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SYNTHESIS OF 2-HYDROXYESTRADIOL-17β[1]

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Abstract—The synthesis of 2-hydroxyestradiol-17β from 19-nortestosterone is described.

RECENTLY, two new metabolites of estrogens, 2-methoxy estrone,[2,3] and 2-methoxyestriol[4] have been isolated from human urine. To explain the formation of these new metabolites a two step mechanism was proposed[2] involving the oxidation of estrogens at C-2 position leading to 2-hydroxyestrogens and subsequent methylation giving rise to 2-methoxy estrogens. For a better understanding of the mechanism it was found necessary to synthesize 2-hydroxy-estrogens and the synthesis of 2-hydroxyestradiol-17β forms the subject of this paper. It may be mentioned in this connexion that the synthesis of 2-hydroxyestradiol has been outlined by a different method[5] without any physical constants for the intermediates or the final compound, and no further detailed publication to this date is available.

17β-Acetoxy-4-estrene-3-one[6] (19-nortestosterone 17β-acetate) (I) was reacted with lead tetra-acetate[7] to give a mixture of two isomeric diacetates in about 20–25 per cent yield from which 2α,17β-diacetoxy-4-estrene-3-one(IIa) m.p. 235–237°, and 2β,17β-diacetoxy-4-estrene-3-one(III) m.p. 185–186° were separated by fractional crystallization; of the two diacetates, compound IIa, m.p. 235–237°, predominated the mixture. The structural proof and configurations assigned to these two diacetates followed from their elemental analysis, and a comparison of their molecular rotations. The molecular rotatory difference (ΔM_D) for the introduction of acetoxy group in IIa is $-48{\cdot}5$ whereas in III ΔM_D is $-522{\cdot}8$. It was shown previously[7d,8] that the 2β-acetoxy group contributes more strongly to the levorotation in Δ^4-3-keto steroids.

When it was attempted to isomerize III to the more stable form IIb by refluxing

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[1] Preliminary communication, *Chem. & Ind.* 1454 (1959).
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[2] S. Kraychy and T. F. Gallagher, *J. Biol. Chem.* **299**, 519 (1957).

[3] L. L. Engel, B. Baggett and P. Carter, *Endocrinology* **61**, 112 (1957).

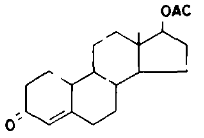
[4] J. Fishman and T. F. Gallagher, *Arch. Biochem. Biophys.* **77**, 511 (1958).

[5] G. C. Mueller, *Nature, Lond.* **176**, 127 (1955).

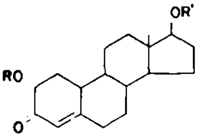
[6] J. A. Hartman, A. J. Tomasewski and A. S. Dreiding, *J. Amer. Chem. Soc.* **78**, 5662 (1956).

[7] [a] G. Ehrhart, H. Ruschig and W. Amuller, *Angew. Chem.* **52**, 363 (1939); [b] T. Reichstein and C. Montigel, *Helv. Chim. Acta* **22**, 1212 (1939); [c] E. Seebeck and T. Reichstein, *Helv. Chim. Acta* **27**, 948 (1944); [d] F. Sondheimer, S. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, *J. Amer. Chem. Soc.* **72**, 4712 (1953); [e] G. Rosenkranz, O. Mancera and F. Sondheimer, *J. Amer. Chem. Soc.* **77**, 145 (1955); [f] R. L. Clarke, K. Dobriner, A. Mooradian and C. M. Martini, *J. Amer. Chem. Soc.* **77**, 661 (1955).

[8] P. Narasimha Rao and L. R. Axelrod, *J. Amer. Chem. Soc.* In press.

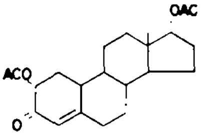


I

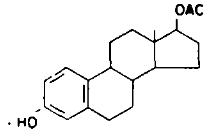


II, (a) R=-COMe, R'=-COMe

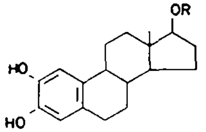
(b) R=H, R'=-COMe



III



IV



V, (a) R=-COMe

(b) R=H

with potassium acetate in glacial acetic acid,[7f] the acetoxy group in III was eliminated, and estradiol 17β-acetate(IV) was obtained by aromatization of ring A.

Controlled hydrolysis of IIa, under a nitrogen atmosphere and at room temperature with exactly one equivalent of potassium hydroxide selectively hydrolysed the C-2 acetate group to give 2α-hydroxy-17β-acetoxy-4-estrene-3-one(IIb). The hydroxyl group in IIb was then oxidized with bismuth trioxide in acetic acid[9] to give the 2,3-dione which immediately aromatized and 2,3-dihydroxy-17β-acetoxyestra 1,3,5(10)-triene(Va) resulted. However, compound Va could be obtained from the mixture of diacetates IIa and III by selective hydrolysis of C-2 acetate group with one equivalent of potassium hydroxide to a mixture of 2α and 2β-hydroxy compounds and then oxidizing the mixture with bismuth trioxide in acetic acid. The 17β-acetoxy compound Va was then hydrolyzed by stirring at room temperature with 1N sodium hydroxide in aqueous ethanol for 18 hours to give 2,3,17β-trihydroxyestra-1,3,5 (10)-triene (2-hydroxy-estradiol-17β (Vb) in excellent yield.

Attempts to oxidize Vb using mild oxidation procedures, in the hope of obtaining 2-hydroxyestrone were not successful.

EXPERIMENTAL[10]

Melting points. All melting points were determined on samples dried under high vacuum at 60° for 24 hr and were uncorrected.

Absorption spectra. The ultraviolet absorption spectra were determined in methanol with a Cary recording spectrophotometer (Model 11 MS). The infrared absorption spectra were determined in potassium bromide disk on a Perkin-Elmer (Model 21) spectrometer.

17β-Acetoxy-4-estrene-3-one(19-nortestosterone 17β-acetate)(I).[11] This compound was prepared as described in the literature.[8]

Reaction of 17β-acetoxy-4-estrene-3-one with lead tetra-acetate

Preparation of (IIa) *and* (III). To a solution of 50 g 17β-acetoxy-4-estrene-3-one in 280 ml glacial acetic acid, 85 g lead tetra-acetate in 560 ml glacial acetic acid was added in one lot and the contents stirred at 85-90°. At the end of 2 hr, (starch-iodide test negative) the acetic acid was removed under vacuum and 4 l. ice cold water added. The residue was dissolved in 250 ml methanol and kept overnight at 10°. The mixture of diacetates (IIa) and (III) (14 g; 24%) m.p. 180–200° fractionally crystallized from methanol as 6·7g *2α,17β-diacetoxy-4-estrene-3-one*(IIa) m.p. 235-237°, $(\alpha)_D^{23\cdot5}$ +24° ($CHCl_3$), M_D +89·9; λ_{max}^{MeOH} 240 mμ, (ε, 14,230); ν_{max}^{KBr} 1732, 1680 and 1614 cm^{-1}. (Found: C, 70·8; H, 8·1. $C_{22}H_{30}O_5$ requires: C, 70·5; H, 8·1 %).

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[9] [a] W. Rigby, *J. Chem. Soc.* 793, (1951); [b] J. S. Baran, *J. Amer. Chem. Soc.* **80**, 1687 (1958).

[10] Analysis were performed by Micro-Tech Laboratories, Skokie, Illinois.

[11] Obtained from Arapahoe Chemicals Inc., Boulder, Colorado.

Isolation of (III). The mother combined liquors from the fractional crystallization were evaporated and the residue crystallized from ethyl acetate. After 3 crystallizations 1·3 g analytically pure *2β,17β-diacetoxy-4-estrene-3-one*, (III) m.p. 185–186°, $(\alpha)_D^{23 \cdot 5}$ −101·6° ($CHCl_3$); M_D −380·5; λ_{max}^{MeOH} 242 mμ (ε, 16,530); ν_{max}^{KBr} 1737, 1727, 1682, and 1624 cm^{-1} was obtained. (Found: C, 70·6; H, 7·9. $C_{22}H_{30}O_5$ requires: C, 70·5; H, 8·1%).

Reaction of III *with potassium acetate in glacial acetic acid.* To a solution of 775 mg 2β,17β-diacetoxy-4-estrene-3-one(III) in 25 ml glacial acetic acid, 4 g anhydrous potassium acetate was added and refluxed in an atmosphere of nitrogen for 15 hr. The ethyl acetate extract was washed with water, 10% aqueous sodium bicarbonate, and again with water until neutral. The product (520 mg) crystallized from methanol as 3-hydroxy-17β-acetoxyestra-1,3,5 (10)-triene(IV) (estradiol 17β-monoacetate) m.p. 217–218°; $(\alpha)_D^{24}$ + 40° λ_{max}^{MeOH} 280 mμ (ε, 2754), λ_{min}^{MeOH} 250 mμ, (ε, 1096), ν_{max}^{KBr} 3475, 1703, 1620, 1585 cm^{-1}; previously reported[12] m.p. 217–219°; $(\alpha)_D^{20}$ + 47°; λ_{max} 280 mμ (log ε 3·42), λ_{min} 250 mμ (log ε 3·03). Acetylation of IV with pyridine and acetic anhydride gave 3,17β-diacetoxy-estra-1,3,5 (10)-triene (estradiol 3,17β-diacetate) m.p. 128–129° and its identity has been established by comparison of the infrared spectrum and mixture m.p. determination with an authentic sample.

2α-Hydroxy-17β-acetoxy-4-estrene-3-one(IIb). Compound IIa 5·78 g in 185 ml absolute methanol were stirred in an atmosphere of nitrogen at 30°. Compound IIa was not completely soluble, and 13·27 ml 0·99 M potassium hydroxide in absolute methanol was added and the stirring continued. The solution gradually turned yellow and after 13 min all solid had dissolved; 180 ml methanol containing 5 ml water was added and the stirring continued for 2 min. It was then acidified with 25 ml 1 N acetic acid and the solvent removed under vacuum at room temp and diluted with water. The product (4·8 g) crystallized from dilute methanol as 3·9 g (77% yield) *2α-hydroxy-17β-acetoxy-4-estrene-3-one*(IIb) m.p. 148–149°. The analytical product m.p. 150–151°, $(\alpha)_D^{23 \cdot 5}$ +76·4 ($CHCl_3$) λ_{max}^{MeOH} 239·5 mμ (ε, 15,920) ν_{max}^{KBr} 3470, 1738, 1673 and 1610 cm^{-1} (Found: C, 72·4; H, 8·4. $C_{20}H_{28}O_4$ requires: C, 72·3; H, 8·5%).

2,3-Dihydroxy-17β-acetoxyestra-1,3,5 (10)-triene (Va). To a solution of 590 mg 2α-hydroxy-17β-acetoxy-4-estrene-3-one in 9 ml glacial acetic acid, 600 mg bismuth trioxide was added and the mixture heated at 100–105° with stirring. After 30 min another 200 mg bismuth trioxide was added and heating continued for 30 min. The ethyl acetate extract was washed with water, saturated sodium bicarbonate solution, and then with water until neutral and dried over sodium sulphate. The product crystallized from benzene to give 340 mg (58% yield) *2,3-dihydroxy-17β-acetoxyestra*-1,3,5 (10)-*triene* (Va), m.p. 180–182°. The analytical product m.p. 183–185°; $(\alpha)_D^{23 \cdot 5}$ +60·3° ($CHCl_3$); λ_{max}^{MeOH} 288 mμ (ε, 4213); λ_{min}^{MeOH} 253 mμ (ε, 413); ν_{max}^{KBr} 3432, 1700, 1600, 1512 and 1446 cm^{-1} (Found: C, 72·75; H, 7·95. $C_{20}H_{26}O_4$ requires: C, 72·7; H, 7·9%).

Preparation of Va *from the mixture of diacetates* IIa *and* III. To a suspension of 12·3 g of mixture of diacetates IIa and III (m.p. 180–200°) in 600 ml absolute methanol, 28·2 ml 0·99 M potassium hydroxide in absolute methanol was added under nitrogen atmosphere and the contents stirred at 30° until all the solid was in solution. It was then diluted with 175 ml methanol containing 5 ml water, stirring continued for 6 min and then acidified with 50 ml 1N acetic acid, most of the solvent was removed under vacuum at room temp and then diluted with water. The crude mixture of 2α and 2β-hydroxy-17β-acetoxy-4-estrene-3-one, 10·2 g was oxidized with bismuth trioxide.

Bismuth trioxide oxidation. The aforementioned solid 10·2 was dissolved in 160 ml glacial acetic acid and 10 g bismuth trioxide added and was heated at 100–105° with stirring for 30 min; another 4 g bismuth trioxide was introduced and the heating and stirring continued for 30 min. The ethyl acetate extract was washed successively with water, saturated sodium bicarbonate solution and again with water until neutral, and dried over sodium sulphate. The product crystallized from benzene as 5·4 g (54%) of 2,3-dihydroxy-17β-acetoxyestra-1,3,5 (10)-triene (Va) m.p. 180–182° identical in all respects with the material obtained by bismuth trioxide oxidation of IIb.

2,3,17β-Trihydroxyestra-1,3,5 (10)-*triene* (*2-hydroxyestradiol*-17β) (Vb). To a solution of 1·2 g Va in 20 ml ethyl alcohol under nitrogen atmosphere, a solution of 1 g sodium hydroxide in 5 ml water was added and the mixture stirred for 18 hr at room temp, then acidified with acetic acid, diluted with water and extracted with ethyl acetate. The ethyl acetate extract was washed with water, 10% aqueous sodium bicarbonate, and again with water, and dried over sodium sulphate. The product 1·1 g crystallized from dilute methanol as *2,3,17β-trihydroxyestra*-1,3,5 (10)-*triene* (*2-hydroxyestradiol*-17β) Vb in light tan colored prisms. The analytical product was obtained with one molecule of solvent of

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[12] C. Djerassi, G. Rosenkranz, J. Romo, S. Kaufman and J. Pataki, *J. Amer. Chem. Soc.* 72, 4534 (1950).

crystallization, m.p. 162–163° (at 102–106° the compound shrinks with loss of solvent of crystallization and melts at 162–163° with slight decomposition), $(\alpha_{\mathrm{D}}^{23\cdot5}$ $+83\cdot2°$ (ethanol), $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ 288 mμ (ε, 4042), $\lambda_{\mathrm{min}}^{\mathrm{MeOH}}$ 253 mμ (ε, 457); $\nu_{\mathrm{max}}^{\mathrm{KBr}}$ 3440 (broad band) 1600, 1512 and 1445 cm^{-1} (Found: C, 71·2; H, 8·8. $C_{18}H_{24}O_3$, CH_3OH requires: C, 71·2; H, 8·8%). The *triacetate* of Vb was prepared with pyridine and acetic anhydride in the cold, and it crystallized from methanol in colorless plates m.p. 168–169°; $(\alpha)_{\mathrm{D}}^{23\cdot5}$ $+52\cdot3$ ($CHCl_3$); $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ 277 mμ (ε, 1480), 271 mμ (ε, 1480), $\lambda_{\mathrm{min}}^{\mathrm{MeOH}}$ 375 mμ (ε, 1344); 248 mμ (ε, 366); $\nu_{\mathrm{max}}^{\mathrm{KBr}}$ 1775, 1722, 1500, 1250 and 1215 (broad) cm^{-1} (Found: C, 69·47; H, 7·37. $C_{24}H_{30}O_6$ requires: C, 69·55; H, 7·29%).