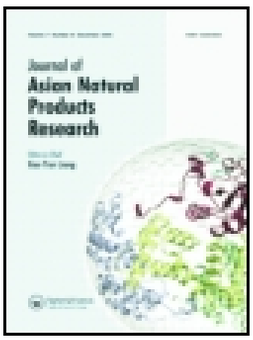

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A new 2-(2-phenylethyl)chromone glycoside in Chinese agarwood "Qi-Nan" from *Aquilaria sinensis*

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ABSTRACT

A new 2-(2-phenylethyl)chromone glycoside, 2-[2-(4-glucosyloxy-3-methoxyphenyl)ethyl]chromone (**1**), was isolated from the high-quality Chinese agarwood "Qi-Nan" originating from *Aquilaria sinensis* (Lour.) Glig. The structure including the absolute configuration of the sugar moiety was elucidated by spectroscopic techniques (UV, IR, 1D and 2D NMR), MS analysis, PMP-labeling HPLC analysis methods, as well as comparison with literature data. To the best of our knowledge, it is the first time that chromone glycoside was discovered in agarwood, or even in the whole *Aquilaria* plants.

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KEYWORDS
Qi-Nan; agarwood; *Aquilaria sinensis*; 2-(2-phenylethyl) chromone derivative; glycoside

1. Introduction

Agarwood is the resinous heartwood from *Aquilaria* or *Gyrinops* species of Thymelaeaceae [1]. It is well known as precious traditional Chinese medicine for its therapeutic effect in stomachache, fever, cough, rheumatism and other illness [2]. Agarwood was also very famous as natural flavor, and was widely used in aromatherapy and religious ceremony. "Qi-Nan" as the most precious kind of agarwood can be smelt without burning for its mysterious and elegant fragrance. Owing to the scarcity and high commercial value, study on its chemical composition has aroused worldwide interest. Japanese researchers have isolated many sesquiterpenes and 2-(2-phenylethyl)chromone derivatives from the "Qi-Nan" of Vietnam originating from *Aquilaria agallocha* Roxb [3,4]. De-lan Yang has isolated a series of 2-(2-phenylethyl)chromone derivatives and sesquiterpenes from Chinese agarwood "Lv Qi-Nan" originating from *Aquilaria sinensis* (Lour.) Glig [5,6]. Our further chemical study on the ethanol extract of this "Lv Qi-Nan" led to the isolation and identification of a new 2-(2-phenylethyl)chromone glycoside (**1**) (Figure 1). Although, the aglycone of **1**, 2-[2-(3-methoxy-4-hydroxyphenyl)ethyl]chromone, has been isolated from the same sample before [5], and 2-(2-phenylethyl)chromone glycoside has ever been found from *Imperata cylindrica* [7], *Ononis vaginalis* [8], *Cucumis melo* L var. *reticulatus* [9], compounds of this

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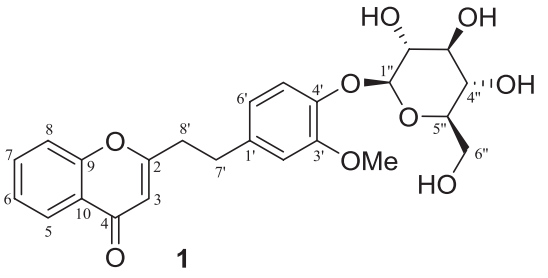

Figure 1. Chemical structure of compound **1**.

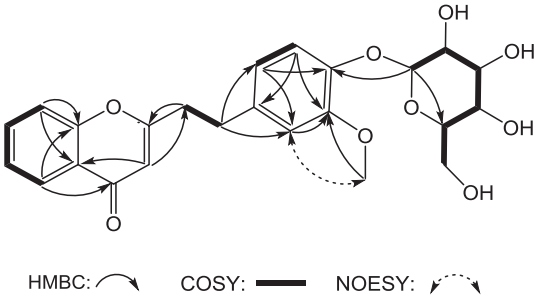

Figure 2. Key HMBC, ROESY, and ^{1}H-^{1}H COSY correlations of compound **1**.

type were discovered in agarwood for the first time in this study. Herein, the isolation and structure elucidation of the new chromone glycoside (**1**) were described.

2. Results and discussion

Compound **1** was obtained as colorless solid. Its molecular formula was deduced to be $C_{24}H_{26}O_9$ on the basis of HR-ESI-MS (*m/z* 481.1466 $[M+Na]^+$). The IR spectrum revealed the presence of hydroxyls (3437 cm^{-1}) and α, β-unsaturated carbonyl group (1641 cm^{-1}) moieties. The ^{1}H NMR spectrum showed the presence of a methoxy group at δ_H 3.71, one olefinic proton at δ_H 6.14 (1H, s, H-3), one set of ABX coupling system at δ_H 6.72 (1H, dd, J = 8.2, 1.8 Hz, H-6′), δ_H 6.83 (1H, d, J = 1.8 Hz, H-2′), and δ_H 7.02 (1H, d, J = 8.2 Hz, H-5′), ascribable to 1,3,4-trisubstituted phenyl moiety. The other set of signals at δ_H 8.05 (1H, dd, J = 7.6, 1.4 Hz, H-5), δ_H 7.43 (1H, dd, J = 7.6, 7.6 Hz, H-6), δ_H 7.75 (1H, ddd, J = 8.4, 7.6, 1.4 Hz, H-7), and δ_H 7.55 (1H, d, J = 8.4 Hz, H-8) revealed the presence of an *ortho*-disubstituted aromatic ring. The presence of two methylene groups and a trisubstituted double bond were evidenced by the ^{13}C and DEPT NMR resonances at δ_C 33.6, 37.1, and δ_C 110.7 (CH), 171.8 (qC), respectively. The signals at δ_C 180.6 and 56.6 confirmed the existence of a conjugated ketone and a methoxy group. In addition, six characteristic oxygenated carbon signals at δ_C 74.9~102.9 in the ^{13}C NMR spectrum, as well as the corresponding ^{1}H NMR signals between 3.31 and 4.79 ppm, were evident for a glucose moiety, indicating the glycosylation of **1**. Except for the additional glucose unit, the remaining NMR resonances of **1** were nearly identical with those of the known compound 2-[2-(3-methoxy-4-hydroxyphenyl)ethyl]chromone [5]. ^{1}H-^{1}H COSY and HMBC data depicted in Figure 2 confirmed

this deduction. The methoxy group was located at C-3′ (δ_C 150.7) by the HMBC correlations from 3′-OCH_3 (δ_H 3.71), H-2′ (δ_H 6.83) and H-5′ (δ_H 7.02) to C-3′ (δ_C 150.7), as well as the ROESY correlation from 3′-OCH_3 (δ_H 3.71) to H-2′ (δ_H 6.83). Furthermore, the coupling constants of H-1″ with H-2″ (J = 7.3 Hz), H-2″ with H-3″ (J = 8.9 Hz), H-3″ with H-4″ (J = 6.8 Hz), and H-4″ with H-5″ (J = 6.8 Hz) in the ^{1}H NMR spectrum were consistent with a β-glucopyranosyl unit [7], which was located at C-4′ based on HMBC correlations from H-1″ (δ_H 4.79) to C-4′ (δ_C 146.6). Acidic hydrolysis of **1** liberated D-glucose, which was determined by PMP-labeling HPLC analysis and by comparison of the optical rotation value ($[\alpha]_D^{25}$ + 54; H_2O) with the standard glucose ($[\alpha]_D^{25}$ + 49; H_2O) and that reported in literature ($[\alpha]_D^{25}$ + 52; H_2O) [10]. Based on these findings, the structure of **1** was determined to be 2-[2-(4-glucosyloxy-3-methoxyphenyl)ethyl]chromone.

Compound **1** was evaluated for the inhibitory activity against AChE by Ellman Method [11] and cytotoxicity against K562, BEL-7402, SGC-7901 cell lines by MTT method [12]. The results showed that compound **1** was inactive against AChE and all the cell lines.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a Rudolph Autopol III polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). UV spectra were recorded on a Shimadzu UV-2550 spectrometer (Beckman, Brea, CA, USA). IR absorptions were obtained on a Nicolet 380 FT-IR instrument (Thermo, Pittsburgh, PA, USA) using KBr pellets. The NMR spectra were recorded on Bruker Avance 500 NMR spectrometers (Bruker, Bremen, Germany), using TMS as an internal standard. HR-ESI-MS were measured with an API QSTAR Pulsar mass spectrometer (Bruker, Bremen, Germany) or Waters Autospec Premier (Waters, Atlanta, GA, USA). Column chromatography was performed with silica gel (60–80, 200–300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), ODS gel (20–45 μm, Fuji Silysia Chemical Co., Ltd, Durham, NC, USA), and Sephadex LH-20 (Merck, Darmstadt, Germany). TLC was carried out on silica gel GF254 (Qingdao Haiyang Chemical Co., Ltd), and spots were detected by spraying with 5% H_2SO_4 in EtOH followed by heating. HPLC purifications were performed on an analytical reversed-phase column (YMC-packed C_{18}, 250 mm × 10 mm, 5 μm) (YMC, Kyoto, Japan) using a G1311C 1260 Quat Pump VL and detected with a G1315D 1260 DAD VL detector (254 nm) (Agilent Technologies 1260 infinity, PaloAlto, California, USA).

3.2. Plant material

Chinese agarwood "Lv Qi-Nan" was donated by Mr. Xiao-Wu Zhang and Mr. Mao-You Guan in July 2011. The specimen was identified as "Lv Qi-Nan" originating from *A. sinensis* by Prof. Hao-Fu Dai and Dr. Xi-Long Zheng. A voucher specimen (QN 20110830) has been deposited at the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **1** in CD_3OD.

Position	δ_C	δ_H	Position	δ_C	δ_H
2	171.8		4′	146.6	
3	110.7	6.14, s	5′	118.1	7.02, d (8.2)
4	180.6		6′	122.0	6.72, dd (8.2, 1.8)
5	126.2	8.05, dd (7.6, 1.4)	7′	33.6	3.00~3.02, m
6	126.6	7.43, dd (7.6, 7.6)	8′	37.1	2.97~3.00, m
7	135.6	7.75, ddd (8.4, 7.6, 1.4)	1″	102.9	4.79, d (7.3)
8	119.3	7.55, d (8.4)	2″	74.9	3.41, dd (7.3, 8.9)
9	158.0		3″	77.8	3.44, dd (8.9, 6.8)
10	124.3		4″	71.3	3.34, t (6.8)
1′	136.2		5″	78.1	3.31~3.33, m
2′	114.0	6.83, d (1.8)	6″	62.4	3.81, br d (12.0)3.63, dd (12.0, 5.2)
3′	150.7		3′-OMe	56.6	3.71, s

3.3. Extraction and isolation

"Lv Qi-Nan" (dry weight 180.2 g) was mixed with 1.5 L of ether by ultrasonic extraction 3 times (30 min/time), and the residue after filtering liquid was extracted with 95% ethanol by heating reflux three times, to get the ethanol extract (15.8 g) by vacuum concentration. The ethanol extract was divided into 11 fractions QE1 ~ QE11 by the reduced pressure chromatography ($CHCl_3$-MeOH, v/v = 1:0–0:1, 1 L). Fraction QE8 (2.6 g) using the ODS column chromatography (MeOH-H_2O, v/v = 3:7–1:0, 1 L) was divided into QE8–1–QE8–16. QE8–9 (42.4 mg) was applied to Sephadex LH-20 (pure MeOH) and silica gel column chromatography ($CHCl_3$-MeOH = 20:1) to obtain compound **1** (3.0 mg).

3.3.1. 2-[2-(4-Glucosyloxy-3-methoxyphenyl)ethyl]chromone (1)

$C_{24}H_{26}O_9$, colorless oil; $[\alpha]_D^{25} + 36$ (*c* 0.05, MeOH); UV (MeOH) λ_{max} (logε): 203 (5.34), 224 (6.25), 296 (4.65) nm; IR (KBr) ν_{max} 3437, 1641, 1016 cm^{-1}; for ^{1}H and ^{13}C NMR spectral data see Table 1; HR-ESI-MS: *m/z* 481.1466 $[M + Na]^+$ (calcd for $C_{24}H_{26}O_9Na$, 481.1469).

3.4. The configuration determination of glucose

3.4.1. Acid hydrolysis of compound 1

Compound **1** (2.0 mg) was refluxed with HCl solution (1 $molL^{-1}$, 1 ml) on a water bath at 70 °C for 5 h. The reaction mixtures were added to saturated solution of Na_2CO_3 for neutralizing the residual acid. The liquid was extracted with ethyl acetate three times. The water layer was then evaporated under vacuum to obtain the dry monosaccharide (0.7 mg).

3.4.2. 1-Phenyl-3-methyl-5-pyrazolone (PMP) derivatization

The authentic D-glucose (2.0 mg) and acid hydrolysis monosaccharides of **1** (0.7 mg) were directly labeled with PMP respectively by adding 120 μl of PMP solution (0.5 M in methanol) and 100 μl of sodium hydroxide solution (0.3 M), vortexing, and refluxing at 70 °C for 1 h. The mixture was then neutralized by adding 100 μl of hydrochloric acid solution (0.3 M). Chloroform (0.5 ml) was added and mixed thoroughly by vortexing and phase separation was enhanced by brief centrifugation for 10 min (13,000 r/min). The organic phase was carefully removed for discarding the remaining PMP. The process of extraction was repeated at least three times. The resulting aqueous phase was mixed with water for HPLC analysis [13]. Chromatographic condition: Mobile phase: acetonitrile-phosphate buffer (KH_2PO_4,

pH 8.3) (17:83, v/v); Chromatographic column: Agilent YMC-packed C_{18} chromatographic column (250 mm × 10 mm, 5 μm); Flow rate: 1.0 ml/min; Column temperature: 30 °C; Sample quantity: 20 μl; Detector: the SPD – 20A UV detector (254 nm) DAD.

Disclosure statement

No potential conflict of interest was reported by the authors.

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