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Iridoid glycosides link with phenylpropanoids from *Rehmannia glutinosa* NATURAL PRODUCT RESEARCH

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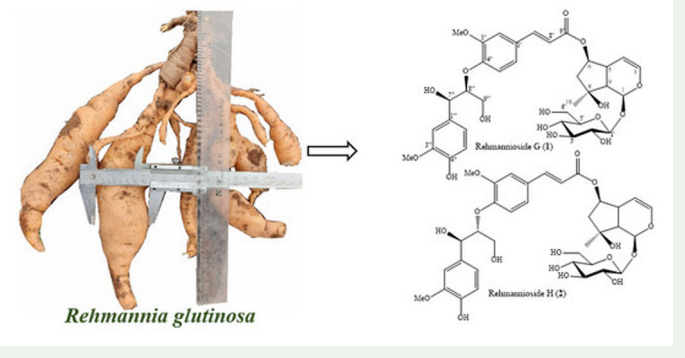
ABSTRACT

Two new iridoid glycosides link with phenylpropanoids, rehmanniosides G (**1**) and H (**2**) along with 11 known compounds, 6-O-(*E*)-caffeoylajugol (**3**), 6-*O*-(*E*)-feruloylajugol (**4**), verbasoside (**5**), jionoside C (**6**), acteoside (**7**), leucosceptoside A (**8**), brachynoside (**9**), jionoside B1 (**10**), jionoside A1 (**11**), isoacteoside (**12**) and isomartynoside (**13**) were isolated from the roots of *Rehmannia glutinosa* (Gaertn.) DC. Their chemical structures were elucidated on the basis of extensive spectroscopic methods, including 1D, 2D NMR and mass spectra. Compounds **7 − 11** showed significant inhibitory α-glucosidase with IC_{50} values ranging from 261.4 to 408.7 μM (acarbose, IC_{50} of 204.2 ± 19.9 μM).



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Rehmannia glutinosa; rehmannioside G; rehmannioside H; α-glucosidase activity

1. Introduction

Rehmannia glutinosa (Gaertn.) DC. (Orobanchaceae), is a famous herbal in oriental medicine (Zhang et al. 2008; Liu et al. 2017). *R. glutinosa* was also one of important

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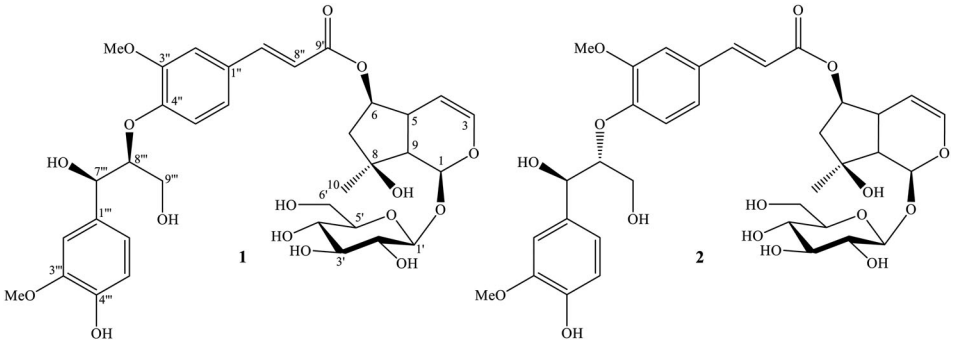

Figure 1. Chemical structures of compounds **1** and **2**.

components of Rehmannia six formula that has been used in Chinese medicine to treat diabetes mellitus (Poon et al. 2011). Phytochemistry of *R. glutinosa* indicated the presence of phenylpropanoid glycosides (Sasaki et al. 1989), iridoids (Morota et al. 1989; Nishimura et al. 1989; Sasaki et al. 1991; Lee et al. 2011), saccharides and amino acids (Zhang et al. 2008). In addition, the extract from *R. glutinosa* exhibited antidiabetic (Zhou et al. 2015; Yan et al. 2018), anti-inflammatory (Kim et al. 1999) and anticancer activities (Xu et al. 2017; Wang and Zhan-Sheng 2018). Interestingly, the roots of *R. glutinosa* were found to inhibit α-glucosidase activity (Jeong et al. 2013). As a part of searching α-glucosidase inhibitors from Vietnamese medicinal plants, we report here the isolation, structural elucidation and α-glucosidase activity of two new iridoid glycosides and eleven known phenylpropanoid glycosides from the roots of *R. glutinosa*.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder and the molecular formula, $C_{35}H_{44}O_{16}$ was indicated by HR ESI MS at *m/z* 743.2521 $[M+Na]^+$ (Calcd. for $[C_{35}H_{44}O_{16}Na]^+$, 743.2522). The ^{1}H NMR spectrum of **1** (in CD_3OD) showed signals for two ABX aromatic rings at δ_H 7.25 (d, $J = 1.5$ Hz), 7.07 (d, $J = 8.0$ Hz), and 7.13 (dd, $J = 1.5$, 8.0 Hz), 7.05 (d, $J = 1.5$ Hz), 6.77 (d, $J = 8.0$ Hz), and 6.88 (dd, $J = 1.5$, 8.0 Hz), four olefinic protons at δ_H 6.47 (d, $J = 16.0$ Hz), 7.66 (d, $J = 16.0$ Hz), 5.52 (d, $J = 2.5$ Hz), and 6.24 (dd, $J = 2.0$, 6.0 Hz), one anomeric proton at δ_H 4.69 (d, $J = 8.0$ Hz), one tertiary methyl group at δ_H 1.41 (3H, s), and two methoxy groups at 3.83 and 3.92 (each 3H, s). The ^{13}C NMR and HSQC spectra (Table S1) showed carbon signals for one carbonyl carbon at δ_C 168.8, 16 olefinic and aromatic carbons (six quaternaries at δ_C 129.7, 133.8, 147.2, 148.9, 151.7 and 152.1 and ten methines at δ_C 104.6, 111.8, 112.4, 115.9, 117.1, 117.6, 120.7, 123.7, 141.1 and 146.3), one non-protonated carbon at δ_C 79.2, eleven methines at δ_C 39.4, 51.7, 71.7, 73.9, 74.8, 78.0, 78.2, 80.5, 86.3, 93.5 and 99.4, three methylenes at δ_C 47.9, 62.1 and 62.9, three methyl (including two methoxy) carbons at δ_C 26.1, 56.4 and 56.7. Extensive analysis of HMBC, COSY and NOESY spectra of **1** provided the assignments of proton and carbon signals. Analysis of the NMR data suggested that structure of **1** contained one iridoid, one sugar and two phenylpropanoid moieties (Figure 1). The structure of **1** was found to be similar to that of 6-O-(*E*)-

caffeoylajugol (**3**) (Harinantenaina et al. 2001) except for an addition of phenylpropanoid moiety at C-4″ of feruloyl. The HMBC correlations (Figure S1) from H-1 (δ_H 5.52) to C-3 (δ_C 141.1), from H-3 (δ_H 6.24) to C-1 (δ_C 93.5)/C-5 (δ_C 39.4), from H-6 (δ_H 4.95) to C-4 (δ_C 104.6)/C-8 (δ_C 79.2)/C-9 (δ_C 51.7) and from H-10 (δ_H 1.41) to C-7 (δ_C 47.9)/C-8 (δ_C 79.2)/C-9 (δ_C 51.7) indicated the positions of the double bond at C-3/C-4, the oxygenated and hydroxyl groups at C-6 and C-8 of iridoid. The α-orientations of H-1, H-6 and H-10 were indicated by the NOESY correlations between H-10 (δ_H 1.41) and H-1 (δ_H 5.52)/H-6 (δ_H 4.95). The ^{13}C NMR chemical shifts of a sugar moiety at δ_C 99.4, 74.8, 78.2, 71.7, 78.0 and 62.9 and multiplicity of anomeric proton at δ_H 4.69 (1H, d, $J = 8.0$ Hz) as well as biosynthesis from *Rehmannia* genus indicated that monosaccharide was β-D-glucopyranosyl (Nishimura et al. 1989, 1990). The HMBC correlation from H-1′ (δ_H 4.69) to C-1 (δ_C 93.5) indicated the position of β-D-glucopyranosyl at C-1. The HMBC correlations between H-2″ (δ_H 7.25)/H-6″ (δ_H 7.13) and C-1″ (δ_C 129.7)/C-4″ (δ_C 151.7) and between H-6 (δ_H 4.95) and C-9″ (δ_C 168.8) and NOESY correlations between methoxy (δ_H 3.92) and H-2″ (δ_H 7.25) indicated the presence of caffeoyl moiety at C-6. The HMBC correlations between H-2‴ (δ_H 7.05)/H-6‴ (δ_H 6.88) and C-1‴ (δ_C 133.8)/C-4‴ (δ_C 148.9)/C-7‴ (δ_C 73.9), between H-7‴ (δ_H 4.91) and C-1‴ (δ_C 133.8)/C-2‴ (δ_C 111.8)/C-6‴ (δ_C 120.7)/C-8‴ (δ_C 86.3)/C-9‴ (δ_C 62.1), and between methoxy group (δ_H 3.83) and C-3‴ (δ_C 147.2) indicated the presence of 1-(3-methoxy-4-hydroxy)phenylpropanetriol. The coupling constant of H-7‴ and H-8‴, $J = 4.5$ Hz possessed an *erytho* relative configuration of these protons (Xiong et al. 2011). The position of this moiety at C-4″ of caffeoyl was proved by the HMBC correlation from H-8‴ (δ_H 4.47) to C-4″ (δ_C 151.7). Based on the above evidence, the structure of **1** was elucidated and named rehmannioside G.

Compound **2** was also obtained as a white amorphous powder. HR-ESI-MS experiment resulted in the same molecular formula as that of **1**. Analysis of the NMR data suggested that structure of **2** contained one iridoid, one sugar and two phenylpropanoid moieties. ^{1}H- and ^{13}C-NMR data of **2** were almost the same as that of rehmannioside G (**1**), suggesting the possibilities of different configurations at the chiral carbons in the 1-(3-methoxy-4-hydroxy)phenylpropanetriol. Moreover, the connection of moieties was proved by the HMBC correlations between H-1′ (δ_H 4.69) and C-1 (δ_C 93.5), H-6 (δ_H 4.96) and C-9″ (δ_C 168.8), and between H-8‴ (δ_H 4.51) and C-4″ (δ_C 151.8). The coupling constant of H-7‴ and H-8‴, $J = 6.0$ Hz possessed a *threo* relative configuration of these protons (Xiong et al. 2011). Consequently, the structure of **2** was elucidated and named rehmannioside H.

The known compounds were characterised as 6-O-(*E*)-caffeoylajugol (**3**) (Harinantenaina et al. 2001), 6-*O*-(*E*)-feruloylajugol (**4**) (Nishimura et al. 1989), verbasoside (**5**) (Nishimura et al. 1990), jionoside C (**6**) (Sasaki et al. 1989), acteoside (**7**) (Sasaki et al. 1989), leucosceptoside A (**8**) (Sasaki et al. 1989), brachynoside (**9**) (Lin and Kuo 1992), jionoside B1 (**10**) (Sasaki et al. 1989), jionoside A1 (**11**) (Calis et al. 1984), isoacteoside (**12**) (Sasaki et al. 1989) and isomartynoside (**13**) (Calis et al. 1984) by analysis of NMR data and in comparison with those reported in the literature (Figure S2).

Compounds **1 − 4** belong to iridoid glycosides link with phenylpropanoids. This compound class has been reported in *Stachys* genus (Venditti et al. 2013) (family Lamiaceae) *Pedicularis* genus (Venditti et al. 2016; Frezza et al. 2017) and also

Rehmannia genus (Xia et al. 2009) (family Orobanchaceae). Thus, this report may contribute to provide evidence for taxonomic markers in families of Lamiales order.

The inhibitory α-glucosidase activity of compounds was screened at a concentration of 500 μM (Table S2). Compounds **7 − 11** exhibited significant α-glucosidase inhibitory activity with inhibitory percentages ranging from 67.0% to 94.0%. These compounds were chosen for further evaluation at concentrations of 500, 200, 100, 20 and 4 μM to obtain IC_{50} values. As results, compounds **7 − 11** showed significant α-glucosidase inhibitory activity with IC_{50} values ranging from 261.4 to 408.7 μM (acarbose, IC_{50} of 204.2 ± 19.9 μM). Of these, aceteoside (**7**), leucosceptoside A (**8**) and isoacteoside (**12**) have been also found as α-glucosidase inhibitors (Liu et al. 2014). Brachynoside (**9**), jionoside B1 (**10**) and jionoside A1 (**11**) have not been evaluated for α-glucosidase inhibitory activity yet. In the structure–activiy relationship of isolated compounds, phenylpropanoid glycosides were found to exhibit the strongest α-glucosidase inhibitory activity. Especially, when the presence of phenylpropanoid moiety (feruloyl/caffeoyl) at C-4 of glucopyarnosyl, the α-glucosidase inhibitory activity increases. In addition, phenylpropanoid glycosides were also the main ingredients of *R. glutinosa*. Thus, these findings indicated phenylpropanoid glycosides could play as main active ingredients of *R. glutinosa*.

3. Experimental

3.1. General

See supporting information

3.2. Plant material

The roots of *R. glutinosa* (Gaertn.) DC., were collected at Viettri, Phutho, Vietnam in March 2020 and identified by one of the authors, Dr. Nguyen The Cuong. 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 three times with hot methanol. The MeOH layer 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 (1/3 of water layer volume) was loaded on a Diaion HP-20 CC to remove 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).

RGW1 was chromatographed on an RP-18 CC eluting with MeOH:H_2O (1/2, v/v) to give four fractions, RGW2A (1.5 g), RGW2B (1.4 g), RGW2C (1.4 g) and RGW2D (0.8 g). RGW2A fraction was chromatographed on a silica gel CC eluting with CH_2Cl_2/MeOH (3/1, v/v) to yield **10** (250 mg). RGW2B was chromatographed on a silica gel CC using solvent eluents of EtOAc/MeOH (8/1, v/v) to yield compounds **7** (18.0 mg) and **13** (10.0 mg). Compound **6** (9.0 mg) was yielded from RGW2C using silica gel CC eluting

with CH_2Cl_2/EtOAc (1/1, v/v). Compounds **9** (11.0 mg) and **12** (20.0 mg) were yielded from RGW2D fraction on a silica gel CC using dichloromethane/acetone (1/1, v/v).

RGW3 fraction was loaded on an RP-18 CC using acetone/water (1/1, v/v) to give fractions, RGW3A–RGW3D. Compound **4** (6.0 mg) was obtained from RGW3C fraction on a silica gel CC eluting CH_2Cl_2/acetone (3/1, v/v). RGW3C fraction was chromatographed on a silica gel CC eluting with CH_2Cl_2/EtOAc (1.5/1, v/v) to yield compounds **1** (5.0 mg) and **2** (5.0 mg). RGW3D (150 mg) was chromatographed on a silica gel CC eluting with EtOAc/MeOH (10/1, v/v) to yield compounds **8** (21.0 mg) and **11** (19.0 mg).

RGW4 was chromatographed on an RP-18 CC eluting with acetone/water (1/1, v/v) to give fractions, RGW4A - RGW4C. RGW4B was chromatographed on a silica gel CC eluting with EtOAc/MeOH (10/1, v/v) to yield compound **5** (16.0 mg). Compound **3** (5.0 mg) was obtained from RGW4C fraction using a silica gel CC (solvent condition: CH_2Cl_2/MeOH (5/1, v/v).

3.3.1. Rehmannioside G (1)

White amorphous powder. $[\alpha]_D^{25} = -46.0$ (*c* 0.1 MeOH). HR-ESI-MS *m/z*: 743.2521 $[M + Na]^+$ (Calcd. for $[C_{35}H_{44}O_{16}Na]^+$, 743.2522). ^1H and ^{13}C NMR (CD_3OD): see Table S1.

3.3.2. Rehmannioside H (2)

White amorphous powder. $[\alpha]_D^{25} = -63.0$ (*c* 0.1 MeOH). HR-ESI-MS *m/z*: 743.2519 $[M + Na]^+$ (Calcd. for $[C_{35}H_{44}O_{16}Na]^+$, 743.2522). ^1H and ^{13}C NMR (CD_3OD): see Table S1.

3.4. α-Glucosidase assay

See reference and see supporting information (Trang et al. 2019)

Disclosure statement

No potential conflict of interest was reported by the authors.

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