Abstract: Five new 9,19-cycloartane triterpene diglycosides, which have been named cimiaceroside C (1), and cimifosides A-D (2-5) together with the known compounds cimiracemoside D (6), cimidahurine (7) and α-D-glucopyranosyl-l-β-D-fructofuranoside (8) were isolated from the rhizome of Cimicifuga foetida. The new triterpene diglycosides 1-5 were identified as cimiacerol-3-O-β-D-xylopyranosyl-(1''→3')-β-D-xylopyranoside, 12β-hydroxycimigenol-3-O-β-D-xylopyranosyl-(1''→3')-β-D-xylopyranoside, 25-O-acetylceimig-enol-3-O-β-D-xylopyranosyl-(1''→3')-β-D-xylopyranoside, 24-acetylhydroshengmanol-3-O-β-D-xylopyranosyl-(1''→3')-β-D-xylopyranoside and 26-deoxyacetyleolacteol-3-O-β-D-xylo- pyranosyl-(1''→3')-β-D-xylopyranoside, respectively, based on analysis of their spectral data and chemical reactions.

Keywords: Cimicifuga foetida, Cycloartane Triterpenoid, Diglycosides, Cimifosides.
Introduction

The rhizome of *Cimicifuga foetida* (family Ranunculaceae) is a popular Chinese Traditional Medicine. Under the trivial name of “Shengma”, it has been used as an antipyretic and analgesic agent since ancient times [1]. Recently, all *Cimicifuga* species were returned to the genus *Actaea* L based on evidence from DNA sequence data [2-4]. In the United States and the European Union, *Actaea racemosa* (L.) Nutt [2-4], commonly known as black cohosh, has been reputed to reduce the frequency and intensity of hot flashes and other menopause symptoms [5, 6]. Chemical constituents of *Cimicifuga* species have been extensively studied by several groups [7-9]. Previous phytochemical studies have revealed that *Cimicifuga* species mainly contain constituents such as chromones, cinnamic acid derivatives, and 9,19-cyclolanostane triterpenes. To date, more than 200 triterpenes have been isolated from the genus [10, 11] and triterpenoid glycosides are considered to be the main active components, which have been used as marker compounds to standardize *Cimicifuga* extracts [12].

In the present study, the constituents of the rhizome of *C. foetida* were investigated. The current paper describes the isolation and identification of the five new cycloartane diglycosides 1-5 (Figure 1), together with the known compounds cimiracemoside D (6) [12], cimidahurine (7) [13] and α-D-glucosyl-β-D-fructofuranoside (8) [14].

**Figure 1.** Structures of triterpenoid diglycoside from the *C. foetida.*
Results and Discussion

Cimiaceroside C (1) was isolated as an amorphous powder. Its molecular formula was determined from its $^{13}$C-NMR and negative FABMS data ($m/z$: 751 for [M-H]) as C$_{40}$H$_{64}$O$_{13}$, and this was confirmed by negative HRFABMS: $m/z$ 751.7352 ([M-H]) (calcd. 751.7362 for C$_{40}$H$_{63}$O$_{13}$). The IR spectrum showed strong hydroxyl bonds at 3376 cm$^{-1}$. The $^{13}$C-NMR spectrum displayed 40 carbons, which included two sugar moieties at $\delta$C 106.3 (C-1'), 74.6 (C-2'), 87.4 (C-3'), 69.4 (C-4'), 67.5 (C-5'), and at $\delta$C 107.2 (C-1''), 75.4 (C-2''), 78.3 (C-3''), 71.0 (C-4''), 66.6 (C-5'') (Table 1). The $^1$H-NMR spectrum showed the presence of a cyclopropane methylene group at $\delta$H 0.15 and 0.44 (each 1H, d, $J$ = 3.6 Hz), seven methyls at $\delta$H 1.19 (3H, s, Me-18), 1.76 (3H, s, Me-26), 1.67 (3H, s, Me-27), 0.83 (3H, s, Me-28), 1.31 (3H, s, Me-29), 1.02 (3H, s, Me-30) and 1.21 (3H, d, $J$ = 6.3 Hz, Me-21), and two sugar anomeric protons at $\delta$H 5.30 (1H, d, $J$ = 7.7 Hz, H-1') and 4.81 (1H, d, $J$ = 7.6 Hz, H-1'') (Table 2). The above spectral evidence suggested 1 was a 9,19-cycloartane triterpenoid diglycoside. By acid hydrolysis, only xylose was identified in the aqueous fraction by TLC comparison with an authentic sample, which indicated the both sugar units in 1 were xylose. The NMR spectroscopic data of 1 showed close resemblance with those of cimiaceroside B [15, 16], except for presence of an additional sugar unit, which suggested 1 had the same aglycone as that of cimiaceroside B. In the HMBC spectrum, the presence of the long-range correlations of $\delta$H 5.30 (H-1') to 88.6 (C-3), and $\delta$H 4.81 (H-1'') to 87.4 (C-3') determined the inner xylose to be connected at C-3, and the terminal xylose to be at C-3' of the inner xylose. The substituent of the xylose sugar unit at C-3' led to significant downfield shift for the $^{13}$C-NMR signal at C-3' and upfield shifts for signals at C-2' and C-4'.

The relative stereochemistry of 1 was determined on the basis of the ROESY experiments (Figure 2). A significant ROESY correlation between H-3 and H-5 suggested a $\beta$-orientation of the substituent at C-3. Moreover, the proton coupling constants of H-1' ($J$ = 7.7 Hz) and H-2'' ($J$ = 7.6 Hz) suggested 1 had a $\beta$-D-xylopyranosyl-(1''$\rightarrow$3')-$\beta$-D-xylopyranoside moiety. Thus, cimiaceroside C (1) was determined to be cimiacerol-3-O-$\beta$-D-xylopyranosyl-(1''$\rightarrow$3')-$\beta$-D-xylopyranoside.

Figure 2. The key HMBC ($\rightarrow$) and ROESY ($\leftarrow\rightarrow$) correlations of compound 1.
Table 1 The $^{13}$C-NMR data of compounds 1-5 in C$_2$D$_2$N (δ in ppm).

| Position | 1      | 2      | 3$^a$   | 4$^b$   | 5$^c$   |
|----------|--------|--------|---------|---------|---------|
| 1        | 32.1 t | 32.4 t | 32.5 t  | 32.4 t  | 31.9 t  |
| 2        | 30.1 t | 30.7 t | 30.9 t  | 30.2 t  | 30.9 t  |
| 3        | 88.6 d | 88.7 d | 88.8 d  | 88.5 d  | 88.3 d  |
| 4        | 41.4 s | 41.3 s | 41.4 s  | 41.4 s  | 41.2 s  |
| 5        | 47.5 d | 47.3 d | 47.6 d  | 47.6 d  | 46.9 d  |
| 6        | 21.0 t | 20.9 t | 21.1 t  | 20.5 t  | 20.2 t  |
| 7        | 26.3 t | 26.1 t | 26.4 t  | 26.6 t  | 25.5 t  |
| 8        | 47.4 d | 47.3 d | 48.7 d  | 49.2 d  | 45.6 d  |
| 9        | 19.7 s | 20.8 s | 20.0 s  | 20.1 s  | 20.1 s  |
| 10       | 26.4 s | 26.6 s | 26.7 s  | 26.7 s  | 26.7 s  |
| 11       | 26.6 t | 40.9 t | 26.5 t  | 26.6 t  | 36.6 t  |
| 12       | 33.5 d | 71.8 d | 34.1 t  | 34.1 t  | 77.1 d  |
| 13       | 46.9 s | 47.9 s | 41.8 s  | 42.0 s  | 48.8 s  |
| 14       | 45.3 s | 48.4 s | 47.2 s  | 46.7 s  | 47.8 s  |
| 15       | 43.3 t | 80.0 d | 80.2 d  | 82.8 d  | 44.1 t  |
| 16       | 72.4 d | 112.4 s| 112.4 s | 103.2 s | 74.5 d  |
| 17       | 52.4 d | 59.7 d | 56.4 d  | 60.5 d  | 56.2 d  |
| 18       | 20.7 q | 12.1 q | 20.0 q  | 20.1 q  | 13.5 q  |
| 19       | 30.0 t | 30.0 t | 30.1 t  | 30.9 t  | 29.5 t  |
| 20       | 34.8 d | 24.1 d | 24.0 d  | 27.7 d  | 23.3 d  |
| 21       | 17.5 q | 21.1 q | 19.5 q  | 21.4 q  | 21.3 q  |
| 22       | 86.9 d | 38.8 t | 38.0 t  | 34.1 t  | 37.3 t  |
| 23       | 106.0 s| 71.1 d | 71.0 d  | 74.7 d  | 105.9 s |
| 24       | 83.3 d | 90.1 d | 86.8 d  | 82.7 d  | 62.5 d  |
| 25       | 83.6 s | 71.0 s | 83.2 s  | 71.2 s  | 62.2 s  |
| 26       | 27.8 q | 25.6 q | 24.0 q  | 27.7 q  | 68.2 t  |
| 27       | 24.9 q | 27.1 q | 21.5 q  | 25.3 q  | 14.3 q  |
| 28       | 19.7 q | 11.9 q | 11.8 q  | 12.0 q  | 19.6 q  |
| 29       | 25.8 q | 25.7 q | 25.6 q  | 25.8 q  | 25.7 q  |
| 30       | 15.5 q | 15.4 q | 15.5 q  | 15.5 q  | 15.3 q  |
| 1$'$     | 106.3 d| 106.3 d| 106.3 d | 106.3 d | 106.3 d |
| 2$'$     | 74.6 d | 74.5 d | 74.5 d  | 74.5 d  | 74.5 d  |
| 3$'$     | 87.4 d | 87.4 d | 87.4 d  | 87.3 d  | 87.4 d  |
| 4$'$     | 69.4 d | 69.4 d | 69.4 d  | 69.4 d  | 69.3 d  |
| 5$'$     | 67.5 t | 67.5 t | 67.4 t  | 67.4 t  | 67.4 t  |
| 1$''$    | 107.2 d| 107.2 d| 107.1 d | 107.6 d | 107.1 d |
| 2$''$    | 75.4 d | 75.4 d | 75.4 d  | 75.6 d  | 75.4 d  |
| 3$''$    | 78.3 d | 78.3 d | 78.3 d  | 78.7 d  | 78.3 d  |
| 4$''$    | 71.0 d | 71.8 d | 71.7 d  | 71.7 d  | 71.0 d  |
| 5$'''$   | 66.6 t | 66.7 t | 66.6 d  | 67.2 t  | 66.8 t  |

The $^{13}$C-NMR spectral data for the ester moiety: a: $\delta_c$ 170.2 s (25-COCH$_3$), 21.5 s (25-COCH$_3$); b: $\delta_c$ 171.4 s (24-COCH$_3$), 21.1 s (24-COCH$_3$); c: $\delta_c$ 170.7 s (12-COCH$_3$), 21.6 s (12-COCH$_3$)
Table 2. The $^1$H-NMR data of compounds 1-5 in C$_5$D$_5$N ($\delta$ in ppm).

| Position | 1     | 2     | 3$^a$ | 4$^b$ | 5$^c$ |
|----------|-------|-------|-------|-------|-------|
| 1        | 1.25 m; 1.52 m | 1.15 m; 1.57 m | 1.36 m; 1.57 m | 1.23 m; 1.51 m | 1.13 m; 1.52 m |
| 2        | 2.25 m; 1.80 m | 2.30 m; 1.79 m | 1.93 m; 2.34 m | 2.29 m; 1.84 m | 2.27 m; 1.81 m |
| 3        | 3.47 dd, 3.8, 11.5 | 3.45 dd, 3.6, 11.5 | 3.54 dd, 4.5, 11.5 | 3.47 dd, 5.0, 9.9 | 3.40 dd, 4.0, 11.6 |
| 5        | 1.32 m | 1.30 m | 1.33 m | 1.32 m | 1.21 m |
| 6        | 1.15 m; 1.31 m | 0.76 dd, 4.5, 12.5 | 0.66 m; 1.30 m | 0.76 dd 4.6, 12.5 | 0.60 m; 1.40 m |
| 7        | 1.01 m; 1.25 m | 1.15 m; 2.10 m | 1.12 m; 2.01 m | 1.18 m; 2.02 m | 0.94 m; 1.22 m |
| 8        | 1.52 m | 1.82 m | 1.67 | 1.90 m | 1.56 dd 4.7, 11.5 |
| 11       | 1.25 m | 1.54 m | 1.06 m; 2.01 m | 1.17 m; 2.11 m | 1.19 dd 4.4, 13.3; 2.68 dd 8.8, 16.0 |
| 12       | 1.51 m | 4.20 m | 1.54 m; 1.64 m | 1.59 m; 1.67 m | 5.08 dd 3.2, 8.5 |
| 15       | 1.68 m; 1.90 m | 4.47 s | 4.14 s | 4.26 m | 1.78 m; 1.87 dd, 7.9, 12.9 |
| 16       | 4.96 dd 8.0, 16.4 | 1.78 m | | | 4.21 dd, 7.1, 14.2 |
| 17       | 1.57 m | 1.81 m | 1.77 m | 1.91 m | 1.76 m |
| 18       | 1.19 s | 1.41 s | 1.22 s | 1.28 s | 1.39 s |
| 19       | 0.15 d 3.6; 0.44 d | 0.34 d 3.6; 0.59 d | 0.27 d 4.0; 0.53 d | 0.25 d 2.9; 0.50 d | 0.15 d 3.8; 0.50 d |
| 20       | 2.29 m | 1.84 m | 1.75 m | 2.10 s | 2.23 m |
| 21       | 1.21 d 6.3 | 1.38 d 5.8 | 0.99 d 5.5 | 0.82 d 4.9 | 1.00 d 6.4 |
| 22       | 3.89 d 10.5 | 1.12 m; 2.38 m | 2.04 m; 2.17 m | 1.27 m; 2.34 dd 4.6, 11.2 | 1.45 m; 1.60 m |
| 23       | | 4.77 d 7.9 | 4.64 d 7.8 | 4.58 d 7.6 |
| 24       | 4.18 s | 3.82 s | 4.10 s | 4.14 s | 3.64 s |
| 26       | 1.76 s | 1.49 s | 1.44 s | 1.63 s | 4.5 |
| 27       | 1.67 s | 1.50 s | 1.47 s | 1.65 s | 1.45 s |
| 28       | 0.83 s | 1.21 s | 1.30 s | 1.18 s | 0.82 s |
| 29       | 1.31 s | 1.29 s | 1.23 s | 1.10 s | 1.27 s |
| 30       | 1.02 s | 1.02 s | 1.21 s | 1.02 s | 0.96 s |
| 1$'$     | 5.30 d 7.7 | 5.29 d 7.7 | 5.32 d 7.6 | 5.32 d 7.2 | 5.27 d 7.6 |
| 2$'$     | 4.10 m | 4.12 d 7.7 | 4.16 m | 4.12 m | 4.17 m |
| 3$'$     | 4.08 m | 4.09 m | 4.11 m | 4.17 m | 4.10 m |
| 4$'$     | 4.09 m | 4.13 m | 4.13 m | 4.16 m | 4.14 m |
| 5$'$     | 3.65 m; 4.30 m | 3.65 m; 4.31 m | 3.65 m; 4.31 m | 3.66 m; 4.31 m | 3.66 m; 4.32 m |
| 1$''$    | 4.81 d 7.6 | 4.81 d 7.6 | 4.83 d 7.6 | 4.80 d 7.6 | 4.77 d 7.5 |
| 2$''$    | 4.04 dd 8.2, 16.1 | 4.04 t 12.7 | 4.04 d 7.1 | 4.04 d 7.1 | 4.06 m |
| 3$''$    | 4.11 m | 4.17 m | 4.13 m | 4.16 m | 4.16 m |
| 4$''$    | 4.12 m | 4.20 m | 4.13 m | 4.13 m | 4.13 m |
| 5$''$    | 3.68 m; 4.35 m | 3.68 m; 4.36 m | 3.71 m; 4.36 m | 3.68 m; 4.34 m | 3.68 m; 4.31 m |

The $^1$H-NMR spectral data for the ester moiety: a: $\delta_H$ 2.01 s (25-COCH$_3$); b: $\delta_H$ 2.11 s (24-COCH$_3$); c: $\delta_H$ 2.11 s (12-COCH$_3$).
Molecules 2008, 13

Cimifoside A (2) was isolated as a white powder. The negative HR-FAB-MS of 2 showed a quasi-molecular ion at m/z 767.4356, corresponding to the molecular formula of C_{40}H_{64}O_{14}. Its $^1$H-NMR spectrum (Table 2) exhibited characteristic cyclopropane methylene signals at $\delta_H$ 0.34 and 0.59 (each 1H, d, $J = 3.6$ Hz), seven methyls at $\delta_H$ 1.41 (3H, s, Me-18), 1.49 (3H, s, Me-26), 1.50 (3H, s, Me-27), 1.21 (3H, s, Me-28), 1.29 (3H, s, Me-29), 1.02 (3H, s, Me-30) and 1.38 (3H, d, $J = 6.3$ Hz, Me-21), and two sugar anomeric protons at $\delta_H$ 5.29 (1H, d, $J = 7.7$ Hz, H-1') and 4.81 (1H, d, $J = 7.6$ Hz, H-1'').

The 13C-NMR spectrum exhibited 40 resonances, of which 30 were attributed to a triterpene skeleton, ten to two sugar units. A comparison of the 1H- and 13C-NMR spectra of 2 with those of cimiside A [17] revealed that 2 has an additional sugar unit. Only xylose was detected in the aqueous fraction of the acid hydrolysis products of 2. In the HMBC spectrum, long-range correlations between $\delta_H$ 5.29 (H-1') and $\delta_C$ 88.7 (C-3), and between $\delta_H$ 4.81 (H-1'') and $\delta_C$ 87.4 (C-3') were observed for 2, which assigned the inner xylose at C-3, and the terminal xylose at C-3' of the inner xylose. The configurations of C(23) and C(24) were ascribed as R and S respectively, by comparing the spectral data of C-23 and coupling constants of H-24 signals of 2 with those of known 9,19-cycloartane triterpene glycosides [18]. Therefore, cimifoside A (2) was determined to be 12β-hydroxycimigenol-3-O-β-D-xylopyranosyl-(1''→3')-β-D-xylopyranoside.

The molecular formula C_{42}H_{66}O_{14} for cimifoside B (3) was established by negative FABMS m/z: 793 [M-H] and HRFABMS 793.4512 (calcd. 793.4573 for C_{42}H_{65}O_{14}). The IR spectrum of 3 showed an absorption at 1739 cm$^{-1}$ due to a carbonyl group. The 1H-NMR spectrum exhibited the cyclopropane methylene signals at $\delta_H$ 0.27 and 0.53 (each 1H, d, $J = 4.0$ Hz), two anomeric proton signals at $\delta_H$ 5.32 (1H, d, $J = 7.6$ Hz, H-1') and 4.83 (1H, d, $J = 7.6$ Hz, H-1'') and a methyl singlet at $\delta_H$ 2.01 (3H). The 13C-NMR spectrum of 3 showed 30 carbon signals for the triterpene skeleton, 10 for two sugar units and two for a carbonyl group. All this evidence suggested that 3 was a 9,19-cycloartane triterpenoid diglycoside. The structure of 3 resembled that of the known compound cimiside B [17]. It differs from cimiside B only by the presence of an acetyl group, which was assigned to C-25 due to dramatic chemical shift changes of C-25 (+12.2 ppm), C-24 (−3.4 ppm), C-26 (−1.4 ppm) and C-27 (−3.9 ppm) (Table 1). The relative configuration of 3 was determined to be the same as that of 2 by comparison with literature data [19]. Thus, the chemical structure of 3 was identified to be 12β-hydroxycimigenol-3-O-β-D-xylopyranosyl-(1''→3')-β-D-xylopyranoside.

Cimifoside C (4) has a molecular formula of C_{42}H_{68}O_{15} as deduced from the negative HRFABMS (m/z 811.4678 [M-H]$^-$; calcd. 811.4653 for C_{42}H_{67}O_{15}), together with the 1H- and 13C-NMR spectra. The IR spectrum of 4 showed absorptions at 3425, 1712 and 1266 cm$^{-1}$ due to hydroxyl and carbonyl groups. The 1H-NMR spectrum of 4 exhibited the cyclopropane methylene signals at $\delta_H$ 0.25 and 0.50 (each 1H, d, $J = 2.8$ Hz), two anomeric proton signals at $\delta_H$ 5.32 (1H, d, $J = 7.2$ Hz, H-1'), 4.80 (1H, d, $J = 7.6$ Hz, H-1''), and a methyl signal at $\delta_H$ 2.11 (3H, s). The 13C-NMR spectrum of 4 showed two anomeric carbons at $\delta_C$ 106.3 (C-1'), 107.2 (C-1'') and a carbonyl group at $\delta_C$ 171.4 s (24 COCH$_3$), and 21.1 s (24 COCH$_3$). By comparison of the 1H- and 13C-NMR spectra with those of 24-acetylhydroshengmanol-3-O-β-D-xylopyranoside [18], 4 was found to have an additional xylose unit. An HMBC correlation between $\delta_H$ 4.85 (H-1'') and 87.3 (C-3') determined the terminal xylose to be connected to C-3' of the inner xylose. On the basis of the above evidence, the chemical structure of 4 was assigned as 24-acetylhydroshengmanol-3-O-β-D-xylopyranosyl-(1''→3')-β-D-xylopyranoside.
Cimifoside D (5) was found to have a molecular formula of $\text{C}_{42}\text{H}_{52}\text{O}_{14}$ from the $^1\text{H}$- and $^{13}\text{C}$-NMR (including DEPT) spectra, which was confirmed by the negative HRFABMS (found 791.4531 for [M-H$^-$], calcd 791.4546 for $\text{C}_{42}\text{H}_{51}\text{O}_{14}$). The $^1\text{H}$- and $^{13}\text{C}$-NMR spectral data of 5 were very similar to those of 23-epi-26-deoxyactin [8], except for an additional xylose. The second xylose was connected to C-3'$\text{'}$ of the first xylose due to an HMBC correlation between $\delta$H 4.77 (H-1'') and 87.4 (C-3'), and the disaccharide unit was further assigned to C-3 by an HMBC correlation of $\delta$H 5.27 (H-1') and 88.3 (C-3'). Hence, 5 was determined to be 23-epi-26-deoxyacetylectol-3-O-$\beta$-D-xylopyranosyl-(1'''→3')-$\beta$-D-xylopyranoside.

Conclusions

Although the rhizome of $C.\text{foetida}$ is a very famous Chinese Traditional Medicine and it has been the subject of extensive phytochemical investigations, its chemical components have not been completely identified yet. Triterpene monoglycosides are considered to be the main components of $\text{Cimicifuga foetida}$, but in our present study a series of new cycloartane diglycosides 1-5 have been isolated and identified, which further clarified the triterpene glycoside components from $C.\text{foetida}$. Moreover this finding will be helpful for identifying extracts of $A.\text{racemosa}$ and $C.\text{foetida}$, as some black cohosh products in America and European market are contaminated with related Asian $\text{Cimicifuga}$ species, such as $C.\text{foetida}$, $C.\text{simplex}$, and $C.\text{dahurica}$.

Experimental

General

IR spectra were recorded on a Shimadzu IR-450 instrument, and are reported in cm$^{-1}$. $^1\text{H}$ (400 and 500 MHz) and $^{13}\text{C}$-NMR (100 and 125 MHz) spectra (all in $\text{C}_5\text{D}_5\text{N}$) were recorded with Bruker AV 400 and DRX500 instruments, using TMS as an internal standard. Silica gel (200-300 mesh, Qingdao Marine Chemical, P.R. China), Lichroprep RP-18 (40-63um, Merck, Darmstadt, Germany) were used for column chromatography (CC). Fractions were monitored by TLC, and spots were visualized by heating TLC sprayed with 10% H$_2$SO$_4$. Mass spectral data were recorded on a VG Autospec 3000 spectrometer.

Plant material

The rhizomes of $\text{Cimicifuga foetida}$ were collected in Lijiang, Yunnan Province, China, in July of 2003 and authenticated by Prof. Zong-Yu Wang (Kunming Institute of Botany, CAS). A voucher specimen (KUN No. 200308025) of the collection has been deposited at State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, the Chinese Academy of Sciences.
Extraction and Isolation

The dried, milled rhizomes of C. foetida (23.4 Kg) were exhaustively extracted with 90% MeOH under reflux. The MeOH extract was evaporated under reduced pressure to yield a syrup (6.2 kg). The syrup was suspended in water-MeOH (9:1), and extracted successively with EtOAc and n-BuOH to give an EtOAc extract (1.8 kg) and an n-BuOH extract (0.5 kg). The n-BuOH extract was subjected to CC (silica gel, CHCl₃-MeOH 20:1, 10:1 gradient) to yield four fractions (Fr.). Fr. 1 was rechromatographed on a column (silica gel, CHCl₃-MeOH 100:1, 80:1, 65:1, 50:1) to obtain 5 (15 mg), 6 (79 mg) and 7 (36 mg). Fr. 2 (1.9 g) was chromatographed repeatedly by CC (silica gel, CHCl₃-MeOH 10:1) to yield 1 and 2. Fr. 3 was subjected to CC (silica gel, CHCl₃-MeOH 10:1) and further purified by Sephadex (MeOH) to give 1 (76 mg) and 2 (43 mg). Fr. 4 was rechromatographed by CC (silica gel, CHCl₃-MeOH 5:1, 0:1) to yield crystals of 8 (20 mg).

Cimiaceroside C (1): white powder; m.p. 292-294 ºC; [α]D⁰⁷ 31.3° [c = 1.60, CHCl₃-EtOH (1:1)]; negative FABMS m/z (%) 751 ([M-H]-, 20), 679 (8), 129 (24); negative HRFABMS: m/z 751.7352 ([M-H]-) (calcd. 751.7362 for C₄₀H₆₃O₁₃); IR (KBr) νmax 3376, 2972, 2940, 2872, 1732, 1639, 1462, 1442, 1382, 1368, 1251, 1209, 1164, 1081, 1052, 1001, 972, 894, 703, 616, 531, 429 cm⁻¹; ¹³C- and ¹H-NMR (500 MHz) data, see Tables 1 and 2.

Cimifoside A (2): white powder; m.p. 287-289 ºC; [α]D⁰⁷ +16.7° [c = 0.60, CHCl₃-EtOH (1:1)]; negative HRFABMS: m/z 767.4366 (calcd. 767.4342 for C₄₀H₆₄O₁₃); IR (KBr) νmax 3431, 2928, 2870, 1631, 1455, 1383, 1363, 1170, 1077, 1040, 563, 470 cm⁻¹; ¹³C- and ¹H-NMR (500 MHz) data, see Tables 1 and 2.

Cimifoside B (3): white powder; m.p. 165-166 ºC; [α]D⁰⁷ -24.2° [c = 0.85, CHCl₃-EtOH (1:1)]; negative HRFABMS: m/z 793.4512 (calcd. 793.4573 for C₄₂H₆₅O₁₄); IR (KBr) νmax 3443, 2963, 2934, 2870, 1739, 1735, 1457, 1239, 1070 cm⁻¹; ¹³C- (100 MHz,) and ¹H-NMR (500 MHz) data, see Tables 1 and 2.

Cimifoside C (4): white powder; m.p. 224-225 ºC; [α]D⁰⁷ -60.0° [c = 1.50, CHCl₃-EtOH (1:1)]; negative HRFABMS: m/z 811.4678, (calcd. 811.4653 for C₄₂H₆₇O₁₅); IR (KBr) νmax 3425, 2940, 2870, 1712, 1632, 1457, 1379, 1266, 1159, 1107, 1072, 1042, 996, 969, 898, 797, 738, 630, 610, 591, 496, 449 cm⁻¹; ¹³C- (100 MHz,) and ¹H-NMR (500 MHz) data, see Tables 1 and 2.

Cimifoside D (5): white powder; m.p. 185-186 ºC; [α]D⁰⁷ 56.2° [c = 0.76, CHCl₃-EtOH (1:1)]; negative HRFABMS: m/z 791.4531 [M-H] (calcd. 791.4546 for C₄₂H₅₅O₁₄); IR (KBr) νmax 3442, 2969, 2937, 2872, 1731, 1633, 1458, 1382, 1366, 1252, 1165, 1042, 984, 898, 841, 804, 677, 629, 602 cm⁻¹; ¹³C- (100 MHz,) and ¹H-NMR (500 MHz) data, see Tables 1 and 2.
**Acid hydrolysis of compounds 1-5**

Compounds 1-5 (6 mg of each) were refluxed with 5% HCl in MeOH (7 mL) for 8 h. Each mixture was diluted with H₂O and neutralized with NaHCO₃. The neutral hydrolysate revealed the presence of only xylose by TLC (n-BuOH-AcOH-H₂O, 4:1:1, Rf = 0.4) upon comparison with the authentic sample.

**Acknowledgements**

We are grateful to the Natural Science Foundation of Yunnan (Project No. 2005C0010z) and Natural Science Foundation of China (Project No.30772636), the Chinese Academy of Science (XiBuZhiGuang Program) for financial support; in addition one of authors (HJ Zhang) gratefully acknowledges the support of The K. C. Wong Education Foundation, Hong Kong.

**References**

1. Pharmacopoeial Commission of the People’s Republic of China. *The Pharmacopoeia of Chinese People’s Republic*; the People’s Health Publishing House & the Chemical Industry Publishing House: Beijing, 2005; p. 50.
2. Compton, J.A.; Culham, A. Phylogeny and circumscription of tribe Actaeae (Ranunculaceae). *Syst. Bot.* **2002**, **27**, 502-511.
3. Compton, J.A.; Culham, A.; Jury, S.L. Reclassification of Actaea to include Cimicifuga and Soullea (Ranunculaceae): phylogeny inferred from morphology, nrDNA ITS, and cpDNA trnL-F sequence variation. *Taxon* **1998**, **47**, 593-634.
4. Compton, J.A.; Culham, A.J.; Gibbings, G.; Jury, S.L. Phylogeny of Actaea including Cimicifuga (Ranunculaceae) inferred from nrDNA ITS sequence variation. *Biochem. Syst. Ecol.* **1998**, **26**, 185-197.
5. Lieberman, S.J. A review of the effectiveness of Cimicifuga racemosa (black cohosh) for the symptoms of menopause. *Women’s Health* **1998**, **7**, 525-529.
6. McKenna, D.J.; Jones, K.; Humphrey, S.; Hughes, K. Black cohosh: efficacy, safety, and use in clinical and preclinical applications. *Altern. Ther.* **2001**, **7**, 93-100.
7. Sun, L.R.; Qing, C.; Zhang, Y.L., Jia, S.Y.; Li, Z.R.; Pei, S.J., Qiu, M.H.; Gross, M.L.; Qiu, S.X. Cimicifoetisides A and B, two cytotoxic cycloartane triterpenoid glycosides from the rhizomes of *Cimicifuga foetida*, inhibit proliferation of cancer cells. *Beilstein J. Org. Chem.* **2007**, **3**, 3-8.
8. Liu, Y.; Chen, D.H.; Si, J.Y.; Tu, G.Z.; An, D.G. Two New Cyclolanostanol Xylosides from the Aerial Parts of *Cimicifuga dahurica*. *J. Nat. Prod.* **2002**, **65**, 1486-1488.
9. Chen, S.N.; Li, W.K.; Fabricant, D.S.; Santarsiero, B.D.; Mesecar, A.; Fitzloff, J.F.; Fong, H.H.; Farnsworth, N.R. Isolation, structure elucidation, and absolute configuration of 26-deoxyactein from *Cimicifuga racemosa* and clarification of nomenclature associated with 27-deoxyactein. *J. Nat. Prod.* **2002**, **65**, 601-605.
10. Li, J.-X.; Yu, Z.-Y. Cimicifugae Rhizoma: from origins, bioactive constituents to clinical outcomes, *Curr. Med. Chem.* **2006**, **13**, 2927-2951.
11. Lin, Y.P.; Qiu, M.H.; Li, Z.R. Studies on the chemical constituents and biologic activities of Cimicifuga. Nat. Prod. Res. Dev. 2002, 14, 58-76.
12. Shao, Y.; Amanda, H.; Wang, M.F.; Zhang, H.J.; Geoffrey, C.; Mika, B.; Lemmo, E. Triterpene glycosides from Cimicifuga racemosa. J. Nat. Prod. 2000, 63, 905-910.
13. Li, C.J.; Chen, D.H.; Xiao, P.G. Phenolic glycosides isolated from Cimicifuga dahurica. Yaoxue Xuebao (Acta Pharm. Sin.) 1994, 29, 195-199.
14. Gong, Y.H. The $^{13}$C-NMR Shifts of Natural Organic Compounds, Yunnan Science and Technology Press: Kunming, 1986; pp. 874-875.
15. Kusano, A.; Takahira, M.; Shibano, M.; Miyase, T.; Okuyama, T.; Kusano, G. Studies on the constituents of Cimicifuga species. XXII. Structure of two new cyclolanostanol xylosides, cimiacerosides A and B. Heterocycles 1998, 48, 1003-1013.
16. Kusano, A.; Shibano, M.; Kusano, G. Studies on the Constituents of Cimicifuga Species. XXVII. Malonyl Cyclolanostanol Glycosides from the Uderground Part of Cimicifuga simplex Wormsk. Chem. Pharm. Bull. 1999, 47, 1175-1179.
17. Li, C.J.; Chen, D.H.; Xiao, P.G. Chemical constituents of Chinese traditional medicine 'Shengma'. Yaoxue Xuebao (Acta Pharm. Sin.) 1993, 28, 777-781.
18. Sakurai, N.; Kimura, O.; Inoue, T.; Nagai, M. Studies on the Chinese Crude Drug "shoma." V. Structures of 24-O-Acetylhydro shengmuanol Xyloside and 22-O-Hydroxycimigenol Xyloside. Chem. Pharm. Bull. 1981, 29, 955-960.
19. Li, J.X.; Kadota, S.; Hattori, M.; Yoshimachi, S.; Shiro, M.; Oogami, N.; Mizuno, H.; Namba, T. Constituents of Cimicifuga Rhizoma. I. Isolation and Characterization of Ten New Cycloartenol Triterpenes from Cimicifuga heracleifolia Komarov. Chem. Pharm. Bull. 1993, 41, 832-841.

© 2008 by the authors; licensee Molecular Diversity Preservation International, Basel, Switzerland. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).