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Two new iridoid-sesquiterpene conjugates from *Rehmannia glutinosa*

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ABSTRACT

Two new iridoid-sesquiterpene conjugates, rehmanniosides E (**1**) and F (**2**) along with eight known compounds, jiocaroteniside A_1 (**3**), 6-*O*-(5″-O-*β*-D-quinovopyranosyl)aeginetoylajugol (**4**), 6-*O*-vanilloylajugol (**5**), 6-O-(*E*)-caffeoylajugol (**6**), 6-O-*trans*-feruloylajugol (**7**), 6-O-(4″-*O*-α-L-rhamnopyranosyl)vanilloylajugol (**8**), rehmaionoside C (**9**), and jioglutoside B (**10**) were isolated from the roots of *Rehmannia glutinosa* (Gaertn.) DC. Their chemical structures were elucidated on the basis of extensive spectroscopic methods, including 1D and 2D NMR, HR ESI MS, and CD spectra.

1. Introduction

Rehmannia glutinosa (Gaertn.) DC., (Orobanchaceae), commonly known as Di-huang, is a famous herb in traditional medicine (Liu et al., 2017; Zhang et al., 2008). Three types of *R. glutinosa* (fresh rehmannia root, dried rhizome, and prepared root) have been used in oriental medicine prescribed for diabetes, anemia, hemoptysis, anticancer, and gynecological diseases (Liu et al., 2017; Zhang et al., 2008). Phytochemical investigations of *R. glutinosa* have shown the presence of iridoids (Lee et al., 2011; Morota et al., 1989; Nishimura et al., 1989; Sasaki et al., 1991) and phenylethanoid glycosides (Sasaki et al., 1989) as the main components. In addition, biological studies on *R. glutinosa* revealed significant pharmacological anticancer (Wang and Zhan-Sheng, 2018; Xu et al., 2017), anti-inflammatory (Kim et al., 1999), and antidiabetes properties (Yan et al., 2018; Zhou et al., 2015). The aim of this study is to isolate compounds from *R. glutinosa.* This paper reports the isolation and structural elucidation of two new iridoid-sesquiterpene conjugates and eight known compounds from *R. glutinosa*.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder and its molecular formula was determined to be $C_{36}H_{56}O_{17}$ by the HR ESI MS ion peak at *m/z* 783.3414 $[M + Na]^+$ (Calcd. for $[C_{36}H_{56}O_{17}Na]^+$, 783.3410). The ^{1}H NMR spectrum of **1** (in CD_3OD) showed proton signals of four tertiary methyl groups at δ_H 1.06, 1.10, 1.39, and 2.34 (each 3H, s) and two anomeric protons at δ_H 4.45 (1H, d, J =8.0 Hz) and 4.68 (1H, d, J =7.5 Hz) suggested the presence of two sugar units. The ^{13}C NMR and DEPT spectra (Table 1) revealed carbon signals of one carbonyl carbon at δ_C 168.7, six olefinic carbons at δ_C 104.8, 118.9, 134.1, 140.9, 141.3, and 154.5, five non-protonated carbons at δ_C 42.3, 79.1, 82.0, and 83.3, fourteen methines at δ_C 39.3, 51.6, 71.7, 72.5, 75.8, 74.8, 77.0, 78.0, 78.2, 78.8, 79.7, 93.4, 97.9, and 99.4, five methylenes at δ_C 18.7, 31.2, 32.7, 48.0, and 62.9, and five methyl carbons at δ_C 14.7, 18.5, 20.5, 21.3, and 25.9. Analysis of the NMR data suggested that the structure of **1** contained one iridoid, one apo carotenoid sesquiterpenoid, and two sugar moieties. The ^{13}C NMR chemical shifts of two sugar moieties at δ_C 99.4, 74.8, 78.0, 71.7, 78.2, and 62.9; 97.9, 75.8, 78.8, 77.0, 72.5, and 18.5; and multiplicity of anomeric protons at δ_H 4.68 (1H, d, J =7.5 Hz) and 4.45 (1H, d, J =8.0 Hz) indicated that the sugar moieties are *β*-D-glucopyranosyl and *β*-D-quinovopyranosyl. These sugar moieties were also reported from *R. glutinosa* (Morota et al., 1989; Nishimura et al., 1989; Sasaki et al., 1991). The HMBC correlations (Fig. 2) between H-1 (δ_H 5.50) and C-3 (δ_C 140.9), H-3 (δ_H 6.23) and C-1 (δ_C 93.4)/C-5 (δ_C 39.3), (δ_H 4.88) and C-4 (δ_C 104.8)/C-8 (δ_C 79.1)/C-9 (δ_C 51.6), and between H-6 H-10 (δ_H 1.39) and C-7 (δ_C 48.0)/C-8 (δ_C 79.1)/C-9 (δ_C 51.6) suggested the

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Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **1** and **2** in CD_3OD.

C	1		2	
	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
1	93.4	5.50 (d, 2.5)	93.4	5.53 (d, 2.0)
3	140.9	6.23 (dd, 2.5, 6.5)	141.1	6.22 (dd, 2.0, 6.0)
4	104.8	5.00 (dd, 2.5, 6.5)	104.6	4.97 (dd, 2.0, 6.0)
5	39.3	2.90 (dd, 2.5, 9.5)	39.4	2.92 (dd, 1.5, 9.5)
6	79.7	4.88 (m)	79.9	4.90 (m)
7	48.0	1.97 (dd, 4.0, 14.0) 2.22 (dd, 6.5, 14.0)	48.0	1.99 (dd, 3.0, 14.0) 2.23 (dd, 6.5, 14.0)
8	79.1	–	79.2	–
9	51.6	2.56 (dd, 2.0, 9.0)	51.5	2.63 (dd, 2.0, 9.5)
10	25.9	1.39 (s)	26.2	1.43 (s)
1′	99.4	4.68 (d, 7.5)	99.4	4.68 (d, 8.0)
2′	74.8	3.21 (m)	74.8	3.21 (m)
3′	78.0	3.39 (t, 9.0)	78.0	3.41 (m)
4′	71.7	3.30 (t, 9.0)	71.9	3.28 (m)
5′	78.2	3.30 (m)	78.2	3.35 (m)
6′	62.9	3.69 (dd, 5.5, 12.0) 3.91 (dd, 1.5, 12.0)	63.0	3.67 (dd, 6.0, 12.0) 3.92 (dd, 2.0, 12.0)
1″	42.3	–	39.8	–
2″	31.2	1.09 (m)/2.20 (m)	37.2	1.17 (m)/1.73 (m)
3″	18.7	1.40 (m)/2.20 (m)	18.7	1.31 (m)/2.12 (m)
4″	32.7	1.73 (m)	32.9	1.73 (m)
5″	83.3	–	83.6	–
6″	82.0	–	80.6	–
7″	141.3	6.87 (d, 16.0)	142.8	6.87 (d, 16.5)
8″	134.1	6.50 (d, 16.0)	127.8	7.69 (d, 16.5)
9″	154.5	–	153.0	–
10″	118.9	5.86 (s)	117.0	5.71 (s)
11″	168.7	–	168.0	–
12″	14.7	2.34 (s)	21.6	2.07 (s)
13″	20.5	1.06 (s)	26.0	1.20 (s)
14″	72.1	3.01 (d, 11.0) 3.64 (d, 11.0)	27.8	0.87 (s)
15″	21.3	1.10 (s)	22.0	1.17 (s)
1‴	97.9	4.45 (d, 8.0)	98.0	4.44 (d, 7.5)
2‴	75.8	3.23 (dd, 8.0, 9.0)	75.8	3.21 (m)
3‴	78.8	3.29 (t, 9.0)	78.8	3.30 (m)
4‴	77.0	3.03 (t, 9.0)	77.1	3.01 (t, 9.0)
5‴	72.5	3.24 (m)	72.6	3.25 (m)
6‴	18.5	1.24 (d, 6.5)	18.4	1.24 (d, 6.0)

Assignments were done by HSQC, HMBC, COSY, and NOESY experiments.

presence of a double bond at C-3/C-4 oxygenated and hydroxyl groups at C-6 and C-8. The NOESY correlations between H-10 (δ_H 1.39) and H-1 (δ_H 5.50)/H-6 (δ_H 4.88) indicated that all these protons were α-orientations. In addition, the HMBC correlations between H-1′ (δ_H 4.68) and C-1 (δ_C 93.4) indicated the position of β-D-glucopyranosyl at C-1′ of iridoid. The HMBC correlations between H-13″ (δ_H 1.06) and C-1″ (δ_C 42.3)/C-2″ (δ_C 31.2)/C-6″ (δ_C 82.0)/C-14″ (δ_C 72.1), H-7″ (δ_H 6.87) and C-1″ (δ_C 42.3)/C-5″ (δ_C 83.3)/C-6″ (δ_C 82.0)/C-7″ (δ_C 141.3), and between H-12″ (δ_H 2.34) and C-8″ (δ_C 134.1)/C-9″ (δ_C 154.5)/C-10″ (δ_C 118.9) indicated the location of hydroxyl groups at C-6″ and C-14″ and double bonds at C-7″/C-8″ and C-9″/C-10″ of the sesquiterpene. The NOESY correlations between H-7″ (δ_H 6.87) and H-12″ (δ_H 2.34)/H-13″ (δ_H 1.06) and between H-14″ (δ_H 3.01 and 3.64)/H-15″ (δ_H 1.10) indicated that the hydroxymethylene group at C-1″, the methyl group at C-5″, and the hydroxyl group at C-6″ were on the same side. The configurations of both double bonds of sesquiterpene at C-7″/C-8″ and C-9″/C-10″ were determined to be *E,* based on the large coupling constant between H-7″ and H-8″, *J* =16.0 Hz and NOESY correlations between H-8″ (δ_H 6.50) and H-10″ (δ_H 5.86). The absolute configurations at C-1″, C-5″, and C-6″ of sesquiterpene were indicated by CD spectroscopy (Fig. 3) with Cotton effects at 226 nm (positive) and 265 nm (negative), similar to those of *sec*-hydroxyjiocarotenoside A_1 (Liu et al., 2016). The position of β-D-quinovopyranosyl at C-5″ of sesquiterpene was confirmed by the HMBC correlation from H-1‴ (δ_H 4.45) to C-5″ (δ_C 83.3). Moreover, the esterification position at C-11″ of sesquiterpene and C-6 of ajugol was confirmed by the HMBC correlation from H-6 (δ_H 4.88) to C-11″ (δ_C 168.7). Based on the above evidence, the structure of **1** was elucidated and named rehmannioside E.

The HR ESI MS of **2** exhibited an ion peak at *m/z* 767.3459 $[M + Na]^+$ corresponding to the molecular formula of $C_{36}H_{56}O_{16}$ (Calcd. for $[C_{36}H_{56}O_{16}Na]^+$, 767.3461). The ^{1}H and ^{13}C NMR spectra of **2** exhibited the signals of one iridoid, one apo carotenoid sesquiterpenoid, and two sugar moieties. The ^{1}H and ^{13}C NMR data of compound **2** were almost the same as those of 6-*O*-(5′′-O-β-D-quinovopyranosyl)aeginetoylajugol (**4**) (Lee et al., 2011), a compound we also isolated from *R. glutinosa*, except for the difference in the chemical shifts of C-8″ and C-12″, suggesting the possibilities of different configurations of the double bond at C-9″/C-10″ of sesquiterpene. This was further confirmed by the NOESY correlations between H-12″ (δ_H 2.07) and H-7″ (δ_H 6.87)/H-10″ (δ_H 5.71). ^{13}C-NMR of C-2 (δ_C 37.2) for compound **2** shifted to low field could be due to methylene group (C-2) near oxygenated groups of sugar and iridoid moieties cause of *E* configuration at C-9″/C-11″. In addition, the absolute configuration at C-6″ of sesquiterpene was proven as *R* by recording the CD spectroscopy with Cotton effects at 230 nm (positive) and 268 nm (negative), similar to those of **1**. The NOESY correlations between H-7″ (δ_H 6.87) and H-13″ (δ_H 1.20) and between H-14″ (δ_H 0.87) and H-15″ (δ_H 1.17) suggested that the configuration of the methyl group at C-5″ was β (*R* configuration at C-5″). Moreover, the connection positions between moieties were proven by the HMBC correlations from H-1′ (δ_H 4.68) to C-1 (δ_C 93.4), H-6 (δ_H 4.90) to C-11″ (δ_C 168.0), and from H-1‴ (δ_H 4.44) to C-5″ (δ_C 83.6). Consequently, the structure of **2** was elucidated and named rehmannioside F.

The known compounds were characterized as jiocaroteniside A_1 (**3**) (Sasaki et al., 1991), 6-*O*-(5′′-O-β-D-quinovopyranosyl)aeginetoylajugol (**4**) (Lee et al., 2011), 6-*O*-vanilloylajugol (**5**) (Akdemir and Tatli, 2004), 6-O-(*E*)-caffeoylajugol (**6**), 6-O-*trans*-feruloylajugol (**7**), 6-O-(4′′-*O*-α-L-rhamnopyranosyl)vanilloylajugol (**8**) (Nishimura et al., 1989), rehmaionoside C (**9**) (Sasaki et al., 1991), and jioglutosideB (**10**) (Morota et al., 1989) by analysis of NMR and mass spectroscopic data and in comparison with the reported literature (Fig. 1).

3. Experimental

3.1. General

All NMR spectra were recorded on a Bruker 500 MHz AVANCE. HR ESI MS were obtained using an AGILENT 6550 iFunnel Q-TOF LC/MS system. CD spectra were recorded on a Chirascan spectrophotometer (AppliedPhotophysics). Optical rotations were determined on a Jasco DIP-370 digital polarimeter. HPLC was carried out using an AGILENT 1200 HPLC system. Column chromatography (CC) was performed on RP-18 gel (30–50 μm, Fuji Silysia Chemical Ltd.) or silica-gel (Kieselgel 60, 230–400 mesh, Merck). For thin layer chromatography (TLC), precoated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ (0.25 mm, Merck) plates were used.

3.2. Plant material

The roots of *Rehmannia glutinosa* (Gaertn.) DC., were collected at Viettri, Phutho, Viet Nam in March 2020 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (RG2003) was deposited at the Hanoi University of Mining and Geology.

3.3. Extraction and isolation

The dried powder roots of *R. glutinosa* (20.0 kg) were sonicated 3 times with hot methanol. The MeOH extract was removed under reduced pressure to yield 2.2 kg of a solid extract. The MeOH extract was suspended in water and successively partitioned with dichloromethane and ethyl acetate (EtOAc) giving dichloromethane extract (RGD, 250 g), EtOAc extract (RGE, 120 g), and water layer (RGW). The RGW was loaded on a Diaion HP-20 column (15 cm ID ×30 cm length), removed

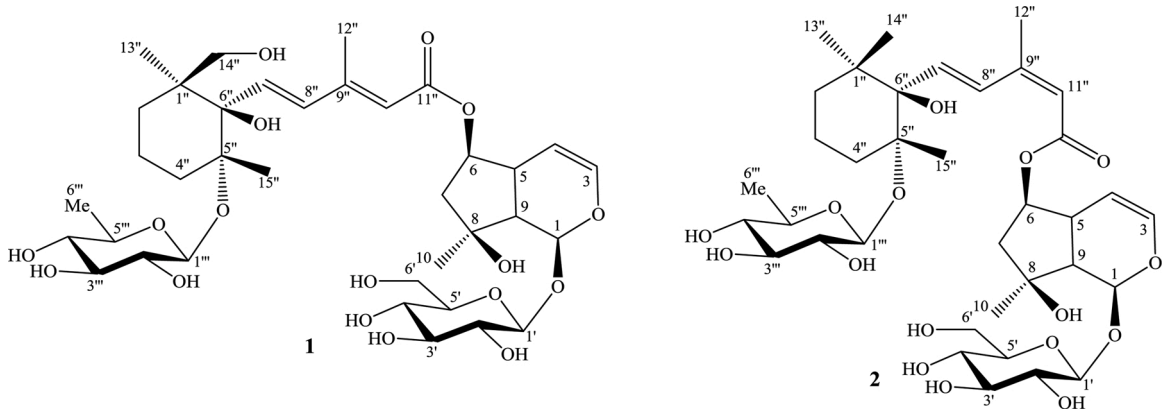

Fig. 1. Chemical structures of compounds **1** and **2**.

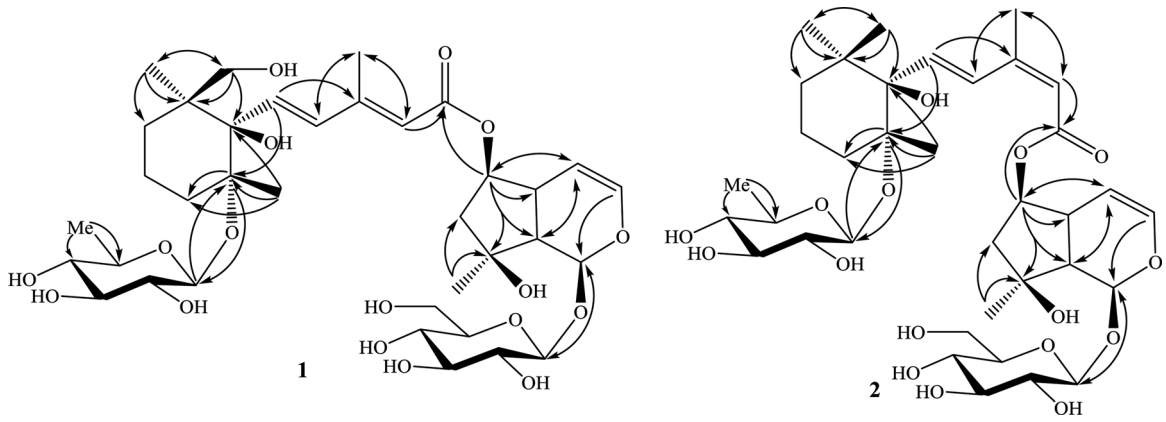

Fig. 2. The key HMBC correlations of compounds **1** and **2**.

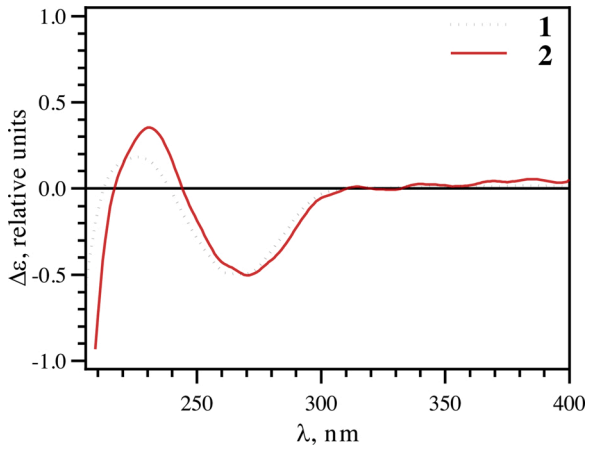

Fig. 3. The CD spectra of compounds **1** and **2** in MeOH.

sugar components by water then increase the concentration of MeOH in water (25, 50, 75, and 100 %, v/v) to obtain RGW1 (80.2 g), RGW2 (7.1 g), RGW3 (10.6 g), and RGW4 (6.3 g).

RGW3 fraction was loaded on an RP-18 column (5 cm ID ×35 cm length), using acetone/water (1/1, v/v) to give four fractions, RGW3A (1.2 g), RGW3B (1.5 g), RGW3C (3.2 g), and RGW3D (2.2 g). RGW3A was chromatographed on a silica gel column (2 cm ID ×40 cm length) eluting with CH_2Cl_2/MeOH (4/1, v/v) to yield compounds **2** (5.0 mg) and **4** (9.0 mg). RGW3B fraction was chromatographed on an RP-18 column (2 cm ID ×40 cm length) using acetone/water (2/1, v/v) to yield compounds **1** (5.0 mg) and **5** (12.0 mg). Compounds **7** (6 mg) and **8** (10.0 mg) were obtained from RGW3C fraction on a silica gel column (3 cm ID ×40 cm length) eluting with CH_2Cl_2/acetone (3/1, v/v).

RGW4 was chromagtographed on an RP-18 column (4 cm ID ×40 cm length) eluting with acetone/water (1/1, v/v) to give fractions, RGW4A (1.2 g), RGW4B (1.9 g), and RGW4C (2.1 g). RGW4B was chromatographed on a silica gel column (3 cm ID ×40 cm length) eluting with EtOAc/MeOH (10/1, v/v) to yield compound **10** (15.0 mg) and RGW4B2 fraction (215 mg). RGW4B2 was chromatographed on a silica column (1.5 cm ID ×60 cm length) eluting with CH_2Cl_2/MeOH (5/1, v/v) to yield compounds **3** (8.0 mg) and **9** (32.0 mg). Compound **6** (5.0 mg) was obtained from RGW4C fraction using a silica gel column (3 cm ID ×40 cm length) (solvent condition: CH_2Cl_2/MeOH (5/1, v/v).

3.3.1. *Rehmannioside E (1)*

White amorphous powder. $[\alpha]_D^{25} = -56.0$ (*c* 0.1 MeOH). HR-ESI-MS *m/z*: 783.3414 $[M + Na]^+$ (Calcd. for $[C_{36}H_{56}O_{17}Na]^+$, 783.3410). CD (rel): 226 (+0.18), 265 (-0.49) ($c = 1.0 \times 10^{-3}$ M, MeOH): see Fig. 3. ^1H and ^{13}C NMR (CD_3OD): see Table 1.

3.3.2. *Rehmannioside F (2)*

White amorphous powder. $[\alpha]_D^{25} = -68.0$ (*c* 0.1 MeOH). HR-ESI-MS *m/z*: 767.3459 $[M + Na]^+$ (Calcd. for $[C_{36}H_{56}O_{16}Na]^+$, 767.3461). CD (rel): 230 (+0.36), 268 (-0.49) ($c = 1.0 \times 10^{-3}$ M, MeOH): see Fig. 3. ^1H and ^{13}C NMR (CD_3OD): see Table 1.

Declaration of Competing Interest

The authors declared no conflict of interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2021.04.008.

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