1970

The structures and reactions of four new 5,10b-ethanophenanthridine alkaloids

Michael Ray Slabaugh
Iowa State University

THE STRUCTURES AND REACTIONS OF FOUR NEW 5,10b-ETHANOPHENANTHRIDINE ALKALOIDS

by

Michael Ray Slabaugh

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Organic Chemistry

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Ames, Iowa

1970

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

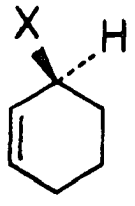
The Amaryllidaceae plant family has been the source of numerous compounds with unique structures and interesting chemistry. With the recent advances in preparative thin-layer chromatography, the isolation and purification of several previously unknown bases has become possible. This thesis describes the isolation and characterization of four new alkaloids which possess the 5,10b-ethanophenanthridine nucleus. The reactions and spectral properties of two of these are compared to several related compounds. Because biosynthetic studies are planned, several deuterium labeled compounds are prepared.

HISTORICAL

The structures and stereochemistry assigned to the new 5,10b-ethanophenanthridine alkaloids are based upon the structures of crinine, powelline, buphanidrine, haemanthidine, 6-hydroxycrinamine, ambelline, and undulatine. A discussion of the chemical and spectral basis for the structures of these reference compounds is followed by a summary of the known chemistry of cotarnine.

The absolute configuration of the alkaloids in this series was assigned originally by the application of Mills' rule. This empirical rule for allylicly substituted cyclohexenes states that the molecular rotation of compounds with configuration 1 are more dextrorotatory at 589 mu than the corresponding epimer (2).[1] Although the validity of this rule in cases where the compounds contain an optically active center adjacent to an aromatic chromophore has been questioned,[2,3] the absolute configuration of all crucial Amaryllidaceae alkaloids assigned by this rule have been verified by x-ray crystallographic data.[4-8]

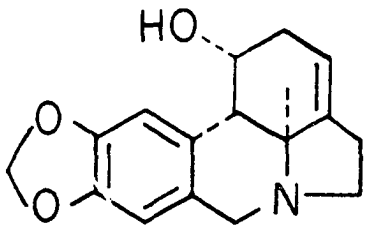
1



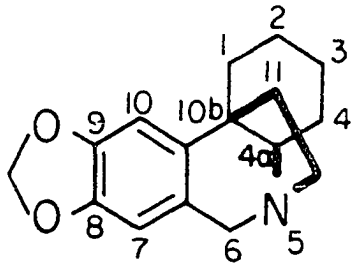
2

Crinine

Crinine was first isolated from two unidentified South African Crinum species in 1955.[9] The structure of crinine is the least complex of the alkaloids containing the 5,10b-ethanophenanthridine nucleus. For this reason, alkaloids possessing the same basic ring system are often referred to as crinine-type alkaloids. Crinine was characterized as a tertiary base, $C_{16}H_{17}NO_3$, containing one hydroxyl group, one reducible double bond, and a methylenedioxy function, but no N-methyl group. Although isomeric with the alkaloid caranine (3) and containing the same functional groups, crinine and its derivatives failed to undergo the reactions characteristic of caranine. Because of this, the spiro ring system (4) was postulated. Subsequent synthesis of 4 confirmed that it was identical to a crinine degradation product.[10,11]

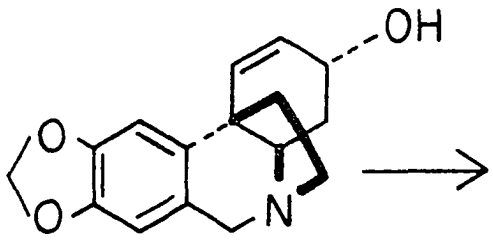


3

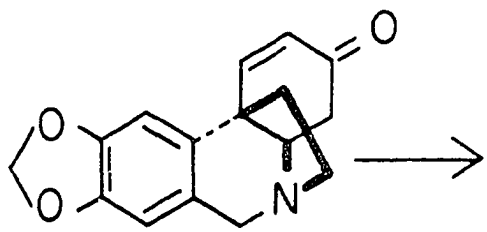


4

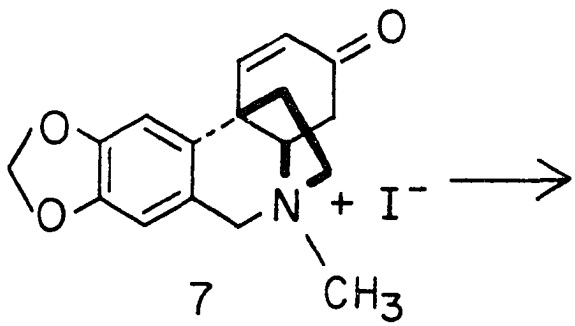
Crinine (5) is readily oxidized by manganese dioxide to oxocrinine (6), indicating that crinine is an allylic alcohol. The location of the hydroxyl group was obtained when oxocrinine methiodide (7) yielded the optically inactive symmetrical dienone (8) upon heating with dilute aqueous alkali.[11]



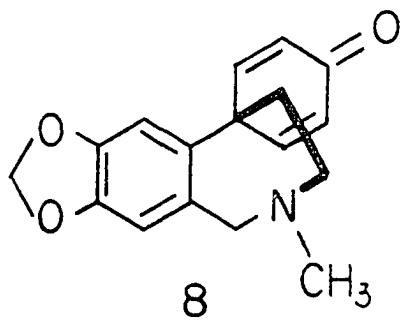
5



6



7

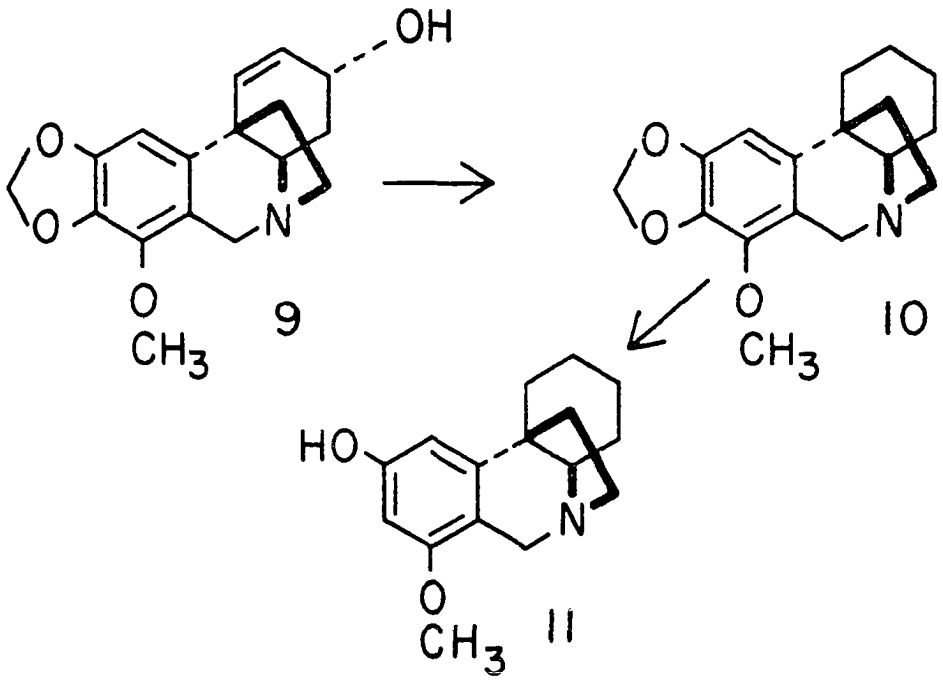


8

Powelline

Powelline (9), originally isolated from Crinum moorei[12] and C. powellii,[13] contains one methoxyl group in addition to the same functional groups as crinine. The close similarity of the reactions of powelline to those of crinine suggested that powelline is an ar-methoxycrinine.[14] This was confirmed by the removal of the methoxyl group by reduction of powelline with sodium and amyl alcohol to give dihydroepicrinine.[15]

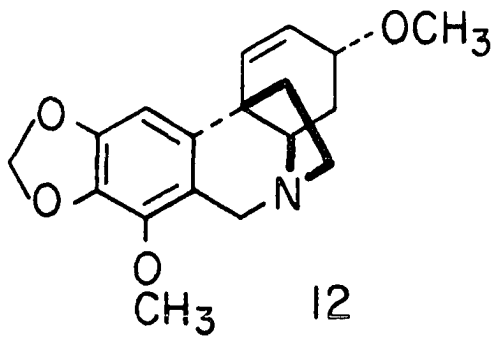
The position of the methoxyl group has been shown to be at C_7 by Birch reduction of powellane (10) to the phenol (11), the methyl ether of which has been synthesized by a route analogous to the synthesis of crinane.[16,17]



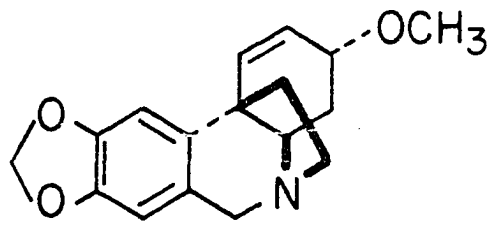
Buphanidrine

Buphanidrine (12), a low melting alkaloid, contains a methylenedioxy group and two methoxyls. One methoxyl could be assigned to the aromatic ring because of strong infrared absorption at 1623 cm^{-1}. When treated with dilute acid, buphanidrine is hydrolyzed to powelline (9).[11,13] Although allylic ethers can undergo rearrangement during acid hydrolysis, O-methylation of powelline with methyl-p-toluenesulfonate afforded buphanidrine.[18] With no evidence that inversion takes place in the methylation step, the configuration of the C_3 hydroxyl and methoxyl groups must be the same in powelline and buphanidrine, respectively. These assignments have been verified by ord and cd data.[2,3]

Removal of the aromatic methoxyl of buphanidrine with sodium and amyl alcohol gives the alkaloid buphanisine (13) which proved identical to the O-methylation product of crinine.[15] Thus crinine also contains the same configuration as powelline and buphanidrine.



12

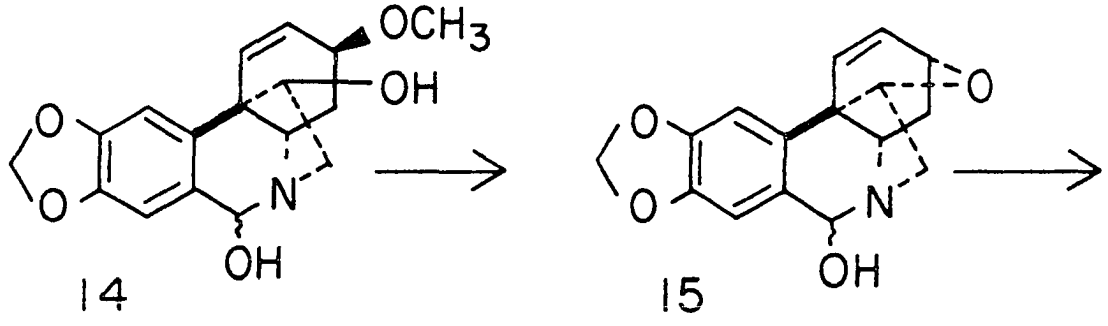


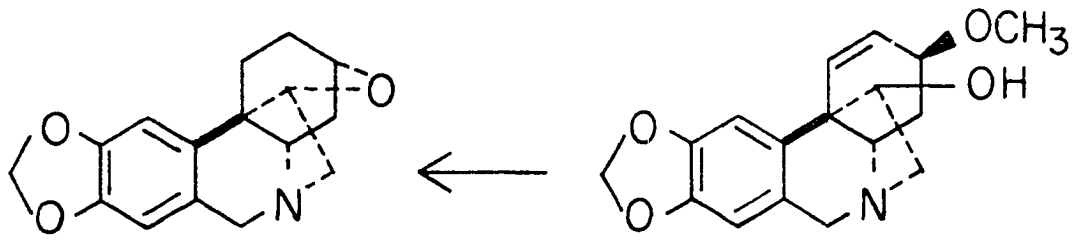
13

Haemanthidine and 6-Hydroxycrinamine

Haemanthidine (14) and its C_3 epimer, 6-hydroxycrinamine, are unique among the crinine-type alkaloids because these two bases alone possess a hydroxyl substituent at C_6. The structure elucidation of these two compounds has enabled the relative stereochemistry of the other 5,10b-ethanophenanthridine alkaloids to be assigned.

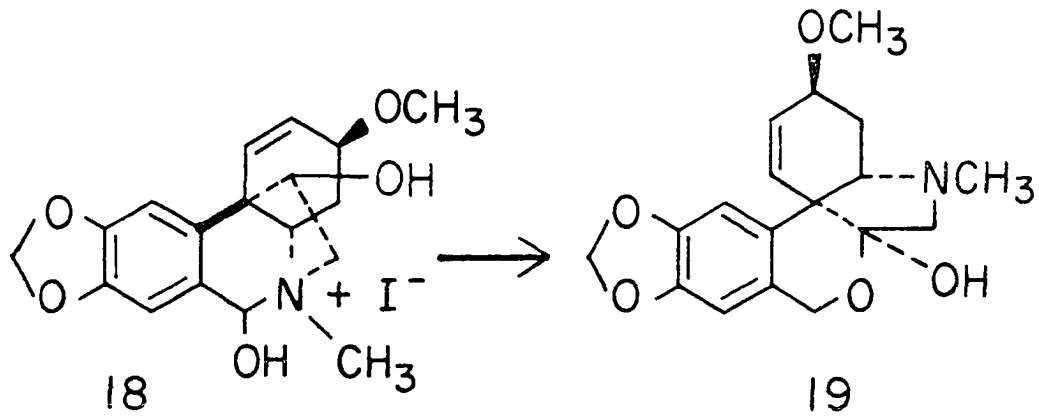
Treatment of haemanthidine with dilute acid converts the base into the ether, apohaemanthidine (15).[19] Catalytic reduction of the double bond and removal of the remaining hydroxyl group of 15 by treatment with thionyl chloride followed by lithium aluminum hydride gave dihydroapohaemanthamine (16), which had previously been prepared from haemanthamine (17).[20] The formation of these apo-bases necessitates a cis axial/equatorial fusion of the pyrrolidine/cyclohexane rings. Since haemanthamine has been related to buphanisine,[18] the previously discussed bases must also contain a cis C/D ring fusion.





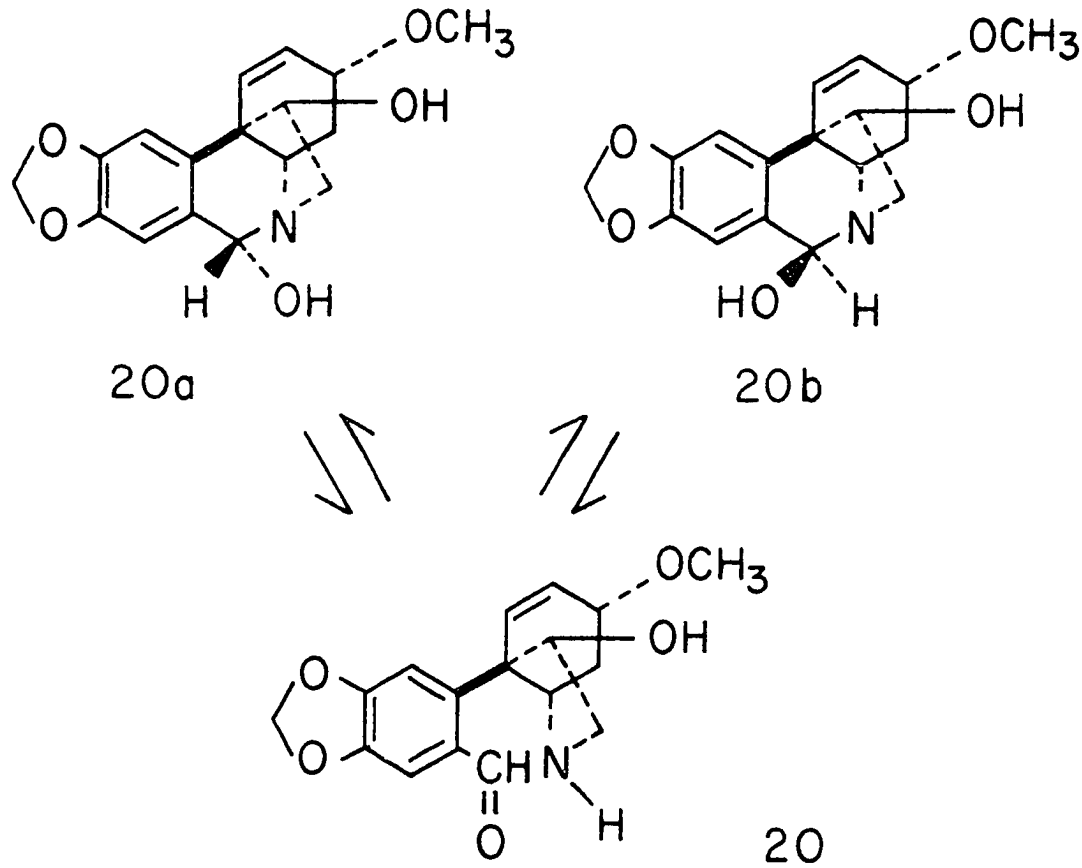
16 17

The treatment of haemanthidine methiodide (18) with strong base affords a quantitative conversion to tazettine (19).[21,22] The structure of 19 and the relative stereochemistry of the allylic methoxyl group with respect to the aromatic ring have been firmly established by degradative and synthetic methods.[23-25] The interrelationship of tazettine to haemanthidine and then ultimately to buphanisine, confirms the <u>cis</u> relationship of the C_3 substituent and the aromatic ring in the earlier compounds.



18 19

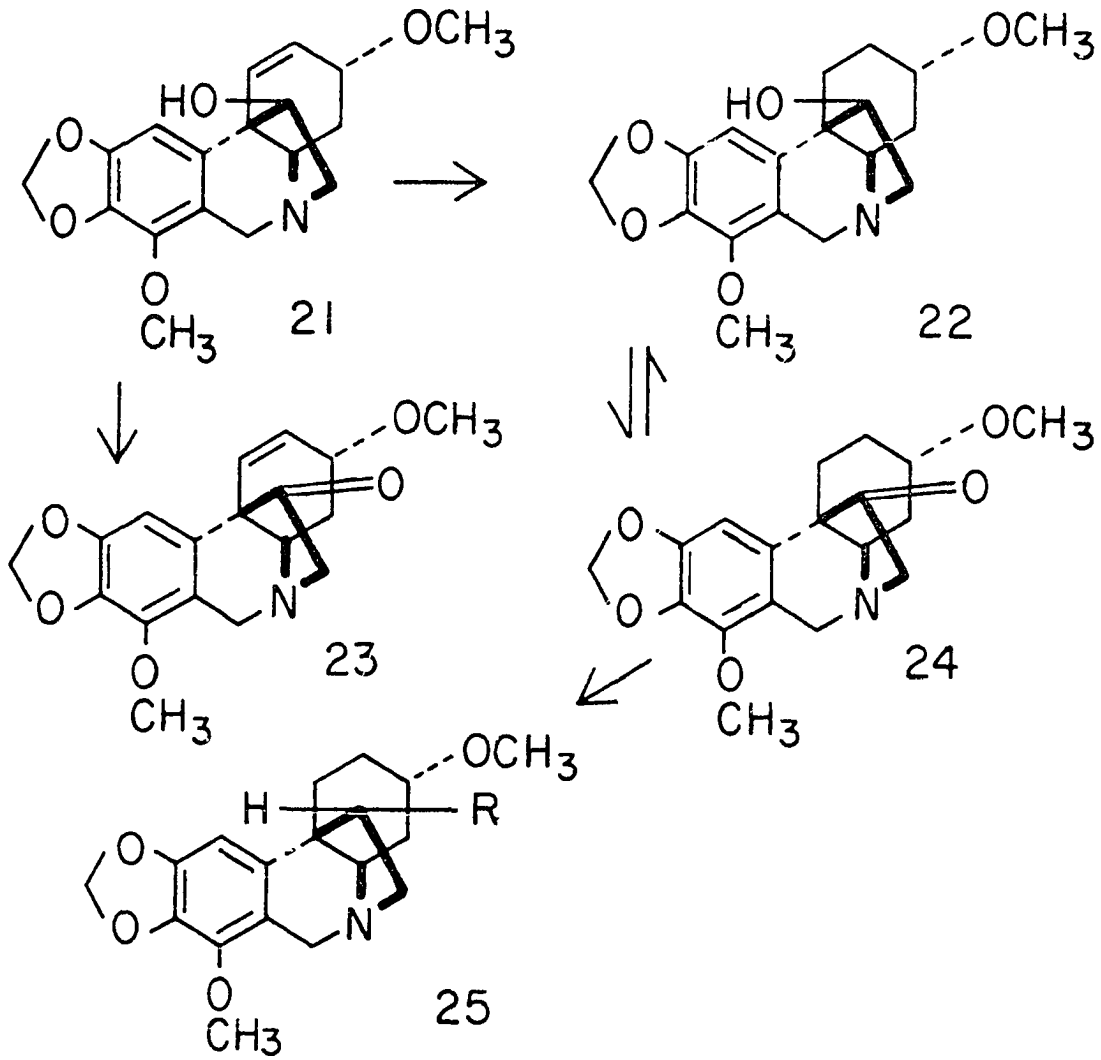
Chemical and spectral data have shown that 6-hydroxycrinamine and haemanthidine exist in solution as an equilibrating mixture of C_6 hydroxyl epimers.[26] This epimerization was envisioned to proceed through the open chain aminoaldehyde (20).[27] In contrast, the x-ray crystallographic analysis of 6-hydroxycrinamine established that in the solid state the C_6 hydroxyl group is <u>trans</u> to the pyrrolidine ring as in 20b.[7] There was no evidence of the epimer (20a) in the solid state.



20a 20b 20

Ambelline

Ambelline (21) was characterized as a tertiary base, $C_{18}H_{21}NO_5$, containing two methoxyl groups, one methylenedioxy group, and one hydroxyl.[28] Catalitic reduction with palladium-on-charcoal provided a single dihydro derivative. The alcohol groups of ambelline and dihydroambelline (22) were oxidized with chromium trioxide and pyridine to give the corresponding ketones (23 and 24).



Reduction of oxodihydroambelline (24) with methanolic sodium borohydride gave a mixture of dihydroambelline (22) and epidihydroambelline (25, R = OH). Reaction of the latter with thionyl chloride followed by lithium aluminum hydride gave dihydrobuphanidrine (25, R = H). This chemical interconversion established the absolute configuration of the basic nucleus as well as the relative configuration of the aliphatic methoxyl.

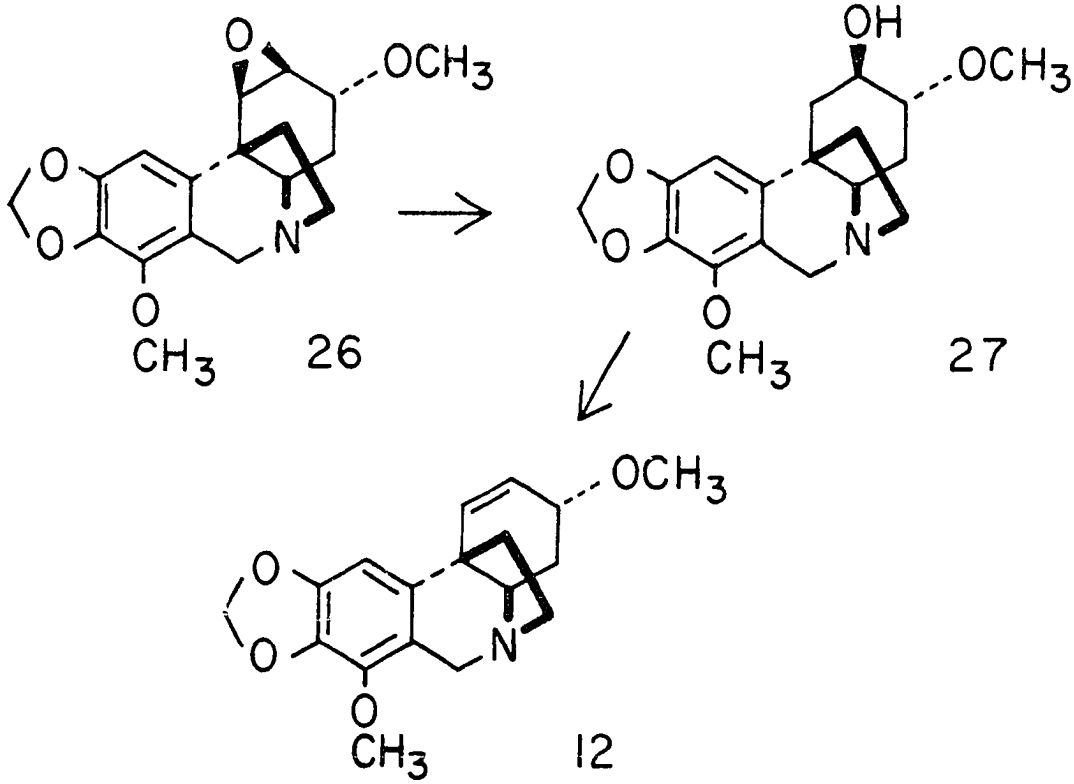
The location of the double bond in ambelline was determined from its nmr spectrum. The two olefinic protons appear as an AB pattern (6.58 and 5.88 ppm). The proton at 5.88 ppm is further split by coupling to a single proton (J = 5 cps) which must be the proton at C_3. This provides positive assignment for the location of the double bond at C_1, C_2.

The position of the hydroxyl group was located in the 5-membered ring from the carbonyl absorption of oxoambelline (5.73 μ). Because the alkaloid is not a carbinolamine, the hydroxyl can only be at C_{11}. The configuration of the hydroxyl group was assigned from hydrogen-bonding studies. Ambelline is strongly hydrogen bonded (3565 cm^{-1}). Reduction of the double bond has no affect upon this absorption indicating the hydrogen is bonded to the π-electrons of the aro-

matic ring. By comparison, hydrogenation of epiambelline shifted the OH band at 3603 cm^{-1} to 3630 cm^{-1}.

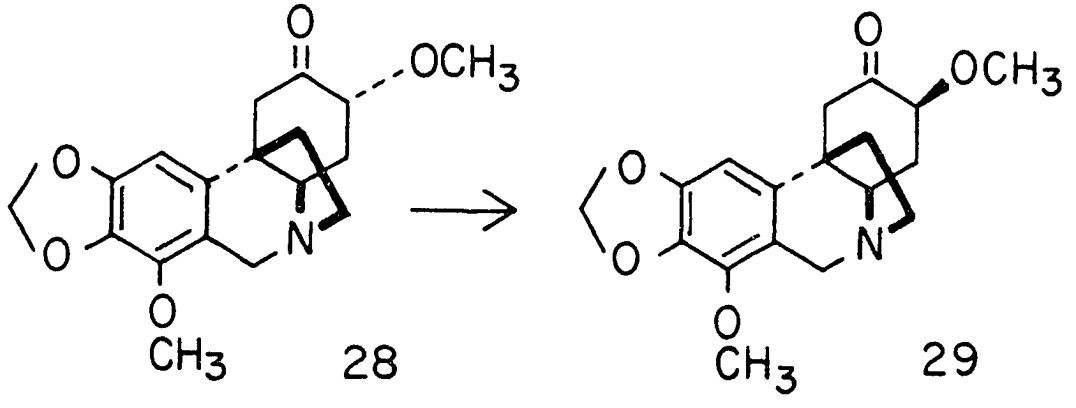
Undulatine

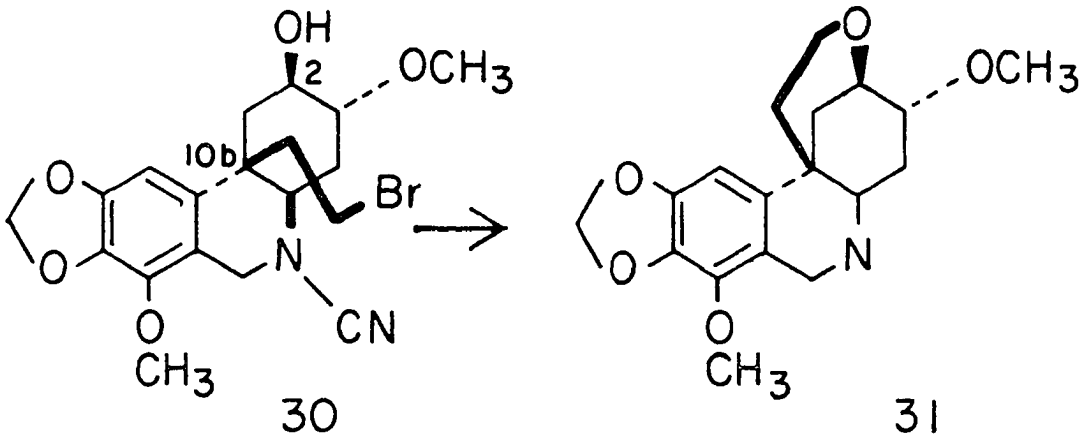
Undulatine (26) was first isolated by Boit from several Nerine species.[12,29,30] The carbon skeleton and many of the features of the compound were demonstrated by the interrelation with buphanidrine (12).[29] Reduction of the alkaloid (26) with lithium aluminum hydride gave a mixture of α-dihydroundulatine (27) and β-dihydroundulatine. The mesylate of α-dihydroundulatine was heated under reflux with potassium t-amyl oxide in t-amyl alcohol to give buphanidrine (12).



Undulatine was shown to be a 1:2-epoxide by a deuterium labeling experiment. Reduction of 26 with lithium aluminum deuteride gave a monodeutero α-dihydroundulatine (27). Oxidation of the deuterated compound gave oxo-α-dihydroundulatine (28). Equilibration of 28 with base in methanol gave epioxo-α-dihydroundulatine (29) which no longer contained deuterium, a result possible only if the deuterium were on the α-carbon of the ketone. Since undulatine did not show the properties of an α-epoxy ether, only structure 26 is permissible.

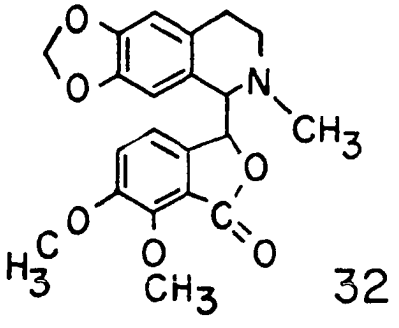
Treatment of α-dihydroundulatine with cyanogen bromide gave a bromo-N-cyano compound (30) which on treatment with base formed the cyclic N-cyanoether (31).[31] The formation of the cyclic ether is sterically possible only if the β-bromoethyl side chain at C_{10b} and the hydroxyl at C_2 are cis. Therefore, α-dihydroundulatine must have the hydroxyl in an axial position and the epoxide of undulatine must be as shown in 26.



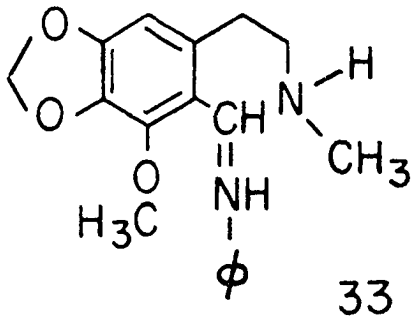


Cotarnine

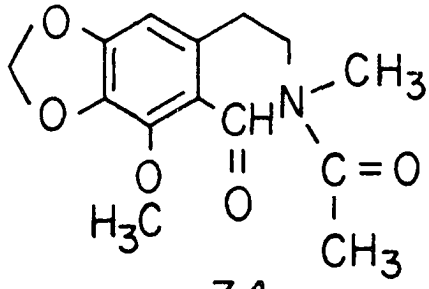
This base was first obtained by Wohler in 1844 from the acidic hydrolysis of narcotine (32).[32] Cotarnine, $C_{12}H_{15}O_5N$, contains one aromatic methoxyl, a methylenedioxy, and an N-methyl substituent. The free base is colorless but readily forms yellow salts with acids by the elimination of water. For this reason, Hantzsch[33] gave the name "pseudo bases" to cotarnine and other carbinolamines such as hydrastinine, berberine, and sanguinarine. The chemical transformations of these "pseudo bases" has been one of the most discussed topics in theoretical organic chemistry since the first publication by Claus and Himmelmann in 1880.[34]



Although cotarnine was successfully synthesized by Salway in 1910,[35] there still remained much controversy over its structure. The free base has been reported to react with aniline as if it were an aromatic aldehyde, giving the anil (33).[36] In addition, cotarnine reacts with acetic anhydride as though it were a secondary amine forming N-acetylcotarnine (34).[37]



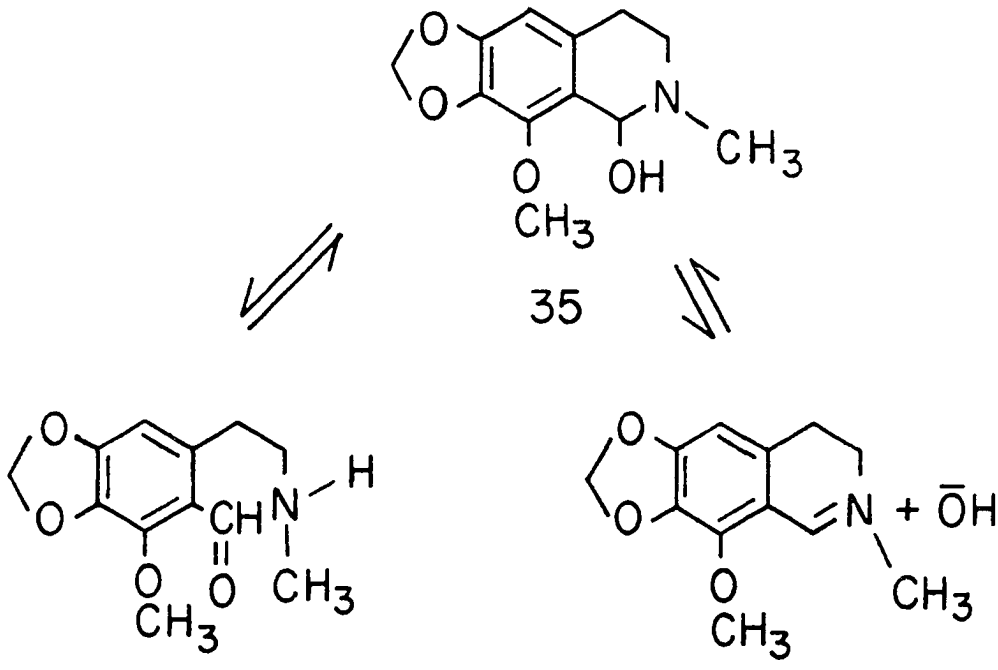
33



34

Roser, therefore, proposed that cotarnine is represented by structure 36 and that its yellow salts are quaternary ring compounds represented by 37.[38] Decker argued that the aldehyde and amine groups would not coexist in close proximity without reacting and suggested that the carbinolamine structure (35) was correct.[39] Gadamer postulated a tautomeric system of the three components, in which the carbinolamine (35), quaternary ammonium hydroxide (37), and the aminoalde-

hyde (36) all exist in equilibrium.[40]



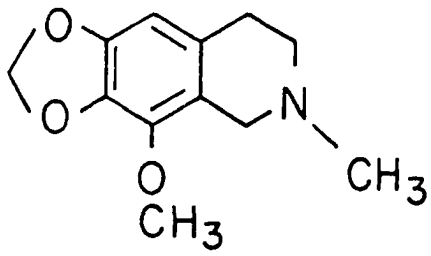
35

36

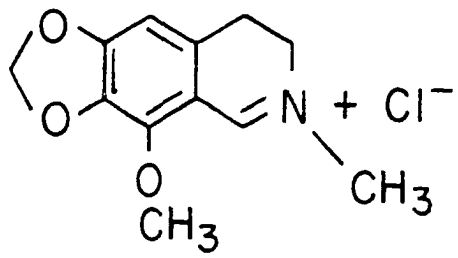
37

Reaction products 33 and 34 tend to support the amino-aldehyde, but physical data for its existence is completely lacking. Infrared spectra indicate crystalline cotarnine to be completely in the carbinolamine form (35).[41] The ultraviolet spectrum of cotarnine in nonpolar solvents is identical with that of hydrocotarnine (38). However, in dilute aqueous or alcoholic solution, the uv spectrum is identical with that of cotarnine chloride (39). Evidence for this equilibrium between 35 and 37 in polar solvents is further strengthened by electrical conductivity measurements and polarographic

investigations.[42]

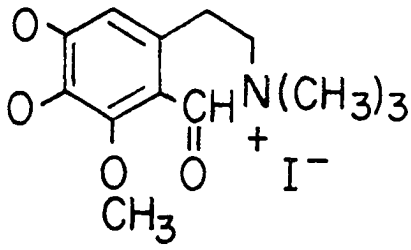


38



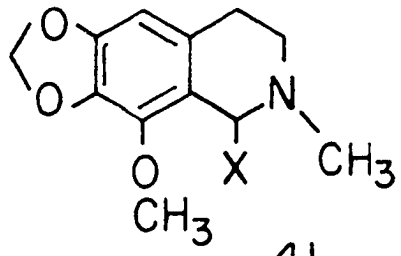
39

Reaction of cotarnine with acylating and alkylating reagents produces derivatives which can only exist as the aminoaldehyde form, such as N-acetylcotarnine (34) and methylcotarnine methiodide (40).[43]



40

By contrast, derivatives (41a-d) formed by reaction with certain nucleophilic reagents, such as alcohols, mercaptans, CN^-, and SO_3H^-, can only exist in a cyclic form.[44]



41

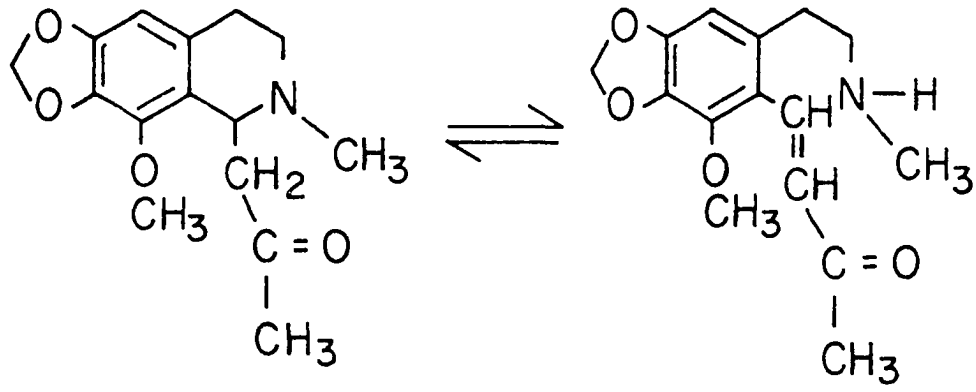
a: X = OR e: X = NHC_6H_5

b: X = SR f: X = NHOH

c: X = CN g: X = CH_2COCH_3

d: X = SO_3H h: X = CH_2NO_2

The situation is similar to cotarnine for certain derivatives obtained by reaction with other nucleophilic reagents such as amino compounds or carbanions derived from carbonyl compounds. These derivatives (41e-h) can also be considered as ring-chain prototropic systems.[42]



41g 42

However, ultraviolet and infrared spectroscopic investigations of these derivatives have shown that the compounds possess the cyclic form (i.e. 41g). In addition, chemical methods of detection were negative in attempts to identify

the open-chain olefinic form (42). Beke concludes that the anil (33) reported 80 years earlier is actually the cyclic structure (41e).[42]

RESULTS AND DISCUSSION

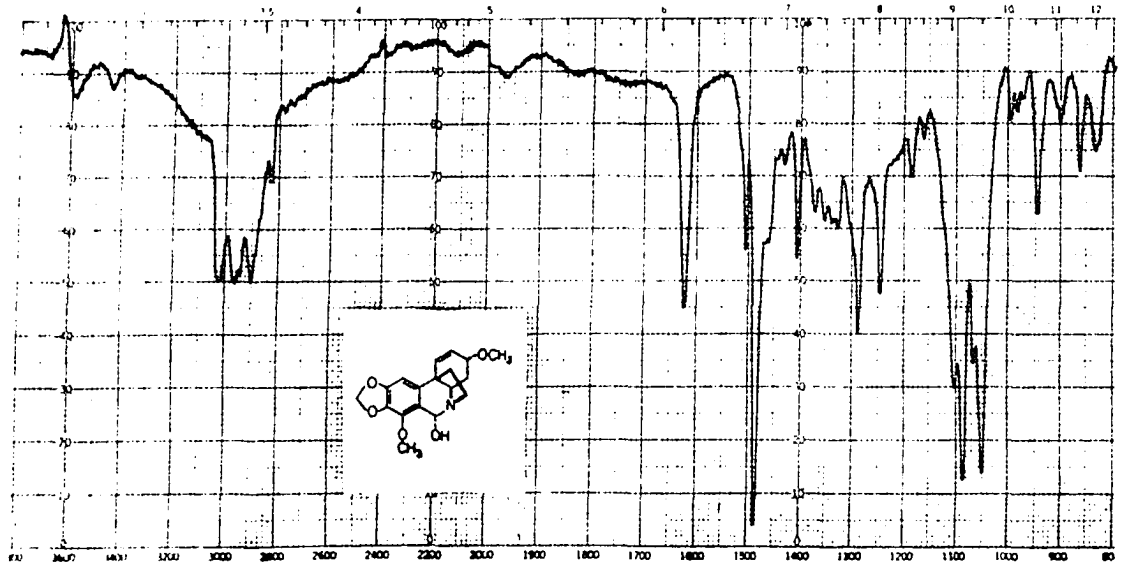
The Structure of 6-Hydroxybuphanidrine

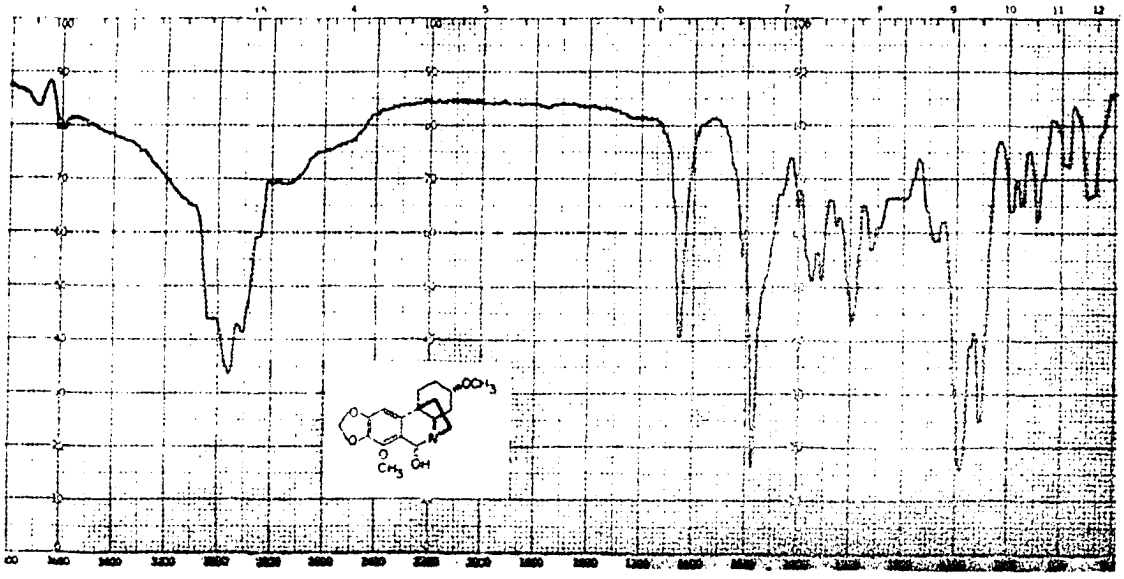
The bulbs of Nerine bowdenii W. Wats. have been found to contain a large number of Amaryllidaceae alkaloids. At present, nineteen alkaloids have been reported to be in the plant.[45,46] Although sixteen alkaloids were isolated from this plant source in 1960,[46] 66% of the total alkaloid fraction remained noncrystalline and uncharacterized. Preliminary fractionation of the alkaloids by column chromatography produced considerable quantities of a nonpolar mixture which contained crinamine and an unknown compound. Thin-layer chromatography of this mixture on a silica gel plate, developed in chloroform-methanol-diethylamine (90:5:5), led to the isolation of 6-hydroxybuphanidrine (43, mp 95-96°, $[\alpha]^{24}_{D}$ -64°). The infrared spectrum of the compound [Fig. 1] indicated the presence of a hydroxyl group (3595 cm^{-1}) and an aromatic ring substituted by both methylenedioxy (945 and 1050 cm^{-1}) and methoxyl (1618 cm^{-1}). The ultraviolet absorption spectrum (λ_{max} 285 mu, ϵ 1880) was in agreement with this formulation.[47] The alkaloid was stable to chemical methods of reduction (e.g. lithium aluminum hydride), but gave a single dihydro derivative upon catalytic reduction. The O-acetyl

Fig. 1. Infrared spectra

a: 6-Hydroxybuphanidrine (43)

b: Dihydro-6-hydroxybuphanidrine





derivative ($C_{20}H_{23}NO_6$) was obtained by treatment of 43 with acetic anhydride and pyridine. The nmr spectrum of 43 [Fig. 2] confirms the presence of a hydroxyl group (5.12 ppm) which disappears upon exchange with deuterium oxide. The spectrum also reveals an aliphatic methoxyl (3.33 ppm), one aromatic proton (6.60 ppm), and two olefinic protons centered at 6.00 and 6.58 ppm. Since the alkaloid contained no N-methyl or N-H groups but possessed two olefinic protons, it could be tentatively assigned the 5,10b-ethanophenanthridine nucleus by a process of elimination.[48]

To verify this conclusion, it seemed desirable to convert the alkaloid to a deoxy compound which might be identical with an alkaloid of known structure. Treatment of 43 with thionyl chloride followed by lithium aluminum hydride gave a compound which by all spectroscopic [Figs. 3 and 4] and chromatographic criteria was identical to the alkaloid buphanidrine (12). This interconversion established the basic nucleus of the alkaloid, the absolute configuration thereof, and the configuration of the aliphatic methoxyl at C_3.

With this information, only the location of the hydroxyl group remained for determination. Substitution at C_1 or C_2 would require the alkaloid to be an enol, a structure incon-

Fig. 2. Nuclear magnetic resonance spectra

a: 6-Hydroxybuphanidrine (43)

b: Dihydro-6-hydroxybuphanidrine

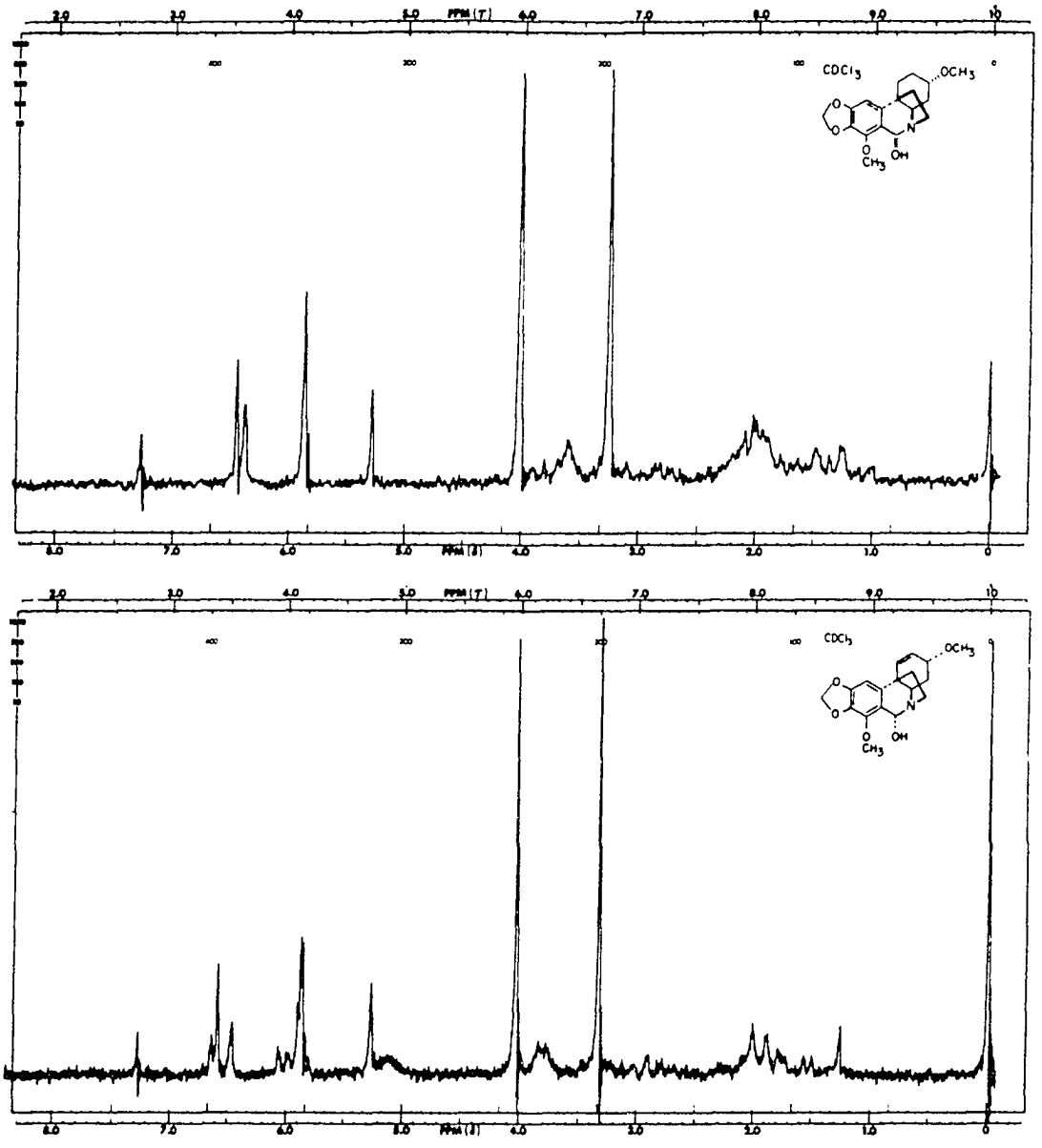
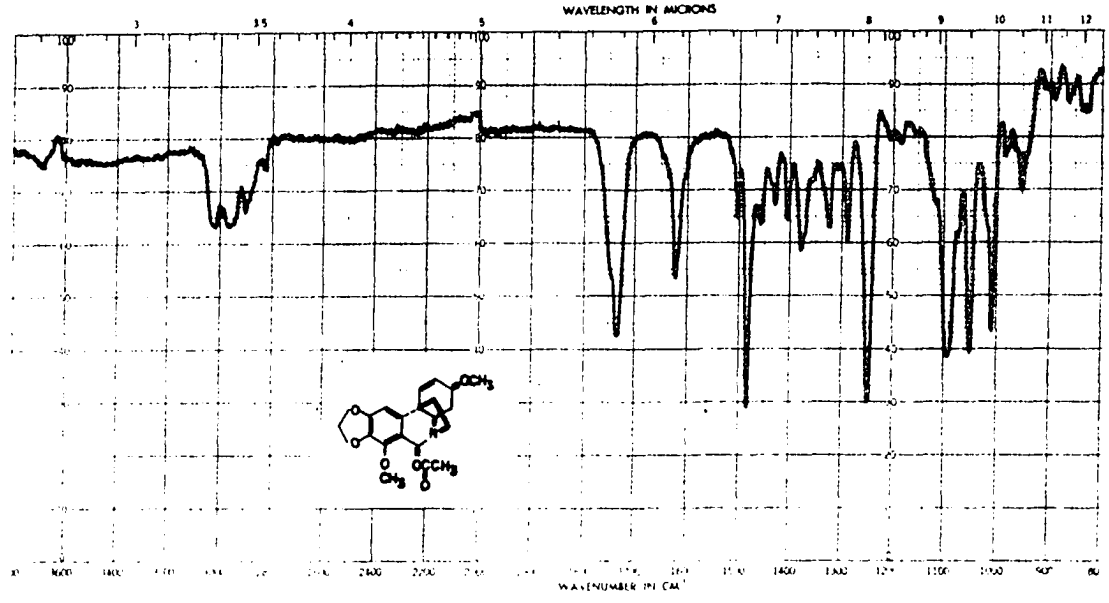

CDCl3
OCH3
OH
CH3
PPM (T)
PPM (δ)

Fig. 3. Infrared spectra

a: 6-O-Acetylbuphanidrine (45)

b: Buphanidrine (12)


WAVELENGTH IN MICRONS
WAVENUMBER IN CM⁻¹

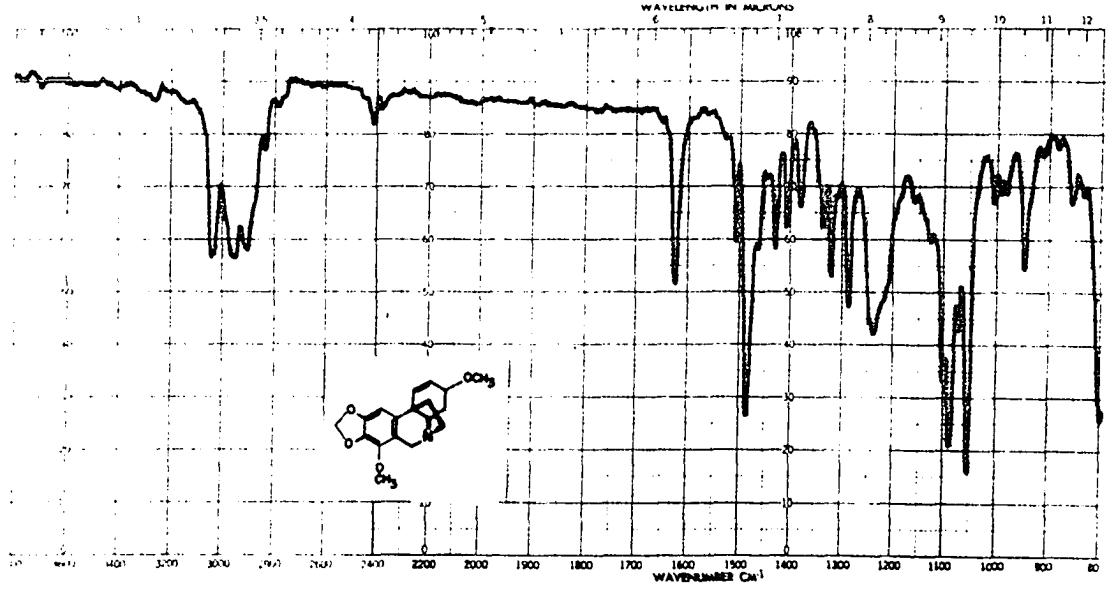
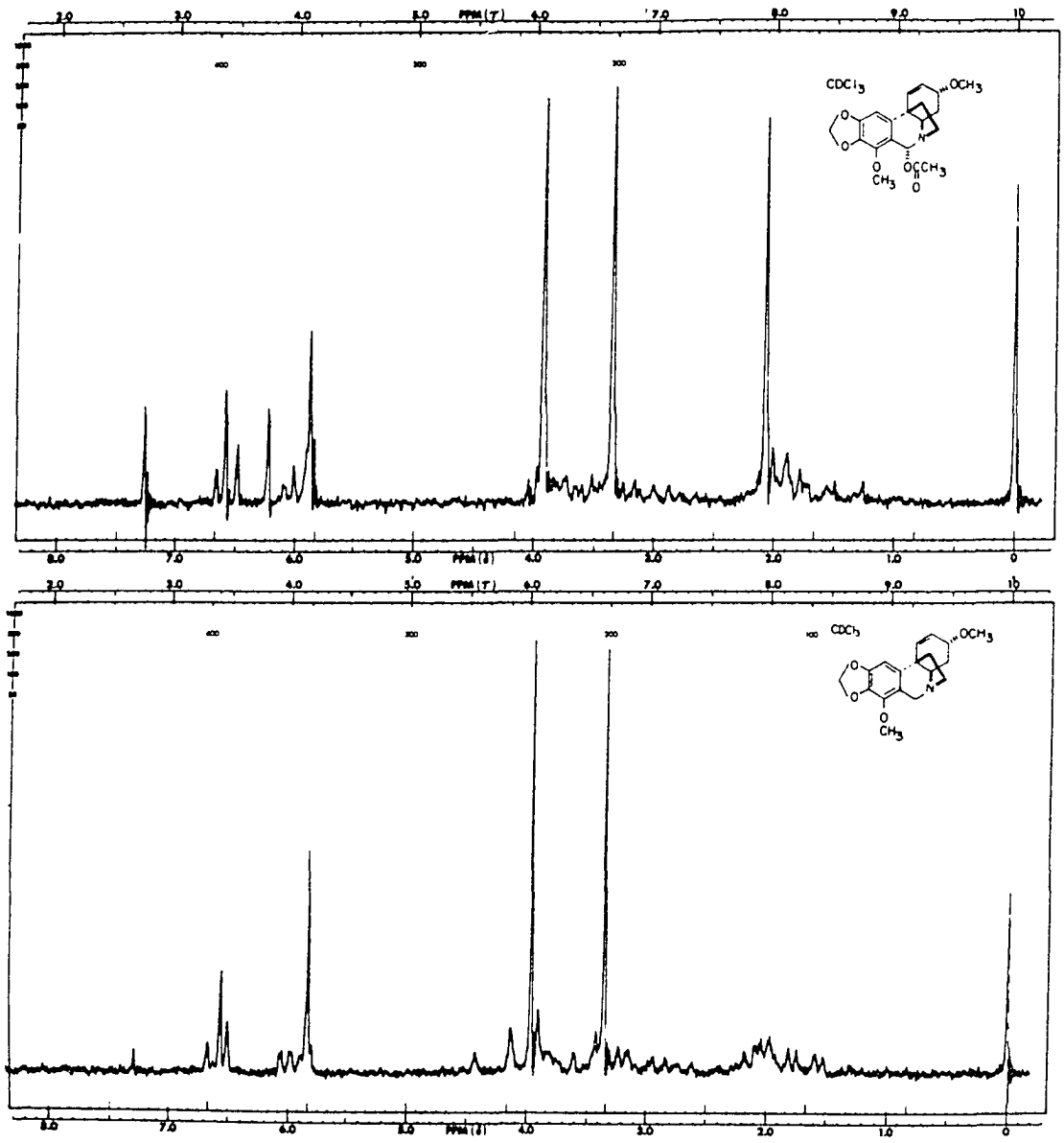
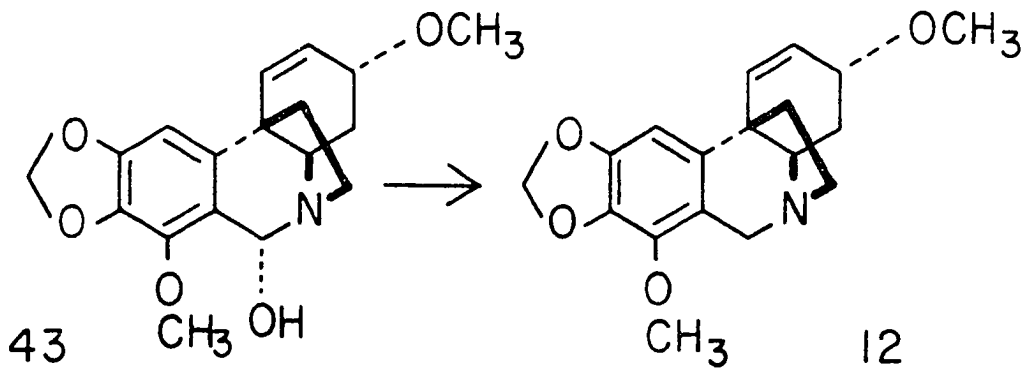

WAVENUMBER CM⁻¹

Fig. 4. Nuclear magnetic resonance spectra

a: 6-O-Acetylbuphanidrine (45)

b: Buphanidrine (12)


CDCl3
OCH3
O
CH3
OCCH3
O
CDCl3
OCH3
O
CH3



sistent with the chemical properties of the compound. Proof that the double bond is located at C_1-C_2 can be obtained from the nmr spectrum [Fig. 2]. The single aromatic proton appeared as a singlet at 6.60 ppm and was shifted to 6.38 ppm in dihydro-6-hydroxybuphanidrine. This upfield shift has been observed in other alkaloids of this series and results from the loss of the deshielding of the C_{10} proton by the C_1,C_2-unsaturation.[49] The two olefinic protons appeared as an AB pattern which overlapped partially with methylenedioxy absorption. The proton at C_2 (6.00 ppm) is further split by coupling to the proton at C_3 (J = 9 cps). Substitution at C_{4a} or C_{12} is unlikely since this type of substitution is unknown in the Amaryllidaceae alkaloids. Also, such hydroxyl substitution is not compatible with the chemical reactivity of the alkaloid. Assignment of the hydroxyl group to C_{11} can

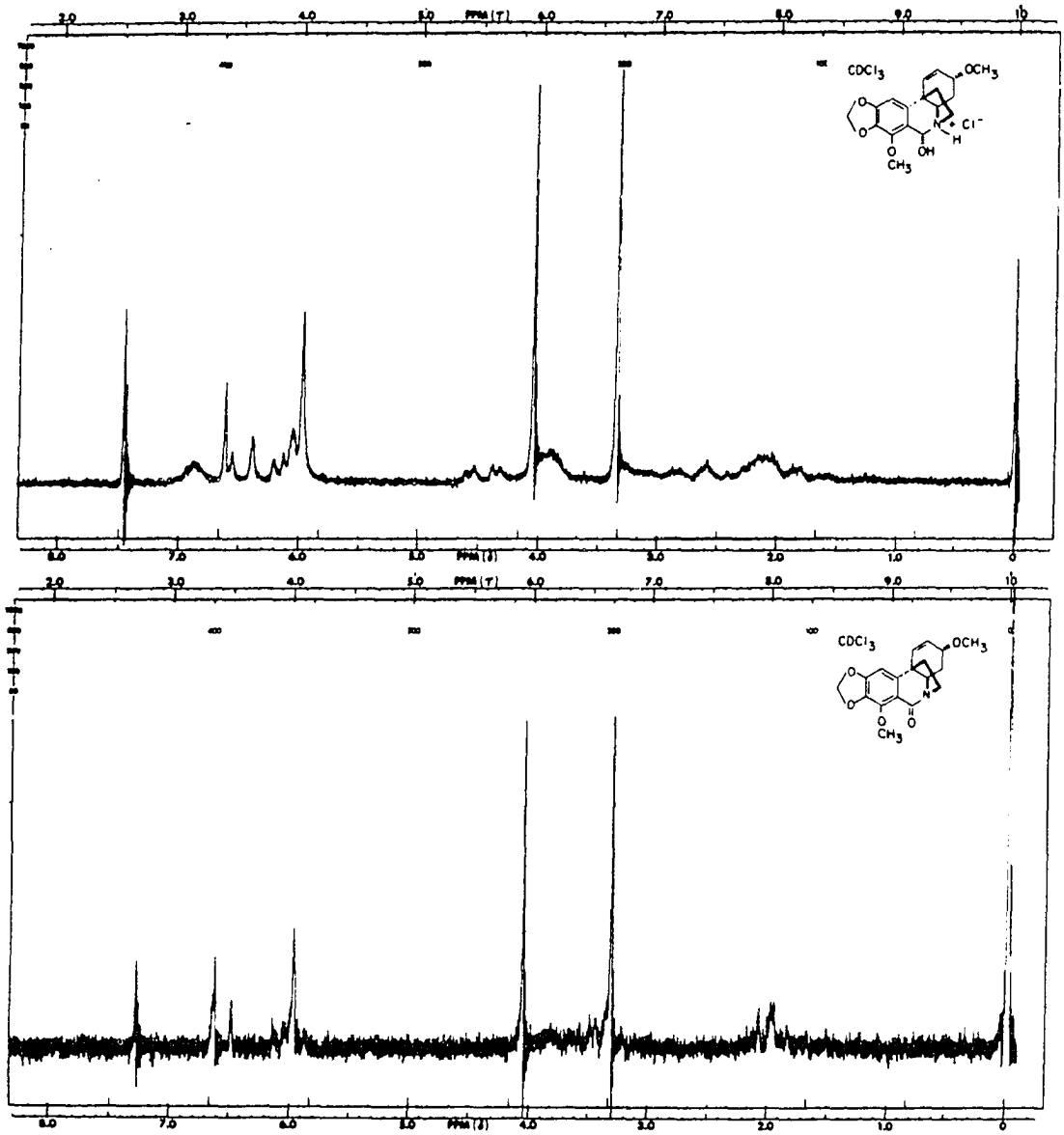
be ruled out since the compound is not identical with either ambelline or epiambelline, both of which are known. The infrared absorption of the hydroxyl group obtained at high dilution in chloroform showed a single band at 3595 cm^{-1}. This is compatible with a hydroxyl function weakly hydrogen bonded to the π-electrons of the aromatic ring and suggested that the hydroxyl group might be located at C_6.[50] Comparable stretching frequencies (3593 and 3597 cm^{-1}) have been reported for the benzylic hydroxyl groups in 6-hydroxycrinamine (20) and haemanthidine (14), respectively.[26]

A very significant aspect of the nmr spectrum of 43 [Fig. 2] is the appearance of a singlet at 5.31 ppm which, by integration, was found to consist of a single proton. It was considered to be a benzylic proton because of the absence of the characteristic AB methylene pattern near 4.0 ppm.[17] It is unusual that this proton shows only one chemical shift since the benzylic protons in 6-hydroxycrinamine (20) and haemanthidine (14), the only two other alkaloids known to contain a C_6 hydroxyl, each showed two chemical shifts.[26,27] Additional support for the assignment of the peak at 5.31 ppm as the benzylic proton was obtained by examining the nmr spectrum [Fig. 5] of 6-hydroxybuphanidrine hydrochloride (44). The

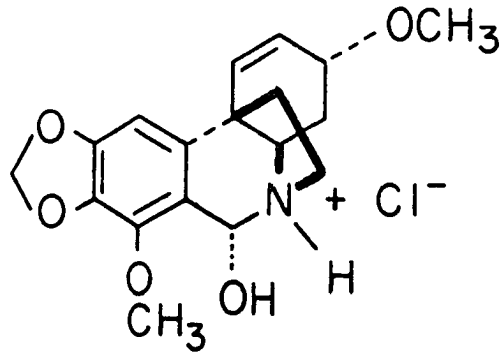
Fig. 5. Nuclear magnetic resonance spectra

a: 6-Hydroxybuphanidrine hydrochloride (44)

b: 6-Oxobuphanidrine (46)


CDCl3
OCH3
Cl-
OH
CH3
CDCl3
OCH3
CH3

singlet was shifted downfield (6.08 ppm), resulting from the deshielding of the proton through the inductive effect of the quaternary nitrogen. The absence of this peak at 5.3 ppm in the nmr spectrum [Fig. 4] of the deoxy compound (12) and the presence of a benzylic AB pattern at 3.95 ppm provide positive proof that 43 is a secondary benzylic alcohol.



44

Reaction of 6-hydroxybuphanidrine with pyridine-chromic oxide reagent and dimethyl sulfoxide-acetic anhydride failed to form an oxidation product. As was found for 6-hydroxycrinamine,[26] this latter oxidation method yielded only the O-acetyl derivative (45). 6-Hydroxybuphanidrine was initially thought to be inert to oxidation with manganese dioxide when stirred in an anhydrous chloroform solution. A significant increase in the reaction time (4 days at room temperature) did permit isolation of the lactam (46) in low yield. 6-Oxo-

buphanidrine has carbonyl absorption in the infrared at 1690 cm^{-1} [Fig. 6] and ultraviolet absorption at 231, 287, and 321 mμ. The nmr spectrum [Fig. 5] confirms the absence of benzylic protons. This spectral evidence supports the proposed structure. The inertness to oxidation of 6-hydroxy-

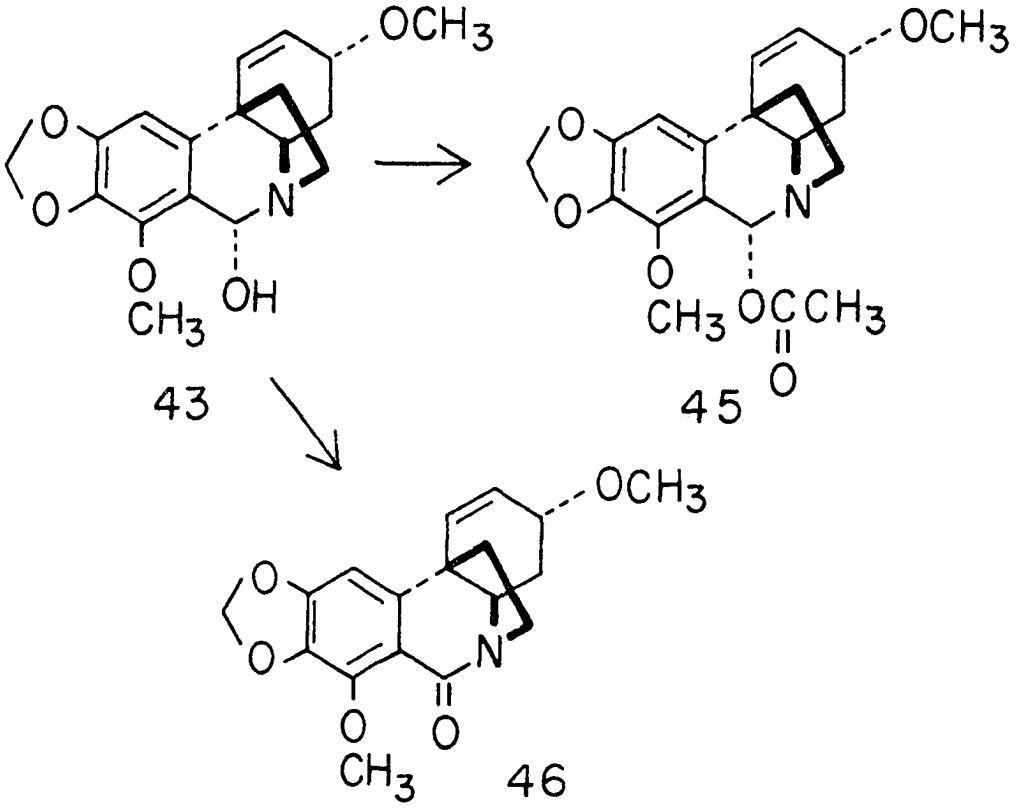
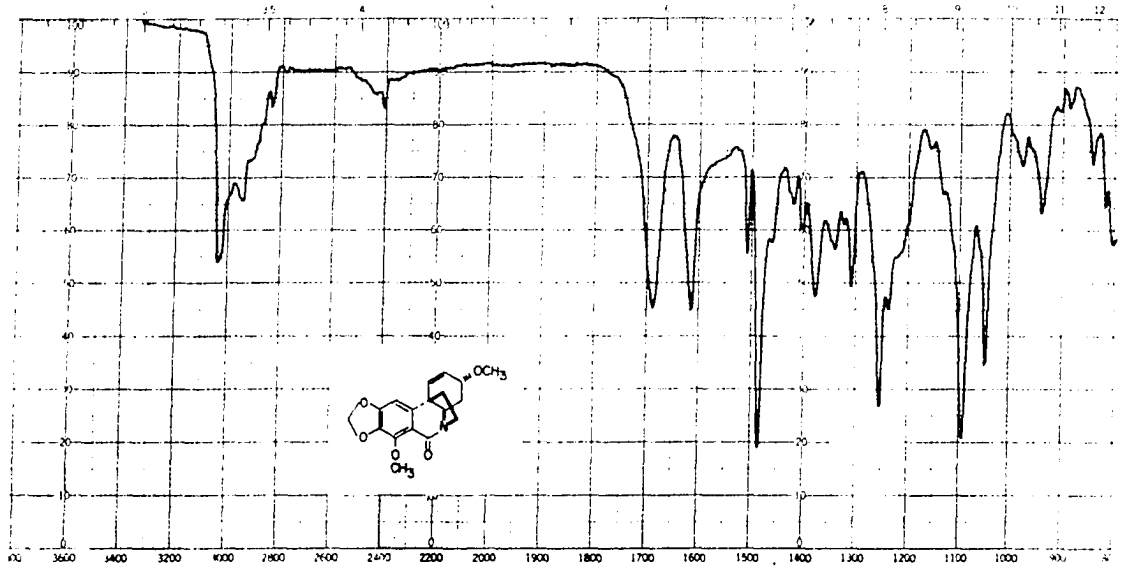
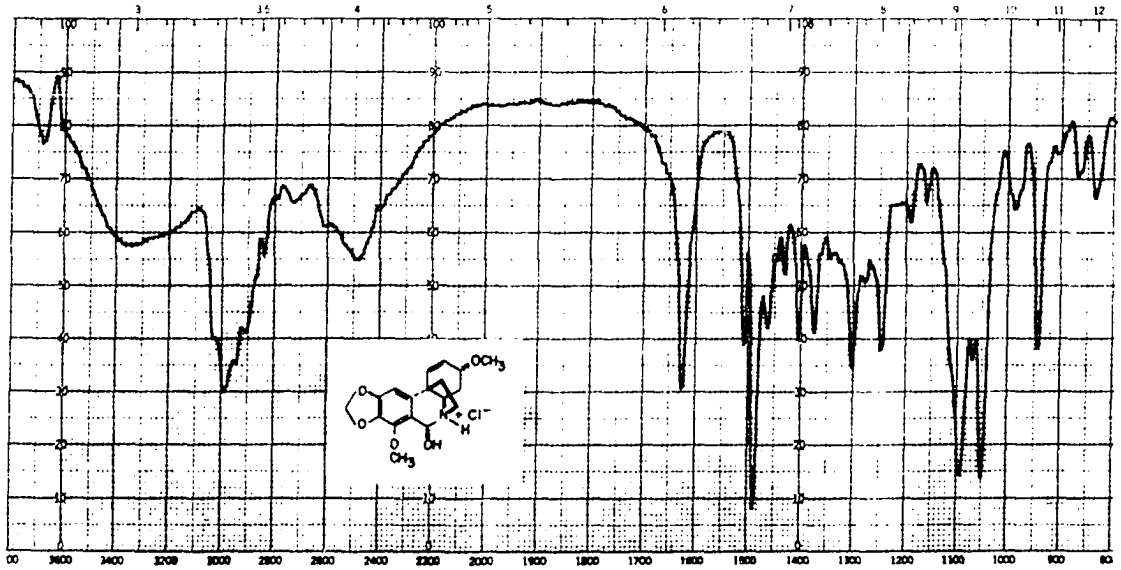


Fig. 6. Infrared spectra

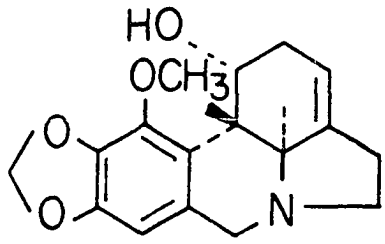
a: 6-Oxobuphanidrine (46)

b: 6-Hydroxybuphanidrine hydrochloride (44)





buphanidrine contrasts with 6-hydroxycrinamine (20) and haemanthidine (14) which are readily oxidized by manganese dioxide. This difference in reactivity is attributed to the steric effect of the aromatic methoxyl group. An analogous case of steric hindrance to oxidation was observed with the base falcatine (47) which proved inter to Oppenauer conditions while caranine (47, no aromatic methanyl) was readily oxidized.[15]



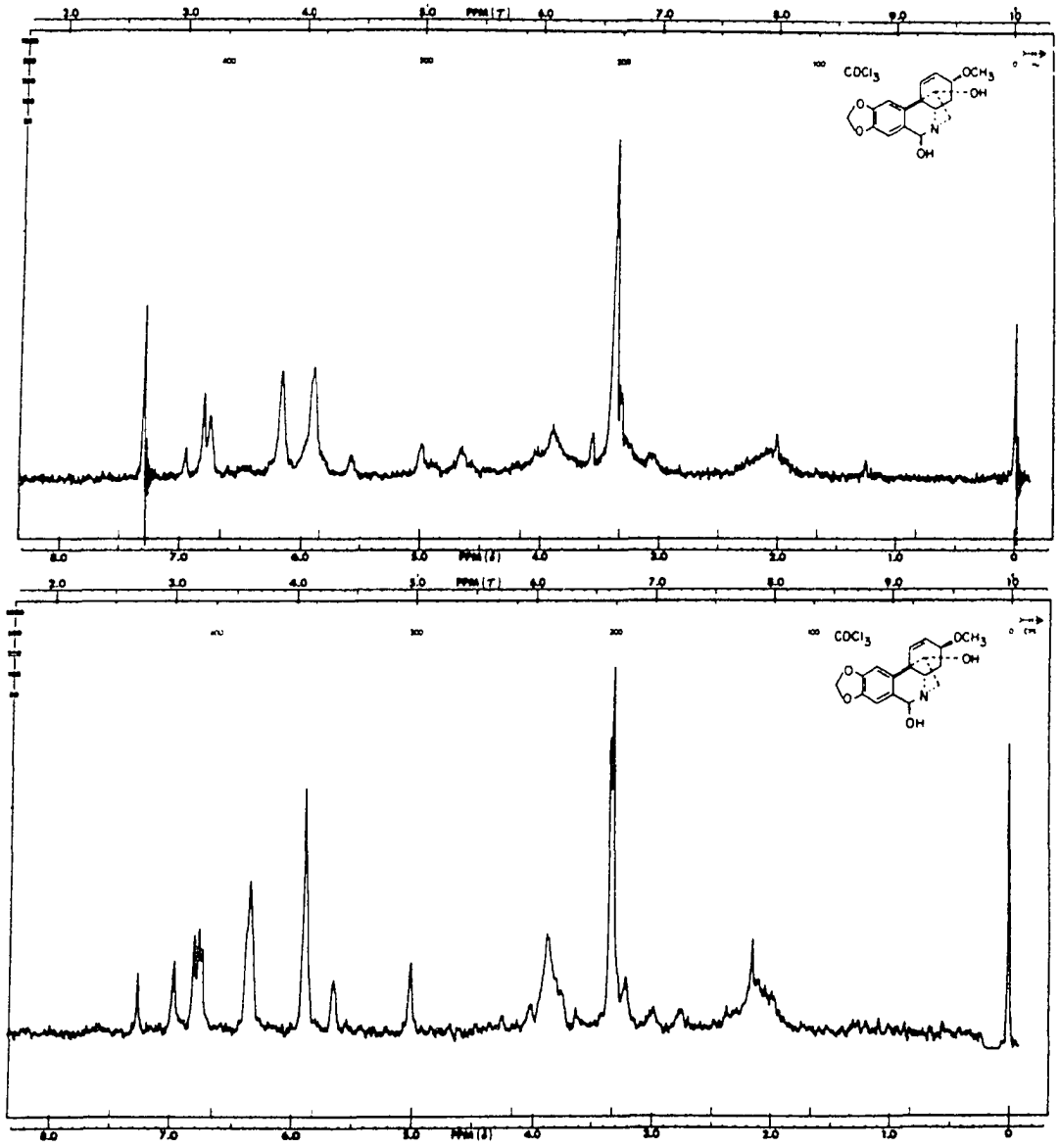
47

Finally, the configuration of the C_6 benzylic hydroxyl group must be considered. It has been shown that haemanthidine (14) and 6-hydroxycrinamine (20) exist in solution as

Fig. 7. Nuclear magnetic resonance spectra

a: 6-Hydroxycrinamine (20)

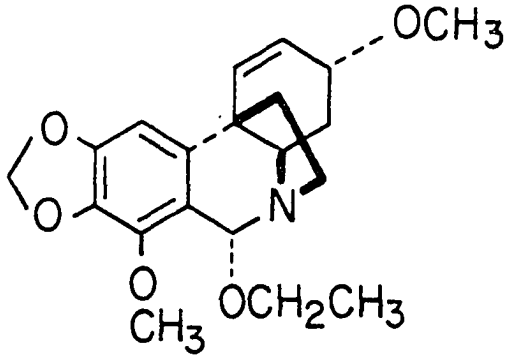
b: Haemanthidine (14)


COCl3
OCH3
OH
N
OH
PPM (δ)
PPM (τ)

a mixture of C_6 epimers. Unlike the nmr spectra of 6-hydroxycrinamine and haemanthidine [Fig. 7], which showed two chemical shifts for the benzylic proton, the spectrum of 6-hydroxybuphanidrine gives no indication of epimeric character. The benzylic proton appears as a singlet at 5.31 ppm. This suggests that either one epimer is present or that two epimers exist which possess the same chemical shift. The latter possibility seems remote in view of the highly different environments present in the rigid ring system and the large difference in chemical shifts reported for the two epimers 20a and 20b. Additional evidence for a single epimer was provided by the O-acetyl and hydrochloride derivatives of 43 which also exhibited singlets for the benzylic proton [Figs. 4 and 5]. In each case, these derivatives melted sharply and were homogeneous by thin-layer chromatography. It is evident that only one C_6 epimer is present in 6-hydroxybuphanidrine.

A most curious aspect of this problem developed when it was discovered that refluxing 6-hydroxybuphanidrine in an acidic ethanol solution produced a small amount of the C_6 ethyl ether. 6-Ethoxybuphanidrine (48) is not crystalline

but is homogeneous by thin-layer chromatography. No hydroxyl absorption is present in the infrared spectrum [Fig. 8], and the nmr spectrum [Fig. 9] reveals an upfield shift of the benzylic proton (4.75 ppm). The triplet corresponding to the methylene protons is clearly visible at 1.25 ppm. Refluxing 6-hydroxybuphanidrine in absolute ethanol without the presence of acid failed to produce the ethyl ether.



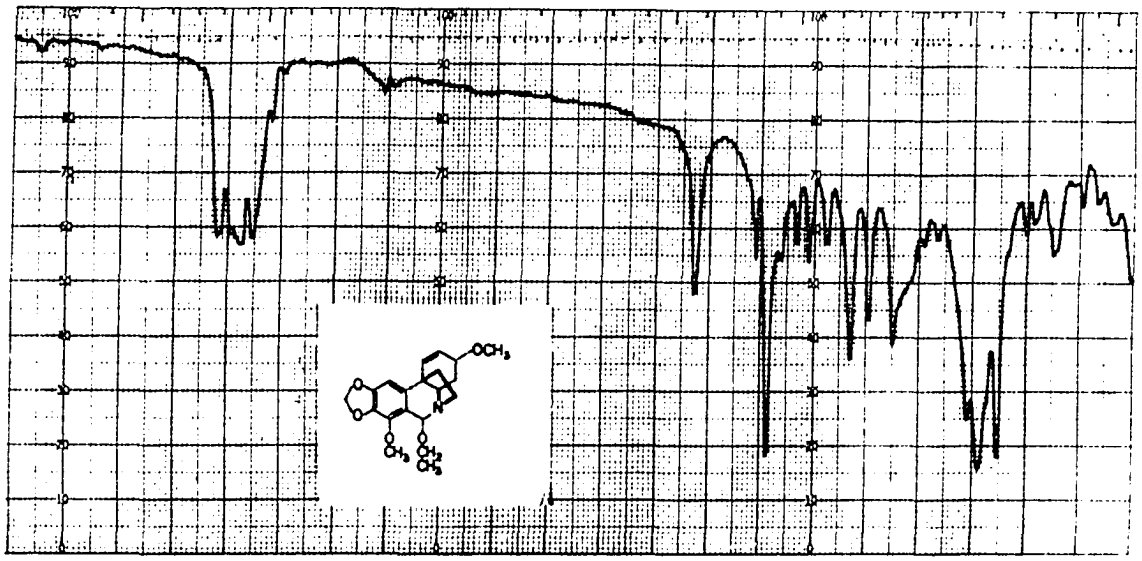
48

This necessity for acid in the reaction suggests that a carbonium ion (49) is obtained by removal of the -OH group followed by reaction with ethanol. The result is very surprising since one would expect the acid to first protonate the tertiary nitrogen. Generation of the dipositive species (50) would then be very unexpected. It is very likely that the benzylic carbonium ion is sufficiently stable so that a small amount of 49 is produced without prior protonation of the nitrogen atom.

Fig. 8. Infrared spectra

a: 6-Ethoxybuphanidrine (48)

b: 6-Methoxybuphanidrine (53)



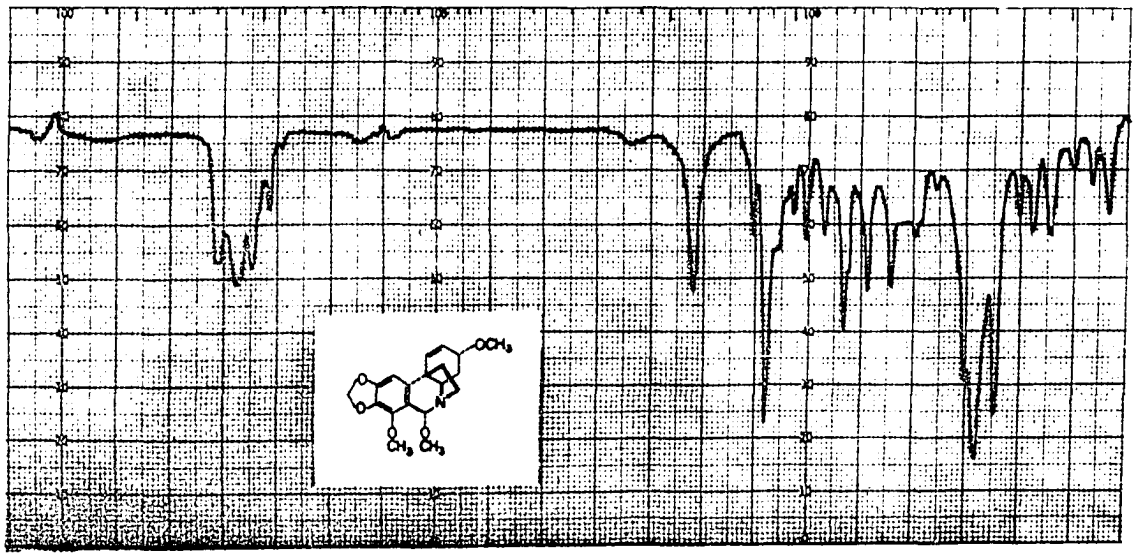
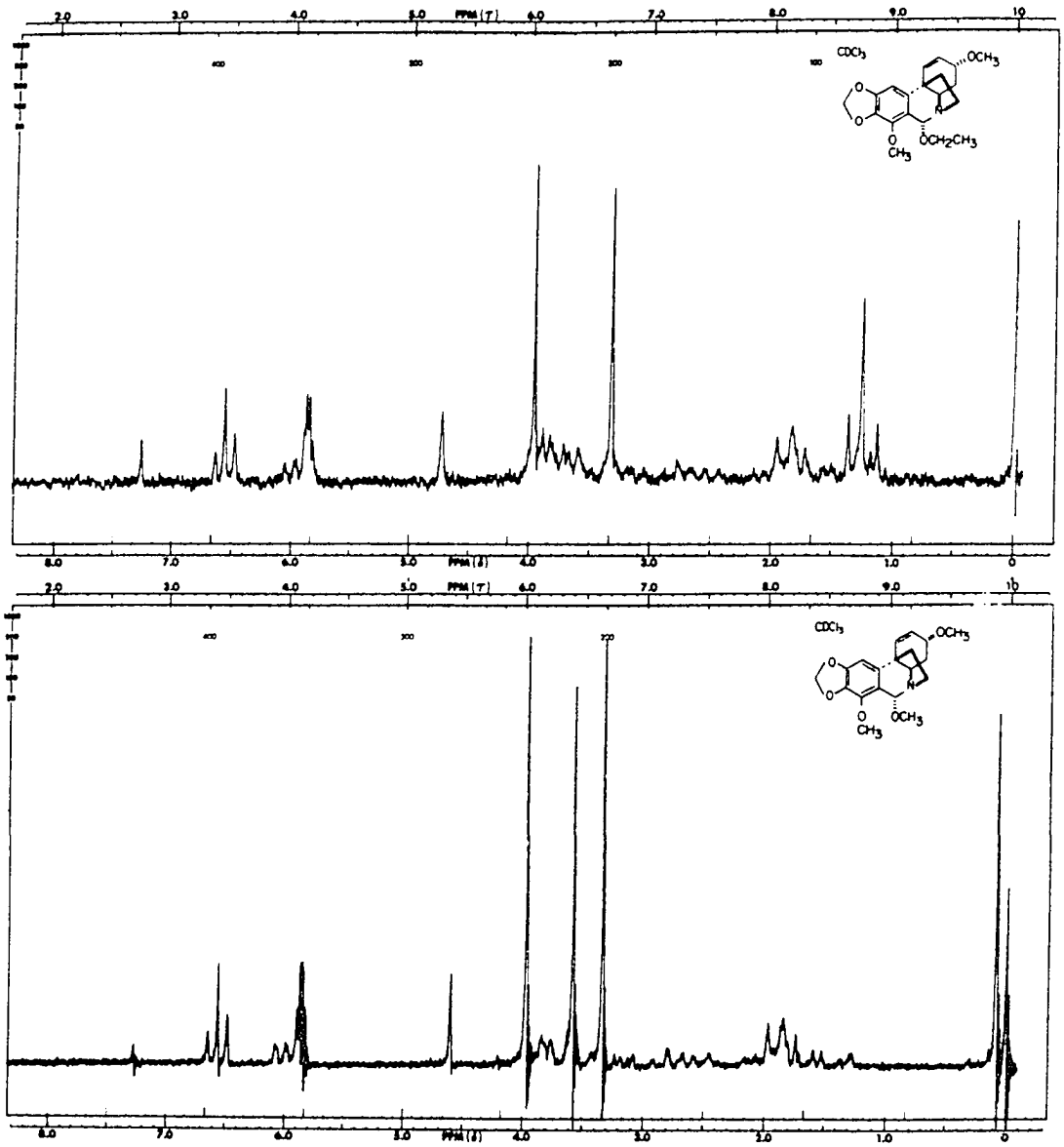
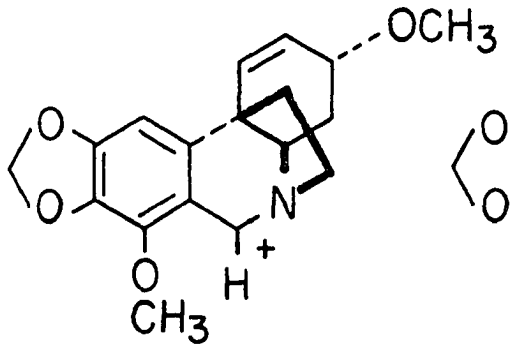


Fig. 9. Nuclear magnetic resonance spectra

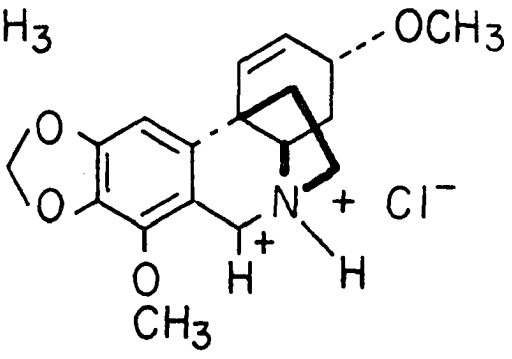
a: 6-Ethoxybuphanidrine (48)

b: 6-Methoxybuphanidrine (53)


CDCl3
OCH3
O
O
N
O
CH3
OCH2CH3
PPM (δ)
CDCl3
OCH3
O
O
N
O
CH3
OCH3
PPM (δ)

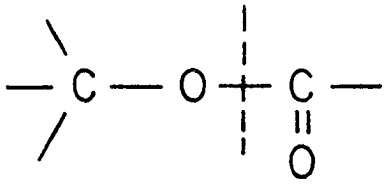


49

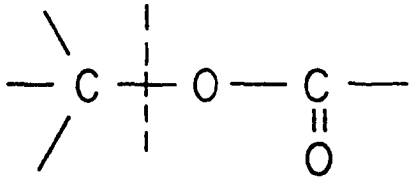


50

The hydrolysis of 6-acetylbuphanidrine in 50% dioxane/water was found to be complete after 10 hours. The product was identical to 6-hydroxybuphanidrine in all respects. Such hydrolytic reactions ordinarily involve breakage of the acyl-oxygen, rather than the alkyl-oxygen bond (that is, 51 rather than 52).[51] The configuration about the asymmetric carbon atom remains unaltered in this step.

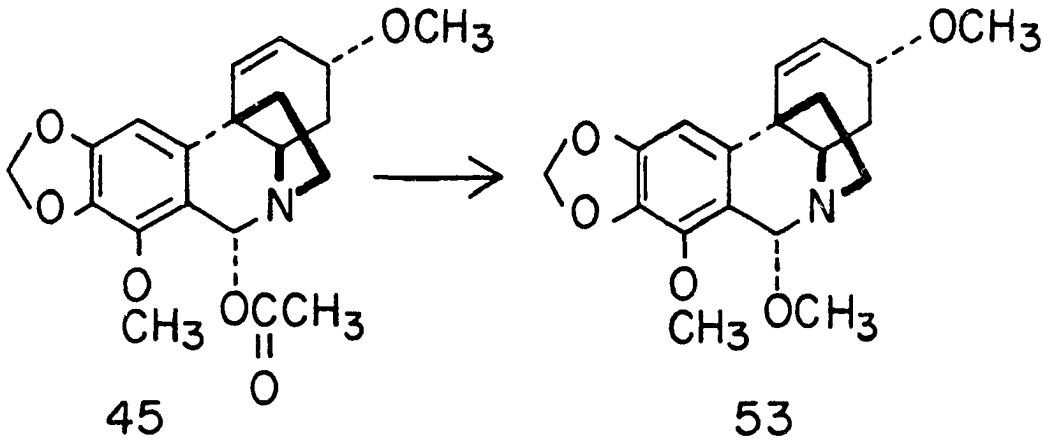


51



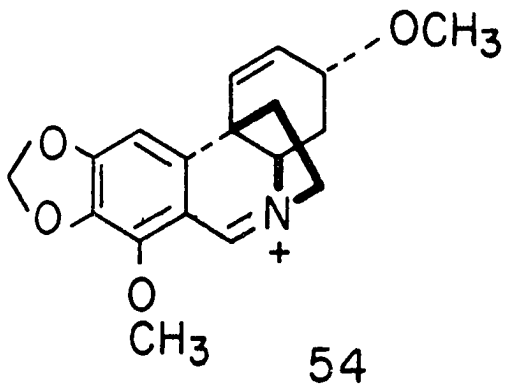
52

It is to be stressed, however, that the reaction might also proceed by a carbonium ion intermediate (49) which would be characterized by the occurrence of alkyl-oxygen fission. A methanolysis of 6-acetylbuphanidrine was carried out to determine the position of bond cleavage. The methanolysis product proved to be the methyl ether of 43. Analogous to the ethyl ether, 6-methoxybuphanidrine (53) proved to be noncrystalline, and the nmr spectrum [Fig. 9] displayed an upfield shift of the benzylic proton (4.60 ppm). The methoxyl protons appeared as a singlet at 3.55 ppm. Thus, alkyl-oxygen fission has occurred showing that the reaction proceeded by a carbonium ion mechanism.



The major factor promoting heterolysis of the alkyl-oxygen bond is conjugative electron release by the benzene ring containing both ortho and para electron releasing substituents. One might also predict assistance by the nitrogen

lone pair electrons. Such an intermediate (54), however, would constitute a violation of Bredt's Rule[52] and, in view of the rigidity of the 1-azabicyclo[3,2,1]octane ring system, is highly unlikely.



54

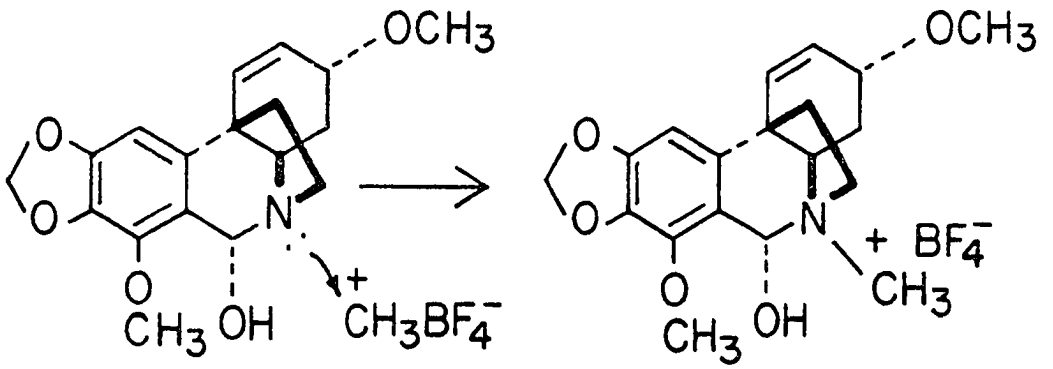
The substituents at C_6 in 6-hydroxybuphanidrine occupy pseudoaxial and pseudoequatorial positions. As a carbonium ion develops at the benzylic position, the sigma electrons of the pseudoaxial bond are more favorably located for orbital overlap with the pi electrons of the benzene ring than those of the corresponding pseudoequatorial bond. As a result, one might expect that formation of a carbonium ion at the benzylic position may involve departure of an a' group more readily than departure of an e' group. The reverse is also true in that one would expect formation of an a' derivative. Therefore, in the hydrolysis reactions one would predict from stereoelectronic factors the formation of pseudoaxial products

as are depicted. Steric control would also predict the same result with attack of the nucleophile on the side opposite the ethano bridge. Thus, a consideration of both stereoelectronic and steric factors would predict the α configuration for 6-ethoxy- and 6-methoxybuphanidrine (48 and 53, respectively).

To demonstrate that 6-hydroxybuphanidrine also possessed the α configuration at C_6, it seemed desirable to prepare the methyl ether by a method in which the C_6-O bond was not broken and compare the derivative to the methyl ether obtained from treatment of 43 with acid and methanol.

Perhaps the simplest method for the formation of methyl ethers involves the use of diazomethane. Although alcohols normally are inert to diazomethane, they can be methylated efficiently by diazomethane under catalysis by fluoroboric acid.[53] A mineral acid, such as hydrochloric acid, is unsatisfactory because it is itself methylated by diazomethane. Fluoroboric acid, on the other hand, could be consumed in the reaction with diazomethane only by some process involving rupture of a B-F bond. The addition of diazomethane to a methylene chloride solution containing 6-hydroxybuphanidrine and a small amount of fluoroboric acid produced a very vigor-

ous reaction. Thin-layer chromatography of the product revealed only considerable amounts of a very polar compound which probably is the salt (55), resulting from attack by the nitrogen lone pair on the methyl carbonium ion.



43 55

The utilization of methyl p-toluenesulfonate provides a method for the methylation of secondary hydroxyl groups which does not quaternize the tertiary amino group at the same time. When methyl p-toluenesulfonate was added to the potassium salt of 6-hydroxybuphanidrine, a 28% yield of the methyl ether (53) was obtained. This product proved identical to 6-methoxybuphanidrine by ir, nmr, and chromatographic data. There was no evidence for the epimeric methyl ether in the methylation reaction products. In order to rule out the possibility of epimerization of the secondary hydroxyl group under the strongly basic conditions encountered, a duplicate experiment

was performed with omission of the methyl p-toluenesulfonate. The reaction afforded only starting material with no evidence of the epimeric alcohol. The analogous reactions with crinine (5) and powelline (9) to give buphanisine (13) and buphanidrine (12), respectively, also proceeded with retention of configuration.[18] Thus, 6-hydroxybuphanidrine also contains the α configuration at C_6. This fact has been verified by an x-ray crystallographic study of the methiodide salt.[8]

In 1960 Boit and Döpke reported the isolation of an unknown alkaloid from Nerine bowdenii which they called base NB.[54] The molecular formula ($C_{18}H_{21}NO_5$) is identical to that of 6-hydroxybuphanidrine. Melting points are not in agreement, however, excluding the possibility that the two alkaloids are the same compound.

The Structure of 6-Hydroxypowelline

Separation of the alkaloid fractions from an unidentified Crinum species with large, strap-shaped petals revealed the presence of buphanidrine, 6-hydroxybuphanidrine, ambelline, crinamidine, and a new alkaloid of unknown structure. The mass spectrum indicated that the compound has a molecular

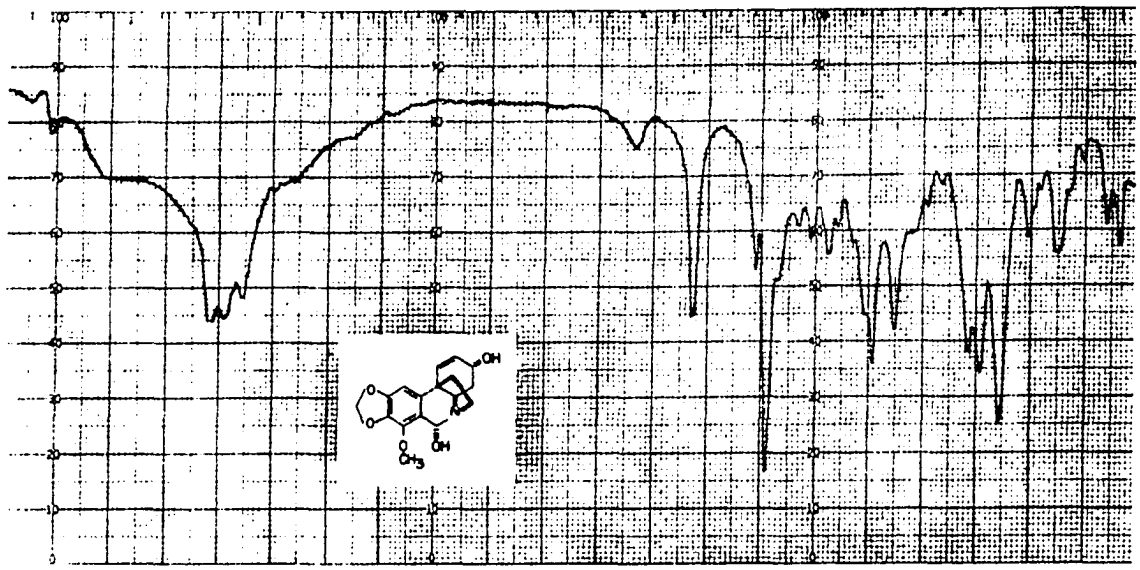
weight of 317. The ultraviolet and infrared spectra [Fig. 10] revealed the presence of an aromatic ring substituted by both methylenedioxy (945 and 1050 cm^{-1}) and methoxyl (1618 cm^{-1}) groups. Of special interest in establishing the structure was the similarity of the nmr spectrum [Fig. 11] to that of 6-hydroxybuphanidrine [Fig. 2]. From this comparison, the alkaloid was tentatively assigned the crinine nucleus. Particularly important in the nmr spectrum was the appearance of the singlet at 5.38 ppm which indicated the existence of a hydroxyl group at C_6. As in the spectrum of 6-hydroxybuphanidrine, there are two olefinic protons which appear as an AB pattern (6.38 and 5.85 ppm). The proton at 5.85 ppm is further split by coupling to a single proton (J = 5 cps) which must be the proton at C_3. Since the alkaloid formed an O,O-diacetyl derivative, the compound was tentatively assigned structure 56 containing hydroxyl groups at C_3 and C_6.

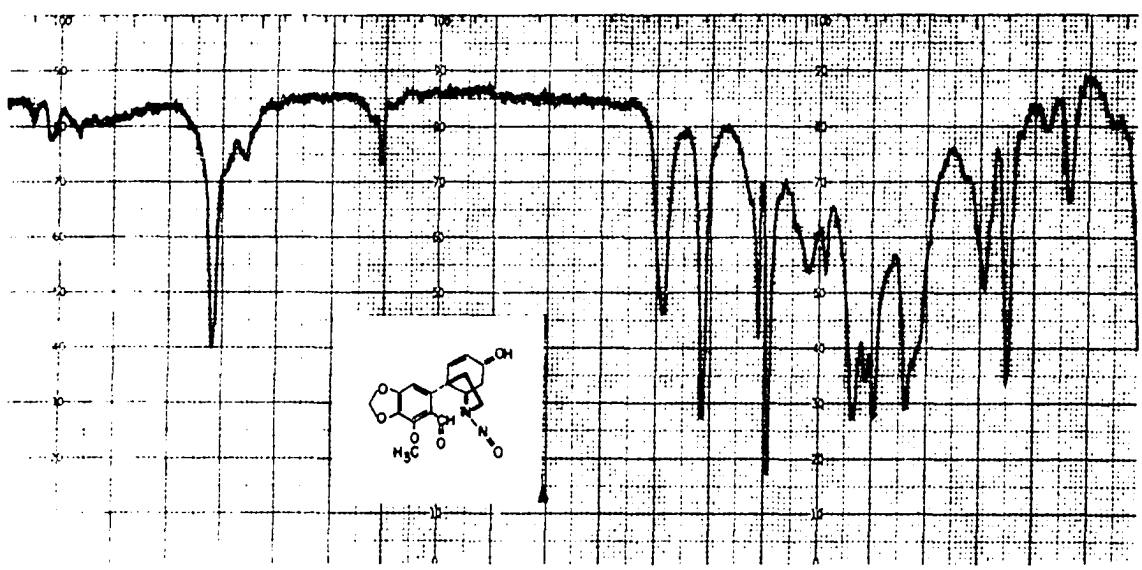
Additional evidence was obtained for location of the hydroxyl at C_6 when sodium nitrite was added to a dilute acetic acid solution of the alkaloid. A product was isolated in high yield which possessed spectral properties consistent with the N-nitroso aldehyde (57). The infrared spectrum [Fig. 10] displayed no hydroxyl absorption but contained a band in

Fig. 10. Infrared spectra

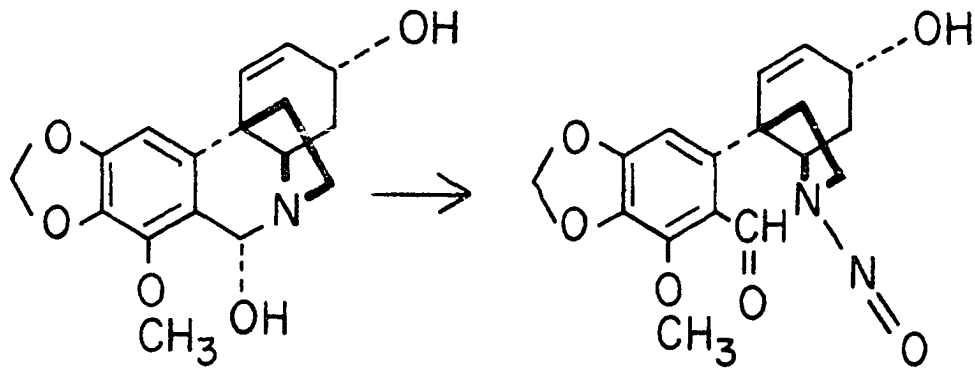
a: 6-Hydroxypowelline (56)

b: N-Nitroso-6-hydroxypowelline (57)





the carbonyl region (1685 cm^{-1}). The nmr spectrum [Fig. 11] confirmed the aldehydic proton (10.3 ppm). This ring opening upon reaction with nitrous acid has also been reported to occur with 6-hydroxycrinamine.[26]



56 57

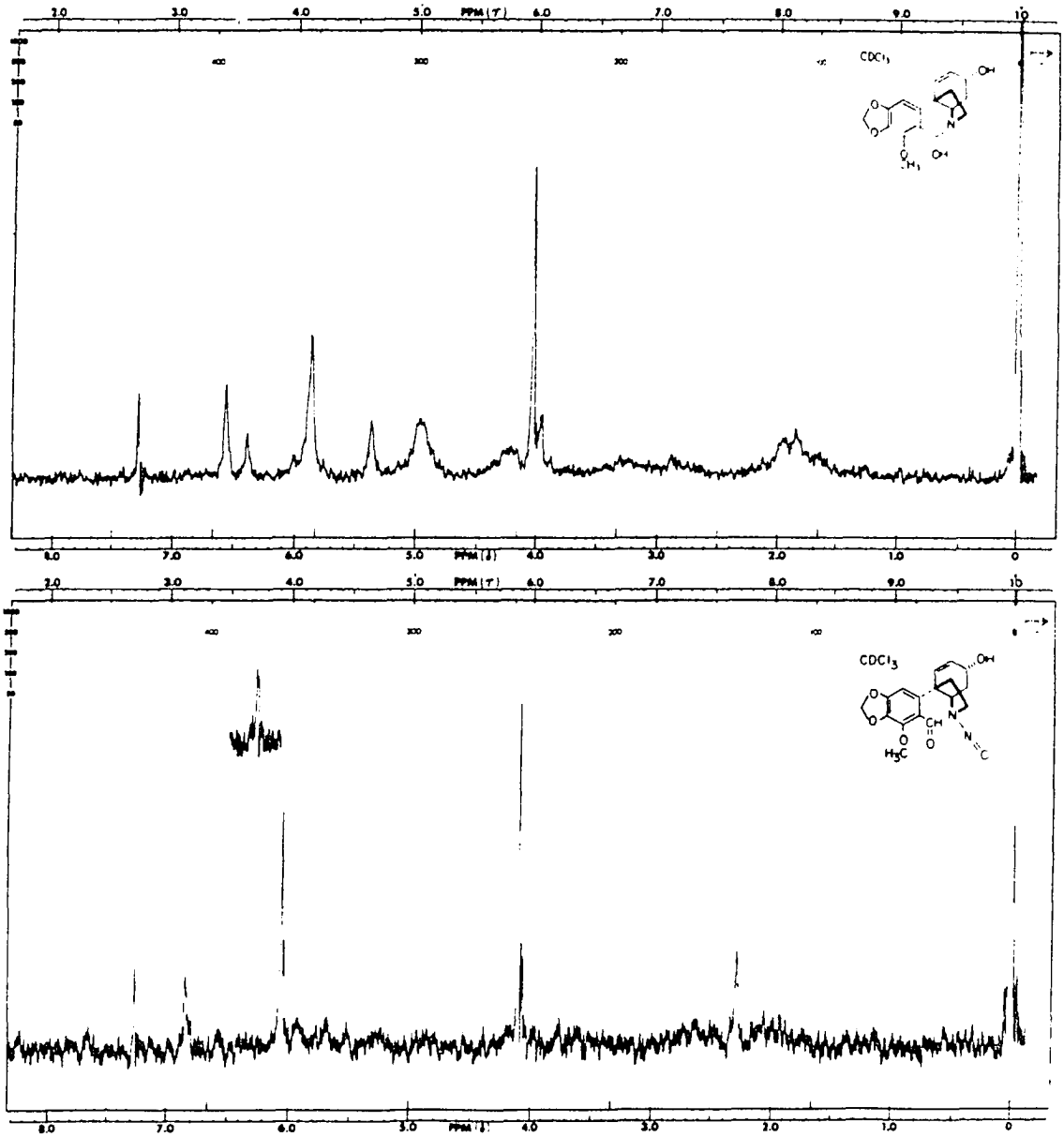
The hydroxyl group at C_6 was assigned the α configuration (as in 56) because the position of the benzylic proton (5.38 ppm) was comparable to the chemical shift of the corresponding proton in 6-hydroxybuphanidrine (5.31 ppm). By analogy with the spectrum of 6-hydroxycrinamine, if the β epimer were present, the benzylic proton should be approximately 0.6 ppm downfield.

To establish the configuration of the C_3 hydroxyl group, it seemed desirable to convert the alkaloid to a known 6-deoxy compound. Treatment of 6-hydroxypowelline (56) with acetic anhydride and pyridine afforded the corresponding O,O-

Fig. 11. Nuclear magnetic resonance spectra

a: 6-Hydroxypowelline (56)

b: N-Nitroso-6-hydroxypowelline (57)


PPM (τ)
PPM (δ)
CDCl3
OH
OH
CDCl3
OH
CH
O
H3C
N
N≡C

diacetyl derivative (58). Purification by thin-layer chromatography showed only one acetylation product, which crystallized easily from acetone. The infrared spectrum [Fig. 12] displayed strong carbonyl absorption (1740 cm^{-1}). Hydroxyl absorption was absent.

Hydrolysis of 3,6-O,O-diacetyl-6-hydroxypowelline (58) with dioxane-water (50:50) produced 3-acetyl-6-hydroxypowelline (59). The position of the hydroxyl group was assigned from the nmr spectra. In the spectrum [Fig. 13] of 3-acetyl-6-hydroxypowelline, the benzylic proton has returned to its upfield position (5.37 ppm) while the rough triplet is still present.

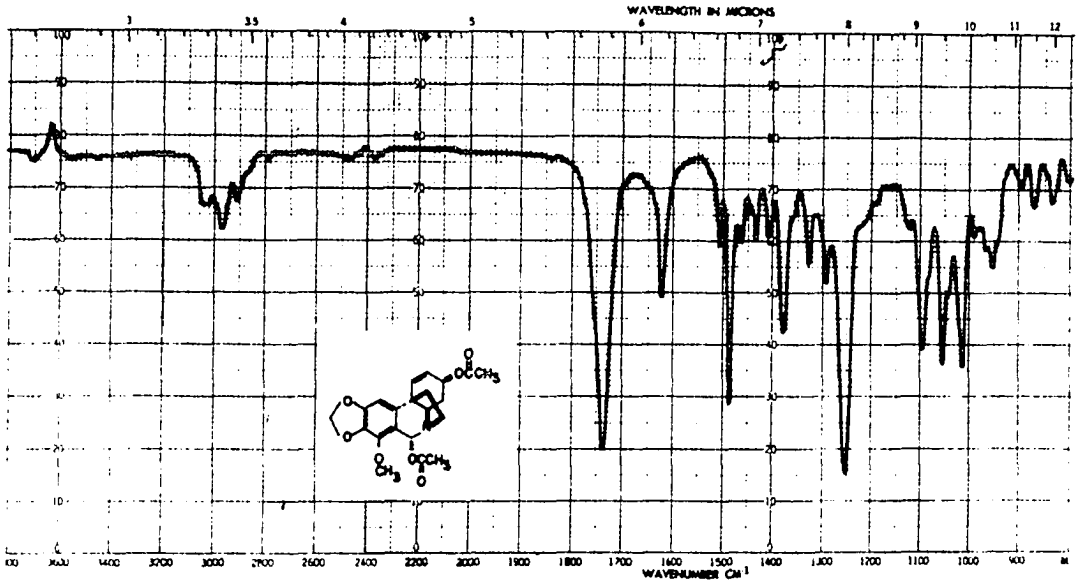
Treatment of 3-acetyl-6-hydroxypowelline with thionyl chloride followed by lithium aluminum hydride produced compound 60. The identity of this product with authentic powelline was proven by mp, mixed mp, spectral [Fig. 14] and chromatographic data. This interconversion established the absolute configuration of the alkaloid and the relative configuration of the C_3 hydroxyl group.

As in the case of 6-hydroxybuphanidrine, 6-hydroxypowelline showed no evidence for the presence of equilibrating epimers at C_6. Both the nmr spectra of 56 and the diacetyl

Fig. 12. Infrared spectra

a: 3,6-O,O-Diacetylpowelline (58)

b: 3-O-Acetyl-6-hydroxypowelline (59)


WAVELENGTH IN MICRONS
WAVENUMBER CM⁻¹

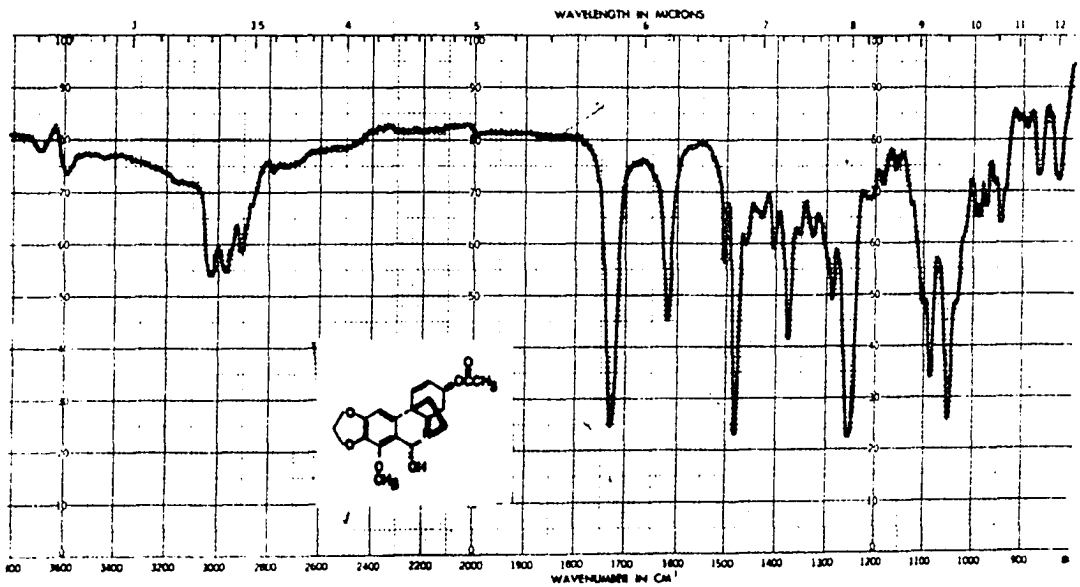

WAVELENGTH IN MICRONS
WAVENUMBER IN CM⁻¹

Fig. 13. Nuclear magnetic resonance spectra

a: 3,6-O,O-Diacetylpowelline (58)

b: 3-O-Acetyl-6-hydroxypowelline (59)

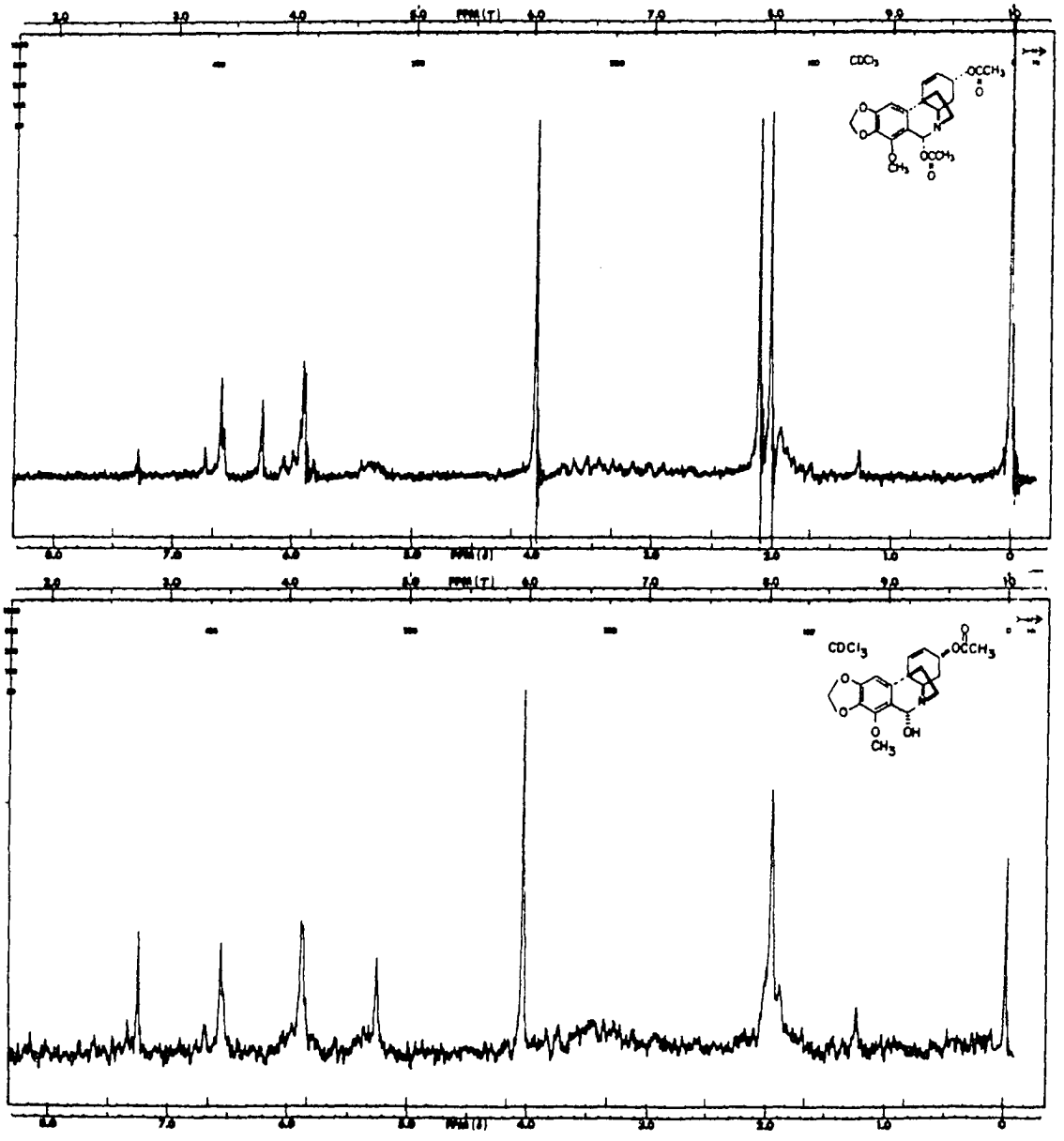
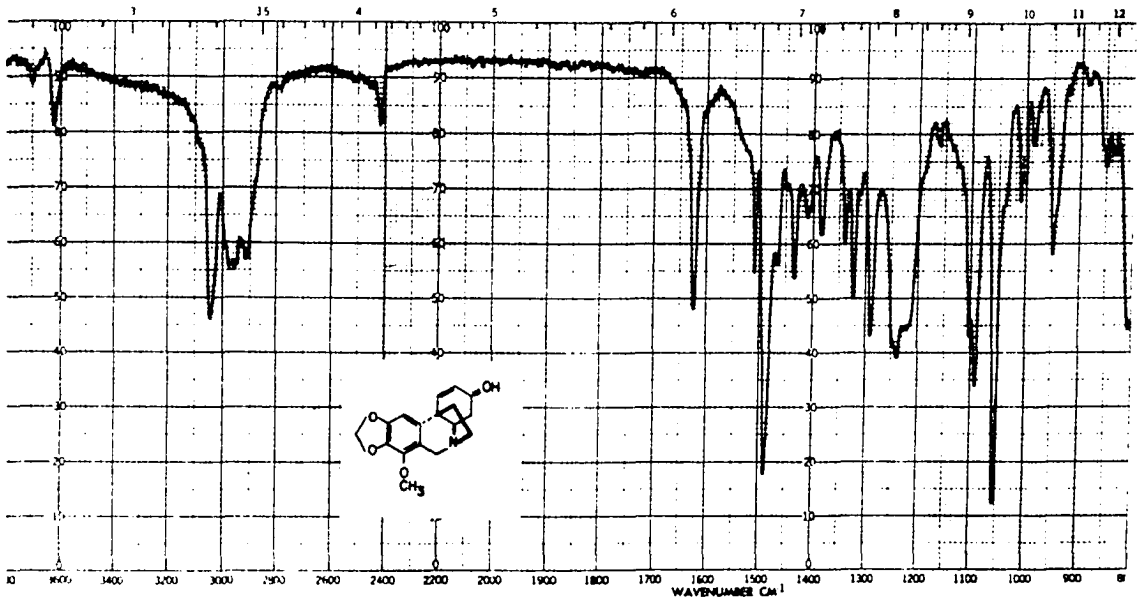
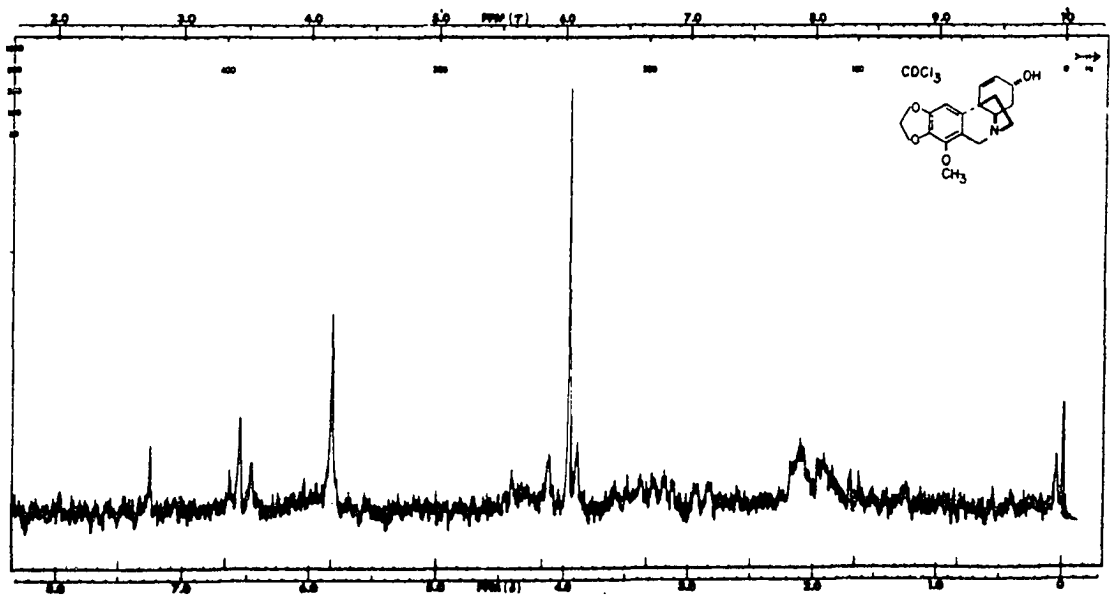

CDCl3
OCCH3
O
N
OCCH3
O
CH3
CDCl3
OCCH3
O
N
OH
CH3

Fig. 14. Powelline (60)

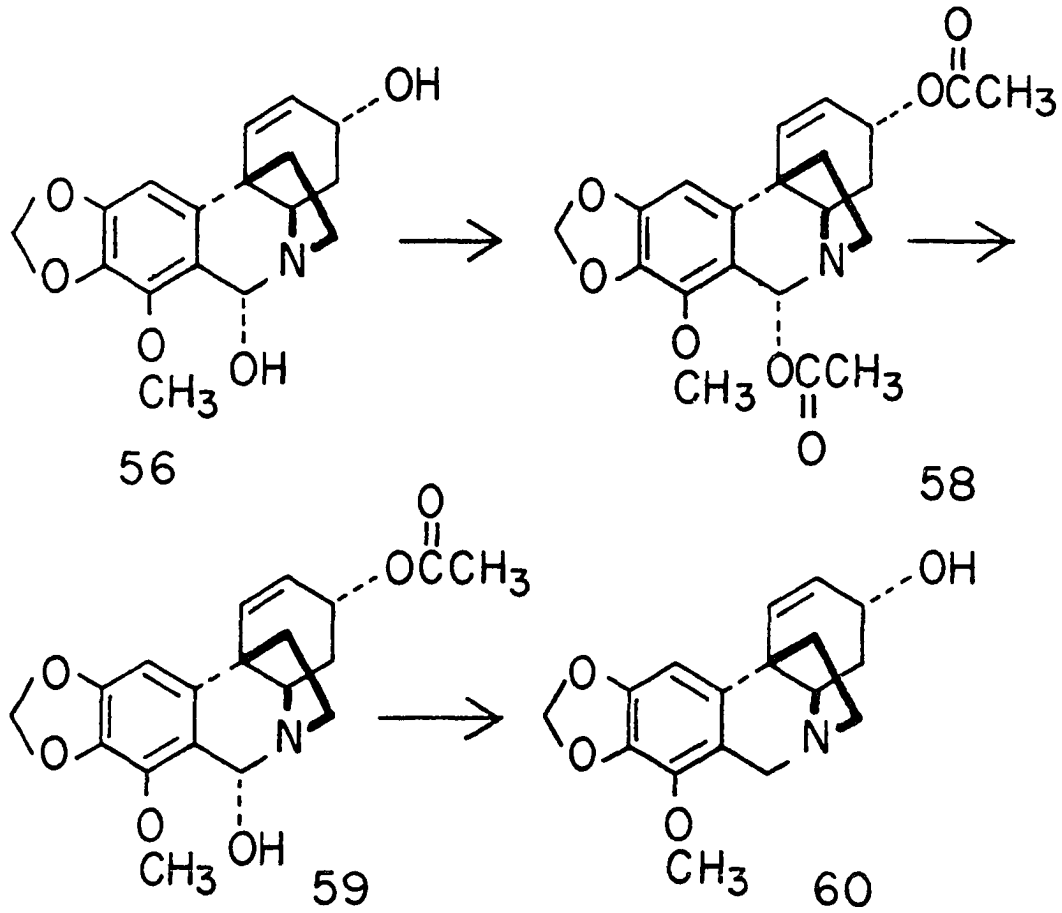
a: Infrared spectrum

b: Nuclear magnetic resonance spectrum


WAVENUMBER CM⁻¹
OH
O
CH3


PPM (T)
CDCl3
OH
O
CH3

derivative displayed a single peak for the benzylic proton.



Deuterium Labeled Derivatives

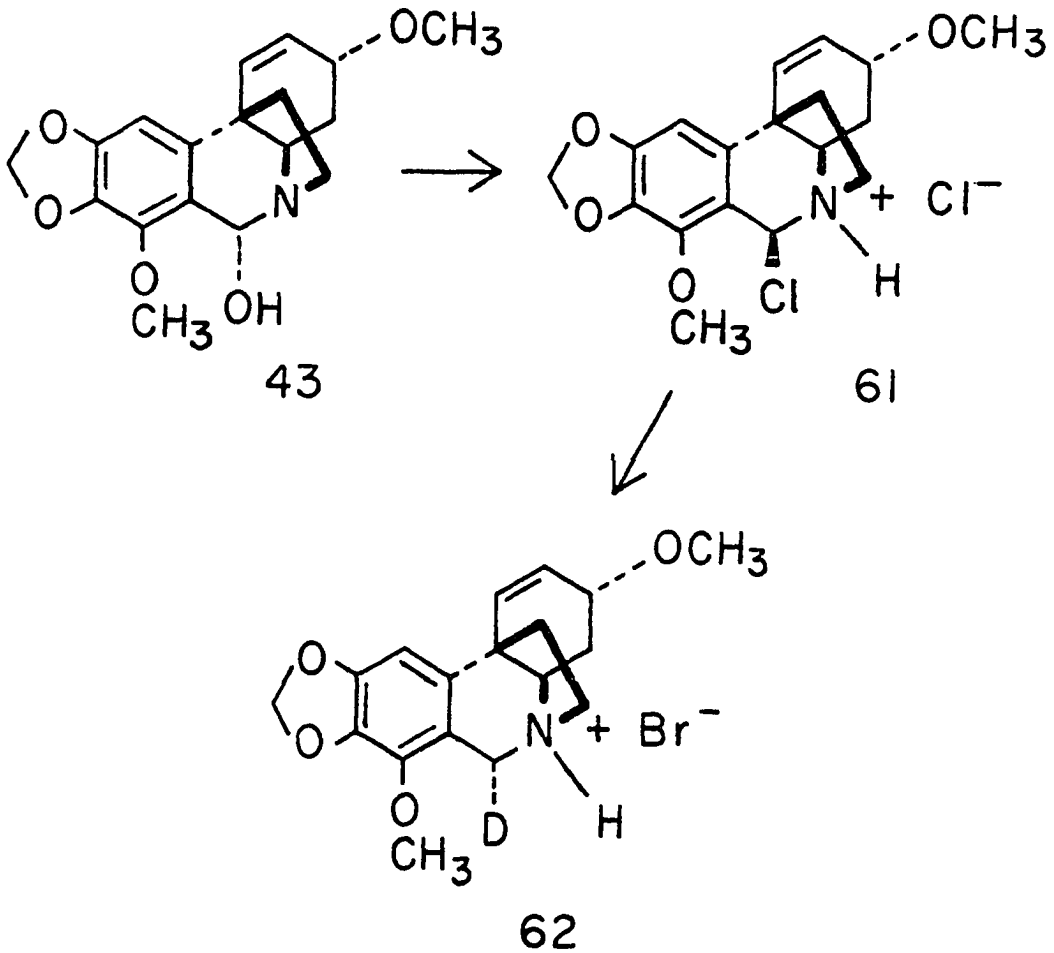
The finding that both 6-hydroxybuphanidrine and 6-hydroxypowelline consist of single epimer at C_6 and are of known configuration provides an excellent opportunity to study the biosynthetic oxidation process at C_6. With this in mind, it seemed desirable to develop a procedure whereby the appropriate tritium labeled precursors could be prepared.

It has become evident from several studies that hydroxylation generally occurs relatively late in the biosynthetic

process.[48] It is reasonable to expect that 6-hydroxybuphanidrine arises from the hydroxylation of buphanidrine. A fundamental problem of biosynthetic importance is the determination of the mechanism of the hydroxylation process. Preparation of the precursors must be carried out with known stereochemistry because it must reflect the process being studied. Preparation of stereospecifically labelled precursors would allow detection of three different mechanisms: a retention mechanism, an inversion mechanism, and an oxidation-reduction mechanism in which there was a lactam intermediate.

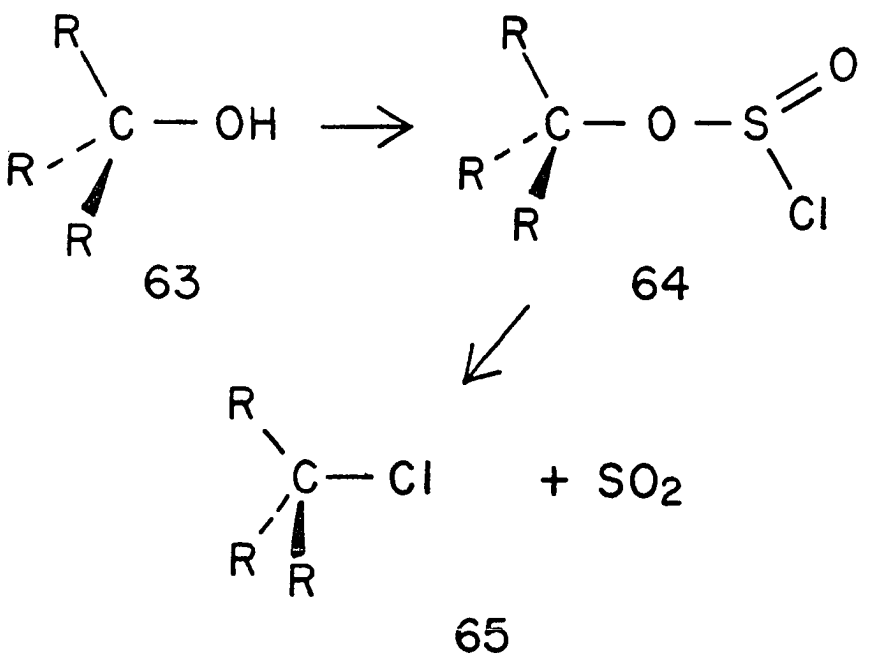
The most attractive route for introduction of a deuterium at the C_6 position in buphanidrine appeared to be from the 6-chloro derivative since the earlier conversion of 6-hydroxybuphanidrine to buphanidrine proceeded in excellent yield. 6-Hydroxybuphanidrine (43) was converted to 6-chlorobuphanidrine hydrochloride (61) by refluxing the alkaloid in thionyl chloride for two hours. Although no attempt was made to purify or characterize the chloride, the compound was believed to be present as the hydrochloride salt because of its solubility characteristics. The chloride (61) was then added to a dry tetrahydrofuran solution, a slight excess of lithium aluminum deuteride added, and the solution refluxed for five

hours. In working with radioactive samples, it is important to have derivatives which can be easily crystallized. Neither buphanidrine or its hydrochloride salt are crystallized without difficulty. The hydrobromide salt, however, can be crystallized from methanol-ether. Thus, it was decided to hydrolyze the reduction mixture with dilute hydrobromic acid. This procedure worked extremely well because the hydrobromide salt (62) was readily soluble in chloroform, and the inorganic precipitates which accompany basic hydrolyses were avoided.

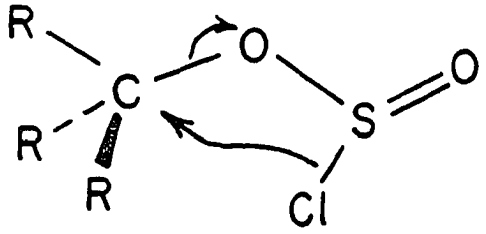


Although the transformation of an alcohol to a chloride with thionyl chloride normally proceeds with retention of configuration, in the presence of pyridine, inversion of configuration has been reported for the process.[55] Since 6-hydroxybuphanidrine also contains a tertiary nitrogen, it seemed desirably to clarify the structure of 6-chlorobuphanidrine and the mechanism of its formation.

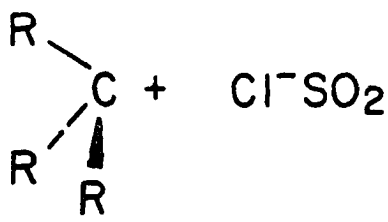
The reaction of an alcohol (63) with thionyl chloride proceeds via a nucleophilic attack by the oxygen atom of the alcohol. The chlorosulfinic ester (64) results and in some cases has been isolated at low temperatures.[55] This intermediate decomposes to form the product (65) and sulfur dioxide. This final step can be viewed as



an internal rearrangement (66) accompanied by retention of configuration or as a charged transition state (67) also giving retention of configuration. In the latter case, the ionic products form a tight ion pair, and the chloride attacks the carbonium ion most readily from the side leading to retention of configuration.[55]

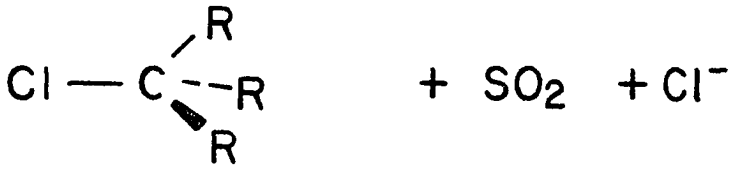


66



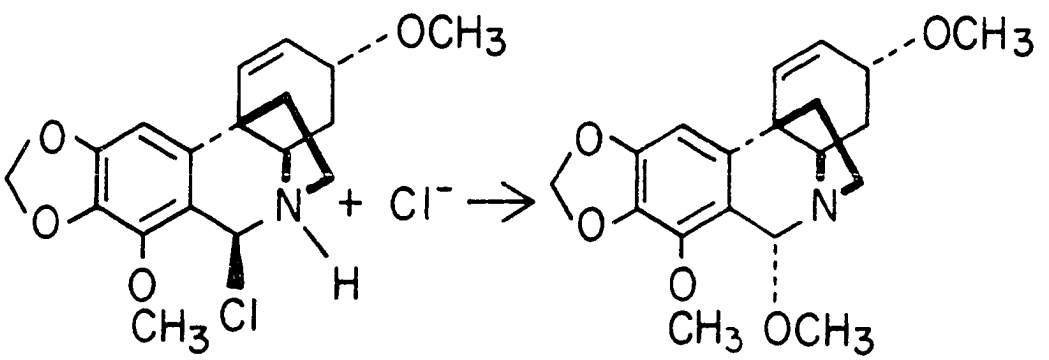
67

In the presence of an amine, the course of the reaction can be altered. When thionyl chloride reacts with an alcohol, hydrogen chloride is generated. The amine removed the hydrogen chloride formed along with the intermediate 64. By doing so, however, it converts the hydrogen chloride to chloride ion which may attack from the rear. The reaction now proceeds to give the inverted product (68).



68

The most attractive method for determining the stereochemistry of the chloro group was to convert the compound to 6-methoxybuphanidrine (53) which earlier had been shown to possess the α-configuration at C_6. This was achieved by treating 61 with sodium methoxide in methanol. The product proved identical to 6-methoxybuphanidrine by all spectroscopic and chromatographic data with no indication for the presence of the epimeric compound. Of particular interest in this comparison was the finding that the benzylic protons in the nmr spectra possessed identical chemical shifts.



61 53

Since the displacement by sodium methoxide undoubtedly proceeds with inversion of configuration, 6-chlorobuphanidrine hydrochloride (61) is assigned the β configuration. Similarly, displacement of the chloride by deuteride would result in inversion of configuration to give the [6α-^{2}H]buphanidrine (62). Thus, the mechanism proposed for the conversion of

6-hydroxybuphanidrine to the chloro derivative involves a nucleophilic displacement of the chlorosulfinate ion by chloride ion as depicted in 64→68. In agreement with this mechanism, when the reaction sequence was carried out on the hydrochloride salt of 6-hydroxybuphanidrine, the product was again 6-methoxybuphanidrine with no evidence for formation of the epimeric derivative.

In agreement with structure 62, the mass spectrum displayed a molecular ion (m/e 316 for the free base) which had increased by one mass unit. The nmr spectrum [Fig. 15] was very definitive when compared to that of buphanidrine hydrobromide [Fig. 15] where the inductive effect of the quaternary nitrogen has shifted the benzylic AB quartet downfield (centered at 4.4 ppm). In the spectrum of [6α-^{2}H] buphanidrine hydrobromide only a singlet is observed (4.24 ppm) corresponding to the one benzylic hydrogen.

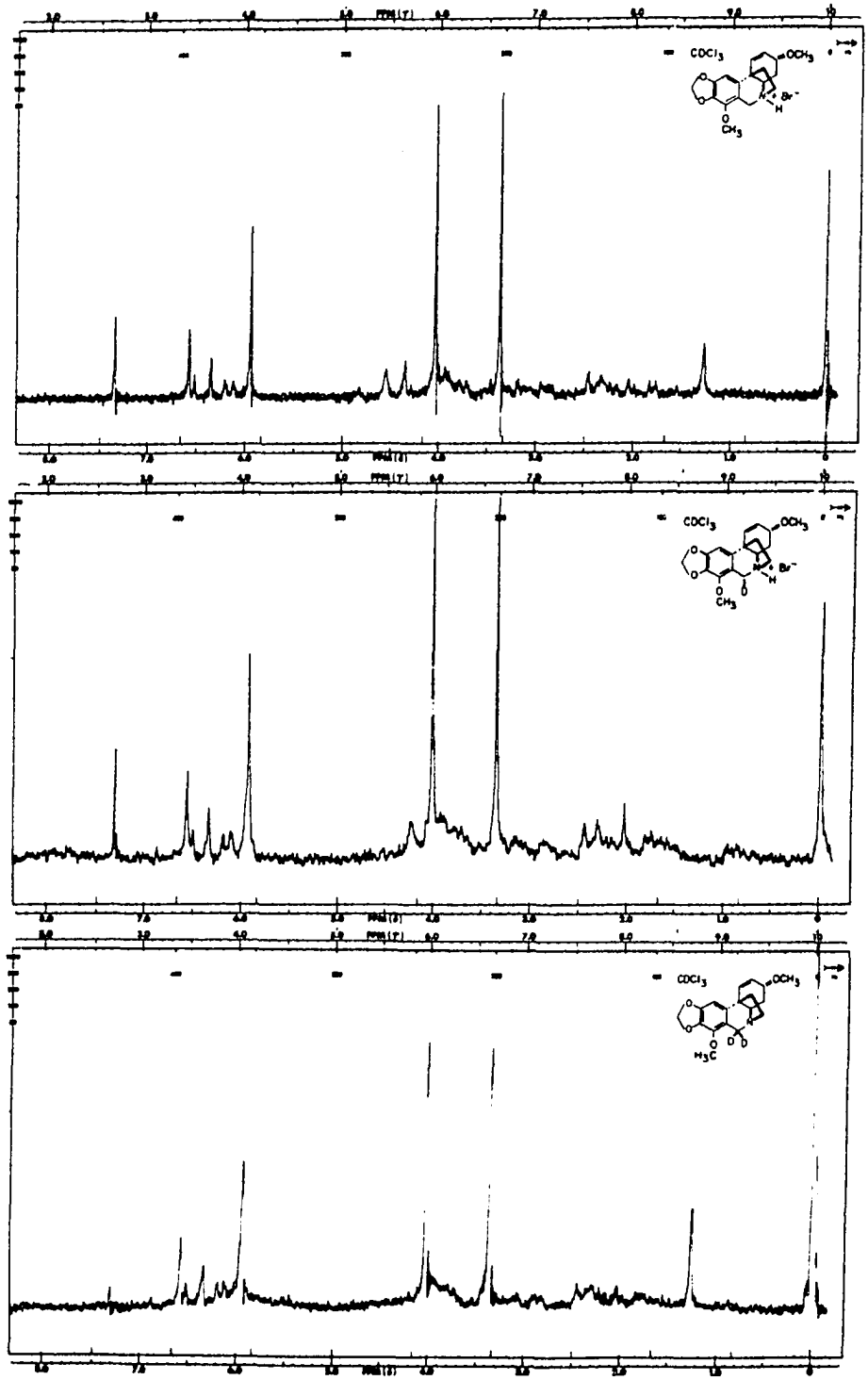
It was decided to also synthesize the doubly-labeled buphanidrine. 6-Oxobuphanidrine (46) was reduced with lithium aluminum deuteride to give the intermediate (69). From the reductions of the lactam (discussed later), it was known that only the α-hydroxy epimer is produced. Treatment of 69 with thionyl chloride followed by lithium aluminum deuteride gave the doubly-labeled buphanidrine (70). The structure was sup-

Fig. 15. Nuclear magnetic resonance spectra

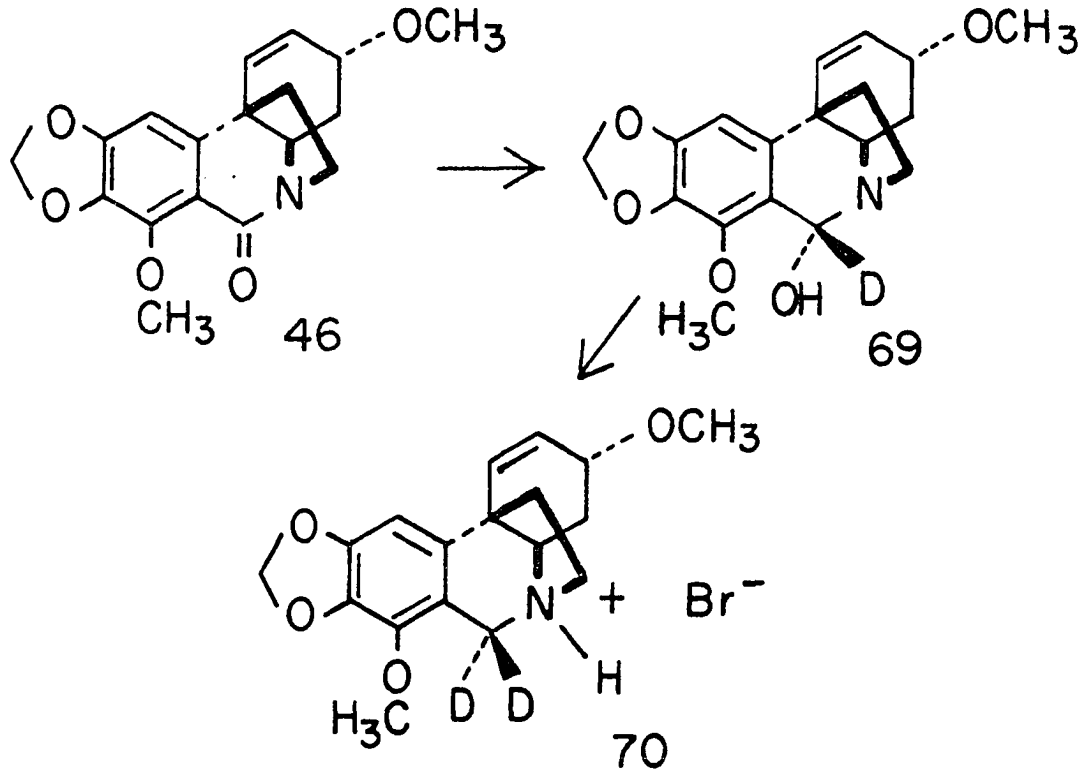
a: Buphanidrine hydrobromide

b: [6α-^{2}H]Buphanidrine hydrobromide (62)

c: [6,6-2^{2}H]Buphanidrine hydrobromide (70)


CDCl3
OCH3
Br-
CH3
CDCl3
OCH3
Br-
CH3
CDCl3
OCH3
H3C

ported by the mass spectrum (m/e 317 for the free base) and the nmr spectrum [Fig. 15] which showed no benzylic protons.



Other Reactions of 6-Hydroxybuphanidrine and Related Compounds

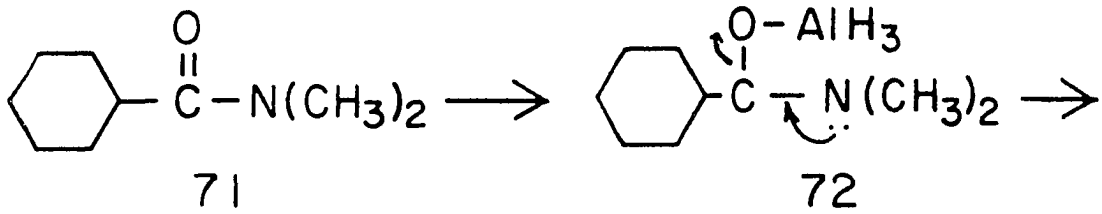
6-Hydroxybuphanidrine (43) and 6-hydroxypowelline (56) are unique among the 5,10b-ethanophenanthridine alkaloids because only these two bases contain a hydroxyl group at C_6 without also containing a substituent at C_{11}. This fact provides an excellent opportunity to study the chemistry of the carbinolamine functionality without the occurrence of rearrangements which characterized the chemistry of 6-hydroxy-

crinamine and haemanthidine.

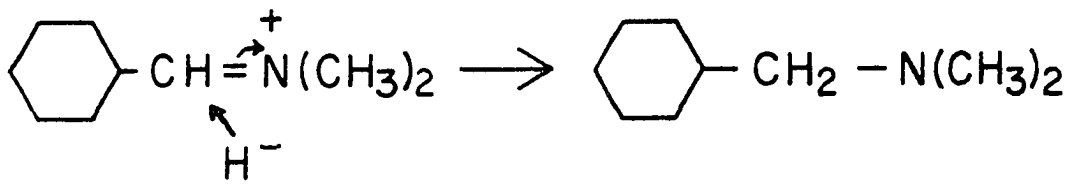
An unusual feature of 6-hydroxybuphanidrine is its failure to react with lithium aluminum hydride. Also of interest is the fact that the reduction product of 6-oxobuphanidrine with lithium aluminum hydride is 6-hydroxybuphanidrine. Normally, the reduction of amides or lactams with lithium aluminum hydride yields the amine.[56] The reaction is believed to proceed by an initial reduction of the amide (e.g. 71) to an amino alcohol derivative (72). The metal oxide complex is displaced by participation of the electron pair on nitrogen to form the imminium ion (73). Further reduction produces the amine (74).

The reactions of 6-hydroxybuphanidrine and 6-oxobuphanidrine toward lithium aluminum hydride are characteristic of an alcohol and ketone rather than a carbinolamine and amide, respectively. This pecularity can be interpreted as the inability of the electrons on the nitrogen to participate in formation of 54. The rigidity inherent in the 1-azabicyclo [3,2,1] octane ring system prevents the nitrogen p orbital from attaining proper alignment. Thus, the reduction stops at the alcohol step. Other examples where reduction is incomplete are the amides of carbazole, N-methylaniline, and

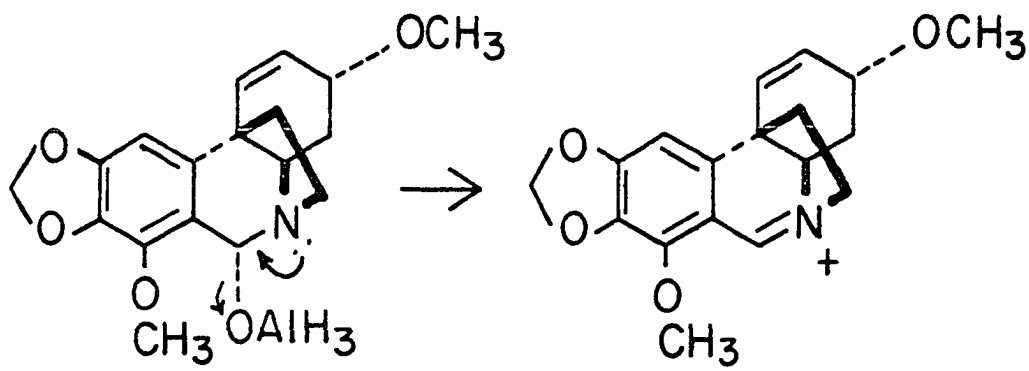
imidazole.[57]



71 72



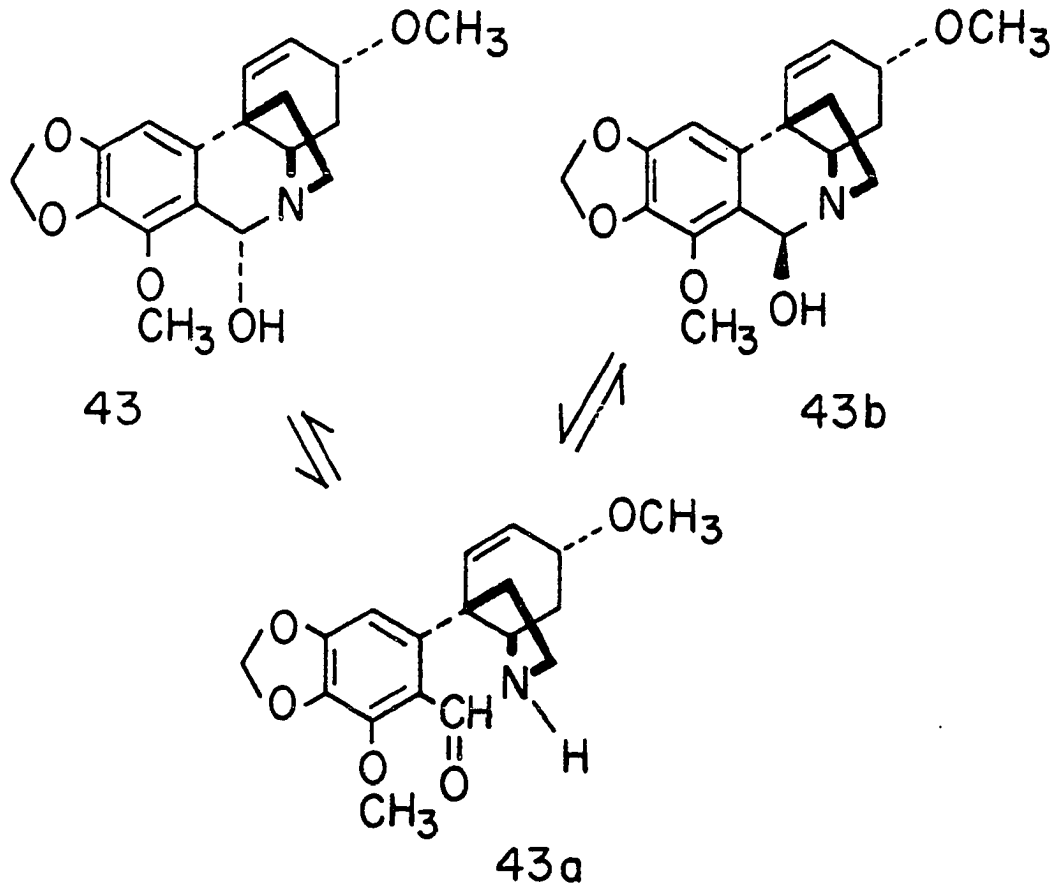
73 74



75 54

The most striking feature of the 6-hydroxy compounds rests with their potential formation of the open-chain amino-aldehyde. The aminoaldehyde (20) has been postulated as an intermediate for the interconversion of the C_6 epimers of

6-hydroxycrinamine. Analogously, one would predict the aminoaldehyde (43a) as an intermediate between the two epimers of 6-hydroxybuphanidrine (43 and 43b).



Infrared spectra of both crystalline 6-hydroxybuphanidrine and in chloroform solution indicate the alkaloid to be in the carbinolamine form. The aldehyde also escapes detection by nmr and ultraviolet spectroscopy. In contrast to haemanthidine and 6-hydroxycrinamine, 6-hydroxybuphanidrine displays no tendency to epimerize at the C_6 position. Refluxing the alkaloid in either acidic or basic media fails to give epimeriza-

tion. The nmr spectrum of 43 in tetrachloroethylene at 100^{o} neglects to show any broadening of the peak assigned to the benzylic proton. Another peak did develop at 5.45 ppm in the spectrum but was assigned to hydroxyl group when it disappeared with the addition of D_2O.

Several attempts were made to prepare the C_6 epimer of 6-hydroxybuphanidrine without success. As noted earlier, reduction of the lactam with lithium aluminum hydride gave 6-hydroxybuphanidrine with no evidence for formation of the epimer. This was not expected since hydride should have attacked from the side opposite the ethano bridge.

It was suggested the tosylate or mesylate of 43 be prepared and then displaced by acetate to give the epimeric derivative. However, all attempts at preparing the tosylate and mesylate were unsuccessful. 6-Chlorobuphanidrine proved fruitless as an intermediate since displacement of the β chloride gave derivatives with overall retention of configuration.

In another effort to prepare a compound which contained a benzylic halogen atom in the α configuration, undulatine (26) was treated with N-bromosuccinimide. Since the benzylic carbonium ion formed pseudoaxial products, a benzylic radical should also form pseudoaxial products. No attempt was made to

isolate the bromo compound which was subsequently treated with base. Purification by thin-layer chromatography afforded 6-hydroxyundulatine (76). The nmr spectrum is shown in Fig. 17 with that of undulatine. Comparison of the two spectra clearly shows the disappearance of the benzylic AB pattern in 6-hydroxyundulatine. The single benzylic proton is shifted downfield (5.20 ppm). However, the position of the proton indicated that the hydroxyl group was α. This was confirmed when the epoxide was removed by Cr^{II} reduction to give 6-hydroxybuphanidrine. These experiments where the β epimer was the expected product (but not observed) suggest that the compound epimerizes before detection.

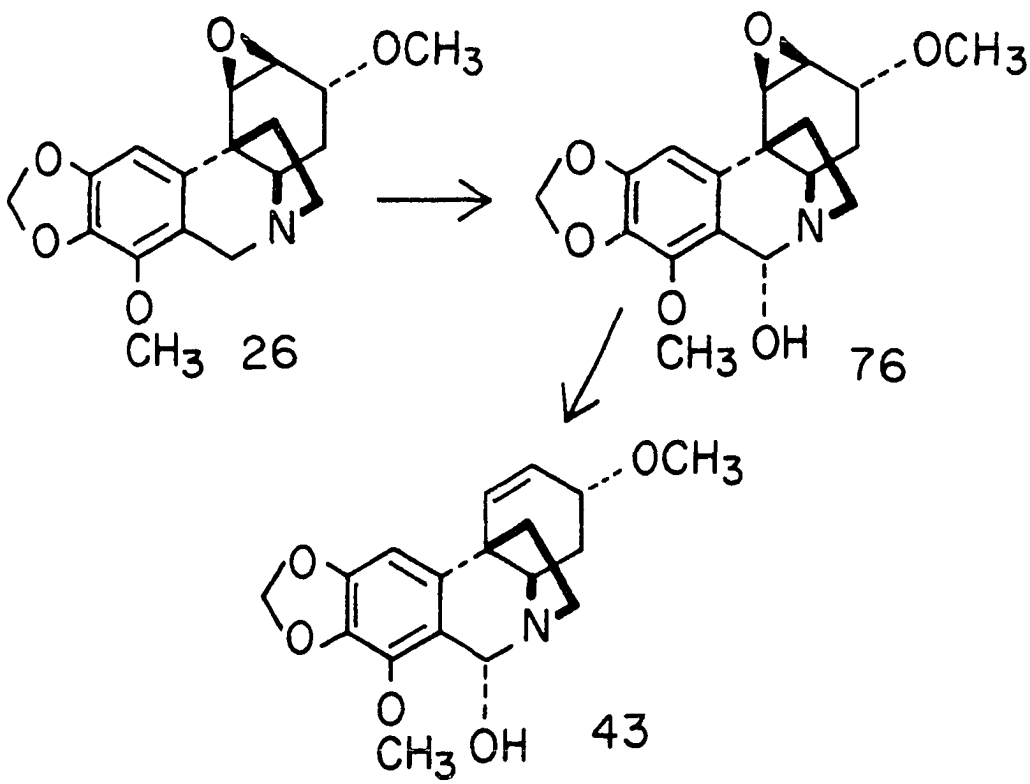
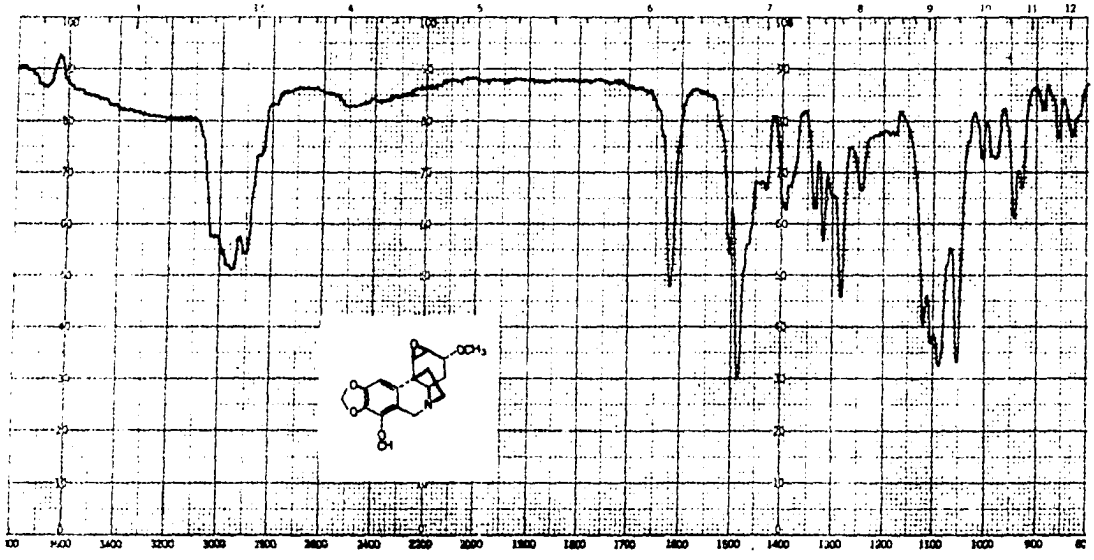


Fig. 16. Infrared spectra

a: Undulatine (26)

b: 6-Hydroxyundulatine (76)



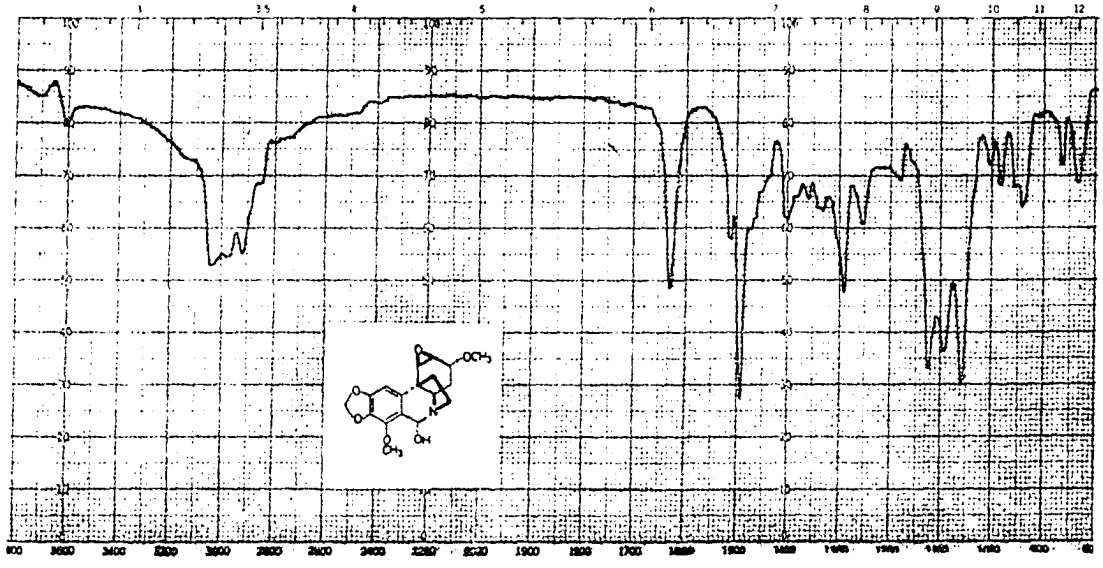
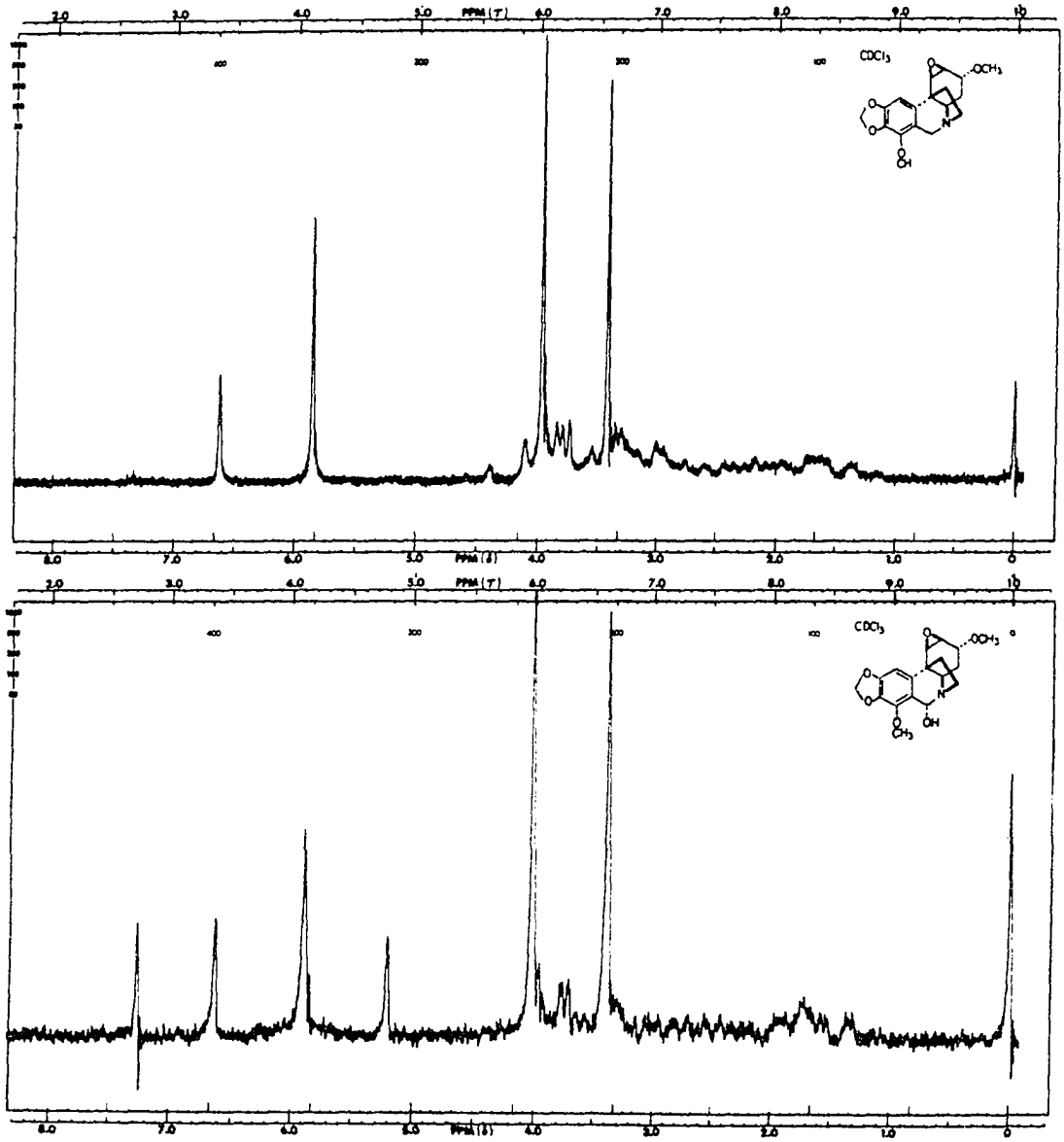


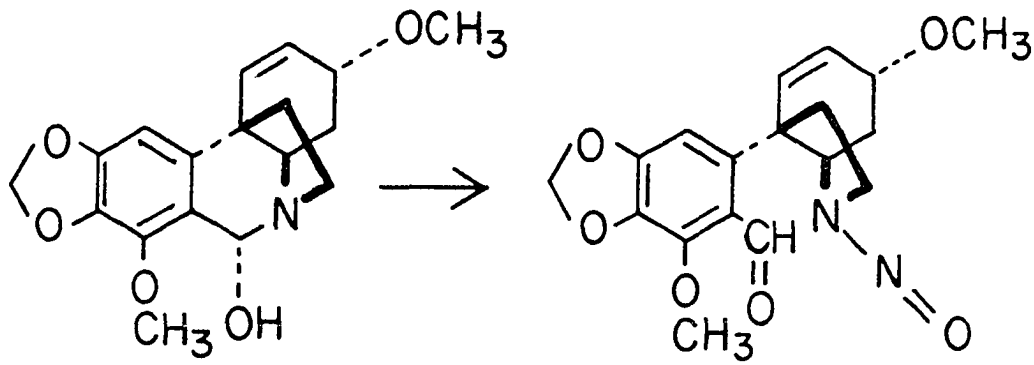
Fig. 17. Nuclear magnetic resonance spectra

a: Undulatine (26)

b: 6-Hydroxyundulatine (76)


CDCl3
OCH3
OH
CDCl3
OCH3
OCH3
OH

As in the case of 6-hydroxypowelline, 6-hydroxybuphanidrine when treated with sodium in dilute acetic acid formed the N-nitroso aldehyde (77). The infrared and nmr spectra of this compound are shown in Figs. 18 and 19. This is a reaction characteristic of secondary amines and may indicate that the aminoaldehyde is present in the solution. However, attack might occur at the nucleophilic nitrogen atom with simultaneous formation of open chain products.



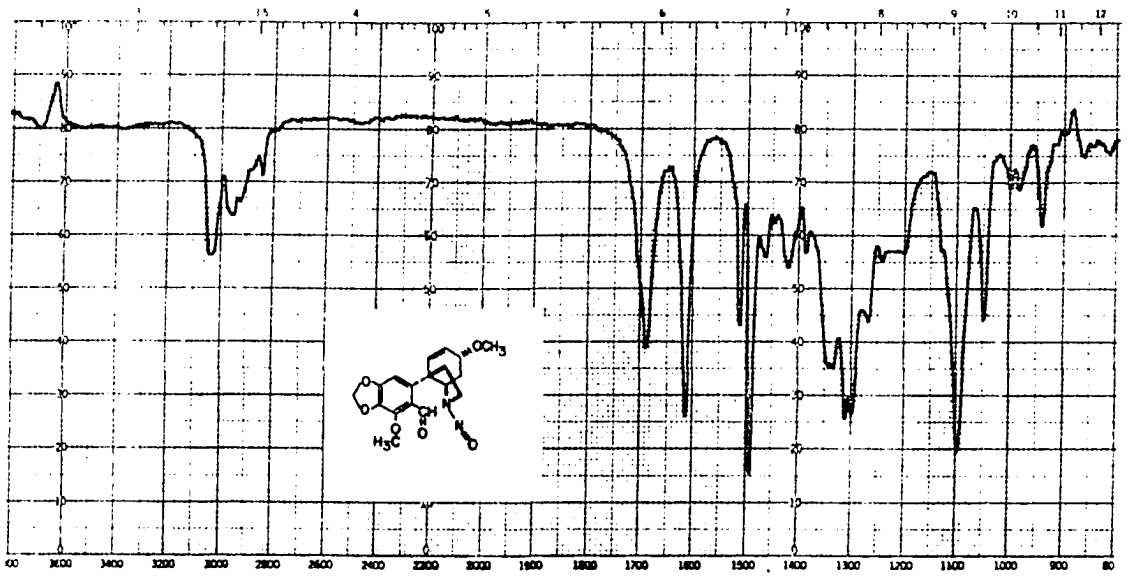
43 77

Ring opening was also observed when 6-hydroxybuphanidrine methiodide (78) was converted to a chloroform-soluble base ($C_{19}H_{23}NO_5$) by treatment with dilute alkali. The infrared [Fig. 18] and ultraviolet spectrum of this product [λ_{max} ($CHCl_3$) 1690 cm^{-1}; λ_{max}($CHCl_3$) 320 mμ (ε1990)] indicated that C_6-N bond cleavage had occurred with the formation of the aminoaldehyde (79). The nmr spectrum showed the N-methyl

Fig. 18. Infrared spectra

a: N-Nitroso-6-hydroxybuphanidrine (77)

b: N-Methyl-6-hydroxybuphanidrine (79)



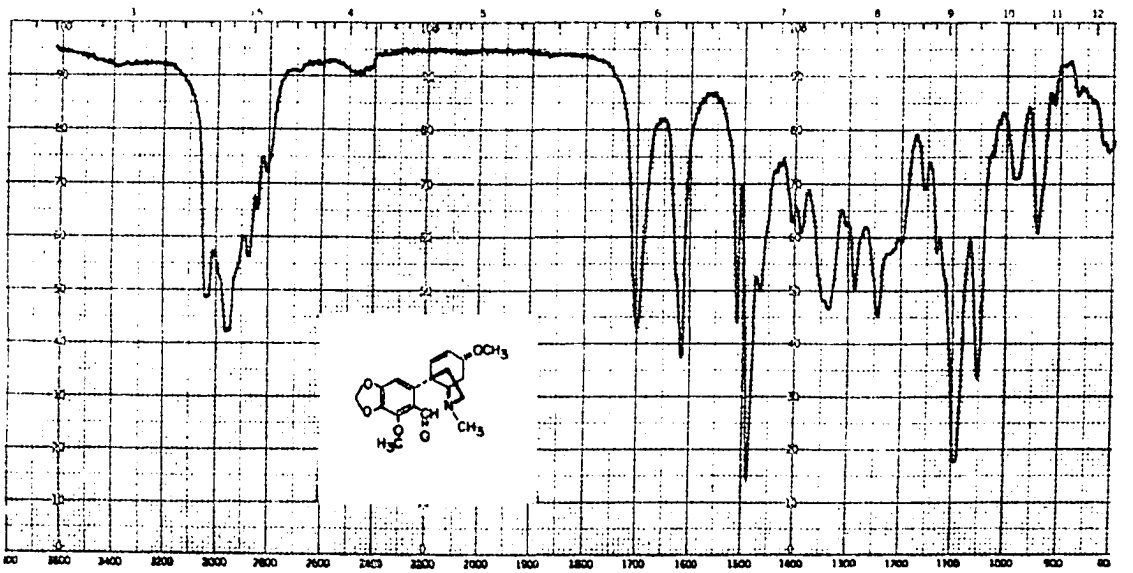
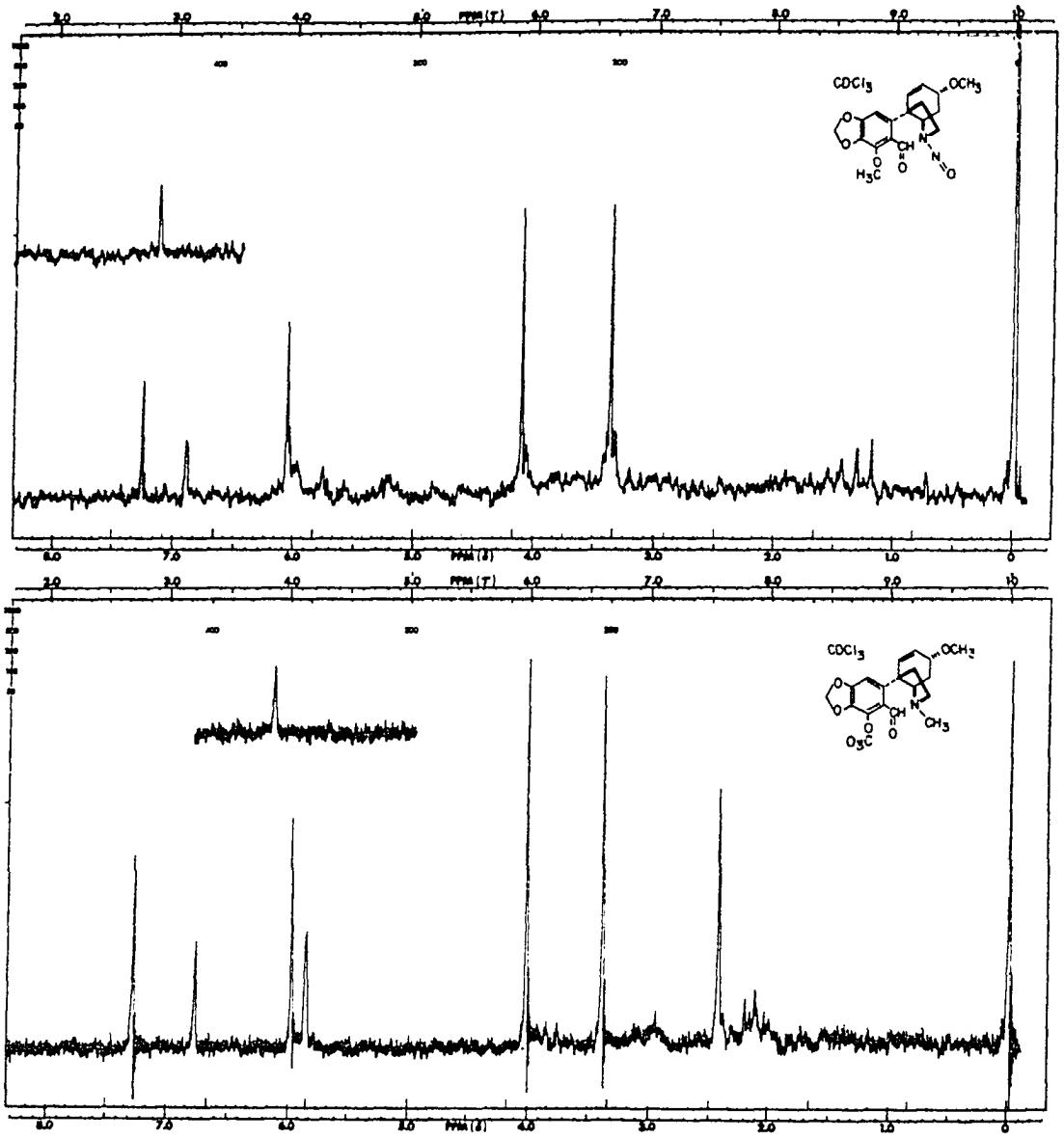


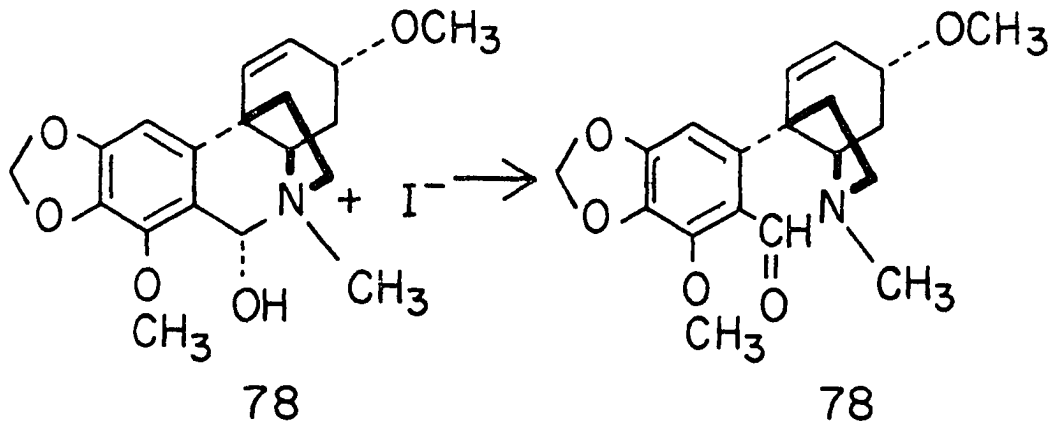
Fig. 19. Nuclear magnetic resonance spectra

a: N-Nitroso-6-hydroxybuphanidrine (77)

b: N-Methyl-6-hydroxybuphanidrine (79)


CDCl3
OCH3
H3C
CDCl3
OCH3
CH3
O3C

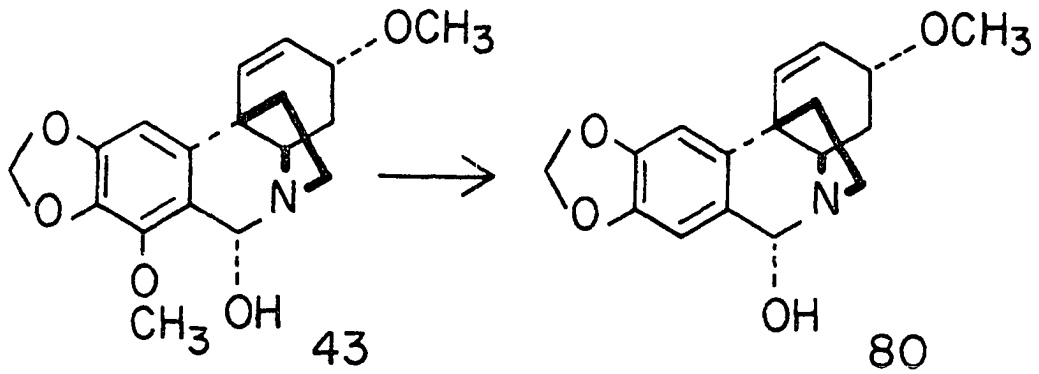
(3.35 ppm) and the aldehydic proton (10.5 ppm) in accord with this structure.



78 78

6-Hydroxybuphanisine

In order to examine the chemistry of 6-hydroxybuphanidrine and 6-hydroxycrinamine in more detail, it seemed desirable to prepare a derivative which contained neither a C_7 atomatic methoxyl nor a C_{11} substituent. The simplest method of accomplishing this appeared to be removal of the aromatic methoxyl of 6-hydroxybuphanidrine. This was accomplished by treating 43 with sodium and isoamyl alcohol in refluxing xylene.



43 80

The spectral and physical properties of 80 were dramatically different from those of 6-hydroxybuphanidrine. The nmr spectrum is shown in Fig. 20. The aromatic region is very similar to that of haemanthidine with the exception that the olefinic protons appear as an AB pattern. The presence of two C_6 epimers is shown by the two benzylic proton peaks (5.10 and 5.70 ppm) and by the two peaks for the C_7 aromatic proton (6.71 and 6.98). At 100° in tetrachloroethylene these peaks broaden suggesting the interconversion of the two epimers.

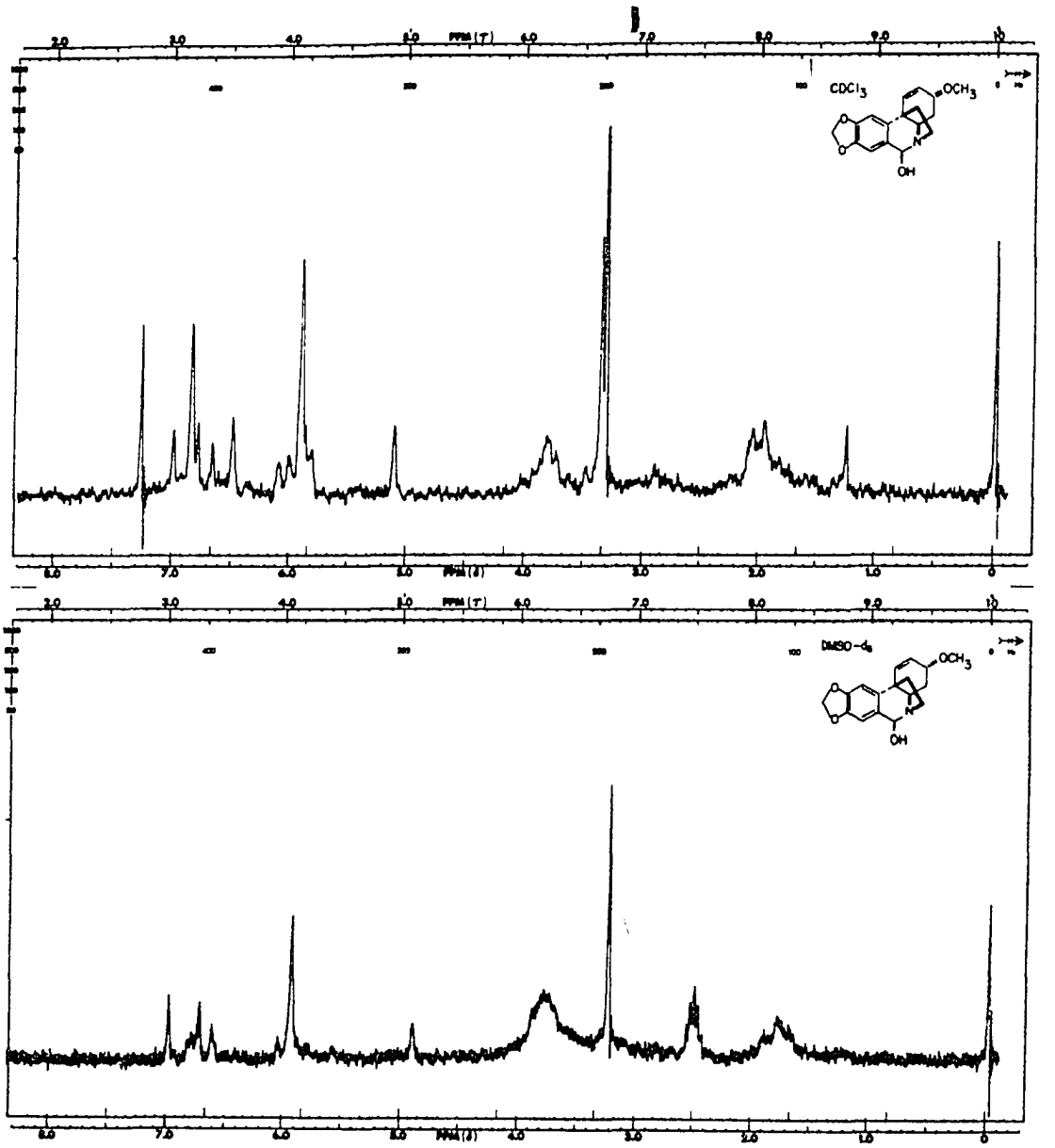
It can be concluded that the C_{11} substituent in 6-hydroxycrinamine and haemanthidine is of no importance in the epimerization process at C_6. Since the only difference in 43 and 80 is the aromatic methoxyl, this group must in some way prevent epimerization in 6-hydroxybuphanidrine. Whether this is a stereoelectronic or steric effect, the phenomena is very difficult to rationalize. The reason may be very subtle since it is noted that haemanthidine has an epimeric ratio of 50:50, whereas in 6-hydroxycrinamine, the ratio has been altered to 70:30. The effect of this small change in structure on the benzylic position would hardly have been predicted.

It is very interesting to note that the C_3 methoxyl in both the spectrum of 6-hydroxybuphanisine and haemanthidine

Fig. 20. Nuclear magnetic resonance spectra

a: 6-Hydroxybuphanisine (80) in $CDCl_3$

b: 6-Hydroxybuphanisine (80) in DMSO-d_6


CDCl3
OCH3
OH
DMSO-d6
OCH3
OH

[Fig. 7] has two chemical shifts. This is in contrast to the spectrum of 6-hydroxycrinamine where a singlet is observed. Another interesting feature is a comparison of the spectrum of 11-O-acetylhaemanthidine and 6,11-O,O-diacetylhaemanthidine [Fig. 21]. In the latter spectrum, only a singlet is observed, whereas in the spectrum of 11-acetylhaemanthidine, two peaks are present. Since any long range coupling seems remote, the C_3 methoxyls (corresponding to the two C_6 epimers) are apparently in different chemical environments. This might be caused by a bimolecular association of the alkaloids through hydrogen bonds, thus reflecting the requirements of a C_6 hydroxyl group. In the case of 6-hydroxycrinamine, the closer proximity of the C_3 methoxyl to the ethano bridge might prevent such a change in environment.

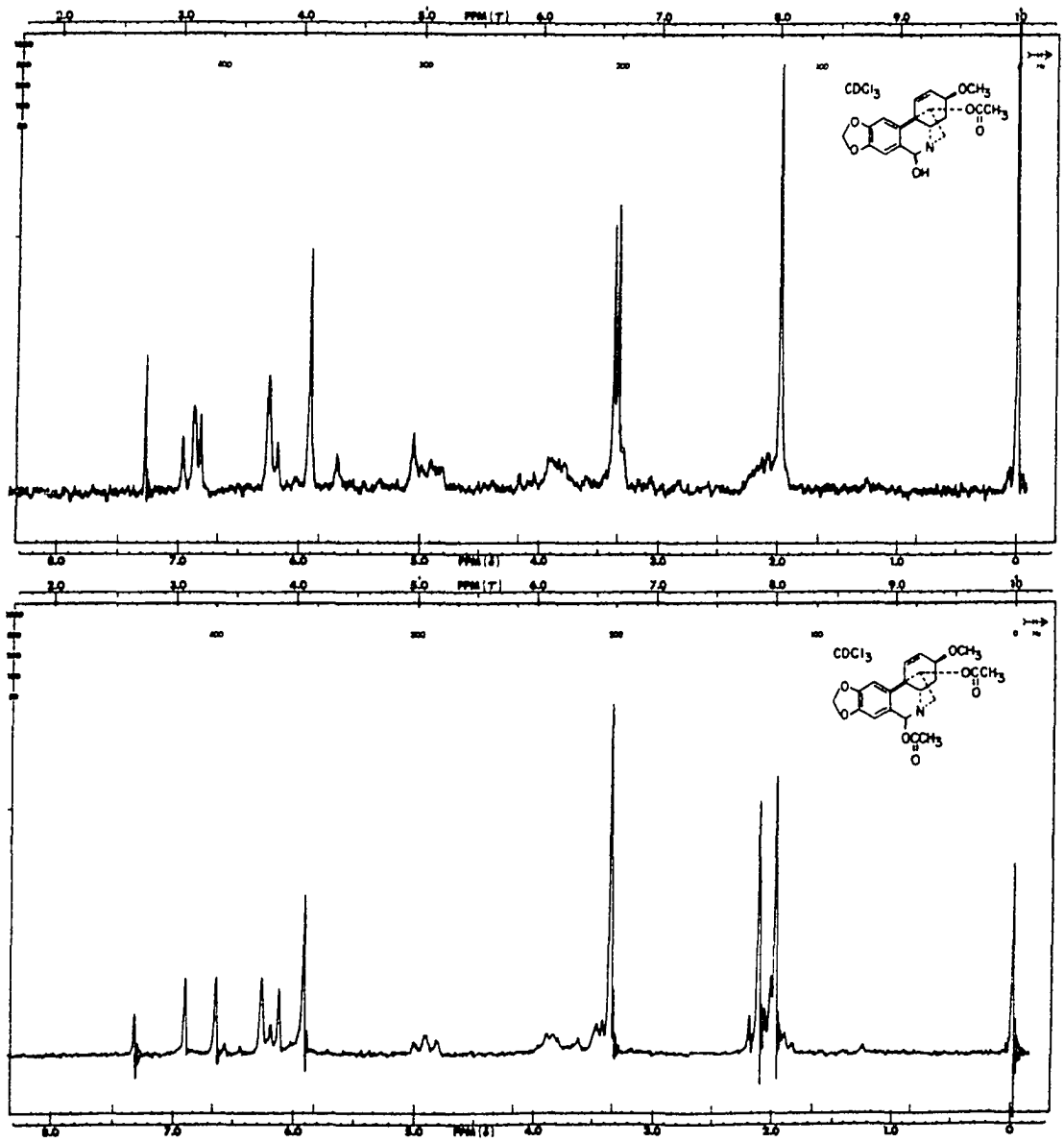
Formation of an oxime derivative

It was noted earlier that haemanthidine (14) and 6-hydroxycrinamine (20) have proven stable to Schiff's and Tollen's reagents, lithium aluminum hydride, sodium borohydride, and hydroxylamine. Quite surprisingly, the reaction of 6-hydroxybuphanidrine (43) with hydroxylamine hydrochloride in refluxing 95% ethanol was found to yield something other than starting material. Purification of the product by thin-

Fig. 21. Nuclear magnetic resonance spectra

a: 11-O-Acetylhaemanthidine

b: 6,11-O,O-Diacetylhaemanthidine


CDCl3
OCH3
OCCH3
O
OH
PPM (T)
PPM (δ)
CDCl3
OCH3
OCCH3
O
OCCH3
O

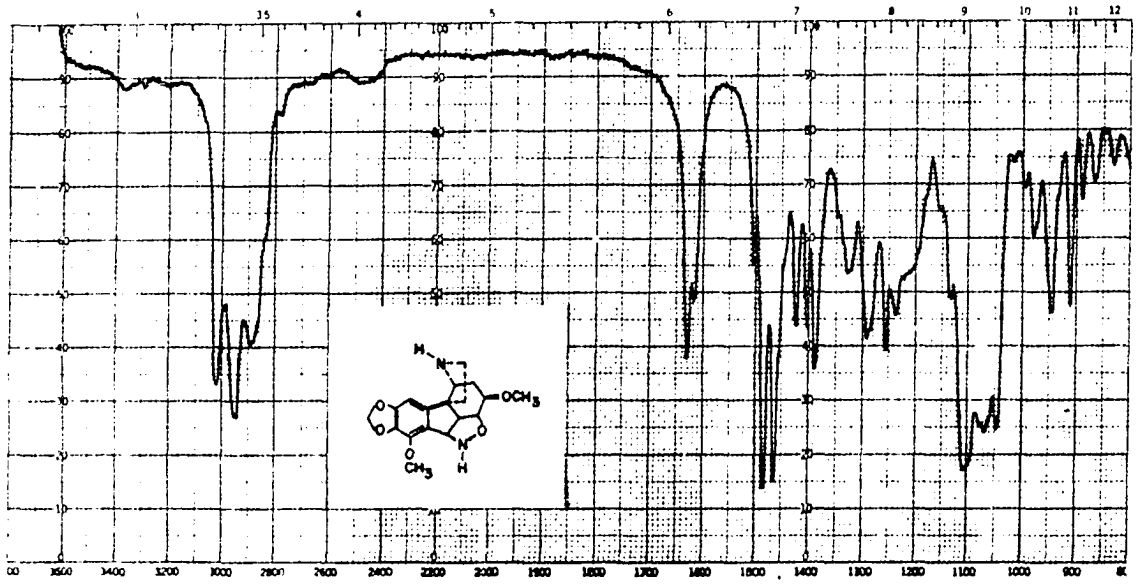
layer chromatography revealed three components: 6-ethoxybuphanidrine (48), starting material, and an unidentified compound. The unknown did not have the expected oxime characteristics. The infrared spectrum [Fig. 22] lacked a C=N stretching band. The ultraviolet spectrum confirmed this fact by its lack of absorption characteristic of benzylic conjugation. Another interesting feature was provided by the nmr spectrum [Fig. 23]. A doublet was located at 5.15 ppm instead of much farther downfield where the benzylic proton was expected for an oxime structure. The aromatic region provided the greatest surprise when it showed the complete absence of olefinic protons. The mass spectrum indicated the compound had a molecular weight of 346, the same molecular weight as the expected oxime derivative.

An important guide to the structure of the hydroxylamine product was obtained when acetylation produced an N,N-diacetyl derivative (83). The infrared spectrum [Fig. 22] showed absorption at 1750 cm^{-1}. The nmr spectrum [Fig. 23] confirmed the N-acetyl groups (2.2 ppm). With the assumption that an oxime was initially formed, the isoxazolidine compounds 82 and 83 are proposed for the hydroxylamine adduct and its acetylated derivative.

Fig. 22. Infrared spectra

a: Hydroxylamine adduct (82)

b: N,N-Diacetylhydroxylamine adduct (83)



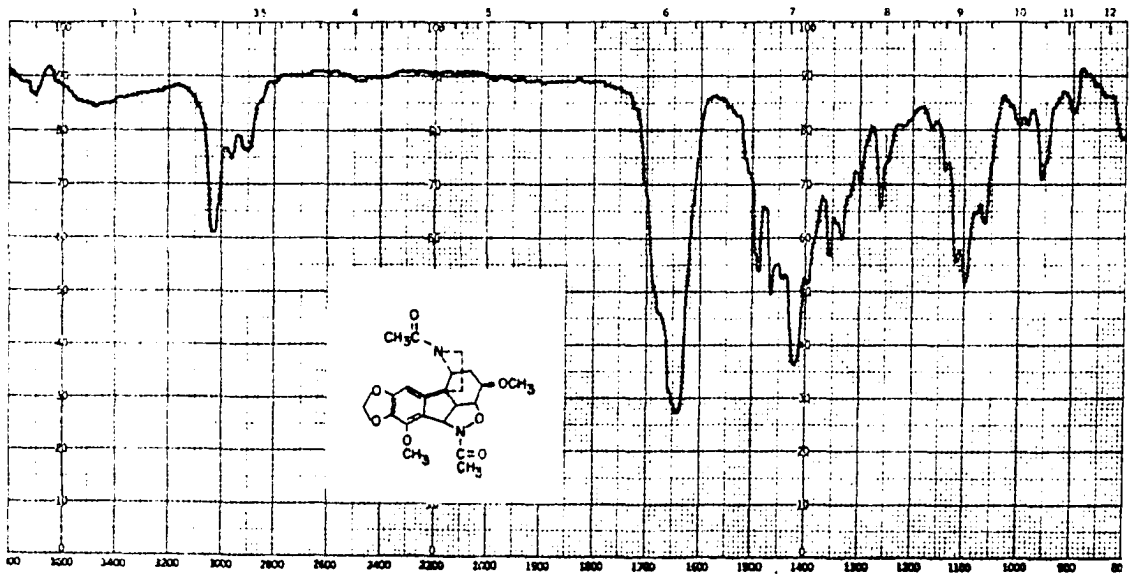
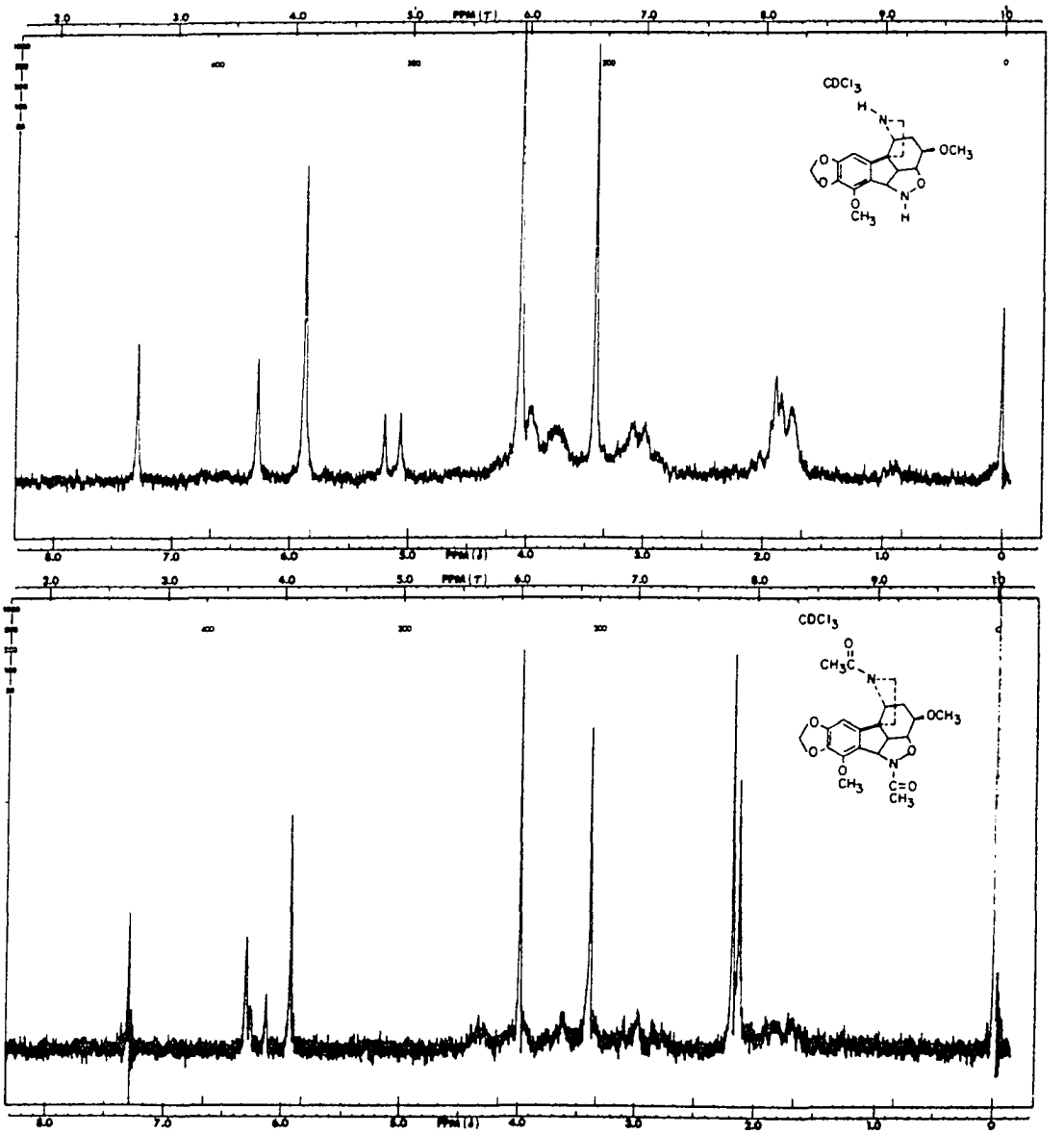
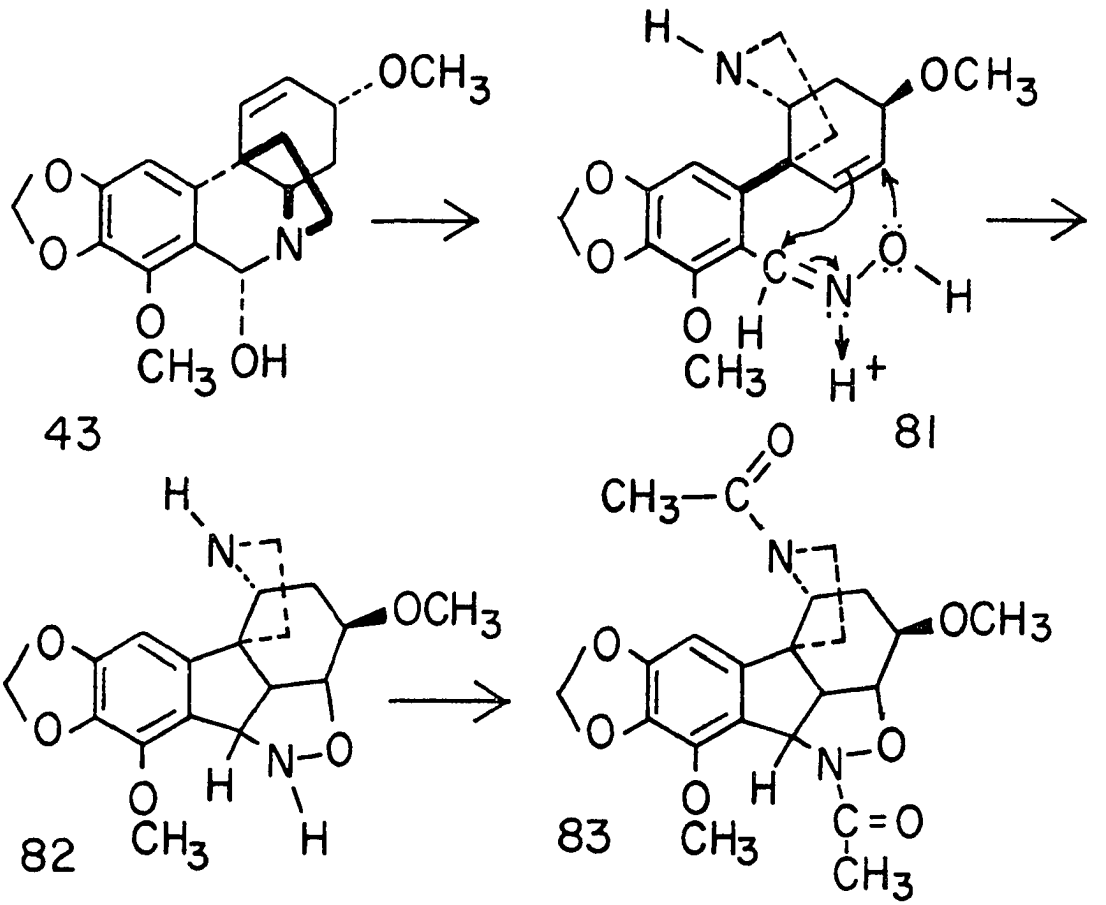


Fig. 23. Nuclear magnetic resonance spectra

a: Hydroxylamine adduct (82)

b: N,N-Diacetylhydroxylamine adduct (83)


CDCl3
OCH3
CDCl3
OCH3

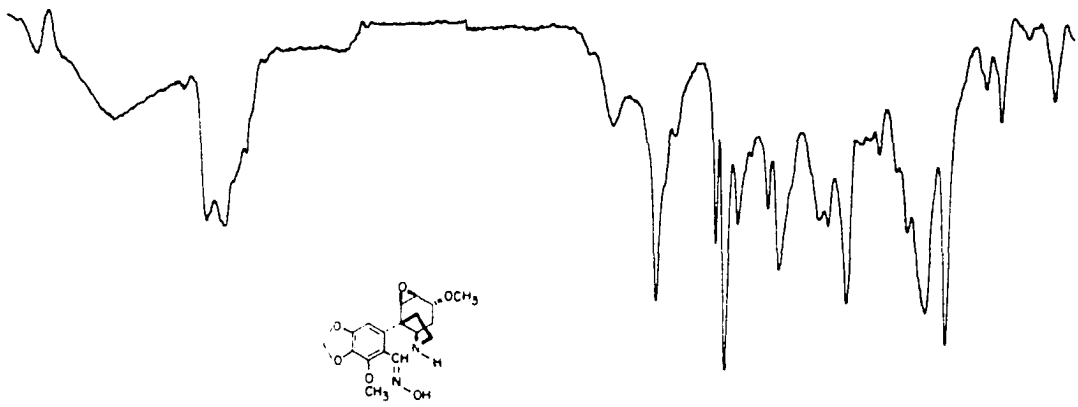


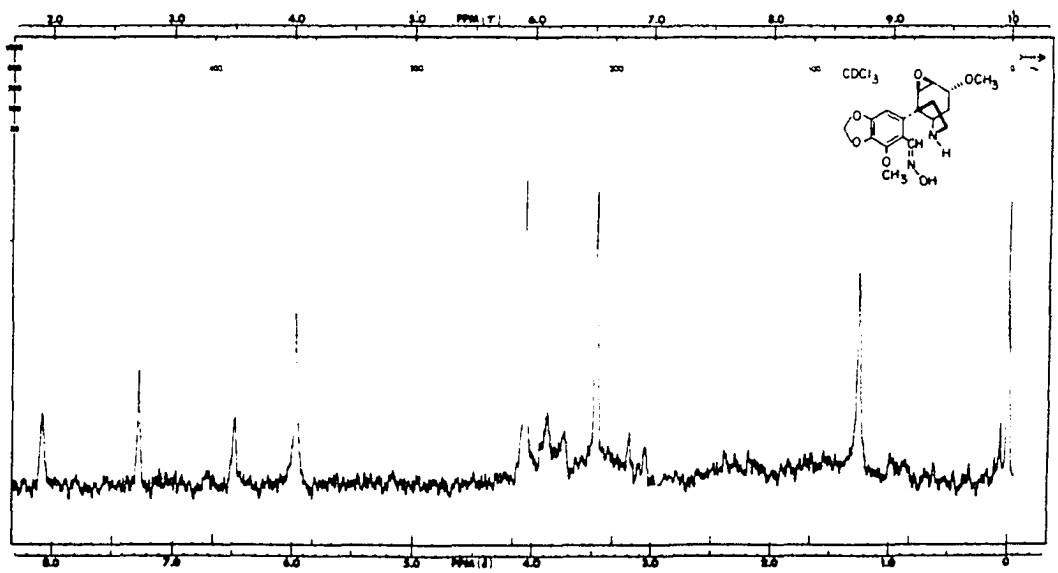
The precedent for addition of oximes to double bonds is well documented.[58] To confirm the intermediacy of the oxime, the reaction was carried out using 6-hydroxyundulatine (76) where there are no olefinic protons. The ultraviolet and infrared spectra [Fig. 24] of the product indicated that C_6-N bond cleavage had occurred with formation of the oxime. The nme spectrum [Fig. 24] confirmed the benzylic proton (8.1 ppm) in accord with this structure.

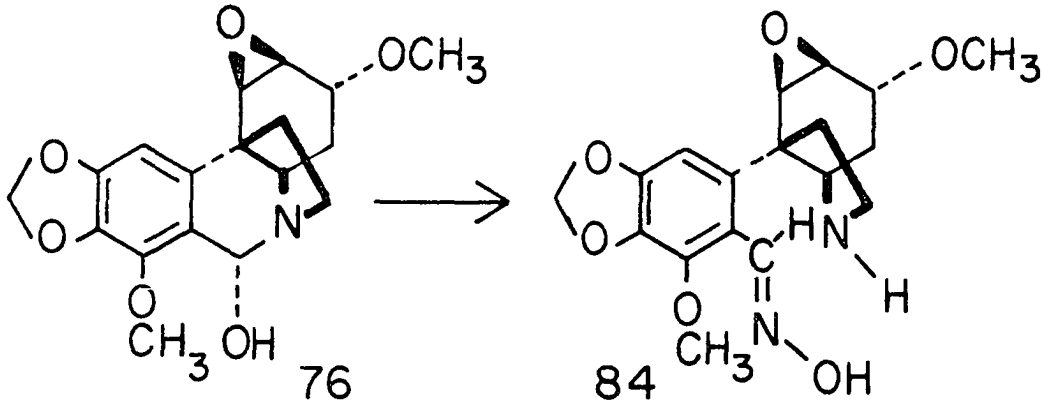
Fig. 24. 6-Hydroxyundulatine oxime (84)

a: Infrared spectrum

b: Nuclear magnetic resonance spectrum

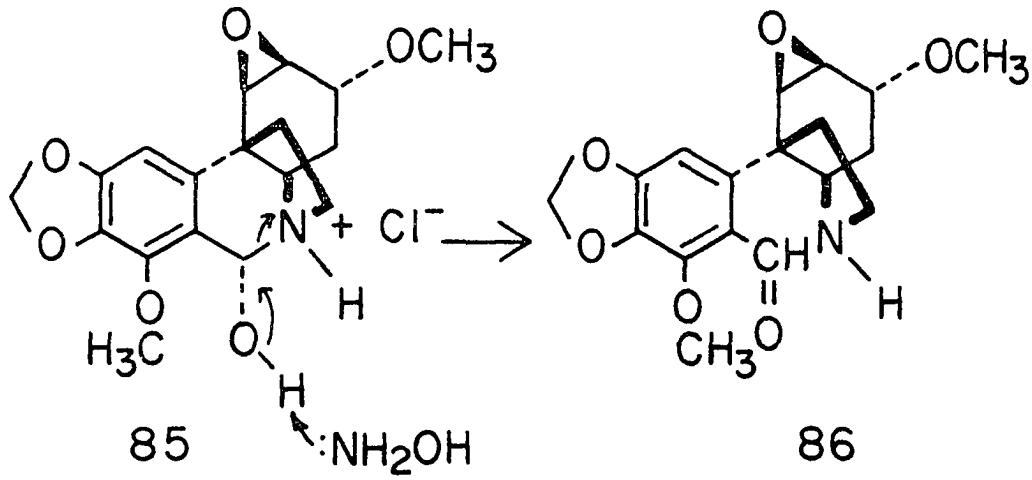

OCH3
CH
N
H
O
CH3
N
OH


CDCl3
OCH3
CH
N
H
O
CH3
N
OH



76 84

One possible mechanism for the oxime formation involves protonation of the nitrogen to give 85. The free hydroxylamine can then abstract the hydroxyl proton with formation of the aminoaldehyde which reacts to form the oxime. This mechanism is identical to that proposed for the base-catalyzed ring opening of 6-hydroxybuphanidrine methiodide (78).

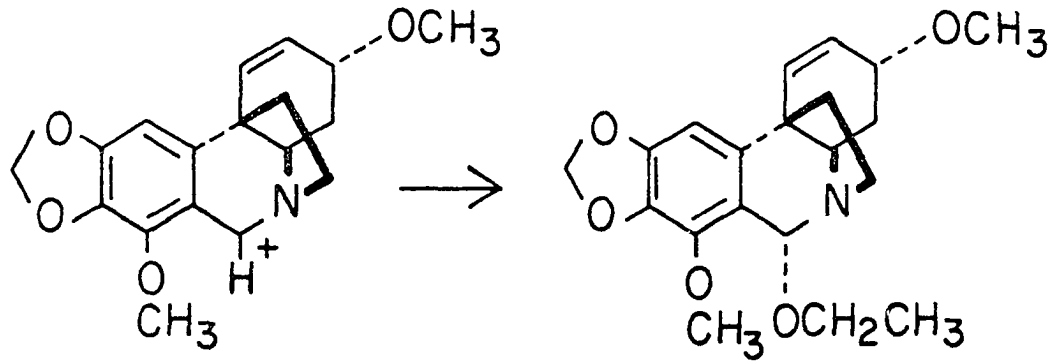


85 86

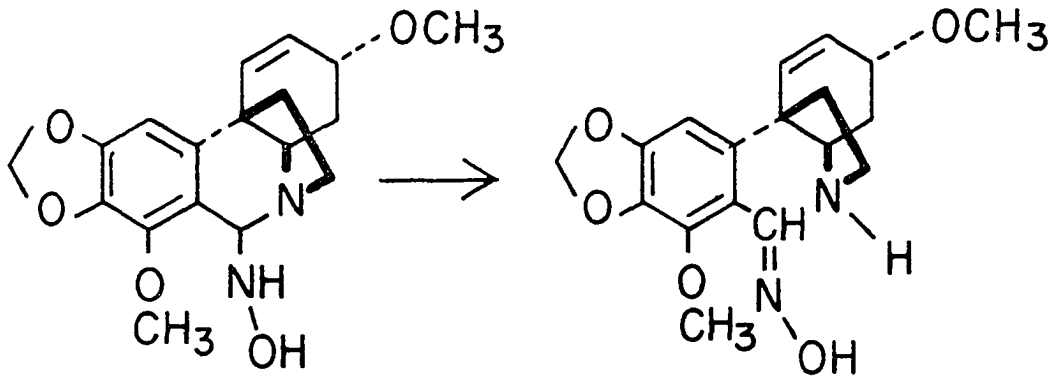
However, this mechanism does not account for the fact that 6-hydroxybuphanidrine reacts with hydroxylamine when 6-hydroxycrinamine and haemanthidine do not. This is

especially curious since one would predict that any chemical evidence for the aminoaldehyde would come from the latter alkaloids where both epimers are present.

In this regard, another mechanism not involving the aminoaldehyde might be considered. It was noted earlier that 6-hydroxybuphanidrine in the presence of acid may form a small amount of the benzylic carbonium ion. Since the hydrochloride salt of hydroxylamine was used in the reaction, it is possible that a small amount of the carbonium ion (54) forms. The isolation of 6-ethoxybuphanidrine (48) from the reaction supports this possibility. The carbonium ion could also react with hydroxylamine to produce 87. In the presence of acid, this hydroxylamine derivative could then isomerize to the oxime (88).



54 48



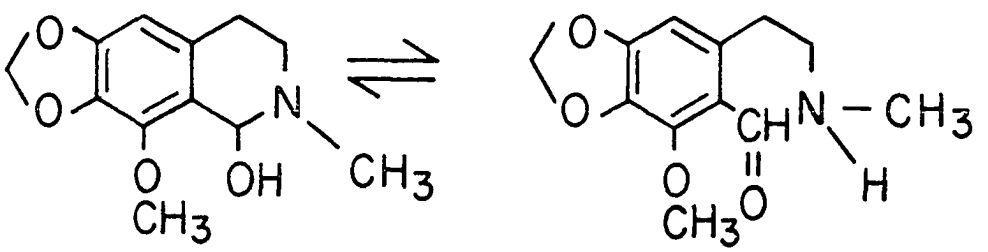
87 88

In addition to the isolation of 6-ethoxybuphanidrine, the following additional observations strongly support the intermediacy of the carbonium ion. When the reaction was performed in a solution buffered with sodium acetate, no isoxazolidine was isolated. The use of dimethyl sulfoxide as the solvent, however, gave an almost quantitative yield of the oxime of 6-hydroxyundulatine.

The inability of 6-hydroxycrinamine and haemanthidine to react reflects the fact that formation of the benzylic carbonium ion is not as favorable without the ortho electron releasing substituent ($-OCH_3$). Thus, both compounds fail to form the C_6-ethyl ether in acidic ethanol. Nor do the C_6-acetyl derivatives undergo methanolysis with cleavage of alkyl-oxygen bond.

Cotarnine

The chemical transformations of cotarnine (35) have been investigated for over a century. Since the majority of this work preceded the modern instrumental methods, it was decided to reexamine the alkaloid. Of special interest was the possibility of detecting the aminoaldehyde form (36) of the carbinolamine. One would predict that the possibility of its existence would be greater than the 6-hydroxy Amaryllidaceae alkaloids since the B ring is not nearly as rigid and the aldehyde can rotate more freely.

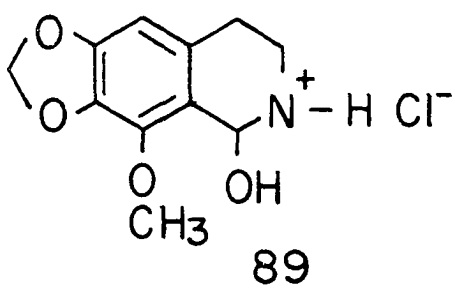


35 36

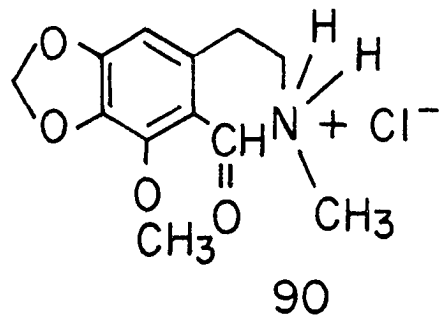
The nmr spectrum of cotarnine is shown in [Fig. 26]. The singlet at 6.31 ppm is assigned to the aromatic proton, and the singlet at 5.85 ppm is produced by the methylenedioxy protons. The peak at 5.39 ppm must be assigned to the benzylic proton. Since there is only one asymmetric center

in cotarnine, there is no possibility for the presence of two epimers at the benzylic position. An examination of the downfield region in the spectrum lacked any indication of an aldehydic proton. The infrared spectrum [Fig. 25] also indicated cotarnine to be completely in the carbinolamine form.

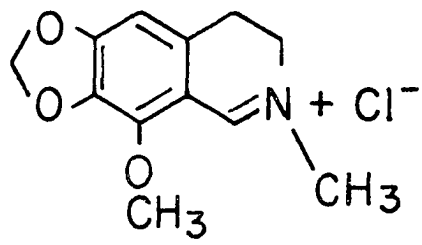
Cotarnine can be easily converted into quaternary salts by the action of dilute acids. The nmr spectrum of cotarnine hydrochloride (39) is shown in Fig. 26. The benzylic proton now appears at 8.88 ppm, and the N-methyl protons have been shifted downfield (3.78 ppm). Although there are two other structures possible for the hydrochloride salt (89 and 90), the mass spectrum indicates that a loss of one molecule of water has taken place. Only structure 39 is consistent with this fact.



89



90

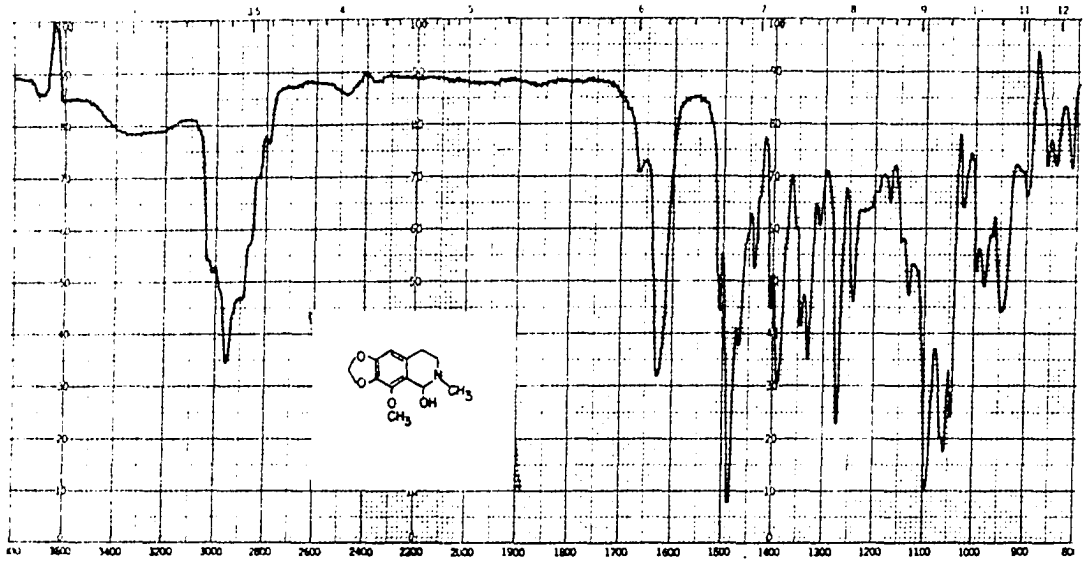


39

Fig. 25. Infrared spectra

a: Cotarnine (35)

b: Cotarnine hydrochloride (39)



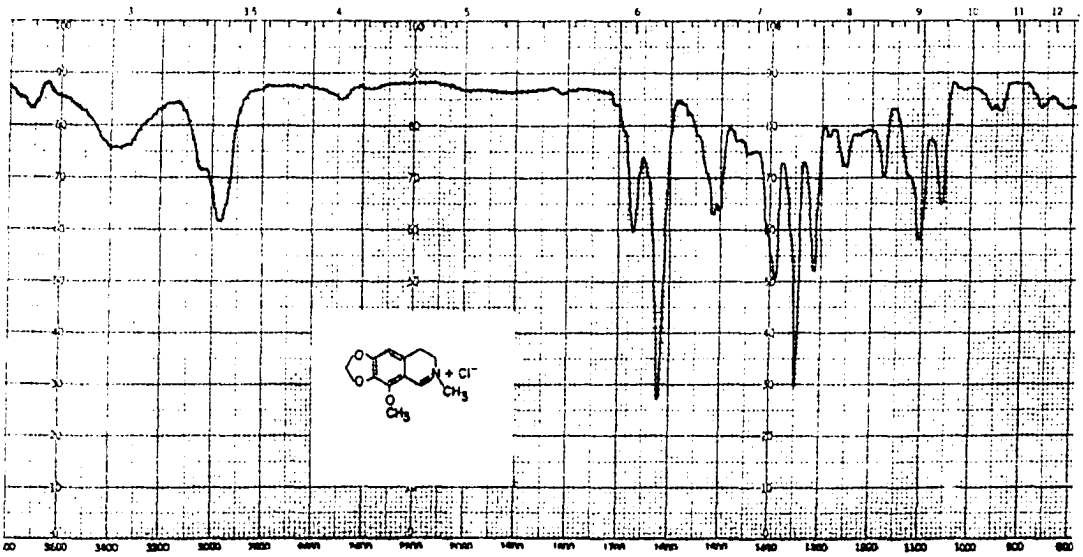
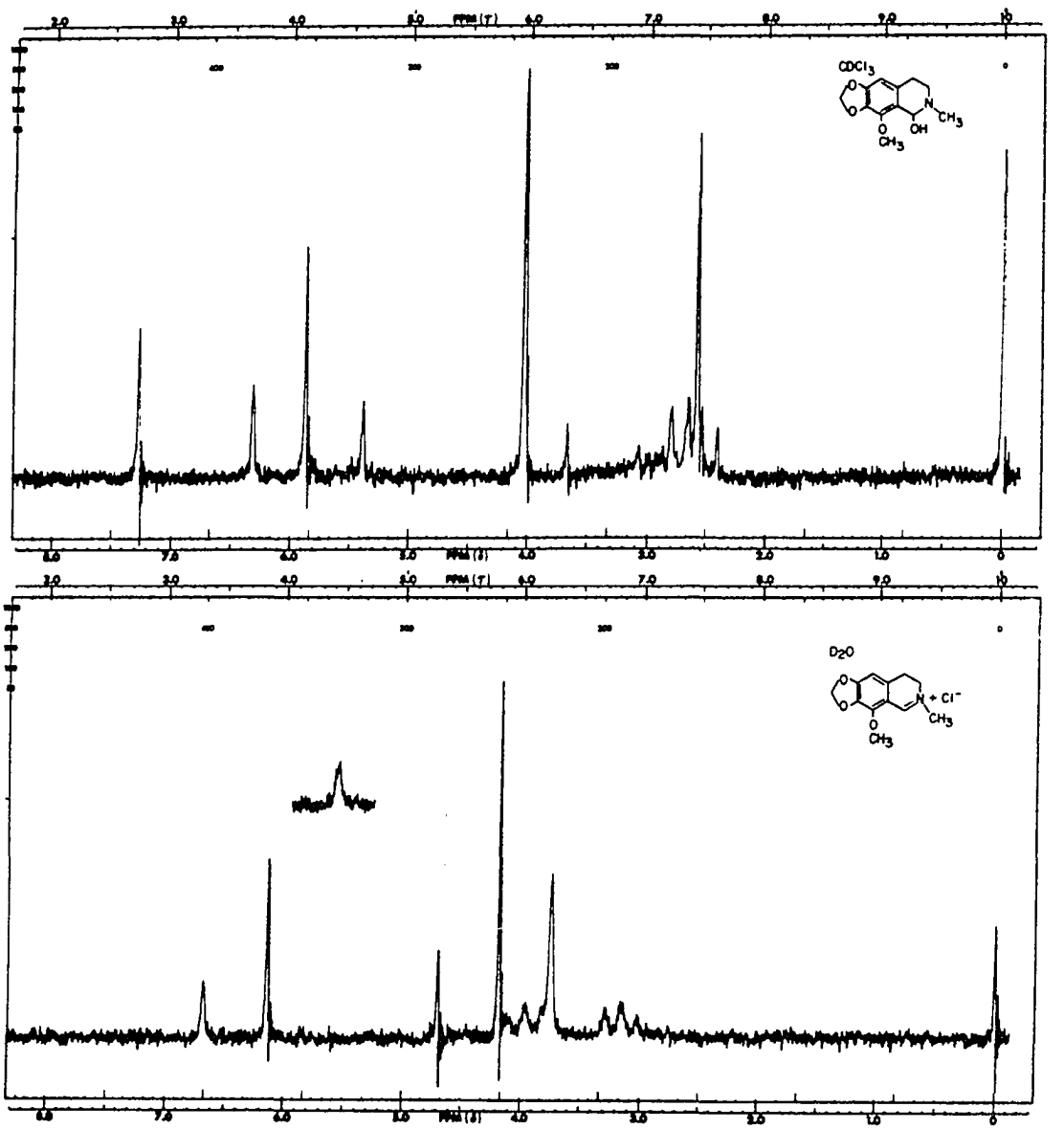


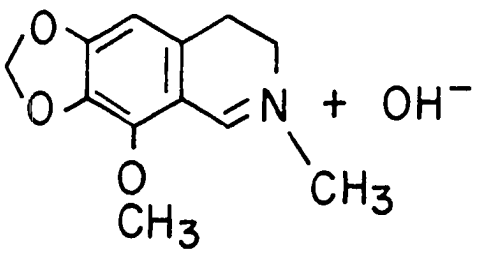
Fig. 26. Nuclear magnetic resonance spectra

a. Cotarnine (35)

b. Cotarnine hydrochloride (39)


CDCl3
OH
CH3
D2O
+ Cl⁻

Several studies have shown the existence of the quaternary ammonium hydroxide form (37) in polar media.[59,60] The ease of formation and stability of this form greatly increases the polarity of the C_1-substituent bond. This fact



37

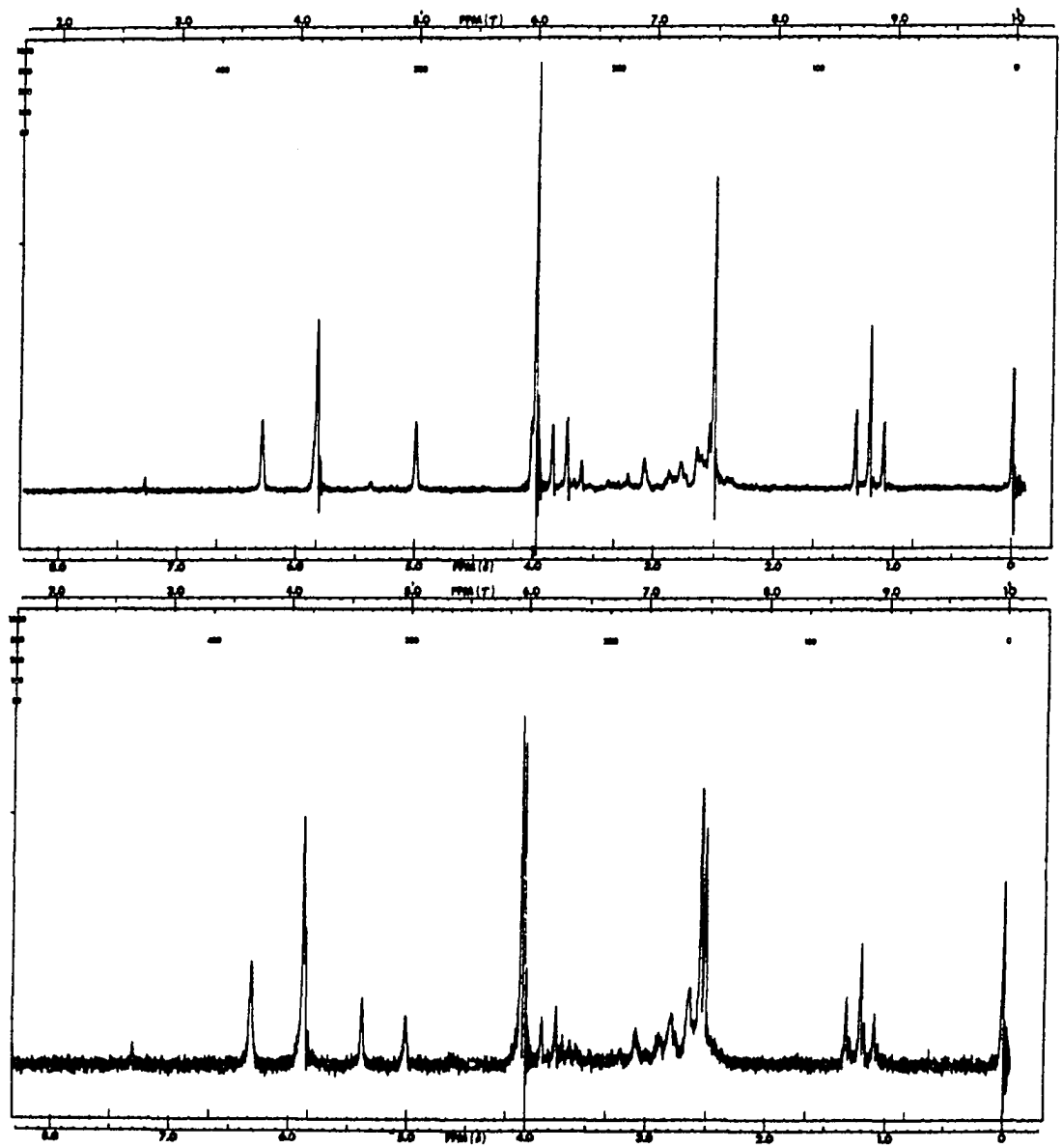
is evident from the facile interconversion of 35 and 91. When pure cotarnine is dissolved in reagent chloroform (containing 1% ethanol), the nmr spectrum of the compound alters considerably [Fig. 27]. Substantial amounts of the 1-ethoxycotarnine are present as shown by the upfield shift of the benzylic proton (4.70 ppm) and by the triplet corresponding to the methylene protons (1.3 ppm). Upon addition of a drop of D_2O to the nmr sample, the equilibrium is shifted back to predominately cotarnine [Fig. 27].

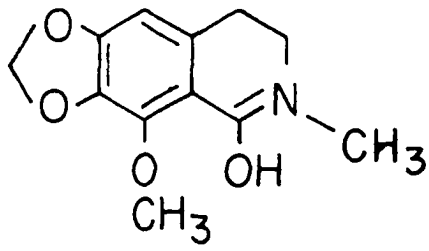
The reaction of cotarnine with hydroxylamine was initially reported to give the oxime (92).[61] Beke, in a review

Fig. 27. Nuclear magnetic resonance spectra

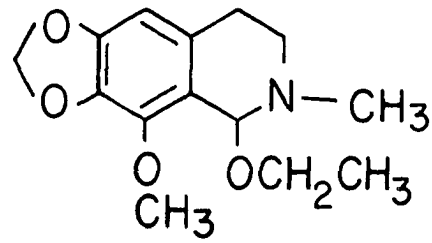
a: Mixture of cotarnine (35) and cotarnine ethyl ether (91)

b: Mixture plus D_2O





35



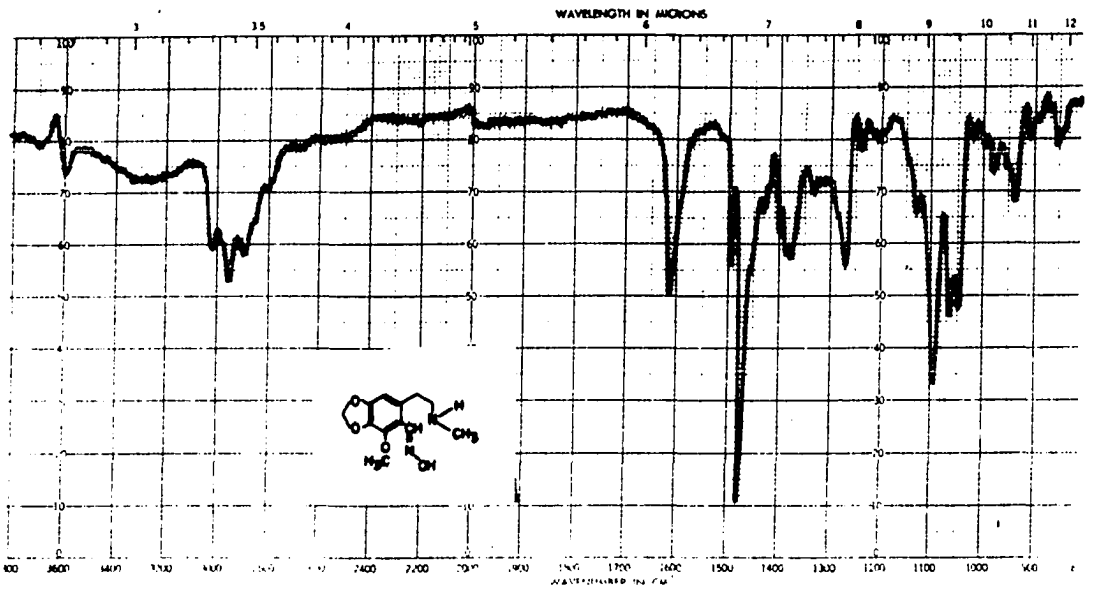
91

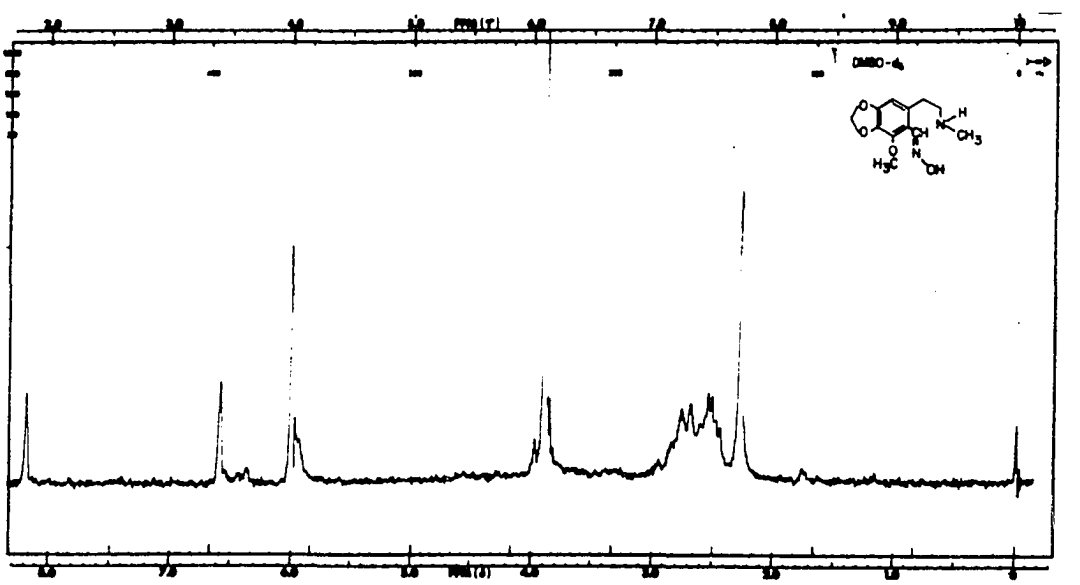
of this work, concluded that the structure assigned was in error and that the compound was actually represented by 93.[62] Since Beke did not report any nmr data and because the earlier work preceded the use of nmr, the reaction was repeated. The product was found to contain a mixture of cotarnine, the ethyl ether, and the oxime. The nmr spectrum of the oxime is shown in Fig. 28. The benzylic proton is found at 8.4 ppm which is much farther downfield than one would predict for the benzylic proton in 93. The ultraviolet spectrum confirmed the benzylic conjugation (344 mu). The same products were isolated whether a buffered or unbuffered ethanolic solution of the hydroxylamine hydrochloride were used. This experiment does not rule out the possibility that Beke may have isolated 93, but it does confirm that the oxime can exist as the open-chain form.

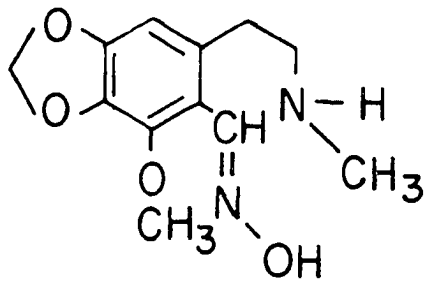
Fig. 28. Cotarnine oxime (92)

a: Infrared spectrum

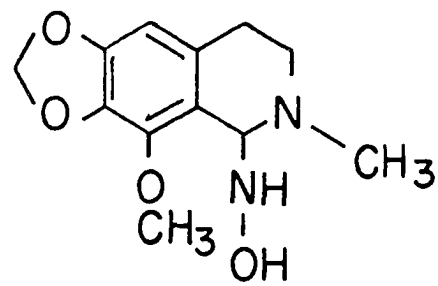
b: Nuclear magnetic resonance spectrum


WAVELENGTH IN MICRONS





92



93

The ease of formation and stability of the quaternary ammonium hydroxide form of cotarnine in polar media would seem to preclude the intermediate aminoaldehyde in this reaction. These two processes appear to be mutually opposing. In the first case, the C-N bond is strengthened, whereas in the second case, the C-N bond is broken. Thus simultaneous existence of these two forms seems remote.

A mechanism similar to that in the formation of the isoxazolidine (82) is suggested. The quaternary form of cotarnine undergoes nucleophilic attack by hydroxylamine to give 93. In the presence of acid or base, 93 isomerizes to the oxime. This alternative mechanism is intended only to show that the formation of the oxime does not necessarily demand an intermediate aminoaldehyde.

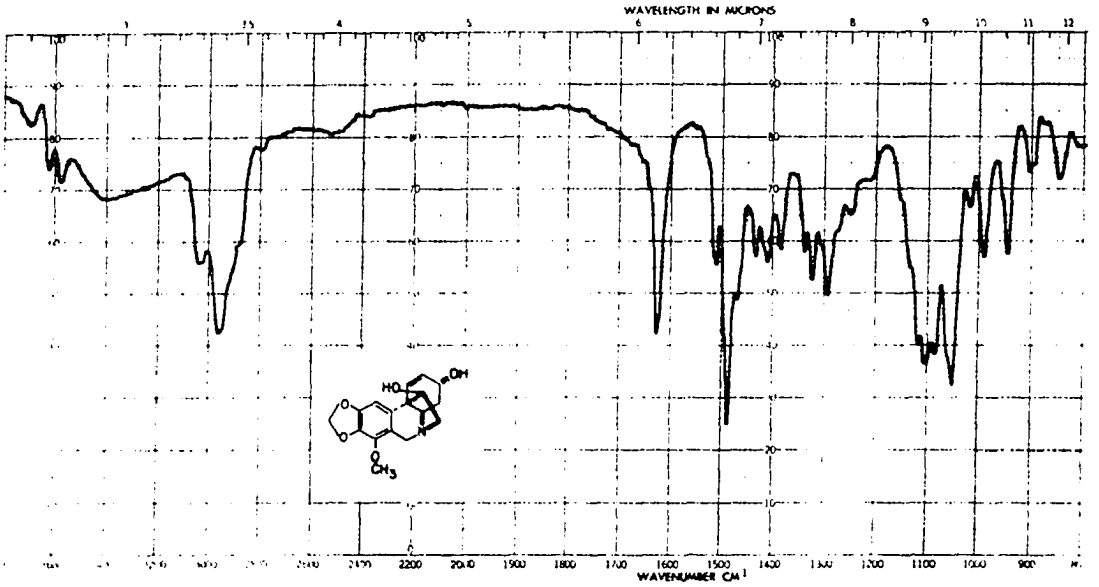
The Structure of 11-Hydroxypowelline

Earlier isolations from Nerine bowdenii have shown the presence of several alkaloids which are quite polar in nature: ambelline, crinamidine, lycorine, methylpseudolycorine, and nerbowdine.[46,54] The use of thin-layer chromatographic techniques led to the isolation of three additional polar compounds which were known but had not been reported to occur in this plant: powelline, coranicine, and 0,0-deacetylbowdensine. In addition, a new alkaloid ($C_{17}H_{19}NO_5$, mp 211-212°, $[\alpha]^{24}_{D}$ - 43°) was isolated which had an R_f 0.2 on a silica gel plate developed in chloroform-methanol-diethylamine (90:5:5). The ultraviolet and infrared spectra [Fig. 29] of the compound (94) indicated that an aromatic methoxyl was present [λ_{max} (95% EtOH) 295 mμ, ε 1450; $\lambda_{max}(CHCl_3)$ 1618 cm^{-1}]. Acetylation afforded a 0,0-diacetyl compound (95). The alkaloid formed a single dihydro derivative upon catalytic reduction. The nmr spectrum [Fig. 30] confirmed C-1, C-2-unsaturation by the presence of two olefinic protons centered at 6.48 and 5.80 ppm with the latter proton further split (J = 5 cps) by the lone hydrogen atom at C-3. The possibility of C-6 substitution was eliminated by the frequently observed benzylic AB pattern centered at 3.95 ppm.

Fig. 29. Infrared spectra

a: 11-Hydroxypowelline

b: 3,11-O,O-Diacetylpowelline (95)


WAVELENGTH IN MICRONS
WAVENUMBER CM⁻¹

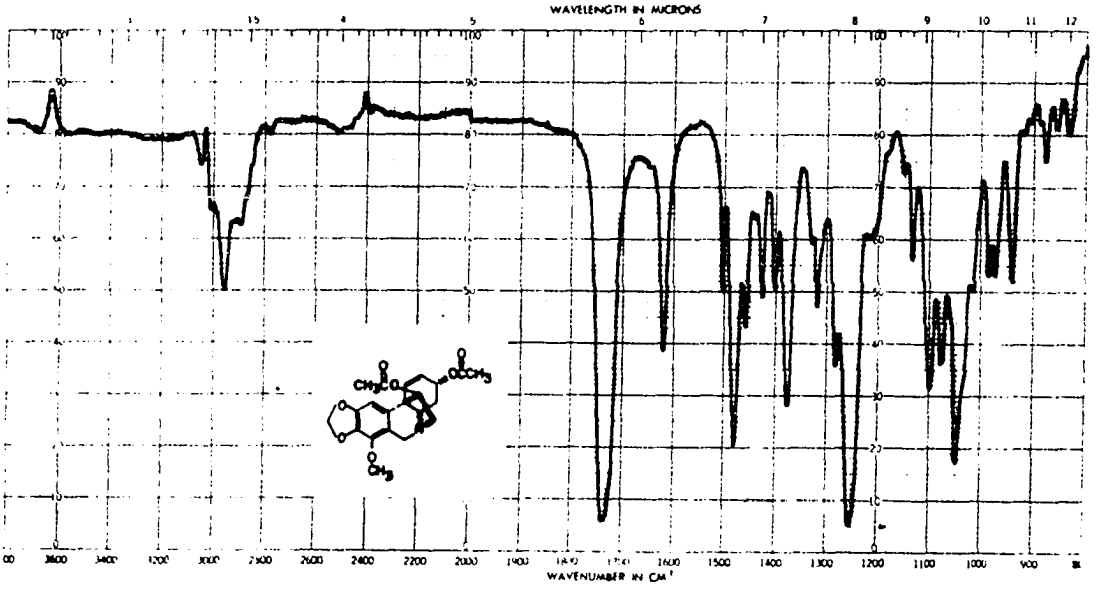

WAVELENGTH IN MICRONS
WAVENUMBER IN CM⁻¹

Fig. 30. Nuclear magnetic resonance spectra

a: 11-Hydroxypowelline (94)

b: 3,11-O,O-Diacetylpowelline (95)

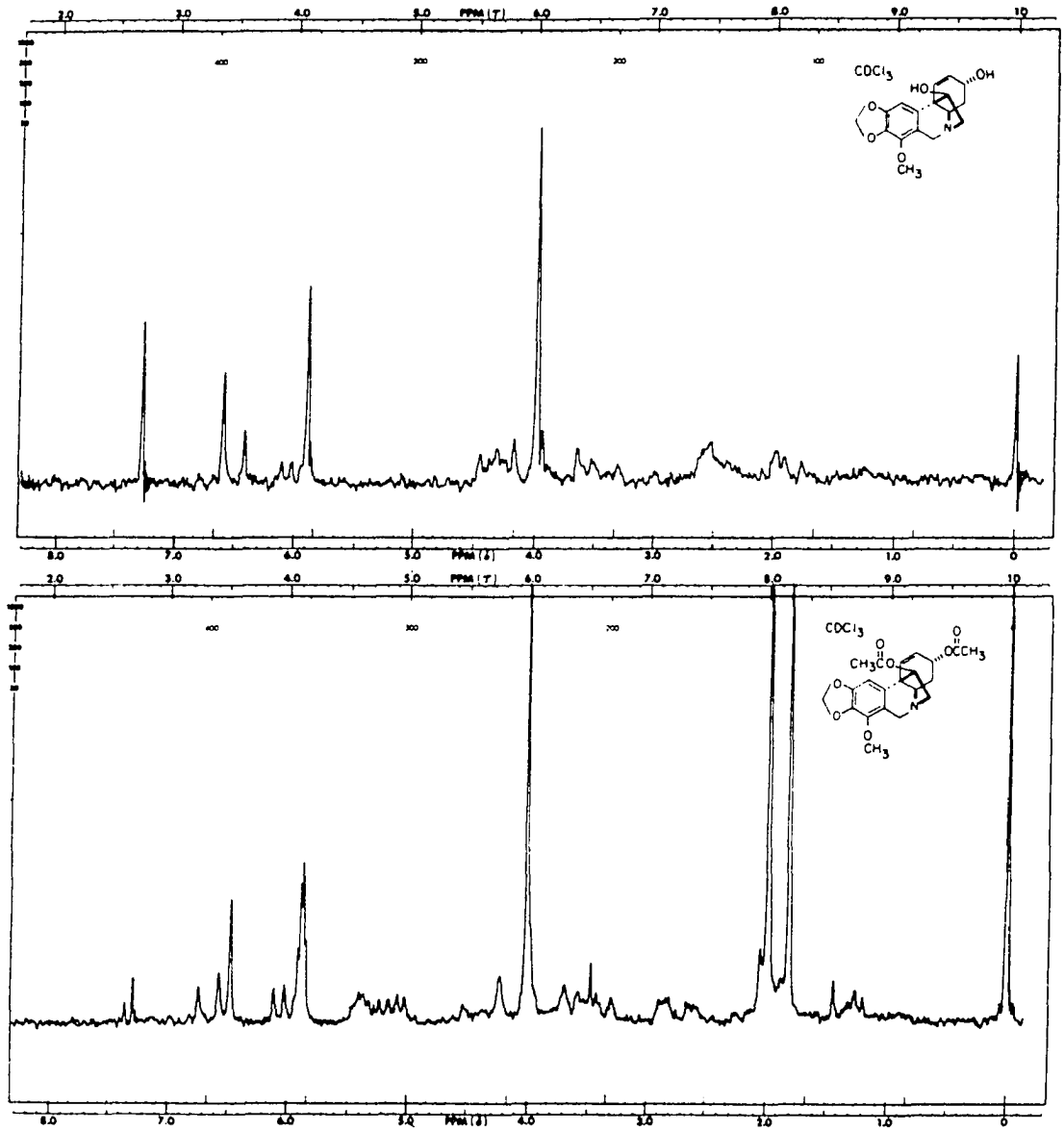
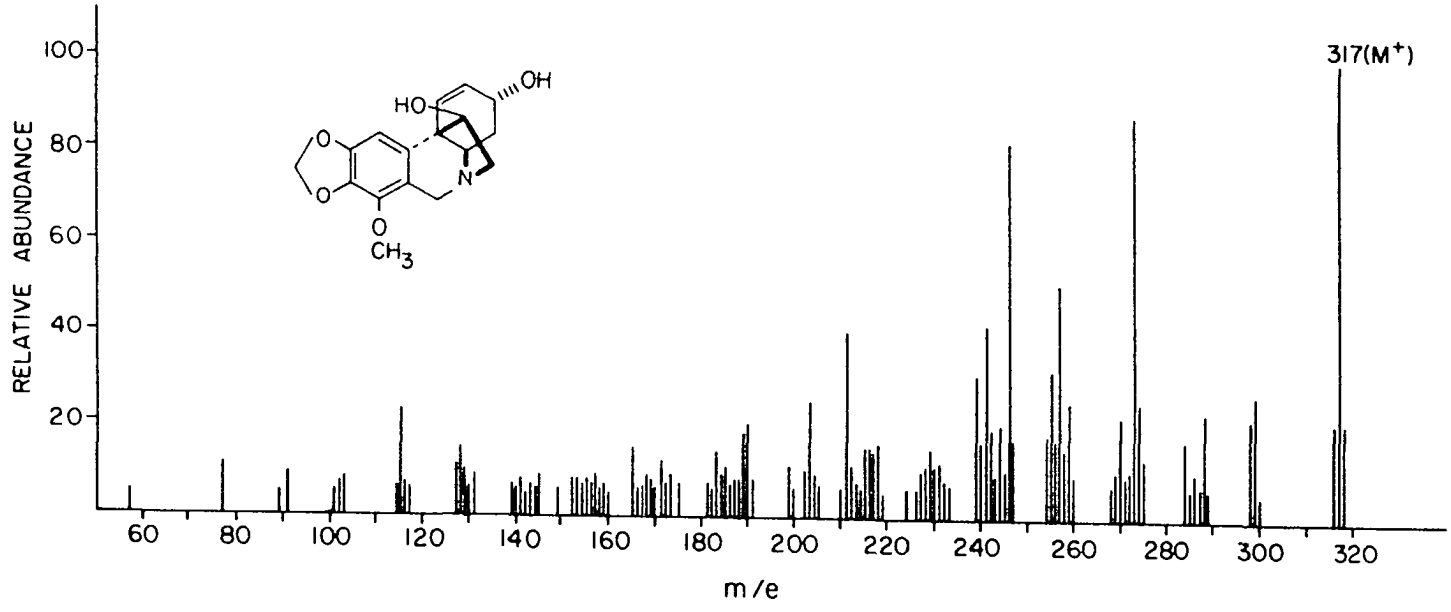
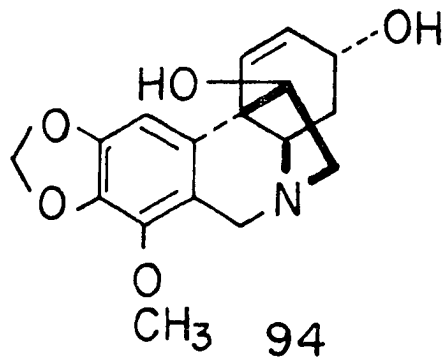
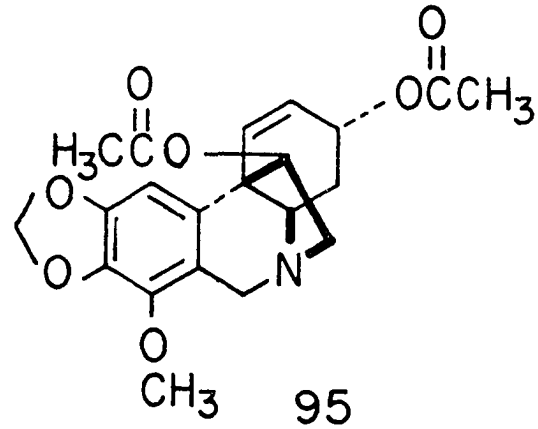

CDCl3
HO
OH
O
CH3
CDCl3
CH3CO
OCCH3
O
CH3

Fig. 31. Mass spectrum of 11-hydroxypowelline (94)


RELATIVE ABUNDANCE
100
80
60
40
20
317(M+)
HO
OH
O
O
N
O
CH3
60
80
100
120
140
160
180
200
220
240
260
280
300
320
m/e

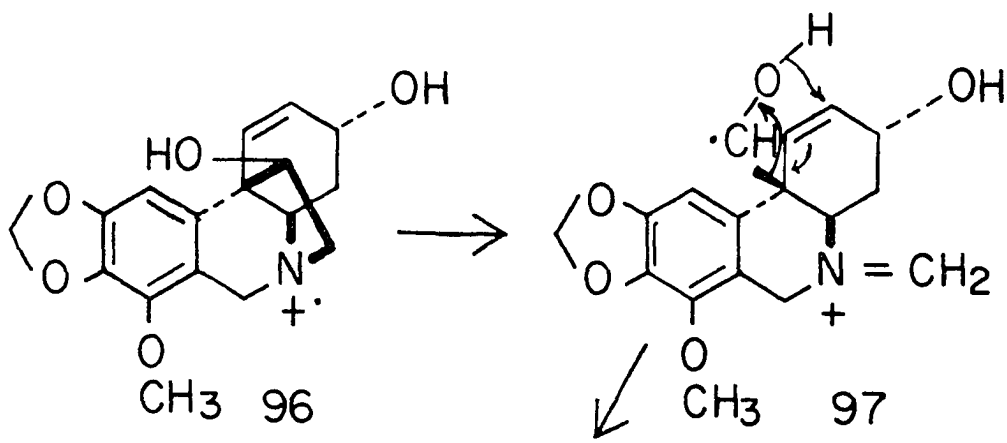


94

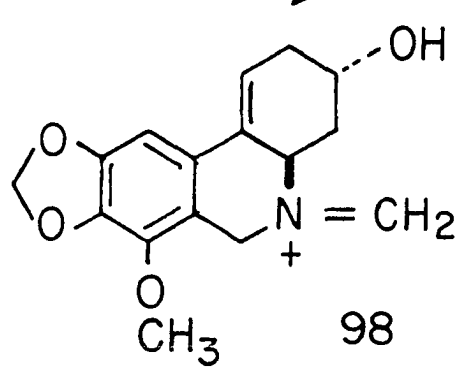


95

An examination of the mass spectrum [Fig. 31] of 94 enabled a tentative assignment of a hydroxyl group on the ethylene bridge to be made. The spectrum contains a peak at m/e 288 (M-29) which could occur through α-cleavage (97) and with the expulsion of CHO to give 98.

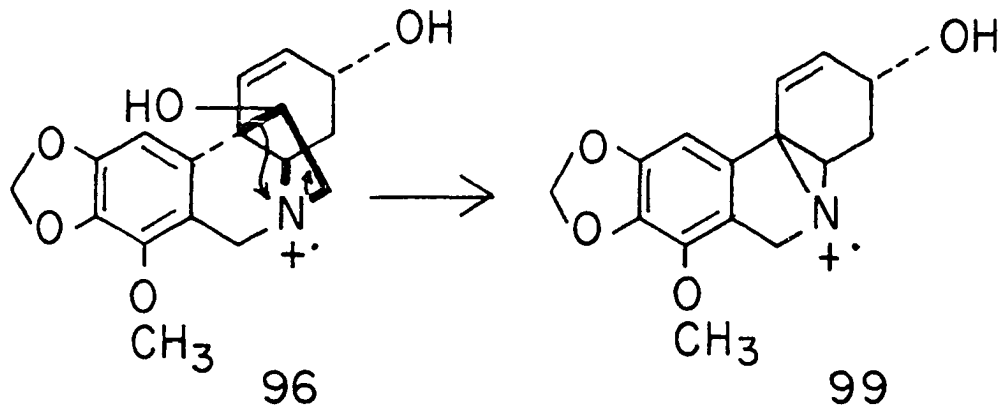


96 97



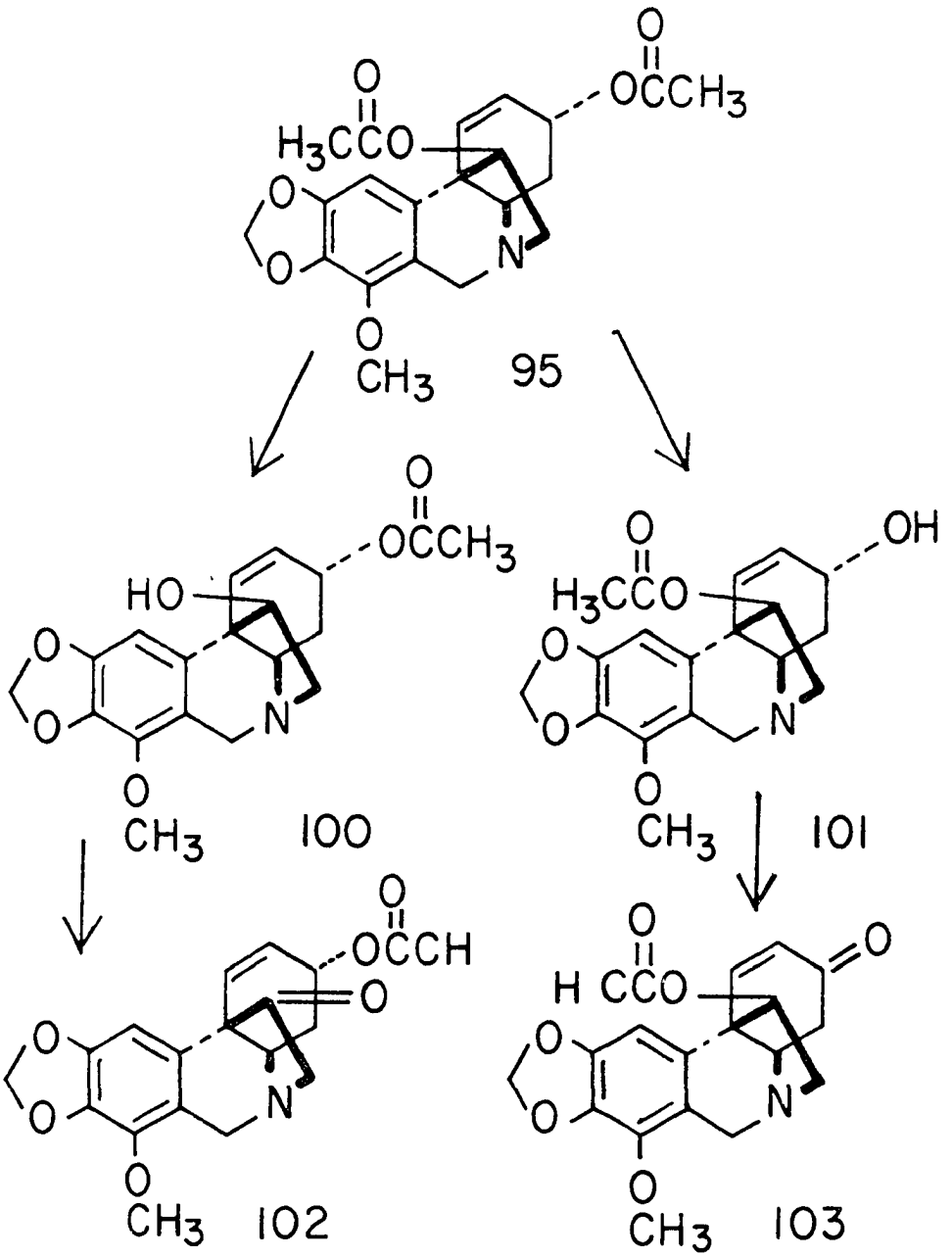
98

The abundant ion at m/e 273 (M-44) in the mass spectrum corresponds to the loss of the hydroxylated ethylene bridge. This elimination can be envisioned to proceed from the molecular ion 96 as shown by the formation of 99.



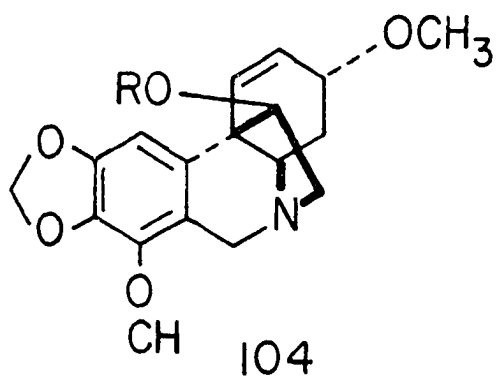
96 99

The 0,0-diacetyl derivative (95) could be hydrolyzed by dilute base to a mixture of 100 and 101. The allylic hydroxyl group of 101 was confirmed by manganese dioxide oxidation to an α,β-unsaturated ketone [113, $\lambda_{max}(CHCl_3)$ 1728 and 1670 cm^{-1}; λ_{max} (95% EtOH) 294 mμ, ε 4500, 315 mμ, ε 950]. The position of the C-11 hydroxyl in 100 was confirmed by oxidation to 102 with dimethyl sulfoxide-acetic anhydride.[63] The carbonyl absorption of 102 at 1745 cm^{-1} located the hydroxyl in the five membered ring. Since the alkaloid is not a carbinol amine, the hydroxyl must be located at C-11.

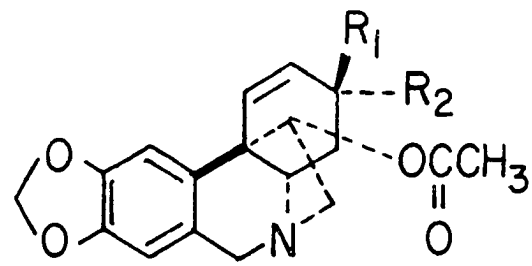


The configuration of the hydroxyl group could be assigned by an examination of the nmr spectra of 94 [Fig. 29] and its 0,0-diacetyl derivative (95). The methylenedioxy protons of 94 appear as a singlet, but in the 0,0-diacetyl compound these protons are split into an AB pattern. Moreover, the

aromatic proton was shifted upfield in the O,O-diacetyl compound. A similar effect has been observed in O-acetylambelline (104, R = $CH_3C{=}O$) and can be ascribed to the C-11 acetyl group possessing a configuration which directs it over the aromatic ring. In comparison, 11-O-acetylhaemanthamine and 11-O-acetylcrinamine (105a and 105b, respectively), do not show any splitting of the methylenedioxy nor a shift of the aromatic protons.



104



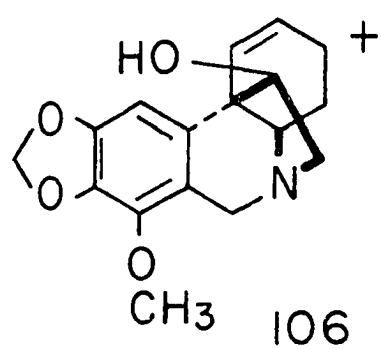
105

a: $R_1 = OCH_3$, $R_2 = H$

b: $R_1 = H$, $R_2 = OCH_3$

To verify structure 94 for the alkaloid, ambelline (104, R = H) was treated with 15% hydrobromic acid in an attempt to cleave the allylic ether located at C-3. The desired diol was obtained and proved identical in all respects with compound 94 by spectroscopic and chromatographic data. Although

acid hydrolysis of allylic ethers can provide rearranged allylic alcohols, the nmr spectrum of the diol indicates that no migration of the double bond has occurred. Furthermore, the crinine nucleus has proved stable to comparable acidic conditions.[11] The hydrolysis did not establish the stereochemistry of the C-3 substituent since the formation of an allylic carbonium ion (106) is very probable in this reaction. However, epimerization of the hydroxyl at C-3 was considered very unlikely since the hydrolysis of buphanidrine (12) and buphanisine (13) formed the expected quasiaxial products with retention of configuration.[11] The relatively small rotation of 94 at 295 mμ (Φ 350°) confirms this assignment. From other compounds of the crinine series, it is known that the C-3-epimer would possess a considerably larger negative optical rotation.



106

It is interesting to note that 11-hydroxypowelline conforms to the trend established by the three other alkaloids which possess a substituent at C-11. When the ethano bridge is α [haemanthamine and crinamine (105a and 105b, respectively)], the C-11 substituent is directed away from the benzene ring. Conversely, when the ethano bridge is β as in the case of ambelline (104, R = H) the substituent is directed toward the benzene ring.

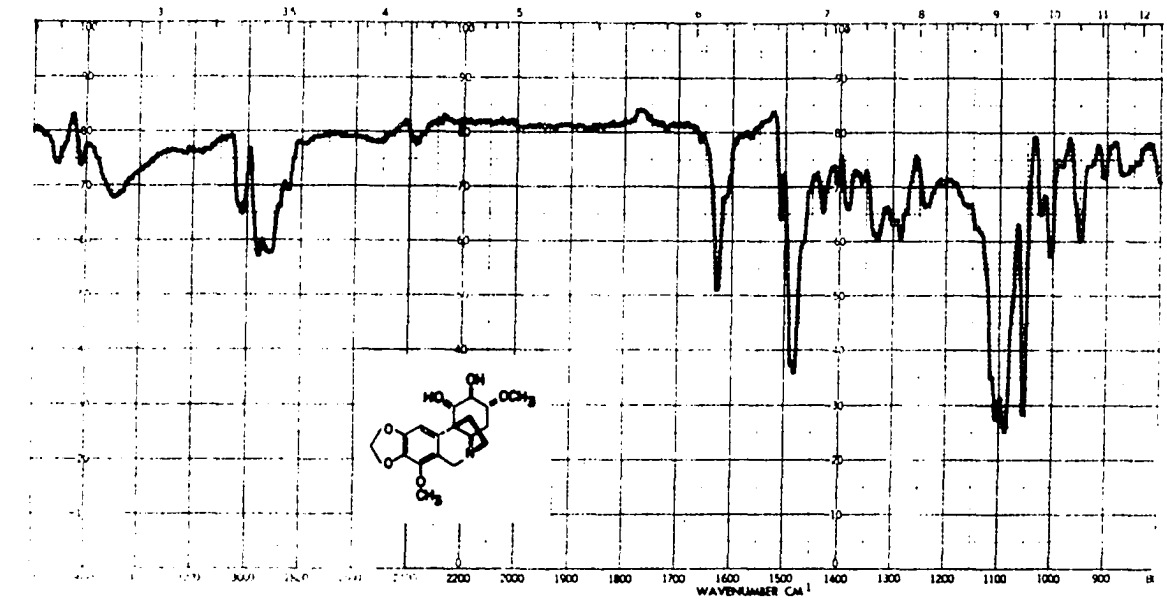
The Structure of 3-Methoxy-O,O-Deacetylbowdensine

The chloroform-insoluble hydrochlorides of Nerine bowdenii contained a majority of the hydroxylic bases. From these chloroform extracts was isolated an alkaloid ($C_{18}H_{27}NO_8$), $[\alpha]^{24}_{\underline{D}}$ - 66°) which had an Rf of 0.1 on a silica gel plate developed in chloroform-methanol-diethylamine (90:5:5). The compound (110) was unique because of its highly polar nature and high melting point, 285-286°. Treatment with pyridine-acetic anhydride afforded a diacetyl derivative ($C_{22}H_{27}NO_8$). The ultraviolet and infrared spectra [Fig. 32] indicated the presence of an aromatic ring substituted by both methylenedioxy (945 and 1050 cm^{-1}) and methoxyl (1620 cm^{-1}). In addition, the nmr spectrum [Fig. 33] revealed the presence

Fig. 32. 3-Methoxy-O,O-deacetylbowdensine (110)

a: Infrared spectrum

b: Nuclear magnetic resonance spectrum


WAVENUMBER CM⁻¹

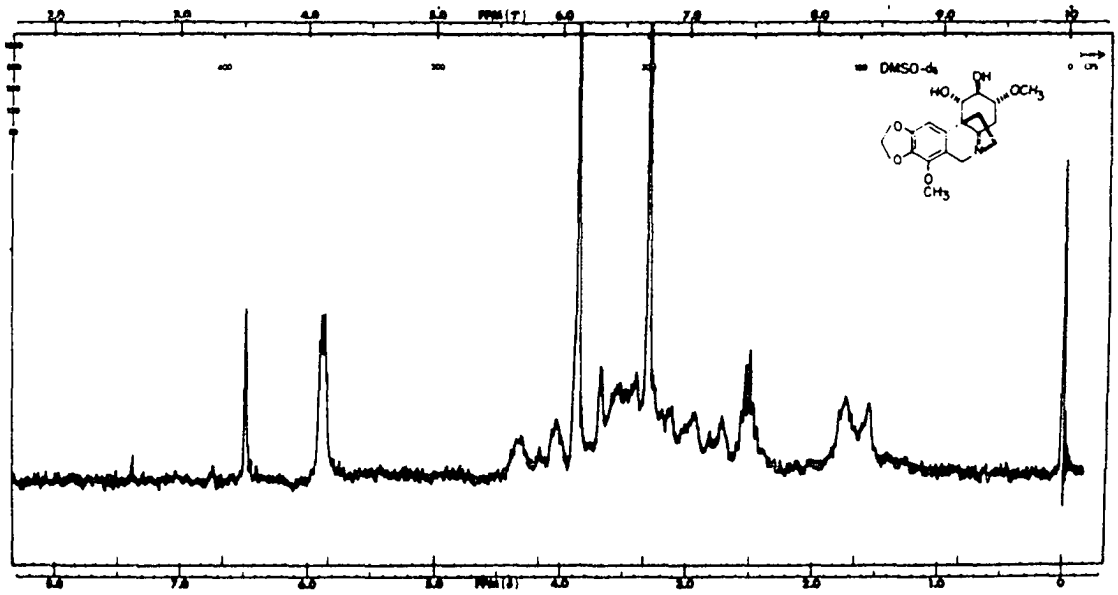
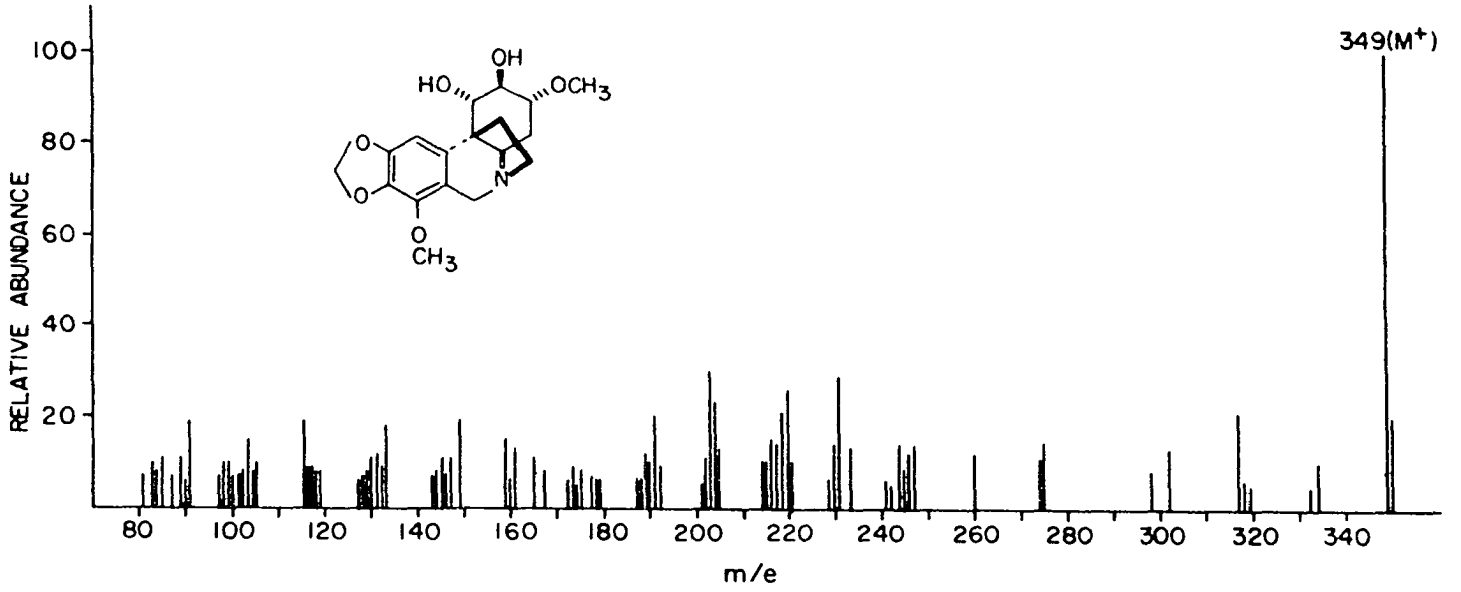
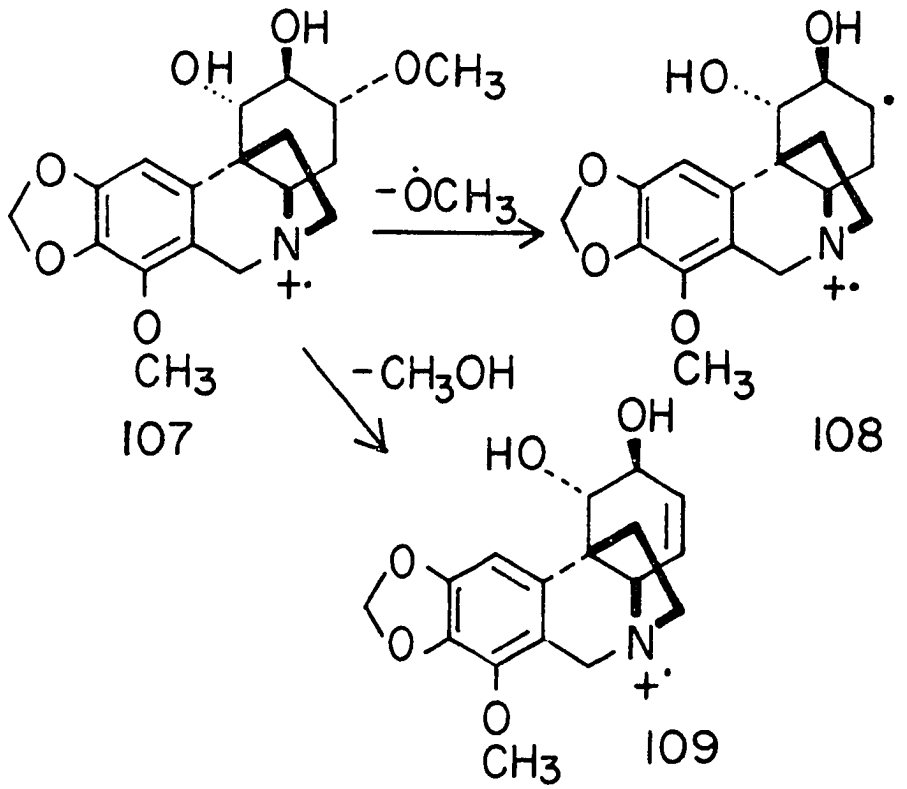

DMSO-d6

Fig. 33. Mass spectrum of 3-methoxy-O,O-deacetylbowdensine (110)

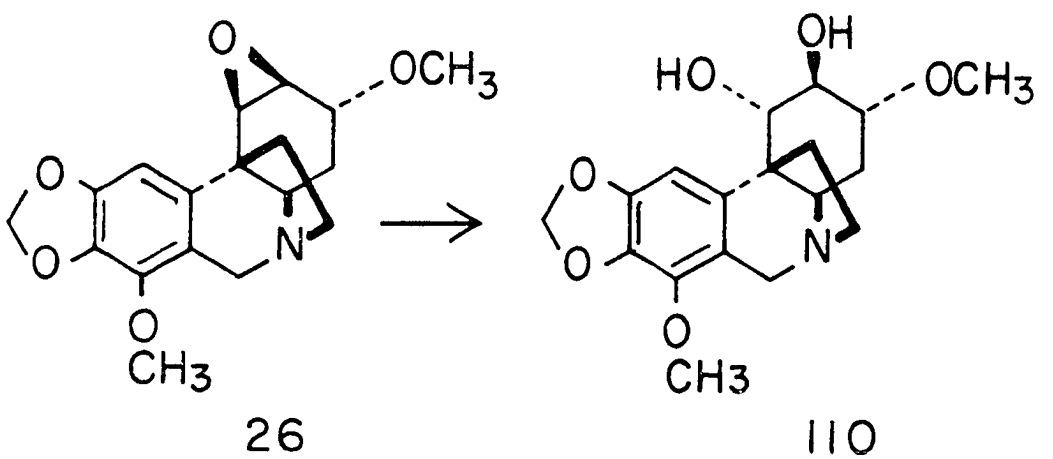

RELATIVE ABUNDANCE
100
80
60
40
20
349(M+)
HO
OH
OCH3
O
O
N
O
CH3
80
100
120
140
160
180
200
220
240
260
280
300
320
340
m/e

of an aliphatic methoxyl at 3.31 ppm. The appearance of the benzylic AB pattern at 4.0 ppm eliminated the possibility of C-6 substituents other than hydrogen. The spectrum also disclosed the absence of any olefinic protons. The mass spectrum [Fig. 33] indicated that the alkaloid had a molecular weight of 349. Loss of a methyl group occurs in the spectrum and can be attributed to the expulsion of a methyl radical from the aliphatic methoxyl group. The fragmentation pattern contains a peak at M-17 which can be assigned to the elimination of a hydroxyl group. Two peaks at m/e 318 (M-31) and m/e 317 (M-32) correspond to the elimination of a methoxyl radical and methanol, respectively. These loses can be formulated from the molecular ion 107 as shown by the formation of 108 (m/e 318) and 109 (m/e 317). The lack of a significant peak at mass 305 (M-44) corresponding to the loss of a hydroxylated ethylene bridge confirms the absence of a C-11 hydroxyl group. From these preliminary data structure 110 was postulated.

To verify this hypothesis, undulatine (26) was refluxed with 10% sulfuric acid for three hours. Complete hydrolysis of the epoxide occurred and the product proved identical with 110 by ir, nmr, and chromatographic data. Structure 110 is



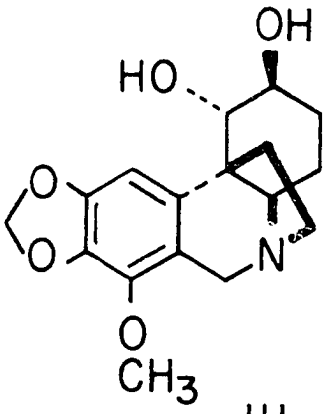
drawn with the stereochemistry depicted since hydrolysis of epoxides normally forms the diaxially substituted products in a rigid molecule.[64,65] The trans hydroxyl group relationship was substantiated by the absence of any hydroxyl absorption between 3500 and 3600 cm^{-1} in dilute chloroform solution.



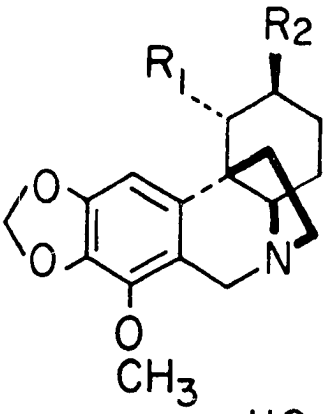
Since undulatine is present in Nerine bowdenii, compound was highly suspect as an artifact. However, attempts to hydrolyze undulatine under conditions employed in the isolation procedure failed. Undulatine also proved to be inert to 5% sodium methoxide in methanol, 20% ethanolic potassium hydroxide, and 10% hydrochloric acid at reflux temperatures.[31] These experiments cast some doubt that 110 is actually an artifact.

The chemical shift of the aromatic proton in the nmr spectrum of 110 is particularly interesting because it demonstrates a useful method for the determination of the hydroxyl configuration at C-1 in alkaloids of this series. Both 110 and O,O-deacetylbowdensine (111) exhibit a singlet at 6.5 ppm for this position. This is in agreement with the chemical shifts observed for dihydrobuphanamine (112a) [Fig. 34] and nerbowdine (113a, 6.54). Each of these four compounds possess an axial hydroxyl group (assuming ring C is in the chair form). An examination of molecular models indicates that the hydroxyl group is directed away from the aromatic proton and is favorably located for hydrogen bond formation with the π-electrons of the aromatic ring.[50] Such bonding is confirmed by infrared hydroxyl stretching absorption frequencies in

dilute chloroform solution which occur near 3600 cm^{-1} in each case. Thus an axial hydroxyl group exerts little influence on the aromatic proton and the expected chemical shift is observed. In contrast, an equatorial hydroxyl group is located in close proximity to the aromatic proton and shows little hydrogen bonding with the aromatic ring. The aromatic protons of both epidihydrobuphanamine (112b) [Fig. 34] and dihydrocrinamidine (113b) have chemical shifts near 7.3 ppm. It is probable that bond anisotropy results in a downfield shift caused by the location of the aromatic proton in the deshielding portion of the induced magnetic field. In every case examined, the axial hydroxyl compounds absorb near 6.5 ppm while the equatorial isomers possess an absorption near 7.3 ppm. This technique should prove to be particularly useful in structural studies of other crinine-type alkaloids with C-1 substituents.



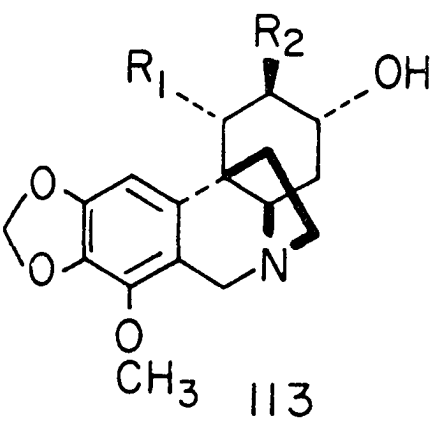
111



112

a: R_1 = OH, R_2 = H

b: R_1 = H, R_2 = OH



113

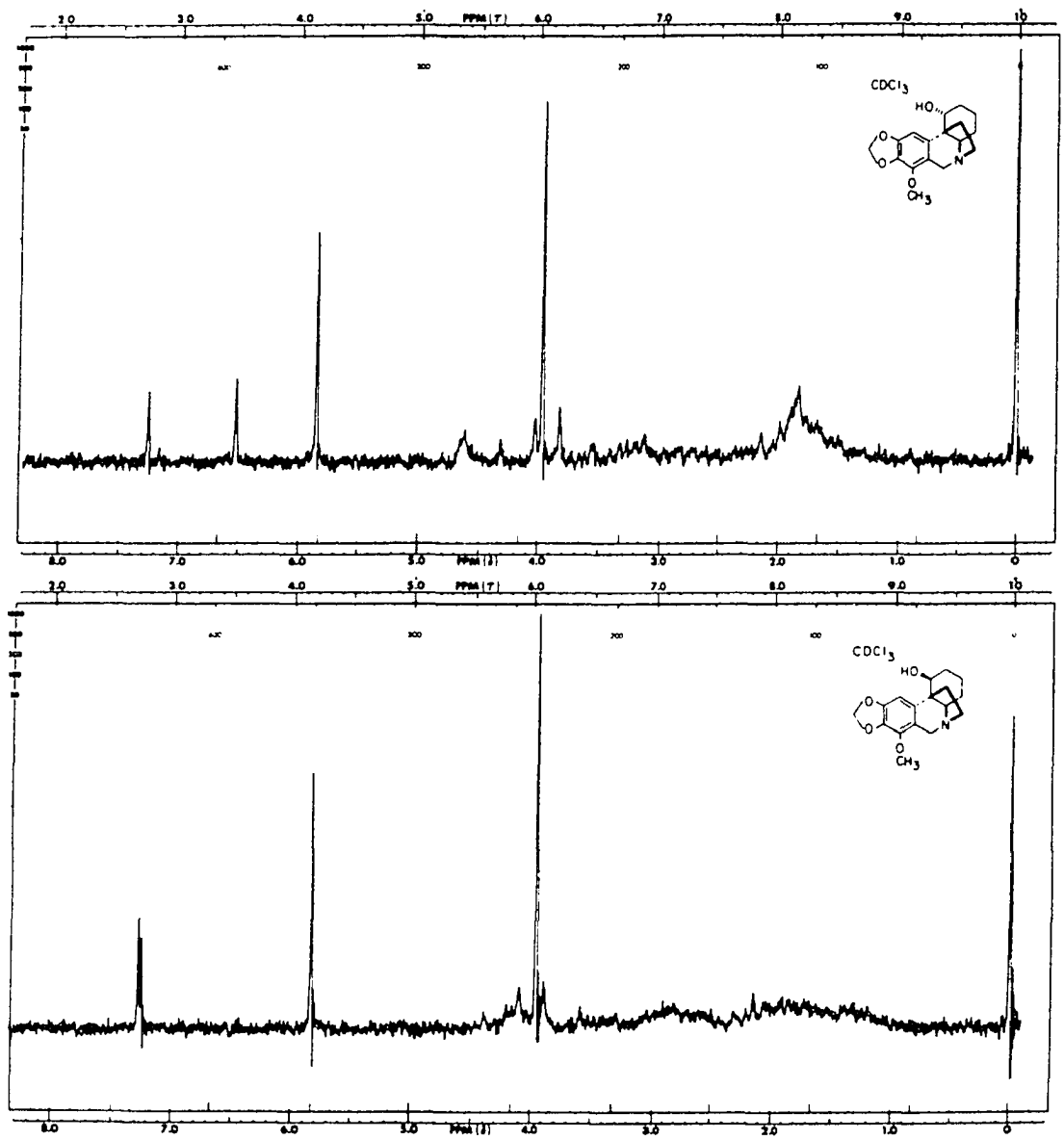
a: R_1 = OH, R_2 = H

b: R_1 = H, R_2 = OH

Fig. 34. Nuclear magnetic resonance spectra

a: Dihydrobuphanamine (112a)

b: Dihydroepibuphanamine (112b)


CDCl3
HO
O
CH3
CDCl3
HO
O
CH3

SUMMARY

Four new Amaryllidaceae alkaloids containing the 5,10b-ethanophenanthridine nucleus have been isolated and their structures established. The chemical and spectral properties of two of these alkaloids, 6-hydroxybuphanidrine and 6-hydroxypowelline, have led to the conclusion that not all 6-hydroxy-5,10b-ethanophananthridine alkaloids exist in solution as a mixture of C_6 epimers as has been predicted. Evidence is presented to show that these two alkaloids possess the alpha-configuration for the C_6-hydroxyl group. A study of the reactivity of the 6-hydroxy alkaloids provided the partial synthesis of 6-hydroxybuphanisine and 6-hydroxyundulatine. Several oxime derivatives of α-aminocarbinol compounds have been prepared and a mechanism is proposed to account for their formation. Because biosynthetic studies are planned, two deuterium-labeled derivatives of buphanidrine are prepared in a stereospecific manner. A valuable nmr technique for determination of C_1-hydroxyl configuration in alkaloids of this ring system is described.

EXPERIMENTAL

Melting points were taken on a Kofler microscope hot stage and are corrected. Ultraviolet spectra were determined in 95% ethanol on a Cary Model 14 spectrometer. Infrared spectra were obtained with a Beckman Model IR-12 spectrophotometer. Hydrogen-bonding studies were carried out in high dilution in carbon tetrachloride unless otherwise noted. Proton nuclear magnetic resonance spectra were recorded with a Varian A-60 spectrometer. Mass spectra were obtained with an Atlas CH-4 mass spectrometer operating at 70 ev. Optical rotations were determined in methanol solution with a Jasco Model 5 optical rotatory dispersion spectrometer. Thin-layer chromatography was performed using Silica Gel PF 254 + 366 (Merck). All comparisons of alkaloids were verified by the identity of the ir spectra, melting points, mixture melting points, and chromatographic data.

Isolation of Alkaloids

Nerine bowdenii W. Wats.

The preliminary isolations from the bulbs of *Nerine bowdenii* W. Wats. have been described in detail previously (46). The method of isolation involved a fractionation of the alkaloids into bases forming chloroform-soluble and

chloroform-insoluble hydrochlorides. Further purification of the alkaloids was achieved by a combination of crystallization and chromatography on Florisil or alumina. Of primary concern were the chloroform-insoluble hydrochlorides which generally have been found to contain the hydroxylic alkaloids. 6-Hydroxybuphanidrine was eluted from a Florisil column with 2% methanolic chloroform to give several viscous fractions which contained as much as 50% crinamine. Thin-layer chromatography of these mixtures on silica gel plates (0.5 mm in thickness) developed in chloroform-methanol-diethylamine (90:5:5) separated the components into two bands: 6-hydroxybuphanidrine (R_f 0.5) and crinamine (R_f 0.4). Ultraviolet light of the appropriate wavelength was used to illuminate the alkaloid bands. The material was recovered by removing the bands from the plate followed by elution of the alkaloids with chloroform. Final elution of the alkaloids forming chloroform-insoluble hydrochlorides with 50% methanolic chloroform produced a large amount of amorphous material.[46] These fractions were found to contain the most polar alkaloids including 11-hydroxypowelline, nerbowdine, 0,0-deacetylbowdensine, coranicine, powelline, and 3-methoxy-0,0-deacetylbowdensine.

Crinum powellii alba

Growing bulbs (19.0 kg) were ground in a Waring Blendor with ten gallons of 95% ethanol. The plant material was allowed to stand at room temperature for two days. The mixture was filtered through cheesecloth, and the solid material was extracted twice using ten gallons of 95% ethanol each time. The ethanolic solutions from the three extractions were combined and concentrated to a volume of approximately three liters in a circulating evaporator. This concentrate was made acidic (pH 4) with tartaric acid and extracted three times with benzene to remove the neutral material. The acidic solution was extracted three times with chloroform to provide 6.0 g of alkaloids forming chloroform-soluble hydrotartrates. The acidic solution was basified with concentrated ammonium hydroxide (pH 8) and extracted three times with chloroform. Finally, the basicity was increased to pH 10 with concentrated ammonium hydroxide, and the solution was extracted three times with chloroform. The chloroform extracts were combined and evaporated to dryness to provide 28.0 g of crude alkaloids. Lycorine (8.5 g) was removed from this fraction by trituration with chloroform and filtration. Thin-layer chromatography of the pH 8-10 fraction on silica

gel plates developed in chloroform-methanol-diethylamine (90:5:5) allowed the isolation of the following alkaloids: buphanidrine (R_f 0.6), 6-hydroxybuphanidrine (R_f 0.5), ambelline (R_f 0.45), crinamidine (R_f 0.4), and 6-hydroxypowelline (R_f 0.35).

6-Hydroxybuphanidrine (43)

The pure alkaloid afforded colorless prisms from chloroform: mp 95-96°; $[\alpha]^{24}_{\underline{D}}$ - 64° (c 0.19, MeOH); λ_{max} (95% EtOH) 215 mu (ε 18,250), 238 mu (ε 7750), 285 mu (ε 1880); ir ($CHCl_3$) [Fig. 1] 1505, 1481, 1050, 945 cm^{-1} (aromatic methylenedioxy), 3595 cm^{-1} (hydroxyl group), 1618 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) [Fig. 2] δ 3.33 ppm (s, 3, aliphatic methoxyl, δ 4.05 ppm (s, 3, aromatic methoxyl), δ 5.31 ppm (s, 1, benzylic proton), δ 5.85 ppm (s, 2, methylenedioxy), δ 5.10 ppm (s, 1, hydroxyl group, δ 6.00 and 6.58 ppm (2, olefinic protons), δ 6.60 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23. Found: C, 65.08; H, 6.57; N, 4.35.

6-Hydroxypowelline (56)

The pure alkaloid afforded colorless prisms from acetone: mp 233-235°; $[\alpha]^{24}_{\underline{D}}$ - 36° (c 0.19, MeOH); λ_{max} (95% EtOH) 218 mu (ε 17,500), 235 mu (ε 6800), 286 mu (ε 2150);

ir ($CHCl_3$) [Fig. 10] 1510, 1490, 1055, 950 cm^{-1} (aromatic methylenedioxy), 3609 cm^{-1} (hydroxyl group), 1623 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) [Fig. 11] δ 4.02 ppm (s, 3, aromatic methoxyl), δ 5.38 ppm (s, 1, benzylic proton), δ 5.88 ppm (2, methylenedioxy), δ 5.85 and 6.38 ppm (2, olefinic protons), δ 6.55 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41. Found: C, 64.17; H, 5.85; N, 4.31.

11-Hydroxypowelline (94)

The alkaloid formed colorless prisms from acetone: mp 211-212°; $[\alpha]^{24}$ D - 43° (c 0.26, MeOH); λ_{max} (95% EtOH) 210 mu (ϵ 33,200), 234 mu (ϵ 8,400), 295 mu (ϵ 1450); ir ($CDCl_3$) [Fig. 29] 1627 cm^{-1} (aromatic methoxyl), 945 and 1050 cm^{-1} (aromatic methylenedioxy); nmr ($CDCl_3$) [Fig. 30] δ 3.98 ppm (s, 3, aromatic methoxyl), δ 5.85 ppm (s, 2, methylenedioxy), δ 6.00 and 6.50 ppm (2 olefinic protons), δ 6.58 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41. Found: C, 64.22; H, 6.14; N, 4.56.

3-Methoxy-O,O-deacetylbowdensine (110)

The alkaloid afforded colorless prisms from chloroform (mp 281-283°). The analytical sample was prepared by sublima-

tion: mp 285-286°; $[\alpha]^{24}$ D - 66° (c 0.20, MeOH); λ_{max} (95% EtOH) 219 mu (ε 20,450), 240 mu (ε 9,400), 288 mu (ε 1675); ir ($CHCl_3$) [Fig. 33] 1625 cm^{-1} (aromatic methoxyl), 945 and 1055 cm^{-1} (aromatic methylenedioxy); nmr (DMSO-d6) [Fig. 33] δ 3.30 ppm (s, 3, aliphatic methoxyl), δ 3.85 ppm (s, 3, aromatic methoxyl), δ 5.85 ppm (s, 2, methylenedioxy), δ 6.50 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01. Found: C, 61.98; H, 6.59; N, 4.01.

Preparation of Derivatives

Dihydro-6-hydroxybuphanidrine

A solution of 65 mg of 6-hydroxybuphanidrine in 10 ml of 95% ethanol was hydrogenated at room temperature and atmospheric pressure with 30 mg of palladium-on-charcoal which had been equilibrated with hydrogen. The reduction stopped after the uptake of 92% of the theoretical amount of hydrogen. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure. Thin-layer chromatography on a silica gel plate (0.5 mm) developed in chloroform-methanol-diethylamine (90:5:5) showed that the residue was pure (R_f 0.7). The band was removed and the material (48 mg) was recovered by extraction with chloroform from a basic suspension

of the silica gel. A pure sample was obtained by sublimation: mp 118-119°; $[\alpha]^{24}$ D - 74° (c 0.21, MeOH); λ_{max} (95% EtOH) 220 mu (ε 19,400), 237 mu (ε 8250), 284 mu (ε 2560); ir ($CDCl_3$) [Fig. 1]; nmr ($CDCl_3$) [Fig. 2] δ 3.29 and 4.02 ppm (2s, 3, $-OCH_3$), δ 5.30 ppm (s, 1, benzylic proton), δ 5.87 ppm (s, 2, methylenedioxy), δ6.38 ppm (s, 1, -OH), δ 6.41 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95; N, 4.20. Found: C, 65.01; H, 6.93; N, 4.20.

6-O-Acetylbuphanidrine (45)

A solution of 58 mg of 6-hydroxybuphanidrine in 1 ml of anhydrous pyridine was treated with 1 ml of acetic anhydride and allowed to stand 24 hr at room temperature. The mixture was diluted with 5 ml of water. Ammonium hydroxide was added until pH 8 was reached, and the solution was extracted four times with chloroform. Evaporation of the chloroform extracts under reduced pressure gave 55 mg of reaction product which was purified by recrystallization from benzene: mp 165-168°; $[\alpha]^{24}$ D - 42° (c 0.23, MeOH); λ_{max} (95% EtOH) 214 mu (ε 17,500), 239 mu (ε 8050), 286 mu (2180); ir ($CHCl_3$) [Fig. 3] 1733 cm^{-1} (c = 0), 1624 cm^{-1} (aromatic methoxyl), 950 and 1050 cm^{-1} (aromatic methylenedioxy); nmr ($CDCl_3$) [Fig. 4]

δ 2.10 ppm (s, 3, CH C = 0), δ 3.33 and 3.90 ppm (2s, 3, $-OCH_3$), δ 5.85 ppm (s, 2, methylenedioxy), δ 6.25 ppm (s, 1, benzylic proton), δ 6.58 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{20}H_{23}NO_6$: C, 64.33; H, 6.21; N, 3.75. Found: C, 64.03; H, 6.12; N, 3.83.

6-Hydroxybuphanidrine hydrochloride (44)

6-Hydroxybuphanidrine afforded an amorphous hydrochloride salt: $[\alpha]^{24}$ D - 22° (c 0.16, MeOH); ir ($CHCl_3$) [Fig. 6]; nmr ($CDCl_3$) [Fig. 5] 3.34 and 4.05 ppm (2s, 3, $-OCH_3$), δ 5.96 ppm (s, 2, methylenedioxy), δ 6.06 ppm (s, 1, benzylic proton), δ 6.62 ppm (s, 1, aromatic proton), δ 6.88 ppm (1, hydroxyl), δ 11.50 ppm (N-H).

Anal. Calcd. for $C_{18}H_{21}NO_5 \cdot HCl$: C, 58.82; H, 5.99; N, 3.81. Found: C, 58.55; H, 6.05; N, 3.96.

Reaction of 6-hydroxybuphanidrine with lithium aluminum hydride

A solution of 42 mg of 6-hydroxybuphanidrine in 2 ml of anhydrous tetrahydrofuran was combined with 50 mg of lithium aluminum hydride. The mixture was refluxed for 16 hr. The complex was destroyed by the addition of ethyl acetate followed by water and 25% sodium hydroxide. The tetrahydrofuran solution was filtered, and the filtrate was dissolved in

dilute hydrochloric acid, washed with benzene, basified with ammonium hydroxide, and extracted with chloroform. The combined tetrahydrofuran and chloroform extracts were evaporated to dryness under reduced pressure to give 31 mg of crude product. This material proved identical with the starting material by ir and chromatographic data. Only trace impurities were detected by thin-layer.

Conversion of 6-hydroxybuphanidrine (43) to buphanidrine (12)

A solution of 75 mg of 6-hydroxybuphanidrine in 10 ml of thionyl chloride was refluxed for 2 hr and then evaporated to dryness. The residue was combined with 15 ml of dry tetrahydrofuran and 300 mg of lithium aluminum hydride. The solution was refluxed for 16 hr. The cooled mixture was hydrolyzed with ethyl acetate followed by water and 25% sodium hydroxide. The tetrahydrofuran solution was filtered and the filtrate washed thoroughly with chloroform. The combined chloroform and tetrahydrofuran extracts were evaporated to dryness. The residue was dissolved in chloroform and chromatographed on silica gel. Only one component with trace impurities was observed. Removal of the band gave 42 mg of material which proved identical to buphanidrine by ir [Fig. 3], nmr [Fig. 4], and tlc comparison.

Attempted oxidation of 43

By chromic anhydride A solution of 50 mg of 6-hydroxybuphanidrine in 1 ml of dry pyridine was added to a solution of 50 mg of chromic anhydride in 1 ml of dry pyridine. The mixture was allowed to stand for 24 hr, diluted with water, and made basic with ammonium hydroxide. The solution was extracted with chloroform. The chloroform extracts were evaporated under reduced pressure to give 43 mg of starting material. Only trace quantities of impurities were present as detected by thin-layer chromatography.

By dimethyl sulfoxide-acetic anhydride A solution of 45 mg of 6-hydroxybuphanidrine in 1 ml of dimethyl sulfoxide and 1 ml of acetic anhydride was allowed to stand at room temperature for 24 hr. The mixture was diluted with water, made basic (pH 8) with ammonium hydroxide, and extracted four times with chloroform. The chloroform extracts were evaporated under reduced pressure to provide a viscous oil. The residual dimethyl sulfoxide was removed by thin-layer chromatography. Elution of the only alkaloid band present on the chromatographic plate with chloroform provided 37 mg of product. This material proved identical by ir, nmr, and chromatographic behavior to 6-O-acetylbuphanidrine.

6-Oxobuphanidrine (46)

A solution of 150 mg of 6-hydroxybuphanidrine in 15 ml of anhydrous chloroform was combined with 700 mg of activated manganese dioxide and allowed to stir for 4 days. The manganese dioxide was removed by filtration, and the solvent was evaporated to dryness under reduced pressure. Separation of the residue by chromatography on silica gel plates developed in chloroform-methanol-diethylamine (90:5:5) allowed the recovery of 14 mg of 6-oxobuphanidrine (R_f 0.7) and 119 mg of 6-hydroxybuphanidrine (R_f 0.5). The material was crystallized from acetone: mp 161-163°; $[\alpha]^{24}$ D - 6° (c 0.25, MeOH); λ_{max} (95% EtOH) 216 mu (ε 20,400), 238 mu (ε 7,200), 287 mu (ε 1500), 321 mu (ε 1350); ir ($CHCl_3$) [Fig. 6] 1690 cm^{-1} (C = O), 1616 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) [Fig. 5] δ 3.30 and 4.05 ppm (2s, 3, $-OCH_3$), δ 5.95 ppm (s, 2, methylenedioxy), δ 6.65 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.26; H, 5.77; N, 4.30.

6-Ethoxybuphanidrine (48)

A solution of 100 mg of 6-hydroxybuphanidrine in 5 ml of 95% ethanol was made acidic (pH 2) with 0.2 ml of 20% hydrochloric acid and refluxed for four hr. The solution was

evaporated to dryness under reduced pressure, and the residue was dissolved in 5 ml of water. The solution was made basic (pH 10) with concentrated ammonium hydroxide and extracted three times with chloroform. The chloroform extracts were evaporated under reduced pressure to give 96 mg of an oil. Separation by thin-layer chromatography provided 22 mg of 6-ethoxybuphanidrine (R_f 0.8) and 68 mg of 6-hydroxybuphanidrine (R_f 0.6). Although chromatographic data indicated the 6-ethoxybuphanidrine was pure, it remained amorphous: $[\alpha]^{24}$ D - 38° (c 0.17 MeOH); λ_{max} (95% EtOH) 240 mu (ε 7940), 287 mu (ε 2130); ir ($CHCl_3$) [Fig. 8]; nmr ($CDCl_3$) [Fig. 9] δ 1.20 ppm (t, 3, CH_3CH_2O-), δ 3.31 and 3.95 ppm (2s, 3, -OCH_3), δ 4.72 ppm (benzylic proton), δ 5.87 ppm (q, 2, methylenedioxy), δ 6.56 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{20}H_{25}NO_5$: C, 66.83; H, 7.01; N, 3.90. Found: C, 66.55; H, 7.07; N, 3.85.

Hydrolysis of 6-O-acetylbuphanidrine (45)

A solution of 40 mg of 6-O-acetylbuphanidrine in 4 ml of water-dioxane (50:50) was allowed to stand at room temperature for 2 days. The solution was extracted three times with chloroform, and the chloroform extracts were evaporated under reduced pressure. Thin-layer chromatography of the residue

on a silica gel plate developed in chloroform-methanol-diethylamine (90:5:5) gave 32 mg of product (R_f 0.6) identical in all respects to 6-hydroxybuphanidrine. The residue crystallized from chloroform (mp 93-95°).

6-Methoxybuphanidrine (53)

From 6-O-acetylbuphanidrine (45) A solution of 45 mg of 6-O-acetylbuphanidrine in 3 ml of absolute methanol was allowed to stand for 4 days at room temperature. The solution was evaporated under reduced pressure. Thin-layer chromatography on silica gel plates revealed the presence of starting material (R_f 0.6) and a second, less polar band (R_f 0.8). Elution of the less polar band with chloroform allowed the recovery of 17 mg of 6-methoxybupahnidrine as an amorphous material: $[\alpha]^{24}$ D - 14° (c 0.18, MeOH); λ_{max} (95% EtOH) 238 mu (ε 7300), 285 mu (ε 2200); ir ($CHCl_3$) [Fig. 8]; nmr ($CDCl_3$) [Fig. 9] δ 3.33, 3.56, and 3.97 ppm (3s, 3, $-OCH_3$), δ 4.61 ppm (s, 1, benzylic proton), δ 5.86 ppm (q, 2, methylenedioxy), δ 6.55 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71, N, 4.06. Found: C, 65.86; H, 6.74; N, 4.19.

From 6-hydroxybuphanidrine (43) treated with methanol By the same procedure which was used to prepare 6-ethoxybuphanidrine from 6-hydroxybuphanidrine (refluxing in acidic

alcohol solution), 100 mg of 6-hydroxybuphanidrine was converted to 27 mg of product. The spectral and chromatographic characteristics of the product were identical to 6-methoxybuphanidrine prepared from 45.

From 6-hydroxybuphanidrine (43) treated with methyl p-toluene-sulfonate To a solution of 100 mg of 6-hydroxybuphanidrine in 40 ml of benzene was added 80 mg of potassium. The solution was refluxed in a nitrogen atmosphere, and the molten potassium was converted to a fine, bluish suspension by high-speed stirring. After 15 minutes the solution was allowed to cool to room temperature, and 100 mg of methyl p-toluenesulfonate in 5 ml of benzene was added dropwise. The mixture was stirred at room temperature for 2 hr, then decomposed with excess ethanol. Evaporation of the solvents left a brown residue which was dissolved in 10% hydrochloric acid and washed with benzene to remove neutral compounds. The solution was made basic (pH 10) with concentrated ammonium hydroxide and extracted three times with chloroform. Evaporation of the chloroform under reduced pressure gave 63 mg of residue. The product was purified by preparative thin-layer chromatography. The major band (R_f 0.8) was recovered as an amorphous material (28 mg) identical in all respects to 6-methoxybuphanidrine.

In order to rule out the possibility of epimerization of the secondary hydroxyl group under the strongly basic conditions, the experiment was repeated with omission of the methyl p-toluene-sulfonate. The reaction afforded only starting material with no formation of the C_6 epimer.

From 6-chlorobuphanidrine hydrochloride (61) A solution of 100 mg of 6-hydroxybuphanidrine in 6 ml of thionyl chloride was refluxed for 2 hr and then evaporated to dryness. The residue was combined with 10 ml of methanol containing 200 mg of sodium methoxide. The solution was allowed to stand at room temperature for 1 hr. The solution was diluted with 10 ml of water and extracted three times with chloroform. Evaporation of the chloroform under reduced pressure gave 88 mg of 6-methoxybuphanidrine which was pure by tlc.

Repetition of the experiment using pyridine as a solvent or with 6-hydroxybuphanidrine hydrochloride as the starting material gave identical results (6-methoxybuphanidrine) with no formation of the C_6 epimer.

Reduction of 6-oxobuphanidrine (46)

A solution of 49 mg of 6-oxobuphanidrine in 2 ml of anhydrous tetrahydrofuran was combined with 50 mg of lithium aluminum hydride. The mixture was refluxed for 12 hr. The

complex was destroyed by the addition of ethyl acetate followed by 25% sodium hydroxide. The tetrahydrofuran solution was filtered, and the filtrate was dissolved in dilute hydrochloric acid, washed with benzene, basified with ammonium hydroxide, and extracted with chloroform. The combined tetrahydrofuran and chloroform extracts were evaporated to dryness under reduced pressure. The product (29 mg) was pure by tlc and crystallized from methanol-ether (mp 94-96°). The material proved identical in all respects to 6-hydroxybuphanidrine.

[6α-^{2}H]Buphanidrine hydrobromide (62)

A solution of 100 mg of 6-hydroxybuphanidrine in 10 ml of thionyl chloride was refluxed for 2 hr and then evaporated to dryness. The residue was combined with 8 ml of dry tetrahydrofuran and 105 mg of lithium aluminum deuteride. The solution was refluxed for 5 hr. The cooled mixture was hydrolyzed with ethyl acetate followed by 5% hydrobromic acid. The solution was extracted four times with chloroform. The chloroform extracts were washed with dilute ammonium hydroxide and evaporated to dryness. The residue (89 mg) was chromatographed on silica gel plates. Removal of the major band provided 76 mg of amorphous [6α-^{2}H]buphanidrine. The mass spectrum of this material contained a molecular ion at m/e 316.

The [6α-^{2}H]buphanidrine afforded a hydrobromide salt which crystallized from methanol-ether: mp 192-194°; $[\alpha]^{24}$ - 18° (c 0.17 MeOH); λ_{max} (95% EtOH) 239 mu (ε 7050), 285 mu (ε 2300); nmr ($CDCl_3$) [Fig. 15] δ 3.35 and 4.00 ppm (2s, 3, $-OCH_3$), δ 4.22 ppm (s, 1, benzylic proton), δ 5.90 ppm (s, 2, methylenedioxy), δ 6.55 ppm (s, 1, aromatic proton).

[6,6-^{2}H]Buphanidrine hydrobromide (70)

A solution of 40 mg of 6-oxobuphanidrine in 3 ml of anhydrous tetrahydrofuran was combined with 40 mg of lithium aluminum deuteride. The mixture was reflexed for 6 hr. The complex was destroyed by the addition of ethyl acetate followed by 5% hydrobromic acid. The solution was extracted four times with chloroform. The chloroform extracts were washed with silute ammonium hydroxide and evaporated to dryness to provide 36 mg of amorphous residue. The material was pure by tlc and possessed identical chromatographic characteristics to 6-hydroxybuphanidrine. The mass spectrum indicated a molecular ion at m/e 332. Following the procedure for preparation of [6α-^{2}H]buphanidrine hydrobromide (62), 32 mg of [6β-^{2}H]6-hydroxybuphanidrine was converted to 24 mg of [6,6-2^2H]buphanidrine. The mass spectrum of the amorphous product contained a molecular ion at m/e 317. The [6,6-2^2H]buphani-

drine afforded a hydrobromide salt which crystallized from methanol-ether: mp 191-194°; $[\alpha]^{24}_{\underline{D}}$ - 19° (c 0.17, MeOH); λ_{max} (95% EtOH) 238 mu (ε 7000), 285 mu (ε 2350); nmr ($CDCl_3$) [Fig. 15] δ 3.36 and 4.02 ppm (2s, 3, $-OCH_3$), δ 5.95 ppm (s, 2, methylenedioxy); δ 6.57 ppm (s, 1, aromatic proton).

N-Nitroso-6-hydroxybuphanidrine (77)

To a solution of 65 mg of 6-hydroxybuphanidrine in 10 ml of 1.5% aqueous acetic acid was added 65 mg of sodium nitride. The reaction mixture was allowed to stand for 8 hr at room temperature. The aqueous solution was extracted four times with chloroform. The chloroform solution was washed with 5% sodium bicarbonate solution and then evaporated under reduced pressure. The residue was chromatographed on a silica gel plate and yielded 17 mg of the starting material and 34 mg of the amorphous N-nitroso-6-hydroxybuphanidrine: $[\alpha]^{24}_{\underline{D}}$ - 7° (c 0.26, MeOH); λ_{max} (95% EtOH) 240 mu (ε 8020), 288 mu (ε 2070), 315 mu (ε 1670); ir($CHCl_3$) [Fig. 18] 1685 cm^{-1} (C = 0); nmr ($CDCl_3$) [Fig. 19] δ3.37 and 4.12 ppm (2s, 3, $-OCH_3$), δ 6.05 ppm (s, 2, methylenedioxy), δ 6.90 ppm (s, 1, aromatic proton), δ 10.40 ppm (s, 1, CHO).

Anal. Calcd. for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59, N, 7.77. Found: C, 59.92; H, 5.17; N, 7.94.

6-Hydroxybuphanidrine methiodide (78)

To a solution of 55 mg of 6-hydroxybuphanidrine in 5 ml of methanol-acetone (1:4) was added 1 ml of methyl iodide. The solution was allowed to stand at room temperature for 2 hr before the solvents were evaporated under reduced pressure. The crude product was dissolved in water, filtered, and the aqueous solution evaporated to dryness under reduced pressure. The material (56 mg) was pure by thin-layer chromatography. Recrystallization from methanol gave fine needles: mp 240-242°; nmr (DMSO - d_6) δ 3.12 ppm (s, 3, N-CH_3), δ 3.29 and 3.97 ppm (2s, 3, -OCH_3), δ 6.09 ppm (s, 2, methylenedioxy), δ 6.98 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{19}H_{24}NO_5I$: C, 48.41; H, 5.10; N, 2.97. Found: C, 48.54; H, 5.22; N, 3.02.

N-Methyl-6-hydroxybuphanidrine (79)

A solution of 48 mg of 6-hydroxybuphanidrine methiodide in 4 ml of water was made basic (pH 10) with ammonium hydroxide. The solution was allowed to stand at room temperature for 3 hr and then extracted four times with chloroform. The chloroform extracts were evaporated under reduced pressure to give 39 mg of an oil. Thin-layer chromatography separated the two components of this mixture. The major band (R_f 0.7)

provided 28 mg of N-methyl-6-hydroxybuphanidrine. The other band (R_f 0.1) was poorly recovered from the silica gel and was presumed to be the starting methiodide. The alkaloid resisted all attempts at crystallization. The sublimed glass gave: $[\alpha]^{24}_{\underline{D}}$ - 14° (c 0.21, MeOH); λ_{max} (95% EtOH) 240 mu (ϵ 8040), 285 mu (ϵ 2010), 320 mu (ϵ 1990); ir ($CHCl_3$) [Fig. 18] 1690 cm^{-1} (C = O); nmr ($CDCl_3$) [Fig. 19] δ 2.42 ppm (s, 3, N-CH_3), δ 3.38 and 4.00 ppm (2s, 3, -OCH_3), δ 5.83 ppm (s, 2, methylenedioxy), δ 5.97 ppm (s, 2, olefinic protons), δ 6.75 ppm (s, 1, aromatic proton), δ 10.5 ppm (s, 1, -CHO).

Anal. Calcd. for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 65.86; H, 6.74; N, 4.19.

Reaction of 6-hydroxybuphanidrine (43) with diazomethane

To a solution of 100 mg of 6-hydroxybuphanidrine in 10 ml of dichloromethane containing 0.2 m. of concentrated fluoroboric acid was slowly added 15 ml of ethereal diazomethane. The solution was allowed to evaporate slowly at room temperature. The residue was dissolved in chloroform, and the solution was washed with water. Evaporation of the chloroform solution under reduced pressure gave 89 mg of residue which was chromatographed on silica gel plates. The major band (R_f 0.1) was removed, treated with 5% hydrochloric

acid, and then basified (pH 10) with concentrated ammonium hydroxide. The solution was extracted three times with chloroform, and the extracts were evaporated under reduced pressure to give 64 mg of amorphous material. The compound proved identical to N-methyl-6-hydroxybuphanidrine (79) by a comparison of spectral and chromatographic data.

6-Hydroxybuphanisine (80)

To a solution of 100 mg of 6-hydroxybuphanidrine in 10 ml of xylene was added 114 mg of sodium. The solution was refluxed under a nitrogen atmosphere, and the sodium was dispersed as a fine suspension by rapid stirring. To the solution was added 1.5 ml of isoamyl alcohol in 5 ml xylene in one portion. After three minutes 0.5 ml of isoamyl alcohol in 1.5 ml of xylene was also added to the reaction. After 10 minutes the solution was allowed to cool to room temperature, and ethanol was added to destroy any remaining traces of sodium. The reaction mixture was diluted with 30 ml of 5% hydrochloric acid. The xylene layer was extracted four times with dilute hydrochloric acid. The combined aqueous solutions were extracted three times with chloroform, and the chloroform extracts were washed with dilute ammonia and then water. Evaporation of the chloroform solution under reduced

pressure provided 68 mg of product. Separation by thin-layer chromatography provided 19 mg of 6-hydroxybuphanidrine (R_f 0.55) and 44 mg of 6-hydroxybuphanisine (R_f 0.50). Although the compound was pure by tlc, it remained amorphous: $[\alpha]^{24}_D$ - 21° (c, 0.20, MeOH); λ_{max} (95% EtOH) 240 mu (ε 5400), 291 mu (ε 3600); ir ($CHCl_3$) 945 and 1050 cm^{-1} (aromatic methylene-dioxy); nmr ($CDCl_3$) [Fig. 20] δ 3.31 ppm (d, 3, $-OCH_3$), δ 5.11 and 5.78 ppm (2s, 1 benzylic proton), δ 5.90 ppm (s, 2, me methylene-dioxy), δ 6.83 ppm (s, 1, C_{10} - aromatic proton), δ 6.78 and 6.98 ppm (2s, 1, C_7 - aromatic proton).

Anal. Calcd. for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.81; H, 6.29; N, 4.68.

Hydroxylamine adduct (82)

A solution of 100 mg of 6-hydroxybuphanidrine in 8 ml of 95% ethanol was combined with 40 mg of hydroxylamine hydrochloride. The solution was refluxed on a steam bath for 4 hr. The solution was reduced in volume under reduced pressure, diluted with 15 ml of water, made basic with ammonium hydroxide, and extracted three times with chloroform. Evaporation of the chloroform extracts under reduced pressure gave 93 mg of residue. The material was chromatographed on silica gel plates developed in chloroform-methanol-diethylamine (90:5:5).

Removal of the three bands present provided 26 mg of 6-ethoxybuphanidrine (R_f 0.8), 38 mg of starting material (R_f 0.6), and 22 mg of the hydroxylamine adduct (R_f 0.5). All attempts to crystallize the adduct were unsuccessful: (amorph); $[\alpha]^{24}_{\underline{D}}$ + 11° [c 0.18, MeOH]; λ_{max} (95% EtOH) 238 mu (ε 8400), 285 mu (ε 2300); ir ($CHCl_3$) [Fig. 22]; nmr ($CDCl_3$) [Fig. 23] δ 3.41 and 4.05 ppm (2s, 3, $-OCH_3$), δ 5.15 ppm (d, 1, benzylic proton), δ 5.90 ppm (s, 2, methylene-dioxy), δ 6.30 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{18}H_{22}N_2O_5$: C, 62.41; H, 6.40; N, 8.09. Found: C, 62.16; H, 6.13; N, 8.00.

N,N-Diacetylhydroxylamine adduct (83)

By the same procedure which was used to prepare 6-O-acetylbuphanidrine from 6-hydroxybuphanidrine, 84 mg of the hydroxylamine adduct (82) was converted to 75 mg of product. The N,N-diacetylhydroxylamine adduct crystallized from ether-acetone: mp 133-135°; $[\alpha]^{24}_{\underline{D}}$ + 18° (c 0.23, MeOH); λ_{max} (95% EtOH) 238 mu (ε 7600), 286 mu (ε 2400); ir($CHCl_3$) [Fig. 22]; nmr ($CDCl_3$) [Fig. 23] δ 2.10 and 2.19 ppm (2s, 3, $CH_3C=O$), δ 3.40 and 3.98 ppm (2s, 3, $-OCH_3$), δ 5.92 ppm (s, 2, methylenedioxy), δ 6.20 ppm (d, 1, benzylic proton), δ 6.30 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{22}H_{26}N_2O_7$: C, 61.38; H, 6.09; N, 6.51. Found: C, 61.15; H, 5.96; N, 6.57.

6-Hydroxyundulatine (76)

To a solution of 259 mg of undulatine in 15 ml of benzene was added 134 mg of N-bromosuccinimide and 30 mg of benzoyl peroxide. The solution was maintained under a nitrogen atmosphere while irradiated with an ultraviolet light for 2 hr and then refluxed on a steam bath for 4 hr. The solution was evaporated to dryness under reduced pressure. The oily residue was dissolved in methanol and treated with 10% sodium hydroxide. The basic solution was allowed to stand at room temperature for 2 hr, diluted with water, and extracted three times with chloroform. Evaporation of the chloroform extracts under reduced pressure gave 214 mg of residue. Thin-layer chromatography of the mixture allowed the recovery of 93 mg of undulatine (R_f 0.7) and 84 mg of 6-hydroxyundulatine (R_f 0.6). The alkaloid crystallized from acetone-ether: mp 211-213°; $[\alpha]^{24}$ D - 43° (c 0.25, MeOH); λ_{max} (95% EtOH) 237 mu (ε 13,900), 292 mu (ε 2410); ir ($CDCl_3$) [Fig. 16]; nmr ($CDCl_3$) [Fig. 17] δ 3.40 and 4.00 ppm (2s, 3, $-OCH_3$), δ 5.20 ppm (s, 1, benzylic proton), δ 5.90 (s, 2, methylenedioxy), δ 6.63 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{18}H_{21}NO_6$: C, 62.24; H, 6.10; N, 4.03. Found: C, 61.96; H, 6.05; N, 4.12.

Preparation of chromium (II)

In a 500 ml 3-neck roundbottom flask equipped with a magnetic stirring bar, nitrogen inlet, and condenser were placed 50 g of chromic sulfate, 320 ml of water, and 13 g of zinc powder. The mixture was stirred overnight at room temperature. The excess zinc powder was allowed to settle. The chromous sulfate-zinc sulfate prepared in this way had a Cr (II) concentration approximately 0.71-0.75 N and indicated a pH of 3.5. The storing flask was equipped with a nitrogen inlet and a serum cap. All transfers were accomplished employing a nitrogen preflushed syringe.

Conversion of 6-hydroxyundulatine (76) to 6-hydroxybuphanidrine (43)

A solution of 100 mg of 6-hydroxyundulatine in 4 ml of dimethylformamide was treated with 3 ml of the chromous sulfate solution prepared above. The reaction was stirred under a nitrogen atmosphere at room temperature for 10 hr. The solution was diluted with water, made basic with ammonium hydroxide, and extracted with chloroform. The chloroform extracts were combined, washed with water, and evaporated under reduced pressure. Chromatography on silica gel plates

developed in chloroform-methanol-diethylamine (90:5:5) gave 22 mg of starting material (R_f 0.65) and 60 mg of 6-hydroxybuphanidrine (R_f 0.60). This material was identical with natural 43 by comparison of ir, nmr, and chromatographic data.

6-Hydroxyundulatine oxime (84)

With 95% ethanol as the solvent By the same procedure cited for the preparation of the 6-hydroxybuphanidrine hydroxylamine adduct (82), 100 mg of 6-hydroxyundulatine was converted to 24 mg of 6-hydroxyundulatine oxime: amorph; $[\alpha]^{24}_{\underline{D}}$ - 18° (c 0.18 MeOH); λ_{max} (95% EtOH) 239 mu (ε 5490), 288 mu (ε 1380), 335 mu (ε 1050); ir ($CHCl_3$) [Fig. 24]; nmr ($CDCl_3$) [Fig. 24] δ 3.48 and 4.06 ppm (2s, 3, $-OCH_3$), δ 5.97 ppm (s, 2, methylenedioxy), δ 6.48 ppm (s, 1, aromatic proton), δ 8.07 ppm (s, 1, benzylic proton).

Anal. Calcd. for $C_{18}H_{22}N_2O_6$: C, 59.66; H, 6.12: N, 7.73. Found: C, 59.75; H, 5.98; N, 7.77.

With dimethyl sulfoxide as the solvent To a solution of 100 mg of 6-hydroxyundulatine in 6 ml of dimethyl sulfoxide was added 50 mg of hydroxylamine hydrochloride. The solution was heated on a steam bath for 6 hr. The cooled solution was diluted with water, made basic with ammonium hydroxide, and extracted with chloroform. The chloroform extracts were evaporated under reduced pressure. The residual dimethyl

sulfoxide was removed by chromatography on silica gel plates. Removal of the major band (R_f 0.5) allowed the recovery of 78 mg of 6-hydroxyundulatine oxime. Although the material remained amorphous, it was pure by tlc criteria and proved identical to 84 prepared above.

Cotarnine (35)

A solution of 150 mg of cotarnine hydrochloride in 5 ml of water was made basic with 5% sodium hydroxide. The precipitate was filtered and recrystallized from acetone to give 126 mg of cotarnine: mp 125-127° (lit[62], mp 132-133°); $[\alpha]^{24}$ + 2° (c 0.22 MeOH), λ_{max} (95% EtOH) 228 mu (ε 6400), 252 mu (ε 2500), 287 mu (ε 1700); ir ($CDCl_3$) [Fig. 25]; nmr ($CDCl_3$) [Fig. 26] δ 2.58 (s, 3, N-CH_3), δ 4.04 ppm (s, 3, -OCH_3), δ 5.40 ppm (s, 1, benzylic proton), δ 5.85 ppm (s, 2, methylenedioxy), δ 6.31 ppm (s, 1, aromatic proton).

Cotarnine oxime (92)

Under the same conditions cited for the preparation of the 6-hydroxybuphanidrine hydroxylamine adduct (82), 70 mg of cotarnine was converted to 36 mg of cotarnine oxime: mp 149-151°, λ_{max} (95% EtOH) 251 mu (ε 6850), 278 mu (ε 1990), 344 mu (ε 1430); ir ($CHCl_3$) [Fig. 28]; nmr ($DMSO_3$) [Fig. 28] δ 2.55 ppm (s, 3, N-CH_3), δ 3.90 ppm (s, 3, aromatic methoxyl), δ 6.00 ppm (s, 2, methylenedioxy), δ 6.58 ppm (s, 1, aromatic

proton), δ 8.20 ppm (s, 1, benzylic proton).

Anal. Calcd. for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.11. Found: C, 57.33; H, 6.25; N, 11.24.

3,6-O,O-Diacetylpowelline (58)

By the procedure cited for the preparation of 6-O-acetylbuphanidrine (45), 100 mg of 58 was converted to 88 mg of crude diacetate. The product was purified by thin-layer chromatography and gave 72 mg of 3,6-O,O-diacetylpowelline after crystallization from acetone: mp 114-117°; $[\alpha]^{24}$ D -26° (c 0.20 MeOH); λ_{max} (95% EtOH) 241 mu (ε 9020), 285 mu (ε 1900); ir($CHCl_3$) [Fig. 12] 1738 cm^{-1} (C = O), 1625 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) [Fig. 13] δ 2.00 and 2.10 ppm (2s, 3, CH_3C = O), δ 3.97 ppm (s, 1, aromatic methoxyl), δ 5.35 ppm (C_3 methine), δ 5.90 ppm (q, 2, methylenedioxy), δ 6.27 ppm (s, 1, benzylic proton), δ 6.60 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{21}H_{23}NO_7$: C, 62.83; H, 5.78; N, 3.49. Found: C, 63.02; H, 5.75; N, 3.36.

3-O-Acetyl-6-hydroxypowelline (59)

A solution of 50 mg of 59 in 5 ml of dioxane-water (50:50) was allowed to stand at room temperature for 24 hr. The solution was evaporated under reduced pressure, and the crude

product was chromatographed on a silica gel plate developed in chloroform-methanol-diethylamine (90:5:5). The major band (R_f 0.6) was recovered as an amorphous material (38 mg). $[\alpha]^{24}$ D - 18° (c 0.25, MeOH), λ_{max} (95% EtOH) 237 mu (ε 7050), 287 mu (ε 1400); ir ($CHCl_3$) [Fig. 12] 1730 cm^{-1} (C = 0); nmr ($CDCl_3$) [Fig. 13] δ 2.03 ppm (s, 3, CH_3C = 0), δ 4.04 ppm (s, 3, $-OCH_3$), δ 5.28 ppm (s, 1, benzylic proton), δ 5.30 (C_3 methine), δ 5.90 ppm (2, methylenedioxy), δ 6.59 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.66; H, 6.01; N, 4.02.

Powelline (60)

A solution of 35 mg of 3-O-acetyl-6-hydroxypowelline in 5 ml of thionyl chloride was refluxed for 2 hr on a steam bath and then evaporated to dryness. The residue was combined with 8 ml of dry tetrahydrofuran and 85 mg of lithium aluminum hydride. The solution was refluxed for 12 hr. The cooled solution was hydrolyzed with ethyl acetate followed by water and 25% sodium hydroxide. The tetrahydrofuran solution was filtered and the filter cake was washed repeatedly with chloroform. The combined chloroform and tetrahydrofuran extracts were evaporated to dryness. The residue was chromatographed

on silica gel plates. Removal of the major band (R_f 0.5) gave 22 mg of material which crystallized from acetone: mp 199-201° (lit.[11] mp 200-201°). The compound showed ir and nmr spectra [Fig. 14] as well as chromatographic characteristics identical with those of powelline.

N-Nitroso-6-hydroxypowelline (57)

To a solution of 47 mg of 6-hydroxypowelline in 6 ml of 1.5% aqueous acetic acid was added 50 mg of sodium nitrite. The reaction mixture was allowed to stand at room temperature for 10 hr. N-Nitroso-6-hydroxypowelline crystallized from the solution as fine needles. The product (28 mg) was filtered from the solution and recrystallized from acetone-ether: mp 156-159°; $[\alpha]^{24}$ D - 14° (c 0.17, MeOH); λ_{max} (95% EtOH) 238 mu (ε 6040), 289 mu (ε 1680), 321 mu (ε 1720); ir ($CHCl_3$) [Fig. 10] 1690 cm^{-1} (C = O), 1615 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) [Fig. 11] δ 4.12 ppm (s, 3, $-OCH_3$), δ 6.08 ppm (s, 2, methylenedioxy), δ 6.07 ppm (s, 2, olefinic protons), δ 6.85 ppm (s, 1, aromatic proton), δ 10.4 ppm (s, 1, CHO).

Anal. Calcd. for $C_{17}H_{18}N_2O_6$: C, 58.95; H, 5.24; N, 8.09. Found: C, 59.12; H, 5.08; N, 7.92.

3,11-O,O-Diacetylpowelline (95)

Under the standard acetylation conditions cited for the preparation of 6-O-acetylbuphanidrine (45), 78 mg of 11-

hydroxypowelline was converted to 73 mg of crude diacetate. Although thin-layer chromatography indicated only one compound was present, it remained amorphous: $[\alpha]^{24}$ D - 57° (c 0.22 MeOH); λ_{max} (95% EtOH) 210 mu (ε 32,700), 290 mu (ε 1340); ir($CHCl_3$) [Fig. 29] 1733 cm^{-1} (C = 0), 1620 cm^{-1} (aromatic methoxyl), 940 and 1050 cm^{-1} (aromatic methylene dioxy); nmr ($CDCl_3$) [Fig. 30] δ 1.85 and 1.97 ppm (2s, 3, CH_3C = 0), δ 4.00 ppm (s, 3, aromatic methoxyl), δ 5.15 ppm (C_3 methine), δ 5.40 ppm (C_{11} methine); δ 5.90 ppm (q, 2, methylenedioxy), δ 6.49 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{21}H_{23}NO_7$: C, 62.83; H, 5.78; N, 3.49. Found: C, 62.69; H, 5.79; N, 3.68.

3-O-Acetyl-11-hydroxypowelline (100) and 11-O-acetylpowelline (101)

To a solution of 135 mg of 3,11-O,O-diacetylpowelline in 5 ml methanol was added 0.2 ml of 0.01 N sodium methoxide in methanol. The reaction was followed by thin-layer chromatography which showed the formation of the two monoacetyl derivatives. Two drops of 0.01 acetic acid in methanol was added to quench the reaction when the mixture contained approximately 50% of the two monoacetyl compounds. The solution was evaporated under reduced pressure to give a residue which was dissolved in 5 ml of 1% hydrochloric acid. The solution was

made basic (pH 8) with ammonium hydroxide and extracted with chloroform. The chloroform extracts were evaporated under reduced pressure to give 128 mg of an oil. Thin-layer chromatography of this mixture on a silica gel plate (0.5 mm) developed in chloroform-methanol-diethylamine (90:5:5) provided 62 mg of 3,11-0,0-diacetylpowelline (R_f 0.9), 32 mg of 11-0-acetylpowelline (R_f 0.5), and 25 mg of 3-0-acetyl-11-hydroxypowelline (R_f 0.45). Crystallization of 3-0-acetyl-11-hydroxypowelline from acetone gave fine needles: mp 180-182°; $[\alpha]^{24}\underline{D}$ - 87° (c 0.18, MeOH); λ_{max} (95% EtOH) 239 mu (ε 10,500) 292 mu (ε 1540); ir($CHCl_3$) 1724 cm^{-1} (C = 0), 1618 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) δ 1.88 ppm (s, 3, CH_3C = 0), δ 3.99 ppm (s, 3, aromatic methoxyl), δ 5.20 ppm (C_3 methine), δ 5.88 ppm (s, 2, methylenedioxy), δ 6.50 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.50; H, 5.95; N, 3.85.

Crystallization of 11-0-acetylpowelline afforded long needles from acetone: mp 178-179°; $[\alpha]^{24}\underline{D}$ - 94° (c 0.24 MeOH); λ_{max} (95% EtOH) 239 mu (ε 9840), 291 mu (ε 1380); ir ($CHCl_3$) 1730 cm^{-1} (C = 0); nmr ($CDCl_3$) 1.95 ppm (s, 3, CH_3C = 0), δ 4.01 ppm (s, 3, aromatic methoxyl), δ 5.40 ppm

(C_{11} methine), δ 5.88 ppm (q, 2, methylenedioxy), δ 6.50 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.44; H, 5.99; N, 3.94.

3-O-Acetyl-11-oxopowelline (102)

A solution of 26 mg of 3-O-acetyl-11-hydroxypowelline in 1 ml of dimethyl sulfoxide and 1 ml of acetic anhydride was allowed to stand at room temperature for 24 hr. The mixture was diluted with water and made basic (pH 8) with ammonium hydroxide. The basic solution was extracted with chloroform and the extracts evaporated under reduced pressure. The residual dimethyl sulfoxide was removed by thin-layer chromatography. Recovery of the material gave 18 mg of crude product. Recrystallization from acetone afforded the pure product: mp 115-116°; $[\alpha]^{24}$ - 46° (c 0.25 MeOH); λ_{max} (95% EtOH) 235 mu (ε 11,500); 290 mu (ε 3900), 315 mu (ε 2450), 325 (ε 2140); ir ($CHCl_3$) 1745 and 1722 cm^{-1} (C = O); nmr ($CDCl_3$) δ 1.90 ppm (s, 3, CH_3C = O), δ 4.00 ppm (s, 3, aromatic methoxyl), δ 5.20 ppm (C_3 methine), δ 5.88 ppm (s, 2, methylenedioxy), δ 6.50 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{19}H_{19}NO_6$: C, 63.86; H, 5.36; N, 3.92. Found: C, 63.51; H, 5.49; N, 4.09.

3-Oxo-11-O-acetylpowelline (103)

A solution of 28 mg of 11-O-acetylpowelline in 3 ml of anhydrous chloroform was stirred with 100 mg of activated manganese dioxide for 6 hr. The manganese dioxide was removed by filtration and the solvent was evaporated under reduced pressure to give 21 mg of crude product. Recrystallization from acetone-ether gave colorless prisms: mp 123-124°; $[\alpha]^{24}\underline{D}$ - 64° (c 0.24, MeOH); λ_{max} (95% EtOH) 242 mu (ε 12,430), 294 mu (ε 4500), 315 mu (ε 950); ir ($CHCl_3$) 1728 and 1670 cm^{-1} (C = O); nmr ($CDCl_3$) δ 1.95 ppm (s, 3, CH_3C = O) δ 3.98 ppm (s, 3, aromatic methoxyl), δ 5.38 ppm (C_{11} methine), δ 5.90 ppm (q, 2, methylenedioxy), δ 6.50 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{19}H_{19}NO_6$: C, 63.86; H, 5.36; N, 3.92. Found: C, 64.11; H, 5.55; N, 4.11.

Hydrolysis of ambelline (104, R = H)

A solution of 100 mg of ambelline in 5 ml of 10% hydrobromic acid was refluxed for 8 hr. The solution was allowed to cool, made basic with concentrated ammonium hydroxide (pH 10), and extracted four times with chloroform. Evaporation of the chloroform gave 93 mg of residue which was chromatographed on silica gel plates. Removal of the alkaloidal

bands allowed the recovery of 63 mg of ambelline (R_f 0.5) and 21 mg of 11-hydroxypowelline (R_f 0.2). The compound crystallized from acetone (mp 208-210°) and proved identical to natural 94 by comparison of spectral and chromatographic data.

3-Methoxybowdensine (110)

By the procedure cited for the preparation of 6-0-acetylbuphanidrine (45), 85 mg of 110 was converted to 72 mg of crude diacetate. The product resisted all attempts at crystallization. The sublimed glass showed: $[\alpha]^{24}$ D + 11° (c 0.25 MeOH); λ_{max} (95% EtOH) 240 mu (ε 7640), 284 mu (ε 2100); ir ($CHCl_3$) 1745 cm^{-1} (C = 0), 1617 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) δ 1.95 and 2.10 ppm (2s, 3, CH_3C = 0), δ 3.48 and 3.97 ppm (2s, 3, $-OCH_3$); δ 5.84 ppm (q, 2, methylenedioxy), δ 6.10 ppm (s, 1, aromatic proton).

Anal. Calcd. for $C_{22}H_{27}NO_8$: C, 60.96; H, 6.28; N, 3.23. Found: C, 61.00; H, 6.42; N, 3.26.

Hydrolysis of undulatine (26)

A solution of 100 mg of undulatine in 5 ml of 10% sulfuric acid was refluxed for 3 hr. The cooled solution was made basic (pH 10) with ammonium hydroxide. Fine needles precipitated (67 mg) and were removed by filtration. The product was recrystallized from chloroform (mp 280-282°).

The infrared spectrum, nmr spectrum, and chromatographic data were identical to that of 3-methoxy-O,O-deacetylbowdensine.

LITERATURE CITED

1. J. A. Mills, J. Chem. Soc., 4976 (1952).

2. G. G. DeAngelis, Dichroism and Dispersion Studies of the Optically Active Aromatic Chromophore, unpublished Ph.D. thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1966.

3. G. G. DeAngelis and W. C. Wildman, Tetrahedron Letters, 5099 (1969).

4. M. Shiro, T. Sato, and H. Koyama, Chem. and Ind., 1415 (1956).

5. D. J. Williams and D. Rogers, Proc. Chem. Soc., 357 (1964).

6. J. C. Clardy, W. C. Wildman, and F. M. Hauser, J. Amer. Chem. Soc., 92, 1781 (1970).

7. J. Karle, J. A. Estlin, and I. L. Karle, J. Amer. Chem. Soc., 89, 6510 (1967).

8. J. C. Clardy, W. C. Wildman, and F. M. Hauser, 6-Hydroxybuphanidrine, unpublished manuscript, W. C. Wildman, Iowa State University of Science and Technology, Ames, Iowa, 1970.

9. L. H. Mason, E. R. Puschett, and W. C. Wildman, J. Amer. Chem. Soc., 77, 1253 (1955).

10. W. C. Wildman, J. Amer. Chem. Soc., 78, 4180 (1956).

11. W. C. Wildman, J. Amer. Chem. Soc., 80, 2567 (1958).

12. H. -G. Boit and H. Ehmke, Chem. Ber., 89, 2093 (1956)

13. H. -G. Boit and H. Ehmke, Chem. Ber., 88, 1590 (1955).

14. W. C. Wildman, Chem. and Ind., 1090 (1956).

15. H. M. Fales and W. C. Wildman, J. Amer. Chem. Soc., 80, 4395 (1958).

16. H. A. Lloyd, E. A. Kielar, R. J. Highet, S. Uyeo, H. M. Fales, and W. C. Wildman, Tetrahedron Letters, 105 (1961).

17. H. A. Lloyd, E. A. Kielar, R. J. Highet, S. Uyeo, H. M. Fales, and W. C. Wildman, J. Org. Chem., 27, 373 (1962).

18. H. M. Fales and W. C. Wildman, J. Amer. Chem. Soc., 82, 3368 (1960).

19. S. Uyeo, H. M. Fales, R. J. Highet, and W. C. Wildman, J. Amer. Chem. Soc., 80, 2590 (1958).

20. H. M. Fales and W. C. Wildman, Chem. and Ind., 561 (1958).

21. H. -G Boit and W. Stender, Chem. Ber., 89, 161 (1956).

22. W. C. Wildman, Chem. and Ind., 123 (1956).

23. T. Ikeda, W. I. Taylor, Y. Tsuda, S. Uyeo, and H. Yajima, J. Chem. Soc., 4749 (1956).

24. H. Irie, Y. Tsuda, and S. Uyeo, J. Chem. Soc., 1446 (1959).

25. S. Uyeo, H. Irie, U. Kitayama, T. Hirose, and A. Yoshitake, Chem. Pharm. Bull. (Tokyo), 12, 489 (1964).

26. C. F. Murphy, The Chemistry of 6-Hydroxycrinamine and Related Compounds, unpublished Ph.D. thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1966.

27. R. W. King, C. F. Murphy, and W. C. Wildman, J. Amer. Chem. Soc., 87, 4912 (1965).

28. P. Naegelli, E. W. Warnhoff, H. M. Fales, R. E. Lyle, and W. C. Wildman, J. Org. Chem., 28, 206 (1963).

29. H. -G. Boit, Chem. Ber., 89, 1129 (1956).

30. H. -G. Boit, Chem. Ber., 90, 369 (1957).

31. E. W. Warnhoff and W. C. Wildman, J. Amer. Chem. Soc., 82, 1472 (1960).

32. F. Wohler, Ann. Chem., 50, 19 (1844).

33. A. Hantzsch, Chem. Ber., 32, 575 (1899).

34. A. Claus and P. Himmelmann, Chem. Ber., 13, 2045 (1880).

35. A. H. Salway, J. Chem. Soc., 97, 1208 (1910).

36. M. Freund and F. Becker, Chem. Ber., 36, 1521 (1903).

37. W. Schneider and B. Mueller, Ann. Chem., 615, 34 (1958).

38. W. Roser, Ann. Chem., 249, 156 (1888).

39. H. Decker, J. Prakt. Chem., 47, 28 (1893).

40. J. Gadamer, J. Prakt. Chem., 84, 817 (1911).

41. D. Beke, D. Korbonits, and R. Kornis-Markovits, Ann. Chem., 626, 225 (1959).

42. D. Beke, Heterocyclic Pseudo Bases, in A. R. Katritzky, Ed., Advances in Heterocyclic Chemistry, Vol. 1, p. 167, Academic Press, London, 1963.

43. W. Roser, Ann. Chem., 254, 334 (1889).

44. D. Beke and M. Barczai-Martos, Acta Chim. Acad. Sci. Hung., 11, 295 (1957). Original not available; abstracted in Chem. Abst., 52, 2874f (1958).

45. H. -G. Boit and H. Ehmke, Chem. Ber., 89, 2093 (1956).

46. R. E. Lyle, E. A. Kielar, J. R. Crowder, H. M. Fales, and W. C. Wildman, J. Amer. Chem. Soc., 82, 2620 (1960).

47. W. C. Wildman and C. J. Kaufman, J. Amer. Chem. Soc., 77, 4807 (1955).

48. W. C. Wildman, The Amaryllidaceae Alkaloids, in F. H. F. Manske, Ed., The Alkaloids, Vol. 11, p. 372, Academic Press, Inc., New York, New York, 1968.

49. A. L. Burlingame, H. M. Fales, and R. J. Highet, J. Amer. Chem. Soc., 86, 4976 (1964).

50. H. M. Fales and W. C. Wildman, J. Amer. Chem. Soc., 85, 784 (1963).

51. E. S. Gould, Mechanism and Structure in Organic Chemistry, Holt, Rinehart, and Winston, Inc., New York, New York, 1964, pp. 239-246.

52. F. S. Fawcett, Chem. Rev., 47, 219 (1950).

53. M. Caserio, J. D. Roberts, M. Neeman, and W. S. Johnson, J. Amer. Chem. Soc., 80, 2584 (1958).

54. H. -G. Boit and W. Dopke, Naturwiss., 47, 109 (1960).

55. E. S. Gould, Mechanism and Structure in Organic Chemistry, Holt, Rinehart, and Winston, Inc., New York, New York, 1964, pp. 294-296.

56. H. O. House, Modern Synthetic Reactions, W. A. Benjamin, Inc., New York, New York, 1965, pp. 34-35.

57. H. A. Staab and H. Braunling, Ann. Chem., 654, 119 (1962).

58. L. A. Paquette, Principles of Modern Heterocyclic Chemistry, W. A. Benjamin, Inc., New York, New York, 1968, pp. 183-190.

59. J. J. Dobbie, A. Lauder, and C. Tinkler, J. Chem. Soc., 83, 598 (1903).

60. W. Schneider and B. Muller, Ann. Chem., 615, 34 (1958).

61. F. Krohnke and I. Vogt, Chem. Ber., 90, 2227 (1957).

62. D. Beke and K. Harsanyi, Zhur. Obshchei Kim., 27, 2108 (1957). Original not available; abstracted in Chem. Abst., 52, 5437 (1958).

63. J. D. Albright and L. Goldman, J. Amer. Chem. Soc., 89, 2416 (1967).

64. A. Furst and P. A. Plattner, Intern. Congress Pure and Applied Chem. Abstract of Papers, 12, 409 (1951).

65. E. L. Eliel in M. Newman, Ed., Steric Effects in Organic Chemistry, pp. 130-134, John Wiley and Sons, Inc., New York, New York, 1956.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Dr. W. C. Wildman for his guidance, patience, and encouragement during the course of this investigation.

Acknowledgement is due the members of Dr. Wildman's research group for many hours of rewarding discussion and for the many suggestions that were so valuable in the course of this research.

The author thanks Verna Thompson for her meticulous typing of this manuscript.

The author wishes to express his deep gratitude to his family for their encouragement throughout the course of his education. Particular thanks are expressed to his wife, Jan, for her endless understanding and inspiration during graduate school.