Phytochemistry, Vol. 25, No. 9, pp. 2227–2231, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

SULPHOXIDES OF THIOBINUPHARIDINE THIOHEMIAMINALS FROM *NUPHAR LUTEA*

AGNIESZKA IWANOW, KRYSTYNA WOJTASIEWICZ and JERZY T. WRÓBEL

University of Warsaw, Department of Chemistry, ul. Pasteura 1, 02-093 Warszawa, Poland

(*Revised received* 24 *September* 1985)

Key Word Index—*Nuphar lutea*; Nymphaeaceae; *Nuphar* alkaloids; sulphoxides of thiobinupharidine; thiohemiaminals; reduction; stereochemistry; NMR.

Abstract—From the rhizomes of *Nuphar lutea* four new alkaloids were isolated. Their structures were established as *syn*-6-hydroxythiobinupharidine sulphoxide, *syn*-6′-hydroxythiobinupharidine sulphoxide, *syn*-6,6′-dihydroxy thiobinupharidine sulphoxide and *anti*-thiobinupharidine sulphoxide.

INTRODUCTION

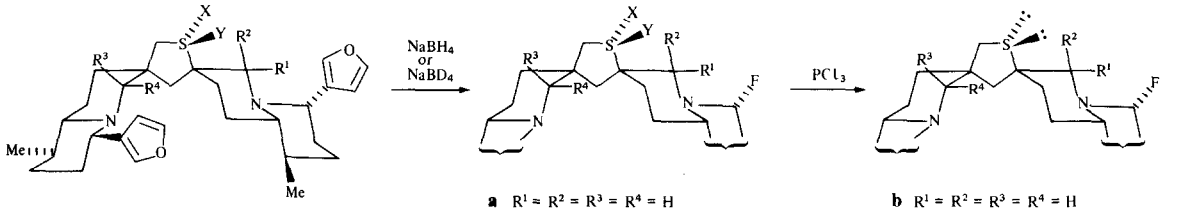
Previously isolated sulphur-containing *Nuphar* alkaloids were recognized as sulphides, thiohemiaminals and sulphoxides [1, 2] showing increasing polarity in the chromatography process. In most polar fractions remaining after isolation of the three mentioned types of alkaloids a new group of *Nuphar* alkaloids was isolated which were found to be sulphoxides of thiochemiaminals.

RESULTS AND DISCUSSION

Two of the new compounds **1** and **3** were isomeric, $C_{30}H_{42}O_4N_2S$ (**1**) amorphous $[\alpha]_D^{20} = +3.4°$, **2** mp 198–204°, $[\alpha]_D^{20} = +9.7°$, whereas the third one (**3**) had a molecular formula $C_{30}H_{42}O_5N_2S$, mp 160–165° and exhibited a rotation of $[\alpha]_D^{20} = +39°$. The ^{1}H NMR spectra of the new alkaloids closely resembled those of previously reported *Nuphar* thiohemiaminals. [1, 3] The mass spectra, in spite of the absence or very low intensity of molecular ions, were in agreement with the previously observed fragmentations for *Nuphar* thiohemiaminals and or sulphoxides [4–6]. Moreover, the IR spectra suggested the presence of S → O and OH groups in all three compounds.

The structures of the novel alkaloids was determined by means of $NaBH_4$ reduction, followed by PCl_3 treatment (Scheme 1). The resulting product which in each case was thiobinupharidine (TBN) **b** supplied the evidence for the total skeleton of **1**, **2** and **3**, and suggested that the compounds isolated are sulphoxides of thiohemiaminals derived from thiobinupharidine. Spectroscopic characteristics of compound **a** (TBN sulphoxide) obtained by $NaBH_4$ reduction of **1**, **2** and **3**, which resembled that previously reported for *syn*-TBN sulphoxide [7] suggested the presence of *syn* configuration of compounds **1–3**.

Further structural determination required the establishment of the number and positions of the hydroxyl groups, their stereochemistry, as well as the stereochemistry of the sulphinyl group. The M_r of the $NaBH_4$ reduction products (which was the same for all compounds derived from **1–3**) was 510 as determined by elemental analysis and mass spectrometry.



1 R^1 = OH, R^2 = R^3 = R^4 = H; X = O, Y = electron pair

2 R^1 = R^2 = R^4 = H, R^3 = OH; X = O, Y = electron pair

3 R^1 = R^3 = OH, R^2 = R^4 = H; X = O, Y = electron pair

a R^1 = R^2 = R^3 = R^4 = H; X = O, Y = electron pair

1a R^1 = D, R^2 = R^3 = R^4 = H; X = O, Y = electron pair

2a R^1 = R^2 = R^4 = H, R^3 = D; X = O, Y = electron pair

3a R^1 = R^3 = D, R^2 = R^4 = H; X = O, Y = electron pair

4 R^1 = R^2 = R^3 = R^4 = H; X = electron pair, Y = O

b R^1 = R^2 = R^3 = R^4 = H

1b R^1 = D, R^2 = R^3 = R^4 = H

2b R^1 = R^2 = R^4 = H, R^3 = D

3b R^1 = R^3 = D, R^2 = R^4 = H

Scheme 1.

Positions and number of hydroxyl groups were determined by examination (^{1}H NMR and mass spectra) of deuterated thiobinupharidines obtained by consecutive reductions of compounds **1–3** with $NaBD_4$ and PCl_3. The course of the $NaBH_4$ reduction showed that **1–3** are carbinolamines. This together with the spectroscopic data for $NaBH_4$ and $NaBD_4$ reduction products excluded other positions than 6 or 6′ for the hydroxyl and hence simplified the problem discussed [1, 3, 4, 5].

The following differences were observed between the mass spectra and ^{1}H NMR (C_6D_6) spectra of thiobinupharidine and deuterated thiobinupharidines **1b**, **2b** and **3b** obtained from **1**, **2** and **3**. In **1b** ([6^{2}H]Tb) the ion at *m/z* 178 drops from 100% to 75% in relative intensity, instead an 100% intensity signal appears at *m/z* 179. In the ^{1}H NMR (C_6D_6) the doublet corresponding to the C-6 axial proton at δ1.92 was not observed whereas the C-6 equatorial proton signal appears as a broad singlet at δ3.10. In **2b** ([6′^{2}H]Tb) the ion at *m/z* 178 was unchanged and the ion at *m/z* 178 was absent. In the ^{1}H NMR (C_6D_6) the absence of a signal for the axial proton at δ1.40 was noticed but the equatorial proton at C-6′ appeared as a broad singlet at δ3.18. In **3b** ([6.6′^{2}H]Tb) the ion at *m/z* 178 shows low intensity whereas the ion at *m/z* 179 appears as 100%. Additionally the ion at *m/z* 230 is shifted to *m/z* 231. In the ^{1}H NMR (C_6D_6) spectrum no signals for axial protons were observed at C-6 or C-6′ but instead the equatorial protons at these carbons gave broad singlets at δ3.10 and 3.18, respectively. Additionally in the mass spectra of **1b**, **2b** and **3b** the main fragments derived from rings A and A′ at *m/z* 136, 107, 94 and 81 remained unchanged. This indicated the position C-6 or C-6′ (in **1** or **2**) or both (in **3**) as the position of the hydroxyl group.

An attempt was made to establish the configuration of the hydroxyl group in alkaloid **1** and 6-OH-TBN obtained fron *Nuphar* species. ^{1}H NMR spectroscopy is not sufficiently informative for this purpose. Instead ^{13}C NMR spectroscopy has been used for the analysis of α, β and γ effects caused by the hydroxyl group and its stereochemistry. On the basis of general rules established earlier in this field [8], the axial hydroxyl group would be expected to cause significant upfield shifts of carbons C-4, C-10 and C-8. These carbons would be in this case in the γ gauche conformation relative to C-17. The latter conformation, being γ *trans* would then hardly affect its chemical shift.

Otherwise the equatorial C-6 hydroxyl group in the 6-OH-TBN skeleton (being γ gauche to the carbons C-17 and C-4 and *trans* to C-10) would cause a much greater upfield shift for C-17 and C-4 than for C-10. Data observed in the ^{13}C NMR spectra for 6-OH-TBN and its *syn* and *anti* sulphoxides are listed in Table 1. ^{13}C NMR chemical shift increments for C-4, C-10 and C-8 are of the order of 6–11 ppm. No increment is observed for C-17. This suggests an axial conformation of the hydroxyl group in both 6-OH-TBN and its sulphoxide **1**.

Stereochemical determination of sulphoxides of *Nuphar* alkaloids has been previously the subject of interest in our laboratory [9–11]. The present results are based on the anisotropic effect of the sulphinyl bond on the C-6 and C-6′ protons $\Delta\delta = \delta^{SO}_{Hi} - \delta^{S}_{Hi}$. In Table 4 are shown the chemical shifts for diagnostic protons (H-6$_{eq}$ and H-6′$_{eq}$) of new alkaloids **1**, **2** and **3** and of isomeric TBN sulphoxides (*syn* and *anti* synthetic models). To identify the signals of proton on C-6 and C-6′ the ^{1}H NMR spectra of deuterated sulphoxides of TBN **1a** [6^{2}H]TBN, **2a** [6′^{2}H]TBN, **3a** [6,6′^{2}H]TBN and of TBN sulphoxides **a** and **4** were analysed. The axial protons on C-4 and C-4′ and one of the unshielded protons on C-6 or C-6′ in the ^{1}H NMR ($CDCl_3$) spectra of both TBN sulphoxides were found as the three proton multiplet at δ2.80–3.20. The remaining shielded protons on C-6 and C-6′ (*anti* to the sulphinyl group) were found as a low intensity doublet of doublets at *ca* δ2.5 (J = 12.5 and 2.5 Hz). In the ^{1}H NMR (C_6D_6) spectra the one proton doublet of doublets (signal of equatorial proton on C-6 or C-6′ for *syn* sulphoxide TNB **a** was found at δ3.25) (J = 12.5 and 2.5 Hz) and in *anti* sulphoxide TBN **4** at δ3.17 (J = 12.5 and 2.5 Hz).

Dreiding models of *syn* **a** and *anti* **4** TBN sulphoxides suggest that the collision complex with benzene shields the proton on C-6′ in *syn* TBN sulphoxide. Therefore the doublet of doublets found in the spectrum of **a** (TBN sulphoxide) at δ3.25 (J = 12.5 and 2.5 Hz; slightly deshielded in comparison with equatorial protons on C-6 and C-6′ in TBN) could be assigned to the C-6 equatorial proton of the *syn* isomer. These deductions are confirmed by (i) the ^{1}H NMR (C_6D_6) spectra of *syn*[6^{2}H]TBN

Table 1. ^{13}C NMR chemical shifts (δ_{Ci}) and increments caused by hydroxyl group ($\Delta\delta_{Ci} = \delta^{OH}_{Ci} - \delta^{H}_{Ci}$) for indicator carbon atoms in 6 OH TBN and its '*syn*' and '*anti*' sulphoxides

	C-4		C-10		C-8		C-17	
Compound	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
TBN	59.68		68.61		39.48		45.07	
6 OH TBN	53.79	−5.89	58.20	−10.41	31.90	−7.58	44.01	−1.06
syn TBN sulphoxide **a**	59.72 or 60.07		68.83		31.72		41.77 or 40.22	
syn 6 OH TBN sulphoxide **1**	53.79	−5.93 or −6.28	58.20	−10.63	25.92	−5.80	40.57 or 39.80	−0.80 or −0.42
anti TBN sulphoxide **4**	58.00		66.31		28.40		41.91 or 38.53	
anti 6 OH TBN sulphoxide (synthetic)	63.53	−5.47	57.86 or 59.68	−8.45 or −6.63	21.75	−7.50	41.22	−0.69 or −2.69

sulphoxide **1a** in which the signal at δ3.25 appears as a broad singlet indicating again the axial conformation of deuterium at C-6; (ii) the ^{1}H NMR spectra of *syn*[6^{2}H]TBN sulphoxide **2a** in which the doublet at δ3.25 (J = 12.5 and 2.5 Hz) is retained.

By spin decoupling it was possible to identify the C-6 axial proton in *syn* TBN sulphoxide at δ2.17 (*d*, J = 12.5 Hz). Another deduction, which can be made using Dreiding models in respect to the changes of chemical shifts by the benzene collision complex, was the probable shielding of the C-6 equatorial proton in *anti* TBN sulphoxide **4**. The one proton signal found at δ3.17 (J = 12.5 and 2.5 Hz) should belong to the C-6′ equatorial proton in *anti* TBN sulphoxide. In Table 2 chemical shifts increments $\Delta\delta_{Hi} = \delta^{SO}_{Hi} - \delta^{S}_{Hi}$ on protons at C-6 and C-6′ are shown.

The anisotropy effect of the sulphinyl group on the C-6 and C-6′ protons in compounds **1**, **2** and **3** as well as the effect on these protons in *syn* TBN sulphoxide prepared from TBN was of the same sign, as that demonstrated by significant deshielding of the proton on C-6 in all compounds discussed. This establishes the configuration around sulphur in all three isolated thiohemiaminals of TNB sulphoxides as '*syn*' to the C-6–C-7 bond. Since the absolute configuration of TBN is known [13] the following assignments could be made for the absolute configuration on sulphur in *syn* TBN sulphoxide **a**, which is *S*, and consequently the *S* configuration could be ascribed to sulphoxides **1**, **2** and **3**.

It has been demonstrated in the case of **1**, that ^{13}C NMR spectroscopy can be used for configurational determination around the sulphur atom in sulphoxides of thiohemiaminals provided that both isomers (*syn* and *anti*) in the basic skeleton are available and the chemical shifts observed for the two compounds can be compared with the examined one. The results obtained shown in Table 3 confirm the '*syn*' configuration of sulphoxide **1**.

It should be noted that all sulphoxides of thiohemiaminals which were isolated from *N. luteum* were of the '*syn*' configuration, no matter the stereochemistry of the skeleton. This is a strong argument that these compounds are not artefacts. All the sulphur-containing *Nuphar* alkaloids when oxidized *in vitro* formed mixtures of *syn* and *anti* sulphoxides.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR (100 MHz) and ^{13}C NMR (22.5 MHz) spectra were determined in $CDCl_3$ and in C_6D_6 using TMS as int. standard. MS (70 eV) direct insertion. IR: $CHCl_3$ and KBr. UV: EtOH and EtOH + conc HCl. Separation by CC was carried out using Al_2O_3 act. II or III (Fluka) and silica gel (MN 100–200 mesh). TLC: (1) silica gel hexane Me_2CO (4:1.5), (2) Al_2O_3–C_6H_6 or $CHCl_3$.

Extraction and isolation. Air dried, powdered rhizome of *N. lutea* (L) Sibth and Sm. (10 kg) (voucher specimens are on deposit at the University of Warsaw, Department of Chemistry Herbarium and were identified by Dr. H. Tomaszewicz (Department of Phytogeography, University of Warsaw) was soaked in 10% NH_3 and extracted with CH_2Cl_2 at room temp. and solvent removed under red. pres. The dark sticky residue (250 g) was dissolved in 1 l. HOAc and poured into 5 l. of H_2O. The ppt was filtered off. The filtrate was extracted with Et_2O and the aq. soln adjusted to pH 10 (NH_4OH or NaOH, and K_2CO_3) and extracted with Et_2O. Solvent was removed under red. pres. to yield crude alkaloid mixture (AM 60 g). This was dissolved in C_6H_6, adsorbed onto an Al_2O_3 column (act. II, 1.5 kg) and eluted with hexane, C_6H_6, Et_2O, $CHCl_3$ and MeOH. The $CHCl_3$ fraction (4 g) was adsorbed onto silica gel 200 g and eluted with petrol (60–80°)–Me_2CO (1–20%) to give: fraction A (10% Me_2CO) 1.2 g, fraction B (15% Me_2CO) 0.5 g and fraction C (20% Me_2CO) 1 g. The eluates were monitored by silica gel TLC in hexane–Me_2CO (4:1.5). Rechromatography of fraction A 1.2 g on silica gel 100 g with hexane–Me_2CO (4:1) gave anti-

Table 2. ^{1}H NMR chemical shifts (δ_{Hi}) and a sulphoxidation increments ($\Delta\delta_{Hi} = \delta^{SO}_{Hi} - \delta^{S}_{Hi}$) for indicator carbon atoms in TBN sulphoxides and thiohemiaminals of TBN sulphoxides

		C-6$_{eq}$		C-6$_{ax}$		C-6′$_{eq}$		C-6′$_{ax}$	
Compound	Solvent	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
TBN **b**	$CDCl_3$	2.80				2.94			
	C_6D_6	3.11		1.92		3.15		1.40	
syn TBN	$CDCl_3$	2.97	+0.19			2.48	−0.46		
sulphoxide **a**	C_6D_6	3.25	+0.12	2.17	+0.25	2.75	−0.40		
anti TBN	$CDCl_3$					2.97	+0.03		
sulphoxide **4**	C_6D_6					3.17	+0.02	1.37	−0.03
6 OH TBN	$CDCl_3$	3.97				2.92			
	C_6D_6	4.25				3.18			
syn 6 OH TBN	$CDCl_3$	4.52	+0.55			2.47	−0.35		
sulphoxide **1**	C_6D_6	4.80	+0.55			2.75	−0.38		
6′ OH TBN	$CDCl_3$	2.83				4.25			
	C_6D_6	3.09				4.33			
syn 6′ OH TBN	$CDCl_3$	3.00	+0.17			3.92	−0.33		
sulphoxide **2**	C_6D_6	3.25	+0.16			4.02	−0.33		
6.6′2 OH TBN	$CDCl_3$	4.00				4.24			
	C_6D_6	4.23				4.35			
syn 6.6′2 OH TBN	$CDCl_3$	4.52	+0.52			3.90	−0.34		
sulphoxide **3**	C_6D_6	4.80	+0.57			4.00	−0.35		

Table 3. ^{13}C NMR chemical shifts δ_{Ci} and sulphoxidation increments ($\Delta\delta_{Ci} = \delta^{SO}_{Ci} - \delta^{S}_{Ci}$) for indicator carbon atom in *syn* **a** and *anti* **4** TBN sulphoxides and *syn* **1** and *anti* 6 OH TBN sulphoxides

Compounds	C-6		C-6		C-8		C-8′	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
TBN	65.79		63.19		39.48		37.32	
syn TBN sulphoxide **a**	56.90	−8.89	64.23	+1.04	31.72	−7.76	40.22	+2.90
anti TBN sulphoxide **4**	59.20	−6.59	64.27	+1.08	28.40	−11.08	41.91	+4.59
6 OH TBN	84.60		62.80		31.90		37.40	
syn 6 OH TBN sulphoxide **1**	81.26	−3.34	64.01	+2.2	25.92	−5.98	39.84 or 40.57	+2.44 or +3.17
anti 6 OH TBN sulphoxide (synthetic)	83.52	−1.08	63.8	+1.0	21.75	−10.15	41.22	+3.82

thiobinupharidine sulphoxide (**4**), glass like compound TLC R_f (1) = 0.5; $[\alpha]_D = -64°$ (95% EtOH; *c* 0.73); UV λ^{EtOH}_{max}: end absorption; IR $\nu^{CHCl_3}_{max}$ cm^{-1}: 2600–2800 (Bohlmann bands), 1030 (S → O), 880 (furan), 610. MS *m/z* (rel. int.): 510 $[M]^+$ (6), 493 $[M - OH]^+$ (100), 357 (4), 230 (54), 178 (6.6), 176 (1.8), 136 (3.1), 107 (16), 94 (28), 81 (12), 79 (14). ^1H NMR (100 MHz, C_6D_6): δ0.8 (6H, *d*, *J* = 2.5 Hz, 2 × CHMe), 1.37 (1H, *d*, *J* = 12.5 Hz, H-6′$_{ax}$), 2.7 (2H, *m*, H-4 and H-4′), 3.17 (1H, *dd*, *J* = 12.5, 2.5 Hz, H-6′$_{eq}$), 6.4 (2H, *m*, β-furan), 7.3 (4H, *m*, α-furan). Irradiation at 3.17 collapsed the doublet at 1.37 into a singlet; irradiation at 1.37 collapsed the doublet at 3.17 into a broad singlet. Found: C, 70.50, H, 8.3; N, 5.40. Calc. for $C_{30}H_{42}O_3N_2S$: C, 70.46; H, 8.20; N, 5.20; S, 6.39. syn-6-Hydroxythiobinupharidine sulphoxide (**1**), TLC R_f(1) = 0.4; $[\alpha]^{20}_D = +3.04$ ($CHCl_3$, *c* 0.85); UV λ^{EtOH}_{max}: end absorption; UV $\lambda^{EtOH+conc\ HCl}_{max}$: end absorption; IR $\nu^{CHCl_3}_{max}$ cm^{-1}: 3300–3500 (bonded OH), 2600–2800 (Bohlmann bands), 1030 (S → O), 880 (furan), 610. MS *m/z* (rel. int.): 526 $[M]^+$ (0.5), 525 (0.4), 524 (0.7), 508 (17.6), 492 (17), 491 (26), 355 (3), 230 (100), 228 (28), 178 (12), 176 (13.5), 136 (8), 107 (22), 94 (41), 81 (19), 79 (28). ^1H NMR (100 MHz, $CDCl_3$): δ0.96 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.75 (1H, *dd*, *J* = 12.5, 2.5 Hz, H-6′$_{eq}$), 2.5 (2H, *br s*, CH_2S). 2.90 (1H, *m*, H-4′), 3.75 (1H, *m*, H-4), 4.15 (1H, *m*, OH exchangeable on addition of D_2O), 4.52 (1H, *s*, H-6, sharpens on addition of D_2O) 6.38 (2H, *m* $W_{1/2}$ = 5 Hz, furan β-H), 7.2–7.4 (4H, *m*, furan αH). ^1H NMR (100 MHz, C_6D_6): δ0.8 (3H, *d*, *J* = 5 Hz, CHMe), 0.82 (3H, *d*, *J* = 5 Hz, CHMe), 2.6–2.8 (2H, *m*, H-6′$_{eq}$ + H-4′), 3.90 (1H, *m*, H-4), 4.80 (1H, *m*, H-6, sharpens on addition of D_2O), 5.80 (1H, *m*, exchangeable on addition of D_2O), 6.35 (1H, *m*, $W_{1/2}$ = 4 Hz, furan βH), 6.5 (1H, *m*, $W_{1/2}$ = 2 Hz, furan βH), 7.15 (3H, *m*, $W_{1/2}$ = 5 Hz, furan αH), 7.68 (1H, *m*, $W_{1/2}$ = 2 Hz, furan αH). Found: C, 68.50; H, 7.81; N, 5.30. $C_{30}H_{42}O_4N_2S$ requires C, 68.46; H, 7.98; O, 12.15; N, 5.32; S, 6.08.

Rechromatography of eluant **B** (0.8 g) on silica gel (50 g) with hexane–Me_2CO (0–50%) gave fraction 20% Me_2CO (200 mg) for which after crystallization from C_6H_6. *syn* 6′-Hydroxythiobinupharidine sulphoxide (**2**) was obtained. Mp 198–204°, R_f (1) = 0.2; $[\alpha]^{20}_D = +9.7°$ (HCl_3; *c* 0.79): UV $\lambda^{EtOH\ and\ EtOH\ +conc.\ HCl}_{max}$: end absorption; IR $\nu^{CHCl_3}_{max}$ cm^{-1}: 3620 (OH), 2600–2880 (Bohlmann band), 1030 (S → O), 880 (furan), 610; MS *m/z* (rel. int.): 526 $[M]^+$ (0.00), 509 (10), 492 (65), 230 (100), 228 (19), 178 (7), 176 (6), 136 (2), 107 (16), 94 (20), 81 (11), 79 (14). ^1H NMR (100 MHz $CDCl_3$): δ0.92 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.18 (2H, AB*q*, centred at 2.18, *J* = 12 Hz, CH_2S), 3.00 (2H, *m*, H-4 + H-6$_{eq}$), 3.4–3.7 (1H, *m*, H-4′), 3.8 (1H, OH exchangeable on addition of D_2O), 3.92 (1H, *br s* sharpens on addition of D_2O, H-6′$_{eq}$), 6.40 (2H, *m*, $W_{1/2}$ = 5 Hz, furan βH), 7.2–7.5 (4H, *m*, furan αH). ^1H NMR (100 MHz, C_6D_6): δ0.85 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.35 (2H, AB*q*, *J* = 12 Hz, CH_2S), 2.8 (1H, *m*, H-4), 3.25 (1H, *dd*, *J* = 12.5, 2.5 Hz, H-6$_{eq}$), 3.7 (1H, *m*, H-4′), 3.92 (1H, *br s*, sharpens on addition of D_2O H-6′$_{eq}$), 6.45 (2H, *m*, $W_{1/2}$ = 5 Hz, furan βH), 7.0–7.5 (4H, *m*, furan αH). Found: C, 68.20; H, 7.80; N, 5.40. $C_{30}H_{42}O_4N_2S$ requires: C, 68.46; H, 7.98; N, 5.32; S, 6.08.

Fraction C gave a crystalline alkaloid *syn* 6,6′-dihydroxythiobinupharidine sulphoxide (**3**). Mp 160–165° (C_6H_6), $[\alpha]^{20}_D$ = +39 ($CHCl_3$ *c* = 0.79) UV $\lambda^{EtOH\ and\ EtOH\ +\ conc\ HCl}_{max}$: end absorption; IR $\nu^{CHCl_3}_{max}$ cm^{-1}: 3620 (OH), 3200–3500 (bonded OH), 1030 (S → O), 880 (furan), 610. MS *m/z* (rel. int.): 542 $[M]^+$ (0.1), 524 (0.8), 522 (0.5), 509 (1), 507 (1.2), 494 (1.3), 493 (1.8), 492 (1.4), 490 (1.8), 446 (22), 445 (10), 230 (100), 229 (34), 216 (20), 178 (2.8), 176 (5), 136 (10), 107 (20), 94 (38), 81 (21), 79 (22). ^1H NMR (100 MHz, $CDCl_3$): δ0.96 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.4 (1H, OH exchangeable on addition of D_2O), 2.64 (2H, AB*q*, *J* = 12 Hz, CH_2S), 3.4–3.9 (2H, *m* H_4 and H4′), 3.7 (1H, *d*, *J* = 5 Hz, OH exchangeable on addition D_2O), 3.90 (1H, *d*, *J* = 3 Hz, sharpens on addition of D_2O, H-6′), 4.52 (1H, *d*, *J* = 5 Hz, sharpens on addition of D_2O, H-6), 6.4 (2H, *m*, $W_{1/2}$ = 5 Hz, furan βH), 7.4–7.5 (4H, *m*, furan αH). ^1H NMR (100 MHz, C_6D_6): δ0.90 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.62 (2H, AB*q*, J_{AB} = 12 Hz, CH_2S), 3.7 (2H, *m*, H-4 and H-4′), 4.0 (1H *br s*, sharpened on addition of D_2O, H-6′), 4.80 (1H, *br s* sharpens on addition of D_2O, H-6), 6.55 (2H, *m*, furan βH), 7.2 (2H, *m*, furan αH), 7.43 (1H, *m*, furan αH), 7.50 (1H, *m*, furan αH). Found: C, 67.00; H, 7.65; N, 5.20. $C_{30}H_{42}O_5N_2S$ requires: C, 66.44; H, 7.74; N, 5.16; S, 5.90.

Reduction of sulphoxides of thiohemiaminals **1**, **2** *and* **3** *with* $NaBH_4$ *or* $NaBD_4$. *General procedure.* Thiohemiaminal sulphoxide **1**, **2** or **3** (50–100 mg) was dissolved in 50 ml of EtOH and treated with 200 mg $NaBH_4$ or $NaBD_4$ in four portions over a period of 24 hr. EtOH was evapd under red. pres. The resulting residue was chromatographed on CC (silica gel, 20 g) using hexane–Me_2CO (0–50%). Eluants were examined by TLC, MS and ^1H NMR. *syn*-Thiobinupharidine sulphoxide **a** obtained from **1**, **2** and **3**. $[\alpha]^{20}_D = -8.8°$ (95% EtOH; *c* 1.62); IR $\nu^{CHCl_3}_{max}$ cm^{-1}: 2600–2800 (Bohlmann bands), 1030 (S → O), 880 (furan), 610. MS *m/z* (rel. int.): 510 $[M]^+$ (20), 493 $[M - OH]^+$ (100), 492 $[M - H_2O]^+$ (40), 357 (5), 280 (1), 262 (2), 230 (95), 178 (10), 176 (18), 136 (5), 107 (26), 94 (35). ^1H NMR (100 MHz, $CDCl_3$): δ0.95 (6H, *d*, *J* = 5 Hz, 2 × CHMe) 2.5 (2H, *s*, CH_2S), 3.0 (2H, *m*, H-4 and H-4′), 2.97 (1H, *dd*, *J* = 12.5, 2.5 Hz, H-6$_{eq}$), 6.38 (2H, *m*, furan βH), 7.25 (4H, *m*, furan αH).

^{1}H NMR (100 MHz, C_6D_6): δ0.80 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.13 (1H, *d*, *J* = 12.5 Hz, H-6_{ax}), 2.6–2.95 (3H, *m*, H-$6'_{eq}$, H-4 and H-4′), 3.25 (1H, *dd*, *J* = 12.5, 2.5 Hz, H-6_{eq}), 6.35 (2H, *m*, furan βH), 7.1 (4H, *m*, furan αH), irradiation at 3.25 collapsed *d* at 2.17 into *s*, irradiation at 2.17 collapsed *d* at 3.25 into *br s*. Found: C, 70.50; H, 8.30; N, 5.40. $C_{30}H_{42}O_3N_2S$ requires: C, 70.45; H, 8.20; N, 5.20; S, 6.39.

syn[6^2H]*Thiobinupharidine sulphoxide* **1a** *from* **1**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2600–2800 (Bohlmann bands), 1030 (S → O), 880 (furan), 610; MS *m/z* (rel. int.): 511 [M]$^+$ (10), 494 [M − OH]$^+$ (100), 493 (67), 492 (21), 358 (3), 231 (39), 230 (66), 179 (5), 178 (4), 177 (2), 176 (2.3), 136 (5), 107 (18), 94 (30), 81 (16), 79 (18). ^{1}H NMR (100 MHz, C_6D_6): δ0.82 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.6 − 2.9 (2H, *m*, H-4, H-4′), 3.25 (0.9H, *br s*, H-6_{eq}), 6.4 (*m*, 1H, furan βH), 6.45 (1H, *m*, furan βH), 7.1–7.2 (4H, *m*, furan αH). syn[$6'^2$H]*Thiobinupharidine sulphoxide* **2a** *from* **2**. IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 2600–2800 (Bohlmann bands), 2040 (C-D), 1030 (S → O), 880 (furan), 610. MS *m/z* (rel. int.): 511 [M]$^+$ (10), 510 (4), 494 (82), 493 (42), 358 (3), 230 (100), 179 (5), 178 (8), 176 (19), 136 (5), 107 (25), 94 (82), 81 (22), 79 (29). ^{1}H NMR (100 MHz, C_6D_6): δ0.81 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.38 (2H, AB*q*, *J* = 12 Hz, CH_2S), 2.17 (1H, *d*, *J* = 12.5 Hz, H-6_{ax}), 2.7–3.0 (2H, *m*, H-4 and H-4′), 3.25 (1H, *dd*, *J* = 12.5, 2.5 Hz, H-6_{eq}).

syn[$6,6'^2H_2$]*Thiobinupharidine sulphoxide* **3a** *from* **3**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2600–2800 (Bohlmann bands), 2040 (C-D), 1030 (S → O), 880 (furan) 610. MS *m/z* (rel. int.): 512 [M]$^+$ (10), 511 (4), 495 (60), 494 (100), 493 (29), 358 (3), 231 (59), 230 (95), 179 (11), 178 (7), 176 (4), 136 (4), 107 (22), 94 (32), 81 (16), 79 (20). ^{1}H NMR (100 MHz, C_6D_6): δ0.8 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.7–3.0 (2H, *m*, H-4 and H-4′), 3.25 (1H, *br s*, H-6_{eq}).

Reduction of sulphoxides of thiobinupharidine **a**, **1a**, **2a** *and* **3a** *with* PCl_3. *General procedure.* A soln of sulphoxide of Tb (60 mg) in EtOAc (4 ml) was treated with PCl_3 (2 ml) and the mixture heated under reflex for 15 min. The mixture was poured into H_2O, made alkaline with aq. KOH and extracted with EtOAc. The EtOAc layer was separated and evapd. The residue was chromatographed on CC Al_2O_3 act II 10 g eluent C_6H_6. Pure thiobinupharidine was obtained, MS, IR, ^{1}H NMR and mmp.

[6^2H]*Thiobinupharidine* **1b** *from* **1**. MS *m/z* (rel. int.): 495 [M]$^+$ (51), 494 (20), 231 (36), 230 (65), 179 (100), 178 (17), 136 (13), 107 (35), 94 (50), 81 (9), 79 (23). ^{1}H NMR (100 MHz, C_6D_6): δ0.8 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 1.4 (1H, *d*, *J* = 12.5 Hz, H-$6'_{ax}$), 2.31 (2H, AB*q*, *J* = 12 Hz, CH_2S), 2.8 (2H, *J* = 10.5 and 3.5 Hz, H-4 and H-4′), 3.10 (0.9H, *br s*, H-6_{eq}), 3.18 (1H, *dd*, *J* = 12.5 Hz, H-$6'_{eq}$).

[$6'^2$H]*Thiobinupharidine* **2b** *from* **2**. MS *m/z* (rel. int.): 495 [M]$^+$ (40), 494 (18), 231 (20), 230 (46), 179 (21), 178 (100), 176 (3), 136 (10), 107 (25), 94 (34), 81 (14), 79 (19). ^{1}H NMR (100 MHz, C_6D_6): δ0.80 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 1.92 (1H, *d*, *J* = 12.5 Hz, H-6_{ax}), 2.31 (2H, AB*q*) *J* = 12 Hz, CH_2S), 2.8 (2H, *dd*, *J* = 12, 2.5 Hz, H-4 and H-4′), 3.11 (1H, *dd*, *J* = 12.5, 2.5 Hz, H-6_{eq}), 3.18 (1H, *br s*, H-$6'_{eq}$).

[$6,6'^2H_2$]*Thiobinupharidine* **3b** *from* **3**. MS *m/z* (rel. int.): 496 [M]$^+$ (40), 495 (26), 231 (42), 230 (40), 179 (100), 178 (44), 176 (35), 136 (15), 107 (39), 94 (38), 81 (21), 79 (26). ^{1}H NMR (90 MHz, C_6D_6): δ0.8 (6H, *d*, *J* = 5 Hz, 2 × CHMe), 2.31 (2H, AB*q*, *J* = 12 Hz, CH_2S), 2.8 (2H, *dd*, *J* = 10.5, 3.5 Hz, H-4 and H-4′), 3.11 (0.9H, *br s*, H-6_{eq}), 3.17 (1H, *br s*, H-$6'_{eq}$).

Acknowledgement—Financial support of the Polish Academy of Sciences (MR.I.12) is gratefully acknowledged.

REFERENCES

1. Wróbel, J. T. (1977) in *The Alkaloids* (Manske, R. H. F., ed.) Vol. XIV, p. 198, Academic Press, New York.
2. Wróbel, J. T., Iwanow, A. and Wojtasiewicz, K. (1976) *Bull. Acad. Pol. Sci. Ser. Chim.* **24**, 99.
3. La Londe, R. T., Wong, Ch. F. and Das, K. C. (1974) *J. Org. Chem.* **39**, 2892.
4. La Londe, R. T., Wong, Ch. F. and Tsai, A. I.-M. (1976) *Org. Mass Spectrom.* **11**, 183.
5. La Londe, R. T., Wong, Ch. F., Woolever, J. T., Auer, E., Das, K. C. and Tsai, A. I-M. (1974) *Org. Mass Spectrom.* **9**, 714.
6. Wróbel, J. T., Iwanow, A., Braeckman-Danheux, C., Martin, T. I. and MacLean, D. B. (1972) *Can. J. Chem.* **50**, 1831.
7. La Londe, R. T. and Wong, C. F. (1978) *Can. J. Chem.* **56**, 56.
8. Wehrli, F. W. and Wirthlin, T. (1978) *Interpretation of Carbon-^{13}C NMR Spectra*, p. 38. Heyden, London.
9. La Londe, R. T., Wong, C. F., Tsai, A. I-M., Wróbel, J. T., Ruszkowska, J., Kabzińska, K., Martin, T. I. and MacLean, D. B. (1976) *Can. J. Chem.* **54**, 3860.
10. Wróbel, J. T., Kabzińska, K., Ruszkowska, J. and Wrona, P. K. (1977) *J. Mol. Struct.* **41**, 287.
11. Wróbel, J. T., Ruszkowska, J. and Bielawska, H. (1979) *Pol. J. Chem.* (*Roczniki Chem.*) **53**, 39.
12. Wróbel, J. T., Bobeszko, B., Martin, T. I., MacLean, D. B., Krishnamachari, N. and Calvo, C. (1973) *Can. J. Chem.* **51**, 2810.