Alkaloids from *Daphniphyllum longeracemosum*

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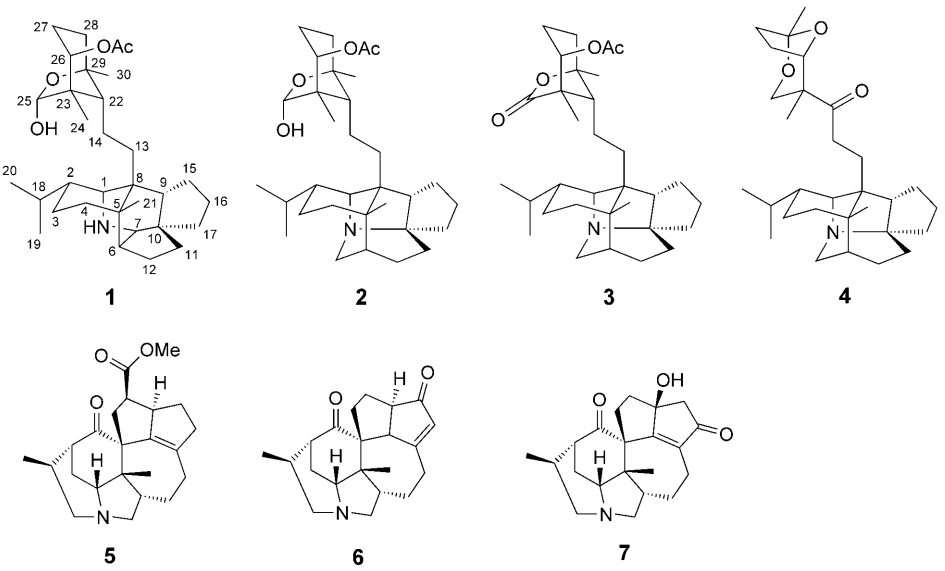
A new alkaloid, daphnilongeridine (**1**), together with six known alkaloids, daphmacropodine (**2**), daphmacrine (**3**), codaphniphylline (**4**), daphniyunnine A (**5**), daphniyunnine C (**6**), and daphniyunnine E (**7**), were isolated from the leaves and stems of *Daphniphyllum longeracemosum*. The so far elusive configuration at C(25) of daphmacropodine (**2**) was assigned on the basis of ROESY correlations.

Introduction. – Phytochemical investigation of *Daphniphyllum* alkaloids has been an attractive project for natural-product chemists [1]. Recently, the groups of *Kobayashi* [2], *Jossang* [3], *Yue* [4], *Bodo* [5], and *Hao* [6] reported a series of new *Daphniphyllum* alkaloids, some of which exhibit cytotoxic activities against several tumor-cell lines [2][3]. *Daphniphyllum longeracemosum* ROSENTH. (Daphniphyllaceae), an evergreen tree, is distributed mainly in Yunnan Province, China [7]. Chemical investigation of this species has previously led to the isolation of several new compounds [4b][6a].

As part of our continuing investigations of *Daphniphyllum* alkaloids [4], we herein report the isolation and structure determination of a new constituent, daphnilongeridine (**1**), which was obtained from the leaves and stems of *D. longeracemosum* together with six known alkaloids: daphmacropodine (**2**) [8], daphmacrine (**3**) [9], codaphniphylline (**4**) [10], daphniyunnine A (**5**) [4a], daphniyunnine C (**6**) [4a], and daphniyunnine E (**7**) [4a]. Also, the configuration at C(25) of daphmacropodine (**2**) could be assigned for the first time on the basis of ROESY correlations.

Results and Discussion. – Daphnilongeridine (**1**) was obtained as a colorless, amorphous, optically active powder, with $[\alpha]_D^{24} = +45.0$ ($c = 0.275$, MeOH). The HR-EI-MS signal at m/z 513.3824 established the molecular formula $C_{32}H_{51}NO_4$ (calc. 513.3818), with seven degrees of unsaturation. The IR spectrum suggested the presence of C=O (1741), secondary amine (3273, very weak), and OH (3396 cm^{-1}) functions. The ^{13}C-NMR (DEPT) spectrum of **1** (*Table*) showed one C=O group (δ(C) 170.4), five sp^3 quaternary C-atoms (36.6, 36.7, 49.7, 50.1, 84.5), six Me (16.6, 20.8, 21.1, 21.4, 21.6 and 26.4), eleven CH_2, and nine CH groups. The ^{1}H-NMR signal at δ(H) 4.85 (*s*) (*Table*), correlated with the signal at δ(C) 99.2 in the HSQC spectrum, indicated that **1** had a hemiacetal function. The ^{1}H- and ^{13}C-NMR data also indicated two nitrogenated methines (δ(C) 48.6, 58.8; δ(H) 3.40, 2.88, resp.), similar to those in the alkaloids secodaphniphylline [11] and caldaphnidine D [4c].



1 **2** **3** **4**

5 **6** **7**

The ^{13}C-NMR data of **1**, regarding the signals for C(1) to C(21), were similar to those of secodaphniphylline [11], which suggested that these two compounds share the same alkaloidal backbone[1]), the only structural difference being at the side chain. Combined analysis of ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments (*Fig. 1*) confirmed the above conclusion. Four of five partial structures (bold lines) in the alkaloid were first established by means of ^{1}H,^{1}H-COSY, and the HMBC correlations were then used to connect these structural subunits, quaternary C-atoms, and a Me *singlet* at δ(H) 0.81 (δ(C) 20.8). The HMBC correlations from H_b–C(13) (δ(H) 1.49–1.56) to C(1), C(8), and C(9), and from H–C(9) (δ(H) 1.74–1.79) to C(1) and C(8) permitted the linkage of C(1), C(9), and C(13) *via* C(8). The strong HMBC correlations from the Me group at δ(H) 0.81 to C(4), C(5), and C(6) demonstrated that C(4), C(6) and Me(21) were attached to C(5), and the HMBC correlation between Me(21) and C(8) enabled us to establish the connectivity of C(5) and C(8). The correlations of CH_2(6)/C(10), H–C(9)/C(10), H_a–C(11)/C(10), and H_a–C(17)/C(10) revealed that C(7), C(9), C(11), and C(17) were connected to C(10). Although there was no HMBC correlation observed to establish the linkage of C(1) and C(7) *via* an N-atom, the typical chemical shifts of C(1) and C(7), and the IR absorption for a secondary amino function, were indicative of this linkage.

Comparison of the ^{1}H- and ^{13}C-NMR data of the side chain of **1** with those of daphnezomine D [11] indicated that both alkaloids bear the same side chain. 2D-NMR ^{1}H,^{1}H-COSY, HSQC, and HMBC analyses further supported this conclusion. In the HMBC spectrum, the Me signal at δ(H) 2.08, correlated with a C=O group at δ(C) 170.4, indicated an Ac group, which was attached at C(26) according to the HMBC cor-

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[1]) Arbitrary atom numbering. For systematic name, see *Exper. Part*.

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. At 400/50 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering.

Atom	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	3.40 (br. *s*)	48.6	3.33 (br. *s*)	63.6
H–C(2)	0.98–1.01 (*m*)	42.7	1.44–1.48 (*m*)	37.9
H_a–C(3)	1.85–1.93 (*m*)	20.3	1.90–1.92 (*m*)	25.7
H_b–C(3)	1.61–1.66 (*m*)		1.27–1.29 (*m*)	
H_a–C(4)	1.59–1.63 (*m*)	38.6	1.96–2.00 (*m*)	36.1
H_b–C(4)	1.24–1.29 (*m*)		1.61–1.62 (*m*)	
C(5)	–	36.6	–	37.1
H–C(6)	2.07–2.09 (*m*)	45.9	1.71–1.76 (*m*)	38.7
H_a–C(7)	2.88 (br. *s*)	58.8	3.54 (br. *d*, J=13.7)	45.7
H_b–C(7)	–		3.34 (br. *d*, J=13.5)	
C(8)	–	36.7	–	47.7
H–C(9)	1.74–1.79 (*m*)	54.2	1.61–1.66 (*m*)	51.8
C(10)	–	49.7	–	77.3
H_a–C(11)	2.08–2.13 (*m*)	35.8	1.86–1.87 (*m*)	21.0
H_b–C(11)	1.55–1.63 (*m*)		1.74–1.79 (*m*)	
H_a–C(12)	1.70–1.74 (*m*)	20.3	2.18 (*dd*, J=15.7, 9.4)	27.7
H_b–C(12)	1.31–1.34 (*m*)		1.63–1.69 (*m*)	
H_a–C(13)	1.63–1.70 (*m*)	22.9	2.08 (*dd*, J=14.0, 9.1)	23.0
H_b–C(13)	1.49–1.56 (*m*)		1.35–1.39 (*m*)	
H_a–C(14)	1.44–1.53 (*m*)	33.8	1.97–1.98 (*m*)	32.6
H_b–C(14)	1.31–1.44 (*m*)		1.00–1.02 (*m*)	
H_a–C(15)	1.80–1.87 (*m*)	30.3	1.90–1.95 (*m*)	29.4
H_b–C(15)	1.73–1.82 (*m*)		1.42–1.44 (*m*)	
H_a–C(16)	1.39–1.47 (*m*)	26.4	1.90–1.94 (*m*)	25.4
H_b–C(16)	1.27–1.29 (*m*)		1.42–1.45 (*m*)	
H_a–C(17)	1.72–1.78 (*m*)	40.3	2.27 (*dd*, J=14.0, 6.3)	39.6
H_b–C(17)	1.53–1.59 (*m*)		1.93–1.96 (*m*)	
H–C(18)	1.73–1.78 (*m*)	28.2	1.91–1.96 (*m*)	29.6
Me(19)	0.94 (*d*, J=6.6)	21.1	0.93 (*d*, J=6.4)	20.7
Me(20)	1.03 (*d*, J=6.4)	21.6	1.10 (*d*, J=6.4)	22.1
Me(21)	0.81 (*s*)	20.8	1.08 (*s*)	25.4
H–C(22)	1.56–1.63 (*m*)	51.4	2.44 (*dd*, J=10.1, 7.2)	50.6
C(23)	–	50.1	–	50.0
Me(24)	1.01 (*s*)	16.6	1.02 (*s*)	17.0
H–C(25)	4.85 (*s*)	99.2	4.83 (*s*)	99.1
H–C(26)	4.76 (*d*, J=5.3)	73.4	4.75 (*d*, J=5.2)	73.8
H_a–C(27)	1.89–1.94 (*m*)	25.7	1.90–1.95 (*m*)	25.6
H_b–C(27)	1.59–1.64 (*m*)		1.60–1.65 (*m*)	
H_a–C(28)	1.51–1.60 (*m*)	27.6	1.44–1.48 (*m*)	27.5
H_b–C(28)	1.31–1.38 (*m*)		1.30–1.36 (*m*)	
C(29)	–	84.5	–	83.8
Me(30)	1.32 (*s*)	26.4	1.29 (*s*)	25.9
Me*C*O	–	170.4	–	169.9
*Me*CO	2.08 (*s*)	21.4	2.05 (*s*)	21.5

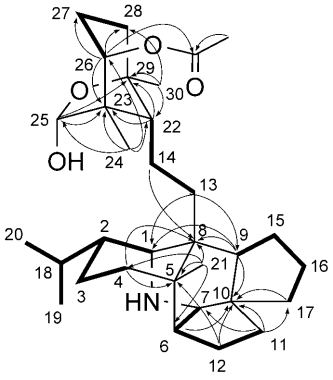

Fig. 1. *$^1H,^1H$-COSY* (▬) *and selected HMBC* (H → C) *correlations* (↔) *of* **1**

relation between H–C(26) and the Ac C=O resonance. The HMBC correlations of Me(24)/C(22), Me(24)/C(23), Me(24)/C(25), Me(24)/C(26) indicated that Me(24), C(25), and C(26) were connected to C(23); similarly, the HMBC correlations of Me(30)/C(22), Me(30)/C(28), and Me/C(29) indicated that C(22), C(28), and Me(30) were bonded to C(29). The HMBC correlation from H–C(25) to C(29) allowed us to make a linkage between C(25) and C(29) *via* an O-atom as part of a furan ring.

The observed ROESY cross-peaks (*Fig. 2*) confirmed the relative configuration of **1**. The ROESY correlations of H_a–C(4)/H–C(2), H_a–C(4)/Me(21), Me(21)/H_b–C(4), Me(21)/H_b–C(12), Me(21)/CH_2(13), Me(21)/H–C(9), and H–C(9)/H_b–C(11) revealed that the relative configuration of the alkaloidal moiety of **1** was identical with that of secodaphniphylline [11]. The ROESY spectrum also showed correlations of H–C(25)/H–C(26), H–C(25)/H_a–C(27), and H–C(22)/H–C(28), thus confirming the configuration at C(25). From these data, the structure of daphnilongeridine (**1**) was fully established.

The NMR data of **2** were essentially the same as those of the known alkaloid daphmacropodine [8]. Nevertheless, we fully assigned all ^{1}H- and ^{13}C-NMR signals (*Table*) by $^1H,^1$H-COSY, HSQC, and HMBC experiments, which confirmed that **2** was, indeed, daphmacropodine. Since the configuration at C(25) of **2** had not been determined previously, we performed a ROESY experiment (*Fig. 3*). The ROESY correlations of H_a–C(27)/H–C(25), H–C(26)/H–C(25), H_a–C(27)/H–C(26), and H_b–C(27)/H–C(26) suggested that the configuration at C(25) in **2** was same as that in **1**, and this was further supported by essentially identical NMR signals for the side chains.

The remaining known alkaloids, daphmacrine (**3**) [9], codaphniphylline (**4**) [10], daphniyunnine A (**5**) [4a], daphniyunnine C (**6**) [4a], and daphniyunnine E (**7**) [4a], were identified by comparison of their NMR data with those reported in the literature.

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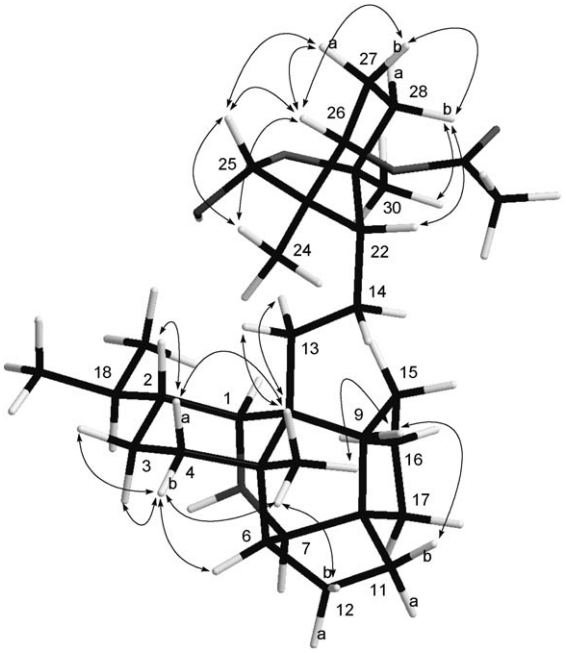

Fig. 2. *Key ROESY correlations* (↔) *of* **1**

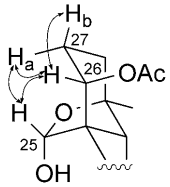

Fig. 3. *Key ROESY correlations* (↔) *near C(25) of* **2**

Experimental Part

General. All solvents were of anal. grade (*Shanghai Chemical Plant*, Shanghai). Column chromatography (CC): silica gel (200–300 mesh), silica gel *H60*. Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectra: *Perkin-Elmer-577* spectrometer; in cm^{-1}. NMR Spectra: *Varian Mercury-400* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. EI-MS (70 eV): *Finnigan MAT-95* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The stems and leaves of *Daphniphyllum longeracemosum* ROSENTH. were collected from Maguan County, Yunnan Province, P. R. China, in November 2004. The plant was identified by Dr. *Qiang Fan*, Institute of Botany, School of Life Sciences, Zhongshan University, China. A voucher specimen (DL-2004-1Y) was deposited at the Shanghai Institute of Materia Medica.

Extraction and Isolation. The dried and powdered stems and leaves (5.2 kg) of *D. longeracemosum* were percolated with 95% EtOH (3×). After solvent removal under reduced pressure, the crude extract (0.8 kg) was suspended in H_2O (1500 ml), and the pH was adjusted to *ca*. 4 by addition of 10% aq. H_2SO_4. The mixture was immediately defatted with AcOEt (4×1000 ml), and the aq. phase was brought to pH 10 with 30% aq. Na_2CO_3 soln., and then exacted with $CHCl_3$ (4×300 ml) to afford crude alkaloids (3.20 g). The crude alkaloids were subjected to column chromatography (CC) (SiO_2; gradient of petroleum ether (PE)/AcOEt/Et_2NH 20:1:0.3 → 1:1:0.3 (*v*/*v*/*v*)) to afford seven major fractions (*Fr. 1–7*). *Fr. 1* (1.3 g) was purified by CC (SiO_2; PE/i-PrOH 1:1) to afford two major fractions, which were resubjected each to

CC (SiO_2; PE/AcOEt/ Et_2NH 10:1:0.3) to yield **3** (0.02 g) and **4** (0.13 g), resp. *Fr. 3* (0.58 g) was crystallized to afford **5** (0.50 g), and the mother liquor was further purified by CC (SiO_2; PE/AcOEt/Et_2NH 6:1:0.1) to afford **1** (50 mg) and **2** (30 mg). *Fr. 6* (0.49 g) was separated by repeated CC (SiO_2, PE/AcOEt/ Et_2NH 3:1:0.3; then SiO_2, $CHCl_3$/MeOH 50:3) to yield **6** (10 mg) and **7** (20 mg).

Daphnilongeridine (=*(1*S*,2*S*,5*S*,7*R*,8*R*)-7-Hydroxy-1,5-dimethyl-8-[2-[(3*aR*,6*S*,6*aS*,9*R*,10*S*,10*aR*,10*bR*)-6a-methyl-9-(1-methylethyl)dodecahydro-10*aH-10,3a,6-(epiminomethanetriyl)benzo[e]azulen-10a-yl]ethyl]-6-oxabicyclo[3.2.1]oct-2-yl Acetate*; **1**). Colorless powder. $[\alpha]_D^{24} = +45.0$ ($c = 0.275$, MeOH). IR (KBr): 3396, 3273, 2947, 2870, 1741, 1589, 1454, 1379, 1244, 1209, 1026, 986, 729. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 513 (87, M^+), 470 (10), 454 (15), 408 (12), 360 (12), 286 (100), 272 (12), 216 (39). HR-EI-MS: 513.3824 (M^+, $C_{32}H_{51}NO_4^+$; calc. 513.3818).

REFERENCES

[1] J. Kobayashi, H. Morita, in 'The Alkaloids', Ed. G. A. Cordell, Academic Press, New York, 2003, Vol. 60, p. 165; S. Yamamura, Y. Hirata, *Tetrahedron Lett.* **1974**, *15*, 3673; C. H. Heathcock, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14323; C. H. Heathcock, *Angew. Chem., Int. Ed.* **1992**, *31*, 665.

[2] H. Morita, N. Ishioka, H. Takatsu, T. Iizuka, J. Kobayashi, *J. Nat. Prod.* **2006**, *69*, 418; H. Morita, N. Ishioka, H. Takatsu, T. Shinzato, Y. Obara, N. Nakahata, J. Kobayashi, *Org. Lett.* **2005**, *7*, 459; H. Morita, J. Kobayashi, *Org. Lett*. **2003**, *5*, 2895; J. Kobayashi, H. Takatsu, Y. C. Shen, H. Morita, *Org. Lett*. **2003**, *5*, 1733; H. Morita, H. Takatsu, J. Kobayashi, *Tetrahedron* **2003**, *59*, 3575.

[3] A. Jossang, H. E. Bitar, V. C. Pham, T. Sévenet, *J. Org. Chem.* **2003**, *68*, 300.

[4] a) H. Zhang, S. P. Yang, C. Q. Fan, J. Ding, J. M. Yue, *J. Nat. Prod*. **2006**, *69*, 553; b) S. P. Yang, H. Zhang, H. D. Cheng, J. M. Yue, *J. Nat. Prod.* **2006**, *69*, 79; c) Z. J. Zhan, C. R. Zhang, J. M. Yue, *Tetrahedron* **2005**, *61*, 11038; d) X. Chen, Z. J. Zhan, J. M. Yue, *Helv. Chim. Acta* **2005**, *88*, 854; e) Z. J. Zhan, S. P. Yang, J. M. Yue, *J. Org. Chem.* **2004**, *69*, 1726; f) S. P. Yang, J. M. Yue, *Org. Lett.* **2004**, *6*, 1401; g) S. P. Yang, J. M. Yue, *J. Org. Chem.* **2003**, *68*, 7961.

[5] H. E. Bitar, V. H. Nguyen, A. Gramain, T. Sévenet, B. Bodo, *Tetrahedron Lett.* **2004**, *45*, 515; H. E. Bitar, V. H. Nguyen, A. Gramain, T. Sévenet, B. Bodo, *J. Nat. Prod.* **2004**, *67*, 1094.

[6] a) Y. T. Di, H. P. He, Y. Lu, P. Yi, L. Li, L. Wu, X. J. Hao, *J. Nat. Prod.* **2006**, *69*, 1074; b) S. Z. Mu, Y. Wang, H. P. He, X. W. Yang, Y. H. Wang, Y. T. Di, Y. Lu, Y. Chang, X. J. Hao, *J. Nat. Prod.* **2006**, *69*, 1065.

[7] M. Zhen, T. L. Min, in 'Chinese Flora' [*Zhongguo Zhiwu Zhi*], Science Press, Beijing, 1980, Vol. 45(1), p. 1.

[8] T. Nakano, M. Hasegawa, Y. Saeki, *J. Org. Chem.* **1973**, *38*, 2404.

[9] T. Nakano, Y. Saeki, *Chem. Commun.* **1968**, 600.

[10] H. Irikawa, N. Sakabe, S. Yamamura, Y. Hirata, *Tetrahedron* **1968**, *24*, 5691.

[11] H. Morita, N. Yoshida, J. Kobayashi, *Tetrahedron* **1999**, *55*, 12549.

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