PHYTOTHERAPY RESEARCH
Phytother. Res. **20**, 945–948 (2006)
Published online 14 August 2006 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.1982

Anticomplement Activity of Cycloartane Glycosides from the Rhizome of *Cimicifuga foetida*

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A tetranor-cycloartane glycoside and two 9,19-cycloartane glycosides were isolated from the EtOAc-soluble fraction of the rhizome of *Cimicifuga foetida*. The structures of the compounds were determined to be cimilactone A (1), 25-*O*-acetylcimigenol 3-*O*-β-D-xylopyranoside (2) and cimigenol 3-*O*-α-L-arabinopyranoside (3), respectively, using spectroscopic analysis. The three compounds were examined for their anticomplement activity against the classical pathway of the complement system. Compound 1 showed significant anticomplement activity with an IC_{50} value of 28.6 μM, whereas compounds 2 and 3 were inactive. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: Cimicifuga foetida; cycloartane-type triterpenoid; anticomplement activity; cimilactone A.

INTRODUCTION

The complement system is a major effector of humoral immunity and is activated by a cascade mechanism via an antigen–antibody mediated process (classical pathway, CP), an antibody independent process (alternative pathway, AP), or through mannan binding lectin/MBL-associated serine protease (MBL/MASP) (Kirschfink, 1997). The proteolytic cascade allows for a very high amplification rate, which in the next step activates the enzymes later in the cascade. This in turn cleaves the non-enzymes, such as C3, C4 and C5. The pathway converges the C3 convertase step leading to C5 convertase and the self-assembly of the membrane attack complex (MAC). In complement activation, the complement components induce the release of mediators from the mast cells and lymphocytes, causing a variety of diseases (i.e. rheumatoid arthritis, osteoarthritis, atopic dermatitis, lung fluid inflammation and atherosclerotic lesion), and as can be fatal if occurring after organ transplantation (Lichtman and Pober, 1997). This effect is normally beneficial for the host, but can also cause adverse effects depending on the site and the extent and duration of complement activation. The modulation of complement activity can be important in the treatment of inflammation.

The complement inhibiting property of the EtOAc-soluble fraction of the rhizome of *Cimicifuga foetida* L. (Ranunculaceae) was examined as part of an ongoing study to detect natural products with anticomplementary activity. The rhizome of this plant is used in Chinese traditional medicine as an antiinflammatory, antipyretic and analgesic (Zhang *et al.*, 2001). Another species, *C. racemosa* (black cohosh), is used primarily as a 'hormone-free' phytomedicine for the treatment of climacteric symptoms related to menopause in Europe and North America (Chen *et al.*, 2002c). A literature survey showed that there were two main classes of compounds isolated from the roots/rhizomes of *Cimicifuga species*, 9,19-cycloartane triterpene glycosides and aromatic acids (Chen *et al.*, 2002a). To date, more than 20 triterpenoid glycosides have isolated from this species, with cimicigenol glycosides (Ye *et al.*, 1999), cycloart-7-enes (Kusano *et al.*, 2001), trinorcycloartane glycosides (Zhang *et al.*, 2001), actaeaepoxide (Wende *et al.*, 2001), acteins (Watanabe *et al.*, 2002), cimiracemosides (Shao *et al.*, 2000; Watanabe *et al.*, 2002; Chen *et al.*, 2002b), cycloartanes (Nishida *et al.*, 2003a), tetranor-cycloartane glycosides (Nishida *et al.*, 2003b) and 15,16-seco-cycloartane glycosides (Nishida *et al.*, 2003c) exhibiting cytotoxic and immunosuppressive activities. This study is part of an ongoing investigation into anticomplementary active compounds from herbal medicines. In this study, a tetranor-cycloartane glycoside and two 9,19-cycloartane glycosides were isolated from the rhizome of *C. foetida*. This paper deals with the structural determination of the cycloartane glycosides along with their anticomplement activity.

MATERIALS AND METHODS

General experimental procedures. Melting points were determined on XRC-1 apparatus and were uncorrected. Optical rotations were obtained on a Jasco-20C digital polarimeter. FABMS was determined on a VG Auto Spec-3000 mass spectrometer. 1D NMR and 2D NMR spectra were measured on a Bruker AV-400 MHz

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Received 6 March 2006
Revised 12 June 2006
Accepted 27 June 2006

instrument, and chemical shifts (δ) were reported with the solvent (pyridine-d_5) as the reference. Silica gel (200–300 mesh and 10–40 μm) was used for column chromatography. Thin-layer chromatography was performed on TLC plates (GF_{254}. Qingdao, People's Republic of China). The spots were visualized by spraying with 20% H_2SO_4 and followed by heating. Reversed-phase column chromatography was carried out on Merck lobar lichroprep RP-18 columns (EM Science, Germany).

Plant material. The rhizome of *C. foetida* was collected in Dali county of Yunnan province, China, in September 2000, and was identified by Professor Wang Z. Y. A voucher specimen (Kun 0273201) was deposited in the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and isolation. The dried rhizome (2.8 kg) of *C. foetida* was extracted exhaustively with 90% MeOH (3 × 10 L) under reflux. The MeOH extract was evaporated to yield a black syrup-like residue (459 g) under reduced pressure. A sample (439 g) of the residue was suspended in water–MeOH (9:1, 1300 mL) and fractionated by successive partition with EtOAc (3 × 1800 mL) and *n*-BuOH (3 × 1800 mL) to give EtOAc-soluble (214 g) and *n*-BuOH-soluble fractions (82 g). A portion (200 g) of the EtOAc-soluble fraction was subjected to silica gel column chromatography (7.5 × 120 cm, 200–300 mesh, 1200 g), eluted with a $CHCl_3$–MeOH (10:0, 9:1, 8:2, 7:3, 6:4 0:10; each 10 L) gradient system to yield six fractions: A (oil), B (14 g), C (9 g), D (88 g), E (43 g) and F (12 g). Fraction D (88 g) was subjected to silica gel CC (7.5 × 120 cm, 200–300 mesh, 800 g), eluted with $CHCl_3$–MeOH (40:1, 25:1, 10:1 each 5 L) to give two subfractions (fr. D_1 and fr. D_2). Fraction D_1 (2.9 g) was rechromatographed over silica gel column (4 × 125 cm, 200–300 mesh, 80 g) eluted with $CHCl_3$–MeOH (40:1, 2.5 L) and RP-18 (3 × 40 cm, 65 g) eluted with MeOH–H_2O (70:30, 2 L) to afford compound **1** (16 mg), and then eluted with MeOH–H_2O (80:20, 2 L) to yield compound **2** (214 mg). Fraction D_2 (27 g) was rechromatographed on silica gel (3 × 40 cm, 400 g) eluted with $CHCl_3$–MeOH (25:1, 1.5 L) and RP-18 (3 × 40 cm, 65 g) and developed with MeOH–H_2O (70:30, 80:20, each 2 L) to provide compound **3** (146 mg).

*Cimilactone A (**1**).* Colorless needles, mp 255–256 °C; $[\alpha]^D$ –36 (*c* 0.75, $CHCl_3$–MeOH, 1:1); FAB-MS: *m/z* 613 $[M+Na]^+$; ^{1}H-NMR (in pyridine-d_5): δ 1.10, 1.49 (each 1H, m, H-1), 1.83 (1H, m, H-2), 2.26 (1H, d, 10.8, H-2), 3.49 (1H, dd, 11.0, 4.0, H-3), 1.23 (1H, m, H-5), 0.70, 1.46 (each 1H, m, H-6), 0.92, 1.24 (each 1H, m, H-7), 1.60 (1H, dd, 11.5, 5.0, H-8), 1.15 (1H, dd, 16.2, 3.1, H-11), 2.71 (1H, dd, 16.2, 8.7, H-11), 5.05 (1H, dd, 9.0, 3.0, H-12), 1.80, 1.96 (each 1H, m, H-15), 4.78 (1H, dd, 14.0, 7.5, H-16), 2.12 (1H, d, 7.5, H-17), 1.23 (3H, s, H-18), 0.19, 0.56 (each 1H, d, 3.4, H-19), 1.99 (1H, m, H-20), 0.95 (3H, d, 6.0, H-21), 2.25, 2.45 (each 1H, m, H-22), 0.83 (3H, s, H-28), 1.25 (3H, s, H-29), 1.00 (3H, s, H-30), 2.12 (3H, s, C$\underline{H}_3$CO), 4.84 (1H, d, 7.5, H-1′), 4.03 (1H, t, 8.0, H-2′), 4.15 (1H, t, 8.5, H-3′), 4.21 (1H, m, H-4′), 3.73 (1H, t, 11.0, H-5′), 4.35 (1H, dd, 11.0, 5.1, H-5′); ^{13}C-NMR (in pyridine-d_5): δ 32.0 (C-1), 29.7 (C-2), 88.2 (C-3), 41.3 (C-4), 47.0 (C-5), 20.4 (C-6), 25.7 (C-7), 46.0 (C-8), 19.6 (C-9), 27.0 (C-10), 36.5 (C-11), 76.8 (C-12), 48.4 (C-13), 47.0 (C-14), 43.9 (C-15), 80.6 (C-16), 53.7 (C-17), 13.3 (C-18), 30.0 (C-19), 27.0 (C-20), 22.1 (C-21), 38.7 (C-22), 174.0 (C-23), 19.6 (C-28), 25.8 (C-29), 15.4 (C-30), 170.9 ($CH_3\underline{C}O$), 21.5 ($\underline{C}H_3CO$), 107.6 (C-1′), 75.7 (C-2′), 78.7 (C-3′), 71.3 (C-4′), 67.2 (C-5′).

*25-O-Acetylcimigenol 3-O-β-D-xylopyranoside (**2**).* White powder, mp 244–246 °C; $[\alpha]^D$ +0.7 (*c* 0.10, $CHCl_3$–eOH, 1:1); FAB-MS: *m/z* 663 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data (in pyridine-d_5) were similar to published values (Kadota *et al.*, 1995).

*Cimigenol 3-O-β-D-arabinopyranoside (**3**).* White powder, mp 257–259 °C; $[\alpha]^D$ +35 (*c* 0.10, MeOH); FAB-MS: *m/z* 621 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data (in pyridine-d_5) were similar to published values (Shao *et al.*, 2000).

Determination of anti-complement activity through the classical pathway (CP). Anti-complement activity was determined by the modified method of Mayer as described previously (Min *et al.*, 2003). For the classical pathway assay, a diluted solution of normal human serum (80 μL) collected from a healthy volunteer (Man) was mixed with gelatin veronal buffer (80 μL) with or without sample. Each sample was dissolved in DMSO, and used as a negative control. The mixture was preincubated at 37 °C for 30 min, and sensitized erythrocytes (sheep red blood cells, 40 μL) were added. After incubation under the same conditions, the mixture was centrifuged (4 °C, 1500 rpm) and the optical density of the supernatant (100 μL) was measured at 405 nm. Tiliroside was employed as a positive control (Jung *et al.*, 1998).

RESULTS AND DISCUSSION

Repeated column chromatography of the EtOAc-soluble fraction of MeOH extract of the rhizome of *C. foetida* subjected to silica gel and reversed phase C-18 column, led to the isolation of one tetranor-cycloartane (**1**) and two 9,19-cycloartane-type triterpee (**2** and **3**) glycosides.

Cimilactone A (**1**) was obtained as colorless needles, mp 255–256 °C. The ^{1}H-NMR spectrum demonstrated the signals for a cyclopropane methylene proton at δ 0.56 (d, J = 3.4 Hz) and 0.19 (d, J = 3.4 Hz), four quaternary methyl protons at δ 1.25, 1.23, 1.00 and 0.83, a secondary methyl proton at δ 0.95 (d, J = 6.0 Hz), an acetyl methyl proton at δ 2.12, and an anomeric proton at δ 4.84 (d, J = 7.5 Hz). These data are very similar to those of acetin except for the side chain (Kusano *et al.*, 1998). A comparative study of the ^{13}C-NMR spectrum of compound **1** with that of acetin indicated that compound **1** was a tetranor-cycloartan 3-*O*-β-D-xylopyranoside with an acetoxyl group at C-12, resulting from a loss of four carbons from acetin, C-24, C-25, C-26 and C-27. The structural assignment was further identified by ^{1}H-^{1}H COSY, HMQC and HMBC experiments. The long-range correlation peaks between the acetyl proton at δ 2.12 and an acetyl carbon at δ 170.9; δ 5.05 (H-12) and an acetyl carbon at δ 170.9; δ 4.78 (H-16) and δ 174.0 (C-23) indicated an acetoxyl group at C-12 and a six-membered lactone ring between C-23 and C-16 (Fig. 1). The connectivity of the sugar

DOI: 10.1002/ptr

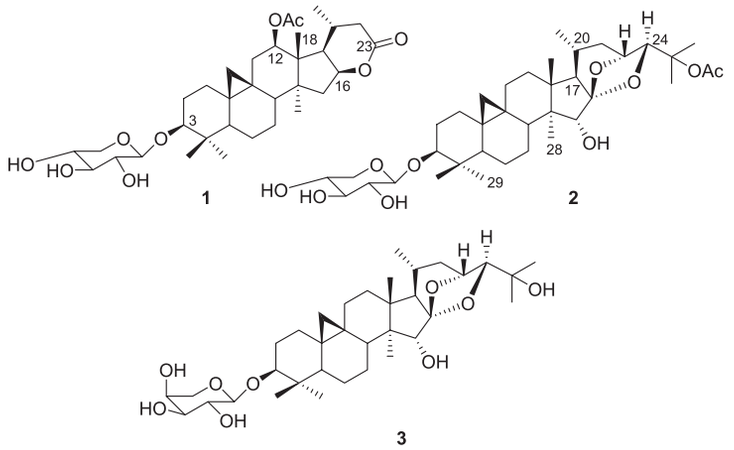

Figure 1. Structures of compounds **1**–**3**.

Table 1. Inhibitory effects of compounds on the classical pathway of the complement system

Compound	IC_{50} (μM)
Cimilactone A (**1**)	28.6
25-*O*-Acetylcimigenol 3-*O*-β-D-xylopyranoside (**2**)	>400
Cimigenol 3-*O*-α-L-arabinopyranoside (**3**)	>400
Tiliroside[a]	78.6

[a] This compound was used as a positive control (Jung *et al.*, 1998).

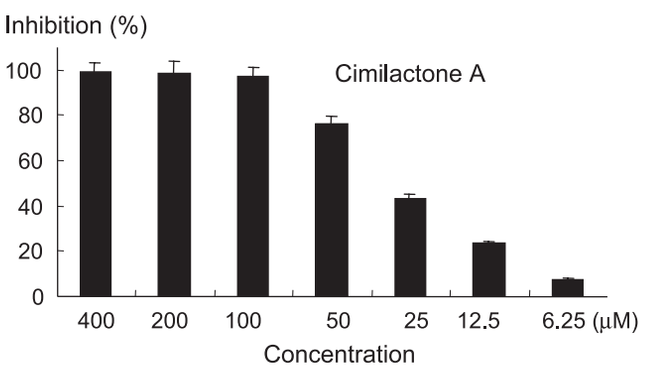

Figure 2. Inhibitory effect of cimilactone A (1) on classical pathway of complement system (mean ± SD, $n = 3$).

was determined by the presence of a correlation peak in the HMBC spectrum between the proton signal at δ 4.84 (H-1′) and a carbon signal at δ 88.2 (C-3). Therefore, compound **1** was assigned as 12β-acetoxy-3β-hydroxy-24,25,26,27-tetranorcycloartan-23,16β-olide 3-*O*-β-D-xylopyranoside, and compared with the published data (Nishida *et al.*, 2003c).

Compounds **2** and **3** were identified as 25-*O*-acetylcimigenol 3-*O*-β-D-xylopyranoside (Kadota *et al.*, 1995) and cimigenol 3-*O*-β-D-arabinopyranoside (Ye *et al.*, 1999), elucidated on the basis of their ^{1}H-, ^{13}C-NMR, and MS data by a comparison with the reported values.

The three cycloartane compounds were tested for their anticomplement activity against the classical pathway of the complement system *in vitro* using the protocol described elsewhere (Min *et al.*, 2003). Table 1 summarizes the inhibitory activity (IC_{50} value). Among them, compound **1** had significant inhibitory activity against the CP of the complement system with an IC_{50} value of 28.6 μM (Fig. 2). On the other hand, compounds **2** and **3** were inactive. It was previously reported that the oleanane-type triterpene saponins, such as coumaroyl maslinic acids from *Zizyphus jujuba* (Lee *et al.*, 2004), gingseng saponins from Korean red ginseng (Kim *et al.*, 1998), kaikasaponins and soyasaponins from *Pueraria lobata* (Oh *et al.*, 2000), bisdesmosidic saponins from *Tiarella polyphylla* (Park *et al.*, 1999) and hederagenin saponins from *Dipsacus asper* (Oh *et al.*, 1999), have significant anti-complement activity. In addition, lanostane-type triterpenes from *Ganoderma lucidum* (Min *et al.*, 2001), prostane-type triterpenes (Matsuda *et al.*, 1998) and damarane-type saponins (Kim *et al.*, 1998) inhibited the hemolytic activity of the complement system. Kim *et al.* reported that oleanane-type saponins have a potent anti-complement activity among the tritepenoids (Kim *et al.*, 1998). Furthermore, the bisdesmosidic oleanane saponins showed significant anti-complement activity compared with that of monodesmosidic saponins (Park *et al.*, 1999). In view of this result, the tetranor-cycloartane glycoside, cimilactone A (**1**), also had a significant anti-complement activity in our assay system. Min *et al.* suggested that the carbonyl group at C-3 of the lanostane-type triterpenes, such as ganoderiol F, ganodermanondiol and ganodermanontriol from *Ganoderma lucidum*, is an essential structure for any inhibitory activity of the complement system (Min *et al.*, 2001). On the other hand, the carboxylic acid of hederagenin and oleanane-type triterpenes was reported to be an essential feature for the anti-complement activity (Oh *et al.*, 1999). Compared with lanostane- and oleanane-type triterpenoids, the terminal ketone at C-23 of compound **1** might be an important moiety for the anti-complement activity. Accordingly, cimilactone A (**1**) might be a good candidate as a compound for improving the unwanted and excessive activation of the complement system. Studies aimed at isolating bisdesmosidic 9,19-cycloartane saponins from this plant and testing it for any anti-complement activity are currently under way.

Acknowledgements

This research was supported by research grants from the Food Drug & Administration, Republic of Korea (2006) and the Catholic University of Daegu in 2005.

DOI: 10.1002/ptr

REFERENCES

Chen SN, Fabricant DS, Lu ZZ *et al.* 2002a. Cimiracemates A-D, phenylpropanoid esters from the rhizomes of *Cimicifuga racemosa*. *Phytochemistry* **61**: 409–413.

Chen SN, Fabricant DS, Lu ZZ *et al.* 2002b. Cimiracemosides I-P, new 9,19-cyclolanostane triterpene glycosides from *Cimicifuga racemosa*. *J Nat Prod* **65**: 1391–1397.

Chen SN, Li W, Fabricant DS *et al.* 2002c. Isolation, structure elucidation, and absolute configuration of 26-deoxyactein from *Cimicifuga racemosa* and clarification of nomenclature associated with 27-deoxyactein. *J Nat Prod* **65**: 601–605.

Jung KY, Oh SR, Park SH *et al.* 1998. Anti-complementary activity of tiliroside from the flower buds of *Magnolia fargesii*. *Biol Pharm Bull* **21**: 1077–1078.

Katoda S, Li JX, Tanaka K, Namba T. 1995. Constituents of Cimicifugae Rhizoma II. Isolation and structures of new cycloartenol triterpenoids and related compounds from *Cimicifuga foetida*. *Tetrahedron* **51**: 1143–1166.

Kim DS, Oh SR, Lee IS *et al.* 1998. Anticomplementary activity of ginseng saponins and their degradation products. *Phytochemistry* **47**: 397–399.

Kirschfink M. 1997. Controlling the complement system in inflammation. *Immunopharmacology* **38**: 51–62.

Kusano A, Shibano M, Tsukamoto D, Kusano G. 2001. Studies on the constituents of *Cimicifuga Species*. XXVIII. Four new cycloart-7-enol glycosides from the underground parts of *Cimicifuga simplex* Wormsk. *Chem Pharm Bull* **49**: 437–441.

Kusano A, Takahira M, Shibano M *et al.* 1998. Studies on the constituents of *Cimicifuga* species. XX. Absolute stereostructures of cimicifugoside and actein from *Cimicifu simples* Wormsk. *Chem Pharm Bull* **46**: 467–472.

Lee SM, Park JG, Min BS *et al.* 2004. Anti-complementary activity of triterpenoids from fruits of *Zizyphus jujuba*. *Biol Pharm Bull* **27**: 1883–1886.

Lichtman AK, Pober JS. 1997. *Cellular and Molecular Immunology.* W. B. Saunders: Philadelphia, 315–338.

Matsuda H, Tomohiro N, Yoshikawa M, Kubo M. 1998. Studies on Alismatis Rhizoma. II. Anti-complementary activities of methanol extract and terpene components from Alismatis Rhizoma (dried rhizome of *Alisma orientale*). *Biol Pharm Bull* **21**: 1317–1321.

Min BS, Gao JJ, Hattori M, Lee HK, Kim YH. 2001. Anticomplement activity of triterpenoids from the spores of *Ganoderma lucidum*. *Planta Med* **67**: 811–814.

Min BS, Lee SY, Lee HK *et al.* 2003. Anti-complement activity of constituents from the stem-bark of *Juglans mandshurica*. *Biol Pharm Bull* **26**: 1042–1044.

Nishida M, Yoshimitsu H, Nohara T. 2003a. Three cycloartane glycosides from Cimicifuga Rhizome and their immunosuppressive activities in mouse allogeneic mixed lymphocyte reaction. *Chem Pharm Bull* **51**: 354–356.

Nishida M, Yoshimitsu H, Nohara T. 2003b. Two new tetranorcycloartane glycosides from Cimicifuga rhizome. *Chem Pharm Bull* **51**: 1117–1118.

Nishida M, Yoshimitsu H, Okawa M, Ikeda T, Nohara T. 2003c. Two new 15,16-seco-cycloartane glycosides from Cimicifuga rhizome. *Chem Pharm Bull* **51**: 1215–1216.

Oh SR, Jung KY, Lee HK *et al.* 1999. *In vitro* anticomplementary activity of hederagenin saponins isolated from roots of *Dipsacus asper*. *Arch Pharm Res* **22**: 317–319.

Oh SR, Kinjo J, Lee HK *et al.* 2000. Effects of triterpenoids from *Pueraria lobata* on immunohemolysis: β-D-glucuronic acid plays an active role in anticomplementary activity *in vitro*. *Planta Med* **66**: 506–510.

Park SH, Oh SR, Lee HK *et al.* 1999. Anticomplement activities of oleanolic acid monodesmosides and bisdesmosides isolated from *Tiarella polyphylla*. *Arch Pharm Res* **22**: 428–431.

Shao Y, Harris A, Cordell GA *et al.* 2000. Triterpene glycosides from *Cimicifuga racemosa*. *J Nat Prod* **63**: 905–910.

Watanabe K, Mimaki Y, Sakagami H, Sashida Y. 2002. Cycloartane glycosides from the rhizomes of *Cimicifuga racemosa* and their cytotoxic activities. *Chem Pharm Bull* **50**: 121–125.

Wende K, Mügge C, Thurow K, Schöpke T, Lindequist U. 2001. Actaeaepoxide 3-*O*-β-D-xylopyranoside, a new cycloartane glycoside from the rhizomes of *Actaea racemosa* (*Cimicifuga racemosa*). *J Nat Prod* **64**: 986–989.

Ye W, Zhang J, Che CT, Ye T, Zhao S. 1999. New cycloartane glycosides from *Cimicifuga dahurica*. *Planta Med* **65**: 770–772.

Zhang QW, Ye WC, Hsiao WWL, Zhao SX, Che CT. 2001. Cycloartane glycosides from *Cimicifuga dahurica*. *Chem Pharm Bull* **49**: 1468–1470.

DOI: 10.1002/ptr