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A New *ent*-Pimarane-Type Diterpenoid Glycoside from *Siegesbeckia pubescens*

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Abstract: A new ent-pimarane-type diterpenoid glucoside, along with eight known same skeleton type were isolated from the ethanol extract of *Siegesbeckia* pubescens Makino by means of various chromatographic techniques (silica gel, RP-8, Sephadex LH-20, Pre-HPLC). Their structures were elucidated on the basis of spectroscopic analyses and the new one identified as ent-15-methylene-2*α*,16,19-trihydroxy-pimar-8(14)-ene-19-O-*β*-D-glucopyranoside.

Keywords: *Siegesbeckia pubescens*; *ent*-pimarane-type diterpenoid; pubeside F.

1. Introduction

The genus *Siegesbeckia* is a small member of Compositae family and only comprises four species, which distributed in tropical, subtropical, and temperate parts of the world [1]. Three species are found in China and have used as "Xi-Xian" included in Chinese Pharmacopoeia for their antirheumatic, lubricate joints and detoxifying properties[2]. Bioactivity studies on extracts or pure components have exhibited multiple positive effects, including antithrombotic, anti-inflammatory, antiallergic, immune-suppressive and so on [3-6]. *Siegesbecia pubescens* Makino, an annual herb plant, is widely growing in the Midlands and the North of China. Previous investigation on *S. pubescens*, ent-kaurane and ent-pimarane diterpenoids were the main compositions of the plant and exhibited antithrombotic activity[5,7-8]. In the present study, we report the isolation and structure eluciation of a new ent-pimarane diterpenoid, together with eight known ones from the-BuOH part of the enthanol extract.

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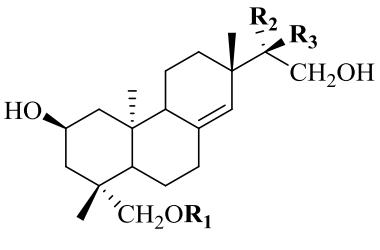
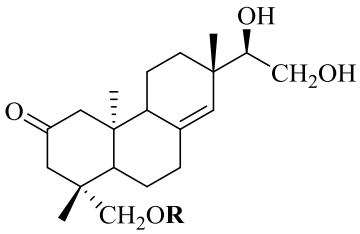
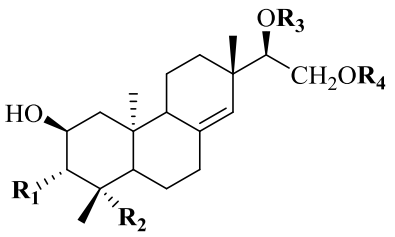


	R_1	R_2	R_3
1	glc	H	H
2	H	OH	H
3	H	H	OH



4 **R** =H
5 **R** = glc



	R_1	R_2	R_3	R_4
6	H	COOH	H	H
7	H	CH2OH	Ac	H
8	H	CH2OH	H	Ac
9	Oglc	Me	H	H

Figure 1. Chemical Structures of compounds **1-9**

2. Materials and Methods

2.1. Material

The aerial of *S. pubescens* Makino was collected from Yuexi County, Anhui Province, China, in October 2009. It was identified by Dr. Qing-Shan Yang, Anhui University of Chinese Medicine. A voucher specimen (XF 201301) was deposited at the deposited at the Laboratory of Phytochemistry, Anhui University of Chinese Medicine.

Optical rotation was recorded on a Jasco P-1020 automatic digital polarimeter. UV spectrum was measured on a Shimadzu UV-2401PC spectrophotometer. IR spectrum was obtained on a Bruker Tensor 27 FT -IR spectrometer with KBr pellet. NMR spectra were recorded on Bruker DRX-400 instruments with TMS as the internal standard. The chemical shifts were given in δ (ppm) scale with reference to the solvent signal. ESI-MS and HR-ESI-MS spectra were acquired on API QSTAR Pulsar i mass spectrometer. Silica gel (200–300 mesh); and Sephadex LH-20 were used for column chromatography (CC). Preparative HPLC was performed on Waters Auto Purification 2545-2489 system equipped with a Shimadzu ODS-18, 9.4 mm × 250 mm column. Fractions monitored by TLC, and spots were visualized by spraying with 10% H_2SO_4 in EtOH, followed by heating.

2.2. Extraction and Isolation

The air-dried and powdered aerial of *S. pubescens* Makino (10.7 kg) was diacolated with 95% ethanol (100 L) and 70% ethanol (30 L) at room temperature. The ethanol extract concentrated in vacuo to give a green crude extract, which was suspended in H_2O and partitioned successively with petroleum ether (PE), EtOAc and *n*-BuOH. The *n*-BuOH part (264.2 g) was chromatographed on silica gel column (2.0 kg, 9.0 × 60 cm) eluting with a CH_2Cl_2-MeOH gradient system (95:5, 90:10, 85:15, 80:20, 70:30 each 20 L, v/v) to afford fraction Fr.1~ Fr.6. Each Fraction was decolorized using MCI gel CHP 20P (0.8 L, 4.0 × 80 cm), eluted with 80% MeOH-H_2O, and then subjected to Sephadex LH-20 (80 g, 2.0 × 150 cm) eluting with MeOH to yield sub-fractions. Fr.2-2 (1.8 g) was separated on silica gel column, eluted with CH_2Cl_2-MeOH (92: 8) to give 7 (83 mg), the rest mix ingredient was purified by preparative HPLC using 35% MeOH-H_2O detected at 215 nm to provide **7** (25 mg) and **8** (54 mg). Fr. 4-2 (8.3 g) was chromatographed on silica gel column eluted with CH_2Cl_2-MeOH (90: 10) to yield **9** (1.26 g). Fr. 4-3 (0.83 g) was subjected to Rp-18 column eluted with 60%MeOH-H_2O, and positive Fr. 4-3-2 (30.6 mg) was purified by preparative HPLC using 45% MeOH-H_2O and provided **2** (7.3 mg) and **3** (11.6 mg). Fr. 4-4 (1.31 g) was subjected to silica gel column eluted with CH_2Cl_2-MeOH (90: 10) to provide **4** (12.8 mg). Fr. 4-4 (0.83 g) was subjected to silica gel CC eluted with CH_2Cl_2-MeOH (85: 15) to obtain **5** (31.8 mg). Fr. 5.2 (1.48 g) was applied an RP-18 column and isocratic elution (60 % MeOH- H_2O) to yield Fr. 5.2.2, which further purified by preparative HPLC (40 % MeOH- H_2O) to afford **6** (8 mg). Compound **1** (13 mg) was isolated from Fr.5.3 using repeated silica gel CC with CH_2Cl_2-MeOH (85: 15) and preparative HPLC with 45 % MeOH- H_2O.

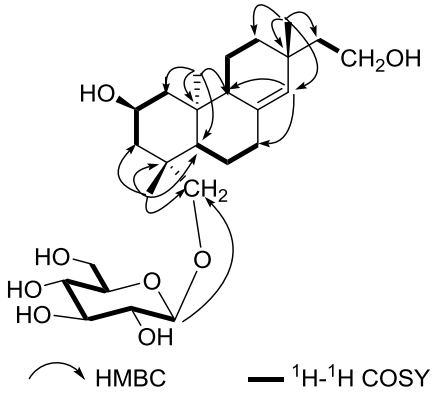


Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of compound **1**

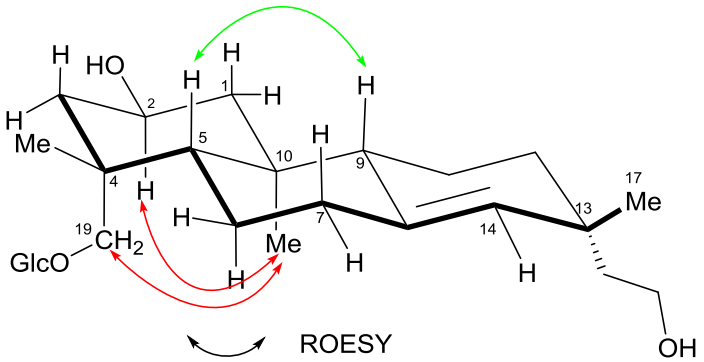


Figure 3. Key ROESY corrections of compound 1

2.3. Spectroscopic Data

*Pubeside F (**1**):* White amorphous power; $[\alpha]_D^{20.0}$ = —32.20 (c 0.001, MeOH); UV (MeOH): λ_{max} (log ε) = 204 (3.75) nm; IR (KBr): υ_{max} = 3416, 2924, 2850, 1645, 1597, 1464, 1375, 1080 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR (MeOD, 400/100 MHz) see Table 1; HR-ESI-MS calcd for $C_{26}H_{44}O_8Na$ $[M + Na]^+$ 507.2934, found 507.2926.

2.4. Acid Hydrolysis

Compound **1** (3 mg) were individually refluxed with 5 % HCl in MeOH (5 mL) for 4 hours. The solution was diluted with H_2O (5 mL) and extracted with EtOAc (10 mL) for 3 times. The aqueous layer was neutralized with $NaHCO_3$ and concentrated in vacumn to give a residue. The residue was purified by RP-18 column, eluted with 20% MeOH-H_2O. The sugar unit was identified as D-glucose on the basis of TLC and optical rotation ($[\alpha]_D^{18.3}$: +40.0 (c 0.05, MeOH)) [9,10].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as a white amorphous power. Its molecular formula was determined to be $C_{26}H_{44}O_8$ with five degrees of unsaturation on the basis of the HR-ESIMS (positive ion): *m/z* 507.2926 $[M + Na]^+$ (calcd. for $C_{26}H_{44}O_8Na$) and the ^{13}C NMR data (Table1). The IR spectrum showed the presence of hydroxyl (3416 cm^{-1}) and double bond (1645 cm^{-1}) functionalities. The ^{1}H NMR spectrum of **1** exhibited three methyl singlet signals at δ_H 0.85, 0.94, 1.09; three oxygenated-

methylene groups [δ_H 4.04, 3.31(1H each, d, 11.6 Hz), 3.61 (2H, m) and 3.87 (1H, dd, 12.0, 6.2 Hz), 3.71 (1H, d, 12.0, 4.8 Hz)] signals; one olefin proton [δ_H 5.24 (s)], and an anoremic proton [δ_H 4.20 (d, J = 7.6 Hz)] signals. The ^{13}C NMR spectrum of **1** displayed 26 carbon resonances, according to three methyl, nine methylene, four methine, four quaternary carbons, and a glucopyranosyl moiety. The NMR characters of **1** were similar to those of ent-2*α*,15,16,19-tetrahydroxypimar-8(14)-en-19-O-*β*-glucopyranoside[11] except for the side chain in position C-13. The HMBC cross-peaks (Figure 2) from δ_H 0.94 (H-17) to C-12, C-13, C-14 and C-15 together with the COSY correlations of H-15/H-16 indicated the carbon signal δ_C 44.5 (t) should be connect to C-13. In addition, the HMBC cross-peaks from the anoremic proton δ_H 4.20 to C-19, and the coupling constant (J = 7.6 Hz) indicated that sugar moiety was attached to C-19 via a *β*-linkage. Furthermore, the key expected correlations were observed as follows: from δ_H 0.85 (20-Me) to C-1, C-5, C-9 and C-10, from δ_H 1.09 (18-Me) to C-3, C-4, C-5 and C-19 in the HMBC spectrum, and of H-1/H-2/H-3, H-5/H-6/H-7, H-9/H-11/H-12 in the ^{1}H-^{1}H COSY spectrum. Based on the above evidences, the planar structure of **1**was established.

The relative configuration of of **1** was established by a ROESY experiment (Figure 3). The correlations H-2 ↔ Me-20 indicated *β*-orientation of 2-OH, and H-19↔Me-20 revealed Me-18 adopted *β*-orientation. Therefore, the structure of compound **1** was identified as ent-15-methylene-2*α*,16,19-trihydroxy-pimar-8(14)-ene-19-O-*β*-D-glucopyranoside, and named pubeside F.

From the NMR and MS data and corresponding with those form literatures, the known ent-pimarame diterpenoids from the plant were identified as ent-2*α*,15R,16,19- tetrahydroxypimar-8(14)-ene (**2**)[12], kirenol (**3**)[13], *ent*-2-oxo-15,16,19-trihydroxypimar-8(14)-ene (**4**)[8], pubeside D (**5**)[8], ent-2*α*,15,16-trihydroxypimar-8(14)-en-19-oic acid (**6**)[8], ent-16-O-acetoxy-2α,16,19-trihydroxypimar-8 (14)-ene (**7**) [14], ent-15-*O*-acetoxy-2*α*,16, 19-trihydroxypimar-8(14)-ene (**8**)[14] and darutoside (**9**)[15].

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

Position	δ_C	δ_H	Position	δ_C	δ_H
1	50.0 (t)	1.99, 1.04 (1H each, m)	14	132.6 (d)	5.24 (1H, s)
2	65.5 (d)	3.85 (1H, m)	15	44.5 (t)	1.64, 1.55 (1H each, m)
3	45.6 (t)	2.35, 0.86 (1Heach, m)	16	60.0(t)	3.61 (2H, m)
4	40.8 (s)		17	29.1 (q)	0.94 (3H, s)
5	56.7 (d)	1.18 (1H, m)	18	28.5 (q)	1.09 (3H, s)
6	23.5 (t)	1.73, 1.31(1Heach, m)	19	74.2 (t)	4.04, 3.31 (1H each, d, 11.6)
7	37.4 (t)	2.26, 2.04 (1H each, m)	20	17.6 (q)	0.85(3H, s)
8	137.3 (s)		1'	105.1 (d)	4.20 (1H, d, 7.6)
9	52.6 (d)	1.81 (1H, m)	2'	75.4 (d)	3.19 (1H, t, 8.4)
10	41.0 (s)		3'	78.4(d)	3.35 (1H, m)
11	20.4 (t)	1.62 (2H, m)	4'	71.8 (d)	3.28 (1H, m)
12	36.5 (t)	1.58, 1.17 (1H each, m)	5'	77.9 (d)	3.27 (1H, m)
13	34.0 (s)		6'	61.8(t)	3.87(1H, dd, 12.0, 6.2) 3.71 (1H, dd, 12.0, 4.8)

*400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR in MeOD in ppm, J in Hz

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/RNP

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