Daphnezomines T—V, Alkaloids from *Daphniphyllum humile*

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Received January 27, 2009; accepted February 21, 2009; published online February 25, 2009

Three new *Daphniphyllum* alkaloids, daphnezomines T—V (1—3), were isolated from the leaves and branches of *Daphniphyllum humile* (Daphniphyllaceae). The structures and relative stereochemistry of 1—3 were elucidated on the basis of spectroscopic data. Daphnezomine T (1) is the first alkaloid without a branched C_1 unit at C-5 among all *Daphiniphyllum* alkaloids reported so far.

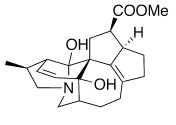
Key words *Daphniphyllum* alkaloid; *Daphniphyllum humile*; daphnezomine T; daphnezomine U; daphnezomine V

Trees of the genus *Daphniphyllum* (Daphniphyllaceae) are known to elaborate structurally diverse group of alkaloids with unique polycyclic fused ring systems.[1,2] These *Daphniphyllum* alkaloids have been attractive targets for biogenetic and synthetic studies.[3—8] Some novel alkaloids with unusual skeletons such as daphnezomines A—S[9—14] have been isolated from *D. humile* at our laboratory. Further investigation of extracts of this plant resulted in the isolation of three new alkaloids, daphnezomines T—V (**1**—**3**). In this paper we describe the isolation and structure elucidation of **1**—**3**.

Results and Discussion

The leaves and branches of *Daphniphyllum humile* were extracted with MeOH, respectively, and each MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$ to give crude alkaloidal fractions. The crude alkaloidal materials prepared from the leaves were subjected to a LH-20 column, followed by an amino silica gel and a silica gel columns to give daphnezomines T (**1**, 0.00063% yield) and U (**2**, 0.00033%). The crude alkaloidal materials prepared from the branches were separated by an amino silica gel column, followed by silica gel column chromatographies to obtain daphnezomine V (**3**, 0.00022%).

Daphnezomine T (**1**) showed the pseudomolecular ion peak at *m/z* 372 $(M+H)^+$ in the electrospray ionization mass spectrometry (ESI-MS), and the molecular formula, $C_{22}H_{29}NO_4$, was established by HR-ESI-MS [*m/z* 372.2180, $(M+H)^+$, Δ +0.5 mmu]. IR absorptions implied the presence of hydroxy (3417 cm^{-1}) and ester carbonyl (1714 cm^{-1}) functionalities. Analyses of ^{1}H- and ^{13}C-NMR data (Table 1) and the heteronuclear multiple quantum coherence (HMQC) spectrum provided evidence that **1** possessed one tetrasubstituted olefin, one disubstituted olefin, one carbonyl, three sp^3 quaternary carbons, five sp^3 methines, seven sp^3 methylenes, and two methyls. Among them, two methylenes (δ_C 62.7, δ_C 55.1) were ascribed to those bearing a nitrogen atom, while one quaternary carbon (δ_C 100.5) was assigned as an aminal carbon.



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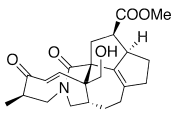

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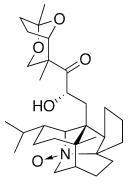

3

Three structural fragments, **a** (C-2 to C-4, C-2 to C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), and **c** (C-13 to C-17), were deduced from the ^{1}H–^{1}H correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY) spectra as shown in Fig. 1. Heteronuclear multiple bond correlations (HMBC) correlations between H_2-7 and C-1, H_2-19 and C-1, and H_2-19 and C-7 suggested that C-1, C-7, and C-19 were connected to each other through a nitrogen atom. Connections between C-4, C-6, C-8, and 5-OH *via* C-5 were implied by HMBC cross-peaks of H-6/C-4, H-6/C-5, 5-OH/C-5, 5-OH/C-6, and 5-OH/C-8. HMBC correlations between H-2 and C-1, H-13 and C-1, and H-13 and C-8 indicated the connectivity of C-1 and C-2. The linkage of units **b** and **c** through C-9 and C-10 was implied by HMBC cross-peaks of H_2-11/C-9, H_2-11/C-10, H-14/C-9, and H_2-17/C-9. In addition, HMBC correlations between H-13 and C-21, and H_3-22 and C-21 suggested that a methoxy group was attached to C-21. The geometry of the disubstituted olefin at C-3 and C-4 was elucidated to be *Z* from ^3J-value for H-3 and H-4 (10.5 Hz). Thus, the gross structure of daphnezomine T was elucidated to be **1**.

The nuclear Overhauser enhancement and exchange spectroscopy (NOESY) cross-peaks of H-3/H-4, H-3/H_3-20, H-4/H-6, H-13a/OH-5, H-13b/H-14, and H-14/H-15 indicated a half-chair form of cyclohexene ring (C-1 to C-5 and C-8) and a chair form of piperidine ring (C-1, N-1, and C-5 to C-8) as well as the relative stereochemistry as shown in Fig. 2.

Daphnezomine U (**2**) showed the pseudomolecular ion peak at *m/z* 422 $(M+Na)^+$ in the ESI-MS, and the molecular formula, $C_{23}H_{29}NO_5$, was established by HR-ESI-MS [*m/z* 422.1954, $(M+H)^+$, Δ +1.1 mmu]. The IR spectrum suggested the presence of hydroxy (3582 cm^{-1}) and carbonyl (1730, 1698, 1642 cm^{-1}) functionalities. The ^{13}C-NMR data revealed twenty-three carbon signals due to one tetrasubstituted olefin, one disubstituted olefin, three carbonyls, two sp^3 quaternary carbons, four sp^3 methines, eight sp^3 methylenes, and two methyls. Among them, two methylenes (δ_C 55.9, 50.0) were ascribed to those bearing a nitrogen atom.

The ^{1}H–^{1}H COSY and TOCSY spectra of **2** revealed connectivities of four partial structures, **a** (C-3 to C-4), **b** (C-6 to C-7 and C-12, and C-11 to C-12), **c** (C-13 to C17), and **d** (C-

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Table 1. ^{1}H- and ^{13}C-NMR Data of Daphnezomines T (**1**) and U (**2**) in $CDCl_3$

Daphnezomine T (**1**)			Daphnezomine U (**2**)		
Position	δ_H	δ_C	Position	δ_H	δ_C
1	—	100.5 s	1	—	171.9 s
2	2.72 (1H, m)	45.8 d	2	—	210.2 s
3	5.62 (1H, dd, 10.5, 3.2 Hz)	125.6 d	3	5.99 (1H, d, 14.3 Hz)	136.4 d
4	5.55 (1H, dd, 10.5, 1.8 Hz)	136.8 d	4	5.51 (1H, d, 14.3 Hz)	135.5 d
5	—	74.8 s	5	—	50.6 s
5-OH	5.47 (1H, s)	—	6	2.77 (1H, m)	36.6 d
6	1.93 (1H, m)	40.9 d	7a	3.70 (1H, dd, 13.8, 9.2 Hz)	50.0 t
7a	3.08 (1H, d, 11.9 Hz)	55.1 t	7b	3.14 (1H, dd, 13.8, 10.3 Hz)	
7b	2.92 (1H, m)		8	—	55.9 s
8	—	53.8 s	9	—	137.2 s
9	—	141.3 s	10	—	135.3 s
10	—	138.9 s	11a	2.31 (1H, m)	24.6 t
11a	2.55 (1H, m)	25.9 t	11b	2.26 (1H, m)	
11b	2.00 (1H, m)		12a	2.40 (1H, m)	24.1 t
12a	2.42 (1H, m)	25.0 t	12b	1.63 (1H, m)	
12b	1.50 (1H, m)		13a	2.77 (1H, dd, 13.2, 8.6 Hz)	33.4 t
13	2.60 (2H, m)	38.6 t	13b	2.31 (1H, dd, 13.2, 6.9 Hz)	
14	2.95 (1H, m)	43.6 d	14	3.17 (1H, m)	42.0 d
15	3.57 (1H, m)	58.4 d	15	3.30 (1H, m)	52.6 d
16a	1.90 (1H, m)	27.7 t	16a	1.90 (1H, m)	27.8 t
16b	1.29 (1H, m)		16b	1.20 (1H, m)	
17a	2.78 (1H, m)	43.3 t	17a	2.47 (1H, m)	42.1 t
17b	2.47 (1H, m)		17b	2.24 (1H, m)	
18	2.67 (1H, m)	34.5 d	18	3.61 (1H, m)	40.8 d
19a	3.54 (1H, dd, 11.9, 9.2 Hz)	62.7 t	19a	4.44 (1H, dd, 13.2, 8.0 Hz)	55.9 t
19b	2.31 (1H, dd, 11.9, 3.7 Hz)		19b	2.48 (1H, dd, 13.2, 10.3 Hz)	
20	1.20 (3H, d, 7.4 Hz)	19.0 q	20	1.01 (3H, d, 6.85 Hz)	13.1 q
21	—	178.5 s	21	4.02 (2H, s)	66.5 t
22	3.70 (3H, s)	51.6 q	22	—	176.4 s
			23	3.67 (3H, s)	51.5 q

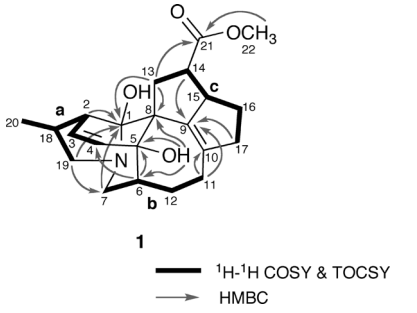

Fig. 1. Selected 2D NMR Correlations for Daphnezomine T (**1**)

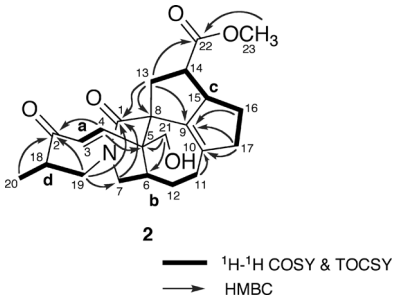

Fig. 3. Selected 2D NMR Correlations for Daphnezomine U (**2**)

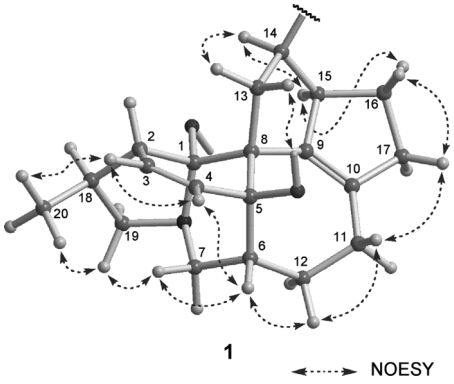

Fig. 2. Selected NOESY Correlations and Relative Stereochemistry for Daphnezomine T (**1**)

C-22–C-23 was omitted.

18 to C-19 and C-20) as shown in Fig. 3. HMBC correlations between H_2-7 and C-1, and H_2-19 and C-1 suggested the connectivities of C-1, C-7, and C-19 through a nitrogen atom, and the presence of amide carbonyl. Connectivities of C-4, C-6 and C-21 *via* C-5 were implied by HMBC cross-peaks of H-4/C-5, H_2-21/C-5, and H_2-21/C-6. HMBC correlations between H_2-13 and C-1, H_2-13 and C-8, and H_2-13 and C-9 disclosed that C-1, C-9, and C-13 were attached to C-8. Connections of C-11 and C-17 to C-9 through C-10 were implied by HMBC cross-peaks of H_2-11/C-10, H_2-16/C-9, H_2-17/C-9, and H_2-17/C-10. HMBC correlations between H-4 and C-20, H_2-19 and C-2, and H_3-20 and C-2 indicated the connectivity of C-3 and C-18 *via* C-2. HMBC correlations observed for H_2-13/C-22 and H_3-23/C-22 suggested that a methoxy group was attached to C-22. The geometry of disubstituted olefin at C-3 and C-4 was assigned as *E* on the basis of ^{3}J-

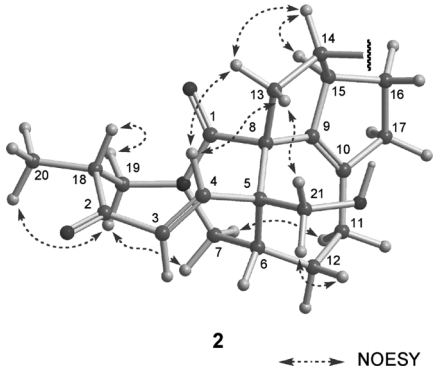

Fig. 4. Selected NOESY Correlations and Relative Stereochemistry for Daphnezomine U (**2**)

C-22–C-23 was omitted.

Table 2. ^{1}H- and ^{13}C-NMR Data of Daphnezomine V (**3**) in CD_3OD

Position	δ_H	δ_C
1	3.75 (1H, br s)	75.2 d
2	1.91 (1H, m)	40.6 d
3a	1.88 (1H, m)	25.8 t
3b	1.43 (1H, m)	
4a	2.07 (1H, m)	36.4 t
4b	1.58 (1H, m)	
5	—	37.8 s
6	1.87 (1H, m)	42.8 d
7a	4.11 (1H, br d, 12.1 Hz)	61.8 t
7a	3.28 (1H, br d, 12.1 Hz)	
8	—	47.6 s
9	2.60 (1H, m)	52.0 d
10	—	90.6 s
11a	2.14 (1H, m)	28.9 t
11b	1.66 (1H, m)	
12	1.75 (2H, m)	22.6 t
13a	2.64 (1H, dd, 16.2, 3.6 Hz)	34.1 t
13b	1.35 (1H, m)	
14	4.84 (1H, dd, 9.6, 3.6 Hz)	72.2 d
15a	2.53 (1H, m)	37.0 t
15b	1.56 (1H, m)	
16a	1.97 (1H, m)	26.9 t
16b	1.84 (1H, m)	
17a	2.21 (1H, m)	32.6 t
17b	1.61 (1H, m)	
18	2.34 (1H, m)	30.2 d
19	0.96 (3H, d, 6.7 Hz)	22.7 q
20	1.17 (3H, d, 6.2 Hz)	21.7 q
21	1.00 (3H, s)	25.1 q
22	—	214.3 s
23	—	50.6 s
24	0.92 (3H, s)	19.4 q
25a	4.54 (1H, d, 12.8 Hz)	66.3 t
25b	3.72 (1H, d, 12.8 Hz)	
26	4.78 (1H, d, 6.6 Hz)	83.0 d
27a	2.02 (1H, m)	24.1 t
27b	1.37 (1H, m)	
28a	2.11 (1H, m)	34.8 t
28b	1.86 (1H, m)	
29	—	106.5 s
30	1.36 (3H, s)	23.7 q

value for H-3 and H-4 (14.3 Hz). Thus, the gross structure of daphnezomine U was elucidated to be **2**.

The relative stereochemistry of **2** was deduced from the NOESY spectrum as shown in Fig. 4. The NOESY correlations observed for H-4/H_2-13, H-7a/H-11a, H-7b/H-19b, H-12b/H_2-21, H-13a/H-14, H-13b/H_2-21, H-14/H-15, H-18/H-19a, and H-19b/H_3-20 disclosed that a conformation of the 1-azabicyclo[5.2.2]undecane ring (C-1 to C-8 and C-18 to C-19) as well as the relative stereochemistry as shown in Fig. 4.

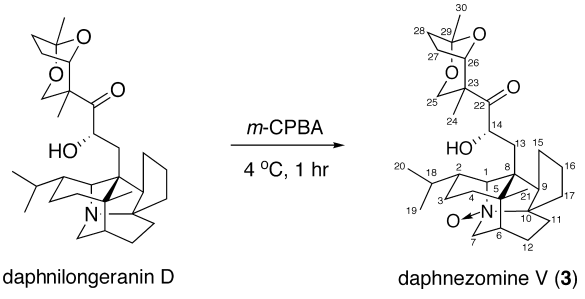

Fig. 5. Chemical Correlation of Daphnilongeranin D to Daphnezomine V (**3**)

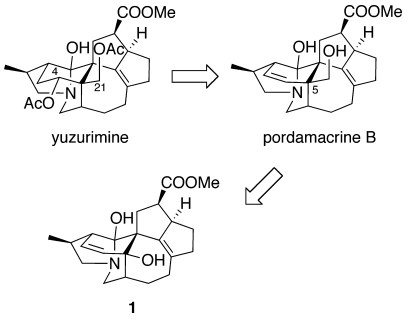

Fig. 6. Plausible Biogenetic Path for Daphnezomine T (**1**)

Daphnezomine V (**3**) showed the pseudomolecular ion peak at *m/z* 502 $(M+H)^+$ in the ESI-MS, and the molecular formula, $C_{30}H_{47}NO_5$, was established by HR-ESI-MS [*m/z* 502.3522, $(M+H)^+$, $\Delta -1.1$ mmu]. IR absorptions implied the presence of hydroxy (3240 cm^{-1}) and ester carbonyl (1704 cm^{-1}) functionalities. ^{1}H- and ^{13}C-NMR, and the HMQC spectra revealed that **3** consisted of one carbonyl carbon, five sp^3 quaternary carbons, seven sp^3 methines, twelve sp^3 methylenes, and five methyls. The chemical shifts of ^{1}H- and ^{13}C-NMR data (Table 2) implied that **3** had the same backbone skeleton as that of daphnilongeranin D.[15] The molecular formula of **3**, which was larger than that of daphnilongeranin D by one oxygen unit, and the down field shifts of chemical shifts for C-1, C-7, and C-10 (δ_C 75.2, 61.8, and 90.6, respectively) of **3** as compared with those of daphnilongeranin D (δ_C 64.2, 48.3, and 74.1, respectively) indicated that **3** was an *N*-oxide form of daphnilongeranin D. Oxidation of daphnilongeranin D with *m*-chloroperoxybenzoic acid (*m*-CPBA) afforded the *N*-oxide derivative, whose spectral data and the $[\alpha]_D$ value {$[\alpha]_D^{22}$ +12.8 (c=0.3, MeOH)} were coincident with those of natural daphnezomine V {**3**, $[\alpha]_D^{22}$ +13.8 (c=0.3, MeOH)}. Thus, the structure of daphnezomine V was concluded to be **3** (Fig. 5).

Daphnezomine T (**1**) is the first alkaloid without a branched C_1 unit at C-5 among all *Daphiniphyllum* alkaloids reported so far. Biogenetically, daphnezomine T (**1**) might be generated from an intermediate like pordamacrine B,[16] which could be derived from yuzurimine[17] by an elimination of acetic acid from C-3–C-4 and hydrolysis of acetyl ester at C-21, through oxidative loss of C_1 branch at C-5 (Fig. 6). Daphnezomine U (**2**) is a rare *Daphiniphyllum* alkaloid

possessing a 1-azabicyclo[5.2.2]undecane moiety,[12,18] while daphnezomine V (**3**) is an *N*-oxide form of daphnilongeranin D. Daphnezomines T—V (**1**—**3**) did not show cytotoxicities against P388 and L1210 murine leukemia, and KB human epidermoid carcinoma cells (IC_{50}>10.0 μg/ml) *in vitro*.

Experimental

General Methods Optical rotation was recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX-600 and a JEOL ECA-500 spectrometers. The 7.26 and 77.0 ppm resonances of residual chloroform and the 3.35 and 49.8 ppm resonances of residual methanol were used as internal references for ^{1}H- and ^{13}C-NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ spectrometer.

Extraction and Separation The leaves and branches of *Daphniphyllum humile* collected at Niigata were extracted with MeOH, respectively, and each MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$ to give crude alkaloidal fractions. A part (2.3 g) of the crude alkaloidal materials prepared from the leaves (1120 g) were separated by a gel filtration on Sephadex LH-20 ($CHCl_3$/MeOH, 9 : 1), followed by an amino silica gel (*n*-hexane/EtOAc→$CHCl_3$/MeOH) and a silica gel ($CHCl_3$/MeOH) column chromatographies to give daphnezomines T (**1**, 0.00063% yield) and U (**2**, 0.00033%) together with known *Daphniphyllum* alkaloids, yuzurimine[17] (0.00049%) and pordamacrine B[16] (0.00033%). The crude alkaloidal materials prepared from the branches (380 g) were separated by an amino silica gel column (*n*-hexane/EtOAc), followed by silica gel column chromatographies (CH_3Cl/MeOH/H_2O) to give daphnezomine V (**3**, 0.00022%) together with known *Daphniphyllum* alkaloid, daphnilongeranin D[15] (0.0015%).

Daphnezomine T (**1**): Colorless amorphous solid; $[\alpha]_D^{22}$ +74.9 (*c*=0.2, $CHCl_3$); IR (neat) ν_{max} 3417, 2949, and 1714 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 1; ESI-MS *m*/*z*: 372 $(M+H)^+$; HR-ESI-MS *m*/*z*: 372.2180 (M+H; Calcd for $C_{22}H_{30}NO_4$, 372.2175).

Daphnezomine U (**2**): Colorless amorphous solid; $[\alpha]_D^{22}$ −72.0 (*c*=0.2, $CHCl_3$); IR (neat) ν_{max} 3582, 2924, 1730, 1698, and 1642 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 1; ESI-MS *m*/*z*: 422 $(M+Na)^+$; HR-ESI-MS *m*/*z*: 422.1954 (M+Na; Calcd for $C_{23}H_{29}NO_5Na$, 422.1943).

Daphnezomine V (**3**): Colorless amorphous solid; $[\alpha]_D^{22}$ +13.8 (*c*=0.3, MeOH); IR (neat) ν_{max} 3240, 2935, 1705, 1704, 1456, 1384, and 1194 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 2; ESI-MS *m*/*z*: 502 $(M+H)^+$; HR-ESI-MS *m*/*z*: 502.3522 (M+H; Calcd for $C_{30}H_{48}NO_5$, 502.3533).

Daphnezomine V (3) Derived from Daphnilongeranin D Daphnilongeranin D (1.0 mg) was treated with CH_2Cl_2 (250 ml) and *m*-CPBA (2.0 mg) at 4 °C for 1 h. The reaction mixture was partitioned between $CHCl_3$ and saturated Na_2CO_3 (aq.), and then the organic layer was evaporated *in vacuo* to afford daphnezomine V (**3**, 1.0 mg), whose spectral data and the $[\alpha]_D$ value {$[\alpha]_D^{22}$ +12.8 (*c*=0.3, MeOH)} were coincident with those of natural specimen.

Acknowledgements The authors thank Ms. S. Oka, Center for Instrumental Analysis, Hokkaido University, for measurements of ESI-MS. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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