CRUDE DRUGS FROM AQUATIC PLANTS. VII.[1] FOUR NEW THIASPIRANE SULFOXIDE TYPE NUPHAR ALKALOIDS, NUPHARPUMILAMINES A, B, C, AND D, FROM CHINESE NUPHARIS RHIZOMA, THE RHIZOMA OF *NUPHAR PUMILUM* (TIMM.) DC. (NYMPHACEAE)†

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Abstract — Following the characterization of thiohemiaminal type nuphar alkaloids with immunosuppressive activity, four new thiaspirane sulfoxide type dimeric sesquiterpene alkaloids, nupharpumilamines A, B, C, and D, were isolated from Chinese Nupharis Rhizoma, the dried rhizoma of *Nuphar pumilum* (TIMM.) DC. The structures of nupharpumilamines A, B, C, and D were determined on the basis of chemical and physicochemical evidence.

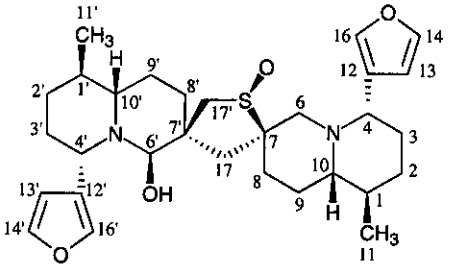
In the course of our studies for the bioactive constituents of natural medicines originating from aquatic plants,[2] we found that the alkaloid fraction from Chinese Nupharis Rhizoma, the dried rhizoma of *Nuphar pumilum* (TIMM.) DC. (Nymphaceae), showed potent inhibitory activity on the *anti*-sheep erythrocyte plaque forming cell formation in mice spleen cells. From the alkaloid fraction, four thiohemiaminal type nuphar alkaloids, 6-hydroxythiobinupharidine (**5**), 6,6'-dihydroxythiobinupharidine (**6**), 6-hydroxythionuphlutine B (**7**), and 6'-hydroxythionuphlutine B (**8**), were isolated as the immunosuppressive principles together with inactive nine known nuphar alkaloids. Furthermore, by examination of the structure requirement for the immunosuppressive activity, the thiohemiaminal structure of those alkaloids was found to be essential to the activity.[1,3] As a continuing study, we have isolated four new thiaspirane sulfoxide type dimeric sesquiterpene alkaloids named nupharpumilamines A (**1**), B (**2**), C (**3**), and D (**4**) from Chinese Nupharis Rhizoma In this paper, we describe the isolation and structure elucidation of nupharpumilamines A (**1**)—D (**4**).[4]

The alkaloid fraction from Chinese Nupharis Rhizoma[1,3] was subjected to normal silica gel and Chromatorex NH column chromatography and finally hplc (Develosil ODS HG-5, MeOH-H_2O-Et_2NH, MeCN-H_2O-Et_2NH) to afford **1** (0 0001%), **2** (0 0002%), **3** (0.0002%), and **4** (0.0005%).

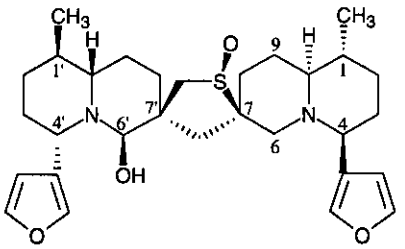
Nupharpumilamine A (1)

Nupharpumilamine A (**1**) was obtained as colorless oil with negative optical rotation ($[\alpha]_D^{25}$ -33.4°). The IR spectrum of **1** showed absorption bands at 3360, 1024, and 874 cm^{-1} ascribable to hydroxyl, sulfinyl, and furan functions and Bohlmann absorptions[5] at 2750—2900 cm^{-1} due to the *trans*-quinolizidine rings. The molecular formula $C_{30}H_{42}N_2O_4S$ of **1** was

† Dedicated to the memory of the late Professor Shun-ichi Yamada



nupharpumilamine A (**1**)



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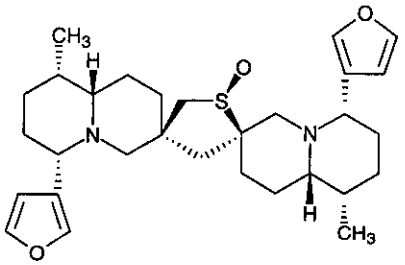
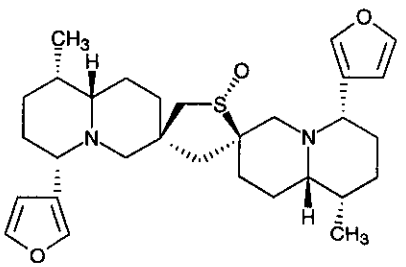


nupharpumilamine C (**3**)



nupharpumilamine D (**4**)

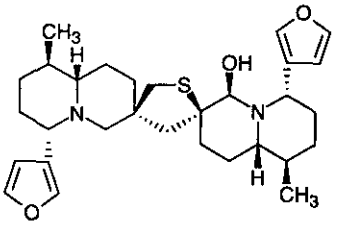
Figure 1

confirmed from the EI-MS and FAB-MS and by high-resolution MS measurement. Namely, the EI-MS and positive-ion FAB-MS of **1** showed dehydration peaks at *m/z* 508 (M^+-H_2O) and 509 (M+H-H_2O)$^+$, respectively, while a quasimolecular ion peak was observed at *m/z* 525 (M-H)$^-$ in the negative-ion FAB-MS of **1**. Furthermore, fragment ion peaks characteristic of thiohemiaminal type nuphar alkaloid[6] were observed at *m/z* 491, 447, 230, 178, and 94 in the EI-MS of **1**. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[7] of **1** showed two tertiary methyls [δ 0.92 (d, *J*=6.4 Hz, 11'-H_3) and 0.98 (d, *J*=6.4 Hz, 11-H_3)], two furans [δ 6.40 (dd, *J*=0.6, 1.5 Hz, 13, 13'-H), 7.37 (dd, *J*=0.6, 1.5 Hz, 16-H), 7.40 (dd, *J*=0.6, 1.5 Hz, 16'-H), and 7.43 (dd, *J*=1.5, 1.5 Hz, 14, 14'-H)], a thiolane sulfoxide [δ 2.02 (d, *J*=15.0 Hz, 17β-H), 2.28 (d, *J*=15.0 Hz, 17α-H), 2.32 (d, *J*=14.3 Hz, 17'α-H), and 2.92 (d, *J*=14.3 Hz, 17'β-H)], a hemiaminal-bearing quinolizidine ring [δ 2.35 (m, 10'-H), 3.68 (dd, *J*=3.4, 10.7 Hz, 4'-H), and 4.14 (s, 6'-H)], and a quinolizidine ring [δ 1.53 (m, 8β-H), 1.64 (m, 10-H), 1.66 (m, 8α-H), 1.89 (dd, *J*=1.2, 11.3 Hz, 6β-H), 2.54 (dd, *J*=2.7, 11.3 Hz, 6α-H), and 3.04 (dd, *J*=3.1, 11.6 Hz, 4-H)]. Comparison of the spectral data for **1** with those for 6-hydroxythiobinupharidine (**5**) and 6,6'-dihydroxythiobinupharidine (**6**)[1,6] led us to presume the thiohemiaminal type dimeric sesquiterpene structure of **1**. Treatment of **1** with sodium borohydride ($NaBH_4$) in methanol furnished *anti*-thiobinupharidine sulfoxide (**9**), whose absolute stereostructure was previously reported.[8]

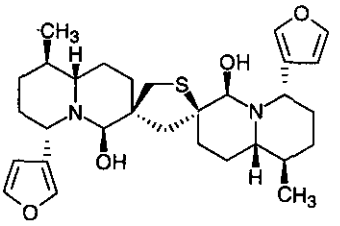
The position of the hydroxyl group in **1** was clarified by a heteronuclear multiple bond correlation (HMBC) experiment. Thus, long-range correlations were observed between the 6'-proton and the 4', 7', 8', 10'-carbons, between the 4'-proton and the 2', 3', 6', 12'-carbons, and between the 10'-proton and the 1', 4', 6'-carbons. The stereostructure of **1** including the configuration of the 6'-hydroxyl group was confirmed by a ^{1}H-NMR nuclear Overhauser and exchange spectroscopy (NOESY) experiment, which showed NOE correlations between the following protons ; 6'-H and 17β, 17'β, 8α-H shown in Figure 3. On the basis of above-mentioned evidence, the structure of nupharpumilamine A (**1**) was determined as shown.

Nupharpumilamine B (2)

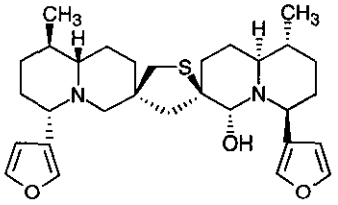
Nupharpumilamine B (**2**) was also isolated as colorless oil with negative optical rotation ($[\alpha]_D^{25}$ -60.1°). The IR spectrum of **2** showed absorption bands due to hydroxyl (3380 cm^{-1}), *trans*-fused quinolizidine ring (Bohlmann absorption, 2950—2750 cm^{-1}), sulfinyl (1021 cm^{-1}), and furan (874 cm^{-1}) functions. The EI-MS and positive-ion FAB-MS of **2** showed dehydration peaks [*m/z* 508 (M^+-H_2O), 509 (M+H-H_2O)$^+$] in addition of fragment ion peaks at *m/z* 491, 230, and 178. In the negative-ion FAB-MS of **2**, a quasimolecular ion peak was observed at *m/z* 525 $(M-H)^-$ and high-resolution MS measurement revealed the molecular formula of **2** to be $C_{30}H_{42}N_2O_4S$, which was identical with that of **1**. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[7] of **2** indicated the presence of two tertiary methyls [δ 0.78 (d, *J*=6.4 Hz, 11'-H_3) and 0.81 (d, *J*=6.4 Hz, 11-H_3)], two furans [δ 6.01 (dd, *J*=0.6, 1.5 Hz, 13'-H), 6.30 (dd, *J*=0.6, 1.8 Hz, 13-H), 6.89 (dd, *J*=1.5, 1.5 Hz, 14'-H), 7.17 (dd, *J*=0.6, 1.5 Hz, 16'-H), 7.31 (dd, *J*=1.5, 1.8 Hz, 14-H), and 7.35 (dd, *J*=0.6, 1.5 Hz, 16-H)], a thiolane sulfoxide [δ



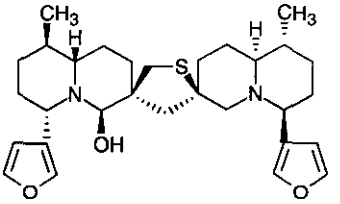
6-hydroxythiobinupharidine (**5**)



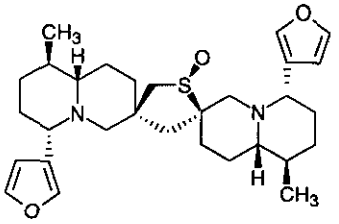
6,6'-dihydroxythiobinupharidine (**6**)



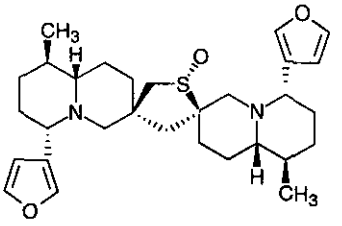
6-hydroxythionuphlutine B (**7**)



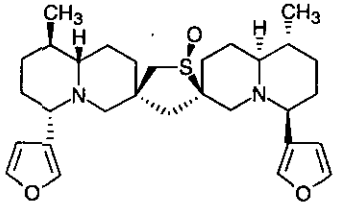
6'-hydroxythionuphlutine B (**8**)



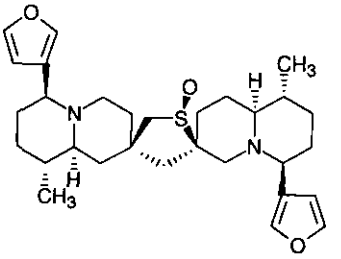
anti-thiobinupharidine sulfoxide (**9**)



syn-thiobinupharidine sulfoxide (**10**)

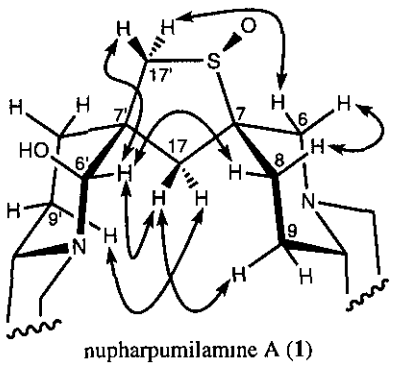


thionuphlutine B β-sulfoxide (**11**)

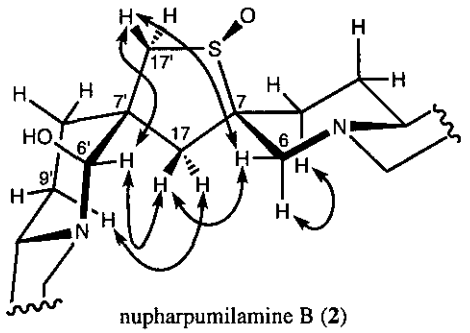


neothiobinupharidine β-sulfoxide (**12**)

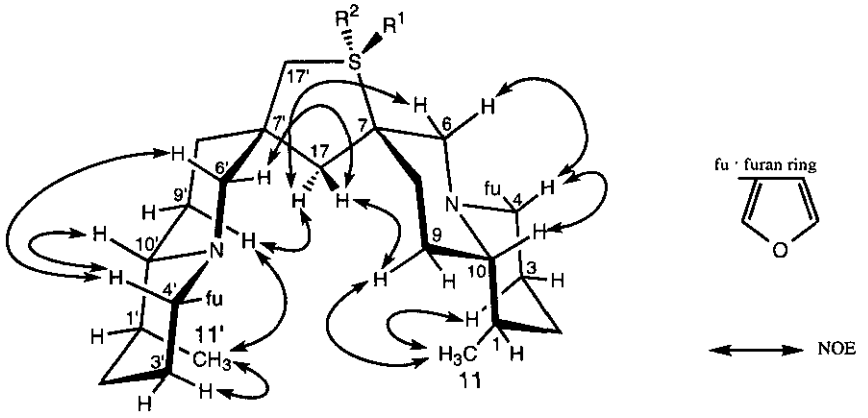
Figure 2



nupharpumilamine A (**1**)



nupharpumilamine B (**2**)



nupharpumilamine C (**3**) : R^1=O
nupharpumilamine D (**4**) : R^2=O

Figure 3. NOE Correlations in the NOESY Spectra of **1**, **2**, **3**, and **4**

1.56 (d, *J*=14.3 Hz, 17α-H), 1.89 (d, *J*=14.3 Hz, 17β-H)] and 2.35 (d, *J*=15.6 Hz, 17'α-H), 2.97 (d, *J*=15.6 Hz, 12'β-H)], a hemiaminal-bearing quinolizidine ring [δ 3.53 (dd, *J*=3.1, 11.6 Hz, 4'-H) and 4.08 (s, 6'-H)], and a quinolizidine ring [δ 1.67 (dd, *J*=0.9, 12.8 Hz, 6α-H), 3.20 (dd, *J*=2.4, 12.8 Hz, 6β-H), and 1.56 (m, 10-H)] From the comparison of the spectral data for **2** with those for **1** and 6'-hydroxythionuphlutine B (**8**), **2** was presumed to be a sulfoxide of **8**. In the HMBC experiment of **2**, long-range correlations were observed between the 6'-proton and the 4', 7', 8', 10'-carbons and between the 4'-proton and 6', 12'-carbons. The NOESY experiment of **2** showed NOE correlations as shown in Figure 3. The configuration of the sulfinyl group was clarified by comparison of the ^{1}H-NMR data for **2** with those for 6'-hydroxythionuphlutine B (**8**),[1,6a] which showed the characteristic anisotropy and proximity effects of the *anti*-sulfinyl group.[9] Finally, the $NaBH_4$ treatment of **2** yielded thionuphlutine B β-sulfoxide (**11**) Consequently, the structure of nupharpumilamine B (**2**) was characterized as shown.

Nupharpumilamine C (3)

Nupharpumilamine C (**3**), obtained as colorless oil with negative optical rotation ($[\alpha]_D^{25}$ -75.1°), showed Bohlmann absorption bands and absorption bands ascribable to sulfinyl and furan functions in the IR spectrum. The molecular formula $C_{30}H_{42}N_2O_3S$ of **3** was confirmed from the quasimolecular ion peak at *m/z* 511 $(M+H)^+$ observed in the positive-ion FAB-MS and by high-resolution MS measurement. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[7] of **3** showed signals

Table 1. ^{13}C-NMR Data for **1**, **2**, **3**, **4**, **9**, **10**, **11**, and **12**

	1[1)]	**2**[1)]	**3**[2)]	**4**[2)]	**9**[2)]	**10**[2)]	**11**[2)]	**12**[2)]
C-1	37.3	37.4	32.3	32.4	36.0	36.0	36.0	36.3
C-2	34.5	34.9	31.9[a)]	32.0[a)]	33.4	33.4	33.6	33.8
C-3	35.7	36.9	29.7	30.2	34.7	35.1[a)]	35.8	35 0[a)]
C-4	60.9	62.6	60.4	60.7	59.7	59.7[b)]	61.1	61.1
C-6	59.7	56.6	59.8	57.2	59.2	56.3	55.9	55.4
C-7	68.2	71.6	66.0	66.3	66.2	66.0	70.2	68.4
C-8	30.8	34.8	28.2	31.5	28.3	31.7	34.3	32.5
C-9	28.5[a)]	29.1	27.3	27.7	27.0	27 4	28.0	28 1
C-10	70.0	69.9	64.6	64.8	68.6	68.8	68.4	68 6
C-11	19.6	19.5	13.3	13.4	18.9	19.1	19.1	19.3
C-12	129 8	129.8	128.9	129.1	128.7	129.0[c)]	128.5	128.8
C-13	110.3	111.2	109.1	109.1	109.2	109.4[d)]	110.2	110 1
C-14	144.6	143.7	142.9	142.9	142.9	142.9	142.3	142.9
C-16	141.5	140.7	139.4	139.4	139.5	139.6[e)]	139.1	139.3
C-17	42.8	47.1	40.9	40.2	41.8	41.1	47.0	49.3
C-1'	38.5	38.6	32.3	32 4	36.1	36.0	36.3	36.6
C-2'	34.3	34.2	31.8[a)]	31.9[a)]	33.4	33.5	33.4	33.6
C-3'	37.2	37.2	29.8	29.8	34.9	34.9[a)]	35.0	36.0[a)]
C-4'	56.0	56.0	60.3	60.4	59.5	60.0[b)]	59.2	59.6
C-6'	96.4	96.6	64.7	64.7	64.2	64.2	65.9	65.4
C-7'	51.7	52.1	43.4	44.4	43.6	44.1	45.9	45.1
C-8'	32.9	33.4	38.2	40.3	38.4	40.2	38.8	39.8
C-9'	29.5a)	30.3	28.0	27.8	27.8	27.5	29.0	28.5
C-10'	59.5	58.8	64.6	64.7	68.6	68.8	68 1	68.5
C-11'	19.4	19 5	13.3	13.3	19.0	19.1	19.0	19.2
C-12'	130.1	129.9	129.1	129.3	128.9	128.8[c)]	128.7	129.6
C-13'	110.4	109.7	109.0	109.2	109.1	109.2[d)]	108.4	109.2
C-14'	144.5	144.4	142.8	142.9	142.8	142.9	143.1	143.5
C-16'	141.4	141.0	139.3	139 4	139.4	139.5[e)]	139.2	139 8
C-17'	58.2	56.9	60.3	59 7	60 0	59.4	59.6	57.5

The spectra were taken with CD_3OD[1)] or $CDCl_3$[2)].

a), b), c), d), e) Assignments may be interchangeable within the same column.

assignable to two tertiary methyls [δ 1.11 (d, *J*=7.0 Hz, 11'-H_3) and 1 13 (d, *J*=7.0 Hz, 11-H_3)], two furans [δ 6.34 (d, *J*=1.5 Hz, 13-H), 6.35 (d, *J*=1.5 Hz, 13'-H), 7.24 (br s, 16, 16'-H), 7.32 (dd, *J*=1.5, 1.5 Hz, 14-H), and 7.33 (dd, *J*=1.5, 1 5 Hz, 14'-H)], a thiolane sulfoxide [δ 1.92 (d, *J*=14.3 Hz, 17α-H), 2.15 (d, *J*=14.3 Hz, 17β-H), 2.43 (d, *J*=13.4 Hz, 17'β-H), and 2 56 (d, *J*=13.4 Hz, 17'α-H)], and two quinolizidine rings [δ 1.48 (d, *J*=11.6 Hz, 6'β-H), 1.69 (m,1'-H), 1 75 (m, 1-H), 1.85 (d, *J*=11.3 Hz, 6β-H), 1.90 (m, 10'-H), 2.01 (m, 10-H), 2.53 (dd, *J*=2.4, 11.3 Hz, 6α-H), 2.80 (dd, *J*=3.4, 11.6 Hz, 4'-H), 2.87 (dd, *J*=3.4, 11.6 Hz, 4-H), 2.90 (dd, *J*=2.4, 11.6 Hz, 6'α-H)]. The proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR spectra of **3** were superimposable on those of *anti*-thiobinupharidine sulfoxide (**9**),[8] except for the C-1—3 and C-1'—3' signals, which showed high-field shift by the axial 1 and 1'-methyl groups. The NOESY experiment of **3** showed NOE

correlations between the following protons : 4-H and 6β, 10-H; 11-H_3 and 3α, 9α-H; 17β-H and 9α, 6'α-H; 17α-H and 6α, 9'α-H; 11'-H_3 and 3'α, 9'α-H; 4'β-H and 6'β, 10'-H, as shown in Figure 3, so that the stereostructure including the C-1 and C-1' configurations of **3** was characterized. Comparison of the ^{1}H-NMR and ^{13}C-NMR data for **3** with those (Table 1) for *anti*- and *syn*-thiobinupharidine sulfoxides (**9**, **10**)[10] and neothiobinupharidine β-sulfoxide (**12**)[11] led us to confirm the structure of nupharpumilamine C (**3**) as shown.

Nupharpumilamine D (4)

Nupharpumilamine D (**4**) was also obtained as colorless oil with positive optical rotation ($[\alpha]_D^{25}$ +20.8°) and its IR spectrum showed Bohlmann absorption and absorption bands due to sulfinyl and furan functions. Here again, the molecular formula $C_{30}H_{42}N_2O_3S$ of **4**, which was identical with that of nupharpumilamine C (**3**), was determined from the quasimolecular ion peak at *m/z* 511 $(M+H)^+$ in the positive-ion FAB-MS and by high-resolution MS measurement. The ^{1}H-NMR and ^{13}C-NMR (Table 1) spectra[7] of **4** indicated the presence of two methyls, two furans, a thiolane sulfoxide, and two quinolizidine rings. The proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR (Table 1) spectra of **4** were very similar to those of *syn*-thiobinupharidine sulfoxide (**10**), except for the C-1—3 and C-1'—3' signals, which were significantly resembled with those of **3**. Furthermore, as is apparent from Figure 3, the NOE correlations in the NOESY experiment of **4** were almost the same as those of **3** On the basis of this evidence, the structure of nupharpumilamine D (**4**) was characterized as shown.

EXPERIMENTAL

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[2]

Isolation of Nupharpumilamine A (1), B (2), C (3), and D (4) from the Alkaloid Fraction of Chinese Nupharis Rhizoma

The *AcOEt soluble fraction (16.3g) from Chinese Nupharis Rhizoma* (Osaka, 7.9 kg) was subjected to ordinary silica gel column chromatography [$CHCl_3$-AcOEt-Et_2NH (20 : 1 : 1)→MeOH-Et_2NH (10 : 1)] to give three fractions [Fr. 1 (9.5 g), Fr. 2 (3.0 g), and Fr. 3 (3.8 g)] as described previously.[1] Fraction 1 (8.7 g) was separated by Chromatorex NH DM1020 (Fuji Silysia Chemical Ltd.) column chromatography [260 g; *n*-hexane-CH_2Cl_2-AcOEt (100 : 100 : 1→20 : 20 : 1)→$CHCl_3$-*MeOH (10 : 1)*] followed by repeated hplc [*Develosil ODS-HG-5, 250x20 mm i.d., i) MeOH-H_2O-Et_2NH* (890 : 10 : 6), ii) MeOH-H_2O-Et_2NH (750 : 150 : 6), iii) MeOH-H_2O-Et_2NH (800 : 100 : 6), iv) MeCN-H_2O-Et_2NH (89 : 1 : 3), flow rate : 8.0 ml/min] to give **3** (10.4 mg) and **4** (17.8 mg). Chromatorex NH DM1020 column chromatography [90 g, *n*-hexane-AcOEt (3 : 1→1 : 5)→AcOEt-acetone (5 : 1→1 : 5)→MeOH-Et_2NH (9 : 1)] of fraction **2** furnished six fractions. Fraction 2-3 (234 mg) was subjected to *HPLC (Develosil ODS-HG-5, 250x20 mm i.d., MeOH-H_2O-Et_2NH (850 : 50 : 6), flow rate :* 8.0 ml/min) to give **1** (17.4 mg). The HPLC (same conditions as described above) of fraction 2-4 (235 mg) yielded **2** (40.6 mg).

Nupharpumilamine A (**1**) : colorless oil, positive for Dragendorff reagent, $[\alpha]_D^{25}$ - 33.4° (*c*=0.6, $CHCl_3$). *High-resolution* EI-MS (*m/z*) : Calcd for $C_{30}H_{40}N_2O_3S$ (M^+-H_2O) : 508.2759; Found . 508.2752. High-resolution negative-ion FAB-MS (*m/z*) : Calcd for $C_{30}H_{41}N_2O_4S$ $(M-H)^-$: 525.2787; Found : 525.2769. IR (film, cm^{-1}) : 3360, 2950—2750, 1501, 1456, 1439, 1159, 1024, 874. ^{1}H-NMR (CD_3OD, 500 MHz, δ) . 0.92 (3H, d, *J*=6.4 Hz, 11'-H_3), 0.98 (3H, d, *J*=6.4 Hz, 11-H_3), 1.33 (1H, m, 9α-H), 1.53 (1H, m, 8β-H), 1.64 (1H, m, 10-H), 1.66 (1H, m, 8α-H), 1.89 (1H, dd, *J*=1.2, 11.3 Hz, 6β-H), 2.02 (1H, d, *J*=15.0 Hz, 17β-H), 2.28 (1H, d, *J*=15.0 Hz, 17α-H), 2.32 (1H, dd, *J*=14.3 Hz, 17'α-H), 2.35 (1H, m, 10'-H),

2.54 (1H, dd, *J*=2.7, 11.3 Hz, 6α-H), 2.92 (1H, d, *J*=14.3 Hz, 17'β-H), 3.04 (1H, dd, *J*=3.1, 11.6 Hz, 4-H), 3.68 (1H, dd, *J*=3.4, 10.7 Hz, 4'-H), 4.14 (1H, s, 6'-H), 6.40 (2H, dd, *J*=0 6, 1.5 Hz, 13, 13'-H), 7.37 (1H, dd, *J*=0.6, 1.5 Hz, 16-H), 7.40 (1H, dd, *J*=0.6, 1.5 Hz, 16'-H), 7.43 (2H, dd, *J*=1.5, 1.5 Hz, 14, 14'-H). ^{13}C-NMR (CD_3OD, 125 MHz, δ_C) : see Table 1. EI-MS (*m/z*) : 508 (M^+-H_2O), 491, 447, 230, 178, 94. Negative-ion FAB-MS (*m/z*) : 525 $(M-H)^-$, 459 $(M-C_4H_3O)^-$, 305 $(M-C_{14}H_{22}NO)^-$. Positive-ion FAB-MS (*m/z*) : 509 $(M+H-H_2O)^+$.

Nupharpumilamine B (**2**) : colorless oil, positive for Dragendorff reagent, $[\alpha]_D^{25}$ - 60.1° (*c*=1.6, $CHCl_3$). High-resolution EI-MS (*m/z*) : Calcd for $C_{30}H_{40}N_2O_3S$ (M^+-H_2O) : 508.2760; Found : 508.2755. High-resolution negative-ion FAB-MS (*m/z*) : Calcd for $C_{30}H_{41}N_2O_4S$ $(M-H)^-$: 525.2787; Found : 525.2806. IR (film, cm^{-1}) : 3380, 2950—2750, 1503, 1439, 1157, 1021, 874. ^{1}H-NMR (CD_3OD, 500 MHz, δ) : 0.78 (3H, d, *J*=6.4 Hz, 11'-H_3), 0.81 (3H, d, *J*=6.4 Hz, 11-H_3), 1.56 (1H, m, 10-H), 1.56 (1H, d, *J*=14.3 Hz, 17α-H), 1.67 (1H, dd, *J*=0.9, 12.8 Hz, 6α-H), 1.89 (1H, d, *J*=14.3 Hz, 17β-H), 2.24 (1H, ddd, *J*=2.4, 8.5, 11.0 Hz, 10'-H), 2.35 (1H, d, *J*=15.6 Hz, 17'α-H), 2.91 (1H, dd, *J*=6.4, 7.3 Hz, 4-H), 2.97 (1H, d, *J*=15.6 Hz, 17'β-H), 3.20 (1H, dd, *J*=2.4, 12.8 Hz, 6β-H), 3.53 (1H, dd, *J*=3.1, 11.6 Hz, 4'-H), 4.08 (1H, s, 6'-H), 6.01 (1H, dd, *J*=0.6, 1.5 Hz, 13'-H), 6.30 (1H, dd, *J*=0.6, 1.8 Hz, 13-H), 6.89 (1H, t, *J*=1.5 Hz, 14'-H), 7.17 (1H, dd, *J*=0.6, 1.5 Hz, 16'-H), 7.31 (1H, dd, *J*=1.5, 1.8 Hz, 14-H), 7.35 (1H, dd, *J*=0.6, 1.5 Hz, 16-H). ^{13}C-NMR (CD_3OD, 125 MHz, δ_C) : see Table 1. EI-MS (*m/z*) : 508 (M^+-H_2O), 491, 230, 178. Negative-ion FAB-MS (*m/z*) : 525 $(M-H)^-$, 459 $(M-C_4H_3O)^-$. Positive-ion FAB-MS (*m/z*) : 509 $(M+H-H_2O)^+$.

Nupharpumilamine C (**3**) : colorless oil, positive for Dragendorff reagent, $[\alpha]_D^{25}$ -75.1° (*c*=0.5, $CHCl_3$), High-resolution FAB-MS (*m/z*) : Calcd for $C_{30}H_{43}N_2O_3S$ $(M+H)^+$: 511.2994; Found : 511.2976. IR (film, cm^{-1}) : 2950—2750, 1501, 1449, 1381, 1159, 1026, 874. ^{1}H-NMR ($CDCl_3$, 500 MHz, δ) : 1.11 (3H, d, *J*=7.0 Hz, 11'-H_3), 1.13 (3H, d, *J*=7.0 Hz, 11-H_3), 1.48 (1H, d, *J*=11.6 Hz, 6'β-H), 1.69 (1H, m, 1'-H), 1.75 (1H, m, 1-H), 1.85 (1H, d, *J*=11.3 Hz, 6β-H), 1.90 (1H, m, 10'-H), 1.92 (1H, d, *J*=14.3 Hz, 17α-H), 2.01 (1H, m, 10-H), 2.15 (1H, d, *J*=14.3 Hz, 17β-H), 2.43 (1H, d, *J*=13.4 Hz, 17'β-H), 2.53 (1H, dd, *J*=2.4, 11.3 Hz, 6α-H), 2.56 (1H, d, *J*=13.4 Hz, 17'α-H), 2.80 (1H, dd, *J*=3.4, 11.6 Hz, 4'-H), 2.87 (1H, dd, *J*=3.4, 11.6 Hz, 4-H), 2.90 (1H, dd, *J*=2.4, 11.6 Hz, 6'α-H), 6.34 (1H, d, *J*=1.5 Hz, 13-H), 6.35 (1H, d, *J*=1.5 Hz, 13'-H), 7.24 (2H, br s, 16, 16'-H), 7.32 (1H, t, *J*=1.5 Hz, 14-H), 7.33 (1H, t, *J*=1.5 Hz, 14'-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, δ_C) : see Table 1. FAB-MS (*m/z*) : 511 $(M+H)^+$.

Nupharpumilamine D (**4**) : colorless oil, positive for Dragendorff reagent, $[\alpha]_D^{25}$ +20.8° (*c*=0.9, $CHCl_3$). High-resolution FAB-MS (*m/z*) ; Calcd for $C_{30}H_{43}N_2O_3S$ $(M+H)^+$: 511 2994; Found . 511.2980. IR (film, cm^{-1}) : 2950—2750, 1501, 1451, 1381, 1159, 1021, 874. ^{1}H-NMR ($CDCl_3$, 500 MHz, δ) : 1.14 (3H, d, *J*=7.0 Hz, 11'-H_3), 1.16 (3H, d, *J*=6.7 Hz, 11-H_3), 1.47 (1H, d, *J*=11.3 Hz, 6'β-H), 1.94 (1H, m, 10'-H), 1.95 (1H, d, *J*=11.3 Hz, 6α-H), 2.03 (1H, m, 10-H), 2.03 (1H, d, *J*=14.3 Hz, 17α-H), 2.33 (1H, d, *J*=14.3 Hz, 17β-H), 2 47 (1H, dd, *J*=2.4, 11 3 Hz, 6'α-H), 2.51 (2H, s, 17'-H_2), 2.83 (1H, dd, *J*=3.4, 11.9 Hz, 4'-H), 2.94 (1H, dd, *J*=3.4, 11.0 Hz, 4-H), 2.97 (1H, dd, *J*=2.4, 11.3 Hz, 6β-H), 6.38 (1H, d-like, 13-H), 6.38 (1H, d-like, 13'-H), 7.25 (1H, br s, 16'-H), 7.28 (1H, br s, 16-H), 7.34 (1H, t-like, 14-H), 7.34 (1H, t-like, 14'-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, δ_C) : Table 1. FAB-MS (*m/z*) : 511 $(M+H)^+$.

Treatment of Nupharpumilamine A (1) with $NaBH_4$ Giving *anti*-Thiobinupharidine Sulfoxide (9)

A solution of nupharpumilamine A (**1**, 2.3 mg) in MeOH (0.5 mL) was treated with $NaBH_4$ (4.6 mg) and the whole mixture was stirred at rt for 30 min. After treatment of the reaction mixture with acetone (0.5 mL), the whole was poured into brine and then it was extracted with AcOEt. The AcOEt extract was washed with brine and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave *anti*-thiobinupharidine sulfoxide (**9**, 2.2 mg), which was identified by HPTLC, HPLC, IR, and ^{1}H-NMR (CD_3OD) spectra comparisons with an authentic sample [1]

Treatment of Nupharpumilamine B (2) with $NaBH_4$ Giving Thionuphlutine B β-Sulfoxide (11)

A solution of nupharpumilamine B (**2**, 3.9 mg) in MeOH (0.5 mL) was treated with $NaBH_4$ (10.0 mg) and the whole mixture was stirred at rt for 30 min. The reaction mixture was worked up as described above to yield thionuphlutine B β-sulfoxide (**11**, 3.8 mg), which was identified by HPTLC, HPLC, IR, and ^{1}H-NMR (CD_3OD) spectra comparisons with an authentic sample.[1]

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