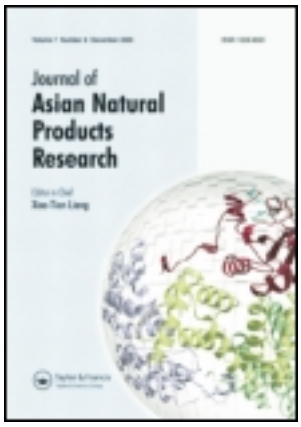

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Triterpenoid glycoside from *Cimicifuga racemosa*

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One new triterpene glycoside, cimiracemoside (**I**), and 14 known triterpene glycosides have been isolated from the rhizome extracts of black cohosh (*Cimicifuga racemosa*). On the basis of spectral and chemical evidence, the structure of the new compound was elucidated to be 12β-acetoxycimigenol-3-*O*-β-D-xylopranoside (**1**), and the known compounds were identified to be 25—acetylcimigenol xyloside (**2**), cimigenol-3-*O*-β-D-xylopyranoside (**3**), acetin (**4**), 27-deoxyacetin (**5**), cimicifugoside H-1 (**6**), 23-*O*-acetylshengmanol 3-*O*-β-D-xylopranoside (7), foetidinol-3-*O*-β-xyloside (**8**), cimicifugoside H-2 (**9**), 25-*O*-methylcimigenol xyloside (**10**), 21-hydroxycimigenol-3-*O*-β-D-xylopyranoside (**11**), 24-epi-7,8—didehydrocimigenol-3-xyloside (**12**), cimidahurinine (**13**), cimidahurine (**14**) and cimifugin (**15**). The compounds **1–5**, **14**, and **15** showed weak antibacterial activities in the agar diffusion assay.

Keywords: *Cimicifuga racemosa*; Ranunculaceae; Cimiracemoside I; Antibacterial activity

1. Introduction

Cimicifuga racemosa has been used to treat a variety of ailments, including diarrhea, sore throat, rheumatism and menopausal disorders [1]. At present, *Cimicifuga* is one of the 'hot' fields of research in the world. The cycloartane triterpenoids are the special constituents of *Cimicifuga* [2,3]. Primary studies have showen that *C. racemosa* possessed significant activities against bacteria. In this paper, 15 compounds were isolated and identified, including 12 cycloartane triterpenoids, 12β-acetoxycimigenol-3-*O*-β-D-xylopyranoside (**1**), 25-acetylcimigenol xyloside (**2**) [4], cimigenol-3-*O*-β-D-xylopyranoside (**3**) [5], actein (**4**) [6], 27-deoxyactein (**5**) [7], cimicifugoside H-1 (**6**) [7], 23-*O*-acetylshengmanol 3-*O*-β-D-xylopranoside (7) [8], foetidinol-3-*O*-β-xyloside (**8**) [9], cimicifugoside H-2 (**9**) [7], 25-*O*-methylcimigenol xyloside (**10**) [10], 21-hydroxycimigenol-3-*O*-β-D-xylopyranoside (**11**) [11], 24-epi-7,8-didehydrocimigenol-3- xyloside (**12**) [2], cimidahurinine (**13**) [12], cimidahurine (**14**) [12]and cimifugin (**15**) [13]. Some compounds showed weak antibacterial activity.

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2. Results and discussion

Compound **1** (figure 1) was isolated as a white powder. The negative HRFAB-MS spectrum showed a quasi molecular ion peak at *m/z* 677.3908 [M − 1] − , in accordance with the molecular formula $C_{37}H_{58}O_{11}$. The IR spectrum showed absorption bands at 3439, 1733 cm^{-1}, assignable a hydroxyl group and a carboxyl group, respectively. The ^1H NMR spectra showed the signals due to cyclopropane protons at δ 0.28 (1H, d, $J = 3.9$ Hz) and 0.56 (1H, d, $J = 3.9$ Hz), a secondary methyl group at δ 0.92 (3H, d, $J = 6.0$ Hz, H-21), six *tert*-methyl groups at δ 1.00, 1.19, 1.27, 1.30, 1.45, 1.48, an acetyl methyl group at δ 2.10 and an anomeric proton at δ 4.81 (1H, d, $J = 7.3$ Hz, H-1′). The ^{13}C NMR spectrum showed the data consistent with a xylose moiety at δ 107.5 d, 75.6 d, 78.6 d, 71.3 d and 67.1 t, which was also supported by the acidic hydrolysis of **1** to give xylose by PC comparison with an authentic sample. All the above evidence suggested that **1** was a highly oxygenated 9,19-cycloartane triterpene monoglycoside. The ^1H and ^{13}C NMR spectra were very similar to those of cimiracemoside D [11] except for the signals attributed to the xylosyl group at C-3 of **1** instead of the arabosyl group in cimiracemoside D, which was fully confirmed by the $^1H-^1H$ COSY, HMQC and HMBC correlations. In the HMBC spectrum, the significant correlation was observed between the signals at δ 4.81 (1H, d, $J = 7.3$ Hz, H-1′) and 88.3 (d, C-3), suggesting that the sugar moiety was located at the C-3 position. The correlation between the signals at δ 5.25 (1H, d, $J = 8.6$ Hz, H-12) and 170.5 (s, OAc) suggested that the acetoxy group was located at the C-12 position. Two hydroxyl groups at the C-15 and C-25 position were confirmed by the correlations between the signals at δ 4.36 (1H, br.s, H-15) with 46.3 (s, C-14), 112.0 (s, C-16), and the signals at δ 1.45 (3H, s, H-27), 1.48 (3H, s, H-26), 3.78 (1H, s, H-24) with 71.0 (s, C-25). Based on the above evidence, the structure of compound **1** was established to be 12β-acetoxycimigenol-3-*O*-β-D-xylopyranoside.

By disk diffusion testing [14], the inhibitory effects of compounds **1–9** and **14–15** against *Shigella flexneri, Shigella dysenteriae, Shigella sonnei, Mycobacterium tuberculosis,* α-Hemolytic *Streptococcus* and *Streptococcus pneumoniae* were observed to behave in a dose-dependent manner. The compounds **1**, **2**, **4**, **5**, **8** and **14**–**15** showed weak antibacterial activities in the agar diffusion assay. No such activities were reported for these compounds before this work.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a XRC-1 micromelting apparatus and are uncorrected. The IR spectra were obtained on a Bio – Rad FTS spectrometer, FAB-MS was performed on an Autospec – 3000 spectrometer under a negative model, and NMR spectra were recorded on a Bruker AM-400 or a Bruker DRX-500 spectrometer in C_5D_5N (δ values with reference to the signal of C_5D_5N) with TMS as an internal standard. Column chromatography was carried out on Qingdao silica (200–300 mesh), MCI gel CHP-20P, and Fuji gel (ODS-Q_3) (Mitsubishi Chemical Co.). TLC was performed on Qingdao precoated plate silica GF_{254} and Merck RP-18 F_{254} plate with the following solvent systems: A, MeOH–$CHCl_3$ (5:95 v/v); B, H_2O–MeOH (10:90 v/v).

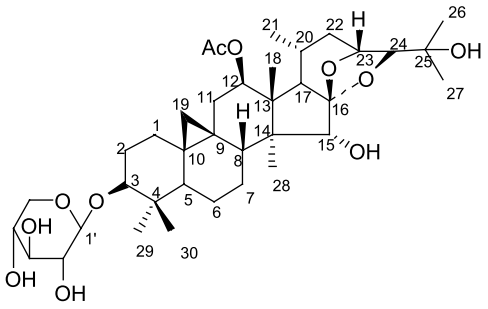

Figure 1. The structure of compound **1**.

3.2 Plant material

The roots of *Cimicifuga racemosa* were collected in Chicago USA. The plant material was identified by Prof. Sheng-Xiang Qiu (College of Pharmacy, University of Illinols at Chicago, Chicago, IL).

3.3 Extraction and isolation

The air-dried roots of *C. racemosa* (5.0 kg) were extracted repeatedly thrice with alcohol (95%) at room temperature. The solvent was evaporated in vacuo at temperature below 50°C to give a deep brown waxy residue, which was suspended in water and participated with $CHCl_3$ (3×2000 ml) and n-BuOH (3×2000 ml). The $CHCl_3$ extract (100 g) was fractionated by column chromatography (silica gel (1500 g, 200 – 300 mesh, $CHCl_3$, $CHCl_3$–MeOH 99:1, 95:5, 90:10 and 80:20) to afford five fractions. Fraction I (3.5 g) was purified by repeated column chromatography ($CHCl_3$–MeOH 95:5, 92:2) and RP-18 column (MeOH–H_2O 8:2) to give a pure compound **15** (820 mg). Fraction II (9.2 g) was purified by repeated column chromatography ($CHCl_3$–MeOH 95:5, 94:6) and RP-18 column (MeOH–H_2O 9:1) to give five pure compounds: **1** (45 mg), **2** (350 mg), **3** (50 mg), **4** (33 mg), **10** (14 mg). Fraction III (8.5 g) was purified by repeated column chromatography ($CHCl_3$–MeOH 92:8, 93:7) and RP-18 column (MeOH–H_2O 75:25) to give three pure compounds: **5** (124 mg), **6** (80 mg), **7** (60 mg). Fraction IV (17.6 g) was purified by repeated column chromatography ($CHCl_3$–MeOH 90:10) and RP-18 column (MeOH–H_2O 6:4) to give six pure compounds: **8** (26 mg), **9** (94 mg), **11** (28 mg), **12** (9 mg), **13** (10 mg), **14** (49 mg).

3.3.1 Cimiracemoside I (1). White powder (45 mg); mp 179–184°C; $[\alpha]_D$ -40 (*c* 0.55, $CHCl_3$); IR (KBr) ν_{max} 3439 (OH), 1733 (C(O) cm^{-1}; negative FABMS *m/z* 677.3908 [M-H] (calcd for $C_{37}H_{58}O_{11}$, 677.3901), ^1H NMR (400 MHz) and ^{13}C NMR (125 MHz) (see table 1).

3.3.2 Acidic hydrolysis. The compound **1** (5 mg) was dissolved in a mixture of MeOH (1.0 ml) and 2 mol/l HCl (1.0 ml) and hydrolysed by refluxing in a boiling water bath for 2 h. The hydrolysate was allowed to cool, diluted twofold with distilled H_2O and partitioned between water and EtOAc. The aqueous. layer was neutralized and concentrated in vacuo to give a residue. The xylose was identified from the residue by paper chromatography comparison with authentic sample with n-BuOH–HAc–H_2O (5:1:5, upper layer).

Table 1. ^{1}H NMR and ^{13}C NMR spectral data of compound **1**.

Position	δ_C	δ_H *(J Hz)*	*Position*	δ_C	δ_H *(J Hz)*
1	32.4 t	1.10 m, 1.53 m	19	30.9 t	0.28 (d, 3.9), 0.56 (d, 3.9)
2	30.1 t	1.86 m, 2.27 m	20	24.1 d	1.65 m
3	88.3 d	3.46 (dd, 11.6, 3.9)	21	21.7 q	0.92 (d, 6.0)
4	41.3 s		22	38.6 t	1.07 m, 2.11 m
5	47.2 d	1.24 m	23	71.5 d	4.72 (d, 9.0)
6	20.7 t	0.73 br q, 1.50 m	24	89.9 d	3.78 s
7	26.0 t	1.06 m, 2.05 m	25	71.0 s	
8	47.2 d	1.65 m	26	25.5 q	1.48 s
9	20.2 s		27	27.1 q	1.45 s
10	26.9 s		28	11.9 q	1.27 s
11	37.5 t	1.15 m, 2.92 m	29	25.7 q	1.19 s
12	77.3 d	5.25 (d, 8.6)	30	15.4 q	1.00 s
13	48.5 s		1′	107.5 d	4.81 (d, 7.3)
14	46.3 s		2′	75.6 d	4.02 (t, 8.6)
15	79.2 d	4.36 s	3′	78.6 d	4.12 (t, 8.9)
16	112.0 s		4′	71.3 d	4.20 m
17	59.2 d	1.53 (d, 12.3)	5′	67.1 t	4.32 (dd, 11.1, 5.1)
18	12.7 q	1.30 s	$COCH_3$	170.5 s	3.45 (dd, 11.1, 5.1)
				20.0 q	2.10 s

Table 2. Inhibitory activity of compounds **1**–**9**, **14**, **15** against tested microorganisms.

	Compounds										
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**14**	**15**
Shigella Flexneri	+	−	−	−	−	−	−	−	−	−	−
Shigella dysenteriae	−	−	−	−	−	−	−	−	−	−	−
Shigella Sonnei	+	−	−	+	−	−	−	−	−	−	−
Mycobacterium tuberculosis	+	+	−	+	+	−	−	−	−	+	−
α-Hemolytic *Streptococcus*	−	−	−	−	−	−	−	−	−	−	−
Streptococcus pneumoniae	+	−	−	−	+	−	−	+	−	−	+

3.4 Antibacteral activity assay

Inhibitory activities of compounds **1**–**9**, **14**–**15** against *Shigella flexneri, Shigella dysenteriae, Shigella sonnei, Mycobacterium tuberculosis,* α-hemolytic *Streptococcus* and *Streptococcus pneumoniae* were determined by the paper disk diffusion assay on agar plates as described [14]. Only compounds **1**, **2**, **4**, **5**, **8** and **14**–**15** showed weak antibacterial activities in the agar diffusion assay (see table 2).

The samples were dissolved in chloroform and applied to a paper disk with a syringe (500 μg each disk), respectively. The disks were dried with flow air and put onto agar media inoculated with the testing organism. Inhibition zones were observed after incubation for 10 h at 42°C.

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