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Daphnezomines L, M, N, and O, new alkaloids from *Daphniphyllum humile*

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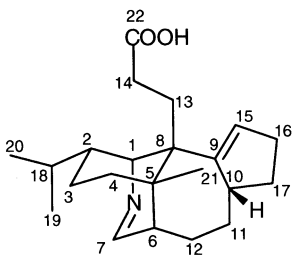
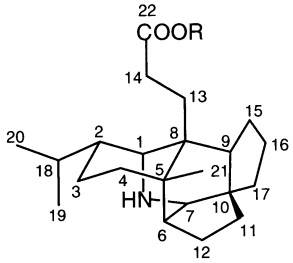
Abstract—Four new alkaloids, daphnezomines L–O (**1**–**4**), have been isolated from the stems of *Daphniphyllum humile*, and the structures and the relative stereochemistry were elucidated on the basis of spectroscopic data. The structure of daphnezomine L (**1**) was close to that of a biogenetic intermediate from secodaphnane to daphnane skeletons proposed previously.

Daphniphyllum alkaloids with unique ring systems have attracted great interests from a biogenetic point of view.[1] Enormous structural diversity of them may be explained by unique biogenetic process involving repeated fission of C–C and/or C–N bonds followed by rearrangements, recyclization, and so on.[2] Recently, some novel alkaloids with unique skeletons such as daphnezomines A, B,[3] F, and G,[4] and daphnicyclidins A–H,[5] J, and K,[6] have been isolated from *D. humile* and *D. teijsmanni*. Our interest has been focused on isolation of structurally interesting alkaloids and biosynthetic intermediates to clarify the biogenetic pathway. Further search for *Daphniphyllum* alkaloids resulted in the isolation of four new alkaloids, daphnezomines L–O (**1**–**4**), from the stems of *D. humile* (Daphniphyllaceae). The structure of **1** was close to those of biogenetic intermediates from secodaphnane to daphnane skeletons proposed previously.[7] In this paper, we describe the isolation and structure elucidation of **1**–**4**.



1



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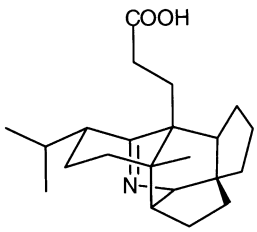
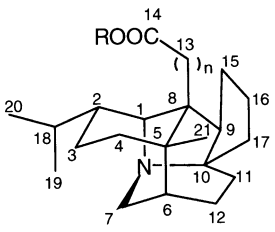
5: R=Me



3



4: R=H, n=1
6: R=Me, n=2

The stems of *D. humile* collected in Sapporo were extracted with MeOH, and the MeOH extract was partitioned between AcOEt and 3% tartaric acid. Water-soluble materials were adjusted at pH 9 with sat. Na_2CO_3 and partitioned with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (MeOH/$CHCl_3$, 1:0⟶0:1) followed by C_{18} HPLC (CH_3CN/0.1%TFA, 1:4) to afford daphnezomines L (**1**, 0.0001%), M (**2**, 0.00007%), N (**3**, 0.00007%), and O (**4**, 0.001%) as colorless solids together with a known related alkaloid, zwitter ionic alkalod[8] (0.0005%).

FABMS data of daphnezomine L (**1**, $[\alpha]_D^{24}=-137°$ (*c* 0.1, MeOH)) showed the pseudomolecular ion peak at *m/z* 344 $(M+H)^+$, and the molecular formula, $C_{22}H_{33}NO_2$, was established by HRFABMS (*m/z* 344.2590 $(M+H)^+$, Δ 0.0 mmu). The ^{13}C NMR (Table 2) spectrum revealed 22 carbon signals due to four quaternary carbons (sp^2×2 and sp^3×2), seven methines (sp^2×2 and sp^3×5), eight methylenes, and three methyls. IR absorptions implied the presence of carboxylate (1585 cm^{-1}) and imine (1670 cm^{-1}) groups. Since three out of seven elements of unsaturation were accounted for, **1** was inferred to possess four rings. Three partial structures **a** (C-1–C-4 and C-18–C-20), **b** (C-6–C-7, C-10–C-12, and C-15–C-17), and **c** (C-13–C-14) were deduced from detailed analyses of 2D NMR data (^1H–^1H COSY, HOHAHA, and HMQC) of **1**.

Keywords: alkaloids; imine; conformation; NMR.

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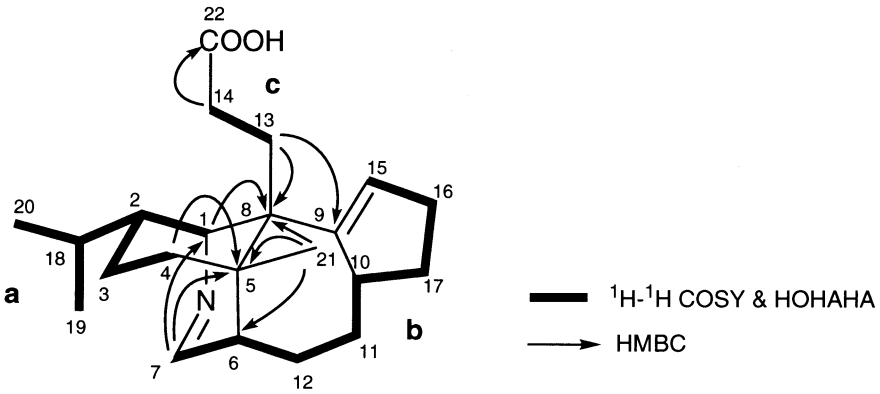


Figure 1. Selected 2D NMR correlations for daphnezomine L (**1**).

The chemical shifts of C-1 (δ_C 66.27) and C-7 (δ_C 173.47) suggested that these carbons were attached to a nitrogen atom, which was also supported by those of H-1 (δ_H 4.37) and H-7 (δ_H 7.95). Connections among C-1, C-4, C-6, and C-21 via two consecutive quaternary carbons C-5 (δ_C 38.02) and C-8 (δ_C 44.36) were implied by HMBC cross-peaks for H-1–C-8, H-4–C-5, H-21–C-5, C-6, and C-8, and H-7–C-5 and C-1, indicating the presence of an azabicyclo[3.3.1] ring system with a methyl at C-5. Long-range C–H couplings for H-13–C-8 and C-9 revealed the connection between units **b** and **c** via C-8 and C-9. The HMBC correlation of H-14–C-22 (δ_C 179.43) revealed that a carboxylate moiety was attached to C-14. Thus, the gross structure of daphnezomine L was elucidated to be **1**, possessing a nitrogen-containing tetracyclic ring system consisting of a 5-membered, two 6-membered, and a 7-membered rings with an isopropyl group at C-2 and a methyl group at C-5 (Fig. 1).

The relative stereochemistry of **1** was elucidated from NOESY correlations as shown in computer-generated 3D drawing (Fig. 2). A chair conformation of a cyclohexane ring (C-1–C-5 and C-8) was suggested by NOESY cross-peaks of H-2/Hb-4 and H-2/H-13.

Daphnezomine M (**2**, $[\alpha]_D^{24}=-21°$ (*c* 0.2, MeOH)) showed the pseudomolecular ion peak at *m/z* 346 $(M+H)^+$ and the molecular formula, $C_{22}H_{35}NO_2$, was established by HRFABMS (*m/z* 346.2733, $(M+H)^+$, Δ −1.3 mmu). IR absorptions implied the presence of amine (3415 cm^{-1}) and carboxylate (1570 cm^{-1}) functionalities. The ^{13}C NMR (Table 2) spectrum showed signals due to four quaternary carbons (sp^2×1 and sp^3×3), six sp^3 methines, nine methylenes, and three methyls. Methylation of **2** with trimethylsilyldiazomethane afforded the methyl ester, whose spectral data and $[\alpha]_D$ value were identical with those of methyl homosecodaphniphyllate (**5**).[9] Thus, the structure and the absolute stereochemistry of daphnezomine M (**2**) were elucidated as shown.

HRFABMS data (*m/z* 344.2606 $(M+H)^+$, Δ +1.6 mmu) of daphnezomine N (**3**) revealed the same molecular formula, $C_{22}H_{33}NO_2$, as that of **1**. The ^{1}H and ^{13}C NMR (Tables 1 and 2) spectra of **3** showed signals due to two sp^2 and three sp^3 quaternary carbons, five sp^3 methines, nine methylenes, and three methyls, suggesting that **3** had a similar backbone skeleton to that of daphnezomine C.[10] The ^{13}C NMR spectrum revealed signals due to a nitrogen bearing carbon (δ_C 71.16, d), an iminium carbon (176.58, s), and a ketone carbon (δ_C 178.71). The gross structure of **3** was elucidated by 2D NMR (^{1}H–^{1}H COSY, HOHAHA, HMQC, and HMBC) data (Fig. 3). The ^{1}H–^{1}H COSY and HOHAHA

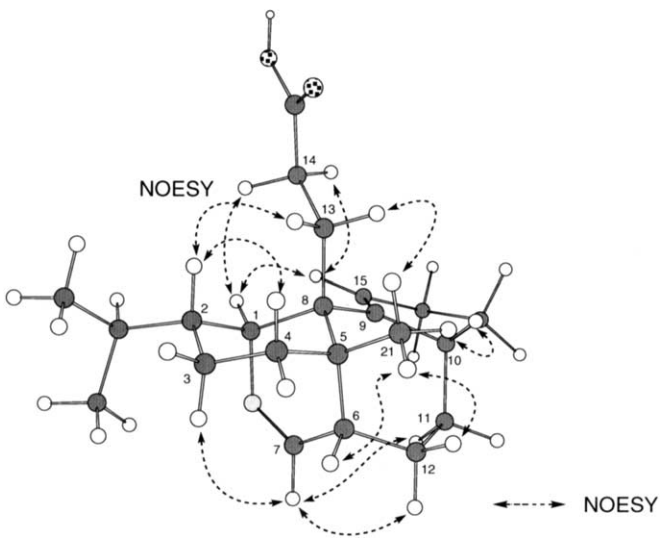


Figure 2. Selected NOESY correlations and relative stereochemistry for daphnezomine L (**1**).

Table 1. ^{1}H NMR data (δ_H (J, Hz)) of daphnezomines L–O (**1**–**4**) in CD_3OD at 300 K

Position	1	2	3	4
1	4.37 (1H, brs)	3.46 (1H, s)		4.08 (1H, brs)
2	1.45 (1H, m)	1.42 (1H, m)	3.26 (1H, dt, 5.2, 10.6)	2.05 (1H, m)
3a	0.94 (1H, brdd, 5.0, 12.0)	1.41 (1H, m)	1.49 (1H, m)	1.76 (1H, m)
3b	1.65 (1H, m)	1.83 (1H, m)	2.57 (1H, m)	2.06 (1H, m)
4a	1.24 (1H, ddd, 1.9, 4.8, 14.3)	1.37 (1H, m)	1.25 (1H, brd, 5.5)	1.60 (1H, m)
4b	1.85 (1H, brdd, 5.4, 14.3)	1.77 (1H, m)	1.97 (1H, m)	2.05 (1H, m)
6	1.91 (1H, m)	2.18 (1H, t, 4.5)	2.15 (1H, m)	1.81 (1H, m)
7a	7.95 (1H, brs)	3.15 (1H, d, 4.5)	4.33 (1H, d, 4.5)	3.14 (1H, d, 13.5)
7b				3.71 (1H, d, 13.5)
9		2.03 (1H, t, 16.0)	1.57 (1H, m)	2.82 (1H, dd, 7.4, 10.6)
10	3.02 (1H, brt, 10.0)			
11a	1.37 (1H, dd, 4.9, 13.4)	1.75 (1H, m)	1.83 (1H, m)	1.50 (1H, m)
11b	1.68 (1H, m)	1.86 (1H, m)	2.05 (1H, m)	1.86 (1H, m)
12a	1.93 (1H, ddd, 2.4, 5.0, 14.5)	1.63 (1H, m)	2.12 (2H, m)	1.78 (1H, m)
12b	1.99 (1H, dd, 4.7, 14.5)	1.84 (1H, m)		2.02 (1H, m)
13a	1.90 (2H, m)	1.67 (1H, m)	2.38 (1H, m)	2.26 (1H, m)
13b		1.78 (1H, m)	2.48 (1H, m)	2.43 (1H, m)
14a	2.27 (1H, m)	2.23 (1H, m)	2.49 (1H, m)	
14b	2.32 (1H, m)	2.43 (1H, m)	2.68 (1H, m)	
15a	5.61 (1H, brs)	1.29 (1H, m)	0.99 (1H, m)	1.82 (1H, m)
15b		1.58 (1H, m)	2.11 (1H, m)	2.16 (1H, m)
16a	2.18 (1H, m)	1.57 (1H, m)	1.52 (1H, m)	1.69 (1H, m)
16b	2.26 (1H, m)	1.82 (1H, m)	1.59 (1H, m)	1.99 (1H, m)
17a	1.50 (1H, brdd, 7.9, 12.4)	1.74 (1H, m)	1.74 (1H, ddd, 6.6, 10.1, 13.1)	1.53 (1H, m)
17b	2.06 (1H, m)	1.81 (1H, m)	1.91 (1H, m)	2.10 (1H, m)
18	1.41 (1H, m)	1.47 (1H, m)	2.20 (1H, m)	1.57 (1H, m)
19	1.12 (3H, d, 6.4)	1.05 (3H, d, 6.0)	1.06 (3H, d, 6.5)	1.02 (3H, d, 6.5)
20	0.89 (3H, d, 6.4)	1.01 (3H, d, 6.0)	1.01 (3H, d, 6.5)	1.08 (3H, d, 6.5)
21	1.19 (3H, s)	0.94 (3H, s)	1.21 (3H, s)	1.03 (3H, s)

spectra revealed connectivities of partial structures **a** (C-2–C-4 and C-18–C-19 and C-20), **b** (C-6–C-7 and C-12, and C-11–C-12), **c** (C-9–C-15 and C-15–C-17), and **d** (C-13–C-14). These four units **a**–**d** were connected to one another on the basis of HMBC correlations of H-2 and H-13–C-1, H-4–C-5, H_3-21–C-5, C-6, and C-8, H-7, H-11, and H-17–C-10, H-9–C-8, and H-13–C-9. The presence of a carboxylate at C-22 was indicated by HMBC correlations of H_2-14–C-22. The relative stereochemistry of **3** was deduced from NOESY correlations (Fig. 3). Reduction of **3** by sodium borohydride gave compound **2**, whose spectral data and $[\alpha]_D$ value were identical with those of daphnezomine M (**2**). Thus, daphnezomine N (**3**) was concluded to be an imine form of **2**.

Table 2. ^{13}C NMR data (δ_C) of daphnezomines L–O (**1**–**4**) in CD_3OD at 300 K

Position	1	2	3	4
1	66.27	52.40	176.58	63.85
2	42.80	42.72	57.59	38.20
3	22.22	21.13	23.77	21.84
4	41.86	38.99	36.95	41.02
5	38.02	37.70	58.55	38.12
6	50.88	46.16	51.95	39.73
7	173.47	59.44	71.16	47.11
8	44.36	38.23	62.74	47.68
9	154.38	53.95	53.49	53.39
10	47.16	50.01	46.09	78.76
11	34.87	41.10	39.42	26.13
12	27.16	24.17	23.08	28.87
13	30.38	28.75	25.90	37.77
14	32.08	30.74	33.04	179.03
15	127.30	30.74	35.73	30.45
16	30.07	27.34	26.58	26.57
17	33.86	36.59	38.28	36.39
18	31.03	29.77	28.78	31.76
19	21.55	21.28	19.28	21.14
20	21.38	21.51	22.68	21.35
21	25.47	21.61	22.44	23.47
22	179.43	177.34	178.71	

The molecular formula of daphnezomine O (**4**) was determined to be $C_{21}H_{33}NO_2$ by HRFABMS (m/z 332.2579 $(M+H)^+$, Δ −1.1 mmu), which was smaller than that of **2** by one methylene unit. The IR spectrum suggested the presence of carboxylate (1585 cm^{-1}) group. The ^{1}H and ^{13}C NMR (Tables 1 and 2) spectra of **4** showed signals due to one carboxylate, five sp^3 methines, three quaternary carbons, nine sp^3 methylenes, and three methyl groups. Comparison of the NMR data of **4** with those of methyl homodaphniphyllate (**6**)[11] suggested that **4** had a daphnane skeleton, while interpreting the ^{1}H–^{1}H COSY and HMBC spectra also revealed the presence of a daphnane-type pentacyclic skeleton. NOESY correlations of **4** indicated the same relative stereochemistry as that of methyl homodaphniphyllate (**6**).[11] Thus, the structure and the relative stereochemistry of daphnezomine O were elucidated to be **4**.

Daphnezomine L (**1**) was close to that of a biogenetic intermediate from secodaphnane to daphnane skeletons. Yamamura et al.[12] suggested that a pentacyclic skeleton like **1** is a possible biogenetic intermediate to daphnane skeleton (**4**), while Heathcock et al.[7] proposed a biogenetic route from secodaphnane (**2**) to daphnane (**4**) skeletons through intermediates **A** and **B** (Scheme 1). The present studies indicate that daphnezomines L (**1**) and O (**4**) might be biosynthesized through intermediates **A** and **B**, while daphnezomine N (**3**) might be generated through intermediate **A**.

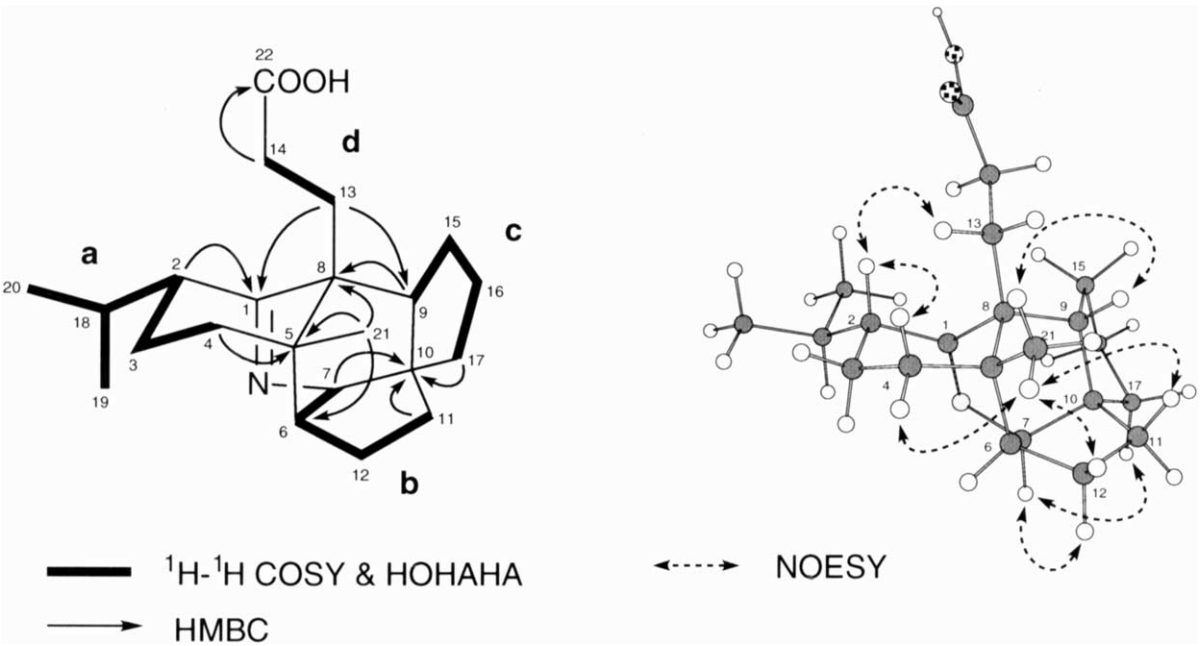


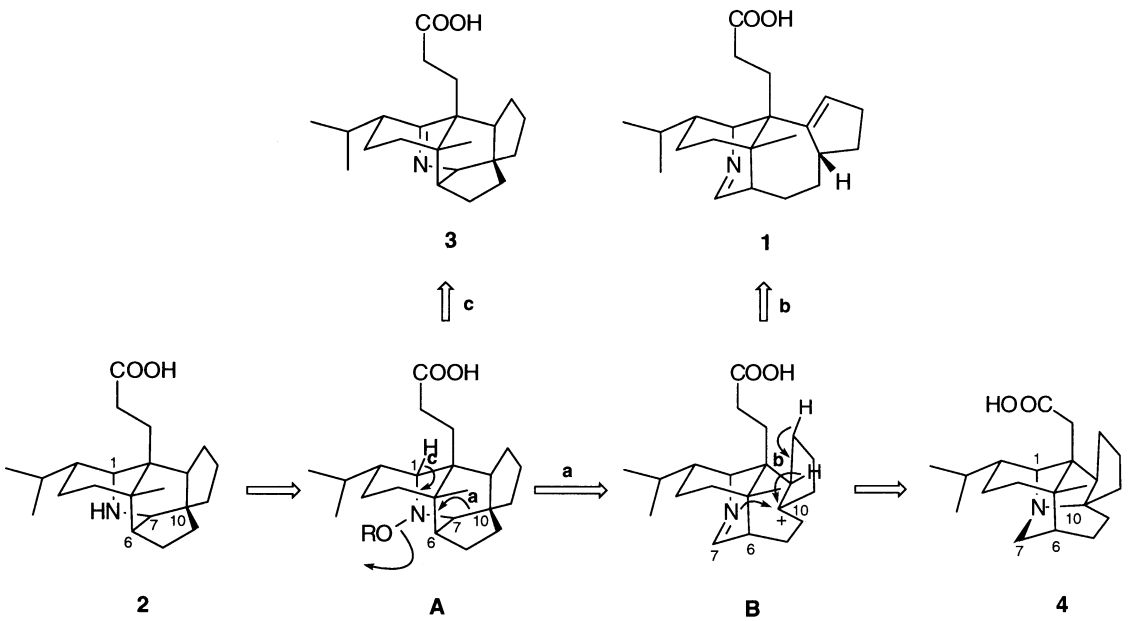
Figure 3. Selected 2D NMR correlations and relative stereochemistry for daphnezomine N (**3**).

Daphnezomines L (**1**) and N (**3**) exhibited cytotoxicity against murine lymphoma L1210 cells in vitro with IC_{50} values of 4.0 and 8.7 μg/mL, respectively, while daphnezomines M (**2**) and O (**4**) did not show such activity (IC_{50}>10 μg/mL).

1. Experimental

1.1. General methods

^{1}H and 2D NMR spectra were recorded on a 600 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. The NMR samples of daphnezomines L–O (**1**–**4**) were prepared by dissolving 1.5 mg in 30 μL of CD_3OD in 2.5 mm micro cells (Shigemi Co. Ltd) and chemical shifts were reported using residual CD_3OD (δ_H 3.31 and δ_C 49.0) as internal standards. Standard pulse sequences were employed for the 2D NMR experiments. COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1 K data points for each of 256 t_1 increments. NOESY and HOHAHA spectra in the phase sensitive mode were measured with a mixing time of 800 and 30 ms, respectively. For HMQC spectra in the phase sensitive mode and HMBC spectra, a total of 256 increments of 1 K data points were collected. For HMBC



Scheme 1.

spectra with *Z*-axis PFG, a 50 ms delay time was used for long-range C–H coupling. Zero-filling to 1 K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation. FABMS was measured by using glycerol as a matrix.

1.2. Material

The stems of *D. humile* were collected in Sapporo in 1998. The botanical identification was made by Mr N. Yoshida, Health Sciences University of Hokkaido. A voucher specimen has been deposited in the herbarium of Hokkaido University.

1.3. Extraction and isolation

The stems (7.5 kg) of *D. humile* were crashed and extracted with MeOH (10 L×3). The crude alkaloidal fraction (14.5 g) of the MeOH extract (477 g) as described in the previous paper[3] was separated by an amino silica gel column chromatography (MeOH/$CHCl_3$, 1:0⟶0:1) followed by C_{18} HPLC (CH_3CN/0.1%TFA, 1:4) to afford daphnezomines L (**1**, 0.0001%), M (**2**, 0.00007%), N (**3**, 0.00007%), and O (**4**, 0.001%), as colorless solids together with a known related alkaloid, zwitter ionic alkaloid[8] (0.0005%).

1.3.1. Daphnezomine L (1). A colorless solid; $[\alpha]_D^{24}=-137°$ (*c* 0.1, MeOH); ^1H and ^{13}C NMR data (Table 1); FABMS *m/z* 344 $(M+H)^+$; HRFABMS *m/z* 344.2590 (M+H; calcd for $C_{22}H_{34}NO_2$, 344.2590); IR (KBr) ν_{max} 3340, 2935, 2865, 1670, 1585, 1380, and 1310 cm^{-1}.

1.3.2. Daphnezomine M (2). A colorless solid; $[\alpha]_D^{24}=-21°$ (*c* 0.2, MeOH); ^1H and ^{13}C NMR data (Table 1); FABMS *m/z* 346 $(M+H)^+$; HRFABMS *m/z* 346.2733 (M+H; calcd for $C_{22}H_{36}NO_2$, 346.2746); IR (KBr) ν_{max} 3415, 2945, 1570, 1465, and 1385 cm^{-1}.

1.3.3. Daphnezomine N (3). A colorless solid; $[\alpha]_D^{24}=-113°$ (*c* 0.3, MeOH); FABMS *m/z* 344 $(M+H)^+$; HRFABMS *m/z* 344.2606 (M+H; calcd for $C_{22}H_{34}NO_2$, 344.2590); IR (KBr) ν_{max} 3450, 2930, 2875, 1675, 1440, 1195, and 1135 cm^{-1}.

1.3.4. Daphnezomine O (4). A colorless solid; $[\alpha]_D^{24}=-22°$ (*c* 0.3, MeOH); ^1H and ^{13}C NMR data; FABMS *m/z* 332 $(M+H)^+$; HRESIMS *m/z* 332.2579 (M+H; calcd for $C_{21}H_{34}NO_2$, 332.2590); IR (KBr) ν_{max} 3325, 2940, 2865, 1585, 1455, and 1380 cm^{-1}.

1.3.5. Methylation of daphnezomine M (2). Trimethylsilyldiazomethane (2.0 M hexane solution, 100 μL) was added to a stirred solution of **2** (1 mg) in methanol (0.2 mL) at room temperature. The mixture was stirred at room temperature for 30 min, and concentrated in vacuo. The residue was subjected to an LH-20 column to give the methyl derivative, whose spectral data and $[\alpha]_D$ value were identical with those of methyl homosecodaphniphyllate (**5**).[10]

1.3.6. Reduction of daphnezomine N (3). To a solution of daphnezomine N (**3**) (1 mg) in CH_3OH (0.1 mL) was added sodium borohydride (1 mg) and was allowed to stand at room temperature for 10 min. The residue was dissolved in $CHCl_3$ and washed with sat. NaCl aq. and then water. After evaporation, the residue was applied to an LH-20 column to give the reductive derivative, whose spectral data and $[\alpha]_D$ value were identical with those of daphnezomine M (**2**).

Acknowledgments

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