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Calycilactone A, a novel hexacyclic alkaloid from *Daphniphyllum calycillum*

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Abstract—A novel *Daphniphyllum* alkaloid with a rearranged fused-hexacyclic ring system, calycilactone A, was isolated from the leaves of *Daphniphyllum calycillum* (Daphniphyllaceae), and the structure and relative stereochemistry of the new compound were elucidated on the basis of spectroscopic data.

Daphniphyllum alkaloids with unique nitrogen-containing polycyclic ring systems have attracted great attention from biogenetic studies as well as challenging targets for total synthesis.[1–3] They were revealed to be derived from six molecules of mevalonic acid via a squalene-like intermediate by radioactive tracer experiments.[4] Heathcock and co-workers have developed an elegant approach to synthesize of their basic structural framework with a breathtaking cascade of biomimetic steps.[3] Recently, many novel *Daphniphyllum* alkaloids have been isolated and identified, which may be explained to be derived from the basic skeleton, followed by unique biogenetic process involving repeated fission of C–C and/or C–N bonds and rearrangements, recyclization, and so on.[5–7]

In the course of our search for *Daphniphyllum* alkaloids with interesting ring system, calycilactone A (**1**), possessing a rearranged daphnilactone B skeleton, together with daphnilactone B (**2**),[8a] calycinine A,[8b] deoxycalyciphylline B,[8c] daphnezomine A,[8d] and yuzurimine A,[1b] were isolated from the leaves of *D. calycillum*. Herein we describe the isolation and structural elucidation of **1**.

Leaves of *D. calycillum*, collected in Dinghushan of Guangdong Province, China, were extracted with 95% EtOH, and the extract was partitioned between EtOAc and 0.001 N HCl. The aqueous layer was then alkalinized to pH 10 with 2 N NaOH followed by exhaustive extraction with $CHCl_3$. The $CHCl_3$-soluble materials were roughly separated by an amino silica gel column chromatography, using $CHCl_3$/MeOH (from 0:1 to 1:0) as eluent, to give 10 fractions. The fraction eluted with $CHCl_3$/MeOH (50:1) was purified by an RP-18 column chromatography (MeOH/H_2O, 5:5 → 1:0) followed by silica gel column chromatography ($CHCl_3$/CH_3COCH_3, 3:1) to afford calycilactone A (**1**, 0.0001%) as a yellowish oil.

Calycilactone A (**1**)[9] was isolated as optically active oil ($[\alpha]_D^{25}$ −128, *c* 0.62, $CHCl_3$) and analyzed for $C_{22}H_{29}NO_3$ by HRESIMS [*m*/*z* $[M+H]^+$ 356.2224; calcd 356.2226], indicating 10 degrees of unsaturation. IR spectrum of **1** showed the presence of ester and ketone carbonyl (1737 and 1706 cm^{-1}, respectively) groups. The ^{13}C NMR spectrum (Table 1) showed 22 carbon signals and DEPT experiments distinguished them as one methyl, five sp^3 methylenes, ten sp^3 methines, two sp^3 quaternary carbons, one trisubstituted olefin (δ_C 127.4 and 149.3), and two carbonyls (one isolated ketone at δ_C 216.2 and one ester at δ_C 175.6). Among them, two methylenes (δ_C 59.1, δ_H 3.54–3.50 and 2.21–2.15; δ_C 50.4, δ_H 2.27–2.20) and one methine (δ_C 71.7, δ_H 3.39) were ascribed to those bearing a nitrogen atom,

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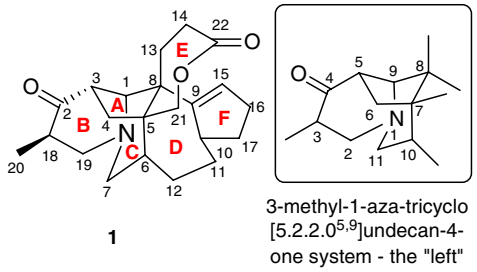
Table 1. ^{1}H [δ_H (J, Hz)] and ^{13}C [δ_C] NMR data of calycilactone A (**1**) in $CDCl_3$ at 300 K

No.	^{13}C (δ_C)	^{1}H (δ_H)	HMBC (H → C)
1	71.7	3.39 (1H, d, 5.0)	4, 5, 7, 8, 13, 19
2	216.2	—	—
3	46.2	2.59–2.53 (1H, m)	1, 2, 4, 5
4a	34.3	1.62–1.56 (1H, m)	2, 3, 6, 8, 21
4b		2.51–2.43 (1H, m)	1, 2, 3, 5, 6
5	47.6	—	—
6	44.1	1.66–1.63 (1H, m)	5, 8
7	50.4	2.27–2.20 (2H, m)	1, 5, 6, 12, 19
8	52.4	—	—
9	149.3	—	—
10	48.9	2.81–2.74 (1H, m)	—
11a	32.6	2.09–2.05 (1H, m)	9, 10, 12
11b		1.52–1.43 (1H, m)	9, 10, 12
12	30.7	1.43–1.36 (2H, m)	6, 10, 11
13	33.5	1.75–1.68 (2H, m)	1, 5, 8, 9, 14, 22
14a	29.6	2.59–2.47 (1H, m)	8, 13, 22
14b		2.42–2.36 (1H, m)	8, 13, 22
15	127.4	5.46 (1H, br s)	8, 9, 10, 16, 17
16	29.7	2.37–2.31 (1H, m)	9, 15
		2.13–2.07 (1H, m)	9, 10, 15
17	31.7	2.19–2.11 (2H, m)	9, 10, 11, 15, 16
18	38.5	2.39–2.29 (1H, m)	2, 19, 20
19a	59.1	3.54–3.50 (1H, m)	2, 6, 7, 18, 20
19b		2.21–2.15 (1H, m)	1, 7, 18, 20
20	14.0	0.94 (3H, d, 7.0)	2, 18, 19
21a	70.6	3.79 (1H, d, 13.5)	5, 6, 8, 22
21b		4.75 (1H, d, 13.5)	4, 5, 8, 22
22	175.6	—	—

while one methylene (δ_C 70.6, δ_H 4.77 and 3.82) was attributed to be connected with an oxygen atom. The above data indicated that **1** possessed hexacyclic structure.

By extensive comparison of ^{13}C NMR data of **1** with those of other *Daphniphyllum* alkaloids,[1,4–8] **1** showed some similarity with daphnilactone B (**2**), especially rings D-F, but one methylene in **2** was replaced by a ketone group in **1** and changes of some chemical shifts were also observed. So, 2D NMR experiments were required to determine the position of the ketone group.



Four structure fragments **a** (C-3 to C-4 and C-1), **b** (C-18 to C-19 and C-20), **c** (C-6 to C-7 and C-12, C-10 to C-12 and C-17, and C-15 to C-17), and **d** (C-13 to C-14), drawn with bold bonds, were established by analysis of 2D NMR data (^{1}H–^{1}H COSY, HMQC, and TOCSY) as shown in Figure 1. The connectivity of fragments (**a**–**d**), heteroatoms and quaternary carbons was furnished by HMBC experiment. In the HMBC spectrum (Table 1), cross-peaks for H-1 and H_2-19 to C-7 indicated that C-1, C-7, and C-19 all connected the nitrogen atom. The two-bond HMBC correlations from H-3 and H-18 to the only ketone group (δ_C 216.2) allowed to locate the ketone at C-2, which was further confirmed by long range correlations (J^3) from H_2-4, H_2-19, and H_3-20 to C-2. The connectivities of C-21 to C-4 (δ_C 34.3) and C-6 (δ_C 44.1) via C-5 (δ_C 47.6) were implied by correlations from H-21a to C-5 and C-6, and H-21b to C-4, and from H-4 and H-6 to C-5. The HMBC correlations from H-1 and H_2-13 to C-8 (δ_C 52.4) suggested that C-1 and C-13 (δ_C 33.5) were connected through C-8. The HMBC cross-peaks from H_2-13 to C-5 and C-9 (δ_C 149.3) indicated the connectivity of C-8 to C-5 and C-9. All the above mentioned correlation data suggested that the 'left' structural moiety of **1** should be a 3-methyl-1-aza-tricyclo[5.2.2.2^{5,9}]undecan-4-one ring system. In addition, the HMBC correlations of H_2-14 to C-22 (δ_C 175.6), H_2-21 to C-8 and C-22, and of H-15 to C-9 and C-10 (δ_C 48.9) revealed the presence of seven-membered lactone ring and cyclopentenyl moiety. Thus, the gross structure of **1**, possessing an unprecedented rearranged daphnilactone B skeleton, was unambiguously established as shown in Figure 1.

The relative stereochemistry of **1** was constructed by analysis of key correlations observed in the ROESY NMR spectrum (Fig. 2). The NOESY correlations of H-21b/H-10, and H-21a/H-6 indicated C-21, H-6, and H-10 were all on the same side toward β-face. Correlations of H-1 with H_2-13 and H-15 indicated H-1 took a β-equatorial conformation, which was further supported by a calculated interatomic distance of 2.3 Å and 2.5 Å, respectively, from the molecular model.

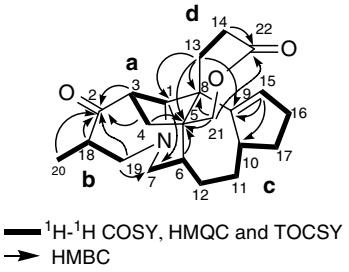


Figure 1. Selected 2D-NMR correlations for **1**.

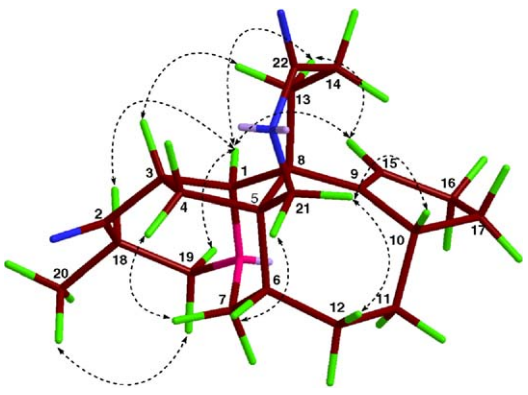
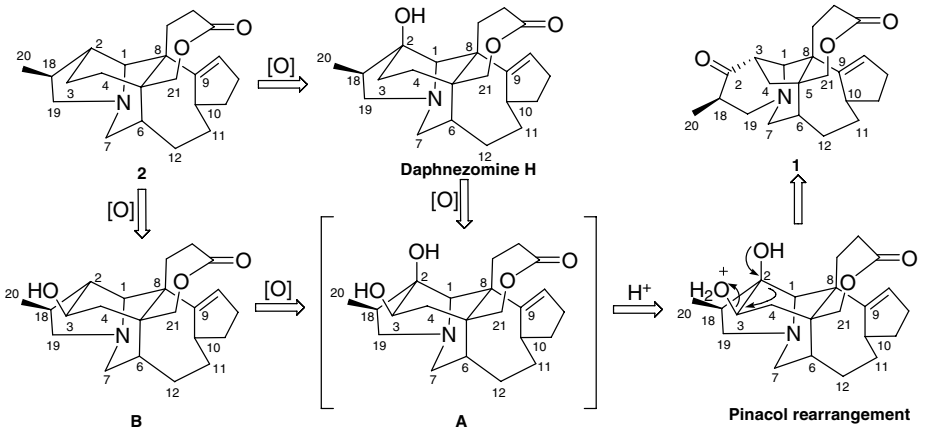


Figure 2. Key NOESY correlations and conformation generated from computer modeling of **1**.



Scheme 1. Biogenetic pathway proposed for **1**.

The additional strong correlation between H-3 and H_2-13 revealed that H-3 also took β-axial configuration. In addition, H-18 taking α-orientation was supported by the correlations of H-18/H-1 and H_3-20/H-19b. The inside direction of nitrogen lone pair was supported by the correlation between H-4a and H-19b.

A plausible biogenetic pathway for calycilactone A (**1**) was proposed as shown in Scheme 1. Calycilactone A (**1**) might be originated from daphnilactone B (**2**), due to their similar structural moiety. The latter was converted to the key intermediate **A** via daphnezomine H[8a] or intermediate **B** by oxidation, followed by Pinacol rearrangement to transform to **1**.

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9. Yellowish oil; $[\alpha]_D^{25}$ −128 (*c* 0.62 $CHCl_3$); IR (KBr) ν_{max} 2928, 1737, 1706 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; ESIMS *m*/*z* $[M+H]^+$ 356; HRESIMS *m*/*z* 356.2224 (calcd for $C_{22}H_{30}NO_3$, $[M+H]^+$ 356.2225).