# Studies on the Constituents of *Cimicifuga* Species. XX.[1] Absolute Stereostructures of Cimicifugoside and Actein from *Cimicifu simplex* WORMSK.

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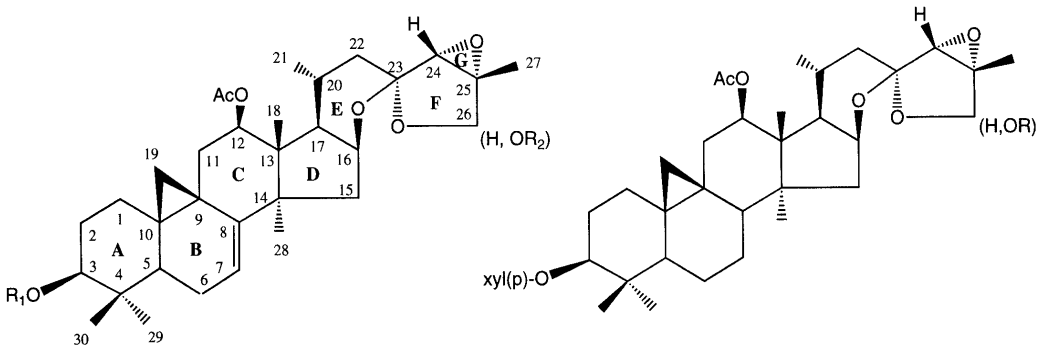
**The absolute stereostructues of cimicifugoside (1) and actein (2) from *Cimicifuga simplex* were established by X-ray crystal analysis of 26(*S*)-*O*-methylcimicifugenin A (1cS) prepared from 1, negative CD curves of 26-*O*-methyl-3-keto-cimicifugenins (1dS, 1dR), comparative analysis of $^1$H- and $^{13}$C-NMR spectra of 1, 2 and their derivatives, and preparation of 2 from 1 by hydrogenation: 20(*R*), 23(*R*), 24(*R*), 25(*S*), 26 (*R*,*S*)-16β : 23; 23 : 26; 24 : 25-triepoxy-12β-acetoxy-3β,26-dihydroxy-9,19-cyclolanost-7-ene 3-*O*-β-D-xylopyranoside for 1 and 20(*R*), 23(*R*), 24(*R*), 25(*S*), 26(*R*,*S*)-16β : 23; 23 : 26; 24 : 25-triepoxy-12β-acetoxy-3β,26-dihydroxy-9,19-cyclolanostane 3-*O*-β-D-xylopyranoside for 2.**

**Key words** *Cimicifuga simplex*; 9,19-cyclolanostane; stereostructure; triepoxyhemiacetal; X-ray analysis

Cimicifugoside (**1**) was isolated from the rhizoma of *Cimicifuga simplex* WORMSK. (Ranunculaceae) collected in Nagano Prefecture and its structure was reported without deciding the stereochemistry of C20, 23, 24, 25 and 26.[2] Actein (**2**), acetylacteol-3-*O*-D-xyloside, was isolated for the first time from *C. racemosa* (L.) NUTT.[3] and its structure was reported.[4,5] Acetylacteol-3-*O*-arabinoside was obtained later from *C. foetida* L. and the stereostructure of the aglycone was reported.[6] Now **1** and **2** have been isolated from the subterranean parts of *C. simplex* grown at Sendai and Shizuoka in Japan, and the absolute stereostructures of **1** and **2** have been investigated.

Cimicifugoside (**1**) and actein (**2**) were identified by direct comparison of TLC, HPLC, and MS data, $[\alpha]_D$, IR and $^1$H-NMR spectra with authentic specimens.[2,7] The $^1$H- and $^{13}$C-NMR signals were assigned using $^1$H–$^1$H correlation spectroscopy (COSY), $^1$H–$^{13}$C COSY, heteronuclear multiple bond connectivity (HMBC), and rotating frame nuclear Overhauser effect (ROE) difference spectroscopy spectra as summarized in Tables 1 and 2.

The $^1$H-NMR spectra (pyridine-$d_5$) of **1** and **2** indicate that these compounds are mixtures of the epimers of C26 in solution, showing distinct signals of the height (3 : 1) of H-7, H-15, H-19, H-21, H-24, H-26, H-27, H-28, H-29 and H-30 due to the convertible hemiacetal groups as indicated with underlines in Table 1. The higher signals were attributed to **1S** and **2S**, while the lower ones were attributed to **1R** and **2R** by the ROEs and in the light of the solvent effect[8]: H-24 ($\delta$ 3.91) and H-27 ($\delta$ 1.78) of **1S** and **2S**, which are located on the same face as the 26 β-hydroxyl group, appeared at a lower field than those ($\delta$ 3.77, 3.76; 1.64, 1.63) of **1R** and **2R**, which are on the opposite face to the reversed 26 α-hydroxyl group. Separation of **1S** and **1R** was achieved by HPLC [column: Cosmosil 10 ph, solvent: 30% $CH_3CN$, 2 ml/min, 40 °C, $t_R$: 22′ and 30′], but evapolation of the solvent of each fraction *in vacuo* again afforded a mixture of the same two compounds (ratio = *ca.* 3 : 1). Then, analysis of the $^1$H- and




**1S** : $R_1$ = β-D-xyl(p), $R_2$=H (26β-OH)
**1R** : $R_1$ = β-D-xyl(p), $R_2$=H (26α-OH)
**1aS** : $R_1$=H, $R_2$=H (26β-OH)
**1aR** : $R_1$=H, $R_2$=H (26α-OH)
**1bS** : $R_1$ = β-D-xyl(p), $R_2$=Me (26β-OMe)
**1bR** : $R_1$ = β-D-xyl(p), $R_2$=Me (26α-OMe)

**2S** : R=H (26β-OH)
**2R** : R=H(26α-OH)
**2aS** : R=Me (26β-OMe)
**2aR** : R= Me(26α-OMe)

Fig. 1. Absolute Stereostructures of **1**, **2** and Their Derivatives

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$^{13}$C-NMR spectra was carried out on the epimeric mixtures. The spectra of **2S** and **2R** were analyzed similarly.

The $^{13}$C-NMR spectra (pyridine-$d_5$) also showed some distinct signals attributed to C-7 (**1S**, **1R**), C-8 (**2S**, **2R**), C-13 (**1S**, **1R**), C-14 (**1S**, **1R**), C-15 (**1S**, **1R**, **2S**, **2R**), C-16 (**2S**, **2R**), C-20 (**1S**, **1R**), C-21 (**1S**, **1R**, **2S**, **2R**), C-22 (**1S**, **1R**) and C-23—C-28 (**1S**, **1R**, **2S**, **2R**) as indicated with underlines in Table 2. The $^1$H-NMR spectra (DMSO-$d_6$) of **1** showed distinct signals of H-24, H-26, and H-27, indicating that epimeric mixtures were also present in the solvent. These signals with such satellites have not been found in the low resolution spectra by us,[2] nor mentioned by other groups.[3-6] These newly isolated compounds and authentic specimens of **1**[2] and **2**[7] showed the same reproducible high resolution spectra as the epimeric mixtures and this is thought to be common for such hemiacetal compounds.

A genuine aglycone (**1a**) was obtained by hydrolysis with cellulase T[Amano]4 and identified by direct comparison with an authentic specimen.[2] The $^1$H- and $^{13}$C-NMR spectra also showed the presence of hemiacetal isomers (**1aS**, **1aR**) in solution (pyridine-$d_5$) and they were assigned similarly to those of **1** as shown in Tables 1 and 2.

Cimicifugoside (**1**) was treated with methanol containing *p*-toluenesulfonic acid to give two epimeric methyl ethers (**1bS**, **1bR**) along with an artifact, 26(*S*)-*O*-methylcimicifugenin A (**1cS**).[2] A major product (**1bS**) was obtained as a colorless powder, mp 207—208 °C, $[\alpha]_D$ −73.6° and the

Table 1. $^1$H-NMR Data of **1**, **2** and Their Derivatives

| | 1S | 1R | 1aS | 1aR | 1bS | 1bR | 1cS | 2S | 2R | 2aS | 2aR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.18, 1.60 | 1.18, 1.60 | 1.20, 1.60 | 1.20, 1.60 | 1.20, 1.60 | 1.20, 1.60 | 1.32, 1.54 | 1.14, 1.54 | 1.14, 1.54 | 1.16, 1.56 | 1.16, 1.55 |
| 2 | 1.89, 2.24 | 1.89, 2.24 | 1.84, 1.94 | 1.84, 1.94 | 1.90, 2.25 | 1.90, 2.27 | 1.65, 1.90 | 1.87, 2.28 | 1.87, 2.28 | 1.87, 2.28 | 1.87, 2.28 |
| 3 | 3.44 | 3.44 | 3.47 | 3.47 | 3.44 dd (11.3, 3.8) | 3.45 dd (11.9, 4.0) | 3.72 | 3.46 | 3.46 | 3.47 dd (12.0, 4.0) | 3.46 dd (11.9, 4.1) |
| 5 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.16 | 1.24 | 1.24 | 1.28 | 1.26 |
| 6 | 1.56, 1.82 | 1.56, 1.82 | 1.60, 1.90 | 1.60, 1.90 | 1.57, 1.87 | 1.58, 1.84 | 1.34, 1.64 | 0.71, 1.44 | 0.71, 1.44 | 0.72, 1.47 | 0.72, 1.48 |
| 7 | 5.07 d (8.0) | 5.16 d (8.0) | 5.10 dd (7.1, 2.0) | 5.19 dd (7.1, 2.0) | 5.15 dd (7.5, 2.0) | 5.17 dd (7.5, 2.0) | 2.32, 1.90 | 0.92, 1.20 | 0.92, 1.20 | 0.97, 1.27 | 0.96, 1.28 |
| 8 | — | — | — | — | — | — | — | 1.60 | 1.64 | 1.63 | 1.66 |
| 11 | 1.26, 2.95 | 1.26, 2.95 | 1.26, 2.96 | 1.26, 2.96 | 1.27<br>2.95 dd (15.6, 8.7) | 1.26<br>2.95 dd (16.0, 8.8) | 2.96, 1.93 | 1.22, 2.72 | 1.22, 2.72 | 1.25 dd (16.0, 4.0)<br>2.73 dd (16.0, 8.5) | 1.22 dd (16.1, 4.0)<br>2.73 dd (16.1, 8.4) |
| 12 | 5.22 d (8.8) | 5.22 d (8.0) | 5.23 | 5.23 | 5.22 d (8.7) | 5.22 d (8.8) | 5.32 dd (7.5, 7.5) | 5.11 dd (8.8, 4.0) | 5.11 dd (8.8, 4.0) | 5.12 dd (8.5, 4.0) | 5.11 dd (8.4, 4.0) |
| 15 | 1.88, 2.00 | 2.00, 2.15 | 1.88, 1.98 | 2.06, 2.16 | 1.99 dd (13.8, 6.8)<br>2.17dd (13.8, 6.8) | 2.01 dd (13.1, 6.8)<br>2.16 dd (13.1, 6.8) | 1.86, 1.92 | 1.56, 1.76 | 1.68, 1.92 | 1.64, 1.93 | 1.67, 1.93 |
| 16 | 4.70 ddd (6.8, 6.8, 6.8) | 4.70 ddd (6.8, 6.8, 6.8) | 4.71 ddd (6.8, 6.8, 6.8) | 4.71 ddd (6.8, 6.8, 6.8) | 4.65 ddd (6.8, 6.8, 6.8) | 4.67 dd (6.8, 6.8, 6.8) | 4.58 ddd (6.8, 6.8, 6.8) | 4.62 ddd (6.8, 6.8, 6.8) | 4.62 dd (6.8, 6.8, 6.8) | 4.56 ddd (6.8, 6.8, 6.8) | 4.58 ddd (6.8, 6.8, 6.8) |
| 17 | 1.78 | 1.78 | 1.78 | 1.78 | 1.80 | 1.80 | 1.76 d (6.3) | 1.80 | 1.80 | 1.82 | 1.82 |
| 18 | 1.41 s | 1.41 s | 1.44 s | 1.44 s | 1.38 s | 1.39 s | 1.15 s | 1.37 s | 1.37 s | 1.35 s | 1.35 s |
| 19 | 0.57 d (4.0)<br>1.09 d (4.0) | 0.59 d (4.0)<br>1.10 d (4.0) | 0.60 d (4.0)<br>1.13 d (4.0) | 0.61 d (4.0)<br>1.13 d (4.0) | 0.57 d (4.0)<br>1.09 d (4.0) | 0.59 d (4.0)<br>1.10 d (4.0) | 3.17 d (13.8)<br>1.79 d (13.8) | 0.25 d (4.0)<br>0.61 d (4.0) | 0.26 d (4.0)<br>0.62 d (4.0) | 0.25 d (4.0)<br>0.61 d (4.0) | 0.26 d (4.0)<br>0.62 d (4.0) |
| 20 | 1.88 | 1.88 | 1.88 | 1.88 | 1.80 | 1.80 | 1.78 | 1.84 | 1.84 | 1.82 | 1.82 |
| 21 | 0.98 d (6.0) | 0.96 d (6.0) | 0.98 d (6.0) | 0.97 d (6.0) | 0.96 d (6.0) | 0.94 d (5.6) | 1.01 d (5.6) | 0.99 d (6.0) | 0.97 d (6.0) | 0.98 d (6.0) | 0.95 d (6.0) |
| 22 | 1.68, 2.20 | 1.68, 2.20 | 1.70, 2.23 | 1.70, 2.23 | 1.65, 2.18 | 1.63, 2.23 | 1.65<br>2.15 dd (13.1, 2.5) | 1.66, 2.22 | 1.66, 2.22 | 1.64<br>2.18 dd (12.0, 3.0) | 1.62<br>2.21 dd (12.5, 3.0) |
| 24 | 3.91 s | 3.77 s | 3.91 s | 3.77 s | 3.79 s | 3.69 s | 3.80s | 3.91 s | 3.76 s | 3.80 s | 3.69 s |
| 26 | 5.73 s | 5.74 s | 5.73 s | 5.73 s | 5.05 s | 5.26 s | 5.04 s | 5.72 s | 5.72 s | 5.05 s | 5.23s |
| 27 | 1.78 s | 1.64 s | 1.78 s | 1.63 s | 1.62 s | 1.55 s | 1.62 s | 1.78 s | 1.63 s | 1.62 s | 1.54 s |
| 28 | 1.01 s | 1.06 s | 1.02 s | 1.07 s | 1.08 s | 1.07 s | 0.95 s | 0.81 s | 0.86 s | 0.88 s | 0.88 s |
| 29 | 1.31 s | 1.32 s | 1.20 s | 1.21 s | 1.31 s | 1.33 s | 0.99s | 1.30 s | 1.31 s | 1.31 s | 1.31 s |
| 30 | 1.02 s | 1.03 s | 1.06 s | 1.07 s | 1.01 s | 1.03 s | 0.97s | 1.00 s | 1.01 s | 1.01 s | 1.01 s |
| $COCH_3$ | 2.18 s | 2.18 s | 2.19 s | 2.19 s | 2.18 s | 2.18 s | 2.13 s | 2.13 s | 2.13 s | 2.13 s | 2.13 s |
| $OCH_3$ | | | | | 3.47 s | 3.58 s | 3.47 s | | | 3.48 s | 3.57 s |
| 1′ | 4.83 d (8.1) | 4.83 d (8.1) | | | 4.83 d (8.1) | 4.83 d (8.1) | | 4.84 d (8.1) | 4.84 d (8.1) | 4.84 d (8.1) | 4.84 d (8.0) |
| 2′ | 4.02 dd (8.8, 8.1) | 4.02 dd (8.8, 8.1) | | | 4.01 dd (8.1, 8.1) | 4.02 dd (8.1, 8.1) | | 4.01 dd (8.8, 8.1) | 4.01 dd (8.8, 8.1) | 4.02 dd (8.1, 8.1) | 4.01 dd (8.0, 8.0) |
| 3′ | 4.13 dd (8.8, 8.8) | 4.13 dd (8.8, 8.8) | | | 4.13 dd (8.7, 8.1) | 4.14 dd (8.8, 8.1) | | 4.14 dd (8.8, 8.8) | 4.14 dd (8.8, 8.8) | 4.14 dd (8.1, 8.1) | 4.13 dd (8.3, 8.0) |
| 4′ | 4.21 ddd (11.1, 8.8, 5.1) | 4.21 ddd (11.1, 8.8, 5.1) | | | 4.20 ddd (10.0, 8.7, 5.0) | 4.21 ddd (10.0, 8.8, 5.0) | | 4.21 ddd (10.0, 8.8, 5.0) | 4.21 ddd (10.0, 8.8, 5.0) | 4.21 ddd (10.0, 8.1, 5.0) | 4.21 ddd (11.5, 8.3, 5.1) |
| 5′ | 3.72 dd (11.8, 11.1)<br>4.34 dd (11.8, 5.1) | 3.72 dd (11.8, 11.1)<br>4.34 dd (11.8, 5.1) | | | 3.71 dd (11.3, 10.0)<br>4.33 dd (11.3, 5.0) | 3.72 dd (11.3, 10.0)<br>4.34 dd (11.3, 5.0) | | 3.73 dd (11.3, 10.0)<br>4.35 dd (11.3, 5.0) | 3.73 dd (11.3, 10.0)<br>4.35 dd (11.3, 5.0) | 3.73 dd (11.3, 10.0)<br>4.35 dd (11.3, 5.0) | 3.72 dd (11.5, 10.8)<br>4.35 dd (10.8, 5.1) |

Obtained on a JEOL α-400 in pyridine-$d_5$. Underlines indicate distinct signals due to the isomers 26*S* and 26*R* in solution.

Table 2. $^{13}$C-NMR Data of **1**, **2** and Their Derivatives

| | 1S | 1R | 1aS | 1aR | 1bS | 1bR | 1cS | 2S | 2R | 2aS | 2aR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 30.27 | 30.27 | 30.63 | 30.63 | 30.29 | 30.26 | 36.68 | 31.93 | 31.93 | 31.95 | 31.95 |
| 2 | 29.48 | 29.48 | 30.55 | 30.55 | 29.48 | 29.48 | 25.84 | 29.88 | 29.88 | 29.89 | 29.89 |
| 3 | 87.89 | 87.89 | 77.58 | 77.58 | 87.90 | 87.87 | 84.87 | 88.11 | 88.11 | 88.13 | 88.11 |
| 4 | 40.40 | 40.40 | 40.19 | 40.19 | 40.41 | 40.40 | 45.35 | 41.21 | 41.21 | 41.22 | 41.21 |
| 5 | 42.50 | 42.50 | 42.28 | 42.28 | 42.48 | 42.48 | 55.04 | 47.03 | 47.03 | 47.04 | 47.03 |
| 6 | 21.85 | 21.85 | 22.13 | 22.13 | 21.87 | 21.85 | 22.96 | 20.40 | 20.40 | 20.42 | 20.44 |
| 7 | 114.00 | 114.10 | 114.14 | 114.23 | 114.08 | 114.08 | 30.92 | 25.63 | 25.63 | 25.71 | 25.71 |
| 8 | 147.79 | 147.79 | 147.77 | 147.77 | 147.77 | 147.77 | 136.84 | 45.63 | 45.70 | 45.66 | 45.70 |
| 9 | 21.30 | 21.30 | 21.31 | 21.31 | 21.30 | 21.30 | 124.21 | 20.18 | 20.18 | 20.20 | 20.20 |
| 10 | 28.35 | 28.35 | 28.68 | 28.68 | 28.37 | 28.37 | 89.59 | 26.84 | 26.84 | 26.83 | 26.85 |
| 11 | 36.68 | 36.68 | 36.81 | 36.81 | 36.69 | 36.64 | 39.80 | 36.69 | 36.69 | 36.70 | 36.67 |
| 12 | 76.81 | 76.81 | 76.89 | 76.89 | 76.78 | 76.73 | 75.20 | 77.08 | 77.08 | 77.06 | 77.01 |
| 13 | 48.08 | 48.10 | 48.09 | 48.09 | 48.05 | 48.09 | 47.49 | 48.01 | 48.01 | 48.75 | 48.80 |
| 14 | 50.59 | 50.66 | 50.62 | 50.69 | 50.72 | 50.66 | 51.72 | 47.92 | 47.92 | 48.05 | 48.02 |
| 15 | 42.40 | 42.50 | 42.44 | 42.50 | 42.48 | 42.48 | 39.32 | 43.59 | 43.70 | 43.68 | 43.65 |
| 16 | 73.14 | 73.14 | 73.15 | 73.15 | 73.28 | 73.32 | 73.22 | 73.00 | 73.10 | 73.15 | 73.21 |
| 17 | 56.85 | 56.85 | 56.87 | 56.87 | 56.81 | 56.73 | 54.80 | 56.43 | 56.43 | 56.43 | 56.31 |
| 18 | 14.80 | 14.80 | 14.82 | 14.82 | 14.78 | 14.78 | 12.50 | 13.48 | 13.48 | 13.45 | 13.46 |
| 19 | 28.87 | 28.87 | 29.02 | 29.02 | 28.88 | 28.86 | 35.17 | 29.48 | 29.48 | 29.47 | 29.50 |
| 20 | 25.83 | 25.55 | 25.83 | 25.56 | 25.77 | 25.44 | 26.10 | 26.00 | 26.00 | 25.68 | 25.63 |
| 21 | 21.00 | 20.99 | 21.02 | 20.94 | 20.93 | 20.90 | 21.40 | 21.02 | 20.95 | 20.42 | 20.92 |
| 22 | 37.36 | 37.00 | 37.39 | 37.00 | 37.14 | 36.64 | 37.24 | 37.60 | 37.60 | 37.38 | 36.90 |
| 23 | 105.88 | 103.45 | 105.88 | 103.46 | 106.23 | 104.05 | 106.17 | 105.80 | 103.41 | 106.19 | 104.01 |
| 24 | 63.42 | 62.86 | 63.43 | 62.88 | 62.33 | 61.84 | 62.25 | 63.46 | 62.93 | 62.36 | 61.90 |
| 25 | 65.60 | 63.93 | 65.60 | 63.95 | 64.64 | 62.54 | 64.61 | 65.54 | 63.90 | 64.59 | 62.51 |
| 26 | 98.47 | 98.20 | 98.47 | 98.12 | 103.98 | 103.29 | 103.94 | 98.45 | 98.20 | 103.96 | 103.29 |
| 27 | 13.07 | 13.15 | 13.07 | 13.15 | 12.67 | 13.06 | 12.66 | 13.06 | 13.15 | 12.66 | 13.06 |
| 28 | 26.74 | 26.78 | 26.79 | 26.84 | 26.82 | 26.77 | 23.50 | 19.50 | 19.60 | 19.55 | 19.56 |
| 29 | 25.73 | 25.73 | 26.14 | 26.14 | 25.71 | 25.72 | 24.93 | 25.71 | 25.71 | 25.65 | 25.63 |
| 30 | 14.24 | 14.24 | 13.58 | 13.58 | 14.23 | 14.24 | 24.01 | 15.30 | 15.30 | 15.30 | 15.32 |
| $\underline{C}OCH_3$ | 170.62 | 170.62 | 170.63 | 170.63 | 170.62 | 170.60 | 170.55 | 170.51 | 170.51 | 170.51 | 170.50 |
| $CO\underline{C}H_3$ | 21.59 | 21.59 | 21.59 | 21.59 | 21.59 | 21.57 | 21.58 | 21.62 | 21.62 | 21.62 | 21.61 |
| $O\underline{C}H_3$ | | | | | 55.04 | 56.32 | 54.99 | | | 54.99 | 56.35 |
| 1′ | 107.42 | 107.42 | | | 107.41 | 107.43 | | 107.47 | 107.47 | 107.48 | 107.48 |
| 2′ | 75.59 | 75.59 | | | 75.59 | 75.59 | | 75.58 | 75.58 | 75.58 | 75.58 |
| 3′ | 78.60 | 78.60 | | | 78.59 | 78.60 | | 78.60 | 78.60 | 78.60 | 78.60 |
| 4′ | 71.25 | 71.25 | | | 71.24 | 71.25 | | 71.26 | 71.26 | 71.26 | 71.26 |
| 5′ | 67.10 | 67.10 | | | 67.10 | 67.11 | | 67.09 | 67.09 | 67.10 | 67.10 |

Measured at 100.4 MHz in pyridine-$d_5$. Underlines indicate distinct signals due to the isomers 26*S* and 26*R* in solution.

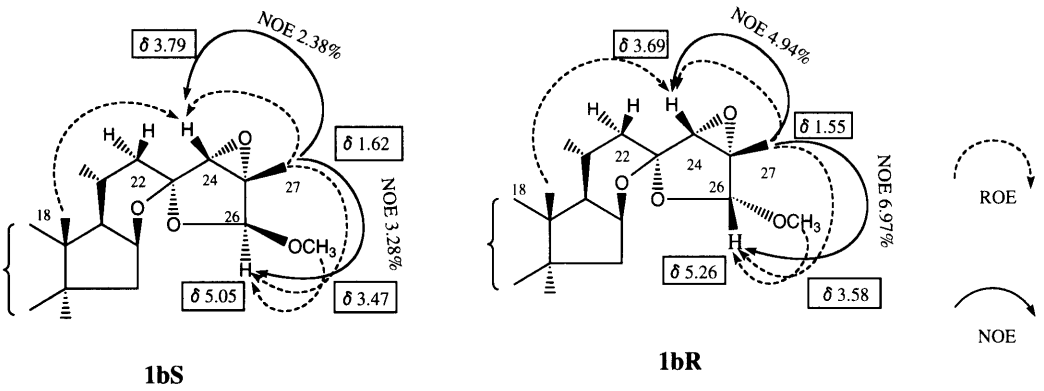
molecular formula was determined to be $C_{38}H_{56}O_{11}$ by positive high resolution secondary ion mass spectroscopy (pos. HR-SI-MS). A minor product (a third of the amount of **1bS**) (**1bR**) was obtained as a colorless powder, mp 194—195 °C, $[\alpha]_D$ −112.8°, and the molecular formula was determined to be $C_{38}H_{56}O_{11}$ by pos. HR-SI-MS.

The $^1$H- and $^{13}$C-NMR signals of **1bS** and **1bR** were assigned as shown in Tables 1 and 2. The relative stereochemistry of the methoxyl group at C26 in **1bS** and **1bR** was determined by comparison of the $^1$H-NMR chemical shifts of H-24, H-26 and H-27 (Me) with those of the artifact (**1cS**), whose structure was determined by X-ray analysis as mentioned later. The chemical shifts of H-24 ($\delta$ 3.79), H-26 ($\delta$ 5.05) and H-27 ($\delta$ 1.62) of **1bS** were similar to those ($\delta$ 3.80, 5.04, 1.62) of **1cS**, but differed from those ($\delta$ 3.69, 5.26, 1.55) of **1bR** as shown in Table 1.

ROEs were observed between H-24 and H-27, H-26 and H-27, H-17 and H-21, and H-21 and H-16$\alpha$ in the ROE difference spectra of **1bS** and **1bR**. The nuclear Overhauser effect (NOE) experiments using a 500 MHz spectrometer showed 3.28% and 2.38% increases in H-26 and H-24 on irradiation of H-27 in **1bS**, while the corresponding increases 6.97% and 4.94% for **1bR** as shown in Fig. 2. These ROEs and NOEs between H-27 and H-26 in both C26 epimeric compounds did not actually clarify the stereochemistry of C25 and C26, although that of acetylacteol-3-*O*-arabinoside has been proposed by the NOE between H-26 and H-27.[6]

The artifact, 26(*S*)-*O*-methylcimicifugenin A (**1cS**), mp 188—189 °C, $C_{33}H_{48}O_7$, was identified as methylcimicifugenin A by direct comparison with an authentic specimen,[2] and subjected to X-ray crystal analysis. There are two crystallographically independent molecules per asymmetric unit in the **1cS** crystal, and the structure of one (B) molecule is illustrated by an ORTEP drawing in Fig. 3. The other (A) molecule was coordinated upside down to the B molecule and both structures were similar with almost the same conformation. Thus, the relative structure of **1cS** was as depicted in Fig. 3.

The torsion angles in the moieties of the rings E, F and G of **1cS** were determined from the X-ray analysis as shown in Table 3. It is notable that the torsion angles, O2–C23–C24–O4 (171.1°), O2–C23–C24–C25 (107.0°) and O3–C23–C24–O4 (53.2°) clarified the $\alpha$-epoxy orientation at C24 : 25, being opposite to the $\beta$ orientation reported on the basis of the NOE data for acetylacteol.[6] The level




**1bS** **1bR**

Fig. 2. ROEs and NOEs in the Ring D and Side-Chain Moieties of **1bS** and **1bR**

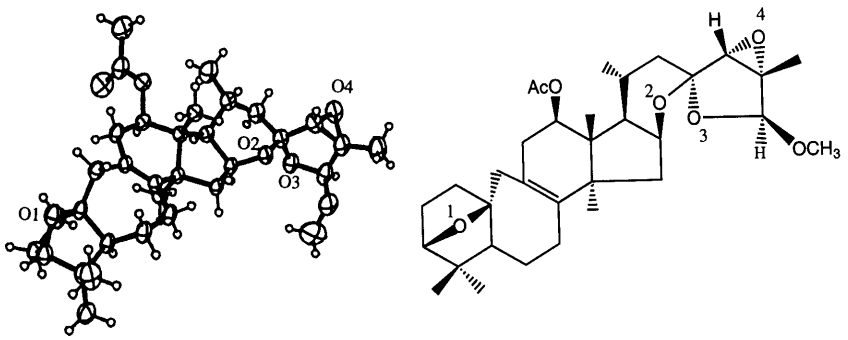



Fig. 3. ORTEP Drawing and Absolute Stereostructure of **1cS**

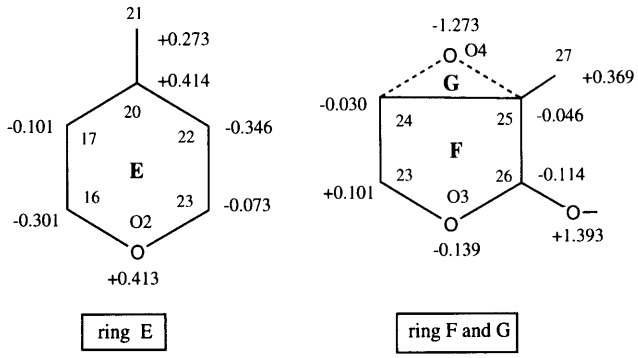



Fig. 4. The Level (Å) of Each Atom of Rings E, F and G from the Best Plane

(Å) of each atom of the rings E, F and G from the best planes calculated according to the plane equations is shown in Fig. 4. The equations were obtained from the results of the X-ray crystal analysis as follows:

$$(-0.52995X)+(0.62810Y)+(0.56978Z)+(-6.66323)=0$$

for the ring E

$$(-0.30795X)+(-0.49178Y)+(0.81444Z)+(-5.07898)=0$$

for the ring F

The semiempirical energy calculations showed that ring E adopted a twisted boat form and ring F a puckered conformation with the α-epoxy ring at C24:25, β-methyl (27) at C25 and β-methoxyl group at C26, as shown at Fig. 4.

The methyl ethers of **1a** were obtained by hydrolysis of an epimeric mixture of **1bS** and **1bR** with cellulase T[Amano]4, and were oxidized with Collin's reagent to provide 3-keto derivatives which were purified by $Al_2O_3$ column chromatography and HPLC. Both 3-keto derivatives of 26(*S*)- and 26(*R*)-*O*-methylcimicifugenin (**1dS**, **1dR**) exhibited the negative CD curves ($\Delta\varepsilon_{295}$: −0.44, $\Delta\varepsilon_{285}$: −0.33), and the basic structure of **1** and its derivatives was proved to have a cycloartane (9,19-cyclolanostane) group.[9] Thus, the absolute stereostructure of **1** was formulated as 20(*R*), 23(*R*), 24(*R*), 25(*S*), 26(*R*,*S*)-16β:23; 23:26; 24:25-triepoxy-12β-acetoxy-3β,

Table 3. Torsion Angles (°) and Standard Deviations Relating to the Stereochemistry of the Ring D and Moieties of the Side-Chain

| Bond | Angle | Bond | Angle |
|---|---|---|---|
| C13–C14–C15–C16 | −38.3 (3) | O4–C24–C25–C26 | −105.1 (4) |
| C14–C15–C16–O2 | 137.4 (3) | O4–C24–C25–C27 | 101.8 (5) |
| C14–C15–C16–C17 | 16.7 (3) | C23–C24–C25–O4 | 103.8 (3) |
| O2–C16–C17–C13 | −105.5 (3) | C23–C24–C25–C26 | −1.3 (3) |
| O2–C16–C17–C20 | 24.6 (3) | C23–C24–C25–C27 | −154.4 (5) |
| C15–C16–C17–C13 | 12.4 (3) | O4–C25–C26–O3 | −49.2 (3) |
| C15–C16–C17–C20 | 142.6 (3) | O4–C25–C26–O5 | −170.4 (4) |
| C13–C17–C20–C21 | −84.3 (4) | C24–C25–C26–O3 | 13.5 (3) |
| C13–C17–C20–C22 | 155.3 (4) | C24–C25–C26–O5 | −107.5 (3) |
| C16–C17–C20–C21 | 154.9 (4) | C27–C25–C26–O3 | 168.3 (4) |
| C16–C17–C20–C22 | 34.6 (3) | C27–C25–C26–O5 | 47.2 (4) |
| C17–C20–C22–C23 | −62.8 (3) | C16–O2–C23–C24 | 170.0 (3) |
| C21–C20–C22–C23 | 174.3 (4) | C26–O3–C23–O2 | −90.8 (3) |
| C20–C22–C23–O2 | 26.8 (3) | C26–O3–C23–C22 | 146.7 (4) |
| C20–C22–C23–O3 | 150.0 (3) | C26–O3–C23–C24 | 22.5 (3) |
| C20–C22–C23–C24 | −94.0 (4) | C23–O3–C26–O5 | 94.0 (4) |
| O2–C23–C24–O4 | 171.1 (3) | C23–O3–C26–C25 | −23.7 (3) |
| O2–C23–C24–C25 | 107.0 (3) | C25–O4–C24–C23 | −99.7 (4) |
| O3–C23–C24–O4 | 53.2 (3) | C24–O4–C25–C26 | 97.1 (4) |
| O3–C23–C24–C25 | −10.8 (3) | C24–O4–C25–C27 | −117.9 (3) |
| C22–C23–C24–O4 | −64.3 (3) | C33–O5–C26–O3 | 62.0 (5) |
| C22–C23–C24–C25 | −128.5 (4) | C33–O5–C26–C25 | 178.7 (5) |

26-dihydroxy-9,19-cyclolanost-7-ene 3-*O*-*β*-D-xylopyranoside, and then, the absolute stereostructure of the above artifact (**1cS**) was established as (3*β*, 10*β*, 12*β*, 16*β*, 20*R*, 23*R*, 24*R*, 25*S*, 26*S*)-3:10; 16:23; 23:26; 24:25-tetraepoxy-12-acetoxy-cycloarta-9,10-seco-8(9)-en-26-ol methyl ether.

Actein (**2**) provided two epimeric methyl ethers (**2a**S and **2a**R) following the same treatment as in **1**, and the signals were assigned as for those of **1b**S and **1b**R. Hydrogenation of **1** using 10% palladium–charcoal in ethanol produced actein (**2**), which was identified by direct comparison of HPLC, mp, MS data, $[\alpha]_D$, IR and $^1$H-NMR spectra. Although $^1$H-NMR data of **2** from *C. racemosa*[4] and from Cimicifugae rhizoma,[5] and $^1$H- and $^{13}$C-NMR data of 23(*R*), 24(*S*), 25(*R*), 27(*S*)-acetylacteol-3-*O*-arabinoside from *C. foetida*[6] have been reported, our assignments were different mainly due to the revised orientation of the 24:25 epoxy moiety and the stereoisomerism of the hemiacetal group in solution, and all the data are summarized in Tables 1 and 2. Thus, the absolute stereostructure of **2** was revised to 20(*R*), 23(*R*), 24(*R*), 25(*S*), 26(*R*,*S*)-16*β*:23; 23:26; 24:25-triepoxy-12*β*-acetoxy-3*β*,26-dihydroxy-9,19-cyclolanostane 3-*O*-*β*-D-xylopyranoside.

## Experimental

**General** The instruments used in this work were as follows: a Yanagimoto micromelting apparatus (for melting points, uncorrected); a JASCO DIP-1000 digital polarimeter (for specific rotation, measured at 23 °C); a JASCO J-500 spectrometer (for CD, measured at 23 °C); a Perkin-Elmer 1720X-FT IR spectrometer (for IR spectra); a Hitachi M-80 spectrometer (for MS spectra); and a Varian Gemini-200, a JEOL *α*-400 and a Varian Unity-INOVA-500 (for NMR spectra, measured in pyridine-$d_5$ containing a few drops of $D_2O$, on the *δ* scale using tetramethylsilane as an internal standard). Column chromatography was carried out on silica-gel (Wakogel C-200) and ODS-A YMC. HPLC was conducted using a Gilson 305 pump equipped with a JASCO 830-RI as a detector. Silica-gel 60 $F_{254}$ (Merck) precoated TLC plates were used and detection was carried out by spraying with 40% $H_2SO_4$ followed by heating.

**Isolation of 1 and 2** *Cimicifuga simplex* was grown at the Experimental Station for Medicinal Plant Studies, Faculty of Pharmaceutical Sciences, Tohoku University for seven years. The underground parts were obtained and dried at 60 °C in the drying room for several days. The powdered materials (100 g) were extracted three times with 300 ml boiling MeOH. After evaporation of the solvent, the extracts were dissolved in water (50 ml) and the mixture was extracted five times with EtOAc–*n*-BuOH (1:1) (100 ml). Afer washing with water and evaporation of the solvent, the residue of the upper layer was chromatographed on octadecylsilanized silicic acid (ODS) (100 g). The fraction eluted with MeOH–$H_2O$ (3:1) was subjected to $SiO_2$ chromatography and the fraction eluted with $CHCl_3$–MeOH (19:1) was subjected to preparative HPLC [column: Develosil PhA-5 (i.d. 10.0×250 mm); solvent: MeOH–$H_2O$–MeCN (10:10:3); effluent speed: 2 ml/min; column temperature: 40 °C]. **1** (colorless prisms, 85 mg) and **2** (colorless prisms, 8 mg) were obtained and identified by direct comparison of TLC, HPLC and spectral data with authentic specimens of **1** and **2** isolated from *C. simplex* (collected at Nagano Prefecture in Japan) and *C. racemosa* (purchased in Switzerland), respectively. **1**: mp 245—246 °C, $[\alpha]_D$ −95.3° [*c*=0.32, MeOH–$CHCl_3$ (2:1)], pos. HR-SI-MS *m/z*: 697.3566 ($C_{37}H_{54}O_{11}$+Na)$^+$, error: 0.5 m.m.u., IR (KBr) cm$^{-1}$: 3050—3700 (OH), 1721 (AcO), $^1$H- and $^{13}$C-NMR (pyridine-$d_5$) *δ*: Tables 1 and 2. $^1$H-NMR (DMSO-$d_6$) *δ*: 0.65 (d, *J*=3.6, H-19), 0.82 (s, H-30), 0.86(d, *J*=6.0, H-21), 0.97 (s, H-28), 0.99 (s, H-29), 1.10 (s, H-18), 1.39 (s, H-27:**1**R), 1.43 (s, H-27:**1**S), 2.00 (s, $COCH_3$), 2.63 (dd, *J*=9.0, 16.8, H-11), 3.45 (s, H-24:**1**R), 3.53 (s, H-24:**1**S), 3.26 (m, H-4′), 3.66 (dd, *J*=5.1, 11.1, H-5′), 4.13 (d,*J*=7.2, H-1′), 4.33 (ddd, *J*=7.2, 7.2, 7.2, H-16), 4.82 (d, *J*=8.0, H-12), 4.98 (s, H-26:**1**R), 5.00 (s, H-26:**1**S), 5.10 (d, *J*=6.6, H-7). **2**: mp 228—229 °C, $[\alpha]_D$ −62.1° [*c*=0.13, MeOH–$CHCl_3$ (2:1)], pos. HR-SI-MS *m/z*: 699.3714 ($C_{37}H_{56}O_{11}$+Na)$^+$, error: −0.3 m.m.u., IR (KBr) cm$^{-1}$: 3300—3600 (OH), 1722 (AcO), $^1$H- and $^{13}$C-NMR (pyridine-$d_5$) *δ*: Tables 1 and 2.

**Methylation of 1** **1** (130 mg) was dissolved in 1% methanolic *p*-toluenesulfonic acid (60 ml) and the solution was stirred at room temperature for 6.5 h. Water was added and the mixture was extracted three times with EtOAc–*n*-BuOH (1:1) . The upper layer was chromatographed on $SiO_2$ (20 g). The fraction eluted with $CHCl_3$–MeOH (10:1) was subjected to HPLC [column: CrestPakC18T-5 (i.d. 7.15×250 mm); solvent: MeOH–$H_2O$–MeCN (10:7:3); effluent speed: 2 ml/min; column temperature: 40 °C] to afford **1b**S (19 mg) and **1b**R (6 mg). **1b**S: Colorless powder, mp 207—208 °C, $[\alpha]_D$ −73.6° (*c*=1.18, MeOH), pos. HR-SI-MS *m/z*: 689.3882 ($C_{38}H_{56}O_{11}$+H)$^+$, error: −1.6 m.m.u., $^1$H- and $^{13}$C-NMR (pyridine-$d_5$) *δ*: Tables 1 and 2. **1b**R: Colorless powder, mp 194—195 °C, $[\alpha]_D$ −112.8° (*c*=0.34, MeOH), pos. HR-SI-MS *m/z*: 689.3904 ($C_{38}H_{56}O_{11}$+H)$^+$, error: 0.7 m.m.u., $^1$H- and $^{13}$C-NMR (pyridine-$d_5$) *δ*: Tables 1 and 2.

The fraction eluted with $CHCl_3$–MeOH (19:1) in the above chromatographic system was rechromatographied on $SiO_2$ (13 g). The hexane–EtOAc (1:1) eluate was purified by HPLC as for **1b**S and **1b**R, except for the solvent: MeOH–$H_2O$–MeCN (12:7:3), and recrystallized from MeOH–EtOAc to afford **1c**S (18.7 mg). **1c**S: Colorless prisms, mp 188—189 °C, $[\alpha]_D$ +20.2° (*c*=1.02, MeOH), pos. HR-SI-MS *m/z*: 557.3485 ($C_{33}H_{48}O_7$+H)$^+$, error: 1.0 m.m.u., IR (KBr) cm$^{-1}$: 1735 (AcO) , $^1$H- and $^{13}$C-NMR (pyridine-$d_5$) *δ*: Tables 1 and 2.

**Enzymatic Hydrolysis of 1** **1** (23 mg) and cellulase T[Amano]4 (from *Trichoderma viride*, 130 mg) were treated as described previously[1] to provide cimicifugenin (**1a**, 15 mg), which was identified by direct comparison with an authentic specimen,[2] mp 165—166 °C, $[\alpha]_D$ −113.3° (*c*=1.01, MeOH), pos. HR-SI-MS *m/z*: 543.3329 ($C_{32}H_{46}O_7$+H)$^+$, error: 1.0 m.m.u., IR ($CHCl_3$) cm$^{-1}$: 3250—3500 (OH), 1731 (AcO), $^1$H- and $^{13}$C-NMR (pyridine-$d_5$) *δ*: Tables 1 and 2.

**Preparation of 1dS and 1dR** A mixture of **1b**S and **1b**R (about 3:1) (42 mg) was treated with cellulase T[Amano]4 (250 mg) as above to provide a mixture of 26(*S*)- and 26(*R*)-methylcimicifugenin (25 mg). Then, they were dissolved in pyridine (1 ml) and 20% $CrO_3$–pyridine solution (1 ml) was added dropwise. After stirring for 1 h at room temperature, the reaction mixture was extracted with EtOAc and subjected to $Al_2O_3$ column chromatography. The eluate with EtOAc provided 26(*S*)-*O*-methyl-3-keto-cimicifugenin (**1d**S) (3.8 mg) and 26(*R*)-*O*-methyl-3-keto-cimicifugenin (**1d**R) (2.5 mg) by HPLC [column: CrestPakC18T-5 (i.d. 7.15×250 mm); solvent: MeOH–$H_2O$–MeCN (13:7:3); effluent speed: 2 ml/min; column temperature: 40 °C] and recrystallized from MeOH . **1d**S: Colorless powder, mp 141—142 °C, EI-MS *m/z*: 554 ($C_{33}H_{46}O_7$)$^+$, 494 (M−AcOH)$^+$, IR ($CHCl_3$) cm$^{-1}$: 1734 (AcO), 1709 (C=O), $^1$H-NMR (pyridine-$d_5$) *δ*: 2.72 (2H, m, H-2), 5.14 (1H, m, H-7), 2.95 (1H, m, H-11), 5.22 (1H, m, H-12), 4.68 (1H, m, H-16), 0.81 (1H, d, *J*=4.0 Hz, H-19), 1.46 (3H, s, H-18), 0.96 (3H, d, *J*=6.0 Hz, H-21), 3.86 (1H, s, H-24), 5.05 (1H, s, H-26), 1.66 (3H, s, H-27), 1.07 (3H, s, H-28), 1.12 (3H, s, H-29), 1.02 (3H, s, H-30), 3.50 (3H, s, $OCH_3$), 2.22 (3H, s, $COCH_3$). CD: $\Delta\varepsilon_{295}$: −0.44 (*c*=3.8×10$^{-4}$, MeOH). **1d**R: Colorless needles, mp 245—246 °C, EI-MS *m/z*: 554 ($C_{33}H_{46}O_7$)$^+$, 494 (M−AcOH)$^+$, IR ($CHCl_3$) cm$^{-1}$: 1735 (AcO), 1709 (C=O), $^1$H-NMR (pyridine-$d_5$) *δ*: 2.72 (2H, m, H-2), 5.16 (1H, m, H-7), 2.97 (1H, m, H-11), 5.22 (1H, m, H-12), 4.70 (1H, m, H-16), 1.45 (3H, s, H-18), 0.85 (1H, d, *J*=4.0 Hz, H-19), 0.96, (3H, d, *J*=6.0 Hz, H-21), 3.79 (1H, s, H-24), 5.26 (1H, s, H-26), 1.60 (3H, s, H-27), 1.06 (3H, s, H-28), 1.13 (3H, s, H-29), 1.03 (3H, s, H-30), 3.61 (3H, s, $OCH_3$), 2.25 (3H, s, $COCH_3$). CD: $\Delta\varepsilon_{285}$: −0.33 (*c*=2.5×10$^{-4}$, MeOH).

**X-Ray Crystal Analysis of 26(*S*)-*O*-Methylcimicifugenin A (1cS)** Prismatic crystals were grown from a mixture of MeOH and EtOAc by slow evaporation at room temperature. Crystal data are as follows: $C_{33}H_{48}O_7$, Mr=556.71, triclinic, space group P1, *a*=13.397(2) Å, *b*=13.827(2) Å, *c*=9.199(1) Å, *α*=92.71(1)°, *β*=105.43(1)°, *γ*=104.86(1)°, *V*=1575.3(3) Å$^3$ (by least-squares refinement on diffractometer angles for 25 automatically centered reflections in the range of 45°<2*θ*<50°), *Z*=2, *D*c=1.174 g·cm$^{-3}$, *λ*(Cu*Kα*)=1.5418 Å, *μ*(Cu*Kα*)=0.65 mm$^{-1}$, *F*(000)=604. A single crystal with the dimensions 0.4×0.2×1.0 mm was used to obtain X-Ray diffraction data on a Rigaku AFC-5 diffractometer employing graphite monochromated Cu*Kα* radiation. A total of 5274 independent reflections were collected with an *ω*-2*θ* scan mode at 293 K; the parameters for data collection were scan speed (in *ω*)=12° min$^{-1}$, scan range (in *ω*)=(1.15+0.15 tan *θ*)°, and data range measured=2°<2*θ*<125°. The stationary background count for 5 s was recorded

on each side of the reflection. Three standard reflections were monitored for every 100 reflection intervals throughout the data collection and showed no significant deterioration. The observed intensities were corrected for Lorentz and polarization effects, but no absorption correction was made.

**Structure Determination and Refinement** The structure was solved by direct methods using the MULTAN program.[10] The positional parameters of non-H atoms were refined by a full-matrix least-squares method with anisotropic thermal parameters using the SHELXL-93 program.[11] The position of the H-atoms was calculated assuming idealized geometries, but not refined. The function $\sum w(Fo^2-Fc^2)^2$ was minimized, and $w=1.0/[\sigma^2Fo^2+(0.082P)^2+0.1877P]$ was used in the final refinement, where $P=(Fo^2+2Fc^2)/3$. Final cycles of least-squares refinement yielded $R=0.043$ and $wR=0.113$ for 5131 observed reflections of $I>2\sigma(I)$.

**Semi-empirical Energy Calculations** The total energy variations of A and B molecules as a function of the torsion angle $\theta$ were calculated by the CNDO/2 method. Table 3 shows the torsion angles for the stereochemistry of rings E, F and G, and Fig. 3 shows the levels of carbon and oxygen atoms of these rings from the best plane calculated as mentioned above.

**Methylation of 2** **2** (55 mg) was treated with 1% methanolic *p*-toluenesulfonic acid (30 ml) as in **1**. **2aS**: Colorless needles (40 mg), mp 245—246 °C, $[\alpha]_D$ −28.4° ($c=0.92$, MeOH), pos. SI-MS *m/z*: 691 $(C_{38}H_{58}O_{11}+H)^+$. $^1$H- and $^{13}$C-NMR (pyridine-$d_5$): Tables 1 and 2. **2aR**: Colorless needles (13.8 mg), mp 225—226 °C, $[\alpha]_D$ −82.1°($c=0.88$, MeOH), pos. SI-MS *m/z*: 691 $(C_{38}H_{58}O_{11}+H)^+$, $^1$H- and $^{13}$C-NMR (pyridine-$d_5$): Tables 1 and 2.

**Hydrogenation of 1 to 2** **1** (20 mg) was dissolved in EtOH (2 ml) and Pd–charcoal (about 20 mg) was added to the solution with stirring under $H_2$ for 24 h. After removal of the catalyst by filtering, the products were subjected to HPLC to afford the starting material (**1**) (8.3 mg) and **2** (7.0 mg). **2** was identified as actein by direct comparison of $^1$H-NMR and MS data, mp, $[\alpha]_D$, and IR of an authentic specimen.

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