# STRUCTURAL STUDIES ON NUPHAR

ALKALOIDS

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By

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A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

September 1974

DOCTOR OF PHILOSOPHY (1974)　　　　　　　　　　　　McMASTER UNIVERSITY
(Chemistry)　　　　　　　　　　　　　　　　　　　　　Hamilton, Ontario.

TITLE:　　　　　　　Structural Studies on <u>Nuphar</u> Alkaloids.

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NUMBER OF PAGES:　　(x), 180.

SCOPE AND CONTENTS:

　　　　The mass spectra of several quinolizidine alkaloids of known
structure, isolated from species of <u>Nuphar luteum</u>, have been studied.
High resolution mass measurements have been used to characterise
fragmentation patterns capable of differentiating between substituents
in rings A and B of the quinolizidine system.

　　　　The stereoisomeric relationship between the thiospiran <u>Nuphar</u>
alkaloids, thiobinupharidine and neothiobinupharidine, has been examined.
A three-dimensional structure has been proposed for thiobinupharidine on
the basis of its spectroscopic properties. An X-ray study has confirmed
this structure and also established the absolute configuration of
thiobinupharidine.

　　　　The structure of three new alkaloids, nupharolutine, thionuphar-
oline, and neothiobinupharidine sulphoxide, have been established on the
basis of mass spectral and p.m.r. data.

The steric course of sodium borodeuteride reduction of hemiaminals of thiobinupharidine in absolute ethanol solution has been investigated. Significant differences have been observed for both the degree of deuterium incorporation and for the stereoselectivity of deuterium incorporation when compared to those results reported for analogous reductions in methanol solution. The transient intermediacy of an alkoxyborodeuteride ion has-been proposed to explain these results.

TO JEN AND "LITTLE JEGS"

ACKNOWLEDGEMENTS

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GENERAL INTRODUCTION

The organic chemist has long found the study of alkaloids challenging, partly because of the many structural and synthetic problems they present, and partly because many of them have been used from ancient times as beneficial drugs or poisons. Seldom has such a multitude of structural types of diverse biological origin been classed under one name.

The term alkaloid or "alkali-like" was first proposed by the pharmacist W. Meissner in 1819. Alkaloids usually have a basic nitrogen atom, as part of one or more heterocyclic ring systems. They often have complex molecular structures and sometimes display significant pharmacological activity. A particular alkaloid is usually restricted to certain families and genera of the plant kingdom.

Disagreement exists regarding the classification of various compounds as alkaloids. Thus Swan has confined his definition of alkaloids to nitrogenous bases of plant origin which are related biosynthetically to amino acids[1]. He therefore excludes heterocyclic nitrogenous compounds based on sesqui- and diterpenes and classes these as "pseudo alkaloids". Other authorities have designated some widely, distributed bases of plant origin, such as methyl, trimethyl and other open-chain simple alkylamines, the cholines and betaines, as "biological amines" or proto alkaloids; the distinction between these and alkaloids is rather arbitrary[2].

1

In this thesis the nitrogenous bases isolated from <u>Nuphar</u> species will be termed alkaloids, even though they are apparently terpenoid in origin[3].

Approximately two thousand alkaloids are now known. The nomenclature of alkaloids has not been systematized because of their complexity and for historical reasons. The two commonly used systems subclassify alkaloids according to the plant genera in which they occur or on the basis of similarity of molecular structure.

Two highly toxic members of the alkaloid family illustrate the variation in complexity typical of these compounds. Coniine, an alkaloid isolated from the poison hemlock, is simply (+)-2-n-propylpiperidine. However strychnine with a relatively simple molecular formula ($C_{21}H_{22}N_2O_2$) has an intricate system of seven fused rings.
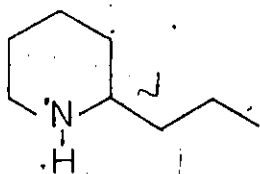
coniine
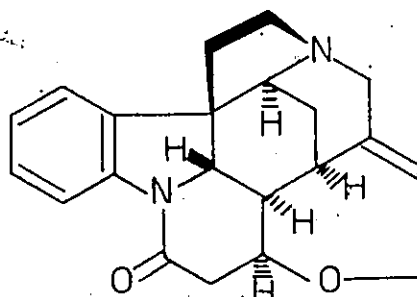
strychnine

Although many alkaloids are toxic to the animal organism, many are useful and show a wide variety of physiological actions. Illustrative of this phenomenon are the uses of atropine in ophthalmology for dilating the pupil and paralysing the muscles of accommodation, and of quinine as an effective drug for the prevention and treatment of malaria.

Morphine has found wide application as an analgesic, and the drug LSD, which is a synthetic derivative of the naturally occurring ergot alkaloids, has been found to cause mental aberrations, similar to schizophrenia, and has been used to study that disease.
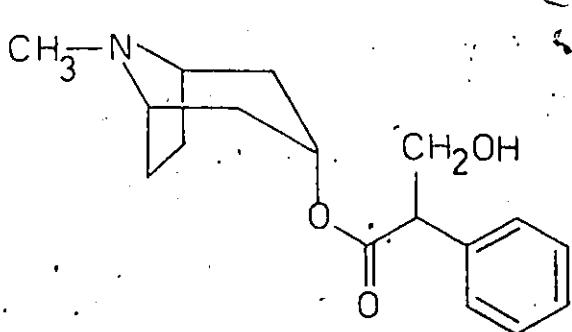
atropine
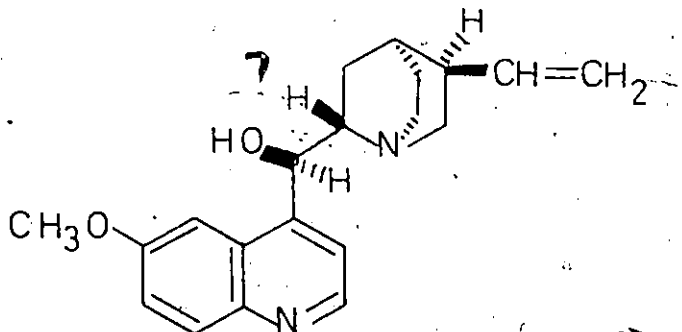
quinine

morphine

LSD
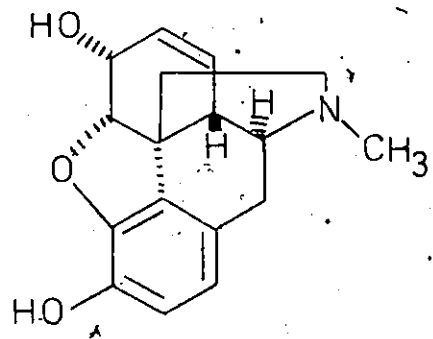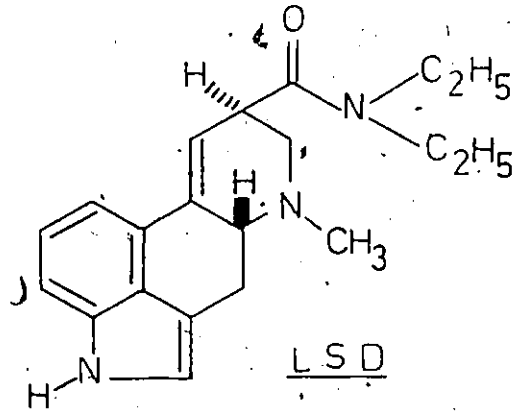
lysergic acid diethylamide

General methods for isolation of alkaloids

Alkaloids are found in various parts of plants, and often occur as salts of inorganic or organic acids. Generally they are separated from the powdered plant material by aqueous or alcoholic extraction. If they occur as the free bases dilute acid extraction may be performed. Alternatively the plant material may be subjected to alkaline treatment

followed by extraction of the free bases with organic solvents. A

crude mixture of alkaloids can often be purified by fractional crystal-

lization of sparingly soluble salts such as hydrochlorides, perchlorates,

etc. Adsorption or partition chromatography may be used to separate

mixtures, as can counter current distribution between an aqueous acid

buffer and an organic phase. The alkaloids whose structures were

elucidated in this present study were isolated from rhizomes of Nuphar

luteum, and its subspecies variegatum, native to Poland. The isolations

were achieved by the group of Professor J.T. Wróbel of the University

of Warsaw and were received from him as reasonably pure bases or salts

of inorganic acids.

General methods for elucidation of molecular structure

This thesis relies heavily on the use of spectroscopic methods,

mainly high resolution mass spectrometry (h.r.m.s.) and high resolution

proton magnetic resonance spectrometry (p.m.r.). It is worthwhile to

make passing reference to some of the older, possibly more tedious, but

not necessarily outdated methods which have been used in the past.

Excellent reviews are available which summarize the chemical methods

for determination of functionality of oxygen atoms, the Kuhn-Roth

procedure for estimation of C-Methyl groups, and methods available for

ascertaining the environment about the nitrogen atom such as the Hofmann,

Emde and von Braun degradative procedures[4].

It is not appropriate that this thesis contain lengthy reviews

concerning the theory of h.r.m.s. and p.m.r. or their application to

structural problems as both techniques are well known and widely accepted. Their particular attributes are manifested in the wealth of literature illustrating the use of these techniques which has appeared over the last decade. It is considered appropriate however to consider the advantages of both techniques which became apparent as our particular studies developed.

High resolution proton magnetic resonance spectrometry (p.m.r.)

This technique has found wide applicability in the elucidation of molecular structures. A short discussion will follow concerning the advantages of employing the 220 MHz p.m.r. spectrometer rather than the more generally used 100 MHz spectrometer. Detailed information about molecular structure can be obtained from a high resolution p.m.r. spectrum, providing it can be analysed in terms of a set of chemical shifts and spin-spin couplings. These chemical shifts arise from differences in the magnetic field strength at different protons in the molecule. They result from variations in the shielding produced by the interactions of the surrounding electrons with the magnetic field. The effect is therefore dependent in magnitude on the applied magnetic field. The spin-spin coupling results from interaction of the magnetic moments of the protons with one another through the intervening electrons and is independent of the magnetic field strength.

The critical parameter determining the appearance of a p.m.r. spectrum is the ratio of the chemical shift differences between the interacting nuclei to the coupling constants between them. The chemical

shift difference between any two nuclei say A and B is usually expressed as $\Delta\nu_{AB}$, in Hertz units, and the ratio referred to above as $\Delta\nu_{AB}/J_{AB}$. If $\Delta\nu/J$ is large (i.e. greater than 10), the resulting spectra consist of well separated symmetrical multiplets (called first order spectra)[5] which lend themselves to facile interpretation. When $\Delta\nu/J$ is small however, as in cases where rather subtle effects of geometric structure or conformation are operative, shifts in the positions of lines and great changes in relative intensities can occur. These spectra are referred to as second order spectra[5], and arise because the coupling represents a perturbation which mixes the energy levels belonging to chemically non-equivalent nuclei. Clearly if $\Delta\nu/J$ can be increased, the analysis of the spectrum may be facilitated. By using a 220 MHz spectrometer, rather than a 100 MHz spectrometer, $\Delta\nu/J$ may be increased by a factor of 2.2.

Accidental near degeneracy is manifested in considerable overlapping of lines or multiplets, resulting in great difficulty in interpretation. The severity of the problem of crowding of many lines into a small region of the spectrum is diminished in proportion to the field strength. High field strengths are also advantageous in giving rise to stronger absorption signals since the overpopulation of the lower spin state, due to the Boltzmann distribution, depends upon H_0. The strength of the absorption signal relative to the irreducible background of radiofrequency noise (signal to noise ratio) varies with approximately the one and one half power of the field strength. Excellent theoretical accounts of this phenomenom have been rendered[6,7].

Consequently an increase in field strength can mean a reduction in the amount of sample required. This was a valuable criterion in these studies. The development of very stable high field strengths is achieved using electromagnets consisting of super conducting solenoids operating in liquid helium cryostats.

The predicted increase in S/N ratio in going from 100 MHz to 220 MHz (a field strength of 23,487 gauss to 51,671 gauss) is approximately 3.25; however as the radiofrequency is increased a corresponding increase in the noise produced in the first r.f. amplifier stage in the detection circuitry occurs. Thus experimentally the improvement in S/N ratio is not as marked as predicted, but is however quite significant.

Mass spectrometry in natural products chemistry

In the field of natural products, mass spectrometry is generally employed for solving one or more of the following problems: determination of molecular weight and of molecular formula, detection of functional groups or substituents, and determination of gross skeletal structure. Sometimes it is possible to elucidate a structure and to obtain stereochemical information. For all these purposes, volatilisation of the substance (or the chemically modified substance) into the electron beam must be achieved. In natural product chemistry the determination of the exact molecular weight is requisite and often difficult by other methods. The detection of differences of $\pm CH_2$ in molecular composition of complex molecules is often impossible by combustion analysis.

The last decade has seen significant progress in the theory and technology of mass spectrometry, and many books have appeared on the interpretation of the mass spectra of complex molecules[8]. Excellent discussions on instrumentation, on techniques of sample handling, and on data acquisition and processing by computer have appeared recently[9].

Advantages of high resolution mass spectrometry (h.r.m.s.)

H.r.m.s. received a great stimulus with the advent of double-focussing spectrometers, which permitted the separation of ions differing in mass by only a few millimass units[10]. In low resolution mass spectrometry only ions of nominal masses are separable; however in h.r.m.s. species of different elemental composition can be resolved even though they have the same nominal mass. Beynon was the first to recognise the potential of accurate mass measurement to determine the molecular formulae of organic compounds[11] and much of the early work is discussed in his book[12].

A knowledge of the elemental composition of the molecular ion and of fragment ions unquestionably aids the interpretation of the mass spectrum. A combustion analysis for C, H, N, and S may require up to 20 mg of pure substance. A h.r.m.s. determination of the molecular formula may be obtained on less than 1 mg of substance, providing it is reasonably pure and exhibits a molecular ion; furthermore, using the photoplate process, or recently developed electronic techniques, it is possible to determine the compositions of all fragment ions using the same sample. Paucity of samples made it desirable to use h.r.m.s. analyses rather than the destructive combustion analyses for many of the

Nuphar alkaloids examined in this study.

It was not possible to incorporate stable isotopes as labels into many of the compounds under investigation in this thesis. In h.r.m.s. however compounds which contain heteroatoms may be thought of as possessing internal labels which greatly aid the interpretation of the spectra. Obviously h.r.m.s. does not represent the ultimate technique, but when used in conjunction with other spectroscopic methods, and when conventional labelling studies are performed where possible, then it becomes a powerful and economical tool for the organic chemist. Further details of the high resolution method used in the studies pertaining to this thesis may be found in the experimental section.

The symbols and abbreviations used in description and discussion of the mass spectra in this thesis conform to those recommended by the editorial board of Organic Mass Spectrometry[13].

Format for structural diagrams

The arabic numerals assigned to the compounds in this thesis conform to the following format. One number is reserved for each individual named compound throughout. Where structural revisions have been made over the years, or where several possible structures have been suggested for the same compound, that number is followed by a lower case letter, a, b, c, etc.

Where structures are represented in two dimensions the conventional format is used, thus the plane of a carbocyclic or heterocyclic ring is depicted as the plane of the paper and substituents above and below this

plane are portrayed by straight solid and straight dotted lines, respectively.

Most of the structures are more conveniently represented in three-dimensions. Here rings are drawn in the chair form with the solid "wedged" side of the ring nearest to the reader indicating that the ring is in a plane perpendicular to the paper. The relative orientation of substituents is depicted by solid and dotted "wedges" indicating that a bond is coming towards or going away from the reader, respectively. A straight line represents a bond in the plane of the paper.

It is felt that these three-dimensional representations serve to show the orientation in space of the substituents and possible inter-actions between them in a more satisfactory manner. Furthermore the origins of the various spin-spin couplings observed in the p.m.r. spectra become more obvious when the molecule is depicted in three-dimensions.

HISTORICAL INTRODUCTION

The family Nymphaeacae, which contains approximately one hundred species, encompasses the genus Nuphar. Two of the most common species of this genus are Nuphar japonicum. DC., and Nuphar luteum. Sibth et Sm., which is the yellow water lily and has two subspecies, variegatum and macrophyllum.

Alkaloids containing 15 carbon atoms

Alkaloids were first detected in the rhizomes of these plants in 1879 by Dragendorff[14]. In the light of present day knowledge however, the key discoveries were undoubtedly those of (+)-nupharidine 1a ($C_{15}H_{23}O_2N$) by Arima and Takahashi[15] and (-)-deoxynupharidine 2a ($C_{15}H_{23}ON$) by Kotake[16]. Both alkaloids were first isolated from Nuphar japonicum, but they have since been found to be ubiquitous in Nuphar species. Reduction of nupharidine with sulphur dioxide or hydrogen iodide gives deoxynupharidine, which can be reconverted to the former alkaloid by oxidation with hydrogen peroxide. Hence nupharidine was formulated as an N-oxide[16,17]. The gross skeletal structures of (-)-deoxynupharidine and hence nupharidine were established, predominantly by Japanese workers[18], using classical chemical methods.

A convincing assignment of the relative configuration and the conformation of (-)-deoxynupharidine was made on the basis of synthesis
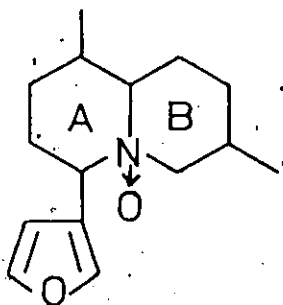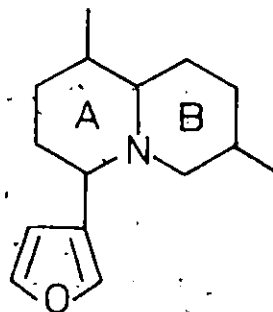
1a.   2a.

and of i.r. studies of model methyl quinolizidines and "deoxynupharidine-like" structures[19,20]. In an early p.m.r. study, Kotake et al.[21] had furnished evidence for an axial methyl group at C-7 and an equatorial methyl group at C-1. A stereospecific assignment of these methyl groups was made possible later[22] by the transformation of nupharamine to deoxynupharidine and its 7-epimer. Since the p.m.r. spectrum of the 7-epimer showed it to have two equatorial methyl groups, then deoxy-nupharidine must have its equatorial methyl group at C-1 and its axial methyl group at C-7[22].

Kotake et al.[21] first proposed the absolute configuration 2b for (-)-deoxynupharidine on the basis that (-)-α-methyladipic acid having the (S)-configuration (and not the (R)-configuration) was obtained upon degradation of deoxynupharidine according to Scheme 1. This configurational assignment was made on the basis of the synthesis of (-)-α-methyl-adipic acid from (-)-α-methyl-γ-butyrolactone, which in turn was correlated with (-)-(S)-methylsuccinic acid[23]*. Turner[24] questioned the assignment of the (S)-configuration to (-)-α-methyladipic acid, since he had

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*This conclusion was uncertain, owing to the very small optical rotation of α-methyladipic acid[23].

2 b.
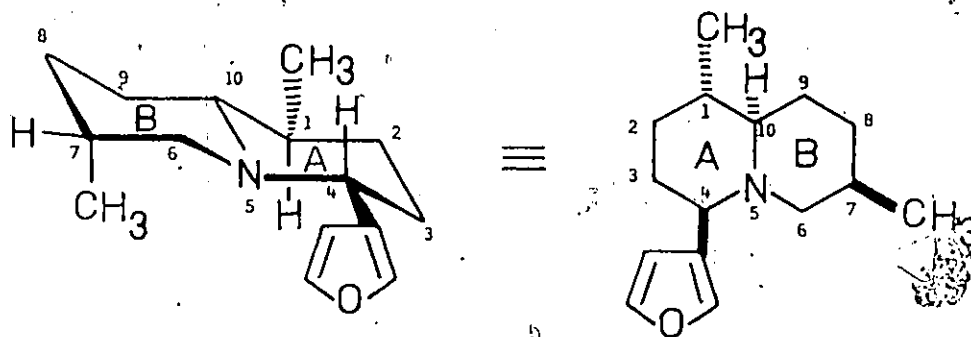
obtained (-)-(R)-α-methylglutaric acid, (+)-(R)-β-methyladipic acid, and
(-)-α-methyladipic acid on oxidative degradation of the cytochalasins A
and B; he therefore suggested that the absolute configuration of (-)-
deoxynupharidine be reversed. Kaneko et al.[23] later isolated (R)-(+)-
methylsuccinic acid from ozonolysis of the aminodiene 3 (Scheme 1), thus
confirming Turner's suggestion.

LaLonde and coworkers[25], however, thought it ambiguous to
assign an absolute configuration to (-)-deoxynupharidine on the basis of
correlation with optically active methylsuccinic acid. He argued that
this optically active acid might arise by ozonolysis of two carbon-carbon
double bonds in 3 (see Scheme 1), thus originating from C-10, C-1, C-2
and C-3. Alternatively, its antipode could arise from ozonolysis of a
double bond and a carbon-nitrogen bond, i.e. the acid would have origin-
ated from C-6, C-7, C-8 and C-9.

He further argued that the origin of (-)-α-methyladipic acid was
unequivocal as it could only arise from C-10, C-1, C-2, C-3, C-4 and the
β-carbon of the furan moiety. Accordingly he synthesized (-)-(R)-α-
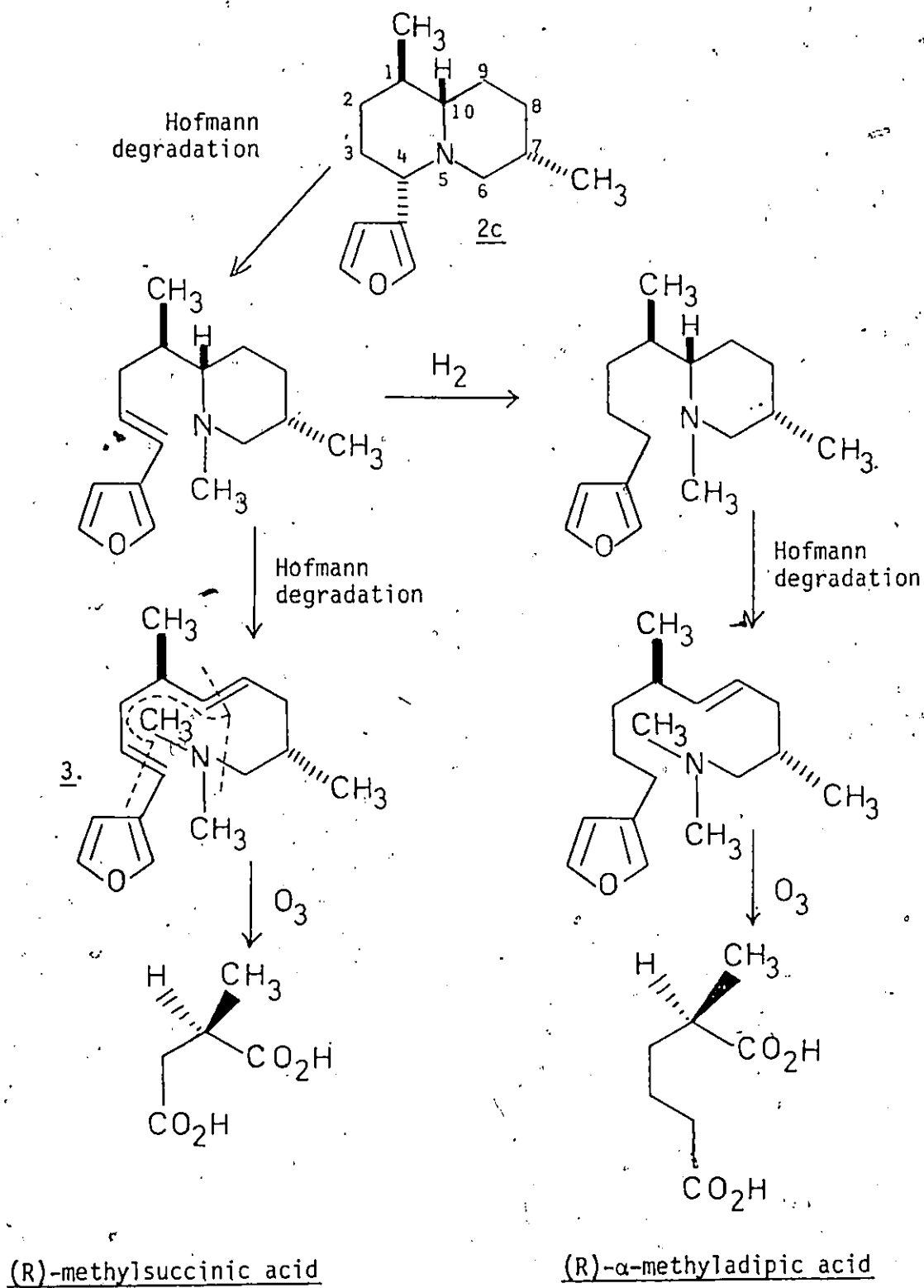
Scheme 1. Chemical degradation of (-)-deoxynupharidine (2c)[23,25]

methyladipic acid from (+)-(R)-3-methylcyclohexanone, and was able to

make a correlation with the (-)-α-methyladipic acid isolated from

degradation of (-)-deoxynupharidine, showing that this had the (R)

configuration and not the (S) as previously reported. Thus the absolute

stereochemistry of (-)-deoxynupharidine is correctly depicted in 2c.
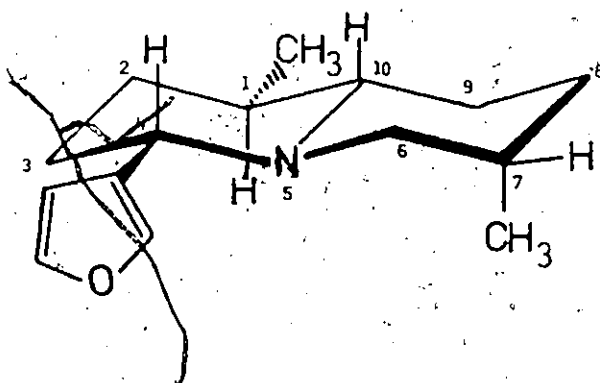
2c. 1R, 4S, 5R, 7S,10S,-(−)-deoxynupharidine.

An independent X-ray study of deoxynupharidine hydrobromide confirmed

this assignment[26]. It was essential that the absolute configuration

of (-)-deoxynupharidine be proven as it had been used to correlate the

absolute configurations of several other Nuphar alkaloids of both the

quinolizidine and piperidine types and in many cases the incorrect

configuration had been assigned. For example, the piperidine alkaloid

nupharamine was first isolated from Nuphar japonicum in 1957 and shown

to have the gross skeletal structure 4a[27,28].

It was later demonstrated that nupharamine could be cyclised in

a number of steps to give a mixture of (-)-deoxynupharidine and its

7-epimer[22]. Consequently, at that time the absolute configuration was
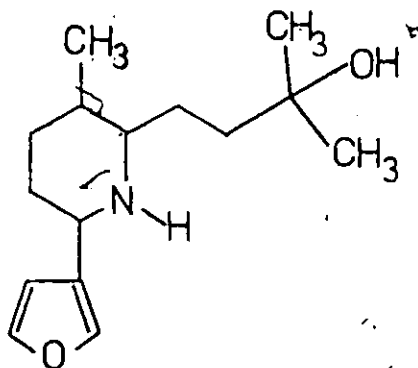
4a.

incorrectly assigned to nupharamine. The correct absolute stereo-
chemistry is that shown as 4b in Figure 1. The structures and absolute
configurations of a number of C_{15} Nuphar alkaloids are now known.
These alkaloids can be subdivided into two structural types, namely the
quinolizidine and the piperidine types. Some representative compounds
are shown in Figures 1 and 2. Examination of the structural features of
the C_{15} alkaloids shows a number of similarities. Notably, all the quino-
lizidine types possess trans-fused rings of the chair form, with the
exception of nupharidine 1b. The latter has been re-examined very
recently by an X-ray study of its hydrobromide, which clearly shows a
cis-fused quinolizidine system with the oxygen of the N-oxide occupying
an equatorial position with respect to ring A[29]. In all quinolizidine
types the 3-furyl group is attached to carbon α to the nitrogen atom and
is always equatorial. The methyl substituents are confined to positions
C-1 and C-7 in accordance with their probable biosynthetic origin[3].

4 b.
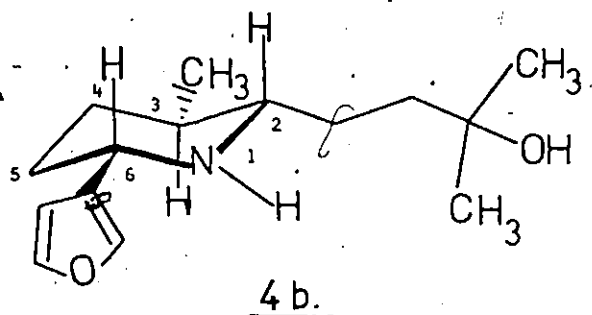
ref. (22,27,28)

nupharamine

5.
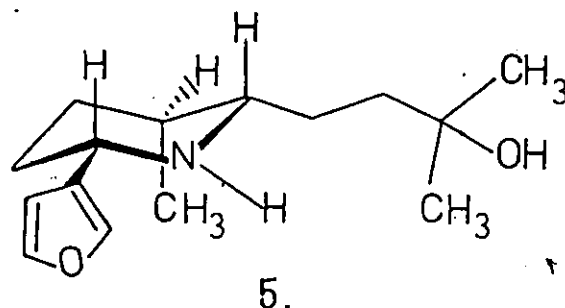
ref. (30)

3-epinupharamine

6.
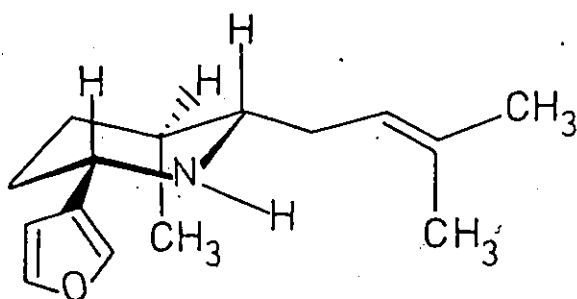
ref. (31,32)

nuphenine

7.
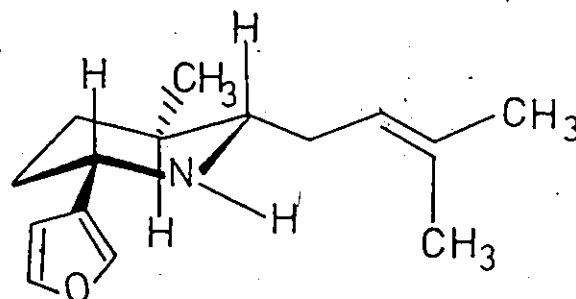
ref. (33)

anhydronupharamine

8.

ref. (34)

nuphamine

9.
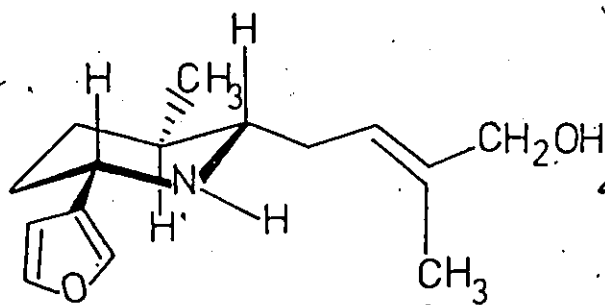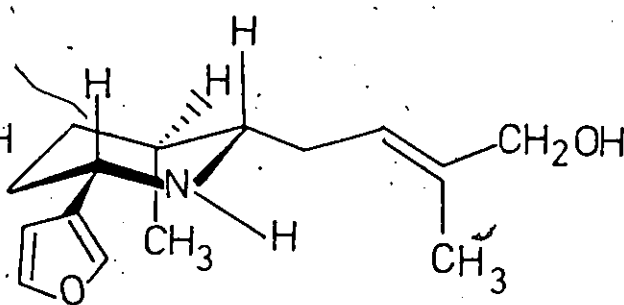
ref. (35)

3-epinuphamine

Figure 1. Representative Nuphar alkaloids: Piperidine type
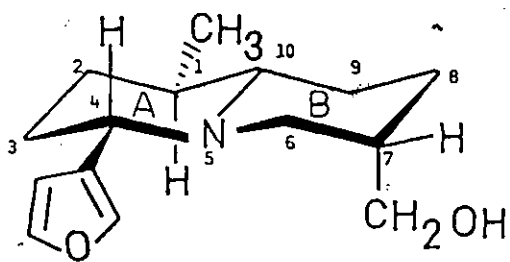
10.

ref. (36,37)

castoramine
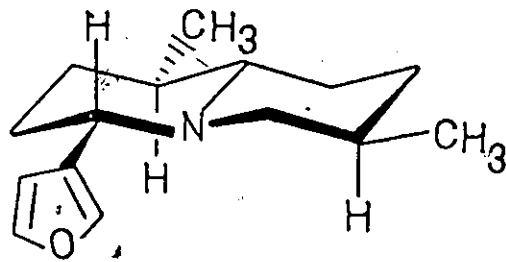
11.

ref. (19,22,38)

7-epideoxynupharidine

12.
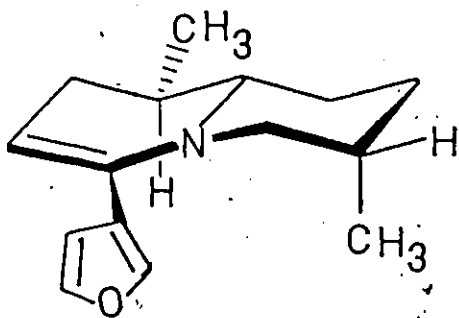
ref. (39)

Δ^3-dehydrodeoxynupharidine
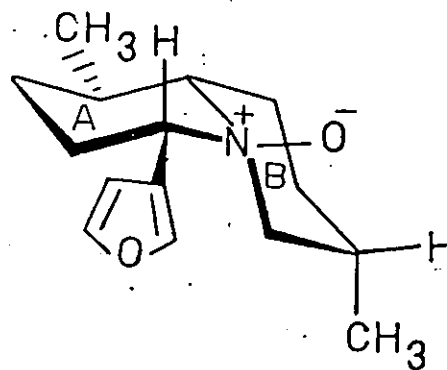
1b.

ref. (29)

nupharidine

Figure 2. Representative Nuphar alkaloids: Quinolizidine type

The relative configuration at C-1 is always (R) but the configuration at C-7 varies. Δ^3-dehydrodeoxynupharidine represents the only example of modification of ring A among the quinolizidine alkaloids isolated from species of Nuphar.

The piperidine alkaloids bear close structural relationships to the quinolizidine alkaloids. Although many of the piperidine alkaloids differ in configuration about C-3, it is evident that all of their structures can be accounted for by modification of ring B of the quinolizidine types, formally by fission of the N-C-6 bond.

Alkaloids containing 30 carbon atoms and sulphur

Four sulphur-containing alkaloids of 30 carbon atoms each were isolated by Achmatowicz and Bellen in 1962 from rhizomes of Nuphar luteum native to Poland[40]. The analysis of the major alkaloid (m.p. 129-130°), named thiobinupharidine 13, agreed with the molecular formula $C_{30}H_{40\rightarrow42}N_2O_2S$. It was recognized that this alkaloid, neglecting sulphur, had almost exactly twice the molecular formula of deoxynuphar-idine ($C_{15}H_{23}NO$). The gross skeletal structure 13a was proposed on the basis of chemical and spectroscopic properties.

Features apparent from the p.m.r. spectrum were the equivalence of the two furan moieties and the presence of a singlet at 0.9δ, suggesting that the methyl groups were attached to quaternary carbon atoms.

The remaining three alkaloids were isolated as their perchlorates. Very little data were reported, but they were named, and the melting points and analyses of their diperchlorates were given as:-
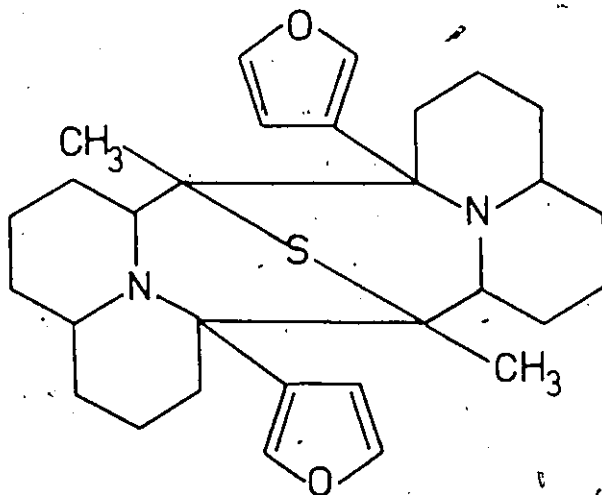
13a.

(i) Allo-thiobinupharidine diperchlorate ($C_{30}H_{42}N_2O_2S.2HClO_4$) m.p. 320-325°.

(ii) Pseudothiobinupharidine diperchlorate ($C_{30}H_{40}N_2O_2S.2HClO_4.2H_2O$) m.p. 173-175°.

(iii) Thiobidesoxynupharidine diperchlorate ($C_{30}H_{40}N_2OS.2HClO_4$) m.p. 225-226°.

Reference to these alkaloids has not appeared again in the literature under these names, but on the basis of the reported melting points of their diperchlorates, it is likely that they are identical with

(i) Neothiobinupharidine diperchlorate[41].

(ii) Diperchlorate of 6-hydroxythiobinupharidine (see page 154).

(iii) Diperchlorate of 6,6'-dihydroxythiobinupharidine (see pages 24 and 157).

The isolation of the crystalline base neothiobinupharidine 14 m.p. 159-160° was reported in 1964[41]. The compound was assigned the molecular formula $C_{30}H_{42}N_2O_2S$ (diperchlorate m.p. 320° $C_{30}H_{42}N_2O_2S \cdot 2HClO_4$). It was suggested that neothiobinupharidine, like thiobinupharidine, was a "dimer" of deoxynupharidine into which a sulphur atom was incorporated. Neothiobinupharidine, however, showed two different p.m.r. absorptions for the β-furan hydrogens and it was suggested it must be a non-symmetrical "dimer". On the other hand thiobinupharidine showed equivalent β-furan hydrogens and it was postulated that the "dimerization" was of a symmetrical nature. The appearance of a broad singlet at 0.87δ was accepted as evidence for methyl groups attached to quaternary carbons and the authors concluded that secondary methyl groups were absent. P.m.r. signals at 3.04, 2.94, 2.83 and 2.69δ were thought to arise from six protons next to nitrogen. By analogy to deoxynupharidine, it was conceded that two more protons α to nitrogen might give rise to absorption in the region 1→2δ. The strong signal at 2.69δ was assigned to a methylene group next to a nitrogen atom which was not contained in a quinolizidine system. A number of these conclusions have since proved to be incorrect.

Shortly afterwards mass spectrometric evidence was reported confirming a relationship between the two sulphur-containing crystalline alkaloids and deoxynupharidine[42]. It was concluded that thiobinupharidine and neothiobinupharidine were stereoisomers as they gave the same fragmentation pattern differing only in the relative intensities of the ions. Oxidation of neothiobinupharidine and thiobinuphar-

idine by the Kühn-Roth method, resulted in 2 moles of acetic acid per

mole of starting material in each case. Since this oxidation is

seldom quantitative, the authors interpreted this result as meaning

that there were at least three and probably four C-methyl groups in

both compounds. Their proposal was that neothiobinupharidine and

thiobinupharidine were therefore stereoisomers of gross skeletal

structure 14a and 13b.
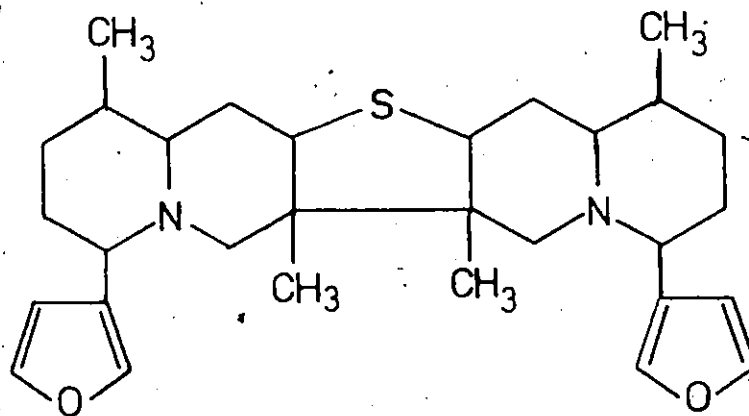
14a & 13b .

Birnbaum's X-ray study of neothiobinupharidine dihydrobromide tetra-

hydrate showed that this structure was incorrect, at least for neothio-

binupharidine. The three dimensional assignment based on this study

is shown as structure 14b[43,44].

Birnbaum's study showed that two "deoxynupharidine-like"

moieties belonging to the same enantiomeric series were linked through

a tetrahydrothiophene ring, however he did not define which enantiomeric

series. His proposed structure was actually the mirror image of 14b.

The structure shown below is based on the assumption that the

14 b. NEOTHIOBINUPHARIDINE.

$(C_{30}H_{42}N_2O_2S)$.
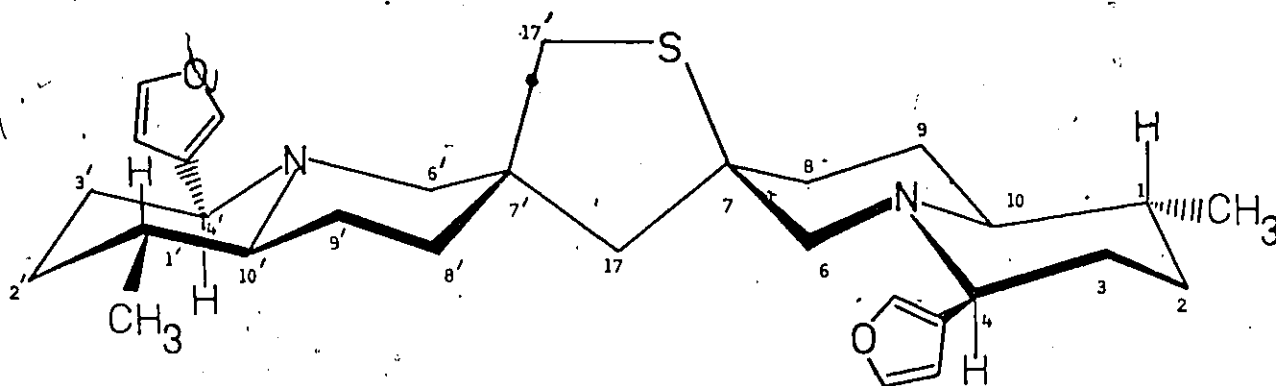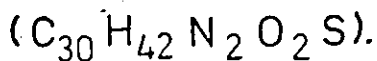
"deoxynupharidine moieties" of the molecule belong to the same enan-
tiomeric series as (-)-deoxynupharidine. It is important to note that
the thiomethylene group and the sulphur atom are bonded axially to
positions C-7' and C-7, respectively. There are elements of "pseudo
symmetry" as replacement of the sulphur atom by methylene would allow
for a C_2 rotation. -

In 1967 a group of Russian workers isolated thiobinupharidine,
neothiobinupharidine, and a new sulphur alkaloid named nuphleine from
rhizomes of N. luteum of East European origin[45;46]. Nuphleine was
isolated as a diperchlorate (m.p. 225-226°). An active hydrogen
determination indicated the presence of two OH groups; thus it was
suggested that it was a dihydroxy derivative of 13b. The significant
discovery that nuphleine was reduced by borohydride in methanol to
thiobinupharidine indicated the presence of hemiaminal functions.
However, the positions of the hydroxy groups were not specified.

As part of his systematic examination of the alkaloid content of North American Nuphar species, LaLonde reported in late 1970 the isolation of two isomeric bishemiaminal sulphur-containing alkaloids, of molecular formula $C_{30}H_{42}N_2O_4S$, from the subspecies macrophyllum[47]. These were originally named 6,6'-dihydroxythionuphlutine-A 16 and -B 17.* Their bishemiaminal character manifested itself in bisimmonium salt formation, and in reduction by sodium borohydride of both into the bisamines, thionuphlutine-A 13 and -B 15,* respectively. LaLonde compared the mass spectrometric fragmentation patterns of the thionuphlutines with those reported by Achmatowicz et al.[42] and concluded that these two bisamines were isomeric with each other and with neothiobinupharidine. Significantly, it was reported at this time that 6,6'-dihydroxythionuphlutine-A formed a diperchlorate, m.p. 226°-228° which was exactly that reported for the diperchlorate of nuphleine[45] and thiobideoxynupharidine[40].

Spectroscopic data suggested that thionuphlutine-A was identical with thiobinupharidine but this was not confirmed until much later when LaLonde investigated the alkaloid content of Nuphar luteum harvested in Poland[48]. He isolated neothiobinupharidine and thionuphlutine-A, neither of which were native to species of North American Nuphar. Thionuphlutine-A was found to be identical with an authentic sample of thiobinupharidine (supplied by Professor J.T. Wröbel) after a comparison of physical and spectroscopic properties. This finding led to the eventual elucidation of the structure of thiobinupharidine.

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* The three-dimensional structures of these alkaloids have now been determined and are shown in Figure 5.

Pharmacological properties

Evidence was furnished in 1945[49] that ointments, extracts and powder prepared from the roots of Nuphar luteum subspecies variegatum had proved useful in the treatment of enteritis, gingivitis, and various skin diseases. In time, groups of Russian workers[50] isolated an alkaloid of unspecified structure or composition, termed "nupharine", which showed very strong antibacterial activity. When tested against forty-five strains of phytopathogenic bacteria it was found that Corynebacterium especially C. michiganense was sensitive to this alkaloidal substance.

A British Patent[51] was obtained in 1964 by Il'inskaya and coworkers which alluded to the protistostatic and spermicidal properties of an alkaloid ($C_{30}H_{42}N_2O_5$, perchlorate m.p. 226°) extracted from Nuphar luteum. The dihydrochloride salt of this alkaloid was reported to arrest the growth of Trichomonas vaginalis in dilutions as low as 1 p.p.m., and killed protista in dilutions of 1:2000 down to 1:5000. It was also found to be active against fungi and gram-positive bacteria. In a later U.S. Patent[52] the indication was given that the molecular formula of the active alkaloid had been incorrectly reported and that it should be $C_{30}H_{42}N_2O_4S$. This was substantiated by spectroscopic studies which however led to incorrect conclusions concerning the structure of the compound (nuphleine: see page 23)[45,46].

Very recently[53] a preliminary report has appeared describing the in vitro antifungal activity of the alkaloid 6,6'-dihydroxythiobinupharidine 16 ($C_{30}H_{42}N_2O_4S$, diperchlorate m.p. 227°). Its structure

and absolute configuration have recently been determined (see discussion) and it is very likely identical with nuphleine[48]. In tests against eight human pathogenic fungi this alkaloid was found to be of low toxicity but of high activity as an inhibitor to the growth of H. capsulatum, B. dermatitidis, and others. This antibiotic activity has elicited considerable interest in these sulphur containing alkaloids as possible agents in water pollution control[54]. C. michiganense was found to be particularly sensitive to the sulphur bearing alkaloids of thirty carbon atoms, whereas this particular bacteria was insensitive to the non-sulphur containing alkaloids deoxynupharidine and nupharidine.

Of considerable interest is the recently reported investigation[55] of twenty-two aquatic plants native to Minnesota, in which only _Nuphar luteum_, subspecies _variegatum_, showed any anti-tumor activity in mice.

Objectives of this research

In 1970 Wróbel first isolated two new sulphur containing alkaloids from species of _Nuphar luteum_. One was named thionupharoline 26[56] (page 85) and the other was simply coded 27 (page 112). Combustion and h.r.m.s. analyses showed that 26 and 27 were isomers of molecular formula $C_{30}H_{42}N_2O_3S$ and hence that they contained one oxygen function more than the bisamines. It was anticipated that they were derivatives of 13, 14 or 15. The objectives of this research were firstly to undertake a detailed study of the fragmentation upon electron impact of several Nuphar alkaloids of known structure using high resolution mass spectrometry. It was anticipated that development of fragmentation patterns might aid the elucidation of the molecular structures of 26 and 27.

Further objectives of this work were to examine the stereoisomeric relationship between 13 and 14 (Figure 5) and to determine the structure of another recently isolated non-sulphur containing Nuphar alkaloid named nupharolutine 18, first isolated in 1969[57].

RESULTS AND DISCUSSION

THE STRUCTURE OF NUPHAROLUTINE (18)

During the examination of the crude bases derived from the rhizomes of Nuphar luteum a new base was isolated to which the name nupharolutine 18 was assigned. Its composition ($C_{15}H_{23}NO_2$) determined by combustion analysis and h.r.m.s. is identical with that of nuphamine 8, 3-epinuphamine 9, nupharidine 1, and castoramine 10. This latter alkaloid was isolated not from the rhizomes of Nuphar, but from the scent gland of the North American Beaver. A very small quantity of the alkaloid 18 was isolated; thus, the study of its structure relied heavily on physical methods.

The infrared spectrum (i.r.) of 18 in carbon tetrachloride shows the presence of bonded (3485 cm^{-1}) and free (3615 cm^{-1}) hydroxyl absorption. The former disappears on dilution indicating that the hydrogen bonding is intermolecular. The i.r. spectrum also shows a strong symmetrical absorption doublet at 2760 cm^{-1} and 2800 cm^{-1}. These so called Bohlmann bands[58] arise because of axial C-H bonds α to the nitrogen atom and anticoplanar to its lone pair of electrons. This intense doublet pattern is inditative of a trans fused quinolizidine system, which contains three such axial C-H bonds[59,60].

Attempts to acetylate the compound with acetic anhydride/pyridine at 20° for 24 hours or at 100° for 2 hours led only to the recovery of starting material, indicating that the hydroxyl function is tertiary.

Examination of the 100 MHz p.m.r. spectrum of 18 in $CDCl_3$ shows several similarities to that reported for deoxynupharidine 2[25], but with one major difference. Deoxynupharidine shows two doublets centred at 0.92δ and 0.99δ which can be assigned to the C-1 equatorial methyl and C-7 axial methyl groups, respectively, on the basis of chemical shift and coupling constant[22]. The correlation of axial methyl groups with lower field signals and larger coupling constants and of equatorial methyl groups with higher field signals and smaller coupling constants is a well-known one in quinolizidine chemistry[61]. The new alkaloid however shows a doublet (3H) at 0.88δ (J = 5.6 Hz) and a singlet (3H) at 1.22δ. The singlet nature of this peak and its chemical shift are compatible with the part structure;

$$
\begin{array}{ccccc}
 & & CH_3 & & \\
 & | & | & | & \\
-\,C & - & C & - & C- \\
 & | & | & | & \\
 & & OH & & \\
\end{array}
$$
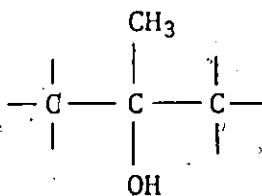
this structure is also in agreement with the acetylation studies. As with deoxynupharidine the spectrum of 18 has signals, attributed to the three protons of a 3-substituted furan moiety, at 7.25δ plus 7.34δ (2H, α-protons) and 6.34δ (1H, β-proton). A poorly resolved quartet of area one centred at 3.03δ (J = 8.3 and 6.0 Hz) resembles a signal at 2.88δ (J = 8.0 and 6.2 Hz) in the spectrum of 2 assigned to H-4a, the methine proton, adjacent both to the nitrogen and the furan ring. A quartet (1H) centred at 2.66δ (J = 11.5 and 2.0 Hz) is assigned by analogy with the spectrum of deoxynupharidine to H-6e. In the latter this signal appears as a quartet centred at 2.70δ (J = 12.5
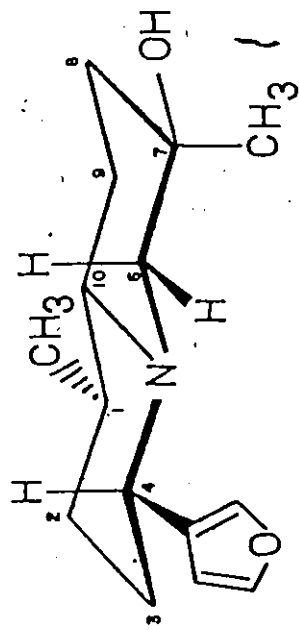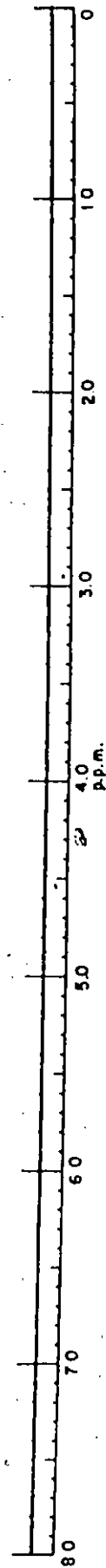
Figure 3. P.m.r. spectrum of (-)-nupharolutine (18) in CDCl₃ recorded at 100 MHz

and 2.5 Hz). The remaining envelope of methylene and methine protons is present between 1.0 and 2.0δ. The 100 MHz spectrum of <u>18</u> is shown in Figure 3 and a comparison of the assignments with those of <u>2</u> is given in Table 1.

The p.m.r. data for <u>18</u> clearly indicate that the ring B opened piperidine structure of <u>8</u> and <u>9</u> may be dismissed because of the lack of signals expected for the part structure,
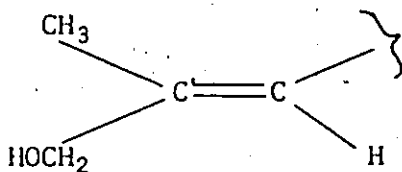
In <u>8</u> and <u>9</u> the vinyl proton appears as a broad singlet at 5.41δ and the vinylic methyl at 1.65δ.

The available data therefore suggest that nupharolutine has one of the following structures:

<u>18a</u> $R_1 + R_2 = CH_3 + OH$.
$R_3 + R_4 = CH_3 + H$

<u>18b</u> $R_1 + R_2 = CH_3 + H$
$R_3 + R_4 = CH_3 + OH$
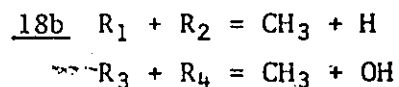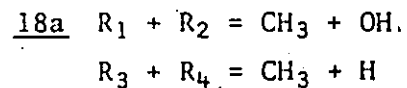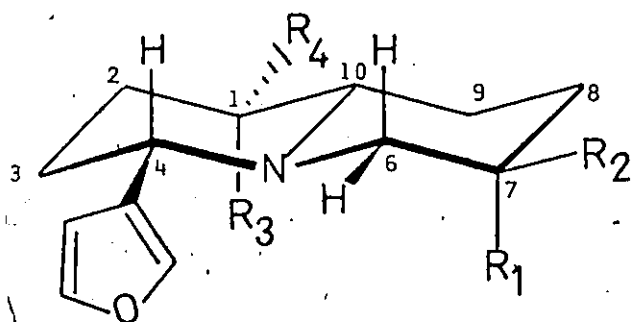
High resolution mass spectrometry was used to resolve this problem.

TABLE 1

A comparison of the 100 MHz p.m.r. assignments for deoxynupharidine and nupharolutine in CDCl$_3$ solution

Proton	deoxynupharidine			nupharolutine		
	Chemical shift (δ)	Multiplicity	Coupling constants J (Hz)	Chemical shift (δ)	Multiplicity	Coupling constants J (Hz)
C-1 methyl (eq)	0.92	doublet	5.6	0.88	doublet	5.6
C-7 methyl (ax)	0.99	doublet	7.0	1.22	singlet	-
Methylene and methine	1→2	envelope	-	1→2	envelope	-
H - 6ax	1.83	quartet	12.5 (6a - 6e) and 2.5 (6a - 7e)	1.6→1.8	obscured by envelope	-
H - 6 eq	2.70	quartet	12.5 (6e - 6a) and 2.5 (6e - 7e)	2.66	quartet	11.5 (6e - 6a) and 2.0 (6e - 8e)
H - 4ax	2.88	quartet	8.0 (4a - 3a) and 6.2 (4a - 3e)	3.03	quartet	8.3 (4a - 3a) and 6.0 (4a - 3e)
furan β - H	6.37	narrow multiplet	-	6.34	narrow multiplet	-
furan α - H	7.24 and 7.31	narrow multiplet	-	7.25 and 7.34	narrow multiplet	-

Development of fragmentation schemes using h..r.m.s.

The mass spectrum of 18 was recorded and compared with those of deoxynupharidine 2, nupharidine 1, and castoramine 10. Plots of the low resolution spectra are shown in Figure 4. The spectrum of 18 resembles those of 2 and 10 but is distinctly different from that of 1. An interpretation of the spectra follows.

The mass spectrum of 2 is identical with that reported earlier[42], showing intense peaks at m/e 233 (M^+) and at m/e 136, 98 and 94. High resolution studies confirm the original compositions assigned to these intense ions and also permit an analysis of the compositions of the less intense ions. This results in a fuller understanding of the fragmentation process, thereby enabling new substituents to be assigned to rings A or B.

There are four positions β to nitrogen in 2 that may undergo fission to yield the molecular ions designated A, B, C, and D in Scheme 2. Of these, C and D should be more stable than the others, because the radical centres are secondary rather than primary. Structures C and D are therefore used to explain the subsequent fragmentation of the molecule. The homologous ions shown in Scheme 3 at m/e 204(a), 190(b) and 176(c) are depicted as being formed from 2C by the loss of the hydrocarbon fragments C_2H_5, C_3H_7, and C_4H_9, respectively, processes accompanied by hydrogen rearrangement, and the ions m/e 162(d) and 148(e) through retro Diels-Alder loss of propylene from m/e 204 and 190, respectively. The ion at m/e 178 (f or g) may arise from 2D or 2C, respectively, by the processes shown in Scheme 3, but the former is
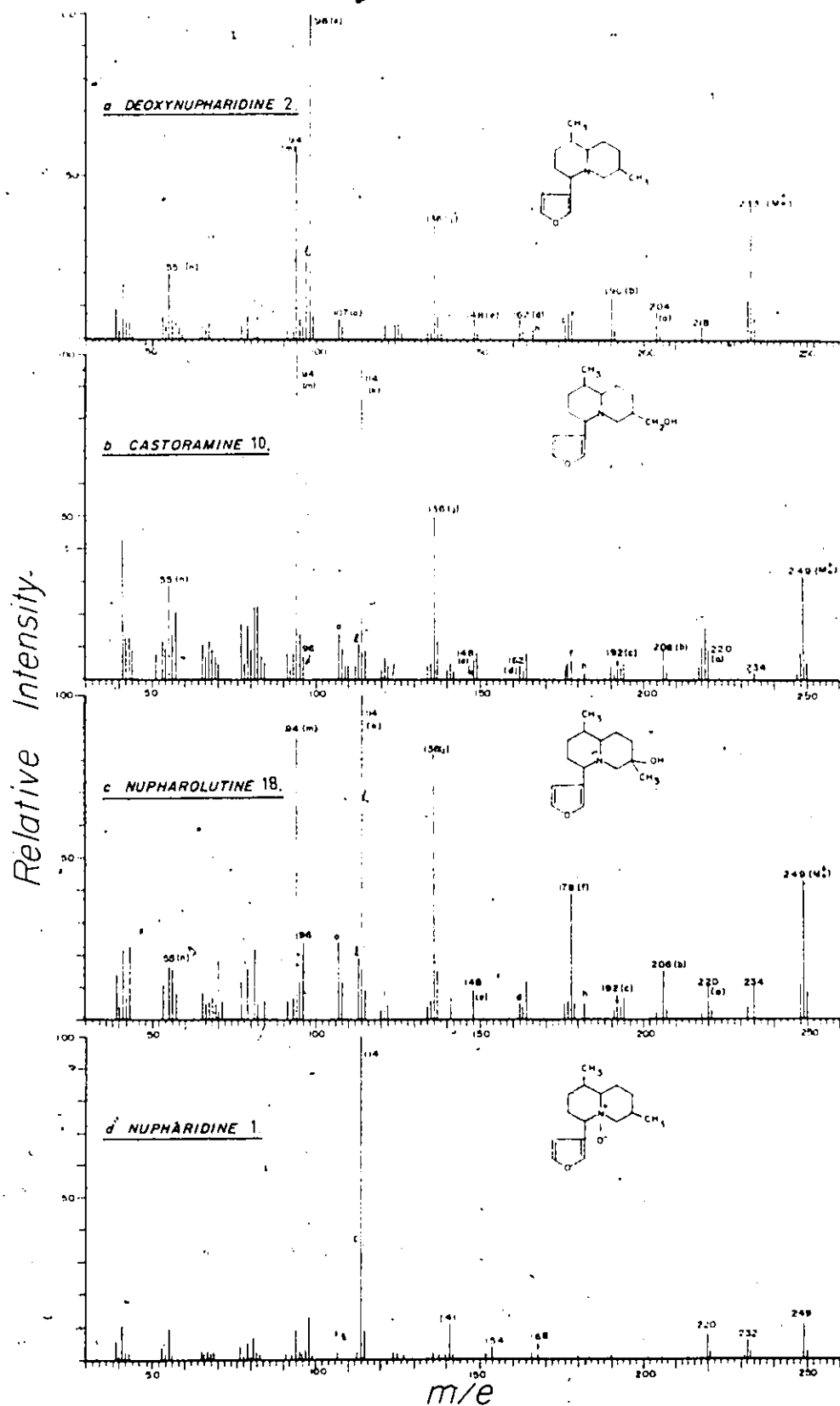
Figure 4.   The mass spectra of (a) deoxynupharidine; (b) castoramine; (c) nupharolutine, and (d) nupharidine.

<u>B</u>

<u>D</u>

<u>A</u>

<u>C</u>

2 m/e 233 (C$_{15}$H$_{23}$NO)

18 and 10 m/e 249 (C$_{15}$H$_{23}$NO$_2$)

2   R$_1$ = CH$_3$; R$_2$ = H

18   R$_1$ = CH$_3$; R$_2$ = OH

10   R$_1$ = CH$_2$OH; R$_2$ = H

Scheme 2

(c)

m/e 176 (C$_{11}$H$_{14}$NO)

m/e 192 (C$_{11}$H$_{14}$NO$_2$)

(h)

2 m/e 166 (C$_{11}$H$_{20}$N)

18 and **10** m/e 182 (C$_{11}$H$_{20}$NO)

C

H transfer

(b)

m/e 190 (C$_{12}$H$_{16}$NO)

m/e 206 (C$_{12}$H$_{16}$NO$_2$).

(e)

2, 18 and **10** m/e 148 (C$_9$H$_{10}$NO)

(g)
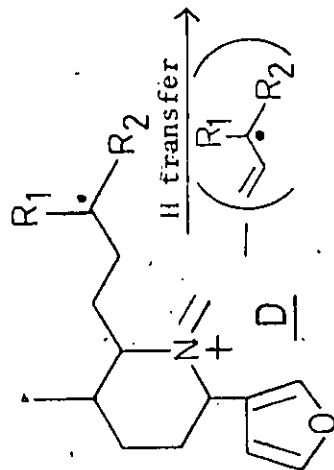
2, 18 and **10** m/e 178 (C$_{11}$H$_{16}$NO)

(f)

2, 18 and **10**

Scheme 3

(a)

2 m/e 204 (C$_{13}$H$_{18}$NO)

18 and **10** m/e 220 (C$_{13}$H$_{18}$NO$_2$)

(d)

2, 18 and **10** m/e 162 (C$_{10}$H$_{12}$NO)

H transfer

D
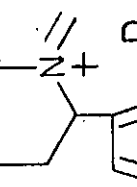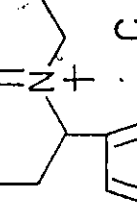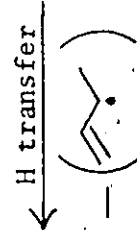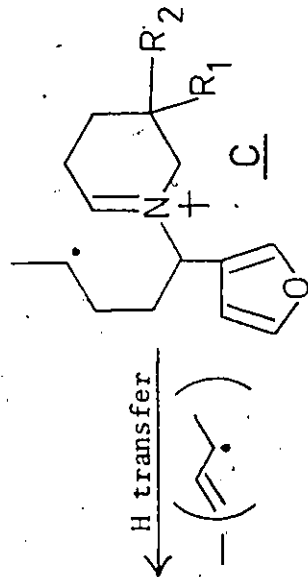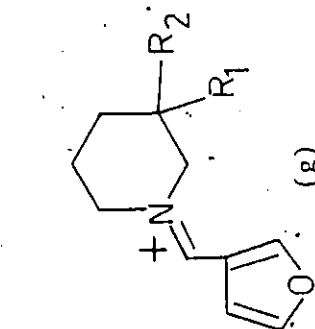
favoured (compare with the spectrum of castoramine below). An ion at
m/e 166(h) has the composition $C_{11}H_{20}N$ and must result from expulsion
of the furan group as illustrated in Scheme 3.

The major ions in the spectrum of $\underline{2}$ appear at m/e 136(j), 98(k),
97(l), 94(m), and 55(n) and a less intense but significant ion at m/e
107(o). In Scheme 4 a proposal for their derivation is outlined. Their
compositions have been established and all may be considered to arise from
the molecular ion $\underline{2C}$. In fragmentation by route 4a the charge is
retained on the furan-containing fragment to give the ion at m/e 136(j),
and in route 4b on the nitrogen containing fragment to give m/e 97(l),
which by a retro Diels-Alder process (route 4c) leads to m/e 55(n).
When fragmentation proceeds with hydrogen transfer as in route 4d the
intense ion m/e 98(k) results. (Hydrogen transfer from C-2 is illustrated
but no evidence exists that transfer occurs from this specific site.)
The further fragmentation of m/e 136 via processes 4e and 4f leads to
ions at m/e 107(o) and 94(m), respectively.

An analysis of the spectrum of castoramine indicates that it
fragments in a manner analogous to deoxynupharidine. Thus ions (a), (b),
(c), and (h) of Scheme 3 contain one oxygen more than those derived from
$\underline{2}$ but are deemed to have the same framework. The ions (d) and (e) are
the same in $\underline{10}$ as in $\underline{2}$ and the ion m/e 178 has the composition $C_{11}H_{16}NO$
and can be represented as (f). Of the ions in Scheme 4, m/e 136(j), 94
(m), 55(n), and 107(o) are identical in composition in both $\underline{2}$ and $\underline{10}$, but
(k) and (l) appearing at m/e 114 and 113, respectively, are shifted
upwards by 16 mass units in $\underline{10}$ from their position in $\underline{2}$, reflecting the
presence of the OH group.
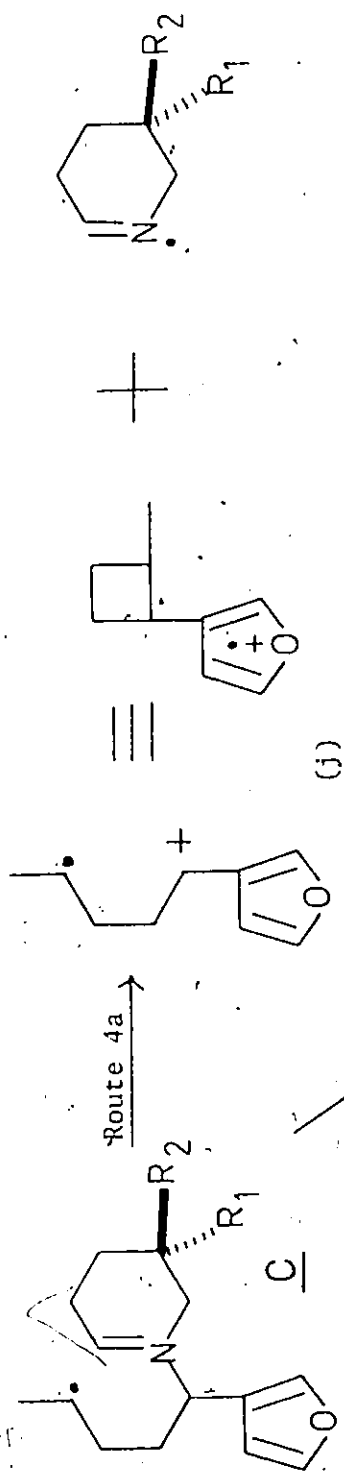
Route 4a

(j)

2, 18 and 10 m/e 136 ($C_9H_{12}O$)

Route 4b

Route 4c

(n)

2, 18 and 10 m/e 55 (C_3H_5N)

(1)
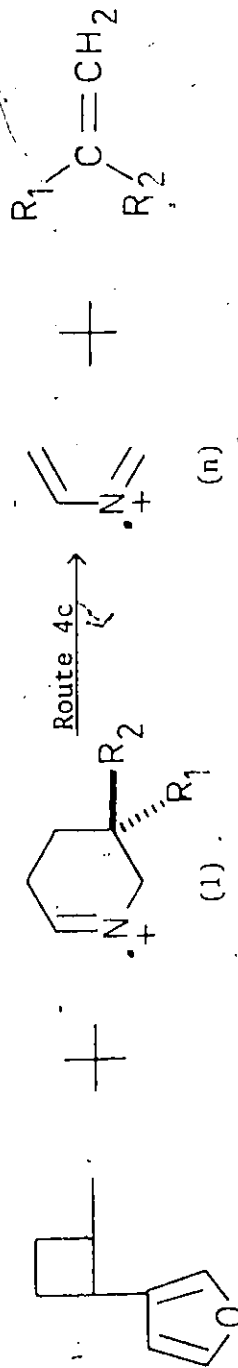
2 m/e 97 ($C_6H_{11}N$)
18 and 10 m/e 113 ($C_6H_{11}NO$)

C

2 m/e 233 ($C_{15}H_{23}NO$)
18 and 10 m/e 249 ($C_{15}H_{23}NO_2$)
2   R_1 = CH_3 ; R_2 = H
18 R_1 = CH_3 ; R_2 = OH
10 R_1 = CH_2OH; R_2 = H

Scheme 4

2, 18 and 10 m/e 107 (C_7H_7O)

(o)

2, 18 and 10 m/e 94 (C_6H_6O)

(m)

(k)

Route 4d →

2 m/e 98 ($C_6H_{12}N$)
18 and 10 m/e 114 ($C_6H_{12}NO$)

— C_2H_5

Route 4e

— C_3H_6

Route 4f

C

2 m/e 233 ($C_{15}H_{23}NO$)
18 and 10 m/e 249 ($C_{15}H_{23}NO_2$)
2 R_1 = CH_3 ; R_2 = H
18 R_1 = CH_3 ; R_2 = OH
10 R_1 = CH_2OH; R_2 = H

(j)

m/e 136 ($C_9H_{12}O$)

Scheme 4 (continued)

The hydroxyl group present in <u>10</u> leads to new ions in its spectrum not present in that of <u>2</u>. Thus an ion at m/e 96 can be ascribed to <u>10</u>k - H_2O and m/e 164 to <u>10</u>h - H_2O. The ions at m/e 218 and 219 correspond to loss of CH_2O and CH_2OH from $M^{+\cdot}$ as shown in Scheme 5.
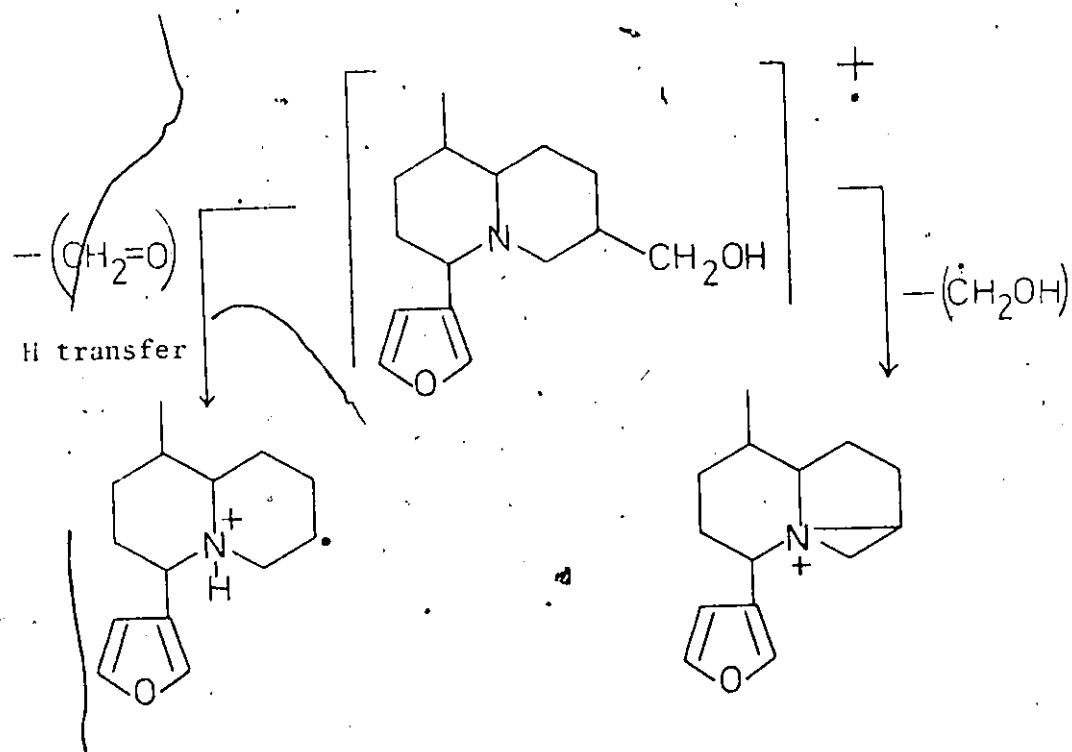
Scheme 5

The spectrum of nupharolutine <u>18</u> has many features in common with that of <u>10</u> but is distinctly different from that of nupharidine <u>1</u>. it is concluded therefore that nupharolutine must have an OH group in ring B and is therefore represented as <u>18a</u>. The structural differences between <u>18</u> and <u>10</u> are reflected in their mass spectra. Thus loss of CH_3 and OH is predicted to be more favoured in <u>18</u> than in <u>10</u> as is the formation of ion (f). The spectra bear this out. The loss of H_2O from

m/e 114
$C_6H_{12}NO$

m/e 141
$C_8H_{15}NO$

m/e 154
$C_9H_{16}NO$

m/e 168
$C_{10}H_{18}NO$

H transfer
(a)

(b)

H transfer
(c)

H transfer
(d)
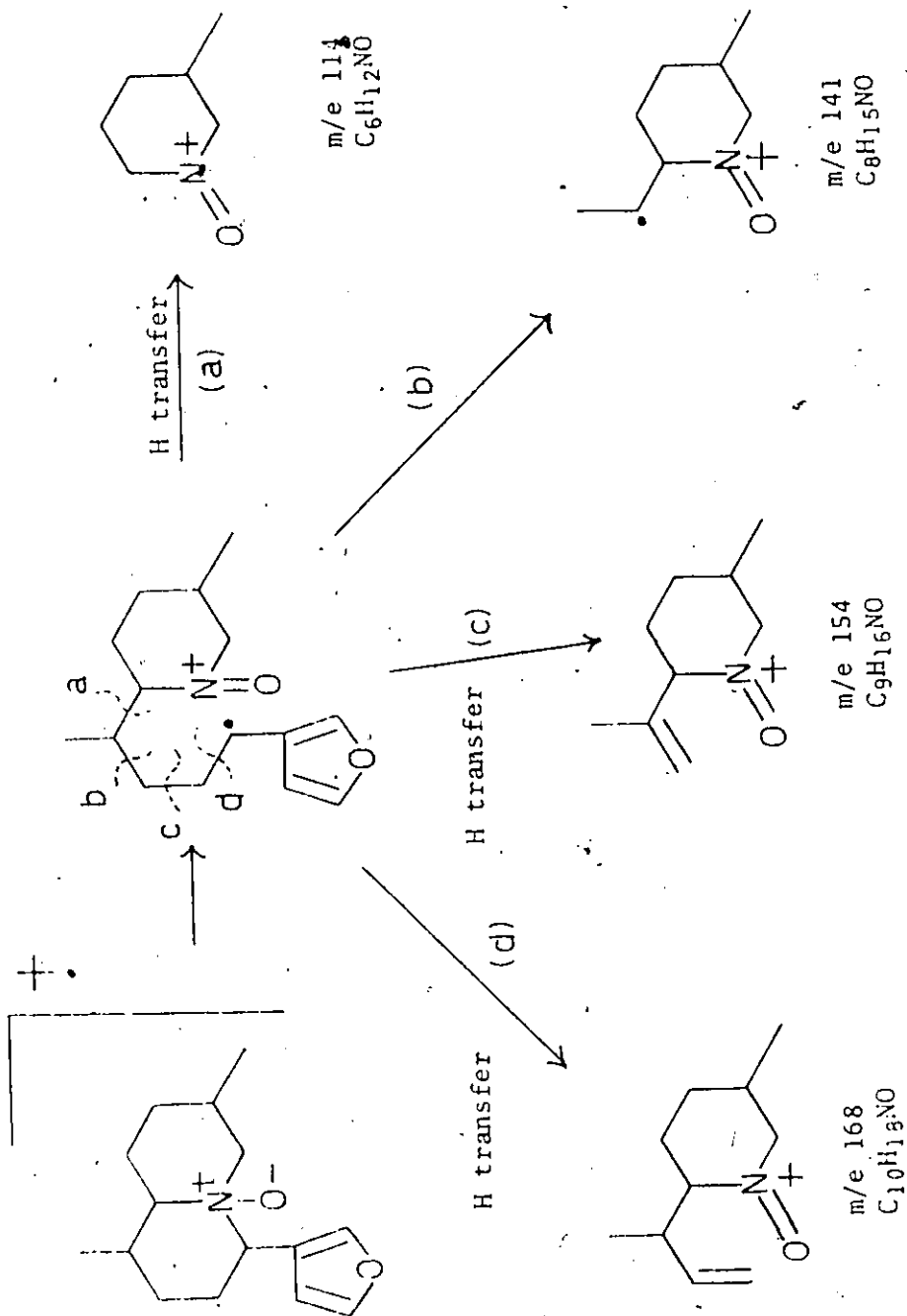
Scheme 6

m/e 114 to form m/e 96 is more pronounced in <u>18</u> the tertiary alcohol, than it is in <u>10</u>, the primary alcohol, an expected result.

The spectrum of nupharidine <u>1</u> is discussed briefly below. Like other N-oxides it suffers loss of oxygen and OH with peaks at m/e 232 and 231[62]. The ion at m/e 220 results from loss of CHO, a fragmentation characteristic of furans[63], and not from loss of an ethyl fragment. Scheme 6 illustrates the proposed derivation of the major ion at m/e 114 and related fragments, based upon a determination of their compositions by h.r.m.s. In the next section it will be shown that the dimeric sulphur containing compounds have many ions in their mass spectra whose formation may be interpreted through Schemes 3 and 4, a factor which lends credibility to them.

Spectroscopic properties of hydroxyquinolizidines

Interest in the hydroxy quinolizidines has been extensive because of their potential biological activity[60]. Aaron has shown[64] that in trans-fused hydroxyquinolizidines, an axial hydroxy group on positions 1 or 3 (see Table 2 for numbering system) will hydrogen bond to the nitrogen lone pair, whereas an equatorial hydroxy group will not. Other workers have observed the same phenomenon with 1-methyl-1-hydroxy-quinolizidines[65]. These results are summarised in Table 2.

Aaron has also studied the trans-fused ► cis-fused isomerisation of the 3-hydroxyquinolizidines which can occur by inversion at the nitrogen atom[64]. His i.r. studies indicate a very strong preference for the trans-fused conformation even though a relatively strong intramolecular hydrogen bond can form in the cis-fused conformation. He estimated the free energy difference between the two fused ring
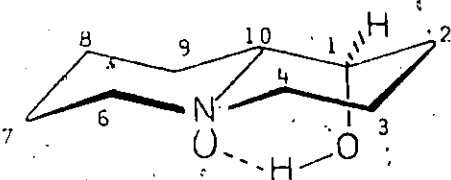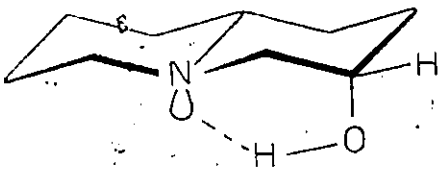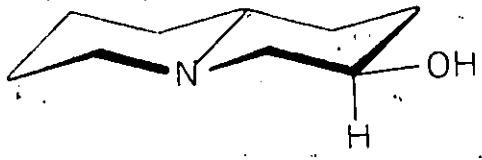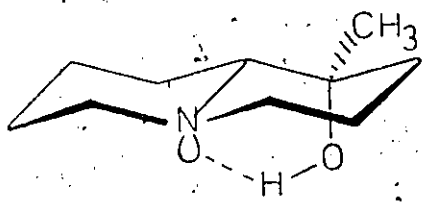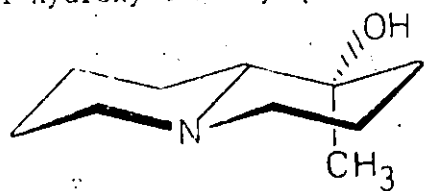
Compound	Bohlmann region (cm^{-1})	Free OH (cm^{-1})	Intramolecularly bonded OH (cm^{-1})

1-hydroxyquinolizidine

| | 2800 2777 | - | 3526 |
| | 2795 2750 | 3618 | - |

3-hydroxyquinolizidine

| | 2797 2757 | - | 3527 |
| | 2800 2762 | 3609 | - |

1-hydroxy-1-methylquinolizidine

| | 2700 - 2800 | - | 3480 |
| | 2700 - 2800 | 3620 | - |

TABLE 2

Infrared Characteristics of Hydroxyquinolizidines

cis-fused

trans-fused
> 99%
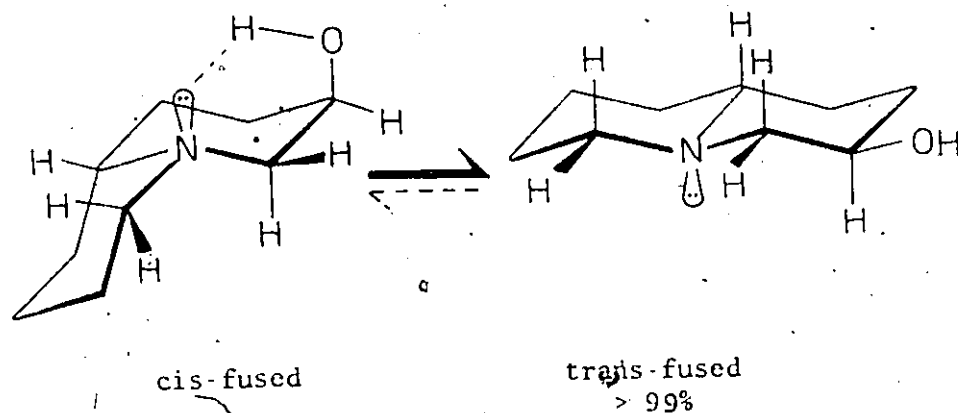
isomers to be 4 → 5 kilocalories per mole.

The spectroscopic and chemical evidence points to structure 18c$\left(R_1 + R_2 = CH_3 + H\right)$ for nupharolutine. The i.r. data is very similar to that reported for the 1 or 3 equatorial hydroxyquinolizidines

18c.
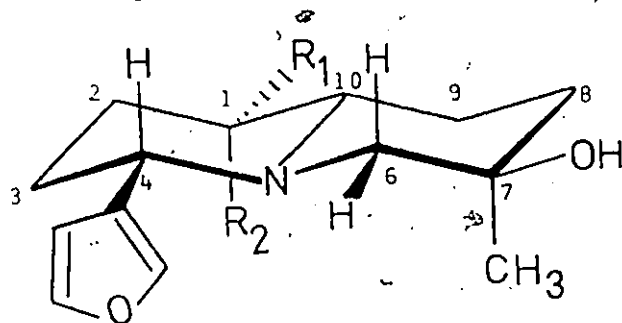
(Table 2). The Bohlmann bands indicate a trans-quinolizidine system and since the tertiary hydroxyl is not internally hydrogen bonded, it must occupy the equatorial position at C-7. P.m.r. studies of deuterio-chloroform solutions of 1-hydroxy-1,7-dimethylquinolizidines possessing the trans-fused ring conformation have shown that an axial methyl group at position 1 absorbs at 1.22 - 1.25δ whereas an equatorial methyl group at position 1 absorbs at 1.08 - 1.11δ[66]. The signal observed

in the $CDCl_3$ solution of nupharolutine is at 1.22δ which is in excellent agreement with an axial methyl at C-7. The p.m.r. data discussed earlier suggest equatorial positions for both the methyl group at C-1 and the 3 furyl group at C-4. The coupling of H-6e in <u>18</u> requires comment since it is almost identical with the corresponding proton in <u>2</u>, where the quartet pattern was explained by a large geminal coupling (H-6e - H-6a) and a smaller coupling (H-6e - H-7e), see Table 1. The geminal coupling also occurs in <u>18</u> but the smaller coupling must be a "W" type coupling (67,68) between H-6e and H-8e, since C-7 is quaternary.

Conclusive proof of the structure would be forthcoming if nupharolutine was converted into deoxynupharidine. Treatment of <u>18</u> with $POCl_3$-pyridine gave a chlorocompound <u>19</u>, separated from the reaction mixture by preparative layer chromatography. The mass spectrum of <u>19</u> determined at low resolution is worthy of comment. It shows the intense ions observed at m/e 136, 107 and 94 in <u>2</u>, <u>18</u>, and <u>10</u>. Ion (k), Scheme 4, appears as an isotopic doublet at m/e 132 and 134, which, through loss of HCl, leads to an ion at m/e 96 also observed in the spectra of 18 and <u>10</u>. Except for the isotopic molecular ions at m/e 267 and 269 the only intense ion in the high mass region appears at m/e 352 ($M^+ - Cl^-$). Compound <u>19</u> was reduced over platinum to a substance whose mass spectrum is identical with <u>2</u>. The substance also has the same retention times as an authentic sample of <u>2</u> on two thin layer chromatograms and on two gas-liquid chromatograms. A comparison of the p.m.r. and optical rotation was not possible owing to lack of material. It was concluded therefore that nupharolutine must be <u>18c</u>, R_1 = CH_3, R_2 = H.

Shortly after completion of this work there appeared in the literature mass spectrometric data on (-)-deoxynupharidine-4β-d$_1$ 20[69] and (-)-deoxynupharidine-6β,7β-d$_2$ 21[70]. Compound 20 was prepared from (+)-nupharidine by conversion into Δ^3-dehydrodeoxynupharidine using the Meisenheimer transformation[71] followed by borodeuteride reduction. Compound 21 was also prepared from (+)-nupharidine by the facile conversion into the Δ^6-dehydrodeoxynupharidine, using the elegant Polonovski transformation[72], followed by catalytic reduction with deuterium over palladium.
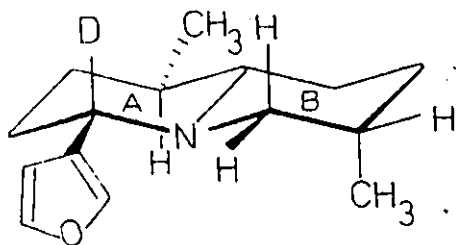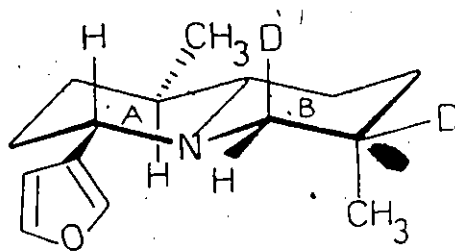
20.   21.

The results of these labelling studies is entirely consistent with the fragmentation mechanisms proposed from the h.r.m.s. data (Scheme 4). Thus the base peak at m/e 98 of deoxynupharidine is shifted to m/e 100 in 21 but retained in 20, whereas m/e 94, 107, and 136 are retained in 21 but each is shifted one mass unit higher in 20. The probable dual nature of m/e 178 (Scheme 3) was also verified.

Later the isolation and elucidation of the structure of a dimeric alkaloid of 6,7β-oxidodeoxynupharidine were reported[73]. This alkaloid of composition $C_{30}H_{42}N_2O_4$ has the structure 22 and is shown to be a bimolecular dehydration product of the diol 23.

22.

23.
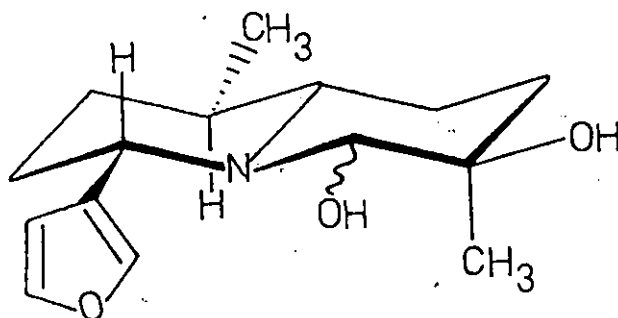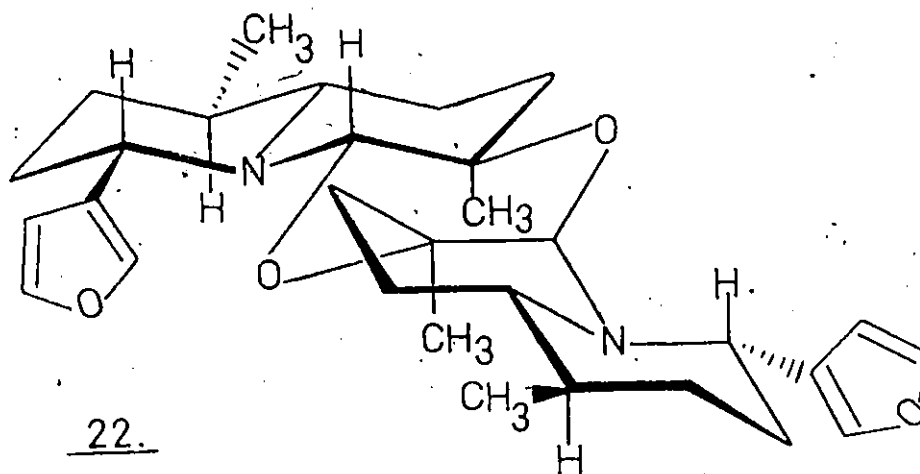
Metal hydride reduction of either 22 or 23 gives deoxynuphari-
dine-7-β-ol, identical with nupharolutine 18c. Subsequently[73] both
deoxynupharidine-7-β-ol and 7-epideoxynupharidine-7-α-ol 24 were
synthesized from Δ6-dehydrodeoxynupharidine by the sequence shown in
Scheme 7. Significantly it was discovered that only deoxynupharidine-
6,7β-ol dehydrated spontaneously to the dimer 22. A comparison of the
physical and spectrometric data of nupharolutine with that of LaLonde's

Δ^6-dehydrodeoxynupharidine

OsO$_4$

NaBH$_4$(D$_4$)

NaBH$_4$(D$_4$)

18 c.

24.
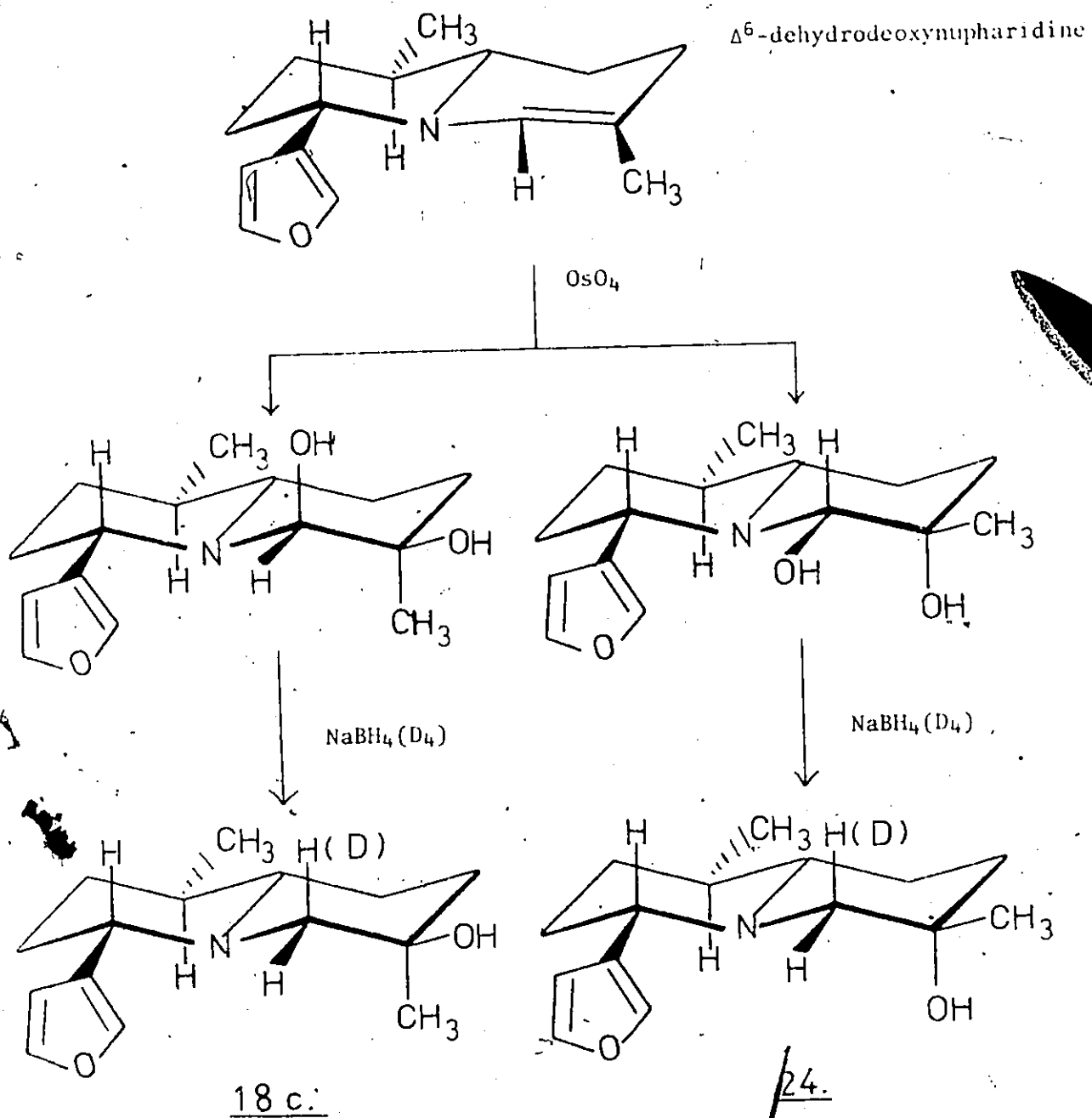
Scheme 7

"synthetic" deoxynupharidine-7β-ol confirmed their identity and established that (-)-nupharolutine belongs to the same enantiomeric series as (-)-deoxynupharidine and therefore has the absolute configuration as shown in 18c.

A gratifying aspect of LaLonde's spectrometric examination was that his conclusions regarding

(i) the origin of ion m/e 114 (115 in the deuterated species),

(ii) the structure of ion m/e 178 as (f) Scheme 3, and

(iii) the "W" coupling between H-6β —— H-8e

were completely consistent with the proposals made above.

THE STRUCTURE AND CONFIGURATION OF THIOBINUPHARIDINE (13c)

The three dimensional structure of neothiobinupharidine 14 has been established[43] and its gross skeletal structure may be represented as 25 (R_1 = R_4 = H). Mass spectrometric evidence[42,47] strongly suggested that thiobinupharidine 13 and thionuphlutine-B 15 are stereo-isomers of each other and of 14 (Figure 5).
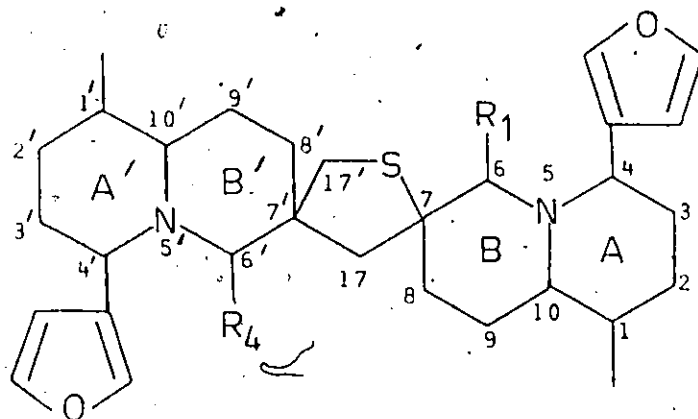
25.

The spectra of the two crystalline bases 13 and 14 were re-examined in order to investigate the nature of their stereoisomeric relationship. The Bohlmann bands exhibited in the i.r. spectra of trans-quinolizidines are evident, as expected, in the spectrum of neothiobinupharidine. When equimolar solutions of 13 and 14 are examined under the same conditions, it is observed that the Bohlmann bands in each spectrum are of nearly equal intensity, although differing slightly in complexity (Figure 6). Thus it seems that 13 like 14 contains two trans-quinolizidine moieties, in which there are six carbon-hydrogen bonds anticoplanar to the non-bonding electron pairs on nitrogen. This observation provides
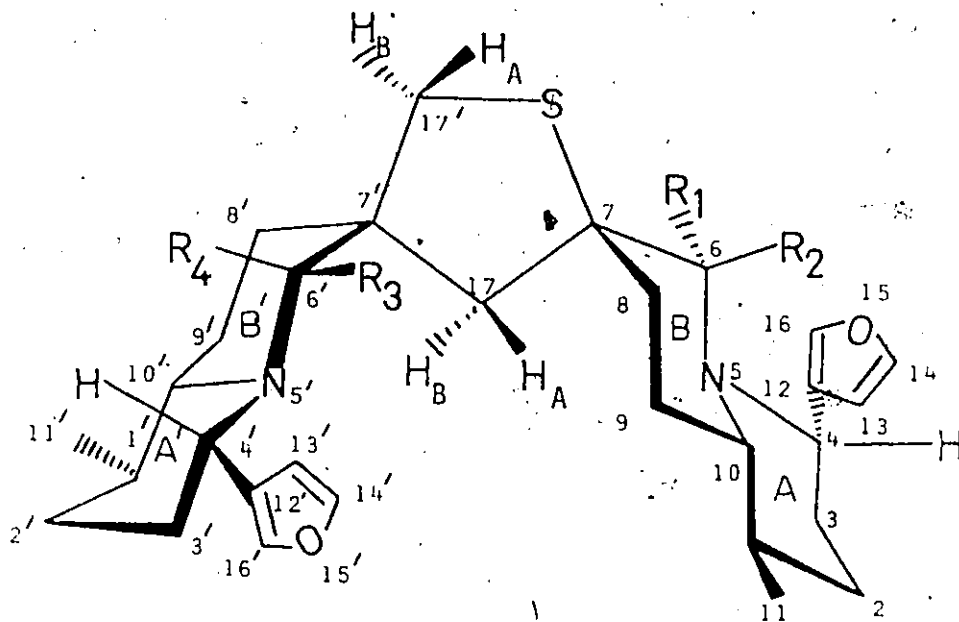
13c $R_1 = R_2 = R_3 = R_4 = H$

16 $R_1 = R_4 = OH$; $R_2 = R_3 = H$

28 $R_1 = R_4 = D$; $R_2 = R_3 = H$ (as interpreted by LaLonde[76])

26 $R_1 = OH$; $R_2 = R_3 = R_4 = H$

31 $R_1 + R_2 = D + H$; $R_3 = R_4 = H$

32 $R_1 + R_2 = D + H$; $R_3 = H$; $R_4 = D$

33 $R_1 = OH$; $R_4 = D$; $R_2 = R_3 = H$

34 $R_4 = D$; $R_1 = R_2 = R_3 = H$
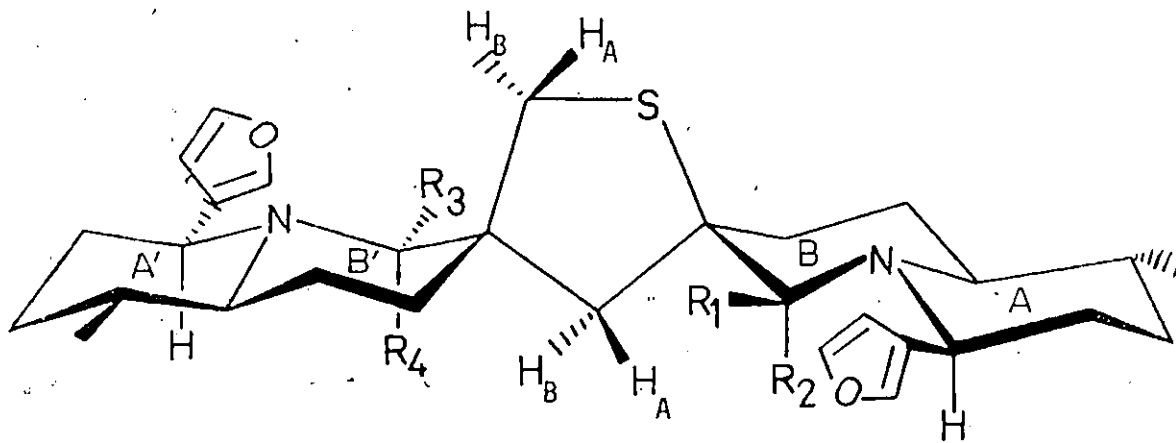
14b $R_1 = R_2 = R_3 = R_4 = H$

Figure 5. Three-dimensional structures of thiospiran Nuphar alkaloids
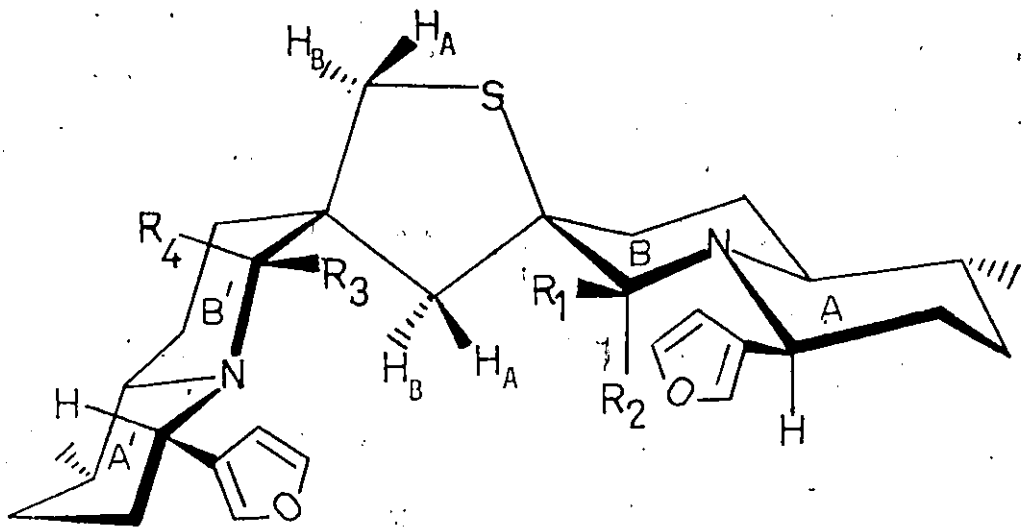
15, $R_1 = R_2 = R_3 = R_4 = H$

17 $R_1 = R_3 = H; \quad R_2 = R_4 = OH$

29 $R_1 = R_3 = H; \quad R_2 = R_4 = D$ (as interpreted by LaLonde[76])
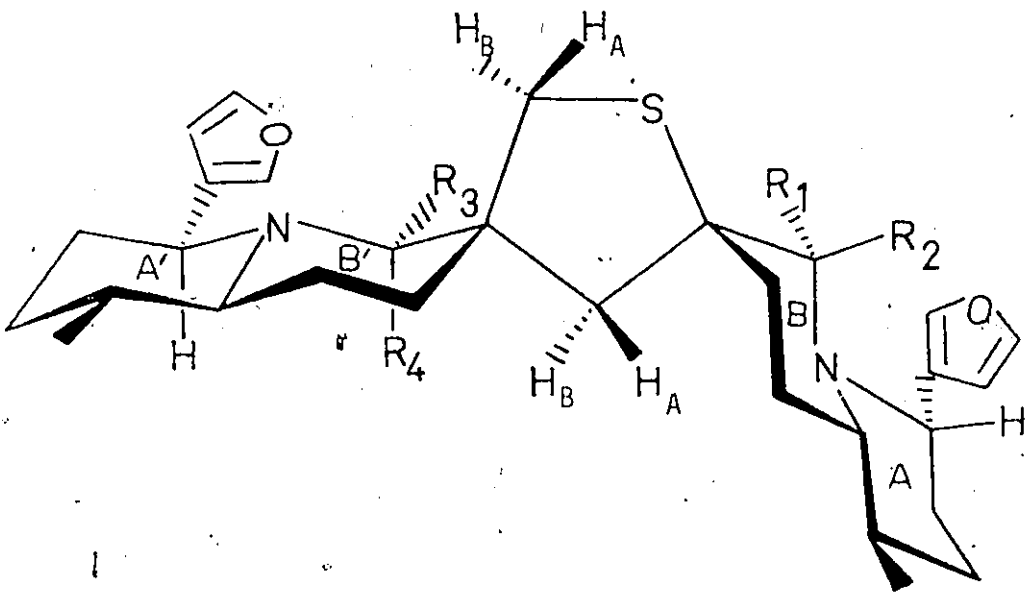
30 $R_1 = R_2 = R_3 = R_4 = H$

Figure 5 (continued)

WAVELENGTH (MICRONS)

THIOBINUPHARIDINE 13c

6.66
(furan)

9.74

3.51(marker)

(furan) 11.45

6.24(marker)

NEOTHIOBINUPHARIDINE 14b

Bohlmann
bands

6.67

9.75

1.45

NEOTHIOBINUPHARIDINE
SULPHOXIDE 27

6.67

O=S
9.62

9.79

11.50

FREQUENCY (CM⁻¹)
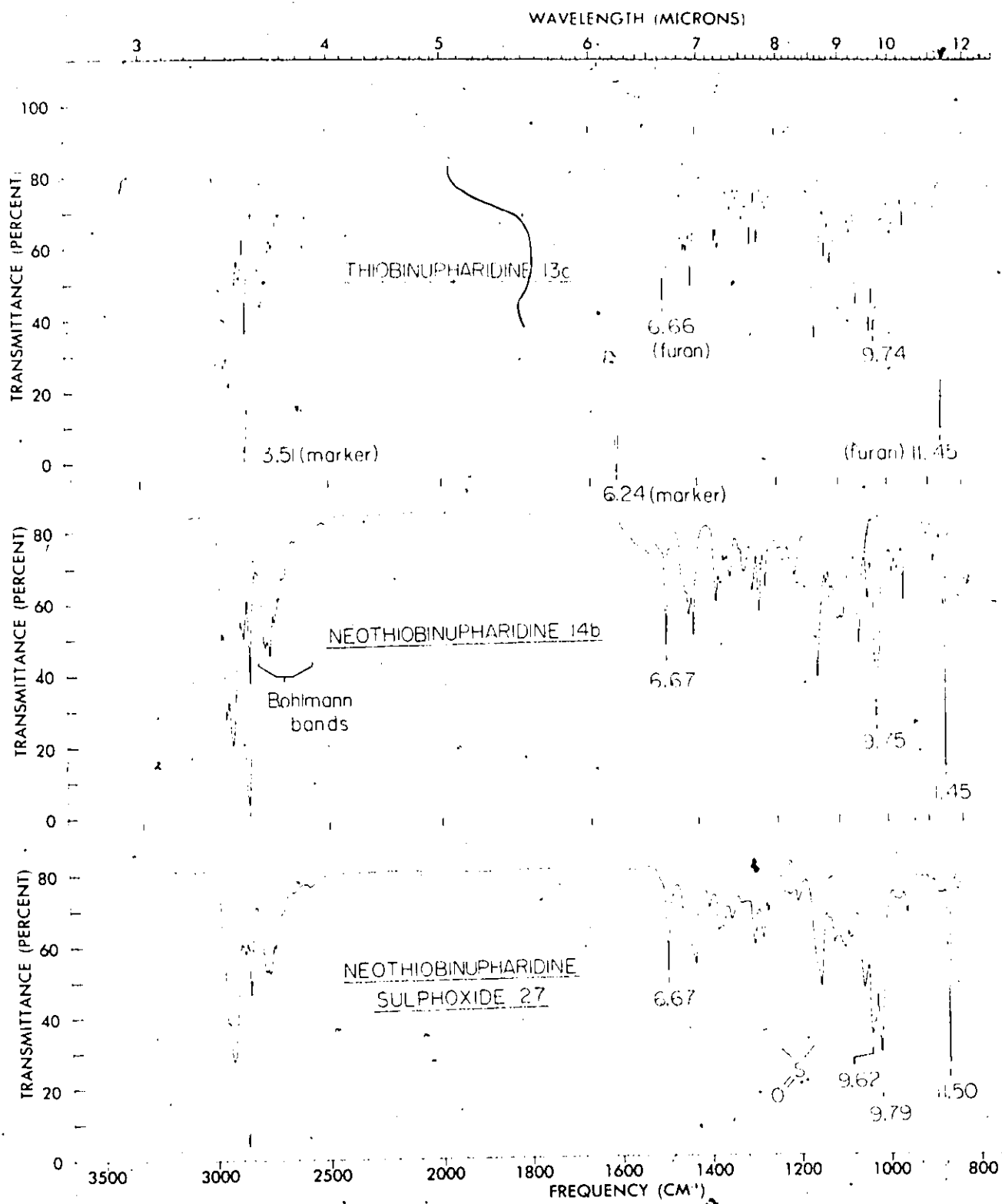
Figure 6. The i.r. spectra of thiobinupharidine (13c); neothiobinupharidine
(14b); and neothiobinupharidine sulphoxide (27), recorded in CCl₄
solution.

preliminary evidence for the equatorial nature of both of the β-furyl

groups in 13.

The mass spectra of 13 and 14 were reported some years ago

and the similarity between their low resolution spectra was observed at

that time. It was suggested that neothiobinupharidine might be distin-

guished from thiobinupharidine by the differences in the relative

intensities of their fragment ions[74]. The mass spectra recorded here

are however almost superimposable when instrumental recording conditions

are standardised. Accurate mass measurements were made to gain further

insight into the fragmentation process, and showed that the compositions

of the major ions in the spectra were identical (Table 3).

Fragmentations proposed for the bisamines 13 and 14

Many of the ions present in the spectra of 13 and 14 are also

prominent in the spectrum of deoxynupharidine 2 shown in Figure 4. In

the high mass region of the spectra of 13 and 14 there are peaks of low

intensity corresponding to the loss of CH_3, C_2H_5, and C_3H_7 from the

molecular ion, just as there are in the spectrum of 2. These fragment-

ations are analogous to those shown in Scheme 3.

Ions at m/e 461 and 447 have no counterparts in the spectrum of

2 for they correspond to the loss of SH and CH_2SH, respectively, from

the molecular ion. The important ion at m/e 359, formed by loss of

$C_9H_{11}O$ from M^{+} may be represented as in Scheme 8, and is entirely

analogous to the formation of ion m/e 98 in the fragmentation of 2. If

hydrogen transfer does not occur and the charge remains with the furan

TABLE 3

Characteristic ions in the m.s. of thiobinupharidine and neothiobinupharidine

m/e	Relative Abundance (%)		Composition	M^+ minus
	Thiobinupharidine	Neothiobinupharidine		
494	30	28	$M^+ C_{30}H_{42}N_2O_2S$	-
479	0.3	0.35	$C_{29}H_{39}N_2O_2S$	CH_3
465	0.75	0.9	$C_{28}H_{37}N_2O_2S$	C_2H_5
461	2.0	2.7	$C_{30}H_{41}N_2O_2$	SH
451	0.5	0.6	$C_{27}H_{35}N_2O_2S$	C_3H_7
447	1.6	2.8	$C_{29}H_{39}N_2O_2$	CH_2SH
427	1.1	1.3	$C_{26}H_{39}N_2OS$	C_4H_3O
359	8.5	10.5	$C_{21}H_{31}N_2OS$	$C_9H_{11}O$
316	0.9	1.2	$C_{19}H_{26}NOS$	$C_{11}H_{16}NO$
264	1.3	1.6	$C_{15}H_{22}NOS$	-
247	1.3	1.2	$M^+/2$	-
231	10	12.5	$C_{15}H_{21}NO$	-
230	35	43	$C_{15}H_{20}NO$	-
178	100	100	$C_{11}H_{16}NO$	-
136	11.5	9.5	$C_9H_{12}O$	-
107	23	20	C_7H_7O	-
94	40	38	C_6H_6O	-

25

13 and 14 (R₁ = R₄ = H)

e

m/e 494
(C₃₀H₄₂N₂O₃S)

H transfer

+ 2e

+
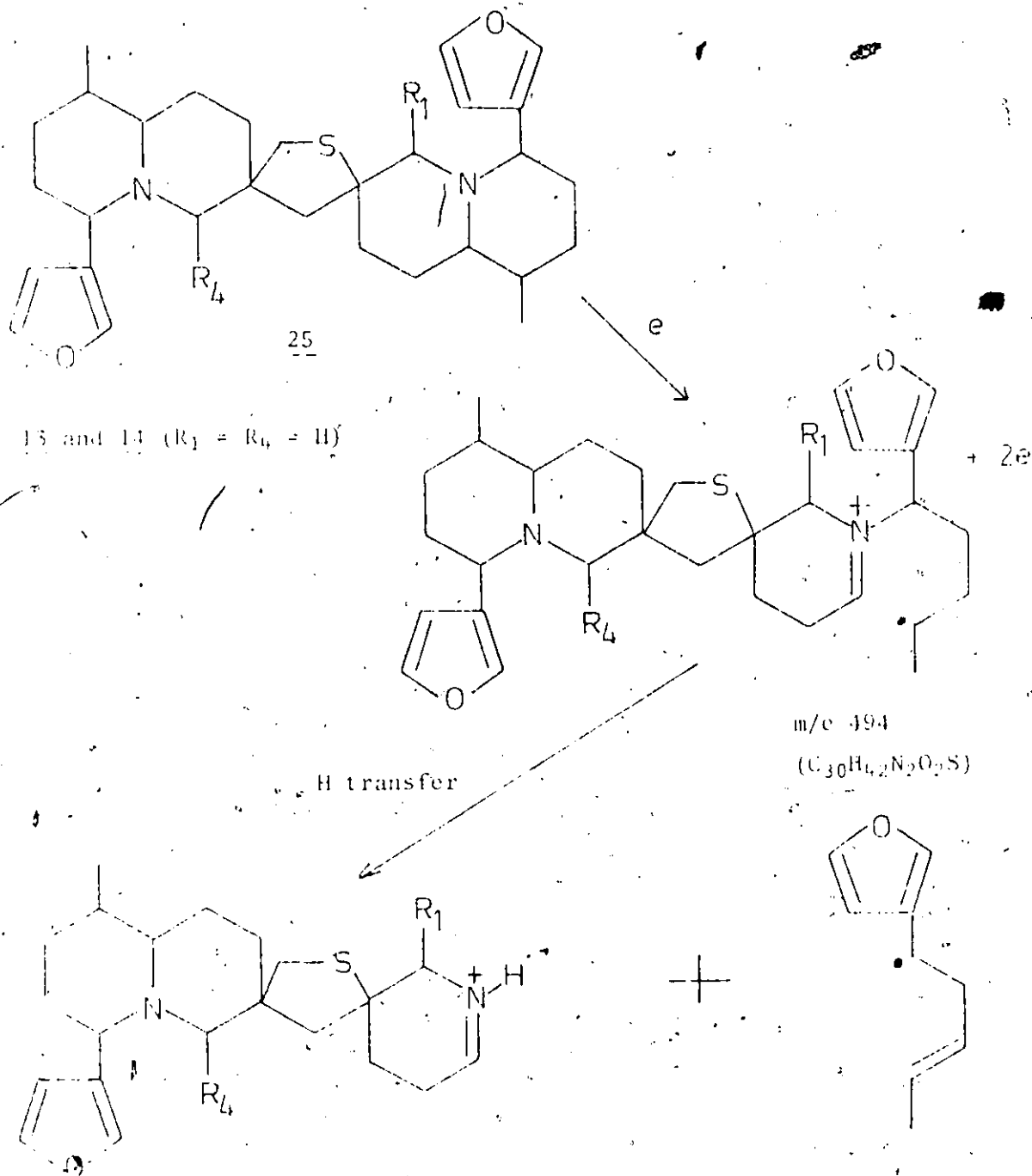
13 and 14 (R₁ = R₄ = H) m/e 359 (C₂₁H₃₁N₂OS)

29 (R₁ = OH, R₄ = H) m/e 375 (C₂₁H₃₁N₂O₂S)

31 (R₁ = D, R₄ = H) m/e 360; 32 (R₁ = R₄ = D) m/e 361

Scheme 8

moiety an ion m/e 136 results of the same mass and composition as in the spectrum of 2 (see Scheme 4).

Other intense ions present in the spectra of 13 and 14 are at m/e 230, 178, 107, and 94. Those at m/e 107 and 94 are also observed in 2 and the scheme given for their derivation may also be applied to the bisamines. The ions at m/e 230 and 178, however, deserve further comment. The former, of composition $C_{15}H_{20}NO$, can be formulated and derived as shown in Scheme 9. If hydrogen is transferred to the sulphur-containing fragment and charge is retained by this fragment, then the appearance of the ion of low intensity at m/e 264 ($C_{15}H_{22}NOS$) can be rationalised. The base peak of the spectrum of 13 and 14 is observed at m/e 178 ($C_{11}H_{16}NO$). A proposal for its derivation is found in Scheme 10. An ion of low intensity present at m/e 316 ($C_{19}H_{26}NOS$) indicates that charge is also carried by the residual fragment. The ion m/e 178 is also observed in the spectrum of 2 but is relatively weak. It seems probable that the quarternary centre present in both 13 and 14 is responsible for promoting fission of the C-6—C-7 bond giving rise to facile formation of this ion.

The fragmentation mechanisms proposed here on the basis of high resolution studies are in agreement with those proposed by LaLonde et al.[47] based on deuterium labelled compounds obtained by reduction of the 6,6'-dihydroxythionuphlutines-A and -B with NaBD$_4$.

It should be stressed that the mass spectrum of 13 indicated only that this bisamine had the same gross skeletal structure as 14 and did not provide evidence for the relative or absolute configuration of 13.

$\underline{25}$
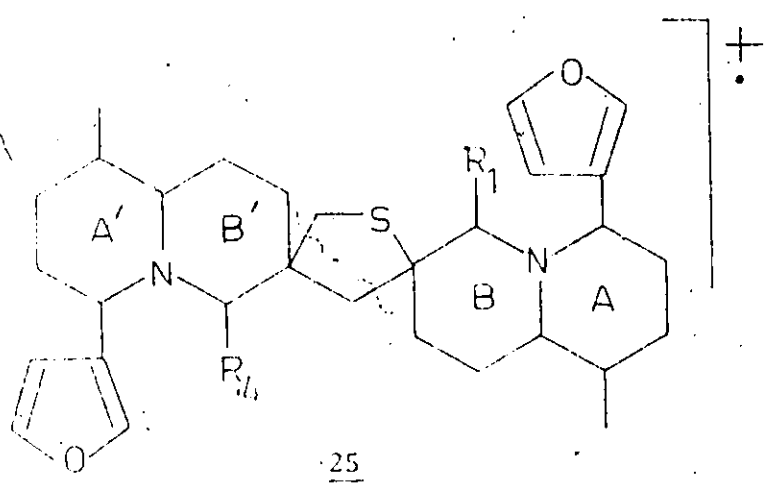
13 and 14 $(R_1 = R_4 = H)$ m/e 494 $(C_{30}H_{42}N_2O_2S)$

26 $(R_1 = OH, R_4 = H)$ m/e 510 $(C_{30}H_{42}N_2O_3S)$

H transfer

−H⋅
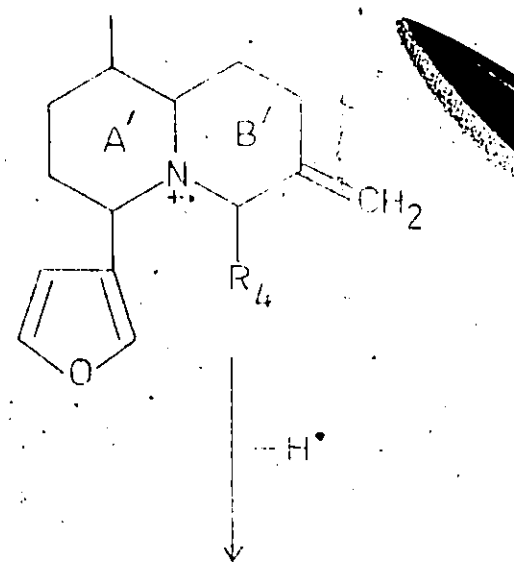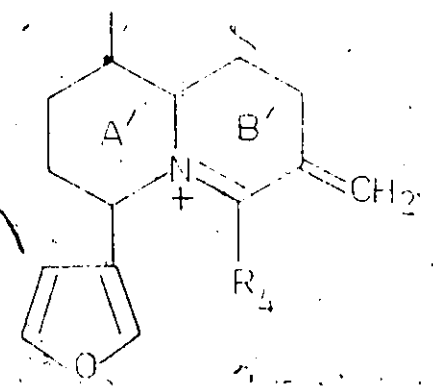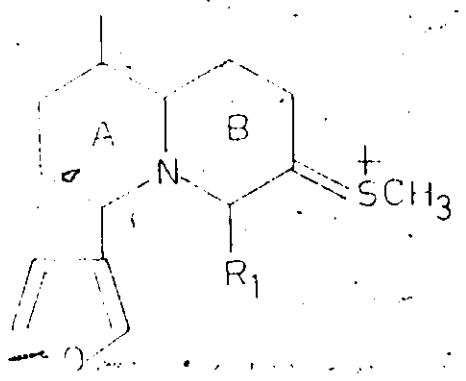
13 and 14 $(R_1 = H)$ m/e 264, $(C_{15}H_{22}NOS)$

26 $(R_1 = OH)$ m/e 280 $(C_{15}H_{22}NO_2S)$ not observed.

13, 14 and 26 m/e 250[#]

$(C_{15}H_{20}NO)$

[#] α-cleavage to sulphur would also give a fragment, m/e 230, containing rings A and B.
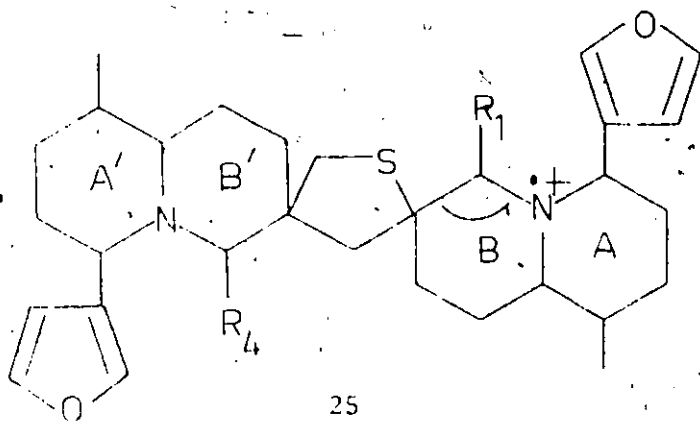
$\underline{13}$ and $\underline{14}$ $(R_1 = R_4 = H)$ m/e 494 $(C_{30}H_{42}N_2O_2S)$

$\underline{26}$ $(R_1 = OH, R_4 = H)$ m/e 510 $(C_{30}H_{42}N_2O_3S)$
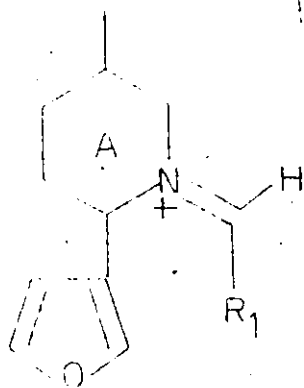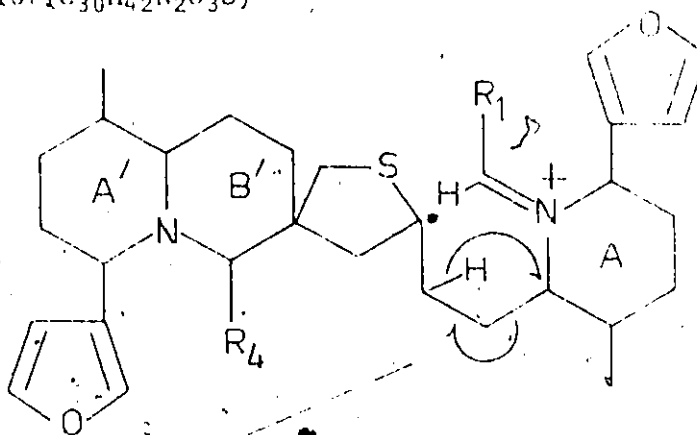
$\underline{13}$ and $\underline{14}$ $(R_1 = H)$ m/e 178 $(C_{11}H_{16}NO)$

$\underline{26}$ $(R_1 = OH)$ m/e 194

$\quad$ $(C_{11}H_{16}NO_2)$ not observed.

$\underline{31}$ and $\underline{32}$ $(R_1 = D)$ m/e 179
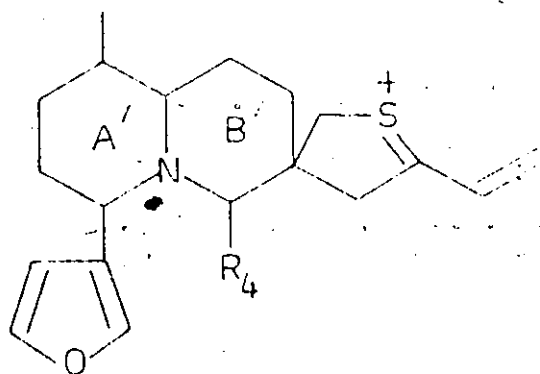
$\underline{13}$ and $\underline{14}$ $(R_4 = H)$ m/e 316 $(C_{19}H_{26}NOS)$

Scheme 10

The fragmentation mechanisms developed here were useful in the elucidation of the molecular structures of the alkaloids 26 (Figure 5) and 27 (Figure 16) as will be shown in the following sections.

The 220 MHz p.m.r. spectra of 13 and 14

The p.m.r. spectra of 13 and 14 unlike their i.r. and mass spectra showed distinct differences. The anomalies of the earlier studies[11] were clarified by recording these spectra in deuteriochloroform solution at 220 MHz (Figures 7 and 8). The interpretation of the spectra was accomplished by referring to the studies on the spectrum of deoxy-nupharidine 2[25] and on the spectra of model sulphur containing quino-lizidines[75]. Although the spectra of 13 and 14 are complex, several assignments are possible.

A doublet of area 6 centred at 0.926 (J = 5.6 Hz) is observed in the spectrum of thiobinupharidine and is assigned to the two equatorial methyl groups at C-1 and C-1'. In neothiobinupharidine a similar doublet (J = 5.5 Hz) is observed at 0.876. These resonances are very similar to that of the equatorial methyl group at C-1 of deoxynupharidine, observed at 0.896 (J = 5.6 Hz) when recorded at 220 MHz in $CDCl_3$ solution. Each of these signals exhibited a solvent-induced upfield shift when the solvent was changed from $CDCl_3$ to C_6D_6, a characteristic shift for the signals of equatorial methyl groups attached to quinolizidine systems[25,69,70]. Contrary to the earlier report there are no signals corresponding to methyl groups at quarternary carbon centres in either 13 or 14, nor are there any other signals attributable to CH_3 groups in the spectra.
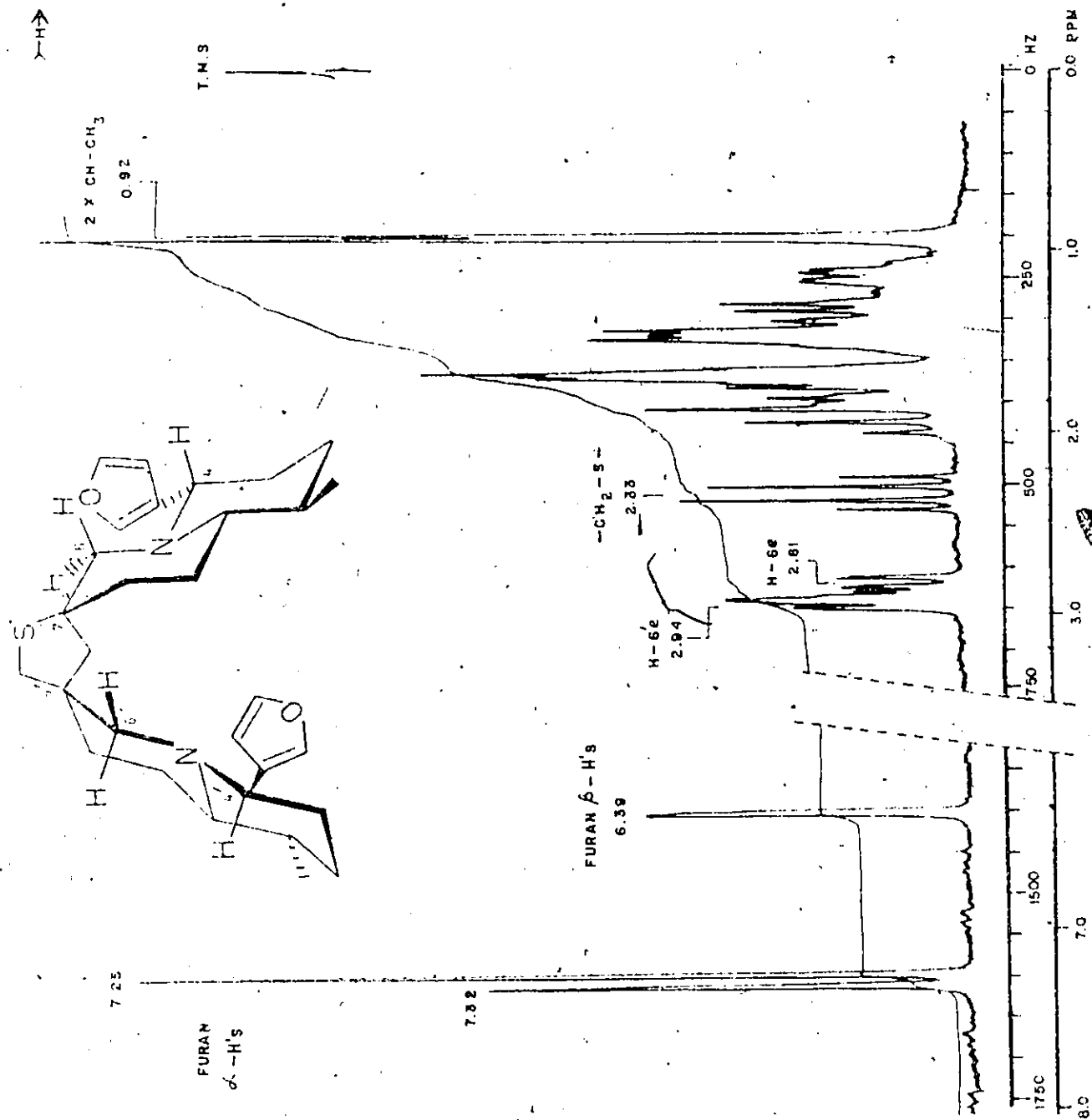
Figure 7. The p.m.r. spectrum of thiobinupharidine (13c) recorded in CDCl₃ solution at 220 MHz.
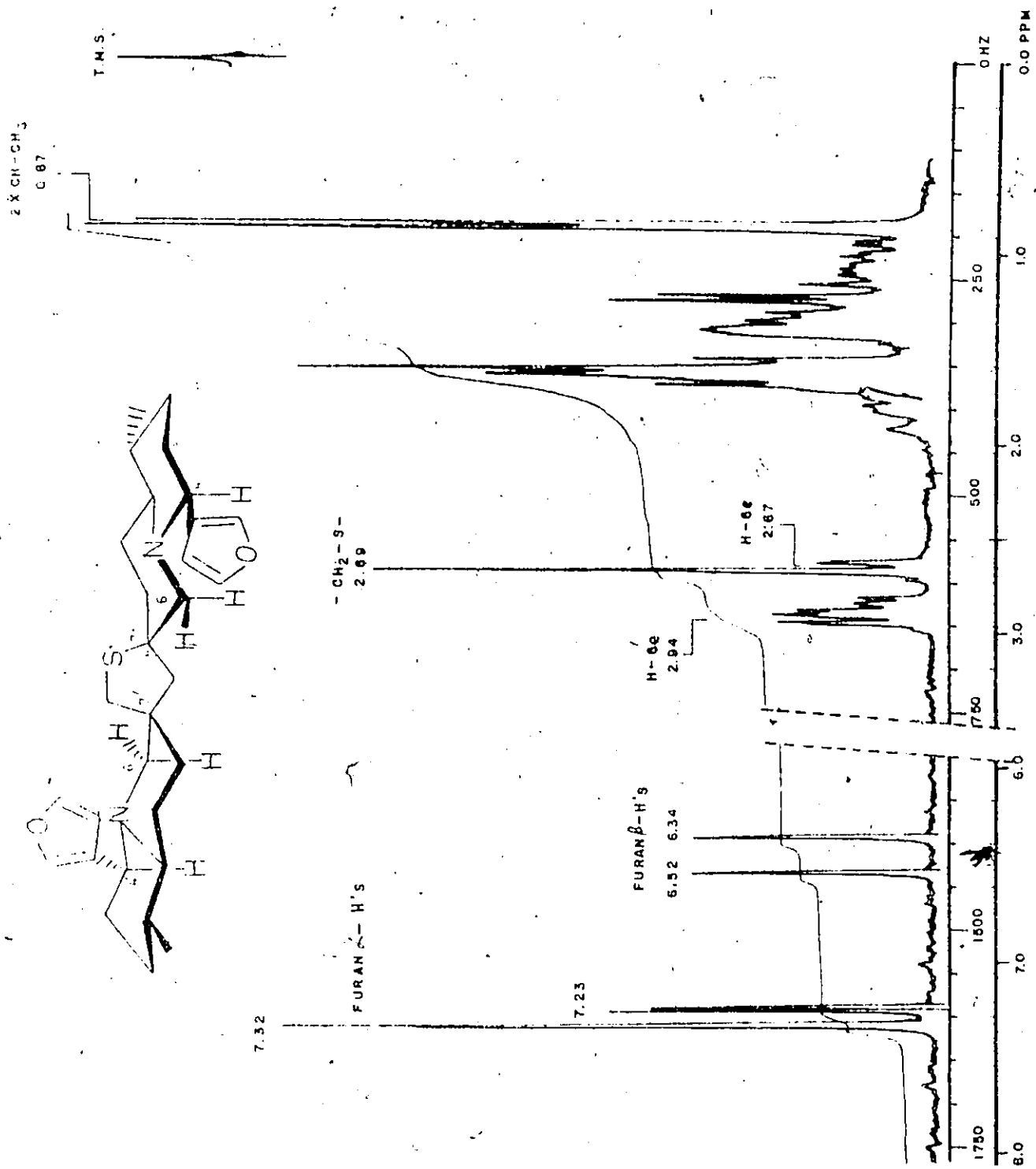
Figure 8. The p.m.r. spectrum of neothiobinupharidine (14b) recorded in CDCl₃ solution at 220 MHz.

The signals corresponding to the furan protons appear for 13 at
6.39δ assigned to the β-protons of the two furan rings and at 7.25δ
and 7.52δ assigned to the four α-protons. In the spectrum of 14 the
furan signals are more complex appearing at 6.34δ (1H) and 6.52δ (1H)
attributed to the β-protons and 7.23δ (1H) and 7.32δ (3H) assigned to
the α-protons. These assignments agreed with those made earlier[41]
when the incorrect conclusion was drawn that 13 must be a more symmetri-
cal molecule than 14.

In the spectrum of 13 there is a multiplet in the region 2.77 -
2.98δ (complex even at 220 MHz) assigned to the two axial protons α to
furan, H-4a and H-4'a, and to the two equatorial protons H-6e and H-6'e.
These assignments are made by analogy to the chemical shifts of the
corresponding protons of 2 in $CDCl_3$ solution (see experimental) which
has been studied in detail[25,69,70]. Close examination of this multi-
plet reveals that it encompasses two quartets (both with J = 11.5 and
2.0 Hz) at 2.81δ and 2.94δ. On the basis of coupling constants and
chemical shifts these are assigned to the equatorial protons H-6e and
H-6'e. Examination of the spectrum of neothiobinupharidine in these
regions shows a similar picture. Thus in the region 2.8 - 3.0δ there
appears a complex multiplet, corresponding to three protons, containing
a discernible quartet (J = 11.5 and 2.0 Hz) which is assigned either to
H-6e or H-6'e. The remainder of this multiplet is assigned to H-4a and
H-4'a combined. The other equatorial proton H-6e or H-6'e is observed
at 2.67δ partly obscured by a sharp singlet (2H) at 2.69δ.

Apart from small chemical shift differences the major difference between the spectra of 13 and 14 is in the signal assigned to the thiomethylene groups. In 13 this appears at 2.33δ as a well-defined AB quartet. In 14 however the sharp singlet at 2.69δ (previously incorrectly assigned to a methylene adjacent to nitrogen, see page 21) is assigned to the methylene adjacent to sulphur. Its singlet nature is surprising in view of the asymmetry of the molecule and must arise from accidental degeneracy of H_A and H_B of the AB system. In their studies on model compounds Lalonde et al.[75] found that axial thiomethylene groups on trans-quinolizidine systems absorb at 2.80δ while equatorial thiomethylene groups absorb at 2.40δ. The thiomethylene of 14 is known to be axial from the X-ray study[43,44] and appears at 2.69δ. The much higher field absorption of the thiomethylene group of 13 at 2.33δ suggests by analogy that it is equatorial with respect to the quinolizidine ring B'. This accounts for one of the stereochemical differences between the two molecules. Assuming that the stereochemical differences between 13 and 14 reside in the tetrahydrothiophene ring, and that the configuration at all other centres is the same (as the p m.r. data and i.r. data suggest) there appeared to be two possible structures for 13, one in which the sulphur linkage to ring B is equatorial 13c, the other in which the sulphur linkage to ring B is axial 15.

In order to solve this problem thiobinupharidine was converted to its crystalline dihydrobromide dihydrate (from which it could be recovered intact by mild basic treatment) and an X-ray study was

initiated. .The results of this study proved that the relative and

absolute configuration of thiobinupharidine is as shown in 13c (Figure 5).

It was while the X-ray study was in progress that thionuphlutine-

A was shown to be identical with thiobinupharidine[48]. LaLonde had

suggested that thionuphlutine-A has the structure 13c based on his

study of the reduction products of the dihydroxythionuphlutines[47,54].

In this study a stereoselective mode of deuterium incorporation was

observed upon sodium borodeuteride reduction of 6,6'-dihydroxythiobinu-

pharidine 16 and 6,6'-dihydroxythionuphlutine-B 17. The p.m.r. spectra

of the reduction products recorded at 60 MHz in CDCl$_3$ showed the incor-

poration of a deuterium into the equatorial position at C-6 of thiobinu-

pharidine-6,6'-d$_2$ 28 but incorporation of a deuterium into the axial

position at C-6 of thionuphlutine-B-6,6'-d$_2$ 29. It was suggested that

the sulphur atom participated in the reduction by directing the attack

of the reducing species on an intermediate immonium ion. The observed

stereoselectivity in the deuterium incorporation was accepted as

evidence for an equatorial sulphur at C-7 of 13c and for an axial sulphur

at C-7 of 15. Further comment on the mechanism and utility of this

stereoselective reduction is found in later sections of this thesis.

Very recently LaLonde et al. have provided further evidence for

the relative[76] and absolute[77] configurations of 13 and 15. Circular

dichroism studies were performed on α-thioimmonium ions derived from

16 and 17 by treatment with perchloric acid. Similar studies were carried

out on α-thioimmonium ions generated from 7-methylthiodeoxynupharidin-

6-ols. The results demonstrated that structures 13c and 15

represent the absolute configurations of thiobinupharidine and thionuphlutine-B, respectively (Figure 5).

It is now apparent that structures 13c and 15 are the result of combination of two "deoxynupharidine-like" quinolizidine moieties, belonging to the same enantiomeric series as either (-)-deoxynupharidine or (-)-7-epideoxynupharidine, through a central tetrahydrothiophene ring to give a gross skeleton as in 25 ($R_1 = R_4 = H$). Moreover 13c and 15 represent two of only four unique diastereomers which can be constructed in this manner. Structure 14b and structure 30 represent the two remaining possible combinations.

It seems very likely that the biosynthetic system constructing these dimeric alkaloids uses the same precursors and it is for this reason that the absolute configuration 14b rather than its antipode is preferred for neothiobinupharidine. The compound of structure 30 has not yet been isolated.

COMPREHENSIVE ASSIGNMENTS IN THE P.M.R. SPECTRA OF THIOBINUPHARIDINE (13c)

AND NEOTHIOBINUPHARIDINE (14b)

Preliminary studies in this laboratory have indicated that the
alkaloids 26 and 27 are oxidised derivatives of 13c and 14b, respectively;
furthermore it seemed probable that conversion to the parent bisamines
by metal-deuteride reduction might be accomplished with concomitant
introduction of deuterium labels. Clearly any assessment of stereo-
selectivity in deuterium incorporation is facilitated by a full assign-
ment of the protons in the p.m.r. spectrum of the reduction product.
For the studies of the reduction of the hemiaminals of thiobinupharidine
it is particularly important to assign chemical shifts to the protons on
the carbons adjacent to the nitrogen atoms of 13c in order to determine
which of these are replaced in the deuterated products.

As the structure of thiobinupharidine is now firmly established
a more detailed analysis of its p.m.r. spectrum was possible. Accordingly
the 220 MHz p.m.r. spectrum of 13c was recorded in C_6D_6 solution, in
which the signals are well resolved[76] (Figure 9) when compared to
their complexity in $CDCl_3$ (Figure 7). The assignments made for 13c are
based on analogy with those of the corresponding protons of deoxynuphar-
idine 2 and 7 epideoxynupharidine 11.

The p.m.r. spectrum of 2 has been studied in detail[25,69,70]
and no ambiguity exists with respect to the signals assigned to the
equatorial methyl at C-1, the axial methyl at C-7 and the protons H-4a,
H-6a, H-6e and H-7e. As the majority of these assignments were made in
deuteriochloroform solution, the spectrum of 2 was recorded in deuterio-
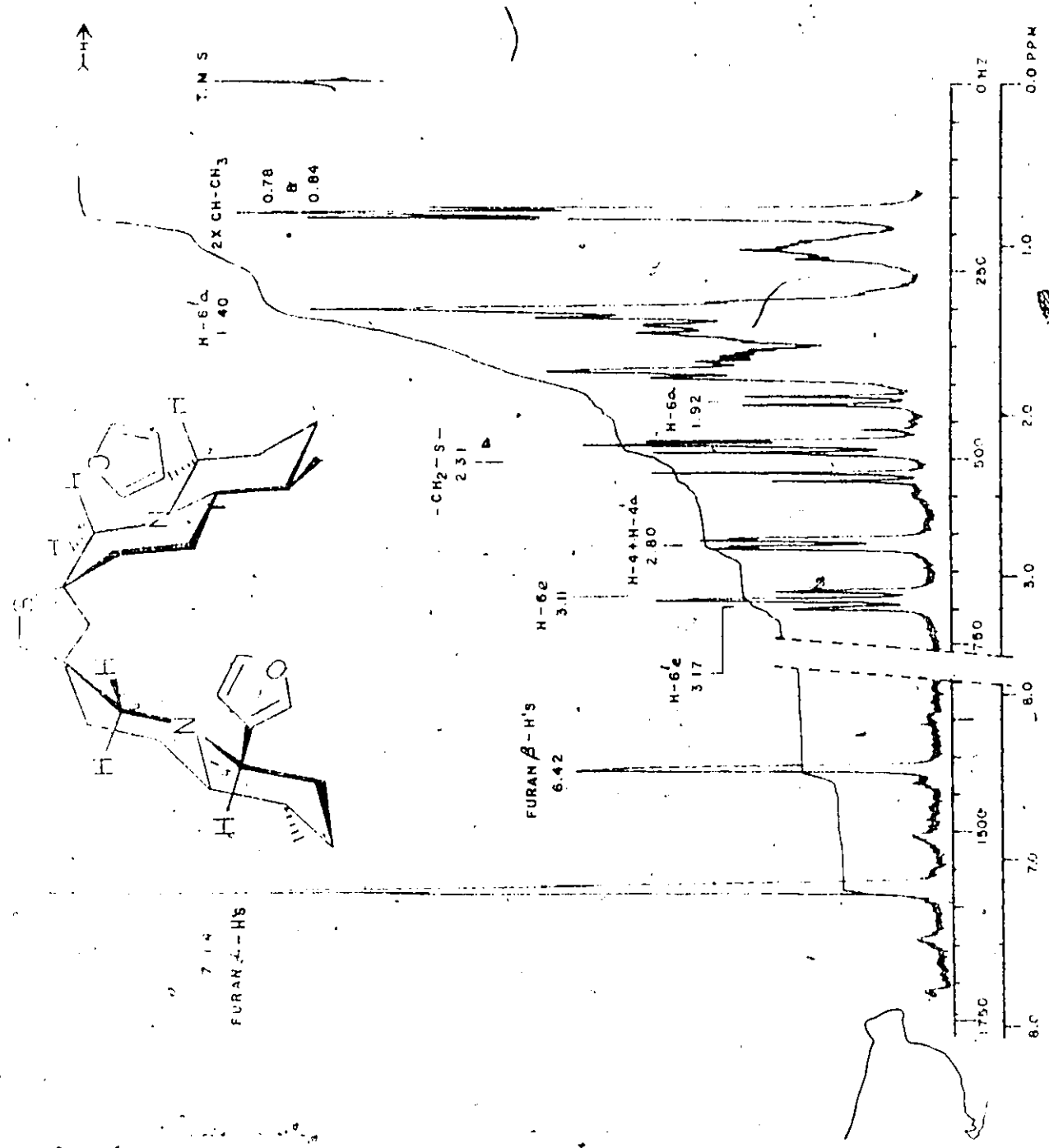
Figure 9. The p.m.r. spectrum of thiobimpharidine (13c) recorded in C_6D_6 solution at 220 MHz.

benzene solution at 220 MHz and the proton assignments were made using spin decoupling techniques. These assignments are shown in Table 4.

The structure and stereochemistry of <u>11</u> follow from the fact that it is formed along with <u>2</u> when Δ^6-dehydrodeoxynupharidine is treated with hydrogen over a Pd catalyst[70]. Its p.m.r. spectrum in $CDCl_3$ and C_6D_6 has been studied but very few resonances were assigned[22,38]. The spectrum of 11 was recorded at 220 MHz in C_6D_6 solution and the assignments shown in Table 5 were made through extensive spin-decoupling experiments.

The chemical shifts and coupling constants of the low field signals in the 220 MHz spectrum of thiobinupharidine in C_6D_6 solution are listed in Table 4. Inspection of Tables 4 and 5 shows that there are many similarities. Thus the quartet at 2.80δ (2H) in the spectrum of 13c can be assigned to H-4a together with H-4'a by analogy to the coupling constants and chemical shifts of H-4a in both <u>2</u> and <u>11</u>. By similar analogy the quartets at 3.11δ and 3.17δ of 13c must be due to H-6e and H-6'e. Spin-decoupling experiments show that the quartets at 3.11 and 3.17δ are coupled to doublets at 1.92δ and 1.40δ, respectively, thus inferring that the latter two signals are those of H-6a and H-6'a. It remains only to identify specifically each of these four protons. This has been achieved, as shown in the next section, through an assessment of the effect that an equatorial alkyl substituent at C-7 has on the protons at C-6.

TABLE 4

Assignment of the signals in the p.m.r. spectra of deoxynupharidine (2c)
and thiobinupharidine (13c) in C_6D_6 solution

13c   2c
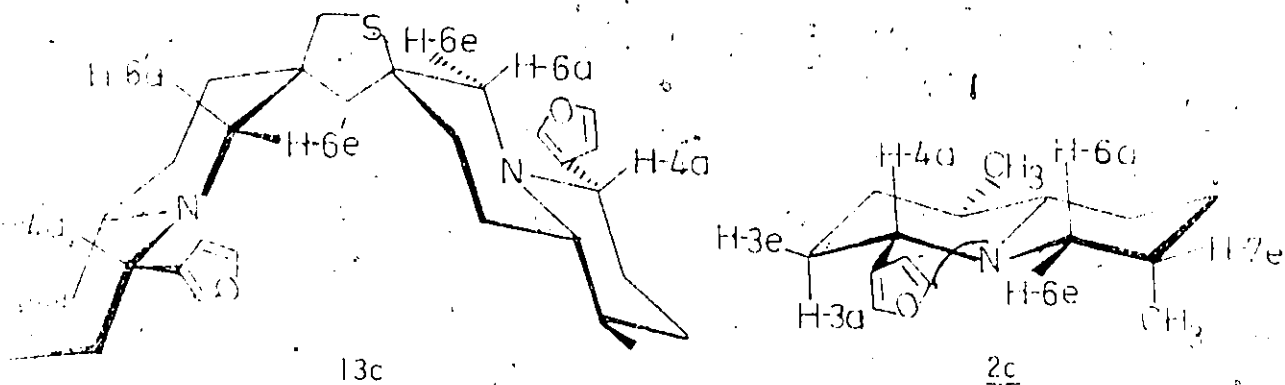
deoxynupharidine

Proton	Chem Shift (δ)	Multiplicity	Coupling Constants J (Hz)
Methyl e	0.81	d	6.0
Methyl a	1.10	d	7.0
H-6a	1.78*	q	11.5 (6a-6e) and 3.0 (6a-7e)
H-6e	2.76	q	11.5 (6e-6a) and 2.5 (6e-7e)
H-4a	2.81	q	11.0 (4a-3a) and 3.0 (4a-3e)

*irradiation at 1.78 collapses quartet at 2.76 into broad singlet

thiobinupharidine

Proton	Chem Shift (δ)	Multiplicity	Coupling Constants J (Hz)
Methyl e	1.40[§]	d	11.5 (6'a-6'e)
Methyl e	1.92[#]	d	11.5 (6a-6e)
H-6a	3.11	q	11.5 (6a-6e); 2.0 (6e-8e)
H-6'a	3.17	q	11.5 (6'a-6'e); 2.5 (6'e-8'e)
H-4a	2.80	q	10.5 (4a-3a); 3.5 (4a-3e)
CH₂	4.18	ABq	J_{AB} = 14.0
CH₂	2.31	ABq	J_{AB} = 11.5

[§] Irradiation at 1.40 collapses q at 3.17 into broad singlet, q at 3.11 unaffected

[#] Irradiation at 1.92 collapses q at 3.11 into broad singlet, q at 3.17 unaffected

TABLE 5

Assignment of the signals in the p.m.r. spectrum of 7-epideoxynupharidine (11) in C$_6$D$_6$ solution
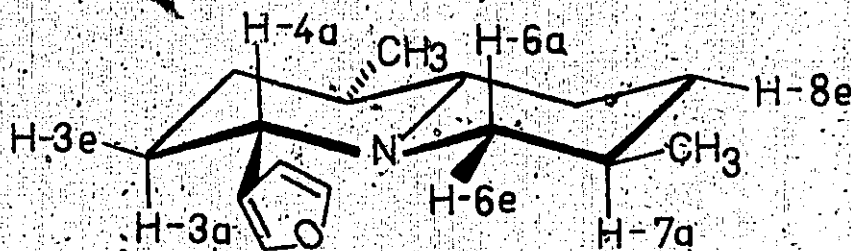
11

Proton	Chem Shift (δ)	Multiplicity	Coupling Constants J (Hz)
C-7 Methyl e	0.68[a]	d	6.5
C-1 Methyl e	0.83	d	6.0
H-6a	1.29[ψ]	t (1:2:1)	11.0 (6a-6e) and 11.0 (6a-7a)
H-7a+H-3e+H-8e	1.64[†]	complex mult	
H-3a	1.77[#]	mult	11.0 (3a-4a) recognisable
H-4a	2.83[¶]	q	11.0 (4a-3a) and 3.5 (4a-3e)
H-6e	3.03[§]	d of q	11.0 (6e-6a) and 3.5 (6e-7a) and 2.0 (6e-8e)

[a] Irradiation at 0.68 induced changes in the region 1.64

[†] Irradiation at 1.64 collapsed doublet at 0.68 into singlet; also collapsed quartet at 2.83 into doublet (J = 11.0 Hz); also collapsed doublet of quartet at 3.03 to doublet (J = 11.0 Hz).

[#] Irradiation at 1.77 collapsed quartet at 2.83 to a doublet (J = 5.5 Hz).

[¶] Irradiation at 2.83 collapsed the 11.0 Hz coupling in signal at 1.77.

[ψ] Irradiation at 1.29 collapsed doublet of quartet at 3.03 to multiplet with couplings 2.0 and 3.5 Hz recognisable.

[§] Irradiation at 3.03 collapsed 1:2:1 triplet at 1.29 to a 1:1 doublet (J = 11.0 Hz).

The differential shielding of the methylene protons adjacent to hetero-atoms and equatorial substituents

Examination of the chemical shifts of the protons of the methyl-ene group adjacent to nitrogen, in both 2 and 11, shows that there is an abnormally large chemical shift difference between the axial and equatorial protons ($\Delta\delta$ = δH-6e - δH-6a). The magnitude of $\Delta\delta$ is large for 2 (0.98 p.p.m.) but significantly larger for 11 (1.74 p.p.m.). There are two $\Delta\delta$ values for thiobinupharidine, established as 1.19 p.p.m. and 1.77 p.p.m. from spin-decoupling studies.

The difference in $\Delta\delta$ values for 2 and 11 is at first sight sur-prising as the only structural difference in the molecules, assuming free rotation of the furyl group, is the relative configuration about C-7. However there is precedence both for the magnitude of $\Delta\delta$ in similar heterocyclic systems and for the effect of an adjacent methyl group on the magnitude of the observed value of $\Delta\delta$.

The abnormally large chemical shift, $\Delta\delta$ = 0.93 p.p.m. between the protons H-4a and H-4e in the p.m.r. spectrum of trans-quinolizidine (78,79) (equivalent to H-6a and H-6e of 2 and 11) has been attributed to shielding of the axial proton because of partial participation of the lone-pair of electrons of nitrogen in a σ^* C-Ha orbital of the adjacent carbon atom[79];
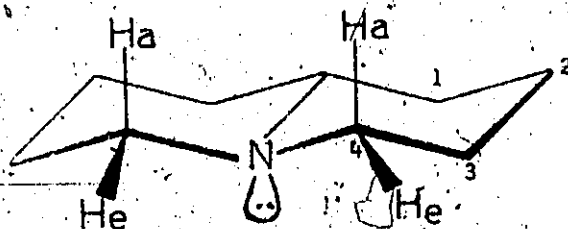
trans-quinolizidine

This leads to an increase in electron density at the axial proton. The value of $\Delta\delta$ should therefore be greatest when the nitrogen lone-pair and the adjacent C-H bond have a trans diaxial relationship. The proposal involving the lone-pair of nitrogen is supported by the decreased shielding observed in protonated salts of quinolizidine, whose $\Delta\delta$ values are about 0.5 p.p.m., comparable to that observed for the axial and equatorial protons in cyclohexane. It has been suggested[79] that the decrease in bond energy of the C-H bond α to nitrogen is responsible for the appearance of the Bohlmann bands in the i.r. spectra of trans-quinolizidines. An explanation of the anti-axial shielding effect of a proton by a lone-pair in terms of a dipole effect has also been offered[80].

Recently[81], an alternative explanation has been given for the large $\Delta\delta$ value for the methylene protons adjacent to nitrogen in the quinolizidine system. Lambert[82], in his studies on the "size of the nitrogen lone-pair" in piperidine and N-alkyl piperidines, has stated that "a small isotropic substituent, e.g. methyl, at the equatorial site of an adjacent atom will have little effect on $\Delta\delta$ since it is almost equivalently positioned with respect to both the equatorial and axial hydrogens of the methylene group". Robinson[81] has strongly disputed this statement on the grounds that it is not supported by all the available evidence. He further states that "it is apparently general that an equatorial methyl group shields the protons of an adjacent methylene group differently, and in all the unambiguous examples, which cover a wide range of environments for methylene groups, the axial hydrogen is

more shielded". Some examples are shown in Table 6 . . Consequently the large chemical shift difference between the methylene protons adjacent to the nitrogen atom in trans-quinolizidine itself may be explained in terms of shielding of the axial proton by the other methylene group attached equatorially to the nitrogen, rather than shielding caused by the lone-pair.

The relative importance of the two shielding mechanisms has recently been discussed by Lambert[83] who has reached the conclusion, as have others[84], that the lone-pair of nitrogen gives rise to a significant amount of the shielding but that equatorial substituents on atoms adjacent to the methylene group must also cause significant shielding of the axial proton.
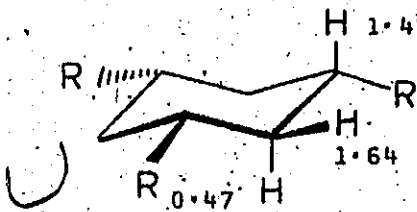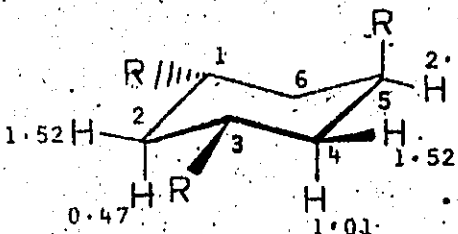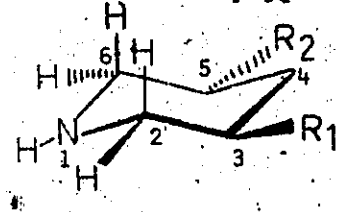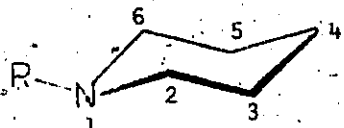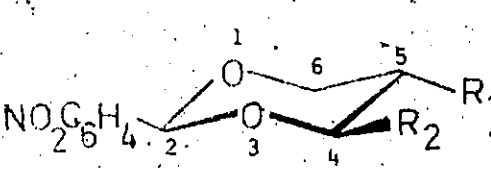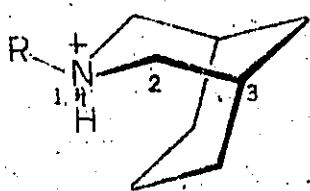
A study of the 3-azabicyclo[3.3.1.]nonane system, in which the nitrogen lone-pair occupies the endo position[81,85], showed that Δδ (C-2) is less than 0.1 p.p.m.[82].
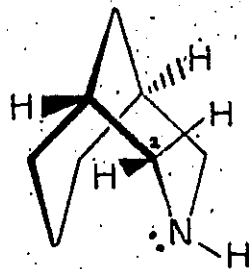
This suggests that an axial methyl or methylene group on C-3 of six-membered nitrogen containing heterocycles will reduce the value of Δδ for the methylene protons adjacent to nitrogen. This phenomenon has also been observed with substituted piperidines having axial methyl substituents[86].

TABLE 6

Differential shielding effects of equatorial methyl groups on the Δδ values of vicinal methylene protons in six membered ring systems

Compound	Δδ	Solvent	Ref.

R = H   0.48 ppm

R = CH$_3$ 1.17 ±0.1 ppm   CCl$_4$ (87)

R = H   0.48 ppm

R = CH$_3$ 1.05 ppm (C$_2$)   CCl$_4$ (87)
         0.51 ppm (C$_4$)

R$_1$ = R$_2$ = H   0.42 - 0.48 ppm   various
         (C$_2$ and C$_6$)

R$_1$ = CH$_3$;   0.79 (C$_2$) and   (82,88)
R$_2$ = H   0.48 (C$_6$)

R$_1$ = R$_2$ = CH$_3$ 0.90 (C$_2$ and C$_6$)   CDCl$_3$ (86)

R = H   0.42 - 0.48 ppm
         (C$_2$ and C$_6$)   various (82)

R = CH$_3$ 0.91 - 1.1
         (C$_2$ and C$_6$)

R$_1$ = R$_2$ = H   0.29 (C$_4$)

R$_1$ = CH$_3$;   0.69 (C$_4$)   (81)
R$_2$ = H

R = H $\overline{0}$ (C$_2$)

R = CH$_3$ 0.38 (C$_2$)   D$_2$O   (85)

Where numbers are placed next to protons this indicates the chemical shifts (δ) of these protons only in those molecules where R = CH$_3$

It is now appropriate to attempt an assessment of the manner in which various substituents present in molecules 2 and 11 affect the Δδ values of their methylene protons at C-6. Both molecules exist in a trans-fused quinolizidine conformation, and H-6a therefore bears a trans diaxial relationship to the nitrogen lone-pair. C-4 of each molecule represents an equatorial substituent on the nitrogen atom. Thus both these effects may be expected to lead to a large value of Δδ because H-6a will be abnormally shielded in both molecules. The furan carbon bonded to C-4 and H-6e have a near 1:3-diequatorial relationship and deshielding of this latter proton is expected by the van der Waal's interaction [89,90], thus increasing the value of Δδ.

The equatorial methyl on C-7 of 11 should shield H-6a resulting in a further increment in Δδ for that compound, whereas the axial methyl on C-7 of 2 is expected to deshield H-6a, leading probably to a small decrease in the magnitude of Δδ. There may be an anisotropic effect from the 3-furyl group on C-4, however this is small at least to the extent that it affects groups at C-7 [75]; furthermore, assuming free rotation of the 3-furyl group about its bond to C-4, any anisotropic effect may be expected to be the same for both 2 and 11.

Thus it seems that the equatorial methyl group on C-7 of 11 is responsible for the larger chemical shift difference (Δδ = 1.74 p.p.m.) observed between the protons H-6e and H-6a when compared to the value of Δδ (0.98 p.p.m.) observed in 2 where the methyl group is axial at C-7.

An examination of the structure of thiobinupharidine, 13c (Figure 5) reveals that the A'B' quinolizidine system bears an equatorial methylene

and an axial methylene at C-7' whereas the AB quinolizidine bears an equatorial sulphur and an axial methylene at C-7. A space filling molecular model shows that the lone-pair electron density of sulphur lies close to H-6a and is expected to deshield this proton relative to H-6'a. Similar effects are observed whenever heteroatoms with unshared electron pairs are close to hydrogen atoms[81] *.

For the reasons stated above the assignments for the protons of thiobinupharidine in C_6D_6 solution are made as shown in Figure 9. Thus H-6'e and H-6'a are assigned to 3.17δ and 1.40δ respectively (Δδ = 1.77 p.p.m.) and the protons H-6e and H-6a are assigned to the signals at 3.11δ and 1.92δ respectively (Δδ = 1.19 p.p.m.). These assignments are in agreement with those made recently on the basis of u.v. and sodium borodeuteride reduction studies of hemiaminals of thiobinupharidine, and p.m.r. studies of model β-thiohemiaminals[76].

Having firmly established the assignments for the protons at C-6 and C-6' of thiobinupharidine in C_6D_6 solution, the spectrum of this compound in $CDCl_3$ was re-examined. Using spin-decoupling techniques and the results of sodium borodeuteride reduction of hemiaminals of thiobinupharidine (see later sections), it was possible to establish specifically the chemical shifts of the protons H-6a, H-6e, H-6'a, and H-6'e in this solvent (Figure 7).

---

* This same effect explains the low field appearance of the signal (2.18δ in C_6D_6) due to the methylene group C-7 - CH_2 - C-7' in thiobinupharidine. A space filling molecular model shows that these protons are "sandwiched" between the lone-pairs of electrons on the nitrogen atoms.

The p.m.r. spectrum of neothiobinupharidine, 14b, was recorded in C_6D_6 solution at 220 MHz. The equatorial and axial protons at C-6 and C-6' were identified by their spin-spin splitting patterns, their chemical shifts and by spin-decoupling techniques. Similarly the signals arising from the axial protons at C-4 and C-4' and the thio-methylene protons were identified (Figure 10 and experimental section).

In contrast to thiobinupharidine (Figure 9) it was observed that the protons H-6a and H-6'a of neothiobinupharidine have similar chemical shifts. This is not unexpected since an examination of the structure of 14b shows that both the AB and A'B' quinolizidine moieties bear an equatorial methylene group, common to both C-7 and C-7', which is expected to shield the axial protons H-6a and H-6'a to approximately the same extent. The values of Δδ (H-6e - H-6a and H-6'e - H-6'a) are similar, 1.33 and 1.50 p.p.m. respectively, whereas they were quite different for thiobinupharidine. The similarity and magnitude of these Δδ values is again an expected result.

The protons H-6'e and H-6e of neothiobinupharidine have quite different chemical shifts (3.21 and 2.88 respectively in C_6D_6), whereas their chemical shifts were similar in 13c. It was anticipated that the chemical shifts of H-6'e and H-6'a, and hence the Δδ value between them, would be similar in both 13c and 14b as the configuration about C-7' is very similar in both molecules. The chemical shift of H-6'e of 13c is established as 3.17δ in C_6D_6 and 2.94δ in $CDCl_3$; furthermore H-6'e of 13c appears downfield from H-6e in both solvents (Figures 7 and 9 J. Thus in the p.m.r. spectra of neothiobinupharidine (Figures 8 and 10),
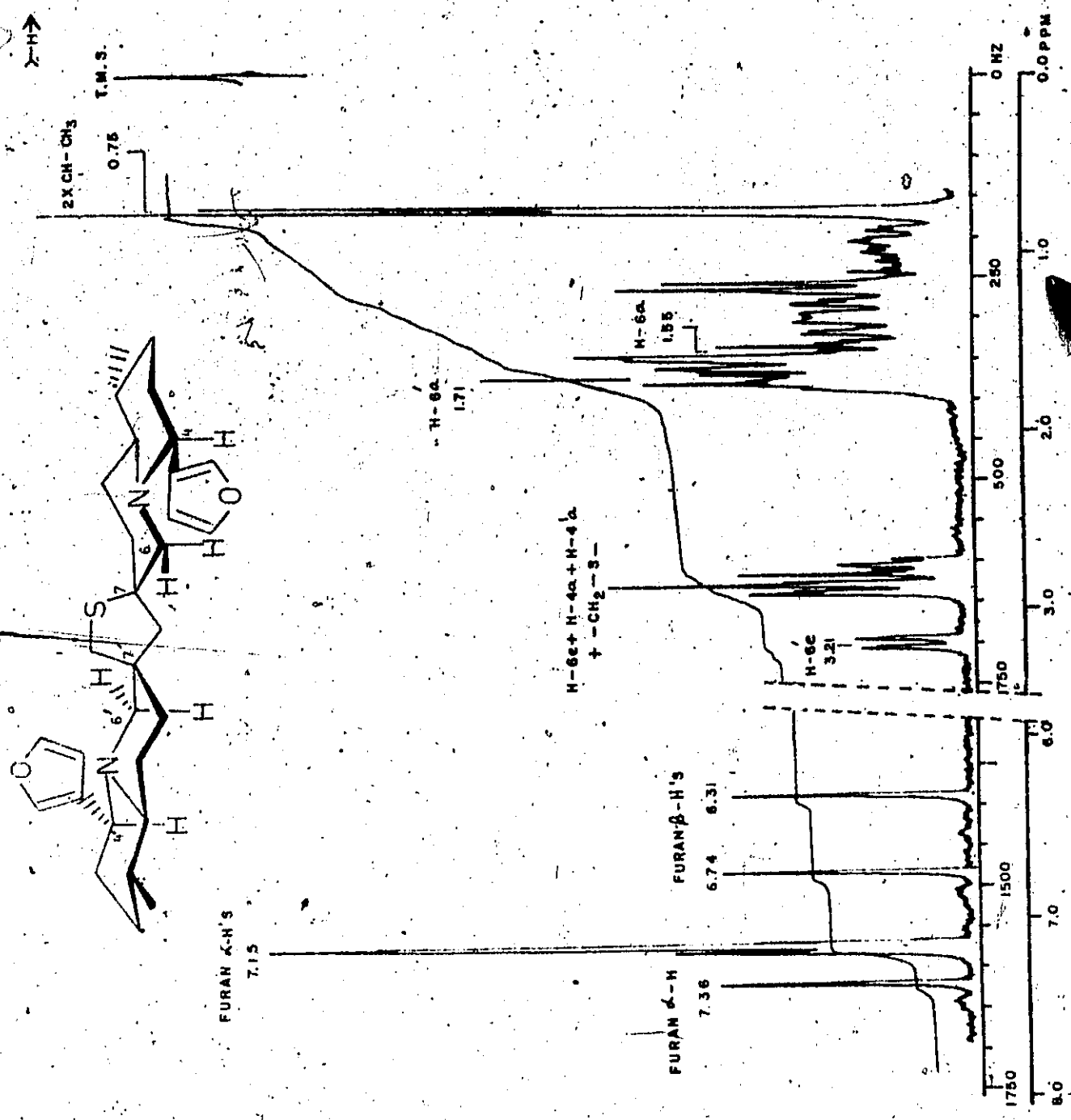
Figure·10. The p.m.r. spectrum of neothiobinupharidine (14b) recorded in C₆D₆ solution at 220 MHz.
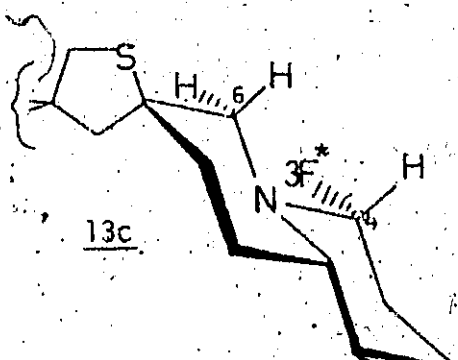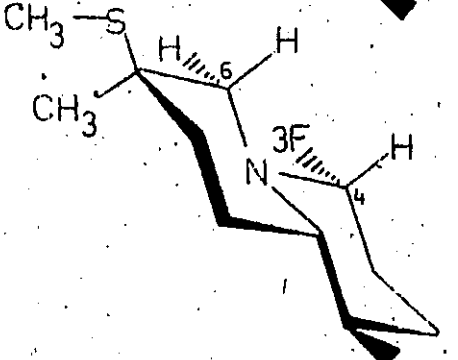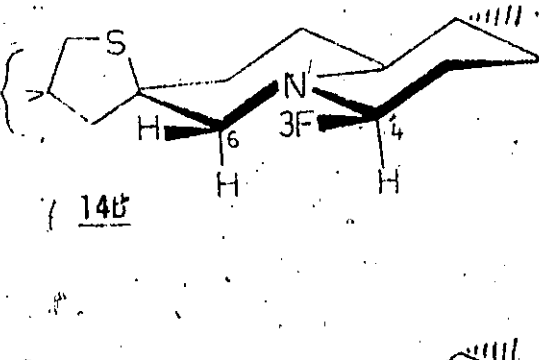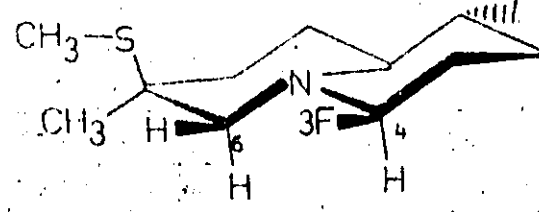
the signals at 3.21δ (C_6D_6) and 2.94δ ($CDCl_3$) are assigned to H-6'e. The chemical shifts of H-6e, H-6'a and H-6a follow from the appearance of their characteristic spin-spin splitting patterns and from spin-decoupling experiments. These assignments are shown in Figures 8 and 10 and are further amplified in the experimental section. The Δδ value H-6'e - H-6'a for neothiobinupharidine in C_6D_6 is therefore established as 1.50 p.p.m. and the magnitude of this value is consistent with the corresponding value observed for these protons in thiobinupharidine (1.71 p.p.m.).

Further evidence that the protons of neothiobinupharidine have been assigned correctly may be found in Table 7 which shows a comparison of the solvent shifts (Δs.s.) observed for the protons H-6a and H-6e of 13c, 14b and model compounds[76] upon changing the solvent from $CDCl_3$ to C_6D_6. The compounds 7-α-methylthio-7-epideoxynupharidine and 7-β-methylthiodeoxynupharidine, shown in Table 7, are suitable models for the AB quinolizidine moieties of neothiobinupharidine and thiobinupharidine respectively. Clearly the solvent shifts observed are consistent with the assignments proposed above. It is not possible, however, to make a straightforward correlation between the chemical shifts observed for H-6a and H-6e of 14b and those observed for the corresponding protons of its model compound.

An examination of the assignments proposed above for 14b shows that H-6e is shielded relative to H-6'e. An attempt was made to use the shielding cones arising from the diamagnetic anisotropy of the oxygen atom of ethers[91] as a model for predicting the effect of the sulphur

TABLE 7

Solvent shifts ($\Delta_{s.s.} = \delta_{CDCl_3} - \delta_{C_6D_6}$) observed for the protons at C-6
of thiospiran alkaloids and model sulphur-containing quinolizidines

Compound		H-6e	H-6a
13c	CDCl$_3$	2.79δ	1.70δ
	C$_6$D$_6$	3.11δ	1.92δ
	$\Delta_{s.s.}$	-0.32 ppm	-0.22 ppm
7-β-methylthiodeoxynupharidine (76)	CDCl$_3$	2.75δ	1.78δ
	C$_6$D$_6$	3.04δ	1.93δ
	$\Delta_{s.s.}$	-0.29 ppm	-0.15 ppm
14b	CDCl$_3$	2.67δ	1.60δ
	C$_6$D$_6$	2.88δ	1.55δ
	$\Delta_{s.s.}$	-0.21 ppm	+0.05 ppm
7-α-methylthio-7-epideoxynupharidine (76)	CDCl$_3$	2.89δ	1.62δ
	C$_6$D$_6$	3.05δ	1.57δ
	$\Delta_{s.s.}$	-0.16 ppm	+0.05 ppm

*3F = 3-furyl group

atom in close proximity to H-6e. The molecule is conformationally labile, to a certain extent, through puckering of the central five-membered thiophane ring; consequently it is not clear exactly to what extent H-6e might lie within a shielding cone of the sulphur atom. The assignments made for H-6e and H-6'e of neothiobinupharidine should, however, be regarded as tentative at this time.

Attempted observation of Nuclear Overhauser Effects (n.O.e.'s)

Extensive measurements of the internuclear distances between the protons of both neothiobinupharidine and thiobinupharidine were made directly from Dreiding models and are recorded in Table 8. Examination of this table shows that in many cases the protons H-6e and H-6'e are in close proximity to certain neighbouring protons. It was anticipated that for these interactions (checked in Table 8) an internal nuclear Overhauser effect[92,93,94] might be observed which would consequently differentiate between H-6e and H-6'e.

Bell and Saunders[94] have defined some criteria for the observation of an internal n.O.e., namely, that the internuclear distance between the protons under consideration, say A and B, should normally not be more than 3.0 Å, that the compounds under investigation should be in a state of strict analytical purity, that the solutions should be dilute in order to minimise molecular association, and that the solutions should be free of paramagnetic oxygen which is capable of participation in a relaxation mechanism. Accordingly 2-3% w/v solutions were prepared in dry C_6D_6 and subjected to six freeze-thaw cycles (liquid N_2) while under high vacuum before sealing the p.m.r. tubes. The solute samples were

TABLE 8

Internuclear distances between protons of importance in
neothiobinupharidine 14b and thiobinupharidine 13c*

Interaction between protons	Internuclear distance (R) Å [#]	
	Neothiobinupharidine 14b	Thiobinupharidine 13c
H-6'e — H_B-17'	2.45 → 2.7 ✓	2.45 → 2.7 ✓
H-6'e — H_A-17'	3.6 → 3.85	3.6 → 3.85
H-6'e — H_B-17	2.35 → 2.8 ✓	2.35 → 2.8 ✓
H-6'e — H_A-17	3.3 → 3.8	3.35 → 3.8
H-6'a — H_B-17	2.45 → 2.75 ✓	2.45 → 2.8 ✓
H-6'a — H_A-17	2.8 → 3.65 ¶	2.8 → 3.65 ¶
H-6e — H_B-17'	4.45 → 5.0	3.9 → 4.0 ✓
H-6e — H_A-17'	3.65 → 4.1	4.55 → 4.9 ¶
H-6e — H_B-17	3.4 → 3.8	2.5 → 2.9 ✓
H-6e — H_A-17	2.35 → 2.7 ✓	3.5 → 3.95
H-6a — H_A-17	2.35 → 2.8 ✓	4.0 → 4.3
H-6a — H_B-17	2.75 → 3.55 ¶	3.7 → 3.75

* The three-dimensional structures are depicted in Figure 5.
Measurements of internuclear distances were made directly from
Dreiding models (average of three readings on two different models).
Values of R were reproducible to ±0.1Å.

[#] The two values of internuclear distance in each column represents
the shortest and longest distance between the two protons under
consideration upon changing the conformation of the central tetra-
hydrothiophene ring.

The values checked (✓) are for those interactions where an internal
n.O.e. should be observed.

The values (¶) are those interactions where an internal n.O.e.
might be observed.

highly crystalline and sharp melting and were further purified by vacuum

sublimation. Nevertheless, all attempts in this laboratory to observe

an enhancement in signal intensity in the spectra of the thiospiran

alkaloids have proved fruitless.

It has been shown that enhancement of the signal intensity of

proton A occurs on irradiation of proton B provided that the main relax-

ation mechanism for A is a direct dipole-dipole interaction with B[92].

If this is the only mechanism a 50% enhancement (n.O.e.) will occur.

Competing mechanisms for relaxation of A (other neighbouring protons,

solvent protons, oxygen, etc.) decrease this n.O.e. Unfortunately all

the protons which would give useful information in the thiospiran alkaloid

systems are attached to carbons carrying other hydrogens capable of relax-

ing any n.O.e. which might otherwise be observed. Furthermore, the

protons attached to C-6 and C-6' are adjacent to a nitrogen atom. The

possibility of a further relaxation mechanism is therefore open to these

protons, namely that of quadrupole relaxation[95].

THE STRUCTURE OF THIONUPHAROLINE (26)

Thionupharoline was first isolated in 1970 from rhizomes of _Nuphar luteum_ harvested in Poland[56]. Purification by molecular distillation under vacuum resulted in a glass-like colourless solid of molecular weight 510. The molecular formula $C_{30}H_{42}N_2O_3S$, established from h.r.m.s. and combustion analyses, suggested that 26 might be a derivative of one of the bisamines 13, 14 or 15. Clearly three questions required answers in order to elucidate completely the structure of 26. These were, (a) What is the nature of the extra oxygen function? (b) Which, if any, bisamine skeleton has been modified to give 26? and (c) Where is the site of the oxygen function? The study of the structure of 26 began with an examination of its spectroscopic properties.

(a) The nature of the oxygen function

The i.r. spectrum of 26 displays a weak absorption at 2.80μ assigned to a hydroxy group. A diperchlorate was prepared by the procedure of Wrobel (see experimental) and the i.r. spectrum (KBr disc) of this salt does not exhibit absorption due to a hydroxy group but displays instead a moderately intense absorption at 6.02μ, characteristic of $\geq C=\overset{+}{N} \leq$, thus indicating that the OH group of 26 must be attached to carbon α to nitrogen.

The p.m.r. spectra of 26 at 220 MHz were recorded in $CDCl_3$ and C_6D_6 solution. The spectrum in benzene (Figure 11) is better resolved and will be discussed here. A signal observed at 2.42δ which disappears on addition of D_2O is assigned to the proton of an OH group. Furthermore, a singlet
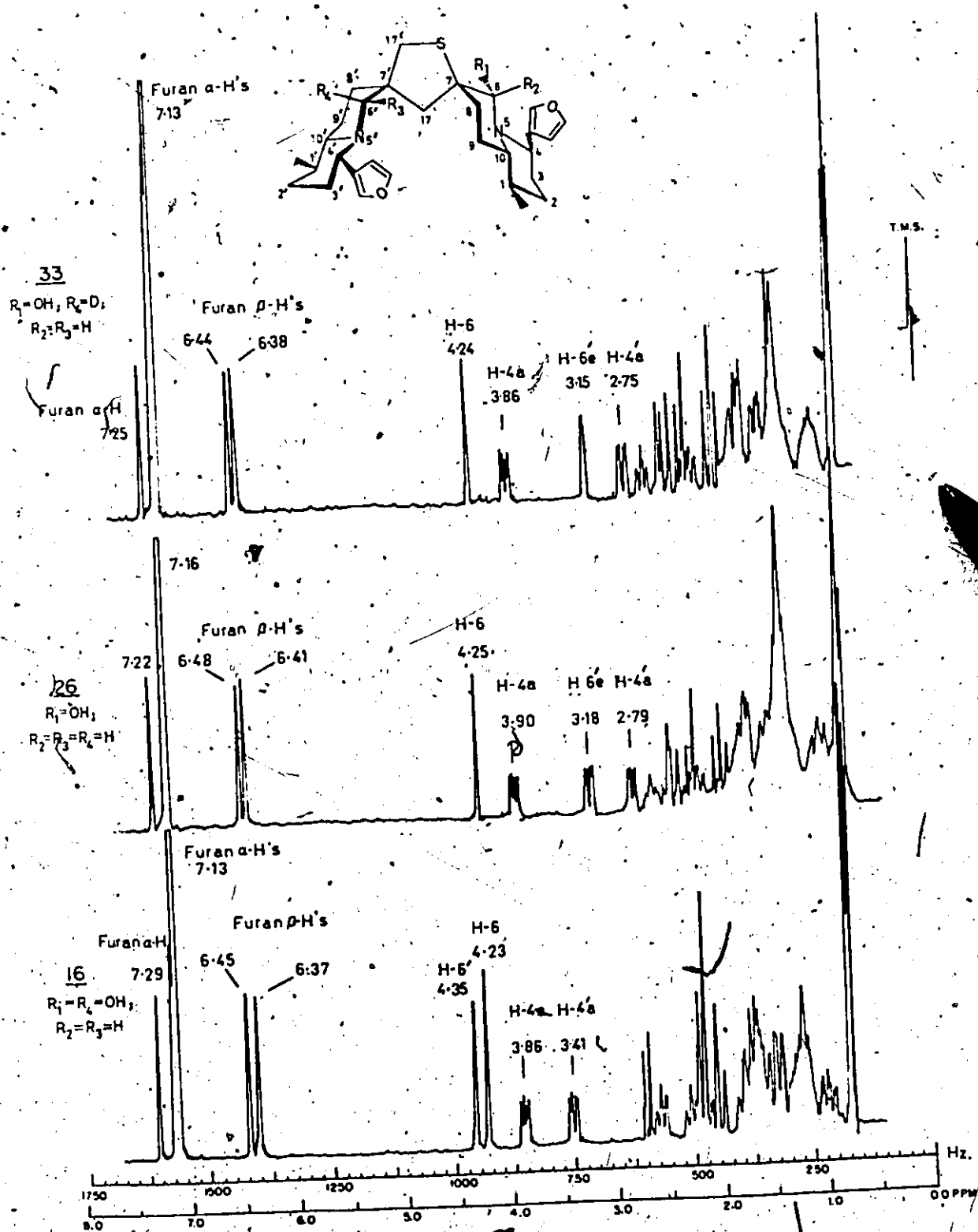
Figure 11. The p.m.r. spectra of 6,6'-dihydroxythiobinupharidine (16), 6-hydroxythiobinupharidine (26), and of 6-hydroxythiobinupharidine-6'-d₁ (33) recorded in C₆D₆ solution at 220 MHz.

observed at 4.25δ (1H) is assigned to a methine proton α to both oxygen and nitrogen atoms, consistent with the presence of a hemiaminal group
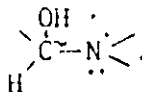
$\overset{\text{OH}}{\underset{\text{H}}{>}}\text{C}-\text{N}<$. Similar low field singlets have been observed in the p.m.r. spectra of the bishemiaminals 16 and 17.[47]

(b) The three-dimensional skeleton

Hemiaminals but not simple alcohols may be reduced with sodium borohydride in alcoholic solution[47]. Reduction of 26 with NaBH₄ in absolute ethanol gave thiobinupharidine 13c identified by a comparison of its optical rotation and its i.r., p.m.r., and mass spectra. A mixed m.p. determination with an authentic sample of 13c showed no depression in melting point, thus establishing that 26 is a monohemiaminal of thiobinupharidine.

(c) The site of the hydroxy group

The singlet observed at 4.25δ in the p.m.r. spectrum of 26, recorded in C_6D_6 at 220 MHz (Figure 11), rules out the possibility that the hydroxy group is located at C-4, C-4', C-10 or C-10' of the thiobinupharidine skeleton, and therefore confines its location to either C-6 or C-6'. A quartet (1H) at 3.18δ (J = 12.0 and 2.5 Hz) may be assigned, by analogy to 13c, to the equatorial proton at the fully reduced C-6 or C-6' position. Similarly the two axial protons at C-4 and C-4', α to both nitrogen and the furan ring, appear as well-resolved quartets at 2.79δ (J = 3.0 and 11.0 Hz) and 3.90δ (J = 4.0 and 10.0 Hz). The former has a chemical shift similar to H-4a and H-4'a in 13c, but the latter appears much further downfield than in the bisamines and must be

deshielded by, and therefore be in close proximity to, the hemiaminal function.

An attempt was made to locate the hemiaminal function by h.r.m.s. and by mass spectrometic studies of borodeuteride reduction products. The mass spectrum of $\underline{26}$ displays a moderately intense molecular ion at m/e 510 ($C_{30}H_{42}N_2O_3S$) and an intense ion at m/e 492 due to loss of H_2O from M^+. The bishemiaminals $\underline{16}$ and $\underline{17}$ show only very weak molecular ions but show intense ions due to the loss of one and two molecules of water. It was anticipated that the presence of the OH group in $\underline{26}$ might be reflected in the appearance of fragment ions at m/e 280 ($C_{15}H_{22}NO_2S$; R_1 = OH) by analogy to Scheme 9, at m/e 332 ($C_{19}H_{26}NO_2S$; R_4 = OH) by analogy to Scheme 10 or at m/e 194 ($C_{11}H_{16}NO_2$; R_1) = OH) also by analogy to Scheme 10. None of these ions is observed however, probably because of facile loss of water either thermally or through electron impact. A weak ion is observed at m/e 246 ($C_{15}H_{20}NO_2$) which is analogous to m/e 230 (Scheme 9), but as m/e 230 can presumably arise from either half of the molecule this does not specifically locate the hydroxy group. Similarly a weak ion at m/e 375, analogous to ion m/e 359 of the bisamines (Scheme 8), does not distinguish between C-6 and C-6' as the two possible locations for the OH group.

The mass spectrum of thiobinupharidine-d_1 $\underline{31}$, obtained by NaBD$_4$ reduction of $\underline{26}$, confirms that the hydroxy group of $\underline{26}$ occupies one of the C-6 positions. The ion m/e 359 (Scheme 8) of $\underline{13c}$ is moved almost exclusively to m/e 360, the ion m/e 178 almost completely to m/e 179 (Scheme 10), and the ion m/e 230 extensively to m/e 231 (Scheme 9),

whereas the ions m/e 136, 107 and 94 of 13c are also present in 31 indicating that the deuterium atom has entered either ring B or ring B' and therefore must be bonded to either C-6 or C-6'.

It seemed that the only way to differentiate between C-6 and C-6' as the site of the hydroxy group was via a determination of the site of deuterium incorporation as a result of borodeuteride reduction of 26. It was while the reduction studies were in progress that it was learned, through a personal communication, that LaLonde and his coworkers had elucidated the structure of 6-hydroxythiobinupharidine, isolated from rhizomes of Nuphar luteum harvested in Poland. A comparison of the u.v., i.r. and p.m.r. spectra reported for 6-hydroxy-thiobinupharidine[76] with those obtained for 26 confirmed their identity.

One apparent anomaly did exist, however, which required clarification. The melting points of the diperchlorate and monoper-chlorate of 6-hydroxythiobinupharidine are reported as 258°-265° and 238°-242°, respectively[76], whereas the diperchlorate of 26 was reported to melt at 172°-174°[56]. The preparation of a diperchlorate and monoperchlorate of 26 was repeated using LaLonde's procedure and products were obtained whose melting points were consistent with his results (see experimental). Moreover, a mixture melting point determination of the diperchlorate of 26, prepared by the LaLonde procedure, and an authentic sample (kindly provided by Professor LaLonde) confirmed that thionupharoline is identical with 6-hydroxythiobinupharidine. It was not possible to investigate fully the anomalous melting behaviour of the diperchlorates because of the paucity of material, but it is

suspected that preparation by the method of Wrobel may lead to a product containing 1 mole of water of crystallization. Alternatively, the diperchlorate may be dimorphic.

THE STERIC COURSE OF BORODEUTERIDE REDUCTION OF HEMIAMINALS

Evidence for sulphur participation

While the studies on the sodium borodeuteride reduction of 16 and 26 were in progress in this laboratory, it was reported[54,76] that the reduction of 6,6'-dihydroxythiobinupharidine 16 and 6,6'-dihydroxy-thionuphlutine-B 17 leads to incorporation of deuterium into the axial position at C-6' with complete stereoselectivity. Sodium borodeuteride reduction at C-6 of 17 was reported to occur so as to incorporate deuterium exclusively into an axial position. This was in contrast to reduction at C-6 of 16 where the deuterium was incorporated exclusively into the equatorial position. Similarly, it was reported at the same time that reduction of 6-hydroxythiobinupharidine 26 (thionupharoline) led to incorporation of deuterium into the equatorial position at C-6 with complete stereoselectivity. Indeed this result, together with u.v. evidence, was the basis for assigning the hydroxy group to the C-6 position rather than the C-6' position. It was also stated that these reductions, performed in methanolic solution, proceeded with about 70% incorporation of deuterium and 30% incorporation of hydrogen, a result that was surprising. The study of the borodeuteride reduction of 26 and 16 in this laboratory was therefore continued as the results differed significantly from those reported.[76]

It was proposed[54, 76] that the sulphur atom interacts with an immonium ion intermediate in the reaction, probably in the form of an episulphonium ion.* This results in a charge distribution between

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* It has been suggested that episulphonium ions may play a role in the addition of electrophilic sulphur reagents to enamines (96).

the sulphur atom and the immonium ion derived from N-5 and C-6. It was suggested that when the sulphur atom occupies an equatorial position with respect to ring B, as in 16 and 26, that this interaction directs the incoming deuterium into an equatorial position at C-6.

equatorial sulphur

3F = (furan structure)

equatorial deuterium

attack on α-face,
opposite sulphur, less
hindered
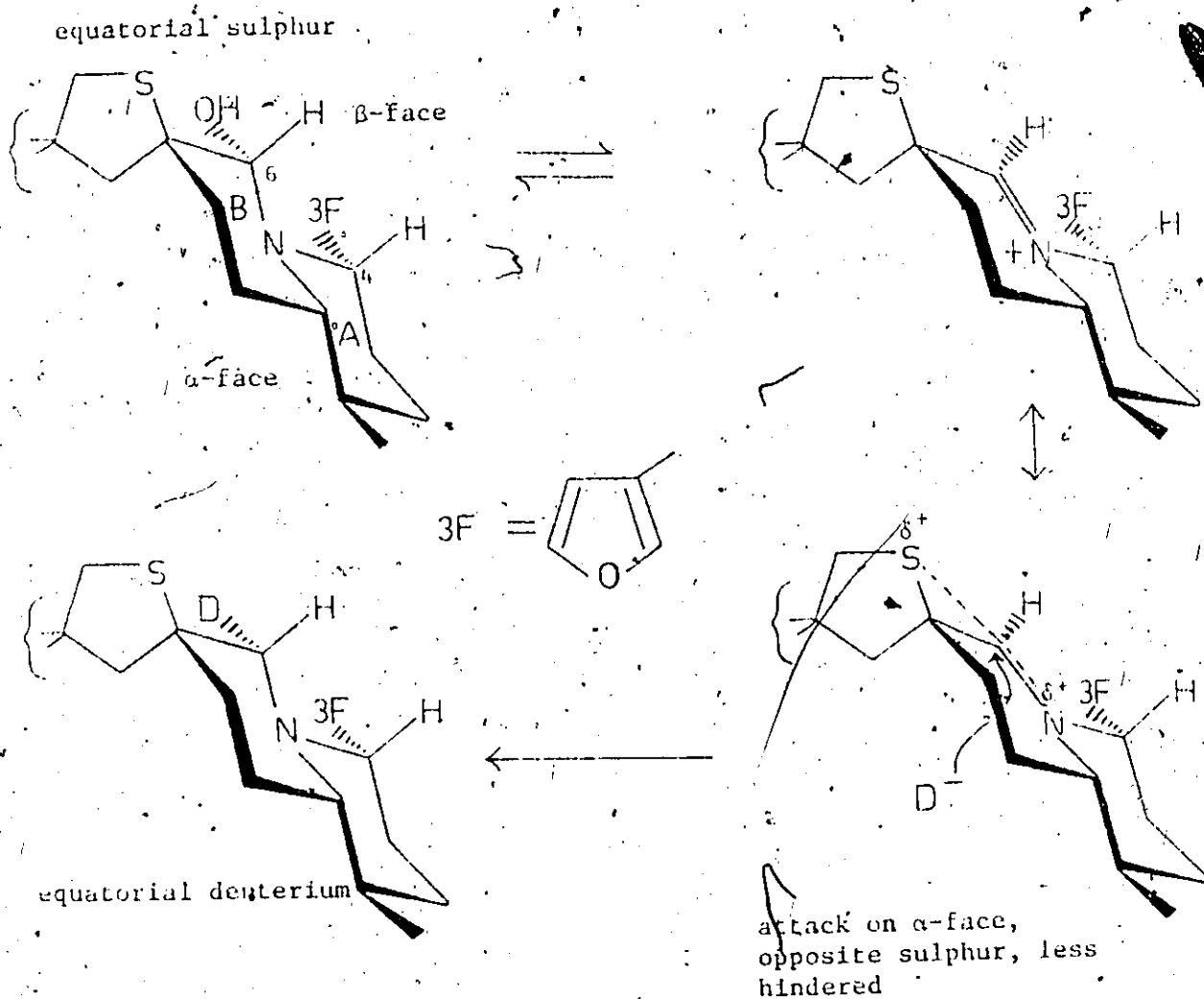
In this case the attack by deuteride ion is on the α face of the quinolizidine system AB. In this discussion the quinolizidine moieties AB and A'B will be considered separately. The β face of each

quinolizidine is defined as the side opposite to the nitrogen lone-pair
and the 3-furyl group. The α face is the same side as the 3-furyl group.
Conversely when the sulphur atom is axial with respect to ring
B as in 17 it was suggested that the incoming deuterium is directed
exclusively into an axial position at C-6.

axial sulphur   α-face

β-face

3F ≡

axial deuterium

attack on β-face, opposite to
sulphur, less hindered
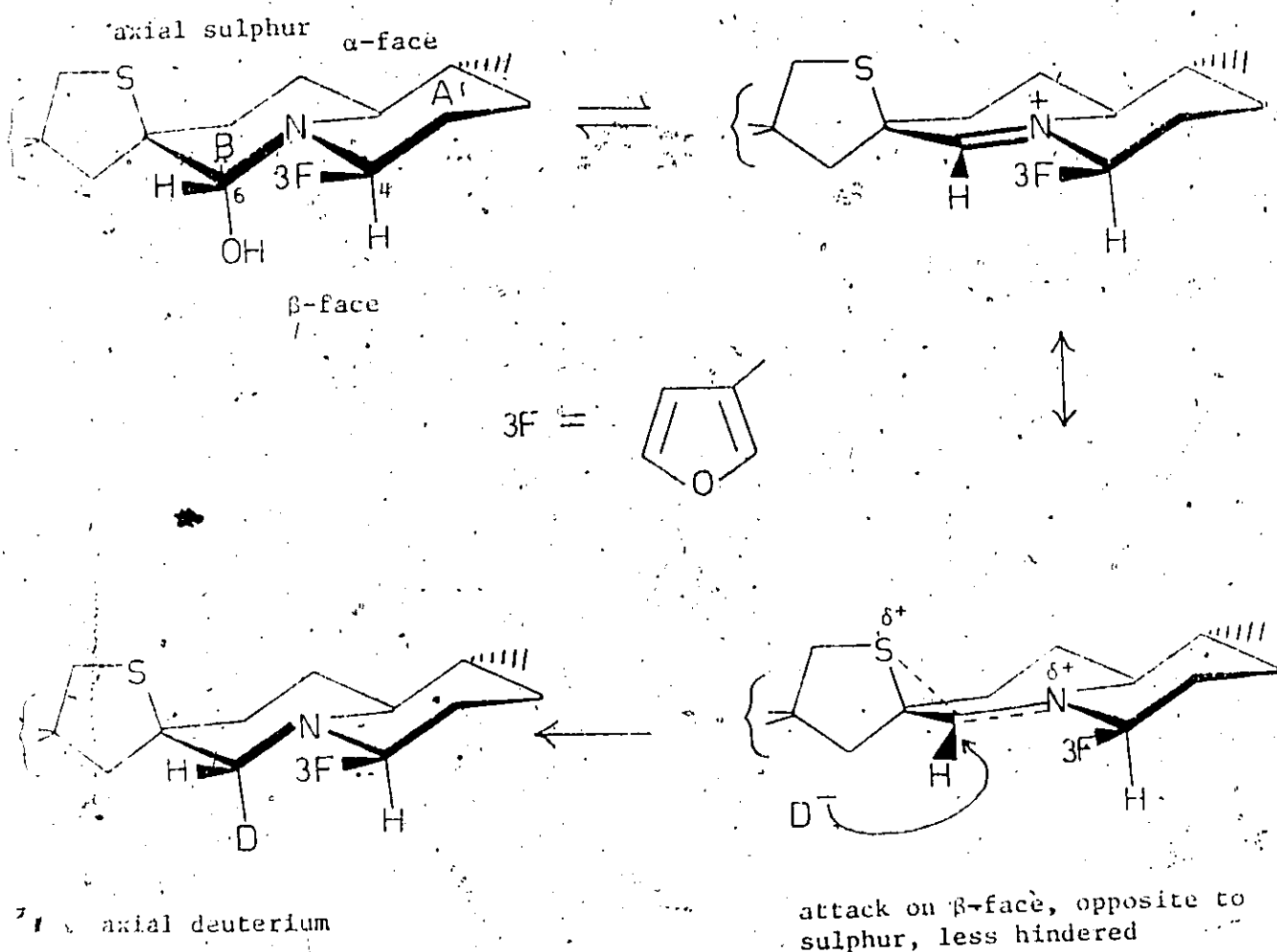
In this case it should be recognised that attack by deuteride
ion is on the β face of the quinolizidine system AB, i.e. on the side

opposite to the 3-furyl group.

In the reduction of the hemiaminal function at N-5' - C-6' of both 16 and 17, where sulphur is less likely to participate because of steric constraints, it was suggested that attack by the reducing species occurs on the less hindered convex β face of the quinolizidine system, A'B', resulting in incorporation of deuterium into an axial position at C-6' of each molecule. Evidence has been presented which indicates that both the enamine, Δ^6-dehydrodeoxynupharidine, and the immonium ion derived from Δ^3-dehydrodeoxynupharidine are reduced by preferential attack on the β side of the quinolizidine systems[69,70]. More recently[76] it has been shown that the 'epi-sulphonium-like" intermediate derived from 7-β-methylthiodeoxynupharidin-6α-ol undergoes selective hydride attack on the α-face and reduction occurs very slowly. In contrast the "epi-sulphonium-like" intermediate from 7-α-methyl thio-7-epideoxy-nupharidin-6β-ol undergoes very rapid selective hydride attack on the β-face.

In summary it appears that the steric course of reduction of the hemiaminal function at N-5' - C-6' of the bishemiaminals is independent of the configuration at C-7, whereas that at N-5 - C-6 is strongly dependent on the configuration at C-7.

U.v. spectra of α-thiohemiaminals

Evidence for an interaction between the sulphur atom and the immonium ion derived from α-thiohemiaminals has been observed in their u.v. spectra[76]. Thus, 6-hydroxythiobinupharidine, 6,6'-dihydroxy-thiobinupharidine and 6,6'-dihydroxythionuphlutine-B, as well as model compounds all display moderately intense absorption maxima at 290-295 nm (ε = 1500 - 3200) in acidic ethanol solution. This band (Figure 12)

Figure 12. The ultraviolet absorption spectra of thiospiran Nuphar alkaloids.
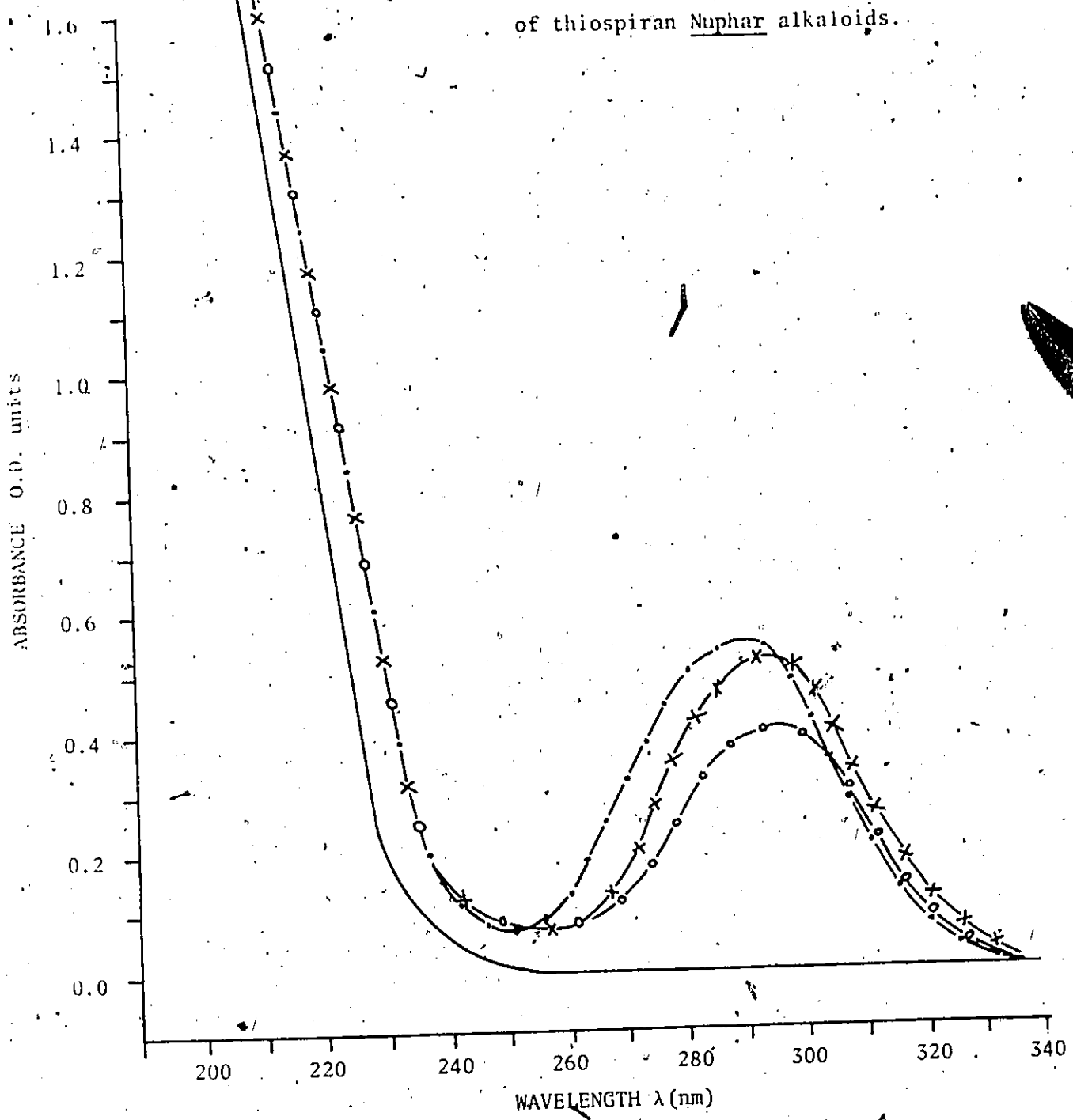
(—o—o—)6,6'-dihydroxythiobinupharidine (16);(—x—x—)6-hydroxy-thiobinupharidine (26);(—•—•—)6-hydroxythiobinupharidine-6'-d_1 (33); in acidic 95% ethanol.

(———)neothiobinupharidine (14b), thiobinupharidine (13c), and neothiobinupharidine sulphoxide (27), in neutral, basic and acidic solution; and of 16, 26 and 33 in neutral and basic 95% ethanol.

disappears on addition of base but reappears on reacidification. The bishemiaminals show only end absorption or high intensity maxima at 210 nm in neutral or basic ethanol. The bisamine thiospiran alkaloids 13, 14 and 15 do not exhibit this 290 - 295 nm absorption band in acidic ethanol and simple immonium ions are reported to show moderate to strong absorption at 222 - 232 nm [97]. The analogy has been drawn [76] between the sulphur-immonium ion interaction observed in the u.v. spectra of α-thiohemiaminals and the sulphur-carbonyl interaction observed in the u.v. spectra of various sulphur containing ketones [98].

The sodium borodeuteride reduction of hemiaminals of thiobinupharidine in absolute ethanol

The reduction of 16 with sodium borodeuteride in ethanol in this laboratory led to recovery of thiobinupharidine-6,6'-d_2 32 and a monohydroxymonodeuteriothiobinupharidine 33. Unlike the published result [76] 32 is >95% dideuterated material. Analysis of the 220 MHz p.m.r. spectrum (C_6D_6) shows that one deuterium atom is incorporated almost exclusively into the axial position at C-6' and that the second deuterium is incorporated to the extent of 40% into the equatorial position at C-6 and 60% into the axial position at C-6. The signal at 1.40δ (H-6'a) in 13c has virtually disappeared in the spectrum of 32 while the signal at 3.16δ (H-6'e) has collapsed to a poorly resolved doublet (1H) apparently coupled only to H-8'e. The signal at 1.93δ (H-6a) is now a broad singlet (0.4H) while the signal at 3.10δ (H-6e) is a poorly resolved doublet (0.6H) coupled only to H-8e. These results are compatible with those of LaLonde et al. with respect to reduction at

C-6' but differ from his with respect to reduction at C-6. Only 40%
introduction of deuterium into the equatorial position at C-6 is
observed and not 100% as reported for the reduction in methanol[54,76].

The second product of the borodeuteride reduction of 16 proved
to be 6-hydroxythiobinupharidine-6'-d$_1$ 33 with greater than 95%
incorporation of deuterium. This compound has a u.v. spectrum identical
with 26 in neutral and acidic ethanol and forms a diperchlorate of the
same melting point as that of 26. This melting point is undepressed in
admixture with the diperchlorate of 26. Compound 33 differs in its
p.m.r. spectrum from that of 26 only in one respect, namely that the
quartet present in 26 at 3.18δ (H-6'e) has collapsed to a poorly
resolved doublet integrating for one proton (Figure 11). Clearly the
deuterium has entered exclusively into an axial position at C-6' and
reduction at C-6' has occurred more rapidly than at C-6. The much
slower attack at C-6 is predictable on the basis of studies on model
compounds[76].

The sodium borodeuteride reduction of 26 in this laboratory
gives thiobinupharidine-6-d$_1$ 31 with deuterium incorporation in excess
of 95%. The p.m.r. spectrum in C_6D_6 solution at 220 MHz shows only
minor differences from the spectrum of 13c. The signal at 1.93δ,
formerly a doublet, now appears as a broad singlet (0.35H) and the
signal at 3.10δ now appears as a broadened singlet (0.65H). Thus
deuterium has entered 65% into the axial position and 35% into the
equatorial position at C-6, a result in clear agreement with the boro-
deuteride reduction of 16 in ethanol solution.

The results of the p.m.r. studies in C_6D_6 are shown in Figure 13. The lower spectrum is that of thiobinupharidine 13c and shows the spin-decoupling experiments performed on that compound. The centre spectrum is that of the product of borodeuteride reduction of 26, and the upper spectrum that of the product of complete borodeuteride reduction of 16. The deuterium incorporation at the various sites is indicated.

The results of the deuterium incorporation studies carried out in ethanol solution in this laboratory were conveyed to Professor LaLonde and he has now reinvestigated the deuterium distribution at C-6 in both thiobinupharidine-6,6'-d_2 and thiobinupharidine-6-d_1[99]. His original results[54,76] were based upon integrations of 60 or 100 MHz p.m.r. spectra recorded mainly in $CDCl_3$ solution. After re-examination of the spectra of the compounds recorded in C_6D_6 at 300 MHz he has revised his results and now indicates 82-84% deuterium incorporated into the equatorial position and 16-18% deuterium incorporated into the axial position at C-6. These results are however still quite different from those achieved in this laboratory which show ∿40% deuterium incorporated into the equatorial position and ∿60% into the axial position at C-6 of the thiobinupharidine skeleton.

An examination of the reaction conditions of LaLonde et al.[54,76,99] shows that there were some minor differences from those used in this laboratory. Thus they used methanol as solvent whereas absolute ethanol was used as the solvent for the reductions carried out in this laboratory. Generally the initial amount of sodium borodeuteride added in my experiments was greater than that added in their experiments; furthermore with the reductions in methanol no further borodeuteride was
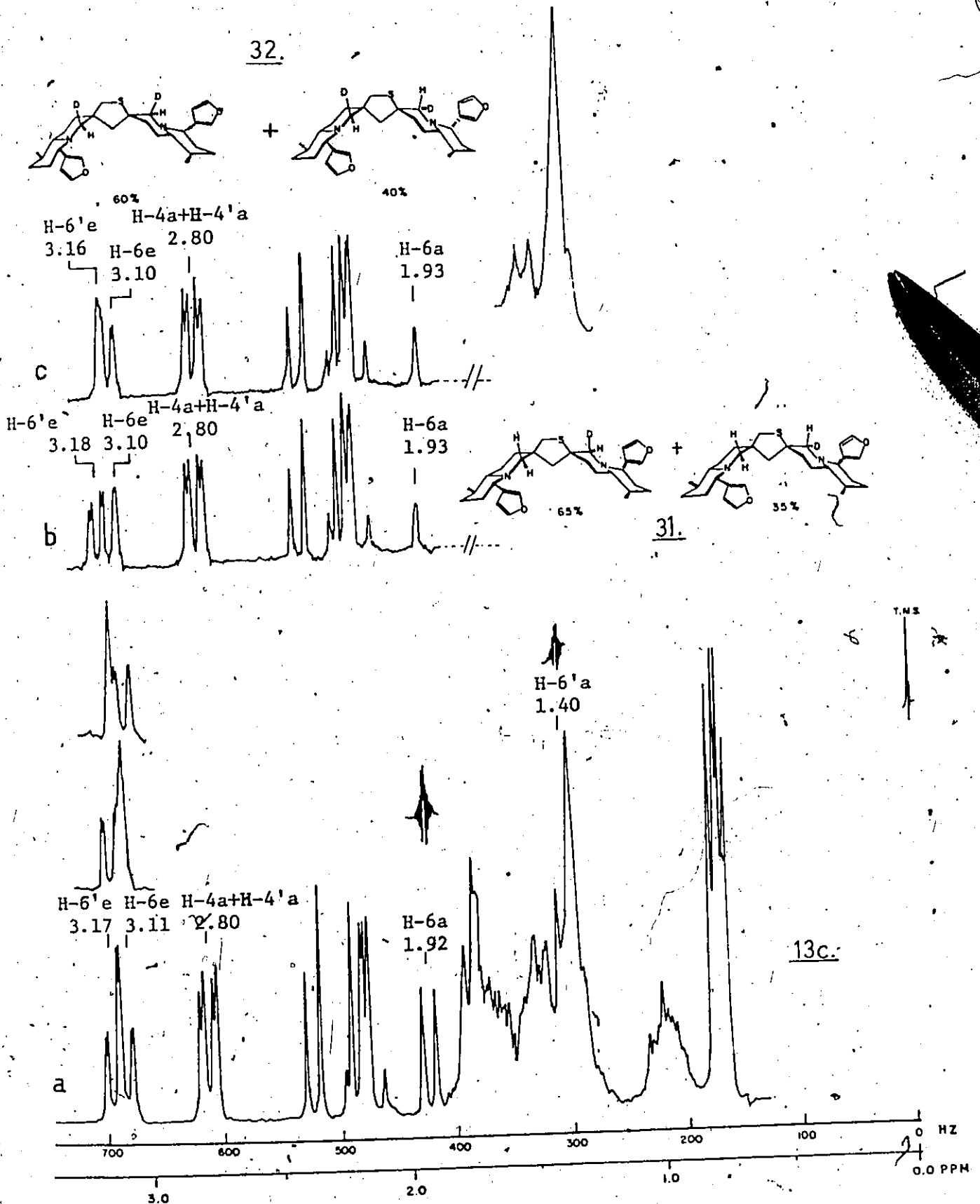
Figure 13. The p.m.r. spectra of thiobinupharidine (a); thiobinupharidine-6-d₁ (b); and thiobinupharidine-6,6-d₂ (c); recorded in C_6D_6 solution at 220 MHz.

added during the course of the reactions, while in my experiments small

portions of borodeuteride were added, to supplement that already present,

during the reaction period. The reaction time and temperature were not

significantly different. Although it may be dangerous to compare

experiments carried out in different laboratories by different personnel,

an attempt will be made to explain the differences observed between the

two sets of experiments.

Reduction at C-6' of a hemiaminal of thiobinupharidine

P.m.r. data from model compounds[76], suggests that the OH group

at C-6' occupies an axial position. The rate of reduction at C-6' is

fast for two reasons, (a) the lone pair on nitrogen has a trans-diaxial

relationship to the hemiaminal hydroxy group and therefore is geometric-

ally arranged to assist the leaving group through anchimeric assistance

in order to form an immonium-ion intermediate, (b) although there is

steric hindrance to α-side attack owing to the 3-furyl group on C-4',

there is no hindrance to attack on the β-face. Thus in both methanol

and ethanol highly stereoselective reduction was observed leading to

incorporation of axial deuterium at this site. Furthermore, from the

studies on model compounds[76] it may be proposed that reduction at

this site is probably complete within 1 hour. It is proposed that the

reducing species at this site is $\bar{B}D_4$.

Reduction at C-6 of a hemiaminal of thiobinupharidine

It is evident that reduction at this site occurs at a much slower

rate than at C-6'. This is predictable from studies on model compounds[76]

and is confirmed by the isolation of much 6-hydroxythiobinupharidine-6'-d_1 from the sodium borodeuteride reduction of 16 even after 24 hrs reaction. P.m.r. studies on the model compounds.[76] also indicate that the hydroxy function occupies an equatorial position at C-6 of both 16 and 26. It is feasible that the sulphur atom through its interaction with the immonium ion may direct the OH group into the equatorial position upon basification of the immonium perchlorate (see experimental). The reasons proposed for the greatly reduced rate of reduction at this site are twofold. Firstly the lone-pair of nitrogen and the oxygen substit- uents at C-6 are not favourably disposed to allow anchimeric assistance in the formation of an immonium-ion intermediate. This process may however be more favourable in methanol (a more ionising solvent) than in ethanol. Secondly approach of a reducing species to both the α and β faces of the quinolizidine moiety AB is hindered. Attack on the α face is hindered in the "normal" manner by the 3-furyl group at C-4 and attack on the β face may be hindered because of participation of sulphur with the intermediate immonium ion derived from N-5 and C-6.

It is proposed therefore that over the course of this slow reduction at C-6, the concentration of some intermediate alkoxyboro- deuteride species may become significant, and that this reducing species may have not only a distinctly different reactivity towards the substrate, but the electronic and steric requirements of the reducing species may also change during the reaction period. It remains to explain the possible origin of such species, as well as their differing stability (lifetimes), reactivity and steric requirement upong changing the solvent from methanol to ethanol.

Origin of intermediate reducing species

The reaction between sodium borohydride and alcohols has been studied by Brown and Ichikawa[100] who have observed that 80% of the available "hydride" is lost within 1 hour in methanol at 0°C whereas at the same temperature in ethanol only 5% of the available "hydride" is lost within 42 hours. For this reason they have advised against the use of methanol as a solvent for borohydride reductions. Davis et al.[101] have shown that the rate of methanolysis of $NaBH_4$ is pH dependent and that added NaOMe will stabilise these solutions; however sodium methoxide is not a product of the methanolysis and the reaction between sodium borohydride and methanol does not change the acidity of the medium.

$$NaBH_4 + 4CH_3OH \longrightarrow NaB(OCH_3)_4 + 4H_2 \quad \ldots \ldots \quad (1)$$
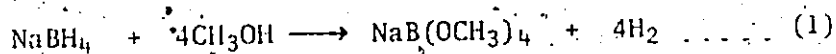
The final product of ethanolysis is also the tetraalkoxyborate anion. Davis has suggested that alkoxyborohydrides such as $CH_3O\bar{B}H_3$, $(CH_3O)_2\bar{B}H_2$, etc. are intermediates in the alkanolysis reaction. Alkoxyborohydrides have been proposed as intermediates in the reduction of ketones with borohydride[100,102,103]. In these instances however the alkoxy group may arise from the substrate as well as the solvent if it be an alcohol.

$$\bar{B}H_4 + \underset{R}{\overset{R}{\diagdown}}C=O \; \underset{k_1}{\rightleftharpoons} \; H_3\bar{B}\text{-}O\text{-}CH\underset{R}{\overset{R}{\diagup}}$$

$$H_3\bar{B}\text{-}OCH\underset{R}{\overset{R}{\diagup}} + \underset{R}{\overset{R}{\diagdown}}C=O \; \underset{k_2}{\rightleftharpoons} \; H_2\bar{B}\text{-}(O\text{-}CH\underset{R}{\overset{R}{\diagup}})_2$$

$$H_2\bar{B}\text{-(OCH}R_2 + \underset{R}{\overset{R}{>}}C=O \underset{}{\overset{k_3}{\rightleftharpoons}} H\bar{B}\text{-(OCH}R)_3$$

$$H\bar{B}\text{-(OCH}R)_3 + \underset{R}{\overset{R}{>}}C=O \underset{}{\overset{k_4}{\rightleftharpoons}} \bar{B}(OCH R)_4$$
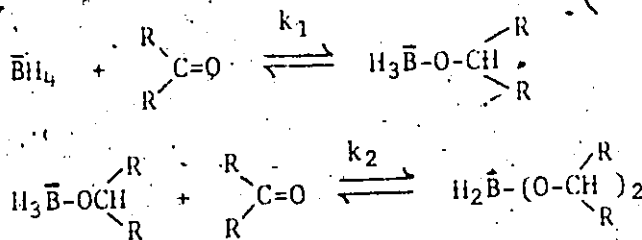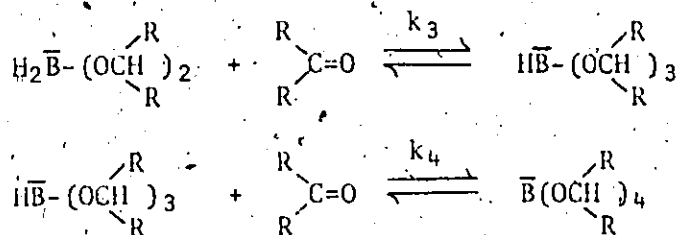
Any of these intermediates may undergo a replacement reaction with alcoholic solvents present in excess,

i.e. $H_3\bar{B}\text{-OCH}R_2$ + $R'OH \rightleftharpoons H_3\bar{B}(OR') + \underset{R}{\overset{R}{>}}CHOH$
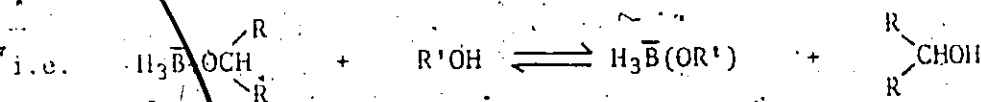
A similar sequence of reactions may be proposed in a reaction between borohydride and an immonium ion.

$$>C=\overset{+}{N}< \; + \; BH_4^- \overset{k_1}{\rightleftharpoons} \; >\overset{H}{\underset{\overset{|}{+}}{C}}-\overset{\bar{B}H_3}{\underset{|}{N}}< \quad etc.$$

$$>\overset{}{C}H-\overset{}{\underset{+}{N}}-\bar{B}H_3 \; + \; ROH \rightleftharpoons \; >CH-N< + R O\bar{B}H_3 \; + \; H^+$$
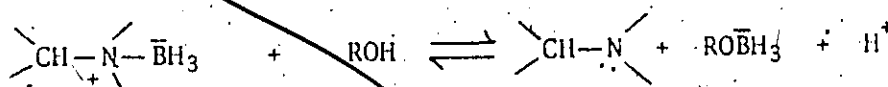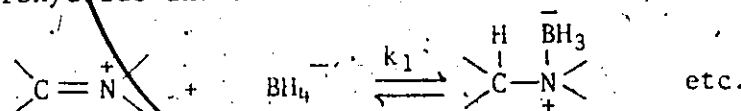
Reactivity of the alkoxyborohydrides

The reaction between substrate and sodium borohydride itself varies according to the solvent[104]. Reduction of ketones in diglyme or triglyme is not observed whereas reduction in alcoholic solvents is fast. The reaction in methanol is probably significantly faster than in ethanol. It has been demonstrated that the rate determining step with substrate is k_1 (see above), i.e. the first hydride transfer, and all subsequent steps, k_2 etc. are faster. The intermediates are more reactive than borohydride both towards reduction of substrate molecules and towards solvents. This has been demonstrated by reduction of acetone

with $NaBH(OCH(CH_3)_2)_3$ in diglyme, where reaction is complete in seconds, at $0°C$. In this solvent no reaction between acetone and sodium boro-hydride is observed after several hours at room temperature[102,104]. An alkoxyborohydride is a more reactive reducing agent because of electron release from the oxygen onto boron which increases the hydridic character of the hydrogen, i.e. BH_3 is a stronger Lewis acid than $B(OR)_3$[102] therefore H^- is more easily lost from $H\bar{B}(OR)_3$ than from $\bar{B}H_4$.

Stability of the alkoxyborodeuterides

Consider now the reaction between $NaBD_4$ and the solvent ROH:

$$BD_4^- + ROH \xrightarrow{k_1} \bar{B}D_3(OR) + HD$$

$$\bar{B}D_3(OR) + ROH \xrightarrow{k_2} \bar{B}D_2(OR)_2 + HD$$

$$\bar{B}D_2(OR)_2 + ROH \xrightarrow{k_3} \bar{B}D(OR)_3 + HD$$

$$\bar{B}D(OR)_3 + ROH \xrightarrow{k_4} \bar{B}(OR)_4 + HD$$
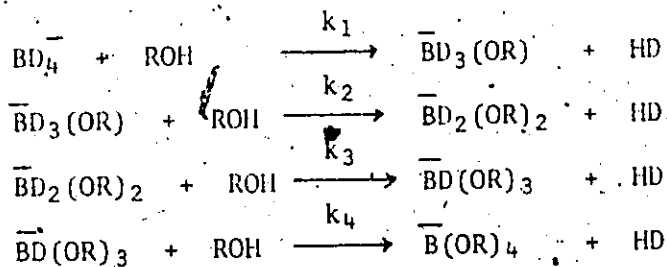
The rate constant k_1 must be greater in methanol than in ethanol and consequently all the subsequent steps, k_2, k_3, k_4 are expected to be not only progressively faster than k_1 but also faster in methanol than in ethanol. An important mode of destruction of $\bar{B}D(OR)_3$ is the disproportionation reaction[105]:

$$4NaBD(OR)_3 \underset{-k_{dis}}{\overset{k_{dis}}{\rightleftharpoons}} NaBD_4 + 3NaB(OR)_4 \quad \_ \_ \_ \_ \_ \_ \_(2)$$
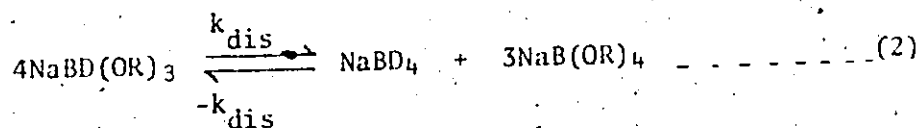
Thus when $NaBH(OCH_3)_3$ is added to diglyme or T.H.F. it dis-proportionates readily. $NaBH(OCH(CH_3)_2)_3$ is stable under these conditions owing to the sterically unfavourable character of the $\bar{B}(OCH(CH_3)_2)_4$ anion.

The disproportionation of $BD(OC_2H_5)_3^-$ also occurs readily[102] but might be expected to be slower than the corresponding methoxy compound.

The solubility of $NaBH_4$ at 20° is reported to be 164 mg/ml in methanol and 40 mg/ml in ethanol[101]. The experiments of LaLonde et al. apparently involved total addition of the borodeuteride in one portion at the beginning of the reaction; furthermore in most cases the amount added was insufficient to exceed the solubility limit. Reaction conditions in this laboratory involved initial addition of sufficient sodium borodeuteride to exceed the solubility limit and further periodic additions in order to replace that lost by reaction with substrate or solvent. It seems feasible that under the conditions of LaLonde et al.[54,76,99] the existence of sodium methoxyborodeuteride intermediates is unlikely, and even if they are formed that they have very short lifetimes before reaction with the solvent (sometimes in vast excess), or before disproportionation.

In the experiments performed in this laboratory there may be factors which stabilise intermediate ethoxyborodeuteride species. Firstly, the reaction with the solvent will be slower, and the solvent was never in vast excess. Secondly, a higher concentration of $NaBD(OC_2H_5)_3$ will be present because the disproportionation of a species such as this according to equation (2) may be minimised. This is because the excess BD_4^- present will affect the equilibrium concentration of the alkoxy-borodeuteride species by increasing k_{-dis}. King et al.[106] have found that $NaBH(OCH_3)_3$ will cause reductive elimination of 1,2-dibromides in diglyme solution. The reaction does not occur with $NaBH_4$ alone, but

occurs readily with a mixture of NaB(OCH$_3$)$_4$ and NaBH$_4$ in the ratio of
3:1, thus indicating that a significant concentration of the trimethoxy-
borohydride species can be achieved through an equilibrium reaction such
as equation (2).

Steric effects resulting from accumulation of an alkoxyborodeuteride species

It is proposed that reduction at C-6 during the initial stages
of the reaction involves very slow attack of BD$_4^-$ on the immonium ion
and that the mode of attack is on the α face of the quinolizidine system
AB, opposite to the sulphur atom, leading to incorporation of deuterium
into the equatorial position at C-6. Over the course of the reduction
the concentration of an intermediate alkoxyborodeuteride species may
become significant. For the reasons cited previously both the lifetime
and concentration of ethoxyborodeuteride species in ethanol solution
are expected to be greater than the corresponding methoxy species in
methanol. It is proposed therefore that the intermediate bulky
ethoxyborodeuteride species has a steric requirement different from BD$_4^-$
and cannot approach the α side of the quinolizidine system AB and must
therefore approach from the β side resulting in incorporation of
deuterium into an axial position. As the alkoxyborodeuteride species
are much superior reducing agents to borodeuteride, a small but finite
amount of this intermediate might lead to a very significant distrib-
ution of deuterium between the axial and equatorial positions even
though α side approach of BD$_4^-$ is occurring very slowly throughout the
course of the reaction. The reduction at C-6 is probably faster in
methanol than in ethanol, the former being a more ionising solvent,

thereby facilitating the formation of an intermediate immonium ion
(indeed, one of the reactions of LaLonde et al, was performed on the
immonium salt). As the lifetime and concentration of intermediate
alkoxyborodeuteride species is not expected to be as great as in ethanol,
the reduction at C-6 is achieved predominantly by the species BD_4^- in
methanol solution leading to predominant incorporation of equatorial
deuterium at this position.

The above proposals are based on very limited data and cannot be
authenticated without a great deal of further work involving detailed
kinetic studies and studies of product distribution versus time. There
is however some precedence for these proposals. Thus Rickborn and
Wuesthoff[107] have implied that there is a significant buildup of a
bulky intermediate alkoxyborohydride intermediate to a finite concentr-
ation in the reduction of alkyl-substituted cyclohexanones. The
ketones gave rise to two isomeric alcohols and the ratio of these
products changed during the course of the reduction. They concluded
that the bulky intermediate·alkoxyborohydride resulted in stereoselective
product formation which was different from the product resulting from
BH_4^- reduction. They also concluded that only a small amount of the
intermediate was required (little more than steady state concentration)
to have a significant stereochemical effect.

Several experiments may be devised to test the proposals suggested
above. It would be of interest to carry out the reduction of the hemiam-
inals of thiobinupharidine using a stable bulky alkoxyborodeuteride as
the reducing reagent. A suitable choice would seem to be $NaBD(OCH(CH_3)_2)_3$

in diglyme solution. Disproportionation of the triisopropoxyboro-deuteride is slow or non-existent in this solvent[108]. Even if some $NaBD_4$ was formed it is unlikely that it will act as a reducing agent in this medium[100]. According to the proposals above exclusive incorporation of deuterium into the axial position at C-6 is expected upon reduction of the hemiaminal function with $NaBD(OCH(CH_3)_2)_3$ in diglyme. An alternative reducing agent which should achieve the same steric result is $NaBD(C_2H_5)_3$ which is easily prepared and stable in solution[104]. The use of this reagent has been suggested in order to achieve steric control of ketone reductions. It would also be of interest to examine the kinetics of the sodium borodeuteride reduction of both the preformed immonium salt and the intact hemiaminals of thiobinupharidine. This should be carried out in a relatively non-ionising solvent such as isopropanol, in which sodium borodeuteride is relatively stable[100], in order to determine whether the rate of immonium ion intermediate formation plays a role in stereoselective product formation.

The anomalously low deuterium content in the singly and doubly labelled thiobinupharidines obtained from sodium borodeuteride reduction of 26 and 16 in methanol solution is puzzling. LaLonde et al.[99] have suggested that the mass spectrometric method used to determine the isotopic compositions may be subject to error because of the inaccuracies associated with comparing peak intensities in labelled and unlabelled samples when relatively intense $M^+ - 1$ peaks are observed. An alternative explanation given was the low reactivity of sodium borodeuteride relative to the reactivity of sodium borohydride. Thus it was suggested[99]

that the 2% NaBH$_4$ present in commercial samples of sodium borodeuteride may react before significant reaction of any of the NaBD$_4$ occurs because of a kinetic isotope effect. As there was a twentyfold excess of reducing agent present this could account for the ratio of hydrogen to deuterium observed in the reduced products.

While the mass spectrometric method may have some intrinsic error the method of calculation appears to be the same in both experiments; furthermore the results obtained in this laboratory were confirmed at least to ±5% by the integrations of the p.m.r. spectra.

In reactions involving hydride transfer from sodium borohydride an inverse isotope effect has been observed. Thus the hydrolysis of, sodium borodeuteride occurs faster than the hydrolysis of borohydride[109], an inverse isotope effect has been observed for the reduction of ketones in alcoholic solution[110] and the methanolysis of lithium borohydride in diglyme also shows an inverse isotope effect[111]. This effect has been ascribed[109] to a secondary isotope effect of the other three hydrogens or deuteriums that are not undergoing the protonolysis reaction in the rate-determining step. The boron-hydrogen bond that is breaking is contributing a small normal primary isotope effect but a large inverse secondary isotope effect makes the ratio, k_H/k_D, less than one. For this reason it does not seem valid to propose a large "normal" isotope effect for the borohydride reduction of hemiaminals in methanolic solution.

THE DETERMINATION OF THE SITE OF A MONOHEMIAMINAL FUNCTION USING MASS SPECTROMETRY

Lengthy experimentation is required in order to differentiate between the two sites, C-6 and C-6', for location of the OH group of a monohemiaminal of the thiospiran alkaloids. The interpretation of the steric course of deuterium introduction, using p.m.r. studies, may prove especially difficult in the case of borodeuteride reduction of monohemiaminals of 14 and 15 where in all cases the entering deuterium is expected to incorporate exclusively into an axial position at C-6 or C-6'. U.v. studies have shown that it may be possible to distinguish between a hemiaminal group at C-6 and one at C-6'. The appearance of a low energy acid-induced absorption band has been attributed to sulphur interaction, through a three-membered ring, with an immonium ion derived from a hemiaminal function at C-6. However, the possibility has been conceded[76] that interaction through a four-membered ring between sulphur and an immonium ion derived from a hemiaminal function at C-6' may be possible, which may also give rise to a high wavelength absorption maximum. Very recently[112] LaLonde and Wong have indicated that circular dichroism studies, performed on solutions of immonium ions derived from monohemiaminals, may be used to differentiate between C-6 and C-6' as the site of the monohemiaminal function of the alkaloids having the bisamine skeleton of 13 or 15.

The results from the mass spectrometric study of the borodeuteride reduction products of 16 and 26 in this laboratory have indicated the presence of a potentially useful diagnostic fragmentation pattern. At

first sight the fragment ion m/e 178 (Scheme 10) may be derived from either side of a molecule with gross structure 25 (R_1 = R_4 = H). The mass spectrum of thiobinupharidine-6-6'-d_2 32 shows that m/e 178 is transposed almost totally to m/e 179, an expected result. The mass spectrum of thiobinupharidine-6-d_1 31, however, also shows that m/e 178 is moved almost totally to m/e 179, whereas the expected result is for m/e 178 and m/e 179 to have comparable relative intensity.

This is interpreted as preferential homolytic cleavage of the C-6—C-7 bond over cleavage of the C-6'—C-7' bond which may occur owing to the particular stability of the resultant tertiary radical centre α to the sulphur atom[113] and/or because the neighbouring polarizable sulphur atom may enhance the rate of homolytic scission of the C-6—C-7 bond. Vastly enhanced rates of homolysis of the O-O bond of peresters have been observed for those cases where a neighbouring sulphur atom is spatially positioned to assist bond cleavage[114].

Thiobinupharidine-6'-d_1 34 was prepared by borohydride reduction of 6-hydroxy-6'-deuteriothiobinupharidine 33 in order to test the above proposal. The mass spectrum of 34 shows that m/e 178 is transposed only to a small extent to m/e 179 and therefore fragmentation of the molecular ion must occur predominantly as shown in Scheme 10.

In summary, it seems that a study of the monodeuterio bisamine, resulting from borodeuteride reduction of the monohemiaminal, using low resolution mass spectrometry, provides an expedient method to distinguish between C-6 and C-6' as the site of the hemiaminal function.

NEOTHIOBINUPHARIDINE SULPHOXIDE (27)

(a) The gross skeletal structure

The other thiospiran alkaloid, of molecular formula $C_{30}H_{42}N_2O_3S$, to be examined in this study was the compound coded 27. Its molecular formula was established by h.r.m.s. and combustion analysis. The i.r. spectrum of 27, unlike that of the monohemiaminal 6-hydroxythiobinuphar-idine 26, displays intense Bohlmann bands in the region 3.60 - 3.91μ. Furthermore, the i.r. spectrum of 27 is very similar to that of neothio-binupharidine, differing only in one respect, namely the presence of one extra absorption at 9.60 - 9.65μ as shown in Figure 6. This is just above the region 9.50μ reported for sulphoxides[115], but considerably below the region 10.30 - 10.50μ reported for N-oxides[116]. An N-oxide also seems unlikely as the Bohlmann bands observed for 27 are comparable in relative intensity with those observed in the i.r. spectra of the bisamine alkaloids. The presence of one N-oxide function and one basic nitrogen, in an alkaloid of this type, should result in a much diminished relative intensity for the Bohlmann bands. No absorption is observed in the region characteristic of hydroxy groups.

The u.v. spectrum of 27 in basic, neutral, and acidic methanol showed only end absorption and was identical with that of the bisamine thiospiran alkaloids (Figure 12). Sulphoxides are reported to exhibit a moderate absorption band at 210 nm in alcoholic solution[117]. This absorption band will therefore be obscured for sulphoxides of the bisamine alkaloids.

The mass spectrum of 27 exhibits ions at m/e 461 and 447 corres-ponding to losses of SOH and CH_2SOH from the molecular ion, paralleling the loss of SH and CH_2SH observed in the mass spectra of the bisamines

13 and 14 (Table 3). The monohemiaminals exhibit an intense peak at m/e 492 corresponding to the loss of H_2O from the molecular ion. The spectrum of 27 on the other hand exhibits an intense ion at m/e 493 corresponding to the loss of OH, a transformation supported by the presence of a "metastable" peak, and this fragmentation is commonly observed in the mass spectra of sulphoxides[118]. This facile loss of OH, rather than H_2O, coupled with the evidence that oxygen is lost together with sulphur on electron impact, suggests that alkaloid 27 is a sulphoxide. The remainder of the mass spectrum of 27 is very similar to that of the bisamines (Schemes 8 - 10). The ions at m/e 230, 178, 136, 107 and 94 are all present and have compositions identical with those found for 13 and 14 (Table 3). Ions of low intensity are present at m/e 280 ($C_{15}H_{22}NO_2S$), 262 (280 - H_2O), 375 ($C_{21}H_{31}N_2O_2S$), and 357 (375 - H_2O). The ion m/e 280 is cognate to ion m/e 230 and is analogous to ion m/e 264 in the spectra of the bisamines 13 and 14, while m/e 375 is cognate to m/e 136-H and is analogous to ion m/e 98 in the spectrum of deoxynupharidine 2 (Scheme 4) and ion m/e 359 in the spectra of the bisamines (Scheme 8).

An N-oxide structure is unlikely from mass spectrometric evidence. The mass spectrum of deoxynupharidine 2 is distinctly different from that of the corresponding N-oxide 1 which is dominated by the ion m/e 114 as shown in Figure 4. If 27 were an N-oxide then its mass spectrum should be similarly dominated by the ion m/e 375 (the ion analogous to m/e 114 in 1). Although m/e 375 ($C_{21}H_{31}N_2O_2S$) is present, it is relatively weak and may also be derived from a sulphoxide structure.

The p.m.r. spectra of 27 recorded in $CDCl_3$ and C_6D_6 solution at 220 MHz are shown in Figures 14 and 15 respectively. Only the region 0.06 - 4.06 is presented here as no other signals appear in the spectra, except those arising from the furan protons (see experimental section for chemical shifts). As there is no low field singlet expected for the part structure $\underset{}{>}N-\overset{OH}{\underset{}{C}}\overset{H}{\underset{}{<}}$, a monohemiaminal function at one of the C-6 positions may be ruled out. These p.m.r. spectra are unchanged after addition of D_2O confirming the absence of OH functions in the molecule.

The p.m.r. spectrum of 27 in $CDCl_3$ (Figure 14 and experimental section) also supports the premise that the alkaloid is not an N-oxide. An examination of the published p.m.r. data[70] for the N-oxide 7-epi-nupharidine shows that the protons H-4a, furan β-H and furan α-H as well as the protons at C-6 of this alkaloid all appear at much lower field than the corresponding protons of 27

Chemical studies have confirmed that 27 is neothiobinupharidine sulphoxide for it may be both prepared from and converted to neothio-binupharidine 14b. The configuration at sulphur, however, was not established at this stage.

When 14b is treated with H_2O_2 in glacial acetic acid it yields an oxidation product identical with the natural base 27. Their identity was established by comparison of their optical rotation, their i.r., u.v., p.m.r., and mass spectra, their melting points, and a mixture melting point determination. Compound 27 is remarkably stable to reducing agents. It is recovered unchanged after treatment with zinc in acetic acid, sulphur dioxide in aqueous solution, and sodium

borohydride in ethanol. This argues further against the possibility of
N-oxide or hemiaminal functions as nupharidine may be reduced to
deoxynupharidine with aqueous SO_2 and hemiaminals may be reduced to
tertiary amines with sodium borohydride.

The natural base 27 yielded a bisamine upon treatment with
phosphorous trichloride in ethyl acetate, a reagent known to convert
sulphoxides to sulphides.[119]. The bisamine was found to be neothio-
binupharidine after a comparison of spectroscopic properties and a
mixed melting point determination as described in the experimental
section.

The alkaloid 27 is therefore a sulphoxide of neothiobinupharidine.
It is the first representative of its class among the sulphur-containing
alkaloids isolated from Nuphar species. Interestingly no hemiaminals
based on the neothiobinupharidine skeleton have been isolated at this
time.

116
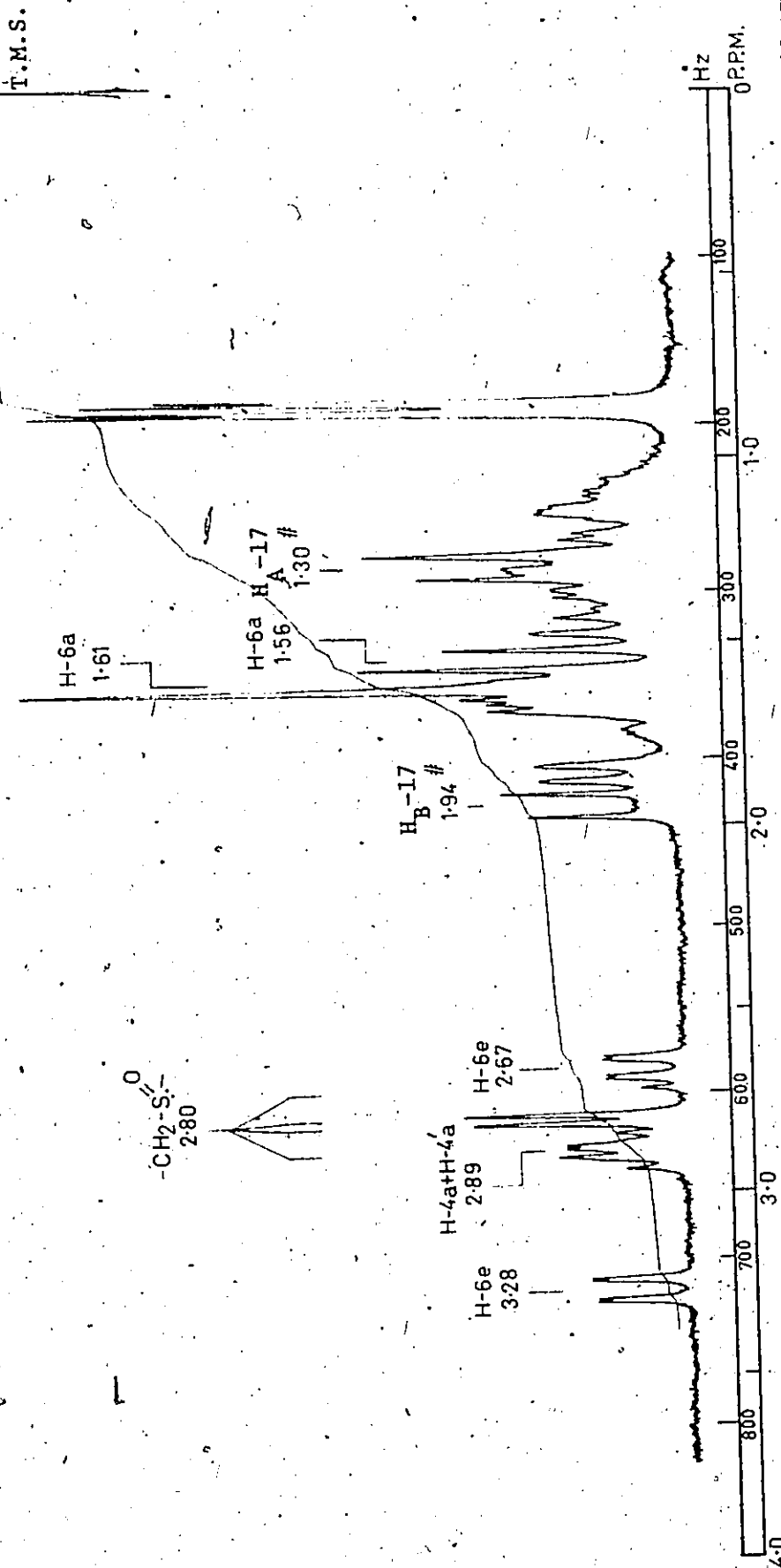
Figure 14. The p.m.r. spectrum of neothiobinupharidine sulphoxide (27) recorded in CDCl₃ solution at 220 MHz. # Assignments applicable to structure 27a only. (Figure 16)
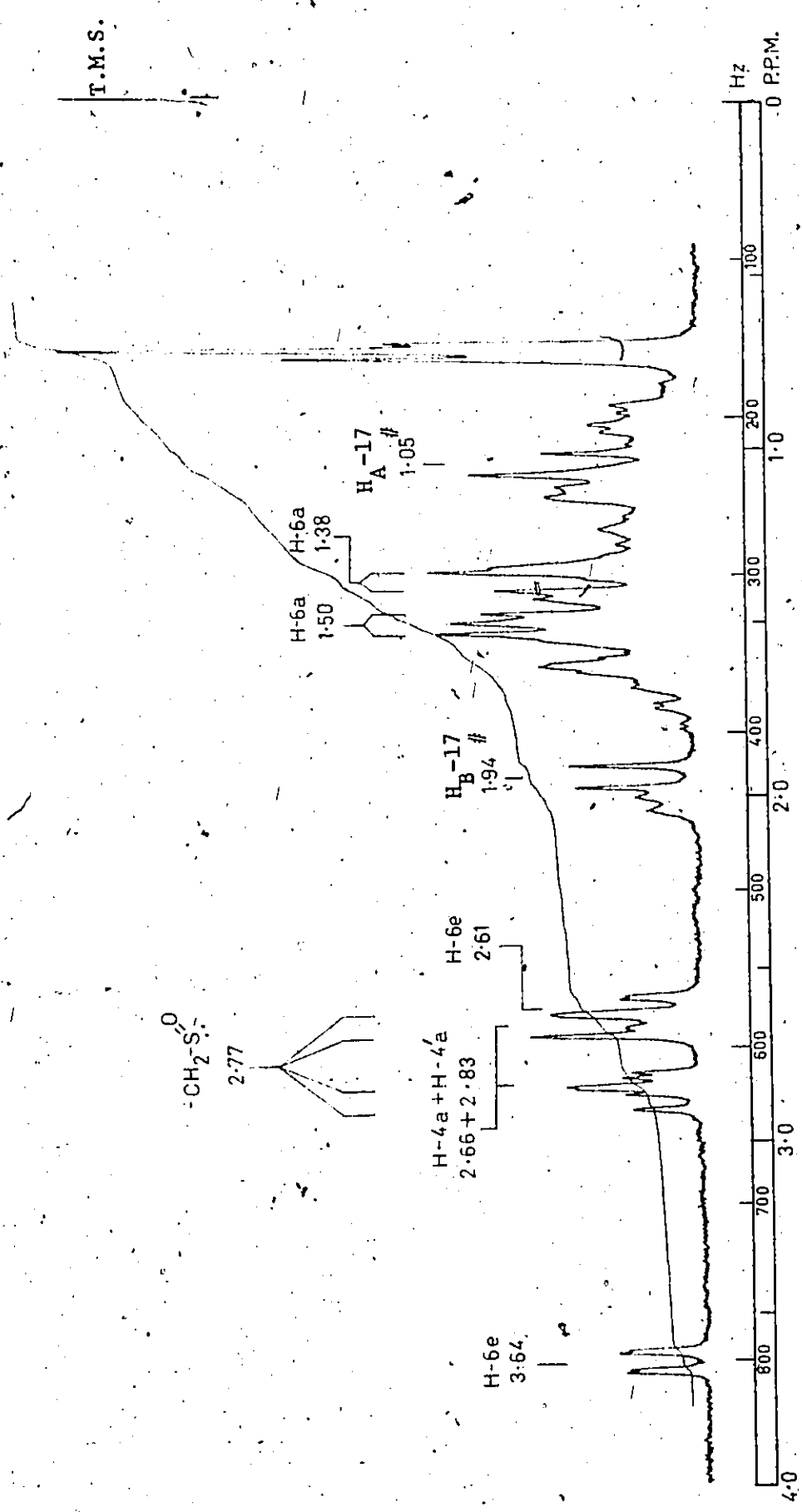
Figure 15. The p.m.r. spectrum of neothioblnupharidine sulphoxide (27) recorded in C_6D_6 solution at 220 MHz.

Assignments applicable to structure 27a only. (Figure 16)

— Assignments applicable to structure 27a only. (Figure 16)

(b) The configuration about sulphur

There are two possible structures for the sulphoxide of neothio-binupharidine and these are represented as <u>27a</u> and <u>27b</u> shown in Figure 16. The compounds <u>27a</u> and <u>27b</u> are diastereomers and in order to distinguish between them a detailed study of the p.m.r. spectra of the sulphoxide was undertaken. The protons at C-6, C-6', C-4, C-4', the two protons at C-17' and the two protons at C-17 were identified from their characteristic chemical shifts, their spin-spin multiplicities and coupling constants and by the use of spin-decoupling techniques (Figures 14, 15 and experimental section).

In order to differentiate between the equatorial proton at C-6 and the equatorial proton at C-6' of <u>27</u>, two basic assumptions were made. The first of these was that the tentative assignments made for these protons in the parent sulphide, neothiobinupharidine, were indeed correct, although it has not been possible to confirm this using n.O.e. studies. (The chemical shifts of the relevant protons of neothiobinupharidine <u>14b</u> together with those of neothiobinupharidine sulphoxide <u>27</u>, in the solvents $CDCl_3$ and C_6D_6, are listed in Table 9.) The second assumption is that a correlation may be drawn between the chemical shifts assigned to H-6a, H-6e, and H-6'a of <u>14b</u> in $CDCl_3$ solution and those observed for the corresponding protons of <u>27</u> in the same solvent. No correlation may be made between the assignments in C_6D_6 solution with any degree of confidence because the solvation of a cyclic sulphoxide by benzene is entirely different from the solvation of the corresponding sulphide[120,121] (see also later in this chapter). From Table 9 it
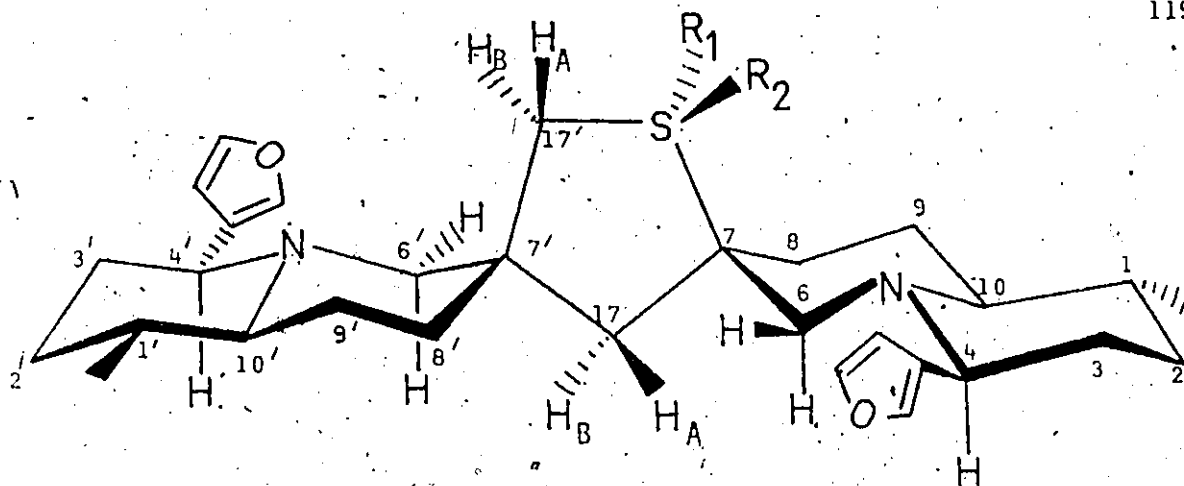
14b R_1 = lone pair; R_2 = lone pair
27a R_1 = O   ; R_2 = lone pair
27b R_1 = lone pair; R_2 = O
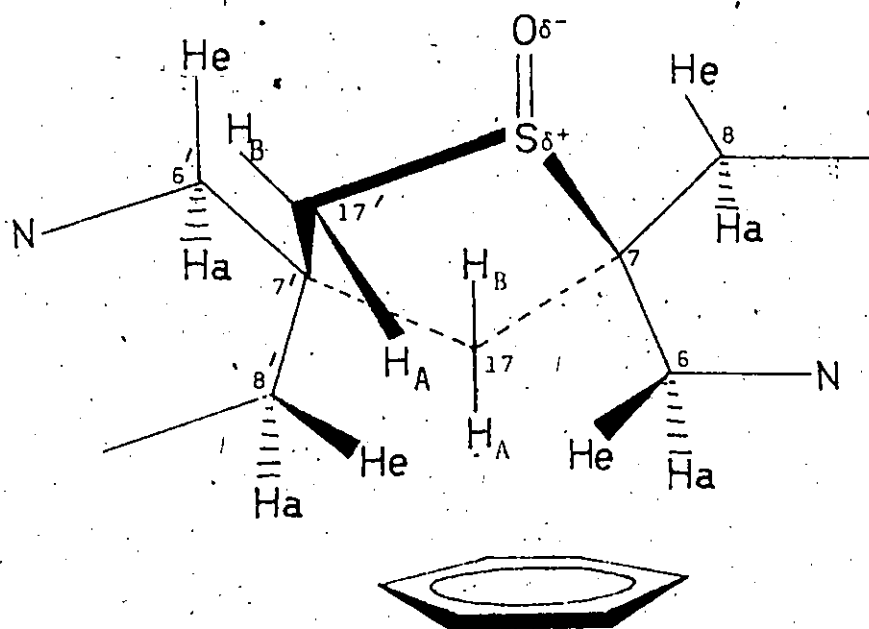
Figure 16. The structures of the two possible diastereomeric forms of neothiobinupharidine sulphoxide 27a and 27b, and the specific solvation by benzene of the S → O bond of 27a.

TABLE 9

Chemical shift and solvent shift data for neothiobinupharidine (14b) and its corresponding sulphoxide (27)

Compound	Solvent				Resonance				
		H-6a	H-6e	H-6'a	H-6'e	H-4a+H-4'a*	H-17A	H-17B	H-17'A+H-17'B*
Neothiobinupharidine (14b)	CDCl₃	1.60	2.67	1.68	2.94	2.82 to 2.95	1.27 or 1.22	1.22 or 1.27	2.69
	C₆D₆	1.55	2.88	1.71	3.21	2.75, 2.89	1.24 or 1.19	1.19 or 1.24	2.79, 2.89
	Δ$_{s.s.}^{sulphide}$	+0.05	-0.21	-0.03	-0.27	+0.04 to +0.18	+0.02 to +0.08	+0.02 to +0.08	-0.10 to -0.20
Neothiobinupharidine Sulphoxide (27)	CDCl₃	1.56	2.67	1.61	3.28	2.89	1.30	1.94	2.77, 2.83
	C₆D₆	1.38	2.61	1.50	3.64	2.66, 2.83	1.05	1.94	2.67, 2.87
	Δ$_{s.s.}^{sulphoxide}$	+0.18	+0.06	+0.11	-0.36	+0.06 to +0.23	+0.25	0	+0.16 to -0.10
Δδ^{obsd} =	CDCl₃	+0.04	0	+0.07	-0.34	-0.07 to +0.04	-0.03 to -0.08	approx. -0.7	-0.08 and -0.14

* It was not possible to differentiate between these two protons.
Chemical shifts (δ) are reported for dilute solutions (2-3% w/v) and are unchanged upon further dilution.

$\Delta\delta^{obsd} = (\delta_{sulphide} - \delta_{sulphoxide})$ calculated for CDCl₃ solutions only.

$\Delta_{s.s.}^{sulphide} = (\delta_{CDCl_3}^{sulphide} - \delta_{C_6D_6}^{sulphide})$; $\Delta_{s.s.}^{sulphoxide} = (\delta_{CDCl_3}^{sulphoxide} - \delta_{C_6D_6}^{sulphoxide})$

is apparent that H-6'e of the sulphoxide is deshielded by 0.34 p.p.m. relative to the parent sulphide.

An examination of a Dreiding model of 27 (as either 27a or 27b) indicated that an n.O.e. might be observed between H-6'e and H_B-17', but not between H-6e and either H_A-17' or H_B-17', for a variety of conformations of the central tetrahydrothiophene ring. However all attempts to observe an n.O.e. proved unsuccessful. Thus the chemical shifts of H-6'e and H-6e of 27 cannot be unambiguously assigned at this time. The discussion is nevertheless continued on the assumption that the assignments shown in Table 9 are correct.

Evidence will be presented based on the anisotropic effect of the S → O bond and based on solvent shift studies, involving specific solvation of the sulphoxide group by benzene, which is consistent with structure 27a, but not for 27b, for neothiobinupharidine sulphoxide.

The anisotropic effect of the sulphoxide S → O bond

There are several methods available for the determination of the configuration of a sulphoxide. The investigation of reactions at the α-carbon[122], such as base catalysed H-D exchange[123-127], necessarily require a reliable method to distinguish between the two α-methylene hydrogens. This was not possible for 27 owing to the lack of any observable n.O.e.'s.

P.m.r. chemical shift values have been employed to assign stereochemistry to both acyclic and cyclic sulphoxides. Significant among the numerous applications to cyclic systems are the recently reported configurational assignments of the sulphoxide groups of thietane-1-

oxides[128,129], episulphoxides[130], 1-4-oxathian-S-oxides[131] and, perhaps most impressively, for the sulphoxides of penicillins[120,132,134]. These studies have revealed that a significant deshielding is observed for protons possessing a cis 1-3-diaxial relationship to the S → O bond of the sulphoxide. This deshielding has been attributed[131a] to a proximity effect[89] and/or acetylenic type anisotropy of the S → O bond (collectively called the syn-axial effect). Other workers[135] have proposed from p.m.r. studies that, qualitatively at least, the screening environment around the S → O bond approximates that of the acetylenic triple bond.

It was of immediate interest, therefore, to note the downfield shift for the signal assigned to H-6'e upon oxidising neothiobinupharidine to its sulphoxide and also the large downfield shift of the signal assigned to H_B-17.

The chemical shift difference between an axial β-proton possessing a syn-axial relationship to an axial S → O bond, and an axial β-proton possessing a syn-axial relationship to the lone pair of the sulphoxide group (S → O bond equatorial) is well-documented[127,129,130], and is of the order of 0.7 → 1.0 p.p.m.
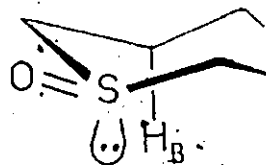
H_A appears ∼0.7 → 1.0 p.p.m. downfield of H_B

However, the effect of a syn-axial S → O bond relative to the parent sulphide is not as well-documented. In 27a it is necessary to know what effect the sulphoxide S → O bond has on the γ-proton H-6'e when compared to the parent sulphide 14b. A suitable model to assess this effect appears to be methyl-3,4-di-O-acetyl-2,6-anhydro-2-thio-α-D-altropyranoside 35 and its corresponding sulphoxide 36[131b].

35   $R_1 = R_2 =$ lone pair

36   $R_1 =$ lone pair; $R_2 = O$
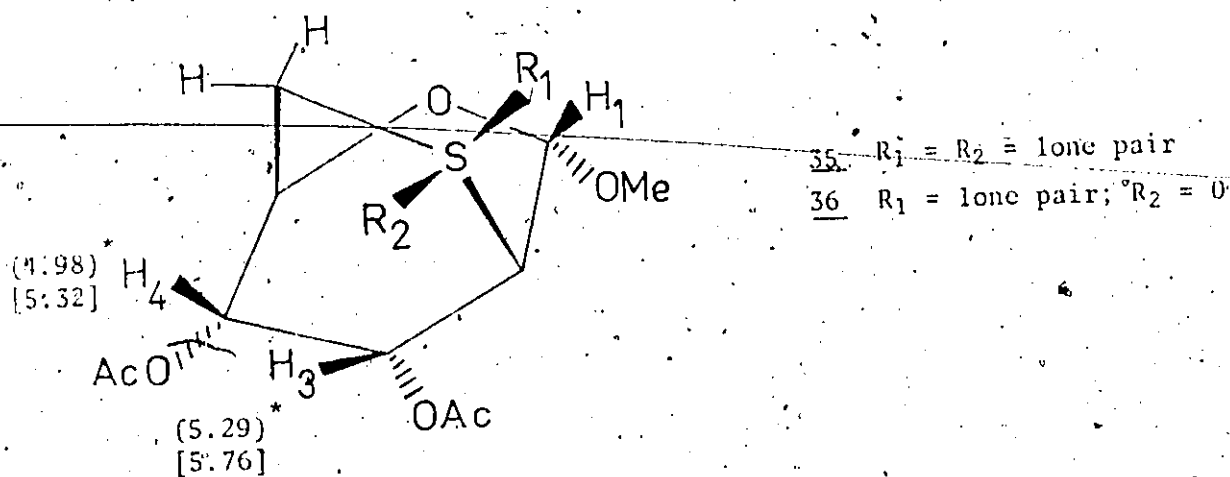
*The figures in round brackets represent the chemical shift (δ) observed for compound 35 in CDCl$_3$ solution and the figures in square brackets represent the chemical shifts of the corresponding protons of the sulphoxide 36 in the same solvent.

In order to compare the model compound 36 to the sulphoxide 27a proposals must be made concerning their preferred conformations in solution. A molecular model of 36 shows that it has a rigid structure and that the sulphoxide bond will probably lie in-between H-3 and H-4 (in order to minimise non-bonded interactions). Neothiobinupharidine sulphoxide, however, is more complicated. The trans-quinolizidine moieties represent fairly rigid sections of the molecule, but the molecule is conformationally labile, to a certain extent, through puckering of

the central tetrahydrothiophene ring.

A study of space-filling and Dreiding models suggests that a favourable conformation is that structure in which C-7', the sulphur atom, C-17 and C-17' are in approximately the same plane with C-7 lying outside this plane by puckering of the central ring, i.e. as the reader views structure 27a, the atoms C-7', C-17', S and C-17 would be approximately in the plane of the paper and C-7 would lie above the paper.* In this conformation the sulphoxide S → O bond bisects the angle between C-7—C-8 and C-7—C-17 and the protons attached to C-17 possess almost fully staggered relationships to the nearby C-C and C-H bonds of C-6, C-7, and C-8. A further consequence is that the S → O bond and the proton H_B-17 are in close proximity. This accounts, in part, for the low-field appearance of the signal assigned to the latter proton (1.94δ in CDCl$_3$) when compared to the same proton of 14b ($\sim$1.25δ); whereas H_A-17 has approximately the same chemical shift in both the sulphide 14b and the sulphoxide 27a. It has been demonstrated that the β-proton cis to the S → O bond appears at lower field than the β-proton trans to the S → O bond in the p.m.r. spectra of tetrahydrothiophene-S-oxide.[121]

Measurements of bond lengths and angles direct from Dreiding models (see also Table 10) shows that the spatial arrangements of the S → O bond and H-6'e of 27a and of the S → O bond and H-4 of the model compound 36 are similar. It should be noted that H-4 of 36 is also a γ-hydrogen and that the downfield shifts observed for H-6'e for the

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* A similar puckering of the central tetrahydrothiophene ring has been observed in the crystal form of neothiobinupharidine dihydrobromide as shown by the X-ray study[44], although in this case it is C-7' which lies outside the plane.

structural transformation 14b → 27a and for H-4 for the transformation 35 → 36 are identical (0.34 p.p.m.).

DeMarco et al.[120] have used the premise that "the electronic distribution about an axis through the sulphur and oxygen atoms of the S → O bond must be symmetrical or very nearly so". Thus they have pointed out that "for bonds which possess axial symmetry, the well-known and extensively invoked McConnell point dipole approximation[136] (Equation 3) is a useful expression relating the sign and magnitude of nuclear screening on a given proton to its spatial position relative to the anisotropic function under consideration".

$$\sigma = \Delta_X \left[\frac{(1 - 3\cos^2\theta)}{3R^3} \right] \quad ----- \quad (3)$$

where R = distance between the proton under study and the electrical centre of gravity of the anisotropic bond (in this case the centre of the S → O bond*), θ is the angle between the direction of R and the symmetry axis of the anisotropic bond, and Δ_X = a constant (anisotropy) characteristic of the bond under consideration*.

It was of interest to discover whether the change in chemical shift ($\Delta\delta^{obsd} = \delta^{obsd}_{sulphide} - \delta^{obsd}_{sulphoxide}$), observed for the various protons in the vicinity of the sulphur atom of 14b upon oxidation to 27a, was in agreement with those values predicted as a result of calculations of the

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* The same assumptions are made in this work as were made by DeMarco and coworkers[120] concerning the electrical centre of gravity of the S → O bond and the values used for Δ_X. The values used are -19.2 and -32.2 x 10^{-30} cm³ molecule^{-1} (the values reported for the acetylenic bond).

McConnell dipole approximation. Using equation (3) and the various parameters measured directly from Dreiding models, the change in chemical shift values were calculated for H-6'e, H-6'a, H-6e, H-6a, the thiomethylene protons at C-17', and the methylene protons at C-17, for the transformations 14b → 27a, 14b → 27b and also for H-3 and H-4 for the transformation 35 → 36.

Examination of Table 10 shows that a reasonable qualitative agreement was obtained in the case of 14b → 27a between predicted and observed values for H-6'e, H-6'a, and H-6a. The predicted change for H-6e was a shift to higher field whereas the observed value shows no change in chemical shift for this proton for the transformation 14b → 27a. However it was pointed out earlier that H-6e did appear to be abnormally shielded in the parent sulphide. Good qualitative agreement was also obtained in the case of the model compounds 35 → 36. The agreement between predicted and observed values for 14b → 27b was not acceptable. As it was not possible to differentiate between the protons H_A-17 and H_B-17' in the p.m.r. spectrum of 27 no comparison between observed and predicted values could be made. Good agreement with the McConnell approximation is not expected for these α-methylene protons, however, as it has been demonstrated[120,129,132-134,137] that protons α-antiaxial to the lone pair of

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* When proposing a preferred conformation for 27b the same non-bonded interactions were considered as for the case of 27a. Thus it seemed that the likely conformation would be that structure in which C-7', C-17, C-17' and the sulphur atom were approximately in the same plane with C-7 lying below this plane (as the reader views 27b). The S → O bond then bisects the angle between C-7—C-6 and C-7—C-17 and H_A-17 lies close to the S → O bond and is therefore assigned the resonance at 1.94δ (CDCl$_3$ and C$_6$D$_6$).

TABLE 10

McConnell Calculations for Screening Effects of S → O bond in the sulphoxides (27a) and (27b) and the model compound (36)

Calculations for the transformation	Proton	θ,deg†	R,Å†	G.F. #	$\Delta\delta^{-19.2}$	$\Delta\delta^{-32.2}$	$\Delta\delta$obsd*
Sulphide (14b) → Sulphoxide (27a)	H-6'e	89	3.2	+0.01016	-0.20	-0.33	-0.34 ✓
	H-6'a	70	4.7	+0.00208	-0.04	-0.06	+0.07 ✓
	H-6e	22	3.6	-0.01128	+0.21	+0.36	0 ✓
	H-6a	42	4.1	-0.00318	+0.06	+0.10	+0.04 ✓
	H_A-17	56	3.75	+0.00039	-0.01	-0.01	-0.03 to ✓, -0.08
	H_B-17	83	2.8	+0.01484	-0.28	-0.48	~-0.7 ✓
	H_A-17'	36	2.8	+0.01463	+0.28	+0.47	-0.08 and -0.14
	H_B-17'	75	2.3	+0.0220	-0.42	-0.70	
Sulphide (14b) → Sulphoxide (27b)	H-6'e	23	4.15	-0.00719	+0.14	+0.23	-0.34 ✓
	H-6'a	48	4.9	-0.00097	+0.02	+0.03	+0.07 ✓
	H-6e	74	2.7	+0.01308	-0.25	-0.42	0 ✓
	H-6a	83	3.65	+0.00655	-0.13	-0.21	+0.04 ✓
	H_A-17	83	2.8	+0.01451	-0.28	-0.47	~-0.7 ✓
	H_B-17	56	3.8	+0.00038	-0.01	-0.01	-0.03 to ✓, -0.08
	H_A-17'	75	2.3	+0.02189	-0.42	-0.70	-0.08 and
	H_B-17'	38	2.8	-0.01233	+0.24	+0.40	-0.14

† Values of θ and R were measured directly from Dreiding models, each value represents the mean of three measurements on two different models. Values of θ and R were reproducible to ±3° and ±0.1 Å, respectively. # G.F. represents the geometric factor of equation (5), i.e. G.F. $= \left[\dfrac{1-3\cos^2\theta}{3R^3}\right]$

* $\Delta\delta$obsd values are taken from Table 9. Checked values (✓) are those considered to show reasonable qualitative agreement with those values predicted.

TABLE 10 (continued)

Calculations for the transformation	Proton	θ,deg†	R,Å†	G.F.#	$\Delta\delta^{-19.2}$	$\Delta\delta^{-32.2}$	$\Delta\delta^{obsd}$*
Sulphide (35) $\Downarrow$ Sulphoxide (36)	H-3	81	2.7	+0.01569	-0.30	-0.50	-0.47 ✓
	H-4	81	2.8	+0.01407	-0.27	-0.45	-0.34 ✓

† Values of θ° and R were measured directly from Dreiding models, each value represents the mean of three measurements on two different models. Values of θ and R were reproducible to ±3° and ±0.1 Å, respectively. # G.F. represents the geometric factor of equation (3), i.e. G.F. = $\left[\dfrac{1-3\cos^2\theta}{3R^3}\right]$

* $\Delta\delta^{obsd}$ values are taken from page 123. Checked values (✓) are those considered to show reasonable qualitative agreement with those values predicted.

the sulphoxide actually experience considerable shielding, which counter-

acts the deshielding expected as a result of the syn-axial S → O bond.

This "lone-pair effect" is analogous to that observed for the nitrogen

lone-pair of piperidines and quinolizidines[83,138].

It is now accepted[132,133,137,139] that for protons on carbon

adjacent to the sulphoxide group the screening environment associated with

the anisotropic function is more complicated than the simple approximation

to an acetylenic bond.

It was possible to identify the protons H_A-17 and H_B-17 of the

sulphide 14b (Table 9) although they could not be differentiated per se.

These protons were also identified in the sulphoxide 27 and it was appar-

ent that one of these protons (assumed to be H_B-17 of 27a) suffers

considerable deshielding in the sulphoxide and it is significant that the

McConnell calculation for this proton predicts the same result.

Solvent-Shift Studies

Aromatic solvent-induced shifts (ASIS)[140] provide further

evidence which is consistent with structure 27a for neothiobinupharidine

sulphoxide. It has been experimentally well-established[141] that aro-

matic systems like benzene are capable of co-ordinating at electron

deficient sites in a solute molecule. Thus, solute protons situated in

the vicinity of a polar group, providing such an electron deficient site,

should experience large screening effects as a result of the anisotropy of

the associated aromatic system. Ledall[142] has proposed a common model

for the benzene-solute complex which presumes that the dipole axis of

the polar functional group in the solute molecule is located along the

sixfold axis of symmetry of the benzene system with the positive end of the polar function nearest and the negative end farthest away. The model has been used with considerable success to confirm the configurations of the sulphoxides of penicillins[120,132], and 2-methyl-thiolanes[139], and to assign chemical shifts to protons of tetrahydro-thiophene-S-oxide[121]. The model predicts that the benzene solvent molecule will approach the structure 27a from the lower front side as shown in Figure 16. The geometry of this complex, together with the anisotropy associated with aromatic systems, necessitates that the protons H-6e, H_A-17, H_A-17', and H-8'e should be shielded, whereas the other protons in the solute molecule be only marginally affected.

Alternatively for the structure 27b the direction of benzene complexation should be from the opposite side of the solute molecule, consequently it is anticipated that H-6'e, H-8e, H_B-17' and H_B-17 might experience strong shielding effects in benzene solution (relative to $CDCl_3$). For large solute molecules with more than one polar site, such as the thiospiran alkaloids studied here, several sites for co-ordination to solvent molecules are possible. Thus benzene solvent shift values ($\Delta_{s.s.}$ values) recorded in Table 9 are the summation of solvent shift contributions resulting from co-ordination of solvent molecules at each polar site within the solute molecule. Consequently, benzene-induced solvent shift values for the different protons, resulting from co-ordination of benzene with the S → O bond only, may be better approximated by subtracting the various $\Delta_{s.s.}$ values recorded (Table 9) for the sulphoxide 27 from the $\Delta_{s.s.}$ values recorded in the same table for the corresponding sulphide 14b. Since both the sulphoxide and the sulphide possess

the same polar functional groups, with the exception of the S → O bond in the former, the net shifts which result from this subtraction should be the solvent shift values (Σ values) which reflect complexation of benzene to the S → O bond.

The results of these calculations are summarized in Table 11. Examination of this table shows that, as expected, H-6e, H_A-17 and one of the protons at C-17' (presumably H_A-17') are shielded by 0.2 → 0.3 p.p.m. while the other protons are only marginally affected. Although H-8'e was not identified in the spectra of neothiobinupharidine, it was discovered that this proton in neothiobinupharidine sulphoxide absorbs at 0.95 p.p.m. in C_6D_6 solution. Thus although the complete solvent shift calculations for H-8'e cannot be made it seems probable from its high field appearance that this proton has also suffered shielding caused by specific benzene solvation of the S → O bond.

The calculations concerning specific benzene solvation are therefore consistent with structure 27a and not 27b for the sulphoxide of neothiobinupharidine. It should be pointed out, however, that all the available p.m.r. evidence rests very heavily on the correct assignment of the protons H-6'e and H-6e of 27. If these assignments are subsequently proven incorrect this would necessarily mean a reversal of the configuration from 27a to 27b.

The use of lanthanide-induced shifts[143] in the p.m.r. spectra of the sulphoxide was contemplated at one point. Although the predominant site of co-ordination is expected to be the oxygen atom of the S → O bond[144] it seemed that the assignment of the protons of a complicated molecule, such as 27 with many polar functions, might present a lengthy

TABLE 11

Benzene induced solvent-shift values $[\Sigma = \Delta_{s.s.}$ (sulphoxide) $- \Delta_{s.s.}$ (sulphide)] for the protons of the thiospiran alkaloid system, resulting from solute-solvent association of benzene with the S → O bond

Reaction	Solvent Shift Parameter	Resonance						
		H-6a	H-6e	H-6'a	H-6'e	H_A-17	H_B-17	H_A-17'+H_B-17'
Neothiobinupharidine 14b ⟹ Neothiobinupharidine sulphoxide 27	$\Delta_{s.s.}^{sulphoxide}$*	+0.18	+0.06	+0.11	-0.36	+0.25	0	-0.10 to +0.16
	$\Delta_{s.s.}^{sulphide}$*	+0.05	-0.21	-0.03	-0.27	+0.02 to +0.08	+0.02 to +0.08	-0.10 to -0.20
	Σ #	+0.13	+0.27	+0.14	-0.09	+0.17 to +0.23	-0.02 to -0.08	0 to +0.36

* From Table 9.

A positive value of Σ indicates that the proton is being shielded by specific benzene solvation of the S → O bond.

and difficult problem.

The stereoselective oxidation of neothiobinupharidine (14b).

It must be considered significant that the oxidation of neothio-binupharidine with peracetic acid gave one major product (excluding recovered substrate) which is identical with the natural sulphoxide 27 and has been assigned the configuration 27a.

Remarkable stereoselectivity in sulphoxide formation from sulphides is not uncommon [120,139,145-147]. Johnson and McCants [145] have put forward three proposals to account for the distribution of isomers obtained upon oxidising a cyclic sulphide to its corresponding sulphoxide. First, the conditions employed may be such that the products formed initially may undergo equilibration so that the final isomer distribution is governed by "thermodynamic product control". Secondly, the product distribution may be controlled by competitive attack from a sterically favoured side as opposed to a sterically hindered side ("steric approach control"). Thirdly, an energetic consideration, termed "product development control", which implies that the same factors influencing the stability of the final products are operational in the transition state (though not necessarily to the same extent) and that these products, once formed, are not further equilibrated.*

The stereoselectivity observed during the oxidation of neothio-binupharidine may be explained if any one or all three of these factors

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* In the case of the penicillins, the overriding factors appear to be thermodynamic product control and product development control owing to the formation of an intermolecular hydrogen bond to the oxidising agent during the reaction and an intramolecular hydrogen bond to the sulphoxide oxygen of the product; this has been termed "reagent approach control" [120].

are in operation. Configuration 27a is very probably thermodynamically more stable than 27b, as in the latter, a considerable repulsive interaction may exist between the π electron density associated with the S $\rightarrow$ O bond (polarised towards the electronegative oxygen atom) and the lone pair of electrons on N-5 and the π system of furan attached to C-4. No similar interaction exists in 27a.

The accepted mechanism for oxidation of sulphides by a peracid is nucleophilic attack of the sulphur atom on the hydroxyl oxygen[120,145].
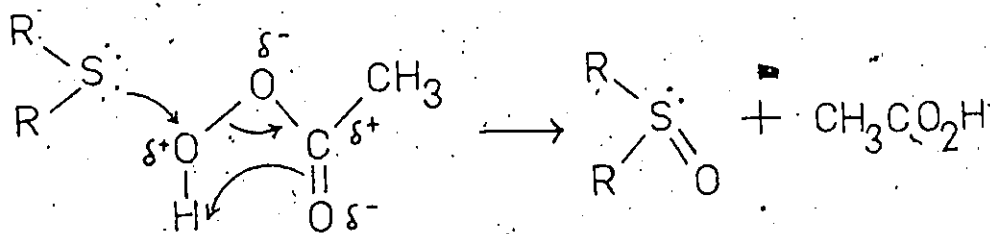
From considerations of steric hindrance it appears more likely that approach of the peracetic acid molecule is less hindered from that side of 14b which results in formation of 27a as opposed to approach from that side which would result in 27b (Figure 16). This is because of the proximity of the β-furyl group on C-4 and because the nitrogen is protonated under these conditions and therefore has an accompanying anion in close proximity. Furthermore, the hydroxyl oxygen of the peracid, which eventually becomes the sulphoxide oxygen atom, must be positively polarised and thus approach to the positively charged nitrogen N-5 is not favoured.

Whatever the reasons for the stereoselectivity observed in the oxidation, it is apparent that the predominant sulphoxide is remarkably stable. Sulphoxides are susceptible to stereomutation[148]; thus, heating to 190-220° or treatment with acid usually results in inversion at the sulphur atom (racemisation of enantiomeric sulphoxides and epimerisation of diastereomers)[148]. Neothiobinupharidine sulphoxide, however, may be sublimed at temperatures as high as 240°, and is also recovered unchanged after treatment with acetic acid. Furthermore, it was apparent that during the preparation of the sulphoxide very little competitive second oxidation stage had occurred, thereby converting the sulphoxide into the sulphone, even though sufficient peracetic acid was present. Usually this further oxidation cannot be avoided[146].

These observations strongly suggest that there is considerable opposition to introduction of an oxygen into that steric environment which would be encountered in configuration 27b. It has been proposed[148] that acid-induced inversion of a sulphoxide is sensitive to steric requirements as it involves an increase in the co-ordination number of sulphur (from three to four) in the rate determining step.

SUGGESTIONS FOR FURTHER WORK

Steric course of borodeuteride reductions of hemiaminals of thiobinupharidine

During the reduction of hemiaminals of thiobinupharidine in alcoholic solution the transient existence of an intermediate alkoxy-borodeuteride species has been proposed. Furthermore, it has been suggested that this species will have different steric and electronic requirements depending on the nature of the solvent. Several experiments to test these hypotheses have already been outlined (page 107).

Unambiguous assignment of chemical shift values to H-6e and H-6'e of neothiobinupharidine sulphoxide

The configuration 27a was assigned to the sulphoxide of neothiobinupharidine on the basis of proton magnetic resonance studies. This conclusion relied heavily on the assumption that the chemical shifts of the protons H-6e and H-6'e of the sulphoxide had been assigned correctly. Unfortunately it was not possible to confirm these assignments using n.o.e. studies, as no signal enhancement was observed.

It is conceivable that the chemical shifts of H-6e and H-6'e of 27 may be confirmed by a study of the natural abundance ^{13}C n.m.r. spectrum of this compound. Similar studies on the sulphoxides of penicillins have met with success[149]. The quaternary carbons C-7 and C-7' should be relatively easily differentiated from other carbons in the molecule by comparing the continuous wave decoupled (C.W.D.) and noise decoupled (N.D.) ^{13}C n.m.r. spectra of 27. As C-7 is α to the S → O group its chemical shift should be considerably downfield from that of C-7'. Similarly it should be possible to rationalise the effect of

the sulphoxide group β to C-6 and γ to C-6', thereby differentiating between these two carbon atoms of 27.

If it does not prove possible to differentiate between C-6 and C-6' through a comparison of their chemical shifts, it may be possible to do so by determining the magnitudes of the ^{13}C-^{1}H couplings. These may be estimated using the additivity relationships of Malinowski[150]. Since J_{C-H} is dependent on the percentage S character at the carbon participating in the C-H bond[151], the magnitude of J_{C-H} in the sulphoxide 27 will be dependent upon the nature of the substituents attached to each carbon (the β substitution pattern is different for C-6 and C-6'). The values of J_{C-H} may be calculated using empirically derived substituent contributions[152]. An example of this type of calculation has been reported for the sulphoxides of penicillins[149]. Providing that the chemical shifts of C-6 and C-6' may be unequivocally assigned, it follows that the protons H-6e and H-6'e may also be so assigned through selective heteronuclear spin-spin decoupling. Fortunately the signals arising from H-6e and H-6'e are well separated in the p.m.r. spectrum of 27 (Figures 14 and 15) thus no experimental difficulties should be encountered when applying double irradiation techniques.

EXPERIMENTAL

Apparatus, Methods and Materials

Mass spectra were determined on a C.E.C. 21-110B double-focusing mass spectrometer. Samples were introduced through a direct inlet system. Relative intensity data were obtained from low resolution mass spectra recorded under standard conditions at an ionising voltage of 70 eV, a trap current of 140 μA, a source temperature of 200°C (unless otherwise stated), and a source pressure of $1 - 2 \times 10^{-6}$ torr.

Low resolution mass spectra are plotted in terms of relative intensity with the most intense peak (base peak) taken as 100%.

Deuterium contents were determined in the following manner. The molecular ion region from M-3 to M+3 of the spectrum was scanned several times using several magnetic sweep velocities. The average peak heights were then determined for the labelled and unlabelled compounds and the deuterium content calculated by the method of Biemann[153].

The high resolution mass spectra (h.r.m.s.) were recorded on Ilford Q-2 photographic plates using perfluorokerosene as an internal marker. The plates were developed in the usual manner while flushing the development tank with a slow fine stream of nitrogen bubbles to ensure mixing[154]. The spectra were then recorded on magnetic tape, using a Gaertner comparator-densitometer linked to a Datex system.

They were then processed on a CDC-6400 computer using a modified version of the HIRES-3 program of Tunnicliff and Wadsworth[155]. The compositions of all ions formed by electron impact and discussed in this thesis were established by high resolution measurements and agreed with the calculated values within the limits ± 5 millimass units.

The early p.m.r. spectra were recorded at 100 MHz using the frequency sweep mode of a Varian HA-100 spectrometer. Samples were dissolved in $CDCl_3$ using added T.M.S. as internal locking signal. Spectra were recorded at 37°C. Chemical shifts were determined using a V4315 frequency counter incorporated in the instrument. Double irradiation was achieved by employing a Hewlett-Packard 201C audio-generator at the desired frequency. The majority of the p.m.r. spectra were recorded at 220 MHz on a Varian 220 HR spectrometer using the field sweep mode. All spectra were recorded at ambient temperature. Samples were dissolved in $CDCl_3$, C_6D_6 or CCl_4 as required using T.M.S. as internal standard. Chemical shifts are reported relative to T.M.S. = 0.06. Typically a sweep width corresponding to 2500 Hz was employed, but sweep widths of 1000, 500 and 250 Hz were sometimes used to determine coupling constants. Symbols s, d, t, q, m, nw, br, and W½ refer to singlet, doublet, triplet, quartet, multiplet, narrow, broad and width at half height, respectively. Spin-decoupling by double irradiation was achieved using a similar technique to that employed for the 100 MHz spectra.

In order to obtain accurate integrations the spectra of the deuterated products were recorded at 220 MHz using the time averaging computation technique (CAT) accumulated over 32 scans. The spectra

were then transferred to chart paper and integrated electronically. The areas under the signals were also compared by the method of "cutting out and weighing". The signal used as a standard integral for one proton was the low field half of the AB quartet arising from the thiomethylene group which in turn was correlated with the quartet (2H) arising from the protons H-4a and H-4'a (Figures 9 and 13).

Infrared spectra were recorded on a Perkin-Elmer 521 spectrometer. Typically, samples of free bases were made up at a concentration of 0.03M in spectroquality carbon tetrachloride or dichloromethane, using cells with KBr windows and a path length of 0.5 mm. Perchlorate salts were made up as KBr discs prepared in the usual manner and the spectra were recorded against air as the reference side.

Ultraviolet spectra were recorded on a Cary 14 spectrometer, at a concentration of 1-2 millimoles/litre, using 1 cm or 0.1 cm quartz cells as appropriate, and 95% ethanol or spectroquality methanol as solvent.

Optical rotation measurements were carried out at 22°C using a Hilger and Watts standard polarimeter equipped with a cell with a path length of 2 decimetres.

Melting points were determined on a Kofler micro-hot-stage and are uncorrected.

Thin layer chromatograms were performed under standard conditions, unless otherwise stated, using 0.5 mm silica gel coated on glass plates, available from Brinkman Instruments, Rexdale, Ontario. Solvent used for development in these cases was hexane:benzene:acetone: = 1:5:1. Development was carried out under conditions of tank saturation in a vessel 25 cm x 20 cm x 7 cm. The spray reagent was Dragendorf reagent[156].

Column chromatography was performed using neutral alumina, Brockman

Activity 1, 80-200 mesh, available from Fischer Scientific, adjusted

to give the required activity[157,158].

Vapour phase chromatography was performed using a Varian-

Aerograph series 200 instrument equipped with stainless steel columns

and flame ionisation detectors. Column lengths and stationary phases

are as described in the experiments concerned.

Sodium borodeuteride-d_4 was purchased from Stohler Isotope

Chemicals, and was certified 99% D. It was stored in its container

under vacuum over KOH as desiccant.

The elemental analyses were performed by A.B. Gygli, Micro-

analyses Laboratory, Toronto, Ontario, Canada.

Preliminary isolation of alkaloids from Nuphar luteum

This work was carried out in the laboratory of Professor

J.T. Wróbel at the University of Warsaw, Poland.

The crude bases extracted from the rhizomes of N. luteum were

adsorbed on neutral alumina (Act III) and the column eluted progress-

ively with benzene, ether, chloroform and methanol.*

The chloroform fraction was rechromatographed on a cellulose

column using a citrate-acetate buffer of pH 4.0 and 1-butanol. The

crude bases 26, 27 and 16 were obtained in this way. The hemiaminals

26 and 16 were separated from 27 and precipitated as their diper-

chlorates. Alkaloid 27 was isolated as a crude colourless solid.

The methanol fraction from the initial separation was

subjected to a 50 transfer counter current distribution using 1-butanol

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*Full details of the isolation procedure will be published elsewhere.

and a citrate-phosphate buffer (pH = 4.0). Fractions 25-35 were combined, the basic fraction recovered and subjected to chromatography on silica gel with chloroform:acetone = 1:1. The middle fractions were combined and evaporation of the solvent led to recovery of crude nupharolutine, 18.

Authentic samples of (+)-nupharidine, (-)-deoxynupharidine perchlorate, (-)-7-epideoxynupharidine perchlorate, (+)-thiobinupharidine, and (-)-neothiobinupharidine were supplied by Prof. J.T. Wróbel and a sample of castoramine hydrochloride[36] was gratefully received from Prof. Z. Valenta of the University of New Brunswick.

Preparation of Castoramine(10)for mass spectrometric study

The free base castoramine was prepared by treatment of the hydrochloride salt with 20% aqueous ammonia followed by extraction with dichloromethane. The extracts were combined, dried over anhydrous Na_2SO_4, and evaporated to yield a colourless solid residue m.p. 65-66° which was used without further purification for the mass spectrometric study.

M.s. m/e (Rel. int. %) 250 (5), 249 M^+ (30), 234 (2), 220 (9), 219 (16), 218 (10), 217 (4), 206 (9), 194 (5), 193 (3.5), 192 (3), 190 (4), 182 (2), 178 (6), 177 (5), 176 (4), 164 (8), 162 (4), 149 (8), 148 (6), 136 (50), 114 (96), 113 (11), 107 (14), 96 (7), 95 (14), 94 (100), 82 (23), 81 (23), 79 (17), 55 (29), 41 (43).

Mass spectrometric study of (+)-Nupharidine (1), m.p. 218°-220°

The sample was examined as received without further purification.

M.s. m/e (Rel. int. %) 249 M^+ (11), 232 (6), 220 (8), 190 (1), 180 (1), 168 (1.5), 166 (2), 154 (3), 141 (11), 136 (2), 115 (8), 114 (100), 113 (2), 107 (3), 98 (13), 94 (9), 81 (7), 55 (10), 41 (11).

Preparation of (-)-Deoxynupharidine (2c) for spectroscopic study

An authentic sample of deoxynupharidine perchlorate, m.p. 238°-243° decomp. (22 mg) was dissolved in 20 ml of 20% aqueous ammonia and stirred for 30 minutes. The aqueous solution was extracted with CH_2Cl_2 (5 x 10 ml), the extracts were combined, dried over anhydrous Na_2SO_4, filtered, and the solvent removed under reduced pressure (40° bath). The resultant colourless oily residue was adsorbed on to 4 g of neutral alumina (Act II) and eluted with 50 ml of 5% ether in hexane. Evaporation of the solvent led to recovery of deoxynupharidine (2c) (14.5 mg, 94%).

p.m.r. 220 MHz $(CDCl_3)$: δ 0.89 (d, J = 5.6 Hz, 3H, C-1-CH_3c) 0.99 (d, J = 7.0 Hz, 3H, C-7-CH_3a) 1.80 (q, J = 11.5 and 2.5 Hz, 1H, H-6a) 2.65 (q, J = 11.5 and 2.0 Hz, 1H, H-6e) 2.92 (q, J = 7.5 and 6.5 Hz, 1H, H-4a) 6.37 (nw.m. $W_{\frac{1}{2}}$ = 3 Hz, 1H, furan β-H) 7.24 and 7.31 (nw.m's. $W_{\frac{1}{2}}$ = 3 Hz, 1H each, furan α-H).

p.m.r. 220 MHz (C_6D_6): δ 0.81 (d, J = 6.0 Hz, 3H, C-1-CH_3c) 1.10 (d, J = 7.0 Hz, 3H, C-7-CH_3a) 1.78 (q, J = 11.5 and 3.0 Hz, 1H, H-6a) 2.76 (q, J = 11.5 and 2.5 Hz, 1H, H-6e) 2.81 (q, J = 11.0 and 3.0 Hz, 1H, H-4a) 6.38 (nw.m. $W_{\frac{1}{2}}$ = 3 Hz, 1H, furan β-H) 7.14 (nw.m. furan β-H).

Irradiation at 1.78δ collapses quartet at 2.76 into broad singlet.

M.s. m/e (Rel. int. %) 234 (7), 233 M$^+$ (41), 232 (12.5), 218 (4), 216 (1), 205 (1.5), 204 (9), 191 (3.5), 190 (13), 178 (6), 177 (8.5), 176 (5), 166 (3), 163 (2.5), 162 (6.5), 149 (2), 148 (6), 138 (2), 137 (7), 136 (35), 135 (2), 134 (2), 126 (2), 125 (5), 124 (4.5), 121 (4.5), 108 (4), 107 (6), 99 (7), 98 (100), 97 (24), 96 (4), 95 (6), 94 (59), 93 (3.5), 91 (2.5), 81 (12), 79 (7), 55 (20), 53 (7), 41 (17), 39 (9).

Preparation of (-)-7-epideoxynupharidine (11) for spectroscopic study

An authentic sample of (-)-7-epideoxynupharidine perchlorate (40 mg), m.p. 237°-241° decomp., was basified and worked up as in the procedure above yielding (-)-7-epideoxynupharidine (11) (25 mg, 90%).

p.m.r. 220 MHz (CDCl$_3$): δ 0.74 (d, J = 6.5 Hz, 3H, C-7-CH$_3$e) 0.91 (d, J = 6.0 Hz, 3H, C-1-CH$_3$e) 2.82 (d of q, J = 11.5 and 3.5 and 2.5 Hz, 1H, H-6e) 2.90 (q, J = 10.5 and 3.5 Hz, 1H, H-4a) 6.45 (nw.m. W½ = 3 Hz, 1H, furan β-H) 7.27 and 7.37 (nw.m's. W½ = 3 Hz, 1H each, furan α-H).

p.m.r. 220 MHz (C$_6$D$_6$): δ 0.68 (d, J = 6.5 Hz, 3H, C-7-CH$_3$e) 0.83 (d, J = 6.0 Hz, 3H, C-1-CH$_3$e) 1.29 (t, (1:2:1), J = 11.0 Hz and 11.0 Hz, 1H, H-6a) 1.64 (complex mult., H-7a + H-3e + H-8e) 1.77 (d of mult., J = 11.0 and 3.5 Hz, distinguishable, H-3a) 2.83 (q, J = 11.0 and 3.5 Hz, 1H, H-4a) 3.03 (d of q, J = 11.0 and 3.5 and 2.0 Hz, 1H, H-6e).

Irradiation at 0.68δ induced changes in the region 1.64δ.

Irradiation at 1.64δ collapsed doublet at 0.68δ into singlet; also collapsed quartet at 2.83δ into doublet (J = 11.0 Hz); also collapsed doublet of quartet at 3.03δ to doublet (J = 11.0 Hz).

Irradiation at 1.77δ collapsed quartet at 2.83δ to a doublet (J = 3.5 Hz).

Irradiation at 2.83δ collapsed the 11.0 Hz coupling in signal at 1.77δ.

Irradiation at 1.29δ collapsed doublet of quartet at 3.03δ to multiplet with couplings 2.0 and 3.5 Hz recognisable.

Irradiation at 3.03δ collapsed 1:2:1 triplet at 1.29δ to a 1:1 doublet (J = 11.0 Hz).

Purification of (-)-nupharolutine (18)

The crude solid base was recrystallised from methanol-acetone (3:1) yielding colourless needles (30 mg), m.p. $96°-98°$; $[\alpha]_D^{22°} = -105°$ (CHCl$_3$) $\underline{c} = 10.84$ mg/ml.

Analysis: Calculated for $C_{15}H_{23}NO_2$ (Mol. wt. 249.173): C, 72.3; H, 9.3; N, 5.6. Found: [249.173 (h.r.m.s.)]: C, 72.4; H, 9.5; N, 5.9.

i.r. (CCl$_4$) 3485 cm^{-1} (bonded OH) and 3615 cm^{-1} (free OH), 2800 and 2760 cm^{-1} (Bohlmann bands).

p.m.r. 100 MHz (CDCl$_3$): δ 0.88 (d, J = 5.6 Hz, 3H, CH-CH$_3$) 1.22 (s, 3H, C(OH)CH$_3$) 2.66 (q, J = 11.5 and 2.0 Hz, 1H, H-6e) 3.03 (q, J = 8.3 and 6.0, 1H, H-4a) 6.34 (nw.m., 1H, furan β-H) 7.25 plus 7.34 (nw.m., 2H, furan α-H).

p.m.r. 220 MHz (CCl$_4$): δ 0.90 (d, J = 6.5 Hz, 3H, CH-CH$_3$).

1.13 (s, C(OH)CH$_3$) 2.54 (q, J = 11.0 and 2.5 Hz, 1H, H-6e)

2.97 (q, J = 8.5 and 5.0 Hz, 1H, H-4a) 6.25 (nw.m., W$\frac{1}{2}$ = 4 Hz, 1H,

furan β-H) 7.16 (nw.m., W$\frac{1}{2}$ = 3 Hz, 1H, furan α-H) 7.20 (nw.m., W$\frac{1}{2}$ = 3 Hz,

1H, furan α-H).

M.s. m/e (Rel. int. %) 250 (8), 249 (43), 234 (10), 220 (11),

218 (2), 207 (3), 206 (15), 204 (2), 194 (7), 193 (4), 192 (4), 191 (3),

182 (5), 178 (39), 176 (5), 164 (12), 163 (4), 162 (5), 148 (9),

141 (7), 136 (82), 115 (9), 114 (100), 113 (19), 107 (24), 96 (24),

95 (12), 94 (87), 93 (7), 91 (6), 81 (22), 79 (16), 55 (17), 53 (11),

43 (23), 41 (22), 39 (14).

Preparation of 7-chlorodeoxynupharidine (19)

Nupharolutine (10 mg) was treated with 1 ml of a solution of

POCl$_3$ in dry pyridine (1:4) and the solution allowed to stand at 0° for

24 hrs with occasional shaking. The resultant light brown solution was

added to ice water (5 ml), basified by addition of 1M NaOH, and extracted

with CH$_2$Cl$_2$ (4 x 10 ml). The extract was dried over anhydrous sodium

sulphate and evaporated to dryness under reduced pressure. The residue

was separated into two components (Rf = 0.60 and 0.45) on a t.l.c. plate

(0.5 mm silica gel) using benzene-acetone = 3:1 as developer. The major

component (Rf = 0.60) was recovered by extraction of the silica gel with

methylene chloride using a small scale Soxhlet apparatus; yield 3.7 mg

of 7-chlorodeoxynupharidine.

M.s. m/e (Rel. int. %) 269 (9), 267 (28), 254 (1), 253 (1),
252 (2), 240 (1), 239 (1.5), 238 (3), 233 (11), 232 (52), 231 (13),
226 (3), 224 (9), 218 (5), 214 (2), 212 (6), 204 (7), 176 (4), 149 (13),
137 (17), 136 (96), 134 (23), 132 (65), 131 (4), 107 (11), 95 (13),
94 (100), 81 (12), 79 (9), 55 (5), 53 (2).

Reduction of 7-chlorodeoxynupharidine to deoxynupharidine (2)

7-chlorodeoxynupharidine (3.4 mg) was dissolved in absolute
ethanol (10 ml) and to the solution 50 mg of solid KOH and 25 mg of
30% Pd on $CaCO_3$ were added. The suspension was shaken with hydrogen
at 45 p.s.i.g. for 30 minutes. The reduction mixture was neutralised
with ethanolic HCl, and the catalyst separated by filtration and washed
with CH_2Cl_2. The ethanolic and dichloromethane solutions were combined
and evaporated to dryness under reduced pressure. The residue was
separated by t.l.c. (silica gel, 0.5 mm) into a major component
(Rf = 0.25) and a minor component (Rf = 0.45 not further examined) with
chloroform-acetone = 5:4. The band, Rf = 0.25, was removed from the
plate and extracted with CH_2Cl_2 yielding a colourless product (2 mg)
which had the same Rf value on t.l.c. as an authentic sample of (-)-deoxy-
nupharidine, 0.5 mm silica gel (chloroform-acetone = 5:4) Rf = 0.25;
0.5 mm silica gel (chloroform:acetone:diethylamine:methanol = 5:4:1:2)
Rf = 0.41. The product had the same retention time as authentic deoxy-
nupharidine on g.l.c. using two different columns. The columns used were
(a) 5% SE30 on 60/80 Chromosorb W (5 ft x 1/8 inch column, 175°, 35
p.s.i.g. He), and (b) 20% DEGS on 60/80 Chromosorb W (5 ft x 1/8 inch
column, 180°, 35 p.s.i.g. He). The mass spectrum of the product was

identical with that of an authentic sample of deoxynupharidine.

Thiobinupharidine (13c)

The sample was obtained from Professor Wrobel as well-formed microscopic colourless crystalline prisms, m.p. 129°-131°. T.l.c. showed only one spot (standard system Rf = 0.51); therefore the sample was examined without further purification. $[\alpha]_D^{22°} = +8°$ (CH₃OH) c = 16 mg/ml. U.v. (neutral and acidic 95% EtOH) end absorption only.

i.r. (CCl₄) 3.59 to 3.88 (Bohlmann bands); 6.66 and 11.45 (furan); and unassigned absorption at 6.88, 6.91, 6.96, 7.23, 7.26, 7.65, 7.76, 8.63, 8.78, 8.89, 9.05, 9.36, 9.65, 9.74 μ.

p.m.r. 220 MHz (CDCl₃): δ 0.92 (d, J = 5.6 Hz, 6H, 2 x CH-CH₃) 1.45 (d, superimposed on envelope, J = 11.5 Hz, H-6'a) 1.72 (d, superimposed on envelope, J = 11.5 Hz, H-6a) 1.90 (ABq, J_{AB} = 14 Hz, 2H, C-7-CH₂-C-7') 2.33 (ABq, J_{AB} = 11.5 Hz, 2H, CH₂-S-) 2.81 (q, J = 11.5 and 2 Hz, 1H, H-6e) 2.94 (q, superimposed on complex multiplet, J = 11.5 and 2 Hz, H-6'e) 6.39 (nw.m., W½ = 6 Hz, 2H, furan β-H) 7.25 + 7.33 (4H furan α-H).

Irradiation at 2.94δ collapses the doublet at 1.45δ into a singlet. Irradiation at 1.45δ collapses signal at 2.94δ into a broad singlet.

Irradiation at 1.72δ collapses signal at 2.81δ into a broad singlet. Irradiation at 2.81δ collapses doublet at 1.72δ into a singlet.

p.m.r. 220·MHz (C_6D_6): δ 0.78 (d, CH-CH$_3$, J = 5.6 Hz),
0.81 (d, J = 6.0 Hz, CHCH$_3$, with 0.78 = 6H) 1.40 (d, J = 11.5 Hz, H-6'a)
1.92 (d, J = 11.5 Hz, 1H, H-6a) 2.18 (ABq, J_{AB} = 14.0 Hz, 2H, C$_7$-CH$_2$-C$_7$')
2.31 (ABq, J_{AB} = 11.5 Hz, 2H, CH$_2$-S) 2.80 (q, J = 10.5 and 3.5 Hz, 2H,
H-4a + H-4'a) 3.11 (q, J = 11.5 and 2 Hz, 1H, H-6e) 3.17 (q, J = 11.5
and 2.5 Hz, 1H, H-6'e) 6.42 (nw.m., 2H, furan β-H) 7.14 (nw.m., 4H,
furan α-H).

Irradiation at 1.92δ collapsed the quartet at 3.11δ into a broad
singlet but the quartet at 3.17δ was unaffected; irradiation at 1.40δ
collapsed the quartet at 3.17δ into a broad singlet but the quartet at
3.11δ was unaffected (Figure 13).

M.s. m/e (Rel. int. %) 495 (10.3), 494 (30), 493 (5.6), 479 (0.3),
465 (0.75), 461 (2.0), 451 (0.5), 447 (1.6), 427 (1.1), 413 (0.5),
360 (2.0), 359 (8.5), 358 (1.9), 357 (2.1), 264 (1.3), 247 (1.3),
231 (10), 230 (35), 180 (10), 179 (12), 178 (100), 136 (11.5), 107 (23),
94 (40).

Neothiobinupharidine (14b)

The sample was obtained from Professor Wróbel as well-formed
microscopic colourless crystalline needles, m.p. 159°-160°. T.l.c.
showed only one spot (standard system Rf = 0.63); therefore the sample
was examined without further purification. U.v. (neutral and acidic
methanol) end absorption only.

i.r. (CCl$_4$) 3.59 - 3.88 intense (Bohlmann bands); 6.67 and
11.45 (furan) and unassigned absorptions at 6.96, 7.22, 7.27, 7.37,
7.66, 7.74, 7.83, 8.64, 8.89, 9.09, 9.40, 9.54, 9.75 and 10.40 μ.

p.m.r. 220 MHz (CDCl$_3$): δ 0.87 (d, J = 5.5 Hz, 6H, 2 x CH-CH$_3$) 1.245 (ABq, ν_A and ν_B = 1.22 and 1.27, J$_{AB}$ = 14.0 Hz, C-7-CH$_2$-C-7') 1.60 (d, superimposed on envelope, J = 11.5 Hz, H-6a) 1.68 (d, super-imposed on envelope, J = 11.5 Hz, H-6'a) 2.67 (q, J = 11.5 and 2.0 Hz, 1H, H-6e) 2.69 (s, 2H, -CH$_2$-S) 2.94 (q, superimposed on complex multiplet due to H-4a and H-4'a, J = 11.5 and 2.0 Hz, H-6'e) 6.34 (nw.m., W$\frac{1}{2}$ = 3 Hz, 1H, furan β-H) 6.52 (nw.m., W$\frac{1}{2}$ = 3 Hz, 1H, furan β-H) 7.23 (nw.m., W$\frac{1}{2}$ = 3 Hz, 1H, furan α-H) 7.32 (nw.m., W$\frac{1}{2}$ = 3 Hz, 3H, furan α-H).

Irradiation at 2.94δ collapses doublet at 1.68δ into broad sing-let with doublet at 1.60δ unaffected. Irradiation at 2.67δ collapses doublet at 1.60δ into broad singlet with doublet at 1.68δ unaffected.

p.m.r. 220 MHz (C$_6$D$_6$): δ 0.75 (d, J = 5.6 Hz, 6H, 2 x CH-CH$_3$) 1.215 (ABq, ν_A and ν_B = 1.19 and 1.24, J$_{AB}$ = 14.0 Hz, C-7-CH$_2$-C-7') 1.55 (d, superimposed on envelope, J = 11.5 Hz, H-6a) 1.71 (d, super-imposed on envelope, J = 11.5 Hz, H-6'a) 2.75 (q, J = 9.5 and 5.0 Hz, 1H, H-4a or H-4'a) 2.84 (ABq, ν_A = 2.79, ν_B = 2.89, J$_{AB}$ = 11.5 Hz, 2H, -CH$_2$-S) 2.88 (q, superimposed on another quartet, J = 11.5 and 2.0 Hz, H-6e) 2.89 (q, J = 9.5 and 5.0 Hz, together with 2.88, 2H, H-4a or H-4'a) 3.21 (q, J = 11.5 and 2.0 Hz, 1H, H-6'e) 6.31 (nw.m., W$\frac{1}{2}$ = 3 Hz, 1H, furan β-H) 6.74 (nw.m., W$\frac{1}{2}$ = 3 Hz, 1H, furan β-H) 7.15 (nw.m., W$\frac{1}{2}$ = 5 Hz, 3H, furan α-H) 7.36 (nw.m., W$\frac{1}{2}$ = 3 Hz, 1H, furan α-H).

Irradiation at 2.88δ collapses doublet at 1.55δ to broad singlet with doublet at 1.71δ unaffected. Irradiation at 3.21δ collapses doublet at 1.71δ to broad singlet with doublet at 1.55δ unaffected.

M.s. m/e (Rel. int. %) 495 (10.2), 494 M$^+$ (28), 493 (5), 479 (0.35), 465 (0.9), 461 (2.7), 451 (0.6), 447 (2.8), 427 (1.3),

415 (0.6), 360 (2.8), 359 (10.5), 358 (3.0), 357 (3.7), 332 (1.5),

329 (1), 325 (0.7), 316 (1.2), 303 (1.2), 302 (1.1), 264 (1.6),

263 (1.5), 262 (1.2), 180 (16), 179 (14), 178 (100), 176 (4), 162 (3),

148 (3), 136 (9.5), 107 (20), 94 (38), 81 (13), 79 (13), 55 (4),

53 (3), 41 (4), 39 (3).

Preparation of Thiobinupharidine Dihydrobromide Dihydrate for X-ray study

An authentic sample of thiobinupharidine (13c) (50 mg) was dissolved in methanol with heating and a 1:1 mixture of methanol and concentrated hydrobromic acid was added dropwise until a pH of 3 was reached. The fine colourless needles that separated on cooling were recrystallised from a 1:1 mixture of acetone and water. The crystals melted at 245°-247°.

Analysis: Calculated for $C_{30}H_{42}N_2O_2S.2HBr.2H_2O$: C, 52.02; H, 6.92; N, 4.04; Br, 23.09. Found: C, 52.06; H, 6.85; N, 4.08; Br, 23.11.

Conversion of the hydrobromide to the free base was achieved by treatment of an aqueous solution of the salt with 20% aqueous ammonia followed by extraction with dichloromethane. Subsequent work-up in the usual way gave a colourless solid which when recrystallised from methanol-acetone at -5° gave off-white needles, m.p. 129°-130°. Mixed m.p. with an authentic sample of thiobinupharidine showed no depression in melting point. M^+ (h.r.m.s. 494.297) Calcd. for $C_{30}H_{42}N_2O_2S$: 494.296.

Details of the X-ray study of thiobinupharidine dihydrobromide dihydrate have been published [159].

Preparation of Thionupharoline (26) from its Perchlorate

A 50 mg sample of thionupharoline diperchlorate, m.p. 172°-174° (see below) was treated with 10 ml of 20% ammonia and stirred for thirty minutes. The resultant oil was extracted with CH_2Cl_2 (5 x 10 ml). The extracts were combined, dried over anhydrous sodium sulphate, and evaporated under reduced pressure to leave a colourless glass on the inside of the vessel. The yield was 34.6 mg of free base which was purified by chromatography from neutral alumina (Act II, 2 g) using 20% ether in hexane as eluant. Thionupharoline 26 (34 mg) was obtained as a glass and showed one spot on t.l.c. Rf = 0.36. Analysis:[56] Calcd. for $C_{30}H_{42}N_2O_3S$ (Mol. wt. 510.291). Found [510.290 (h.r.m.s.)]. U.v. (95% EtOH, neutral), end absorption only; u.v. (95% EtOH, acidic) λ_{max_1} 208 nm (ϵ 20,000), λ_{max_2} 293 nm (ϵ = 2540).

i.r. (CH_2Cl_2) 2.80 (OH), (weak), 3.59 (Bohlmann band weak), 6.65 and 11.45 (furan) and unassigned bands at 6.90, 7.26, 8.66, 9.08, 9.40, 9.67, 9.74 μ.

p.m.r. 220 MHz ($CDCl_3$): δ 0.88 (d, J = 5 Hz, 6H, 2 x $CHCH_3$) 2.20 (ABq, J_{AB} = 12 Hz, 2H, CH_2-S) 2.26 (OH exchangeable on addition of D_2O) 2.89 (q, J = 5.6 and 8 Hz, 1H, H-4'a) 2.92 (q, J = 11.5 and 2 Hz, 1H, H-6'e) 3.70 (q, J = 7.5 and 7 Hz, 1H, H-4a) 3.97 (s, 1H, H-6, sharpens on addition of D_2O) 6.34 (nw.m., $W\frac{1}{2}$ = 7 Hz, 2H, furan β-H) 7.21 (nw.m., 1H, furan α-H) 7.30 (nw.m., $W\frac{1}{2}$ = 4 Hz, 3H, furan α-H).

p.m.r. 220 MHz (C_6D_6): δ 0.75 (two superimposed doublets J = 6 Hz, 6H, 2 x $CHCH_3$), 2.11 (ABq, J_{AB} = 12 Hz, 2H, CH_2-S)

2.42 (OH exchangeable with D_2O) 2.79 (q, J = 3 and 11 Hz, 1H, H-4'a)

3.18 (q, J = 2.5 and 12 Hz, 1H, H-6'e) 3.90 (q, J = 4 and 10 Hz, 1H,

H-4a) 4.25 (s, 1H, H-6) 6.41 (nw.m., furan β-H) 6.48 (nw.m., 2H, with

6.41 furan β-H) 7.16 (nw.m., 3H, furan α-H) 7.22 (nw.m., 1H, furan α-H).

M.s. (230°) m/e (Rel. int. %) 510 M^+ (15); 494 (19), 493 (31),

492 (72), 481 (1), 477 (2), 475 (0.8), 464 (4), 459 (3), 445 (6),

431 (1.5), 425 (1), 397 (1.5), 383 (1), 375 (8), 359 (2), 357 (2),

304 (4), 262 (5); 246 (5), 244 (4), 231 (24), 230 (100), 229 (28),

228 (45), 215 (9), 178 (24), 176 (54), 136 (12), 107 (26), 94 (50).

Monoperchlorate of Thionupharoline

Thionupharoline (21.6 mg, 0.0424 mM) was dissolved in 5 ml

MeOH and treated with 2.23 ml of 0.019 M $HClO_4$ (0.0424 mM). The

solution was warmed for a few minutes on a steam bath and the solvent

removed under reduced pressure leaving 27 mg of colourless semi-

crystalline solid. Recrystallization from ether containing sufficient

acetone to effect solution gave 25 mg colourless prismatic needles.

Thionupharoline monoperchlorate, m.p. 240°-243°, i.r. (KBr disc) 5.62

(weak Bohlmann band); 6.03 and 6.06 (C = N^+<); 6.66 and 11.45 μ (furan).

Treatment of the monoperchlorate with aqueous ammonia led to

recovery of the free base, thionupharoline, characterized by its

p.m.r. and mass spectra.

Diperchlorate of Thionupharoline

Method 1

Thionupharoline 5.1 mg (0.01 mM) was dissolved in 1 ml of anhydrous ethanol and treated with 1 ml 0.02 M (0.02 mM) perchloric acid. Anhydrous ether (5 ml) was added and the solution was set aside to crystallize. The crystals, well-formed prisms, were filtered and washed several times with anhydrous ether, m.p. $172°-174°$, i.r. (KBr disc) 6.02 ($>C = N<$); 6.65 and 11.46 (furan). Other unassigned absorptions were present at 6.88, 6.95, 7.06, 7.24, 7.32, 7.52, 7.84, 8.09 µ.

Method 2

Thionupharoline 5.1 mg (0.01 mM) was treated directly with 1 ml of 0.02 M (0.02 mM) perchloric acid and a few ml of water were added to ensure that all the glassy base was in contact with the perchloric acid solution. After 30 minutes the solution was evaporated to dryness under reduced pressure ($50°C$ bath), and the residue was dissolved in a minimum of boiling anhydrous methanol. After storage at $-5°$ for 2 days crystallization had occurred. The crystals were washed twice with anhydrous methanol leaving 4 mg of colourless needles, m.p. $260°-263°$ (softening), $267°-269°$ (main melting), $270°$ (all liquid), $278°$ (decomposition). A mixture m.p. (50:50 mixture) with a sample of the diperchlorate of 6-hydroxythiobinupharidine obtained from Professor R.T. LaLonde, (m.p. $258°$ softens) $263-266.5°$ (liquid) $276°$ (decomposition), showed no depression in melting point.

i.r. (KBr disc) 6.03 and 6.06 (>C = N$^+$<); 6.66 and 11.47 (furan);

8.1 to 10.2 (ClO$_4^-$) and unassigned absorption at 3.27, 6.85, 6.89, 6.96,

7.08, 7.21, 7.25, 7.47, 7.52, 7.85, 10.38, 10.62, 10.76, 12.35, 13.65 µ.

The i.r. spectrum was identical with the i.r. spectrum of the

sample of the diperchlorate of 6-hydroxythiobinupharidine.

Reduction of Thionupharoline (26) with Sodium Borohydride

Thionupharoline (10 mg) was dissolved in 2 ml of absolute

ethanol and treated with 100 mg of NaBH$_4$. Further portions of 25 mg

each of NaBH$_4$ were added four times over the course of 24 hours. The

slurry thus obtained was stirred occasionally. The mixture was then

diluted with water and extracted with CH$_2$Cl$_2$ (3 x 10 ml). The extracts

were combined, dried over anhydrous sodium sulphate, filtered and

evaporated under reduced pressure. A colourless residue resulted which

was adsorbed onto a narrow column of alumina (neutral, Act II, 1.5 g).

The column was then eluted successively with (i) 50 ml n-hexane and

(ii) 50 ml 10% anhydrous ether in n-hexane. The latter solvent eluted

thiobinupharidine (13c) (6 mg) from the column: $[\alpha]_D^{22°}$ = +8° (MeOH)

$\underline{c}$ = 6 mg/ml; i.r. (CCl$_4$); p.m.r. (220 MHz, CDCl$_3$ and C$_6$D$_6$); M.s.;

identical with the spectra of an authentic sample of 13c.

Reduction of Thionupharoline with Sodium Borodeuteride

Thionupharoline (20.6 mg) was dissolved in 2 ml of absolute

ethanol and treated with 100 mg of sodium borodeuteride. A further

100 mg were added in 4 portions of 25 mg each over a period of 24 hours.

The slurry was stirred occasionally and kept at room temperature.

After this time the reaction was worked up in the same manner as for the borohydride reduction. The resultant colourless residue was adsorbed onto a narrow column of neutral alumina (Act II, 3 g). This was eluted with (i) 50 ml of n-hexane, (ii) 50 ml 10% ether in n-hexane, (iii) 50 ml 20% ether in n-hexane, (iv) 50 ml 50% ether in n-hexane and (v) 50 ml 100% ether. These eluants were examined by t.l.c. and M.S.

Eluant (i) gave no product, eluant (ii) gave pure monodeutero-thiobinupharidine (31) M^+ 495 (Rf = 0.51, yield 8 mg), eluant (iii) gave predominantly the mono ethyl ether of thionupharoline (2 mg), Rf = 0.42; M.S., M^+ 538, intense ion at 222; along with traces of slower and faster moving components, eluants (iv) and (v) gave unreacted thionupharoline 26 (8 mg), Rf = 0.36.

The thiobinupharidine-6d (31) had 2% d_0, 94% d_1, and 4% d_2 by mass spectrometry.

p.m.r. 220 MHz ($CDCl_3$): δ 0.92 (d, J = 5.6 Hz, 6H, 2 x CHCH$_3$) 1.45 (d, J = 11.5 Hz, H-6'a) 1.70 (br. s superimposed on envelope, H-6a) 1.90 (ABq, J_{AB} = 14 Hz, 2H, C-7-CH$_2$-C-7') 2.33 (ABq, J_{AB} = 11.5 Hz, 2H, CH$_2$-S) 2.79 (d, J = 2 Hz, 0.6H, H-6e) 2.93 (q, J = 11.5 and 2 Hz, H-6'e superimposed on multiplet assigned to H-4a and H-4'a) 6.39 (nw.m., W½ = 7 Hz, 2H, furan β-H) 7.25 and 7.33 (nw.m., 4H, furan α-H). There was a slight deuterium isotope shift in signals at 1.70 and 2.79δ.

p.m.r. 220 MHz (C_6D_6): δ 0.78 (d, J = 5.6 Hz, CH-CH$_3$), 0.81 (d, J = 6.0 Hz, CH-CH$_3$, with 0.78, 6H) 1.40 (d, J = 11.5 Hz, H-6'a) 1.93 (s, 0.35 H, H-6a) 2.18 (ABq, J_{AB} = 14.0 Hz, 2H, C-7-CH$_2$-C-7') 2.31 (ABq, J_{AB} = 11.5 Hz, 2H, CH$_2$-S) 2.80 (q, J = 10.5 and 3.5 Hz, 2H,

H-4'a and H-4a) 3.10 (broadened singlet, 0.65H; H-6e) 3.18 (q, J = 11.5

and 2 Hz, 1H, H-6'e) 6.42 (nw.m., 2H, furan β-H) 7.14 (nw.m., 4H,

furan α-H).

M.s. m/e (Rel. int. %) 496 (26), 495 (72), 494 (13.5),

480 (0.5), 466 (1.1), 462 (3), 452 (0.7), 448 (2.7), 428 (1.5),

414 (0.7), 361 (5), 360 (17.5), 359 (4.5), 358 (4.5), 265 (1.0),

264 (1.3), 247.5 (2.6), 232 (10), 231 (33), 230 (28), 180 (15),

179 (100), 178 (9.5), 136 (8.5), 107 (18), 94 (28).

6,6'-Dihydroxythiobinupharidine (16) from its Diperchlorate

The diperchlorate of 6,6'-dihydroxythiobinupharidine (35 mg),

m.p. 225°-227°[76], was stirred with 15 ml of 20% ammonia for 30

minutes. The resultant oily base was extracted with CH_2Cl_2 (5 x 10 ml).

These extracts were combined, dried over anhydrous sodium sulphate,

filtered and evaporated to give a glassy residue, (22 mg). This was

adsorbed on 4 g of neutral alumina (Act II) and eluted with 100 ml of

20% ether in benzene to give 17.3 mg of 6,6'-dihydroxythiobinupharidine

16, Rf = 0.18. $[\alpha]_D^{22°}$ = +80° (CH_2Cl_2), c = 17.3 mg/ml. U.v.: λ_{max}

(neutral 95% EtOH) 208 nm, ε = 12,000; (acidic 95% EtOH), λ_{max_1}, 208 nm,

ε = 16,120; λ_{max_2}, 294 nm, ε = 1,890. I.r. (CCl_4) 2.75 nw. and 2.83 br.

(OH); no Bohlmann band at 3.59; 6.66 and 11.45 μ (furan).

p.m.r. 220 MHz ($CDCl_3$): δ 0.91 (d, J = 6.0 Hz, CH-CH₃)

0.93 (d, J = 6.5 Hz, CH-CH₃, with 0.91, 6H) 1.85 (ABq, J_{AB} = 14.5 Hz,

C-7-CH₂-C-7') 2.33 (br.s., exchangeable with D_2O, OH) 2.44 (ABq, J_{AB} =

12 Hz, 2H, CH₂-S) 3.58 (q, J = 6 and 8 Hz, 1H, H-4'a) 3.75 (q, J = 7

and 7.5 Hz, 1H, H-4a) 3.98 (s, 1H, H-6) 4.24 (s, 1H, H-6').

6.35 (nw.m., $W_{\frac{1}{2}}$ = 7 Hz, 2H, furan β-H) 7.25 (nw.m., $W_{\frac{1}{2}}$ = 5 Hz, 2H,

furan α-H) 7.35 and 7.37 (nw.m., $W_{\frac{1}{2}}$ = 3Hz, 2H, furan α-H).

p.m.r. 220 MHz (C_6D_6 + D_2O): δ 0.77 (d, J = 6.0 Hz, $CHCH_3$)

0.80 (d, J = 6.5 Hz, $CH-CH_3$, with 0.77 = 6H) 2.11 (ABq, J_{AB} = 14.5 Hz,

2H, C-7-CH_2-C-7') 2.46 (ABq, J_{AB} = 12 Hz, 2H, CH_2-S) 3.41 (q, J = 3.5,

and 10.5 Hz, 1H, H-4'a) 3.86 (q, J = 4 and 10 Hz, 1H, H-4a)

4.23 (s, 1H, H-6) 4.35 (s, 1H, H-6') 6.37 (s, $W_{\frac{1}{2}}$ = 3 Hz, 1H, furan

β-H) 6.45 (s, $W_{\frac{1}{2}}$ = 3 Hz, 1H, furan β-H) 7.13 (nw.m., $W_{\frac{1}{2}}$ = 3 Hz, furan

α-H) 7.29 (s, $W_{\frac{1}{2}}$ = 3 Hz, furan α-H).

M.s. (230°) m/e (Rel. int: %) 526 (<0.1), 509 (2.5), 508 (7),

507 (2.5), 494 (0.3), 493 (0.3), 492 (0.3), 491 (0.2), 490 (0.2),

480 (0.7), 479 (0.5), 475 (1.5), 461 (0.5), 447 (2.5), 446 (5), 445 (2),

371 (3), 302 (1), 262 (1), 261 (1), 248 (7), 231 (20), 230 (100),

229 (1.5), 228 (1.1), 216 (1.5), 178 (0.7), 176 (1), 136 (1), 107 (5),

94 (7).

Reduction of 6,6'-dihydroxythiobinupharidine (16) with Sodium Borohydride

6,6'-dihydroxythiobinupharidine (16) (100 mg) was dissolved in

10 ml methanol and treated with 100 mg sodium borohydride. The slurry

was left to stand for 16 hours, evaporated to dryness and 5 ml of water

was added. The aqueous solution was extracted with ether. The ethereal

extracts were dried over anhydrous potassium carbonate and evaporated

to dryness giving 80 mg of crude reduction product. The product was

adsorbed onto neutral alumina (Act IV, 5 g) and eluted with 100 ml of

benzene to give thiobinupharidine (13c) (53 mg) which was recrystallized

from methanol:acetone (1:1) at -5°, m.p. 129°-130°. $[\alpha]_D^{22°}$ = +8° (MeOH),

$\underline{c}$ = 12 mg/ml; p.m.r. (220 MHz CDCl$_3$) identical with the spectrum of an
authentic sample of $\underline{13c}$.

Reduction of 6,6'-dihydroxythiobinupharidine (16) with Sodium Borodeuteride

6,6'-dihydroxythiobinupharidine(16)(17 mg) was dissolved in 2 ml
absolute ethanol and treated with 100 mg sodium borodeuteride. A
further 4 portions of 25 mg each of borodeuteride were added over a
period of 24 hrs. The slurry was stirred occasionally at room temper-
ature for this period, then worked up in the usual manner to give
14.5 mg of a colourless glass. T.l.c. showed three spots, Rf = 0.50
(intense), Rf = 0.27 - 0.42 (intense), and Rf = 0.15 (trace), presumed
to be starting material. The product was dissolved in n-hexane
containing the minimum amount of ether to complete dissolution, and
was adsorbed onto a narrow column of neutral alumina (Act II, 1.5 g).
The column was eluted with (i) 50 ml n-hexane, (ii) 50 ml 5% ether in
hexane, (iii) a further 50 ml 5% ether in hexane, (iv) 50 ml 25% ether
in n-hexane, (v) 50 ml 50% ether in n-hexane. All samples were
analysed by t.l.c.

Eluant (i) gave 4.5 mg of thiobinupharidine-6,6'-d$_2$, $\underline{52}$, pure
by t.l.c., Rf = 0.5. Eluant (ii) gave a weak spot (Rf = 0.5) and an
intense spot with Rf = 0.25 - 0.40. Eluants (iii) and (iv) gave
intense spots with Rf = 0.26 - 0.42. Eluant (v) showed traces of
starting material at Rf = 0.16. Eluants (ii), (iii) and (iv) were
combined to give 9 mg which was adsorbed onto a further 2 g of neutral
alumina (Act II) using hexane as solvent. The column was eluted with
50 ml of n-hexane to give Fraction (vi) (1.5 mg), 100 ml of 20% ether

in hexane to give Fraction (vii) (6.6 mg), and finally with 50 ml. of

ether to give Fraction (viii) (less than 1 mg). Fraction (vii)

showed only one spot on t.l.c., Rf = 0.34 (centre of spot). This was

the sample used for spectroscopic analysis and deduced to be 6-hydroxy-

thiobinupharidine-6'-d_1 (33). Fractions (vi) and (viii) were not

further examined.

Fraction (i), Thiobinupharidine-6,6'-d_2 (32)

Mass spectrometric analysis gave the following: d_0 = 1%,

d_1 = 1%, and d_2 = 98%.

p.m.r. 220 MHz (CDCl$_3$): δ 0.92 (J = 5.6 Hz, 6H, 2 x CHCH$_3$)

1.45 (H-6'a, essentially disappeared) 1.70 (br.s., superimposed on

envelope, H-6a) 1.90 (ABq, J_{AB} = 14 Hz, 2H, C-7-CH$_2$-C-7') 2.33 (ABq,

J_{AB} = 11.5 Hz, 2H, -CH$_2$-S) 2.79 (d, J = 2 Hz, ~0.6H, H-6e)

2.85 - 3.0 (complex m. not well resolved, ca. 3 protons, H-4a + H-4'a

+ H-6'e) 6.39 (nw.m., W$\frac{1}{2}$ = 7 Hz, 2H, furan β-H) 7.25 + 7.33 (nw.m.,

4H, furan α-H).

p.m.r. 220 MHz (C$_6$D$_6$): δ 0.78 (d, J = 5.6 Hz, CH-CH$_3$)

0.81 (d, J = 6.0 Hz, CH-CH$_3$, 6H with 0.78) 1.40 (H-6'a, essentially

disappeared) 1.93 (s, 0.4H, H-6a) 2.18 (ABq, J_{AB} = 14.0 Hz, 2H,

C-7-CH$_2$-C-7') 2.31 (ABq, J_{AB} = 11.5 Hz, 2H, CH$_2$-S) 2.80 (q, J = 10.5

and 3.5 Hz, 2H, H-4'a + H-4a) 3.10 (poorly resolved doublet, J = 2 Hz,

0.6H, H-6e) 3.16 (poorly resolved doublet, J = 2 Hz, 1H, H-6'e).

M.s. m/e (Rel. int. %) 497 (17), 496 (47), 495 (9), 481 (0.3),

467 (0.9), 463 (1.9), 453 (0.5), 449 (1.6), 429 (1.1), 415 (0.45),

362 (3), 361 (14), 360 (3), 359 (3), 248 M$^+$/2 (2.5), 232 (10), 231 (32),

230 (16), 181 (10), 180 (13.5), 179 (100), 178 (4.5), 136 (8), 107 (17.5), 94 (24).

Fraction (vii), 6-hydroxythiobinupharidine-6'-d(33)

Mass spectrometric analysis showed 2% d_0 and 98% d_1.

U.v.: (95% EtOH neutral) end absorption only; (95% acidic EtOH) λ_{max_1} 208 nm, ε = 21,000; λ_{max_2} 289 nm, ε = 2640.

The diperchlorate salt was prepared as for thionupharoline (method 2) and recrystallized from MeOH at -5°C, m.p. 260° (softens), 266°-269° (liquid), 278° (decomposes).

p.m.r. 220 MHz (CDCl$_3$): δ 0.88 (d, J = 5.0 Hz, 6H, 2 x CHCH$_3$) 2.21 (ABq, J_{AB} = 12 Hz, 2H, CH$_2$-S), 2.26 (s, 1H, exchangeable with D$_2$O, OH) 2.90 (q, J = 5.6 and 8 Hz, H-4'a) 2.92 (br.s. superimposed on q at 2.90, total 2H, H-6'e) 3.77 (q, J = 7.5 and 7 Hz, 1H, H-4a) 3.97 (s, 1H, H-6) 6.34 (nw.m., W½ = 7 Hz, 2H, furan β-H) 7.21 (nw.m., 1H, furan α-H) 7.30 (nw.m., W½ = 4 Hz, 3H, furan α-H).

p.m.r. 220 MHz (C$_6$D$_6$): δ 0.75 (two superimposed doublets, J = 6.0 Hz, 6H, 2 x CHCH$_3$) 2.11 (ABq, J_{AB} = 12 Hz, 2H, CH$_2$-S) 2.42 (br.s., 1H, exchangeable with D$_2$O, OH) 2.75 (q, J = 5.5 and 10.5 Hz, 1H, H-4'a) 3.15 (d, J = 2.0 Hz, 1H, H-6'e) 3.86 (q, J = 10 and 4 Hz, 1H, H-4a) 4.24 (s, 1H, H-6) 6.38 (nw.m., W½ = 3 Hz, 1H, furan β-H) 6.44 (nw.m., 1H, W½ = 3 Hz, furan β-H) 7.13 (nw.m., 3H, furan α-H) 7.25 (nw.m., W½ = 3 Hz, 1H, furan α-H).

M.s. (200°) m/e (Rel. int. %) 511 (72), 510 (9), 495 (12), 494 (52), 493 (90), 482 (6), 478 (4), 465 (7), 460 (4), 446 (6), 452 (2), 376 (22), 360 (10), 305 (11), 231 (52), 230 (100), 229 (22), 228 (20),

179 (26), 178 (52), 176 (30), 136 (20), 107 (37), 94 (48).

Reduction of 6-hydroxythiobinupharidine-6'-d_1 (33) with sodium borohydride

6-hydroxythiobinupharidine-6'-d_1 (33) (2 mg) was dissolved in 1 ml absolute ethanol and treated with sodium borohydride (50 mg). The suspension was left to stand at room temperature for 96 hours with addition of a further 25 mg of borohydride every 24 hours. The solvent was removed under reduced pressure and the resultant residue was treated with 5 ml of water. The whole was extracted with CH_2Cl_2 (5 x 5 ml). These extracts were dried over anhydrous Na_2SO_4 and evaporated to leave a yellowish oil ($\sim$ 1 mg). This was transferred to a p.l.c. plate (0.5 mm silica gel) which was developed in the usual manner. The band of Rf = 0.50 (fully-reduced material) was removed and extracted with CH_2Cl_2. This extract was then evaporated to dryness and rechromato-graphed on 2 g of neutral alumina (Act II) and eluted with benzene (50 ml). This eluant gave thiobinupharidine-6'-d_1 (34) ($\sim$ 1 mg) as a colourless glass which was examined by t.l.c. and M.S.

T.l.c.: Rf = 0.50 only, no trace of non-reduced substances of lower Rf value. Analysis: Calc. for $C_{30}H_{41}N_2O_2SD$ (h.r.m.s. 495.303) Found: (495.301). Mass spectrometry showed 9% d_0, 90% d_1, 1% d_2.

M.s. m/e (Rel. int. %) 496 (12.4), 495 M^+ (35), 494 (9.8), 480 (0.4), 466 (1), 462 (2.2), 452 (0.7), 448 (2), 428 (1.3), 361 (2), 360 (9), 359 (3), 358 (2), 247.5 $M^+/2$ (1.5), 231 (28.5), 230 (41), 180 (5), 179 (23), 178 (100), 148 (4.5), 136 (9.5), 107 (20), 94 (26).

Purification of Neothiobinupharidine sulphoxide (27)

The crude solid base (20 mg) from the initial isolation procedure was examined on the usual t.l.c. system and showed an elongated spot (Rf = 0.15) with traces of slower moving impurities. The alkaloid was adsorbed onto 4 g of neutral alumina (Act II) and eluted progressively with (i) 75 ml benzene, (ii) 75 ml 10% chloroform in benzene, (iii) 75 ml 50% chloroform in benzene, (iv) a further 75 ml 50% chloroform in benzene, (v) 75 ml chloroform. Fraction (iii) gave a colourless semi-crystalline product (18 mg). Recrystallization without loss from anhydrous methanol at -10°C gave colourless clusters of fine needles (18 mg), m.p. 221°-223°.

Analysis: Calc. for $C_{30}H_{42}N_2O_3S$ (Mol. wt. 510.291): C, 70.6; H, 8.2; N, 5.5. Found: [510.286 (h.r.m.s.)]: C, 70.2; H, 8.3; N, 5.3. $[\alpha]_D^{22°} = /-174°$ (CHCl$_3$), $\underline{c}$ = 4.7 mg/ml. U.v.: (neutral and acidic methanol) end absorption only.

i.r. (CCl$_4$) 3.60 to 3.91 intense (Bohlmann bands) 6.67 and 11.50 (furan) 9.60 - 9.65 (:S=O) and unassigned absorption at 6.96, 7.22, 7.27, 7.37, 7.66, 7.77, 8.65, 8.87, 9.10, 9.21, 9.43, 9.79 and 10.42μ.

p.m.r. 220 MHz (CDCl$_3$): δ 0.86 (d, J = 6.0 Hz, CH-CH$_3$) 0.87 (d, J = 6 Hz, CH-CH$_3$, with 0.86, 6H) 1.30 (d, J = 14.0 Hz, one half of ABq of C-7-CH$_2$-C-7') 1.56 (d, superimposed on envelope, J = 11.5 Hz, H-6a) 1.61 (d, superimposed on envelope, J = 13.0 Hz, H-6'a) 1.94 (d, J = 14.0 Hz, one half of ABq of C-7-CH$_2$-C-7') 2.67 (q, J = 11.5 and 2.0 Hz, 1H, H-6e) 2.80 (ABq, ν_A and ν_B = 2.77 and 2.83, J_{AB} = 16.0 Hz, 2H, -CH$_2$-S$\overset{O}{\underset{..}{\ll}}$) 2.89 (mult., 2H, H-4a and H-4'a) 3.28 (q, J = 13.0 and 2.0 Hz, 1H, H-6'e) 6.35 (nw.m.; W$\frac{1}{2}$ = 3 Hz, 1H, furan β-H) 6.43 (nw.m., W$\frac{1}{2}$ = 3 Hz, 1H, furan β-H). 7.22 + 7.29 + 7.33 (nw.m's., W$\frac{1}{2}$ = 3 Hz, 4H, furan α-H's).

Irradiation at 2.67δ collapses doublet at 1.56δ into broad singlet with the doublet at 1.61δ unaffected.

Irradiation at 3.28δ collapses doublet at 1.61δ into broad singlet but doublet at 1.56δ unaffected.

Irradiation at 1.30δ collapses doublet at 1.94δ to singlet; irradiation at 1.94δ collapses doublet at 1.30δ to singlet.

p.m.r. 220 MHz (C_6D_6): δ 0.72 (d, J = 5.0 Hz, CH-CH$_3$) 0.73 (d, J = 5.0 Hz, CH-CH$_3$, with 0.72, 6H) 1.05 (d, J = 14.0 Hz, one half of ABq of C-7-CH$_2$-C-7') 1.38 (d, superimposed on envelope, J = 11.5 Hz, H-6a) 1.50 (d, superimposed on envelope, J = 13.0 Hz, H-6'a) 1.94 (d, J = 14.0 Hz, one half of ABq of C-7-CH$_2$-C-7') 2.61 (q, superimposed on multiplet, J = 11.5 and 2.0 Hz, H-6e) 2.66 (mult., H-4a or H-4'a) 2.77 (ABq, v_A and v_B = 2.67 and 2.87, J_{AB} = 15.0 Hz, 2H, CH$_2$-S$\overset{O}{\underset{}{\lessgtr}}$) 2.83 (q, J = 10.5 and 3.5 Hz, 1H, H-4a or H-4'a) 3.64 (q, J = 13.0 Hz and 2.0 Hz, 1H, H-6'e) 6.17 (nw.m., W½ = 3 Hz, 1H, furan β-H) 6.70 (nw.m., W½ = 3 Hz, 1H, furan β-H) 7.04, 7.07, 7.18 and 7.51 (nw.m's, W½ = 3 Hz, 1H each, furan α-H's).

Irradiation at 2.61δ collapses doublet at 1.38δ to broad singlet with doublet at 1.50δ unaffected.

Irradiation at 3.64δ collapses doublet at 1.50δ to a broad singlet with doublet at 1.38δ unaffected.

Irradiation at 1.05δ collapses doublet at 1.94δ into singlet; irradiation at 1.94δ collapses doublet at 1.05δ into singlet.

Addition of D_2O to either $CDCl_3$ solution or C_6D_6 solution has no effect on the appearance of the spectra.

M.s. (260°) m/e (Rel. int. %) 511 (3.6), 510 (10), 495 (6.2), 494 (21), 493 (52), 492 (3.5), 481 (0.2), 465 (0.15), 464 (0.22),

463 (0.25), 462 (0.2), 461 (0.5), 460 (0.35), 459 (0.9), 449 (0.35),

448 (1.0), 447 (1.1), 446 (0.4), 445 (0.9), 385 (0.3), 375 (0.3), 373 (0.3),

359 (1.0), 358 (1.3), 357 (5), 325 (0.5), 311 (0.5), 302 (0.5), 280 (2),

264 (1), 263 (1), 262 (1.5), 244 (2.5), 231 (20), 230 (100), 229 (6),

228 (5.5), 216 (3.5), 214 (2), 202 (3.0), 200 (2.5), 178 (10), 176 (7),

162 (4.5), 149 (3), 148 (4), 136 (5.5), 135 (4), 134 (4.5), 122 (5.5),

121 (4), 120 (4.5), 108 (8), 107 (23), 96 (10.5), 95 (13), 94 (87),

93 (7.5), 91 (8), 81 (21), 79 (22), 55 (7), 53 (5).

"Metastable peak" observed centred at m/e 477. Calculated for

$$m/e\ 510 \xrightarrow[-\ OH\cdot]{} m/e\ 493.$$

$$m^* = \frac{(493.283)^2}{510.291} = 476.8$$

Conversion of Neothiobinupharidine (14b) to Neothiobinupharidine sulphoxide (27)

A solution of neothiobinupharidine (14b) (110 mg) in glacial acetic

acid (4 ml) was treated with 30% hydrogen peroxide (54 mg) at 20° for 1.5

hrs. The solution was poured into water (20 ml), made alkaline with

potassium hydroxide, and extracted repeatedly with benzene. Evaporation

of the benzene gave an off-white solid examined on t.l.c. Two major spots

of Rf = 0.63, Rf = 0.26, and a third elongated spot of Rf = 0.1 - 0.2, were

observed, together with traces of slower moving components. The crude

solid was dissolved in a minimum of benzene, adsorbed onto a column of

15 g of neutral alumina (Act II) and eluted with (i) 200 ml benzene, (ii)

200 ml 10% chloroform in benzene, (iii) 200 ml 50% chloroform in benzene,

(iv) a further 200 ml 50% chloroform in benzene, (v) 100 ml chloroform.

Fractions (i) and (ii) were combined, evaporated under reduced pressure and the resultant solid was dissolved in a minimum of acetone and cooled to -10° for 24 hrs, resulting in crystallization of neothiobinupharidine (22 mg), m.p. 159°-160° (colourless needles). Fraction (iii) gave a colourless oily product (20 mg) not further examined. Fraction (iv) gave a crystalline residue, on evaporation of solvent, which was recrystallized from anhydrous methanol at -10°, yielding 68 mg of colourless needles, m.p. 221°-223°. This substance has spectroscopic properties identical with the natural compound, and behaved identically on two t.l.c. systems other than the standard system. These were 0.2 mm alumina (chloroform:benzene = 1:1) Rf = 0.12; and 0.5 mm silica gel (methanol:acetone:benzene = 1:1:1) Rf = 0.73.

A mixed m.p. determination of the natural sulphoxide 27 and the oxidation product of neothiobinupharidine showed no depression in melting point. Both the natural product and the oxidation product of neothiobinupharidine were recovered unchanged after sublimation at 180° (0.005 mm Hg) (also at 240° under the same conditions).

Conversion of Neothiobinupharidine sulphoxide (27) to Neothiobinupharidine (14b)

A solution of 27 (4 mg) in ethyl acetate (4 ml) was treated with PCl_3 (3-4 drops) and the mixture was heated under reflux for 15 minutes, poured into ice-water (5 ml) and basified with aqueous KOH. The organic layer was separated and the aqueous solution extracted with CH_2Cl_2 (5 x 5 ml). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The resultant crystalline residue was recrystallized from a minimum of acetone at -10°,

yielding neothiobinupharidine (3 mg) identified by a comparison of its
spectroscopic properties, by a mixed melting point determination
(159°-160°) and t.l.c. comparison with an authentic sample of 14b.
T.l.c. 0.2 mm alumina (ether:n-hexane = 1:4) Rf = 0.23; 0.5 mm silica
gel (standard system) Rf = 0.63.

SUMMARY

The quinolizidine alkaloids, deoxynupharidine 2c, castoramine 10,
and nupharidine 1b, isolated from species of Nuphar luteum of Polish
origin, have been examined using high resolution mass spectrometry.
Accurate mass measurements have demonstrated that a characteristic pattern
of fragmentation upon electron impact can be defined. Consequently it
has proved possible to differentiate between rings A and B of the
quinolizidine system as the site of new substituents. The structure of
a new quinolizidine Nuphar alkaloid, nupharolutine 18c, has been
established on the basis of h.r.m.s. and p.m.r. data.

The stereoisomeric relationship between thiobinupharidine 13c,
and neothiobinupharidine 14b has been re-examined using spectroscopic
techniques. The three-dimensional structure proposed for thiobinuphar-
idine has been confirmed by an X-ray study which has also established the
absolute configuration of this alkaloid.

The p.m.r. spectra of 13c and 14b, recorded at 220 MHz, have
been examined in some detail. Attempts have been made to observe nuclear
Overhauser effects in the p.m.r. spectra of these alkaloids but these have
proved unsuccessful. However, it has been possible to assign chemical
shifts to many of the protons of these thiospiran alkaloids.

The structures of two new isomeric thiospiran Nuphar alkaloids
26 and 27, of molecular formula $C_{30}H_{42}N_2O_3S$, have also been established
on the basis of spectroscopic properties and chemical interconversions.

Thionupharoline 26 has been shown to be identical with the monohemiaminal 6'-hydroxythiobinupharidine. The alkaloid 27 is a sulphoxide of neothio-binupharidine, and the configuration about sulphur has been tentatively deduced following a detailed study of the p.m.r. spectra of 14b and 27.

The sodium borodeuteride reduction of hemiaminals of thiobinuphar-idine in absolute ethanol solution has been extensively studied. The observed stereoselectivity of deuterium incorporation in these reductions is significantly different to that reported for analogous reductions in methanol solution. The intermediacy of an alkoxyborodeuteride reducing species has been proposed to explain this dichotomy.

The development of a characteristic mass spectrometric fragment-ation scheme for the monodeuterated reduction products of monohemiaminals of thiobinupharidine has resulted in a quick and simple method to distinguish between C-6 and C-6' as the site of the original hydroxy group. This method promises to show wide applicability to any mono-hemiaminal (located at one of the C-6 positions) derived from one of the bisamine thiospiran alkaloids of gross skeletal structure 25.

Finally some suggestions have been made concerning further work required in this field.

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