Alkaloids from Fruits of *Daphniphyllum oldhamii*

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Seventeen *Daphniphyllum* alkaloids, including two new pentacyclic alkaloids, yuzuric acid (**1**) and daphnezomic acid (**2**), and 15 known ones, were isolated from the fruits of *Daphniphyllum oldhamii*. The structures and configuration of the two new alkaloids were determined on the basis of spectroscopic methods, especially 2D NMR techniques.

Introduction. – The genus *Daphniphyllum* (Daphniphyllaceae) comprises about 30 plant species distributed mainly in the southeast of Asia. Ten of them were found in southern China [1]. The highly complex *Daphniphyllum* alkaloids have been the attracting research programs of natural-products chemistry. A series of structurally diverse *Daphniphyllum* alkaloids have been isolated from different plant parts of *Daphniphyllum* species in our research group [2].

Daphniphyllum oldhamii (HEMSL.) ROSENTH. is a small evergreen tree. Its leaves and roots were applied in traditional Chinese medicine for the treatment of fever, snakebite, and fractures [3]. Our previous chemical investigation [4] on the leaves of *D. oldhamii* showed the presence of six polycyclic alkaloids, and subsequent studies on its aerial parts of fresh saplings and roots by *Hao* and co-workers [5] resulted in the isolation of 13 *Daphniphyllum* alkaloids. The fruits of this plant have not been chemically studied previously. In the present study, two new pentacyclic *Daphniphyllum* alkaloids, yuzuric acid (**1**) and daphnezomic acid (**2**), together with 15 known ones, daphnigraciline, daphnezomine R, daphnigracine, a zwitterionic alkaloid (O^{20}-deacetyl-5-de(acetyloxy)-8,9-didehydro-2-deoxy-8,12-dihydro-9,22-secoyuzurimin-23-oic acid), daphnezomine S, yuzurimic acid B, daphnilactone B and its methyl ester, daphnezomine H, yuzurimine B, paxdaphnines A and B, deoxyisocalyciphylline B, deoxycalyciphylline B, and calyciphylline B were isolated from the EtOH extract of the fruits of *D. oldhamii*. The structures of the new alkaloids were elucidated on the basis of spectroscopic methods, especially 2D NMR techniques.

Results and Discussion. – Alkaloid **1** was isolated as a white amorphous solid. Its molecular formula, $C_{23}H_{35}NO_4$, was determined on the basis of the molecular-ion peak at *m*/*z* 389.2545 in the HR-EI-MS. The IR spectrum showed absorption bands between 3000 and 2500 cm^{-1}, and at 1711 cm^{-1}, typical for the presence of a carboxylic acid. The ^{1}H- and ^{13}C-NMR (*Table*), HMBC (*Fig. 1*), and ROESY data (*Fig. 2*) showed that

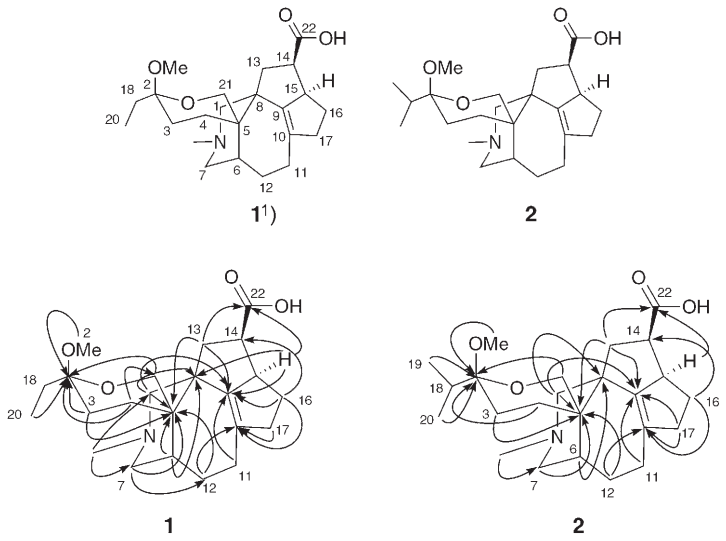

Fig. 1. *Key HMBC correlations* (H → C) *of compounds* **1** *and* **2**

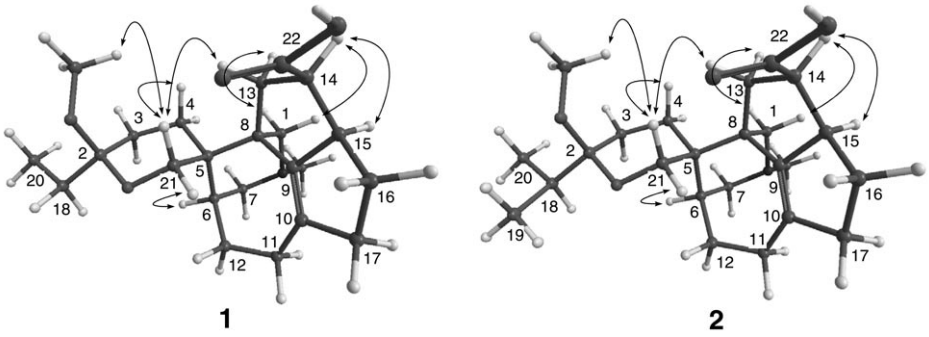

Fig. 2. *3D Structures and key ROESY correlations* (H ↔ H) *of compounds* **1** *and* **2**

compound **1** was the ester hydrolysate of yuzurine [6]. Compound **1** was named yuzuric acid.

The ^{1}H-NMR spectrum of **1** showed two geminal protons at δ(H) 4.25 (*d*, $J = 12.8$ Hz) and 3.82 (*dd*, $J = 12.8$, 2.2 Hz) assignable to CH_2(21), which is diagnostic for a yuzurine-type skeleton of a *Daphniphyllum* alkaloid. The resonances at δ(H) 3.19 (*s*) and 2.73 (*s*) were attributed to an MeO and a MeN group, respectively. A signal at δ(H) 0.85 (*t*, $J = 7.4$ Hz) was assigned to Me(20), indicating that an Et group was attached to C(2). The ^{13}C-NMR (with DEPT) spectrum confirmed the yuzurine-type skeleton of **1**. The signals at δ(C) 146.1 (*s*, C(9)) and 136.6 (*s*, C(10)) stem from a tetrasubstituted C=C bond. The resonance at δ(C) 100.8 (*s*, C(2)) revealed the presence of the ketal group. A C=O signal at δ(C) 181.2 (*s*, C(22)) confirmed the presence of a COOH group. Except for the presence of a C=C bond and the COOH group, 7 degrees of unsaturation in the molecule required a pentacyclic skeleton for

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

Table. *^{1}H- and ^{13}C-NMR* (with DEPT) *Data* (CD_3OD, 400 and 100 MHz), resp. *of Alkaloids* **1** *and* **2**[1]). δ in ppm, J in Hz.

	1		**2**	
	δ (H)	δ (C)	δ (H)	δ (C)
CH_2(1)	2.95 (br. *s*)	59.7 (*t*)	2.73 (*d*, J = 12.2, H_α), 2.67 (*d*, J = 12.2, H_β)	60.7 (*t*)
C(2)		100.8 (*s*)		102.7 (*s*)
CH_2(3)	1.64 – 1.67 (*m*)	29.3 (*t*)	1.72 – 1.76 (*m*, H_a), 1.36 (br. *d*, 10.3, H_b)	23.4 (*t*)
CH_2(4)	1.92 – 1.98 (*m*, H_α), 1.69 – 1.71 (*m*, H_β)	24.1 (*t*)	1.94 – 1.96 (*m*, H_α), 1.66 – 1.69 (*m*, H_β)	23.7 (*t*)
C(5)		37.9 (*s*)		37.9 (*s*)
H–C(6)	2.55 (br. *s*)	34.4 (*d*)	2.33 – 2.37 (*m*)	34.5 (*d*)
CH_2(7)	3.30 – 3.32 (*m*, H_a), 3.24 (*dd*, J = 13.1, 5.4, 1 H_b)	56.7 (*t*)	3.09 (br. *d*, J = 12.8, H_a), 2.97 – 2.99 (*m*, H_b)	57.1 (*t*)
C(8)		47.9 (*s*)		48.1 (*s*)
C(9)		146.1 (*s*)		147.2 (*s*)
C(10)		136.6 (*s*)		135.4 (*s*)
CH_2(11)	2.35 – 2.42 (*m*, H_a), 2.24 – 2.29 (*m*, H_b)	27.5 (*t*)	2.39 – 2.44 (*m*, H_a), 2.20 (*dd*, J = 16.2, 5.4, H_b)	27.9 (*t*)
CH_2(12)	1.74 – 1.76 (*m*)	27.5 (*t*)	1.68 – 1.71 (*m*)	28.0 (*t*)
CH_2(13)	1.61 – 1.64 (*m*, H_α), 2.82 – 2.87 (*m*, H_β)	41.4 (*t*)	1.55 – 1.58 (*m*, H_α), 2.80 – 2.85 (*m*, H_β)	41.7 (*t*)
H–C(14)	3.48 (br. *s*)	56.3 (*d*)	3.44 (br. *s*)	56.4 (*d*)
H–C(15)	2.76 – 2.79 (*m*)	46.0 (*d*)	2.74 – 2.77 (*m*)	46.6 (*d*)
CH_2(16)	1.88 – 1.93 (*m*, H_a), 1.53 – 1.61 (*m*, H_b)	30.1 (*t*)	1.86 – 1.90 (*m*, H_a), 1.57 – 1.62 (*m*, H_b)	30.4 (*t*)
CH_2(17)	2.60 – 2.67 (*m*, H_a), 2.32 – 2.38 (*m*, H_b)	44.1 (*t*)	2.55 – 2.60 (*m*, H_a), 2.29 – 2.35 (*m*, H_b)	44.0 (*t*)
CH_2(18) or H–C(18)	1.71 – 1.74 (*m*, H_a), 1.39 – 1.46 (*m*, H_b)	30.1 (*t*)	2.01 – 2.08 (*m*, 1 H)	33.0 (*d*)
Me(19)	–	–	0.92 (*d*, J = 6.4)	18.2 (*q*)
Me(20)	0.85 (*t*, 7.4)	8.5 (*q*)	0.84 (*d*, J = 7.0)	17.3 (*q*)
CH_2(21)	3.82 (*dd*, J = 12.8, 2.2, H_α), 4.25 (*d*, J = 12.8, H_β)	64.3 (*t*)	3.86 (*dd*, J = 12.5, 2.9, H_α), 4.21 (*d*, J = 12.9, H_β)	64.7 (*t*)
C(22)		181.2 (*s*)		182.3 (*s*)
Me–O	3.19 (*s*)	48.3 (*q*)	3.18 (*s*)	47.5 (*q*)
MeN	2.73 (*s*)	46.6 (*q*)	2.52 (*s*)	47.0 (*q*)

alkaloid **1**. A HMBC experiment confirmed the constitution of **1** (*Fig. 1*), in which the key correlations CH_2(13)/C(22) and H–C(15)/C(22) revealed that COOH was located at C(14); the correlations of MeO/C(2), Me(20)/C(2), and CH_2(21)/C(2) confirmed the presence of the ketal group and the location of the Et group; the position of the MeN group was confirmed by the correlations MeN/C(1) and MeN/C(7). The C(9)=C(10) bond was established by the mutual HMBC correlations CH_2(13)/C(9), CH_2(1)/C(9), H–C(15)/C(9), CH_2(12)/C(10), CH_2(16)/C(10), and CH_2(17)/C(10). The HMBC cross-peaks CH_2(13)/C(8), CH_2(11)/C(9), CH_2(7)/C(12) further supported the proposed constitutional formula of **1**. The relative configuration of **1** was determined to be the same as in yuzurine by the ROESY correlations CH_2(1)/H–C(14), H–C(14)/H–C(15), and CH_2(1)/H_a–C(13) which showed that these protons were on the same side of the ring and were arbitrarily assigned to be α-oriented. The ROESY correlations of

MeO/H_β–C(21), H_β–C(21)/H_β–C(4) showed that they were on the other side of the yuzurine-type ring system and were thus assigned the β-configuration.

Alkaloid **2** was obtained as a white amorphous solid. The HR-EI-MS molecular-ion peak at *m/z* 403.2725 revealed its molecular formula as $C_{24}H_{37}NO_4$, which is 14 mass units more than **1**, and 14 mass units less than daphnezomine R [7]. The IR spectrum also exhibited typical absorption bands between 3000 and 2500 cm^{-1} and at 1705 cm^{-1} indicating the presence of a carboxylic acid. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table*) showed high similarity to those of compound **1**. Comparison with the ^{1}H-NMR data of daphnezomine R indicated that **2** was likely the ester hydrolysate of daphnezomine R [7]. The HMBC and ROESY data (*Figs. 1* and *2*) confirmed the proposed structure of **2** which was named daphnezomic acid.

In the ^{1}H-NMR spectrum of **2**, two Me groups resonated at δ(H) 0.92 (*d*, *J* = 6.4 Hz) and 0.84 (*d*, *J* = 7.0 Hz) and a CH group at δ(H) 2.06 (*m*, 1 H), typical of an isopropyl group, which was tentatively placed at C(2). Compared to daphnezomine R, the MeO, signal of the COOMe group was absent in the ^{1}H-NMR spectrum of **2**. The HMBC plot revealed the key correlations Me(19)/C(2), Me(20)/C(2), MeO/C(2), CH_2(21)/C(2), and CH_2(3)/C(2), showing the existence of and ^{i}Pr, MeO, and ketal group and their connectivity (*Fig. 1*). The presence of a COOH group at C(14) was confirmed by the correlations CH_2(13)/C(22) and H–C(15)/C(22), and the correlations CH_2(1)/C(9) and CH_2(12)/C(10) further indicated the presence of a C(9)=C(10) bond. Thus, the constitution of **2** was elucidated. The relative configuration of **2** was identical with that of daphnezomine R as established by a ROESY experiment, in which the key correlations H_a–C(1)/H–C(14), H–C(14)/H–C(15), H_b–C(1)/H_α–C(13), H–C(6)/H_α–C(21), H_β–C(13)/H_β–C(21), and MeO/H_β–C(21) were observed (*Fig. 2*).

The chemical shifts in the vicinity of the N-atom of alkaloids **1** and **2** as compared with those of their corresponding methyl esters [6][7], and also their IR absorptions clearly indicated that yuzuric acid (**1**) and daphnezomic acid (**2**) were present as shown rather than in the form of the corresponding zwitterions.

On the basis of the ^{1}H- and ^{13}C-NMR and MS data, the fifteen remaining alkaloids were shown to be already known and identified as daphnigraciline [6][8], daphnezomine R [7], daphnigracine [6][8], a zwitterionic alkaloid [9] (O^{20}-deacetyl-5-de(acetyloxy)-8,9-didehydro-2-deoxy-8,12-dihydro-9,22-secoyuzurimin-23-oic acid), daphnezomine S[7], yuzurimic acid B [10], daphnilactone B and its methyl ester [11], daphnezomine H [11c], yuzurimine B [12], paxdaphnine A and B [13], deoxyisocalyciphylline B [2a], deoxycalyciphylline B [2a], and calyciphylline B [14].

Financial support from the *National Natural Science Foundation* (No. 20472093 and Key Project No. 30630072) and the *Shanghai Municipal Scientific Foundation* (Grant No. 06DZ22028) of the People's Republic of China is gratefully acknowledged. We thank professor *S.-Q.* Tang for the collection and identification of the plant material.

Experimental Part

General. All solvents were of anal. grade (*Shanghai Chemical Plant*, Shanghai, People's Republic of China). Column chromatography (CC): silica gel (200–300 mesh), C_{18} reversed-phase silica gel (150–200 mesh; *Merck*), *MCI* gel (*CHP20P*, 75–150 μm; *Mitsubishi Chemical Industries Ltd.*), or *Sephadex-LH-20* gel (*Amersham Biosciences*). TLC: precoated silica gel *GF254* plates (*Qingdao Haiyang Chemical Plant*, Qingdao, People's Republic of China). Semi-prep. HPLC; *Waters 515* pump; *Waters 2487* detector; *YMC-Pack-ODS-A* column (250 × 10 mm, S-5 μm, 12 nm). Optical rotations:

Perkin-Elmer 341 polarimeter. IR Spectra: *Perkin-Elmer 577* spectrometer; KBr disks, in cm^{-1}. NMR Spectra: *Bruker AM-400, Varian Inova-400,* or *Varian Inova-600* spectrometer; $SiMe_4$ as internal standard. EI-MS (70 eV) and ESI-MS: *Finnigan MAT95* and *Finnigan LC-Q^{DECA}* instrument, resp. in *m*/*z* (rel. %).

Plant Material. The fruits of *D. oldhamii* (HEMSL.) ROSENTH. was collected from Guangxi Province of P. R. China and authenticated by Prof. *Shao-Qing Tang*, Guangxi Normal University. A voucher specimen has been deposited at the Institute of Materia Medica, SIBS, Chinese Academy of Sciences (accession number: DO-T-frt-zg1Y).

Extraction and Isolation. The fresh fruits of *D. oldhamii* (4.9 kg) were extracted with 95% EtOH at r.t. to give a crude extract (135 g), which was dissolved in H_2O (1 l) to form a suspension and adjusted to pH *ca.* 3 with 2M H_2SO_4. The acidic suspension was first partitioned with petroleum ether (3 × 1 l) and AcOEt (3 × 1 l) to remove the neutral components. The aq. phase was then basified to pH *ca.* 10 with sat. Na_2CO_3 soln. and extracted with AcOEt (3 × 800 ml) to obtain 49 g of crude alkaloids. The latter were then subjected to CC (*MCI*): *Fractions* A_1–A_3. *Fr.* A_1 was further separated by CC (*Sephadex-LH-20*, 100% EtOH): *Fr.* A_{1a} and A_{1b}. *Fr.* A_{1a} was then separated by CC (silica gel, AcOEt/EtOH/Et_2NH 10:1:0.1) to give daphnilactone B (200 mg) and its methyl ester (15 mg), daphnezomine H (15 mg), as well as an alkaloid mixture, which was further purified by prep. HPLC (20% MeCN with 0.05% Et_2NH): **1** (11 mg) and **2** (9 mg). *Fr.* A_{1b} was purified by prep. HPLC (8% MeCN with 0.05% Et_2NH): yuzurimic acid B (8 mg). *Fr.* A_2 was subjected to CC (*Sephadex-LH-20*, 100% EtOH): *Fr.* A_{2a} and A_{2b}. *Fr.* A_{2a} was purified by prep. HPLC (12% MeCN): zwitter-ionic alkaloid (O^{20}-deacetyl-5-de(acetyloxy)-8,9-didehydro-2-deoxy-8,12-dihydro-9,22-secoyuzurimin-23-oic acid) (15 mg) and daphnezomine S (12 mg). *Fr.* A_{2b} was separated by CC (silica gel, CH_2Cl_2/MeOH/Et_2NH 30:1:0.1): paxdaphnine B (4 mg), yuzurimine B (9 mg), and daphnigracine (11 mg). *Fr.* A_3 was separated by CC (silica gel, AcOEt/MeOH 25:1): *Fr.* A_{3a}–A_{3d}. Each of the latter was then purified by CC (amino silica gel, cyclohexane/CHCl 20:1). *Fr.* A_{3a} afforded daphnigraciline (7 mg) and daphnezomine R (9 mg), *Fr.* A_{3b} deoxyisocalyciphylline B (9 mg) and deoxycalcyciphylline B (5 mg), *Fr.* A_{3c} calyciphylline B (8 mg), and *Fr.* A_{3d} paxdaphnine A (5 mg).

Yuzuric Acid (=*rel-(3′*R,*4*R,*6′*R,*8a*S,*9*S,*10a*S*)-6′-Ethyl-2,3,4,5,5′,6,6′,7,8,8a,9,10-dodecahydro-6′-methoxy-2-methyl(spiro[1*H*-4,10a-methanopentaleno[1,6-*cd*]azonine-11,3′(4′*H*)-[2*H*]pyran]-9-carboxylic Acid*; **1**): White amorphous solid. $[\alpha]_D^{20} = -23.6$ ($c = 0.59$, MeOH). IR (KBr): 3431, 2937, 2833, 2779, 1711, 1633, 1570, 1466, 1379, 1180, 1043, 891. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS (70 eV): 389 (4, M^+), 374 (12), 357 (15), 329 (8), 300 (21), 273 (10), 58 (100). HR-EI-MS: 389. 2545 (M^+, $C_{23}H_{35}NO_4^+$; calc. 389.2566).

Daphnezomic Acid (=*rel-(3′*R,*4*R,*6′*S,*8a*S,*9*S,*10a*S*)-2,3,4,5,5′,6,6′,7,8,8a,9,10-Dodecahydro-6′-methoxy-2-methyl-6′-(1-methylethyl)spiro[1*H*-4,10a-methanopentaleno[1,6-*cd*]azonine-11,3′(4*H*)-[2*H*]pyran]-9-carboxylic Acid*; **2**): White amorphous solid. $[\alpha]_D^{20} = -24.9$ ($c = 0.45$, MeOH). IR (KBr): 3440, 2958, 2835, 1705, 1581, 1468, 1367, 1209, 1099, 1036, 899. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS (70 eV): 403 (2, M^+), 388 (16), 371 (30), 328 (12), 300 (36), 58(100). HR-EI-MS: 403.2725 (M^+, $C_{24}H_{37}NO_4$; calc. 403.2723).

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Received June 28, 2007