Scyphiphorins A and B, Two New Iridoid Glycosides from the Stem Bark of a Chinese Mangrove *Scyphiphora hydrophyllacea*

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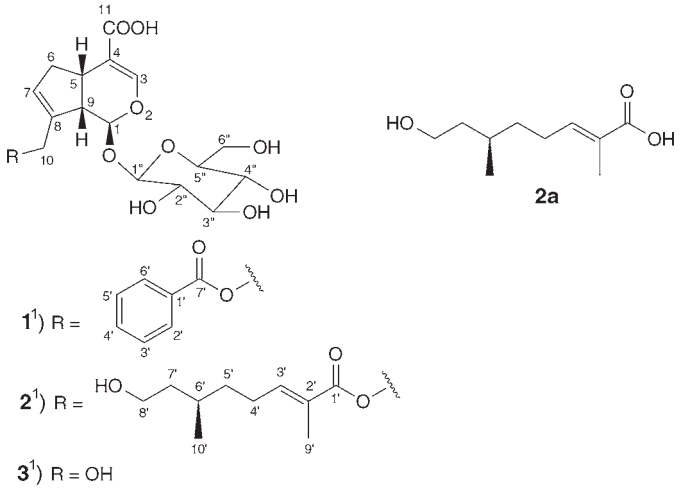
Two new iridoid glycosides, named scyphiphorins A (**1**) and B (**2**), together with four known compounds, geniposidic acid (=(1*S*,4a*S*,7a*S*)-1-(β-D-glucopyranosyloxy)-1,4a,5,7a-tetrahydro-7-(hydroxymethyl)cyclopenta[*c*]pyran-4-carboxylic acid; **3**), 4-(4-hydroxy-3-methoxybenzyl)butan-2-one, oleanolic acid (=(3β)-3-hydroxyolean-12-en-28-oic acid), and stigmasterol β-D-glucoside (=(3β,22*E*)-stigmasta-5,22-dien-3-yl β-D-glucopyranoside), were isolated for the first time from the stem bark of a Chinese mangrove, *Scyphiphora hydrophyllacea* GAERTN. f. The structures of compounds **1** and **2** were determined as 10-*O*-benzoylgeniposidic acid and 10-*O*-[(2*E*,6*R*)-8-hydroxy-2,6-dimethyl-1-oxooct-2-en-1-yl]geniposidic acid, respectively, on the basis of spectroscopic data and chemical methods, including 2D NMR techniques.

Introduction. – Mangrove plants are distributed in tidelands of tropical and semitropical areas. The genus *Scyphiphora* (Rubiaceae) has only one species, *Scyphiphora hydrophyllacea* GAERTN. f., mainly distributed along the seashore of India, Malaysia, Australia, and Hainan Island of China. In continuation of our studies on the chemical diversity of this plant, two new iridoid glycosides, scyphiphorins A (**1**) and B (**2**), have been isolated from the stem bark of *S. hydrophyllacea*, together with four known compounds including geniposidic acid (=(1*S*,4a*S*,7a*S*)-1-(β-D-glucopyranosyloxy)-1,4a,5,7a-tetrahydro-7-(hydroxymethyl)cyclopenta[*c*]pyran-4-carboxylic acid; **3**) [1], 4-(4-hydroxy-3-methoxybenzyl)butan-2-one [2], oleanolic acid (=(3β)-3-hydroxyolean-12-en-28-oic acid) [3], and stigmasterol β-D-glucoside (=(3β,22*E*)-stigmasta-5,22-dien-3-yl β-D-glucopyranoside) [4]. Herein, the isolation and structure elucidation of compounds **1** and **2** are presented.

Results and Discussion. – The EtOH extract of the stem bark of *S. hydrophyllacea* was subjected to extraction and solvent partitioning as described in the *Exper. Part.* The resulting BuOH extract was subjected to column chromatography to yield the two new compounds **1** and **2**, and four known compounds. Compound **2**, named scyphiphorin B, is an iridoid glucoside with a monoterpene substituent. To date, iridoid glucosides with this monoterpene substituent have been reported mainly from a few genera of plant families, including scrophulariaceae [5–8], verbenaceae [9–12], and oleaceae [13].



Scyphiphorin A[1]) (**1**), a white amorphous powder, had the molecular formula $C_{23}H_{26}O_{11}$ as established by HR-ESI-MS ($[M + Na]^+$ at m/z 501.1365). Its IR absorption bands at 3500–2500, 1718, 1701, 1602, 1578, and 1509 cm^{-1} indicated the existence of OH and C=O groups and of an aromatic ring. Analysis of the 1D and 2D NMR data and comparison with those of geniposidic acid (**3**) enabled us to assign the structure of **1** as 10-*O*-benzoylgeniposidic acid, *i.e.*, as *rel*-(1*R*,4a*R*,7a*R*)-7-[(benzoyloxy)methyl]-1-(β-D-glucopyranosyloxy)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylic acid.

Compound **1** had 11 degrees of unsaturation as deduced from the molecular formula $C_{23}H_{26}O_{11}$. The ^{1}H- and ^{13}C-NMR data (*Table*) indicated that seven units of the 11 unsaturations come from five C=C bonds and two C=O groups. Therefore, the molecule was tetracyclic. In the ^{1}H-NMR spectrum of **1** (*Table*), the presence of an anomeric proton at δ 4.78 (*d*, J = 8.0 Hz), protons of a monosubstituted phenyl ring (δ 7.61 (*t*, J = 7.5 Hz), 7.50 (*t*, J = 7.5 Hz, 2 H), and 8.05 (*d*, J = 7.5 Hz, 2 H)), and two further olefinic protons (δ 7.55 (*s*) and 5.94 (*s*)) were recognized. Moreover, the ^{13}C-NMR spectrum (*Table*) showed the presence of two C=O groups (δ 170.9 (*s*, COOH) and 167.9 (*s*, COOR)), eight olefinic C-atoms, and six C-atoms belonging to a β-D-glucopyranosyl moiety. The ^{1}H- and ^{13}C-NMR data were similar to those of geniposidic acid (**3**) [1] obtained from the same plant, indicating that compound **1** might have the same iridoid glycoside nucleus. However, signals of a benzoyl group (δ(H) 7.61 (*t*, J = 7.5 Hz), 7.50 (*t*, J = 7.5 Hz, 2 H), 8.05 (*d*, J = 7.5 Hz, 2 H), and δ(C) 131.3 (*s*), 130.6 (*d*), 129.6 (*d*), 134.3 (*d*), and 167.9 (*s*)) appeared. Comparison of the NMR data of **1** with those of **3** showed that C(8) was shifted upfield by 5.3 ppm, while CH_2(10) and C(10) were shifted downfield by 0.75–0.80 and 2.8 ppm, respectively. This suggested the benzoyl group to be attached at O–C(10) of the iridoid glycone. Confirming evidence was obtained from the HMBC correlations (*Fig.*) CH_2(10)/C(8), C(7), and C(7′). The strong NOE interactions H–C(5)/H–C(9), H–C(5)/H_β–C(6), and H–C(9)/H_β–C(6) confirmed the *cis* orientation of these protons as in **3**.

Scyphiphorin B[1]) (**2**) was isolated as a white amorphous powder. Its molecular formula was established as $C_{26}H_{38}O_{12}$ by HR-ESI-MS ($[M - H]^-$ at m/z 541.2271). The

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

Table. *[1]H- and [13]C-NMR Data* (500 and 125 MHz, resp.; CD_3OD) *for Compounds* **1**–**3**[1]). δ in ppm, J in Hz.

	1		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	5.24 (*d*, *J* = 7.5)	98.4	5.21 (*d*, *J* = 7.6)	98.3	5.16 (*d*, *J* = 7.5)	98.2
H–C(3)	7.55 (*s*)	153.4	7.54 (*s*)	153.4	7.51 (*s*)	152.9
C(4)		112.6		112.7		113.4
H–C(5)	3.21–3.25 (*m*)	36.5	3.21–3.23 (*m*)	36.5	3.17–3.23 (*m*)	36.8
CH_2(6)	2.15–2.19 (*m*, H_α), 2.89–2.92 (*m*, H_β)	40.0	2.14–2.19 (*m*, H_α), 2.86–2.91 (*m*, H_β)	40.0	2.10–2.15 (*m*, H_α), 2.83–2.89 (*m*, H_β)	39.8
H–C(7)	5.94 (*s*)	131.5	5.86 (*s*)	131.0	5.82 (*s*)	128.4
C(8)		139.5		139.9		144.8
H–C(9)	2.82–2.86 (*m*)	47.5	2.76–2.80 (*m*)	47.6	2.71–2.74 (*m*)	47.0
CH_2(10)	5.10, 5.02 (2*d*, *J* = 13.9)	64.3	4.92, 4.85 (2*d*, *J* = 13.8)	63.9	4.35, 4.22 (2*d*, *J* = 14.4)	61.5
C(11)		170.9		170.9		171.9
C(1′)		131.3		169.6		
H–C(2′) or C(2′)	8.05 (*d*, *J* = 7.5)	130.6		128.6		
H–C(3′)	7.50 (*t*, *J* = 7.5)	129.6	6.84 (*t*, *J* = 7.2)	144.5		
H–C(4′) or CH_2(4′)	7.61 (*t*, *J* = 7.5)	134.3	2.24–2.30 (*m*)	27.2		
H–C(5′) or CH_2(5′)	7.50 (*t*, *J* = 7.5)	129.6	1.49–1.53 (*m*), 1.31–1.36 (*m*)	37.0		
H–C(6′)	8.05 (*d*, *J* = 7.5)	130.6	1.61–1.66 (*m*)	30.5		
C(7′) or CH_2(7′)		167.9	1.61–1.66 (*m*), 1.38–1.40 (*m*)	40.6		
CH_2(8′)			3.58–3.62 (*m*)	61.0		
Me(9′)			1.88 (*s*)	12.6		
Me(10′)			0.95 (*d*, *J* = 6.5)	19.8		
H–C(1″)	4.78 (*d*, *J* = 8.0)	100.5	4.74 (*d*, *J* = 7.9)	100.6	4.74 (*d*, *J* = 7.5)	100.3
H–C(2″)	3.28–3.32 (*m*)	74.8	3.23–3.27 (*m*)	74.9	3.24–3.27 (*m*)	74.9
H–C(3″)	3.42–3.44 (*m*)	77.9	3.38–3.42 (*m*)	78.0	3.40–3.44 (*m*)	78.1
H–C(4″)	3.34–3.36 (*m*)	71.4	3.30–3.33 (*m*)	71.5	3.31–3.33 (*m*)	71.8
H–C(5″)	3.34–3.36 (*m*)	78.3	3.30–3.33 (*m*)	78.5	3.31–3.33 (*m*)	78.3
CH_2(6″)	3.87–3.89, 3.66–3.70 (2*m*)	62.8	3.86–3.90, 3.64–3.69 (2*m*)	62.9	3.87–3.89, 3.66–3.69 (2*m*)	62.7

NMR data of **2** (*Table*) were similar to those of geniposidic acid (**3**), except for the presence of one more monoterpene moiety. The structure of **2** was finally determined as 10-*O*-[(2*E*,6*R*)-8-hydroxy-2,6-dimethyl-1-oxooct-2-en-1-yl]geniposidic acid, *i.e.*, as *rel*-(1*R*,4a*R*,7a*R*)-1-(*β*-D-glucopyranosyloxy)-1,4a,5,7a-tetrahydro-7-{{[(2*E*,6*R*)-8-hydroxy-2,6-dimethyl-1-oxooct-2-en-1-yl]oxy}methyl}cyclopenta[*c*]pyran-4-carboxylic acid, on the basis of NMR data, including 2D-NMR techniques, and hydrolysis. Alkaline hydrolysis of **2** with aqueous 20% Na_2CO_3 solution afforded **2a**, which was identified as 8-hydroxy-2,6-dimethyloct-2-enoic acid (HDOA) by its ^{1}H-NMR and ESI-MS data [14]. The absolute configuration of C(6) of **2a** was determined as (*R*) by comparison of its optical-rotation value ($[\alpha]_D^{20} = +8.9$) with those of (*S*)-HDOA ($[\alpha]_D^{20} = -8.3$) and (*R*)-HDOA ($[\alpha]_D^{20} = +5.6$) [14].

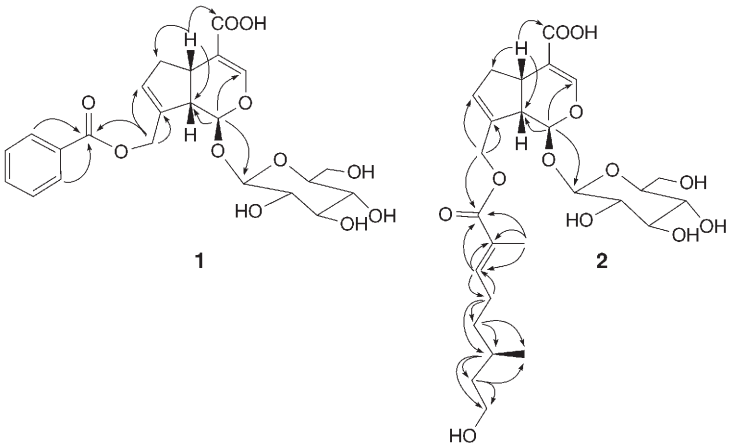


Figure. *Selected HMBC correlations* (→) *of* **1** *and* **2**

Compound **2** had eight degrees of unsaturation as deduced from the molecular formula $C_{26}H_{38}O_{12}$. The ^{1}H- and ^{13}C-NMR data (*Table*) indicated that five of the eight elements of unsaturation come from three C=C bonds and two C=O groups. Therefore, the molecule was tricyclic. In the ^{1}H-NMR spectrum of **2** (*Table*), the presence of an anomeric proton (δ 4.74 (*d*, J = 7.9 Hz)), two Me groups (δ 1.88 (*s*) and 0.95 (*d*, J = 6.5 Hz)), and two olefinic protons (δ 7.54 (*s*) and 5.86 (*s*)) were recognized. Moreover, the ^{13}C-NMR spectrum of **2** (*Table*) showed the presence of a COOH group (δ 170.9 (*s*)) and four olefinic C-atoms for the aglycone of geniposidic acid and six C-atoms for a β-D-glucopyranosyl moiety. In addition, ten C-atoms (δ 169.6 (*s*), 144.5 (*d*), 128.6 (*s*), 61.0 (*t*), 40.6 (*t*), 37.0 (*t*), 30.5 (*d*), 27.2 (*t*), 19.8 (*q*), and 12.6 (*q*)) were assigned to a monoterpene moiety, namely to an 8-hydroxy-2,6-dimethyl-1-oxooct-2-enyl substituent [14]. Comparison of the NMR data of **2** with those of geniposidic acid (**3**) showed that C(8) was shifted upfield by 4.9 ppm, while CH_2(10) and C(10) were shifted downfield by 0.57–0.63 and 2.4 ppm, respectively. This indicated that the monoterpene moiety was located at O–C(10) of the iridoid glycone. Confirming evidence was obtained from the HMBC correlations (*Fig.*) CH_2(10)/C(8) and C(1′). Moreover, the HMBC correlations from Me(9′)/C(1′), C(2′), and C(3′) indicated that the C=C bond of the monoterpene moiety was located between C(2′) and C(3′). Extensive analysis of the HMBC and NOESY data further confirmed the structural assignment of the monoterpene moiety. In particular, the NOESY interactions H–C(9′)/CH_2(4′), H–C(3′)/CH_2(5′) showed the configuration of C(2′)=C(3′) to be (*E*).

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Experimental Part

General. Column chromatography (CC): macroporous resin (*D101*; *Nankai University Chemical Plant*, Nankai, P. R. China), silica gel (200–300 mesh; *Qingdao Haiyang Chemical Plant*, Qingdao, P. R. China), *RP-18* silica gel (40–60 mesh; *Merck*). TLC: precoated silica gel *G* plates (*Qingdao Haiyang Chemical Plant*, Qingdao, P. R. China). HPLC: *ODS* column (*YMC-Pack ODS-5-A*, 250 × 10 mm i.d., 5 μm; *YMC*); *Waters-600* HPLC system equipped with a *Waters-996* photodiode array detector. Optical rotation: *Polaptronic-HNQW5* high-resolution polarimeter. UV Spectra: *Varian-Cary-100* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker Vector-22* IR spectrophotometer; in cm^{-1}. NMR Spectra: *Bruker DRX-500* spectrometer; $SiMe_4$ as internal standard; δ in ppm, J in Hz. MS: *VG Auto-Spec-3000* spectrometer for HR ESI and *Finnigan MAT-90* instrument for ESI; positive or negative mode; in *m*/*z*.

Plant Material. The stem bark of *Scyphiphora hydrophyllacea* GAERTN. f. was collected in April 2005 from Wenchang, Hainan Province, P. R. China. The specimen was identified by Professor *Si Zhang*, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen has been deposited in the South China Sea Institute of Oceanology, Chinese Academy of Sciences (accession number: GKLMMM017).

Extraction and Isolation. The air-dried and powdered plant material (10 kg) was extracted with 95% EtOH (40 l) at r. t. for four times (4 × 6 days). After evaporation of the EtOH, the viscous residue (560 g) was suspended in H_2O (1 l) and extracted successively with petroleum ether, AcOEt, and BuOH for three times each, and with 2 l of solvent each time. The BuOH extract (150 g) was fractionated by CC (macroporous resin, 0 → 100% H_2O/EtOH): *Fractions 1–4. Fr. 3* (20 g) was fractionated by CC (*RP-18*, 40 → 100% H_2O/MeOH): *Fr. 3a–3d. Fr. 3c* (3.3 g) was then purified by CC (silica gel, 30 → 50% $CHCl_3$/MeOH): **1** (250 mg) and **2** (40 mg). *Fr. 3d* (2.5 g) was separated by CC (silica gel, $CHCl_3$/MeOH 8:1): **3** (60 mg). *Fr. 4* (14.5 g) was separated by CC (silica gel, 0 → 50% $CHCl_3$/MeOH): *Fr. 4a–4f. Fr. 4a* (2.4 g) was purified by CC (silica gel, 0 → 30% $CHCl_3$/MeOH): 4-(4-hydroxy-3-methoxybenzyl)butan-2-one (20 mg) and oleanolic acid (40 mg). *Fr. 4d* (1.6 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 10:1): stigmasterol β-D-glucoside (110 mg).

Alkaline Hydrolysis of **2**. Compound **2** (10 mg) was dissolved in dioxane (5 ml) and refluxed with 20% aq. Na_2CO_3 soln. (5 ml) at 100° for 5 h. The mixture was neutralized with 6M HCl to pH 7.0 and then extracted with $CHCl_3$ (50 ml). The $CHCl_3$ extract was concentrated and the residue then purified by HPLC (250 × 10 mm i.d., 5 μm, MeOH/H_2O 40:60): *(2*E*,6*R*)-8-hydroxy-2,6-dimethyloct-2-enoic acid* ((*R*)-HDOA; **2a**; 1 mg). Colorless oil. $[\alpha]_D^{20} = +8.9$ ($c = 1.0$, $CHCl_3$). ^{1}H-NMR: 6.84 (br. *t*, $J = 7.3$); 3.70 (*m*, 2 H); 2.20 (*m*, 2 H); 1.88 (*s*, 3 H); 1.63 (*m*, 2 H); 1.43 (*m*); 1.41 (*m*); 1.26 (*m*); 0.95 (*d*, $J = 6.5$, 3 H). ESI-MS: 185 ($[M-H]^-$), 209 ($[M+Na]^+$).

Scyphiphorin A (= rel-*(1*R*,4a*R*,7a*R*)-7-[(Benzoyloxy)methyl]-1-(β-D-glucopyranosyloxy)-1,4a,5,7a-tetrahydrocyclopenta[c]pyran-4-carboxylic Acid*; **1**): White amorphous powder. $[\alpha]_D^{20} = +20.26$ ($c = 7.7$, MeOH). UV (MeOH): 231 (4.38). IR: 3422, 2920, 1718, 1701, 1602, 1578, 1509, 1277, 1073, 757, 714, 686. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 501.1365 ($[M+Na]^+$, $C_{23}H_{26}NaO_{11}^+$; calc. 501.1372).

Scyphiphorin B (= rel-*(1*R*,4a*R*,7a*R*)-1-(β-D-glucopyranosyloxy)-1,4a,5,7a-tetrahydro-7-{[(2*E*,6*R*)-8-hydroxy-2,6-dimethyl-1-oxooct-2-en-1-yl]oxy}methyl}cyclopenta[c]pyran-4-carboxylic Acid*; **2**): White amorphous powder. $[\alpha]_D^{20} = +21.4$ ($c = 5.0$, MeOH). UV (MeOH): 224 (4.74). IR (KBr): 3415, 2950, 1721, 1705, 1642, 1456, 1387. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 541.2271 ($[M-H]^-$, $C_{26}H_{37}O_{12}^-$; calc. 541.2285).

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