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First Diamino *Daphniphyllum* Alkaloid, Daphnipaxinin, with an Unprecedented Heterohexacyclic Skeleton from *Daphniphyllum paxianum*

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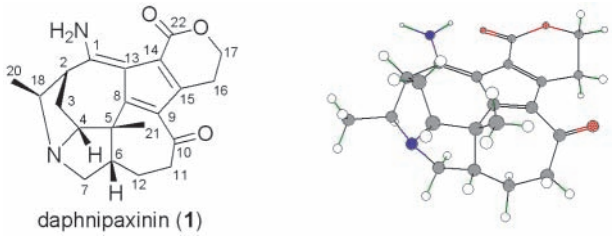
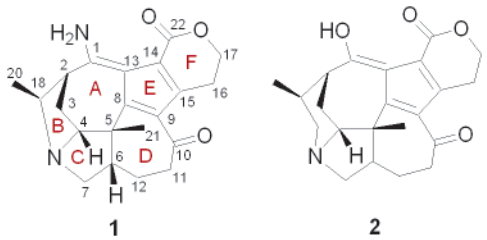
ABSTRACT



The first diamino *Daphniphyllum* alkaloid, daphnipaxinin (1), with an unprecedented hexacyclic fused skeleton, along with a known alkaloid, daphnicyclidin A (2), was isolated from the stem of *Daphniphyllum paxianum* Rosenth. The structure and absolute stereochemistry of 1 were established by spectral methods, especially two-dimensional NMR techniques and CD analysis.

The genus *Daphniphyllum* (Daphniphyllaceae) produces structurally astonishing secondary metabolites,[1] a group of highly complex polycyclic *Daphniphyllum* alkaloids, which have been challenging subjects of natural products, and biogenetic synthesis programs. *Daphniphyllum* alkaloids reported previously were classified into six main skeleton types.[2] Radioactive tracer experiments have demonstrated biogenetically that these alkaloids were generated from six molecules of mevalonic acid via a squalene-like intermediate.[3] Heathcock and co-workers reported a series of synthetic work on *Daphniphyllum* alkaloids in which one exceptionally efficient route of biomimetic total synthesis of several polycyclic *Daphniphyllum* alkaloids was noted.[4] Recently, about 30 additional new *Daphniphyllum* alkaloids were reported by Kobayashi, Morita, Jossang, Yue, and their co-workers.[5] *Daphniphyllum* alkaloids are neurotoxic and target directly the central nervous system, resulting in depression of voluntary movement as well as respiratory function.[1a] A few *Daphniphyllum* alkaloids also showed potent cytotoxicity against several human tumor cell lines.[5b−k]



Daphniphyllum species such as *D. calycinum*, *D. macropodum*, and *D. oldhami* are used as traditional Chinese medicine with several indications.[6] *Daphniphyllum paxianum* Rosenth, an evergreen tree, is only distributed in southern

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Table 1. ^{1}H and ^{13}C NMR Data, HMBC, and NOESY Correlations of **1**[a]

	δ_C	δ_H, multi, J (Hz)	HMBC H→C	NOESY H→H
1	179.55			
2	56.17	2.89 (dd, 8.2, 1.4)	20, 18, 14,[b] 13, 4, 3, 1	20 (m), 18 (w), 3α (m), 3β (w)
3	30.20	α 2.46 (ddd, 13.9, 8.2, 7.1)	5, 4, 2, 1	4 (m), 3β (s), 2 (m)
		β 2.27 (d, 13.9)	18, 5, 4, 2, 1	21 (w), 4 (w), 3α (s), 2 (w)
4	80.56	3.40 (d, 7.1)	21, 18, 9,[b] 8, 6, 5, 3, 2	21 (m), 6 (m), 3α (m), 3β (w)
5	52.90			
6	54.76	2.48 (m)	21, 12, 8, 7, 5, 4	21 (w), 12β (m), 7β (m), 7α (w), 4 (m)
7	65.01	β 3.54 (dd, 10.8, 7.7)	18, 6, 5, 4	7α (s), 6 (m)
		α 2.33 (dd, 10.8, 10.6)	18, 12, 6, 4	18 (s), 12α (w), 7β (s), 6 (w)
8	147.76			
9	130.31			
10	207.90			
11	41.28	β 2.74 (ddd, 19.6, 13.8, 1.6)	12, 10, 6	21 (w), 11α (s)
		α 2.49 (ddd, 19.8, 5.7, 1.7)	12, 10, 9, 6	12β (m), 11β (s)
12	28.97	β 2.07 (dddd, 13.7, 5.7, 5.7, 1.6)	11, 10, 6, 5	12α (s), 11α (m), 6 (m)
		α 1.43 (dddd, 13.8, 13.7, 11.2, 1.7)	11, 10, 7, 6, 5	12β (s), 7α (w)
13	113.81			
14	111.38			
15	146.70			
16	25.95	β 3.17 (ddd, 17.9, 10.4, 5.3)	22,[b] 17, 15, 14, 9, 8[b]	17β (m), 17α (m), 16α (s)
		α 2.72 (ddd, 17.9, 5.3, 4.4)	22,[b] 17, 15, 14, 9, 8[b]	17β (w), 17α (w), 16β (s)
17	69.86	β 4.41 (ddd, 10.5, 5.3, 5.3)	22, 16, 15, 9[b]	17α (s), 16β (m), 16α (w)
		α 4.26 (ddd, 10.5, 10.4, 4.4)	22, 16, 15	17β (s), 16β (m), 16α (w)
18	76.00	3.12 (qd, 6.7, 1.4)	7, 3, 2, 1	20 (m), 7α (s), 2 (w)
20	26.08	1.26 (3H, d, 6.7)	18, 2	18 (m), 2 (m)
21	34.02	1.34 (3H, s)	8, 6, 5, 4	11β (w), 6 (w), 4 (m), 3β (w)
22	170.45			

[a] Measured in CD_3OD. [b] J^4 correlation; s = strong, m = moderate, w = weak.

China. Its leaves and stems are used as antiinflammation herbs in Chinese folk medicine.[7] A diamino alkaloid, daphnipaxinin (**1**), with an unprecedented hexacyclic fused skeleton, together with a known alkaloid daphnicyclidin A (**2**), was isolated from the stem of *D. paxianum*. Daphnipaxinin **1** is the first diamino daphniphyllum alkaloid.

The plant material was collected from Hainan Island of China. The air-dried stem powder of *D. paxianum* was percolated with 95% ethanol, and the crude extract was partitioned with EtOAc. The aqueous phase containing alkaloids was evaporated to dryness and subjected to a silica gel column chromatography ($CHCl_3/CH_3OH$, 10/1) to enrich the alkaloids. The crude alkaloid was then separated by a silica gel column ($CHCl_3/CH_3OH/Et_2NH$, 35/1/0.1) to give two major fractions, each of which was further purified by a column of Sephadex LH-20 (CH_3OH) to yield daphnipaxinin (**1**, 0.00024%) and daphnicyclidin A (**2**, 0.0010%), respectively.

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The structural types of daphniphyllum alkaloids[8] of *D. paxianum* collected from Guangdong Province of the Peoples Republic of China are absolutely different from those isolated from *D. paxianum* from Hainan Island.

Daphnipaxinin (**1**)[9] was obtained as an optically active ($[\alpha]^{20}_D = +148°$) tiny fiber (in acetone). The molecular formula of **1** was established as $C_{21}H_{24}N_2O_3$ by HR-EIMS at *m*/*z* 352.1794 $[M]^+$ and the positive mode of HR-ESIMS at *m*/*z* 353.1877 $[M + H]^+$. Daphnipaxinin (**1**) also exhibited a deprotonated molecular ion at *m*/*z* 351 $[M - H]^-$ in the negative mode of ESIMS to confirm the assignment for the molecular formula with 11 degrees of unsaturation. All 21 carbon atoms in the molecule were resolved in the ^{13}C NMR spectrum (DEPT experiments) as 21 carbon signals comprising eight sp^2 and one sp^3 quaternary carbons, four tertiary carbons, six secondary carbons, and two methyls. Eight sp^2 quaternary carbons were attributable to one ester carbonyl at δ 170.45, one ketone carbonyl at δ 207.90, and three persubstituted double bonds (Table 1). Twenty-two proton signals were detected in the ^1H NMR spectrum (Table 1) and assigned on the basis of the HMQC spectrum to each of the carbon atoms. According to the molecular formula, two proton atoms were missed in the ^1H NMR spectrum and were considered to be exchangeable protons, which were most likely attached to the nitrogen atoms (IR at 3406 and 3250 cm^{-1}). The ester carbonyl, ketone group, and three double bonds accounted for 5 out of the 11 degrees of unsaturation, the remaining 6 degrees of unsaturation were assumed for the presence of a hexacyclic system in **1**.

Three structural fragments, **a** (C-2−C-4, C-18, and C-20), **b** (C-6, C-7, C-11, and C-12), and **c** (C-16 and C-17), drawn with bold bonds, were clearly established by $^1H-^1H$ COSY and coupling constants (Figure 1 and Table 1). The connectivity of three fragments (**a**−**c**), quaternary carbons, and heteroatoms was furnished by HMBC experiment[10] (Figure 1). In the HMBC, the proton signals at δ 4.41 and 4.26 assigned for H_2-17 showed correlations with C-22 at δ 170.45. The Δ^{14} double bond was assignable on the basis of the correlations pairs of H_2-16/C-14, H_2-16/C-15, and H_2-17/C-15. Correlations between H_2-16 and C-9 (^3J) revealed the connectivity of C-15 and C-9. The ketone group assigned to C-10 was linked with C-9 as judged from the correlation between H-11α and C-9 (^3J). Two proton signals at δ 2.74 and 2.49 attributable to H_2-11 showed cross-peaks with C-10 to link C-10 and C-11. The linkage of structural fragments **a** and **b** via a nitrogen atom and a quaternary carbon (C-5) was achieved by chemical shifts of C-4 (δ 80.56), C-7 (δ 65.01), and C-18 (δ 76.00), by the HMBC correlations (^2J) of H-4/C-5 and H-6/C-5, and by the HMBC correlations (^3J) of H_2-7/C-18, H_2-7/C-4, H-18/C-7, and H-4/C-18. The proton signal assigned for H_3-21 correlated with C-5 to place C-21 at C-5. The linkage of C-5 to C-8 of Δ^8 was revealed by HMBC correlations (^3J) of C-8 with H-4, H-6, and H_3-21, as well as HMBC correlations (^4J) of H-4/C-9 and H_2-16 (δ 3.17 and 2.72)/C-8. The $\Delta^{1(13)}$ double bond was located by the HMBC correlations of C-1 with H-18, H_2-3, and H-2 and HMBC correlation between H-2 and C-13. The ^4J correlations between H-2 and C-14 inferred the linkage of C-13 and C-14. Although the linkages between C-13 and C-8 and between C-22 and C-14 could not be resolved directly by HMBC correlations, with the determination of all the other linkages, these linkages could be tentatively assigned and rationalized from a biogenetic perspective (Scheme 1). Comparison of the ^1H and ^{13}C NMR data of the E and F rings in **1** with those of daphnicyclidin A (**2**) also supported the assignment for the highly unsaturated

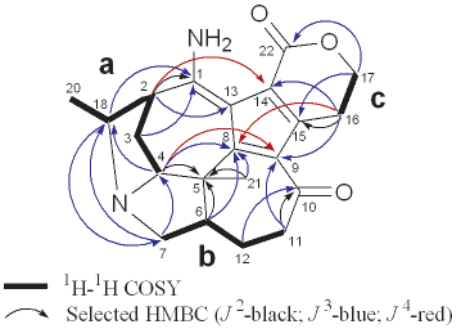
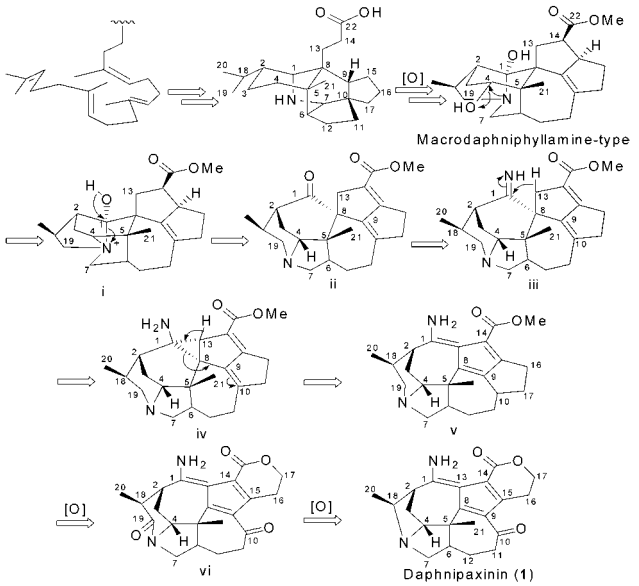


Figure 1. Selected two-dimensional NMR correlations of daphnipaxinin (**1**).

Scheme 1. Biogenetic Pathway Proposed for Daphnipaxinin (**1**)



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(9) **Daphnipaxinin (1):** tiny fiber (in acetone); mp = 207 °C dec.; $[\alpha]^{20}_D = +148°$ (*c* 0.28, CH_3OH); UV λ 371 (logϵ = 4.27), 295 (logϵ = 4.46) nm; IR (KBr) ν_{max} 3406, 3250, 2926, 1614, 1456, 1346, 1298, 1051 and 868 cm^{-1}; ^1H NMR see Table 1; ^{13}C NMR see Table 1; EIMS 70 eV *m*/*z* (rel int) 353 $[C_{20}{}^{13}CH_{24}N_2O_3]^+$ (22), 352 $[M]^+$ (100), 338 (5), 337 (24), 324 (15), 308 (6) and 173 (9); ESIMS (negative) $[M - H]^-$, *m*/*z* 351; ESIMS (positive) $[M + H]^+$, *m*/*z* 353; HR-EIMS *m*/*z* 352.1794 $[C_{21}H_{24}N_2O_3]^+$ (calcd 352.1787); HR-ESIMS (positive) $[M + H]^+$, *m*/*z* 353.1877 $[C_{21}H_{25}N_2O_3]^+$ (calcd 353.1865).

system.[5h] The remaining amino group was only assignable to the C-1 at δ 179.55, and the downfield shifted C-1 carbon signal was considered to be caused by the deshielding effect of the system of conjugated double bonds and carbonyls.[5h] The planar structure of daphnipaxinin is, therefore, outlined.

The relative stereochemistry of daphnipaxinin was fixed by NOESY spectrum (Figure 2). The NOESY interaction

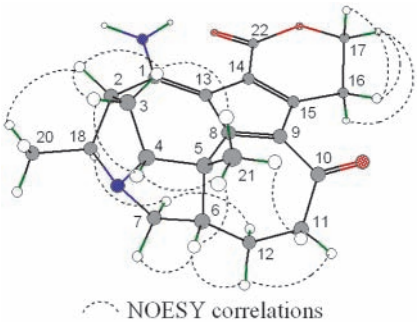

Figure 2. Key NOESY correlations and conformation generated from computer modeling of daphnipaxinin (**1**).

pairs of H_3-21/H-6 and H_3-21/H-4 indicated that CH_3-21, H-6, and H-4 were β-oriented. The correlation between H-18 and H-7α revealed that H-18 was α-oriented. The H-2 correlated to H_3-20, suggesting that both were on the same side. The H-3 at δ 2.27 correlated with the H-2, H-4, and H_3-21 was assigned β-orientation. The three-dimensional structure (Figure 2) of **1** was generated by computer modeling (CS Chem 3D Pro Version 6.0, using MM2 force field calculations for energy minimization). The relative stereochemistry and a favorable conformation of **1** offered by computer modeling were consistent with those of **1** assigned by NOESY spectrum. Thus, the structure of daphnipaxinin was unambiguously elucidated as **1**.

The absolute stereochemistry of daphnipaxinin (**1**) was determined on the basis of the CD spectrum (Figure 3). The Cotton effects (290 and 320 nm) centered at 295 nm corresponding to the UV maximum of a cyclopentene group conjugated with the carbonyl at C-22.[5h] The positive Cotton effect at 320 nm caused by the transition between the conjugated cyclopentene moiety and the ketone group at C-10 established the absolute configuration of **1**, which was identical to that of the known alkaloid, daphnicyclidin A (**2**).[5h]

A plausible biogenetic pathway for daphnipaxinin (**1**) is proposed as shown in Scheme 1. The biogenetic origin of **1**

(10) Daphnipaxinin could not be crystallized to give a high-quality crystal for X-ray analysis, and HMBC experiment was thus performed (J_{CH} = 7.5 Hz) to figure out the planar structure of **1**.

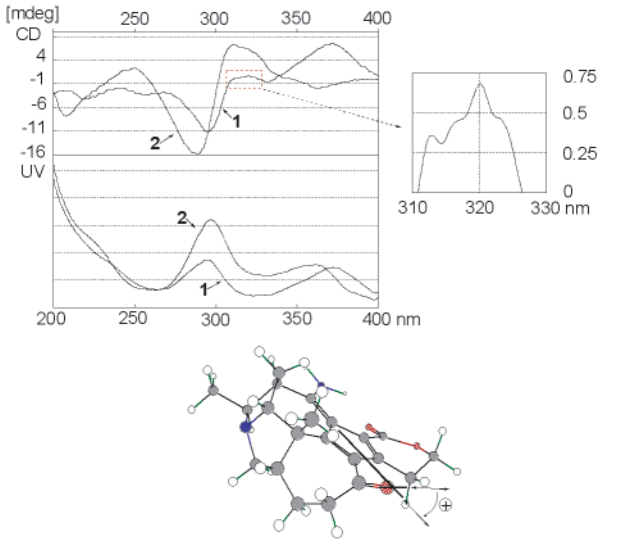

Figure 3. CD and UV spectra of **1** and **2** and chiral analysis of daphnipaxinin (**1**). Bold lines denote the electric transition dipole of the chromophore.

seems to be macrodaphniphyllamine-type alkaloids. The formation of the C-4−N bond and the release of one molecular water yielded intermediate i, which underwent the cleavage of the C-1−N bond to produce intermediate ii. The addition of one molecular ammonia to the deoxycalyciphyline A-type intermediate ii formed key intermediate iii, much like the pairs of structurally related keto and imino alkaloids, megistosarconine and megistosarcimine, isolated from a single plant.[11] The intermediate iii was subsequently rearranged to give key intermediate iv, which further rearranged to form the intermediate v. After oxidation, the intermediate v was transformed to intermediate vi, which finally underwent an oxidative degradation of C-19 carbon atom to produce the novel alkaloid, daphnipaxinin (**1**).

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Supporting Information Available: Experimental section, one- and two-dimensional NMR, MS, and IR spectra of daphnipaxinin (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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