[Chem. Pharm. Bull. 25(12)3182—3189(1977)]

UDC 547.918.02 : 581.192

Studies on the Constituents of *Cimicifuga* spp. XIII.[1] Structure of Cimicifugoside[2]

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(Received April 4, 1977)

Cimicifugoside (I), mp 237—238°, $C_{37}H_{54}O_{11}$ was isolated along with friedelin, β-sitosterol, β-sitosteryl glucoside, 25-O-methylcimigenol, cimigenol and cimigenyl xyloside from the roots of *Cimicifuga simplex*. The structure (I) was proposed for cimicifugoside on the basis of chemical and physicochemical data. The major topic is chemistry of a hydrolysis product, cimicifugenin A (IV) and its derivatives.

Keywords——*Cimicifuga simplex*; triterpenoid xyloside; triterpenoids; ^{1}H- and ^{13}C-NMR spectra; cleavage of cyclopropane ring

In the course of our continuing studies on the constituentes of *Cimicifuga* species, a new glycoside, named cimicifugoside (I), was obtained along with friedelin (XVI), β-sitosterol (XVII), stigmasterol (XVIII), campesterol (XIX), cimigenol (XX), 25-O-methylcimigenol (XXI), cimigenyl xyloside (XXII) and β-sitosteryl glucoside (XXIII) from the rhizoma of *Cimicifuga simplex*. Cimicifugenol,[4] cimigol,[5] dahurinol, dehydroxydahurinol, isodahurinol,[6] acerinol, acerionol, 24-O-acetylacerionol,[1] kehllol, ammiol, caffeic acid dimethyl ether and cimifugin,[7] have been isolated from the same plant.

Cimicifugoside (I), mp 237—238°, $C_{37}H_{54}O_{11}$ was obtained as colorless prisms from the methanolic extract of the rhizoma of *Cimicifuga simplex* WORMSK. The infrared (IR) spectrum shows hydroxyl bands at 3500 and 1000 cm^{-1} and acetyl bands at 1720 and 1260 cm^{-1}. After hydrolysis of cimicifugoside with 1% sulfuric acid, xylose was confirmed by paper chromatography (PPC) and thin-layer chromatography (TLC) of the sugar fraction and by gas chromatography (GC) of the trimethylsilyl ether.

The nuclear magnetic resonance (NMR) spectrum (in pyridine-d_5) of cimicifugoside (I) suggests the presence of a cyclopropane ring (1H, d, $J=4$ Hz at 0.57 ppm) and an acetoxyl group (3H, s at 2.12 ppm and 1H, t, $J=6$ Hz at 5.06 ppm). Since a tetraacetate (III), mp 166—168°, $C_{45}H_{62}O_{15}$, was produced on acetylation of cimicifugoside (I), it was suggested that cimicifugoside had at least four hydroxy groups in the molecule. Three of them are ones of xylose and the fourth is thought to be in the aglycone moiety. A singlet (1H) at 5.63 ppm in the NMR spectrum of I displayed a shift to 6.39 ppm in that of the acetate (III). From the chemical shift of these singlets in the NMR spectra of I and III, and from the data mentioned later, the fourth hydroxy group is assigned to that of a hemiketal group.

Although cimicifugoside (I) is sensitive to mineral acids to produce many kinds of products which are hard to separate, it was hydrolysed with 50% acetic acid to provide an

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[1] Part XII: G. Kusano, H. Uchida (née Hanasaka), Y. Murakami, N. Sakurai (née Yamamoto) and T. Takemoto, *Yakugaku Zasshi*, **96**, 321 (1976).

[2] This work was presented partially at the 11th symposium on the chemistry of natural products. Symposium papers, p. 338, 1967, Kyoto.

[3] Location: *a*) *Aobayama, Sendai*; *b*) *Yamashirocho, Tokushima.*

[4] T. Takemoto, G. Kusano, and N. Yamamoto, *Yakugaku Zasshi*, **90**, 68 (1970).

[5] G. Kusano and T. Takemoto, *Yakugaku Zasshi*, **95**, 1133 (1975).

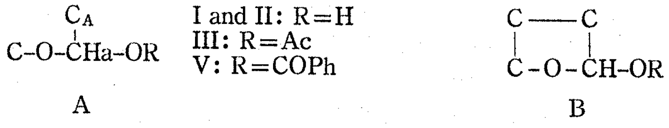
[6] G. Kusano, Y. Murakami, N. Sakurai, and T. Takemoto, *Yakugaku Zasshi*, **96**, 82 (1976).

[7] Y. Kondo and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 1940 (1972).

aglycone, cimicifugenin A (IV), mp 211—212°, $C_{32}H_{46}O_7$. The IR spectrum shows hydroxyl bands at 3585 and 2445 cm^{-1}, and an acetyl band at 1725 cm^{-1}. The NMR spectrum of IV showed singlet (3H) at 2.02 ppm and a triplet (1H) at 5.03 ppm, due to an acetate Me group and C$\underline{H}$OAc, as well as that of I, but no signals of a cyclopropane ring.

Cimicifugenin A (IV) gave a monobenzoate (V), mp 262—263°, $C_{39}H_{50}O_8$, the IR spectrum of which has no hydroxyl bands. A singlet at 5.06 ppm in the NMR spectrum of IV displayed a shift to 6.37 ppm in that of benzoate (V). The similar shift was observed in the NMR spectra of cimifugoside (I) and its acetate (III) as mentioned above. These results suggest a partial structure (A) for I, III, IV and V.

On oxidation with chromium trioxide in pyridine, cimicifugenin A (IV) afforded a lactone (VI), mp 243—244°, $C_{32}H_{44}O_7$. The IR spectrum shows a γ-lactone band at 1782 cm^{-1}, but the NMR spectrum has no signal of the lactonic hydrogen. Therefore, the partial structure (A) is developed to the structure (B). The mass spectrum of the lactone (VI) showed the molecular peak at *m/e* 540 and the base peak at *m/e* 480 (M^+—AcOH) along with the peaks at *m/e* 524 (M^+—15), 465 (M^+—AcOH—15), 462 (M^+—AcOH—18) and 447 (M^+—AcOH—15—18), indicating $C_{32}H_{44}O_7$ for the molecular formula of the compound (VI).



$C\text{-}O\text{-}\overset{C_A}{\overset{|}{C}}H_a\text{-}OR$

I and II: R=H
III: R=Ac
V: R=COPh

$\begin{array}{l} C\text{——}C \\ |\qquad\ | \\ C\text{-}O\text{-}CH\text{-}OR \end{array}$

A B

Chart 1

On treatment of cimicifugoside (I) with 50% ethanolic acetic acid or on treatment of cimicifugenin A (IV) with 1% ethanolic hydrochloric acid, an ethyl ether (VII), mp 215—216°, $C_{34}H_{50}O_7$ was obtained. VII gave a desacetyl derivative (VIII), mp 201—202°, $C_{32}H_{48}O_6$ on treatment with 1% methanolic sodium hydroxide. Under treatment of VII with *m*-chloroperbenzoic acid, an epoxide (IX), mp 197—199°, $C_{34}H_{50}O_8$ was produced. IX provided a diene (X), mp 244—245°, $C_{34}H_{48}O_7$ under reflux with 0.1% ethanolic hydrochloric acid. These results and the absence of the signals of vinyl hydrogens in the NMR spectra of the compounds (IV—VIII) suggest that these compounds contain a tetrasubstituted double bond in the molecules.

After all, cimicifugenin A (IV) is thought a tetracyclic triterpene, which has four intramolecular ether linkages, one acetoxy group, one hydroxy group and a tetrasubstituted double bond. One ether linkage group and one hydroxy group among these functional groups are pariticipated in the partial structure (B).

The characteristic singlets corresponding to one methyl group in the NMR spectra of I, IV and VI [1.72 ppm in I (pyridine-d_5), 1.60 ppm in IV ($CDCl_3$), 1.62 ppm in VI ($CDCl_3$)] are assigned to a tertiary methyl group on an epoxide ring.

The properties attributed to the partial structure (B) and the NMR signals of the tertiary methyl group on the epoxide ring are similar to those of acetylacteol (XIV), which is a

Table I. Comparison of NMR Spectra ($CDCl_3$) of Cimicifugenin A (IV) and Acetylacteol (XIV)

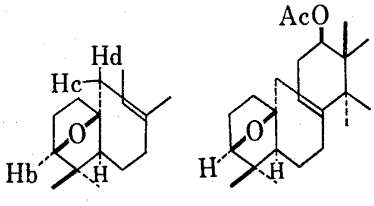
	IV	XIV
C_{12}-H	5.06(t.)	4.88(q.)
C_{16}-H	4.40(m.)	4.40(m.)
C_{24}-H	3.43(s.)	3.45(s.)
C_{25}-CH_3	1.60(s.)	1.63(s.)
C_{26}-H	5.06(s.)	5.08(s.)

genin of actein (XIII), obtained from *Cimicifuga racemosa*.[8] The signals assigned to the side chain moiety (C_{16}-H, C_{24}-H, C_{25}-CH_3 and C_{26}-H) of acetylacteol (XIV) are recognized in the NMR spectrum of cimicifugenin A (IV) (Table I).

On treatment of cimicifugenin A (IV) with sodium borohydride in methanol, a deoxy derivative (XI), mp 105—106° (dec.), $C_{32}H_{46}O_6$, was prepared. A similar result of the reaction was reported in the work of the structurel elucidation, in which acetyl acteol (XIV) provided the deoxy derivative (XV).[8]

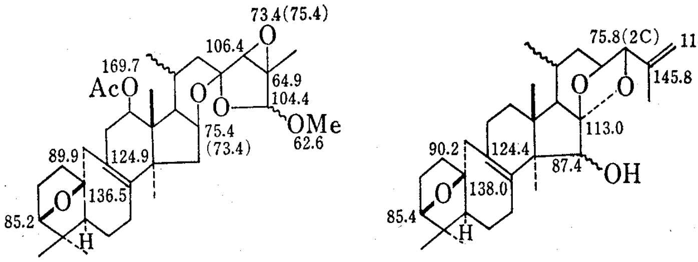
On the other hand, a series of signals (1H, each) at 3.70, 3.08 and 1.62 ppm in the NMR spectrum of cimicifugenin A (IV), are not detected in that of cimicifugoside (I). Therefore, the partial structure correspoding to these signals is thought to be created in the course of the change from I to IV and to have some relation to the cyclopropane ring and the xyloside linkage moiety of cimicifugoside (I). The series of the similar signals have been found in the NMR spectra of acerinol (XVI) and its related compounds,[1] and they have been assigned to Hb, Hc and Hd of the partial structure (C).

In the NMR spectrum of the diene (X), the signals of three hydrogens are found in the field lower than 5 ppm, and two of them (1H, t, $J=7$ Hz at 5.64 ppm and 1H, s at 5.30 ppm) are assigned to the hydrogens on a conjugated diene system and the rest (1H, s, 5.22 ppm) is assigned to $\mathrm{C\underline{H}OAc}$, while the corresponding hydrogen gives a triplet at 5.05 ppm on the NMR spectrum of IV. The UV spectrum of the diene derivative (X) showed the maxima at 242, 249 and 258 nm and it is more similar to that of $\Delta^{7,9(11)}$-lanostadiene (237, 244, 251 nm)[9] rather than to that of $\Delta^{7,9(11)}$-euphdienol (233, 240, 247.5 nm).[10] Moreover, the M_D increment for conversion of 26-O-ethylcimicifugenin A (VII) to the diene (X) is +98.6°, as it is about +100° in lanosterol series, while in the euphenol series the shift is about −600°.[11] Therefore, the partial structure (C) is developed to (D) in cimicifugenin A (IV). Inspection of the molecular formula in the light of the partial structures (B) and (D), the structure of acetylacteol, and general biosyntheses of tetracyclic triterpenes indicated the structure (IV) for cimicifugenin A.



C D

Chart 2



26-O-methylcimicifugenin A acerinol dehydrate

Chart 3. ^{13}C–NMR Data (Benzene-d_6)

The ^{13}C–NMR spectrum of 26-O-methylcimicifugenin A (XII), mp 214—215°, $C_{33}H_{48}O_7$, which was prepared on treatment of cimicifugoside (I) with methanolic hydrochloric acid, was analysed reasonably by comparison with ^{13}C–NMR spectra of acerinol and its related compounds (Chart 3).[1]

When cimicifugoside was hydrolysed with cellulase, a genin (II), mp 251—253°, $C_{32}H_{48}O_7$, was obtained as colorless needles. The NMR spectrum of the genin has a doublet (1H, $J=4$ Hz at 0.58 ppm) assigned to a hydrogen of methylene of 1,1,2,2-tetrasubstituted cyclopropane ring and the signals assigned to C_{12}-H (1H, t, $J_1=J_2=10$ Hz at 5.00 ppm), C_{16}-H (1H, octet,

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8) S. Corsano, H. Linde, G. Piancatelli, and L. Parizzi, *Chimia*, **21**, 130(1967).
9) C. Dorée, J.F. McGhie, and F. Kurzer, *J. Chem. Soc.*, **1947**, 1467.
10) J.B. Barbour, R.N.E. Bennet, and F.L. Warren, *J. Chem. Soc.*, **1951**, 2540.
11) L.F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corporation, New York, 1959, p. 390.

$J_1=6$, $J_2=13$, $J_3=2$ Hz at 4.49 ppm), C_{24}-H (1H, s at 3.45 ppm), C_{25}-CH_3 (3H, s at 1.60 ppm) and C_{26}-H (1H, s at 5.11 ppm). A broad singlet (1H) at 5.16 ppm is assigned to a hydrogen on a trisubstituted double bond.

On the basis of these properties, especially those of cimicifugenin A (IV) and the genuine genin (II), the structure (I) was proposed for cimicifugoside. The mechanism of the formation of cimicifugenin A from cimicifugoside is supposed as shown in Chart 4. The structure (I) is closely related to that of actein (XIII) and cimicifugoside is supposed as a Δ^7-derivative of actein (XIII). Since cimicifugenol (Δ^7-cycloartenol) was obtained from *C. simplex*,[4] it is interesting to suspect that actein (XIII) is biosynthesized from cycloartenol in *Cimicifuga racemosa*, while cimicifugoside (I) is biosynthesized from cimicifugenol in *C. simplex*. Further studies for structural elucidation of other reaction products of cimicifugoside (I) are going on and it is expected that the data would provide the strict structure including the whole stereochemistry of cimicifugoside (I).

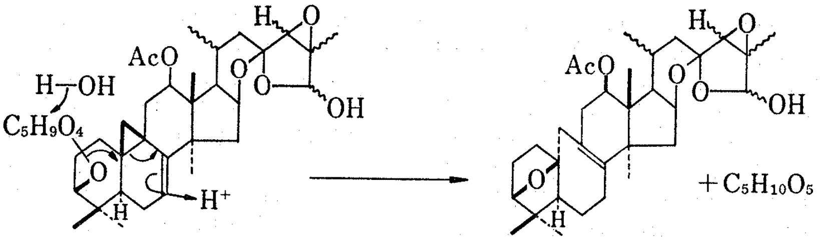
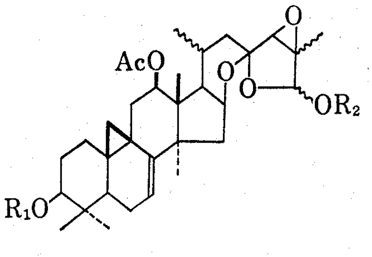
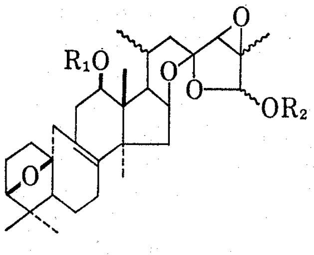


Chart 4

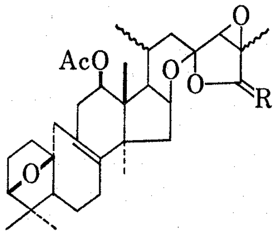
In the silica gel chromatography, where cimicifugoside (I) was isolated, friedelin (XVI), mp 250—251°, β-sitosterol (XVII), stigmasterol (XVIII), campesterol (XIX) and cimigenol (XX),[12] mp 224—226°, were obtained.



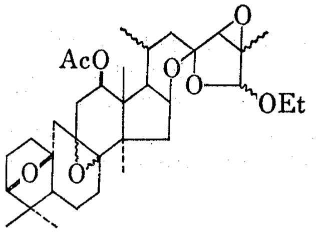
I : R_1=xylosyl; R_2=H
II : $R_1=R_2$=H
III : R_1=tri-O-acetyl-xylosyl; R_2=Ac



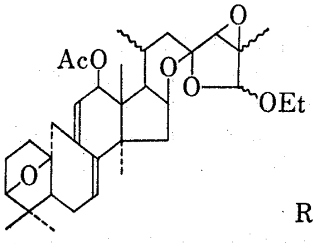
IV : R_1=Ac; R_2=H
V : R_1=Ac; R_2=COPh
VII : R_1=Ac; R_2=Et
VIII : R_1=H; R_2=Et
XII : R_1=Ac; R_2=Me



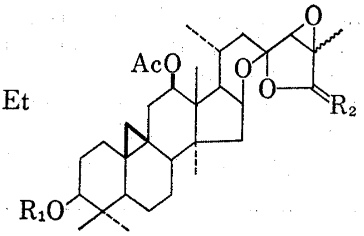
VI : R=O
XI : $R=H_2$



IX



X



XIII : R_1=xylosyl; R_2=OH, H
XIV : R_1=H; R_2=OH, H
XV : R_1=H; $R_2=H_2$

Ph=phenyl

Chart 5

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[12] T. Takemoto and G. Kusano, *Yakugaku Zasshi*, **87**, 1569 (1967).

The residue after the removal of cimicifugoside and the eluates with ethyl acetate–methanol (10: 1—2) in the silica gel chromatography were treated with methanolic hydrochloric acid. The ethyl acetate extract of the hydrolysis products was chromatographed on silica gel, where cimigenol (XX), 25-O-methylcimigenol (XXI),[13] mp 235—236°, cimigenyl xyloside (XXII),[14] mp 271—273°, and β-sitosteryl glucoside (XXIII), mp 291—295°, were obtained along with two new triterpenes, XXIV, mp 249—250°, $C_{31}H_{46}O_5$ and XXV, mp 269—270°, $C_{30}H_{46}O_4$. The work for the structural elucidation of the compounds XXIV and XXV is going on.

Experimental[15]

Isolation Procedure——The dried rhizoma (6.8 kg) of *Cimicifuga simplex*, which were collected in the mountainous area on the outskirts of Sendai city, were extracted with hot MeOH (50 l) for 6—7 hr. This extraction was undertaken 5 times and the solution was concentrated *in vacuo* to the volume of 1.5 l. The concentrate was extracted with AcOEt (1.5 l, each time) 3 times and the AcOEt fraction was concentrated to afford the residue (630 g). The last extract was dissolved to MeOH (2 l) and silica gel (2 kg) was added to the MeOH solution. The solvent was evaporated under reduced pressure. The dried mixture of silica gel and the AcOEt extract was put on the top of a silica gel column (3.2 kg) and eluted in turn with benzene, benzene–AcOEt mixture, AcOEt, AcOEt–MeOH mixture. The first of the eluates with benzene was refluxed in 2% methanolic NaOH (100 ml) for 2 hr, acidified with dil. HCl and extracted with AcOEt. After usual treatment, the residue (4.1 g) was chromatographed on Al_2O_3 (40 g). A benzene eluate gave colorless needles (XVI, 37 mg) after recrystallization from AcOEt, mp 250—251°, IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1710 (C=O). By comparison of mp and IR spectrum of an authentic specimen, XVI was identified to friedelin.

The second (8.2 g) of the eluates with benzene in the silica gel chromatography provided colorless plates (0.75 g) after recrystallization from AcOEt. TLC: *Rf* 0.29 [solvent: benzene–AcOEt (9: 1)]. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3440, 1645. MS *m/e*: 414, 412, 400, 399, 397, 396, 385, 382. A gas chromatography (GC) of the trimethylsilyl (TMS) ethers [column: 3% OV-101 on chromosorb W-HP (80—100 mesh), 1 m, column temp. 230°, N_2 (carrier) 1 kg/cm^2] showed Rt 7.2 min [campesterol (XIX), 5%], 8.2 [stigmasterol (XVIII), 5—7%], 9.7 [β-sitosterol (XVII), 88—90%].

After the elution of phytosterol mixture, the silica gel column was eluted with benzene–AcOEt (5: 1) and colorless needles (XX: 0.7 g), mp 224—226°, TLC: *Rf* 0.35 [benzene–AcOEt (4: 1)], IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3430, 1150, were obtained. XX was identified to cimigenol by comparison of mp, TLC and IR spectrum with an authentic specimen. XX (0.5 g) was acetylated with Ac_2O in pyridine at room temperature. After usual treatment, the products were chromatographed on SiO_2 (10 g), where an eluate with benzene–AcOEt (10: 1) afforded colorless needles (380 mg) after recrystallization from EtOH, mp 201—202°, NMR ($CDCl_3$) δ: 0.39 ppm (1H, d, $J=4$ Hz, H on a cyclopropane ring), 0.64 (1H, d, $J=4$ Hz, H on a cyclopropane ring), 0.83 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.89 (3H, d, $J=6$ Hz, CH-$C\underline{H}_3$), 1.07 (3H, s, CH_3), 1.11 (6H, s, $2\times CH_3$), 1.16 (3H, s, CH_3), 2.03 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 3.37 (1H, s, C_{24}–H), 4.36 (1H, unresolved d, $J=9$ Hz, C_{23}–H), 4.55 (1H, m, C_3–H), 5.17 (1H, s, C_{15}–H). After comparison of the NMR spectrum with that of an authentic specimen, this was identified as 3,15-O-diacetylcimigenol.

In the first silica gel chromatography, an eluate AcOEt provided colorless prisms (I, 2.2 g) after recrystallization from EtOH, mp 237—238°, *Anal.* Calcd. for $C_{37}H_{54}O_{11}\cdot C_2H_5OH$: C, 64.98; H, 8.39. Found: C, 64.97; H, 7.97. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3500, 1000 (OH), 1720, 1260 (OAc). NMR (C_5D_5N) δ: 0.57 ppm (1H, d, $J=4$ Hz, H on a cyclopropane ring), 1.72 (3H, s, CH_3 on an epoxide ring), 2.20 (3H, s, $COCH_3$), 3.83 (1H, s, H on an epoxide ring), 4.69 (1H, d, $J=6$ Hz, anomeric H), 5.06 (1H, t, $J=6$ Hz, $C\underline{H}OAc$), 5.35 (1H, bs, C=CH–), 5.59 (1H, s, C–$C\underline{H}$(OR)OH).

The residue (80 g) after the removal of cimicifugoside (I) showed several spots on TLC, and a portion (50 g) was refluxed in 10% H_2SO_4 (150 ml)–MeOH (350 ml) for 4.5 hr. The reaction solution was concentrated to the half volume and water was added to the concentrate. This mixture was extracted with AcOEt, and after usual treatment, the residue (40 g) was chromatographed on SiO_2 (440 g). An eluate with benzene–AcOEt (10: 1) gave colorless needles (XXII, 519 mg) after recrystallization from AcOEt, mp 235—236°,

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[13] T. Takemoto and G. Kusano, *Yakugaku Zasshi*, **88**, 623 (1968).

[14] T. Takemoto, G. Kusano, and M. Kawahara, *Yakugaku Zasshi*, **90**, 64 (1970).

[15] Melting points were determined on a Yanagimoto hot stage and uncorrected. Microanalyses were performed by the Analysis center of this institute. IR spectra were obtained with Shimazu IR-27G photometer. ^{1}H-NMR spectra were determined on Hitachi Perkin-Elmer R-20 and JEOL PS-100 spectrometers using tetramethylsilane as internal standard. ^{13}C-NMR spectra were recorded on a JEOL-100 NMR spectrometer, equipped with a PFT-100 pulsed-Fourier *trans*-form spectrometer and ^{13}C-accessories, in combination with JEC-60 spectrum computer.

IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3500, 3310 (OH). NMR ($CDCl_3$) δ: 0.36 ppm (1H, d, $J=4$ Hz, H on a cyclopropane ring), 0.64 (1H, d, $J=4$ Hz, H on a cyclopropane ring), 3.21 (3H, s, OCH_3), 3.46 (1H, s, C_{24}–H), 3.87 (1H, s, C_{15}–H), 4.43 (1H, bd, $J=9$ Hz, C_{23}–H). By comparison of mp, IR- and NMR-spectrum with those of an authentic specimen, XXI was identified as 25-O-methylcimigenol. An eluate with benzene–AcOEt (10:3) provided cimigenol (XX, 3.9 g) as colorless needles after recrystallization from AcOEt. An eluate with AcOEt–MeOH (10:1) afforded colorless powder (1.5 g), which showed three spots on TLC [$CHCl_3$–MeOH (10:1)]. After acetylation of this mixture, two kinds of the products were isolated by preparative TLC.

Cimigenyl Xyloside Tetraacetate: Colorless powder by recrystallization from petroleum benzine, mp 137—140°. NMR ($CDCl_3$) δ: 0.35 ppm (1H, d, $J=4$ Hz, H on a cyclopropane ring), 0.60 (1H, d, $J=4$ Hz, H on a cyclopropane ring), 0.74 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.88 (3H, d, $J=6$ Hz, CH–C$\underline{H}_3$), 1.05 (3H, s, CH_3), 1.10 (6H, s, $2\times CH_3$), 1.14 (3H, s, CH_3), 2.02 (9H, s, $3\times OCOCH_3$), 2.06 (3H, s, $OCOCH_3$), 3.1 (1H, m, C_3–H), 3.23 (1H, q, $J_1=12$, $J_2=2$ Hz, $C_{5'}$–H), 4.08 (1H, q, $J_1=12$, $J_2=5$ Hz, $C_{5'}$–H), 4.34 (1H, bd, $J=9$ Hz, C_{23}–H), 4.46 (1H, d, $J=7$ Hz, anomeric H), 4.9 (1H, m, $C_{4'}$–H), 4.91 (1H, t, $J_1=J_2=7$ Hz, $C_{2'}$–H), 5.07 (1H, t, $J_1=J_2=7$ Hz, $C_{3'}$–H), 5.15 (1H, s, C_{15}–H). The NMR spectrum was identical with that of an authentic specimen of cimigenyl xyloside tetraacetate, which was prepared from an authentic specimen of cimigenyl xyloside obtained from *C. acerina* and identified on the basis of the results of chemical reactions and spectroscopic data.14) After this acetate (30 mg) was refluxed in 1% methanolic NaOH for 1 hr, the solution was acidified with dil. HCl. The precipitate was filtered and recrystallization from MeOH to provide colorless needles (XXII, 17 mg), mp 271—273°. XXII (5 mg) was refluxed in 3% HCl ($MeOH$–$H_2O=1:1$) for 1 hr. After removal of cimigenol, the aqueous solution was passed through a column of Amberlite IR-4B. The passed fraction was concentrated to examine on TLC [BuOH: AcOH: H_2O (4:1:1)], *Rf* 0.45 (an authentic specimen of D-xylose, *Rf* 0.44). GC of TMS-derivative gave two peaks of t_R 4.6 and 5.7 min (column: 1.5% OV-1 on chromosorb, column temp, 180 °C, N_2 (carrier), 1.0 kg/cm^2). From these data, the glycoside (XXII) was identified to cimigenyl xyloside.

β-Sitosteryl Glucoside Acetate: After the acetate (50 mg) was refluxed in 1% methanolic NaOH for 1 hr, the solution was acidified with dil. HCl to provide precipitates. The dried preparation was recrystallized from MeOH to provide colorless powder (XXIII, 37 mg), mp 291—295°. *Rf* 0.41 [$CHCl_3$–MeOH (10:1), an authentic specimen: *Rf* 0.41]. XXIII was refluxed in 3% HCl (MeOH: $H_2O=1:1$) for 2 hr and then MeOH was evaporated. The concentrate was passed to a column of Amberlite IR-4B and the passed fraction was condensed to examine on TLC: *Rf* 0.40 [D-glucose: 0.40 (BuOH: AcOH: $H_2O=4:1:1$)]. The IR spectrum of XXIII was identical with that of an authentic specimen of β-sitosteryl glucoside.

In the first silica gel chromatography, eluates with AcOEt–MeOH (10:1—2) (100 g) were refluxed in 3% methanolic H_2SO_4 for 4 hr. MeOH was evaporated and the residue was extracted with AcOEt. The AcOEt soluble fraction (65 g) was chromatographed on SiO_2 (700 g). Eluates with benzene–AcOEt (10:1) gave colorless needles (XXIV, 70 mg) after recrystallization from AcOEt, mp 249—250°. The mass spectrum showed the parent peak at *m/e* 498, that suggested the molecular formula, $C_{31}H_{46}O_5$. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3520 (OH), 1620, 845 (C=CH–), 1100, 958. NMR ($CDCl_3$) δ: 0.05 ppm (1H, q, $J_1=10$, $J_2=5$ Hz, H on a cyclopropane ring), 0.72 (6H, s, $2\times CH_3$), 0.83 (3H, s, CH_3), 0.97 (3H, d, $J=6$ Hz, CH–C$\underline{H}_3$), 1.00 (3H, s, CH_3), 1.60 (3H, s, CH_3 on an epoxide ring), 3.33 (1H, q, $J_1=12$, $J_2=6$ Hz, C$\underline{H}$OH), 3.40 (3H, s, OCH_3), 3.42 (1H, s, H on an epoxide ring), 4.35 (1H, m, H on an epoxide ring), 4.75 (1H, s, OC$\underline{H}$OCH_3), 5.02 (1H, bd $J=5$ Hz, C=CH–).

Eluates with benzene–AcOEt(5:1) provided colorless needles (XXV, 400 mg) after recrystallization from AcOEt, mp 268—269°. *Anal.* Calcd. for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85. Found: C, 76.20; H, 9.82. NMR ($CDCl_3+C_5D_5N$) δ: 0.00 ppm (1H, q, $J_1=10$, $J_2=5$ Hz, H on an cyclopropane ring), 0.64 (3H, s, CH_3), 0.75 (3H, s, CH_3), 0.85 (3H, d, CH–C$\underline{H}_3$), 1.04 (6H, s, $2\times CH_3$), 1.20 (3H, s, CH_3), 1.22 (3H, s, CH_3), 3.30 (1H, q, $J_1=12$, $J_2=6$ Hz, C$\underline{H}$OH), 3.32 (1H, s, C$\underline{H}$OR), 4.43 (1H, bd, $J=9$ Hz, C$\underline{H}$OR), 5.05 (1H, bd, $J=5$ Hz, C=CH–). XXVI (0.3 g) was acetylated with Ac_2O in pyridine at room temperature. After usual treatment, the acetate (270 mg) was obtained as colorless needles, mp 203—204°. *Anal.* Calcd. for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C, 74.86; H, 9.60. MS *m/e*: 512 (M^+). NMR ($CDCl_3$) δ: 0.01 ppm (1H, q, $J_1=9$, $J_2=4$ Hz, H on a cyclopropane ring), 0.51 (3H, s, CH_3), 0.64 (3H, s, CH_3), 0.75 (3H, s, CH_3), 0.85 (3H, d, $J=6$ Hz, CH–C$\underline{H}_3$), 1.03 (9H, s, $3\times CH_3$), 1.91 (3H, s, $OCOCH_3$), 3.24 (1H, s, CHOR), 4.29 (1H, bd, $J=9$ Hz, CHOR), 4.46 (1H, q, $J_1=12$, $J_2=7$ Hz, CHOAc), 4.98 (1H, bd, $J=5$ Hz, C=CH–).

Hydrolysis of Cimicifugoside (I) with aq. AcOH——I (2 g) was refluxed in AcOH–H_2O (1:1) for 20 hr and the products were extracted with AcOEt to chromatograph on SiO_2 (50 g). Eluates with benzene–AcOEt (10:1) gave colorless needles (IV, 360 mg), mp 211—212°, *Anal.* Calcd. for $C_{32}H_{46}O_7$: C, 70.82, H, 8.54. Found: C, 70.63 H, 8.61. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3560 (OH), 1740, 1228 (Ac). NMR ($CDCl_3$) δ: 1.60 ppm (3H, s, CH_3 on an epoxide ring), 2.02 (3H, s, $OCOCH_3$), 1.62 (1H, d, $J=14$ Hz, =C–C$\underline{H}$H–C–), 3.08 (1H, d, $J=14$ Hz, =C–CH$\underline{H}$–C–), 3.43 (1H, s, C(OR)H), 3.70 (1H, bd, $J=6$ Hz, C(OR)H), 4.40 (1H, m, C(OR)H), 5.06 (1H, t, $J_1=J_2=7.5$ Hz, CHOAc), 5.06 (1H, s, O–CH–OR).

Benzoate (V) of Cimicifugenin A (IV)——IV (49 mg) was dissolved to pyridine (2 ml) and 5 drops of benzoyl chloride were added to the solution. After leaving at room temperature over night, water was added and the mixture was extracted with AcOEt. The AcOEt extract was chromatographed on Al_2O_3 and the eluate with benzene–AcOEt (5:1) was recrystallized from AcOEt.

Benzoate (V, 52 mg): Colorless needles, mp 262—263°. *Anal.* Calcd. for $C_{39}H_{50}O_8$: C, 72.42; H, 7.79. Found: C, 72.16; H, 7.80. IR ν_{max}^{KBr} cm^{-1}: 1725, 1270, 1250 ($OCOCH_3$, OCOPh), 3050, 1600, 715 (phenyl). NMR ($CDCl_3$) δ: 1.65 (3H, s, CH_3 on an epoxide ring), 2.07 (3H, s, $OCOCH_3$), 3.14 (1H, d, $J=14$ Hz, =C–C$\underline{H}$H–C–), 3.57 (1H, s, C(OR)H), 3.60 (1H, bd, $J=6$ Hz, CHOR), 4.35 (1H, m, CHOR), 5.05 (1H, t, $J_1=J_2=7$ Hz, CH–OAc), 6.37 (1H, s, OCHOCOPh).

Oxidation of Cimicifugenin A——IV (100 mg) was dissolved to pyridine (2 ml) and pyridine–CrO_3 solution was added dropwise with stirring at room temperature for 5 hr. AcOEt was added and the solution was passed through Al_2O_3. The passed fraction was recrystallized from AcOEt, to provide colorless needles (VI, 77 mg), mp 243—244°. *Anal.* Calcd. for $C_{32}H_{44}O_7$: C, 71.08; H, 8.20. Found: C, 70.91; H, 8.20. IR ν_{max}^{KBr} cm^{-1}: 1788 (γ-lactone), 1735, 1240 (OAc). NMR ($CDCl_3$) δ: 1.62 (3H, s, CH_3 on an epoxide ring), 2.05 (3H, s, $OCOCH_3$), 3.07 (1H, d, $J=14$ Hz, =C–C$\underline{H}$H–C–), 3.70 (1H, bd, $J=6$ Hz, C(OR)H), 3.79 (1H, s, C(OR)H), 4.44 (1H, m, C(OR)H), 5.05 (1H, t, $J=8$ Hz, CH–OAc). MS *m/e*: 540 (M^+), 525 (M^+-CH_3), 480 (M^+-AcOH), 465 (M^+-AcOH–CH_3), 462 (M^+-AcOH–H_2O), 447 (M^+-AcOH–CH_3–H_2O).

Cimicifugenin A Ethyl Ether (VII)——Cimicifugoside (I, 2 g) was refluxed in 50 ml of AcOH–EtOH (1:1) for 2 days. The solution was concentrated and water was added to the concentrate. The precipitates were filtered, dried and chromatographed on SiO_2 (50 g). Eluates with benzene–AcOEt (10:1) provided colorless needles (VII), mp 215—216°, after recrystallization from AcOEt. *Anal.* Calcd. for $C_{34}H_{50}O_7$: C, 71.55; H, 8.80. Found: C, 71.59; H, 9.00. $[\alpha]_D$ $-53.3°$ ($CHCl_3$, $c=1.5$), $[M]_D$ $-303.8°$. IR ν_{max}^{KBr} cm^{-1}: 1725, 1250 (OAc). NMR ($CDCl_3$) δ: 1.64 (3H, s, CH_3 on an epoxide ring), 2.10 (3H, s, $OCOCH_3$), 3.16 (1H, d, $J=14$ Hz, =C–C$\underline{H}$H–C–), 3.52 (1H, s, C(OR)H), 3.73 (2H, q, $J=6$ Hz, OC$\underline{H}_2$CH$_3$), 3.77 (1H, d, $J=6$ Hz, C(OR)H), 4.43 (1H, m, C(OR)H), 4.92 (1H, s, O–CH–OR), 5.21 (1H, q, $J_1=J_2=6$ Hz, CH–OAc). Cimicifugenin A (IV, 200 mg) was dissolved to 1% EtOH–HCl (10 ml) to leave at room temperature for 20 hr. Water was added and the precipitates were filtered. The major product was purified by silica gel chromatography and identified as cimicifugenin A ethyl ether (VII).

Hydrolysis of VII——Cimicifugenin A ethyl ether (VII, 300 mg) was refluxed in 1% methanolic NaOH (10 ml) for 3 hr. Water was added and the mixture was extracted with AcOEt. The residue after removal of the solvent was recrystallized from AcOEt to give colorless needles (VIII, 254 mg), mp 201—202°. *Anal.* Calcd. for $C_{32}H_{48}O_6$: C, 72.69; H, 9.15. Found: C, 72.23; H, 8.95. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH). NMR ($CDCl_3$) δ: 0.87 ppm (6H, s, $2\times CH_3$), 0.92 (3H, s, CH_3), 0.98 (3H, s, CH_3), 1.20 (3H, t, $J=7$ Hz, OCH$_2$C$\underline{H}_3$), 1.55 (3H, s, C(OR)CH_3), 3.11 (1H, d, $J=14$ Hz, =C–C$\underline{H}$H–C–), 3.40 (1H, s, C(OR)H), 3.69 (1H, d, $J=6$ Hz, C(OR)-H), 3.74 (2H, q, $J=7$ Hz, OC$\underline{H}_2$CH$_3$), 4.35 (1H, m, C(OR)H), 4.83 (1H, s, O–CHOR).

Epoxidation of Cimicifugenin A Ethyl Ether (VII)——VII (173 mg) was dissolved to $CHCl_3$ (10 ml) and *m*-chloroperbenzoic acid (115 mg) was added with ice-cooling at room temperature. $CHCl_3$ (20 ml) was added and the solution was passed to Al_2O_3 (10 mg). The passed fraction was concentrated and the residue was recrystallized from MeOH to yield colorless needles (IX, 170 mg), mp 197—199°. *Anal.* Calcd. for $C_{34}H_{50}O_8$: C, 69.59; H, 8.59. Found: C, 69.36; H, 8.56.

Preparation of Diene (X)——The epoxide (IX, 100 mg) was refluxed in 0.1% ethanolic HCl for 5 hr. Water was added and the mixture was extracted with AcOEt. The soluble fraction was chromatographed on SiO_2 (10 g), where benzene–AcOEt (20:1) eluted the major product (X, 70 mg) as colorless needles after recrystallization from AcOEt, mp 207—209°. *Anal.* Calcd. for $C_{34}H_{48}O_7$: C, 71.80; H, 8.51. Found: C, 71.84; H, 8.31. $[\alpha]_D$ $-36°$ ($CHCl_3$, $c=1.3$), $[M]_D$ $-205.2°$. UV λ_{max}^{MeOH} (log ε): 242 nm (4.32), 249 (4.36), 258 (4.16). NMR ($CDCl_3$) δ: 0.87 (3H, s, CH_3), 1.03 (6H, s, $2\times CH_3$), 0.97 (3H, s, CH_3), 1.26 (3H, t, $J=7$ Hz, OCH$_2$C$\underline{H}_3$), 1.61 (3H, s, CH_3 on an epoxide ring), 2.14 (3H, s, $COCH_3$), 2.55 (1H, d, $J=14$ Hz, =C–C$\underline{H}$H–C–), 3.22 (1H, d, $J=14$ Hz, =C–CH$\underline{H}$–C–), 3.48 (1H, s, C(OR)H), 3.71 (2H, q, $J=7$ Hz, OC$\underline{H}_2$CH$_3$), 3.80 (1H, d, $J=4$ Hz, C(OR)H), 4.54 (1H, m, C(OR)H), 4.91 (1H, s, O–CH–OR), 5.21 (1H, bs, CHOAc), 5.30 (1H, bs, C=CH–), 5.64 (1H, t, $J=6$ Hz, C=C$\underline{H}$–CH$_2$).

Reduction of Cimicifugenin A (IV) with $NaBH_4$——IV (120 mg) was dissolved to MeOH (50 ml) and $NaBH_4$ (30 mg) was added to the solution with stirring at room temperature for 30 min. AcOH (0.1 ml) was added and the solution was concentrated *in vacuo*. The concentrate was extracted with AcOEt and the extract was chromatographed on SiO_2 (5 g). An eluate with benzene–AcOEt (9:1) was recrystallized from CCl_4 to provide colorless needles (XI, 71 mg), mp 105—106° (dec.). *Anal.* Calcd. for $C_{32}H_{46}O_6$: C, 72.97; H, 8.80. Found: C, 72.77; H, 8.68. NMR ($CDCl_3$) δ: 1.55 ppm (3H, s, C_{25}–CH_3), 2.01 (3H, s, $OCOCH_3$), 3.07 (1H, d, $J=14$ Hz, C_{19}–H), 3.28 (1H, s, C_{24}–H), 3.45 (1H, d, $J=11$ Hz, C_{26}–H), 3.68 (1H, bd, $J=6$ Hz C_3–H), 3.98 (1H, d, $J=11$ Hz, C_{26}–H), 4.10 (1H, m, C_{16}–H), 5.03 (1H, t, $J_1=J_2=8$ Hz, C_{12}–H). Deoxycimicifugenin A (XI, 60 mg) was dissolved to EtOH (5 ml) and $NaBH_4$ (50 mg) was added while stirring at room temperature. After leaving for two days, the reaction mixture was treated as described in the reduction of IV and the products were chromatographed on SiO_2. An eluate with benzene–AcOEt (5:1) afforded a desacetyl derivative as colorless needles (27 mg), mp 269—270°. *Anal.* Calcd. for $C_{30}H_{44}O_5$: C, 74.34; H, 9.15. Found: C, 73.92; H, 9.16. IR ν_{max}^{KBr} cm^{-1}: 3440 (OH). NMR ($CDCl_3$) δ: 0.85 ppm (3H, s, CH_3), 0.92 (6H, s, $2\times CH_3$). 0.98 (3H, s, CH_3), 1.49 (3H, s, C_{25}–CH_3), 3.08 (1H, d, $J=16$ Hz, C_{19}–H), 3.28 (1H, s, C_{24}–H), 3.47 (1H, d, $J=10$ Hz, C_{26}–H), 3.67 (1H, bd, $J=6$ Hz, C_3–H), 3.97 (1H, d, $J=10$ Hz, C_{26}–H), *ca.* 4 ppm (2H, m, C_{12}– and C_{16}–H).

Acetate of Cimicifugoside (I)——I (200 mg) was dissolved to pyridine (10 ml) and Ac_2O (1 ml) mixture to leave at room temperature over night. Water was added and the mixture was extracted with AcOEt. After usual treatment, the major product (III) was obtained as colorless powder through recrystallization from petroleum benzine, mp 166—168°. *Anal.* Calcd. for $C_{45}H_{62}O_{15}$: C, 64.11; H, 7.41. Found: C, 63.81; H, 7.22. NMR (pyridine) δ: 0.54 ppm (1H, d, $J=4$ Hz, H on a cyclopropane ring), 1.54 (3H, s, C_{25}–CH_3), 1.93 (3H, s, $OCOCH_3$), 1.99 (3H, s, $OCOCH_3$), 2.08 (6H, s, $OCOCH_3 \times 2$), 2.11 (3H, s, $OCOCH_3$), 3.83 (1H, s, C_{24}–H), 6.39 (1H, s, C_{26}–H).

Hydrolysis of Cimicifugoside (I) with Cellulase——I (3.0 g) was dissolved to hot EtOH (50 ml) and water was added dropwise with stirring. After cooling to room temp., cellulase (Lot 14C-0330, E.C.NO.3.2.1.4, from *Aspergillus niger*, practical grade Type II, 500 mg) was added to leave at 40° for one month. AcOEt was added to the solution after shaking for 1 month, and the AcOEt soluble fraction was chromatographed on SiO_2 (50 g). An eluate with benzene–AcOEt (5: 1) gave colorless needles (II, 0.5 g) after recrystallization from EtOH, mp 251—252°. *Anal.* Calcd. for $C_{32}H_{48}O_7$: C, 70.56; H, 8.88. Found: C, 70.47; H, 8.80. NMR ($CDCl_3$) δ: 0.58 (1H, d, $J=4$ Hz, H on a cyclopropane ring), 0.84 (3H, s, CH_3), 0.99 (6H, s, $2\times CH_3$), 1.15 (3H, s, CH_3), 1.60 (3H, s, C_{25}–CH_3), 2.02 (3H, s, $OCOCH_3$), 2.81 (1H, q, $J_1=9$, $J_2=16$ Hz, C_{11}–H), 3.3 (1H, m, C_3–H), 3.45 (1H, s, C_{24}–H), 4.49 (1H, m, C_{16}–H), 5.00 (1H, t, $J_1=J_2=10$ Hz, C_{12}–H), 5.11 (1H, s, C_{26}–H), 5.16 (1H, bd, $J=6$ Hz, C_7–H).

Acknowledgement The authors are grateful to the members of Central Analysis Room of this institute.