

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

Fengqing Xu¹*, Huaping Hu¹, Ying Li¹, Yashuo Ren¹, Hongsu Zhao¹, Qi
Huang¹ and Jutao Wang¹*

School of Pharmacy, Anhui University of Chinese Medicine; Anhui Innovative Team from Colleges for Scientific Research's Platform -The Innovative Team in Researching the Key Technologies concerning the Integration of Processing Chinese Medicine Decoction Pieces in Producing Area, Hefei 230012, P. R. China

(Received November 22, 2017; Revised December 15, 2017; Accepted December 16, 2017)

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. © 2018 ACG Publications. All rights reserved.

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]. *Siegesbeckia 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 elucidation of a new *ent*-pimarane diterpenoid, together with eight known ones from the-BuOH part of the ethanol extract.

* Corresponding authors: E-mail: hfglnds@sina.com (Fengqing Xu) and wjt591@163.com (Jutao. Wang), Phone: +86 551 68129167.

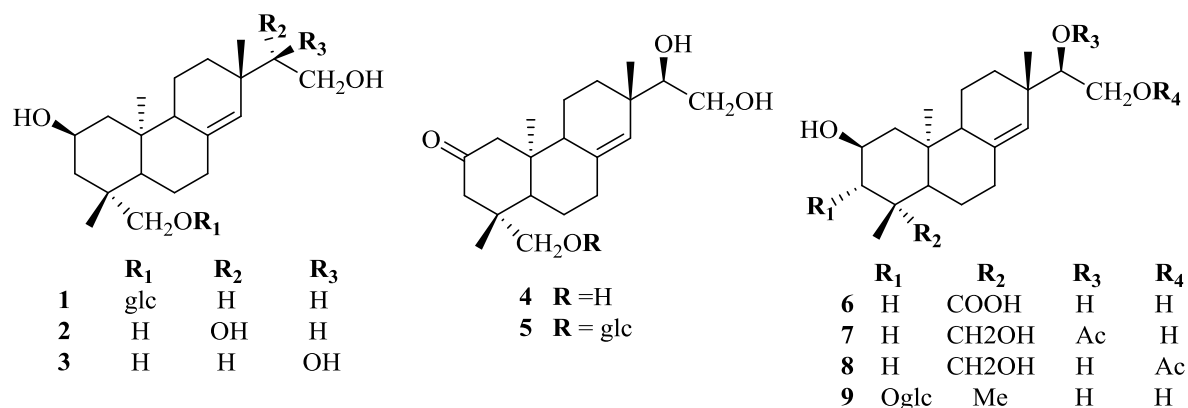


Figure 1. Chemical Structures of compounds 1-9

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 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 \times 250 mm column. Fractions monitored by TLC, and spots were visualized by spraying with 10% H₂SO₄ 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 H₂O 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 \times 60 cm) eluting with a CH₂Cl₂-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 \times 80 cm), eluted with 80% MeOH-H₂O, and then subjected to Sephadex LH-20 (80 g, 2.0 \times 150 cm) eluting with MeOH to yield sub-fractions. Fr.2-2 (1.8 g) was separated on silica gel column, eluted with CH₂Cl₂-MeOH (92: 8) to give 7 (83 mg), the rest mix ingredient was purified by preparative HPLC using 35% MeOH-H₂O 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 CH₂Cl₂-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-H₂O, and positive Fr. 4-3-2 (30.6 mg) was purified by preparative HPLC using 45% MeOH-H₂O 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 CH₂Cl₂-MeOH (90: 10) to provide 4 (12.8 mg). Fr. 4-4 (0.83 g) was subjected to silica gel CC eluted with CH₂Cl₂-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- H₂O) to yield Fr. 5.2.2, which further purified by preparative HPLC (40 % MeOH- H₂O) to afford 6 (8 mg). Compound 1 (13 mg) was isolated from Fr.5.3 using repeated silica gel CC with CH₂Cl₂-MeOH (85: 15) and preparative HPLC with 45 % MeOH- H₂O.

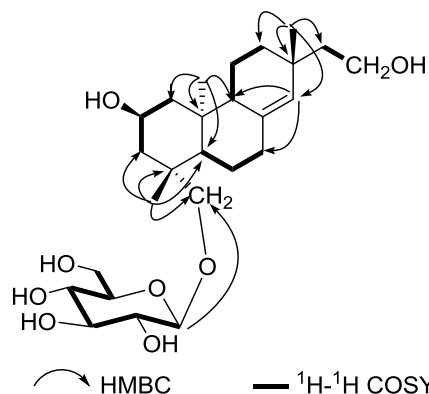


Figure 2. Key ^1H - ^1H COSY and HMBC correlations of compound **1**

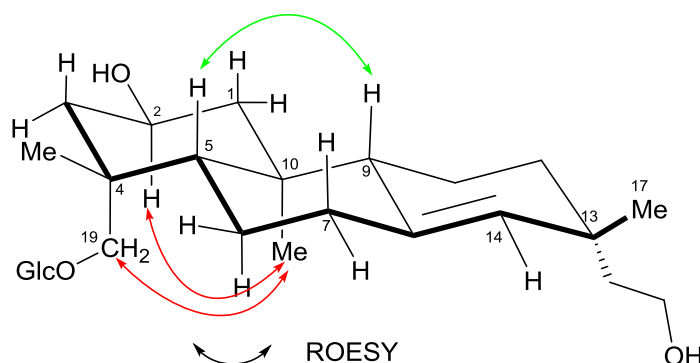


Figure 3. Key ROESY correlations of compound **1**

2.3. Spectroscopic Data

Pubeside F (1): White amorphous powder; $[\alpha]_{\text{D}}^{20.0} = -32.20$ (c 0.001, MeOH); UV (MeOH): λ_{max} (log ϵ) = 204 (3.75) nm; IR (KBr): $\nu_{\text{max}} = 3416, 2924, 2850, 1645, 1597, 1464, 1375, 1080 \text{ cm}^{-1}$; ^1H -NMR and ^{13}C -NMR (MeOD, 400/100 MHz) see Table 1; HR-ESI-MS calcd for $\text{C}_{26}\text{H}_{44}\text{O}_8\text{Na}$ $[\text{M} + \text{Na}]^+$ 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 vacuum 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 ($[\alpha]_{\text{D}}^{18.3}: +40.0$ (c 0.05, MeOH)) [9,10].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined to be $\text{C}_{26}\text{H}_{44}\text{O}_8$ with five degrees of unsaturation on the basis of the HR-ESIMS (positive ion): m/z 507.2926 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{26}\text{H}_{44}\text{O}_8\text{Na}$) and the ^{13}C NMR data (Table 1). The IR spectrum showed the presence of hydroxyl (3416 cm^{-1}) and double bond (1645 cm^{-1}) functionalities. The ^1H 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-tetrahydroypimar-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 ^1H - ^1H COSY spectrum. Based on the above evidences, the planar structure of **1** was established.

The relative configuration of **1** was established by a ROESY experiment (Figure 3). The correlations H-2 \leftrightarrow Me-20 indicated β -orientation of 2-OH, and H-19 \leftrightarrow 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 from literatures, the known *ent*-pimarane diterpenoids from the plant were identified as *ent*-2 α ,15R,16,19-tetrahydroypimar-8(14)-*ene* (**2**)[12], kirenol (**3**)[13], *ent*-2-oxo-15,16,19-trihydroypimar-8(14)-*ene* (**4**)[8], pubeside D (**5**)[8], *ent*-2 α ,15,16-trihydroypimar-8(14)-*en*-19-*oic* acid (**6**)[8], *ent*-16-*O*-acetoxy-2 α ,16,19-trihydroypimar-8(14)-*ene* (**7**) [14], *ent*-15-*O*-acetoxy-2 α ,16,19-trihydroypimar-8(14)-*ene* (**8**)[14] and darutoside (**9**)[15].

Table 1. ^1H 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 (1H each, 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(1H each, 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 ^1H NMR and 100 MHz for ^{13}C NMR in MeOD in ppm, J in Hz

Acknowledgments

This research was financially supported by Anhui Province College Excellent Young Talents Fund (2013SQRL041ZD), postdoctoral Start-up Fund of Anhui University (j01002021), and postdoctoral fund of Anhui Province (2016B129). We also thank the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences for all spectra tests.

Supporting Information

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

ORCID Fengqing Xu: [0000-0001-5956-1556](https://orcid.org/0000-0001-5956-1556)Huaping Hu: [0000-0001-6035-9574](https://orcid.org/0000-0001-6035-9574)Ying Li: [0000-0002-3582-0573](https://orcid.org/0000-0002-3582-0573)Yashuo Ren: [0000-0002-3031-8103](https://orcid.org/0000-0002-3031-8103)Hongsu Zhao: [0000-0001-9269-7893](https://orcid.org/0000-0001-9269-7893)Qi Huang: [0000-0002-4252-9557](https://orcid.org/0000-0002-4252-9557)Jutao Wang: [0000-0001-8258-7658](https://orcid.org/0000-0001-8258-7658)**References**

- [1] Flora of China Editorial Committee (1979). *Flora Reipublicae Popularis Sinicae*. Beijing: Science Press. Vol. **75**, 339-340.
- [2] Chinese Pharmacopoeia (2015). Chinese Pharmacopoeia Commission. Beijing: Chinese Medical Science and Technology Press. Vol. **I**, 368.
- [3] J. P. Wang, H. X. Xu, Y. X. Wu, Y. J. Ye, J. L. Ruan, C. M. Xiong, and Y. L. Cai (2011). Ent-16 beta,17-dihydroxy-kauran-19-oic acid, a kaurane diterpene acid from *Siegesbeckia pubescens*, presents antiplatelet and antithrombotic effects in rats, *Phytomedicine* **18**, 873-878
- [4] Z. M. Wang, S. G. Zhu, Z. W. Wu, Y. Lu, H. Z. Fu and R. Q. Qian (2011). Kireinol upregulates nuclear Annexin-1 which interacts with NF-kappa B to attenuate synovial inflammation of collagen-induced arthritis in rats, *J. Ethnopharmacol.* **137**, 774-782.
- [5] J. Xiao, R. B. Yang, L. Yang, X. H. Fan, W. W. Liu and W. B. Deng (2015). Kireinol attenuates experimental autoimmune Encephalomyelitis by Inhibiting Differentiation of Th1 and Th17 Cells and Inducing Apoptosis of Effector T Cells, *Sci. Rep.* **5**, 1-8.
- [6] H. M. Kim, C. Y. Kim, M. H. Kwon, T. Y. Shin and E. J. Lee (1997). Suppression of anaphylactic reaction in murine by *Siegesbeckia pubescens*, *Arch. Pharmacol. Res.* **20**, 122-127.
- [7] G. X. Chou, R. M. Jin, Z. T. Wang and C. X. Chen (2005). Study on antithrombotic fraction of herb *Siegesbeckia*, *Acta Univ Tradit Med Sin Pharmacol Shanghai.* **19**, 39-41.
- [8] R. Wang, W. H. Chen and Y. P. Shi (2010). Ent-kaurane and ent-pimarane diterpenoids from *Siegesbeckia pubescens*, *J. Nat. Prod.* **73**, 17-21.
- [9] C. Wang, F. Q. Xu, J. H. Shang, H. Xiao, W. W. Fan, F. W. Dong, J. M. Hu and J. Zhou (2013). Cycloartane triterpenoid saponins from water soluble of *Passiflora edulis* Sims and their antidepressant-like effects, *J. Ethnopharmacol.* **148**, 812-817.
- [10] L. L. Pan, P. L. Fang, X. J. Zhang, W. Ni, L. Li, L. M. Yang, C. X. Chen, Y. T. Zhang, C. T. Li, X. J. Hao and H. Y. Liu (2011). Tiglane-type diterpenoid glycosides from *Euphorbia fischeriana*, *J. Nat. Prod.* **74**, 1508-1512.
- [11] Y. Xiang, H. Zhang, C. Q. Fan and J. M. Yue (2004). Novel diterpenoids and diterpenoid glycosides from *Siegesbeckia orientalis*, *J. Nat. Prod.* **67**, 1517-1521.
- [12] J. B. Wang, H. Q. Duan, Y. Wang, B. Pan, C. Gao, C. Y. Gai, Q. Wu and H. Z. Fu (2017). Ent-Strobane and ent-pimarane diterpenoids from *Siegesbeckia Pubescens*, *J. Nat. Prod.* **80**, 19-29.
- [13] K. Liu and E. Rödler (1991). Diterpene aus *Siegesbeckia glabrescens*, *Planta Med.* **57**, 395-396.
- [14] J. Xiong, Y. B. Ma and Y. L. Xu (1997). The constituents of *Siegesbeckia orientalis*, *Nat. Prod. Sci.* **3**, 14-18.
- [15] J. H. Kim, K. D. Han, K. Yamasaki and O. Tanaka (1979). Darutoside, a diterpenoid from *Siegesbeckia pubescens* and its structure revision, *Phytochemistry* **18**, 894-895.

ACG
publications

© 2018 ACG Publications