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Daphmanidins C and D, Novel Pentacyclic Alkaloids from *Daphniphyllum teijsmanii*

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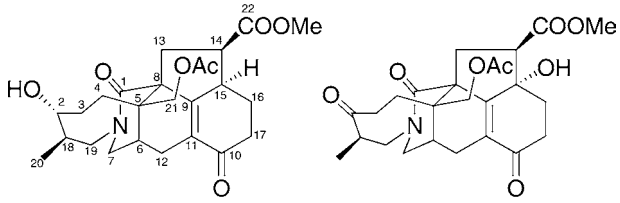
ABSTRACT



daphmanidin C (**1**) daphmanidin D (**2**)

Two novel alkaloids with an unprecedented fused-pentacyclic skeleton, daphmanidins C (1) and D (2), have been isolated from the leaves of *Daphniphyllum teijsmanii*, and the structures were elucidated on the basis of spectroscopic data. The relative stereochemistry of 1 and 2 was assigned by combination of NOESY correlations and a simulation analysis. Daphmanidin C (1) elevated activity of NGF biosynthesis.

Daphniphyllum alkaloids are a family of fused-heterocyclic natural products elaborated by trees of the genus *Daphniphyllum* (Daphniphyllaceae).[1,2] These ring systems have attracted great interest as challenging targets for total synthesis[3] as well as biosynthetic studies.[4]

In our search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids,[5] two novel fused-pentacyclic alkaloids, daphmanidins C (**1**) and D (**2**), consisting of 1-azabicyclo[5.2.2]undecane, hexahydronaphthalen-1-one, and cyclopentane rings were isolated from the leaves of *Daphniphyllum teijismanni*. In this paper,

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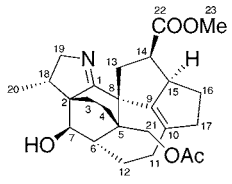
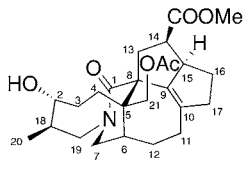
Table 1. ^{1}H and ^{13}C NMR Data of Daphmanidins C (**1**)[a] and D (**2**)[b] in CD_3OD at (a) 315 and (b) 300 K

	1			**2**		
	δ_H	δ_C	HMBC (^{1}H)	δ_H	δ_C	HMBC (^{1}H)
1		172.7	7a, 13b, 19a, 19b		172.7	7a, 13a
2	3.47 (1H, ddd, 3.2, 3.2, 10.2)	77.3	18, 19b		217.2	4a, 20
3a	1.81 (1H, brd, 17.5)	34.1		2.45 (1H, ddd, 2.0, 10.1, 14.2)	42.6	
3b	1.94 (1H, m)			2.74 (1H, dd, 1.8, 12.0)		
4a	1.77 (1H, brs)	31.8	21b	2.14 (1H, m)	32.8	21a, 21b
4b	2.03 (1H, m)			2.20 (1H, m)		
5		30.7	7a, 13b, 21b		30.7	
6	2.79 (1H, brd, 7.7)	34.1	21b	2.61 (1H, m)	33.1	21a
7a	2.98 (1H, brd, 13.1)	56.2	6, 19a	2.99 (1H, dd, 2.7, 14.6)	54.5	6
7b	3.81 (1H, dd, 10.5, 14.2)			3.35 (1H, dd, 10.1, 14.6)		
8		41.9	13b		42.0	
9		162.8	12b, 13b, 15		159.4	12a
10		200.1	12b, 16a		199.7	12a, 17a
11		130.2	6, 12b		131.2	12a
12a	2.09 (1H, m)	28.6		2.24 (1H, dd, 2.1, 18.9)	29.5	7a
12b	2.23 (1H, ddd, 2.5, 2.5, 18.3)			2.09 (1H, m)		
13a	2.04 (1H, m)	31.8		2.77 (1H, m)	29.9	
13b	2.63 (1H, dd, 8.0, 12.8)			1.94 (1H, dd, 12.0, 12.0)		
14	3.61 (1H, ddd, 8.7, 11.3, 11.3)	45.5		3.60 (1H, dd, 8.3, 10.1)	56.4	15
15	3.21 (1H, m)	42.0			77.7	
16a	1.58 (1H, ddd, 5.7, 12.5, 24.8)	27.8		1.89 (1H, ddd, 4.8, 13.1, 13.1)	33.8	15
16b	2.05 (1H, m)			2.10 (1H, m)		
17a	2.43 (1H, m)	38.4		2.37 (1H, ddd, 1.7, 14.6, 17.8)	33.9	
17b	2.46 (1H, m)			2.80 (1H, m)		
18	2.40 (1H, m)	38.4	2	3.50 (1H, m)	41.1	3a, 19a, 20
19a	2.52 (1H, dd, 11.6, 13.5)	55.5	18	2.51 (1H, dd, 10.2, 13.1)	53.1	20
19b	3.85 (1H, brd, 10.8)			4.31 (1H, dd, 7.6, 13.1)		
20	0.95 (3H, d, 6.7)	17.1		0.99 (3H, d, 6.6)	13.9	
21a	3.75 (1H, d, 11.0)	69.7	4a	3.71 (1H, d, 11.0)	70.2	
21b	3.98 (1H, d, 11.0)			3.98 (1H, d, 11.0)		
22		175.6	14, 23		174.7	14, 23
23	3.71 (3H, s)	52.2		3.75 (3H, s)	52.3	
24		172.8	25		172.6	25
25	2.07 (3H, s)	20.7		2.08 (3H, s)	20.6	

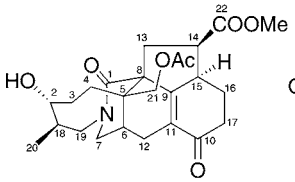
we describe the isolation and structural elucidation of **1** and **2**.



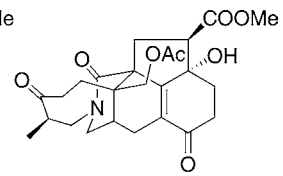
daphmanidin A



daphmanidin B



daphmanidin C (**1**)



daphmanidin D (**2**)

The leaves of *D. teijsmanii* were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (hexane/AcOEt, 1:0 → 0:1, and then $CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with $CHCl_3$ was purified by a silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1) followed by C_{18} HPLC (25% CH_3CN/0.1% TFA) to afford daphmanidins C (**1**, 0.6 mg, 0.00005% yield) and D (**2**, 0.6 mg, 0.00005%) as TFA salts together with known alkaloids, daphmanidins A and B,[5f] and yuzurimine E.[2a]

Daphmanidin C (**1**)[6] showed the pseudomolecular ion peak at *m*/*z* 482 $(M + Na)^+$ in the ESIMS, and the molecular formula, $C_{25}H_{33}NO_7$, was established by HRESIMS [*m*/*z* 482.2177, $(M + Na)^+$, Δ +2.2 mmu]. IR absorptions implied the presence of hydroxyl (3370 cm^{-1}) and carbonyl functionalities, including esters, conjugated ketone, and amide (1738, 1728, 1665, and 1645 cm^{-1}, respectively). The ^{13}C NMR spectra of **1** at 300 K in CD_3OD gave partially broad signals for a part of the molecule, which might be due to conformational exchange.[7] The broadening observed for the ^{13}C NMR spectrum was slightly overcome by measuring the NMR spectrum at 315 K. ^{13}C NMR data at 315 K (Table 1) revealed 25 carbon signals due to one tetrasubstituted olefin,

(6) Daphmanidin C (**1**): colorless solid; $[\alpha]_D$ −15° (*c* 0.1, CH_3OH); IR (neat) ν_{max} 3420, 2940, 1738, 1728, 1665, 1645, 1235, 1050, and 750 cm^{-1}; UV (MeOH) λ_{max} 250 nm (ϵ 12 000); ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 482 $(M + Na)^+$; HRESIMS *m*/*z* 482.2177 (M + Na; calcd for $C_{25}H_{33}NO_7Na$, 482.2155).

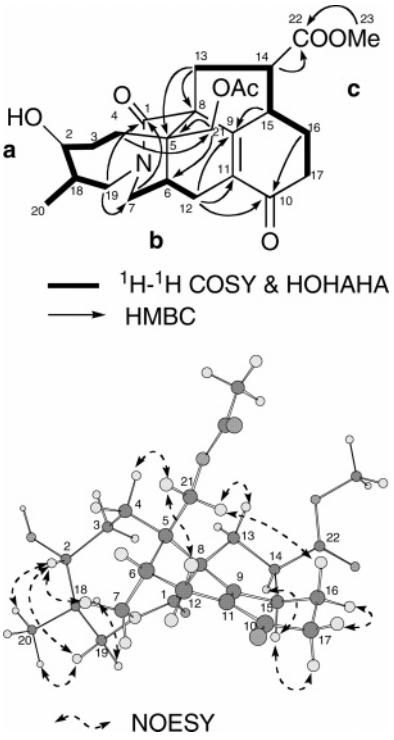


Figure 1. Selected two-dimensional NMR correlations for daphmanidin C (**1**).

one carbonyl, two ester carbonyls, one amide carbonyl, two sp^3 quaternary carbons, five sp^3 methines, nine sp^3 methylenes, one methyl, one methoxy, and one acetoxy group. Among them, two methylenes (δ_C 56.2, δ_H 2.98 and 3.81; and δ_C 55.5, δ_H 2.52 and 3.85) were ascribed to those bearing a nitrogen, while one methine (δ_C 77.3, δ_H 3.47) and one methylene (δ_C 69.7, δ_H 3.75 and 3.98) were those bearing an oxygen.

The ^{1}H−^{1}H COSY and HOHAHA spectra revealed connectivities of three partial structures **a** (C-2−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** (C-13 to C-17) as shown in Figure 1. HMBC correlations were observed for H-19a to C-1 (δ_C 172.7) and C-7 (δ_C 56.2), and H-7b to C-1, suggesting that C-1, C-7, and C-19 were connected to each other through a nitrogen atom. The connectivities from C-4 and C-6 to C-21 with an acetoxy group and C-13 through quaternary carbons at C-5 and C-8 were implied by long-range correlations for H-4b to C-21, H-21 to C-5 and C-6, and H-13b to C-5 and C-8. These correlations indicated that partial structures **a** and **b** constitute a 1-azabicyclo[5.2.2]undecane ring with a hydroxyl at C-2, a methyl at C-18, a ketone at C-1, and an acetoxy methyl at C-5. In partial structure **c** revealed by the COSY and HOHAHA spectra, a methoxycarbonyl group was connected to C-14 from HMBC correlations for H-14 and H_3-23 to C-22. The connectivity from C-13 to C-9 through

(7) Broad signals of C-3, C-5, C-6, C-8, C-12, C-19, and C-21 located at or near the 1-azabicyclo[5.2.2]undecane ring at 300 K were slightly changed to sharp ones at 315 K.

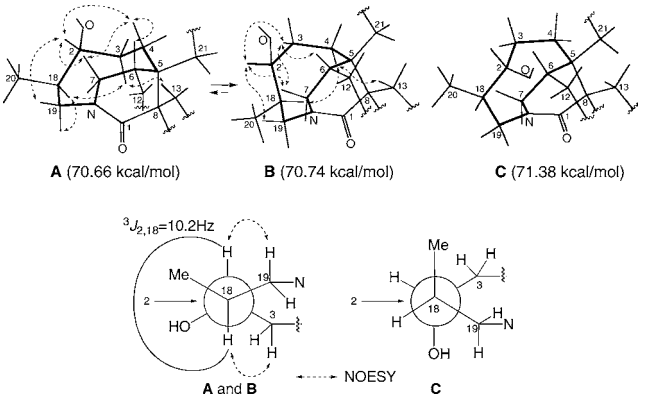


Figure 2. Three representative stable conformers (A−C) for daphmanidin C (**1**) analyzed by Monte Carlo simulation followed by minimization and clustering analysis (upper) and rotation models for the C-2−C-18 bond of **1** (lower).

a quaternary carbon at C-8 was implied by long-range correlations for H-13b to C-8 and C-9. The presence of α,β-unsaturated ketone between partial structures **b** and **c**, constructing a hexahydronaphthalen-1-one and a cyclopentane ring system, was shown by HMBC cross-peaks for H-12b and H-16b to C-10, H-12b to C-11, and H-12b and H-15 to C-9. Homoallyl couplings between H-15 and H_2-12 also supported this partial structure. Thus, the gross structure of daphmanidin C was assigned as **1**, having an unprecedented fused-pentacyclic ring system consisting of an 1-azabicyclo[5.2.2]undecane ring with a ketone at C-1, a hydroxyl at C-2, an acetoxy methyl group at C-5, and a methyl at C-18, a hexahydronaphthalen-1-one, and a cyclopentane ring with a methoxy carbonyl at C-14 as shown in Figure 1.

NOESY correlation of H-2/H-7b indicated that the hydroxyl at C-2 was of α-configuration. Furthermore, the relative configurations at C-5, C-6, C-8, C-14, C-15, and C-18 were deduced from correlations observed in the phase-sensitive NOESY spectrum as shown in computer-generated three-dimensional drawing (Figure 1). Conformational calculations using the MMFF force field[8] implemented in the Macromodel program[9] suggested that a part (C-2∼C-7) of the nine-membered rings with a chair conformation (**A**) and a twist-chair conformation (**B**) was stable, whereas that with a twist-boat conformation (**C**) had relatively higher energy (Figure 2). For the C-2−C-18 bond, the ^3J (H-2, H-18) (10.2 Hz) and the NOESY correlations implied that H-2 had anti relation to H-18 and compound **1** underwent a conformational change between conformers A and B.[10]

Daphmanidin D (**2**)[11] showed the pseudomolecular ion peak at *m*/*z* 496 $(M + Na)^+$ in the ESIMS spectrum, and the molecular formula, $C_{25}H_{31}NO_8$, was established by HRESIMS [*m*/*z* 496.1949, $(M + Na)^+$, Δ +0.2 mmu]. IR

(8) Halgren, T. *J. Am. Chem. Soc.* **1990**, *112*, 4710−4723.
(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.

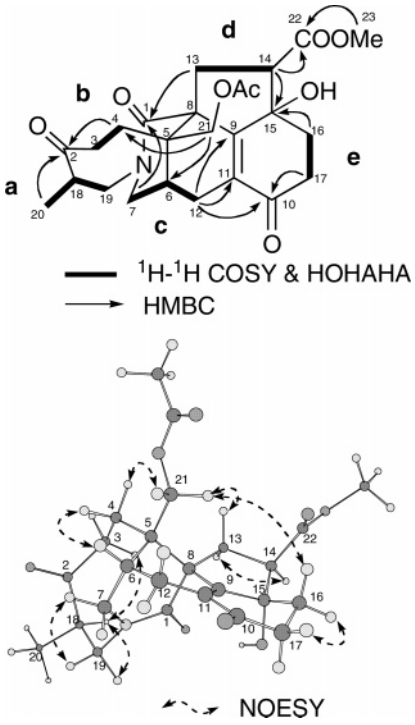
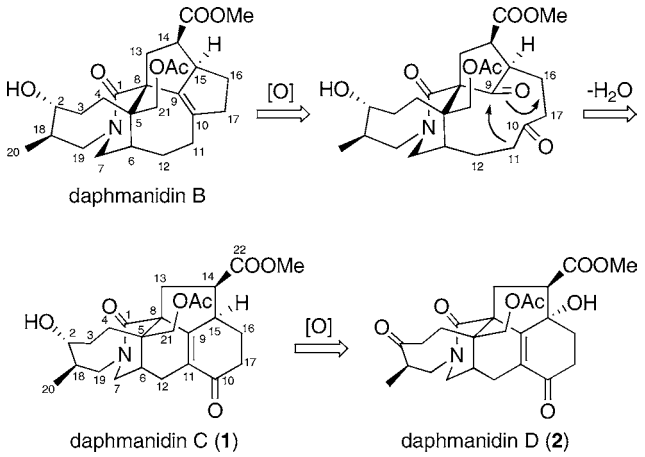


Figure 3. Selected two-dimensional NMR correlations for daphmanidin D (**2**).

absorptions implied the presence of hydroxyl (3415 cm^{-1}), ester carbonyl (1740 and 1725 cm^{-1}), ketone (1705 cm^{-1}), unsaturated ketone (1680 cm^{-1}), and amide (1645 cm^{-1}) functionalities. The ^{13}C NMR (Table 1) spectrum of **2** at 300 K showed sharp signals due to one tetrasubstituted olefin, two carbonyls, two ester carbonyls, one amide carbonyl, three sp^3 quaternary carbons, three sp^3 methines, nine sp^3 methylenes, one methyl, one methoxy, and one acetoxy group. Among them, two methylenes (δ_C 54.5, δ_H 2.99 and 3.35; and δ_C 53.1, δ_H 2.51 and 4.31) were ascribed to those bearing a nitrogen, while one sp^3 quaternary carbon (δ_C 77.7) and one methylene (δ_C 70.2, δ_H 3.71 and 3.98) were those bearing an oxygen. The structure of **2** was elucidated by two-dimensional NMR (^{1}H−^{1}H COSY, HOHAHA, HMQC, and HMBC) data and comparison with the spectroscopic data of **1**. The ^{1}H−^{1}H COSY and HOHAHA spectra revealed connectivities of five units **a** (C-18 to C-19 and C-20), **b** (C-3−C-4), **c** (C-6 to C-7 and C-12), **d** (C-13−C-14), and **e** (C-16−C-17). (Figure 3). These five units were connected to one another on the basis of HMBC correlations as shown in Figure 3. The presence of an amide carbonyl at C-1 was revealed by HMBC correlations of H-7b and H-13b to C-1.

Scheme 1. Plausible Biogenetic Pathway for Daphmanidins C (**1**) and D (**2**)



Thus, the structure of daphmanidin D was elucidated to be **2**, consisting of a 1-azabicyclo[5.2.2]undecane ring with ketones at C-1 and C-2, an acetoxymethyl at C-5, and a methyl at C-18, a hexahydronaphthalen-1-one, and a cyclopentane ring with a methoxy carbonyl at C-14 and a hydroxyl at C-15, whose skeleton was the same as daphmanidin C (**1**).

The relative stereochemistry of **2** was deduced from NOESY correlations (Figure 3). The conformation of the 1-azabicyclo[5.2.2]undecane moiety of **2** taking a twist-chair form as shown in Figure 3 was energetically more stable than that of **1** by a hydrogen bond between the amide carbonyl oxygen at C-1 and the hydroxyl at C-15 with α-configuration. These data were also consistent with the results of a conformational search using the MMFF force field[8] implemented in the Macromodel program.[9]

A plausible biogenetic pathway for daphmanidins C (**1**) and D (**2**) is proposed as shown in Scheme 1. Daphmanidins C (**1**) and D (**2**) might be derived through oxidative C−C bond fission followed by aldol-type condensation from daphmanidin B.[5f]

Daphmanidin C (**1**) elevated activity of NGF biosynthesis.[12]

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Supporting Information Available: One- and two-dimensional NMR spectra for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Determination of the absolute configuration at C-2 of **1** by using modified Mosher's method failed (all of the $\Delta\delta_S-\Delta\delta_R$ values were positive). Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(11) Daphmanidin D (**2**): colorless solid; $[\alpha]_D$ −18° (*c* 0.3, CH_3OH); IR (neat) ν_{max} 3415, 2925, 1740, 1725, 1705, 1680, 1645, 1515, 1235, and 1050 cm^{-1}; UV (MeOH) λ_{max} 246 nm (ϵ 13 000); ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 496 $(M + Na)^+$; HRESIMS *m*/*z* 496.1949 (M + Na; calcd for $C_{25}H_{31}NO_8Na$, 496.1947).

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