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IRIFLOPHENONE GLYCOSIDES FROM *Aquilaria sinensis*

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Five iriflophenone glycosides were obtained from the stems of Aquilaria sinensis *by phytochemical methods. Their structures were determined by spectroscopic methods and comparison with literature data. As a novel compound, compound **1** was given a trivial name aquilarinoside B.*

Keywords: iriflophenone glycosides, *Aquilaria sinensis*, aquilarinoside B.

Agarwood (Chen-xiang in Chinese), which is also called eaglewood, is the resinous heartwood from *Aquilaria* species formed in response to injury from cutting, holing, burning, or incursion of moth and microorganism [1]. In China, agarwood is highly valued for its uses in traditional Chinese medicine as a sedative, analgesic, and digestive agent [2]. *Aquilaria sinensis* (Lour.) Gilg, the only plant resource in China for agarwood, is mainly cultivated in Yunnan, Guangdong, Guangxi, Hainan, Taiwan, and Fujian Provinces [3, 4]. In the course of continuous research on the chemical constituents of *A. sinensis*, five iriflophenone glycosides (**1** – **5**) were obtained and elucidated from the stems of *Aquilaria sinensis* by phytochemical methods and modern spectroscopic methods such as UV, IR, MS, HR-ESI-MS, and 1D NMR and 2D NMR. Among them, compound **1** is a novel compound, named aquilarinoside B.

Compound **1** was obtained as primrose crystalline powder with $[\alpha]_D^{25}$ +42.5° (*c* 1.3, MeOH). The molecular formula of **1** was determined as $C_{21}H_{22}O_{12}$ by HR-ESI-MS at *m/z* 465.0981 ($[M - H]^-$, calcd for $C_{21}H_{21}O_{12}$, 465.1036) and DEPT spectra. The 11 degrees of unsaturation indicated that **1** might contain two benzene rings. The positive Molisch reaction suggested that **1** had a glycoside moiety, which was supported by the NMR data. The UV spectrum showed maximum absorbance at 206 nm (0.60), 225 nm (0.71), and 270 nm (0.54), which indicated an α,β-unsaturated carbonyl group. The IR spectrum gave absorptions for hydroxyl (3435 cm^{-1}), the aromatic group (1605, 1587, 1521 and 1450 cm^{-1}), and the carbonyl bond (1615 cm^{-1}). The ^1H and ^{13}C NMR (Table 1) were similar to those of iriflophenone 3,5-*C*-β-diglucopyranoside [5], indicating that **1** might be a derivative of an iriflophenone. In the ^1H NMR spectrum, the presence of a *para*-substituted symmetric phenolic ring with two equivalent pairs of *ortho*-coupled protons was indicated by the four proton signals at δ 7.72 (2H, d, J = 8.6, H-2′, 6′) and 6.83 (2H, d, J = 8.6, H-3′, 5′); the three singlets at δ 10.52 (1H, s), 10.31 (1H, s), and 10.05 (1H, s) were assigned to three phenolic-hydroxyl groups; a signal at δ 4.05 (3H, s, $COOCH_3$) exhibited one methoxyl group; and a glucose group exhibited proton signals at δ 3.40 to 5.10, including one signal of anomeric proton at δ 4.86 (1H, d, J = 9.2 Hz, H-1″). Moreover, no proton signals on the *meta*-substituted benzene ring were found in the ^1H NMR data, which suggested that the *meta*-substituted benzene ring was fully substituted. The ^{13}C NMR and DEPT spectra revealed two carbonyl groups at δ 197.3 (C-7) and 171.2 (C-8), one oxygenated methyl group at δ 53.0 (OCH_3), one glucose moiety from δ 62.5 to 82.1, together with two phenyl rings corresponding to the remaining 12 signals. The complete assignment of **1** was performed by ^1H–^1H COSY, HSQC, and HMBC. The anomeric proton at δ 4.86 (1H, d, J = 9.2 Hz, H-1″) was located on the carbon at δ 76.8 (C-1″) in the HSQC spectrum.

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TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Compound **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	–	109.6	5′	6.83 (1H, d, J = 8.6)	116.3
2	–	163.4	6′	7.72 (1H, d, J = 8.6)	133.0
3	–	105.8	Glc		
4	–	164.1	1″	4.86 (1H, d, J = 9.2)	76.8
5	–	94.7	2″	3.78 (1H, m)	73.9
6	–	160.9	3″	3.45 (1H, m)	79.8
7	–	197.3	4″	3.43 (1H, m)	71.4
8	–	171.2	5″	3.41 (1H, m)	82.1
OCH_3	4.05 (3H, s)	53.0	6″	3.82 (1H, dd, J = 12.2, 2.5);	62.5
1′	–	131.5		3.71 (1H, dd, J = 12.2, 2.5)	
2′	7.72 (1H, d, J = 8.6)	133.0	OH	10.52 (1H, s)	
3′	6.83 (1H, d, J = 8.6)	116.3	OH	10.31 (1H, s)	
4′	–	164.1	OH	10.05 (1H, s)	

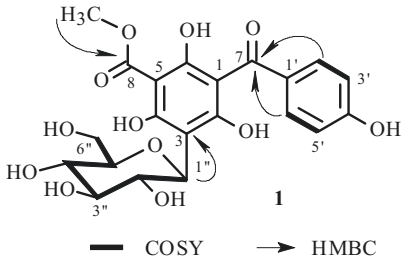
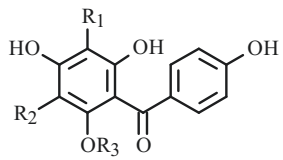


Fig. 1. Selective correlations of HMBC and COSY of compound **1**.

In the HMBC spectrum, correlations from δ 7.72 (H-2′, 6′) to δ 197.3 (C-7) showed that the *para*-substituted benzene ring was attached to the carbonyl group, correlations from H-1″ (δ 4.86) to C-3 (δ 105.8) confirmed the location of the glucose moiety at C-3, and correlations from δ 4.05 (OCH_3) to δ 171.2 (C-8) verified the existence of the methyl formic group. Considering the *para*-substituted benzene ring and three *meta*-substituted hydroxyls, the linkage of the methyl formic group was only at C-5. The *β*-configuration of the sugar unit was deduced based on the coupling constants of the anomeric protons H-1″ (J = 9.2 Hz). Thus, the structure of **1** was determined to be iriflophenone 5-formic methyl-3-*C*-*β*-D-glucose, assigned the trivial name aquilarinoside B (Fig. 1).

Compounds **2**–**5** were elucidated to be iriflophenone 3-*C*-*β*-D-glucopyranoside (**2**) [6], iriflophenone 2-*O*-*α*-L-rhamnopyranoside (**3**) [5], iriflophenone 3,5-*C*-*β*-diglucopyranoside (**4**) [5], and aquilarisinin (**5**) [7] by comparison of their spectral data with the reported literature.



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2: $R_1 = R_3 = H$, R_2 = Glc*p*; **3:** $R_1 = R_2 = H$, R_3 = Rha*p*
4: $R_1 = R_2$ = Glc*p*; $R_3 = H$; **5:** $R_1 = R_2 = H$, R_3 = Glc*p*→Rha*p*

EXPERIMENTAL

General Experimental Procedures. Optical rotation was taken on a JASCO P-1020 polarimeter. UV spectra were detected in MeOH by a UV-2401 PC spectrometer. The IR spectra were measured on a PerkinElmer Fourier transform infrared spectroscopy (FTIR) spectrometer with KBr discs. ^{1}H NMR and ^{13}C NMR, HMQC, HMBC, and COSY spectra were analyzed by a Bruker AV 500 instrument, with TMS as internal standard. Mass spectra were performed on an MS Agilent 1100 Series

LC/MS Trap mass spectrometer (ESI-MS). HR-ESI-MS was recorded on a Micro Q-TOF MS (HR-ESI-MS). Gas chromatography (GC) analysis was obtained on a Fuji GC-9790 gas chromatograph equipped with H_2 flame ionization detector. Gas chromatography was performed on an OV-17 column using Silanox (0.3 mm × 50 m). Column chromatography (CC) was performed on silica gel (200–300 mesh; Qingdao Ocean Chemical Group Corporation, Qingdao, China), macroporous resin D101 (Chemical Reagent Factory, Tianjin, China), MCI gel (Mitsubishi Chemical Corporation, Tokyo, Japan), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, US), and reversed-phase C_{18} silica gel (Merck, Darmstadt Germany). Thin-layer chromatography (TLC) was performed on silica GF_{254} precoated plates (Merck), RP-18 F_{254} precoated plates (Merck), and polyamide plates (from Nanjing). The authentic D-glucose and L-rhamnose were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other chemical reagents were of analytical grade.

Plant Material. Stems of *A. sinensis* were collected in Qingyuan, Guangdong Province of China in July 2012 and were authenticated by Prof. Deyun Wang, Institute of Traditional Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University, where a voucher specimen (No. 20120702) was deposited.

Extraction and Isolation. The dried stems of *A. sinensis* (5 kg) were crushed and extracted with 95% (v/v) ethanol (90 L, 1 d × 2) and 80% (v/v) ethanol (75 L, 1 d × 1) by diacolation at room temperature, followed by removal of the combined solvent in vacuum at 50°C to afford a brown crude concentrate (0.57 kg). The concentrate was then partitioned successively with petroleum ether (PE, 60–90°C), dichloromethane, ethyl acetate, and *n*-butanol to yield four fractions. The *n*-BuOH fraction (196 g) was subjected to silica gel CC with CH_2Cl_2–MeOH–H_2O (49:1:0.1–1:1:0.1, v/v) to give five fractions (Fr. 1–Fr. 5). Fraction 3 (35 g) was chromatographed on a silica gel column eluted with CH_2Cl_2–MeOH–H_2O (98:1:0.1–1:1:0.1, v/v) to yield seven fractions (Fr. 3A–Fr. 3G). Fraction 3F (6 g) was decolorized on a Sephadex LH-20 column with CH_2Cl_2–MeOH (1:1, v/v) to give six subfractions (Fr. 3F1–3F6). Fraction 3F2 was isolated on an RP-C_{18} open column eluted with a step gradient of acetone–H_2O (1:9 to 9:1, v/v) to afford five subfractions (Fr. 3F2a–3F2e). Fraction 3F2b was separated by repeated preparative TLC with CH_2Cl_2–MeOH–H_2O (35:2:0.1, v/v) and then eluted on Sephadex LH-20 CC by MeOH to give **1** (12 mg) and **2** (10 mg). Fraction 4 was separated over a silica gel column eluted with CH_2Cl_2–MeOH–H_2O (29:1:0.1–1:1:0.1, v/v) to give five fractions (Fr. 4A–Fr. 4E). Subfraction 4B was applied to an RP-C_{18} gel column eluted with gradient of MeOH–H_2O (1:9 to 9:1, v/v) to afford five subfractions 4B1 to 4B5. Fraction 4B2 was applied to a silica gel H column with CH_2Cl_2–MeOH–H_2O (79:10:1, v/v) and separated by preparative TLC repeatedly with acetone–MeOH–H_2O (12:1:0.1, v/v) and *n*-BuOH–AcOH–H_2O (4:5:1), then decolorized on a Sephadex LH-20 column with MeOH (1:1, v/v) to give **3** (14 mg). Fraction 4B5 was chromatographed on a silica gel column eluted with CH_2Cl_2–MeOH–H_2O (19:1:0.1–1:1:0.1, v/v) to afford four subfractions (4B5a to 4B5d). Subfraction 4B5d was separated by preparative TLC repeatedly with CH_2Cl_2–MeOH–H_2O (65:35:10, v/v) and *n*-BuOH–AcOH–H_2O (4:5:1) to yield **4** (8 mg) and **5** (11 mg).

Hydrolysis and GC Chromatography. Compounds **2** and **4** (5 mg) were hydrolyzed in 10 mL 2M HCl for 5 h at 100°C. After cooling to room temperature, the mixture was neutralized with saturated $NaHCO_3$ and extracted with EtOAc (2 mL × 3). The aqueous layer was evaporated to dryness. The gas-liquid chromatography experiment was performed following the reported method [8]. The residue obtained was coincident with an authentic sample α-L-rhamnose and β-D-glucose.

Aquilarinoside B (1), $C_{21}H_{22}O_{12}$, primrose crystalline powder. UV spectrum (MeOH, λ_{max}, nm): 206 (0.60), 225 (0.71), and 270 (0.54). IR spectrum (KBr, ν_{max}, cm^{-1}): 3435, 1615, 1605, 1587, 1521, and 1450. ESI-MS *m/z* 465 $[M - H]^-$; HR-ESI-MS *m/z* 465.0981 ($[M - H]^-$, calcd for $C_{21}H_{21}O_{12}$, 465.1036). For ^1H NMR (500 MHz, DMSO-d_6) spectral data and ^{13}C NMR (125 MHz, DMSO-d_6) spectral data, see Table 1.

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