PROPERTIES OF SULFUR CONTAINING NUPHAR ALKALOIDS

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Abstract - The various types of *Nuphar* alkaloids are noted and thereafter
attention is focused on one of these types, the thiaspirane *Nuphar*
alkaloids. A brief discussion of the spectral characteristics useful in
ascertaining the gross structure is followed by a more detailed dis-
cussion of the spectral properties, primarily ^{1}H and ^{13}C NMR and the CD,
and the chemistry employed to establish the stereochemistry of the
thiaspiranes. Emphasis is given to sulfur atom-immonium ion inter-
actions which are revealed in studies of metal hydride reduction and
the UV and CD of α- and β-thiohemiaminals. Finally an indication of the
biological activities of thiohemiaminal thiaspirane and synthetic analogs
is presented.

INTRODUCTION

Nuphar luteum is a species of the aquatic family Nymphaeaceae. It is unique in producing a
group of alkaloids characterized by structures possessing a regular sesquiterpenic skeleton
incorporated into 3-furyl substituted piperidines, **1**, or quinolizidines, **2**. C_{30} alkaloids
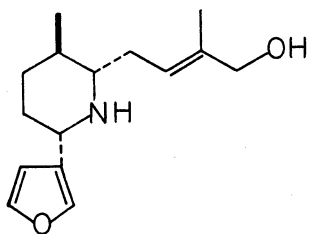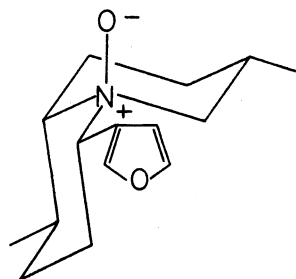

Nuphamine

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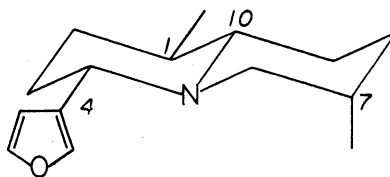
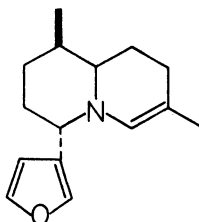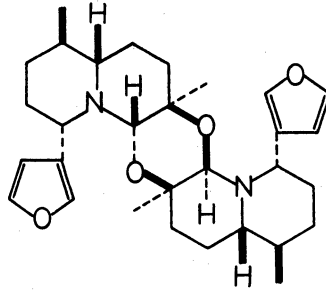Deoxynupharidine

2

are produced also by *N. luteum*, presumably by the combination of two appropriately ela-
borated C_{15} alkaloids. Indeed, the laboratory conversion of nupharidine, **3**, to the enamine,
4, (1), and subsequent oxidation of the latter leads to the C_{30} alkaloid, **5**, which has been
uncovered in extracts of *N. luteum*, subsp. *macrophyllum* (2).

3

4

5

The thiaspiranes, 6, comprise the second class of C_{30} *Nuphar* alkaloids. They are mevalonate in origin (3). Linking two (-)-deoxynupharidine units, as depicted in Fig. 1, was the
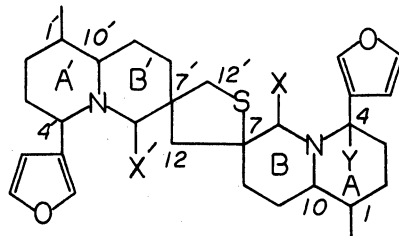
6, X=X'=Y=H
7, X=OH; X'=Y=H
8, X'=OH; X=Y=H
9, X=X'=OH; Y=H
10, X=Y=OH; X'=H

central idea for a proposed biogenesis scheme involving a series of immonium ion-enamine combinations (4). However, this proposal remains untested by experiment.

Fig. 1. The combination of two deoxynupharidine units with sulfur giving a thiaspirane.

The presence of sulfur containing bases in *Nuphar* was first reported by Achmatowicz and co-
workers (5) in the early 1960's following several published indications that various *Nuphar*
preparations possessed antibiotic properties (6). These unusual sulfur containing bases
are the subject matter for discussion here. Primarily our discussion will deal with the
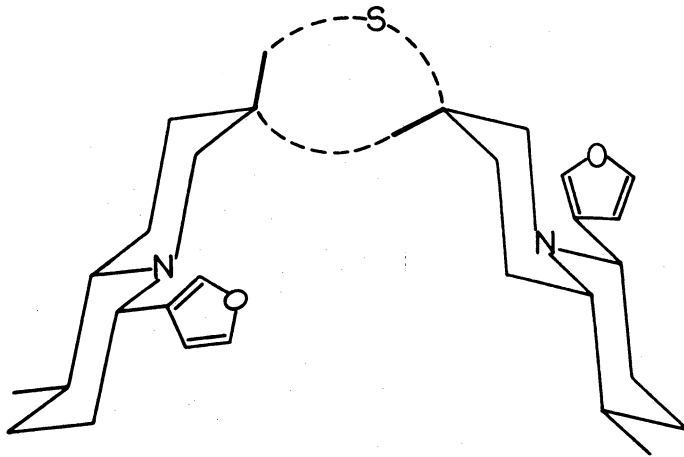chemical and physical properties which manifest the structure of the thiaspiranes, but it
will conclude by noting briefly the biological activities of these and similar compounds.
The authors are honored and pleased to report on their contributions to *Nuphar* alkaloid
chemistry in the setting where these sulfur containing alkaloids were discovered.

SPECIAL CHARACTERISTICS AND GROSS STRUCTURE

The thiaspirane *Nuphar* alkaloids have been isolated from nature as the bisamines, 6, mono-
hemiaminals, 7 and 8, or bishemiaminals 9. Because most have been isolated in our labora-
tory in 1-10 mg quantities, it has been necessary to rely on methods of structural analysis
consuming very small amounts of material. Accordingly the electron-impact mass spectro-
metry (MS) of *Nuphar* alkaloids has received attention (7). The appearance of the parent
ion, m/e 494 (M^+), associated with ions corresponding to the loss of CH_2S, CH_3S, CH_3SH and
SH is a good first indication for the thiaspirane bisamine, 6. The latter series of peaks
seldom exceed 10% relative intensity, but are distinctive because they fall within a broad
spectral region free of other ions. Another indication of the bisamine is the appearance
of m/e 230 and 178 whose origins are shown in Fig. 2. These are always intense, one or the
other usually being the base peak. The series m/e 136, 107, 94 and 81 is present in the
spectra of all *Nuphar* quinolizidines. Its appearance and the presence of m/e 359 is a
further indication of the thiaspirane bisamine.

447 (449) [448] [448]'

176* 81 (81) [81] [81]'
      +H

94 (94) [94] [94]'

107 (107) [107] [107]'
      -H

136 (136) [136] [136]'

178 (179) [179] [178]'
  +H

359 (361) [360] [360]'

230 ——— 230
(231) -H   -HS (231)
[231]   [231]

X = X' = H : m/e
X = X' = D : (m/e)
X = D; X' = H : $[m/e]$
X = H; X' = D : $[m/e]$'
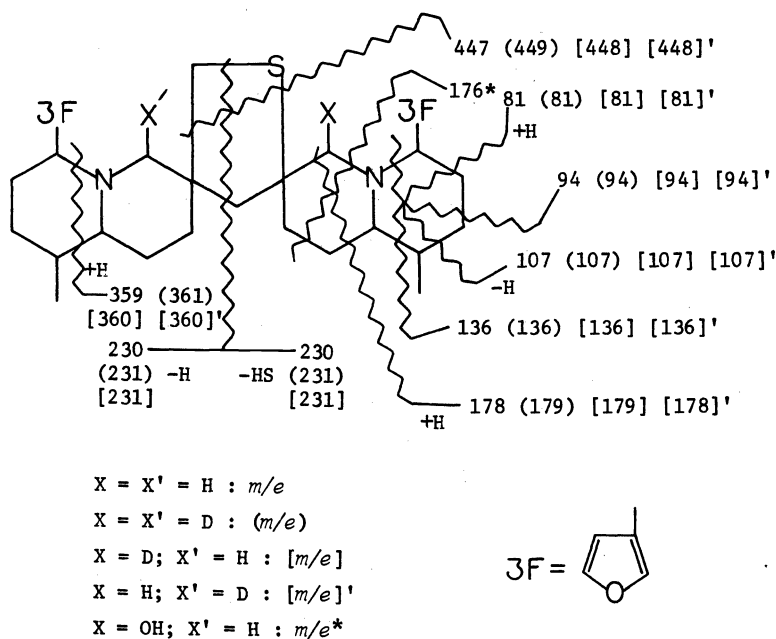X = OH; X' = H : m/e*

$3F = $

Fig. 2. Mass spectral fragmentation pattern of thiaspirane *Nuphar*
alkaloids with corresponding peaks expressed as m/e.

The M^+ for bishemiaminals (m/e 526) and monohemiaminals (m/e 510) is sometimes not observed, but ions corresponding to M^+-H_2O, OH and CH_3S, CH_2S, CH_3SH and SH always are. The presence of a C-6 hemiaminal, as in 7, 8 or 9, always gives strong peaks at m/e 176 and 228, peaks which are virtually absent in the spectra of the bisamines. In the case of C-6 and C-6' monohemiaminals (7 and 8), a distinction between a pair is sometimes indicated by the relatively greater intensity of m/e 176 in the spectrum of the C-6 hemiaminal.

In our work with hemiaminal thiaspiranes, hemiaminal presence has been confirmed by sodium borodeuteride reduction followed by a MS analysis of the resulting deuterium labelled bisamine. Incorporation of one and two deuterium atoms signifies that the labelled bisamine was derived from mono- and bishemiaminal respectively. However the MS of the labelled bisamine has additional value in distinguishing a C-6 from a C-6' hemiaminal since the singly labelled bisamine from the former gives a spectrum in which m/e 178 has been shifted to m/e 179, in accord with the fragmentation depicted in Fig. 2. In the MS of the C-6' labelled bisamine, m/e 178 is retained (8). The sodium borodeuteride reduction followed by MS analysis has served recently to identify highly polar 4,6-dihydroxyneothiobinupharidine (10) which develops by air oxidation of 6-hydroxyneothiobinupharidine on standing. Thus the observation of the m/e 178→180 shift and the shift, one mass unit higher, of each of the peaks in the m/e 136, 107, 94 series, located the hemiaminal functions in the C-6 and C-4 positions (9).

The dual hemiaminal-amine character of the monohemiaminals is signaled also by the infrared spectrum in conjunction with prior knowledge of the facile metal hydride reduction. Bohlmann infrared (IR) bands at 3.5-3.6 μm are characteristic of quinolizidine systems possessing at least two α-hydrogen atoms anticoplanar to the nitrogen lone pair. The bishemiaminals (9) do not give these bands. Therefore the appearance of IR Bohlmann bands in conjunction with the metal hydride reduction of a hydroxyl group is supporting evidence for monohemiaminal character. Regarding the location of the hemiaminal hydroxyl among the possible C-4, C-6 and C-10 positions, the ^{1}H nuclear magnetic resonance (NMR) allows a simple distinction since only a C-6 hemiaminal will possess a carbinyl proton, which characteristically falls in the δ 3.92-4.57 region.

Finally the C-6 and C-6' hemiaminals can be distinguished by a study of their acidic solution ultraviolet (UV) spectra. Both the C-6 thiaspirane hemiaminal (7), an α-thiohemiaminal, and the C-6' counterpart (8), a β-thiohemiaminal, gave only UV end absorption in neutral solution. However in acidic solution, the α-thiohemiaminals displayed an absorption band in the 290-310 nm region while the β-thiohemiaminals absorbed in the shorter wavelength region of 270-285 nm. Upon basification, these bands disappeared but reappeared on reacidification. Such absorption was absent in the acidic solution spectra of deoxynupharidine (2) and the bisamines (6). Simple immonium ions showed moderate to strong absorption in a shorter wavelength region of 220-235 nm (12). Similarly, the immonium perchlorates 11 (13) and 12 (9) displayed bands at 230 nm (13). Therefore the appearance of the acidic solution
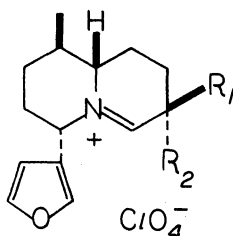
11, R_1=OH; R_2=CH_3
12, R_1=CH_3; R_2=OH

thiohemiaminal bands has been attributed to sulfur atom-immonium ion interaction (13). Another line of evidence for this interaction is the stereospecificity of α-thiohemiaminal reduction, a topic considered in a later section.
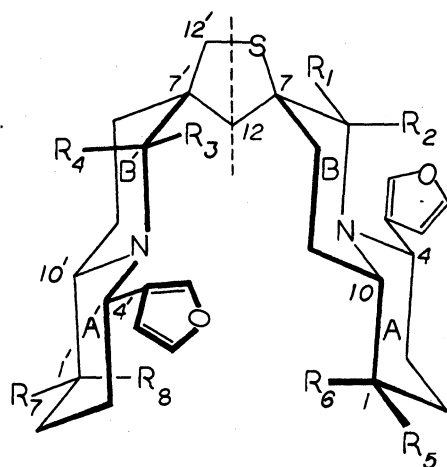
<u>13</u>, $R_1=R_2=R_3=R_4=R_6=R_8=H$; $R_5=R_7=CH_3$

<u>17</u>, $R_1=R_2=R_3=R_4=R_5=R_7=H$; $R_6=R_8=CH_3$

<u>18</u>, $R_1=R_2=R_3=R_4=R_5=R_8=H$; $R_6=R_7=CH_3$

<u>19</u>, $R_1=R_2=R_3=R_4=R_6=R_7=H$; $R_5=R_8=CH_3$

<u>25</u>, $R_1+R_2=H+OH$; $R_3+R_4=H+OH$; $R_5=R_7=CH_3$; $R_6=R_8=H$

<u>27</u>, $R_1=R_4=D$; $R_2=R_3=R_6=R_8=H$; $R_5=R_7=CH_3$

<u>29</u>, $R_1+R_2=H+OH$; $R_3=R_4=R_6=R_8=H$; $R_5=R_7=CH_3$

<u>30</u>, $R_1=D$; $R_2=R_3=R_4=R_6=R_8=H$; $R_5=R_7=CH_3$

<u>39</u>, $R_1+R_2=H+OH$; $R_3=R_6=R_8=H$; $R_4=D$; $R_5=R_7=CH_3$

<u>45</u>, $R_1=R_2=R_6=R_8=H$; $R_3+R_4=H+OH$; $R_5=R_7=CH_3$
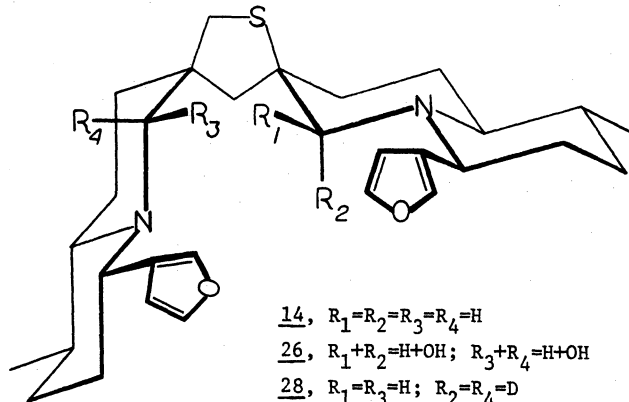
<u>14</u>, $R_1=R_2=R_3=R_4=H$

<u>26</u>, $R_1+R_2=H+OH$; $R_3+R_4=H+OH$

<u>28</u>, $R_1=R_3=H$; $R_2=R_4=D$
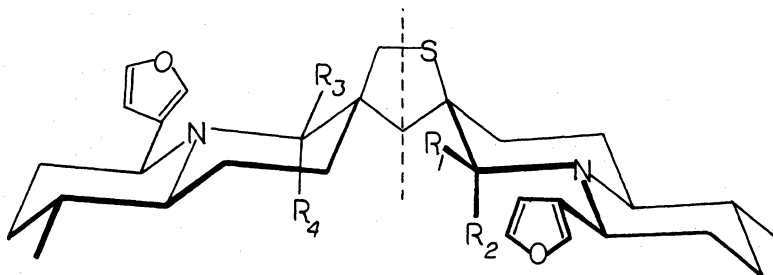
<u>15</u>, $R_1=R_2=R_3=R_4=H$

<u>40</u>, $R_1=R_2=H$; $R_3+R_4=H+OH$

<u>41</u>, $R_1=R_2=R_3=H$; $R_4=D$

<u>46</u>, $R_1+R_2=H+OH$; $R_3=R_4=H$

16, $R_1=R_2=R_3=R_4=H$
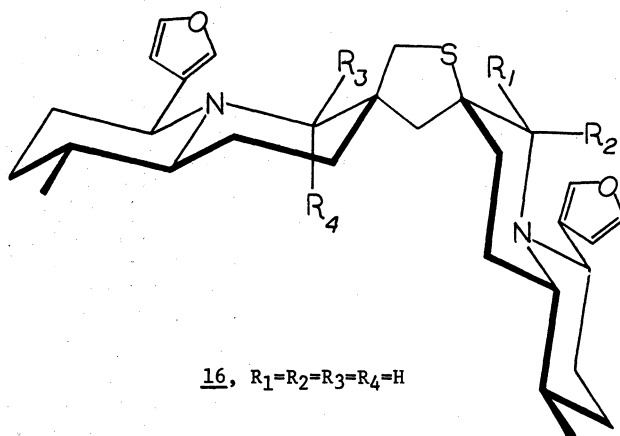
CHEMICAL AND SPECTRAL PROPERTIES AND STEREOCHEMISTRY

There are four ways possible for combining two (-)-deoxynupharidine units in the manner illustrated in Fig. 1. Such combination produces the four stereochemical structures 13-16, three of which represent known thiaspirane bisamines. These three are: thiobinupharidine (13), thionuphlutine B (14), and neothiobinupharidine (15). The fourth, 16, has eluded discovery. The stereochemical assignments have been based on many different lines of evidence, not the least important of which are the X-ray crystallographic studies of 13 (14) and 15 (15). Undoubtedly, the X-ray study of the latter is a landmark because it yielded the first structure of the C_{30} sulfur containing *Nuphar* alkaloids. Notwithstanding the importance of these X-ray studies, this discussion will be limited to representing the several different lines of evidence obtained in the investigations of the small samples available in the authors' laboratory.

In an earlier report (13), the evidence for the stereochemistry of thiobinupharidine and thionuphlutine B was organized and presented as answers to three questions: 1) is the configuration at C-1 (C-1'), C-4 (C-4'), and C-10 (C-10') in the thiaspiranes the same as that in (-)-deoxynupharidine, 2? 2) What is the stereochemistry of the CH_2S attachment to the A'B' quinolizidine moiety? 3) What is the stereochemistry of the sulfur atom attachment to the AB quinolizidine moiety? The same organization will be used here in dealing with evidence used in the determination of stereochemistry.

Configurations at C-1 (C-1'), C-4 (C-4') and C-10 (C-10'). Equal normal solutions of thiobinupharidine (13), thionuphlutine B (14) and (-)-deoxynupharidine (2) exhibited IR Bohlmann bands of equal intensity and complexity (13). This observation not only indicated the *trans* fused quinolizidine systems in 13 and 14 but also pointed to the equatorial 3-furyl group attached to C-4 (C-4'). It was argued that had the 3-furyl group been axial, its strong equatorial preference (16) would have resulted in the inversion of nitrogen and the resulting conversion of a *trans* to a *cis* fused quinolizidine system with accompanying loss in Bohlmann band intensity. Since no loss in Bohlmann intensity was observed, the 3-furyl groups must be equatorial in *trans* fused quinolizidines.

Aromatic solvent induced ^{1}H NMR shifts were employed to ascertain the conformation of the C-1 methyl groups. A related study of methyl quinolizidines revealed that changing the solvent from deuterochloroform to benzene resulted in the shielding of all methyl groups except 1- and 3-axial methyl groups which became deshielded (17). The C-1 methyl groups in the thiaspiranes underwent upfield shifts and accordingly were assigned an equatorial conformation. Thus all the evidence pointed to a relative configuration of the quinolizidine moieties being the same in the thiaspiranes and deoxynupharidine. More recently the thiaspiranes 13, 14, and 15, deoxynupharidine, 2, and 7-*epi*-deoxynupharidine have been examined by ^{13}C NMR (18). This examination has revealed that in no case do the chemical shift ranges for C-1, C-4 and C-10 exceed 1.1 ppm. This result lends additional support for the same relative configuration of all deoxynupharidine moieties since ^{13}C NMR is very sensitive to stereochemical changes. The power of ^{13}C NMR in dealing with problems of thiaspirane stereochemistry is exemplified in the case of the 1-*epi*-1'-*epi*-thiobinupharidine (17), 1-*epi*-thiobinupharidine (18), and 1'-*epi*-thiobinupharidine (19), the last two existing in an inseparable two-component mixture (19). The appearance of the coincidental methyl resonances of 17 at 13.4 ppm, some 5.8 ppm upfield from the methyl resonances of thiobinupharidine (13), was a clear indication of the axial disposition of these methyl groups, when compared with the similar chemical shift increment of methyl groups in the pair of 1-methylquinolizidines 20 and 21. Moreover the chemical shift increments of α,β and γ carbons in
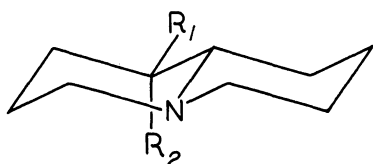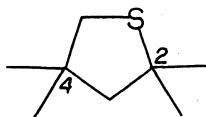
20, R_1=CH₃; R_2=H
21, R_1=H; R_2=CH₃
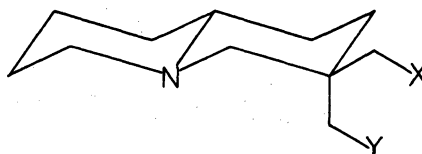
22, X=H,Y=SCH₃
23, X=SCH₃,Y=H

24

13 and 17 closely matched those of α,β and γ carbons in the pair 20 and 21. The presence of one equatorial and one axial methyl in 18 and 19 was revealed in the display of both the 13.4 and 19.2 ppm resonances. That the mixture consisted of 18 and 19 and not 13 and 17 was clear from other resonances in the spectra and obvious differences in chromatographic behavior.

The stereochemistry of the CH_2S attachment to the A'B' quinolizidine moiety. This aspect of thiaspirane stereochemistry is evident from data provided from ¹H (20) and ¹³C NMR (18), the X-ray crystallographic studies of thiobinupharidine (13) and neothiobinupharidine (14) and the circular dichroism (CD) of the various C-6' hemiaminal derivatives. The discussion of the last named is included within a general discussion of hemiaminal CD in a section below.

A ¹H NMR study of a pair of stereochemically defined 3-methyl-3-methylthiomethylquinolizidines, 22 and 23, revealed that the axial CH_2S resonance of 22 appeared 23 Hz (at 60 MHz) downfield from the equatorial CH_2S resonance of 23. This difference is well known for axial and equatorial methyl groups attached to the C-3 position of quinolizidine ring systems. In comparison, the thiomethylene resonances were observed at δ 2.32, 2.33 and 2.70 respectively for 13, 14 and 15. Since the CH_2S resonance of 15 was 27 Hz downfield from that of 13 and 14, the CH_2S in 15 must be axial, but equatorial in 13 and 14. These assignments are consistent with the X-ray structures of 13 and 15.

Because both the CH_2S (C-12') and the isolated CH_2 (C-12) resonances were clearly observed in the ¹³C NMR (18), the latter yielded more information than ¹H NMR regarding the steric disposition of the quinolizidine systems relative to the thiolane ring. Employing the same model pair 22 and 23, the axial-equatorial increment for a CH_2S group was established, the axial CH_2S in 22 being shielded by 6.0 ppm relative to the equatorial CH_2S in 23. The associated study of the tetramethylthiolane 24 proved that the isolated CH_2 would appear downfield from the CH_2S. These latter results assisted the C-12' resonance assignments which are compared, along with C-12 resonances, in Fig. 3 for the three thiaspiranes, 13, 14 and 15, and the model compounds 22, 23 and 24. For two thiaspiranes, 13 and 14, the CH_2S resonance was the same and downfield by 3.6 ppm from that in the third thiaspirane, 15. Therefore thiaspiranes 13 and 14 must have an equatorial CH_2S attached to C-7' and 15 must possess an axial CH_2S at C-7', conclusions which are consistent with the ¹H NMR results.
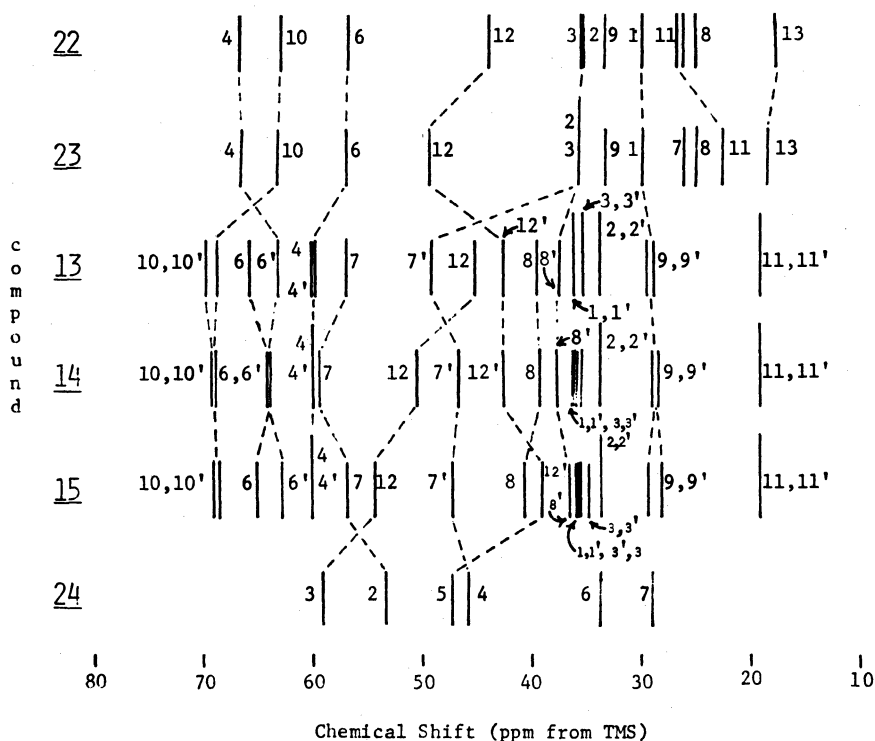
Fig. 3. ^{13}C NMR chemical shift correlation of selected resonances of: 3β-methyl-3α-methylthiomethylquinolizidine, 22; 3α-methyl-3β-methyl-thiomethylquinolizidine, 23; thiobinupharidine, 13; thionuphlutine B, 14; neothiobinupharidine, 15; 2,2,4,4-tetramethylthiolane, 24.

Figure 3 also reveals the regular progression of the isolated CH_2(C-12) chemical shifts in passing from one thiaspirane to another. This observation confirms the stereochemistry of C-12 assigned on the basis of studies described in the following section. The highest field chemical shift for C-12 occurred for thiobinupharidine and indicates the axial conformation of C-12 relative to two quinolizidine systems. The lowest field chemical shift occurred for neothiobinupharidine and indicates the equatorial conformation of C-12 relative to two quinolizidines. The intermediate chemical shift must be one in which C-12 is axial to the one quinolizidine system but equatorial to the other.

The stereochemistry of the sulfur atom attachment to the AB quinolizidine moiety. Manifes-tations of intramolecular sulfur-immonium ion interactions, one of the more interesting aspects of thiaspirane chemistry, first came to light in solving the problem of C-7 con-figuration. The sulfur atom stereochemistry at C-7 was established in our laboratory initially by examining the steric mode of metal deuteride reductions of the thiaspirane hemiaminals and related model thiohemiaminals (13, 21, 22). Reduction of 6,6'-dihydroxy-thiobinupharidine, 25, and 6,6'-dihydroxythionuphlutine B, 26, with sodium borodeuteride in methanol gave bisamines doubly labelled at C-6 and C-6' as expected. However the thiobinu-pharidine-6,6'-d_2, 27, contained an axial deuterium at C-6' and, to the extent of 82%, equatorial deuterium at C-6. This finding contrasted with that obtained from 6,6'-dihydroxy-thionuphlutine B which produced thionuphlutine B-6,6'-d_2, 28, containing axial deuterium at both C-6 and C-6'. Like the bishemiaminal 25, the monohemiaminal, 6-hydroxythiobinuphari-dine (29), underwent reduction with incorporation of 84% equatorial deuterium and 16% axial deuterium at C-6. These findings were based on the following observations. The ^1H NMR of unlabelled thiobinupharidine revealed four protons, two C-6, C-6' equatorial and two C-4, C-4' protons, in the δ 2.7-3.2 region. The δ 1.7-1.8 region contained the C-6, C-6' axial protons. The ^1H NMR of the unlabelled thionuphlutine B was similar. The ^1H NMR of the doubly labelled thiobinupharidine showed essentially two C-4 and one C-6 equatorial protons in the δ 2.7-3.2 region and one C-6 axial proton in the δ 1.7-1.8 region. In contrast, the NMR of the doubly labelled thionuphlutine B showed essentially two C-4 and two C-6 equa-torial protons in the δ 2.42-3.15 region, while the δ 1.0-2.0 region contained two fewer protons. The C-6 axial protons at δ 1.7-1.8 were best accounted for by integrating over the entire δ 1.0-2.0 region. The analysis of the steric disposition of deuterium in the labelled thiobinupharidines was extended to a 300 MHz ^1H NMR examination of the doubly

labelled 27 and the singly labelled 30 in deuterobenzene, this solvent being particularly
advantageous in effecting the increased separation of resonance lines as had been demon-
strated much earlier in cases of both piperidine (23) and quinolizidine (11) *Nuphar* alka-
loids. The pertinent regions of the spectra are illustrated in Fig. 4. Using one of the
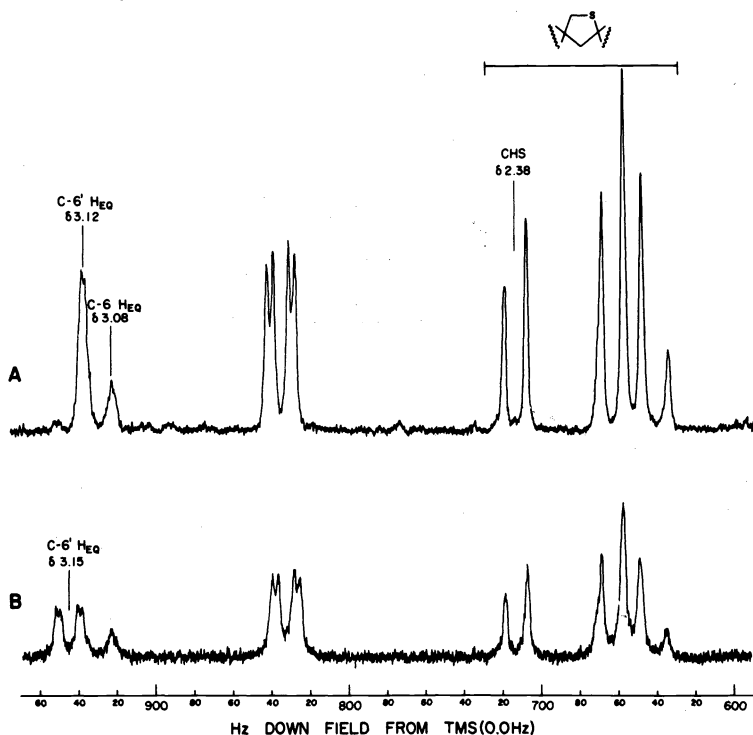

Fig. 4.   300 MHz ^{1}H NMR spectra of: (A) thiobinupharidine-6,6'-d$_2$, 27;
(B) thiobinupharidine-6-d$_1$, 30.

two thiomethylene protons (δ 2.38) as the standard for integration, the singly labelled 30
contained 1.13 protons in the δ 3.05-3.20 region, the proton in excess of 1.00 being the
signal at δ 3.08 which arose in part from the labelled species containing a C-6 equatorial
hydrogen and a C-6 axial deuterium. The doublet of a doublet at δ 3.15 represented the
C-6' equatorial proton. Employing the same method of analysis for the doubly labelled 27,
1.14 protons are observed in the 3.05-3.20 region. The doublet at δ 3.12 represents the
C-6' equatorial proton, W- coupled only to the C-8 equatorial proton since the C-6' axial
protium is now replaced with deuterium. It is through these analyses that the 82 and 84%
equatorial deuterium incorporation values, referred to above, were determined. Clearly the
^{1}H NMR study of the CH$_2$S attachment to C-7' had indicated that the configuration of C-7' was
the same in thiobinupharidine and thionuphlutine B and therefore it was reasoned that C-6'
in both bishemiaminals underwent reduction with incorporation of an axial deuterium. It
followed that C-6 must incorporate deuterium in two steric modes, largely equatorial in the
case of thiobinupharidine but axial in the case of thionuphlutine B.

The variable steric mode of reduction at C-6 was attributed to the interaction of the C-7
sulfur atom with an intermediate immonium ion. Deuterium incorporation at C-6 was occur-
ring predominantly from the side of the molecule opposite the interacting sulfur. Incor-
poration of equatorial deuterium at C-6, as was observed for thiobinupharidine, must signify
that the interacting sulfur atom at C-7 was equatorial giving structures 27 and 30. Incor-
poration of axial deuterium at C-6, as was observed for thionuphlutine B, must signify that
the interacting sulfur atom at C-7 was axial giving structure 28.

The stereospecificity found in the sodium borodeuteride reductions of C-6 in the pair of
thiaspirane bishemiaminals 13 and 14 has been observed also for various α-thiohemiaminals
derived from deoxynupharidine (13, 22). α-Thiohemiaminals 31 and 35, wherein the sulfur
atom attached to C-7 is equatorial, gave respectively thioamines 33 and 37 containing
equatorial deuterium at C-6, as determined by ^{1}H NMR studies. In contrast, α-thiohemi-
aminals 32 and 36, possessing axial sulfur functions at C-7, afforded respectively thio-
amines 34 and 38 containing axial deuterium at C-6. These reductions, like those of 13
and 14, were also carried out in methanol.
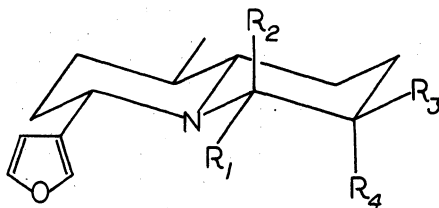
31, $R_1+R_2=OH+H$; $R_3=SCH_3$; $R_4=CH_3$ **37**, $R_1=D$; $R_2=H$; $R_3=SC_6H_5$; $R_4=CH_3$

32, $R_1+R_2=OH+H$; $R_3=CH_3$; $R_4=SCH_3$ **38**, $R_1=H$; $R_2=D$; $R_3=CH_3$; $R_4=SC_6H_5$

33, $R_1=D$; $R_2=H$; $R_3=SCH_3$; $R_4=CH_3$ **42**, $R_1+R_2=OH+H$; $R_3=S-\underline{p}-C_6H_4=CH_3$; $R_4=CH_3$

34, $R_1=H$; $R_2=D$; $R_3=CH_3$; $R_4=SCH_3$ **43**, $R_1=R_2=H$; $R_3=SCH_3$; $R_4=CH_3$

35, $R_1+R_2=OH+H$; $R_3=SC_6H_5$; $R_4=CH_3$ **44**, $R_1=R_2=H$; $R_3=CH_3$; $R_4=SCH_3$

36, $R_1+R_2=OH+H$; $R_3=CH_3$; $R_4=SC_6H_5$ **50**, $R_1+R_2=H+OH$; $R_3=CH_3$; $R_4=OH$

51, $R_1+R_2=H+OH$; $R_3=S-p-C_6H_4C\ell$; $R_4=CH_3$

The incorporation of equatorial deuterium at C-6 is remarkable in view of the steric shielding by the 3-furyl group of the side of the molecule to which the 3-furyl is attached. This effect is demonstrated in the catalytic addition of deuterium to 6-dehydrodeoxynuphari-dine, **4**, which occurred to the extent of 90% from the side opposite the 3-furyl group (1). Any amount of equatorial reduction in the case of the thiobinupharidine α-thiohemiaminals is all the more remarkable since the substrate possesses near C_2 symmetry, and accordingly the axial reduction which occurs at C-6' could also be expected at C-6, rather than the equatorial reduction which in fact occurs and does so from the direction of the more hindered concave face of the molecule.

Likely it is the influence of the 3-furyl group which accounts for the observation that reductions of isomeric C-7 α-thiohemiaminals occurred at different rates; the thiohemiaminal **32**, possessing an axial sulfur at C-7, underwent reduction nearly 800 times faster than **31**, wherein the sulfur function at C-7 is equatorial (13). The enhanced rate of axial sodium borohydride reduction is reflected in the reductions of the thiaspirane hemiaminals themselves. When 6,6'-dihydroxythiobinupharidine was treated carefully with limited amounts of sodium borodeuteride in methanol, 6-hydroxythiobinupharidine-6'-d_1 was the only monohemi-aminal detected (8). But under similar conditions, 6,6'-dihydroxythionuphlutine B, which underwent reduction only in the axial mode, gave a mixture of both 6 and 6'-monohemiaminals, the latter predominating (8). Similarly 6,6'-dihydroxyneothiobinupharidine gave a mixture of monohemiaminals rich in the C-6' isomer (9).

The stereochemistry of α-thiohemiaminal reduction appears to be sensitive to the medium and the metal hydride reducing agent. Reduction of 6,6'-dihydroxythiobinupharidine in dry ethanol occurred with incorporation of 40% equatorial deuterium at C-6 (24) rather than the 82% observed when the reduction was carried out in methanol. Similarly when the deoxynu-pharidine derived α-thiohemiaminal **42** was reduced with lithium aluminium deuteride in dry ether, an equal amount of axial and equatorial deuterium was incorporated at C-6 (9).

The stereochemistry of β-thiohemiaminal reduction is independent of the configuration of C-7' and the stereochemistry of the sulfur atom; this finding contrasts with α-thiohemi-aminal reduction, as noted in the paragraphs above. The β-thiohemiaminal **40**, wherein the C-7' thiomethylene is axial, underwent axial incorporation of deuterium at C-6' as evidenced by the ^{1}H NMR spectrum of the labelled compound **41** which showed no δ 1.55 doublet for the C-6' axial proton as did the unlabelled neothiobinupharidine (25). But as already noted, both 6,6'-dihydroxythiobinupharidine and 6,6'-dihydroxythionuphlutine B, wherein the C-7' thiomethylenes are equatorial, also underwent reduction with incorporation of axial deuterium at C-6' (13).

The picture which seems to be developing from these various reduction results is the following. α-Thiohemiaminal reductions carried out in methanol are highly stereospecific, but β-thiohemiaminal reductions are not. The stereochemistry of α-thiohemiaminal reduction is influenced by two structural features of the substrate: the stereochemistry of the sulfur atom at C-7 and the presence of the 3-furyl group substituted at C-4. In methanol solution, the stereochemistry of the sulfur atom controls the direction of deuteride attack. When the latter occurs from the direction opposite the 3-furyl group, such that axial deuterium is introduced, the rate is greater than that of C-6' reduction. However when deuteride attack must occur from the same side as the 3-furyl group, such that equatorial deuterium is intro-duced, then the rate is less than that of C-6' reduction. The stereochemistry of β-thio-hemiaminal reduction is influenced only by the presence of the 3-furyl group.

The CD of hemiaminals in acid solution has emerged as the most convenient method for studying these compounds, once the relative stereochemistry of thiobinupharidine and thio-nuphlutine B became secure. However the absolute configuration of both of these molecules had not been established; therefore it was necessary to correlate C-7 configuration with the sign and wavelength of the CD bands (26). This was achieved by studying the pair of (-)-deoxynupharidine derived hemiaminals 31 and 32 (13). Reduction converted 31 and 32 respectively to amines 43 and 44 whose ^{1}H NMR were compared. Since the C-7 methyl resonance of 43 appeared downfield from that of 44, the former was attributed to an axial methyl and the latter to an equatorial methyl in quinolizidine systems displaying full intensity Bohlmann IR bands. These relative stereochemical assignments were confirmed by the chloroform to benzene induced solvent shifts (*vide ultra*), the C-7 methyl resonance of 43 moving downfield and that of 44 upfield. Finally the stereochemistry was supported by ^{13}C NMR results (18).

The α-thiohemiaminals 31 and 32 were converted to their respective immonium perchlorates. The 95% ethanol solution of 32 perchlorate displayed a positive CD band at 298 nm while 31 perchlorate gave a negative CD band at 300 nm. Since the absolute configuration of (-)-deoxynupharidine had been determined (10), the CD curves of 31 and 32 established the corre-lation between the sign and wavelength of the CD bands and the configuration at C-7.

Both of the immonium perchlorates derived from 25 and 29 displayed a positive CD band at 296 nm while the immonium perchlorate of 6,6'-dihydroxythionuphlutine B, 26, gave a negative band at 308 nm. These results, in comparison with the CD of immonium perchlorates from 31 and 32 established the absolute configurations of the thiaspirane hemiaminals. In agreement with the structures, the immonium perchlorates of the bishemiaminals also gave positive bands in the 270-280 nm region, in addition to the bands in the 295-310 nm region. The immonium perchlorate of 25 gave a 265 nm band and that from 26 gave a 275 nm band.

Finally the CD of four thiaspirane monohemiaminals were compared (Fig. 5). The compounds were: 6-hydroxythiobinupharidine, 29; 6'-hydroxythiobinupharidine, 45; 6-hydroxyneothiobi-nupharidine, 46; and 6'-hydroxyneothiobinupharidine, 40, the last two being newly isolated natural products whose structures were to be confirmed (25). The four possible configura-tions of C-7 and C-7' are represented in these four monohemiaminals. In acidic solutions

Fig. 5. The circular dichroism in acid solution of: 6-hydroxy-thiobinupharidine (29) (-·-··-); 6'-hydroxy-thiobinupharidine (45) (— · —); 6-hydroxy-neothiobinupharidine (46) (———); 6'-hy-droxyneothiobinuphari-dine (40) (---).

the α-thiohemiaminals containing 7S (29) and 7R (46) configurations gave respectively positive and negative CD bands near 300 nm. The β-thiohemiaminals possessing 7'S (45) and 7'R (40) configurations gave respectively positive and negative CD bands in the shorter wavelength 275-285 nm region.

In concluding this section we note that there are two properties of α-thiohemiaminals which can be rationalized by a sulfur-immonium ion interaction: the stereospecificity of α-thiohemiaminal reduction and the appearance of the UV and CD bands in acidic solution. In the case of the β-thiohemiaminals, the thioimmonium absorption is observed but the stereospecificity of reduction is not. These findings suggest that the β-thioimmonium ion interaction is sufficiently strong to generate the absorption but not properly disposed to control the stereochemistry of sodium borodeuteride reduction.

BIOLOGICAL ACTIVITY

The literature records for over 30 years the biological activity of various *Nuphar* preparations. Seemingly some of this activity can be linked to the presence of thiaspirane hemiaminals now that a few specific activities of these and similar compounds have been established. 6,6'-Dihydroxythiobinupharidine, 25, was active against *Corynebacterium michiganense* (27), and at levels of 40 μg/ml this same compound inhibited the growth of the human pathogenic fungi *Histoplasma capsulatum* and *Blastomyces dermatitidis* (28). At levels of 100 μg/ml, 25 suppressed the growth of *Microsporum gypseum, M. canis, Trichophyton mentagrophytes* and *T. tonsurans*.

Having discovered the activities of thiohemiaminals, an attempt was made to synthesize simple analogs containing the essential structural and functional features of 25. These features were an α-thiohemiaminal and a 3-furyl group incorporated into a quinolizidine ring system. The compounds took form in the deoxynupharidine derived α-thiohemiaminals such as 31 and 32, and 37 and 38. These and similar compounds were prepared by the reaction of 6-dehydrodeoxynupharidine, 4, with an electrophilic thialating agent; alkyl and aryl arene thiosulfonates, aryl sulfenyl chlorides and succinimides, and aryl disulfides have been used for this purpose (13, 22, 29). An α-thiohemiaminal lacking the 3-furyl group, 49, was prepared by starting with 3-methyl-4-ketoquinolizidine, 47, which was reduced with diisobutyl aluminium hydride. The resulting enamine, 48, was treated with methyl *p*-toluenethiosulfonate to obtain 49 (22).

47   48   49

The α-arylthiohemiaminals 37, 42 and 51, the α-alkylthiohemiaminals 31, 32 and 49, the α-hydroxyhemiaminal 50, and the α-*tert*-amino sulfides 43 and 44 and the pair obtained by reducing a mixture of 35 and 36 are among the several compounds tested against a number of human pathogenic fungi. All the α-thiohemiaminals except 49 were active at 20 to 40 μg/ml, the aryl substituted the more so. These concentrations are the same or slightly less than the active concentration of 6,6'-dihydroxythiobinupharidine. Since α-thiohemiaminals undergo acid promoted conversion to α-thioimmonium ions and tests were performed in the pH range of 5.0-6.5, the above results indicate the structural and functional components required for activity are the ring A methyl and 3-furyl groups and the functionality capable of generating an α-thioimmonium ion.

More recently in preliminary cytotoxicity screening using strain L (Earle) cells, 35 and 51 at 1.25 to 2.5 μg/ml have been found to inhibit cell growth to the extent of 50% of control (30).

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