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Daphenylline, a New Alkaloid with an Unusual Skeleton, from *Daphniphyllum longeracemosum*

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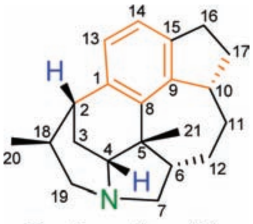
ABSTRACT



Daphenylline (**1**)

A new alkaloid, daphenylline (1), with an unprecedented rearranged 22-nor-calyciphylline skeleton, was isolated from the fruits of *Daphniphyllum longeracemosum*. Its structure and stereochemistry were elucidated on the basis of spectroscopic and computational approaches. A plausible biosynthetic pathway of 1 was also proposed.

Daphniphyllum alkaloids are a family of natural products with complex and diversified structures elaborated by trees of the genus *Daphniphyllum*.[1] In recent years, many new *Daphniphyllum* alkaloids have been discovered from this genus, some of which possessed new carbon skeletons.[2] Those fused-heterocyclic systems have attracted great interest as challenging targets for total synthesis[3] as well as biosynthesis.[4] The previous work carried out by our group on the alkaloids of the genus *Daphniphyllum* led to a series of novel alkaloids with highly complex polycyclic systems.[5] In a continuing search for structurally unique and biogenetically interesting alkaloids, the chemical constituents in *Daphniphyllum longeracemosum* Rosenth have been further in-

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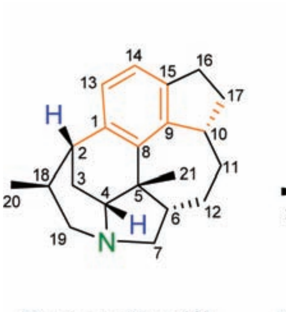
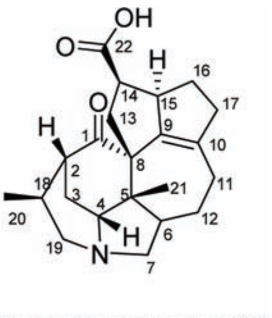
vestigated.[6] A novel alkaloid, named daphenylline (**1**), was isolated from the fruits and possessed an expanded neohexatomic ring with C-13 connected to C-1 instead of C-8 as usual. In this paper, the isolation and structure elucidation of **1** are described.



Daphenylline (**1**)



Daphnilongeranin C (**2**)

The fruits (60 kg) of *D. longeracemosum* were extracted with 95% EtOH, and the crude extract was adjusted to pH 2 with 2% HCl. After extraction with petroleum ether and chloroform, the aqueous layer was then basified to pH 10 with 3% NaOH, followed by exhaustive extraction with chloroform and *n*-BuOH. The *n*-BuOH fraction was separated on silica gel eluted by acetone, methanol, and methanol/diethylamine (20:1) separately to afford three parts, the third of which was further chromatographed over a series of ion-exchange resin, C_{18} silica gel, and silica gel to yield daphenylline (**1**, 44 mg, 0.000073%) and a known calyciphylline A-type alkaloid daphnilongeranine C (**2**, 1.5 g, 0.0025%).[6c]

Daphenylline (**1**),[7] a colorless oil, showed the pseudomolecular ion peak at *m/z* 294 $[M + H]^+$ in the FABMS and ESIMS, and the molecular formula of **1**, $C_{21}H_{27}N$, was established by HRESIMS [*m/z* 294.2219, $(M + H)^+$, calcd. 294.2221] requiring nine degrees of unsaturation. ^{13}C NMR and DEPT revealed 21 carbon signals due to four sp^2 quaternary carbons, two sp^2 methines, one sp^3 quaternary carbon, five sp^3 methines, seven sp^3 methylenes, and two methyl groups. Among them, two methylenes (δ_C 58.0 and δ_C 50.0) and one methine (δ_C 65.4) were suggested to be attached to a nitrogen atom. Furthermore, the six sp^2 carbon signals mentioned above, the ^1H NMR doublet peaks at δ_H 7.05 and 6.87 with coupling constant 7.6 Hz, and the characteristic IR absorptions at 1639 and 1563 cm^{-1} implied the presence of a 1,2,3,4-tetrasubstituted benzene ring moiety in the structure. Apart from three degrees of unsaturation occupied by three double bonds of the benzene ring, the remaining six degrees of unsaturation indicated that **1** should possess a hexacyclic system.

Table 1. ^1H, ^{13}C, and DEPT NMR Data of Daphenylline (**1**) in $CDCl_3$

	δ_H (mult. Hz)	δ_C
1		143.8 (s)
2	2.75 (1H, m)	36.5 (d)
3α	2.37 (1H, m)	17.8 (t)
3β	1.95 (1H, m)	
4	3.73 (1H, m)	65.4 (d)
5		45.4 (s)
6	2.45 (1H, m)	47.2 (d)
7α	3.74 (1H, m)	58.0 (t)
7β	2.46 (1H, m)	
8		132.7 (s)
9		144.6 (s)
10	3.43 (1H, m)	42.9 (d)
11α	2.04 (1H, m)	28.3 (t)
11β	1.57 (1H, m)	
12α	1.86 (1H, m)	27.7 (t)
12β	1.30 (1H, m)	
13	6.87 (1H, d, 7.6 Hz)	127.0 (d)
14	7.05 (1H, d, 7.6 Hz)	124.1 (d)
15		137.4 (s)
16α	2.76 (1H, m)	31.0 (t)
16β	2.68 (1H, m)	
17α	1.60 (1H, m)	36.0 (t)
17β	2.33 (1H, m)	
18	1.87 (1H, m)	32.8 (d)
19α	2.71 (1H, m)	50.0 (t)
19β	2.97 (1H, dd, 13.2, 6.0)	
20	1.26 (1H, d, 7.2)	18.0 (q)
21	1.45 (1H, s)	26.0 (q)

The $^1H-^1H$ COSY revealed that **1** possessed three 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, C-10 to C-12 and C-17, and C-16 to C-17), and **c** (C-13 to C-14), as shown in Figure 1. Further detailed HMBC studies established the connections among the three fragments, the quaternary carbons, and a nitrogen atom. HMBC correlations of H-4 to C-19 (δ_C 50.1), H-19 to C-7 (δ_C 58.1), and H-7 to C-4 (δ_C 65.5) confirmed that C-4, C-7, and C-19 were linked at the nitrogen atom. Connectivity of C-21 to C-4, C-6, and C-8 through C-5 was indicated by HMBC correlations of H-4 to C-8 and C-21, H-21 to C-5, C-6, and C-8. The presence of a tetrasubstituted benzene ring, composed of C-1, C-8, C-9, C-13, C-14, and C-15, was suggested by HMBC cross-peaks for H-13 to C-8, C-9 (J^4) and C-15, and H-14 to C-1. Furthermore, HMBC correlations of H-17 to C-9 and H-16 to C-14 indicated the connectivity of fragment **b** and the benzene ring through bonds C-15/C-16 and C-9/C-10. Meanwhile, the fragment **a** and the benzene ring were connected through bond C-1/C-2, which was supported by the HMBC correlations of H-3 to C-1. Thus, the gross structure of daphenylline was assigned as **1** with an unusual fused-

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(7) **Daphenylline**: colorless oiliness; $[\alpha]_D^{16} = -45.6$ (*c* 0.19, MeOH); UV (MeOH) λ_{max} nm 204.4; IR (KBr) ν_{max}: 2922, 2848, 2388, 2283, 1639, 1563, 1458, 1412, 1383, 1292, 1035, 846 and 799 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); FABMS and ESIMS *m/z* 294 $[M + H]^+$; HRESIMS *m/z* 294.2219 (calcd for $[M + H]^+$ 294.2221).

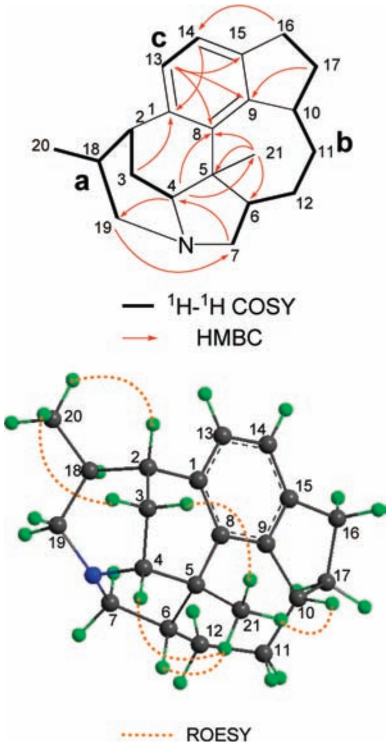


Figure 1. Selected two-dimensional NMR correlations of daphenylline (**1**).

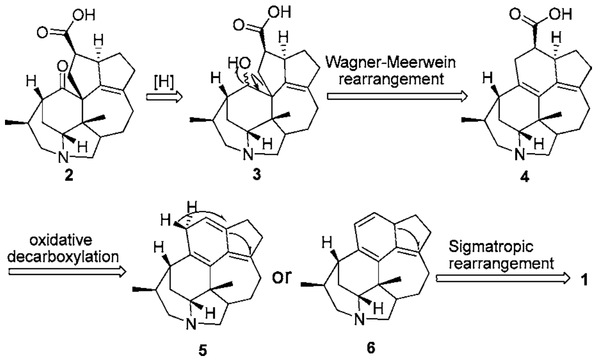
hexacyclic ring system (two five-, three six-, and one seven-membered rings).

The relative stereochemistry of **1** was fixed by ROESY as shown in Figure 1. The ROESY interaction pairs of H_3-21/H-4, H_3-21/H-6, and H_3-21/H-10 indicated their β-oriented configuration. Interaction pairs of H-2/H_3-20, H_3-20/H_α-3, and H_β-3/H_3-21 suggested both H-2 and H_3C-20 were β-oriental.

To assign the absolute configuration, the optical rotation (OR) values of **1** and its enantiomer were calculated by the density functional theory (DFT) methods[8] in the Gaussian 03 program package.[9] The minimum geometries of **1** and its enantiomer were optimized by B3LYP/6-311G(d, p). The OR values were calculated by B3LYP/6-311G+(d, p) under the Self-Consistent Reaction Field model of solvation: for **1** it was −64.3, and for its enantiomer was +64.3. The former was close to the experimental value of −45.6, which indicated the absolute configuration of daphenylline as given in structure **1**. This conclusion was also in accord with the previously hypothetical stereochemistry of the calyciphylline A-type alkaloids based on their biogenetic pathways.[10]

A plausible biogenetic pathway for **1** was proposed as shown in Scheme 1. The biogenetic origin of **1** should be calyciphylline A-type alkaloids such as **2** which was also acquired from the title plant in quantity. Alkaloid **2** might be reduced and dehydrated to form a ring expanded intermediate **4** via the Wagner−Meerwein rearrangement. Then the intermediate **4** could be involved in the elimination of the carboxyl group at C-21 by decarboxylase to generate **5** or **6** with a double bond between C-13 and C-14 or between C-14 and C-15, followed by one or two steps of syn-[1,3] sigmatropic rearrangement to yield **1**.

Scheme 1. Plausible Biosynthetic Pathway of **1**



Daphenylline (**1**) was evaluated for cytotoxic activities against the human tumor cell lines (HL-60, SMMC-7721, A-549, SK-BR-3). The results indicated that **1** was inactive against the above cancer cells (IC_{50} >40 μM).[11]

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Supporting Information Available: Experimental section, optical rotation calculation, one- and two-dimensional NMR spectra, and mass spectrum for daphenylline **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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[11] DDP was used as the positive control compound, and the IC_{50} values against the cell lines (HL-60, SMMC-7721, A-549, and SK-BR-3) were 3.99, 11.65, 25.67, and 13.70 μg/mL, respectively.