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Steroid Saponins and Sapogenins of Underground Parts of *Trillium kamtschaticum* PALL. II.¹⁾ Pennogenin- and Kryptogenin 3-O-Glycosides and Related Compounds

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Of twelve compounds so far isolated from the underground parts of *Trillium kamtschaticum* PALL., eight steroid saponins, Ta, Td, Tb, Tc, Tg, Te, Tk and Tf, were characterized, respectively, as follows: prosapogenin A (VII) of dioscin, mp 242—246° (decomp.), $[\alpha]_D -108.3^\circ$; a furostanol bisglycoside (VIII) corresponding to VII, mp 265—271° (decomp.), $[\alpha]_D -83.1^\circ$; pennogenin 3-O- α -L-rha.pyr-(1→2)- β -D-glc.pyranoside (X), mp 273—276° (decomp.), $[\alpha]_D -117.8^\circ$; pennogenin 3-O- β -chacotrioside (XI), mp 297—299° (decomp.), $[\alpha]_D -126.7^\circ$; pennogenin 3-O- α -L-rha.pyr-(1→4)- α -L-rha.pyr-(1→4)-[α -L-rha.pyr-(1→2)]- β -D-glc.pyranoside (XII), mp 224—228° (decomp.), $[\alpha]_D -136^\circ$; the proto-type compound (XIX) of X, mp 275—280° (decomp.), $[\alpha]_D -84.6^\circ$; kryptogenin 3-O- β -D-glc.pyranoside (XXIII), mp 238—241° (decomp.), $[\alpha]_D -129.4^\circ$; 26-O- β -D-glc.pyr-17(20)-dehydrokryptogenin 3-O- α -L-rha.pyr-(1→2)- β -D-glc.pyranoside (XXIV), mp 265—268° (decomp.), $[\alpha]_D -80.1^\circ$.

X, XI and XII are the first pennogenin glycosides ever reported and XXIII has never been isolated either. Marker's "nolonin" (VI) was presumed to be a furostanol 3,26-O-bisglycoside such as XIX corresponding to X, and the structures of nologenin (V) and VI were supposed to be represented as V' and VI', respectively.

XXIV was regarded as an artefact yielded from XIX during chromatography over silica gel.

In the preceding paper¹⁾ it was reported that the methanol extracts of the title plant materials were hydrolyzed with hydrochloric acid to give pennogenin (I), kryptogenin (II), 26-chloro-26-deoxykryptogenin, and bethogenin (III) along with diosgenin (IV), and that the structure of pennogenin is represented as I. Since Marker, *et al.*³⁾ regarded I—III as artefacts yielded from "nolonin" (VI), a glycoside of nologenin (V), during acid hydrolysis (Chart 1), the above result suggests existence, in the extracts, of "nolonin" which was found and assigned a tentative structure VI by Marker, *et al.*³⁾ but has not been investigated ever since. Moreover, as mentioned previously,¹⁾ if "nolonin" is assumed to be the furostanol 3,26-O-bisglycoside related to a pennogenin 3-O-glycoside, the latter could also be coexistent.

This paper concerns isolation of twelve compounds, conventionally named Ta—h, Tk and Tx—z, and characterization of eight (Ta—g, Tk) of them, which include pennogenin- and kryptogenin 3-O-glycosides and the furostanol 3,26-O-bisglycosides corresponding to one of the formers.

The materials employed were the same as those described in the preceding paper¹⁾ and the procedure of extraction, separation and purification of the respective compound are shown in Chart 2.

1) Part I: T. Nohara, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1772 (1974).

2) Location: 3-1-1, Maedashi, Higashi-ku, Fukuoka, 812, Japan.

3) a) R.E. Marker, R.B. Wagner, P.R. Ulshafer, E.L. Wittbecker, D.P. J. Goldsmith, and C.H. Ruof, *J. Am. Chem. Soc.*, **65**, 1205 (1943); R.E. Marker, R.B. Wagner, D.P.J. Goldsmith, P.R. Ulshafer, and C.H. Ruof, *ibid.*, **65**, 1248 (1943); R.E. Marker and J. Lopez, *ibid.*, **69**, 2386, 2395 (1947); b) R.E. Marker, R.B. Wagner, P.R. Ulshafer, E.L. Wittbecker, D.P.J. Goldsmith, and C.H. Ruof, *ibid.*, **69**, 2167 (1947).

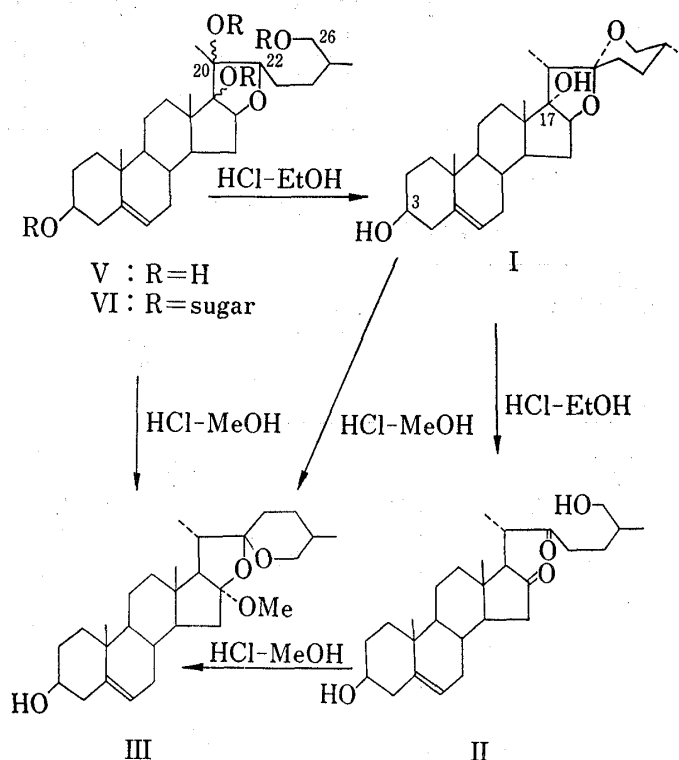
Compound Ta (VII), mp 242—246° (decomp.), $[\alpha]_D -108.3^\circ$, was negative to the Ehrlich test,⁴⁾ showed the 25D-spiroketal absorptions⁴⁾ on the infrared (IR) spectrum, and was acid hydrolyzed to give IV, D-glucose and L-rhamose. VII acetate, mp 204—207°, $[\alpha]_D -62.5^\circ$, gave on its mass spectrum the peak of molecular ion at m/e 974 and those of peracetylated methylpentosyl-hexose⁵⁾ and terminal methylpentose⁵⁾ residues at 561 and 273, respectively. VII methylate, mp 165—166°, $[\alpha]_D -92.3^\circ$, showed on the nuclear magnetic resonance (NMR) spectrum one proton doublet ($J=7$ Hz) at 4.35 ppm and one proton broad singlet at 5.21 ppm ascribable to anomeric protons of D-glucose and L-rhamnose, respectively. The methylate was methanolized to give methyl pyranosides of 3,4,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose.

All the above data indicate that VII is diosgenin 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside, that is, prosapogenin A of dioscin.^{6,7)}

Td (VIII); mp 265—271° (decomp.). $[\alpha]_D -83.1^\circ$, positive to the Ehrlich test, showed no spiroketal absorptions on IR but a methoxy signal on NMR spectra, suggesting the 22-methoxyfurostanol structure.⁴⁾ Hydrolysis with almond emulsin gave glucose and a spirostanol glycoside which was identified with VII (Chart 3). VIII acetate, $[\alpha]_D -41.4^\circ$, exhibited on the mass spectrum the peak of M^+-MeOH ion⁴⁾ and those of peracetylated methylpentosyl-hexose, terminal hexose⁵⁾ and methylpentose residues at m/e 1304, 561, 331 and 273, respectively.

Accordingly VIII is defined as 26-O- β -D-glucopyranosyl-22-methoxyfurost-5-ene-3 β ,26-diol 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside.

When VIII was boiled with aqueous acetone, a compound (Td') (IX), mp 266—270° (decomp.), was provided. IX was more polar than VIII on thin-layer chromatogram (TLC), showed no methoxy signal on the NMR spectrum, and was converted back to VIII on refluxing in methanol. Therefore, in analogy to proto-dioscin,⁴⁾ IX is considered to be the 22-hydroxy



4) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1162 (1968); T. Kawasaki, T. Komori, K. Miyahara, T. Nohara, I. Hosokawa, and K. Mihashi, *ibid.*, **22**, 2164 (1974).

5) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, 1964, pp. 203—227; T. Kawasaki, T. Komori, Y. Ida, Y. Inatsu, K. Miyahara, and T. Nohara, Preprints, International Conference on Mass Spectroscopy, Kyoto, September, 1969, p. 221; T. Komori, Y. Ida, Y. Muto, K. Miyahara, T. Nohara, and T. Kawasaki, *Biomed. Mass.*, in the Press.

6) T. Tsukamoto, T. Kawasaki, and T. Yamauchi, *Pharm. Bull.* (Japan), **4**, 35 (1956); T. Kawasaki, and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1070 (1968).

7) VII and its acetate showed the R_f values on TLC and melting points (alone and on admixture), respectively, identical with those of the samples obtained from dioscin. The optical rotations of prosapogenin A of dioscin and its acetate and the melting point of the methylate reported earlier⁶⁾ should be corrected as given here.

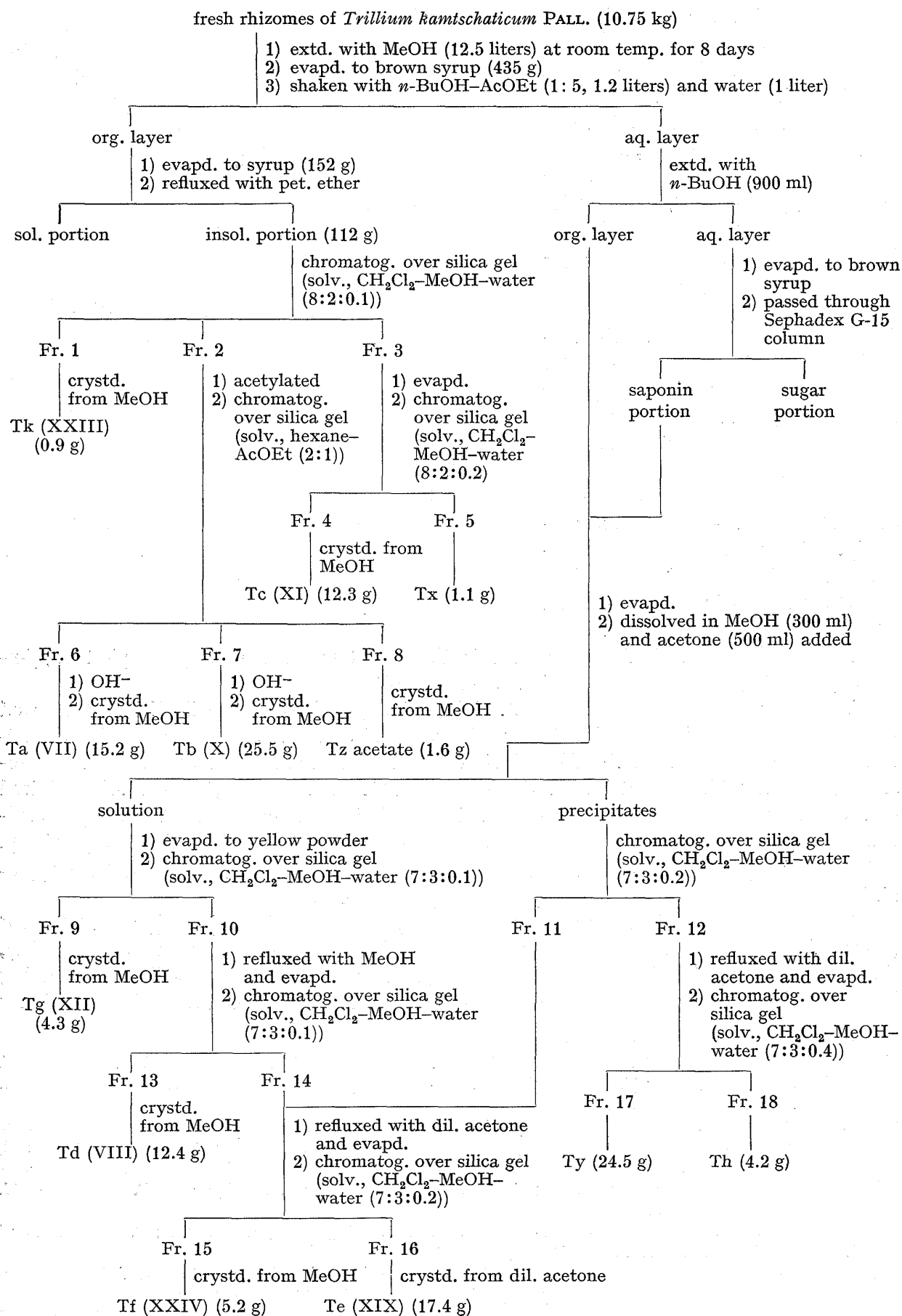
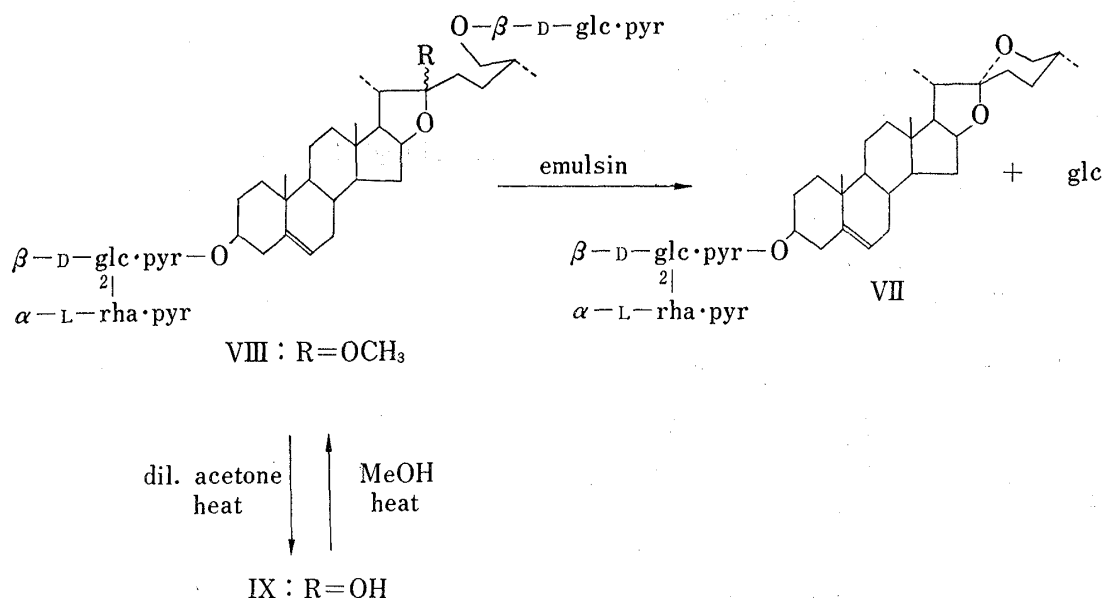


Chart 2



compound of VIII which is originally existing in the plant and regarded as the proto-type compound of VII.

Tb (X), mp 273—276° (decomp.), $[\alpha]_D -117.8^\circ$, Tc (XI), mp 297—299° (decomp.), $[\alpha]_D -126.7^\circ$, and Tg (XII), mp 224—228° (decomp.), $[\alpha]_D -136.0^\circ$, were all negative to the Ehrlich test and showed the 25D-spiroketal absorptions on their IR spectra. They seemed homogeneous on TLC, but were hydrolyzed with hydrochloric acid in methanol equally to give I, II and III together with D-glucose and L-rhamnose. It is then conceivable that X—XII are glycosides of either V, as claimed by Marker, *et al.*,³⁾ or I.³⁾ The formers are, however, excluded on the basis of their IR spectra, and hence X—XII are considered respectively as a pure glycoside consisting of I, D-glucose and L-rhamnose.

X acetate (XIII), mp 195—197°, $[\alpha]_D -51.8^\circ$, showed on its mass spectrum the molecular ion at m/e 990, the fragment ions due to aglycone I at 412, 394, and those of peracetylated methylpentosyl-hexose and terminal methylpentose residues at 561 and 273, respectively. The above data and the elemental analysis of the free glycoside indicate that X is a L-rhamnosyl-D-glucoside of I, C₃₉H₆₂O₁₃. X methylate (XIV), mp 176—178°, $[\alpha]_D -90.1^\circ$, gave the mass spectrum consistent with that of XIII and was methanolized to yield the same two methyl pyranosides as those from VII methylate. The prosapogenin (XV) obtained from X was identified with synthetic 3-O-β-D-glucopyranoside of I (Chart 4) and the molecular rotation difference⁸⁾ between X and XV suggested α conjugation of the L-rhamnose unit. Therefore the sugar moiety of X is identical with that of VII, and X is defined as pennogenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside. The NMR spectra (Fig. 1) of XIII and VII acetate were almost the same except a triplet at 3.94 ppm which appeared in that of XIII and was ascribable to the proton at C₁₆ of the aglycone I.

XI acetate (XVI), mp 112—115°, $[\alpha]_D -47.7^\circ$, gave the mass spectrum in which the molecular peak (m/e 1220), fragment ions due to aglycone I (412, 394), and that of peracetylated terminal methylpentose residue (273) were observed. It suggests XI to consist of I, one mole of D-glucose and two moles of L-rhamnose, one or both of which is located at the terminal. Methanolysis of XI methylate provided methyl pyranosides of 2,3,4-tri-O-methyl-L-rhamnose and 3,6-di-O-methyl-D-glucose. The NMR spectra of XVI and dioscin (3-O-β-chacotrioxide of IV) acetate⁹⁾ were almost identical except the triplet due to the proton at C₁₆ observed in the former.

8) W. Klyne, *Biochem. J.*, **47**, xli (1950).

9) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull. (Tokyo)*, **16**, 1692 (1968).

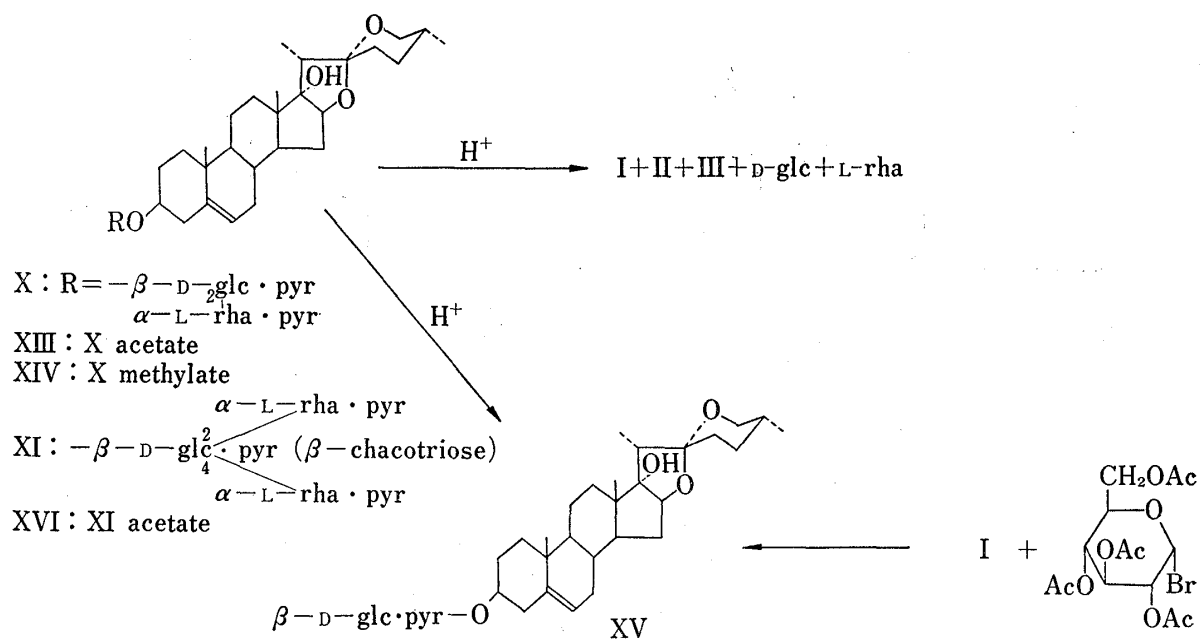


Chart 4

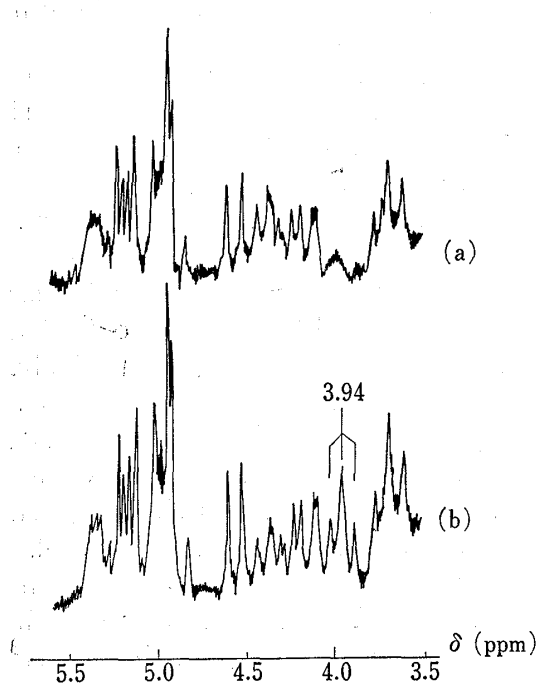
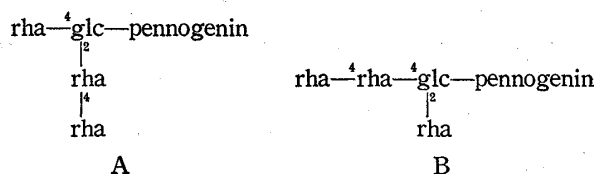


Fig. 1. NMR Spectra of VII Acetate (a) and XIII (b) (CDCl_3 , 100 MHz)



Formulae 1

Therefore, in analogy to X, XI is regarded as pennogenin 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 4)]- β -D-glucopyranoside (β -chacotriose).

XII methylate (XVII), mp 172–175°, $[\alpha]_D -97.1^\circ$, showed on its mass spectrum the peaks of molecular ion and permethylated methylpentosyl-methylpentose⁵⁾ and terminal methylpentose⁵⁾ residues at m/e 1170, 363 and 189, respectively. The above data and elemental analysis of XII indicate that XII has the molecular formula $\text{C}_{51}\text{H}_{82}\text{O}_{21}$, consisting of I, one mole of D-glucose and three moles of L-rhamnose, and that only rhamnose unit(s) is (are) located at the

terminal. Methanolysis of XVII gave methyl pyranosides of 3,6-di-O-methyl-D-glucose and 2,3,4-tri-O-methyl- and 2,3-di-O-methyl-L-rhamnoses. The sugar moiety of XII is then to have the sequence either A or B (Formulae 1). However, since the NMR spectrum of XVII was, similarly to the cases of XIII and XVI, almost the same as that of the methylate of diosgenin glycoside Pb (XVIII),¹⁰⁾ the tetrasaccharide moiety of XII was thought to have the A sequence and to be fully identical with that of XVIII. Subsequently, conversion of XII to the corres-

10) T. Nohara, H. Yabuta, M. Suenobu, R. Hida, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull. (Tokyo)*, **21**, 1240 (1973).

ponding diosgenin glycoside and comparison with XVIII were undertaken. Taking account of the glycoside nature of XII and a successful preliminary experiment with I acetate, XVII was subjected to a series of reactions shown in Chart 5. The glycoside methylate thus obtained, mp 137—139°, $[\alpha]_D -108.5^\circ$, was identical in all respects with an authentic sample of XVIII methylate.

In consequence, XII is pennogenin 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside.

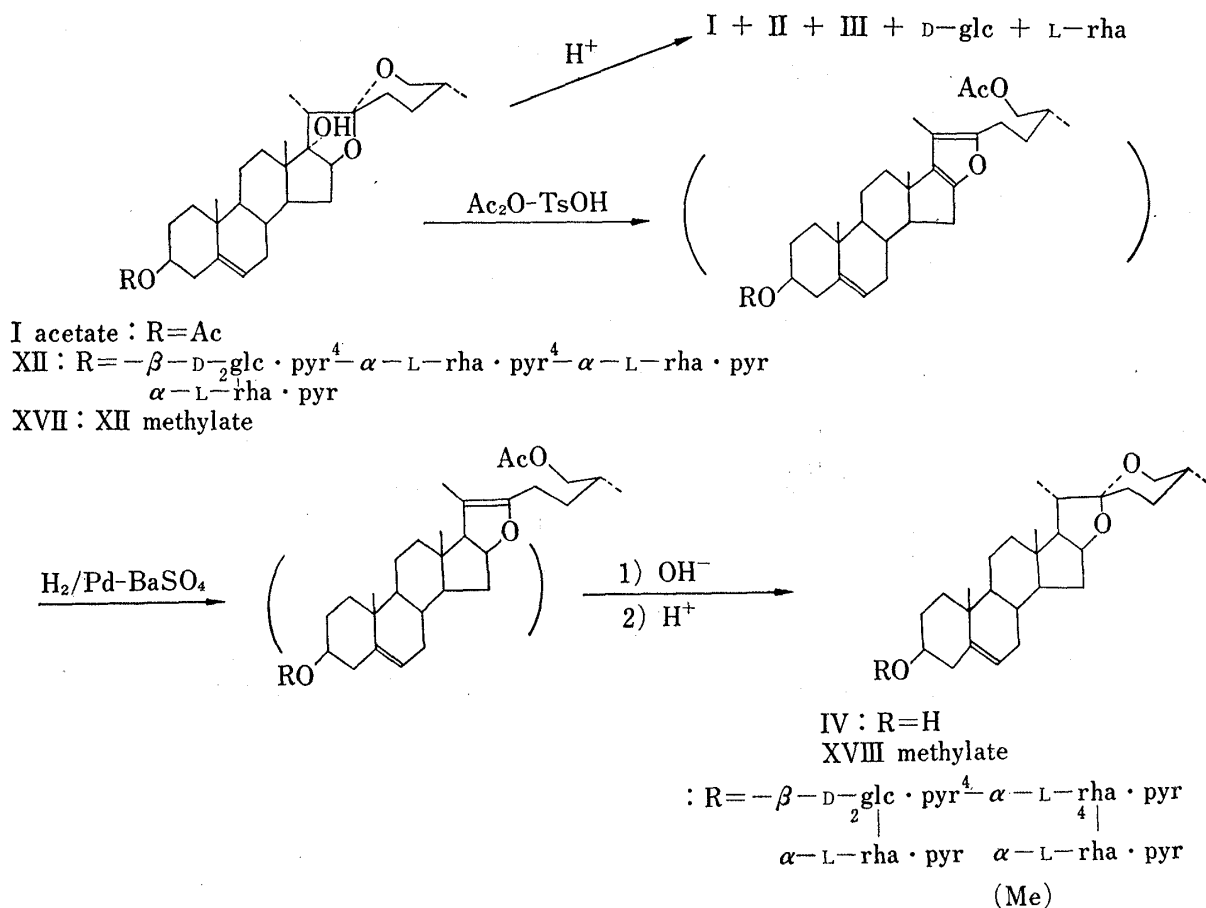


Chart 5

Te (XIX), mp 275—280° (decomp.), $[\alpha]_D -84.6^\circ$, was boiled with methanol to yield a less polar compound Te' (XX), mp 272—279° (decomp.), $[\alpha]_D -83.1^\circ$, which was converted back to XIX on refluxing in water. Although both XIX and XX were negative to the Ehrlich test, no spiroketal absorptions on their IR spectra and the above interconversion suggest⁴⁾ XIX and XX to be the glycosides of 22-hydroxy- and 22-methoxyfurostane derivatives, respectively, and it was supported by the fact that they were treated with acid alike to give I, rhamnose and glucose, and with almond emulsin to afford X and glucose. While XIX acetate, $[\alpha]_D -38.7^\circ$, showed no methoxy signal on its NMR spectrum, XX acetate, mp 131—135°, $[\alpha]_D -42.1^\circ$, exhibited the signal and on the mass spectrum it gave the peak of $M^+ - \text{MeOH}$ ion at m/e 1320 and those of peracetylated methylpentosyl-hexose, terminal hexose and methylpentose units at 561, 331 and 273, respectively. From these data XIX is presumed to be 26-O- β -D-glucopyranosyl-furost-5-ene-3 β ,17 α ,22,26-tetraol 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside and XX as its 22-methoxy analog. The structure XX was corroborated, in the same manner as in proto-dioscin,⁴⁾ by Baeyer-Villiger oxidation which provided 5 α -pregnane-3 β ,5 α ,6 β ,16 β ,17 α ,20 α -hexaol (XXI) and methyl γ -methyl- δ -hydroxypentanoate β -D-glucopyranoside (XXII) (Chart 6).

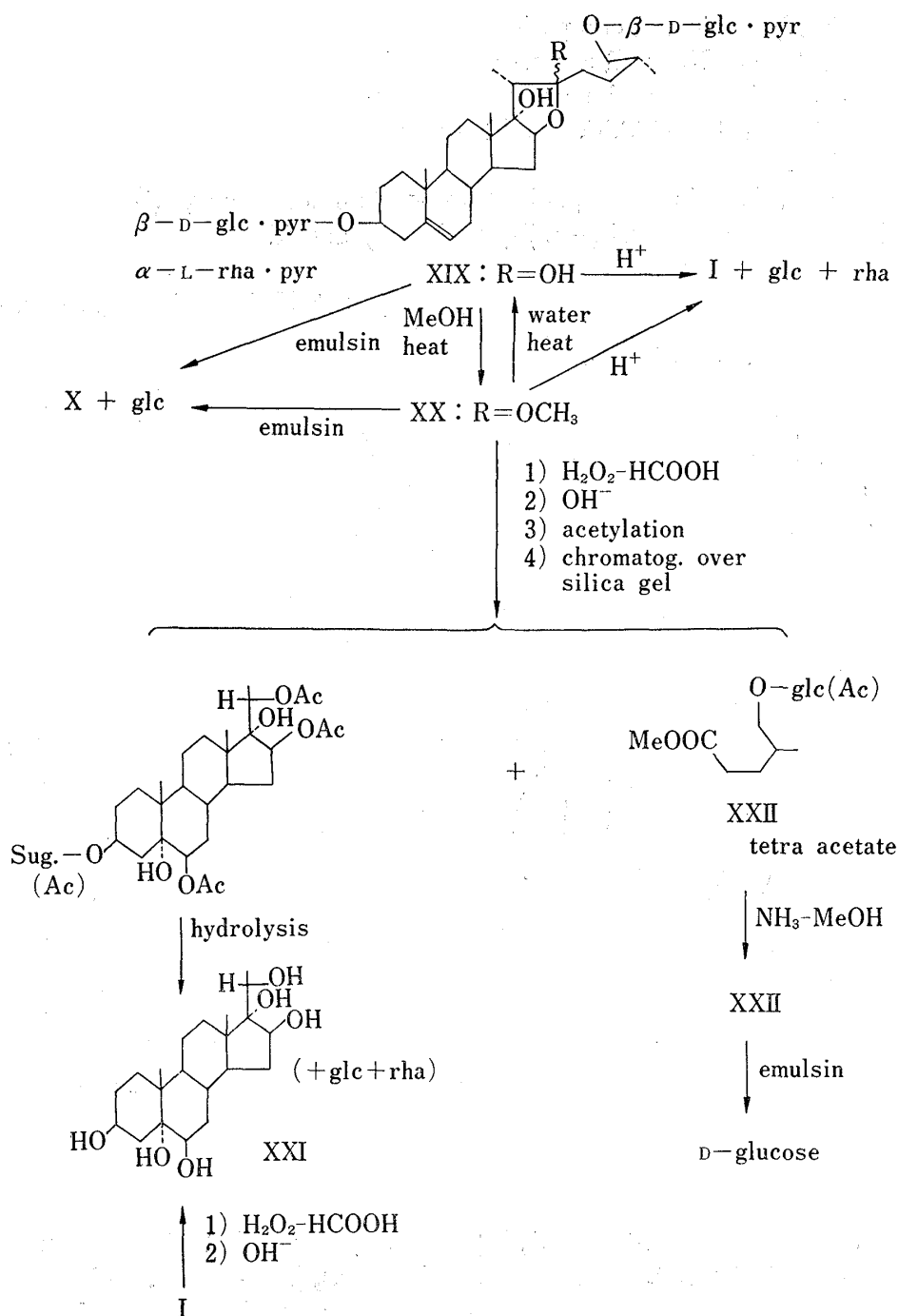


Chart 6

XIX is regarded as the proto-type compound of X, and XX as an artefact formed from XIX during the procedures of extraction and separation using methanol.

Tk (XXIII), mp 238—241° (decomp.), $[\alpha]_{\text{D}} -129.4^\circ$, gave on acid hydrolysis II and glucose indicating it to be a glycoside of any of I, II and V.³⁾ However, its IR spectrum showed no spiroketal but two carbonyl absorptions, and hence I and V are ruled out as the aglycone. XXIII acetate, mp 181—182°, $[\alpha]_{\text{D}} -114.6^\circ$, showed an optical rotatory dispersion (ORD) curve quite similar to that of II diacetate (Fig. 2), and on its mass spectrum the peaks of molecular ion and peracetylated terminal hexose residue were observed at m/e 802 and 331, respectively. From the above data it is evident that XXIII is a kryptogenin monoglucoside, $\text{C}_{33}\text{H}_{52}\text{O}_9$. When XXIII was reduced with sodium borohydride and subsequently acidified,

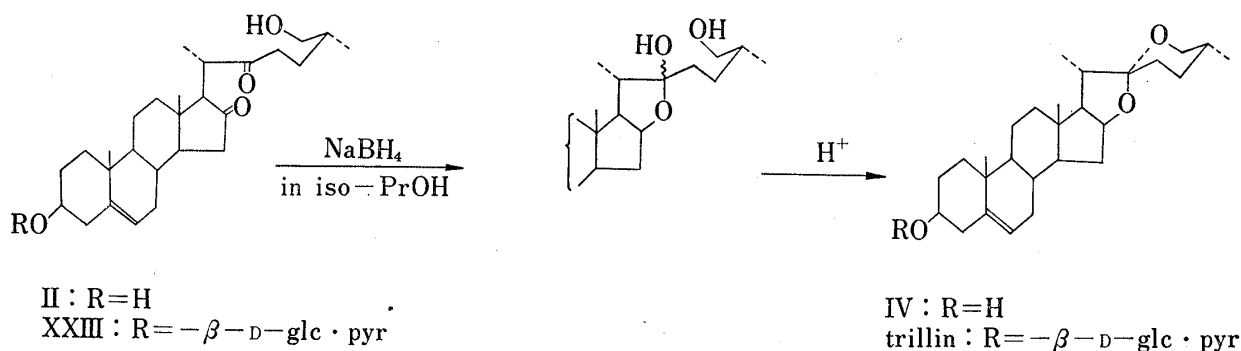


Chart 7

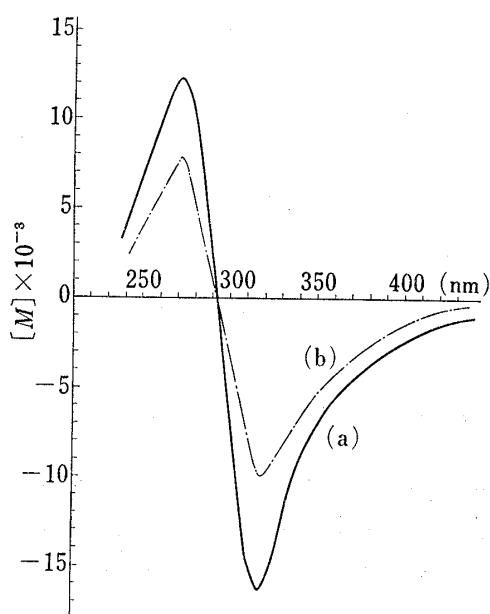


Fig. 2. ORD Curves of II Diacetate (a) and XXIII Acetate (b)

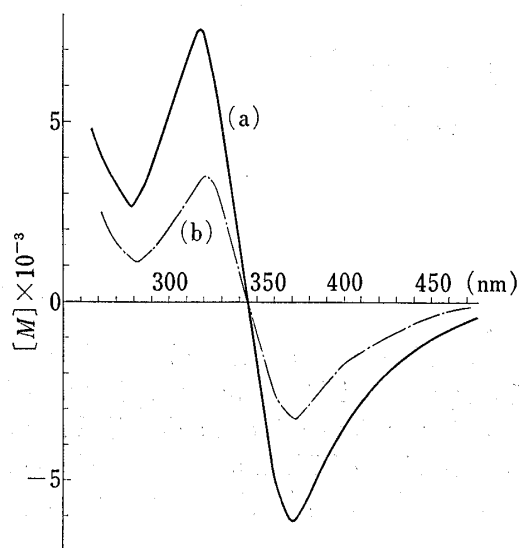


Fig. 3. ORD Curves of XXVI Diacetate (a) and XXV (b)

according to the Uhle method¹¹⁾ which converts II to IV (Chart 7), a glycoside, mp 264–266° (decomp.), $[\alpha]_D -102.3^\circ$, was provided. It was fully identical with an authentic sample of trillin (3-O- β -D-glucopyranoside of IV).⁶⁾

Now it follows that XXIII is kryptogenin 3-O- β -D-glucopyranoside.

Tf (XXIV), mp 265–268° (decomp.), $[\alpha]_D -80.1^\circ$, was acid hydrolyzed to provide glucose, rhamnose and five kinds of ether-soluble compound which were detected on TLC but could not be identified. The IR, UV and ORD spectra of XXIV indicated the presence of a conjugated enone system, and the NMR spectrum showed a three proton singlet at 1.95 ppm probably due to a methyl group attached to a substituted olefinic carbon atom and the signals ascribable to 18- and 19-methyl groups on steroid nucleus. XXIV acetate (XXV), mp 190–192°, $[\alpha]_D -67.1^\circ$, showed on its mass spectrum the peak of molecular ion at m/e 1318 and those of peracetylated methylpentosyl-hexose, terminal hexose and methylpentose residues at 561, 331 and 273, respectively. The UV and ORD (Fig. 3) spectra of XXV were quite similar to those of the diacetate of 17(20)-dehydrokryptogenin (XXVI) derived^{3b,12)} from I via pseudokryptogenin diacetate (XXVII) (Chart 8).

11) F.C. Uhle, *J. Org. Chem.*, **31**, 4193 (1966).

12) A. Sandoval, J. Romo, G. Rosenkranz, Sr. Kaufmann, and C. Djerassi, *J. Am. Chem. Soc.*, **73**, 3820 (1951).

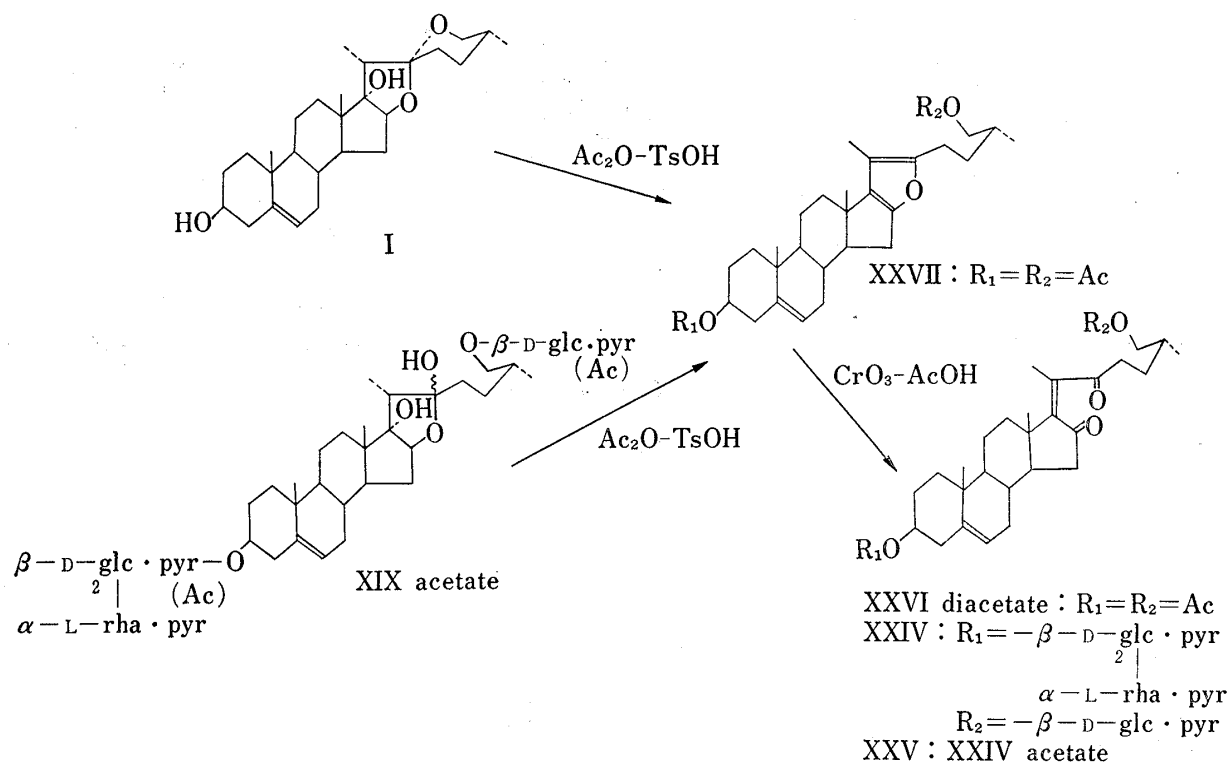


Chart 8

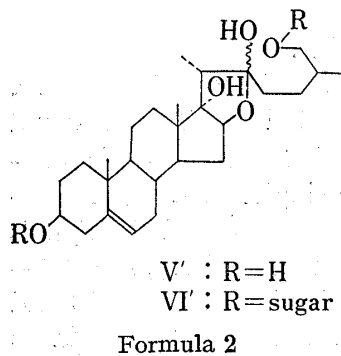
On the basis of all the above data XXIV was presumed to be 26-O-glucosyl-17(20)-dehydrokryptogenin 3-O-rhamnopyranosyl-glucoside.

In order to determine the detailed structure of the sugar moiety and to confirm the aglycone, conversion of XIX acetate to the corresponding glycoside acetate of XXVI was then carried out (Chart 8), in the analogous way to that in I, and the product was identified with XXV.

Consequently XXIV is defined as 26-O- β -D-glucopyranosyl-17(20)-dehydrokryptogenin 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside.

Since XIX was easily converted to XXIV in a solution in the presence of silica gel, XXIV is considered as an artefact yielded from XIX by dehydration¹³⁾ followed by air oxidation during the procedure of chromatographic separation.

Tb (X), Tc (XI) and Tg (XII) are the first pennogenin glycosides ever reported. Their occurrence and, in particular, actual isolation of Te (XIX) (and Tf (XXIV)), the proto-type compound of X, suggest the existence also of the furostanol bisglycosides related to XI and XII, and may support the assumption¹⁾ that Marker's "nolonin" (VI) is one of the furostanol 3,26-O-bisglycosides corresponding to pennogenin 3-O-glycosides. The hydroxy group at C_{20} in nologenin (V) is then most likely to be replaced to C_{22} (V') and the structure of "nolonin" (VI) might be represented as VI'. Occurrence of the first kryptogenin glycoside Tk (XXIII) is also noted.



Experimental

Melting points were determined on a Kofler block and a micromelting point apparatus (an air-bath type) and are uncorrected. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter at 15–25°

13) cf. H. Hirschmann and F.B. Hirschmann, *Tetrahedron*, **3**, 243 (1958).

unless otherwise indicated and the values for reducing sugars are those shown in an aged solution. ORD curves were measured with a JASCO ORD/UV-5 recording spectropolarimeter. IR and UV spectra were obtained with a JASCO IR-G and a Shimadzu SV-50-A spectrometers, respectively, and NMR spectra were taken at 60 MHz on a JEOL C-60H spectrometer and chemical shifts are given in δ (ppm) scale with tetramethylsilane as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet). Mass spectra were recorded on a JMS-01SG mass spectrometer with an accelerating potential of 4.6 or 6.4 kV, an ionizing potential of 30–75 eV and a source temperature of 150–230°. Gas-liquid chromatography (GLC) was run on a Yanagimoto GCG 550F with flame ionization detector using glass column (4 mm \times 1.7 m) packed with 5% 1,4-butanediol succinate on Chromosorb W (60–80 mesh): for dimethyl ethers of methyl rhamnoside, column temp. 178°, N₂ 60 ml/min; for trimethyl ethers of methyl rhamnoside, column temp. 108°, N₂ 73 ml/min; for dimethyl ethers of methyl glucoside, column temp. 198°, N₂ 55 ml/min; for trimethyl ethers of methyl glucoside, column temp. 149°, N₂ 80 ml/min. Paper partition chromatography (PPC) for sugars was conducted on Tōyō Roshi No. 50 in double ascending method using upper layer of *n*-BuOH–pyridine–water (6:2:3) + pyridine (1) as solvent and aniline hydrogen phthalate for staining. All *R_f* values given are on TLC which was performed on Kieselgel G nach Stahl (Merck) using Ehrlich reagent, anisaldehyde reagent and 10% H₂SO₄ as the detector.⁴⁾ Column chromatography was carried out with Kieselgel (0.05–0.2 mm) (Merck) and “Kanto” silica gel (100–200 mesh) in thirty to fifty times quantity of the material. Unless otherwise specified, solvents employed both in TLC and column chromatography were as follows: solv. 1. CHCl₃–MeOH–water (7:3:0.5); solv. 2, hexane–AcOEt (1:1); solv. 3, hexane–AcOEt (1:2).

The ratios of solvents and reagents in mixture are given in v/v.

Extraction and Isolation of Steroid Glycosides—The plant materials employed were same as those described in the preceding paper¹⁾ and the procedure is shown in Chart 2.

Ta (VII)—Colorless needles, mp 242–246° (decomp.), $[\alpha]_D -108.3^\circ$ ($c=0.97$, pyridine), -121.6° ($c=0.66$, MeOH), -101.0° ($c=0.97$, dioxane). Ehrlich test:⁴⁾ negative. IR ν_{\max}^{KBr} cm⁻¹: 3600–3200 (OH), 982, 920, 900, 865 (intensity 900 > 920, 25D-spiroketal).⁴⁾ Anal. Calcd. for C₃₉H₆₂O₁₂: C, 64.82; H, 8.58. Found: C, 64.53; H, 8.70. Hydrolysis of VII (2.5 g) with 1N HCl in MeOH (45 ml) gave diosgenin (IV) (320 mg) (identified by mixed mp and by comparison of *R_f* values and IR spectra with those of an authentic sample) and a sugar portion (glucose and rhamnose being detected on PPC). The latter was passed through a Sephadex LH-20 column, the MeOH eluate was evaporated and the residue was rechromatographed over silica gel by using CH₂Cl₂–MeOH–water (7:3:0.4) as eluent. The first fraction gave a syrup (180 mg), $[\alpha]_D +6.5^\circ$ ($c=1.25$, water), identical with L-rhamnose on PPC, and the second provided another syrup (210 mg), $[\alpha]_D +55.9^\circ$ ($c=1.23$, water), identified as D-glucose. Acetylation of VII on heating with Ac₂O–pyridine (1:1) gave VII acetate as colorless needles (from MeOH), mp 204–207°, $[\alpha]_D -62.5^\circ$ ($c=1.09$, CHCl₃), -60.5° ($c=1.50$, CHCl₃), no hydroxy absorptions on the IR spectrum. Mass Spectrum *m/e*: 974 (M⁺), 561 (C₂₄H₃₃O₁₅⁺), 273 (C₁₂H₁₇O₇⁺). NMR (CDCl₃) (100 MHz, JEOL PS-100): 0.80 (3H, s, 18-CH₃), 1.03 (3H, s, 19-CH₃), 1.21 (3H, d, *J* = 7 Hz, 6-CH₃ of rhamnose), 1.99–2.10 (18H, AcO \times 6), 4.58 (1H, d, *J* = 7 Hz, C₁-H of glucose), 4.98 (1H, d, *J* = 1.5 Hz, C₁-H of rhamnose) (Fig. 1). Anal. Calcd. for C₅₁H₇₄O₁₈: C, 62.83; H, 7.60. Found: C, 62.81; H, 7.65. Complete methylation of VII (260 mg) by the Hakomori method¹⁴⁾ provided VII methylate (180 mg) as colorless needles (from MeOH), mp 165–166°, $[\alpha]_D -92.3^\circ$ ($c=1.10$, CHCl₃), no hydroxy absorptions on the IR spectrum. Mass Spectrum *m/e*: 806 (M⁺), 393 (C₁₈H₃₃O₉⁺), 189 (C₉H₁₇O₄⁺). NMR (CDCl₃): 1.23 (3H, d, *J* = 6 Hz, 6-CH₃ of rhamnose), 4.35 (1H, d, *J* = 7 Hz, C₁-H of glucose), 5.21 (1H, broad s, C₁-H of rhamnose). The methylate (110 mg) was methanolized on refluxing with 2N HCl in MeOH (15 ml) for 2 hr. The reaction mixture was diluted with MeOH and passed through an Amberlite A-400 column. The eluate was evaporated and the residue was examined by GLC. Two peaks were detected and identified as those of methyl pyranosides of 3,4,6-tri-O-methyl- α -D-glucose and 2,3,4-tri-O-methyl- α -L-rhamnose by comparison with the authentic samples¹⁵⁾ and the reference compounds¹⁵⁾ such as methyl pyranosides of 2,3,4-, 2,3,6- and 2,4,6-tri-O-methyl- α -D-glucoses.

Td (VIII)—Colorless needles, mp 265–271° (decomp.), $[\alpha]_D -83.1^\circ$ ($c=1.03$, pyridine), no spiroketal absorptions on the IR spectrum and positive to the Ehrlich test. NMR (C₅D₅N): 3.15 (3H, s, OCH₃). VIII (200 mg) in water (15 ml) was incubated with almond emulsin¹⁶⁾ (25 mg) overnight at 37°. The precipitates were collected by filtration and crystallized from MeOH to give colorless needles, mp 240–245° (decomp.), $[\alpha]_D -102.4^\circ$ ($c=1.21$, pyridine), which were identified (TLC, mixed mp, IR) with an authentic sample of VII. The filtrate was concentrated *in vacuo* and examined by PPC. Only glucose was detected. VIII was boiled with Ac₂O–pyridine (1:2) for 1.5 hr and the product was crystallized from hexane to give VIII peracetate as a white powder, $[\alpha]_D -41.4^\circ$ ($c=1.14$, CHCl₃). Mass Spectrum *m/e*: 1304 (M⁺–MeOH), 561 (C₂₄H₃₃O₁₅⁺), 331 (C₁₄H₁₉O₉⁺), 273 (C₁₂H₁₇O₇⁺).

Td' (IX)—VIII (210 mg) in water (10 ml) and acetone (20 ml) was refluxed for 7 hr and left stand in a refrigerator to give IX (25 mg) as colorless needles, mp 266–270° (decomp.), $[\alpha]_D -85.2^\circ$ ($c=0.96$, pyridine),

14) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

15) H. Okabe, N. Koshito, K. Tanaka, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2394 (1971).

16) Sigma Chem. Co.

Rf 0.35 (VIII 0.39, solv. 1), no methoxy signal on a NMR spectrum (in C_5D_5N). On refluxing with MeOH VIII was regenerated.

Tb (X)—Colorless needles, mp 273–276° (decomp.), $[\alpha]_D -117.8^\circ$ ($c=0.87$, pyridine), $[M]_x -870^\circ$, negative to the Ehrlich test. IR ν_{max}^{KBr} cm^{-1} : 3600–3200 (OH), 980, 920, 900, 890 (intensity 900 > 920, 25D-spiroketal). *Anal.* Calcd. for $C_{39}H_{62}O_{13} \cdot 2H_2O$: C, 60.46; H, 8.52. Found: 60.47, H, 8.50.

Hydrolysis of X—X (4.52 g) was boiled with 1.5N HCl in MeOH (60 ml) for 2 hr, the solution was cooled and diluted with water (100 ml).

The precipitates were collected by filtration, dried and chromatographed over silica gel (hexane–AcOEt (3:1)) to give three fractions. Fr. 1 was crystallized from MeOH to give colorless needles (320 mg), mp 161–163°, $[\alpha]_D -99.8^\circ$ ($c=0.82$, $CHCl_3$), which were identified as bethogenin (III) by comparison (mixed mp, IR, TLC) with an authentic sample.¹⁾ Fr. 2 gave on crystallization from MeOH colorless needles (440 mg), mp 234–237°, $[\alpha]_D -110.1^\circ$ ($c=1.12$, $CHCl_3$), identical with pennogenin (I)¹⁾ and Fr. 3 afforded colorless needles (from MeOH) (360 mg), mp 187–189°, $[\alpha]_D -198.2^\circ$ ($c=1.00$, $CHCl_3$), which were identified as kryptogenin (II).¹⁾

The filtrate of the hydrolysate was treated in the same way as the sugar portion from VII to give two syrups. One (260 mg), $[\alpha]_D +7.4^\circ$ ($c=2.50$, water), was identical with L-rhamnose on PPC, and the other (230 mg), $[\alpha]_D +56.2^\circ$ ($c=2.36$, water), was identified as D-glucose.

X Acetate (XIII)—Prepared on heating X (120 mg) with Ac_2O –pyridine (1:1) (5 ml) for 2 hr. Colorless needles (from MeOH), mp 195–197°, $[\alpha]_D -51.8^\circ$ ($c=1.21$, $CHCl_3$). Mass Spectrum m/e : 990 (M^+), 972 (M^+-H_2O), 561 ($C_{24}H_{33}O_{15}^+$), 412 ($C_{27}H_{40}O_3^+$), 394 ($C_{27}H_{38}O_2^+$), 273 ($C_{12}H_{17}O_7^+$). NMR ($CDCl_3$) (100 MHz, JEOL PS-100): 0.80 (3H, s, 18- CH_3), 1.01 (3H, s, 19- CH_3), 1.20 (3H, d, $J=7$ Hz, 6- CH_3 of rhamnose), 1.98–2.10 (18H, $AcO \times 6$), 3.94 (1H, t, $J=7$ Hz, $C_{16}-H$), 4.56 (1H, d, $J=7$ Hz, C_1-H of glucose), 4.96 (1H, d, $J=1.5$ Hz, C_1-H of rhamnose) (Fig. 1). *Anal.* Calcd. for $C_{51}H_{74}O_{19} \cdot 1/2H_2O$: C, 61.26; H, 7.51. Found: C, 61.31; H, 7.52.

X Methylate (XIV)—X (120 mg) was methylated by the Hakomori method and the product was crystallized from MeOH to give XIV (43 mg) as colorless needles, mp 176–178°, $[\alpha]_D -90.1^\circ$ ($c=1.02$, $CHCl_3$). Mass Spectrum m/e : 822 (M^+), 804 (M^+-H_2O), 412 ($C_{27}H_{40}O_3^+$), 394 ($C_{27}H_{38}O_2^+$), 393 ($C_{18}H_{33}O_9^+$), 189 ($C_9H_{17}O_8^+$). NMR ($CDCl_3$): 1.23 (3H, d, $J=6$ Hz, 6- CH_3 of rhamnose), 4.35 (1H, d, $J=7$ Hz, C_1-H of glucose), 5.22 (1H, d, $J=1.5$ Hz, C_1-H of rhamnose). XIV (20 mg) was methanolized, worked up and the sugar portion was examined by GLC in the same way as in VII methylate to give the same result.

Prosapogenin (XV) of X—X (1.6 g) was hydrolyzed with 0.5N H_2SO_4 in 50% EtOH (50 ml) on a water-bath for 35 min. The hydrolysate was neutralized with 5% KOH in MeOH, concentrated *in vacuo*, diluted with water and extracted with BuOH (40 ml). The BuOH layer was evaporated and the residue was chromatographed over silica gel (CH_2Cl_2 –MeOH–water (8:2:0.1)). A fraction showing one spot (*Rf* 0.71, solv. 1) was crystallized from MeOH to give XV as colorless needles (210 mg), mp 275–278° (decomp.), $[\alpha]_D -117.7^\circ$ ($c=1.23$, pyridine), $[M]_{XV} -697^\circ$. $[M]_X - [M]_{XV} -173^\circ$ ($[M]$ of methy L-rhamnopyranoside: α , -111° ; β , $+170^\circ$). Identified by mixed mp and on TLC with 3-O- β -D-glucopyranoside of I synthesized as below.

3-O- β -D-Glucopyranoside (XV) of I—I (210 mg) and 1 α -bromo-1-deoxy-D-glucopyranose tetraacetate (350 mg) in $CHCl_3$ (20 ml) were stirred with Ag_2O (850 mg) at room temperature for 15 hr (Königs-Knorr method).¹⁷⁾ The precipitates were filtered off, 5% KOH in MeOH (15 ml) was added to the filtrate and the mixture was refluxed for 30 min. The solvent was evaporated *in vacuo*, the residue was treated with water and with benzene. The insoluble product was crystallized from MeOH to give XV as colorless needles, mp 273–277° (decomp.), $[\alpha]_D -117.2^\circ$ ($c=0.94$, pyridine). XV was acetylated in the same way as X to give the tetraacetate as colorless needles (from MeOH), mp 205–208°, $[\alpha]_D -63.9^\circ$ ($c=1.44$, $CHCl_3$). Mass Spectrum m/e : 760 (M^+), 412 ($C_{27}H_{40}O_3^+$), 394 ($C_{27}H_{38}O_2^+$), 331 ($C_{14}H_{19}O_9^+$). XV (160 mg) was methylated by the Kuhn method¹⁸⁾ to give the tetramethyl ether as colorless leaflets (from MeOH) (55 mg), mp 174–175°, $[\alpha]_D -82.6^\circ$ ($c=0.99$, $CHCl_3$). NMR ($CDCl_3$): 0.80 (3H, s, 18- CH_3), 1.00 (3H, s, 19- CH_3), 3.52–3.61 (12H, $OMe \times 4$), 3.97 (1H, t, $J=7$ Hz, $C_{16}-H$), 4.31 (1H, d, $J=7$ Hz, C_1-H of glucose), 5.31 (1H, m, C_6-H).

Tc (XI)—Colorless needles, mp 297–299° (decomp.), $[\alpha]_D -126.7^\circ$ ($c=0.86$, pyridine), negative to the Ehrlich test. IR ν_{max}^{KBr} cm^{-1} : 3600–3400 (OH), 980, 920, 900, 890 (intensity 900 > 920, 25D-spiroketal). *Anal.* Calcd. for $C_{45}H_{72}O_{17} \cdot H_2O$: C, 59.87; H, 8.21. Found: C, 59.70; H, 8.32. Acid hydrolysis of XI provided, in common with X, glucose, rhamnose and three kinds of aglycone, I, II and III.

XI Acetate (XVI)—Prepared in the same way as XIII and crystallized from MeOH. Colorless needles, mp 112–115°, $[\alpha]_D^{25} -47.7^\circ$ ($c=1.06$, $CHCl_3$). Mass Spectrum m/e : 1220 (M^+), 1202 (M^+-H_2O), 412 ($C_{27}H_{40}O_3^+$), 394 ($C_{27}H_{38}O_2^+$), 273 ($C_{12}H_{17}O_7^+$). NMR ($CDCl_3$): 0.81 (3H, s, 18- CH_3), 1.00 (3H, s, 19- CH_3), 1.16 (6H, d, $J=6$ Hz, 6- CH_3 of rhamnose $\times 2$), 1.92–2.10 (24H, $AcO \times 8$), 3.94 (1H, t, $J=7$ Hz, $C_{16}-H$), 5.00 (2H, broad s, C_1-H of rhamnose $\times 2$) (dioscin acetate:⁹⁾ 0.80 (3H, s, 18- CH_3), 1.00 (3H, s, 19- CH_3), 1.17 (6H, d, $J=6$ Hz, 6- CH_3 of rhamnose $\times 2$), 1.93–2.11 (24H, $AcO \times 8$), 5.01 (2H, broad s, C_1-H of rhamnose $\times 2$).

17) W. Königs and E. Knorr, *Chem. Ber.*, **34**, 957 (1901).

18) R. Kuhn, I. Löw, and H. Trischmann, *Chem. Ber.*, **88**, 1492, 1690 (1955).

XI Methylate—XI (2.1 g) was methylated as in the case of X and the product was purified by chromatography over silica gel using solv. 2 as eluent to give the methylate (1.7 g) as a white solid, $[\alpha]_D -82.8^\circ$ ($c=0.93$, CHCl_3), homogeneous on TLC (solv. 2). The methylate (1.6 g) was methanolized on refluxing with 2N HCl in MeOH (20 ml) for 2.5 hr. The mixture was diluted with water, the precipitates were filtered off and the filtrate was extracted with AcOEt (100 ml). The organic layer was washed with water, dried and evaporated to a syrup. A part of the residue was examined by GLC to show two peaks, which were identified as those of methyl pyranosides of 3,6-di-O-methyl-glucose¹⁹ and 2,3,4-tri-O-methyl-rhamnose. The remaining syrup was hydrolyzed on boiling with 0.5N HCl for 2.5 hr, and the hydrolysate was neutralized with 5% KOH and placed on a Sephadex LH-20 column. The MeOH eluate was evaporated and the residue was chromatographed over silica gel (CH_2Cl_2 -MeOH-water (8:2:0.1)) to give 2,3,4-tri-O-methyl-L-rhamnopyranose, a syrup, $[\alpha]_D +24.8^\circ$ ($c=0.69$, water), (lit.¹⁹) $[\alpha]_D +27^\circ$, and 3,6-di-O-methyl-D-glucopyranose, colorless needles (from AcOEt), mp 113–115°, $[\alpha]_D +55.2^\circ$ ($c=0.64$, water) (lit.¹⁹) mp 113–116°, $[\alpha]_D +62^\circ$.

Tg (XII)—Colorless needles, mp 224–228° (decomp.), $[\alpha]_D -136.0^\circ$ ($c=0.65$, pyridine), negative to the Ehrlich test. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400–3200 (OH), 980, 920, 900, 890 (intensity 900>920, 25D-spiroketal). Anal. Calcd. for $\text{C}_{51}\text{H}_{82}\text{O}_{21} \cdot 3\text{H}_2\text{O}$: C, 56.45; H, 8.12. Found: C, 56.55; H, 8.11. Acid hydrolysis gave the same result as in X and XI.

XII Methylate (XVII)—XII (280 mg) was methylated by the Hakomori method and the product was crystallized from MeOH to give XVII (121 mg) as colorless needles, mp 172–175°, $[\alpha]_D -97.1^\circ$ ($c=0.52$, CHCl_3). Mass Spectrum m/e : 1170 (M^+), 1152 ($\text{M}^+-\text{H}_2\text{O}$), 412 ($\text{C}_{27}\text{H}_{40}\text{O}_3^+$), 394 ($\text{C}_{27}\text{H}_{38}\text{O}_2^+$), 363 ($\text{C}_{17}\text{H}_{31}\text{O}_8^+$), 189 ($\text{C}_9\text{H}_{17}\text{O}_4^+$). NMR (CDCl_3): 0.83 (3H, s, 18- CH_3), 1.04 (3H, s, 19- CH_3), 1.26 (9H, d, $J=6$ Hz, 6- CH_3 of rhamnose $\times 3$), 3.40–3.60 (OCH_3), 4.40 (1H, d, $J=7$ Hz, C_1 -H of glucose), 5.03 (1H, broad s, C_1 -H of rhamnose), 5.22 (2H, broad s, C_1 -H of rhamnose $\times 2$) (methylate of Pb (XVIII): 0.80 (3H, s, 18- CH_3), 1.03 (3H, s, 19- CH_3), 1.25 (9H, d, $J=6$ Hz, 6- CH_3 of rhamnose $\times 3$), 3.40–3.60 (OCH_3), 4.40 (1H, d, $J=7$ Hz, C_1 -H of glucose), 5.03 (1H, broad s, C_1 -H of rhamnose) 5.21 (2H, broad s, C_1 -H of rhamnose $\times 2$)). Methanolysis of XVII and examination of the resulting methylated sugars were carried out in the same way as in XIV. Methyl pyranosides of 3,6-di-O-methyl-glucose, and 2,3,4-tri-O-methyl- and 2,3-di-O-methyl-rhamnoses¹⁹ were detected on GLC.

Conversion of XVII to Pb (XVIII) Methylate¹⁰ (Chart 5)—XVII (130 mg) in Ac_2O (6 ml) was refluxed with *p*-toluenesulfonic acid (2 mg) for 30 min.^{3b} The reaction mixture was poured into water, extracted with ether and the ether layer was evaporated to give an oil (110 mg). It was hydrogenated over Pd/BaSO₄ (30 mg) in AcOEt (35 ml) for 30 min.^{3b,20} catalysts were filtered off, the filtrate was evaporated to dryness, and the residue (95 mg) was refluxed with 5% KOH in MeOH (20 ml) for 20 min. The mixture was cooled, acidified with 1N HCl^{3b} and extracted with ether. The ether-soluble fraction showed two spots (*Rf* 0.36, 0.34; solv. 2) on TLC and was chromatographed over silica gel by using the above solvent as eluent. The first fraction (*Rf* 0.36) was crystallized from MeOH to give XVIII methylate (10.7 mg) as colorless needles, mp 137–139°, $[\alpha]_D -108.5^\circ$ ($c=0.36$, CHCl_3). Identified with an authentic sample,¹⁰ mp 138–140°, $[\alpha]_D -109.8^\circ$ ($c=0.54$, CHCl_3), by direct comparison (*Rf*, mixed mp, IR, NMR).

Te (XIX)—Colorless needles, mp 275–280° (decomp.), $[\alpha]_D -84.6^\circ$ ($c=1.02$, pyridine), *Rf* 0.31 (solv. 1), negative to the Ehrlich test and no spiroketal absorptions on the IR spectrum. XIX acetate (white powder) prepared in an usual way showed $[\alpha]_D -38.7^\circ$ ($c=0.84$, CHCl_3), *Rf* 0.21 (solv. 3) and no methoxy signal on the NMR spectrum. Hydrolysis of XIX with 1N H_2SO_4 in 50% EtOH gave I (identified by mixed mp with an authentic sample), glucose and rhamnose (PPC). XIX (2.1 g) in water (25 ml) was incubated with almond emulsin (100 mg) at 37° overnight. White precipitates were collected by filtration and crystallized from MeOH to give X (410 mg) as colorless needles, mp 274–278° (decomp.), $[\alpha]_D -116.2^\circ$ ($c=1.21$, pyridine), (acetate, mp 196–197°, $[\alpha]_D -53.2^\circ$ ($c=0.82$, CHCl_3)), which were identified with the authentic samples by direct comparison. The filtrate of hydrolysate was evaporated and the residue was placed on a silica gel column. The eluate (CHCl_3 -MeOH (1:1)) was evaporated to give a syrup, $[\alpha]_D +62.2^\circ$ ($c=1.8$, water), identified with D-glucose on PPC.

Te' (XX)—XIX (1.1 g) in MeOH (100 ml) was refluxed for 3.5 hr. The solution was concentrated, left stand and the white precipitates were collected and crystallized from MeOH to give XX as colorless needles, mp 272–279° (decomp.), $[\alpha]_D -83.1^\circ$ ($c=0.96$, pyridine), *Rf* 0.39 (solv. 1), negative to the Ehrlich test and no spiroketal absorptions on the IR spectrum. Hydrolysis with acid and emulsin gave the same results as in XIX.

XX Acetate—XX (400 mg) was acetylated with Ac_2O -pyridine (1:2) on a water-bath for 1.5 hr. The product could not be crystallized and was precipitated from a hexane solution to give the acetate as a white powder, mp 131–135°, $[\alpha]_D -41.2^\circ$ ($c=1.01$, CHCl_3), *Rf* 0.38 (solv. 3). NMR (CDCl_3): 3.17 (3H, s, OCH_3). Mass Spectrum m/e : 1320 (M^+-MeOH), 561 ($\text{C}_{24}\text{H}_{38}\text{O}_{15}^+$), 412 ($\text{C}_{27}\text{H}_{40}\text{O}_3^+$), 394 ($\text{C}_{27}\text{H}_{38}\text{O}_2^+$), 331 ($\text{C}_{14}\text{H}_{19}\text{O}_9^+$), 273 ($\text{C}_{12}\text{H}_{17}\text{O}_7^+$).

19) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **10**, 703 (1962).

20) St. Kaufmann and G. Rosenkranz, *J. Am. Chem. Soc.*, **70**, 3502 (1948).

Baeyer-Villiger Oxidation of XX (Chart 6)—XX (1.1 g) suspended in $(\text{CH}_2\text{Cl})_2$ (22 ml) was oxidized with 90% formic acid (30 ml) and 30% H_2O_2 (3.2 ml) and the product was treated with alkali and acetylated in the same way as reported⁴⁾ for proto-dioscin acetate. The crude acetate (0.76 g) was chromatographed over silica gel by using hexane-AcOEt (2:1) as eluent.

Methyl γ -Methyl- δ -hydroxypentanoate β -D-Glucopyranoside (XXII): The first fraction gave the tetraacetate of XXII as a colorless oil (30 mg), $[\alpha]_D^{25} -18.7^\circ$ ($c=1.28$, CHCl_3). NMR and mass spectra were identical with those of the authentic sample⁴⁾ obtained from proto-dioscin acetate. The acetate was saponified with NH_3 in MeOH and treated in water with almond emulsin to give glucose (PPC).

5 α -Pregnane-3 β ,5 α ,6 β ,16 β ,17 α ,20 α -hexaol (XXI): The second fraction in the chromatography provided a white solid (240 mg). It was boiled with 1.5N H_2SO_4 in 50% EtOH (40 ml) for 3 hr. The reaction mixture was neutralized with 5% KOH in EtOH, evaporated and shaken with BuOH and water. BuOH layer was evaporated to dryness and the residue was chromatographed over silica gel. A fraction eluted with CH_2Cl_2 -MeOH-water (7:2:0.1) was crystallized from dil. MeOH to give XXI as colorless needles, mp 282–285° (decomp.), $[\alpha]_D^{25} -2.1^\circ$ ($c=0.61$, pyridine). XXI was acetylated on heating with Ac_2O -pyridine (2:1) to provide the tetraacetate as a white powder, mp 145–148°, $[\alpha]_D^{25} -15.2^\circ$ ($c=0.72$, CHCl_3). NMR (CDCl_3): 0.90 (3H, s, 18- CH_3), 1.15 (3H, s, 19- CH_3), 1.25 (3H, d, $J=6$ Hz, 21- CH_3), 1.93–2.05 (12H, OAc $\times 4$).

XXI from I: To a solution of I (0.9 g) in $(\text{CH}_2\text{Cl})_2$ (20 ml) were added 90% formic acid (25 ml) and 30% H_2O_2 (3 ml), and the mixture was heated at 50° for 30 min. Solvent being removed, the residue was treated with 3% KOH in MeOH on a water-bath for 10 min, the mixture was neutralized with dil. HCl, evaporated, and shaken with water and BuOH. BuOH soluble product was crystallized from dil. MeOH to give XXI (230 mg) as colorless needles, mp 282–284° (decomp.), $[\alpha]_D^{25} -2.7^\circ$ ($c=1.21$, pyridine). XXI prepared from I was identified with that from XX by mixed mp and by comparison of the NMR spectra.

Tk (XXIII)—Colorless needles, mp 238–241° (decomp.), $[\alpha]_D^{30} -129.4^\circ$ ($c=0.51$, EtOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600–3200 (OH), 1740, 1710 (C=O), no spiroketal absorptions. Anal. Calcd. for $\text{C}_{33}\text{H}_{52}\text{O}_9$: C, 66.89; H, 8.78. Found: C, 66.81; H, 8.77. XXIII (210 mg) was boiled with 1N H_2SO_4 in 50% EtOH (15 ml) for 1.5 hr to give an aglycone and glucose (PPC). The former was acetylated to give colorless plates (21 mg) (from MeOH), mp 150–152°, $[\alpha]_D^{25} -148.2^\circ$ ($c=0.73$, CHCl_3), identical (mixed mp, IR and mass spectra) with an authentic sample⁴⁾ of II diacetate.

XXIII Acetate—Prepared by usual acetylation of XXIII. Colorless leaflets (from MeOH), mp 181–182°, $[\alpha]_D^{30} -114.6^\circ$ ($c=0.96$, CHCl_3). Mass Spectrum m/e : 802 (M^+), 394 ($\text{C}_{27}\text{H}_{38}\text{O}_2^+$), 376 ($\text{C}_{27}\text{H}_{36}\text{O}^+$), 331 ($\text{C}_{14}\text{H}_{18}\text{O}_9^+$). ORD ($c=0.064$, EtOH) $[M]$ (nm): +7980° (271) (peak), –10260° (316) (trough) (II diacetate, ORD ($c=0.070$, EtOH) $[M]$ (nm): +12030° (271) (peak), –16450° (316) (trough)) (Fig. 2).

Conversion¹¹⁾ of XXIII to Trillin (3-O- β -D-Glucopyranoside of IV) (Chart 7)—XXIII (110 mg) in iso-PrOH (25 ml) was reduced with NaBH_4 (36 mg) at room temperature for 1.5 hr. The mixture was acidified with 1N HCl in MeOH, left stand for 1 hr, neutralized with 5% KOH in MeOH and evaporated. Water was added to the residue, the insoluble white precipitates were collected and crystallized from MeOH to give trillin as colorless needles, mp 264–266° (decomp.), $[\alpha]_D^{25} -102.3^\circ$ ($c=1.01$, pyridine), –93.2° ($c=0.93$, dioxane). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3600–3200 (OH), 979, 918, 899, 862 (intensity 899>918, 25 β -spiroketal). It was identified with an authentic sample⁶⁾ by mixed mp and by comparison of the IR spectra.

Tf (XXIV)—Colorless needles, mp 265–268° (decomp.), $[\alpha]_D^{30} -80.1^\circ$ ($c=0.73$, pyridine). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600–3300 (OH), 1720–1700 (C=O), 1630 (C=C). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 242 ($\epsilon=8250$). ORD ($c=0.103$, EtOH) $[M]$ (nm): +5140° (322) (peak), –4760° (370) (trough). NMR ($\text{C}_5\text{D}_5\text{N}$): 0.95 (3H, s, 18- CH_3), 1.02 (3H, s, 19- CH_3), 1.95 (3H, s, $\text{CH}_3\text{-C(R)=C<}$). Hydrolysis with 2N H_2SO_4 in 50% EtOH gave glucose, rhamnose and an ether-soluble product which showed on TLC five spots, R_f 0.83, 0.65, 0.63, 0.23 and 0.20 (solvent 2).

XXIV Acetate (XXV)—XXIV (50 mg) was acetylated on heating with Ac_2O -pyridine (1:1) and the product was crystallized from dil. MeOH to give XXV as colorless needles, mp 190–192°, $[\alpha]_D^{30} -67.1^\circ$ ($c=0.83$, CHCl_3). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 243 ($\epsilon=10300$). ORD ($c=0.081$, EtOH) $[M]$ (nm): +3500° (322) (peak), –3240° (372) (trough) (Fig. 3). Mass Spectrum m/e : 1318 (M^+), 561 ($\text{C}_{24}\text{H}_{33}\text{O}_{15}^+$), 392 ($\text{C}_{27}\text{H}_{36}\text{O}_2^+$), 331 ($\text{C}_{14}\text{H}_{18}\text{O}_9^+$), 273 ($\text{C}_{12}\text{H}_{17}\text{O}_7^+$).

Conversion of I to 17(20)-Dehydrokryptogenin (XXVI) Diacetate via Pseudokryptogenin Diacetate (XXVII) (Chart 8)—I (120 mg) in Ac_2O (10 ml) was refluxed with p -toluenesulfonic acid (3 mg) for 15 min.^{3b)} The mixture was poured into water, extracted with ether and the ether layer was evaporated. The residue was chromatographed over silica gel by using hexane-AcOEt (4:1) as eluent to give XXVII as colorless oil. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 228.5 ($\epsilon=18000$). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 1750 (OAc), 1580 (enol). NMR (CDCl_3): 0.90 (3H, s, 19- CH_3), 0.95 (3H, d, $J=6$ Hz, 27- CH_3), 1.10 (3H, s, 18- CH_3), 1.92 (3H, s, 21- CH_3), 2.00 (3H, s, OAc), 2.02 (3H, s, OAc), 3.90 (2H, d, $J=6$ Hz, $\text{C}_{26}\text{-H}_2$), 4.65 (1H, m, $\text{C}_3\text{-H}$). XXVII (65 mg) was oxidized¹²⁾ with CrO_3 (10 mg) in AcOH (5 ml) at room temperature under stirring for 1 hr and worked up as usual to give XXVI diacetate as colorless needles (from dil. MeOH), mp 109–110°, $[\alpha]_D^{25} -182.3^\circ$ ($c=1.02$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1740–1730 (OAc), 1717, 1704 (C=O), 1632 (C=C). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 245 ($\epsilon=12000$). ORD ($c=0.057$, EtOH) $[M]$ (nm): +7580° (318) (peak), –6120° (370) (trough) (Fig. 3). NMR (CDCl_3): 0.95 (3H, d, $J=6$ Hz, 27- CH_3), 1.08 (3H, s, 19- CH_3), 1.12 (3H, s, 18- CH_3), 1.95 (3H, s, 21- CH_3), 2.04 (3H, s, OAc), 2.07 (3H, s, OAc), 3.93

(2H, d, $J=6$ Hz, $C_{26}-H_2$), 4.55 (1H, m, C_8-H), 5.37 (1H, m, C_6-H). *Anal.* Calcd. for $C_{31}H_{44}O_6$: C, 72.62; H, 8.65. Found: C, 72.73; H, 8.52.

Conversion of XIX Acetate to Corresponding Glycoside Acetate of XXVI (Chart 8)—XIX acetate (120 mg) was treated in the same way as in I described above. The ether-soluble crude product (syrup) was directly oxidized with CrO_3 (10 mg) in AcOH (4 ml) at room temperature for 1.5 hr. Excess CrO_3 was decomposed with MeOH, the mixture was diluted with water and extracted with ether. Ether-soluble product was chromatographed over silica gel by using solv. 2 as eluent to give XXVI glycoside acetate as colorless needles (from dil. MeOH), mp 189–191°, $[\alpha]_D^{25} -69.2^\circ$ ($c=0.73$, $CHCl_3$). UV λ_{max}^{EtOH} nm: 243 ($\epsilon=11500$). ORD ($c=0.092$, EtOH) $[M]$ (nm): +4200° (322) (peak), -3700° (373) (trough). It was identified with XXV by direct comparison (mixed mp, IR, NMR).

Conversion of XIX to XXIV—To a solution of XIX (160 mg) in $CHCl_3$ -MeOH-water (7:5:0.4) was added Kieselgel (0.05–0.2 mm) (Merck) (250 mg) and the mixture was heated on a water-bath for 1.5 hr. Kieselgel was filtered off, the filtrate was evaporated and the residue was crystallized from MeOH to give XXIV as colorless needles, mp 263–268° (decomp.), $[\alpha]_D -82.4^\circ$ ($c=1.02$, pyridine), R_f 0.39 (XIX, R_f 0.31; solv. 1). IR ν_{max}^{KBr} cm^{-1} : 3600–3300 (OH), 1720–1700 (C=O), 1630 (C=C). UV λ_{max}^{EtOH} nm: 242 ($\epsilon=8640$). XXIV thus obtained was boiled with water without any change (TLC) and the possible contamination of XX was excluded.²¹⁾ It was identified with XXIV isolated from the plant extracts by mixed mp and on TLC.

In another run, XIX (180 mg) and Kieselgel (280 mg) in dioxane or acetone-water (2:1) were heated on a water-bath for 3 hr. The residue provided by evaporation of the filtrate showed one spot (R_f 0.39) and was crystallized from MeOH to give the same product as above.

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21) XIX might be converted in the procedure using MeOH to XX which shows the same R_f value as that of XXIV. However, when XX is boiled with water XIX should be regenerated.