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Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

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To cite this article: Hong-Biao Chu, Nan-Nan Li, Zong-Ping Zhang, Xiao-Yue Hu, Cai-Yun Yu & Lei Hua (2019): Steroidal glycosides from the underground parts of *Hosta ventricosa* and their anti-inflammatory activities in mice, Natural Product Research, DOI: 10.1080/14786419.2019.1638379

To link to this article: https://doi.org/10.1080/14786419.2019.1638379

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Steroidal glycosides from the underground parts of *Hosta ventricosa* and their anti-inflammatory activities in mice

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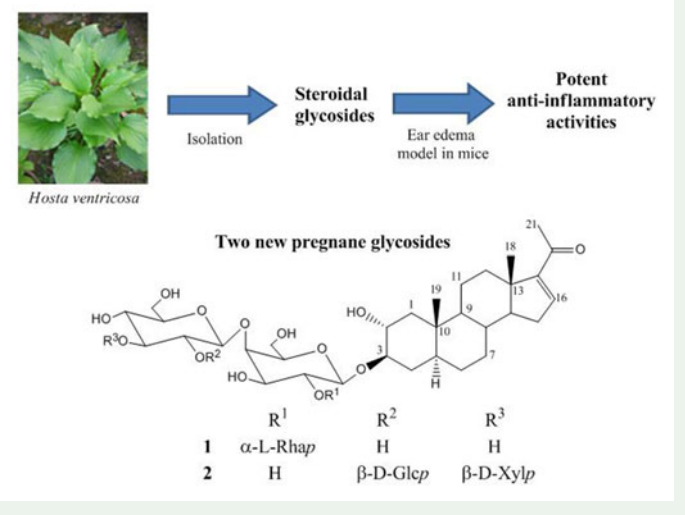
ABSTRACT

Two new pregnane glycosides, 2α, 3β-dihydroxy-5α-pregn-16-en-20-one-3-O-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside} (**1**) and 2α, 3β-dihydroxy-5α-pregn-16-en-20-one-3-O-{β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}(**2**), have been isolated along with two known spirostanol saponins from the underground parts of *Hosta ventricosa*. Their structures were elucidated on the basis of chemical and spectral evidence. The anti-inflammatory activities of these steroidal glycosides were evaluated using a xylene-induced ear edema model. Our results indicated that the compounds exhibited promising anti-inflammatory activities.



ARTICLE HISTORY
Received 31 March 2019
Accepted 25 June 2019

KEYWORDS
Hosta ventricosa; steroidal glycosides; pregnane glycosides; anti-inflammatory

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2019.1638379.

1. Introduction

The genus *Hosta* belongs to the family Liliaceae, with approximately 40 species distributed in the temperate and subtropical zones of Asia (Liu et al. 2011). Previous phytochemical studies on *Hosta* have afforded structurally-diverse and biologically-active compounds, such as steroids, alkaloids, flavonoids, and monoterpenes, with some of them showing potent anti-inflammatory, cytotoxic, antibacterial, antiviral, and antioxidant activities (Budzianowski 1990; Mimaki et al. 1996, 1997, 1998; Wang et al. 2007a, 2007b; Yada et al. 2010; Wang et al. 2017; Yang et al. 2017; He et al. 2018). The ethnopharmacological significance of the genus *Hosta* led us to investigate the chemical constituents of one of its species, *Hosta ventricosa*. This plant is widely cultivated in China and Western countries not only for its ornamental value but also for its use as a medicinal herb and economic value as a food source (Schmid 1991). This paper describes the isolation and structural elucidation of two new pregnane compounds along with two known steroidal compounds isolated from the underground parts of *H. ventricosa*. In addition, the anti-inflammatory activities of these compounds were determined using a xylene-induced ear edema model.

2. Results and discussion

A 95% ethanol extract obtained from the underground parts of *H. ventricosa* was concentrated with a rotary evaporator, suspended in water, and then successively partitioned with petroleum ether and *n*-butanol. The *n*-butanol extract was subjected to column chromatography using silica-gel, Sephadex LH-20, MCI gel, and ODS-C_{18} on a reversed-phase HPLC system, which afforded four compounds including two new pregnane glycosides **1** and **2** (Figure 1). The known compounds were identified as (25 R)-2α, 3β-dihydroxy-5α-spirost-9-en-12-one-3-O-{β-D-glucopyranosyl(1→2)-[β-D-xylopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside} (**3**) and (25 R)-3β-hydroxy-5α-spirost-9-en-12-one-3-O-{β-D-glucopyranosyl(1→2)-[β-D-xylopyranosyl (1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside} (**4**) by comparing their MS and NMR data with those reported in the literature (Mimaki et al. 1995).

Compound **1** was obtained as a white amorphous powder. The ESI-MS spectrum gave a quasi-molecular ion peak at *m/z* 825.4348 $[M + Na]^+$ and HR-TOF-MS suggested a molecular formula of $C_{39}H_{62}O_{17}$ (*m/z* 801.3914, calcd. 801.3909, $[M–H]^-$). The IR spectrum (KBr) showed characteristic absorption bands at 3418 and 1661 cm^{-1} corresponding to hydroxyl and α, β-unsaturated carbonyl groups, respectively. The ^{1}H-NMR spectrum (Table S1) of **1** revealed the presence of three methyl groups at δ_H 0.89 (3H, s, CH_3-18), 0.87 (3H, s, CH_3-19), and 2.21 (3H, s, CH_3-21), two oxymethines at δ_H 4.05 (1H, overlapped, H-2) and 3.82 (1H, m, H-3), one olefin at δ_H 6.57 (1H, dd, J = 3.1, 1.7 Hz, H-16), and three anomeric proton doublets at δ_H 4.92 (1H, d, J = 7.6 Hz, Gal, H-1), 5.15 (1H, d, J = 7.8 Hz, Glc, H-1″), and 6.22 (1H, d, J = 1.2 Hz, Rha, H-1‴). This information was supported by the ^{13}C-NMR data obtained for compound **1**. The ^{13}C-NMR spectrum of **1** (Table S2) showed signals for three methyl groups (δ_C 13.2, 15.9, and 26.9), two carbons bearing a hydroxyl group (δ_C 70.3 and 84.9), one olefin carbon (δ_C 144.5), and three anomeric carbons (δ_C 100.8, 107.0, and 102.1). Acid hydrolysis of **1** using 2 M hydrochloric acid gave aglycone **1a** ($C_{21}H_{32}O_3$), D-galactose, D-glucose, and

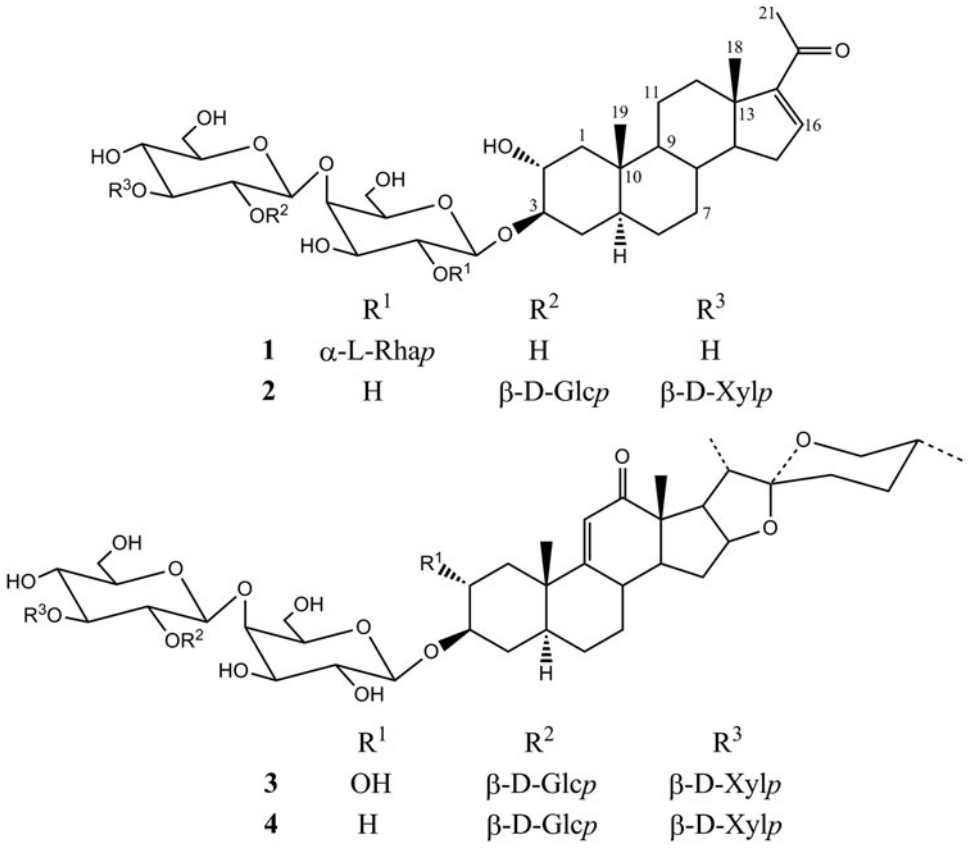

Figure 1. Structures of compounds **1–4**.

L-rhamnose in a 1:1:1 ratio as the carbohydrate compounds. The absolute configurations of the sugar units were assigned using HPLC analysis of the chiral derivatives of the sugars in the acid hydrolysate. An α-anomeric configuration for the rhamnose unit was concluded from the multiplicity of the anomeric ^{1}H NMR signal (d, J = 1.2 Hz) of rha and the chemical shift of rha C-5‴ (δ_C 69.2). The β-anomeric configuration for both galactose and glucose was assigned from their coupling constants ($J_{1,2}$ >7.0 Hz) (Agrawal et al. 1985; Agrawal 1992). In the HMBC spectrum (Figure S7), cross-peaks due to long-range correlations between gal H-1′ (δ_H 4.92) of galactose and C-3 (δ_C 84.9) of the aglycone, rha H-1‴ (δ_H 6.22) of rhamnose and C-2′ (δ_C 76.6) of galactose, glc H-1″ (δ_H 5.15) of glucose and C-4′ (δ_C 81.1) of galactose indicated that a trisaccharide moiety, -3-O-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside was linked to the aglycone at C-3. In addition, we concluded that the trisaccharide was linked to the C-3 hydroxyl position of the aglycone because in the ^{13}C NMR spectrum of **1**, the signal due to C-3 was shifted down-field by 8.5 ppm, whereas the signals due to C-2 and C-4 were shifted up-field by 2.5 and 1.8 ppm, respectively, when compared to those of **1a**.

Upon comparison of the ^{13}C NMR data with those of steroidal saponins (Mimaki et al. 1997; Yada et al. 2010), the ^{13}C signals due to parts A and B, including C-19,

were superimposable on those of steroidal saponins, indicating the presence of a 2α, 3β - hydroxyl group bearing a triglycoside at C-3. The 16-en-20-one structure of **1** was in good agreement between the NMR data obtained for the D-ring, C-18 methyl and acetylmethyl groups, and those previously reported for pregnane glycoside (Mimaki et al. 1997). This data implies that compound **1** was a triglycoside of a pregnane with a 16-en-20-one structure derived from a spirostanol via Marker's degradation (Gould et al. 1952). The relative configuration of compound **1** was determined from the NOESY spectrum (Figure S9). The correlation of δ 0.87 (H-19) to δ 4.05 (H-2) suggested the relative configuration at H-19 and H-2 to be β orientation, while, δ 3.82 (H-3) to δ 0.97 (H-5), suggested the relative configuration at H-3 and H-5 to be α orientation. On the basis of the above-mentioned evidence, compound **1** was identified to be 2α, 3β-dihydroxy-5α-pregn-16-en-20-one-3-O-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside}.

Compound **2** was obtained as a white amorphous powder. The ESI-MS spectrum gave a quasi-molecular ion peak at *m/z* 973.4245 $[M + Na]^+$ and HR-TOF-MS suggested a molecular formula of $C_{44}H_{70}O_{22}$ (*m/z* 949.4286, calcd. 949.4280, $[M–H]^-$). The IR spectrum (KBr) showed characteristic absorption bands at 3407 and 1649 cm^{-1} corresponding to hydroxyl and α, β-unsaturated carbonyl groups. The ^{13}C and ^{1}H NMR spectra (Table S1 and Table S2) of **2** were closely related to those of **1** except for the sugar moieties, indicating that **2** was also a pregnane-type glycoside with an identical aglycone to that found in compound **1**. The ^{13}C and ^{1}H NMR spectra showed the presence of galactose, two glucose, and xylose moieties in **2**, instead of galactose, glucose, and rhamnose found in **1**. This was confirmed by acid hydrolysis of **2** which gave **1a**, D-galactose, D-glucose, and L-xylose in a 1:2:1 ratio. The absolute configurations of the sugar units were assigned using HPLC analysis of the chiral derivatives of the sugars in the acid hydrolysate. The HMBC cross-peaks (Figure S19) due to the long-range correlations between gal H-1′ (δ_H 4.91) of galactose and C-3 (δ_C 84.0) of the aglycone, glc H-1″ (δ_H 5.58) of glucose and C-4′ (δ_C 79.5) of galactose, glc H-1‴ (δ_H 5.21) of glucose and C-2″ (δ_C 81.2) of glucose, and xyl H-1⁗ (δ_H 5.23) of xylose and C-3″ (δ_C 87.1) of glucose indicate that a tetrasaccharide moiety, -3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, was linked to the aglycone at C-3. The relative configuration of compound **2** was determined from the NOESY spectrum (Figure S21). The correlation of δ 0.69 (H-19) to δ 4.08 (H-2) suggested the relative configuration at H-19 and H-2 to be β orientation, while, δ 3.86 (H-3) to δ 1.02 (H-5), suggested the relative configuration at H-3 and H-5 to be α orientation. Consequently, **2** was determined to be 2α, 3β-dihydroxy-5α-pregn-16-en-20-one-3-O-{β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}.

The effects of the four compounds identified in the present study on edema formation in mice were investigated using a xylene-induced ear edema model. The application of xylene in mouse ears brought about a significant increase in ear weight in the xylene control group (Table S3). In contrast, the control group mice treated with aspirin (500 mg/kg) showed significant ($p < 0.01$) inhibition (60.38%) compared to that of the saline-control group. The animals in the groups treated with compound **1** (25 mg/kg), **2** (50 mg/kg), **3** (12.5 mg/kg), and **4** (25 mg/kg) showed significant

inhibition of ear edema of 66.97%, 70.74%, 60.66%, and 79.24%, respectively (for all, $p < 0.01$ versus the saline control group). The results suggest that compounds **1**–**4** have anti-edematogenic effects.

3. Experimental

3.1. General experimental procedures

Column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co., Ltd., Sweden), and MCI–gel CHP 20P (75–150 mm, Mitsubishi Chemical, Tokyo, Japan). 1 D NMR [^{1}H (600 MHz) and ^{13}C (150 MHz)] and 2 D NMR [^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY] spectroscopy was recorded on a Bruker Avance600 instrument (Bruker, Switzerland) at the Centre for Analysis Facilities at Nanchang University. Optical rotations were measured on a WZZ-2S polarimeter (Shanghai Physics and Optics Instrument Inc., China). IR spectroscopy was obtained on a Nicolet 5700 spectrophotometer using KBr pellets (Nicolet, USA). HR-TOF-MS was recorded on a Trip TOFTM 5600 spectrometer (Sciex, USA).

3.2. Plant material

Plant material was collected from Wugongshan, Jiangxi Province of China in October 2014 and identified by Prof. Zhaochang Liang (School of Medicine, Jinggangshan University, Ji'an, China). A voucher specimen (JU 0810021) was deposited in the herbarium of Jinggangshan University.

3.3. Animals

Kunming mice (18–22 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd, and fed a commercial diet with water ad libitum. The animals were housed in cages maintained on a natural 12 h of light and dark cycle. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee, School of Medicine, Jinggangshan University.

3.4. Extraction and isolation

Dried and powdered plant material (9 kg) obtained from the underground parts of *H. ventricosa* was extracted three times with 95% ethanol heated under reflux. The residue was suspended in water and partitioned with petroleum ether and *n*-butanol, respectively. The *n*-butanol portion (800 g) was separated into 6 fractions (Frs A–F) using a silica gel column eluted with EtOAc–MeOH (30:1 to 6:1). Fr. D (17.9 g) was subjected to MCI-gel column chromatography using 20, 40, 60, 80 and 100% MeOH as the eluent (each concentration gradient for 1.5 L). The 80% MeOH fraction (10.4 g) was further purified using a Sephadex LH-20 column chromatography to afford a crude mixture (6.2 g). Compounds **1** (645 mg, t_R 26.5 min), **2** (897 mg, t_R 32.5 min), **3**

(1690 mg, t_R 51.3 min), and **4** (1587 mg, t_R 60.0 min) were obtained from the crude mixture using semi-preparative HPLC (Zorbax ODS-C_{18}, MeOH–H_2O = 7:3).

3.4.1. 2α, 3β-dihydroxy-5α-pregn-16-en-20-one-3-O-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside} (1)

White amorphous powder. $[\alpha]_D^{20} = -29.5^\circ$ ($c = 0.1$, CH_3OH). IR (KBr): 3418, 2933, 1661, 1372, and 1073 cm^{-1}. HR-TOF-MS: *m/z* 801.3914 (calcd. 801.3909 for $C_{39}H_{61}O_{17}$, $[M–H]^-$). ^1H NMR (600 MHz, pyridine-d_5) δ: 6.57 (1H, dd, J = 3.1, 1.7, H-16), 6.22 (1H, d, J = 1.2, H-1‴ of Rha), 5.15 (1H, d, J = 7.8, H-1″ of Glc), 4.92 (1H, d, J = 7.6, H-1′ of Gal), 4.81 (1H, m, H-5‴), 4.76 (1H, m, H-2‴), 4.55 (1H, overlapped, H-3‴), 4.53 (1H, d, J = 6.0, H-6b), 4.52 (1H, overlapped, H-4′), 4.51 (1H, m, H-6″b), 4.50 (1H, m, H-2′), 4.26 (1H, m, H-4‴), 4.25 (1H, m, H-6b), 4.24 (1H, m, H-3′), 4.19 (1H, overlapped, H-3″), 4.17 (1H, overlapped, H-6″a), 4.07 (1H, overlapped, H-2″), 4.05 (1H, overlapped, H-2), 4.04 (1H, m, H-4″), 3.96 (1H, m, H-5′), 3.92 (1H, m, H-5″), 3.82 (1H, m, H-3), 2.51 (1H, m, H-12b), 2.21 (3H, s, H-21), 2.18 (1H, m, H-1b), 2.10 (1H, m, H-15b), 1.98 (1H, m, H-4b), 1.81 (1H, m, H-15a), 1.78 (1H, m, H-4a), 1.58 (3H, d, J = 6.0, H-6‴), 1.51 (1H, m, H-11b), 1.49 (1H, m, H-7b), 1.35 (1H, m, H-8), 1.29 (1H, m, H-11a), 1.28 (1H, m, H-12a), 1.24 (1H, m, H-6b), 1.24 (1H, m, H-14), 1.19 (1H, m, H-6a), 1.09 (1H, m, H-1a), 0.97 (1H, m, H-5), 0.89 (3H, s, H-18), 0.87 (3H, s, H-19), 0.83 (1H, m, H-7a), 0.63 (1H, m, H-9). ^{13}C NMR (150 MHz, pyridine-d_5) δ: 196.1 (C-20), 155.2 (C-17), 144.5 (C-16), 107.0 (C-1″ of Glc), 102.1 (C-1‴ of Rha), 100.8 (C-1′ of Gal), 84.9 (C-3), 81.1 (C-4′) , 78.7 (C-3″), 78.4 (C-5″), 76.6 (C-2′) , 76.2 (C-3′) , 75.4 (C-2″), 75.3 (C-5′), 73.9 (C-4‴), 72.6 (C-3‴), 72.2 (C-2‴), 71.9 (C-4″), 70.3 (C-2), 69.2 (C-5‴), 62.8 (C-6″), 60.7 (C-6′) , 56.1 (C-14), 54.7 (C-9), 46.3 (C-13), 45.3 (C-1), 44.7 (C-5), 36.7 (C-10), 35.0 (C-12), 33.2 (C-4), 33.0 (C-8), 32.0 (C-15), 31.7 (C-7), 27.9 (C-6), 26.9 (C-21), 21.2 (C-11), 18.3 (C-6‴), 15.9 (C-19), 13.2 (C-18).

3.4.2. 2α, 3β-dihydroxy-5α-pregn-16-en-20-one (1a)

White amorphous powder. ESI-MS: *m/z* 333 ($[M + H]^+$). ^1H NMR (600 MHz, pyridine-d_5) δ: 6.58 (1H, dd, J = 3.1, 1.7, H-16), 4.01 (1H, m, H-2), 3.82 (1H, m, H-3), 2.54 (1H, m, H-12b), 2.21 (3H, s, H-21), 2.18 (1H, m, H-1b), 2.10 (1H, m, H-15b), 1.88 (1H, m, H-4b), 1.85 (1H, m, H-15a), 1.83 (1H, m, H-4a), 1.67 (1H, m, H-11b), 1.57 (1H, m, H-7b), 1.54 (1H, m, H-8), 1.34 (1H, m, H-11a), 1.31 (1H, m, H-12a), 1.28 (1H, overlapped, H-14), 1.27 (1H, overlapped, H-6b), 1.22 (1H, m, H-6a), 1.19 (1H, m, H-1a), 1.19 (1H, m, H-5), 0.88 (3H, s, H-18), 0.87 (1H, m, H-7a), 0.83 (3H, s, H-19), 0.70 (1H, m, H-9). ^{13}C NMR (150 MHz, pyridine-d_5) δ: 196.1 (C-20), 155.2 (C-17), 144.5 (C-16), 76.4 (C-3), 72.8 (C-2), 56.1 (C-14), 54.9 (C-9), 46.3 (C-13), 46.0 (C-1), 45.2 (C-5), 37.4 (C-10), 37.0 (C-12), 35.0 (C-4), 33.0 (C-8), 32.0 (C-15), 31.8 (C-7), 28.1 (C-6), 26.9 (C-21), 21.3 (C-11), 16.0 (C-19), 13.4 (C-18).

3.4.3. 2α, 3β-dihydroxy-5α-pregn-16-en-20-one-3-O-{β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (2)

White amorphous powder. $[\alpha]_D^{20} = -39.0^\circ$ ($c = 0.1$, CH_3OH). IR (KBr): 3407, 2929, 1649, and 1069 cm^{-1}. HR-TOF-MS: *m/z* 949.4286 (calcd. 949.4280 for $C_{44}H_{69}O_{22}$, $[M–H]^-$). ^1H NMR (600 MHz, pyridine-d_5) δ: 6.62 (1H, dd, J = 3.1, 1.7, H-16), 5.58 (1H, d, J = 7.8, H-1″ of Glc), 5.23 (1H, m, H-1‴′ of Xyl), 5.21 (1H, m, H-1‴ of Glc), 4.91 (1H, d, J = 7.8, H-1′ of

Gal), 4.60 (1H, overlapped, H-4′), 4.59 (1H, overlapped, H-6′b), 4.56 (1H, m, H-6‴b), 4.48 (1H, m, H-2′), 4.48 (1H, m, H-6″b), 4.35 (1H, m, H-2″), 4.33 (1H, m, H-6‴a), 4.22 (1H, overlapped, H-6′a), 4.22 (1H, overlapped, H-3‴), 4.22 (1H, overlapped, H-5⁗b), 4.20 (1H, m, H-4‴), 4.15 (1H, m, H-3′), 4.10 (1H, overlapped, H-3″), 4.09 (1H, overlapped, H-4⁗), 4.08 (1H, m, H-2), 4.06 (1H, m, H-6″a), 4.04 (1H, m, H-3⁗), 3.97 (1H, m, H-5′), 3.94 (1H, m, H-2‴), 3.91 (1H, m, H-2⁗), 3.86 (1H, overlapped, H-3), 3.85 (1H, m, H-5″), 3.85 (1H, m, H-5‴), 3.82 (1H, m, H-4″), 3.66 (1H, m, H-5⁗a), 2.51 (1H, m, H-12b), 2.25 (3H, s, H-21), 2.15 (1H, m, 1 b), 2.09 (1H, m, H-15b), 1.90 (1H, m, H-4b), 1.84 (1H, m, H-15a), 1.69 (1H, m, H-4a), 1.51 (1H, m, H-11b), 1.49 (1H, m, H-7b), 1.35 (1H, m, H-8), 1.31 (1H, m, H-11a), 1.27 (1H, m, H-6b), 1.26 (1H, overlapped, H-12a), 1.25 (1H, overlapped, H-14), 1.16 (1H, m, H-6a), 1.09 (1H, m, H-1a), 1.02 (1H, m, H-5), 0.88 (3H, s, H-18), 0.87 (1H, m, H-7a), 0.69 (3H, s, H-19), 0.63 (1H, m, H-9). ^{13}C NMR (150 MHz, pyridine-d_5) δ: 196.5 (C-20), 155.4 (C-17), 144.9 (C-16), 104.9 (C-1″ of Glc), 104.8 (C-1‴ of Glc), 104.7 (C-1⁗ of Xyl), 103.2 (C-1′ of Gal), 87.1 (C-3″), 84.0 (C-3), 81.2 (C-2″), 79.5 (C-4′), 78.7 (C-5‴), 78.5 (C-3⁗), 78.2 (C-3‴), 77.6 (C-5″), 76.1 (C-3′), 75.8 (C-5′), 75.5 (C-2‴), 75.2 (C-2⁗), 72.5 (C-2′), 71.4 (C-4‴), 70.8 (C-4″), 70.5 (C-2), 70.5 (C-4⁗), 67.3 (C-5⁗), 62.9 (C-6‴), 62.7 (C-6″), 60.7 (C-6′), 56.3 (C-14), 54.9 (C-9), 46.5 (C-13), 45.4 (C-1), 44.8 (C-5), 37.0 (C-10), 35.2 (C-12), 34.1 (C-4), 33.2 (C-8), 32.3 (C-15), 31.9 (C-7), 28.1 (C-6), 27.2 (C-21), 21.4 (C-11), 16.2 (C-19), 13.3 (C-18).

3.5. Acid hydrolysis of compounds 1 and 2

A solution of **1** (50 mg) in 2 M HCl (dioxane–H_2O = 1:1, 8 mL) was heated at 100 °C for 2 h. After cooling, the reaction mixture was neutralized upon the addition of NH_4OH and extracted with EtOAc. The combined organic layers were evaporated and isolated using semi-preparative HPLC to afford aglycone **1a** (21 mg), which was identified using MS and NMR spectroscopy. The aqueous layer was evaporated to dryness to obtain the sugar fractions. A 2- mg portion of the sugar residue was dissolved in H_2O (1 mL) and mixed with EtOH (1 mL) containing (s)-α-methylbenzylamine (17 μL) and $NaBH_3CN$ (8 mg), respectively. The mixture was agitated at 40 °C for 4 h, glacial acetic acid (0.2 mL) added, and then concentrated in vacuo. Acetic anhydride (0.3 mL) and pyridine (0.3 mL) were added to the resulting residue at room temperature for 24 h. After the pyridine was removed, an aqueous solution of the reaction mixture was subjected to column chromatography on a Cleanert C_{18}-N column (Agela) using H_2O, 20 and 50% CH_3CN (15, 15, and 10 mL) as the eluent. The 50% CH_3CN fraction was analyzed using HPLC under the following conditions: Agilent SB-C_{18} column (4.6 × 250 mm, 5 μm); mobile phase, 40% CH_3CN; flow rate, 0.8 mL/min; and DAD detection, 230 nm. The absolute configuration of the monosaccharides was determined by comparing the retention time t_R (min) of their derivatives with those of authentic samples: 19.51 min (D-galactose derivative), 22.77 min (D-glucose derivative), and 24.93 min (L-rhamnose derivative).

A solution of **2** (10 mg) in 2 M HC1 (dioxane–H_2O = 1:1, 2 mL) was treated identically to that of compound **1** to give **la** (2.4 mg) and the saccharide fraction. D-galactose, D-glucose, and D-xylose were identified in a 1: 2: 1 ratio in the saccharide fraction of **2** upon conversion into their 1-[(S)-N-acetyl-α-methylbenzylamino]-1-deoxyalditol

acetate derivatives followed by HPLC analysis: 19.48 min (D-galactose derivative), 22.82 min (D-glucose derivative), and 18.12 min (D-xylose derivative).

3.6. Xylene-induced ear edema

The xylene-induced ear edema test was performed as previously described (Tang et al. 1984). Briefly, a total 140 mice were randomly divided into fourteen groups consisting of 5 males and 5 females in each group. The different groups were treated with saline, compounds **1**–**4** (HPLC purity $\geq$ 98%, 12.5, 25, and 50 mg/kg), and aspirin (500 mg/kg). The mice were orally administrated once a day for 5 successive days. On the fifth day, after administration for 1 h, the mice in each group were daubed with 0.05 mL xylene on two sides of the right ear and the left ear was used as the control. After the application of xylene for 0.5 h, all mice were euthanized under ether anesthesia and both ears were cut down along the auricle baseline. The round pieces of the ears were sampled using a punch (diameter = 8 mm) and weighed on an electronic balance.

3.7. Statistical analysis

All data were expressed as the mean ± standard error of mean (per group n = 10). The normally distributed data were subjected to two-way ANOVA followed by Dunnett's test. $p < 0.05$ was considered statistically significant.

4. Conclusions

In conclusion, two new pregnane glycosides, 2α, 3β-dihydroxy-5α-pregn-16-en-20-one-3-O-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside} (**1**) and 2α, 3β-dihydroxy-5α-pregn-16-en-20-one-3-O-{β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**2**) along with two known spirostanol saponins (**3** and **4**) were isolated and characterized on the basis of chemical and spectral evidence (^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY). All the compounds exhibited promising anti-inflammatory activities. Our present investigation demonstrates that *H. ventricosa* is a potential source of anti-inflammatory natural products.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by the National Natural Science Foundation of China (31260084) and the Scientific Research Fund of Jiangxi Provincial Education Department (GJJ150769).

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