Studies on the Constituents of *Cimicifuga* Species. XVII.¹⁾ Four New Glycosides from the Aerial Parts of *Cimicifuga simplex* WORMSK.

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Four new glycosides (1–4) were isolated from the aerial parts of *Cimicifuga simplex* (Ranunculaceae), and their structures were determined to be 12 β -hydroxycimigenol-3-*O*- β -D-xylopyranoside (1), 12 β -hydroxycimigenol-3-*O*- α -L-arabinopyranoside (2), 7 β -hydroxycimigenol-3-*O*- β -D-xylopyranoside (3) and 25-*O*-acetyl-7 β -hydroxycimigenol-3-*O*- β -D-xylopyranoside (4).

Key words *Cimicifuga simplex*; 12 β -hydroxycimigenol; β -D-xyloside; α -L-arabinoside; cycloartane; 12 β -hydroxycimigenol

We recently reported on the isolation of three new triterpenic glycosides from the aerial parts of *Cimicifuga simplex*.¹⁾ In our continuing work, we isolated four new glycosides (1–4) from the same herb. This paper deals with the isolation and the structural elucidation of these glycosides.

Compounds 1–4 were obtained as described in the experimental section by repeated chromatographies on

Diaion HP-20, octadecylsilanized silicic acid (ODS) and silica gel columns, and HPLC of the aqueous fraction after removal of the *n*-BuOH fraction from MeOH extracts of the aerial parts.

Compound 1 was obtained as colorless needles, mp 286–287°C, $[\alpha]_D +6.4^\circ$ ($c=0.44\%$, MeOH), and the molecular formula was determined to be C₃₅H₅₆O₁₀ by positive high resolution secondary ion mass spectroscopy

Table 1. ¹H-NMR Data of 1–4 and Their Aglycones (1a, 3a, 4a) in Pyridine-*d*₅

	1a ^{a)}	1	2	3a	3	4a	4
1	1.25, 1.57	1.20, 1.55	1.20, 1.55	1.20, 1.60	1.25, 1.58	1.20, 1.60	1.25, 1.58
2	1.85, 1.97	1.80, 2.12	1.80, 2.14	1.92, 2.35	1.92, 2.35	1.95, 2.35	1.94, 2.37
3	3.53 dd (11.5, 4.0)	3.45 dd (11.5, 4.2)	3.46 dd (11.5, 4.2)	3.57 dd (11.6, 4.5)	3.52 dd (11.6, 4.3)	3.57 dd (11.5, 4.3)	3.53 dd (11.6, 4.2)
5	1.30 dd (12.0, 3.8)	1.31	1.31	1.55	1.60	1.55	1.58
6	0.82 ddd (12.0, 12.0, 12.0)	0.72 ddd (12.5, 12.5, 12.5)	0.72 ddd (12.5, 12.5, 12.5)	1.20, 2.05	1.20, 2.04	1.20, 2.05	1.20, 2.04
7	1.60 1.25, 2.17	1.58 1.25, 2.26	1.60 1.25, 2.28	3.66 ddd (11.5, 10.5, 3.0)	3.67 ddd (12.0, 10.0, 3.0)	3.68 ddd (12.0, 10.0, 3.0)	3.68 ddd (12.0, 10.0, 3.0)
8	1.85	1.80	1.79	1.82 d (10.0)	1.80 d (10.0)	1.83 d (10.0)	1.82 d (10.0)
11	1.50 2.83 dd (16.0, 7.7)	1.50 2.78 dd (15.5, 9.0)	1.50 2.80 dd (15.5, 9.0)	1.10 2.08	1.08 2.08	1.10 2.08	1.10 2.06
12	4.23 dd (7.7, 2.5)	4.20 bd (9.0)	4.20 bd (9.0)	1.50, 1.65	1.55, 1.65	1.55, 1.65	1.55, 1.65
15	4.44 s	4.42 s	4.40 s	4.46 s	4.43 s	4.46 s	4.44 s
17	1.84 d (9.2)	1.81 d (9.3)	1.82 d (9.3)	1.51 d (10.8)	1.52 d (10.8)	1.52 d (11.0)	1.51 d (11.0)
18	1.45 s	1.41 s	1.43 s	1.19 s	1.18 s	1.21 s	1.19 s
19	0.46 d (4.0) 0.67 d (4.0)	0.40 d (4.0) 0.58 d (4.0)	0.42 d (4.0) 0.58 d (4.0)	0.37 d (4.0) 0.67 d (4.0)	0.35 d (4.2) 0.66 d (4.2)	0.39 d (4.2) 0.69 d (4.2)	0.36 d (4.2) 0.67 d (4.2)
20	1.85	1.85	1.86	1.58	1.60	1.60	1.60
21	1.39 d (6.0)	1.35 d (5.7)	1.37 d (5.8)	0.88 d (6.5)	0.87 d (6.4)	0.89 d (6.4)	0.88 d (6.5)
22	1.15, 2.41	1.12, 2.38	1.13, 2.39	1.10, 2.30	1.10, 2.28	1.04, 2.30	1.10, 2.28
23	4.77 d (9.2)	4.74 d (8.8)	4.76 d (8.8)	4.73 d (9.0)	4.72 d (8.8)	4.65 d (9.0)	4.63 d (8.8)
24	3.85 s	3.84 s	3.86 s	3.86 s	3.81 s	4.19 s	4.17 s
26	1.53 s	1.52 s	1.55 s	1.56 s	1.52 s	1.78 s	1.75 s
27	1.50 s	1.49 s	1.51 s	1.52 s	1.49 s	1.74 s	1.73 s
28	1.24 s	1.28 s	1.26 s	1.33 s	1.34 s	1.32 s	1.35 s
29	1.20 s	1.21 s	1.23 s	1.23 s	1.30 s	1.23 s	1.30 s
30	1.07 s	1.01 s	0.99 s	1.10 s	1.07 s	1.11 s	1.08 s
1'		4.82 d (7.6)	4.81 d (7.2)		4.84 d (7.5)		4.86 d (7.5)
2'		4.00 dd (8.5, 7.6)	4.40 dd (8.5, 7.2)		4.02 dd (8.5, 7.5)		4.05 dd (8.0, 7.5)
3'		4.16 dd (8.6, 8.5)	4.20 dd (8.5, 3.0)		4.17 dd (8.8, 8.5)		4.19 dd (8.8, 8.0)
4'		4.24 ddd (10.5, 8.6, 5.0)	4.35 brs		4.23 ddd (11.0, 8.8, 5.0)		4.26 ddd (10.0, 8.8, 5.0)
5'		3.71 dd (10.5, 10.5)	3.80 d (11.0)		3.73 dd (11.0, 11.0)		3.75 dd (11.0, 10.0)
COCH ₃		4.35 dd (10.5, 5.0)	4.30 dd (11.0, 3.0)		4.36 dd (11.0, 5.0)	1.98 s	4.38 dd (11.0, 5.0) 1.98 s

a) Obtained on a General Electric GN-400. The other data were obtained on a Varian XL-300.

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(pos. HR-SIMS) $[(M+H)^+, m/z 637.3957]$, pos. FAB-MS $[(M+H)^+, m/z 637, (M+Na)^+, m/z 659]$ and the data of the ^{13}C -NMR spectrum. The IR spectrum showed strong hydroxyl bands at $3550\text{--}3250\text{ cm}^{-1}$. The ^1H -NMR (Table 1) and the ^{13}C -NMR (Table 2) spectra were similar to those of cimigenol and its related triterpene glycosides previously isolated from the *Cimicifuga* species.¹⁻⁵⁾

Compound **1** was hydrolyzed with Cellulase T [Amano] **4** to afford **1a**, mp $216\text{--}217^\circ\text{C}$, $[\alpha]_D^{25} +10.1^\circ$ ($c=0.71\%$, MeOH), $\text{C}_{30}\text{H}_{48}\text{O}_6$. The positive high resolution electron impact mass spectroscopy (pos. HR-EI-MS) showed a M^+ ion at $m/z 504.3447$, clarifying the molecular formula. Signals due to 45 Hs and 30 Cs were assigned by the ^1H - ^1H shift correlation spectroscopy (^1H - ^1H COSY) spectrum coupled with the ^{13}C - ^1H COSY and heteronuclear multiple bond correlation (HMBC) spectra as summarized in Tables 1 and 2 (3 Hs of 48 Hs were exchanged by D_2O). A long-range correlation, especially among the quaternary carbons (C-4, 9, 10, 13, 14, 16, 25) and hydrogens, was found in the HMBC spectrum in assigning the H signals of the tertiary methyl groups (18- H_3 : δ 1.45 ppm, 26- H_3 : 1.53, 27- H_3 : 1.50, 28- H_3 : 1.24, 29- H_3 : 1.20, 30- H_3 : 1.07) as shown in Fig. 1 and Table 1.

The nuclear Overhauser effect (NOE) difference spectra afforded the definitive formulation of **1a** as 12 β -hydroxycimigenol, as shown in Fig. 2. Irradiation of the 28- H_3 signal line increased the 12 α -H signal at δ 4.23 by 2.8%. This irradiation increased 11 α -H (δ 2.83) by 1.4%, 7 α -H (δ 2.17) by 1.0% and 17-H (δ 1.84) by 3.4% at the same time. These data clarified the presence of a 12 β -hydroxy group in **1a**.

Compound **1** was hydrolyzed with 0.5 N HCl to afford 12 β -hydroxycimigenol as an aglycone (**1a**), along with D-xylose, which was detected by HPLC (Shodex RS-Pak DC-6143, 80% CH_3CN , 1 ml/min., 70°C) equipped with a chiral detection Shodex OR-1.⁶⁾

The ^1H -NMR spectrum of **1** showed 1'-H (δ 4.82, d, $J=7.6\text{ Hz}$), 2'-H (δ 4.00, dd, $J=8.5, 7.6\text{ Hz}$), 3'-H (δ 4.16, dd, $J=8.6, 8.5\text{ Hz}$), 4'-H (δ 4.24, ddd, $J=10.5, 8.6, 5.0\text{ Hz}$), 5'-H (δ 3.71, dd, $J=10.5, 10.5\text{ Hz}$) and 5'-H (δ 4.35, dd, $J=10.5, 5.0\text{ Hz}$), suggesting the presence of a β -D-xylopyranosyl group. On the other hand, the ^{13}C -NMR

spectrum showed that C-3 appeared at δ 88.71 by the glycosylation shift of 10.94 ppm from **1a**. Thus, the structure of **1** should be 12 β -hydroxycimigenol-3-O- β -D-xylopyranoside, as shown in Fig. 3.

Compound **2** was obtained as colorless needles, mp

Table 2. ^{13}C -NMR Chemical Shifts of **1**—**4** and Their Aglycones (**1a**, **3a**, **4a**) in Pyridine- d_5

	1a ^{a)}	1	2	3a	3	4a	4
1	32.54	32.42	32.06	30.72	30.18	30.08	30.50
2	30.86	29.99	29.53	30.31	29.98	30.08	29.88
3	77.77	88.71	88.18	77.68	88.28	77.28	88.13
4	40.84	41.23	40.84	40.81	41.03	40.39	40.19
5	47.04	47.35	46.92	46.08	46.28	45.71	46.14
6	21.01	20.80	20.47	32.46	32.29	32.13	32.19
7	26.09	26.08	25.71	69.42	69.29	69.02	69.15
8	47.30	47.35	46.92	56.16	56.28	55.83	56.18
9	20.47	20.53	20.25	18.90	18.94	18.50	18.85
10	26.47	26.49	26.14	27.20	27.17	27.04	27.09
11	40.65	40.63	40.24	26.58	26.57	26.26	26.47
12	72.66	72.89	72.39	33.92	33.96	33.59	33.85
13	47.84	47.85	47.44	42.25	42.37	41.99	42.23
14	48.14	48.27	47.86	47.80	47.76	47.44	47.71
15	79.68	79.93	79.40	79.12	79.15	78.67	78.93
16	112.15	112.53	111.90	111.86	111.72	111.92	112.14
17	59.63	59.81	59.36	59.82	59.85	59.38	59.60
18	11.96	12.04	11.70	19.61	19.63	19.23	19.47
19	30.83	30.75	30.38	30.79	30.64	30.53	30.50
20	23.89	24.00	23.64	23.96	24.00	23.51	23.75
21	20.94	21.03	20.69	19.61	19.63	19.23	19.47
22	38.64	38.78	38.39	38.08	38.10	37.51	37.75
23	71.61	71.89	71.40	71.75	71.70	71.12	71.36
24	89.88	90.22	89.65	90.29	90.30	86.38	86.63
25	70.97	71.33	70.81	71.22	71.18	83.29	83.42
26	25.37	25.43	25.10	25.49	25.54	20.92	21.15
27	26.67	26.49	26.14	26.03	26.37	23.08	23.34
28	11.74	11.81	11.48	11.95	11.94	11.68	11.88
29	26.00	25.71	25.34	26.03	25.65	25.71	25.54
30	14.68	15.32	14.96	14.72	15.29	14.37	15.17
1'		107.60	106.79		107.45		107.78
2'		75.41	72.22		75.31		75.27
3'		78.33	73.95		78.28		78.27
4'		71.11	68.84		71.01		70.96
5'		67.00	66.08		66.96		66.90
CO						170.21	170.23
CH ₃						22.04	22.22

a) Measured at 100.5 MHz. The others: at 75.4 MHz.

Fig. 1. HMBC of 12 β -Hydroxycimigenol (**1a**)

Fig. 2. ^1H -NMR (Normal and NOE) Spectra of 12 β -Hydroxycimigenol (**1a**)

a) Normal spectrum. b) NOE difference spectrum on irradiation at δ 1.24 (C-28).

Fig. 3. The Structures of **1**–**4** and Their Aglycones (**1a**, **3a**, **4a**)

267–268 °C, $[\alpha]_D +15.3^\circ$ ($c=0.38\%$, MeOH), and the molecular formula was determined to be $\text{C}_{35}\text{H}_{56}\text{O}_{10}$ by the pos. HR-SIMS $[(\text{M}+\text{H})^+]$, m/z 637.3954]. The IR-spectrum showed strong hydroxyl bands at 3550–3200 cm^{-1} .

Compound **2** was hydrolyzed with 0.5 N HCl to afford 12 β -hydroxycimigenol (**1a**) as an aglycone and L-arabinose as a sugar detected by HPLC equipped with Shodex OR-1, as in **1**. The ^1H -NMR spectrum showed the presence of an α -L-arabinopyranosyl moiety (1'-H: δ 4.81, d, $J=7.2$; 2'-H: δ 4.40, dd, $J=8.5, 7.2$ Hz; 3'-H: δ 4.20, dd, $J=8.5, 3.0$ Hz; 4'-H: δ 4.35, br s; 5'-H: δ 3.80, d, $J=11.0$ Hz; 5'-H: δ 4.30, dd, $J=11.0, 3.0$ Hz). The glycosylation position of 3-O was clarified by the ^{13}C -NMR data (δ 88.18 ppm for 3-C). Thus, the structure of **2** should be 12 β -hydroxycimigenol-3-O- α -L-arabinopyranoside, as shown in Fig. 3.

Compound **2** seems to be the same compound isolated from the roots of *Cimicifuga dahurica* by Kondo *et al.*⁷⁾ They reported it as a 12-hydroxycimigenol arabinoside at

the 96th Annual Meeting of the Japan Pharmaceutical Society (1976). We looked for a direct comparison, but neither the authentic sample nor the spectral data were available. Therefore, in this report we treated **2** as a new compound.

Compound **3** was obtained as colorless needles, mp 310 °C, $[\alpha]_D +14.7^\circ$ ($c=1.17\%$, MeOH), and the molecular formula was determined to be $\text{C}_{35}\text{H}_{56}\text{O}_{10}$ by the pos. HR-SIMS $[(\text{M}+\text{H})^+]$, m/z 637.3947]. The IR-spectrum showed strong hydroxyl bands at 3450–3200 cm^{-1} .

Compound **3** was hydrolyzed with 0.5 N methanolic HCl to afford 7 β -hydroxycimigenol (**3a**), mp 255–256 °C as an aglycone and D-xylose ($[\alpha]_D +17.4^\circ$) as a sugar. The structure of **3a** was reported previously and the primary ^1H -NMR data in CDCl_3 were described.²⁾ In this report, the detailed data of ^1H - and ^{13}C -NMR spectra (pyridine- d_5) of this aglycone are described (Tables 1, 2). The glycosylation position of 3-O was clarified by the ^{13}C -NMR data (δ 88.28 ppm for 3-C). Thus, the structure of

3 should be 7 β -hydroxycimigenol-3-*O*- β -D-xylopyranoside, as shown in Fig. 3.

Compound **4** was obtained as colorless needles, mp 252–253 °C, $[\alpha]_D + 14.0^\circ$ ($c=0.41\%$, MeOH), and the molecular formula was determined to be $C_{37}H_{58}O_{11}$ by the pos. HR-SIMS $[(M+H)^+]$, m/z 679.4053. The IR-spectrum showed strong hydroxyl bands at 3560–3200 cm^{-1} and an acetyl band at 1720 cm^{-1} . The 1H -NMR (Table 1) and the ^{13}C -NMR (Table 2) spectra were similar to those of 25-*O*-acetylcimigenol.^{2,5)} An acetyl group (δ_H 1.98, 3H, s, δ_C 170.23, 22.22 ppm), 25-dimethyl groups (δ_H 1.75, 1.73 ppm, 26, 27-3H, s), 24-H (δ_H 4.17 ppm, 1H, s) and 25-C (δ_C 83.42 ppm) are characteristic to **4**.

Compound **4** was hydrolyzed with Cellulase T [Amano] **4** to afford 25-*O*-acetyl-7 β -hydroxycimigenol (**4a**), mp 160–161 °C, $[\alpha]_D + 22.6^\circ$ ($c=0.93$, MeOH), and the molecular formula was determined to be $C_{32}H_{50}O_7$ by the pos. HR-SIMS $[(M+H)^+]$, m/z 547.3627.

Compound **4** was hydrolyzed with 1% Na_2CO_3 to afford **3**. Thus, the structure of **4** should be 25-*O*-acetyl-7 β -hydroxycimigenol-3-*O*- β -D-xylopyranoside, as shown in Fig. 3.

Experimental

General The instruments used in this work were as follows: Yanagimoto micromelting apparatus (melting points), Perkin-Elmer 1720X-FT-IR spectrometer (IR spectra), Varian Gemini-200, Varian XL-300, and General Electric GN-400 (NMR spectra). Hitachi M-80 and JMS-DX-300 spectrometers (MS spectra). Melting points are uncorrected. NMR spectra were measured in pyridine- d_5 solution, and chemical shifts are expressed 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 on a Gilson 305 pump equipped with a Shodex refractometer as a detector.

Extraction of 1–4 The residue (185 g) after the isolation of cimigenol diglycosides from the aerial parts of *Cimicifuga simplex*¹⁾ was chromatographed on SiO_2 (1.0 kg, i.d. 6.0 $\times$ 40.0 cm). Elution fractions with $CHCl_3$ -MeOH (10–9:1) were chromatographed on ODS (100 g, i.d. 3.5 $\times$ 24 cm). Elution with MeOH-H₂O (2:1) afforded the mixtures of **1** and **2**. HPLC [column: Crest Pak C18T-5 (5 μm , i.d. 4.6 mm $\times$ 250 mm); solvent, MeOH-H₂O-CH₃CN (10:13:3); effluent speed: 1 ml/min; column temperature, 40 °C] and recrystallization from MeOH afforded **1** as colorless needles (28 mg), **2** as colorless needles (25 mg). Elution with MeOH:H₂O (3:1) afforded **3** and (5:1) **4**. HPLC [same condition as in **1**, **2** except the solvent: MeOH-H₂O-CH₃CN (10:7:3)] and recrystallization from CH_3CN -CH₃OH afforded **3** as colorless needles (60 mg) and **4** as colorless needles (65 mg).

1, mp 286–287 °C, $[\alpha]_D + 6.4^\circ$ ($c=0.44\%$, MeOH), $C_{35}H_{56}O_{10}$. Pos. HR-SIMS: m/z 637.3957 $[(M+H)^+]$, error: 0.9 (m mass). Pos. FAB-MS: m/z 637 $[(M+H)^+]$, m/z 659 $[(M+Na)^+]$. IR (KBr) cm^{-1} : 3550–3250 (OH). 1H - and ^{13}C -NMR (pyridine- d_5) δ : Tables 1 and 2.

2, mp 267–268 °C, $[\alpha]_D + 15.3^\circ$ ($c=0.38\%$, MeOH), $C_{35}H_{56}O_{10}$. Pos. HR-SIMS: m/z 637.3954 $[(M+H)^+]$, error: 0.6 (m mass), pos. SIMS: m/z 637 $[(M+H)^+]$, m/z 659 $[(M+Na)^+]$. IR (KBr) cm^{-1} : 3550–3200 (OH). 1H - and ^{13}C -NMR (pyridine- d_5): Tables 1 and 2.

3, mp 310 °C, $[\alpha]_D + 14.7^\circ$ ($c=1.17\%$, MeOH), $C_{35}H_{56}O_{10}$. Pos. HR-SIMS: m/z 637.3947 $[(M+H)^+]$, error: –0.1 (m mass). IR (KBr) cm^{-1} : 3450–3200 (OH). 1H - and ^{13}C -NMR (pyridine- d_5): Tables 1 and 2.

4, mp 252–253 °C, $[\alpha]_D + 14.0^\circ$ ($c=0.41\%$, MeOH), $C_{37}H_{58}O_{11}$. Pos. HR-SIMS: m/z 679.4053 $[(M+H)^+]$, error: –0.1 (m mass). IR (KBr) cm^{-1} : 3560–3200 (OH), 1720 (CH_3CO). 1H - and ^{13}C -NMR (pyridine- d_5): Tables 1 and 2.

Hydrolysis of 1 with Cellulase T [Amano] 4 **1** (19 mg) was dissolved in 1% ethanolic AcOH (20 ml), then water (40 ml) was added on stirring and the solution was adjusted to pH 4.5 by the dropwise addition of

AcOH. Cellulase T [Amano] **4** (from *Trichoderma viride*, 200 mg) was added. The solution was stirred for 2 d at room temperature. Then, the reaction solution was shaken with EtOAc (30 ml $\times$ 3), and after washing the joined EtOAc layer with water and drying it over Na_2SO_4 , the solvent was evaporated *in vacuo*. The residue was chromatographed on SiO_2 (12 g) and eluted with *n*-hexane-EtOAc (1:1) to afford **1a** as colorless needles (7 mg) by recrystallization from EtOAc. **1a**: mp 216–217 °C, $[\alpha]_D + 10.1^\circ$ ($c=0.71\%$, MeOH), $C_{30}H_{48}O_6$. SIMS: m/z 505 $[(M+H)^+]$. Pos. EI-HR-MS: m/z 504.3447 $[(M)^+]$, error: –0.2 (m mass). IR (KBr) cm^{-1} : 3500–3100 (OH). 1H - and ^{13}C -NMR (pyridine- d_5) δ : Tables 1 and 2. HMBC: Fig. 1. NOE: Fig. 2.

Acidic Hydrolysis of 1 with 0.5N HCl **1** (5.8 mg) was treated in the same method as reported previously.¹⁾ The water layer and the water washings after the extraction of the aglycone (**1a**) were joined and passed to an Amberlite IR-35 column. The passed fraction was subjected to HPLC (column: Shodex RS-Pak DC-613, solvent: 80% CH_3CN , effluent speed: 1 ml/min, column temperature: 70 °C) equipped with a chiral detection OR-1: t_R 11 min, +15° (D-xylose).

Acidic Hydrolysis of 2 with 0.5N HCl **2** (20 mg) was treated as above to afford **1a** (7.2 mg) as an aglycone. The water layer was passed to an Amberlite IR-35 column and the passed fraction was subjected to HPLC equipped with OR-1 as in **1**. L-arabinose: t_R 13.5 min. +10°.

Hydrolysis of 4 with 1% Na_2CO_3 **4** (4.7 mg) was dissolved in MeOH (2 ml) and 1% Na_2CO_3 (2 ml) was added on stirring for 15 h at 25 °C. After neutralization with 5% AcOH, the mixture was shaken with EtOAc (20 ml $\times$ 3) and washed with water. The product was chromatographed on SiO_2 (10 g) and recrystallized from CH_3CN -MeOH to afford colorless needles (3.2 mg). The 1H -NMR spectrum (pyridine- d_5) was identical with that of the isolated compound **3**.

Hydrolysis of 3 with 0.5N HCl **3** (20.5 mg) was treated as above, and the product (10 mg) from the EtOAc layer was identified with 7 β -hydroxycimigenol (**3a**) by comparison of the 1H -NMR spectrum. D-Xylose, $[\alpha]_D + 17.4^\circ$ ($c=0.31\%$, 50% MeOH) was isolated from the water layer by HPLC [column: LiChrosorb NH₂ (5 μm , i.d. 4.6 mm $\times$ 250 mm) solvent: CH_3CN -H₂O (4:1), effluent speed: 1 ml/min, column temperature: 40 °C, t_R 5:40 min].

Hydrolysis of 4 with Cellulase T [Amano] 4 **4** (27.4 mg) was treated as in **1** with Cellulase T [Amano] **4** (200 mg) to afford **4a** as colorless needles (12 mg), mp 160–161 °C, $[\alpha]_D + 22.6^\circ$ ($c=0.93\%$, MeOH), $C_{32}H_{50}O_7$. Pos. HR-SIMS: m/z 547.3627 $[(M+H)^+]$, error: –0.5 (m mass). SIMS: m/z 547 $[(M+H)^+]$, 569 $[(M+Na)^+]$. IR ($CHCl_3$) cm^{-1} : 3500–3200 (OH), 1733 (OAc). 1H - and ^{13}C -NMR (pyridine- d_5) δ : Tables 1 and 2.

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References and Notes

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