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Steroid Saponins and Sapogenins of Underground Parts of *Trillium kamtschaticum* Pall. I. Component Sapogenins and Structure of Pennogenin

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From the rhizomes of *Trillium kamtschaticum* Pall. diosgenin, pennogenin (II″), kryptogenin (III), bethogenin (IV) and a new compound (XI), mp 149—151°, $[\alpha]_D$ −192.7°, were obtained. XI was assigned the structure, 26-chloro-26-deoxykryptogenin, and thought to be an artefact formed during hydrolysis with hydrochloric acid of a glycoside of kryptogenin or a related sapogenin. Of the two formulae II (Marker, *et al.*) and II′ (Heusler, *et al.*) of pennogenin, II was favored on the basis of NMR spectral and chemical evidences, and pennogenin is represented as 25D-spirost-5-ene-3β,17α-diol (II″) having the same configurations at C-16, 17, 20, and 22 as usual steroid sapogenins.

In 1942 Noller, *et al.*[2] isolated from the rhizomes of *Trillium erectum* (Liliaceae) (beth roots) a new steroid sapogenin bethogenin, and the next year Marker, *et al.*[3] obtained from the same source, along with diosgenin, four additional new sapogenins named nologenin, pennogenin, kryptogenin and fesogenin. These sapogenins and a nologenin glycoside "nolonin" were also found[4] in the rhizomes of *Dioscorea mexicana* later in 1947. Marker, *et al.*[3–5] assigned the structures I—V to nologenin, pennogenin, kryptogenin, bethogenin and fesogenin, respectively, and clarified their chemical relationship with one another as shown in Chart 1. Thus, "nolonin" which was given a tentative structure VI was regarded as a parent compound from which pennogenin (II), kryptogenin (III) and bethogenin (IV) were yielded secondarily during acid hydrolysis.

Recently, following the discovery by Schreiber, *et al.*[6] of a new furostanol 26-O-glucoside jurubine (VII), Tschesche, *et al.*[7] have isolated and characterized a furostane-3,22,26-triol 3,26-O-bisglycoside sarsaparilloside (VIII) and found that VIII provides on acid and enzymatic hydrolysis the corresponding spirostanol sarsasapogenin (IX) and its 3-O-monoglycoside parillin (X), respectively. Subsequently several kinds of analogous glycosides have been obtained[8] and it has also been demonstrated[8a] that the fresh materials of some Liliaceae

1) Location: *Katakasu, 812, Fukuoka.*

2) S. Liebermann, F.C. Chang, M.R. Barusch, and C.R. Noller, *J. Am. Chem. Soc.*, **64**, 2581 (1942); C.R. Noller and M.R. Barusch, *ibid.*, **65**, 1435 (1943).

3) 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).

4) R.E. Marker and J. Lopez, *J. Am. Chem. Soc.*, **69**, 2386, 2395 (1947).

5) R.E. Marker, R.B. Wagner, P.R. Ulshafer, E.L. Wittbecker, D.P.J. Goldsmith, and C.H. Ruof, *J. Am. Chem. Soc.*, **69**, 2167 (1947).

6) K. Schreiber and H. Ripperger, *Tetrahedron Letters*, **1966**, 5997; H. Ripperger, H. Budzikiewicz, and K. Schreiber, *Chem. Ber.*, **100**, 1725 (1967).

7) R. Tschesche, G. Lüdke, and G. Wulff, *Tetrahedron Letters*, **1967**, 2785; *idem*, *Chem. Ber.*, **102**, 1253 (1969).

8) *a*) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1162 (1968); *b*) R. Tschesche, B.T. Tjoa, G. Wulff, and R.V. Noronha, *Tetrahedron Letters*, **1968**, 5141; R. Tschesche, L. Seidel, S.C. Sharma, and G. Wulff, *Chem. Ber.*, **105**, 3397 (1972); J. Petricic and A. Radosevic, *Farmac. Glasnik*, **25**, 91 (1969) [*C.A.*, **71**, 64049 (1969)]; H. Sato and S. Sakamura, *Agr. Biol. Chem.*, 37, 225 (1973).

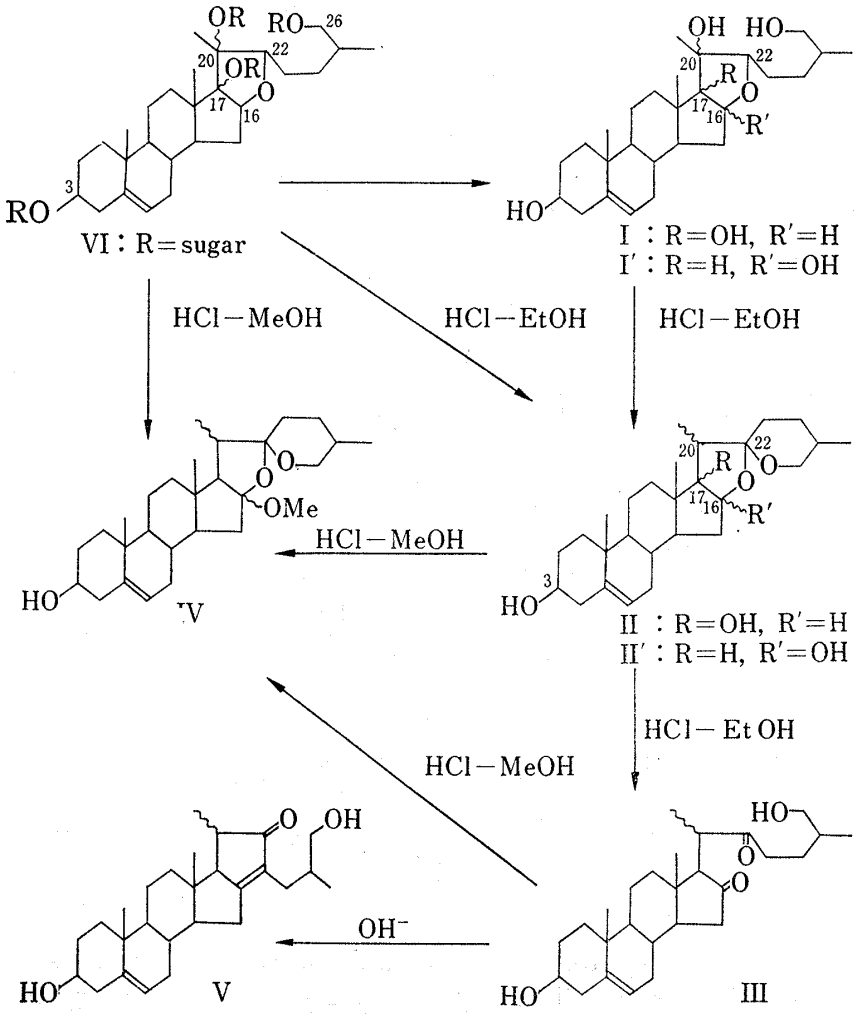


Chart 1

and Dioscoreaceae plants contain predominantly the furostanol bisglycosides, while in the stored materials the spirostanol glycosides are major.

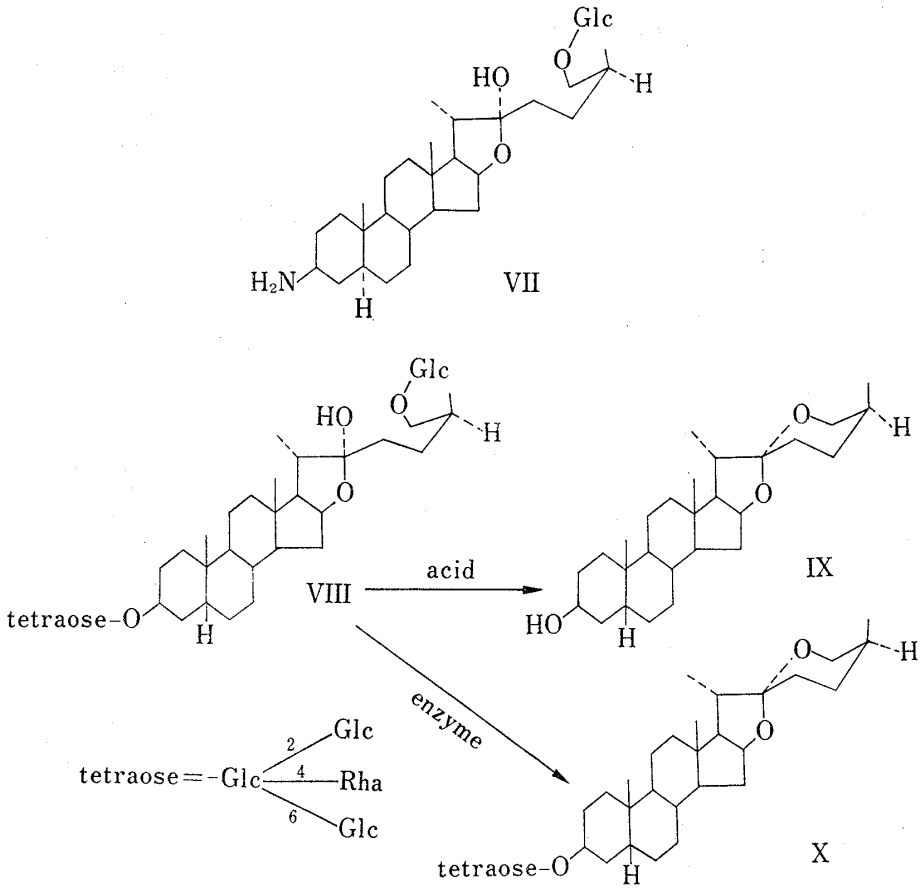
In consideration of these new findings together with the structural relationship of "nolonin" (VI), nologenin (I) and pennogenin (II), "nolonin" seems very likely to be a furostanol 3,26-O-bisglycoside corresponding to a pennogenin 3-O-monoglycoside. Accordingly the hydroxyl group at C-20 of I and VI might be at C-22 and II could be existent in the plants as its 3-O-glycosides. Furthermore nologenin and pennogenin were given, by Heusler, *et al.*,[9] the alternative structures I' and II',[10] respectively, where a tertiary hydroxyl group is located at C-16.

During these twenty years a number of papers[11] have appeared which concern the isolation of pennogenin from several different species of Liliaceae plants. However, in all cases it was

9) K. Heusler and A. Wettstein, *Chem. Ber.*, **87**, 1301 (1954).

10) Pennogenin is represented as II' in some literatures (R.N. Jones, E. Katzenellenbogen, and K. Dobriner, *J. Am. Chem. Soc.*, **75**, 158 (1953); C. Djerassii and R. Ehrlich, *ibid.*, **78**, 440 (1956); J. Elks, "Rodd's Chemistry of Carbon Compounds," 2nd ed., Vol. II, Part E, ed. by S. Coffey, Elsevier Publishing Co., Amsterdam, 1971, p. 45). Lowegenin isolated from *Tamus edulis* Lowe (Dioscoreaceae) was assigned the structure 3β,16-dihyroxy-25D-spirost-5-ene-11-one and assumed to be related to 11-ketonologenin (R.F. Barreira, A.G. Gonzalez, J.A.S. Rocio, and E.S. Lopez, *Phytochemistry*, **9**, 1641 (1970)).

11) For example, *a*) O.S. Girdano and A.G. Gonzalez, *An. Real Soc. Espan. Fis. Quim.*, Ser. B, **63**, 945 (1967) [*C.A.*, **68**, 6143 (1968)]; *b*) Wei-Kuang Huang, *Yao Hsueh Hsueh Pao*, **12**, 657 (1965) [*C.A.*, **64**, 11553 (1966)]; *c*) K. Takeda, A. Shimaoka, M. Iwasaki, and H. Minato, *Chem. Pharm. Bull.* (Tokyo), **13**, 691 (1965); *d*) T. Okanishi, A. Akahori, and I. Yasuda, *Ann. Rept. Shionogi Res. Lab.*, **10**, 395 (1960).



obtained from the acid hydrolysate of the extractives, and neither the second isolation of "nolonin" and nologenin nor the discovery of any glycoside of pennogenin has ever been reported. The chemical confirmation of the structures of nologenin and pennogenin has not been made either.[12]

In a hope to confirm the above assumption and attain a chemical proof for the structures of pennogenin and nologenin, a study has been carried out in this laboratory on the steroid saponins and sapogenins in some Liliaceae plants from which "nolonin", nologenin and pennogenin seem promising to be obtained.

This paper deals with isolation from the title plant materials of expected pennogenin together with diosgenin, kryptogenin (III), bethogenin (IV), and a new compound 26-chloro-26-deoxykryptogenin (XI), and with chemical evidence for the structure of pennogenin in favor of the Marker's formula (II).

The whole underground parts collected during June and packed in sawdust were sliced after about two weeks and immediately extracted with refluxing methanol. The glycoside fraction of the extractives was hydrolyzed with hydrochloric acid in methanol and treated as shown in Chart 2 to give five kinds of homogeneous compounds. Four of them were identified as diosgenin, pennogenin (II or II'), kryptogenin (III) and bethogenin (IV). The remaining compound (XI), mp 149—150°, $[\alpha]_D$—192.7°, showed an infrared (IR) spectrum and an optical rotatory dispersion (ORD) curve quite similar to those of III, but contained a halogen atom (positive Beilstein reaction). On a mass spectrum (Fig. 1) the peaks at *m/e* 448 (450),[13] 325, 297, 133 (135),[13] and 105 (107)[13] were respectively assigned to the molecular

12) An attempted synthesis of pennogenin from 17(20)-dehydrotigogenin was discontinued (M.J. Thompson, J.A. Moore, and E. Mosettig, *J. Org. Chem.*, **27**, 4108 (1962)).

13) Isotopic ion.

Sliced rhizomes of *Trillium kamtschaticum* PALL. (2.1 kg)
1) exted. with refluxing MeOH (2 liters)
2) evapd.
MeOH extractives (77.2 g)
partitioned between *n*-BuOH and water
org. layer | aq. layer
1) evapd. to brown powder (49.8 g)
2) refluxed with *n*-hexane
insol. portion (42.1 g) | sol. portion
1) hydrolyzed with 2N HCl in 50% MeOH (320 ml) for 4 hr
2) water added
3) extracted with ether
org. layer | aq. layer
1) evapd. to brown syrup (6.2 g)
2) treated with active charcoal
3) chromatog. on silica gel, (solv., *n*-hexane–AcOEt, 3:1, 5:2, 2:1, 1:1; v/v)

Fr. 1	Fr. 2	Fr. 3	Fr. 4	Fr. 5
needles (360 mg) diosgenin	needles (220 mg) bethogenin (IV)	needles (320 mg) pennogenin (II′)	plates (30 mg) 26-chloro-26-deoxy-kryptogenin (XI)	plates (420 mg) kryptogenin (III)

Chart 2

ion $C_{27}H_{41}O_3Cl^+$ and the fragments $C_{22}H_{29}O_2^+$, $C_{21}H_{29}O^+$, $C_6H_{10}OCl^+$, and $C_5H_{10}Cl^+$, indicating that XI is the 26-chloro-26-deoxy analog of III (M^+ 430). XI was acetylated in an usual manner to give a monoacetate $C_{29}H_{43}O_4Cl$, and while a nuclear magnetic resonance (NMR) spectrum of XI exhibited one proton multiplet due to C_3–H and two proton doublet (J=4.5 Hz) ascribable to C_{26}–H_2 both at 3.50 ppm, on that of the acetate the former one proton signal was shifted to 4.60 ppm and the latter remained at the same field. From the above data XI was defined as 3β-hydroxy-26-chlorocholest-5-ene-16,22-dione(26-chloro-26-deoxykryptogenin). It is thought to be an artefact possibly produced during hydrolysis with hydrochloric acid of a glycoside of kryptogenin or a related sapogenin.

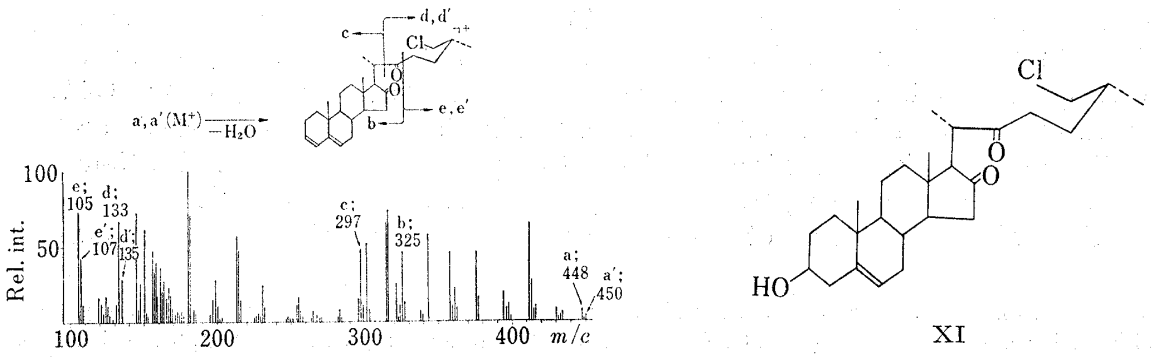


Fig. 1. Mass Spectrum of 26-Chloro-26-deoxykryptogenin

As for the structure of pennogenin, Marker's formula (II) was deduced from the fact[4] that it was correlated with nologenin which was degraded *via* 16-acyloxy-17-ol-20-one (XII) to give 3-hydroxypregn-5-ene-16,20-dione (XIII). On the other hand, Heusler, *et al.*[9] proposed

the formula II′ on the basis of an analogous experiment with a model compound 16β-acetoxy-17α-ol-20-one (XIV) which gave, on the same treatment with acid and alkali as Marker's, no XIII but the D-homo compound (s) (XV and/or XVI and/or XVII) (Chart 3).

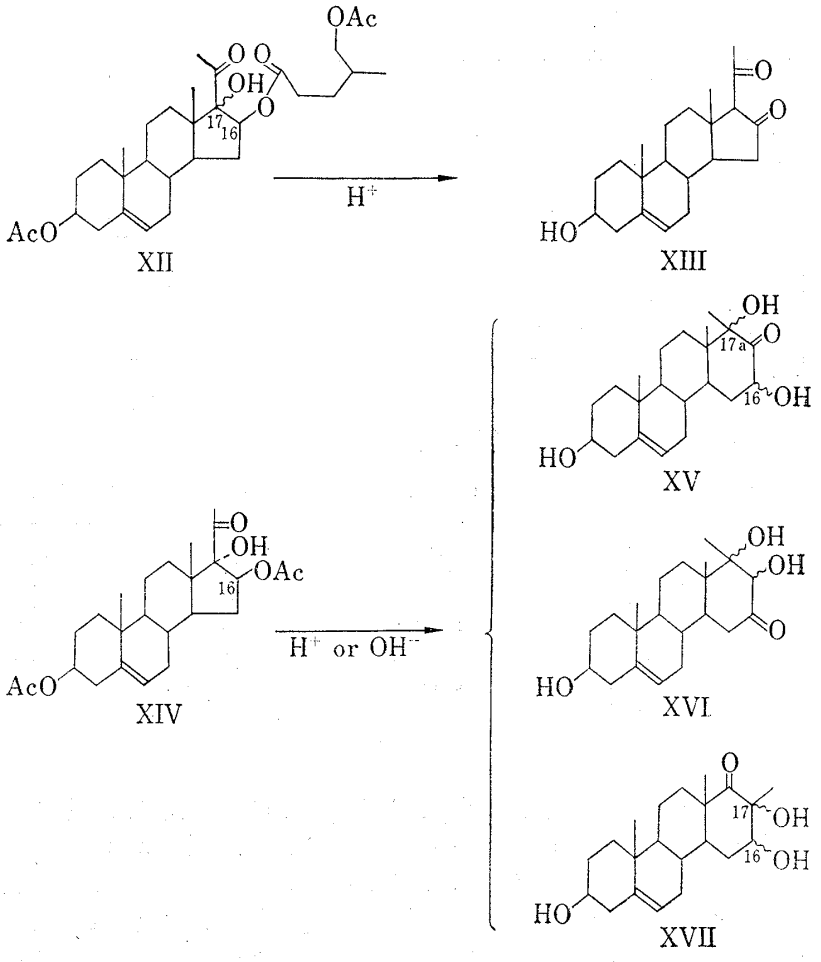


Chart 3

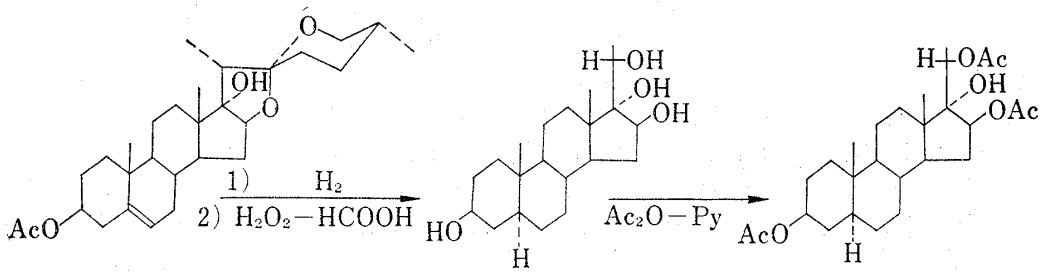
Although in the present study neither nologenin nor enough amounts of pennogenin could be obtained so as to reexamine the Marker's result, a repetition of the Heusler's experiment gave a crude product[14] in which XIII was not detected even on thin-layer chromatogram (TLC) run in parallel with an authentic sample.[15] The result seemed to be in favor of the Heusler's proposal. However, as reported by Tori, *et al.*,[16] on a NMR spectrum of pennogenin, the 18-methyl and 21-methyl signals appeared at lower and higher fields, respectively, than the corresponding values for those of diosgenin, suggesting a hydroxyl group at C-17. Moreover the one proton triplet at 3.96 ppm observed in a spectrum of pennogenin acetate but not in that of diosgenin acetate is ascribable to C_{16}-hydrogen only coupled with 15-methylene. The above spectral data support conversely the Marker's formula (II).

A chemical corroboration of the structure II was now undertaken as follows.

14) It showed on TLC two spots and the major component was isolated by column chromatography over silica gel as colorless needles (from $CHCl_3$), mp 240—242° (diacetate, mp 251—254°, $[\alpha]_D$ −28.4° ($CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3559 (OH), 1748 (OAc), 1702 (C=O)). This compound was assumed to be XV (16β, 17aβ-di-OH). The 16α-epimer of XIV was reported (V.A. Dubrovskii, A.A. Akhrem, and A.V. Kamernitskii, *Bull. Acad. Sci. USSR, Div. Chem. Sci.*, **1964**, 87) to give, on a similar treatment, XV (16α,17aα-di-OH) and XVII (16α,17α-di-OH).

15) Synthesized from diosgenin according to the method of Morita, *et al.*[18] (mp 180—182°, UV λ_{max}^{EtOH} nm: 283.5; lit.: mp 180—182°, UV λ_{max}^{EtOH} nm: 285;[18] mp 186—188°[17]).

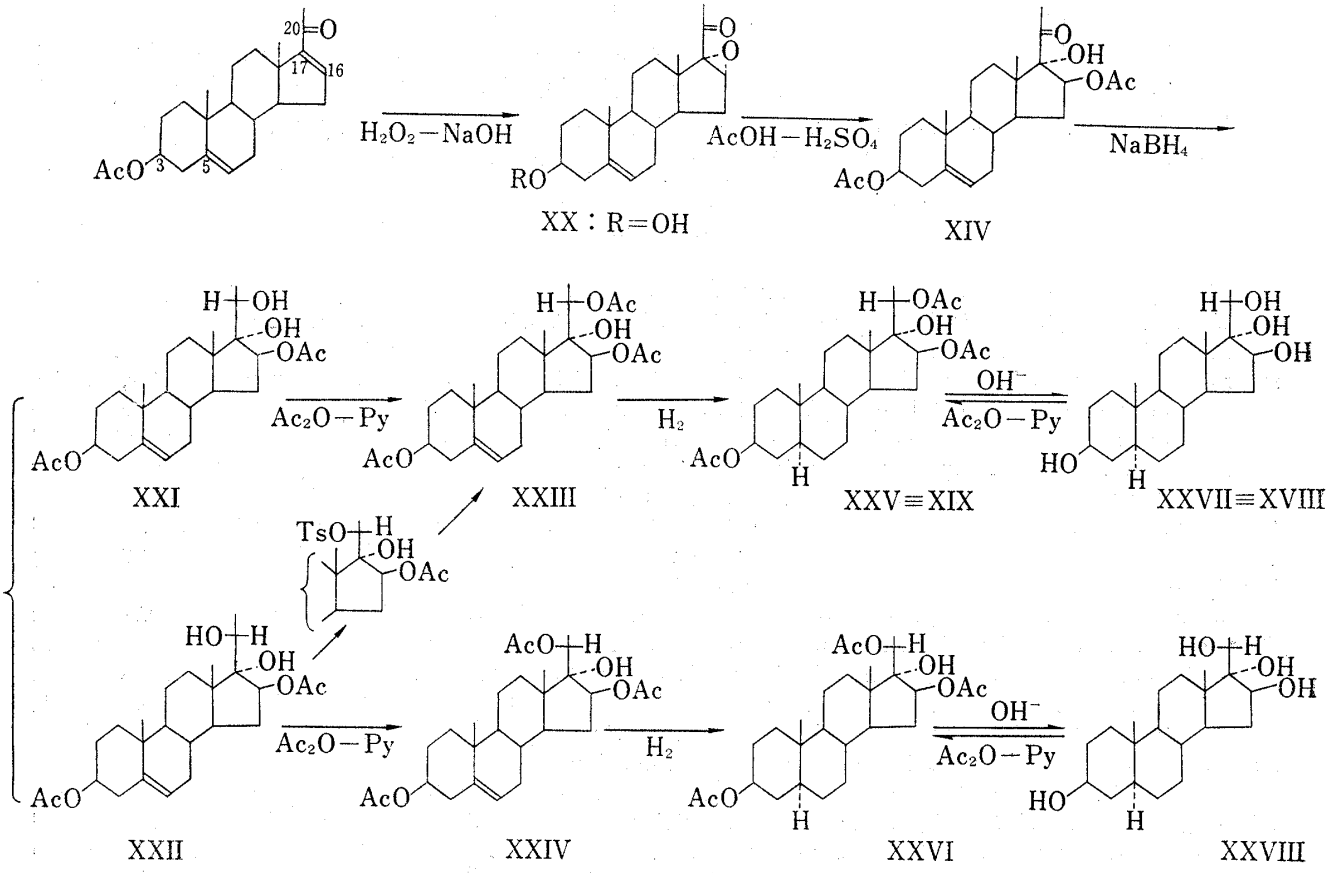
16) K. Tori and K. Aono, *Ann. Rept. Shionogi Res. Lab.*, **13**, 109 (1963).



II″ acetate XVIII XIX

Chart 4

When pennogenin (II″) acetate was hydrogenated to 5α-dihydro compound and subsequently subjected to the Baeyer-Villiger reaction in an analogous manner to those of Marker, *et al.*[17] and Morita, *et al.*,[18] a tetraol (XVIII), mp 236—238°, $[\alpha]_D$ +11.4°, $C_{21}H_{36}O_4$, was obtained (Chart 4). XVIII showed no carbonyl absorptions on IR spectrum and was acetylated with acetic anhydride-pyridine to give a triacetate (XIX), mp 196—198°, $[\alpha]_D$ +10.8°.



XX : R=OH XIV

XXI XXIII XXV≡XIX XXVII≡XVIII

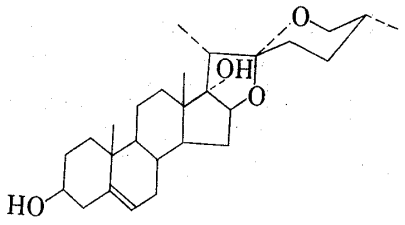
XXII XXIV XXVI XXVIII

Chart 5

An unequivocal synthesis of two epimeric 5α-pregnane-3β,16β,17α,20-tetraols (XXVII and XXVIII) from 3β-hydroxy pregna-5,16-dien-20-one was then carried out according to a series of established methods shown in Chart 5. Of the two pregnane-3β,16β,17α,20-tetraol 3,16-diacetates, the one (XXI), mp 242—244°, $[\alpha]_D$ —17.7°, was acetylated to give a 3,16,20-triacetate

17) R.E. Marker and D.L. Turner, *J. Am. Chem. Soc.*, **62**, 2540 (1940).
18) K. Morita, S. Noguchi, H. Kono, and T. Miki, *Chem. Pharm. Bull.* (Tokyo), **11**, 90, 144 (1963); T. Miki, K. Morita, S. Noguchi, T. Kishi, K. Hiraga, and H. Nawa, *ibid.*, **11**, 95 (1963); M. Uchibayashi, A. Okabori, K. Morita, and T. Miki, *ibid.*, **11**, 103, 139 (1963).

(XXIII), mp 228—229°, $[\alpha]_D$ −19.2°, and the other (XXII), mp 219—221°, $[\alpha]_D$ −5.1°, yielded a triacetate (XXIV), mp 192—194°, $[\alpha]_D$ +11.0°. XXI was more levorotatory than XXII and the molecular rotation difference ($[M]_{XXIII}-[M]_{XXI}$) of the former pair was minus, whereas that of the latters was plus. Therefore[19] XXI is regarded as 20α-ol, and hence the hydrogenation product (XXV), mp 195—197°, $[\alpha]_D$ +11.1°, of the triacetate (XXIII) is the corresponding 5α-pregnane derivative and the subsequent saponification product (XXVII), mp 234—238°, $[\alpha]_D$ +12.6°, is 5α-pregnane-3β,16β,17α,20α-tetraol. The tetraol (XVIII) and its acetate (XIX) derived from pennogenin were identical with XXVII and XXV, respectively. Accordingly it is chemically proved that pennogenin has the tertiary hydroxyl group at C-17 as Marker, *et al.* proposed and not at C-16.



II″

Since the Baeyer-Villiger reaction on the spiroketal side chain is known[18] not to affect the configuration at C-20 and the IR[20] and NMR[21] spectra of pennogenin showed the 25D-spirostane structure, pennogenin is represented as 25D-spirost-5-ene-3β,17α-diol (II″) having the same configurations at C-16, 17, 20 and 22 as usual steroid sapogenins.

Taking into account of the relationship with pennogenin, nologenin is presumed also to have a hydroxyl group at C-17 (I). The difference in behavior against acid and alkali of 16-acyloxy compound (XII) from 16-acetoxy compound (XIV) is tentatively assumed to be due to the kind of acyl group, but still remains a matter of concern.

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 20—25° in a chloroform solution unless otherwise specified. ORD were measured using a JASCO ORD/UV-5 recording spectropolarimeter. IR spectra were obtained with a JASCO IR-G spectrophotometer. NMR spectra were recorded at 60 MHz on a JEOL JNM C-60H and a Varian Model A-60 spectrometers in a deuteriochloroform solution and chemical shifts are given in δ scale with tetramethylsilane as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet). Mass spectra were recorded on a JEOL JMS-O1SG mass spectrometer with an accelerating potential of 5.0—6.0 kV, an ionizing potential of 75 eV and a source temperature of 150—200°. TLC and column chromatographies were carried out, respectively, on Kieselgel G nach Stahl (Merck), and with Kieselgel (0.05—0.2 mm) (Merck) and "Kanto" silica gel (100—200 mesh) in fifty to one hundred times quantity of the material.

Isolation of Steroid Sapogenins——The whole underground parts (2.1 kg) of *Trillium kamtschaticum* PALL. collected during June in the suburb of Sapporo and packed in sawdust were sliced after about two weeks, immediately extracted with refluxing methanol and the extracts were treated as shown in Chart 2.

Diosgenin: Fr. 1 was crystallized from MeOH to give colorless needles (360 mg), mp 198—200°, $[\alpha]_D$ −119.4° (c=1.02), which was identified with an authentic sample of diosgenin by comparison of their IR and NMR spectra and by mixed melting point determination.

Bethogenin (IV): Fr. 2 was crystallized from MeOH to give colorless needles (220 mg), mp 157—159°, $[\alpha]_D$ −103.2° (c=0.78) (lit.:[11c] mp 160—164°), which was recrystallized from 2% KOH–MeOH to afford colorless needles, mp 189—190° (lit.: mp 192—194°, $[\alpha]_D$ −96.0°,[11c] mp 191—193°[5]). Mass Spectrum *m/e*: 444 (M^+), 430 (M^+-CH_2), 412 (M^+—MeOH), 394 (M^+—MeOH–H_2O). *Anal.* Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.90; H, 9.96. Usual acetylation gave an acetate as colorless prisms (from MeOH), mp 208—209°, $[\alpha]_D$ −100.2° (c=0.49). NMR: 3.34 (3H, s, $-OCH_3$). Respectively identified (TLC, NMR, mixed mp) with the samples of bethogenin (mp 158—160°) and its acetate (mp 208—210°) prepared from pennogenin according to the Marker's procedure.[4,5]

19) L.F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Co., New York, N.Y., 1959, p. 179, 180, 614, 615.
20) M.E. Wall, C.R. Eddy, M.L. McClennan, and M.E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); C.R. Eddy, M.E. Wall, and M.K. Scott, *ibid.*, **25**, 266 (1953); E.S. Rothman, M.E. Wall, and C.R. Eddy, *J. Am. Chem. Soc.*, **74**, 4013 (1952).
21) J.P. Kutney, *Steroids*, **2**, 225 (1963).

Pennogenin (II''): Fr. 3 was crystallized from MeOH to give colorless needles (320 mg), mp 232—234°, $[\alpha]_D$ −104.3° (c=1.02). IR ν_{max}^{KBr} cm^{-1}: 3600—3400 (OH), 980, 920, 900, 890 (intensity; 920<900, 25D-spiroketal side chain). NMR: 0.82 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 0.90 (3H, d, J=6 Hz, 21-CH_3), 3.40 (2H, m, C_{26}–H_2), 3.95 (1H, t, J=7 Hz, C_{16}–H), 5.30 (1H, m, C_6–H) (diosgenin; 0.80 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 1.97 (3H, d, J=6 Hz, 21-CH_3), 3.40 (2H, m, C_{26}–H_2), 4.40 (1H, m, C_{16}–H), 5.32 (1H, m, C_6–H)). Mass Spectrum m/e: 430 (M^+), 412 (M^+-H_2O), 396 (M^+-2H_2O). *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.17; H, 9.74. Identified with an authentic sample of pennogenin,[22] mp 234—236°, $[\alpha]_D$ −104.9° (c=0.82), by comparison of their IR and NMR spectra and by mixed melting point determination. Usual acetylation gave an acetate as colorless needles (from MeOH), mp 197—198°, $[\alpha]_D$ −101.0° (c=0.69) (lit.: mp 199°,[4] mp 194—196°, $[\alpha]_D$ −100° (c=1.24)[11a]). NMR: 3.96 (1H, t, J=7 Hz, C_{16}–H).

26-Chloro-26-deoxykryptogenin (XI): Fr. 4 was crystallized from MeOH to give colorless prisms (30 mg), mp 149—150°, $[\alpha]_D$ −192.7° (c=0.44). Beilstein reaction: positive. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1735 (five-membered ring ketone), 1715 (aliphatic ketone), no spiroketal absorptions. ORD (c=0.06, EtOH) $[M]$(nm): +9800 (270) (peak), −12800 (316) (trough). NMR: 0.80 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 3.50 (1H, m, C_3–H), 3.50 (2H, d, J=4.5 Hz, C_{26}–H_2), 5.32 (1H, m, C_6–H). Mass Spectrum (Fig. 1) m/e: 448 (450)[13] (M^+), 325 ($C_{22}H_{29}O_2^+$), 297 ($C_{21}H_{29}O^+$), 133 (135)[13] ($C_6H_{10}OCl^+$), 105 (107)[13] ($C_5H_{10}Cl^+$). Acetylation with Ac_2O-pyridine gave a monoacetate as colorless plates (from MeOH), mp 132—133°, $[\alpha]_D$ −191.7° (c=1.13). Beilstein reaction: positive. NMR: 2.01 (3H, s, –OCOCH$_3$), 3.50 (2H, d, J=4.5 Hz, C_{26}–H_2), 4.60 (1H, m, C_3–H), 5.37 (1H, m, C_6–H). Mass Spectrum m/e: 490 (492)[13] (M^+), 325 ($C_{22}H_{29}O_2^+$), 297 ($C_{21}H_{29}O^+$), 133 (135)[13] ($C_6H_{10}OCl^+$), 105 (107)[13] ($C_5H_{10}Cl^+$). *Anal.* Calcd. for $C_{29}H_{43}O_4Cl$: C, 71.02; H, 8.78. Found: C, 71.23; H, 8.82.

Kryptogenin (III): Fr. 5 was crystallized from MeOH to give colorless plates (420 mg), mp 186—188°, $[\alpha]_D$ −209.6° (c=1.12). IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1735 (five-membered ring ketone), 1715 (aliphatic ketone), no spiroketal absorptions. ORD (c=0.08, EtOH)$[M]$(nm): +10200 (271) (peak), −13200 (316) (trough). NMR: 3.50 (1H, m, C_3–H), 3.51 (2H, d, J=4.5 Hz, C_{26}–H_2), 5.36 (1H, m, C_6–H). Mass Spectrum m/e: 430 (M^+), 325 ($C_{22}H_{29}O_2^+$), 297 ($C_{21}H_{29}O^+$). Acetate, mp 149—151°, $[\alpha]_D$ −152.8° (c=1.02). NMR: 2.03 (3H, s, –OCOCH$_3$), 2.05 (3H, s, –OCOCH$_3$), 3.93 (2H, d, J=7 Hz, C_{26}–H_2), 4.55 (1H, m, C_3–H), 5.35 (1H, m, C_6–H). Mass Spectrum m/e: 514 (M^+), 385 ($C_{24}H_{33}O_4^+$), 307 ($C_{23}H_{33}O_3^+$), 325 ($C_{22}H_{29}O_2^+$), 297 ($C_{21}H_{29}O^+$). *Anal.* Calcd. for $C_{31}H_{40}O_6$: C, 72.34; H, 9.01. Found: C, 72.39; H, 9.06. Respectively identified (TLC, NMR, mixed mp) with the samples of kryptogenin (mp 187—189°) and its acetate (mp 150—151°) prepared from pennogenin according to the Marker's procedure.[5]

Degradation of Pennogenin Acetate into 5α-Pregnane-tetraol (XVIII) (Chart 4)——Pennogenin acetate (220 mg) was hydrogenated over 5% Pd-charcoal (30 mg) in EtOH (50 ml) to give 5α,6-dihydro compound (200 mg). 35% H_2O_2 (0.5 ml) was added to a solution of the dihydro compound in ethylene chloride (2 ml) and 99% formic acid (4 ml),[17,18] the mixture was kept at 50° for 1 hr, poured into water (20 ml) and extracted with CH_2Cl_2. The organic layer was washed and evaporated to dryness. The residue (150 mg) was dissolved in MeOH (3 ml), 5% NaOH aq. (1 ml) was added, the mixture was left stand overnight at room temperature and poured into a large amount of water. The white precipitates were collected, dried and crystallized from MeOH to give XVIII as colorless needles (105 mg), mp 236—238°, $[\alpha]_D$ +11.4° (c=0.30, EtOH). *Anal.* Calcd. for $C_{21}H_{36}O_4$: C, 71.55; H, 10.29. Found: C, 71.64; H, 10.18. IR ν_{max}^{Nujol} cm^{-1}: 3509, 3356 (OH), no carbonyl absorptions. XVIII was heated with Ac_2O-pyridine on a water bath for 1 hr and an usual work-up and crystallization of the product from MeOH gave a triacetate (XIX) as colorless needles, mp 196—198°, $[\alpha]_D$ +10.8° (c=0.59, EtOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3630 (OH), 1745, 1733 (OAc). *Anal.* Calcd. for $C_{27}H_{42}O_7$: C, 67.75; H, 8.85. Found: C, 67.35; H, 8.95.

Synthesis of 5α-Pregnane-3β,16β,17α,20α- and β-tetraols (XXVII and XXVIII) from 3β-Hydroxypregna-5,16-dien-20-one (Chart 5)——16α,17α-Epoxy-3β-hydroxypregn-5-en-20-one (XX):[23] To a solution of hydroxypregnadienone (3 g) in MeOH (200 ml) were added 4N NaOH aq. (6 ml) and 30% H_2O_2 (12 ml), the mixture was kept at 50° overnight and poured into water (800 ml). White precipitates were collected by filtlation, washed, dried and crystallized from MeOH to give XX as colorless needles (2.67 g), mp 188—190°, $[\alpha]_D$ +1.5° (c=0.82) (lit.[23]: mp 187—190°). IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1715 (carbonyl), 960—800 (epoxide). *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.17; H, 9.14. Acetate prepared by usual method was colorless needles (from MeOH), mp 159—161°, $[\alpha]_D$ −31.0° (c=0.81) (lit.[23a]: mp 156—158°). IR ν_{max}^{Nujol} cm^{-1}: 1734 (OAc), 1696 (carbonyl), 970—800 (epoxide). *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.34; H, 8.73.

3β,16β,17α-Trihydroxypregn-5-en-20-one 3,16-diacetate (XIV)[9,24]: To a solution of XX acetate (500 mg) in AcOH (10 ml), conc. H_2SO_4 (1 ml) in AcOH (10 ml) was added in dropwise during 3 min. The mix-

22) Kindly provided by Dr. K. Takeda of Shionogi Res. Lab.

23) *a*) B. Löken, S. Kaufmann, G. Rosenkranz, and F. Sondheimer, *J. Am. Chem. Soc.*, **78**, 1738 (1956); *b*) P.L. Julian, E.W. Meyer, W.J. Karpel, and I.R. Waller, *ibid.*, **72**, 5145 (1950).

24) J. Romo and A. Romo de Vivar, *J. Org. Chem.*, **21**, 902 (1956).

ture was left stand at room temperature for 30 min and then poured into ice-water (100 ml). The product was extracted with ether, the organic layer was washed successively with $NaHCO_3$ aq. and water, dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from benzene–*n*-hexane–MeOH mixture (1: 1: 2, v/v) to give XIV as a white solid (210 mg), mp 164—167°. Recrystallization from MeOH gave colorless needles (130 mg), mp 169—171°, $[\alpha]_D$ −31.0° (c=0.79) (lit.[9]: mp 169—171°, $[\alpha]_D$ −38.0° (c=0.78)). IR ν_{max}^{Nujol} cm^{-1}: 3348 (OH), 1736, 1709 (OAc and C=O). *Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.36; H, 8.59.

Pregn-5-ene-3β,16β,17α,20α- and β-tetraol 3,16-diacetates (XXI and XXII): Triol-20-one diacetate (XIV) (1 g) in MeOH (20 ml) was reduced with $NaBH_4$ (200 mg) under ice-cooling and stirring. After 3 min the mixture was neutralized with AcOH, concentrated to one third volume and diluted with water to give white precipitates, which showed on TLC (solv., cyclohexane–AcOEt, 1: 1, v/v) two spots (*Rf* 0.59, 0.55). They were collected, washed with water, dried and fractionated over silica gel column by using *n*-hexane–AcOEt (2: 1, v/v) as eluent. The first fraction (*Rf* 0.59) was crystallized from MeOH to give XXI (210 mg)[25] as colorless needles, mp 242—244°, $[\alpha]_D$ −17.7° (c=0.26, EtOH) ([*M*] −76.9°). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3534 (OH), 1739 (OAc). *Anal.* Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81. Found: C, 68.92; H, 8.85. The second fraction (*Rf* 0.55) was crystallized from *n*-hexane to give XXII (110 mg)[23] as colorless needles, mp 219—221°, $[\alpha]_D$ −5.1° (c=0.93, EtOH) ([*M*] −22.2°). IR ν_{max}^{Nujol} cm^{-1}: 3650 (OH), 1736 (OAc). *Anal.* Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81. Found: C, 68.98; H, 8.78.

Pregn-5-ene-3β,16β,17α,20α- and β-tetraol 3,16,20-triacetates (XXIII and XXIV): XXI was acetylated with Ac_2O-pyridine to give 3β,16β,17α,20α-tetraol 3,16,20-triacetate (XXIII), colorless needles (from MeOH), mp 228—229°, $[\alpha]_D$ −19.2° (c=0.67, EtOH) ([*M*] −91.5°, molecular rotation difference between XXIII and XXI, −14.6°). *Anal.* Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46. Found: C, 67.69; H, 8.39. XXII gave, in the same way as above, the epimeric tetraol triacetate (XXIV), colorless needles (from *n*-hexane), mp 192—194°, $[\alpha]_D$ +11.0° (c=0.59, EtOH) ([*M*] +74.6°, molecular rotation difference between XXIV and XXII, +96.8°). *Anal.* Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46. Found: C, 67.78; H, 8.43.

5α-Pregnane-3β,16β,17α,20α- and β-tetraol 3,16,20-triacetates (XXV and XXVI): XXIII (500 mg) in EtOH (50 ml) was shaken with PtO_2 (10 mg) at room temperature in a hydrogen atmosphere. Usual work-up and crystallization of the product from MeOH gave dihydro compound (XXV) (480 mg) as colorless needles, mp 195—197°, $[\alpha]_D$ +11.1° (c=0.98, EtOH). *Anal.* Calcd. for $C_{27}H_{42}O_7$: C, 67.75; H, 8.85. Found: C, 67.38; H, 8.96. Identical with XIX derived from pennogenin in all respects (TLC, IR, NMR, mixed mp). XXIV was hydrogenated as above to give XXVI as colorless needles, mp 190—192°, $[\alpha]_D$ +37.6° (c=0.43, EtOH). *Anal.* Calcd. for $C_{27}H_{42}O_7$: C, 67.75; H, 8.85. Found: C, 67.41; H, 8.91.

5α-Pregnane-3β,16β,17α,20α- and β-tetraols (XXVII and XXVIII): XXV (30 mg) was saponified with 5% KOH in MeOH (5 ml) at 50—60° for 10 min. The reaction mixture was poured into water, the white precipitates were collected, washed with water and crystallized from MeOH to give XXVII as colorless needles, mp 234—238°, $[\alpha]_D$ +12.6° (c=0.42, EtOH). Identified with XVIII derived from pennogenin by direct comparison (TLC, IR, NMR, mixed mp). XXVI gave, in the same way as above, XXVIII as colorless needles, mp 306—307°, $[\alpha]_D$ +17.2° (c=0.67, EtOH).

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25) It is known (L.F. Fieser and M. Fieser, "Steroids", Reinhold Publishing Co., New York, N.Y., 1959, p. 567) that a reduction of 20-ketone with metal hydride, particularly with $NaBH_4$, gives 20β-ol as major product. Benn (W.R. Benn, *J. Org. Chem.*, **28**, 3557 (1963)) reported that 3β-hydroxy pregna-5,16-dien-20-one acetate (or 3,20-dione 3-ethyleneketal) was reduced with $LiAlH_4$ to give a molecular complex of the corresponding 20α- and β-ols (1: 1), which shows mp, $[\alpha]_D$, and *Rf* value on TLC similar to those of the pure 20α-epimer. In order to examine whether the major product XXI is homogeneous or a mixture of two 20-epimers, XXII was converted *via* 20β-tosylate to the 20α-acetate by the Fukushima's method (D.K. Fukushima, N.S. Leeds, H.L. Bradlow, T.H. Kritchevsky, M.B. Stoken, and T.F. Gallagher, *J. Biol. Chem.*, **212**, 449 (1955)). The product, mp 227—228°, $[\alpha]_D$ −17.5°, was identical with XXI acetate (XXIII).