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SCYPHIPHORINS C AND D, TWO NEW IRIDOID GlYCOSIDES FROM THE CHINESE MANGROVE *SCYPHIPHORA HYDROPHYLLACEA*

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Abstract – Two new iridoid glycosides with a lignan substituent, named scyphiphorins C (**1**) and D (**2**), were isolated from the stem bark of a Chinese mangrove *Scyphiphora hydrophyllacea* Gaertn. f., together with five known iridoid glycosides, 10-*O*-acetylgeniposidic acid (**3**), 7-deoxy-8-*epi*-loganic acid (**4**), mussaenoside (**5**), 7-deoxygardoside (**6**), and 10-deoxygeniposidic acid (**7**). The structures of compounds **1** and **2** were elucidated on the basis of spectroscopic data, especially 2D NMR techniques.

Rubiaceae family of mangrove plants has only one species, *Scyphiphora hydrophyllacea* Gaertn. f. that mainly distributed along the seashore of India, Malaysia, Australia and Hainan Island of China. Pharmacological studies revealed that the ethanol extract of the twigs of *S. hydrophyllacea* showed inhibitory effect towards human hepatoma SMMC-7721 cell line with IC_{50} value of 15.1 μg/ml.[1] And previous chemical investigations on this plant uncovered over twenty compounds, including iridoids, triterpenoids, steroids, flavonoids, coumarins.[1-5] Further investigation on the stem bark of the same plant resulted in the discovery of two new iridoid glycosides with a lignan substituent, scyphiphorins C (**1**) and D (**2**), together with five known iridoid glycosides, 10-*O*-acetylgeniposidic acid[6] (**3**), 7-deoxy-8-*epi*-loganic acid[7] (**4**), mussaenoside[7] (**5**), 7-deoxygardoside[8] (**6**), and 10-deoxygeniposidic acid[9] (**7**) (Figure 1). To date, iridoid glycosides with such kind of lignan substituent haven't been reported. The structure arguments for the two new compounds **1** and **2** are detailed below.

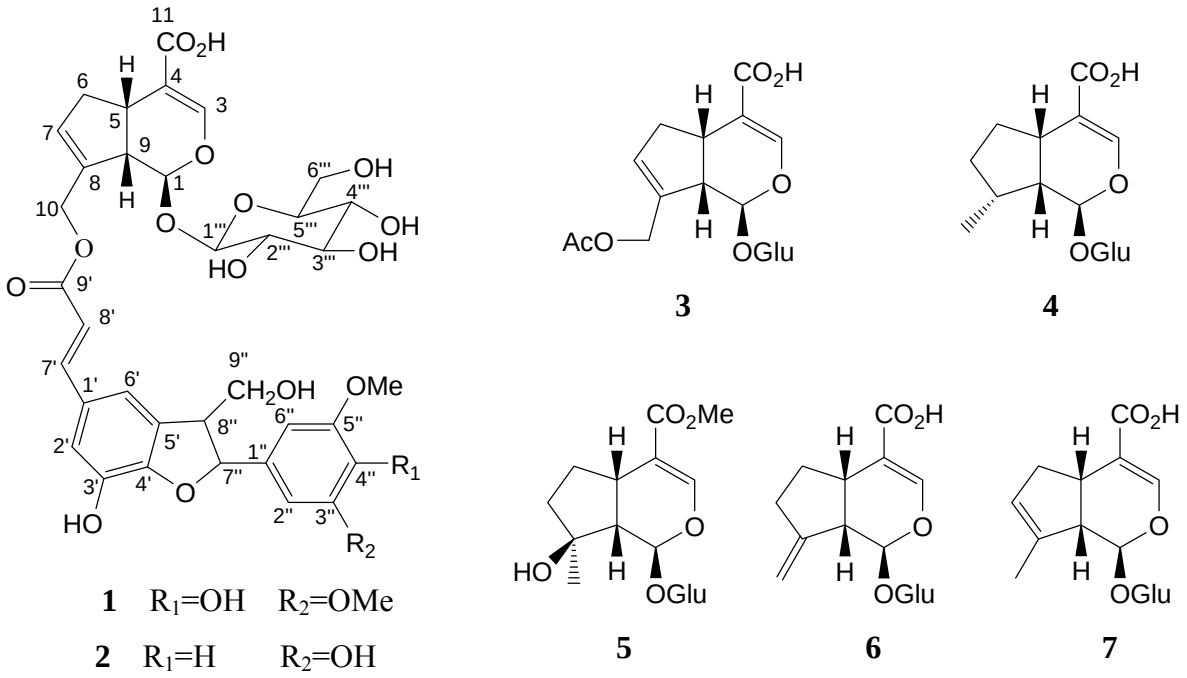

Figure 1. The Structures of Compounds **1-7**

The ethanolic extract of the stem bark of *S. hydrophyllacea* was subjected to sequential extraction with petroleum ether, EtOAc, and *n*-BuOH . The resulting *n*-BuOH extract was chromatographed on silica gel and followed by preparative reverse-phase C18 HPLC to yield compounds **1-7**.

Compound **1** was obtained as white amorphous powder, and had a molecular formula of $C_{36}H_{40}O_{17}$ established by HR-ESI-MS ($[M+Na]^+$, *m/z* 767.2134). Consequently, **1** had 17 degrees of unsaturation. The IR (KBr) absorption bands at 3420, 1715, 1709, 1605, and 1495 cm^{-1} indicated the existence of hydroxyl, carbonyl group, and aromatic ring. The ^1H and ^{13}C NMR data (Table 1) indicated that 11 units of the 17 unsaturations came from nine carbon-carbon double bonds, and two carbonyl groups. Therefore, the other six units of unsaturations came from six rings. In the ^1H NMR spectrum of **1**, the presence of a trans carbon-carbon double bond [δ_H 7.63, 6.37 (each 1H, *d*, *J* = 15.9 Hz)], another six olefinic protons [δ_H 7.54, 7.11, 7.03, 5.90 (each 1H, *s*), and 6.72 (2H, *s*)], two methoxy groups [δ_H 3.83 (6H, *s*)] were recognized. DEPT experiments revealed that **1** had two tertiary methyls (two methoxy), four methylenes, eighteen methines (eight olefinic) and twelve quaternary carbons (including two carbonyls). Among these signals, six carbons （δ_C 100.6 *d*, 78.4 *d*, 78.0 *d*, 74.9 *d*, 71.6 *d*, 62.9 *t*） belonging to a glucopyranosyl moiety. And the signal at δ_H 4.75 (1H, *d*, *J* = 7.8 Hz) suggested the relative configuration of the glucopyranosyl moiety was β-configuration.

Table1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1** and **2**
(500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR)

position	**1** (in Methanol-d_4)		**2** (In Pyridine-d_5)	
	δ_H	δ_C	δ_H	δ_C
1	5.22 (1H, *d*, *J* = 7.6 Hz)	98.4	5.59 (1H, *d*, *J* = 7.6 Hz)	97.9
3	7.54 (1H, *s*)	153.2	7.91 (1H, *s*)	151.8
4		113.0		112.7
5	3.21 (1H, *m*)	36.7	3.55 (1H, *m*)	36.0
6α	2.17 (1H, *m*)	40.0	2.31 (1H, *m*)	39.5
6β	2.90 (1H, *m*)		3.10 (1H, *m*)	
7	5.90 (1H, *s*)	131.4	5.80 (1H, *s*)	130.0
8		139.9		139.3
9	2.80 (1H, *m*)	47.5	2.98 (1H, *m*)	47.1
10	4.95 (1H, *d*, *J* = 14.3 Hz) 4.85 (1H, *d*, *J* = 14.3 Hz)	63.7	5.14 (1H, *d*, *J* = 13.5 Hz) 5.25 (1H, *d*, *J* = 13.5 Hz)	62.7
11		171.2		169.5
1′		129.7		128.9
2′	7.03 (1H, *s*)	117.1	7.38 (1H, *s*)	116.9
3′		142.8		143.2
4′		151.2		151.2
5′		130.9		131.0
6′	7.11 (1H, *s*)	117.9	7.53 (1H, *s*)	117.7
7′	6.37 (1H, *d*, *J* = 15.9 Hz)	115.6	6.66 (1H, *d*, *J* = 15.9 Hz)	115.3
8′	7.63 (1H, *d*, *J* = 15.9 Hz)	147.2	7.99 (1H, *d*, *J* = 15.9 Hz)	146.1
9′		169.1		167.2
1″		133.7		133.1
2″	6.72 (1H, *s*)	104.3	7.20 (1H, *s*)	119.9
3″		149.5		148.3
4″		136.6	7.21 (1H, *s*)	116.6
5″		149.5		148.9
6″	6.72 (1H, *s*)	104.3	7.30 (1H, *s*)	110.9
7″	5.62 (1H, *d*, *J* = 6.3 Hz)	89.7	6.13(1H, *d*, *J* = 6.8 Hz)	89.1
8″	3.55 (1H, *m*)	55.3	4.0 (1H, *m*)	54.5
9″	3.88 (1H, *m*)	64.8	4.30 (1H, *m*)	64.0
Glu-1‴	4.75 (1H, *d*, *J* = 7.8 Hz)	100.6	5.45 (1H, *d*, *J* = 7.8 Hz)	101.2
2‴	3.26 (1H, *m*)	74.9	4.02 (1H, *m*)	75.0
3‴	3.41 (1H, *m*)	78.0	4.30 (1H, *m*)	78.4
4‴	3.32 (1H, *m*)	71.6	4.16 (1H, *m*)	71.5
5‴	3.32 (1H, *m*)	78.4	4.27 (1H, *m*)	78.8
6‴	3.91 (1H, *m*) 3.67 (1H, *m*)	62.9	4.52 (1H, *m*) 4.36 (1H, *m*)	62.6
OCH_3	3.83 (3H, *s*)	56.9	3.61(3H, *s*)	55.8

Moreover, Ten signals at δ_C 153.2 *d*, 139.9 *s*, 131.4 *d*, 113.0 *s*, 98.4 *d*, 63.7 *t*, 47.5 *d*, 40.0 *t*, 36.7 *d*, were similar to those of 7-deoxy-8-*epi*-loganic acid[7] (**4**), mussaenoside[7] (**5**), 7-deoxygardoside[8] (**6**),

10-deoxygeniposidic acid[9] (**7**), and especially 10-*O*-acetylgeniposidic acid[6] (**3**), which indicated that **1** should have the same geniposidic acid nucleus with a special substituent at C-10.

In addition, the NMR data showed the signals of two tetrasubstituted phenyl rings [δ_H 7.11, 7.03 (each 1H, *s*), and 6.72 (2H, *s*)], one trans carbon-carbon double bond [δ_H 7.63, 6.37 (each 1H, *d*, *J* = 15.9 Hz)], two methines [δ_H 5.62 (1H, *d*, *J* = 6.3 Hz), 3.55 (1H, *m*); δ_C (89.7 *d*, 55.3 *t*)] and one methylene [δ_H 3.88 (2H, *m*); δ_C 64.8 *d*) that attributed to an aliphatic –O-CH-CH-CH_2-O– link. These NMR data were similar to those of cassyformin[10] and cimicifugic acid,[11] which suggested that **1** had a dihydrobenzofuran type neolignan moiety. In the HMBC spectrum (Figure 2), the correlations between H-7′ and C-9′, C-1′, H-8′ and C-9′, C-2′or C-6′, H-7″ and C-1″, C-2″or C-6″, C-8″, C-9″, H-8″ and C-5′, C-6′, C-9″, C-7″, C-1″ confirmed the determination of the neolignan moiety. Furthermore, the HMBC correlations between H-10 and C-8, C-7, C-9′ showed that the neolignan moiety was located at C-10 of the iridoid aglycone. In particular, the NOESY interactions between H-7″ and H-9″, H-8″ and H-2″/6″ indicated *trans* configuration of the dihydrofuran ring. Based on the above results, the structure of **1**, named scyphiphorin C, was elucidated as shown in Figure 1.

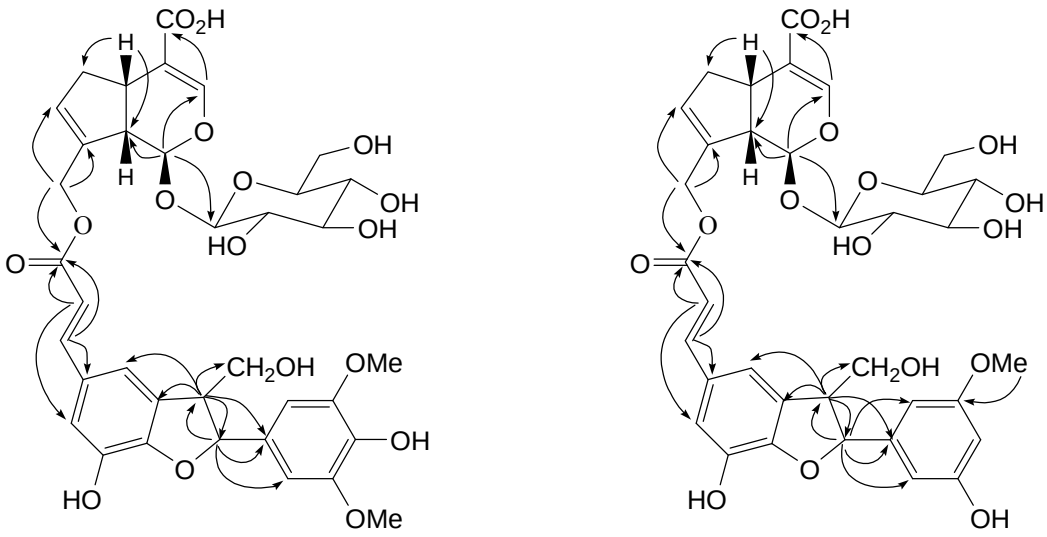

Figure 2. Selected HMBC Correlations of Compounds **1** and **2**

Compound **2** was isolated as a white amorphous powder. Its molecular formula was established as $C_{35}H_{38}O_{16}$ by HR-ESI-MS ([*M*+*Na*]$^+$, *m/z* 737.2028). The ^{1}H and ^{13}C NMR spectral data of **2** (Table 1) were very similar to those of **1**, except for the presence of a 1,3,5-trisubstituted phenyl ring [δ_H 7.30, 7.21, 7.20 (each 1H, *s*), δ_C 148.9 *s*, 148.3 *s*, 133.1 *s*, 119.9 *d*, 116.6 *d*, 110.9 *d*] instead of a 1,3,4,5-tetrasubstituted phenyl ring in compound **1**. The NMR data of the 1,3,5-trisubstituted phenyl ring and the HMBC

correlations between methoxy proton and C-5″， H-7″ and C-1″, C-2″, C-6″ suggested that there was a 3-OH-5-OCH_3-trisubstituted phenyl ring attached to C-7″. More confirming evidences about the structural elucidation of **2** were shown in its HMBC spectrum (Figure 2). The NOESY interactions between H-7″ and H-9″, H-8″ and H-2″/6″ also indicated the 7″/8″-*trans* configuration. Thus the structure of **2**, named scyphiphorin D, was characterized as shown in Figure 1.

EXPERIMENTAL

General NMR spectra were recorded in methanol-d_4 or pyridine-d_5 using a Bruker AV-500 spectrometer (500MHz for ^{1}H NMR and 125MHz for ^{13}C NMR) with tetramethylsilane as the internal standard. UV spectra were obtained on a Varian cary 100 conc UV-Visible spectrophotometer and IR spectra were mesured on a Bruker VECTOR22 infrared spectrophotometer. HR-ESI-MS spectral were recorded on a Bruker Bio TOF Q MS spectrometer in positive ion mode. Optical rotation: *POLAPTRONIC HNQW5* high resolution polarimeter. Preparative HPLC was carried out on ODS colums (250 × 10 mm i.d., Phenomenex luna) with a Waters 996 photodiode array detector. For Column Chromatography (CC), silica gel (200-300 mesh) (Qingdao Mar. Chem. Ind. Co. Ltd.), macroporous resin (D101)(Nankai University Chemical Plant), Sephadex LH-20 (Pharmacia), and RP-18 silica gel (40-60 mesh) (Merck) were used.

Plant Material The stem bark of *Scyphiphora hydrophyllacea* Gaertn. f. was collected in April 2005 from Wenchang, Hainan province, P. R. China. The identification of the plant was performed by Professor *Si Zhang*, the South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen (No. GKLMMM017) is deposited in the South China Sea Institute of Oceanology, Chinese Academy of Sciences.

Extraction and isolation The dried stem bark (10 kg) of *S. hydrophyllacea* was extracted with 95% ethanol at rt for four times (6 days per time). The extract was concentrated under reduced pressure. The viscous residue (560 g) was suspended in water and extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH extract (150 g) was chromatographed on silica gel column and eluted using $CHCl_3$/MeOH system (100:0—1:1) to yield Fractions 1-9. Fr.1 was refined in $CHCl_3$/MeOH (1:1) to yield **3** (70 mg). Fr. 4 was separated by CC (RP-18 Si gel, H_2O/MeOH 50:50) to yield **4** (7 mg). Fr. 7 was purified with preparative HPLC (H_2O/MeOH 55:45) to yield **1** (8 mg) and **2** (10 mg). Fr. 8 was subjected to CC (Si gel, $CHCl_3$/MeOH 5:1—1:1) to give **5** (30 mg), **6** (180 mg). Fr. 9 was purified by CC (RP-18 Si gel, H_2O/MeOH 50:50) to afford **7** (25 mg).

Scyphiphorin C (1): White amorphous powder. $[\alpha]_D^{20}$ +28.0 (*c* 8.0, MeOH); UV λmax (MeOH) nm (log ε): 230 (4.29), 329 (4.10). IR (KBr) cm^{-1}: 3420, 2960, 1715, 1709, 1605, 1495, 1509, 1268, 1073. ^{1}H and ^{13}C NMR (see Table 1). HR-ESI-MS *m/z* 767.2134 ([*M*+*Na*]$^+$, $C_{36}H_{40}NaO_{17}^+$, Calcd. 767.2158).

Scyphiphorin D (2): White amorphous powder. $[\alpha]_D^{20}$ +27.0 (*c* 10.0, C_5H_5N). UV λmax (MeOH) nm (log ε): 228 (4.20), 331 (3.96). IR (KBr) cm^{-1}: 3425, 2950, 1716, 1710, 1603, 1504, 1274, 1065. ^1H and ^{13}C NMR (see Table 1). HR-ESI-MS *m/z* 737.2028 ($[M+Na]^+$, $C_{35}H_{38}NaO_{16}^+$, Calcd 737.2052).

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