

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

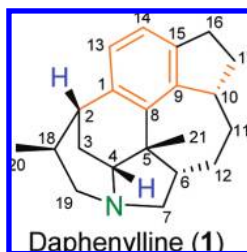
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ABSTRACT



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 synthesis³ 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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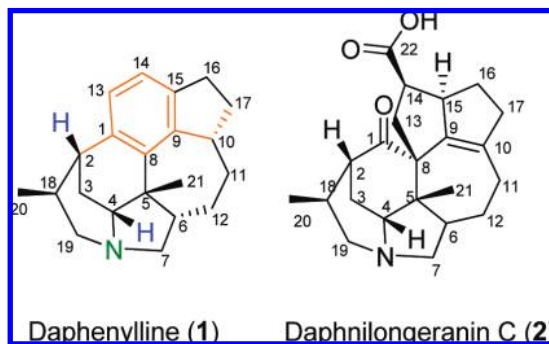
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vestigated.⁶ A novel alkaloid, named daphenylline (**1**), was isolated from the fruits and possessed an expanded neo-hexameric ring with C-13 connected to C-1 instead of C-8 as usual. In this paper, the isolation and structure elucidation of **1** are described.



The fruits (60 kg) of *D. longracemosum* 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, C₁₈ silica gel, and silica gel to yield daphenylline (**1**, 44 mg, 0.00073%) and a known calyciphylline A-type alkaloid daphnilongeranin 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**, C₂₁H₂₇N, was established by HRESIMS $[m/z$ 294.2219, $(M + H)^+$, calcd. 294.2221] requiring nine degrees of unsaturation. ¹³C NMR and DEPT revealed 21 carbon signals due to four sp² quaternary carbons, two sp² methines, one sp³ quaternary carbon, five sp³

Table 1. ¹H, ¹³C, and DEPT NMR Data of Daphenylline (**1**) in CDCl₃

	δ_H (mult. Hz)	δ_C
1		143.8 (s)
2	2.75 (1H, m)	36.5 (d)
3 α	2.37 (1H, m)	17.8 (t)
3 β	1.95 (1H, m)	
4	3.73 (1H, m)	65.4 (d)
5		45.4 (s)
6	2.45 (1H, m)	47.2 (d)
7 α	3.74 (1H, m)	58.0 (t)
7 β	2.46 (1H, m)	
8		132.7 (s)
9		144.6 (s)
10	3.43 (1H, m)	42.9 (d)
11 α	2.04 (1H, m)	28.3 (t)
11 β	1.57 (1H, m)	
12 α	1.86 (1H, m)	27.7 (t)
12 β	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)
16 α	2.76 (1H, m)	31.0 (t)
16 β	2.68 (1H, m)	
17 α	1.60 (1H, m)	36.0 (t)
17 β	2.33 (1H, m)	
18	1.87 (1H, m)	32.8 (d)
19 α	2.71 (1H, m)	50.0 (t)
19 β	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)

methines, seven sp³ 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 sp² 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.

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 ¹³C NMR data (Table 1); FABMS and ESIMS m/z 294 $[M + H]^+$; HRESIMS m/z 294.2219 (calcd for $[M + H]^+$ 294.2221).

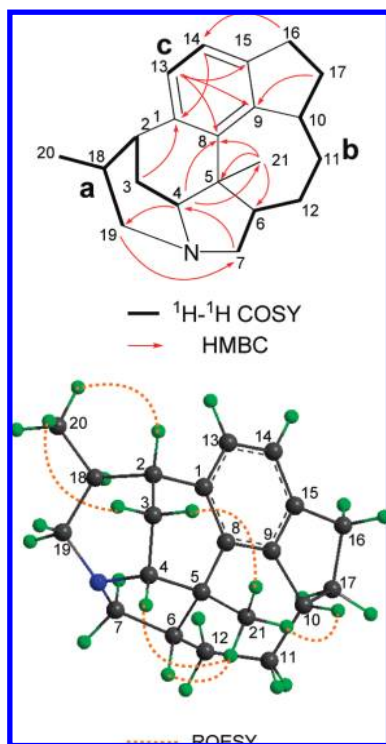


Figure 1. Selected two-dimensional NMR correlations of daphenylline (**1**).

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

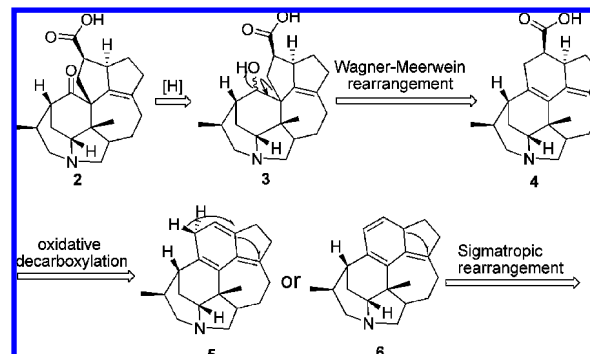
The relative stereochemistry of **1** was fixed by ROESY as shown in Figure 1. The ROESY interaction pairs of H₃-21/H-4, H₃-21/H-6, and H₃-21/H-10 indicated their β -oriented configuration. Interaction pairs of H-2/H₃-20, H₃-20/H α -3, and H β -3/H₃-21 suggested both H-2 and H₃C-20 were β -oriental.

To assign the absolute configuration, the optical rotation (OR) values of **1** and its enantiomer were calculated by the density functional theory (DFT) methods⁸ 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

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



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**.

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 ($IC_{50} > 40 \mu M$).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 $\mu g/mL$, respectively.

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