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Synthesis of Ecdysone. III.[1] A Novel Synthesis of 14α-Hydroxy-7-en-6-oxo Steroids

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A novel synthetic method of 14α-hydroxy-6-oxo-7-ene steroid from 6-oxo-7-ene steroid was developed. The enol acetylation of 7-en-6-one with acetic anhydride in the presence of perchloric acid afforded 6-acetoxy-6,8(14)-diene, which on treatment with monoperphthalic acid gave 14α-hydroxy-7-en-6-one. 2β,3β,14α-Trihydroxy-5β-cholest-7-en-6-one (XXVIII), the nuclear structure of which is the same as that of ecdysone, was prepared by this method, and the stereochemistry of XXVIII and related compounds was studied.

In the previous papers of this series, a novel synthesis of 2β,3β-dihydroxy steroids[3] and 2β,3β-dihydroxy-6-oxo steroids,[4] which have a partial structure of the insect moulting hormone ecdysone, have been reported. This paper describes a novel synthesis of 14α-hydroxy-7-en-6-oxo steroids, a partial structure of ecdysone in ring B and C, and also synthesis of 2β,3β,14α-trihydroxy-7-en-6-oxo steroids, nuclear structure of ecdysone.

14-Hydroxy-7-en-6-oxo compound was prepared by Zürcher, *et al.*[5] in 1954, that is, 3β-acetoxy-5α-hydroxyergosta-7,22-dien-6-one (I) was treated with selenium dioxide to

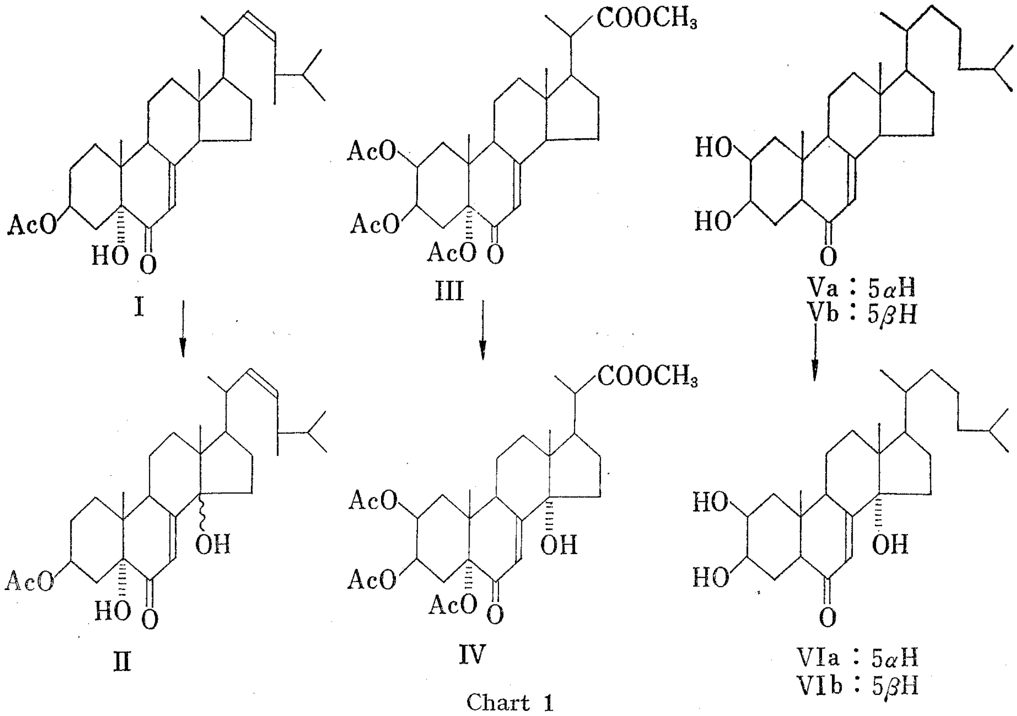

Chart 1

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[1] Part II: *Chem. Pharm. Bull.* (Tokyo), **15**, 466 (1967).

[2] Location: *1604 Shimosakunobe, Kawasaki-shi.*

[3] H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **15**, 460 (1967).

[4] H. Mori, K. Tsuneda, K. Shibata, and M. Sawai, *Chem. Pharm. Bull.* (Tokyo), **15**, 466 (1967).

[5] A. Zürcher, H. Heusser, O. Jeger, and P. Geislich, *Helv. Chim. Acta*, **37**, 1562 (1954).

give 3β–acetoxy–5α,14–dihydroxyergosta–7,22–dien–6–one (II), in which the configuration of the hydroxyl group newly introduced was not clarified. The same type of reaction (III→IV) was used by Sidall, *et al.*[6] in their synthesis of ecdysone,[7] and the hydroxyl group introduced was found to be α–oriented. In synthesis of ecdysone by Schering and F. Hoffmann–La Roche group,[8–11] Furlenmeier, *et al.*[10] described that various 7–en–6–oxo steroids, in which the configuration of hydrogen at C–5 is α– or β–oriented, could be also transformed into 14α–hydroxy–7–en–6–oxo compounds by selenium dioxide, for example, Va and Vb were introduced into VIa and VIb, respectively. Selenium dioxide oxidation was applied by two groups in their synthesis of ecdysone as a key reaction of introduction of hydroxyl group at C–14, and this appeared to be only one method of synthesis of 14α–hydroxy–7–en–6–oxo steroid. It was desirable to develop a novel synthetic method of the functional group in steroid.

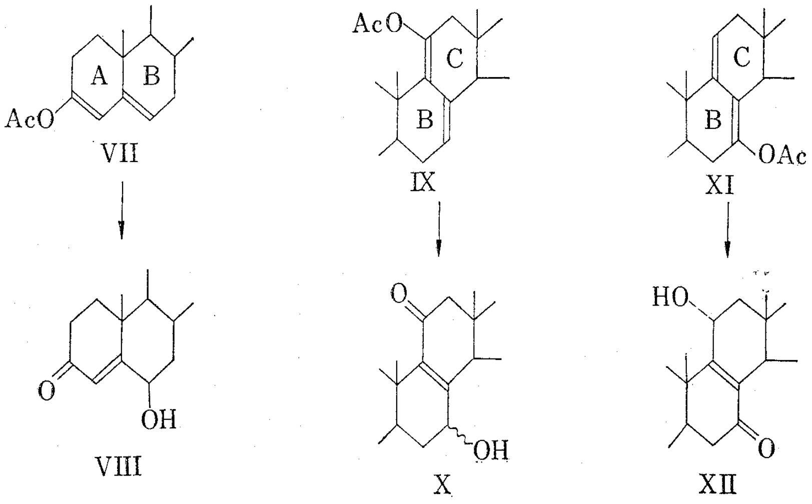

Chart 2

In some cases, the reaction of an enol acetate of an α,β–unsaturated ketone with peracid affords γ–hydroxy–α,β–unsaturated ketone. It was reported in steroid series that enol acetates of 4–en–3–one[12,13] 8–en–11–one[14] and 8–en–7–one[14,15] (VII, IX and XI) were transformed into the corresponding γ–hydroxy–α,β–unsaturated ketones (VIII, X and XII) by monoperphthalic acid. The application of this reaction for 7–en–6–oxo steroid was considered to be very attractive for our purpose.

The treatment of 3β–acetoxy–5α–cholest–7–en–6–one (XVIIa) with acetic anhydride in ethyl acetate in the presence of catalytical amount of perchloric acid at room temperature[16]

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[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).
[7] J.B. Siddall, A.D. Cross, and J.H. Fried, *J. Am. Chem. Soc.*, **88**, 862 (1966).
[8] U. Kerb, P. Hocks, R. Wiechert, A. Furlenmeier, A. Fürst, A. Langemann, and G. Waldvogel, *Tetrahedron Letters*, **1966**, 1387.
[9] R. Wiechert, U. Kerb, P. Hocks, A. Furlenmeier, A. Fürst, A. Langemann, and G. Waldvogel, *Helv. Chim. Acta*, **49**, 1581 (1966).
[10] A. Furlenmeier, A. Fürst, A. Langemann, G. Waldvogel, U. Kerb, P. Hocks, and R. Wiechert, *Helv. Chim. Acta*, **49**, 1591 (1966).
[11] U. Kerb, G. Schulz, P. Hocks, R. Wiechert, A. Furlenmeier, A. Fürst, A. Langemann, and G. Waldvogel, *Helv. Chem. Acta*, **49**, 1604 (1966).
[12] J. Romo, G. Rosenkranz, C. Djerassi, and F. Sondheimer, *J. Org. Chem.*, **19**, 1509 (1954).
[13] H. Mori, *Chem. Pharm. Bull.* (Tokyo), **9**, 328 (1961).
[14] O. Mancera, L. Miramontes, G. Rosenkranz, F. Sondheimer, and C. Djerassi, *J. Am. Chem. Soc.*, **75**, 4428 (1953).
[15] L.F. Fieser and W.Y. Huang, *J. Am. Chem. Soc.*, **75**, 5356 (1953).
[16] B.E. Edwards and P.N. Rao, *J. Org. Chem.*, **31**, 324 (1966).

yielded an enol acetate in high yield. Possible structures for this enol acetate are 6,8(14)-diene (XVIII), 6,8-diene (XX) and 5,7-diene (XXI). The structure of the enol acetate was given immediately from ultraviolet absorption spectrum ($\lambda_{\max}^{\mathrm{MeOH}}$ 253 mμ, e=18,400). Structure XX and XXI could be ruled out, because characteristic absorption of such homoannular diene must be observed at higher wavelength with somewhat lower absorbance,[17,18] and the ultraviolet data can be explained reasonably by heteroannular diene structure (XVIII).

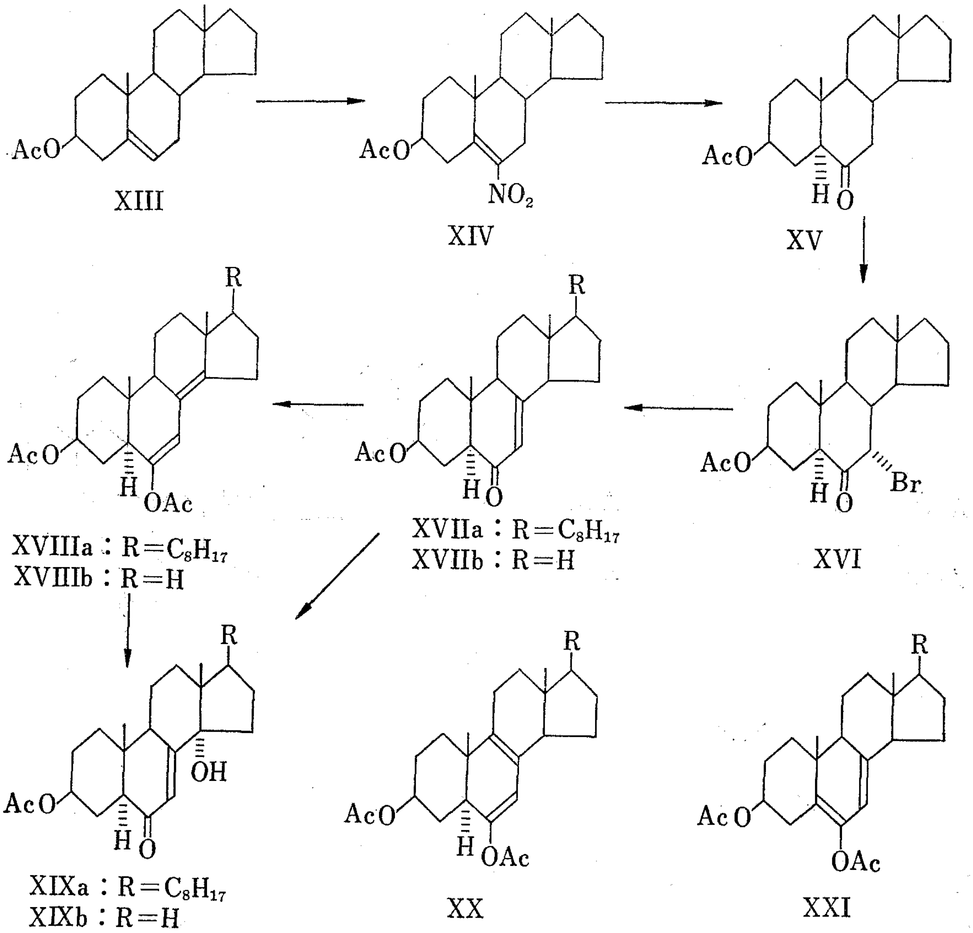

Chart 3

The enol acetate (XVIIIa) was oxidized by 1.0—1.3 equivalent volume of monoperphthalic acid in ether to afford a hydroxylated α,β-unsaturated ketone. The hydroxyl group introduced by this reaction appeared to locate at C-14, and, in fact, that this is the case and the configuration is α was shown by nuclear magnetic resonance. Methyl signals of compounds in cholestane series under consideration were considerably complex and assignment of signals was difficult only from these spectra. In order to assign signals in cholestane series with certainty, the corresponding compounds in androstane series were prepared and their nuclear magnetic resonance were measured. Nitration of 5-androsten-3β-ol acetate (XIII) with fuming nitric acid[19] in ether yielded 6-nitro compound (XIV), which was treated with zinc dust and acetic acid to give 3β-acetoxy-5α-androstan-6-one (XV). Monobromination of XV in acetic acid and ether, and successive reflux gave 7α-bromo compound (XVI), the assignment of the structure being based upon analogy with the corresponding bromination

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17) L.F. Fieser and M. Fieser, "Steroids," Reingold Publishing Co., N.Y., 1959, p. 15.
18) L. Dorfman, *Chem. Revs.*, **53**, 47 (1953).
19) C.E. Anagnostopoulos and L.F. Fieser, *J. Am. Chem. Soc.*, **76**, 532 (1954).

in cholestane series.[20] The bromo compound (XVI) was dehydrobrominated by lithium carbonate and dimethylformamide[21] to afford 7–en–6–oxo compound (XVIIb). XVIIb was converted into the enol acetate (XVIIIb) and the hydroxylated 7–en–6–oxo compound (XIXb) by the similar treatment described above. Nuclear magnetic resonance spectra of XVIIa, XVIIb, XIXa and XIXb were measured and methyl signals (18–Me and 19–Me) were assigned as shown in Table I by comparison of four spectra. The possibility that a hydroxyl group was introduced at C–5 or C–9 was readily ruled out, because 19–methyl signal must be shifted by such introductions.[22] Hence, it is concluded that a hydroxyl group is introduced at C–14. As being apparent from Table I, the introduction of a hydroxyl group at C–14 caused at downfield shift of C–18 angular methyl group. From this observation, the configuration of the hydroxyl group should be considered α–oriented.[22] Moreover, selenium dioxide oxidation of XVIIa gave the compound identical with that obtained from XVIIIa by monoperphthalic acid.

As it became apparent that the reactions proceeded as expected, this method was applied to 2β,3β–diacetoxy compounds. 2β,3β–Diacetoxy–5α–cholestan–6–one (XXII)[4] was bromi-

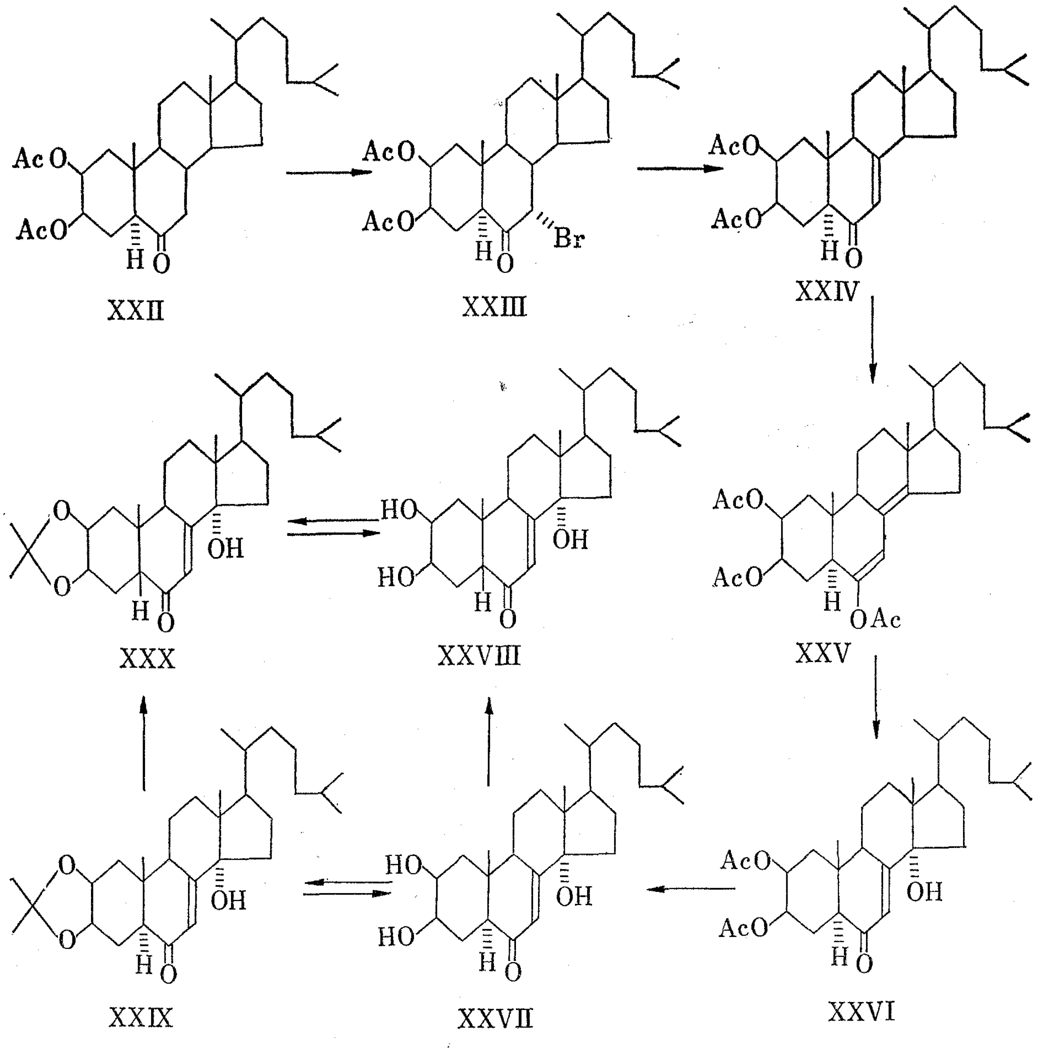

Chart 4

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20) E.J. Corey and A. Sneen, *J. Am. Chem. Soc.*, **78**, 6269 (1956).

21) M.P. Hartshorn and A.F.A. Wallis, *J. Chem. Soc.*, **1962**, 3839.

22) N.S. Bhacca, D.H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden–Day, Inc., San Francisco, 1964, p. 13.

nated by the procedure reported by Corey and Sneen[20] to give 7α–bromo compound (XXIII)[23] and without purification, dehydrobromination of the crude bromo compound (XXIII) by lithium carbonate in dimethylformamide yielded 2β,3β–diacetoxy–5α–cholest–7–en–6–one (XXIV).[23] XXIV was treated with acetic anhydride in the presence of perchloric acid by the method reported by Edwards and Rao[16] to afford an enol acetate (XXV). The assignment of the structure was made by ultraviolet, infrared, nuclear magnetic resonance spectrum and molecular rotation differences, which support A/B trans structure. Oxidation of XXV with 1.3 equivalent volume of monoperphthalic acid afforded 14α–hydroxy compound (XXVI) with 40—50% yield. *m*–Chloroperbenzoic acid could be also used as oxidation reagent instead of monoperphthalic acid with satisfactory result, yield being somewhat higher. It is of interest to point out that triplet signals were observed for 7–proton resonances in 7–en–6–oxo compounds (XVIIa, XVIIb and XXIV), while doublet signals in 14–substituted compounds (XVIIIa, XVIIIb, XIXa, XIXb, XXV and XXVI) (Table I). Thus, proton at C–7 clearly couples with proton at C–14 and also proton at C–5 or C–9. That long–range coupling between protons at C–7 and C–9 is the case was shown by Nakanishi and his co-workers.[24]

TABLE I. Nuclear Magnetic Resonance Spectral Data[a] of 7–En–6–oxo Steroids and Derivatives

Compound	18–CH_3	19–CH_3	7–H
XVIIb	0.65	0.89	5.73(t), *J*2.5
XVIIIb	0.98	0.77	5.93(d), *J*2.5
XIXb	0.74	0.89	5.93(d), *J*2.5
XVIIa	0.61	0.89	5.75(t), *J*2.5
XVIIIa	0.94	0.76	5.87(d), *J*2.5
XIXa	0.68	0.89	5.89(d), *J*2.5
XXIV	0.59	1.01	5.74(t), *J*2.5
XXV	0.92	0.82	5.91(d), *J*2.5
XXVI	0.67	1.00	5.90(d), *J*2.5

a) The spectra were obtained at 60 Mcps on Hitachi H–60 spectrometer in $CDCl_3$ containing tetramethylsilane as an internal standard. Chemical shifts are quoted as ppm downfield from (0.00 ppm). Coupling constants are given in cps. Abbreviations used are d=doublet and t=triplet.

Hydrolysis of XXVI with dilute sodium hydroxide in dioxane at room temperature afforded 2β,3β,14α–trihydroxy–7–en–6–one (XXVII). In the second paper of this series we reported that the ratios of 5β– and 5α–compound in equilibrium mixtures of 2β,3β–dihydroxycholestan–6–ones and their acetonides were found to be 2:3 and 0:1, respectively. 1,3–Diaxial interaction between 2β–hydroxyl and methyl group in 5α–compound must be considered as an instability factor of 5α–compound, and, accordingly, ratio of isomers described above is very different from that observed in general 6–oxo steroids.[25–27] From similar point of view, the isomerization experiments in 2β,3β,14α–trihydroxy–7–en–6–ones and their derivatives are particularly interesting. Treatment of XXVII with 0.33% potassium carbonate in 90% methanol at reflux temperature led to an equilibrium mixture, from which pure 5β–isomer (XXVIII) was isolated by preparative thin–layer chromatography. Analysis of the equilibrium mixture showed that the ratio of 5β– and 5α–compound is 4.5:1. The same equilibrium

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23) XXIII and XXIV were prepared by the same procedure by Furlenmeier, *et al.*[10], and unambiguous proof for structures of these compounds were given therein.
24) K. Nakanishi, M. Koreeda, S. Sasaki, M.L. Chang, and H.Y. Hsu, *Chem. Commun.*, **1966**, 915.
25) R.B. Turner, *J. Am. Chem. Soc.*, **74**, 5362 (1952).
26) H.B. Henbest and T.I. Wrigley, *J. Chem. Soc.*, **1957**, 4569.
27) J.F. Biellmann and W.S. Johnson, *Bull. Soc. Chim. France*, **1965**, 3500.

mixture was also obtained from 5β-isomer by the same treatment. Treatment of 5α- and 5β-compound (XXVII and XXVIII) with acetone and acid led to the corresponding acetonides (XXIX and XXX), which on hydrolysis recovered the corresponding starting materials. The ratio of 5-isomers in acetonide series was found to be 2:1 (5β:5α). It is of interest that ratio of 5β-isomer is larger than that observed in saturated compounds.

Experimental(28)

3β-Acetoxy-6-nitro-5-androstene (XIV)——Fuming nitric acid (120 ml) was added dropwise to a solution of 3β-acetoxy-5-androstene (XIII, 20 g) in anhydrous ether (240 ml) at 5—10° over a period of 1 hr, and the solution was stirred for another 1 hr. Ice-cold 5% NaOH was added, the product was extracted with ether, and the organic layer was washed with 4% NaOH and water, and dried (Na_2SO_4). The solvent was removed *in vacuo* at room temperature to give crystalline material. Recrystallization from MeOH-CH_2Cl_2 afforded 6-nitro compound (XIV, 14.65 g), mp 156—158°. An analytical sample was obtained by further recrystallization from the same solvent as colorless needles. mp 157—158.5°, $[\alpha]_D^{19}$ −125° (c=0.92), UV λ_{max} (ε) 261 mμ (1770). *Anal.* Calcd. for $C_{21}H_{31}O_4N$: C, 69.80; H, 8.40. Found; C, 69.77; H, 8.65.

3β-Acetoxy-5α-androstan-6-one (XV)——To a solution of 3β-acetoxy-6-nitro-5-androstene (XIV, 1.0 g) in AcOH (45 ml) and water (5 ml) was added Zn dust (4.0 g) in small portions, and the suspension was refluxed for 3.5 hr. Precipitates were removed by filtration, ice-cold water was added and the product was extracted with ether. The ether layer was washed with 5% Na_2CO_3 and water, and dried (Na_2SO_4). The removal of the solvent by distillation afforded an oily substance, which on recrystallization from MeOH gave 6-oxo compound (XV, 0.55 g), mp 112—115°. An analytical sample was obtained by further recrystallization from MeOH as colorless needles. mp 115—117°, $[\alpha]_D^{19}$ −52° (c=1.13). *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 76.14; H, 9.73. Found: C, 75.86; H, 9.70.

3β-Acetoxy-7α-bromo-5α-androstan-6-one (XVI)——To a solution of 3β-acetoxy-5α-androstan-6-one (XV, 8.5 g) in anhydrous ether (109 ml) and AcOH (22 ml) was added dropwise a solution of bromine (1.35 ml, 1.03 equivalent) in AcOH (36 ml), and the solution was refluxed for 22 hr. After removal of most of ether at reduced pressure, water was added, and the product was extracted with ether. The ether solution was washed with 5% $NaHCO_3$ and water, and dried (Na_2SO_4). The removal of the solvent by distillation afforded crystalline material, which was recrystallized from MeOH to give 7α-bromo compound (XVI, 6.9 g), mp 158—161°. Further recrystallization from MeOH gave an analytical sample as colorless needles. mp 160.5—162.5°, $[\alpha]_D^{19}$ +41° (c=1.28). *Anal.* Calcd. for $C_{21}H_{31}O_3Br$: C, 61.16; H, 7.36. Found: C, 61.26: H, 7.60.

3β-Acetoxy-5α-androst-7-en-6-one (XVIIb)——A suspension of 3β-acetoxy-7α-bromo-5α-androstan-6-one (XVI, 5.9 g) and Li_2CO_3 (21.6 g) in N,N-dimethylformamide (300 ml) was refluxed for 5 hr in N_2 atmosphere. Li_2CO_3 was removed by filtration, ice-water was added, and the product was extracted with ether. The ether solution was washed with 10% HCl, 5% Na_2CO_3 and water, and dried (Na_2SO_4). The removal of the solvent by distillation afforded crystalline material, which on recrystallization from MeOH gave 7-en-6-oxo compound (XVIIb, 2.3 g), mp 158—161°. Further 7-en-6-oxo compound (XVIIb, 0.56 g) was obtained by recrystallization of mother liquor from *n*-hexane. An analytical sample was obtained by further recrystallization from *n*-hexane as colorless plates. mp 160.5—162.5°, $[\alpha]_D^{23}$ −44° (c=1.25), UV λ_{max} (ε) 244 mμ (13200). *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.23; H, 8.97.

3β,6-Diacetoxy-5α-cholesta-6,8(14)-diene (XVIIIa)——3β-Acetoxy-5α-cholest-7-en-6-one (XVIIa, 2.0 g) was dissolved in 0.001 M solution of $HClO_4$ in EtOAc (180 ml) and Ac_2O (20 ml), and the solution was allowed to stand for 30 min at room temperature with stirring. Ether was added, and the solution was washed with 5% $NaHCO_3$ and water. After drying (Na_2SO_4), the solvent was removed by distillation to give oily substance, which on recrystallization from acetone MeOH afforded enol acetate (XVIIIa, 1.75 g), mp 90—93°. Further recrystallization from the same solvent gave an analytical sample as colorless prisms. mp 91—93°, $[\alpha]_D^{18}$ −32° (c=1.58). UV λ_{max} (ε) 254 mμ (18400). *Anal.* Calcd. for $C_{31}H_{48}O_4$: C, 76.81; H, 9.98. Found: C, 77.10; H, 10.25.

3β-Acetoxy-14α-hydroxy-5α-cholest-7-en-6-one (XIXa)——a) **From XVIIIa by Monoperphthalic Acid**: To a solution of 3β,6-diacetoxy-5α-cholesta-6,8(14)-diene (XVIIIa, 1.75 g) in ether (200 ml) was added monoperphthalic acid in ether (0.0744 g/ml, 10 ml, 1.13 equivalent), and the solution was allowed to stand at dark place at 25° for 38 hr. The solution was washed with 4% NaOH and water, and dried (Na_2SO_4). The evaporation of the solvent left crystalline material, which was chromatographed on Florisil (30 g). Elution with benzene-EtOAc (19:1) afforded 14α-hydroxy compound (XIXa, 0.83 g), which on recrystallization form MeOH-acetone afforded an analytical sample was colorless prisms. mp 184—186°, $[\alpha]_{18}^{D}$ +35° (c=1.21), UV λ_{max} (ε) 242 mμ (11900). *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.88; H, 10.05.

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28) All melting points were uncorrected. Optical rotations were measured in chloroform, ultraviolet spectra in ethanol unless otherwise stated.

b) **From XVIIa by SeO$_2$**: A suspension of 3β–acetoxy–5α–cholest–7–en–6–one (XVIIa, 0.4 g) and SeO$_2$ (0.8 g) in dioxane (30 ml) was stirred at 80° for 1 hr in N$_2$ atmosphere. After cooling, precipitates were removed by filtration, and the filtrate was poured into water. Product was extracted with ether and the ether layer was washed with water and dried (Na$_2$SO$_4$). Removal of the solvent gave crystalline material which was recrystallized from acetone to give 14α–hydroxy compound (XIXa, 0.2 g), mp 179—183°. Repeated recrystallization from acetone afforded pure compound, which was identical with the sample obtained above in all respects.

3β,6-Diacetoxy-5α-androsta-6,8(14)-diene (XVIIIb)——3β–Acetoxy–5α–androst–7–en–6–one (XVIIb, 1.5 g) was dissolved in 0.001 M solution of HClO$_4$ in EtOAc (135 ml) and Ac$_2$O (15 ml), and the solution was allowed to stand for 20 min ether was added, and organic layer was washed with 5% NaHCO$_3$ and water, and dried (Na$_2$SO$_4$). The removal of the solvent afforded yellow gum, which was chromatographed on Florisil. Colorless oily substance eluted by *n*–hexane–EtOAc (19:1) was used in the following reaction. An analytical sample was obtained by recrystallization from MeOH as colorless needles. mp 130—132°, $[\alpha]_D^{23}$ $-32°$ (c=1.24), UV λ_{max} (ε) 253 mμ (19500). *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.54; H, 8.40. Found: C, 74.16; H, 8.66.

3β-Acetoxy-14α-hydroxy-5α-androst-7-en-6-one (XIXb)——The crude enol acetate obtained above (1.49 g) was dissolved in ether (200 ml) and 1.1 equivalent volume of monoperphthalic solution acid in ether was added. After standing at 25° in dark place for 48 hr., the solution was washed with 4% NaOH and water, and dried (Na$_2$SO$_4$). The removal of the solvent afforded oily substance, which was chromatographed on Florisil. The starting material was recovered by elution with benzene. The substance eluted by benzene–EtOAc (19:1) was recrystallized from acetone–*n*–hexane to give 14α–hydroxy compound (XIXb, 0.35 g), mp 168—171°. Further recrystallization gave an analytical sample as colorless needles. mp 174—176°, $[\alpha]_D^{23}$ $+15°$ (c=1.01), UV λ_{max} (ε) 241.5 mμ (11600). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.52; H, 8.52. Found: C, 72.80; H, 8.73.

2β,3β-Diacetoxy-7α-bromo-5α-cholestan-6-one (XXIII)——To a solution of 2β,3β–diacetoxy–5α–cholestan–6–one (XXII, 10 g) in AcOH (200 ml) was added dropwise a solution of Br$_2$ (1.024 ml) in AcOH (40 ml) at 35° over a period of 30 min, and the solution was stirred at 50° for 2 hr. Water was added, and the product was washed with 5% Na$_2$CO$_3$ and water, and dried (Na$_2$SO$_4$). The solvent was removed by evaporation to give yellow oily substance (10.0 g), which was submitted to the following reaction without purification.

2β,3β-Diacetoxy-5α-cholest-7-en-6-one (XXIV)——A suspension of crude 7α–bromo compound (10.0 g) obtained above and Li$_2$CO$_3$ (5.0 g) in N,N–dimethylformamide (100 ml) was refluxed for 3 hr. After Li$_2$CO$_3$ was removed by filtration, water was added and the product was extracted with ether. The ether layer was washed with 10% HCl, 5% Na$_2$CO$_3$ and water, and dried (Na$_2$SO$_4$). The solvent was removed by evaporation *in vacuo* to give crystalline substance, which on recrystallization from MeOH afforded 7–en–6–oxo compound (XXIV, 4.3 g). An analytical sample was obtained by further recrystallization from MeOH as colorless needles. mp 208—210°, $[\alpha]_D^{18}$ $+30°$ (c=1.98), UV λ_{max} (ε) 245 mμ (14400). *Anal.* Calcd. for $C_{31}H_{48}O_5$: C, 74.36; H, 9.66. Found: C, 74.65; H, 9.53.

2β,3β,6-Triacetoxy-5α-cholesta-6,8(14)-diene (XXV)——2β,3β–Diacetoxy–5α–cholest–7–en–6–one (XXIV, 6.4 g) was dissolved in 0.001 M solution of HClO$_4$ in EtOAc (575 ml) and Ac$_2$O (64 ml), and the solution was allowed to stand at room temperature for 30 min. Water was added, and the product was extracted with ether. The ether layer was washed with 5% Na$_2$CO$_3$ and water, and dried (Na$_2$SO$_4$). The removal of the solvent by distillation left oily substance (7.6 g), which was used to the next reaction without purification. An analytical sample was obtained by chromatographed on Florisil (benzene–EtOAc 19:1) followed by recrystallization from MeOH as colorless needles. mp 76—78°, $[\alpha]_D^{18}$ $-14°$ (c=1.99), UV λ_{max} (ε) 254 mμ (20100). *Anal.* Calcd. for $C_{33}H_{50}O_6$: C, 73.03; H, 9.29. Found: C, 73.14; H, 9.22.

2β,3β-Diacetoxy-14α-hydroxy-5α-cholest-7-en-6-one (XXVI)——a) **By Monoperphthalic Acid**: The crude enol acetate obtained above (7.6 g) was dissolved in a solution of 1.3 equivalent volume of monoperphthalic acid solution in ether (600 ml), and the solution was allowed to stand at 25° overnight at dark place, and then washed with 4% NaOH and water. After drying (Na$_2$SO$_4$), the solvent was removed by distillation to give crystalline material, which was washed with *n*–hexane and recrystallized from MeOH to give 14α–hydroxy compound (XXVI, 2.8 g), mp 221—224°. An analytical sample was obtained by further recrystallization from MeOH as colorless needles. mp 224—226°, $[\alpha]_D^{16}$ $+75°$ (c=0.87), UV λ_{max} (ε) 242 mμ (12800). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.04: H, 9.33. Found: C, 72.06; H, 9.36.

b) **By *m*-Chloroperbenzoic Acid**: The crude enol acetate (XXV, 0.85 g) and 79.5% *m*–chloroperbenzoic acid (0.35 g) was dissolved in anhydrous ether (65 ml) and the solution was allowed to stand at 25° and dark place overnight. The similar treatment with the preceding experiment gave colorless needles (XXVI, 0.418 g), mp 223—225°, identical with the sample obtained above in all respects.

2β,3β,14α-Trihydroxy-5α-cholest-7-en-6-one (XXVII)——a) **By Hydrolysis of XXVI**: A solution of 2β,3β–diacetoxy–14α–hydroxy–5α–cholest–7–en–6–one (XXVI, 0.597 g) in dioxane (36 ml) and 0.6 N KOH (12 ml) was allowed to stand at room temperature for 50 min. After addition of water, product was extracted with *n*–BuOH and the organic layer was washed with water. The solvent was removed *in vacuo* to leave crystalline material, which on recrystallization from MeOH afforded XXVII (0.304 g), mp 240—245°. An analytical sample was obtained by further recrystallization from MeOH as colorless needles. mp 249—252°, $[\alpha]_D^{16}$ $+63°$

(c=0.473, dioxane), UV $\lambda_{\max}$ (ε) 241 mμ (11000). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.83; H, 10.03.

b) **By Hydrolysis of XXIX**: A solution of 2β,3β,14α-trihydroxy-5α-cholest-7-en-6-one 2,3-acetonide (XXIX, 15 mg) in 0.1 N HCl in 90% tetrahydrofuran (0.5 ml) was allowed to stand at room temperature for 5 hr. After addition of water, product was extracted with BuOH and the organic layer was washed with 5% $NaHCO_3$ and water. Evaporation of the solvent *in vacuo* gave crystalline material, the infrared spectrum of which was identical with the sample obtained above.

2β,3β,14α-Trihydroxy-5β-cholest-7-en-6-one (XXVIII)——a) **By Isomerization of XXVII**: A solution of 2β,3β,14α-trihydroxy-5α-cholest-7-en-6-one (XXVII, 0.24 g) in 0.33% K_2CO_3 in 90% MeOH was refluxed for 40 min, and poured into water. The product was extracted with *n*-BuOH and the organic layer was washed with water. The solvent was removed by distillation *in vacuo* to yield oily substance, which was submitted to preparative thin-layer chromatography on silica gel Merck GF_{254} (0.5 mm plates. developing system CH_2Cl_2—acetone—EtOH (80:20:5). The fraction of low *Rf* value was recrystallized from acetone—hexane to afford 5β-isomer (XXVIII, 0.108 g), mp 190—195°. An analytical sample was obtained by further recrystallization from MeOH as colorless prisms. mp 194—198°, $[\alpha]_D^{16}$ +78° (c=0.98), UV $\lambda_{\max}$ (ε) 244 mμ (13600). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.93; H, 10.25. Found: C, 74.86; H, 10.44.

From the fraction of high *Rf* value was isolated 5α-isomer (XXVII, 28 mg). Analysis by ultraviolet spectra showed that the equilibrium mixture consisted of 5β- and 5α-isomer at a ratio of 4.5:1. The equilibrium mixture obtained by the similar treatment of pure 5β-isomer (XXVIII) was found to have the same composition.

b) **By Hydrolysis of XXVI accompanied by Isomerization**: A solution of 2β,3β-diacetoxy-14α-hydroxy-5α-cholest-7-en-6-one (XXVI, 0.30 g) in 0.6% K_2CO_3 in 90% MeOH (60 ml) was stirred at room temperature, and then refluxed for 30 min. Water was added and the product was extracted with BuOH. After washing with water, the solvent was removed by distillation *in vacuo* to afford oily substance. Worked up as the above experiment, 5β-isomer (XXVIII, 0.119 g) was obtained by preparative thin-layer chromatography.

c) **By Hydrolysis of XXX**: A solution of 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one 2,3-acetonide (XXX, 15 mg) in 0.1 N HCl in 90% tetrahydrofuran (0.5 ml) was allowed to stand at room temperature for 4 hr, and poured into water. The product was extracted with BuOH and the organic layer was washed with water. Evaporation of the solvent *in vacuo* afforded 5β-isomer (XXVIII), the infrared spectrum of which was identical with that of the sample obtained above.

2β,3β,14α-Trihydroxy-5α-cholest-7-en-6-one 2,3-Acetonide (XXIX)——A suspension of 2β,3β,14α-trihydroxy-5α-cholest-7-en-6-one (XXVII, 0.29 g) in acetone (23 ml) and 5% phosphomolybdic acid in acetone (4.7 ml) was stirred at room temperature (Crystals were soon dissolved.) for 20 min. After addition of 30% NH_4OH and water, product was extracted with ether-CH_2Cl_2, and the organic layer was washed with 5% Na_2CO_3 and water, and dried (Na_2SO_4). After evaporation of the solvent, the residue was recrystallized from acetone hexane to give acetonide (XXIX, 0.177 g) as colorless leaflets. mp 246—249°, $[\alpha]_D^{16}$ +67° (c=2.27), UV $\lambda_{\max}$ (ε) 242 mμ (12700). *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.17; H, 10.30.

2β,3β,14α-Trihydroxy-5β-cholest-7-en-6-one 2,3-Acetonide (XXX)——A solution of 2β,3β,14α-trihydroxy-5β-cholest-7-en-6-one (XXVIII, 0.142 g) in acetone (11.4 ml) and 5% phosphomolybdic acid in acetone (2.2 ml) was stirred at room temperature for 20 min. After addition of 30% NH_4OH and water, product was extracted with ether-CH_2Cl_2 and the organic layer was washed with 5% Na_2CO_3 and water, and dried (Na_2SO_4). Evaporation of the solvent afforded crystalline material, which on recrystallization from acetone—hexane gave acetonide (XXX, 0.070 g) as colorless needles. mp 182—184.5°, $[\alpha]_D^{16}$ +50° (c=0.99), UV $\lambda_{\max}$(ε) 243 mμ (12600). *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.83; H, 10.00.

Equilibrating Experiment of Acetonides XXIX and XXX——The acetonide (XXIX or XXX, 5.0 mg) was dissolved in 0.33% K_2CO_3 in 90% MeOH (1.5 ml) and the solution was refluxed for 1.5 hr. Water was added, and product was extracted with ether-CH_2Cl_2. After washing with water and drying (Na_2SO_4), the solvent was evaporated to afford crystalline residue, which was submitted to preparative thin-layer chromatography on silica gel Merck GF_{254} (0.5 mm, developing system: $CHCl_3$—acetone (4:1)). Fraction of 5α-isomer (XXIX, low *Rf* value) and 5β-isomer (XXX, high *Rf* value) were analyzed by ultraviolet absorption spectra. Ratio of 5β- and 5α- compound was found to be 2:1 in both experiments (from XXIX, and XXX).

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