6α- and 6β-Hydroxyestriol. Synthesis, Configurational Assignments, and Spectral Properties[1]

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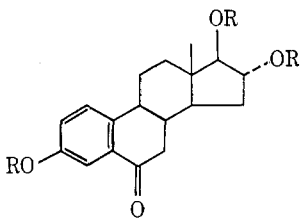
Received May 12, 1972

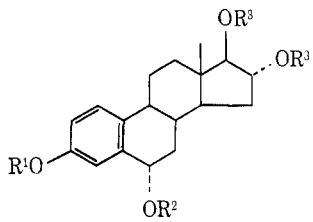
Preparation and properties of both epimers of 6-hydroxyestriol, an important metabolite in human pregnancy urine, are described. Configurational assignments are made on the basis of nmr spectral data. CD spectra for estradiol, estriol, 6-oxoestriol, 6-oxoestriol triacetate, and 6α- and 6β-hydroxyestriol are reported.

Of the two hydroxyestriols[5] present in significant amounts in human late pregnancy urine, one has been conclusively identified as 15α-hydroxyestriol.[6] The second urinary metabolite was shown to be a 6-hydroxyestriol of undefined stereochemistry.[7] A 6-hydroxyestriol had been synthesized by sodium borohydride reduction of 6-oxoestriol[8] and was designated as "6α" on the basis of a thermodynamic–steric argument advanced by Wintersteiner and Moore in the closely related case of the 6-hydroxyestradiols.[9,10] No spectral data were given for the "6α"-hydroxyestriol from borohydride reduction, or for the urinary metabolite. Identification of the latter was based on identical mobilities in a number of paper chromatographic and tlc systems and on dehydration to 6-dehydroestriol. 6β-Hydroxyestriol has not been reported or described.

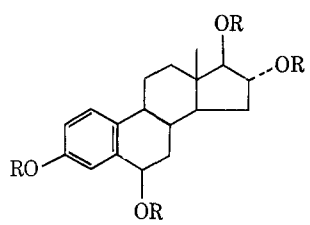
We here report the synthesis of both 6-hydroxyestriols, and assign the configurations by comparison of spectral data.



1a, R = Ac
b, R = H



2a, $R^1 = R^3 = Ac$; $R^2 = H$
b, $R^1 = R^2 = H$; $R^3 = Ac$
c, $R^1 = R^2 = R^3 = H$
d, $R^1 = R^2 = R^3 = Ac$



3a, R = H
b, R = Ac

Results and Discussion

Sodium borohydride reduction of 6-oxoestriol triacetate[8] (**1a**) at 0° gave a readily separable mixture of two acetates, later identified as 6α-hydroxyestriol 3,16,17-triacetate (**2a**) and 6α-hydroxyestriol 16,17-diacetate (**2b**). Phenolic acetates are known to be readily hydrolyzed by methanolic borohydride solutions.[11,12] In this case, while **2b** was the major product (65:35) in methanol, **2a** predominated (85:15) in ethanol. Mild alkaline hydrolysis of **2a** or **2b** gave the tetrol **2c**, whose physical constants agreed with those previously reported for "6α"-hydroxyestriol.[8]

Triacetate **1a** was hydrolyzed to the known[8] 6-oxoestriol (**1b**) which was hydrogenated over platinum to the tetrol **3a**. Tetrols **2c** and **3a** each showed a single spot on tlc but were not separable from one another in the systems used; however, mixture melting points were depressed by 20–40°. From the spectral data presented below we may conclude that, while a few per cent of the other isomer would be undetected, samples of **2c** and **3a** obtained in this manner are at least 95% configurationally pure.[12a]

We sought to establish the configurations of the tetrols **2c** and **3a** by a comparison of their C-6 proton nmr signals. The spectrum of **3a** (determined in methanol-d_4 because the tetrols are insoluble in chloroform, benzene, and acetone) showed clearly the C-17 proton signal as a doublet at 3.47 ppm and the C-16 proton signal as a multiplet at 4.07 ppm, but the C-6 proton signal at *ca.* 4.6 ppm was largely obscured by the huge CD_3OH peak. Consequently, tetraacetates **2d** and **3b** were prepared and their spectra were determined in deuteriochloroform. Both displayed identical signals for the C-17 and C-16 protons, the former a doublet at 4.98 ppm, the latter a broad unsymmetrical triplet at 5.19 ppm. The C-6 proton signals (at 6.02 ppm in both spectra), however, differed significantly. That of **2d** was a broad, poorly defined triplet ($J \approx 8$ Hz, with further splitting) while that of **3b** was a narrow doublet ($J = 2.5$ Hz, with further splitting). The widths at half height were, respectively, 17 and 5 Hz. Thus proof of the orientation of the C-6 proton as pseudoaxial in **2d** and pseudoequatorial in **3b** conclusively establishes the configuration of the hydroxyl group at C-6 as α (pseudoequatorial) in **2d** and β (pseu-

(1) Supported in part by the Agency for International Development under Contract AID/csd 2491 administered by the Population Council.

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(4) Supported by NIH Grant HD-05797.

(5) Estriol is the trivial name for 1,3,5(10)-estratriene-3,16α,17β-triol.

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

(7) J. Breuer, F. Breuer, H. Breuer, and R. Knuppen, *Z. Physiol. Chem.*, **346**, 279 (1966).

(8) G. F. Marrian and A. Sneddon, *Biochem. J.*, **74**, 430 (1960).

(9) Estradiol is the trivial name for 1,3,5(10)-estratriene-3,17β-diol.

(10) O. Wintersteiner and M. Moore, *J. Amer. Chem. Soc.*, **81**, 442 (1959).

(11) K. Tsuneda, K. Yasuda, and N. Yamada, Japanese Patent 20,163 (1964); *Chem. Abstr.*, **62**, 10484*h* (1965).

(12) J. S. Elce, J. G. D. Carpenter, and A. E. Kellie, *J. Chem. Soc. C*, 542 (1967).

(12a) NOTE ADDED IN PROOF.—Tetrols **2c** and **3a** are readily separable by liquid chromatography using a Waters Model ALC-202. By this method the samples were shown to be at least 99% configurationally pure. Conditions were 6 ft × 2 mm i.d. Corasil/C_{18}, 9:1 water–acetonitrile, flow rate 1.0 ml/min. Retention times of **2c** and **3a** were 11.9 and 10.3 min, respectively.

doaxial) in **3b**. Accordingly, borohydride reduction does give the thermodynamically favored product, and on catalytic hydrogenation the less sterically hindered α side of the steroid does face the catalyst.

Comparison of the acetyl methyl group resonances in the nmr spectra of tetraacetates **2d** and **3b** showed another significant difference. Singlets at 2.06 and 2.09 ppm were assigned to the C-16, C-17 acetyl groups because identical signals also appeared at these positions in the spectra of triacetate **2a** and diacetate **2b**. In the spectrum of the 6β-tetraacetate **3b** the C-6 acetyl signal was also at 2.06 ppm, resulting in a six-proton singlet.[13] The C-6 acetyl signal of **2d** was shifted downfield 7 Hz relative to that of **3b**, resulting in three three-proton singlets at 2.05, 2.09, and 2.13 ppm.

Investigation of the CD spectra of the estriol derivatives here described (Table I and Figure 1) revealed a dramatic effect due to the 6-hydroxyl substituent. Estradiol and estriol showed nearly identical spectra with two weak negative maxima at 290 and 282 (283) nm and a strong positive maximum at 230 (229) nm. The C-16 hydroxyl group is so remote as to have little effect on either the CD absorption within the ^1L_b band or within the ^1L_a band.[14] However, the effect of the 6-hydroxyl group is strong and configurationally specific on both bands. In 6α-hydroxyestriol (**2c**) the intensity of the negative maxima at 288 and 283 nm was more than doubled relative to estriol, and the ^1L_a band CD showed a strong *negative* maximum. In 6β-hydroxyestriol (**3a**) the ^1L_b band CD maxima were weakly positive and the ^1L_a band maximum was very strongly positive ([θ] +20,000 compared to [θ] +12,000 in the case of estriol). Thus, while estradiol and estriol display ^1L_b and ^1L_a Cotton effects of opposite sign, both Cotton effects are negative for **2c** and positive for **3a**.

We had hoped, on the basis of the observed correlation of spectral data with configuration for the 6-hydroxyestriols, to be able to make unambiguous assignment of configuration to the 6-hydroxyestradiols.[10] Crabbé and Klyne reported[15] that the ORD spectrum of Wintersteiner's "6β"-hydroxyestradiol showed a weak negative Cotton effect (*a* −18) centered at 272 nm and a strong positive Cotton effect centered at 221 nm. This prompted us to determine the ORD spectrum of **3a**. It displayed a weak positive Cotton effect (*a* +9) centered at 280 nm (superimposed on a strongly positive background curve), in full accord with its CD spectrum. Consequently the question of configuration of the 6-hydroxyestradiols cannot be resolved without determination of the CD (or ORD) spectrum of a sample of demonstrated purity.

Regarding configurational identification of the 6-hydroxyestriol from pregnancy urine, our observation

TABLE I

SPECTRAL DATA FOR ESTRADIOL DERIVATIVES IN ABSOLUTE ETHANOL[a]

Compd	—Uv maxima— λ, nm	(ε)	—CD maxima— λ, nm	([θ])
Estradiol	287[b]	(1900)	290	(−310)
	282	(2100)	282	(−520)
	230[b]	(4800)	230	(+11,000)
	222	(7000)		
Estriol	287[b]	(1900)	290	(−540)
	282	(2100)	283	(−700)
	229[b]	(6100)	229	(+12,000)
	222	(8400)		
6-Oxoestriol triacetate (**1a**)			366	(+1500)
			352	(+4700)
			338	(+8900)
			326	(+10,000)
	298	(2300)	296	(−15,000)
	247	(11,000)	247	(−15,000)[c]
6-Oxoestriol (**1b**)	327	(3100)	345	(+23,000)[d]
			310	(−21,000)[d]
	256	(9300)	252	(−11,000)[e]
	222	(21,000)	222	(+27,000)
6α-Hydroxyestriol (**2c**)	288[b]	(1900)	288	(−1700)
	283	(2100)	283	(−1700)
	230[b]	(6000)	229	(−5200)
	222	(7700)		
6β-Hydroxyestriol (**3a**)	288[b]	(1900)	288	(+550)
	282	(2100)	280	(+620)
	228[b]	(6200)	227	(+20,000)
	222	(7500)		

[a] *c* 0.0028–0.021 g/100 ml; *l* = 1 cm; temperature, 25°. [b] Shoulder. [c] Negative minimum, $[\theta]_{268}$ −3000. [d] Reported for 6-oxoestradiol, $[\theta]_{345}^{MeOH}$ +24,982 (max), $[\theta]_{310}^{MeOH}$ −20,573 (max): R. C. Cambie, L. N. Mander, A. K. Bose, and M. S. Manhas, *Tetrahedron*, **20**, 409 (1964). Spectrum below 280 nm not described. [e] Negative minimum, $[\theta]_{275}$ −2500.

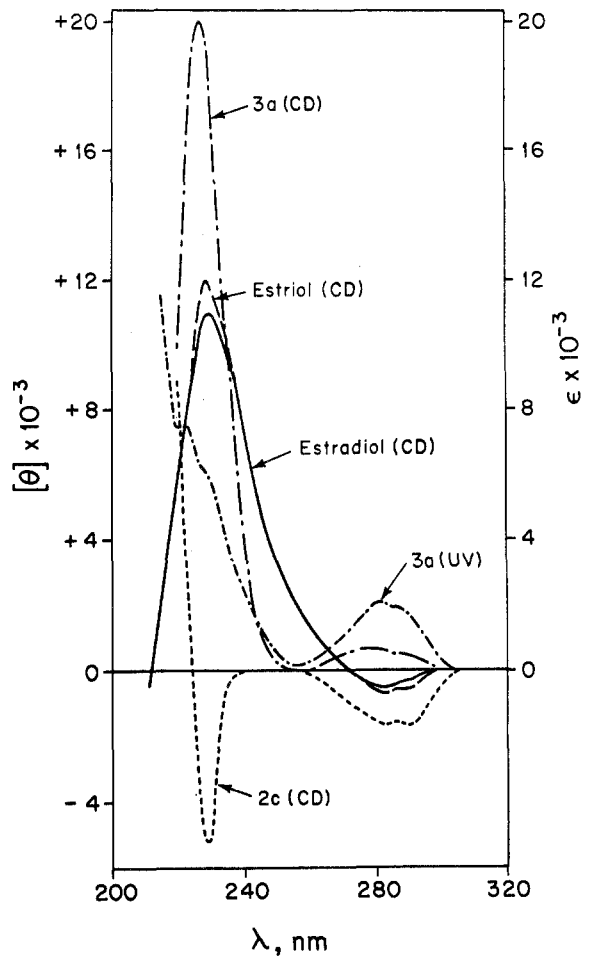

Figure 1.—Circular dichroism (CD) and ultraviolet (uv) absorption spectra of estradiol, estriol, 6α-hydroxyestriol (**2c**), and 6β-hydroxyestriol (**3a**) in absolute ethanol.

(13) Slow scan on expanded scale revealed two singlets separated by 0.5 Hz.

(14) G. Snatzke and P. C. Ho, *Tetrahedron*, **27**, 3645 (1971).

(15) P. Crabbé and W. Klyne, *ibid.*, **23**, 3449 (1967).

that the epimers were not separable by tlc renders spectral determinations essential.[12a]

Experimental Section

Melting points were taken in open capillary tubes and are corrected. Elemental analyses were done by Galbraith Laboratories, Knoxville, Tenn. Tlc systems (silica gel HF-254) were the following: system 1, 9:1 benzene–ethyl acetate; system 2, 9:1 benzene–methanol; system 3, 4:1 benzene–methanol; system 4, 19:1 ethyl acetate–absolute ethanol. Nmr spectra were determined with a Varian XL-100-15 spectrometer and chemical shifts (δ) are reported in parts per million downfield from TMS. A Beckman IR-10 spectrophotometer was used for ir spectra, and a Cary Model 14 spectrophotometer for uv spectra. CD (in absolute ethanol) and ORD (in methanol) spectra were measured using a Cary Model 60 spectropolarimeter equipped with a CD Model 6001 accessory.

6-Oxoestriol Triacetate (1a).—To a stirred, ice-cooled solution of estriol triacetate (1.96 g, 4.73 mmol) in glacial acetic acid was added during 10 min a solution of chromium trioxide (1.45 g, 14.5 mmol) in water (1 ml) and glacial acetic acid (9 ml). The ice bath was removed and stirring was continued for 24 hr. The mixture was diluted with water (300 ml) and extracted with ether (150 ml). The ether layer was washed with water followed by small portions of 2% sodium bicarbonate until the aqueous layer had pH 8 and was pink in color, and finally was washed with water. Chromatography of the contents of the dried ($MgSO_4$) ether solution (1.85 g) on silica gel (38 g, packed in benzene and eluted with 50:1 benzene–ether) gave unchanged estriol triacetate (145 mg, 7%) (R_f 0.6, system 1) followed by crude **1a** (516 mg, oil). The yield of pure **1a** (R_f 0.25, system 1; R_f 0.9, system 2), mp 137–139°, $[\alpha]^{25}D$ −48° (*c* 0.58, absolute C_2H_5OH) [lit.[8] mp 137–139°, $[\alpha]^{16.5}D$ −41° (*c* 0.493, C_2H_5OH)], crystallized from methanol, was 312 mg (15%).

Sodium Borohydride Reductions of 6-Oxoestriol Triacetate (1a).—To a stirred, ice-cooled solution of **1a** (180 mg, 0.42 mmol) in methanol (10 ml) was added sodium borohydride (60 mg, 1.6 mmol) and the mixture was stirred for 40 min at 0°. Acetic acid was added dropwise to pH 5 (moist Hydrion paper) and the mixture was evaporated to dryness. The residue was partitioned between ethyl acetate (20 ml) and water (5 ml). The ethyl acetate was washed with small portions of 1% sodium bicarbonate until the aqueous phase had pH 8 and then twice with water. Chromatography of the dried ($MgSO_4$) product so obtained (180 mg) on silica gel (3.5 g, packed in benzene and eluted with benzene–ether mixtures gradually progressing from 50:1 to 1:1) yielded 6α-hydroxyestriol 3,16,17-triacetate (**2a**) (35%, oil) (R_f 0.7, system 2) and 6α-hydroxyestriol 16,17-diacetate (**2b**) (65%, oil) (R_f 0.35, system 2). **2b** had nmr ($CDCl_3$) δ 0.84 (s, 3, C-18 H), 2.06 (s, 3, C-16 or C-17 CH_3CO), 2.09 (s, 3, C-16 or C-17 CH_3CO), 3.78 (br s, 1, disappears with D_2O, C-6 OH), 4.76 (m, 1, C-6 H), 4.96 (d, 1, J = 6 Hz, C-17 H), 5.17 (br t, 1, C-16 H), 6.37 (br s, 1, disappears with D_2O, C-3 OH), 6.69 (q, 1, J = 3 and 9 Hz, C-2 H), and 7.06 ppm (m, 2, C-1 and C-4 H). The spectrum of **2a** was identical except for the absence of the phenolic proton at 6.37 ppm, the presence of a third CH_3CO singlet at 2.28 ppm, and the positions of the aromatic quartet (6.91 ppm) and multiplet (7.25 ppm, partially obscured by $CHCl_3$).

The reduction was carried out as described above for 1 hr using 211 mg (0.49 mmol) of **1a** and 75 mg (2 mmol) of sodium borohydride in absolute ethanol (20 ml), and was worked up in a similar manner. A similar chromatographic separation (4 g of silica gel) yielded **2a** (85%) and **2b** (15%).

6α-Hydroxyestriol (2c).—A solution of diacetate **2b** (50 mg) in 0.2 *N* 95% methanolic potassium hydroxide (1 ml) was allowed to stand for 5 hr at room temperature. Most of the solvent was removed under a stream of nitrogen, and the residue was diluted with water (1 ml) and acidified with 5% hydrochloric acid. The resulting crystalline solid was collected, washed well with water, and dried at reduced pressure. **2c** so obtained (23 mg, 59%) had mp 238–240°, $[\alpha]^{25}D$ +80° (*c* 0.547, absolute C_2H_5OH) [lit.[8] mp 242–245°, $[\alpha]^{14}D$ +84° (*c* 0.498, C_2H_5OH)], ir (KBr) 920, 1305, 1325, and 1610 cm^{-1}, and was homogeneous on tlc (R_f 0.4, systems 3 and 4).

6β-Hydroxyestriol (3a).—A trial saponification of **1a** (19 mg) in 0.5 *N* 95% methanolic potassium hydroxide (0.5 ml) at room temperature for 18 hr followed by a work-up similar to that described above for **2c** yielded pure 6-oxoestriol (**1b**) (10 mg, 77%; R_f 0.55, system 3; R_f 0.5, system 4). A sample recrystallized from methanol had mp 241–242° (lit.[8] mp 240–242°). A 115-mg sample of **1a** was similarly saponified and the resulting product was dissolved in absolute ethanol (15 ml) and hydrogenated for 9 hr over platinum (from 45 mg of platinum oxide). Tlc of the residue (68 mg, 83% from **1a**) after filtration through celite and evaporation of the solvent revealed a small amount of unchanged **1b** in addition to a major component of R_f 0.4 (systems 3 and 4). The mixture was unchanged on further hydrogenation over fresh platinum, but recrystallization from methanol yielded 40 mg (47% from **1a**) of **3a**: mp 255–260° dec; mixture with **2c**, mp 218–230° dec; $[\alpha]^{25}D$ +24° (*c* 0.536, absolute C_2H_5OH); ir (KBr) 945, 1165, 1290, 1350, 1580, and 1620 cm^{-1}; nmr (CD_3OD) δ 0.83 (s, 3, C-18 H), 3.47 (d, 1, J = 6 Hz, C-17 H), 4.07 (m, 1, C-16 H), 6.68 (q, 1, J = 3 and 9 Hz, C-2 H), 6.79 (d, 1, J = 3 Hz, C-4 H), and 7.13 ppm (d, 1, J = 9 Hz, C-1 H). Absorption at *ca.* 4.6 ppm due to the C-6 proton was largely obscured by CD_3OH.

Anal. Calcd for $C_{18}H_{24}O_4 \cdot {}^1/_2CH_3OH$: C, 69.35; H, 8.18. Found: C, 69.10; H, 7.95.

6α- and 6β-Hydroxyestriol Tetraacetates (2d and 3b).—Treatment of **2c** and **3a** with acetic anhydride in pyridine at room temperature overnight gave the respective tetraacetates **2d** and **3b** as oils (lit.[8] for **2d**, mp 159–161°) inseparable by tlc (R_f 0.3, system 1; R_f 0.95, system 2) and with essentially identical ir spectra. **2d** had nmr ($CDCl_3$) δ 0.86 (s, 3, C-18 H), 2.05 (s, 3, C-16 or C-17 CH_3CO), 2.09 (s, 3, C-16 or C-17 CH_3CO), 2.13 (s, 3, C-6 CH_3CO), 2.28 (s, 3, C-3 CH_3CO), 4.97 (d, 1, J = 6 Hz, C-17 H), 5.19 (t, 1, C-16 H), 6.02 (br t, 1, J = 8 Hz, C-6 H), and 6.96 and 7.3 ppm (two m, partially obscured by $CHCl_3$, C-1, C-2, and C-4 H). **3b** had nmr ($CDCl_3$) δ 0.90 (s, 3, C-18 H), 2.06 (s, 6, C-16 or C-17 and C-6 CH_3CO), 2.09 (s, 3, C-16 or C-17 CH_3CO), 2.26 (s, 3, C-3 CH_3CO), 4.99 (d, 1, J = 6 Hz, C-17 H), 5.19 (t, 1, C-16 H), 6.02 (d, 1, J = 2.5 Hz, C-6 H), and 7.03 and 7.3 ppm (two m, partially obscured by $CHCl_3$, C-1, C-2, and C-4 H).

Registry No.—**1a**, 36614-98-9; **1b**, 7323-86-6; **2a**, 36615-00-6; **2b**, 36615-01-7; **2c**, 7291-49-8; **2d**, 36615-03-9; **3a**, 36615-04-0; **3b**, 36615-05-1; estradiol, 50-28-2; estriol, 50-27-1.