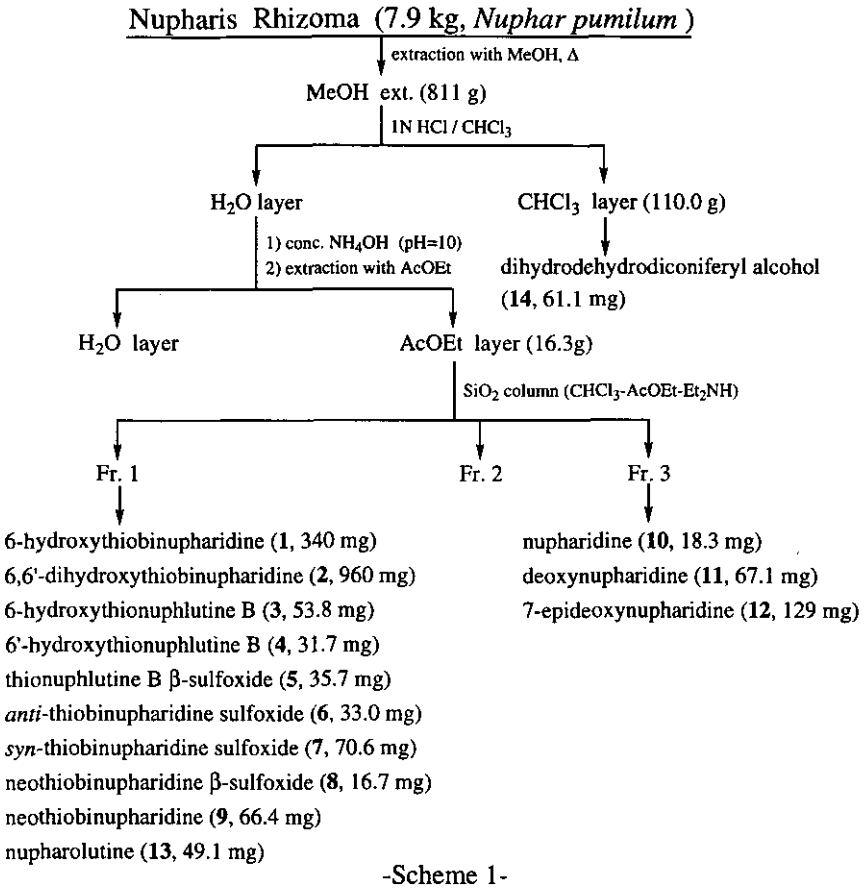
CRUDE DRUGS FROM AQUATIC PLANTS. VI.[1] ON THE ALKALOID CONSTITUENTS OF CHINESE NUPHARIS RHIZOMA, THE DRIED RHIZOMA OF *NUPHAR PUMILUM* (TIMM.) DC. (NYMPHACEAE) : STRUCTURES AND REARRANGEMENT REACTION OF THIOHEMIAMINAL TYPE NUPHAR ALKALOIDS

Masayuki Yoshikawa,* Toshiyuki Murakami, Shuji Wakao, Atsushi Ishikado, Nobutoshi Murakami, Johji Yamahara, and Hisashi Matsuda

Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan

Abstract — From Chinese Nupharis Rhizoma, the dried rhizoma of *Nuphar pumilum* (TIMM.) DC. (Nymphaceae), four thiohemiaminal type nuphar alkaloids with potent immunosuppressive activity, 6-hydroxythiobinupharidine, 6,6'-dihydroxythiobinupharidine, 6-hydroxythionuphlutine B, 6'-hydroxythionuphlutine B, have been isolated together with nine inactive nuphar alkaloids. The configurations of the 6- and 6'-hydroxyl groups in those thiohemiaminal type nuphar alkaloids were confirmed on the basis of their 2D-NMR analysis. During the course of chemical elucidation of those thiaspirane type dimeric sesquiterpene alkaloids, new rearrangement reaction of the thiaspirane ring in thiohemiaminal type alkaloids with 6-hydroxyl group was found and the reaction pathway was discussed. By using this rearrangement reaction, 6-hydroxythionuphlutine B was chemically related with 6-hydroxythiobinupharidine, so that the absolute stereostructures of thionuphlutine B and its related compounds such as 6-hydroxythionuphlutine B, 6'-hydroxythionuphlutine B, and thionuphlutine B β-sulfoxide, were characterized.

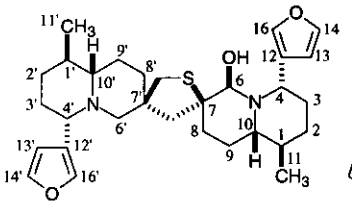
Nupharis Rhizoma [Japanese name "Senkotsu (川骨)"], which is a natural medicine indigenous to Japan, has been combined in Japanese traditional preparations used as an antipyretic, analgesic, and anti-inflammatory. The botanical origin of this natural medicine was prescribed as the dried rhizome of an aquatic plant *Nuphar japonicum* DC. (Nymphaceae) in Japanese Pharmacopea XIII. On the other hand, Chinese Nupharis Rhizoma [Chinese name "Ping peng cao gen (萍蓬草根)"], which was prepared from the rhizoma of *Nuphar pumilum* (TIMM.) DC., has been used as a tonic and diuretic and also for treatment of a menstrual disorder and bloodstasis syndrome in Chinese traditional medicine. In recent years, due to the poor supply of Nupharis Rhizoma, Chinese Nupharis Rhizoma has been imported and commonly used in the traditional preparations. In regard to chemical studies on the constituents of Nupharis Rhizoma, alkaloids and tannins have been isolated from the rhizoma of *Nuphar japonicum*.[2,3] Especially, the alkaloid constituents, which are the principal ingredients of this natural medicine, have been the subjects of many investigations and various monomeric sesquiterpen alkaloids such as

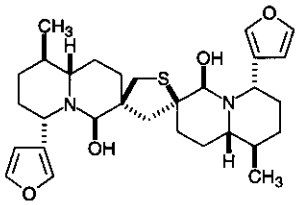


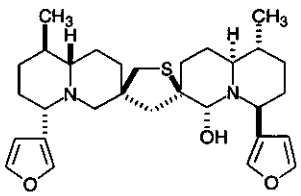
-Scheme 1-

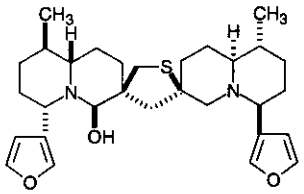
nupharidine (**10**) and deoxynupharidine (**11**) have been characterized.[2] However, as the pharmacological study of Nuphar alkaloids, only the central paralysis effect of deoxynupharidine (**11**) has been reported so far[4] and also no report on Chinese Nupharis Rhizoma has been published to date as far as we know.

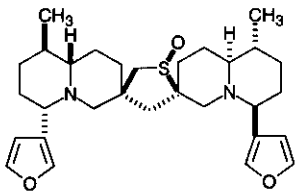
In the course of our studies for the bioactive constituents of natural medicines originating from aqueous plants,[1,5] we found that the alkaloid fraction from Chinese Nupharis Rhizoma showed potent immunosuppressive activity. From the alkaloid fraction, four thiohemiaminal type dimeric sesquiterpene alkaloids, 6-hydroxythiobinupharidine (**1**), 6,6'-dihydroxythiobinupharidine (**2**), 6-hydroxythionuphlutine B (**3**), and 6'-hydroxythionuphlutine B (**4**), were isolated as the immunosuppressive principles[6] together with inactive nine nuphar alkaloids, thionuphlutine B β-sulfoxide (**5**), *anti*-thiobinupharidine sulfoxide (**6**), *syn*-thiobinupharidine sulfoxide (**7**), neothiobinupharidine β-sulfoxide (**8**), neothiobinupharidine (**9**), nupharidine (**10**), deoxynupharidine (**11**), 7-epideoxynupharidine (**12**), and nupharolutine (**13**). 6-Hydroxythiobinupharidine (**1**), 6,6'-dihydroxythiobinupharidine (**2**), and 6-hydroxythionuphlutine B (**3**) have already been isolated from the rhizoma of *Nuphar luteum* and also 6'-hydroxythionuphlutine B (**4**) has been derived from 6,6'-dihydroxythionuphlutine B by partial reduction with sodium borohydride.[7,8] The absolute stereostructures of **1** and **2** have been reported on the basis of chemical and physicochemical evidence except for the configurations of the 6- and 6'-hydroxyl groups in the thiohemiaminal moiety.[7] Recently, the configurations of the 6- and 6'-hydroxyl groups in **1** and **2** were reported on the basis of the detailed ^{13}C-NMR analysis of 6-hydroxythiobinupharidine sulfoxide.[9] On the other hand, the absolute stereostructures and configurations of the thiohemiaminal hydroxyl groups in **3** and **4** were left uncharacterized.[8] This paper

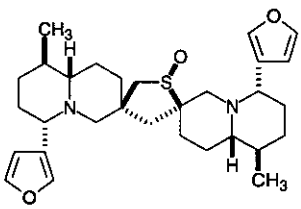

6-hydroxythiobinupharidine (**1**)

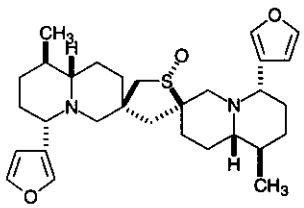

6,6'-dihydroxythiobinupharidine (**2**)

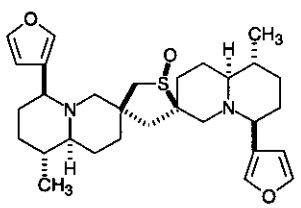

6-hydroxythionuphlutine B (**3**)

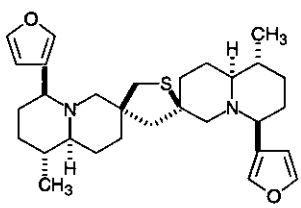

6'-hydroxythionuphlutine B (**4**)

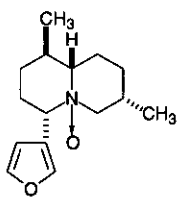

thionuphlutine B β-sulfoxide (**5**)

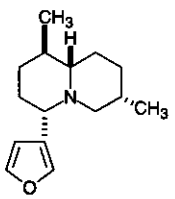

anti-thiobinupharidine sulfoxide (**6**)

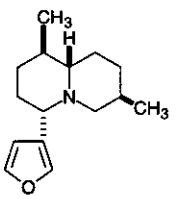

syn-thiobinupharidine sulfoxide (**7**)

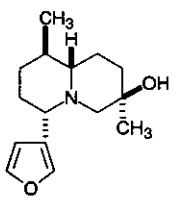

neothiobinupharidine β-sulfoxide (**8**)

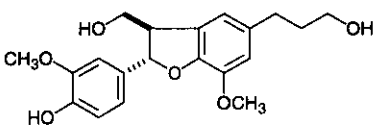

neothiobinupharidine (**9**)


nupharidine (**10**)


deoxynupharidine (**11**)


7-epideoxynupharidine (**12**)


nupharolutine (**13**)


dihydrodehydrodiconiferyl alcohol (**14**)

-Scheme 2-

deals with the isolation of alkaloid constituents from Chinese Nupharis Rhizoma and reinvestigation of the thiohemiaminal structures in **1**, **2**, **3**, and **4** using 2D-NMR experiment. In addition, we describe new rearrangement reaction of the thiaspirane ring peculiar to thiohemiaminal type nuphar alkaloids with 6-hydroxyl group such as **1**, **2**, and **3**.[10]

Chinese Nupharis Rhizoma was extracted with methanol under reflux and the methanolic extract was partitioned into a 1 N aqueous HCl-chloroform mixture. The acidic aqueous phase was made to basic with concentrated ammonium hydroxide and then extracted with ethyl acetate. Since the ethyl acetate-soluble portion (so-called alkaloid fraction) showed inhibitory activity on the anti-sheep erythocyte (SRBC)-plaque forming cell (PFC) formation in mice spleen cells,[6] it was subjected to normal-

Table 1. ^{1}H-NMR Data of **1**, **2**, **3**, and **4**

	1	**2**	**3**	**4**
1-H	1.26*	1.26*	1.18*	1.47*
2α-H	1.64*	1.62*	1.56*	1.70*
2β-H	1.09*	1.10*	1.04*	1.17*
3-H_2	1.66*	1.65*	1.48*, 1.60*	1.66*, 1.76*
4-H	3.63 (dd, *J*=4.5, 10.5 Hz)	3.70 (dd, *J*=5.0, 8.0 Hz)	3.63 (dd, *J*=3.0, 11.5 Hz)	3.12 (dd-like)
6α-H	3.77 (s)	3.77 (s)	3.85 (s)	1.83 (d, *J*=11.5 Hz)
6β-H				2.77 (d, *J*=11.5 Hz)
8α-H	1.31*	1.40*	1.70*	1.56*
8β-H	1.84*	1.86*	1.88*	1.88*
9α-H	1.26*	1.21*	1.77*	1.94*
9β-H	1.80*	1.82*	1.53*	1.41*
10-H	2.28*	2.32*	2.31*	1.77*
11-H_3	0.90 (d, *J*=6.0 Hz)	0.92 (d, *J*=6.5 Hz)	0.87 (d, *J*=6.5 Hz)	0.92 (d, *J*=6.5 Hz)
13-H	6.43 (d, *J*=1.7 Hz)	6.43 (d, *J*=1.0 Hz)	6.44 (d, *J*=1.5 Hz)	6.44 (d, *J*=1.2 Hz)
14-H	7.41 (t, *J*=1.7 Hz)	7.45 (t, *J*=1.0 Hz)	7.40 (t, *J*=1.5 Hz)	7.38 (t-like)
16-H	7.35 (br s)	7.43 (br s)	7.41 (br s)	7.33 (br s)
17α-H	1.63 (d, *J*=13.5 Hz)	1.79 (d, *J*=13.0 Hz)	1.62 (d-like)	1.61 (s)
17β-H	2.07 (d, *J*=13.5 Hz)	1.84 (d, *J*=13.0 Hz)	1.69 (d-like)	1.61 (s)
1'-H	1.50 (d, *J*=2.9 Hz)	1.28*	1.36*	1.17*
2'α-H	1.72*	1.62*	1.63*	1.59*
2'β-H	1.16*	1.10*	1.11*	1.07*
3'-H_2	1.66*, 1.79*	1.65*	1.58*	1.50*, 1.62*
4'-H	2.92 (d-like)	3.65 (dd, *J*=5.0, 8.0 Hz)	2.92 (dd, *J*=3.5, 10.5 Hz)	3.63 (dd, *J*=3.0, 11.0 Hz)
6'α-H	2.92 (d, *J*=11.5 Hz)	3.93 (s)	2.68 (d, *J*=11.0 Hz)	3.57 (s)
6'β-H	1.52 (d, *J*=11.5 Hz)		1.53 (d, *J*=11.0 Hz)	
8'α-H	1.53*	1.60*	1.20*	1.73*
8'β-H	1.19*	1.27*	1.76*	1.58*
9'α-H	1.31*	1.21*	1.20*	1.16*
9'β-H	1.89*	1.79*	1.88*	1.84*
10'-H	1.53*	2.35*	1.53*	2.32*
11'-H_3	0.94 (d, *J*=6.5 Hz)	0.93 (d, *J*=6.5 Hz)	0.90 (d, *J*=6.5 Hz)	0.88 (d, *J*=6.5 Hz)
13'-H	6.41 (d, *J*=1.7 Hz)	6.42 (d, *J*=1.0 Hz)	6.23 (d, *J*=1.5 Hz)	6.26 (d, *J*=1.2 Hz)
14'-H	7.46 (t, *J*=1.7 Hz)	7.45 (t, *J*=1.0 Hz)	7.17 (t, *J*=1.5 Hz)	7.36 (t-like)
16'-H	7.43 (br s)	7.43 (br s)	7.27 (br s)	7.33 (br s)
17'α-H	2.25 (d, *J*=12.0 Hz)	2.19 (d, *J*=12.0 Hz)	2.20 (d, *J*=11.5 Hz)	2.30 (d, *J*=12.5 Hz)
17'β-H	2.30 (d, *J*=12.0 Hz)	2.64 (d, *J*=12.0 Hz)	2.39 (d, *J*=11.5 Hz)	2.54 (d, *J*=12.5 Hz)

*Signals were overlapped

phase silica gel and Chromatorex NH column chromatography and finally HPLC to afford **1** (0.019%), **2** (0.020%), **3** (0.003%), and **4** (0.002%) together with **5** (0.0005%), **6** (0.0005%), **7** (0.001%), **8** (0.0003%), **9** (0.001%), **10** (0.0002%), **11** (0.002%), **12** (0.0008%), and **13** (0.004%). The chloroform-soluble portion was separated by repeated normal-phase silica gel and reversed-phase silica gel column chromatography and HPLC to give dihydrodehydrodiconiferyl alcohol (**14**, 0.001%).

Table 2. ^{13}C-NMR data for **1**, **2**, **3**, and **4**

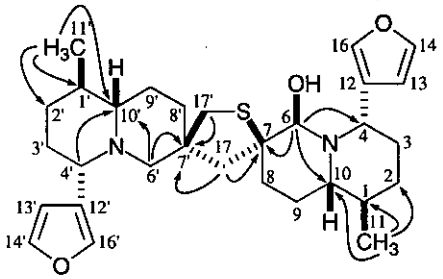
	1	**2**	**3**	**4**
C-1	38.4	38.3	38.3	36.6
C-2	34.4	34.4	34.5	34.4
C-3	37.2	37.3[a]	37.8	35.6
C-4	56.1	55.9[b]	56.3	61.8
C-6	97.5	96.9	98.2	64.0
C-7	64.1	63.5	64.5	59.5
C-8	34.7	34.8	33.3	39.2
C-9	30.4	30.3	29.7	29.8
C-10	59.8	59.9	60.1	70.7
C-11	19.6	19.7	19.6	19.3
C-12	130.2	130.3[c]	130.4	130.2
C-13	110.7	110.8	111.1	110.5
C-14	144.2[a]	144.4[d]	144.0	144.3
C-16	141.1[b]	141.6[e]	140.9	141.2
C-17	45.8	45.9	49.9	51.5
C-1'	37.3	38.5	37.3	38.5
C-2'	34.8	34.4	34.6	34.3
C-3'	35.8	37.1[a]	36.0	37.3
C-4'	61.6	56.1[b]	61.1	55.9
C-6'	64.3	96.0	66.0	97.1
C-7'	49.6	55.4	48.4	52.9
C-8'	38.2	31.8	37.8	32.4
C-9'	29.5	29.6	29.5	29.1
C-10'	71.3	60.7	70.7	60.2
C-11'	19.6	19.8	19.5	19.6
C-12'	130.2	130.1[c]	130.3	130.2
C-13'	110.6	110.8	109.8	110.5
C-14'	144.4[a]	144.3[d]	144.3	144.5
C-16'	141.5[b]	141.5[e]	140.7	141.2
C-17'	42.9	40.8	43.1	41.9

The spectra were taken with CD_3OD.

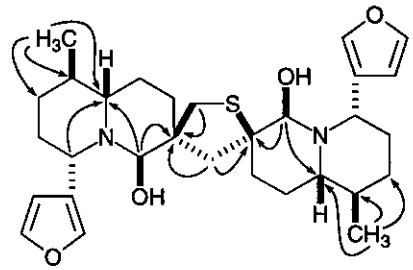
a, b, c, d, e Assignments may be interchangeable within the same column.

Configurations of the Thiohemiaminal Hydroxyl Groups in 1, 2, 3, and 4

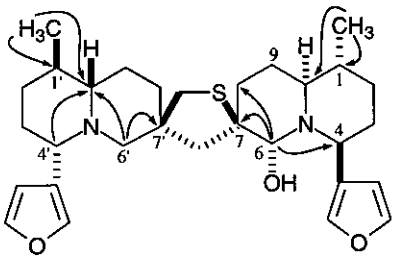
In order to confirm the identification of **1**, **2**, **3**, and **4**, those alkaloids were subjected to the reductive dehydroxylation with sodium borohydride.[7,8] Namely, treatment of **1** and **2** with sodium borohydride in methanol yielded thiobinupharidine (**15**),[11] respectively, while **3** and **4** gave thionuphlutine B (**16**)[7a] by the same treatment. The ^{1}H-NMR (Table 1) and ^{13}C-NMR (Table 2) of **1**, **2**, **3**, and **4** were assigned with the aid of homo and hetero correlation spectroscopy (^{1}H-^{1}H, ^{1}H-^{13}C COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond correlation (HMBC), which showed long-range correlations between the protons and carbons in Figure 1. In the ^{1}H-NMR nuclear Overhauser effect spectroscopy (NOESY) of **1**, **2**, **3**, and **4**, NOE correlations were observed between the 6-equatorial proton and 17α, 17'α, 8'-



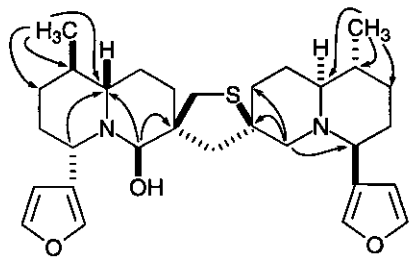
6-hydroxythiobinupharidine (**1**)



6,6'-dihydroxythiobinupharidine (**2**)



6-hydroxythionuphlutine B (**3**)



6'-hydroxythionuphlutine B (**4**)

Figure 1. HMBC Correlations of **1**, **2**, **3**, and **4**

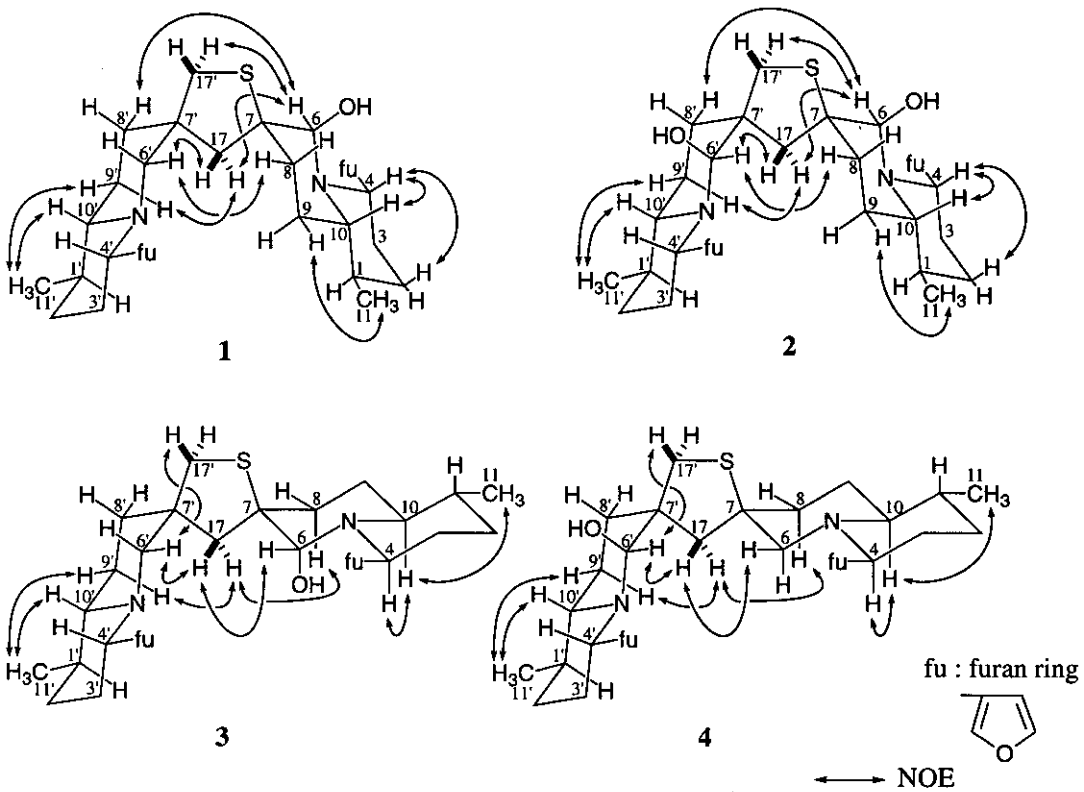


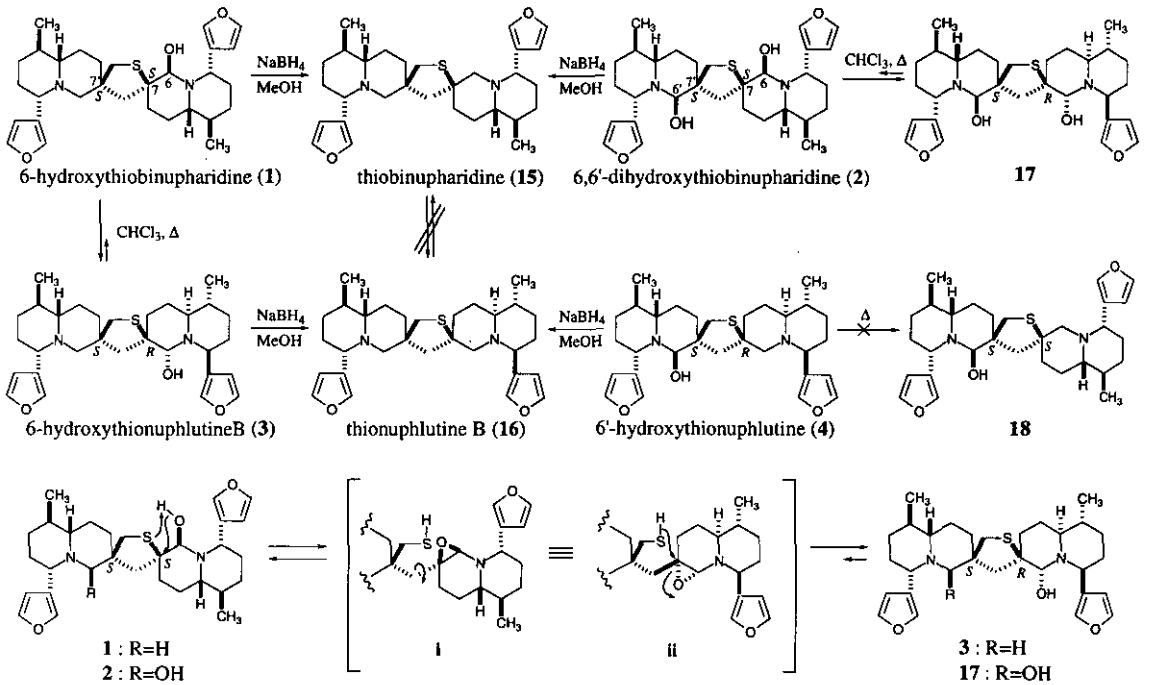
Figure 2. NOESY Correlations of **1**, **2**, **3**, and **4**

equatorial protons in **1** and **2**, between the 6'-equatorial proton and the 17β, 8-equatrial protons in **2**, between the 6-equatorial proton and 17β-proton in **3**, and between the 6'-equatorial proton and the 17β, 17'β-protons in **4** as shown in Figure 2. On the basis of the above mentioned 2D-NMR evidence, the configurations of the thiohemiaminal hydroxyl groups in **1** and **2** were confirmed, and also those of **3** and **4** were characterized.

Rearrangement of the Thiaspirane Ring Specific to Thiohemiaminal Type Nuphar Alkaloids with 6-Hydroxyl Group

During the course of structure elucidation of thiohemiaminal type nuphar alkaloids, we have found that **1** partly changes to **3** in the meantime of the NMR measurement. After preliminary examination of the rearrangement condition, thermal treatment of **1** in chloroform solution furnished a mixture of **1** and **3** (*ca.* 6 : 4), while **3** changed to **1** in a small amount to furnish a mixture of **1** and **3** (*ca.* 1 : 9) by the same treatment. The thermal treatment of **2** gave a mixture of **2** and 6,6'-dihydroxythionuphlutine B (**17**) (*ca.* 65 : 35), while **17** was found to change to **2** slightly by the thermal treatment.

In order to shed light on the reaction pathway for the rearrangement of the thiaspirane ring in **1**, **2**, and **3**, we examined the thermal treatment of some dimeric sesquiterpene nuphar alkaloids with thiaspirane ring. In the case of thiobinupharidine (**15**) and thionuphlutine B (**16**), which lacked the hemiaminal hydroxyl group of **1**, **2**, and **3**, the rearrangement was not observed and each starting alkaloids (**15**, **16**) were recovered. Furthermore, 6'-hydroxythionuphlutine B (**4**) was also found to give no rearranged product (**18**). This finding led us to confirm that this rearrangement of the thiaspirane ring was specific to the thiohemiaminal type nuphar alkaloids with 6-hydroxyl group and, although some other pathways could be considered, the rearrangement reaction could presumably be explained by the pathway shown in Scheme 3. Namely, the thio-ether group in **1** and **2** may initially abstract a hydrogen atom from the neighboring 6-hydroxyl group to form a epoxide intermediate (**i**), which would gave the rearranged product (**3** and **17**) though the attack of thiol group to the epoxide in **i**.



-Scheme 3-

Reduction of **1** and **2** with sodium borohydride yielded thiobinupharidine (**15**), whose absolute stereostructure was determined by X-Ray crystal analysis,[11] whereas reduction of **3** and **4** gave thionuphlutine B (**16**). Since **3** was related with **1** by this rearrangement reaction, the absolute stereostructures of **16** and its related compounds such as **3**, **4**, and **5** were determined.

EXPERIMENTAL

The instruments used to obtain physical data and experimental conditions for chromatography were the same as described in our previous paper.[1]

Isolation of Alkaloid Constituents (1-13) and a Phenyl Propanoid (14) from Chinese Nupharis Rhizoma

Chinese Nupharis Rhizoma (7.9 kg, imported from China through Shinwa Bussan & Co., Ltd., Osaka) were cut finely and then extracted three times with MeOH (8 L) under reflux. After removal of the solvent from the MeOH solution under reduced pressure, the extract (811 g) was partitioned into 1 N aqueous HCl-$CHCl_3$ (1 : 1) solution (8 L). The 1 N aqueous HCl solution was made to a pH of about 10 with conc. NH_4OH and then extracted with AcOEt. After removal of the solvent under reduced pressure from the AcOEt-soluble portion, the AcOEt extract (16.3 g) was subjected to ordinary-phase silica gel column chromatography [60 g, $CHCl_3$-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)]. Fraction 1 (8.7 g) was further 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)] to furnish six fractions [Fr. 1-1 (3.0 g), Fr. 1-2 (390 mg), Fr. 1-3 (790 mg), Fr. 1-4 (370 mg), Fr. 1-5 (2.1 g), Fr. 1-6 (1.5 g)]. Fraction 1-2 (390 mg) was purified with HPLC [Develosil ODS-HG-5 (Nomura Chemical Co., 250x20 mm i.d.), MeOH-H_2O-Et_2NH (890 : 10 : 6, v/v)] to give **1** (330 mg) and **3** (53.8 mg). Repeated HPLC [Develosil ODS-HG-5, i) MeOH-H_2O-Et_2NH (890 : 10 : 6, v/v), ii) MeCN-H_2O-Et_2NH (89 : 1 : 3, v/v)] of fraction 1-3 (370 mg) afforded **4** (31.7 mg). Fraction 1-4 (2.1 g) was subjected to repeated HPLC (the same conditions as the case of fraction 1-3) to give **2** (960 mg), **5** (35.7 mg), **6** (33.0 mg), **7** (70.6 mg), **8** (16.7 mg), **9** (66.4 mg), and **13** (49.1 mg). Fraction 3 (2.8 g) was separated with Chromatorex NH DM1020 [150g, $CHCl_3$-AcOEt-Et_2NH (60 : 2 : 5)→$CHCl_3$-MeOH-Et_2NH (10 : 1 : 1)→MeOH] followed by ordinary-phase silica gel column chromatography [150 g, $CHCl_3$-MeOH (30 : 1→20 : 1→10 : 1)→MeOH] to provide **10** (18.3 mg), **11** (67.1 mg), and **12** (129 mg). After removal of the solvent under reduced pressure from the $CHCl_3$-soluble portion, the $CHCl_3$ extract (110.0 g) was subjected to repeated ordinary-phase silica gel column chromatography [i) 2.0 kg, $CHCl_3$-MeOH (50 : 1→5 : 1)→$CHCl_3$-MeOH-H_2O (6 : 4 : 1)→MeOH, ii) 300 g, $CHCl_3$-MeOH-H_2O (30 : 3 : 1, lower phase)→$CHCl_3$-MeOH (1 : 1)→MeOH] followed by reversed-phase silica gel column chromatography [50 g, MeOH-H_2O (4 : 6→5 : 5→6 : 4)→MeOH] and HPLC [YMC-Pack R&D ODS-5A (YMC Co., Ltd., 250x20 mm i.d.), MeOH-H_2O (50 : 50, v/v)] to yield **14** (61.1 mg). Those known compounds obtained from Chinese Nupharis Rhizoma were identified by comparison of their physical data with reported values [thionuphlutine B β-sulfoxide (**5**),[12] *anti*-thiobinupharidine sulfoxide (**6**),[9] *syn*-thiobinupharidine sulfoxide (**7**),[9] neothiobinupharidine β-sulfoxide (**8**),[13] neothiobinupharidine (**9**),[14] nupharidine (**10**),[14b,15] deoxynupharidine (**11**),[14b,16] 7-epideoxynupharidine (**12**),[14b,17] nupharolutine (**13**),[14b,18] and dihydrodehydrodiconiferyl alcohol (**14**)[19]].

6-Hydroxythiobinupharidine. (**1**) : Colorless oil, Dragendorff reagent : +, $[\alpha]_D^{25}$ +39.2° (*c*=3.0, CH_2Cl_2), lit.,[7b] $[\alpha]_D^{25}$ +33.0° (*c*=3.6, CH_2Cl_2). High-resolution EI-MS (*m/z*) : Calcd for $C_{30}H_{40}N_2O_2S$ (M^+-H_2O) : 492.2810; Found: 492.2815.

IR (film, cm^{-1}) : 3500, 2950-2750, 1501, 1157, 874. ^{1}H-NMR (CD_3OD, 500 MHz, δ) : given in Table 1. ^{13}C-NMR (CD_3OD, 125 MHz, δc) : given in Table 2. EI-MS (*m/z*) : 492 (M^+-H_2O).

6,6'-Dihydroxythiobinupharidine (**2**) : Colorless oil, Dragendorff reagent : +, $[\alpha]_D^{25}$ +78.0° (*c*=1.5, CH_2Cl_2), lit.,[7c] $[\alpha]_D^{25}$ +80.0° (*c*=1.73, CH_2Cl_2). High-resolution EI-MS (*m/z*) : Calcd for $C_{30}H_{38}N_2O_2S$ (M^+-$2H_2O$) : 490.2654; Found : 490.2639. IR (film, cm^{-1}) : 3500, 2950-2750, 1501, 1159, 1065, 874. ^{1}H-NMR (CD_3OD, 500 MHz, δ) : given in Table 1. ^{13}C-NMR (CD_3OD, 125 MHz, δc) : given in Table 3. EI-MS (*m/z*) : 490 (M^+-$2H_2O$).

6-Hydroxythionuphlutine B (**3**) : Colorless oil, Dragendorff reagent : +, $[\alpha]_D^{25}$ -37.2° (*c*=1.6, $CHCl_3$). High-resolution EI-MS (*m/z*) : Calcd for $C_{30}H_{40}N_2O_2S$ (M^+-H_2O) : 492.2811; Found : 492.2823. IR (film, cm^{-1}) : 3450, 2950-2750, 1501, 1456, 1157, 1065, 874. ^{1}H-NMR (CD_3OD, 500 MHz, δ) : given in Table 1. ^{13}C-NMR (CD_3OD, 125 MHz, δc) : given in Table 2. EI-MS (*m/z*) : 492 (M^+-H_2O).

6'-Hydroxythionuphlutine B (**4**) : Colorless oil, Dragendorff reagent : +, $[\alpha]_D^{25}$ +55.2° (*c*=1.6, $CHCl_3$). High-resolution EI-MS (*m/z*) : Calcd for $C_{30}H_{40}N_2O_2S$ (M^+-H_2O) : 492.2811; Found : 492.2810. IR (film, cm^{-1}) : 3430, 2950-2750, 1505, 1456, 1156, 874. ^{1}H-NMR (CD_3OD, 500 MHz, δ) : given in Table 1. ^{13}C-NMR (CD_3OD, 125 MHz, δc) : given in Table 2. EI-MS (*m/z*) : 492 (M^+-H_2O).

Treatment of 6-Hydroxythiobinupharidine (1) and 6,6'-Dihydroxythiobinupharidine (2) with $NaBH_4$ Giving Thiobinupharidine (15)

A solution of **1** (20.0 mg) or **2** (30.0 mg) in MeOH (2.0 mL for **1**, 3.0 mL for **2**) was treated with $NaBH_4$ (40.0 mg for **1**, 120.0 mg for **2**) 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 $MgSO_4$, then filtered. Removal of the solvent under reduced pressure from the filtrate gave **15** (19.4 mg from **1**, 28.2 mg from **2**), which was identified by comparison of the physical data with the reported values.[11]

Treatment of 6-Hydroxythionuphlutine B (3) and 6'-Hydroxythionuphlutine B (4) with $NaBH_4$ Giving Thionuphlutine B (16)

A solution of **3** (13.4 mg) or **4** (13.0 mg) in MeOH (1.0 mL) was treated with $NaBH_4$ (50.0 mg) and the whole mixture was stirred at rt for 30 min. The reaction mixture was worked up as described above to yield **16** (13.0 mg from **3**, 12.6 mg from **4**), which was identified by comparison of the physical data with the reported values.[7a]

Rearrangement Reaction of 6-Hydroxythiobinupharidine (1) and 6-Hydroxythionuphlutine B (3)

1) A solution of **1** (8.3 mg) in $CHCl_3$ (1.0 mL) was heated under reflux for 24 h. After removal of the solvent under reduced pressure, the residue was separated with HPLC [Develosil ODS-HG-5, MeOH-H_2O-Et_2NH (890 : 10 : 6, v/v)] to give **1** (2.6 mg) and **3** (1.7 mg), which were identified with authentic 6-hydroxythiobinupharidine and 6-hydroxythionuphlutine B by TLC, HPLC, and ^{1}H-NMR spectrum comparisons.

2) A solution of **3** (14.1 mg) in $CHCl_3$ (1.0 mL) was heated under reflux for 24 h and the reaction solution was worked up as described above to give **1** (1.2 mg) and **3** (10.1 mg), which were identified with authentic samples as described above.

Rearrangement Reaction of 6,6'-Dihydroxythiobinupharidine (2) and 6,6'-Dihydroxythionuphlutine B (17)

A solution of **2** (11.8 mg) in $CHCl_3$ (1.0 mL) was heated under reflux for 24 h and the reaction solution was worked up as described above to give **2** (6.5 mg) and **17** (3.4 mg). Recovered **2** was identified with an authentic 6,6'-dihydroxy-thiobinupharidine as described above and **17** was identified by comparison of the physical data with the reported values.[7a]

A solution of **17** (1.0 mg) in $CHCl_3$ (0.5 mL) was heated under reflux for 24 h. The reaction solution was concentrated under reduced pressure to give a product, which was subjected to HPLC [Develosil ODS-HG-5 (Nomura Chemical Co., 250x4.6 mm i.d.), MeOH-H_2O-Et_2NH (890 : 10 : 6, v/v), 1.0 ml/min] to detect **17** and **2** (trace); t_R **17** : 11.8 min, **2** : 9.2 min.

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