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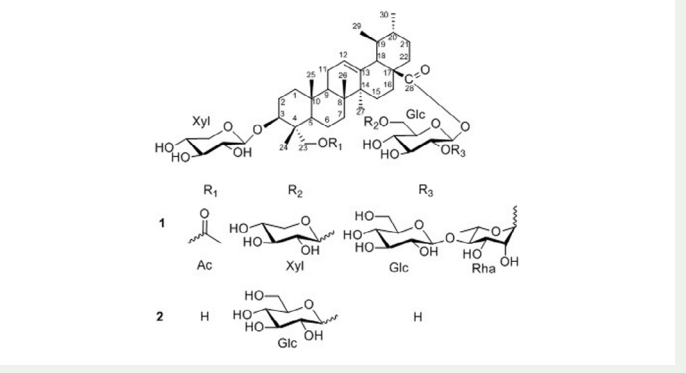
Ursane-type triterpenoid saponins from *Elsholtzia bodinieri*

Lunli Xiang[#], Lu Zhang[#], Xuanqin Chen, Xueshan Xia, Rongtao Li and Jindong Zhong

Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, P. R. China

ABSTRACT

Investigation of the n-BuOH extract of the aerial parts of *Elsholtzia bodinieri* led to the isolation of two new ursane-type triterpenoid saponins, bodiniosides O (**1**) and P (**2**), along with five known saponins, rotungenoside (**3**), 3,28-*O*-bis-*β*-D-glucopyranosides of 19*α*-hydroxyarjunolic acid (**4**), oblonganosides I (**5**), rotungenic acid 28-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**6**), and bodinioside M (**7**) isolated from the species. The structures of compounds **1** and **2** were characterized by spectroscopic data as well as acid hydrolysis and GC analysis as 3-*O*-*β*-D-xylopyranosyl-23-acetoxy-urs-12(13)-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside and 3-*O*-*β*-D-xylopyranosyl-23-hydroxy-urs-12(13)-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside. Compounds **1** and **2** exhibited potent anti-HCV activities *in vitro* with a selective index of 30.63 and 9.08, respectively.



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1. Introduction

Elsholtzia bodinieri Van't (Labiatae), an annual herbaceous plant, was widely distributed in the northwest and southwest districts of China, especially in Yunnan and Guizhou Provinces. The aerial part of the species was usually used as herbal tea or traditional folk medicine for the

CONTACT Jindong Zhong jindongzhongkm@163.com
[#]These authors contributed equally to this work.

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prophylaxis and treatment of cough, headache, pharyngitis, fever and hepatitis (College Jiangsu New Medical 1985). The chemical compositions of *E. Bodinieri* are previously reported to mainly contain triterpenoid saponins (Zhu et al. 2002; Li et al. 2005; Hu et al. 2007a), sesquiterpene glycosides (Hu et al. 2007b), clerodane diterpenoid glycosides (Hu et al. 2008), and phenolic constituents (Hu et al. 2007c). Some of the compounds from the species were revealed to possess antiviral and antibacterial activity (Guo et al. 2012). In a continuation of our study on the medicinal plants of *Elsholtzia* family (Li et al. 2005, 2008; Zhao et al. 2015; Zhong et al. 2016a, 2016b, 2016c), we have investigated the n-BuOH soluble fraction of *E. bodinieri* crude acetonic extract. In this paper, we describe the isolation, biological activity to anti-HCV, and structure elucidation of two new ursane-type triterpenoid saponins designated bodinioside O (**1**) and bodinioside P (**2**), along with five known triterpenoid saponins: rotungenoside (**3**), 3,28-*O*-bis-*β*-D-glucopyranosides of 19*α*-hydroxyarjunolic acid (**4**), oblonganosides I (**5**), rotungenic acid 28-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**6**) and bodinioside M (**7**) from the aerial parts of this plant.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{60}H_{96}O_{27}$ according to the positive HR-ESI-TOF-MS, which exhibited a pseudomolecular ion peak $[M - H]^-$ at *m/z* 1247.6067 (calcd for $C_{60}H_{95}O_{27}$ 1247.6061), indicating thirteen degrees of unsaturation. It exhibited UV maximum absorption at 202.8 nm. Its IR spectrum revealed absorption typical of hydroyl (3441 cm^{-1}) and carbonyl (1721 cm^{-1}).

The ^1H NMR spectrum showed four tertiary methyl and two secondary methyl signals at δ_H 0.84 (3H, s), 0.95 (3H, s), 1.02 (3H, s), 1.17 (3H, s), 0.97 (3H, d, $J = 6.1$ Hz) and 1.19 (3H, d, $J = 8.9$ Hz), correlating with carbons at δ_C 13.0 (C-24), 17.8 (C-26), 16.3 (C-25), 23.4 (C-27), 21.4 (C-30) and 17.1 (C-29) in the HSQC spectrum, respectively. The signal at δ_H 5.45 (1H, br. s), corresponding to the carbon at δ_C 126.2 (C-12) which coupled with δ_C 138.1 (C-13) in the ^{13}C NMR spectrum, indicated the existence of a double bond. On the basis of the above spectroscopic data, compound **1** was suggested to possess an urs-12-ene skeleton. Comparison of its ^1H and ^{13}C NMR spectroscopic data with those of cynarasaponins D (Shimizu et al. 1988), suggested that they had the same aglycone, 3,23-dihydroxyurs-12-en-28-oic acid, but differ in the sugar moiety and an acetoxy group due to signals of δ_C 170.8 and 20.7 and δ_H 2.12 (3H, s).

The ^1H NMR spectrum also displayed five anomeric signals at 6.49 (1H, br. s), 6.03 (1H, d, $J = 8.2$ Hz), 5.13 (1H, d, $J = 7.6$ Hz), 4.87 (1H, d, $J = 7.5$ Hz), and 4.84 (1H, d, $J = 7.5$ Hz), which correlated with anomeric carbon signals at δ_C 100.8 (d), 94.4 (d), 107.0 (d), 107.2 (d) and 105.6 (d) in the HSQC spectrum, respectively, suggesting the presence of five sugar moieties. Acid hydrolysis of **1** with 1 M HCl produced L-rhamnose, D-glucose and D-xylose units as sugar residues as determined by GC analysis of their corresponding trimethylsilylated L-cysteine derivatives. Since NMR signals of five sugar units have undesirable overlapped effects, the HMQC-TOCSY experiment was successfully used to distinguish and assign the ^1H and ^{13}C NMR signals of each sugar moiety. The correlations from the anomeric proton signal at δ_H 4.87 to five carbon signals at δ_C 107.2 (anomeric carbon), 75.3, 78.2, 71.0, and 67.0, as well as from five proton signals at δ_H 4.87, 4.00, 3.79, 4.23, and 4.37 to the anomeric carbon, suggested the presence of D-xylopyranose (Xyl). In a similar way, the ^1H and ^{13}C NMR signals for D-glucopyranosyl (Glc) and L-rhamnopyranosyl (Rha) were assigned. The *β* anomeric

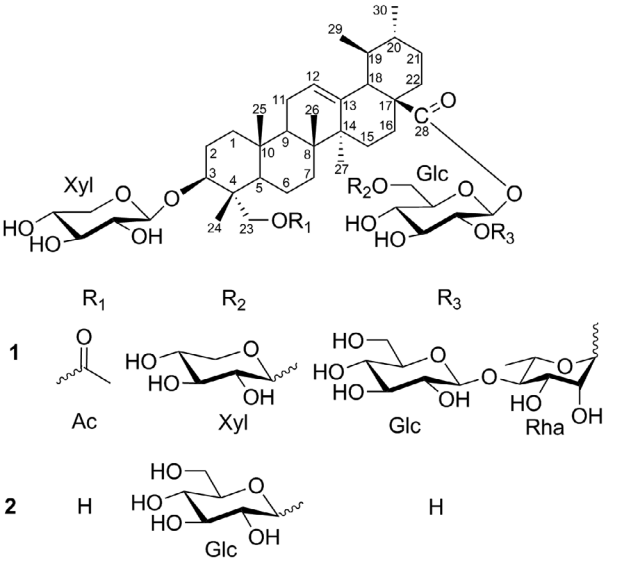

Figure 1. The structure of compounds **1** and **2**.

configurations for the xylopyranosyl and glucopyranosyl were determined by means of their $^{3}J_{H1,H2}$ coupling constants (^{3}J = 7.5, 8.2 and 7.6 Hz, respectively). Comparison of the ^{13}C signals and observation of a broad singlet for H-1 deduced the α anomeric configuration for L-rhamnopyranosyl (Li et al. 2012b).

The sequence of the glycoside chains connected to C-3 and C-28 was established by analysis of the HMBC correlations. The absence of any glycosidation shift for Xyl suggested that Xyl was a terminal sugar unit attached at C-3 of the aglycone, which was further confirmed by HMBC correlation of H_{Xyl}-1 (δ_H 4.87) of C-3 (Figure 1). A series of HMBC correlations from H_{Glc}-1 (δ_H 6.03) to C-28, from H_{Rha}-1 (δ_H 6.49) to C_{Glc}-2 (δ_c 74.7), from $H_{Xyl'}$-1 (δ_H 4.84) to C_{Glc}-6 (δ_c 68.8), and from H_{Glc}'-1 (δ_H 5.13) to C_{Rha}-4 (δ_c 85.7), unambiguously clarified the sequence of the second osidic part at C-28. In addition, HMBC correlation between H-23 (δ_H 4.55, 4.45) and the carbonyl carbon (δ_C 170.8) suggested that the acetoxy group was attached at C-23.

Based on the above evidence, the structure of compound **1** was established as 3-*O*-*β*-D-xylopyranosyl-23-acetoxy-urs-12(13)-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside, a new ursane-type saponin, and named as bodinioside O.

Bodinioside P (**2**), a white amorphous powder, was assigned the molecular formula of $C_{47}H_{76}O_{18}$, deduced from the pseudomolecular ions $[M - H]^-$ and $[M + COOH]^-$ at *m/z* 927.5 and 973.5, respectively, in the negative ESI-MS, and further confirmed by the positive HR-ESI-MS (*m/z* 973.5002, $[M + COOH]^+$), requiring nine degrees of unsaturation. A comprehensive analysis of ^{1}H and ^{13}C NMR spectral data suggested that **2** was also a saponin, with urs-12-ene as the aglycone. Detailed comparison of the ^{1}H and ^{13}C NMR spectral data of **2** with those of **1** indicated they are highly structurally similar, except for the signal absence of acetyl group in **1** and the difference of monosaccharides attached to C-28. Acid hydrolysis of **2** also yielded two D-glucose and a D-xylose as sugar residues as determined by GC analysis.

Table 1 Anti-HCV activities of compounds **1** and **2** from *E. bodinieri*.

	CC_{50} (nM)	EC_{50} (nM)	SI (CC_{50}/EC_{50})
IFNα-1b	n.d.	1.08[a]	–
Ribavirin	95.94	9.57	10.03
1	12.56	0.41	30.63
2	14.35	1.58	9.08

[a]The unit of IFN is IU/mL.

In the ^{1}H NMR spectrum of **2**, three anomeric protons at δ_H 5.03 (d, J = 6.2 Hz), 6.15 (d, J = 5.8 Hz) and 4.99 (d, J = 5.8 Hz) also indicated the presence of three sugar residues: a xylopyranosyl (Xyl) and two glucopyranosyl (Glc) units. The Xyl unit was still deduced to link to C-3 of the aglycone based on the HMBC correlation between δ_H 5.03 (H-1) of Xyl and δ_C 82.0 (C-3). Whereas, HMBC correlations from δ_H 6.15 (H-1) of Glc to δ_C 176.4 (C-28) of the aglycone and from δ_H 4.99 (H-1) of Glc′ to δ_C 69.2 (C-6) of Glc, enabled the sugar chain at *C*-28 to be assigned as *β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranosyl. Thus, the structure of **2** was adequately illustrated as 3-*O*-*β*-D-xylopyranosyl-23-hydroxy -urs-12(13)-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside, and named bodinioside P.

The five known ursane-type triterpenoid saponins were determined as *β*-D-glucopyranosyl 3*β*,19*α*,23-trihydroxyurs-12-en-28-oate (rotungenoside, **3**) (Nakatani et al. 1989), 3,28-*O*-bis-*β*-D-glucopyranosides of 19α-hydroxyarjunolic acid (**4**) (Higuchi et al. 1982), 3-*O*-*β*-D-xylopyranosyl-3*β*,19*α*,23-trihydroxyurs-12(13)-en-28-oic acid-28-*O*-*β*-D-glucopyranosyl ester (oblonganosides I, **5**) (Wu et al. 2007), rotungenic acid 28-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**6**) (Taketa and Schenkel 1994) and 3-*O*-*β*-D-xylopyranosyl-19α-hydroxy-23-acetoxy-urs-12(13)- en-28-oic acid 28-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (bodinioside M, **7**) (Zhong et al. 2016b) by comparison of the spectroscopic data with those reported, respectively.

The anti-HCV activities of compounds **1** and **2** were evaluated, and their cytotoxicity was measured in parallel with the determination of antiviral activity, using ribavirin as positive control (CC_{50} = 95.94 nM, EC_{50} = 9.57 nM). Among the tested compounds, compound **1** exhibited potent anti-HCV activity, with an EC_{50} value of 0.41 nM, a CC_{50} value of 12.56 nM, and a SI value of 30.63. Moreover, compound **2** showed moderate anti-HCV activity, with an EC_{50} value of 1.58 nM, a CC_{50} value of 14.35 nM, and a SI value of 9.08 (Table 1). The isolated compounds (**1–7**) were also evaluated for anti-HIV-1 effects and anti-influenza A virus activities, but they were found to be inactive.

3. Experimental

3.1. General experimental procedures

Optical rotations were obtained using a Jasco DIP-370 digital polarimeter. UV spectra were run on a UV-210A spectrophotometer. IR spectra were recorded on a Bio-Rad FtS-135 spectrophotometer in a KBr disk. NMR spectra were recorded using Bruker AM-400 and DRX-600 instruments with tetramethylsilane (TMS) as an internal standard. EIMS were measured on a VG Auto-Spec-3000 spectrometer. ESIMS and HRESIMS were obtained with an API-Qstar-TOF instrument. GC analysis was run on Agilent Technologies HP5890 gas chromatograph with flame ionization detector. Semi-preparative HPLC was performed on an Agilent 1200 liquid chromatograph with a ZORBAX SB-C18 (5, 9.4 × 250 mm) column. Column

chromatography (CC) was carried out on silica gel (200–300 mesh, 100–200 mesh, 80–100 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Diaion HP-20SS (63–150 mm Mitsubishi Fine Chemical Industries Co., Ltd., Tokyo, Japan), ODS-C_{18} (75 μm, YMC Co., Ltd., Japan) and Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden). Fractions were monitored by TLC plates (Si gel G, Qingdao Marine Chemical Factory, Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4–EtOH.

3.2. Materials

The aerial parts of *E. bodinieri* Van't were collected from Yuxi, Yunnan Province, P. R. China, in May 2016, and identified by Dr Xuanqing Chen. A voucher specimen (KMUST 20160005) was deposited at the Laboratory of Phytochemistry, Faculty of Life Science and Technology, Kunming University of Science and Technology. Ribavirin and IFNα-1b were purchased from Sigma-Aldrich, St. Louis, MO, USA. Human hepatoma Huh7.5.1 cells were a gift from Prof. Ping Zhao (Second Military Medical University). The purity (>95%) of compounds **1** and **2** used for biological assay was determined by HPLC.

3.3. Extraction and isolation

The dried aerial parts of *E. bodinieri* (15 kg) were powdered and extracted with 75% aq. Me_2CO (3 × 35 L, 24 h, each) at room temperature, then concentrated in vacuo to yield an extract, which was suspended in H_2O, and successively partitioned with $CHCl_3$, EtOAc, and *n*-BuOH.

The *n*-BuOH extract (300.0 g) was separated over macroporous resin CC (Diaion HP-20SS) eluting with MeOH/H_2O (gradient 10, 30, 60, 90, and 100%, each 2.5 L) to afford five fractions (*Fr.* A~E). *Fr.* B (50 g) was subjected to Sephadex LH-20 gel CC, eluting with MeOH/H2O (gradient 10, 30, 50, 70, 90, and 100%, each 1 L) to obtain subfractions B-1 ~ B-4. *Fr*. B-2 (7.9 g) was chromatographed successively over silica gel CC (chloroform/MeOH/H_2O, gradient 20:1:0.1→5:5:0.5) to obtain subfractions B-2–1 ~ B-2–4. *Fr*. B-2–2 (1.2 g) was separated by ODS (MeOH/H_2O, gradient 10→100%) to obtain subfractions B-2–2-1 ~ B-2–2-4. *Fr*. B-2–2-2 (166.0 mg) was purified over semi-preparative HPLC (26% CH_3CN, 3 mL/min) to give **6** (t_R = 17.5 min, 7.7 mg), **4** (t_R = 20.3 min, 15.6 mg), and **3** (t_R = 22.0 min, 13.7 mg). *Fr*. B-2–2-3 (125.0 mg) was further purified on semi-preparative HPLC (25% CH_3CN, 3 mL/min) to obtain compounds **2** (t_R = 22.6 min, 6.9 mg) and **5** (t_R = 24.3 min, 18.3 mg). **1** (t_R = 20.8 min, 5.6 mg) and **7** (t_R = 22.7 min, 13.6 mg) were prepared from *Fr*. B-2–2-4 (185.0 mg) on semi-preparative HPLC (23% CH_3CN, 3 mL/min).

Bodinioside O (**1**): white amorphous powder. $[\alpha]_D^{26.6} = -9.33$ (c = 0.10, MeOH). IR (KBr): 3441, 2931, 1721, 1632, 1386, 1255, 1074 cm^{-1}; UV λ_{max} (MeOH) nm (logε): 206.8 (2.16); ESI-MS (*pos.*) *m/z*: 1271 $[M + Na]^+$); HR-ESI-MS (*pos.*) *m/z*: 1247.6067 $[M - H]^-$ (Calcd for $C_{60}H_{95}O_{27}$, 1247.6061). ^{1}H NMR (600 MHz, pyridine-d_5): δ 1.50 (1H, m, H-1a), 0.87 (1H, m, H-1b), 2.06 (1H, m, H-2a), 1.29 (1H, m, H-2b), 3.93 (1H, m, H-3), 1.23 (1H, m, H-5), 1.56 (1H, m, H-6a), 1.39 (1H, m, H-6b), 1.58 (2H, m, H-7), 1.60 (1H, m, H-9), 2.00 (2H, m, H-11), 5.45 (1H, br. s, H-12), 1.50 (2H, m, H-15), 2.08 (1H, m, H-16a), 1.89 (1H, m, H-16b), 2.56 (1H, d, J = 10.9 Hz, H-18), 1.30 (1H, m, H-19), 1.42 (1H, m, H-20), 2.16 (2H, m, H-21), 2.08 (1H, m, H-22a), 1.78 (1H, m, H-22b), 4.55 (1H, m, H-23a), 4.45 (1H, m, H-23b), 0.84 (3H, s, H-24), 1.02 (3H, s, H-25), 0.95 (3H, s, H-26), 1.17 (3H, s, H-27), 1.19 (3H, d, J = 8.9 Hz, H-29), 0.97 (3H, d, J = 6.1 Hz, H-30), 23-OAC: 2.12 (3H,

s); 3-*O*-sugar: Xyl 4.87 (1H, d, *J* = 7.5 Hz, H-1), 4.00 (1H, m, H-2), 3.79 (1H, m, H-3), 4.23 (1H, m, H-4), 4.37 (1H, m, H-5a), 3.75 (1H, m, H-5b); 28-*O*-sugar: Glc 6.03 (1H, d, *J* = 8.2 Hz, H-1), 4.30 (1H, m, H-2), 4.19 (1H, m, H-3), 4.29 (1H, m, H-4), 3.96 (1H, m, H-5), 4.60 (1H, m, H-6a), 4.23 (1H, m, H-6b), Rha 6.49 (1H, br. s, H-1), 4.72 (1H, m, H-2), 4.61 (1H, m, H-3), 4.38 (1H, m, H-4), 4.59 (1H, m, H-5′), 1.87 (3H, d, *J* = 6.2 Hz, H-6), Xyl′ 4.84 (1H, d, *J* = 7.5 Hz, H-1), 3.98 (1H, m, H-2), 3.76(1H, m, H-3), 4.20 (1H, m, H-4), 4.28 (1H, m, H-5a), 3.62(1H, m, H-5b), Glc′ 5.13 (1H, d, *J* = 7.6 Hz, H-1), 4.02 (1H, m, H-2), 4.12 (1H, m, H-3), 4.13 (1H, m, H-4), 4.14 (1H, m, H-5), 4.47 (1H, m, H-6a), 4.30 (1H, m, H-6b). ^{13}C NMR (150 MHz, pyridine-d_5): 38.5 (C-1), 26.0 (C-2), 82.0 (C-3), 42.3 (C-4), 48.3 (C-5), 18.3 (C-6), 33.3 (C-7), 40.1 (C-8), 48.5 (C-9), 36.6 (C-10), 23.5 (C-11), 126.2 (C-12), 138.1 (C-13), 42.3 (C-14), 28.3 (C-15), 24.6 (C-16), 48.1 (C-17), 53.0 (C-18), 38.1 (C-19), 39.3 (C-20), 30.7 (C-21), 36.6 (C-22), 64.6 (C-23), 13.0 (C-24), 16.3 (C-25), 17.8 (C-26), 23.4 (C-27), 176.5 (C-28), 17.1 (C-29), 21.4 (C-30), 23-OAC 170.8 (–C=O), 20.7 (–CH3); 3-*O*-sugar: Xyl 107.2 (C-1), 75.3 (C-2), 78.2 (C-3), 71.0 (C-4), 67.0 (C-5); 28-*O*-sugar: Glc 94.4 (C-1), 74.7 (C-2), 79.6 (C-3), 70.6 (C-4), 77.2 (C-5), 68.8 (C-6), Rha 100.8 (C-1), 71.5 (C-2), 72.4 (C-3), 85.7 (C-4), 68.0 (C-5), 18.8 (C-6), Xyl′ 105.6 (C-1), 74.6 (C-2) 78.0 (C-3), 70.9 (C-4), 67.0 (C-5), Glc′ 107.0 (C-1), 76.2 (C-2), 78.5 (C-3), 71.8 (C-4), 78.4 (C-5), 63.0 (C-6).

Bodinioside P (**2**): white amorphous powder. $[\alpha]_D^{24.9} = -4.29$ (*c* = 0.25, MeOH). ESI-MS (*neg.*) *m/z*: 927.5 $[M-H]^-$, 973.5 $[M + COOH]^-$; HR-ESI-MS (*pos.*) *m/z*: 973.5002 $[M + COOH]^+$ (Calcd for $C_{47}H_{76}O_{18}$, 928.5032). ^{1}H NMR (400 MHz, pyridine-d_5): δ 1.57 (1H, m, H-1a), 0.78 (1H, m, H-1b), 2.22 (2H, m, H-2), 4.24 (1H, m, H-3), 1.23 (1H, m, H-5), 1.63 (1H, m, H-6a), 1.31 (1H, m, H-6b), 1.63 (1H, m, H-7a), 1.31 (1H, m, H-7b), 1.65 (1H, m, H-9), 1.91 (2H, m, H-11), 5.42 (1H, br. s, H-12), 2.37 (1H, m, H-15a), 1.04 (1H, m, H-15b), 2.00 (1H, m, H-16a), 1.89 (1H, m, H-16b), 2.47 (1H, d, *J* = 11.2 Hz, H-18), 1.30 (1H, m, H-19), 1.02 (1H, m, H-20), 1.30 (1H, m, H-21a), 1.18 (1H, m, H-21b), 1.90 (1H, m, H-22a), 1.74 (1H, m, H-22b), 4.27 (1H, m, H-23a), 3.67 (1H, m, H-23b), 0.92 (3H, s, H-24), 0.97 (3H, s, H-25), 1.14 (3H, s, H-26), 1.11 (3H, s, H-27), 0.88 (3H, d, *J* = 5.9 Hz, H-29), 0.82 (3H, d, *J* = 6.1 Hz, H-30); 3-*O*-sugar: Xyl 5.03 (1H, d, *J* = 6.2 Hz, H-1), 3.98 (1H, m, H-2), 3.85 (1H, m, H-3), 4.19 (1H, m, H-4), 4.25 (1H, m, H-5a), 3.61 (1H, m, H-5b); 28-*O*-sugar: Glc 6.15.(1H, d, *J* = 5.8 Hz, H-1), 3.99(1H, m, H-2), 4.18 (1H, m, H-3), 4.17 (1H, m, H-4), 4.06 (1H, m, H-5), 4.67 (1H, m, H-6a), 4.27 (1H, m, H-6b), Glc′ 4.99 (1H, d, *J* = 5.8 Hz, H-1), 4.06 (1H, m, H-2), 4.16 (1H, m, H-3), 4.15 (1H, m, H-4), 4.05 (1H, m, H-5), 4.45 (1H, m, H-6a), 4.30 (1H, m, H-6b). ^{13}C NMR (100 MHz, pyridine-d_5): 38.7 (C-1), 26.0 (C-2), 82.0 (C-3), 42.3 (C-4), 47.2 (C-5), 17.9 (C-6), 32.9 (C-7), 39.9 (C-8), 47.9 (C-9), 36.6 (C-10), 23.5 (C-11), 126.2 (C-12), 138.6 (C-13), 43.2 (C-14), 28.5 (C-15), 24.3 (C-16), 48.2 (C-17), 53.0 (C-18), 38.8 (C-19), 39.1 (C-20), 30.5 (C-21), 36.5 (C-22), 64.1 (C-23), 13.5 (C-24), 16.2 (C-25), 17.5 (C-26), 23.5 (C-27), 176.4 (C-28), 27.2 (C-29), 21.1 (C-30); 3-*O*-sugar: Xyl 106.9 (C-1), 75.4 (C-2), 78.1 (C-3), 70.9 (C-4), 66.9 (C-5); 28-*O*-sugar: Glc 95.7 (C-1), 74.9 (C-2), 78.4 (C-3), 70.6 (C-4), 77.6 (C-5), 69.2 (C-6), Glc′ 105.4 (C-1), 73.5 (C-2), 78.3 (C-3), 71.2 (C-4), 78.2 (C-5), 62.3 (C-6).

3.4. Acid hydrolysis for sugar analysis

A solution of **1** and **2** (1.0 mg for each compound) in 1 M HCl (0.4 mL) was heated at 90–100 °C in a screw-capped vial for 5 h. The mixture was neutralized by addition of Amberlite IRA400 (OH^- form) and then filtered. The filtrate was dried in vacuo, dissolved in 0.2 mL of pyridine containing L-cysteine methyl ester (10 mg/mL) and reacted at 60 °C for 1 h. A solution (0.2 mL) of trimethylsilylimidazole in pyridine (10 mg/mL) was added to this mixture, and it was heated at 60 °C for 1 h. The final mixture was directly analyzed by GC [30QC2/AC-5 quartz

capillary column (30 m × 0.32 mm) with the following conditions: column temperature: 180/280 °C; programmed increase 3 °C/min; carrier gas: N_2 (1 mL/min); injection and detector temperature: 250 °C; injection volume: 4 μL; split ratio: 1/50]. The authentic samples D- and L-glucose, D- and L-xylose, and L-rhamnose were treated in the same manner. Under these conditions, the retention times of authentic samples D- and L-glucose, D- and L-xylose, and L-rhamnose were 18.29, 18.87, 13.35, 14.01, and 14.97 min, respectively. During our studies, D-glucose, D-xylose and L-rhamnose were detected in **1**. D-glucose and D-xylose were detected in **2**.

3.5. Anti-HCV activity

Inhibitor preparation. For the inhibitory activity assays, compounds **1** and **2** were dissolved and then serially diluted with DMSO, using DMSO as blank, ribavirin and IFNα-1b as positive controls.

Cell line and cell culture. Stocks of infectious hepatitis C virus (HCV) J6/JHH-1 viral particles were generated as previously described (Li et al. 2012a) and aliquoted for storage at −80 °C. The HCV load (RNA copies) in stocks was measured with real-time quantitative RT-PCR.

Anti-HCV assay *in vitro*. Anti-HCV assay was carried out in clear-bottomed 96-well plates. Huh7.5.1 cells were seeded at a density of 1.5×10^4 cells/well in 100 μL of DMEM culture medium and incubated overnight, and virus stocks were added into the wells and incubated for 8 h. Before the samples were added, the supernatants were removed and cells were washed with completed medium five times. The samples or controls were serially diluted in DMEM and then added to the appropriate wells. After incubation for 3 days, the supernatants were collected to determine their viral load with real-time quantitative RT-PCR.

Cytotoxicity assay. The toxicities of the compounds were assayed by a modified MTT method (Li et al. 2012a). In brief, the test samples were prepared at different concentrations. After Huh7.5.1 cells had been seeded in a 96-well microplate for 4 h, the samples (20 μL) were placed in each well and incubated for 3 days at 37 °C; then, 0.1 mL MTT was added for 4 h. After removal of the MTT medium, DMSO (100 μL/well) was added onto the microplate for 10 min. The formazan crystals were dissolved, and the absorbance was measured on a microplate reader at 490 nm.

4. Conclusion

Our present work on the plants of *E. bodinieri* yielded two new ursane-type triterpenoid saponins, along with five known saponins. Two new compounds **1** and **2** exhibited potent anti-HCV activities *in vitro* with a selective index of 30.63 and 9.08, respectively. This investigation should provide valuable information for further understanding of the *E. bodinieri*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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