[Chem. Pharm. Bull. 17(4) 690—698 (1969)]

UDC 547.924.07 : 591.169

Synthesis of Ecdysone. IV.[1] A Novel Synthesis of the Side Chain Structure of Ecdysone

Hiromu Mori, Kenyu Shibata, Kiyoshi Tsuneda, and Masanobu Sawai

Research Laboratory, Teikoku Hormone Mfg. Co., Ltd.[2]

(Received May 7, 1968)

A novel synthetic method of preparation of the side chain of ecdysone, 22,25-dihydroxycholestane side chain was developed. The sequence of this method was summarized as the procedures XI→XII→XIII→XIV→XVI→XVII (Chart 3)→XXV→XXVII→XXIX (Chart 5).

The previous papers of this series described novel preparative methods of 2β,3β-dihydroxy[3] and 14α-hydroxy-7-en-6-one steroids,[4] both of which have partial structures of ecdysone (I). It was also described in the third paper of this series to synthesize the nuclear structure of ecdysone in cholestane series, namely, 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one (II).[4] This paper describes the synthesis of 22,25-dihydroxycholestane side chain structure, the other partial structure of ecdysone.

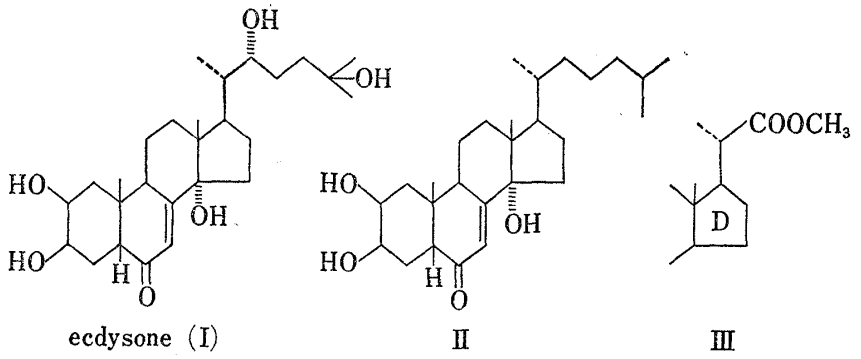

Chart 1

It was already reported by Karlson and his co-workers[5] that the side chain of ecdysone is unstable to mineral acid to afford dehydrated compound. Thus, the synthesis of ecdysone by the reaction sequence of the construction of side chain structure followed by the introduction of functional groups in a steroid nucleus seems very difficult. Probably from this reason, bisnorcholan-22-oic acid methyl ester derivatives (III) were selected as key intermediates, nuclear structure was constructed in these compounds, and then the synthesis of side chain

1) Part III: H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **16**, 1593 (1968).
2) Location: *Shimosakunobe, Kawasaki.*
3) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **15**, 460 (1967); H. Mori, K. Tsuneda, K. Shibata, and M. Sawai, *ibid.*, **15**, 466 (1967).
4) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **16**, 1593 (1968).
5) P. Karlson, H. Hoffmeister, H. Hummel, P. Hocks, and G. Spiteller, *Chem. Ber.*, **98**, 2394 (1965).
6) J.B. Siddall, J.P. Marshall, A. Bowers, A.D. Cross, J.A. Edwards, and J.H. Fried, *J. Am. Chem. Soc.*, **88**, 379 (1966); J.B. Siddall, A.D. Cross, and J.H. Fried, *ibid.*, **88**, 862 (1966).
7) U. Kerb, P. Hocks, R. Wiechert, A. Furlenmeier, A. Fürst, A. Langemann, and G. Waldvogel, *Tetrahedron Letters*, **1966**, 1387; R. Wiechert, U. Kerb, P. Hocks, A. Furlenmeier, A. Fürst, A. Langemann, and G. Waldvogel, *Helv. Chim. Acta*, **49**, 1581 (1966); A. Furlenmeier, A. Fürst, A. Langemann, G. Waldvogel, U. Kerb, P. Hocks, and R. Wiechert, *ibid.*, **49**, 1591 (1966); U. Kerb, G. Schulz, P. Hocks, R. Wiechert, A. Furlenmeier, A. Fürst, A. Langemann, and G. Waldvogel, *ibid.*, **49**, 1604 (1966).

structure was completed in both independent synthesis of ecdysone by two groups.[6,7] Moreover, 14α-hydroxyl group was also not stable to mineral acid.[5] This observation suggests that the synthesis of side chain structure by drastic condition would not be able to use in the synthesis of ecdysone. In such a circumstance, it seemed for us to be a promising idea that if γ-lactone (22-hydroxyhomocholan-25-oic acid 25→22-lactone) (V) is prepared from easily obtainable aldehyde (IV), the Grignard reaction of γ-lactone (V) would afford the side chain structure. γ-Lactone (V) would probably be stable to various reagents; accordingly, the reactions in construction of nuclear structure reported in above-mentioned papers[3,4] would proceed without any trouble. Finally, the construction of side chain would be completed by Grignard reaction. In fact, the synthesis of ecdysone succeeded by using γ-lactone (V) as a key intermediate.[8]

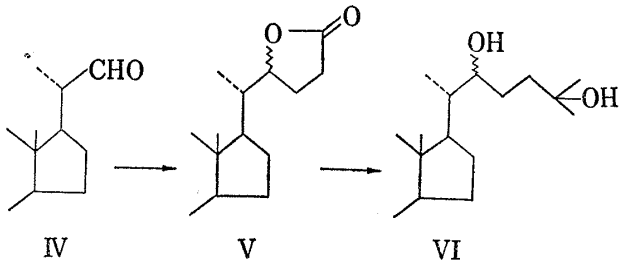

Chart 2

3,3;6,6-Bisethylenedioxybisnor-5α-cholan-22-al (XI) was selected as a starting aldehyde, not only because its nuclear structure is fully protected from reactions described below, but

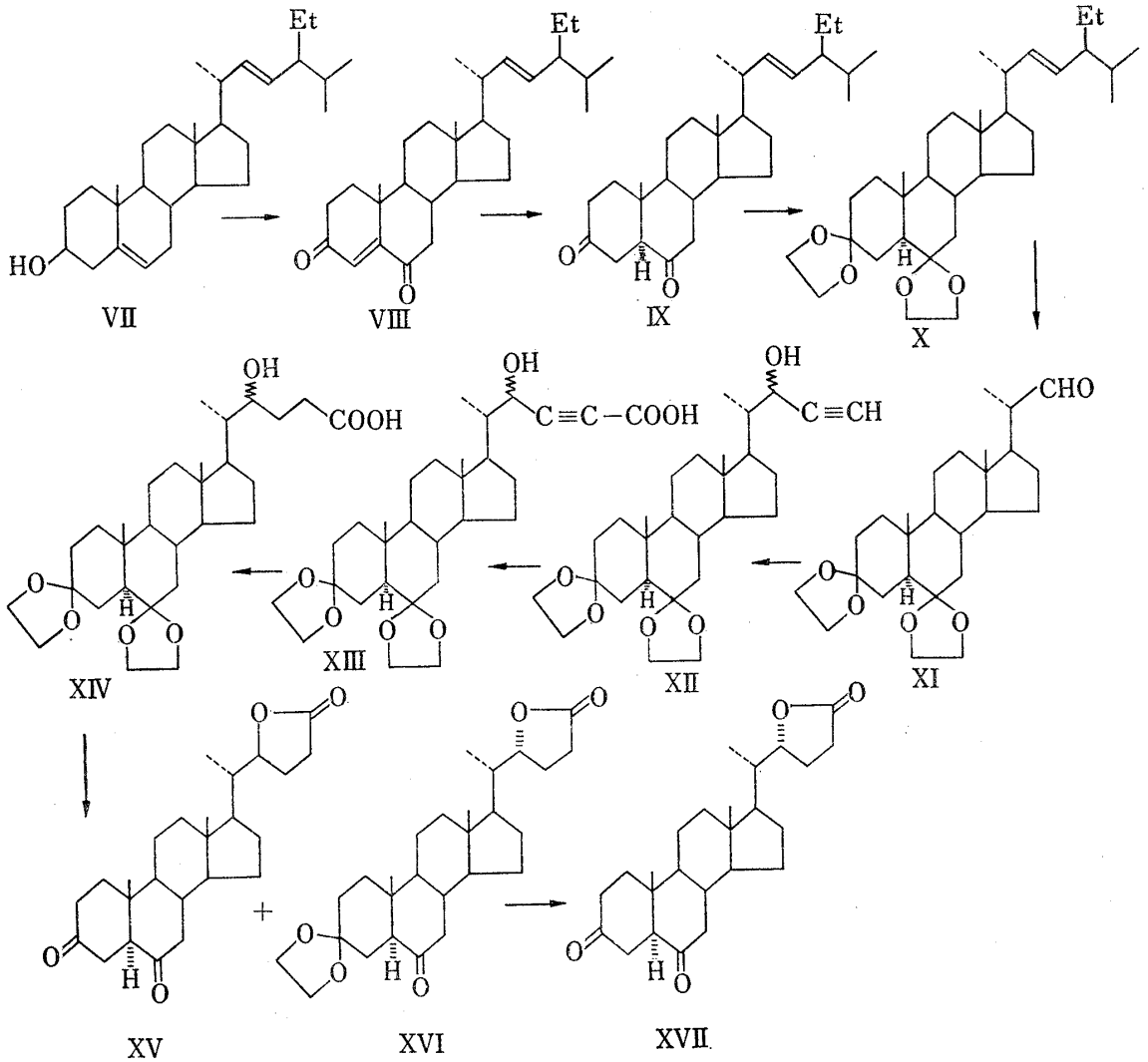

Chart 3

8) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **16**, 563 (1968).

also because XI would be directly used as a starting material for the synthesis of ecdysone. This aldehyde (XI) was prepared from stigmasterol (VII) as follows. Marker and his co-workers[9] reported that the oxidation of stigmasterol (VII) with chromium trioxide in acetic acid gave 4-ene-3,6-dione (VIII), which on reduction with zinc dust in aqueous acetic acid led to the dione (IX) in 53% yield. On the other hand, Nishina and Kimura[10] described that cholesterol was converted to 4-cholestene-3,6-dione by oxidation with Jones reagent in high yield. The oxidation of stigmasterol (VII) with both methods were tried, proving that the oxidation with Jones reagent gave the desired 4-ene-3,6-dione (VIII) with somewhat higher yield. The reduction of VIII with zinc dust afforded 3,6-dione (IX) as described in the literature,[9] which was converted to the diketal (X) by the usual method. The controlled ozonolisis of X was made by the modification of the method by Slomp and Johnson[11] for 4,22-stigmastadien-3-one. The reaction was stopped at the point that the band at 975 cm^{-1} in infrared (IR) spectrum of the reaction solution mostly disappeared, and treatment of the reaction mixture with zinc dust and acetic acid afforded the desired aldehyde (XI) with satisfactory yield.

At first attempt to prepare γ-lactone, the ethynylation of the aldehyde (XI) with acetylene in the presence of potassium *tert*-butoxide was tried to afford a mixture of epimeric ethynyl compound (XII). Without isolation of any epimeric compound in pure state, the mixture was treated with methylmagnesium bromide and shaken in carbon dioxide atomosphere. The resulting ethynyl carboxylic acid (XIII) was catalytically hydrogenated with 10% palladium on charcoal and then treated with acid. The IR spectrum showed that the reactions proceeded as expected, and, in fact, a lactone (XV) was isolated in almost pure state[12] by recrystallization. The thin-layer chromatography of the mother liquor, however, showed that 20-methyl group was partly isomerized probably in the first reaction. Thus, it was desirable to prepare ethynyl compound (XII) without isomerization at C-20. Ethynylmagnesium bromide[13] was found to be a satisfactory reagent; the replacement of ethynylpotassium to ethynylmagnesium bromide followed by the similar treatment described above yielded dioxolactone mixture without isomerization C-20. The ethynylation was not stereospecific and the ratio of isomers at C-20 was estimated by IR absorption to be XV: XVII=2:1.[14] The recrystallization of the reaction mixture yielded XV (90% purity) in about 30% yield. The thin-layer chromatography and IR spectrum of the mother liquor showed that the principal ingredient was still dioxolactone and the ratio of isomers was about 1:1. The separation of the lactone (XV) from the mother liquor succeeded partly by chromatography on silica gel, but the other isomer (XVII) could not be isolated. An effective and practical method of isolation could be developed, in which both isomers were separated in pure state with good yield. The mixture was again transformed into 3,6-diketal with the usual manner, and partially hydrolyzed with dilute hydrochloric acid. Recrystallization afforded a sparlingly soluble material with high melting point, which was formulated 3-monoketal of another isomer of lactone (XVI) on the basis of spectral and chemical evidences as shown below. The residue after isolation of XVI was found to be 3,6-dioxolactone (XV) containing small amount of isomeric 3,6-dioxolactone (XVII), and recrystallization of the

9) R.E. Marker, H.M. Crooks, Jr., E.M. Jones, and E.L. Wittbecker, *J. Am. Chem. Soc.*, **64**, 219 (1942).
10) T. Nishina and M. Kimura, *Yakugaku Zasshi*, **84**, 390 (1964).
11) G. Slomp, Jr. and J.L. Johnson, *J. Am. Chem. Soc.*, **80**, 915 (1958).
12) An analytical pure sample was effectively obtained as follows. Crude lactone obtained above was reduced by sodium borohydride to 3β, 22-dihydroxy-6-oxohomo-5α-cholan-25-oic acid 25→22-lactone (This selective reduction was reported by H. Mori, K. Shibata, K. Tsuneda, and M. Sawai (*Chem. Pharm. Bull.* (Tokyo), **16**, 2416 (1968)). After the ketol was purified by recrystallization, oxidation with Jones reagent and recrystallization afforded pure sample of XV.
13) L. Skattebøl, E.R.H. Jones, and M.C. Whiting, *Org. Syn.*, Coll. Vol. **4**, 792 (1963).
14) Analysis was made by a characteristic absorption at 975 cm^{-1} for the isomer (XV) and 1013 cm^{-1} for another isomer (XVII).

residue gave almost pure XV. The configuration of oxygen atom at C–22 was given by the synthesis of ecdysone from the isomer XVII,[8] that is, the configuration of XV and XVII at C–22 is α_F- and β_F- oriented, respectively.

Now we wish to describe observations concerning XVI. The IR spectrum showed the presence of lactone group (1768 cm^{-1}) and six–membered carbonyl group (1710 cm^{-1}). The optical rotatory dispersion exhibited a negative Cotton effect as shown in Fig. 1, which was superimposable with the usual 6-oxo steroid in 5α-series,[15] supporting the structure of 3-monoketal (XVI) rather than 6-monoketal. The hydrolysis of XVI with acid afforded 3,6-dioxolactone (XVII) isomeric with XV. Further unequivocal proof was given by the synthesis of 3,6-dioxolactone 6-monoketal,[8] the optical rotatory dispersion curve of which was possitive one as observed in 3-oxo steroid in 5α-series. It is of interest to point out that the 6-ketal functional group was hydrolyzed more rapidly to 6-oxo group, because 6-oxo is considered to be more sterically hindered than 3-oxo group and, accordingly, pratially hydrolyzed compound should be predicted to

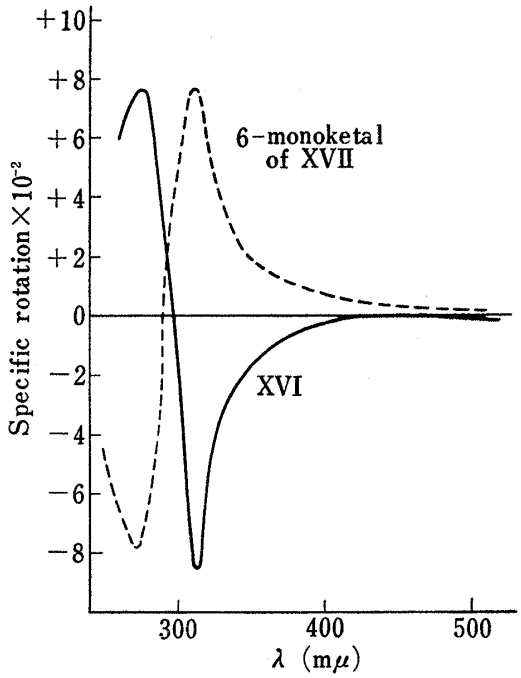

Fig. 1. Optical Rotaory Dispersion Curves of XVI and 6-Monoketal of XVII

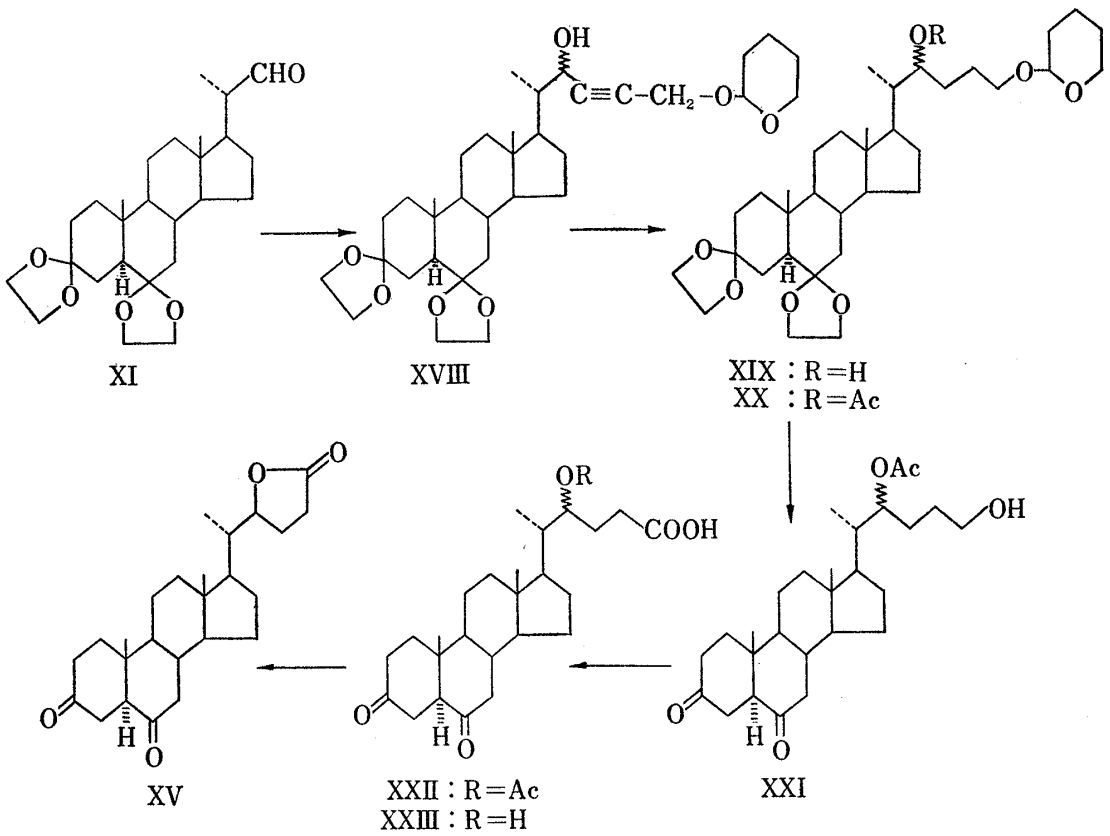

Chart 4

15) C. Djerassi, "Optical Rotatory Dispersion," MacGraw-Hill Book Co., Inc., New York, N.Y., 1960 p. 42.

be 6-monoketal from steric point of view. A reasonable explanation of this fact is that 1,3-diaxial interaction between 6β-oxygen atom in 3,6-diketal and 19-methyl group unstabilizes 6-ketal group and probably this is a driving force for the rapid hydrolysis of 6-ketal.[16]

The mixture of dioxolactones (XV and XVII) was prepared with low yield from the aldehyde (XI) by the different route as shown in Chart 4. The reaction of the aldehyde (XI) with Grignard reagent prepared from propagyl alcohol tetrahydropyranyl ether and methylmagnesium bromide gave ethynyl carbinol (XVIII) as a mixture of C–22 isomers. The catalytic hydrogenation of the ethynyl carbinol (XVII) to XIX, followed by acetylation,

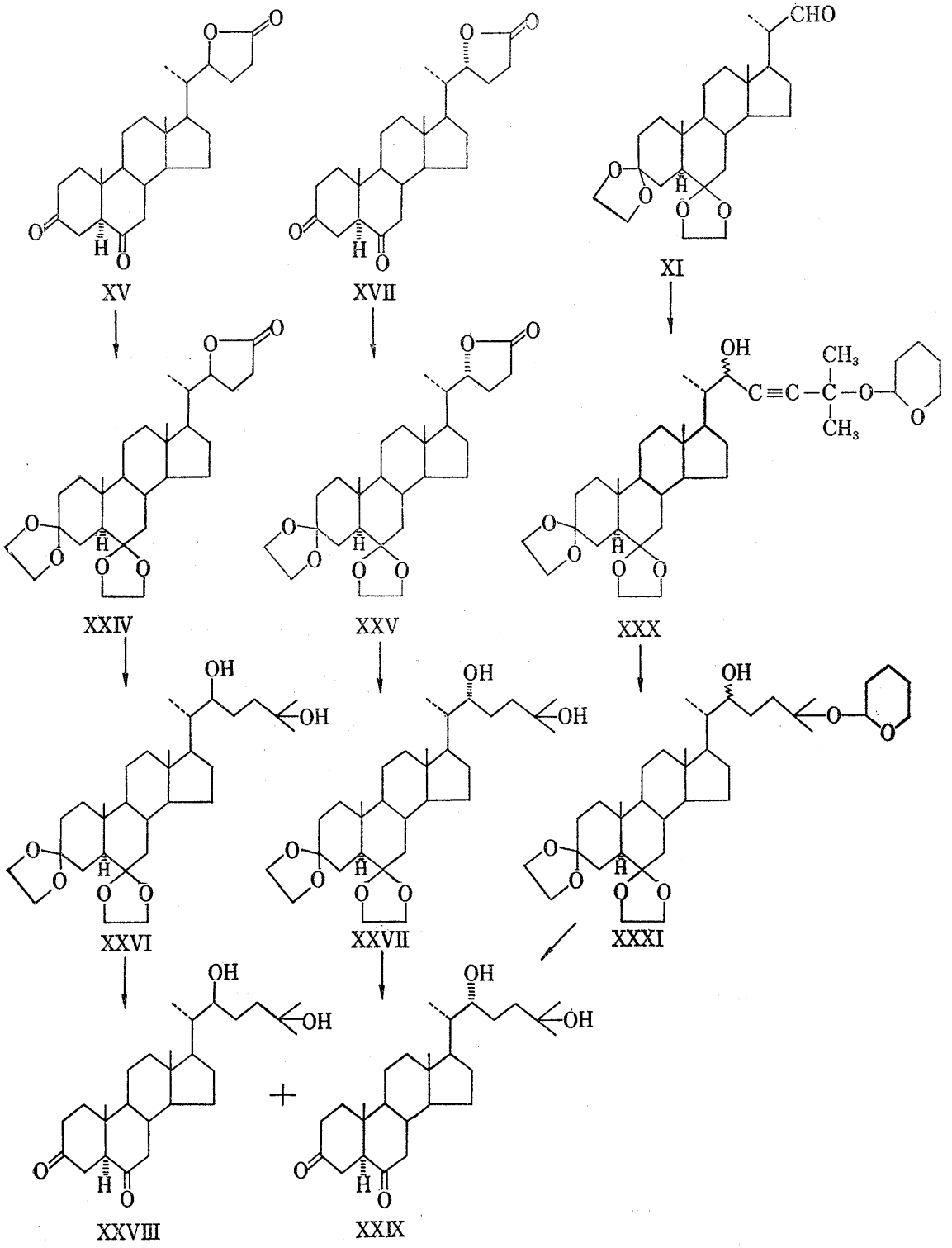

Chart 5

afforded the acetate (XX). The hydrolysis of the acetate (XX) with acetic acid gave 25-hydroxy-3,6-dione (XXI), which on Jones oxidation yielded the carboxylic acid (XXII). Hydrolysis of XXII and treatment of the resulting hydroxycarboxylic acid (XXIII) with acid gave a mixture of 3,6-dioxolactones, from which XV was isolated in almost pure state. The low yield of dioxolactones was apparently due to the production of carboxylic acid which could not be introduced into lactone, but no further attempt to improve the yield was made.

It was now shown that the lactones, XV and XVII, could be converted into the compounds having the side chain structure of 22,25-dihydroxycholestane. Carbonyl groups of 3,6-dioxolactones, XV and XVII, were protected by ketalization to give the corresponding 3,6-diketals, XXIV and XXV, respectively. The Grignard reaction of XXIV and XXV with methylmagnesium bromide afforded XXVI and XXVII as oily products, respectively. Treatment of oils with *p*-toluenesulfonic acid–methanol–acetone–water system led to the corresponding dihydroxydiones, XXVIII and XXIX. Drastic hydrolysis with acid gave complex mixture owing to easily dehydrated characteristics of 25-hydroxyl group. The same 22,25-dihydroxy-5α-cholestane-3,6-dione, XXVIII and XXIX were synthesized by the different route. As this type of reactions were used in the synthesis of ecdysone by Kerb, *et al.*[7] and Harrison, *et al.*[17] an indirect proof of the side chain structure of XXVIII and XXIX was given. The aldehyde (XI) was treated with Grignard reagent prepared from 3-methyl-1-butyn-3-ol tetrahydropyranyl ether and methylmagnesium bromide to yield the ethynyl carbinol (XXX) as a mixture of 22-isomers. The mixture was catalytically hydrogenated to the saturated mixture (XXXI), which on hydrolysis with *p*-toluenesulfonic acid gave a mixture of epimeric dihydroxydiones. The separation of epimers succeeded easily by recrystallization; XXVIII and XXIX, which were identical with those obtained as described above, were isolated in about 30 and 15%, respectively. It is interesting to show that 22α_F-isomer is superior product in the reactions of aldehyde (XI) with both Grignard reagents.

Experimental[18]

4,22-Stigmastadiene-3,6-dione (VIII)——To a solution of stigmasterol (VII, 200 g, Upjohn Co., *ca.* 95% purity) in acetone (8000 ml) and tetrahydrofuran (6000 ml) was added dropwise 8N chromium trioxide solution[19] (600 ml) at 0°. The mixture was stirred at 0° for 1 hr, diluted with water containing sodium bisulfite (100 g) and extracted with ether. The organic layer was washed with 5% sodium carbonate and water, and dried (sodium sulfate). The evaporation of the solvent gave a crystalline material, which on recrystallization from ethanol yielded 4-ene-3,6-dione (VIII, 146.2 g), mp 143—147° (reported,[20] mp 155°).

5α-Stigmast-22-ene-3,6-dione (IX)——To a solution of 4-ene-3,6-dione (VIII, 121.8 g) in acetic acid (3650 ml) and water (240 ml) was added zinc dust (121.8 g) and the mixture was refluxed for 5 hr. After removal of zinc dust by decantation, water was added and the product was extracted with ether–dichloromethane mixture. The organic layer was washed with water, 5% sodium carbonate and water, and dried (sodium sulfate). The evaporation of the solvent left a crystalline material, which was recrystallized from acetone to yield 3,6-dione (IX, 100 g), mp 190—195° (reported,[19] mp 197°).

3,3;6,6-Bisethylenedioxy-5α-stigmast-22-ene (X)——A mixture of 3,6-dione (IX, 50 g) in benzene (2000 ml) and ethylene glycol (800 ml) was slowly distilled in order to remove a trace of water. After *p*-toluenesulfonic acid (8.0 g) was added, distillation was continued for 6 hr, during which dry benzene was added to maintain about constant volume. After cooling, powdered sodium bicarbonate was added, and the solution was washed with 5% sodium bicarbonate and water, and dried (sodium sulfate). After pyridine (5—6 drops) was added, the solvent was evaporated to dryness to give a crystalline material, which on recrystallization from ethanol containing a trace of pyridine yielded the diketal (X, 55.9 g), mp 117—120°. Further

16) This explanation was suggested from Prof. M. Shiota (Ochanomizu University).
17) I.T. Harrison, J.B. Siddall, and J.H. Fried, *Tetrahedron Letters*, **1966**, 3457.
18) All melting points were uncorrected. Optical rotations were measured in chloroform unless otherwise stated.
19) A solution of chromium trioxide (26.72 g) in sulfuric acid (23 ml) diluted with water at a volume of 100 ml was used.
20) E. Fernholz, *Ann.*, **508**, 215 (1934).

recrystallization from the same solvent gave an analytical sample as colorless needles. mp 119—121°, $[\alpha]_D^{26}$ −3° (c=1.36). *Anal.* Calcd. for $C_{33}H_{54}O_4$: C, 76.99; H, 10.57. Found: C, 77.16; H, 10.84.

3,3;6,6-Bisethylenedioxybisnor-5α-cholan-22-al (XI)——To a solution of the diketal (X, 18.0 g) in dichloromethane (600 ml) and pyridine (2.82 ml) was bubbled oxygen containing ozone, until the band at 975 cm^{-1} in IR absorption was mostly disappeared. Zinc dust (22.5 g) and acetic acid (45 ml) were added and the mixture was stirred at room temperature in nitrogen atmosphere for 2 hr. After removal of zinc dust, the solution was washed with 2% sodium hydroxide and water, and dried (sodium sulfate). The solvent was evaporated and α-ethylvaleroaldehyde was removed *in vacuo* to yield a crystalline material, which on recrystallization from dichloromethane–ether–hexane mixture afforded the aldehyde (XI, 12.6 g), mp 160—164°. Further recrystallization from the same solvent afforded an analytical sample. mp 172—175°, $[\alpha]_D^{31}$ −2° (c=1.04). *Anal.* Calcd. for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 71.85; H, 9.46.

Preparation of a Mixture of 22-Epimeric 22-Hydroxy-3,6-dioxohomo-5α-cholan-25-oic Acid 25→22-Lactones (XV and XVII) by Ethynylmagnesium Bromide——Acetylene gas was bubbled to an ice-cold solution of Grignard reagent prepared from magnesium (30 g), methyl bromide (60 ml) and tetrahydrofuran (1000 ml). A solution of the aldehyde (XI, 40 g) in tetrahydrofuran (400 ml) was dropwise added to the solution of ethynylmagnesium bromide over a period of 1 hr and the mixture was stirred for another 1 hr. After the addition of ice to decompose excess Grignard reagent, ether was added and the organic layer was washed with 10% ammonium chloride, 5% sodium bicarbonate and water, and dried (sodium sulfate). The removal of the solvent by evaporation yielded a mixture of 22-epimeric ethynyl carbinols (XII, 47 g) as oily substance.

A solution of XII (47 g) in tetrahydrofuran (200 ml) was added to a Grignard reagent prepared from magnesium (26 g), methyl bromide (50 ml) and tetrahydrofuran (800 ml) with stirring and the mixture was heated under reflux for 6 hr. The mixture was cooled by ice–water and stirred in carbon dioxide atomosphere. Ice–water was added to the resulting suspension, and the product was extracted with dichloromethane–ether mixture. The desired ethynyl carboxylic acid was extracted with 2% potassium hydroxide, and pH was adjusted to 2.0—3.0 by the addition of 2N hydrochloric acid. After extraction with ether, washing with water and drying (sodium sulfate), the solvent was evaporated to dryness to give a mixture of 22-epimeric ethynyl carboxylic acids (XIII, 44 g) as oily substance.

A solution of XIII (44 g) in dioxane (660 ml) and piperidine (22 ml) was shaken in hydrogen atomosphere with 10% palladium on charcoal (6.6 g) to absorb two moles of hydrogen. After the removal of the catalyst by filtration, water was added and pH was adjusted to 2.0—3.0 by 2N hydrochloric acid. The product was extracted with ether, and the organic layer was washed with water, and dried (sodium sulfate). The evaporation of the solvent gave a mixture of 22-epimeric hydroxycarboxylic acids (XIV) as oily substance. A solution of XIV in 70% aqueous acetic acid (460 ml) was heated at 90° for 1 hr. After cooling, the resulting precipitates were collected by filtration to yield a mixture of 22-epimeric dioxolactones (XV and XVII, 26.58 g). The analysis by IR spectrum showed that the product had 90% purity as γ-lactone and the ratio of 22α_F-lactone (XV) was 67%.

Separation of 22α_F- and 22β_F-Hydroxy-3,6-dioxohomo-5α-cholan-25-oic Acid 25→22-Lactone (XV and XVII)——i) 22α_F-Hydroxy-3,6-dioxohomo-5α-cholan-25-oic Acid 25→22-Lactone (XV): Twice recrystallizations of the lactone mixture (26.58 g) obtained as described above afforded XV (7.9 g) of 90% purity (from IR), mp 250—266°. The thin-layer chromatography (silicagel; benzene–acetone 9:1) showed that the impurity was 22β_F-lactone (XVII). Further recrystallization or chromatography did not afford a pure sample. An analytical sample was obtained as follows. XV obtained as described above was reduced with sodium borohydride in ethanol–dichloromethane mixture[12] to 3β,22α_F-dihydroxy-6-oxohomo-5α-cholan-25-oic acid 25→22-lactone, which could be easily purified by recrystallization. The Jones oxidation of the pure ketol gave an analytical sample as colorless needles. mp 270—277°, $[\alpha]_D^{31}$ +28° (c=0.92). *Anal.* Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.48; H, 8.89.

ii) 22β_F-Hydroxy-3,6-dioxohomo-5α-cholan-25-oic Acid 25→22-Lactone (XVII): From the residue derived from the mother liquor after the isolation of 22α_F-lactone (XV) was isolated 22β_F-lactone (XVII) as follows. The ketalization of the residue, followed by partial hydrolysis, gave sparlingly soluble 3-monoketal of 22β_F-lactone, which could be easily purified by recrystallization. The hydrolysis and recrystallization gave a pure 22β_F-lactone (XVII). About 60—70% of 22β_F-lactone (XVII) could be isolated by once treatment. When this treatment was repeated twice or three times, almost all 22β_F-lactone (XVII) could be isolated. From the mother liquor was isolated further 22α_F-lactone (XV). A typical example of isolation was shown as follows.

Concentrated hydrochloric acid (15 ml) and tetrahydrofuran (1500 ml) were added and the mixture was stirred at 20° for 1.5 hr. After the addition of powdered sodium bicarbonate (30 g), ether and water were added and the organic layer was dried (sodium sulfate). After the evaporation of the solvent, the residue was recrystallized from dichloromethane–hexane afforded the 3-monoketal (XVI, 3.78 g), mp 287—296°. Further recrystallization from dioxane gave an analytical sample as colorless needles. mp 314—317°, $[\alpha]_D^{16}$ −9° (c=1.46), ORD (c=0.419, $CHCl_3$). $[\alpha]_D^{26}$ (mμ): −5° (700), −5° (589), −106° (350), −642° (314)

(trough), +1° (295), +642° (276) (peak), +495° (260). *Anal.* Calcd. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 72.68; H, 9.13.

A mixture of the 3-monoketal (XIV, 3.0 g) and *p*-toluenesulfunic acid (3.0 g) in acetone (600 ml) and water (150 ml) was heated under reflux for 2 hr and poured into water. The product was extracted with dichloromethane–benzene–ether mixture and the organic layer was washed with 5% sodium bicarbonate and water, and dried (sodium sulfate). The evaporation of the solvent afforded a crystalline material, which on recrystallization from methanol–acetone gave 22β_F-lactone (XVII, 2.36 g) as colorless leaflets. mp 243—246°, $[\alpha]_{578}^{20}$ +20° (c=0.675). *Anal.* Calcd. for $C_{25}H_{36}O_5$: C, 74.96; H, 9.06. Found: C, 75.18; H, 9.43.

Preparation of a Mixture of Epimeric 22-Hydroxy-3,6-dioxohomo-5α-cholan-25-oic Acid 25→22-Lactones by Ethynylpotassium——Potassium (12 g) was dissolved in a mixture of *tert*-butanol (270 ml) and tetrahydrofuran (360 ml) in nitrogen atomosphere and to the ice–cold solution was bubbled acetylene gas. A solution of the aldehyde (XI, 20 g) in tetrahydrofuran (200 ml) was dropwise added to the ethynylpotassium solution for 30 min and the solution was stirred for 1.5 hr, during which acetylene gas was bubbled. After the addition of water, the product was extracted with ether, and the organic layer was washed with water, and dried (sodium sulfate). The evaporation of the solvent afforded a mixture of ethynyl carbinols, which was submitted to the following reactions as the same manner described above, 22α_F-lactone (XV, 6.8 g) of 90% purity was obtained. The residue obtained from the mother liquor was not a crystalline material, the IR spectrum of which was very similar to that of 22α_F- or 22β_F-lactone (XV or XVIII). Further two spots near 22α_F- and 22β_F-lactone (XV and XVII) were observed in its thin-layer chromatography.

Preparation of a Mixture of Epimeric 22-Hydroxy-3,6-dioxohomo-5α-cholan-25-oic Acid 25→22-Lactones by 3-(2-tetrahydropyranyloxy)propynylmagnesium Bromide——A solution of propagyl alchol tetrahydropyranyl ether (15 g) in tetrahydrofuran (50 ml) was dropwise added to a Grignard reagent prepared from magnesium (2.45 g), ethyl bromide (11.0 g) and tetrahydrofuran (150 ml) and the mixture was stirred for 1 hr at room temperature. A solution of the aldehyde (XI, 10 g) in tetrahydrofuran (60 ml) was added to the ice–cold Grignard reagent prepared as described above, and stirring was continued for 1.5 hr. After the decomposition of excess Grignard reagent by the addition of 10% ammonium chloride, the product was extracted with ether and the organic layer was washed with 10% ammonium chloride and water, and dried (sodium sulfate). The oily residue obtained by evaporation of the solvent was chromatogarphied on Florisil (200 g). After propagyl alcohol tetrahydropyranyl ether was eluted by hexane (3000 ml), a mixture of 22-epimeric ethynyl carbinols (XVIII, 14.18 g) was obtained from the fraction eluted by ether (3000 ml) as an oily substance.

A solution of XVIII (14.18 g) in ethanol (500 ml) was shaken in hydrogen atmosphere with 10% palladium on charcoal (5.0 g) for 1.5 hr. After the removal of the catalyst by filtration, the solvent was evaporated to dryness to give the saturated compound (XIX, 14.2 g).

A solution of XIX (4.7 g) in acetic anhydride (20 ml) and pyridine (40 ml) was allowed to stand overnight at room temperature. Water was added and the product was extracted with ether. The organic layer was washed with water, 5% sodium carbonate and water, and dried (sodium sulfate). The solvent was evaporated to dryness to yield the acetate (XX, 4.27 g).

A solution of XX (3.07 g) in methanol (150 ml) and 50% acetic acid (150 ml) was heated at 80° for 2 hr. After cooling, water was added and the organic layer was washed with water, 5% sodium bicarbonate and water, and dried (sodium sulfate). The evaporation of the solvent gave 25-hydroxy-3,6-dione (XXI). To a solution of XXI in 70% acetic acid (35 ml) was added a solution of chromium trioxide (1.41 g) in 90% acetic acid (10 ml) and the solution was stirred for 6 hr at 35°. Methanol (2 ml) and water were added and the product was extracted with ether–dichloromethane mixture. The organic layer was washed with water and shaken with 30% potassium hydroxide solution several times. The combined alkaline solution was heated at 80° for 30 min in nitrogen atmosphere, and acidified by the addition of 2N hydrochloric acid. The precipitates were collected by filtration and dissolved in methanol (15 ml). Concentrated hydrochloric acid (2.1 ml) was added and the solution was allowed to stand for 5 min. Water was added and the resulting precipitates were collected by filtration to give the crude lactone (1.77 g), which was dissolved in dichloromethane and passed through a short column of alumina. Recrystallization from ethanol–dichloromethane afforded a mixture of dioxolactones (XV and XVII (6:4), 466 mg), which on further recrystallization from acetone yielded 22α_F-lactone (XV, 24 mg) in almost pure state.

22α_F-Hydroxy-3,3;6,6-bisethylenedioxyhomo-5α-cholan-25-oic Acid 25→22-Lactone (XXIV)——The ketalization of 22α_F-lactone (XV, 180 mg) was made on the similar manner described above by benzene (36 ml), ethylene glycol (9 ml) and *p*-toluenesulfonic acid (26 mg). Recrystallization of the product (crystalline material, 239 mg) from methanol containing a trace of pyridine gave an analytical sample as colorless needles. mp 246—250°, $[\alpha]_D^{28}$ +34° (c=1.06). *Anal.* Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08. Found: C, 71.38; H, 9.04.

22β_F-Hydroxy-3,3;6,6-bisethylenedioxyhomo-5α-cholan-25-oic Acid 25→22-Lactone (XXV)——The ketalization of 22β_F-lactone (XVII, 95 mg) was made on the similar manner described above by benzene (30 ml), ethylene glycol (8 ml) and *p*-toluenesulfonic acid (30 mg). Twice recrystallizations of the product from

methanol containing a trace of pyridine afforded an analytical sample (91 mg) as colorless needles. mp 247—252°, $[\alpha]_D^{16}$ +11° (c=0.98). *Anal.* Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08. Found: C, 71.53; H, 8.78.

22α_F, 25- and 22β_F, 25-Dihydroxy-5α-cholestane-3,6-dione (XXVIII and XXIX)——To an ice-cold solution of Grignard reagent prepared from magnesium (2.92 g), ethyl bromide (13.08 g) and tetrahydrofuran (180 ml) was dropwise added a solution of 3-methyl-1-butyn-3-ol tetrahydropyranyl ether (25 g) in tetrahydrofuran (60 ml), and the mixture was stirred at room temperature for 1 hr. A solution of the aldehyde (XI, 10.0 g) in tetrahydrofuran (100 ml) was dropwise added to the ice-cold mixture obtained as described above. After stirring at room temperature for 1 hr, excess Grignard reagent was destroyed by the addition of 10% ammonium chloride. The product was extracted with ether, and the organic layer was washed with 10% ammonium chloride, 5% sodium carbonate and water, and dried (sodium sulfate). After the addition of piperidine (3 drops), the solvent was removed by evaporation to give an oily substance, which was chromatographed on alumina (200 g). After the elution with hexane (3-methyl-1-butyn-3-ol tetrahydropyranyl ether), the addition product (XXX, 14.1 g) was obtained from the fraction eluted by ether as an oily substance. This product was found to be a mixture of 22-epimers by thin-layer chromatography, but the separation of these epimers was unsuccessful.

The addition product (XXX, 14.1 g) was catalytically hydrogenated in pre-reduced mixture of 10% palladium on charcoal (4.0 g) in ethanol (1000 ml) containing piperidine (5 drops) at room temperature. The absorption of hydrogen was completed for 30 min. After 1 hr, the catalyst was removed by filtration and the solvent was removed by evaporation *in vacuo* to obtain the saturated product (XXXI, 14.5 g) as an oily substance. The separation of epimers was here again unsuccessful.

The saturated compound (XXXI, 14.5 g) obtained as described above was heated under reflux with *p*-toluenesulfonic acid (0.73 g), methanol (200 ml), acetone (200 ml) and water (40 ml) for 1 hr. Water was added, and the product was extracted with ether–dichloromethane mixture. After washing with 5% sodium carbonate and water, and dried (sodium sulfate), the solvent was evaporated to dryness to give a crystalline residue. Recrystallization from methanol afforded 22α_F, 25-dihydroxy-5α-cholestane-3,6-dione (XXVIII, 3.7 g), mp 183—186°. An analytical sample was obtained by further recrystallization from methanol as colorless needles. mp 183—185.5°, $[\alpha]_D^{26}$ −9° (c=0.97). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.67; H, 10.43.

Recrystallization of the mother liquor from ethyl acetate afforded 22β_F, 25-dihydroxy-5α-cholestane-3,6-dione (XXIX, 1.7 g), mp 198—202°. An analytical sample was obtained by further recrystallization from ethyl acetate as colorless needles. mp 203—206°, $[\alpha]_D^{26}$ +7°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.31; H, 10.07.

22α_F,25-Dihydroxy-5α-cholestane-3,6-dione (XXVIII)——A solution of the diketal (XXIV, 100 mg) in tetrahydrofuran (20 ml) was dropwise added to an ice-cold solution of Grignard reagent prepared from magnesium (1.0 g), methyl bromide and ether (60 ml). After heating under reflux for 3 hr, excess Grignard reagent was decomposed by the addition of water. The product was extracted with ether and the organic layer was washed with 10% ammonium chloride, 5% sodium carbonate and water, and dried (sodium sulfate). The evaporation of the solvent afforded an oily substance.

The oily material described above was heated under reflux with *p*-toluenesulfonic acid (30 mg), methanol (9 ml), acetone (9 ml) and water (2 ml) for 1 hr. After the addition of water, the product was extracted with ether–dichloromethane mixture and the organic layer was washed with 5% sodium carbonate and water, and dried (sodium sulfate). The evaporation of the solvent gave a crystalline material. Recrystallization from methanol afforded 22α_F, 25-dihydroxy-5α-cholestane-3,6-dione (XXVIII), the thin-layer chromatography and infrared spectrum of which was identical with those of the compound obtained by another route.

22β_F, 25-Dihydroxy-5α-cholestane-3,6-dione (XXIX)——The diketal (XXV, 100 mg) was treated as the same manner in the preceding experiment to give 22β_F, 25-dihydroxy-5α-cholestane-3,6-dione (XXIX), the thin-layer chromatography and infrared spectrum of which was identical with those of the compound (XXIX) obtained by another route.

Acknowledgement The authors are indebted to Prof. M. Shiota (Ochanomizu University) and Prof. U. Mizuhara (Keio University) for their interest and discussion, and also Drs. I. Chuman, H. Ando and S. Wada (this company) for their support and encouragement throughout this work. Thanks are due to Mr. W. Murakami and Mrs. T. Hosoyama for their technical help.