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# 57. Hiromu Mori, Kenyu Shibata, Kiyoshi Tsuneda, and Masanobu Sawai: Synthesis of Ecdysone. I. A Novel Synthesis of 2β,3β-Dihydroxy Steroids.

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A novel synthesis of 2β,3β-dihydroxy steroids from 3-oxo compounds in 5α-series was developed. Autoxidation of 3-oxo compound afforded an enol mixture of 2,3-dioxo compound, which on reduction with sodium borohydride yielded 2β,3β-dihydroxy steroid. It was found that 2β,3β-dihydroxy steroid was obtained with high stereospecificity by the reduction of 2-hydroxy-1-en-3-oxo steroid.

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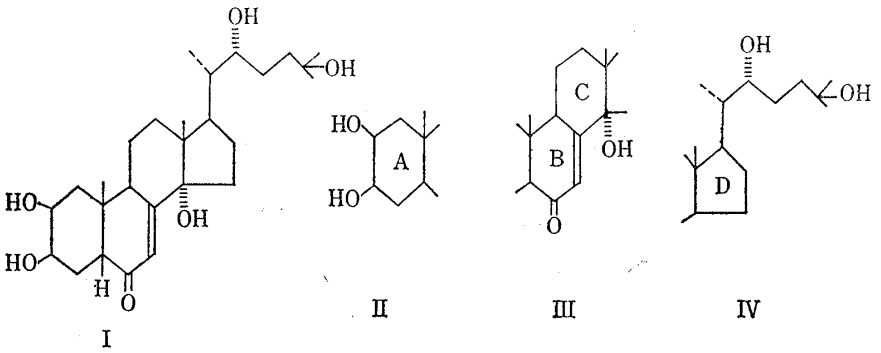
An insect hormone, ecdysone, was first isolated by Butenandt and Karlson[1] in 1954 from pupae of silkworm *Bombyx mori*. However, the elucidation of its chemical structure was very difficult problem, not only because only a small amount of material could be isolated from natural sources, but also because it has an unique structure. About one decade later, Huber and Hoppe[2] succeeded in the elucidation of the structure by X-ray diffraction analysis, the structure being a highly hydroxylated steroid, 2β,3β,14α,22β$_F$,25-pentahydroxy-5β-cholest-7-en-6-one (I). Our program to




I II III IV

Chart 1.

synthesize ecdysone being now in progress, two groups[3,4] have reported synthesis of ecdysone independently only one year after the elucidation of the structure. In our schedule, the structure of ecdysone was divided in three partial structures, namely, A-ring structure (II), B,C-ring structure (III) and side chain structure (IV), and synthetic methods of these partial structures were studied indevidedly. Then, the process of synthesis of ecdysone was arranged by combination of methods studied. In our study on 2β,3β-dihydroxy steroids, a novel synthetic method was developed, which will be described in this paper.

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1) A. Butenandt, P. Karlson: Z. Naturforsch., **9b**, 389 (1954).
2) R. Huber, W. Hoppe: Chem. Ber., **98**, 2403 (1965).
3) J. B. Sidall, J. P. Marshall, A. Bowers, A. D. Cross, J. A. Edwards, J. H. Fried: J. Am. Chem. Soc., **88**, 379 (1966); J. B. Sidall, A. D. Cross, J. H. Fried: *Ibid.*, **88**, 862 (1966).
4) U. Kerb, P. Hocks, R. Wiechert, A. Furlenmeier, A. Fürst, A. Langemann, G. Waldvogel: Tetrahedron Letters, **1966**, 1387.

All four isomers of 5α-cholestane-2,3-diol have been already prepared and their stereochemical problems have been fully studied.[5,6] The preparation of 5α-cholestane-2β,3β-diol was first made by Henbest and Smith[6] and Shoppee and his co-workes[5] independently almost at the same time from 5α-cholest-2-ene by treatment with silver actate, iodine and moist acetic acid. This seemed to be only one method of the synthesis of 2β,3β-dihydroxy steroid for preparative purpose.*2 Accordingly, it is very desirable to establish a novel and better synthetic method of 2β,3β-dihydroxy steroid.

2,3-Dioxo steroid was very attractive material for the preparation of 2,3-dihydroxy steroid, because an elegant synthesis of 2,3-dioxo steroid from 3-oxo steroid by autoxidation in the presence of potassium *t*-butoxide in *t*-butanol was developed.[7~9] Sodium borohydride reduction of 2,3-dioxo steroids was studied in the hope that 2β,3β-dihydroxy steroids would be obtained, and in fact, it was found that 2β,3β-dihydroxy steroids are major products in these reactions.

The reduction of 2,17β-dihydroxy-4,4-dimethylandrosta-1,5-dien-3-one (V)[9] with sodium borohydride in methanol gave an isomer of 2,3-diol in high yield. This diol yielded an acetonide on treatment with acetone in the presence of hydrogen chloride, showing that *cis* diol was obtained. The diol was recovered on the hydrolysis of the acetonide (Ⅶ) by hydrochloric acid. Sodium borohydride reduction of 3β,17β-dihydroxy-4,4-dimethylandrost-5-en-2-one (X) obtainable from V by three steps of reactions (V→Ⅷ→Ⅸ→X)[10] afforded the diol identical with that obtained above. Consequently, the diol must be formulated as 4,4-dimethylandrost-5-ene-2β,3β,17β-triol (Ⅵ).

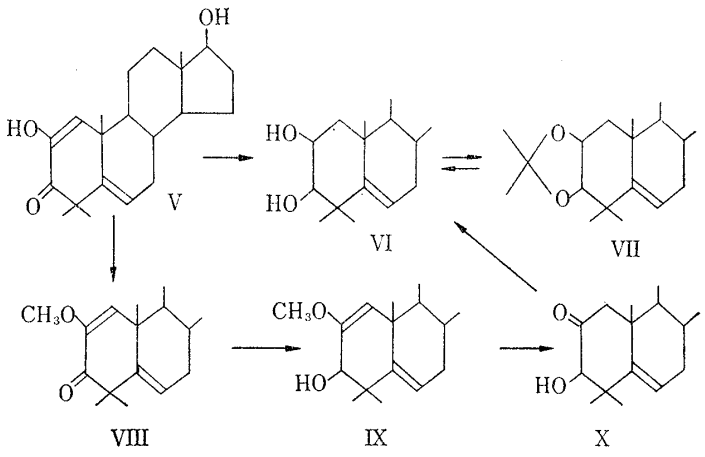


Chart 2.

The success in the preparation of 2β,3β,-diol in this special case prompted us further study on reduction of the enol forms of steroidal 2,3-dione. The autoxidation of 5α-cholestan-3-one (Ⅺ) by the method developed by Barton and his co-workers afforded the mixture of two possible enol forms of 5α-cholestane-2,3-dione (Ⅻ). This mixture was reduced with sodium borohydride in methanol without isolation of enol forms in pure state to give a mixture of 2,3-diols, which was treated with acetone containing hydrogen chloride and chromatographed on Florisil. 5α-Cholestane-2β,3β-diol acetonide (XIV)[11] was first eluted, the yield of which was 30~35% based on Ⅺ. Two *trans* diols, 5α-cholestane-2α,3β-diol (XVa) and 2β,3α-diol (XVIa) were isolated by further elution of the column as minor products. The structures of these compounds were confirmed by comparison of physical constants of free diols and their acetates with those reported in literature.[5] It should be noted to describe that the stereochemical course of reduction in enol mixture of 5α-cholestane-2,3-dione (Ⅻ) is very different

*2 In synthesis of ecdysone in reference 4), it was reported that the reaction of 2α-bromo-3β-acetoxy steroid with silber acetate gave 2β,3β-diacetoxy steroid, but detailed experimental data was not given.

5) C. W. Shoppee, D. N. Jones, G. H. R. Summers : J. Chem. Soc., **1957**, 3100.
6) H. B. Henbest, M. Smith : *Ibid.*, **1957**, 926.
7) D. H. R. Barton, S. K. Pradhan, S. Sternhell, J. F. Templeton : J. Chem. Soc., **1961**, 255.
8) B. Camerino, B. Patelli, R. Sciaky : Tetrahedron Letters, **1961**, 554.
9) H. Mori, V. S. Gandhi, E. Schwenk : This Bulletin, **10**, 842 (1962).
10) K. Shibata, J. Yamada, H. Mori : *Ibid.*, **15**, 186 (1967).
11) H. B. Henbest, R. A. L. Wilson : J. Chem. Soc., **1957**, 1958.

from that in V. It was expected that the enol compound (XIX), in which 2-oxo group is enolized, would be reduced to 2β,3β-diol stereospecifically, while the reduction of the other enol compound (XVIII), in which 3-oxo group in enolzied, would be not so stereospecific; as a consequence, the reduction of the enol mixture would give 2β,3β-diol with considerable amount of 2α,3β- and 2β,3α-diol. In order to ascertain whether this is the case or not, the stereochemical course of reduction in two pure diosphenols, XVIII and XIX was investigated.

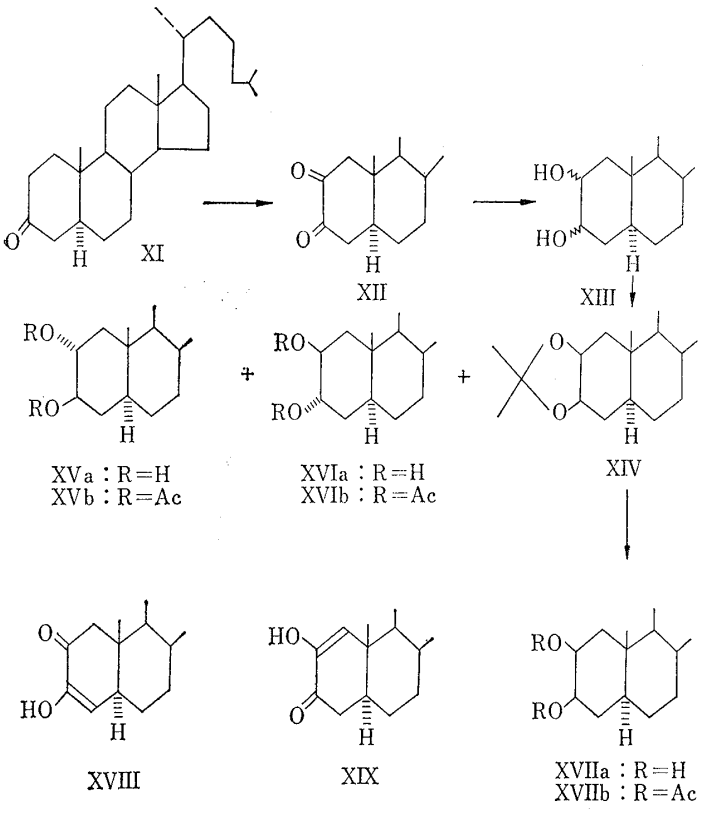



Chart 3.

From the enol mixture of 5α-cholestane-2,3-dione described above, the pure diosphenols (XVIII and XIX) were isolated by the procedure reported by Stiller and Rosenhelm.[12] These two diosphenols were reduced and the reduction products were submitted to gas chromatography as trimethyl silyl ether. As shown in Fig. 1-A and 2-A, two peaks were observed in their chromatogram. 2α,3β-Diol and 2β,3β-diol should be considered not to be separated in Fig. 1-A and 2-A, because trimethyl silyl ether of 5α-cholestane-2α,3β-diol, 2β,3α-diol and 2β,3β-diol were found to show retention times of 3.85, 2.64 and 3.70 (5α-cholestane=1.00) respectively. In order to obtain clearly separated chromatograms, the reduction products were treated with acetone containing hydrogen chloride, and *cis*-diol acetonide and *trans*-diol fraction were separated by chromatography on Florisil. After hydrolysis of *cis*-diol acetonide fractions, *cis*- and *trans*-diol fractions were submitted to gas chromatography as trimethyl silyl ether, and fully separated chromatograms were obtained (Fig. 1-B, 1-C, 2-B and 2-C). Although 5α-cholestane-2α,3α-diol could not be isolated in large scale experiment on the reduction of enol mixture, it is apparently detected in Fig. 1-C. It should be noted that 2α,3α-diol was produced only in reduction of the diosphenol (XVIII). The ratios of diols in reduction products were calculated as shown in Table I. From these observations it is concluded that the

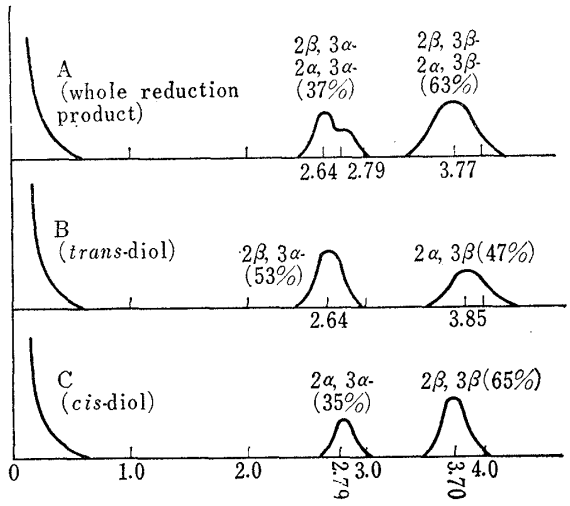
Table I. Ratio of Reduction Product in Diosphenols (XVIII and XIX)

| Compound reduced | Ratio of reduction product (%)[a] | | | |
|---|---|---|---|---|
| | 2β,3α | 2α,3α | 2β,3β | 2α,3β |
| XVIII | 22 | 20 | 38 | 20 |
| XIX | 6 | 0 | 89 | 5 |

a) These values are calculated from weights of *cis*- and *trans*-diol fractions and data from chromatogram shown in Fig. 1-B, 1-C, 2-B and 2-C.

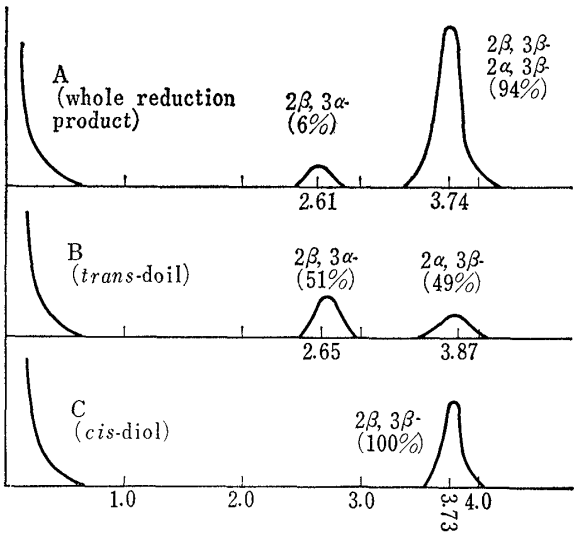
12) E. T. Stiller, O. Rosenhelm : J. Chem. Soc., **1938**, 353.

ratios of products are very different; 2β,3α, : 2α,3α : 2β,3β : 2α,3β≒ 1:1:2:1 (from 3-enol compound (XVIII)), 2β,3α : 2β,3β : 2α,3β ≒ 1:19:1 (from 2-enol compound (XIX)). This result clearly supports the concept described above.




Relative Retention Time
(5α-Cholestane=1.00)

Fig. 1. Gas Chromatogram of Reduction Product from XVIII




Relative retention time
(5α-Cholestane=1.00)

Fig. 2. Gas Chromatogram of Reduction Product from XIX

Table II. Difference of Frequecies between Free and Hydrogen-bonded OH Absorption

| Compound | Conformation | $\Delta\nu$ ($cm^{-1}$) | $L$ (Å) | $\phi$ (°C) |
|---|---|---|---|---|
| 2β,3β-diol (XVIIa) | a,e-*cis* | 45 | 2.10 | 41 |
| 2α,3β-diol (XVa) | e,e-*trans* | 31 | 2.38 | 60 |

Infrared spectra were measured in concentration of 1/300$M/L$. in $CCl_4$ by 3 mm. NaCl cell. (Prism: NaCl)

The differenece in ratios of diols between these diosphenols can be explained reasonably as follows. The first reduction by sodium borohydride should be considered to be taken place at the non-enolized carbonyl group. It was known that 3-oxo steroids in 5α-series are reduced by metal hydride to 3β-hydroxy compounds with high stereospecificity.[13] Thus, stereospecific reduction to 3β-hydroxy compound in the diosphenol (XIX) is expected. The ketol (3β-hydroxy-2-one) thus obtained was reduced again to 2β,3β-diol in high yield, because 3β-hydroxy group probably disturbs β-side attack of the reagent at the carbonyl group. On the other hand, the metal hydride reduction of 2-oxo steroids in 5α-series are not so stereospecific as that of 3-oxo steroids. Accordingly, the diosphenol (XVIII) must be considered to be reduced at first stage ketol mixture (2-hydroxy-3-ones) without high stereospecificity. As a result, such a stereospecificity in another diosphenol (XIX) would not be observed in this case.

Infrared absorption spectra of 5α-cholestane-2α,3β-diol (XVa), 2β,3α-diol (XVIa) and 2β,3β-diol (XVIIa) in OH stretching region were measured. Intramolecular hydrogen-bonding was observed in 2α,3β-diol (XVa) and 2β,3β-diol (XVIIa), while not in 2β,3α-diol

13) L. F. Fieser, M. Fieser: "Steroids," p. 268. Reinhold Publishing Co., N. Y. (1959).

(XVIa). It is quite reasonable that axial-axial *trans*-diol is not intramolecular hydrogen-bonded. The difference of frequencies between free and hydrogen-bonded hydroxy absorption ($\Delta\nu$) in XVa and XVIIa are shown in Table II. Kuhn[14] has reported an empirical relationship between $\Delta\nu$ and hydrogen-bonded distance ($L$) as follows.

$$\Delta\nu = \frac{250}{L} - 74$$

$L$ values in XVa and XVIIa are calculated as 2.38 Å and 2.10 Å by applying this equation, showing that dihedral angles of oxygen atoms at C-2 and C-3 are 60° and 41°. Kuhn[14] has described that $\Delta\nu$ value of *trans*- and *cis*-cyclohexane-1,2-diol are 33 $cm^{-1}$ and 38 $cm^{-1}$. In the case of XVa, the value listed in Table II is consistent with that of *trans*-cyclohexane-1,2-diol within error. On the other hand, $\Delta\nu$ value of 2β,3β-diol (XVIIa) is considerably large comparing with that of *cis*-cyclohexane-1,2-diol. It should be considered that 2β-hydroxy group in XVIIa is deformed so as to diminish the dihedral angle due to 1,3-diaxial interaction with 19-methyl group.

## Experimental*3

**4,4-Dimethylandrost-5-ene-2β,3β,17β-triol (VI)**——a) From V: To a solution of 2,17β-dihydroxy-4,4-dimethylandrosta-1,5-dien-3-one (V, 5.0 g.) in MeOH (100 ml.) was added $NaBH_4$ (2.5 g.) and the solution was refluxed for 1 hr. After decomposition of excess $NaBH_4$ by addition of AcOH, $H_2O$ was added and precipitates were collected by filtration, washed with $H_2O$, and dried. Recrystallization from MeOH afforded the triol (VI, 4.55 g.), m.p. 267~271°. An analytical sample was obtained by further recrystallization from the same solvent as colorless needles. m.p. 268~270°, $[\alpha]_D^{27}$ −53°(c=0.98, dioxane). *Anal.* Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.23; H, 10.34.

b) From VII: A solution of 4,4-dimethylandrost-5-ene-2β,3β,17β-triol 2,3-acetonide (VII, 50 mg.) in dioxane (3.5 ml.) and 2% HCl (3.5 ml.) was refluxed for 2.5 hr., and poured into $H_2O$. The product was extracted with ether and after washing with $H_2O$ and drying ($Na_2SO_4$), the solvent was removed by distillation. The residue was recrystallized from MeOH to give the triol (VI), the infrared spectrum of which was identical with that of the compound obtained above.

c) From X: 3β,17β-Dihydroxy-4,4-dimethylandrost-5-en-3-one (X, 100 mg.) was reduced with $NaBH_4$ (10 mg.) in MeOH (100 ml.), and usual treatment of isolation yielded the triol (VI) identical with the compound obtained above.

**4,4-Dimethylandrost-5-ene-2β,3β,17β-triol 2,3-Acetonide (VII)**——4,4-Dimethylandrost-5-ene-2β,3β,17β-triol (VI, 1.0 g.) was suspended in acetone (25 ml.) and saturated HCl solution in acetone (25 ml.) was added. After standing overnight at room temperature, the solution was poured into 5% $Na_2CO_3$, and the product was extracted with ether. After washing with $H_2O$ and drying ($Na_2SO_4$), the solvent was removed by distillation. The residue was recrystallized from acetone to give the acetonide (VII, 0.957 g.), m.p. 179~181°. An analytical sample was obtained by further recrystallization from the same solvent as colorless prisms. m.p. 179~181°, $[\alpha]_D^{27}$ −48°(c=0.86). *Anal.* Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 77.38; H, 10.36.

**Reduction of Enol Mixture of 5α-Cholestane-2,3-dione (XII)**——5α-Cholestan-3-one (XI, 7.0 g.) was added to *t*-BuOH (280 ml.) in which K (8.4 g.) was dissolved beforehand, and the suspension was stirred under oxygen atmosphere at 19°, for 30 min., during which 470 ml. of oxygen was absorbed. The resulting solution was poured into $H_2O$, and acidified with 10% HCl. The product was extracted with ether and the ether solution was shaken with 40% KOH. The ether layer was removed by decantation and the precipitated K salt was suspended in ice-cold $H_2O$. After acidification with 10% HCl, the free diosphenol was extracted with ether, and the ether layer was washed with 5% $Na_2CO_3$ and $H_2O$, and dried ($Na_2SO_4$). Ether was removed by distillation to afford white solid (XII).

The suspension of the solid obtained above in MeOH (280 ml.) and $NaBH_4$ (3.5 g.) was stirred at 5° for 30 min., and the resulting solution was refluxed for 2 hr. After decomposition of excess $NaBH_4$ by addition of AcOH, $H_2O$ was added and the product was extracted with ether. The ether solution was washed with $H_2O$ and dried ($Na_2SO_4$). Ether was removed by distillation to give a mixture of 5α-cholestane-2,3-diols (XIII) as white solid.

*3 All melting points were uncorrected, and optical rotations were measured in chloroform unless otherwise described.

14) L. P. Kuhn: J. Am. Chem. Soc., **74**, 2492 (1952); **76**, 4323 (1954).

The suspension of the mixture (XIII) in acetone (175 ml.) and acetone (175 ml.) saturated with HCl was allowed to stand overnight at room temperature. The solid in the suspension was collected by filtration and recrystallized from MeOH to give 5α-cholestane-2α,3β-diol (XVa, 287 mg.), m.p. 204~208°. The filtrate was poured into ice-cold 5% $Na_2CO_3$ and the product was extracted with ether. After washing with $H_2O$ and drying ($Na_2SO_4$), the solvent was evaporated to dryness. The residue was chromatographed on Florisil (140 g.). Recrystallization of the material eluted by *n*-hexane-EtOAc (9:1) afforded 5α-cholestane-2β,3β-diol acetonide (XIV, 2.34 g.), m.p. 110~113°, $[\alpha]_D^{18}$ +47°(c=1.01) (reported,[11] m.p. 117°, $[\alpha]_D$ +40°). The material eluted earlier with *n*-hexane-EtOAc (1:1) was recrystallized from MeOH to yield 5α-cholestane-2β,3α-diol (XVIa, 203 mg.), m.p. 202~206°, $[\alpha]_D^{18}$ +34°(c=1.05) (reported,[5] m.p. 200~202°, $[\alpha]_D$ +33°). The material obtained by further elution with the same solvent was acetylated with $Ac_2O$-pyridine at room temperature, and 5α-cholestane-2β,3α-diol diacetate (XVIb, 296 mg.), m.p. 133~135°, $[\alpha]_D^{18}$ +57°(c=1.13) (reported,[5] m.p. 133~135°, $[\alpha]_D$ +56°) was obtained three times recrystallization from acetone-MeOH. The substance eluted with *n*-hexane-EtOAc (1:4) and EtOAc was recrystallized from MeOH to give 5α-cholestane-2α,3β-diol (XVa, 174 mg.), m.p. 210~212°, $[\alpha]_D^{18}$ +16°(c=1.14) (reported,[5] m.p. 204~205°, $[\alpha]_D$ +17°).

**5α-Cholestane-2β,3β-diol (XVIIa)**——A solution of 5α-cholestane-2β,3β-diol acetonide (XIV, 1.0 g.) in dioxane (70 ml.) and 2% HCl (70 ml.) was refluxed for 4 hr., and poured into $H_2O$. The product was extracted with ether and after washing with 5% $Na_2CO_3$ and $H_2O$, and drying ($Na_2SO_4$), the solvent was evaporated to dryness. The residue was recrystallized from MeOH to give the diol (XVIIa, 0.7 g.), m.p. 170~174°, $[\alpha]_D^{18}$ +40°(c=1.00) (reported,[5] m.p. 176~177°, $[\alpha]_D$ +38°).

**5α-Cholestane-2β,3β-diol Diacetate (XVIIb)**——A solution of 5α-cholestane-2β,3β-diol (XVIIa, 0.2 g.) in $Ac_2O$ (5.0 ml.) was refluxed for 2 hr., and poured into $H_2O$. The product was extracted with ether and the ether layer was washed with 5% $Na_2CO_3$ and $H_2O$, and dried ($Na_2SO_4$). After removal of the solvent, the residue was recrystallized from acetone-MeOH to give the acetate (XVIIb, 0.19 g.), m.p. 118~119°, $[\alpha]_D^{18}$ +37°(c=0.98) (reported,[5] m.p. 119°, $[\alpha]_D$ +38°).

**5α-Cholestane-2α,3β-diol Diacetate (XVb)**——5α-Cholestane-2α,3β-diol (XVa, 100 mg.) was acetylated by $Ac_2O$ (4.0 ml.) and pyridine (4.0 ml.) at room temperature. The diacetate (XVb, 89 mg.), m.p. 108~110°, $[\alpha]_D^{18}$ −30°(c=1.03) (reported,[5] m.p. 106~107°, $[\alpha]_D$ −27°) was obtained.

**2-Hydroxy-5α-cholest-1-en-3-one (XIX) and 3-Hydroxy-5α-cholest-3-en-2-one (XVIII)**——From the enol mixture obtained by autoxidation of 5α-cholestane-3-one as the procudure described above, the separation of pure diosphenols (XVIII and XIX) was carried out by the procedure reported by Stiller and Rosenhelm.[12]

**Reduction of 2-Hydroxy-5α-cholest-1-en-3-one (XIX)**——A mixture of XIX (101.8 mg.) and $NaBH_4$ (100 mg.) in MeOH (30 ml.) was stirred at 0° for 30 min., and then refluxed for 3 min. After cooling, AcOH (5 drops) was added and the mixture was stirred for 1 hr. The solution was poured into $H_2O$, and the product was extracted with ether. After washing with 5% $Na_2CO_3$ and $H_2O$, and drying ($Na_2SO_4$), the solvent was removed by distillation to give crystalline solid (Fig. 2-A, 100.5 mg.).

To a suspension of the solid (Fig. 2-A, 118 mg.) in acetone (20 ml.) was added 5% phosphomolybdic acid in acetone (2.4 ml.). After standing at room temperature for 15 min, the mixture was poured into 5% $NH_4OH$. The product was extracted with ether, and after washing with 5% $Na_2CO_3$ and $H_2O$, and drying ($Na_2SO_4$), the solvent was removed by distillation. The residue was chromatographed on Florisil (4.0 g.). Acetonide fraction (100.0 mg.) was eluted with *n*-hexane-EtOAc (9:1), and *trans*-diol fraction (Fig. 2-B, 12.6 mg.) was obtained by elution with EtOAc.

A mixture of the acetonide fraction in EtOH (15 ml.) and 10% $H_3PO_4$ (2.4 ml.) was refluxed for 30 min., and poured into $H_2O$. The product was extracted with ether, and after washing with 5% $Na_2CO_3$ and $H_2O$, and drying ($Na_2SO_4$), the solvent was removed by distillation to give *cis*-diol fraction (Fig. 2-C, 94.9 mg.).

Fig. 2-A, 2-B and 2-C were transformed into trimethyl silyl ether by usual manner respectively, and submitted to gas chromatography.

**Reduction of 3-Hydroxy-5α-cholest-3-en-2-one (XVIII)**——XVIII (100.4 mg.) was reduced by the same procedure described above to give crystalline solid (Fig. 1-A, 98.0 mg.). The separation of *trans*-diol (Fig. 1-B, 36.1 mg.) and *cis*-diol (Fig. 1-C, 50.0 mg.) fraction was carried out as described above. Fig. 1-A, 1-B and 1-C were submitted to gas chromatography as trimethyl silyl ether.

Gas chromatography was carried out an Shimadzu GC-B1. Column : 1% SE-52 on Chromsorb W, 4 mm. 2.25 m. Carrier gas : $N_2$ 40 ml./min. Column temperature : 236.5°.

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