Alkaloids from the Leaves and Stems of *Daphniphyllum calycinum*

by **Zha-Jun Zhan**[a]), **Gui-Wei Rao**[a])[b]), **Xiao-Rong Hou**[a]), **Cheng-Ping Li**[b]), and **Wei-Guang Shan***[a])

[a]) College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310014, P. R. China (phone: +86-571-88871030; e-mail: tianranyaowu@zjut.edu.cn)

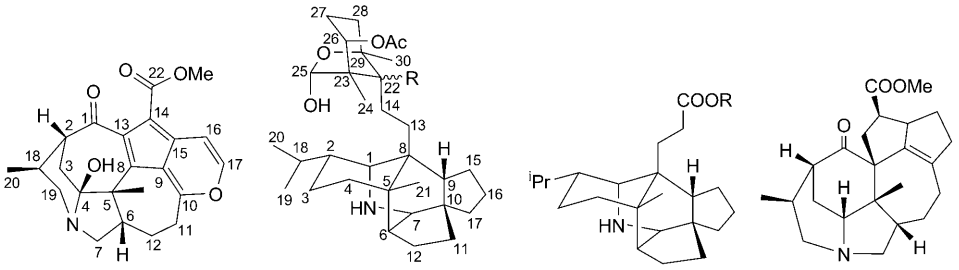
[b]) College of Biology and Environment Engineering, Zhejiang Shuren University, Hangzhou 310015, P. R. China

One new alkaloid, named caldaphnidine H (**1**), together with eight known ones, daphnioldhanin G (**2a**), methyl homosecodaphniphyllate (**3**), daphnezomine M (**4**), daphniyunine A (**5**), calyciphylline A (**6**), deoxycalyciphylline B (**7**), deoxyisocalyciphylline B (**8**), and calyciphylline B (**9**) was isolated from the leaves and stems of *Daphniphyllum calycinum*. The structure of **1** was established by spectral methods, especially 2D-NMR techniques. The structure of daphnioldhanin G (**2b**) reported previously was revised to **2a** mainly by single-crystal X-ray diffraction and spectral analysis.

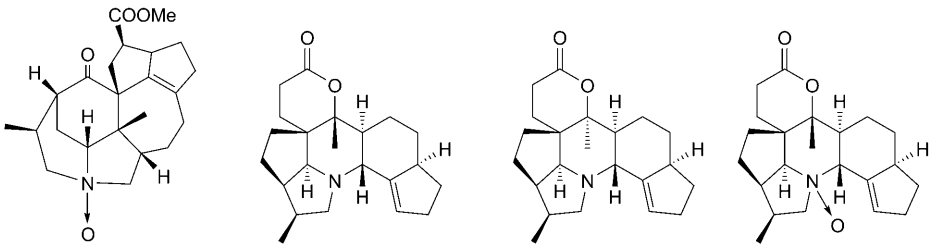
Introduction. – The genus of *Daphniphyllum* (Daphniphyllaceae) comprising about 30 species, is endemically distributed over the southeast of Asia, and 10 species grow in southern China [1]. They are distinguished for producing a group of highly complex and diversified polycyclic alkaloids that have been challenging subjects in natural products and biogenetic and synthetic programs. After the first *Daphniphyllum* alkaloid daphnimacin discovered from *D. macropodum* in 1966, more than 130 *Daphniphyllum* alkaloids have been isolated from this genus to date [2].

D. calycinum BENTH., an evergreen shrub, is native to the south of China. Its leaves and seeds are used as traditional Chinese medicine with several indications, such as their use as antipyretic and anti-inflammatory and curing influenza [1]. The previous studies on this species resulted in the isolation of a series of *Daphniphyllum* alkaloids [3–17], some of which showed cytotoxic activity [12][14]. A few flavonoid glycosides with antioxidative activity were also isolated from this plant [18]. In our continuing search for the structurally unique and biogenetically interesting *Daphniphyllum* alkaloids [4][10]][19], a new highly unsaturated hexacyclic alkaloid, caldaphnidine H (**1**), was isolated from the leaves and stems of *D. calycinum*, together with eight known ones, *i.e.*, daphnioldhanin G (**2a**) [20], methyl homosecodaphniphyllate (**3**) [21], daphnezomine M (**4**) [22], daphniyunine A (**5**) [23], calyciphylline A (**6**) [12], deoxycalyciphylline B (**7**), deoxyisocalyciphylline B (**8**) [24], and calyciphylline B (**9**) [12]. The structure **2b** was first reported by *Hao* and co-workers [20] for daphnioldhanin G isolated from *D. oldhami*. The extensive spectroscopic analysis, especially single-crystal X-ray diffraction, showed that this structure should be revised to **2a**. In this article, we report the isolation and structure elucidation of **1**, and the structure revision of daphnioldhanin G.

Results and Discussion. – Caldaphnidine H (**1**) was obtained as an optically active, pale yellow powder. The molecular formula of **1**, as determined by HR-ESI-MS (*m*/*z* at



1[1]) **2a**[1]) R = α-H **2b**[1]) R = β-H **3** R = Me **4** R = H **5**



6 **7** **8** **9**

396.1806 ($[M+H]^+$), is $C_{23}H_{25}NO_5$ with 12 double-bond equivalents. The IR absorptions revealed the presence of ester C=O, ketone, and OH functionalities (1711, 1666, and 3434 cm^{-1}, resp.), which were confirmed by ^{13}C-NMR signals at δ(C) 209.9 and 166.4 (*Table 1*). The ^{1}H-NMR spectrum of **1** displayed signals for two Me groups (δ(H) 0.91 (*s*) and 1.54 (*s*)), two olefinic H-atoms (δ(H) 7.67 (*s*) and 7.66 (*s*)), and a MeO group (δ(H) 3.82 (*s*)) (*Table*). In the ^{1}H-NMR spectrum, a *s* at δ(H) 5.77 showing no correlation in the HMQC spectrum, was indicative of an exchangeable H-atom, such as OH. In accord with the molecular formula of **1**, a total of 23 signals were discernible in the ^{13}C-NMR spectrum, and categorized by DEPT experiments as two C=O groups, three tetrasubstituted C=C bonds, two sp^2 secondary C-atoms, two sp^3 quaternary C-atoms, and three sp^3 CH, five sp^3 CH_2, and three Me groups. Among them, two sp^3 secondary C-atoms (δ(C) 57.7 and 50.7) were ascribed to those bearing an N-atom, and the downfield quaternary C-atom signal at δ(C) 93.8 was due to a hemiaminal C-atom. The established functionalities accounted for six degrees of unsaturation, and a hexacyclic core was thus required for **1** to account for the remaining six double-bond equivalents. The data mentioned above showed that **1** is a daphnicyclidin A-type alkaloid [25][26].

The scaffold of **1** was constructed by comprehensive collation of the 2D-NMR data including ^{1}H,^{1}H-COSY, HMQC, and HMBC data. The ^{1}H- and ^{13}C-NMR and HMQC spectra allowed to attribute all H-atoms to their respective bonding C-atoms. Three H-atom-bearing and spin-coupling subunits, drawn with bold bonds, were established by

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$; 500 and 125 MHz, resp.) *of Compound* **1**[1]). δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
C(1)	–	209.9	C(13)	–	96.4
H–C(2)	3.02–3.06 (*m*)	47.5	C(14)	–	122.1
CH_2(3)	2.49–2.54 (*m*, H_β),	37.2	C(15)	–	132.6
	2.14 (*d*, J = 15.3, H_α)		H–C(16)	7.66 (*s*)	110.7
C(4)	–	93.8	H–C(17)	7.67 (*s*)	143.3
C(5)	–	51.1	H–C(18)	2.50–2.55 (*m*)	37.4
H–C(6)	2.93–2.97 (*m*)	40.9	CH_2(19)	3.25 (*dd*, J = 15.5, 10.4, H_α),	50.7
CH_2(7)	3.04–3.08 (*m*, H_β),	57.7		2.69 (*dd*, J = 15.5, 3.1, H_β)	
	2.24–2.20 (*m*, H_α)		Me(20)	0.91 (*d*, J = 6.8)	13.2
C(8)	–	141.5	Me(21)	1.54 (*s*)	25.3
C(9)	–	126.2	C(22)	–	166.4
C(10)	–	165.1	MeO	3.82 (*s*)	51.2
CH_2(11)	3.57–3.64 (*m*, H_β),	29.9	OH	5.77 (*s*)	–
	2.95–2.99 (*m*, H_α)				
CH_2(12)	2.49–2.55 (*m*, H_β),	26.5			
	1.60–1.67 (*m*, H_α)				

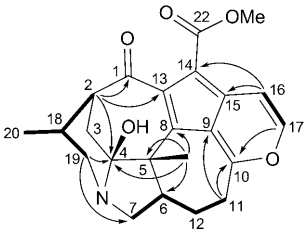


Fig. 1. *Selected ^{1}H,^{1}H-COSY* (—) *and HMBC* (H → C) correlations of **1**[1])

the ^{1}H,^{1}H-COSY data (*Fig. 1*). The assemblage of all C-atoms, including quaternary C-atoms and hetero atoms was mainly made by means of a HMBC experiment (*Fig. 1*). The linkages of C(19), C(7), and C(4) to the only N-atom were established by the HMBCs H–C(19)/C(7) and H–C(19)/C(4). Connections between C(4), C(6), C(8), and Me(21) *via* C(5) were suggested by the HMBC cross-peaks Me(21)/C(4), C(5), C(6), and C(8). The HMBCs H–C(2)/C(1) and C(13) were indicative of the linkage of C(1) and C(13). The HMBC H–C(17)/C(10) indicated that C(10) and C(17) are linked through an O-atom. One MeO group connected to C(22) was indicated by the HMBC MeO/C(22). The connections C(8)–C(9), C(13)–C(14), and C(14)–C(22) were suggested by the characteristic UV maxima at 360 and 292 nm [26][27]. Thus, the constitutional formula of **1** was established.

The relative configuration of **1** was deduced by a NOESY analysis (*Fig. 2*). The NOEs Me(21)/H–C(6), H–C(6)/H_β–C(7), and Me(21)/OH–C(4) indicated that Me(21), H–C(6), H_β–C(7), and OH–C(4) were on the same side of the molecular plane, tentatively assumed as the β-side. As a consequence, the NOE correlations H_β–C(7)/H_β–C(19), H_β–C(19)/Me(20), and Me(20)/H–C(2) indicated that H–C(2) and Me(20) were also β-configured. Thus, the relative configuration of caldaphnidine H (**1**) was established. Complete ^{1}H- and ^{13}C-NMR assignments (*Table*) were achieved by

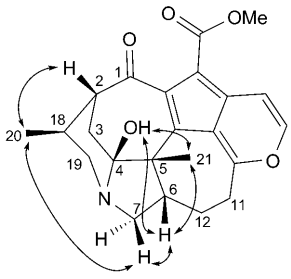


Fig. 2. *Key NOESY correlations and relative configuration of* **1**[1])

a combination of two-dimensional NMR techniques, including ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY.

Compound **2a** was obtained as optically active colorless crystals. It has the molecular formula $C_{32}H_{51}NO_4$ by HR-ESI-MS analysis (*m/z* 395.1474 ($[M+Na]^+$)). Comparison of the ^{1}H- and ^{13}C-NMR and optical-rotation data with those reported in the literature showed that the structure of **2a** was identical with the one of daphnioldhanin G to which structure **2b** had been assigned [20]. The overlapping H-atom signals gave rise to some uncertainty concerning the relative configuration of **2a** as suggested from the NOESY data. Therefore, a single-crystal X-ray diffraction analysis was carried out to confirm the configuration of **2a**. A recrystallization of **2a** from $CHCl_3$ afforded colorless orthorhombic crystals, which were used for this experiment, the result of which is shown in *Fig. 3*[2]). Thus, the structure of daphnioldhanin G should be revised to **2a** with an α-positioned H-atom at C(22)[1]) (= H_{anti}–C(8) with respect to the 6-oxabicyclo[3.2.1]octane skeleton). The relative configuration of H–C(22) in daphnioldhanins D–F is the same as that of daphnioldhanin G as deduced by chemical correlations [20], so the structures of daphnioldhanins D–F should also be revised as to structures with H–C(22) in α-position.

The known alkaloids methyl homosecodaphniphyllate (**3**) [21], daphnezomine M (**4**) [22], deoxycalyciphylline B (**7**), and deoxyisocalyciphylline B (**8**) [24] were identified by spectral data (EI-MS and ^{1}H- and ^{13}C-NMR) and comparison with authentic samples (co-TLC). The other known alkaloids were identified as daphnioldhanin G (**2a**) [20], daphniyunine A (**5**) [23], calyciphylline A (**6**), and calyciphylline B (**9**) [12] by comparison of their NMR data with those reported in the literature.

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[2]) Crystallographic data of **2a**: $C_{65}H_{103}Cl_3N_2O_8$, M_r 1146.90, orthorhombic system, space group $P2_12_12_1$; $a = 10.6412$ (3), $b = 16.9126$ (5), $c = 35.4598$ (10) Å, $V = 6381.7$ (3) Å^3, $Z = 4$, $D_x = 1.194$ g/cm^3. A crystal of dimensions $0.37 \times 0.32 \times 0.17$ mm was used for measurements on a *Rigaku/MSC* four-circle diffractometer. CCDC-715081 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif.

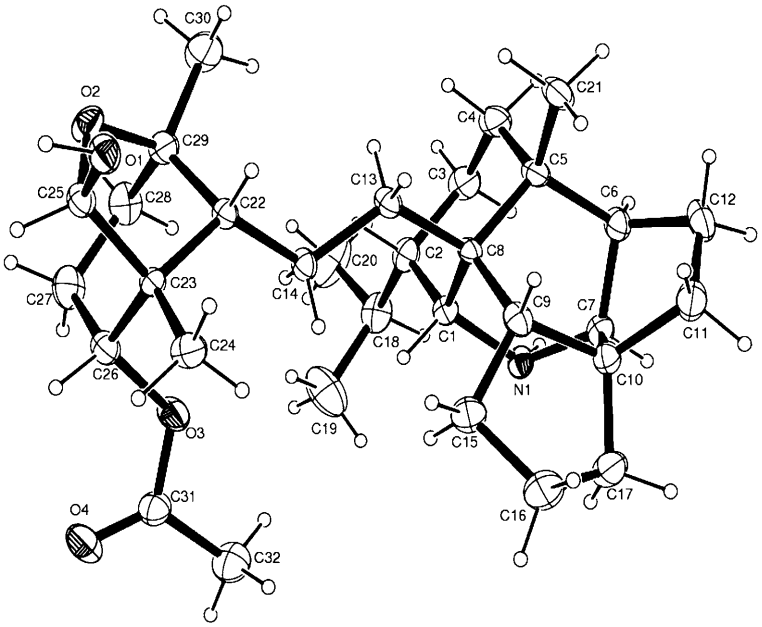

Fig. 3. *Single-crystal X-ray structure of* **2a**[1])

Experimental Part

General. All solvents used were of anal. grade (*Hangzhou Gaojing Fine Chemical Plant*); Thin-layer chromatography (TLC): pre-coated silica gel (SiO_2) GF_{254} plates (*Qingdao Haiyang Chemical Plant*). Column chromatography (CC): SiO_2 (230–400 mesh), SiO_2 *H-60*, *MCI-CHP20P* gel (75–150 μm; *Mitsubishi Chemical Industries Ltd.*), *Toyopearl-HW-40C* gel (*Tosoh Corporation*), and amino SiO_2 (*NH-DM 1020*, 20–45 μm; *Fuji Silysia Chemical Ltd.*). Optical rotations: *Rudolph-Autopol-IV* polarimeter. UV Spectra: *Shimadzu-UV-2450* spectrometer. IR Spectra: *Thermo-Nicolet-6700* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-AM-500* apparatus; δ in ppm rel. to Me_4Si, J in Hz. ESI-MS: *Agilent-6210-Lc/Tof* mass spectrometer; in *m/z*.

X-Ray Crystallography. Cell constants were determined by a least-squares fit to the setting parameters of 25 independent reflections measure with a *Rigaku-AFC7R* four-circle diffractometer by means of graphite-monochromated MoK_α radiation (λ 0.71073 Å) and operating in the $\varphi-\omega$ scan mode.

Plant Material. The plant material used for this study was collected from Guangxi Province, P. R. China, in June 2007, and identified by Prof. *Lan Tang* of the Zhejiang University of Technology, P. R. China. A voucher specimen (No. 20070614T) was deposited with the Zhejiang University of Technology.

Extraction and Isolation. The powdered stems and leaves of *D. calycinum* (35 kg) were extracted with 95% aq. EtOH (3×). After solvent removal, the crude extract (2500 g) was suspended in H_2O (12 l), and the pH adjusted to *ca.* 4 with 0.2M H_2SO_4. The acidic mixture was immediately extracted with AcOEt (5 × 2 l) to remove nonalkaloidal components. The aq. phase was brought to pH *ca.* 10 by addition of 1N Na_2CO_3 and extracted with $CHCl_3$ (6 × 500 ml) to give the crude alkaloid mixture (37.4 g). The crude alkaloids were then subjected to CC (SiO_2, petroleum ether/AcOEt 4:1 → 0:1, then $CHCl_3$/MeOH 4:1 → 0:1): *Fractions 1–6*. *Fr. 1* (3.0 g) was purified by CC (SiO_2, $CHCl_3$/MeOH 30:1): **3** (5.5 mg). *Fr. 2* (3.4 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH/Et_2NH 25:1:0.1): **5** (400 mg). Compound **2a** (1.87 g) was recrystallized from *Fr. 3* (3.1 g) with $CHCl_3$. *Fr. 4* (5.8 g) was separated by CC (amino SiO_2, $CHCl_3$/MeOH 20:1) and then purified by CC (*Toyopearl HW-40C*, MeOH): **7** (88 mg), **8** (101 mg), and **1** (12.6 mg). *Fr. 5* (2.72 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 30:1 → 15:1): **9**

(101 mg) and **6** (10.5 mg). *Fr. 6* (3.64 g) was separated by CC (*MCI-CHP20P* gel, MeOH/H_2O 6:4 → 0:1), and the fraction eluted with MeOH/H_2O 6:4 was recrystallized from MeOH; **4** (5.8 mg).

Caldaphnidine H (= rel-*(7a*R,*11*R,*12*S,*13a*R,*13b*R*)-6,7,7a,8,10,11,12,13,13a,13b-Decahydro-13a-hydroxy-11,13b-dimethyl-14-oxo-1,12-methanopyrano[4′,3′,2′:1,8]azuleno[4,5-*a*]indolizine-2-carboxylic Acid Methyl Ester*; **1**): Pale yellow powder. UV (MeOH): 360 (4.2), 292 (3.9) 273 (4.2). $[\alpha]_D^{20} = -198.0$ ($c = 0.8$, $CHCl_3$). IR (KBr): 3434 (OH), 2924, 1711 (C=O), 1666, 1629, 1440, 1383, 1316, 1234, 971. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 396 ($[M+H]^+$). HR-ESI-MS: 396.1806 ($[M+H]^+$, $C_{23}H_{26}NO_5^+$; calc. 396.1811).

'*Daphnioldhanin G* (= rel-*(1*R,*2*R,*5*R,*7*S,*8*R*)-8-{2-[(3*aS,*4*S,*5a*R,*6*S,*9*R,*9a*S,*9b*S,*10*R*)-Decahydro-9-methyl-6-(1-methylethyl)-3a,4,9-propan[1]yl[3]ylidene-3a*H*-cyclopenta[*c*]quinolin-9a(4*H*)-yl]ethyl}-1,5-dimethyl-6-oxabicyclo[3.2.1]octane-2,7-diol 2-Acetate*; **2a**). Colorless orthorhombic crystals from $CHCl_3$. $[\alpha]_D^{20} = -42.3$ ($c = 1.1$, $CHCl_3$). ESI-MS (pos.): 514 ($[M+H]^+$). HR-ESI-MS: 514.3902 ($[M+H]^+$, $C_{32}H_{52}NO_4^+$; calc. 514.3896).

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