Two New Alkaloids from *Daphniphyllum angustifolium* HUTCH.

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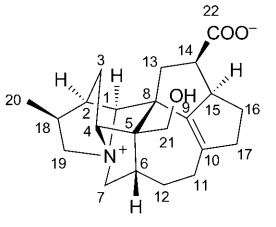
Daphangustifolines A and B (**1** and **2**), two new *Daphniphyllum* alkaloids were isolated from the whole plant of *Daphniphyllum angustifolium* HUTCH. Their structures were established on the basis of spectroscopic data. Their cytotoxicity against HL-60, MCF-7, and A549 cell lines was also evaluated.

Introduction. – *Daphniphyllum* alkaloids are a family of structurally diverse natural products with complex polycyclic systems elaborated by plants of the genus *Daphniphyllum* [1]. Their unique structural features have attracted great attention to their total synthesis and biosynthetic research [2]. In recent years, a series of novel *Daphniphyllum* alkaloids have been isolated and identified by our research group, which may be explained by the unique biogenetic process involving repeated fissions of C–C or C–N bonds followed by rearrangements and recyclization [3].

Daphniphyllum angustifolium HUTCH. (Daphniphyllaceae), an evergreen tree, is distributed mainly in the southern area of China [4]. The previous studies on the chemical constituents of this species resulted in the isolation of a few flavonoids. Up to now, the alkaloids of *D. angustifolium* have not been reported. In our ongoing search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids, two new alkaloids, named daphangustifoline A[1] (**1**) and B[1] (**2**) were isolated from the whole plant of *D. angustifolium*. Compound **1** is the fourth daphniglaucin-A-type *Daphniphyllum* alkaloid containing a 1-azoniatetracyclo$[5.2.2.0^{1,6}.0^{4,9}]$undecane ring system, while **2** is the first alkaloid with a Bz group in the genus of *Daphniphyllum*. This



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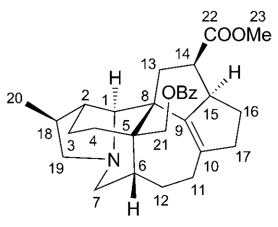

2[1]

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

article describes the isolation and structural elucidation of **1** and **2**, and their cytotoxicity against HL-60, MCF-7, and A549 cell lines were also evaluated.

Results and Discussion. – Daphangustifoline A (**1**) was obtained as an optically active white powder. The molecular formula of **1** was established as $C_{22}H_{29}NO_3$ by HR-ESI-MS (*m/z* 356.2228 ([*M* + H]$^+$), calc. 356.2225), with nine degrees of unsaturation. The IR absorption at 1569 cm^{-1} implied the presence of a COO^- anion functionality. ^{13}C-NMR and DEPT Spectra of **1** (*Table*) revealed 22 C-atoms due to one

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

	1			**2**	
	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.88–3.86 (*m*)	89.2	H–C(1)	3.59 (br. *s*)	68.7
H–C(2)	2.66–2.61 (*m*)	44.2	H–C(2)	2.81–2.73 (*m*)	36.4
H_a–C(3)	2.23–2.15 (*m*)	19.6	H_a–C(3)	2.17–2.13 (*m*)	33.3
H_b–C(3)	1.99–1.93 (*m*)		H_b–C(3)	1.87–1.83 (*m*)	
H–C(4)	3.96 (*d*, *J* = 6.0)	82.8	CH_2(4)	1.87–1.81 (*m*)	21.7
C(5)	–	59.1	C(5)	–	40.0
H–C(6)	3.01–3.06 (*m*)	41.4	H–C(6)	2.78–2.73 (*m*)	37.4
CH_2(7)	3.64–3.62 (*m*)	51.6	CH_2(7)	3.73 (br. *d*, *J* = 4.0)	57.5
C(8)	–	48.4	C(8)	–	44.5
C(9)	–	145.5	C(9)	–	143.2
C(10)	–	134.6	C(10)	–	137.8
H_a–C(11)	2.22–2.13 (*m*)	26.3	H_a–C(11)	2.48–2.38 (*m*)	25.5
H_b–C(11)	2.22–2.13 (*m*)		H_b–C(11)	2.27–2.21 (*m*)	
H_a–C(12)	2.18–2.15 (*m*)	22.5	H_a–C(12)	1.41–1.35 (*m*)	28.1
H_b–C(12)	1.92–1.85 (*m*)		H_b–C(12)	1.70–1.67 (*m*)	
H_a–C(13)	2.37–2.28 (*m*)	38.0	H_a–C(13)	2.79 (*d*, *J* = 2.0)	40.2
H_b–C(13)	2.17–2.11 (*m*)		H_b–C(13)	2.27–2.23 (*m*)	
H–C(14)	2.76–2.68 (*m*)	45.3	H–C(14)	3.04–2.94 (*m*)	44.1
H–C(15)	3.33–3.29 (*m*)	54.0	H–C(15)	3.67–3.62 (*m*)	55.5
H_a–C(16)	1.98–1.88 (*m*)	30.2	H_a–C(16)	2.17–2.13 (*m*)	28.2
H_b–C(16)	1.58–1.48 (*m*)		H_b–C(16)	1.94–1.90 (*m*)	
H_a–C(17)	2.58–2.51 (*m*)	42.5	H_a–C(17)	2.73–2.67 (*m*)	44.1
H_b–C(17)	2.34–2.27 (*m*)		H_b–C(17)	2.48–2.38 (*m*)	
H–C(18)	2.67–2.64 (*m*)	35.3	H–C(18)	2.73–2.68 (*m*)	38.9
H_a–C(19)	3.70–3.63 (*m*)	58.4	H_a–C(19)	3.98–3.92 (*m*)	65.4
H_b–C(19)	3.11–3.07 (*m*)		H_b–C(19)	2.98–2.94 (*m*)	
Me(20)	1.13 (*d*, *J* = 6.4)	13.0	Me(20)	1.18 (*d*, *J* = 6.4)	14.1
CH_2(21)	3.88 (*d*, *J* = 12.0)	56.5	CH_2(21)	4.70 (*dd*, *J* = 11.6, 15.4)	70.3
C(22)	–	181.5	C(22)	–	176.4
			Me(23)	3.68 (*s*)	51.9
			21-OBz:		
			C=O	–	167.8
			C(1′)	–	131.4
			H–C(2′,4′)	8.02 (*d*, *J* = 8.0)	130.5
			H–C(3′,5′)	7.51 (*t*, *J* = 7.6)	129.7
			H–C(6′)	7.64 (*t*, *J* = 7.6)	134.4

tetrasubstituted C=C bond (δ(C) 145.5 and 134.6), one C=O group (δ(C) 181.5), two sp^3 quaternary C-atoms, and nine sp^3 CH$_2$, seven sp^3 CH, and one Me group. Of these, two CH$_2$ groups (δ(C) 82.8 and δ(H) 3.96; δ(C) 89.2 and δ(H) 3.88–3.86) were ascribed as being attached to an N-atom, while one CH$_2$ group (δ(C) 56.5 and δ(H) 3.88) was ascribed as bearing an O-atom. One C=C bond and one C=O group accounted for two degrees of unsaturation; the remaining seven could only be attributed to the existence of a heptacyclic ring system. The data mentioned above implied that compound **1** is a daphniglaucin-A-type *Daphniphyllum* alkaloid. The ^{13}C-NMR data of **1** were similar to those of the known alkaloid daphniglaucin A [5], suggesting that the two alkaloids share the same basic skeleton. Further comparison of the NMR data of **1** with those of daphniglaucin A revealed that the main difference was the absence of a Me group in the carboxylate moiety at C(14) of **1**. Detailed analysis of the 2D-NMR data, including the HMQC, ^{1}H,^{1}H-COSY, and HMBC plots, confirmed the above deduction. The 1D-NMR and HMQC spectra allowed to attribute all H-atoms to their respective bonding C-atoms. Three structural units, **a** – **c**, drawn with bold bonds in *Fig. 1*, were established by the ^{1}H,^{1}H-COSY experiment. The assemblage of all C-atoms including the hetero atoms was mainly made by means of a HMBC experiment (*Fig. 1*). The location of the COO$^-$ group was determined by the HMBC H–C(14) (δ(H) 2.76–2.68)/C(22) (δ(C) 181.5). The structure of daphangustifoline A (**1**) was determined as the COO$^-$ anion form of daphniglaucin A. The relative configuration of **1** was elucidated to be the same as a boat form for daphniglaucin A from the NOESY data as shown in the computer-generated three-dimensional drawing (*Fig. 1*) [6]. The NOESY correlation H$_b$–C(3)/H$_a$–C(13) indicated that the cyclohexane ring C(1)–C(5) and C(8) supported by a *W*-type long-range coupling between H–C(1) and H–C(4), both equatorial, through an N-atom.

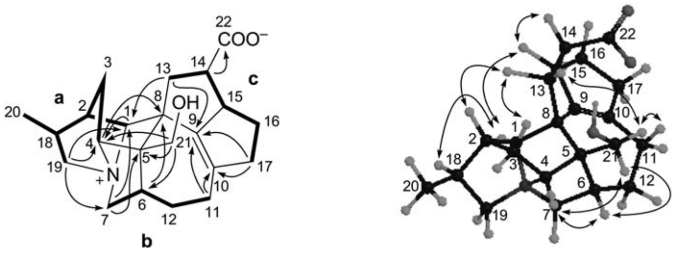

Fig. 1. *Key ^{1}H,^{1}H-COSY* (—), *HMBC* (H → C), *and NOESY* (H ↔ H) *features of* **1**

Daphangustifoline B (**2**) was obtained as optically active white amorphous powder. Its molecular formula was established as C$_{30}$H$_{37}$NO$_4$ by its HR-ESI-MS (*m/z* 476.2793 ([*M* + H]$^+$), with 13 degrees of unsaturation. IR Absorptions at 1721 and 1601 cm^{-1} and UV absorptions at 201 (3.7) and 228 (3.8) nm suggested the presence of an ester C=O and a Ph group. The ^{13}C-NMR and DEPT spectra of **2** (*Table*) revealed 30 signals due to a tetrasubstited C=C bond (δ(C) 143.2 and 137.8), a Bz group (δ(C) 167.8, 131.4, 130.5 (2 C), 129.7 (2 C), and 134.4), an ester C=O group (δ(C) 176.4), two sp^3 quaternary C-atoms, six sp^3 CH, ten sp^3 CH$_2$, and two Me groups including one MeO group. Among them, one CH (δ(C) 68.7) and two CH$_2$ groups (δ(C) 57.5 and 65.4) were typical for N-substituted groups, and one CH_2 groups (δ(C) 70.3) was attributed to be connected with an O-atom. Extensive comparison of the NMR data of **2** (*Table*) with those of other *Daphniphyllum* alkaloids implied that **2** has a yuzurimine-type skeleton; the data showed high similarity to those of the known compound macrodaphniphyllidine, except that the AcO group in macrodaphniphyllidine was replaced by a BzO group in **1** [5]. This assignment of the BzO group to C(21) was confirmed by the HMBC CH_2(21)/C=O of BzO (δ(C) 167.8). Further 2D-NMR experiments including HMQC, ^{1}H,^{1}H-COSY, and HMBC as shown in *Fig. 2*, confirmed the above elaboration. The relative configuration of **2** was established from the NOESY correlations Me(20)/H_a–C(19), H–C(2)/H_a–C(13) and H_a–C(4), H_a–C(4)/H_b–C(13), and CH_2(21)/H–C(14), which suggested the β-orientation of Me(20) and H_a–C(19) and the chair conformations of a cyclohexane (C(1) to C(5) and C(8)) and a piperidine ring (N, C(1), C(8), and C(5) to C(7)) of the 2-azabicyclo[3.3.1]nonane moiety (*Fig. 2*). Thus, the structure of **2** was determined as 21-*O*-benzoylyuzurimine B, named daphangustifoline B.

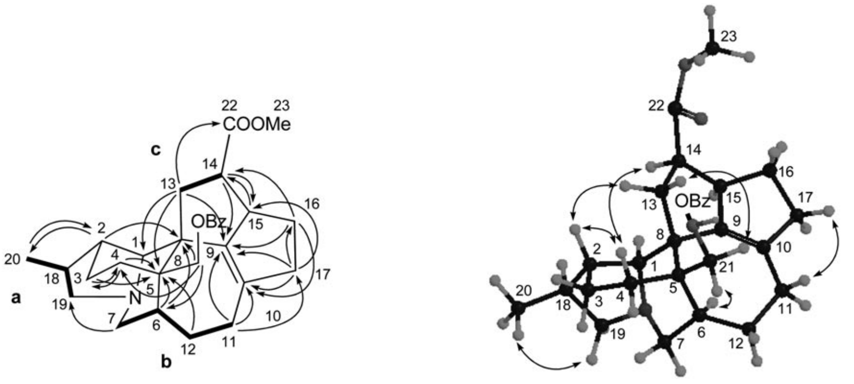

Fig. 2. *Key ^{1}H,^{1}H-COSY* (—), *HMBC* (H → C), *and NOESY* (H ↔ H) *features of* **2**

Daphangustifolines A and B (**1** and **2**) were evaluated for their cytotoxic activities against human tumor cell lines. At the $1.00 \cdot 10^{-5}$ M concentration value, compound **2** showed weak inhibition of tumor growth against the HL-60, MCF-7, and A549 cell lines, with the values 28.64 ± 13.56%, 11.80 ± 17.17%, and 53.25 ± 1.68%, respectively, while compound **1** was inactive against all of these cell lines.

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Experimental Part

General. All solvents used for extraction and isolation were distilled prior to use. Column chromatography (CC): silica gel (SiO_2; 200–300 and 300–400 mesh; *Qingdao Haiyang Chem. Ind. Ltd.*, P. R. China), SiO_2 *H* (10–40 μm mesh; *Qingdao*), amino SiO_2 (90–140 μm; *Fuji*, *Merck*), and *Lichroprep-RP-18* gel (40–63 μm; *Merck*). MPLC: *Büchi* pump module *C-605* and *Büchi* pump manager

C-615. TLC: visualization by spraying with *Dragendoff*'s reagent. Optical rotations: *Jasco-DIP-370* digital polarimeter. IR Spectra: *Bio-Rad-FTS-135* spectrometer; KBr discs; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-AM-400* instrument; at 400 (^{1}H) and 100 MHz (^{13}C); δ in ppm rel. to residual solvent signals, *J* in Hz. ESI- and HR-ESI-MS: *VG-Autospec-3000* spectrometers; in *m*/*z*.

Plant Material. The whole plants of *D. angustifolium* Hutch. were collected in Guangzhou, in October 2008. Voucher specimens were deposited with the South China Botanical Garden, Chinese Academy of Sciences, and were identified by Prof. *Huagu Ye.*

Extraction and Isolation. The air-dried and powdered whole plants of *D. angustifolium* (10.0 kg) were extracted three times with 95% EtOH (5, 4, and 4 h, resp.), and the crude extract was partitioned between AcOEt and an acidic liquor at pH 3. The acidic aq. layer was then basified to pH 10 with sat. Na_2CO_3 soln., followed by exhaustive extraction with $CHCl_3$ and BuOH to obtain the corresponding extracts. The BuOH-soluble material was subjected to repeated CC (*RP-18*): daphangustifoline A (**1**; 30 mg). The $CHCl_3$-soluble material was subjectd to CC (amino SiO_2, $CHCl_3$/MeOH 1 : 0 → 0 : 1): *Frs. 1–5*. *Fr. 3* (14.5 g) was subjected to CC (*RP-18*, MeOH/H_2O 1 : 9 → 1 : 0): *Frs. 3.1–3.7*. *Fr. 3.1* (220.0 mg) was subjected to repeated CC (SiO_2, petroleum ether/Et_2NH): daphangustifoline B (**2**; 40 mg).

Daphangustifoline A (= rel-*(2*R*,2a*R*,6a*S*,8*S*,10*S*,11*R*,12a*R*,12b*R*,12c*R*,13*S*)-2-Carboxy-2,2a,3,4,5,6,6a,7,9,10,11,12,12a,12b-tetradecahydro-12b-(hydroxymethyl)-10-methyl-8,11,12c-methano-1*H*-cyclopent[1,8]azuleno[4,5-*a*]indolizinium Inner Salt*; **1**): White amorphous powder. $[\alpha]_D^{16.6} = -607.3$ ($c = 0.10$, MeOH). IR (KBr): 3416, 2924, 1569, 1397, 1319, 1040. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 356 ($[M+H]^+$). HR-ESI-MS: 356.2228 ($[M+H]^+$, $C_{22}H_{30}NO_3^+$; calc. 356.2225).

Daphangustifoline B (= rel-*(1*R*,3*R*,3a*R*,5a*R*,6*S*,10a*R*,11*R*,12a*S*,12b*S*)-5a-[(Benzoyloxy)methyl]-2,3,3a,5,5a,6,7,8,9,10,10a,11,12,12b-tetradecahydro-3-methyl-4*H*-1,6-methanocyclopent[1,8]azuleno[4,3a-*g*]indole-11-carboxylic Acid Methyl Ester*; **2**): White amorphous powder. $[\alpha]_D^{16.3} = -12.9$ ($c = 0.25$, MeOH). UV (MeOH): 201 (3.7), 228 (3.8). IR (KBr): 2952, 2929, 1721, 1602, 1450, 1273, 1172, 1115. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 476 ($[M+H]^+$). HR-ESI-MS: 476.2793 ($[M+H]^+$, $C_{30}H_{38}NO_4^+$; calc. 476.2800).

REFERENCES

[1] J. Kobayashi, H. Mrita, in 'The Alkaloids', Ed. G. A. Cordell, Academic Press, New York, 2003, Vol. 60, p. 165; S. Yamamura, in 'The Alkaloids', Ed. A. Brossi, Academic Press, New York, 1986, Vol. 29, p. 265; S. Yamamura, Y. Hirata, in 'The Alkaloids', Ed. R. H. F. Manske, Academic Press, New York, 1975, Vol. 15, p. 41.

[2] G. A. Wallace, C. H. Heathcock, *J. Org. Chem.* **2001**, *66*, 450; C. H. Heathcock, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14323; C. H. Heathcock, D. Joe, *J. Org. Chem.* **1995**, *60*, 1131.

[3] J. Kobayashi, T. Kubota, *Nat. Prod. Rep.* **2009**, *26*, 936; M. Dong, M. L. Zhang, Q. W. Shi, J. C. Gu, H. Kiyota, *Curr. Org. Chem.* **2009**, *13*, 646.

[4] M. Zhen, M. T. Min, 'Flora of China', Science Press, Beijing, 1980, Vol. 45, pp. 1–11.

[5] H. Sakurai, N. Sakabe, Y. Hirata, *Tetrahedron Lett.* **1966**, *7*, 6309; T. Nakano, B. Nilsson, *Tetrahedron Lett.* **1969**, *10*, 2883.

[6] J. Kobayashi, H. Takatsu, Y. C. Shen, H. Morita, *Org. Lett.* **2003**, *5*, 1733.

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