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Chemical Constituents of *Geum urbanum* L. Roots

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ABSTRACT

A new dehydrodigallic acid derivative (**1**), along with 19 known compounds, including 6 phenolic derivatives, 2 steroids, and 11 triterpenoids were isolated from the ethanol extract of the root of *Geum urbanum*. Herein, there is the first report of steroid and triterpene in *Geum urbanum*. The purified metabolites were characterized by NMR spectroscopic and mass spectrometric analyses. The identification of the known compounds (**2-20**) was based on the comparison of their NMR spectroscopic features with previously published data. The structural characteristics of compound **1** were elucidated by comprehensive 1D and 2D NMR spectroscopic methods, and acid hydrolysis.

Keyword: dehydrodigallic acid derivative, triterpenoid, *Geum urbanum*, ellagic acid derivatives

1. Introduction

Geum urbanum L. (Wood Aven) is a perennial pubescent herb with a short, thick rhizome and a member of Rosaceae family, native to Eurasian Temperate Zone. It is commonly distributed throughout the British Isles but absents from the Shetland and parts of northern Scotland. The species spreads from southern Norway, Sweden, Finland, and Russia to the Mediterranean. Outside Europe, it exists eastwards to Asia, western Siberia, and the Himalaya, and expands along northwest African coast from west of Algiers to the Tunisia (Taylor 1997). *Geum urbanum* has long been used in traditional European medicine for treatment of stomach complaints (Fournier 1947), gingivitis and inflammation of mucous membranes (Fournier 1947, Menković et al. 2011).

Main compounds, isolated from the roots of this herb, were ellagitannins (tellimagrandin, stachyurin, casuarnin and gemin A, and ellagic acid derivatives), procyanidins (procyanidin B3, procyanidin C2, and catechin derivatives) (Granica et al. 2016, Piwowski et al. 2014), gallic acid, vanillic acid (Granica, Kłębowska, Kosiński, Piwowski, Dudek, Kaźmierski and Kiss 2016), gein, and vicianose (Pšenák et al. 1972). In this report, monophenolic compounds, gein, and ellagic acid derivatives were also collected. However, one new dehydrodigallic acid derivative, steroids, and triterpenoids were isolated for the first time in *Geum* and their structures were identified based on NMR spectroscopic methods.

2. Results and discussion

By implementing repeatedly silica gel and Sephadex LH-20 column chromatographic separation, and preparative HPLC on CHCl₃ and EtOAc fractions from the original ethanol extract of *Geum urbanum* roots, a new compound (**1**) and 19 known (**2-20**) phenolics, steroids, and triterpenoids of the oleanane, ursane types have been isolated. The new compound (**1**) was identified as a dehydrodigallic derivative as supported by, acid hydrolysis and 1D and 2D NMR spectroscopic, and mass spectrometric techniques (Table S1, Figure 1). The known compounds

were identified as gallic acid (**2**) (Eldahshan 2011), vanillic acid (**3**) (Phan Duc et al. 2016), isovanillic acid (**4**) (Ding et al. 2000), gein (**5**) (Shahani et al. 2012), 3,3',4'-tri-*O*-methylellagic acid (**6**) (Gao et al. 2014), 3,3',4'-tri-*O*-methylellagic acid 4-*O*- β -D-glucopyranoside (**7**) (Gao, Wu, Zou and Dai 2014), pomonic acid (**8**) (Cheng and Cao 1992), 19 α -hydroxy-3-oxoursa-1,12-dien-28-oic acid (**9**) (Traoré-Coulibaly et al. 2009), ursolic acid (**10**) (Othman et al. 1996), 2 α ,3 α ,24-trihydroxyursa-12,18-dien-28-oic acid (**11**) (Yuan et al. 2008), rubuside A (**12**) (Li et al. 2009), rubuside D (**13**) (Li, Fu, Bai, Sasaki, Kato and Koike 2009), β -sitosterol (**14**) (Atiku et al. 2009), β -sitosterol 3-*O*- β -D-glucopyranoside (**15**) (Mizushima et al. 2006), oleanolic acid (**16**) (Guvenalp et al. 2006), niga-ichigoside F1(**17**) (Shigenaga et al. 1985), arjunglucoside I (**18**) (Honda et al. 1976), 2 α ,3 α ,24-trihydroxyursa-12,18-dien-28-oic acid 28-*O*- β -D-glucopyranoside (**19**) (Li, Fu, Bai, Sasaki, Kato and Koike 2009), and 2 α ,3 β ,23-trihydroxyursa-12,19(29)-dien-28-oic acid 28-*O*- β -D-glucopyranoside (**20**) (Li, Fu, Bai, Sasaki, Kato and Koike 2009) (Figure S2) by comparison of their spectroscopic data and literature values.

Compound **1** was obtained as a white powder. The molecular formula of C₁₅H₁₂O₁₀ was assigned for this compound based on HR-ESI-MS analysis ([M + Na]⁺ *m/z* 375.0338, calcd 375.0328). The ¹H-NMR spectrum (Table S1) displayed resonances for three aromatic protons δ_H 7.22 (1H, *d*, 2.0, H-6), 7.06 (1H, *s*, H-4'), 6.72 (1H, *d*, 2.0, H-2), and a methoxy group δ_H 3.68 (3H, *s*). The ¹³C-NMR along with DEPT-NMR indicated the existence of fifteen carbons, among which there were two carboxyl groups at δ_C 170.1 and δ_C 167.6, and one methoxy group at δ_C 52.2. The HMBC correlations between H-2/C-1, H-2/C-3, H-2/C-4, H-2/C-6, H-2/C=O, H-6/C-2, H-6/C-4, H-6/C-5, and H-6/C=O were observed (Figure S1), which indicated the presence of a tetrasubstituted benzene ring. Moreover, the pentasubstituted aromatic ring was deduced based on the HMBC correlations between H-6'/C-1', H-6'/C-2', H-6'/C-3', H-6'/C-4', and H-6'/C=O. The position of the methoxy group was assigned to be at 1'-C=O by the observed HMBC correlations. The ¹H and ¹³C NMR spectra of **1** resembled closely those of

dehydrodigallic acid derivatives, isolated from *Geum urbanum* (Piwowarski, Granica, Kosiński and Kiss 2014, Yoshida et al. 1985). Additionally, normal acid hydrolysis (HCl 2N, 15 mins, 100°C) yielded dehydrodigallic acid with melting point of 362°C (Nawwar et al. 2009). Thus, the structure of mono-methyl dehydrodigallate (**1**) was concluded as shown (Figure 1).

[table S1 and figure 1 near here]

In the genus *Geum*, almost of triterpenoids, belong to olean and ursane skeleton, were evaluated biological activities such as antiviral, anticoagulant, and myogenic effects (Cheng et al. 2011). Nigaichigoside F1, ursane-type triterpenoid, was isolated from *Geum japonicum*, which stimulates the regenerative potential of a damaged muscle (Cheng et al. 2006), and hence it could be treat myocardial infarctions. From the EtOAc extract of *Geum japonicum*, 2 α ,3 β ,19 α ,23-tetrahydroxyurs-12-en-28-oic acid showed potent anticoagulant effect (Zeng et al. 1998); and two ursane-type triterpenes (2*R*,19*R*-dihydroxy-3-oxo-12-ursen-28-oic acid and ursolic acid) and an olean-type one (maslic acid) exhibited significant HIV-1 protease inhibitory activity (Xu et al. 2000, Xu et al. 1996). Oleanolic acid and arjunolic acid (two olean-type triterpenes) showed antitumor activity against Ehrlich and intestinal cells, respectively (Elsherbiny and Al-Gayyar 2016, Liu 1995). Thus, all isolated triterpenoids from the chloroform and ethyl acetate extracts of *Geum urbanum* could be showed potent biological activities.

3. Experimental

3.1. General Experimental Procedures

The HR-ESI-MS was determined with a MicrOTOF QII mass spectrometer (Bruker Daltonics). The NMR spectra were recorded on a Bruker Avance III 500 spectrometer with TMS as an internal standard, and the chemical shifts are expressed as δ values. Column chromatography was carried out using silica gel 60, 0.06–0.2 mm (Scharlau, Barcelona, Spain) and LiChroprep[®] RP-18, 40–63 μ m (Merck KGaA, Darmstadt, Germany). Analytical and

preparative TLC was carried out on precoated Kieselgel 60F₂₅₄ or RP₁₈ plates (Merck KGaA, Darmstadt, Germany). Other chemicals were of the highest grade available.

3.2. *Plant Material*

The roots of *Geum urbanum* L. were collected at Roskilde University, Denmark in August 2010. The plant was identified by botanist Hoang Viet. A voucher specimen (BP002) was deposited in the herbarium of the Department of Organic Chemistry, VNUHCM–University of Science.

3.3. *Extraction and Isolation*

Dried root *Geum urbanum* (3.0 kg) was exhausted with ethanol, the ethanol solution was evaporated under reduced pressure. The ethanol residue was suspended in H₂O and successively partitioned with *n*-hexane (H), chloroform (C), ethyl acetate (EA), to yield the *n*-hexane (7.2 g), chloroform (50.1 g), ethyl acetate (58.7 g) and aqueous (108.1 g) extracts, respectively.

The ethyl acetate extract (58.7 g) was subjected to silica gel column chromatography and eluted with H-EA mixtures (7:3 to 0:10, v/v) and EA:MeOH (10:0 to 0:10, v/v), to afford six fractions (EA1-6). Fraction EA1 (4.0 g) was chromatographed over silica gel column with H-EA (8:2 to 0:10, v/v), to yield five subfractions (EA1.1-EA1.5). Subfraction EA1.2 (760 mg) was subjected to ODS column, eluted with MeOH–H₂O (6:4, v/v), to afford **8** (8.0 mg), and **9** (11.0 mg). Subfraction EA1.3 (830 mg) purified by preparative TLC with H–EA-MeOH (4:6:1), to yield **2** (10.0 mg), and **11** (10.0 mg). Fraction EA3 (7.74 g) was subjected to silica gel column chromatography, eluted with EA-MeOH (10:0 to 0:10, v/v), to yield five subfractions (EA3.1-EA3.5). Subfraction EA3.1 (590 mg) were subjected to silica gel column chromatography, eluted with C:MeOH (9:1 to 0:10), to yield **1** (10.0 mg), **12** (5.0 mg), **13** (4.3 mg), and **15** (4.0 mg). Subfraction EA3.5 (180 mg) were chromatographed over silica gel column with C:MeOH (8:2 to 0:10), to afford three subfractions **17** (20.0 mg), and **18** (11.0 mg).

The chloroform extraction (50.1 g) was subjected to silica gel column chromatography, eluted with gradient H-EA (10:0-0:10, v/v) and EA-MeOH (10:0-0:10, v/v), to afford thirteen fractions (C1-13). Fraction C5 (1.29 g) was separated over a sephadex LH-20 column with C:MeOH (5:5, v/v), to yield seven subfractions (C5.1- C5.7). Subfraction C5.4 (381.1 mg) was subjected to silica gel column chromatograph with C:EA (8:2 to 0:10, v/v), to afford **4** (15.7 mg), and **6** (3.2 mg). Fraction C10 (1.37 g) was subjected to silica gel column chromatography, eluted with H-EA mixtures (9:1-0:10), to yield nine subfractions (C10.1– C10.9). Subfraction C10.3 (125.4 mg) purified by preparative TLC with H–C–MeOH (4:6:0,5), to afford **10** (5,3 mg), **3** (6,6 mg). Fractions C11 (1.95 g) was subjected to silica gel column chromatography, and eluted gradient C-MeOH (9:10-0:10), to yield six subfractions (C11.1-6). Subfraction C11.4 (265.8 mg) were chromatographed over silica gel column with C-EA-MeOH (5:4:1), to yield **6** (4,7 mg), **7** (5,6 mg) and **5** (5,8 mg).

3,4-Dihydroxy-5-(2,3,6-trihydroxy-5-methoxycarbonylphenoxy)benzoic acid (1): white powder; ¹H-NMR (CD₃OD, 500 MHz), δ (ppm): 7.22 (1H, *d*, 2.0, H-6), 7.06 (1H, *s*, H-5'), 6.72 (1H, *d*, 2.0, H-2), 3.68 (3H, *s*, 6'-COOCH₃); ¹³C-NMR (CD₃OD, 125 MHz), δ (ppm): 170.1 (1-COOH), 167.6 (6'-COO), 148.5 (C-3), 146.7 (C-5), 143.8 (C-4'), 140.9 (C-3'), 140.9 (C-2'), 140.5 (C-4), 138.0 (C-1'), 121.8 (C-1), 115.1 (C-6'), 112.3 (C-6), 109.9 (C-5'), 108.3 (C-2), 52.2 (6'-OCH₃); (+)-HRESIMS *m/z* 375.0338 [M + Na]⁺ (calcd for C₁₅H₁₂NaO₁₀, 375.0328).

Supplementary material

1D and 2D NMR and (+)-HRESIMS spectra for the new compound **1**.

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