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Daphniglaucin C, a novel tetracyclic alkaloid from *Daphniphyllum glaucescens*

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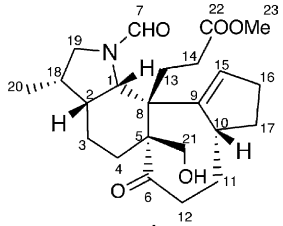
Abstract—A novel *Daphniphyllum* alkaloid with an unprecedented tetracyclic ring system consisting of an octahydroindole and a hexahydroazulene rings, daphniglaucin C (**1**), has been isolated from the leaves of *Daphniphyllum glaucescens* and the structure and relative stereochemistry were elucidated on the basis of spectroscopic data. Daphniglaucin C (**1**) inhibited the polymerization of tubulin.

Plants of *Daphniphyllum* species produce structurally diverse alkaloids with unusual polycyclic skeletons.[1,2] These unique ring systems have attracted great interest as challenging targets for total synthesis and biosynthetic studies.[3] Heathcock and co-workers have proposed a biogenetic pathway for *Daphniphyllum* alkaloids and demonstrated a biomimetic total synthesis of several *Daphniphyllum* alkaloids.[3,4]

Recently, we have isolated some novel types of *Daphniphyllum* alkaloids[5–12] such as daphnezomines A and B[5] with a unique aza-adamantane core and daphnezomines F and G[6] with a 1-azabicyclo[5.2.2]undecane ring system as well as daphnicyclidins A–H,[8] J, and K[9] with unique hexa- or pentacyclic ring system, and daphmanidin A[10] with an unprecedented fused-hexacyclic skeleton from the leaves and stems of *D. teijismanni* and/or *D. humile*, daphniglaucin A[11] with a fused-heptacyclic skeleton and a quaternary nitrogen from the leaves of *D. glaucescens*, and calyciphyllines A and B[12] with a novel hexacyclic skeleton from the leaves of *D. calycinum*. In our continuing search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids, daphniglaucin C (**1**), a novel tetracyclic alkaloid consisting of an octahydroindole and a hexahydroazulene rings, was isolated from the leaves of *D. glaucescens*. This paper describes the isolation and structural elucidation of **1**.

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 satd Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, 9:1 → 1:1 and then $CHCl_3$/MeOH, 1:0 → 0:1), from which a fraction eluted with $CHCl_3$/MeOH (7:3) was purified by C_{18} HPLC (30% CH_3CN/0.1% TFA) to afford daphniglaucin C[13] (**1**, 0.009% yield) together with a known alkaloid, macrodaphniphyllidine.[14]



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Daphniglaucin C (**1**) showed the pseudomolecular ion peak at m/z 404 $(M+H)^+$ in the FABMS, and the molecular formula, $C_{23}H_{33}NO_5$, was established by HRFABMS [m/z 404.2438, $(M+H)^+$, Δ +0.1 mmu]. IR

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absorptions implied the presence of hydroxyl (3370 cm^{-1}) and three carbonyl functionalities including ester, ketone, and amide (1730, 1695, and 1635 cm^{-1}, respectively). ^{13}C NMR data (Table 1) revealed 23 carbon signals due to one trisubstituted olefin, one carbonyl, one ester carbonyl, one amide carbonyl, two sp^3 quaternary carbons, four sp^3 methines, ten sp^3 methylenes, one methyl, and one methoxy group. Among them, one methylenes (δ_C 51.7; δ_H 2.96 and 3.61) and one methine (δ_C 66.3; δ_H 4.15) were ascribed to those bearing a nitrogen, while one methylene (δ_C 65.4; δ_H 3.81 and 4.49) was 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-10 to C-12, C-10 to C-17, and C-15 to C-17), and **c** (C-13 to C-14) as shown in Figure 1. HMBC correlations were observed for H-19b to C-1 (δ_C 66.3) and H-7 to C-19 (δ_C 51.7), suggesting that C-1, C-7, and C-19 were connected to each other through a nitrogen atom. Long-range couplings for H-7 to H_2-19 and H-1 indicated the presence of an *N*-formyl group (δ_H 8.08; δ_C 165.3). The connectivity of C-1 and C-13 through C-8 was implied by HMBC correlations for H-1 and H-13 to C-8 (δ_C 46.8). HMBC correlations for H-4 to C-5 (δ_C 57.6) and H_2-21 to C-5 and C-6 (δ_C 216.0) indicated that C-21 was connected to C-4 and C-6 through C-5. HMBC cross-peaks for H_2-13 to C-5 and C-9 (δ_C 150.6) indicated connectivities of C-8 to C-5 and C-9, constructing an octahydroindole ring system. The connectivity of C-6 to C-12 was implied by the HMBC correlation for H-12b to C-6. In addition, the HMBC correlation for H-17 to C-9 indicated the connectivity of C-9 to C-10, constructing a hexahydroazulene ring system. A methoxy group was attached to C-22 by HMBC correlations for H_3-23 and H_2-14 to C-22 (δ_C 175.6). Thus, the gross structure of daphniglaucin C was assigned as **1** having an unprecedented fused-tetracyclic ring system consisting of an octahydroindole ring with an *N*-formyl group and a methyl group (C-20) at C-18 and a hexahydroazulene ring with a ketone group at C-6 and a methoxycarbonyl ethyl group (C-13, C-14, C-22, and C-23) at C-8 as shown in Figure 1.

Table 1. ^1H [δ_H (*J*, Hz)] and ^{13}C [δ_C] NMR data of daphniglaucin C (**1**) in CD_3OD at 300 K

	δ_H	δ_C	HMBC (^1H)
1	4.15 (1H, d, 4.1)	66.3	13, 19b
2	2.24 (1H, m)	40.4	18, 19b, 20
3a	1.65 (1H, m)	16.9	—
3b	1.76 (1H, m)	—	—
4a	1.60 (1H, m)	26.7	21
4b	2.22 (1H, m)	—	—
5	—	57.6	4b, 13, 21
6	—	216.0	12b, 21
7	8.08 (1H, s)	165.3	—
8	—	46.8	1, 13
9	—	150.6	13, 15, 16a, 17a
10	3.16 (1H, br t, 11.5)	47.1	12a, 15, 17b
11a	1.55 (1H, m)	32.3	17b
11b	1.88 (1H, m)	—	—
12a	2.32 (1H, m)	42.9	—
12b	3.20 (1H, dt, 5.9, 13.2)	—	—
13	2.01 (2H, t, 7.8)	31.3	14
14	2.25 (2H, t, 7.8)	30.6	13
15	5.74 (1H, s)	132.0	16a, 17a
16a	2.44 (1H, m)	30.1	15, 17b
16b	2.30 (1H, m)	—	—
17a	1.74 (1H, m)	34.5	15, 16b
17b	2.16 (1H, m)	—	—
18	2.29 (1H, m)	34.9	19a, 20
19a	3.61 (1H, t, 11.5)	51.7	7, 20
19b	2.96 (1H, t, 11.5)	—	—
20	1.09 (3H, d, 6.7)	12.8	19a
21	4.49 (1H, d, 10.6)	65.4	—
	3.81 (1H, d, 10.6)		
22	—	175.6	13, 14, 23
23	3.65 (3H, s)	52.3	—

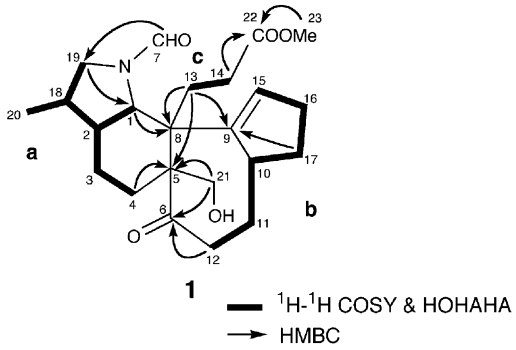


Figure 1. Selected 2D NMR correlations for daphniglaucin C (**1**).

The relative stereochemistry of **1** was deduced from correlations observed in the phase sensitive NOESY spectrum as shown in computer-generated 3D drawing (Fig. 2). NOESY correlations of H-2/H-4a and H-21b/H-4a and a W-type long-range coupling between H-1 and H-3b, both equatorial indicated that the cyclohexane ring (C-1 to C-5 and C-8) took a chair form.

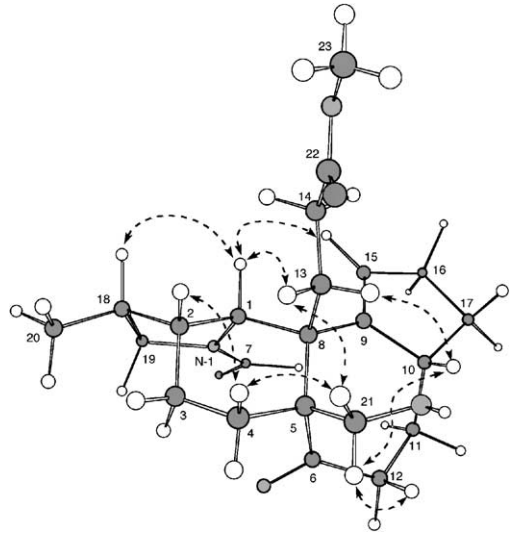
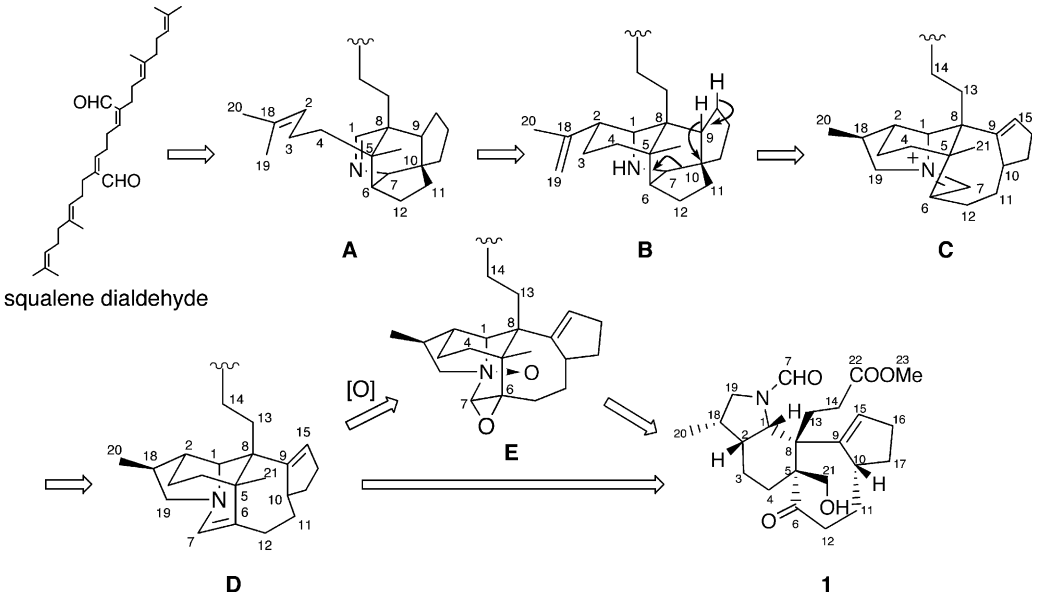


Figure 2. Selected NOESY correlations (dotted arrows) and relative stereochemistry for daphniglaucin C (**1**).



Scheme 1. Plausible biogenetic path for daphniglaucin C (**1**).

A plausible biogenetic pathway for daphniglaucin C (**1**) is proposed as shown in Scheme 1. The biogenetic origin of daphniglaucin C (**1**) seems to be an imine intermediate **C**, which might be produced through fragmentation reaction of a secodaphnane skeleton (**B**) derived from an imine intermediate **A** proposed by Heathcock et al.[3] Oxidation of N-1, C-6, and C-7 of the intermediate **D** and cleavage of C-6 to C-7 bond of an intermediate **E** by Polonovski-type reaction[15] will give the skeleton of daphniglaucin C (**1**), although an alternative path through oxidative cleavage of C-6 to C-7 bond is also possible.

Daphniglaucin C (**1**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50}, 0.1 μg/mL) in vitro and inhibited the polymerization of tubulin[16,17] (IC_{50}, 25 μM).

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