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Cytotoxic Chemical Constituents from the Roots of *Cimicifuga fetida*

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Seven new 9,19-cycloartane triterpene glycosides, 25-*O*-acetylcimigenol-3-*O*-[2′-*O*-(*E*)-2-butenoyl]-*β*-D-xylopyranoside (**1**), 25-*O*-acetylcimigenol-3-*O*-[4′-*O*-(*E*)-2-butenoyl]-*β*-D-xylopyranoside (**2**), 25-*O*-acetylcimigenol-3-*O*-[3′-*O*-acetyl]-*β*-D-xylopyranoside (**3**), 25-*O*-acetylcimigenol-3-*O*-[4′-*O*-acetyl]-*β*-D-xylopyranoside (**4**), 25-*O*-acetyl-12*β*-acetoxycimigenol-3-*O*-*β*-D-xylopyranoside (**5**), 3′-*O*-acetylactein (**6**), and 3′-*O*-acetyl-23-*epi*-26-deoxyactein (**7**), together with eight known compounds (**8**−**15**), were isolated from the roots of *Cimicifuga fetida*. Their structures were established by spectroscopic and chemical methods. Most of these compounds showed more selective and higher cytotoxicity against the human HepG2 cell line than against the MCF7, HT29, and MKN28 cell lines. Compounds **2**, **3**, and **7** exhibited significant cytotoxicity against HepG2 cells, with IC_{50} values of 1.29, 0.71, and 1.41 μM, respectively.

The *Cimicifuga* (now *Actaea*)[1,2] species have a long history of use as a medicinal herb.[3] In Europe and the United States, *C. racemosa*, commonly called black cohosh, is a well-known dietary supplement for women's health in alleviating menstrual pain and menopausal disorders.[4,5] In China, the roots of *C. fetida* are an important traditional Chinese medicine and have been officially listed in the Chinese Pharmacopoeia as a cooling and detoxifying remedy.[6] Chemical and pharmacological studies on *C. fetida* have shown that it contains a series of bioactive constituents such as chromones, caffeic acid derivatives, and 9,19-cyclolanostane triterpenoid glycosides.[7−14] Previously, we carried out studies on this plant collected from Dali and Lijiang Counties in Yunnan Province and reported a new triterpene alkaloid[15] and a series of cycloartane triterpenoid glycosides, as well as their antitumor and anticomplement activities.[16−18]

Our continuing investigation on the roots of *C. fetida* collected from Heze County, Guizhou Province, has led to the isolation of seven new 9,19-cycloartane triterpene glycosides (**1**−**7**), together with eight known compounds, acteinol (**8**), asiaticoside A (**9**), actrin-3-one (**10**), 26-deoxyacteinol (**11**), 25-*O*-acetylcimigenol (**12**), 12-*β*-acetoxycimigenol (**13**), cimigenol-3-*O*-α-L-arabinoside (**14**), and norcimifugin (**15**). All compounds were tested for their cytotoxicities against the human HepG2, MCF7, HT29, and MKN28 cancer cell lines using the MTT assay. Described herein are the isolation, structure elucidation, and biological activities of these compounds.

Results and Discussion

Compound **1** was obtained as a white powder, showing an $[M + Na]^+$ ion at *m/z* 753.4169 in the HRTOF-ESIMS consistent with the empirical molecular formula $C_{41}H_{62}O_{11}$ (calcd 753.4189), requiring 11 sites of unsaturation. The IR spectrum showed absorptions for hydroxy groups at 3461 cm^{-1} and carbonyl groups at 1731 cm^{-1}. The assignment of ^1H and ^{13}C NMR spectroscopic data of **1** (Tables 1 and 2) was based on HSQC, HMBC (Figure 1), and $^1H-^1H$ COSY data. In the ^1H NMR spectrum (Table 1), the characteristic cyclopropane methylene resonances at δ_H 0.21 and 0.43 (1H each, d, $J = 4.0$ Hz), an anomeric proton at δ_H 4.87 (d, $J = 8.0$ Hz), two olefinic protons at δ_H 6.07 (1H, dd, $J = 1.6$, 15.6 Hz) and 7.09 (1H, m), an acetyl methyl group at δ_H 1.95, two secondary methyl resonances at δ_H 0.82 (d, $J = 6.4$ Hz) and 1.65 (d, $J = 7.2$ Hz), and six tertiary methyl groups at δ_H 0.82−1.66 were observed. The ^{13}C NMR and DEPT spectra of **1** (Table 2) showed resonances ascribable to the methylene carbon of the

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Table 1. ^{1}H NMR Data of Compounds **1**−**7** in Pyridine-d_5

proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	1.55 m	1.58 m	1.59 m	1.56 m	1.54 m	1.52 m	1.49 m
	1.21[a]	1.22[a]	1.21[a]	1.20[a]	1.10 m	1.14[a]	1.10 m
2	1.88 m	1.94 m	1.92[a]	1.93 m	1.86 m	1.82 m	1.82 m
	2.25 m	2.30 m	2.30 m	2.29 m	2.27 m	2.23 m	2.21 m
3	3.39 dd (4.4, 11.6)	3.51 dd (4.0, 11.6)	3.47 dd (4.0, 12)	3.51 dd (4.4, 11.6)	3.46 m	3.41 dd (4.0, 11.6)	3.39 dd (4.4, 11.6)
4							
5	1.29[a]	1.30[a]	1.29[a]	1.32[a]	1.26[a]	1.16[a]	1.18[a]
6	0.66 m	0.70 m	0.67 m	0.72 m	0.68 m	0.62 m	0.55 m
	1.51 m	1.52 m	1.52 m	1.52 m	1.49 m	1.36[a]	1.36[a]
7	1.08[a]	1.11 m	1.07 m	1.11 m	1.08 m	0.87 brd (6.8)	0.89[a]
	2.07 m	2.08 m	2.10 m	2.11 m	2.12[a]	1.16[a]	1.22[a]
8	1.65[a]	1.68[a]	1.66[a]	1.69[a]	1.72[a]	1.54 m	1.58 m
9							
10							
11	1.08[a]	1.08 m	1.16 m	1.19 m	1.13[a]	1.16[a]	1.17 m
	2.13 m	2.11 m	2.10 m	2.11 m	2.92 dd (9.6, 16.0)	2.70 dd (8.4, 15.6)	2.69 dd (8.4, 16.0)
12	1.51 m	1.56 m	1.55 m	1.52 m	5.23 d (7.6)	5.05[a]	5.07[a]
	1.64[a]	1.67[a]	1.67[a]	1.68[a]			
13							
14							
15	4.25 s	4.27 brs	4.26 s	4.29 s	4.38 s	1.52 m	1.73 m
						1.71 m	1.85 m
16						4.60 dd (7.6, 14.6)	4.22 m
17	1.45 d (11.2)	1.44 d (10.8)	1.46 m	1.47 m	1.62 brs	1.84 m	1.77 m
18	1.11 s	1.14 s	1.13 s	1.15 s	1.30 s	1.34 s	1.39 s
19	0.21 d (4.0)	0.27 d (4.0)	0.26 d (3.6)	0.29 d (4.0)	0.28 d (4.0)	0.20 d (4.4)	0.16 d (4.0)
	0.43 d (4.0)	0.50 m	0.49 d (3.6)	0.53 d (4.0)	0.56 brs	0.53 d (4.4)	0.48 d (4.0)
20	1.63[a]	1.65[a]	1.67[a]	1.69[a]	1.62 brs	1.81 m	2.21 m
21	0.82 d (6.4)	0.84 d (6.8)	0.82 d (6.4)	0.85 d (6.0)	0.91 d (6.4)	0.94 d (6.4)	0.99 d (6.4)
22	0.97 m	0.98 m	0.97 m	0.98 m	0.97[a]	1.65 m	1.42[a]
	2.25 m	2.27 m	2.24 m	2.26 dd (3.5, 11.2)	2.27 m	2.22 m	1.58 m
23	4.58 d (8.8)	4.60 d (9.2)	4.58 d (8.8)	4.61 d (9.2)	4.58 d (8.8)		
24	4.09 brs	4.11 brs	4.10 brs	4.13 brs	4.08 brs	3.93 brs	3.65 brs
25							
26	1.66 s	1.69 s	1.68 s	1.70 s	1.68 s	5.74[a]	3.60 d (10.4)
							4.04 d (10.4)
27	1.64 s	1.66 s	1.65 s	1.68 s	1.66 s	1.77 s	1.45 s
28	1.16 s	1.19 s	1.17 s	1.20 s	1.19 s	0.77 s	0.82 s
29	1.08 s	1.31 s	1.25 s	1.32 s	1.28 s	1.23 s	1.23 s
30	0.95 s	1.04 s	0.99 s	1.05 s	1.01 s	0.93 s	0.91 s
3-Xyl							
1′	4.87 d (8.0)	4.90 d (7.6)	4.84 d (7.6)	4.88 d (7.6)	4.83 d (7.6)	4.82 d 7.6	4.81 d 7.6
2′	5.64 t (8.0)	4.10 t (8.4)	4.05 t (8.6)	4.07 t (8.8)	4.03 t (8.0)	4.04 t 8.4	4.04 t 8.4
3′	4.23 m	4.33 t (8.8)	5.73 t (9.2)	4.29 t (9.2)	4.17 m	5.74 t 10.0	5.74 t 9.2
4′	4.23 m	5.47 m	4.22 m	5.43 ddd (5.6, 10.0, 12.4)	4.21 m	4.22 m	4.21 m
5′	3.70 t (10.0)	3.64 t (10.4)	3.71 t (10.8)	3.61 t (10.4)	3.71 t (10.4)	3.72 t (11.2)	3.71 t (10.8)
	4.32 dd (4.4, 11.0)	4.37 dd (5.6, 11.2)	4.33 dd (4.2, 11.4)	4.35 dd (5.0, 11.2)	4.33 dd (4.8, 10..8)	4.33 dd (5.6, 11.6)	4.33 dd (5.6, 11.2)
12-OCOC$\underline{H}_3$					2.11 s	1.96 s	2.11 s
25-OCOC$\underline{H}_3$	1.95 s	1.98 s	1.94 s	1.98 s	1.97 s		
3′-OCOC$\underline{H}_3$			1.96 s			2.13 s	1.95 s
4′-OCOC$\underline{H}_3$				1.98 s			
2(4)′-OCOC$\underline{H}$=CH-CH$_3$	6.07 dd (1.6, 15.6)	5.86 d (15.2)					
2(4)′-OCOCH=C$\underline{H}$-CH$_3$	7.09 m	6.97 m					
2(4)′-OCOCH=CH-C$\underline{H}_3$	1.65 d (7.2)	1.58 d (7.2)					

[a] Signals overlapped.

cyclopropane ring at δ_C 30.9 (C-19), oxymethine carbons at δ_C 88.8 (C-3), 86.8 (C-24), 80.2 (C-15), and 71.7 (C-23), two oxygen-bearing quaternary carbons at δ_C 112.4 (C-16) and 83.2 (C-25), and a carbonyl group at δ_C 170.2 for the aglycone moiety. The ^{13}C NMR spectrum also revealed carbons assignable to a 2-butenoyl moiety at δ_C 165.8 (s), 123.6 (d), 144.8 (d), and 17.8 (q) and to a glycosidic moiety at 104.8 (d), 75.6 (d), 76.4 (d), 71.4 (d), and 67.2 (t). In the HMBC spectrum (Figure 1), a correlation was observed between the proton at δ_H 4.87 (H-1′, 1H, d, J = 8.0 Hz) and the methine carbon at δ_C 88.8 (C-3), suggesting that the sugar moiety was located at C-3. The sugar obtained after acid hydrolysis was identified as D-xylose by comparing its TLC and specific rotation with an authentic sample. The ^{1}H and ^{13}C NMR spectroscopic data of **1** were found similar to those of 25-*O*-acetylcimigenol-3-*O*-β-D-xylopyranoside (**16**),[19] except for the resonances of the sugar moiety. In **16**, the H-2′ resonance was observed at δ_H 4.02, whereas in **1** it shifted downfield to δ_H 5.64. In addition, the C-1′ resonances at δ_C 107.5 and C-3′ at δ_C 78.6 in **16** shifted upfield to δ_C 104.8 and 76.4, respectively, in **1**, which may be explained by the presence of the C-2′ 2-butenoyl group of the xylose unit.

Table 2. ^{13}C NMR Data of Compounds **1**−**7** in Pyridine-d_5

C	1	2	3	4	5	6	7
1	32.1 t	32.2 t	32.5 t	32.4 t	32.4 t	31.9 t	31.9 t
2	30.0 t	30.0 t	30.1 t	30.0 t	30.1 t	29.9 t	29.9 t
3	88.8 d	88.7 d	88.9 d	88.6 d	88.3 d	88.3 d	88.4 d
4	41.1 s	41.4 s	41.4 s	41.3 s	41.3 s	41.2 s	41.1 s
5	47.5 d	47.6 d	47.6 d	47.6 d	47.2 d	47.0 d	47.0 d
6	21.1 t	21.1 t	21.1 t	21.1 t	20.9 t	20.3 t	20.3 t
7	26.4 t	26.4 t	26.4 t	26.3 t	26.1 t	25.7 t	25.6 t
8	48.7 d	48.7 d	48.7 d	48.6 d	47.3 d	45.7 d	45.6 d
9	20.1 s	20.1 s	20.1 s	20.0 s	20.2 s	20.1 s	20.2 s
10	26.6 s	26.7 s	26.7 s	26.7 s	26.8 s	26.7 s	26.7 s
11	26.3 t	26.5 t	26.5 t	26.4 t	37.6 t	36.7 t	36.6 t
12	34.0 t	34.1 t	34.1 t	34.0 t	77.3 d	77.1 d	77.1 d
13	41.8 s	41.8 s	41.9 s	41.8 s	46.2 s	48.8 s	48.8 s
14	47.2 s	47.3 s	47.3 s	47.2 s	48.4 s	47.8 s	47.9 s
15	80.2 d	80.2 d	80.2 d	80.2 d	79.1 d	43.6 t	44.2 t
16	112.4 s	112.5 s	112.5 s	112.4 s	112.5 s	73.0 d	74.5 d
17	59.4 d	59.5 d	59.5 d	59.4 d	59.1 d	56.4 d	56.2 d
18	19.5 q	19.5 q	19.6 q	19.5 q	12.8 q	13.5 q	13.5 q
19	30.9 t	30.9 t	30.9 t	30.9 t	30.9 t	29.5 t	29.4 t
20	23.9 d	23.9 d	24.0 d	24.0 d	24.0 d	26.0 d	23.3 d
21	19.5 q	19.5 q	19.5 q	19.5 q	19.9 q	21.0 q	21.2 q
22	37.9 t	37.9 t	37.9 t	37.9 t	38.4 t	37.6 t	37.6 t
23	71.7 d	71.8 d	71.8 d	71.7 d	71.4 d	105.8 s	105.9 s
24	86.8 d	86.8 d	86.8 d	86.8 d	86.7 d	63.5 d	62.3 d
25	83.2 s	83.2 s	83.2 s	83.1 s	83.2 s	65.6 s	62.5 s
26	23.4 q	23.4 q	23.4 q	23.4 q	23.4 q	98.4 d	68.1 t
27	21.5 q	21.6 q	21.6 q	21.6 q	21.5 q	13.1 q	14.3 q
28	11.8 q	11.8 q	11.9 q	11.8 q	11.9 q	19.5 q	19.6 q
29	25.5 q	25.7 q	25.7 q	25.7 q	25.7 q	25.6 q	25.6 q
30	15.3 q	15.5 q	15.4 q	15.4 q	15.5 q	15.2 q	15.2 q
3-Xyl							
1′	104.8 d	107.4 d	107.2 d	107.3 d	107.6 d	107.2 d	107.2 d
2′	75.6 d	75.8 d	73.2 d	75.7 d	75.6 d	73.2 d	73.1 d
3′	76.4 d	75.1 d	79.4 d	74.9 d	78.6 d	79.4 d	79.3 d
4′	71.4 d	73.0 d	69.3 d	73.2 d	71.2 d	69.3 d	69.2
5′	67.2 t	63.4 t	66.8 t	63.2 t	67.1 t	66.8 t	66.8 t
12-O$\underline{C}$OCH$_3$					170.6 s	170.8 s	170.6 s
12-OCO$\underline{C}$H$_3$					21.7 q	21.6 q	21.6 q
25-O$\underline{C}$OCH$_3$	170.2 s	170.3 s	170.2 s	170.2 s	170.3 s		
25-OCO$\underline{C}$H$_3$	22.3 q	22.3 q	22.3 q	21.1	22.4 q		
3′-O$\underline{C}$OCH$_3$			170.9 s			170.6 s	170.8 s
3′-OCO$\underline{C}$H$_3$			21.6 q			21.2 q	21.3 q
4′-O$\underline{C}$OCH$_3$				170.6 s			
4′-OCO$\underline{C}$H$_3$				21.6			
2(4)′-O$\underline{C}$OCH=CH-CH$_3$	165.8 s	166.2 s					
2(4)′-OCO$\underline{C}$H=CH-CH$_3$	123.6 d	122.9 d					
2(4)′-OCOCH=$\underline{C}$H-CH$_3$	144.8 d	145.4 d					
2(4)′-OCOCH=CH-$\underline{C}$H$_3$	17.8 q	17.7 q					

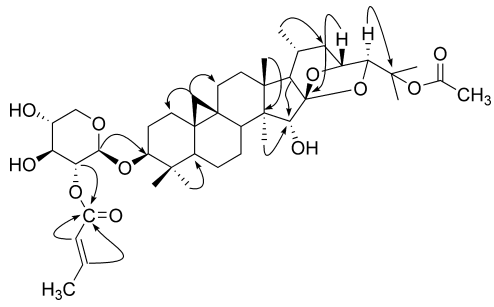


Figure 1. Major long-distance $^1H-^{13}C$ correlations of **1** observed by HMBC (pyridine-d_5).

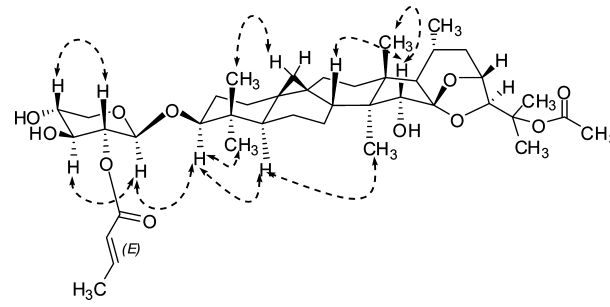
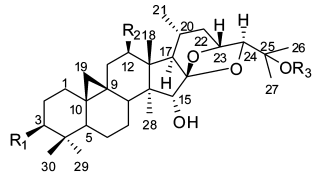


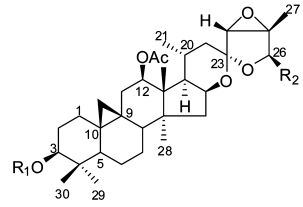
Figure 2. Key ROESY correlations of compound **1**.

This deduction was confirmed by the HMBC correlation observed between H-2′ (δ_H 5.64) and the ester carbonyl carbon (δ_C 165.8). The coupling constant ($J = 15.6$ Hz) of the two olefinic protons at δ_H 6.07 and 7.09 confirmed the *E*-geometry of the double bond of the C-2′ side chain. In the ROESY spectrum (Figure 2), associations of H-3 with H-5 and H-29 suggested a β-orientation of the substituent at C-3, whereas the associations of H-15 with H-8 and H-18 suggested an α-orientation of the C-15 hydroxy group. The configurations at C-23 and C-24 were assigned as *R* and *S*, respectively, by comparing the coupling constants of the H-23 and H-24 of **1** with those of known 9,19-cyclolanostane triterpene glycosides.[20] Therefore, the structure of **1** was elucidated as 25-*O*-acetylcimigenol-3-*O*-[2′-*O*-(*E*)-2-butenoyl]-β-D-xylopyranoside.

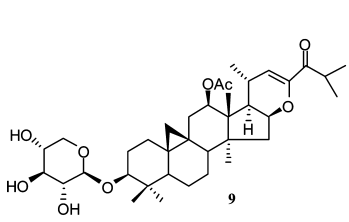
Compound **2**, a white powder, showed a pseudomolecular ion at *m/z* 753 $[M + Na]^+$ in the positive ESIMS. The ^{13}C NMR and HRTOF-ESIMS (*m/z* 753.4193 $[M + Na]^+$) determined its mo-



1 R_1 = -O-2'-O-(*E*)-2-butenoyl-*β*-D-xylose, R_2 = H, R_3 = Ac
2 R_1 = -O-4'-O-(*E*)-2-butenoyl-*β*-D-xylose, R_2 = H, R_3 = Ac
3 R_1 = -O-3'-O-acetyl-*β*-D-xylose, R_2 = H, R_3 = Ac
4 R_1 = -O-4'-O-acetyl-*β*-D-xylose, R_2 = H, R_3 = Ac
5 R_1 = -O-*β*-D-xylose, R_2 = OAc, R_3 = Ac
10 R_1 = =O, R_2 = H, R_3 = H
12 R_1 = OH, R_2 = H, R_3 = Ac
13 R_1 = OH, R_2 = OAc, R_3 = H
14 R_1 = -O-*α*-L-arabinose, R_2 = H, R_3 = H



6 R_1 = 3'-O-acetyl-*β*-D-xylose, R_2 = OH
8 R_1 = H, R_2 = OH
11 R_1 = H, R_2 = H



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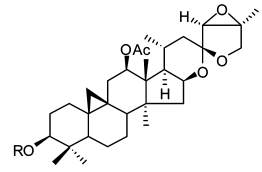
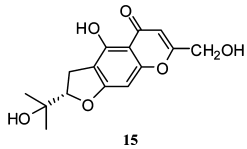


7 R = 3'-O-acetyl-*β*-D-xylose



15

lecular formula as $C_{41}H_{62}O_{11}$, which is identical to compound **1**. The IR and NMR spectroscopic data of **2** were also similar to those of **1**. The major difference in the ^{1}H NMR spectrum of the two compounds involves the H-2′ resonance of **1** at δ_H 5.64 (t, J = 8.0 Hz), which was shifted upfield to δ_H 4.10 (t, J = 8.4 Hz) in **2**. The H-4′ resonance was shifted downfield from δ_H 4.23 in **1** to δ_H 5.47 in **2**. The downfield shift of H-4′ in **2** can be explained by the attachment of the (*E*)-2-butenoyl group at C-4′. Supportive evidence was obtained from the HMBC spectrum, which showed a correlation between H-4′ (δ_H 5.47) and the carbonyl group at δ_C 166.2. Thus, compound **2** was characterized as 25-*O*-acetylcimigenol-3-*O*-[4′-*O*-(*E*)-2-butenoyl]-*β*-D-xylopyranoside.

The spectroscopic features of compounds **3** and **4** were very similar. The HRTOF-ESIMS of both compounds exhibited a sodiated molecular ion at *m*/*z* 727.40 [M + Na]$^+$ (**3**, *m*/*z* 727.4033; **4**, *m*/*z* 727.4033) indicating the same molecular formula of $C_{39}H_{60}O_{11}$. The IR spectra showed hydroxy and carbonyl absorptions, respectively, at 3460 and 1721 cm^{-1} for **3** and 3458 and 1728 cm^{-1} for **4**. Compounds **3** and **4** are similar to 2′,25-*O*-diacetylcimigenol-3-*O*-*β*-D-xylopyranoside (**17**),[21] with the major differences in the resonances assigned to the sugar moiety. In the ^{13}C NMR spectrum (Table 2), the C-3′ resonance of **17** exhibited a downfield shift from δ_C 76.3 to 79.4 in **3**. The C-2′ and C-4′ resonances showed characteristic upfield shifts from δ_C 75.6 and 71.4 in **17** to δ_C 73.2 and 69.3 in **3**, respectively. On the basis of this evidence, we deduced that an acetyl group is attached at C-3′ in **3** instead of at C-2′ in **17**, which was further confirmed by the presence of the HMBC correlation between H-3′ (δ_H 5.73) and the carbonyl carbon at δ_C 170.9. Thus, the structure of **3** was assigned as 25-*O*-acetylcimigenol-3-*O*-[3′-*O*-acetyl]-*β*-D-xylopyranoside. In the same way, an acetyl group was determined to be at C-4′ for **4**, which was also confirmed by the presence of the HMBC correlation between H-4′ (δ_H 5.43) and the carbonyl carbon at δ_C 170.6. Therefore, compound **4** was identified as 25-*O*-acetylcimigenol-3-*O*-[4′-*O*-acetyl]-*β*-D-xylopyranoside.

Compound **5** was isolated as a white powder. The HRTOF-ESIMS showed a quasi-molecular ion at *m*/*z* 743.4001 [M + Na]$^+$ for a molecular formula of $C_{39}H_{60}O_{12}$. The IR spectrum indicated the presence of hydroxy and carbonyl groups at 3457 and 1729 cm^{-1}, respectively. The NMR spectra (Tables 1 and 2) of **5** resemble those of 25-*O*-acetylcimigenol-3-*O*-*β*-D-xylopyranoside (**18**),[19] with the exception of an additional acetyl group, which was assigned to C-12 on the basis of the correlation of H-12 (δ_H 5.23) with the acetyl carbonyl carbon at δ_C 170.6 in the HMBC spectrum and the correlation of H-12 with H_2-11 (δ_H 1.13, 2.92) in the ^{1}H−^{1}H COSY spectrum. Significant ROESY correlations of H-12 with H-17 and H-28 indicated a *β*-orientation of the substituent at C-12. Thus, **5** was elucidated as 25-*O*-acetyl-12*β*-acetoxycimigenol-3-*O*-*β*-D-xylopyranoside.

Compound **6** was isolated as a white powder. The positive HRTOF-ESIMS established the molecular formula of $C_{39}H_{58}O_{12}$. The IR spectrum showed absorptions of hydroxy and carbonyl groups at 3465 and 1741 cm^{-1}, respectively. The ^{1}H NMR spectrum (Table 1) displayed cyclopropane methylene resonances at δ_H 0.20 and 0.53 (each 1H, d, J = 4.4 Hz), six methyl groups at δ_H 0.77, 0.93, 1.23, 1.34, 1.77, and 0.94 (d, J = 6.4 Hz), two acetyl methyls at δ_H 1.96 and 2.13, and an anomeric proton at δ_H 4.82 (d, J = 7.6 Hz), which suggested **6** to be a 9,19-cyclolanostane triterpene glycoside with two acetyl groups. In addition, diagnostic HMBC correlations observed from H-26 (δ_H 5.74) to two quaternary carbon resonances at δ_C 105.8 (C-23) and 65.6 (C-25) and from the methyl resonance at δ_H 1.77 (Me-27) to a quaternary carbon resonance at δ_C 65.6 (C-25) and two methine carbon resonances at δ_C 98.4 (C-26) and 63.5 (C-24) indicated the aglycone of **6** was acteol.[22] It showed identical NMR spectroscopic data to those of actein (**19**),[23] except for the differences in the chemical shifts of the sugar moiety. In the ^{1}H NMR spectrum, a downfield resonance was observed at δ_H 5.74 (t, J = 10.0 Hz), which showed correlations with the methine resonance at δ_H 4.22 (H-4′) and with the methine resonance at δ_H 4.04 (H-2′), which, in turn, showed a correlation with an anomeric proton at δ_H 4.82 in the ^{1}H−^{1}H COSY spectrum. From this evidence, an acetyl group at C-3′ in **6** was concluded. In the ^{13}C NMR spectrum (Table 2), the sugar resonances of **6** at δ_C 107.2, 73.2, 79.4, 69.3, and 66.8 were the same as those of **3**, which confirmed a xyloside moiety with an acetyl group attached at C-3′ in **6**. Therefore, **6** was deduced to be 3′-*O*-acetylactein.

Compound **7** was also isolated as a white powder. The combination of its HRTOF-ESIMS (*m*/*z* 725 [M + Na]$^+$) and ^{13}C NMR spectroscopic data led to the determination of its formula as $C_{39}H_{58}O_{11}$. A comparison of the spectroscopic data of **7** with those of 23-*epi*-26-deoxyactein (**20**)[24] showed that, structurally, **7** closely resembles **20** except for an additional acetyl group attached at C-3′ in **7**. This conclusion was confirmed by analysis of the HMBC spectrum, which showed a correlation between H-3′ and the carbonyl carbon at δ_C 170.8. The structure of **7** was thus elucidated as 3′-*O*-acetyl-23-*epi*-26-deoxyactein.

The known compounds acteinol (**8**),[7] asiaticoside A (**9**),[25] actrin-3-one (**10**),[26] 26-deoxyacteinol (**11**),[7] 25-*O*-acetylcimigenol (**12**),[27] 12-*β*-acetoxycimigenol (**13**),[28] cimigenol-3-*O*-*α*-L-arabinoside (**14**),[29] and norcimifugin (**15**)[12] were identified by comparing their physical and spectroscopic data with reported data.

As noted in the introduction, roots of *C. fetida* have been employed as cooling and detoxification agents by Chinese people since ancient times. In the theory of Chinese Medicine, a tumor is a kind of toxin,[14] so it is of interest to investigate the antitumor activity of this plant. Our research group has previously reported

Table 3. Cytotoxicity of Compounds from the Roots of *Cimicifuga foetida* (IC_{50} values; μM)

compound	HepG2	MCF7	HT29	MKN28
1	6.37	>100	78.49	53.78
2	1.29	>100	>100	>100
3	0.71	>100	28.89	>100
4	2.80	>100	26.12	>100
5	>100	47.54	>100	>100
6	32.08	>100	50.15	53.37
7	1.41	>100	35.68	>100
8	2. 56	>100	>100	>100
9	4.02	>100	49.41	54.76
10	5.51	>100	15.37	71.98
11	27.73	>100	35.36	82.20
12	20.30	>100	>100	>100
13	43.06	>100	>100	>100
14	20.42	>100	>100	>100
15	5.55	>100	>100	>100
cisplatin	1.73	<0. 3	4.66	4.26

two cytotoxic cycloartane triterpenoid glycosides from *C. fetida*.[16] Another report also indicated that the triterpene glycosides from *C. fetida* showed moderate cytotoxicity against the R-HepG2 drug-resistant hepatocarcinoma cell line.[13] The compounds isolated in the present study were screened against the human HepG2, MCF7, HT29, and MKN28 cancer cell lines using the MTT assay. Most of these compounds exhibited more selective and higher cytotoxicity against the human HepG2 cell line than against the MCF7, HT29, and MKN28 cell lines (Table 3). The new compounds **2**, **3**, and **7** exhibited significant cytotoxicity against HepG2 cells, having IC_{50} values of 1.29, 0.71, and 1.41 μM, respectively. Compounds **1** and **4**, along with the known compounds **8**−**10** showed notable cytotoxicity against HepG2 cells, with IC_{50} values of 6.37, 2.80, 2.56, 4.02, and 5.51 μM, respectively. This is the first report of the activity of norcimifugin (**15**) (IC_{50}, 5.55 μM), one of the chromones of *Cimiciguga* species, against the HepG2 cell line. These data suggest that some of the chemical constituents from *C. fetida* might be valuable antitumor promoters and show supportive evidence for the theory of Chinese Medicine about cancer.

Experimental Section

General Experimental Procedures. Optical rotations were obtained with a Horiba SEAP-300 polarimeter. ^{1}H and ^{13}C NMR spectra were measured on Bruker AV-400 and DRX-500 instruments (Bruker, Zürich, Switzerland) using TMS as internal standard for chemical shifts. Chemical shifts (δ) were expressed in ppm with reference to the TMS resonance. ESIMS and HRTOF-ESIMS data were recorded on a VG Autospec-300 spectrometer. Infrared spectra were recorded on a Shimadzu IR-450 instrument by using KBr pellets. TLC was performed on precoated TLC plates (200−250 μm thickness, F_{254} Si gel 60 and F_{254} RP-18 Si gel 60, Qingdao Marine Chemical, Inc.) with compounds visualized by spraying the dried plates with 10% aqueous H_2SO_4 followed by heating until dryness. Silica gel (200−300 mesh, Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40−63 μm, Merk), Amberlite IR-35 (10 mL) columns, and Sephadex LH-20 (20−150 μm, Pharmacia) were used for column chromatography.

Cytotoxicity Bioassay. The assay for cytotoxicity against HepG2, MCF7, HT29, and MKN28 cancer cell lines was performed as previously described.[30–32] (Supporting Information).

Plant Material. Roots of *C. fetida* were collected from Heishitou, Heze County, Guizhou Province, China, in August 2006. The material was identified by Prof. Baogui Li, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Science. A voucher specimen (KUN No. 200608028) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Kunming, China.

Extraction and Isolation. The dried and milled roots of *C. fetida* (10 kg) were extracted with Me_2CO (3 × 20 L × 24 h) at room temperature to give a residue (603 g) after evaporating under vacuum at 50 °C. This residue was suspended in H_2O (1500 mL) and then extracted successively with petroleum ether (3 × 2 L), EtOAc (3 × 2 L), and *n*-BuOH (3 × 2 L) to give a petroleum ether-soluble portion (45 g), an EtOAc-soluble portion (158 g), and an *n*-BuOH-soluble portion (180 g). The EtOAc extract (158 g) was chromatographed over a silica gel column (900 g) and eluted with $CHCl_3$−MeOH [100:0 (1.5 L), 50:1 (2 L), 20:1 (8 L), 10:1 (10 L)] to afford fractions A (13 g), B (8 g), C (34 g), and D (37 g). Fraction B (8 g) was subjected to column chromatography on silica gel (50 g). Gradient elution with $CHCl_3$−MeOH (60:1 to 40:1, 2.5 L) gave fractions B-1, B-2, and B-3. Fraction B-2 (3.8 g) was chromatographed on an RP-18 column [120 g, MeOH−H_2O (7:3), 4 L] and then purified on Sephadex LH-20 (150 g, MeOH, 3 L) to afford **8** (23 mg), **10** (21 mg), **11** (31 mg), **12** (19 mg), **13** (17 mg), and **15** (120 mg). Fraction C (34 g) was subjected to column chromatography on silica gel (250 g) and eluted with $CHCl_3$−MeOH (gradient polarity from 40:1 to 20:1, 8 L) to yield fractions C-1, C-2, and C-3. Fraction C-2 (2.6 g) was subjected to column chromatography [silica gel 20 g, $CHCl_3$−Me_2CO (4:1), 1 L; then RP-18 120 g, MeOH−H_2O (3:2), 4.5 L] to yield **1** (18 mg), **2** (19 mg), **3** (17 mg), **4** (20 mg), **5** (21 mg), **6** (19 mg), and **7** (18 mg). Fraction C-3 (16 g) was chromatographed on a chromatography column [silica gel 60 g, $CHCl_3$−Me_2CO (3:1 to 2:1), 5 L; then RP-18, 120 g, MeOH−H_2O (3:2), 3 L] to afford fractions C-3-1 and C-3-2. Fraction C-3-1 (2.5 g) was subjected to column chromatography on an RP-18 column [120 g, MeOH−H_2O (3:2), 3 L], then purified on a Sephadex LH-20 column (150 g, MeOH, 1 L) to afford **9** (21 mg) and **14** (276 mg).

2′-*O*-(*E*)-2-Butenoyl-25-*O*-acetylcimigenol-3-*O*-β-xylopyanoside (1): white powder; $[\alpha]^{25}_D$ 51.04 (*c* 0.21, MeOH); IR (KBr) ν_{max} 3461, 2932, 2873, 1731, 1632, 1472, 1243, 1071, 987 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ESIMS *m/z* 753 $[M + Na]^+$; HRTOF-ESIMS at *m/z* 753.4169 $[M + Na]^+$ (calcd for $C_{41}H_{62}O_{11}Na$, 753.4189).

4′-*O*-(*E*)-2-Butenoyl-25-*O*-acetylcimigenol-3-*O*-β-D-xylopyranoside (2): white powder; $[\alpha]^{25}_D$ 28.71 (*c* 0.24, MeOH); IR (KBr) ν_{max} 3456, 2950, 2853, 1740, 1638, 1464, 1086, 1043 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ESIMS *m/z* 753 $[M + Na]^+$; HRTOF-ESIMS at *m/z* 753.4193 (calcd for $C_{41}H_{62}O_{11}Na$, 753.4189).

3′,25-*O*-Diacetylcimigenol-3-*O*-β-D-xylopyranoside (3): white powder; $[\alpha]^{25}_D$ 41.67 (*c* 0.16, MeOH); IR (KBr) ν_{max} 3460, 2929, 2872, 1721, 1461, 1250, 1042 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ESIMS *m/z* 727 $[M + Na]^+$; HRTOF-ESIMS at *m/z* 727.4027 (calcd for $C_{39}H_{60}O_{11}Na$, 727.4033).

4′,25-*O*-Diacetylcimigenol-3-*O*-β-D-xylopyranoside (4): white powder; IR (KBr) ν_{max} 3458, 2936, 2870, 11728, 1462, 1248, 1058, 978 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ESIMS *m/z* 727 $[M + Na]^+$; HRTOF-ESIMS at *m/z* 727.4018 (calcd for $C_{39}H_{60}O_{11}Na$, 727.4033).

12β,25-*O*-Diacetylcimigenol-3-*O*-β-D-xylopyranoside (5): white powder; IR (KBr) ν_{max} 3457, 2935, 2872, 1743, 1457, 1372, 1244, 1044, 987 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ESIMS *m/z* 743 $[M + Na]^+$; HRTOF-ESIMS at *m/z* 743.4001 (calcd for $C_{39}H_{60}O_{12}Na$, 743.3982).

3′-*O*-Acetylactein (6): white powder; $[\alpha]^{25}_D$ −38.69 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3465, 2932, 2873, 1741, 1472, 1243, 1071, 987 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ESIMS *m/z* 741 $[M + Na]^+$; HRTOF-ESIMS at *m/z* 741.3819 (calcd for $C_{39}H_{58}O_{12}Na$, 741.3825).

3′-*O*-Acetyl-23-*epi*-26-deoxyactein (7): white powder; $[\alpha]^{25}_D$ 0 (*c* 0.12, MeOH); IR (KBr) max 3455, 2930, 2849, 1737, 1457, 1376, 1244, 1033 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; positive ESIMS *m/z* 725 $[M + Na]^+$; HRTOF-ESIMS at *m/z* 725.3863 (calcd for $C_{39}H_{58}O_{11}Na$, 725.3876).

Hydrolysis and Identification of the Sugar Moieties in Compounds 1−7. Compounds **1**−**7** (15 mg) were separately dissolved in MeOH (15 mL); 4% K_2CO_3 (for **1** and **2**, 15 mL, for other compounds, 10 mL) was added, and the solution was stirred at room temperature overnight. The solution was neutralized with 10% HOAc and extracted with EtOAc (3 × 30 mL). After removal of the solvent, the EtOAc extract was dissolved in MeOH (10 mL) and refluxed with 0.5 N HCl (3 mL) for 4 h. Each reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The water layer was applied on an Amberlite IR-35 (10 mL) column, and the resultant fraction was concentrated *in vacuo* to give a monosaccharide, which had an R_f (EtOAc −$CHCl_3$−MeOH−H_2O, 3:2:2:1) and specific rotation ($[\alpha]^{25}_D$ +32.1 (*c* 0.16, H_2O)) comparable to those of D-xylose (Sigma-Aldrich).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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