

Article

New Flavonoid Glycosides from *Elsholtzia rugulosa* Hemsl.

Gaimei She ¹, Zhiqin Guo ¹, Haining Lv ¹ and Dongmei She ^{2,*}

¹ School of Chinese Pharmacy, Beijing University of Chinese Medicine, Beijing 100102, China; E-Mails: shegaimei@126.com (G.S.); medicine_guo@163.com (Z.G.); seagullning@163.com (H.L.)

² The Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100049, China

* Author to whom correspondence should be addressed; E-Mails: dongmeishe@yahoo.com.cn; Tel.: +86-10-84738628; Fax: +86-10-84738611.

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Abstract: *Elsholtzia rugulosa* Hemsl. is known in China as a local herbal tea, medicinal herb and honey plant. Chemical examination of *E. rugulosa* led to the isolation of two new flavonoid glycosides, apigenin 4'-O- α -D-glucopyranoside (**1**) and 5,7,3',4'-tetrahydroxy-5'-C-prenylflavone-7-O- β -D-glucopyranoside (**2**), together with nine known flavonoids. Their structures were elucidated on the basis of spectroscopic evidence.

Keywords: *Elsholtzia rugulos*; flavonoid glycosides; apigenin 4'-O- α -D-glucopyranoside; 5,7,3',4'-tetrahydroxy-5'-C-prenylflavone-7-O- β -D-glucopyranoside

1. Introduction

Elsholtzia rugulosa Hemsl. (Lamiaceae), which is distributed in the Yunnan, Sichuan and Guizhou provinces of China, is known as a local herbal tea, medicinal herb and honey plant [1]. In these regions, the title plant is also widely used by local people in the treatment of colds, headaches, coughs, pharyngitis and fever [2]. Several flavonoids, maltol glycosides and cyanogenic glycosides have been isolated from *E. rugulosa* [3,4]. The antiviral activities of these flavonoids were also reported [4]. As a part of our systematical investigation of Chinese tea and herbal tea plants, and in the search for biologically active flavonoids from plants sources, a detailed study on ethanol extracts of *E. rugulosa* was carried out [5,6,7]. This led to the isolation of two new flavonoids glycosides, apigenin 4'-O- α -D-

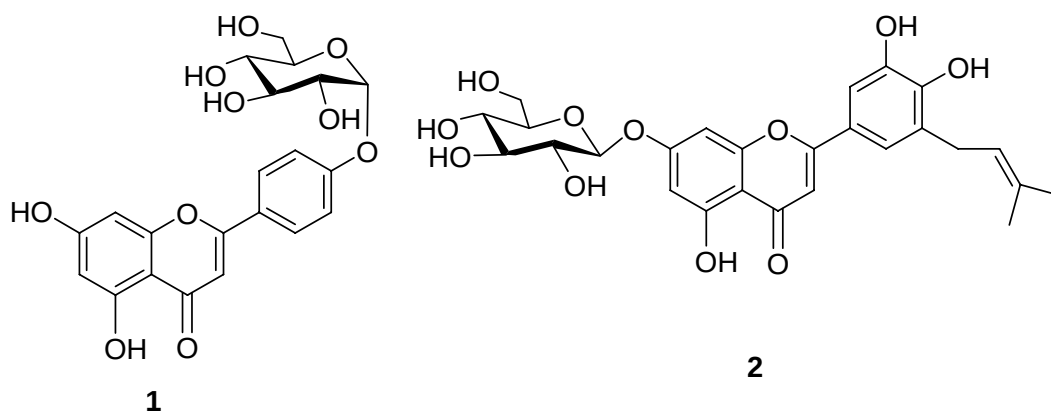
glucopyranoside (**1**) and 5,7,3',4'-tetrahydroxy-5'-C-prenylflavone 7-*O*- β -D-glucopyranoside (**2**), together with nine known flavonoids **3–11**. Herein, we present the details of this study.

2. Results and Discussion

Repeated column chromatography (CC) of the chlorophyll-free fraction of an ethanol extract of *E. rugulosa* on Dianion HP 2MGL, Sephadex LH-20, MCI-gel CHP-20P, and silica gel, resulted in the isolation of 11 compounds, of which nine known flavonoids were identified as luteolin (**3**) [8] luteolin 7-*O*- β -D-glucoside (**4**) [8], luteolin 3'-*O*- β -D-glucuronide-6"-methylester (**5**) [9], apigenin (**6**) [10], apigenin 7-*O*- β -D-glucoside (**7**) [11], quercetin 3-*O*- β -D-glucuronide-6"-methylester (**8**) [12], kaempferol (**9**) [13], 3',4',5,7-tetrahydroxy-8-prenyl-flavone (**10**) [14] and 7,4-dimethylkaempferol (**11**) [15], respectively, by direct comparison with authentic samples or comparison of the spectroscopic data with reported literature values. Among them, compounds **7–10** were isolated for the first time from *E. rugulosa*. The two new compounds were identified as apigenin 4'-*O*- α -D-glucopyranoside (**1**) and 5,7,3',4'-tetrahydroxy-5'-C-prenylflavone 7-*O*- β -D-glucopyranoside (**2**), and their structures were elucidated as follows.

Compound **1** was obtained as a yellow amorphous powder, and had a molecular formula $C_{21}H_{20}O_{10}$, derived from its negative HR-FAB-MS (m/z 431.1280 [M-H][−]) and ¹³C-NMR spectrum. Comparison of the NMR data with those of apigenin [10], and the further 2D-NMR spectral data allow elucidation the structure of compound **1** as shown in Figure 1.

Figure 1. Structures of compounds **1** and **2**.



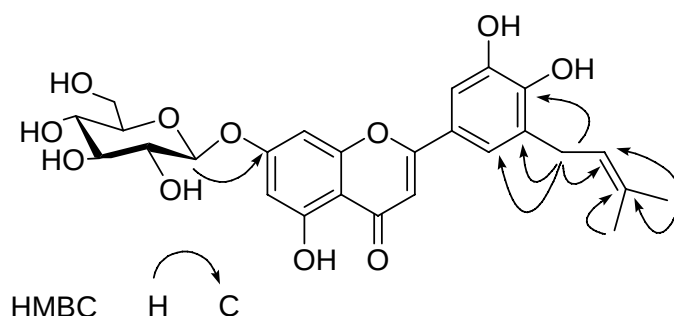
The UV spectrum exhibited absorption maxima at 265 nm (band II) and 331 nm (band I), that are characteristic flavone skeleton bands. The IR spectrum of **1** indicated the presence of hydroxyl ($3,376\text{ cm}^{-1}$) and carbonyl functions ($1,640\text{ cm}^{-1}$). The occurrence of a flavone skeleton in the molecule could be easily deduced from the ¹H-NMR spectrum, in which compound **1** showed the signals for an exchangeable proton at δ 12.95 (1H, s), A₂B₂-type aromatic protons at δ 7.94 (d, H2', 6') and 6.92 (d, H3', 5') on B-ring, two doublets at δ 6.43 (d, H6) and 6.82 (d, H8) on A-ring, together with an olefinic proton at δ 6.86 (s, H3) on a flavone C-ring. In addition, the ¹H-NMR also exhibited signals due to one α -glucopyranosyl unit [δ 5.42 (d, $J = 3.7\text{ Hz}$, H1'')]. The J value (3.7 Hz) of the anomeric proton indicated the α -configuration of the glucose moiety [16]. This was supported by the IR spectrum

showing a strong band at 770, 780 cm^{-1} , probably due to one glucosyl unit, and the enzymatic hydrolysis displaying the R_f values consistent with those of a standard sample of D-glucose, as well as anomeric carbon signal δ 99.9 (C(1')) of α -D-glucosyl group observed, in accord with those of literature values [17,18]. The ^{13}C -NMR spectrum of **1** exhibited 21 carbons whose aglycon chemical shift were in good agreement with those of apigenin and the sugar moiety chemical shifts were in good agreement with those of α -D-glucosyl moiety [18]. The attachment of the glucopyranosyl moiety was deduced to be at C-4' according to glycosylation rule. The conclusion was further confirmed by the HMBC spectrum in which the anomeric proton of the glucopyranosyl moiety at δ 5.42 (d, H1'') showed long range correlation with C(4') (δ 161.1). Therefore, the structure of **1** was determined to be apigenin 4'-O- α -D-glucopyranoside.

Compound **2** was obtained as a pale yellow amorphous powder. The molecular formula $\text{C}_{26}\text{H}_{28}\text{O}_{11}$ was derived by negative ion HR-FAB-MS (m/z : 515.1913 $[\text{M}-\text{H}]^-$) in combination with the presence of 26 carbon signals in its ^{13}C -NMR spectrum, and the further 2D-NMR spectral data allow to elucidate the structure of compound **2** as shown in Figure 1.

The signals at δ 6.78 (s, H3) ascribable to C₃- proton on a flavone C-ring, and two aromatic proton signals at δ 6.61 (d, H6) and 6.48 (d, H8) due to H-6, 8 on A-ring protons, respectively, two broad singlet signals at high field in the aromatic region [δ 7.40 (d, H2'), 6.95 (d, H6')] on B-ring were observed in ^1H -NMR spectrum, which suggested the occurrence in the molecule of a flavone skeleton with a tetra-substituted B-ring. In addition, one glucopyranosyl unit [$\delta(\text{H})$ 5.08 (d, H1''), $\delta(\text{C})$ 101.9 C(1'')] was evident in the ^1H - and ^{13}C -NMR of **2**. On enzymatic hydrolysis, compound **2** liberated D-glucose and the J value (8.1 Hz) of the anomeric proton concluded the β -configuration of the D-glucose moiety. The HMBC correlations of glucosyl H-1'' [δ 5.08 (d, H1'')] in **2** with the C(7) (δ 166.7) confirmed that the location of glucopyranosyl groups were at C-7 in **2**. These NMR features were resembled to those of luteolin 7-O- β -D-glucoside (**4**) [8], except for the existence of an additional set of signals arising from a prenyl group in **2**. Characteristic signals of prenyl group were observed at δ 1.68 (s, H5'''), 1.62 (s, H4'''), 3.62 (m, H1'''), 5.16 (br. t, H2'''), confirming that **2** was a prenylated flavone glycoside [20]. The downfield chemical shift of C(5') of **1** at δ 127.1 indicated that the additional prenyl group was linked at the C(5') position, which was further confirmed by the HMBC correlations of H1''' (δ 3.62) of prenyl unit with the carbon at δ 127.1 C(5')) of the flavone glycoside (Figure 2). On the basis of the above evidence, the structure of **2** was elucidated as 5,7,3',4'-tetrahydroxy-5'-C-prenylflavone 7-O- β -D-glucopyranoside.

Figure 2. Key HMBC correlations of **2**.



3. Experimental

3.1. General

Column chromatography (CC) was performed on Dianion *HP 2MGL* (Mitsubishi Chemical Co.), Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.), MCI-gel CHP20P (Mitsubishi Chemical Co.) and silica gel (Qingdao Haiyang Chemical Co.). TLC was carried on silica gel G precoated plates (Qingdao Haiyang Chemical Co.) with CHCl_3 -MeOH- H_2O (9:1:0.1 or 7:3:0.5). The spots were detected by spraying with 10% H_2SO_4 ethanol solution, followed by heating. UV spectra were obtained on a UV 210A Shimadzu spectrometer (Shimadzu, Kyoto, Japan). IR spectra were recorded on a Shimadzu IR-450 spectrometer as KBr pellets. ^1H - and ^{13}C -NMR, HSQC and HMBC spectra were recorded with Bruker AM-400 and DRX-500 spectrometers operating at 500 and 400 MHz for ^1H , and 125 and 100 MHz for ^{13}C , respectively. FABMS and HRFABMS were recorded on an AutoSpec 3000 spectrometer (VG, Manchester, UK) with glycerol as the matrix.

3.2. Plant Material

The aerial parts of *E. rugulosa* were collected from Yunnan Province, China. The voucher specimen (No. 0215159) was deposited in the KUN Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and Isolation

Dried plant material (400 g) of *E. rugulosa* was refluxed four times with ethanol (4.0 L) for 3 h. After removal of the organic solvent under reduced pressure, the aqueous solution afforded precipitates, which were removed by filtration, and the filtrate was partitioned with ethyl ether to yield ethyl ether and aqueous fractions. The aqueous fraction was concentrated to a small volume (120 mL) and applied to a Dianion *HP 2MGL* column, eluting with H_2O -MeOH (1:0-0:1) to afford five fractions (fr. 1-5). Fr. 2 (0.4 g) was subjected to CC on silica gel (CHCl_3 -MeOH- H_2O , 9:1:0.1-7:3:0.5), Sephadex LH-20 and MCI-gel CHP-20P, eluting with H_2O -MeOH (1:0-0:1) to afford compounds **2** (6 mg) and **5** (15 mg). Fr. 3 (1.7 g) was subjected to CC on silica gel (CHCl_3 -MeOH- H_2O , 9:1:0.1-7:3:0.5), Sephadex LH-20 (H_2O -MeOH, 1:0-0:1) and MCI-gel CHP-20P (H_2O -MeOH, 1:0-0:1) to afford **1** (8 mg), **3** (34 mg), **4** (59 mg) and **11** (16 mg). Repeated CC on Sephadex LH-20 and MCI-gel CHP-20P, eluting with H_2O -MeOH (1:0-0:1), respectively, gave **7** (9 mg), **8** (10 mg) and **9** (50 mg) from Fr. 4 (1.7 g), and **6** (22 mg) and **10** (24 mg) from Fr. 5 (0.7 g).

Compound 1: Yellow amorphous powder. UV-Visible λ_{max} (nm) MeOH: 265, 296, 331; IR (KBr, cm^{-1}): 3.376, 1.640, 1.613, 1.508, 1.055, 780, 770; ^1H NMR (500 MHz, $\text{DMSO}-d_6$): 7.94 (d, $J = 8.6$ Hz, H2', H6'), 6.92 (d, $J = 8.6$ Hz, H3', H5'), 6.86 (s, H3), 6.82 (d, $J = 1.6$ Hz, H8), 6.43 (d, $J = 1.6$ Hz, H6), 5.42 (d, $J = 3.7$ Hz, H1"), 5.10 (d, $J = 3.9$, 12.5 Hz, Ha6"), 5.05 (dd, $J = 3.9$, 12.5 Hz, Hb6"), 4.63 (m, H2"), 3.71 (m, H4"), 3.17-3.58 (m, H3", H5") ppm; ^{13}C -NMR (125 MHz, $\text{DMSO}-d_6$): 181.9 (s, C-4), 162.9 (s, C-2), 161.4 (s, C-5), 161.1 (s, C-4'), 156.9 (s, C-9), 128.6 (d, C-2', C-6'), 121.0 (s, C-1'),

116.0 (d, C-3', 5'), 105.4 (s, C-10), 103.1 (d, C-3), 99.9 (d, C-1''), 99.5 (d, C-6), 94.8 (d, C-8), 77.2 (d, C-3''), 76.3 (d, C-5''), 73.1 (d, C-2''), 69.5 (d, C-4''), 60.6 (t, C-6''); HR-FAB-MS (neg.): 431.1280 [M-H]⁻ (calcd. for C₂₁H₁₉O₁₀ 431.1102).

Compound 2: Yellow amorphous powder. UV-Visible $\lambda_{\max}$ (nm) MeOH: 256, 267, 346; IR (KBr, cm⁻¹): 3,450, 2,920, 1,650, 1,573, 1,515, 990-600; ¹H-NMR (500 MHz, MeOH+DMSO-d₆): 7.40 (d, *J* = 1.9 Hz, H2'), 6.95 (d, *J* = 1.9 Hz, H6'), 6.78 (s, H3), 6.61 (d, *J* = 1.8 Hz, H6), 6.48 (d, *J* = 1.8 Hz, H8), 5.16 (br t, *J* = 6.7 Hz, H2'''), 5.08 (d, *J* = 8.1 Hz, H1''), 3.78-4.40 (m, H2'', H3'', H4'', H5''), 3.62 (m, H1'''), 1.68 (s, H5'''), 1.62 (s, H4''') ppm; ¹³C-NMR (125 MHz, MeOH+DMSO-d₆): 183.7 (s, C-4), 166.7 (s, C-7), 162.1 (s, C-5), 158.9 (s, C-9), 151.1 (s, C-4'), 147.3 (s, C-3'), 130.1 (s, C-3''), 123.7 (s, C-1'), 127.1 (s, C-5'), 122.1 (s, C-2''), 117.6 (d, C-6'), 114.6 (d, C-2'), 107.1 (s, C-10), 104.5 (s, C-3), 101.9 (d, C-1''), 101.0 (d, C-6), 96.1 (d, C-8), 78.5 (d, C-3''), 77.9 (d, C-5''), 74.7 (d, C-2''), 71.2 (d, C-4''), 62.9 (t, C-6''), 28.4 (t, C-1'''), 25.6 (q, C-5'''), 17.8 (q, C-4'''); HR-FAB-MS (neg.): 515.1913 [M-H]⁻ (calcd for C₂₆H₂₇O₁₁ 515. 1657).

3.4. Enzymatic hydrolysis of compounds 1 and 2

An aqueous solution of **1** (3 mg) and maltase (1 mg) was incubated at 37 °C for 80 h. The solution was extracted with CHCl₃ and aglycone produced was identified as apigenin by comparison with compound **6** on silica gel TLC using CHCl₃-MeOH-H₂O (8:2:0.2), *R_f* = 0.68. The aqueous layer was concentrated to a residue, which was dissolved by water and examined for identification of the component sugar, and D-glucose was identified by direct comparison on silica gel TLC with an authentic sample, using CHCl₃-MeOH-H₂O (7:3:0.5). *R_f* = 0.23.

A solution of **2** (2 mg) in H₂O (1 mL) were treated with crude cellulase (7 mg) at 37 °C for 60 h. The reaction mixture was diluted with H₂O (2 mL), and extracted with CHCl₃ (3 mL × 2). The aqueous layer was concentrated to a residue, which was dissolved by water and examined for identification of the component sugar, and D-glucose was identified by direct comparison on silica gel TLC with an authentic sample, using CHCl₃-MeOH-H₂O (7:3:0.5). *R_f* = 0.23.

4. Conclusions

A detailed phytochemical investigation on *E. rugulosa* led to the isolation of two new flavonoid glycosides, apigenin 4'-O- α -D-glucopyranoside (**1**) and 5,7,3',4'-tetrahydroxy-5'-C-prenylflavone-7-O- β -D-glucopyranoside (**2**), together with nine known compounds (**3-11**). Among them, compounds **7-10** were isolated for the first time from *E. rugulosa*.

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Sample Availability: Samples of the compounds are available from the authors.

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