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Synthesis of Ecdysone. V.[1] Synthesis of 22-Isoecdysone

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22-Isoecdysone (XXIX) was synthesized from 22α_F-hydroxy-3,6-dioxohomo-5α-cholan-25-oic acid 25→22-lactone (II) by 14 steps. Novel methods of synthesis of 2β,3β-dihydroxy and 14α-hydroxy-6-oxo-7-ene function reported in this series of papers could be used very efficiently.

In this series of papers, novel synthetic methods of partial structures of ecdysone (I), A-ring (2β,3β-diol),[3] B,C-ring (14α-hydroxy-7-en-6-one)[4] and side chain structure (22,25-dihydroxy cholestane side chain)[5] were already described. The problem arisen at this point was how to combine these methods for synthesis of ecdysone, which will be described in this paper. In the fourth paper of this series,[5] the synthesis of two 22-hydroxy-3,6-dioxohomo-5α-cholan-25-oic acid 25→22-lactones isomeric at C–22 was described. The isomer (II) now formulated as 22α_F-hydroxy compound was first considered to have the same configuration (22β_F) as ecdysone in oxygen function at C–22 on the basis of following evidences. The configuration of 22-hydroxyl group in II was proved to be different from that of 22-hydroxycholesterol[6] isolated from natural product by chemical transformations, which will be reported elsewhere. It was described by Klyne and Stokes[7] that 22-hydroxyl group in natural 22-hydroxycholesterol should be considered 22α_F-oriented. For this reason, 3,6-dioxolactone (II) was first picked up as a starting material for synthesis of ecdysone. The compound derived from II was, however, not ecdysone but 22-isoecdysone. As a result, the assignment of the configuration at C–22 in 22-hydroxycholesterol, which was first suggested by Klyne and Stokes and supported by Tsuda and Hayatsu,[8] should be revised. Although the synthesis of ecdysone was not succeeded in this paper because of the wrong assignment of the configuration in 3,6-dioxolactone (II), it is strongly suggested that the synthesis of ecdysone can be accomplished by the similar procedures described in this paper on another 3,6-dioxolactone isomeric at C–22.

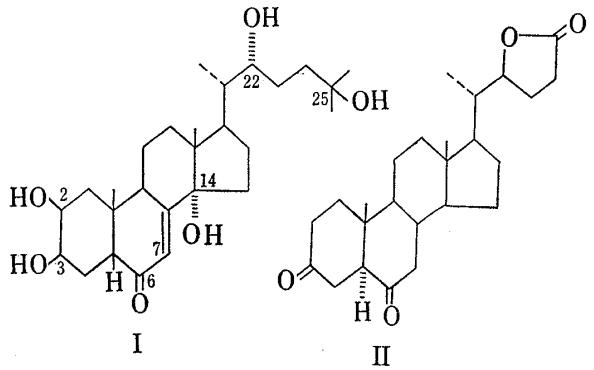

Chart 1

1) Part IV: *Chem. Pharm. Bull.* (Tokyo), to be printed.
2) Location: *Shimosakunobe, Kawasaki.*
3) *a*) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **15**, 460 (1967); *b*) 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) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), to be printed.
6) A. Stabursvik, *Acta Chem. Scand.*, **7**, 1220 (1953).
7) W. Klyne and M. Stokes, *J. Chem. Soc.*, **1954**, 1979.
8) K. Tsuda and R. Hayatsu, *Chem. Pharm. Bull.* (Tokyo), **6**, 580 (1958); *idem*, *J. Am. Chem. Soc.*, **81**, 5987 (1959).

The structure of lactone in the side chain of II was considered to be stable to reactions used in the synthesis of 2β,3β-dihydroxy[3] and 14α-hydroxy-7-en-6-one structure,[4] and, in fact, the construction of nuclear structure was succeeded by the similar route reported in cholestane derivatives.[3,4] It was described in the second paper[3b] that 3β-hydroxy-5α-cholestan-6-one can be used as a suitable starting material for synthesis. The corresponding ketol (III) was prepared by the selective reduction of II with sodium borohydride in high

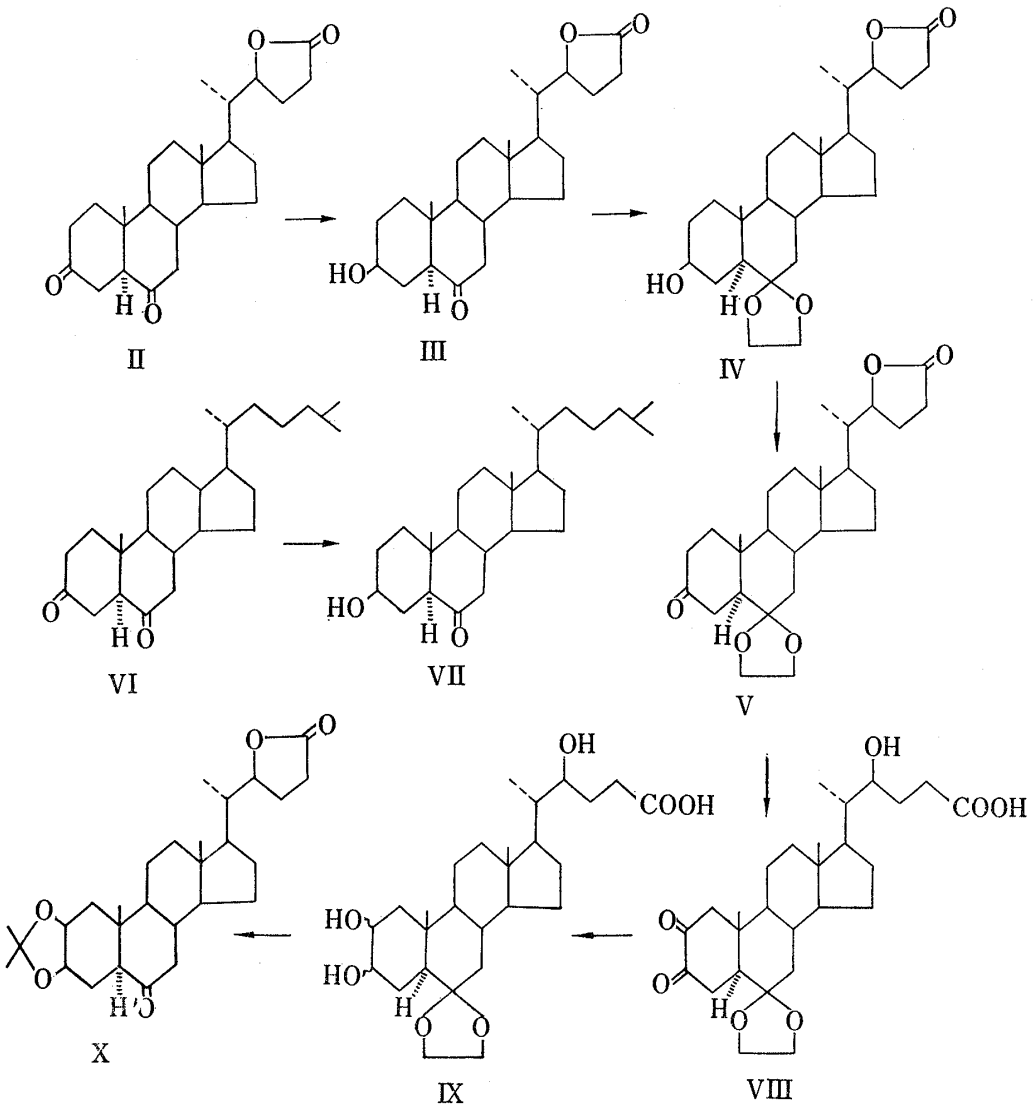

Chart 2

yield. The reduction was made with one mole of sodium borohydride at −5——10° to afford the desired ketol (III) accompanied with a small amount of over-reduced compound. If more excess reagent was used or reaction temperature was too high, considerable amount of 3,6-dihydroxy compound was produced. The evidence of the structure was based on analogy with the observation that 5α-cholestane-3,6-dione (VI) was transformed into 3β-hydroxy-5α-cholestan-6-one (VII) identical with an authentic sample by the similar treatment. Moreover, the optical rotatory dispersion curve of III was found to be negative one generally observed in 6-oxo steroids[9] as shown in Fig. 1. The ketol (III) was converted into the 6-ketal (IV) by the usual method of ketalization with satisfactory result. The ketal (IV) was oxidized with pyridine–chromium trioxide complex[10] to the 3,6-dione 6-monoketal (V), for which a positive Cotton effect curve typical for 3-oxo steroids in 5α-series[9] was obtained in optical rotatory

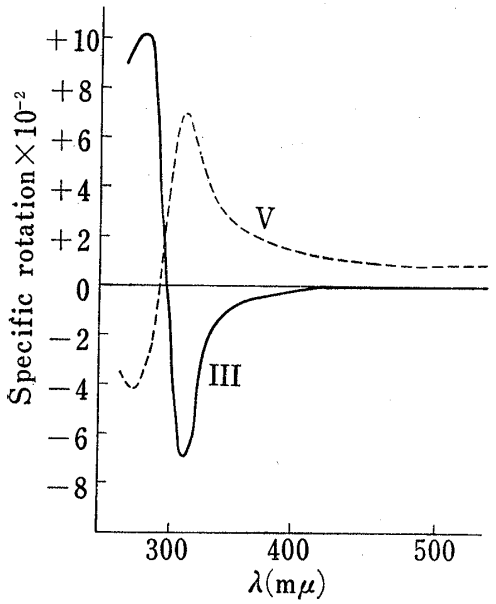

Fig. 1. Optical Rotatory Dispersion Curves of III and V

dispersion (Fig. 1). For preparative purpose, the isolation of the ketal (IV) in pure state was not necessary.

The autoxidation of the 6-monoketal by the same manner reported in the preceding papers[3] afforded unsatisfactory result because of low solubility in *tert*-butanol. Tetrahydrofuran was found to be a suitable solvent in this case. Thus, the 6-monoketal (V) was autoxidized in a mixture of tetrahydrofuran and *tert*-butanol in the presence of potassium *tert*-butoxide and isolation was made after careful acidification of the reaction mixture with dilute hydrochloric acid. The infrared and ultraviolet absorption spectrum suggested that the lactone group in V was opened to 22-hydroxycarboxylic acid and the structure of A-ring was a mixture of two possible enol forms of 2,3-diketone as shown in cholestane series[3a,11]; this mixture is formulated as VIII in Chart 2 for convenience' sake. Without purification, the mixture (VIII) was reduced with sodium borohydride in methanol to afford a mixture of 2,3-diols (IX). The mixture IX was treated with acetone in the presence of phosphomolybdic acid and the chromatography of the product on Florisil yielded the acetonide (X). It was evident from infrared spectrum that γ-lactone formation from 22-hydroxycarboxylic acid structure presented in side chain and the hydrolysis of 6-ketal were occurred during this acetonide formation reaction. The hydrolysis of the acetonide (X) with phosphoric acid yielded the diol (XI), from

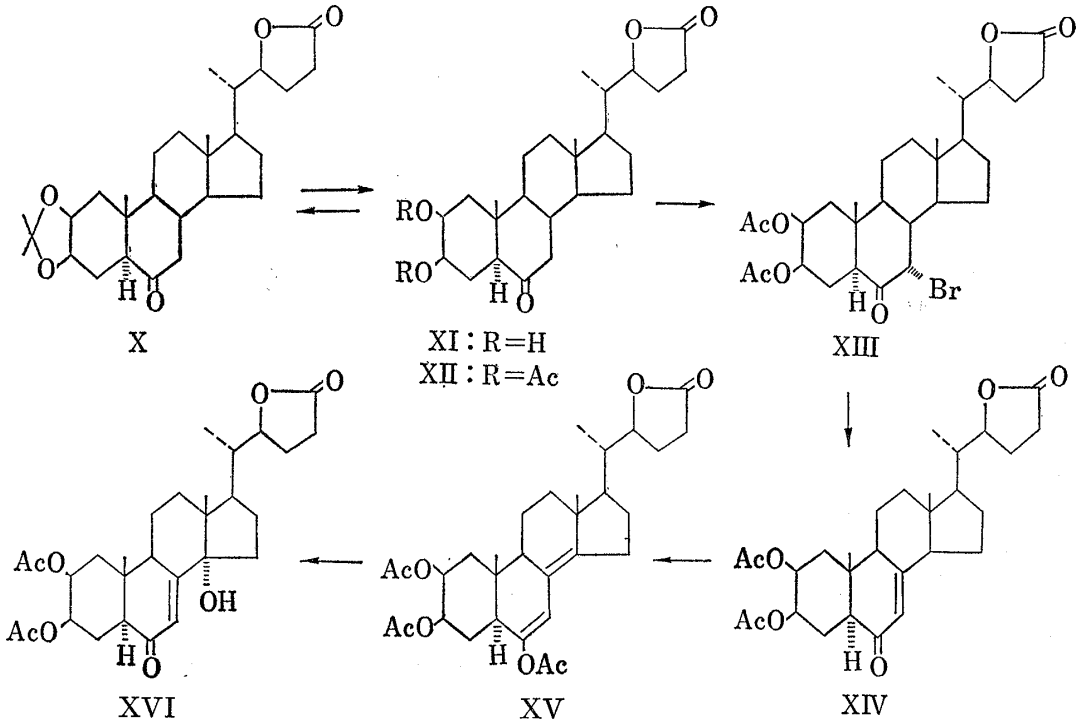

Chart 3

9) C. Djerassi, W. Closson, and A.E. Lippman, *J. Am. Chem. Soc.*, **78**, 3163 (1956).
10) C. Djerassi, R.H. Shapiro, and M. Vandewalle, *J. Am. Chem. Soc.*, **87**, 4892 (1965).
11) E.T. Stiller and O. Rosenheim, *J. Chem. Soc.*, **1938**, 353.

which the acetonide could be recovered by treatment with acetone and phosphomolybdic acid; this observation also supports the structure of the acetonide (X). The assignment of the structure of the acetonide (X) was, however, mainly based upon analogy with transformations for cholestane series, where absolute proof of the structure was given.[3b]

The acetylation of the diol (XI) with boiling acetic anhydride gave the diacetate (XII). The bromination of the diacetate (XII) in acetic acid with one mole of bromine, followed by warming at 50° for 2 hours, led to 7α-bromo compound (XIII).[12] The bromination of 6-oxo steroid was fully studied[13]; bromination is first occurred at C–5 and 5α-bromo compound thus obtained is rearranged by warming in the presence of hydrogen bromide to give 7α-bromo compound. α-Orientation of the bromine atom in the compound under consideration was supported by infrared spectrum; the carbonyl bands of C–6 oxo group in XII and XIII were observed at 1718 and 1722 cm^{-1} (carbon tetrachloride) respectively, showing that the bromine atom in XIII is axial.[14] That XIII is in fact 7α-bromo compound was shown by the transformation of XIII into 6-oxo-7-ene compound (XIV) by treatment with lithium carbonate and dimethylformamide at reflux temperature.[15]

The introduction of 14α-hydroxyl group in XIV was made by the method reported in the preceding paper.[4] The enol acetylation of XIV with acetic anhydride in the presence of catalytic amount of perchloric acid[16] afforded an enol acetate, the ultraviolet spectrum of which supported the structure of 6,8(14)-dien-6-ol acetate (XV). The maximum absorption was observed at 254 mμ; possible other enol acetates (5,7-diene and 6,8-diene) would not show such an absorption. The enol acetate (XV) was treated with 1.2—1.3 moles of perphthalic acid in tetrahydrofuran and ether at 25° to yield 14α-hydroxy-7-en-6-one (XVI). The overall yield of XVI from XIV was about 45%. *m*-Chloroperbenzoic acid could be used as an excellent oxidation agent to yield XVI with rather higher yield. Data of nuclear magnetic resonance spectra were shown in Table I.

Table I. Nuclear Magnetic Resonance Spectral Data[a] of 6-Oxo-7-ene Steroids and Derivatives

Compound	18–CH_3	19–CH_3	7–H
2β,3β-Diacetoy-5α-cholest-7-en-6-one	0.59	1.01	5.74 (t), J 2.5
2β,3β-Diacetouy-14α-hydroxy-5α-cholest-7-en-6-one	0.67	1.00	5.90 (d), J 2.5
XIV	0.62	1.01	5.82 (t), J 2.5
XVI	0.69	1.00	5.99 (d), J 2.5

a) The spectra were obtained at 60 Mcps on Hitachi H–60 spectrometer for the first two compounds and at 100 Mcps on JNM–4H–100 spectrometer for XIV and XVI in $CDCl_3$ containing TMS as an internal standard. Chemical shifts are quoted as ppm downfield from TMS (0.00 ppm). Coupling constants are given in cps. Abbreviations used are d=doublet and t=triplet.

As being apparent from Table I, shifts of angular methyl signals were very consistent with those observed in cholestane series,[4] showing that the reaction proceeded as expected.

Now, attention was turned to how to introduce XVI into 2β,3β,14α,22,25-pentahydroxy-5β-cholest-7-en-6-one. It was already reported form this laboratory[5] that the Grignard reaction of lactone structure, 22-hydroxyhomocholan-25-oic aicd 25→22-lactone, led to the side chain structure of ecdysone (22,25-dihydroxy cholestane). The problem arisen at this

12) A. Furlenmeier, A. Fürst, A. Langemann, G. Waldvogel, U. Kerb, P. Hocks, and R. Wiechert, *Helv. Chim. Acta*, **49**, 1591 (1966).
13) I.M. Heilbron, E.R.H. Jones, and F.S. Spring, *J. Chem. Soc.*, **1937**, 801; D.R. James and C.W. Shoppee, *ibid.*, **1954**, 4224; E.J. Corey, *J. Am. Chem. Soc.*, **76**, 175 (1954).
14) L.J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen & Co., Ltd., London, 1956, pp. 139–141.
15) M.P. Hartshorn and A.F.A. Wallis, *J. Chem. Soc.*, **1962**, 3839.
16) B.E. Edwards and P.N. Rao, *J. Org. Chem.*, **31**, 324 (1966).

point is that XVI must be transformed into the compound, the nuclear structure of which is inactive to Grignard reagent. At first, it was considered that 6-oxo group must be protected from Grignard reagent. From this point of view, it seemed to be the most promising way that 6-oxo group in 14α-hydroxy-6-oxo-7-ene compound (XVII) would be reduced to 6,14α-dihydroxy-7-ene compound (XVIII), and after the construction of the side chain structure

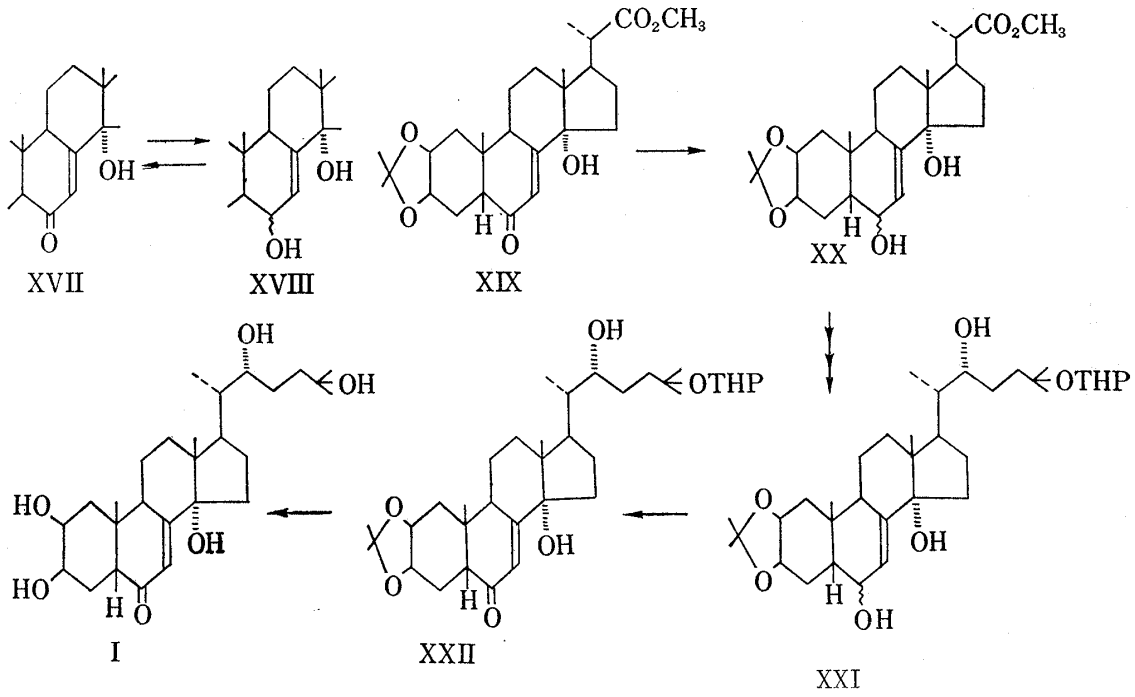

Chart 4

for XVIII, allylic oxidation by a suitable reagent such as manganese dioxide would give the desired compound. In fact, Syntex group[17] succeeded in the synthesis of ecdysone by the similar idea; the route was shown in Chart 4 (XIX→XX→XXI→XXII→I). Some model experiments on 2β,3β,14α-trihydroxy-7-cholesten-6-ones and their derivatives made it clear that tri(*tert*-butoxy)lithium aluminum hydride is a suitable reduction reagent, which is recommended by Syntex group,[17b] and the allylic oxidation of 5α-cholest-7-ene-2β,3β,6,14α-tetrol with manganese dioxide gave an unsatisfactory result because of the absorption of the compound to manganese dioxide.[18] It was also found that 6-oxo group of 2β,3β,14α-trihydroxy-5α(or 5β)-cholest-7-en-6-one could not be reduced by treatment with tri(*tert*-butoxy)lithium aluminum hydride at room temperature overnight in tetrahydrofuran, while 2,3-diacetate or 2,3-acetonide of 2β,3β,14α-trihydroxy-5α(or 5β)-cholest-7-en-6-one was completely reduced to 6-hydroxy compound by the same treatment. It is of interest to point out that the reactivities of 6-oxo group are very different among these compounds in which only minor differences are presented in ring A. This observation seemed to suggest the possibility that the Grignard reaction of 2β,3β,14α,22-tetrahydroxy-6-oxohomo-7-cholen-25-oic acid 25→22-lactones would give the desired compound where only the side chain structure (lactone group) is reacted. In order to ascertain whether this is possible or not, the Grignard reactions of 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one (XXIII)[4,12] and 22α_R-hydroxy-3,6-dioxohomo-5α-cholan- 25-oic acid 25→22-lactone 3,6-diethylene ketal (XXIV)[5] were examined. The reactions were

17) *a*) J.B. Siddall, A.D. Cross, and J.H. Fried, *J. Am. Chem. Soc.*, **88**, 862 (1966); *b*) I.T. Harrison, J.B. Siddall, and J.H. Fried, *Tetrahedron Letters*, **1966**, 3457.
18) D. Burn, V. Petrow, and C.O. Weston, *Tetrahedron Letters*, **1960**, 14.

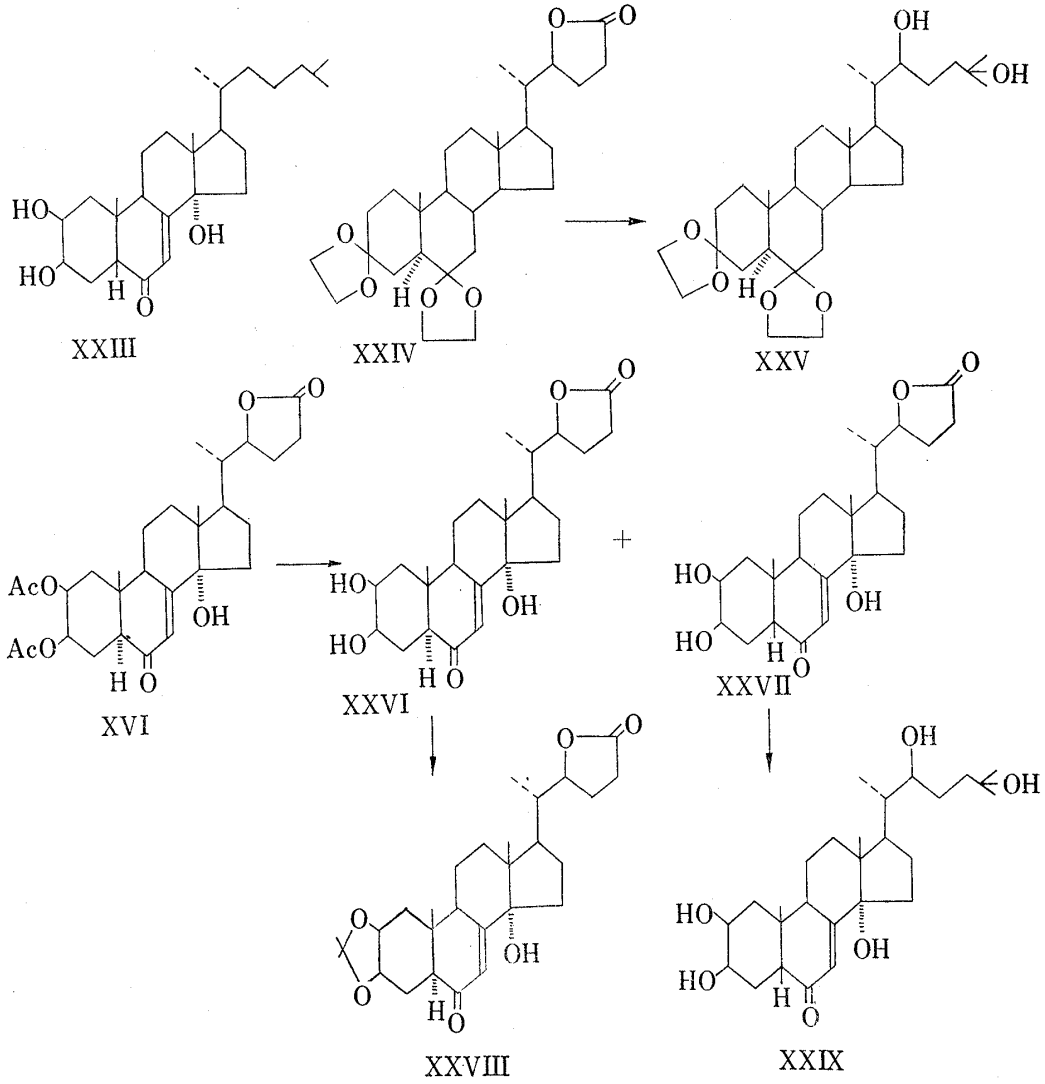

Chart 5

taken place with methylmagnesium bromide in tetrahydrofuran at 0—5° for 30 minutes; the starting material was recovered in the case of XXIII, while 22α_F,25-dihydroxy compound (XXV) was obtained from XXIV.[5] Thus, the selective Grignard reaction seemed to be quite promising, and, in fact, 22-isoecdysone (XXIX) was synthesized by this way as shown below.

The lactone (XVI) was hydrolyzed with potassium hydroxide in aqueous dioxane at room temperature, and treated with acetone in the presence of phosphomolybdic acid to yield the acetonide (XXVIII). As it was reported that A/B ring juncture is not epimerized in these reactions (hydrolysis and acetonide formation),[4] the acetonide (XXVIII) should be considered to belong to A/B trans series. The lactone (XVI) was hydrolyzed by treatment with 0.6% potassium carbonate in 90% aqueous methanol[19] at 30—40° for 30 minutes, and the resulting solution was refluxed for 30 minutes. As the same treatment of 2β,3β-diacetoxy-14α-hydroxy-5α-cholest-7-en-6-one afforded an equilibrium mixture of hydrolyzed compounds,[4] hydrolysis and equilibration were expected in this reaction described above. In addition to such reactions, it was found from infrared spectrum that γ-lactone grouping was also opened to γ-hydroxycarboxlic acid. Thus, the treatment of the product with acid afforded a mixture

19) 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).

which mainly consisted of XXVI and XXVII at a ratio of 1:4 (5α: 5β), which was in agreement with the equilibrium ratio reported in the corresponding compounds in cholestane series.[4] The isomers at C–5, XXVI and XXVII were separated in pure state by preparative thin-layer chromatography. The Grignard reaction of XXVII was made with large excess methylmagnesium bromide in tetrahydrofuran at 0—5° for 30 minutes. The infrared and ultraviolet spectrum of the product showed that lactone group was completely reacted, and 14α-hydroxy-7-en-6-oxo group unaltered. The preparative thin-layer chromatography of the product and recrystallization afforded 22-isoecdysone. Melting point, optical rotation, and nuclear magnetic resonance spectrum of 22-isoecdysone obtained here were identical with those reported by two groups[17,20] which was summarized in Table II. The fact, that the synthesis of ecdysone was succeeded by similar route for 22β_F-hydroxy-3,6-dioxohomo-5α-cholan-25-oic acid 25→22-lactone which is isomeric at C–22 in dioxolactone (II),[21] strongly supports the structure of XXIX.

Table II. A Comparison of Physical Properties of XXIX with Those of Ecdysone and 22-Isoecdysone

	Ecdysone[a]	XXIX	22-Isoecdysone[b]
Melting point	235—237°	241—245°	251—254°
UV λ_{max}^{EtOH} mμ (ε)	244 (12400)	243 (11600)	244 (11850)
Optical rotation	$[\alpha]_D$ +58.5°	$[\alpha]_{578}$ +4°	$[\alpha]_D$ −3°
NMR[c] 18–H	0.74	0.79	0.79
19–H	1.07	1.06	1.06
21–H	1.28	1.23	—
26,27–H	1.38	1.41	1.41

a) A. Butenandt and P. Karlson, *Z. Naturforsch.*, **9b**, 389 (1954)
b) lit. 17*b*)
c) Chemical shifts are quoted as ppm downfield from TMS (0.00 ppm). All data are obtained in C_5D_5N.

Experimental[22]

3β,22α_F-Dihydroxy-6-oxohomo-5α-cholan-25-oic Acid 25→22-Lactone (III)——To a solution of 22α_F-hydroxy-3,6-dioxohomo-5α-cholan-25-oic acid 25→22-lactone (II, 1.6 g) in dichloromethane (80 ml) and ethanol (80 ml) was added sodium borohydride (0.152 g) at −3——5°. After stirring for 15 min at the same temperature, acetone was added to decompose excess sodium borohydride, and the mixture was shaken with ether and 10% hydrochloric acid. The organic layer was washed with 10% hydrochloric acid, 5% sodium bicarbonate and water, and dried (sodium sulfate). Evaporation of the solvent left a crystalline material, which on recrystallization from methanol afforded the lactone (III, 1.32 g), mp 242—248°. An analytical sample was obtained by further recrystallization from methanol as colorless needles. mp 249—255°, $[\alpha]_D^{28}$ +15° (c=0.72), ORD (c=0.156, $CHCl_3$) $[\alpha]^{25}$ (mμ): −15° (700), −9° (400), −74° (350), −698° (314) (trough), −161° (300), +1060° (276) (peak), 898° (260). *Anal.* Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.41; H, 9.40.

3β,22α_F-Dihydroxy-6,6-ethylenedioxyhomo-5α-cholan-25-oic Acid 25→22-Lactone (IV)——A solution of the ketal (III, 2.5 g) in benzene (180 ml) and ethylene glycol (18 ml) was distilled slowly to remove a trace of water. *p*-Toluenesulfonic acid (60 mg) was added and the solution was distilled for 5 hr, during which benzene was added to maintain a constant volume. After cooling, pyridine and then ether–dichloromethane mixture were added and the solution was washed with water, and dried (sodium sulfate). Evaporation of the solvent afforded a crystalline material (IV, 2.92 g), which was used to the next procedure without purification.

22α_F-Hydroxy-6,6-ethylenedioxy-3-oxohomo-5α-cholan-25-oic Acid 25→22-Lactone (V)——A solution of the ketal (IV, 2.92 g) described above in pyridine (50 ml) was added to pyridine–chromium trioxide

20) U. Kerb, G. Schulz, P. Hocks, R. Wiechert, A. Fürlenmeier, A. Fürst, A. Langemann, and G. Waldvogel, *Helv. Chim. Acta*, **49**, 1601 (1966).
21) H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **16**, 563 (1968).
22) All melting points were uncorrected. Optical rotations were measured in chloroform, and ultraviolet spectra in ethanol unless otherwise stated.

complex prepared from pyridine (60 ml) and chromium trioxide (6.0 g) at 0—5°, and the mixture was stirred for 2 hr at the same temperature. After standing overnight at 25°, ether–dichloromethane mixture was added and the insoluble material was filtered off on Celite. The filtrate was washed with water, dried (sodium sulfate) and passed through Florisil column. The solvent was removed by distillation to yield a crystalline material (V, 3.02 g), which was used to the next procedure without purification. An analytical sample was obtained by twice recrystallizations from methanol containing a trace of pyridine as colorless needles. mp 225—230°, $[\alpha]_D^{28}$ +51°(c=1.08), ORD (c=0.233, $CHCl_3$) $[\alpha]^{25}$ (mμ): +47° (700), +150° (400), +264° (350), +698° (312) (peak), 422° (300), −417° (270) (trough), −354° (260). *Anal.* Calcd. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 73.10; H, 9.21.

22α_F-Hydroxy-2β,3β-isopropylidenedioxy-6-oxohomo-5α-cholan-25-oic Acid 25→22-Lactone (X)——a) From V: The crude ketal (V, 3.87 g) prepared from the ketol (III, 3.78 g) was dissolved in tetrahydrofuran (100 ml), and the solution was added to *tert*-butanol (400 ml) in which potassium(12 g) was dissolved beforehand. The solution was shaken in oxygen atmosphere at 20—23° for 10 min during which 210 ml of oxygen was absorbed, and poured into water. 10% Hydrochloric acid was dropwise added until pH became 2.0—3.0, and the product was extracted with ether–dichloromethane mixture. The organic layer was washed with water, and dried (sodium sulfate). The solvent was removed by distillation gave an oily substance (VIII).

To a solution of the product (VIII) in methanol (400 ml) was added sodium borohydride (4.0 g), and the solution was refluxed for 30 min. After addition of 50% acetic acid (400 ml), the solution was heated under reflux for 2 hr. Water was added, and the product was extracted with ether–dichloromethane mixture. The organic layer was washed with water, 5% sodium bicarbonate and water, and dried (sodium sulfate). Evaporation of the solvent gave an yellow crystalline substance (IX, 3.38 g).

To a suspension of the material described above (IX, 3.38 g) in acetone (670 ml) was added 5% phosphomolybdic acid in acetone (50 ml), and the resulting solution was allowed to stand at room temperature for 15 min. After addition of 30% ammonium hydroxide and then water, the product was extracted with ether–dichloromethane mixture and the organic layer was washed with 5% sodium bicarbonate and water, and dried (sodium sulfate). Evaporation of the solvent afforded a crystalline material, which was chromatographed on Florisil (160 g). The material, eluted with benzene–ether (4:1) and ether, was recrystallized from acetone to give the acetonide (X, 1.34 g), mp 264—270° as colorless needles. $[\alpha]_D^{28}$ +31° (c=0.79). *Anal.* Calcd. for $C_{28}H_{42}O_5$: C, 73.32; H, 9.23. Found: C, 73.23; H, 9.25.

b) From XI: 2β,3β,22α_F-Trihydroxy-6-oxohomo-5α-cholan-25-oic acid 25→22-lactone (XI, 20 mg) was treated with acetone and phosphomolybdic acid as the same manner described above. The acetonide (X), mp 263—269°, the infrared spectrum of which was identical with that of the compound described above, was obtained.

2β,3β,22α_F-Trihydroxy-6-oxohomo-5α-cholan-25-oic Acid 25→22-Lactone (XI)——A solution of the acetonide (X, 1.34 g) in ethanol (500 ml) and 10% phosphoric acid (90 ml) was heated under reflux for 2 hr. After dilution with water, the product was extracted with ether–dichloromethane mixture and the organic layer was washed with 5% sodium bicarbonate and water, and dried (sodium sulfate). The solvent was evaporated to give a crystalline material (XI, 1.18 g), which was used to the next procedure without purification. An analytical sample was obtained by recrystallization from methanol as colorless needles. mp 257—263°, $[\alpha]_D^{20}$ +37° (c=0.84). *Anal.* Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.63; H, 9.08.

2β,3β-Diacetoxy-22α_F-hydroxy-6-oxohomo-5α-cholan-25-oic Acid 25→22-Lactone (XII)——A solution of the diol described above (XI, 1.18 g) in acetic anhydride (50 ml) was heated under reflux for 2 hr, and water was added. After decomposition of acetic anhydride, the product was extracted with ether–dichloromethane mixture and the organic layer was washed with 5% sodium bicarbonate and water, and dried (sodium sulfate). Evaporation of the solvent afforded a crystalline material, which on recrystallization from methanol gave the diacetate (XII, 1.23 g), mp 231—235°. An analytical sample was obtained by further recrystallization from methanol as colorless needles. mp 239—244°, $[\alpha]_D^{28}$ +22° (c=1.11). *Anal.* Calcd. for $C_{29}H_{42}O_7$: C, 69.29; H, 8.42. Found: C, 69.01; H, 8.39.

2β,3β-Diacetoxy-22α_F-hydroxy-7α-bromo-6-oxohomo-5α-cholan-25-oid Acid 25→22-Lactone (XIII)——To a solution of the diacetate (XII, 1.94 g) in acetic acid (40 ml) containing a trace of hydrogen bromide was added a solution of bromine (0.635 g) in acetic acid (2.0 ml). The solution was warmed to 50°, during which the color of bromine was disappeared. After stirring for 30 min at 50°, water was added and the product was extracted with ether–dichloromethane mixture. The organic layer was washed with water, 5% sodium bicarbonate and water, and dried (sodium sulfate). The solvent was evaporated to dryness to give a crystlline material, which on recrystallization from acetone–hexane afforded the bromo compound (XIII, 1.73 g), mp 234° (decomp.). An analytical sample was obtained by further recrystallization from the same solvent as colorless needles. mp 234° (decomp.), $[\alpha]_{578}^{20}$ +74° (c=1.07). *Anal.* Calcd. for $C_{29}H_{41}O_7Br$: C, 59.89; H, 7.11. Found: C, 59.70; H, 7.16.

2β,3β-Diacetoxy-22α_F-hydroxy-6-oxohomo-5α-chol-7-en-25-oic Acid 25→22-Lactone(XIV)——The bromo compound (XIII, 390 mg) was heated under reflux with dimethylformamide (4.0 ml) and lithium carbonate (200 mg) for 2 hr under nitrogen atmosphere. After addition of 1% hydrochloric acid, the product was extracted with ether–dichloromethane mixture and the organic layer was washed 10% hydrochloric acid,

5% sodium bicarbonate and water, and dried (sodium sulfate). Evaporation of the solvent left a crystalline material, which on recrystallization from methanol afforded 6-oxo-7-en compound (XIV, 239 mg), mp 249—252°. An analytical sample was obtained by further recrystallization from methanol as colorless needles. mp 256—260°, $[\alpha]_{578}^{20}$ +63° (c=0.76), UV λ_{max} mμ (ε): 244 (13000). *Anal.* Cacld. for $C_{29}H_{40}O_7$: C, 69.57; H, 8.05. Found: C, 69.41; H, 7.88.

2β,3β-Diacetoxy-14α,22α_F-dihydroxy-6-oxohomo-5α-chol-7-en-25-oic Acid 25→22-Lactone (XVI)——a) By Monoperphthalic Acid: The 6-oxo-7-ene compound (XIV, 701 mg) was dissolved in 0.001 M solution of perchloric acid in ethyl acetate (63 ml) and acetic anhydride (7 ml), and the solution was allowed to stand at room temperature for 30 min. Water was added and the mixture was stirred for 1 hr to decompose acetic anhydride. The product was extracted with ether–dichloromethane mixture and the organic layer was washed with 5% sodium bicarbonate and water, and dried (sodium sulfate). Evaporation of the solvent afforded the enol acetate (XV).

To a solution of the enol acetate described above (XV) in anhydrous ether (35 ml) and anhydrous tetrahydrofuran (35 ml) was added a solution of monoperphthalic acid in ether (3.86 ml; 0.0813 g/ml; 1.2 equivalent) and the solution was allowed to stand at 25° in dark place for 2 days. Further monoperphthalic acid solution (0.4 ml) was added and the solution was allowed to stand for another 2 days. Ether–dichloromethane mixture was added and the solution was washed with 5% sodium bicarbonate and water, and dried (sodium sulfate). Evaporation of the solvent left a crystalline material, which was submitted to preparative thin-layer chromatography on silica gel, Merck GF_{254} (0.5 mm plates; developing system, chloroform–acetone 7:3). The material obtained from main fraction was recrystallized from methanol–acetone to afford 14α-hydroxy compound (XVI, 316 mg), mp 263—268°. An analytical sample was obtained by further recrystallization from the same solvent as colorless needles. mp 268—272°, $[\alpha]_{578}^{20}$ +92° (c=0.95), UV λ_{max} mμ (ε): 241 (11800). *Anal.* Calcd. for $C_{29}H_{40}O_8$: C, 67.42; H, 7.80. Found: C, 67.56; H, 7.81.

b) By *m*-Chloroperbenzoic Acid: The enol acetate obtained from 6-oxo-7-ene compound (XIV, 102 mg) as the same manner described above was dissolved in chloroform (3 ml) and a solution of *m*-chloroperbenzoic acid (47.3 mg) (purity 78.5%; 1.03 equivalent) in chloroform (1 ml) was added. The solution was washed with 5% sodium bicarbonate and water, and dried (sodium sulfate). Evaporation of the solvent afforded a crystalline material, which on recrystallization from ether–hexane and then methanol gave 14α-hydroxy compound (XVI, 60 mg), mp 261—269°.

Hydrolysis and Isomerization of 2β,3β-Diacetoxy-14α,22α_F-dihydroxy-6-oxohomo-5α-chol-7-en-25-oic Acid 25→22-Lactone (XVI)——A solution of the diacetate (XVI, 191 mg) in 0.6% potassium carbonate in 90% aqueous methanol (40 ml) was warmed at 30° for 30 min and then heated under reflux for 30 min. Water was added and pH was adjusted to 2.0 by addition of dilute hydrochloric acid. The proruduct was extracted with butanol and the organic layer was washed with water. The solvent was removed by distillation *in vacuo* to afford an oily material. The oily material was dissolved in methanol (40 ml) containing *p*-toluenesulfonic acid (50 mg) and the solution was allowed to stand at room temperature for 10 min. Water was added and the product was extracted with butanol–ether mixture. The organic layer was washed with 5% sodium bicarbonate and water. The solvent was evaporated *in vacuo* to yield a crystalline material, which was submitted to preparative thin-layer chromatography on silica gel, Merck GF_{254} (0.5 mm plates; developing system, dichloromethane–acetone–ethanol 80:20:5). The material obtained from polar fraction was recrystallized from methanol to afford 2β,3β,14α,22α_F-tetrahydroxy-6-oxohomo-5β-chol-7-en-25-oic acid 25→22-lactone (XXVII, 86 mg) as colorless needles. mp 255—257°, $[\alpha]_{578}^{20}$ +122° (c=0.39, ethanol), UV λ_{max} mμ (ε): 243 (11100). *Anal.* Calcd. for $C_{25}H_{36}O_6$ (2β,3β,14α,22α_F-tetrahydroxy-6-oxohomo-5β-chol-7--25 oic acid 25→22-lactone): C, 69.42; H, 8.39. Found: C, 69.30; H, 8.21.

The material obtained from nonpolar fraction was recrystallized from methanol to afford 2β,3β,14α,22α_F-tetrahydroxy-6-oxohomo-5α-chol-7-en-25-oic acid 25→22-lactone (XXVI, 18 mg) as colorless needles. mp 263—265°, $[\alpha]_{578}^{20}$ +111°, $[\alpha]_{D}^{20}$ +119° (c=0.33, pyridine), UV λ_{max} mμ (ε): 241 (11800). *Anal.* Calcd. for $C_{25}H_{36}O_6 \cdot H_2O$ (2β,3β,14α,22α_F-tetrahydroxy-6-oxohomo-5α-chol-7-en-25-oic acid 25→22-lactone): C, 66.64; H, 8.50. Found: C, 66.81; H, 8.39.

The analysis of fractions of thin-layer chromatography by ultraviolet absorption showed that the equilibrium mixture consisted of 5α- and 5β-compound (XXVI and XXVII) at a ratio of 1:4.

14α,22α_F-Dihydroxy-2β,3β-isopropylidenedioxy-6-oxohomo-5α-chol-7-en-25-oic Acid 25→22-Lactone (XXVIII)——A solution of the diacetate (XVI, 96 mg) in 0.6 N potassium hydroxide (2 ml) and dioxane (6 ml) was allowed to stand at room temperature for 1 hr. Ice-cold water was added and pH was adjusted to 3.0 by addition of dilute hydrochloric acid. The product was extracted with butanol and the organic layer was washed with water. The solvent was evaporated *in vacuo* to yield a crystalline material.

To a suspension of the product described above in acetone (20 ml) was added 5% phosphomolybdic acid in acetone (1.5 ml) and the resulting solution was allowed to stand at room temperature for 20 min. After addition of 30% ammonium hydroxide and then water, the product was extracted with dichloromethane–ether mixture and the organic layer was washed with 5% sodium bicarbonate and water, and dried (sodium sulfate). After evaporation of the solvent, a crystalline material was submitted to preparative thin-layer chromatography on silica gel, Merck GF_{254} (0.5 mm plates, developing solvent, chloroform–acetone 7:3). The material obtained from the main fraction was recrystallized from acetone to yield the acetonide

(XXVIII, 21 mg) as colorless needles. mp 255—257°, $[\alpha]_{578}^{20}$ +80° (c=0.53), UV λ_{max} mμ (ε): 241 (11400). *Anal.* Calcd. for $C_{28}H_{40}O_6$: C, 71.16; H, 8.53. Found: C, 70.95; H, 8.73.

2β,3β,14α,22α_F,25-Pentahydroxy-5β-cholest-7-en-6-one (XXIX) (22-Isoecdysone)——To an ice-cold solution of the triol (XXVII, 29 mg) in anhydrous tetrahydrofuran (15 ml) was added Grignard reagent prepared from magnesium (400 mg), methyl bromide and tetrahydrofuran (15 ml). The solution was stirred at 0—5°, for 30 min and ice was added to decompose Grignard reagent. After addition of 10% ammonium hydroxide and water, the product was extracted with butanol and the organic layer was washed with 10% ammonium hydroxide, 5% sodium carbonate and water. After removal of the solvent by distillation *in vacuo*, the residue was submitted to preparative thin-layer chromatography on silica gel, Merck GF_{254} (0.5 mm plates; developing solvent, ethyl acetate-methanol 4:1). The material obtained from the main fraction was recrystallized from methanol-ethyl acetate to give 22-isoecdysone (XXIX) as colorless needles. mp 241—245°,$[\alpha]_{578}^{25}$ +4° (c=0.50, ethanol), UV λ_{max} mμ (ε): 243 (11600).

3β-Hydroxy-5α-cholestan-6-one (VII)——To a solution of 5α-cholestane-3,6-dione (VI, 1.0 g) in dichloromethane (30 ml) and ethanol (50 ml) was added sodium borohydride (95 mg) at 0——5°. After stirring for 30 min at the same temperature, acetone was added to decompose excess sodium borohydride and the mixture was shaken with ether and 10% hydrochloric acid. The organic layer was washed with 10% hydrochloric acid, 5% sodium bicarbonate and water, and dried (sodium sulfate). The solvent was evaporated to dryness and the residue was recrystallized from methanol to give 3β-hydroxy-5α-cholestan-6-one (VII, 0.42 g), mp 138—140°, which was identical with an authentic sample.

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