CHARACTERISATION OF THE PRODUCTS FROM THE OXIDATION OF PROGESTERONE WITH OSMIUM TETROXIDE

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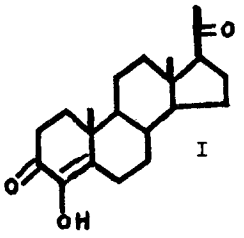
ABSTRACT

The nature of the compounds resulting from treatment of progesterone and 6β-hydroxy-4-pregnene-3,20-dione with hydrogen peroxide and catalytic quantities of osmium tetroxide was investigated. This reagent gives mixtures of 4,5-cis-glycols and the structures were assigned using kinetic dehydration studies, optical rotatory dispersion curves and nuclear magnetic resonance spectra.

Butenandt and Wolz (1) and Eastham et al (2) have investigated the reaction of 4-cholesten-3-one with osmium tetroxide in the presence of excess hydrogen peroxide. Eastham et al obtained two products and they assigned structures to the two 4ξ,5-dihydroxy-5ξ-cholestan-3-ones on the basis of degradation and kinetic dehydration studies.

Application of the reaction to progesterone gave two glycols 4ξ,5-dihydroxy-5ξ-pregnane-3-ones {cf. (3)} and this paper is concerned with the characterisation of these compounds and the extension of the reaction to some 6-oxygenated steroids.

Hydroxylation of olefins with equimolar proportions of osmium tetroxide is known to yield cis-glycols (4). That the glycols described in this paper, obtained using the hydrogen peroxide-osmium tetroxide reagent, were cis-glycols was confirmed by treating



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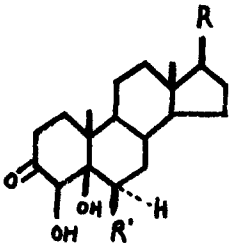
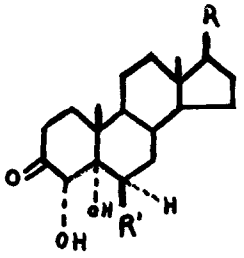


IIa R = C_8H_{17}, R' = H

IIb R = $COCH_3$, R' = H

IIc R = $COCH_3$, R' = OH

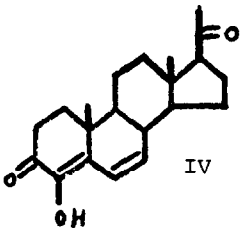


IIIa R = C_8H_{17}, R' = H

IIIb R = $COCH_3$, R' = H

IIIc R = $COCH_3$, R' = OH

IIId R = $COCH_3$, R' = $OCOCH_3$



IV

progesterone with an equimolar quantity of osmium tetroxide and decomposing the precipitated osmate ester, when fractional crystallisation gave the same two isomers. Both isomers gave 4-hydroxy-4-pregnene-3,20-dione (I) (3, 5) on dehydration and on acetylation gave mono- and di-acetates.

The optical rotatory dispersion (ORD) curves of the glycols from 4-cholesten-3-one confirmed the assignments given by Eastham (2). 4β,5-dihydroxy-5β-cholestan-3-one (IIa) (cis-ring A/B junction) gave a negative Cotton effect and 4α,5-dihydroxy-5α-cholestan-3-one

(IIIa) (trans-ring A/B) gave a positive Cotton effect (6, 7, 8). The isomers from progesterone both show a positive Cotton effect due to the C-20 carbonyl, but one is stronger than the other. That with the stronger Cotton effect is designated 4α,5-dihydroxy-5α-pregnane-3,20-dione (IIIb) since the Cotton effects for the two carbonyl systems would enhance one another. The other isomer is 4β,5-dihydroxy-5β-pregnane-3,20-dione (IIb) since the Cotton effect for the 3-carbonyl system is negative.

Comparison of the rates of dehydration of the compounds confirmed the above designations. The ratio of the rate constants ($^{K\beta}/_{K\alpha}$) for the glycols from 4-cholesten-3-one (IIa) and (IIIa) was 4.3 and from progesterone (IIb) and (IIIb) was 5.1.

The nuclear magentic resonance (NMR) spectra of the compounds were compared with those of 5-hydroxy-5β-cholestan-3-one and 5-hydroxy-5α-cholestan-3-one (9) and supported the above designations (Table 1). The β-glycols (IIa) and (IIb) and 5-hydroxy-5β-cholestan-3-one have almost the same chemical shift for the C-19 protons (61-63 c.p.s.). Similarly the α-glycols (IIIa) and (IIIb) on comparison with 5-hydroxy-5α-cholestan-3-one show almost the same chemical shift for the C-19 protons (71-75 c.p.s.).

The hydroxylation reaction was extended to 6β-hydroxy-4-pregnene-3,20-dione, giving two isomers. That with the stronger

Cotton effect was reasoned to be 4α,5,6β-trihydroxy-5α-pregnane-3,20-dione (IIIc) the other being 4β,5,6β-trihydroxy-5β-pregnane-3,20-dione (IIc). In agreement with this, kinetic dehydration studies showed the 4β,5β,6β-triol to dehydrate faster than the 4α,5α,6β-triol ($^{K\beta}/_{K\alpha}$ = 33).

Hydroxylation of 6β-hydroxy-4-pregnene-3,20-dione acetate gave one major product which is designated as 4α,5,6β-trihydroxy-5α-pregnane-3,20-dione 6-acetate (IIId), since on hydrolysis it gave 4α,5,6β-trihydroxy-5α-pregnane-3,20-dione (IIIc). Dehydration of the triols (IIIc) and (IIc) and the 6-acetate (IIId) gave 4-hydroxy-4,6-pregnadiene-3,20-dione (IV) (10).

Addition of chemical shift increments for 6β-hydroxyl and 6β-acetoxyl (11) to the C-19 proton values for the glycols from progesterone, that is (IIb) and (IIIb), gave good agreement with the experimental values (Table II) obtained for the triols (IIc) and (IIIc) and the 6-acetate (IIId). Also, in the compounds 4α,5,6β-trihydroxy-5α-pregnane-3,20-dione (IIIc) and its 6-acetate (IIId) the 4β-proton is shifted downfield by 36 and 30 c.p.s. respectively, relative to that of 4α,5-dihydroxy-5α-pregnane-3,20-dione (IIIb), confirming the 1:3 diaxial relationship of this proton with the 6β-oxygen function (12).

EXPERIMENTAL

Melting points were determined on a microscope hot stage and are uncorrected. Infrared spectra were determined on potassium bromide pellets on Perkin-Elmer 137 and Beckman IR7. Ultraviolet absorption spectra were determined on a Beckman Model D.U. spectrophotometer. NMR spectra were obtained using a Varian 4300 NMR spectrometer at a frequency of 60 Mc.p.s., the samples being dissolved in duteriochloroform and peak positions reported in c.p.s. downfield from tetramethylsilane (internal reference). Optical rotatory dispersion curves were determined in dioxane with an automatic recording spectropolarimeter (O.C. Rudolph and Sons Inc.).

Hydroxylation of Progesterone (a) Progesterone (2.1 g) was dissolved in t-butanol (40 ml), osmium tetroxide (104 mg) in t-butanol (20 ml) was added and the mixture treated with hydrogen peroxide (5 ml of 30%). The reaction was allowed to proceed at room temperature, samples being removed at intervals for ultraviolet analysis. After 24 hours the crystalline solid which separated out was filtered off, washed with t-butanol, dried in vacuum, 4α,5-dihydroxy-5α-pregnane-3,20-dione (IIIb) (540 mg), m.p. 242-250°. Analytical sample had m.p. 248-256°, $\{\alpha\}_D$ +84° (c, 0.31 dioxane) $\gamma_{max.}^{KBr}$ 3510 (Hydroxyl), 3460 (Hydroxyl), 2950, 2855, 1720 (C-20 carbonyl), 1680 (C-3 carbonyl), 1380, 1360, 1330, 1230, 1155, 1110 and 800 $cm.^{-1}$. (Found: C, 72.3; H, 9.35. $C_{21}H_{32}O_4$ requires: C, 72.4; H, 9.25%). Optical rotatory dispersion: in dioxane, {M} +9,160 (309 mμ, peak), -9,610 (261 mμ, trough), +260 (589 mμ), +181° (700 mμ). Acetylation of a portion of the 4α,5α-diol (50 mg) in acetic-anhydride-pyridine overnight at room temperature gave the 4-monoacetate (40 mg), fine needles, m.p. 233-236°, $\{\alpha\}_D$ +77° (c, 0.41 dioxane), $\gamma_{max.}^{KBr}$ 3420, 2940, 2350, 1735 (ester carbonyl), 1719 (carbonyl), 1702 (carbonyl), 1232, 11222, 1075, 1068 and 1042 $cm.^{-1}$ (Found: C, 70.45; H, 8.65. $C_{23}H_{34}O_5$ requires: C, 70.7; H, 8.8%). Acetylation of a further portion (100 mg) with isopropenyl acetate and p-toluene sulphonic acid under reflux for 2 hours gave the diacetate (90 mg), needles, m.p. 175-178°, $\{\alpha\}_D$ +78° (C, 1.29 dioxane), $\gamma_{max.}^{KBr}$ 3000, 1760 (ester carbonyl), 1720 (carbonyl), 1460, 1380, 1240, 1050 and 935 $cm.^{-1}$. (Found: C, 69.3; H, 8.6. $C_{25}H_{36}O_6$ requires: C, 69.4; H, 8.4%).

The mother liquor and washings were concentrated under vacuum to give a brown semi-crystalline solid (1.5 g), a portion of which (510 mg) was chromatographed on a partition column (300 g celite 545, 200 ml of methanol:water (4:1)). Elution with

hexane:benzene (4:1) gave progesterone (35 mg) m.p. 131-133°, infrared spectrum identical with that of an authentic specimen. Hexane-benzene (3:2) eluted 4α,5-dihydroxy-5α-pregnane-3,20-dione (IIIb) (68 mg), identical in respect of melting point and I.R. spectrum with the above. Further elution with hexane-benzene (3:2) gave a mixture (119 mg), then 4β,5-dihydroxy-5β-pregnane-3, 20-dione (IIb) (230 mg), m.p. 169-174°. Two crystallisations from ethyl acetate-methanol gave the pure compound, m.p. 174-177°, $\{\alpha\}_D$ +85° (c, 0.31 dioxane) $\gamma_{max.}^{KBr}$ 3500 (Hydroxyl), 2950, 2850, 1720 (C-20 Carbonyl), 1680 (C-3 Carbonyl), 1440, 1380, 1175, 1110 and 1070 $cm.^{-1}$. (Found: C, 72.5; H, 9.2. $C_{21}H_{32}O_4$ requires: C, 72.4; H, 9.25%). ORD: in dioxane, {M } +5,920 (310-312 mμ, peak), -4080 (269 mμ, trough), +509 (589 mμ), +360° (700 mμ). Acetylation of a portion of the 4β,5β-diol (50 mg) in acetic anhydride-pyridine overnight at room temperature gave the 4-monoacetate (48 mg), fine needles, m.p. 211-214°, $\{\alpha\}_D$ +88° (C, 0.32 in dioxane), $\gamma_{max.}^{KBr}$ 3420 (Lydroxyl), 2940, 2870, 1750 (ester carbonyl), 1725 (carbonyl), 1704 (carbonyl), 1225, 1085, 1070, 1057 and 1038 $cm.^{-1}$ (Found: C, 70.9; H, 8.5. $C_{23}H_{34}O_5$ requires: C, 70.7; H, 8.8%). Acetylation of a further portion (52 mg) with isopropenyl acetate and p-toluenesulphonic acid under reflux for 2 hrs. gave the diacetate (50 mg), m.p. 195-197°, $\{\alpha\}_D$ +96° (C, 0.64 in dioxane), $\gamma_{max.}^{KBr}$ 3200, 1760 (ester carbonyl), 1730 (carbonyl), 1375, 1240, 1075 and 1020 $cm.^{-1}$ (Found: C, 69.1; H, 8.5. $C_{25}H_{36}O_6$ requires: C, 69.4; H, 8.4%). Elution with hexane-benzene (1:1) and benzene gave 3,4-seco-4-nor-5,20-dioxopregnane-3-carboxylic acid (50 mg), m.p. 180-183°, I.R. spectrum identical to that of an authentic sample.

(b) Progesterone (200 mg) was dissolved in dioxane (4 ml), osmium tetroxide (130 mg) and pyridine (0.4 ml) were added. After 2 hours, crystals began to separate from the brown solution. After 18 hours the mixture was treated with sodium bisulphite (0.24 g), water (4 ml) and pyridine (3 ml) and stirred for two hours. The dark brown solution was extracted with chloroform and the extracts washed with water, dried (Na_2SO_4) and taken down under reduced pressure, giving a yellow gum. Fractional crystallisation from methanol gave first 4α,5-dihydroxy-5α-pregnane-3,20-dione (IIIb) (94 mg), m.p. 247-252°, I.R. spectrum identical to that of an authentic sample, and second 4β,5-dihydroxy-5β-pregnane-3,20-dione (IIb) (57 mg), m.p. 170-174°, I.R. spectrum identical to that of an authentic sample.

Hydroxylation of 4-cholesten-3-one. 4-cholesten-3-one (1 g) was hydroxylated using method (a) above, giving a crystalline product

Fractional crystallisation from ethanol separated 4α,5-dihydroxy-5α-cholestan-3-one (IIIa) (440 mg), white plates, m.p. 209-212°, I.R. spectrum identical to that of an authentic sample, ORD: in dioxane, {M} +3,620 (291 mμ, shortest wavelength measured), +57 (589 mμ), +29° (700 mμ). 4β,5-dihydroxy-5β-cholestan-3-one (IIa) (256 mg) was obtained from the mother liquor as plates which changed to needles at 125°, m.p. 135-136°, I.R. spectrum identical to that of an authentic sample, ORD: in dioxane {M} -211 (297 mμ, trough), +364 (275 mμ, shortest wavelength measured), +167 (589 mμ), +136° (700 mμ).

4-Hydroxy-4-pregnene-3,20-dione (I) (a) 4α,5-Dihydroxy-5α-pregnane-3,20-dione (IIIb) (45 mg) was dissolved in glacial acetic acid (8 ml), concentrated hydrochloric acid (0.2 ml) was added and the mixture heated at 70° in a nitrogen atmosphere. After 2 hours, the yellow mixture was concentrated under vacuum, diluted with ethyl acetate and the solution washed with water, aqueous sodium bicarbonate and dried (Na_2SO_4). Evaporation and crystallisation from ethyl acetate gave 4-hydroxy-4-pregnene-3, 20-dione (I) (32 mg), m.p. 235-238°, I.R. spectrum identical with that of an authentic sample (1, 5).

(b) 4β,5-dihydroxy-5β-pregnane-3,20-dione (IIb) (22 mg) was dehydrated as described above. The product (15 mg) was crystallised from ethyl acetate, m.p. 234-236°, I.R. spectrum identical with that of the above material.

Measurement of Dehydration Rates Stock solutions of the compounds (0.02 g/100 ml) and of hydrochloric acid (1.5 M) were made up in glacial acetic acid. At time zero for a kinetic run, a 2 ml aliquot of steroid solution and 2 ml of the hydrochloric acid solution were combined and diluted to 10 ml in a volumetric flask with glacial acetic acid. The ultraviolet light absorption of the reaction solution was measured by comparison with an acetic acid blank prepared with hydrochloric acid alone. Optical density (D_t) was measured at timed intervals at the position of maximum absorption of the 4-hydroxy-4-pregnene-3,20dione (I) in acid solution (279 mμ). After several hours the rate of change in optical density decreased. First order rate constants were taken as the slope of the straight line obtained by a plot of 2.3 log ($D_\alpha/D_\alpha-D_t$) against t (time in minutes).

Hydroxylation of 6β-Hydroxy-4-pregnene-3,20-dione 6β-Hydroxy-4-pregnene-3,20-dione (250 mg) was dissolved in warm t-butanol (5 ml).

The solution was cooled to room temperature and hydrogen peroxide (0.7 ml of 30%) and osmium tetroxide (33 mg) were added. The reaction mixture was kept at room temperature in darkness and after two days hydrogen peroxide (0.7 ml) was added. After seven days some crystalline material had separated, and the mixture was blown down to approximately 3 ml, diluted with saturated sodium chloride solution and sodium sulphite (2.5 g) was added in portions with cooling. The solution was extracted with methylene chloride, the extracts washed with saturated sodium chloride solution and dried (Na_2SO_4). Evaporation under reduced pressure gave a yellowish semi-solid (273 mg). Crystallisation from methylene chloride-methanol gave 4α,5,6β-trihydroxy-5α-pregnane-3,20-dione (IIIc) (97 mg), prisms, m.p. 265-269°. Two crystallisations from methanol-ethyl acetate gave the analytical sample, m.p. 266-269°, $\gamma_{max.}^{KBr}$ 3500 (hydroxyl), 3405 (hydroxyl), 2960, 2930, 2870, 1706 (C-20 carbonyl), 1680 (C-3 carbonyl), 1092, 1085, 1054, 1030 and 1011 $cm.^{-1}$, (Found: C, 69.1; H, 8.6. $C_{21}H_{32}O_5$ requires: C, 69.2; H, 8.85%). ORD: in dioxane, {M} +8,730 (303 mμ, peak), -3,900 (275 mμ, shortest wavelength measured), +122 (589 mμ), +132° (700 mμ). The mother liquor from the above triol gave a yellow syrup which was combined with similar material from a subsequent experiment (460 mg), and chromatographed on a partition column (116 g Silica gel 70, 116 ml of methanol-water (4:1)). Hexane-ethyl acetate (2:1) eluted a crystalline mixture, fractional crystallisation of which from methanol gave the above triol (100 mg) and 4β,5,6β,trihydroxy-5β-pregnane-3,20-dione (IIc) (230 mg), plates, m.p. 215-223°. Two crystalliations from methanol gave the pure triol, m.p. 218-225°, $\gamma_{max.}^{KBr}$ 3540 (Hydroxyl), 2940, 2860, 1729, 1705, 1090, 1072, 1039 and 1019 $cm.^{-1}$, (Found: C, 68.9; H, 8.9. $C_{21}H_{32}O_5$ requires: C, 69.2; H, 8.85%). ORD: in dioxane, {M} +5,340 (309 mμ, peak), -2,820 (267 mμ, trough), +103 (589 mμ), +184° (700 mμ).

Hydroxylation of 6β-Hydroxy-4-pregnene-3,20-dione acetate

6β-Hydroxy-4-pregnene-3,20-dione acetate (220 mg) was dissolved in warm t-butanol (5 ml). The solution was cooled to room temperature and hydrogen peroxide (0.7 ml of 30%) and osmium tetroxide (40 mg) was added. The reaction mixture was kept at room temperature in darkness and after three days hydrogen peroxide (0.7 ml) was added. Three days later aqueous sodium chloride was added and the mixture extracted with ethyl acetate. The extracts were washed with aqueous sodium bisulphite (5%), aqueous sodium bicarbonate and dried (Na_2SO_4). Evaporation under reduced pressure gave a colourless syrup (170 mg) which was chromatographed on silica gel (20 g). Benzene-ethyl acetate (9:1) eluted 4α,5,6β-trihydroxy-5α-

pregnane-3,20-dione 6-acetate (IIId) (106 mg), prisms, from ethyl acetate, m.p. 187-190°, $\nu_{max.}^{KBr}$ 3550 (Hydroxyl), 3445 (Hydroxyl), 2950, 2850, 1729 (carbonyl), 1718 (carbonyl), 1700 (carbonyl), 1240, 1057, 1043, 1030 and 1009 cm.$^{-1}$. (Found: C, 67.7; H, 8.4. $C_{23}H_{34}O_6$ requires: C, 68.0; H, 8.4%). ORD: in dioxane, {M} +6,810 (307 mμ, peak), -9,800 (260 mμ, trough), -9160 (263 mμ, shoulder), +25 (589 mμ), +19° (700 mμ). Elution with benzene-ethyl acetate (3:1) gave a syrup (12 mg) which was crystallised from ethyl acetate, m.p. 258-264°. This material was not investigated further.

4-Hydroxy-4,6-pregnadiene-3,20-dione (IV) (a) 4α,5,6β-trihydroxy-5α-pregnane-3,20-dione (IIIc) (125 mg) was dissolved in glacial acetic acid (8 ml), concentrated hydrochloric acid (0.5 ml) was added and the mixture kept at room temperature, samples being removed at intervals for UV analysis. After 50 hours the reaction mixture was blown down and the residue crystallised from methanol. Two crystallisations from methanol gave pure 4-hydroxy-4,6-pregnadiene-3,20-dione (83 mg) (IV), m.p. 239-241°, $\lambda_{max.}^{CH_3OH}$ 316 mμ ($\varepsilon_{max.}$ 21,200), $\lambda_{max.}^{0.2M\ KOH}$ 360 mμ ($\varepsilon_{max.}$ 15,600), $\lambda_{max.}^{0.3M\ HCl}$ 321 mμ ($\varepsilon_{max.}$ 20,000), $\nu_{max.}^{KBr}$ 3350 (Hydroxyl), 2940, 2860, 1690 (carbonyl), 1640, 1610, 1370, 1180, 1145 and 1100 cm.$^{-1}$, (Found: C, 76.8; H, 8.4. $C_{21}H_{28}O_3$ requires: C, 76.8; H, 8.6%).

(b) 4β,5,6β-trihydroxy-5β-pregnane-3,20-dione (IIc) (25 mg) was dehydrated as described above. The product was crystallised from methanol, (16 mg) m.p. 240-242°, $\lambda_{max.}^{CH_3OH}$ 317 mμ ($\varepsilon_{max.}$ 22,000), infrared spectrum identical with that of the above material.

(c) 4α,5,6β-trihydroxy-5α-pregnane-3,20-dione 6-acetate (IIId) (15 mg) was dehydrated as described above. The product was crystallised from methanol, (9 mg) m.p. 237-241°, $\lambda_{max.}^{CH_3OH}$ 316 mμ ($\varepsilon_{max.}$ 18,500), infrared spectrum identical with that of the above material.

Hydrolysis of 4α,5,6β-Trihydroxy-5α-pregnane-3,20-dione 6-acetate (IIId) The steroid (20 mg) was dissolved in methanol (1 ml) and aqueous potassium bicarbonate (10 mg in 0.3 ml) was added. The mixture was kept at room temperature for 17 hours, blown down to about 0.5 ml and the crystalline solid filtered off and dried (12 mg). Two crystallisations from methanol gave 4α,5,6β-trihydroxy-5α-pregnane-3,20-dione (IIIc), m.p. 260-264°, infrared spectrum identical with that of an authentic sample.

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Table 1

Nuclear Magnetic Resonance Maxima

Compound	Functional Group c.p.s.			
	C_4-H	C_4-OH	C_{19}-H	C_{18}-H
5-hydroxy-5β-cholestan-3-one	-	-	62.5	43.0
4β,5-dihydroxy-5β-cholestan-3-one (IIa)	270.4[a]	224.5[a]	62.5	42.5
4β,5-dihydroxy-5β-pregnane-3,20-dione (IIb)	263.8[a]	218.8[a]	61.3	38.0
4β,5,6β-trihydroxy-5β-pregnane-3,20-dione (IIc)	259.1[a]	235.7[a]	72.5	40.0
5-hydroxy-5α-cholestan-3-one	-	-	71.0	41.5
4α,5-dihydroxy-5α-cholestan-3-one (IIIa)	246.3[a]	223.8[a]	75.0	41.0
4α,5-dihydroxy-5α-pregnane-3,20-dione (IIIb)	243.8[a]	221.3[a]	73.3	37.5
4α,5,6β-trihydroxy-5α-pregnane-3,20-dione (IIIc)	280.0	232.5	87.5	40.0
4α,5,6β-trihydroxy-5α-pregnane-3,20-dione 6β-acetate	273.8[a]	236.3[a]	84.0	40.8

[a]-doublet J = 2.5 c.p.s.

Table 11

Calculated and observed values for C_{19}-protons

Compound or Substituent	Increment c.p.s.	C_{19}-H c.p.s. Calculated	Observed
4α,5-dihydroxy-5α-pregnane-3,20-dione (IIIb)			73.3
6β-hydroxyl in 5α-steroid	13.5		
4α,5,6β-trihydroxy-5α-pregnane-3,20-dione (IIIc)		86.8	87.5
4β,5-dihydroxy-5β-pregnane-3,20-dione (IIb)			61.3
6β-hydroxyl in 5β-steroid	11.5		
4β,5,6β-trihydroxy-5β-pregnane-3,20-dione (IIc)		72.8	72.5
4α,5-dihydroxy-5α-pregnane-3,20-dione (IIIb)			73.3
6β-acetoxy in 5α-steroid	11.0		
4α,5,6β-trihydroxy-5α-pregnane-3,20-dione (IIId)		84.3	84.0