The Hydride Reduction of Hemiaminals of Thiobinupharidine and the Structure of Thionupharoline

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Thionupharoline has been shown to be identical with 6-hydroxythiobinupharidine. The hydride reduction of hemiaminals of thiobinupharidine has been examined in ethanol solution. The steric course of reduction at C-6 is not nearly as selective as it has been reported to be in solution in methanol.

T. I. MARTIN, D. B. MACLEAN, J. T. WRÓBEL, A. IWANOW et W. STARZEC. Can. J. Chem. **52**, 2705 (1974).

On montre que la thionupharoline est identique à l'hydroxy-6 thiobinupharidine. L'étude de la réduction par un hydrure des hémiaminals de la thiobinupharidine a été effectuée en solution dans l'éthanol. Le développement stérique de la réduction en C-6 n'est pas aussi sélectif que ce qui a été rapporté quand le solvant est le méthanol. [Traduit par le journal]

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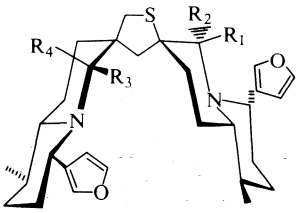
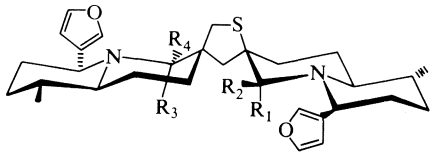
In 1970 Wróbel reported the isolation of a new sulfur-containing alkaloid from the yellow water lily (1). He named the new alkaloid thionupharoline ($C_{30}H_{42}N_2O_3S$, **1**) and suggested that it was related structurally to the known isomeric sulfur containing alkaloids of this series, thiobinupharidine **2** and neothiobinupharidine **3**, of composition $C_{30}H_{42}N_2O_2S$, but unlike the others to contain a hydroxyl group. In the same year LaLonde *et al.* (2) reported the isolation from the same species of the isomeric bishemiaminals of composition, $C_{30}H_{42}N_2O_4S$, that were named 6,6′-dihydroxythionuphlutine-A, **4**, and 6,6′-dihydroxythionuphlutine-B, **5**. Later (3) it was shown that thionuphlutine-A was identical with thiobinupharidine and the latter name having precedence in the literature is used for this compound.

The structure of **3** has been known since 1965 (4) but it is only in the last year that the structures of thiobinupharidine (5, 6, 7) and of thionuphlutine-B, **6** (6, 7), have been resolved.

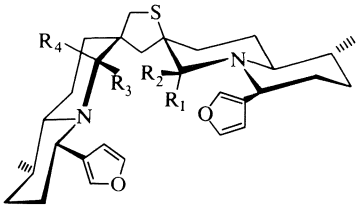
Very recently LaLonde *et al.* (6) reported the isolation and identification of a monohemiaminal, 6-hydroxythiobinupharidine. Here we report the identity of thionupharoline and 6-hydroxythiobinupharidine and describe p.m.r. and reduction studies in this series that differ from those reported (6).



1 $R_1 + R_2 = H + OH$; $R_3 = R_4 = H$
2 $R_1 = R_2 = R_3 = R_4 = H$
4 $R_1 + R_2 = R_3 + R_4 = H + OH$



3 $R_1 = R_2 = R_3 = R_4 = H$



5 $R_1 + R_2 = R_3 + R_4 = H + OH$
6 $R_1 = R_2 = R_3 = R_4 = H$

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Reduction of **1** in sodium borohydride yielded thiobinupharidine. Since hemiaminals but not simple alcohols are known to undergo reduction with this reagent, it was established that **1** was a hemiaminal and that it must be either 6- or 6′-hydroxythiobinupharidine. This conclusion was substantiated by the presence of a singlet in the p.m.r. spectrum of **1** measured in benzene at 4.25 δ assigned to the methine proton adjacent to both oxygen and nitrogen.

We found that the u.v., i.r., p.m.r., and mass spectra of **1** corresponded with those reported by LaLonde *et al.* (6) for 6-hydroxythiobinupharidine.[1] We prepared a monoperchlorate and a diperchlorate of **1** according to the procedure described by LaLonde *et al.* (6) and found them to be identical with the corresponding salts of 6-hydroxythiobinupharidine (6). The diperchlorate of **1** described by Wróbel was reported to melt at 170–172°, nearly 100° lower than that of 6-hydroxythiobinupharidine described by LaLonde. We repeated the preparation according to the original procedure confirming the original result. It is apparent that the diperchlorate is dimorphic or that it crystallizes with a mole of water when prepared by the original procedure of Wróbel. Because of the paucity of **1** we were unable to resolve this problem to our satisfaction.

Before we became aware of the results of LaLonde *et al.* (6) we attempted to resolve the structural problem through p.m.r. studies and mass spectrometry. This led us to examine the p.m.r. spectra of **1** and **2** at 220 MHz and to prepare 6-deuterio- and 6,6′-dideuteriothiobinupharidine for p.m.r. and mass spectrometric studies. The results that we obtained are sufficiently different from those already reported (6) that they merit discussion.

We found that the spectra of **1** and **2** are much better resolved in perdeuteriobenzene than they are in deuteriochloroform. We therefore set about to assign as many of the signals as possible in the spectrum of **2** recorded in benzene solution. Our assignments are based on analogy with those of the corresponding protons of deoxynupharidine **7** and 7-epideoxynupharidine **8**.

The p.m.r. spectrum of **7**, whose structure and absolute configuration are known (8), has been studied in detailed by LaLonde and co-workers (9–11). As a result of their work no ambiguity exists with respect to the assignment of signals of the equatorial methyl group at C-1, the axial methyl group at C-7, and the hydrogens, H-4a, H-6a, H-6e, and H-7e. The majority of the prior work was carried out in $CDCl_3$. We have repeated this work in benzene solution at 220 MHz and have obtained the results shown in Table 1.

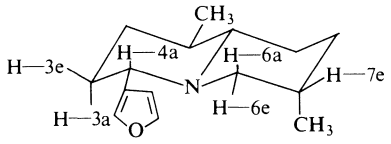
7-Epideoxynupharidine was prepared synthetically (12, 13) before it was isolated from nature (14). Its structure and stereochemistry follow from the fact that it is formed along with **7** when $\Delta^{6,7}$-dehydrodeoxynupharidine is treated with hydrogen over a Pd catalyst (10). Its p.m.r. spectrum in deuteriochloroform and in benzene has been examined but only a few resonances have been assigned (14). We have re-examined the spectrum in benzene and through decoupling experiments have made the assignments shown in Table 2.

In the first p.m.r. study of thiobinupharidine (15) the signals of individual protons other than the *C*-methyl and the furan protons were not assigned but in a later study (2) the absorption of the methylene protons adjacent to sulfur were assigned. The latter assignment has been placed on a firm foundation through work on model systems (16). Assignment of the hydrogens at the axial and equatorial positions at C-6 and C-6′ have been made through a study of the deuterated compounds obtained on reduction of 6,6′-dihydroxythiobinupharidine (6). The axial hydrogens at C-4 and C-4′ have also been identified in the spectrum (5, 6). Much of this work has been carried out in $CDCl_3$ where there is much overlap of the signals attributed to the protons at C-4, C-4′, C-6e, and C-6′e (see for example the published spectrum of **2**, (5)) but better separation of the signals is obtained in perdeuteriobenzene (6). The assignments that we have made from 220 MHz studies are shown in Table 3.

Inspection of the three tables shows that the chemical shifts of H-6a and H-6e of **2** differ from one another by approximately the same amount as the corresponding protons of **7**. Similarly the chemical shifts of H-6′a and H-6′e differ from one another by approximately the same amount as the corresponding protons of **8**. The protons at C-4 and C-4′ of **2** absorb in the same region and have virtually the same chemical shift as

[1]We thank Professor LaLonde for disclosing his results to us prior to their publication and for providing spectra and samples of his compounds for comparison.

TABLE 1. Proton magnetic resonance spectrum of deoxynupharidine in C_6D_6

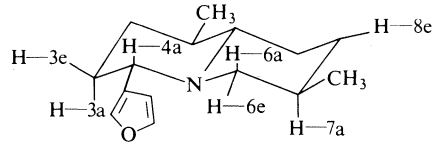


7

Proton	Chemical shift (δ)	Multiplicity	Coupling constants J (Hz)
C-1 methyl e	0.81	d	6.0
C-7 methyl a	1.10	d	7.0
H-6a	1.78[a]	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)

[a]Irradiation at 1.78 collapses quartet at 2.76 into broad singlet.

TABLE 2. Proton magnetic resonance spectrum of 7-epideoxynupharidine in C_6D_6



8

Proton	Chemical 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[e]	t (1:2:1)	11.0 (6a-6e) and 11.0 (6a-7a)
H-7a, H-3e, H-8e	1.64[b]	Complex multiplet	
H-3a	1.77[c]	Multiplet	11.0 (3a-4a) recognizable
H-4a	2.83[d]	q	11.0 (4a-3a) and 3.5 (4a-3e)
H-6e	3.03[f]	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.
[b]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).
[c]Irradiation at 1.77 collapsed quartet at 2.83 to a doublet (J = 3.5 Hz).
[d]Irradiation at 2.83 collapsed the 11.0 Hz coupling in signal at 1.77.
[e]Irradiation at 1.29 collapsed doublet of quartet at 3.03 to multiplet with couplings 2.0 and 3.5 Hz recognizable.
[f]Irradiation at 3.03 collapsed 1:2:1 triplet at 1.29 to a 1:1 doublet (J = 11.0 Hz).

the protons at C-4 in **7** and **8**. It is apparent that the equatorial alkyl substituent causes a shielding of H-6′a of **2** and H-6a of **8** relative to the corresponding proton of **7**. The protons H-6a and H-6e of **2** are deshielded, presumably by the equatorial sulfur, relative to the corresponding protons of **7**.

When the p.m.r. spectra of **1** and **2** were compared we found it impossible to assign definitively the hydroxy group to C-6 or to C-6′. The presence of the hydroxy function introduced too many changes in the spectrum. We turned therefore to examine the products of the borodeuteride reduction of 6,6′-dihydroxythiobinupharidine **4** and of **1** in the expectation that an examination of these compounds by p.m.r. or mass spectrometry might lead to a resolution of the problem. While our work was in progress it was reported (6) that the reduction of **4** with $NaBD_4$ led to the incorporation of deuterium into the axial position at C-6′ and into an equatorial position at C-6 with complete stereoselectivity. It was also stated that the reduction proceeded with about 70% incorporation of deuterium and 30% incorporation of hydrogen, a result that was surprising.

We reduced **4** with sodium borodeuteride in ethanol and obtained a dideuteriothiobinu-

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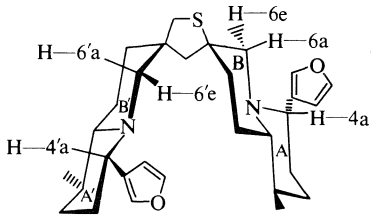
TABLE 3. Proton magnetic resonance spectrum of thiobinupharidine in C_6D_6



2 Thiobinupharidine ($C_{30}H_{42}N_2O_2S$)

Proton	Chemical shift (δ)	Multiplicity	Coupling constants *J* (Hz)
H-6'a	1.40[a]	d	11.5 (6'a-6'e)
H-6a	1.92[b]	d	11.5 (6a-6e)
H-6e	3.11	q	11.5 (6a-6e); 2.0 (6e-8e)
H-6'e	3.17	q	11.5 (6'a-6'e); 2.5 (6'e-8'e)
H-4a + H-4'a	2.80	q	10.5 (4a-3a); 3.5 (4a-3e)
C-7-CH_2-C-7'	2.18	ABq	J_{AB} = 14.0
-S-CH_2-	2.31	ABq	J_{AB} = 11.5

[a]Irradiation at 1.40 collapses q at 3.17 into broad singlet; q at 3.11 unaffected.
[b]Irradiation at 1.92 collapses q at 3.11 into broad singlet; q at 3.17 unaffected.

pharidine and a monohydroxymonodeuteriothiobinupharidine. Unlike the published result (6) our dideuterio compound was approximately 95% dideuterated material. The 220 MHz spectrum in perdeuteriobenzene showed that one deuterium was incorporated, almost exclusively, into the axial position at C-6′ and that the second deuterium was incorporated to the extent of 40% into the equatorial position and 60% into the axial position at C-6. The signal observed at 1.40 δ (H-6′a) in **2** had virtually disappeared in the spectrum of the dideuterated compound while the signal at 3.16 δ (H-6′e) had collapsed to a poorly resolved doublet, apparently coupled only to H-8′e. The signal at 1.93 δ (H-6a) was now a broad singlet (0.4 H) whereas the signal at 3.10 δ (H-6e) was a poorly resolved doublet (0.6H) coupled to H-8e. Our results are compatible with those of LaLonde *et al.* (6) with respect to reduction at C-6′ but they differ from his with respect to reduction at C-6. We actually observe only 40% introduction of deuterium into the equatorial position at C-6 and not 100% as they observed. They attributed the stereoselectivity to sulfur interaction with an immonium salt intermediate. They proposed that the presence of an equatorial sulfur as in **2** resulted in preferential introduction of deuterium into an equatorial position whereas the presence of an axial sulfur as in thionuphlutine-B led to preferential introduction of deuterium into an axial position (6). Our results show that sulfur in an equatorial position influences the reaction but not to the same extent as it does when the reaction is conducted in methanol solution. Other studies have shown that enamines in the monomeric system are normally attacked on the β face of the molecule to give axial introduction of hydrogen or deuterium at the carbons adjacent to nitrogen (10, 11).

The second product of the borodeuteride reduction proved to be 6′-deuterio-6-hydroxythiobinupharidine with greater than 95% incorporation of deuterium. This compound had a u.v. spectrum identical with **1** in acid and neutral media and formed a diperchlorate of the same melting point as **1**. Its melting point was undepressed in admixture with the diperchlorate of **1**. It differed in its 220 MHz spectrum from that of **1** only in one obvious respect, namely that the quartet present in **1** at 3.18 δ (H-6′e) had collapsed to a poorly resolved doublet integrating for one proton. It is clear that the deuterium has entered axially at C-6′ and that reduction at C-6′ has occurred more rapidly than at C-6. The more rapid attack at C-6′ in this system is predictable on the basis of studies on model compounds (6).

Reduction of **1** with borodeuteride gave 6-deuteriothiobinupharidine with deuterium incorporation in excess of 95%. The 220 MHz spectrum of this compound in benzene showed

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only minor differences from the spectrum of **2**. The signal at 1.93 δ, formerly a doublet, now appeared as a broad singlet (0.35 H) and the signal at 3.10 δ now appeared as a broadened singlet (0.65 H). Thus deuterium had entered 65% into the axial position and 35% into the equatorial position, a result in clear agreement with the borodeuteride reduction of **4**.

The results of the p.m.r. studies on the borodeuteride reduction products of **1** and **4** are illustrated in Fig. 1. The lower spectrum is that of thiobinupharidine recorded at 220 MHz in C_6D_6 and shows the double irradiation experiments conducted on that compound. The centre spectrum is that of the product of borodeuteride reduction of **1** and the upper spectrum that of the product of complete borodeuteride reduction of **4**. The spectra of the deuterated compounds were recorded with 5–10 mg samples under the same conditions as the spectrum of **2**. The deuterium incorporation at the various sites is indicated.

This work has established the identity of thionupharoline and 6-hydroxythiobinupharidine. It has shown that borohydride reduction in ethanol solution of hemiaminals of thiobinupharidine that carry a hydroxyl group at C-6 does not occur with high stereoselectivity. Our results contrast with those reported before (6) where the reduction was effected in methanol. We found that incorporation of deuterium was nearly complete in the borodeuteride reduction whereas only about 70% incorporation has been observed in an earlier study (6).

Experimental

Apparatus, Methods, and Materials

Infrared spectra were recorded on a Perkin-Elmer 521 spectrometer. Typically, samples of free bases were made up at a concentration of 0.03 *M* in spectroquality carbon tetrachloride or dichloromethane, using cells with KBr windows and a path length of 0.1 mm. Perchlorate salts were made up in KBr discs prepared in the usual manner and the spectra were run against air as the reference side.

Proton magnetic resonance spectra were recorded at 220 MHz on a Varian 220 HR spectrometer using a field sweep mode. All spectra were recorded at ambient temperature. Samples were dissolved in $CDCl_3$ or C_6D_6 as required using added TMS as internal standard. Chemical shifts are reported relative to TMS = 0.0 δ. Typically a sweep width of 2500 Hz was employed but sweep widths of 1000, 500, and 250 Hz facilitated the determination of coupling constants. Symbols s, d, q, m, nw, br, and $W_{1/2}$ refer to singlet, doublet, quartet, multiplet, narrow, broad, and width at half height, respectively. Ultraviolet spectra were recorded on a Cary 14 spectrometer using 1 or 0.1 cm quartz cells as appropriate.

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

Thin-layer chromatograms were performed under standard conditions using 0.5 mm silica gel coated on glass plates available from Brinkman Instruments, Rexdale, Ontario. Solvent used for development in all cases was hexane–benzene–acetone, 1:5:1. Development was carried out under conditions of tank saturation in a vessel 25 cm × 20 cm × 7 cm. The spray reagent was Dragendorf reagent (17). Column chromatography was performed using neutral alumina, Brockman Activity 1, 80–200 mesh, available from Fischer Scientific, adjusted to give the required activity (18, 19). 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.

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

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 the unlabelled compounds and the deuterium content calculated by the method of Biemann (20).

Isolation of Thionupharoline 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 30 min. The resultant oil was extracted with CH_2Cl_2 (5 × 10 ml). The extracts were combined, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to leave a colorless glass on the inside of the vessel. The yield was 34.6 mg of free base which was purified by chromatography from neutral alumina (Activity II, 2 g) using 20% ether in hexane as eluant. The thionupharoline (34 mg) obtained as a glass, showed one spot on t.l.c. R_f 0.36; u.v. (95% EtOH, neutral), end absorption only; u.v. (95% EtOH, acidic) λ_{max_1} 208 nm (*q* 20 000), λ_{max_2} 293 nm (*q* 2540); i.r. (CH_2Cl_2) 2.80 (OH), (weak), 3.59 (Bohlmann band), and unassigned bands at 6.65, 6.90, 7.26, 8.66, 9.08, 9.40, 9.67, 9.74, 11.45 μ; p.m.r. (220 MHz $CDCl_3$): δ 0.88 (d, J = 5 Hz, 6 H, 2 × CHCH_3), 2.20 (ABq, J_{AB} = 12 Hz, 2 H, CH_2—S), 2.26 (OH exchangeable on addition of D_2O), 2.89 (q, J = 5.6 and 8 Hz, 1 H, H-4′), 2.92 (quartet J = 11.5 and 2 Hz, 1 H, H-6′e), 3.70 (q, J = 7.5 and 7 Hz, 1 H, H-4), 3.97 (s, 1 H, H-6, sharpens on addition of D_2O), 6.34 (nw m, $W_{1/2}$ = 7 Hz, 2 H, furan β H), 7.21 (nw m, 1 H, furan α H), 7.30 (nw m, $W_{1/2}$ = 4 Hz, 3 H, furan α H); p.m.r. (220 MHz C_6D_6): δ 0.75 (two superimposed doublets J = 6 Hz, 6 H, 2 × CHCH_3), 2.11 (AB quartet, J_{AB} = 12 Hz, 2 H, CH_2—S), 2.42 (OH, exchangeable with D_2O), 2.79 (q, J = 3 and 11 Hz, 1 H, H-4′), 3.18 (q, J = 2.5 and 12 Hz, 1 H, H-6′e), 3.90 (q, J = 4 and 10 Hz, 1 H, H-4), 4.25 (s, 1 H, H-6), 6.41 (nw m, furan β H), 6.48 (nw m, 2 H, with 6.41 furan β H),

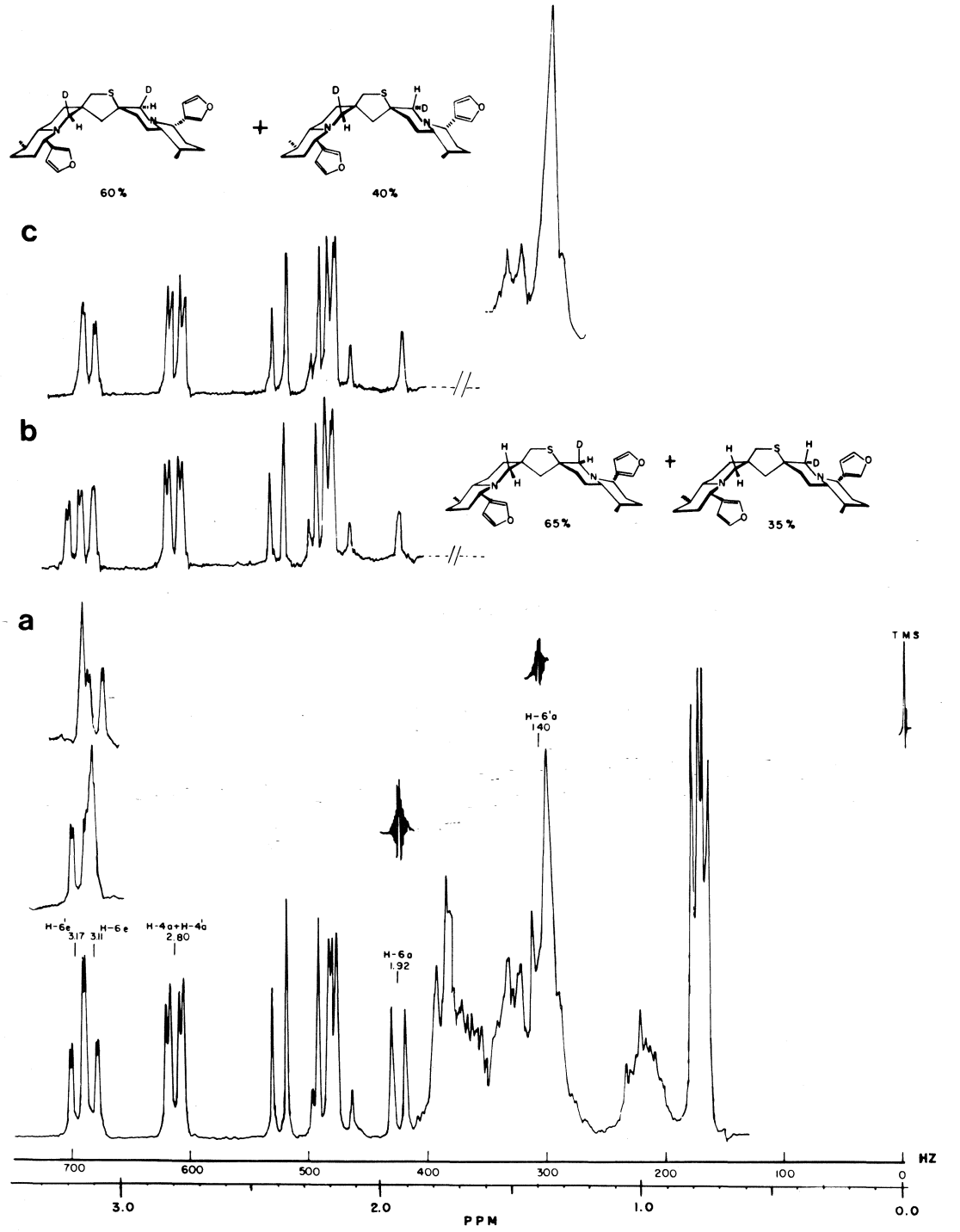

FIG. 1. The 220 MHz spectra in C_6D_6 of thiobinupharidine (a), the product of borodeuteride reduction of **1** (b), and the product of complete borodeuteride reduction of **4** (c).

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7.16 (nw m, 3 H, furan α H), 7.22 (nw m, 1 H, furan α H); mass spectrum (230°) *m/e* (relative intensity, %) 510 (15) M^+, 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 m*M*) was dissolved in 5 ml MeOH and treated with 2.23 ml of 0.019 *M* $HClO_4$ (0.0424 m*M*). 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) 3.62 (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 m*M*) was dissolved in 1 ml of anhydrous ethanol and treated with 1 ml 0.02 *M* (0.02 m*M*) 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 m*M*) was treated directly with 1 ml of 0.02 *M* (0.02 m*M*) perchloric acid and a few milliliters of water were added to ensure that all the glassy base was in contact with the perchloric acid solution. After 30 min the solution was evaporated to dryness under reduced pressure (50 °C bath) and the residue was dissolved in a minimum of boiling anhydrous MeOH. After storage at −5° for 2 days crystallization had occurred. The crystals were washed twice with anhydrous MeOH 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), 8.1–10.2 (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 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 h. The slurry thus obtained was stirred occasionally. The mixture was then diluted with water and extracted with CH_2Cl_2 (3 × 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, Activity 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 6 mg of thiobinupharidine from the column, $[\alpha]_D^{22} + 8°$ (CH_3OH); i.r. (CCl_4) 3.59–3.88 (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 $CDCl_3$): identical with an authentic sample of **2** (5), δ 0.92 (d, J = 5.6 Hz, 6 H, 2 × CH–CH_3), 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, 2 H, C-7-CH_2-C-7′), 2.33 (ABq, J_{AB} = 11.5 Hz, 2 H, CH_2-S-), 2.81 (q, J = 11.5 and 2 Hz, 1 H, H-6e), 2.94 (q, superimposed on complex multiplet, J = 11.5 and 2 Hz, H-6′e), 6.39 (nw m, $W_{1/2}$ = 6 Hz, 2 H, furan β H), 7.25 + 7.33 (4 H furan α H), irradiation at 2.94 collapses the doublet at 1.45 into a singlet, irradiation at 1.45 collapses signal at 2.94 into a broad singlet, irradiation at 1.72 collapses signal at 2.81 into a broad singlet, irradiation at 2.81 collapses doublet at 1.72 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 = 6 H), 1.40 (d, J = 11.5 Hz, H-6′a), 1.92 (d, J = 11.5 Hz, 1 H, H-6a), 2.18 (ABq, J_{AB} = 14.0 Hz, 2 H, C_7-CH_2-C_7′), 2.31 (ABq, J_{AB} = 11.5 Hz, 2 H, CH_2-S), 2.80 (q, J = 10.5 and 3.5 Hz, 2 H, H-4a + H-4′a), 3.11 (q, J = 11.5 and 2 Hz, 1 H, H-6e), 3.17 (q, J = 11.5 and 2.5 Hz, 1 H, H-6′e), 6.42 (nw m, 2 H, furan β-H), 7.14 (nw m, 4 H, furan α H); irradiation at 1.92 collapsed the quartet at 3.11 into a broad singlet but the quartet at 3.17 was unaffected; irradiation at 1.40 collapsed the quartet at 3.17 into a broad singlet but the quartet at 3.11 was unaffected; mass spectrum *m/e* (relative abundance): 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).

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 four portions of 25 mg each over a period of 24 h. 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 (Activity 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 mass spectrometry.

Eluant (*i*) gave no product, eluant (*ii*) gave pure monodeuteriothiobinupharidine M^+ 495 (R_f 0.51, yield, 8 mg), eluant (*iii*) gave predominantly the monoethyl

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ether of thionupharoline (2 mg), mass spectrum M^+ 538, intense ion at 222 (R_f 0.42, along with traces of slower and faster moving components), eluants (*iv*) and (*v*) gave thionupharoline (8 mg) (R_f 0.36). The thiobinupharidine-6*d*, 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, 6 H, 2 × $CHCH_3$), 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, 2 H, C-7-CH_2-C-7′), 2.33 (ABq, J_{AB} = 11.5 Hz, 2 H, CH_2-S), 2.79 (d, J = 2 Hz, 0.6 H, 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, $W_{1/2}$ = 7 Hz, 2 H, furan β H), 7.25 and 7.33 (nw m, 4 H, furan α H). There was a slight deuterium isotope shift in signals at 1.70 and 2.79 δ; 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, 6 H), 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, 2 H, C-7-CH_2-C-7′), 2.31 (ABq, J_{AB} = 11.5 Hz, 2 H, CH_2-S), 2.80 (q, J = 10.5 and 3.5 Hz, 2 H, H-4′a and H-4a), 3.10 (broadened singlet, 0.65 H, H-6e), 3.18 (q, J = 11.5 and 2 Hz, 1 H, H-6′e), 6.42 (nw m, 2 H, furan β H), 7.14 (nw m 4 H, furan α H); mass spectrum *m/e* (relative abundance): 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 from its Diperchlorate

The diperchlorate of 6,6′-dihydroxythiobinupharidine (35 mg), m.p. 225–227° (2), was stirred with 15 ml of 20% ammonia for 30 min. The resultant oily base was extracted with CH_2Cl_2 (5 × 10 ml). These extracts were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to give 22 mg of glassy residue. This was adsorbed on 4 g of neutral alumina (Activity II) and eluted with 100 ml of 20% ether in benzene to give 17.3 mg of 6,6′-dihydroxythiobinupharidine, R_f 0.18; $[\alpha]_D^{25}$ +80° (*c* 1.73, CH_2Cl_2) (LaLonde *et al.* (2) report +44.5°); u.v. λ_{max} (neutral 95% EtOH) 208 nm, ε 12 000; (acidic 95% EtOH), λ_{max}, 208 nm, ε 16 120; λ_{max_2}, 294 nm, ε 1890; 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-CH_3), 0.93 (d, J = 6.5 Hz, CH-CH_3, with 0.91, 6 H), 1.85 (ABq, J_{AB} = 14.5 Hz, C-7-CH_2-C-7′), 2.33 (br s, exchangeable with D_2O, OH), 2.44 (ABq, J_{AB} = 12 Hz, 2 H, CH_2-S), 3.58 (q, J = 6 and 8 Hz, 1 H, H-4′a), 3.75 (q, J = 7 and 7.5 Hz, 1 H, H-4a), 3.98 (s, 1 H, H-6), 4.24 (s, 1 H, H-6′), 6.35 (nw m, $W_{1/2}$ = 7 Hz, 2 H, furan β H), 7.25 (nw m, $W_{1/2}$ = 5 Hz, 2 H, furan α H), 7.35 and 7.37 (nw m, $W_{1/2}$ = 3 Hz, 2 H, 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 = 6 H), 2.11 (ABq, J_{AB} = 14.5 Hz, 2 H, C-7-CH_2-C-7′), 2.46 (ABq, J_{AB} = 12 Hz, 2 H, CH_2-S), 3.41 (q, J = 3.5 and 10.5 Hz, 1 H, H-4′), 3.86 (q, J = 4 and 10 Hz, 1 H, H-4), 4.23 (s, 1 H, H-6), 4.35 (s, 1 H, H-6′), 6.37 (s, $W_{1/2}$ = 3 Hz, 1 H, furan β H), 6.45 (s, $W_{1/2}$ = 3 Hz, 1 H, furan β H), 7.13 (nw m, $W_{1/2}$ = 3 Hz, furan α H), 7.29 (s, $W_{1/2}$ = 3 Hz, furan α H); mass spectrum (230°) *m/e* (relative abundance: 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 with Sodium Borohydride

6-6′-Dihydroxythiobinupharidine (100 mg) was dissolved in 10 ml methanol and treated with 100 mg sodium borohydride. The slurry was left to stand for 16 h, 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 (5 g Activity IV) and eluted with 100 ml of benzene to give 53 mg of thiobinupharidine identified by comparison of its p.m.r. spectrum and its melting point with an authentic sample of thiobinupharidine.

Reduction of 6,6′-Dihydroxythiobinupharidine with Sodium Borodeuteride

6,6′-Dihydroxythiobinupharidine (17 mg) was dissolved in 2 ml absolute ethanol and treated with 100 mg sodium borodeuteride. A further four portions of 25 mg each of borodeuteride were added over a period of 24 h. The slurry was stirred occasionally at room temperature 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, R_f 0.50 (intense), 0.27 → 0.42 (intense), and 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 (Activity 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, pure by t.l.c., R_f 0.5. Eluant (*ii*) gave a weak spot (R_f 0.5) and an intense spot with R_f 0.25 → 0.40. Eluants (*iii*) and (*iv*) gave intense spots with R_f 0.26 → 0.42. Eluant (*v*) showed traces of starting material at R_f 0.16. Eluants (*ii*), (*iii*), and (*iv*) were combined to give 9 mg which was adsorbed onto a further 2 g of neutral alumina (Activity II) using hexane as solvent. The column was eluted with 50 ml of *n*-hexane to give 1.5 mg (fraction *vi*), 100 ml of 20% ether in hexane to give 6.6 mg (fraction *vii*), and finally with 50 ml of ether to give less than 1 mg (fraction *viii*). Fraction *vii* showed only one spot on t.l.c., R_f 0.34 (center of spot). This was the sample used for spectroscopic analysis and deduced to be 6-hydroxy-6′-deuteriothiobinupharidine; fractions *vi* and *viii* were not further examined.

Fraction i, Thiobinupharidine-6,6′-d_2

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, 6 H, 2 × $CHCH_3$), 1.45 (H-6′a, essentially disappeared), 1.70 (br s, superimposed on envelope, H-6a), 1.90 (ABq, J_{AB} = 14 Hz, 2 H, C-7-CH_2-C-7′), 2.33 (ABq, J_{AB} = 11.5 Hz, 2 H, -CH_2-S), 2.79 (d, J = 2 Hz, ~0.6 H, 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, $W_{1/2}$ = 7 Hz, 2 H, furan β H), 7.25 + 7.33 (nw m, 4 H, furan α H); 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, 6 H with 0.78), 1.40 (H-6′a, essentially disappeared), 1.93 (s, 0.4 H, H-6a), 2.18 (ABq, J_{AB} = 14.0 Hz, 2 H, C-7-CH_2-C-7′), 2.31 (ABq, J_{AB} = 11.5 Hz, 2 H, CH_2-S), 2.80 (q, J = 10.5 and 3.5 Hz, 2 H, H-4′a + H-4a),

3.10 (poorly resolved doublet $J \simeq 2$ Hz, 0.6 H, H-6e), 3.16 (poorly resolved doublet, $J \simeq 2$ Hz, 1 H, H-6′e); mass spectrum *m/e* (relative abundance): 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

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 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, 6 H, 2 × $CHCH_3$), 2.21 (ABq, $J_{AB} = 12$ Hz, 2 H, CH_2-S), 2.26 (s, 1 H, exchangeable with D_2O, OH), 2.90 q, $J = 5.6$ and 8 Hz, H-4′a), 2.92 (br s superimposed on q at 2.90, total 2 H, H-6′e), 3.70 (q, $J = 7.5$ and 7 Hz, 1 H, H-4), 3.97 (s, 1 H, H-6), 6.34 (nw m, $W_{1/2} = 7$ Hz, 2 H, furan β H), 7.21 (nw m, 1 H, furan α H), 7.30 (nw m, $W_{1/2} = 4$ Hz, 3 H, furan α H); p.m.r. 220 MHz (C_6D_6): δ 0.75 (two superimposed doublets, $J = 6.0$ Hz, 6 H, 2 × $CHCH_3$), 2.11 (ABq, $J_{AB} = 12$ Hz, 2 H, CH_2-S), 2.42 [br s, 1 H (exchangeable with D_2O), OH] 2.75 (q, $J = 3.5$ and 10.5 Hz, 1 H, H-4′), 3.15 (d, $J = 2.0$ Hz, 1 H, H-6′e), 3.86 (q, $J = 10$ and 4 Hz, 1 H, H-4), 4.24 (s, 1 H, H-6), 6.38 (nw m, $W_{1/2} = 3$ Hz, 1 H, furan β H), 6.44 (nw m, 1 H, $W_{1/2} = 3$ Hz, furan β H), 7.13 (nw m, 3 H, furan α H), 7.25 (nw m, $W_{1/2} = 3$ Hz, 1H, furan α H); mass spectrum (200°) *m/e* (relative abundance): 511 (72), 510 (9), 495 (12), 494 (52), 493 (90), 482 (6), 478 (4), 465 (7), 460 (4), 446 (6), 432 (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).

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1. J. T. Wróbel. Rocz. Chem. **44**, 457 (1970).
2. R. T. LaLonde, C. F. Wong, and W. P. Cullen. Tetrahedron Lett. 4477 (1970).
3. R. T. LaLonde and C. F. Wong. Phytochemistry, **11**, 3305 (1972).
4. G. I. Birnbaum. Tetrahedron Lett. 4149 (1965).
5. J. T. Wróbel, B. Bobeszko, T. I. Martin, D. B. MacLean, N. Krishnamachari, and C. Calvo. Can. J. Chem. **51**, 2810 (1973).
6. R. T. LaLonde, C. F. Wong, and K. C. Das. J. Am. Chem. Soc. **95**, 6342 (1973).
7. C. F. Wong and R. T. LaLonde. J. Org. Chem. **38**, 3225 (1973).
8. K. Oda and H. Koyama. J. Chem. Soc. B, 1450 (1970).
9. C. F. Wong, E. Auer, and R. T. LaLonde. J. Org. Chem. **35**, 517 (1970).
10. R. T. LaLonde, E. Auer, C. F. Wong, and V. P. Muralidharan. J. Am. Chem. Soc. **93**, 2501 (1971).
11. R. T. LaLonde, J. T. Woolever, E. Auer, and C. F. Wong. Tetrahedron Lett. 1503 (1972).
12. F. Bohlmann, E. Winterfeldt, P. Studt, H. Laurent, G. Boroschewski, and K. Kleine. Chem. Ber. **94**, 202 (1961).
13. I. Kawasaki, S. Matsutani, and T. Kaneko. Bull. Chem. Soc. Japan, **36**, 1474 (1963).
14. C. F. Wong and R. T. LaLonde. Phytochemistry, **9**, 659 (1970).
15. O. Achmatowicz and J. T. Wróbel. Tetrahedron Lett. 129 (1964).
16. R. T. LaLonde, C. F. Wong, and H. G. Howell. J. Org. Chem. **36**, 3703 (1971).
17. M. Murrier. Bull. Soc. Chim. Fr. **33**, 846 (1951).
18. B. L. Loev and M. M. Goodman. Chem. Ind. 2026 (1967).
19. B. L. Loev and K. M. Snader. Chem. Ind. 15 (1965).
20. K. Biemann. Mass spectrometry: organic chemical applications. McGraw-Hill Book Co., Inc., New York. 1962.

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