

Two New Noriridoids from *Scyphiphora hydrophyllacea*

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Chemical investigation of the EtOH extract of the aerial parts of *Scyphiphora hydrophyllacea* Gaertn. F. collected from Hainan resulted in the isolation of the two new noriridoids hydrophylin A (**1**) and hydrophylin B (**2**). The structures were elucidated by a study of their physical and spectral data.

Key words: *Scyphiphora hydrophyllacea*, Noriridoid, Hydrophylin A, Hydrophylin B

Introduction

Mangrove plants are woody plants growing in tropical and subtropical intertidal habitats, containing plenty of new and bioactive secondary metabolites due to their special eco-environment [1–3]. *Scyphiphora hydrophyllacea* Gaertn. F. (Rubiaceae), one of the mangrove plants, is distributed from south to south-east Asia, Caroline Islands, Australia, and New Caledonia [4]. In our screening for cytotoxic agents from mangrove plants, the ethanol extract from the twigs of *S. hydrophyllacea* showed an inhibitory effect towards the human hepatoma SMMC-7721 cell line with an IC_{50} value of $15.1 \mu\text{g mL}^{-1}$. Previous research has revealed that this plant mainly contains known flavones and triterpenoids, among which betulone shows inhibitory activity towards the human hepatoma SMMC-7721 cell line with an IC_{50} value of $12.5 \mu\text{g mL}^{-1}$ [5,6]. Further investigation on the ethanol extract of this plant resulted in the isolation of the two new noriridoids **1** and **2**, named hydrophylin A and B. However, neither of them showed obvious cytotoxicity against the SMMC-7721 cell line by the MTT method. The IC_{50} values of the two compounds against the SMMC-7721 cell line were more than $100 \mu\text{g mL}^{-1}$. In this paper, we describe the isolation and the structure elucidation of **1** and **2** (Fig. 1).

Results and Discussion

The EtOH extract of *S. hydrophyllacea* was subjected to repeated chromatography to afford **1** and **2**.

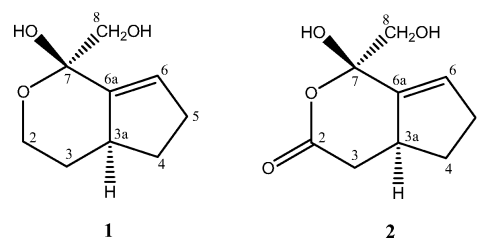
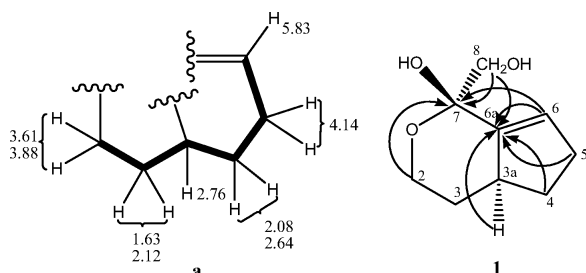


Fig. 1. The structures of compounds **1** and **2**.

The molecular formula of **1** was established as $\text{C}_9\text{H}_{14}\text{O}_3$ with three degrees of unsaturation according to the high-resolution ESI-MS data at $m/z = 193.0838$ (calcd. 193.0840 for $\text{C}_9\text{H}_{14}\text{O}_3\text{Na}$, $[\text{M}+\text{Na}]^+$), which was supported by ^{13}C NMR and DEPT spectral data. The ^{13}C NMR and DEPT spectra of **1** presented nine carbon signals, which analyzed as five methylenes ($\delta_{\text{C}} = 36.3, 38.2, 59.3, 66.2, 67.8$) including two oxygen-bearing carbons, two methines ($\delta_{\text{C}} = 44.5, 131.4$) including one olefinic carbon, and two quaternary carbons ($\delta_{\text{C}} = 99.5, 143.9$) including one olefinic carbon and one oxygen-bearing carbon. Interpretation of 2D NMR data, especially from ^1H - ^1H COSY and HMQC spectra, allowed us to construct the partial structure **a** (Table 1 and Fig. 2). HMBC correlations of C-6a with H-3a, H-4, H-5, and H-6 suggested that C-6a was connected with C-3a and C-6. Atom C-7 was connected with C-2 through an oxygen atom on the basis of HMBC correlations between C-7 and H-2. Furthermore, the observation of HMBC correlations from C-7 to H-6 and H-8 indicated that C-7 was connected

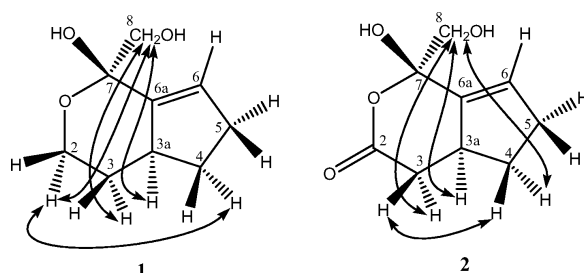
Table 1. ^1H and ^{13}C NMR data of **1** (CD_3OD , δ in ppm, J in Hz).

	δ_{C}	δ_{H}	^1H - ^1H COSY selected	HMBC selected
2 (CH_2)	67.8	3.88 (m, H- α), 3.61 (dd, 8.8, 14.6, H- β)	H-3	H-3
3 (CH_2)	36.3	1.63 (m, H- α), 2.12 (m, H- β)	H-2, 3a	H-2, 4
3a (CH)	44.5	2.76 (m)	H-3, 4	H-2, 3, 4, 6
4 (CH_2)	38.2	2.08 (m, H- α), 2.64 (m, H- β)	H-3a, 5	H-3, 3a, 6
5 (CH_2)	59.3	4.14 (m)	H-4, 6	H-6
6 (CH)	131.4	5.83 (br s)	H-5	H-3a, 4, 5
6a	143.9			H-3a, 4, 5, 6, 8
7	99.5			H-2, 6, 8
8 (CH_2)	66.2	3.67 (d, 11.5), 3.61 (d, 11.6)		

Fig. 2. Selected 2D NMR correlations for **1**. Bonds in bold indicate ^1H - ^1H COSY, arrows indicate HMBC.

with C-6a and C-8 (Fig. 2). The chemical shifts of C-7 ($\delta_{\text{C}} = 99.5$) revealed that C-7 was not only connected with C-2 through an oxygen atom, but also substituted by one hydroxy group. The relative stereochemistry at the stereocenters in compound **1** was supported by the ROESY spectrum (Fig. 3). The NOE interactions from H-3a to H-8 showed H-3a and H-8 at the same side. When they are assigned α orientation, the hydroxy group at C-7 is in β orientation. Thus, the structure of compound **1** was confirmed and named hydrophylin A.

Compound **2**, obtained as a brown gel, has a molecular formula $\text{C}_9\text{H}_{12}\text{O}_4$ based on its HR-ESI-MS ($m/z = 207.0636$; calcd. 207.0633 for $\text{C}_9\text{H}_{12}\text{O}_4\text{Na}$, $[\text{M}+\text{Na}]^+$), indicating that **2** possesses four degrees of unsaturation. The ^{13}C - and DEPT-NMR spectra of **2** revealed the presence of nine carbon signals, which analyzed as four methylenes ($\delta_{\text{C}} = 38.4, 38.7, 59.0, 65.3$) including two oxygen-bearing carbons, two methines ($\delta_{\text{C}} = 40.0, 133.4$) including one olefinic carbon, and three quaternary carbons ($\delta_{\text{C}} = 102.4, 143.3, 179.6$) including one oxygen-bearing carbon, one olefinic carbon, and one carbonyl carbon. Comparison of the ^{13}C NMR and DEPT spectra of **2** with those of **1** showed that **2** had one quaternary carbon more and one methylene carbon less than **1** (Table 2). The chemical shift of C-2 of **2** was shifted downfield to $\delta = 179.6$,

Fig. 3. Key ROESY correlations and relative configurations assigned for **1** and **2**.

which suggested that C-2 was changed to be a carbonyl group. The ROESY experiment showed the cross peaks from H-3a to H-8, which suggested that H-3a and H-8 in **2** are at the same side as in **1** (Fig. 1). When they were assigned α orientation, the hydroxy group at C-7 was in β orientation. Thus, the structure of compound **2** was confirmed and named hydrophylin B.

Experimental Section

General

TLC: precoated TLC plates (Si gel G) from Qingdao Marine Chemical Factory, Qingdao, P. R. China. Column chromatography (CC): silica gel (200–300 and 80–100 mesh) from Qingdao Marine Chemical Factory, Qingdao, P. R. China. Sephadex LH-20 from Amersham Bioscience. Optical rotations: Jasco DIP-370 digital polarimeter. IR spectra: Bio-Rad FTS-135 IR spectrometer; KBr pellets; in cm^{-1} . NMR spectra: Bruker AM-400 or DRX-500 instruments; SiMe_4 as internal standard; δ in ppm, J in Hz. MS: VG Auto-Spec-3000 and APIQSTAR-Pulsar-i spectrometer.

Plant material

The aerial parts of *Scyphiphora hydrophyllacea* Gaertn. F. were collected in Wenchang county (Nov. 2004), Hainan Province of P. R. China. It was identified by associate Prof. Zheng-Fu Dai of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural

Table 2. ^1H and ^{13}C NMR data of **2** (CD_3OD , δ in ppm, J in Hz).

	δC	δH	^1H - ^1H COSY selected	HMBC selected
2	179.6			H-3
3 (CH_2)	38.4	2.97 (dd, 10.4, 18.0, H- α), 2.40 (dd, 5.2, 18.4, H- β)	H-3a	H-3a, 4
3a (CH)	40.0	3.10 (m)	H-3, 4	H-3, 6
4 (CH_2)	38.7	2.79 (ddd, 2.0, 8.4, 15.2, H- β), 2.24 (dt, 2.4, 17.6, H- α)	H-3a, 5	H-3
5 (CH_2)	59.0	4.18 (m)	H-4, 6	H-6
6 (CH)	133.4	5.99 (br s)	H-5	H-3a, 5, 4
6a	143.3			H-3a, 4, 5, 6, 8
7	102.4			H-6, 8
8 (CH_2)	65.3	3.94 (d, 12.4), 3.64 (d, 12.0)		

Sciences, where a voucher specimen (SH20051112) is deposited.

Extraction and isolation

The dried, milled aerial parts of *Scyphiphora hydrophyllacea* Gaertn. F. (17.6 kg) were exhaustively extracted with 95 % EtOH (3×30 L) at r. t. After evaporation, the residue was suspended in H_2O and partitioned with light petroleum to give a light petroleum fraction (687.0 g). The H_2O part was applied to a D101 reticular resin column eluted with H_2O and MeOH. The H_2O eluent was not further fractionated because the major components were sugars. The MeOH eluent was concentrated *in vacuo* to give a residue (421.0 g), which was chromatographed on a silica gel column (200–300 mesh) with CHCl_3 -MeOH [50:1 (2.6 L), 20:1 (21.5 L), 10:1 (17.5 L), 5:1 (21.5 L), 2:1 (21.0 L)] to give 26 fractions. Fraction 2 (1.96 g) was subjected to column chromatography over silica gel eluted with light petroleum-AcOEt (5:5) to afford 9 further fractions. Sub-fractions 6–8 (230.8 mg) were then rechromatographed on a silica-gel column with light petroleum-AcOEt (6:4) to afford compound **1** (83.0 mg). Fraction 6 (5.29 g) was subjected to column chromatography over silica gel eluted with light petroleum-AcOEt (3:7) to afford 14 further fractions. Sub-fractions 10–11 (187.8 mg) were fractionated by column chromatography (Sephadex LH-20) eluted with 95 % EtOH and further purified by silica gel CC eluted with light petroleum-AcOEt (4:6) to afford compound **2** (29.2 mg).

Hydrophylin A (**1**)

Brown gel. $[\alpha]_{\text{D}}^{16.5} = +27.49$ ($c = 0.009$, MeOH). – IR (KBr): $\nu = 3503, 3397$ (OH), 1410, 1368, 1265, 1236, 1097, 1052 (C–O) cm^{-1} . – ^1H - and ^{13}C NMR: Table 1. – HR-ESI-MS: $m/z = 193.0838$ (calcd. 193.0840 for $\text{C}_9\text{H}_{14}\text{O}_3\text{Na}$, $[\text{M}+\text{Na}]^+$).

Hydrophylin B (**2**)

Brown gel. $[\alpha]_{\text{D}}^{16.5} = +5.75$ ($c = 0.01$, MeOH). – IR (KBr): $\nu = 3503, 3396$ (OH), 1413, 1370, 1267, 1234, 1096, 1051 (C–O), 3193, 2966, 2947, 1336, 1712 (C=O) cm^{-1} . – ^1H - and ^{13}C NMR: Table 2. – HR-ESI-MS: $m/z = 207.0636$ (calcd. 207.0633 for $\text{C}_9\text{H}_{12}\text{O}_4\text{Na}$, $[\text{M}+\text{Na}]^+$).

Cytotoxic activity

Compounds **1** and **2** were examined for their cytotoxic activity against the human hepatoma SMMC-7721 cell line. Cancer cells were incubated for 3 d at 37 °C in the presence of various concentrations of compounds from DMSO-diluted stock solutions. The growth inhibitory property was determined by *in vitro* treatment of respective cell lines using the MTT assay.

Acknowledgement

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