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Daphniglaucins A and B, Novel Polycyclic Quaternary Alkaloids from *Daphniphyllum glaucescens*

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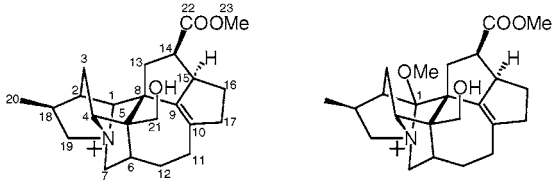
ABSTRACT



Two cytotoxic quaternary *Daphniphyllum* alkaloids with an unprecedented fused-polycyclic skeleton containing a 1-azoniatetracyclo[5.2.2.0.^{1,6}0.^{4,9}]-undecane ring system, daphniglaucins A (1) and B (2), have been isolated from the leaves of *Daphniphyllum glaucescens*. Their structures and relative stereochemistry were elucidated on the basis of spectroscopic data.

Daphniphyllum alkaloids are a structurally diverse group of natural products that are elaborated by the oriental tree "Yuzuriha" (*Daphniphyllum macropodum*; Daphniphyllaceae), which is a type of dioecious evergreen trees and shrubs native to Japan.[1,2] These unusual ring systems have attracted great interest as challenging targets for total synthesis and for biosynthetic studies.[3] Heathcock and coworkers have proposed a biogenetic pathway for the *Daphniphyllum* alkaloids and demonstrated a biomimetic total synthesis of several *Daphniphyllum* alkaloids.[3,4]

Recently, some novel types of *Daphniphyllum* alkaloids[5−10] such as daphnezomines A and B[5] with a unique aza-adamantane core, daphnezomines F and G[6] with an 1-azabicyclo[5.2.2]undecane ring system, daphnicyclidins A−H,[8] J, and K[9] with a unique hexa- or pentacyclic ring system, and daphmanidin A[10] with an unprecedented fused-hexacyclic skeleton were isolated from the leaves and stems

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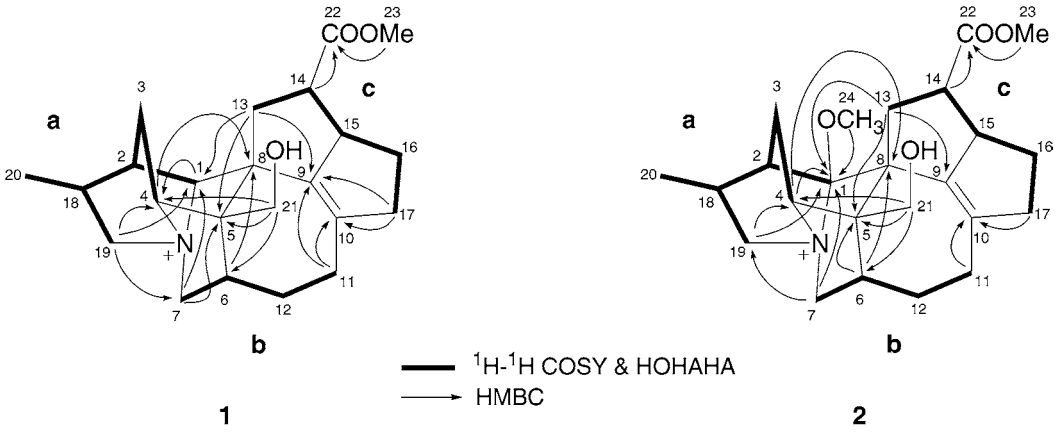

Figure 1. Selected two-dimensional NMR correlations for daphniglaucins A (**1**) and B (**2**).

of *Daphniphyllum humile* and/or *Daphniphyllum teijismanni*. In a continuing search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids, daphniglaucins A (**1**) and B (**2**), two quaternary alkaloids with an unprecedented fused-polycyclic skeleton with a 1-azoniatetracyclo-[5.2.2.0.^{1,6}0.^{4,9}]undecane ring system, were isolated from the leaves of *D. glaucescens*. This paper describes the isolation and structural elucidation of **1** and **2**.

The leaves of *D. glaucescens* were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 9 with saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1), from which a fraction was eluted with $CHCl_3$/MeOH (7:3) and purified by C_{18} HPLC (30% CH_3CN/0.1% TFA) to afford daphniglaucins A[11] (**1**, 0.009% yield) and B[12] (**2**, 0.002%) together with a known alkaloid, daphnilactone B.[13]

Daphniglaucin A (**1**) showed a molecular ion peak at *m*/*z* 370 $(M)^+$ in the FABMS, and the molecular formula, $C_{23}H_{32}NO_3$, was established by HRFABMS [*m*/*z* 370.2398, $(M)^+$, Δ +1.6 mmu]. IR absorptions implied the presence of hydroxyl and ester carbonyl (3385 and 1730 cm^{-1}, respectively) functionalities. ^1H and ^{13}C NMR data (see Supporting Information) revealed 23 carbon signals due to one tetrasubstituted olefin, one carbonyl, two sp^3 quaternary carbons, seven sp^3 methines, eight sp^3 methylenes, one oxymethylene, and one methoxy group. Among them, two methylenes (δ_C 51.5; δ_H 3.64, δ_C 58.4; δ_H 3.10 and 3.68) and two methines (δ_C 89.1; δ_H 3.86, δ_C 82.8; δ_H 3.99) were ascribed to those bearing a nitrogen, while the methylene (δ_C 56.2; δ_H 3.80 and 3.88) was ascribed to that bearing an oxygen.

The $^1H-^1H$ COSY and HOHAHA spectra revealed connectivities of three partial structures **a** (C-1 to 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-11 to C-12), and **c** (C-13 to C-17) as shown in Figure 1. HMBC correlations were observed for H-19b to C-7 (δ_C 51.5) and H_2-7 to C-1 (δ_C 89.1), the last of which was also correlated to H_2-19, suggesting that C-1, C-7, and C-19 were connected to each other through a nitrogen atom. The connectivity of C-4 to a nitrogen atom was implied by the HMBC correlation for H-1 to C-4 (δ_C 82.8). The chemical shifts of C-1, C-4, C-7, and C-19 indicated the presence of a neighboring quaternary nitrogen.[14] The ^{15}N NMR chemical shift (δ_N 99.2) of N-1, which was assigned by $^1H-^{15}N$ HMBC correlations from H-2, H-3b, H-7, and H-18, also supported the presence of the quaternary nitrogen.[15] HMBC cross-peaks for H-13 to C-1 and C-5 (δ_C 59.2) and for H-4 to C-8 (δ_C 48.4) indicated connectivities of C-1 to C-13 through C-8 and of C-4 to C-8 through C-5. The connectivity of C-21 to C-4 and C-6 through C-5 was implied by HMBC correlations for H_2-21 to C-4, C-5, and C-6 (δ_C 40.9). In addition, HMBC correlations for H-13a, H_2-11, and H-17a to C-9 (δ_C 144.0) and for H_2-11 and H-17a to C-10 (δ_C 136.1) indicated connectivities of C-8 to C-11 through C-9 and C-10 and of C-10 to C-17. A methoxy group was attached to C-22 by HMBC correlations for H_3-23 and H-14 to C-22 (δ_C 176.1). Thus, the gross structure of daphniglaucin A was assigned as **1** having a unique fused polycyclic ring system containing a 1-azoniatetracyclo[5.2.2.0.^{1,6}0.^{4,9}]undecane ring (N-1, C-1−C-8, C-18, and C-19) as shown in Figure 1.

The relative stereostructure of **1** was deduced from correlations observed in the phase-sensitive NOESY spectrum as shown in the computer-generated three-dimensional drawing (Figure 2). The NOESY correlation of H-3b/H-13a

(11) Daphniglaucin A (**1**): colorless solid; $[\alpha]_D$ −51° (*c* 1.0, CH_3OH); IR (neat) ν_{max} 3385, 2930, 1730, 1688, 1200, and 1128 cm^{-1}; ^1H and ^{13}C NMR data (see Supporting Information); FABMS *m*/*z* 370 $(M)^+$; HRFABMS *m*/*z* 370.2398 (M; calcd for $C_{23}H_{32}NO_3$, 370.2382).

(12) Daphniglaucin B (**2**): colorless solid; $[\alpha]_D$ −30° (*c* 0.6, CH_3OH); IR (KBr) ν_{max} 3385, 2935, 1736, 1685, 1200, and 1130 cm^{-1}; ^1H and ^{13}C NMR data (see Supporting Information); FABMS *m*/*z* 400 $(M)^+$; HRFABMS *m*/*z* 400.2502 (M; calcd for $C_{24}H_{34}NO_4$, 400.2488).

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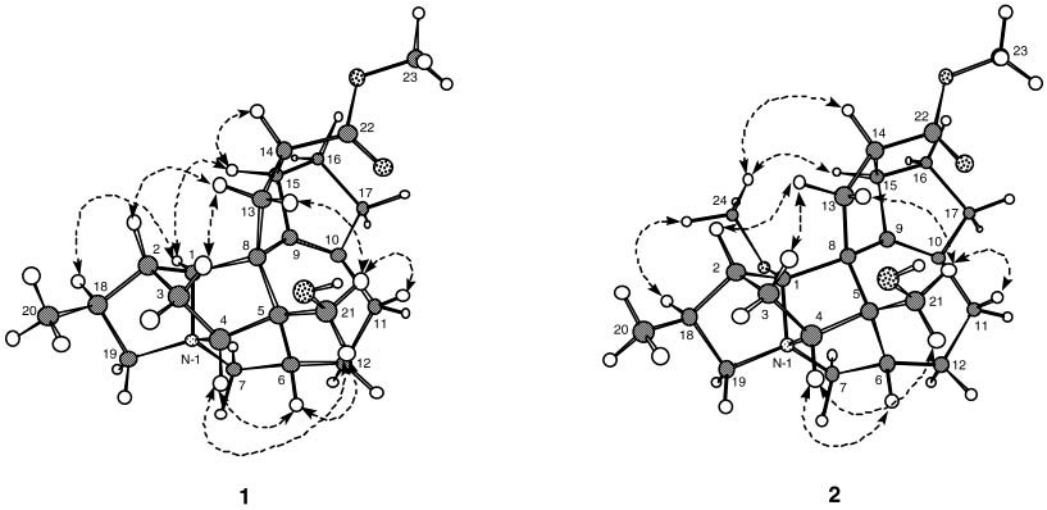


Figure 2. Selected NOESY correlations (dotted arrows) and relative stereochemistry for daphniglaucins A (**1**) and B (**2**).

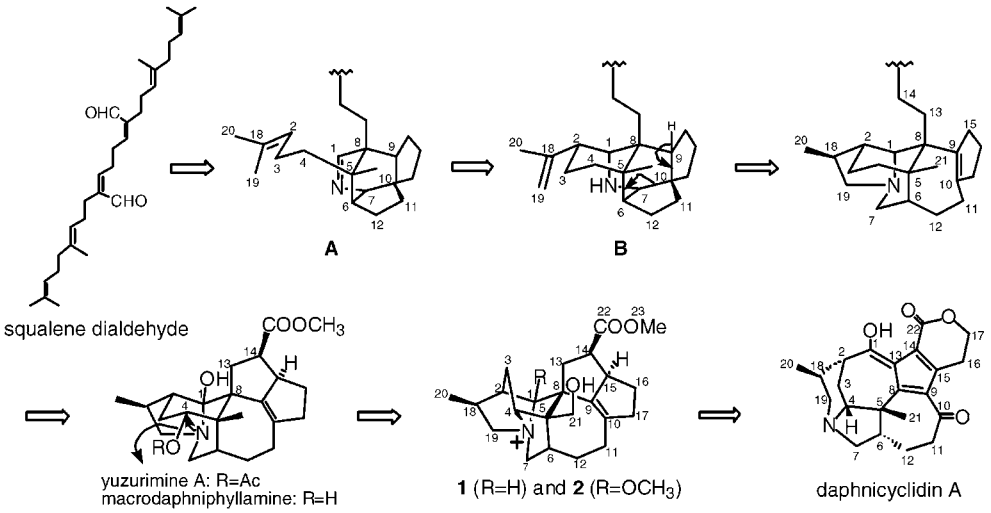
indicated that the cyclohexane ring (C-1−C-5 and C-8) took a boat form, which was supported by a W-type long-range coupling between H-1 and H-4, both equatorial, through a nitrogen.

Daphniglaucin B (**2**) was shown to have the molecular formula of $C_{24}H_{34}NO_4$ by HRFABMS [*m*/*z* 400.2502, $(M)^+$, Δ +1.4 mmu], which was larger than that of **1** by a CH_2O unit. The ^1H and ^{13}C NMR data (see Supporting Information) of **2** were analogous to those of **1** except for the following observation: a methoxy signal (δ_H 3.70) lacking in **1** appeared for **2**, while a methine signal [δ_H 3.86 (H-1)] observed for **1** was absent for **2**. One quaternary carbon (δ_C 119.4) was assigned as an amino acetal carbon.[16] The HMBC spectrum showed correlations for H_3-24 to C-1 (δ_C 119.4) through an oxygen, suggesting that a methoxy group was connected to C-1. HMBC correlations as shown in Figure 1 gave rise to connectivities of partial structures **a**−**c**. Thus, daphniglaucin B (**2**) was assigned as the methoxy form at C-1 of daphniglaucin A (**1**).

The relative stereochemistry of **2** was elucidated from NOESY correlations as shown in Figure 2. The NOESY correlation of H-11b/H-21b (Figure 2) indicated that the seven-membered ring (C-5−C-6 and C-8−C-12) took a twist boat conformation similar to that of the crystal structure of daphnicyclidin A.[8]

A plausible biogenetic pathway for daphniglaucins A (**1**) and B (**2**) is proposed as shown in Scheme 1. Daphniglaucins A (**1**) and B (**2**) might be generated from yuzurimine-type alkaloids such as yuzurimine A[17] and macrodaphniphyllamine[18] through a common imine intermediate **A**, which has been proposed as a precursor of the secodaphniphylline-type skeleton (**B**) by Heathcock *et al.*[3] Loss of the leaving group

Scheme 1. Plausible Biogenetic Pathway for Daphniglaucins A (**1**) and B (**2**)



at C-4 by attack of the nitrogen to form the N-1−C-4 bond will give daphniglaucins A (**1**) and B (**2**). Furthermore, daphniglaucins A (**1**) and B (**2**) may be biogenetically related to daphnicyclidins.[8]

Daphniglaucins A (**1**) and B (**2**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50} 2.7 and 3.9 μg/mL, respectively) and human epidermoid carcinoma KB cells (IC_{50} 2.0 and 10.0 μg/mL, respectively) in vitro.[19]

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Supporting Information Available: One- and two-dimensional NMR spectra and ^1H and ^{13}C NMR data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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