

Cytotoxic Cycloartane Triterpenes of the Traditional Chinese Medicine “Shengma” (*Cimicifuga dahurica*)

Authors

Yin Nian^{1,2}, Hai-Yan Wang^{1,2}, Lin Zhou¹, Jia Su^{1,2}, Yan Li¹, Ming-Hua Qiu¹

Affiliations

¹ State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, PR China

² Graduate School of the Chinese Academy of Sciences, Beijing, PR China

Key words

- Ranunculaceae
- *Cimicifuga dahurica*
- cycloartane triterpenes
- cytotoxicity
- structure-activity relationships

Abstract

Twelve new 9,19-cycloartane triterpenes (**1–12**), together with fourteen known compounds (**13–26**), were isolated from the roots of *Cimicifuga dahurica*. Their structures were determined by application of spectroscopic analyses and chemical methods. Biological evaluation of the compounds against human HL-60, SMMC-7721, A549, MCF-7, and SW-480 cell lines indicated that cimigenol-type glycosides (**1–3**, **19**, and **20**) showed broad-spectrum and moderate cytotoxicities, with IC₅₀

values ranging from 4.2 to 14.5 μM. Meanwhile, cimigenol-type aglycones (**6–8**, **15**, **16**, and **18**) exhibited broad-spectrum and weak cytotoxicities, having IC₅₀ values around 20 μM. In addition, the key points of the structure-activity relationships of aglycones with a cimigenol skeleton were discussed.

Supporting information available online at <http://www.thieme-connect.de/ejournals/toc/plantamedica>

received June 7, 2012
revised October 22, 2012
accepted October 29, 2012

Bibliography

DOI <http://dx.doi.org/10.1055/s-0032-1328019>
 Published online December 5, 2012
 Planta Med 2013; 79: 60–69
 © Georg Thieme Verlag KG
 Stuttgart · New York ·
 ISSN 0032-0943

Correspondence

Prof. Ming-Hua Qiu

State Key Laboratory of Phytochemistry and Plant Resources in West China
 Kunming Institute of Botany
 Graduate School of the Chinese Academy of Sciences
 132 Lanhei Road
 Kunming 650204
 PR China
 Phone: + 86 87 15 22 33 27
 Fax: + 86 87 15 22 32 55
 mhchiu@mail.kib.ac.cn

Introduction

Cancer has become one of the major causes of mortality in humans throughout the world. In 2007, 7.6 million people died from cancer. Significantly, 27 million new cancer cases and 17.5 million cancer deaths are projected to occur in the world by 2050 [1]. Natural products have been a rich source of antitumor agents, and approximately 60% of currently available drugs are natural compounds or are related to them (from 1940 to 2010) [2].

As one of the three ancient medicinal systems, traditional Chinese medicine (TCM), has gained increasing acceptance and has been recognized by pharmaceutical enterprises as a fountainhead of antitumor drugs [3]. According to the theories of TCM, cancer is caused by imbalances between endogenous physical conditions of the body and exogenous pathogenic factors, including accumulated toxins, heat, and blood stasis [3]. Therefore, the roots of *Cimicifuga foetida*, belonging to the family Ranunculaceae and officially listed in the Chinese Pharmacopoeia with the name “shengma” as a cooling and detoxicating agent [4], were chosen as the object to investigate potential antitumor constituents. As a result, a number of cytotoxic 9,19-cycloartane triterpenes were suc-

cessively isolated from this herb medicine [5–7]. In addition, the preliminary structure-activity relationships (SAR) of the triterpenes with a cimigenol skeleton were proposed [8].

In the Chinese Pharmacopoeia, the roots of *C. dahurica* are another element of “shengma” [4]. Chemical studies on *C. dahurica* showed that it also mainly contains 9,19-cyclolanostane triterpenes [9–16]. Recently, other research groups paid attention to chemical and pharmaceutical properties of the aerial parts of *C. dahurica* and reported that the ethyl acetate fraction of an 80% ethanol extract and three isolated cycloartane triterpenoids displayed growth inhibitory activities against several human tumor cell lines [17, 18]. However, little is known about the cytotoxic triterpenes of the roots of *C. dahurica* [19].

Inspired by the described observations concerning *C. foetida*, we undertook phytochemical and pharmacological investigations on the roots of *C. dahurica*. Twelve new 9,19-cyclolanostane triterpenes (**1–12**), together with fourteen known compounds, cimigenol (**13**) [20], 7(8)-en-cimigenol (**14**) [21], 25-*O*-acetyl-7(8)-en-cimigenol (**15**) [21], cimigenol-3-one (**16**) [22], 24-*epi*-cimigenol-7(8)-en-3-one (**17**) [21], cimigenol-1(2)-en-3-one (**18**) [23], 25-*O*-acetylcimigenol-3-*O*-[2'-*O*-acetyl]- α -L-arabinopyranoside (**19**) [4], 25-anhy-

drocimigenol-3-*O*-[2'-*O*-acetyl]- β -D-xylopyranoside (**20**) [24], 23-*epi*-26-deoxyactein (**21**) [25], actein (**22**) [26], 24-*O*-acetyl-7(8)-*en*-hydroshengmanol (**23**) [27], 24-*O*-acetylisodahurinol-3-*O*-[2'-*O*-acetyl]- α -L-arabinopyranoside (**24**) [25], 23-*O*-acetylshengmanol-3-*O*- β -D-xylopyranoside (**25**) [28], and cimiraemonol B (**26**) [29], were isolated and identified (● Fig. 1). The isolated compounds were evaluated for their cytotoxicities against human HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines, using the MTT method. Described herein are the isolation, structure elucidation, and biological activities of the compounds.

Materials and Methods

General experimental procedures

Optical rotations were measured in MeOH with a Horiba SEAP-300 polarimeter. ^1H and ^{13}C NMR spectra were recorded in pyridine- d_5 on Bruker DRX-500 and Avance III-600MHz spectrometers (Bruker). Unless otherwise specified, chemical shifts (δ) are expressed in ppm with respect to the solvent signals. ESIMS and HR-TOF-ESIMS data were obtained using a VG Autospec-3000 spectrometer. Infrared spectra were recorded on a Shimadzu IR-450 instrument with KBr pellets. Thin-layer chromatography was performed on precoated TLC plates (200–250 μm thickness, silica gel 60 F₂₅₄; Qingdao Marine Chemical, Inc.), and spots were visualized by heating after spraying with 10% aq. H₂SO₄ sol. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a YMC-Pack Pro C18 RS 10 mm \times 250 mm column. Silica gel (200–300 mesh; Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40–63 μm ; Merck), and Sephadex LH-20 (20–150 μm ; Pharmacia) were used for column chromatography (cc).

Plant material

The roots of *Cimicifuga dahurica* M. (0.9 kg) were collected from Qingyuan County, Liaoning Province, China, in September 2006 and identified by Prof. Shengji Pei, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KUN No. 200609004) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, PR China.

Extraction and isolation

The dried and milled roots of *Cimicifuga dahurica* (0.9 kg) were extracted with MeOH (3 \times 3 L \times 24 h) at room temperature to give a residue (106 g) after evaporating *in vacuum* at 50 °C. The extract was subjected to silica gel cc (2 kg, 10 \times 150 cm) and eluted with CHCl₃-MeOH [100:0 (2 L), 50:1 (4 L), 20:1 (5 L), 10:1 (4 L), 0:100 (3 L)] to afford fractions **A** (21.5 g), **B** (13.1 g), **C** (14.5 g), **D** (16.8 g), and **E** (16.2 g). Fraction **B** (13.1 g) was divided into five sub-fractions (**B.1–B.5**) after performing RP-18 cc (180 g, 5 \times 25 cm), eluting with MeOH-H₂O (gradient from 60:40 to 100:0, 10 L). Fraction **B.3** (1.5 g) was subjected to repeated silica gel cc (40 g, 4 \times 40 cm) eluted with CHCl₃-Me₂CO (gradient from 20:1 to 10:1, 4 L) and then to repeated semipreparative HPLC (eluted with CH₃CN-H₂O, gradient from 60:40 to 85:15) to yield **4** (3.0 mg), **5** (3.0 mg), **6** (2.0 mg), **13** (4.0 mg), **16** (4.7 mg), and **26** (3.6 mg). Compounds **7** (2.8 mg), **14** (3.2 mg), **15** (3.5 mg), **17** (2.3 mg), **18** (2.5 mg), and **23** (2.2 mg) were purified from fraction **B.4** (1.8 g) by silica gel cc (30 g, 3.5 \times 40 cm) eluted with CHCl₃-Me₂CO (20:1, 3 L), followed by repeated semipreparative HPLC (eluted with CH₃CN-H₂O, gradient from 65:35 to 85:15). Fraction **B.5** (0.9 g) was applied to a silica gel column (30 g,

3.5 \times 40 cm) eluted with CHCl₃-Me₂CO (20:1, 3 L), then purified over semipreparative HPLC (eluted with CH₃CN-H₂O, gradient from 65:35 to 90:10) to afford **8** (2.7 mg), **9** (1.2 mg), **10** (1.9 mg), and **11** (1.6 mg). Fraction **C** (14.5 g) was fractionated into four subfractions (**C.1–C.4**) by performing RP-18 cc (180 g, 5 \times 25 cm) eluted with MeOH-H₂O (gradient from 60:40 to 90:10, 12 L). Fraction **C.3** (2.8 g) was subjected to silica gel cc (50 g, 4 \times 40 cm) eluted with CHCl₃-Me₂CO (gradient from 10:1 to 5:1, 4 L), then to repeated semipreparative HPLC (eluted with CH₃CN-H₂O, gradient from 60:40 to 75:25) to give **1** (5.5 mg), **2** (4.8 mg), **3** (4.3 mg), **12** (5.3 mg), **19** (3.3 mg), and **24** (28 mg). Fractions **C.1** and **C.2** (4.8 g) were chromatographed on silica gel cc (50 g, 4 \times 40 cm), eluting with CHCl₃-Me₂CO (10:1, 8 L) to yield **20** (5.2 mg), **21** (6.0 mg), **22** (5.2 mg), and **25** (4.2 mg).

*Cimigenol-3-*O*-[2'-*O*-(*E*)-2-butenoyl]- α -L-arabinopyranoside (**1**):* A white powder; $[\alpha]_D^{24} = 18.67$ (c 0.10, MeOH); IR (KBr): $\nu_{\text{max}} = 3452, 2931, 2870, 1727, 1632, 1458, 1383, 1044, 977 \text{ cm}^{-1}$; ^1H (C₅D₅N, 500 MHz) and ^{13}C NMR (C₅D₅N, 150 MHz) spectra, see ● Tables 1 and 2; ESIMS: $m/z = 711$ [M + Na]⁺; HR-TOF-ESIMS: $m/z = 711.4065$ (calc. for C₃₉H₆₀O₁₀Na, 711.4084).

*25-*O*-Acetylcimigenol-3-*O*-[4'-*O*-acetyl]- α -L-arabinopyranoside (**2**):* A white powder; $[\alpha]_D^{24} = 6.67$ (c 0.06, MeOH); IR (KBr): $\nu_{\text{max}} = 3472, 2968, 2870, 1739, 1458, 1374, 1072, 972 \text{ cm}^{-1}$; ^1H (C₅D₅N, 500 MHz) and ^{13}C NMR (C₅D₅N, 125 MHz) spectra, see ● Tables 1 and 2; ESIMS: $m/z = 727$ [M + Na]⁺; HR-TOF-ESIMS: $m/z = 727.4029$ (calc. for C₃₉H₆₀O₁₁Na, 727.4033).

*25-*O*-Acetylcimigenol-3-*O*-[3'-*O*-acetyl]- α -L-arabinopyranoside (**3**):* A white powder; $[\alpha]_D^{24} = -6.80$ (c 0.05, MeOH); IR (KBr): $\nu_{\text{max}} = 3467, 2935, 2870, 1741, 1458, 1375, 1067, 972 \text{ cm}^{-1}$; ^1H (C₅D₅N, 500 MHz) and ^{13}C NMR (C₅D₅N, 125 MHz) spectra, see ● Tables 1 and 2; ESIMS: $m/z = 703$ [M - H]⁻; HR-TOF-ESIMS: $m/z = 703.4055$ (calc. for C₃₉H₅₉O₁₁, 703.4057).

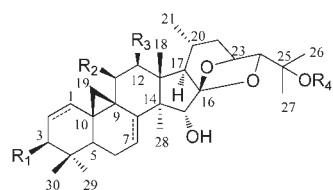
*12 β -Hydroxy-7(8)-*en*-cimigenol (**4**):* A white powder; $[\alpha]_D^{24} = 3.20$ (c 0.10, MeOH); IR (KBr): $\nu_{\text{max}} = 3423, 2931, 2872, 1638, 1447, 1381, 1025, 979 \text{ cm}^{-1}$; ^1H (C₅D₅N, 500 MHz) and ^{13}C NMR (C₅D₅N, 125 MHz) spectra, see ● Tables 1 and 2; ESIMS: $m/z = 525$ [M + Na]⁺; HR-TOF-ESIMS: $m/z = 525.3192$ (calc. for C₃₀H₄₆O₆Na, 525.3192).

*11 β -Hydroxy-7(8)-*en*-cimigenol (**5**):* A white powder; $[\alpha]_D^{24} = 0.47$ (c 0.10, MeOH); IR (KBr): $\nu_{\text{max}} = 3441, 2997, 2882, 1634, 1447, 1380, 1027, 973 \text{ cm}^{-1}$; ^1H (C₅D₅N, 500 MHz) and ^{13}C NMR (C₅D₅N, 125 MHz) spectra, see ● Tables 1 and 2; ESIMS: $m/z = 525$ [M + Na]⁺; HR-TOF-ESIMS: $m/z = 525.3199$ (calc. for C₃₀H₄₆O₆Na, 525.3192).

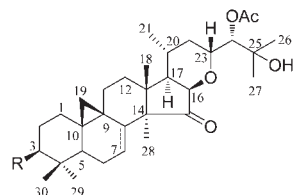
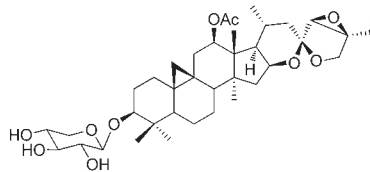
*11 β -Hydroxy-15-deoxycimigenol-7(8)-*en*-3-one (**6**):* A white powder; $[\alpha]_D^{24} = -23.33$ (MeOH, c 0.05); IR (KBr): $\nu_{\text{max}} = 3441, 2969, 2869, 1711, 1633, 1382, 975 \text{ cm}^{-1}$; ^1H (C₅D₅N, 500 MHz) and ^{13}C NMR (C₅D₅N, 150 MHz) spectra, see ● Tables 1 and 2; ESIMS: $m/z = 507$ [M + Na]⁺; HR-TOF-ESIMS: $m/z = 507.3090$ (calc. for C₃₀H₄₄O₅Na, 507.3086).

*Cimigenol-7(8)-*en*-3-one (**7**):* A white powder; $[\alpha]_D^{24} = -21.67$ (c 0.10, MeOH); IR (KBr): $\nu_{\text{max}} = 3492, 2984, 2839, 1709, 1637, 1452, 1382, 1024, 987 \text{ cm}^{-1}$; ^1H (C₅D₅N, 500 MHz) and ^{13}C NMR (C₅D₅N, 125 MHz) spectra, see ● Tables 1 and 2; ESIMS: $m/z = 507$ [M + Na]⁺; HR-TOF-ESIMS: $m/z = 507.3086$ (calc. for C₃₀H₄₄O₅Na, 507.3086).

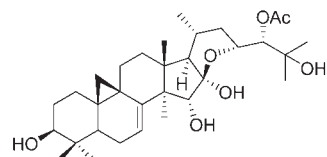
*Cimigenol-1(2),7(8)-*dien*-3-one (**8**):* A white powder; $[\alpha]_D^{24} = -60.60$ (c 0.10, MeOH); IR (KBr): $\nu_{\text{max}} = 3432, 2947, 2852, 1693, 1637, 1460, 1382, 1070, 978 \text{ cm}^{-1}$; ^1H (C₅D₅N, 500 MHz) and ^{13}C NMR (C₅D₅N, 125 MHz) spectra, see ● Tables 1 and 2; ESIMS: $m/z = 505$ [M + Na]⁺; HR-TOF-ESIMS: $m/z = 505.2944$ (calc. for C₃₀H₄₂O₅Na, 505.2929).



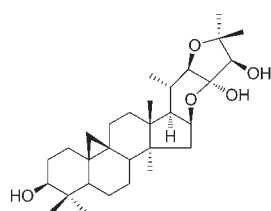
- 1 $R_1 = O\text{-}2'\text{-}O\text{-}(E)\text{-}2\text{-butenyl-}\alpha\text{-L-arabinose}$, $R_2 = H$, $R_3 = H$, $R_4 = H$, 24 *S*
 2 $R_1 = O\text{-}4'\text{-}O\text{-acetyl-}\alpha\text{-L-arabinose}$, $R_2 = H$, $R_3 = H$, $R_4 = Ac$, 24 *S*
 3 $R_1 = O\text{-}3'\text{-}O\text{-acetyl-}\alpha\text{-L-arabinose}$, $R_2 = H$, $R_3 = H$, $R_4 = Ac$, 24 *S*
 4 $R_1 = OH$, $R_2 = H$, $R_3 = OH$, $R_4 = H$, Δ 7(8), 24 *S*
 5 $R_1 = OH$, $R_2 = OH$, $R_3 = H$, $R_4 = H$, Δ 7(8), 24 *S*
 7 $R_1 = O$, $R_2 = H$, $R_3 = H$, $R_4 = H$, Δ 7(8), 24 *S*
 8 $R_1 = O$, $R_2 = H$, $R_3 = H$, $R_4 = H$, Δ 1(2), Δ 7(8), 24 *S*
 13 $R_1 = OH$, $R_2 = H$, $R_3 = H$, $R_4 = H$, 24 *S*
 14 $R_1 = OH$, $R_2 = H$, $R_3 = H$, $R_4 = H$, Δ 7(8), 24 *S*
 15 $R_1 = OH$, $R_2 = H$, $R_3 = H$, $R_4 = Ac$, Δ 7(8), 24 *S*
 16 $R_1 = O$, $R_2 = H$, $R_3 = H$, $R_4 = H$, 24 *S*
 17 $R_1 = O$, $R_2 = H$, $R_3 = H$, $R_4 = H$, Δ 7(8), 24 *R*
 18 $R_1 = O$, $R_2 = H$, $R_3 = H$, $R_4 = H$, Δ 1(2), 24 *S*
 19 $R_1 = O\text{-}2'\text{-}O\text{-acetyl-}\alpha\text{-L-arabinose}$, $R_2 = H$, $R_3 = H$, $R_4 = Ac$, 24 *S*
 27 $R_1 = O\text{-}\alpha\text{-L-arabinose}$, $R_2 = H$, $R_3 = H$, $R_4 = H$, 24 *S*

10 $R = H$ 11 $R = H$, Δ 7(8)24 $R = O\text{-}2'\text{-}O\text{-acetyl-}\alpha\text{-L-arabinose}$ 29 $R = O\text{-acetyl-}\alpha\text{-L-arabinose}$ 

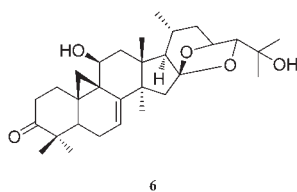
21



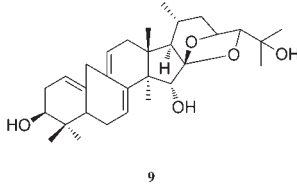
23



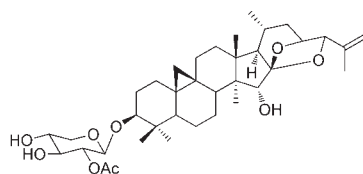
26



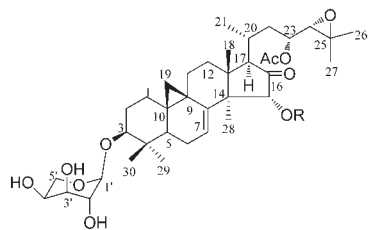
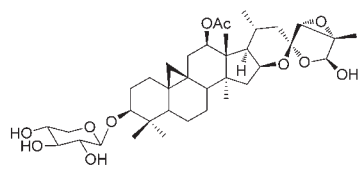
6



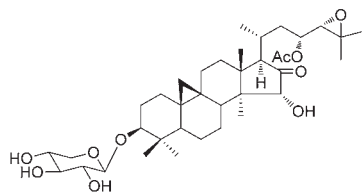
9



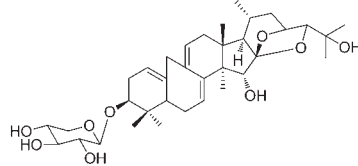
20

12 $R = Ac$ 30 $R = H$ 

22



25



28

Fig. 1 Structures of compounds 1–30.

Table 1 ^1H NMR data of compounds **1–12** in pyridine- d_5 at 500 MHz.

Proton	1	2	3	4	5	6	7	8	9	10	11	12
1	1.53 ^a 1.17 ^a	1.54 m 1.22 ^a	1.54 m 1.16 ^a	1.70 m 1.35 m	2.78 m 1.71 m	2.99 m 1.88 m	1.85 m 1.54 m	6.71 d (10.0)	5.54 brs	1.53 m 1.14 m	1.68 m 1.24 m	1.64 m 1.30 ^a
2	2.28 m 1.87 m	2.34 m 1.96 ^a	2.34 dd (4.0, 16.0) 1.95 ^a	1.92 m 1.86 brs	2.11 m 1.93 m	2.84 m 2.35 m	2.75 ddd (6.5) 2.29 m	6.16 d (10.0)	2.52 m 2.26 m	1.99 m 1.86 dd (3.2, 10.0)	1.95 m 1.89 m	2.36 m 1.95 m
3	3.39 dd (4.0, 11.5)	3.50 dd (5.0, 14.5)	3.47 dd (5.0, 14.2)	3.50 dd (3.5, 11.0)	3.57 dd (5.0, 10.5)				3.78 m	3.48 m	3.51 m	3.49 dd (3.5, 10.5)
4												
5	1.28 dd (3.0, 12.3)	1.30 ^a	1.29 m	1.25 dd (5.0, 12.0)	1.31 dd (5.0, 12.0)	1.64 m	1.54 m	1.84 dd (5.0, 12.5)	2.36 m	1.25 dd (3.2, 10.0)	1.20 ^a	1.27 ^a
6	1.48 ^a 0.66 m	1.52 m 0.70 m	1.49 m 0.69 m	1.94 m 1.68 m	1.92 m 1.77 m	1.82 m 1.64 m	1.70 m 1.54 m	1.75 m 1.60 m	2.64 m 2.36 m	1.51 m 0.65 m	1.93 m 1.56 m	1.76 m 1.43 m
7	2.06 m 1.11 ^a	2.07 m 1.07 ^a	2.09 m 1.04 ^a	6.20 d (7.0)	6.21 d (6.5)	5.23 m	6.05 brd (7.0)	6.11 d (7.0)	5.52 brd (8.0)	2.27 m 1.95 m	6.36 brd (6.5)	5.33 d (7.0)
8	1.65 ^a	1.66 ^a	1.66 ^a									
9										1.72 brd (10.4)		
10												
11	2.06 m 1.01 m	2.07 m 1.15 ^a	2.09 m 1.15 ^a	2.96 dd (9.0, 15.0) 1.54 ^a	4.58 brd (8.5)	4.53 m	2.13 m 1.16 ^a	2.23 m 1.40 m	5.39 brd (5.0)	2.23 m 1.05 ^a	1.98 m 1.13 ^a	2.11 m 1.16 m
12	1.65 ^a 1.53 ^a	1.66 ^a 1.52 m	1.64 ^a 1.52 m	4.36 t (6.5)	2.74 m 2.11 m	2.73 m 1.99 m	1.81 m 1.68 m	1.81 m 1.70 m	2.21 m 1.94 dd (6.0, 12.5)	1.58 m 1.37 m	1.65 m 1.29 m	1.89 m 1.78 m
13												
14												
15	4.26 brs	4.27 brs	4.25 brs	4.76 brs	4.62 s	2.46 d (13.0) 2.28 d (13.0)	4.55 brd (8.0)	4.54 d (9.0)	2.36 m 2.09 m			5.92 s
16										3.79 d (9.6)	3.83 d (11.2)	
17	1.48 ^a	1.44 brd (14.0)	1.45 brd (14.0)	1.86 brs	1.48 ^a	1.59 d (12.0)	1.50 ^a	1.49 ^a	1.53 ^a	1.52 m	1.20 m	2.34 d (8.0)
18	1.13 s	1.13 s	1.13 s	1.51 s	1.27 s	1.26 s	1.16 s	1.10 s	0.81 brs	1.18 s	1.20 s	1.29 s
19	0.45 brs 0.21 brs	0.50 d (4.0) 0.25 d (4.0)	0.52 d (4.0) 0.28 d (4.0)	1.19 ^a 0.73 d (4.0)	1.97 d (4.0) 1.07 brd (2.5)	2.11 d (4.0) 1.11 d (4.0)	1.19 ^a 0.67 d (4.0)	1.52 d (4.8) 1.04 d (4.0)	3.24 d (13.5) 3.15 d (13.5)	0.52 d (3.6) 0.29 d (3.6)	1.07 ^a 0.48 d (4.0)	0.99 ^a 0.51 d (3.5)
20	1.65 ^a	1.67 ^a	1.59 m	1.86 m	1.64 brd (10.5)	1.66 brd (9.5)	1.70 m	1.68 m	1.60 m	1.80 m	1.81 m	2.10 m
21	0.84 d (7.0)	0.83 d (6.4)	0.84 d (6.4)	1.41 d (5.5)	0.82 d (6.0)	0.83 d (6.0)	0.89 d (6.5)	0.89 d (7.0)	0.81 brs	0.91 d (7.2)	0.93 d (6.5)	1.19 d (6.5)
22	2.26 m 1.01 m	2.25 m 0.97 m	2.26 m 0.95 m	2.42 m 1.16 ^a	2.22 m 0.99 m	2.24 m 0.99 t (11.5)	2.30 m 1.07 m	2.28 m 1.07 m	2.21 m 0.96 ^a	1.72 brd (10.4) 1.44 m	1.73 m 1.47 m	2.77 t (12.0) 1.68 m
23	4.74 d (9.0)	4.59 d (11.5)	4.59 brd (12.0)	4.80 d (9.0)	4.73 d (9.0)	4.79 d (8.5)	4.78 brd (9.0)	4.77 t (8.0)	4.77 brd (9.0)	4.25 brd (11.2)	4.27 d (11.5)	5.38 brt (9.5)
24	3.76 brs	4.11 brs	4.11 brs	3.87 s	3.77 s	3.71 s	3.80 brs	3.80 s	3.69 brs	5.31 d (1.6)	5.33 d (2.0)	3.03 d (8.0)
25												cont.

Table 1 Continued

Proton	1	2	3	4	5	6	7	8	9	10	11	12
26	1.48 s	1.68 s	1.68 s	1.52 s	1.47 s	1.51 s	1.48 s	1.49 s	1.52 s	1.61 s	1.62 s	1.25 s
27	1.46 s	1.66 s	1.66 s	1.51 s	1.45 s	1.46 s	1.49 s	1.48 s	1.47 s	1.61 s	1.63 s	1.39 s
28	1.16 s	1.18 s	1.18 s	1.46 s	1.36 s	1.39 s	1.38 s	1.36 s	1.28 s	1.01 s	1.13 s	1.34 s
29	1.08 s	1.31 s	1.22 s	1.17 s	1.19 s	1.16 s	1.04 s	1.15 s	1.31 s	1.19 s	1.21 s	1.31 s
30	0.95 s	1.04 s	1.00 s	1.07 s	1.14 s	1.12 s	1.07 s	0.99 s	0.98 s	1.05 s	1.07 s	1.02 s
3-Ara												
1'	4.79 d (7.5)	4.79 d (9.0)	4.88 d (8.0)									4.79 d (7.0)
2'	5.99 t (8.5)	4.38 t (10.0)	4.63 brd (6.0)									4.18 m
3'	4.23 brd (9.5)	4.24 m	5.48 dd (4.5, 11.0)									4.47 t (8.0)
4'	4.30 brs	5.50 brs	4.57 brs									4.33 brs
5'	4.28 m 3.79 brd (12.0)	4.24 m 3.81 d (16.0)	4.30 m 3.85 d (13.5)									4.31 brd (9.5) 3.79 d (12.5)
15-OCOCH ₃												2.26 s
23-OCOCH ₃												2.02 s
24-OCOCH ₃										2.16 s	2.18 s	
25-OCOCH ₃		1.96 s	1.95 s									
3'-OCOCH ₃			1.96 s									
4'-OCOCH ₃		1.96 s										
4'- OCOCH=C- H-CH ₃	6.05 d (15.5)											
4'- OCOCH=C- H-CH ₃	7.09 m											
4'- OCOCH=C- H-CH ₃	1.63 d (6.5)											

Chemical shifts are in δ scale with J values in parentheses. ° Signals overlapped

9,10-Seco-1(10),7(8),9(11)-trien-cimigenol (9): A white powder; $[\alpha]_D^{24} = 5.73$ (c 0.05, MeOH); IR (KBr): $\nu_{\max} = 3432, 2947, 2852, 1624, 1460, 1383, 1070, 978 \text{ cm}^{-1}$; ^1H ($\text{C}_5\text{D}_5\text{N}$, 500 MHz) and ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 150 MHz) spectra, see **Tables 1 and 2**; ESIMS: $m/z = 469$ [$\text{M} + \text{H}$] $^+$; HR-TOF-ESIMS: $m/z = 469.3311$ (calc. for $\text{C}_{30}\text{H}_{45}\text{O}_4$, 469.3317).

24-O-Acetylisdahurinol (10): A white powder; $[\alpha]_D^{24} = 2.09$ (c 0.07, MeOH); IR (KBr): $\nu_{\max} = 3473, 2946, 2862, 1738, 1471, 1377, 1024, 994 \text{ cm}^{-1}$; ^1H ($\text{C}_5\text{D}_5\text{N}$, 500 MHz) and ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 125 MHz) spectra, see **Tables 1 and 2**; ESIMS: $m/z = 553$ [$\text{M} + \text{Na}$] $^+$; HR-TOF-ESIMS: $m/z = 553.3494$ (calc. for $\text{C}_{32}\text{H}_{50}\text{O}_6\text{Na}$, 553.3505).

24-O-Acetyl-7(8)-en-isdahurinol (11): A white powder; $[\alpha]_D^{24} = -4.80$ (c 0.05, MeOH); IR (KBr): $\nu_{\max} = 3437, 2920, 2875, 1736, 1640, 1472, 1375, 1026, 996 \text{ cm}^{-1}$; ^1H ($\text{C}_5\text{D}_5\text{N}$, 500 MHz) and ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 125 MHz) spectra, see **Tables 1 and 2**; ESIMS: $m/z = 551$ [$\text{M} + \text{Na}$] $^+$; HR-TOF-ESIMS: $m/z = 551.3346$ (calc. for $\text{C}_{32}\text{H}_{48}\text{O}_6\text{Na}$, 551.3348).

23-O-Diacetyl-7(8)-en-shengmanol-3-O- α -L-arabinopyranoside (12): A white powder; $[\alpha]_D^{24} = -7.69$ (MeOH, c 0.07); IR (KBr): $\nu_{\max} = 3473, 2942, 2871, 1736, 1643, 1458, 1376, 1024, 972 \text{ cm}^{-1}$; ^1H ($\text{C}_5\text{D}_5\text{N}$, 500 MHz) and ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 150 MHz) spectra,

see **Tables 1 and 2**; ESIMS: $m/z = 725$ [$\text{M} + \text{Na}$] $^+$; HR-TOF-ESIMS: $m/z = 725.3895$ [$\text{M} + \text{Na}$] $^+$ (calc. for $\text{C}_{39}\text{H}_{58}\text{O}_{11}\text{Na}$, 725.3876).

Hydrolysis and identification of the sugar moieties in compounds 1, 2, 3, and 12

Compound **1** (4.0 mg) together with **2** and **3** (3.0 mg of each) were individually dissolved in MeOH (5 mL), then 4% K_2CO_3 (5 mL) was added, and each solution was stirred at rt overnight. Each solution was neutralized by 10% HOAc and extracted with EtOAc ($3 \times 15 \text{ mL}$). Each EtOAc extract, after removal of solvent, was dissolved in MeOH (5 mL) and refluxed with 0.5 N HCl (3 mL) for 4 h [7]. Compound **12** (4.0 mg), by contrast, was directly dissolved in MeOH (5 mL) and refluxed with 0.5 N HCl (3 mL) for 4 h. Each reaction mixture was diluted with H_2O and extracted with CHCl_3 ($3 \times 10 \text{ mL}$). Each aqueous layer was then neutralized by Ag_2CO_3 , and the formed precipitation was filtered to give a monosaccharide, which had an R_f (EtOAc- CHCl_3 -MeOH- H_2O , 3:2:2:1) and specific rotation $[\alpha]_D^{20} + 82.78$ (c 0.05, MeOH) corresponding to those of L-arabinose (Sigma-Aldrich).

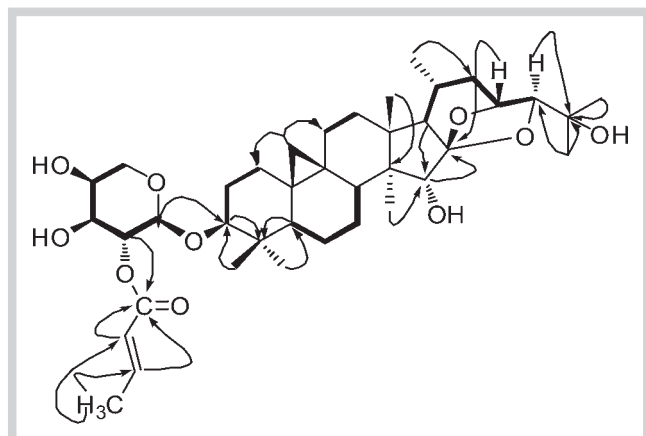


Fig. 2 Major HMBC (→) and ^1H - ^1H COSY (---) correlations of compound **1**.

Cytotoxicity bioassay

Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480, were used in the cytotoxic assay. Cells were cultured in DMEM medium (Hyclone) supplemented with 10% fetal bovine serum (Hyclone), in 5% CO_2 at 37°C . The cytotoxicity assay was performed according to the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] method in 96-well microplates [32,33]. Briefly, $100\ \mu\text{L}$ of adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before drug addition with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compounds (dissolved in DMSO, then diluted by DMEM medium) at concentrations of 0.064, 0.32, 1.6, 8, and $40\ \mu\text{M}$ in triplicate for 48 h, with cisplatin (Sigma) and culture solution as positive and negative controls, respectively. After compound treatment, cell viability was detected, and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method [34].

Supporting information

The NMR spectra of compounds **1–12**, as well as ESI and HR-TOF-ESI data of **1–12** are available as Supporting Information.

Results and Discussion

Compound **1** was obtained as white powder. Its molecular formula ($\text{C}_{39}\text{H}_{60}\text{O}_{10}$) was deduced from the analysis of ^{13}C NMR and HR-TOF-ESIMS data $\{m/z: 711.4065\ [\text{M} + \text{Na}]^+ \text{ (calc. for } \text{C}_{39}\text{H}_{60}\text{O}_{10}\text{Na, } 711.4084)\}$. The IR spectrum showed absorptions for hydroxy groups at $3452\ \text{cm}^{-1}$, carbonyl groups at $1727\ \text{cm}^{-1}$, and double bonds at $1632\ \text{cm}^{-1}$, respectively. The ^1H -NMR spectrum (Table 1) showed the presence of the characteristic cyclopropane methylene signals at δ_{H} 0.21 and 0.45 (each 1H, brs), an anomeric proton at δ_{H} 4.79 (d, $J = 7.5\ \text{Hz}$), two olefinic protons at δ_{H} 6.05 (1H, d, $J = 15.5\ \text{Hz}$) and 7.09 (1H, m), two secondary methyl signals at δ_{H} 0.84 (d, $J = 7.0\ \text{Hz}$) and 1.63 (d, $J = 6.5\ \text{Hz}$), and six tertiary methyl groups at δ_{H} 0.95–1.48. In the ^{13}C and DEPT NMR spectra of **1** (Table 2), the signals ascribable to an α,β -unsaturated ketone moiety at δ_{C} 166.3, 122.3, and 145.2 were observed. A comparison of the spectroscopic data of **1** with those of cimige-

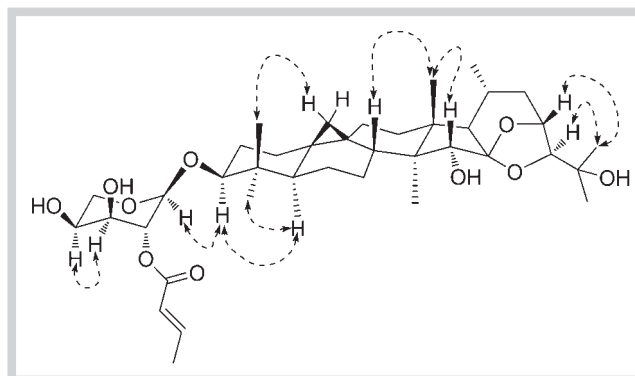


Fig. 3 Key NOESY correlations of compound **1**.

nol-3-*O*- α -L-arabinopyranoside (**27**) [15] showed that, structurally, **1** closely resembles **27**, with the main differences of the sugar unit and the presence of another tetra-carbon unit, including the α,β -unsaturated ketone resonances. In the ^1H - ^1H COSY spectrum (Fig. 2), a correlation was observed between the secondary methyl signal at δ_{H} 1.63 (d, $J = 6.5\ \text{Hz}$) and the olefinic proton at δ_{H} 7.09 (m), which indicated the tetra-carbon unit to be a 2-butenoyl. In addition, the coupling constant ($J = 15.6\ \text{Hz}$) of the two olefinic protons at δ_{H} 6.05 and 7.09 confirmed the *E*-geometry of a double bond in the 2-butenoyl moiety. In the HMBC spectrum (Fig. 2), a correlation was observed between the anomeric proton at δ_{H} 4.79 (H-1', 1H, d, $J = 7.5\ \text{Hz}$) and the methine signal at δ_{C} 89.2 (C-3), suggesting that a sugar unit was attached at the C-3. The sugar obtained after acid hydrolysis was identified as α -L-arabinose by comparing its TLC and specific rotation with the standard. In the ^1H NMR spectrum, a downfield resonance was observed at δ_{H} 5.99 (t, $J = 8.5\ \text{Hz}$), which showed correlations with the methine resonance at δ_{H} 4.30 (H-3') and the anomeric proton at δ_{H} 4.79 in the ^1H - ^1H COSY spectrum (Fig. 2). Furthermore, the HMBC (Fig. 2) correlation between the carbonyl group (δ_{C} 166.3) and the proton resonance (δ_{H} 5.99, t, $J = 8.5\ \text{Hz}$) indicated the (*E*)-2-butenoyl unit was attached at C-2'. In the ROESY spectrum (Fig. 3), H-3 showed a correlation with H-5 suggesting an α -orientation of the H-3, while H-15 showed a correlation with Me-18, indicating an α -orientation of the hydroxyl group at C-15. The configuration of C-23 and C-24 was deduced as *R* and *S*, respectively, by comparison of the coupling constant of H-23 and H-24 with those of cimigenol-type compounds [6,22,26]. Therefore, the structure of **1** was determined as cimigenol-3-*O*-[2'-*O*-(*E*)-2-butenoyl]- α -L-arabinopyranoside.

Compounds **2** and **3** were determined to have the same molecular formula $\text{C}_{39}\text{H}_{60}\text{O}_{11}$ by HR-TOF-ESIMS (m/z 727.4029 [$\text{M} + \text{Na}]^+$ and 703.4055 [$\text{M} - \text{H}$], respectively). The NMR spectroscopic data (Tables 1 and 2) of **2** and **3** were similar to those of 25-*O*-acetyl-cimigenol-3-*O*-[2'-*O*-acetyl]- α -L-arabinopyranoside (**19**), except for the sugar moiety. In the ^1H -NMR spectrum (Table 1) of **2**, the signal due to H-4' showed a downfield shift from δ_{H} 4.16 to 5.50. Meanwhile, the signal of H-2' was shifted from δ_{H} 5.89 to δ_{H} 4.38. In addition, the signal due to C-4' exhibited a downfield shift from δ_{C} 69.8 to 72.3 in the ^{13}C NMR spectrum (Table 2). The changes of these chemical shifts may be explained by the *O*-acetyl group being attached to C-4 of the sugar unit, which was further confirmed by the presence of the HMBC correlation between the H-4' signal at δ_{H} 5.50 and the carbonyl group signal at δ_{C} 170.8. The sugar obtained after acid hydrolysis was confirmed

as α -L-arabinopyranose by comparing its TLC and specific rotation with the standard. The configurations of C-23 and C-24 are proposed as *R* and *S*, respectively, by the same way as that of **1**. Thus, the structure of **2** was assigned as 25-*O*-acetylcimigenol-3-*O*-[4'-*O*-acetyl]- α -L-arabinopyranoside. In the same way, an acetoxy group was determined to be at C-3' for **3**, which was further confirmed by the presence of the HMBC correlation between the H-3' signal at δ_{H} 5.48 and the carbonyl group signal at δ_{C} 170.8. Therefore, **3** was identified as 25-*O*-acetylcimigenol-3-*O*-[3'-*O*-acetyl]- α -L-arabinopyranoside.

Compound **4** gave a molecular formula of $\text{C}_{30}\text{H}_{46}\text{O}_6$ as established by HR-TOF-ESIMS (m/z 525.3192 [$\text{M} + \text{Na}$] $^+$), which is 16 Da more than those of 7(8)-en-cimigenol (**14**). The ^1H NMR spectrum (Table 1) displayed downfield cyclopropane methylene signals at δ_{H} 0.73 (1H, *d*, $J = 4.0$ Hz) and 1.19 (overlapped), seven methyl groups at δ_{H} 1.07–1.52, and an olefinic proton at δ_{H} 6.20 (1H, *d*, $J = 7.0$ Hz), respectively, suggesting **4** to be a 9,19-cyclolanostane aglycone with a pair of olefinic carbons close to the cyclopropane methylene at C-19 [20]. The NMR data of **4** showed a close resemblance with those of **14**, except that the methylene signal at δ_{C} 33.7 (C-12) was absent, showing instead a hydroxymethine at δ_{C} 72.5. On the basis of the above observations, it was reasonable to deduce that **4** was a 12-hydroxy derivative of **14**, which was also supported by the HMBC correlations of H-12 at δ_{H} 4.36 with C-11 (δ_{C} 40.3), C-13 (δ_{C} 47.1), and CH_3 -18 (δ_{C} 13.2), as well as by the downfield shift of C-11 about 14.9 ppm in the ^{13}C spectrum (Table 2). Significant ROESY correlations of H-12 with H-5, and CH_3 -28 suggested a β -orientation of the substituent at C-12. Therefore, **4** was elucidated as 12 β -hydroxy-7(8)-en-cimigenol.

Compound **5** was assigned as $\text{C}_{30}\text{H}_{46}\text{O}_6$, as deduced from the HR-TOF-ESIMS (m/z 525.3199 [$\text{M} + \text{Na}$] $^+$), which is identical to that of compound **4**. The NMR data of **5** were similar to that of **4** with the major difference being that a hydroxyl group was shifted from C-12 to C-11. In the ^{13}C NMR spectrum (Table 2) of **5**, the signal due to C-12 exhibited an upfield shift from δ_{C} 72.5 to 49.4, while C-11 showed a downfield shift from δ_{C} 40.3 to 63.5, further confirming the deduction. The relative configuration of the hydroxyl group at C-11 was proposed as β -orientated by analyses of the ROESY spectrum. Accordingly, compound **5** was characterized as 11 β -hydroxy-7(8)-en-cimigenol.

Compound **6** had the molecular formula $\text{C}_{30}\text{H}_{44}\text{O}_5$ from its positive mode HR-TOF-ESIMS (507.3090 [$\text{M} + \text{Na}$] $^+$). In the ^{13}C and DEPT spectra (Table 2), **6** exhibited signals very similar to those of **5** except that hydroxy methine signals due to C-3 and C-15 were absent, whereas a carbonyl carbon at δ_{C} 216.1 and a downfield methylene at δ_{C} 45.8 were observed. In addition, HMBC correlations of H-2, CH_3 -29, and CH_3 -30 with the carbonyl group at δ_{C} 216.1 and protons (δ_{H} 2.46 and 2.28, each 1H) due to the methylene at δ_{C} 45.8 with C-14, C-16, and CH_3 -28 indicated that a carbonyl carbon replaced a hydroxyl group at C-3, and the methine at C-15 was transformed to a methylene in **6**. Ultimately, **6** was elucidated as 11 β -hydroxy-15-deoxycimigenol-7(8)-en-3-one.

The HR-TOF-ESIMS (m/z 507.3086 [$\text{M} + \text{Na}$] $^+$) of compound **7** determined its molecular formula as $\text{C}_{30}\text{H}_{44}\text{O}_5$, which is identical with 24-*epi*-cimigenol-7(8)-en-3-one (**17**). The NMR data (Tables 1 and 2) of **7** resembled those of **17** with major differences at C-22 (δ_{C} 38.0), C-23 (δ_{C} 72.1), C-24 (δ_{C} 90.3), and C-25 (δ_{C} 70.9), which are similar to the key structural differences between cimigenol-type and 24-*epi*-cimigenol-type triterpenes [21]. By comparing the coupling constant of H-24 (0 Hz) with those of cimigenol-type compounds (0 Hz) and 24-*epi*-cimigenol-type constituents (4 Hz), the configuration of C-24 of **7** was determined to

be *S* [6,21,25]. Thus, **7** was elucidated as cimigenol-7(8)-en-3-one.

The molecular formula of compound **8** was determined as $\text{C}_{30}\text{H}_{42}\text{O}_5$ from the HR-TOF-ESIMS (m/z 505.2944 [$\text{M} + \text{Na}$] $^+$). Its NMR data (Tables 1 and 2) were similar to those of **7** except for the signals of ring A. Unsaturated carbon signals at δ_{C} 152.6 and 127.2 were observed in **8**, whereas signals of two methylenes due to C-1 and C-2 were absent. Significant HMBC correlations were observed between the carbonyl C-atom at δ_{C} 203.8 and the olefinic protons at δ_{H} 6.16 and 6.71 (each 1H, *d*, $J = 10.0$ Hz). The above evidence suggested that compound **8** is transformed from **7** through dehydrogenation between C-1 and C-2. Therefore, compound **8** was characterized as cimigenol-1(2),7(8)-dien-3-one.

Compound **9** was assigned a molecular formula of $\text{C}_{30}\text{H}_{46}\text{O}_4$ from its HR-TOF-ESIMS (m/z 469.3311 [$\text{M} + \text{H}$] $^+$). In the ^1H NMR spectrum, signals for the significant downfield cyclopropane methylene at δ_{H} 3.15 and 3.24 (1H each, *d*, $J = 14.0$ Hz), seven methyl groups at δ_{H} 0.81–1.52, and three olefinic protons at δ_{H} 5.39, 5.52, and 5.54 were observed, suggesting **9** is a 9,10-*seco*-9,19-cycloartane triterpene [30]. The ^{13}C NMR and DEPT spectroscopic data of **9** were identical with the aglycone resonances of cimicinal (**28**) [31], except for the upfield shift of the C-3 by 9.7 ppm, which could be explained by the absence of a sugar unit at C-3. Therefore, **9** was elucidated as 9,10-*seco*-1(10),7(8),9(11)-trien-cimigenol.

The molecular formula of compound **10** was established as $\text{C}_{32}\text{H}_{50}\text{O}_6$ on the basis of HR-TOF-ESIMS (m/z 553.3494 [$\text{M} + \text{Na}$] $^+$). In the IR spectrum, absorption bands at 3473 and 1733 cm^{-1} for hydroxyl and carbonyl groups were observed. In the ^{13}C and DEPT NMR spectra, the signals ascribable to the methylene carbon of the cyclopropane ring at δ_{C} 31.3 (C-19), four oxygen-bearing methine carbons at δ_{C} 84.3 (C-16), 79.8 (C-24), 79.1 (C-23), and 77.9 (C-3), as well as two carbonyl carbons at δ_{C} 213.9 (C-15) and 171.1 (C-24-acetoxy) were observed, suggesting that **10** was a highly oxygenated 9,19-cycloartane triterpene aglycon with an *O*-acetyl group. By comparison of NMR spectroscopic data, **10** was determined to be the aglycon of 24-*O*-acetylisodahurinol-3-*O*- α -L-arabinopyranoside (**29**) [7]. The configuration of C-24 was deduced as *S* by comparison of the coupling constants of H-24 (1.6 Hz) with those of dahuriny diacetate (9 Hz) and isodahuriny diacetate (2 Hz) [29]. Accordingly, compound **10** was characterized as 24-*O*-acetylisodahurinol.

Compound **11** gave a molecular formula of $\text{C}_{32}\text{H}_{48}\text{O}_6$ by HR-TOF-ESIMS at m/z 551.3346 [$\text{M} + \text{Na}$] $^+$. The NMR spectroscopic data (Tables 1 and 2) of **11** resembled those of **10** except for major differences at C-7 (δ_{C} 115.0) and C-8 (δ_{C} 141.9) due to dehydrogenation at these positions. This deduction was confirmed by the correlations of δ_{H} 6.36 (H-7) with δ_{H} 1.56 and 1.93 (H-6) in the ^1H - ^1H COSY spectrum. Accordingly, compound **11** was characterized as 24-*O*-acetyl-7(8)-en-isodahurinol.

Compound **12** exhibited the molecular formula $\text{C}_{39}\text{H}_{58}\text{O}_{11}$, as established by HR-TOF-ESIMS at m/z 725.3895 [$\text{M} + \text{Na}$] $^+$. The molecular weight of **12** is 42 Da more than that of 23-*O*-acetyl-7(8)-en-shengmanol-3-*O*- α -L-arabinopyranoside (**30**) [27], which may be due to an acetyl group. When its spectroscopic data (Tables 1 and 2) were compared with those of **30** [27], an additional *O*-acetyl group was assigned to C-15 on the basis of the upfield shift of the carbonyl carbon (C-16) from δ_{C} 220.3 to 214.2, the downfield shift of H-15 from δ_{H} 4.56 to 5.92, as well as the HMBC correlation of H-15 and the carbonyl group signal at δ_{C} 170.9. Significant ROESY correlations of H-15 with CH_3 -18 sug-

Table 3 Cytotoxicity^a (IC₅₀, $\mu\text{M} \pm \text{SD}$) of compounds isolated from the roots of *C. dahurica*.

Compounds	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	4.2 \pm 0.4	13.4 \pm 1.1	13.8 \pm 0.8	14.2 \pm 0.7	11.8 \pm 1.3
2	8.1 \pm 0.5	12.2 \pm 1.2	14.5 \pm 0.7	12.7 \pm 1.4	13.1 \pm 0.9
3	5.8 \pm 0.2	8.7 \pm 0.3	10.3 \pm 1.1	12.6 \pm 0.5	11.3 \pm 1.2
4	> 40	> 40	> 40	> 40	> 40
5	> 40	> 40	> 40	> 40	> 40
6	18.9 \pm 1.6	19.5 \pm 0.7	21.1 \pm 1.8	23.7 \pm 1.5	20.3 \pm 1.7
7	21.1 \pm 1.5	18.3 \pm 1.7	20.5 \pm 1.0	19.2 \pm 0.9	> 40
8	21.9 \pm 2.2	17.4 \pm 1.8	19.5 \pm 0.8	17.5 \pm 1.3	22.5 \pm 1.2
9	> 40	> 40	> 40	> 40	> 40
10	> 40	> 40	> 40	> 40	> 40
11	> 40	> 40	> 40	> 40	> 40
12	> 40	> 40	> 40	> 40	> 40
13	> 40	> 40	> 40	> 40	> 40
14	> 40	> 40	> 40	> 40	> 40
15	10.5 \pm 0.9	22.5 \pm 1.3	19.1 \pm 2.1	20.6 \pm 1.4	13.8 \pm 0.8
16	20.2 \pm 0.7	22.9 \pm 1.5	20.4 \pm 1.7	21.1 \pm 1.2	21.3 \pm 1.4
17	> 0	> 40	> 40	> 40	> 0
18	20.2 \pm 0.8	22.9 \pm 1.7	20.4 \pm 1.3	15.1 \pm 1.2	> 40
19	5.8 \pm 0.7	8.7 \pm 0.8	10.3 \pm 2.1	12.6 \pm 1.7	11.3 \pm 0.7
20	8.1 \pm 0.6	12.2 \pm 1.4	14.5 \pm 1.2	12.7 \pm 2.3	13.1 \pm 1.5
21	> 40	> 40	> 40	> 40	> 40
22	> 40	> 40	> 40	> 40	> 40
23	> 40	> 40	> 40	> 40	> 40
24	> 40	> 40	> 40	> 40	> 40
25	> 40	> 40	> 40	> 40	> 40
26	> 40	> 40	> 40	> 40	> 40
Cisplatin	0.52 \pm 0.05	13.4 \pm 0.6	12.4 \pm 0.7	15.0 \pm 1.4	14.4 \pm 1.1

^a Cytotoxicity is the average (n = 3) of calculated IC₅₀s; the purity of compounds **1–26** is greater than 95% and of cisplatin greater than 99%

gested an α -orientation of the substituent at C-15. The configurations of C-23 and C-24 were considered to be *R* and *S*, respectively, by comparing coupling constants of H-23 and H-24 with those of known 9,19-cyclolanostane triterpene glycosides [25]. In the ¹³C NMR spectrum (Table 2), compound **12** showed resonances corresponding to an α -L-arabinose moiety at δ_{C} 108.1 (d), 73.4 (d), 75.2 (d), 70.2 (d), and 66.5 (t) [8,29], which was further confirmed by comparing its TLC and specific rotation with the standard after hydrolysis. Ultimately, **12** was elucidated as 15,23-O-diacetyl-7(8)-en-shengmanol-3-O- α -L-arabinopyranoside.

All isolated compounds were screened for their *in vitro* antitumor activities. As summarized in Table 3, the new compounds **1–3** and the known compounds **19** and **20** showed broad-spectrum and moderate cytotoxicities against human HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines, with IC₅₀ values ranging from 4.2 to 14.5 μM . In addition, the new compounds **6–8** and the known compounds **15**, **16**, and **18** exhibited broad-spectrum and weak cytotoxicities, having IC₅₀ values around 20 μM . Based on the above results, we suggest that the roots of *C. dahurica* may be another potential resource for promising antitumor agents.

In the present study, structural and bioactive properties of five cimigenol-type glycosides (**1–3**, **19**, **20**) are completely in accordance with the SAR we proposed before. Thus, the SAR proposed in our previous studies may be used for the design of more potent lead compounds. Furthermore, six cimigenol-type aglycons (**6–8**, **15**, **16**, **18**) exhibited broad-spectrum and weak cytotoxicities. The main structural characters of these compounds are: (1) the configurations of C-23 and C-24 are *R* and *S*, respectively; (2) carbonyl and acetoxy groups instead of a hydroxyl group at C-3 or C-25. Previously, we reported that cimigenol-type aglycone actrin-

3-one has potent and moderate activities against human HepG-2 and HT 29 cell lines, respectively. Meanwhile, 25-O-acetylcimigenol exhibited moderate activity against the human HepG-2 cell line [7]. Based on the analyses of these data, we may propose that for cimigenol-type aglycones, hydrophobic groups, such as carbonyl and acetoxy, instead of a hydroxyl group at C-3 or C-25 are essential for cytotoxicity.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (No. U1132604), Knowledge Innovation Program of the CAS (Grant No. KZCX2-XB2-15-03, KSCX2-EW-R-15), as well as Top Talents Program of Yunnan province (2009C1120), and the Foundation of State Key Laboratory of Phytochemistry and Plant Resources in West China (P2010-ZZ14).

Conflict of Interest

All authors declare that there are no conflicts of interest. The isolation and structural elucidations were accomplished by Yin Nian, Hai-Yan Wang, Lin Zhou, and Ming-Hua Qiu, and the cell culture and cytotoxicity assay were performed by Jia Su and Yan Li. All the authors knew about this manuscript and had no objection to submitting it.

References

- Pan L, Chai HY, Kinghorn AD. The continuing search for antitumor agents from higher plants. *Phytochem Lett* 2010; 3: 1–8
- Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 2012; 75: 311–335
- Hsiao WLW, Liu L. The role of traditional Chinese herbal medicines in cancer therapy from TCM theory to mechanistic insights. *Planta Med* 2010; 76: 1118–1131
- Chinese Pharmacopoeia Commission. The Pharmacopoeia of Chinese People's Republic. 2010 edition. Beijing: The Chemical Industry Publishing House; 2010: 68–69
- Sun LR, Qing C, Zhang YL, Ji SY, Li ZR, Pei SJ, Qiu MH, Gross ML, Qiu SX. Cimicifugosides A and B, two cytotoxic cycloartane triterpenoid glycosides from the rhizomes of *Cimicifuga foetida*, inhibit proliferation of cancer cells. *Beilstein J Org Chem* 2007; 3: 1–6
- Lu L, Chen JC, Song HJ, Li Y, Nian Y, Qiu MH. Five new triterpene bisglycosides with acyclic side chains from the rhizomes of *Cimicifuga foetida* L. *Chem Pharm Bull* 2009; 58: 729–733
- Nian Y, Zhang YL, Chen JC, Lu L, Qing C, Qiu MH. Cytotoxic chemical constituents from the roots of *Cimicifuga foetida*. *J Nat Prod* 2010; 73: 93–98
- Nian Y, Zhang XM, Li Y, Wang YY, Chen JC, Lu L, Zhou L, Qiu MH. Cycloartane triterpenoids from the aerial parts of *Cimicifuga foetida* Linnaeus. *Phytochemistry* 2011; 72: 1473–1481
- Sakurai N, Inoue T, Nagai M. Studies on the Chinese crude drug “Sho-ma.” II. Triterpenes of *Cimicifuga dahurica* MAXIM. *Yakugaku Zasshi* 1972; 92: 724–728
- Kimura O, Sakurai N, Inoue T. Studies on the Chinese crude drug “Sho-ma.” VII. Isolation and determination of genuine natural products, acetyl shengmanol xyloside, 24-O-acetylhydroshengmanol xyloside, and shengmanol xyloside, in *Cimicifuga dahurica* and the other *Cimicifuga* plants. *Yakugaku Zasshi* 1983; 103: 293–299
- Li CJ, Chen DH, Xiao PG. Studies on the chemical constituents from the aerial parts of *Cimicifuga dahurica*. *Acta Pharm Sin* 1993; 28: 777–781
- Li CJ, Chen DH, Xiao PG. Chemical constituents of traditional Chinese drug “sheng-ma” (*Cimicifuga dahurica*). II. chemical structure of cimicifugamide. *Acta Chim Sin* 1994; 52: 296–300
- Li CJ, Chen DH, Xiao PG. Chemical constituents of traditional Chinese drug “sheng-ma” (*Cimicifuga dahurica*) III structures of cimicide C and cimicide D. *Acta Chim Sin* 1994; 52: 722–726
- Zhang QW, Ye WC, Hsiao W, Zhao SX, Che CT. Cycloartane glycosides from *Cimicifuga dahurica*. *Chem Pharm Bull* 2001; 49: 1468–1470
- Ye WC, Zhang JW, Che CT, Ye T, Zhao SX. Cycloartane glycosides from *Cimicifuga dahurica*. *Planta Med* 1999; 65: 770–772
- Liu Y, Chen DH, Si JY, Tu GZ, An DG. Two new cyclolanostanol xylosides from the aerial parts of *Cimicifuga dahurica*. *J Nat Prod* 2002; 65: 1486–1488
- Tian Z, Yang MS, Huang F, Li KG, Si JY, Shi L, Chen SB, Xiao PG. Cytotoxicity of three cycloartane triterpenoids from *Cimicifuga dahurica*. *Cancer Lett* 2005; 226: 65–75
- Tian Z, Si JY, Chang Q, Zhou L, Chen SL, Xiao PG, Wu E. Antitumor activity and mechanisms of action of total glycosides from aerial part of *Cimicifuga dahurica* targeted against hepatoma. *BMC Cancer* 2007; 7: 237–246
- Einbonda LS, Ye WC, Hec K, Wu HL, Cruz E, Rollerc M, Kronenberg M. Growth inhibitory activity of extracts and compounds from *Cimicifuga* species on human breast cancer cells. *Phytomedicine* 2007; 15: 504–511
- Sun LR, Yan J, Pei SJ, Qiu MH. A new cycloartane triterpenoid from the rhizome of *Cimicifuga foetida* collected in Dali. *Acta Bot Yunn* 2005; 27: 331–336
- Li JX, Kadota S, Hattori M, Yoshimachi S, Shiro M, Oogami N, Mizuno H, Namba T. Isolation and characterization of ten new cycloartenol triterpenes from *Cimicifuga heracleifolia* Komarov. *Chem Pharm Bull* 1993; 41: 832–841
- Gao JC, Zhang JC, Lu ZJ, Zhu GY, Yang MS, Xiao PG. Chemical constituents of *Actaea asiatica* Hara and their anti-osteoporosis activities. *Biochem Syst Ecol* 2006; 34: 710–713
- Kusano A, Takahira M, Shibano M, Miyase T, Okuyama T, Kusano G. Studies on the constituents of *Cimicifuga* species. XXII. Structure of two new cyclolanostanol xylosides, cimiacerosides A and B. *Heterocycles* 1998; 48: 1003–1013
- Zhou L, Yang JS, Tu GZ, Zou JH. Cyclolanostane triterpene glycosides from *Souliea vaginata*. *Chem Pharm Bull* 2006; 54: 823–826
- Chen SN, Fabricant DS, Lu ZZ, Fong HHS, Farnsworth NR. Cimicacemosides I–P, new 9,19-cyclolanostane triterpene glycosides from *Cimicifuga racemosa*. *J Nat Prod* 2002; 65: 1391–1397
- Kusano A, Takahira M, Shibano M, In Y, Ishida T, Miyase T, Kusano G. Studies on the constituents of *Cimicifuga* species. XX. Absolute stereostructures of cimicifugoside and actein from *Cimicifuga simplex* WORMSK. *Chem Pharm Bull* 1998; 46: 467–472
- Kusano A, Shibano M, Kitagawa S, Kusano G. Studies on the constituents of *Cimicifuga* species. XIX. Eight new glycosides from *Cimicifuga simplex* WORMSK. *Chem Pharm Bull* 1996; 44: 2078–2085
- Kusano A, Shibano M, Kitagawa S, Kusano G. Studies on the constituents of *Cimicifuga* species. XV. Two new diglycosides from the aerial parts of *Cimicifuga simplex* WORMSK. *Chem Pharm Bull* 1994; 42: 1940–1943
- Shao Y, Harris A, Wang M, Zhang H, Cordell GA, Bowman M, Lemmo E. Triterpene glycosides from *Cimicifuga racemosa*. *J Nat Prod* 2000; 63: 905–910
- Ali Z, Khan SI, Fronczek FR, Khan IA. 9,10-seco-9,19-cyclolanostane arabinosides from the roots of *Actaea podocarpa*. *Phytochemistry* 2007; 68: 373–382
- Kadota S, Li JX, Tanaka K, Namba T. Constituents of *Cimicifugae* rhizoma II. Isolation and structures of new cycloartenol triterpenoids and related compounds from *Cimicifuga foetida* L. *Tetrahedron* 1995; 51: 1143–1166
- Mossmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Immunol Methods* 1983; 65: 55–63
- Alley MC, Scudiero DA, Monks A. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res* 1988; 48: 589–601
- Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. *Am J Hyg* 1938; 27: 493–497