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Steroid Saponins and Sapogenins of Underground Parts of *Trillium kamtschaticum* Pall. IV.[1] Additional Oligoglycosides of 18-Norspirostane Derivatives and Other Steroidal Constituents

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Three oligoglycosides of 18-norspirostane derivatives, Ty-2, Ty-3, and Ty-4, along with two steroidal compounds, Tm and Tj, were isolated from the underground parts of *Trillium kamtschaticum* Pall. These five compounds and two other compounds, Th and Tz acetate, which had been obtained previously but remained unidentified, were characterized as follows. Ty-2, mp 235—240° (dec.), $[\alpha]_D$ −110°: deapio-trillenoside A (II) (named trillenoside B). Ty-3, mp 182—185° (dec.), $[\alpha]_D$ −98.7°: 3,21-di-O-acetyl-24-epitrillenogenin 1-O-(2′,3′,4′-tri-O-acetyl-α-L-rha·pyr)-(1—2)-α-L-ara·pyranoside (IV) (epitrillenoside C-PA). Ty-4, mp 226—230° (dec.), $[\alpha]_D$ −105°: 21-deoxytrillenoside A (VII) (deoxytrillenoside A). Tm, mp 326—329° (dec.), $[\alpha]_D$ −99.4°: 24β-hydroxypennogenin (V). Tj, mp 205—212° (dec.), $[\alpha]_D$ −66.5°: prototype compound (VIII) of pennogenin 3-O-β-chacotrioside. Th, mp 194—200° (dec.), $[\alpha]_D$ −90°: prototype compound (IX) of pennogenin 3-O-α-L-rha·pyr-β-chacotrioside. Tz acetate: crude ecdysterone triacetate (X).

This is the first report of the natural occurrence of these compounds, except for IX and X; the occurrence of IV is particularly interesting. VIII and IX had been expected to coexist with the corresponding pennogenin glycosides.

Keywords——oligoglycosides of 18-norspirostane derivatives; trillenoside B; epitrillenoside C-PA; deoxytrillenoside A; 24β-hydroxypennogenin; prototype compounds of pennogenin glycosides; ecdysterone; structure determination; *Trillium kamtschaticum* Pall.

In the preceding paper[1] of this series, it was reported that compound Ty (see "Chart 2" in Part II)[3] was separated to give two homogeneous compounds, Ty-1 (I) and Ty-2 (II), named trillenosides A and B, respectively. Further, I was shown to be an apiose-containing branched-chain tetraglycoside of trillenogenin (III), a novel type (18-nor) of spirostane derivative.

The petroleum ether-soluble portion in "Chart 2" mentioned above was also treated with hexane and the insoluble part was fractionated to afford compounds Ty-3 (IV) and Tm (V), together with pennogenin (VI).[4] A less polar fraction (Fr. 1′) in column chromatography of the petroleum ether-insoluble portion in "Chart 2" also provided IV. Furthermore, a fraction (Fr. 11′) between Fr. 11 and 12 was separated to afford two compounds, Ty-4 (VII) and Tj (VIII).

This paper concerns the characterization of II, IV, V, VII, and VIII, as well as of compounds Th (IX) and Tz acetate (X), which had previously been obtained[3] from Fr. 18 and 8, respectively, in "Chart 2", but which had remained unidentified.

Ty-2 (trillenoside B) (II), mp 235—240° (dec.), $[\alpha]_D$ −110°, was acid-hydrolyzed to give III, rhamnose, xylose, and arabinose. II showed the same *Rf* value on thin-layer chromatography (TLC) and the same ultraviolet (UV), circular dichroism (CD), infrared (IR), and proton magnetic resonance (PMR) spectra as a prosapogenin (XI), 1-O-α-L-thamnopyranosyl-(1-2)-[β-D-xylopyranosyl-(1-3)]-α-L-arabinopyranoside of III,[1] formed by partial hydrolysis of I. II permethylate, $[\alpha]_D$ −99.8°, was also identical with XI permethylate.[1] Thus, II is a prosapogenin (XI) of I.

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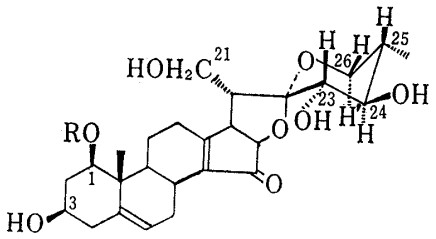

I (trillenoside A): R = β-D-api · fur—[3]α-L-rha · pyr[2] / β-D-xyl · pyr[3] α-L-ara · pyr—

II=XI (trillenoside B): R = α-L-rha · pyr[2] / β-D-xyl · pyr[3] α-L-ara · pyr—

III (trillenogenin): R = H

Formulae 1

Ty-3 (IV), mp 182—185° (dec.), $[\alpha]_D$ −98.7°, which showed hydroxy, ester, and enone absorptions in its infrared (IR) spectrum, was acid-hydrolyzed to give a compound (XII), mp 286—288° (dec.), $[\alpha]_D$ −210.1°, along with L-arabinose and L-rhamnose. The UV, CD, IR, and electron impact ionization mass spectra of XII were very similar to those of III,[1] suggesting a close structural resemblance of the compounds. The PMR spectrum of XII acetate (XIII) (Fig. 1) was also similar to that of III pentaacetate (XIV)[1] except for the signals assignable to 23-H (4.96 ppm, d, J=3.5 Hz in XIII and 4.98 ppm, d, J=9.5 Hz in XIV) and 24-H (5.26 ppm, dd, J=2.5, 3.5 Hz in XIII and 5.13 ppm, dd, J=9.5, 9.5 Hz in XIV), both being geminal to the acetoxy group. The coupling patterns of 23-H and 24-H of XIII indicate that the 24-H has β (equatorial) configuration and the 24-acetoxy group has α configuration. Furthermore, the signals ascribable to 26-H and -H′ are indicative of β (axial) configuration of 25-H and hence α (equatorial) configuration of the methyl group at C_{25}. Treatment of XII with acetone and *p*-toluenesulfonic acid yielded a product (XV) whose acetate (XVI) was shown to be a monoacetonide triacetate by PMR spectroscopy. Thus, the 23- and 24- hydroxy groups in XII should have the same configuration (α). Therefore XII is the 24-diastereoisomer of III; it was named epitrillenogenin.[5]

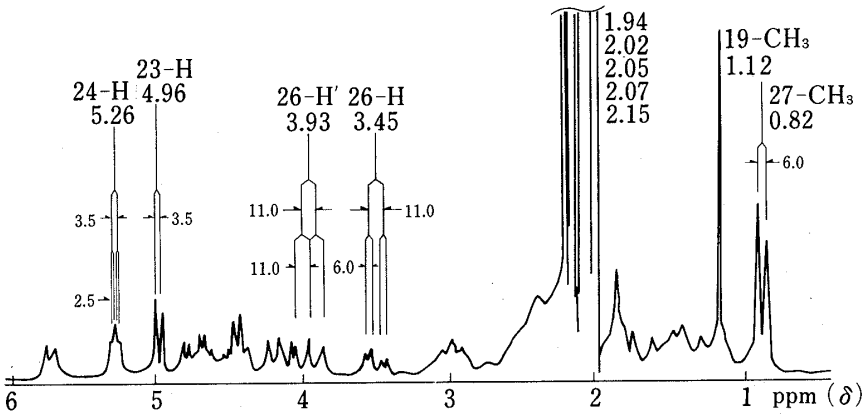

Fig. 1. PMR Spectrum of Epitrillenogenin Acetate (XIII)

When IV was hydrolyzed with alkali, a glycoside (XVII), mp 211—219° (dec.), $[\alpha]_D$ −109.2°, was formed. XVII was acid-hydrolyzed in the same way as IV to give XII and a mixture of arabinose and rhamnose. Methanolysis of XVII permethylate (XVIII), $[\alpha]_D$ −116.2°, provided methyl pyranosides of 2,3,4-tri-O-methyl-L-rhamnose and 3,4-di-O-methyl-

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L-arabinose together with an aglycone (XIX), $[\alpha]_D$ −198.2°. XIX was acetylated to give an acetate (XX), whose PMR spectrum showed, like that of the 3,21,23,24-tetra-O-methyl ether 1-O-acetate of III,[1] four methoxy and one acetoxy signals, and a double doublet at 4.65 ppm due to one proton geminal to the acetoxy group, ascribable to 1α-H. Therefore XX has a 1β-acetoxy group and hence XVII has a biose linked to the 1β-hydroxy group of XII. The molecular rotation differences[6] between XVII and the prosapogenin (XXI), $[\alpha]_D$ −138.2°, obtained by partial hydrolysis of XVII, and between XXI and XII suggested α-linkage of both the rhamnose and arabinose units (Table I). Accordingly, XVII is the 1-O-α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranoside of XII.

TABLE I. Molecular Rotation Differences[1] of XVII-XXI and XXI-XII

	$[M]_D$	$\Delta[M]_D$
XII—L-ara · pyr^2—L-rha · pyr (XVII)	−876°	−36°
XII—L-ara · pyr (XXI)	−840°	+160°
Epitrillenogenin (XII)	−1000°	

Compound IV showed five acetoxy signals in the PMR spectrum, and two series of fragment peaks in its mass spectrum (Fig. 2), namely, at *m/z* 273, 231, 189, and at 459, 417, 381, 339, 321. The former series of fragments is regarded as originating from the terminal tri-O-acetyl-rhamnose residue[3] and the latter from the 3,21-di-O-acetate of XII.

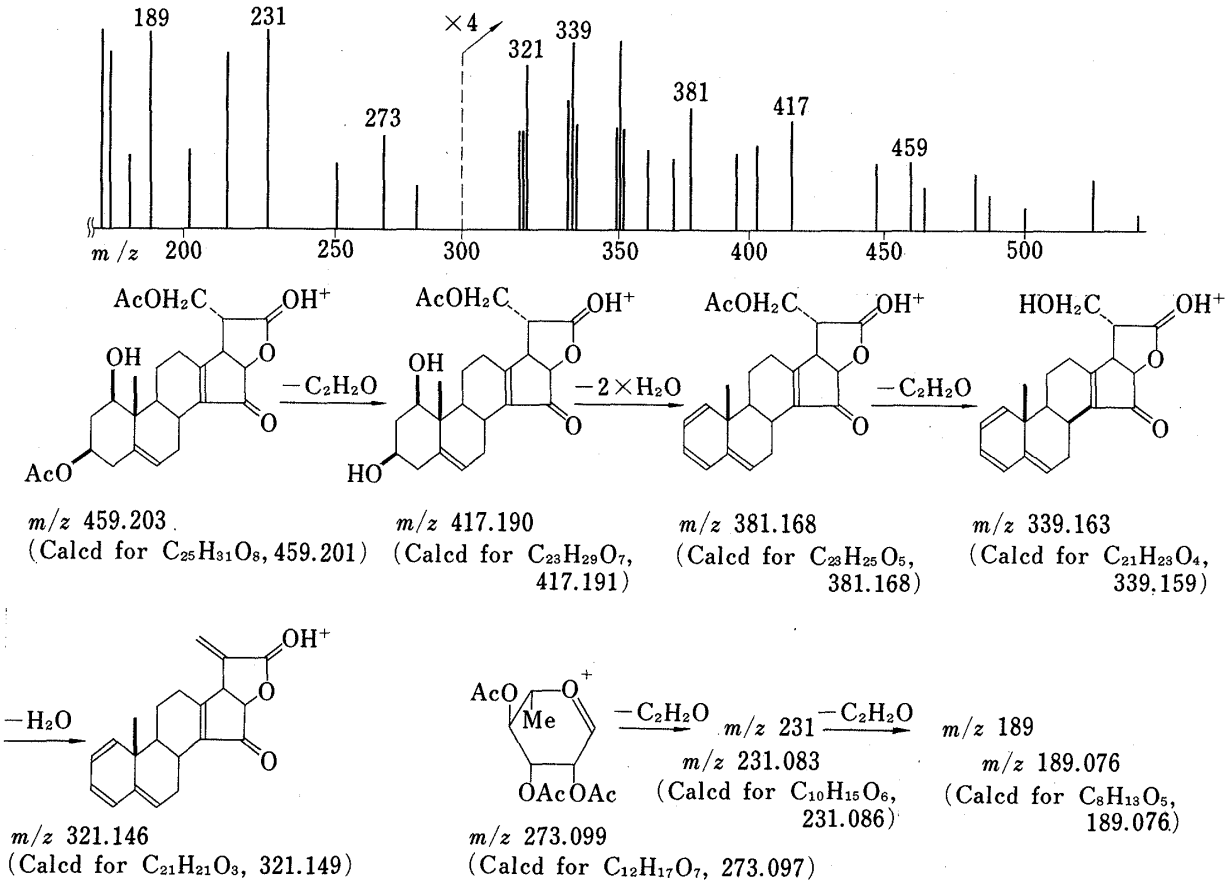

Fig. 2. Mass Spectrum of Epitrillenoside C-PA (IV)

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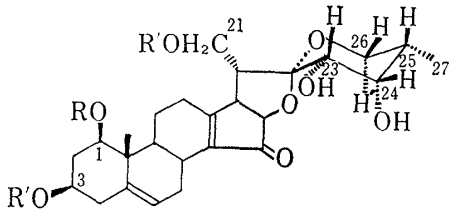

IV (epitrillenoside C-PA): R=tri-O-Ac-α-L-rha·pyr$\overset{2}{-}$α-L-ara·pyr—
R′=Ac

XII (epitrillerogenin): R=R′=H

XVII: R=α-L-rha·pyr$\overset{2}{-}$α-L-ara·pyr—
R′=H

XXI: R=α-L-ara·pyr—
R′=H

Formulae 2

Consequently, IV is formulated as 3,21-di-O-acetyl-epitrillenogenin 1-O-(2′,3′,4′-tri-O-acetyl-α-L-rhamnopyranosyl)-(1-2)-α-L-arabinopyranoside, and designated as epitrillenoside C-PA.[5]

Tm (V), mp 326—329° (dec.), $[\alpha]_D$ −99.4°, showed in the mass spectrum (Fig. 3) the molecular ion at *m/z* 446 and fragment peaks at *m/z* 428, 410, 171, 169, and 142, which are 16 mass units heavier than those (*m/z* 430, 412, 394, 155, 153, 126) observed in the spectrum (Fig. 4) of pennogenin (VI).[4] The above data suggest that V has another hydroxy group on the F-ring of VI. V was acetylated to give the diacetate (XXII), $C_{31}H_{46}O_7$, whose PMR spectrum (Fig. 5) resembled that of VI acetate (Fig. 6), but was discriminated therefrom by the presence of an additional acetoxy signal and the different patterns of signals due to the F-ring protons. Two double doublets at 3.50 (J=11.5, 11.5 Hz) and 3.64 ppm (J=3.5, 11.5 Hz) are assignable to 26-H_2 vicinal to 25β(axial) -H, and a doublet of doublets of doublets at 4.88 ppm is ascribable to a proton geminal to the acetoxy group. Its coupling constants (J=11, 11, and 5.5 Hz) are understandable only if the proton is located at C_{24} in the α (axial) configuration and is vicinal to 25β(axial) and 23-methylene protons. Thus, V is concluded to be 24β-hydroxypennogenin, that is, 25D-spirost-5-ene-3β,17α,24β-triol.

Ty-4 (VII), mp 226—230° (dec.), $[\alpha]_D$ −105°, showed IR absorptions due to hydroxy and enone groups, and in its PMR spectrum, signals ascribable to the 21-methyl group and to four anomeric protons of the sugar units were observed. It was acid-hydrolyzed to yield apiose, xylose, arabinose, and rhamnose together with an aglycone (XXIII), mp 282—286°,

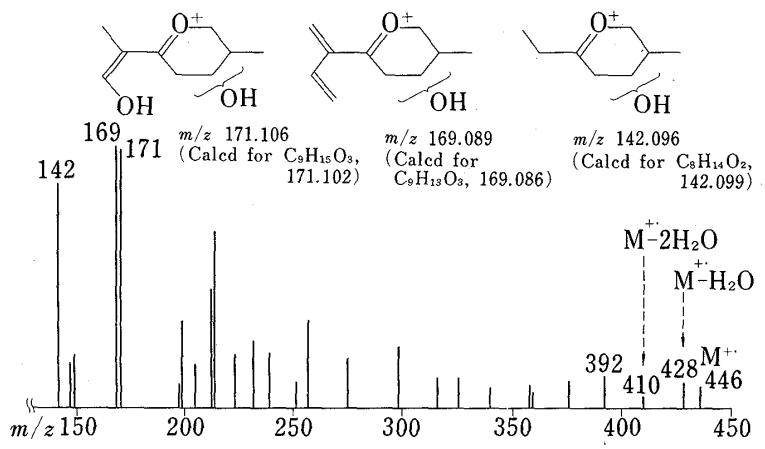

Fig. 3. Mass Spectrum of 24-Hydroxypennogenin (V)

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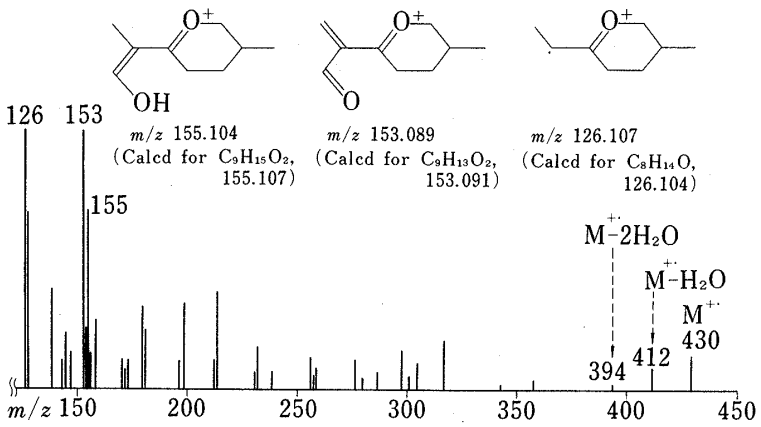

Fig. 4. Mass Spectrum of Pennogenin (VI)

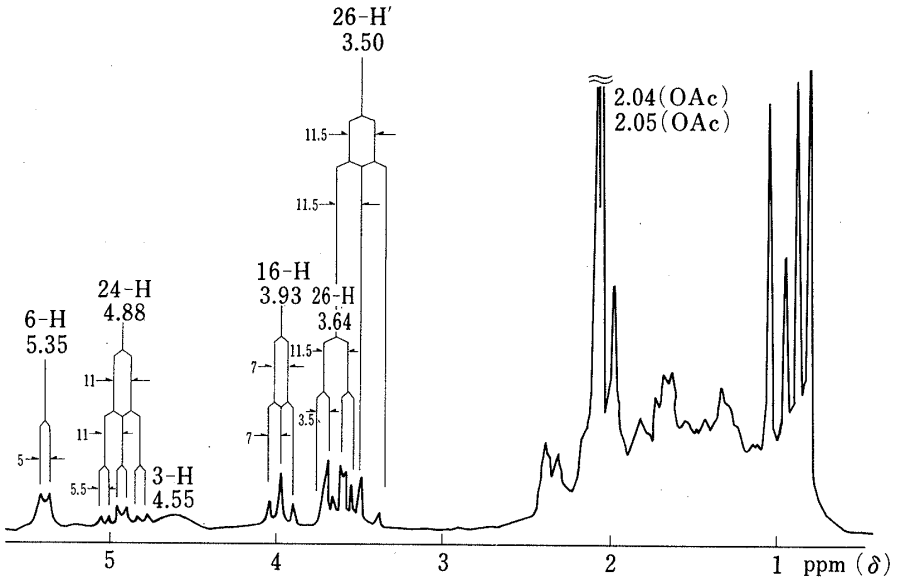

Fig. 5. PMR Spectrum of 24β-Hydroxypennogenin Acetate (XXII)

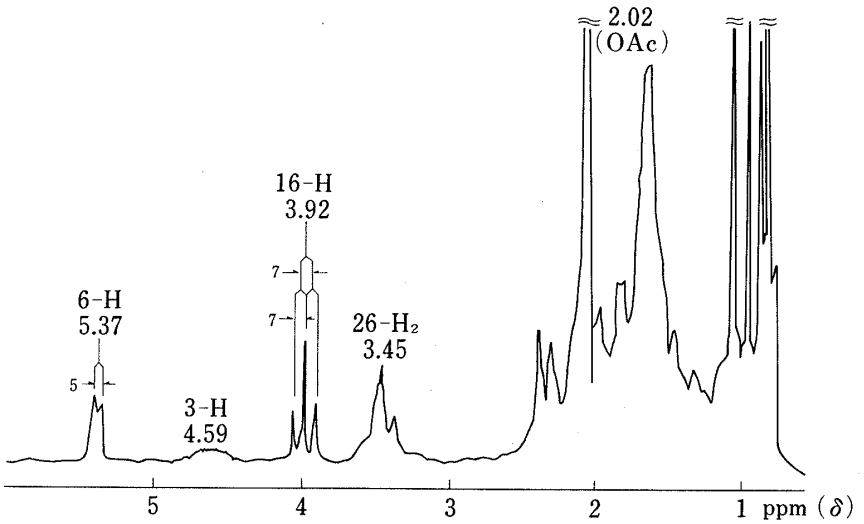

Fig. 6. PMR Spectrum of Pennogenin (VI) Acetate

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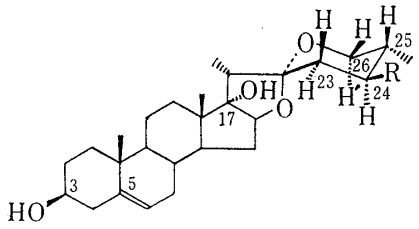
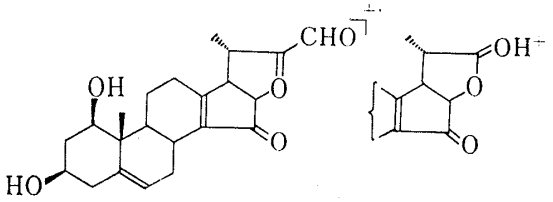
$[\alpha]_D$ −210.8°, $C_{26}H_{36}O_7$. The mass spectrum of XXIII showed peaks at *m/z* 460 ($M^{+\cdot}$), 372, and 359, each being 16 mass units less than those of *m/z* 476, 388, and 375 observed in the spectrum of III.[1] The latter two peaks were presumed to be due to the fragments shown in Fig. 7. When the PMR spectrum of XXIII acetate (XXIV) was compared with that of III acetate,[1] they were similar, except that a doublet at 1.14 ppm ascribable to 21-methyl protons and four acetoxy signals were observed in the former in place of two double doublets at 4.09 and 4.43 ppm attributable to 21-methylene protons and five acetoxy signals in the latter. These data suggest that XXIII is the 21-deoxy analog of III, namely 21-deoxytrillenogenin.



V (24β-hydroxypennogenin): R=OH
VI (pennogenin): R=H

Formulae 3



m/z 372.195 (Calcd for $C_{22}H_{28}O_5$, 372.193)

m/z 359.186 (Calcd for $C_{21}H_{27}O_5$, 359.185)

Fig. 7. Probable Structures of Fragments (*m/z* 372 and 359) Seen in the Mass Spectrum of 21-Deoxytrillenogenin (XXIII)

VII was methylated to provide the permethylate (XXV), whose methanolysis yielded an aglycone (XXVI) and four kinds of methylated sugars identical to those from I permethylate.[1] XXVI acetate (XXVII), mp 194—195°, $[\alpha]_D$ −190.7°, gave a PMR spectrum which was similar to that[1] of the 3,21,23,24-tetra-O-methyl ether 1-O-acetate of III, except in showing three methoxy and 21-methyl signals. The above results indicate that the sugar moiety and its site of linkage to the aglycone in VII are the same as in I, and that XXIII is the 21-deoxy analog of III.

When the prosapogenins (XXVIII, XXIX, and XXX) afforded by partial hydrolysis of VII and XXVIII were methylated and methanolyzed, the methylated sugars obtained were identical with those[1] from the permethylates of the corresponding prosapogenins of I, and in all cases the aglycone was XXVI. The molecular rotation differences[6] (Table II) between XXV—XXVIII permethylate, XXVIII—XXIX, XXIX—XXX, and XXX—XXIII suggest that the anomeric configurations of the component sugars are also the same as in I.

Table II. Molecular Rotation Differences[1] of XXV—XXVIII Permethylate, XXVIII—XXIX, XXIX—XXX, and XXX—XXIII

		$[M]_D$	$\Delta[M]_D$
XXIII—L-ara·pyr^2—L-rha·pyr^3—D-api·fur |^3 D-xyl·pyr permethylate	(XXV)	−1060°	
XXIII—L-ara·pyr^2—L-rha·pyr |^3 D-xyl·pyr permethylate	(XXVIII permethylate)	−976°	−84°
XXIII—L-ara·pyr^2—L-rha·pyr |^3 D-xyl·pyr	(XXVIII)	−969°	
XXIII—L-ara·pyr^2—L-rha·pyr	(XXIX)	−901°	−68°
XXIII—L-ara·pyr	(XXX)	−856°	−45°
Deoxytrillenogenin	(XXIII)	−968°	+112°

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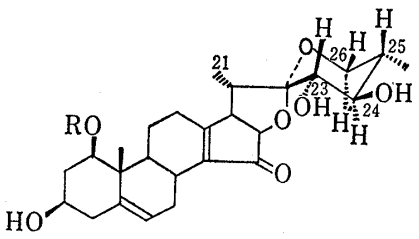

VII (deoxytrillenoside A): R= β-D-api·fur[3]—α-L-rha·pyr[2]
α-L-ara·pyr—
β-D-xyl·pyr[3]

XXIII (21-deoxytrillenogenin): R=H

XXVIII: R= α-L-rha·pyr[2]
α-L-ara·pyr—
β-D-xyl·pyr[3]

XXIX : R=α-L-rha·pyr[2]—α-L-ara·pyr—

XXX : R=α-L-ara·pyr—

Formulae 4

Consequently, VII is the 21-deoxy compound of I; it was named deoxytrillenoside A.

Tj (VIII), mp 205—212° (dec.), $[\alpha]_D$ −66.5°, showed no spiroketal absorptions in its IR spectrum, and no methoxy group signal was observed in the PMR spectrum. It was refluxed with methanol to give a less polar compound (VIII′) having one methoxy group, as indicated by PMR spectroscopy. VIII′ was converted back to VIII on boiling with water. Incubation of VIII with a commercial glycosidase gave D-glucose and a glycoside which was identical with compound Tc (XXXI),[3] pennogenin 3-O-β-chacotrioside.

Accordingly VIII was presumed to be the furostanol bisglycoside corresponding to XXXI. The structure, particularly the linkage of one mole of β-D-glucopyranose to the 26-hydroxy

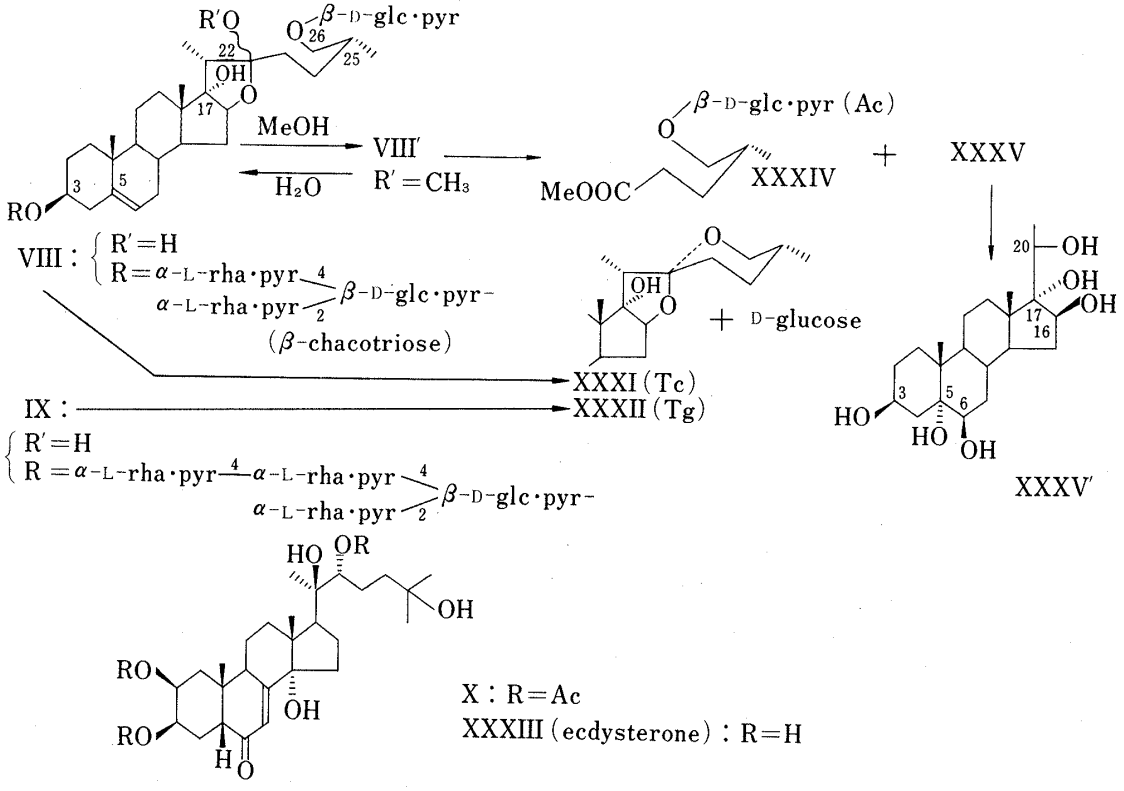

Chart 1

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group of the aglycone was confirmed by Baeyer–Villiger oxidation followed by hydrolysis and identification of the products in the manner described previously for compound Te'.[3]

Thus, VIII is 26-O-β-D-glucopyranosyl 25D-furost-5-ene-3β,17α,22ξ,26-tetraol 3-O-β-chacotrioside, the prototype compound of Tc (XXXI).[3]

Th (IX), mp 194—200° (dec.), $[\alpha]_D$ −90°, was identical with compound Hd,[7] the prototype compound of Tg (XXXII),[3] pennogenin 3-O-α-L-rhamnopyranosyl-β-chacotrioside, as judged by direct comparison with an authentic specimen.

Tz acetate (X) was presumed to be crude ecdysterone triacetate on the basis of its spectral data.[8] X was saponified to afford a compound, mp 236—238° (dec.), identical with an authentic sample of ecdysterone (XXXIII).[8a] No X but only XXXIII was detected on TLC of Fr. 2 in "Chart 2," indicating that XXXIII is originally present in the underground parts.[9]

II, IV, and VII are additional oligoglycosides of a novel type (18-nor) of spirostane derivative; IV is particularly interesting in that it is partially acetylated in both the sugar and aglycone moieties. V is the first reported monohydroxypennogenin, and VIII and IX are compounds which had been expected to coexist with XXXI and XXXII.

Experimental

Melting points were determined on a micro melting point apparatus (an air-bath type) and are uncorrected. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter (cell: 0.2 or 0.1 dm) at 18—24°. ORD curves and CD spectra were measured with a JASCO ORD/UV-5 recording spectropolarimeter. IR and UV spectra were obtained with JASCO IR-G and Shimadzu SV-50-A spectrometers, respectively. PMR spectra were taken at 100 MHz on a JEOL PS-100 spectrometer, and chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet). Mass spectra were recorded on a JEOL JMS-01SG mass spectrometer (electron impact ionization) with direct insertion of the probe into the ion source. In measurements of high resolution spectra by the photo plate method, Ilford type Q_2 thin glass was used as a dry plate and perfluorokerosene was employed as an internal calibration standard. The accelerating potential and ionizing potential were 4.4—6.5 kV and 25—75 eV, respectively, and the source temperature was 135—210°. All *Rf* values given are for TLC performed on Kieselgel G nach Stahl (Merck) using the Ehrlich reagent, anisaldehyde reagent, and 10% H_2SO_4 as detectors.[10] Column chromatography (on silica gel) was carried out with Kieselgel (0.05—0.2 mm) (Merck) and "Kanto" silica gel (100—200 mesh) in 30- to 50-fold excess. Unless otherwise specified, solvents employed in TLC and column chromatography were as follows: solv.(a), $CHCl_3$–MeOH–water = 65: 35: 10 v/v (lower phase); (b), 70: 30: 5; (c), 70: 30: 3; (d), 70: 30: 2; (e), 80: 20: 2; (f), 80: 20: 1; (g), benzene–acetone = 1: 1 v/v; (h), 2: 1; (i), 3: 1; (j), 5: 1.

Isolation of Ty-3 (IV), Tm (V) and Pennogenin (VI)——The petroleum ether-soluble portion (18 g) ("Chart 2" in Part II[3]) was refluxed with hexane and the insoluble part (4 g) was subjected to column chromatography on silica gel (solv. $CHCl_3$–MeOH = 50: 1→20: 1 v/v) to give three fractions, which were crystallized from MeOH or dil. acetone to afford IV (0.18 g), V (0.15 g), and VI (0.1 g) (identical with an authentic sample[4] by direct comparison (mixed mp, TLC, IR)). IV (0.3 g) was also obtained by crystallization of Fr. 1', a fraction prior to Fr. 1 in "Chart 2."

Isolation of Ty-4 (VII) and Tj (VIII)——Fr. 11', a fraction between Fr. 11 and 12 in "Chart 2," was refluxed with dil. acetone, then the solvent was removed *in vacuo*, and the residue was chromatographed on silica gel (solv. (c)) to give two homogeneous (TLC, solv. (a), (b)) compounds, which were crystallized from dil. acetone to provide VII (1.5 g) and VIII (0.4 g).

Ty-2 (II)[1]——A white powder (2.5 g) (from MeOH–acetone), *Rf* 0.20 (solv. (a)), mp 235—240° (dec.), $[\alpha]_D$ −110° (c=0.90, MeOH). *Anal.* Calcd for $C_{42}H_{62}O_{20}\cdot H_2O$: C, 55.74; H, 7.13. Found: C, 55.37; H, 7.03. II (200 mg) in 1 N HCl–MeOH (15 ml) was refluxed for 2.5 hr, then the solution was diluted with water (20 ml), concentrated *in vacuo* to 1/2 volume and again heated on a boiling water bath for 1.5 hr. The reaction mixture was neutralized with 4% KOH–MeOH, concentrated, and the precipitates were filtered off. The filtrate was evaporated to dryness and the residue was passed through a Sephadex LH-20 column with MeOH as an eluent. The eluate was chromatographed on silica gel (solv. (b)) to give III (identical (mixed mp, $[\alpha]_D$, TLC, IR, MS) with an authentic sample[1]) and a mixture of arabinose, xylose, and rhamnose (paper partition chromatography (PPC)[1]). II was shown to be identical with a prosapogenin (XI)[1] of I by direct comparison ($[\alpha]_D$, TLC, IR). Methylation of II by the Kuhn method[11] provided the permethylate, amorphous, $[\alpha]_D$ −99.8° (c=0.55, $CHCl_3$), MS m/z: 1040 ($M^{+\cdot}$). *Anal.* Calcd for $C_{53}H_{84}O_{20}$: C, 61.13; H, 8.13. Found: C, 60.92; H, 8.11. It was identical with XI permethylate[1] with respect to *Rf* value on TLC, IR, PMR and mass spectra and methanolysis products.

Ty-3 (IV)——A white powder (from dil. acetone), *Rf* 0.77 (solv. (e)), mp 182—185° (dec.), $[\alpha]_D$ −98.7° (c=0.92, MeOH). *Anal.* Calcd for $C_{47}H_{64}O_{21}\cdot H_2O$: C, 57.42; H, 6.77. Found: C, 57.18; H, 6.75. IR ν_{max}^{KBr}

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cm^{-1}: 3480 (OH), 1745 (ester), 1710 and 1628 (enone), 999, 979, 951, 933, 912, 866. UV λ_{max}^{EtOH} nm (ε): 248 (7940). CD (c=0.063, EtOH) [θ] (nm): +4300 (316) (positive maximum). PMR ($CDCl_3$–CD_3OD=3:1) δ: 0.82 (3H, d, J=7 Hz, 27-CH_3), 1.68 (3H, s, 19-CH_3), 2.03, 2.18, 2.21 (each 3H, s, OAc×3), 2.11 (6H, s, OAc×2). MS: Fig. 2.

Acid Hydrolysis of IV——IV (190 mg) was hydrolyzed and the reaction mixture was worked up in the same way as for II. The product was chromatographed on silica gel (solv. (d)→(b)) to provide an aglycone (XII) (30 mg), L-rhamnose, $[\alpha]_D$ +9.2° (c=2.52, water, after 14 hr), and L-arabinose, $[\alpha]_D$ +98.2° (c=2.43, water, after 14 hr) (PPC).[1]

Aglycone (XII)——Colorless needles (from dil. MeOH), *Rf* 0.72 (solv. (e)), mp 286—288° (dec.), $[\alpha]_D$ −210.1° (c=0.46, MeOH). *Anal.* Calcd for $C_{26}H_{36}O_8$: C, 65.53; H, 7.62. Found: C, 65.26; H, 7.69. UV λ_{max}^{EtOH} nm (ε): 247.5 (8900). CD (c=0.05, EtOH) [θ] (nm): +5940 (318) (positive maximum). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1703 and 1634 (enone), 1000, 985, 980, 943, 905, 865.

XII Acetate (XIII)——XII (25 mg) was acetylated with Ac_2O–pyridine (each 4 ml) on heating for 1 hr. The product was crystallized from EtOH to give XIII as colorless prisms (18 mg), mp 176—177°, $[\alpha]_D$ −161.2° (c=0.38, $CHCl_3$). *Anal.* Calcd for $C_{36}H_{46}O_{13}$: C, 62.96; H, 6.75. Found: C, 62.63; H, 6.81. MS m/z: 686 ($M^{+\cdot}$). PMR: Fig. 1.

XII Acetonide (XV)——A solution of XII (40 mg) and *p*-toluenesulfonic acid (10 mg) in acetone (10 mg) was stirred at room temperature for 48 hr. The reaction mixture was evaporated to dryness and the residue was passed through a Sephadex LH-20 column (solv. MeOH). The eluate was evaporated to dryness to give a solid which was chromatographed on silica gel (solv. (f)) to yield XV (19.5 mg) as a white powder, $[\alpha]_D$ −154.2° (c=0.97, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3600—3150 (OH), 1700 and 1635 (enone). MS m/z: 516 ($M^{+\cdot}$). XV (10 mg) was acetylated in the usual way to give the acetate (XVI) (7.3 mg) as a white powder. PMR ($CDCl_3$) δ: 1.34, 1.54 (3H each, s, $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!C\!<\!\!\begin{matrix}O-\\O-\end{matrix}$), 2.03 (3H, s, OAc), 2.08 (6H, s, OAc×2).

Alkaline Hydrolysis of IV——A solution of IV (350 mg) in 3% KOH–MeOH (15 ml) was refluxed for 15 min. The solution was neutralized with 1N HCl–MeOH, and evaporated to dryness *in vacuo*. The residue was treated with water and the soluble part was passed through a Sephadex LH-20 column (solv. MeOH) to give a glycoside (XVII) as a white powder (250 mg) (from MeOH–acetone), *Rf* 0.44 (solv. (b)), mp 211—219° (dec.), $[\alpha]_D$ −109.2° (c=0.71, MeOH). *Anal.* Calcd for $C_{37}H_{54}O_{16}\cdot 2H_2O$: C, 56.19; H, 7.39. Found: C, 56.39; H, 7.41. IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1690 and 1622 (enone), 900, 945, 934, 915, 870, 854. Acid hydrolysis of XVII in the manner described for II provided XII and a mixture of arabinose and rhamnose.

XVII Permethylate (XVIII)——XVII (230 mg) was methylated twice by the Kuhn method,[11] and the product was chromatographed on silica gel (solv. (i)) to give XVIII (165 mg) as a white powder, $[\alpha]_D$ −116.2° (c=1.01, $CHCl_3$). *Anal.* Calcd for $C_{46}H_{72}O_{16}$: C, 62.71; H, 8.24. Found: C, 62.60; H, 8.36. IR: no OH. MS m/z: 880 ($M^{+\cdot}$), 349(permethylated methylpentosyl-pentosyl cation, $C_{16}H_{29}O_8$), 189(permethylated methylpentosyl cation, $C_9H_{17}O_4$).[1]

Methanolysis of XVIII——XVIII (150 mg) was methanolyzed with 1N HCl–MeOH (5 ml) in the usual manner to yield an aglycone (XIX) (30 mg), $[\alpha]_D$ −198.2° (c=0.46, $CHCl_3$) and a mixture of methylated sugars, which was found to consist of methyl 2,3,4-tri-O-methyl-α-L-rhamnopyranoside and methyl 3,4-di-O-methyl-L-arabinopyranoside by TLC and gas liquid chromatography (GLC).[1]

XIX Acetate (XX)——XIX (25 mg) was acetylated with Ac_2O–pyridine to give the acetate (XX) as colorless plates (from EtOH–hexane), mp 172—173°, $[\alpha]_D$ −204.2° (c=0.41, $CHCl_3$). *Anal.* Calcd for: $C_{32}H_{46}O_9$: C, 66.87; H, 8.07. Found: C, 66.59; H, 8.12. IR ν_{max}^{KBr} cm^{-1}: 1725 (OAc), 1702, 1630 (enone), 1140, 1080 (ether). PMR ($CDCl_3$) δ: 0.93 (3H, d, J=6 Hz, 27-CH_3), 1.11 (3H, s, 19-CH_3), 2.12 (3H, s, OAc), 3.37 (6H, s, OCH_3×2), 3.43, 3.60 (3H each, s, OCH_3×2), 4.30 (1H, d, J=6 Hz, 16-H), 4.65 (1H, dd, J=4.5, 11.5 Hz, 1-H), 5.65 (1H, broad d, J=6 Hz, 5-H).

Partial Hydrolysis of XVII—— A solution of XVII (160 mg) in 0.5N HCl–MeOH (12 ml) was refluxed for 15 min. Usual work-up and chromatography of the product on silica gel (solv. (b)) provided a prosapogenin (XXI) as a white powder (from MeOH–acetone), $[\alpha]_D$ −138.2° (c=0.34, MeOH). *Anal.* Calcd for $C_{31}H_{44}O_{12}$: C, 61.17; H, 7.29. Found: C, 60.91; H, 7.32. Acid hydrolysis in the same way as for IV yielded XII and arabinose.

Tm (V)——Colorless needles (from dil. MeOH), *Rf* 0.23 (solv. (c); VI, *Rf* 0.45), mp 326—329° (dec.), $[\alpha]_D$ −99.4° (c=0.45, MeOH). *Anal.* Calcd for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.34; H, 9.47. IR ν_{max}^{KBr} cm^{-1}: 3500—3200 (OH), 983, 962, 919, 909, 890, 837, 817. MS: Fig. 3.

V Diacetate (XXII)——V (30 mg) was acetylated with Ac_2O–pyridine to yield the acetate (XXII) as colorless needles (18 mg) (from MeOH), mp 218—219°, $[\alpha]_D$ −61.5° (c=0.45, $CHCl_3$). *Anal.* Calcd for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74. Found: C, 70.01; H, 8.69. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1735 (ester), 994, 982, 959, 908, 888. MS m/z: 470.302 ($M^{+\cdot}$–AcOH=$C_{29}H_{42}O_5$, 470.303), 410.287 ($C_{27}H_{38}O_3$, 410.282). PMR: Fig. 5.

Ty-4 (VII)——A white powder (from MeOH–acetone), *Rf* 0.27 (solv. (a); I, *Rf* 0.16), mp 226—230° (dec.), $[\alpha]_D$ −105° (c=1.00, $CHCl_3$). *Anal.* Calcd for $C_{47}H_{70}O_{23}\cdot 2H_2O$: C, 54.32; H, 7.18. Found: C. 54.10; H, 7.16. IR ν_{max}^{KBr} cm^{-1}: 3500—3200 (OH), 1688 and 1622 (enone). PMR ($CDCl_3$–CD_3OD+CF_3COOH):[12] 0.96 (3H, d, J=6 Hz, 27-CH_3), 1.12 (3H, d, 19-CH_3), 1.18 (3H, d, J=6 Hz, 21-CH_3), 1.33 (1H, d, J=6 Hz, 6-CH_3 of rhamnose), 4.36 (1H, multiplet, 1-H of arabinose), 4.43 (2H, d, J=6 Hz, 16-H and 1-H of xylose),

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5.22 (1H, d, $J=3$ Hz, 1-H of apiose), 5.38 (1H, broad s., 1-H of rhamnose).

Acid Hydrolysis of VII——A solution of VII (200 mg) in 1 N H_2SO_4–50% EtOH (15 ml) was refluxed for 1.5 hr, then the solution was concentrated to 1/2 volume and diluted with water (20 ml). The solution was again heated on a boiling water bath for 1.5 hr, then neutralized with 3% KOH–MeOH. The resulting precipitates were filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel (solv. (b)) to afford an aglycone (XXIII) and a sugar mixture consisting of arabinose, xylose, apiose, and rhamnose (identified by PPC).[1]

Aglycone (XXIII)——Colorless prisms (from dil. MeOH), *Rf* 0.57 (solv. (e), III, 0.44; XI, 0.50), mp 282—286°, $[\alpha]_D$ −210.5° ($c=1.01$, MeOH). *Anal.* Calcd for $C_{26}H_{36}O_7 \cdot H_2O$: C, 65.25; H, 8.00. Found: C, 65.42; H, 7.98. IR ν_{max}^{KBr} cm^{-1}: 3650—3100 (OH), 1700 and 1630 (enone). UV λ_{max}^{EtOH} nm (ε): 250 (9496). CD ($c=0.023$, EtOH) [θ] (nm): +5700 (317) (positive maximum). MS *m/z*: 460.246 ($M^{+\cdot}=C_{26}H_{36}O_7$, 460.246), 372.195 ($C_{22}H_{28}O_5$, 372.193), 359.186 ($C_{21}H_{27}O_5$, 359.185).

XXIII Acetate (XXIV)——XXIII (28 mg) was acetylated with Ac_2O–pyridine (2 ml each) and the product was crystallized from MeOH to give XXIV as colorless plates (16 mg), mp 170—171°, $[\alpha]_D$ −141° ($c=0.65$, $CHCl_3$). *Anal.* Calcd for $C_{34}H_{44}O_{11}$: C, 64.95; H, 7.05. Found: C, 65.21; H, 7.11. IR ν_{max}^{KBr} cm^{-1}: 1770—1740 (OAc), 1720, 1645 (enone). PMR ($CDCl_3$) δ: 0.85 (3H, d, $J=6$ Hz, 27-CH_3), 1.13 (3H, s, 19-CH_3), 1.14 (3H, d, $J=7$ Hz, 21-CH_3), 1.98, 2.00, 2.02, 2.08 (3H each, s, OAc×4), 4.36 (1H, d, $J=6.5$ Hz, 16-H), 4.71 (1H, d d, $J=4$, 11.5 Hz, 1-H), 4.86 (1H, d, $J=10$ Hz, 23-H), 5.19 (1H, d d, $J=9.5$ Hz, 24-H). MS *m/z*: 628 ($M^{+\cdot}$).

VII Permethylate (XXV)——VII (900 mg) was methylated three times by the Kuhn method.[11] The reaction mixture was worked up as usual and the crude product was chromatographed on silica gel (solv. (i)) to give XXV as a white powder (320 mg), $[\alpha]_D$ −90.6° ($c=4.02$, $CHCl_3$). *Anal.* Calcd for $C_{59}H_{94}O_{23}$: C, 60.49; H, 8.09. Found: C, 60.71; H, 8.01. IR: no OH. MS *m/z*: 1170 ($M^{+\cdot}$), 484, 384, 354, 349, 249, 175.

Methanolysis of XXV——A solution of XXV (300 mg) in 1 N HCl–MeOH (20 ml) was refluxed for 2 hr, then neutralized with 3% KOH–MeOH and evaporated to dryness *in vacuo*. The residue was treated with MeOH and the soluble part was passed through a Sephadex LH-20 column (solv. MeOH). The eluate was evaporated to dryness and the residue was chromatographed on silica gel (solv. (j)) to afford a mixture of methylated glycosides and an aglycone (XXVI), a white solid, $[\alpha]_D$ −196.2° ($c=0.54$, $CHCl_3$). MS *m/z*: 502 ($M^{+\cdot}$). In the manner described for the methanolysis of I permethylate,[1] the above mixture of methyl glycosides was examined by TLC and GLC, and further hydrolyzed with 1 N HCl to yield free sugars, which were separated by chromatography on silica gel (solv. (h)→(g)) to give four kinds of sugars. The methyl glycosides detected and the free sugars isolated were identical (GLC, TLC) to those in the case of I permethylate.

XXVI Acetate (XXVII)——Usual acetylation of XXVI provided an acetate (XXVII) as a crystalline solid (from MeOH), mp 194—195°, $[\alpha]_D$ −190.7° ($c=0.82$, $CHCl_3$). *Anal.* Calcd for $C_{31}H_{44}O_8$: C, 68.36; H, 8.14. Found: C, 68.11; H, 8.09. IR ν_{max}^{KBr} cm^{-1}: 1725 (OAc), 1705, 1630 (enone), 1135, 1076 (ether). MS *m/z*: 544 ($M^{+\cdot}$). PMR ($CDCl_3$) δ: 0.92 (3H, d, $J=7$ Hz, 27-CH_3), 1.10 (3H, s, 19-CH_3), 1.12 (3H, d, $J=7$ Hz, 21-CH_3), 2.09 (3H, s, OAc), 3.33, 3.47, 3.54 (3H each, s, OCH_3×3), 4.24 (1H, d, $J=6$ Hz, 16-H), 4.62 (1H, d d, $J=4$, 12 Hz, 1-H), 5.62 (1H, broad d, $J=6$ Hz, 6-H).

Partial Hydrolysis of VII——VII (1.1 g) was refluxed with 0.5 N HCl–MeOH (40 ml) for 20 min, then worked up as usual. The product was chromatographed on silica gel (solv. (d)) to give a prosapogenin (XXVIII) (470 mg), a white powder, $[\alpha]_D$ −111.4° ($c=1.03$, MeOH). XXVIII (100 mg) was methylated in the same way as VII to give the permethylate (46 mg), a white powder, $[\alpha]_D$ −96.6° ($c=2.28$, $CHCl_3$). It was methanolyzed to yield the aglycone XXVI and three kinds of methyl glycosides which were identical (GLC, TLC) to those from the permethylate of II (=XI).[1]

Partial Hydrolysis of XXVIII——XXVIII (300 mg) was hydrolyzed with 0.5 N HCl–MeOH (12 ml) for 20 min and worked up as usual. The product was chromatographed on silica gel (solv. (e)→(d)) to give two prosapogenins (XXIX) (20 mg) and (XXX) (35 mg), *Rf* 0.31 and 0.40, respectively (solv. (a)). On acid hydrolysis, XXIX, a white solid, $[\alpha]_D$ −122.1° ($c=0.72$, MeOH), yielded XXIII (identified by direct comparison (mp, TLC, IR) with an authentic sample) and a mixture of arabinose and rhamnose (PPC), while XXX, a solid, $[\alpha]_D$ −144.6° ($c=0.91$, MeOH), provided XXIII and arabinose.

Permethylation and Methanolysis of XXIX——These procedures were carried out as described for XXV, and the products were identified (TLC, IR) as XXVI, methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside and methyl 3,4-di-O-methyl-L-arabinopyranoside (TLC, GLC).[1]

Tj (VIII)——A white powder (from dil.acetone), *Rf* 0.14 (solv. (b)), $[\alpha]_D$ −66.5° ($c=1.05$, pyridine). IR: no spiroketal absorptions. Ehrlich test:[10] negative. PMR: no OCH_3 signal. VIII was boiled with MeOH and the resulting solution was evaporated to dryness. The residue was chromatographed on silica gel (solv. (b)) to give a compound (VIII′), *Rf* 0.19 (solv. (b)), $[\alpha]_D$ −62.2° ($c=1.18$, pyridine). PMR (pyridine-d_5) δ: 3.27 (3H, s, OCH_3). A solution of VIII′ in water was refluxed for 30 min and evaporated to dryness to regenerate VIII (TLC). A solution of VIII (50 mg) in water (2 ml) was incubated with a commercial glycosidase[13] (30 mg) at 39° for 3 hr. The solution was evaporated to dryness *in vacuo* and the MeOH–soluble portion of the residue was chromatographed on silica gel (solv. (e)) to give a solid (25 mg) and a syrup (15 mg). The former was crystallized from MeOH to afford colorless needles, mp 294—298° (dec.), $[\alpha]_D$ −129° ($c=0.55$,

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pyridine). This product was identical with compound Tc (pennogenin 3-O-β-chacotrioside) (XXXI)[3] as judged by direct comparison (mixed mp, TLC, $[\alpha]_D$, IR). The latter syrup, $[\alpha]_D$ +43.1° (c=1.02, water, after 14 hr), was identified as D-glucose by PPC and TLC.

Baeyer-Villiger Oxidation of VIII'——In the manner described for compound Te',[3] VIII' (300 mg) was oxidized with 30% H_2O_2 and 90% HCOOH at 50° for 30 min. The product was treated with 3% KOH–MeOH at 50° for 20 min, and then acetylated. The crude acetate (200 mg) was chromatographed on silica gel (solv. hexane–AcOEt=2:1 v/v) to give methyl γ-methyl-δ-hydroxypentanoate β-D-glucopyranoside tetraacetate (XXXIV) (identical (PMR, IR, MS) with an authentic sample[3]) and a compound (XXXV) as a white powder. XXXV was refluxed with 1.5 N H_2SO_4–50% EtOH for 3 hr to give colorless needles (from dil.MeOH), mp 285—288° (dec.). This product was identical (mixed mp, IR) with an authentic sample of 5α-pregnane-3β,5α,6β,16β,17α,20α-hexaol (XXXV').[3]

Th (IX)——A white powder (from dil.acetone), mp 194—200° (dec.), $[\alpha]_D$ −90° (c=1.05, pyridine). PMR: no OCH_3. This product was identical (*Rf* value on TLC, IR, PMR spectra and enzymatic hydrolysis products (Tg (XXXII)[3] and D-glucose)) with compound Hd[6] from *Heloniopsis orientalis*.

Tz Acetate (X)——Colorless needles (from dil.MeOH), mp 162—165°, $[\alpha]_D$ +59° (c=1.37, $CHCl_3$). The UV, IR, PMR, and mass spectra were similar to those[8] of ecdysterone triacetate. Alkaline hydrolysis of X provided a compound (XXXIII). It was recrystallized from dil.MeOH to give colorless needles, mp 236—238°(dec.), which were identical with an authentic specimen of ecdysterone[8] (*Rf* value on TLC, IR, ORD, PMR).

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