New Monoterpene Lactones from *Actaea cimicifuga*

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Abstract

Three new monoterpene lactones, cimicifugolides A–C (**1**–**3**), along with a known one (**4**), were identified from the dried rhizome of *Actaea cimicifuga* L. that was used as traditional Chinese medicine for thousands of years with the Chinese common name of *shengma*. The structures of the new isolates were established using spectroscopic methods, including NMR, mass, UV, and IR spectra. The inhibition activity of compounds **1, 2**, and **4** against pancreatic lipase was evaluated.

Key words

Actaea cimicifuga L. · *shengma* · Ranunculaceae · monoterpene · cimicifugolides A–C · traditional Chinese medicine

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Actaea cimicifuga L. (syn. *Cimicifuga foetida* L.) is an Asian perennial herb in the buttercup family (Ranunculaceae) and a sister member of black cohosh (*Actaea racemosa* L.). The dried roots and rhizomes of *A. cimicifuga*, known as *shengma* in the Chinese Pharmacopoeia, have been used as a traditional Chinese medicine (TCM) for clearing excessive heat, detoxifying the body, and relieving exterior syndrome by dispersion [1]. Our field work in Northwest Yunnan (October–November, 2009) showed that some ethnic minorities in China, such as the Tibetan, Yi, and Bai people have used *A. cimicifuga* for a very long time in the treatment of headaches, toothaches, sore throats, measles, swelling, and gynecological diseases.

Previous studies on *A. cimicifuga* have revealed the occurrence of 9,19-cyclolanostane triterpenoid glycosides and cinnamic acid derivatives with a wide range of biological activities such as anti-tumor, anti-human immunodeficiency virus (HIV), anti-complement, and anti-inflammation [2–8]. In recent TCM practices, *shengma* is used for improving lipid profiles and cardiovascular health, and isoferulic acid isolated from *A. cimicifuga* showed activity in reducing blood glucose concentration of diabetic mice [9]. However, the biologically active constituents related with lowering serum cholesterol and triglycerides, as well as with resistance to atherosclerosis are not clear.

In our research, three new monoterpene lactones, cimicifugolides A–C (**1**–**3**), and one known monoterpene (3*S*)-4-*α*-hydroxy-3-(2-hydroxyethylidene)-5-*β*-(2-methylprop-1-enyl) dihydrofuran-2-one (**4**) were isolated from roots and rhizomes of *A. cimicifuga* (**Fig. 1**). It is the first time that monoterpenes have been identified in this species. In this paper, we describe the isolation and structural elucidation of the three new monoterpene lactones as well as their ability to inhibit pancreatic lipase *in vitro*.

The molecular formula $C_{10}H_{14}O_4$ of compound **1** was assigned from HRESIMS *m/z* 221.0790 ([M + Na]$^+$), indicating 4 degrees of unsaturation. Its IR spectrum indicated the presence of hydroxyl (3431 cm^{-1}) and lactone carbonyl (1746 cm^{-1}) groups. The ^{13}C NMR spectrum (**Table 1**) of **1** showed ten signals for one ester carbonyl group, two trisubstituted double bonds, three oxygenated sp3 carbons, and two methyl groups. Because three degrees of unsaturation were accounted for one carbonyl and two double bonds, the molecular structure of **1** must possess one ring, which implied that **1** might be a monoterpene lactone. Comparison of NMR data with those of the known compound **4** and the aglycon of kodemariosides C, D, and F revealed that **1** was likely the analogue of compound **4** [10] with an *E*-configured exocyclic double bond. The planar structure of **1** was confirmed by the HMBC spectrum of **1** (**Fig. 2**). The *trans* configuration of H-4 and H-5 was elucidated by the correlations of H_3-10/H-4 and H-4/H-6 in the ROESY spectrum (**Fig. 3**). On the basis of the above results, the structure of **1** was assigned as rel-(3*R*,4*S*,*E*)-3-hydroxy-2-hydroxyethylidene-6-methylhept-5-en-1,4-olide, and **1** was given the trivial name cimicifugolide A.

The molecular formula $C_{11}H_{18}O_5$ of compound **2** was assigned from HREIMS *m/z* 230.1143, indicating 3 degrees of unsaturation. Its IR spectrum indicated the presence of hydroxy (3407 cm^{-1}) and lactone carbonyl (1749 cm^{-1}) groups. The ^{13}C NMR spectrum (**Table 1**) of **2** showed the presence of 11 carbon signals, namely, one ester carbonyl group, one trisubstituted double bond, five oxygenated sp^3 carbons including one methoxy group, and two methyl groups. The NMR data of **2** were similar to those of **1** except that the signals for one double bond had disappeared in **2** and three signals for two methine and one methoxy groups were observed in the ^{13}C NMR spectrum of **2**. A molecular fragment from C-1 to C-6 was easily established by the ^{1}H–^{1}H COSY spectrum of **2** (**Fig. 2**). Furthermore, the planar structure of **2** was elucidated as shown in **Fig. 2** by its HMBC spectrum. The *trans*-configuration of H-4 and H-5 was deduced by the cor-

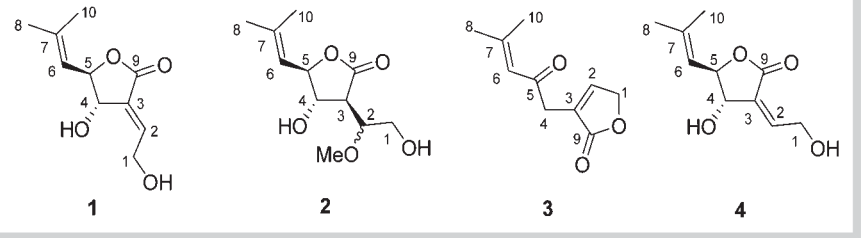

Fig. 1 Chemical structures of **1**–**4**.

Table 1 ^{1}H and ^{13}C NMR data for **1**–**3** in $CDCl_3$ (δ in ppm, J in Hz).

Position	1		2		3	
	δ_C[a]	δ_H[b]	δ_C[c]	δ_H[d]	δ_C[a]	δ_H[b]
1	61.5 (CH_2)	4.60 (2H, m)	62.2 (CH_2)	4.10 (1H, dd, 12.1, 2.7) 3.62 (1H, d, 12.1)	70.7 (CH_2)	4.80 (2H, m)
2	142.6 (CH)	6.88 (1H, dd, 5.8, 3.0)	76.7 (CH)	3.87 (1H, br s)	148.1 (CH)	7.42 (1H, br s)
3	130.4 (qC)		53.4 (CH)	2.71 (1H, dd, 9.4, 2.2)	127.7 (qC)	
4	72.6 (CH)	4.82 (1H, br s)	72.2 (CH)	4.30 (1H, dd, 9.4, 8.6)	39.3 (CH_2)	3.39 (2H, m)
5	81.8 (CH)	5.02 (1H, dd, 9.1, 4.1)	79.5 (CH)	4.85 (1H, t, 8.6)	194.7 (qC)	
6	120.8 (CH)	5.15 (1H, d, 9.1)	120.4 (CH)	5.24 (1H, d, 8.6)	122.9 (CH)	6.07 (1H, br s)
7	141.5 (qC)		142.9 (qC)		158.2 (qC)	
8	25.8 (CH_3)	1.78 (3H, s)	25.9 (CH_3)	1.80 (3H, s)	27.8 (CH_3)	1.86 (3H, br s)
9	170.4 (qC)		174.3 (qC)		174.2 (qC)	
10	18.6 (CH_3)	1.79 (3H, s)	18.6 (CH_3)	1.79 (3H, s)	21.0 (CH_3)	2.10 (3H, br s)
OMe			58.1 (CH_3)	3.40 (3H, s)		

[a] Measured at 125 MHz; [b] measured at 500 MHz; [c] measured at 100 MHz; [d] measured at 400 MHz

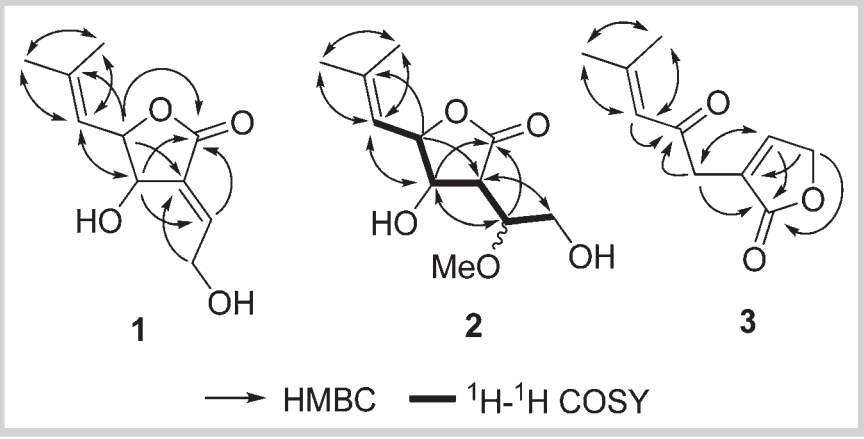

Fig. 2 Key 2D NMR correlations for **1**–**3**.

relations of H_3-10/H-5 and H-4/H-6 in the ROESY spectrum (**Fig. 3**). Additionally, the *cis*-configuration of H-3 and H-5 was determined by the correlations between H-3 and H-5 (**Fig. 3**). Thus, the structure of **2** (cimicifugolide B) was assigned as rel-(2*R*,3*S*,4*R*)-3-hydroxy-2-(hydroxy-1-methoxyethyl)-6-methyl-hept-5-en-1,4-olide.

The molecular formula $C_{10}H_{12}O_3$ of compound **3** was assigned from HREIMS *m/z* 180.0783, indicating 5 degrees of unsaturation. The ^{13}C NMR (**Table 1**) spectrum revealed 10 carbon signals: two carbonyl groups, two trisubstituted double bonds, two methylene groups, and two methyl groups. Compound **3** also possessed the characteristics of monoterpene lactones. Based on its HMBC spectrum (**Fig. 2**), the structure of **3** (cimicifugolide C) was assigned as 2-(4-methyl-2-oxopent-3-enyl)-but-2-en-1,4-olide.

The ability of cimicifugolides A and B, and (3*S*)-4-α-hydroxy-3-(2-hydroxyethyl-idene)-5-β-(2-methylprop-1-enyl) dihydrofuran-2-one to inhibit pancreatic lipase was examined in this study. The standard anti-obesity drug, orlistat, displayed an IC_{50} of 1.60 ± 0.18 µg/mL in the lipase assay, while cimicifugolide A and (3*S*)-4-α-hydroxy-3-(2-hydroxyethyl-idene)-5-β-(2-methylprop-1-enyl) dihydrofuran-2-one, which are configurational isomers, showed IC_{50} values of 130.07 ± 10.14 µg/mL and 356.28 ± 43.67 µg/mL, respectively. Cimicifugolide B showed an even lower lipase inhibitory activity with IC_{50} value of 1749.82 ± 27.31 µg/mL. Although the lipase-inhibitory activity of these compounds is not strong, it might be interesting to investigate their potential involvement in the recent popular TCM practices of *shengma* to improve lipid profiles [11].

Materials and Methods

Optical rotations were determined on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu double-beam 210A spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 infrared spectrophotometer with KBr disks. ESIMS and HR-ESIMS analyses were carried out on an API Qsta Pulsar 1 instrument. EIMS and HREIMS were carried out on a Waters Autospec Premier P776 mass spectrometer. 1D and 2D NMR spectra were recorded on Bruker AM-400, 500 MHz and DRX-500 spectrometers with TMS as an internal standard. Column chromatography was performed over silica gel (80–100, 200–300, and 300–400 mesh; Qingdao Makall Group Co., Ltd.), Sephadex LH-20 (40–70 µm; Amersham Pharmacia Biotech AB), C_{18} silica gel (40 µm; Fuji Silysia Chemical Ltd.), and MCI gel CHP 20P (polystyrene type, 75–150 µm; Mitsubishi Chemical Corporation). TLC was conducted on precoated silica gel plates GF 254 (Qingdao). TLC spots were visualized under UV light and detected by spraying with 5% H_2SO_4 in EtOH, followed by heating.

Roots and rhizomes of *A. cimicifuga* were collected from Shangri-La, Yunnan Province, China, in November 2009. The plant specimen was collected and identified by Professor Chunlin Long, and

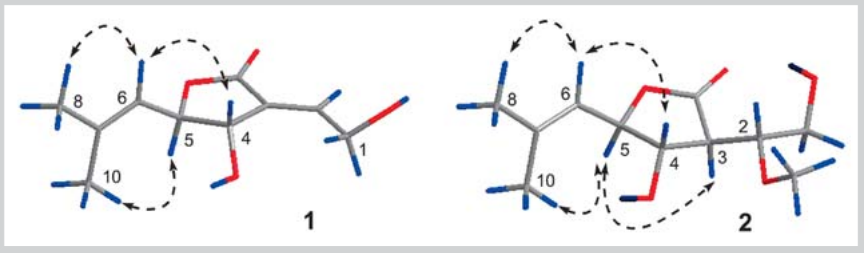

Fig. 3 Key ROESY correlations for **1** and **2**. (Color figures available online only.)

a voucher specimen was deposited at the herbarium at the Kunming Institute of Botany, Chinese Academy of Sciences (shortly KIB) (LCL-0931). Three *A. cimicifuga* plants from the same plant population were grown in the botanical garden at KIB to verify their identification.

The air-dried, milled roots and rhizomes of *A. cimicifuga* (5.5 kg) were extracted exhaustively with MeOH (3 × 15 L, 4, 3, and 3 h, respectively) under reflux. The extracts were evaporated to give a residue, which was suspended with water and then successively partitioned with ethyl acetate (600 mL) three times. The EtOAc-soluble part (339 g) was separated over a silica gel column chromatography (80 × 10 cm, 80–100 mesh), using a $CHCl_3$–MeOH gradient (v : v: 1 : 0, 20 : 1, 10 : 1, 8 : 1, 5 : 1, 3 : 1, 0 : 1, 3 L each). The fractions Fr. B ($CHCl_3$–MeOH 20 : 1, between 2.1 L and 3.0 L) and Fr. C ($CHCl_3$–MeOH 10 : 1, between 0 L and 1.0 L) were combined as Fr. $(BC)_1$ (27 g) and then subjected to MCI gel CHP 20P column chromatography (80 × 10 cm, polystyrene type, 75–150 µm) eluted with H_2O–MeOH gradient (v : v: 100 : 0, 20 : 80, 10 : 90, 0 : 100, 1.5 L each). The fraction Fr. $(BC)_{12}$ (9.5 g, H_2O–MeOH 20 : 80, between 0 L and 0.5 L) was subjected to C_{18} silica gel column chromatography (4 × 50 cm, LiChroprep RP–18, 40 µm), using a H_2O–MeOH gradient (v : v: 95 : 5, 90 : 10, 85 : 15, …→5 : 95, 0.8 L each), and yield the fraction Fr. $(BC)_{12(3)}$ (0.451 g, MeOH–H_2O 25 : 75, between 0.4 L and 0.8 L). Fr. $(BC)_{12(3)}$ was further fractionated by Sephadex LH-20 column chromatography (2.5 × 200 cm, Sephadex LH-20, 1 L) in MeOH to give two subfractions, Fr. $(BC)_{12(3)1}$ (0.101 g, between 0.1 L and 0.2 L) and Fr. $(BC)_{12(3)2}$ (0.127 g, between 0.25 L and 0.35 L). Fr. $(BC)_{12(3)1}$ was subjected to silica gel column chromatography (40 × 2 cm, 200–300 mesh) eluted with $CHCl_3$–Me_2CO gradient (v : v: 50 : 1, 45 : 1, 10 : 1, 8 : 1, 5 : 1, 2 : 1, 0.1 L each) to give compounds **2** (13.0 mg; $CHCl_3$–Me_2CO 8 : 1, between 0.02 L and 0.05 L) and **3** (3.4 mg, $CHCl_3$–Me_2CO 50 : 1, between 0.05 L and 0.07 L). Fr. $(BC)_{12(3)2}$ was subjected to silica gel column chromatography (40 × 2 cm, 200–300 mesh) eluted with $CHCl_3$–MeOH gradient (v : v 150 : 1, 120 : 1, 80 : 1, 50 : 1, 20 : 1, 0.1 L each) to give compounds **1** (50.0 mg, $CHCl_3$–MeOH 80 : 1, between 0.02 L and 0.065 L) and **4** (13.0 mg, $CHCl_3$–MeOH 20 : 1, between 0.03 L and 0.06 L). The purity of compounds **1**–**4** was greater than 95% as determined by TLC and NMR.

Isolates

Cimicifugolide A (**1**): pale yellow oil; $[\alpha]_D^{25}$ + 16.5 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.61) nm; IR (KBr) ν_{max} 3431, 2925, 1746, 1639, 1321, 1207, 1033, 778 cm^{-1}; ^1H and ^{13}C NMR data, see **Table 1**; ESIMS *m/z* 221 $[M + Na]^+$; HRESIMS *m/z* 221.0790 $[M + Na]^+$ (calcd. for $C_{10}H_{14}O_4Na$, 221.0789).

Cimicifugolide B (**2**): yellow oil; $[\alpha]_D^{25}$ − 19.0 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.26) nm; IR (KBr) ν_{max} 3407, 2919, 1749, 1378, 1198, 1123, 1033, 967 cm^{-1}; ^1H and ^{13}C NMR data, see **Table 1**; EI *m/z* (%) 230 (9) $[M]^+$, 156 (35), 138 (47), 123 (27), 99 (58), 87 (77), 69 (12), 55 (100); HREIMS *m/z* 230.1143 (calcd. for $C_{11}H_{18}O_5$, 230.1154).

Cimicifugolide C (**3**): yellow oil; ^1H and ^{13}C NMR data, see **Table 1**; EI *m/z* (%) 180 (24) $[M]^+$, 163 (52), 97 (57), 83 (97), 67 (59); HREIMS *m/z* 180.0783 (calcd. for $C_{10}H_{12}O_3$, 180.0786).

Lipase inhibition activity assay was done according to the method described by Liu et al. [12] with minor modifications which were described in Supporting Information.

Supporting information

1D and 2D NMR spectra for the new compounds (**1**–**3**), the lipase inhibition activity assay, and a flowchart for the isolation of chemical constituents from *Actaea cimicifuga* are available as Supporting Information.

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Conflict of Interest

There were no conflicts of interests among all authors in this manuscript.

References

1 The Pharmacopoeia of People's Republic of China. Beijing: The Chemical Industry Publishing House; 2000: 55
2 *Cao P, Pu XF, Peng SL, Zhang XR, Ding LS.* Chemical constituents from *Cimicifuga foetida*. J Asian Nat Prod Res 2005; 7: 145–149
3 *Sun LR, Qing C, Zhang YL, Jia SY, Li ZR, Pei SJ, Qiu MH, Gross ML, Qiu SX.* Cimicifoetisides A and B, two cytotoxic cycloartane triterpenoid glycosides from the rhizomes of *Cimicifuga foetida*, inhibit proliferation of cancer cells. Beilstein J Org Chem 2007; DOI: 10.10.1186/1860-5397-3-3
4 *Qiu M, Kim JH, Lee HK, Min BS.* Anticomplement activity of cycloartane glycosides from the rhizome of *Cimicifuga foetida*. Phytother Res 2006; 20: 945–948
5 *Tian Z, Pan RL, Chang Q, Xiao PG, Wu E. Cimicifuga foetida* extract inhibits proliferation of hepatocellular cells via induction of cell cycle arrest and apoptosis. J Ethnopharmacol 2007; 114: 227–233
6 *Li JX, Yu ZY.* Cimicifugae rhizoma: from origins, bioactive constituents to clinical outcomes. Curr Med Chem 2006; 13: 2927–2951

7 *Ou S, Kwok KC.* Ferulic acid: pharmaceutical functions, preparation and applications in foods. J Sci Food Agric 2004; 84: 1261–1269
8 *Yamahara J, Kobayashi M, Kimura H.* Biologically active principles of crude drugs. The effect of *Cimicifugae* rhizoma and constituents in preventive action on the carbon tetrachloride-induced liver disorder in mice. Shoyakugaku Zasshi 1985; 39: 80–84
9 *Liu IM, Chi TC, Hsu FL, Chen CF, Cheng JT.* Isoferulic acid as active principle from the rhizome of *Cimicifuga dahurica* to lower plasm a glucose in diabetic rats. Planta Med 1999; 65: 712–714
10 *Yoshida K, Hishida A, Iida O, Hosokawa K, Kawabata J.* Highly oxygenated monoterpene acylglucosides from *Spiraea cantoniensis*. J Nat Prod 2010; 73: 814–817
11 *Zhang HJ.* Which traditional Chinese medicine can reduce blood sugar? News Paper Med Health Care 2004: 10
12 *Liu DZ, Wang F, Liao TG., Tang JG, Steglich W, Zhu HJ, Liu JK.* Vibralactone: a lipase inhibitor with an unusual fused beta-lactone produced by cultures of the basidiomycete *Boreostereum vibrans*. Org Lett 2006; 8: 5749–5752

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Bibliography
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