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Two new epimeric pairs of iridoid from mangrove plant *Scyphiphora hydrophyllacea*

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Abstract

Two new epimeric pairs of iridoid scyphiphin A_1 (**1a**), A_2 (**1b**) and scyphiphin B_1 (**2a**), B_2 (**2b**) were isolated from *Scyphiphora hydrophyllacea* Gaertn. F. Their structures were elucidated by spectroscopic methods. The mixture of scyphiphin B_1 and scyphiphin B_2 showed moderate cytotoxicity against human hepatoma SMMC-7721 cell line *in vitro* by MTT method.

Keywords: *Scyphiphora hydrophyllacea* Gaertn. F.; Iridoid; Cytotoxicity; SMMC-7721

Mangrove plants are woody plants growing in tropical and subtropical intertidal habitats [1]. It was reported that many secondary metabolites isolated from mangrove plants possessed anti-tumor, anti-HIV, anti-bacterial, anti-proliferative, and anti-estrogenic activities which are due to their containing of terpenoid, steroid, alkaloid and polysaccharide [2,3]. In our screening for cytotoxic agents from mangrove plants in Hainan, the ethanol extract of *Scyphiphora hydrophyllacea* Gaertn. F. (Rubiaceae) showed inhibitory activity towards human hepatoma SMMC-7721 and B_{16} cell lines. Bioassay-guided fractionation led to the isolation of two epimeric pairs of iridoid from the aerial part of *Scyphiphora hydrophyllacea*, named scyphiphin A_1, A_2 and scyphiphin B_1, B_2. The mixture of scyphiphin B_1 and scyphiphin B_2 showed moderate cytotoxicity against human hepatoma SMMC-7721 cell line *in vitro* by MTT method with the IC_{50} value of 59.1 μg/mL.

Compounds **1a** and **1b** (Fig. 1) were isolated as an inseparable mixture of isomers in the proportion of 3:1, white amorphous powder, $[\alpha]_{20}^{D}$ + 2.5 (*c* 2.0, MeOH). The molecular formula of the isomers (**1a** and **1b**) was established as $C_{10}H_{14}O_4$ according to the high-resolution ESI-MS spectrometric data at *m/z* 221.0789 $[M + Na]^+$ (calcd. for $C_{10}H_{14}O_4Na$, 221.0787), which was supported by ^{13}C NMR and DEPT spectra. The ^{13}C NMR and DEPT spectra of **1a** presented ten carbon signals for one methyl (δ 25.2), two methylenes (δ 30.2, 40.2), five methines (δ 31.4, 53.4, 96.5, 164.3, 193.0) including one oxygenated carbon, one olefinic carbon and one aldehyde carbon, and two quaternary carbons (δ 80.9, 125.1) including one olefinic carbon. The ^{13}C NMR spectrum of **1a** was similar to that of

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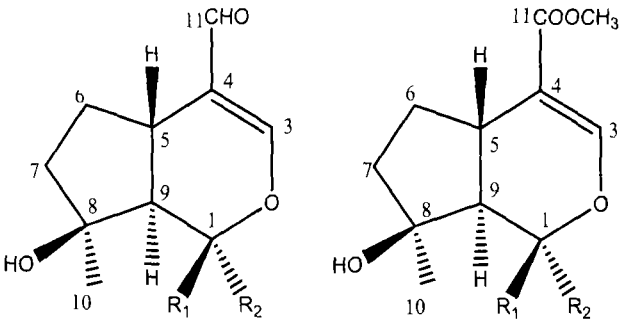

1a R_1=H, R_2=OH
1b R_1=OH, R_2=H

2a R_1=H, R_2=OH
2b R_1=OH, R_2=H

Fig. 1. The structures of **1a**, **1b**, **2a** and **2b**.

Table 1
The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **1a** and **1b** in CD_3OD (δ ppm, J Hz)

No.	**1a**		**1b**	
	δ_C	δ_H	δ_C	δ_H
1	96.5	5.20 d (5.8)	95.8	5.61 d (2.6)
3	164.3	7.39 s	163.7	7.36 s
4	125.1		125.8	
5	31.4	3.18 m	31.2	3.18 m
6	30.2	2.32 m	30.2	2.32 m
7	40.2	2.00 m, 1.72 m	40.8	2.00 m, 1.72 m
8	80.9		80.7	
9	53.4	2.02 t (6.5)	53.2	2.01 t (overlapped)
10	25.2	1.34 s	25.1	1.39 s
11	193.0	9.18 s	193.6	9.23 s

the known compound ixoroside [4] except for the lack of a β-D-glucopyranosyl group at C-1 and the difference of stereochemistry at C-1, C-5, and C-9. The ^{13}C NMR data δ 53.4 (C-9) suggested the hydroxyl group at C-8 was in β-orientation [5], so the methyl group (C-10) at C-8 was in α-orientation. The ROESY experiment showed that δ 1.34 (H-10) correlated with δ 2.02 (H-9), while did not correlate with δ 5.20 (H-1) and 3.18 (H-5). This result indicated that the H-9 was in α-orientation, while H-1 and H-5 were in β-orientation. Thus, the structure of **1a** was confirmed. Comparison of the ^{1}H and ^{13}C NMR spectra of **1b** with those of **1a** (Table 1) showed the clear different chemical shift and constant coupling of H-1 in **1a** [δ 5.20 (5.8)] and **1b** [δ 5.61 (2.6)], which suggested **1b** was an epimer of **1a** at C-1. Both **1a** and **1b** were new compounds, which were named scyphiphin A_1 and scyphiphin A_2, respectively.

Compounds **2a** and **2b** were isolated as an inseparable mixture of isomers in the proportion of 2:1, white amorphous powder, $[\alpha]_{20}^{D}$ + 29.0 (*c* 1.0, MeOH). Their molecular formula $C_{11}H_{16}O_5$ was established according to the high-resolution ESI-MS spectrometric data at *m/z* 251.0899 $[M + Na]^+$ (calcd. for $C_{11}H_{16}O_5Na$, 251.0895). This formula can also be validated through ^{13}C NMR, ^{1}H NMR, and DEPT spectra. The ^{13}C NMR spectrum of **2a** was similar to that of **1a** except for the presence of a carbomethoxy group (δ 169.8) instead of the aldehyde group (δ 193.0) at C-4. Elucidation of the relative configuration for **2a** was generally similar to **1a**. The chemical shift of C-9 (δ 53.6) suggested the methyl group (C-10) at C-8 was in α-orientation [5]. The ROESY experiment showed that δ 1.35 (H-10) correlated with δ 1.98 (H-9), while did not correlate with δ 5.02 (H-1) and 3.19 (H-5). This result indicated that the H-9 was in α-orientation, while H-1 and H-5 were in β-orientation. Thus, the structure of **2a** was confirmed. The different chemical shift and constant coupling of H-1 in **2a** [δ 5.02 (6.6)] and **2b** [δ 5.47 (3.4)] (Table 2) indicated that **2b** was an epimer of **2a** at C-1. Thus, the structures of **2a** and **2b** were confirmed. Both **2a** and **2b** were new compounds, named scyphiphin B_1 and scyphiphin B_2, respectively.

Table 2
The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **2a** and **2b** (in CD_3OD, δ ppm, J Hz)

No.	**2a**		**2b**	
	δ_C	δ_H	δ_C	δ_H
1	94.9	5.02 d (6.6)	93.1	5.47 d (3.4)
3	153.1	7.42 d (0.9)	152.2	7.39 d (1.2)
4	112.0		112.1	
5	33.6	3.19 m	33.0	3.18 m
6	31.2	1.35 m, 2.31 m	32.2	1.35 m, 2.31 m
7	39.8	1.72 t (7.5)	40.5	1.72 (overlapped)
8	81.1		82.6	
9	53.6	1.98 dd (6.8, 7.9)	52.2	1.98 (overlapped)
10	25.3	1.35 s	23.7	1.41 s
11	169.8		171.2	
OCH_3	51.6	3.68 s	51.6	3.69 s

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