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Calyciphylline G, a novel alkaloid with an unprecedented fused-hexacyclic skeleton from *Daphniphyllum calycinum*

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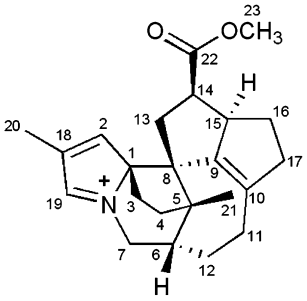
Abstract—Calyciphylline G (1), a novel *Daphniphyllum* alkaloid with an unprecedented fused-hexacyclic skeleton containing a 5-azatricyclo[6.2.1.0^{1,5}]undecane ring, has been isolated from the stem of *Daphniphyllum calycinum* (Daphniphyllaceae), and the structure and relative stereochemistry were elucidated on the basis of spectroscopic data.

Plants of the genus *Daphniphyllum* (Daphniphyllaceae) produce structurally diverse alkaloids with unusual fused-heterocyclic skeletons.[1–6] These unique ring systems have been challenging targets for total synthesis as well as biosynthetic studies.[7] Recently, we have isolated some novel types of *Daphniphyllum* alkaloids such as calyciphyllines A–D[2a,b,h] from the leaves of *Daphniphyllum calycinum*. In our search for structurally unique and biogenetically interesting alkaloids, calyciphylline G (**1**),[8] a novel alkaloid with an unprecedented hexacyclic skeleton containing a 5-azatricyclo[6.2.1.0^{1,5}]undecane ring, was isolated from the stems of *D. calycinum*. This Letter describes the isolation and structure elucidation of **1**.



1

The stem of *D. calycinum* was extracted with MeOH, and the MeOH 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/EtOAc and then $CHCl_3$/MeOH), in which a fraction eluted with (hexane/EtOAc, 9:1) was purified by repeated silica gel columns ($CHCl_3$/MeOH/TFA) to afford calyciphylline G (**1**, 0.00076% yield).

Calyciphylline G (**1**) showed the molecular ion peak at *m*/*z* 352 $(M)^+$ in the ESIMS, and the molecular formula, $C_{23}H_{30}NO_2$, was established by HRESIMS [*m*/*z* 352.2261, $(M)^+$, Δ −1.6 mmu]. The IR absorption at 1730 cm^{-1} suggested the presence of ester carbonyl functionality. Analyses of ^1H and ^{13}C NMR data (Table 1) revealed that **1** possessed 23 carbons due to one ester carbonyl, three sp^2 quaternary carbons, two sp^2 methines, three sp^3 quaternary carbons, three sp^3 methines, eight sp^3 methylenes, and three methyls. It was suggested that C-7 (δ_C 51.5) was adjacent to a nitrogen atom from the chemical shift.

The gross structure of **1** was elucidated by analyses of 2D NMR data, including the ^1H–^1H COSY, HMQC, and HMBC spectra in CD_3OD (Fig. 1). The ^1H–^1H COSY spectrum of **1** revealed connectivities of three structural fragments, **a** (C-3 to C-4), **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 from H_3-21 to C-4 (δ_C 39.7), C-5 (δ_C 49.2), C-6 (δ_C 47.9) and C-8 (δ_C 67.6) suggested that C-4, C-6, C-8, and C-21 were attached

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Table 1. ^{1}H and ^{13}C NMR Data of Calyciphylline G (**1**) in CD_3OD

Position	δ_H	δ_C	
1		94.5	s
2	7.72 (1H, s)	156.0	d
3a	2.73 (1H, m)	29.2	t
3b	1.95 (1H, m)		
4	2.26 (2H, m)	39.7	t
5		49.2	s
6	2.36 (1H, m)	47.9	d
7a	4.51 (1H, br d, 14.9)	51.5	t
7b	4.38 (1H, dd, 14.9, 7.4)		
8		67.6	s
9		142.3	s
10		141.3	s
11	2.14 (2H, m)	26.3	t
12a	2.12 (1H, m)	27.7	t
12b	1.74 (1H, m)		
13a	2.93 (1H, dd, 15.5, 2.9)	41.0	t
13b	2.54 (1H, dd, 15.5, 9.2)		
14	3.06 (1H, dd, 9.2, 2.9)	44.8	d
15	2.99 (1H, m)	59.9	d
16a	1.85 (1H, m)	30.3	t
16b	1.31 (1H, m)		
17a	2.65 (1H, m)	44.8	t
17b	2.36 (1H, m)		
18		139.6	s
19	8.84 (1H, s)	169.7	d
20	2.18 (3H, s)	13.0	q
21	1.44 (3H, s)	22.3	q
22		177.0	s
23	3.66 (3H, s)	52.5	q

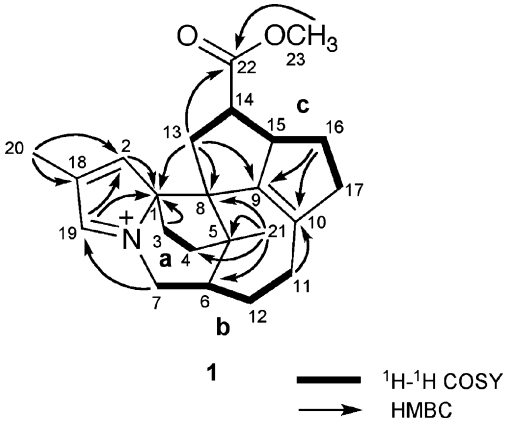

Figure 1. Selected 2D NMR correlations for calyciphylline G (**1**).

to C-5. HMBC cross-peaks for H_2-13 to C-1 (δ_C 94.5), C-8, and C-9 (δ_C 142.3) indicated connectivities of C-1, C-5, C-9, and C-13 through C-8. Connections of C-9, C-11, and C-17 via C-10 were implied by HMBC cross-peaks for H_2-11 to C-10 (δ_C 141.3), and H_2-16 to C-9 and C-10. HMBC correlations for H_2-13 and H_3-23 to C-22 (δ_C 177.0) suggested that a methoxy group was attached to C-22. HMBC correlations observed for H_2-7 to C-19 and H-19 to C-1 indicated that C-1, C-7, and C-19 were connected to each other through a nitrogen atom (N-1). The presence of an imino group at C-19 was supported by the chemical shift of C-19 (δ_C 169.7) and the IR absorption at 1680 cm^{-1}. HMBC correlations for H_3-20 to C-2 (δ_C 156.0) and

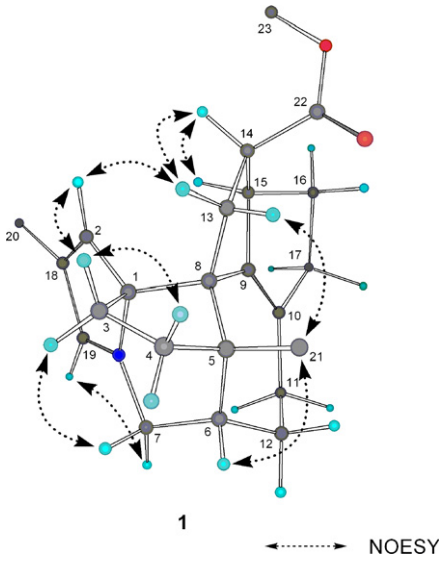

Figure 2. Selected NOESY correlations and relative stereochemistry of calyciphylline G (**1**) (hydrogen atoms of methyl groups were omitted).

C-18 (δ_C 139.6), H-19 to C-2, and H-2 to C-1 indicated the presence of a 2*H*-pyrrolium ring. The likage of C-1 and C-3 was implied by an HMBC cross-peak for H-3 to C-1. Thus, the gross structure of calyciphylline G was elucidated to be **1**.

The relative stereochemistry of **1** was deduced from NOESY correlations as shown in Figure 2. These NOESY correlations indicated the relative configurations at C-1, C-5, C-6, C-8, C-14, and C-15 and the conformation of 5-azatricyclo[6.2.1.$0^{1,5}$]undecane ring (C-1 to C-8, N-1, and C-18 to C-19).

Calyciphylline G (**1**) is a novel *Daphniphyllum* alkaloid with an unprecedented fused-hexacyclic skeleton containing a 5-azatricyclo[6.2.1.$0^{1,5}$]undecane ring. Calyciphylline G (**1**) showed cytotoxicity against L1210 murine leukemia cells (IC_{50}, 9 μg/mL) in vitro.

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8. Calyciphylline G (**1**): Colorless amorphous solid; $[\alpha]_D^{21}$ +8.5 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 2960, 1730, 1680, 1440, 1210, and 1140 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS *m*/*z* 352 $(M)^+$; HRESIMS (*m*/*z* 352.2261 $[(M)^+$; Calcd for $C_{23}H_{30}NO_2$, 352.2277]).