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THE STRUCTURE OF LYCODINE

(PART ONE)

and

THE PREPARATION AND PROPERTIES OF SOME PHENANTHROLINES

(PART TWO)

BY

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A thesis submitted in partial fulfillment
of the requirements of the degree of
Doctor of Philosophy

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PREFACE

Lycodine, isolated by Dr. C.R. Eves in 1958, is one of the few alkaloids of Lycopodium annotinum L. which contain two nitrogens. The presence of a 2,3-disubstituted pyridine ring in lycodine has been established by the work of Dr. Eves. The present investigation was designed to elucidate the complete structure and stereochemistry of lycodine and was undertaken at the suggestion of Dr. F.A.L. Anet. This is described in Part I. The work presented in Part II, arose in connection with the degradation of lycodine, and describes the preparation of several phenanthrolines and their properties particularly the ultraviolet and NMR spectra.

The author's sincere thanks are due to Dr. F.A.L. Anet for the stimulating guidance and enlightening discussions at every stage of the work and for his kindness in providing financial support from his research grants.

The author is grateful to Dr. L. Marion for a gift of the crude alkaloid mixture used in this study and to Dr. K. Biemann for the measurement and discussion of the mass spectrum of lycodine.

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ABSTRACT

PART I

Lycodine (1), an alkaloid of Lycopodium annotinum L., has been isolated by an improved procedure. The structure of lycodine has been deduced from physical and degradative studies. One of the dehydrogenation products of lycodine has been identified as 5-isobutyl-1,7-phenanthroline and has been synthesised.

The stereochemistry and the structure of lycodine have been deduced by relating the alkaloid to lycopodine, another Lycopodium alkaloid, whose structure (2) and stereochemistry (3) are known.

PART II

Several alkyl-1,7-phenanthrolines have been prepared as model compounds for the dehydrogenation products of lycodine. A few alkyl-4,7-phenanthrolines and 2,7-diazaphenanthrene have also been prepared. The ultraviolet and NMR spectra of the phenanthrolines have been measured and the effect of alkyl substitution on the spectral characteristics has been studied. These spectra have been found to be useful in identifying the ring system and the nature and location of the substituents in this series of compounds.

PART I

THE STRUCTURE OF LYCODINE

INTRODUCTION

Scope of the problem.

With the elucidation, in 1957, of the unusual structure of annotinine (4), an alkaloid of *Lycopodium annotinum L.*, interest in the structures of other *Lycopodium* alkaloids has been growing rapidly.

This part of the thesis is concerned with the structure and sterochemistry of lycodine, an alkaloid of *L. annotinum L.*, isolated by Anet and Eves in 1958 (1). It was shown by these authors that lycodine, m.p. 118°, $C_{17}H_{24}N_2$*, is a tetracyclic diacidic base, containing a C-methyl group, a secondary nitrogen, and a 2,3-disubstituted pyridine ring.

Review of pertinent literature.

Lycopodium annotinum L., "Stiff Club Moss" is one of about a hundred species of plants belonging to the *Lycopodium* genus. These diminutive plants belong to the vast class of vascular cryptogams and are botanically quite primitive, as evidenced by their appearance in fossils. They occupy a mediaeval position in the evolutionary history of plants, linking the algae and fungi with the highly evolved flowering plants. It must be mentioned here that

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* This has since been revised to $C_{16}H_{22}N_2$

nicotine, an alkaloid hitherto found only in flowering plants, has also been detected in certain Lycopodium species (5).

Although various Lycopodium plants have been investigated for alkaloidal constituents since the nineteenth century (6, 7, 8), the first report on the alkaloids of Lycopodium annotinum L. appeared only in 1943 (9). In this year, Manske and Marion isolated, from L. annotinum L., annotinine ($C_{16}H_{21}O_3N$), the principal alkaloid of the species, the previously known lycopodine ($C_{16}H_{25}ON$) (6, 7), the obscurines, and several other alkaloids which were not well characterised, hence designated by the letter L. followed by a number e.g. L-8 ($C_{16}H_{25}O_2N$). Other alkaloids isolated from certain sub-species of L. annotinum L. include acrifoline ($C_{16}H_{23}O_2N$), annotine ($C_{16}H_{21}O_3N$), nicotine ($C_{10}H_{14}N_2$), and isolycopodine ($C_{16}H_{25}ON$), (10, 11, 12). In the past, the methods of isolation and purification were mostly restricted to fractional distillation, fractional crystallisation of the bases in the case of the more abundant alkaloids and fractional crystallisation of various salts of the alkaloids in the case of the minor alkaloids. These methods were slow and not always reproducible. Anet and Eves (1) developed suitable paper-chromatographic procedures for the detection and assay of the minor alkaloids and introduced counter current distribution as an

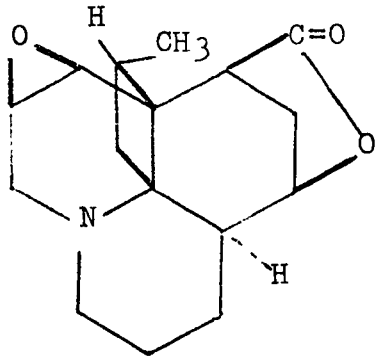
efficient and reproducible technique for the concentration and purification of particular alkaloids detected by paper-chromatography. Employing these methods, Anet and Eves (1) isolated lycodine, an alkaloid which occurs only in minute quantities in the crude alkaloids of L. annotinum L. . Anet and Khan (13) perfected these methods and isolated four other alkaloids, annofoline ($C_{16}H_{25}O_2N$), lycofoline ($C_{16}H_{25}O_2N$), lofoline ($C_{18}H_{29}O_3N$) and fawcettiine ($C_{18}H_{29}O_3N$). Exhaustive reviews on the isolation, distribution and characterisation of alkaloids of the various Lycopodium species are available (8, 11, 14).

Chemistry of Lycopodium alkaloids.

At the start of the work described herein, only the structure of annotinine had been established. In addition, studies on the functional groups and degradation of several other alkaloids including the partial structure of lycodine had been reported. These have been reviewed previously (8, 11, 14, 15). Since then, a considerable amount of work in the field has been published leading to the elucidation of the structure, interrelation, and stereo-chemistry of several Lycopodium alkaloids. In the following pages of this section a brief discussion of the structural studies and reactions of some of the more important Lycopodium alkaloids is given. In view of the relation of lycodine to

lycopodine, and thus to annofoline and acrifoline, these alkaloids are of particular relevance. The chemistry of these and otherwise interesting alkaloids is discussed in some detail. Annotinine, an important alkaloid, on the other hand has a carbon skeleton different from the others, and has not been so far related to any of the other alkaloids. The chemistry of annotinine is not included in this discussion. However there are excellent summarising publications and reviews on the chemistry of annotinine.

Annotinine, $C_{16}H_{21}O_3N$, the principal alkaloid of Lycopodium annotinum L., was isolated by Manske and Marion (9). Extensive degradation work on annotinine (for references see ref. 4, 16) and X-ray crystallographic studies of Przybylska and Marion (17) on annotinine bromohydrin have culminated in the structure (I) for annotinine.



(I)

The X-ray crystallographic work also established the relative stereochemistry implied in structure I for

annotinine. Wiesner et al have recently deduced the absolute configuration (I) of annotinine (18).

Lycopodine, $C_{16}H_{25}ON$, m.p. 116°, is the major alkaloid of many Lycopodium species and has been detected in almost all the species. It was first isolated by Bödeker (6); but its correct molecular formula was assigned by Achmatovicz et al. (7), who isolated it from Lycopodium clavatum C. and also reported the absence of $-OCH_3$, $-N-CH_3$, or active hydrogen in lycopodine.

Preliminary studies had shown that lycopodine contains a tertiary nitrogen (19), a C-methyl group, a carbonyl group (20), and no other functional groups or unsaturation (19, 20, 21). Hence it was inferred to be tetracyclic.

Manske and Marion (19) dehydrogenated lycopodine with selenium and isolated among others, two products that were identified as 7-methylquinoline and 5,7-dimethylquinoline. The former was also obtained under milder conditions of catalytic dehydrogenation with palladium on barium sulfate. Thus there might be present, in the alkaloid, a hydrogenated quinoline system.

MacLean, Manske, and Marion (20) confirmed the presence of a carbonyl group in lycopodine and showed it to be a keto group, by reacting lycopodine with phenyl-lithium to obtain a tertiary alcohol. Reduction of lycopodine

with lithium aluminum hydride gave dihydrolycopodine, from which lycopodine could be regained by chromic acid oxidation. Dehydration of dihydrolycopodine gave anhydrodihydrolycopodine, which was identical with a naturally occurring Lycopodium alkaloid L-14, previously isolated by Manske and Marion (21).

Lycopodine reacted with cyanogen bromide, in a Von Braun degradation, to form two isomeric addition-cleavage products designated as α- and β-cyanobromolycopodine ($C_{17}H_{25}ON_2Br$). The α- cyanobromolycopodine was formed in predominant amount, and could be converted back into lycopodine by treatment with potassium acetate and acetic acid. Both α- and β-cyanobromolycopodine cyclised under basic conditions, with loss of hydrogen bromide, to form isomeric compounds $C_{17}H_{24}ON_2$. The product from the α- isomer is formed presumably by internal alkylation at a carbon atom adjacent to the keto group. The product from the β- isomer appeared to be an inert enol ether (20, 23).

The location of the keto group relative to the nitrogen could be studied with the Von Braun degradation products (22, 23). Both α- and β-cyanobromolycopodine have been converted to acids with the same number of carbon atoms by the displacement of the bromine atom by acetate, hydrolysis to the alcohol, followed by chromic acid oxidation of the alcohols, proving thereby that both isomers are primary

halides. Reduction of the acid from the β-isomer by sodium borohydride resulted in a lactone, which from infrared spectral evidence appeared to be a five or six membered ring lactone. It followed therefore that the nitrogen and the carbonyl group are separated by three or four carbon atoms in the direction of β-cleavage. Whereas the acid from the α-isomer, upon reduction with sodium borohydride, gave a hydroxy acid which showed no tendency to lactonise, showing thereby that the nitrogen atom and the keto group are far removed from each other tracing the direction of α-cleavage. The "α-" hydroxy acid could be hydrolysed, to remove the cyanogroup, to give an amino acid hydrochloride. Treatment of this with diazomethane gave, among other products, a lactam which from infrared spectral evidence appeared to be a six membered or larger ring lactam. Lithium aluminum hydride reduction of the lactam gave dihydrolycopodine. This indicated that there were no rearrangements involved in the above sequence of reactions.

Both α- and β- cyanobromolycopodine, upon catalytic hydrogenolysis in alkali yielded the corresponding α- and β-cyanolycopodine, by replacement of the bromine atom with hydrogen. Reduction with sodium borohydride gave α- and β-cyanodihydrolycopodine. Both these compounds upon oxidation by a modified Kuhn-Roth procedure gave acetic, propionic, and butyric acids, whereas lycopodine itself gave

only acetic acid. This indicated that both α- and β-cyanodihydrolycopodine contained a propyl group (24). Therefore lycopodine has a partial structure:

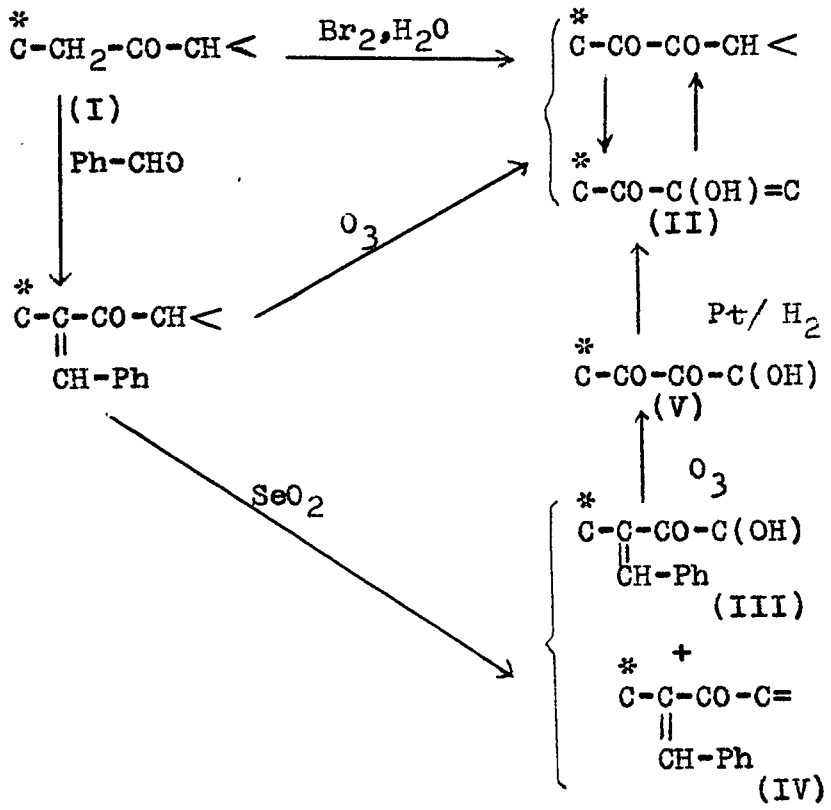
$-CO-(C)_n-(CH_2)_3-N-(CH_2)_3-$, where n = 0 or 1

The vicinity of the keto group could be studied well in α-cyanolycopodine. It gave a benzylidene derivative (22). Bromination of α-cyanolycopodine (I) followed by hydrolysis gave a diketone (II) with enolic properties. These results showed that there is a methylene group adjacent to the keto group and further that a grouping of the type: $CH-CH_2-CO-$, or $-CH_2-CO-CH$ should be present to allow the formation of an enolisable diketone. Deuterium exchange studies (24) on α-cyanolycopodine showed the presence of three enolisable hydrogen atoms; this, along with the further evidence described below, showed that the latter of the two alternative groupings was definitely present.

The benzylidene derivative of α-cyanolycopodine on ozonolysis gave the diketone (II) described above. Selenium dioxide oxidation of the benzylidene derivative gave two neutral oxidation products: a hydroxy compound (III) and an olefinic compound (IV). Ozonolysis of the hydroxy compound gave a yellow compound (V) which was a diketo alcohol. It had, unlike the diketone (II), no enolic

properties. Catalytic hydrogenation of the diketo alcohol (V) gave the diketone (II). These results and the fact that α-cyanolycopodine has only three enolisable hydrogens permit further inferences regarding the structure around the keto group.

It is significant that the diketone (II) has an enolisable hydrogen which is replaced by a hydroxy in the diketoalcohol (V). Since the hydroxyl group was introduced when the methylene group was protected by the benzylidene group, the methylene group and the -CH group are on either side of the keto group. Also in the diketoalcohol (V) there is a sequence of a tertiary hydroxyl, two keto groups, and a quaternary center or a bridgehead -CH group after the second keto group, to preclude its enolisation. These considerations allowed the assignment of the following partial structures for α-cyanolycopodine (I) and the products (II to V).



* indicates a quaternary center or a bridgehead -CH< group.

Since there is a quaternary center or a bridgehead on the methylene side of the keto group, the previously mentioned three carbon residue (23, 24) connecting the nitrogen and the keto group in the " β " direction, might be on the side of the -CH< group. Hence the partial structure deduced previously could be elaborated to look like

$$\overset{*}{C}\text{-}CH_2\text{-}CO\text{-}CH\text{-}(CH_2)_3\text{-}\overset{\beta}{N}\text{-}(CH_2)_3\text{-}$$

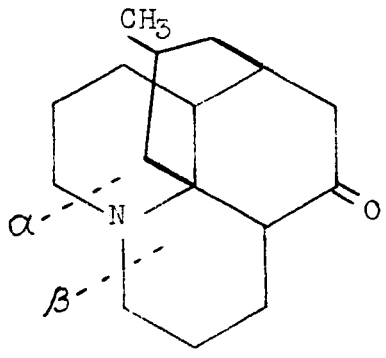
(VI) α

where * indicated a quaternary or a bridgehead -CH< group.

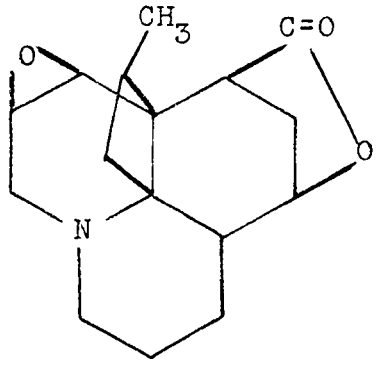
The partial structure (VI) accounts for ten out of the sixteen carbon atoms in lycopodine. The C-methyl group present in lycopodine does not figure in the above partial structure.

Previously Barclay and MacLean (22) had compared the pKas of lycopodine and several of its α- and β-cleavage products. The pKas did not show any significant variation in both the α- and β-cleavage products. But the brominated products did show lower basicities. From this they had concluded that the keto group and the nitrogen atom have a close relationship which is not disturbed either by the α- or the β-cleavage of the molecule.

On the strength of the above evidence, Harrison and Maclean have proposed structure (VII) for lycopodine (24).



(VII)

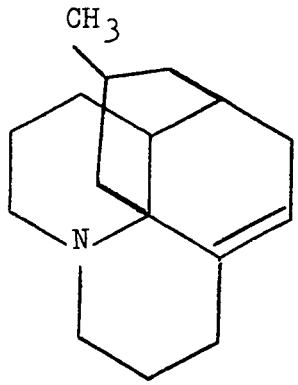


(VIII)

The structure accounts for all the known reactions of lycopodine and its degradation products, a C-methyl group and for the formation of 7-methylquinoline and 5,7-dimethylquincline on dehydrogenation. The structure postulates without rigorous proof a hexahydrojulolidine ring system resembling in that respect the annotinine (VIII) skeleton; but departing from the annotinine skeleton in having a six membered ring instead of a four membered ring.

Lycopodine has since been related to annofoline, another Lycopodium alkaloid, whose structure has been arrived at by independent considerations (25). This has provided additional confirmation of structure (VII) of lycopodine. The stereochemistry of these alkaloids has been deduced (3). This will be presented in detail after the discussion of the chemistry of annofoline.

Alkaloid L-14, a liquid alkaloid, $C_{16}H_{25}N$ (perchlorate, m.p. 238°, $[\alpha]_D$,-107° in methanol), was isolated by Manske and Marion (26). As has been mentioned in the section under lycopodine, it was identical with the dehydration product of dihydrolycopodine. Therefore alkaloid L-14 is anhydrodihydrolycopodine (21). The NMR spectrum of L-14 showed a single olefinic proton. Therefore the dehydration had proceeded forming the double bond in the direction of the ring junction (3). The following structure was therefore appropriate.



L-14

Acrifoline, $C_{16}H_{23}O_2N$, m.p. 99-104°, was isolated from the plant Lycopodium annotinum var. acrifolium Fern. (10). Earlier reports (11, 12) on the functional groupings in acrifoline were based mainly on negative findings and have since been proved to be erroneous in some respects. However it was shown positively that acrifoline reacted with phenyllithium to give a C_{22} compound and yet did not show any tendency to form a benzylidene derivative. It followed therefore acrifoline had a keto group with no adjacent methylene group. Acrifoline also formed a methiodide. As will be evident from what follows, the functional groups in acrifoline do not show normal reactivity due to certain peculiarities of structure.

Perry and MacLean (27) have studied the nature of the functional groups in acrifoline. The infrared spectrum of acrifoline in nujol showed a band at 3310cm^{-1}, and weak bands at 1700cm^{-1} and at 1670cm^{-1} which have been

assigned to a hydroxyl group, a keto group and unsaturation respectively. In chloroform solution there was a strong band at 1700cm^{-1}. Catalytic hydrogenation of acrifoline gave dihydroacrifoline ($C_{16}H_{25}O_2N$), and lithium aluminum hydride reduction of the latter gave α-dihydroacrifolinol ($C_{16}H_{27}O_2N$). This showed hydroxyl absorption (3300cm^{-1}) but no carbonyl absorption or unsaturation in the infrared spectrum. An isomer, β-dihydroacrifolinol was prepared by reduction of acrifoline, first with lithium aluminum hydride, followed by catalytic reduction. Acetylation of acrifoline gave a monoacetylacrifoline, the infrared spectrum of which showed bands at 1740cm^{-1} (acetoxy group) and 1700cm^{-1} (ketone), but no band in the hydroxyl region. These studies confirmed the presence of a double bond, a hydroxyl group, a tertiary nitrogen, and a keto group in acrifoline.

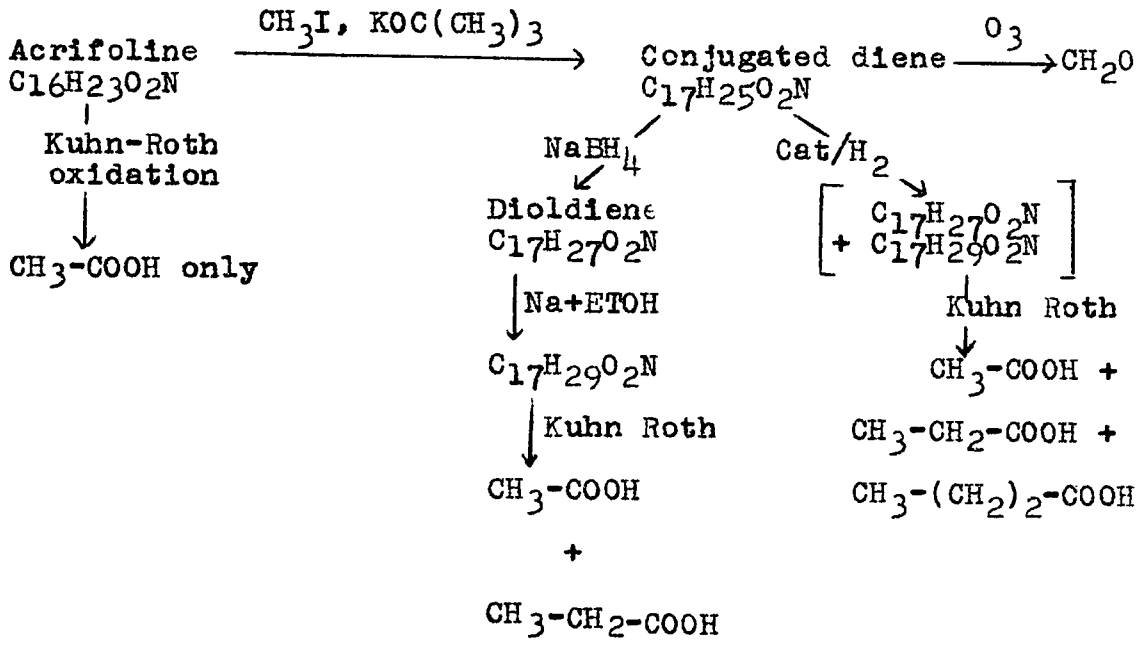
Dihydroacrifoline (pKa 9.13) is a considerably stronger base than acrifoline (pKa 8.34). This has been taken to be an indication of a -C=C-C-N (allylamine) structure in acrifoline (27).

The NMR spectrum of acrifoline indicated the presence of a CH.Me group (Doublet at δ = 3.70 p.p.m. from water), confirmed the presence of a keto group (no resonance due to aldehydic proton at low field) a secondary hydroxyl (-CHOH absorption at δ = 0.85 p.p.m.) and a

tri-substituted double bond (δ = -0.46) (28).

As mentioned earlier, acrifoline showed weak or no absorption in the carbonyl region of the infrared spectrum, measured in nujol mull, but showed a strong band 1700cm^{-1} when measured as a chloroform solution. This difference in behaviour in the solid state and a solution has been taken as evidence for the presence of a hemiketal form in the solid state. This means that the keto group and the hydroxyl carbon are separated by two or three atoms (28).

Hoffmann degradation of acrifoline methiodide ($C_{17}H_{26}O_2NI$) with potassium t-butoxide gave a conjugated diene ($C_{17}H_{25}O_2N$). French and MacLean have carried out experiments on this compound, designed to give information on the relationship of the double bond and the nitrogen in acrifoline. These are shown in the following flow sheet.



As the C-methyl group in acrifoline has been shown (cf. below) to be elsewhere in the molecule, these results indicate that a $C = CH-(CH_2)_2-N$ grouping is present in acrifoline.

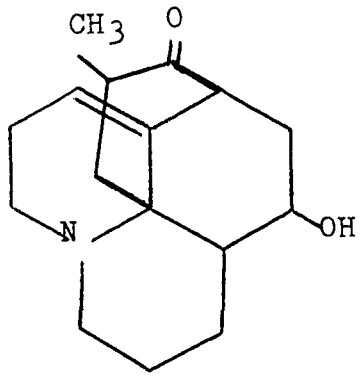
Acrifoline reacted with cyanogen bromide to give two cleavage products. One of them was shown to be formed by the cleavage at the same C-N bond as in the Hoffmann degradation, as its quaternary trimethylammonium bromide ($C_{20}H_{32}O_2N_3Br$) gave a conjugated diene ($C_{17}H_{22}O_2N_2$) upon treatment with strong base. The second product gave a noncrystalline quaternary salt with trimethylamine, which in turn gave an oily neutral product with base. The neutral product had two isolated double bonds showing that the cleavage in this case was of a second C-N bond. Ozonolysis of the neutral product gave formaldehyde. Catalytic reduction of the neutral product followed by oxidation yielded butyric, propionic and acetic acids. From the combined evidence the following partial structure emerged for acrifoline:
$-(CH_2)_3-N-(CH_2)_2-CH=C-$

Oxidation of acrifoline with selenium dioxide gave a conjugated carbonyl compound ($C_{16}H_{21}O_2N$), (infrared spectrum of its perchlorate had a band at 1690cm^{-1}; u.v. $\lambda_{max.}$ 243 mμ ; 5300). The NMR spectrum indicated a methyl group at a lower field δ= 3.00 and an olefinic

proton at $\delta = -2.27$ from water. Hydrogenation of the compound gave dihydroacrifoline ($C_{16}H_{25}O_2N$). Acrifoline has, therefore, a $-CO-CH(Me)-CH_2-$ system. The conjugated carbonyl compound could be cyclised by base to an isomeric compound, which showed a carbonyl band at 1720cm^{-1} in the infrared spectrum. This could be rationalised as a base-catalysed addition of the hydroxyl to the conjugated double bond. Therefore the hydroxyl carbon and the carbon atom β to the carbonyl group are separated by two or three atoms.

Dihydroacrifolinol ($C_{16}H_{27}O_2N$) underwent Oppenauer oxidation to yield a diketone ($C_{16}H_{23}O_2N$), the infrared spectrum of which showed strong absorption at 1700cm^{-1} and at 1420cm^{-1} for a carbonyl and a methylene adjacent to a keto group. Such a methylene group band is absent in the spectrum of acrifoline. Therefore in acrifoline there is a $-CH_2-CH(OH)-$ group. The hydroxyl group in acrifoline could not be replaced by halogen under mild conditions and hence it is not an allylic hydroxyl group.

French and MacLean (28) have proposed the following structure for acrifoline to explain the above results.



Acrifoline

Annofoline, $C_{16}H_{25}O_2N$, m.p. 156-157°, was isolated by Anet and Khan from Lycopodium annotinum L. (13). It was a strong base (pKa, 9.15) and formed a methiodide, indicating the presence of a tertiary nitrogen. It analysed for a single C-methyl group.

The infrared spectrum of annofoline in nujol mull indicated a hydroxyl group (band at 3400cm^{-1}) and a carbonyl group (1700cm^{-1}), probably a ketone in a six membered ring. However, the infrared spectrum in carbon tetrachloride solution showed a much weaker carbonyl band at 1705cm^{-1} (25). This behaviour is reminiscent of acrifoline, which, it has been shown, can exist as an internal hemiketal. Therefore in a solution of annofoline, there exists an equillibrium between the hydroxy ketone and the hemiketal forms. The NMR spectrum of

annofoline showed a doublet at 8.93 τ indicating that the methyl group was present as a >CH.Me group. The presence of a keto group has been confirmed by the reduction of annofoline by sodium borohydride to two isomeric diols, $C_{16}H_{27}O_2N$, α-dihydroannofoline and β-dihydroannofoline (25). Later work has shown that β-dihydroannofoline is not a genuine reduction product of annofoline, but of an isomeric ketone (3).

Selenium dioxide oxidation of annofoline gave an $\alpha\beta$-unsaturated ketone, $C_{16}H_{23}O_2N$, (λ max. 241 mμ ; log ϵ = 3.8 in EtOH; νmax. 1685cm^{-1} in CCl_4). The NMR spectrum of the compound showed a sharp band at 2.74 τ attributed to an olefinic proton β-to the keto group and a singlet at 8.18 τ for the methyl group. Therefore it was concluded that there was a -CO-C(Me)=CH- system in the compound. Also as the band for the olefinic proton was unsplit, it was probable that there was a quaternary carbon atom adjacent to the =CH group. Therefore annofoline contained a -CO-CH(Me)-CH_2-C*- structure (where C* is a quaternary center).

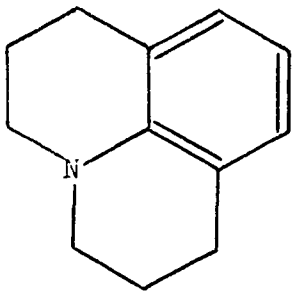
Annofoline reacted with ethanethiol to give a single product, which analysed for $C_{18}H_{29}ONS$. The infra-red spectrum showed a weak band at 1625cm^{-1} (double bond) and a band at 2600cm^{-1} (hydroxyl) but no band in the carbonyl region. The NMR spectrum showed that there were

no olefinic protons, but that the methyl group was now attached to a doubly bonded carbon. It followed therefore that the compound was an ethyl thioenol ether and had a C_2H_5S-C=C-Me system. Thus both in this reaction and in the selenium dioxide oxidation the keto group in annofoline preferred to enolise in the direction of the methyl group.

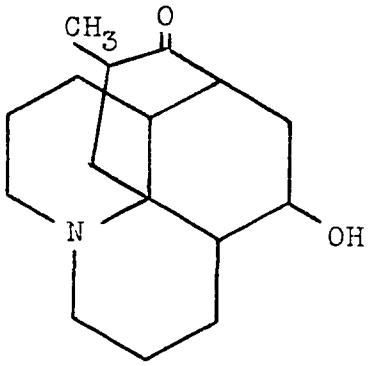
Wolff-Kishner reduction of annofoline gave dihydrodeoxyannofoline ($C_{16}H_{27}ON$). The oxygen atom in this compound was present as a hydroxyl function. Chromic acid oxidation gave deoxyannofoline, $C_{16}H_{25}ON$, m.p. 88-92°, isomeric with lycopodine, but not identical with it. Selenium dioxide oxidation of this ketone gave an α-diketone, which showed enolic properties, hence a $-CH_2-CH(OH)-CH$ or a $-CH-CH_2-CH(OH)-$ group was present in annofoline.

Annofoline reacted with t-butyl nitrite in t-butanol in the presence of potassium hydroxide to give an amphoteric compound. Dehydrogenation of this compound with palladium-charcoal gave a compound identified as julolodine (I). The formation of the amphoteric compound proceeds via an intermediate nitroso ketone which is cleaved by base at the -CO-CH(Me) bond. It followed therefore that annofoline contained a hexahydrojulolidine ring system, and that the $-CO-CH(Me)-CH_2$ group present in

annofoline was lost during the dehydrogenation step.



(I)



(II)

Anet and Khan (25), have proposed the structure (II) for annofoline, to explain the above reactions of annofoline. The structure bears a similarity with the proposed structures of lycopodine and acrifoline and to some extent to that of annotinine. Annofoline has since been related directly to acrifoline and indirectly to lycopodine. This will be discussed in detail later, while considering the stereochemistry of these alkaloids.

<u>α- and β-Obscurine</u>. Manske and Marion (29) first isolated "obscurine" from <u>Lycopodium obscurum L.</u>. Moore and Marion (30) showed that "obscurine" was a mixture of two alkaloids, α-obscurine, $C_{17}H_{26}ON_2$, m.p. 283-284° (dec.) and β-obscurine $C_{17}H_{24}ON_2$, m.p. 322-323° (dec.). These authors studied the functional groups in both alkaloids and also the degradation of α-obscurine. They reported the absence of N-methyl groups in the

alkaloids. But later workers have shown that both the alkaloids do contain an N-methyl group.

The infrared spectrum of α-obscurine measured in chloroform solution was indicative of an -NH-group (band at 3411cm^{-1}) and a carbonyl group (band at 1667cm^{-1}), while that of β-obscurine, showed an -NH- group (band 3385cm^{-1}) and an α-pyridone system (strong double peaks at 1659cm^{-1} and 1651cm^{-1} and smaller peaks at 1620cm^{-1} and 1613cm^{-1} (30). The ultraviolet spectrum of β-obscurine also showed an α-pyridone chromophore; but that of α-obscurine showed absorption ($\lambda_{max.}$ 255 mμ ; log ε, 3.75), but did not show such a chromophore. Moore and Marion, considered among other possibilities, the likelihood that α-obscurine contained a partially reduced α-pyridone system, which would make it a dihydro-β-obscurine. But an attempt to dehydrogenate partially α-obscurine to β-obscurine by bromination and dehydrobromination was not successful (30).

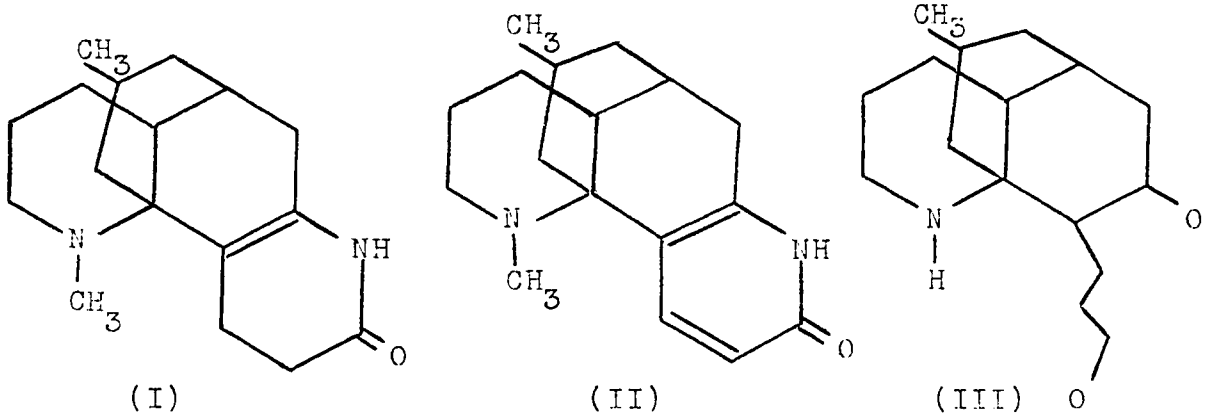
Dehydrogenation of α-obscurine with palladium-charcoal gave 7-methylquinoline and 6-methyl-2-pyridone (30).

β-obscurine was resistant to acetylation. From this Anet and Eves concluded that the basic nitrogen in β-obscurine was tertiary (1).

Ayer and Iverach (31) upon reinvestigation, found that both α- and β-obscurine contained an N-methyl group and a C-methyl group as indicated by the NMR spectra and analysis. This meant that the ring system in both the bases contained only sixteen carbon atoms. The ultraviolet absorption spectrum of α-obscurine, was interpreted by these workers, to be more consistent with a 3,4-dihydropyridone than with a 5,6-dihydropyridone type. The infrared spectrum of α-obscurine showed in addition to previously mentioned bands a medium intensity band 1700cm^{-1}. These authors assigned this band to a -C=C- stretching vibration of the 3,4-dihydro-2-pyridone system the abnormally high frequency of which had a parallel in structurally similar enolic δ-lactones. Further, although α-obscurine had an -NH- group, the base could not be acetylated. Therefore the -NH- group must be present as an amide. Thus it seemed probable that α-obscurine was simply dihydro- β-obscurine. This was confirmed by dehydrogenation of α-obscurine to β-obscurine by treatment with N-bromosuccinimide (31), and by the conversion of β-obscurine into α-obscurine by reduction with lithium in liquid ammonia.

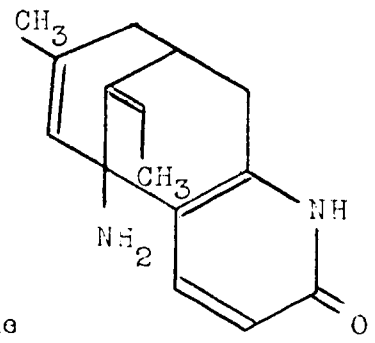
The two dehydrogenation products of α-obscurine, i.e. 7-methylquinoline and 6-methyl-2-pyridone (30) and

the N-methyl group accounted for all the seventeen carbon atoms in α-obscurine. On the basis of the above evidence and on biogenetic grounds Ayer and Iverach have proposed the structures I and II for α-and β-obscurine respectively. They have also postulated that a biogenetic precursor such as III could give rise to I and II by the inclusion of a second nitrogen followed by cyclisation, N-methylation, and related biological processes and could give lycopodine by a mere cyclisation.



(I) (II) (III)

Selagine, $C_{15}H_{18}ON_2$, m.p. 224-226°, isolated by Valenta and co-workers from L. Selago, has been assigned the structure I (p.) as a result of investigations by two groups of workers (32).



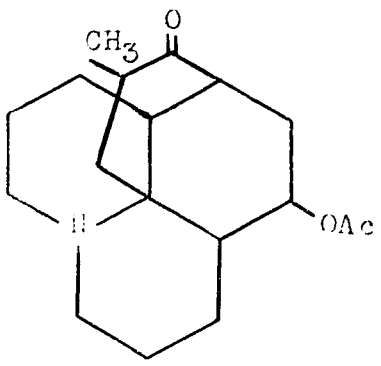
Selagine

(I)

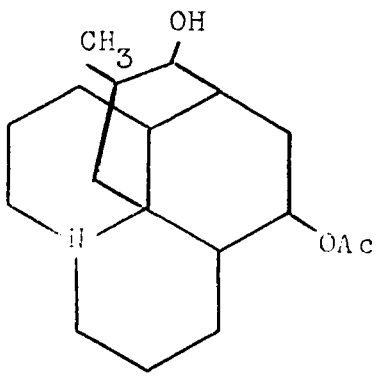
Structure (I) shows an interesting biogenetic relationship to lycopodine (24) and α- and β-obscurine. It has been suggested that α- and β-obscurine are biogenetic links between lycopodine and selagine (32).

Fawcettiine, (13, 33); β-lofoline; $C_{18}H_{29}O_3N$, m.p. 166-167°, was simultaneously isolated by two groups of workers. It has been shown to contain a hydroxyl group, an O-acetyl group, one C-methyl group apart from the one in the acetyl group, and a tertiary nitrogen.

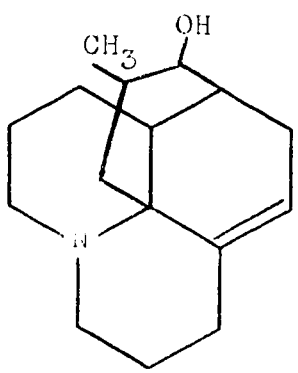
On hydrolysis fawcettiine afforded a diol, deacetylfawcettiine, $C_{16}H_{27}O_2N$, which on dehydration gave anhydrodeacetylfawcettiine, $C_{16}H_{25}ON$ (34). The latter although it contained a hydroxyl group was resistant to further dehydration (34). Oxidation of fawcettiine with chromium trioxidepyridine gave dehydrofawcettiine, $C_{18}H_{27}O_3N$, which could be hydrolysed by base to annofoline. As has been shown later (3) this was not a simple hydrolysis but one in which isomerisation accompanied deacetylation. Since the structure of annofoline was known the gross structure of dehydrofawcettiine and therefore of fawcettine follow from this to be I and II respectively.



(I)



(II)



(III)

The NMR spectrum of anhydrodeacetyl fawcettiine showed only one olefinic proton and also a $-CH.CH_3$ group. Therefore anhydrodeacetyl fawcettiine must be represented by III.

Lycodine, m.p. 118°, $[\alpha]_D$,-10°, is a minor alkaloid of Lycopodium annotinum L. and occurs only in minute quantities in the plant. It was isolated by Anet and Eves, from the crude alkaloidal residue, by successive countercurrent distribution, followed by chromatography on alumina (1). These authors assigned the molecular formula, $C_{17}H_{24}N_2$, for lycodine. This has, however, been revised to $C_{16}H_{22}N_2$, by Ayer and Iverach (35), in a work contemporaneous with the work described in this thesis.

The ultraviolet spectra of lycodine in neutral solution (λ max. 268.2 mμ , logϵ , 3.728; 275.7 mμ logϵ , 3.615) and in acid (λ max. 272 mμ logϵ 3.926) showed great similarity with the spectra of the unconjugated pyridine chromophore as base and salt respectively. Further, a comparison of the spectra of lycodine, 2,3-lutidine and various 2,3-polymethylenepyridines revealed that in this respect lycodine showed the greatest similarity with 2,3-tetramethylene pyridine (5,6,7,8-tetrahydroquinoline). Thus it was probable that lycodine has a saturated six membered ring fused 2,3- to the

pyridine ring (1).

The infrared spectrum of lycodine, in nujol mull, showed bands indicative of an N-H group (3270cm^{-1}), and a pyridine ring (1580cm^{-1}). The spectrum in carbon tetrachloride indicated a C-methyl group (1382cm^{-1}).

Lycodine could be acetylated to N-acetyl lycodine which was a considerably weaker base and showed only one basic centre by titration. The pKa of N-acetyl lycodine was 4.95 whereas the pKa's of lycodine were 3.97 and 8.08. Hydrolysis of N-acetyl lycodine, gave back lycodine.

The NMR spectrum of lycodine showed three peaks at low field, assignable to the protons on the pyridine ring. A broad peak at highest field corresponded to the protons on methyl group. The presence of a C-methyl group in lycodine has been confirmed also by Kuhn-Roth analysis and the infrared spectrum. The absence of splitting of the methyl peak was taken to be indicative of the quaternary nature of the carbon bearing the methyl group. This has been shown not to be the case by later work (35).

The NMR spectrum, at high resolution, showed each of the three peaks at low field, as quartets, and was similar to the spectrum of 2,3-lutidine and 5,6,7,8-

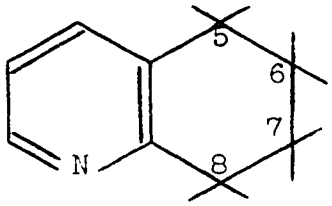
tetrahydroquinoline. The coupling constants were of the same order of magnitude. However, the γ-proton of lycodine was at lower field than the γ-proton in 2,3-lutidine and 5,6,7,8-tetrahydroquinoline. The three peaks at low field have been assigned from low field to high field to the α, γ and the β-protons of the pyridine ring.

The abnormal chemical shift of the γ-proton, was explained as being due to the nearness of the secondary nitrogen atom to the γ-proton and the pyridine ring. However from a comparison of the pKas of lycodine, (3.97 and 8.08) and nicotine (3.35 and 7.70) Anet and Eves suggested that the secondary nitrogen might be separated from the pyridine ring by more than one carbon atom to explain the higher pKa of the pyridine nitrogen in lycodine.

Anet and Eves also recognised the possibility that β-obscurine ($C_{17}H_{24}ON_2$) might be closely related to lycodine. β-obscurine has been shown to have an α-pyridone system. But it was found that in β-obscurine the basic nitrogen could not be acetylated and hence was tertiary, whereas in lycodine there is a secondary nitrogen atom. Thus β-obscurine was not a simple α-pyridone analogue of lycodine.

Apart from the pyridine ring, lycodine did not show any other unsaturation and hence was shown to be tetracyclic.

The results of Anet and Eves, were summarised in the following partial structure.



NH not* attached to C_5 or C_8.

$-CH_3$ attached to a quartenary* carbon atom.

+ 7C* + 17H* + 2 rings

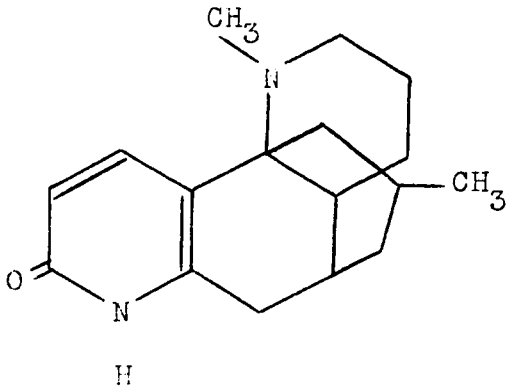
* These findings have been shown to be incorrect by contemporary work including the work described in this thesis.

The report of Anet and Eves (1) on lycodine, was all that was known about lycodine at the start of this work.

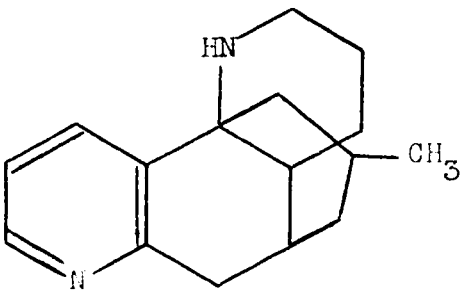
During the course of this investigation, there appeared two important reports on the structure of lycodine.

Ayer and Iverach (31) deduced the structure of α- and β-obscurine and from a biogenetic consideration suggested that lycodine might be the pyridine analog of demethylated β-obscurine.

In a later publication, Ayer and Iverach confirmed the molecular formula of lycodine to be $C_{16}H_{22}N_2$ and related lycodine to β-obscurine (35). Lycodine was methylated to N-methyl lycodine, by refluxing with formaldehyde and formic acid. N-methyl lycodine, m.p. 91-92° analysed for $C_{17}H_{24}N_2$. N-methyl lycodine was obtained from β-obscurine as follows: Treatment of β-obscurine with phenylphosphonic dichloride at 200-210° gave an oily chloro compound. The ultraviolet spectrum of the crude compound showed $\lambda_{max.}$ 275 mμ and the infrared spectrum showed bands at 3330cm^{-1} and 1565cm^{-1} indicating an NH- group and a pyridine ring. The crude chloro compound was hydrogenated in glacial acetic acid solution in presence of Adam's catalyst. The product on chromatography over alumina gave N-methyl lycodine and a small amount of lycodine. Since the structure of β-obscurine is known to be I, the structure of lycodine must be II. (35).



(I)



(II)

Interrelation and stereochemistry.

With the elucidation of the gross structures of lycopodine (24), acrifoline (28), annofoline (25), and fawcettiine (34) to be I, II, III and IV (Fig. 1) respectively, it became apparent that there must be a close relationship between these alkaloids. Interrelation of these alkaloids has since been done (3, 34) and the stereochemistry of these alkaloids has been deduced from these and other studies by Anet (3).

Catalytic hudrogenation of acrifoline (II) gave, in addition to the previously reported dihydroacrifoline, a small yield of annofoline (III) (3). Thus annofoline is a "dihydroacrifoline" and is epimeric with the previously known dihydroacrifoline at C_4 (see Fig. 1 for numbering used).

Attempts to prepare lycopodine (I) from annofoline (III) had been unsuccessful. Anet and Khan (15, 25) obtained, by Wolff-Kishner reduction of annofoline, followed by chromic acid oxidation, a compound designated deoxyannofoline, $C_{16}H_{25}ON$, m.p. 88-92°, which had the gross structure I, but was not identical with lycopodine, m.p. 115°. This was indicative of the fact that more than one stereochemical configuration was involved. It has been shown by Anet (3) that lycopodine and deoxyannofoline differ only in the

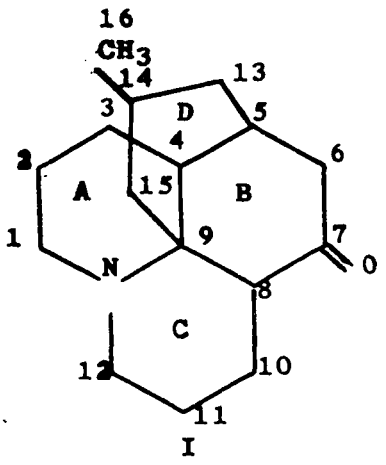
configuration of C_{14} atom bearing the methyl group.

The O-acetyl derivative of annofoline (III) has the same gross structure as dehydrofawcettiine, the chromium trioxide oxidation product from fawcettiine (IV). But these two compounds have been shown to be not identical (3)(comparison of the hydrobromides). However, both O-acetylannofoline and dehydrofawcettine, on hydrolysis with base, furnished annofoline (3, 34). This apparent discrepancy can be explained only if there was an isomerisation during the alkaline hydrolysis of dehydrofawcettine, as it is known that during acetylation and chromium trioxide oxidation no isomerisation are likely. Thus it is clear that annofoline and fawcettiine have opposite stereochemistry at C_{14} and also that annofoline is more stable than the hydrolysis product from dehydrofawcettiine, i.e. C_{14} epimer of annofoline (3).

Reduction of annofoline (III) with sodium borohydride in aqueous ethanol was reported to give a mixture of α- and β-dihydroannofoline (25). Later work showed that these were not epimeric alcohols at C_{13}, as might be thought. Reduction with lithium aluminum hydride or with sodium borohydride in neutral solution gave only the α-isomer. Sodium borohydride reduction in the presence of sodium hydroxide gave as

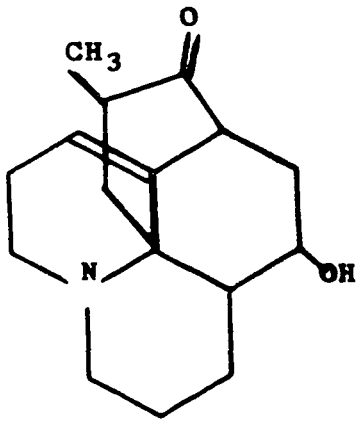
much as 50% of the β-isomer. Thus β-dihydroannofoline is not a genuine reduction product of annofoline, but of a ketone having the methyl group on C_{14} in the opposite configuration. On this premise, the identity of β-dihydroannofoline and deacetylfawcettiine, reported previously, (34) could be readily understood. The reduction of annofoline could be in two steps, initial equilibration between annofoline and the less stable ketone by alkali, followed by faster reduction of the less stable ketone. The difference in the speeds of reduction of the two isomers can be understood on the basis of the proposed stereochemistry of these compounds, discussed below.

Lycopodine has been related to annofoline by the following series of reactions, which allow the assignment of the configuration at C_{14} in lycopodine (3). Deacetylfawcettiine (identical to β-dihydro-annofoline) was dehydrated to anhydrodeacetylfawcettiine. Chromic acid oxidation of anhydrodeacetylfawcettiine gave an oily ketone, which was reduced by the Wolff-Kishner method to yield a compound $C_{16}H_{25}N$, proved to be identical with anhydrodihydrolycopodine (naturally occurring alkaloid L-14) previously prepared from lycopodine (21). Lycopodine, therefore, resembles fawcettiine in the configuration at C_{14} and not annofoline.



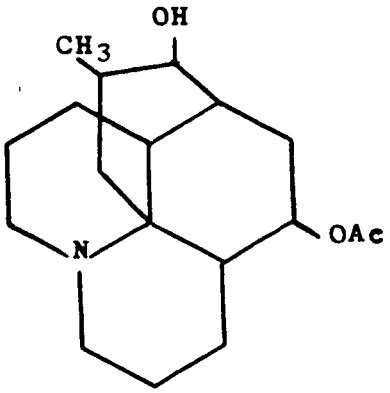
I

Lycopodine



II

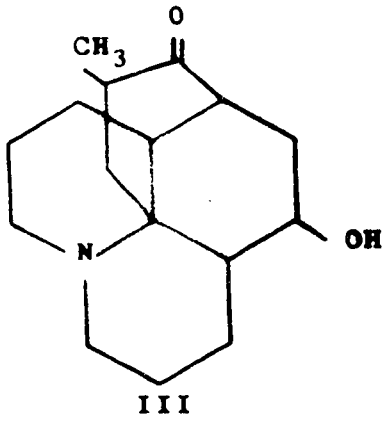
Acrifoline



IV

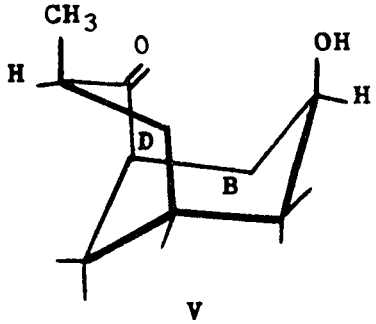
Fawcettiine

H_2/PtO_2

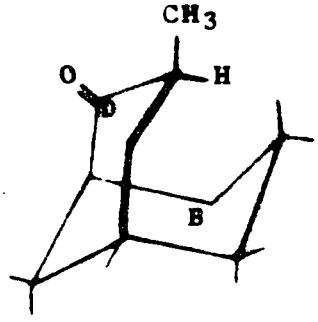


III

Annofoline



V



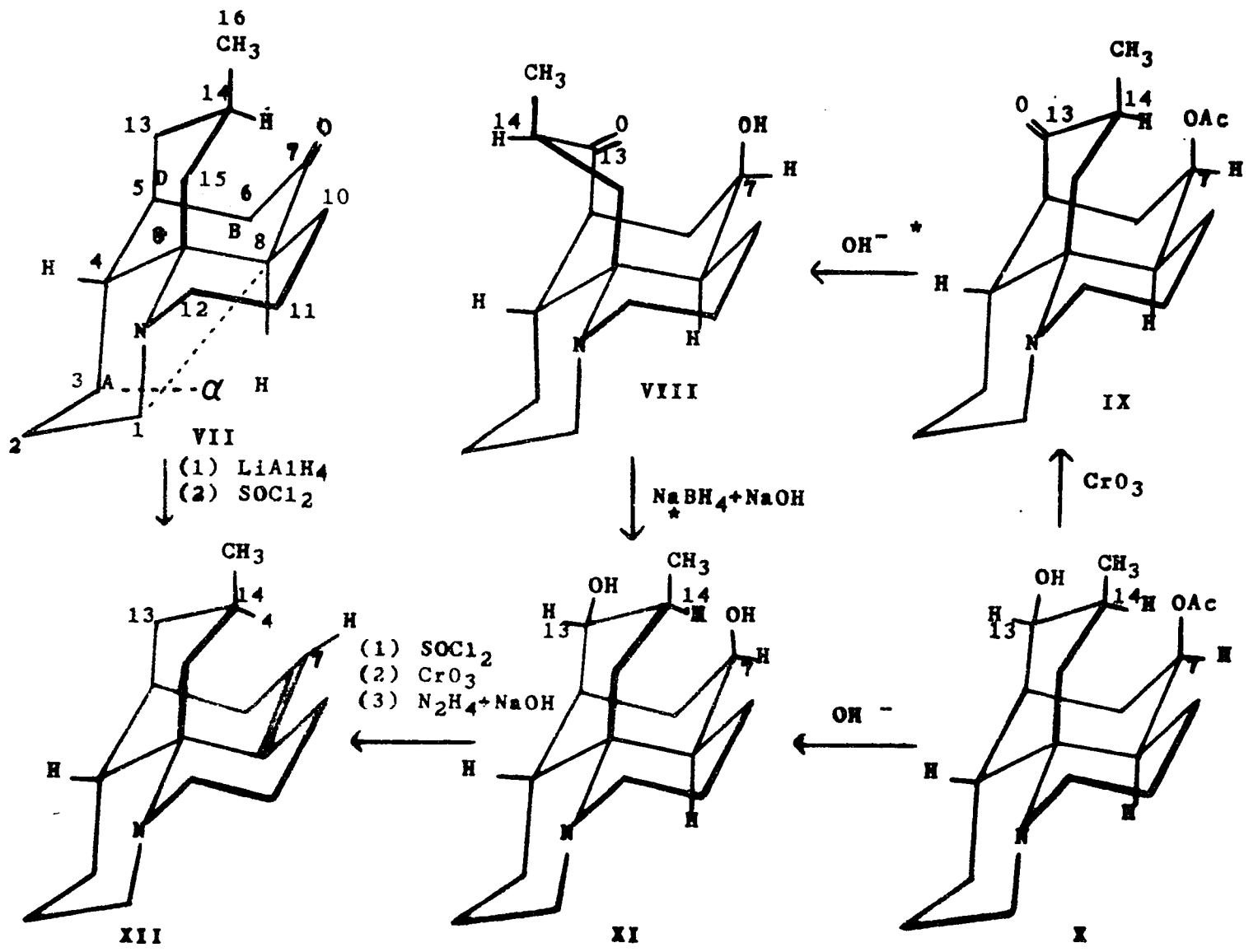
VI

Figure 1. Interrelation and stereochemistry of some Lycopodium alkaloids-I.

Both annofoline and acrifoline exist as mixtures of hemiketal and internally hydrogen bonded hydroxy ketone forms. This means that the [3, 1, 3]-bicyclononane system comprising rings B and D must conform to a chair and a boat and the ring D must exist in a boat form to allow for the hemiketal formation and hydrogen bonding. Also for the same reason the hydroxyl group on C_7 must be axial. The partial conformation shown V must therefore be appropriate for both annofoline and acrifoline. The conformation with ring D in the chair form would be destabilised due to strong repulsion between C_{14} and the axial substituent on C_7. Since annofoline has been shown to be the stable isomer at C_{14}, the methyl group would have to be equatorial on ring D in the boat form as in V. However if the interaction between C_{14} and C_7 substituents is sufficiently relieved the chair form of ring D should become the preferred conformation and in such a case the isomer with the C_{14} methyl group equatorial on ring D would be more stable. The configuration of the methyl group is now the opposite of that in the previous case as in VI. The stability of configuration on C_{14} depends on whether the ring D is in a chair or boat form and this in turn is decided by the bulk of the substituents on C_7.

Lycopodine and deoxyannofoline were both stable to base showing that rings B and C are trans fused. The ready dehydration of dihydrolycopodine confirmed this conclusion. Rings A and B were shown to be fused cis from facts already mentioned; α-cyanobromolycopodine was known to be cyclised by base by internal alkylation at C_8 (20, 23). This would be feasible if the ring residue in α-cyanobromolycopodine was axial on ring B. The catalytic hydrogenation of acrifoline gave dihydroacrifoline in major yield and annofoline in smaller yield. This would be expected to be so, since hydrogenation from the less hindered side would be expected to give a trans fusion of rings A and B and therefore annofoline, being the C_4 epimer, would have rings A and B cis fused.

On the basis of the above evidence, the stereochemistry of lycopodine and annofoline have been assigned as in VII and VIII (3). The interrelation of fawcettiine, annofoline and lycopodine is shown graphically in figure 2. The proposed stereochemistry of these compounds, is also in agreement with several other facts observed. As expected, Wolff-Kishner reduction of dehydrofawcettiine (IX) gave largely the isomerised product, and no detectable amounts of dihydrolycopodine. Fawcettiine (X) and therefore deacetylfawcettine (XI)



* indicates that the step involves isomerisation at C_{14}

Figure 2. Interrelation and stereochemistry of some <u>Lycopodium</u> alkaloids - II

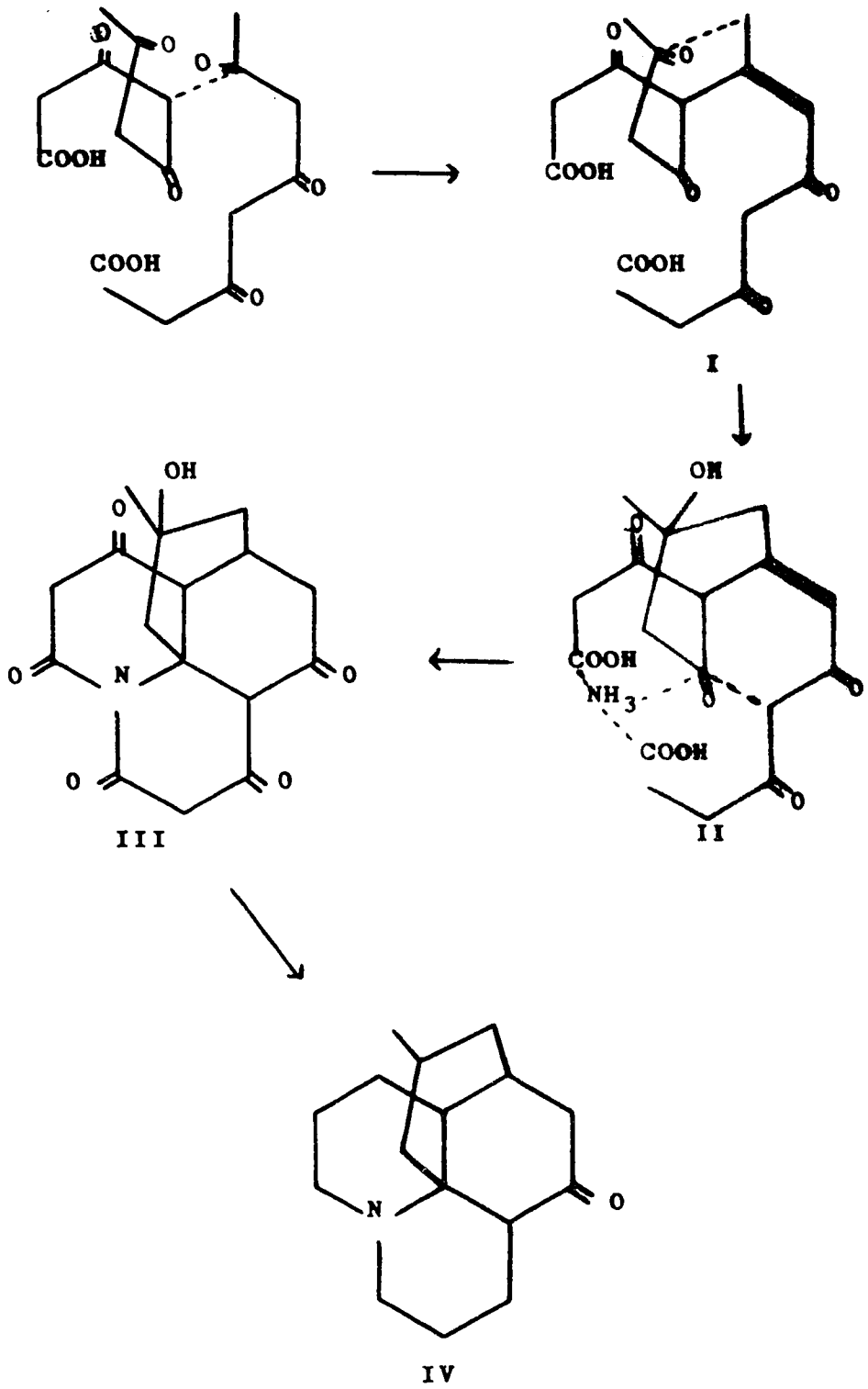


Figure 3. Conroy's scheme for the biogenesis of lycopodine.

must have the ring D in the chair form, as in the boat form the methyl group would be in the unfavourable flag-pole position. This is borne out by the fact that neither fawcettiine (X) nor lofoline, which are epimeric alcohols at C_{13}, show evidence of hydrogen bonding in dilute carbon tetrachloride solution. Anhydrodeacetyl-fawcettiine was resistant to further dehydration and therefore the hydroxyl at C_{13} must be <u>trans</u> to the methyl group, in agreement with the proposed stereo-chemistry for deacetylfawcettiine (XI) (3).

<u>Biogenesis of Lycopodium alkaloids</u>.

When the structure of annotinine was established, Leete (36) suggested a possible pathway for the biogenesis of annotinine, from $\beta\delta$-diketocaproic acid, mevalonic lactone, and the dialdehyde amine $HN-(CH_2-CH_2-CHO)_2$.

After the elucidation of the structure of lycopodine, a more attractive biogenetic scheme has been put forward by Conroy (37).

Conroy's scheme (Fig. 3), postulated a condensation of two molecules of 3, 5, 7-triketo-octanoic acid (or equivalent) to give the basic skeleton I. This condensation, involves biogenetically feasible aldol condensation and dehydration. A second aldol condensation gives II. The double bond is reduced at this stage. A Mannich condensation in visualised

for the incoroporation of nitrogen. The subsequent steps include lactam formation to give III, followed by reduction to give lycopodine, IV.

The intermediate I may be the precursor to several Lycopodium alkaloids. Suitable pathways for the biogenesis of the obscurines, lycodine, selagine, and annotinine from I, have been suggested (37). The structures of the alkaloids, acrifoline, annofoline and fawcettiine, subsequently elucidated, are also such that these may well be resulting from a similar biogenetic route. The scheme also provides for the observed location of the oxygenated and doubly bonded carbon atoms and such other details, in almost all the Lycopodium alkaloids whose structures are known to date.

EXPERIMENTAL

Description of general methods.

The melting points were determined on a "Lietz" hot-stage apparatus. The infrared spectra of solutions were measured in a microcavity cell (ca. 1 mm path length; 0.04 ml. volume) on a Perkin Elmer "Infracord" double beam instrument. The ultraviolet spectra were recorded using a Beckmann DK-2 spectrophotometer in ethanol solution in 1 cm. cells. The nuclear magnetic resonance (NMR) spectra were recorded on a Varian Model V-4302 spectrometer operating at 60 Mc/s. Nylon plugs were used in the case of smaller samples (containing less than 20 mg/0.2 ml of solvent) as described in the Varian technical bulletin. The spectra were calibrated by the side band technique using tetramethysilane as the internal standard. The coupling constants are expressed in cycles per second (cps) and the proton chemical shifts are expressed in τ values (39). τ in p.p.m. = $10.0 - 10^6 (H_{Me_4Si} - H_{obs}) / H_{Me_4Si}$; where H = magnetic field at resonance.

Elementary analyses were kindly performed by Miss E. Busk.

Paper chromatography, unless otherwise stated, was carried out according to Anet and Khan (13), on Whatmann No. 1 paper impregnated with a citrate-phosphate

buffer. The chromatogram was developed by n-butanol saturated with water and the spots were revealed by spraying with a modified Dragendorff reagent (38).

Isolation of lycodine.

The material available for this work was the mother liquor remaining after the crystallisation of annotinine from the crude alkaloids of L. annotinum L. (1). Lycodine was separated from this in several steps.

(a) Distillation of the crude alkaloids.

The alkaloidal material (50g) from the mother liquor was mixed with dibutyl phthalate (50ml), octyl phthalate (45ml) and castor oil (85ml) and the mixture was circulated continuously (22 hors) in a falling film molecular still (Distillation Products Inc., Rochester, N.Y. Type CMS-1000) with a preheat temperature of 50-55°, column temperature from 80° to 130° and a pressure of 0.001mm. (40). The distillate, containing the alkaloids and the volatile esters, was dissolved in ether (300ml) and extracted with dilute hydrochloric acid. The acid extract was washed with ether twice, and made alkaline with aqueous sodium hydroxide. This was extracted with chloroform exhaustively. The combined chloroform extract was dried with anhydrous sodium sulfate and evaporated to dryness. Recovery of a faintly colored alkaloid material was 39.5g. Paper chromatography of the distilled alkaloids and the crude alkaloids,

on paper buffered to pH 6 was carried out and all the bases present in the crude material appeared to be present in the distilled material. The residue in the still was treated similarly, to isolate its basic content and paper chromatography revealed that very little alkaloid material was present in it.

(b) Preliminary separation of "chloroform soluble" alkaloids from "buffer (pH 7.0) soluble" alkaloids.

The distilled alkaloid mixture (40g) was partitioned repeatedly between chloroform and a buffer of pH 7.0, using the extraction pattern employed by Anet and Khan previously (13). The chloroform fractions were combined and worked up to yield 12.0g of alkaloid material. Considerable amounts of fairly pure annotinine crystallised from this fraction from a methanolic solution. The mother liquors from this were used later for the isolation of lycodine.

(c) Countercurrent distribution of "chloroform soluble" alkaloids with the system chloroform-buffer pH 5.2.

A "chloroform soluble" alkaloid fraction (18.0g) similar to one in (b) above, but from a batch of undistilled material, was available to us from previous work in this department (13). A part of this (6.2g) was dissolved in chloroform (120ml) and placed in the first three tubes of a sixty tube Craig countercurrent distribution apparatus (41),

and partitioned between chloroform and citrate-phosphate buffer of pH 5.2, using fifty seven transfers. Aliquots (2ml) of each phase from twenty representative tubes were withdrawn and the alkaloid from each tube was isolated and weighed. The weight distribution curve, so obtained, is shown in figure 4. The pattern of distribution was also studied by paper chromatography (pH 6.0), and ultraviolet spectrophotometry. From these studies it was learnt that lycodine was present in tubes 19-36 with a maximum in tubes between 28-31. The contents of the tubes 19-36 were mixed together and the alkaloid material (1.3g) from these was isolated. Tubes 0-18 contained mainly annotinine and tubes 33-45 contained mainly lycopodine. The tubes 45-59 contained a mixture of several alkaloids (1.6g) in small quantities and were not investigated further.

(d) Acetylation of the lycodine fraction.

Paper chromatography of the alkaloid mixture obtained from tubes 19-36, on paper buffered to pH 7.0, revealed that lycodine (Rf 0.85) was present along with lycopodine (Rf 0.55) and small amounts of annotinine (0.8). The mixture (1.3g) was acetylated by refluxing with an excess of acetic anhydride (4ml), under nitrogen for two hours (1). The solution was cooled and excess methanol (20ml) was added to react with the remaining acetic anhydride. The methyl acetate formed and the methanol

were removed under reduced pressure. The residue was acidified with 5% hydrochloric acid and washed with ether. The washed acid extract was made basic with aqueous sodium hydroxide and extracted with ether to obtain the mixture of N-acetyl lycodine and other alkaloids. Paper chromatography, using paper buffered at pH 3.0 revealed that all the lycodine (Rf 0.35 at pH 3.0) had been converted to N-acetyl lycodine (Rf 0.65).

(e) Separation of N-acetyl lycodine.

(i) Preliminary experiments leading to a choice of conditions. Two experiments were carried out separately in the following manner. The mixture (5mg) containing N-acetyl lycodine was in each case dissolved in chloroform (3ml) and the chloroform solution was washed with a citrate-phosphate buffer (3ml) of pH 3.0 in one case and pH 4.0 in the second experiment. The ultraviolet spectra of both fractions from each of the above experiments were measured. It was inferred from the absorption ratios, that N-acetyl lycodine had a partition coefficient of 15 ($CHCl_3$: buffer pH 3.0) and greater than 20 ($CHCl_3$: buffer pH 4.0) respectively. By means of paper chromatography it was found that the chloroform extract washed with buffer pH 3.0 contained purer N-acetyl lycodine than in the latter case.

(ii) In view of the above results, the

bulk of the acetylated mixture was dissolved in chloroform and partitioned between chloroform and buffer of pH 3.0 three times on a countercurrent basis. The base was isolated from the combined chloroform fractions and subjected to evaporative distillation (120-125°/0.02mm) to give a colorless viscous liquid (0.22g). The infrared spectrum of the compound showed a band at 1661cm^{-1} and paper chromatography on paper buffered at pH 3.0 showed a single spot at Rf 0.65 in agreement with properties reported for N-acetyl lycodine. The ultraviolet spectra of the compound and its salt were similar to the reported spectra of lycodine and its salt (1).

(f) Hydrolysis of N-acetyl lycodine.

N-acetyl lycodine was refluxed with hydrochloric acid (1:1) under nitrogen for six hours. The solution was cooled and made basic with aqueous sodium hydroxide and extracted with ether. The ether extract was washed with water dried over anhydrous sodium sulfate and evaporated to dryness. The residue was submitted to evaporative distillation (80-90°/0.02mm). The colorless distillate was crystallised from ether-petroleum ether to obtain colorless prisms (0.15g). Recrystallisation from the same solvent mixture gave prisms of lycodine (0.12g), m.p. 118°. The infrared spectrum, the ultraviolet spectrum (λ max. 268.2, 275.9) and the paper chromatographic

behaviour of the alkaloid were identical to that reported by Anet and Eves (1). The NMR spectrum of lycodine was measured at 60 Mc/s and is shown in figure 5. The mass spectrum of lycodine was determined later in the course of this work and from this the molecular weight of lycodine was found to be 242.

Improved separation of lycodine.

With a second lot of material, the following method of isolation was used successfully. The "chloroform soluble" alkaloid mixture, obtained in (b) above, was acetylated directly omitting the countercurrent distribution with the Craig apparatus. The mixture, now containing a comparatively much smaller proportion of N-acetyl lycodine, was partitioned between a buffer of pH 3.0 and benzene or warm petroleum ether (b.p. 60-80°). The petroleum ether fraction gave relatively pure N-acetyl lycodine. Evaporative distillation, followed by hydrolysis gave pure lycodine. This method was much quicker although the recovery of lycodine might have been slightly lower than in the more elaborate method used above. A total of 0.32g of lycodine was isolated during the later part of this work by this procedure.

N-Acetyl lycodine.

This was prepared from pure lycodine by the

method already mentioned (1). The NMR spectrum of N-acetyl lycodine is shown in figure 6.

Selenium dioxide oxidation of lycodine.

Several small scale experiments to determine suitable conditions, were carried out initially in each of which lycodine (2-3mg) and selenium dioxide (2-6mg) in a solvent (2ml. dioxane, ethanol, or n-amyl alcohol) were refluxed for varying durations. Examination of the reaction product by means of infrared, ultraviolet and paper chromatographic methods indicated that lycodine had remained largely unchanged. The precipitated selenium was also very little. Experiments in which more vigorous conditions were used, are described below.

(a) Lycodine (5mg) and resublimed selenium dioxide (10mg) in xylene (2ml) were refluxed under nitrogen for six hours. The selenium was filtered off and the solution was washed with a small amount of 4N sodium hydroxide. The solvent was removed and the residue was distilled (100-110°/0.02mm) to obtain a viscous liquid (3mg). Paper chromatography (pH 3.0) of the product revealed a new spot (Rf 0.83) apart from that due to unchanged lycodine (Rf 0.39). The infrared spectrum of the mixture in carbon tetrachloride showed a somewhat wide, and intense band at 1725cm^{-1}. The product was separated from

unchanged lycodine, by partitioning the crude product between chloroform and buffer of pH 4.0 and distilling the chloroform fraction. The ultraviolet spectrum of the distillate showed an absorption maximum at 272 mμ and more intense absorption at wavelengths lower than 250 mμ.

(b) Lycodine (10mg) and selenium dioxide (20mg) in dioxane (3ml) containing about 5% water, were heated in a sealed tube at 160° for fifteen hours. The contents of the tube were cooled and worked up as in (i) to obtain 4mg of an oily distillate. The infrared spectrum in carbon tetrachloride solution showed two bands at 1725cm^{-1} and 1700cm^{-1}. Paper chromatography (pH 3.0) showed the presence of two new compounds (Rf 0.53 and 0.83) along with a trace of lycodine (Rf 0.35). The product having Rf 0.53 was formed in larger proportion than the one having Rf 0.83. The latter, being weakly basic, was separated as in (i) and it appeared to be identical with the product obtained in (i). The product having Rf of 0.53 sublimed (0.02mm) slower than lycodine and thus was separated and obtained crystalline (ca 2mg). The infrared spectrum of the compound in carbon tetrachloride showed an intense sharp band at 1700cm^{-1} indicative of a ketone group. The ultraviolet spectrum showed absorption at λmax 280 mμ, changed in acid to λ max 276.5 with slightly diminished intensity. Further experiments were not carried out as the yields were not encouraging.

Selenium dioxide oxidation of 5,6,7,8-tetrahydroquinoline.

5,6,7,8-Tetrahydroquinoline (50mg) and sublimed selenium dioxide (50mg) in dioxane (10ml: 95%) were refluxed under nitrogen for four hours. At the end of this period, the mixture was cooled and the crude material was worked up for basic products as above, to obtain 15mg of a colorless distillate. Chromatography on paper buffered at pH 3.0 revealed two new compounds (Rf 0.25 and 0.88) in addition to unchanged tetrahydroquinoline (Rf 0.45). The infrared spectrum of the mixture in chloroform solution showed a band at 1700cm^{-1} which was not present in the spectrum of tetrahydroquinoline. The ultraviolet spectrum of the mixture showed an inflexion due to absorption between 280-285 mμ in addition to the bands at 268 mμ and 255 mμ due to tetrahydroquinoline. Attempts to separate the product (Rf 0.88) from the rest, were not successful.

Oxidation of lycodine with potassium permanganate.

Lycodine (3mg) in acetone (0.1ml) was mixed with a solution of potassium permanganate (6mg) in water (3ml) and shaken at room temperature for fifteen minutes. Methanol (1ml) was added and the mixture was taken to dryness. Extraction with methanol followed by evaporative distillation (0.02mm) gave a colorless distillate (ca 1mg). Paper chromatography (pH 3.0), showed the presence of two products (Rf 0.55 and 0.83) and the absence of lycodine.

The infrared spectrum of the mixture in carbon tetrachloride showed bands at 1725cm^{-1} and 1695cm^{-1}. In view of the formation of a mixture in low yield this was not investigated further.

Deuterium exchange experiments on 2,3-lutidine.

In each of the following experiments the NMR spectrum of the product was compared with that of a control solution of 2,3-lutidine. In the NMR spectrum of 2,3-lutidine, the resonance due to the 2-methyl group occurred approximately at 7.6 τ and that due to the 3-methyl group occurred at 7.8 τ. The intensities of the two bands were equal.

(a) 2,3-lutidine (60mg), pyridine (350mg) and sodium deuteroxide (NaOD) in heavy water, obtained from sodium (55mg) and heavy water (1ml), were heated together in a sealed tube at water bath temperature for one hour. the NMR spectrum showed that the intensities of the two methyl bands were equal.

(b) 2,3-lutidine (46mg) and potassium-t-butoxide in deuterated t-butanol (910mg) were heated together in a sealed tube for two hours. the NMR spectrum showed diminished intensity for the band at lower (7.6 τ) field as compared with the band at 7.8 τ.

After prolonged heating (15 hours) at 190° in a furnace the methyl band at 7.6 τ showed greatly diminished

intensity (approximately 1:15) compared to the band at 7.8 τ.

Dehydrogenation of lycodine.

(a) Preliminary experiments leading to a choice of conditions.

In the following experiments lycodine, a dehydrogenation catalyst and an aromatic solvent were heated together at reflux for varying durations. The catalyst was filtered off and washed with ether. The filtrate and the ether washings were combined together and extracted exhaustively with dilute acid. The combined acid extract was washed with ether, made basic with aqueous sodium hydroxide and extracted with chloroform. The chloroform extractive was submitted to evaporative distillation. The distillate was examined by means of paper chromatography. Ultraviolet spectra were studied in cases where there was evidence of dehydrogenation.

(i) Lycodine (3mg) and palladium-charcoal catalyst (5%, 3mg), in p-cymene (1.5ml) were heated together at reflux temperature under nitrogen for two hours. The reaction product was isolated as mentioned above. Paper chromatography (pH 3.0) and ultraviolet spectrum indicated that the product was largely unchanged lycodine.

When the refluxing was carried out for six to nine hours, the product showed on paper chromatography mainly unchanged lycodine along with two new compounds in

small amounts.

(ii) Dehydrogenation of lycodine with palladium-charcoal catalyst in boiling naphthalene in the same way as in (i) resulted in a slightly greater proportion of the product to lycodine.

(iii) In an attempt to attain a higher temperature of dehydrogenation, diphenyl ether was used as the solvent. Lycodine (3mg) was dehydrogenated with palladium-charcoal catalyst (3mg) in diphenyl ether (1.5ml), by refluxing under nitrogen for ten hours. The product was isolated as usual. Paper chromatography of the distilled product on paper buffered at pH 3.0 showed the presence of two spots (Rf 0.75 and 0.86) and a small spot due to unchanged lycodine (Rf 0.35).

When the duration of heating was increased to eighteen to twenty hours, there was only a tra ce of unchanged lycodine and after heating for periods over twenty-five hours, there was no unchanged lycodine in the reaction product.

(b) <u>The dehydrogenation products of lycodine</u>.

(i) <u>Preliminary studies</u>:

The ultraviolet spectrum of the crude product obtained as in (a.iii) above showed λ max 270 mμ (s), 230 mμ (v.s.), 343 mμ (w) and 325 mμ (w). Weak inflexions at 275 mμ and 268 mμ were also present probably due to

traces of lycodine. The product was freed from lycodine by washing a chloroform solution of the mixture with a buffer of pH 4.0 counter-currentwise. Paper chromatography (pH 3.0) of the purified products showed two spots, at Rf 0.75 and 0.86. The ultraviolet spectrum showed absorption at λmax 230 mμ (v.s.), 270 mμ (s), 325 mμ (w), 343 mμ (w) and λmin 248 mμ. The crude mixture gave no color with ferrous sulfate in the "ferroin test" for 1,10-phenanthrolines. The mixture of dehydrogenation products was separated, by preparative paper chromatography on Whatman No. 3MM paper at pH 3.0, into two fractions, one having Rf 0.86 and a second one having Rf 0.75. The ultraviolet spectrum of the fraction having Rf 0.86 showed the main band absorption at λmax 271.5 mμ. In acid (HCl) the absorption was slightly enhanced and was shifted to longer wavelength at 276.5 mμ. The ultraviolet spectrum of the second fraction (Rf 0.75) was similar to the first one in shape but showed the main band at λmax 269 mμ intensified and shifted in acid to 275.5 mμ. These spectra were closely similar to the spectra of 1,7-phenanthroline and its salts, published by Krumholz (42), both in shape and magnitude of intensities, but differed slightly in the positions of the maxima.

In a large scale experiment, lycodine (53mg) was dehydrogenated in diphenyl ether for twenty hours as in (a.iii) and the products (19mg) were separated by preparative

paper chromatography as above. The ultraviolet spectra were measured. The fraction of Rf 0.86 now showed the main band absorption at λmax 271 mμ and the second fraction (Rf 0.75) showed the main band at 267 mμ.

(ii) Improved paper chromatographic separation of dehydrogenation products.

The mixture of dehydrogenation products was applied on an unbuffered Whatman No. 1 paper and developed by butanol: water: HCl mixture (80ml of butanol saturated with water + 20ml of conc. HCl forming a single phase). The spots were revealed as usual by spraying with a modified Dragendorff reagent (38). There appeared four different spots due to the dehydrogenation products (Rf 0.52, 0.40, 0.28 and 0.19). The fraction having Rf 0.52 was in predominant amount and will be designated as "dehydrogenation product A" in the following pages. The second major product designated dehydrogenation product B, was the one with Rf 0.19. The compounds with Rf 0.40 and 0.28 were present only in small amounts. Both 1,7-phenanthroline and 4,7-phenanthroline run on the same paper showed Rf value of 0.18, and methyl-1,7-phenanthrolines had Rf values less than 0.30.

Dehydrogenation products A and B were isolated in small amounts by preparative paper chromatography on Whatman No. 3MM (thick) paper using the single phase system butanol, water and hydrochloric acid. These were freed from

non-basic impurities from the paper, by washing an acid solution with chloroform followed by evaporative distillation. Both A and B were obtained as oily liquids (ca 2mg each). A small quantity of B was also obtained as crystalline prisms, m.p. 138-140°.

The ultraviolet spectrum of dehydrogenation product A showed absorption at λmax 234 mμ (v.s.) 271.5 mμ (s), 326 mμ (w) and 342 mμ (w). In 0.1N HCl the spectrum showed intensification of the main band with a shift to 276 mμ from 271 mμ. Also, the changes in the spectrum of the compound in solutions of various acidities were indicative of the presence of two protonated species. The shapes, relative intensities of the bands and the spectral behaviour of the compound in acid were reminiscent of the reported spectra of 1,7-phenanthroline and its salts (42), shown in figure 24. The maxima of absorption were similar to those in the spectrum of 5-methyl-1,7-phenanthroline one of the model compounds prepared by us (cf. Part II, table VIII). The ultraviolet spectrum of dehydrogenation product B was very similar to that of A but the maximum position of the main band was at 266 mμ instead of 271 mμ. The maxima in the spectrum of B did not correspond to the maxima of any of the simpler alkyl-1,7-phenanthrolines prepared as model compounds (cf. Part II).

(iii) Chromatography of the crude dehydrogenation products on alumina.

The mixture of dehydrogenation products obtained as in (a.iii) was dissolved in petroleum ether containing a little chloroform and adsorbed on an alumina column. The column was eluted with a 3:7 mixture of chloroform-petroleum ether (b.p. 30-60°). Twenty-eight fractions of 2ml each were collected. The solvent from these fractions was removed and the elution pattern was followed by paper chromatography and ultraviolet spectra. The results are shown in table I.

(c) Isolation of dehydrogenation product A.

Two lots of lycodine were dehydrogenated in each of which lycodine (60mg), palladium-charcoal (80mg) and diphenyl ether (10ml) were refluxed for twenty hours. The crude product was freed from unchanged lycodine as usual and distilled (60-80°/0.02mm). The distillate (45mg) was chromatographed on alumina using a 1:9 mixture of redistilled chloroform-petroleum ether (b.p. 30-60°) for elution. Thirty one fractions, of 3ml each, were collected. The ultraviolet spectra of the fractions were measured. The eluate was divided into three main fractions; (1) fractions showing λ max 270.5-272 mμ were pooled together (Fraction I), (2) those showing λ max 269-270.5 mμ (Fraction II) and (3) the remaining fractions showing absorption (Fraction III).

Fraction No.	Rf values of the constituents. Main constituent underlined			λmax mμ
9	-	-	-	( )
10	-	0.60	-	272.5+
11	0.51	<u>0.60</u>	-	272.5
12	<u>0.52</u>	( )	-	271.5
13	<u>0.51</u>	-	-	271
14	<u>0.51</u>	0.40		271
15	<u>0.52</u>	0.40		270.5
16	<u>0.52</u>	<u>0.40</u>		270.5
17	0.52	0.40	<u>0.19</u>	269
18		( )	<u>0.19</u>	268.5
19		( )	<u>0.19</u>	268.5
20		( )	0.19	268
21	-	(-)	(-)	( )
D. Product "A"	0.52			
D. Product "B"			0.19	
Others		0.40	0.30	

Table I. Elution pattern obtained in the alumina chromatography of crude dehydrogenation products.

Fraction I was submitted to evaporative distillation (70-80°/0.02mm) and a colorless oil (24mg) was obtained. The NMR spectrum of the substance was very similar to that of 5-methyl-1,7-phenanthroline in the region 0-3.0 τ (see figure 18, and table VI, Part II). The high field part of the spectrum of Fraction I showed doublets at 7.06 τ and 8.98 τ. Several other peaks between 7.8-8.8 τ were also present but these were found to be due to impurities, as these were eliminated upon purification. Fraction I was dissolved in ether petroleum-ether (1ml) and cooled by immersion in a dry ice-acetone bath, when a small amount of a waxy material separated. The clear solution was quickly decanted, concentrated and cooled again. Colorless crystals were deposited, which were quickly freed from the mother liquor. Recrystallisation from ether-petroleum ether was affected more readily by cooling in a refrigerator. The colorless prisms (12mg) of dehydrogenation product A, so obtained, melted at 80-82°. The NMR spectrum of dehydrogenation product A is shown in figure 9. The low field (0-3.0 τ) region of the NRM spectrum was very similar to that of 5-methyl-1,7-phenanthroline and different from that of 6-methyl-1,7-phenanthroline (Fig. 18, Part II). The high field part of the spectrum, consisting of a doublet (7.07 τ) of relative area of two protons, a barely visible multiplet (nine visible lines centred at 8.01 τ seen at faster

sweep rate) of relative area of one proton and an intense doublet (8.96 τ) of relative area of six protons, showed similarity with the high field spectrum of isobutylbenzene (cf. Part II). The NMR and ultraviolet spectra of dehydrogenation product A were identical with those of 5-isobutyl-1,7-phenanthroline prepared subsequently (cf. Part II; figure 21 and tables VI and VIII). The compound showed no depression of melting point upon admixture with 5-isobutyl-1,7-phenanthroline. The infrared spectrum of dehydrogenation product A (figure 10) showed all the bands present in that of 5-isobutyl-1,7-phenanthroline (figure 25).

The NMR spectra of Fractions II and III were measured. The spectrum of Fraction II showed, in addition to all the peaks shown by Fraction I, several small peaks in the aromatic region. Fraction III contained very small amount of material and hence was not investigated in detail.

Conversion of lycopodine into lycodine.

(a) Preparation of β-cyanobromolycopodine (20).

A solution of lycopodine (3g) in dry benzene (30ml) was added gradually with stirring to a solution of cyanogen bromide (10g) in dry benzene (30ml). The mixture was allowed to stand overnight. The solvent and excess cyanogen bromide were removed under reduced pressure. The residue was dissolved in chloroform, washed with dilute acid, water and dilute aqueous sodium bicarbonate. The

chloroform solution was concentrated and adsorbed on a column of alumina. The column was eluted with chloroform and the first eluate was worked up for α- and β-cyanobromolycopodine. The two compounds were separated and obtained pure by fractional crystallisation following the procedure reported by previous workers (20). The major product α-cyanobromolycopodine, melted at 140-141°; β-cyanobromolycodine, m.p. 106-108°, was obtained in much smaller amount. About 0.2g of β-cyanobromolycopodine was obtained pure, by recrystallisation from ether containing a little chloroform, for use in the subsequent steps. The infrared spectrum of the compound in carbon tetrachloride showed bands at 2200cm^{-1} (N-CN) and 1700cm^{-1} (C=O).

(b) Reaction of β-cyanobromolycopodine with sodium azide in acetone.

β-cyanobromolycopodine (80mg) in acetone (3ml) and excess sodium azide (50mg) were refluxed for twenty two hours. The solvent was removed under reduced pressure. The residue was dissolved in carbon tetrachloride, and the solution was washed with water, dried over anhydrous sodium sulfate and evaporated to remove the solvent. β-cyanoazidolycopodine was obtained as an oily liquid. The aqueous washings gave a positive test for bromide ion. The infrared spectrum of the oily liquid in carbon tetrachloride showed bands at 2200cm^{-1} (-N-CN), 2095 ($-N_3$) and 1700 (C=O).

(c) Catalytic reduction of β-cyanoazidolycopodine.

β-cyanoazidolycopodine (50mg) in ethanol (5ml) containing a little HCl, was shaken with hydrogen in the presence of palladium-charcoal catalyst (20mg, 5%) for eight hours. The catalyst was filtered off and the ethanol was removed under reduced pressure. The residue was dissolved in dilute hydrochloric acid, washed with chloroform, made alkaline and extracted exhaustively with chloroform. The chloroform extract was dried with anhydrous sodium sulfate and evaporated to yield an oily base (35mg). The infrared spectrum of the base in chloroform showed strong absorption at 2195cm^{-1} (N-CN), 1690cm^{-1} (C=O) and a weak absorption at 3390cm^{-1} and 1605cm^{-1}, but no absorption at 2095cm^{-1} (azide). The product was crystallised from benzene-methanol, to give a crystalline material m.p. 150-155°. The infrared spectrum of the crystalline material in chloroform solution showed an intense band at 2195cm^{-1}, a less intense band at 1690cm^{-1} and a weaker band at 1650cm^{-1}. The crystalline material was subjected to evaporative distillation (90-100°/0.02mm). The colorless distillate partially crystallised. The infrared spectrum of the sublimed compound in carbon tetrachloride showed an intense band at 2200cm^{-1}, a weaker band at 1660cm^{-1} and extremely weak band at 1700cm^{-1}. The ultraviolet spectrum showed no pyridine absorption in the region 250-280 mμ. As small quantities

of the compound were available, and also as the compound was not very stable, no analysis was carried out. The sublimed material was directly used in the next step.

(d) Dehydrogenation with palladium-charcoal in p-cymene.

The sublimed base (50mg) obtained as in (c) above was dissolved in p-cymene (3ml). Palladium-charcoal catalyst (20mg) was added and the mixture was refluxed for one and a half hours. The catalyst was filtered off and washed with a small amount of benzene. The combined filtrate was extracted with dilute hydrochloric acid. The acidic extract was washed with ether, made basic and extracted with chloroform exhaustively. The combined chloroform extract was dried over anhydrous sodium sulfate and taken to dryness. The infrared spectrum of the oily residue showed a band at 2200cm^{-1}. The ultraviolet spectrum showed the pyridine-like chromophore characteristic of lycodine at λmax 268 mμ and 276 mμ. The substance was sparingly soluble in ether and carbon tetrachloride, unlike lycodine.

(e) Hydrolysis of N-cyanolycodine.

The crude compound (22mg) obtained in (d) was hydrolysed by heating it with 1:1 hydrochloric acid (3ml) for six hours. The solution was cooled, washed with ether, made alkaline and extracted with redistilled ether. The ether extract was dried over anhydrous sodium sulfate and

taken to dryness. Paper chromatography, on paper buffered at pH 3, showed the presence of a spot with Rf value 0.36 identical to that of lycodine (Rf 0.36). Crystallisation from ether-pentane gave prisms (12mg) of a compound, m.p. 118°. The m.p. of the compound was not depressed upon admixture with an authentic sample of lycodine (m.p. 118°) isolated from L. annotinum L. (1). The infrared and ultra-violet spectra of the two bases were identical.

Miscellaneous experiments.

(1) Reaction of α-cyanobromolycopodine with potassium hydroxide (20).

α-cyanobromolycopodine (0.45g) was refluxed (6 hrs) with potassium hydroxide (1g) in methanol (15ml). The solvent was removed under reduced pressure and the residue was diluted with water causing the separation of a solid. The mixture was extracted with ether and the extract was washed with dilute hydrochloric acid, followed by water and dried over sodium sulfate. Concentration of the ether solution yielded a crystalline solid, m.p. 143-144°. Lit. m.p. 143-144°. The compound gave a negative Beilstein halogen test and depressed the melting point of the starting material. The infrared spectrum of the compound in carbon tetrachloride showed a band at 1415cm^{-1} ($-CH_2-CO$) which was also present in the spectra of lycopodine and α-cyanobromolycopodine.

(2) Attempts to prepare lycopodine from anhydrodihydrolycopodine (L-14).

Preparation of anhydrodihydrolycopodine (21).

Dihydrolycopodine (200mg), prepared according to the procedure of Maclean, Manske and Marion (20), was dissolved in dry benzene (15ml) and purified thionyl chloride (1.5ml) was added. The whole was refluxed for two hours. The solution was cooled and water was added to react with the excess thionyl chloride. The mixture was extracted with dilute hydrochlorid acid. The acid extract was made alkaline and was extracted with ether. Drying, followed by removal of ether, gave an oily base, which was obtained colorless by evaporative distillation. The perchlorate of the base melted at 238°. Lit. m.p. 238° (21). The infrared spectrum of anhydrodihydrolycopodine in carbon tetrachloride showed a weak band at 1660cm^{-1}, but no hydroxyl absorption. The NMR spectrum of anhydrodihydrolycopodine in carbon tetrachloride showed a poorly resolved quartet at 4.66 τ attributable to a single olefinic proton.

(a) Attempted Brown hydration (43, 44) of anhydrodihydrolycopodine.

(1) A solution of lithium aluminum hydride (60mg) in anhydrous ether (3ml) was added dropwise to a solution containing anhydrodihydrolycopodine (60mg) and boron trifluoride etherate (1ml) in ether (7ml), under

nitrogen. After one and a half hours at room temperature, the mixture was treated with saturated sodium sulfate solution followed by solid sodium sulfate and solid sodium hydroxide. The mixture was extracted with chloroform. The residue from the chloroform extract was boiled with hydrogen peroxide (1.7ml 30%) and sodium hydroxide (0.13g) in ethanol (5ml, 90%) for fifteen minutes. The mixture was taken to dryness, and aqueous sodium hydroxide was added. The whole was extracted with chloroform; the extract was dried and the chloroform was removed. The liquid residue was submitted to evaporative distillation. The infrared spectrum of the liquid distillate was similar to that of anhydrodihydrolycopodine. The recovered anhydrodihydrolycopodine accounted for the majority of the starting material.

(ii) Diborane generated from lithium aluminum hydride (230mg in 30ml of ether) and boron trifluoride etherate (1.2ml in 5ml of ether) was passed into a solution of anhydrodihydrolycopodine (100mg) in ether (3ml). After standing (1 hour) the latter solution was worked up for the product and oxidised as in (i) by boiling with alkaline hydrogen peroxide for half an hour. The product was worked up as in (i). A small amount of crystalline material was obtained from an ether solution on cooling. The infrared spectrum of the compound in carbon tetrachloride solution showed a sharp band at 3650cm^{-1} (probably free hydroxyl)

and a broad wide band between 3400-3200cm^{-1} the pattern of which was not affected by dilution. The residue from the mother liquor showed an infrared spectrum quite similar to that of anhydrodihydrolycopodine.

(b) Oxidation of anhydrodihydrolycopodine with osmium tetroxide.

The conditions used by Pinder et al (45) for the oxidation of tropidine were adapted for this reaction. To a solution of osmium tetroxide (200mg) in dry ether (5ml) was added during ten minutes, with shaking and ice cooling a solution of anhydrodihydrolycopodine (175mg) in ether (5ml). The solution was set aside for two days after which the dark precipitate was separated and refluxed (30 min.) with a solution of sodium sulfite (2g) in water (25ml). The cooled solution was saturated with potassium carbonate and extracted continuously with ether for twenty four hours. The extract was dried and evaporated to yield a viscous material (100mg). This was submitted to evaporative distillation to yield a glassy material (65mg). The infrared spectrum (CCl_4) showed a sharp band at 3600cm^{-1}, a broad band between 3500-3300cm^{-1}, and an intense band at 1725cm^{-1}. Paper chromatography (pH 3.0) revealed that the product was a mixture of at least three compounds. Heating the material with H_2SO_4 (20%) caused the appearance of a band at 1700cm^{-1} in the infrared spectrum (CCl_4) of

the product, and of a spot with Rf value equal to that of lycopodine in the paper chromatogram of the product. No further investigations were carried out due to lack of starting material.

(c) Oxidation of anhydrodihydrolycopodine with potassium permanganate.

(i) Anhydrodihydrolycopodine (100mg) was emulsified with water (10ml). A solution of potassium permanganate (350mg) in water (25ml) was added gradually (10 min.) with stirring. The solution was stirred for a further ten minutes and methanol (2 ml) was added. The mixture was treated with sodium hydroxide and extracted exhaustively with chloroform. The extract was dried and the solvent was removed. The residue, upon recrystallisation from ether gave colorless crystals (40mg). This substance was insoluble in acid. The infrared spectrum in nujol mull showed bands at 3550cm^{-1} (sharp), 3320cm^{-1} (wide) and at 1600cm^{-1}. The band at 1600cm^{-1} was also somewhat broad, but intense.

(ii) Reduction with lithium aluminum hydride.

The crystalline compound from (d) was suspended in ether (5ml) and a solution of lithium aluminum hydride (100mg) in ether (5ml) was added slowly and the mixture was refluxed for two hours. Aqueous methanol was added and the resultant mixture was worked up for basic material. The infrared spectrum (nujol) of the product showed bands

in the hydroxyl region but no bands at 1600cm^{-1}. The product was soluble in acid.

(iii) The product from (e) was heated with sulfuric acid (65%) for two hours under nitrogen. The solution was cooled, diluted with water, made basic and extracted with chloroform. The extract was dried and the solvent was removed. The residue was subjected to evaporative distillation (80-100°/0.02mm). The infrared spectrum (CCl_4) of the distillate showed a band at 1700cm^{-1} which was also present in the spectrum of lycopodine (C=O). Paper chromatography (pH 4.0) revealed the presence, among others, of a compound with an Rf value identical to that of lycopodine. Further investigation of this conversion could not be carried out due to lack of material.

DISCUSSION

Isolation of lycodine.

Lycodine is present only in small amounts in the crude bases of L. annotinum L. Anet and Eves employed successive countercurrent distribution and chromatography for the isolation of lycodine. During the earlier part of the present work, preliminary handling of the crude alkaloid mixture was carried out essentially according to the procedure of Anet and Eves (1). The crude alkaloids remaining after the crystallisation of annotinine, were partitioned between chloroform and buffer pH 7.0 (13) and divided into three fractions (1) "chloroform soluble" alkaloids (2) "Buffer soluble" alkaloids and (3) Middle fraction containing alkaloids having nearly equal solubility in both phases. A chloroform soluble alkaloid fraction expected to contain lycodine was available to the author from previous work in this laboratory. This was submitted to a second countercurrent distribution in a sixty-tube Craig apparatus (41) with the system chloroform-buffer pH 5.2. The pattern of weight distribution so obtained is shown in figure 4. Further analysis of the contents of the various tubes by paper-chromatography, ultraviolet and infrared spectra revealed the presence of annotinine in tubes 0-15, lycodine in tubes 19-36 and lycopodine in tubes 33-45. The tubes 46-59 contained

mixtures of hitherto uncharacterised bases in minute quantities. Thus lycodine was the only alkaloid of interest in the chloroform fraction, since the other alkaloids in the fraction were either available in abundance from other sources or were present only in trace amounts.

Tubes 19-36 contained mainly lycodine, small amounts of lycopodine and nonalkaloidal matter. Anet and Eves separated lycodine from this, by chromatography on alumina, but lycodine so obtained did not crystallise readily and had to be isolated through picrate formation. Therefore, purification of lycodine in the present instance was carried out by a different method depending on the following considerations.

It had been previously established that lycodine was a diacidic base (pKa 3.97 and 8.08) due to a pyridine ring and an -NH- function. Acetylation of lycodine gave N-acetyl lycodine which contained only one basic nitrogen (pKa 4.95) and was a weaker base than lycodine. This difference in basicities of lycodine and N-acetyllycodine was useful for the separation of lycodine. Although lycodine and lycopodine have nearly the same partition-coefficients in a variety of systems, the weakly basic acetyl lycodine and lycopodine should have different partition coefficients.

Accordingly the mixture from tubes 19-36 was

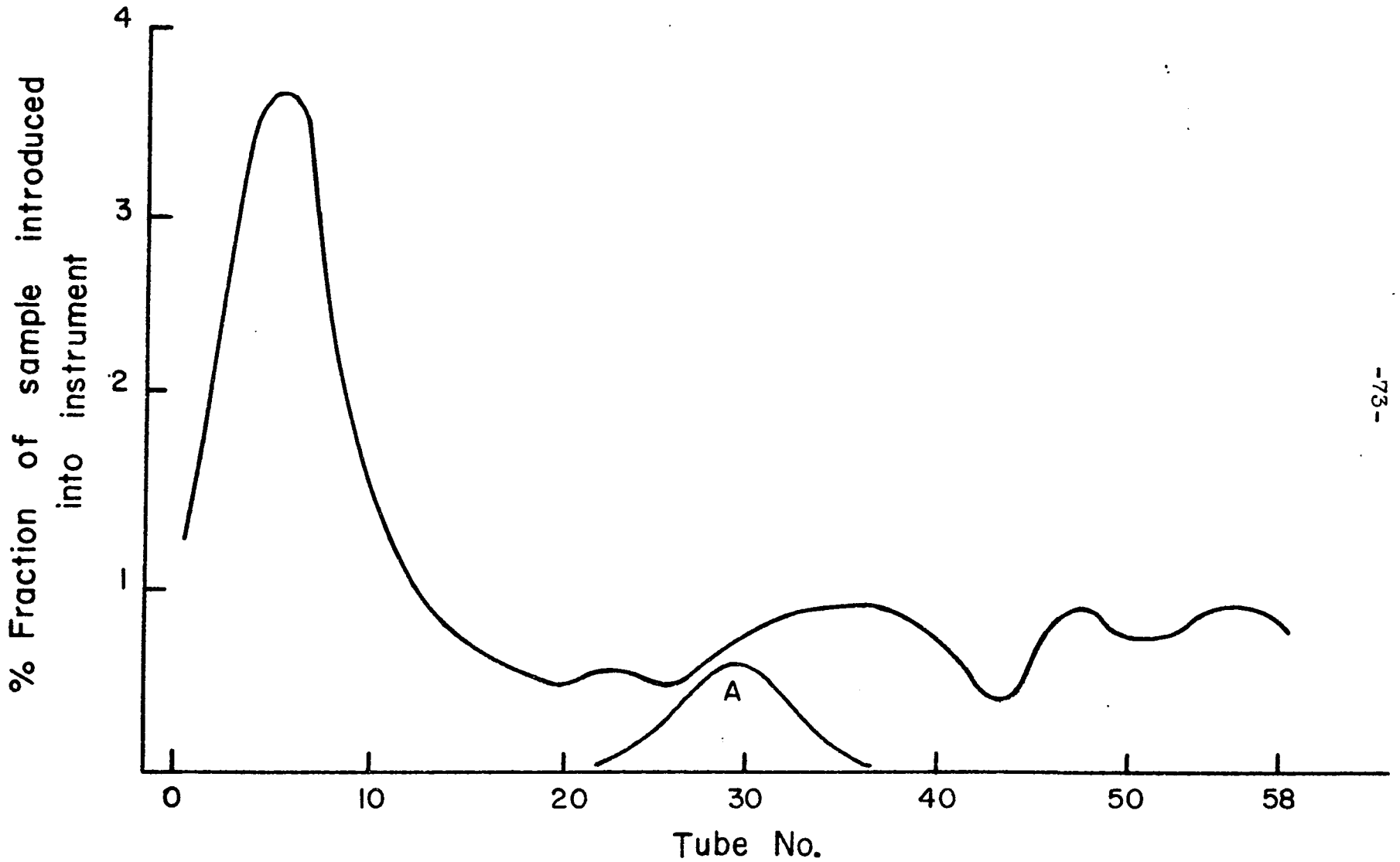


Fig. 4 Countercurrent distribution of "Chloroform soluble" alkaloid fraction with the system Chloroform - buffer pH 5.2 . (A) Location of the Lycodine peak

acetylated to obtain N-acetyl lycodine. From a preliminary experiment it was found that N-acetyl lycodine had a partition coefficient of 20 with the system chloroform-buffer pH 4.0 and of 15 with the system chloroform-buffer pH 3.0. The latter system was preferred because the N-acetyl lycodine obtained in this way was purer. The bulk of the acetylated mixture was similarly partitioned between chloroform and buffer of pH 3.0, thrice on a counter-current basis. Lycopodine and any other strongly basic alkaloids present remained completely in the buffer phase. The residue from the chloroform phase, upon evaporative distillation, furnished N-acetyl lycodine. Lycodine was obtained from this by hydrolysis and evaporative distillation. Lycodine obtained in this way crystallised readily and was found pure by paper chromatography and other physical properties.

Improved isolation of lycodine.

The isolation procedure used by previous workers although efficient and generally applicable, presented the following unsatisfactory features. (1) The crude bases tended to give stable emulsions during the various extraction procedures, especially so with the lycodine containing "chloroform soluble" fraction because the non-alkaloidal impurities invariably persisted in the chloroform phase and caused trouble in the Craig countercurrent

distribution. (2) Lycopodine and lycodine had nearly equal partition coefficients in a variety of systems and necessitated further purification by chromatogrphy even after the Craig counter current distribution. (3) Recovery of lycodine via its dipicrate was not convenient and lycodine obtained did not crystallise readily and (4) The proce dures were time consuming.

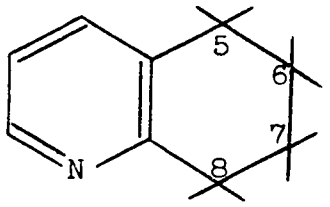
Isolation of lycodine through its acetyl derivative, described already, remedied the difficulties mentioned in (2) and (3) above. The following modifications of the isolation procedure were carried out successfully on a small scale and later adopted for handling the crude material. The crude bases were distilled in a molecular still (80-130°/0.001mm). The distilled material was faintly colored and presented no difficulties during extractions. The distilled mixture was partitioned between chloroform and buffer pH 7.0 according to the procedure of Anet and Khan (13). The "chloroform soluble" fraction so obtained contained all the lycodine, along with mainly annotinine and lycopodine. As already shown, this fraction did not contain any new alkaloids in isolable quantities. Therefore counter current distribution of this in a Craig apparatus did not offer any advantages. It was, therefore, treated directly with acetic anhydride to convert the lycodine present into N-acetyl lycodine. Thc mixture containing N-acetyl lycodine was partitioned between benzene

and a buffer of pH 3.0, on a counter current basis with three transfers. The benzene phase yielded N-acetyl lycodine. In a later experiment warm petroleum ether was substituted for benzene quite successfully. Lycodine was obtained by hydrolysis as usual and it crystallised readily. In this way, the tedious Craig counter current distribution and chromatographic procedures were avoided. A total of 440mg of lycodine in all was isolated for use in this work.

Structure of lycodine.

The NMR spectra of lycodine and its derivatives.

The partial structure (I) of lycodine was suggested by Anet and Eves (1) mainly on physical evidence such as infrared and NMR spectra.



(I)

+

NH not attached to C_5 or C_8

$-CH_3$ attached to quaternary carbon atom

7C + 17H + 2 rings

In the above, the conclusion that the methyl group in lycodine was attached to a quaternary carbon atom arose from NMR spectral evidence consisting of an apparently sharp and unsplit methyl resonance at high field. However the reported spectrum, measured at 40 Mc/s, showed a methyl signal which was too wide to be due to a methyl group on a

quaternary carbon atom. It was therefore thought necessary to re-examine the NMR spectrum of lycodine.

Indeed, when the NMR spectrum of lycodine was measured at 60 Mc/s frequency and under somewhat better resolution (Fig. 5) the methyl band (9.18 τ) appeared as a wide band, which was just discernibly a doublet of spacing 1.9 cps. The spectrum of N-acetyl lycodine (Figs. 6,7) shows the methyl band (9.12 τ) which is unsplit but nevertheless very wide compared to the sharp $-CO-CH_3$ band (7.87 τ). Thus it could be concluded that the methyl group in lycodine was a $-CH-CH_3$ group. Such peculiar effects in the NMR spectra of $-CH-CH_3$ groups in saturated systems have been observed in a number of cases by Anet (46). The methyl band due to such a group can be a doublet, or a doublet with additional lines or in extreme cases a multiplet observable only as a wide band, depending on whether the -CH- proton is additionally coupled to protons with large or small chemical shifts with respect to the -CH- group. Normally a -CH- group is sufficiently chemically shifted from methylene and methyl groups (47) and occurrs at lower field. But in lycodine the -CH- group carrying the methyl group is presumably shifted up-field by the magnetic anisotropy of the pyridine ring (48, 104). It has also been pointed out that in cases where a "poor doublet" is obtained, the spacing is less than the true coupling constant (46). The normal coupling constant of

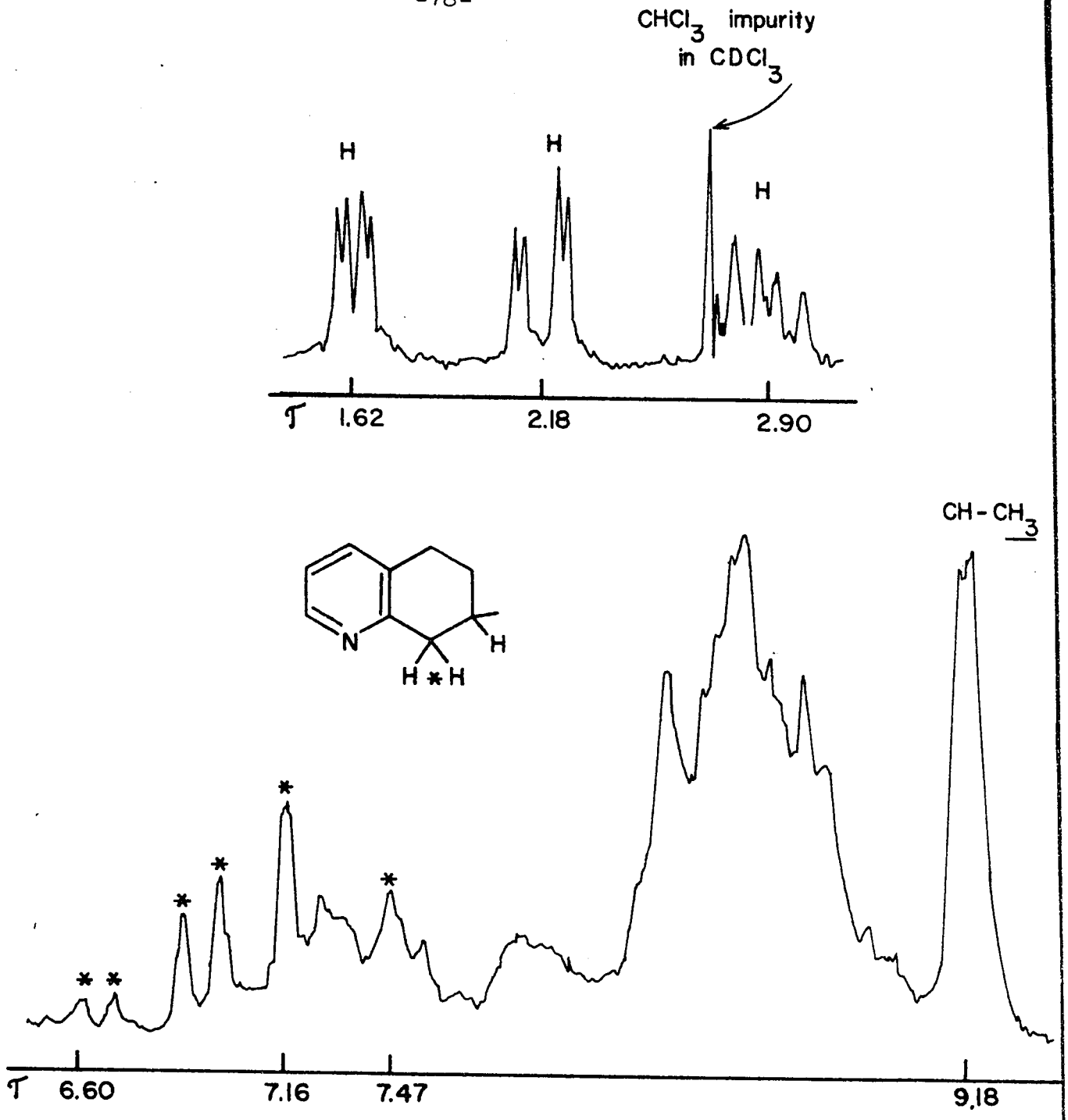


Figure 5. The NMR spectrum of Lycodine in $CDCl_3$

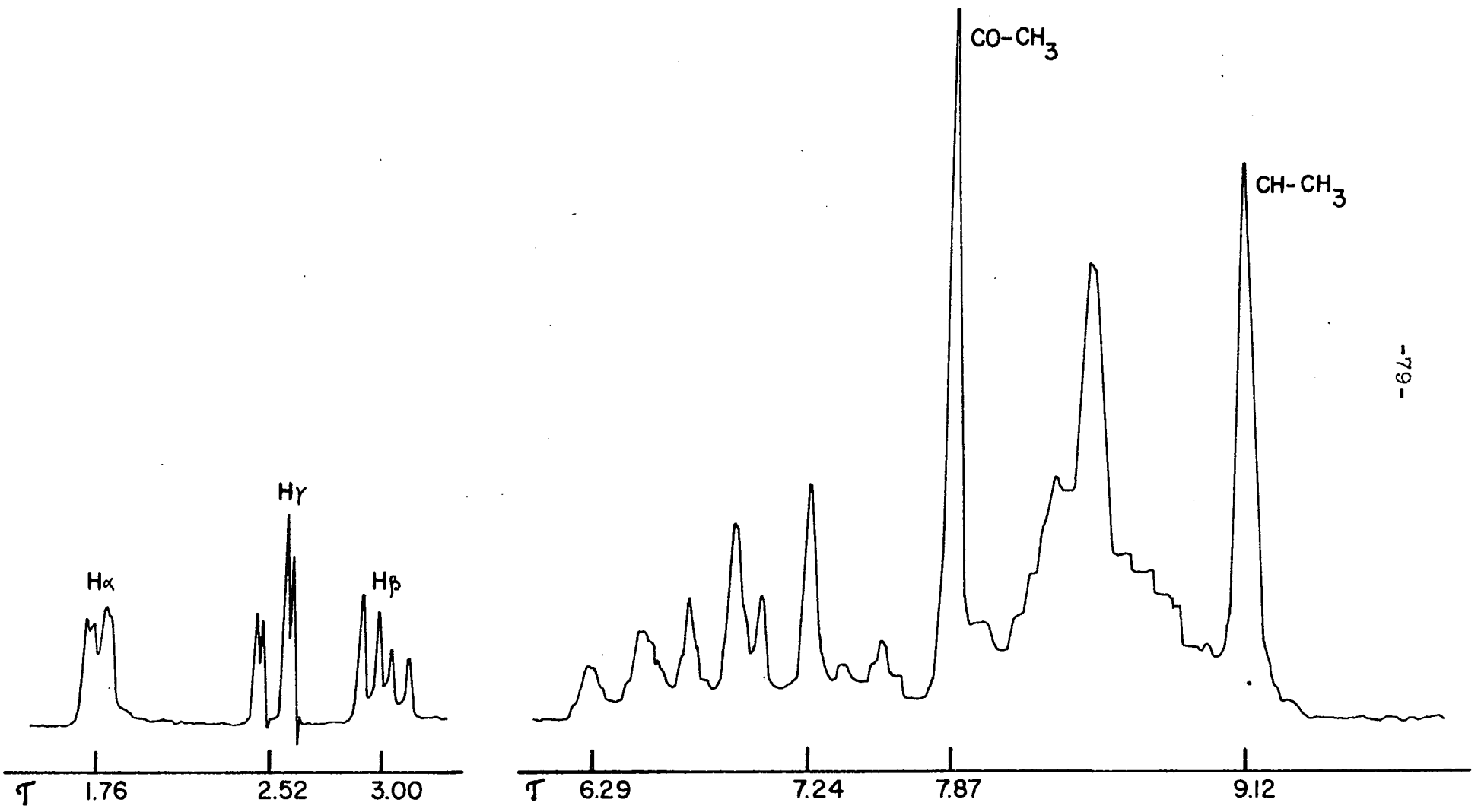


Fig.6. The NMR Spectrum of N-Acetyl Lycodine in Carbon Tetrachloride

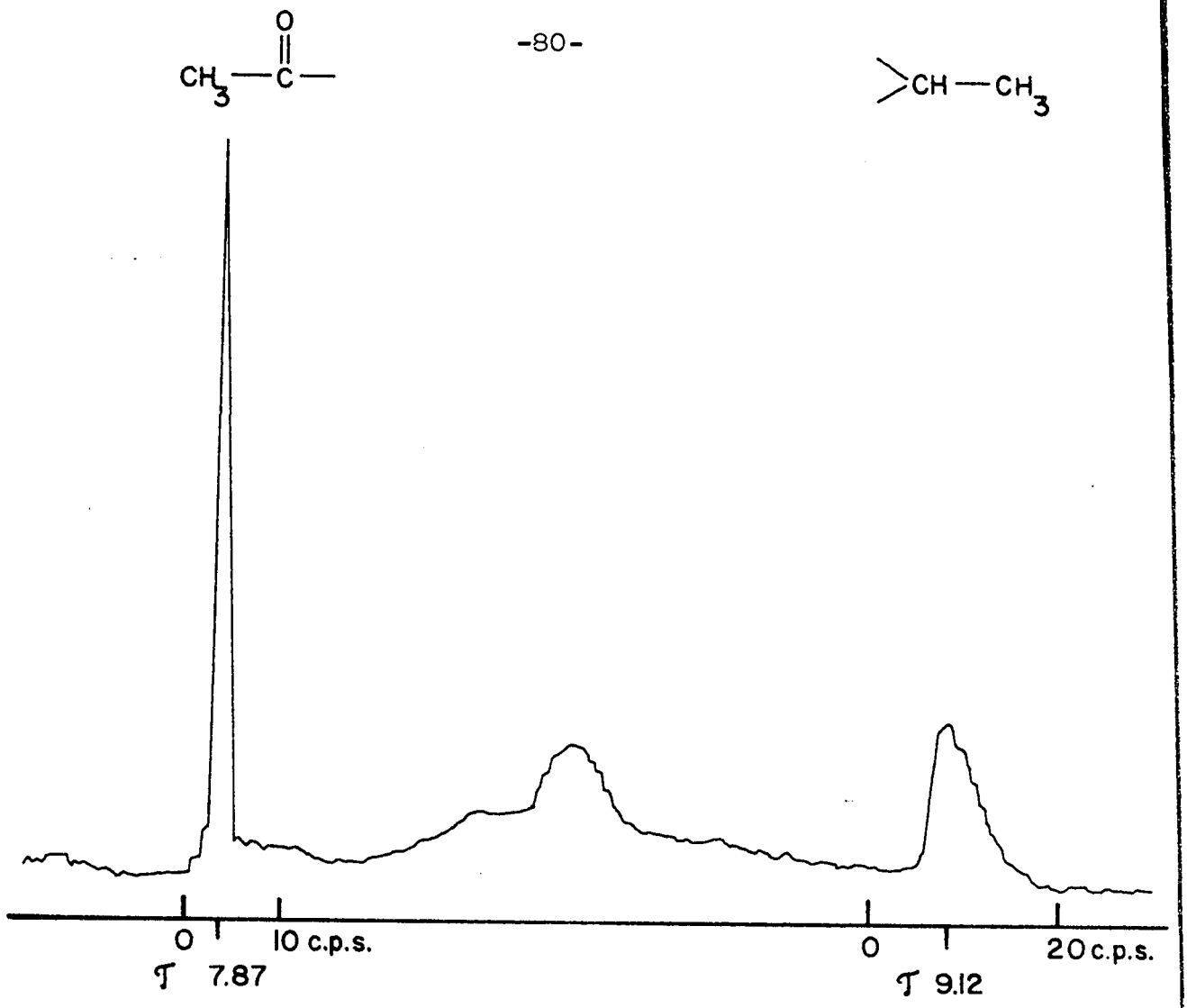


Figure 7. Slow sweep of the Methyl groups of N-Acetyl Lycodine

$-CH_3$ protons to a proton on an adjacent carbon atom is about 7 cps and to a proton four bonds remote is about 0.4-0.5 cps or less (48). Thus the spacing of 1.9 cps in the methyl band of the lycodine is quite consistent with a $-CH-CH_3$ group.

The region 6.6-7.5 τ in the spectrum of lycodine showed, among others, six lines of total area approximately equal to two protons. This six line pattern (shown by asterisks in Fig. 5) remained remarkably unchanged in the spectra of lycodine in $CDCl_3$, carbon tetrachloride, and in trifluoroacetic acid. The line separations were also of an unmistakable pattern showing two spacings of 6.5 cps each (Lines 1, 2 and 3, 4 from left to right) and three spacings of 18.4 cps (Lines 1,3; 2, 4 and 5, 6) not disturbed by change of solvent. The spectrum of N-acetyl lycodine also shows a similar pattern, but is somewhat complicated by other bands shifted down-field presumably due to the acetyl group. In view of the similarity of these chemical shifts to those of benzylic protons (47) and on the basis of partial structure (I) of lycodine, these six lines in the spectrum of lycodine can be most reasonably assigned to two protons present either on C8 or C5 (structure I, Page 76). Since it will be shown later that C5 is attached to the second nitrogen atom of lycodine, the two protons in question should therefore be attached to C8. The presence of six lines can be rationalised as

being due to coupling with a single proton attached to C7. The geminal protons on C8 are nonequivalent as one of them (A) is in the plane of the pyridine ring and would be expected at lower field than the other (B) which is not in the plane of the aromatic ring (48). Models (49) of 5,6,7, 8-tetrahydroquinoline system showed that when the half chair composed of the carbocyclic ring, is held rigid in one of its favoured conformations, the two protons on C8 are situated as stated above. Further, one of them (A) forms a 30° dihedral angle with the proton on C_7 (X) while the other (B) forms a nearly 90° dihedral angle. So the six lines (Fig. 5) would be the AB part of an ABX spectrum where J_{AB} = ca 18 cps, J_{AX} = ca 6 cps, J_{BX} = ca 0 and δ_{AB} = 0.68 ppm. The value of 18 cps for the coupling constant between two gem protons (AB) is somewhat high although values up to 22 cps have been previously reported (50). The coupling constants 6.5 cps and ca 0 cps for J_{AX} and J_{BX} are quite consistent with the dihedral angles concerned, according to the Karplus' theory (51) of angular dependence of coupling. Thus the α-position of the pyridine carries a $-\overset{8}{C}H_2-\overset{7}{C}H$ group. We have found that the spectrum of N-methyl lycodine in benzene solution shows similar six lines uncomplicated by other signals (Fig. 8; shown by vertical lines above the spectrum).

The NMR spectrum of lycodine does not show any resonance in the region 5.0-6.5 τ. In the spectrum of

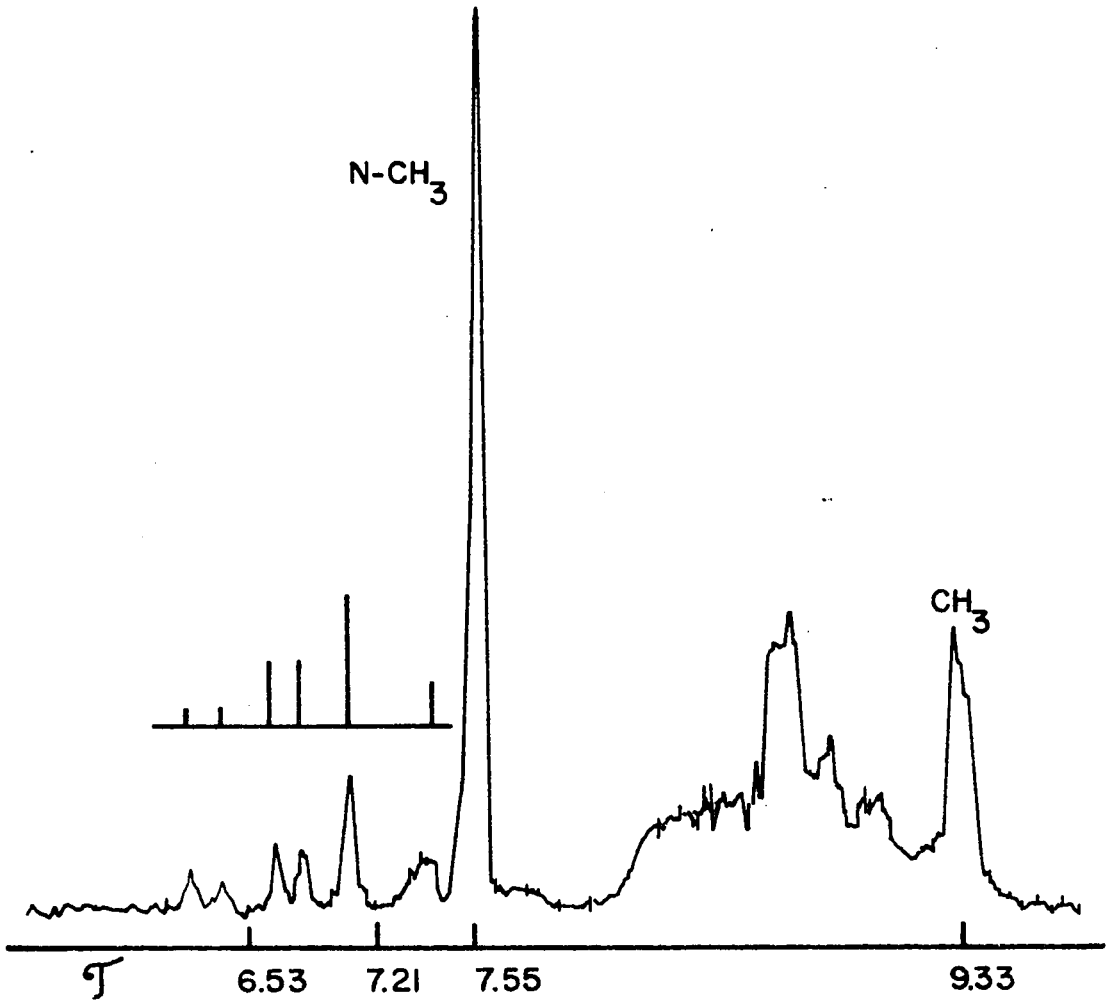


Figure 8. The NMR spectrum of N-Methyl Lycodine in Benzene

nicotine the -CH-group, attached to the pyridine ring as well as the nitrogen atom of the pyrrolidine ring, shows a band at 6.47. Since it will be shown that in lycodine C5 is attached to a nitrogen atom as well as the pyridine ring, the absence of any bands between 5.0τ and 6.5τ is indicative of the fact that C5 does not carry a hydrogen atom.

The abnormal chemical shift of the γ-proton (Fig. 5) in the pyridine ring of lycodine as compared to those observed in pyridine and 2,3-lutidine, has been attributed to the nearness of the secondary nitrogen atom and the γ-proton (1). The influence of the nitrogen on the chemical shift of the γ-proton can be seen readily from the large difference (0.34 p.p.m.) in the chemical shifts of the γ-proton in lycodine (Fig. 5) and N-acetyl lycodine (Fig. 6). This was further evidence for the fact that the secondary nitrogen in lycodine is very close to the γ-proton.

Chemical studies.

Since only a small quantity of lycodine (ca 0.4g) was available for this investigation, physical methods were used whereever possible and chemical investigations were kept to a minimum. In the experiments described below, the amount of lycodine used for preliminary experiments was of necessity restricted to milligram quantities (usually 2-3mg)

and the product was usually examined by paper chromatography and absorption spectra. A reaction was studied in detail only if the information obtainable from it warranted the study on a reasonable scale (10-50mg). In some cases, although valuable information could be obtained, the reaction had to be given up because low yield or a mixture of products was obtained. Absorption spectra mentioned in the following pages, were determined on compounds shown to be homogeneous and pure by paper chromatography.

Attempted Selenium dioxide oxidation of lycodine.

Previously Eves (14) had obtained spectral evidence for the formation of benzylidene derivative from lycodine. It was therefore suspected that a methylene group was attached to the α-position of the pyridine ring in lycodine. In order to confirm the presence of such a methylene group, selenium dioxide oxidation of lycodine was attempted. This reagent is known to oxidise compounds containing reactive methylene groups to ketones (52) and has been used successfully for the oxidation of 2-picoline to 2-pyridinecarboxaldehyde and 5,6-benzo-7-azahydrindene to 1-keto-5,6-benzo-7-azahydrindene (52). Oxidation of lycodine should therefore produce a ketone, conjugated with the pyridine ring, which could be readily identified by spectral properties.

In view of the variety of oxidations and dehydrogenation brought about by selenium dioxide (52), the oxidation of lycodine was first attempted under mild conditions. But lycodine was found to be quite stable to oxidation under the usual conditions using a solvent like dioxane or ethanol. When the oxidation was carried out in refluxing xylene, there was evidence of oxidation. The reaction mixture still contained some unchanged lycodine as shown by paper chromatography. Examination of the product showed that it was a weaker base than lycodine, as the product could be freed from lycodine by washing a chloroform solution of the reaction product with a buffer of pH 4.0, but it nevertheless gave a positive Dragendorff test. The substance showed a band at 1725cm^{-1} (CCl_4) in the carbonyl region of its infrared spectrum. The weak basicity of the product and other properties indicated that the oxidation must have taken place in the neighbourhood of the strongly basic -NH- in lycodine rather than near the pyridine ring.

Oxidation of lycodine with selenium dioxide in dioxan, in a sealed tube at a higher temperature, gave two products in low yield. One of them appeared to be identical with that formed in oxidation in xylene above. The second product was a stronger base. It showed a band at 1700cm^{-1} in its infrared spectrum, assignable to a ketone formed by

the oxidation at the methylene group attached to the pyridine ring. The ultraviolet spectrum showed a band at λmax 280 mμ shifted in acid to 276.5 mμ with slightly diminished intensity where as the spectrum of lycodine itself showed the main band at λmax 268.2 mμ, shifted in acid to 272 mμ, with an increase in intensity. The shift to longer wavelength seen in the spectrum of the product was indicative of the fact that the metylene group adjacent to the pyridine ring had been oxidised to a keto group thus extending the chromophore. Selenium dioxide oxidation of 5,6,7,8-tetrahydroquinoline gave a compound which showed a band at 1700cm^{-1} in the infrared spectrum and a band λmax 280-285 mμ in the ultraviolet spectrum and in this respect showing a similarity with oxidation product from lycodine. Neither of these reactions was studied further as the yields were generally low.

It should be possible to exchange the hydrogens on the methylene group with deuteriums and the presence or absence of an α-methylene group can be confirmed from the NMR spectra of the deuterated and undeuterated compounds. But a model experiment with 2,3-lutidine showed that quite vigorous conditions were necessary to deuterate the 2-methyl group. Therefore deuteration of lycodine was not attempted. However, meanwhile, the presence of a -CH_2-CH- group adjacent to the pyridine ring in lycodine, was confirmed from the

60 Mc/s NMR spectra of lycodine as previously mentioned.

An attempted oxidation of lycodine with potassium permanganate gave low yields of two products and therefore was not considered a good way of degrading lycodine.

Dehydrogenation of lycodine.

Dehydrogenation experiments have been often used in structure determination of alkaloids, and the use of chemical as well as catalytic dehydrogenation in the degradation of annotinine, lycopodine and α-obscurine has been discussed in the introduction. In these instances, however, the dehydrogenation was accompanied by considerable degradation, which was no doubt mainly due to the peculiarities of structure of the alkaloid, and partly due to the fact that drastic conditions and high temperatures were used. In the following experiments with lycodine, a catalytic dehydrogenation agent palladium-charcoal was preferred, and relatively low temperature and solvent were used. It is known that, palladium-charcoal is a mild aromatising agent (53) and does not generally bring about loss of non-angular alkyl groups (54), although rare instances of rearrangement have been recorded (53).

From preliminary experiments, it was learnt that lycodine was relatively stable to dehydrogenation by palladium-charcoal catalyst in refluxing p-cymene or naphthalene. After considerable experimentation, it was found

that dehydrogenation by palladium-charcoal in refluxing diphenyl ether (18-20 hours) gave good yields of the dehydrogenation products and these conditions were used in the preparative experiments.

The dehydrogenation products were freed from unchanged lycodine by partitioning the crude reaction product between chloroform and a buffer of pH 4.0. The chloroform soluble matter upon evaporative distillation gave lycodine-free dehydrogenation products. This incidentally showed that dehydrogenation products were weakly basic.

Paper chromatography of the product on paper buffered to pH 3.0 showed two spots (Rf 0.75 and 0.86). By means of preparative paper chromatography, the "compounds" responsible for the two spots were separated in small quantities. The ultraviolet spectra of these and the crude product were all similar and consisted of bands at λ max 230 mμ (very strong), about 270 mμ (s), 325 mμ (w) and 342 mμ (w).

In acid solution, the main band (270 mμ) was intensified and shifted to longer wavelength (276 mμ). The spectra showed close similarity with those of 1,7-phenanthroline and their salts, published by Krumholz (42). It was soon evident that the "compounds" separated by preparative paper chromatography as above, were not homogeneous

as the ultraviolet spectra of these compounds from two different runs were not identical. Thus, an improved method of separation was necessary before the spectral properties could be accepted.

Paper chromatography on unbuffered paper, using an n-butanol: water: HCl mixture as the developing solvent, was found to be very satisfactory for separating the mixture of dehydrogenation products. With this system, the dehydrogenation product gave four spots (Rf 0.52, 0.40, 0.28 and 0.19) instead of two. Of these the compound with Rf value 0.52 was present as the major ingredient and was designated; dehydrogenation product A. The compound with Rf 0.19 was the next in order of abundance and was designated as dehydrogenation product B. The other two were present in smaller amounts and have not been studied. The ultraviolet spectra of each of the compounds (Rf 0.52 and 0.19) from different runs were identical and the compounds appeared to be homogeneous.

The ultraviolet spectrum of dehydrogenation product A showed absorption at λ max 234 mμ (s) 271 mμ (s), 326 mμ (w) 342 mμ (w). In acid solution, the main band at 271 mμ was intensified and shifted to 276 mμ showing evidence of protonation.

The changes in the spectrum of the compound in solutions of different acidities were indicative of the

presence of two protonated species (cf. for comparison the spectra of 1,7-phenanthroline and its salts; Fig. 24). Since the compound has been shown to be homogeneous, it could be concluded from this evidence that the compound was probably a diacidic base. The shape and intensities of spectra of dehydrogenation product A in ethanol and in acid showed close similarity with the reported spectra of 1,7-phenanthroline and its salts (42). The compound might therefore be a substituted 1,7-phenanthroline.

It must be mentioned here that the spectra of 1,10- and 4,7-phenanthroline (42) are also very similar to the spectra of 1,7-phenanthroline. But for reasons discussed below, these possibilities were discarded.

Unlike 1,10-phenanthroline and derivatives, dehydrogenation product A was a diacidic base and gave a negative "ferroin" test (55) and therefore was not likely to be a 1,10-phenanthroline derivative.

The spectra of the salts of 1,7-phenanthroline and 4,7-phenanthroline show certain points of difference. The monoprotonated form of 4,7-phenanthroline shows a more intense main band than the diprotonated form and both of these show a more intense main band at longer wave lengths than the main band of 4,7-phenanthroline. On the other hand, the monoprotonated form of 1,7-phenanthroline shows a spectrum less intense than the diprotonated form and only

slightly more intense than the base itself. Dehydrogenation product A resembled 1,7-phenanthroline in this respect rather than 4,7-phenanthroline. Besides, the shapes and the relative intensities of the other bands showed closer resemblance to the spectra of 1,7-phenanthroline. Other evidence in favour of the 1,7-phenanthroline system came from previous work on lycodine discussed below.

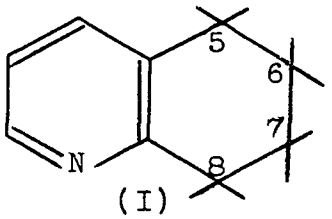
It had been observed that in the NMR spectrum of lycodine the γ-proton of the pyridine ring showed an abnormal chemical shift and occurred at much lower field than in the spectra of pyridine and 2,3-lutidine (1). Anet and Eves recognized this as being due to the proximity of the secondary nitrogen to the γ-proton. The abnormal chemical shift could be best understood if the secondary nitrogen atom was attached to C5 (structure I, Page 76). This would also explain the large difference (0.34 ppm) in the positions of the γ-protons of lycodine (Fig. 5) and N-acetyl lycodine (Fig. 6). A similar shift to lower field is shown by H10 (0.56 τ) in the spectrum of 1,7-phenanthroline, as compared with H10 (1.23 τ) in the spectrum of 4,7-phenanthroline and must be due to the proximity of the nitrogen atom in 1 position (cf. Part II). All these facts point to the probability that the secondary nitrogen atom in lycodine is attached to C5 (Structure I, Page 76) and therefore dehydrogenation product A must be a 1,7-phenanthroline derivative.

In the light of these findings the conclusion of Anet and Eves, that the secondary nitrogen of lycodine is not attached to C5, must be held erroneous. The only argument presented in favour of this conclusion was the difference in the pKa s of the pyridine nitrogens in lycodine (3.97 and 8.08) and nicotine (3.35 and 7.70). However, nicotine is not an adequate model for lycodine, as in the former the pyrrolidine nitrogen can assume a position quite near to the pyridine nitrogen due to "free rotation". In a hydrogenated 1,7-phenanthroline system such as one postulated at present for lycodine, the secondary nitrogen atom will be held rigidly in the furthest position from the pyridine nitrogen. For this reason the pyridine nitrogen in lycodine can have a higher pKa than that of nicotine, even if the secondary nitrogen is attached to C5.

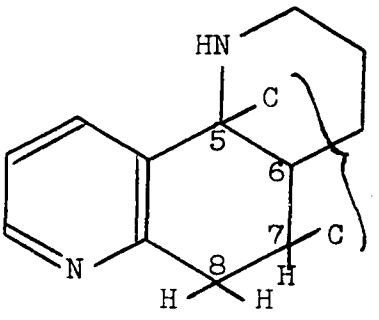
Finally the postulation of a reduced 1,7-phenanthroline ring system for lycodine is biogenetically more attractive, since such a system can be realised by biogenetically feasible pathways from the oxygenated perhydrojulolidine system which has been shown to be present in Lycopodium alkaloids; annotinine and lycopodine (16, 23).

The above findings permitted us to elaborate the partial structure of Anet and Eves and speculate in terms of the complete structure of lycodine. Since it has already been shown that in partial structure I, C5 carries

a nitrogen atom and is tetrasubstituted, C_7 is trisubstituted and C8 is a methylene group, the 1,7-phenanthroline



(I)



(II)

system can be obtained only by fusing a piperidine ring across C5 and C6. A 1,7-phenanthroline ring system accounts for 12 carbon atoms and three out of the four rings in lycodine. The fourth ring must therefore be across C5 and C7 as in II. A structure such as II would be expected to give rise to a 5-substituted 1,7-phenanthroline upon dehydrogenation, assuming that no rearrangements occurred.

Dehydrogenation product A was obtained relatively pure in small quantity (ca 2.0mg) by chromatography on alumina. Its ultraviolet spectrum was found to be almost identical with that of 5-methyl-1,7-phenanthroline in the positions of absorption maxima, but different from those of several other alkylphenanthrolines prepared as model compounds (see part II). Dehydrogenation product A was not identical with 5-methyl-1,7-phenanthroline.

At this stage, Ayer and Iverach (31) deduced the structures of α- and β-obscurine and suggested from biogenetic considerations that the molecular formula of

lycodine might be $C_{16}H_{22}N_2$ rather than $C_{17}H_{24}N_2$. Lycodine would, in such a case, be the pyridine analog of demethyl-β-obscurine and thus also closely related to lycopodine (31). Our experimental results were in agreement with this view.

The NMR spectrum of dehydrogenation product A is shown in figure 9. The region 0-3.0 τ of the spectrum differed from that of 1,7-phenanthroline (Fig. 17, Part II) only in that the signal area of the bands in the region 2.0-2.25 τ in the former was half of that in the latter. These lines in the spectrum of 1,7-phenanthroline have been assigned to the benzene ring protons (H5 and H6) (see Part II). Therefore dehydrogenation product A must be a 1,7-phenanthroline substituted in the benzene ring (at 5 or 6 position). The position of the substituent was seen to be in the 5 position from the similarity of the low field (0- 3.0 τ) part of the spectrum of dehydrogenation product A with the like region in that of 5-methyl-1,7-phenanthroline and the dissimilarity with that of 6-methyl-1,7-phenanthroline (Fig. 18, Part II). The band at lowest field in the spectrum of 5-methyl-1,7-phenanthroline has been assigned to the proton in the 10 position (see Part II) and consists at high resolution and slow sweep, of eight lines due to coupling with H9, H8 and H6. The long range coupling (0.7 cps) between H10 and H6 is of a type observed by Anet (56) in the spectra of quinolines in which H4 is

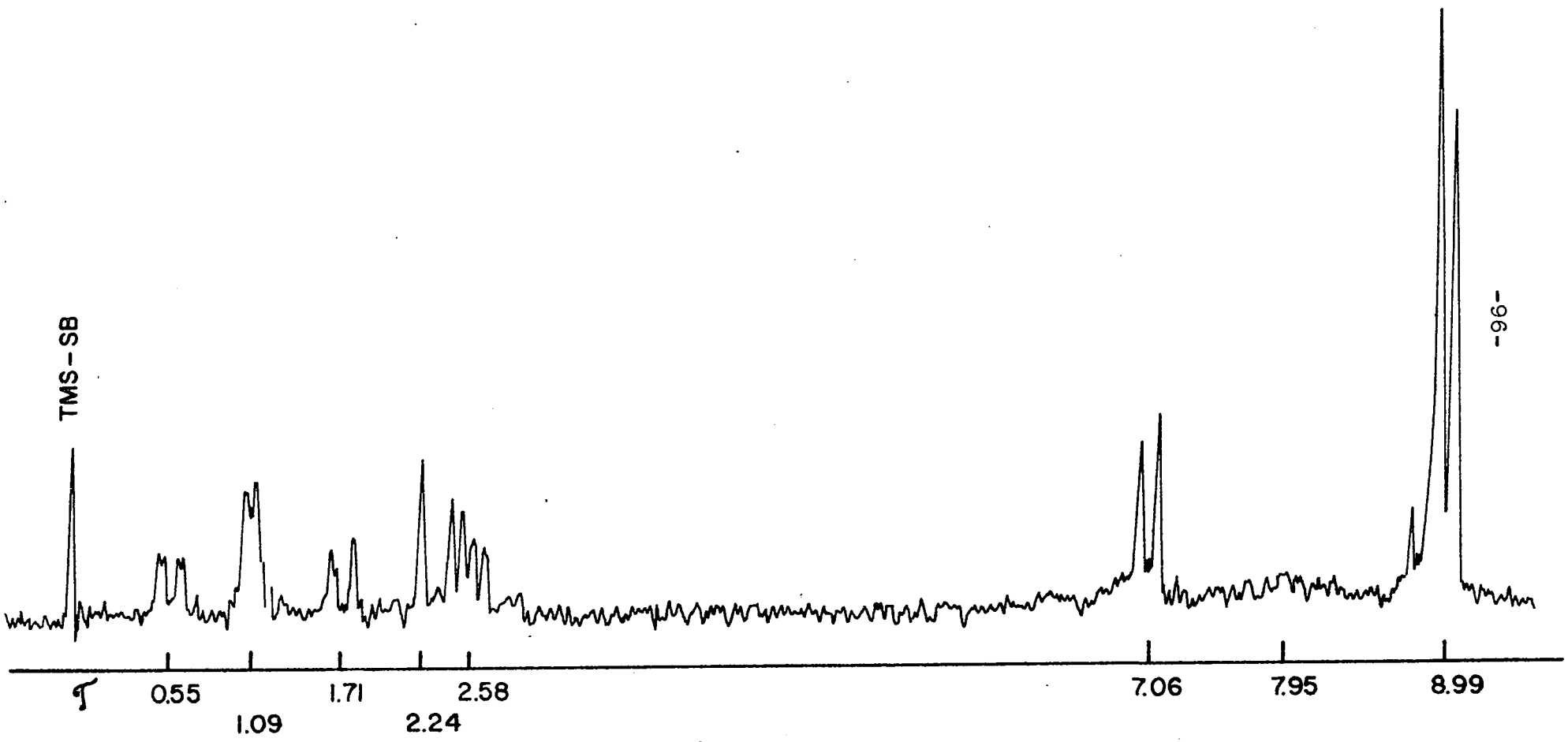


Fig.9.

The NMR spectrum of dehydrogenation product A from Lycodine in Carbon Tetrachloride

coupled to H8 by about 0.8 cps. With a faster sweep, even at high resolution, the H10 band does not show the fine coupling clearly and consists of four lines which are not very sharp (see figure). In contrast the spectrum of 6-methyl-1,7-phenanthroline shows the H10 band as four sharp lines even under high resolution and either slow or fast sweep, since in this case there is no proton in position 6. In the spectrum of dehydrogenation product A the H10 band (0.55τ) appears as four lines which are not very sharp and show evidence of additional splitting. This spectrum could not be obtained at a slow sweep owing to the limited amount of the compound available.

The high field region (7.0 -9.0τ) of the spectrum of dehydrogenation product A (Fig. 9), consisting of a doublet (7.07τ ; J = 6.3 cps), a multiplet ($\sim$8.01τ), and an intense doublet (8.96τ ; J = 6.3 cps), was readily recognized as being due to an isobutyl group, from an inspection of the chemical shifts, areas and the multiplicity, and from its general similarity to the like region in the spectrum of isobutylbenzene (see Part II). Therefore dehydrogenation product A must be 5-isobutyl-1,7-phenanthroline (Fig. 11).

Dehydrogenation product A (ca 10mg) was obtained crystalline only after considerable difficulty. It melted at 80-82° and was identical with synthetic 5-isobutyl-1,-7-phenanthroline (m.p. 84-85°) with respect to mixed

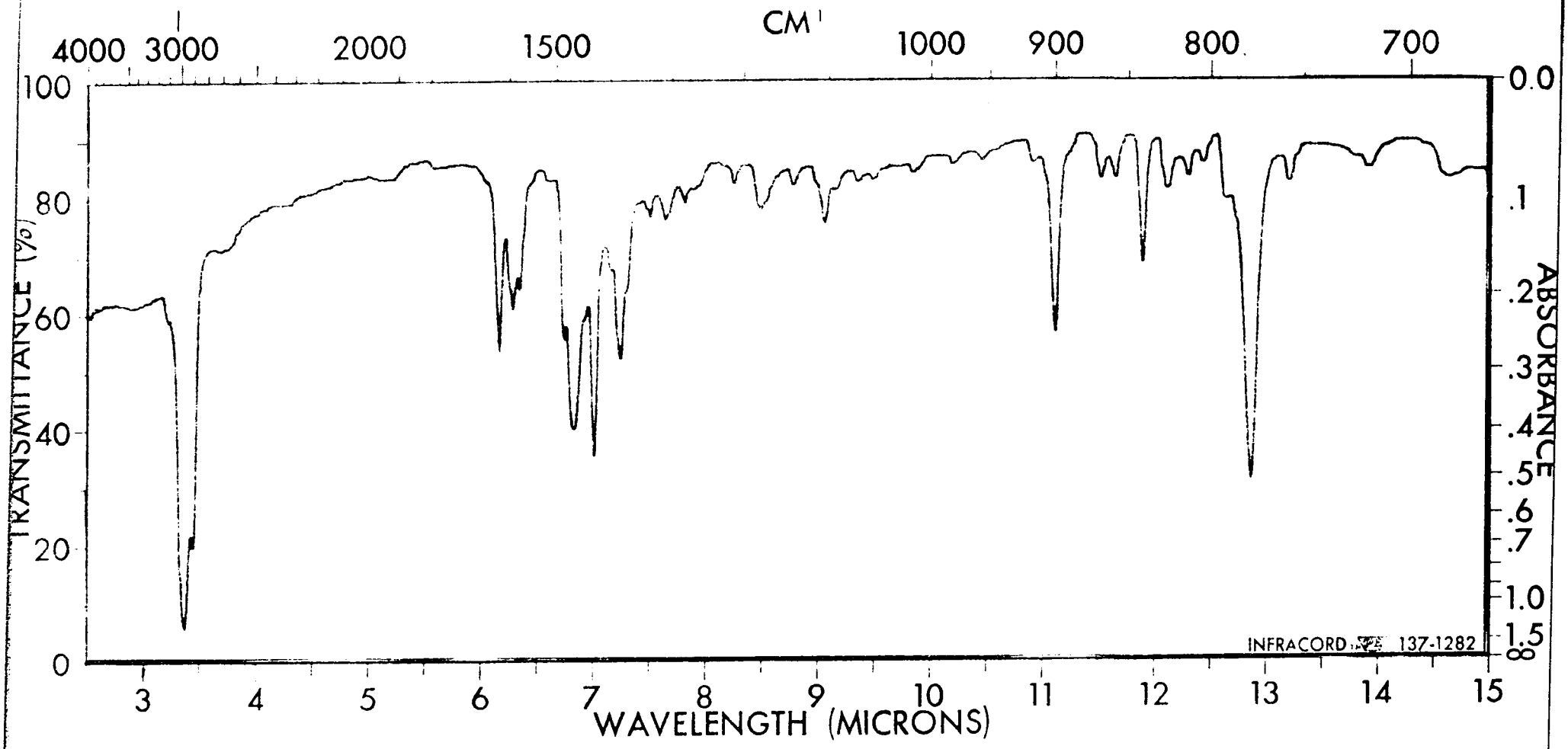


Fig.10. The infrared spectrum of dehydrogenation product A in nujol mull.

melting point, ultraviolet and NMR spectra (Figs. 9 and 21). The infrared spectrum (Fig. 10) of dehydrogenation product A showed all the bands present in the spectrum (Fig. 25, Part II) of 5-isobutyl-1,7-phenanthroline. However both the NMR and infrared spectra indicated that the dehydrogenation product was still slightly impure. Further purification was not carried out as only small amounts of the compound were available.

Dehydrogenation product B also appears to be a 1,7-phenanthroline derivative. It shows the ultraviolet absorption maximum of the main band at 266 mμ which is at shorter wavelength than the corresponding band (267 mμ) of 1,7-phenanthroline itself. Alkyl substitution causes usually a bathochromic shift of the main band of many aromatic systems (except in non-alternant aromatic systems where substitution in certain positions causes a hypsochromic shift) (57). However we have found that 10-methyl-1,7-phenanthroline did show the main band at 264.5 mμ as compared to 267 mμ in the spectrum of the parent compound itself. This may presumably be due to steric factors (see Part II for more examples and discussion). However 10-methyl-1,7-phenanthroline is not likely to be formed from lycodine. But it is quite possible that the substitution in dehydrogenation product B is such as to cause steric strain and the attendant shift of the main band to lower wavelengths. It might be speculated that a 1,7-

phenanthroline in which positions 5 and 6 or 5 and 4 are joined by ring formation might cause a shift to shorter wavelengths in the spectrum due to steric effects. But it was found that, in the case of an open 4,5-disubstitution namely 4,5-dimethyl-1,7-phenanthroline, the main band was shifted to longer wavelength.

Molecular weight and molecular formula of lycodine.

The molecular weight of lycodine was confirmed to be 242, from its mass spectrum, obtained through the kindness of Dr. Biemann. This confirms the revised formulation of lycodine as $C_{16}H_{22}N_2$. The fragmentation pattern in the mass spectrum presents certain points of interest and these are discussed in relation to the complete structure of lycodine, at the end of this section.

Interrelation of lycodine and lycopodine.

Speculations as to a possible biogenetic relationship between lycodine and lycopodine arose while the dehydrogenation experiments were still in progress and have been discussed previously in considering the ring system in the dehydrogenation product. Confirmation was sought by chemical correlation of the two alkaloids and was achieved in a five-step synthesis of lycodine from lycopodine. The details of this conversion are shown in Figures 11 and 12.

β-cyanobromolycopodine (20) available as the minor product from the reaction of lycopodine (Fig. 11) with

cyanogen bromide was heated with a suspension of sodium azide in acetone. The product showed intense bands, at 1700cm^{-1} (ketone), 2095cm^{-1} (azide group) and at 2200cm^{-1} (cyanamide) in the infrared spectrum, and was directly reduced to a base by means of hydrogen in the presence of palladium charcoal in acidic ethanol. This indirect method was chosen as it was known (20, 23) that β-cyanobromolycopodine underwent ready cyclisation under alkaline conditions to form an inert enol ether. The base, which partially cyclised under the isolation conditions, was dehydrogenated under mild conditions (boiling p-cymene and palladium charcoal for 1.5 hours, conditions under which lycodine was known to be stable) and then heated with hydrochloric acid to remove the cyano group. The base, obtained in fair yield, crystallised readily and was identified as lycodine by m.p., mixed m.p., paper chromatography, ultraviolet and infrared spectra.

Therefore lycodine has the structure and stereochemistry shown in (Fig. 11). Since β- and α-obscurine have been shown to be respectively α-pyridone and 3,4 dihydropyridone analogues of N-methyl lycodine (35) the stereochemistry of these alkaloids is also established by this work.

<u>The mass spectrum of lycodine</u>.

The fragmentation of lycodine shows a pattern which

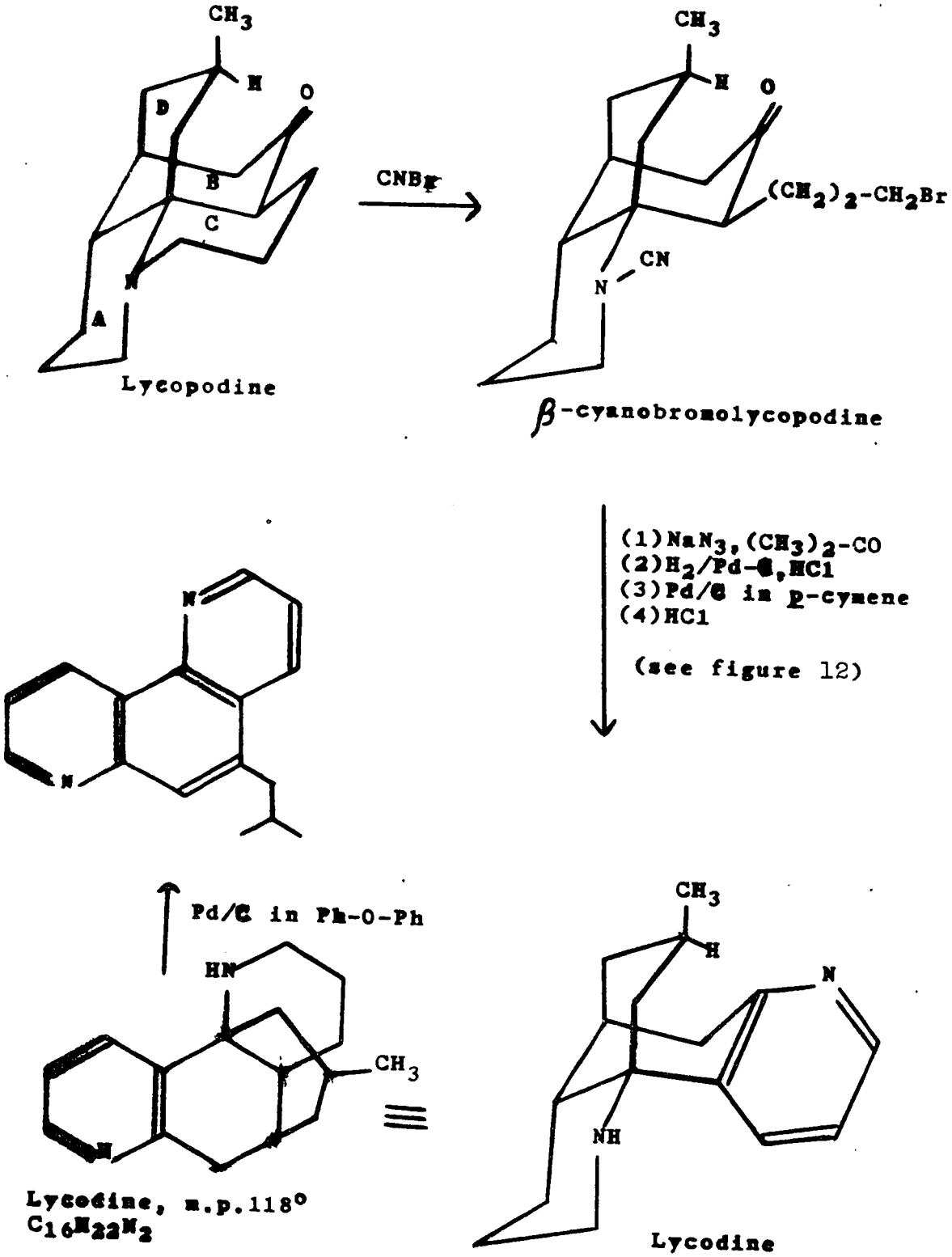


Figure 11. Structure of lycodine and dehydrogenation product A; conversion of Lycopodine into lycodine.

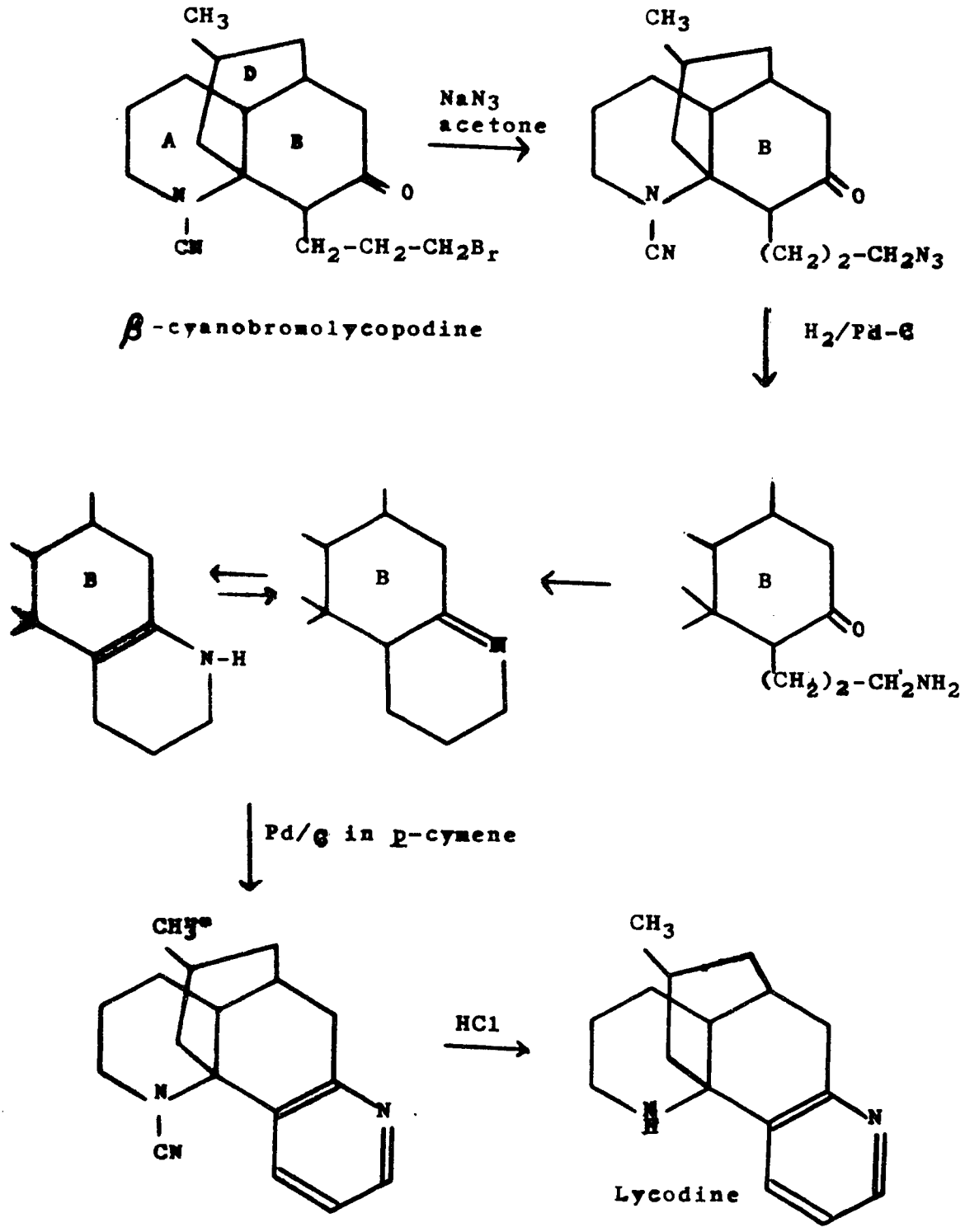


Figure 12. Probable intermediates in the conversion of lycopodine to lycodine.

is very consistent with the assigned structure. In addition to the main (molecular weight peak) peak at 242, the mass spectrum shows other major peaks showing the abundance of ions of mass 199, 185, and 157. The probable mechanism of fragmentation and the intermediacy of these particular ions can be best explained as in Fig. 13. Loss of an electron from lycodine (from the secondary N atom) might give rise to the "aminium ion radical" (242) which stabilises itself by forming the immonium ion radical (also 242) with the fission of a carbon-carbon bond. The latter could give either the immonium ion (199) and a propyl radical or the immonium ion (185) and a butyl radical by internal abstraction of a hydrogen atom, as shown in figure 13.

The immonium ion (185) might be further visualised to give, by a type of reverse Diels and Alder reaction, the completely conjugated immonium ion (157) and ethylene. In the figure, shifts of one electron are indicated by a dotted arrow and two-electron shifts are shown by a full arrow. It is interesting that the first C-C bond to undergo scission in the above, is the same as the one to break first in the dehydrogenation of lycodine.

Miscellaneous experiments.

It was reported (20) that when α-cyanobromolycopodine ($C_{17}H_{25}ON_2Br$) is treated with methanolic potassium

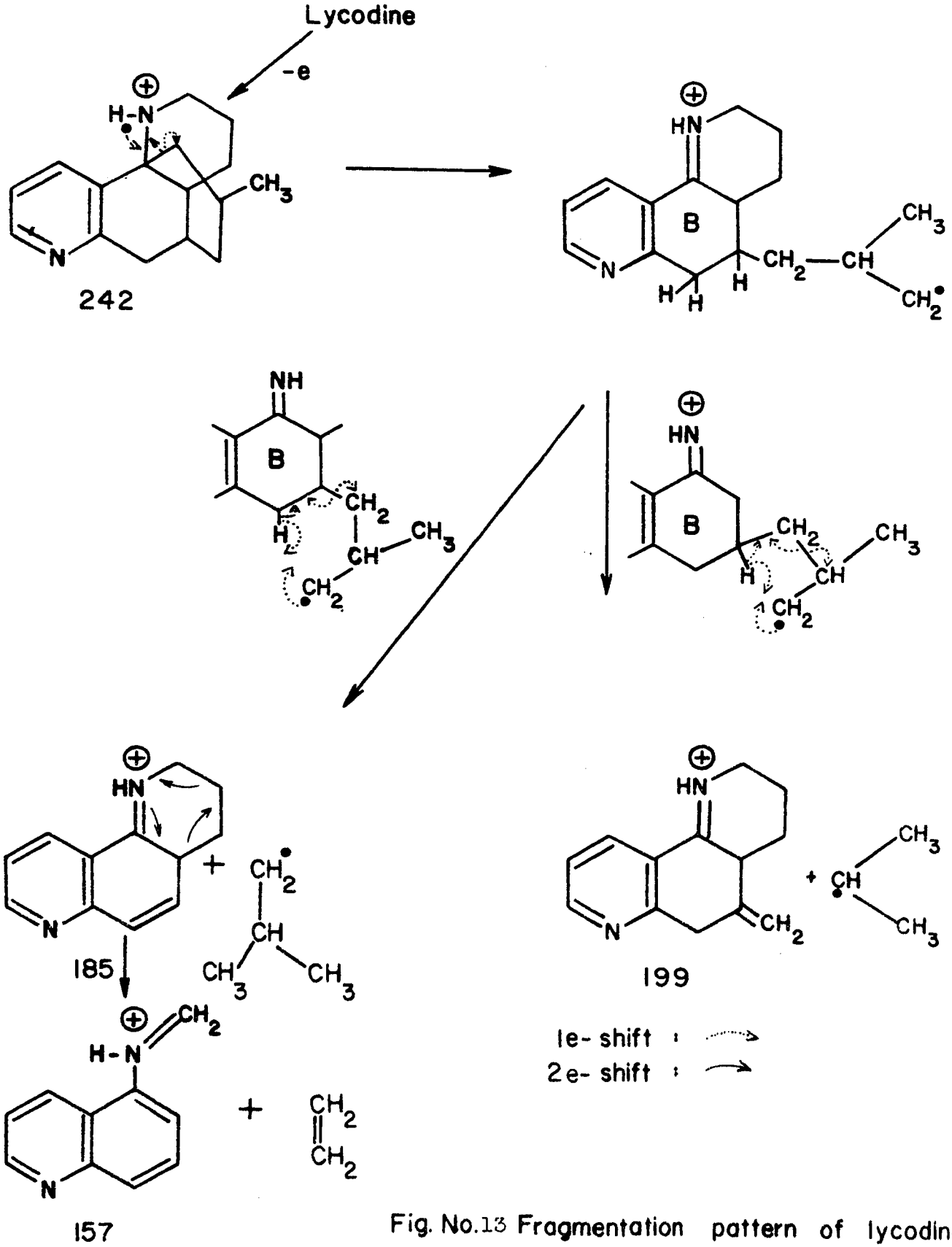


Fig. No.13 Fragmentation pattern of lycodine

hydroxide an inert compound ($C_{17}H_{24}ON_2$) resulted. The compound was resistant to oxidation and hydrogenation. Although the structure of this has not been studied as such, it has been suggested that it is formed by internal alkylation (with loss of HBr) at either the -CH- or the $-CH_2-$ group adjacent to the carbonyl group (23). The infrared spectrum of the compound in carbon tetrachloride solution showed a band at 1415cm^{-1} (CH_2-CO) indicating that the alkylation has not taken place at the methylene group adjacent to the carbonyl group. The 1415cm^{-1} band is present also in the spectrum of lycopodine and has been previously assigned to the methylene group adjacent to the carbonyl group (23).

Attempted conversion of anhydroidihydrolycopodine (L14) to lycopodine.

Anhydrodihydrolycopodine (L14) occupes a key position in the interrelation of Lycopodium alkaloids. Although it has been obtained from both annofoline and lycopodine (3), conversion of L14 into either lycopodine or annofoline has not been accomplished. In view of the work going on in this department towards the synthesis of annofoline and acrifoline it was of interest to complete the sequence anhydrodihydrolycopodine to lycopodine. A synthesis of annofoline would then be a synthesis of lycopodine and also lycodine. Although the conversion was not accomplished, the approaches attempted will be described

here briefly.

Attempted Brown hydration of anhydrodihydrolycopodine (L14) did not give the expected "epidihydrolycopodine"; but the bulk of the starting material was recovered. Dr. Ayer has also confirmed that L14 was unreactive to the hydroboration reaction (58). Experience has shown that reactivity of a double bond in the hydroboration reaction is roughly paralleled by the reactivity in hydrogenation reactions (59). It is therefore not surprising that the double the bond in L14 was unreactive to hydroboration since it was also resistant to hydrogenation.

Osmium tetroxide oxidation of L14 gave, in low yield, a hydroxyl containing compound in a mixture of three compounds. The reaction was therefore not suitable for working with small quantities.

Hydroxylation of L14 with potassium permanganate gave a neutral compound which appeared to be a diol lactam (infrared: band at 3550cm^{-1}, and wide band at 3320cm^{-1} for hydroxyl and an intense broad band at 1600cm^{-1}, probably a hydrogen bonded amide carbonyl). Lithium aluminum hydride reduction of this gave a basic compound. When the base was heated with acid a second compound was obtained. The latter compound, like lycopodine, showed a band at 1700cm^{-1} and had an Rf value identical to that of lycopodine. This series of reactions, although promising, could not be repeated for confirmation due to lack of L14.

PART II

THE PREPARATION AND PROPERTIES OF SOME PHENANTHROLINES.

INTRODUCTION

The Scope of the Problem.

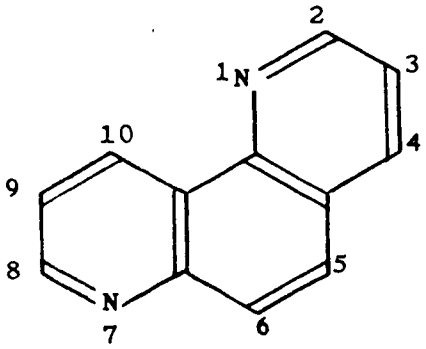
The work described in this part of the thesis stemms from our experiments on the dehydrogenation of lycodine, reported in Part I. The dehydrogenation products from lycodine were believed to be alkylphenanthrolines, from their ultraviolet spectra and other properties. As the supply of lycodine and the yield of the dehydrogenation products were meagre, identification of the dehydrogenation products could be made easier by studies on model compounds. A survey of the literature revealed that only a few methylphenanthrolines had been prepared. It was therefore found necessary to prepare and study several alkylphenanthrolines, as model compounds. The actual identification and later the synthesis of one of the dehydrogenation products was thus realised. Apart from this topical interest,it was found that the ultraviolet and NMR spectra of this series of compounds were quite characteristic and interesting, and in most cases allowed an assignment of structure from spectral properties.

Review of Pertinent Literature.

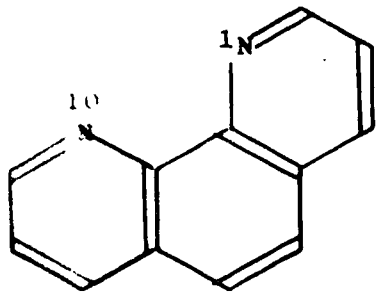
The general term - diazaphenanthrene - which includes all phenanthrolines, has been applied to azalogs of phenanthrene, in which any two -CH- groups have been

replaced by -N- groups. But in the Chemical Abstracts and other chemical journals the name phenanthroline appears to have been restricted to diazaphenanthrenes containing a nitrogen in each of the terminal rings. However occasionally, it has also been applied to other diazaphenanthrenes (60, 61). In this thesis, the term phenanthroline is used in the sense intended in the Chemical Abstracts, and can be described aptly as diazaphenanthrenes which contain a dipyridyl system. The specific nomenclature and numbering in the phenanthroline series has been somewhat confused in the past, and the system followed in the Chemical Abstracts, is used herein. Thus the three phenanthrolines derived from m-phenylenediamine, o-phenylenediamine and p-phenylenediamine are referred to as 1,7-phenanthroline (I), 1,10-phenanthroline (II), and 4,7-phenanthroline (III) respectively. The structures are given in figure 14.

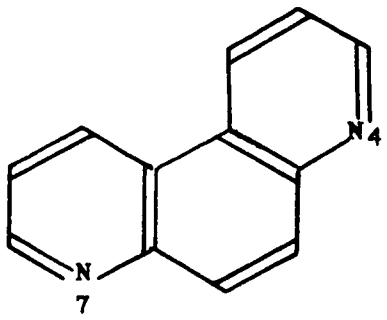
1,7-phenanthroline, the first known member of the series, was prepared by Skraup and Vortmann (62) in 1882. Blau synthesised 1,10-phenanthroline in 1898 and observed that it formed complexes with many metal salts. There was not much work done in the field, until Walden (63) in 1931 made use of the complexing properties of 1,10-phenanthroline, in analytical chemistry. Since then several substituted 1,10-phenanthrolines have been prepared for use as complexing agents (64). However this



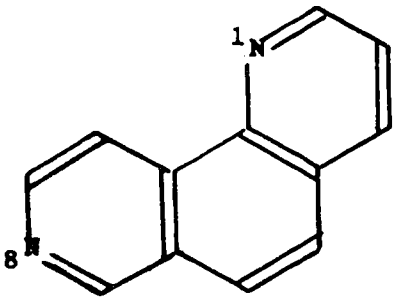
1, 7-Phenanthroline
(meta-Phenanthroline)
I



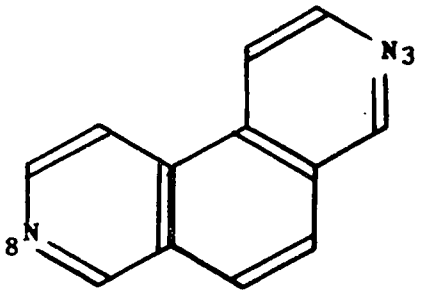
1, 10-Phenanthroline
(ortho-Phenanthroline)
II



4, 7-Phenanthroline
(para-Phenanthroline)
III



1, 8-Phenanthroline



3, 8-Phenanthroline

Figure 14. The Phenanthrolines.

activity has been limited to the field of 1,10-phenanthrolines and relatively little attention has been paid to 1,7-phenanthroline, 4,7-phenanthroline and their alkyl derivatives.

(a) Methods of preparation.

The Skraup and related syntheses have been used, almost invariably, in the preparation of phenanthrolines and alkyl phenanthrolines of the quinoline type. The parent compounds, 1,7-, 1,10-, and 4,7-phenanthroline have been prepared by reacting meta-, ortho- and para-phenylenediamine respectively with glycerol or acrolein, in a Skraup reaction (65). Substitution in the heterocyclic rings can be achieved by replacing acrolein with a suitable $\alpha\beta$-unsaturated carbonyl compound of the general type R^{I}-CH=C(R^{II})-CO-R^{III} (or equivalent), where R^{I}, R^{II}, and R^{III} respectively are the desired substituents located α, β and γ to the nitrogen (64, 66). Phenanthrolines substituted in the middle ring, as well as the terminal rings can be obtained by a Skraup reaction on suitably substituted aminoquinoline or phenylenediamine and glycerol.

A problem of orientation arises when an aminoquinoline, with two positions adjacent to the amino group available for cyclisation, is subjected to a quinoline synthesis. The product may be either an angular diazaphenanthrene or a linear diazaanthracene. But generally in the Skraup and related reactions, angular cyclisation has been

observed almost exclusively (65, 67). Reasons for the preferential angular or linear cyclisation have been discussed (67, 68).

(b) General properties.

1,7-Phenanthroline has been prepared from m-phenylenediamine by a double Skraup reaction (62). It has also been prepared from 7-aminoquinoline. The following alkyl, 1,7-phenanthrolines have been reported; 2-methyl-, 4-methyl-, 6-methyl-, 8-methyl-, 2,4-dimethyl- and 2,8-dimethyl-1,7-phenanthroline. The methods of preparation and properties of these have been reviewed (65, 66).

1,7-phenanthroline is a weak diacidic base. The dissociation constants of the 1,7-phenanthrolinium ions have been determined to be 10^{-4} and 10^{-1} approximately (in water at 20°) (42). Since the 1-nitrogen is somewhat hindered, the possibility of preferential mono-salt formation has been suggested; but has not been studied. However there is evidence that, in the quaternisation of 1,7-phenanthroline, only one of the nitrogens is preferentially quaternised (65). 1,7-Phenanthroline and many alkyl-1,7-phenanthrolines crystallise from aqueous solvents with water of crystallisation (65).

1,10-Phenanthroline has been prepared from o-phenylenediamine (69) and from 8-aminoquinoline by the Skraup reaction (70). The four methyl-1,10-phenanthrolines and many dimethyl- and trimethyl- 1,10-phenanthrolines have

been reported (64, 71).

1,10-phenanthroline is a weak monoacidic base although it contains two nitrogens. The dissociation constant of the 1,10-phenanthrolinium ion has been determined to be 1.1×10^{-5} at 25° (72). It has been suggested that steric and/or electrostatic effects, due to the proximity of the two nitrogen atoms, might prevent the protonation of the second nitrogen (72).

The main interest in 1,10-phenanthrolines arises from its property of forming complexes with many metal salts. The complexes with ferrous salts are of importance in analytical chemistry. These complexes, in which three molecules of a 1,10-phenanthroline is bound to one molecule of a ferrous salt, are brightly colored and can be oxidised to the corresponding faintly colored ferric complexes. This forms the basis of the well known "ferroin test" and has found use in analytical chemistry for indicating end points in redox reactions (55).

4,7-phenanthrolines: The parent compound was synthesised by Skraup and Vortmann (73) in 1883. 4,7-Phenanthrolines with methyl substituents in 1-, 3-, 1,3-, and 3,8-positions have been reported previously. The preparation and properties of these have been reviewed (65).

4,7-Phenanthroline behaves as a weak diacidic base. Krumholz (42) has determined spectrometrically the dissociation constants of 4,7-phenanthrolinium ions to be

10^{-4} and 4×10^{-3}, approximately (in water at 20°).

1,8-Phenanthroline, m.p. 111-111.5°, has been prepared in about 5% yield by the Skraup reaction on 5-aminoisoquinoline. 2-Methyl-1,8-phenanthroline has been prepared indirectly from 4-chloro-2-methyl-1,8-phenanthroline by catalytic reduction (74).

3,8-Phenanthroline, m.p. 225° (75) has been prepared by a modification of the isoquinoline synthesis of Pomeranz. The Schiff base obtained from terephthalaldehyde and aminoacetal, was reduced to protect the double bonds and cyclised by sulfuric acid. The product underwent simultaneous dehydrogenation and yielded 3,8-phenanthroline.

(c) The ultraviolet absorption spectra of the phenanthrolines.

Krumholz (42) has reported the ultraviolet spectra of 1,7-, 1, 10-, and 4,7-phenanthroline and their salts. The spectra of the three bases closely resembled each other and consisted in each case of two pricipal regions of absorption around 230 mμ and 270 mμ . The mono- and di-protonated forms of 1,7- and 4,7-phenanthroline showed decreased intensity in the 230 mμ region and increased intensity with a shift to longer wavelengths of the bands in the 270 mμ region. Further, the spectrum of the mono-protonated form in each case showed distinct differences in shape, maxima and intensities from the corresponding di-protonated form and thus both species could be detected

by adjusting the acidity to obtain the predominance of a particular species. In the case of 1,10-phenanthroline, there was a similar variation of the spectrum in acid, but only one protonated species could be detected, consistent with the fact that 1,10-phenanthroline was a monoacidic base. Krumholz has also pointed out the resemblance of the spectra of phenanthrolines to that of phenanthrene, particularly in the salts of 4,7-phenanthroline which show a third region of absorption at long wavelengths around 330 mμ . The details of the spectra reported by Krumholz are included in Tables VIII and IX along with our own results. The spectrum of 1,7-phenanthroline is reproduced in figure 24.

The ultraviolet spectrum of 1,10-phenanthroline has been reported also by Badger (76) in a comparative study of the spectra of phenanthrene and its various azalogs. Both 1,10-phenanthroline and phenanthrene showed three main regions of absorption, and general similarity of detail. However, there were significant differences between the two spectra. The main differences, which seem to be general for a large number of aromatic azahydrocarbons and their carbocyclic analogs, have been discussed by Badger and may be summarised as follows. The absorption spectra of aromatic azahydrocarbons show, (1), intensified absorption in the region 314-345 mμ ; (2), considerable loss of fine structure and (3), absorption extending to

longer wavelengths, as compared with their carbocyclic analogs.

(d) The nuclear magnetic resonance spectra of phenanthrolines.

There is a single report in the literature on the NMR spectra of this series of compounds. Ito, Isobe and Sone (77) have measured the NMR spectra of 1,10-phenanthroline and a few methyl substituted 1,10-phenanthrolines and have provided a complete assignment of the spectra. The spectrum of 1,10-phenanthroline showed from low to high field two quadruplets due to H2, 9 and H4, 7 respectively, a single band due to H5, 6 followed by a quartet due to H3, 8. The protons on the hetero ring were analysed as an ABX type of spectrum. The coupling constants of these were of the same order of magnitude as those of the ring protons in pyridine and 2,3-lutidine. There was no significant coupling between the protons on the middle ring and those on the hetero rings. The relative chemical shifts and the coupling constants reported by these authors are given in Tables IV and V, along with results obtained by us.

EXPERIMENTAL

Description of general methods.

The methods described in Part I - experimental were used. The NMR spectra were measured in methylene chloride solution unless mentioned otherwise.

1,7-Phenanthroline.

Commercially available "m-phenanthroline" (Aldrich Chemical Co. Inc.) was recrystallised from benzene-petroleum ether and sublimed at 60-65°/0.02mm. M.P. 78°. The u.v. spectrum is shown in figure 24. The NMR spectrum is shown in figure 17.

3,5-Dinitro-4-aminotoluene.

This was obtained according to the procedure of Brady *et al.* (78).

p-Acetylaminotoluene (20.0g) was added in small portions at a time to fuming nitric acid (200ml). The solution was poured into ice water and the precipitated pale yellow 3,5-dinitro-4-acetylaminotoluene was filtered and thoroughly washed with water. The precipitate, freed from water as far as possible, was hydrolysed by heating at 90° for one hour with sulfuric acid (50ml) and water (50ml). The deep yellow 3,5-dinitro-4-aminotoluene was filtered through glass wool, washed with a small quantity of fifty percent sulfuric acid, followed by water and dried

in a vacuum desiccator. Yield 16.0g. M.P. 165-166°.

3,5-Dinitrotoluene.

The procedure described by Cohen et al. (79) was found to be very satisfactory for this preparation. 3,5-Dinitro-4-aminotoluene (10.0g), ethanol (200ml) and concentrated sulfuric acid (50ml) were heated together on a water bath and dry, powdered sodium nitrite (30.0g) was gradually added. The mixture was heated until the effervescence ceased. Water was then added to precipitate the 3,5-dinitro-toluene, which was filtered and recrystallised from ethanol. Yield, 8.2g. M.p. 92°. The NMR spectrum of the compound showed a triplet at 1.25 τ (H4) and a doublet at 1.61 τ in the aromatic region.

5-Methyl-1,7-Phenanthroline.

3,5-Diaminotoluene was prepared by the reduction of 3,5-dinitrotoluene with hydrazine hydrate and Raney nickel. To a solution of 3,5-dinitrotoluene (1.0g) in ethanol (50ml) was added an excess of hydrazine hydrate (98%; 6ml). A deep wine red solution resulted. To this was added Raney nickel (0.1g) and the mixture, after standing for ten minutes, was refluxed for an hour on a steam bath. An additional amount of Raney nickel (0.1g) was added and the mixture refluxed for an additional half hour to destroy the excess hydrazine hydrate. The mixture was cooled and filtered. The filtrate on evaporation left

a colorless oil, which darkened somewhat upon exposure. The diaminotoluene was used in the following Skraup reaction without any further purification.

3,5-Diaminotoluene (from 1.0g of 3,5-dinitrotoluene), arsenic pentoxide (2.8g), glycerol (5ml), sulfuric acid (4ml) and water (1ml) were mixed well and heated for one hour at 110-120° and for three hours at 140-150°. The mixture was cooled and poured into cold water. Aqueous sodium hydroxide was added to obtain a pH of between 5 and 6. The whole was extracted with benzene and the extract was washed by shaking with aqueous sodium hydroxide. The washed solution was dried with anhydrous sodium sulfate and evaporated to dryness. The brown residue was subjected to evaporative distillation under reduced pressure whereupon 5-methyl-1,7-phenanthroline distilled between 70-80°/0.02mm and solidified. The crystalline material was recrystallised from benzene-petroleum ether to give colorless prisms (0.04g), m.p. 113-114°

Calcd. for $C_{13}H_{10}N_2$:	C, 80.38;	H, 5.19;
Found:	C, 80.77;	H, 5.24.

3-Amino-5-nitrotoluene (78).

Ammonia (28%; 8ml) was mixed with water (22ml) and the mixture was saturated with hydrogen sulfide gas. 3,5-Dinitrotoluene (5.0g) in ethanol (50ml) was added and the mixture was boiled under reflux for one hour. The hot solution was filtered and diluted with water. The precipi-

tate obtained was extracted repeatedly with boiling hydrochloric acid, the combined acid extract was washed once with chloroform and was made alkaline with ammonia. The precipitated brown material was recrystallised from ethanol-water. Yield of 3-amino-5-nitrotoluene, m.p. 98°, was 3.0g.

5-Nitro-7-methylquinoline.

3-Amino-5-nitrotoluene (1.52g), arsenic pentoxide (1.6g), glycerol (2.7ml), sulfuric acid (d. 1.84; 2.3ml), and water (0.75ml) were mixed and heated under reflux for one hour at 110-120° and for three hours at 140-150°. The resulting mixture was poured into water, made alkaline with aqueous sodium hydroxide and extracted with benzene. The benzene extract was dried with anhydrous sodium sulfate, and evaporated to dryness and chromatographed over alumina using benzene-petroleum ether (1:1) as solvent and eluent. The light colored fraction of this eluate was evaporated to dryness to give cream colored needles. Recrystallisation from ethanol gave cream colored needles, m.p. 135-136° (0.9g).

Calcd. for $C_{10}H_8N_2O_2$:	C, 63.8;	H, 4.29;
Found:	C, 63.79;	H, 4.18.

5-Amino-7-methylquinoline.

5-Nitro-7-methylquinoline (0.7g), methanol (15ml), and palladium charcoal catalyst (5%; 0.2g) were

cautiously mixed together and shaken with hydrogen at atmospheric pressure. When the required amount of hydrogen was absorbed, the catalyst was filtered off and the residue from the filtrate was sublimed (70-80°/0.02mm). The sublimate was recrystallised from ethanol to give pale yellow needles of 5-amino-7-methylquinoline (0.5g). M.p. 155-156°.

Calcd. for $C_{10}H_{10}N_2$:	C, 75.92;	H, 6.37;
Found:	C, 75.58;	H, 6.15.

4,5-Dimethyl-1,7-phenanthroline.

The method described by Campbell et al. for the preparation of 4-methylquinoline was adapted successfully for making this compound. A mixture of 5-amino-7-methyl-quinoline hydrochloride (from 0.16g of the amine), ferric chloride hexahydrate (0.43g), anhydrous zinc chloride (0.02g) and ethanol (2.0ml) was warmed short of reflux and methyl vinyl ketone (0.1g) was added dropwise. The mixture was then refluxed with stirring for three hours and left overnight at room temperature. The alcohol was evaporated off and aqueous sodium hydroxide was added. The alkaline solution was extracted several times with benzene and the combined benzene extract was dried and evaporated to yield a brownish solid. This on evaporative distillation (80-90°/0.02mm), recrystallisation from benzene-petroleum ether, and sublimation gave 4,5-dimethyl-1,7-phenanthroline

(0.08g) as colorless prisms, m.p. 122-124°.

Calcd. for $C_{14}H_{12}N_2$: C, 80.74; H, 5.81;

Found: C, 81.06; H, 6.23.

6-Methyl-1,7-phenanthroline. (80).

2,4-Diaminotoluene (1.3g), glycerol (5.5ml), arsenic pentoxide (3.0g), sulfuric acid (8.0ml) and water (2.0ml) were mixed well and heated with reflux at 120° for one hour and at 140-150° for three hours. The resulting dark mixture was worked up as usual for the isolation of the phenanthroline. 6-Methyl-1,7-phenanthroline was recrystallised from benzene-petroleum ether (b.p. 60-80°) and sublimed at reduced pressure (90-100°/0.02mm). M.p. 95-96°. Yield of pure product 0.4g.

Bromination of 6-methyl-1,7-phenanthroline with N-bromosuccinimide.

6-Methyl-1,7-phenanthroline (0.06g), N-bromosuccinimide (0.05g) freed from volatile impurities in high vacuum (81), and benzoyl peroxide (trace) were refluxed with carbon tetrachloride (6.0ml) for three hours. The resulting mixture was cooled, filtered and the solvent was removed. The crystalline residue was recrystallised from benzene-petroleum ether to give colorless prisms (0.05g), m.p. 118-120° not identical with the starting material. The NMR spectrum in methylene chloride showed no resonance due to a methyl group; but the low field part

of the spectrum showed all the signals shown by 6-methyl-1,7-phenanthroline (Table VI) with only minor variations in chemical shifts. The resonance due to the methylene group of relative intensity of two protons was only slightly separated from the solvent peak and could not be seen at low resolution.

Attempted alkylation of 6-Bromomethyl-1,7-phenanthroline with isopropyl magnesium bromide.

Isopropyl magnesium bromide was prepared from magnesium metal (0.1g), isopropyl bromide (0.38ml) in dry ether (3.0ml). 6-Bromomethyl-1,7-phenanthroline (0.05g), in ether (4.0ml) was added and the mixture was refluxed for two hours. The resulting dark solution was treated with acetone to destroy the excess reagent, and then with water. The resultant was worked up for basic material, which upon distillation under high vacuum did not amount to much. There was, however, left a considerable amount of dark residue in the distillation bulb.

5-Nitroquinoline and 7-nitroquinoline.

These were prepared by the Skraup reaction on m-nitroaniline (15.0g) and separated to obtain 5-nitroquinoline and 7-nitroquinoline, as described by Rowe et al. (82).

5-Nitroquinoline (8.0g), m.p. 71°,

7-Nitroquinoline (2.2g), m.p. 136°.

5-Aminoquinoline.

5-Nitroquinoline was reduced catalytically with hydrogen and palladium charcoal catalyst (5%), in dilute methanolic solution at atmospheric pressure by a procedure similar to one described by Long et al. (83) for the reduction of alkylnitroquinolines. 5-Aminoquinoline, m.p. 110°, was obtained in good yields. Lit. m.p. 110° (84).

7-Aminoquinoline.

This was prepared by reduction of 7-nitroquinoline with hydrazine hydrate (98%) and Raney nickel in ethanolic solution in the usual manner. 7-Aminoquinoline (0.6g) m.p. 93-94°, was obtained from 1.0g of the nitroquinoline and was obtained pure by sublimation at 50-60°/0.02mm. Lit. m.p. 93-94° (84).

4-Methyl-1,7-phenanthroline. (66)

A mixture of 5-aminoquinoline hydrochloride (from 0.43g of the amine), ferric chloride hexahydrate (1.3g) and zinc chloride (0.05g) in ethanol (3.0ml) was warmed to 60° and methyl vinyl ketone (0.2g) was added dropwise. The mixture was refluxed with stirring for two hours and allowed to stand over night at room temperature. The ethanol was removed on a rotary evaporator. Aqueous alkali was added and the basic material was extracted with benzene. The dried (Na_2SO_4) benzene extract was taken to

dryness when a brownish residue was obtained. Crystallisation from acetone, followed by sublimation, gave 4-methyl-1,7-phenanthroline (0.25g), m.p. 103-104°.

Ethyl vinyl ketone.

This was prepared essentially as described by McMahon et al. (85). Chlroform was substituted for carbon disulfide, as the solvent for the Friedel and Crafts reaction. Propionyl chloride (30.0g) aluminum chloride (46.0g) and chloroform (100ml) were used in the initial step. Yield of ethyl vinyl ketone, b.p. 102°, was 18.0g.

4-Ethyl-1,7-phenanthroline.

The procedure followed was similar to that used for 4-methyl-1,7-phenanthroline, ethyl vinyl ketone being substituted for the methyl vinyl ketone. 5-Aminoquinoline hydrochloride (from 0.6g of the amine), ferric chloride hexahydrate (1.8g), zinc chloride (0.07g), ethyl vinyl ketone (0.32g) and ethanol (3.6ml) were used. The reaction mixture was worked up in the same way to give 4-ethyl-1,7-phenanthroline (0.4g) m.p., 127-128°.

Calcd. for $C_{14}H_{12}N_2$:	C, 80.74;	H, 5.81;
Found:	C, 80.71;	H, 5.63.

10-Methyl-1,7-phenanthroline.

7-Aminoquinoline (0.29g) as the hydrochloride, ferric chloride hexahydrate (0.87g) and zinc chloride (0.04g) in ethanol (2.0ml) were reacted with methyl vinyl

ketone (0.1g) in the usual manner. The crude product was chromatographed over alumina and eluted with benzene-petroleum ether (1:1) and repeatedly crystallised from acetone, benzene-petroleum ether, and ether. Evaporative distillation (70-80°/0.02mm) followed by crystallisation from acetone gave colorless prisms (0.05g) m.p. 102-103°.

Calcd. for $C_{13}H_{10}N_2$:	C, 80.38;	H, 5.19;
Found:	C, 78.03;	H, 5.69.

Isobutyryl chloride.

This was conveniently made using the method of Brown (86). Isobutyric acid (44.0g) and benzoylchloride (71.0g) were used. Yield of isobutyryl chloride, b.p. 92°, was 32.0g.

Isobutyrophenone.

Following the procedure of Vogel (87) for the preparation of butyrophenone, isobutyryl chloride (35.0g), benzene (88ml) and aluminum chloride (47.0g) were reacted in a Friedel and Crafts reaction to obtain isobutyrophenone, (35.0g), b.p. 92-98°/10mm.

Isobutylbenzene.

Using essentially the procedure of Brady et al. (88) for the Clemmensen reduction of p-methylacetophenone, isobutyrophenone (34.0g) was reduced with mossy zinc (200.0g), mercuric chloride (40.0g) in water (800ml), and concentrated hydrochloric acid (1L) in water (250ml).

Yield of pure isobutylbenzene, b.p. 172°, was 23.0g. The NMR spectrum showed a doublet at 7.59 τ (CH_2) nine visible lines at 8.17 τ(CH) and an intense doublet at 9.15 τ(gem CH_3).

p-Acetylaminoisobutylbenzene.

Isobutyl benzene (20.0g) was added dropwise to an ice cooled mixture of sulfuric acid (48ml; d. 1.84) and nitric acid (18ml; d. 1.42), with efficient stirring, the temperature not being allowed to rise above 30°. The mixture was stirred for twenty minutes and the oily nitration product (18.0g) was separated from the nitrating mixture. The nitration product was washed with water, alkali, and again with water and dried (Na_2SO_4).

Reduction of the nitro compounds (12.0g) was carried out with hydrazine hydrate (35ml) and Raney nickel (0.6g) in ethanol (400ml) by refluxing the mixture of these, as usual, for an hour. Raney nickel (0.2g) was further added to decompose the unreacted hydrazine. The cooled mixture was filtered to remove the catalyst. The ethanol was removed on a rotary evaporator under reduced pressure. The liquid amine was converted to the acetyl derivative as follows. The crude amine was dissolved in slightly more than the required amount of hydrochloric acid (7.5ml) in water (500ml). Acetic anhydride (7.0ml) followed by sodium acetate (9.0g) in water (40ml) were

added, the solution was cooled in ice with vigorous stirring. Almost colorless acetyl derivative crystallised out. Recrystallisation from aqueous ethanol gave colorless plates (7.5g). The compound upon heating, softens around 125° but soon recrystallises to melt again at a temperature above 150°. Hickinbottom et al. have reported a melting point 127-128° for this compound (89). The NMR spectrum of the compound (acetone) showed two doublets at 2.25 τ (H2,6) and at 3.19 τ (H3,5); $J_{2,3}$ = 8.2 cps. The high field spectrum ($CHCl_3$) showed the isobutyl group and the $-CO-CH_3$ group.

3,5-Dinitro-4-acetylaminoisobutylbenzene.

Following essentially the procedure employed by Brady and Cunningham (90) for the preparation of a lower homologue, p-acetylaminoisobutylbenzene (3.2g) was added slowly with stirring to fuming nitric acid (30ml; d. 1.5) cooled to -10 to -5° with ice-salt mixture. The temperature was not allowed to rise above 0° during the addition. The mixture was kept at 0° for another half hour with stirring and then poured into ice water containing a little urea, stirred vigorously, filtered, and washed with plenty of water immediately. The pale yellow product, sucked dry and then recrystallised from ethanol gave pale yellow 3,5-dinitro-4-acetylamincisobutylbenzene (2.1g), m.p. 159-161°.

Calcd. for $C_{12}H_{15}N_3O_5$: C, 51.24; H, 5.38;
Found: C, 50.55; H, 5.25.

The NMR spectrum of the compound ($CHCl_3$) showed a single line at 1.89 τ (H2,6), a doublet at 7.3 τ (CH_2), a multiplet at 7.9 τ (CH), a single line at 7.7 τ ($-COCH_3$) and a doublet at 9.05 τ (gem dimethyl), all of right intensities.

3,5-Dinitroisobutylbenzene.

3,5-Dinitro-4-acetylaminoisobutylbenzene (2.0g) was hydrolysed by heating with concentrated sulfuric acid (5ml) and water (5ml) for one hour at 90°; when the pale yellow acetyl compound was replaced by a golden yellow amino compound. Water was added and the mixture was filtered; the amine was washed with water and dried over calcium chloride in a vacuum desiccator to constant weight. The dried amine (1.5g), dissolved in sulfuric acid (8ml) and absolute ethanol (30ml) was heated on a water bath and dry powdered sodium nitrite (3.5g) was added in small portions, sufficient time being allowed between additions, for the effervescence to slacken. After the addition, the mixture was heated for an additional 15 minutes and then poured into cold water. This was extracted with light petroleum and the dried (Na_2SO_4) extract on evaporation gave a yellow oil. The material could not be induced to crystallise from ether, alcohol, or petroleum ether. It

was therefore distilled cautiously at high vacuum, making no attempts at total recovery. About three fourths of the material was collected, at 80-90°/0.02mm. 3,5-Dinitroisobutylbenzene (0.8g) was obtained as an oily liquid, yellow in color. It gave a purple coloration with acetone and sodium hydroxide and a deep wine red color with hydrazine, in ethanolic solution; tests which were also answered by other meta-dinitrobenzene type of compounds. The NMR spectrum of the compound showed a triplet at 1.18 τ (H4) and a doublet a 1.6 τ in the aromatic region and the isobutyl group bands at high field, all bands showing the right relative intensity.

5-Isobutyl-1,7-phenanthroline.

To a solution of 3,5-dinitroisobutylbenzene (0.6g) in ethanol (30ml) was added hydrazine hydrate (98%, 5ml). A deep wine red colored solution resulted. Raney nickel (0.1g) was then added and the mixture was refluxed on a steam bath for one and a half hours. The reaction mixture was then cooled, filtered to remove the catalyst and evaporated under reduced pressure to remove the solvents. The residual liquid, which was light in color, darkened somewhat upon exposure. This was directly used in the Skraup reaction.

The 3,5-diaminoisobutylbenzene was mixed with arsenic pentoxide (1.5g), glycerol (2.4ml), sulfuric acid

(3.7ml) and water (0.9ml) and heated at 110-120° for one hour and then the temperature was raised slowly to 150° and maintained as such for three additional hours. The mixture was cooled and mixed with cold water. Crude 5-isobutyl-1,7-phenanthroline was extracted with warm petroleum ether after partial basification as usual. The compound was first obtained crystalline, from an ether-hexane solution, by cooling in dry-ice; but later recrystallisation could be done from petroleum ether-benzene, and ether-petroleum ether mixtures. The substance was subjected to evaporative distillation (80-90°/0.02mm) and three recrystallisations, when colorless prisms of 5-isobutyl-1,7-phenanthroline (0.1g) were obtained, m.p. 84-84.5°.

Calcd. for $C_{16}H_{16}N_2$:	C, 81.32;	H, 6.83;
Found:	C, 80.91;	H, 6.68.

The u.v. spectral data of the compound is shown in table VIII. The NMR spectrum is shown in figure 21.

4,7-Phenanthroline.

This was prepared according to the procedure of Haskelberg (91). p-Nitroaniline (17.0g), glycerol (6.5g), ferrous sulfate (4.2g), nitrobenzene (7.5g), and sulfuric acid (40.0g) were used in the double Skraup cyclisation. Yield of 4,7-phenanthroline, m.p. 173°, was 8.0g. The NMR spectrum of 4,7-phenanthroline is shown in figure 20.

6-Aminoquinoline.

To a solution of 6-nitroquinoline (4.0g) in ethanol (40ml) was added hydrazine hydrate (98%; 6ml) followed palladium charcoal catalyst (5%; 0.2g). The whole was refluxed on a steam bath for an hour. The catalyst was filtered off, the ethanol and hydrazine were removed at water pump pressure on a rotary evaporator and the product was recrystallised. Sublimation (60°/ 0.04mm) gave anhydrous 6-aminoquinoline (3.1g), m.p. 114°.

1-Methyl-4,7-phenanthroline, (66).

6-Aminoquinoline (0.3g) as its hydrochloride, ferric chloride hexahydrate (0.9g), zinc chloride (0.05g) in ethanol (2ml) were warmed to 60° and methyl vinyl ketone (0.12g) was added dropwise into the mixture. The whole was refluxed for two hours with stirring and left standing overnight. The ethanol was evaporated down and the basic product was extracted with benzene, from a basified aqueous solution. 1-Methyl-4,7-phenanthroline was recrystallised from benzene-petroleum ether in colorless needles, m.p. 105-106°.

1-Ethyl-4,7-phenanthroline.

Using ethyl vinyl ketone instead of methyl vinyl ketone, in a reaction analogous to that for the preparation of 1-methyl-4,7-phenanthroline, the above compound

was obtained in good yield. 6-Aminoquinoline (0.6g), ferric chloride hexahydrate (1.7g), zinc chloride (0.07g), ethanol (3.6ml) and ethyl vinyl ketone (0.32ml) were used. 1-Ethyl-4,7-phenanthroline was purified by recrystallisation from ether-petroleum ether and sublimation (70-80°/0.02mm) and obtained as needles, m.p. 106-107°.

Calcd. for $C_{14}H_{12}N_2$:	C, 80.74;	H, 5.81;
Found:	C, 80.85;	H, 5.77.

2-Nitro-5-aminotoluene (92).

This was made according to the procedure recommended by McGookin et al. starting from "acet-m-toluidide" (3-acetylaminotoluene). Acet-m-toluidide (12.5g) was nitrated with a mixture of nitric acid (34ml), sulfuric acid (38ml) and acetic acid (50ml) by stirring at 15° for one and a half hours. The mixture was poured into cooled water and the precipitated nitration product was recrystallised from ethanol to give 2-nitro-5-acetylaminotoluene as pale yellow prisms, m.p. 99-101°. This was hydrolysed by heating with sulfuric acid (40ml 50% $^{v}/_{v}$) on a water bath for four hours. The mixture, poured into ice cold water, gave 2-nitro-5-aminotoluene as a yellow precipitate, which was filtered, washed and sucked dry. Yield of the compound, m.p. 134°, was 6.5g.

2,5-Diaminotoluene.

2-Nitro-5-aminotoluene (6.0g) was reduced with hydrazine hydrate (98%, 9ml) and palladium charcoal catalyst (5%, 0.3g) in ethanol (100ml), the mixture being refluxed for one and a half hours. Filtration to remove the catalyst, followed by evaporation of the solvent and hydrazine gave a somewhat dark colored, oily liquid, which was converted to the diacetyl derivative. Recrystallisation from aqueous ethanol gave colorless prisms of 2,5-diacetylaminotoluene (3.5g), m.p. 220°.

5-Methyl-4,7-phenanthroline.

2,5-Diacetylaminotoluene (3.0g), arsenic pentoxide (3.2g), glycerol (5ml), sulfuric acid (5ml) and water (1.2ml) were mixed and heated to raise the temperature gradually to 110° in one hour, maintained at 110-120° for one hour and then at 140-150° for three hours. The whole was poured into water and the mixture worked up for the phenanthroline in the usual manner. 5-Methyl-4,7-phenanthroline (0.6g) was obtained pure by successive sublimation, chromatography over alumina with benzene-petroleum ether for elution, and crystallisation from benzene-petroleum ether. M.p. 103-104°.

Calcd. for $C_{13}H_{10}N_2$:	C, 80.38;	H, 5.19;
Found:	C, 79.82;	H, 5.13.

4,8-Dimethyl-6-nitroquinoline.

Commercially available (Eastman Kodak) 2-Methyl-5-

nitroaniline (9.5g) as its hydrochloride, ferric chloride hexahydrate (27.0g), zinc chloride (1.5g) in ethanol (50ml) were warmed short of reflux and methyl vinyl ketone (1ml) was added drop by drop so that the temperature did not exceed 70°. The mixture was refluxed for three hours and allowed to stand overnight. The ethanol was removed on a rotary evaporator and the residue was treated with aqueous sodium hydroxide. The precipitated material was filtered washed with water and recrystallised from benzene-petroleum ether. Yield of colorless needles of 4,8-dimethyl-6-nitroquinoline, m.p. 163-164°, was 4.9g.

Calcd. for $C_{11}H_{10}O_2N_2$;	C, 65.33;	H, 4.98;
Found:	C, 65.44;	H, 5.22.

6-Amino-4,8-dimethylquinoline.

To a solution of 4,8-dimethyl-6-nitroquinoline (4.0g) in ethanol (50ml) was added hydrazine hydrate (7ml) followed by palladium charcoal catalyst (5%, 0.2g) and the mixture was refluxed for one and a half hours. The catalyst was filtered off and the filtrate was evaporated down to dryness. The residue, recrystallised from benzene-petroleum ether gave colorless 6-amino-4,8-dimethylquinoline (2.8g), m.p. 195-196°.

Calcd. for $C_{11}H_{12}N_2$;	C, 76.71;	H, 7.02;
Found:	C, 76.87;	H, 7.13.

1,5-Dimethyl-4,7-phenanthroline.

6-Amino-4,8-dimethylquinoline (0.54g), arsenic pentoxide (2.4g), glycerol (1.2ml), sulfuric acid (4.5ml) and water (1.1ml) were mixed well and heated at 110-120° for one hour and then the temperature was raised to 140-150° and maintained at this temperature for four hours. At the end of this period the reaction mixture was cooled and poured into ice water. Aqueous sodium hydroxide was added to obtain a pH of between 5 and 6 and the mixture was extracted with benzene. The dried (sodium sulfate) benzene extract was evaporated and the product was sublimed (80-90°/0.02mm). The crystalline sublimate was recrystallised from benzene-petroleum ether. Yield of colorless needles of 1,5-dimethyl-4,7-phenanthroline, m.p. 117-118°, was 0.3g.

Calcd. for $C_{14}H_{12}N_2$;	C, 80.74;	H, 5.81;
Found:	C, 80.40;	H, 6.03.

1,10-Phenanthroline:

Commercially available "O-phenanthroline" (Fisher Scientific Co., certified reagent) was sublimed (70-80°/0.02mm) and the anhydrous material melting at 117° was used to measure the spectra.

1,2,3,4-Tetrahydroisoquinoline.

Isoquinoline (100g), copper chromite catalyst (10g) (93) and absolute ethanol (200ml) were placed in the

bomb of a high pressure hydrogenator and reduced according to the procedure of Cram *et al.* (94) at a pressure of 1800 p.s.i. and a temperature of 180°. The product (95g) was purified by distillation. B.p. 234-236°.

1,2,3,4-Tetrahydro-7-nitroisoquinoline hydrochloride (95).

Tetrahydroisoquinoline (10.5g) was converted into the sulphate by dissolving it in a theoretical amount of 5N sulfuric acid and evaporation to dryness. The powdered salt was added to a solution of potassium nitrate (10g) in sulfuric acid (d 1.84, 50ml) with ice cooling and after being kept overnight at room temperature was poured on crushed ice and cautiously neutralised with aqueous ammonia. The bases were extracted with chloroform and the dried (potassium carbonate) extract was evaporated to dryness at room temperature and reduced pressure. The residue was extracted with warm light petroleum and the extract evaporated as above. The free bases were converted to the hydrochlorides, which recrystallised from aqueous acetone gave 1,2,3,4-tetrahydro-7-nitroisoquinoline hydrochloride (7.0g), m.p. 259-260°.

1,2,3,4-Tetrahydro-7-aminoisoquinoline.

To a suspension of 1,2,3,4-tetrahydro-7-nitroisoquinoline hydrochloride (4.0g) in ethanol (40ml) was added hydrazine hydrate (98%; 7.0ml) followed by

palladium on charcoal catalyst (5%, 0.2g). The mixture was allowed to stand for 10 minutes and then refluxed for one hour on a steam bath. The catalyst and solvent were removed by filtration and evaporation under reduced pressure respectively and a chloroform solution of the residue was washed twice with small amounts of water. The dried (sodium sulfate) chloroform solution was evaporated to dryness on a rotary evaporator. 1,2,3,4-Tetrahydro-7-aminoisoquinoline (2.3g) crystallised in prisms, from benzene-petroleum ether. M.p. 121-123°. Lit. m.p. 120-121° (95).

7-Aminoisoquinoline.

1,2,3,4-Tetrahydro-7-aminoisoquinoline (1.2g) was dehydrogenated by heating with palladium charcoal catalyst (5%; 0.1g) and p-cymene (30ml) at 120-130° for two hours. After cooling, the catalyst was removed by filtration and the filtrate was extracted thrice with dilute hydrochloric acid. The combined acid extract was washed twice with small amounts of ether, made alkaline with sodium hydroxide solution and extracted with chloroform. The dried chloroform extract upon evaporation gave a solid residue, which was recrystallised from benzene-petroleum ether to give 7-aminoisoquinoline (0.8g), m.p. 201-202°. Lit. m.p. 204° (96). The ultraviolet absorption spectrum in ethanol was identical with the spectrum reported in the literature (97).

2,7-Diazaphenanthrene.

7-Aminoisoquinoline (1.0g), glycerol (2.1g), arsenic pentoxide (0.8g), sulfuric acid (d. 1.85; 2.1g) and water (0.5g) were heated together at 110-120° for one hour and at 130-150° for three hours. The reaction mixture was cooled and poured into ice cold water, basified to a pH between 5 and 6 (pH paper). The mixture was extracted with chloroform several times, and the combined extract was dried (sodium sulfate) and evaporated to dryness. The residue was sublimed at a pressure of 0.02mm and 90-100° (air bath). The crystalline sublimate was recrystallised from ether-petroleum ether and resublimed. 2,7-Diazaphenanthrene was obtained as prisms (0.5g), m.p. 144-145°.

Calcd. for $C_{12}H_8N_2$: C, 79.98; H, 4.48%

Found: C,79.81; H, 4.40%

The u.v. absorption spectral data is shown in table IX. The NMR spectrum is shown in figure 23.

Attempted Skraup reaction with 1,2,3,4-tetrahydro-7-aminoisoquinoline.

1,2,3,4-Tetrahydro-7-aminoisoquinoline (1.0g), glycerol (2.0g), arsenic pentoxide (0.8g), sulfuric acid (d. 1.85; 2.1g) and water (0.5g) was heated at 130-140° for four hours. The mixture was poured on ice and made alkaline with aqueous sodium hydroxide, and extracted with chloroform. The dried (sodium sulfate) extract was

evaporated to dryness. The residue was chromatographed on alumina using benzene for developing and benzane-petroleum ether (b.p. 30-60°) for eluting. The only crystalline compound isolated from eluate was identical with the product from the Skraup reaction on 7-amino-isoquinoline. The remaining fractions from the chromatogram gave on evaporation dark colored residues, which could not be induced to crystallise. Attempts to distill the fractions resulted in a dark tar.

DISCUSSION

a) The preparation of the various phenanthrolines.

The Skraup and related reactions offer the most convenient route to alkylphenanthrolines of the quinoline type. The introduction of the alkyl group can be accomplished at different stages of the preparation, the deciding factors being convenience, position of the desired group and the probable orientation effects of the alkyl group on the course of the subsequent reactions. The preparations described in the present work followed, up to a point, well established routes and on the whole presented no difficulties except for the labour involved.

In Table II, the starting materials and procedure for preparing the various alkyl-1,7-phenanthrolines are listed. Table III gives the details for the preparation of alkyl-4,7-phenanthrolines. In these tables, procedure I refers to the usual Skraup conditions wherein the amine and the enone (or equivalent) are reacted together with sulfuric acid as the condensing agent and arsenic acid as the oxidising agent. Procedure II refers to an adaptation of the method of Campbell and Schaffner for the synthesis of lepidines (98) wherein zinc chloride and hydrogen chloride are used as the condensing agents and ferric chloride as the oxidising agent, in ethanolic solution. The procedure II

Table II

-1,7-Phenanthroline	Amine	Second Component	Procedure used
2-Methyl- (65)	5-Aminoquinoline (84)	C	I
3-Methyl-	"	MA	I
4-Methyl- (66)	"	MVK	II
4-Ethyl-	"	EVK	II
5-Methyl-	3,5-Diaminotoluene	G	I*
5-Isobutyl-	3,5-Diamino-++ isobutylbenzene	G	I*
4,5-Dimethyl-	5-Amino-7-++ methyl-quinoline	MVK	II
6-Methyl- (80)	2,4-Diaminotoluene	G	I
10-Methyl-	7-Aminoquinoline (84)	MVK	II

C = Crotonaldehyde
MA = Methylacrolein
MVK = Methyl vinyl ketone
EVK = Ethyl vinyl ketone
G = Glycerol
* = See figure
++ = See text

Table III

-4,7-Phenanthroline	Amine	Second Component	Procedure used
1-Methyl- (66)	6-Aminoquinoline	MVK	II
1-Ethyl-	"	EVK	II
1,5-Dimethyl-	4,8-Dimethyl-6-$^{++}$ aminoquinoline	G	I
5-Methyl-	2,5-Diaminotoluene	G	I
3-Methyl- (65)	6-Aminoquinoline	C	I

See Table II for abbreviations.

was used for the lepidine type of syntheses, since the Skraup reaction was known to give low yields of the products in these cases (64, 71).

Several of the amines referred to in Tables II and III were previously known, and were obtained by established routes. Preparations of amines which were not known previously are discussed below.

3,5-Diaminoisobutylbenzene was obtained in several steps from p-acetylaminoisobutylbenzene. The preparation of this is shown in Figure15 (R = $-CH_2-CH(CH_3)_2$). Nitration of isobutylbenzene followed by reduction and acetylation gave predominantly p-acetylaminoisobutylbenzene, which has been obtained previously by another route (89). The melting point of our compound showed slight discrepancy from that reported in the literature. But the structure of the compound was confirmed from its NMR spectrum which showed, in addition to the isobutyl and the $-CO-CH_3$ groups, an uncomplicated four line spectrum (due to an AB system), in the aromatic region, expected of a p-disubstituted benzene. Nitration of this gave 3,5-dinitro-4-acetylaminoisobutylbenzene which was fully characterised by analysis and the NMR spectrum,the aromatic region of which showed a single line of area equal to two protons, in addition to the isobutyl and the $-CO-CH_3$ groups at higher field. Hydrolysis and deamination gave 3,5-dinitro-

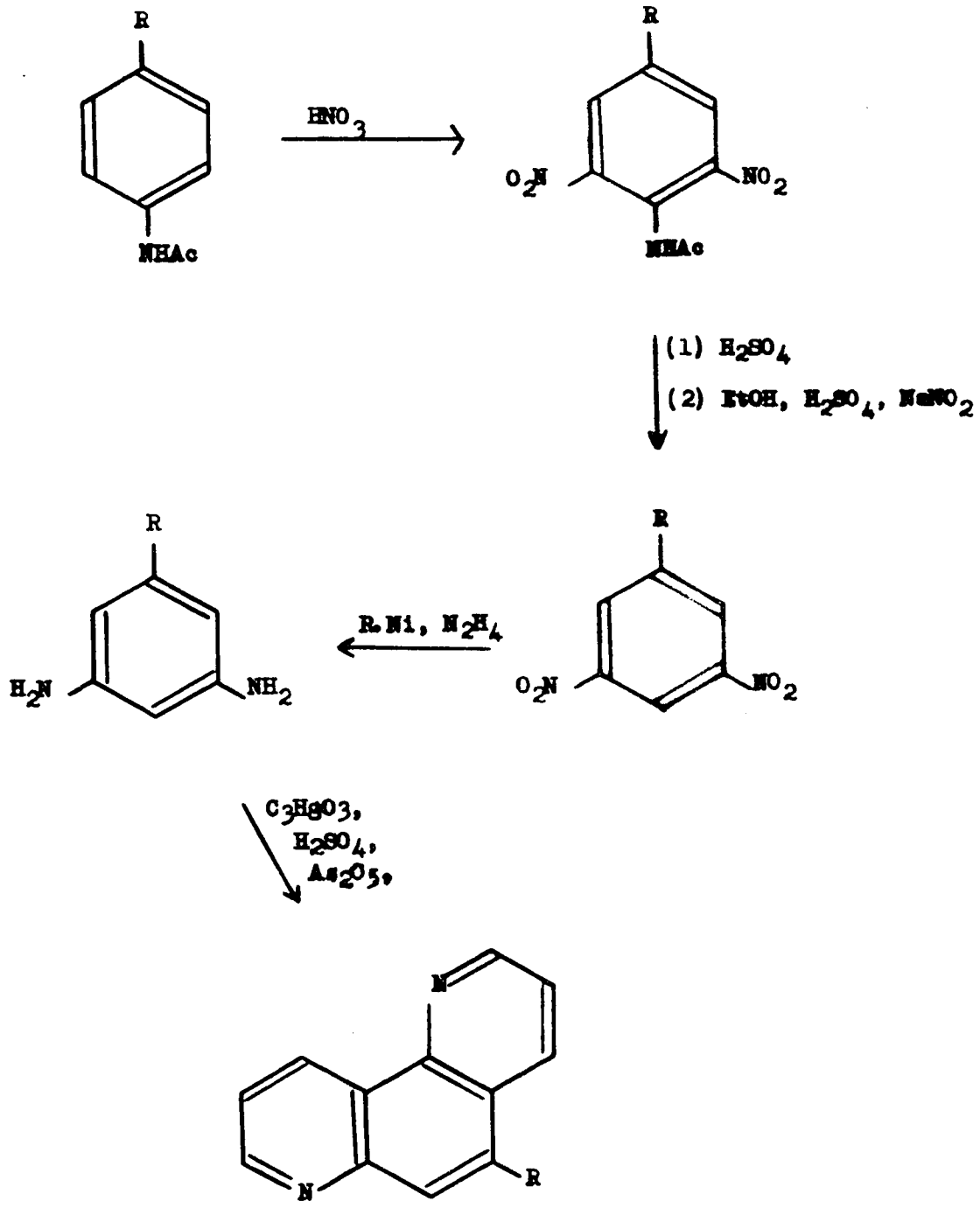


Figure 15. Preparation of 5 -alkyl-1 ,7-phenanthro lines.

isobutylbenzene obtained as a viscous liquid. The aromatic region of the NMR spectrum of the compound was similar to the like region in the spectrum of 3,5-dinitrotoluene and different from that of 2,4-dinitrotoluene. The high field part of the spectrum showed the isobutyl group.

Reduction of 3,5-dinitroisobutylbenzene gave the diamine which was submitted to a Skraup reaction to give 5-isobutyl-1,7-phenanthroline. In the above sequence of preparations, only the final compound and one intermediate were analysed and the other intermediates were characterised by their NMR spectra.

5-Amino-7-methylquinoline was obtained by a Skraup synthesis on 3-amino-5-nitrotoluene followed by the reduction of the nitro compound. The Skraup reaction may be expected to give either a 5-nitro-7-methylquinoline or 7-nitro-5-methylquinoline or a mixture of the two compounds. The major and the only isolated product from this reaction was confirmed to be 5-nitro-7-methylquinoline, since on reduction it gave an amine which showed an ultraviolet spectrum similar to that of 5-aminoquinoline and different from that of 7-aminoquinoline (97) and a quinoline synthesis with methyl vinyl ketone gave 4,5-dimethyl-1,7-phenanthroline the structure of which was independently proved by its NMR spectrum. The formation of 5-nitro-7-methylquinoline as the major product is consistent with

the conclusions of Rowe *et al.* who have studied the orientation effects in the Skraup reaction with various *meta* substituted anilines (82).

4,8-Dimethyl-6-aminoquinoline was obtained by a quinoline synthesis with 3-nitro-6-aminotoluene and methyl vinyl ketone, followed by reduction.

In the preparations described herein, the nitro compounds were usually reduced to the amines with hydrazine hydrate and palladium-charcoal or Raney Nickel (99) as this method was preferable to the methods used previously and gave usually good yields of pure products.

The preparation of 2,7-diazaphenanthrene is shown graphically in Fig. 16. 7-Aminoisoquinoline has been prepared in the past by a Bucherer reaction on 7-hydroxyisoquinoline (96) or by the reduction of 7-nitroisoquinoline (95). The route used in the present work was found to be more suitable on a preparative scale.

(b) *General properties of alkylphananthrolines.*

All the alkylphenanthrolines described in this study were colorless and crystalline solids melting under 150° and could be readily sublimed under reduced pressure at temperatures between 60° and 120°. Sublimation was invariably used for the purification of the compounds from the crude reaction products. Many of the alkylphenanthro-

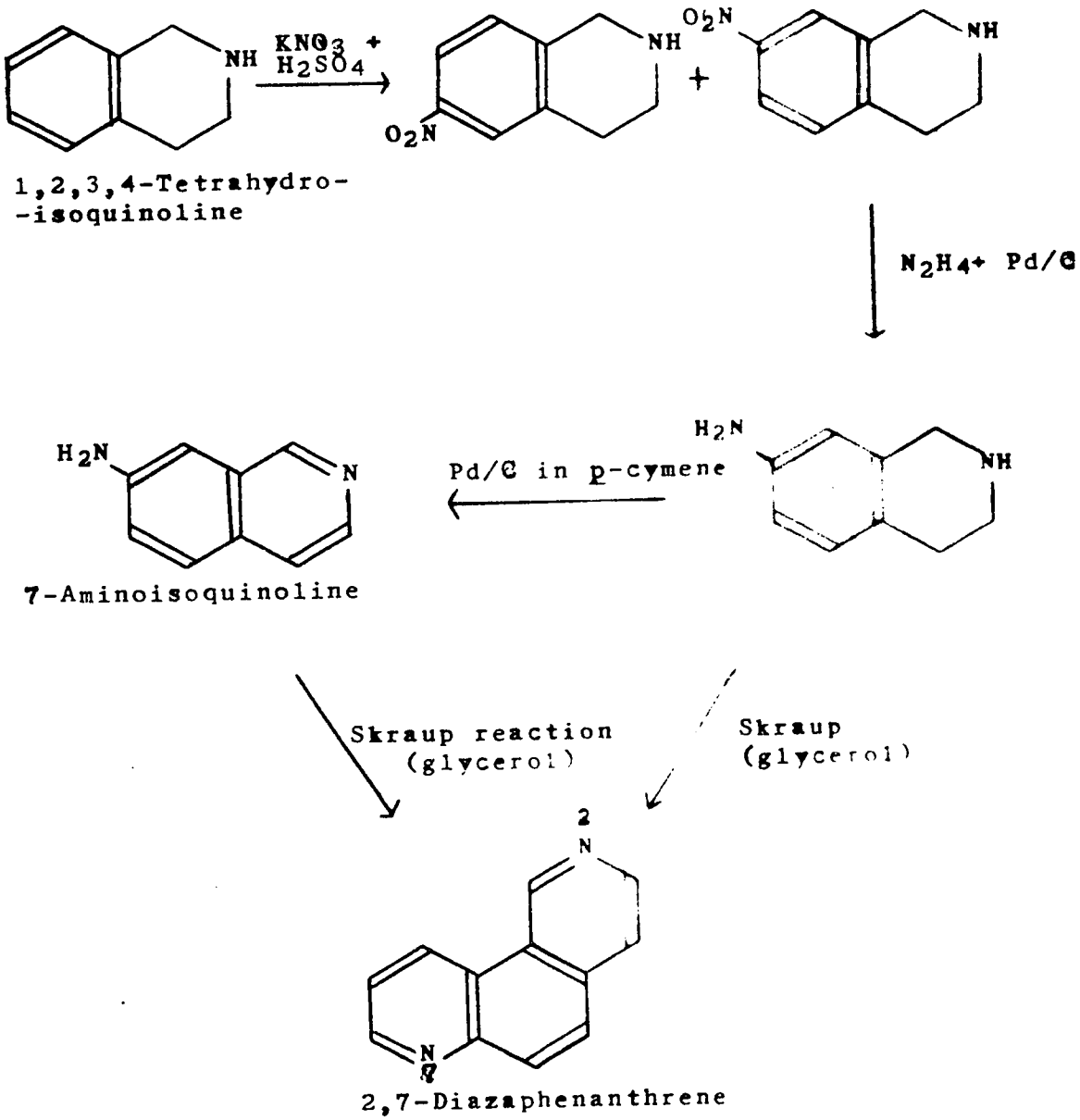


Figure 16. Preparation of 2,7-diazaphenanthrene

lines appear to form hydrates, but the phenomenon of hydration was not studied as such in the present investigation. All the compounds were sublimed at high vacuum for the purposes of characterisation and study. Coincidentally several of the alkylphenanthrolines melt around 100° and thus the melting point is not a useful property for rapid characterisation. The compounds were all sparingly soluble in carbon tetrachloride with the exception of 5-isobutyl-1,7-phenanthroline which was quite soluble. However all the compounds are fairly soluble in ether and acetone and quite soluble in benzene, alcohol, chloroform and methylene chloride.

The alkylphenanthrolines were weak bases like the parent phenanthrolines and purification was often affected by extraction after partial basification (pH 4 -5). They were adsorbed weakly on alumina and could be separated from stronger bases also by chromatography.

With the exception of 10-methyl-1,7-phenanthroline which yellowed quickly on standing, all the alkylphenanthrolines were quite stable in air.

(c) The NMR spectra of the phenanthrolines.

The spectra were measured at 60 M/cs in a methylene chloride solution. The $-CH_2-$ band of methylene chloride does not interfere with any of the bands of alkyl-

phenanthrolines and the chemical shifts in this solvent were comparable to those in a carbon tetrachloride solution. Acetone is also a satisfactory solvent for these compounds; but the acetone band (and the spinning side bands) interfere with the alkyl group bands and also the chemical shifts between the various aromatic protons of the phenanthrolines are often too small in acetone solution. Acetone or a mixture of acetone and methylene chloride was sometimes used as the solvent to separate overlapping bands. Such solvent effects on the position of bands have been observed recently by other workers also (100).

(i) Coupling constants.

It has been established by previous work on pyridines and quinolines (48, 101) that the coupling constants $J_{\alpha\beta}$, $J_{\alpha\gamma}$ and $J_{\beta\gamma}$ are respectively 4-6 cps, 1.7-2.0cps and 7-9 cps. Similar magnitudes were obtained for the coupling constants of the pyridine ring protons in the phenanthroline series. In Table IV the coupling constants between the various protons in the parent phenanthrolines are listed. The benzene ring protons (H5 and H6) in phenanthrolines show an observable coupling of 8-11 cps when they are nonequivalent and sufficiently chemically shifted and thus parallel the ortho protons in benzene derivatives in the magnitude of coupling. The type of long range coupling (0.8 cps) observed between

4 and 8 protons in quinoline systems (56) is also observed in the spectra of 1,7-phenanthrolines between the analogous H10 and H6 protons in all the compounds studied. This phenomenon is of value in the assignment of bands in the spectrum of 1,7-phenanthroline. In the case of 4,7-phenanthrolines also such a coupling of H1 and H5 is no doubt present, but it manifests itself in mere line broadening, probably due to the small or zero chemical shifts between H5 and H6.

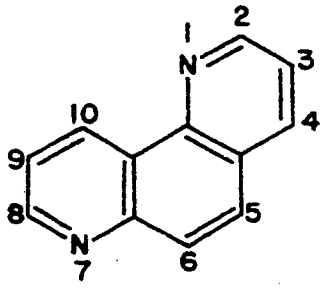
Phenanthroline	$J_{\alpha\beta}$	$J_{\alpha\gamma}$	$J_{\beta\gamma}$	$J_{5,6}$	$J_{6,10}$
1,7-	4.6	1.8 1.9	7.9 8.1	9.6	0.7
1,10-	4.4	1.8	8.1		-
4,7-	4.5	1.8	8.6		
2,7-	4.4 5.0	1.9	8.8	8.9	

Table IV. The coupling constants of the various protons of the phenanthrolines.

Coupling between neighbouring protons of different rings (other than H10 and H6) was not observable. The bands due to the protons α- to the nitrogen atoms, were somewhat broad and this must be due to spin coupling with the nitrogen (102).

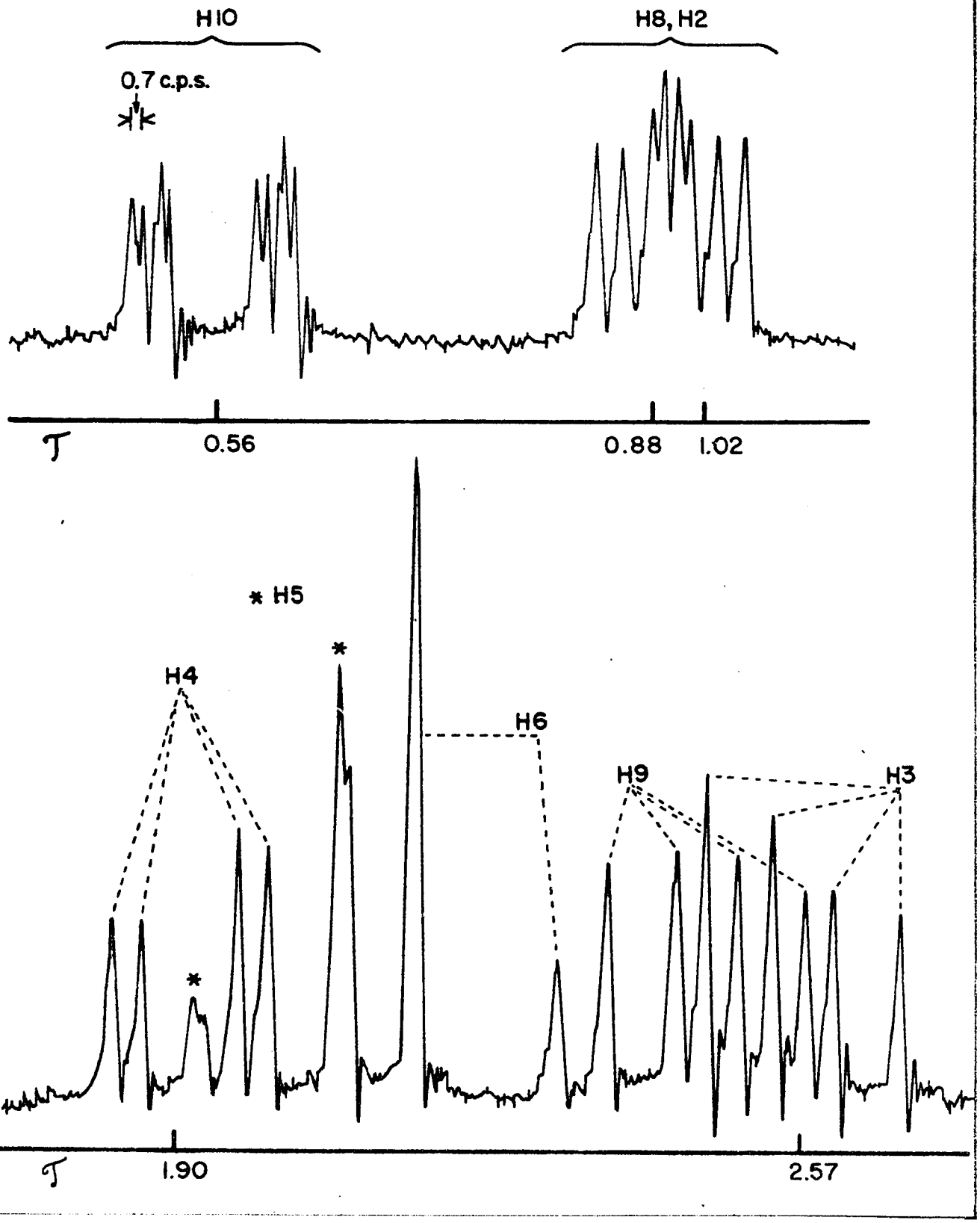
(ii) The spectra of the parent phenanthrolines

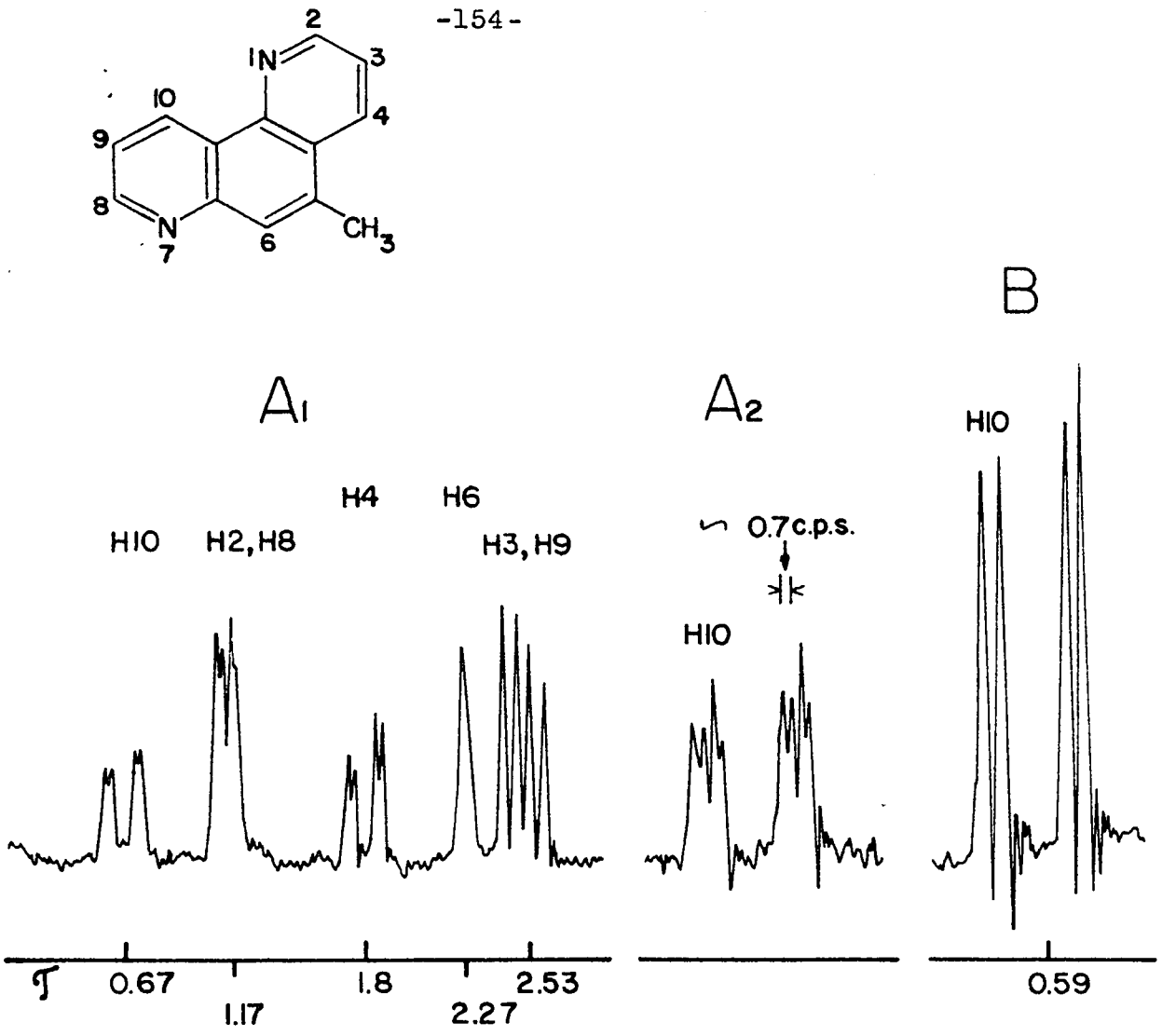
1,7-Phenanthroline: The NMR spectrum of 1,7-phenanthroline is shown in Fig. 17 and Table V. The assignments of H4, H5, and H6 bands (see Table VI) follow from the disappearance of these bands in the compounds substituted in the respective positions. Theoretically H6 band would be expected to occur at lower field than H5 due its proximity to the nitrogen in the 7 position. Therefore the assignment of the two lines at lower field to H6 and the two lines at higher field to H5 is justified. The band consisting of eight lines at the lowest field (0.56 τ) is assigned to H10 in view of its expected low field position due to the large ring current effect at the angular position and due to its nearness to the nitrogen in position 1. This assignment is supported by the multiplicity of the band, which appears as eight lines singularly consistant with its expected coupling with H9, H8 and H6. Such fine coupling (0.7 cps) of H10 and H6 is absent in 6-methyl-1,7-phenanthroline (Fig. 18) and present in other alkylphenanthrolines. The eight lines at the highest field must be due to H3 and H9, and the eight lines centred at 1.0 τ must be due to H2 and H8. Actual assignments in these cases are not obvious but can be made with certainty for H3 and H9 and tentatively for H2 and H8 from a consideration of the intensities of the lines. The bands due to H3 and H9 are separated only



The NMR spectrum of 1.7-Phenanthroline in Methylene Chloride

Figure 17.





The NMR spectrum of 5 Methyl-1,7-Phenanthroline in Methylene Chloride A_1, ring protons, A_2, H10 at slow sweep.

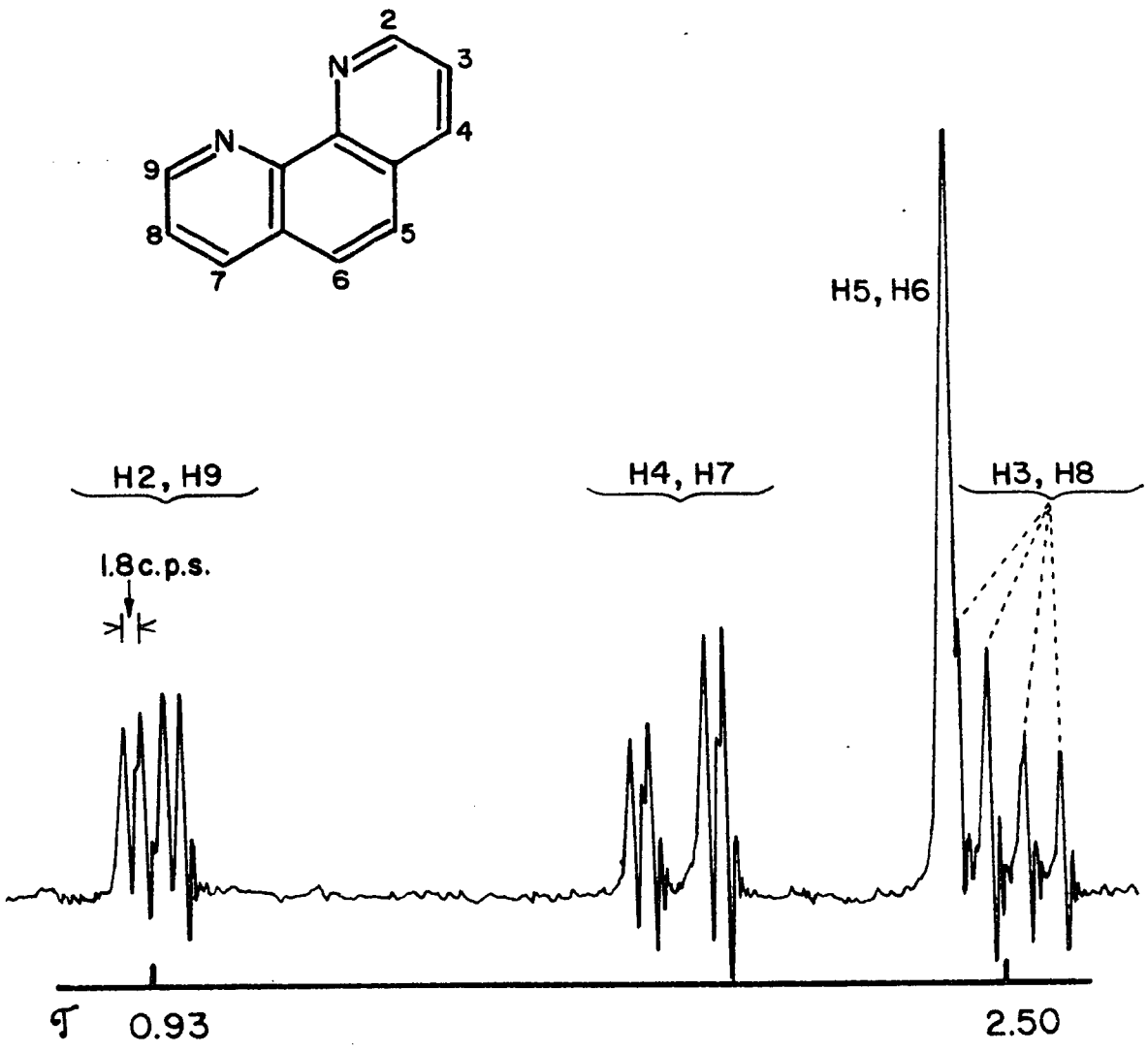
B, Bands of H10 of 6-Methyl-1,7-Phenanthroline at slow sweep.

Figure 18.

slightly from H4 band and well separated from the H10 band (see figure). But H3 and H4 are coupled strongly to each other and to the same extent as are H9 and H10. In view of the small chemical shift and strong coupling between H3 and H4 the lines due to these would be expected to be skewed, with the intensity increasing towards the centre of the H3, H4 spectrum. Therefore the four skewed lines at the higher field having the right spacings (see figure) are assigned to H3, and the four lines showing even intensities to H9. In agreement, the H4 band shows skewed intensity whereas H10 band shows even intensity distribution in the four lines. The position regarding H2 and H8 is less certain as the intensities in this case are not clearly different. Alkyl substitution would not be of any help, as it is known to disturb slightly the chemical shifts of the bands. However the four lines centred at lower field (0.99τ) are tentatively assigned to H8 and those at higher field (1.02τ) to H2, from the intensity distributions in the spectra of 1,7-phenanthroline and of 6-methyl-1,7-phenanthroline. In the latter the intensity differences are better observable. In the spectrum of 1,7-phenanthroline in acetone solution, the benzene protons (H5 and H6) are not chemically shifted from each other and also H2 and H8 were not chemically shifted from each other.

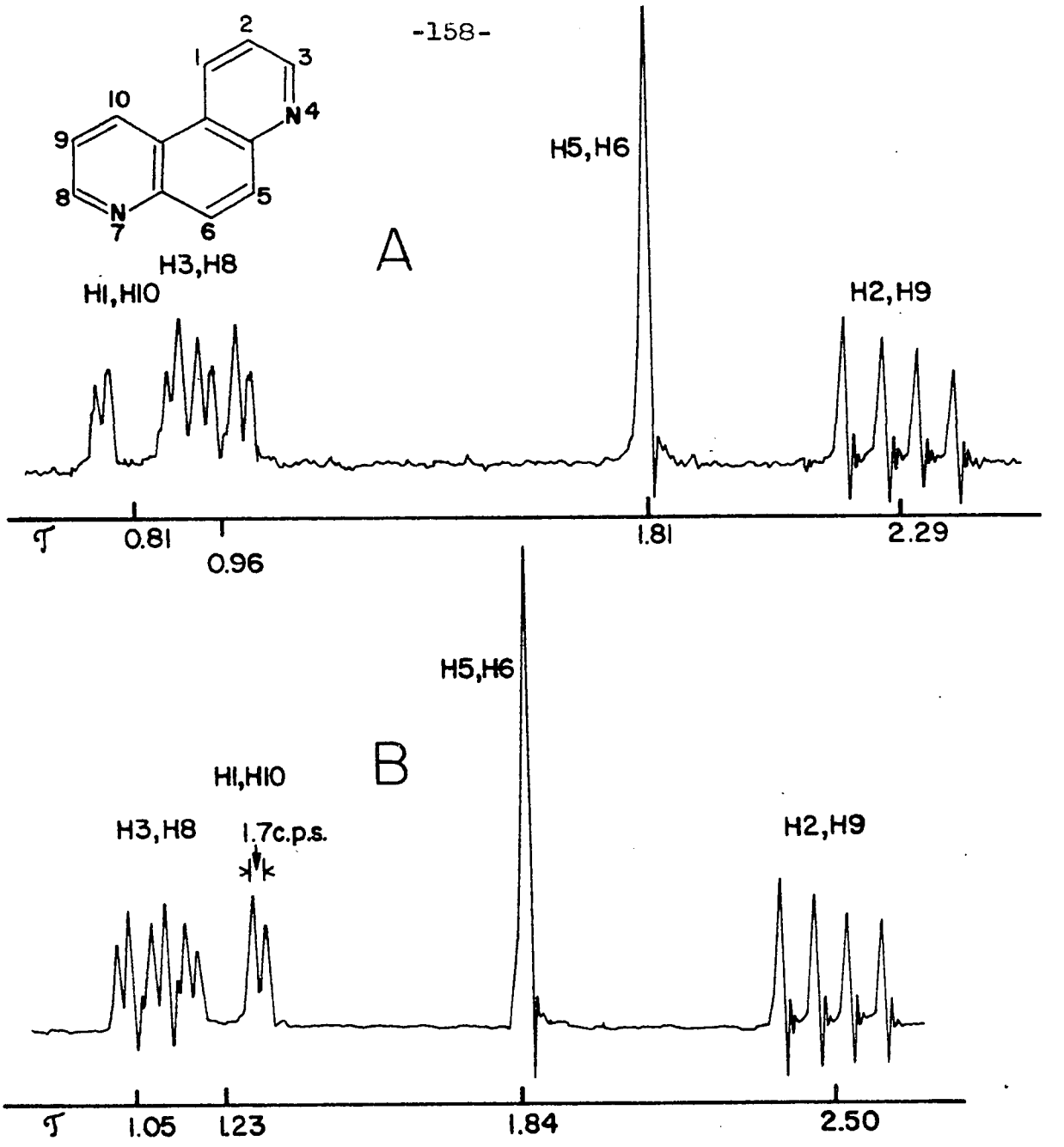
1,10-Phenanthroline: The NMR spectrum of 1,10-Phenanthroline in methylene chloride and the assignments are shown in figure 19 and Table V. The relative chemical shifts in this solvent are nearly the same as in an acetone solution reported by previous workers (77). The assignments and the chemical shifts in τ values shown in Table V confirm the assignment of these workers. However it should be pointed out that the negative value for $J_{2,4}$ (-4.0 cps) quoted by these authors (77) is not consistent with their published spectrum.

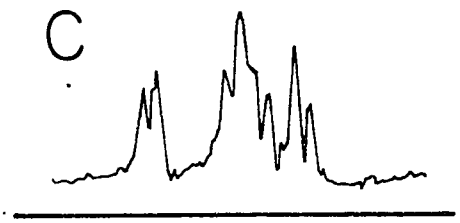
4,7-Phenanthroline: The NMR spectrum and the assignments of 4,7-phenanthroline, in methylene chloride, acetone, and in a mixture of the two solvents are shown in figure 20. The assignments in each case follow from the area, multiplicity of the bands and the spacings associated with the quartets. The bands due to the H3 and H1 protons show a remarkable change of chemical shifts with solvent. The H1 band occurs at higher field than the H3 band in methylene chloride solution and at lower field in acetone solution. In a mixture of the two solvents the two bands are overlapping (see figure 20). Assuming that coupling constants $J_{1,2}$ and $J_{3,2}$ do not change appreciably with the solvent, these spectra show that $J_{1,2}$ and $J_{3,2}$ have the same sign. If the coupling constants were of opposite sign, the intensities of some of the lines would be very low



The NMR spectrum of 1,10-phenanthroline in methylene chloride

Figure 19.





The NMR spectrum of 4.7- Phenanthroline

A, in Acetone

B, in Methylene Chloride

C, Bands of H1, H10, H3, H8, in CH_2Cl_2 & CH_3COCH_3

Figure 20.

in the spectrum in the mixed solvent (see figure). Similar use of solvents has been made for the determination of relative signs of the coupling constants in an ABX system, by Schneider (103).

(iii) The chemical shifts.

The chemical shifts of the individual protons of the parent phenanthrolines along with those of the ring protons of 2,3-lutidine and of 4 and 9 protons of phenanthrene (protons 1 and 5 if numbered as for diazaphenanthrene) are listed in Table V. The data in the table refer to the spectra in methylene chloride solutions unless otherwise stated and the chemical shifts in methylene chloride are considered for comparison in the following discussion. It must be noted that the numbering does not necessarily refer to analogous protons in the various compounds.

(a) The pyridine ring protons in the phenanthrolines.

It is known from previous work on pyridines that the order of appearance of bands due to the ring protons is α, γ, and β from low field to high field (48, 101). The chemical shifts between these protons have been rationalised on the basis of the electron density at the point of attachment. In considering the chemical shifts of the pyridine ring protons in the phenanthrolines, the following additional factors need to be taken into account. (1a) the

Compound	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
5,6-Dimethylpyridine (2,3-Lutidine)		1.72	3.02	2.62						
Phenanthrene (numbering as for phenanthrolines)	1.35				2.34	2.34				
1,7-Phenanthroline (CH_2Cl_2)	-	1.02	2.57	1.90	2.20	2.02	-	0.99	2.47	0.56
" (acetone)	-	0.88	2.25	1.51	1.89	1.89	-	0.88	2.20	0.42
1,10-Phenanthroline (CH_2Cl_2)	-	0.93	2.50	1.89	2.39					-
" (acetone)	-	0.83	2.25	1.54	2.08					
4,7-Phenanthroline (CH_2Cl_2)	1.23	2.50	1.05	-	1.84					
" (acetone)	0.81	2.29	0.96	-	1.81					
2,7-Diazaphenanthrene (CH_2Cl_2)	0.11	-	1.21	2.18	2.09	1.85	-	0.99	2.28	0.99
" (acetone: CH_2Cl_2 5:1)	-0.09	-	1.25	2.11	1.96	1.86	-	1.00	2.26	0.72

Table V. The chemical shifts (in τ values) of the various protons of the phenanthrolines, phenanthrene and 2,3-lutidine.

ring current effect due to the three aromatic rings which deshields all the protons to some extent in all the compounds (48, 104), (1b) the particularly large ring current effect which deshields the protons attached in the positions 1 and 10 (comparable to position 4 in phenanthrene) (48, 105), and (2) the deshielding influence of the nitrogen on protons of other rings, which is of a particularly large magnitude in the case of H10 in 1,7-phenanthrolines due to the nearby nitrogen in 1 position. Thus taking the chemical shifts of the ring protons of 2,3-lutidine as points of reference, the spectrum of 1,10-phenanthroline can be explained on the basis of effect (1a) (77). Except for the generally lower chemical shifts, the spectrum still resembles that of 2,3-lutidine. In the spectrum of 4,7-phenanthroline, in addition to (1a), the effect of the (1b) type is operating and this shifts the H1 band so far down-field that the bands of H1 and H3 are very close to each other. But the spectrum in methylene chloride still retains a resemblance to that of 2,3-lutidine. In acetone solution, the H1 band actually occurs at lower field than the H3 band. The spectrum of 1,7-phenanthroline illustrates the effect (2). In this case (1b) and (2) shift the H10 band to the lowest field (0.56 τ). However that part of the spectrum due to H2, H3 and H4 is still similar to the spectrum of 2,3-lutidine (Figure 17 and Table V).

(b) The benzene ring protons (H5 and H6).

In the present instance, the chemical shifts of protons 9 and 10 in the spectrum of phenanthrene serve as a point of reference. The only deshielding influence on these protons (H5 and H6) is noticeable when the nitrogens are three bonds apart from the protons. Thus 1,10-phenanthroline, in which the nitrogens are far removed from H5 and H6, shows only a very small departure from phenanthrene itself (see Table). In 1,7-phenanthroline, the 7-nitrogen shifts H6 noticeably down-field and in 4,7-phenanthroline both H5 and H6 are shifted to lower field.

The NMR spectra of alkylphenanthrolines.

The chemical shifts of the various protons of the alkyl-1,7-phenanthrolines and 1,7-phenanthroline are listed in Table VI. A comparison of the spectrum of the parent compound with that of the substituted compound reveals the position of the substituent, since substitution causes the disappearance of the signal due to the ring proton which is replaced and also changes the multiplicity of the bands due to protons coupled with the replaced proton. Coupling between the alkyl group protons and the adjacent ring protons is very small (< 0.2 cps) and does not complicate the spectrum noticeably. Another effect of alkyl substitution is the shifting of the protons on the substituted ring to higher field than that observed in the

-1,7-Phenanthroline	H2	H3	H4	H5	H6	H8	H9	H10	Others
Unsubstituted	1.02	2.57	1.90	2.20	2.02	0.99	2.47	0.56	
4-Ethyl-	1.20	2.62	-	2.04	1.84	1.02	2.44	0.51	CH_2,6.80;CH_3,8.62
5-Methyl-	1.17	2.53	1.80	-	2.27	1.17	2.53	0.67	CH_3,7.40
6-Methyl-	1.14	2.53	1.96	2.31	-	1.03	2.53	0.59	CH_3,7.28
10-Methyl-	1.02	2.50	1.83	2.15	1.98	1.23	2.60	-	CH_3,6.68
4,5-Dimethyl-	1.23	2.67	-	-	2.20	1.07	2.47	0.50	CH_3s,7.07,7.07
5-Isobutyl-	1.01	2.44	1.57	-	2.13	1.01	2.41	0.52	CH_2, 7.0 ;CH,7.97;$(CH_3)_2$8.99
" (CCl_4)	1.12	2.61	1.74	-	2.25	1.12	2.57	0.56	CH_2, 7.07;CH,7.91;$(CH_3)_2$8.99
Dehydrogenation Product A in CCl_4	1.09	2.54	1.71	-	2.24	1.09	2.58	0.55	CH_2, 7.06;CH,7.95;$(CH_3)_2$8.96

Table VI. The chemical shifts (in τ values) of the protons of the various 1,7-phenanthrolines. The data are for the spectra of a methylene chloride solution unless otherwise stated.

spectrum of 1,7-phenanthroline itself (see Table). The simpler alkyl-1,7-phenanthrolines can be readily identified by the NMR spectrum since the low-field spectrum reveals the position of the alkyl group and the high field spectrum reveals the nature of the alkyl group. The spectra of many alkyl groups are known from previous work (106).

The environmental factors which influence the chemical shifts of ring protons also influence, although to a smaller extent, the chemical shifts of the methyl or methylene groups attached to the ring. The τ values of the methyl bands in 5-methyl-(7.4), 6-methyl-(7.28), and 10-methyl-1,7-phenanthroline (6.68) show the same order of shielding as H5 (2.20), H6 (2.02), and H10 (0.56) in 1,7-phenanthroline.

The chemical shifts of the various protons of alkyl-4,7-phenanthrolines are shown in Table VII. Although alkyl substitution destroys the symmetry of the 4,7-phenanthroline nucleus, it has only a slight effect on the chemical shifts of the ring protons. Thus analogous protons, although nonequivalent are still not well separated. But under high resolution and slow sweep the bands due to the various protons can be picked out. The effect of alkyl substitution, otherwise, is the same as in the case of 1,7-phenanthrolines.

-4,7-Phenanthroline	H1	H2	H3	H5	H6	H8	H9	H10	Others
Unsubstituted	1.23	2.50	1.05	1.84	1.84	1.05	2.50	1.23	
1-Ethyl-	-	2.60	1.18	1.84	1.84	1.07	2.48	1.03	CH_2,6.63;CH_3,8.52
5-Methyl-	1.25	2.52	1.07	-	1.99	1.02	2.46	1.29	CH_3,7.25
1,5-Dimethyl-	-	2.69	1.25	-	2.05	1.16	2.62	1.12	1-CH_3,7.09 5-CH_3,7.26

Table VII The chemical shifts (in τ values) of the protons of the various 4,7-phenanthrolines.

(v) Structure determination with the NMR spectra.

The compound suspected to be 4,5-dimethyl-1,7-phenanthroline was prepared by a quinoline synthesis with methyl vinyl ketone and a methylquinolylamine which was either 5-amino-7-methylquinoline or 5-methyl-7-amino-quinoline. The compound therefore could be either 4,5-dimethyl-1,7-phenanthroline or 5,10-dimethyl-1,7-phenanthroline. A comparison of the spectra of 1,7-phenanthroline and of the compound in question, showed that bands assigned to H4 and H5 (or H6) in the former were absent in the latter (see Table VI). The H10 band in the latter showed fine splitting due to H6 and therefore the substituents must be attached at positions 4 and 5. This also proved that the methyl-quinolylamine of the previous step was 5-amino-7-methylquinoline.

The compound 10-methyl-1,7-phenanthroline was unstable in air and gave a rather poor analysis; but its structure was confirmed by its NMR spectrum (Figure 22) and its phenanthroline-like ultraviolet spectrum.

The NMR spectra of 5-methyl-and 5-isobutyl-1,7-phenanthrolines are shown in figures 18 and 21. The use of the NMR spectra in the identification of the dehydrogenation product of lycodine has been discussed in Part I.

(vi) The NMR spectrum of 2,7-diazaphenanthrene.

The spectrum of 2,7-diazaphenanthrene is shown

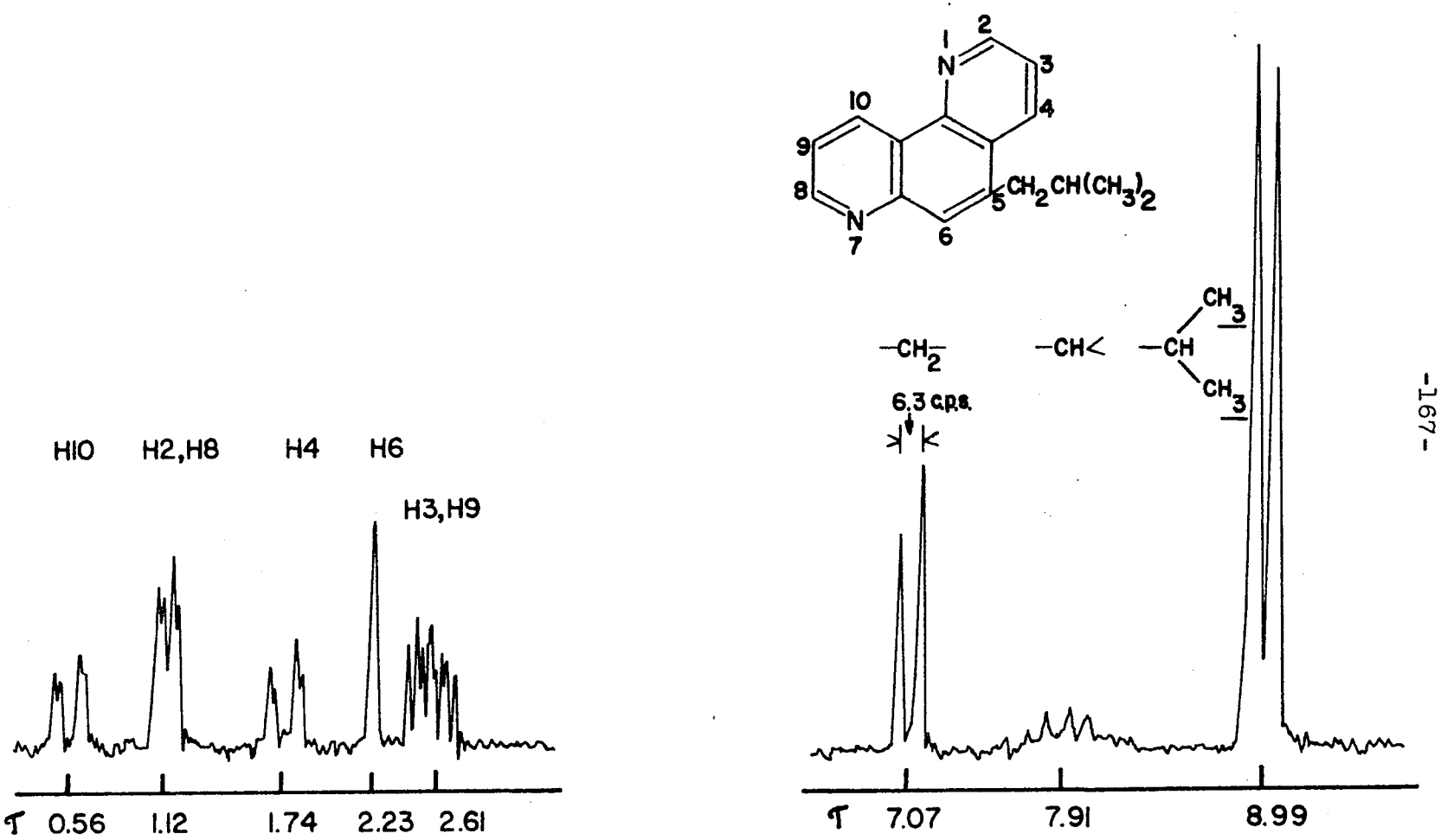
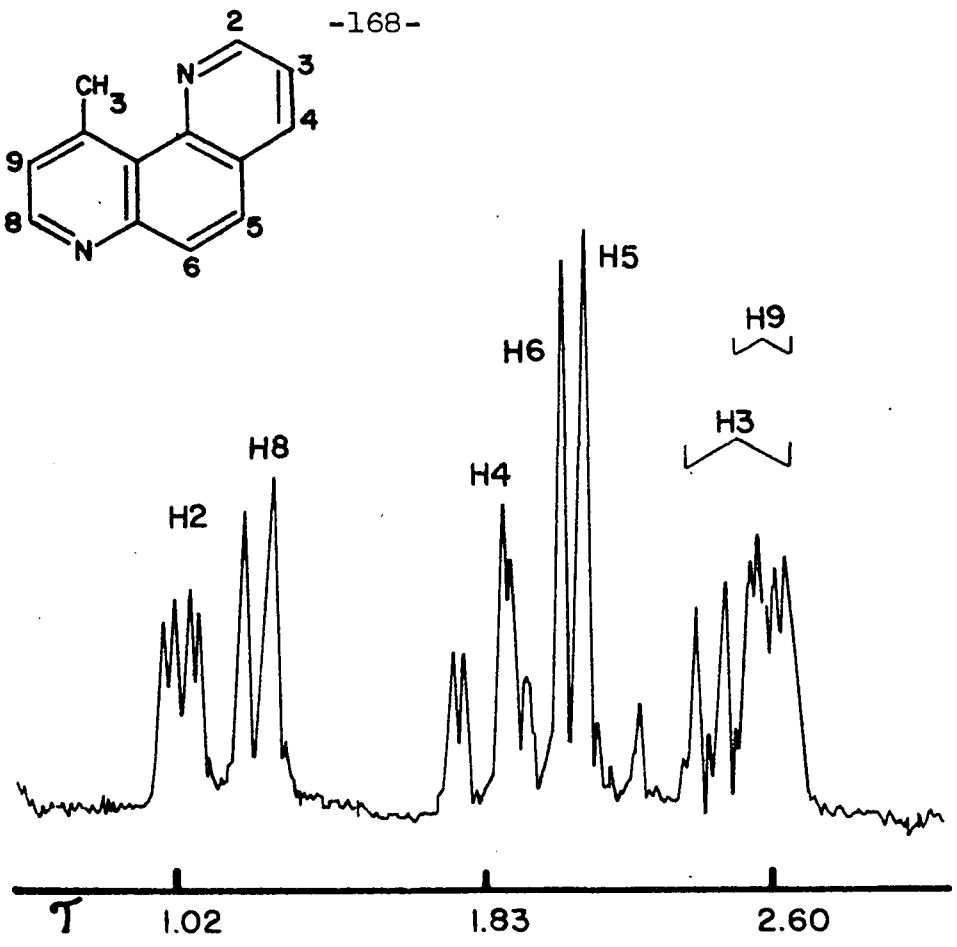


Figure 21. The N M R spectrum of 5-Isobutyl-1,7-Phenanthroline in Carbon Tetrachloride

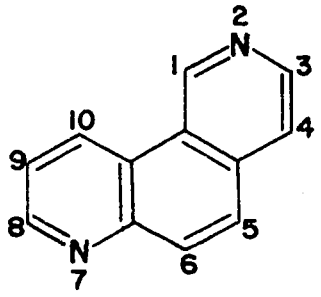


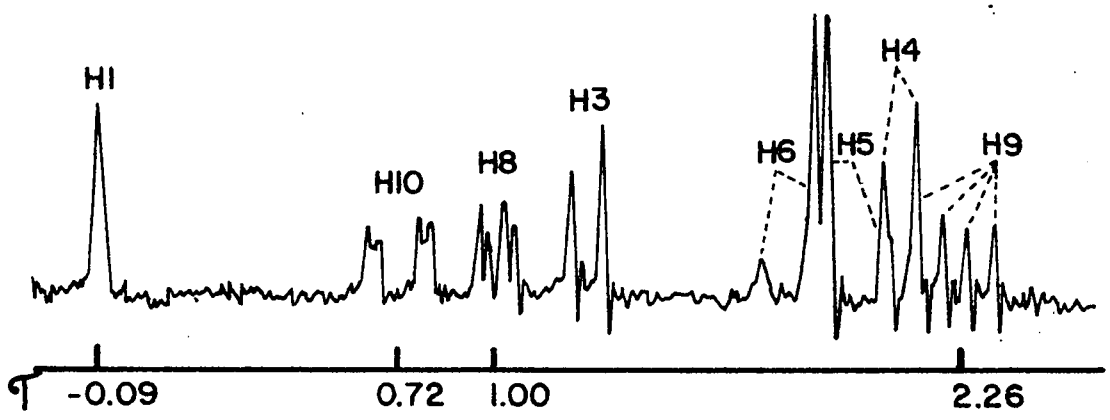
The NMR spectrum of the ring protons of 10-Methyl-1,7-Phenanthroline in Methylene Chloride

Figure 22.

in Figure 23. As in other cases the spectrum of this compound shows considerable variations with change of solvent. A mixture of solvents was used to obtain the best possible separation of the signals. Assignments of H1, H3 and H4 (see figure) are straightforward and arise from the line positions and multiplicity. H5 and H6 being nonequivalent show a recognisible AB system centred at 1.91 τ with J_{AB} = 9.4 cps and ν_{AB} = 6.3 cps. The H6 band must be the one at lower field, since H6 and the nitrogen at 7 position are very close to each other. The assignments of the remaining three quartets to H10, H8 and H9 are readily seen from their chemical shifts and the spacings associated with the quartets ($J_{\beta\gamma} > J_{\alpha\beta} > J_{\alpha\gamma}$). The occurrence of the H10 band at lower field than the H8 band, derives a ready explanation from the ring current effect due to the three rings. In pure methylene chloride solution H10 and H8 are hardly separated although H5 and H6 are very well separated. In acetone on the other hand H5 and H6 are not separated at all although H10 and H8 were very well separated. A mixture of the two solvents gave a satisfactory separation of all the bands. The occurrence of the H1 band at low field is understandable, as it is an α-pyridine proton attached at the 1 position.

Incidentally, the large coupling (8.9 cps) between the benzene ring protons (H5 and H6) shows that





The NMR spectrum of 2,7-Diazaphenanthrene in Acetone-Methylene Chloride (5:1)

Figure 23.

these are ortho to each other and therefore the compound must be the angular diazaphenanthrene and not the linear diazaanthracene in which the benzene ring protons would be para to each other. The angularity of such tricyclic systems prepared by the Skraup and related reactions has been ascertained in the past by chemical degradation to to bipyridyls or by their ultraviolet spectra (68, 70, 76). The NMR spectra would be useful in clarifying this point, in cases where the protons on the middle ring are chemically shifted.

(d) The ultraviolet spectra of phenanthrolines.

The ultraviolet absorption spectra of 1,7-, 1,10- and 4,7-phenanthrolines and their salts have been discussed in the introduction. The spectra of 1,7-phenanthroline and its salts, reported by Krumholz, are reproduced in Figure 24. The absorption maxima and the intensities of the spectra of the various 1,7-phenanthrolines are listed in Table VIII, and those of the other phenanthrolines are listed in Table IX.

In addition to the bands reported by Krumholz (42), the ultraviolet spectra of 1,7- 1,10- and 4,7-phenanthrolines, all contain a low intensity band system at longer wavelengths (350-320 mμ), which is noticeable only at higher concentrations (see table; see also the spectrum, of 1,10-phenanthroline reported by Badger et al.(76)

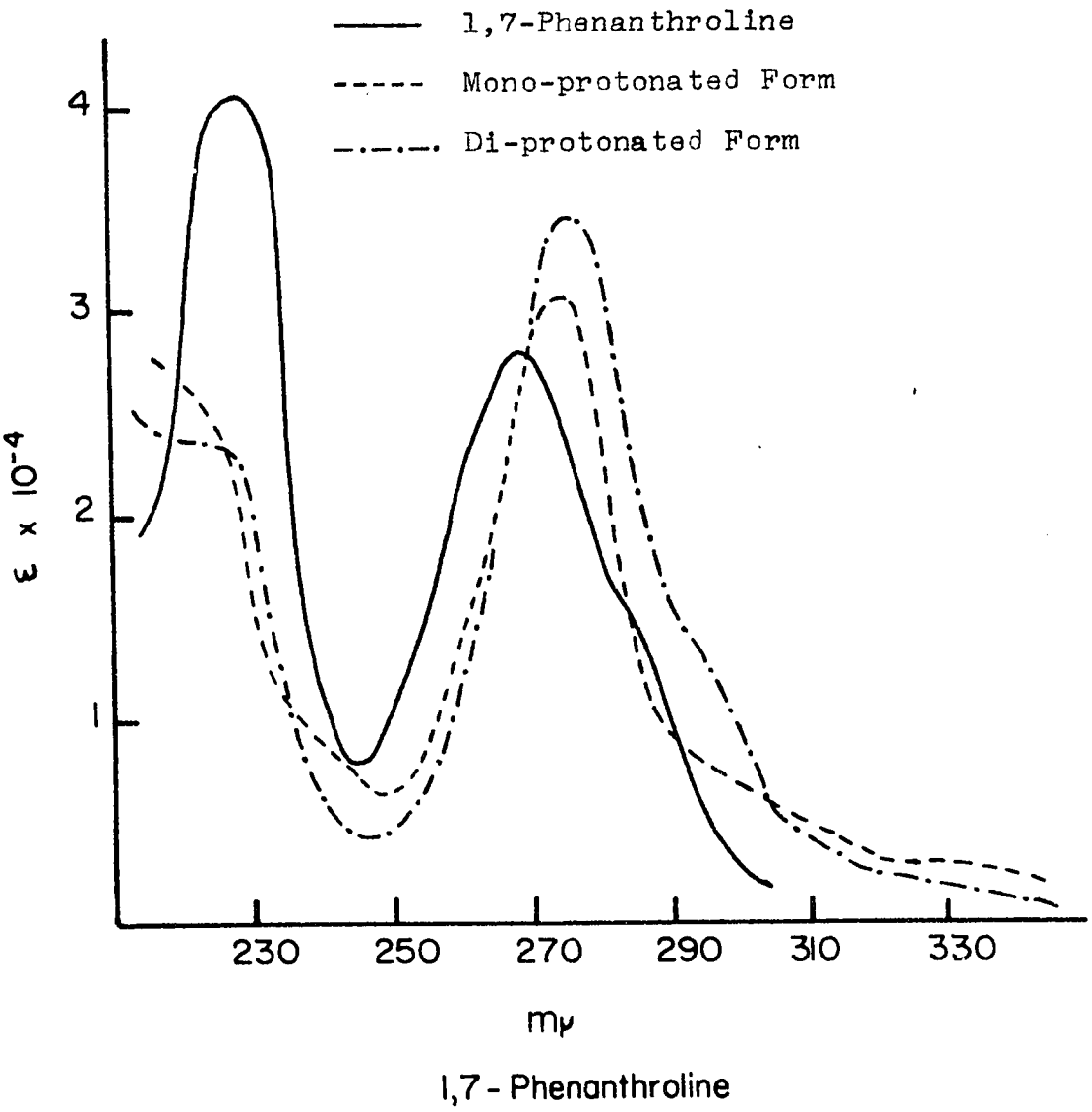


Figure 24. The Ultraviolet Absorption Spectra.

for comparison). Such a fine structure was first noticed in the spectrum of the dehydrogenation product of lycodine and was useful in confirming the ring system in the dehydrogenation product. This long wavelength fine structure is present in the spectra of all the substituted phenanthrolines and is quite intense in some of them, particularly in that of 4,5-dimethyl-1,7-phenanthroline.

The main band which occurrs around 279 mμ in all the compounds of the series is quite characteristic. The variations in the position and intensity of this band in the spectra of parent phenanthrolines, upon protonation have been studied (42) and have been discussed in the introduction (see also Figure 24). We have studied the effect of alkyl substitution on the position of the main band (Table VIII and IX). In 1,7- and 4,7-phenanthrolines, an alkyl substituent can cause either a bathochromic shift (shift to longer wavelengths) or a hypsochromic shift (shift to shorter wavelengths) of the main band in the ultraviolet spectrum, depending on the position of the substituent. Substitution in the "angular" (1 or 10 position) position shifts the maximum of the main band to shorter wavelength. Thus 10-methyl-1,7-phenanthroline shows the main band at 264.5 mμ as compared to 267.5 mμ shown by the parent phenanthroline. 1-Methyl- and 1-ethyl-4,7-phenanthrolines also show the main band at shorter

-1,7-Phenanthroline	λ max mμ (ε max)				
Unsubstituted	231.5(46600)	267(28000)	321.5(670)	336.5(260)	227.5(45500) 307 (960)
5-Methyl-	233.5(46900)	271.5(30500)	325.5(810)	341.5(450)	
5-Isobutyl-	234 (46400)	271.5(30000)	325.5(850)	341.5(480)	
4-Ethyl-	230.5(37600)	268 (29900)	323 (670)	338 (300)	295 (7710)
4,5-Dimethyl-	238.5(39600)	273 (26000)	329 (1390)	345 (1080)	299.5(7810)
6-Methyl-	233.5(43900)	270 (28700)	325.5(510)	342 (120)	
10-Methyl-	235.5(41300)	264.5(27200)	323 (510)	338 (200)	231 (37800)
2-Methyl	233	270.5	325	340	
3-Methyl		269	323	338	
4-Methyl-	230.5	268	323	338	

Table VIII. The ultraviolet absorption spectra of 1,7-phenanthrolines.

wavelength (266 mμ and 267 mμ) than the parent compound (270.5 mμ). In the other alkylphenanthrolines studied, the main band, in each case, was at longer wavelength than the parent compound. Thus the situation is different from that observed in the case of phenanthrene and many other alternant aromatic systems, where alkyl substitution causes only a shift to longer wavelengths (57, 107). Even in the case of 4-methyl-phenanthrene, the main band does not occur at shorter wavelength than in phenanthrene. Such bathochromic shifts have been attributed to hyperconjugative extension of the chromophore (108). However alkyl substitution in the ortho positions of diphenyl systems is known to cause hypsochromic shifts in the absorption bands. This has been attributed to the nonplanarity of the substituted system and the resulting steric inhibition of resonance (57). A closer parallel to the case in question is seen in the spectrum of 1,3-dimethylbenzo (f) quinoline, the main band of which occurrs at shorter wavelength (266 m) than that of benzo (f) quinoline (267 m) and of 2,3-dimethylbenzo(f)quinoline (274 m) (68). The hypsochromic shift observed in the spectra of 10-methyl-1,7-phenanthroline and 1-methyl-4,7-phenanthroline is no doubt of the same type as that observed in 1,3-dimethylbenzo(f)quinoline and must be due to the methyl group in the angular (1 or 10) position, which causes some degree of nonplanarity of

Phenanthroline	λ max mμ (ε max)									
4,7-	232	(47400)	270.5	(26700)	320	(740)	335	(460)	227 305	(44300) (1050)
1-Methyl-4,7-	236		266.5		323		338		293	
1-Ethyl-4,7-	235.5	(37800)	267	(25400)	323	(600)	338	(310)	293	(8900)
1,5-Dimethyl-4,7-	237	(43700)	270	(25200)	326	(780)	341	(500)	296	(9800)
5-Methyl-4,7-	234	(47800)	273	(26000)	324	(800)	339.5	(450)		
3-Methyl-4,7-	233		274.5		322		337			
1,10-	229.5	(41500)	264	(27500)	324	(560)	338	(110)	308	(950)
2,7-	237	(40900)	267	(11400)	325.5	(6600)	341.5	(8070)	279 298 306 311 319	(6800) i (1520) (1510) (3030) (2520)

Table IX. The ultraviolet absorption spectra of the phenanthrolines. i = inflection.

the otherwise planar aromatic system. Disubstitution in adjacent peri positions might also be expected to result in steric strain. But the strain in this case does not result in a hypsochromic shift, but in increased intensities and fewer bands in the long wavelength band system (350-300 mμ). This is illustrated by the spectrum of 4,5-dimethyl-1,7-phenanthroline the main band of which occurss at 273 mμ . As mentioned earlier, the low intensity fine structure of this compound is more intense than of others (Table VIII).

Thus the data presented in Tables VIII and IX may be summarised as follows; the position of the main band in the phenanthrolines is shifted to shorter wavelength by substitution in the angular (1 or 10) positions and longer wavelengths by substitution in other positions. Another characteristic of the spectra of phenanthrolines substituted in the pyridine ring is a band of medium intensity between 285-300 mμ , which is present only as a smooth inflexion in the parent compounds and other substituted phenanthrolines.

The spectrum of 2,7-diazaphenanthrene (Table IX) shows increased and more intense fine structure in the long wavelength band system but is otherwise similar to the spectra of the phenanthrolines. In regard to the fine structure, it resembles the spectra of benzo(f)

quinoline and phenanthridine (107) more than those of the phenanthrolines. It might be mentioned here that the ultraviolet spectrum of isoquinoline shows increased and more intense fine structure than that of quinoline (107) and the enhanced fine structure shown by 2,7-diazaphenanthrene is therefore understandable. The absence of absorption at wavelengths longer than 350 mμ in the spectrum of 2,7-diazaphenanthrene is indicative of its angular structure since diazaanthracenes and other linear tricyclic systems show intense absorption in this region (68, 76). That the compound is a diazaphenanthrene and not a diazaanthracene has been confirmed also by its NMR spectrum.

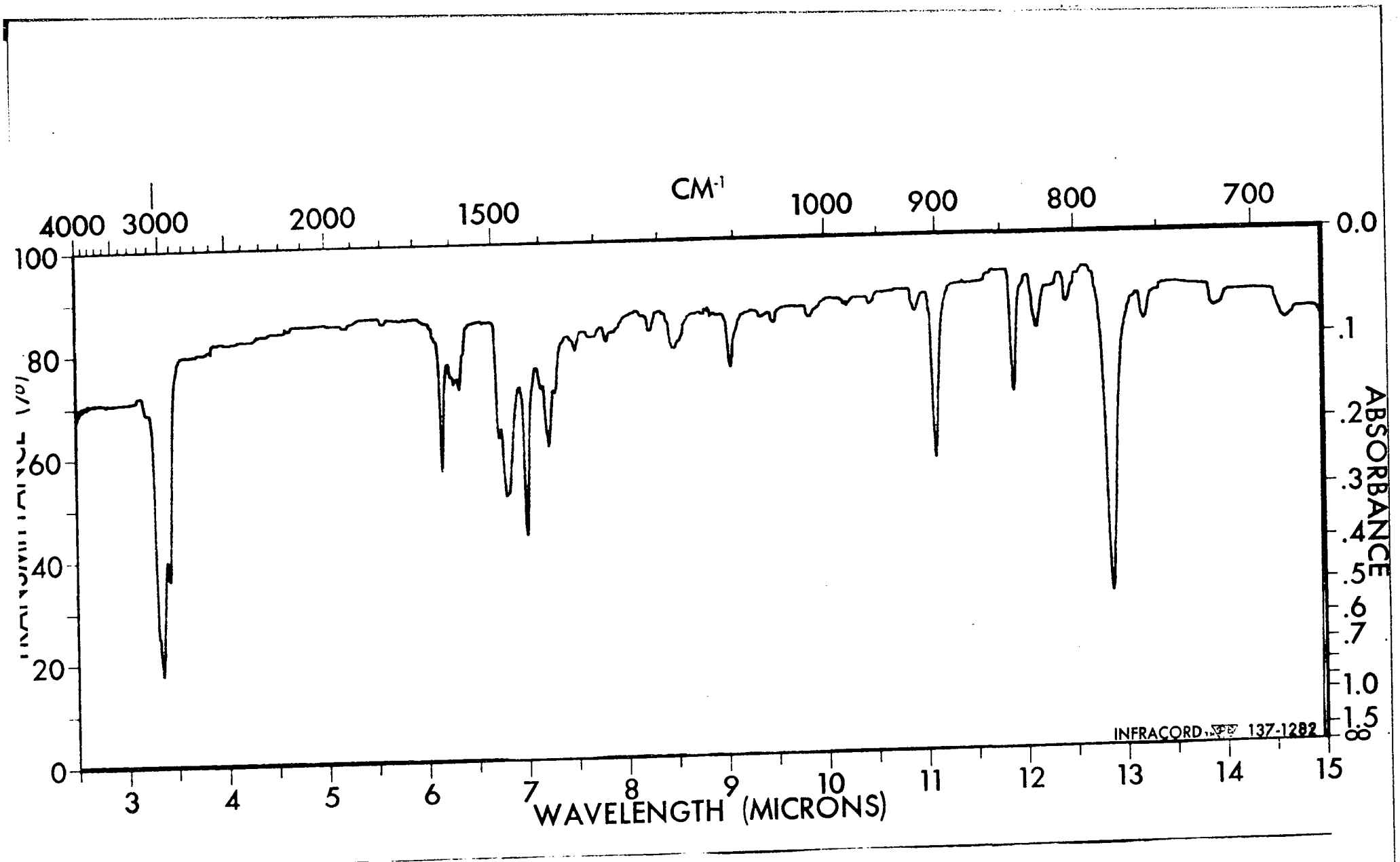


Figure 25. The infrared spectrum of 5-isobutyl-1,7-phenanthroline in nujol mull.

CLAIMS TO ORIGINAL RESEARCH

1. Lycodine has been isolated from *L. annotinum L.* by an improved procedure.
2. The structure of lycodine has been established by physical and degradative studies.
   (a) It was shown from NMR spectral evidence that the methyl group in lycodine is a $-CH-CH_3$ group and that the pyridine ring of lycodine carried a $-CH_2-CH-$ group in the 2-position and a carbon carrying no hydrogen in the 3-position.
   (b) The dehydrogenation products of lycodine were shown to be substituted 1,7-phenanthrolines from their ultraviolet absorption spectra.
   (c) One of the dehydrogenation products of lycodine has been identified as 5-isobutyl-1,7-phenanthroline by its NMR spectrum and its structure has been proved by synthesis.
3. Lycodine was related to lycopodine, another alkaloid of *L. annotinum L.*, and the structure and stereochemistry of lycodine have been deduced from its relation to lycopodine.
4. The following new alkylphenanthrolines have been prepared and characterised:

5-Methyl-1,7-phenanthroline

5-Isobutyl-1,7-phenanthroline

4,5-Dimethyl-1,7-phenanthroline

4-Ethyl-1,7-phenanthroline

10-Methyl-1,7-phenanthroline

1-Ethyl-4,7-phenanthroline

5-Methyl-4,7-phenanthroline

1,5-Dimethyl-4,7-phenanthroline

5. 2,7-Phenanthroline (2,7-diazaphenanthrene) has been prepared for the first time. The ultraviolet and NMR spectra of the compound have been measured. A complete assignment of the various bands in the NMR spectrum has been made. The NMR spectrum also showed that the compound is a diazaphenanthrene and not the linear diazaanthracene.

6. The following new compounds have been prepared and characterised as intermediates in the preparation of the phenanthrolines.

5-Nitro-7-methylquinoline

5-Amino-7-methylquinoline

3,5-Dinitro-4-acetylaminoisobutylbenzene

4,8-Dimethyl-6-nitroquinoline

4,8-Dimethyl-6-aminoquinoline

7. The ultraviolet absorption spectra of the phenanthrolines have been measured and the effect of alkyl

substitution on the main band has been studied. In 1,7- and 4,7-phenanthrolines alkyl substitution in 1 or 10 positions caused the maximum of the main band to shift to shorter wavelength and substitution in other positions studied caused the main band maximum to shift to longer wavelengths than those shown by the parent phenanthrolines.

8. The NMR spectra of 1,7- 4,7-, and 1,10-phenanthroline have been measured and complete assignments of the bands in these spectra have been made. The assignments have been confirmed from a study of the spectra of suitable alkyl substituted phenanthrolines. The possibility of determining the structure of an alkyl-1,7-phenanthroline from its NMR spectrum has been demonstrated in the case of the dehydrogenation product of lycodine, 4,5-dimethyl-1,7-phenanthroline and 10-methyl-1,7-phenanthroline.

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