Two New Noriridoids from *Scyphiphora hydrophyllacea*

Yan-Bo Zeng[a], Wen-Li Mei[a], You-Xing Zhao[b], and Hao-Fu Dai[a]

[a] State Key Laboratory of Tropical Crops Biotechnology, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, Hainan, P. R. China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P. R. China

Reprint requests to Prof. Dr. H. F. Dai. Fax: +86-898-66960599. E-mail: hfdai2001@yahoo.com.cn

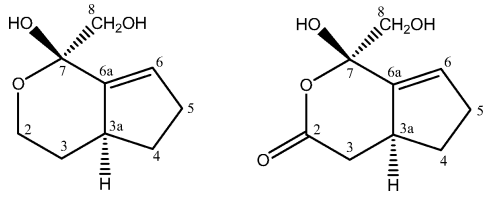
Z. Naturforsch. **2008**, *63b,* 108 – 110; received July 13, 2007

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 southeast 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 $\mu g\,mL^{-1}$. 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 $\mu g\,mL^{-1}$ [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 cytotoxity 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 $\mu g\,mL^{-1}$. In this paper, we describe the isolation and the structure elucidation of **1** and **2** (Fig. 1).



1 **2**

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

Results and Discussion

The EtOH extract of *S. hydrophyllacea* was subjected to repeated chromatography to afford **1** and **2**. The molecular formula of **1** was established as $C_9H_{14}O_3$ with three degrees of unsaturation according to the high-resolution ESI-MS data at *m/z* = 193.0838 (calcd. 193.0840 for $C_9H_{14}O_3Na$, $[M+Na]^+$), 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 (δ_C = 36.3, 38.2 59.3, 66.2, 67.8) including two oxygen-bearing carbons, two methines (δ_C = 44.5, 131.4) including one olefinic carbon, and two quaternary carbons (δ_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

0932–0776 / 08 / 0100–0108 $ 06.00

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

	δC	δH	^{1}H-^{1}H 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)		

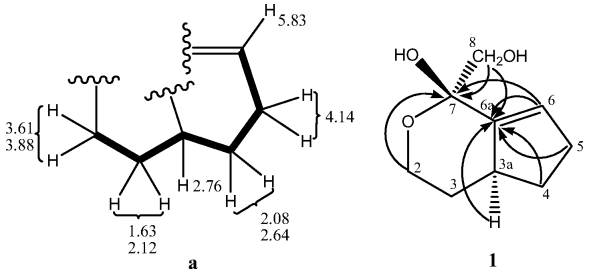


Fig. 2. Selected 2D NMR correlations for **1**. Bonds in bold indicate ^{1}H-^{1}H COSY, arrows indicate HMBC.

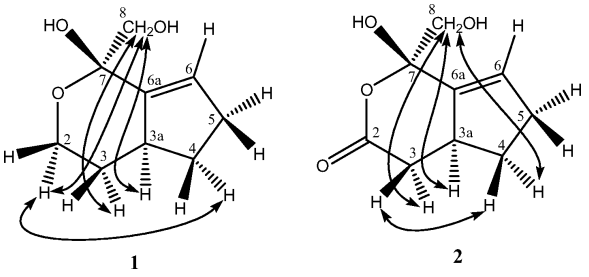


Fig. 3. Key ROESY correlations and relative configurations assigned for **1** and **2**.

with C-6a and C-8 (Fig. 2). The chemical shifts of C-7 (δ_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 stereomeric centers 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 $C_9H_{12}O_4$ based on its HR-ESI-MS (*m/z* = 207.0636; calcd. 207.0633 for $C_9H_{12}O_4Na$, $[M+Na]^+$), 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 (δ_C = 38.4, 38.7, 59.0, 65.3) including two oxygen-bearing carbons, two methines (δ_C = 40.0, 133.4) including one olefinic carbon, and three quaternary carbons (δ_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 δ = 179.6, 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. ^{1}H and ^{13}C NMR data of **2** (CD_3OD, δ in ppm, J in Hz).

	δC	δH	^{1}H-^{1}H 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. $[\alpha]_D^{16.5}$ = +27.49 (c = 0.009, MeOH). – IR (KBr): ν = 3503, 3397 (OH), 1410, 1368, 1265, 1236, 1097, 1052 (C–O) cm^{-1}. – ^{1}H- and ^{13}C NMR: Table 1. – HR-ESI-MS: *m/z* = 193.0838 (calcd. 193.0840 for $C_9H_{14}O_3Na$, $[M+Na]^+$).

*Hydrophylin B (**2**)*

Brown gel. $[\alpha]_D^{16.5}$ = +5.75 (c = 0.01, MeOH). – IR (KBr): ν = 3503, 3396 (OH), 1413, 1370, 1267, 1234, 1096, 1051 (C–O), 3193, 2966, 2947, 1336, 1712 (C=O) cm^{-1}. – ^{1}H- and ^{13}C NMR: Table 2. – HR-ESI-MS: *m/z* = 207.0636 (calcd. 207.0633 for $C_9H_{12}O_4Na$, $[M+Na]^+$).

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

This project was supported by the National Basic Research Program of China (2007CB116306) and the Natural Science Foundation of Hainan (20502).

[1] B. S. Wang, S. C. Liang, W. Y. Zhang, Q. J. Zan, *Acta Botanica Sinica* **2003**, *45*, 644 – 653.
[2] Y. Zhao, Y. W. Guo, *Chin. J. Nat. Med.* **2004**, *2*, 137 – 140.
[3] Y. S. Wang, L. He, Q. J. Wang, S. Zhang, *Chin. J. Mar. Drugs* **2004**, *23*, 26 – 31.
[4] The Chinese Academy of Science Flora of China Editional Board, *Flora Reipublicae Popularis Sinicae*, Tomus 71, Science Press, Beijing, **1999**, pp. 368 – 370.
[5] Y. B. Zeng, W. L. Mei, L. Zhuang, K. Hong, H. F. Dai, *J. Trop. Subtrop. Bot.* **2007**, *15*, 249 – 252.
[6] H. F. Dai, W. L. Mei, J. Wu, X. M. Li, B. G. Wang, *Chin. Pharm. J.* **2006**, *41*, 1452 – 1454.