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Calyciphyllines H–M, new *Daphniphyllum* alkaloids from *Daphniphyllum calycinum*

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Abstract

Six new *Daphniphyllum* alkaloids, calyciphyllines H–M (**1**–**6**), were isolated from the leaves and stems of *Daphniphyllum calycinum* (Daphniphyllaceae). The structures and relative stereochemistry of **1**–**6** were elucidated on the basis of spectroscopic data, and the absolute stereochemistry of **3** was assigned by PGME method.

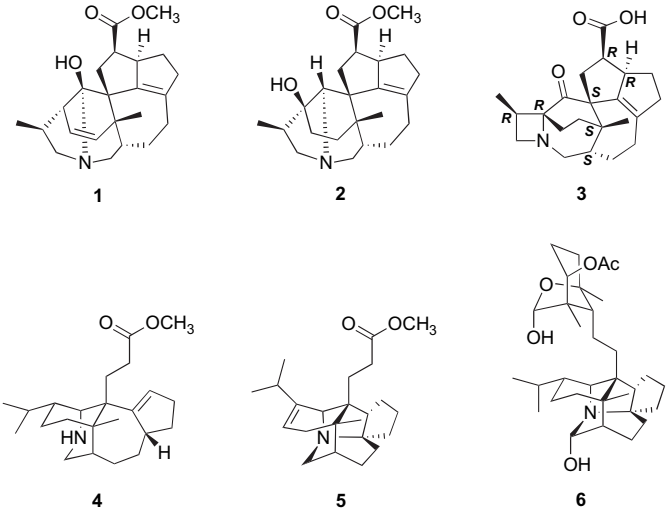
Keywords: *Daphniphyllum calycinum*; *Daphniphyllum* alkaloid; Calyciphyllines H–M

1. Introduction

Trees of the genus *Daphniphyllum* (Daphniphyllaceae) are known to elaborate structurally diverse group of alkaloids with unique polycyclic fused ring systems.[1–6] These *Daphniphyllum* alkaloids have been attractive targets for biogenetic and synthetic studies.[7] Recently, some novel alkaloids with unusual skeletons such as calyciphyllines C–G[2a–d] have been isolated from *Daphniphyllum calycinum* at our laboratory. Further investigation of extracts of this plant resulted in the isolation of six new alkaloids, calyciphyllines H–M (**1**–**6**). In this paper we describe the isolation and structural elucidation of **1**–**6**.

2. Results and discussion

The leaves and stems of *D. calycinum* were extracted with MeOH separately, and each 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 $CHCl_3$ to give crude alkaloidal fractions. The crude alkaloidal materials prepared from the leaves were subjected to an amino silica gel column (hexane/EtOAc and then $CHCl_3$/MeOH), in which a fraction eluted with hexane/EtOAc (6:4) was purified by a silica gel column ($CHCl_3$/MeOH) to give calyciphyllines H (**1**, 0.00007% yield), I (**2**, 0.00009%), K (**4**, 0.00003%), and L (**5**, 0.00018%). The crude alkaloidal materials prepared from the stems were separated by the same procedure as described above to yield calyciphyllines J (**3**, 0.00013%) and M (**6**, 0.00018%).



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Calyciphylline H (**1**) showed the pseudomolecular ion peak at *m/z* 370 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{23}H_{31}NO_3$, was established by HRESIMS [*m/z* 370.2387, $(M+H)^+$, Δ +0.5 mmu]. IR absorption at 1734 cm^{-1} suggested the presence of the ester carbonyl functionality. Analyses of ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum provided evidence that **1** possessed one tetrasubstituted olefin, one disubstituted olefin, one carbonyl, three sp^3 quaternary carbons, five sp^3 methines, seven sp^3 methylenes, and three methyls. Among them, two methylenes (δ_C 54.7, δ_C 61.8) were ascribed to those bearing a nitrogen atom, while one quaternary carbon (δ_C 103.2) was assigned as an aminal carbon.

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), were deduced from the $^1H-^1H$ COSY and TOCSY spectra as shown in Figure 1. HMBC correlations of H_2-7 to C-19 (δ_C 61.8) and H_2-19 to C-1 (δ_C 103.2) suggested that C-1, C-7, and C-19 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-4 to C-5 (δ_C 41.8), and H_3-21 to C-4 (δ_C 138.0) and C-6 (δ_C 39.8). HMBC correlations for H-4 to C-8 (δ_C 51.6), and H_2-13 to C-1, C-8, and C-9 (δ_C 141.9) indicated connectivities of C-1, C-5, C-9, and C-13 via C-8. The linkage of units **b** and **c** through C-10 was implied by HMBC cross-peaks for H_2-17 to C-10 (δ_C 137.4) and C-11 (δ_C 24.9). In addition, HMBC correlations for H_3-23 and H-14 to C-22 (δ_C 175.5) suggested that a methoxy group was attached to C-22. Thus, the gross structure of calyciphylline H was elucidated to be **1**.

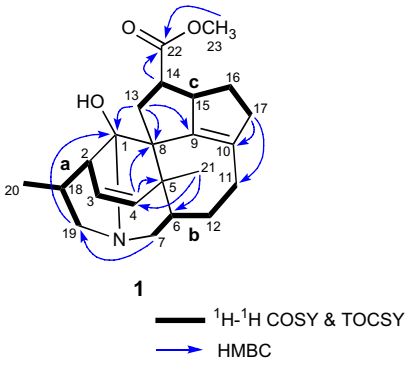

Figure 1. Selected 2D NMR correlations for calyciphylline H (**1**).

Table 1
^1H and ^{13}C NMR data of calyciphyllines H–J (**1**–**3**)

	1[a]		**2**[a]		**3**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		103.2 s	3.89 (1H, br s)	64.9 d		212.8 s
2	2.99 (1H, m)	46.4 d		76.8 s		75.2 s
3	5.70 (1H, dd, 10.0, 3.5)	125.0 d	1.96 (1H, m)	29.3 t	2.32 (1H, m)	23.0 t
			1.80 (1H, m)		1.38 (1H, m)	
4	5.49 (1H, dd, 10.0, 2.0)	138.0 d	1.96 (1H, m)	35.7 t	1.92 (1H, m)	35.1 t
			1.59 (1H, m)		1.82 (1H, m)	
5		41.8 s		34.4 s		39.4 s
6	1.80 (1H, m)	39.8 d	2.13 (1H, m)	41.3 d	2.06 (1H, m)	48.9 d
7	3.46 (1H, dd, 13.5, 5.5)	54.7 t	3.76 (1H, m)	55.8 t	2.96 (1H, dd, 12.0, 4.6)	54.7 t
	3.31 (1H, m)		3.37 (1H, m)		2.58 (1H, m)	
8		51.6 s		44.9 s		59.9 s
9		141.9 s		141.9 s		142.3 s
10		137.4 s		136.0 s		133.6 s
11	2.61 (1H, m)	24.9[b] t	2.28 (1H, m)	24.3 t	2.06 (2H, m)	25.4 t
	2.14 (1H, m)		2.18 (1H, m)			
12	2.14 (1H, m)	25.1[b] t	2.18 (1H, m)	27.5 t	2.06 (1H, m)	25.8 t
	1.59 (1H, m)		1.53 (1H, m)		1.59 (1H, m)	
13	2.61 (1H, m)	39.3 t	2.95 (1H, m)	34.4 t	2.74 (1H, dd, 13.7, 6.3)	40.1 t
	2.38 (1H, dd, 15.0, 10.0)		2.53 (1H, m)		1.92 (1H, m)	
14	2.29 (1H, m)	42.5 d	2.91 (1H, m)	41.5 d	3.11 (1H, m)	42.1 d
15	3.76 (1H, m)	58.1 d	3.50 (1H, m)	53.5 d	4.01 (1H, m)	56.4 d
16	1.91 (1H, m)	29.2 t	1.87 (1H, m)	27.8 t	2.02 (1H, m)	29.5 t
	1.42 (1H, m)		1.24 (1H, m)		1.47 (1H, m)	
17	2.61 (1H, m)	43.2 t	2.53 (1H, m)	42.3 t	2.61 (1H, m)	42.5 t
	2.31 (1H, dd, 15.0, 8.3)		2.28 (1H, m)		2.27 (1H, m)	
18	3.16 (1H, m)	32.4 d	2.46 (1H, m)	44.0 d	3.05 (1H, m)	25.9 d
19	4.20 (1H, m)	61.8 t	4.13 (1H, m)	61.1 t	3.10 (2H, m)	56.8 t
	2.61 (1H, m)		2.53 (1H, m)			
20	1.25 (3H, d, 7.3)	18.3 q	1.14 (3H, d, 6.9)	12.4 q	1.12 (3H, br d, 6.3)	14.2 q
21	1.38 (3H, s)	22.6 q	1.19 (3H, s)	24.3 q	1.19 (3H, s)	26.3 q
22		175.5 s		175.6 s		178.9 s
23	3.62 (3H, s)	51.0 q	3.63 (3H, s)	51.3 q		

[a] Measured in $CDCl_3$.
[b] Assignments are interchangeable.

The relative stereochemistry of **1** was deduced from the NOESY spectrum as shown in Figure 2. NOESY correlations of H-3/H-4, H-3/H_3-20, H-4/H-6, H-4/H_3-21, and H-13a/H_3-21 indicated a pseudo boat conformation of cyclohexene ring (C-1 to C-5 and C-8) and a chair conformation of piperidine ring (C-1, N-1, and C-5 to C-8). Orientations of H-14 and H-15 were provided from NOESY correlations of H-13b/H-14 and H-14/H-15.

Calyciphylline I (**2**) showed the pseudomolecular ion peak at *m/z* 372 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{23}H_{33}NO_3$, was established by HRESIMS [*m/z* 372.2552, $(M+H)^+$, Δ +1.3 mmu]. The IR spectrum (1734 cm^{-1}) suggested the presence of ester carbonyl group. The ^{13}C NMR data revealed 23 carbon signals due to one tetrasubstituted olefins, one carbonyl, three sp^3 quaternary carbons, five sp^3 methines, nine sp^3 methylenes, and three methyls. Among them, one methine (δ_C 64.9) and two methylenes (δ_C 55.8, δ_C 61.1) 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-3 to C-4), **b** (C-6 to C-7 and C-12, and C-11 to C-12), **c** (C-13 to C17), and **d** (C-18 to C-19 and C-20) as shown in Figure 3. HMBC correlations of H_2-19 to C-1 (δ_C 64.9) and H_2-7 to C-19 (δ_C 61.1) suggested that C-1, C-7, and C-19 were connected to each other through a nitrogen atom. Connectivities of C-4, C-6, and C-21 via C-5 were implied by HMBC cross-peaks of H_3-21 to C-4 (δ_C 35.7), C-5 (δ_C 34.4), and C-6 (δ_C 41.3). HMBC correlations for H_2-13 to C-1, C-8 (δ_C 44.9), C-9 (δ_C 141.9) and H_3-21 to C-8 suggested that C-1, C-5, C-9, and C-13 were attached to C-8. Connections of C-11 and C-17 to C-9 through C-10 were implied by HMBC cross-peaks for H_2-16 to C-9, C-10 (δ_C 136.0), and H_2-12 to C-10.

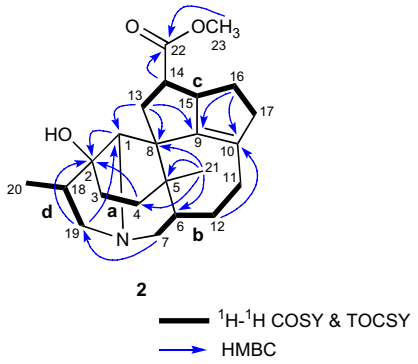

Figure 3. Selected 2D NMR correlations for calyciphylline I (**2**).

HMBC correlations of H-1, H_2-4, and H_2-19 to C-2 (δ_C 76.8) indicated the linkage of C-1 and C-3, C-18 via C-2. HMBC correlations for H_3-23 and H-14 to C-22 (δ_C 175.6) suggested that a methoxy group was attached to C-22. Thus, the gross structure of calyciphylline I was elucidated to be **2**.

The relative stereochemistry of **2** was elucidated from NOESY correlations as shown in Figure 4. Chair conformations of a cyclohexane ring (C-1 to C-5 and C-8) and a piperidine ring (C-1, N-1, and C-5 to C-8) were deduced from NOESY correlations of H_2-3/H_3-20, H-4b/H-6, H-6/H-7b, H-6/H_3-21, H-7b/H-19b, and H-19b/H_3-20. NOESY correlations of H-1/H-15, H-13a/H-14, and H-14/H-15 indicated α-orientations of H-14 and H-15.

Calyciphylline J (**3**) was obtained as a colorless amorphous solid, and the molecular formula was established as $C_{22}H_{29}NO_3$ by HRESIMS [*m/z* 356.2237, $(M+H)^+$, Δ +1.1 mmu]. ^1H and ^{13}C NMR data of **3** (Table 1) were similar to those of calyciphylline C,[2d] except for the absence of a methoxy group. Detailed analyses of 2D NMR data indicated that **3** was the free acid form of calyciphylline C (Fig. 5).

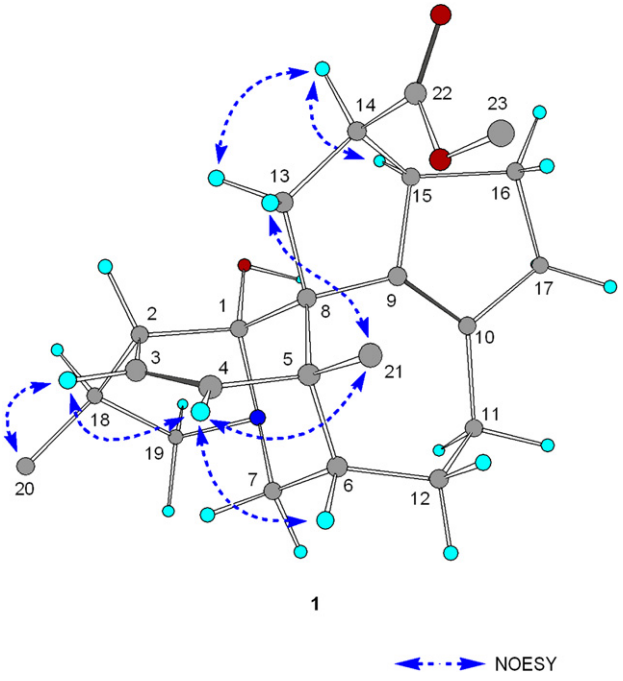

Figure 2. Selected NOESY correlations and relative stereochemistry of calyciphylline H (**1**) (hydrogen atoms of methyl groups were omitted).

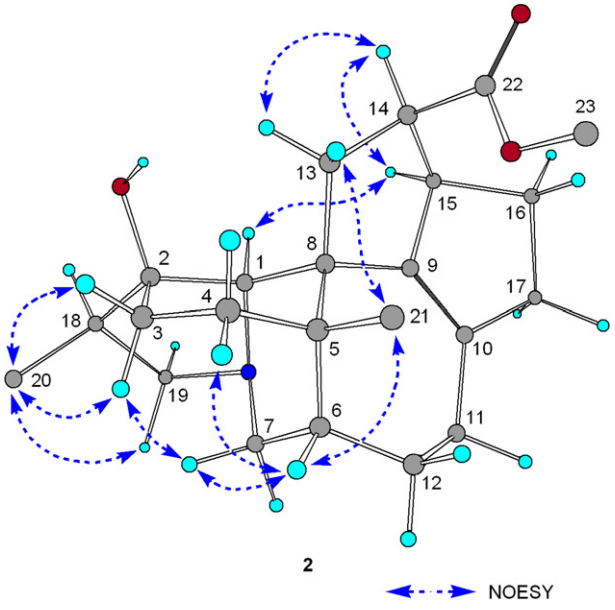

Figure 4. Selected NOESY correlations and relative stereochemistry of calyciphylline I (**2**) (hydrogen atoms of methyl groups were omitted).

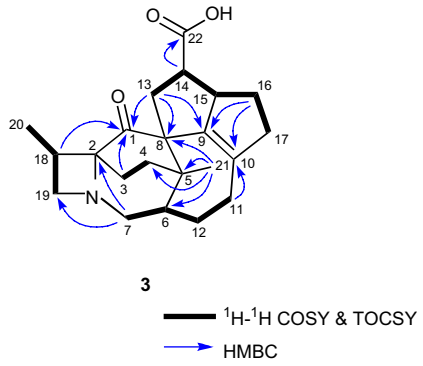

Figure 5. Selected 2D NMR correlations for calyciphylline J (**3**).

The relative stereochemistry of **3** was elucidated to be the same as that of calyciphylline C on the basis of NOESY data (Fig. 6).

To elucidate the absolute configuration at C-14, **3** was converted into its (*S*)- and (*R*)-phenylglycine methyl ester (PGME) amides of the carboxy group at C-14. The $\Delta\delta$ [δ (*S*-PGME amide)$-\delta$ (*R*-PGME amide)] values obtained from the ^{1}H NMR spectra of the PGME amides suggested that the absolute configuration at C-14 in **3** was *R* (Fig. 7).[8] Thus, the absolute stereochemistry of **3** was elucidated as shown in Figure 7.

Calyciphylline J (**3**) was treated with trimethylsilyl diazomethane to afford calyciphylline C, whose spectral data and $[\alpha]_D$ value {$[\alpha]_D^{20}$ −78.7 (*c* 0.1, MeOH)} were coincident with those of natural calyciphylline C.[2d,9] Thus, the absolute stereochemistry of calyciphylline C was concluded to be the same as **3**.

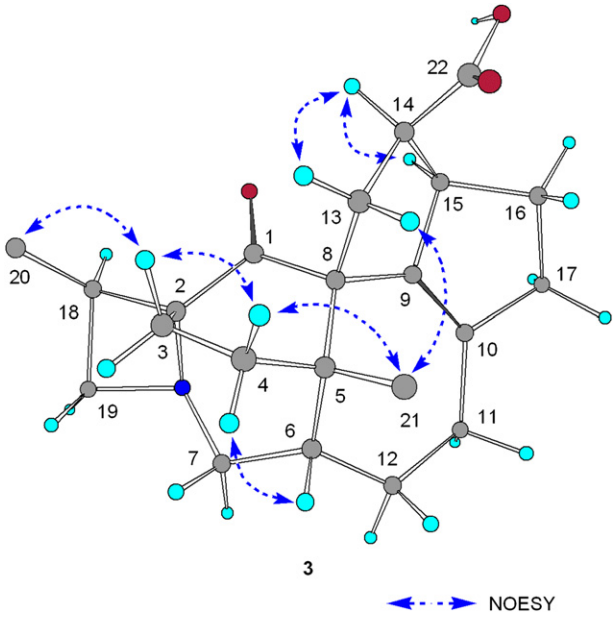

Figure 6. Selected NOESY correlations and relative stereochemistry of calyciphylline J (**3**) (hydrogen atoms of methyl groups were omitted).

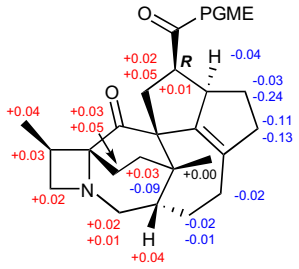

Figure 7. $\Delta\delta$ values [$\Delta\delta$ (in ppm)] $\delta_S-\delta_R$] obtained for the (*S*)- and (*R*)-PGME amides of calyciphylline J (**3**).

Calyciphylline K (**4**) showed the pseudomolecular ion peak at *m/z* 360 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{23}H_{37}NO_2$, was established by HRESIMS [*m/z* 360.2905, $(M+H)^+$, Δ +0.2 mmu]. The IR absorption at 1735 cm^{-1} suggested the presence of ester carbonyl functionality. The ^{13}C NMR (Table 2) spectrum of **4** gave signals due to one trisubstituted olefin, one carbonyl, two sp^3 quaternary carbons, five sp^3 methines, nine sp^3 methylenes, and four methyls, implying that the structure of **4** was similar to that of daphnezomine L.[10]

The chemical shifts of C-1 (δ_C 60.9) and C-7 (δ_C 47.5) in **4** suggested that these carbons were attached to a nitrogen atom and the connectivity of C-1 and C-7 via a nitrogen was implied by HMBC cross-peaks of H_2-7 to C-1. The ^{1}H−^{1}H COSY and TOCSY spectra of **4** 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, C-10 to C-12 and C-17, and C-15 to C-17), and **c** (C-13 to C14), which were connected to each other on the basis of HMBC correlations as shown in Figure 8. HMBC correlations for H_3-23 and H-14 to C-22 (δ_C 175.0) suggested that a methoxy group was attached to C-22. Thus, the gross structure of calyciphylline K (**4**) was elucidated to be *N*,7-dihydro daphnezomine L methyl ester.

The relative stereochemistry of **4** was elucidated from NOESY correlations as shown in Figure 9. Chair forms of a cyclohexane ring (C-1 to C-5 and C-8) and a piperidine ring (C-1, N-1, and C-5 to C-8), and a β-orientation of H-10 were suggested by NOESY cross-peaks of H-3b/H-7a and H-6/H_3-21 and H-10/H_3-21. An α-orientation of H-15 was suggested by NOESY cross-peaks of H-1/H-15 and H-1/H_3-20.

Calyciphylline L (**5**) showed the pseudomolecular ion peak at *m/z* 358 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{23}H_{35}NO_2$, was established by HRESIMS [*m/z* 358.2740, $(M+H)^+$, Δ −0.6 mmu]. The IR absorption at 1734 cm^{-1} suggested the presence of ester carbonyl functionality. The ^{13}C NMR data revealed 23 carbon signals due to one trisubstituted olefin, one ester carbonyl, three sp^3 quaternary carbons, four sp^3 methines, nine sp^3 methylenes, and four methyls. The chemical shifts of ^{1}H and ^{13}C NMR data (Table 2) suggested that the structure of **5** was close to that of methyl homodaphniphyllate.[11]

The ^{1}H−^{1}H COSY and TOCSY spectra of **5** revealed connectivities of five structural fragments, **a** (C-3 to C-4), **b** (C-6 to C-7 and C-12, and C-11 to C-12), **c** (C-9 to C-15, and C-15 to C17), **d** (C-13 to C-14), and **e** (C-18 to C-19 and C-20) as

Table 2
^{1}H and ^{13}C NMR data of calyciphyllines K–M (**4**–**6**)

	4[a]		**5**[b]		**6**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.73 (1H, d, 3.5)	60.9 d	3.91 (1H, br s)	66.9 d	3.44 (1H, m)	65.6 d
2	1.40 (1H, m)	41.0 d		136.0 s	1.44 (1H, m)	38.2 d
3	1.80 (1H, m)	27.1 t	6.31 (1H, m)	135.4 d	1.87 (1H, m)	26.0 t
	1.55 (1H, m)				1.44 (1H, m)	
4	1.96 (1H, m)	41.0 t	2.48 (1H, m)	43.5 t	1.98 (1H, m)	36.1 t
	1.40 (1H, m)		2.18 (1H, m)		1.65 (1H, m)	
5		37.9 s		39.5 s		38.2 s
6	1.49 (1H, m)	44.1 d	1.76 (1H, m)	42.3 d	1.88 (1H, m)	45.5 d
7	3.44 (1H, dd, 14.7, 7.6)	47.5 t	3.58 (1H, br d, 12.6)	44.6 t	5.74 (1H, br d, 3.4)	81.2 d
	2.60 (1H, br d, 14.7)		3.14 (1H, br d, 12.6)			
8		46.6 s		49.1 s		47.1 s
9		155.5 s	2.70 (1H, m)	51.9 d	2.48 (1H, m)	51.5 d
10	3.02 (1H, m)	48.2 d		80.3 s		76.7 s
11	1.72 (1H, m)	34.7 t	2.07 (1H, m)	28.9 t	2.76 (1H, m)	28.7 t
	1.43 (1H, m)		1.83 (1H, m)		1.64 (1H, m)	
12	1.90 (1H, m)	32.9 t	2.07 (1H, m)	22.1 t	2.04 (1H, m)	16.4 t
	1.39 (1H, m)		1.83 (1H, m)		1.80 (1H, m)	
13	2.13 (2H, m)	31.7 t	1.82 (1H, m)	27.1 t	2.10 (1H, m)	22.9 t
			1.54 (1H, m)		1.39 (1H, m)	
14	2.32 (1H, m)	31.7 t	2.63 (1H, m)	32.6 t	1.94 (1H, m)	32.3 t
	1.63 (1H, m)		2.52 (1H, m)		1.00 (1H, m)	
15	5.49 (1H, br s)	126.5 d	2.01 (1H, m)	31.0 t	1.96 (1H, m)	29.4 t
			1.75 (1H, m)		1.52 (1H, m)	
16	2.37 (1H, m)	29.8 t	2.03 (1H, m)	26.9 t	1.90 (1H, m)	25.1 t
	2.18 (1H, m)		1.62 (1H, m)		1.52 (1H, m)	
17	2.15 (1H, m)	32.6 t	2.12 (1H, m)	40.9 t	2.16 (1H, m)	39.4 t
	1.49 (1H, m)		1.92 (1H, m)		1.90 (1H, m)	
18	1.67 (1H, m)	31.1 d	2.32 (1H, m)	34.9 d	1.70 (1H, m)	29.8 d
19	0.93 (3H, d, 6.9)	20.9 q	1.16 (3H, d, 6.4)	22.2 q	0.94 (3H, d, 6.3)	20.9 q
20	0.92 (3H, d, 6.9)	20.9 q	1.14 (3H, d, 6.4)	23.1 q	1.00 (3H, d, 6.3)	21.7 q
21	1.08 (3H, s)	27.5 q	1.17 (3H, s)	24.3 q	1.17 (3H, s)	26.0 q
22		175.0 s		176.1 s	1.59 (1H, m)	52.0 d
23	3.66 (3H, s)	51.5 q	3.69 (3H, s)	53.1 q		50.0 s
24					1.05 (3H, s)	17.0 q
25					4.88 (1H, br s)	99.2 d
26					4.79 (1H, br d, 5.2)	73.6 d
27					1.95 (1H, m)	25.6 t
					1.62 (1H, m)	
28					1.53 (1H, m)	27.5 t
					1.35 (1H, m)	
29						84.2 s
30					1.33 (3H, s)	26.0 q
31						169.9 s
32					2.09 (3H, s)	21.6 q

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

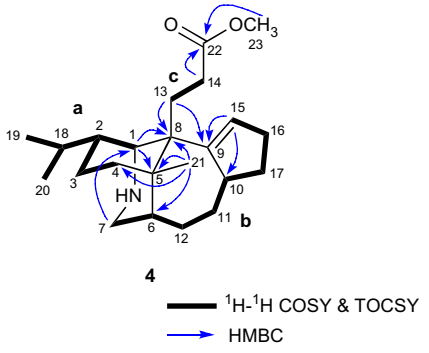

Figure 8. Selected 2D NMR correlations for calyciphylline K (**4**).

shown in Figure 10. Connections between C-1, C-3, and C-18 via C-2 were implied by HMBC cross-peaks for H-3 to C-1 (δ_C 66.9) and C-18 (δ_C 34.9), and H_3-19 to C-2 (δ_C 136.0). The connectivities of five partial structures **a**–**e** were revealed on the basis of HMBC correlations as shown in Figure 10. In addition, HMBC correlations for H_3-23 and H_2-14 to C-22 (δ_C 176.1) suggested that a methoxy group was attached to C-22. Thus, the gross structure of calyciphylline L (**5**) was elucidated to be 2,3-dehydro methyl homodaphniphyllate.

The relative stereochemistry of **5** was elucidated from NOESY correlations as shown in Figure 11. A pseudo boat conformation of a cyclohexene ring (C-1 to C-5 and C-8),

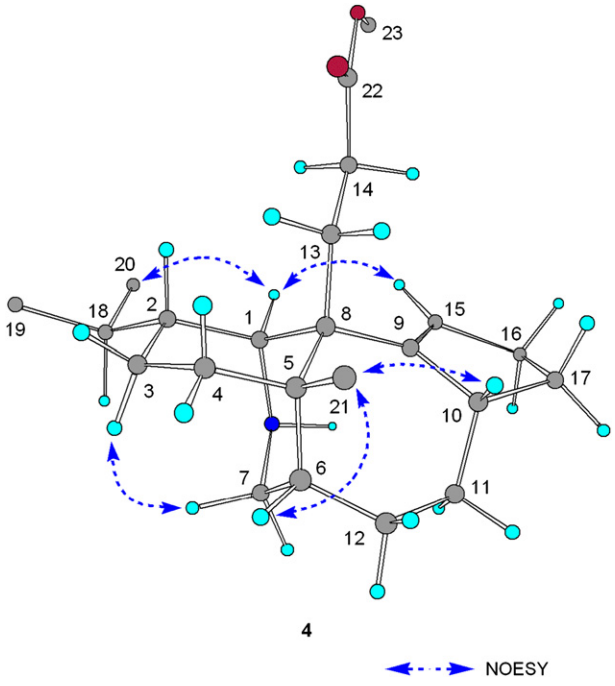

Figure 9. Selected NOESY correlations and relative stereochemistry of calyciphylline K (**4**) (hydrogen atoms of methyl groups were omitted).

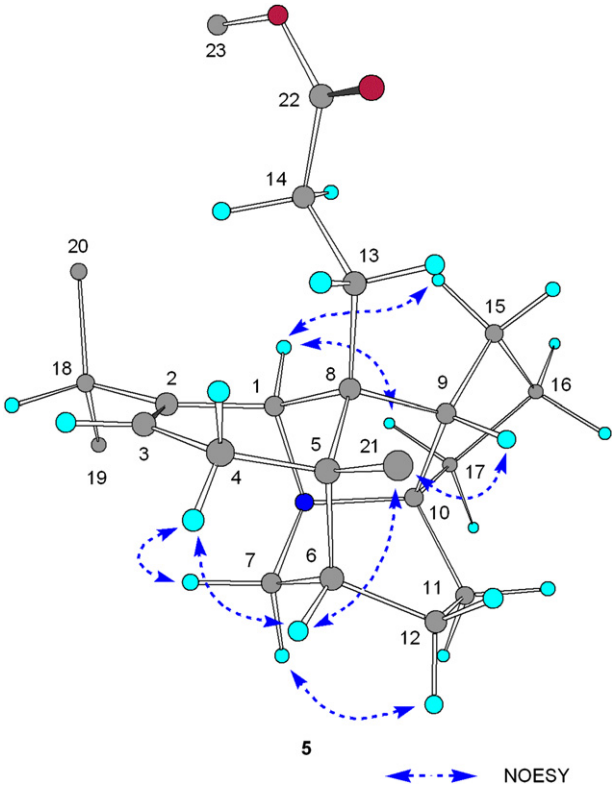

Figure 11. Selected NOESY correlations and relative stereochemistry of calyciphylline L (**5**) (hydrogen atoms of methyl groups were omitted).

a chair conformation of a piperidine ring (C-1, N-1, and C-5 to C-8), and a β-orientation of H-9 were deduced from NOESY cross-peaks of H-4b/H-6, H-4b/H-7a, H-6/H_3-21, and H-9/H_3-21.

The molecular formula of calyciphylline M (**6**) was determined to be $C_{32}H_{51}NO_5$ by HRESIMS [*m/z* 530.3850, $(M+H)^+$, Δ +0.5 mmu]. The IR spectrum suggested the presence of hydroxyl (3300 cm^{-1}) and ester carbonyl (1731 cm^{-1}) groups.

^1H and ^{13}C NMR, and the HMQC spectra revealed that **6** possessed 23 carbons due to one ester carbonyl, five sp^3 quaternary carbons, nine sp^3 methines, 11 sp^3 methylenes, and six methyls. The chemical shifts of ^1H and ^{13}C NMR data (Table 2) implying that **6** had the same fused-pentacyclic backbone skeleton as that of daphnimacropine[12] with the side chain consistent with that of daphmacropodine.[13] Detailed analyses of 2D NMR data revealed that **6** was the 7-hydroxy form of daphmacropodine (Figs. 12 and 13).

Effects of calyciphyllines H–M (**1**–**6**) on neurotrophic factor biosynthesis in 1321N1 human astrocytoma cells were examined by a semiquantitative RT-PCR method.[14,15] Among compounds **1**–**6**, it was found that the mRNA expressions for NGF were significantly enhanced by **4**.

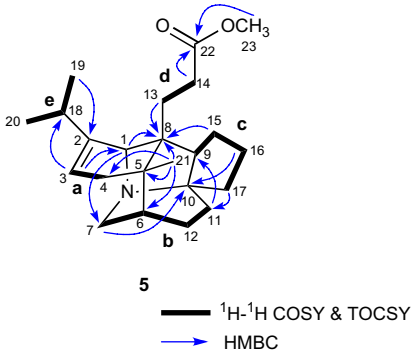

Figure 10. Selected 2D NMR correlations for calyciphylline L (**5**).

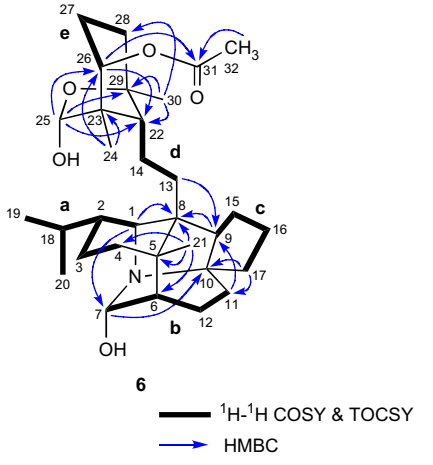

Figure 12. Selected 2D NMR correlations for calyciphylline M (**6**).

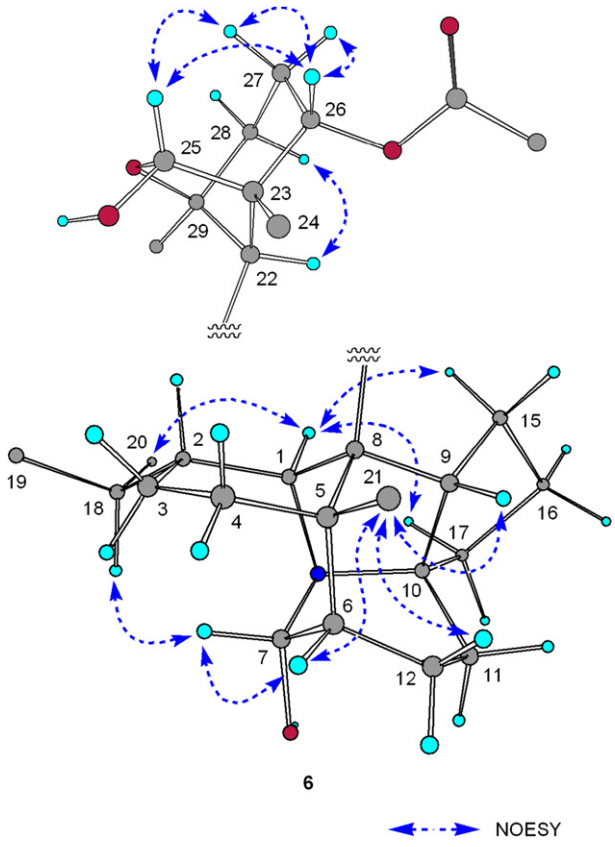

Figure 13. Selected NOESY correlations and partial relative stereochemistry of calyciphylline M (**6**) (hydrogen atoms of methyl groups were omitted).

3. Experimental

3.1. General experimental procedures

Optical rotation was recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 and a JEOL ECA-500 spectrometers. The 7.26 and 77.0 ppm resonances of residual $CDCl_3$ and the 3.35 and 49.8 ppm resonances of residual CD_3OD were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ spectrometer.

3.2. Isolation

The leaves and stems of *D. calycinum* were extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which are adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$ to give crude alkaloidal fractions. The crude alkaloidal fraction prepared from the leaves was 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 calyciphyllines H (**1**, 0.00007% yield), I (**2**, 0.00009%), K (**4**, 0.00003%), and L (**5**, 0.00018%). The crude alkaloidal fraction prepared from the stems was separated by the same procedure as described above to yield calyciphyllines J (**3**, 0.00013%) and M (**6**, 0.00018%).

3.2.1. Calyciphylline H (*1*)

Colorless amorphous solid; $[\alpha]_D^{22}$ +36.6 (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3649, 2928, 1734, 1669, 1457, and 1200 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m/z* 370 $(M+H)^+$; HRESIMS *m/z* 370.2387 (M+H; calcd for $C_{23}H_{32}NO_3$, 370.2382).

3.2.2. Calyciphylline I (*2*)

Colorless amorphous solid; $[\alpha]_D^{22}$ −9.2 (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3394, 2964, 1734, 1673, 1457, 1200, and 1134 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 372 $(M+H)^+$; HRESIMS *m/z* 372.2552 (M+H; calcd for $C_{23}H_{34}NO_3$, 372.2539).

3.2.3. Calyciphylline J (*3*)

Colorless amorphous solid; $[\alpha]_D^{22}$ −45.0 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3420, 2931, 1735, 1705, 1456, 1384, and 1194 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 356 $(M+H)^+$; HRESIMS *m/z* 356.2237 (M+H; calcd for $C_{22}H_{30}NO_3$, 356.2226).

3.2.4. (R)- and (S)-PGME amides of calyciphylline J (*3*)

To an ice cooled DMF solution (500 μL) of **3** (0.4 mg) and (*S*)-PGME (0.5 mg) were added PyBOP (1.0 mg), HOBt (0.5 mg), and *N*-methylmorpholine (25 μL), and stirring was continued at rt for 27 h. After addition of 5% HCl (1 mL), the mixture was extracted with EtOAc (2.5 mL). The extract was washed with saturated $NaHCO_3$ aq (1 mL) and brine (1 mL), and then concentrated in vacuo to afford the (*S*)-PGME amide of **3** (0.6 mg). The (*R*)-PGME amide of **3** was prepared according to the same procedure as described above.

3.2.5. (R)-PGME amide of calyciphylline J (*3*)

^{1}H NMR (600 MHz in $CDCl_3$) δ 7.33 (5H, m, phenyl), 6.39 (1H, d, 7.2, NH), 5.57 (1H, d, 7.2, C*H*NH), 3.96 (1H, m, H-15), 3.72 (3H, s, OCH_3), 3.06 (2H, m, H_2-19), 3.01 (1H, m, H-18), 2.94 (1H, m, H-14), 2.80 (2H, m, H-7, H-13), 2.59 (1H, m, H-17), 2.53 (1H, m, H-7), 2.27 (1H, dd, 15.0, 8.5, H-17), 2.23 (1H, m, H-3), 2.00 (3H, m, H_2-11, H-12), 1.98 (1H, m, H-4), 1.95 (1H, m, H-16), 1.90 (1H, m, H-6), 1.82 (1H, m, H-13), 1.73 (1H, m, H-4), 1.66 (1H, m, H-12), 1.45 (1H, m, H-16), 1.33 (1H, m, H-3), 1.19 (3H, s, H_3-21), 1.03 (3H, d, 6.2, H_3-20); ESIMS *m/z* 503 $(M+H)^+$; HRESIMS *m/z* 503.29018 (M+H; calcd for $C_{31}H_{39}N_2O_4$, 503.29098).

3.2.6. (S)-PGME amide of calyciphylline J (*3*)

^{1}H NMR (600 MHz in $CDCl_3$) δ 7.34 (5H, m, phenyl group), 6.35 (1H, d, 6.5, NH), 5.54 (1H, d, 7.2, C*H*NH), 3.92 (1H, m, H-15), 3.71 (3H, s, OCH_3), 3.08 (2H, m, H_2-19), 3.04 (1H, m, H-18), 2.95 (1H, m, H-14), 2.82 (2H, m, H-7, H-13), 2.54 (1H, m, H-7), 2.48 (1H, m, H-17), 2.26 (1H, m, H-3), 2.14 (1H, dd, 14.9, 8.4, H-17), 1.98 (3H, m,

H_2-11, H-12), 1.94 (1H, m, H-6), 1.89 (1H, m, H-4), 1.87 (1H, m, H-13), 1.76 (1H, m, H-4), 1.65 (2H, m, H-12, H-16), 1.38 (1H, m, H-3), 1.21 (1H, m, H-16), 1.19 (3H, s, H_3-21), 1.07 (3H, d, 6.5, H_3-20); ESIMS *m/z* 503 $(M+H)^+$; HRESIMS *m/z* 503.28919 (M+H; calcd for $C_{31}H_{39}N_2O_4$, 503.29098).

3.2.7. Chemical conversion of calyciphylline J (*3*) to calyciphylline C

To a MeOH solution (300 μL) of **3** (0.4 mg) was added two drops of trimethylsilyl diazomethane (2.0 M solution in diethyl ether), and stirring was continued at rt for 2 h. The mixture was concentrated in vacuo, and the residue was purified by a silica gel column ($CHCl_3$/MeOH, 1:0→0:1) to afford calyciphylline C (0.2 mg).

3.2.8. Calyciphylline K (*4*)

Colorless amorphous solid; $[\alpha]_D^{19}$ −38.1 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 2923, 1735, 1543, 1508, 1457, and 1169 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 360 $(M+H)^+$; HRESIMS *m/z* 360.2905 (M+H; calcd for $C_{23}H_{38}NO_2$, 360.2903).

3.2.9. Calyciphylline L (*5*)

Colorless amorphous solid; $[\alpha]_D^{22}$ −3.5 (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 2961, 1734, 1672, 1458, 1323, 1199, and 1130 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 358 $(M+H)^+$; HRESIMS *m/z* 358.2740 (M+H; calcd for $C_{23}H_{36}NO_2$, 358.2746).

3.2.10. Calyciphylline M (*6*)

Colorless amorphous solid; $[\alpha]_D^{17}$ +1.1 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3300, 2961, 1731, 1665, 1456, 1242, 1201, and 1136 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 530 $(M+H)^+$; HRESIMS *m/z* 530.3850 (M+H; calcd for $C_{32}H_{52}NO_5$, 530.3845).

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