Hemiaminal Derivatives of Neothiobinupharidine[1]

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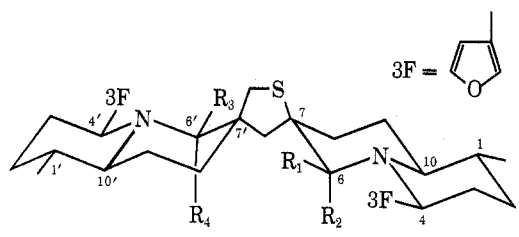
6- and 6′-hydroxyneothiobinupharidine were isolated from *Nuphar luteum* from Poland. The presence of infrared Bohlmann bands, hydroxyl groups reduced by sodium borodeuteride, and the appearance of the pairs of peaks at *m/e* 230 and 228 and 178 and 176 in the mass spectrum indicated the dual hemiaminal–amine nature of each of the two alkaloids. The ^{1}H NMR signals at δ 4.57 given by the one isomer and at δ 4.08 given by the other demonstrated that the hemiaminal hydroxyl was located at a C-6 or C-6′ position. On sodium borodeuteride reduction, singly labeled neothiobinupharidines were formed, that from the C-6 hemiaminal giving *m/e* 179 but that from the C-6′ hemiaminal giving *m/e* 178. The absence of the C-6 axial proton and the appearance of the C-6 equatorial proton as a singlet in the ^{1}H NMR indicated the presence of an axial deuterium at C-6 in neothiobinupharidine-6-d_1. Similar evidence indicated that the deuterium atom in neothiobinupharidine-6′-d_1 was axial also. The CD of both hemiaminals in acid gave negative bands, the one from the C-6 hemiaminal appearing at 295 nm, and the one from the C-6′ hemiaminal appearing at 279 nm.

6,6′-Dihydroxythiobinupharidine, one of the several hemiaminals recently isolated from *Nuphar,* possesses activity against human pathogenic fungi.[2] This finding, along with the discovery of the similar activity of synthetically derived deoxynupharidine α-thiohemiaminals,[3] has led us to extend our search for other *Nuphar* hemiaminals. The C_{30} thiaspiran hemiaminals previously studied belonged to the thiobinupharidine and thionuphlutine B stereochemical families.[4] This paper reports the detection, isolation, and structure determination of the hemiaminals of the neothiobinupharidine family.

Both 6- (1) and 6′-hydroxyneothiobinupharidine (2) were isolated from extracts of *Nuphar luteum* of Polish origin. A mixture of the two had been obtained and was the subject of an earlier report.[5] Since the separation of the components in the original mixture proved refractory, only the hemiaminal 1 was removed from this mixture and attention was focused on another fraction for the source of hemiaminal 2. Noteworthy in regard to the purification of



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

the hemiaminals is the thin layer chromatographic appearance of 1. When freshly chromatographed from a column of alumina, this hemiaminal appeared as a single spot but material which was aged but a few hours showed an additional somewhat more mobile second spot. The 6′ isomer, 2, did not exhibit this behavior. Both freshly chromatographed and aged samples of 1 yielded neothiobinupharidine on reduction. Consequently we propose that the appearance of two spots results from epimeric hydroxyls at C-6.

Evidence adduced for establishing the dual hemiaminal–amine character within a C_{30} thiaspiran structure was similar to that offered previously in assigning structures of the monohemiaminals of thiobinupharidine and thionuphlutine B.[4d] Thus satisfactory values for the molecular weights corresponding to thiaspiran monohemiaminals were observed in the high-resolution mass spectra. Moreover, the pairs of mass spectral peaks *m/e* 230 and 228 and 178 and 176, in which the intensity of the peak of lower mass was equal to or greater than that of the higher mass peak of the pair, indicated[6] the presence of one fully reduced quinolizidine moiety and one C-6 hemiaminal quinolizidine moiety in each of the two alkaloids. The appearance of infrared Bohlmann bands and the facile borodeuteride reduction of the hemiaminal hydroxyl, coupled with the previous finding that bishemiaminal quinolizidines do not give Bohlmann bands,[4d] supported the monohemiaminal–amine character of the two new alkaloids.

6- and 6′-hydroxyneothiobinupharidine exhibited respectively ^{1}H NMR singlets at δ 4.57 and 4.08, the chemical shifts being consistent with those displayed by the C-6 and C-6′ monohemiaminals of thiobinupharidine and thionuphlutine B.[4d] Accordingly these resonances were assigned to the C-6 or C-6′ hemiaminal carbinyl protons. Hemiaminals located at C-4 or C-10 would show no carbinyl protons.

Each of the monohemiaminals was reduced with sodium borodeuteride to a singly deuterium labeled neothiobinupharidine identified by comparison to authentic unlabeled sample. These reductions not only established the stereochemical type of thiaspiran to which the hemiaminals belonged but also confirmed the monohemiaminal presence as well as its location. Earlier work[4d] demonstrated that deuterium located at C-6 in the AB quinolizidine system produced a shift of *m/e* 178 to 179 resulting from the fragmentation depicted in Figure 1. In contrast, when deuterium was located at C-6′, in the A′B′ quinolizidine system, *m/e* 178 was maintained. The mass spectrum of the deuterium labeled neothiobinupharidine (3), obtained from 6-hydroxyneothiobinupharidine, showed that *m/e* 178 was shifted to *m/e* 179 to the extent of 80% whereas the deuterium labeled counterpart, 4, from the 6′ isomer exhibited only *m/e* 178.

In our studies of naturally occurring thiohemiaminals, two procedures have emerged for differentiating a C-6 hemiaminal (i.e., an α-thiohemiaminal) from a C-6′ hemiaminal (i.e., a β-thiohemiaminal).[4d] Employment of these procedures is especially advantageous in the examination of small amounts of *Nuphar* hemiaminals such as are the subjects of this report. One procedure consists in a sodium borodeuteride reduction followed by mass spectral analysis to locate the deuterium location. The examination of 1 and 2 by this procedure has just been described above.

The second procedure relies on the acidic solution ab-

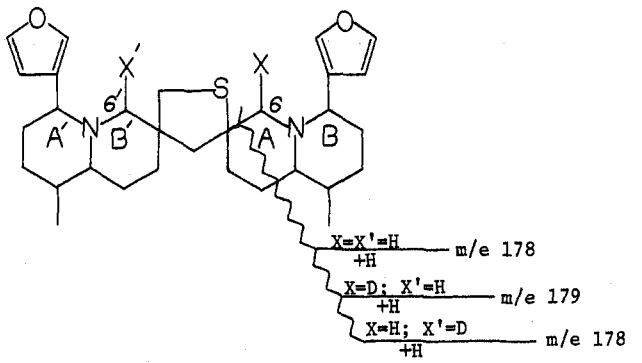
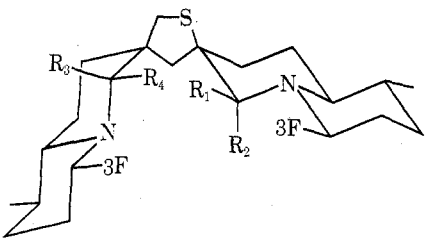


Figure 1. Mass spectral fragmentations of neothiobinupharidines labeled at C-6 and C-6′.

sorption of thiohemiaminals in the 270–310-nm region.[4a,4d,7] This procedure is more readily executed than the first and, when advantage is taken of the circular dichroism (CD), yields more information since not only are the C-6 and C-6′ hemiaminal positions differentiated but the stereochemical disposition of the thiohemiaminals can be ascertained as well.[7] α-Thiohemiaminals in acid solution absorb in the 290–300-nm region whereas β-thiohemiaminals absorb in a somewhat lower region, 270–280 nm. The α-thiohemiaminal **1** in acid produced an ultraviolet absorption maximum at 298 nm whereas the β-thiohemiaminal **2** gave a maximum at 275 nm. These observations support the positional assignments of the hemiaminal function.

The CD of 6-hydroxyneothiobinupharidine (**1**) in neutral solution exhibited a weak negative band at 255 nm and a weak positive band at 239 nm. In acid solution, a considerably stronger negative band is produced at 295 nm, as is depicted in Figure 2. These CD properties are virtually the same as those exhibited by 6-hydroxythionuphlutine B (**5**),



5, $R_1 + R_2 = H + OH$; $R_3 = R_4 = H$
9, $R_1 + R_2 = H + OH$; $R_3 + R_4 = H + OH$

which exhibits a negative band at 298 nm,[4d] and 7α-methylthiodeoxynupharidin-6-ol,[7] whose relative stereochemistry is secure and is derived from (−)-deoxynupharidine whose absolute configuration has been established. Contrasting with the CD properties of **1** and **5** are those of 6-hydroxythiobinupharidine (**6**), which gives a positive band at 296 nm (Figure 2),[7,4d] and 7β-methylthiodeoxynupharidin-6-ol, which, like the 7α isomer just mentioned, is also derived from (−)-deoxynupharidine. Therefore the acid solution CD properties of **1** are consistent with a 7*R* configuration, the same configuration being found in **5**.

The CD of 6′-hydroxyneothiobinupharidine (**2**) in neutral solution was much like that of the C-6 isomer, since **2** showed a weak negative band at 253 nm and a weak positive band at 239 nm. However, in acid solution, a negative band was produced at 279 nm. These acid-induced CD properties should be compared, as has been done in Figure 2, with those of 6′-hydroxythiobinupharidine (**7**), which, in acid solution, gives a positive CD band at 280 nm.[4d] The sign of the CD band given by **2** and position of the band, relative to that exhibited by **1**, is consistent with a β-thiohemiaminal possessing the 7′*S* configuration.

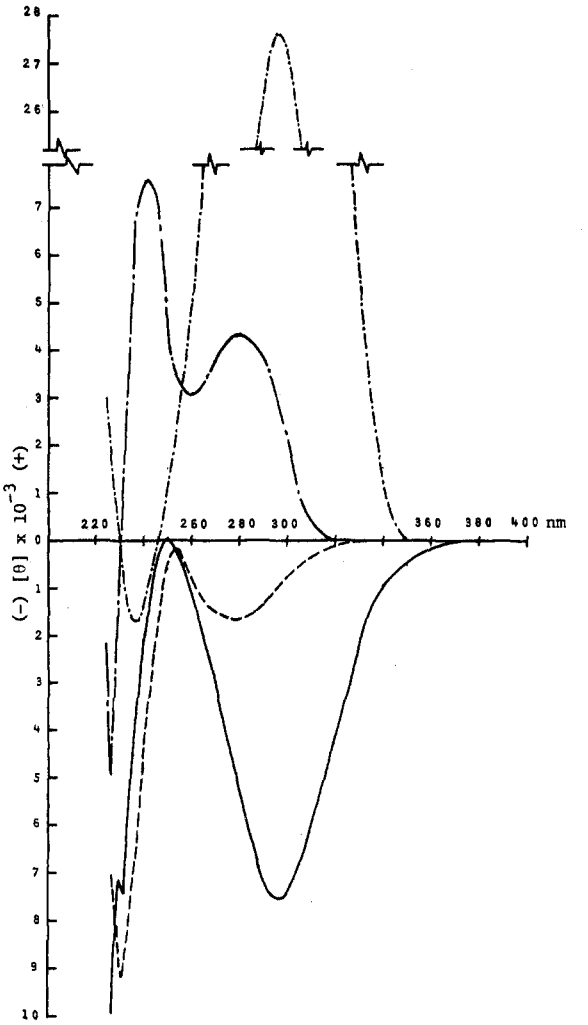
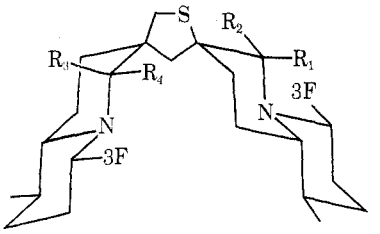


Figure 2. The circular dichroism in acid solution of 6-hydroxythiobinupharidine (**6**) (- - - -); 6′-hydroxythiobinupharidine (**7**) (— · —); 6-hydroxyneothiobinupharidine (**1**) (——); 6′-hydroxyneothiobinupharidine (**2**) (- - - -).

The examination of the CD properties of 6′-hydroxyneothiobinupharidine (**2**) completes the series of 7*R*, 7*S*, 7′*R*, and 7′*S* α- and β-thiohemiaminals. In summary, the results of the present and preceding CD studies show that in acid solution the thiaspiran α-thiohemiaminals possessing 7*S* and 7*R* configurations give respectively positive and negative CD bands near 300 nm. The thiaspiran β-thiohemiaminals possessing 7′*R* and 7′*S* configurations give respectively positive and negative CD bands in the 275–285-nm region. Consistent with this generalization of the acid solution CD properties are those exhibited by the known bishemiaminals, 6,6′-dihydroxythiobinupharidine (**8**) and 6,6′-dihydroxythionuphlutine B (**9**).[7]

One other aspect of these results worthy of comment is the stereochemistry of the sodium borodeuteride reduction



6, $R_1 + R_2 = H + OH$; $R_3 = R_4 = H$
7, $R_1 = R_2 = H$; $R_3 + R_4 = H + OH$
8, $R_1 + R_2 = H + OH$; $R_3 + R_4 = H + OH$

of the α- and β-thiohemiaminals. Both 6-hydroxythiobinupharidine (6) and 6,6′-dihydroxythiobinupharidine (8) possess an equatorial sulfur atom at C-7 and undergo reduction with the incorporation of both axial and equatorial deuterium at C-6,[8] although in one study the incorporation was observed to be predominantly equatorial.[4b,9] However, 6,6′-dihydroxythionuphlutine B (9)[4b] and 6-hydroxyneothiobinupharidine (1) possess an axial sulfur atom at C-7 and undergo reduction with the incorporation of only axial deuterium at C-6. In the case of 1, the NMR of the labeled product (3) reveals no doublet at δ 1.71, which corresponds to the signal of the C-6 axial proton of unlabeled neothiobinupharidine. The C-6 equatorial proton at δ 3.20 appears as a broad singlet.

A number of model α-thiohemiaminals, derived from (−)-deoxynupharidine and possessing axial and equatorial C-7 sulfide groups, exhibit the same stereospecificity in reduction as the thiaspiran α-thiohemiaminals.[4b,3] Clearly the steric mode of sodium borodeuteride reduction is influenced by the stereochemistry of the C-7 sulfur atom, as previously discussed.[4b,9]

Contrasting with the steric course of α-thiohemiaminal reduction, the stereochemistry of β-thiohemiaminal reduction is independent of the configuration of C-7′ and the stereochemistry of the sulfur atom. β-Thiohemiaminal, 2, wherein the C-7′ thiomethylene is axial, undergoes axial incorporation of deuterium at C-6′ as evidenced by the ^{1}H NMR spectrum of the labeled compound 4, which shows no δ 1.55 doublet for the C-6′ axial proton as did the unlabeled neothiobinupharidine. However, both 6,6′-dihydroxythionuphlutine B (9) and 6,6′-dihydroxythiobinupharidine (8), wherein the C-7′ thiomethylenes are equatorial, also undergo reduction with incorporation of axial deuterium at C-6′.[4b]

The stereospecificity of α-thiohemiaminal reduction and the appearance of the 290–310-nm absorption bands of acidic α-thiohemiaminal solutions have been attributed[4b,7] to the interaction of sulfur with immonium ion. In the case of the β-thiohemiaminals, the thioimmonium absorption is observed but stereospecificity of reduction is not. This disparity of behavior suggests that the β-thioimmonium ion interaction, though sufficient to generate the absorption, is improperly disposed to direct the stereochemistry of sodium borodeuteride reduction.

Experimental Section

Spectra were determined as follows: proton nuclear magnetic resonance (^{1}H NMR) in solution specified, 2% Me_4Si (δ 0.00 ppm), on Varian XL-100-15 operating in the pulsed FT mode unless indicated otherwise, symbols br, s, d, q, and m refer to broad, singlet, doublet, quartet, and multiplet, respectively; infrared spectra (ir) on Perkin-Elmer 137 in solvents as indicated, symbols st, wk, and m refer to strong, weak, and moderate intensity, respectively; mass spectra (MS) on a Hitachi Perkin-Elmer RMU-6E using a direct inlet probe, 70 eV and 120°; high-resolution mass spectra were determined at the High Resolution Mass Spectrometry Laboratory, Battelle's Columbus Laboratories, Columbus, Ohio, on an AEI MS-9 using a direct inlet probe, 70 eV and 150 and 180°; circular dichroism (CD) on a Jasco Model 5 spectropolarimeter in solution at the concentrations indicated.

Optical rotations were determined in solution as indicated on a Perkin-Elmer 141 polarimeter. Thin layer chromatography was carried out on microscope slides uniformly coated with 0.25 mm of alumina GF_{254} and using the solvent systems specified. Elution chromatography employed neutral alumina in all cases. The sodium borodeuteride was purchased from Merck Sharp and Dohme and contained a minimum of 98% deuterium.

Isolation of 6-Hydroxyneothiobinupharidine (1). Chromatography of the combined mixture of two fractions, previously designated A-8 and A-9 and originating from *N. luteum* of Polish origin,[4d] was carried out on 20 g of alumina (4.5% water) using the eluting solvents in the volumes indicated and giving the fractions in amounts as follows: hexane, fraction A′1, 0 mg; hexane–Et_2O (9:1), 50 ml, fraction A′2, 2 mg; hexane–Et_2O (8:1), 50 ml, fraction A′3, 67 mg; hexane–Et_2O (8:2), 50 ml, fraction A′4, 59 mg; hexane–Et_2O (8:2), 50 ml, fraction A′5, 38.5 mg; hexane–Et_2O (8:2), 50 ml, fraction A′6, 21 mg; Et_2O, 100 ml, fraction A′7, 67 mg; MeOH, 100 ml, fraction A′8, 4 mg. Fraction A′7 was rechromatographed on 15 g of alumina (activity 3) using pyridine–Et_2O–hexane (3:10:37). After Dragendorf active eluate was first detected, five-drop fractions were taken and thereafter were collected several five-drop fractions totaling about 25 ml, the latter group of fractions and those preceding it being monitored by TLC [alumina, pyridine–Et_2O–hexane (3:10:37)]. Combination of fractions 1–8 gave combined fraction B′1 (46.8 mg); fractions 9–21 gave fraction B′2 (10 mg); and the last 25 ml of eluate gave fraction B′3 (18 mg) consisting of the previously described mixture of 6- and 6′-hydroxyneothiobinupharidine.[5] After standing at 5° for 5 months, B′3 became colored and therefore was chromatographed on 6 g of alumina which was eluted with two 30-ml portions of hexane, to obtain fractions B′4 (2 mg) and B′5 (11 mg), and 20 ml of Et_2O, to obtain fraction B′6 (0.5 mg). Fraction B′5 was chromatographed on 2 g of alumina (activity 2) with 75 ml of benzene to obtain fraction C′1 (9.4 mg) consisting of a mixture of 6- and 6′-hydroxyneothiobinupharidine: TLC (hexane–Et_2O, 9:1) R_f 0.26 and 0.36 (6-hydroxythiobinupharidine), 0.51 (6′-hydroxythiobinupharidine); high-resolution MS, obsd/calcd mass (formula) 510.2814/510.2915 ($C_{30}H_{42}N_2O_3S$), 509.2762/509.2838 ($C_{30}H_{41}N_2O_3S$), 508.2714/508.2759 ($C_{30}H_{40}N_2O_3S$). Fraction C′1 was chromatographed on 3 g of alumina (activity 3) eluting with hexane–Et_2O (9:1), the first 50 ml of which produced 8 mg of fraction D′1 [TLC (hexane–EtOAc, 9:1) R_f 0.26, 0.36, 0.51] and the second 30 ml of which yielded 1.4 mg of fraction D′2 consisting of 6-hydroxyneothiobinupharidine (1): TLC (hexane–EtOAc, 9:1) R_f 0.26 and on standing 0.26 and 0.36; CD (acidic 95% EtOH) $[\theta]_{298}$ −7500°.

To obtain a larger amount of 1, fraction D′1 was chromatographed on 10 g of alumina eluting first with CH_2Cl_2 in 16-ml and 12-ml portions to obtain fractions E′1 [1.6 mg, TLC (CH_2Cl_2) R_f 0.75] and E′2 [2 mg, TLC (CH_2Cl_2) R_f 0.45, 0.60, 0.75], respectively. Continued elution with 100 ml of CH_2Cl_2–MeOH (95:5) produced 5.8 mg of fraction E′3 consisting of 5.8 mg of 1: TLC (CH_2Cl_2) R_f 0.45 (major), 0.60 (minor), (hexane–EtOAc, 9:1) R_f 0.26 (major), 0.36 (minor), (hexane–EtOAc 8:2, twice developed) R_f 0.30 (major), 0.45 (minor), (CH_2Cl_2 and 1 drop MeOH) R_f 0.80; uv (acidic 95% EtOH) λ_{max} 298 nm (ε 2700); ir ($CDCl_3$) 3.1 (br, wk, OH), 2.65 (wk Bohlmann bands), 11.45 μ (st, 3-furyl); ^{1}H NMR (*c* 2.5 mg/0.3 ml $CDCl_3$) δ 8.32 (m, 4 H, 3-furyl β-H), 7.57 and 7.33 (br s, 2 H, 3-furyl β-H), 4.57 (s, 1 H, C-6 H), 3.96 (m, 1 H, C-4 H), 2.62 (br s, 2 H, CH_2S), 0.86 (d, 6 H, C-1 and C-1′ CH_3); high-resolution MS, obsd/calcd mass (formula) 510.2883/510.2916 ($C_{30}H_{42}N_2O_3S$), 509.2831/509.2838 ($C_{30}H_{41}N_2O_3S$), 508.2764/508.2766 ($C_{30}H_{40}N_2O_3S$), 492.2744/492.2810 ($C_{30}H_{40}N_2O_2S$), 230.1529/230.1545 ($C_{15}H_{20}NO$), 228.1380/228.1388 ($C_{15}H_{18}NO$), 178.1216/178.1232 ($C_{11}H_{16}NO$), 176.1057/176.1075 ($C_{11}H_{14}NO$); CD (*c* 0.7 mg/ml, neutral 95% EtOH) $[\theta]_{290}$ ±0°, $[\theta]_{255}$ −474°, $[\theta]_{248}$ ±0°, $[\theta]_{239}$ +1750°, $[\theta]_{233}$ ±0°, $[\theta]_{230}$ −1640°, $[\theta]_{229}$ −801°; CD (*c* 0.7 mg and 1 drop of 0.2 *M* $HClO_4$ in 1 ml of 95% EtOH) $[\theta]_{370}$ ±0°, $[\theta]_{295}$ −7500°, $[\theta]_{250}$ ±0°, $[\theta]_{236}$ −4520°, $[\theta]_{232}$ −7430°, $[\theta]_{230}$ −7210°, $[\theta]_{227}$ −9910°.

Conversion of 6-Hydroxyneothiobinupharidine to Neothiobinupharidine-*6-d*$_1$ (3). A solution of 1.8 mg of 6-hydroxyneothiobinupharidine in 10 drops of MeOH was treated with 10 mg of sodium borodeuteride at ambient temperature for 30 min and thereafter the solvent was removed by vacuum evaporation, giving a residue whose TLC showed R_f 0.3 (hexane–Et_2O, 8:2) and 0.2 (benzene–CH_2Cl_2, 9:1). Chromatography of the residue on 2 g of alumina (activity 2) using benzene gave fraction 1 (3 ml, 0 mg), fraction 2 (10 ml, 0.3 mg), and fraction 3 (20 ml, 1.3 mg). Fractions 2 and 3 were combined to give 1.6 mg of neothiobinupharidine-*6-d*$_1$ (3): $[\alpha]^{25}D$ −175° (*c* 1.3 mg/ml, 95% EtOH); ^{1}H NMR (deuteriobenzene) δ 3.20 (s, C-6 H eq), 1.71 (d, J = 11.5 Hz, C-6 H ax) observed in the spectrum of neothiobinupharidine was absent; MS *m/e* (rel intensity) 497 (3), 496 (9), 495 (24), (15% d_0, 84% d_1, 1% d_2), 494 (8), 360 (9), 231 (18), 230 (27), 170 (100), 178 (26), 136 (11), 107 (22), 94 (31), 81 (18), 79 (16).

Isolation of 6′-Hydroxyneothiobinupharidine (2). Chromatography of a 333-mg fraction, previously designated A 60[4a] and originating from *N. luteum* of Polish origin, was carried out on 15 g of alumina (activity 2) eluting with hexane–EtOAc (9:1) in 45 (fraction F′1, 113 mg), 45 (fraction F′2, 187 mg), and 100 ml (fraction F′3, 10 mg) and finally with 50 ml of MeOH (fraction F′4, 10 mg). Fraction F′1, giving a TLC (hexane–EtOAc, 9:1) R_f 0.24 and

0.33, was chromatographed on 15 g of alumina (activity 2) by eluting with hexane–EtOAc (95:5) in two 50-ml portions, fractions G'1 and G'2, respectively (0 and 98 mg), and thereafter one 100-ml portion, fraction G'3 (34 mg). Fraction G'3, giving a TLC (hexane–EtOAc, 9:1) R_f 0.24 and 0.33, was chromatographed on 15 g of alumina (activity 2) by eluting with hexane–EtOAc (9:1) in 7 (fraction H'1, 0 mg), 4 (fraction H'2), 2 (fraction H'3), and 24 ml (fraction H'4, 69 mg). Fractions H'2 and H'3 were combined to give, after evaporation of solvent, 3.4 mg of 6'-hydroxyneothiobinupharidine (**2**): TLC (hexane–EtOAc, 9:1) R_f 0.33; uv (acidic 95% EtOH) λ_{max} 275 nm (ϵ 470); ir (CCl_4) 2.85 (wk OH), 3.7 (m, Bohlmann bands), 11.45 μ (st, 3-furyl); ^{1}H NMR (3 mg/0.3 ml $CDCl_3$) δ 7.3 (m, 4 H, 3-furyl α-H), 6.53 and 6.24 (br singlets, 2 H, 3-furyl β-H), 4.34 (br s, ~1 H, OH), 4.08 (br s, 1 H, C-6' H), 3.54 (m, C-4' H), 0.86 (d, 6 H, C-1 CH_3); high-resolution MS, obsd/calcd mass (formula) 510.2935/510.2916 ($C_{30}H_{42}N_2O_3S$), 509.2868/509.2838 ($C_{30}H_{41}N_2O_3S$), 508.2774/508.2759 ($C_{30}H_{40}N_2O_3S$), 492.2755/492.2810 ($C_{30}H_{40}N_2O_2S$), 230.1513/230.1545 ($C_{15}H_{20}NO$), 228.1367/228.1388 ($C_{15}H_{18}NO$), 178.1198/178.1233 ($C_{11}H_{16}NO$), 176.1047/176.1075 ($C_{11}H_{14}NO$); CD (*c* 0.34 mg/ml, neutral 95% EtOH) $[\theta]_{270}$ ±0°, $[\theta]_{253}$ −750°, $[\theta]_{246}$ ±0°, $[\theta]_{239}$ +1690°, $[\theta]_{234}$ ±0°, $[\theta]_{228}$ −4050°, $[\theta]_{227}$ +2850°; CD (*c* 0.34 mg and 1 drop of 0.2 *M* $HClO_4$ in 1 ml of 95% EtOH) $[\theta]_{330}$ ±0°, $[\theta]_{285}$ −1500°, $[\theta]_{280}$ −1650; $[\theta]_{278}$ −1650°, $[\theta]_{270}$ −1500°, $[\theta]_{253}$ −375°, $[\theta]_{231}$ −9150°, $[\theta]_{228}$ −7430°.

Conversion of 6'-Hydroxyneothiobinupharidine to Neothiobinupharidine-6'-d_1 (4). A solution of 1.5 mg of 6'-hydroxyneothiobinupharidine in 5 drops of MeOH was treated with 10 mg of sodium borodeuteride for 1 hr at ambient temperature. Thereafter the solvent was evaporated under a stream of nitrogen and the residue was digested with 5 ml of CH_2Cl_2. The solvent was evaporated from the resulting extract and the residual oil was chromatographed on 2 g of alumina (activity 2) eluted with hexane–Et_2O (8:2), in 10- and 40-ml portions, fractions 1 and 2, and thereafter with 20 ml of benzene which gave fraction 3 comprised of 1.4 mg of neothiobinupharidine-6'-d_1 (**4**): $[\alpha]^{25}D$ −160° (*c* 1.3 mg/ml, 95% EtOH); ir (CCl_4) 3.60 (st, Bohlmann band), 4.90 (wk, C–D), 11.45 μ; ^{1}H NMR (deuteriobenzene) δ 3.21 (d of d, J = 2 and 11.5 Hz, C-6 H eq), 2.60–3.05 (overlapping multiplets, 5 H, C-6' H eq, C-4 and C-4' H, CH_2S), 1.71 (d, J = 11.5 Hz, C-6 H ax), and the 1.55 (d, J = 11.5 Hz, C-6' H ax) observed in the spectrum of neothiobinupharidine was absent; MS *m/e* (rel intensity) 497 (2), 496 (6), (25.6% d_0, 73% d_1, 1.4% d_2), 495 (16.5), 494 (7.5), 360 (7), 231 (14), 230 (26), 179 (17), 178 (100), 136 (8), 94 (22), 81 (11), 79 (10).

Registry No.—**1**, 55869-57-3; **2**, 55869-58-4; **3**, 55869-59-5; **4**, 55869-60-8; **6**, 50478-55-2; **7**, 52002-85-4; neothiobinupharidine, 4850-09-3.

References and Notes

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Fluorescent Modification of Guanine. Reaction with Substituted Malondialdehydes

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Six representative tricyclic 1,N^2-(allylidene)guanine derivatives (**3a–f**), or 10-oxo-9,10-dihydropyrimido[1,2-*a*]purines, which bear a variety of substituents at position 7 (the second carbon of the allylidene bridge), have been prepared by reaction of the corresponding malondialdehydes (**1a–f**) with guanine (**2**) in aqueous 1 *N* HCl at 40°. The substituted malondialdehydes, by pK_a determination, show acidities similar to those of substituted acetic acids and, by their ultraviolet absorption spectra, show amphoteric behavior. The guanine products (**3**), structural analogues of the naturally occurring Y bases, are compared in terms of their NMR, ultraviolet, and fluorescence spectroscopic properties. The three ring protons of the tricyclic 1,N^2-(2-R-allylidene)guanine system show proton magnetic resonance signals at low field in trifluoroacetic acid indicative of aromatic ring current. The ultraviolet spectra of the products (**3**) exhibit long-wavelength absorption in aqueous acidic, neutral, and alkaline solution where guanine does not absorb, and their fluorescence spectra exhibit solvent dependence. In general, 1,N^2-[2-(*p*-methoxyphenyl)allylidene]guanine has the most favorable ultraviolet absorption and fluorescence emission properties, which suggests the potential utility of *p*-methoxyphenylmalondialdehyde in reactions with more complex guanine derivatives.

Recent investigations in our laboratory have been directed toward the preparation of modified tRNA bases[1] or tRNA base analogues[2–4] which are fluorescent. In considering reactions which involve modification of the existing tRNA bases, we have endeavored to devise or elaborate selective reactions which can be carried out under mild aqueous conditions compatible with the stability of nucleosides, nucleotides, coenzymes, and nucleic acids. Using these guidelines, we have turned our attention to the preparation of fluorescent guanine derivatives.

Structural elucidation of the "Y" bases (or "Wye" bases, imidazo[1,2-*a*]purines),[5–9] as naturally occurring tricyclic guanine derivatives found in $tRNA^{Phe}$ from yeast, wheat germ, and other sources, has stimulated the synthesis of nucleosides having related structures.[10,11] The naturally occurring Y bases and the recently prepared synthetic Y-base analogues are fluorescent. Consideration of these findings led us to conclude that reagents capable of cyclization reactions involving the 1-NH and the exocyclic 2-NH_2 substituent of guanine and providing three ring atoms and two double bonds would give convenient access to fluorescent guanine derivatives.[1] Earlier reports have indicated that