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Calycinumines A and B, Two Novel Alkaloids from *Daphniphyllum calycinum*

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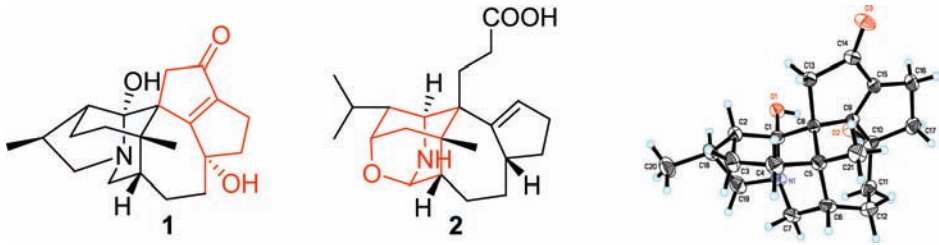
ABSTRACT



Two novel alkaloids, calycinumines A (1) and B (2), were isolated from the twigs of *Daphniphyllum calycinum*. Calycinumine A (1) is the first example of C-22-nor yuzurimine-type alkaloids, and calycinumine B (2) features an unprecedented heteroatom-containing adamantane-like western hemisphere of the alkaloid. Their structures were elucidated by spectroscopic methods, and that of 1 was confirmed by a single-crystal X-ray diffraction.

The genus of *Daphniphyllum* (Daphniphyllaceae) is a well-known source for biosynthesizing structurally diverse *Daphniphyllum* alkaloids with highly complex polycyclic skeletons. *Daphniphyllum* alkaloids have been challenging projects for natural products and synthetic chemistry for several decades.[1] Recently, quite a number of *Daphniphyllum* alkaloids have been isolated, some of which exhibited cytotoxic activity against tumor cell lines.[2] The genus of *Daphniphyllum* comprised of about 30 species is endemically distributed over southeast Asia. There are 10 species of this plant genus growing in southern China,[3] some of which, such as *D. calycinum*, *D. macropodum*, and *D. oldhami*, have been traditionally applied as folk medicine for the treatment of asthma,[4] cough, rheumatism, inflammation, fever, and snakebites.[5]

Previous studies on different parts of *D. calycinum* collected from several locations by us and the other research groups have resulted in the isolation of a series of

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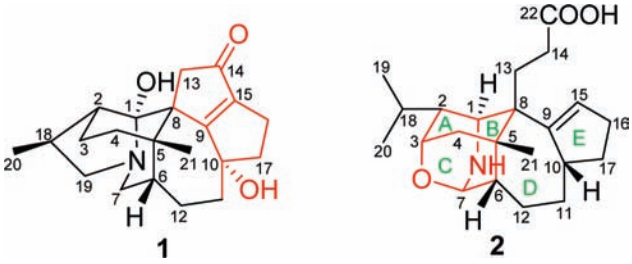
alkaloids,[1g,2a,c,4,6] and a few flavonoid glycosides.[7] In a continuation of our research for structurally unique *Daphniphyllum* alkaloids, calycinumine A (**1**), the first C-22-nor yuzurimine-type alkaloid, and calycinumine B (**2**) featuring an unprecedented heteroatom-containing adamantane-like western hemisphere of the alkaloid, were isolated from the ethanolic extract of *D. calycinum*. Their structures were elucidated by spectroscopic methods, especially two-dimensional NMR techniques, and the structure of **1** was also confirmed by the performance of a single-crystal X-ray diffraction. We present herein the isolation and structural elucidation of these two alkaloids.



The powdered dried twigs (3 kg) of *D. calycinum* were extracted with 95% ethanol at room temperature three times. After removal of the solvent under reduced pressure, the crude extract (600 g) was dissolved in 2 L of H_2O to form a suspension and adjusted with 2 N H_2SO_4 to pH $\approx$ 4. The acidic mixture was defatted with EtOAc (800 mL $\times$ 4), and the aqueous phase was basified with 30% Na_2CO_3 in water to pH $\approx$ 10 and extracted with $CHCl_3$ (500 mL $\times$ 5) to obtain 15.4 g of crude alkaloids. The crude alkaloids were then subjected to a silica gel column eluted with $CHCl_3/CH_3OH/Et_2NH$ (200:1:0.1 to 5:1:0.1) to give three major fractions 1−3. Fraction 1 (7.9 g) was chromatographed on a silica gel column eluted with petroleum/EtOAc/Et_2NH (from 8:1:0.1 to 4:1:0.1) to afford six fractions (F1a−F1f). Fraction F1c was chromatographed over a silica gel column eluted with $CHCl_3/CH_3OH/Et_2NH$ (100:1:0.1) to give a major component, which was then purified by a Sephadex LH-20 gel column eluted with CH_3OH to give **1** (7 mg). Fraction 3 (3.4 g) was subjected to a MCI gel column eluted with MeOH−H_2O (3:7 to 7:3) to give four subfractions (F3a−F3d). Fraction F3b was separated on a silica gel column eluted with $CHCl_3/CH_3OH$ (30:1) to give a major alkaloid, which was purified by a Sephadex LH-20 gel column eluted with CH_3OH to yield **2** (11 mg).

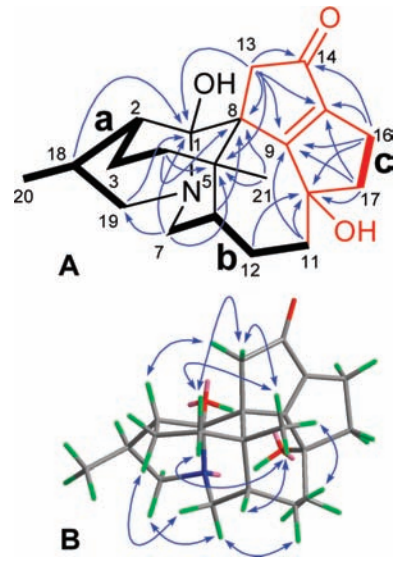


Figure 1. Key HMBC (**A**: →) and ROESY (**B**: ↔) correlations of **1**.

Calycinumine A (**1**),[8] obtained as a colorless crystal, possessed a molecular formula of $C_{21}H_{29}NO_3$ as determined by HREIMS at *m/z* 343.2150 $[M]^+$ (calcd 343.2147) with eight degrees of unsaturation. The UV absorption band at 242 nm (log ε 3.77) and IR absorptions at 3406, 3304, 1703, 1676, and 1628 cm^{-1} implied the presence of hydroxyl and α,β-unsaturated ketone groups. The ^{13}C NMR spectrum (Table 1) combining with DEPT experiments displayed 21 carbon resonances, including two methyls, nine methylenes (for those resonated at δ_C 64.2 and 61.6 being linked with the N-atom or O-atom), three methines, and seven quaternary carbons (two olefinics at δ_C 188.3 and 153.4, one ketone carbonyl at δ_C 207.0, and two oxygenated ones at δ_C 96.5, 83.7). The α,β-unsaturated ketone system accounted for two out of eight degrees of unsaturation, and the remaining six degrees of unsaturation required alkaloid **1** being hexacyclic. Three structural fragments **a** (C-2 to C-4 and C-18 to C-20), **b** (C-6 to C-7, and C-11 and C-12), and **c** (C-16 and C-17) as drawn with bold bonds were readily established by using a combination of 2D NMR spectra (HSQC, $^1H-^1H$ COSY, and HMBC). The linkages of components **a**−**c** with the quaternary carbons and heteroatoms were finally achieved by the HMBC experiment. A quaternary carbon at δ_C 96.5 was assigned to the C-1 of a N-atom containing semiketal, indicating that the N-atom and one hydroxyl were located at C-1. Two methylenes of CH_2-7 (δ_H 3.43, dd, J = 12.7, 7.4 Hz; δ_H 3.17, brd, J = 12.7 Hz) and CH_2-19 (δ_H 3.57, dd, J = 11.6, 9.7 Hz; δ_H 2.30, m) were attributed to those

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(8) White amorphous powder: $[\alpha]^{20}_D$ +18 (*c* 0.23, MeOH); mp 173−174 °C; UV (MeOH) λ_{max} (log ε) 242 (3.77) nm; IR (KBr) ν_{max} 3406, 3304, 2922, 2856, 1703, 1676, 1628, 1446, 1381, 1244, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 343 $[M]^+$ (5), 325 (100), 297 (64), 282 (27), 254 (12), 185 (30), 96 (16), 55 (17); HREIMS *m/z* 343.2150 $[M]^+$ (calcd for $C_{21}H_{29}NO_3$ 343.2147).

attached to the N-atom, suggesting that two partial structures **a** and **b** were connected via the N-atom. This was confirmed by the HMBC correlations of H_2-7/C-19, H_2-7/C-1, H_2-19/C-1, and H-18/C-1. The linkages of C-21, C-4, and C-6 to C-5 were fixed by the HMBC correlations from H_3-21, H_2-4, and H_2-7 to C-5. The attachment of C-1, C-5, and C-13 to C-8 was made by the HMBC correlations of H_3-21/C-8, H_2-4/C-8, H-6/C-8, H_2-13/C-1, H_2-13/C-5, and H_2-13/C-8. Two secondary carbons C-11 and C-17 were attached to the oxygenated quaternary carbon C-10 (δ_C 83.7) bearing a hydroxyl by the key HMBC correlations from H_2-11 and H_2-17 to C-10. The connection between C-15 and C-16 was established by the HMBC correlations from H_2-16 and H_2-17 to C-15. The linkages of C-8, C-10, and C-15 to C-9 were tentatively made by the HMBC correlations of H_2-13/C-9, H_2-13/C-15, H_2-11/C-9, H_2-16/C-9, and H_2-17/C-9. The quaternary carbon signal at δ_C 207.0 for a ketone carbonyl was assigned to C-14 by the HMBC correlations from H_2-13 and H_2-16 to C-14. The planar structure of **1** was thus outlined. The relative configuration of **1** as assigned by ROESY experiment (Figure 1) seems consistent with those of yuzurimine-type *Daphniphyllum* alkaloids except that the 10-OH was left unassigned by the available data.

The structure of **1** was finally confirmed by a single-crystal X-ray diffraction (Figure 2), which also fixed the 10α-OH, which is beyond the common expectation for a 10β-OH since all the yuzurimine-type alkaloids normally possess a 10β-H to satisfy the stereospecific requirement. Calycinumine A (**1**) is the first example of C-22-nor yuzurimine-type *Daphniphyllum* alkaloids.

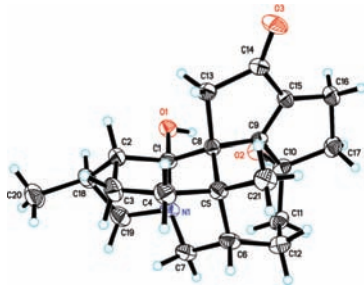

Figure 2. X-ray structure of **1**.

Calycinumine B (**2**),[9] obtained as a colorless oil, showed a molecular ion at *m*/*z* 359.2457 in HREIMS corresponding to the molecular formula of $C_{22}H_{33}NO_3$ (calcd 359.2460) requiring seven degrees of unsaturation. The ^{13}C NMR spectrum with the assistance of DEPT experiments (Table 1) resolved 22 carbon resonances comprising three methyls, seven methylenes, eight methines, (one olefinic at δ_C 125.2, one being linked with the N-atom at δ_C 61.1, and two oxygenated ones at δ_C 84.1 and 68.7), and four quaternary carbons (one olefinic at δ_C 155.4 and one carbonyl at δ_C 179.5). The carbonyl group and the double bond occupied two of the seven degrees of unsaturation, and the remaining five degrees of unsaturation required **2** being pentacyclic. Three structural fragments **a** (C-1 to C-4 and C-18 to C-20), **b** (C-6 and C-7, C-10 to C-12, and C-15 to C-17), and **c** (C-13 and C-14) were deduced as drawn in bold lines (Figure 3) by using a combination of 2D NMR spectra (HSQC, $^1H-^1H$ COSY, and HMBC). The overlapping proton signals gave rise to some uncertainty in establishing the structural fragments **a**−**c** only from the HSQC and $^1H-^1H$ COSY spectra, so the HMBC spectrum was further applied to secure the rightness. The linkages of the three structural fragments **a**−**c** with the quaternary carbons and heteroatoms were achieved by examination of HMBC spectrum (Figure 3). The quaternary carbon signal at δ_C 179.5 was allocated to C-22 by the strong correlations from H_2-13 and H_2-14 to C-22. The two methines of CH-1 (δ_H 2.79, brs) and CH-7 (δ_H 4.36, brs) were attributed to those linking to the N-atom on the basis of chemical shifts, indicating that two partial structures **a** and **b** were connected via the N-atom, which was confirmed by the mutual HMBCs of H-1/C-7 and H-7/C-1. The attachment of C-21, C-4, and C-6 to C-5 was established by the HMBC correlations from H_3-21, H_2-4, and H-7 to C-5, respectively. The linkages of C-10 and C-15 with C-9 were furnished by the HMBCs from H_2-11, H-15, H_2-16, and H_2-17 to C-9. The C-1 and C-13 were attached to C-8 by the key HMBCs from H-1 and H_2-13 to C-8, respectively. The crucial linkages of C-5 and C-9 to C-8 were made by the multiple HMBC correlations of H-1/C-5, H_2-13/C-5, H_3-21/C-8, H-6/C-8, H-15/C-8, H-1/C-9, and H_2-13/C-9. Two oxygenated methines at δ_C 68.7 (C-3) and at δ_C 84.1 (C-7)

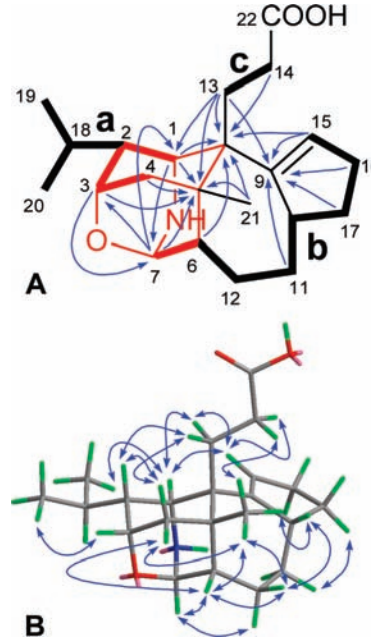

Figure 3. Key HMBC (**A**: →) and ROESY (**B**: ↔) correlations of **2**.

(9) Colorless oil: $[\alpha]^{20}_D$ −23.2 (*c* 0.32, MeOH); IR (KBr) ν_{max} 2956, 2850, 1714, 1624, 1558, 1468, 1387, 1261, 1088, 1024, 802, 754, 665 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 359 $[M]^+$ (55), 344 (12), 316 (11), 300 (15), 286 (31), 252 (29), 147 (40), 139 (54), 111 (100), 91 (34), 79 (25), 55 (21); HREIMS *m*/*z* 359.2457 $[M]^+$ (calcd for $C_{22}H_{33}NO_3$ 359.2460).

Table 1. ^{1}H and ^{13}C NMR Spectrosopic Data of **1** and **2**

	1[a]		**2**[b]	
position	δ_C	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)
1	96.5		61.1	2.79 (brs)
2	45.6	2.05 (m)	43.9	1.05 (m)
3a	23.6	1.79 (m)	68.7	4.08 (brt, 1.8)
3b		1.60 (m)		
4a	38.5	1.77 (m)	45.0	1.99 (m)
4b		1.66 (m)		1.76 (m)
5	41.8		37.8	
6	45.1	1.95 (m)	50.4	1.84 (m)
7a	61.6	3.43 (dd, 12.7, 7.4)	84.1	4.36 (brs)
7b		3.17 (brd, 12.7)		
8	56.7		47.2	
9	188.3		155.4	
10	83.7		48.5	2.89 (brs)
11a	37.6	2.29 (m)	33.9	1.94 (m)
11b		1.90 (m)		1.50 (m)
12a	30.1	1.80 (m)	28.5	1.84 (m)
12b		1.63 (m)		1.62 (m)
13a	49.0	2.86 (d, 18.1)	32.4	2.19 (m)
13b		2.75 (d, 18.1)		1.71 (m)
14a	207.0		32.3	2.45 (m)
14b				2.39 (m)
15	153.4		125.2	5.43 (brd, 1.4)
16a	45.7	2.38 (m, 2H)	30.0	2.35 (m)
16b				2.27 (m)
17a	23.3	2.47 (m)	32.4	2.19 (m)
17b		2.27 (m)		1.46 (m)
18	37.1	2.82 (m)	25.2	2.15 (m)
19a	64.2	3.57 (dd, 11.6, 9.7)	20.4	0.93 (d, 6.8, 3H)
19b		2.30 (m)		
20	15.3	1.09 (d, 7.4, 3H)	21.0	1.00 (d, 6.8, 3H)
21	24.0	0.84 (s, 3H)	25.6	1.08 (s, 3H)
22			179.5	

[a] Data were measured in CD_3OD at 400 MHz (^{1}H) and 100 MHz (^{13}C). [b] Data were measured in $CDCl_3$ at 400 MHz (^{1}H) and 100 MHz (^{13}C). Chemical shifts (δ) are in ppm being relative to TMS.

shared the remaining one oxygen atom to form an unprecedented 1,3-oxazinane ring as judged from the HMBC correlations of H-3/C-7 and H-7/C-3. The planar structure of **2** was thus elucidated.

The relative configuration of **2** was assigned by a performance of a ROESY experiment (Figure 3), in which the ROESY correlations from H_3-21 to H-6, H-10, H-12a, and H-13a indicated that they were cofacial and arbitrarily assigned in a β-configuration. The ROESY correlations of H-13a/H-2, H-13a/H-4a, H-2/H-4a, H-1/H-13b, H-1/H-15, H-6/H_3-21, and H-6/H-4b indicated that all the A, B, and C rings took the chair conformation to favor the stereospecific requirement of the heteroatom-containing adamantane-like western hemisphere of alkaloid **2**. The ROESY correlations of H_3-21/H-6, H_3-21/H-10, H_3-21/H-12a, and H-12a/H-10 suggested that the seven-membered D ring also took a chairlike conformation. The five-membered E ring was tentatively furnished in the envelope conformation.

Calycinumine B (**2**) features an unprecedented heteroatom-containing adamantane-like western hemisphere of the molecule.

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Supporting Information Available: Experimental procedures and physical and spectroscopic data of calycinumines A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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