Actaeaepoxide 3-*O*-*β*-D-Xylopyranoside, a New Cycloartane Glycoside from the Rhizomes of *Actaea racemosa* (*Cimicifuga racemosa*)

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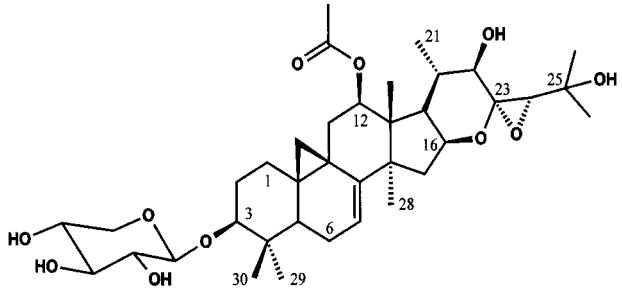
A new cycloartane glycoside (**1**) was obtained from a minor triterpene fraction of the rhizome extract of *Actaea racemosa* (synonym: *Cimicifuga racemosa*) along with a known compound, cimigenol 3-*O*-*β*-D-xylopyranoside. The structure of **1** was elucidated as 20(*S*),22(*R*),23(*R*),24(*S*)-12*β*-acetoxy-16*β*:23,23α:24-diepoxy-3*β*,22*β*,25-trihydroxy-9,19-cyclolanost-7-ene 3-*O*-*β*-D-xylopyranoside (actaeaepoxide 3-*O*-*β*-D-xylopyranoside) on the basis of spectral and chemical evidence.

Cimicifuga Wernisch is one of the smallest genera in the family Ranunculaceae. It comprises about 25 species distributed throughout East Asia, Europe, and North America. Recently, all *Cimicifuga* species were returned to the genus *Actaea* L.[1] *Actaea racemosa* L. [synonym: *Cimicifuga racemosa* (L.) Nutt.] (black cohosh, black snakeroot) is the most commonly known among six North American species.[2,3] The rhizome of *C. racemosa* was once used to treat a variety of diseases in traditional American medicine. In the middle of the 20th century it was introduced to several countries in western Europe and has gained increasing importance for the treatment of menopausal complaints (e.g., hot flashes or depression) and dysmenorrhea.

From the rhizomes of *A. racemosa*, several 9,19-cyclolanostane-type triterpenoids (e.g., actein, 27-deoxyactein, and several cimiracemosides)[4−10] and phenylpropanoid derivatives (e.g., fukinolic acid and several cimicifugic acids) were isolated and recognized as the major constituents.[11] Additionally, the constituents of the rhizomes and aerial parts of the Asian species of the genus, including *C. simplex, C. dahurica, C. foetida*, and *C. heracleifolia*, are well characterized chemically, and many triterpene glycosides,[12−14] phenolic acid derivatives,[15] and some minor components[16] have been described.

In the present study the triterpenoids of the underground parts of *A. racemosa* were investigated. The paper describes the isolation and structure elucidation of a new 9,19-cyclolanostane-type triterpene glycoside (**1**). Compound **1** was accompanied by the known substance cimigenol 3-*O*-*β*-D-xylopyranoside (**2**).

Compound **1** was isolated from an aqueous-2-propanolic extract of the rhizomes of *A. racemosa*. The extract was dried and subjected to a liquid−liquid partition between water and dichloromethane. The organic phase was dried and fractionated on a Si gel column with dichloromethane/MeOH as mobile phase. The fractions obtained were screened by TLC on Si gel and RP-18 Si gel using actein and 27-deoxyactein as references. On the basis of these data the fractions were grouped into five groups, two containing no triterpenoids (G1 and G5), the 27-deoxyactein group (G2), the actein group (G3), and a group containing unidentified triterpenoids (G4). The latter was separated by repeated MPLC on a RP-18 column with MeOH−water as the mobile phase and by HPLC on a RP-18 column with MeCN−water as eluent to give compound **1**.



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Electrospray ionization mass spectrometry (ESIMS) of compound **1** showed a molecular ion peak $[M - H]^-$ at *m*/*z* 675, which was in agreement with the molecular formula $C_{37}H_{56}O_{11}$. High-resolution electron impact mass spectrometry (HREIMS) of **1** afforded an ion at *m*/*z* 598.3506 (calcd 598.3506), which is in agreement with the molecular formula $C_{35}H_{50}O_8$ [M − acetic acid − H_2O]. The ESIMS of compound **2** afforded an ion $[M - H]^-$ at *m*/*z* 619; HREIMS an ion at *m*/*z* 620.3963 (calcd 620.3925), giving the molecular formula $C_{35}H_{56}O_9$.

The ^{1}H NMR spectrum of **1** displayed characteristic signals for cyclopropane methylene protons at *δ* 0.50 and 0.98 (each 1H, d, $J = 4.0$ Hz), six tertiary methyl groups at *δ* 1.77, 1.69, 1.41, 1.33, 1.07, and 1.03 (each 3H, s), and one secondary methyl group at *δ* 1.31 (3H, d, $J = 6.4$ Hz). Additionally, signals for an anomeric proton at *δ* 4.84 (1H, d, $J = 7.6$ Hz) and for an acetyl group at *δ* 2.15 (3H, s) were observed. The ^{13}C NMR spectrum of **1** exhibited 37 signals, of which 30 were attributed to the aglycon, five to a pentose residue, and two to an acetyl group. These data indicated that compound **1** is an acetylated 9,19-cyclolanostane-type triterpene monoglycoside. Acid hydrolysis of the compound, transformation of the hydrolyzed monosaccharide to the methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylate, and gas chromatography (GC) of the trimethylsilylated product showed that D-xylose is the

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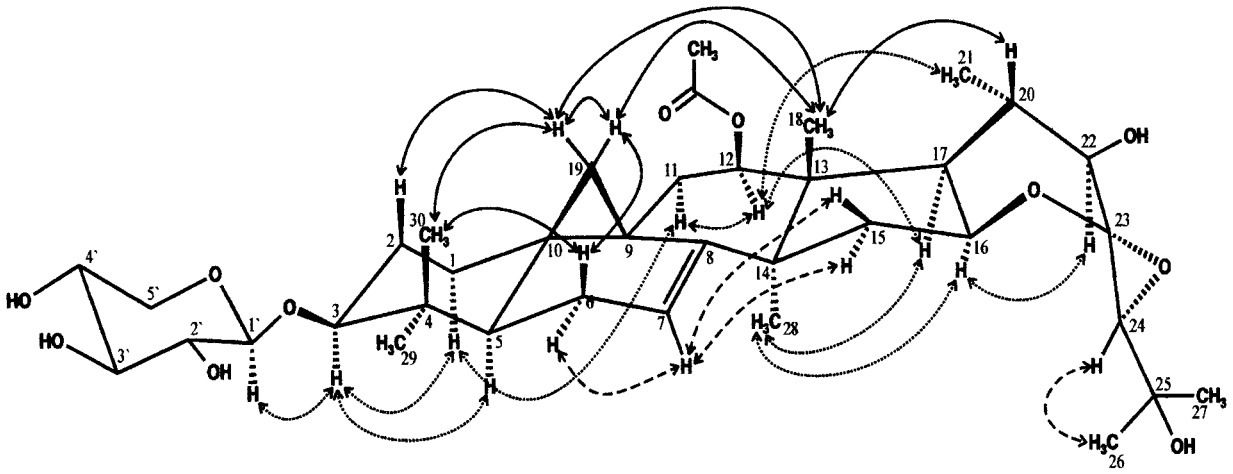

Figure 1. ROESY correlations of compound **1**.

Table 1. ^{1}H and ^{13}C NMR Data (δ) of the Aglycon of **1** on the Basis of COSY, TOCSY, ROESY, HMQC, and HMBC Spectra[a]

proton	δ	COSY	TOCSY	HMQC	HMBC
H-1ax	1.56	2.23, 1.15	3.43, 2.23, 1.87, 1.15	30.2	
H-1eq	1.15	2.23, 1.56	3.43, 2.23, 1.87, 1.56	30.2	
H-2ax	1.87	3.43, 2.23	3.43, 2.23, 1.56, 1.15	29.4	28.2
H-2eq	2.23	3.43, 1.87, 1.56, 1.15	3.43, 1.87, 1.56, 1.15	29.4	
H-3ax	3.43 (dd, 4.0/11.5)	2.23, 1.87	2.23, 1.87, 1.56, 1.15	87.9	107.2, 40.3,[b] 25.7, 14.2
H-5ax	1.18		5.12, 1.85, 1.54	42.4	40.3, 28.2, 14.2
H-6ax	1.54		5.12, 1.85, 1.18	21.7	
H-6eq	1.85	5.12	5.12, 1.54, 1.18	21.7	147.5
H-7	5.12	1.85	1.85, 1.54, 1.18	113.9	50.9, 42.4, 21.7, 21.1
H-11ax	1.20 (dd, 8.4, 16.0)	2.93	5.23, 2.93	36.6	28.2, 21.1
H-11eq	2.93	5.23, 1.20	5.23, 1.20	36.6	
H-12ax	5.23 (d, 8.4)	2.93	2.93, 1.20	76.8	171.0, 21.1, 15.0
H-15A[c]	2.12	5.09, 2.06	5.09, 2.30	41.8	
H-15B[c]	2.06	5.09, 2.12	5.09, 2.30, 1.80	41.8	50.9, 26.7
H-16ax	5.09	2.12, 2.06, 1.80	2.30, 2.12, 2.06, 1.80	72.2	48.7
H-17ax	1.80 (dd, 1.6/8.0)	5.09, 2.30	5.09, 3.94, 2.30, 2.06, 1.31	53.2	50.9, 48.7, 34.1, 17.4, 15.0
H-20ax	2.30 (s)	5.09, 2.30	5.09, 3.94, 2.30, 2.06, 1.31	34.1	53.2, 17.4
H3-21	1.31	2.30	3.94, 2.30, 1.80	17.4	86.6, 53.2, 34.1
H-22ax	3.94	2.30	2.30, 1.80, 1.31	86.6	105.6, 83.7, 17.4
H3-18	1.41			15.0	76.8, 53.2, 50.9, 48.7
H-19A[c]	0.97 (d, 4.0)	0.50	0.50	28.7	
H-19B[c]	0.50 (d, 4.0)	0.97	0.97	28.7	42.4, 28.2
H-24eq	4.29 (s)			82.9	105.6, 86.6, 83.7, 24.9
H3-26	1.77 (s)			27.9	83.7, 82.9, 24.9
H3-27	1.69 (s)			24.9	83.7, 82.9, 27.9
H3-28	1.07 (s)			26.7	147.5, 50.9, 48.7, 41.8
H3-29	1.31 (s)			25.7	87.9, 42.4, 40.3, 14.2
H3-30	1.03 (s)			14.2	87.9, 42.4, 40.3, 25.7

[a] Horizontal lines indicate separate proton spin systems. [b] Low intensity. [c] Due to the ring conformation, protons are not present in axial or equatorial position.

Table 2. ^{13}C NMR Data of Compound **1** in Pyridine-d_5

position	δ	position	δ	position	δ
C-1	30.2	C-16	72.2	Xyl C-1	107.2
C-2	29.4	C-17	53.2	Xyl C-2	75.3
C-3	87.9	C-18	15.0	Xyl C-3	78.2
C-4	40.3	C-19	28.7	Xyl C-4	71.0
C-5	42.4	C-20	34.1	Xyl C-5	66.9
C-6	21.7	C-21	17.4		
C-7	113.9	C-22	86.6		
C-8	147.5	C-23	105.6		
C-9	21.1	C-24	82.9		
C-10	28.2	C-25	83.7		
C-11	36.6	C-26	27.9		
C-12	76.8	C-27	24.9		
C-13	48.7	C-28	26.7		
C-14	50.9	C-29	25.7		
C-15	41.8	C-30	14.2		

sugar component of compound **1**.[17] The NMR data of **1** were assigned on the basis of the 2D NMR spectra (COSY, TOCSY, ROESY, HMQC, and HMBC) as shown in Tables 1 and 2. From these data it was evident that the acetylated, xylose-bearing 16:23,23:24-diepoxy-3,12,22,25-tetrahydroxy-9,19-cyclolanost-7-ene was the aglycon of compound **1**. The relative stereochemistry of **1** was identified on the basis of ^{1}H NMR coupling constants (Table 1) and ROESY data which are summarized in Figure 1. These data prove unambiguously that the substituents at C-3, C-20, C-22 and the proton at C-24 are in equatorial positions, while the epoxy ring between carbons 23 and 24 and the hydroxyl group at C-12 are in axial positions. These data also prove that the absolute configuration of C-20 is *S* and that of C-22 is *R*, while C-23 and C-24 should be present in *R*- and *S*-configurations, respectively.

Cross-peaks in the HMBC spectrum between C-3 of the aglycon (δ 87.9) and H-1 of xylose (δ 4.82) and reverse cross-peaks between C-1 of xylose (δ 107.2) and H-3 of the aglycon (δ 3.43) established that the sugar is attached to the aglycon at C-3. This finding was further supported by a cross-peak in the ROESY spectrum between H-3 of the aglycon (δ 3.43) and H-1 of xylose (δ 4.82). The location of the acetyl moiety was also identified on the basis of the HMBC spectrum, where a cross-peak between the carbonyl carbon of the acid (δ 171.0) and H-12 of the aglycone (δ

5.23) showed that the acid is located at C-12. The H_1-H_1 coupling constant of 7.6 Hz indicated that xylose is present as the β-anomer in the $^4C_1$ configuration. Consequently, the structure of **1** was elucidated as 20(*S*),22(*R*),23(*R*),24-(*S*)-12β-acetoxy-16β:23β,23α:24α-diepoxy-3β,22β,25-trihydroxy-9,19-cyclolanost-7-ene 3-*O*-β-D-xylopyranoside.

The NMR data of compound **2** were assigned on the basis of the same 1D and 2D NMR spectra as described for compound **1** (see Experimental Section), showing that **2** is 3β,15α,25-trihydroxy-16β:23α,16α:24-diepoxy-9,19-cyclolanostane 3-*O*-β-D-xylopyranoside (cimigenol 3-*O*-β-D-xylopyranoside).

As previously mentioned, the presence of numerous highly hydroxylated 9,19-cyclolanostane glycosides is a common feature of plants of the genus *Actaea* (*Cimicifuga*). The hydroxyl functions are prevalent in ring D and on the side chain at C-17. Due to the loss of water, they give heterocyclic systems consisting of at least one pyran ring (16:23-epoxy) and additional furan (16:23;16:24-diepoxy, 22:25-epoxy, or 23:26-epoxy) and/or epoxy rings usually formed between C-24 and C-25.[9,10,12,18] We now have described for the first time a cyclolanotane glycoside possessing an aglycon having the epoxy bridge between carbons 23 and 24. The aglycon part of compound **1** was named acetaeaepoxide. In contrast, compound **2** has been already obtained from *C. acerina*, *C. dahurica*, *C. japonica*,[19] and *C. racemosa*.[10]

Experimental Section

General Experimental Procedures. Melting points were determined on a Mel-Temp II, Laboratory Devices Inc., Holliston, MA. Optical rotations were obtained on a JASCO DIP-140 digital polarimeter (cell length 10 cm). IR spectra were recorded on a Perkin-Elmer series 1600 infrared spectrometer. ^1H and ^{13}C NMR as well as all 2D spectra were measured in pyridine-d_5 at 300 K on a Bruker DPX 300 (^{13}C NMR and DEPT) and a AMX-600 NMR (^1H NMR, COSY, TOCSY, ROESY, HMQC, HMBC) spectrometer (^1H, 600.13 MHz; ^{13}C, 150.92 MHz). ESIMS data were recorded on a Micromass Quadro 2 mass spectrometer in the negative mode. HREIMS data were obtained on an AMD 402 sector field instrument. Gas chromatography was performed on a Fisons 8000 series gas chromatograph equipped with a Fisons MD800. Thin-layer chromatography was performed on Merck TLC-plates precoated with Si_{60} F_{254} or Si_{60} RP18 F_{254}, mobile phase $CHCl_3$–MeOH–H_2O (8:2.5:1), with a visualization carried out by spraying with 6% $SnCl_4$ in acetic acid–chloroform (1:1), followed by heating. HPLC was carried out on a Kontron Instruments system (HPLC pump 422/422S, diode array detector KI DAD 440) using a Merck LiChrospher60 RP18-column as stationary phase (250 mm × 10 mm i.d., 10 μm particle size) for preparative HPLC and a Merck LiChrosorb RP-18-column, 5 μm, 250 × 4 mm i.d., AcCN–H_3PO_4 0.1%, 35–41% in 60 min, 1 mL/min for analytical HPLC. Medium-pressure liquid chromatography (MPLC) was performed on a Büchi 688-system (Büchi 688 chromatography pump, Büchi 687 gradient former) equipped with a Pharmacia Uvicord S II UV-detector and a Büchi column (500 mm × 35 mm i.d.) filled with LiChroprep60 RP-18 (15–25 μm). Silica gel 60 (40–63 μm) was used for open column chromatography.

Plant Material. A standardized propan-2-ol/water extract (lot no. 704510, 26.05.97) of *Actaea racemosa* was used for this investigation. The extract was obtained from Schaper & Brümmer GmbH & Co. KG, Salzgitter, Germany. A HPLC trace of the extract is submitted as Supporting Information.

Extraction and Isolation. An aliquot of 1 L of the liquid extract of *A. racemosa* was dried in vacuo followed by freeze-drying to give 100.4 g of a brown powder. The dried extract was suspended in water and extracted with CH_2Cl_2. The organic layer was dried (11.5 g) and then subjected to open column chromatography on Si gel. The mobile phase consisted of CH_2Cl_2–MeOH (93:7, 88:12) and MeOH (2 L each). The fractions were monitored by TLC and combined to give five fractions. TLC further indicated that fractions 2 (1.3 g), 3 (2.2 g), and 4 (4.1 g) contained triterpenoids. Aliquots of 0.5 g each of fraction 4 were subjected to MPLC by using MeOH–water (6:4, 2:1) and MeOH as mobile phase to afford six fractions. The fractions obtained from each of the runs were combined. Fractions 2 (83 mg) and 4 (49 mg) were rechromatographed using the same system, giving a whitish powder. HPLC purification (MeCN–water, 37:63) of the product obtained from fraction 2 gave 25 mg of compound **1**, and purification of the product obtained from fraction 4 afforded 18 mg of compound **2**.

Actaeaepoxide 3-*O*-β-D-xylopyranoside (1): white, amorphous powder; mp 197 °C (dec); $[\alpha]_D$ +26.3° (*c* 0.32, i-PrOH–MeOH, 7:5); TLC R_f 0.53 (RP-18, MeOH–H_2O, 8:2); HPLC t_R 11.6 min; IR (KBr) ν_{max} 3412, 2935, 2359, 1730, 1649 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) δ 4.82 (1H, d, J = 7.6 Hz, H-1′), 4.36 (1H, dd, J = 5.1, 11.2 Hz, H-5′a), 4.23 (1H, dd, J = 5.1, 9.0 Hz, H-4), 4.20 (1H, dd, J = 8.3, 9.0 Hz, H-3), 4.04 (1H, t, J = 8.3 Hz, H-2′), 3.75 (1H, dd, J = 10.0, 11.2 Hz, H-5′b), aglycon, see Table 1; ^{13}C NMR, see Table 2; ESIMS *m*/*z* 675 $[M - H]^-$; HREIMS *m*/*z* 598.3506 (calcd for $C_{35}H_{50}O_8$, 598.3506).

Cimigenol 3-*O*-β-D-xylopyranoside (2): white, amorphous powder; mp 235 °C; $[\alpha]_D$ +551.9° (*c* 0.091, i-PrOH–MeOH, 45:75); TLC R_f 0.15; HPLC t_R 47.5 min; IR (KBr) ν_{max} 3432, 2935, 2870, 1457, 1383, 1071 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) δ 4.87 (1H, d, J = 7.6 Hz, H-1′), 4.75 (1H, d, J = 9.0 Hz, H-23), 4.38 (1H, dd, J = 4.9, 11.3 Hz, H-5′a), 4.29 (1H, s, H-15), 4.25 (1H, overlapped, H-4′), 4.23 (1H, overlapped, H-3′), 4.05 (1H, t, J = 7.6, H-2′), 3.84 (1H, s, H-24), 3.51 (1H, dd, J = 9.9, 11.3 Hz, H-5′b), 3.51 (1H, dd, J = 4.2, 11.7 Hz, H-3), 2.33 (1H, overlapped, H-2a), 2.32 (1H, overlapped, H-22a), 2.10 (2H, overlapped, H-7a and H-11a), 1.95 (1H, overlapped, H-2b), 1.68 (2H, overlapped, H-8 and H-12a), 1.66 (1H, overlapped, H-20), 1.58 (1H, overlapped, H-1a), 1.56 (1H, overlapped, H-12b), 1.55 (3H, s, H-26), 1.53 (1H, overlapped, H-6a), 1.50 (1H, overlapped, H-17), 1.50 (3H, s, H-27), 1.32 (3H, s, H-29), 1.30 (1H, overlapped, H-5), 1.26 (1H, overlapped, H-1b), 1.23 (3H, s, H-28), 1.16 (3H, s, H-18), 1.14 (1H, overlapped, H-7b), 1.08 (1H, overlapped, H-11b), 1.07 (1H, overlapped, H-22b), 1.07 (3H, s, H-30), 0.87 (1H, overlapped, H-6b), 0.87 (3H, d, J = 6.5 Hz, H-21), 0.51 (1H, d, J = 4.0 Hz, H-19a), 0.29 (1H, d, J = 4.0 Hz, H-19b); ^{13}C NMR (pyridine-d_5, 150 MHz) δ 112.0 (C-16), 107.3 (C-1′), 90.2 (C-24), 88.6 (C-3), 80.1 (C-15), 78.1 (C-3′), 75.3 (C-2′), 71.9 (C-23), 71.2 (C-25), 71.0 (C-4′), 68.2 (C-5′), 59.5 (C-17), 48.6 (C-8), 47.5 (C-5), 47.2 (C-14), 41.8 (C-13), 41.3 (C-4), 38.1 (C-22), 34.0 (C-12), 32.4 (C-1), 30.9 (C-19), 30.0 (C-2), 28.2 (C-10), 26.4 (C-26), 26.3 (C-11), 26.3 (C-7), 25.7 (C-29), 25.3 (C-27), 24.0 (C-20), 21.0 (C-6), 19.9 (C-9), 19.6 (C-21), 19.5 (C-18), 15.4 (C-30), 11.1 (C-28); ESIMS *m*/*z* 619 $[M - H]^-$; HREIMS *m*/*z* 620.3963 (calcd for $C_{35}H_{56}O_9$, 620.3925).

Identification of the Sugar Component of 1 and Determination of its Absolute Configuration. The determination was performed according to ref 17 using about 4 mg of **1**. Due to poor water solubility, the compound was dissolved in a mixture of dioxane–hydrochloric acid (3:7). Reference compounds were prepared in an identical manner. GLC conditions: column DB-5 column (15 m × 0.25 mm i.d., film thickness 0.25 μm, oven temperature 180 °C, injection port temperature 250 °C, carrier gas helium, 10 psi, injection volume 1 μL, split ratio 1:50). Retention times: D-xylose 3.50 min, L-xylose 3.92 min.

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Supporting Information Available: HPLC trace of a standardized propan-2-ol/water extract. This material is available free of charge via the Internet at http://pubs.acs.org.

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