate, methyl propionate, and methyl butyrate in the molar ratio of 77:19:4 respectively.

Reaction of Acrifoline with Cyanogen Bromide

(a) Isolation of β-Cyanobromoacrifoline (V)

A solution of acrifoline (1.0 g) and cyanogen bromide (1.0 g) in 15 ml of anhydrous benzene was allowed to stand at room temperature for 40 hours. The solvent and excess cyanogen bromide were removed under reduced pressure and the residue taken up in chloroform, washed with dilute acid, water and aqueous sodium bicarbonate. Acrifoline (0.13 g) was recovered from the acid extract. The chloroform solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving 1.0 g of a pale yellow oil which was taken up in acetone-ether and placed in the refrigerator. After several hours, 0.41 g (30%) of colorless crystals melting at 153-154° were removed by filtration. Recrystallization from acetone-ether raised the melting point to 154.5°.

Calc. for $C_{17}H_{23}O_2N_2Br$: C, 55.6; H, 6.31; N, 7.63%.

Found: C, 56.0; H, 6.67; N, 7.92%.

The infrared spectrum of this material in nujol showed cyanamide absorption at 2200 cm^{-1} and hydroxyl absorption at 3300 cm^{-1} but no absorption in the carbonyl region. The infrared spectrum in chloroform showed hydroxyl absorption at 3580 cm^{-1}, cyanamide absorption at 2205 cm^{-1}, weak carbonyl absorption at 1710 cm^{-1} and double bond absorption at 1670 cm^{-1}.

(b) Isolation of α-Cyanobromoacrifoline (IV) as its Quaternary Trimethylammonium Bromide (VI)

The filtrate from the separation of the crystalline bromide (V) was evaporated to dryness yielding an oil which was dissolved in 5 ml of absolute ethanol containing trimethylamine (0.00075 equiv. per ml). After the solution had remained for 24 hours at room temperature, the solvent and excess trimethylamine were removed under reduced pressure. The residue partially crystallized on addition of acetone and on filtration yielded 0.364 g (22% based on the weight of acrifoline used) of colorless crystals melting at 276.0-276.5°. Recrystallization from methanol-acetone raised the melting point to 276.5-277.0°.

Calc. for $C_{20}H_{32}O_2N_3Br$: C, 56.3; H, 7.57; N, 9.86%.

Found: C, 56.5; H, 7.57; N, 9.66%.

The infrared spectrum showed hydroxyl absorption at 3300 cm^{-1}, cyanamide absorption at 2210 cm^{-1}, double bond absorption at 1655 cm^{-1} and weak carbonyl absorption at 1700 cm^{-1}.

Treatment of the Quaternary Ammonium Bromide (VI) with Potassium Tertiary Butoxide

A mixture of the quaternary ammonium bromide (VI) (0.250 g) and potassium tertiary butoxide (0.50 g) in 25 ml of tertiary butanol and 1 ml of benzene was heated under reflux for 2 hours. The mixture was cooled, water added, and the organic solvent removed under reduced pressure. The resulting alkaline aqueous mixture was extracted several times with chloroform. The combined chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure yielding a colorless

oil which crystallized from petroleum ether. The first crop of compound VII weighed 0.135 g (80%) and melted at 183.0-183.5°. Recrystallization from ether-petroleum ether did not raise the melting point.

Calc. for $C_{17}H_{22}O_2N_2$: C, 71.3; H, 7.75; N, 9.78%.

Found: C, 71.4; H, 7.67; N, 9.86%.

The infrared spectrum in nujol showed terminal methylene absorption at 900 cm^{-1} with an overtone at 1800 cm^{-1}, double bond absorption at 1640 cm^{-1} and 1590 cm^{-1}, cyanamide absorption at 2210 cm^{-1} and hydroxyl absorption at 3300 cm^{-1}. The infrared spectrum in chloroform showed carbonyl absorption at 1705 cm^{-1} and hydroxyl absorption at 3480 cm^{-1} as well as the absorptions due to unsaturation and cyanamide. The ultraviolet spectrum had λ_{max} at 2400Å, ϵ=24,800.

Hydrogenation of Dehydrobromo-α-cyanobromoacrifoline (VII)

Dehydrobromo-α-cyanobromoacrifoline (0.087 g) was dissolved in 20 ml of methanol and treated with hydrogen (50 psig) and platinum (0.050 g PtO_2) for 15 hours. The catalyst was removed by filtration and and the solvent evaporated under reduced pressure. The colorless residue was dissolved in chloroform, and the chloroform solution washed first with dilute hydrochloric acid, then with aqueous sodium bicarbonate, and dried over anhydrous sodium sulphate. Evaporation of the chloroform under reduced pressure left a colorless oil that partially crystallized from ether. A total of 0.045 g (50%) of crystalline product (VIII) melting at 154-155° was obtained. This material was sublimed at 0.001 mm and 140° for analysis.

Calc. for $C_{17}H_{24}O_2N_2$: C, 70.8; H, 8.39%.

Found: C, 70.3; H, 8.37%.

The infrared spectrum of this compound had broad hydroxyl absorption in the 3400 cm^{-1} region, weak carbonyl absorption at 1710 cm^{-1}, and cyanamide absorption at 2240 cm^{-1}. In chloroform solution, the infrared spectrum of compound VIII showed hydroxyl absorption at 3650 cm^{-1}, weak carbonyl absorption at 1710 cm^{-1}, cyanamide absorption at 2240 cm^{-1}, and strong ether absorption at 1100 cm^{-1}.

Modified Kuhn-Roth Oxidation of the 1, 2-Reduced α-Cyano Compound (VIII)

Oxidation of 0.0134 g (0.0465 millimole) of compound VIII yielded 0.0676 milliequiv. (73%) of volatile carboxylic acids. Vapor phase chromatography of their methyl esters indicated the presence of methyl acetate and methyl propionate in the molar ratio of 69:31 respectively.

Modified Kuhn-Roth Oxidation of Crude Hydrogenated α-Cyano Compound

The filtrate obtained from the isolation of crystalline 1, 2-reduced α-cyano compound (VIII) was taken to dryness and the residual oil distilled at 0.001 mm and 130-140°. Chromic acid oxidation of a portion of this material (0.012 g, 0.045 millimole) yielded 0.0608 milliequiv. (68%) of volatile carboxylic acids. Vapor phase chromatography of their methyl esters indicated the presence of methyl acetate, methyl propionate, and methyl butyrate in the molar ratios of 77:21:2 respectively.

Removal of Hydrogen Bromide from β-Cyanobromoacrifoline (V)

A solution of β-cyanobromoacrifoline (V) (0.20 g) in 7 ml of absolute ethanol containing trimethylamine (0.00075 equiv. per ml) was allowed to stand for 40 hours at room temperature. The solvent and

excess trimethylamine were removed under reduced pressure leaving an oil (IX) which could not be induced to crystallize.

A solution of this non-crystalline quaternary ammonium bromide (IX) in 20 ml of tertiary butanol and 1 ml of benzene was heated under reflux with potassium tertiary butoxide (0.60 g) for 3 hours. The mixture was cooled, water added, and the organic solvents removed under reduced pressure. The resulting aqueous mixture was extracted several times with chloroform. The combined chloroform extract was washed with dilute aqueous acid and aqueous sodium bicarbonate, dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual colorless oil (X) (0.090 g, 58%) could not be induced to crystallize even after purification by chromatography on alumina or by distillation in vacuo.

The infrared spectrum (film) of the oil (X) showed carbonyl absorption at 1705 cm^{-1}, double bond absorption at 1635 cm^{-1}, terminal double bond absorption at 910 cm^{-1} with an overtone at 1820 cm^{-1}, hydroxyl absorption at 3490 cm^{-1}, and cyanamide absorption at 2200 cm^{-1}. The crude oil showed no absorption in the ultraviolet region of the spectrum other than weak absorption owing to the carbonyl group.

Ozonolysis of Compound X

A solution of compound X (0.048 g) in 10 ml of glacial acetic acid was treated with an excess of ozone. Zinc dust (0.30 g) was added to the solution and the mixture steam distilled. The steam distillate (30 ml) was neutralized with sodium hydroxide, made slightly acid with acetic acid, and treated with 5 ml of dimedone solution. Five hours

later, 0.044 g of needles melting at 165-175° were removed by filtration. Recrystallization gave 0.025 g of dimedone derivative with a sharp melting point of 190°. A mixture melting point with authentic formaldehyde dimedone derivative showed no depression.

Hydrogenation of Dehydrobromo-β-cyanobromoacrifoline (X)

Compound X, prepared as above from 0.205 g of compound V, was dissolved in methanol and treated with hydrogen (50 psig) and platinum (0.050 g of PtO_2) for 10 hours. The catalyst was removed by filtration and the solvent by evaporation under reduced pressure. The residual colorless oil was taken up in ether from which colorless crystals separated after several hours. The crystalline product (XI) amounted to 0.039 g (24% based on the weight of β-cyanobromoacrifoline (V) used) and melted at 155.5-157.5°. This material was sublimed at 100-130° and 0.001 mm for analysis.

Calc. for $C_{17}H_{26}O_2N_2$: C, 70.3; H, 9.03; N, 9.65%.

Found: C, 70.4; H, 8.91; N, 9.43%.

The infrared spectrum of this material (XI) had a sharp absorption at 3370 cm^{-1} owing to hydroxyl, strong absorption at 2200 cm^{-1} owing to cyanamide but no absorption in the 1600-1800 cm^{-1} region owing to carbonyl or unsaturation.

Modified Kuhn-Roth Oxidation of Dihydro-β-cyanoacrifoline (XI)

Oxidation of 0.019 g (0.0655 millimole) of dihydro-β-cyanoacrifoline (XI) yielded 0.086 milliequiv. (66%) of volatile carboxylic acids. Vapor phase chromatography of their methyl esters revealed the presence of methyl acetate, methyl propionate, and methyl butyrate in the molar ratios of 42:14:44 respectively.

Reaction of Acrifoline with Phenyllithium

Phenyllithium was prepared from 0.1 g of lithium and 1.14 g of bromobenzene in 20 ml of anhydrous ether. To the phenyllithium solution was slowly added 0.60 g of acrifoline in 20 ml of ether-toluene (1:1). The reaction mixture was heated under reflux for 1.5 hours following the addition of acrifoline, then cooled and decomposed in cold dilute hydrochloric acid. The organic layer was separated and washed with dilute hydrochloric acid. The acid washings and original aqueous portion were combined, made alkaline with ammonia, and extracted several times with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a residual light pink oil which crystallized on the addition of ether. Recrystallization from acetone-methanol gave a total of 0.54 g (70%) of colorless needles melting at 236.5-237.0°. Bertho and Stoll (24) report a melting point of 230-231° for this compound.

Calc. for $C_{22}H_{29}O_2N$: C, 77.8; H, 8.61; N, 4.13%.

Found: C, 77.7; H, 8.63; N, 3.96%.

The infrared spectrum of compound XII showed hydroxyl absorption at 3250 cm^{-1}, double bond absorption at 1670 cm^{-1} and phenyl absorption at 1595 cm^{-1}. The ultraviolet spectrum in ethanol showed benzenoid absorption with λ_{max} at 2580Å, ϵ =228.

Preparation of Phenylacrifoline Methiodide

A solution of phenylacrifoline (0.150 g) in 10 ml of hot acetone was treated with 1 ml of methyl iodide. Crystals began to separate from the solution within 1 minute, and the mixture was let stand a further hour at room temperature. Filtration of the mixture yielded 0.197 g

(93%) of product melting at 290-291° (with decomposition). Recrystallization from methanol-acetone raised the melting point to 297° (with decomposition).

Calc. for $C_{22}H_{29}O_2N.CH_3I$: C, 57.4; H, 6.70; N, 2.91%.

Found: C, 57.6; H, 6.81; N, 3.47%.

Dehydration of Phenylacrifoline

A solution of phenylacrifoline (0.150 g) in 6 ml of 85% phosphoric acid was heated to 145-150° for 2.5 hours. The solution was cooled, diluted by pouring into cold water, made alkaline with ammonia, and extracted several times with chloroform. The combined chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving 0.15 g of pale yellow oil (XIII). The infrared spectrum of this oil (film) showed complete absence of hydroxyl absorption. The oil could not be induced to crystallize but a crystalline methiodide was readily prepared. The methiodide melted above 310°.

Calc. for $C_{22}H_{27}ON.CH_3I$: C, 59.6; H, 6.53; N, 3.02%.

Found: C, 60.1; H, 6.52; N, 3.34%.

Dehydration of Phenylacrifoline via its O-Acetyl Derivative

A solution of phenylacrifoline (0.150 g) in 2 ml of acetic anhydride was heated under reflux for 2 hours and during this time the reaction mixture became very dark in color. The mixture was cooled and shaken with cold water in order to decompose excess acetic anhydride. The resulting aqueous acid solution was extracted several times with ether, made alkaline with ammonia and extracted several times with chloroform. The combined chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving 0.170 g

of a pale yellow oil (XIV). The infrared spectrum of this oil showed sharp hydroxyl absorption at 3540 cm^{-1}, ester absorption at 1740 cm^{-1}, double bond absorption at 1670 cm^{-1}, and phenyl absorption at 1500 cm^{-1}.

The acetyl derivative obtained above (XIV) was heated under reflux with 2 ml of phosphorus oxychloride containing a drop of 37% hydrochloric acid. (In other experiments, it was found that no dehydration took place when pure phosphorus oxychloride, 37% hydrochloric acid, or thionyl chloride were used.) The solution was heated for 3 hours, cooled, and cautiously poured into cold water to destroy excess phosphorus oxychloride. The resulting aqueous acid solution was extracted with ether, made alkaline with ammonia and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a residual oil (0.150 g) which could not be induced to crystallize. The infrared spectrum (film) of this oil (XV) did not show any hydroxyl absorption, but showed ester absorption at 1720 cm^{-1} and phenyl absorption at 1500 cm^{-1}. The ultraviolet spectrum exhibited styrenoid absorption with λ_{max} at 2420Å, $E^{1\%}_{1\ cm}$ = 117 (ϵ ca 4200).

The dehydrated acetyl compound (XV) was hydrolyzed by heating in a water-ethanol solution of potassium hydroxide for 15 hours. The solution was cooled, diluted with water, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual oil (XVI), which did not crystallize, was treated with methyl iodide in acetone to form a crystalline methiodide. The recovered methiodide weighed 0.070 g and

melted at 282° after recrystallization from methanol-acetone.

Calc. for $C_{22}H_{27}ON.CH_3I$: C, 59.6; H, 6.53; N, 3.02%.

Found: C, 59.8; H, 6.47; N, 3.50%.

Treatment of Phenylacrifoline Methiodide with Potassium Tertiary Butoxide

A mixture of phenylacrifoline methiodide (0.155 g) and potassium tertiary butoxide (0.70 g) in 15 ml of tertiary butanol was heated under reflux. After 4 hours, the mixture was cooled, water was added and the organic solvent removed under reduced pressure. The resulting alkaline aqueous mixture was extracted three times with chloroform. The combined extract was washed with water, dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a colorless oil which slowly solidified. Recrystallization from ether-petroleum ether gave 0.081 g (71%) of colorless crystals that melted at 194-195°. Bertho and Stoll (24) report a melting point of 192-193° for this compound.

Calc. for $C_{23}H_{31}O_2N$: C, 78.1; H, 8.84; N, 3.96%.

Found: C, 77.3; H, 8.66; N, 4.00%.

The infrared spectrum of this compound (XVII) had a broad hydroxyl absorption at 3200-3300 cm^{-1}, $=CH_2$ absorption at 900 cm^{-1} with an overtone at 1800 cm^{-1}, and phenyl absorption at 1600 cm^{-1}. The ultraviolet absorption spectrum had λ_{max} at 2430Å, ϵ=27,000.

Selenium Dioxide Oxidation of Acrifoline

A stirred solution of 0.50 g of selenium dioxide and 0.50 g of acrifoline in 25 ml of aqueous dioxane (10% water) was heated under reflux under an atmosphere of nitrogen for 8 hours. During this period, black selenium metal precipitated and the solution became deep yellow in color.

The solution was cooled, diluted with 50 ml of water, and excess selenium dioxide destroyed by reduction with sulphur dioxide. The precipitated metallic selenium was removed by filtration through a sintered glass funnel and the clear, yellow acidic filtrate washed with chloroform, made alkaline with ammonia and extracted several times with chloroform. The latter chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual yellow oil (0.415 g) was chromatographed on alumina with benzene. The first band, eluted with benzene, was a colorless oil (XVIII) that formed 0.045 g (6.5%) of a crystalline perchlorate melting at 270-271° (with decomposition).

Calc. for $C_{16}H_{21}O_2N.HClO_4$: C, 53.4; H, 6.16; N, 3.89%.

Found: C, 53.4; H, 6.12; N, 4.05%.

The infrared spectrum of this compound had carbonyl absorption at 1725 cm^{-1}, double bond absorption at 1680 cm^{-1}, but had no absorption in the hydroxyl region. The ultraviolet absorption spectrum showed only slight absorption at 2900Å corresponding to a carbonyl group.

A second band was eluted from the column with chloroform. Evaporation of the solvent left a light yellow oil that partially formed a crystalline perchlorate from acetone-ether. The perchlorate (0.20 g (30%), m.p. 267-269°) was purified for analysis by two recrystallizations from methanol-acetone. It finally melted at 273.5-274.0°.

Calc. for $C_{16}H_{21}O_2N.HClO_4$: C, 53.4; H, 6.16; N, 3.89%.

Found: C, 53.6; H, 6.17; N, 3.97%.

The infrared spectrum of the perchlorate had hydroxyl absorption at 3530 cm^{-1}, carbonyl absorption at 1690 cm^{-1}, and a conjugated double

bond absorption at 1627 cm^{-1}. The ultraviolet absorption spectrum had λ_{max} at 2430Å, ϵ=5,330.

The base recovered from the filtrate of the above perchlorate preparation weighed 0.150 g but was not characterized.

Preparation of the Methiodide of the Selenium Dioxide Oxidation Product (XIX)

The perchlorate (0.20 g), prepared as above from the unsaturated carbonyl product (XIX) was converted to the free base and treated with 1 ml of methyl iodide in 15 ml of acetone. The crystalline methiodide separated almost immediately, and the mixture was let stand overnight at room temperature. Filtration yielded 0.20 g (90%) of a compound that melted at 303° (with decomposition) after crystallization from methanol-acetone.

Calc. for $C_{16}H_{21}O_2N.CH_3I$: C, 50.9; H, 6.03; N, 3.49%.

Found: C, 50.9; H, 6.20; N, 3.63%.

The infrared spectrum of the methiodide had hydroxyl absorption at 3320 cm^{-1}, carbonyl absorption at 1685 cm^{-1}, and enhanced double bond absorption at 1635 cm^{-1}.

Conversion of the Unsaturated Carbonyl Compound (XIX) to the Saturated Carbonyl Compound (XVIII)

A solution of the perchlorate of the unsaturated carbonyl compound (0.020 g) in 6 ml of 95% ethanol containing sodium hydroxide (0.05 g) was allowed to stand at room temperature for 24 hours. The solution was diluted with water and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness leaving

a colorless oil that formed 0.015 g (75%) of crystalline perchlorate. The infrared spectrum of this salt was identical with that of the perchlorate of the saturated carbonyl compound (XVIII) obtained from the selenium dioxide reaction.

In another experiment, the crude product from the selenium dioxide oxidation was treated with an alcoholic sodium hydroxide solution at room temperature for 20 hours. The base isolated from the latter reaction was chromatographed on alumina with ether-petroleum ether yielding 38% of crystalline perchlorate whose infrared spectrum was identical with that of the perchlorate of the saturated carbonyl compound (XVIII).

Oppenauer Oxidation of Acrifolinol

A solution of acrifolinol (0.050 g), prepared by reduction of acrifoline with lithium aluminum hydride (3), and aluminum iso-propoxide (0.30 g) in 5 ml of toluene and 1 ml of cyclohexanone was heated under reflux for 33 hours. The mixture was decomposed in cold dilute hydrochloric acid and the toluene layer removed. The aqueous acid solution was made alkaline with sodium hydroxide solution, and extracted several times with chloroform. The dried chloroform extract yielded a pale yellow oil whose infrared spectrum (film) showed strong absorption at 1700 cm^{-1} (carbonyl) as well as absorption in the hydroxyl region. The oil readily formed 0.040 g (52%) of crystalline methiodide from an acetone-methyl iodide solution. Recrystallization from acetone yielded a product that melted at 289-290°.

Calc. for $C_{16}H_{23}O_2N.CH_3I$: C, 50.6; H, 6.47%.

Found: C, 50.8; H, 6.40%.

The infrared spectrum of the methiodide showed carbonyl absorption at 1700 cm^{-1} and broad hydroxyl absorption. The spectrum was distinctly different from that of acrifoline methiodide.

Oppenauer Oxidation of Dihydroacrifolinol

A solution of dihydroacrifolinol (0.170 g) and aluminum tertiary butoxide (0.50 g) in 2 ml of cyclohexanone and in 25 ml of toluene was heated under reflux for 7 hours, then poured into cold dilute hydrochloric acid and the organic layer extracted several times with dilute acid. The aqueous extract was made alkaline with sodium hydroxide solution and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a pale yellow oil whose infrared spectrum (film) showed two intense absorptions of equal magnitude at 1705 cm^{-1} and 1735 cm^{-1} in the carbonyl region, and absorption at 3500 cm^{-1} in the hydroxyl region.

The crude base obtained above was chromatographed on alumina with 25% chloroform in benzene. Fractions 26-28 (80-100 ml fractions) yielded a colorless oil that crystallized in poor yield from ether-petroleum ether giving 0.016 g of solid (XXI) melting at 128-129°. The infrared spectrum of this solid (XXI) showed a single strong carbonyl absorption at 1705 cm^{-1} and no hydroxyl absorption. A sharp peak at 1420 cm^{-1} was indicative of a $-CH_2-$ group adjacent to a carbonyl. Fractions 29-37 yielded the same base with only a single carbonyl absorption. The entire pure product obtained by chromatography was converted to the hydrobromide salt which was recrystallized twice from methanol-acetone for analysis. The total yield of salt (m.p. above 315°) was 0.10 g (45%).

Calc. for $C_{16}H_{23}O_2N.HBr$: C, 56.1; H, 7.07; N, 4.09%.

Found: C, 56.2; H, 7.12; N, 4.11%.

The infrared spectrum of the hydrobromide showed a single strong carbonyl absorption at 1700 cm^{-1} but no hydroxyl absorption. A sharp peak at 1420 cm^{-1} was again indicative of a $-CH_2-$ group adjacent to a carbonyl. There was no strong absorption in the ultraviolet spectrum.

A second product of the Oppenauer oxidation containing carbonyl absorption at 1735 cm^{-1} in the infrared spectrum was not isolated.

Oxidation of Dihydroacrifoline with Copper Sulphate

A solution of dihydroacrifoline (0.5 g) and $CuSO_4.5H_2O$ (1.2 g) in 12 ml of pyridine and 4 ml of water was heated on the steam bath for 24 hours. The deep blue solution was cooled, poured into water and extracted several times with chloroform. The combined chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a yellow oil. Chromatography of this oil with chloroform on alumina yielded a colorless oil that readily formed 0.51 g (78%) of a crystalline hydrobromide (m.p. above 315°) from acetone.

Calc. for $C_{16}H_{23}O_2N.HBr$: C, 56.1; H, 7.07; N, 4.09%.

Found: C, 55.8; H, 6.87; N, 4.11%.

The infrared spectrum showed strong carbonyl absorption at 1720 cm^{-1} but no absorption in the hydroxyl region.

A sample of the hydrobromide of compound XXIV was converted to the perchlorate salt (m.p. above 315°) for analysis.

Calc. for $C_{16}H_{23}O_2N.HClO_4$: C, 53.1; H, 6.68; N, 3.87%.

Found: C, 53.4; H, 6.83; N, 3.99%.

The perchlorate salt showed strong absorption at 1720 cm^{-1} in the carbonyl region of the infrared spectrum, but no absorption in the hydroxyl region. Likewise, the infrared spectrum of the free base (XXIV) showed only carbonyl absorption and no hydroxyl absorption when measured either as a film or in chloroform solution.

Oxidation of Dihydroacrifoline with Peroxytrifluoroacetic Acid

A stock solution of trifluoroperoxyacetic acid in methylene chloride was prepared in the following manner: a solution of trifluoroacetic anhydride (6.3 ml) in 10 ml of methylene chloride was added to a cold stirred suspension of 1 ml of 90% hydrogen peroxide in 10 ml of methylene chloride, the mixture stirred until homogeneous, and then diluted to 100 ml with methylene chloride. To 35 ml of this solution was added 0.10 g of dihydroacrifoline and 3.0 g of Na_2HPO_4. The mixture was heated under reflux for 16 hours, cooled and poured into an aqueous solution of sulphur dioxide. After 30 minutes, the aqueous phase was made alkaline with ammonia and extracted several times with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving 0.050 g of colorless oil. This oil formed hydrobromide and perchloric salts whose infrared spectra were identical with those of the corresponding salts of the product (XXIV) isolated from the oxidation of dihydroacrifoline with copper sulphate.

Reduction of Compound XXIV

(a) With Sodium Borohydride

A solution of the hydrobromide of XXIV (0.20 g) and sodium borohydride (0.20 g) in 25 ml of absolute ethanol was heated under reflux

for 1 hour. The mixture was cooled and acetone was added to destroy excess sodium borohydride. Water was then added, and the organic solvents removed under reduced pressure. The resulting aqueous mixture was extracted with chloroform, and the dried chloroform extract evaporated under reduced pressure. Crystallization of the residual oil from ether-petroleum ether gave 0.115 g (75%) of product (XXV) that melted at 182-183°. This material was sublimed at 0.001 mm and 110° for analysis. It melted at 182-183°.

Calc. for $C_{16}H_{25}O_2N$: C, 73.0; H, 9.56; N, 5.32%.

Found: C, 73.1; H, 9.75; N, 4.65%.

The infrared spectrum of compound XXV showed hydroxyl absorption at 3380 cm^{-1}, no carbonyl absorption, and peaks at 1070 cm^{-1} and 1100 cm^{-1} attributed to ether absorption.

The mother liquor from the separation of the crystalline base was acidified with hydrobromic acid, and yielded 0.040 g (20%) of hydrobromide salt of compound XXV. The hydrobromide was crystallized twice from methanol-acetone for analysis. It melted above 310°.

Calc. for $C_{16}H_{25}O_2N.HBr$: C, 55.8; H, 7.61; N, 4.07%.

Found: C, 56.1; H, 7.45; N, 4.45%.

The infrared spectrum showed hydroxyl absorption at 3300 cm^{-1} and ether absorption at 1075 cm^{-1} and 1105 cm^{-1}.

(b) With Lithium Aluminum Hydride

A solution of 0.058 g of the copper sulphate product (XXIV) in 15 ml of anhydrous ether was added to a suspension of 0.15 g of lithium aluminum hydride in 15 ml of anhydrous ether. The mixture was heated

under reflux for 9 hours, excess hydride destroyed with wet ether, and water added. The ether layer was decanted and the aqueous slurry extracted several times with ether. The combined ether solutions were dried over anhydrous sodium sulphate and evaporated to dryness. The colorless residue crystallized from ether-petroleum ether giving 0.030 g (52%) of product that melted at 183°. A mixed melting point with the sodium borohydride product (XXV) was not depressed. The filtrate yielded 0.030 g (40%) of hydrobromide whose infrared spectrum was identical with that of the hydrobromide of the borohydride product (XXV).

(c) With Sodium Methoxide

A solution of 0.10 g of the hydrobromide of XXIV and sodium methoxide in methanol (prepared by dissolving 0.15 g of sodium in 5 ml of anhydrous methanol) was heated in a sealed tube for 16 hours at 175°. The contents were cooled, poured into water and the aqueous solution extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and then evaporated under reduced pressure. The residual colorless oil crystallized from ether-petroleum ether giving 0.065 g (85%) of product melting at 181-182°. The melting point of a mixture of this compound with the sodium borohydride reduction product or with the lithium aluminum hydride reduction product was not depressed. The filtrate yielded 0.010 g (10%) of hydrobromide salt whose infrared spectrum was the same as the spectra of the hydrobromides formed above.

The reduction product (XXV) was also obtained in 85% yield when the copper sulphate product (XXIV) was heated with barium hydroxide in 50% methanol at 175° for 16 hours. Similarly, heating with sodium

ethoxide in ethanol gave the reduction product (XXV) in 90% yield. No reaction occurred when the copper sulphate product (XXIV) was heated under reflux with sodium methoxide in methanol, or with barium hydroxide in aqueous methanol.

Preparation of the Acetate of the Reduced Copper Sulphate Product (XXV)

A solution of 0.030 g of the reduced copper sulphate product (XXV) in the mixed anhydride of trifluoroacetic acid and acetic acid (prepared by mixing 0.10 ml of glacial acetic acid and 0.16 ml of trfluoroacetic anhydride) was let stand overnight at room temperature. The solution was poured into a dilute aqueous ammonia solution, and then extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a colorless oil that slowly solidified. The solid, compound XXVI, was sublimed at 75° and 0.001 mm for analysis, and melted at 148-149°.

Calc. for $C_{18}H_{27}O_3N$: C, 70.8; H, 8.91; N, 4.59%.

Found: C, 71.1; H, 8.75; N, 4.88%.

The infrared spectrum of XXVI had no hydroxyl absorption but had strong acetate absorption at 1730 cm^{-1} and 1235 cm^{-1}. A strong peak at 1042 cm^{-1} was attributed to ether absorption.

A similar reaction using 0.060 g of reduced copper sulphate product (XXV) in 0.20 ml of glacial acetic acid and 0.32 ml of trifluoroacetic anhydride yielded 0.043 g (50%) of hydrobromide salt of the acetate (XXVI). The hydrobromide was crystallized twice from methanol-acetone for analysis, and melted above 310°.

Calc. for $C_{18}H_{27}O_3N.HBr$: C, 56.0; H, 7.31%.

Found: C, 56.0; H, 7.19%.

The infrared spectrum of this compound had acetate absorption at 1735 cm^{-1} and 1235 cm^{-1} and had no hydroxyl absorption.

Oxidation of Compound XXV to the Copper Sulphate Product (XXIV) with Chromic Acid

A solution of 0.025 g of the reduced copper sulphate product (XXV) in 25 ml of 85% acetic acid-water was cooled to -15°, and treated with 0.10 g of chromic anhydride. The slushy mixture was stirred at -15° for two hours, and then for four hours at -5°. Methanol was added to destroy excess chromic acid, and the solution let stand overnight in the refrigerator. Water was added and the organic solvents removed under reduced pressure. The resulting aqueous solution was acidified with hydrochloric acid, extracted with ether, made alkaline with ammonia and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a colorless oil. The infrared spectrum of this oil (film) was identical with that of the copper sulphate product (XXIV). The oil yielded 0.028 g (86%) of hydrobromide salt whose infrared spectrum was identical with that of the hydrobromide of the copper sulphate product (XXIV).

Reaction of Compound XXIV with Phenyllithium

To a solution of phenyllithium in ether (prepared from 0.10 g of lithium and 1.14 g of bromobenzene in 20 ml of ether) was added 0.202 g of the copper sulphate product (XXIV) in 15 ml of ether. The solution was heated under reflux for two hours, let stand overnight, and poured

ino cold dilute hydrochloric acid. The aqueous solution was extracted several times with ether, made alkaline with ammonia and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residual pale yellow oil partially solidified but a crystalline product was not obtained in good yield. The base was dissolved in acetone and treated with hydrobromic acid, yielding 0.289 g (89%) of colorless crystals melting above 315°. The salt was crystallized twice from methanol-acetone for analysis.

Calc. for $C_{22}H_{29}O_2N.HBr$: C, 62.9; H, 7.19; N, 3.33%.

Found: C, 62.9; H, 7.26; N, 3.32%.

The infrared spectrum of this compound had hydroxyl absorption at 3330 cm^{-1}, no carbonyl absorption, and weak phenyl absorption at 1600 cm^{-1} and 1500 cm^{-1}. The ultraviolet spectrum showed benzenoid absorption with λ_{max} at 2580Å, ϵ=237.

The same compound (XXVII) was obtained in 86% yield by the addition of a phenyllithium solution in ether to an ethereal solution of the copper sulphate product cooled in ice. The mixture was worked up five minutes after the addition of phenyllithium was complete.

Reaction of Compound XXIV with Methyllithium

A solution of the copper sulphate product (0.10 g) in 10 ml of ether was added to a benzene-ether solution of methyllithium prepared from 1.6 g of methyl iodide and 0.174 g of lithium. The solution was heated under reflux for 30 minutes, cooled, and excess methyllithium decomposed by the dropwise addition of water. The organic phase was washed with water, dried over anhydrous sodium sulphate and evaporated

under reduced pressure. The residual oil yielded 0.065 g (61%) of colorless crystals melting at 211-213° from ether. A second crop of compound XXVII amounted to 0.017 g (16%), and melted at 208-211°. The combined product was crystallized twice from ether-petroleum ether for analysis and melted at 216°.

Calc. for $C_{17}H_{27}O_2N$: C, 73.6; H, 9.81; N, 5.05%.

Found: C, 73.7; H, 9.82; N, 5.11%.

The infrared spectrum of this compound (XXVII) had hydroxyl absorption at 3370 cm^{-1}, and ether absorption at 1100 cm^{-1} and 1125 cm^{-1}.

Preparation of Methylacrifoline (XXXII)

Methyllithium was prepared by allowing 3.6 g of methyl iodide and 0.35 g of lithium to react in 20 ml of ether under an atmosphere of nitrogen. The reaction was vigorous when the lithium was added as a thin foil. When the reaction had apparently ceased (no more lithium being consumed), benzene was added, and excess methyl iodide and ether removed by distillation. Unreacted lithium was removed by filtration through a glass wool-sintered glass filter under an atmosphere of nitrogen.

To the benzene solution of methyllithium was added 0.40 g of acrifoline in 10 ml of benzene. The solution was heated under reflux for 5 hours, cooled, and excess methyllithium destroyed by the dropwise addition of water. The organic phase was washed with water, dried over anhydrous sodium sulphate and evaporated under reduced pressure. The resulting yellow oil gave 0.250 g (59%) of orange crystals melting at 177.0-178.5° from ether-petroleum ether. The base was purified by sublimation at 150° and 0.001 mm, and then crystallizedfrom acetone-ether. The colorless product (XXXII) melted at 180-181°.

Calc. for $C_{17}H_{27}O_2N$: C, 73.6; H, 9.81; N, 5.05%.

Found: C, 73.8; H, 9.82; N, 5.00%.

The infrared spectrum of this compound had a sharp hydroxyl absorption at 3300 cm^{-1} with broad hydrogen-bonded hydroxyl absorption in the 2400-3200 cm^{-1} region.

The filtrate from the isolation of the above base yielded 0.112 g (21%) of hydrobromide salt which melted above 300°. The salt was recrystallized twice from methanol-acetone for analysis.

Calc. for $C_{17}H_{27}O_2N.HBr$: C, 57.0; H, 7.87; N, 3.91%.

Found: C, 57.0; H, 7.90; N, 3.89%.

The infrared spectrum of the hydrobromide showed peaks at 3280 and 3350 cm^{-1} in the hydroxyl region.

Oxidation of Methylacrifoline (XXXII)

(a) With Chromic Acid

A solution of methylacrifoline (0.035 g) in 10 ml of 10% aqueous acetic acid was cooled to -10° and stirred with 0.10 g of chromic anhydride for 6 hours. Excess oxidant was destroyed with methanol, water was added and the solution partially evaporated under reduced pressure. The resulting aqueous solution was made strongly acidic with dilute hydrochloric acid, extracted with chloroform, then made alkaline with ammonia and again extracted with chloroform. The latter chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a colorless oil. The hydrobromide salt of the oil (XXXIII) was gelatinous at first but crystallized on scratching to give 0.017 g (40%) of product which melted above 310°. The hydrobromide was recrystallized twice from methanol-acetone for analysis.

Calc. for $C_{17}H_{25}O_2N.HBr$: C, 57.3; H, 7.36%.

Found: C, 57.5; H, 7.31%.

The infrared spectrum of this compound had a sharp hydroxyl absorption at 3300 cm^{-1} and no carbonyl absorption, and was distinctly different than that of methylacrifoline hydrobromide. The free base obtained from the hydrobromide did not exhibit carbonyl absorption when measured as a film or in chloroform solution. Absorption due to hydrogen bonding, which was present in methylacrifoline, was absent in the free base of this compound.

(b) By the Oppenauer Procedure

A solution of methylacrifoline (0.050 g) and aluminum tertiary butoxide (1.0 g) in 10 ml of toluene and 2 ml of cyclohexanone was heated under reflux for 65 hours. The solution was cooled, poured into aqueous hydrochloric acid and the organic phase extracted with aqueous acid. The aqueous extracts were made alkaline with aqueous sodium hydroxide and extracted with chloroform. The dried chloroform extract yielded a colorless oil on evaporation under reduced pressure. The hydrobromide of this oil was gelatinous when first prepared but crystallized on scratching to give 0.020 g (31%) of product whose infrared absorption spectrum was identical with that of the hydrobromide of compound (XXXIII) obtained from chromic acid oxidation of methylacrifoline.

Preparation of Methyldihydroacrifoline (XXXIV)

(a) From Dihydroacrifoline and Methyllithium

A solution of dihydroacrifoline (0.50 g) in 10 ml of benzene was added to a benzene-ether solution of methyl-lithium prepared from 5.4 g

of methyl iodide and 0.522 g of lithium. The solution was heated under reflux for 90 minutes, cooled, and excess methyllithium decomposed by the dropwise addition of water. The organic phase was washed with water, dried over anhydrous magnesium sulphate and evaporated under reduced pressure. The residual colorless oil yielded 0.340 g (64%) of crystalline product (XXXIV) that melted at 189.5-190.0°. Crystallization from ether-petroleum ether raised the melting point to 190.5°.

Calc. for $C_{17}H_{29}O_2N$: C, 73.1; H, 10.5; N, 5.01%.

Found: C, 73.1; H, 10.6; N, 5.40%.

The infrared spectrum of this compound had broad hydroxyl absorption in the 3050-3200 cm^{-1} region.

The filtrate from the crude product obtained above formed 0.110 g (17%) of crystalline hydrobromide which melted above 310° and which was recrystallized twice from methanol-acetone for analysis.

Calc. for $C_{17}H_{29}O_2N.HBr$: C, 56.7; H, 8.39; N, 3.89%.

Found: C, 56.8; H, 8.51; N, 3.99%.

(b) By Hydrogenation of Methylacrifoline (XXXII)

A solution of methylacrifoline (0.020 g) in 10 ml of methanol was treated with hydrogen (30 psig) and platinum (0.015 g PtO_2) for 7 hours. Removal of the catalyst by filtration and solvent by evaporation left a colorless oil that yielded 0.022 g (85%) of crystalline hydrobromide. The infrared spectrum of this compound was identical with that of methyl-dihydroacrifoline hydrobromide prepared from dihydroacrifoline and methyllithium.

Oxidation of Methyldihydroacrifoline (XXXIV)

(a) By the Oppenauer Procedure

A solution of methyldihydroacrifoline (0.20 g) and aluminum tertiary butoxide (1.0 g) in 25 ml of toluene and 2 ml of cyclohexanone was heated under reflux for 7 hours. The solution was cooled, poured into dilute hydrochloric acid and the organic phase extracted with aqueous acid. The combined aqueous extract was made alkaline with sodium hydroxide and extracted with chloroform. The chloroform extract yielded a colorless oil that crystallized from ether-petroleum ether giving 0.140 g (70%) of product (XXXV) melting at 147-149°. This material was purified for analysis by sublimation at 150° and 0.001 mm (m.p. 150-151°).

Calc. for $C_{17}H_{27}O_2N$: C, 73.6; H, 9.81; N, 5.05%.

Found: C, 73.2; H, 9.90; N, 4.89%.

The infrared spectrum of this compound had a broad hydroxyl absorption in the 2900-3000 cm^{-1} region, and ether absorption at 1100 cm^{-1} and 1120 cm^{-1}, but no carbonyl absorption. In chloroform solution, free hydroxyl absorption occurred at 3580 cm^{-1}, bonded hydroxyl in the 3300-3450 cm^{-1} region, and strong ether absorption at 1105 cm^{-1}.

The hydrobromide salt of the compound XXXV was gelatinous when first prepared, but slowly crystallized on standing or scratching. The salt was crystallized from methanol-acetone for analysis.

Calc. for $C_{17}H_{27}O_2N.HBr.H_2O$: C, 54.3; H, 8.04; N, 3.72%.

Found: C, 54.6; H, 8.28; N, 3.77%.

(b) With Chromic Acid

A solution of methyldihydroacrifoline (0.030 g) in 10 ml of 10% aqueous acetic acid was cooled to -10° and stirred with 0.10 g of chromic anhydride for 7 hours. Excess oxidant was destroyed with methanol, water was added and the solution partially evaporated under reduced pressure. The resulting solution was made strongly acidic with hydrochloric acid and extracted with chloroform, then made alkaline with ammonia and again extracted with chloroform. The latter chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a colorless oil. The hydrobromide salt of this oil (0.018 g, 54%) had an infrared spectrum that was identical with that of the hydrobromide of the Oppenauer oxidation product (XXXV) of methyldihydroacrifoline.

Treatment of Oxidized Methyldihydroacrifoline (XXXV) with Hydrochloric Acid

A solution of 0.058 g of oxidized methyldihydroacrifoline (XXXV) in 7 ml of methanol and 3 ml of 37% hydrochloric acid was heated under reflux for 24 hours. The solution was cooled, diluted with water, made alkaline with ammonia and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure leaving a colorless oil. The oil formed 0.038 g (53%) of crystalline hydrobromide that melted above 310°. The salt was crystallized twice from methanol-acetone for analysis.

Calc. for $C_{17}H_{27}O_2N.HBr$: C, 57.0; H, 7.87%.

Found: C, 56.9; H, 7.92%.

The infrared spectrum of the hydrobromide had a small sharp absorption at 3330 cm^{-1} owing to hydroxyl, and a strong absorption at 1720 cm^{-1} owing to carbonyl.

Methyldihydroacrifoline was unchanged on similar treatment with hydrochloric acid, indicating that the above reaction was characteristic of oxidized dihydromethylacrifoline.

No reaction was observed when oxidized dihydromethylacrifoline was treated with sodium methoxide in methanol.

SUMMARY

Acrifoline ($C_{16}H_{23}O_2N$), a minor alkaloid of Lycopodium annotinum, had previously been shown to contain a hydroxyl group, a carbonyl group, a double bond, and a tertiary nitrogen atom, and therefore to be tetracyclic. In the present investigation, nuclear magnetic resonance spectroscopy, as well as chemical evidence, has shown that the alcohol is secondary, the carbonyl group is ketonic, and the double bond carries only one hydrogen atom. Further, a methyl group was found to be present as $>CHCH_3$.

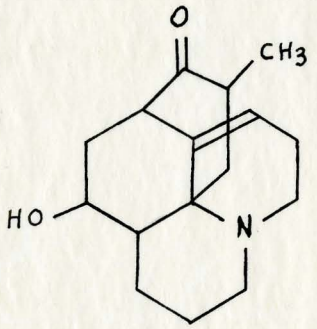
A study of the products of Hofmann and von Braun degradations of acrifoline has shown the presence of the sequence $>C{=}CHCH_2CH_2\overset{+}{N}CH_2CH_2CH_2C$ in the molecule.

A study of the products of selenium dioxide oxidation of acrifoline established the presence of a $-COCH(CH_3)CH_2-$ system in the molecule with the $-CH_2-$ group separated by two or three atoms from the carbon atom carrying the hydroxyl group.

Oppenauer oxidation of dihydroacrifolinol gave a diketone that had both carbonyl groups in rings that were six-membered or larger. The nature of this product indicated that the carbon atom carrying the hydroxyl group and the carbonyl group in acrifoline were separated by two atoms, and that a $-CH_2-$ unit was adjacent to the -CHOH- function in acrifoline.

Oxidation of dihydroacrifoline with either copper sulphate or peroxytrifluoroacetic acid yielded a product that had an ether linkage between the carbon atom originally carrying the hydroxyl function and the carbon atom bearing the methyl group.

Evidence presented in this thesis indicates that acrifoline has the following structure:



APPENDIX

The Nuclear Magnetic Resonance Spectra of Acrifoline and its Derivatives

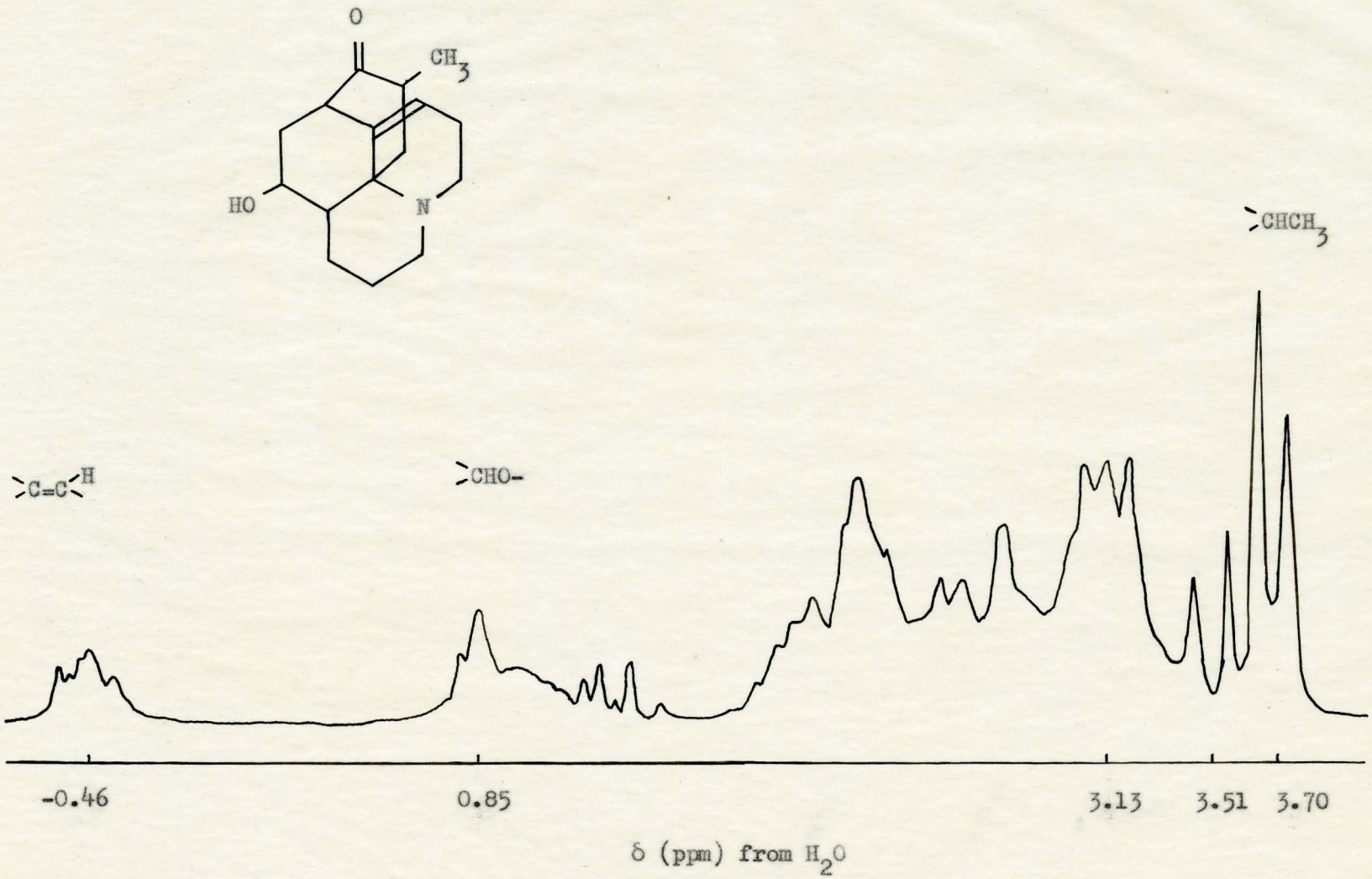


Figure 7. The NMR Spectrum of Acrifoline.

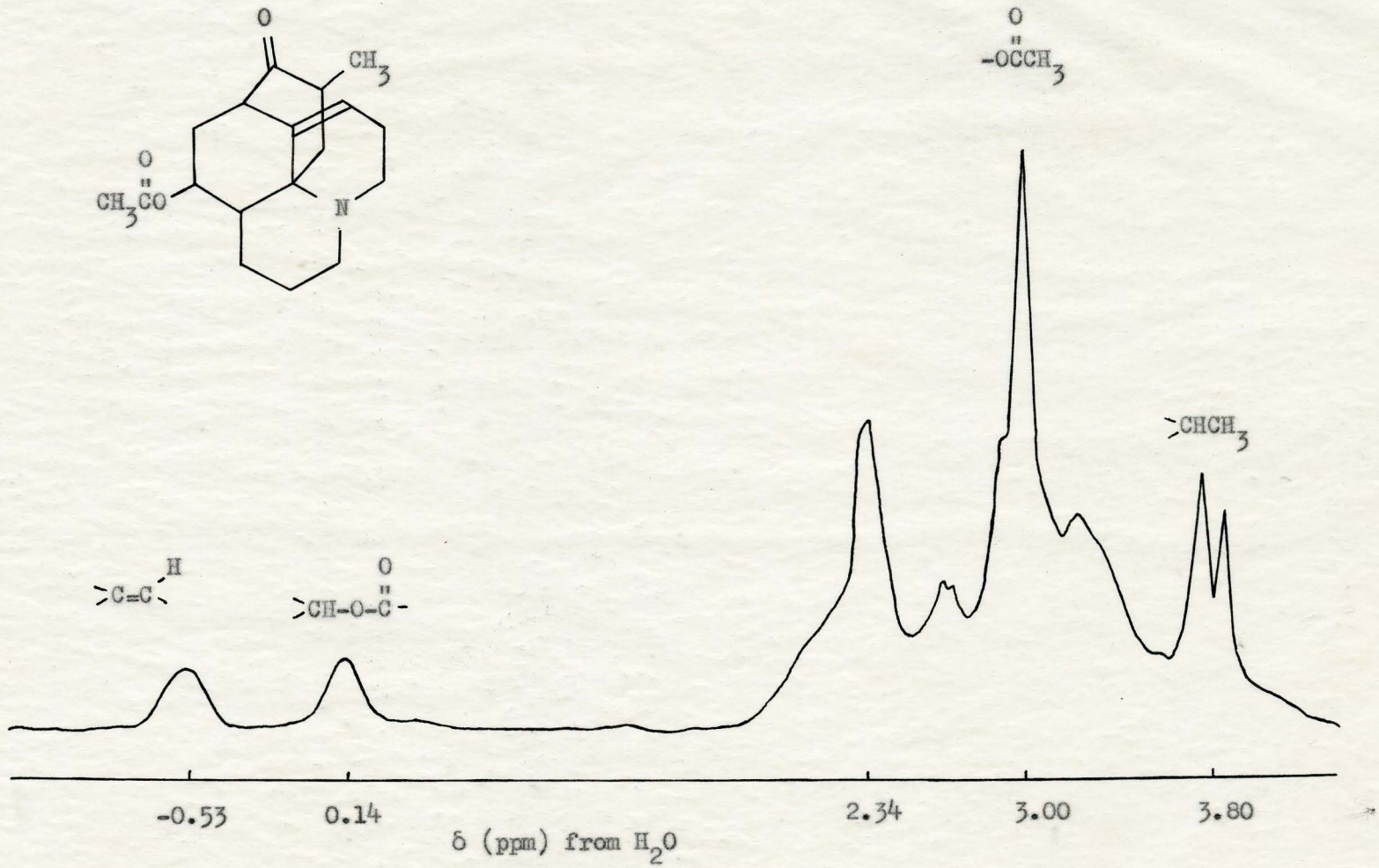


Figure 8. The NMR Spectrum of O-Acetylacrifoline.

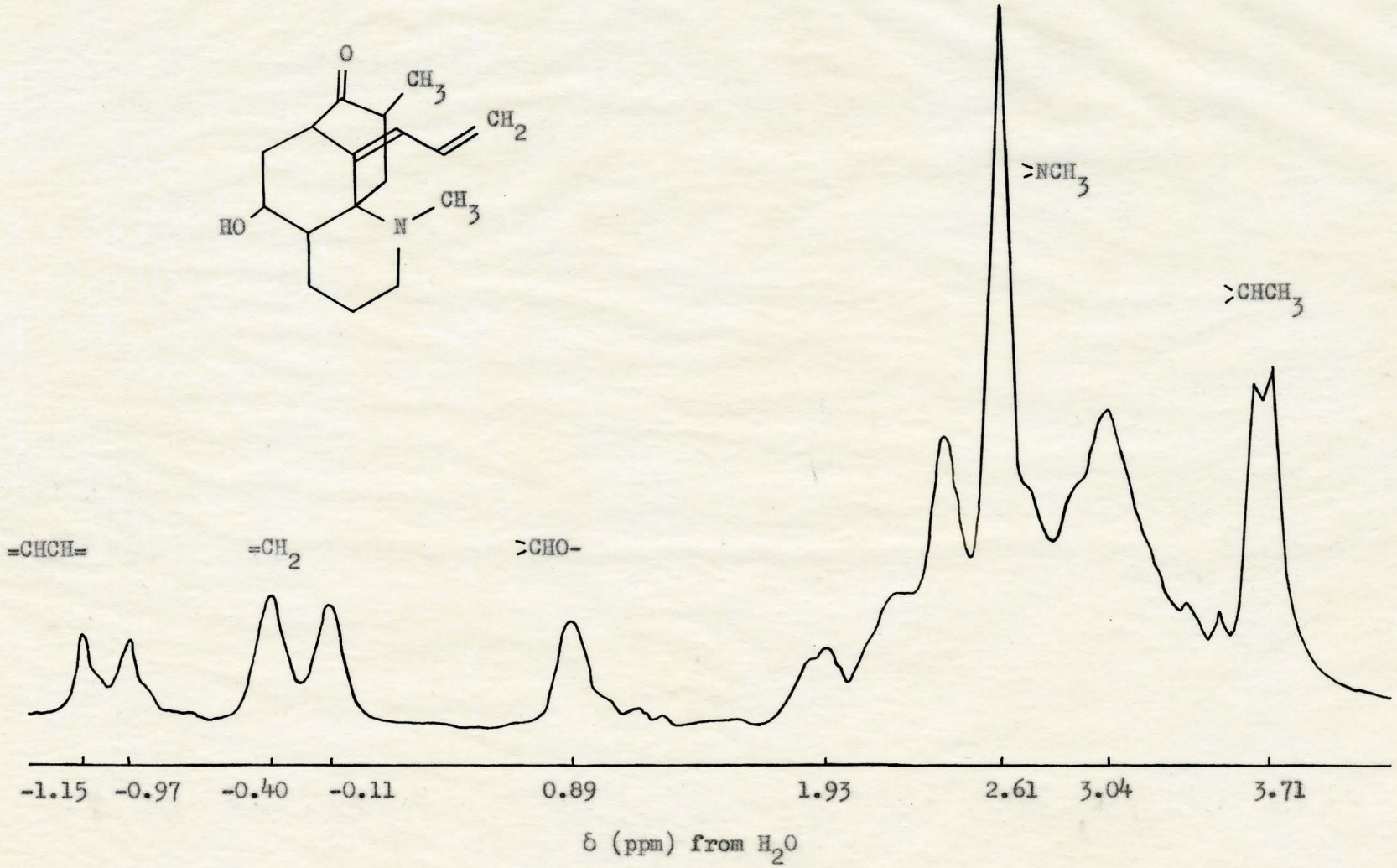


Figure 9. The NMR Spectrum of Acrifoline Hofmann Product (I).

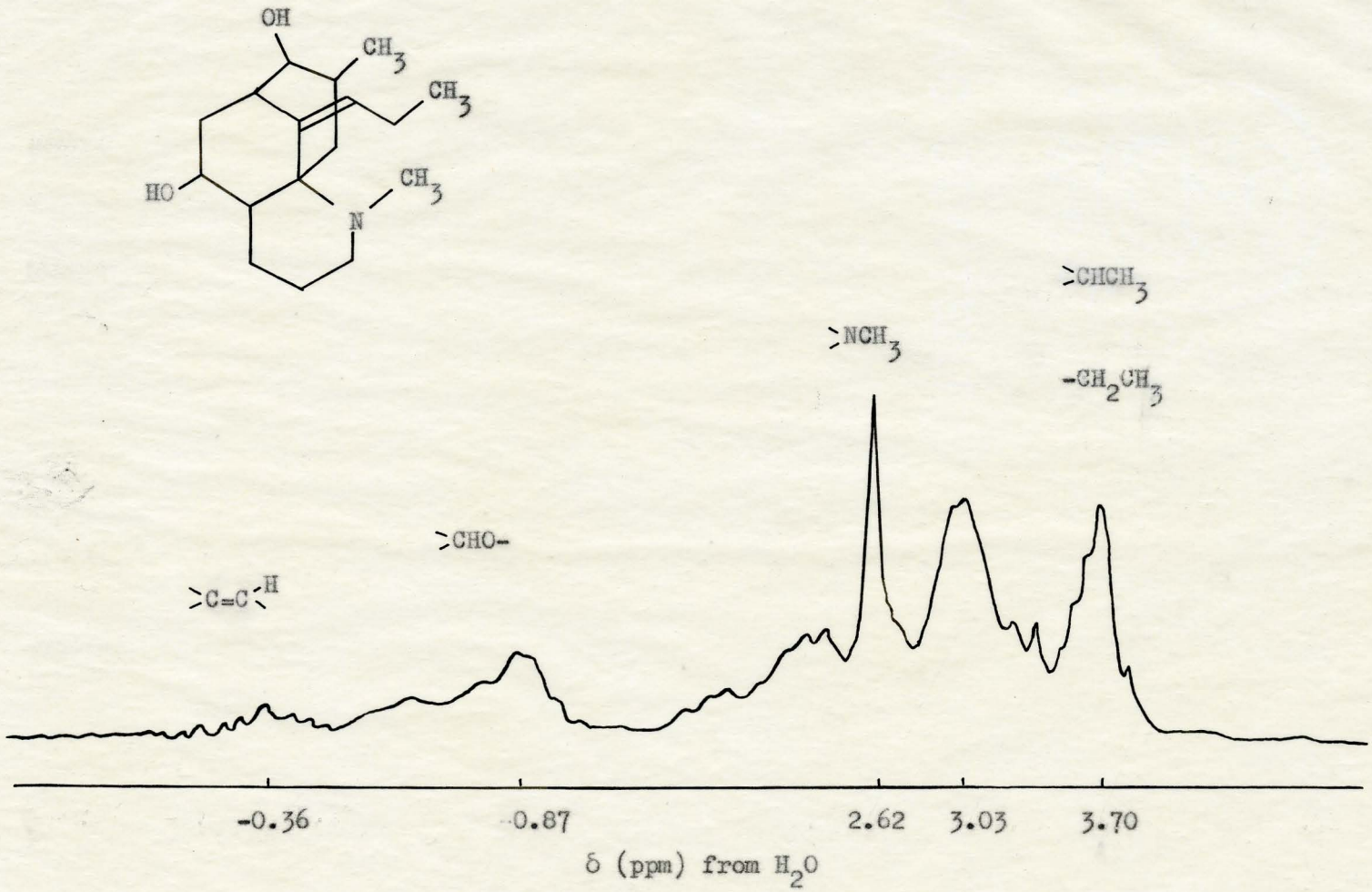


Figure 10. The NMR Spectrum of the 1, 2-Reduced Hofmann Product Diol (III).

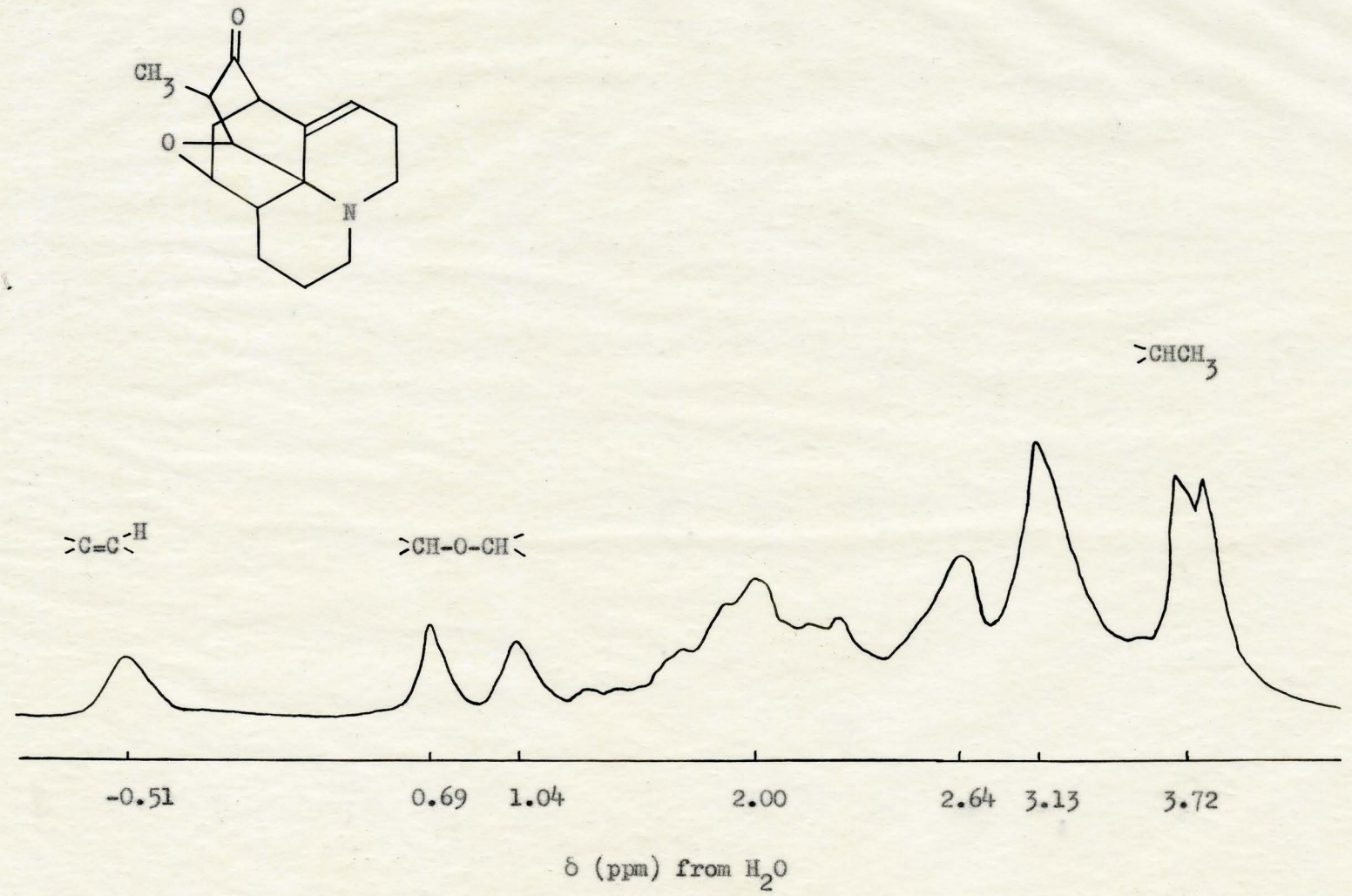


Figure 11. The NMR Spectrum of the Selenium Dioxide Oxidation Product (XVIII).

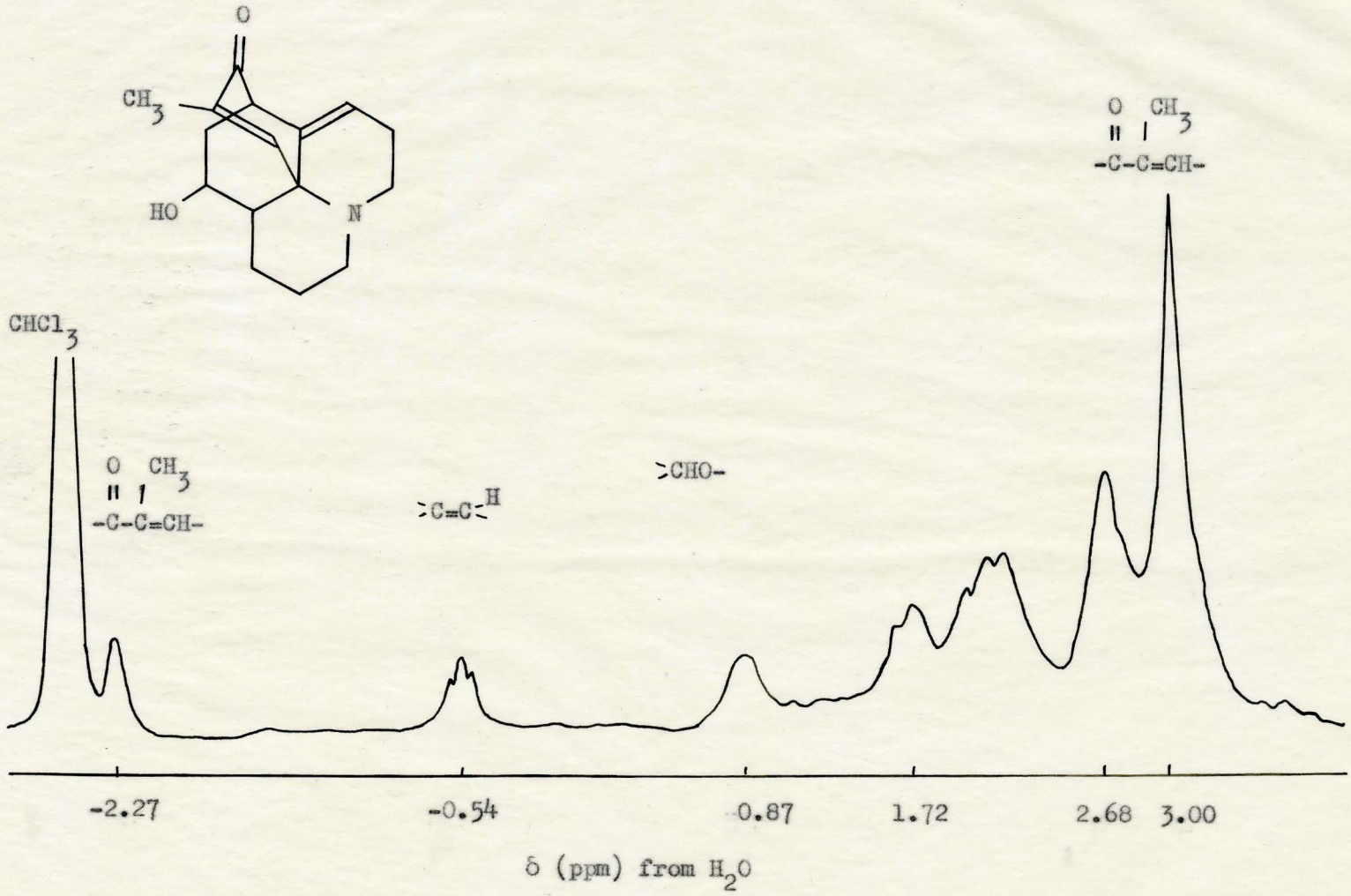


Figure 12. The NMR Spectrum of the Selenium Dioxide Oxidation Product (XIX).

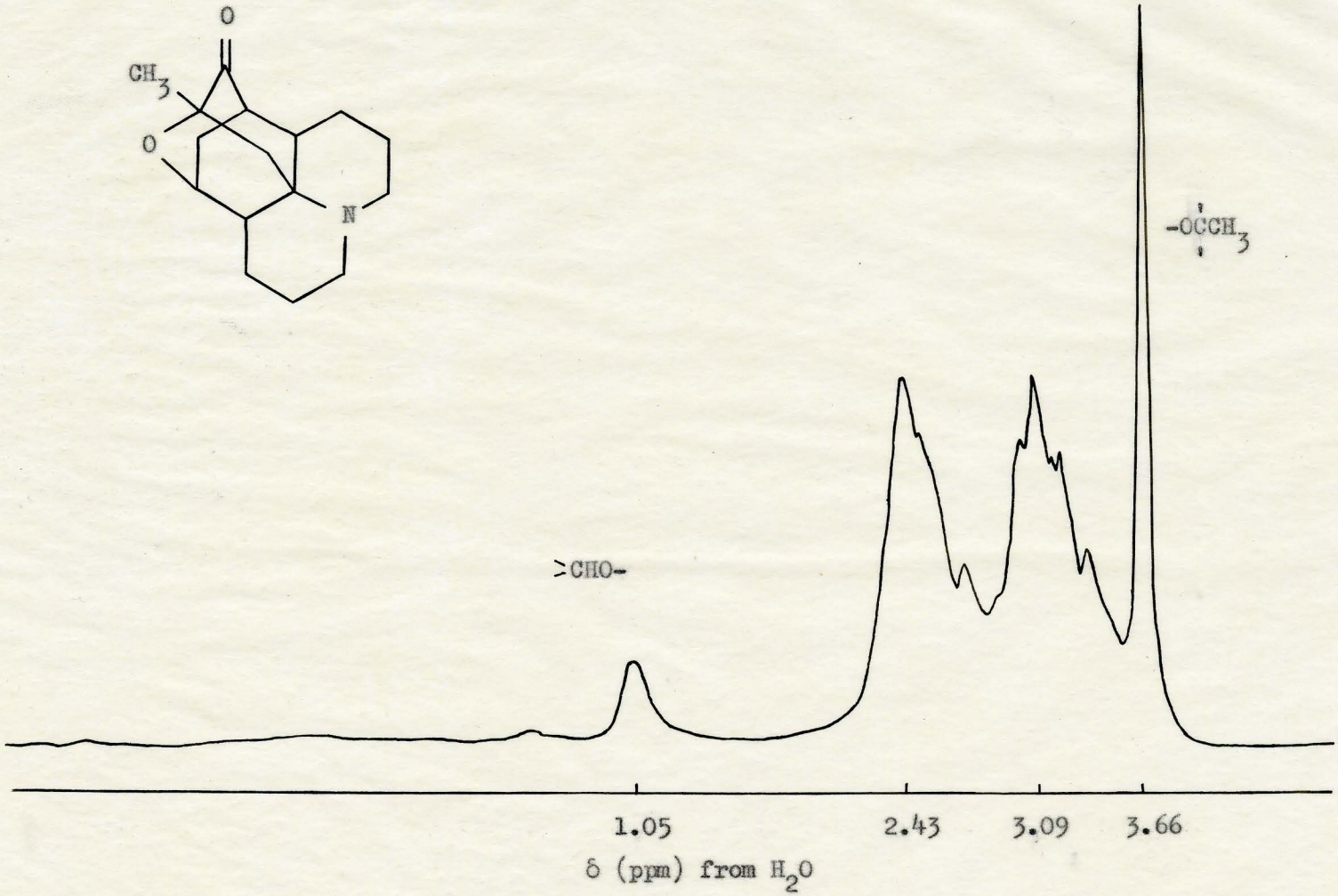


Figure 13. The NMR Spectrum of the Copper Sulphate Product (XXIV).

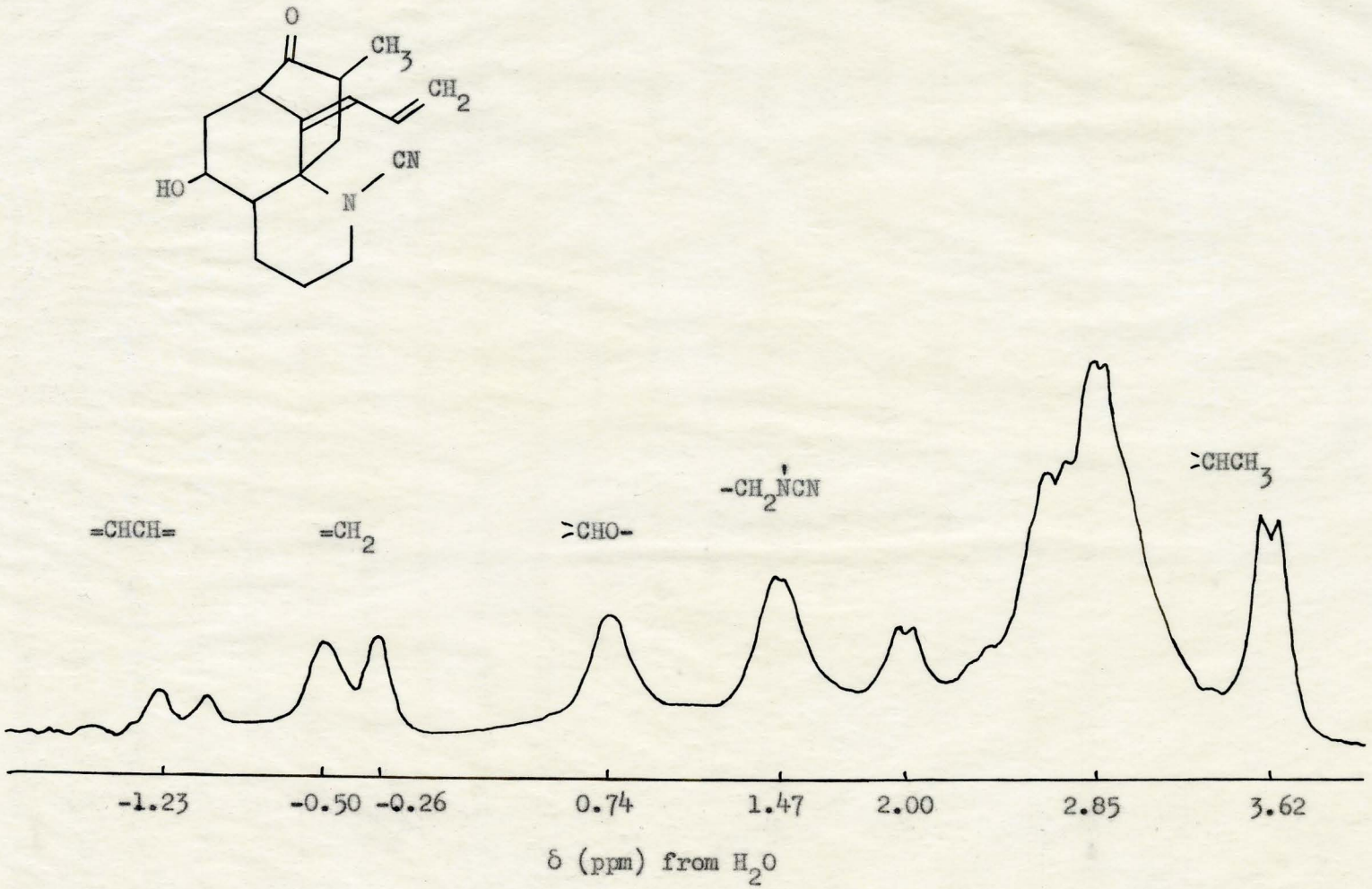


Figure 14. The NMR Spectrum of Dehydrobrominated α-cyanobromoacrifoline (VII).

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