Daphnezomines T—V, Alkaloids from *Daphniphyllum humile*

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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 C₁ unit at C-5 among all *Daphniphyllum* 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 Na₂CO₃, were extracted with CHCl₃ 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, C₂₂H₂₉NO₄, 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 ¹H- and ¹³C-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 aminor carbon.

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 ¹H–¹H correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY) spectra as shown in Fig. 1. Heteronuclear multiple bond correlations (HMBC) correlations between H₂-7 and C-1, H₂-19 and C-1, and H₂-19 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 H₂-11/C-9, H₂-11/C-10, H-14/C-9, and H₂-17/C-9. In addition, HMBC correlations between H-13 and C-21, and H₃-22 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 ³*J*-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/H₃-20, 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, C₂₃H₂₉NO₅, 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 ¹³C-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 ¹H–¹H 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 C-17), and **d** (C-

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Table 1. ^1H - 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 3J -

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

C-22–C-23 was omitted.

Table 2. ^1H - 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
7b	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/H₂-13, H-7a/H-11a, H-7b/H-19b, H-

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

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

12b/H₂-21, H-13a/H-14, H-13b/H₂-21, H-14/H-15, H-18/H-19a, and H-19b/H₃-20 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.

Daphnezomine V (**3**) showed the pseudomolecular ion peak at m/z 502 ($\text{M}+\text{H}$)⁺ in the ESI-MS, and the molecular formula, $\text{C}_{30}\text{H}_{47}\text{NO}_5$, was established by HR-ESI-MS [m/z 502.3522, ($\text{M}+\text{H}$)⁺, Δ −1.1 mmu]. IR absorptions implied the presence of hydroxy (3240 cm^{-1}) and ester carbonyl (1704 cm^{-1}) functionalities. ^1H - 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 ^1H - 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 $[\alpha]_{\text{D}}^{22}$ value $\{[\alpha]_{\text{D}}^{22} + 12.8$ ($c=0.3$, MeOH) $\}$ were coincident with those of natural daphnezomine V (**3**, $[\alpha]_{\text{D}}^{22} + 13.8$ ($c=0.3$, 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 *Daphniphyllum* alkaloids reported so far. Biogenetically, daphnezomine T (**1**) might be generated from an intermediate like pordamacrine B,¹⁶⁾ which could be derived from yuzurimine¹⁷⁾ 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 *Daphniphyllum* 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 (IC₅₀ > 10.0 μ g/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. ¹H- and ¹³C-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 ¹H- and ¹³C-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 Na₂CO₃, were extracted with CHCl₃ 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 (CHCl₃/MeOH, 9:1), followed by an amino silica gel (*n*-hexane/EtOAc $\rightarrow$ CHCl₃/MeOH) and a silica gel (CHCl₃/MeOH) column chromatographies to give daphnezomines T (**1**, 0.00063% yield) and U (**2**, 0.00033%) together with known *Daphniphyllum* alkaloids, yuzurimine¹⁷⁾ (0.00049%) and por-damacrine B¹⁶⁾ (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 (CH₃Cl/MeOH/H₂O) to give daphnezomine V (**3**, 0.00022%) together with known *Daphniphyllum* alkaloid, daphnilongeranin D¹⁵⁾ (0.0015%).

Daphnezomine T (**1**): Colorless amorphous solid; [α]_D²² +74.9 (*c*=0.2, CHCl₃); IR (neat) $\nu_{\max}$ 3417, 2949, and 1714 cm⁻¹; ¹H- and ¹³C-NMR, see Table 1; ESI-MS *m/z*: 372 (M+H)⁺; HR-ESI-MS *m/z*: 372.2180 (M+H; Calcd for C₂₂H₃₀NO₄, 372.2175).

Daphnezomine U (**2**): Colorless amorphous solid; [α]_D²² -72.0 (*c*=0.2, CHCl₃); IR (neat) $\nu_{\max}$ 3582, 2924, 1730, 1698, and 1642 cm⁻¹; ¹H- and ¹³C-NMR data see Table 1; ESI-MS *m/z*: 422 (M+Na)⁺; HR-ESI-MS *m/z*: 422.1954 (M+Na; Calcd for C₂₃H₂₉NO₅Na, 422.1943).

Daphnezomine V (**3**): Colorless amorphous solid; [α]_D²² +13.8 (*c*=0.3, MeOH); IR (neat) $\nu_{\max}$ 3240, 2935, 1705, 1704, 1456, 1384, and 1194 cm⁻¹; ¹H- and ¹³C-NMR data see Table 2; ESI-MS *m/z*: 502 (M+H)⁺; HR-ESI-MS *m/z*: 502.3522 (M+H; Calcd for C₃₀H₄₈NO₅, 502.3533).

Daphnezomine V (3**) Derived from Daphnilongeranin D** Daphnilongeranin D (1.0 mg) was treated with CH₂Cl₂ (250 ml) and *m*-CPBA (2.0 mg) at 4 °C for 1 h. The reaction mixture was partitioned between

CHCl₃ and saturated Na₂CO₃ (aq.), and then the organic layer was evaporated *in vacuo* to afford daphnezomine V (**3**, 1.0 mg), whose spectral data and the [α]_D²² value {[α]_D²² +12.8 (*c*=0.3, MeOH)} were coincident with those of natural specimen.

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