9. Tokuo Kubota, Keiji Yoshida, Fumiko Hayashi, and
Ken'ichi Takeda: Studies on A-Norsteroids. I.

Synthesis of A-Nor- $\Delta^{3(5)}$ -1,2-dioxo Steroids.*¹

(Shionogi Research Laboratory, Shionogi & Co., Ltd.*²)

During the course of the structural investigation on kogagenin, 25D-spirostane-1 β ,2 β ,3 α ,5 β -tetrol, it has been found¹⁾ that the treatment of 25D-spirost-4-ene-1 β ,2 β ,3 α -triol (I) with manganese dioxide yielded A-nor-25D-spirost-3(5)-ene-1,2-dione (IIIa). The formation of this A-norsteroid has been explained to proceed through benzilic acid type rearrangement of an intermediate enetrione, which would probably arise from further oxidation of the primarily produced 1 β ,2 β -dihydroxy- Δ^4 -3-one (II).

In connection with recent findings²⁾ that some derivatives of A-norsteroids possess

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*² Fukushima-ku, Osaka (久保田徳夫, 吉田圭治, 林富美子, 武田健一).

1) T. Kubota, K. Takeda: *Tetrahedron*, **10**, 1 (1960).

2) a) L. J. Lerner, A. Bianchi, A. Borman: *Proc. Soc. Exp. Med. Biol.*, **103**, 172 (1960). b) J. Jacques, G. Pincus: *Excerpta Medica*, **51**, 3 (1962).

interesting physiological activities, it was of interest to evaluate biological activities of steroidal hormone analogs having the novel A-nor- $\Delta^{3(5)}$ -1,2-dioxo structure.

As manganese dioxide is a mild oxidizing agent, it is anticipated that this reagent gives no effect on functional groups, such as isolated carbonyl and hydroxyl groups, other than the 1,2-dihydroxy- Δ^4 -3-oxo moiety in steroids. Thus the oxidative ring contraction with manganese dioxide would probably be applicable to preparation of the modified hormone analogs. On the other hand, the 1,2-dihydroxy- Δ^4 -3-ones would certainly be converted into the A-norsteroids regardless of the configuration of hydroxyl groups at C₁ and C₂.

In order to prepare 1,2-dihydroxy- Δ^4 -3-oxo derivatives, treatment of $\Delta^1,4$ -3-oxo steroids with osmium tetroxide was chosen as the most desirable approach. The $\Delta^1,4$ -3-oxo steroids (Va~f), except for commercially available prednisolone acetate (Vg), were conveniently prepared by 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) oxidation³⁾ of the parent Δ^4 -3-ones, *i.e.* 25D-spirost-4-en-3-one (Va), cholest-4-en-3-one (Vb), testosterone propionate (Vc), progesterone (Ve) and deoxycorticosterone acetate (Vf).

Hirschmann, *et al.*^{4,5)} have carried out hydroxylation of the $\Delta^1,4$ -3-oxo group in a few steroid derivatives with osmium tetroxide and they have got various results, in which 1,2- or 4,5-diol alone, or both were obtained. In this investigation, osmium tetroxide oxidation of the $\Delta^1,4$ -3-oxo steroids (Va~g) followed essentially the procedure of Hirschmann.^{4,5)} The compounds were allowed to react with osmium tetroxide in pyridine and the osmates were decomposed with hydrogen sulfide. In every case, the crude hydroxylated products were predicted to consist of two isomeric diols, from their

Chart 2.

3) D. Burn, D. N. Kirk, V. Petrow : Proc. Chem. Soc., 1960, 14.

4) R. Hirschmann, G. A. Bailey, R. Walker, J. M. Chemerda : J. Am. Chem. Soc., 81, 2822 (1959).

5) R. Hirschmann, N. G. Steinberg, R. Walker : J. Am. Chem. Soc., 84, 1270 (1962).

thin-layer chromatographies. Separations of the mixtures, except for those from spirostadienone (Va) and cholestadienone (Vb), were performed effectively by column chromatography over silica gel to isolate the first eluted 4,5-diols (VIc~g) and the next eluted 1,2-diols (Vlc~g). The unseparable mixtures obtained from Va and Vb were subjected to an acetonide formation. Refluxing in acetone containing a catalytic amount of *p*-toluenesulfonic acid brought on the formation of only the 1,2-diol acetonides (VIIa, b), which could be isolated from the unreacted 4,5-diols (VIIa, b) by chromatography on silica gel. Hydrolysis of the acetonides with refluxing aqueous acetic acid regenerated the pure 1,2-diols (Va) and (Vb). The physical constants of the 1,2- and 4,5-diols are listed in Table I. Their ultraviolet absorption data thoroughly support the assigned structures and more definitive evidence was provided with the nuclear magnetic resonance spectra. The 1,2-dihydroxy- Δ^4 -3-one (Va) of spirostane series showed a singlet signal at 4.18 τ due to the olefinic proton at C-4, while the 4,5-dihydroxy- Δ^1 -3-one (VIIa) exhibited the C-1,2 olefinic proton signals as two doublets at 3.28 τ ($J=11$ c.p.s.) and 3.97 τ ($J=11$ c.p.s.).

TABLE I.

1 α ,2 α -Dihydroxy- Δ^4 -3-ones				4 α ,5 α -Dihydroxy- Δ^1 -3-ones			
	m.p.	$[\alpha]_D$	$\lambda_{\max}$ m μ (ϵ)		m.p.	$[\alpha]_D$	$\lambda_{\max}$ m μ (ϵ)
Va	247~248	- 14	238 (11,600)	VIIa	241~245	- 15	230 (8,200)
Vb	156~157	+ 77	241 (12,700)	VIIb	149~150	+ 82	231 (9,000)
Vc	222~224	+ 81	240 (13,900)	VIIc	168~170	+ 81	231 (9,450)
Vd	235~237	+ 97	241 (13,700)	VIIId	202~203	+ 80	231 (9,150)
Ve	183~184	+183	241 (13,800)	VIIe	222~224	+154	231 (9,550)
Vf	195~197	+171	240 (13,700)	VIIIf	198~199	+157	231 (9,200)
Vg	248~250	+170 (dioxane)	241 (13,300)	VIIIf	189~191	+122 (dioxane)	231 (9,550)

The hydroxyl groups in these diols were inferred to be α -configuration on the assumption that the addition of osmium tetroxide to the double bond should occur more readily from the less hindered α -side of the steroid molecule. This conjecture was supported by the optical rotatory dispersions (ORD). The 1,2-dihydroxy- Δ^4 -3-ones (Va) and (Vb) exhibited negative Cotton curves as in 2 α -hydroxy- Δ^4 -3-ones but did not the reverse curve as observed in 2 β -hydroxy- Δ^4 -3-ones.⁶⁾ In the 4,5-dihydroxy- Δ^1 -3-ones, the C₄-hydroxyl group should be equatorial, regardless of its configuration, in view of the fact that the hydroxylation of a double bond with osmium tetroxide gives a *cis* glycol. Hence the Cotton effect of the Δ^1 -3-oxo moiety should not be greatly affected by the C₄-hydroxyl group but by the A/B ring juncture. The ORD of VIIa and VIIb did not exhibit a positive Cotton effect, which has been shown in the circular dichroism⁷⁾ of 17 β -acetoxy-5 β -androst-1-en-3-one, but were the negative Cotton curves as described⁸⁾ for cholest-1-en-3-one. Actually, the 1,2-diol obtained from the osmylation of 25D-spirosta-1,4-dien-3-one (Va) was proved to be the 1 α ,2 α -dihydroxy- Δ^4 -3-one (Va) by correlation with the 1 β ,2 β -dihydroxy- Δ^4 -3-one (II)¹⁾ previously derived from kogagenin. The both are entirely different from each other but on alkaline treatment yielded the identical 2-hydroxy- $\Delta^{1,4}$ -3-one (X).⁹⁾ In analogy, it is most probable that all the 1,2- and 4,5-diols isolated in the other series have the same α -configuration of the hydroxyl groups.

6) K. Kuriyama, E. Kondo, K. Tori: Tetrahedron Letters, 1963, 1485.

7) L. Velluz, M. Legrand: Angew. Chem., 73, 603 (1961).

8) C. Djerassi, R. Riniker, B. Riniker: J. Am. Chem. Soc., 78, 6377 (1956).

9) K. Takeka, T. Kubota, A. Shimaoka: Tetrahedron, 7, 62 (1959).

Although Hirschmann have described⁵⁾ that the hydroxylation of prednisolone-BMD with osmium tetroxide was accompanied with the oxidation of the 11β -hydroxyl group to afford 1,2-dihydroxycortisone-BMD, in this experiment, osmium hydroxylation of prednisolone acetate (Vg) yielded the corresponding 1,2-diol (Vg)*³ and 4,5-diol (VIg) with the intact 11β -hydroxyl group, in the same way as in the other examples.

Treatment of $1\alpha,2\alpha$ -dihydroxy-25D-spirost-4-en-3-one (Va) with manganese dioxide was carried out in the same manner as the previous experiment on the $1\beta,2\beta$ -dihydroxy epimer (II)¹⁾ and gave A-nor-25D-spirost-3(5)-ene-1,2-dione (IIIa) identical with the product from the latter. Thus all of the other $1\alpha,2\alpha$ -dihydroxy derivatives (Vb~g) were subjected to the oxidative ring contraction with manganese dioxide.

As previously described, manganese dioxide was prepared according to the direction of O. Mancera, *et al.*¹⁰⁾ Although chloroform has previously been used as the reaction solvent, the reaction could be proceeded also in acetone, *tert*-butanol, benzene or carbon tetrachloride. When progresses of the reaction on Vb, d and e were followed with ultraviolet spectra,¹⁾ it was shown that acetone was superior as the reaction solvent to chloroform in the reaction rates. Thus, the 1,2-diols (Vb~g) were treated with manganese dioxide in acetone and smoothly converted into the corresponding A-nor- $\Delta^3(5)$ -1,2-diones (IIIb~g), as expected. The structures were established by the satisfying analytical results and by the typical ultraviolet and infrared absorptions.¹⁾

TABLE II. A-Nor- $\Delta^3(5)$ -1,2-diones

	m.p.	$[\alpha]_D$	$\lambda_{\max}$ m μ (ϵ)	$\nu_{\max}^{\text{Nujol}}$ cm ⁻¹
IIIa ¹⁾	228~230	-118	282(5,600)	1756, 1720, 1604
IIIb	119~120	-26	284(5,400)	1755, 1715, 1602
IIIc	160~162	-26	283(6,200)	1744, 1712, 1599
IIId	229~230	-27	284(5,900)	1755, 1700, 1588
IIIe	180~181	+75	283(6,150)	1753, 1714, 1696, 1603
IIIf	218~220	+91	283(6,200)	1753, 1746, 1730, 1710, 1600
IIIg	199~201	+100	282(5,600)	1758, 1740, 1724, 1706, 1650, 1593

(dioxane)

On the other hand, the $4\alpha,5\alpha$ -dihydroxy- Δ^1 -3-ones were found to be stable to manganese dioxide. Thus, a mixture of the isomeric diols, derived from cholestadienone (Vb), was subjected to the oxidation with manganese dioxide, without isolation of the 1,2-diol (Vb). The A-norsteroid (IIIb) arising from the oxidation of the 1,2-diol could be readily separated from the unreacted 4,5-diol (VIb) by chromatography over silica gel.

Experimental

All melting points were uncorrected. Unless otherwise stated, optical rotations were measured in CHCl_3 solutions at ca. 25° with a Rudolf Photoelectric Polarimeter Model 200. UV spectra were taken in 95% EtOH solutions with a Hitachi Recording Spectrophotometer EPS-2. IR spectra were recorded on a Koken Infrared Spectrophotometer Model DS 301. NMR spectra were determined at 60 Mc. in CDCl_3 solutions containing tetramethylsilane as an internal standard using a Varian A-60 analytical NMR spectrometer. ORD curves were recorded in dioxane solutions with a Rudolf Recording Spectropolarimeter.

General Procedure for the Δ^1 -Dehydrogenation of a Δ^1 -3-Oxo Steroid—A mixture of a Δ^1 -3-oxo steroid and DDQ (1.2 equiv.) in dry benzene (40~65 ml./1 g. of the steroid) was heated under reflux for 25~30 hr. The precipitated hydroquinone was removed by filtration and the filtrate was passed through a column of Al_2O_3 (10 g./1 g. of the steroid) in order to remove the excess DDQ. The Al_2O_3 was washed with CHCl_3 and the combined eluates were evaporated under a reduced pressure and the residue was recrystallized from a solvent.

*³ This compound is indeed different from $1\alpha,2\alpha$ -dihydroxycortisone 21-acetate, m.p. 244.5~246°, $[\alpha]_D +163^\circ$, UV $\lambda_{\max}^{\text{EtOH}}$ m μ (ϵ): 235(13,200), obtained by osmylation of prednisone acetate. (Unpublished work in this Laboratory).

10) O. Mancera, G. Rosenkranz, F. Sondheimer: J. Chem. Soc., 1953, 2189.

Cholesta-1,4-dien-3-one (Vb)—Cholest-4-en-3-one (Vb) (7.69 g.) was dehydrogenated with DDQ (5.44 g.) in dry benzene (400 ml.) by the general procedure described above. Recrystallization of the crude product (5.70 g.) from MeOH gave Vb (4.89 g.) as needles, m.p. 111~112°, $[\alpha]_D + 26^\circ$ (c=1.01), (reported¹¹) m.p. 110~112°, $[\alpha]_D + 31^\circ$. UV: $\lambda_{\max}$ 247 m μ (ϵ 15,800). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1666, 1626, 1605, 883. *Anal.* Calcd. for C₂₇H₄₂O: C, 84.75; H, 11.07. Found: C, 84.47; H, 11.14.

17 β -Hydroxyandrosta-1,4-dien-3-one Propionate (Vc)—By the general procedure described previously, testosterone propionate (Vc) (27.6 g.) was dehydrogenated with DDQ (21.8 g.) in dry benzene (1.2 L.). Recrystallization of the crude product (24.6 g.) from Me₂CO-petr. ether afforded scales of Vc (14.6 g.), m.p. 138~140°, $[\alpha]_D + 34^\circ$ (c=1.03). UV: $\lambda_{\max}$ 245 m μ (ϵ 17,100). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1733, 1667, 1623, 1605, 1186, 875. *Anal.* Calcd. for C₂₂H₃₀O₃: C, 77.15; H, 8.83. Found: C, 77.21; H, 8.86.

17 β -Hydroxyandrosta-1,4-dien-3-one (Vd)—The foregoing propionate (Vc) (19.55 g.) was saponified by heating with a 1.5% KOH-MeOH solution (600 ml.) for 1 hr. The mixture was neutralized with dil. HCl and concentrated and then H₂O was added. Precipitated crystals were collected, dried (16.09 g.) and recrystallized from Me₂CO giving Vd (14.77 g.), m.p. 167~169°, $[\alpha]_D + 22^\circ$ (c=1.06), (reported¹²) m.p. 168~170°, $[\alpha]_D + 23^\circ$. UV: $\lambda_{\max}$ 246 m μ (ϵ 15,400), IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3480, 1663, 1622, 1600, 883. *Anal.* Calcd. for C₁₉H₂₆O₂: C, 79.68; H, 9.15. Found: C, 79.93; H, 9.16.

Pregna-1,4-diene-3,20-dione (Ve)—Progesterone (Ve) (4.718 g.) in dry benzene (300 ml.) was dehydrogenated with DDQ (4.08 g.) as described in the general procedure. Recrystallization of the crude product (3.658 g.) from Me₂CO gave Ve (2.833 g.) as prisms, m.p. 146~150°. Further recrystallization from Me₂CO raised the m.p. to 150~151°, $[\alpha]_D + 122^\circ$ (c=1.09), (reported¹³) m.p. 152~153°, $[\alpha]_D + 120^\circ$. UV: $\lambda_{\max}$ 246 m μ (ϵ 16,300). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1695, 1660, 1626, 1602, 882. *Anal.* Calcd. for C₂₁H₂₈O₂: C, 80.73; H, 9.03. Found: C, 80.55; H, 9.10.

21-Hydroxypregna-1,4-diene-3,20-dione Acetate (Vf)—By the general procedure described previously, desoxycorticosterone acetate (Vf) (11.18 g.) was transformed with DDQ (8.17 g.) in dry benzene (600 ml.). The crude product (9.80 g.) was recrystallized from Me₂CO yielding Vf (8.80 g.) as prisms, m.p. 201~202°, $[\alpha]_D + 136^\circ$ (c=1.04), (reported¹⁴) m.p. 202~204°, $[\alpha]_D + 143^\circ$. UV: $\lambda_{\max}$ 245 m μ (ϵ 16,200). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1745, 1725, 1667, 1626, 1605, 890. *Anal.* Calcd. for C₂₃H₃₀O₄: C, 74.56; H, 8.16. Found: C, 74.89; H, 8.32.

General Procedure for the Hydroxylation of a $\Delta^{1,4}$ -3-Oxo Steroid—A solution of a $\Delta^{1,4}$ -3-oxo steroid in pyridine was cooled in an ice-bath and OsO₄ was added. The mixture was allowed to stand at room temperature for 7 days in a dark place and poured into a large volume of petr. ether. The precipitated crude osmate was separated from the solvent by decantation, washed with petr. ether to free from the pyridine and dissolved in a mixture of dioxane and CHCl₃. A stream of H₂S was bubbled through the solution kept in an ice-bath for 30 min. After standing overnight, a precipitate was filtered off and the filtrate was evaporated under reduced pressure. The crude product was subjected to chromatography on a column of silica gel.

Hydroxylation of 25D-Spirosta-1,4-dien-3-one (Va) with Osmium Tetroxide—A solution of Va (2.00 g.), prepared¹⁵ by DDQ dehydrogenation of 25D-spirost-4-en-3-one, in pyridine was treated with OsO₄ (1.6 g.) by the general procedure described above. The resulting crude product (2.45 g.) was chromatographed over a column of silica gel (35 g.) to separate into two fractions, those are the eluates (220 mg.) with benzene-CHCl₃ (4:1 to 2:1) and the eluates (729 mg.) with benzene-CHCl₃ (2:1) to CHCl₃ alone.

Crystallization of the former fraction from MeOH gave prisms (150 mg.) of 4 α ,5 α -dihydroxy-25D-spirost-1-en-3-one (VIIa), m.p. 241~245°. Further recrystallization from MeOH did not change the m.p., $[\alpha]_D - 15^\circ$ (c=1.14). UV: $\lambda_{\max}$ 230 m μ (ϵ 8,200). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3510, 1695, 1610. NMR (τ): 3.28 (doublet, J=11 c.p.s., C₁-proton), 3.97 (doublet, J=11 c.p.s., C₂-proton), 5.40 (singlet, C₄-proton), 8.73 (singlet, C₁₉-CH₃), 9.18 (singlet, C₁₈-CH₃). ORD (c=0.5878): $[\alpha]_{348}^{\text{trough}}$ -449°, $[\alpha]_{280} + 1565^\circ$, $[\alpha]_{260} + 3440^\circ$. *Anal.* Calcd. for C₂₇H₄₀O₅: C, 72.94; H, 9.07. Found: C, 73.25; H, 9.13.

The latter fraction of the above chromatography was dissolved in Me₂CO (80 ml.) containing *p*-toluenesulfonic acid (80 mg.). The mixture was refluxed for 5 hr., neutralized with 5% NaHCO₃ and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel (15 g.) and the fractions (172 mg.) eluted with benzene-CHCl₃ (4:1) on crystallization from hexane afforded 1 α ,2 α -dihydroxy-25D-spirost-4-en-3-one acetone (VIIIa) (112 mg.) as needles, m.p. 191~193°, $[\alpha]_D - 35^\circ$ (c=0.85, dioxane). UV: $\lambda_{\max}$ 244.5 m μ (ϵ 15,100). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1676, 1625. *Anal.* Calcd. for C₃₀H₄₄O₅: C, 74.34; H, 9.15. Found: C, 74.43; H, 9.19. Elution of the chromatography with benzene-CHCl₃ (4:1 to 1:1) and crystallization of the eluate (160 mg.) from MeOH gave the above-mentioned 4,5-diol (VIIa) (55 mg.), m.p. 234~242°. The

11) A. L. Wilds, C. Djerassi: J. Am. Chem. Soc., **68**, 1712 (1946).

12) H. J. Ringold, G. Rosenkranz, F. Sondheimer: J. Org. Chem., **21**, 239 (1956).

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14) H. L. Herzog, C. C. Payne, M. T. Hughes, M. J. Gentles, E. B. Hershberg, A. Nobile, W. Charney, C. Federbush, D. Sutter, P. L. Perlman: Tetrahedron, **18**, 581 (1962).

15) K. Takeda, T. Okanishi, K. Igarashi, A. Shimaoka: Tetrahedron, **15**, 183 (1961).

fractions (436 mg.) eluted further with CHCl_3 were assumed to be a mixture consisted of the unreacted 1,2-diol (VIa) and 4,5-diol (VIIa), on the basis of the thin-layer chromatogram.

1 α ,2 α -Dihydroxy-25D-spirost-4-en-3-one (VIa)—A mixture of the foregoing acetone (VIIa) in 50% aq. AcOH (5 ml.) was heated at 70–80° for 1 hr. and then evaporated *in vacuo* to dryness. Crystallization of the residue (94 mg.) from MeOH afforded needles (67 mg.) of VIa, m.p. 247–248°, $[\alpha]_D -14^\circ$ ($c=1.07$). UV: λ_{max} 238 m μ (ϵ 11,600). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3566, 3472, 1680, 1620. NMR (τ): 4.17 (singlet, C₄-proton), 5.67 (doublet, $J=3$ c.p.s., C₂-proton), 5.97 (doublet, $J=3$ c.p.s., C₁-proton), 8.72 (singlet, C₁₉-CH₃), 9.18 (singlet, C₁₈-CH₃). ORD ($c=0.1158$): $[\alpha]_{700} -86^\circ$, $[\alpha]_{346}^{\text{rough}} -1192^\circ$, $[\alpha]_{300} +2997^\circ$, $[\alpha]_{281} +3739^\circ$, $[\alpha]_{275} +4275^\circ$. Anal. Calcd. for C₂₇H₄₀O₅: C, 72.94; H, 9.07. Found: C, 73.16; H, 9.20.

Treatment of VIa with Alkali—A mixture of the above 1 α ,2 α -diol (VIa) (30 mg.) in MeOH (7 ml.) and 10% KOH (1 ml.) was refluxed for 30 min. The reaction mixture was diluted with H₂O, acidified with dil. HCl and extracted with CHCl_3 -Et₂O (1:4). The organic layer was washed with H₂O, dried and evaporated under diminished pressure leaving an oil (30 mg.). Crystallization from Me₂CO afforded scales (17 mg.), m.p. 224–227°, which was identical with a sample of 2-hydroxy-25D-spirosta-1,4-dien-3-one (X)⁹ previously derived from kogagenin in all respects.

Treatment of VIa with Manganese Dioxide—To a solution of the 1 α ,2 α -diol (VIa) (8 mg.) in CHCl_3 (1 ml.), MnO₂ (100 mg.) prepared by the procedure of Mancera, *et al.*¹⁰ was added. The mixture was stirred at 32° for 6 hr. and the MnO₂ was removed by filtration. Evaporation of the filtrate left a yellow solid, which was recrystallized from Me₂CO giving yellow prisms, m.p. 227–231°. Identity with an authentic sample of A-nor-25D-spirost-3(5)-ene-1,2-dione (IIIa)¹ was established by comparison of the IR spectra and a mixed melting point determination.

Hydroxylation of Cholesta-1,4-dien-3-one (Vb) with Osmium tetroxide—To a solution of Vb (5.50 g.) in pyridine (60 ml.) was added OsO₄ (4 g.). The crude product (5.0 g.), obtained after working up by the general procedure, was chromatographed on silica gel (70 g.). The elution with benzene- CHCl_3 (1:1) and CHCl_3 gave a mixture (3.69 g.) of the isomeric diols as a gelatinous solid.

a) A part (2.88 g.) of the foregoing mixture was dissolved in Me₂CO (576 ml.) and *p*-toluenesulfonic acid (288 mg.) was added. The solution was refluxed for 5 hr., neutralized with 5% NaHCO₃ and evaporated. The residue was taken up with Et₂O and the Et₂O solution was washed with H₂O, dried and evaporated to dryness. The residue (3.20 g.) was chromatographed over silica gel (70 g.). The fractions (1.60 g.) eluted with benzene- CHCl_3 (9:1) gave no crystals on attempts to crystallize it from solvents and appeared to be a mixture of the 1,2-diol acetone and the unreacted 4,5-diol by thin-layer chromatography. The mixture was subjected to preparative thin-layer chromatography using plates (20 × 20 cm.) spread Merck's Silica Gel GF₂₅₄ in thickness of 0.5 mm. After development with benzene-AcOEt (3:1), the two fractions detected by UV light were scraped, extracted with CHCl_3 and there were obtained the more mobile fraction (670 mg.) and the less mobile one (445 mg.). Recrystallization of the former fraction from petr. ether gave 1 α ,2 α -dihydroxycholest-4-en-3-one acetone (VIIIb) (365 mg.) as plates, m.p. 134–136°. Concentration of the mother liquor afforded the second crop (60 mg.), m.p. 132–135°. The first crop showed the following constants, $[\alpha]_D +51^\circ$ ($c=0.55$). UV: λ_{max} 245 m μ (ϵ 14,600). IR $\nu_{\text{max}}^{\text{CCl}_4}$ cm^{-1} : 1683, 1630, 1382, 1374. Anal. Calcd. for C₃₀H₄₀O₃: C, 78.89; H, 10.59. Found: C, 79.18; H, 10.73.

The less mobile fraction of the preparative thin-layer chromatography was combined with the eluates (1.23 g.) of the above column chromatography with benzene- CHCl_3 (9:1 to 1:1). An attempt to crystallize from abs. EtOH afforded a gelatinous solid (1.470 g.), which was acetylated by standing with Ac₂O (15 ml.) and pyridine (20 ml.) at room temperature. The crude product (1.675 g.), extracted with AcOEt in the usual manner, was chromatographed over silica gel (40 g.). Elution with benzene- CHCl_3 (9:1 to 4:1) gave an oily fraction (142 mg.), which resisted to crystallization. Further elution with benzene- CHCl_3 (4:1) to CHCl_3 afforded a crystalline fraction (1.476 g.), which on recrystallization from Me₂CO gave needles (977 mg.), m.p. 224–226° (decomp.). This substance was identified with an authentic sample of 4 α ,5 α -dihydroxycholest-1-en-3-one 4-acetate described below by a mixed melting point determination and the IR spectra.

b) The remainder (810 mg.) of the mixture of the isomeric diols was dissolved in Me₂CO (81 ml.) and stirred together with MnO₂ (8.1 g.) at 30–32° for 6 hr. The mixture was filtered and the filtrate was evaporated to give the yellow crystalline residue (645 mg.), which was chromatographed on silica gel (20 g.). The yellow fractions (225 mg.) eluted with benzene to benzene- CHCl_3 (9:1) yielded, on recrystallization from Me₂CO, yellow plates (150 mg.), m.p. 119–120°. This substance was identical with an authentic sample of A-norcholest-3(5)-ene-1,2-dione (IIIb) described below in all respects.

The fractions (357 mg.) eluted the above chromatography with benzene- CHCl_3 (1:1) were purified further by the preparative thin-layer chromatography, in the manner as described in a). A gelatinous solid (240 mg.), obtained from the main spots, on recrystallization from abs. EtOH afforded 4 α ,5 α -dihydroxycholest-1-en-3-one (VIIb) (176 mg.) as clusters of small plates, m.p. 149–150°, $[\alpha]_D +82^\circ$ ($c=1.11$). UV: λ_{max} 231 m μ (ϵ 9,000). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3576, 3490, 1685, 1618. ORD ($c=0.2262$): $[\alpha]_{349}^{\text{rough}} -257^\circ$, $[\alpha]_{300} +2185^\circ$, $[\alpha]_{287} +2490^\circ$, $[\alpha]_{275} +2968^\circ$. Anal. Calcd. for C₂₇H₄₄O₃: C, 77.83; H, 10.65. Found: C, 77.64; H, 10.54.

1 α ,2 α -Dihydroxycholest-4-en-3-one (VIb)—A mixture of the above-mentioned 1,2-diol acetone (VIIb) (330 mg.) and 70% AcOH (20 ml.) was heated at 80° for 1 hr. The solution was diluted with H₂O and extracted thoroughly with AcOEt. The organic layer was washed with 5% Na₂CO₃ and H₂O, dried and evaporated giving the crystalline residue (297 mg.), m.p. 149~152°. Recrystallization from Me₂CO afforded plates (158 mg.) of VIb, m.p. 156~157°. Concentration of the mother liquor gave the second crop (100 mg.), m.p. 155~156°. $[\alpha]_D^{25} + 77^\circ$ (c=1.05). UV: $\lambda_{\max}$ 241 m μ (ϵ 12,700). IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3582, 3490, 1680, 1618. ORD (c=0.2926): $[\alpha]_{470} + 103^\circ$, $[\alpha]_{347}^{\text{rough}} - 943^\circ$, $[\alpha]_{300} + 3746^\circ$. Anal. Calcd. for C₂₇H₄₄O₃: C, 77.83; H, 10.65. Found: C, 77.87; H, 10.68.

4 α ,5 α -Dihydroxycholest-1-en-3-one 4-Acetate—A mixture of the pure 4,5-diol (VIIb) (50 mg.), Ac₂O (1 ml.) and pyridine (1 ml.) was allowed to stand at room temperature overnight. The product, isolated with AcOEt in the usual manner, was recrystallized from Me₂CO to give the monoacetate (36 mg.) as needles, m.p. 224~226° (decomp.), $[\alpha]_D^{25} + 83^\circ$ (c=0.99). UV: $\lambda_{\max}$ 231 m μ (ϵ 9,950). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3353, 1754, 1692, 1623, 1224. Anal. Calcd. for C₂₉H₄₆O₄: C, 75.94; H, 10.11. Found: C, 76.21; H, 10.25. Concentration of the above mother liquor gave the second crop (13 mg.), m.p. 222~224° (decomp.).

A-Norcholest-3(5)-ene-1,2-dione (IIIb)—A mixture of VIb (100 mg.) and MnO₂ (1.0 g.) in Me₂CO (10 ml.) was stirred at 30~32° for 4 hr. The MnO₂ was filtered off and the filtrate was evaporated to dryness. The yellow crystalline residue (84 mg.) was recrystallized from Me₂CO giving IIIb (60 mg.) as yellow plates, m.p. 119~120°, $[\alpha]_D^{25} - 26^\circ$ (c=1.09). UV: $\lambda_{\max}$ 284 m μ (ϵ 5,400). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1755; 1715, 1602. Anal. Calcd. for C₂₆H₄₀O₂: C, 81.20; H, 10.48. Found: C, 81.11; H, 10.66.

Hydroxylation of 17 β -Hydroxyandrost-1,4-dien-3-one Propionate (Vc) with Osmium Tetroxide—To a solution of Vc (12.8 g.) in pyridine (125 ml.) was added OsO₄ (10.0 g.). The reaction mixture was processed by the general procedure described above. Chromatography of the crude product over silica gel (125 g.) was eluted with benzene-CHCl₃ (1:1) to give a fraction (3.66 g.), which on recrystallization from Me₂CO-petr. ether afforded prisms (2.55 g.) of 4 α ,5 α ,17 β -trihydroxyandrost-1-en-3-one 17-propionate (VIIc), m.p. 168~170°, $[\alpha]_D^{25} + 81^\circ$ (c=1.03). UV: $\lambda_{\max}$ 231 m μ (ϵ 9,450). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3578, 1727, 1675, 1620, 1277, 1215. Anal. Calcd. for C₂₈H₃₈O₅: C, 70.18; H, 8.57. Found: C, 70.28; H, 8.59.

The next fractions (5.19 g.) eluted the above chromatography with CHCl₃ gave, on recrystallization from MeOH, 1 α ,2 α ,17 β -trihydroxyandrost-4-en-3-one 17-propionate (VIIc) (3.93 g.), m.p. 219~222°. Further recrystallization from MeOH gave the pure sample, m.p. 222~224°, as needles, $[\alpha]_D^{25} + 81^\circ$ (c=1.06). UV: $\lambda_{\max}$ 240 m μ (ϵ 13,900). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3570, 1710, 1668, 1615, 1275, 1245. Anal. Calcd. for C₂₈H₃₈O₅: C, 70.18; H, 8.57. Found: C, 70.47; H, 8.61.

17 β -Hydroxy-A-norandrost-3(5)-ene-1,2-dione Propionate (IIIc)—A mixture of the foregoing VIIc (1.50 g.) and MnO₂ (15 g.) in Me₂CO (150 ml.) was stirred at 30~32° for 4 hr. After removals of the MnO₂ and the solvent, the yellow crystalline product (1.03 g.) was recrystallized from Me₂CO-petr. ether giving yellow scales (850 mg.) of IIIc, m.p. 160~162°, $[\alpha]_D^{25} - 26^\circ$ (c=1.06). UV: $\lambda_{\max}$ 283 m μ (ϵ 6,200). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1744, 1712, 1599, 1201. Anal. Calcd. for C₂₁H₂₈O₄: C, 73.22; H, 8.19. Found: C, 73.07; H, 8.19.

Hydroxylation of 17 β -Hydroxyandrost-1,4-dien-3-one (Vd) with Osmium Tetroxide—A mixture of Vd (10.93 g.) and OsO₄ (10 g.) in pyridine (135 ml.) was processed by the general procedure described previously. The crude product was chromatographed on silica gel (130 g.). The fractions (3.48 g.), eluted with CHCl₃, were crystallized from Me₂CO-petr. ether giving prisms (2.906 g.) of 4 α ,5 α ,17 β -trihydroxyandrost-1-en-3-one (VIIId), m.p. 200~203° (decomp.). Further recrystallization from Me₂CO gave an analytical sample, m.p. 202~203° (decomp.), $[\alpha]_D^{25} + 80^\circ$ (c=1.02). UV: $\lambda_{\max}$ 231 m μ (ϵ 9,150). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3430, 1673, 1616. Anal. Calcd. for C₁₉H₂₆O₄: C, 71.22; H, 8.81. Found: C, 71.47; H, 8.83.

Further elution of the chromatography with CHCl₃-MeOH (100:1) gave a crystalline fraction (3.39 g.). Recrystallization from MeOH afforded needles (1.945 g.) of 1 α ,2 α ,17 β -trihydroxyandrost-4-en-3-one (VId), m.p. 230~232°. Concentration of the mother liquor yielded the second crop (990 mg.) m.p. 223~227°. For analysis, the first crop was recrystallized further from MeOH. m.p. 235~237°, $[\alpha]_D^{25} + 97^\circ$ (c=0.95). UV: $\lambda_{\max}$ 241 m μ (ϵ 13,700). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3458, 1675, 1623. Anal. Calcd. for C₁₉H₂₆O₄: C, 71.22; H, 8.81. Found: C, 71.36; H, 8.82.

17 β -Hydroxy-A-norandrost-3(5)-ene-1,2-dione (IIId)—A mixture of the foregoing VId (1.7 g.) and MnO₂ (17 g.) in Me₂CO (170 ml.) was stirred at 30~32° for 6 hr. The MnO₂ was filtered off and the filtrate was evaporated. The yellow crystalline residue (1.17 g.) on recrystallization from Me₂CO-petr. ether gave IIId (870 mg.) as yellow needles, m.p. 229~230°, $[\alpha]_D^{25} - 27^\circ$ (c=0.98). UV: $\lambda_{\max}$ 284 m μ (ϵ 5,900). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3515, 1755, 1700, 1588. NMR (τ): 3.43 (singlet, C₃-proton), 8.72 (singlet, C₁₉-CH₃), 9.20 (singlet, C₁₈-CH₃). Anal. Calcd. for C₁₈H₂₄O₃: C, 74.97; H, 8.39. Found: C, 74.71; H, 8.36.

Hydroxylation of Pregna-1,4-diene-3,20-dione (Ve) with Osmium Tetroxide—By the general procedure described above, Ve (2.23 g.) was treated with OsO₄ (2 g.) in pyridine (30 ml.). The crude product was chromatographed on silica gel (60 g.). The fractions (609 mg.) eluted with benzene-CHCl₃ (1:1) were recrystallized from Me₂CO to give prisms (453 mg.) of 4 α ,5 α -dihydroxypregn-1-ene-3,20-dione (VIId), m.p. 222~224° (decomp.), $[\alpha]_D^{25} + 154^\circ$ (c=1.06). UV: $\lambda_{\max}$ 231 m μ (ϵ 9,550). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3524, 1693, 1676, 1622. Anal. Calcd. for C₂₁H₃₀O₄: C, 72.80; H, 8.73. Found: C, 72.74; H, 8.83.

The next fractions (530 mg.) eluted with benzene-CHCl₃ (1:1) to CHCl₃ were shown to be a mixture of

the 1,2-diol (Vle) and the 4,5-diol (Vle) by thin-layer chromatography. Recrystallization from Me₂CO resulted in the isolation of the 4,5-diol (Vle) (133 mg.), m.p. 219~223° (decomp.).

Further elution with CHCl₃ to CHCl₃-MeOH (50:1) gave a fraction (588 mg.), which on crystallization from Me₂CO afforded prisms (160 mg.) of 1 α ,2 α -dihydroxypregn-4-ene-3,20-dione (Vle), m.p. 183~184°. Concentration of the mother liquor yielded additional crystals (249 mg.), m.p. 179~181°. The first crop showed the following constants. $[\alpha]_D + 183^\circ$ (c=1.06). UV: $\lambda_{\max}$ 241 m μ (ϵ 13,800). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3543, 1689, 1626. Anal. Calcd. for C₂₁H₃₀O₄: C, 72.80; H, 8.73. Found: C, 72.74; H, 8.83.

A-Norpregn-3(5)-ene-1,2,20-trione (IIIe)—A mixture of the foregoing Vle (80 mg.), MnO₂ (800 mg.) and Me₂CO (8 ml.) was stirred at 30~32° for 4 hr. After removal of the MnO₂, the filtrate was evaporated to give the yellow crystalline residue (52 mg.), which was purified by chromatography on silica gel. The eluate (43 mg.) with benzene-CHCl₃ (1:1) gave, on recrystallization from Me₂CO-petr. ether, yellow needles (37 mg.), m.p. 179~181°. Further recrystallization from Me₂CO-petr. ether yielded the pure sample of IIIe, m.p. 180~181°, $[\alpha]_D + 75^\circ$ (c=1.03). UV: $\lambda_{\max}$ 283 m μ (ϵ 6,150). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1753, 1714, 1696, 1603. NMR (τ): 3.43 (singlet, C₃-proton), 7.91 (singlet, C₂₁-CH₃), 8.73 (singlet, C₁₉-CH₃), 9.33 (singlet, C₁₈-CH₃). Anal. Calcd. for C₂₀H₂₆O₃: C, 76.40; H, 8.34. Found: C, 76.31; H, 8.44.

Hydroxylation of 21-Hydroxypregna-1,4-diene-3,20-dione Acetate (Vf) with Osmium Tetroxide—To a solution of Vf (2.65 g.) in a mixture of pyridine (60 ml.) and benzene (10 ml.), OsO₄ (2.0 g.) was added. Working up by the general procedure described above gave the crude product as a brittle foam, which was chromatographed over silica gel (40 g.). The fractions (865 mg.), eluted with benzene-CHCl₃ (1:1) and CHCl₃, gave plates (744 mg.) of 4 α ,5 α ,21-trihydroxypregn-1-ene-3,20-dione 21-acetate (VIIf), m.p. 194~195°, by recrystallization from Me₂CO-petr. ether. Further recrystallization from Me₂CO raised the m.p. to 198~199°. $[\alpha]_D + 157^\circ$ (c=1.08). UV: $\lambda_{\max}$ 231 m μ (ϵ 9,200). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3570, 1738, 1708, 1695, 1620, 1237. Anal. Calcd. for C₂₃H₃₂O₆: C, 68.29; H, 7.97. Found: C, 68.13; H, 8.05.

The next fractions (643 mg.), eluted the chromatography with CHCl₃, were recrystallized from Me₂CO yielding 1 α ,2 α ,21-trihydroxypregn-4-ene-3,20-dione 21-acetate (VIIf) (408 mg.) as needles, m.p. 193~196°. Further recrystallization from the same solvent raised the m.p. to 195~197°, $[\alpha]_D + 171^\circ$ (c=1.05). UV: $\lambda_{\max}$ 240 m μ (ϵ 13,700). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3585, 3525, 1743, 1724, 1693, 1630, 1235, 1218. Anal. Calcd. for C₂₃H₃₂O₆: C, 68.29; H, 7.97. Found: C, 68.38; H, 7.99.

21-Hydroxy-A-norpregn-3(5)-ene-1,2,20-trione Acetate (IIIIf)—A mixture of VIIf (210 mg.) and MnO₂ (2.1 g.) in CHCl₃ (21 ml.) was stirred at 30~32° for 6 hr. Removals of the MnO₂ and the solvent left a yellow crystalline material (135 mg.). Recrystallization from Me₂CO-petr. ether gave IIIIf (95 mg.) as yellow scales, m.p. 218~220°, $[\alpha]_D + 91^\circ$ (c=1.05). UV: $\lambda_{\max}$ 283 m μ (ϵ 6,200). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1753, 1746, 1730, 1710, 1600. Anal. Calcd. for C₂₂H₂₈O₅: C, 70.94; H, 7.58. Found: C, 71.00; H, 7.67.

Hydroxylation of Prednisolone Acetate (Vg) with Osmium Tetroxide—Prednisolone acetate (Vg) (2.88 g.) was hydroxylated with OsO₄ (2 g.) in pyridine (30 ml.) by the general procedure described above. The resulting crude product was chromatographed on silica gel (40 g.). The fractions (300 mg.) eluted with CHCl₃-MeOH (100:1 to 50:1) were recrystallized from Me₂CO-petr. ether to recover the starting material as prisms, m.p. 238~241°. The fractions (600 mg.), eluted further with CHCl₃-MeOH (50:1), on two recrystallization from Me₂CO gave needles (127 mg.) of 4 α ,5 α ,11 β ,17 α ,21-pentahydroxypregn-1-ene-3,20-dione 21-acetate (VIIf), m.p. 189~191°, $[\alpha]_D + 122^\circ$ (c=1.03, dioxane). UV: $\lambda_{\max}$ 231 m μ (ϵ 9,550). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3500, 1723, 1695, 1676, 1616, 1268, 1246. Anal. Calcd. for C₂₃H₃₂O₈· $\frac{1}{2}$ H₂O: C, 62.03; H, 7.46. Found: C, 62.13; H, 7.63.

The fractions (820 mg.) eluted with CHCl₃-MeOH (9:1) were recrystallized from Me₂CO-petr. ether giving 1 α ,2 α ,11 β ,17 α ,21-pentahydroxypregn-4-ene-3,20-dione 21-acetate (VIIf) (685 mg.), m.p. 246~247°, as plates. Further recrystallization from Me₂CO-petr. ether gave the pure sample with the following constants: m.p. 248~250°, $[\alpha]_D + 170^\circ$ (c=1.06, dioxane). UV: $\lambda_{\max}$ 241 m μ (ϵ 13,300). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3536, 1729, 1713, 1674, 1620, 1247. Anal. Calcd. for C₂₃H₃₂O₈: C, 63.28; H, 7.39. Found: C, 63.53; H, 7.59.

11 β ,17 α ,21-Trihydroxy-A-norpregn-3(5)-ene-1,2,20-trione 21-Acetate (IIIIf)—A mixture of VIIf (71 mg.) and MnO₂ (710 mg.) in Me₂CO (7 ml.) was stirred for 4 hr. at 30~32°. The MnO₂ was filtered off and the filtrate was evaporated. The yellow crystalline residue (52 mg.) was chromatographed over silica gel (1 g.). The eluate with CHCl₃-MeOH (100:1) gave, on recrystallization from Me₂CO-petr. ether, yellow needles (25 mg.) of IIIIf, m.p. 199~201°, $[\alpha]_D + 100^\circ$ (c=0.58, dioxane). UV: $\lambda_{\max}$ 282 m μ (ϵ 5,600). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3604, 3576, 3465, 3354, 1758, 1740, 1724, 1706, 1650, 1593, 1279, 1270, 1245. Anal. Calcd. for C₂₂H₂₈O₇·H₂O: C, 62.54; H, 7.16. Found: C, 62.64; H, 7.19.

Summary

A number of steroidal $\Delta^1,4$ -3-ones (Va~g) were hydroxylated with osmium tetroxide affording the 1 α ,2 α -dihydroxy- Δ^1 -3-ones (VIa~g) and the 4 α ,5 α -dihydroxy- Δ^1 -3-ones (VIIa~g). The former compounds (VIa~g) were converted into the A-norsteroids having $\Delta^{3(5)}$ -1,2-dioxo structure (IIIa~g) by treatment with manganese dioxide at room temperature.

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