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Calyciphyllines E and F, novel hepta- and pentacyclic alkaloids from *Daphniphyllum calycinum*

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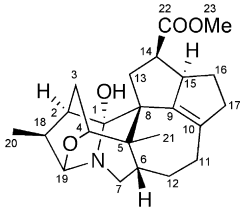
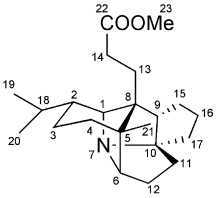
Abstract—Two new *Daphniphyllum* alkaloids, calyciphyllines E (**1**) and F (**2**), have been isolated from the leaves of *Daphniphyllum calycinum* (Daphniphyllaceae), and the structures and relative stereochemistry were elucidated on the basis of spectroscopic data.

Daphniphyllum alkaloids are a structurally diverse group of natural products with unique fused-heterocyclic ring systems, which are elaborated by trees of the genus *Daphniphyllum* (Daphniphyllaceae).[1–7] These unusual ring systems have been attractive targets for total synthesis as well as biosynthetic studies.[8] Recently, we have isolated novel types of *Daphniphyllum* alkaloids such as calyciphyllines A–C[2a,g] from the leaves of *Daphniphyllum calycinum*. In our search for structurally unique and biogenetically interesting alkaloids, two new *Daphniphyllum* alkaloids, calyciphyllines E (**1**)[9] and F (**2**),[10] were isolated from the leaves of *D. calycinum*. In this Letter, we describe the isolation and structure elucidation of **1** and **2**.



1



2

The leaves of *D. calycinum* were 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, 1:0→4:6, and then $CHCl_3$/MeOH, 1:0→0:1), in which a fraction eluted with hexane/EtOAc (6:4) was purified by a silica gel column ($CHCl_3$/MeOH, 1:0→0:1) to give calyciphylline E (**1**, 1.9 mg, 0.00011% yield). The fraction eluted with hexane/EtOAc (7:3) in the previous amino silica gel column was further purified by a silica gel column ($CHCl_3$/MeOH, 1:0→0:1) followed by C_{18} HPLC (CH_3CN/0.1% TFA, 1:1) to afford calyciphylline F (**2**, 3.6 mg, 0.0002% yield).

Calyciphylline E (**1**) showed the pseudomolecular ion peak at *m/z* 386 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{23}H_{31}NO_4$, was established by HRESIMS [*m/z* 386.2303, $(M+H)^+$, Δ −2.8 mmu]. IR absorption at 1730 cm^{-1} suggested the presence of ester carbonyl functionality. ^{13}C NMR (Table 1) data revealed 23 carbon signals due to one tetrasubstituted olefin, one carbonyl, three sp^3 quaternary carbons, seven sp^3 methines, seven sp^3 methylenes, and three methyls.

The gross structure of **1** was elucidated by analyses of 2D NMR data including the ^1H–^1H COSY, HMQC, and HMBC spectra in $CDCl_3$ (Fig. 1). The ^1H–^1H COSY and TOCSY spectra of **1** revealed connectivities of three structural fragments, **a** (C-2 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 2. The chemical shift of C-7 (δ_C 43.9) suggested that the carbon was adjacent to a nitrogen atom, while those of C-1 (δ_C 95.8) and C-19 (δ_C 96.0) indicated that these carbons were aminal and/or acetal carbons. HMBC correlations of H-19 to C-1 and C-7 suggested

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Table 1. ^{1}H and ^{13}C NMR Data of calyciphyllines E (**1**) and F (**2**)

1[a]				**2**[b]			
Position		δ_H	δ_C	Position		δ_H	δ_C
1			95.8	1	3.65	(1H, d, 1.8)	69.6
2	2.16	(1H, m)	46.5	2	1.53	(1H, m)	39.8
3a	2.03	(1H, m)	23.5	3a	1.99	(1H, m)	24.9
3b	1.75	(1H, dd, 13.7, 6.4)		3b	1.50	(1H, m)	
4	3.21	(1H, m)	83.0	4a	1.97	(1H, m)	35.0
				4b	1.58	(1H, m)	
5	—		45.8	5	—		44.7
6	2.49	(1H, m)	38.7	6	4.18	(1H, t, 5.2)	70.6
7a	3.44	(1H, dd, 14.3, 9.8)	43.9	7	—		
7b	3.02	(1H, dd, 14.3, 8.9)					
8	—		53.9	8	—		53.1
9	—		143.0	9	2.42	(1H, m)	52.3
10	—		135.4	10	—		85.2
11a	1.97	(1H, m)	22.8	11a	2.31	(1H, m)	35.0
11b	1.60	(1H, m)		11b	2.08	(1H, m)	
12	2.16	(2H, m)	25.9	12	2.18	(2H, m)	23.8
13a	2.49	(1H, m)	37.7	13a	2.12	(1H, m)	21.8
13b	2.28	(1H, dd, 13.6, 8.6)		13b	1.82	(1H, ddd, 16.4, 11.8, 4.4)	
14	3.14	(1H, dt, 11.4, 8.4)	42.4	14a	2.56	(1H, ddd, 16.4, 11.8, 5.8)	31.4
				14b	2.38	(1H, m)	
15	3.71	(1H, m)	54.6	15a	2.05	(1H, m)	30.2
				15b	1.66	(1H, m)	
16a	1.84	(1H, m)	28.6	16a	2.02	(1H, m)	27.4
16b	1.20	(1H, m)		16b	1.66	(1H, m)	
17a	2.49	(1H, m)	42.3	17a	2.17	(1H, m)	37.6
17b	2.20	(1H, m)		17b	1.89	(1H, m)	
18	2.41	(1H, m)	35.8	18	1.52	(1H, m)	33.9
19	4.30	(1H, br s)	96.0	19	1.06	(3H, d, 6.0)	22.0[c]
20	1.14	(3H, d, 6.9)	10.6	20	1.04	(3H, d, 6.0)	21.6[c]
21	0.97	(3H, s)	20.6	21	1.10	(3H, s)	19.6
22	—		176.4	22	—		175.9
23	3.63	(3H, s)	51.1	23	3.72	(3H, s)	53.2

[a] In $CDCl_3$.
[b] In CD_3OD.
[c] Assignments are interchangeable.

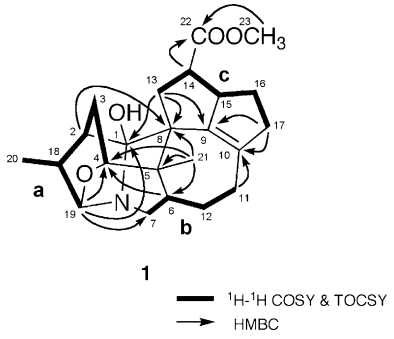

Figure 1. Selected 2D NMR correlations for calyciphylline E (**1**).

that C-1, C-7, and C-19 were connected to each other through a nitrogen atom. The linkage of C-4 and C-19 via an oxygen atom was deduced from the HMBC correlation of H-19 to C-4 (δ_C 83.0). HMBC correlations from H_3-21 to C-4, C-5 (δ_C 45.8), C-6 (δ_C 38.7) suggested that C-4, C-6, and C-21 were attached to C-5, and the correlation for H_3-21 to C-8 (δ_C 53.9) indicated the connection between C-5 and C-8. The linkage of C-2 and C-8 via C-1 was implied by an HMBC cross-peak for H-2 to C-8. HMBC correlations for H_2-13 to C-1,

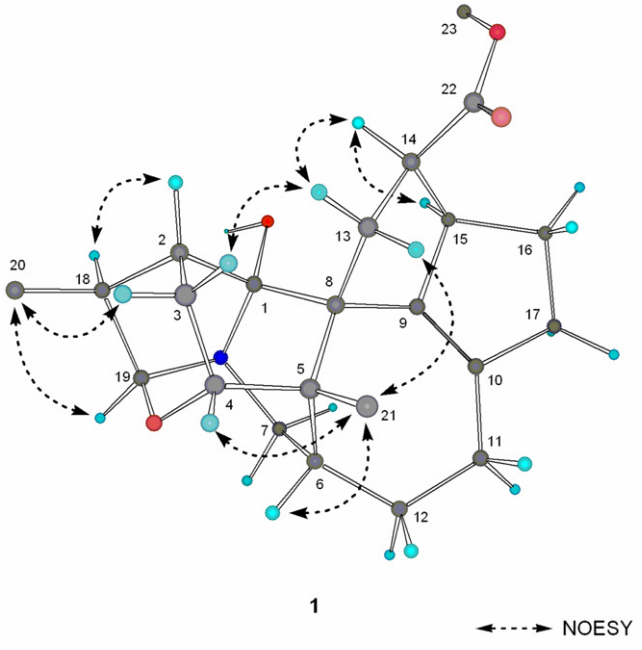

Figure 2. Selected NOESY correlations and relative stereochemistry for calyciphylline E (**1**) (hydrogen atoms of methyl groups were omitted).

C-8, and C-9 (δ_C 143.0) indicated the connectivities of C-1 and C-13 to C-9 through C-8. The connection of C-9, C-11, and C-17 via C-10 was implied by HMBC cross-peaks for H_2-11 and H_2-17 to C-10 (δ_C 135.4) and H-17 to C-9. HMBC correlations for H_3-23 and H-14 to C-22 (δ_C 176.4) suggested that a methoxy group was attached to C-22. Thus, the gross structure of calyciphylline E was elucidated to be **1**.

The relative stereochemistry of **1** was deduced from NOESY correlations as shown in Figure 2. These NOESY correlations suggested that the cyclohexane ring (C-1 to C-5 and C-8) and the piperidine ring (C-1, C-5 to C-8 and N-1) took boat forms, and that the tetrahydropyran ring (C-2 to C-4, C-18 to C-19, and O-4) took a chair form.

Calyciphylline F (**2**) showed the pseudomolecular ion peak at *m/z* 346 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{22}H_{35}NO_2$, was established by HRESIMS [*m/z* 346.2741, $(M+H)^+$, Δ −0.5 mmu]. IR absorptions implied the presence of ester carbonyl (1740 cm^{-1}) functionality. The ^{13}C NMR (Table 1) spectrum of **2** gave signals including one ester carbonyl, three sp^3 quaternary carbons, five sp^3 methines, nine sp^3 methylenes, and three methyls. Among them, two methines (δ_C 69.6 and 70.6) and one quaternary carbon (δ_C 85.2) were ascribed to those bearing a nitrogen atom.

The ^1H–^1H COSY and TOCSY spectra of **2** revealed connectivities of four 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-12, and C-11 to C-12), **c** (C-13 to C-14), and **d** (C-9 to C-15, and C-15 to C-17) as shown in Figure 3. HMBC correlations of H-1 to C-6 (δ_C 70.6) and H-6 to C-10 (δ_C 85.2) suggested that C-1, C-6, and C-10 were connected to each other through a nitrogen atom. Connections between C-4, C-6, and C-21 via C-5 were implied by HMBC cross-peaks for H_3-21 to C-4 (δ_C 35.0), C-5 (δ_C 44.7) and C-6. HMBC correlations for H-9 to C-1 (δ_C 69.6), H_2-13 to C-9 (δ_C 52.3), and H_3-21 and H_2-14 to C-8 (δ_C 53.1), indicated connectivities of C-1, C-5, C-9, and C-13 via C-8. The linkage of units **b** and **d** through C-10 was implied by HMBC cross-peaks for H_2-11 to C-17 (δ_C 37.6) and C-9, and H_2-16 to C-10. In addition, HMBC correlations for H_3-23 and H_2-14 to C-22 (δ_C 175.9) suggested that a methoxy group was attached to C-22. Thus, the gross structure of calycipylline E was elucidated to be **2**.

The relative stereochemistry of **2** was deduced from NOESY correlations as shown in Figure 4. NOESY correlations of H-1/H-15b, H-2/H-4a, H-9/H_3-21, and H-3b/H-6 indicated the relative stereochemistry of the fused-pentacyclic ring and a chair form of the cyclohexane ring (C-1 to C-5 and C-8).

Calyciphylline E (**1**) is a new *Daphniphyllum* alkaloid with an unprecedented fused-heptacyclic ring system, while calyciphylline F (**2**) is a new *Daphniphyllum* alkaloid possessing a unique fused-pentacyclic skeleton containing a 8-azatricyclo[4.2.1.0.^{4,8}]nonane ring system.

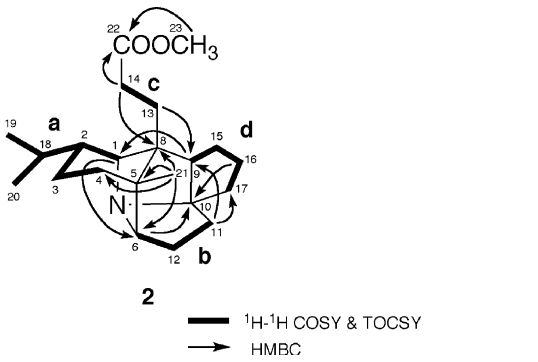

Figure 3. Selected 2D NMR correlations for calyciphylline F (**2**).

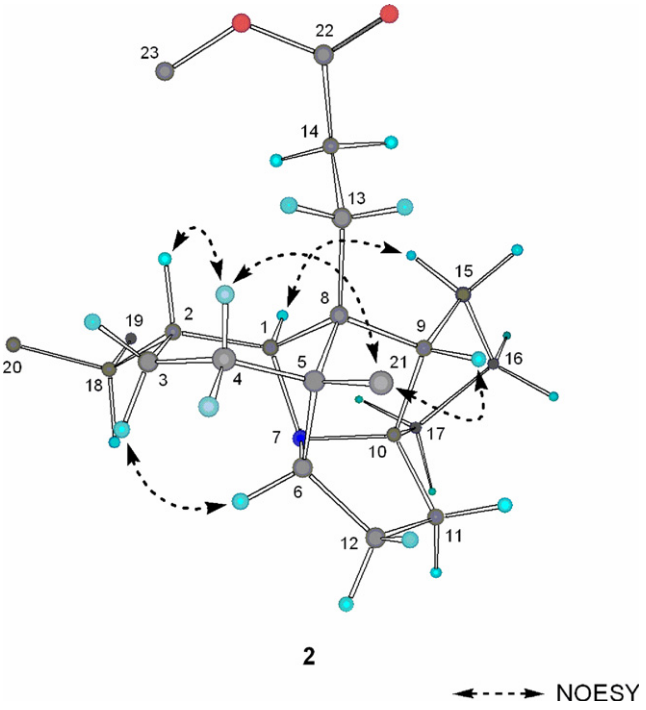

Figure 4. Selected NOESY correlations and relative stereochemistry for calyciphylline F (**2**) (hydrogen atoms of methyl groups were omitted).

Investigations on the absolute stereochemistry of **1** and **2** are currently carried out.

Acknowledgments

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9. *Calyciphylline E* (**1**): A colorless amorphous solid; $[\alpha]_D^{21}$ −39 (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3650, 2920, 1730, 1460, and 1170 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 386 $(M+H)^+$; HRESIMS *m/z* 386.2303 (M+H; calcd for $C_{23}H_{32}NO_4$, 386.2331).
10. *Calyciphylline F* (**2**): A colorless amorphous solid; $[\alpha]_D^{16}$ −37 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 2960, 1740, 1670, 1200, and 1130 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 346 $(M+H)^+$; HRESIMS *m/z* 346.2741 (M+H; calcd for $C_{22}H_{36}NO_2$, 346.2746).