H, 5.22; N, 36.20. Found : C, 51.44; H, 5.54; N, 36.07.

The action of the compounds on lactic acid bacteria was measured as described earlier.[1] In this experiment three different media, OFA, PFA, and FA, were used for *L. casei*. The content of folic acid in FA medium increased to 10 mγ/cc.

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Summary

Action of aminated azaindolizines on *Lactobacillus casei* and *Streptococcus faecalis* was compared with that of several diaminopyrimidine compounds. All compounds exhibited more pronounced effect on *St. faecalis* than on *L. casei*. Growth inhition caused by these compounds was reversed in the decreasing order by thymine, leucovorin, and folic acid. These results suggested that C=N bond between 1- and 8a-positions in 1-azaindolizine molecule might have an effect similar to amino group on the lactic acid bacteria.

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185. Kaname Hamamoto : Studies on the Steroidal Components of Domestic Plants. XXIII.[1] Structure of Metagenin. (3).[1]

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In the previous papers[1,2] from this laboratory, metagenin was assigned the structure of 5β,25D-spirostane-2β,3β,*x*-triol (*x*=7 or 11) (I) and the location of the third hydroxyl group had remained still undetermined. The present paper is concerned with the elucidation of the properties and conformation of the undecided hydroxyl group.

Metagenone (IIa), prepared[1] with chromium trioxide oxidation of metagenin 2,3-acetonide[2] (Va) or 2,3-diacetate,[1] afforded a new triol (IIIa), epimetagenin, m.p. 134~136°, when it was treated with sodium borohydride, while metagenin (I) was regenerated in a good yield with sodium and isopropanol. In the case of reduction with lithium aluminium hydride, metagenone diacetate (IIb) gave epimetagenin as a main product together with a small amount of metagenin.

The activities of the unknown hydroxyl groups of both isomers (I) and (IIIa) were compared by acylation reactions. Epimetagenin gave only a diacetate (IIIb), m.p. 175°, by the action of acetic anhydride and pyridine at room temperature. Epimetagenin acetonide (IV), m.p. 218~221°, prepared from epimetagenin, was not affected by cathylation reaction[3] with ethyl chlorocarbonate. On the other hand, metagenin acetonide (Va) readily gave an acetonide cathylate (Vb), m.p. 159°, which turned into metagenin monocathylate (VIa), m.p.

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*1 192 Imafuku, Amagasaki, Hyogo-ken (浜元 要).

1) Part XXII, Part (2) : K. Takeda, K. Hamamoto : This Bulletin, 8, 1004(1960).
2) Part (1) : K. Takeda, T. Okanishi, K. Hamamoto, A. Shimaoka, N. Maezono : Yakugaku Zasshi, **77**, 175(1957).
3) L. F. Fieser, J. E. Herz, M. W. Klohs, M. A. Romers, T. Utne : J. Am. Chem. Soc., **74**, 3309(1952).

218°, by the deacetonization with dilute acetic acid. A similar reaction course was described in the previous paper[1] for the formation of metagenin acetonide acetate (Vc) and metagenin monoacetate (VIb). The third hydroxyl group of epimetagenin (IIIa) was shown to be very unstable to acids. Namely, when epimetagenin diacetate (IIIb) was saponified with acids, or epimetagenin was heated directly in an acid solution, a dehydration took place affording a spirostene-diol (VIIa), m.p. 173~176°, and its diacetate (VIIb), m.p. 192~193°, having an absorption maximum probably due to 9—11 double bond (λ_{max}^{EtOH} 206 mμ (ε 3900)). Thus it is concluded that the unknown hydroxyl group in metagenin and its epimeric one in epimetagenin have an equatorial and an axial conformation, respectively.

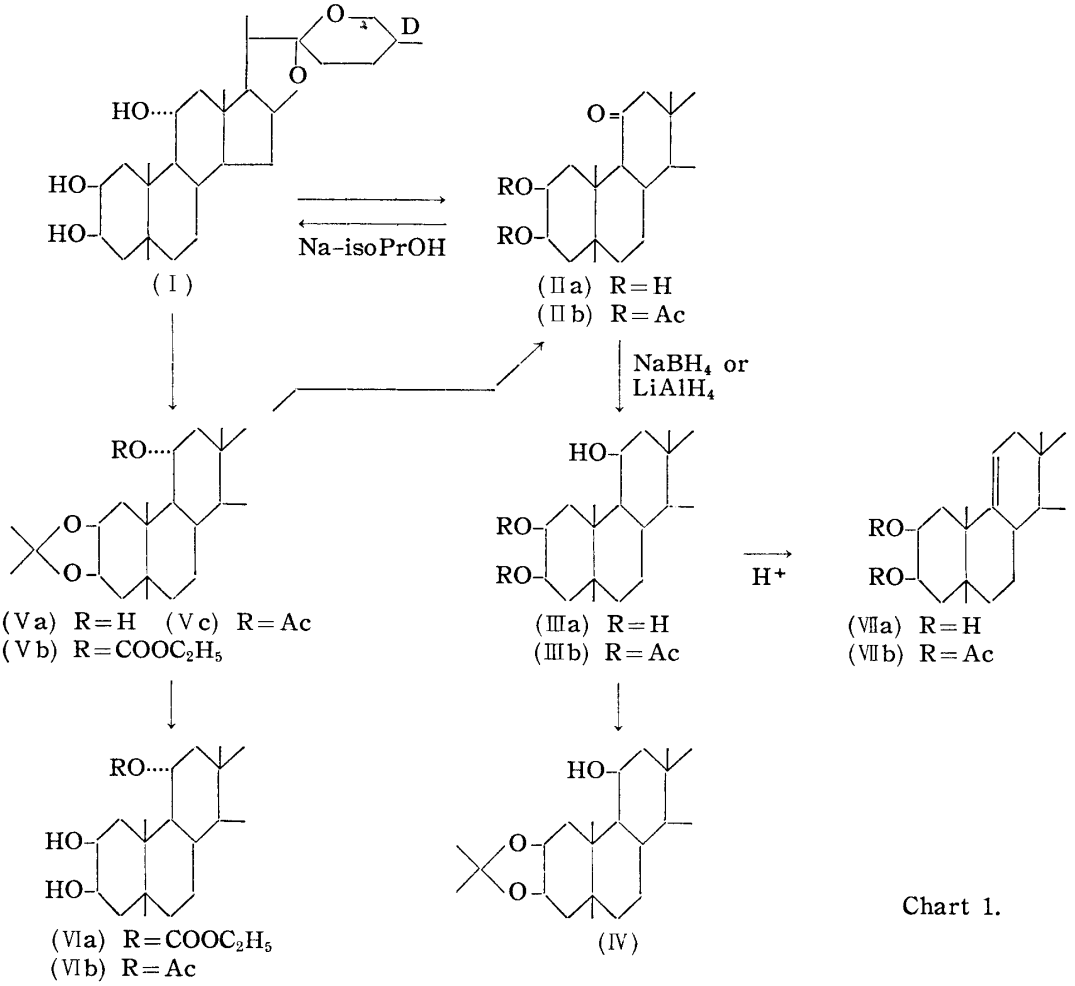

Chart 1.

In order to determine the position and configuration of the unknown hydroxyl group, metagenin was converted to its A-seco acid, keeping the hydroxyl group unchanged. According to a molecular model, metagenin should afford 11α-hydroxy-δ-lactone (Xa) or 7α-hydroxy-δ-lactone (XVI) via 2,3-seco acid, if it has 7- or 11-hydroxyl group in α-side and, in the case of β-orientation it must be almost impossible to form such a lactone ring.

Chromium trioxide oxidation of metagenin x-monocathylate (VIa) or x-monoacetate (VIb) gave ethoxycarbonyl-A-seco acid (VIIIa), m.p. 274° (decomp.), or acetyloxy-A-seco acid (VIIIb), m.p. 282° (decomp.), respectively. Acetyloxy-A-seco acid gave its dimethyl ester (IX) melting at 106~108°. These A-seco acids readily afforded the same compound (Xa), m.p. 248°, by saponification with alkali and it was esterified with diazomethane or dimethyl sulfate to give a monomethyl ester (Xb), m.p. 172°. Analytical data and infrared absorption spectrum of each compound (Xa or Xb) indicate no hydroxy-A-seco acid structure

like that of (XIIIa), but a δ-lactone monocarboxylic acid (Xa) $C_{27}H_{40}O_6$ (λ_{max}^{Nujol} μ : 5.75 (δ-lactone); 5.81 (COOH))[4] and its monomethyl ester (Xb), $C_{28}H_{42}O_6$ (λ_{max}^{Nujol} μ : 5.78 (δ-lactone); 5.83 ($COOCH_3$)). The same δ-lactone (Xb) was obtained from dimethyl metagenate (XIb)[2] by reduction with sodium and isopropanol, and also directly from metagenin itself with chromium trioxide in acetic acid at 15° as a by-product of metagenic acid (XIa). This δ-lactone (Xb) afforded a triol (XII), m.p. 208°, with lithium aluminium hydride. As the same triol (XII) was also obtained from the dimethyl ester (IX) with the same reagent, it is clear that no epimerization occurred in the course of the lactone formation.

On the other hand, sodium borohydride reduction of dimethyl metagenate (XIb) afforded an epimeric hydroxy-A-seco acid dimethyl ester (XIIIa), m.p. 163°, which was resistant to

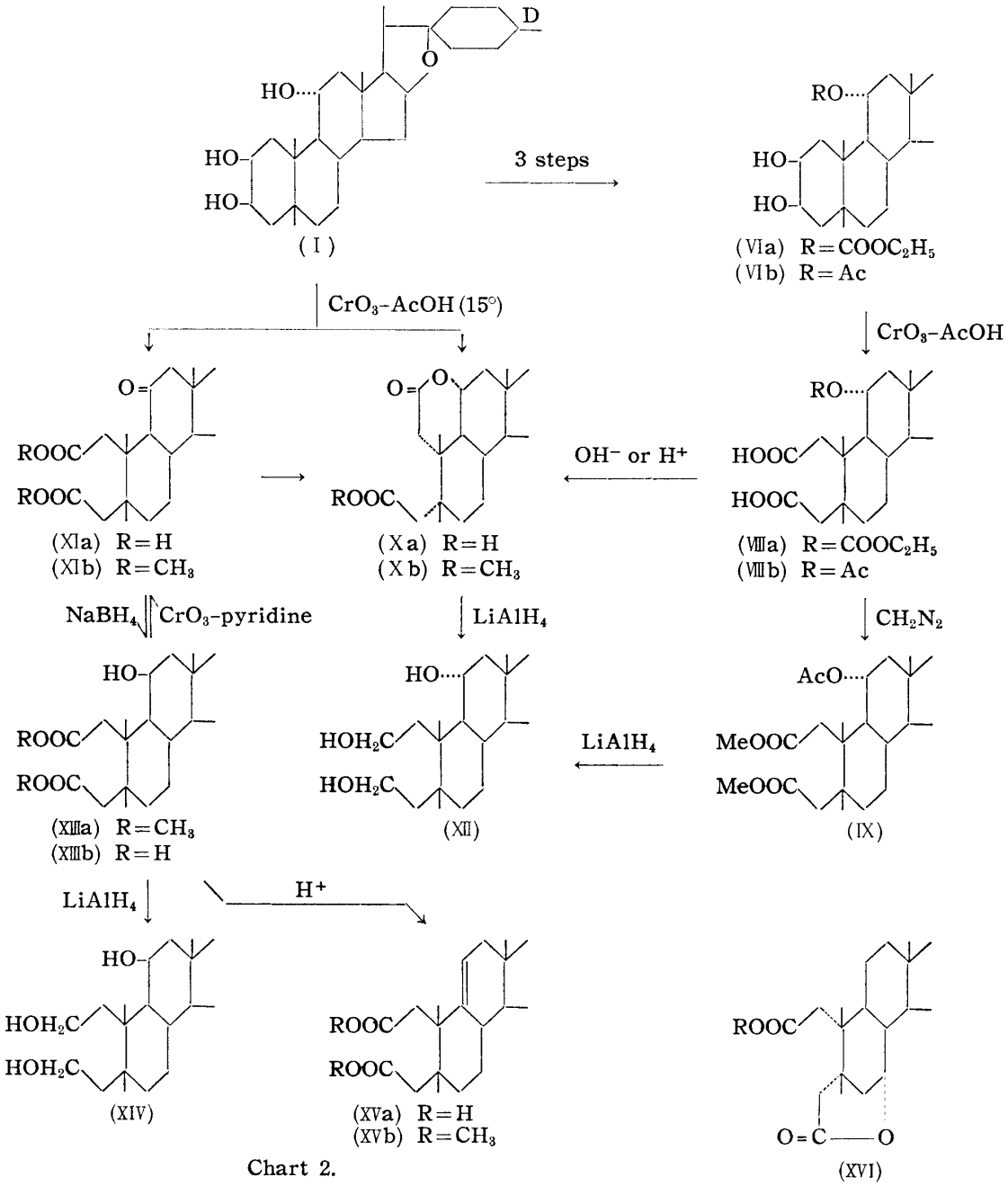

Chart 2.

4) R. N. Jones : "Chemical Applications of Spectroscopy," 455(1956), Interscience Publishers Inc., New York.

δ-lactone formation and gave a free dioic acid (XIIIb), m.p. 222° (decomp.), by alkaline hydrolysis. By an acid treatment, it was dehydrated to unsaturated A-seco acid (XVa), m.p. 246°, and its ester (XVb), m.p. 136~137°. The parent ketone (XIb) was regenerated from the ester (XIIIa) by chromium trioxide in pyridine at room temperature. The hydroxyl group of the ester (XIIIa) was not affected by mild acetylation as in the case of epimetagenin. The dimethyl ester (XIIIa) gave a triol (XIV), m.p. 216~217°, as expected, not identical with the above-mentioned triol (XII).

These reaction sequences indicate that the unknown hydroxyl group in metagenin should be located on the same side as the acetic acid residue of samogenic acid; in other words, this hydroxyl group must be α-oriented. Furthermore, the hydroxyl group has an equatorial conformation, C-11 being the most probable position for this group. Comparison of the molecular rotation values of the corresponding metagenin and epimetagenin derivatives also supports this assignment[5] (see Table I).

Table I. Molecular Rotation Values of Metagenin and Epimetagenin Derivatives

Derivatives	β-OH (Epimetagenin series)		α-OH (Metagenin series)		M_D (β-OH) M_D (α-OH)
	$[\alpha]_D$	M_D	$[\alpha]_D$	M_D	
Sapogenin	−47°	−212°	−82°	−368°	+156°
2,3-Diacetate	−50°	−264°	−67°	−359°	+ 95°
2,3-Acetonide	−56°	−272°	−86°	−419°	+147°
2,3-Seco-triol	−40°	−184°	−60°	−270°	+ 86°
7-Hydroxy-5β-steroids[5]		+110°		− 79°	+189°
11-Hydroxy-5β-steroids[5]		+ 96°		− 29°	+125°

Experimental

All melting points are uncorrected. Unless otherwise noted, rotations were measured in $CHCl_3$ solution.

Epimetagenin (IIIa)—a) Reduction of Metagenone (IIa) with $NaBH_4$: A solution of 200 mg. of metagenone (IIa) in 18 cc. of MeOH was reduced with 0.2 g. of $NaBH_4$ for 2 hr. at room temperature. The precipitate separated by addition of water was dissolved in Et_2O, the Et_2O solution was dried, and evaporated to give an oily residue (0.2 g.), which was chromatographed over Al_2O_3. The eluate with MeOH-$CHCl_3$ (2:98) afforded crude crystals (165 mg.), purified from 50% MeOH-H_2O to prisms, m.p. 134~136°, $[\alpha]_D^{25}$ −47.3°. *Anal.* Calcd. for $C_{27}H_{44}O_5 \cdot \frac{1}{2}H_2O$: C, 70.86; H, 9.91. Found: C, 70.64; H, 9.88. IR: λ_{max}^{Nujol} 2.92 μ (OH).

b) Reduction of Metagenone Diacetate (IIb) with $LiAlH_4$: A solution of 300 mg. of metagenone diacetate (IIb) in 10 cc. of tetrahydrofuran was refluxed for 2 hr. with 0.1 g. of $LiAlH_4$. Addition of Na_2SO_4 solution and extraction with Et_2O afforded oily residue (280 mg.), which was refluxed with 10% KOH-EtOH for 1 hr. to complete saponification of the acetyl groups. The crude product, obtained in the usual manner, was chromatographed over Al_2O_3. The eluate with MeOH-$CHCl_3$ (2:98) yielded 220 mg. of epimetagenin, which was purified from 50% MeOH-H_2O to prisms, m.p. 134~136°, identical with the product obtained in a). The eluate with MeOH-$CHCl_3$ (20:80) gave 20 mg. of metagenin (I), m.p. 268~270°; an admixture with the sample obtained in a previous work[2] showed no depression.

Epimetagenin Diacetate (IIIb)—A mixture of 80 mg. of epimetagenin with 2 cc. Ac_2O and 1 cc. of pyridine was left at room temperature overnight. The crude product, processed in the usual manner, was purified from petr. ether-Et_2O, yielding 80 mg. of diacetate, m.p. 174~175°, $[\alpha]_D^{25}$ −48.8°. *Anal.* Calcd. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 69.90; H, 9.09. IR λ_{max}^{Nujol} μ: 8.18, 7.93, 7.88 (5β; 2β,3β-diacetate); 2.85 (OH).

Metagenin (I) from Metagenone Diacetate (IIb)—To a solution of 74 mg. of metagenone diacetate (IIb) in 5 cc. of iso-PrOH 200 mg. of Na was added under refluxing, which was continued until complete dissolution of Na (about 30 min.). Dilution with water and filtration afforded 63 mg. of metagenin (I), m.p. 267~270°, proved to be identical with the sample[2] by a mixed m.p.

Epimetagenin Acetonide (IV)—A solution of 100 mg. of epimetagenin (IIIa) and 10 mg. of *p*-TsOH in 18 cc. of Me_2CO was refluxed for 6 hr. After Na_2CO_3 was added, the volume was reduced to ca.

5) L. F. Fieser, M. Fieser: "Steroids," 179(1959). Reinhold Publishing Corp., New York.

8 cc. and the reaction mixture was extracted with Et_2O. The solvent was evaporated and the residue was chromatographed over Al_2O_3. The eluate with Et_2O was recrystallized from MeOH yielding 90 mg. of acetonide (IV), m.p. 217~221°, $[\alpha]_D^{20}$ −55.6°. *Anal.* Calcd. for $C_{30}H_{48}O_5$: C, 73.44; H, 9.98. Found: C, 73.50; H, 10.20. IR : λ_{max}^{Nujol} 2.86 μ (OH).

This (IV) resisted cathylation reaction and was recovered unchanged from the reaction mixture [cf. (Vb)].

Metagenin Acetonide Cathylate (Vb)—To a solution of 350 mg. of metagenin acetonide (Va) in a mixture of 8 cc. of dioxane and 4 cc. of pyridine, an excess ClCOOEt (1 g.) was added at 0° and the reaction mixture was left overnight. Dilution with H_2O containing HCl, extraction with Et_2O, and evaporation of the solvent furnished an oily residue (350 mg.), which was chromatographed over Al_2O_3. The eluate with petr. ether yielded 300 mg. of crude cathylate (Vb), m.p. 162~166°, which recrystallized from Et_2O-petr. ether to needles, m.p. 166~167°, $[\alpha]_D^{24}$ −84.2°. *Anal.* Calcd. for $C_{33}H_{52}O_7$: C, 70.68; H, 9.35. Found : C, 70.52; H, 9.41.

The eluate with Et_2O yielded 50 mg. of the starting material (Va), proved by a mixed m.p.

Metagenin Monocathylate (VIa)—Metagenin acetonide cathylate (Vb) (400 mg.) was warmed at 55° for 1 hr. with 10 cc. of 50% AcOH and the reaction mixture was evaporated under a reduced pressure. The oily residue was recrystallized from MeOH-$CHCl_3$, yielding 350 mg. of (VIa), m.p. 217~218°, $[\alpha]_D^{23}$ −84.4°. *Anal.* Calcd. for $C_{30}H_{48}O_7 \cdot 2H_2O$: C, 64.71; H, 9.42. Found : C, 64.80; H, 9.44.

5β,25D-Spirost-9(11)-ene-2β,3β-diol (VIIa)—Epimetagenin diacetate (IIIb) (100 mg.) was refluxed with a mixture of 5 cc. of 35% HCl and 13 cc. of MeOH for 4.5 hr., diluted with H_2O, and extracted with Et_2O. After washing with 10% Na_2CO_3 solution and H_2O, the solvent was evaporated and the residue (82 mg.) was purified over Al_2O_3. The eluate with MeOH-$CHCl_3$ (5:95) yielded 72 mg. of (VIIa), m.p. 173~176° (from $CHCl_3$-hexane), $[\alpha]_D^{20}$ −79.8°. *Anal.* Calcd. for $C_{27}H_{42}O_4 \cdot \frac{1}{2}H_2O$: C, 73.76; H, 9.83. Found : C, 73.47; H, 10.01. IR λ_{max}^{Nujol} μ : 6.12 (double bond); 3.04, 2.93 (OH). UV : $\lambda_{max}^{95\%EtOH}$ 260 mμ. (ε 4200).

5β,25D-Spirost-9(11)-ene-2β,3β-diol Diacetate (VIIb)—The above-mentioned diol (VIIa) (10 mg.) was refluxed with 1 cc. of Ac_2O for 1 hr. and processed in the usual manner. The crude product was purified from $CHCl_3$-MeOH to crystals of m.p. 193~194°, $[\alpha]_D^{22}$ −49.3°. *Anal.* Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found : C, 72.36; H, 9.03. IR λ_{max}^{Nujol} μ : 8.18, 7.99 (5β; 2β,3β-diacetate); 5.77 (AcO), 6.04 (double bond at 9(11). UV : $\lambda_{max}^{95\%EtOH}$ 206 mμ (ε 3900).

11α-Acetoxy-2,3-seco-5β,25D-spirostane-2,3-dioic Acid (11α-Acetoxysamogenic Acid) (VIIIb)—A solution of 0.5 g. of metagenin 11-monoacetate (VIb) in 50 cc. of AcOH was oxidized with 0.1 g. of CrO_3 in 20 cc. of 90% AcOH at 15° for 30 min., diluted with H_2O, and extracted with Et_2O. After washing the Et_2O layer with 5% NaOH solution, the alkaline solution was acidified with AcOH and the precipitate was collected. The crude product (0.5 g.) was recrystallized from $CHCl_3$-MeOH to a crystalline powder, m.p. 282° (decomp.), $[\alpha]_D^{20}$ −67.6°. *Anal.* Calcd, for $C_{29}H_{44}O_8$: C, 66.90; H, 8.52. Found : C, 66.73; H, 8.58.

11α-Ethoxycarbonyl-2,3-seco-5β,25D-spirostane-2,3-dioic Acid (11α-Ethoxycarbonylsamogenic Acid) (VIIIa)—The oxidation of metagenin 11-monocathylate (VIa) (350 mg.) was carried out exactly as described above for 11-monoacetate (VIb) and yielded 250 mg. of 2,3-dioic acid (VIIIa), m.p. 274° (decomp.) (from MeOH-$CHCl_3$), $[\alpha]_D^{20}$ −56.2°. *Anal.* Calcd. for $C_{30}H_{46}O_9$: C. 65.43; H, 8.42. Found : C, 65.26; H, 8.40.

Dimethyl 11α-Acetoxysamogenate (IX)—A solution of 400 mg. of 11α-acetoxysamogenic acid (VIIIb) in a mixture of 20 cc. of Et_2O and 20 cc. of MeOH was methylated with CH_2N_2-Et_2O solution. After processing in the usual manner, the oily residue was chromatographed over Al_2O_3. The eluate with benzene-EtOH (9:1) furnished 300 mg. of ester (IX), m.p. 106~108° (from $CHCl_3$-MeOH), $[\alpha]_D^{32}$ −58.3°. *Anal.* Calcd. for $C_{31}H_{48}O_8$: C, 67.85; H, 8.82. Found : C, 67.65; H, 8.70.

11α-Hydroxy-2,3-seco-5β,25D-spirostane-2,3-dioic Acid δ-Lactone (Xa)—a) From 11α-Acetoxysamogenic acid (VIIIb) : 11α-Acetoxysamogenic acid (VIIIb) (400 mg.) in 40 cc. of 5% KOH-EtOH was stood for 36 hr. at room temperature. The solution was evaporated, diluted with H_2O, and acidified with AcOH. The crude product, extracted with Et_2O, was recrystallized from $CHCl_3$-MeOH, yielding 300 mg. of the lactone monoacid (Xa), m.p. 247~248°, $[\alpha]_D^{20}$ −53.4°. *Anal.* Calcd. for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75. Found : C, 70.17; H, 8.64. IR λ_{max}^{Nujol} μ : 5.81 (COOH); 5.75 (δ-lactone).

b) From 11α-Ethoxycarbonylsamogenic Acid (VIIIa) : 11α-Ethoxycarbonylsamogenic acid (VIIIa) (200 mg.) was refluxed with 10 cc. of 10% KOH-EtOH for 30 min. The reaction mixture was processed as mentioned in a) to give crystals of (Xa), m.p. 246~247°, identical with the sample obtained in a).

11α-Hydroxy-2,3-seco-5β,25D-spirostane-2,3-dioic Acid δ-Lactone Methyl Ester (Xb)—a) From δ-Lactone Monoacid (Xa) with CH_2N_2 : δ-Lactone monoacid (Xa) (100 mg.) was left in Et_2O solution of CH_2N_2 overnight in an ice-chamber and processed in the usual manner. Recrystallization from Et_2O-MeOH yielded 80 mg. of (Xb), m.p. 171~172°, $[\alpha]_D^{20}$ −43.1°. *Anal.* Calcd. for $C_{28}H_{42}O_6$: C, 70.85; H, 8.92. Found : C, 70.72; H, 8.94. IR λ_{max}^{Nujol} μ : 5.83 ($COOCH_3$); 5.78 (δ-lactone).

b) From (Xa) with Me_2SO_4 : δ-Lactone monoacid (Xa) (300 mg.) was dissolved in NaOEt solution

(Na 0.1 g. in 18 cc. of EtOH), evaporated to 2/3 of the original volume, and diluted with 20 cc. of benzene. To the Na salt suspension 1 g. of Me_2SO_4 was added and the reaction mixture was stirred for 5 hr. at 60~65°. The product, m.p. 169~171°, obtained in the usual manner, was identical with the sample from a).

c) From 11α-Acetoxysamogenic Acid (VIIIb) with HCl-MeOH : 11α-Acetoxysamogenic acid (VIIIb) (20 mg.) was refluxed with 3.5 cc. of 10% HCl-MeOH for 30 min. and the solvent was evaporated. The residue was extracted with Et_2O, which was washed with 10% Na_2CO_3 solution, and evaporated, giving crystals of (Xb), m.p. 164~168°, identical with the samples mentioned above.

d) From Metagenin by CrO_3-AcOH Oxidation : A solution of 1.5 g. of metagenin (I) in 150 cc. of AcOH was oxidized at 15° for 30 min. with a solution of 0.3 g. of CrO_3 in 30 cc. of 80% AcOH. The reaction mixture was diluted with H_2O, extracted with Et_2O, and washed with 10% NaOH solution, which was acidified with AcOH. The crude product, obtained by Et_2O extraction and evaporation, was methylated with CH_2N_2-Et_2O solution. The oily residue (1.0 g), obtained in the usual manner, was chromatographed over Al_2O_3. The eluate with petr. ether-benzene (1:9) and benzene furnished 0.6 g. of crude crystals, m.p. 98~111°, which were purified from $CHCl_3$-MeOH to give dimethyl metagenate (XIb), m.p. 114~115°, proved to be identical with the sample mentioned in Part (1).[2] The eluate with Et_2O gave 0.2 g. of the lactone monoester (Xb), m.p. 162~165°. Further recrystallization raised the m.p. to 171~172°, identical with the product obtained in a).

e) From Dimethyl Metagenate (XIb) with Na in iso-PrOH : To a solution of 100 mg. of dimethyl metagenate (XIb) in 6 cc. of iso-PrOH 200 mg. of Na was added under refluxing. The reaction mixture was diluted with H_2O, extracted with Et_2O, and the extract was washed with 5% NaOH solution. The alkaline layer was acidified with AcOH and extracted with Et_2O. The oily residue was methylated with CH_2N_2 and processed in the usual manner. The product, m.p. 167~169°, was shown to be identical with (Xb) by a mixed m.p.

2,3-Seco-5β,25D-spirostane-2,3,11α-triol (XII)—a) From δ-Lactone Monomethyl Ester (Xb) : A solution of 100 mg. of the δ-lactone monomethyl ester (Xb) in 3 cc. of tetrahydrofuran was refluxed with 80 mg. of $LiAlH_4$ for 3 hr. and processed in the usual manner. The crude product was recrystallized from $CHCl_3$-MeOH to 75 mg. of prisms, m.p. 207~208°, $[\alpha]_D^{23}$ −59.9° ($CHCl_3$-MeOH 1:1). *Anal.* Calcd. for $C_{27}H_{46}O_5$: C, 71.96; H, 10.29. Found : C, 72.19; H, 10.54. IR λ_{max}^{Nujol} μ : 3.08, 2.99 (OH).

b) From Dimethyl 11α-Acetoxysamogenate (IX) : A solution of 100 mg. of dimethyl 11α-acetoxysamogenate (IX) in 5 cc. of tetrahydrofuran was refluxed with 80 mg. of $LiAlH_4$ for 3 hr. and processed as described in a). The product, m.p. 207~208°, was identified by mixed m.p. with the sample from a).

Dimethyl 11β-Hydroxy-2,3-seco-5β,25D-spirostane-2,3-dioate (Dimethyl 11β-Hydroxysamogenate) (XIIIa)—To a solution of 300 mg. of dimethyl metagenate (XIb) in a mixture of 20 cc. of Et_2O and 20 cc. of MeOH, a solution of 100 mg. of $NaBH_4$ in 5 cc. of MeOH was added. The reaction mixture was left at 20° for 2 hr., evaporated to dryness, and diluted with H_2O. The crude product, obtained by extraction with Et_2O and evaporation of the solvent, was chromatographed over Al_2O_3. The eluate with benzene yielded 250 mg. of needles, m.p. 162~163° (from $CHCl_3$-MeOH), $[\alpha]_D^{32}$ −17.8°. *Anal.* Calcd. for $C_{29}H_{46}O_7$: C, 68.74; H, 9.19. Found : C, 68.83; H, 9.11. IR λ_{max}^{Nujol} μ : 5.81, 5.77 ($COOCH_3$); 2.85 (OH).

This substance (XIIIa) resisted acetylation reaction with Ac_2O and pyridine, and was recovered unchanged.

11β-Hydroxy-2,3-seco-5β,25D-spirostane-2,3-dioic Acid (11β-Hydroxysamogenic Acid) (XIIIb)—The dimethyl ester (XIIIa) was refluxed for 1 hr. with 1.5 cc. of 10% KOH-MeOH, the reaction mixture was diluted with H_2O, and acidified with AcOH. Extraction with EtOAc and evaporation of the solvent afforded free dioic acid (XIIIb), m.p. 222° (decomp.) (from MeOH), $[\alpha]_D^{18}$ −15.4° ($CHCl_3$-MeOH 1:1). *Anal.* Calcd. for $C_{27}H_{42}O_7 \cdot \frac{1}{2}H_2O$: C, 66.50; H, 8.89. Found : C, 66.15; H, 8.55. IR λ_{max}^{Nujol} μ : 5.93, 5.88 (COOH); 2.78 (OH).

It gave the original ester (XIIIa), m.p. 161~163°, with a solution of CH_2N_2 in Et_2O.

Dimethyl Metagenate (XIb) from Dimethyl 11β-Hydroxysamogenate (XIIIa)—A solution of 100 mg. of dimethyl 11β-hydroxysamogenate (XIIIa) in 2 cc. of pyridine was left with a solution of 75 mg. of CrO_3 in 1 cc. of pyridine for 17 hr. at room temperature and processed in the usual manner. The crude product was chromatographed over Al_2O_3 to give 60 mg. of dimethyl metagenate (XIb), m.p. 114~115°, identical with the sample described in part (1).[2]

2,3-Seco-5β,25D-spirostane-2,3,11β-triol (XIV)—A solution of 50 mg. of dimethyl 11β-hydroxysamogenate (XIIIa) was refluxed with 40 mg. of $LiAlH_4$ for 3 hr. and processed as described for (XII), giving crystals of m.p. 216~217°, $[\alpha]_D^{32}$ −40.0°. *Anal.* Calcd. for $C_{27}H_{46}O_5 \cdot \frac{1}{2}H_2O$: C, 70.55; H, 10.31. Found : C, 70.48; H, 10.36. IR : λ_{max}^{Nujol} 2.98 μ (OH).

2,3-Seco-5β,25D-spirost-9(11)-ene-2,3-dioic Acid (XVa) and Dimethyl Ester (XVb)—Dimethyl 11β-hydroxysamogenate (XIIIa) (95 mg.) was refluxed with a mixture of 4 cc. of 35% HCl and 10 cc. of MeOH for 4 hr. and diluted with water. Extraction with Et_2O and washing with 5% NaOH solution furnished two fractions, the Et_2O solution containing neutral substances and the alkaline solution. The

latter was acidified with AcOH, extracted with EtOAc, dried, and was evaporated to give 18 mg. of 2,3-dioic acid (XVa), m.p. 246° (from $CHCl_3$-MeOH). *Anal.* Calcd. for $C_{27}H_{40}O_6$ (XVa) : C, 70.40; H, 8.75. Found : C, 70.20; H, 8.89.

Neutral Et_2O solution was evaporated to give 75 mg. of dimethyl ester (XVb), m.p. 136~137° (from petr. ether-Et_2O), $[\alpha]_D^{21}$ −14.8°. *Anal.* Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08. Found : C, 71.15; H, 8.96. IR λ_{max}^{Nujol} μ : 6.11 (double bond); 5.78 ($COOCH_3$). UV : $\lambda_{max}^{95\%EtOH}$ 207 mμ (ε 2800).

This ester (XVb) gave the free dioic acid (XVa), m.p. 246°, when refluxed with 10% KOH-MeOH for 1 hr.

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Summary

The properties of the third hyhroxyl group in metagenin (I) and epimetagenin (IIIa) were examined. From the results of acylation reaction and acid treatment, it was confirmed that the third hydroxyl group in metagenin had an equatorial and the epimeric one in epimetagenin had an axial conformation. Metagenin formed a δ-lactone ring by the opening of A-ring and was approximately confirmed as 5β,25D-spirostane-2β,3β,11α-triol.

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