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A NEW FLAVANONE GLUCOSIDE FROM *Oroxylum indicum*

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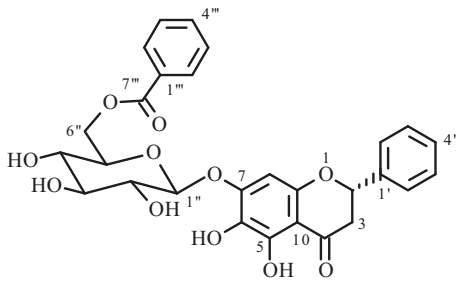
*A new flavanone glucoside, (2*S*)-dihydrobaicalein 7-*O*-(6″-benzoylglucopyranoside) (**1**), along with the known compounds scutellarein 4′-methyl ether (**2**), pectolinarigenin (**3**), and* β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**4**), were isolated from the MeOH extract of* Oroxylum indicum *stem bark. The structure of the new compound was established on the basis of detailed spectroscopic and chemical studies.*

Keywords: *Oroxylum indicum*, Bignoniaceae, flavonoids.

Oroxylum indicum Vent. (Bignoniaceae) is a small tree growing in India, China, and Southeast Asia [1, 2]. The stem bark, root, and root bark of this plant have been used as traditional medicine in India, Thailand, and other Asian countries for the treatment of rheumatism, diarrhea, dysentery, and scorpion sting [3]. The seeds of this plant have been used as traditional Chinese medicine for the treatment of cough, bronchitis, pharyngitis, pertussis, and other respiratory problems [4]. The plant possesses antimicrobial [5], anti-inflammatory [6], anticancer [7, 8], antioxidant [6], gastroprotective [9], and immuno-stimulant [10] activities. Three flavonoids, baicalein, oroxylin A, and chrysin, isolated from its stem bark exhibited significant inhibitory activity against proprotein convertases, which play an important role in cancer and viral and bacterial infections [11]. Baicalein also exhibited significant antileprotic activity against camptothecin-resistant *Leishmania donovani* strain [12] and antitumor activity against colorectal carcinoma CT-26 cells [13]. Previous chemical studies on different parts of the plant reported the isolation of flavonoids [9, 14–17], pterocarpans [18], naphthoquinones [19], anthraquinone [20], prenylated glycosides, and cyclohexyl ethanoids [21].

Our further investigation on the stem bark of *Oroxylum indicum* led to the isolation of three more minor flavonoids (**1**–**3**) and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**4**). These minor flavonoids are (2*S*)-dihydrobaicalein 7-*O*-(6″-benzoylglucopyranoside) (**1**), scutellarein 4′-methyl ether (**2**), and pectolinarigenin (= 5,7 dihydroxy-6,4′-dimethoxyflavone) (**3**). Compound **1** is new and the known compounds **2**–**4** are reported for the first time from this plant.



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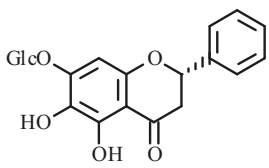

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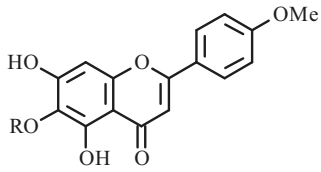

2, 3

2: R = H; **3:** R = Me

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TABLE 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_H	δ_C (DEPT)	C atom	δ_H	δ_C (DEPT)
2	5.64 (dd, J = 12.5, 2.5)	79.8 (CH)	1″	4.86 (d, J = 7.5)	101.1 (CH)
3	2.78 (dd, J = 16.8, 2.5), 3.16 m*	42.6 (CH_2)	2″	3.10–3.78	72.8 (CH)
4	–	196.8 (C)	3″	3.10–3.78	76.2 (CH)
5	–	150.1 (C)	4″	3.10–3.78	69.8 (CH)
6	–	128.2 (C)	5″	3.10–3.78	78.2 (CH)
7	–	154.8 (C)	6″	5.12 (br.d, J = 11.5)	64.8 (CH_2)
8	6.22 s	96.2 (CH)	1‴	4.98 (br.d, J = 11.5)	131.3 (C)
9	–	153.4 (C)	2‴, 6‴	7.98 (dd, J = 8.5, 1.8)	130.8 (CH)
10	–	103.8 (C)	3‴, 5‴	7.39 m	130.0 (CH)
1′	–	138.0 (C)	4‴	7.39 m	134.8 (CH)
2′, 6′	7.58 (br.d, J = 8.5)	126.4 (CH)	7‴	–	167.0 (C)
3′, 5′	7.29 m	128.1 (CH)	5-OH	12.37 s	–
4′	7.29 m	128.4 (CH)	6-OH	8.06 br.s	–

*Signal was overlapped with solvent signal.

The positive mode FAB-MS of compound **1** exhibited a molecular ion at *m/z* 539 $[M + H]^+$ corresponding to its molecular formula $C_{28}H_{26}O_{11}$. This was also supported by its negative mode FAB-MS, which showed a molecular ion at *m/z* 537 $[M - H]^-$. The UV spectrum in MeOH displayed absorption maxima, λ_{max} 242 sh, 285 and 348 nm, characteristic of flavanones [22]. In the presence of $AlCl_3$, the UV spectrum exhibited bathochromic shifts at λ_{max} 248 sh, 312, 314, and 443 sh, indicating the presence of a free hydroxyl group at the C-5 position [22]. The IR spectrum showed the bands for hydroxyl (3427 cm^{-1}), *α,β*–unsaturated carbonyl (1653 cm^{-1}), and aromatic (1611 and 1578 cm^{-1}) and glycosidic (1169 and 1032 cm^{-1}) functions. The ^{1}H NMR spectrum (Table 1) displayed signals for one aromatic proton [δ 6.22(1H, s, H-8)], an unsubstituted B ring of the flavone moiety [δ 7.58 (2H, br.d, J = 8.5 Hz, H-2′, 6′) and 7.29 (3H, m, H-3′, 4′, 5′)], one methine proton [δ 5.64 (1H, dd, J = 12.5 and 2.5 Hz, H-2)], one methylene proton [δ 2.78 (1H, dd, J = 16.8 and 2.5 Hz, H-3) and 3.16 (1H, overlapped with solvent signal, H-3)], one anomeric sugar proton [δ 4.86 (1H, d, J = 7.5 Hz, H-1″)], and two phenolic hydroxyl protons [δ 12.37 (1H, s, 5-OH) and 8.06 (1H, br.s, 6-OH)], supporting its dihydrobaicalein glucoside structure. In addition, the ^{1}H NMR spectrum showed the signals for five aromatic protons of a benzoyl moiety [δ 7.98 (2H, dd, J = 8.5 and 1.8 Hz, H-2‴, 6‴) and 7.39 (3H, m, H-3‴, 4‴, 5‴)] indicating the presence of a benzoyl group as an acylating unit. The ^{13}C NMR spectrum (Table 1) with DEPT experiments revealed the signals for two methylenes, 17 methines, and nine quaternary carbons. Possibly some carbon signals represent more than one carbon as the molecule contains 28 carbons. The carbon resonance values were very similar to those reported for dihydrobaicalein 7-*O*-glucoside (**1a**) [23]. The high chemical resonance of the C-6″ sugar carbon (δ_C 64.8 ppm) and the downfield chemical resonance of H-6″ protons [δ_H 5.12 and 4.98] confirmed the location of a benzoyl group at the C-6″ position of the glucose moiety. FAB-MS of the compound showed mass ions at *m/z* 435 $[C_{21}H_{23}O_{10}]^+$, indicating the presence of the dihydrobaicalein hexoside moiety in it. Its EI-MS showed mass ions at *m/z* 272, $[C_{15}H_{12}O_5]^+$, 168 $[C_7H_4O_5]^+$, and 104 $[C_8H_8]^+$, indicating its flavanone skeletal structure with three hydroxyl groups in the A-ring and an unsubstituated B ring. The mass ion at *m/z* 105 $[C_7H_5O]^+$ suggested the presence of a benzoyl group as the acyl moiety. Saponification of the compound with NaOH solution afforded benzoic acid, confirming the attachment of a benzoyl group as the acyl moiety. Acid hydrolysis with 2 M HCl afforded D-glucose, suggesting the presence of a glucose moiety as hexose sugar. The configuration of the C-2 position was assigned as (*S*) from its CD spectrum in MeOH, which displayed positive Cotton effects at 338 and 214 nm and a strong negative Cotton effect at 290 nm [24]. Therefore, the structure of the compound was elucidated as (2*S*)-dihydrobaicalein 7-*O*-(6″-benzoylglucopyranoside)[=(*2S*),5,6,7-trihydroxyflavanone 7-*O*-(6″-benzoylglucopyranoside)] (**1**).

EXPERIMENTAL

General. Melting points were determined with a Koffler apparatus and are uncorrected. UV-Vis spectra were recorded with a PerkinElmer Lamda 25 spectrometer. IR spectra were obtained on a PerkinElmer FTIR-100 spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Varian XL- 400 and Brucker 600 spectrometers with TMS as internal reference.

FAB and EI-MS were recorded on a JEOL JMS-AX505 HA spectrometer. CD spectrum was measured on a Jasco J805 spectropolarimeter.

Extraction and Isolation. Air-dried powdered stem bark (2.0 kg) of *O. indicum* collected from Agartala was extracted with MeOH (3 × 5 L). The MeOH extract was evaporated under reduced pressure to a thick condensate and dissolved in H_2O (0.15 L). The aqueous solution was extracted three times each with hexane, EtOAc, and *n*-BuOH. The EtOAc extract on repeated column chromatography (CC) over silica gel afforded **2** (15 mg) and **3** (9 mg). The BuOH extract on repeated CC over Si gel afforded **1** (25 mg) and **4** (38 mg).

(2*S*)-Dihydrobaicalein 7-*O*-(6″-Benzoyl-*β*-D-glucopyranoside) (1). Pale yellow amorphous powder, mp 176–178°C. $C_{28}H_{26}O_{11}$. (M^+ 538). CD (MeOH, *c* 0.02 mg mL^{-1}): $[\theta]_{338}$ + 7862, $[\theta]_{290}$ –32150, $[\theta]_{214}$ +88724.

Alkaline Hydrolysis of 1. Compound **1** (3 mg) was refluxed with 0.1 M methanolic NaOH solution (5 mL) for 1 h. The reaction mixture was concentrated, diluted with H_2O, and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated to dryness to a residue, which was identified as benzoic acid by comparing its mass spectrum and co-TLC with an authentic sample.

Acid Hydrolysis of 1. Compound **1** (4 mg) was refluxed with 2 M methanolic HCl (5 mL) for 2 h. The reaction mixture was cooled, concentrated, diluted with H_2O, neutralized with Ag_2CO_3, and filtered. The aqueous filtrate was concentrated. The concentrated solution indicated the presence of D-glucose on co-TLC with a standard sample of D-glucose.

Scutellarein 4′-Methyl Ether (2). Yellow amorphous powder, mp 187–189°C. $C_{16}H_{12}O_6$ (M^+ 300). ^1H and ^{13}C NMR spectral data were similar to that of published data [23].

Pectolinarigenin (3). Yellow needles, mp 209–210°C, $C_{17}H_{14}O_6$ (M^+ 314). Physical constants and spectral data were identical with the literature data [25].

***β*-Sitosterol Glucoside (4)**. White amorphous powder, mp 284–285°C, $C_{35}H_{60}O_6$ (M^+ 576). The spectral data were identical with the literature data [26].

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