Cimiracemoside A: A New Cyclolanostanol Xyloside from the Rhizome of *Cimicifuga racemosa*

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A new 9,19-cyclolanostane-type triterpene xyloside (1), from the rhizomes of *Cimicifuga racemosa*, has been isolated together with four known saponins; cimiaceroside A, 25-*O*-methylcimigenol-3-*O*-β-D-xylopyranoside, 27-deoxyactein and 23-*O*-acetylshengmanol-3-*O*-β-D-xylopyranoside. The structure of the new compound was established as 16β,23 : 22β,25-diepoxy-12-acetoxy-3β,23,24β-trihydroxy-9,19-cyclolanost-7-ene-3-*O*-β-D-xylopyranoside. For the structure elucidation, 1D- and 2D-NMR experiments and high resolution electrospray ionization Fourier transformation mass spectrometry (HRESIFTMS) were used.

Key words *Cimicifuga racemosa*; 9,19-cyclolanostanol; Ranunculaceae

Cimicifuga (*C.*), one of the smallest genus in the family Ranunculaceae, comprises about 25 species distributed throughout North America, Europe and East Asia. This genus is represented by 6 species in the flora of North America. *Cimicifuga racemosa*, best known as black cohosh (formerly black snakeroot), is the most common American species.[1,2] The rhizomes of *C. racemosa* have been used in European phytotherapy for the treatment of menopausal symptoms for over 50 years. Pharmacological and controlled clinical studies have confirmed that *C. racemosa* preparations are a safe and effective alternative for hormone replacement therapies in the treatment of menopause.[2] The rhizomes of Asian species, *C. simplex*, *C. dahurica*, *C. foetida* and *C. heracleifolia*, have been used as anti-inflammatory, antipyretic and analgesic agents in traditional Japanese and Chinese medicine. Generally, Asian species of *Cimicifuga* are chemically much better characterized than North American species, including *C. racemosa*. During a series of chemical investigations of *Cimicifuga* species, 9,19-cyclolanostane-type triterpenoids, fukiic acid esters, piscidic acid esters, caffeic acid derivatives, phenolic acid derivatives and chromones have been isolated.[3—8]

In this study, *C. racemosa* was investigated for its triterpene glycoside content, and here we describe the isolation and structure elucidation of a new 9,19-cyclolanostane-type triterpene xyloside (**1**). The related known xylosides, cimiaceroside A,[9] 25-*O*-methylcimigenol-3-*O*-β-D-xylopyranoside,[4] 27-deoxyactein[11] and 23-*O*-acetylshengmanol-3-*O*-β-D-xylopyranoside[10] were also isolated.

The IR spectrum of **1** exhibited a hydroxyl absorption band (3413 cm^{-1}), and an ester carbonyl absorption band (1729 cm^{-1}).

High resolution electrospray ionization Fourier transformation mass spectrometry (HRESIFTMS) of **1** showed an ion peak for $[M+Na]^+$ at *m/z* 699.2665, in agreement with the molecular formula $C_{37}H_{56}O_{11}$.

The ^{1}H-NMR spectrum of **1** (Table 1) displayed signals characteristic of cyclopropane-methylene protons as an AX system (δ 0.48, 1.02, J=3.8 Hz), an acetyl group [δ 2.10 ($COC\underline{H}_3$)], and six tertiary (1.77, 1.69, 1.40, 1.33, 1.06 and 1.02) and a secondary methyl (δ 1.31, d, J=6.6 Hz) group. Additionally, an anomeric proton signal was observed at δ 4.80 (d, J=7.3 Hz). Thus, compound **1** was considered to be

Table 1. ^{1}H- and ^{13}C- Assignments of **1**,[a] (in Pyridine, at 500 and 125 MHz, respectively)

C/H	δ_C	δ_H (J Hz)
1	31.36 t	1.22 m, 1.55 m
2	30.50 t	1.84 m, 2.27 m
3	89.03 d	3.44 dd (4.0, 11.7)
4	41.54 s	
5	43.58 d	1.26[b]
6	22.97 t	1.56 m, 1.84 m
7	115.04 d	5.11 d (8.0)
8	149.80 s	
9	22.33 s	
10	29.40 s	
11	37.83 t	1.26[b], 2.62 dd (16.0, 9.1)
12	77.82 d	5.25 d (8.7)
13	49.88 s	
14	52.10 s	
15	43.09 t	2.05 dd (13.1, 7.5), 2.15 dd (12.7, 8.0)
16	73.40 d	5.05 dd (13.1, 7.8)
17	54.42 d	1.77[b]
18	16.18 q	1.40 s
19	29.86 t	0.48 (3.8), 1.00 (3.8)
20	35.37 d	2.29 m
21	18.68 q	1.31 d (6.6)
22	87.80 d	3.90 d (10.5)
23	106.84 s	
24	84.40 d	4.23 s
25	84.80 s	
26	29.11 q	1.77 s
27	26.03 q	1.69 s
28	27.86 q	1.06 s
29	26.03 q	1.33 s
30	15.38 q	1.02 s
1′	108.57 d	4.80 d (7.3)
2′	76.72 d	4.01 dd (7.3, 7.6)
3′	79.73 d	4.14 dd (7.6, 8.2)
4′	72.37 d	4.23[b]
5′	68.24 t	3.70 dd (10.0, 11.3), 4.31 dd (5.1, 11.3)
$COCH_3$	171.78 s	2.10 s

a) Assignments confirmed by COSY, HMQC and HMBC experiments. *b*) Signal pattern was unclear due to overlapping.

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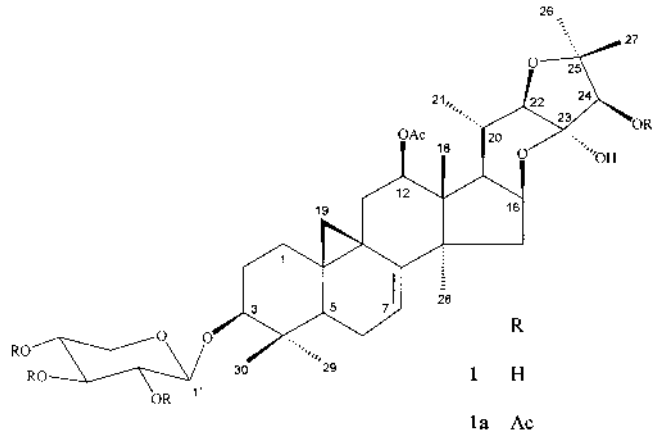

Chart 1. Structures of **1** and **1a**

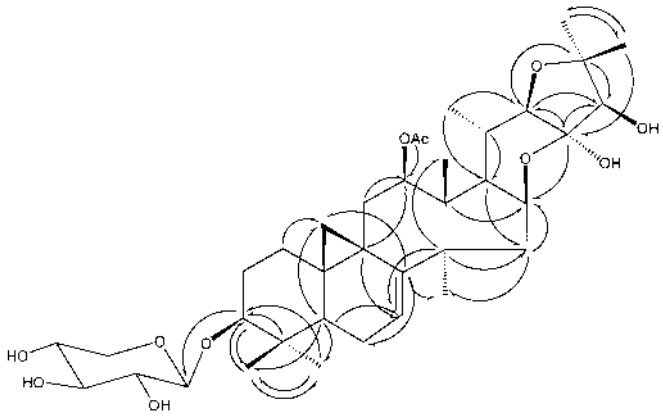

Chart 3. Structure and HMBC For **1**

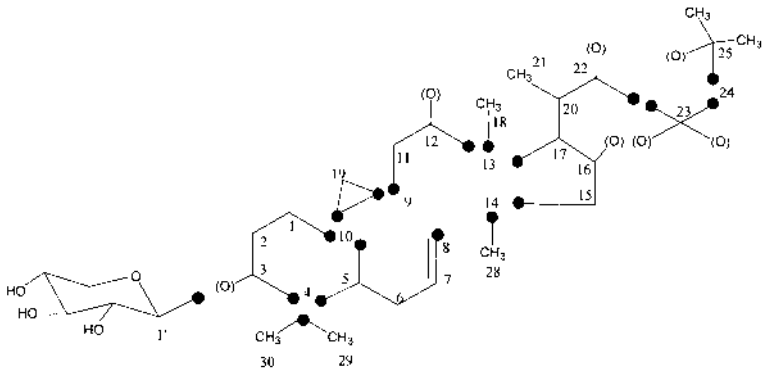

Chart 2. Partial Structures of **1** Deduced from 2D-NMR Measurements

a 9,19-cyclolanostane-type triterpene monoglycoside.

The ^{13}C-NMR spectrum of **1** exhibited 37 signals. Thirty signals were accounted for by the aglycone moiety. The remaining signals were in accord with the presence of one pentose and an acetyl unit. The ^{1}H- and ^{13}C-NMR data (Table 1) supported the assignment of the sugar moiety in **1** as β-D-xylopyranose.[3—5)]

Beside glycosidic carbons, the remaining downfield signals displayed nine signals due to five oxymethine carbon atoms (δ 89.03, d, C-3; δ 87.80, d, C-22; δ 84.40, d, C-24; δ 77.82, d, C-12; δ 73.40, d, C-16), two quaternary carbon atoms, one of which is a ketal (δ 106.84, s, C-23; δ 84.80, s, C-25), as well as one endocyclic double bond (δ 115.04, d, C-7; δ 149.8, s, C-8). Taking into account the results from our comprehensive 1D and 2D-NMR studies and previous knowledge derived from metabolites isolated from the genus *Astragalus*,[12—14)] it suggests that **1** possesses a highly oxidized triterpene nucleus. These data, together with the information from the ^{1}H–^{1}H-correlation spectroscopy (COSY) and ^{1}H–^{13}C-correlation spectroscopy (HMQC) experiments indicated the presence of the partial structures shown in Chart 2. In order to establish the interfragment relationship, a heteronuclear multiple bond correlation experiment (HMBC) was performed (Chart 3).

Acetylation of **1** yielded a tetraacetate, **1a**. From the HRESIFTMS of **1a**, which displayed an $[M+Na]^+$ ion at *m/z* 867.3791, a molecular formula of $C_{45}H_{64}O_{15}$ was proposed. The IR spectrum of **1a** still displayed a free hydroxyl absorption band after acetylation (3433 cm^{-1}), indicating the presence of the tertiary hydroxyl group at C-23. In the ^{1}H-NMR spectrum of **1a**, H-24 was observed at δ 5.60 (s), showing the expected downfield shift in comparison to **1**.

The relative stereochemistry of **1** was resolved by a combination of two dimensional nuclear Overhouser enhancement spectroscopy (2D-NOESY) data, an analysis of the coupling constants, and comparison with analogous compounds. The cross peaks observed in the NOESY spectrum between H-16, H-12 and CH_3-26; H-12, CH_3-21, CH_3-28 and H-22; H-24 and H-17, implied that these protons were cofacial (α). Based on these findings and a comparison of ^{1}H- and ^{13}C-NMR data with those of cimiaceroside A,[9)] the orientation of O(H)-groups were determined to be 3β, 12β, 16β, 22β, 24β.

Consequently, the structure of **1** was elucidated as 16β,23;22β,25-diepoxy-12β-acetoxy-3β,23,24β-trihydroxy-9,19-cyclolanost-7-ene-3-*O*-β-D-xylopyranoside.

Cimiaceroside A,[9)] 25-*O*-methylcimigenol-3-*O*-β-D-xylopyranoside,[4)] 23-*O*-acetylshengmanol-3-*O*-β-D-xylopyranoside[10)] and 27-deoxyactein[11)] were also isolated from the rhizomes of *C. racemosa* and identified on the basis of their NMR (^{1}H- and ^{13}C-) data, in comparison with literature values.

As far as could be ascertained, this is the first report of cimiaceroside A,[9)] 25-*O*-methylcimigenol-3-*O*-β-D-xylopyranoside,[4)] and 23-*O*-acetylshengmanol-3-*O*-β-D-xylopyranoside[10)] from *C. racemosa*.

Experimental

General Experimental The IR spectra were recorded with an ATI Mattson Genesis Series Fourier transform (FT)-IR spectrophotometer. The 1D- and 2D-NMR spectra were obtained on a Bruker® Avance DRX 500 FT spectrometer operating at 500 and 125 MHz, respectively. The chemical shift values are reported as parts per million (ppm) units relative to tetramethylsilane (TMS) for ^{1}H- and ^{13}C-; and the coupling constants are in Hz (in parentheses). For the ^{13}C-NMR spectra, multiplicities were determined by a distortionless enhancement by polarization transfer (DEPT) experiment. HRESIFTMS were obtained using a Bruker BioApex FT-MS in ESI mode.

Chromatographic Conditions TLC, precoated Si 250F plates (Baker); developing system, ethyl formate–toluene–formic acid (50 : 50 : 15); visualization, 50% H_2SO_4. Column chromatography, silica gel 230—400 mesh (Merck).

Plant Material The powdered rhizome material of *C. racemosa* was purchased from Frontier Natural Products Co., 3021 78th Street, Norway, IA 52318, product code/lot number: 957.9012.

Extraction and Isolation Plant material (900 g) was extracted with dichloromethane (600 ml) for 8 h and the extract was filtered. The filtrate was concentrated to dryness *in vacuo* (12.08 g). An aliquot of the extract (10.80 g) was chromatographed on a silica gel column (500 g). Elution with increasing amounts of EtOAc in hexane (15—60% EtOAc) yielded 23 fractions (frs. 1—23). Fraction 22 (1.34 g), rich in saponins, was subjected to flash column chromatography using reversed-phase material (C-18, 150 g). H_2O–MeOH mixtures (40 : 60, 30 : 70 and 20 : 80) and MeOH were used as the eluents, to yield **1** (fr. A, 43.5 mg) and frs. B—K. Further purification of the frs. B—K by normal, and reversed-phase column chromatography led to the isolation of four compounds (Cimiaceroside A, 23.0 mg; 25-*O*-methylcimigenol-3-*O*-*β*-D-xylopyranoside, 101.0 mg; 23-*O*-acetylshengmanol-3-*O*-*β*-D-xylopyranoside, 88.6 mg; and 27-deoxyactein, 57 mg).

Compound **1**: 16*β*,23;22*β*,25-diepoxy-12-*β*-acetoxy-3*β*,23,24*β*-trihydroxy-9,19-cyclolanost-7-ene-3-*O*-*β*-D-xylopyranoside: IR (KBr) ν_{max}: 3413, 2968, 2359, 1729, 1461 cm^{-1}. ^{1}H- and ^{13}C-NMR: see Table 1. HRESIFTMS *m/z* : 699.2665 $[M+Na]^+$.

Acetylation of **1**: Treatment of compound **1** (5 mg), with Ac_2O (1 ml) and pyridine (1 ml) at room temperature overnight followed by the usual workup yielded compound **1a** (4 mg).

Cimiracemoside A tetraacetate (**1a**): IR (KBr) ν_{max}: 3433, 2961, 2359, 1726, 1624, 1458 cm^{-1}. ^{1}H- NMR: ($CDCl_3$) δ 5.60 (1H, s, H-24), 5.15 (1H, t, J=9.0 Hz, H-3′), 5.08 (1H, d, J=8.9 Hz, H-12), 4.95 (3H, H-16, H-7 and H-2′, overlapped), 4.49 (1H, d, J=7.1 Hz, H-1′), 4.47 (1H, overlapped, H-4′), 4.08 (1H, dd, J=10.2, 5.1 Hz, H-5′a), 3.70 (1H, d, J=11.1 Hz, H-22), 3.29 (1H, dd, J=9.9, 9.5 Hz, H-5′b), 3.12 (1H, dd, J=11.2, 4.0 Hz, H-3), HRESIFTMS *m/z* 867.3791 $[M+Na]^+$.

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