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The Epimeric 6-Hydroxy-3,17 β -estradiols

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The preparation of the epimeric 6-hydroxy-3,17 β -estradiols from 6-keto-3,17 β -estradiol diacetate is described. Reduction with sodium borohydride affords one of these as the sole product and in good yield, and on this basis this epimer is tentatively assigned the 6 α (quasi-equatorial) configuration, and the other epimer, which results from catalytic reduction with platinum oxide in ethanol, the 6 β -configuration. Meerwein-Ponndorf reduction leads to both epimers in about equal amounts.

In 1939, the senior author prepared from 6-keto-3,17 β -estradiol¹ small amounts of the 6-epimeric 6-hydroxy-3,17 β -estradiols with the intent of exploring their use as intermediates in the synthesis of 6-dehydroestrone. Since the latter compound was shortly afterward obtained by another route,² the work on these new triols was discontinued at that time but was resumed more recently when it became apparent that they might have some biological interest.³

6-Ketoestradiol diacetate was used as the starting material in all experiments. The method originally explored, namely, Meerwein-Ponndorf reduction with aluminum isopropylate, afforded both epimers in about equal amounts, but in low yield. To effect their separation from each other and from unreduced ketone the crude product was acetylated and chromatographed on alumina. The pure triol triacetates thus obtained, provisionally designated 6'' α '' and 6'' β '' (6'' α ''-hydroxy-3,17 β -estradiol triacetate, m.p. 144–145°, [α]_D +39°; 6'' β ''-hydroxy-3,17 β -estradiol triacetate, m.p. 176–178°, [α]_D +53°) on alkaline hydrolysis at room temperature yielded, respectively, 6'' α ''-hydroxy-3,17 β -estradiol (m.p. 249–251°, [α]_D +78° in ethanol) and 6'' β ''-hydroxy-3,17 β -estradiol (m.p. 191–195°, [α]_D +29° in ethanol).⁴ On reacetylation the triols gave the respective triacetates in yields indicating that little or no epimerization had occurred during the hydrolysis of the triacetates.

When the reduction was carried out with sodium borohydride in methanol, the 6'' α ''-triacetate could be obtained in good yield by direct crystallization of the acetylated reduction product. Chromatography of the mother liquor failed to yield any of the 6'' β ''-triacetate. That little, if any, of the latter epimer is formed in this type of reduction follows also from the fact that substantially pure 6'' α ''-triol could be secured by subjecting the reduction product, which consists of a mixture of the 3,17-diacetate and the 17-monoacetate, directly to alkaline hydrolysis.

In contrast, catalytic reduction with platinum oxide in ethanol gave a mixture which after acetylation and chromatography afforded 6'' β ''-triacetate as the sole crystallizable product, but in only about

20% of the theoretical yield. The low rate of conversion is in part due to the fact that even when the hydrogen uptake approached one mole (which was not always the case) a good portion of the starting ketone (20–40%) remained unreduced, so that Girard reagent had to be used for its removal. Evidently, the reduction is accompanied by some hydrogenolysis such as is likely to occur with an α -tetralone, although no estradiol diacetate could be found in the non-ketonic fraction.

The data on hand do not permit the definite assignment of absolute configurations to the two epimers. Thus, while metal hydride reduction and catalytic reduction in a neutral medium of unhindered steroidal ketones both generally lead to the same (the equatorial) alcohol,⁵ this was not the case with 6-ketoestradiol. It is of course possible that this generalization does not apply to keto groups which occupy one of the terminal positions in the planar half of a semi-chair and are activated by the adjacent ethylenic bond (or, in the present case, a benzene ring) imparting planarity on that half. Moreover, since in the catalytic reduction the yield of the 6'' β ''-epimer accounted for less than half of the ketone actually reduced, the possibility that substantial amounts of the 6'' α ''-epimer may have been formed but then selectively hydrogenolyzed cannot be disregarded. Actually a case of sorts can be made for the designations chosen by us on the following grounds: The epimer formed in good yield on reduction with sodium borohydride should be the thermodynamically more stable one, and in the present case this would be the 6'' α ''-epimer with quasi-equatorial hydroxyl. The (apparently) preferential formation of the 6'' β ''-epimer in the catalytic reduction could be explained by approach of the catalyst from the relatively less hindered α -face of the molecule. On the other hand, the sequence of elution of the two triacetates after adsorption on alumina (6'' α '' before 6'' β ''') is the opposite of what one would expect if the former were the quasi-equatorial, and the latter the quasi-axial, epimer.⁵ Pending the acquisition of more decisive configurational evidence we think it proper to retain the quotes over α and β to indicate that the above assignments should be regarded as tentative.

The biological properties of the epimeric triols will be reported elsewhere.

Experimental

The melting points were taken in open Pyrex glass capillaries and are corrected for stem exposure. The rotation measurements were carried out in a 1-dm. semi-micro tube, with chloroform as the solvent, unless indicated otherwise. The ultraviolet spectra were measured in absolute ethanol

(1) B. Longwell and O. Wintersteiner, *J. Biol. Chem.*, **133**, 219 (1940).

(2) W. H. Pearlman and O. Wintersteiner, *ibid.*, **132**, 605 (1940).

(3) Cf. for instance the report by G. C. Mueller and G. Rudeon (THIS JOURNAL, **79**, 1004 (1957)) on the conversion by mouse liver microsomes of estradiol to 6-ketoestradiol and the triol described here as 6'' α ''-hydroxyestradiol, but in their paper designated, in accordance with the terminology then used by us, 6'' β ''.

(4) Both triols tend to show variable and unsharp melting points due to solvent retention (cf. Experimental). The values given above are the highest observed.

(5) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).

in a Cary self-recording instrument model 11M. The infrared spectra were determined on Nujol mulls in the Perkin-Elmer double beam self-recording spectrophotometer model 21. The characteristics of the infrared bands are expressed in the text as follows: (s) strong, (m) medium, (l) low, (br) broad, (sh) shoulder.

6-Keto-3,17 β -estradiol was prepared from estradiol diacetate as previously described.¹ Substitution of *t*-butyl chromate⁶ for chromium trioxide as the oxidant resulted in inferior yields.

Meerwein-Ponndorf Reduction.—6-Keto-3,17 β -estradiol diacetate (499 mg.) dissolved in isopropyl alcohol freshly distilled from sodium (27 ml.) was added to a solution of aluminum isopropylate (1.7 g.) in dry benzene (20 ml.). The mixture was boiled under reflux for 20 hours, when the nitroprusside test for acetone (carried out at 5-hour intervals on 15-ml. portions of distillate with subsequent replacement of the lost volume by fresh isopropyl alcohol) had become negative. The turbid mixture was distributed between ether and 5% hydrochloric acid, and the ether phase was washed twice with new portions of hydrochloric acid, then with sodium bicarbonate solution and water. The residue from the dried and evaporated extract, which to judge from its ultraviolet spectrum still contained about 20% of unreduced ketone, was acetylated (acetic anhydride, pyridine, room temp. 16 hours) and the resulting product (518 mg.) was chromatographed in benzene-hexane 1:5 on a column (17 \times 95 mm.) of Merck acid-washed alumina, using for elution 60-ml. portions of benzene-hexane 1:3 (360 ml.), 1:1 (960 ml.), benzene (1080 ml.) and ether-benzene 1:9 (420 ml.). The 1:1 benzene-hexane eluates (109 mg., m.p. 125–138°) were combined, and on two recrystallizations from methanol yielded 52 mg. of 6'' α ''-hydroxy-3,17 β -estradiol triacetate (small rods, m.p. 143–144°, $[\alpha]^{25D} +40^\circ$ (*c* 0.944); $\lambda_{\max}^{\text{alc}}$ 268 m μ (ϵ 730), 275 m μ (ϵ 670); $\lambda_{\min}^{\text{alc}}$ 248 m μ (ϵ 335); $\lambda_{\max}^{\text{Nujol}}$ 5.67 (m, phenolic acetate), 5.80 (s, acetate), 6.71 (m, 1 aromatic C=C), 8.12 (s, ester) μ).

Anal. Calcd. for C₂₄H₃₀O₈ (414.2): C, 69.53; H, 7.30. Found: C, 69.26; H, 7.27.

The first benzene eluate (44 mg.) on recrystallization from methanol likewise yielded pure 6'' α ''-triacetate (25 mg.). The 7 benzene fractions following the second showed melting points in the range 163–172°. They were combined (127 mg.) and recrystallized twice from methanol. The 6'' β ''-hydroxy-3,17 β -estradiol triacetate thus obtained (rosettes of platelets, 50 mg.) melted at 176–178° and showed $[\alpha]^{25D} +53^\circ$ (*c* 0.860); the ultraviolet spectrum was identical with that of the 6'' α ''-triacetate. This was also true for the infrared spectrum between 2 and 9 μ ; however, there were marked differences in the 9–12 μ region.

Anal. Calcd. for C₂₄H₃₀O₈ (414.2): C, 69.53; H, 7.30. Found: C, 69.72; H, 7.22.

A solution of the 6'' α ''-triacetate (99 mg.) in 5% methanolic potassium hydroxide solution (16 ml.) was allowed to stand under nitrogen at room temperature for 20 hours, and then worked up in the usual manner. The partly crystalline product (80 mg.) was recrystallized twice from acetone-ether from which it formed tiny rectangular platelets (28 mg.) of 6'' α ''-hydroxy-3,17 β -estradiol, m.p. 233–246°, $[\alpha]^{25D} +78^\circ$ (*c* 0.746 in ethanol); $\lambda_{\max}^{\text{alc}}$ 282 m μ (ϵ 2350), sh. 287 (ϵ 2130); $\lambda_{\max}^{\text{Nujol}}$ 2.95 (s), 6.18 (l), 6.31 (m), 6.70 (m), 6.79 (m) μ . The analytical sample was dried at 137° (1 mm.) for 3 hours and then melted at 239–249°.

Anal. Calcd. for C₁₈H₂₂O₃ (288.4): C, 74.97; H, 8.39. Found: C, 75.20; H, 8.10.

Other preparations recrystallized from acetone, acetone-ether or 90% aqueous ethanol likewise melted over a wide range (4–14°), and often at lower temperatures than given above (for instance 207–211°, 228–242°). Moreover, recrystallization, or drying at 110° *in vacuo*, often resulted in a drop of the melting range. The specimen prepared in 1939, recrystallized from dry ether, showed the highest and sharpest melting point ever observed, namely, 249–251°, but this mode of recrystallization is not practicable for larger amounts.

(6) R. V. Oppenauer and H. Oberrausch, *Ann. de la Asociacion Quimica Argentina*, **27**, 246 (1949); K. Heusler and A. Wettstein, *Helv. Chim. Acta*, **35**, 284 (1952).

6'' β ''-Hydroxy-3,17 β -estradiol was obtained in the same manner from the 6'' β ''-triacetate. In an experiment starting with 70 mg. of the latter the crude product (50 mg.) was recrystallized first from ether and then from a few drops of methanol and excess ether. The resulting needles (24 mg.) showed a double melting point: 126–134° and 191–195° after resolidification, virtually unchanged after drying at 110° (1 mm.), $[\alpha]^{25D} +29^\circ$ (*c* 0.487 in ethanol); $\lambda_{\max}^{\text{Nujol}}$ 295 (sh., s), 3.10 (s), 6.21 (m), 6.32 (l), 6.69 (m), 6.80 (l) μ . The analytical sample was dried at 110° (1 mm.) for 3 hours.

Anal. Calcd. for C₁₈H₂₄O₃· $\frac{1}{2}$ CH₃OH (304.4): C, 72.98; H, 8.61. Found: C, 72.66; H, 8.70.

The specimen prepared in 1939, recrystallized from ethanol-ether, melted sharply at 200–201° after drying. Nevertheless the analysis indicated the presence of 0.5 mole of ethanol of crystallization.

Reduction with Sodium Borohydride.—A solution of 6-ketoestradiol diacetate (378 mg.) in methanol (30 ml.) was added dropwise with stirring to methanol (23 ml.) containing 243 mg. of sodium borohydride. After standing for 2 hours the solution was made acidic with 10% aqueous acetic acid and taken to dryness *in vacuo*. The product was recovered in the usual way by ether extraction and acetylated. The acetylated material (394 mg.) yielded on recrystallization from 50% and then 90% aqueous ethanol 261 mg. of 6'' α ''-triacetate, m.p. 143–144° (62% of theoretical yield).

In a subsequent experiment with 250 mg. of the ketone the reduced material was hydrolyzed with alkali as described for the triacetate. After two recrystallizations from 90% ethanol the 6'' β ''-triol (128 mg.) thus obtained melted at 244–251°. In another run starting with 345 mg. of the ketone the crude reduction product itself was subjected to fractional crystallization from ethyl acetate. The material passing into the top fraction after 4 crystallizations (32 mg. of rosettes of elongated prisms, m.p. 173–177° after drying at 110°, $[\alpha]^{25D} +46^\circ$ (*c* 0.747)) was identified as 6'' α ''-hydroxy-3,17 β -estradiol 17-monoacetate by analysis and spectral properties ($\lambda_{\max}^{\text{alc}}$ 282 m μ , (ϵ 2150), sh. 287 m μ (ϵ 1980); $\lambda_{\max}^{\text{Nujol}}$ 2.84 (sh, m), 2.94 (m), 5.79 (s), 6.22 (m), 6.68 (m), 6.79 (l), 8.02 (s) μ).

Anal. Calcd. for C₂₀H₂₆O₄ (330.4): C, 72.70; H, 7.93. Found: C, 72.71; H, 7.85.

From the ethyl acetate mother liquors there was obtained a homogeneous-looking product, m.p. 191–194°, which exhibited a similar ultraviolet spectrum but analyzed for a mixture of mono- and diacetate. The two crystalline products as well as the remaining mother liquor fractions on acetylation all yielded 6'' β ''-triacetate, m.p. 142–144°.

Catalytic Reduction.—A solution of 6-keto-3,17 β -estradiol (338 mg.) in absolute ethanol (34 cc.) was shaken in a hydrogen atmosphere with pre-reduced platinum oxide (250 mg.). The uptake came to a standstill after 6 hours with 0.93 molar equivalent of the gas consumed. The reduced material, which on the basis of its ultraviolet spectral characteristics (intensity at 300 m μ peak) contained 38% unreduced starting material, was separated with Girard reagent T into ketonic (122 mg.) and non-ketonic (124 mg.) fractions in the usual way. The non-ketonic fraction was acetylated and chromatographed on alumina, yielding in the 1:4 and 1:1 benzene-hexane eluates crystalline products with m.p. >160°, from which after purification 64 mg. of substantially pure 6'' β ''-hydroxyestradiol triacetate m.p. 174–176°, $[\alpha]^{25D} +49^\circ$, was obtained (17% of theoretical yield). In another experiment, with 102 mg. of ketone, in which only 16% of the latter remained unreduced, the yield of triacetate was only slightly better (25%). There was no evidence in either case for the presence of 6'' α ''-triacetate or of estradiol diacetate in the non-ketonic fraction.

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