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Daphnezomines H, I, J, and K, New Daphnilactone-type and Yuzurimine-type Alkaloids from *Daphniphyllum humile*

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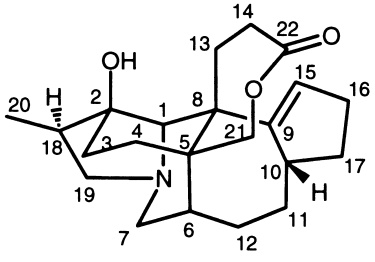
Abstract—Daphnezomines H (**1**), I (**2**), J (**3**), and K (**4**), four new alkaloids possessing a daphnilactone-type (**1** and **2**) or a yuzurimine-type skeleton (**3** and **4**) have been isolated from the leaves (**1**), stems (**2**) and fruits (**3** and **4**) of *Daphniphyllum humile*, respectively, and the structures including relative stereochemistry were elucidated on the basis of spectroscopic data.

Daphniphyllum alkaloids with unique nitrogen-containing polycyclic ring systems have attracted great interests from a biogenetic point of view.[1] Recently we have isolated five novel alkaloids, named daphnezomines A–E, from the leaves and stems of *Daphniphyllum humile*.[2] Our continuing search for biogenetic intermediates of *Daphniphyllum* alkaloids resulted in the isolation of new alkaloids with a daphnilactone-type skeleton, daphnezomines H (**1**) and I (**2**), and those with a yuzurimine-type skeleton, daphnezomines J (**3**) and K (**4**). In this paper we describe the isolation and structure elucidation of **1**–**4**.



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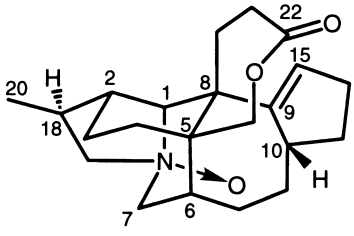

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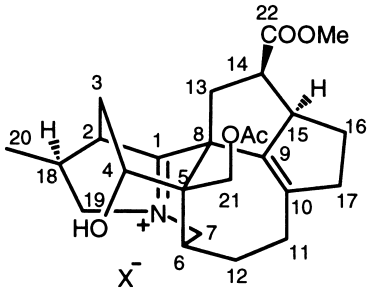

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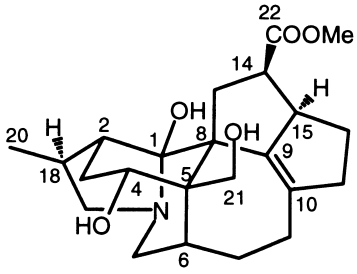

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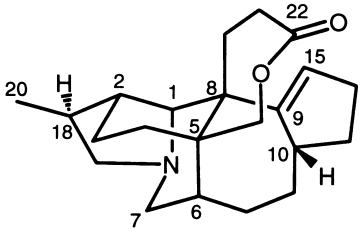

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Keywords: alkaloids; imines; conformation; NMR.

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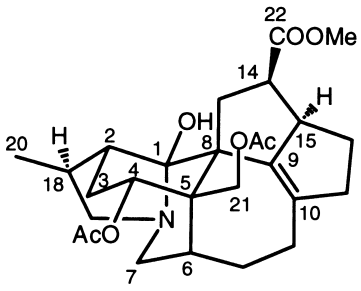

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The leaves, stems and fruits of *D. humile* collected in Sapporo were extracted with MeOH. Each MeOH extract from the leaves and stems was partitioned between EtOAc and 3% tartaric acid, and then each water-soluble material was adjusted at pH 9 with sat. Na_2CO_3 and partitioned with $CHCl_3$. Each $CHCl_3$-soluble material from the leaves and stems was subjected to a C_{18} column (CH_3CN/0.1% TFA, 3:7→7:3) followed by gel filtration on Sephadex LH-20 (MeOH/$CHCl_3$, 1:1) to afford daphnezomines H (**1**, 0.0002%) and I (**2**, 0.005%), respectively, together with the known related alkaloids, daphnilactone B (**5**, 0.002%)[3] and yuzurimine (**6**, 0.01%),[4] from the leaves. The MeOH extract from the fruits was subjected to a C_{18} column (CH_3CN/0.1%TFA, 1:9→9:1) followed by C_{18} HPLC (CH_3CN/0.1%TFA, 1:4→3:7) to afford daphnezomine J (**3**, 0.002%) as unspecified salt and daphnezomine K (**4**, 0.002%).

Table 1. ^1H and ^{13}C NMR data of daphnezomine H (**1**) in $CDCl_3$ at 300 K

Assignment	δ_H	δ_C	HMBC (^1H)
1	3.86 (1H, s)	71.86	7a
2		76.55	1, 3, 4a, 20
3a	1.88 (1H, dt, 6.5, 15.8)	27.80	1
3b	1.94 (1H, dt, 6.1, 15.8)		
4a	1.45 (1H, m)	29.72	21
4b	2.54 (1H, m)		
5		38.30	1
6	2.29 (1H, m)	41.11	
7a	3.67 (1H, d, 14.4)	56.44	1, 12b, 19
7b	3.30 (1H, dd, 10.3, 14.4)		
8		42.70	13, 21
9		148.72	10
10	3.07 (1H, m)	49.51	15
11a	1.74 (1H, m)	31.51	
11b	2.22 (1H, m)		
12a	1.45 (1H, m)	29.68	7
12b	1.68 (1H, m)		
13	2.52 (2H, m)	29.89	
14a	1.76 (1H, m)	32.56	
14b	3.06 (1H, t, 13.5)		
15	6.03 (1H, s)	134.23	
16a	2.24 (1H, m)	29.58	15, 17
16b	2.45 (1H, m)		
17a	1.70 (1H, m)	32.01	15, 16
17b	1.76 (1H, m)		
18	2.41 (1H, m)	43.77	20
19a	2.42 (1H, m)	62.73	7b, 20
19b	4.29 (1H, m)		
20	1.11 (3H, d, 6.5)	11.81	
21a	3.74 (1H, d, 13.1)	73.00	
21b	4.94 (1H, d, 13.1)		
22		174.80	13, 14, 21

Daphnezomine H {**1**, $[\alpha]_D^{23}=-59°$ (*c* 0.4, $CHCl_3$)} was obtained as a colorless solid, and the molecular formula was established as $C_{22}H_{31}NO_3$ by HRFABMS [*m/z* 358.2374, $(M+H)^+$, Δ −0.8 mmu]. IR absorptions implied that **1** possessed hydroxyl (3400 cm^{-1}) and ester carbonyl (1735 cm^{-1}) groups. The analysis of ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum provided evidence that **1** possessed one methyl, one ester carbonyl, one oxymethylene, one trisubstituted olefin, 10 methylenes, four methines and three quaternary carbons, one of which bore an oxygen atom. The ^1H–^1H COSY and HOHAHA spectra revealed connectivities of C-3 to C-4, C-18 to C-19 and C-20, C-6 to C-7 and C-12, C-10 to C-12 and C-17, C-15 to C-17, and C-13 to C-14, as shown in Fig. 1. The chemical shifts of C-1 (δ 71.86), C-7 (δ 56.44) and C-19 (δ 62.73) suggested that these carbons were adjacent to a nitrogen atom, while those of C-2 (δ 76.55) and C-21 (δ 73.00) indicated that these carbons were attached to an oxygen atom. In the HMBC (Table 1) spectrum, long-range ^1H–^{13}C correlations of H_2-14 to C-22 (δ 174.80), H_2-13 to C-8, H_2-21 to C-8 and C-22 revealed the presence of a seven-membered lactone ring like daphnilactone B (**5**) (Fig. 1). Connections between C-10 and C-15 via C-9, and between C-21 and C-4 via C-5 were implied by the HMBC cross-peaks for H-10 to C-9, H-15 to C-10, and H_2-21 to C-4. HMBC correlations of H-1, H_2-3 and H_3-20 to C-2 (δ 76.55) revealed that a hydroxyl group was attached to C-2, while correlations of H-1 and H_2-19 to C-7 indicated the connection among C-1, C-7 and C-19 through N-1. These HMBC correlations containing that of H-1 to C-5 provided connectivities among all the units, indicating the presence of a nitrogen-containing hexacyclic daphnilactone-type skeleton with a hydroxyl at C-2 and a methyl group at C-18. Thus the structure of daphnezomine H was elucidated to be **1**.

The relative stereochemistry of **1** was deduced from NOESY correlations as shown in computer-generated 3D drawing (Fig. 2). These NOESY correlations indicated the relative configurations at C-2, C-10 and C-18 and the chair forms of the cyclohexane ring (C-1–C-5 and C-8) and the piperidine ring (N-1, C-1, C-8 and C-5–C-7) in the 2-azabicyclo[3.3.1]nonane moiety.

The molecular formula of daphnezomine I (**2**) was determined as $C_{22}H_{31}NO_3$ by HRFABMS [*m/z* 358.2373, $(M+H)^+$, Δ −0.9 mmu], which has the same molecular formula as that of **1**. The IR spectrum suggested the

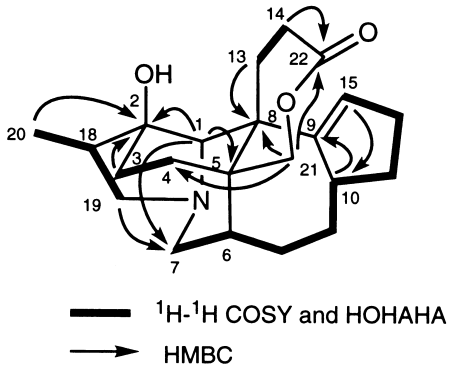

Figure 1. Selected 2D NMR data of daphnezomine H (**1**).

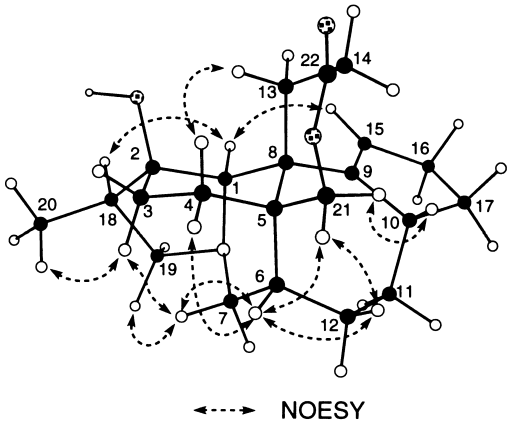

Figure 2. Relative stereochemistry of daphnezomine H (**1**).

presence of ester carbonyl group (1730 cm^{-1}). ^1H and ^{13}C NMR data revealed the presence of 4 quaternary carbons (sp^2×2 and sp^3×2), 6 methines (sp^2×1 and sp^3×5), 11 methylenes and 1 methyl, implying that **2** had a daphnilactone-type skeleton like **1**, whereas the carbon signal due to an sp^3 methine (δ 36.72) at C-2 was observed in place of the oxygenated quaternary carbon (C-2, δ 76.55) in **1**. A comparison of the ^{13}C chemical shifts of C-1, C-7 and C-19 (δ 84.58, 73.77 and 81.99, respectively) in **2** with those (δ 72.27, 57.82 and 65.22, respectively) of daphnilactone B (**5**) indicated the presence of an N-oxide group attached to these carbons. Treatment of daphnilactone B (**5**) with *m*-chloroperbenzoic acid (*m*-CPBA) gave the N-oxide of **5**, the spectral data and the $[\alpha]_D$ value of which were identical with those of daphnezomine I (**2**). Thus daphnezomine I (**2**) was concluded to be the N-oxide form of daphnilactone B (**5**).

Table 2. ^1H and ^{13}C NMR data of Daphnezomine J (**3**) in $CDCl_3$ at 300 K

Assignment	δ_H	δ_C	HMBC (^1H)
1		156.44	
2	2.46 (1H, br s)	43.01	20
3a	2.29 (1H, dd, 2.7, 15.0)	19.02	
3b	2.39 (1H, dd, 4.0, 15.0)		
4	4.29 (1H, m)	62.34	21
5		54.06	
6	2.84 (1H, m)	43.87	21
7a	2.73 (1H, m)	56.77	
7b	3.61 (1H, m)		
8		60.00	
9		140.56	16b
10		138.64	11, 17a
11a	2.05 (1H, m)	25.90	
11b	2.11 (1H, m)		
12a	1.74 (1H, m)	28.30	
12b	1.92 (1H, m)		
13a	2.25 (1H, dd, 8.4, 14.5)	40.13	
13b	2.95 (1H, dd, 5.0, 14.5)		
14	2.81 (1H, m)	41.94	
15	3.43 (1H, m)	53.52	16a
16a	1.34 (1H, m)	26.91	
16b	1.98 (1H, m)		
17a	2.42 (1H, m)	41.28	
17b	2.71 (1H, m)		
18	2.73 (1H, m)	29.20	20
19a	3.06 (1H, m)	49.10	20
19b	3.09 (1H, m)		
20	1.19 (3H, d, 6.9)	18.82	
21a	4.30 (1H, d, 12.0)	65.29	
21b	4.64 (1H, d, 12.0)		
22		174.18	14, 23
22-OMe	3.67 (3H, s)	51.72	
24		170.96	21, 25
25	2.13 (3H, s)	20.76	

Daphnezomine J {**3**, $[\alpha]_D^{23}=-20°$ (*c* 0.9, MeOH)} showed the molecular ion at *m/z* 428 $(M)^+$ and the molecular formula, $C_{25}H_{34}NO_5$, was established by HRFABMS [*m/z* 428.2440, $(M)^+$, Δ +0.3 mmu]. IR absorptions implied the presence of hydroxyl (3445 cm^{-1}), ester carbonyl (1735 cm^{-1}) and imine (1680 cm^{-1}) functionalities. Analyses of ^1H and ^{13}C NMR data (Table 2) and the HMQC spectrum provided evidence that **3** possessed one acetoxy group, one tetrasubstituted olefin, one methoxycarbonyl group, one oxymethine, five methines, one oxymethylene, eight methylenes, two quaternary carbons and one methyl group. Detailed analysis of the ^1H–^1H COSY and HOHAHA spectra of **3** implied connectivities of C-2 to C-4, C-18 to C-2, C-19, and C-20, C-6 to C-7 and C-12, C-11 to C-12, and C-13 to C-17. From HMBC correlations of H-14 and H_3-OMe to C-22 (δ 174.18), and H_2-21 and acetyl methyl proton to acetyl carbonyl carbon (δ 170.96), the methoxy carbonyl group was attached to C-14 and the acetoxy group was attached to C-21. HMBC correlations from H_2-16 to C-9 (δ 140.56) and H_2-11 to C-10 (δ 138.64) indicated the position of the tetrasubstituted olefin. These data indicated that **3** possessed a yuzurimine-type skeleton and functionalities. The ^1H and ^{13}C chemical shifts of C-4 (δ_H 4.29; δ_C 62.34) suggested the presence of a hydroxyl group at C-4 (Figs. 3 and 4).

The IR absorption at 1680 cm^{-1} supported the imine functionality. The carbon chemical shifts of C-1 (δ 156.44), C-2 (δ 43.01), C-8 (δ 60.00), C-7 (δ 56.77) and C-19 (δ 49.10) indicated the presence of the imino group at C-1 and N-1. Thus the structure of daphnezomine J was assigned as **3**.

The relative stereochemistry was elucidated to be the same as that of yuzurimine (**6**) on the basis of NOESY data of **3**. The conformations of the cyclohexane ring (C-1–C-5 and C-8) and the piperidine ring (N-1, C-1, C-8 and C-5–C-7) in the 2-azabicyclo[3.3.1]nonane moiety with anti-Bredt rule imine[5] were assigned as both twist boat forms to release the ring strain from NOESY correlations of Hβ-3/Hα-13 and H-2/Hα-13. These ring conformations were coincident with

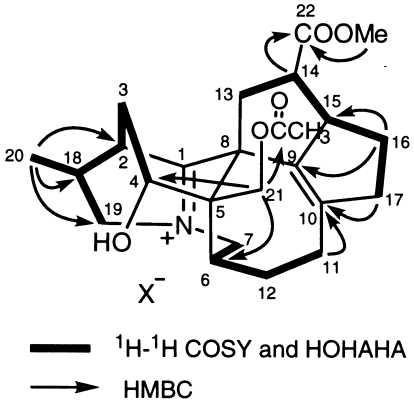

Figure 3. Selected 2D NMR data of daphnezomine J (**3**).

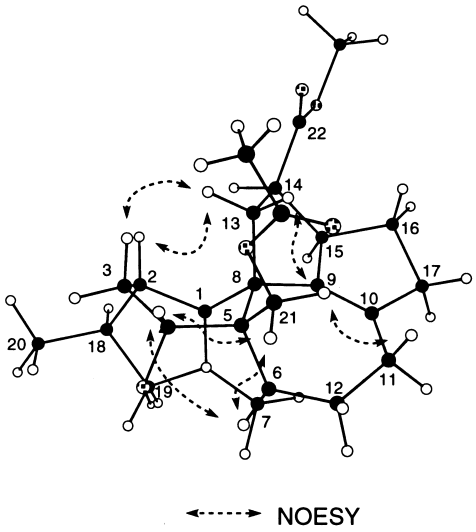

Figure 4. Relative stereochemistry of daphnezomine J (**3**).

the energy-minimized conformation calculated from conformational search as shown in Fig 5.

Daphnezomine K {**4**, $[\alpha]_D^{23}=-15°$ (*c* 0.3, MeOH)} showed the pseudomolecular ion at *m/z* 404 $(M+H)^+$ and the molecular formula, $C_{23}H_{33}NO_5$, was established by HRESIMS [*m/z* 404.2415, $(M+H)^+$, Δ −2.2 mmu]. IR absorptions implied the presence of hydroxyl (3420 cm^{-1}) and ester (1730 cm^{-1}) functionalities. The ^{13}C NMR spectrum containing 6 quaternary carbons (sp^2×3 and sp^3×3), 6 sp^3 methines, 9 methylenes and 2 methyls implied that **4** was structurally related to yuzurimine (**6**).[4] Detailed analyses of the 2D NMR data indicated that **4** was deacetylated yuzurimine. Hydrolysis of yuzurimine (**6**) with 10% HCl/MeOH afforded the deacetylated derivative, whose spectral data and the $[\alpha]_D$ value were identical with those of natural daphnezomine K (**4**). Thus daphnezomine K (**4**) was concluded to be the deacetylated form of yuzurimine (**6**).

Daphnezomine I (**2**) is the first N-oxide alkaloid having a daphnilactone-type skeleton, while daphnezomine J (**3**) is the first alkaloid possessing a yuzurimine-type skeleton with an anti-Bredt-rule imine.[5] Biogenetically daphnezomine I (**2**) may be derived from daphnilactone B (**5**) through oxidation at N-1, while daphnezomine J (**3**) may be generated from yuzurimine (**6**) through dehydroxylation at C-1.

Daphnezomine J (**3**) exhibited cytotoxicity against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells with IC_{50} values of 7.3 and 9.7 μg/mL, respectively, in vitro, while daphnezomines H (**1**), I (**2**) and K (**4**) did not show such cytotoxicity (IC_{50} >10 μg/mL).

Experimental

General methods

^1H and 2D NMR spectra were recorded in $CDCl_3$ on a 600 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 125 MHz spectrometer. Chemical shifts were reported using residual $CDCl_3$ (δ_H 7.26 and δ_C 77.03) as internal standards. Standard pulse sequences were employed for the 2D NMR experiments. HMBC spectra were recorded using a 50 ms delay time for long-range C–H coupling with *Z*-axis PFG. NOESY spectra were measured with a mixing time of 800 ms. FABMS was measured by using glycerol matrix.

Material

The stems and leaves of *Daphniphyllum humile* were collected in Sapporo in 1998 and the fruits in 1999. The botanical identification was made by Mr N. Yoshida, Faculty of Pharmaceutical Sciences, Hokkaido University. A voucher specimen has been deposited in the herbarium of Hokkaido University.

Extraction and isolation

The leaves (4.0 kg), stems (7.5 kg) and fruits (300 g) of *Daphniphyllum humile* were crushed and extracted with MeOH (10 L×3, 20 L×3 and 1 L×3, respectively) to give each MeOH extract (230, 477 and 23 g, respectively). The MeOH extract from the leaves was treated with 3% tartaric acid to adjust at pH 2 and then partitioned with EtOAc. The aqueous layer was treated with sat. Na_2CO_3 aq. to adjust at pH 9 and extracted with $CHCl_3$ to give a crude alkaloidal fraction (7.6 g), which was subjected to C_{18} column chromatography (CH_3CN/0.1%TFA, 3:7→7:3) followed by gel filtration on Sephadex LH-20 (MeOH/$CHCl_3$, 1:1) to afford daphnezomine H (**1**, 0.0002%) as colorless solid together with a known related alkaloid, daphnilactone B (**5**, 0.002%) and yuzurimine (**6**, 0.01%). The crude alkaloidal

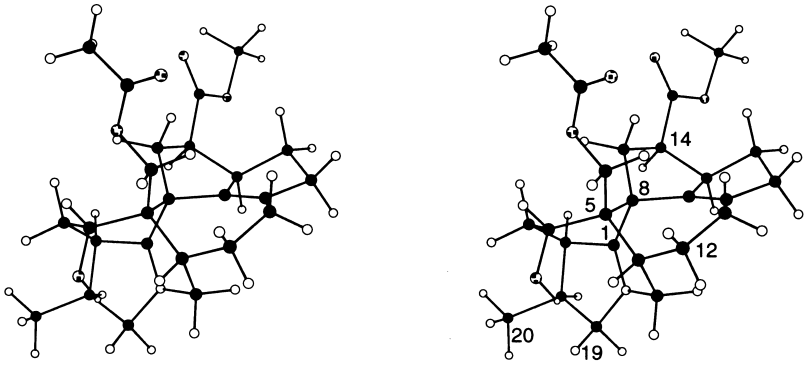

Figure 5. Stereoscopic view of energy-minimized 3D structure of daphnezomine J (**3**).

fraction (34.0 g) prepared from the stems was separated by the same procedure described for **1** to yield daphnezomine I (**2**, 0.005%). The MeOH extract from the fruits was subjected to C_{18} column chromatography (CH_3CN/0.1% TFA, 1:9→9:1) followed by C_{18} HPLC (CH_3CN/0.1% TFA, 1:4→3:7) to give daphnezomines J (**3**, 0.002%) and K (**4**, 0.002%).

Daphnezomine H (1). A colorless solid; $[\alpha]_D^{23}=-59°$ (*c* 0.4, $CHCl_3$); ^1H and ^{13}C NMR data (Table 1); FABMS *m/z* 358 $(M+H)^+$; HRFABMS *m/z* 358.2374 (M+H; calcd for $C_{22}H_{32}NO_3$, 358.2382); IR (neat) ν_{max} 3400, 2970, 1735 and 1685 cm^{-1}.

Daphnezomine I (2). A colorless solid; $[\alpha]_D^{23}=-22°$ (*c* 0.6, $CHCl_3$); FABMS *m/z* 358 $(M+H)^+$; HRFABMS *m/z* 358.2373 (M+H; calcd for $C_{22}H_{32}NO_3$, 358.2382); IR (neat) ν_{max} 2930, 1730 and 1680 cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.78 (1H, d, 2.7, H-1), 2.73 (1H, m, H-2), 1.61 (1H, m, H-3a), 1.92 (1H, m, H-3b), 1.52 (1H, dt, 15.5, 8.1, H-4a), 2.27 (1H, m, H-4b), 2.34 (1H, m, H-6), 4.11 (1H, dd, 8.8, 15.5, H-7a), 4.18 (1H, d, 15.5, H-7b), 3.11 (1H, m, H-10), 1.61 (1H, m, H-11a), 2.28 (1H, m, H-11b), 1.58 (1H, m, H-12a), 1.68 (1H, m, H-12b), 2.02 (1H, dd, 7.7, 14.1, H-13a), 2.19 (1H, m, H-13b), 2.49 (1H, dd, 7.6, 15.0, H-14a), 2.63 (1H, t, 15.0, H-14 b), 5.90 (1H, s, H-15), 2.31 (1H, m, H-16a), 2.42 (1H, m, H-16b), 1.70 (1H, m, H-17a), 2.15 (1H, m, H-17b), 3.07 (1H, m, H-18), 3.72 (1H, dd, 9.4, 12.0, H-19a), 4.61 (1H, t, 12.0, H-19b), 1.05 (3H, d, 6.5, H-20), 3.79 (1H, d, 13.2, H-21a) and 4.84 (1H, d, 13.2, H-21b); ^{13}C NMR ($CDCl_3$) δ 84.58 (C-1), 36.72 (C-2), 19.62 (C-3), 30.88 (C-4), 37.87 (C-5), 43.46 (C-6), 73.77 (C-7), 42.73 (C-8), 146.31 (C-9), 50.02 (C-10), 32.55 (C-11), 29.75 (C-12), 29.67 (C-13), 33.93 (C-14), 133.36 (C-15), 30.17 (C-16), 30.85 (C-17), 32.43 (C-18), 81.99 (C-19), 13.05 (C-20), 72.46 (C-21) and 174.10 (C-22).

Daphnezomine J (3). A colorless solid; $[\alpha]_D^{23}=-20°$ (*c* 0.9, MeOH); ^1H and ^{13}C NMR data (Table 2); FABMS *m/z* 428 $(M)^+$; HRFABMS *m/z* 428.2440 (M; calcd for $C_{25}H_{34}NO_5$, 428.2437); IR (neat) ν_{max} 3445, 2925, 1735 and 1680 cm^{-1}.

Daphnezomine K (4). A colorless solid; $[\alpha]_D^{23}=-15°$ (*c* 0.3, MeOH); ^1H and ^{13}C NMR data; ESIMS *m/z* 404 $(M+H)^+$; HRESIMS *m/z* 404.2415 (M+H; calcd for $C_{23}H_{34}NO_5$, 404.2437); IR (neat) ν_{max} 3420, 2930, 1730 and 1670 cm^{-1}. ^1H NMR ($CDCl_3/CD_3OD$ 9:1) δ 2.37 (1H, m, H-2), 1.60 (1H, m, H-3a), 2.05 (1H, m, H-3b), 4.38 (1H, dd, 7.3, 11.8, H-4), 2.69 (1H, t, 7.1, H-6), 3.50 (1H, dd, 8.9, 15.0, H-7a), 3.61 (1H, d, 15.0, H-7b), 2.10 (1H, dd, 4.3, 17.7, H-11a), 2.50 (1H, m, H-11b), 1.46 (1H, m, H-12a), 1.91 (1H, m, H-12b), 2.34 (1H, dd, 9.8, 15.1, H-13a), 2.94 (1H, dd, 2.1, 15.1, H-13b), 3.04 (1H, m, H-14), 3.77 (1H, m, H-15), 1.07 (1H, m, H-16a), 1.93 (1H, m, H-16b), 2.27 (1H, dd, 8.2, 15.1, H-17a), 2.53 (1H, m, H-17b), 3.15 (1H, m, H-18), 2.58 (1H, dd, 6.4, 11.6, H-19a), 4.13 (1H, t, 11.6, H-19b), 1.11 (3H, d, 7.3, H-20), 3.68 (1H, d, 11.9, H-21a), 4.19 (1H, d, 11.9, H-21b) and 3.64 (3H, s, OMe); ^{13}C NMR ($CDCl_3/CD_3OD$ 9:1) δ 100.87 (C-1), 41.76 (C-2), 29.83 (C-3), 69.44 (C-4), 45.54 (C-5), 30.74 (C-6), 57.88 (C-7), 50.87 (C-8), 141.66 (C-9), 139.12 (C-10), 24.65 (C-11), 26.18 (C-12), 36.34 (C-13), 43.22 (C-14), 57.30 (C-15), 29.91 (C-16), 43.22 (C-17), 32.49 (C-18), 63.12 (C-19), 14.19 (C-20), 65.25 (C-21), 177.57 (C-22) and 51.67 (OMe).

Daphnilactone B (5). A colorless solid; $[\alpha]_D^{23}=-63°$ (*c* 0.8, $CHCl_3$); FABMS *m/z* 342 $(M+H)^+$; IR (neat) ν_{max} 2930, 1730 and 1680 cm^{-1}. ^{13}C NMR ($CDCl_3$) δ 72.27(C-1), 37.46 (C-2), 20.55 (C-3), 31.51 (C-4), 38.16 (C-5), 41.75 (C-6), 57.82 (C-7), 42.23 (C-8), 147.58 (C-9), 49.38 (C-10), 34.48 (C-11), 29.95 (C-12), 29.74 (C-13), 31.54 (C-14), 134.72 (C-15), 29.89 (C-16), 32.02 (C-17), 35.71 (C-18), 65.22 (C-19), 13.66 (C-20), 72.91 (C-21) and 174.25 (C-22).

Oxidation of daphnilactone B (5). *m*-Chloroperbenzoic acid (2 mg) was added to a stirred solution of daphnilactone B (**5**, 2.0 mg) in CH_2Cl_2 (0.2 ml) at room temperature. The mixture was stirred at room temperature for 1 day, and washed with 20% Na_2SO_3 (2 mL) and then H_2O (4 mL), and concentrated to give a pale yellow oil (2.2 mg). The oil was subjected to silica gel column chromatography ($CHCl_3$/EtOAc/MeOH, 10:1:1) to give the *N*-oxide derivative (1.6 mg), whose spectral data and $[\alpha]_D$ value were identical with those of **2**.

Hydrolysis of yuzurimine (6). A solution of yuzurimine (**6**) in 10% HCl/MeOH was heated at 100°C for 12 h in a sealed tube. After cooling, the solution was concentrated to dryness to give the hydrolysate, whose spectral data and $[\alpha]_D$ values were identical with those of daphnezomine K (**4**).

Computational methods

Conformational searching for daphnezomine J (**3**) was carried out using the Pseudo Monte Carlo simulation in Macromodel program (v6.0).[6] The closure bonds were chosen at C-2–C-3 and C-7–N-1 with the closure limit from 1 to 4 Å, while the other closure bond was chosen at C-11–C-12. Each conformer was finally minimized by molecular mechanics calculation of MMFF force field.[7] Five thousand Monte Carlo steps were performed, yielding 26 unique conformations in the energy region of 0–10 kcal/mol and the lowest energy conformation, which took a twist boat–twist boat conformation in 2-azabicyclo[3.3.1]nonan ring as depicted in Fig. 5.

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