Triterpene Glycosides from *Cimicifuga racemosa*

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Eight new triterpene glycosides named cimiracemosides A−H, respectively, and eight known triterpene glycosides were isolated from the rhizome extracts of black cohosh (*Cimicifuga racemosa*). The new compounds were determined by spectral data to be 21-hydroxycimigenol-3-*O*-α-L-arabinopyranoside (**1**), 21-hydroxycimigenol-3-*O*-*β*-D-xylopyranoside (**2**), cimigenol-3-*O*-α-L-arabinopyranoside (**3**), 12*β*-acetoxycimigenol-3-*O*-α-L-arabinopyranoside (**4**), 24-acetylisodahurinol-3-*O*-*β*-D-xylopyranoside (**5**), 20(*S*),22(*R*),23(*S*),24(*R*)-16*β*:23;22:25-diepoxy-12*β*-acetoxy-3*β*,23,24-trihydroxy-9,19-cycloanost-7-ene-3-*O*-*β*-D-xylopyranoside (**6**), 20(*S*),22(*R*),23(*S*),24(*R*)-16*β*:23;22:25-diepoxy-12*β*-acetoxy-3*β*,23,24-trihydroxy-9,19-cycloanost-7-en-3-*O*-α-L-arabinopyranoside (**7**), and 20(*S*),22(*R*),23(*S*),24(*R*)-16*β*:23;22:25-diepoxy-12*β*-acetoxy-3*β*,23,24-trihydroxy-9,19-cycloanostane-3-*O*-*β*-D-xylopyranoside (**8**).

Cimicifuga racemosa (Ranunculaceae), commonly known as black cohosh, is an herb native to North America. First introduced to Western medicine by Native American groups, its rhizomes have been used to treat a variety of ailments, including diarrhea, sore throat, and rheumatism. However, it is best known for its health benefit in treating painful menstrual periods and menopausal disorders.[1]

Although black cohosh has been sold as a dietary supplement and an over-the-counter medication all over the world, its chemical components are not completely known. Triterpene glycosides are considered to be the main components of black cohosh[2] and are used as marker compounds to standardize black cohosh extracts. Until now, however, only actein, 27-deoxyactein, and cimicifugoside (cimigenol-3-*O*-*β*-D-xylopyranoside) were isolated from black cohosh, and the structure of actein was only fully solved in 1998.[3,4] With the increasing market demand for black cohosh, it became necessary to clarify the triterpene glycoside components of black cohosh. In addition, comparison with other *Cimicifuga* genus plants, such as *C. simplex*, from which more than 50 triterpene glycosides have been isolated.[4−14] and *C. foetida* L., from which more than 10 triterpene glycosides have been identified,[15,16] suggests that the triterpene glycoside constituents of black cohosh (*C. racemosa*) deserve further study.

In this study, the triterpene glycoside components of black cohosh were systematically investigated. We now report the isolation and structure elucidation of eight new 9,19-cycloartane triterpene glycosides, cimiracemosides A−H (**1**−**8**), and eight known triterpene glycosides, including 27-deoxyactein,[17] 26-deoxycimicifugoside,[13] actein,[4] acetyl shengmanol xyloside,[6] cimicifugoside (cimigenol-3-*O*-*β*-D-xylopyranoside),[18] cimiaceroside A,[11] 12*β*-hydroxycimigenol-3-*O*-*β*-D-xylopyranoside,[8] and 12*β*-hydroxycimigenol-3-*O*-α-L-arabinopyranoside.[8]

Results and Discussion

Cimiracemoside A (**1**) was isolated as a white powder. In the negative ESIMS, it showed a significant molecular ion peak at *m*/*z* 635 [M − 1]$^-$. Its molecular formula $C_{35}H_{56}O_{10}$ was deduced from ^{13}C NMR and MS data. The IR showed an absorption band at 3448 cm^{-1} for OH groups. The ^{1}H NMR spectra showed the signals due to a cyclopropane methylene at *δ* 0.27 and 0.54 (each 1H, d, *J* = 3.6 Hz); six *tert*-methyl groups at *δ* 1.04, 1.24, 1.29, 1.30, 1.50, and 1.50; and an anomeric proton at *δ* 4.82 (d, *J* = 7.5 Hz). The ^{13}C NMR spectrum showed data consistent with an arabinose moiety at *δ* 107.8 (d), 75.0 (d), 73.3 (d), 69.9 (d), and 67.1 (t)[8] and seven oxygenated carbon signals at *δ* 112.7 (s), 90.3 (d), 88.9 (d), 80.8 (d), 72.5 (d), 71.3 (s), and 64.3 (t). All of the above evidence suggested that **1** was a highly oxygenated 9,19-cycloartane triterpene monoglycoside. Comparing the ^{1}H NMR and other data with those of known 9,19-cycloartane triterpene glycosides,[6,8,19] the aglycon of **1** should be very similar to that of cimicifugoside (cimigenol-3-*O*-*β*-D-xylopyranoside). In cimicifugoside, the signal for CH_3-21 was observed at *δ* 0.84 (d, *J* = 6.4 Hz), while in **1**, the signal for this characteristic secondary methyl group disappeared, and instead signals for a $-CH_2O-$ group at *δ* 3.97 (1H, m), 3.74 (1H, m) were observed. The ^{13}C NMR spectrum of **1**, in which a signal at *δ* 64.3 (t) was observed for a $-CH_2O-$ rather than a secondary methyl group, also supported this structural modification. Through the combination of ^{1}H−^{1}H COSY, HMQC, and HMBC experiments, the ^{1}H and ^{13}C signals for compound **1** were fully assigned (Table 1 and Table 2). In the ^{1}H−^{1}H COSY spectrum of **1**, the signal assigned to 20-H at *δ* 2.15 was correlated with ^{1}H signals at *δ* 3.97 and 3.74, additionally demonstrating that a $-CH_2OH$ group was attached at the C-20 position. In the HMBC spectrum, a significant correlation was observed between the ^{1}H signal at *δ* 4.82 (1H, d, *J* = 7.5 Hz, H-1$_{ara}$) and the ^{13}C signal at *δ* 88.9 (d, C-3), suggesting that the sugar moiety was located at the C-3 position. The relative stereochemistry of **1** was determined on the basis of the coupling constants from the proton and ROESY experiments. In the ROESY spectrum, CH_3-18 at *δ* 1.30 showed correlations with H-12, H-20, and H-15; CH_3-30 was correlated to H-19 and CH_3-29; CH_3-29 was correlated to H-3 and CH_3-30; and H-8 showed correlations with H-15 and H-19. Based on these observations, H-3 and CH_3-28 were assigned as α-configurations and CH_3-18, H-15, H-8, and H-20 were assigned as *β*-configurations. The configu-

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Table 1. The ^{1}H NMR Data for Compounds **1**−**8** in Pyridine-d_5[a]

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	1.23, 1.60	1.20, 1.58	1.24, 1.56	1.10, 1.50	1.17, 1.56	1.18, 1.60	1.15, 1.57	1.13, 1.52
2	1.95, 2.35	1.95, 2.35	1.96, 2.38	1.86, 2.30	1.92, 2.35	1.86, 2.27	1.90, 2.28	1.85, 2.30
3	3.50 dd (3.9, 11.4)	3.49 dd (4.4, 11.7)	3.50 dd (4.0, 11.4)	3.45 dd (4.2, 11.7)	3.50 dd (4.3, 11.7)	3.46 dd (4.0, 12.0)	3.42 dd (4.2, 11.6)	3.49 dd (4.2, 11.7)
5	1.35	1.33	1.32	1.22	1.30	1.20	1.20	1.25
6	0.73, 1.53	0.71, 1.50	0.72, 1.52	0.70, 1.45	0.59, 1.47	1.57, 1.87	1.57, 1.87	0.73, 1.45
7	1.20, 2.10	1.18, 2.08	1.08, 2.10	1.08, 2.10	1.00, 1.60	5.15	5.12	0.95, 1.23
8	1.75	1.72	1.73	1.73	1.28			1.62
11	1.10, 2.10	1.13, 2.10	1.09, 2.07	1.14 dd (16.0, 3.0) 2.92 dd (16.0, 9.4)	1.03, 2.23	1.23, 2.95 dd (16.0; 9.0)	1.23, 2.92 dd (16.1; 9.0)	1.28, 2.75 dd (16.2, 9.0)
12	1.69, 1.88	1.69, 1.83	1.68, 1.54	5.27 dd (9.4, 3.0)	1.48, 1.65	5.26 d (8.6)	5.23 d (8.6)	5.18 dd (3.6, 9.0)
15	4.35	4.33	4.35	4.36		2.05, 2.15	2.03, 2.13	1.92, 1.77
16					3.78 d (11.6)	5.09 dd (7.6, 15.6)	5.09	5.02
17	2.13	2.12	1.50	1.70	1.58	1.77	1.83	1.77
18	1.30 s	1.24 s	1.15 s	1.31 s	1.15 s	1.41 s	1.39 s	1.38 s
19	0.27 d (3.6), 0.54 d (3.6)	0.25 d (3.9), 0.50 d (3.9)	0.29 d (3.0), 0.52 d (3.0)	0.28 d (4.2), 0.56 d (4.2)	0.24 d (4.2), 0.47 d (4.2)	0.50 d (4.0) 1.01 d (4.0)	0.47 d (4.1), 1.00 d (4.1)	0.21 d (3.9), 0.54 d (3.9)
20	2.15	2.15	1.66	1.66	1.80	2.30	2.31	2.31
21	3.74, 3.97	3.72, 3.94	0.86 d (6.0)	0.92 d (6.0)	0.90 d (6.4)	1.33 d (6.5)	1.31 d (6.4)	1.38 d (3.3)
22	1.75, 2.70	1.72, 2.70	1.03, 2.29	1.03, 2.09	1.47, 1.70	3.91 d (10.5)	3.91 d (10.7)	3.92 d (10.8)
23	4.92 d (9.0)	4.87 d (9.0)	4.75 d (8.7)	4.74 d (8.7)	4.25			
24	3.91 s	3.91 s	3.78 s	3.78 s	5.31 d (2.4)	4.23 s	4.22 s	4.24 s
26	1.50 s	1.46 s	1.49 s	1.49 s	1.61 s	1.78 s	1.76 s	1.79 s
27	1.50 s	1.48 s	1.47 s	1.47 s	1.61 s	1.70 s	1.68 s	1.72 s
28	1.24 s	1.20 s	1.19 s	1.25 s	1.00 s	1.08 s	1.05 s	0.87 s
29	1.30 s	1.28 s	1.28 s	1.21 s	1.30 s	1.34 s	1.28 s	1.35 s
30	1.04 s	1.04 s	1.03 s	0.98 s	1.02 s	1.02 s	0.97 s	1.02 s
1′	4.82 d (7.5)	4.84 d (7.5)	4.81 d (7.1)	4.77 d (7.1)	4.85 d (7.5)	4.85 d (7.5)	4.75 d (7.2)	4.88 d (7.5)
2′	4.45	4.02	4.45	4.45	4.03 dd (8.6, 7.5)	4.05	4.44	4.07
3′	4.13 dd (8.6, 2.5)	4.15 t (8.7)	4.16 dd (8.8, 2.6)	4.16 dd (8.7, 2.6)	4.16 t (8.6)	4.16 t (8.6)	4.14 dd (8.9, 3.3)	4.19 t (8.7)
4′	4.33	4.22	4.30	4.30 br.d	4.22	4.24	4.29 br.s	4.22
5′	3.80, 4.31	3.72 dd (11.2, 5.1), 4.31 dd (10.0, 11.2)	3.80, 4.30	3.80, 4.30	3.73 dd (11.2, 10.0), 4.35 dd (5.1, 11.2)	3.73 dd (11.1, 11.2) 4.34 dd (11.1, 5.1)	3.75 d (10.7) 4.34 dd (10.7, 2.6)	3.76 t (11.1) 4.39 dd (11.1, 4.8)
CH_3CO				2.10 s	2.15 s	2.13 s	2.11 s	2.11 s

[a] Chemical shifts (δ) in ppm, coupling constants (Hz) in parentheses.

Table 2. The ^{13}C NMR Data (δ, ppm) for Compounds **1**−**8** in Pyridine-d_5

position	1	2	3	4	5	6	7	8
1	32.7 (t)	32.5 (t)	32.7 (t)	32.4 (t)	32.5 (t)	30.5 (t)	30.2 (t)	32.2 (t)
2	30.4 (t)	30.2 (t)	30.3 (t)	30.0 (t)	30.2 (t)	29.8 (t)	29.5 (t)	30.0 (t)
3	88.9 (d)	88.6 (d)	88.9 (d)	88.3 (d)	88.4(d)	88.2 (d)	87.9 (d)	88.3 (d)
4	41.7 (s)	41.4 (s)	41.6 (s)	41.3 (s)	41.4 (s)	40.7 (s)	40.4 (s)	41.5 (s)
5	47.9 (d)	47.6 (d)	47.9 (d)	47.2 (d)	47.4 (d)	42.7 (d)	42.4 (d)	47.3 (d)
6	21.4 (t)	21.1 (t)	21.4 (t)	20.8 (t)	20.8 (t)	22.1 (t)	21.8 (t)	20.8 (t)
7	26.7 (t)	26.4 (t)	26.6 (t)	26.0 (t)	25.9 (t)	114.2 (d)	113.9 (d)	26.0 (t)
8	49.1 (d)	48.8 (d)	48.9 (d)	47.3 (d)	43.6 (d)	148.0 (s)	147.7 (s)	46.0 (d)
9	20.4 (s)	20.1 (s)	20.3 (s)	20.1 (s)	20.1 (s)	21.5 (s)	21.2 (s)	20.3 (s)
10	27.0 (s)	26.7 (s)	26.9 (s)	26.8 (s)	27.1 (s)	28.6 (s)	28.3 (s)	27.0 (s)
11	26.8 (t)	26.5 (t)	26.7 (t)	37.5 (t)	26.1 (t)	37.0 (t)	36.7 (t)	37.1 (t)
12	34.1 (t)	33.8 (t)	34.4 (t)	77.3 (d)	31.2 (t)	77.0 (d)	76.7 (d)	75.9 (d)
13	42.4 (s)	42.1 (s)	42.1 (s)	48.5 (s)	40.0 (s)	49.1 (s)	48.8 (s)	49.6 (s)
14	47.7 (s)	47.4 (s)	47.6 (s)	46.3 (s)	55.1 (s)	51.3 (s)	51.0 (s)	48.4 (s)
15	80.8 (d)	80.5 (d)	80.5 (d)	79.2 (d)	214.1 (s)	42.3 (t)	42.0 (t)	43.3 (t)
16	112.7 (s)	112.4 (s)	112.2 (s)	112.0 (s)	84.3 (d)	72.6 (d)	72.3 (d)	72.3 (d)
17	53.6 (d)	53.3 (d)	59.8 (d)	59.3 (d)	52.3 (d)	53.6 (d)	53.3 (d)	52.8 (d)
18	20.4 (q)	19.9(q)	19.9 (q)	12.7(q)	20.3 (q)	15.4 (q)	15.1 (q)	14.1 (q)
19	31.1 (t)	30.9 (t)	31.2 (t)	30.9 (t)	31.4 (t)	29.0 (t)	28.7 (t)	30.3 (t)
20	32.7 (d)	32.4 (d)	24.4 (d)	24.1 (d)	33.2 (d)	34.6 (d)	34.3 (d)	34.8 (d)
21	64.3 (t)	64.0 (t)	19.8 (q)	21.7 (q)	21.1 (q)	17.9 (q)	17.6 (q)	17.8 (q)
22	33.5 (t)	33.2 (t)	38.4 (t)	38.6 (t)	38.7 (t)	87.0 (d)	86.7 (d)	86.9 (d)
23	72.5 (d)	72.1(d)	72.1 (d)	71.5 (d)	79.0 (d)	106.0 (s)	105.7 (s)	105.8 (s)
24	90.3 (d)	90.0 (d)	90.4 (d)	90.0 (d)	79.9 (d)	83.6 (d)	83.3 (d)	83.6 (d)
25	71.3 (s)	71.1 (s)	71.2 (s)	71.0 (s)	72.1 (s)	84.0 (s)	83.7 (s)	83.8 (s)
26	25.7 (q)	25.4 (q)	25.7 (q)	25.5 (q)	26.8 (q)	28.3 (q)	28.0 (q)	28.1 (q)
27	27.6 (q)	27.1 (q)	26.0 (q)	27.1 (q)	28.3 (q)	25.2 (q)	25.0 (q)	25.2 (q)
28	12.2 (q)	12.0 (q)	12.1 (q)	11.9 (q)	17.6 (q)	27.0 (q)	26.7 (q)	20.0 (q)
29	26.1 (q)	25.8 (q)	27.5 (q)	25.7 (q)	25.7 (q)	26.0 (q)	25.8 (q)	25.2 (q)
30	15.8 (q)	15.5 (q)	15.7 (q)	15.4 (q)	15.5 (q)	14.5 (q)	14.2 (q)	15.7 (q)
1′	107.8 (d)	107.6 (d)	107.7 (d)	107.5 (d)	107.6 (d)	107.8 (d)	107.5 (d)	107.8 (d)
2′	73.3 (d)	75.6 (d)	73.2 (d)	73.0 (d)	75.6 (d)	75.9 (d)	73.0 (d)	75.9 (d)
3′	75.0 (d)	78.6 (d)	74.9 (d)	74.7 (d)	78.7 (d)	79.0 (d)	74.7 (d)	78.9 (d)
4′	69.9 (d)	71.3 (d)	69.7 (d)	69.6 (d)	71.3 (d)	71.6 (d)	69.7 (d)	71.5 (d)
5′	67.1 (t)	67.1 (t)	67.0 (t)	66.8 (t)	67.2 (t)	67.5 (t)	66.9 (t)	67.4 (t)
CO				170.6 (s)	171.2 (s)	170.9 (s)	170.7 (s)	170.6 (s)
CH_3				20.0 (q)	20.0 (q)	21.9 (q)	21.7 (q)	22.0 (q

rations of C-23 and C-24 were assigned as *R* and *S*, respectively, by comparing the coupling constants of the C-23 and C-24 proton signals of **1** with those of known 9,19-cycloartane triterpene glycosides.[8,17] Thus, this compound was elucidated as 21-hydroxycimigenol-3-*O*-α-L-arabinopyranoside.

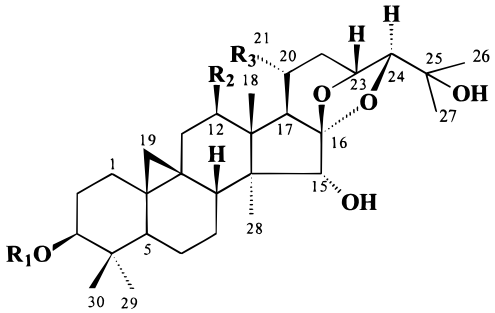
Cimiracemoside B (**2**) was isolated as a white powder, and its molecular formula, $C_{35}H_{56}O_{10}$, was deduced from negative ESIMS and the ^{13}C NMR of **2**. Compound **2** showed a very similar IR pattern to that of **1**. In the ^1H NMR spectrum, compound **2** showed the signals for a cyclopropane methylene at δ 0.25 and 0.50 (each 1H, d, J = 3.9 Hz), six *tert*-methyl groups at δ 1.04, 1.20, 1.24, 1.28, 1.46, and 1.48; a $-CH_2OH$ group at δ 3.94 and 3.72; and an anomeric proton resonance at δ 4.84 (d, J = 7.5 Hz), suggesting this compound was also a cyclotriterpene glycoside. In the ^{13}C NMR, compound **2** showed the signals for a xylose moiety at δ 107.6 (d), 78.6 (d), 75.6 (d), 71.3 (d), and 67.1 (t).[8,17] The remaining 30 carbon signals were identical with the aglycon signals of **1**, therefore **2** was elucidated as 21-hydroxycimigenol-3-*O*-β-D-xylopyranoside.

Cimiracemoside C (**3**) was isolated as a white powder. Its molecular formula, $C_{35}H_{56}O_{9,}$ was deduced from negative ESIMS and ^{13}C NMR data. The IR spectrum of **3** showed an absorption band at 3418 cm^{-1} (OH). In the ^1H NMR spectrum, **3** showed characteristic 9,19-cycloartane triterpene signals at δ 0.29 and 0.52 (each 1H, d, J = 3.0 Hz); six *tert*-methyl groups at δ 1.03, 1.15, 1.19, 1.28, 1.47, and 1.49; and a secondary methyl group at δ 0.86 (d, J = 6.0 Hz). In the ^{13}C NMR spectrum, compound **3** showed the signals for an arabinose moiety at δ 107.7, 74.9, 73.2, 69.7, and 67.0.[8] The remaining 30 carbon signals were identical with the aglycon signals of cimicifugoside (cimigenol-3-*O*-β-D-xylopyranoside), so this compound was elucidated as cimigenol-3-*O*-α-L-arabinopyranoside (**3**).

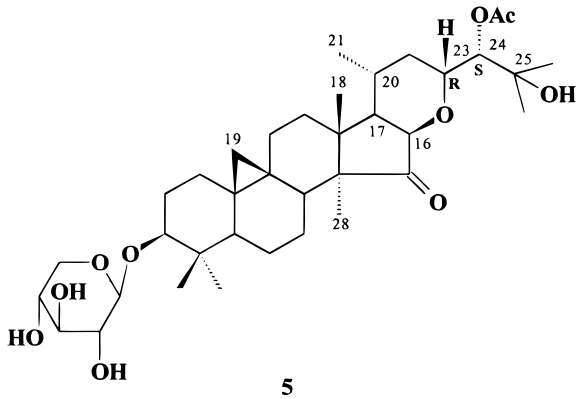
The negative ESIMS of **4** gave a molecular ion peak at *m*/*z* 677 $[M - 1]^-$, corresponding to the molecular formula $C_{37}H_{58}O_{11}$, which differs from **3** by containing an additional acetoxy group. The ^1H NMR spectrum showed the presence of a cyclopropane methylene at δ 0.28 and 0.56 (each 1H, d, J = 4.2 Hz); a secondary methyl group at δ 0.92 (3H, d, J = 6.0 Hz), six tertiary methyl groups at 0.98, 1.21, 1.25, 1.31, 1.47, and 1.49; and an anomeric proton at δ 4.77 (1H, d, J = 7.1 Hz). The ^1H and ^{13}C NMR data were assigned with the aid of $^1H-^1H$ COSY and HMQC experiments and by comparison with those of cimiracemoside C. The data were similar to those of cimiracemoside C, except for the signals for positions 11, 12, 13, and 18 and one extra acetoxy group. The ^1H signals at δ 1.14 (1H, dd, J = 16.0, 3.0 Hz), 2.92 (1H, dd, J = 16.0, 9.4 Hz), 5.27 (1H, dd, J = 9.4, 3.0 Hz), and 1.31 (3H, s) and ^{13}C signals at δ 37.5 (t), 77.3 (d), 48.5 (s), and 12.7 (q) were assigned to positions 11, 12, 13, and 18 for a 12β-acetoxycimigenol moiety, by comparison with those of 12β-acetoxy 9,19-cycloartane triterpene glycosides.[4,13] Therefore, **4** was elucidated as 12β-acetoxycimigenol-3-*O*-α-L-arabinopyranoside.

Cimiracemoside E (**5**) was isolated as a white powder. Its molecular formula $C_{37}H_{58}O_{10}$ was deduced from negative ESIMS, which showed a molecular peak at *m*/*z* 661 $[M - 1]^-$. In the IR spectrum of **5**, absorption bands for OH and carbonyl groups were observed. In the ^1H NMR, **5** showed signals due to a cyclopropane methylene at δ 0.24 and 0.47 (each 1H, d, J = 4.2 Hz); six *tert*-methyl groups at δ 1.00, 1.02, 1.15, 1.30, 1.61, and 1.61 (s); a secondary methyl

group at δ 0.90 (3H, s, J = 6.4 Hz); and an anomeric proton at δ 4.85 (d, J = 7.5 Hz). In the ^{13}C NMR spectrum, compound **5** showed signals due to a xylose moiety at δ 107.6, 78.7, 75.6, 71.3, and 67.2.[8] The corresponding ^{1}H signals for a xylose moiety were assigned as δ 4.85 (H-1′), 4.03 (H-2′), 4.16 (H-3′), 4.22 (H-4′), 4.35 (H-5′), and 3.73 (H-5′). Based on these observations, compound **5** was identified as a 9,19-cycloartane triterpene monoglycoside. ^{1}H−^{1}H COSY, HMQC, and HMBC spectra led to the full assignment of the ^{1}H and ^{13}C NMR data for the aglycon. In the HMBC spectrum, significant correlations were observed between CO-15 and H-16; between CO-15 and the 28-methyl group; between C-23 and H-16; and between the acetyl carbonyl group and H-24. The aglycon was, therefore, elucidated as 24-acetyldahurinol or 24-acetylisodahurinol (the difference between dahurinol and isodahurinol is the configuration for C-24, one *R* and one *S*).[20] The configuration of C-24 was elucidated as *S* by comparison of the coupling constants of H-24 (2.4 Hz) with those of dahurinyl diacetate (9 Hz) and isodahurinyl diacetate (2 Hz). Thus, compound **5** was elucidated as 24-acetylisodahurinol-3-*O*-β-D-xylopyranoside.



1 R_1 = α-L-arabinopyranosyl, R_2=H, R_3=CH_2OH
2 R_1 = β-D-xylopyranosyl, R_2=H, R_3=CH_2OH
3 R_1 = α-L-arabinopyranosyl, R_2=H, R_3=CH_3
4 R_1 = α-L-arabinopyranosyl, R_2=OAc, R_3=CH_3



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Cimiracemoside F (**6**) was isolated as a white powder. Its molecular formula was determined as $C_{37}H_{56}O_{11}$ on the basis of negative ESIMS and data from the ^{13}C NMR spectrum. The IR spectrum showed a strong hydroxyl band and a strong carbonyl band. In the ^{1}H NMR, **6** showed the signals for a cyclopropane methylene at δ 0.50 and 1.01 (each 1H, d, J = 4.0 Hz), a secondary methyl group, and an anomeric proton at δ 4.85 (1H, d, J = 7.5 Hz). Like **5**, the ^{13}C NMR spectrum of compound **6** showed signals for a xylose moiety at δ 107.8 (d), 79.0 (d), 75.9 (d), 71.6 (d), and 67.5 (t).[8] The remaining 32 carbon signals were assigned with the aid of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. The aglycon was very similar to that of cimiaceroside A,[11] with slight differences at C-11, C-12, C-13, and C-18. The chemical shifts for these positions were at δ 37.0 (C-11), 77.0 (C-12), 49.1 (C-13), and 15.4 (C-18), instead of δ 25.5 (C-11), 33.5 (C-12), 44.9 (C-13), and 23.3 (C-18) as in cimiaceroside A. The ^{13}C NMR data also showed an additional acetyl group [δ 170.9 (CO), 21.9 (CH_3)]. Based on these data, the structure of **6** was assigned as 12-acetoxycimiaceroside A. The relative stereochemisty of **6** was determined on the basis of NOESY experiments, the coupling constants of the protons, and comparison of the ^{1}H and ^{13}C NMR data with those of cimiaceroside A.[11] In the NOESY spectrum, important correlations were observed between CH_3-18/12β-OAc, CH_3-18/H-20, H-22/CH_3-21, H-22/CH_3-26, and H-3/CH_3-29. Because the chemical shifts of positions 16, 20, 22, 23, and 24 were identical with those of cimiaceroside A, compound **6** should have the same configurations at these positions. Therefore, **6** was identified as 20(*S*),22(*R*),23(*S*),24(*R*)-16β:23;22:25-diepoxy-12β-acetoxy-3β,23,24-trihydroxy-9,19-cycloanost-7-ene-3-*O*-β-D-xylopyranoside.

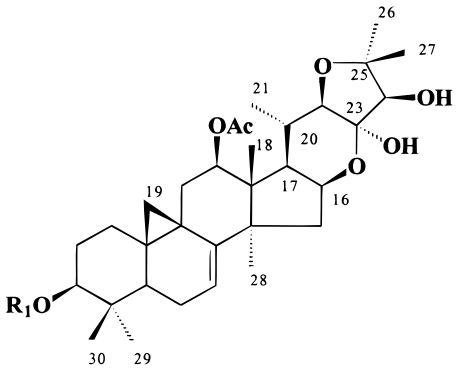
Cimiracemoside G (**7**) showed the same molecular weight in the ESIMS as **6**. In the ^{1}H NMR, compound **7** showed characteristic 9,19-cycloartane triterpene signals; signals at δ 0.97, 1.05, 1.28, 1.39, 1.68, and 1.76 for six *tert*-methyl groups; and a signal at δ 1.31 for a secondary methyl group. Comparison of the ^{13}C NMR data of **7** with those of cimiracemoside F, revealed that only the sugar moiety was different. Instead of a xylose moiety, an arabinose moiety was found in compound **7**. Consequently, **7** was elucidated as 20(*S*),22(*R*),23(*S*),24(*R*)-16β:23;22:25-diepoxy-12β-acetoxy-3β,23,24-trihydroxy-9,19-cycloanost-7-en-3-*O*-α-L-arabinopyranoside.

Cimiracemoside H (**8**) had molecular formula $C_{37}H_{58}O_{11}$ on the basis of negative ESIMS and ^{13}C NMR data, and the IR spectrum showed OH and carbonyl bands. The ^{1}H and ^{13}C NMR data were very similar to those of **6**, except for the absence of a 7(8)-double bond and the signals shifts due to the neighboring effects of the double bond (H-5, H-6, H-7, H-8, H-15, H-19, H-28, C-5, C-7, C-8, C-14, C-15, C-18, C-19, C-28); therefore, **8** was elucidated as 20(*S*),22(*R*),-23(*S*),24(*R*)-16β:23;22:25-diepoxy-12β-acetoxy-3β,23,24-trihydroxy-9,19-cycloanostane-3-*O*-β-D-xylopyranoside. Evaluation of the estrogen-receptor-binding activity of these compounds is in progress.

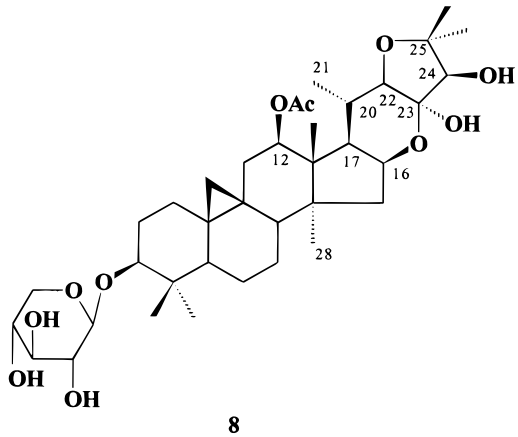
Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover "unimelt" apparatus. Optical rotations were obtained on Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet 5000 infrared spectrometer. ^{1}H and ^{13}C NMR were measured on a Bruker Avance 500 MHz NMR or 300 MHz instrument, and all 2D spectra NMR were run on a Bruker 500 MHz NMR spectrometer. MS data were recorded on a Micromass LC−MS system. Thin-layer chromatography was performed on Whatman TLC plates (250 μm thickness, K6F Si gel 60A), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in EtOH. Semipreparative HPLC was carried out on a Varian Pro-Star system equipped with UV−vis detector and a Dynamax C_{18} semipreparative column (21.4 × 250 mm). Reversed-phase chromatography was carried out on a Merck Lobar Lichroprep RP_{18} column. Si gel (Whatman, 230−400 mesh) and Sephadex LH-20 were used for column chromatography.

Plant Material. The standardized black cohosh (*C. racemosa*) extract (lot number: 98122590) was a gift from Finzelberg GmbH & Co. KG, Andernach, Germany. A voucher specimen was deposited in laboratory of the Solgar Research



6 R_1=β-D-xylopyranosyl
7 R_1=α-L-arabinopyranosyl



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Center for Excellence, Whitehall-Robins Pharmaceutical R & D, Richmond, USA.

Extraction and Isolation. The black cohosh powdered extract (1 kg) was re-extracted three times over a one-week period using 10 L of MeOH. The extracts were evaporated under reduced pressure to afford a residue (200 g). A sample (190 g) of the residue was directly subjected to column chromatography on Si gel and eluted with $CHCl_3$−MeOH with increasing MeOH content [first 4 L $CHCl_3$, then 4 L $CHCl_3$−MeOH (100:1), then $CHCl_3$−MeOH (50:1, 30:1, 20:1, 15:1, 14:1, 12:1, 10:1, 8:1, 6:1, 4:1, 3:1, each 2000 mL), and finally 2000 mL MeOH]. A total of 28 fractions was collected.

Fraction 9 was subjected to a normal-phase Si gel column eluted with EtOAc−MeOH−H_2O (40:1:1) to obtain three fractions (I−III). Fraction II was subjected to a RP_{18} column eluted by MeOH−H_2O (1:1, 500 mL, 3:2, 500 mL, 7:3, 100 mL) to get five subfractions (IIA−IIE). Subfraction IIB was subjected to semipreparative HPLC (MeCN−H_2O, 35:65, flow rate 10 mL/min) to yield pure 27-deoxyactein (100 mg) and 26-deoxycimicifugoside (13 mg). Acetin (260 mg) was crystallized from subfraction IIC (using MeOH). Subfraction IID was separated by semipreparative HPLC (MeCN−H_2O, 35:65, flow rate 10 mL/min) to afford acetylshengmanol xyloside (15 mg). Subfraction IIE was subjected to semipreparative HPLC (MeCN−H_2O, 35:65, flow rate 10 mL/min) to yield pure **5** (11 mg).

Fraction 13 was dissolved in MeOH, from which crystals (1.1 g) were obtained. A sample (300 mg) of the crystals was subjected to semipreparative HPLC (MeCN−H_2O, 40:60, flow rate 10 mL/min) to afford **3** (33 mg) and cimicifugoside (200 mg).

Fractions 14−17 were combined and subjected to a Sephadex LH-20 column and eluted with MeOH to provide two fractions. Fraction II was subjected to a RP_{18} column and eluted with MeCN−H_2O (30:70) to yield four subfractions (IIA−IID). Fraction IIB was subjected to a RP_{18} column and eluted with MeCN−H_2O (25:75) to give **6** (340 mg) and **7** (43 mg). Fraction IIC was rechromatographed on a RP_{18} column eluted with MeCN−H_2O (30:70) and then separated further by semipreparative HPLC (MeCN−H_2O, 30:70, flow rate 10 mL/min) to afford **4** (7 mg), **8** (11 mg), and cimiaceroside A (11 mg).

Fractions 19−22 were combined and subjected to a Sephadex LH-20 column, eluted with MeOH to separate phenolic compounds. The triterpene fraction was subjected to a normal-phase Si gel column, using $CHCl_3$−MeOH(12:1) to provide three fractions (I−III). Fraction II was subjected to a RP_{18} column and eluted with MeCN−H_2O (25:75) to yield two subfractions (IIA and IIB). Subfraction IIA was subjected to semipreparative HPLC and eluted with MeCN−H_2O (25:75, flow rate 10 mL/min) to afford pure 12β-hydroxycimigenol-3-*O*-β-D-xylopyranoside (20 mg) and 12β-hydroxycimigenol-3-*O*-α-L-arabinopyranoside (33 mg). Fraction III was subjected to semipreparartive HPLC to give pure **1** (70 mg) and **2** (62 mg).

Cimiracemoside A (1): white powder (70 mg); mp 285−287 °C; $[\alpha]_D$ +24.0° (*c* 0.13, MeOH); IR (film) ν_{max} 3448 (OH) cm^{-1}; negative ESIMS *m/z* 635 $[M - 1]^-$; ^1H (500 MHz) and ^{13}C (125 MHz) NMR, Tables 1 and 2, respectively.

Cimiracemoside B (2): white powder (62 mg); mp 288−290 °C; $[\alpha]_D$ +8.3° (*c* 0.24, MeOH); IR (film) ν_{max} 3411 (OH) cm^{-1}; negative ESIMS *m/z* 635 $[M - 1]^-$; ^1H (500 MHz) and ^{13}C (125 MHz) NMR, Tables 1 and 2, respectively.

Cimiracemoside C (3): white powder (33 mg); mp 258−260 °C; $[\alpha]_D$ +39.0° (*c* 0.11, MeOH); IR (film) ν_{max} 3418 (OH) cm^{-1}; negative ESIMS *m/z* 619 $[M - 1]^-$; ^1H (300 MHz) and ^{13}C (75 MHz) NMR, Tables 1 and 2, respectively.

Cimiracemoside D (4): white powder (7 mg); mp 165−168 °C; $[\alpha]_D$ −20.0° (*c* 0.05, MeOH); IR (film) ν_{max} 3430 (OH), 1730 (CO) cm^{-1}; negative ESIMS *m/z* 677 $[M - 1]^-$; ^1H (500 MHz) and ^{13}C (125 MHz) NMR, Tables 1 and 2, respectively.

Cimiracemoside E (5): white powder (15 mg); mp 232−234 °C; $[\alpha]_D$ +40.0° (*c* 0.07, MeOH); IR (film) ν_{max} 3426 (OH), 1744 (CO), 1727 (CO) cm^{-1}; negative ESIMS *m/z* 661 $[M - 1]^-$; ^1H (500 MHz) and ^{13}C (125 MHz) NMR, Tables 1 and 2, respectively.

Cimiracemoside F (6): white powder (340 mg); mp 266−270 °C; $[\alpha]_D$ −56.8° (*c* 0.28, MeOH); IR (film) ν_{max} 3432 (OH), 1734 (CO) cm^{-1}; negative ESIMS *m/z* 675 $[M - 1]^-$; ^1H (500 MHz) and ^{13}C (125 MHz) NMR, Tables 1 and 2, respectively.

Cimiracemoside G (7): white powder (43 mg); mp 198−201 °C; $[\alpha]_D$ −59.1° (*c* 0.11, MeOH); IR (film) ν_{max} 3441(OH), 1733 (CO) cm^{-1}; negative ESIMS *m/z* 675 $[M - 1]^-$; ^1H (500 MHz) and ^{13}C (125 MHz) NMR, Tables 1 and 2, respectively.

Cimiracemoside H (**8**): white powder (11 mg); mp 189−192 °C; $[\alpha]_D$ −30° (*c* 0.06, MeOH); IR (film) ν_{max} 3433 (OH), 1735 (CO) cm^{-1}; negative ESIMS *m/z* 677 $[M - 1]^-$; ^1H (300 MHz) and ^{13}C (75 MHz) NMR, Tables 1 and 2, respectively.

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