Three New Flavonoid Glycosides Isolated from *Elsholtzia bodinieri*

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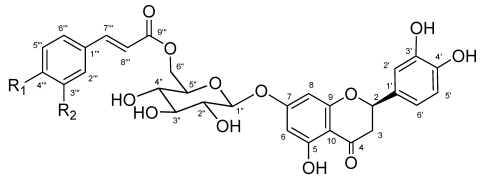
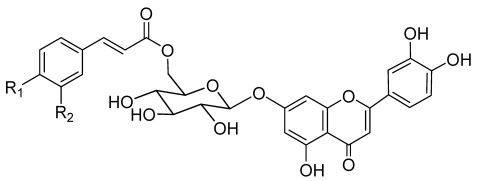
Three new flavonoid glycosides, eriodictyol 7-*O*-(6″-feruloyl)-β-D-glucopyranoside (1), eriodictyol 7-*O*-[6″-(3‴-hydroxy-4‴-methoxy cinnamoyl)]-β-D-glucopyranoside (2), and luteolin 7-*O*-[6″-(3‴-hydroxy-4‴-methoxy cinnamoyl)]-β-D-glucopyranoside (3), and eight known flavonoids were isolated from the whole plants of *Elsholtzia bodinieri*. The structures of the 3 new compounds were elucidated on the basis of extensive spectroscopic analysis.

Key words *Elsholtzia bodinieri*; Labiatae; flavonoid

Elsholtzia bodinieri Vaniot, belonging to the taxonomically diverse group of the family Labiatae, is a medicinal plant growing in Yunnan and Guizhou Provinces in China. It is commonly known as "yashuacao" and has been used as a traditional Chinese medicine for the treatment of cough, headache, pharyngitis, fever and hepatitis.[1)] An earlier study on this plant led to the isolation of two new triterpene glycosides, hederagenin 3-*O*-β-D-xylopyranoside and dodecandral 3-*O*-β-D-xylopyranoside.[2)] Inspired by the efficacious treatment for influenza[3)] and by lack of systematic investigation on the chemical constituents on this plant, we investigated the EtOAc extract of the whole plants of *E. bodinieri*. In a preceding paper, we have described the elucidation of two novel triterpenoid glycosides, bodiniosides A and B.[4)] We report herein on the isolation and structural determination of three new flavonoid glycosides, eriodictyol 7-*O*-(6″-feruloyl)-β-D-glucopyranoside (**1**), eriodictyol 7-*O*-[6″-(3‴-hydroxy-4‴-methoxy cinnamoyl)]-β-D-glucopyranoside (**2**), and luteolin 7-*O*-[6″-(3‴-hydroxy-4‴-methoxy cinnamoyl)]-β-D-glucopyranoside (**3**), together with eight known flavonoids, luteolin 7-*O*-(6″-feruloyl)-β-D-glucopyranoside (**4**), eriodictyol, miscanthoside, luteolin, glucoluteolin, apigenin-7-glucoside, chrysin and apigenin from the same extract.



1 R_1 = OH R_2 = OCH_3
2 R_1 = OCH_3 R_2 = OH



3 R_1 = OCH_3 R_2 = OH
4 R_1 = OH R_2 = OCH_3

Fig. 1. Structures of Compounds **1**—**4**

Results and Discussion

Compound **1** was isolated as yellow amorphous powder, whose molecular formula of $C_{31}H_{30}O_{14}$ was determined on the basis of HR-FAB-MS (negative-ion mode) analysis ($[M-H]^-$, 625.1543) and ^{13}C-NMR spectrum, indicating 17 degrees of unsaturation. The IR spectrum indicated the presence of OH (3420 cm^{-1}), α,β-unsaturated carbonyl (1690 cm^{-1}) and aromatic rings (1642, 1516, 1453 cm^{-1}). The UV spectrum exhibited maximun absorptions at 327, 285 and 203 nm. Examination of the ^{1}H- and ^{13}C-NMR spectral data of **1** (Tables 1, 2) indicated that the molecule consisted of a flavanone, a glucose, a 1,3,4-trisubstituted benzene ring, a *trans*-α,β-unsaturated ester, and a methoxyl moieties.

The ^{1}H-NMR spectum suggestd that **1** has an aglycon eriodictyol. Signals at δ_H 7.51 (br s, H-2′), 7.28 (d, J=8.0 Hz, H-5′), and 7.03 (br d, J=8.0 Hz, H-6′) are characteristic for a 3,4-disubstituted B ring of an eriodictyol unit. Two sets of doublets at δ_H 6.69 (d, J=1.5 Hz) and 6.46 (d, J=1.5 Hz) originated from the signals of H-6 and H-8 in ring A of an eriodictyol. This was in sound agreement with the ^{13}C-NMR spectrum. The six carbon signals of the sugar moiety were at δ_C 101.6, 78.3, 75.8, 74.7, 71.3 and 64.4, suggesting that **1** was an eriodictyol glycoside. The presence of the β-D-glucopyranosyl moiety was supported by the ^{13}C-NMR data and further confirmed by the acid hydrolysis of **1**, which resulted in a release of glucose identified by HPTLC comparisons of the hydrolyzate with an authentic sugar sample. The configuration of the glucopyranosyl was assigned to be β-D based on the coupling constant of the anomeric proton. The glucosyl residue was located at the 7-*O*-position of the aglycon eriodictyol by the appearance of HMBC cross peaks of the glucosyl anomeric proton H-1″ (δ_H 5.70, d, J=7.6 Hz) with the carbon signal at δ_C 166.5 (C-7).

From these data, the substituent at C-7 of the aglycon moiety gave a pattern of the ^{13}C-NMR signals similar to those in miscanthoside, except for the presence of an additional 3,4-substituted coumaroyl unit, which was confirmed by the HMBC correlations shown in Fig. 2. The *trans*-substitution at the olefinic protons (H-7‴ and H-8‴) of 3,4-substituted coumaroyl was established from the large coupling (J= 15.9 Hz). Compared with the ^{13}C-NMR data of miscanthoside, the C-5″ resonance of **1** was shifted upfield by 3.2 ppm,

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Table 1. ^{1}H-NMR Data of Compounds **1**—**3**[a)]

Position	**1**	**2**	**3**
2	5.40 (d, J=14.6)	5.38 (d, J=14.6)	—
3a	2.86 (d, J=17.1)	2.85 (d, J=17.1)	6.83 (s)
3b	3.23 (dd, J=17.1, 14.6)	3.22 (dd, J=17.1, 14.6)	—
6	6.69 (d, J=1.5)	6.71 (d, J=2.3)	6.95 (d, J=2.5)
8	6.46 (d, J=1.5)	6.44 (d, J=2.3)	6.90 (d, J=2.5)
2′	7.51 (br s)	7.50 (br s)	7.86 (d, J=2.2)
5′	7.28 (d, J=8.0)	7.27 (d, J=8.0)	7.12 (d, J=8.8)
6′	7.03 (br d, J=8.0)	7.05 (br d, J=8.0)	7.48 (dd, J=8.8, 2.2)
1″	5.70 (d, J=7.6)	5.71 (d, J=7.6)	5.79 (d, J=7.8)
2″–5″	4.26—4.42 (m)	4.24—4.40 (m)	4.26—4.89 (m)
6″a	5.11 (d, J=11.8)	5.13 (d, J=12.1)	5.20 (d, J=11.8)
6″b	4.90 (dd, J=11.8, 6.0)	4.92 (dd, J=12.1, 5.8)	4.86 (dd, J=11.8, 5.3)
2‴	7.27 (s)	7.05 (d, J=1.5)	6.97 (s)
5‴	7.21 (d, J=6.0)	6.74 (d, J=5.6)	7.30 (d, J=4.0)
6‴	7.21 (d, J=6.0)	7.18 (d, J=5.6)	7.13 (overlap)
7‴	7.93 (d, J=15.9)	7.95 (d, J=15.7)	7.88 (d, J=15.9)
8‴	6.69 (d, J=15.9)	6.70 (d, J=15.7)	6.68 (d, J=15.9)
5-OH	12.6 (s)	12.6 (s)	13.5 (s)
–OCH_3	3.86 (s)	3.89 (s)	3.80 (s)

a) Data were determined at 400 MHz in C_5D_5N, chemical shifts are in ppm and coupling constant J in Hz.

Table 2. ^{13}C-NMR Data of Compounds **1**—**3**[a)]

Position	**1**	**2**	**3**
2	80.0 (d)	80.0 (d)	164.0 (s)
3	43.5 (t)	43.5 (t)	104.0 (d)
4	197.5 (s)	197.5 (s)	183.0 (s)
5	164.7 (s)	164.7 (s)	162.8 (s)
6	97.6 (d)	97.6 (d)	100.7 (d)
7	166.5 (s)	166.5 (s)	165.5 (s)
8	96.8 (d)	96.9 (d)	95.7 (d)
9	163.6 (s)	163.6 (s)	157.8 (s)
10	104.5 (s)	104.5 (s)	106.7 (d)
1′	130.5 (s)	130.5 (s)	122.6 (s)
2′	115.4 (d)	115.4 (d)	114.7 (d)
3′	147.6 (s)	147.6 (s)	149.1 (s)
4′	149.1 (s)	149.4 (s)	152.0 (s)
5′	116.7 (d)	116.7 (d)	116.9 (d)
6′	118.9 (d)	118.9 (d)	119.8 (d)
1″	101.6 (d)	101.6 (d)	101.9 (d)
2″	74.7 (d)	74.7 (d)	74.8 (d)
3″	78.3 (d)	78.3 (d)	78.5 (d)
4″	71.3 (d)	71.3 (d)	71.6 (d)
5″	75.8 (d)	75.8 (d)	75.8 (d)
6″	64.4 (t)	64.4 (t)	64.6 (t)
1‴	126.6 (s)	125.3 (s)	125.2 (s)
2‴	111.6 (d)	106.9 (d)	106.7 (d)
3‴	151.3 (s)	149.4 (s)	149.1 (s)
4‴	148.1 (s)	140.8 (s)	140.7 (s)
5‴	116.9 (d)	115.4 (d)	115.2 (d)
6‴	123.8 (d)	124.0 (d)	124.0 (d)
7‴	146.1 (d)	146.4 (d)	146.2 (d)
8‴	115.1 (d)	115.1 (d)	115.2 (d)
9‴	167.7 (s)	167.7 (s)	167.6 (s)
–OCH_3	56.1 (q)	56.6 (q)	56.0 (q)

a) Data were determined at 100 MHz in C_5D_5N, chemical shifts are in ppm; assignments were confirmed by ^{1}H–^{1}H COSY, HMQC and HMBC.

while the signal attributable to the adjacent C-6″ was shielded downfield by 2.2 ppm, which indicated that the 3,4-substituted coumaroyl was attached to C-6″. This was confirmed by observation of a HMBC cross peak (Fig. 2) between H_2-6″ (δ_H 5.11, 4.90) and C-9‴ (δ_C 167.7).

HMBC cross peaks from H-5‴ (δ_H 7.21, d, J=6.0 Hz) to C-4‴ and C-6‴ (δ_C 123.8), and from H-7‴ (δ_H 7.93, d, J=15.9 Hz) to C-2‴ (δ_C 111.6), C-6‴, C-8‴ (δ_C 115.1), and C-9‴ (δ_C 167.7) were also observed. In addition, a cross-peak correlation was observed between the OCH_3 (δ_H 3.86) and C-3‴. This information led to the conclusion that the OCH_3 was located at C-3‴, and the OH at C-4‴. This assignment was also supported by the NOESY correlation between the OCH_3 and H-2‴.

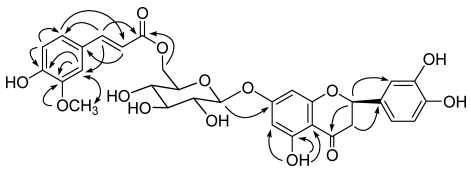

Fig. 2. The Key HMBC and NOESY Correlations for Compound **1**
→ HMBC, ↔ NOESY.

To determine the absolute configuration of the C2-phenyl group of **1**, the circular dichroism (CD) absorption value was examined. The CD spectrum showed positive Cotton effects around 300 nm and a negative Cotton effect around 350 nm, suggesting that **1** has the C2-phenyl group in the β-orientation and is therefore designated as R.[5)] Thus, the structure of **1** was elucidated as eriodictyol 7-*O*-(6″-feruloyl)-β-D-glucopyranoside.

Compound **2** was analyzed for $C_{31}H_{30}O_{14}$ by negative HR-FAB-MS (m/z 625.1538 $[M-H]^-$, the same as that of **1**. The ^{1}H- and ^{13}C-NMR spectral data of **2** (Tables 1, 2) were strikingly similar to those of **1**. These similarities, together with the fact that **1** and **2** possess identical molecular formulae, suggested a close relationship between these two compounds. In fact, in the NMR spectra of **2**, chemical shift differences were only observed for C-2‴ (δ_H 7.05, δ_C 106.9), C-3‴ (δ_C 149.4), C-4‴ (δ_C 140.8), and C-5‴ (δ_H 6.74, δ_C 115.4) in the 3,4-substituted coumaroyl uint. Therefore, it is tentatively assumed that **2** was proposed to exist as an isomer of **1** with different substituted location of OCH_3 and OH at C-3‴ and C-4‴ in the 3,4-substituted coumaroyl unit.

The proton H-2‴ (δ_H 7.05, d, J=1.5 Hz) of **2** provided a

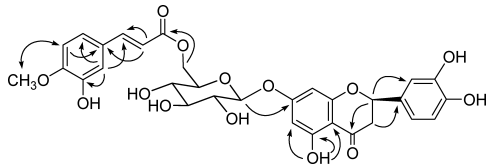

Fig. 3. The Key HMBC and NOESY Correlations for Compound **2**

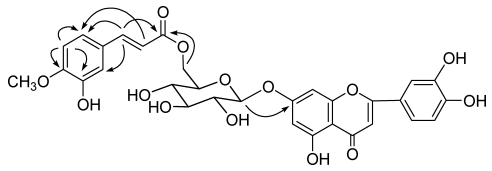

Fig. 4. The Key HMBC Correlations for Compound **3**

starting point to assign the signals of C-1‴ (δ_C 125.3), C-3‴ (δ_C 149.4), C-4‴ (δ_C 140.8), C-6‴ (δ_C 124.0), and C-7‴ (δ_C 146.4) through the information derived from HMBC correlations (Fig. 3) between H-2‴ and C-3‴, C-6‴, and C-7‴, between H-5‴ (δ_H 6.74, d, J=5.6 Hz) and C-1‴, and between the OCH_3 (δ_H 3.89, s) and C-4‴. These findings established the structure of **2** as eriodictyol 7-*O*-[6″-(3‴-hydroxy-4‴-methoxy cinnamoyl)]-β-D-glucopyranoside.

Compound **3** was obtained as yellow amorphous powder. Its empirical formula was comfirmed as $C_{31}H_{28}O_{14}$ by negative HR-FAB-MS and ^{13}C-NMR spectroscopic data. A closer comparison of the NMR spectra of **3** with those of **2** indicated that they are closely related (Tables 1, 2). However, the ^{13}C-NMR spectrum of **3** lacked the oxygenated methylene and methine signals at C-2 and C-3 (δ_C 80.0, d; 43.5, t, in **2**), showing instead two olefinic carbons at δ_C 164.0 (s) and 104.1 (d). These chemical shifts were in agreement with **3** as a derivative of luteolin glycosides with acylated with 3‴-hydroxy-4‴-methoxy-cinnamic and ferulic acids. HMBC correlations (Fig. 4) of H_2-6″ (δ_H 5.20, 4.86) with C-9‴ (δ_C 167.6), of H-7‴ (δ_H 7.88, d) with C-2‴ (δ_C 106.7), C-6‴ (δ_C 124.0) and C-9‴, of H-5‴ (δ_H 7.30, d) with C-3‴ (δ_C 149.1), and of H-8‴ (δ_H 6.68, d) with C-6‴ (δ_C 124.0) further confirmed the above assignments. From these data, compound **3** was concluded to be luteolin 7-*O*-[6″-(3‴-hydroxy-4‴-methoxy cinnamoyl)]-β-D-glucopyranoside (**3**).

The structure characterizations of the known compounds, luteolin 7-*O*-(6″-feruloyl)-β-D-glucopyranoside (**4**),[6] eriodictyol,[7] miscanthoside,[8] luteolin,[7] glucoluteolin,[8] apigenin-7-glucoside,[9] chrysin,[10] and apigenin[11] were accomplished by direct comparison of their spectroscopic data (^{1}H-, ^{13}C-NMR, and MS) with those reported in the literature.

Experimental

General Procedure Optical rotations were obtained using a JASCO DIP-370 digital polarimeter. UV spectra were determined on a UV 210A spectrometer. IR spectra were recorded on a Bio-Rad PTS-135 spectrophotometer with KBr pellets. Negative FAB-MS were measured with a VG Auto Spec-3000 spectrometer. 1D- and 2D-NMR spectra were run on Bruker AM-400 and DRX-500 instruments with $SiMe_4$ as an internal standard. Column chromatography was performed on silica gel and silica gel H (200—300 mesh, 10—40 μm, Qingdao Marine Chemical Inc., China).

Plant Material The Pharmaceutical Factory of Yunnan Institute of Materia Medica provided the samples of *E. bodinieri*.

Extraction and Isolation The powdered air-dried whole plants (6.0 kg) were extracted with 70% aq. Me_2CO (26 l×3) at r.t. overnight. The extract was partitioned between H_2O and EtOAc. The EtOAc layer (610 g) was chromatographed on MCI-gel CHP 20P (90% MeOH–H_2O→MeOH) to afford Fractions A and B. Fraction A (90% MeOH, 500 g) was subjected to CC over Si gel eluting with a $CHCl_3$–MeOH 1 : 0→0 : 1 gradient system to give Fractions 1—15. Fraction 4 (56 g) was further purified by CC over Si gel using $CHCl_3$–acetone (20 : 1→10 : 3) as eluent to yield chrysin (16 mg) and apigenin (50 mg). Eriodictyol (9 mg) and luteolin (35 mg) were isolated and purified from Fr. 5 (1 g) over Si gel developing with $CHCl_3$–MeOH 20 : 1. Fraction 6 was subjected to column chromatography on silica gel ($CHCl_3$–MeOH 8 : 2), then semi-preparative HPLC (MeOH–H_2O 37 : 63) to give **1** (65 mg), **2** (30 mg) and glucoluteolin (4.91 g). Fraction 7 (34 g) was chromatographed on Si gel ($CHCl_3$–MeOH–H_2O 8 : 1 : 0.1→3 : 1 : 0.1), then semi-preparative HPLC (MeOH–H_2O 40 : 60) to afford **3** (10 mg), **4** (9 mg) and apigenin-7-glucoside (31 mg). Fraction 12 was further purified using CC on RP-18 by eluting with 30% MeOH–H_2O to yield miscanthoside (324 mg).

Eriodictyol 7-*O*-(6″-Feruloyl)-β-D-glucopyranoside (**1**): Yellow amorphous powder; $[\alpha]_D^{20.9}$ −68.3° (c=0.27, C_5H_5N); IR (KBr) cm^{-1}: 3420, 2926, 1690, 1642, 1516, 1453, 1272, 1194, 1066; UV λ_{max} (MeOH) nm (log ε): 203 (4.82), 285 (4.43), 327 (4.32); FAB-MS (neg.) m/z: 625 (100, $[M-H]^-$); HR-FAB-MS (neg.) m/z: 625.1543 (Calcd for $C_{31}H_{29}O_{14}$, 625.1557); CD (c=1.05, MeOH) (mdeg): 354 (−4.09), 302 (+2.27). ^{1}H- and ^{13}C-NMR, see Tables 1 and 2, respectively.

Eriodictyol 7-*O*-[6″-(3‴-Hydroxy-4‴-methoxy cinnamoyl)]-β-D-glucopyranoside (**2**): Yellow amorphous powder; $[\alpha]_D^{20.9}$ −67.9° (c=0.21, C_5H_5N); IR (KBr) cm^{-1}: 3425, 2920, 1688, 1635, 1515, 1458, 1270, 1195, 1060; UV λ_{max} (MeOH) nm (log ε): 206 (4.72), 280 (4.38), 328 (4.29); FAB-MS (neg.) m/z: 625 (100, $[M-H]^-$); HR-FAB-MS (neg.) m/z: 625.1538 (Calcd for $C_{31}H_{29}O_{14}$, 625.1543); CD (c=1.10, MeOH) (mdeg): 352 (−4.38), 299 (+2.08). ^{1}H- and ^{13}C-NMR, see Tables 1 and 2, respectively.

Luteolin 7-*O*-[6″-(3‴-Hydroxy-4‴-methoxy cinnamoyl)]-β-D-glucopyranoside (**3**): Yellow amorphous powder; $[\alpha]_D^{22.0}$ −100.0° (c=0.09, C_5H_5N); IR (KBr) cm^{-1}: 3425, 2924, 1689, 1657, 1608, 1515, 1499, 1374, 1261, 1179, 1081; FAB-MS (neg.) m/z: 623 (100, $[M-H]^-$); HR-FAB-MS (neg.) m/z: 623.125 (Calcd for $C_{31}H_{27}O_{14}$, 623.100); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2, respectively.

Acid Hydrolysis of Compounds 1—3 on HPTLC Plate Compounds **1**—**3** and authentic sugar were individually spotted on a HPTLC precoated plate, and hydrolyzed with concentrated HCl vapor (80 °C water bath for 20 min) followed by co-TLC with authentic sugar developing with $CHCl_3$–MeOH–H_2O–HOAc (7 : 3 : 0.5 : 1). The HPTLC plate was sprayed with 10% H_2SO_4 for detection. The β-D-glucose was detected with an Rf value of 0.42.

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