Pordamacrines A and B, Alkaloids from *Daphniphyllum macropodum*

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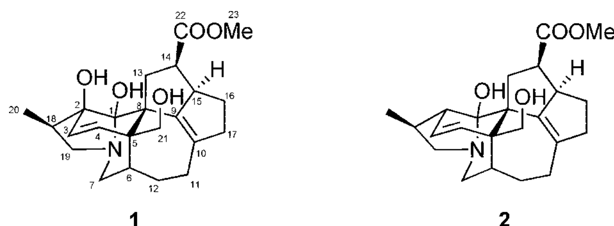
The new *Daphniphyllum* alkaloids, pordamacrines A (**1**) and B (**2**), have been isolated from the leaves of *Daphniphyllum macropodum*, and their structures were elucidated on the basis of interpretation of spectroscopic data and by the single-crystal X-ray diffraction analysis of **2**. Pordamacrines A (**1**) and B (**2**) exhibited moderate vasorelaxant effects on the rat aorta.

Daphniphyllum alkaloids are a family of fused-heterocyclic natural products elaborated by species of the genus *Daphniphyllum* (Daphniphyllaceae).[1] Heathcock and co-workers have reported biomimetic synthesis of the 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. Investigation of extracts of the leaves of *Daphniphyllum macropodum* Miq. resulted in the isolation of pordamacrines A (**1**) and B (**2**), which inhibited vasocontraction induced by norepinephrine (NE) on the rat aorta. This paper describes the isolation and structure elucidation of **1** and **2**, which exhibited a moderate vasorelaxant activity.

The leaves of *D. macropodum* 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 aqueous Na_2CO_3, were extracted with CH_2Cl_2. The CH_2Cl_2-soluble materials were subjected to purification over an amino silica gel column (hexane/EtOAc → $CHCl_3$/MeOH), followed by silica gel column chromatography (1:1 $CHCl_3$/MeOH), to afford pordamacrines A (**1**, 0.002%) and B (**2**, 0.00006%).



Pordamacrine A (**1**) exhibited a pseudomolecular ion peak at m/z 402 $[M + H]^+$, and the molecular formula $C_{23}H_{31}NO_5$ was established by HRESIMS (m/z 402.2270, $[M + H]^+$, Δ −1.0 mmu). IR absorptions implied the presence of hydroxyl (3448 cm^{-1}) and carbonyl (1741 cm^{-1}) functionalities. Analysis of ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum of **1** revealed the presence of three sp^2 and four sp^3 quaternary carbons, two sp^3 and four sp^3 methines, eight sp^3 methylenes, and two methyl groups. Among them, two sp^3 methylene (δ_C 48.3, δ_H 2.86 and 3.46 and δ_C 58.1, and δ_H 2.25 and 3.03) and one sp^3 carbon (δ_C 95.3) with a hydroxyl were ascribed to those bearing a nitrogen atom, while one carbonyl carbon (δ_C 178.2), one sp^3 quaternary carbon (δ_C 82.1), and one sp^3 methylene (δ_C 64.9) were assigned to those bearing an oxygen atom.

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

position	δ_H **1**	δ_H **2**	δ_C **1**	δ_C **2**
1			95.3	101.5
2		2.49(1H,m)	82.1	50.7
3	5.50(1H,d,10.3)	5.61(1H,dd,10.2,2.7)	124.3	125.6
4	5.99(1H,d,10.3)	5.81(1H,dd,10.2,1.8)	137.9	135.0
5			46.8	47.1
6	2.13(1H,m)	2.09(1H,m)	33.6	35.9
7a	2.86(1H,dd,14.4,8.9)	2.93(1H,dd,13.0,8.3)	48.3	51.3
7b	3.46(1H,dd,14.4,9.3)	3.28(1H,dd,13.0,5.5)		
8			51.4	49.9
9			143.8	143.7
10			136.9	136.8
11a	2.31(2H,m)	2.15(1H,m)	26.6	26.4
11b		2.45(1H,m)		
12a	1.47(1H,m)	1.51(1H,m)	24.6	25.6
12b	2.31(1H,m)	2.16(1H,m)		
13a	2.02(1H,m)	2.35(1H,m)	33.5	38.5
13b	2.96(1H,dd,13.7,8.3)	2.50(1H,m)		
14	3.23(1H,m)	3.01(1H,ddd,4.8,10.4,10.4)	43.4	43.4
15	3.52(1H,m)	3.47(1H,m)	56.2	56.9
16a	1.27(1H,m)	1.42(1H,m)	31.0	29.5
16b	1.83(1H,m)	1.82(1H,m)		
17a	2.18(1H,m)	2.32(1H,m)	43.0	43.5
17b	2.49(1H,m)	2.49(1H,m)		
18	2.01(1H,m)	2.40(1H,m)	40.3	33.4
19a	2.25(1H,dd,10.6,7.9)	2.40(1H,m)	58.1	63.1
19b	3.03(1H,t,7.2)	3.17(1H,m)		
20	0.96(3H,d,6.9)	1.10(3H,d,6.8)	11.0	15.9
21a	3.68(1H,d,10.7)	3.78(1H,d,11.2)	64.9	64.4
21b	3.90(1H,d,10.7)	3.92(1H,d,11.2)		
22			178.2	177.8
23	3.61(3H,s)	3.64(3H,s)	51.6	51.6

[a] δ in parts per million.

Four partial structures (**a**–**d**) were deduced from extensive analysis of the two-dimensional (2D) NMR data of **1**, including the ^1H–^1H COSY, HOHAHA, and HMQC spectra in CD_3OD (Figure 1). The ^1H and ^1C NMR data are presented in Table 1. HMBC correlations for H-18 and H-4 of C-2 (δ_C 82.1) gave rise to the connectivity between C-3 and C-18 through C-2. HMBC correlations from H-7a to C-1 (δ_C 95.3) and C-19 (δ_C 58.1) and from H-19b to C-1 and C-7 suggested that C-1, C-7, and C-19 are connected in each other through a nitrogen atom. Connectivities among C-4, C-6, and C-21 through C-5 were

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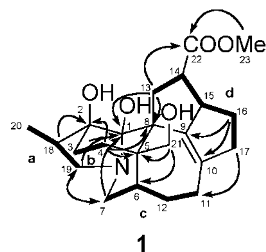

Figure 1. Selected 2D NMR correlations for pordamacrine A (**1**).

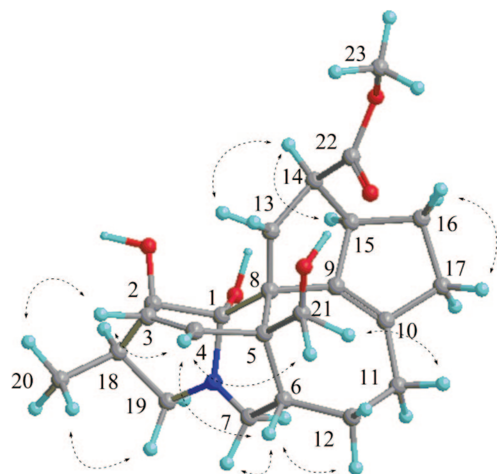

Figure 2. Selected NOESY correlations and relative stereochemistry for pordamacrine A (**1**).

elucidated by HMBC cross-peaks from H-4 and H_2-21 to C-5 (δ_C 46.8) and H_2-21 to C-6 (δ_C 33.6). Connectivities among C-1, C-5, and C-13 through C-8 were provided by HMBC correlations for H_2-13 of C-1 and C-8 (δ_C 51.4) and H-4 of C-8. HMBC correlations for H-16b of C-9 (δ_C 143.8) and C-10 (δ_C 136.9) and H-17a of C-11 (δ_C 26.6) and the chemical shifts of C-9 (δ_C 143.8) and C-10 (δ_C 136.9) suggested this tetrasubstituted olefin connected fragments **c** and **d**, which thus constitute a bicyclo[3,3,0]oct-1-ene unit (C-8–10 and C-13–17). The presence of a methoxy carbonyl group at C-14 was deduced from HMBC correlations for H_3-23 and H_2-13 of C-22 (δ_C 178.2). Thus, the gross structure of pordamacrine A was elucidated as **1** with a hexacyclic ring system, based on a yuzurimine-type skeleton. The relative configuration of **1** was elucidated by NOESY correlations as depicted in a computer-generated three-dimensional drawing (Figure 2). NOESY correlations of H-4/H-6 and H-3/H_3-20 indicated that H-6 and H_3-20 were in the β-orientation. The α-orientation of H-14 and H-15 was suggested by NOESY correlations of H-13b/H-14 and H-14/H-15.

Pordamacrine B (**2**) was crystallized from methanol and water as colorless plates (53 °C dec) and exhibited a pseudomolecular ion peak at m/z 386 $[M + H]^+$ via ESIMS, corresponding to the molecular formula $C_{23}H_{31}NO_4$ (m/z 386.2326, $[M + H]^+$, Δ −0.5 mmu). IR absorptions implied the presence of hydroxyl (3367 cm^{-1}) and ester carbonyl (1741 cm^{-1}) functionalities. The ^1H and ^1C NMR (Table 1) spectra of **2** were analogous to those of **1** expect for the following observation: one sp^3 carbon (δ_C 50.7) for **2** was observed in place of one sp^3 carbon (δ_C 82.1) bearing an oxygen atom in **1**. The structure and relative stereochemistry were elucidated by using single-crystal X-ray diffraction analysis. An ORTEP drawing of **2** is shown in Figure 3. Thus, pordamacrine B (**2**) was assigned as the 2-deoxy form of pordamacrine A (**1**).

Pordamacrines A (**1**) and B (**2**) exhibited a slow relaxation activity against norepinephrine (NE, 3×10^{-7} M)-induced contrac-

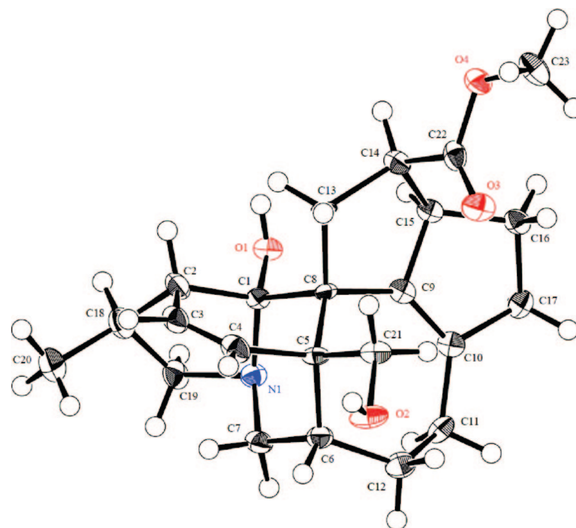

Figure 3. ORTEP drawing for pordamacrine B (**2**).

tions of thoracic rat aortic rings with endothelium (50.0 and 47.1% at 10^{-4} M, respectively).

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 are reported using residual CD_3OD (δ_H 3.31 and δ_C 49.0) as an internal standard. Standard pulse sequences were employed for the 2D NMR experiments. Mass spectra were recorded on a Micromass LCT spectrometer.

Plant Material. The leaves of *D. macropodum* (Daphniphyllaceae) were collected in Tokyo in 2005. The botanical identification was made by N. Yoshida. Voucher specimens (catalog no. 251101) have been deposited in the herbarium of Hoshi University.

Extraction and Isolation. The leaves of *D. macropodum* (2 kg) were crushed and extracted with MeOH (2 × 20 L). 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 aqueous Na_2CO_3 to pH 10 and extracted with CH_2Cl_2 to give a crude alkaloidal fraction (2.7 g). This fraction was passed over an amino silica gel column (1:0 hexane/EtOAc → 0:1 $CHCl_3$/MeOH) and then subjected to silica gel column chromatography ($CHCl_3$/MeOH, 0:1 → 1:0) to afford pordamacrines A (**1**, 48.7 mg, 0.002% yield) and B (**2**, 15.6 mg, 0.0008% yield).

Pordamacrine A (1): colorless solid; $[\alpha]^{27}_D$ −27 (c 0.2, MeOH); IR (neat) λ_{max} 3448, 2931, 1741 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS m/z 402 $[M + H]^+$; HRESIMS m/z 402.2270 (M + H; calcd for $C_{23}H_{32}NO_5$, 402.2281).

Pordamacrine B (2): colorless solid; $[\alpha]^{20}_D$ +37 (c 0.3, MeOH); IR (neat) λ_{max} 3367, 2971, 1741 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS m/z 386 $[M + H]^+$; HRESIMS m/z 386.2326 (M + H; calcd for $C_{23}H_{32}NO_4$, 386.2331).

X-Ray Analysis of Pordamacrine B (2). Pordamacrine B (**2**) was crystallized from MeOH and H_2O to give colorless needles (53 °C dec). Crystal data: $C_{24}H_{35}NO_5$, crystal dimensions of 0.20 mm × 0.20 mm × 0.10 mm, space group $P2_12_12_1$ (#19), a = 7.71062 (14) Å, b = 14.9044 (3) Å, c = 18.3639 (3) Å, V = 2110.42(7) Å^3, Z = 4, D_{calc} = 1.314 g/cm^3. All measurements were taken on a Rigaku RAXIS RAPID imaging plate area detector with graphite-monochromated Cu Kα radiation (λ = 1.54187 Å). The data were collected at −175 ± 1 °C to a maximum 2θ value of 136.4°. A total of 90 oscillation images were collected. A sweep of data was done using ω scans from 80.0 to 260.0° in 10.0° steps, at χ = 0.0° and ϕ = 0.0°. The exposure rate was 12.0 s/deg. A second sweep was performed using ω scans from 80.0 to 260.0° in 10.0° steps, at χ = 54.0° and ϕ = 0.0°. The exposure rate was 12.0 s/deg. Another sweep was performed using ω scans from

80.0 to 260° in 10.0° steps, at $\chi = 54.0°$ and $\phi = 180.0°$. The exposure rate was 12.0 s/deg. Another sweep was performed using ω scans from 80.0 to 260° in 10.0° steps, at $\chi = 54.0°$ and $\phi = 270.0°$. The exposure rate was 12.0 s/deg. The crystal–detector distance was 127.40 mm. Readout was performed in the 0.100 mm pixel mode. Of the 23952 reflections that were collected, 2199 were unique ($R_{int} = 0.028$); equivalent reflections were merged. The linear absorption coefficient, μ, for Cu Kα radiation is 7.358 cm^{-1}. An empirical absorption correction was applied which resulted in transmission factors ranging from 0.842 to 0.929. The data were corrected for Lorentz and polarization effects.

The structure was determined by direct methods and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement on F^2 was based on 2199 observed reflections and 278 variable parameters and converged (largest parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors of $R_1 = \sum F_o - F_c/\sum F_o = 0.0310$ [$I > 2.00\sigma(I)$] and $wR_2 = [\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2]^{1/2} = 0.0804$. The standard deviation of an observation of unit weight was 1.04. Unit weights were used. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.23 and −0.14 $e^-/Å^3$, respectively.

Neutral atom scattering factors were taken from Cromer and Waber.[11] Anomalous dispersion effects were included in F_c; the values for D_f' and D_f'' were those of Creagh and McAuley.[12] The values for the mass attenuation coefficients are those of Creagh and Hubbell.[13] All calculations were performed using the CrystalStructure crystallographic software package[14] except for refinement, which was performed using SHELXL-97. The Flack parameter refined by using 1620 Friedel pairs was 0.1(3) so that Friedel-pair reflections were merged before the last refinement. The refined fractional atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC).

Vasodilation Assay.[15] A male Wistar rat weighing 260 g was sacrificed by bleeding from carotid arteries under anesthesia. 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 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 being treated 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 regarded as tissues with endothelium. When the NE-induced contraction reached a 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.

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