Two New Iridoid Alkaloids, Daphmacropodosidines A and B, from *Daphniphyllum macropodum*

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Two new iridoid alkaloids, daphmacropodosidines A (**1**) and B (**2**), together with two known ones, daphcalycinosidine B and caldaphnidine F, were isolated from the fruits of *Daphniphyllum maropodum*. Their structures were established by spectral methods, especially 2D NMR spectra (^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY), as well as chemical means.

1. Introduction. – *Daphniphyllum* alkaloids with highly complex polycyclic structures are the secondary metabolites elaborated by plants of the genus *Daphniphyllum* [1]. Radioactive tracer experiments revealed that *Daphniphyllum* alkaloids were generated from six molecules of mevalonic acid *via* a squalene-like intermediate [2]. *Heathcock* and co-workers have done an outstanding work on a biomimetic total synthesis of several *Daphniphyllum* alkaloids [3]. In recent years, more than 60 new *Daphniphyllum* alkaloids were reported by *Kobayashi*, *Bodo*, *Yue*, *Hao*, and their co-workers [4–7]. However, only six iridoid alkaloids have been elucidated since the first one was reported by *Kobayashi* in 2003 [5][6c][8][9]. In our continuous research on structurally and biogenetically interesting *Daphniphyllum* alkaloids, two new iridoid alkaloids, daphmacropodosidines A (**1**) and B (**2**) (*Fig. 1*), together with two known ones, daphcalycinosidine B [5] and caldaphnidine F [6c], were isolated from the fruits of *Daphniphyllum macropodum*.

2. Results and Discussion. – Daphmacropodosidine A (**1**) was obtained as optically active, colorless crystals. Its molecular formula was established as $C_{40}H_{57}NO_{13}$ by HR-ESI-MS ($[M+H]^+$ at m/z 760.3910), indicating the presence of 13 degrees of unsaturation. The IR spectrum of **1** exhibited absorptions for OH (3422 cm^{-1}), C=O (1729 and 1643 cm^{-1}), and C=C functions. The UV absorption at 307.2 nm suggested the presence of a conjugated system.

Hydrolysis of **1** gave D-glucose and geniposidic acid. The ^{1}H- and ^{13}C-NMR (*Table*), ^{1}H,^{1}H-COSY (*Fig. 2*), HMQC, HMBC (*Fig. 2*), and NOESY data allowed to assign the structure and the absolute configuration of **1** as shown in *Fig. 1*. Daphmacropodosidine A (**1**) is the first example of an iridoid alkaloid in which the alkaloid moiety is located at C(3′) of the β-D-glucose moiety instead of C(6′) in all iridoid alkaloids reported.

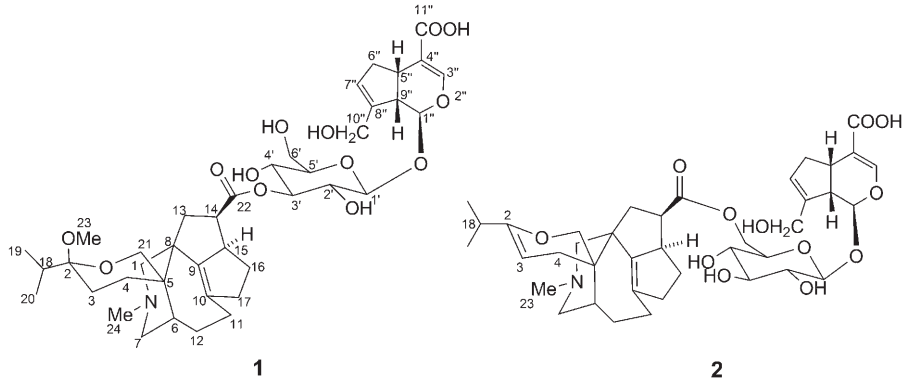

Fig. 1. *Daphmacropodosidine A* (**1**) *and B* (**2**). Trivial atom numbering.

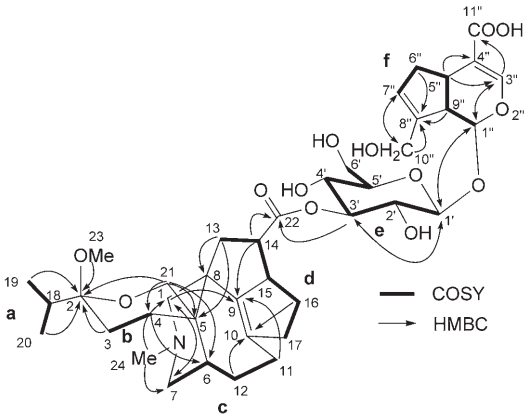

Fig. 2. *Selected 2D-NMR correlations for Daphmacropodosidine A* (**1**)

The ^{13}C-NMR spectrum of **1** showed 40 C-signals, attributed to 9 quaternary C-atoms (6 sp^2, 3 sp^3) and 14 CH (2 sp^2, 12 sp^3), 13 CH_2 (sp^3), and 4 Me (including 1 MeO and 1 MeN moieties). Among the 13 degrees of unsaturation, 2 were assigned to the O-bearing C=O (δ(C) 175.9 and 175.7) and 3 to C=C bonds (δ(C) 118.5 and 148.7, δ(C) 128.4 and 145.0, and δ(C) 135.4 and 146.5), thus **1** was inferred to possess 8 rings. Analysis of the ^{1}H,^{1}H-COSY (*Fig. 2*) and HMQC data of **1** led to the identification of the six partial structures **a** (C(18) to C(20)), **b** (C(3)–C(4)), **c** (C(6)–C(7) and C(11)–C(12)), **d** (C(13) to C(17)), **e** (C(1′) to C(6′), and **f** (C(5″) to C(7″) and C(9″) as well as three isolated CH_2, *i.e.*, CH_2(1), CH_2(21), and CH_2(10″)[1]). Further analysis of the HMBC plot allowed to attribute these six partial structures and three isolated CH_2 to three substructures: an alkaloid moiety, a glucose moiety, and a de-*O*-methylgenipin moiety, which were consistent with those of daphcalycinosidine B [5]. However, comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of daphcalycinosidine B revealed differences. In the ^{1}H-NMR spectrum, the signals of H–C(2′), H–C(3′), and H–C(4′) were shifted downfield from δ(H) 3.23, 3.40, and 3.30 in daphcalycinosidine B to δ(H) 3.35, 4.95, and 3.46 in **1**, and the signals of H–C(5′), H_a–C(6′), and H_b–C(6′) were shifted upfield from δ(H) 3.48, 4.48, and 4.14 in daphcalycinosidine B to δ(H) 3.32, 3.84, and 3.66 in **1**. Furthermore, in the ^{13}C-NMR spectra, the signals

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

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** and **2**. δ in ppm, J in Hz.

	1 (CD_3OD)		**2** (C_5D_5N)	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	2.55, 2.43 (each *d*, *J* = 10.8)	61.2 (*t*)	2.32, 2.01 (each *d*, *J* = 10.4)	64.4 (*t*)
C(2)		102.3 (*s*)		159.6 (*s*)
CH_2(3) or H−C(3)	1.73, 1.35 (2*m*)	22.8 (*t*)	4.45 (br. *d*, *J* = 4.2)	91.2 (*d*)
CH_2(4)	1.96, 1.58 (2*m*)	23.2 (*t*)	2.26 (*dd*, *J* = 16.8, 4.2), 1.88 (*d*, *J* = 16.8)	27.6 (*t*)
C(5)		37.7 (*s*)		37.2 (*s*)
H−C(6)	2.31 (*m*)	34.1 (*d*)	2.09 (*m*)	34.8 (*d*)
CH_2(7)	2.90 (*d*, *J* = 12.4), 2.77 (overlap)	56.7 (*t*)	2.63 (*d*, *J* = 11.6), 2.46 (*m*)	55.5 (*t*)
C(8)		47.3 (*s*)		46.8 (*s*)
C(9)		146.5 (*s*)		147.3 (*s*)
C(10)		135.4 (*s*)		133.5 (*s*)
CH_2(11)	2.11, 1.65 (2*m*)	27.8 (*t*)	2.64, 2.40 (2*m*)	27.6 (*t*)
CH_2(12)	2.45, 2.27 (2*m*)	27.8 (*t*)	1.97, 1.62 (2*m*)	27.7 (*t*)
CH_2(13)	2.76, 1.68 (2*m*)	40.5 (*t*)	2.47, 1.54 (2*m*)	40.0 (*t*)
H−C(14)	3.00 (*m*)	44.2 (*d*)	2.95 (*m*)	49.9 (*d*)
H−C(15)	3.55 (*m*)	56.0 (*d*)	3.53 (*m*)	55.5 (*d*)
CH_2(16)	1.83, 1.73 (2*m*)	29.0 (*t*)	1.96, 1.56 (2*m*)	28.9 (*t*)
CH_2(17)	2.57, 2.33 (2*m*)	43.6 (*t*)	2.48, 2.36 (2*m*)	43.0 (*t*)
H−C(18)	2.04 (*m*)	32.5 (*d*)	2.33 (*m*)	32.6 (*d*)
Me(19)	0.84 (*d*, *J* = 6.9)	16.7 (*q*)	1.08 (*d*, *J* = 6.8)	20.7 (*q*)
Me(20)	0.93 (*d*, *J* = 6.7)	17.7 (*q*)	1.08 (*d*, *J* = 6.8)	20.8 (*q*)
CH_2(21)	4.06, 3.88 (each *d*, *J* = 12.4)	64.3 (*t*)	4.72, 4.38 (each *d*, *J* = 11.6)	70.1 (*t*)
C(22)		175.9 (*s*)		175.3 (*s*)
Me(23)O or Me(23)N	3.17 (*s*)	47.2 (*q*)	2.11 (*s*)	46.6 (*q*)
Me(24)N	2.34 (*s*)	46.8 (*q*)		
H−C(1′)	4.77 (*d*, *J* = 7.6)	100.2 (*d*)	5.39 (*d*, *J* = 7.8)	100.7 (*d*)
H−C(2′)	3.35 (*m*)	73.2 (*d*)	4.06 (*m*)	74.9 (*d*)
H−C(3′)	4.95 (*t*, *J* = 7.0)	78.4 (*q*)	4.25 (*t*, *J* = 8.9)	78.2 (*d*)
H−C(4′)	3.46 (*m*)	69.7 (*d*)	3.99 (*t*, *J* = 8.9)	71.6 (*d*)
H−C(5′)	3.32 (*m*)	78.3 (*q*)	4.09 (*m*)	75.8 (*d*)
CH_2(6′)	3.84 (*d*, *J* = 12.0), 3.66 (*dd*, *J* = 12.0, 5.1)	62.2 (*t*)	5.01 (*d*, *J* = 11.3), 4.55 (*dd*, *J* = 11.3, 6.7)	64.6 (*t*)
H−C(1″)	5.05 (*d*, *J* = 7.5)	97.8 (*d*)	5.68 (*d*, *J* = 7.3)	97.7 (*d*)
H−C(3″)	7.22 (*s*)	148.7 (*d*)	7.94 (*s*)	151.9 (*d*)
C(4″)		118.5 (*s*)		113.2 (*s*)
H−C(5″)	3.21 (*m*)	37.7 (*d*)	3.57 (*m*)	36.2 (*d*)
CH_2(6″)	2.83, 2.06 (2*m*)	40.0 (*t*)	3.15, 2.44 (2*m*)	39.6 (*t*)
H−C(7″)	5.76 (*s*)	128.4 (*d*)	6.02 (*s*)	127.2 (*d*)
C(8″)		145.0 (*s*)		145.6 (*s*)
H−C(9″)	2.63 (*m*)	47.5 (*d*)	3.13 (*m*)	47.0 (*d*)
CH_2(10″)	4.27, 4.16 (each *d*, *J* = 13.2)	61.6 (*t*)	4.77, 4.65 (each *d*, *J* = 14.2)	61.0 (*t*)
C(11″)		175.7 (*s*)		169.8 (*s*)

of C(3′) and C(5′) were shifted downfield from δ(C) 77.6 and 75.8 in daphcalycinosidine B to δ(C) 78.4 and 78.3 in **1**, while the signals of C(2′), C(4′), and C(6′) were shifted upfield from δ(C) 74.8, 71.6, and 64.5 in daphcalycinosidine B to δ(C) 73.2, 69.7, and 62.2 in **1**. These differences suggested that **1** and daphcalycinosidine B were connected at different positions of the alkaloid and the glucose moieties. Moreover, in the HMBC of **1**, no correlation CH_2(6′)/C(22) was observed, indicating that the alkaloid moiety was not located at C(6′), while the presence of the strong correlation of H–C(3′) at δ(H) 4.95 with C(22) and anomeric C(1′) established the connection of the alkaloid moiety to C(3′) in **1**. In the NOESY plot, the strong correlations H–C(14)/H–C(15) and H–C(5″)/H–C(9″) indicated that **1** possessed the same relative configuration as daphcalycinosidine B.

Daphmacropodosidine B (**2**) was isolated as optically active white amorphous powder exhibiting UV absorptions at 308.6 (2.52) and 377.8 (2.26) nm. The HR-ESI-MS ($[M+H]^+$ at m/z 728.3626) suggested a molecular formula $C_{39}H_{53}NO_{12}$ with 14 degrees of unsaturation. Extensive analysis of the ^{1}H- and ^{13}C-NMR (*Table*), ^{1}H,^{1}H-COSY, HMBC, and NOESY data as well as their comparison with those of caldaphnidine F [6c], elucidated **2** as a dehydration derivative of caldaphnidine F.

The ^{13}C-NMR signals for C(2) and C(3) were shifted downfield from δ(C) 97.8 and 26.0 in caldaphnidine F to δ(C) 159.6 and 91.2 in **2**, which indicated the presence of a C=C bond between C(2) and C(3) in **2**. Furthermore the chemical shift of H–C(3) at δ(H) 4.45 was typical for an olefinic proton, and in addition, the HMQC data showed that the C(3) signal was arising from a methine moiety. The correlations H–C(3)/CH_2(4) in the ^{1}H,^{1}H COSY plot and H–C(3)/C(2), C(3), C(4), C(5), and C(18) in the HMBC plot supported this assignment.

Two known iridoid alkaloids were identified as daphcalycinosidine B [5] and caldaphnidine F [6c] by comparing the spectral data with those reported in the literature and with those of authentic samples.

Experimental Part

General. Optical rotations: *Jasco-DIP-370* digital polarimeter. M.p.: *YuHua-X-4* apparatus. IR Spectra: *Bio-Rad FTS-135* infrared spectrometer; KBr pellets; in cm^{-1}. UV Spectra: *UV-210A* spectrometer; λ_{max} (log ε) in nm. 1D and 2D-NMR Spectra: *DRX-500* spectrometers; δ in ppm, J in Hz; Me_4Si as internal standard, measured in CD_3OD or C_5D_5N. HPLC: *Agilent-1100* series; *Waters Spherisorb® NH$_2$* (5 μm, 4.6 × 250 mm) anal. column; *LiChroprep® Rp-18* (43–63 μm; *Merck*). Column chromatography (CC): silica gel (200–300 mesh; *Qindao Marine Chemical Factory*, Qindao, China).

Plant Material. The fresh fruits of *D. macropodum* were collected in Lushan of Jiangxi Province, P. R. China, in November 2005, and identified by Prof. *Xun Gong* of the Kunming Institute of Botany, CAS.

Extraction and Isolation. The fresh fruits of *D. macropodum* were extracted with 95% EtOH (3 × 40 l) for 4, 3, and 2 h at refluxing temp. The combined extract was concentrated, the residue suspended in H_2O, and after acidification with tartaric acid to pH 3, extracted with $CHCl_3$. The H_2O-soluble fraction was basified to pH 9 with 10% aq. NH_3 soln. and extracted with $CHCl_3$ and BuOH. The BuOH fraction was concentrated and afforded a mixtures of H_2O-soluble alkaloids (53 g), which was purified by CC (silica gel, $CHCl_3$/MeOH 99 : 1 → 1 : 1, then MeOH): *Fractions A–E*. *Fr. C* (12.8 g) was subjected to prep. HPLC on *RP-18*, 0 → 40% MeOH/H_2O): *Fr. C1–C5*. *Fr. C3* (2.1 g) was divided into *Fr. D1–D4* by CC (silica gel, AcOEt/MeOH 9 : 1 → 6 : 4, then MeOH). Daphmacropodosidine A (14 mg, **1**) was obtained from *Fr. D3* (33 mg) by prep. TLC ($CHCl_3$/MeOH 4 : 1). Daphmacropodosidine B (8 mg; **2**), daphcalycinosidine B (27 mg), and caldaphnidine F (16 mg) were isolated from 1/12 of *Fr. C4* (300 mg) by anal. HPLC (MeCN/H_2O 2 : 8).

Daphmacropodosidine A (= *1*S,*4a*S,*7a*S)*-1-{[3*-O*-{[(3′*S,*4*S,*6′*S,*8a*R,*9*R,*10a*R)*-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-yl]carbonyl}-β-*D*-glucopyranosyl}oxy}-1,4a,5,7a-tetrahydro-7-(hydroxymethyl)cyclopenta[*c*]pyran-4-carboxylic Acid*; **1**): Colorless crystals. M.p. 176–178° (MeOH). $[\alpha]_D^{29.3} = -43.5$ ($c = 0.84$, MeOH). UV (MeOH): 307.2 (2.73), 374.4 (2.42). IR (KBr): 3422, 2930, 1729, 1643, 1549, 1458, 1400, 1158, 1082, 1042. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 760.3910 ($[M+H]^+$, $C_{40}H_{58}NO_{13}^+$; calc. 760.3908).

Daphmacropodosidine B (= *(1*S*,4a*S*,7a*S*)-1-{[6*-O*-{[(3′*S*,4*S*,8a*R*,9*R*,10a*R*)-2,3,4,5,6,7,8,8a,9,10-Decahydro-2-methyl-6′-(1-methylethyl)spiro[1*H*-4,10a-methanopentaleno[1,6-*cd*]azonine-11,3′(4′*H*)-[2*H*]pyran]-9-yl]carbonyl}-β-*D*-glucopyranosyl}oxy}-1,4a,5,7-tetrahydro-7-(hydroxymethyl)cyclopenta[*c*]pyran-4-carboxylic Acid*; **2**): White amorphous powder. M.p. 196–200° (MeOH). $[\alpha]_D^{29.3} = 0.0$ ($c = 0.26$, MeOH). UV (MeOH): 308.6 (2.52), 377.8 (2.26). IR (KBr): 3425, 2927, 1735, 1641, 1549, 1452, 1397, 1169, 1146, 1086, 1044. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 728.3626 ($[M+H]^+$, $C_{39}H_{54}NO_{12}^+$; calc. 728.3646).

Hydrolysis of Daphmacropodosidine A (**1**) *and Daphmacropodosidine B* (**2**). A soln. of **1** (1 mg) in 1% KOH soln. (1 ml) was stirred at r.t. for 4 h. Then the mixture was acidified with 1M HCl. D-Glucose and geniposidic acid were identified by co-TLC with authentic samples.

Hydrolysis of **2** (1 mg) by the same procedure afforded also D-glucose and geniposidic acid.

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