Thiobinupharidines Epimeric at C-1 and C-1′

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Thiobinupharidine epimers possessing axial methyl groups at C-1 and C-1′ have been isolated from extracts of *Nuphar luteum*. ^{13}C chemical shift data for the methyl and ring carbons demonstrate that both methyl groups are axial in 1-*epi*-1′-*epi*-thiobinupharidine and that one of two methyls is axial in 1-*epi*- and in 1′-*epi*-thiobinupharidine.

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On a isolé à partir d'extraits du *Nuphar luteum* des épimères du thiobinupharidine possédant des groupes méthyles axiaux en C-1 et en C-1′. Les données de la résonance magnétique nucléaire du ^{13}C pour les groupes méthyles et les carbones du cycle démontrent que chacun des groupes méthyles est axial dans l'*épi*-1 *épi*-1′ thiobinupharidine et qu'un des deux méthyles est axial dans l'*épi*-1 et l'*épi*-1′ thiobinupharidine.

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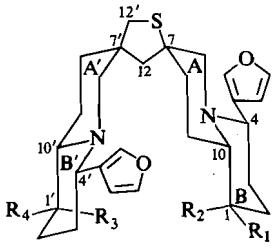
In the course of a search for hemiaminal derivatives of neothiobinupharidine (1) we reduced a chromatographic fraction with sodium borodeuteride for the purpose of ascertaining the position of the hemiaminal hydroxyl group. Comparative thin layer chromatography of the product mixture indicated the presence of thiobinupharidine, **1**, neothiobinupharidine, and a component believed to be deoxynupharidine, but since determination of the position of the deuterium label was desired, the components were separated and their mass spectra were determined. Surprisingly, the component possessing the thin layer chromatographic behavior of deoxynupharidine (molecular weight 231) gave a mass spectrum showing a parent ion at *m/e* 495, a mass number consistent with a singly labelled thiaspirane Nuphar alkaloid. These observations led us to reexamine fractions of earlier separations containing the 'deoxynupharidine spot'. The reexamination has led to the isolation of 1-*epi*-1′-*epi*-thiobinupharidine, **2**, and a mixture of 1-*epi*-, **3**, and 1′-*epi*-thiobinupharidine, **4**. This paper discloses the details of isolation and the evidence for the structures, the latter relying heavily on ^{13}C n.m.r. and previous ^{13}C studies of quinolizidine compounds (2, 3).

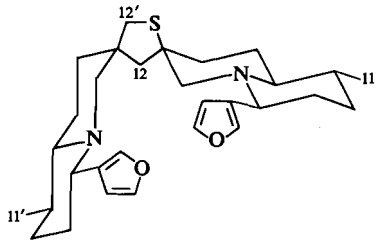


1 $R_1 = CH_3$(C-11); $R_4 = CH_3$(C-11′); $R_2 = R_3 = H$
2 $R_1 = R_4 = H$; $R_2 = CH_3$(C-11); $R_3 = CH_3$(C-11′)
3 $R_1 = R_3 = H$; $R_2 = CH_3$(C-11); $R_4 = CH_3$(C-11′)
4 $R_1 = CH_3$(C-11); $R_3 = CH_3$(C-11′); $R_2 = R_4 = H$

The mass spectra of **2** was typical of the C_{30} thiaspirane Nuphar alkaloids (4) and thus indicated the general skeletal characteristics. However, the presence of some of the separate structural features was clearly evident from other spectral characteristics. The i.r. absorption at 877 cm^{-1} and the ^{13}C n.m.r. signals in the 109–143 p.p.m. region (see Experimental) demonstrated the incorporation of two 3-furyl groups (3). The doublets at 1.11 and 1.14 p.p.m. in the ^1H n.m.r. and the separate lines at 13.6 and 13.7 p.p.m. in the ^{13}C n.m.r. indicated the presence of two methyls, involved as $HCCH_3$, the stereochemistry of which is discussed below. The AB quartet at 2.34 p.p.m. and the broad singlet found at 1.90 p.p.m. in the ^1H spectra were characteristic of methylenes in the thiolane ring (5). The ^1H n.m.r. absorption in the region 2.7–3.1 was typical of C-6 equatorial and C-4 axial protons in *trans*-fused quinolizidines that are substituted at C-4 by a 3-furyl group.

The stereochemical features of the ring systems were ascertained as follows. The i.r. Bohlmann bands, whose intensities were equivalent to those exhibited by thiobinupharidine, showed that *trans* quinolizidines were involved. The 2.34 p.p.m. AB quartet, attributed to the thiomethyl-

ene group, indicated that the new alkaloid belonged to either the thiobinupharidine or thionuphlutine B (**5**) families of thiaspiranes but not



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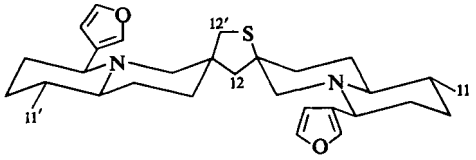

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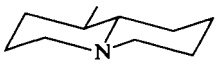
the neothiobinupharidine (**6**), nor a fourth as yet undiscovered stereochemical type, since neothiobinupharidine possesses an axial thiomethylene attached to the A′B′ quinolizidine moiety and this thiomethylene appears at 2.72 p.p.m. The thiomethylene in the fourth stereochemical possibility would also be axial and would be expected to appear in the 2.7 p.p.m. region but not the 2.3 p.p.m. region. However, the positions of the C-12 and C-12′ ^{13}C resonances, 44.4 and 42.6 p.p.m. respectively, are uniquely consistent with the thiobinupharidine lines appearing at 45.3 and 42.7 p.p m. (Fig. 1). The line positions of the C-12 and C-12′ methylenes are related to the number of axial and equatorial attachments in which these methylenes are involved (3). Thus the C-12′ thiomethylene carbon in neothiobinupharidine is axial with respect to the A′B′ quinolizidine ring and appears upfield at 39.1 p.p.m. The C-12 methylene in thiobinupharidine is axial to both the AB and A′B′ quinolizidines and is found further upfield than the corresponding signals of thionuphlutine B (50.6 p.p.m.) or neothiobinupharidine (54.5 p.p.m.) in which the C-12 methylenes are respectively equatorial to one or both quinolizidine systems. Therefore, the evidence from ^{1}H and ^{13}C n.m.r. establishes that the new alkaloid **2** belongs to the thiobinupharidine stereochemical family of thiaspirane Nuphar alkaloids.

The structural difference between **2** and thiobinupharidine, **1**, resides in the configuration of the C-1 and C-1′ methyls. The methyl ^{1}H n.m.r. signals appear somewhat downfield in **2** relative to the spectrum of **1** which exhibits them at 0.93 p.p.m. This observation in conjunction with the known chemical shift dependence (6) on the axial–equatorial disposition of the methyl group attached to the quinolizidine ring was the first indication that the C-1 methyls were axial.

In our earlier Nuphar alkaloid work, we employed an aromatic solvent induced shift of the methyl ^{1}H resonances to determine the axial or equatorial orientation of the methyl groups. Equatorial 1- and 3-methyl quinolizidines undergo upfield shifts whereas the axial methyls meet with a downfield shift (7). No significant solvent shift of methyl groups in **2** was observed and consequently no confirmation of methyl conformation was afforded. However, the ^{13}C n.m.r. spectrum in comparison with that of **1** clearly indicated the methyls in **2** were axial. Figure 1 shows that the highest field resonance of **2**, attributed to the coincidental methyl groups, is considerably shielded relative to the methyls of **1**. This chemical shift increment and those for the α, β and γ carbons are given in Table 1 which also compares the increments for corresponding carbon centers in model quinolizidines **7** and **8**.



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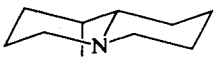

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Table 1 illustrates that the shieldings of all of the various carbons, represented by the sign of the increments, is consistent with the known shift differences resulting from equatorial to axial changes of substituent groups in piperidine and quinolizidine rings (2, 8).

All of the spectral evidence important in determining that **2** belongs to the thiobinupharidine stereochemical family is given also (see Experimental) by the mixture of **3** and **4**, the components of which defied separation by conventional elution and thin layer chromatographic methods. Clearly the comparison of ^{13}C spectra given in Fig. 1 demonstrates that the mixture contains components with both axial and equatorial C-1 and C-1′ methyls. That the mixture consists of **3** and **4** and not **1** and **2** is apparent

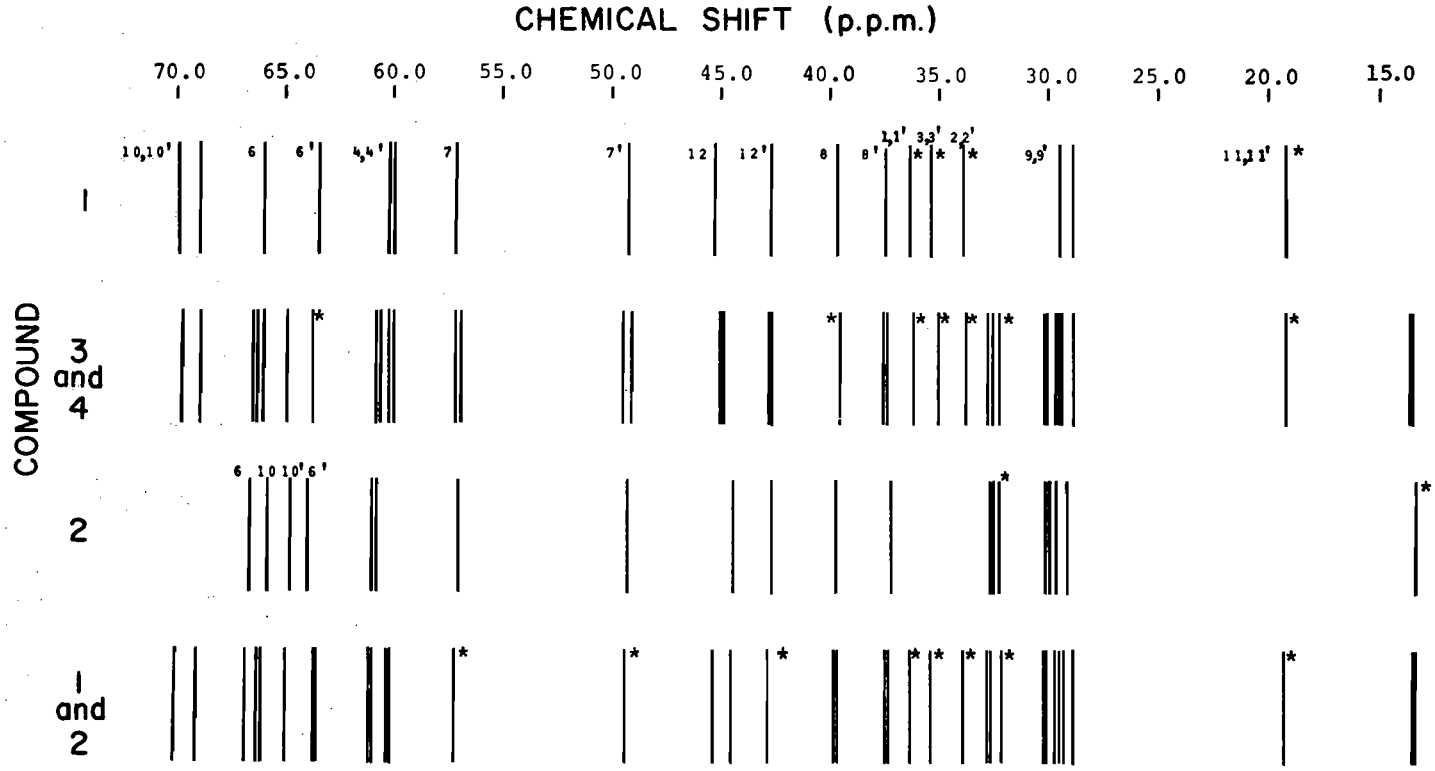

FIG. 1. ^{13}C n.m.r. line positions for saturated carbons in thiobinupharidine (**1**), 1-*epi*-1′-*epi*-thiobinupharidine (**2**), and 1-*epi*- (**3**) and 1′-*epi*-thiobinupharidine (**4**) based on correlation with thiobinupharidine (3), the axial methyl effect in quinolizidine systems (2) and, in the case of **1**, an off-resonance spectrum (the asterisk indicates intensity resulting from more than one carbon based on the average peak height and peak width at half height of C-8 and C-8′ in thiobinupharidine). Chemical shifts quoted in p.p.m. from TMS.

TABLE 1. Saturated carbon shieldings and a comparison of the stereochemical effect of axial and equatorial C-1 methyl groups in quinolizidine models (**7** and **8**) and C_{30} thiaspirane Nuphar alkaloids (**1** and **2**)

Effect at carbon	Chemical shifts (δ)[a,b] and increments (Δδ)[c]					
	Model compounds[d]			Thiaspirane alkaloids		
	7 (C-1, eq. Me)	**8** (C-1, ax. Me)	Δδ	**1**[e,f] (C-1, eq. Me)	**2**[f] (C-1, ax. Me)	Δδ
α						
C-1	36.6	33.3	−3.3	36.3	32.5	−3.8
					32.6	−3.7
β						
C-2	34.2	32.4	−1.8	33.8	32.2	−1.6
C-10	69.5	65.9	−3.6	69.9	65.7	−4.2
					64.6	−4.3
γ						
C-3	25.9	20.9	−5.0	35.3	29.9	−5.4
					30.1	−5.2
C-9	29.9	30.3	+0.4	28.8	29.1	+0.3
Me	18.7	13.8	−4.9	19.2	13.4	−5.8

[a]δ in p.p.m. from TMS.
[b]Chemical shift assignments for **2** are tentative and are based on the best fit of increment values to those of the model pair as well as the line separations of pairs of primed and non-primed carbon numbers in **1** (*e.g.* 10 and 10′, 6 and 6′ etc.).
[c]$\Delta\delta = \delta^{13C}$ (axial Me) − δ^{13C} (equatorial Me).
[d]Chemical shifts are taken from ref. 2.
[e]Chemical shifts are taken from ref. 3.
[f]Chemical shifts are given for primed and non-primed carbon numbers when separate resonances are observed.

from comparing the ^{13}C n.m.r. of these mixtures, especially in the regions occupied by C-7, C-7′, C-12, and C-12′ resonances. Moreover, the thin layer chromatographic behavior of thiobinupharidine, **1**, and the mixture of **3** and **4** are clearly distinguishable, the spot representing **1** being less mobile and widely separated from the spot, or on occasion the two spots, representing **3** and **4**. Therefore the isolated sample containing both axial and equatorial C-1 and C-1′ methyl groups must consist of two epimeric thiobinupharidines, the one possessing C-1 axial, C-1′ equatorial methyls, **3**, and the other C-1 equatorial and C-1′ axial methyls, **4**.

Evidence, primarily from ^{1}H n.m.r., indicates that the three piperidine Nuphar alkaloids nuphenine (9), 3-epinuphamine (10), and 3-epinupharamine (11) possess axial C-1 methyl groups. The newly isolated **2**, **3**, and **4** are the first quinolizidine Nuphar alkaloids possessing axial C-1 methyl groups. Also noteworthy is the previously mentioned isolation of the deuterium labelled material possessing both the mass spectral and thin layer chromatographic properties of the 1-*epi*- and 1′-*epi*-thiobinupharidines. This finding means that the 1-*epi*-thiobinupharidines are also present in the plant extracts as hemiaminals.

Experimental

Measurements and Methods

Spectra were determined as follows: mass spectra (m.s.) on a Hitachi–Perkin-Elmer RMU6E using a direct inlet probe, 70 eV and 120 °C; high resolution mass spectra (h.r.m.s.) were determined at the High Resolution Mass Spectrometry Laboratory, Battelle's Columbus Laboratories, Columbus, Ohio, using an AEI MS-9 spectrometer employing a direct inlet probe, 70 eV and 200 °C; infrared spectra (i.r.) in CCl_4 solution on Perkin-Elmer 137 and 621 spectrometers; ^{1}H n.m.r. in $CDCl_3$ and C_6D_6 (1% TMS, δ 0.00) as indicated on Varian A 60 and XL-100-15, the latter controlled by a VFT-100L computer and operating in the FT mode (m, s, d, t, q, and br referring to multiplet, singlet, doublet, triplet, quartet, and broad); ^{13}C n.m.r. in 5 mm tubes in $CDCl_3$, used also as a secondary reference (77.2 p.p.m. from TMS at δ 0.00 p.p.m.), on a Varian XL-100-15 operating at 25.16 MHz in the FT absorption mode employing 8192 data points. Field frequency lock was established on the deuterium resonance of $CDCl_3$. Fully decoupled spectra of **2** and the mixture of **3** and **4** were obtained from 71.5 and 74.4 K transients respectively. Chemical shifts are accurate to 0.1 p.p.m. Thin layer chromatography (t.l.c.) was performed on microscope slides uniformly coated (0.25 mm) with Alumina GF_{254} and using the solvent systems indicated.

Detection of Monodeuterated epi-Thiobinupharidines in a Reduction Mixture

A 2.1 mg chromatography fraction originating from *Nuphar luteum* from Poland had been obtained in the course of purifying 6- and 6′-hydroxythiobinupharidine

(1). This fraction was dissolved in 5 drops of methanol and the resulting solution was treated with 10 mg of sodium borodeuteride at ambient temperature for 30 min at the end of which time the product mixture was processed by the usual procedure to obtain 2 mg of colorless oil: t.l.c. (Et_2O–hexane, 1:9) R_f 0.12 (neothiobinupharidine), 0.39 (thiobinupharidine), and 0.56 ('Deoxynupharidine' (*epi*-thiobinupharidine)).

Chromatography of the above described mixture was effected on a column of 6 g of neutral Alumina (5% H_2O) eluted first with 30 ml of Et_2O–hexane (5:95); thereafter several 5-drop fractions of the same solvent were taken. Combined fractions 1–10 yielded ~1 mg of *epi*-thiobinupharidines: t.l.c. (Et_2O–hexane, 1:9) R_f 0.56; m.s. *m/e* (relative intensity, %) 495(21), 494(18), 360(11), 359(8), 231(43), 230(53), 179(100), 178(82), 136(21), 107(56), 94(91).

Isolation of 1-epi-1′-epi-Thiobinupharidine, 2, and a Mixture of 1-epi- and 1′-epi-Thiobinupharidine, 3 and 4

Three chromatographic fractions (5) which had been eluted with hexane, and previously designated A1, A2, and A3, were combined (219 mg) and chromatographed on 5 g of neutral alumina (activity 2) that was first eluted with 75 ml of hexane, to obtain 140 mg of u.v. active but Dragendorf inactive material, and then with 30 ml of Et_2O–hexane (5:95) to obtain 75 mg of material. The latter was dissolved in 15 ml of Et_2O and the resulting solution was shaken with 15 ml of 0.01 *N* aqueous HCl and then with water. The aqueous acid and water layers were combined, made alkaline with powdered sodium hydroxide, saturated with salt, and finally extracted several times with small portions of methylene chloride. The combined extracts were dried (Na_2SO_4) and the solvent removed at the rotary evaporator to obtain 58 mg of a colorless oily residue which was chromatographed on a column (1 cm o.d. × 15.5 cm) containing 20 g of neutral alumina (5% water) by eluting with: 75 ml of hexane (fraction A′1, 0 mg); Et_2O–hexane in eight 20-drop portions (combined fraction A′2, 4.4 mg); Et_2O–hexane in eighteen 30-drop portions (combined fractions A′3, 25 mg); 150 ml of Et_2O–hexane (fraction A′4, 20 mg). This chromatography and the one below were monitored by t.l.c. (Alumina GF_{254}, 0.25 mm). Combined fractions A′2 and A′3 were admixed and chromatographed on a similar column by eluting with: 10 ml of hexane (fraction B′1, 0 mg); six 20-drop fractions of hexane (combined fraction B′2, 9.3 mg); three 20-drop fractions of hexane (combined fraction B′3, 3.7 mg); ten 20-drop fractions of hexane (combined fraction B′4, 8.3 mg); 20 ml of methylene chloride (fraction B′5, 0.2 mg). Fraction B′2 was pure 1-*epi*-1′-*epi*-thiobinupharidine, **2**: t.l.c. (eluted three times with Et_2O–hexane, 5:95) R_f 0.65 (R_f thiobinupharidine, 0.33); $[\alpha]_D^{25}$ 30.9 (*c* 9 mg/ml, 95% EtOH); i.r. 2810–2490 cm^{-1} (Bohlmann band) and 877 cm^{-1} (3-furyl); ^1H n.m.r. (100 MHz, $CDCl_3$) δ 2.7–3.1 (m, 4H, C-6 eq., C-6′ eq., C-4, and C-4′), 2.34 (AB q, J = 12.5 Hz, 2H, CH_2S) 1.90 (br s, 2H, C-7 CH_2), and 1.11 and 1.14 (2d, 6H, C-1 and C-1′ CH_3); ^1H n.m.r. (100 MHz, C_6D_6) δ 3.12 (2d, J = 12.5 Hz, 2H, C-6 eq., and C-6′ eq.), 2.70 (m, 2H, C-4, and C-4′), 2.29 (AB q, J = 13 Hz, 2H, CH_2S), 2.13 (AB q, J = 13 Hz, 2H, C-7 CH_2), 1.13 (2d, 6H, C-1, and C-1′); ^{13}C n.m.r. (7 mg in 0.3 ml $CDCl_3$) δ 29.6, 37.2, 39.7, 42.6, 44.4, 49.2, 56.9, 60.6, 60.8, 63.8, 66.5, 109.7 (C-15 or C-15′) (3), 109.7 (C-15′ or C-15), 129.7 (C-13 or C-13′), 129.9 (C-13′ or C-13), 139.7 (C-14 and C-14′), 143.0 (C-16 and C-16′) and assigned resonances given in Table 1; h.r.m.s. obsvd/calcd mass (formula) 494.2957/494.2967 ($C_{30}H_{42}N_2O_2S$), 447.3021/447.3011 ($C_{29}H_{39}N_2O_2$), 359.2140/359.2157 ($C_{21}H_{31}N_2OS$), 230.1523/230.1545 ($C_{15}H_{20}NO$), 136.0868/136.0888 ($C_9H_{12}O$), 107.0497/107.0497 (C_7H_7O), 94.0413/94.0419 (C_6H_6O), and 81.0337/81.0340 (C_5H_5O).

Fractions A′4 and B′4 were combined to give 27 mg of a mixture of 1-*epi*- and 1′-*epi*-thiobinupharidine: t.l.c. (eluted three times with Et_2O–hexane, 5:95) R_f 0.61 (R_f thiobinupharidine, 0.33; R_f 1-*epi*-1′-*epi*-thiobinupharidine, 0.65); $[\alpha]_D^{25}$ 23.9 (*c* 27 mg/ml, 95% EtOH); i.r. 2810–2490 (Bohlmann bands, intensities equivalent to those exhibited by thiobinupharidine), 877 cm^{-1} (3-furyl); ^1H n.m.r. (60 MHz, $CDCl_3$) δ 2.6–3.15 (m, 4H, C-6 eq., C-6′ eq., C-4, and C-4′), 2.31 (AB q, J = 12.5 Hz, 2H, —CH_2S—), 1.88 (br s, 2H, C-7 CH_2), 1.13 (2d, J = 6.5 Hz, 3H), and 0.94 (2d, 3H); ^1H n.m.r. (60 MHz, C_6D_6) δ 3.2 (m, C-6 eq. and C-6′ eq.), 2.75 (m, C-4 and C-4′), 2.33 (AB q, J = 12.5 Hz, 2H, CH_2S), 2.17 (AB q, J = 12.5 Hz, 2H, C-7 CH_2), 1.12 (2d, J = 6.5 Hz, 3H), 0.84 (2d, 3H); ^{13}C n.m.r. (27 mg in 0.3 ml $CDCl_3$) δ 13.6, 13.7, 19.2, 28.8, 29.3, 29.4, 29.6, 30.0, 30.1, 32.2, 32.5, 32.7, 33.7, 35.2, 36.1, 37.3, 37.5, 39.5, 42.6, 42.7, 44.8, 44.9, 49.0, 49.4, 56.8, 57.1, 59.9, 60.1, 60.5, 60.7, 63.6, 64.8, 65.9, 66.2, 66.4, 68.8, 69.7, 109.7, 109.8, 129.6, 129.9, 139.7, and 143.0; h.r.m.s. obsvd/calcd mass (formula) 494.2950/494.2967 ($C_{30}H_{42}N_2O_2S$), 447.2976/447.3011 ($C_{29}H_{39}N_2O_2$), 359.2094/359.2157 ($C_{21}H_{31}N_2OS$), 230.1520/230.1545 ($C_{15}H_{20}NO$), 136.0907/136.0888 ($C_9H_{12}O$), 107.0497/107.0497 (C_7H_7O), 94.0414/94.0419 (C_6H_6O), and 81.0339/81.0340 (C_5H_5O).

Mixture of Thiobinupharidine, 1, and 1-epi-1′-epi-Thiobinupharidine, 2

^{13}C n.m.r. (5 mg of **1** and 5 mg of **2** in 0.3 ml $CDCl_3$) δ 13.4, 13.5, 19.3, 28.8, 29.3, 29.5, 29.7, 30.1, 30.2, 32.1, 32.4, 32.6, 32.8, 33.9, 35.4, 36.3, 37.3, 37.5, 39.7, 39.8, 42.8, 44.5, 45.3, 49.3, 57.1, 60.0, 60.2, 60.8, 61.0, 63.4, 64.0, 64.8, 65.9, 66.1, 66.6, 68.9, 109.9, 129.7, 130.5, 140.0, and 143.2.

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