Synthesis of Epimeric 15-Hydroxyestriols, New and Potential Metabolites of Estradiol[1]

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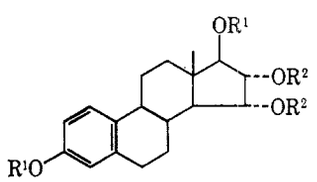
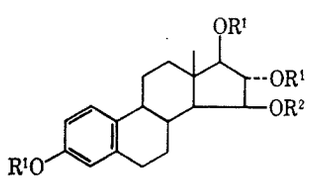
The synthesis of estra-1,3,5(10)-triene-3,15α,16α,17β-tetrol, a new metabolite of estradiol, and of estra-1,3,5(10)-triene-3,15β,16α,17β-tetrol, a potential metabolite, are described. The nuclear magnetic resonance spectra of the corresponding tetraacetates which were instrumental in assigning the correct structures are discussed.

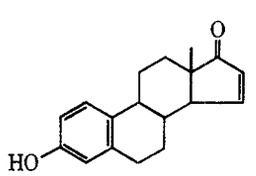
A new polar metabolite of estradiol was isolated from both pregnancy and neonatal urine.[2-4] It constitutes the main product of fetal metabolism and apparently is not produced at all by the adult.[3] Initial chemical characterization on the very small amounts available indicated the compound to be a tetrol, but the location of the hydroxyl groups was undetermined. It was apparent that a definite structure assignment for this novel and interesting metabolite required the synthesis of possible estrogen tetrols for comparison purposes. A most likely candidate was estra-1,3,5,(10)-triene-3,15α,16α,17β-tetrol (Ia) and the synthesis of this compound was undertaken. The epimeric 15β-hydroxytetrol represents another potential natural substance and the preparation of estra-1,3,5(10)-triene-3,15β,-16α,17β-tetrol (IIa) was also initiated. The successful synthesis of 15α-hydroxyestriol Ia described below permitted its comparison[5] with the unknown metabolite and thus their complete identity was established. The structure of the major fetal metabolite of estradiol could therefore be described as the tetrol Ia.



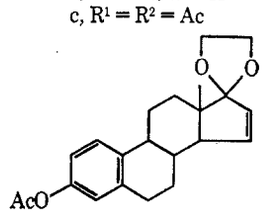
Ia, $R^1 = R^2 = H$
b, $R^1 = Ac$; $R^2 = H$
c, $R^1 = R^2 = Ac$



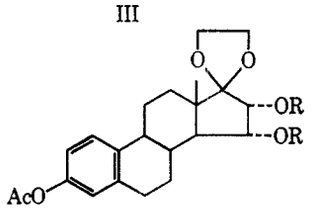
IIa, $R^1 = R^2 = H$
b, $R^1 = Ac$; $R^2 = H$
c, $R^1 = R^2 = Ac$



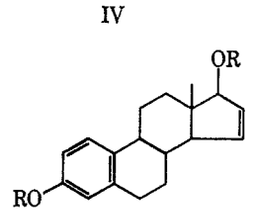
III



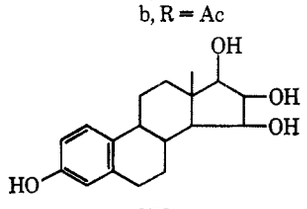
IV



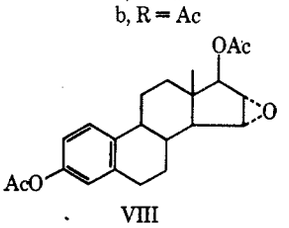
Va, R = H
b, R = Ac



VIa, R = H
b, R = Ac



VII



VIII

The initial route to estra-1,3,5(10)-triene-3,15α,-16α,17β-tetrol (Ia) involved *cis* hydroxylation of the double bond in the known α,β-unsaturated ketone III.[6] The advantage of this particular starting material resided in the possibility of preparing the two C-17 epimeric tetrols upon reduction of the 17-ketone intermediate. Because of the instability of the highly strained cyclopentenone structure[7] with the attendant possibilities of double-bond migration prior to hydroxylation the dioxolane derivative IV was chosen as the substrate for the OsO_4 oxidation. Oxidation of IV gave the 17,17-ethylenedioxyestra-1,3,5(10)-triene-3,15α,16α-triol 3-acetate (Va) as the major product, isolated as the triacetate Vb. The orientation of the D-ring hydroxyl groups was confirmed as α on the basis of nmr evidence presented later. Attempts to remove the dioxolane group of Vb with toluenesulfonic acid in acetone at room temperature failed completely. When aqueous sulfuric acid in warm dioxane was used, hydrolysis of the acetate groups took place and the resultant rearrangements gave a mixture containing a multitude of products. The stability of Vb is in contrast to the dioxolane derivatives of both the α,β-unsaturated and saturated 17 ketones which react readily under mild conditions. The apparent cause is the presence of substituent at C-16 since the 16α-bromo-17-diethylene ketal derivative[6] is similarly inert to mild deketalizing conditions. Whether this effect[8] is limited to 16α substituents only or is also produced by 16β substitution requires further study.

Oxidation of the Δ^{15} double bond by OsO_4 in the presence of a preformed 17β-hydroxy group was the next method of choice. Reaction of the 3-hydroxyestra-1,3,5(10),15-tetraen-17-one (III) with $NaBH_4$ even under the mildest conditions resulted in reduction of the double bond to give estradiol-17β as the sole product. $LiAlH_4$ at 0° in ether, however, gave the desired allylic alcohol, estra-1,3,5(10),15-tetraene-3,-17β-diol (VIa), isolated as the diacetate VIb, in sat-

(1) Part of this work has been reported in a preliminary form: J. Fishman and H. Guzik, *Tetrahedron Lett.*, 2929 (1967).

(2) A. A. Hagen, M. Barr, and E. Diczfalusy, *Acta Endocrinol.* (Copenhagen), **49**, 207 (1965).

(3) E. Gurpide, J. Schwers, M. T. Welch, and S. Lieberman, *J. Clin. Endocrinol. Metab.*, **26**, 1355 (1966).

(4) J. Schwers, E. Gurpide, R. L. Vande Wiele, and S. Lieberman, *ibid.*, **27**, 1403 (1967).

(5) G. Zucconi, B. P. Lisboa, E. Simonitsch, L. Roth, A. A. Hagen, and E. Diczfalusy, *Acta Endocrinol.*, **56**, 413 (1967).

(6) E. W. Cantrall, R. Littell, and S. Bernstein, *J. Org. Chem.*, **29**, 214 (1964).

(7) W. S. Johnson and W. F. Johns, *J. Amer. Chem. Soc.*, **79**, 2005 (1955).

(8) This effect is not likely to be due to steric hindrance but may involve a greater preference for a tetrahedral C-17 in the presence of a C-16 substituent: M. M. Kreevoy, C. R. Morgan, and R. W. Taft, Jr., *ibid.*, **82**, 3064 (1960).

isfactory yield.[9] Reduction of the 17 ketone in III led stereoselectively to the β alcohol at C-17 since catalytic hydrogenation of VIb gave only estradiol 17β-diacetate with no evidence for the presence of estradiol 17α-diacetate.

Oxidation of the allylic diacetate VIb with OsO_4 produced as the major product estra-1,3,5(10)-triene-3,15α,16α,17β-tetrol 3,17-diacetate (Ib). A small amount of another product tentatively assigned the isomeric 15β,16β-diol structure VII, was isolated by preparative thin layer chromatography; the amount of material obtained prevented further characterization. The desired estrogen tetrol Ia was obtained from either the diacetate Ib or the tetraacetate Ic by heating with K_2CO_3 in methanol; more drastic alkaline or acid conditions or reductive cleavage with $LiAlH_4$ resulted in poorer yields of the free tetrol.

Estra-1,3,5(10),15-tetraene-3,17β-diol diacetate (VIb) also served as the starting material for the preparation of the isomeric tetrol IIa. Reaction of VIb with per acid gave 15α,16α-epoxyestra-1,3,5(10)-triene-3,17β-diol diacetate (VIII) as the main product. The location and orientation of the epoxide oxygen in VIII was confirmed by $LiAlH_4$ cleavage which gave estra-1,3,5(10)-triene-3,16α,17β-triol as the only product. The opening of the 15α,16α-epoxide with $LiAlH_4$ adheres to the established preference for axial hydroxyl generation[10] in that the bisectional 16α-hydroxyl rather than the pseudo-equatorial 15α-hydroxyl is produced.

In view of the direction of reductive opening of the epoxide in VIII it was expected that acetic acid would also yield the axial product. In fact the only identified product of the reaction of VIII with glacial acetic acid was estra-1,3,5(10)-triene-3,15β,16α,17β-tetrol 3,15,17-triacetate (IIb), isolated as the tetraacetate IIc. Hydrolysis of IIc with K_2CO_3 in aqueous methanol at room temperature gave the tetrol IIa. The milder hydrolytic conditions were required in view of the demonstrably lesser stability of the 15β-tetrol IIa compared with that of the 15α-tetrol Ia.

Nmr spectroscopy was instrumental in assigning the orientation of the newly introduced hydroxyl groups. *A priori* one may expect that the 15β substituent would produce a much larger effect on the chemical shift of the C-18-methyl group than a corresponding 15α substituent.[11,12] This follows not only from the β orientation but also from 1,3 diaxial relationship of the C-18 methyl with the 15β substituent. The C-18-methyl shifts of the various compounds of interest are listed in Table I. It is apparent from these data that the chemical shifts are fully consistent with the assigned orientations. It has been emphasized[13] that the assignment of ring-D substituent orientations on the basis of C-18-methyl shifts is hazardous. This is true with respect to the bisectional C-16 substituents, but C-15 substituents which have pseudo- axial and -equatorial conformations can be firmly assigned on this basis.

The nmr of the C-15, -16, and -17 protons in the isomeric tetrol acetates Ic and IIc further confirms their structures. The first-order analysis of the spectrum of the 15α-acetoxy compound Ic permits assignment of a doublet at 303 cps ($J \sim 6$ cps) to the 17α-H. A pair of doublets ($J \sim 6$, 8 cps) centered at 325 cps represents the 16β-H, while a multiplet at 310 cps is assigned to the pseudo-axial 15β-H, and reflects its coupling with both the 16β and 14α protons. The spectrum of the 15β-acetoxy compound IIc presents a substantially different picture. The 17α-H resonance appears as a doublet at 292 cps owing to coupling with the 16β-H with an apparent coupling constant of 6 cps. The 16β proton now appears as a doublet (J = 6 cps at 312 cps) on which is superimposed a broad singlet at 310 cps representing the 15α proton. The assignments in both spectra were confirmed wherever possible by double resonance studies. The significant feature of these spectra is the identical coupling of the 17α-H in both indicating the same orientation of the 16α-acetoxy substituent in both compounds. Also significant is the greatly decreased coupling of the 15 proton in IIc in accord with its equatorial α orientation. These findings are pertinent since on the basis of the C-18-methyl chemical shifts alone (64 cps observed *vs.* 59 cps calculated) the possibility of opening the epoxide in VIII to give a 15α,16β,17β product would have to be considered.

The demonstrated dextorotatory effect of 15α substituents and the levorotatory shift of 15β substituents[14] permitted a further confirmation of the orientation of the C-15 substituents in the new tetrols. The molecular rotation differences calculated for compounds Ia and IIa show ΔM_D +226 for Ia and ΔM_D −61 for IIa and are in accord with the assigned structures.

TABLE I
CHEMICAL SHIFTS OF C-18-METHYLS

Compound	C-18 methyl[a] Obsd	Calcd
Estra-1,3,5(10)-triene-3,16α,17β-triol triacetate	51	...
Estra-1,3,5(10)-triene-3,15α,16α,17β-tetrol tetraacetate (Ic)	56	55
Estra-1,3,5(10)-triene-3,15β,16α,17β-tetrol tetraacetate (IIc)	64	64
Estra-1,3,5(10)-triene-3,16β,17β-triol triacetate	55	...
Estra-1,3,5(10)-triene-3,15α,16β,17β-tetrol tetraacetate	...	59
17,17-Ethylenedioxyestra-1,3,5(10)-trien-3 ol	54	...
17,17-Ethylenedioxyestra-1,3,5(10)-triene-3,15α,16α-triol triacetate (Vb)	63	61

[a] The chemical shift values are in cycles per second downfield from tetramethylsilane.

Experimental Section[15]

17,17-Ethylenedioxyestra-1,3,5(10)-triene-3,15α,16α-triol Triacetate (Vb).—A 0.5-g sample of 17,17-ethylenedioxyestra-1,3,5(10),15-tetraen-3-ol acetate (IV) dissolved in 12 ml of benzene and 1 ml of pyridine was treated with 0.5 g of osmium-tetroxide for 45 hr at room temperature. The solvents were removed *in vacuo* and the residue was stirred at room temperature for 4 hr in 34 ml of water, 12 ml of benzene, and 23 ml of methanol containing 3 g of sodium sulfite and 3 g of potassium bicarbonate. Chlorofom was then added, the mixture was filtered,

(9) The use of hydrocarbon solvents to prevent coreduction of the double bond in α,β-unsaturated ketones has been reported recently: E. I. Snyder, *J. Org. Chem.*, **32**, 3531 (1967).

(10) A. Furst and A. Plattner, *Helv. Chim. Acta*, **32**, 275 (1949); A. Furst, and R. Scotoni, *ibid.*, **36**, 1332 (1953).

(11) R. F. Zurcher, *Helv. Chim. Acta*, **46**, 2054 (1963).

(12) L. L. Smith, *Steroids*, **4**, 395 (1964).

(13) A. D. Cross and C. Beard, *J. Amer. Chem. Soc.*, **86**, 5317 (1964).

(14) E. W. Cantrall, R. Littell, and S. Bernstein, *J. Org. Chem.*, **29**, 64 (1964).

(15) Melting points were determined on a hot-stage apparatus and are corrected. Nmr spectra were obtained on a Varian A-60 instrument in deuteriochloroform with tetramethylsilane as an internal standard. Analyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich.

and the precipitate was washed with hot chloroform. The combined filtrates were washed with saturated sodium chloride solution to neutrality, dried, and evaporated. The oily residue was acetylated with acetic anhydride in pyridine and after work-up was chromatographed on 40 g of acid-washed alumina. Elution with ether–chloroform 1:1 gave 186 mg of material which was crystallized from ether–petroleum ether (bp 40–60°) and melted at 145–147°. The nmr spectrum in deuteriochloroform showed C-18 absorption at 63 cps. The acetate bands appeared at 122 (6 H) and 136 cps (3 H). The analytical sample melted at 146–148°.

Anal. Calcd for $C_{26}H_{32}O_8$: C, 66.08; H, 6.83. Found: C, 66.12; H, 6.97.

Attempted Deketalization of Vb.—A 10-mg sample of Vb was stirred at room temperature overnight in 1 ml of acetone containing 2 mg of *p*-toluenesulfonic acid. Thin layer chromatography of the reaction mixture in ethyl acetate–cyclohexane 1:1 showed the presence of only starting material. Similar results were obtained using either glacial acetic acid or *p*-toluenesulfonic acid in dioxane at room temperature. When the reaction was attempted with 10% sulfuric acid in dioxane–water 1:1 at 50° for 4 hr, a mixture of at least six compounds was obtained, none of which provided sufficient material for further work.

Estra-1,3,5(10),15-tetraene-3,17β-diol Diacetate (VIb).—A solution of 387 mg of 3-hydroxyestra-1,3,5(10),15-tetraen-17-one (III) in 400 ml of ether was stirred at 0° with 100 mg of $LiAlH_4$ for 1 hr. The excess reagent was decomposed by the addition of water and the mixture was acidified with ice-cold 5% sulfuric acid. The ether layer was washed with water, dried, and evaporated. The residue was acetylated with acetic anhydride in pyridine to give after the usual work-up 340 mg of oil. Chromatography on 20 g of alumina and elution with petroleum ether–benzene 8:2 gave 285 mg of VIb which crystallized from petroleum ether and melted at 90–92°. The nmr spectrum revealed C-18-methyl absorption at 54 cps and acetate methyls at 128 and 138 cps, $[\alpha]^{26}D$ −19° ($CHCl_3$).

Anal. Calcd for $C_{22}H_{26}O_4$; C, 74.55; H, 7.39. Found: C, 74.86; H, 7.26.

Hydrogenation of VIb.—A 10-mg sample of VIb was dissolved in 5 ml of ethanol and an equal weight of 10% Pd on charcoal was added. After hydrogenation had proceeded for 30 min, filtration and evaporation of solvent gave 10 mg of crystals, mp 120–124. The infrared spectrum was identical with that of estradiol 17β-diacetate.

Estra-1,3,5(10)-triene-3,15α,16α,17β-tetrol 3,17-Diacetate (Ib).—A 260-mg sample of the allylic diacetate VIb was oxidized with 250 g of OsO_4. The reaction and work-up conditions were as described above for the oxidation of IV. The product, an oil weighing 245 mg, was chromatographed on 15 g of alumina. Elution with benzene afforded 123 mg of Ib which crystallized from acetone–hexane and melted at 186–190°. The analytical sample obtained by further crystallization had a melting point of 189–192°. The nmr spectrum showed the C-18-methyl resonance at 51 cps and acetate methyls at 130 and 138 cps.

Anal. Calcd for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 67.82; H, 7.06.

The tetraacetate Ic was obtained from Ib with acetic anhydride and pyridine. Crystallization from methanol gave material melting at 174–178°, $[\alpha]^{23}D$ +92° ($CHCl_3$).

The nmr spectrum of the tetraacetate Ic exhibited C-18-methyl resonance at 58 and acetate methyl absorptions at 122 (3 H), 125 (6H), and 138 cps (3 H). The 17α hydrogen appeared as a doublet ($J \sim 6$ cps) at 303 cps. A pair of doublets at 325 cps was assigned to the 16β-H, while a multiplet at 310 cps was assigned to the 15β-H. Irradiation at 325 cps caused collapse of the 303-cps doublet to a singlet.

Anal. Calcd for $C_{26}H_{32}O_8$: C, 66.08; H, 6.83. Found: C, 66.28; H, 6.23.

Estra-1,3,5(10)-triene-3,15α,16α,17β-tetrol (Ia).—A 100-mg sample of tetrol diacetate Ib obtained directly from the alumina column was dissolved in 20 cc of methanol containing 20 mg of anhydrous potassium carbonate. The mixture was refluxed under N_2 for 2 hr, cooled, filtered, and taken down to dryness. The residue was separated by preparative thin layer chromatography on silica gel in the system 10% CH_3OH–90% ethyl acetate into two crystalline products. The less polar one (2 mg, mp 240–242°) was tentatively assigned the structure of the 3,15β,-16β,17β-tetrol (VII). The more polar material (22 mg) was crystallized from ethyl acetate (mp 230–235°) and represented the desired α-tetrol Ia, $[\alpha]^{25}D$ +135° (EtOH). The analytical sample melted at 234–236°.

Anal. Calcd for $C_{18}H_{24}O_4$; C, 71.02; H, 7.05. Found: C, 69.54; H, 7.86.

15α,16α-Epoxyestra-1,3,5(10)-triene-3,17β-diol Diacetate (VIII).—A solution of 400 mg of the allylic diacetate VIb in 6 ml of $CHCl_3$ was mixed with 300 mg of *m*-chloroperbenzoic acid in 8 ml of $CHCl_3$ and allowed to stand at 5° for 40 hr. After dilution with 100 ml of $CHCl_3$ the solution was washed with 5% sodium bicarbonate and then water, dried, and evaporated. The residue was chromatographed on 40 g of alumina. Elution with benzene–petroleum ether 1:1 yielded 215 mg of crystalline material, mp 165–171°. Recrystallization from acetone–petroleum ether gave the analytical sample of VIII which melted at 168–172°. The nmr spectrum showed the C-18-methyl resonance at 62 and two acetate methyl bands at 128 and 136 cps.

Anal. Calcd for $C_{22}H_{26}O_5$: C, 71.33; H, 7.08. Found: C, 70.67; H, 6.87.

$LiAlH_4$ Reduction of VIII—Reduction of 20 mg of VIII with excess $LiAlH_4$ in refluxing ether gave, after the usual work-up, 7 mg of estra-1,3,5(10)-triene-3,16α,17β-triol, mp 248°. The material, which was isolated by preparative thin layer chromatography on silica gel in ethyl acetate, was identical by mixture melting point and infrared spectral comparison with authentic estriol.

Acetolysis of VIII.—A solution of 165 mg of the epoxide VIII in 10 ml of glacial acetic acid was refluxed for 18 hr. The acetic acid was removed *in vacuo* and the residue, by thin layer chromatography, consisted of a mixture of tri- and tetraacetates IIb and IIc, respectively. The material was therefore acetylated with acetic anhydride in pyridine. After work-up and preparative thin layer chromatography in the system 1:1 cyclohexane–ethyl acetate a homogenous tetraacetate IIa was obtained, which, however, resisted crystallization. The nmr spectrum of IIc showed the C-18-methyl singlet at 64 and the acetate methyls appeared at 122 (6 H), 133 (3 H), and 138 cps (3 H). The methine hydrogens were assigned as follows: 17α-H at 292 (doublet, $J \sim 6$ cps), 16β-H at 312 (doublet, $J \sim 6$ cps), and 15α-H at 310 cps (singlet). Irradiation at 312 cps collapsed the 17α-H doublet to a singlet.

Estra-1,3,5(10)-triene-3,15β,16α,17β-tetrol (IIa).—A solution of 23 mg of the oily tetraacetate IIc in 5 ml of methanol containing 5 mg of potassium carbonate was stirred overnight at room temperature under N_2. The solution was neutralized with dilute acetic acid, and the solvents were removed *in vacuo*. The residue was taken up in chloroform–ethanol 4:1, washed with water, dried, and evaporated. The solid residue was purified by preparative thin layer chromatography in the system 10% methanol–90% ethyl acetate. The material obtained weighed 6 mg and melted at 250–257°. Recrystallization from acetone–petroleum ether gave the analytical sample of IIa: mp 257–262°; $[\alpha]^{24}D$ +40° (EtOH).

Anal. Calcd for $C_{18}H_{24}O_4 \cdot H_2O$: C, 67.06; H, 8.13. Found: C, 67.47; H, 8.03.

Registry No.—Ia, 15183-37-6; Ib, 16127-99-4; Ic, 16934-35-3; IIa, 16934-36-4; IIc, 16960-04-6; Vb, 16127-97-2; VIb, 16127-98-3; VIII, 16934-39-7.

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