A novel alkaloid from the seeds of *Daphniphyllum calycinum*

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One novel alkaloid, named as caldaphnidine G (**1**), and two known alkaloid calycicine A (**2**), daphnezomine L (**3**) were isolated from the seeds of *Daphniphyllum calycinum*. The structure of **1** was established by spectral methods, especially 2D NMR techniques.

Keywords: *Daphniphyllum calycinum*, alkaloids, Chinese medicine

Plants of the genus *Daphniphyllum* are known to produce structurally diverse *Daphniphyllum* alkaloids which are biosynthesised from six molecules of mevalonic acid via a squalene-like intermediate. The alkaloid possess a highly complex polycyclic, fused heterocyclic skeleton. More than 130 *Daphniphyllum* alkaloids have been reported from this genus.[1,2] Because of their unusual structures, the *Daphniphyllum* alkaloids have drawn the attention of synthetic groups.[3-7]

D. calycinum Benth.(Daphniphyllaceae), an evergreen shrub, is native to the south of China. Its leaves and seeds are used as traditional Chinese medicine for several indications, such as antipyretic, anti-inflammatory and influenza. Previous studies on the constituents of this species resulted in the isolation of a number of *Daphniphyllum* alkaloids,[1,2,8-12] and a few antioxidant flavonoid glycosides.[13] In our continuing search for the structurally unique and biogenetically interesting *Daphniphyllum* alkaloids,[14,15] one novel and two known alkaloids were isolated from the seeds of *D. calycinum*. We report herein the isolation and structural determination of these compounds from *D. calycinum*.

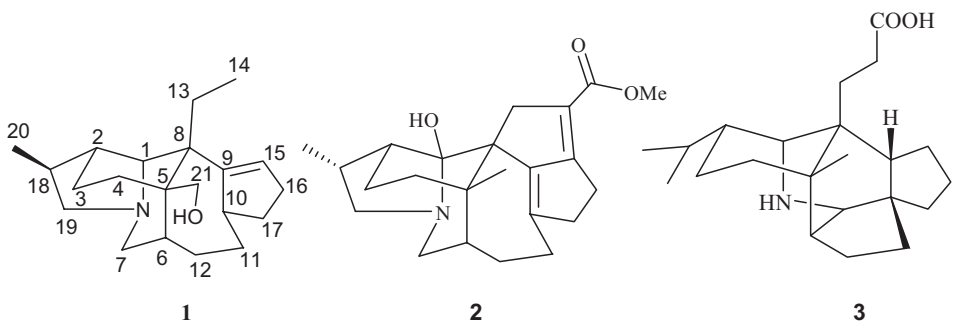
Caldaphnidine G (**1**) was obtained as an amorphous powder. It showed a pseudomolecular ion peak at *m/z* 338 $[M + Na]^+$ in the ESIMS. The molecular formula, $C_{21}H_{33}NO$, was established by HR-ESIMS [*m/z* 338.2455; calcd 338.2460], implying the existence of six degrees of unsaturation. A strong IR absorption band at 3350 cm^{-1} was attributed to the presence of one OH group. Twenty-one carbon signals comprising three quaternary carbons, six methines, 10 methylenes, and two methyls were evident from its ^{13}C NMR and DEPT spectra. The following functionalities functional groups were identified in one trisubstituted double bond (δ_C 151.5, C-9; 131.7, C-15), an oxygenated methylene (δ_C 67.1, C-21), one nitrogenated methine carbons (δ_C 74.0, C-1), and two nitrogenated methylene (δ_C 65.4, C-19; 59.0, C-7). The ^1H NMR spectrum (Table 1) of **1** displayed proton signals for two methyls at δ 1.13 (3H, d, 7.0 Hz), 1.35 (3H, t, J = 7.5 Hz), and a hydroxymethyl (δ 4.27 1H, d, J = 10.5 Hz; δ 3.61 1H, d, J = 10.5 Hz). Combined with the ^{13}C NMR spectrum, the signal at δ 5.88 (brs) was defined as proton signal of H-15, unambiguously. Besides the one degree of unsaturation occupied by the double bond, the remaining five degrees of unsaturation were accounted for in a pentacyclic ring system in **1**.

Table 1 ^1H and ^{13}C NMR data of 1

No.	**1** (δ_H)	**1** (δ_C)
1	3.88 (1H, d, 4.0)	74.0
2	2.56 (1H, m)	37.4
3	1.81 (2H, m)	20.9
4	2.12 (1H, m) 1.58 (1H, m)	34.1
5	–	42.2
6	2.50 (1H, m)	39.5
7	3.58 (1H, dd, 14.5, 6.5) 3.50 (1H, d, 14.5)	59.3
8	–	42.5
9	–	151.5
10	3.0 (1H, m)	49.0
11	1.80 (1H, m) 1.55 (1H, m)	33.6
12	1.48 (1H, m) 2.02 (1H, m)	33.5
13	1.82 (1H, m) 1.89 (1H, m)	31.5
14	1.35 (3H, t, 7.5)	23.6
15	5.88 (1H, brs)	131.7
16	2.41 (1H, m) 2.20 (1H, m)	30.6
17	1.68 (1H, m) 2.16 (1H, m)	33.9
18	2.70 (1H, m)	38.4
19	a: 4.02 (1H, t, 11.5) b: 2.85 (1H, dd, 11.3, 8.5)	65.4
20	1.13 (3H, d, 7.0)	13.5
21	4.27 (1H, d, 10.5) 3.61 (1H, d, 10.5)	67.1

[a]Measured in CD_3OD at 500 MHz.

After linking all the protons with their directly bonded carbon partners via a HMQC measurement, it was possible from the HMBC spectrum to deduce the planar structure of **1**. The chemical shifts of the CH-1 methine (δc 74.0; δ_H 3.88), CH_2-7 methylene (δc 59.3; δ_H 3.58 and 3.50) and CH_2-19 methylene (δc 65.8; δ_H 4.02 and 2.85) indicated the connectivity of C-1, C-7 and C-19 via the nitrogen atom. This was confirmed by the HMBC correlations between H-1 and C-7, and between H_2-19 and C-7. In the HMBC, the C-8 (δc 42.5) correlating with the H-1 (δ 3.88) and H_2-13 (δ 1.89 and 1.82) indicated that the linkage of C-1 and C-13 via C-8; the C-4, C-6 and C-21 were attached to the C-5 as judged by the



1 **2** **3**

Fig. 1 Structure of compounds **1**, **2** and **3**.

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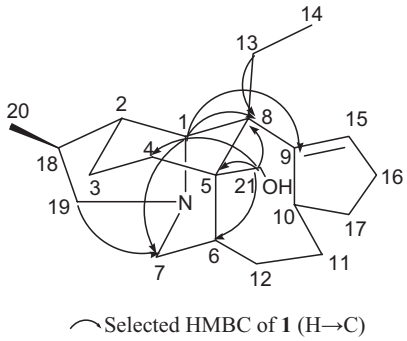

Fig. 2 Selected 2D NMR correlations of **1**.

strong HMBC correlation pairs of H-4/C-5, H-6/C-5 and H_2-21/C-5, respectively. The linkage of C-5 and C-8 could be tentatively established by the HMBC correlations of H_2-21/C-5 and H_2-21/C-8. Two quaternary carbons C-8 and C-9 could also be connected by the HMBC correlations of H-1/C-8, H-1/C-9; The planar structure of **1** was thus established.

The ^{1}H and ^{13}C NMR data of **1** exhibited similarity with those of caldaphinidine C,[15] except for the loss of carboxyl and the presence of an additional hydroxyl at C-21. These data indicated that compound **1** was an analogue of caldaphinidine C, and had the same relative stereochemistry with caldaphinidine C, which was confirmed by the NOESY spectrum. In the NOESY spectrum, the proton signal of H-21 showed correlations with the signals of H-6 and H-10, indicating that H-6, H-10, and H-21 were on the same side of the molecule and given a *β*-orientation; the correlation pairs of H-1/H-2, and H-2/H-18 indicated that the H-1, H-2, and H-18 were in the α-configuration, and as a consequence, H_3-20 was put in placed a β-orientation. The ^{1}H, ^{13}C NMR spectral data and 2D NMR experiments support the assignment of structure **1** to the new compound which is named caldaphnidine G. It may arise from decarboxylation and hydroxylation of methyl at C-21 of caldaphinidine C.

Compounds **2** and **3** were identified as calycicine A, as daphnezomine L by comparison NMR data with literature data,[16,17] respectively.

Experimental

Optical rotations were determined on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet 6700 spectrometer with KBr disks. NMR spectra were measured on a Bruker Avance-500 spectrometer with TMS as internal standard. ESIMS was recorded on a Finnigan LCQ^{DECA} Mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China). Silica gel (200–300 mesh) was used for column chromatography, and a precoated silica gel GF_{254} plate (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) was used for TLC. Amino silica gel (NH-DM 1020, 20–45 μm, Fuji Silysia Chemical Ltd.) was used for column chromatography.

Plant material. The seeds of *Daphniphyllum calycinum* were collected from GuangXi Province of P. R. China and identified by Lan Tang of the Zhejiang University of Technology. A voucher specimen (ZJUT 07616) was deposited at Zhejiang University of Technology, P.R. of China.

Extraction and isolation

The dry seeds (600 g) of *D. calycinum* were ground and percolated with 95% ethanol. After removal of the ethanol under reduced pressure, the crude extract was adjusted with 0.5 N H_2SO_4 to pH≈5. The acidic mixture was extracted with ethyl acetate (6 × 300 ml) to remove the non-alkaloid components. The aq. phase was brought to pH≈10 by addition of 1N Na_2CO_3 and partitioned with chloroform (6 × 300 ml) to give the crude alkaloids (1.3 g). The crude alkaloids were then subjected to a silica gel column (2.5 × 45 cm) eluted with $CHCl_3$/MeOH (40 : 1–10 : 1) to collect two major fractions 1 and 2. Fraction 1 (130 mg) was separated by column chromatography packed with silica gel and eluted with $CHCl_3$/MeOH (10:1) to yield alkaloid **1** (14 mg) and **3** (12 mg). Fraction 2 (80 mg) was also chromatographied (Amino silica gel; cyclohexane/EtOAc 2:1) to afford alkaloid **2** (26 mg).

Caldaphnidine G **(1)**, amorphous powder, $[\alpha]^{20}_{D}$ −7.3° (*c* 0.82, CH_3OH); IR (KBr): 3350, 2973, 2824, 1655, 1466, 1392, 1159, 1048, 805 cm^{-1}; ESIMS *m/z*: 338 $[M + Na]^+$; HR-ESIMS *m/z*: 338.2455 $[M + Na]^+$ (Calcd. for $C_{21}H_{33}NaNO$ 338.2460). ^{1}H NMR and ^{13}C NMR data: see Table 1.

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