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Paxiphyllines A and B, new alkaloids from *Daphniphyllum paxianum*

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Abstract—Two new *Daphniphyllum* alkaloids with daphnicyclidin-type skeleton, paxiphyllines A (**1**) and B (**2**) were isolated from the twigs and the leaves of *Daphniphyllum paxianum*. Paxiphylline A (**1**) has an unprecedented additional carbon from a biogenetic perspective. The structures and relative configurations were elucidated on the basis of spectroscopic data.

Daphniphyllum alkaloids, with highly complex polycyclic systems, have been the attractive targets for total synthesis as well as biosynthetic studies.[1–8] *Daphniphyllum paxianum* Rosenth. is an evergreen tree mainly distributed in southern China.[9] Several new *Daphniphyllum* alkaloids have been isolated from *Daphniphyllum paxianum* by Yue and co-workers.[4a,c,d] In our further search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids,[2] two new daphnicyclidin-type alkaloids, paxiphyllines A (**1**) and B (**2**) were isolated from the twigs and the leaves of *Daphniphyllum paxianum*. Paxiphylline A (**1**) was a novel C-23 *Daphniphyllum* alkaloid with an unprecedented additional carbon, which was biogenetically different from those reported.[2c,3c,8d] In this Letter we describe the isolation and structure elucidation of **1** and **2**.

The twigs and the leaves of *D. paxianum* were extracted with 95% EtOH, and the crude extract was adjusted with saturated tartaric acid to pH ~ 2. The acidic mixture was defatted with petroleum ether (PE), and then extracted with $CHCl_3$. The aqueous phase was basified to pH ~ 10 with saturated Na_2CO_3 and extracted with $CHCl_3$ to obtain crude alkaloids. The crude alkaloids were then subjected to a silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with $CHCl_3$/MeOH (20:1) was chromatographed over a series of silica gel column ($CHCl_3$/acetone and $CHCl_3$/MeOH) to afford paxiphyllines A (**1**, 9.6 mg) and B (**2**, 11.2 mg).

Paxiphylline A (**1**)[10] was obtained as an optically active light yellow solid, $[\alpha]_D^{23}$ −221.7 (*c* 0.40, CH_3OH). The ESIMS spectrum showed the pseudomolecular ion $[M+H]^+$ at *m*/*z* 394, and the molecular formula, $C_{24}H_{27}NO_4$, was established by HRESIMS (*m*/*z* 394.2012, $[M+H]^+$; calcd: 394.2018), indicating twelve degrees of unsaturation. IR absorptions implied the presence of two conjugated carbonyl (1718 cm^{-1}, 1629 cm^{-1}) functionalities. ^{13}C and DEPT spectroscopy (Table 1) revealed 24 carbon signals due to ten sp^2 carbon atoms at low field and fourteen sp^3 carbon atoms ($1\times C$, $4\times CH$, $6\times CH_2$, $2\times CH_3$ and $1\times OCH_3$). In addition, the ten sp^2 carbon atoms were attributable to two carbonyls, three tetrasubstituted double bonds and one exocyclic double bond. Besides six degrees of unsaturation belonging to two carbonyl groups and four double bonds, respectively, six degrees of unsaturation were due to six rings in the molecule.

Keywords: *Daphniphyllum paxianum*; Alkaloids; Paxiphylline.
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Table 1. ^{1}H and ^{13}C NMR data of paxiphyllines A (**1**) and B (**2**) in $CDCl_3$

No.	Paxiphylline A (**1**)		Paxiphylline B (**2**)	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1	—	199.9 s	—	211.4 s
2	2.55 (1H, m)	49.1 d	—	76.2 s
3a	2.30 (1H, m)	18.3 t	2.28 (2H, d, 6.4)	30.7 t
3b	2.14 (1H, m)			
4	3.04 (1H, m)	66.8 d	3.57 (1H, m)	66.3 d
5	—	52.1 s	—	45.5 s
6	2.37 (1H, m)	51.0 d	2.58 (1H, m)	45.3 d
7α	2.36 (1H, m)	60.0 t	3.12 (1H, m)	59.2 t
7β	3.49 (1H, m)		2.34 (1H, m)	
8	—	142.4 s	—	125.1 s
9	—	122.7 s	—	122.1 s
10	—	168.8 s	—	165.2 s
11	—	139.7 s	3.59 (1H, m)	29.9 t
			2.94 (1H, m)	
12α	2.19 (1H, m)	41.1 t	2.56 (1H, m)	27.5 t
12β	2.82 (1H, m)		1.67 (1H, m)	
13	—	134.8 s	—	141.9 s
14	—	124.4 s	—	108.6 s
15	—	129.8 s	—	132.1 s
16a	3.01 (1H, m)	23.2 t	7.69 (1H, br s)	110.8 d
16b	2.83 (1H, m)			
17a	4.67 (1H, dd, 4.8, 10.4)	68.8 t	7.69 (1H, br s)	143.4 d
17b	4.14 (1H, ddd, 3.6, 10.4, 18.4)			
18	2.27 (1H, m)	30.2 d	2.71 (1H, m)	35.9 d
19α	2.98 (1H, m)	53.2 t	3.11 (1H, m)	51.9 t
19β	2.53 (1H, br)		2.62 (1H, m)	
20	1.24 (3H, d, 4.5)	18.5 q	0.87 (3H, d, 6.8)	13.6 q
21	1.22 (3H, s)	33.4 q	1.45 (3H, s)	28.2 q
22	—	167.4 s	—	166.6 s
23	3.81 (3H, s)	51.7 q	3.84 (3H, s)	51.2 q
24a	5.95 (1H, s)	123.5 t		
24b	5.63 (1H, s)			

[a] Measured at 400 MHz.
[b] Measured at 100 MHz.

Three partial structural units **a** (C-2 to C-4 and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12) and **c** (C-16 to C-17), drawn with bold bonds in Figure 1, were deduced from the analysis of the 2D NMR spectra including HSQC, ^{1}H–^{1}H COSY, and HMBC. HMBC correlations of H_b-7 to C-4 (δ_C 66.8) and C-19 (δ_C 53.2) suggested that C-4, C-7, and C-19 were connected to each other through a nitrogen atom. HMBC cross-peaks of H_3-21 to C-4, C-5 and C-6 implied that C-4, C-6 and C-21 were connected to C-5 (δ_C 52.1), and the correlations of H_3-21 to C-8 (δ_C 142.4) indicated the connection between C-5 and C-8. HMBC correlations of H-2 and H_2-3 to C-1 (δ_C 199.9) implied a ketone group was adjacent to C-2. The linkage of C-10 and C-17 (δ_C 68.8) via an oxygen atom was deduced from the HMBC correlations of H_2-17 to C-10 (δ_C 168.8). Meanwhile, connections among C-12, C-24 (δ_C 123.5) and C-10 via C-11(δ_C 139.7) was deduced by the correlations of H_2-12 and H_2-24 to C-11 and C-10, respectively. HMBC correlations of H_3–OCH_3 to C-22 (δ_C 167.4) suggested that a methoxyl group was connected to C-22. Additionally, the conjugated cyclopentadiene unit of C-8 to C-9 and

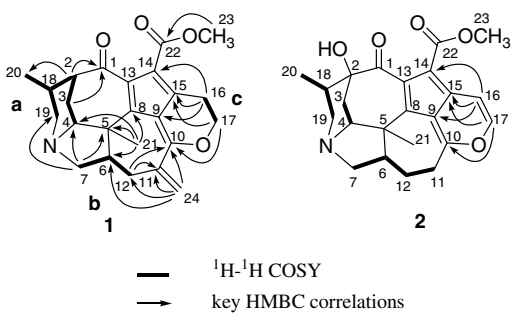


Figure 1. Selected 2D NMR correlations for **1** and **2**.

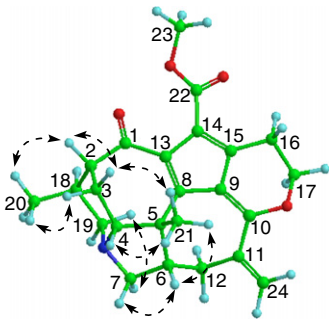
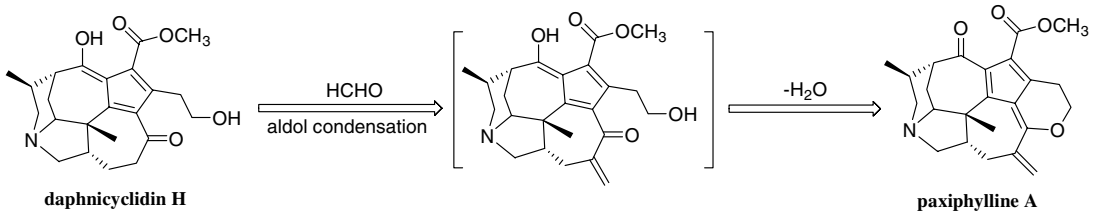


Figure 2. Key ROESY correlations and relative stereochemistry of **1**.



Scheme 1. Biogenetic pathway proposed for paxiphylline A (**1**).

C-13 to C-14 and C-15 was suggested by the HMBC correlations of H_2-16 to C-9, C-14 and C-15, H_2-17 to C-15, and H_3-21 to C-8, respectively, which was also supported by UV absorptions (275 and 354 nm).[3d,e] Thus, the planar structure of paxiphylline A (**1**) was established, possessing a methylidene group at C-11 (Fig. 1).

The relative stereochemistry of **1** was elucidated by using ROESY spectrum as shown in the computer-generated 3D drawing (Fig. 2). The ROSEY correlations of H_3-21/H-4, H_3-21/H-6, and H-6/H-7β implied that H_3-21, H-4 and H-6 took β-orientation, while the correlations of H_3-21/H-3 β, H-3β/H-2 and H-2/H_3-20 implied that H-2 and H-20 also took β-orientation.

Biogenetically, paxiphylline A (**1**) should be derived from daphnicyclidin H[3e] as described in Scheme 1. The 'extra' carbon (C-24) might be provided by formaldehyde through aldol condensation, which differ from other biosynthetic hypotheses of Mannich-type cyclization accounted for 'extra' carbon.[2c,3c,8d]

Paxiphylline B (**2**)[11] was obtained as an optically active ($[\alpha]_D^{22}$ −326.7 (*c* 0.175, MeOH)) light yellow solid. Its molecular formula was inferred as $C_{23}H_{25}NO_5$ by HRESIMS (*m*/*z* 396.1818, $[M+H]^+$, calcd: 396.1810). The IR spectrum indicated the presence of OH (3431 cm^{-1}) and two conjugated carbonyl groups (1711 and 1629 cm^{-1}, respectively). The ^1H and ^{13}C NMR data of **2** (Table 1) revealed 23 carbon signals due to four double bonds, two carbonyls, two sp^3 quaternary carbons, three sp^3 methines, five sp^3 methylenes, two methyls, and one methoxy group. UV absorptions (275 and 331 nm) suggested the existence of the conjugated fulvene.[3d,e]

The NMR spectral data of **2** is strikingly similar to daphnicyclidin F,[3e] except for the presence of signals corresponding to an additional aromatic proton at δ_H (7.69) instead of two methylene groups (δ_H 2.76 and 3.19, δ_H 4.15 and 4.67). The additional aromatic protons in **2** were placed at C-16 and C-17 positions based on the HMBC correlations from H-16 to C-9, C-15 and C-14, and from H-17 to C-15 and C-10. The β-orientation of H-4, H-6, H_3-20 and H_3-21 in **2** was deduced from the NOESY spectrum, which was similar to daphnicyclidin F. B (**2**) could be considered as the oxidative derivative of daphnicyclidin F.

A cytotoxicity assay showed that compounds **1** and **2** were not active against the acute myelogenous leukemia (HL60) and human lung cancer (A549) cell lines ($ED_{50} > 10$ μg/ml).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.10.136.

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10. *Paxiphylline A* (**1**): A light yellow solid; $[\alpha]_D^{23}$ −221.7 (*c* 0.40, CH_3OH); IR (KBr) ν_{max} 3440, 2926, 1718, 1629, 1564 and 1124 cm^{-1}; UV (MeOH) λ_{max} 275 (ε 9707), 354 nm (10962); ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 394 $[M+H]^+$; HRESIMS *m*/*z* 394.2012 (M+H; calcd for $C_{24}H_{27}NO_4$, 394.2018).
11. *Paxiphylline B* (**2**): A light yellow solid; $[\alpha]_D^{22}$ −326.7 (*c* 0.175, CH_3OH); IR (KBr) ν_{max} 3431, 2930, 1711, 1677, 1629 and 1442 cm^{-1}; UV (MeOH) λ_{max} 275 (ε 26346), 331 nm (4476); ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 396 $[M+H]^+$; HRESIMS *m*/*z* 396.1818 (M+H; calcd for $C_{23}H_{25}NO_5$, 396.1810).