(50 ml.). After standing overnight the solution deposited crystals (0.52 g.), which were filtered off and recrystallized several times from toluene as slightly yellow rods, m.p. 264~265° (decomp.), UV $\lambda_{max}^{CHCl_3}$ 32 mμ (log ε 4.41) (an inflection : 341 mμ (log ε 4.22)). *Anal.* Calcd. for $C_{23}H_{15}O_2N_3S$: C, 69.50; H, 3.80; N, 10.57; S, 8.07. Found : C, 69.46; H, 3.78; N, 10.29; S, 8.28. Major IR absorptions (Nujol) were at 1727, 1645, 1597, 1566, 1493, 1326, 1309, 1250, 1072, 890, 829, and 683 cm^{-1}.

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Tokuo Kubota, Keiji Yoshida, and Fumihiko Watanabe : The Configuration of the Products obtained from Hydroxylation of Steroidal $\Delta^{1,4}$-3-Ketones with Osmium Tetroxide.

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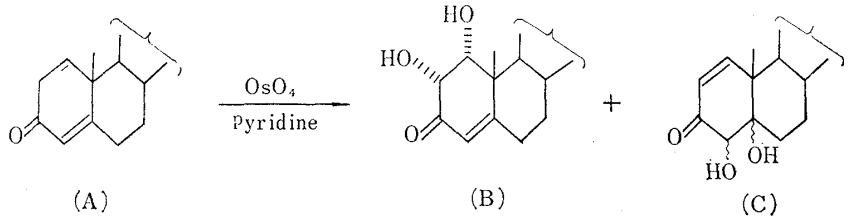
Previously, in connection with thes ynthesis of A-norsteroid derivatives,[1] several steroidal $\Delta^{1,4}$-3-ketones (A) have been hydroxylated with osmium tetroxide in pyridine. Each of the compounds afforded the corresponding two isomeric products, 1,2-dihydroxy-Δ^4-3-one (B) and 4,5-dihydroxy-Δ^1-3-one (C), for which the α-configuration of the introduced hydroxyl groups had been assigned.

Out of these assignment, the 1α,2α-dihydroxyl configuration in the former isomers (B) was firmly settled by the optical rotatory dispersions (ORD), which have been studied thoroughly by Kuriyama, *et al.*,[2] and by the chemical correlation of the derivative from 25D-spirosta-1,4-dien-3-one with the previously known epimer, 1β,2β-dihydroxy-25D-spirost-4-en-3-one.[3]



(A) (B) (C)

Chart 1.

In analogy, the hydroxy groups of the 4,5-dihydroxy-Δ^1-3-ones (C) had been assigned as α-configuration, based on the assumption that bulky osmium tetroxide should approach the double bond from the less hindered α-side. Although this was supported by the ORD curves showing negative Cotton effect which resembles to that of cholest-1-en-3-one, no example sufficient for discussion was existed and some doubt had been remained on the assign of α-configuration in (C).

For the purpose of preparing 4α,5α,17β-trihydroxyandrostan-3-one in the subject of other investigation, the 4,5-dihydroxy derivative (Id) prepared previously from osmylation of 17β-hydroxyandrosta-1,4-dien-3-one, was subjected to catalytic hydrogenation on palladium charcoal. There was obtained a dihydro product, m.p. 164~166°,

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1) T. Kubota, K. Yoshida, F. Hayashi, K. Takeda : This Bulletin, **13**, 50 (1965).
2) K. Kuriyama, E. Kondo, K. Tori : Tetrahedron Letters, **1963**, 1485.
3) T. Kubota, K. Takeda : Tetrahedron, **10**, 1 (1960).

$[\alpha]_D$ +31.4°, which on examination of ORD exhibited unexpectedly a negative Cotton curve suggesting to be the 3-ketone with 5β-configuration (IId).

With this surprising result differing from the 1,2-dihydroxylated derivatives (B), other 4,5-dihydroxy derivatives, Ia, Ib-acetate, and Ic, which have previously been prepared,[1] were hydrogenated in the same way as above. All of the hydrogenated products showed negative Cotton curves.

Since it is well known that ORD of saturated ketones depends on the octant rule but the sign was affected by neither hydroxyl vicinal to ketone nor hydroxyl on ring juncture,[4] it is almost certain that these hydrogenated products, IIa, IIb-acetate, IIc, and IId, have the 5β-configuration. Accordingly, the precursors (C) should be the 4β, 5β-dihydroxy-Δ^1-3-ones in view of *cis*-hydroxylation with osmium tetroxide.

However, one of the hydrogenated products, 4β,5β-dihydroxycholestan-3-one 4-monoacetate (IIb-acetate), m.p. 197~199°, $[\alpha]_D$ +55.2°, has already been known and its m.p. is somewhat distant from that previously described,[5] m.p. 185~186°, $[\alpha]_D$ +52.5°

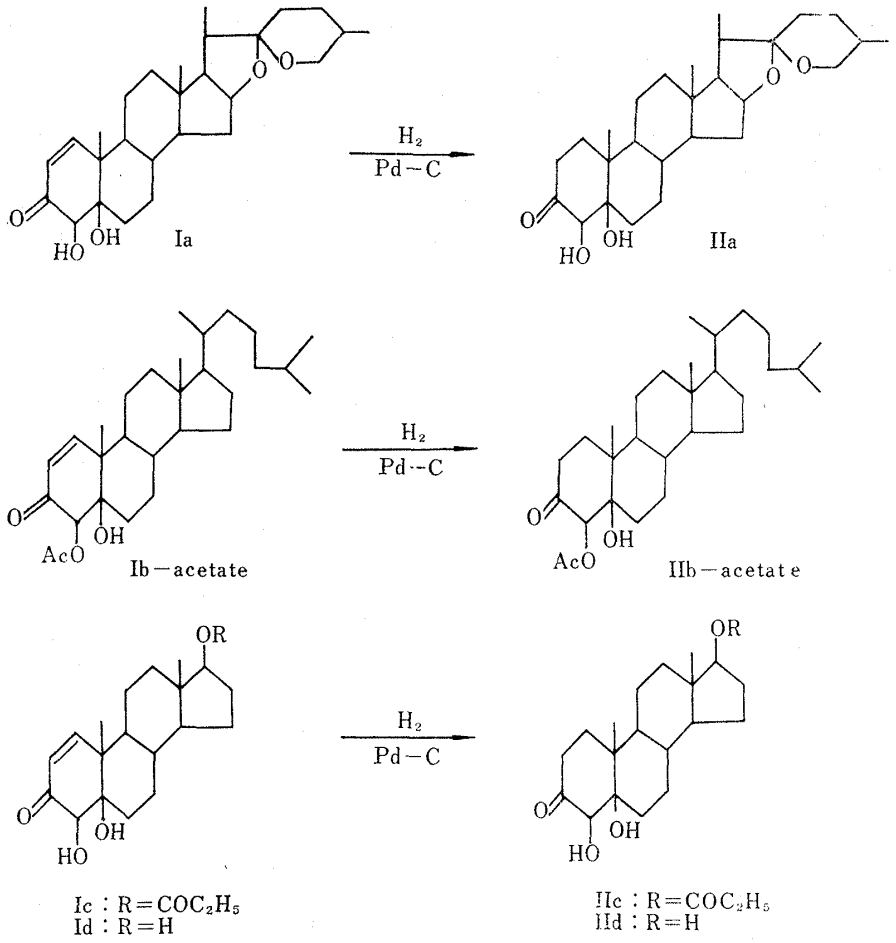

Chart 2.

(the α-isomer, m.p. 223~225°, $[\alpha]_D$ +22.4°*[2]). In order to eliminate any doubt on the assign of β-configuration and on the purity of the products, preparation of the pairs of the 4β,5β- and 4α,5α-dihydroxy-3-ones was undertaken on the cholestane and androstane series.

*[2] Dr. Eastham kindly informed, that the sign of $[\alpha]_D$ in the literature[5] should read +22.4° for −22.4°, to our inquiry.

4) C. Djerassi: "Optical Rotatory Dispersion," 53, 111 (1960), McGraw-Hill Book Co., Inc., New York.

5) J. F. Eastham, G. B. Miles, C. A. Kranth: J. Am. Chem. Soc., **81**, 3114 (1959).

Cholest-4-en-3-one was hydroxylated with hydrogen peroxide and osmium tetroxide according to the procedure of Eastham, *et al.*[5] Fractional recrystallization of the crude product afforded two isomers, m.p. 207~208° and m.p. 116~117°, in good agreement with those reported, m.p. 206~208° and 112~112.5°. The former isomer, which have been formulated as 4α,5α-dihydroxy-3-one (Ⅳb), on acetylation yielded Ⅳb-acetate, m.p. 227~229°, $[\alpha]_D$ +20.5°. The acetate on the ORD determination showed a positive Cotton curve reversed to that of the hydrogenated product from the 4,5-dihydroxy-Δ^1-3-one (Ib-acetate). On the other hand, the β-isomer, m.p. 116~117°, on acetylation gave the acetate, which on examination by thin-layer chromatography (TLC) was found to accompany a weak spot corresponding to that of the α-isomer (Ⅳb-acetate). Purification by preparative TLC yielded the pure Ⅱb-acetate, m.p. 197~199°, which was identical with the specimen obtained from hydrogenation of Ib-acetate in all respects.

Testosterone propionate (Ⅲc), after treatment by the same procedure as above, yielded in pure state only an isomer, m.p. 199~200°, $[\alpha]_D$ +17.8, different from Ⅱc. The product exhibited a positive Cotton curve reversed to that of Ⅱc and was recognized as the α-isomer (Ⅳc). Attempts to resolve the β-isomer (Ⅱc) from the mother liquor were unsuccessful. Treatment of testosterone propionate (Ⅲc) with osmium tetroxide in pyridine resulted in the exclusive formation of another isomer, m.p. 171~173°, which was identical with the product from hydrogenation of 4,5-dihydroxy-Δ^1-3-one (Ic), in all respects.

The above results have now confirmed that the hydroxy groups in 4,5-dihydroxy-Δ^1-3-ones (C), prepared by hydroxylation of $\Delta^{1,4}$-dien-3-ones (A) with osmium tetroxide

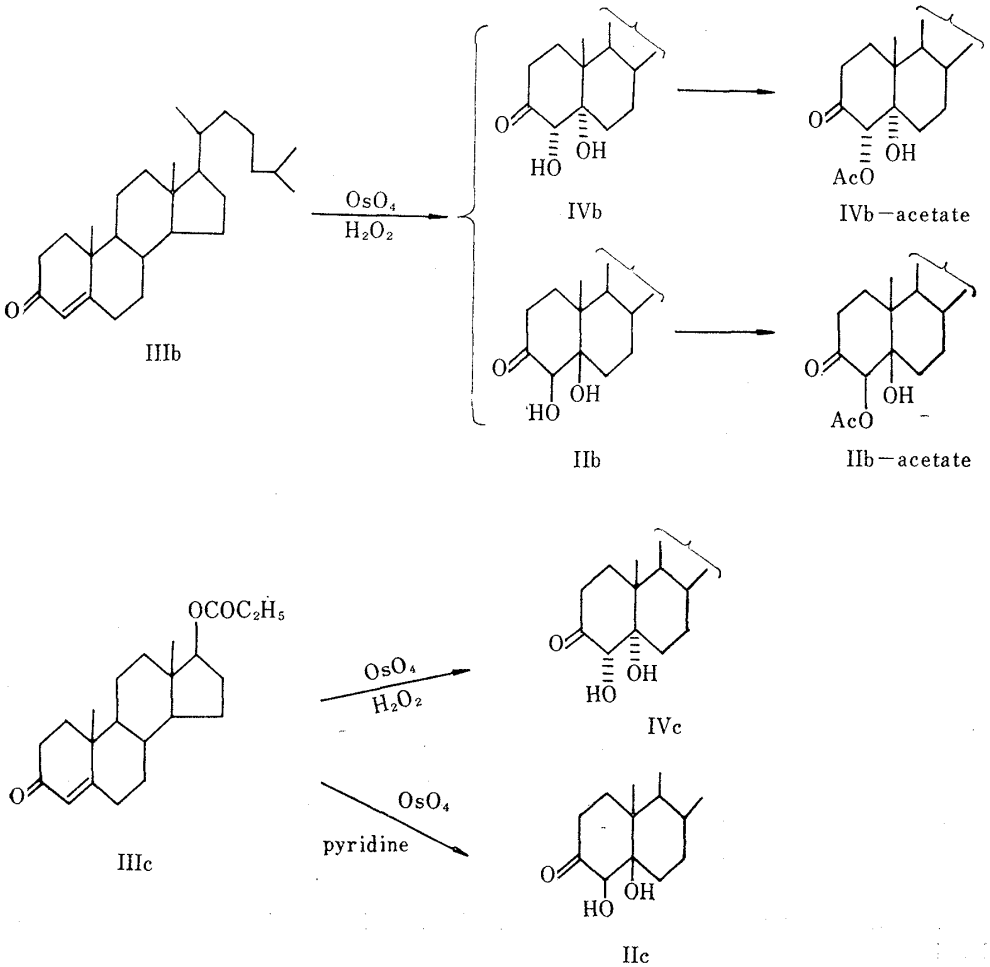

Chart 3.

in pyridine, are oriented to β-configuration. Therefore, the formulae of the 4α,5α-hydroxyl groups described for seven analogues of 4,5-dihydroxy-Δ^1-3-ones (C) (Ⅶa～g with the numbering used in the previous paper[1]) should now be corrected to the 4β, 5β-dihydroxy-Δ^1-3-ones. It is of interest that, in a conjugated system, $\Delta^{1,4}$-3-keto steroids, addition of osmium tetroxide to the Δ^1-double bond occurred with approach from the α-side of the molecule whereas attack from the β-side resulted in the addition to the Δ^4-double bond.

With the above results, the circular dichroisms (CD) of 4β,5β-dihydroxycholest-1-en-3-one and its 4-acetate were measured.*3 The former free diol showed, in some solvents, a broad negative CD, which gave no indication for the C_5-configurations. The 4-acetate (Ib-acetate) on determination in dioxane exhibited a negative CD with two bands at 334.5 and 323.5 mμ. These bands are in good agreement with the bands, 334 and 322 mμ, described[6] for 5β-steroid Δ^1-3-ones but located at shorter wavelengthes about 8 mμ than those (343 and 330 mμ) for 5α-steroid Δ^1-3-ones.

Experimental

All melting points were uncorrected. Optical rotations were measured in $CHCl_3$ solutions at ca. 25°. ORD curves were determined in MeOH solutions. IR spectra were recorded in Nujol mull.

4β,5β-Dihydroxy-25D-spirostan-3-one (IIa)——The previously obtained Ia, m.p. 241～245°, $[\alpha]_D$ −15°,[1] (65 mg.) in AcOEt (7 ml.) was shaken with 5% Pd-C (65 mg.) in H_2. After removals of the catalyst and solvent, the residue was recrystallized from MeOH giving needles (40 mg.) of IIa, m.p. 222～224°, $[\alpha]_D$ −44.8° (c=1.05). ORD (c=0.05518): $[\varphi]_{298.5}$ −2286°, $[\varphi]_{258}$ +1534°. IR ν_{max} cm^{-1}: 3489, 3439, 1715. *Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.57; H, 9.51.

4β,5β-Dihydroxycholestan-3-one 4-Acetate (IIb-acetate)——The previously obtained Ib-acetate, m.p. 224～226° (decomp.), $[\alpha]_D$ +83°,[1] (100 mg.) in AcOEt (10 ml.) was shaken with 5% Pd-C (100 mg.) in H_2. After removals of the catalyst and solvent, the residue was recrystallized from EtOH affording IIb-acetate (78 mg.) as needles, m.p. 197～199°. Further recrystallization from EtOH did not alter the m.p. $[\alpha]_D$ +55.2° (c=0.98). ORD (c=0.2797): $[\varphi]_{297}$ +276°, $[\varphi]_{244}$ +2915° IR ν_{max} cm^{-1}: 3355, 1750, 1730. *Anal.* Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.42; H, 10.50.

4β,5β,17β-Trihydroxyandrostan-3-one 17-Propionate (IIc)——The previously obtained Ic, m.p. 168～170°, $[\alpha]_D$ +81°,[1] (500 mg.) in MeOH (50 ml.) was shaken with 5% Pd-C (500 mg.) in H_2. After removals of the catalyst and solvent, the residue was recrystallized from aq. MeOH giving plates (328 mg.) of IIc, m.p. 170～172°. Concentration of the mother liquor gave an additional crop (88 mg.), m.p. 169～171°. The first crop was recrystallized from aq. MeOH yielding an analytical sample, m.p. 171～173°, $[\alpha]_D$ +28.4° (c=0.52). ORD (c=0.2824): $[\varphi]_{299}$ −1245°, $[\varphi]_{257}$ +3017°. IR ν_{max} cm^{-1}: 3447, 1724, 1721, 1208. *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81, H, 9.05. Found: C, 69.99, H, 9.02.

4β,5β,17β-Trihydroxyandrostan-3-one (IId)——The previously obtained Id, m.p. 202～203°, $[\alpha]_D$ +80°,[1] (70 mg.) in MeOH (7 ml.) was shaken with 5% Pd-C (70 mg.) in H_2 atmosphere. After filtration and evaporation of the filtrate, the residue was recrystallized from acetone-petr. ether affording plates (43 mg.), m.p. 162～165°. Further recrystallization from aq. MeOH gave pure IId, m.p. 164～166°, $[\alpha]_D$ +31.4° (c=0.56). ORD (c=0.3135): $[\varphi]_{298.5}$ −1198°, $[\varphi]_{258}$ +2865°. IR ν_{max} cm^{-1}: 3515, 3445, 1727. *Anal.* Calcd. for $C_{19}H_{30}O_4$: C, 70.77; H, 9.38. Found: C, 70.69; H, 9.55

Hydroxylation of Cholest-4-en-3-one (IIIb)——A solution of IIIb in ether was treated with OsO_4 and H_2O_2, in the manner described by Eastham, *et al.*[5] Crystallization of the crude product from EtOH afforded the first crop as needles, m.p. 204～207°, which on recrystallization from EtOH gave needles of pure 4α,5α-dihydroxychlolestan-3-one (Ⅳb), m.p. 207～208°, $[\alpha]_D$ +34.4° (c=1.08). (reported[5] m.p. 206～208°, $[\alpha]_D$ + 30.9°). ORD (c=0.05704): $[\varphi]_{298}$ +5136°, $[\varphi]_{258.5}$ −5429°. IR ν_{max} cm^{-1}: 3420, 1723. *Anal.* Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.17; H, 11.03.

The foregoing Ⅳb (70 mg.) was acetylated with Ac_2O and pyridine at room temp. The product, isolated by the usual manner, was recrystallized from EtOH giving plates (56 mg.) of the 4-acetate (Ⅳb-acetate), m.p. 227～229°, $[\alpha]_D$ +20.5° (c=1.03). (reported[5] m.p. 223～225°, $[\alpha]_D$ +22.4°*2). ORD (c=0.05954): $[\varphi]_{298}$ +3715, $[\varphi]_{260}$ −3019. IR ν_{max} cm^{-1}: 3430, 1743, 1724, 1239. *Anal.* Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.63; H, 10.56.

*3 The CD curves were determined through kind offices of Dr. G. Snatzke, Universität Bonn.

6) G. Snatzke, H. W. Fehlhaber: Tetrahedron, **20**, 1243 (1964).

The mother liquor, removed the above-mentioned first crop from the crude hydroxylation product, was diluted with AcOEt and washed with H_2O. The organic layer was dried on Na_2SO_4 and evaporated to give a resinous residue, which was chromatographed over silica gel. Elution with benzene-AcOEt (3:1) yielded crystals, which was recrystallized from EtOH and the precipitated crystals was removed by filtration. The filtrate was evaporated and the residue was recrystallized twice from MeOH yielding plates of fairly pure IIb, which sinters at 116~117° and melts at 140~148°. (reported[5] m.p. 125~126° after moisten at 112°). IR ν_{max} cm^{-1}: 3470, 1725.

The foregoing IIb (37 mg.) was acetylated with Ac_2O (1 ml.) and pryidine (1.5 ml.) at room temp. in the usual manner. The acetylated product (41 mg.) was subjected to preparative TLC developing with benzene-AcOEt (3:1) on silica gel G plates. The more mobile fraction (3 mg.), m.p. 225~228°, was identified with the above-mentioned IVb-acetate by IR spectra. The less mobile fraction (36 mg.) on recrystallization from EtOH gave needles of IIb-acetate, m.p. 194~196°. (reported[5] m.p. 185~186°). This compound was identical with a sample, obtained from hydrogenation of Ib-acetate, in all respects.

Hydroxylation of Testosterone Propionate (IIIc)——a) With OsO_4 and H_2O_2 in ether. A solution of IIIc (1.6 g.) in ether was treated with OsO_4 and H_2O_2, in the manner described in the preceding experiment. Crystallization of the crude product from MeOH gave crystals (150 mg.), m.p. 188~200°, which was recrystallized twice from MeOH yielding IVc (51 mg.) as plates, m.p. 199~200°, $[\alpha]_D$ +17.8° (c=0.99). ORD (c=0.2634): $[\varphi]_{297}$ +4598°, $[\varphi]_{257}$ −5245°. IR ν_{max} cm^{-1}: 3440, 1730, 1190. *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 70.00; H, 9.20.

The mother liquor of the first crystallization was evaporated and the resinous residue (1.7 g.) was chromatographed on silica gel. The fractions (252 mg.) eluted with $CHCl_3$, was recrystallized from aq. MeOH yielding crystals, m.p. 144~145°. Although this fraction was supposed to be a mixture of IIc and IVc, attempts to resolve into respective components were unsuccessful.

b) With OsO_4 in pyridine. A solution of IIIc (1.23 g.) and OsO_4 (1.0 g.) in pyridine (13 ml.) was allowed to stand in a dark place at room temp. for 3 days. To the reaction mixture was added petr. ether (140 ml.) and the precipitated osmate was separated by decantation, washed with petr. ether and dissolved in dioxane (70 ml.). A stream of H_2S was bubbled through the solution kept in an ice bath. The mixture was filtered and the filtrate was evaporated to give a dark crystalline residue (1.225 g.). Recrystallization from EtOH gave needles (324 mg.), m.p. 171~173°, which was identical with a sample of IIc obtained from catalytic reduction of Ic. Concentration of the mother liquor afforded a second crop (88 mg.) of IIc, m.p. 165~170°.

The authors wish to thank Dr. G. Snatzke for the CD determinations and Dr. K. Kuriyama of this laboratory for the ORD measurements.

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Tatsuzo Fujii*1 and Tomio Fujii*2 : Aggregation of High-density Lipoproteins from Egg Yolk.

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In the course of preparation of lipovitellin, a high-density lipoprotein of hen egg yolk, Joubert and Cook[1] reported the occurrence of a minor component which sedimented faster than lipovitellin upon ultracentrifugation. A similar component was also found by one of the present authors[2] in lipovitellin preparations from the eggs of frog, trout and dog-fish, having a sedimentation constant of 14~15 S as compared with 10 S of lipovitellin. Later Radomski and Wallace[3] and Wallace[4] investigated on such

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1) F. J. Joubert, W. H. Cook: Can. J. Biochem. Physiol., **36**, 389 (1958).
2) T. Fujii: Acta Embryol. Morphol. Exptl., **3**, 260 (1960).
3) M. W. Radomski, R. A. Wallace, W. H. Cook: Biochim. et Biophys. Acta, **70**, 600 (1963).
4) R. A. Wallace: *Ibid.*, **74**, 495 (1963).