Daphnezomines A and B, Novel Alkaloids with an Aza-adamantane Core from *Daphniphyllum humile*

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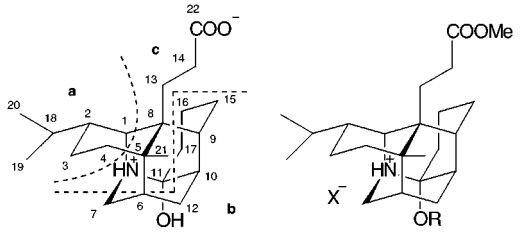
Two novel alkaloids with a unique aza-adamantane core, daphnezomines A (**1**) and B (**2**), have been isolated from the leaves of *Daphniphyllum humile*, and the structures including relative stereochemistry were elucidated on the basis of spectroscopic data and chemical means. The absolute configuration of **2** was established by X-ray crystallographic analysis.

Daphniphyllum alkaloids such as daphniphylline and yuzurimine possess unique ring systems and have attracted great interest from a biogenetic point of view.[1,2] These stimulating polycyclic skeletons have prompted extensive synthetic work. Heathcock and co-workers demonstrated an extraordinary transformation into these alkaloids by using three elementary reagents, potassium hydroxide, ammonia, and acetic acid.[3] These results indicate that a similar process might be an important step in the biosynthesis of the *Daphniphyllum* alkaloids. In our project on a search for biogenetic intermediates of *Daphniphyllum* alkaloids, daphnezomines A (**1**) and B (**2**), two novel alkaloids possessing an azaadamantane core containing an amino ketal bridge were isolated from the leaves of *Daphniphyllum humile*.[4] This paper describes the isolation and structural elucidation of **1** and **2**.



1

2: R=H
3: R=Ac

The leaves of *D. humile* collected in Sapporo were extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 9 with saturated Na_2CO_3, were partitioned with $CHCl_3$, and $CHCl_3$-soluble materials were subjected to a C_{18} column (CH_3CN/0.1% TFA 3:7) to afford daphnezomine A (**1**, 0.01% yield) as colorless needles. EtOAc-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH 99:1 → 1:1), in which a fraction eluted with $CHCl_3$/MeOH (9:1) was purified by a C_{18} column (CH_3CN/0.1% TFA 1:4) followed by gel filtration on Sephadex LH-20 (MeOH) to give daphnezomine B (**2**, 0.008% yield) as an unspecified salt.

Daphnezomine A (**1**) showed a pseudomolecular ion at *m/z* 362 $(M + H)^+$ in the FABMS, and the molecular formula, $C_{22}H_{35}NO_3$, was established by HRFABMS [*m/z* 362.2697, $(M + H)^+$, Δ +0.2 mmu]. The IR absorptions implied the presence of OH (3600 cm^{-1}), NH (3430 cm^{-1}), and carboxylate (1570 and 1390 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectra revealed signals due to one carbonyl, three quaternary carbons, six methines, nine methylenes, two secondary methyls, and one tertiary methyl. Among them, one methine (δ_C 59.82; δ_H 3.79) and one methylene (δ_C 45.99; δ_H 3.44 and 3.97) were ascribed to those bearing a nitrogen, while one quaternary carbon (δ_C 89.64) was assigned as an amino ketal carbon.[5]

The three partial structures (**a**−**c**) were deduced from extensive analyses of the 2D NMR data of **1** including COSY, HOHAHA,[6] HMQC, and HMBC[7] spectra in $CDCl_3$−CD_3OD (9:1). The ^1H and ^{13}C NMR data and HMBC correlations are presented in Table 1. Interpreting

(1) For reviews of the *Daphniphyllum* alkaloids, see: (a) Yamamura, S.; Hirata, Y. In *The Alkaloids*; Manske, R. H. F., Ed.; Academic Press: New York, 1975; Vol. 15, p 41. (b) Yamamura, S. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1986; Vol. 29, p 265.

(2) (a) Hao, X.; Zhou, J.; Node, M.; Fuji, K. *Yunnan Zhiwu Yanjiu* **1993**, *15*, 205−207. (b) Arbain, D.; Byrne, L. T.; Cannon, J. R.; Patrick, V. A.; White, A. H. *Aust. J. Chem.* **1990**, *43*, 185−190. (c) Yamamura, S.; Lamberton, J. A.; Niwa, M., Endo, K., Hirata, Y. *Chem. Lett.* **1980**, 393−396. (d) Yamamura, S.; Toda, M.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 839. (e) Yamamura, S.; Lamberton, J. A.; Irikawa, H.; Okumura, Y.; Hirata, Y. *Chem. Lett.* **1975**, 923−926. (f) Yamamura, S.; Irikawa, H.; Okumura, Y.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2120−2123. (g) Yamamura, S.; Hirata, Y. *Tetrahedron Lett.* **1974**, *42*, 3673−3676. (h) Yamamura, S.; Sasaki, K.; Toka, M.; Hirata, Y. *Tetrahedron Lett.* **1974**, 2023−2026 and references therein.

(3) (a) Heathcock, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14323−14327. (b) Heathcock, C. H.; Joe, D. *J. Org. Chem.* **1995**, *60*, 1131−1142. (c) Heathcock, C. H.; Kath, J. C.; Ruggeri, R. B. *J. Org. Chem.* **1995**, *60*, 1120−1130. (d) Heathcock, C. H. *Angew. Chem.* **1992**, *104*, 675−691; *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 665−681 and references therein.

(4) (a) In the previous study, yuzurimine was isolated as a major alkaloid together with secodaphniphylline, daphniteijsmine, deoxyyuzurimine, and isodaphnilactone-B: Yamamura, S.; Terada, Y. *Chem. Lett.* **1976**, 1381−1382. (b) A disease with jaundice, colic, and photophobia as main symptoms that had broken out among grazing cattle in Hokkaido was demonstrated to be a plant poisoning caused by *D. humile* which contained a toxic substance affecting the liver specifically: Sonoda, M.; Tasaka, M.; Takahashi, K.; Koiwa, M.; Minami, S.; Iwasa, M.; Yashiro, K. *J. Jpn. Vet. Med. Assoc.* **1978**, *31*, 140−144.

(5) The ^{13}C NMR signals for sp^3 quaternary carbons located at a ketal are observed at δ_C 95−110 ppm (Wiemer, D. F.; Wolfe, L. K.; Fenical, W.; Strobel, S. A.; Clardy, J. *Tetrahedron Lett.* **1990**, *31*, 1973−1976), whereas those located at an amino ketal resonate at δ_C 85−95 ppm: Lindquist. N.; Fenical, W. *Tetrahedron Lett.* **1990**, *31*, 2521−2524.

(6) Edwards, M. W.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 918−923.

(7) (a) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094. (b) Bax, A.; Aszalos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056−8063.

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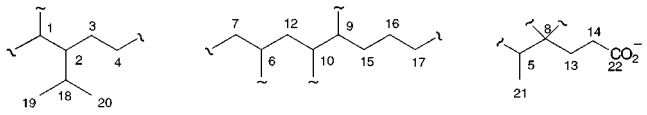
Table 1. ^{1}H and ^{13}C NMR Data of Daphnezomine A (1) in $CDCl_3-CD_3OD$ (9:1)

position	H(*J*, Hz)	C	HMBC (^{1}H)
1	3.79 d (3.4)	59.82	3b, 7a, 13a, 18
2	1.67 m	39.48	1, 4a, 19
3a	1.56 m	26.13	1, 4a
3b	1.92 m		
4a	1.51 m	34.74	21
4b	2.02 m		
5		36.01	1, 7a, 12a, 13a, 21
6	1.41 br s	38.67	4b, 12a, 21
7a	3.97 d (13.0)	45.99	1, 12b
7b	3.44 d (13.0)		
8		39.21	1, 4a, 6, 7a, 13b, 15b, 21
9	2.39 br s	34.33	12a, 13b
10	1.81 m	38.18	6
11		89.64	7b, 9, 12b, 15b, 16b
12a	2.17 m	26.40	7b
12b	1.81 m		
13a	1.24 m	29.12	14a, 14b
13b	2.13 m		
14a	2.16 m	32.82	
14b	2.26 m		
15a	1.72 m	24.59	17a
15b	1.81 m		
16a	2.08 m	34.49	
16b	2.34 m		
17a	1.70 m	21.24	9, 16b
17b	1.81 m		
18	1.88 m	28.61	19, 20
19	0.90 d (6.6)	21.08	20
20	0.91 d (6.2)	21.87	19
21	1.03 s	25.55	1, 4b
22		180.64	13a, 13b, 14a, 14b

the COSY and HOHAHA spectra easily revealed proton connectivities from C-1 to C-4 with an isopropyl group (C-18−C-20) at C-2, corresponding to partial structure **a**.



For partial structure **b** (C-7, C-6, C-12, C-10, C-9, C-15, C-16, and C-17), connectivities from C-7 to C-12 and from C-9 to C-16 were clearly revealed by the COSY spectrum. It was, however, difficult to obtain unambiguous evidence for connecting the remaining methine (C-10, δ_C 38.18) and methylene (C-17, δ_C 21.24) carbons, due to the heavy overlapping of these proton signals. The connectivity of unit **b** was completed by HSQC-HOHAHA[8] correlations for H_a-12/C-10, H-9/C-10, and H-9/C-17.

The methyl group (C-21) and two consecutive methylenes (C-13 and C-14) in partial structure **c** (C-21, C-5, C-8, C-13, C-14, and C-22) were located on C-5 and C-8, respectively, by HMBC cross-peaks for H_b-13/C-8, H_a-13/C-5, H_2-14/C-13, and H_3-21/C-5. The carboxylate group was inferred to be attached to C-14 by HMBC correlations for H_2-14/C-22. The connectivity among three units **a**−**c** was provided by the following HMBC correlations (Table 1).

The H_2-7 showed HMBC correlations for C-1 (δ_C 59.82), thus giving rise to the connectivity (C-1−N-1−C-7) of partial structures **a** and **b** through a nitrogen atom (N-1). In addition, HMBC correlations for H_a-7/C-5, H_a-12/C-5, H_b-15/C-8, H-1/C-8, and H_3-21/C-4 suggested that

(8) Lerner, L.; Bax, A. *J. Magn. Reson.* **1986**, *69*, 375−380.

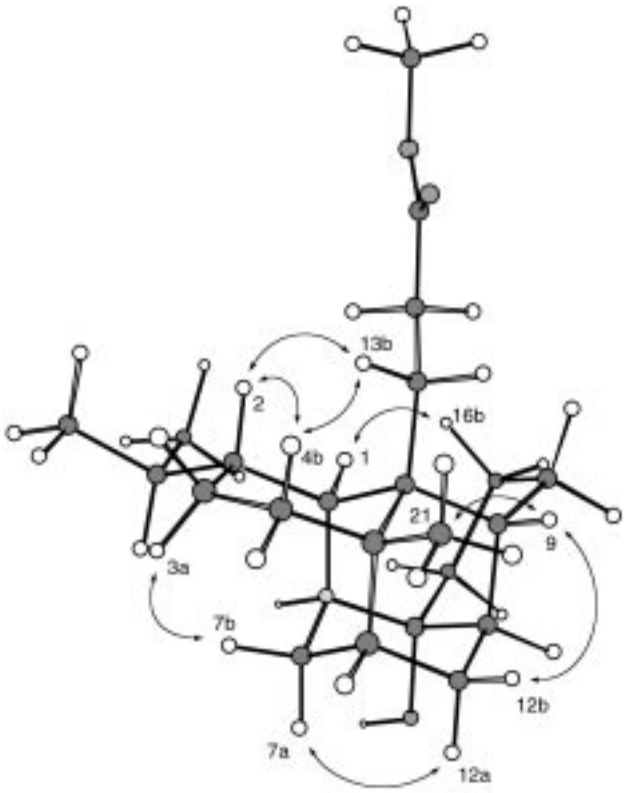


Figure 1. Key NOESY correlations (arrows) and relative configurations assigned for daphnezomine A (**1**).

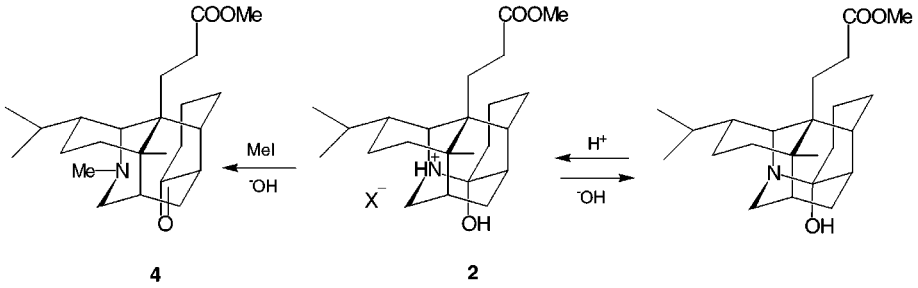
partial structures **a**−**c** were connected through C-5−C-6, C-8−C-9, C-1−C-8, and C-4−C-5 bonds. In the HMBC spectrum, H_b-7, H-9, H_b-12, and H_b-16 showed correlations to C-11 through N-1−C-11, C-10−C-11, and C-11−C-17 bonds. Thus, the remaining amino ketal carbon was assigned as C-11 to complete the gross structure of **1**, consisting of an aza-adamantane core (N-1, C-1, and C-5−C-12) fused to a cyclohexane ring (C-1−C-5 and C-8) and another cyclohexane ring (C-9−C-11 and C-15−C-17), and three substituents at C-2, C-5, and C-8.

The phase-sensitive NOESY spectrum of **1** showed cross-peaks as shown in computer-generated 3D drawing (Figure 1).[9] NOESY correlations of H_b-13/H-2, H_b-13/H_b-4, and H-2/H_b-4 indicated that H-2 and C-13 were β-oriented. The relative configurations at C-1, N-1, C-5, and C-6 were elucidated by the NOESY correlation of H_a-3/H_b-7.[10] The cis-ring junction at C-10 and C-11 was deduced from the NOESY correlation of H_b-16/H-1.[10] NOESY correlations of H-9/H_3-21 and H-9/H_b-12 argued well for the stereochemistry of the aza-adamantane skeleton. A chair form of the piperidine ring (C-7, C-6, C-12, C-10, C-11, and N-1) was verified by the NOESY correlation of H_a-7/H_a-12 and the long-range W-coupling between H_b-7 and H_b-12. The NMR data facilitated by the NOESY spectra provided additional proof corroborating chair conformations for all six-membered rings in **1**. Thus, the relative stereostructure of daphnezomine A (**1**) was concluded as shown in Figure 1.

(9) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(10) Two NOE correlations (H_a-3/H_b-7 and H_b-16/H-1) are indicative of the chair−chair form in two azabicyclo[3.3.1]nonane parts (N-1 and C-1−C-8, and N-1, C-1, C-8−C-11, and C-15−C-17): Appleton, R. A.; Egan, S. C.; Evans, J. M.; Graham, S. H.; Dixon, J. R. *J. Chem. Soc. C* **1968**, 1110−1115.

Scheme 1



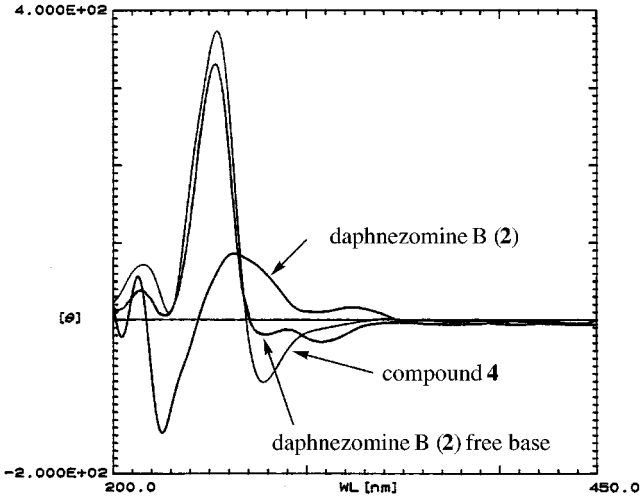


Figure 2. CD spectra of daphnezomine B (**2**), daphnezomine B (**2**) free base, and compound **4**.

HRFABMS data [*m*/*z* 376.2852, $(M + H)^+$, Δ 0.0 mmu] of daphnezomine B (**2**) established the molecular formula, $C_{23}H_{37}NO_3$, which was larger than that of **1** by a CH_2 unit. The NMR data of **2** were analogous to those of **1** except for the following observation: a methoxy signal (δ_H 3.67) lacking in **1** appeared for **2**. Acetylation of **2** afforded the monoacetate (**3**), in which the axially oriented tertiary hydroxyl group was acetylated. When daphnezomine B (**2**) was treated with aqueous Na_2CO_3, it was converted into its free base, showing spectroscopic anomalies[11] (Scheme 1). When the free base was treated with acetic acid, it was easily converted into **2** again. Compound **4** was obtained by treatment of **2** with CH_3I/K_2CO_3 (Scheme 1).[2e] Production of **4** would have resulted from N-methylation of the free base followed by the alkaline-induced N−C-11 bond cleavage to generate a ketone at C-11. In the CD spectra (Figure 2), the structural similarity between the free bases of **2** showing spectroscopic anomalies[11] and **4** were obtained by the CD spectra (MeOH) [free base of **2**: λ_{max} 255 (θ +400) and 280 (−80) nm; **4**: λ_{max} 260 (θ +350), 280 (−20), and 305 (−30) nm], showing CD curves different from that of **2** [λ_{max} 225 (θ −150) and 265 (+100) nm]. These data indicated that the balance of the amino ketal in free base of **2** declined in keto form in solution. Thus, the structure of daphnezomine B was elucidated to be **2**. The spectral data and the $[\alpha]_D$ value of the methyl ester of **1**, which was obtained by treatment of **1** with trimethylsilyldiazomethane, were in complete agreement with those of natural daphnezomine B (**2**).

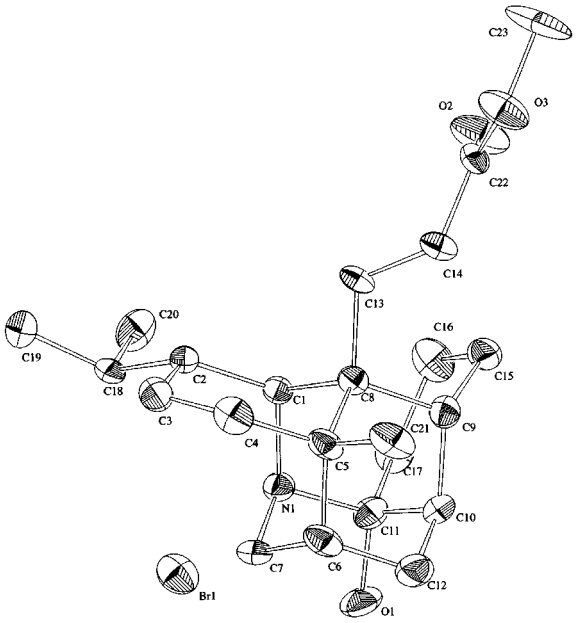


Figure 3. Molecular structure of daphnezomine B (**2**) hydrobromide obtained by X-ray analysis (ORTEP drawing; ellipsoids are drawn at the 30% probability level). Hydrogen atoms are omitted for clarity.

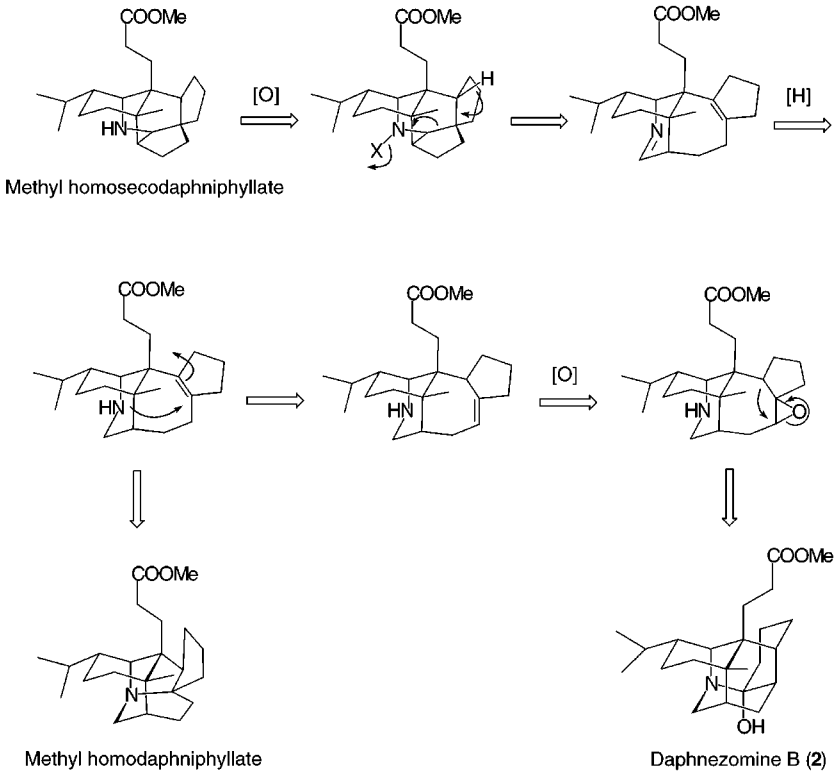
To determine the absolute stereochemistry of **1** and **2**, a crystal of the hydrobromide of **2** generated from MeOH/acetone (1:9) was submitted to X-ray crystallographic analysis. The crystal structure containing the absolute configuration, which was determined through the Flack parameter,[12] $\chi = -0.02(2)$, was shown in Figure 3.

Daphnezomines A (**1**) and B (**2**) consisting of all six-membered rings are the first natural products containing an aza-adamantane core[13] with an amino ketal bridge,[14−18] although there are reports on a number of *Daphniphyllum* alkaloids containing five-membered rings, which may be generated from a nitrogen-involved squalene intermediate via secodaphnane skeleton.[1] A biogenetic pathway for daphnezomine B (**2**) is proposed in Scheme 2. Daphnezomines A (**1**) and B (**2**) might be generated through ring expansion accompanying backbone rearrangement of a common fragmentation intermediate. Daphnezomine B (**2**) exhibited cytotoxicity against mu-

(11) The ^{13}C signals (C-9, C-12, C-16, C-17, and C-18) of the free base showed extreme broadening, while the quaternary carbon (C-11) was not observed. NMR evidence indicating a differently directed nitrogen lone pair were obtained by the ^{15}N NMR spectra (**2**: δ_N 93.7; free base of **2**: δ_N 63.0). On the other hand, in the IR spectrum, the close proximity of the carbonyl and the nitrogen permits pronounced interaction of these two functions to result in lower shift of the carbonyl peak (ν_{max} 1680 cm^{-1}) for **4**, whereas it was not observed for the free base of **2**. To examine the spectroscopic anomalies, yuzurimine free base with the similar amino ketal functionality was prepared under the same conditions, but such spectroscopic anomalies were not observed.

(12) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876−881.

Scheme 2



rine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro with IC_{50} values of 0.46 and 8.5 μg/mL, respectively, while **1** was not cytotoxic (>10 μg/mL).

Experimental Section

General Procedures. ^{1}H, ^{13}C, and ^{15}N NMR spectra were recorded on 600 MHz and 500 MHz spectrometers, with chemical shifts (δ) reported in ppm. The spectra were recorded at 300 K. FABMS was measured by using glycerol matrix.

Material. The leaves of *D. humile* were collected in Sapporo in 1998. 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.

Isolation. The leaves of *D. humile* (4 kg) were crushed and extracted with MeOH (3 L) three times to give a MeOH extract (230 g), which was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with saturated Na_2CO_3 (aq) to pH 9 and extracted with $CHCl_3$ to give a crude alkaloidal fraction (7.7 g), which was subjected to ODS column chromatography (CH_3CN/0.1% TFA, 3:7) to give daphnezomine A (**1**, 400 mg, 0.01%, wet weight) as colorless needles. The EtOAc-soluble fraction (20 g) was partially subjected to a silica gel column eluted with $CHCl_3$/MeOH (99:1 → 1:1). Part (0.3 g) of the fraction (0.8 g) eluted with $CHCl_3$/MeOH (9:1) was purified by a C_{18} column (CH_3CN/0.1% TFA, 1:4) followed by gel filtration on Sephadex LH-20 (MeOH) to afford daphnezomine B (**2**, 45 mg, 0.008%, wet weight).

Daphnezomine A (1): colorless needles; mp 218−220 °C (CH_3CN-H_2O); $[\alpha]_D$ −30° (*c* 0.1, $CHCl_3$/MeOH 1:1); ^{1}H and ^{13}C NMR data (Table 1); FABMS *m/z* 362 $(M + H)^+$; HRFABMS *m/z* 362.2697 (M + H; calcd for $C_{22}H_{36}NO_3$, 362.2695); IR (KBr) ν_{max} 3600, 3430, 2950, 2580, 1570, and 1390 cm^{-1}.

Daphnezomine B (2): colorless powder; $[\alpha]_D$ −31° (*c* 0.3, $CHCl_3$); FABMS *m/z* 376 $(M+H)^+$; HRFABMS *m/z* 376.2852 (M + H; calcd for $C_{23}H_{38}NO_3$, 376.2852); IR ($CHCl_3$) ν_{max} 3580, 2960, 2660, 1735, 1670, 1430, 1390, 1200, and 1130 cm^{-1}. ^{1}H NMR (500 MHz in $CDCl_3$) δ 3.92 (d, 3.2, H-1), 1.67 (m, H-2), 1.61 (m, H-3a), 2.01 (m, H-3b), 1.61 (m, H-4a), 2.01 (m, H-4b), 1.52 (br s, H-6), 4.08 (d, 13.3, H-7a), 3.55 (br d, 13.3, H-7b), 2.42 (br s, H-9), 1.94 (m, H-10), 2.30 (dt, 13.4, 2.9, H-12a), 1.84 (m, H-12b), 1.46 (m, H-13a), 2.18 (m, H-13b), 2.22 (m, H-14a), 2.46 (m, H-14b), 1.81 (m, H-15), 2.39 (m, H-16a), 2.50 (m, H-16b), 1.80 (m, H-17a), 1.91 (m, H-17b), 1.86 (m, H-18), 0.95 (d, 6.7, H_3-19), 0.96 (d, 6.9, H_3-20), 1.08 (s, H_3-21), 3.71 (s, OMe); ^{13}C NMR (125 MHz in $CDCl_3$) δ 59.70 (C-1), 39.15 (C-2), 25.64 (C-3), 34.53 (C-4), 35.79 (C-5), 38.30 (C-6), 46.13 (C-7), 39.01 (C-8), 34.09 (C-9), 37.90 (C-10), 89.70 (C-11), 26.08 (C-12), 27.36 (C-13), 29.72 (C-14), 24.35 (C-15), 34.14 (C-16), 20.83 (C-17), 28.52 (C-18), 20.70 (C-19), 20.95 (C-20), 25.47 (C-21), 173.67 (C-22), 51.97 (OMe); CD (MeOH) λ_{max} 225 (θ −150) and 265 (+100) nm.

Acetylation of 2. Daphnezomine B (**2**, 2 mg) was treated with 0.5 mL of acetic anhydride and 0.5 mL of pyridine at 50 °C for 6 h. After the usual workup, the monoacetate (**3**, 2.2 mg) was given: colorless powder; $[\alpha]_D$ −26° (*c* 0.2, $CHCl_3$); FABMS *m/z* 418 $(M + H)^+$; HRFABMS *m/z* 418.2962 (M + H; calcd for $C_{25}H_{40}NO_4$, 418.2957); IR (film) ν_{max} 2920, 2850, 1738, 1460, 1370, 1250, 1170, 1020, and 960 cm^{-1}. ^{1}H NMR (500 MHz in $CDCl_3$) δ 0.87 (3H, d, 6.6), 0.98 (3H, d, 6.4), 0.99 (3H, s), 2.10 (3H, s), 2.30−2.44 (5H, m), 2.69 (1H, m), 3.30 (3H, m), 3.68 (3H, s); ^{13}C NMR (125 MHz in $CDCl_3$) δ 20.47 (t), 20.72 (q), 21.33 (q), 22.57 (q), 23.45 (t), 25.99 (d), 27.24 (t), 27.53 (t),

(13) 1-Aza-adamantanes have explicitly been used as rigid models for studies on heterolytic fragmentation (Grob, C. A.; Bolleter, M.; Kunz, W. *Angew. Chem.* **1980**, *92*, 734−735), intramolecular charge-transfer phenomena (Worrell, C.; Verhoeven, J. W.; Speckamp, W. N. *Tetrahedron* **1974**, *30*, 3525−3531), and gas-phase basicity: Dekkers, A. W. J. D.; Nibbering, N. M. M.; Speckamp, W. N. *Tetrahedron* **1972**, *28*, 1829.

(14) There are a few biologically interesting natural products that possess heteroadamantane skeletons such as tetrodotoxin,[15] muamvatin,[16] narelin,[17] and neohobartine.[18]

(15) Goto, T.; Kishi, Y.; Takahashi, S.; Hirata, Y. *Tetrahedron Lett.* **1964**, 779−786; *Tetrahedron* **1965**, *21*, 2059−2088. Woodward, R. B. *Pure Appl. Chem.* **1964**, *9*, 49−74.

(16) Roll, D. M.; Biskupiak, J. E.; Mayne, C. L.; Ireland, C. M. *J. Am. Chem. Soc.* **1986**, *108*, 6680−6682.

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28.91 (t), 30.37 (d), 30.72 (t), 32.59 (d), 33.20 (t), 35.13 (d), 35.76 (t), 36.77 (s), 38.10 (s), 39.03 (d), 39.56 (d), 47.35 (t), 51.68 (q), 57.01 (d), 97.08 (s), 169.70 (s), 174.72 (s).

Methylation of 1. Trimethylsilyldiazomethane (2.0 M hexane solution, 100 μL) was added to a stirred solution of **1** (1 mg) in methanol (0.5 mL) at room temperature. The mixture was stirred at room temperature for 5 min and concentrated in vacuo. The residue was subjected to LH-20 column chromatography to give the methyl derivative, whose spectral data were identical with those of **2**.

Methylation of 2 with MeI. A solution of **2** (2 mg), MeI (0.5 mL), and K_2CO_3 (2 mg) in acetone (0.5 mL) was heated under reflux for 12 h and then concentrated under reduced pressure. The residue was dissolved in $CHCl_3$, washed with saturated NaCl (aq), and then dried over anhydrous Na_2SO_4. Removal of the solvent afforded the compound **4** (2 mg) as colorless powder: $[\alpha]_D$ −40° (*c* 0.4, $CHCl_3$); FABMS *m*/*z* 390 $(M + H)^+$; HRFABMS *m*/*z* 390.3009 (M + H; calcd for $C_{24}H_{40}NO_3$, 390.3008); IR (film) ν_{max} 2950, 1730, 1680, 1460, 1440, 1270, 1220, and 1170 cm^{-1}. ^1H NMR (600 MHz in $CDCl_3$) δ 3.13 (br s, H-1), 1.42 (m, H-2), 1.61 (m, H-3a), 1.81 (m, H-3b), 1.45 (m, H-4a), 1.90 (m, H-4b), 1.23 (br s, H-6), 2.41 (m, H-7a), 3.26 (ddd, 2.9, 20.3, 29.3, H-7b), 2.54 (m, H-9), 1.41 (m, H-10), 2.58 (dd, 3.0, 14.0, H-12a), 1.67 (m, H-12b), 1.51 (m, H-13a), 2.04 (ddd, 4.5, 12.2, 16.3, H-13b), 2.37 (ddd, 5.0, 12.3, 15.5, H-14a), 2.49 (ddd, 4.5, 12.8, 15.4, H-14b), 1.79 (m, H-15a), 1.84 (m, H-15b), 2.16 (m, H-16a), 2.16 (m, H-16b), 1.84 (m, H-17a), 1.84 (m, H-17b), 1.88 (m, H-18), 0.84 (d, 6.5, H_3-19), 1.02 (d, 6.4, H_3-20), 0.97 (s, H_3-21), 3.69 (s, OMe), 2.18 (s, NMe); ^{13}C NMR (125 MHz in $CDCl_3$) δ 59.44 (C-1), 46.03 (C-2), 27.52 (C-3), 37.55 (C-4), 35.19 (C-5), 41.32 (C-6), 53.76 (C-7), 43.60 (C-8), 36.43 (C-9), 46.70 (C-10), 201.18 (C-11), 25.05 (C-12), 30.49 (C-13), 31.58 (C-14), 24.82 (C-15), 38.64 (C-16), 21.92 (C-17), 31.35 (C-18), 22.00 (C-19), 22.14 (C-20), 25.48 (C-21), 174.72 (C-22), 51.70 (OMe), 41.19 (NMe); CD (MeOH) λ_{max} 260 (θ +350), 280 (−20), and 305 (−30) nm.

Crystal Data of Daphnezomine B (2) Hydrobromide. Daphnezomine B (**2**, 5 mg) was dissolved in acetone, and then 50% aqueous HBr (1 drop) was added to the ice-cold solution. The mixture was evaporated under reduced pressure, and the residue was crystallized from MeOH/acetone (1:9) to give the hydrobromide of daphnezomine B (**2**) as colorless plates (mp 233−234 °C). Crystal data: $C_{23}H_{38}NO_3Br$, $M_r = 456.46$, crystal dimensions 0.30 × 0.30 × 0.20 mm, orthorhombic, space group $P2_12_12_1$ (no. 19), $a = 13.870(2)$ Å, $b = 15.616(2)$ Å, $c = 10.059(1)$ Å, $V = 2178.6(4)$ Å^3, $Z = 4$, $D_{calc} = 1.392$ g/cm^3. All measurements were made on a Rigaku AFC7R diffractometer with graphite-monochromated Mo Kα radiation ($\lambda = 0.71069$ Å) and a 18 kW rotating anode generator. The data were collected at 23 ± 1 °C by using the $\omega - 2\theta$ scan technique to a maximum 2θ value of 55.0°. A total of 2816 reflections was collected. The intensities of three representative reflections were measured after every 150 reflections. No decay correction was applied. The linear absorption coefficient, μ, for Mo Kα radiation was 19.2 cm^{-1}. Azimuthal scans of several reflections indicated no need for an absorption correction. The data were corrected for the Lorentz and polarization effects. A correction for secondary extinction was applied (coefficient = 4.75660×10^{-7}).

The structure was solved by SIR92 and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 1557 observed reflections ($I > 1.50\sigma(I)$) and 255 variable parameters and converged with unweighted and weighted agreement factors of $R = 0.040$, $R_w = 0.051$. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corp.

Molecular Mechanics Calculations. Molecular mechanics and dynamics calculations were performed by using MM2* force field in the MacroModel (v6.0) on an IRIS 4D computer in vacuo. Each conformation generated by a high-temperature molecular dynamics simulation (100 ps at 1000 K) was minimized to reduce the gradient rms to less than 0.001 kcal/Å·mol. Inspection of the minimized structures provided the lowest energy conformation.

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Supporting Information Available: 1D and 2D NMR spectra for compounds **1** and **2** and X-ray crystallographic data of **2** hydrobromide. This material is available free of charge via the Internet at http://pubs.acs.org.

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