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Studies on the Chinese Crude Drug "Shoma." V.[1] Structures of 24-O-Acetylhydroshengmanol Xyloside and 22-Hydroxycimigenol Xyloside[2]

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Two new xylosides were isolated from the underground part of *Cimicifuga japonica*—24-O-acetylhydroshengmanol xyloside (**2**), $C_{37}H_{60}O_{11}$, mp 235—237°, $[\alpha]_D^{22}$ +6.6° and 22-hydroxycimigenol xyloside (**3**), $C_{35}H_{56}O_{10}\cdot H_2O$, mp 282—284°, $[\alpha]_D^{17}$ +17.7°.

The xyloside (**2**) afforded, on acid hydrolysis in aq. methanol, cimigenol (**4**) and isodahurinol (**5**). Enzymatic hydrolysis of **2** afforded its genuine aglycone 24-O-acetylhydroshengmanol (**8**), $C_{32}H_{52}O_7$, mp 200—202°, $[\alpha]_D^{28}$ +9.0°. The xyloside (**2**) and its genin (**8**) showed a mutarotation. When treated at room temperature with sulfuric acid in aq. methanol, **2** changed firstly into 25-O-acetylcimigenol xyloside (**6**) and finally into cimigenol xyloside (**7**).

On the basis of chemical and spectral data, the structure of 24-O-acetylhydroshengmanol xyloside (**2**) was proposed to be (23*R*,24*S*)-3β,15α,16ξ,25-tetrahydroxy-24-acetoxy-16,23-epoxy-9,19-cyclolanostane 3-O-β-D-xylopyranoside.

Another glycoside (**3**) showed, in its ^{13}C-nuclear magnetic resonance (NMR) spectrum, a pattern of chemical shifts very similar to that of cimigenol xyloside (**7**), but a downfield shift of C-22 by 44.2 ppm was observed, suggesting oxygenation of C-22 in the glycoside (**3**). The xyloside (**3**) afforded, on both acidic and enzymatic hydrolyses, its aglycone (**13**), $C_{30}H_{48}O_6$, mp 274—276°, $[\alpha]_D^{19}$ +45.0°. Huang-Minlon reduction of a diketo derivative (**15**) of **13** provided a didesoxy compound (**16**), $C_{30}H_{48}O_4$, mp 202.5—203.5°, which was identical with the 3-desoxy derivative of cimigenol. The stereochemistry of the hydroxyl group at C-22 of **13** was assigned as *R* on the basis of its NMR spectrum. The structure of 22-hydroxycimigenol xyloside (**3**) was established as (22*R*)-22-hydroxycimigenol 3-O-β-D-xylopyranoside.

Keywords——*Cimicifuga japonica*; Ranunculaceae; 24-O-acetylhydroshengmanol xyloside; hemiketal; 22-hydroxycimigenol xyloside; 9,19-cyclolanostane; transformation by acid; precursor of *Cimicifuga* glycosides

In the preceding,[1] we reported the structure elucidation of acetylshengmanol xyloside (**1**) isolated from the fresh underground part of *Cimicifuga japonica* (Thunb.) Sprengel and its facile transformation into some known *Cimicifuga* glycosides, and we also suggested that the genin of **1** is a probable precursor of some aglycones isolated from several *Cimicifuga* plants. This report deals with the isolation and the structure elucidiaton of two new xylosides from the ethyl acetate-soluble fraction of the methanolic extract of *C. japonica*—24-O-acetylhydroshengmanol xyloside (**2**), $C_{37}H_{60}O_{11}$, mp 235—237°, $[\alpha]_D^{22}$ +6.6° and 22-hydroxycimigenol xyloside (**3**), $C_{35}H_{56}O_{10}\cdot H_2O$, mp 282—284°, $[\alpha]_D^{17}$ +17.7°.

24-O-Acetylhydroshengmanol xyloside (**2**) is a monoacetate, since it showed bands at 1703 and 1260 cm^{-1} in its infrared (IR) spectrum and a three-proton singlet at δ_H 2.03 ppm in its proton nuclear magnetic resonance (^{1}H-NMR) spectrum. It exhibited a mutarotation and there was a weak negative Cotton effect $[\theta]_{314}$ -2.00×10^3 in the circular dichroism (CD) spectrum. A single carbonyl signal at δ_C 171.1 ppm in its carbon 13 NMR (^{13}C-NMR) spectrum was ascribable to the ester carbonyl.

On hydrolysis with sulfuric acid in aqueous methanol under heating, **2** yielded cimigenol (**4**)[3] and a smaller amount of isodahurinol (**5**)[4] as the aglycone, and xylose as the sugar moiety. On the other hand, when treated with sulfuric acid in aqueous methanol at room temperature, **2** changed firstly into 25-O-acetylcimigenol xyloside (**6**),[5] and finally into cimigenol xyloside (**7**),[3] indicating that 24-O-acetylhydroshengmanol xyloside (**2**) has a structure that is easily

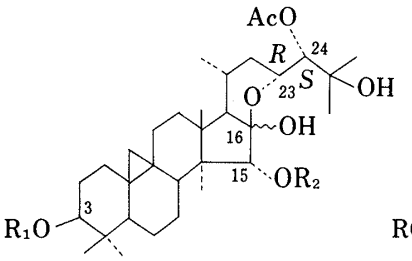
changeable into 25-O-acetylcimigenol xyloside (**6**) under acidic conditions. Enzymatic hydrolysis of the xyloside (**2**) using crude hesperidinase[6] afforded its genuine aglycone (**8**), designated now as 24-O-acetylhydroshengmanol, $C_{32}H_{52}O_7$, mp 200—202°, $[\alpha]_D^{28}$ +9.0°. The genin (**8**) also showed a mutarotation, an acetoxyl band in its IR spectrum, and a weak negative Cotton effect in its CD curve. In nuclear magnetic double resonance (NMDR) experiments, a doublet at δ_H 4.73 ppm ascribable to the proton on C-24 bearing an acetoxyl group changed into a singlet upon irradiation of a multiplet at δ_H 4.26 ppm due to the proton on C-23 bound to an oxygen function. When treated with sulfuric acid in aqueous methanol, 24-O-acetylhydroshengmanol (**8**) changed, analogously to its parent glycoside (**2**), firstly into 25-O-acetylcimigenol (**9**),[5] and finally into cimigenol (**4**). On acetylation with acetic anhydride in pyridine, the aglycone (**8**) afforded a triacetate (**10**) and a tetraacetate (**11**). The former acetate (**10**) was easily found to be the 3,15,23-triacetate (see data in "Experimental"). The latter acetate (**11**) has a hydroxyl group and unexpectedly a ketonic function exhibiting a negative Cotton effect $[\theta]_{313}$ -1.14×10^4 in its CD curve. This negative Cotton effect is as strong as that observed in the case of acetyl shengmanol (**12**),[1] indicating that the ketonic function of the acetate (**11**) is located at C-16.[7] In the ^{1}H-NMR spectrum of **11**, signals due to four protons on carbons bearing an acetoxyl group appeared at δ_H 4.56 (3-H, m), 5.26 (15-H, s), 5.46 (23-H, br d), and 4.78 ppm (24-H, d). In NMDR experiments the doublet at δ_H 4.78 ppm, corresponding to the doublet at δ_H 4.73 ppm in the ^{1}H-NMR spectrum of **8**, changed to a singlet on irradiation of the broad doublet at δ_H 5.46 ppm (23-H), which was observed at δ_H 4.26 ppm as a multiplet in the genin (**8**).

We assumed structure **11** for the tetraacetate (**11**) and structure **3** for 24-O-acetylhydroshengmanol xyloside (**3**). These assumptions seem to account well for the chemical and physicochemical features of **3** as follows.

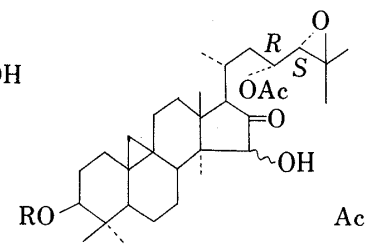
When 24-O-acetylhydroshengmanol xyloside (**2**) was treated with sulfuric acid, migration of the acetyl group at C-24 to the hydroxyl group at C-25 and subsequent dehydration between the two hydroxyl groups at C-16 and C-24, explain satisfactorily the formation of a ketal compound, 25-O-acetylcimigenol xyloside (**6**). Slow chemical equilibration between the hemiketal form (**2**) and its keto-alcohol form (**2b**) in solution occurs, and this explains the mutarotation and the weak Cotton effect in the CD observed in both the xyloside (**2**) and its aglycone (**8**). In fact, acetylation of **8** afforded two products, one an acetylation product (**10**) of **2**, and the other, the acetate (**11**) with 16-oxo structure as an acetylation product of **2b**.

On the basis of the above evidence and discussion, we propose a hemiketal structure **2** for 24-O-acetylhydroshengmanol xyloside (**2**), namely (23*R*, 24*S*)-3β,15α,16ξ,25-tetrahydroxy-24-acetoxy-16,23-epoxy-9,19-cyclolanostane 3-O-β-D-xylopyranoside. We consider that both acetyl shengmanol xyloside (**1**) and 24-O-acetylhydroshengmanol xyloside (**2**) are biosynthetic precursors of some *Cimicifuga* glycosides such as cimigenol xyloside (**7**).

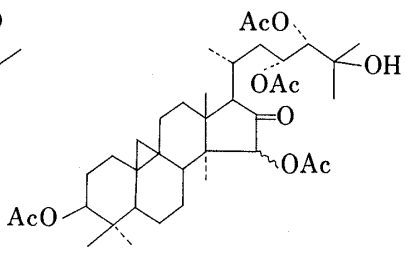
Another new glycoside 22-hydroxycimigenol xyloside (**3**) showed, in its ^{13}C-NMR spectrum, a pattern of chemical shifts very similar to that of cimigenol xyloside (**7**), except for the signals due to C-20, -22 and -23. In particular, C-22 of **3** was observed at δ_C 82.2 ppm instead of the signal at δ_C 38.0 ppm due to C-22 in the spectrum of **7**,[8] suggesting that **3** is a xyloside of 22-monohydroxylated cimigenol. The xyloside (**3**) afforded its aglycone (**13**), $C_{30}H_{48}O_6$, mp 274—276°,[9] $[\alpha]_D^{19}$ +45.0° on both acidic and enzymatic hydrolyses. Acetylation of **13** provided a triacetate (**14**), 202—203.5°, which has a further hydroxyl group. Oxidation of the aglycone (**13**) with chromic acid in pyridine, afforded a diketone (**15**), mp 253—254°, in whose ^{1}H-NMR spectrum the protons observed as two doublets at δ_H 3.69 (24-H, J=1.2 Hz) and 4.53 ppm (23-H, J=1.2 Hz) were ascribable to ones on carbons bearing ether oxygen. These doublets were obviously coupled with each other but with no other protons as judged from NMDR experiments, suggesting that the diketone (**15**) has a carbonyl group at C-22. On Huang-Minlon reduction,[10] **15** afforded a didesoxy compound (**16**), $C_{30}H_{48}O_4$, mp 202.5—203.5°, which was identical with 3-desoxycimigenol (**16**) obtained by a similar reduction of the 3-keto deri-



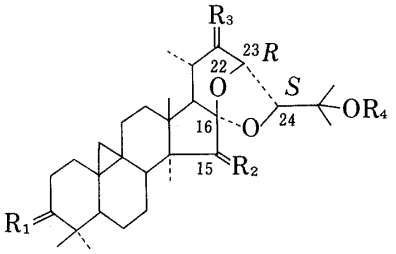
2: $R_1=\beta$-D-xyl(p), $R_2=H$
8: $R_1=R_2=H$
10: $R_1=R_2=COCH_3$



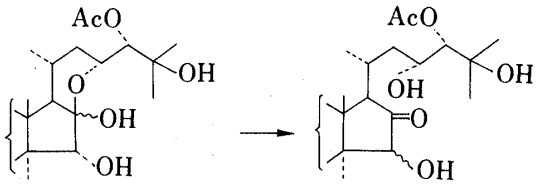
1: $R=\beta$-D-xyl(p)
12: $R=H$



11



3: $R_1=\langle^{O\cdot xyl(p)}_{H}$, $R_2=\langle^{H}_{OH}$, $R_3=\langle^{OH}_{H}$, $R_4=H$
4: $R_1=\langle^{OH}_{H}$, $R_2=\langle^{H}_{OH}$, $R_3=H_2$, $R_4=H$
6: $R_1=\langle^{O\cdot xyl(p)}_{H}$, $R_2=\langle^{H}_{OH}$, $R_3=H_2$, $R_4=COCH_3$
7: $R_1=\langle^{O\cdot xyl(p)}_{H}$, $R_2=\langle^{H}_{OH}$, $R_3=H_2$, $R_4=H$
9: $R_1=\langle^{OH}_{H}$, $R_2=\langle^{H}_{OH}$, $R_3=H_2$, $R_4=COCH_3$
13: $R_1=R_3=\langle^{OH}_{H}$, $R_2=\langle^{H}_{OH}$, $R_4=H$
14: $R_1=R_3=\langle^{OCOCH_3}_{H}$, $R_2=\langle^{H}_{OCOCH_3}$, $R_4=H$
15: $R_1=R_3=O$, $R_2=\langle^{H}_{OH}$, $R_4=H$
16: $R_1=R_3=H_2$, $R_2=\langle^{H}_{OH}$, $R_4=H$
17: $R_1=O$, $R_2=\langle^{H}_{OH}$, $R_3=H_2$, $R_4=H$



2 **2b**

Chart 1

vative of cimigenol (**4**). The stereochemistry of the hydroxyl group at C-22 of the aglycone (**13**) was assigned as *R* on the basis of a Dreiding model of **13** and the coupling constant of 22-H (d, $J=12$ Hz) in its ^{1}H-NMR spectrum. If the configuration of C-22 of the genin (**13**) is *S*, the coupling constants of 22-H with 20-H and 23-H are calculated to be $J_1=4.4$ Hz and $J_2=7.5$ Hz, and if it is *R*, they are calculated to be $J_1=8.6$ Hz and $J_2=-0.10$ Hz from the Karplus equation.[11] The sugar moiety of 22-hydroxycimigenol xyloside (**3**) was proved to be attached to C-3 of the genin (**13**) as β-D-xylopyranose by comparison of the ^{13}C-NMR chemical shifts of the anomeric carbon and C-3 in the xyloside (**3**) with those of cimigenol xyloside (**7**).[8] Application of Klyne's rule[12] to the xyloside (**3**) also supported the β-D-xylopyranoside structure. (Molecular rotation difference at 589 nm of the xyloside (**3**) and its aglycone (**13**): $-111°$. Methyl β-D-xylopyranoside: M_D $-108°$.).[13]

Accordingly, the structure of 22-hydroxycimigenol xyloside (**3**) was established as (22*R*)-22-hydroxycimigenol 3-O-β-D-xylopyranoside.

Experimental

All melting points were determined on a Shimadzu micro melting point determination apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter in a 1 dm tube. NMR spectra were recorded at 100 MHz with a JEOL JNM FX-100 spectrometer. Tetramethylsilane was used as the internal standard. Chemical shifts are given on the δ scale (ppm). The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. Coupling constants (J values) are given in Hz. CD spectra were measured with a JASCO J-40 machine. Mass spectra (MS) were recorded with a JEOL JMS-D 300 spectrometer. Gas–liquid partition chromatography (GLC) was run on a Shimadzu GC-4A chromatograph with a hydrogen flame ionization detector. Columns for chromatography were prepared with silica gel (Kanto Chemical Co. Inc., 100 mesh, or Merck, 70—230 mesh). Silica gel 60 F_{254} (Merck) precoated TLC plates were used, and detection was carried out by spraying 10% H_2SO_4 followed by heating.

Isolation of 24-O-Acetylhydroshengmanol Xyloside (2) and 22-Hydroxycimigenol Xyloside (3)——An EtOAc-soluble fraction (32 g) of the MeOH extract (139 g) from the fresh underground part (2 kg) of *C. japonica* was subjected to column chromatography on silica gel. The fractions eluted with benzene–EtOAc (1:2) were rechromatographed on silica gel. The fractions eluted with $CHCl_3$–MeOH (93:7) were combined and concentrated, affording crude 24-O-acetylhydroshengmanol xyloside (**2**) (0.5 g) as colorless needles. The fractions eluted with $CHCl_3$–MeOH (9:1) were combined and concentrated, affording crude 22-hydroxycimigenol xyloside (**3**) (0.01 g).

Properties of 24-O-Acetylhydroshengmanol Xyloside (2)——Pure **2** was obtained as colorless needles after recrystallization from EtOH, mp 235—237°, $[\alpha]_{589}^{22}$ +6.6° (+4.4°), $[\alpha]_{577}^{22}$ +5.7° (+4.1°), $[\alpha]_{546}^{22}$ +6.8° (+4.4°), $[\alpha]_{435}^{22}$ +7.6° (−0.53°), $[\alpha]_{365}^{22}$ +1.5° (−33.3°) (c=0.7, $CHCl_3$–MeOH (1:1)) (figures in parenthesis give specific rotation after 24 hr). *Anal.* Calcd for $C_{37}H_{60}O_{11}$: C, 65.27; H, 8.88. Found: C, 65.47; H, 8.81. IR ν_{max}^{KBr} cm^{-1}: 3600—3200, 1040 (OH), 1703, 1260 ($OCOCH_3$). ^{13}C-NMR (pyridine-d_5) δ_C: 30.8 (C-19), 71.1 (C-25), 74.5 (C-23), 82.3 (C-15), 88.2 (C-24), 102.9 (C-16), 107.2 (anomeric C), 171.1 ($O\underline{C}OCH_3$). CD (c=6.61×10^{-4}, MeOH) $[\theta]^{22}$ (nm): -2.00×10^3 (314) (negative maximum).

Acid Hydrolysis of 24-O-Acetylhydroshengmanol Xyloside (2)——A solution of **2** (28 mg) in 5% H_2SO_4 in 50% MeOH (20 ml) was refluxed for 4 hr. The MeOH was removed *in vacuo*. The precipitates were collected and washed with water. Crystallization of the precipitates from EtOH provided cimigenol (**4**) as colorless needles (4 mg) which were identified by comparison with an authentic sample of cimigenol (**4**) (mixed mp, TLC and IR). The filtrate was extracted with ether. The ether solution was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The residue was found by TLC to contain components identical with authentic samples of cimigenol (**4**) and isodahurinol (**5**). The water-soluble fraction was treated with Dowex 2-X8 (OH^-) (1 g), and concentrated under reduced pressure. The residue was subjected to PPC (Toyo Filter Paper, No. 50, *n*-BuOH–AcOH–H_2O (6:1:2), coloring with aniline H_3PO_4), and xylose was identified.

Treatment of 24-O-Acetylhydroshengmanol Xyloside (2) with 5% H_2SO_4——a) A solution of **2** (32 mg) in MeOH (6 ml) was treated with 5% H_2SO_4 in 50% MeOH (1.5 ml) and the total mixture was kept for 5 hr at room temperature. After removal of the MeOH, the residue was diluted with water, and extracted with EtOAc. The EtOAc layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed over silica gel (5 g). Elution with benzene–EtOAc (1:2) gave 25-O-acetylcimigenol xyloside (**6**) which was identical with an authentic sample as judged by mixed mp, IR and TLC.

b) A solution of **2** (2 mg) in 5% H_2SO_4 in 50% MeOH (0.5 ml) was kept for 26 hr at room temperature. The MeOH was removed *in vacuo* and the residue was diluted with water, and extracted with EtOAc. The EtOAc layer was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The product was identified as cimigenol xyloside (**7**) by TLC.

Enzymatic Hydrolysis of 24-O-Acetylhydroshengmanol Xyloside (2)——A solution of 24-O-acetylhydroshengmanol xyloside (**2**) (300 mg) in a mixture of EtOH (150 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0) (300 ml) was treated with crude hesperidinase (300 mg, Tanabe Pharm. Co., Lot. No. 71) in H_2O (150 ml) and the total mixture was kept for 39 hr with gentle stirring at 37°. The incubation mixture was concentrated, and extracted with ether. The ether layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed over silica gel (3 g). Elution with benzene–EtOAc (3:1) gave 24-O-acetylhydroshengmanol (**8**) as colorless needles (93.9 mg), mp 200—202° (from $(CH_3)_2$-CO), $[\alpha]_{589}^{28}$ +9.0° (+3.8°), $[\alpha]_{577}^{28}$ +8.7° (+3.6°), $[\alpha]_{546}^{28}$ +8.8° (+3.1°), $[\alpha]_{435}^{28}$ +0.7° (−15.8°), $[\alpha]_{365}^{28}$ −60.6° (−117°) (c=0.7, MeOH) (specific rotation after 6 hr). *Anal.* Calcd for $C_{32}H_{52}O_7$: C, 70.04; H, 9.55. Found: C, 70.15; H, 9.63. MS m/e: 548 (M^+), 530 (M^+-H_2O), 470 ($M^+-CH_3COOH-H_2O$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1738 ($OCOCH_3$). ^{1}H-NMR ($CDCl_3$) δ_H: 0.35, 0.60 (each 1H, d, J=4.8 Hz, cyclopropane methylene), 0.80, 0.88, 0.96, 1.12, 1.17, 1.31 (each 3H, s, 6×*tert.* CH_3), 2.18 (3H, s, $OCOCH_3$), 2.33(1H, d, J=9.0 Hz, OH, disappeared on addition of D_2O), 2.87 (1H, s, OH, disappeared on addition of D_2O), 3.2 (1H, m, 3-H), 3.65 (1H, d, J=9.0 Hz, 15-H, varied to a singlet on addition of D_2O), 3.96 (1H, s, OH, disappeared on addition of D_2O), 4.26 (1H, m, $W_{h/2}$=20.0 Hz, 23-H), 4.73 (1H, d, J=5.0 Hz, 24-H). CD (c=6.61×10^{-4}, MeOH) $[\theta]^{22}$ (nm): -1.69×10^3 (314) (negative maximum).

Acid Treatment of 24-O-Acetylhydroshengmanol (8)——A solution of **8** (2 mg) in 5% H_2SO_4 in 50% MeOH (1 ml) was refluxed for 2 hr. The MeOH was removed *in vacuo*. The resulting solution was extracted with ether. The ether solution was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The products were identified as cimigenol (**4**) and isodahurinol (**5**) by TLC comparison with authentic samples.

Acetylation of 24-O-Acetylhydroshengmanol (8)——24-O-Acetylhydroshengmanol (**8**) (55 mg) was acetylated overnight with Ac_2O (0.3 ml) in pyridine (1 ml) at room temperature. After work-up in the usual manner, the product was passed through a silica gel column (1.2 g). The fraction eluted with benzene–EtOAc (19: 1) afforded a triacetate (**10**) as colorless needles (5 mg), mp 181—182° (from EtOH–benzene), $[\alpha]_D^{22}$ −5.3° (c=0.5, $CHCl_3$–MeOH (3: 7)). *Anal.* Calcd for $C_{36}H_{56}O_9$: C, 68.32; H, 8.92. Found: C, 68.68; H, 8.61. IR ν_{max}^{KBr} cm^{-1}: 3540, 1042 (OH), 1748, 1720, 1260 ($OCOCH_3$). ^{1}H-NMR ($CDCl_3$) δ_H: 0.35, 0.62, (each 1H, d, J=4.8 Hz, cyclopropane methylene), 0.83, 0.87, 1.03, 1.17, 1.19, 1.24 (each 3H, s, 6×*tert.* CH_3), 2.04, 2.10, 2.15 (each 3H, s, 3×$OCOCH_3$), 3.9 (1H, m, 23-H), 4.02 (1H, s, OH, disappeared on addition of D_2O), 4.5 (1H, m, 3-H), 4.82 (1H, d, J=8.1 Hz, 24-H), 4.97 (1H, s, 15-H). CD ($c=7.39\times10^{-3}$, $CHCl_3$–MeOH (3: 7)) $[\theta]^{21}$ (nm): -4.87×10^2 (312) (negative maximum). The fraction eluted with benzene–EtOAc (9:1) afforded another acetate (**11**) as an amorphous powder (25 mg), $[\alpha]_D^{22}$ +16.2° (c=0.3, MeOH). High resolution MS *m/e*: Calcd for $C_{38}H_{58}O_{10}$ (M^+) 674.403, $C_{38}H_{56}O_9$(M^+-H_2O) 656.392, $C_{37}H_{53}O_9$ ($M^+-H_2O-CH_3$) 641.369, $C_{36}H_{54}O_8$ (M^+-CH_3COOH) 614.382. Found: 674.400, 656.387, 641.362, 614.376. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3650, 1040 (OH), 1762, 1742, 1230 (CO, $OCOCH_3$). NMR ($CDCl_3$) δ_H: 0.44, 0.66 (each 1H, d, J=4.8 Hz, cyclopropane methylene), 0.85, 0.88, 1.00, 1.18, 1.24, 1.29 (each 3H, s, 6×*tert*-CH_3), 2.05, 2.07, 2.12, 2.19 (each 3H, s, 4×$OCOCH_3$), 4.56 (1H, m, 3-H), 4.78 (1H, d, J=1.5 Hz, 24-H), 5.26 (1H, s, 15-H), 5.46 (1H, br d, $W_{h/2}$= 12.0 Hz, 23-H). CD ($c=2.23\times10^{-3}$, MeOH) $[\theta]^{21}$ (nm): -1.14×10^4 (313) (negative maximum).

Properties of 22-Hydroxycimigenol Xyloside (3)——22-Hydroxycimigenol xyloside (**3**) has mp 282—284°, and $[\alpha]_D^{17}$ +17.7° (c=0.6, $CHCl_3$–MeOH (1: 1)). *Anal.* Calcd for $C_{35}H_{56}O_{10}\cdot H_2O$: C, 64.20; H, 8.93. Found: C, 64.00; H, 8.68. IR ν_{max}^{KBr} cm^{-1}: 3640—3100, 1060 (OH). ^{1}H-NMR (pyridine-d_5) δ_H: 0.99, 1.15, 1.16, 1.38 (each 3H, s, 4×*tert.* CH_3), 1.25 (6H, s, 2×*tert.* CH_3), 4.74 (1H, d, J=6.5 Hz), ^{13}C-NMR (pyridine-d_5) δ_C: 34.3 (C-20), 70.9 (C-25), 79.8 (C-15, C-23), 82.2 (C-22), 86.4 (C-24), 88.5 (C-3), 107.4 (anomeric C), 112.6 (C-16).

Acid Hydrolysis of 22-Hydroxycimigenol Xyloside (3)——A solution of **3** (400 mg) in 5% H_2SO_4 in 50% MeOH (300 ml) was refluxed for 4 hr. The MeOH was removed *in vacuo*. The resulting solution was diluted with H_2O, and extracted with ether. The ether solution was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The crude products (253 mg) were chromatographed over silica gel (9.0 g). Elution with benzene–EtOAc (10: 11) gave 22-hydroxycimigenol (**13**) (62.1 mg), mp 274—276° (from EtOAc) or mp 226—229° (from EtOAc), $[\alpha]_D^{19}$ +45.0° (c=0.4, $CHCl_3$–MeOH (1: 1)). *Anal.* Calcd for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.12; H, 9.80. IR ν_{max}^{KBr} cm^{-1}: 3620—3130, 1060 (OH). ^{1}H-NMR (pyridine-d_5) δ_H: 0.36, 0.60 (each 1H, d, J=4.2 Hz, cyclopropane methylene), 3.6 (1H, m, 3-H), 3.64 (1H, d, J=1.2 Hz, 24-H), 3.68 (1H, d, J=12 Hz, 22-H), 3.99 (1H, d, J=1.2 Hz, 23-H), 4.40 (1H, s, 15-H).

Enzymatic Hydrolysis of 22-Hydroxycimigenol Xyloside (3)——A solution of **3** (100 mg) in a mixture of EtOH (100 ml) and 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0) (200 ml) was treated with the crude α-glucosidase (2.5 ml) in H_2O (100 ml), and the total mixture was kept for 40 hr with gentle stirring at 38°. The EtOH in the mixture was evaporated off and the solution was extracted with EtOAc. The EtOAc layer was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The residue (69.3 mg) was chromatographed over silica gel (2.5 g). Elution with benzene–EtOAc (10: 11) gave 22-hydroxycimigenol (**13**) (15.7 mg), mp 274—276° (from EtOAc) or mp 226—229° (from EtOAc), which was identical with the acid hydrolysis product described above as judged by TLC, IR and ^{1}H-NMR.

Acetylation of 22-Hydroxycimigenol (13)——22-Hydroxycimigenol (**13**) (55 mg) was acetylated overnight with Ac_2O (0.2 ml) in pyridine (0.3 ml) at room temperature. After work-up in the usual manner, the product was passed through a silica gel column (1 g) (eluent, benzene–EtOAc (8: 2)). After crystallization from EtOAc, the triacetate (**14**) was obtained as colorless needles (22 mg), mp 202—203.5°, $[\alpha]_D^{20}$ +92.7° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3540, 1065 (OH), 1730, 1220 ($OCOCH_3$). ^{1}H-NMR ($CDCl_3$) δ_H: 3.67 (1H, d, J=1.2 Hz, 24-H), 4.18 (1H, d, J=10.8 Hz, 22-H), 4.23 (1H, d, J=1.2 Hz, 23-H), 4.6 (1H, m, 3-H), 5.12 (1H, s, 15-H). High resolution MS *m/e*: Calcd for $C_{36}H_{54}O_9$ (M^+) 630.377, $C_{35}H_{51}O_9$ (M^+-CH_3) 615.353. Found: 630.380, 615.352.

Oxidation of 22-Hydroxycimigenol (13)——A solution of **13** (24 mg) in pyridine (5 mg) was treated with a solution of CrO_3 (30 mg) in pyridine (5 ml). The total mixture was allowed to stand overnight at 5°, then EtOAc (50 ml) was added. The mixture was passed through an Al_2O_3 column (20 g), and eluted with EtOAc (100 ml). The solvent was removed *in vacuo* and the residue was passed through a silica gel column (1.5 g). Crystallization of the residue obtained from the benzene–EtOAc (7: 3) eluate gave the diketone (**15**), as colorless needles (4.8 mg), mp 253—254° (from EtOH), $[\alpha]_D^{20}$ +70.2° (c=0.2, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3480, 1065 (OH), 1722, 1685 (CO). ^{1}H-NMR ($CDCl_3$) δ_H: 0.66, 0.84 (each 1H, d, J=4.5 Hz, cyclopropane methylene), 3.69 (1H, d, J=1.2 Hz, 24-H), 4.13 (1H, d, J=12.8 Hz, 15-H changed to a singlet on addition of D_2O), 4.53 (1H, d, J=1.2 Hz, 23-H). High resolution MS *m/e*: Calcd for $C_{30}H_{44}O_6$ (M^+) 500.314, $C_{30}H_{42}O_5$(M^+-H_2O) 482.303, $C_{30}H_{40}O_4$ (M^+-2H_2O) 464.293. Found: 500.317, 482.303, 464.297.

Reduction of 3,22-Diketone (15) with Hydrazine Hydrate——A solution of **15** (4.8 mg) in diethylene glycol (5 ml) was treated with KOH (0.26 mg) and 90% hydrazine hydrate (0.1 ml). The total mixture was refluxed

at 145° for 1.5 hr, then the condenser was removed to allow the aqueous liquor to evaporate off and the temperature of the reaction mixture to rise to 195°. After refluxing at 195° for 2 hr, the reaction mixture was cooled, diluted with H_2O, and extracted with Et_2O. The ether layer was washed with 0.1 N H_2SO_4 and then with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The residue (3.4 mg) was passed through a silica gel column (0.6 g). Elution with benzene–EtOAc (8: 2) gave 3-desoxycimigenol (**16**) (1.5 mg), mp 202.5—203.5° (from EtOH), $[\alpha]_D^{30}$ +40.4° (c=0.57, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3530, 1070 (OH). ^{1}H-NMR ($CDCl_3$) δ_H: 0.34, 0.59 (each 1H, d, J=3.9 Hz, cyclopropane methylene), 2.53 (1H, d, J=8.5 Hz, OH, disappeared on addition of D_2O), 3.45 (1H, d, J=1.2 Hz, 24-H), 3.91 (1H, d, J=8.5 Hz, 15-H, changed to a singlet on addition of D_2O), 4.46 (1H, d-d, J_1=9.3 Hz, J_2=1.2 Hz, 23-H). High resolution MS m/e: Calcd for $C_{30}H_{48}O_4$ (M^+) 472.355. Found: 472.358.

Reduction of Cimigen-3-one (17) with Hydrazine Hydrate——A solution of **17** (70 mg) in diethylene glycol (10 ml) was treated with KOH (0.53 g) and 90% hydrazine hydrate (0.5 ml). After treatment as described above, the crude product (39.1 mg) was passed through a silica gel column (2.0 g). Elution with benzene–EtOAc (8: 2) gave 3-desoxycimigenol (**16**) (8.8 mg), mp 202.5—203.5°. High resolution MS m/e: Calcd for $C_{30}H_{48}O_5$ (M^+) 472.355. Found: 472.352. The samples of 3-desoxycimigenol derived from cimigenol and 22-hydroxycimigenol were shown to be identical by mixed mp, IR, TLC and GLC [1.5% OV-17 on Shimalite W (80—100 mesh), 4 mm × 1.5 m, column temp. 274°, carrier gas N_2 (1 kg/cm^2), t_R(min): 6.0].

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