Daphmanidins E and F, Alkaloids from *Daphniphyllum teijsmannii*[⊥]

Hiroshi Morita,*[,†] Nozomi Ishioka,[‡] Hiroshi Takatsu,[†,‡] Toru Iizuka,[†] and Jun'ichi Kobayashi*[,‡]

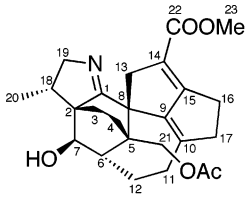
Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41 Shinagawa, Tokyo 142-8501, Japan, and Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Received September 29, 2005

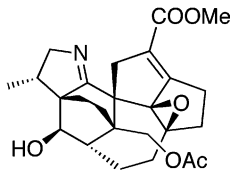
Two new *Daphniphyllum* alkaloids, daphmanidins E (**1**) and F (**2**), have been isolated from the leaves of *Daphniphyllum teijsmannii*, and the structures were elucidated on the basis of spectroscopic data. Daphmanidins E and F showed a moderate vasorelaxant effect on rat aorta.

Daphniphyllum alkaloids are a family of fused-heterocyclic natural products elaborated by trees of the genus *Daphniphyllum* (Daphniphyllaceae).[1] Heathcock and co-workers have reported biomimetic synthesis of daphnane and secodaphnane type skeletons of *Daphniphyllum* alkaloids.[2] In a search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids, we isolated previously new types of polycyclic *Daphniphyllum* alkaloids[3−8] such as a series of daphnezomines,[3−5] daphnicyclidins,[6,7] daphmanidins,[8] calyciphyllines,[9] and daphniglaucins[10] from various *Daphniphyllum* species. Recently, we isolated two novel alkaloids, daphmanidins C and D,[11] consisting of a novel 1-azabicyclo[5.2.2]undecane, a hexahydronaphthalen-1-one, and a cyclopentane ring, from *D. teijsmannii* Zoll. Further investigation on extracts of the leaves of *D. teijsmannii* resulted in the isolation of daphmanidins E (**1**) and F (**2**), which inhibited vasocontraction induced by norepinephrine (NE) on rat aorta. This paper describes the isolation and structural elucidation of **1** and **2** with moderate vasorelaxant activity.

The leaves of *D. teijsmannii* were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 10 with saturated Na_2CO_3(aq), were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to passage over an LH-20 column ($CHCl_3$/MeOH, 1:1), followed by a C_{18} column (30% MeOH → 80% MeOH) and then C_{18} HPLC (25% CH_3CN/0.1% TFA) to afford daphmanidins E (**1**, 0.0001%) and F (**2**, 0.00003%) with a known related alkaloid, daphmanidin A (**3**).[8]



1



2

Daphmanidin E (**1**) showed a pseudomolecular ion peak at *m*/*z* 426 $(M + H)^+$, and the molecular formula, $C_{25}H_{31}NO_5$, was established by HRFABMS [*m*/*z* 426.2273, $(M + H)^+$, Δ −0.8 mmu]. IR absorptions implied the presence of hydroxyl (3620 cm^{-1}) and carbonyl (1735 and 1685 cm^{-1}) functionalities. Analysis of ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum of **1** revealed the presence of seven sp^2 and three sp^3 quaternary carbons, three sp^3 methines, nine sp^3 methylenes, and three methyl groups.

Table 1. ^1H and ^{13}C NMR Data of Daphmanidins E (**1**) and F (**2**) in CD_3OD at 300 K[a]

	δ_H		δ_C	
position	**1**	**2**	**1**	**2**
1			205.1	201.0
2			58.2	57.9
3a	2.58 (1H, m)	2.46 (1H, m)	23.3	23.9
3b	1.59 (1H, m)	1.51 (1H, m)		
4a	1.84 (2H, m)	2.07 (1H, m)	27.3	27.2
4b		1.81 (1H, m)		
5			42.5	48.6
6	2.16 (1H, m)	1.90 (1H, m)	51.8	51.4
7	4.15 (1H, m)	3.85 (1H, m)	67.5	69.5
8			49.6	45.4
9			152.9	74.9
10			149.3	84.5
11a	2.24 (1H, m)	2.15 (2H, m)	24.3	27.4
11b	2.42 (1H, m)			
12a	1.94 (1H, m)	1.23 (1H, m)	25.4	25.1
12b	1.87 (1H, m)	1.91 (1H, m)		
13a	3.15 (1H, d, 16.8)	3.59 (1H, d, 17.2)	46.7	47.0
13b	3.55 (1H, d, 16.8)	2.22 (1H, d, 17.2)		
14			117.5	125.8
15			169.5	159.5
16a	2.95 (2H, m)	2.17 (1H, m)	43.0	34.4
16b		2.30 (1H, m)		
17a	2.77 (2H, m)	2.77 (1H, dd, 9.1, 19.0)	26.5	23.6
17b		2.16 (1H, m)		
18	2.50 (1H, dd, 7.3, 16.8)	2.47 (1H, m)	37.7	37.5
19a	3.79 (1H, m)	3.80 (1H, m)	61.2	62.1
19b	4.28 (1H, dd, 9.0, 16.8)	4.35 (1H, dd, 8.9, 13.8)		
20	1.27 (3H, d, 7.3)	1.21 (3H, d, 7.4)	14.2	13.6
21a	4.10 (2H, d, 16.1)	3.98 (1H, d, 12.0)	65.6	66.7
21b		4.19 (1H, d, 12.0)		
22			167.2	165.2
23	3.67 (3H, s)	3.78 (3H, s)	51.6	52.3
24			172.3	172.1
25	2.03 (3H, s)	2.00 (3H, s)	20.6	20.6

[a] δ in ppm.

Among them, one sp^3 methylene (δ_C 61.2; δ_H 3.79 and 4.28) and one sp^2 iminium carbon[12] (δ_C 205.1) were ascribed to those bearing a nitrogen, while two carbonyl carbons (δ_C 167.2 and 172.3), one sp^3 methine (δ_C 67.5), and one sp^3 methylene (δ_C 65.6) were ascribed to those bearing an oxygen atom.

Four partial structures (**a**−**d**) were deduced from extensive analyses of the 2D NMR data of **1** including the $^1H-^1H$ COSY, HOHAHA, HMQC, and HMBC spectra in CD_3OD (Figure 1). The ^1H and ^{13}C NMR data are presented in Table 1. HMBC correlations for H-18 and H_2-3 of C-2 (δ_C 58.2) gave rise to the connectivity between C-3 and C-18 through C-2. HMBC correlations for H_2-19 and H-3a to C-1 (δ_C 205.1) showed the presence of a dihydropyrrole ring (C-1, C-2, C-18, C-19, and N-1). Connectivities between C-7

[⊥] Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

* To whom correspondence should be addressed. (H.M.) Tel: (03)5498-5778. Fax: (03)5498-5778. E-mail: moritah@hoshi.ac.jp. (J.K.) Tel: (011)-706-4985. Fax: (011)706-4989. E-mail: jkobay@pharm.hokudai.ac.jp.

[†] Hoshi University.

[‡] Hokkaido University.

10.1021/np0503799 CCC: $33.50
Published on Web 01/05/2006

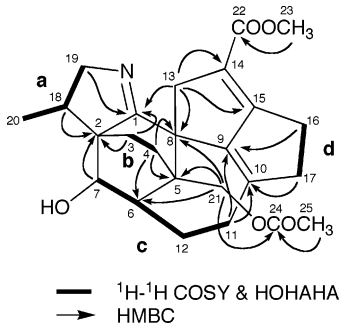


Figure 1. Selected two-dimensional NMR correlations for daphmanidin E (**1**).

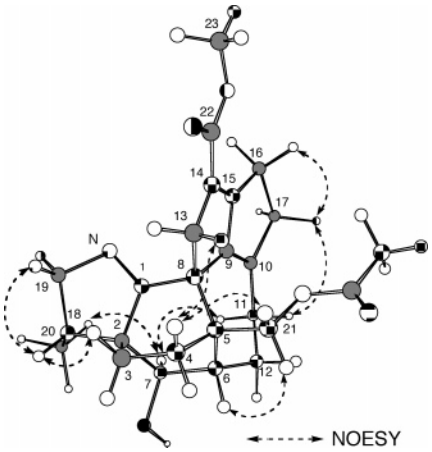
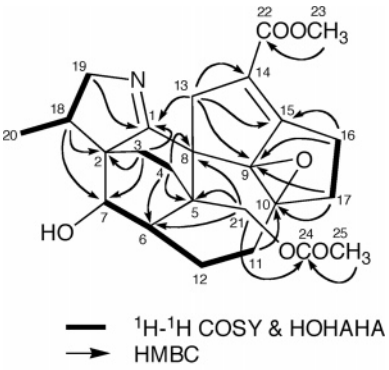


Figure 2. Selected NOESY correlations and relative stereochemistry for daphmanidin E (**1**).

and C-2 and between C-4 and C-6 through C-5 were elucidated by HMBC cross-peaks for H-7 to C-2 and for H_2-4 to C-5 (δ_C 42.5) and C-6 (δ_C 51.8). Connectivities among C-1, C-5, and C-13 through C-8 were provided by HMBC correlations for H_2-13 to C-1 and C-8 (δ_C 49.6) and H_2-4 to C-8. HMBC correlations for H_2-13 to C-14 (δ_C 117.5) and C-15 (δ_C 169.5) and the chemical shifts of C-14 (δ_C 117.5) and C-15 (δ_C 169.5) suggested this tetrasubstituted olefin was conjugated with a carbonyl group. Connectivities among C-8, C-11, C-13, C-16, and C-17 through a conjugated diene (C-9 and C-10, and C-14 and C-15) were implied by long-range correlations for H_2-11 and H_2-17 to C-10 (δ_C 149.3) and for H_2-11, H_2-13, and H_2-16 to a conjugated diene. So, the presence was suggested of a bicyclo[3.3.0]2-carboxy-1,5-octadiene unit. HMBC cross-peaks for H_2-21 and H_3-25 to C-24 (δ_C 172.3) indicated that an acetoxy group was attached to C-21, which was connected to C-5 by those for H_2-21 to C-5, C-6, and C-8. The presence of a methoxy carbonyl group at C-14 was deduced from HMBC correlations for H_3-23 to C-22 (δ_C 167.2). Thus, the gross structure of daphmanidin E was elucidated to be **1** with a hexacyclic ring system and a diene moiety. The relative stereochemistry of **1** was elucidated by NOESY correlations as depicted in the computer-generated 3D drawing (Figure 2).[13] A bicyclo[2.2.2]octane ring (C-1−C-8) took the boat-boat form, resulting from correlations for H-4a and H-13a, H-3a and H-18, and H-6 and H-21. The C-7 hydroxyl group had a β-configuration from the correlations for H-7 and H_3-20 and for H-7 and H-11a.

Daphmanidin F (**2**) showed a pseudomolecular ion peak at *m*/*z* 442 $(M + H)^+$ and 464 $(M + Na)^+$ in the FABMS spectrum, and the molecular formula, $C_{25}H_{31}NO_6$, was established by HRFABMS [*m*/*z* 442.2236, $(M + H)^+$, Δ +0.6 mmu, and *m*/*z* 464.2046, $(M + Na)^+$, Δ −0.3 mmu]. IR absorptions implied the presence of hydroxyl (3610 cm^{-1}) and ester carbonyl (1740 and 1685 cm^{-1}) functionalities. The ^1H and ^{13}C NMR (Table 1) spectra of **2** were analogous to those of **1** expect for the following observation: two



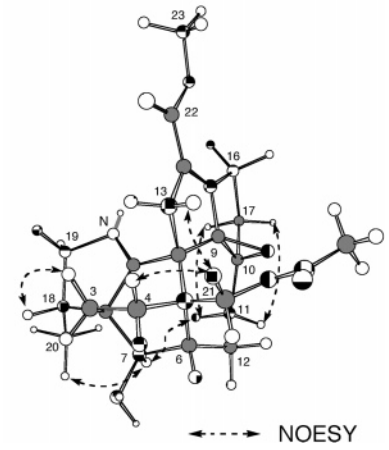


Figure 3. Selected 2D NMR correlations and relative stereochemistry for daphmanidin F (**2**).

sp^3 quaternary carbons (δ_C 74.9 and 84.5) bearing an oxygen atom for **2** were observed in place of the olefin carbons of **1**. Figure 3 showed 2D NMR correlations for daphmanidin F (**2**). The positions of these quaternary carbons were deduced to be C-9 and C-10 by HMBC correlations for H_2-13, H_2-16, and H_2-17 to C-9, and H_2-11 and H_2-17 to C-10.

The relative stereochemistry of **2** was deduced from NOESY correlations (Figure 3). The β-configuration of the epoxide at C-9 and C-10 affected the nonequivalency of the chemical shifts of methylene protons at C-16, C-17, and C-21. In addition, NOESY correlations between H-13a and H-21a, H-11a and H-17a, and H-11b and H-17b supported the presence of the β-epoxide.

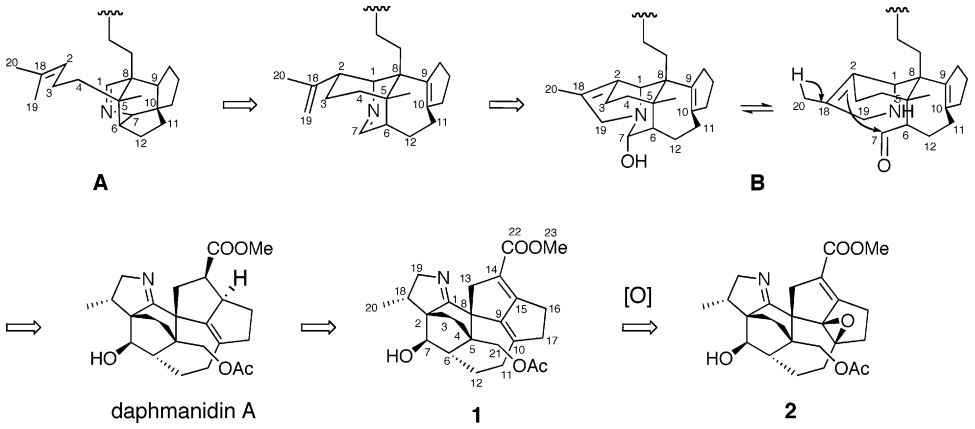
A plausible biogenetic pathway for daphmanidins E (**1**) and F (**2**) is proposed as shown in Scheme 1. Daphmanidin E (**1**) might be generated from a common imine intermediate, **A**, which has been proposed as a precursor of the secodaphniphylline-type skeleton by Heathcock et al.[2] Formation of the C-1−C-2 bond and N-1−C-19 bond followed by oxidation at C-7 will give the intermediate **B**. Then, subsequent cleavage of the N-1−C-7 bond followed by formation of the C-2−C-7 bond will afford daphmanidins A, E (**1**), and F (**2**).

When norepinephrine (NE) at 3×10^{-7} M was applied to thoracic aortic rings after achieving a maximal response, daphmanidins A and F (**2**) at 10^{-5} M showed slow vasorelaxant actions (37.5% and 45.8%), whereas the vasorelaxant effect (34.9%) of daphmanidin E (**1**) was comparable to those of daphmanidins A and F (**2**) at 10^{-4} M.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-4 polarimeter. UV spectra were recorded on a Shimadzu UV1600PC spectrophotometer and IR spectra on a Perkin-Elmer 1710 spectrophotometer. ^1H and 2D NMR spectra in methanol-d_4 were recorded on a 600 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. Chemical shifts

Scheme 1. Plausible Biogenesis of Daphmanidins E (**1**) and F (**2**)



were reported using residual CD_3OD (δ_H 3.31 and δ_C 49.0) as internal standard. Standard pulse sequences were employed for the 2D NMR experiments. FAB and high-resolution mass spectra were recorded on a VG Autospec instrument by using a glycerol matrix.

Plant Material. The leaves of *Daphniphyllum teijsmannii* (Daphniphyllaceae) were collected in Hiroshima in 2000. The botanical identification was made by Dr. S. Mukai, Miyajima Natural Botanical Garden, Hiroshima University. Voucher specimens (no. 201101) have been deposited in the herbarium of Hokkaido University.

Extraction and Isolation. The leaves of *D. teijsmannii* (2 kg) were crushed and extracted with MeOH (20 L × 2). The MeOH extract (273 g) 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 10 and extracted with $CHCl_3$ to give a crude alkaloidal fraction (2.7 g). This fraction was subjected to an LH-20 column ($CHCl_3$/MeOH, 1:1) and then C_{18} column chromatography (30% MeOH → 80% MeOH), in which a fraction eluted with 50% MeOH was purified by C_{18} HPLC (YMC-Pack, Polymer C_{18}, 5 μm, YMC Co., Inc., 10 × 250 mm; eluent, 25% CH_3CN/0.1% TFA; flow rate, 2 mL/min; UV detection at 205 nm) to afford daphmanidins E (**1**, 2.2 mg, 0.0001% yield) and F (**2**, 0.6 mg, 0.00003% yield) as TFA salts, together with daphmanidin A (**3**, 0.8 mg, 0.00004%).

Daphmanidin E (1): colorless solid; $[\alpha]^{20}_D$ +11 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 296 nm (ϵ 8200); IR (neat) ν_{max} 3620, 2930, 1735, 1685, and 1200 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); FABMS *m*/*z* 426 $(M + H)^+$; HRFABMS *m*/*z* 426.2273 (M + H; calcd for $C_{25}H_{32}NO_5$, 426.2281).

Daphmanidin F (2): colorless solid; $[\alpha]^{20}_D$ +25 (*c* 1.0, MeOH); UV (MeOH) λ_{max} 235 nm (ϵ 6200) and 291 nm (ϵ 4200); IR (neat) ν_{max} 3610, 2920, 1740, 1685, and 1240 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); FABMS *m*/*z* 442 $(M + H)^+$; HRFABMS *m*/*z* 442.2236 (M + H; calcd for $C_{25}H_{32}NO_6$, 442.2230) and 464 $(M + Na)^+$; HRFABMS *m*/*z* 464.2046 (M + Na; calcd for $C_{25}H_{31}NO_6Na$, 464.2049).

Vasodilator Assay.[14] A male Wistar rat weighting 340 g was sacrificed by bleeding from carotid arteries under anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS: 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparations 3 mm in length. The tissue was placed in a well-oxygenated (95% O_2, 5% CO_2) bath of 10 mL of KHS solution at 37 °C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.

After equilibration, each aortic ring was contracted by treatment with 3×10^{-7} M norepinephrine (NE). The presence of functional endothelial cells was confirmed by demonstrating relaxation to 10^{-5} M acetylcholine (Ach), and aortic rings in which 80% relaxation occurred were regard as tissues with endothelium. When the NE-induced contraction reached plateau, each sample was added.

These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References and Notes

(1) Kobayashi, J.; Morita, H. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, *The Daphniphyllum Alkaloids*; 2003; Vol. 60, p 165.
(2) Heathcock, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14323−14327, and references therein.
(3) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **1999**, *64*, 7208−7212.
(4) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **2000**, *65*, 3558−3562.
(5) (a) Morita, H.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **1999**, *55*, 12549−12556. (b) Morita, H.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **2000**, *56*, 2641−2646. (c) Morita, H.; Kobayashi, J. *Tetrahedron* **2002**, *58*, 6637−6641.
(6) Kobayashi, J.; Inaba, Y.; Shiro, M.; Yoshida, N.; Morita, H. *J. Am. Chem. Soc.* **2001**, *123*, 11402−11408.
(7) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **2002**, *67*, 2278−2282.
(8) Kobayashi, J.; Ueno, S.; Morita, H. *J. Org. Chem.* **2002**, *67*, 6546−6549.
(9) Morita, H.; Kobayashi, J. *Org. Lett.* **2003**, *5*, 2895−2898.
(10) (a) Takatsu, H.; Morita, H., Ya-Ching, S.; Kobayashi, J. *Tetrahedron* **2004**, *60*, 6279−6284. (b) Morita, H.; Takatsu, H.; Ya-Ching, S.; Kobayashi, J. *Tetrahedron Lett.* **2004**, *45*, 901−904. (c) Kobayashi, J.; Takatsu, H.; Ya-Ching S.; Morita, H. *Org. Lett.* **2003**, *5*, 1733−1736. (d) Morita, H.; Takatsu, H.; Kobayashi, J. *Tetrahedron* **2003**, *59*, 3575−3579.
(11) Morita, H.; Ishioka, N.; Takatsu, H.; Shinzato, T.; Hoshino, T.; Obara, Y.; Nakahata, N.; Kobayashi, J. *Org. Lett.* **2005**, *7*, 459−462.
(12) The iminium carbon (C-1) observed at δ 202.93 for the TFA salt of **1** was shifted to δ 188.45 for free base. Some examples: Uemura, D.; Chou, T.; Haino, T.; Nagatsu, A.; Fukuzawa, S.; Zheng, S.-z.; Chem, H.-s. *J. Am. Chem. Soc.* **1995**, *117*, 1155−1156. Chou, T.; Haino, T.; Kuramoto, M.; Uemura, D. *Tetrahedron Lett.* **1996**, *37*, 4027−4030.
(13) Conformational search and molecular mechanics calculations were conducted with the Macromodel program: 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.
(14) Nagai, M.; Noguchi, M.; Iizuka, T.; Otani, K.; Kamata, K. *Biol. Pharm. Bull.* **1996**, *19*, 228−232.

NP0503799