[Chem. Pharm. Bull. 23(11)2550—2555(1975)]

UDC 547.92.02.05 : 581.192

A New Spirostanol, 19-Hydroxyyonogenin, from the Aerial Parts of *Dioscorea tokoro* Makino*

Kazumoto Miyahara, Etsuko Kanezaki, and Toshio Kawasaki

Faculty of Pharmaceutical Sciences, Kyushu University[1]

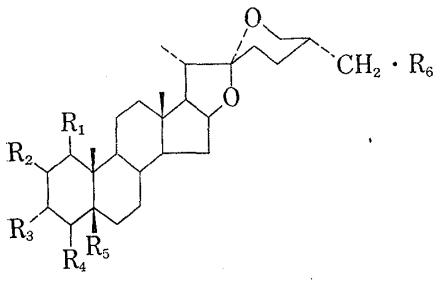
(Received April 30, 1975)

A new spirostanol (I), mp 249—250°, $[\alpha]_D$ −15.5°, was isolated from a crude sapogenin mixture obtained by acid hydrolysis of the methanol extracts of the aerial parts of *Dioscorea tokoro* Makino. The structure of I was established as (25*R*)-5β-spirostane-2β,3α,19-triol, that is 19-hydroxy-yonogenin. This is the first spirostane derivative having an oxygen function at C_{19}, and an additional example of the unusual spirostanol carrying the α-hydroxyl group at C_3.

The steroid sapogenins in the plant kingdom have been extensively surveyed and a large number of spirostane derivatives have been documented.[2] However, in contrast to the cardiac aglycones and the steroid alkamines among which some are known[3] to have the 19- or 18-methyl group of the steroid nucleus oxygenated, no spirostane bearing an oxygen function at the angular methyl groups has so far been reported.

This paper concerns the first isolation and characterization of a new spirostanol (I) which has the hydroxyl group at C_{19}.

The aerial parts of *Dioscorea tokoro* Makino have attracted a special interest in that they contain a series of unusual 3α-hydroxy-(25*R*)-5β-spirostane derivatives[4] such as yonogenin (II), tokorogenin (III), kogagenin (IV), isodiotigenin (V), igagenin (VI) and isotenuipegenin (tetraol). In an attempt to isolate IV from a crude sapogenin mixture obtained by acid hydrolysis of the methanol extracts, the fraction showing one spot of IV on thin-layer chromatogram (TLC) was acetylated to give a product which was unexpectedly composed of IV triacetate[5] and an unknown minor compound. Isolation of an appreciable amount of the minor ingredient was then undertaken, and it was successfully obtained in a chromatographically homogeneous state according to the procedure shown in Chart 1. On saponification it provided a compound (I) contaminated with a small amount of III, which was purified by repeated chromatography over silica gel followed by recrystallization to give pure I, mp 249 —250°, $[\alpha]_D$ −15.5°, $C_{27}H_{44}O_5$. I was considered to be a (25*R*)-spirostanetriol based on the



II : $R_1=R_4=R_5=R_6=H$, $R_2=R_3=OH$
III : $R_4=R_5=R_6=H$, $R_1=R_2=R_3=OH$
IV: $R_4=R_6=H$, $R_1=R_2=R_3=R_5=OH$
V : $R_1=R_5=R_6=H$, $R_2=R_3=R_4=OH$
VI: $R_1=R_4=R_5=H$, $R_2=R_3=R_6=OH$

Formulae 1

* Dedicated to the memory of Prof. Eiji Ochiai.

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

2) R. Tschesche and G. Wulff, "Fortschritte der Chemie organischer Naturstoffe," Vol. 30, ed. by W. Herz, H. Grisebach, and G.W. Kirby, Springer-Verlag, Wien, 1973, p. 480.

3) P.G. Marshall, "Rodd's Chemistry of Carbon Compounds," 2nd. ed., Vol. II_D, ed. by S. Coffey, Elsevier Publishing Co., Amsterdam, 1970, p. 362; S.M. Kupchan and A.W. By, "The Alkaloids," Vol. X, ed. by R.H.F. Manske, Academic Press, New York, 1968, p. 194.

4) K. Takeda, "Progress in Phytochemistry," Vol. 3, ed. by L. Reinhold and Y. Liwschitz, Interscience Publishers, London, 1972, p. 300.

5) K. Takeda, T. Kubota, and A. Shimaoka, *Tetrahedron*, 7, 62 (1959).

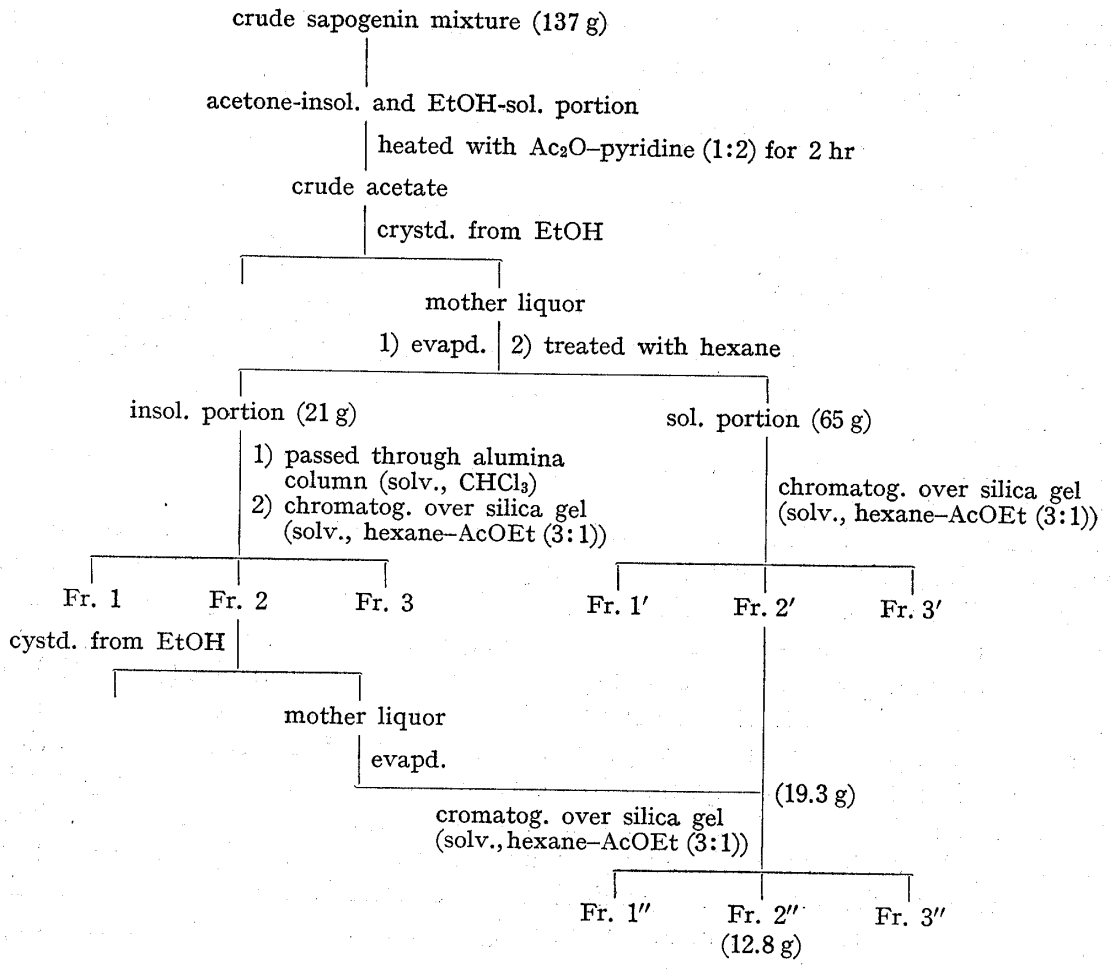


Chart 1

infrared (IR)[6] and the mass spectral[7] data and its molecualr formula, but the physical constants were different from those[4] of known triols III, V, and VI. Subsequently, assuming it to be a new spirostanol, the structure elucidation was conducted.

I was acetylated with acetic anhydride–pyridine (1: 1, v/v) overnight to give the triacetate (VII), mp 104—106°, $[\alpha]_D$ −1.9°, $C_{33}H_{50}O_8$. Its nuclear magnetic resonance (NMR) spectrum was consistent with the structure, 16-unsubstituted (25*R*)-spirostane-triol triacetate, and showed a two-proton broad multiplet ($W_{h/2}$=15 Hz) at 4.90 ppm and a two-proton double doublet (J=12 Hz) at 3.98 and 4.38 ppm. The former is assigned to the axial protons of two methine groups carrying an acetoxyl function, while the latter is ascribable to the angular acetoxymethylene protons. Furthermore, the spectrum exhibited not four but three methyl signals which are assignable to the 18-, 21-, and 27-methyl groups by comparing with those[8a] of gitogenin diacetate, II diacetate and III triacetate.

From these data I is thought to contain two equatorial secondary hydroxyl groups and to have the 19-methyl group hydroxylated.

6) 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).

7) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, 1964, p. 110; W.H. Faul and C. Djerassi, *Organic Mass Spectrometry*, **3**, 1187 (1970).

8) *a*) K. Tori and K. Aono, *Ann. Repts. Shionogi Res. Lab.*, **14**, 136 (1964); *b*) J.P. Kutney, *Steroids*, **2**, 225 (1963); G.F.H. Green, J.E. Page, and S.E. Staniforth, *J. Chem. Soc.* (*B*), **1966**, 807.

Although neither oxidation of I with sodium metaperiodate in aqueous acetone solution nor acetonide formation with acetone and *p*-toluenesulfonic acid was accomplished, I was treated with periodic acid in methanol solution[9] to give a product (VIII), and with 2,2-dimethoxypropane[10] and *p*-toluenesulfonic acid in dimethylformamide solution to provide two compounds (IX and X).

VIII, mp 156—158°, $[\alpha]_D$ −13.4°, showed no hydroxyl absorptions on its IR, three methoxyl signals on the NMR and the molecular ion peak at *m/e* 506 on the mass spectra, suggesting that it is very likely to be a diacetal formed methanol and the oxidation product (a seco-dialdehyde) having the same number of carbon atoms as that of I. IX, mp 135—139°, $[\alpha]_D$ −35.9°, $C_{34}H_{56}O_6$, and X, mp 192—195°, $[\alpha]_D$ −29.1°, $C_{30}H_{48}O_5$, are respectively regarded as a diacetonide and a monoacetonide on the basis of their IR and NMR spectral data and molecular formulae.

Therefore the two equatorial secondary hydroxyl groups in I are adjacent to each other in *trans* configuration.[11]

When I was treated with acetic anhydride–pyridine (10: 1, v/v) for 6 hr, two diacetates were afforded along with triacetate VII, and they were converted by the Jones oxidation into the corresponding keto-diacetates (XI and XII). XI, mp 220—222°, $[\alpha]_D$ −99°, exhibited on the NMR spectrum a two-proton double doublet (J=11 Hz) at 4.10 and 4.44 ppm, an one-proton sharp quartet (J=5, 13 Hz) at 5.24 ppm and an one-proton triplet (J=16 Hz) at 2.88 ppm. The first one is due to the angular acetoxymethylene protons, the second is assigned to the axial proton geminal to an acetoxyl function and α to a methylene group, and the third one to the axial proton of a methylene α to a carbonyl and to the methine group bearing an axial proton. Another keto-diacetate XII (a white powder) showed, together with the signal due to the angular acetoxymethylene protons, an one-proton broad triplet (J=8 Hz) at 5.25 ppm attributable to the axial proton geminal to an acetoxyl function and α to a methylene group, and a two-proton singlet at 2.53 ppm assignable to the methylene protons adjacent to a carbonyl and to a tertiary carbon atom.

The above data indicate that XI and XII have the partial structures, $-CH_2-CH(OAc)-CO-CH_2-CH<$ and ■$-CH_2-CO-CH(OAc)-CH_2-$, respectively, and consequently the vicinal *trans*-glycol grouping in I is to be placed between two methylene groups. Such a structure for I is conceivable on the spirostane framework only when the two hydroxyl groups are attached to C_2 and C_3. Accordingly XI is regarded as 3-oxo-2,19-diol diacetate and XII as the isomeric 2-oxo-3,19-diol diacetate.

Since XI showed a negative Cotton curve on the optical rotatory dispersion (ORD) spectrum, the A/B ring juncture of XI and hence of I is *cis* (5β),[12] so that the two equatorial hydroxyl groups at C_2 and C_3 of I should have β and α configurations, respectively.

Finally the presence of the third hydroxyl group at C_{19} was supported by the NMR spectrum of VIII. Thus, on the spectrum were observed, in addition to three methoxyl signals, a two-proton double doublet (J=8 Hz) at 4.10 and 3.59 ppm and an one-proton quartet (J=2, 5.5 Hz) at 4.93 ppm. The former is considered to be due to the angular methylene protons next to an ether–oxygen atom and the latter is ascribable to the cyclic acetal proton adjacent to two ether–oxygen atoms and α to a methylene group. These data imply that VIII con-

9) I was oxidized with periodic acid also in aqueous dioxane solution (*cf.* K. Takeda, T. Okanishi, K. Sasaki, and A. Shimaoka, *Chem. Pharm. Bull.* (Tokyo), **9**, 631 (1961)), but the product could not be isolated due to its secondary alterations during the work-up procedure. In a hope to convert the resulting unstable aldehyde immediately into the stable acetal, the oxidation was carried out in methanol solution.

10) A. Hasegawa and H.G. Fletcher, *Jr.*, *Carbohydrate Research*, **29**, 209 (1973); M.L. Wolfrom, A.B. Diwadkar, J. Gelas, and D. Horton, *ibid.*, **35**, 87 (1974).

11) K. Takeda, T. Okanishi, and A. Shimaoka, *Chem. Pharm. Bull.* (Tokyo), **6**, 532 (1958); J. Pataki, G. Rosenkranz, and C. Djerassi, *J. Am. Chem. Soc.*, **73**, 5375 (1951).

12) T. Kawasaki and K. Miyahara, *Tetrahedron*, **21**, 3633 (1965).

tains a cyclic acetal as well as a methyl acetal grouping in the molecule. Since the formation of VIII from I, that is 2,3,18- or 2,3,19-triol, is thought to have proceeded *via* 2,3-seco-2,3-dialdehyde, taking into account the unlikeliness of ring formation of the 2- or 3-aldehyde function with the hydroxyl group at C_{18} in the presence of methanol, VIII should be represented by the 2,19-oxide structure VIIIa or the 3,19-oxide VIIIb.

In consequence, I has the 19-methyl group hydroxylated and is defined as (25*R*)-5β-spirostane-2β,3α,19-triol, namely 19-hydroxy-yonogenin.

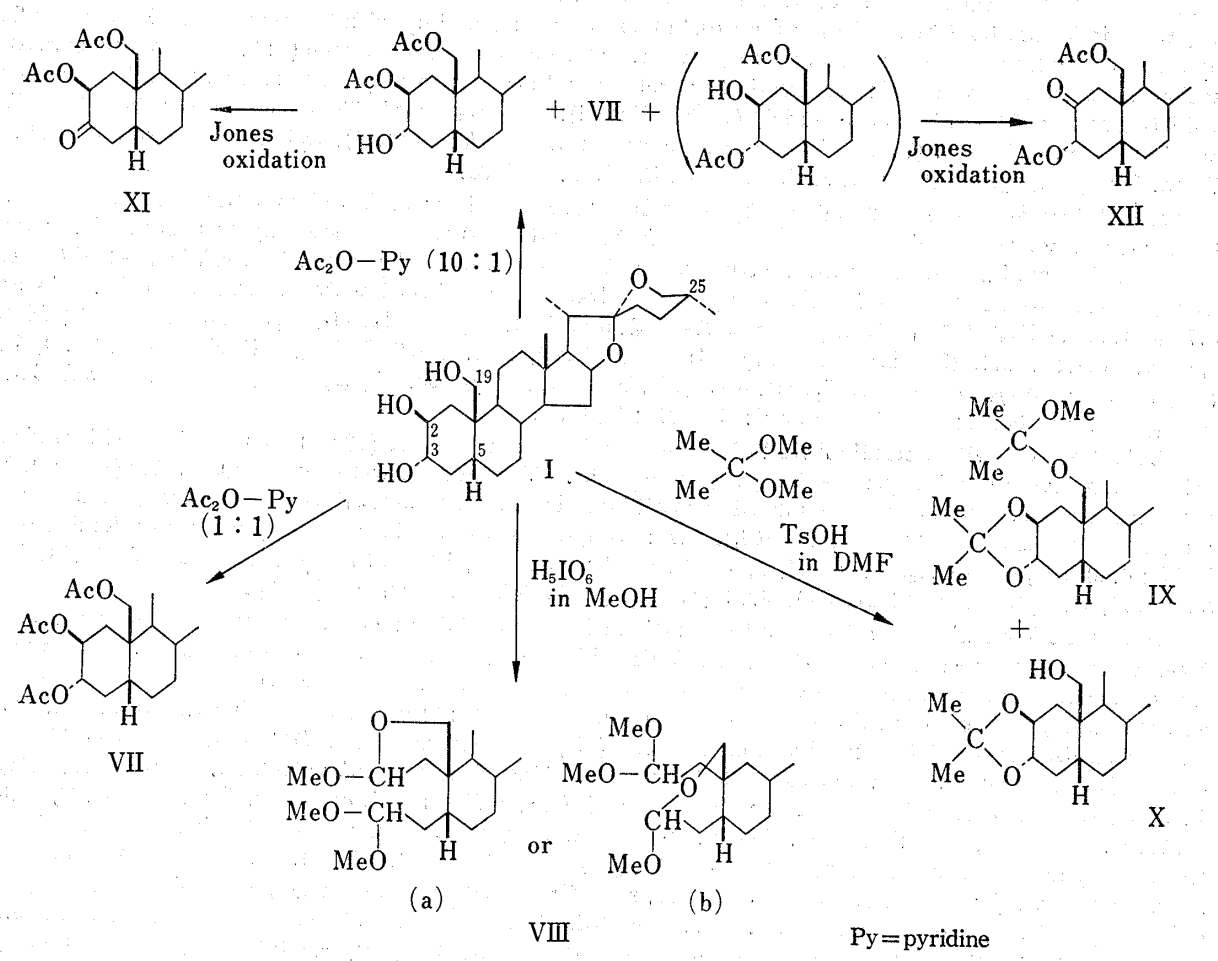


Chart 2

Experimental

All 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 at room temperature (16—20°). IR and ORD spectra were recorded with a JASCO IR-G spectrometer in nujol mulling and a JASCO ORD/UV-5 recording spectropolarimeter, respectively. NMR spectra were taken in $CDCl_3$ solution at 100 MHz on a JEOL PS-100 spectrometer and chemical shifts are given in δ (ppm) scale with tetramethylsilane as internal standard. Signal multiplicities are represented by s(singlet), d(doublet), dd(double doublet), t(triplet), q(quartet) and m(multiplet). Mass spectra were recorded on a JMS-01SG mass spectrometer with an accelerating potential of 4.5—6.7 kV, an ionizing potential of 75 eV and a source temperature of 95—145°. TLC was performed on Kieselgel G nach Stahl (Merck) using 10% H_2SO_4 (spraying followed by heating) as a detector. Column chromatography was carried out with Kieselgel (0.05—0.2 mm) (Merck) in 50—150 times quantity of the material. The ratios of reagents and solvents in mixture are given in v/v.

13) Kindly furnished by Dr. K. Takeda of Shionogi Research Laboratory. A black solid obtained by acid hydrolysis of the MeOH extracts of the whole aerial parts collected at the end of summer.

Isolation of 19-Hydroxy-yonogenin (I)——A crude sapogenin mixture[13] from the aerial parts of *Dioscorea tokoro* MAKINO showed on TLC (solvent, AcOEt) several spots including those of yonogenin (II), tokorogenin (III), and kogagenin (IV). In a preliminary experiment attempting to isolate IV, it was treated with acetone and the insoluble part was extracted with MeOH. The extractives were repeatedly chromatographed over silica gel (solvent, AcOEt) to give a fraction exhibiting on TLC only one spot corresponding to that of IV. The fraction was acetylated on boiling with Ac_2O–pyridine (1: 2) for 2 hr to give IV triacetate[5] obviously being accompanied with a new compound (=I triacetate (VII)), which was considerably less polar than IV triacetate and slightly more polar than III triacetate on TLC (solvent, hexane–AcOEt (2: 1)).

Subsequently the sapogenin mixture (137 g) was fractionated, under monitoring by TLC (solvent; AcOEt for free sapogenin, hexane–AcOEt (2: 1) and $CHCl_3$–MeOH (9: 1) for acetate), as shown in Chart 1. Fraction 2″ (12.8 g) was free from IV acetate and seemed to be homogeneous VII. It was treated with boiling 5% KOH in MeOH for 1 hr to yield crude I (9.4 g) contaminated mainly with less polar III. It was purified by repeated chromatography over silica gel (solvent, AcOEt and benzene-acetone (1: 2)) followed by crystallizations successively from AcOEt, methyl ethyl ketone and acetone to provide pure I as a white crystalline solid (1.2 g), mp 249—250°, $[\alpha]_D$ −15.5° (c=0.6, EtOH). IR ν_{max} cm^{-1}: 3200—3500 (OH), 980, 920, 897, 862 (intensity, 920<897, (25*R*)-spiroketal[6]). Mass Spectrum *m/e*: 448 (M^+), 139, 115 (spiroketal).[7] *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.21; H, 9.98.

Acetylation of I——I (100 mg) in Ac_2O–pyridine (1: 1) (5 ml) was allowed to stand overnight at room temperature, poured into ice-water and the precipitates (108 mg) were collected by filtration. They were crystallized from dil.MeOH to afford the triacetate (VII) as a white powder, mp 104—106°, $[\alpha]_D$ −1.9° (c=1.1, $CHCl_3$). IR ν_{max} cm^{-1}: 1745 (AcO), no hydroxyl absorptions. Mass Spectrum *m/e*: 574 (M^+). NMR[8]: 0.75 (3H, s, 18-CH_3), 0.77 (3H, d, J=7 Hz, 27-CH_3), 0.96 (3H, d, J=6 Hz, 21-CH_3), 2.00, 2.02, 2.07 (3H×3, s, each, AcO×3), 3.42 (2H, broad m, $W_{h/2}$=16 Hz, 26-CH_2), 3.98, 4.38 (2H, dd, J=12 Hz, 19-C$\underline{H}_2$OAc), 4.38 (1H, broad m, $W_{h/2}$=*ca.* 20 Hz, C_{16}-H), 4.90 (2H, broad m, $W_{h/2}$=*ca.* 15 Hz, >C$\underline{H}$OAc×2). *Anal.* Calcd. for $C_{33}H_{50}O_8$: C, 68.96; H, 8.77, Found: C, 68.92; H, 8.72.

Oxidation of I with Periodic Acid in MeOH——I (70 mg) in MeOH (10 ml) was oxidized with periodic acid (20 mg) under stirring at room temperature for 3 hr. The mixture was diluted with water (150 ml), the precipitates were collected and crystallized from dil.MeOH to give a product (VIII) as colorless plates (73 mg), mp 156—158°, $[\alpha]_D$ −13.4° (c=1.2, $CHCl_3$). IR: no hydroxyl absorptions. Mass Spectrum *m/e*: 506 (M^+). NMR: 0.80 (3H, s, 18-CH_3), 0.97 (3H, d, J=6 Hz, 21-CH_3), 3.35, 3.36, 3.37 (3H×3, s, each, MeO×3), 4.10, 3.59 (2H, dd, J=8 Hz, 19-CH_2O–), 4.93 (1H, q, J=2, 5.5 Hz, cyclic-C$\underline{H}$$(OR)_2$).

Acetonide Formation from I——To the solution of I (270 mg) in dimethylformamide (15 ml) were added 2,2-dimethoxypropane (3 ml) and *p*-toluenesulfonic acid (20 mg). The mixture was stirred at room temperature overnight, poured into water, extracted with AcOEt, and the organic layer was evaporated. The residue was chromatographed over silica gel by using hexane–AcOEt (2: 1) as eluent to give two fractions. One was crystallized from MeOH to give a diacetonide (IX) as colorless needles (132 mg), mp 135—139°, $[\alpha]_D$ −35.9° (c=1.6, $CHCl_3$). IR: no hydroxyl absorptions. Mass Spectrum *m/e*: 560 (M^+). NMR: 0.74 (3H, s, 18-CH_3), 1.34, 1.44 (6H×2, s, each, $Me_2C<$×2), 3.21 (3H, s, MeO). *Anal.* Calcd. for $C_{34}H_{56}O_6$: C, 72.82; H, 10.05. Found: C, 72.62; H, 10.00. Another fraction was crystallized from MeOH to yield a monoacetonide (X) as colorless needles (174 mg), mp 192—195°, $[\alpha]_D$ −29.1° (c=1.3, EtOH). IR ν_{max} cm^{-1}: 3500 (OH). Mass Spectrum *m/e*: 488 (M^+). NMR: 0.75 (3H, s, 18-CH_3), 1.42, 1.45 (3H×2, s, each, $Me_2C<$), 3.57, 3.95 (2H, dd, J=11 Hz, 19-C$\underline{H}_2$OH). *Anal.* Calcd. for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90. Found: C, 73.45; H, 9.97.

Partial Acetylation of I——I (200 mg) in Ac_2O–pyridine (10: 1) (10 ml) was left stand at room temperature for 6 hr. The solution was poured into ice-water, the precipitates were collected by filtration and washed with water. They were chromatographed over silica gel (solvent, hexane–AcOEt (1: 1)). The first fraction gave a white powder (120 mg), which was identified with VIII by comparisons of their *Rf* values on TLC and NMR spectra. The second fraction was crystallized from hexane to give a diacetate (=2,19-diacetate) as fine needles (90 mg), mp 184.5—185.5°, $[\alpha]_D$ −17.8° (c=0.9, $CHCl_3$). IR ν_{max} cm^{-1}: 3530 (OH), 1740, 1726 (AcO). Mass Spectrum *m/e*: 532 (M^+). NMR: 2.08 (6H, s, AcO×2), 4.02, 4.38 (2H, dd, J=11.5 Hz, 19-C$\underline{H}_2$OAc), 4.70 (1H, octet, J=4, 11, 12 Hz, >C$\underline{H}$OAc), 3.64 (1H, broad t, >C$\underline{H}$OH).

Oxidation of Diacetates——To a solution of the above diacetate (50 mg) in acetone (10 ml) were added five drops of the Jones reagent (CrO_3 0.7 g, water 5 ml, H_2SO_4 0.6 ml). The mixture was allowed to stand at room temperature for 20 min, poured into water and the precipitates were collected. They were crystallized from MeOH to give a keto-diacetate (XI) as colorless prisms, mp 220—222°, $[\alpha]_D$ −99° (c=1.0, $CHCl_3$). IR ν_{max} cm^{-1}: 1750 (C=O), 1735 (AcO), no hydroxyl absorptions. Mass Spectrum *m/e*: 530 (M^+). NMR: 0.78 (3H, s, 18-CH_3), 2.05, 2.15 (3H×2, s, each, AcO×2), 2.88 (1H, t, J=16 Hz, C_4–H), 4.10, 4.44 (2H, dd, J=11 Hz, 19-C$\underline{H}_2$OAc), 5.24 (1H, q, J=5, 13 Hz, C_2–H). ORD (c=0.2, MeOH) [*M*] (nm): −380° (302) (trough), −320° (258) (peak). The mother liquor of crystallization of the second fraction in chromatography of partial acetylation product was evaporated and the residue (45 mg) was oxidized in the similar way as above. The product was chromatographed over silica gel by using hexane–AcOEt (3: 1) as eluent to give three fractions. The first one was crystallized from MeOH to provide colorless prisms (13 mg) identical (mp, NMR) with XI, and the third one yielded another keto-diacetate (XII) as a homogeneous (TLC) white powder (25 mg).

NMR: 0.73 (3H, s, 18-CH_3), 0.95 (3H, d, J=6 Hz, 21-CH_3), 2.10, 2.15 (3H×2, s, each, AcO×2), 2.53 (2H, s, 1-CH_2), 3.94, 4.45 (2H, dd, J=11 Hz, 19-$C\underline{H}_2OAc$), 5.25 (1H, broad t, J=8 Hz, C_3-H).

Acknowledgement The authors are grateful to Dr. K. Takeda of Shionogi Research Laboratory for the crude sapogenin mixture from *D. tokoro* and for the authntic samples of yonogenin, tokorogenin and kogagenin. Thanks are also due to Misses Y. Inatsu and M. Kawamura for the mass spectra, and to the member of the Central Analysis Room of this University for microanalysis.