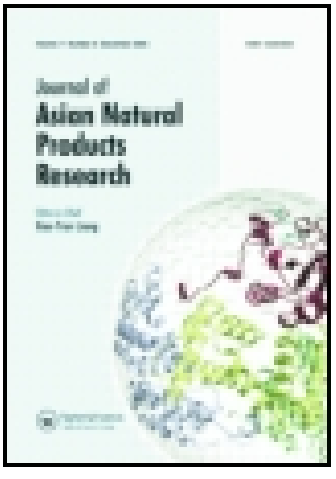

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A new flavonoid glucoside from *Rhododendron seniavinii*

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The leaves of *Rhododendron seniavinii* Maxim with little phytochemical information are used as folk remedies for the treatment of acute and chronic bronchitis in China. In our pursuing for the biologically active chemical constituents in the leaves, a new flavonoid glycoside 5,7,3′-trimethoxy-quercetin-3-*O*-β-D-glucopyranoside (**1**) was isolated from the water extract of its leaves, together with two known compounds 5,7,3′-trimethoxy-quercetin (**2**) and ovafolinin B-9′-*O*-β-D-glucopyranoside (**3**). The structures of the new flavonoid glucoside as well as two known compounds were elucidated by spectroscopic and chemical methods.

Keywords: *Rhododendron seniavinii*; flavonoid glucoside; 5,7,3′-trimethoxy-quercetin-3-*O*-β-D-glucopyranoside

1. Introduction

Rhododendron seniavinii Maxim (Ericaceae), a traditional medicinal plant, is widely distributed in the southern provinces of China. This plant is locally known as "Man Shang Bai," and its leaves have been used as folk medicine for the treatment of acute and chronic bronchitis [1]. Several Chinese patent drugs containing this plant, such as Man-Shang-Bai syrup, were produced and sold now. Although pharmacological studies on this plant had demonstrated its effects on respiratory system and analgesic and anti-inflammatory aspects, there were few reports about its chemical constituents [2]. In order to better understand the biological substances existed in the leaves, the chemical constituents of the leaves from the titled plant were investigated, and one new flavonoids glycoside (Figure 1) together with two known compounds were isolated from the water-soluble extract of the leaves of *R. seniavinii*, except for a series of other known compounds [3]. Herein, we report the isolation and structural elucidation of the new compound.

2. Results and discussion

Compound **1** was obtained as a light yellow amorphous powder. Its molecular formula was determined to be $C_{24}H_{26}O_{12}$ by HR-ESI-MS, which showed a $[M + H]^+$ peak at *m/z* 507.1496. The IR spectra showed a strong absorption band at 1631 cm^{-1} for a conjugated carbonyl group, and an intense broad band centered at 3383 cm^{-1} from the vibration (ν_{OH}) of hydroxyl groups. The UV absorption maxima occurred at 250 nm, implying the presence of long-conjugated double bond systems in the molecule. The ^1H NMR spectrum showed signals for three methoxyl groups (δ_H 3.88, 3.84, and 3.84) and five aromatic protons. Among these aromatic protons, a set of three protons was resolved as one ABX spin-coupling system at δ_H 7.98 (d, $J = 1.8$ Hz) for H-

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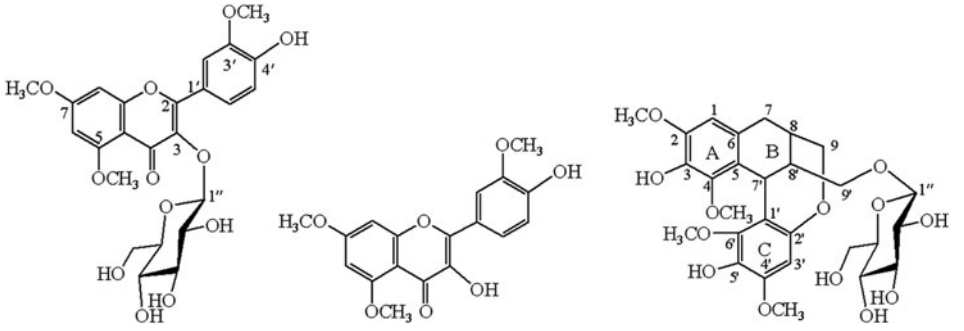

Figure 1. Chemical structures of compounds **1**–**3**.

2′, 6.90 (d, $J = 8.4$ Hz) for H-5′, and 7.53 (dd, $J = 8.4$, 1.8 Hz) for H-6′ in a flavonol; the other AX system at δ_H 6.49 (br s) and δ_H 6.79 (br s) was assigned to H-6 and H-8 protons, respectively. The methoxyl groups were attached at C-5, 7, and 3′ on the basis of the HMBC correlations between methyl protons at δ_H 3.88, 3.84, and 3.84 with carbons at δ_C 160.3 (C-5), 163.9 (C-7), and 146.9 (C-3′), respectively. The NOE correlations between H-8 and 7-OCH_3, H-6 and 5-OCH_3, and H-2′ and 3′-OCH_3 further confirmed the locations of three methoxyl groups (Table 1). Hence, the flavonoid part has the basic structure of a known compound 5,7,3′-trimethoxy-quercetin. The anomeric proton of the sugar appeared at δ_H 5.42 (d, $J = 7.2$ Hz) suggested that it be a glycoside. The ^{13}C NMR spectrum (Table 1) of **1** exhibited signals of six carbons at δ_C 101.2 (C-1″), 77.4 (C-2″), 74.5 (C-3″), 69.9 (C-4″), 76.6 (C-5″), and 60.7 (C-6″), which were in good accordance with the presence of a glucosyl unit [3]. The coupling constant value ($J = 7.2$ Hz) of the anomeric proton confirmed the β-linkage of the sugar moiety [4]. A long-range correlation between the anomeric proton at δ_H 5.42 (d, $J = 7.2$ Hz, H-1″) and the carbon at δ_C 135.6 (C-3) of the flavonoid skeleton in HMBC spectrum determined the attachment of the sugar at C-3 of the aglycone (Figure 2). Acid hydrolysis of **1** afforded D-glucose, the structure of which was confirmed by HPLC analysis referenced to the method [5] reported previously. Thus, the structure of **1** was determined as 5,7,3′-trimethoxy-quercetin-3-*O*-β-D-glucopyranoside.

Table 1. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of compound **1** (δ in ppm, J in Hz).

	1*	
No.	^1H	^{13}C
2	–	153.2
3	–	135.6
4	–	172.2
5	–	160.3
6	6.49 d (2.1)	96.0
7	–	163.9
8	6.79 d (2.1)	92.9
9	–	158.2
10	–	108.2
1′	–	121.2
2′	7.98 d (1.8)	113.4
3′	–	146.9
4′	–	149.0
5′	6.90 d (8.4)	115.1
6′	7.53 dd (8.4, 1.8)	121.6
1″	5.42 d (7.2)	101.2
2″	3.21–3.23 m	74.5
3″	3.22–3.25 m	76.6
4″	3.07–3.10 m	69.9
5″	3.05–3.09 m	77.4
6″	3.58 (d, 13.1), 3.36–3.39 (m)	60.7
5-OCH_3	3.84 s	56.2
7-OCH_3	3.88 s	55.7
3′-OCH_3	3.84 s	56.1

Note: Solvent: *DMSO-d_6.

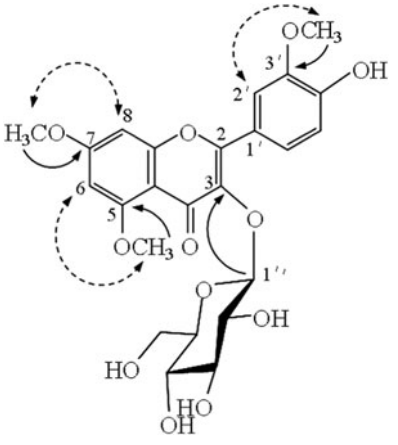

Figure 2. Key HMBC (H → C) and NOE (H···H) correlations for structural determination of compound **1**.

Compounds **2** and **3** were obtained as an amorphous yellow or yellowish powder. Their structures were, respectively, determined to be 5,7,3′-trimethoxy-quercetin [5–7] and ovafolinin B-9′-*O*-β-D-glucopyranoside [8] on the basis of NMR and MS data, as compared with the literature.

3. Experimental

3.1 General experimental procedures

Optical rotations were carried out using a JASCO P-1020 automatic digital polarimeter (JASCO Corporation, Tokyo, Japan). UV spectra were recorded on a JASCO V-550 UV/VIS spectrometer (JASCO Corporation). IR spectra were obtained using a JASCO FT/IR-480 plus spectrometer (JASCO Corporation) with KBr pellets. HR-ESI-MS were acquired using Agilent 6210 LC/MSD TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Measurement of 1D and 2D NMR spectra was run with a Bruker AV-300 spectrometer (Bruker Corporation, Fallanden, Switzerland) with TMS as the internal standard, and chemical shifts were expressed in δ values (ppm). An Agilent 1200 Series HPLC instrument (Agilent Technologies) equipped with a quaternary pump, a multiple wavelength detector, an AutoSampler, and an Ultimate™ XB-C18 column (5 μm, 4.6 × 250 mm, Welch, Potamac, MA, USA) were used for analytical HPLC. Semi-preparative HPLC was carried out on an Agilent 1200 Series HPLC instrument (Agilent Technologies) equipped with a variable wavelength detector and a reversed-phase column (Ultimate™ XB-C18, 5 μm, 10 × 250 mm, Welch). Open column chromatography (CC) was performed by D101 macroporous resin (Tianjin Agricultural Pesticide Corporation, Tianjin, China), polyamide plates (80–100 mesh; Taizhou Luqiao Plaschemgel, Taizhou, China), precoated silica-gel plates (GF_{254}, Yantai Chemical Industry Research Institute, Yantai, China), ODS (50 μm, YMC, Tokyo, Japan), and Sephadex LH-20 (Phamacia Biotech, Zurich, Switzerland). Thin-layer chromatography was performed by precoated TLC spraying with 10% H_2SO_4 followed by heating. All the reagents were purchased from Tianjin Damao Chemical Company (Tianjin, China). Standard sugars D-glucose and L-glucose, and L-cysteine methyl ester in the HPLC analysis were purchased from Adamas-Beta Company (Basel, Switzerland). *O*-Tolyl isothiocyanate was purchased from Sigma Company (Santa Clara, CA, USA).

3.2 Plant material

The water extract of the leaves of *R. seniavinii* was prepared and provided by the Jiangxi Zixi Pharmaceutical Factory (Fuzhou, China). The original plant *R. seniavinii* and the crude leaf material from the plant were authenticated by the technician Mr GJ Lee majored in medicinal plants at quality control department in the factory. The material for extraction was collected in Zixi County, Jiangxi Province in 2008 summer. A voucher specimen with accession no. RS 201001

has been deposited in the herbarium of the College of Pharmacy, Jinan University.

3.3 Extraction and isolation

The water extract (324 g) of the leaves of *R. seniavinii* was subjected to D101 macroporous absorption resin column (80 × 20 cm) eluting with EtOH–H_2O (0 → 30 → 50 → 70 → 95) to afford five fractions (Fr A–E). Fr C (15.6 g) was subjected to CC (column chromatography) on polyamide eluting with MeOH/H_2O (1:4 → 1:0) to yield 10 fractions (Fr C_1–C_{10}). Fr C_3 (2.3 g) was subjected to CC (ODS, MeOH/H_2O 1:5 → 1:0) to yield subfractions Fr C_3-a–C_3-g. Fr C_3-e (300 mg) was fractionated by CC (Sephadex LH-20, MeOH/H_2O 50:50) to yield Fr C_3-e_1–C_3-e_8. Fr C_3-e_4 (100 mg) was finally purified by semi-preparative HPLC (flow rate; 4.0 ml/min; Rt: 19.3 min) with CH_3CN/H_2O (30:70) as an eluant to obtain compound **1** (15.1 mg). Fr B_3-d (58 mg) was finally purified by CC (Sephadex LH-20, MeOH/H_2O 30:70 as an eluting solvent) to obtain compound **3** (9.8 mg). Fr E (1 g) was subjected to CC (Sephadex LH-20, MeOH) to yield subfractions Fr E_1–E_{10}. Fr E_7 (120 mg) was finally purified by semi-preparative HPLC (flow rate; 4.0 ml/min; Rt: 23.5 min) with MeOH–H_2O (70:30) as an eluting system to obtain compound **2** (16.3 mg).

3.3.1 5,7,3′-Trimethoxy-quercetin-3-O-β-D-glucopyranoside (1)

Light-yellow amorphous powder; $[\alpha]_D^{27}$ −6.7 ($c = 0.10$, MeOH); UV (MeOH) λ_{max}: 206, 250, 265 (sh) nm; IR (KBr) ν_{max}: 3383, 1631, 1603, 1517.3, 1457.9, 1431.1, 1357.9, 1287.1, 1214.9, 1107.9, 1065.4, 1030.1, 824.4 cm^{-1}; for ^1H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectral data, see Table 1; ESI-MS: *m/z* 529 $[M + Na]^+$, 505 $[M-H]^-$; HR-ESI-MS: *m/z* 507.1496 $[M + H]^+$ (calcd for $C_{24}H_{27}O_{12}$, 507.1497).

3.4 Acid hydrolysis and HPLC analysis

The absolute configuration of the sugar moiety in the structures was determined by the method of Tanaka *et al.* [5]. Compound **1** (2 mg) was hydrolyzed with 10 ml of 2 M HCl for 6 h at 90°C. The mixture was evaporated to dryness under vacuum and then it was extracted by $CHCl_3$–H_2O (1:1) twice to afford the aqueous phase, which was evaporated to dryness under vacuum. The residue was dissolved in pyridine (2 ml) containing L-cysteine methyl ester (2 mg) and heated at 60°C for 1 h. Then, *O*-tolyl isothiocyanate (10 μl) was added to the mixture, which was heated at 60°C for 1 h. The reaction mixture was directly analyzed by reversed-phase HPLC. Analytical HPLC was performed on the column at 30°C with isocratic elution of 25% CH_3CN containing 0.08% formic acid for 40 min and subsequent washing of the column with 90% CH_3CN at a flow rate of 1 ml/min. Peaks were detected by a UV detector at 250 nm. The peak of the derivative of **1** was observed at t_R 17.940 (D-Glc) min. The standard sugars D-glucose and L-glucose were subjected to the same method. The peaks of standard sugar derivatives were recorded at t_R 17.816 (D-Glc) and 16.459 (L-Glc) min. Following the above procedure, the derivative of **3** also gave a peak at t_R 17.910 (D-Glc) min.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita, *Florae Reipublicae Popularis Sinicae* (Science Press, Beijing, 1999), Vol. 57.

[2] D.P. Lu, *Mod. Chin. Med.* **10**, 14 (2008).
[3] Q.Q. Wang, Y. Zhang, W.C. Ye, and G.X. Zhou, *China J. Chin. Mater. Med.* **38**, 366 (2013).
[4] C. Wu, Y.H. Duan, M.M. Li, W. Tang, X. Wu, G.C. Wang, W.C. Ye, G.X. Zhou, and Y.L. Li, *Planta Med.* **79**, 1348 (2013).
[5] T. Tanaka, T. Nakashima, T. Ueda, K. Tomii, and I. Kouno, *Chem. Pharm. Bull.* **55**, 899 (2007).
[6] H. Ishida, T. Umino, K. Tsuji, and T. Kosuge, *Chem. Pharm. Bull.* **36**, 4414 (1988).
[7] K.R. Markham, B. Ternai, R. Stanley, H. Geiger, and T.J. Marbry, *Tetrahedron* **34**, 1389 (1978).
[8] K.L.M. Yang, L.J. Zhang, H.T. Huang, Z.H. Lin, C.C. Liaw, H.L. Cheng, K.H. Lee, N.S.L. Marris, Y.H. Kuo, and H.O. Ho, *J. Nat. Prod.* **76**, 580 (2013).