[CONTRIBUTION FROM THE DIVISION OF STEROID METABOLISM AND BIOCHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Synthesis of 1,3,5(10)-Estratriene-3,16β,17α-triol[1]

JACK FISHMAN AND WARREN R. BIGGERSTAFF[2]

Received February 10, 1958

The preparation of 1,3,5(10)-estratriene-3,16β,17α-triol is described. The 16α and 16β bromo epimers of estrone were also prepared and some of their reactions were studied.

Of the four possible estriols isomeric at carbons 16 and 17 only three are known. They include the original estriol, or 1,3,5(10)-estratriene-3,16α,17β-triol (I), and the two cis isomers, 1,3,5(10)-estratriene-3,16β,17β-triol (II) and 1,3,5(10)-estratriene-3,16α,17α-triol (III). All three have been synthesized[3] and the former two have also been identified as metabolites of estradiol in the human[4]. Our interest in the metabolism of estrogens and in the chemistry of these stereoisomers prompted us to undertake the preparation of the remaining isomer 1,3,5(10)-estratriene-3,16β,17α-triol (IV).

The initial attempts were directed towards reduction of the readily available 3,16β-dihydroxy-1,3,5(10)-estratriene-17-one (XVa), or its acetate,[5] with less stereospecific reagents than lithium aluminum hydride. All the reductants tried, however, gave only 1,3,5(10)-estratriene-3,16β,17β-triol (II). The preparation of IV by the action of acetic acid on the 16α,17α oxide XIVa was then studied. Despite the previous failure to isolate any products from this reaction,[3a] we were able to obtain a small amount of the new estriol. Since, however, the α-oxide is not easily accessible, synthesis of the desired estriol isomer in more substantial quantities required another approach. The work of Fajkos[6] in the androstane series appeared to offer an attractive route to IV.

Bromination of estrone enol diacetate V gave 16α-bromoestrone acetate (VIa), a compound previously prepared[7] but in which the orientation of the bromine had not been defined. From analogy with the androstane series[6] the α-orientation was assumed, and this was borne out by subsequent reactions. Reduction at 0° of 16α-bromoestrone acetate (VIa) with lithium aluminum hydride gave the *trans* bromohydrin VIII, which was directly transformed to 16β,17β-epoxy-1,3,5(10)-estratriene-3-ol (XII) by refluxing in alkaline solution. The structure of the new oxide XII was established by reduction with lithium aluminum hydride to yield 16β-estradiol (XVb), identical (infrared spectrum and mixture melting point comparison) with a sample prepared from 1,3,5(10)-estratriene-16-one (XVI) by sodium borohydride reduction.[8] Refluxing the oxide XII with acetic acid followed by hydrolysis of the products gave a mixture of the two isomeric *trans* estriols I and IV, from which the new 1,3,5(10)-estratriene-3,16β,17α-triol (IV) was obtained in about 50% yield by chromatography and fractional crystallization.

It was of interest to prepare 16β-bromoestrone acetate (VIIa) and to compare the reactions of the two bromoketones epimeric at C-16. Although acid hydrolysis of VIa at room temperature gave 16α-bromoestrone (VIb), hot acid hydrolysis resulted in epimerization to give predominantly 16β-bromoestrone (VIIb). Acetylation of VIIb gave 16β-bromoestrone acetate (VIIa). VIIa could also be obtained directly from VIa in lower yield by partial epimerization on alumina, followed by careful chromatography and fractional crystallization. The two epimeric bromoestrones, VIb and VIIb, had the same melting point with little depression on admixture. They were not separated by alumina or chromatography on paper in several systems, but were separated by fractional crystallization. The specific rotation was found to be an acceptable criterion of purity. The acetates VIa and VIIa showed similar behavior although the mixture melting point depression was considerable. Lithium aluminum hydride reduction followed directly by alkaline reflux was then carried out on both epimers VIb and VIIb. The 16α-bromoketone VIa afforded in addition to the 16β, 17β-oxide XII, about 10% of estrone (XIII) derived from the *cis* 16α, 17α-bromohydrin

(1) This investigation was supported in part by a grant from the American Cancer Society and a research grant (CY-3207) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

(2) Present address: Fresno State College, Fresno, Calif.

(3) (a) V. Prelog, L. Ruzicka, and P. Wieland, *Helv. Chim. Acta*, **28**, 250 (1945). (b) M. N. Huffman and H. H. Darby, *J. Am. Chem. Soc.*, **66**, 150 (1944). (c) M. N. Huffman and W. R. Miller, *Science*, **100**, 312 (1944); A. Butenandt and E. L. Schäffler, *Z. Naturforsch*, **1**, 82 (1946); N. S. Leeds, D. K. Fukushima, and T. F. Gallagher, *J. Am. Chem. Soc.*, **76**, 2943 (1954).

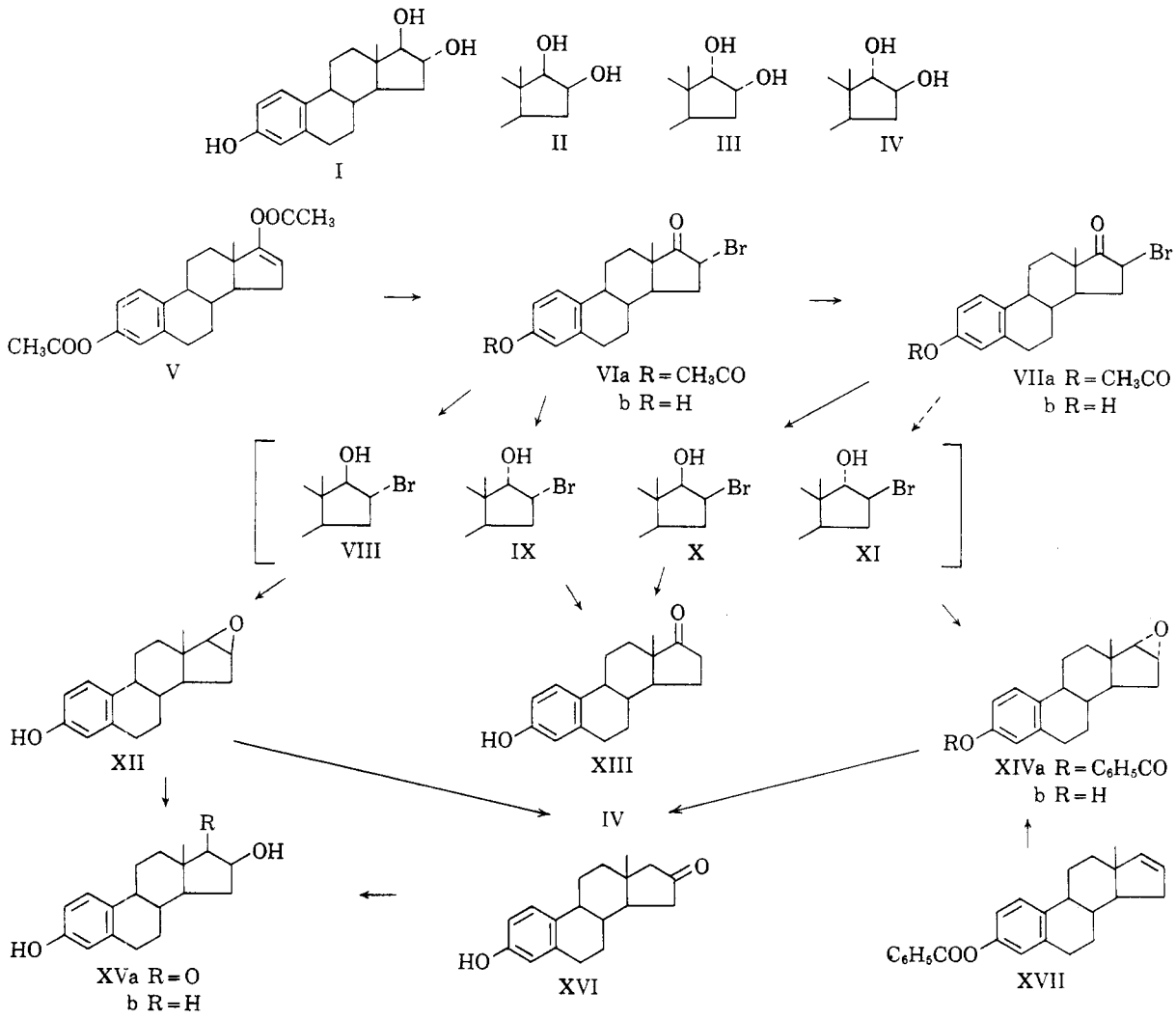
(4) (a) G. F. Marrian, *Biochem. J.*, **24**, 435, 1021 (1930). (b) G. F. Marrian, E. J. D. Watson, and M. Panattoni, *Biochem. J.* **65**, 12 (1957).

(5) W. R. Biggerstaff and T. F. Gallagher, *J. Org. Chem.*, **22**, 1220 (1957).

(6) J. Fajkos, *Collection Czechoslav. Chem. Commun.*, **20**, 312, 1478 (1957).

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

(8) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **213**, 343 (1955).



IX formed during the reduction.[9] The 16β-bromoestrone (VIIb) gave rise only to estrone derived from the *cis*-16β,17β-bromohydrin X; none of the 16α,17α-oxide XIVb expected from the *trans* bromohydrin XI was found. These results not only confirm the assignment of the bromine orientation in the two isomers but also support the previous finding[5] that a 16β-substituent results in stereospecific β reduction of the 17-ketone while a 16α-substituent makes the reduction only stereoselective, with about 10-15% of α-reduction.

The contribution of the new estriol to the metabolism of estradiol in man is at present being investigated.

EXPERIMENTAL[10]

16α-Bromoestrone acetate (VIa). One gram of estrone enol diacetate (V) in carbon tetrachloride containing some potassium carbonate was treated with one equivalent of bromine in carbon tetrachloride following the procedure of Johnson and Johns.[7] The usual work-up gave 700 mg. of product of m.p. 163–167°. Four recrystallizations from methanol gave 16α-bromoestrone acetate, m.p. 169–171°; $[\alpha]_D^{24}$ +119° (chloroform). Literature m.p. 168–170°.

16α-Bromoestrone (VIb). A solution of 300 mg. of the acetate VIa in 4% ethanolic sulfuric acid was allowed to stand for 20 hr. at room temperature. Dilution with water and extraction with chloroform gave 243 mg. of 16α-bromoestrone, crystallized from benzene as long needles, m.p. 225–228°; $[\alpha]_D^{24}$ +120° (chloroform).

Anal. Calcd. for $C_{18}H_{21}O_2Br$: C, 61.89; H, 6.02. Found: C, 62.33; H, 6.28.

Acetylation with acetic anhydride and pyridine regenerated VIa.

16β-Bromoestrone acetate (VIIa). Five hundred milligrams of the crude α-bromoacetate VIa in the minimum amount of 1:1 benzene-petroleum ether mixture was adsorbed on a column of 100 g. of alumina. After standing overnight the column was eluted in 100 cc. of fractions first with 3:2 benzene–petroleum ether and then with the same solvents in 4:1 ratio. The various fractions were combined on the basis of their melting points. The first 5 fractions gave on crystallization from methanol 0.23 g. of plates of pure 16α-bromoestrone acetate (VIa). Fractions 6–10 were mixtures.

(9) The estrone could also be derived from the β-*cis* bromohydrin (X) which could result by epimerization of the α-bromine prior to reduction. This, however, is unlikely in view of the low temperature, inert solvent, and the rapidity of the reduction.

(10) Melting points were obtained on a Kofler hot stage apparatus and are corrected. Analyses are by Spang Microanalytical Laboratories.

Fractions 10 to 14 crystallized from methanol to give 47 mg. of β-bromoestrone acetate (VIIa) as needles, m.p. 166–169°. The analytical sample melted at 170–173°; $[\alpha]_D^{25}$ +156° (chloroform).

Anal. Calcd. for $C_{20}H_{23}O_3Br$: C, 61.38; H, 5.92. Found: C, 61.45; H, 5.88.

Subsequent fractions eluted from the column with more polar solvents proved to be a mixture of the hydrolyzed α and β isomers. A mixture melting point of VIIa with the 16α isomer (VIa) showed a depression of 40°. The infrared spectra of the two compounds in carbon disulfide were different in the 1400–650 cm.$^{-1}$ region, but there was no difference in the position of the ketone band at 1758 cm.$^{-1}$ Paper chromatography in several systems failed to separate the two isomers.

16β-Bromoestrone (VIIb). Room temperature hydrolysis of 16β-bromoestrone acetate VIIa with 4% ethanolic sulfuric acid for 20 hr. gave the free phenol VIIb, which crystallized from benzene as short needles, m.p. 224–227° with sublimation. Mixed melting point with the α-isomer VIb was 213–220°. The infrared spectra of the two compounds in chloroform showed significant differences in the 1150–800 cm.$^{-1}$ region. Paper chromatography in various systems again failed to separate the two compounds. The analytical sample melted at 225–228°; $[\alpha]_D^{24}$ +154° (chloroform).

Anal. Calcd. for $C_{18}H_{21}O_2Br$: C, 61.89; H, 6.02. Found: C, 62.06; H, 6.03.

The same compound could be obtained by refluxing the α-bromoestrone acetate VIa with 4% ethanolic sulfuric acid overnight. The resultant mixture was predominantly 16β-bromoestrone (VIIb), which could be obtained pure by fractional crystallization. Acetylation with acetic anhydride in pyridine gave 16β-bromoestrone acetate (VIIa).

16β,17β-Epoxy-1,3,5(10)-estratriene-3-ol (XII). One gram of α-bromoestrone acetate VIa was stirred for 2 hr. at 0° with an excess of lithium aluminum hydride in anhydrous ether. The excess reagent was destroyed with water; acidification with dilute hydrochloric acid and evaporation of the organic phase gave 0.78 g. of a gum, the infrared spectrum of which lacked any carbonyl absorption. Without further purification the material was refluxed for 4 hr. with 5% ethanolic potassium hydroxide. Dilution with water and extraction with chloroform gave 0.58 g. of solid which was chromatographed on 50 g. of alumina. A total of 0.24 g. of the 16β,17β-oxide (XII) was eluted first with 9:1 benzene–petroleum ether. Subsequent fractions contained 92 mg. of estrone. The oxide crystallized from benzene–petroleum ether as short needles, m.p. 198–202°. The analytical sample melted 200–204°; $[\alpha]_D^{25}$ +119° (chloroform).

Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.25. Found: C, 80.15; H, 8.22.

Reduction of the oxide (XII) with lithium aluminum hydride gave 1,3,5(10)-estratriene-3,16β-diol (XVb), m.p. 224–226° identical with a sample prepared by sodium borohydride reduction of 1,3,5(10)-estratriene-16-one (XVI).

When 150 mg. of the 16β-bromoestrone acetate (VIIa) was reduced under identical conditions with lithium aluminum hydride followed by heating with alkali 94 mg. of estrone was obtained. No 16α,17α-oxide XIVb was isolated.

1,3,5(10)-Estratriene-3,16β,17α-triol (IV). (a) A solution of 300 mg. of the β oxide XII in 30 cc. of glacial acetic acid was refluxed for 4 hr. Removal of the acetic acid under vacuum left an oily residue, which was refluxed in 6% ethanolic potassium hydroxide for 1.5 hr. Dilution and acidification with dilute hydrochloric acid and thorough extraction with chloroform gave 300 mg. of a white solid which was chromatographed on 25 g. of alumina. The first fractions eluted with 900 cc. of chloroform containing 3% methanol were combined to give 124 mg. of material which crystallized from benzene-methanol as prisms, m.p. 245–248°. Further recrystallization raised the melting point to 248–250°; $[\alpha]_D^{25}$ +61° (ethanol).

Anal. Calcd. for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 74.74; H, 8.30.

The subsequent fractions eluted weighed 64 mg. and proved to be the other *trans* isomer, 1,3,5(10)-estratriene-16α,17β-triol (I).

The infrared spectrum of the new estriol in potassium bromide showed differences from the other three estriol isomers. Paper chromatography in a benzene-methanol: water-ethyl acetate system separated the new estriol from its isomers. The compound was somewhat less polar than 1,3,5(10)-estratriene-3,16α,17β-triol (I), but considerably more polar than the two *cis* triols in the solvent system used.

(b) A solution of 100 mg. of 1,3,5(10),16-estratetraene-3-ol benzoate (XVII), m.p. 161–166° in ether was treated with perbenzoic acid. On working up the reaction, 111 mg. of crude 16α,17α-epoxy-1,3,5(10)-estratriene-3-ol benzoate (XVIa) was obtained. Without further purification this material was refluxed for 2 hr. in 3 cc. of glacial acetic acid under nitrogen. The acetic acid was removed and the residue refluxed in 8% ethanolic potassium hydroxide for 1.5 hours. The isolated product was a yellow semisolid weighing 73 mg. The material was decolorized with charcoal and crystallized from acetone–petroleum ether to give 23 mg. of crystals, m.p. 200–235°. It was then dissolved in benzene containing 10% ethyl acetate and chromatographed through a 1 × 10 cm. silica column. The combined material eluted with 50% ethyl acetate-benzene weighed 12 mg., m.p. 228–240°. One recrystallization raised the melting point to 244–246°. Mixture melting point determination and infrared spectra comparison established the identity with the 1,3,5(10)-estratriene-3,16β,17α-triol (IV) prepared from the β oxide XII.

Acknowledgment. The authors wish to thank Dr. T. F. Gallagher for his advice and interest in this problem, and Dr. D. K. Fukushima for his helpful discussion. They also wish to thank Dr. Glyn Roberts and staff for the determination of the infrared spectra. The technical assistance of Mrs. Maria Tomasz and Mrs. Rosemarie Lehman is gratefully acknowledged.

New York, N. Y.