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Structure of Diotigenin.[1,3] (1)

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The structure of a new steroidal sapogenin, diotigenin (Ia), isolated from *Dioscorea tenuipes* complex, was investigated, and it was assumed to be 25L-2β,3α,4β-trihydroxy-5β-spirostane.

As already reported,[3,4] the steroidal components of *Dioscorea tenuipes* complex (Himedokoro in Japanese) have been investigated and a new steroidal sapogenin, diotigenin, has been isolated. We now wish to report the results of its structural investigation.

Diotigenin (Ia), $C_{27}H_{44}O_5$, mp 280—281°, $[\alpha]_D$ −59.8°, afforded the triacetate (Ib), mp 218—219°, on acetylation with acetic anhydride in pyridine at room temperature, indicating the presence of three primary or secondary hydroxyl groups in the molecule.

As the infrared spectrum shows the characteristic bands[5] corresponding to the E- and the F-rings of the 25L-spirostane, diotigenin is a 25L-trihydroxyspirostane. When diotigenin

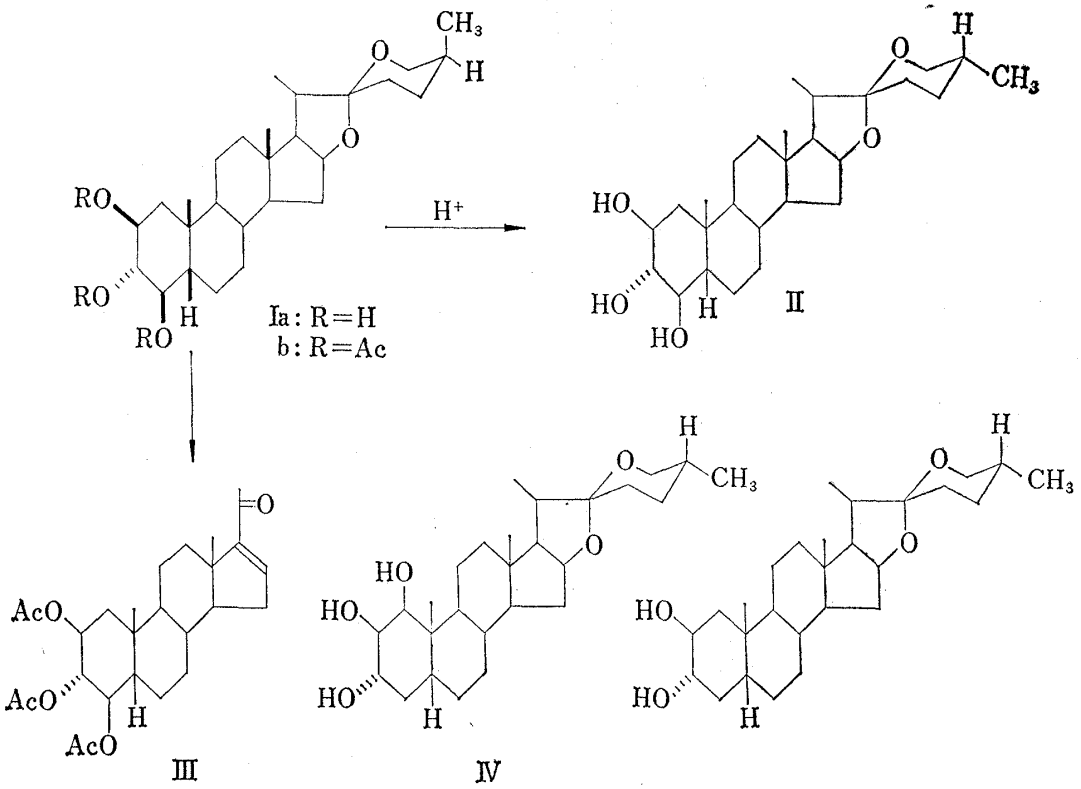

Chart 1

1) Studies on the Steroidal Components of Domestic Plants. LII.
2) Location: 2-47, *Sagisu*, *Fukushima-ku*, *Osaka*.
3) Part LI: A. Akahori, F. Yasuda, and T. Okanishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 498 (1968).
4) A. Akahori, *Phytochemistry*, **4**, 97 (1965).
5) R.N. Jones, *J. Am. Chem. Soc.*, **75**, 158 (1953). M.E. Wall, C.R. Eddy, M.L. Clenman, and M.E. Klump, *Analyt. Chem.*, **24**, 1337 (1952).

was heated under reflux with 4.5% hydrochloric acid in methanol for 72 hr, it gave the 25D–spirostane, isodiotigenin (II), mp 266—268°, $[\alpha]_D$ −48.6°.

Oxidative cleavage of the E– and the F–rings of diotigenin (Ia) under usual conditions afforded a pregnenolone derivative (III), mp 191—193°, which had three acetoxyl groups. The three hydroxyl groups of diotigenin are therefore situated in A–, B–, C–, and D–rings.

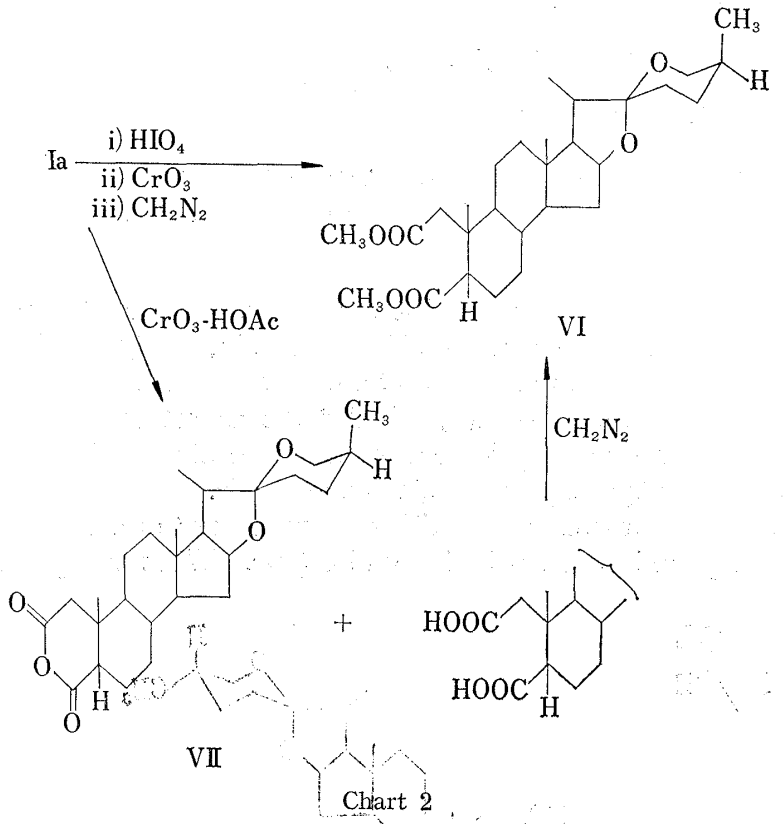

Chart 2

Assuming that the three hydroxyl groups of diotigenin are all in A–ring on the basis of coexistence[3,4] of diotigenin and tokorogenin (IV) in this plant, it was oxidized with periodic acid. The oxidation product was further oxidized with chromium trioxide followed by esterification with diazomethane, to give a dimethyl ester (VI), mp 147—148°, which has the empirical formula, $C_{28}H_{44}O_6$. Therefore it was elucidated that diotigenin lost one carbon atom by periodic acid oxidation. Moreover, on oxidation of diotigenin with chromium trioxide in acetic acid, it gave an acid anhydride (VII), mp 215—219°, and the above–mentioned dimethyl ester (VI) by esterification of the acid fraction. This acid anhydride corresponds to $C_{26}H_{38}O_5$ and is a derivative losing one carbon atom from diotigenin.

These results lead to the conclusion that three secondary hydroxyl groups are adjacent and all situated in the A–ring.

As formation of acetonide gives a valuable information about the configurations of vicinal hydroxyl groups, diotigenin (Ia) was heated under reflux with toluene–*p*–sulphonic acid in acetone for 5 hr. However, its acetonide was not obtained, but the starting material was recovered in quantitative yield. It may therefore be concluded that the three hydroxyl groups are all in *trans*–relation to each other. Moreover, as it has been observed on our earlier studies[4,6] on the components of *Dioscorea* sp. that the 5*β*–spirostanes having two, three or four hydroxyl groups in the A–ring are simultaneously isolated from one plant of *Dioscorea*

Table I. 19–Methyl Siganls (c/sec)

	In $CHCl_3$		In pyridine	
	c/sec	Acetylation effect (Ic)−(Ia) c/sec	c/sec	Acetylation effect (Ic)−(Ia) c/sec
Diotigenin (Ia)	62	−1[a]	61	+3[a]
Diotigenin triacetate (Ic)	63		58	

a) Plus sign represents an upfield shift.

6) T. Okanishi and A. Shimaoka, *Ann. Rept. Shionogi Res. Lab.*, **6**, 78 (1956); A. Akahori, *ibid.*, **10**, 1411 (1960); **11**, 93 (1961); 13, 68 (1963).

genus, diotigenin (Ia) is assumed to be a 5β-spirostane in accordance with the fact that the 5β-spirostanes, tokorogenin (IV) and yonogenin (V), coexist in this plant.[3,4] Accordingly diotigenin may be an 1β,2α,3β-, an 1α,2β,3α-, a 2β,3α,4β- or a 2α,3β,4α-trihydroxy compound. Nuclear magnetic resonance (NMR) spectra of diotigenin and its triacetate are shown in Table I. The difference between the value of 19-methyl signal of diotigenin and that of its triacetate is −1 c/sec in chloroform or +3 c/sec in pyridine.

On 5β-spirostanes, Tori and Aono[7] reported the facts that the acetylation effect of the 1β-hydroxyl group on 19-methyl signal is +9 c/sec (in $CHCl_3$) or +17.4 c/sec (in pyridine), whereas that of the 2α-,2β-,3α-, or 3β-hydroxy group is −2.4—+1.2 c/sec (in $CHCl_3$) or +1.8—+3 c/sec (in pyridine).

Therefore, the 1β,2α,3β-trihydroxy compound is excluded for diotigenin, and we tried to synthesise the 1α,2β,3α-trihydroxy-5β-compound (XIVa).

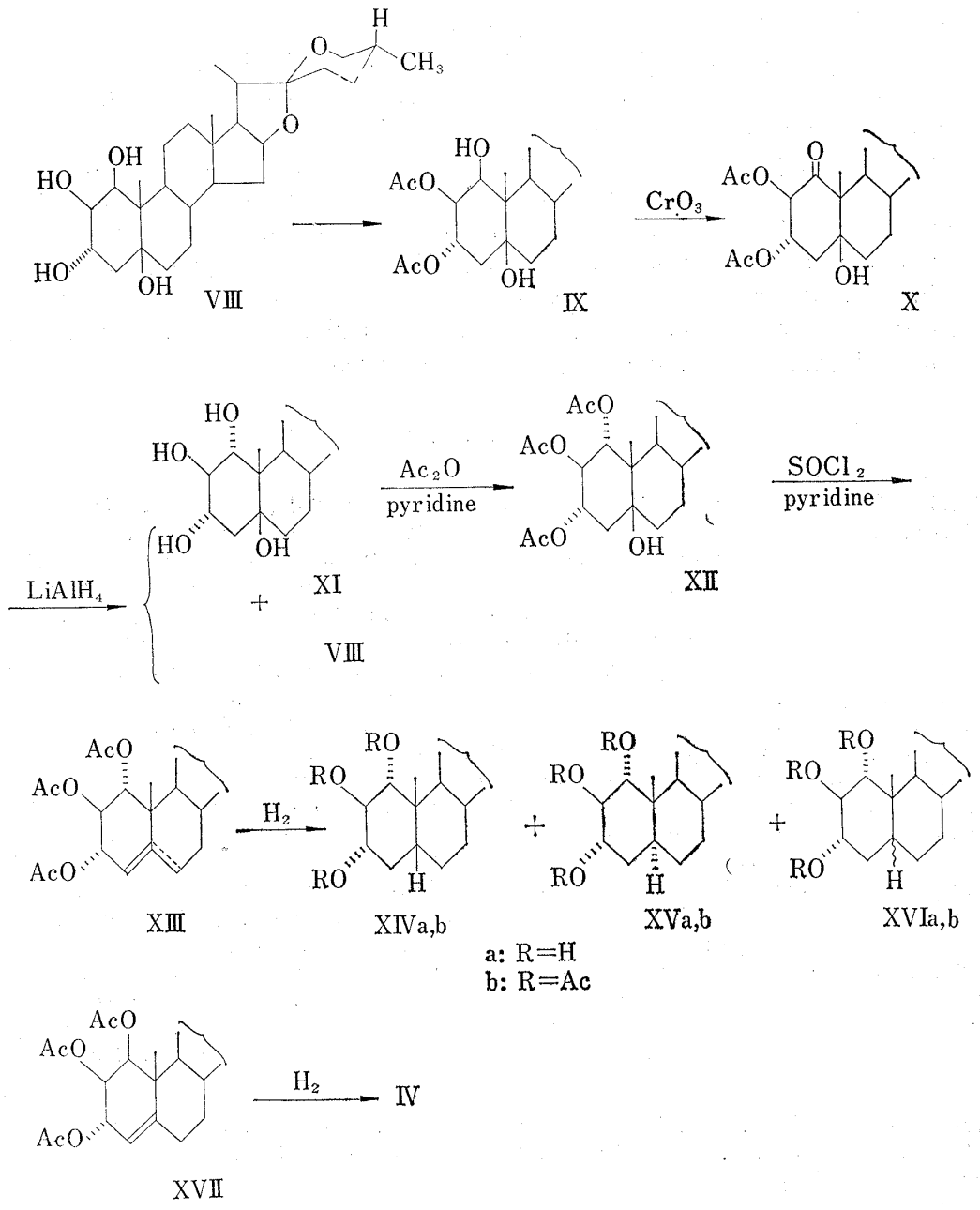

Chart 3

7) K. Tori and K. Aono, *Ann. Rept. Shionogi Res. Lab.*, **14**, 136 (1964).

According to Kubota's method,[8] kogagenin (VIII) was converted into a ketone (X) *via* the diacetate (IX). When the ketone (X) was reduced with lithium aluminium hydride, kogagenin (VIII) and its 1-epimer (XI), mp 307—308°, were obtained. The triacetate (XII) of the latter was treated with thionyl chloride–pyridine to eliminate the tertiary hydroxyl group at C-5. The product (XIII) consisting of the double bond isomers was hydrogenated with Adam's catalyst in ether containing acetic acid, to give a triacetate (XIVb), mp 187—188°, as a major product and a small amount of a triacetate (XVb), mp 170—175°, together with a diacetate[9] (XVIb), mp 203—205°. On saponification of these acetates, they afforded respective trihydroxy compounds (XIVa), mp 215—217°, $[\alpha]_D$ −55.7°, and (XVa), mp 255—257°, and a dihydroxy compound (XVIa), mp 200—202°, respectively. As reported earlier,[8] anhydrokogagenin triacetate (XVII) gave an A/B *cis*-derivative (tokorogenin, IV) as a major product, therefore in this case the major product (XIVa) may probably be an A/B *cis*- and the minor one (XVa) a *trans*-compound. Neither the compound (XIVa or XVa) nor its acetate (XIVb or XVb) was identical with isodiotigenin (II) or its acetate. From these results, 1α,2β,3α-trihydroxy compounds (XIVa and XVa) also should be excluded for diotigenin, and consequently diotigenin is assumed to be a 2,3,4-trihydroxy compound.

A considerable amount of diotigenin was isolated as a free sapogenin from this plant. The facts that the only spirostane existing as the free sapogenin in the plant body is one having an α-hydroxyl group at C-3 and the 5β-hydrogen on the basis of our earlier studies,[4,6] and that tokorogenin (IV) and yonogenin (V) are obtained from this plant, lead to the assumption that diotigenin is a 3α-hydroxy compound. Moreover, as the precipitation reaction[4,10] of diotigenin with digitonin is negative after 24 hr, this assumption is also supported. Now, we wish to propose the formula (Ia) for the structure of diotigenin. Further structural confirmation of diotigenin is now under investigation in our laboratory.

Experimental[11]

Diotigenin[3] (Ia)——Colorless needles (from Me_2CO), mp 280—281°, $[\alpha]_D^{24}$ −59.8° (c=1.062, MeOH); triacetate[4] (Ib), colorless needles (from MeOH), mp 218—219°, $[\alpha]_D^{24}$ −25.0° (c=1.172).

Conversion of Diotigenin (Ia) into Isodiotigenin (II)——A solution of Ib (230 mg) in 95% EtOH (30 ml) and 35% HCl (5 ml) was heated under reflux for 72 hr, and was evaporated and extacted with ether. The extract was washed with 2N Na_2CO_3, dried (Na_2SO_4), and evaporated leaving a residue (175 mg). The residue was recrystallized from acetone to give isodiotigenin (II), colorless needles, mp 266—268°, $[\alpha]_D^{25}$ −48.6° (c=1.064), ν_{max}^{Nujol} 3445, 3340, 981, 925, 900, and 865 cm^{-1}. *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.05; H, 9.83. Triacetate, colorless needles, mp 212.5—213.5° (from MeOH), $[\alpha]_D^{25}$ −26.9° (c=0.834). *Anal.* Calcd. for $C_{33}H_{50}O_8$: C, 68.98; H, 8.76. Found: C, 68.99; H, 8.87.

Triacetoxypregn-16-en-20-one (III) from Diotigenin (Ia)——A mixture of Ia (500 mg) in Ac_2O (30 ml) was heated in a sealed tube at 200° for 10 hr and extracted with ether to give a product (690 mg). The product was dissolved in AcOH (15 ml) and oxidized with a solution of CrO_3 (500 mg) in 90% AcOH (5 ml) at 25° for 1 hr. The reaction mixture was extracted with ether, washed with 5% KOH and water, dried (Na_2SO_4), and evaporated leaving a residue (520 mg). The residue was dissolved in benzene (50 ml) and chromatographed on alumina (20 g) to give triacetoxypregn-16-en-20-one (III, 176 mg) as colorless needles (from n-hexane), mp 191—193°, $[\alpha]_D^{24}$ +74.2° (c=1.13), λ_{max} 240 mμ (ε 9300), ν_{max}^{Nujol} 1745, 1669, 1592 and 1334 cm^{-1}. *Anal.* Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07. Found: C, 68.58; H, 8.07.

Oxidation of Diotigenin (Ia) with Periodic Acid——A solution of Ia (503 mg) and $HIO_4 \cdot 2H_2O$ (330 mg) in MeOH (150 ml) and water (20 ml) was left for 20 hr at room temperature, and then the solvent was evaporated *in vacuo* and the residue was extracted with ether. The ether solution was washed with 5% $NaHCO_3$, dried (Na_2SO_4), and evaporated leaving a residue (508 mg). The residue was dissolved in acetone (50 ml)

8) T. Kubota, *Chem. Pharm. Bull.* (Tokyo), **7**, 898 (1959). K. Takeda, T. Kubota, and A. Shimaoka, *Tetrahedron*, **7**, 62 (1959). T. Kubota and K. Takeda, *ibid.*, **10**, 1 (1960).
9) It may be obtained by hydrogenolysis.
10) E. Fernholz, *Z. Physiol. Chem.*, **232**, 97 (1935).
11) Unless otherwise specified, UV spectra were taken in 95% EtOH and rotations in $CHCl_3$. NMR spectra were taken with a Varian A-60 spectrometer. Melting points were measured on a Kofler block and are corrected.

and oxidized with chromium trioxide solution[12] (1.2 ml) at 20° for 2 min. The mixture was extracted with ether, and the ether solution was extracted with 5% KOH. The ether layer gave a neutral oil (61 mg), and the aqueous layer was acidified with AcOH and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated leaving an acid residue (418 mg), which was immediately esterified with diazomethane to give a methyl ester (444 mg). The residue was chromatographed on alumina to give a dimethylester (VI, 112 mg) as colorless needles (from MeOH), mp 147—148°, $[\alpha]_D^{24}$ −46.7° (c=1.103), $\nu_{max}^{CHCl_3}$ 1744 cm^{-1}. *Anal.* Calcd. for $C_{28}H_{44}O_6$: C, 70.55; H, 9.31. Found: C, 70.39; H, 9.28.

Oxidation of Diotigenin (Ia) with Chromium Trioxide——A solution of CrO_3 (500 mg) in 80% AcOH (15 ml) was added to a solution of Ia (500 mg) in AcOH (80 ml) with stirring at room temperature and stirred for 3 hr at the same temperature. EtOH (5ml) was added to this mixture and the solvent was evaporated *in vacuo*. The residue was extracted with ether, and the ether solution was extracted with 5% KOH. The ether layer was washed with water, dried (Na_2SO_4), and evaporated leaving a neutral residue (213 mg), which was recrystallized from EtOH to give an acid anhydride (VII, 105 mg) as colorless needles, mp 215—219°, $[\alpha]_D^{24}$ −67.4° (c=0.526), $\nu_{max}^{CHCl_3}$ 1806 and 1759 cm^{-1}. *Anal.* Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.38; H, 8.89. The 5% KOH solution was acidified with AcOH, extracted with ether, washed with water, dried (Na_2SO_4), and evaporated leaving an acid residue (192 mg). Esterification of the residue with diazomethane gave VI.

Oxidation of Kogagenin Diacetate (IX) to X——A solution of kogagenin diacetate[8] (IX, 100 mg) in acetone (20 ml) was oxidized with chromium trioxide solution[12] (0.2 ml) at 20—22° for 3 min. The mixture was extracted with ether, washed with 10% Na_2CO_3, dried (Na_2SO_4), and evaporated leaving a residue (77 mg), which was recrystallized from MeOH to give a ketone (X, 52 mg) as colorless needles, mp 274—275°, ν_{max}^{Nujol} 3395, 1745, 1734, and 1714 cm^{-1}. *Anal.* Calcd. for $C_{31}H_{46}O_8$: C, 68.10; H, 8.48. Found: C, 68.23; H, 8.49.

1-Epikogagenin (XI)——A solution X (1.13 g) in tetrahydrofuran (100 ml) was added to a suspension of $LiAlH_4$ (1 g) in tetrahydrofuran (50 ml) with stirring for 30 min at room temperature and stirred for additional 1.5 hr under reflux. Water (10 ml) was added to this mixture with stirring in an ice-bath and extracted with ether, and the extract was washed with water, dried (Na_2SO_4), and evaporated leaving a residue (672 mg). The residue was chromatographed on "Florisil" (50 g) to give kogagenin (VIII, 41 mg) and 1-epikogagenin (XI, 481 mg), colorless plates (from MeOH), mp 307—308°, $[\alpha]_D^{25}$ −47.6° (c=0.662, MeOH) (*Anal.* Calcd. for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55. Found: C, 69.78; H, 9.53). I-Epikogagenin triacetate (XII), colorless needles (from MeOH), mp 243—245°, ν_{max}^{Nujol} 3460, 1745, and 1720 cm^{-1}. *Anal.* Calcd. for $C_{33}H_{50}O_9$: C, 67.09; H, 8.53. Found: C, 67.24; H, 8.42.

Conversion of XII into 1α,2β,3α-Trihydroxy Spirostanes (XIVa and XVa)——A solution of $SOCl_2$ (500 mg) in pyridine (2 ml) was added to a solution of XII (342 mg) in pyridine (5 ml) with stirring for 10 min in an ice-bath and left for 45 min at room temperature. The mixture was poured onto ice-water and extracted with ether. The extract was washed with 5% H_2SO_4, 5% Na_2CO_3, and water, dried(Na_2SO_4), and evaporated leaving a residue (XIII, 312 mg). The residue (298 mg) was dissolved in ether (75 ml) and AcOH (3ml) and hydrogenated with Adams' catalyst (300 mg). The mixture was filtered, and the filtrate was washed with water and 5% Na_2CO_3, dried (Na_2SO_4), and evaporated leaving a crystalline residue, mp 170—180° (282 mg). The residue was chromatographed on alumina (10 g) to give diacetate (XVIb, 74 mg), colorless needles (from MeOH), mp 203—205°, $\nu_{max}^{CS_2}$ 1742 and 1250 cm^{-1}, a triacetate (XIVb, 146 mg), colorless needles (from MeOH), mp 187—188°, $\nu_{max}^{CS_2}$ 1750 and 1227 cm^{-1} (*Anal.* Calcd. for $C_{33}H_{50}O_8$: C, 68.96; H, 8.77. Found: C, 69.18; H, 8.85), and a triacetate (XVb, 31 mg), colorless needles (from MeOH) ,mp 170—175°, $\nu_{max}^{CS_2}$ 1745, 1242, and 1225 cm^{-1}.

XIVa, colorless needles (from MeOH), mp 215—217°, $[\alpha]_D^{25}$ −55.7° (c=0.673, MeOH). *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 71.87; H, 9.99.

XVa, colorless needles (from MeOH), mp 255—257°. *Anal.* Found: C, 71.92; H, 9.67.

XVIa, colorless plates (from MeOH), mp 200—202°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.63; H, 10.27.

Precipitation Reaction of Ia with Digitonin——A solution of digitonin (10 mg) in 90% EtOH (1ml) was added to a solution of Ia (10 mg) in 95% EtOH (1.5 ml) and left for 24 hr at room temperature. This recation was negative.

Acetonide of Ia——A solution of Ia (400 mg) and *p*-toluenesulphonic acid (250 mg) in acetone (250 ml) was heated under reflux in a steam bath for 5 hr. The mixture was evaporated *in vacuo* and extracted with ether. The extract was washed with 5% $NaHCO_3$, dried (Na_2SO_4), and evaporated leaving the starting material (Ia, 394 mg).

12) CrO_3 (2.67 g) and conc. H_2SO_4 (2.3 ml) in water (10 ml).