Daphniyunnines A−E, Alkaloids from *Daphniphyllum yunnanense*

Hua Zhang, Sheng-Ping Yang, Cheng-Qi Fan, Jian Ding, and Jian-Min Yue*

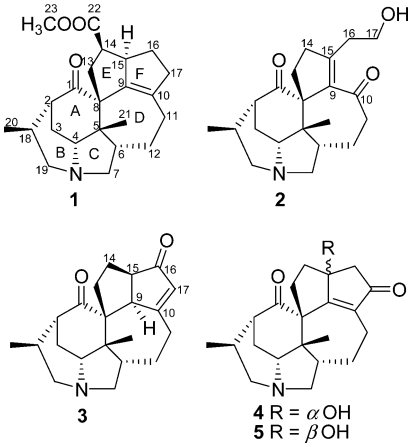
State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhangjiang Hi-Tech Park, Shanghai, 201203, People's Republic of China

Received November 26, 2005

The first chemical study on the stems and leaves of *Daphniphyllum yunnanense* led to the isolation of five new alkaloids, daphniyunnines A−E (**1**−**5**). Daphniyunnines B−E (**2**−**5**) are four unusual C-22 nor-*Daphniphyllum* alkaloids. The structures of these alkaloids were characterized by spectroscopic methods, especially 2D NMR techniques. A single-crystal X-ray diffraction analysis was used to confirm the structure of **1**. Daphniyunnine D (**4**) showed cytotoxicity against two tumor cell lines, P-388 and A-549, with IC_{50} values of 3.0 and 0.6 μM, respectively.

The *Daphniphyllum* genus, the only one in the Daphniphyllaceae family, is known for its structurally diversified and fascinating *Daphniphyllum* alkaloids.[1] From 2003 until now, about 45 additional new *Daphniphyllum* alkaloids were reported by Kobayashi, Jossang, Bodo, Yue, and their co-workers.[2] Some *Daphniphyllum* alkaloids also exhibited moderate cytotoxicity against several tumor cell lines.[1,2b,c,f−i]

Daphniphyllum yunnanense, as shrubs or small trees, is endemic to the southeast of Yunnan Province, People's Republic of China,[3] and its chemical constituents have not been investigated previously. As a continuation of our research on structurally and biogenetically interesting *Daphniphyllum* alkaloids,[2j−p] five new alkaloids, daphniyunnines A−E (**1**−**5**), were isolated from *D. yunnanense*. The structures of these alkaloids were elucidated on the basis of their 1D and 2D NMR spectra, and the structure of **1** was further confirmed by a single-crystal X-ray diffraction determination. It is notable that daphniyunnines B−E (**2**−**5**) represent four rare C-22 nor-*Daphniphyllum* alkaloids, and they all possess an α,β-unsaturated ketone group. Compounds **1**−**5** were evaluated in bioassays for antitumor activity. Daphniyunnine D (**4**) showed moderate cytotoxic activity against two tumor cell lines, P-388 and A-549, with IC_{50} values of 3.0 and 0.6 μM, respectively. This paper describes the isolation and characterization of these new alkaloids.



Results and Discussion

Daphniyunnine A (**1**) was obtained as colorless rhomboid crystals (acetone) with $[\alpha]^{20}_D$ −130.5 (*c* 0.11, $CHCl_3$). Its molecular formula was determined as $C_{23}H_{31}NO_3$ by HREIMS at *m*/*z* 369.2303 (calcd 369.2304) with nine degrees of unsaturation. IR absorption bands at 1728 and 1686 cm^{-1} suggested the presence of ester and ketone carbonyls, respectively. Its ^{13}C NMR data (Table 2) showed 23 carbon signals, which were classified into primary, secondary, tertiary, and quaternary carbons by DEPT experiments. Two carbonyls and one double bond distinguished by ^{13}C NMR spectroscopy accounted for three degrees of unsaturation. The remaining six degrees of unsaturation required the presence of a hexacyclic system in **1**.

Four partial structures, **a** (C-2 to C-4), **b** (C-18 to C-19 and C-20), **c** (C-6 to C-7 and C-12, and C-11 to C-12), and **d** (C-13 to C-17), were deduced from $^1H-^1H$ COSY as shown in Figure 1A. The linkage of the four structural fragments **a**−**d** with quaternary carbons and heteroatoms was achieved by examination of HMBC data (Figure 1A, Supporting Information Figure S9), which enabled us to establish the planar structure of daphniyunnine A (**1**).

The relative configuration of **1** was fixed by NOESY data as shown in Figure 1B. The cross-peaks observed between the proton pairs H_3-21/H-4, H_3-21/H-6, H_3-21/H-13β, and H-4/H-6 indicated that CH_3-21, H-4, H-6, and CH_2-13 were coplanar and were arbitrarily assigned β-orientations. H-7β (δ_H 2.88) showed a key correlation with H-6 (δ_H 2.25), and in consequence, the correlations of H-7α/H-18 and H_3-20/H-2 indicated that H-18 (δ_H 2.78) had an α-orientation, while H-2 (δ_H 2.16) had a β-orientation. H-14 (δ_H 2.79) and H-15 (δ_H 3.38) were assigned α-orientations on the basis of the strong NOESY correlations of H-13α/H-14 and H-14/H-15. The NOESY correlation pairs of H-13α/H-3β and H-3α/H-19β implied that both the A- and B-rings adopted boat conformations. The structure and the relative configuration of **1** were later confirmed by single-crystal X-ray diffraction (Figure 2).

Daphniyunnine B (**2**) showed a molecular formula of $C_{21}H_{29}NO_3$ as determined by HREIMS at *m*/*z* 343.2147 (calcd 343.2147) with eight degrees of unsaturation. IR absorptions indicated the presence of hydroxyl (3425 cm^{-1}), ketone (1701 cm^{-1}), and α,β-unsaturated ketone (1678 and 1660 cm^{-1}) groups. All 21 carbon signals, which were assignable to two ketone carbonyls, one tetrasubstituted double bond, two quaternary carbons, four methines, nine methylenes, and two methyls, were resolved in its ^{13}C NMR (DEPT, Table 2) spectrum. Among them, one methylene (δ_C 59.86, δ_H 3.78 and 3.67) was ascribed as bearing a hydroxyl group. Two carbonyls and the only double bond accounted for three degrees of unsaturation; the remaining five degrees of unsaturation were ascribed to the existence of a pentacyclic ring system in **2**.

The patterns and chemical shifts of ^1H and ^{13}C NMR data (Tables 1 and 2) of the A- to D-rings in **2** were similar to those (Tables 1 and 2) of **1**, suggesting that the two alkaloids shared the same basic skeleton. Extensive analysis of 2D NMR spectra (HMQC, $^1H-^1H$ COSY, and HMBC) (Figure 3) allowed the definition of the A- to E-ring connectivities for alkaloid **2**. Compared with alkaloid **1**, the main differences were the loss of the C-22 ester group, the presence

* Corresponding author. Tel: +86-21-50806718. Fax: +86-21-50806718. E-mail: jmyue@mail.shcnc.ac.cn.

10.1021/np050490e CCC: $33.50
Published on Web 03/10/2006

Table 1. ^{1}H NMR Data of Daphniyunnines A−E (**1**−**5**)

	1[a]	**2**[b]	**3**[c]	**4**[c]	**5**[c]
no.	mult, *J* (Hz)	mult, *J* (Hz)	mult, *J* (Hz)	mult, *J* (Hz)	mult, *J* (Hz)
2	2.16 (m)	2.06 (m)	2.17 (br d, 3.9)	2.34 (br d, 4.0)	2.16 (m)
3	α 1.98 (m)	α 2.05 (m)	α 2.08 (m)	α 2.11 (m)	α 2.05 (m)
	β 2.15 (m)	*β* 2.41 (m)	*β* 2.35 (br dd, 15.4, 4.8)	*β* 2.28 (m)	*β* 2.19 (m)
4	3.21 (br d, 5.0)	3.50 (br d, 3.9)	3.62 (br d, 5.0)	3.59 (br d, 3.0)	3.43 (br d, 4.6)
6	2.25 (m)	2.32 (m)	2.45 (m)	2.43 (m)	2.36 (m)
7	α 2.66 (dd, 11.2, 9.9)	α 2.83 (m)	α 2.54 (dd, 12.6, 9.8)	α 2.92 (m)	α 2.89 (m)
	β 2.88 (dd, 9.9, 7.1)	*β* 2.78 (m)	*β* 3.31 (dd, 9.8, 6.2)	*β* 3.11 (m)	*β* 2.82 (m)
9			4.12 (br d,5.2)		
11	α 2.09 (m)	α 2.53 (m)	2.89 (2H, m)	α 2.07 (m)	α 1.89 (ddd, 16.8, 13.3, 2.1)
	β 2.03 (m)	*β* 2.35 (m)		*β* 2.46 (ddd, 15.9, 5.7, 2.9)	*β* 2.29 (ddd, 16.8, 4.7, 2.5)
12	α 1.65 (m)	α 1.79 (m)	α 1.74 (m)	α 1.87 (m)	α 1.81 (m)
	β 1.94 (m)	*β* 1.96 (m)	*β* 2.04 (m)	*β* 1.72 (m)	*β* 1.64 (m)
13	α 2.32 (m)	α 1.81 (m)	α 1.90 (m)	α 2.23 (m)	α 2.11 (dd, 13.0, 6.3)
	β 2.76 (m)	*β* 2.66 (m)	*β* 1.77 (m)	*β* 2.63 (dd, 12.5, 8.0)	*β* 2.90 (m)
14	2.79 (m)	2.48 (2H, m)	α 1.25 (m)	α 2.23 (m)	α 1.41 (td, 12.9, 6.3)
			β 1.88 (m)	*β* 1.43 (td, 14.7, 8.0)	*β* 2.01 (dd, 12.9, 5.4)
15	3.38 (m)		2.73 (dd, 9.7, 5.6)		
16	α 1.87 (m)	a 2.66 (m)		α 2.72 (d, 17.7)	2.52 (2H, s)
	β 1.27 (m)	b 2.39 (m)		*β* 2.44 (d, 17.7)	
17	α 2.68 (m)	a 3.78 (m)	6.08 (d, 1.5)		
	β 2.35 (m)	b 3.67 (td, 9.7, 4.2)			
18	2.78 (m)	2.87 (m)	2.85 (m)	2.83 (m)	2.80 (m)
19	α 2.78 (m)	α 2.80 (m)	α 2.95 (dd, 13.9, 7.7)	α 2.96 (dd, 14.0, 7.4)	α 2.81 (m)
	β 2.48 (m)	*β* 2.52 (m)	*β* 2.64 (dd, 13.9, 10.5)	*β* 2.68 (m)	*β* 2.51 (dd, 16.9, 13.1)
20	1.01 (3H, d, 6.5)	0.97 (3H, d, 6.6)	1.08 (3H, d, 6.6)	1.08 (3H, d, 6.8)	1.00 (3H, d, 6.4)
21	1.33 (3H, s)	1.31 (3H, s)	1.23 (3H, s)	1.25 (3H, s)	1.29 (3H, s)
23	3.62 (3H, s)				

[a] In CD_3OD. [b] In $CDCl_3$ (containing 5% CD_3OD, v/v). [c] In $CDCl_3$.

Table 2. ^{13}C NMR Data of Daphniyunnines A−E (**1**−**5**)

no.	**1**[a]	**1**[b]	**2**[c]	**3**[b]	**4**[b]	**5**[b]
1	213.74	216.61	218.51	215.69	216.05	212.83
2	45.63	44.13	43.96	43.33	43.39	44.17
3	21.02	20.45	20.42	19.16	19.16	20.87
4	68.60	66.31	65.41	67.21	65.38	64.52
5	53.50	51.86	53.37	50.22	51.22	48.19
6	52.80	51.27	48.87	48.91[d]	51.17	50.99
7	57.29	56.51	53.55	57.44	53.80	53.82
8	63.13	61.87	71.59	63.74	63.33	64.95
9	143.05	141.12	140.15	48.88[d]	174.89	178.57
10	139.99	138.65	206.79	182.17	142.17	142.76
11	26.74	25.46	36.41	26.86	18.75	17.27
12	28.40	26.86	19.26	23.73	23.41	24.01
13	41.58	40.00	33.49	30.82	36.39	36.13
14	43.43	41.88	35.58	24.56	36.58	33.41
15	55.11	53.27	154.82	49.70	82.02	82.36
16	29.18	28.10	32.85	210.52	48.59	49.67
17	42.71	41.53	59.86	132.28	207.06	207.70
18	34.35	33.68	32.09	31.64	32.36	32.54
19	50.95	49.43	49.79	48.40	49.06	49.56
20	18.91	18.52	18.72	18.97	18.63	18.89
21	25.07	24.42	22.17	25.98	21.89	23.02
22	176.81	175.15				
23	52.02	51.27				

[a] In CD_3OD. [b] In $CDCl_3$. [c] In $CDCl_3$ (containing 5% CD_3OD, v/v). [d] Signals may be exchanged.

of an α,*β*-unsaturated ketone, and the opening of the F-ring. In the HMBC spectrum, the correlations of H_2-11 (δ_H 2.53 and 2.35) to C-10 (δ_C 206.79), and H_2-14 (δ_H 2.48) to C-9 (δ_C 140.15) and C-15 (δ_C 154.82), indicated the location of the α,*β*-unsaturated ketone; the correlations of H-16b (δ_H 2.39) to C-15 and H_2-17 (δ_H 3.78 and 3.67) to C-16 (δ_C 32.85) linked C-16 to C-15 and assigned the hydroxyl at C-17.

The relative configuration of **2** was deduced from its NOESY spectrum. The configurations at C-2, C-4, C-5, C-6, C-8, and C-18 were consistent with those of **1**. The A- and B-rings also had the same conformations as those in **1**, as deduced from the NOESY correlations of H-13α/H-3*β* and H-3α/H-19*β*. The structure of daphniyunnine B was thereby elucidated as **2**.

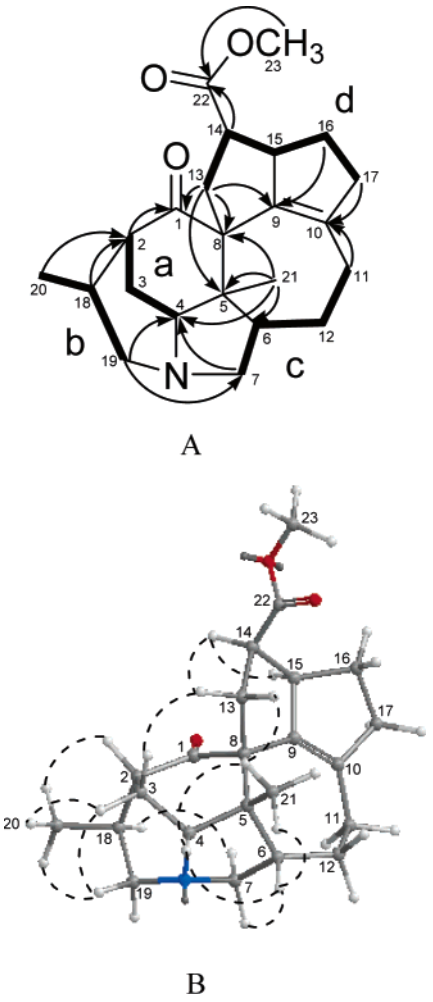


Figure 1. (A) ^{1}H−^{1}H COSY (bold) and HMBC (arrow, H → C) correlations of **1**. (B) NOESY (dashed) correlations of **1**.

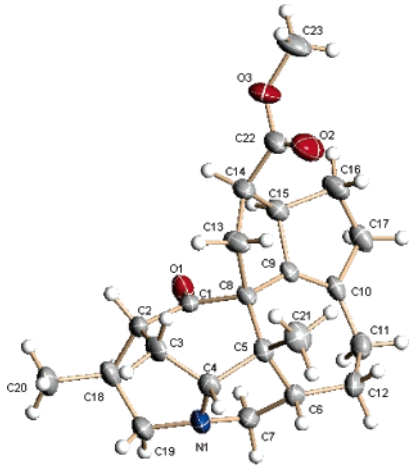


Figure 2. Single-crystal X-ray structure of **1**.

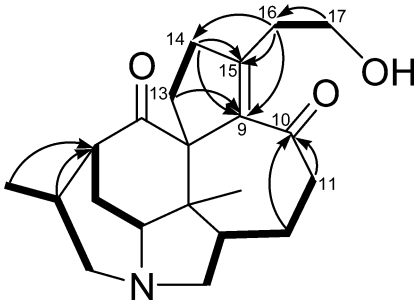


Figure 3. $^1H-^1H$ COSY (bold) and HMBC (arrow, H → C) correlations of **2**.

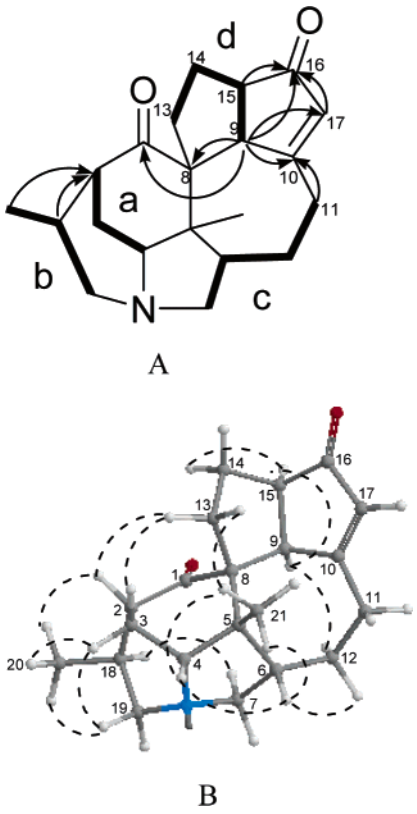


Figure 4. (A) $^1H-^1H$ COSY (bold) and HMBC (arrow, H → C) correlations of **3**. (B) NOESY (dashed) correlations of **3**.

Daphniyunnine C (**3**) had a molecular formula of $C_{21}H_{27}NO_2$ as determined by HREIMS (*m/z* 325.2052, calcd 325.2042), with nine degrees of unsaturation. Its IR spectrum showed a very strong and broad absorption at 1693 cm^{-1}, implying the presence of ketone group(s). Its UV absorptions at 209 (4.247) and 241 (4.024) nm suggested the presence of an α,*β*-unsaturated ketone. The ^{13}C NMR (DEPT, Table 2) spectrum revealed the presence of two ketone carbonyls, one trisubstituted double bond, two quaternary carbons, six methines, seven methylenes, and two methyls. The unsaturations required a hexacyclic ring system in **3**.

1D and 2D NMR (HMQC, $^1H-^1H$ COSY, and HMBC) spectra (Figure 4A) showed that alkaloid **3** is also a C-22 nor-*Daphniphyllum* alkaloid. By comparing with **1**, the major differences were the loss of the methoxycarbonyl group at C-14 and the presence of an α,*β*-unsaturated ketone in the F-ring. The location of the α,*β*-unsaturated ketone moiety was assigned by the HMBC correlations of H-9 (δ_H 4.12) with C-10 (δ_C 182.17), C-16 (δ_C 210.52), and C-17 (δ_C 132.28), H-15 (δ_H 2.73) with C-16, and H-17 (δ_H 6.08) with C-16. The allylic couplings of H-17 with both H-9 and H_2-11 (δ_H 2.89) were also observed in the $^1H-^1H$ COSY spectrum to support the above assignments.

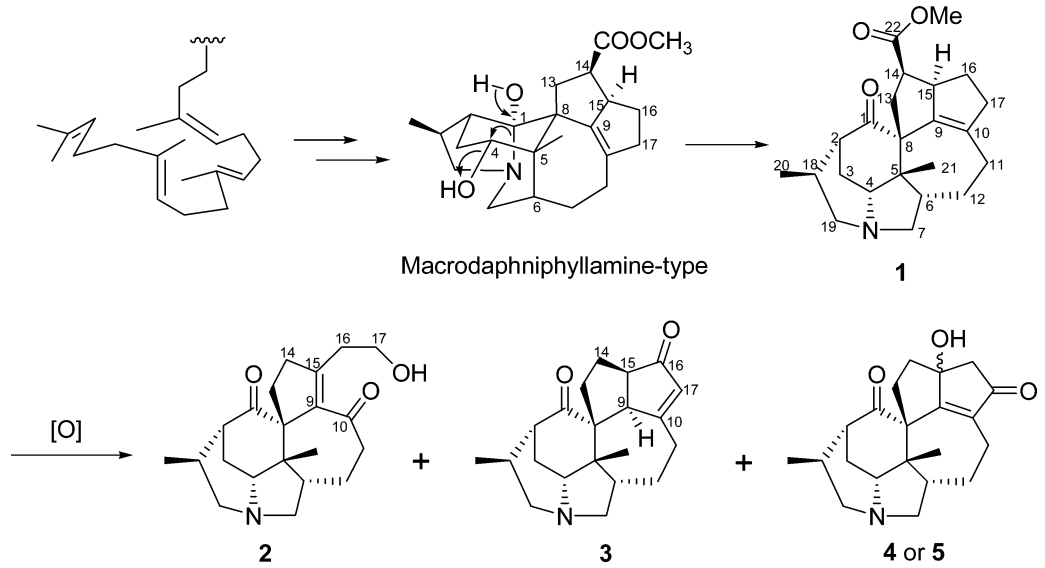
The relative configurations at C-2, C-4, C-5, C-6, C-8, and C-18 of **3** were consistent with those of **1** as assigned by NOESY spectroscopy (Figure 4B). The strong NOESY correlations of H-6/H-12*β*, H-12α/H-9, and H-9/H-15 suggested that H-9 and H-15 were α-oriented. The remarkable NOESY correlations of H-13α/H-3*β* and H-3α/H-19*β* indicated that both the A- and B-rings possessed boat conformations. The structure of daphniyunnine C was thus elucidated as **3**.

Daphyniyunnine D (**4**) showed a molecular formula of $C_{21}H_{27}NO_3$ as assigned by HREIMS at *m/z* 341.1996 (calcd 341.1991). Its IR spectrum exhibited the absorption bands of hydroxyl (3419 cm^{-1}), ketone (1709 cm^{-1}), and α,*β*-unsaturated ketone (1674 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra (Table 2) revealed 21 carbon signals due to two ketone carbonyls, one tetrasubstituted double bond, three quaternary carbons, four methines, eight methylenes, and two methyls. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **4** indicated that its structure was closely related to that of **3**, especially at the A- to C-rings. The main structural changes occurred at the F-ring, which affected some chemical shifts of both proton and carbon signals in the D- and E-rings. Analysis of 2D NMR (HMQC, $^1H-^1H$ COSY, and HMBC) (Figure 5A) spectra showed that **4** shared the same carbon backbone as that of **3**, and the differences were the location of the α,*β*-unsaturated ketone moiety and the presence of one hydroxyl at C-15. The location of the α,*β*-unsaturated ketone unit was determined by HMBC correlations of H-11*β* (δ_H 2.46) with C-9 (δ_C 174.89), C-10 (δ_C 142.17), and C-17 (δ_C 207.06) and H-13*β* (δ_H 2.63) with C-9. The only hydroxyl group was linked to C-15 at δ_C 82.02, as judged by the HMBC cross-peaks from H-14*β* (δ_H 1.43) and H_2-16 (δ_H 2.72 and 2.44) to C-15.

The relative configuration of **4** was fixed by a NOESY experiment as shown in Figure 5B. The configurations at C-2, C-4, C-5, C-6, C-8, and C-18 of **4** were the same as in **1**. The strong NOESY correlations of H_3-21/H-13*β*, H-13*β*/H-14*β*, and H-14*β*/H-16*β* implied that CH_2-14 and CH_2-16 approached each other above the molecular plane due to the presence of an α-oriented hydroxyl at C-15. Both the A- and B-rings also adopted boat conformations. The structure of daphniyunnine D was thereby elucidated as **4**.

Daphniyunnine E (**5**) had the same molecular formula of $C_{21}H_{27}NO_3$ as **4** determined by HREIMS at *m/z* 341.2006 (calcd 341.1991). The ^1H and ^{13}C NMR (Tables 1 and 2) spectra and EIMS fragmentation pattern were closely related to those of **4**, implying that they likely shared the same planar structure and were stereoisomers. This was confirmed by 2D NMR (HMQC, $^1H-^1H$ COSY, and HMBC) spectra. The key NOESY (Figure 6) correlations of **5** showed that its relative configurations except for C-15 were identical to those of compound **4**. The hydroxyl group at C-15 in **5** was assigned a *β*-orientation on the basis of the following observations. H-14α (δ_H 1.41), as distinguished by its NOESY

Scheme 1. Plausible Biogenetic Pathway Proposed for Alkaloids **1**−**5**



correlation with H-13α (δ_H 2.11), showed a crucial NOESY cross-peak with H-16α (δ_H 2.52), indicating that CH_2-14 and CH_2-16 approached each other below the molecular plane due to the presence of 15β-OH. Thus, the structure of daphniyunnine E was established as **5**.

Plausible Biogenetic Pathway Proposed for Daphniyunnines A−E (1−5). A possible biosynthetic pathway for alkaloids **1**−**5** is shown in Scheme 1. The biogenetic origin of these alkaloids seems to be macrodaphniphyllamine-type alkaloids, which would undergo a series of rearrangements to give daphniyunnine A (**1**). Daphniyunnine A (**1**) would be then transformed into daphniyunnines B−E (**2**−**5**) by enzyme catalytic reactions, such as decarboxylation, oxidation, and ring opening.

Cytotoxicity Evaluation of Daphaniyunnines A−E (1−5). Daphaniyunnines A−E (**1**−**5**) were evaluated in bioassays for antitumor activity according to standard protocols,[4] and pseudolaric acid B[5] was used as a positive control. Only daphniyunnine D (**4**) showed cytotoxic activity against two tumor cell lines, P-388 and A-549, with IC_{50} values of 3.0 and 0.6 μM, respectively.

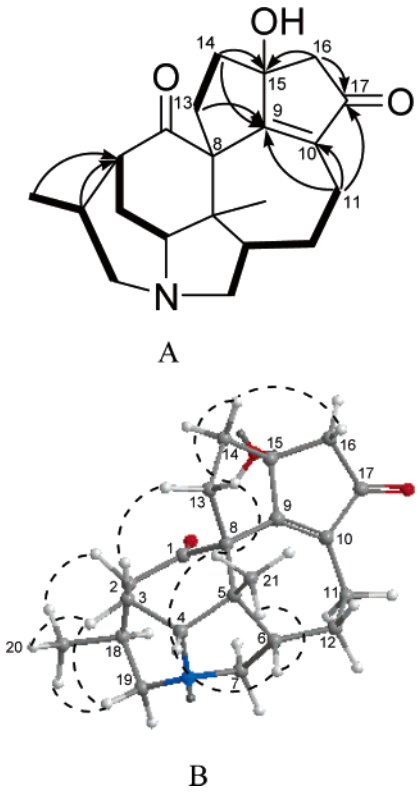


Figure 5. (A) $^1H-^1H$ COSY (bold) and HMBC (arrow, H → C) correlations of **4**. (B) NOESY (dashed) correlations of **4**.

Experimental Section

General Experimental Procedures. Melting points were measured on a SWG X-4 melting instrument and are uncorrected. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Hitachi U-2010 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. NMR spectra were measured on a Bruker AM-400 spectrometer. EIMS and HREIMS (70 eV) were recorded on a Finnigan MAT 95 mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Reagents Company, Ltd.). Silica gel (200−300 mesh, Qingdao Haiyang Chemical Company, Ltd.) was used for column chromatography, and precoated silica gel GF254 plates (Yantai Huiyou Silica Gel Exploitation Company, Ltd.) were used for TLC. RP-18 silica gel (150−200 mesh, Merck) was also used for column chromatography.

In X-ray crystallography, cell constants were determined by a least-squares fit to the setting parameters of 25 independent reflections measure on a Rigaku AFC7R four-circle diffractometer employing graphite-monochromated Mo Kα radiation (0.71073Å) and operating in the $\varphi-\omega$ scan mode. Data reduction and empirical absorption corrections (ψ-scans) were performed with the SHELXS-97 package.[6]

Plant Material. *D. yunnanense* was collected from Pingbian County of Yunnan Province, People's Republic of China, in November 2004 and was identified by Dr. Qiang Fan of the Institute of Botany, School of Life Sciences, Zhongshan University. A voucher specimen was deposited in Shanghai Institute of Materia Medica (accession number: DY-2004-1Y).

Extraction and Isolation. The air-dried powder of the stems and leaves of *D. yunnanense* (2.0 kg) was extracted with 85% EtOH at

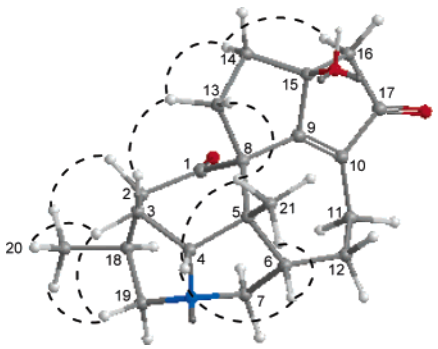


Figure 6. NOESY correlations (dashed) of **5**.

room temperature three times (each time for 4 days). After removal of the solvent under reduced pressure, the residue (108 g) was suspended in 1.0 L of acidic water (adjusted with 2 N H_2SO_4 to about pH 2−3). The acidified suspension was partitioned with Et_2O (500 mL × 5) to remove the nonalkaloids. Then it was carefully made basic with 5% Na_2CO_3 to pH 9−10 and extracted with $CHCl_3$ (500 mL × 3) to obtain 1.05 g of crude alkaloids.

The crude alkaloids were chromatographed on a silica gel column eluted with petroleum ether−EtOAc−Et_2NH (15:1:0.3 to 1:1:0.3) to give five fractions, F1−F5. F2 was subjected to a silica gel column ($CHCl_3$−MeOH, 40:1) to give alkaloid **1** (102 mg). F3 was subjected to a RP-18 silica gel column (MeOH−H_2O, 3:2) to yield **3** (20 mg) and **4** (7 mg). F4 was first subjected to a silica gel column ($CHCl_3$−MeOH, 10:1) and then further separated on a RP-18 silica gel column (MeOH−H_2O, 2:3) to yield **2** (12 mg) and **5** (10 mg).

Daphniyunnine A (1): colorless rhomboid crystals (acetone); mp 171−172 °C; $[\alpha]^{20}_D$ −130.5 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (3.962) nm; IR (KBr) ν_{max} 2960, 2928, 2810, 1728, 1686, 1475, 1450, 1435, 1362, 1333, 1281, 1259, 1190, 1163, 1134, 972, 807 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 369 $[M]^+$ (65), 340 (9), 326 (5), 310 (100), 292 (11), 110 (45); HREIMS *m*/*z* 369.2303 (calcd for $C_{23}H_{31}NO_3$, 369.2304).

Daphniyunnine B (2): white amorphous powder; $[\alpha]^{20}_D$ +67.0 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 246 (3.755) nm; IR (KBr) ν_{max} 3425, 2920, 2866, 2505, 1701, 1678, 1660, 1616, 1437, 1387, 1338, 1281, 1281, 1236, 1171, 1055, 1009, 887, 563 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 343 $[M]^+$ (7), 325 (9), 297 (4), 282 (4), 216 (5), 110 (100); HREIMS *m*/*z* 343.2147 (calcd for $C_{21}H_{29}NO_3$, 343.2147).

Daphniyunnine C (3): white amorphous powder; $[\alpha]^{20}_D$ −147.1 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (4.247), 241 (4.024) nm; IR (KBr) ν_{max} 3446 (water), 2951, 2511, 1693, 1634, 1605, 1458, 1419, 1385, 1331, 1290, 1259, 1194, 1169, 1099, 986, 893, 750, 550 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 325 $[M]^+$ (26), 300 (14), 293 (8), 257 (7), 181 (24), 178 (19), 160 (21), 149 (43), 110 (100); HREIMS *m*/*z* 325.2052 (calcd for $C_{21}H_{27}NO_2$, 325.2042).

Daphniyunnine D (4): white amorphous powder; $[\alpha]^{20}_D$ −140.0 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 213 (3.925), 230 (3.902) nm; IR (KBr) ν_{max} 3419, 2924, 2854, 2521, 1709, 1674, 1456, 1375, 1284, 1261, 1178, 1095, 1022, 750 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 341 $[M]^+$ (41), 323 (7), 313 (13), 298(11), 258 (6), 218 (7), 156 (30), 149 (15), 110 (100); HREIMS *m*/*z* 341.1996 (calcd for $C_{21}H_{27}NO_3$, 341.1991).

Daphniyunnine E (5): white amorphous powder; $[\alpha]^{20}_D$ −28.8 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 245 (3.904) nm; IR (KBr) ν_{max} 3431, 2956, 2924, 2868, 1701, 1670, 1441, 1375, 1263, 1207, 1171, 1070, 974, 729 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 341 $[M]^+$ (100), 326 (56), 313 (6), 298(52), 258 (9), 243 (7), 110 (81); HREIMS *m*/*z* 341.2006 (calcd for $C_{21}H_{27}NO_3$, 341.1991).

CCDC 286208 contains the supplementary crystallographic data for daphniyunnine A (**1**), and these data can be obtained free of charge from the Cambridge Crystallographic Data Center via http://www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgment. Financial support of the National Natural Science Foundation (Grant No. 20472093), Shanghai Municipal Scientific Foundation (Grant No. 04XD14019), and the foundation from the Ministry of Science and Technology (Grant No. 2002CB512807) of People's Republic of China is gratefully acknowledged. We thank Dr. Q. Fan for the collection and identification of the plant material.

Supporting Information Available: EIMS, IR and 1D and 2D NMR spectra of daphniyunnines A−E (**1**−**5**) are supplied in the supplementary data, and this material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) For a latest review of *Daphniphyllum* alkaloids, see: Kobayashi, J.; Morita, H. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 2003; Vol. 60, pp 165−205, and reference therein.

(2) (a) Morita, H.; Ishioka, N.; Takatsu, H.; Shinzato, T.; Obara, Y.; Nakahata, N.; Kobayashi, J. *Org. Lett.* **2005**, *7*, 459−462. (b) Bitar, H. E.; Nguyen, V. H.; Gramain, A.; Sévenet, T.; Bodo, B. *Tetrahedron Lett.* **2004**, *45*, 515−518. (c) Morita, H.; Takatsu, H.; Shen, Y. C.; Kobayashi, J. *Tetrahedron Lett.* **2004**, *45*, 901−904. (d) Takatsu, H.; Morita, H.; Shen, Y. C.; Kobayashi, J. *Tetrahedron* **2004**, *60*, 6279−6284. (e) Bitar, H. E.; Nguyen, V. H.; Gramain, A.; Sévenet, T.; Bodo, B. *J. Nat. Prod.* **2004**, *67*, 1094−1099. (f) Kobayashi, J.; Morita, H. *Org. Lett.* **2003**, *5*, 2895−2898. (g) Kobayashi, J.; Takatsu, H.; Shen, Y. C.; Morita, H. *Org. Lett.* **2003**, *5*, 1733−1736. (h) Morita, H.; Takatsu, H.; Kobayashi, J. *Tetrahedron* **2003**, *59*, 3575−3579. (i) Jossang, A.; Bitar, H. E.; Pham, V. C.; Sévenet, T. *J. Org. Chem.* **2003**, *68*, 300−304. (j) Chen, X.; Zhan, Z. J.; Yue, J. M. *Helv. Chim. Acta* **2005**, *88*, 854−860. (k) Chen, X.; Zhan, Z. J.; Yue, J. M. *Chem. Biodivers.* **2004**, *1*, 1513−1518. (l) Yang, S. P.; Yue, J. M. *Org. Lett.* **2004**, *6*, 1401−1404. (m) Zhan, Z. J.; Yang, S. P.; Yue, J. M. *J. Org. Chem.* **2004**, *69*, 1726−1729. (n) Yang, S. P.; Yue, J. M. *J. Org. Chem.* **2003**, *68*, 7961−7966. (o) Zhan, Z.-J.; Zhang, C.-R.; Yue, J.-M. *Tetrahedron* **2005**, *61*, 11038−11045. (p) Yang, S.-P.; Zhang, H.; Zhang, C.-R.; Chen, H.-D.; Yue, J.-M. *J. Nat. Prod.* **2005**, *68*, in press.

(3) Zheng, M.; Min, T. L. In *Chinese Flora* (*Zhongguo Zhiwu Zhi*); Science Press: Beijing, 1980; Vol. 45 (1), pp 8−10.

(4) Pauwels, B.; Korst, A. E. C.; De Pooter, C. M. J.; Pattyn, G. G. O.; Lambrechts, H. A. J.; Baay, M. F. D.; Lardon, F.; Vermorken, J. B. *Cancer Chemother. Pharm.* **2003**, *51*, 221−226.

(5) Pan, D. J.; Li, Z. L.; Hu, C. Q.; Chen, K.; Chang, J. J.; Lee, K. H. *Planta Med.* **1990**, *56*, 383−385.

(6) Sheldrick, G. M. *SHELX-97: Program for Crystal Structure Refinement*; University of Gottingen: Gottingen, Germany, 1997.

NP050490E