Stereochemical Studies on 6,7-Substituted Derivatives of Estra-3,17β-diol

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In an attempt to establish conclusively the configurations tentatively assigned[1] to the epimeric 6-hydroxyestra-3,17β-diols IIa and IIIa, 6β-hydroxy-7α-bromoestradiol 3,17-diacetate (Va) was prepared from 6-dehydroestradiol diacetate (IV) with N-bromoacetamide and converted *via* the 7α-bromo 6-ketone VIa to its 6α-epimer VIIa. On removal of the bromine atom by catalytic hydrogenation the latter bromohydrin gave as expected 6α-hydroxyestradiol 3,17-diacetate (IIc). However, IIc was also the main product obtained in this reaction from the 6β-hydroxy-7α-bromo isomer Va, while the expected 6β-hydroxyestradiol 3,17-diacetate (IIIc) was formed in small amounts only. The cause of this anomaly is obscure, as no inversion occurs when 6β-hydroxyestradiol diacetate is treated in the same manner. That Va is correctly formulated as the *trans* diaxial bromohydrin follows *inter alia* from its convertibility with anhydrous base to 6β,7β-oxidoestradiol diacetate (IXa). This epoxide reacts abnormally in steric regard in that on reduction with lithium aluminum hydride it yields the equatorial alcohol, 7β-hydroxyestradiol (XIb), and on hydrolysis the 6ξ,7β-glycol (tentatively formulated as the 6α,7β-diequatorial glycol XIVa), whereas the 6α,7α-epoxide X is opened to the normal diaxial 6β,7α-glycol XIIa. The n.m.r spectra of the fully acetylated compounds bearing 7α-substituents or hydrogen only at C-7 indicate that ring B in these is in the hemichair form, while in those with a 7β-acetoxy group (XIc and XIVb) it appears to assume a more planar conformation intermediate between hemichair and boat.

In a previous publication[1] we described the preparation from 6-ketoestradiol diacetate (Ia) of the 6-epimeric 6-hydroxy-17β-estradiols and their triacetates. The higher melting triol (m.p. 249–251°, triacetate m.p. 144–145°) was tentatively assigned the 6α-configuration (IIa) and the lower melting triol (m.p. 191–195°, triacetate m.p. 176–178°) the 6β-configuration (IIIa) on the basis of the stereochemical criteria then available. The objective of the work reported in the present paper was originally to adduce conclusive proof for these assignments. To this end we explored the reaction of 6-dehydroestradiol diacetate (IV)[2] with N-bromoacetamide in the hope that the resulting bromohydrin would be the 6β-hydroxy-7α-bromo isomer, which was then expected to yield, on reductive removal of the bromine and acetylation, the 6β-triol triacetate IIIb. The reaction of IV with N-bromoacetamide proceeded smoothly to give in almost quantitative yield a bromohydrin (m.p. 186–187°), which indeed carried the new hydroxyl group at C-6, since on Jones oxidation[3] it afforded a bromo ketone exhibiting ultraviolet absorption characteristics similar to those of 6-ketoestradiol diacetate (Ia). The same bromo ketone was also obtained as the sole product when Ia was brominated in acetic acid containing a small amount of hydrobromic acid. The carbonyl stretching vibration band in the infrared spectrum appears at 5.91 μ, while that of the parent ketone Ia is located at 5.94 μ. In the spectra of the corresponding 17-monoacetates the positions of these bands are 5.92 and 5.96 μ, respectively (measurements on chloroform solutions; in Nujol mulls considerably higher values for Δ, namely 5.95 → 5.90 μ for the diacetates, and 6.01 → 5.93 μ for the 17-monoacetates, were observed). These hypsochromic shifts, corresponding to +8 and +11 cm.$^{-1}$, respectively, on the frequency scale, are smaller than those usually associated with the introduction of an equatorial bromine atom in the α-position of saturated cyclic ketones (average +20 cm.$^{-1}$).[4] In view of these ambiguous results, and since there was no assurance that the above shift rule derived from measurements on unconjugated ketones[4] would hold for the α-tetralone system of Ia, recourse was taken to optical rotatory dispersion measurements.[5] The curves of Ia and of the derived bromo ketone in dioxane were rather similar in that they both showed a positive Cotton effect with peaks of moderate amplitudes at 357 mμ, respectively (*cf.* Experimental). The smallness of the bathochromic shift and the *decrease* in amplitude ([α] 2013 → 1833°; [α] of peaks 553 → 576°) in going from Ia to the bromo ketone seemed to indicate that the bromine substituent was equatorial rather than axial.[6] Examination of the n.m.r. spectra of the two ketones, however, led to the opposite conclusion. The spectrum of the bromo ketone exhibits a doublet at τ 5.50 (J = 2 c.p.s.) which is absent in that of Ia and hence must represent the 7-proton coupled with the axial 8β-proton. The small value of J indicates a dihedral angle between these two protons of not less than 60° and not more than 120°.[7] Now, the scale models of Ia with ring B in either the hemichair or the hemiboat form show that the only one of the four dihedral angles in question which falls into this range is ϕ (7βH–8βH, hemichair) $\cong$ 75°, meaning that the bromine atom must be α and axial in a hemichair. Since this conclusion is also supported, as will appear farther below, by chemical evidence relating to the reactions of the bromohydrin, the bromo ketone must be formulated as VIa, and the bromohydrin, on the basis of the rule of diaxial addition and of the chemical findings mentioned, as the 6β-hydroxy-7α-bromo stereoisomer Va.

It is clear from the above facts that the generalizations regarding the changes in peak positions and amplitude of O.R.D. curves following the introduction

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

(2) S. Kaufmann, J. Pataki, G. Rosenkranz, J. Romo, and C. Djerassi, *ibid.*, **72**, 4531 (1950).

(3) K. Bowden, I. M. Heilbron, E. R. H. Jones, and C. L. Weston, *J. Chem. Soc.*, 39 (1946); C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(4) R. N. Jones, D. A. Ramsay, F. Herling, and K. Dobriner, *J. Am. Chem. Soc.*, **74**, 2828 (1952).

(5) These measurements were carried out in the laboratory of Dr. Franz Sondheimer, Syntex S. A., Mexico City, who informed us that he had independently prepared the bromo ketone VIa from the enol acetate of Ia and determined the infrared spectra and O.R.D. curves of the two ketones. The measurements were then repeated on our samples in two different solvents. We are greatly indebted to Dr. Sondheimer for putting these data at our disposal for publication.

(6) C. Djerassi and W. Klyne, *J. Am. Chem. Soc.*, **79**, 1506 (1957); C. Djerassi, J. Osiecki, R. Riniker, and B. Riniker, *ibid.*, **80**, 1216 (1958).

(7) H. Conroy, "Advances in Organic Chemistry," Vol. 2, Interscience Publishers, Inc., New York, N. Y., 1960, p. 311; see ref. 25c also.

of an axial α-bromine substituent in conformationally rigid cyclohexanones[6] do not hold for the α-tetralone system present in Ia. This is not surprising, as these generalizations, and their extension in the "axial halo ketone rule,"[6] were not meant, and in fact have never been shown, to apply even to transoid α'-halo-α,β-unsaturated octalones, the closest analogs that could be substituted in this context for the bromo ketone VIa.

Since VIa was obtained from Ia by bromination in the presence of hydrobromic acid, it should be the thermodynamically more stable isomer. Attempts to prepare the epimeric 7-bromo ketone by bromination in the presence of sodium acetate, that is, under conditions of kinetic control, failed, as only VIa could be isolated, although in smaller yield than in the runs with excess hydrobromic acid. This was also the case in equilibration experiments in which VIa was treated with hydrobromic acid at room temperature, and even when a solution of the bromo ketone in acetic acid containing 5% *p*-toluenesulfonic acid was heated under reflux for 24 hr.[8] All that happened in the latter reaction was the loss of the 3-acetyl group, so that the product isolated was 7α-bromo-6-ketoestradiol 17-monoacetate (VIb). It must be concluded from these results that the 7α-bromo compound, as the thermodynamically favored isomer, preponderates under conditions of kinetic control as well. It is of interest in this connection that 3β-acetoxy-7α-bromo-6-ketocholestane, the infrared[9] and ultraviolet[10] spectra of which leave no doubt about the axial character of its bromine substituent, is quite stable in acetic acid containing hydrobromic acid at 90° (the conditions under which it is formed from the 5α-bromo isomer),[11] and that its 7β-epimer is unknown.

In various attempts to remove the bromine atom of the bromohydrin Va or its triacetate Vb with zinc in ethanol, these compounds remained either unchanged or reverted to 6-dehydroestradiol diacetate. Reduction with Raney nickel appeared unpromising since Iriarte, *et al.*,[12] had found that the use of this reagent on 6α,7α-oxidoestradiol and 6α,7α-dihydroxyestradiol resulted in hydrogenolysis at both C-6 and C-7 with the formation of estradiol. Of the catalytic hydrogenation experiments carried out with noble metal catalysts, only that with palladium on calcium carbonate in ethanol was successful in the sense that the bromine was eliminated quantitatively after the uptake of 1 mole of hydrogen. There was obtained in about 50% of the theoretical yield a compound, m.p. 140–142°, $[\alpha]_D$ +53°, which had the composition and spectral properties of a 6-hydroxyestradiol 3,17-diacetate, and on oxidation with chromium trioxide gave 6-ketoestradiol diacetate. However, the triacetate it yielded on acetylation was not the expected 6$''\beta''$-hydroxyestradiol triacetate, m.p. 178°, but slightly impure (m.p. 140°) 6$''\alpha''$-hydroxyestradiol triacetate. In later hydrogenation runs the diacetate always turned up in form of a lower melting polymorphous modification melting at 128–129°. This was also the case in experiments in which the crude product was subjected to thin layer chromatography on acid-washed alumina; in addition the chromatogram yielded small amounts of estradiol diacetate and 17-monoacetate, and of a triol diacetate, m.p. 160°, which was subsequently identified as 6$''\beta''$-hydroxyestradiol 3,17-diacetate.

It proved possible to secure the 3,17-diacetates of the 6-epimeric 6-hydroxyestradiols by modifying the conditions originally employed for the preparation of the two epimeric triols from 6-ketoestradiol diacetate. Thus, by conducting the reduction with sodium borohydride at 0° for only 15 min.,[13] and chromatographing the crude product, the lower melting modification of 6$''\alpha''$-hydroxyestradiol 3,17-diacetate was obtained in good yield together with a smaller amount of the 17-monoacetate; and 6$''\beta''$-hydroxyestradiol 3,17-diacetate, m.p. 162°, was the sole product when ethyl acetate was substituted for ethanol as the solvent in the catalytic reduction of the ketone with platinum oxide as the catalyst.

While this work was in progress, Breuer, Knuppen, and Pangels[14] succeeded in bringing about incontrovertible proof for the configuration at C-6 of the 6-hydroxyestradiols by correlating them with the 6-epimeric 6-hydroxyandrostenediones by means of the aromatizing placental enzyme of Ryan.[15] The results showed that our tentative assignments were correct. The triol diacetate (m.p. 142°), which is the main product in the reductive debromination of the bromohydrin, is thus indeed 6α-hydroxyestradiol 3,17-diacetate (IIc), and the diacetate (m.p. 162°), formed only in small amounts in that reaction, is the 6β-epimer IIIc.

Since at the time when this work came to our attention the configuration of the bromohydrin Va was still in question, it appeared from the result of the reductive debromination experiment (formation from Va of 6α-hydroxyestradiol 3,17-diacetate as the main product) that the bromohydrin was either the 6α-hydroxy-7α-bromo-*cis* isomer VIIa, or the diequatorial 6α-hydroxy-7β-bromo isomer VIII. The former could conceivably have arisen from IV *via* a 6α,7α-bromonium ion passing into a 6-carbonium ion, which then added hydroxyl ion from the less hindered α-side. This would parallel the behavior of certain β-substituted styryl oxides,[16] stilbene oxides,[17] and indene oxides,[18] which undergo ring opening by aqueous acids or hydrohalides predominantly or exclusively with retention of configuration at the reacting benzylic carbon atom. Alternatively, an initially formed 6β,7β-bromonium ion may have by the same mechanism produced the diequatorial isomer VIII.

Chemical proof that the stereochemistry of the bromohydrin, m.p. 187°, is correctly represented by Va rather than by VIIa or VIII was obtained as follows.

(8) Using these conditions G. P. Mueller and W. F. Johns [*J. Org. Chem.* **26**, 2403 (1961)] were able to effect the conversion of 3-methoxy-17α-brom oestra-1,3,5(10)-trien-16-one to its 17β-epimer.

(9) E. J. Corey, *J. Am. Chem. Soc.*, **76**, 175 (1954).

(10) E. Cookson, *J. Chem. Soc.*, 282 (1954).

(11) I. M. Heilbron, E. R. H. Jones, and F. S. Spring, *ibid.*, 801 (1937).

(12) J. Iriarte, H. Ringold, and C. Djerassi, *J. Am. Chem. Soc.*, **80**, 6105 (1958).

(13) In this manner 6-ketoestrone acetate could be reduced to 6α-hydroxyestradiol 3-monoacetate (personal communication by Professor H. Breuer, Bonn).

(14) H. Breuer, R. Knuppen, and G. Pangels, *Nature*, **190**, 720 (1961); *Biochem. Biophys. Acta*, **65**, 1 (1962).

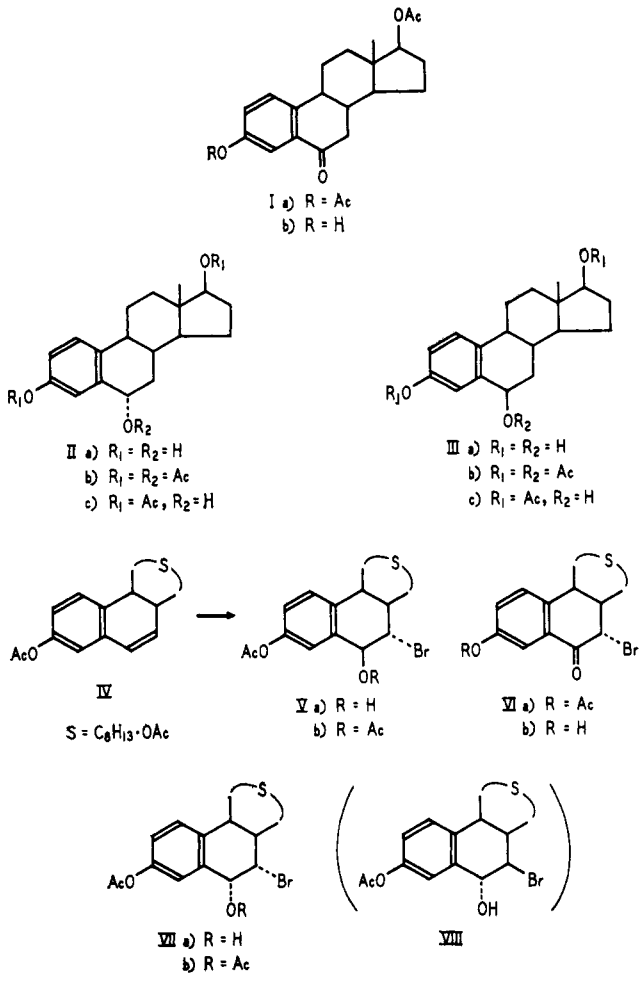
(15) K. J. Ryan, *J. Biol. Chem.*, **234**, 268 (1959).

(16) R. Kuhn and F. Ebel, *Ber.*, **58**, 919 (1925); H. Wasserman and N. E. Aubrey, *J. Am. Chem. Soc.*, **78**, 1726 (1956).

(17) J. Boeseken and G. Eisen, *Rec. trav. chim.*, **47**, 694 (1928); J. Boeseken and G. C. C. Schneider, *J. prakt. Chem.*, **131**, 285 (1931); J. H. Brewster, *J. Am. Chem. Soc.*, **78**, 4061 (1956).

(18) J. Boeseken, *Rec. trav. chim.*, **41**, 199 (1922).

SCHEME I



On reduction with sodium borohydride in methanol (15 min., 0°) the bromo ketone VIa afforded in excellent yield and as the sole product a new bromohydrin diacetate (m.p. 170–172°), which was further characterized by conversion to the triacetate (m.p. 228–230°). The highly stereospecific character of this reaction (and of Ia → IIc) is noteworthy insofar as, in contrast, 3β-acetoxy-7α-bromo-6-ketocholestane on reduction by this agent gives rise to a mixture of the 6-epimeric alcohols.[19] On short treatment with potassium *t*-butoxide in *t*-butyl alcohol at room temperature, the bromohydrin (m.p. 172°) yielded 6-ketoestradiol diacetate (Ia), showing that the two ring B substituents are *cis* to each other, and consequently *trans* in its 6-epimer, the bromohydrin (m.p. 187°) obtained from 6-dehydroestradiol diacetate. The conclusion that the latter bromohydrin is the diaxial 6β-hydroxy-7α-bromo isomer Va (rather than the diequatorial isomer VIII), and the bromohydrin (m.p. 172°) is, therefore, the 6α-hydroxy-7α-bromo isomer VIIa, rests on the formation from the former on treatment with potassium *t*-butoxide of the 6β,7β-epoxide which depending on conditions is obtained either as the 3,17-diacetate IXa or the 17-monoacetate IXb.

That the oxiran ring in these compounds is β-oriented follows from two facts. (1) The diacetate (m.p. 126°) was not identical with the 6α,7α-oxidoestradiol diacetate X (m.p. 169°) which Iriarte, Ringold, and Djerassi[12] had obtained with perphthalic acid from 6-dehydroestradiol diacetate and had reduced with lithium aluminum hydride to 7α-hydroxyestradiol (XIa). (2) Application of the latter reaction to the new oxidoestradiol diacetate (m.p. 126°) gave 7β-hydroxyestradiol (XIb), identical with the triol resulting from the reduction of 7β-hydroxyestrone[20,21] with sodium borohydride. It should be noted that this result, while proving that the parent oxide is the β,β isomer, is anomalous, since the reduction of steroid expoxides normally leads to the axial alcohol, and hence in the present case should have yielded 6β-hydroxyestradiol (IIIa). Obviously, the partial positive charge on the benzylic atom directs the hydride ions delivered by the reducing agent to this site.

The hydrolysis of the oxide IXa by acid (perchloric acid in boiling aqueous ethanol) likewise took a sterically abnormal course. The hydrolysis product was amorphous and hence had to be characterized as the tetraacetate (m.p. 217°, $[\alpha]_D$ −1°). Had the opening of the oxiran ring taken the normal course, this compound should be the tetraacetyl derivative of the diaxial *trans* glycol, 6β,7α-dihydroxyestradiol tetraacetate (XIIb), and hence should be identical with the tetraacetate derivable from the 6α,7α-oxidoestradiol diacetate X of Iriarte, *et al.* Since the Syntex authors had not studied the hydrolysis of X [although they did assign the 6β,7α-dihydroxyestradiol 3,17-diacetate structure XIIa—erroneously, as will be shown further below—to a crystalline product (m.p. 88°) they had isolated from the mother liquors of X], we prepared the oxide by their procedure and subjected it to hydrolysis with perchloric acid. In this case the hydrolysis product itself, a 6,7-dihydroxyestradiol 3,17-diacetate (m.p. 171°), was crystalline, and on acetylation yielded a new tetraacetate (m.p. 136°, $[\alpha]_D$ +68°). There can be no doubt that these compounds are, respectively, the 3,17-diacetate XIIa and tetraacetate XIIb of 6β,7α-dihydroxyestradiol, the isomer formed by normal *trans* diaxial opening of the oxide X, for the following reasons. (1) As already mentioned, X is reduced by lithium aluminum hydride to the normal product, the axial alcohol 7α-hydroxyestradiol. (2) Iriarte, *et al.*,[12] found that when 6α,7α-dihydroxyestrone, prepared from 6-dehydroestrone with osmium tetroxide, was treated with methanolic hydrogen chloride, it was isomerized to its 6-epimer, the *trans* diaxial glycol 6β,7α-dihydroxyestrone (XIIc), also obtained from 6α,7α-oxidoestrone by acid hydrolysis, and hence configurationally analogous to XIIa and b in the estradiol series. Since this unusual inversion must have involved, as the Syntex authors suggest, the intermediary formation of a carbonium ion at the benzylic carbon atom, there can be no doubt that the glycol formed on hydrolysis of 6α,7α-oxido-

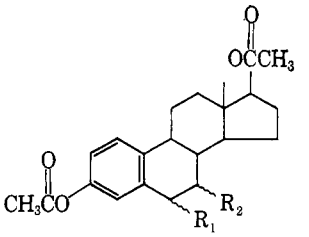
(19) D. R. James and C. W. Shoppee, *J. Chem. Soc.*, **1954**, 4224.

(20) W. H. Pearlman and O. Wintersteiner, *J. Biol. Chem.*, **132**, 605 (1940).

(21) The 7-configurations of the two epimeric 7-hydroxyestrones and of 7α-hydroxyestradiol (XIa) were inferred by Iriarte, *et al.*,[12] from the fact that 7β-hydroxyestrone had been prepared by Pearlman and Wintersteiner[20] by catalytic reduction of 7-hydroxy-6-dehydroestrone diacetate (enol acetate of 7-ketoestrone acetate) in which catalyst approach was assumed by the Syntex authors to have occurred from the α-side. The possibility that this may not be so when, as in estrogens, the 19-methyl group is absent was not considered. However, our n.m.r. data on 7β-hydroxyestradiol triacetate (Table I) leave no doubt about the correctness of the above configurational assignments.

TABLE I

N.M.R. SPECTRA OF FULLY ACETYLATED DERIVATIVES OF 6-HYDROXY-, 6-HYDROXY-7-BROMO-, AND 6,7-DIHYDROXYESTRADIOLS

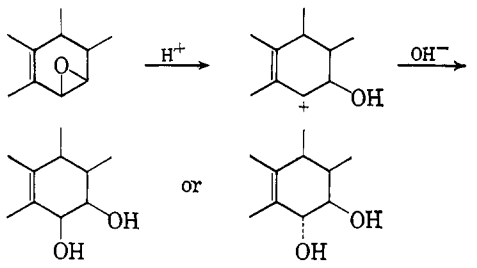


Compound	R_1	R_2	τ Values for CH_3COO— C-3	C-6	C-7	C-17	C-6 H, τ[a]	C-7 H, τ[a]	C-18 CH_3, τ
IIb	α-OAc		7.73	7.88		7.96	3.98 (d, d, 8.5, 5.5)		9.18
IIIb	β-OAc		7.73	7.95		7.95	3.99 (d, ~3)		9.14
VIIb	α-OAc	α-Br	7.69	7.72		7.95	3.93 (d, ~4)	5.23 (d, 4)	9.14
Vb	β-OAc	α-Br	7.69	7.95		7.95	3.85 (d, ~2)	5.72 (d, ~2)	9.11
XIIIb	α-OAc	α-OAc	7.72	7.89	7.96	7.97	3.95 (d, 4)	4.51 (d, 4)	9.16
XIIb	β-OAc	α-OAc	7.73	7.93	7.95	7.97	4.13 (d, 2)	4.89 (m)	9.13
XIc		β-OAc	7.73		7.98	7.96	7.16 (d, 7)		
							6.97 (d, 6.5)	4.93 (m)	9.16
XIV[b]	α-OAc	β-OAc	7.72	7.94	7.94	7.95	3.97 (d, 6)	4.78 (m)	9.16

[a] Number of peaks in parentheses, J in c.p.s., s = singlet, d = doublet, m = multiplet.

estrone and, consequently, XIIa and b are correctly formulated as the *trans* diaxial 6β,7α isomers.

The Syntex authors also applied the osmium tetroxide reaction to 6-dehydroestradiol and thus obtained 6α,7α-dihydroxyestradiol (XIIIa), the tetraacetate XIIIb of which melts at 229° and has $[\alpha]_D$ +17°. Clearly, then, the tetrol tetraacetate (m.p. 217°) obtained from the 6β,7β-oxide IX, since it is different from the two tetraacetates XIIb and XIIIb having the 7α-configuration, must have the β-configuration at C-7, and, therefore, must be derived either from the diequatorial 6α,7β- or the axial–equatorial 6β,7β-glycol. The latter could have arisen by the carbonium ion mechanism shown below which is analogous to the one postulated by Cookson and Hudec[22] to explain the conversion by acid of 3β-phenyl-2α,3α-oxidocholestane to 3β-phenyl-2α,3α-dihydroxycholestane.



The same mechanism could, of course, be invoked for the formation of the 6α,7β-dihydroxy isomer since in the second step the hydroxyl ion could just as well, or perhaps even more readily, add from the unhindered α-side. Alternatively, it could be assumed that ring B in the oxide exists in the boat conformation, in which case likewise, as in the formation of diequatorial 17α-hydroxy-16β-bromo derivative in the reaction of 3α-acetoxy-11,17α-diketo-D-homo-5β-androstane 16α,17α-oxide with hydrobromic acid,[23] the diequatorial 6α,7β-glycol would result.

(22) R. C. Cookson and J. Hudec, *Proc. Chem. Soc.* 24 (1957).

(23) N. L. Wendler, D. Taub, S. Dobriner, and D. K. Fukushima, *J. Am. Chem. Soc.*, **78**, 5027 (1956); N. L. Wendler, *Chem. Ind.* (London), 1662 (1958).

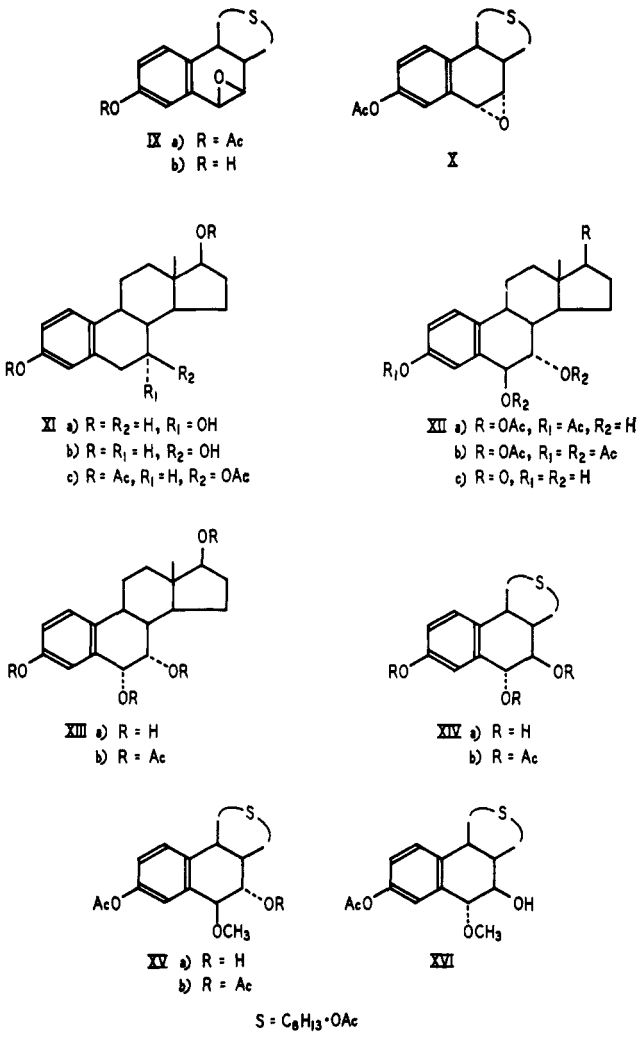
We were unable to resolve this question by chemical means, but from a consideration of the coupling constants for protons 6 and 7 and of the chemical shift of the 6-acetoxy signal in the n.m.r. spectra of the tetraacetate in question (see Table I and discussion) have come to favor the 6α,7β-configuration for this hydrolysis product, and hence tentatively write it as XIVa, and its tetraacetate (m.p. 217°) as XIVb.

One of the unsuccessful attempts to establish the configuration of the 6-acetoxy group in the tetraacetate (m.p. 217°) involved the perchloric acid-catalyzed methanolysis of both the 6α,7α-oxide X and the 6β,7β-oxide IXa. It was reasoned that in both cases the methanolysis would take the same steric course as hydrolysis; *i.e.*, replacement of the oxidic linkage by methoxyl ion would in both compounds occur at the benzylic C-6 atom. Thus X would give rise to the diaxial isomer, 6β-methoxy-7α-hydroxyestradiol 3,17-diacetate (XVa), and IXa either to the diequatorial isomer, 6α-methoxy-7β-hydroxyestradiol 3,17-diacetate (XVI), or to the latter's 6-epimer, 6β-methoxy-7β-hydroxyestradiol 3,17-diacetate. Oxidation of the two methanolysis products should then lead either to the same 7-ketone or to two different 7-ketones, depending on whether the methanolysis product of IXa (and hence also the tetraacetate, m.p. 217°) has the 6β- or the 6α-configuration.

The methanolysis product of the 6α,7α-oxide X, undoubtedly representing the diaxial 6β-methoxy-7α-hydroxy isomer XVa, turned out to be identical with the compound (m.p. 88°), which, as has been mentioned by Iriarte, *et al.*,[12] had been obtained from methanolic mother liquors of X and erroneously assumed to be the glycol XIIa. [We had likewise encountered it in this source, but then found that on acetylation it formed a triacetate (XVb) instead of a tetraacetate, and that it contained methoxyl.]

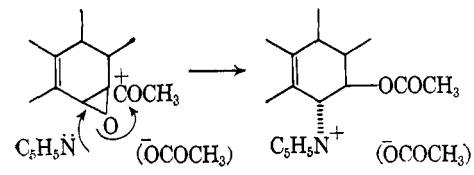
The methanolysis product (m.p. 178°) obtained in the same manner from the 6β,7β-oxide IXa can then be formulated, on the basis of the configurations suggested by the n.m.r. data for the tetraacetate (m.p. 217°), as the diequatorial isomer XVI. (See Scheme II.)

SCHEME II



The two methanolysis products XVa and XVI were subjected to chromic acid oxidation under a variety of conditions. In no case, however, could the 7-ketone be obtained, either as such or in the form of a crystalline derivative, from either substance. It is possible that this failure has its cause in the tendency of the incipient 7-keto group to enolize towards C-6, the partial positive charge on this carbon atom being reinforced by the electron-withdrawing effect of the O-methyl group, facilitating the formation of the enolic double bond and ultimately resulting in the loss of the O-methyl group from the enol ether formed when acidic conditions prevailed.

It is noteworthy that the 6β,7β-oxide IX deviates from other steroid epoxides also in its purely chemical behavior. It was, for instance, not possible to convert the 17-monoacetate IXb into the 3,17-diacetate IXa by acetylation with acetic anhydride and pyridine, because it was transformed by these reagents (but not by pyridine alone) to water-soluble, highly polar products which are in all probability 7β-acetoxy-6-pyridinium acetates formed by the concerted mechanism shown below (or the equivalent 6-carbonium ion mechanism). The conversion of IXb into IXa can be effected, however, with acetic anhydride alone. Furthermore, the oxide is readily hydrolyzed by water near neutrality, as shown by the following observations. When the



trans-bromohydrin Va was dissolved in aqueous methanol containing 1% potassium carbonate, it rapidly eliminated bromine ion and yielded after neutralization with acetic acid an amorphous product having the composition and spectral properties of a dihydroxyestradiol 17-monoacetate (XIVa) which on acid-catalyzed acetylation afforded the tetraacetate XIVb (m.p. 217°) in good yield. The amorphous monoacetate XIVa convertible to the tetraacetate XIVb was also obtained when in the preparation of the oxide IX from the *trans*-bromohydrin Va with potassium *t*-butoxide, 1 equiv. (in respect to the latter) of acetic acid was added prior to working up the reaction mixture with ether and water. Lastly, all attempts to convert the oxide IX to a bromohydrin by treatment with hydrobromic acid in acetic acid or dioxane were unsuccessful. Under the usual conditions for bromohydrin formation (acetic acid, 1 hr. at room temperature) almost no bromide ion was consumed, and the oxide appeared to remain intact, as shown by the formation of water-soluble products from the recovered material after acetylation in pyridine. When the reaction time was extended to 24 hr., some reaction did occur in acetic acid, but the only crystalline product which could be isolated by chromatography from the material acetylated with acetic anhydride and sodium acetate was a small amount of impure tetrol tetraacetate XIVb (m.p. 207°, identified by infrared spectrum). Clearly hydrolysis or acetolysis had supervened over bromohydrin formation.

There remains the question why 6β-hydroxy-7α-bromoestradiol diacetate (Va) yielded on catalytic hydrogenation with palladium–calcium carbonate as the main product 6α-hydroxyestradiol 3,17-diacetate (IIc) and only a small amount of its 6-epimer IIIc. Numerous attempts were made to eliminate the bromine atom from Va as the triacetate Vb by other reductive procedures, catalytic and chemical. In no case, however, was either IIc or IIIc or the corresponding 17-monoacetates encountered at all. In the catalytic hydrogenation experiments, hydrogen uptake was generally sluggish and often incomplete, and the prevailing reaction was usually hydrogenolysis at both C-6 and C-7, resulting in the formation of estradiol diacetate and leaving some of the bromohydrin unattacked. This was also the course of events when the triacetate Vb was hydrogenated in the presence of palladium–calcium carbonate.

The hydrogenation with palladium–calcium carbonate of the 6-epimeric bromohydrin, 6α-hydroxy-7α-bromoestradiol diacetate (VIIa), proceeded with far less facility than with Va, presumably because catalyst approach from the rear was impeded by the 6α-hydroxy group. It did afford, however, though in poor yield (since some starting material always remained unattacked) the expected product, 6α-hydroxyestradiol 3,17-diacetate (IIc), thus confirming the configurational proof of Breuer, *et al.*[14]

In view of the possibility that 6β-hydroxyestradiol diacetate (IIIc) might be the primary product formed in the catalytic reduction with palladium–calcium carbonate of the *trans*-bromohydrin Va, and then be epimerized to the quasi-equatorial isomer IIc, the diacetate IIIc was subjected for 8 hr. to the conditions of the hydrogenation experiment, except that 1 equiv. of hydrobromic acid was added at the start. However, save for estradiol diacetate formed by hydrogenolysis, only IIIc was recovered. It appears, therefore, that the inversion of the 6β-hydroxy group of Va is dependent on the simultaneous reductive removal of the 7-bromine atom.

Nuclear Magnetic Resonance Spectra.—In all but one (XIV) of the compounds measured the configurations of the 6- and/or 7-substituents have been established by chemical means. It was then of interest to ascertain by means of n.m.r. measurements[24] how these substituents affect the conformation of ring B.

Conformational assignments have been made[25] from a study of n.m.r. spectra, particularly from a consideration of the proton–proton coupling constants arising from the spin interaction of protons on adjacent carbon atoms. The quantitative relationship between the dihedral angle and the magnitude of the coupling constant J proposed by Karplus[25] and modified by Williamson and Johnson[25] has been used by Tadanier and Cole[25] for the assignment of the B-ring chair conformation and the configuration of the 6-substituent of the epimeric 6-acetoxy-17-ethylenedioxy-3α,5α-cycloandrostanes.

The pertinent data are shown in Table I. The epimeric 6-hydroxyestradiol triacetates IIb and IIIb will be considered first. It is evident from the fact that the spectrum of the 6α-epimer IIb is of the ABX type and that of the 6β-epimer IIIc of the AX type, and furthermore from the much larger coupling constant of the 6β-proton of IIb as compared with that of the 6α-proton in IIIb, that the 6-acetoxy group in IIb and IIIb must be quasi-equatorial and quasi-axial, respectively, and that consequently ring B in both epimers must be in the hemichair form.[26] (If ring B were in the boat form, the values of the coupling constants would be reversed for the two compounds.) This conclusion is furthermore supported by the differences in the chemical shift of the 6-acetoxyl groups in the two epimers, the deshielding effect of the benzene ring on the equatorial 6α-acetoxy group of IIb being responsible for the larger downfield shift observed with this epimer.

Similar relationships in regard to the magnitude of the coupling constants of the 6-protons and the chemical shifts of the 6-acetoxy signals were obtained with the 6-epimeric bromohydrin triacetates Vb and VIIb and the tetrol tetraacetates XIIIb and XIIb,[27] and it, therefore, appears that in these compounds ring B is also in the hemichair form.

The C-6 protons of 7β-hydroxyestradiol triacetate (XIc) appear as four equal intensity peaks between 167 and 185 c.p.s. downfield of the internal reference. Since the ratio, $J_{AB}/(\delta_B - \delta_A)$, is approximately one, the AB spectrum would appear as two intense inner peaks and two weak outer peaks. At the concentration used, the first-order assignment of the coupling pattern is that of an AX and BX spectrum. The coupling constants of the 6-protons with the 7α-proton in XIc are 6.5 and 7 c.p.s., corresponding to dihedral angles of approximately 35 and 130°.[28] These angles cannot be well accommodated in either the ring B hemichair or the boat form of XIc, and suggest that ring B in this compound assumes a more planar conformation intermediate between these forms.

In the tetrol tetraacetate XIVb, the only other compound with a 7β-substituent, the configuration of the 6-acetoxy group is not known. However, a case of sorts can be made for this group having the α-configuration on the following grounds. The position of the signal for this group (τ 7.94) conforms with those for the axial 6β-acetoxy groups in IIIb, Vb, and XIIb, and hence would indicate that this group, too, is β and axial, provided ring B in XIVb is in the hemichair form. However the dihedral angles (39 or 128°) calculated from the coupling constant $J_{6\xi\text{-H}-7\beta\text{-H}} = 6.0$ c.p.s. do not agree with those measured on the model for 6α-H to 7α-H in the hemichair (165°), nor for 6β-H to 7α-H in the boat (75°) (in which latter case the 6-acetoxy group would, of course, be α-oriented but likewise axial). Rather, the coupling constant indicates, as with XIc, that ring B is in a conformation intermediate between hemichair and boat, and, when this form is approximated in the model (with 6α–7α = 30°, or 6β–7α = 145°), it appears that a 6α-acetoxy group would have somewhat more axial character and, therefore, be less deshielded by the aromatic ring A than a 6β-acetoxy group. We, therefore, tentatively assign to this group in XIVb the α-configuration.

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. semimicrotube, with chloroform as the solvent, unless indicated otherwise. The ultraviolet spectra were measured in absolute ethanol in a Cary self-recording instrument, Model 11 M. 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, (vl) very low, (br) broad, (sh) shoulder. The analytical samples were dried at 110° (2 mm.) unless indicated otherwise.

6α-Hydroxyestradiol 3,17-Diacetate (IIc).—6-Ketoestradiol diacetate (1 g.) was dissolved in reagent methanol (400 ml.),

(24) The spectra were obtained with a Varian Associates Model A-60 n.m.r. spectrometer of samples dissolved in deuteriochloroform containing tetramethylsilane as an internal reference and were run by A.I.C.

(25)(a) R. U. Lemieux, R. K. Kullnig, and R. Y. Moir, *J. Am. Chem. Soc.*, **80**, 2237 (1958); (b) K. L. Williamson and W. S. Johnson, *ibid.*, **83**, 4623 (1961); (c) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); (d) J. Tadanier and W. Cole, *J. Org. Chem.*, **27**, 4610 (1962).

(26) The coupling pattern and the magnitude of the coupling constant of the 6α-proton of IIIb are similar to those of the 6α-proton of 6β-acetoxy-17-ethylenedioxy-3α,5α-cycloandrostane assigned the chair conformation by Tadanier and Cole.[25d] The corresponding 6α-acetoxy derivative and IIb show similar coupling patterns of the 6β-proton but different values of the coupling constants because the B-ring of IIb is more planar than that of the 3α,5α-cycloandrostane derivative.

(27) Williamson and Johnson[25b] found that the 2β-proton signal in 2α-bromocholestan-3-one exhibits the same coupling constants as in the 2α-acetoxy derivative. It should be also noted that the greater chemical shift of the 6α-acetoxy group in the *cis*-bromohydrin triacetate VIIb reflects additional deshielding by the 7α-bromine atom.

(28) The dihedral angles calculated from the equation of Karplus[25c] for the compounds of this series should be regarded as approximations in the sense recently stressed by this author [*J. Am. Chem. Soc.*, **85**, 2870 (1963)], and hence conclusions regarding gross deviations from the normal hemichair conformation of ring B are justified only if the differences between the calculated angles and the normal angles are large, as in the case of XIc and XIVb.

magnetically stirred, and cooled to 0°. A solution of sodium borohydride (1 g.) in reagent methanol (100 ml.) cooled to 0° was added dropwise under anhydrous conditions. After 15 min. the reaction mixture was acidified to about pH 5 with cold 10% acetic acid and diluted with water (1.5 l.). The mixture was extracted with three portions of ether. The combined ether extracts were washed with sodium bicarbonate and twice with water. Chromatography of the residue of the dried ether solution on acid-washed alumina yielded slightly impure 6α-hydroxyestradiol diacetate (482 mg.) in the 1:3 ether–benzene eluates. After two crystallizations from ethyl acetate–hexane the compound (334 mg.) melted at 128–129.5°; $[\alpha]^{21}D$ +64° (*c* 0.987); λ_{max}^{alc} 267 mμ (ε 760), 275 (644), 263 sh (658); λ_{max}^{Nujol} 2.90 (m), 5.67 (s), 5.85 (s), 8.20 (s) μ.

Anal. Calcd. for $C_{22}H_{28}O_5$ (372.4): C, 70.94; H, 7.58. Found: C, 70.97; H, 7.59.

6β-Hydroxyestradiol 3,17-Diacetate (IIIc).—A solution of 6-ketoestradiol diacetate (410 mg.) in ethyl acetate (20 ml.) was added to pre-reduced platinum dioxide (250 mg.) in ethyl acetate (23 ml.) and shaken with hydrogen at atmospheric pressure. When the uptake of hydrogen ceased (1.17 equiv.) the catalyst was removed by filtration and the solution was evaporated to dryness *in vacuo*. The residue crystallized from ethyl acetate–hexane as plates (341 mg.) which melted at 161–162°; $[\alpha]^{21}D$ +5° (*c* 1.008); λ_{max}^{alc} 267mμ (ε 502), 275 (450), 215 sh (9700); λ_{max}^{Nujol} 2.86 (m), 5.64 (s), 5.83 (s), 7.94 (s) μ.

Anal. Calcd. for $C_{22}H_{28}O_5$ (372.4): C, 70.94; H, 7.58. Found: C, 70.58; H, 7.87

6β-Hydroxy-7α-bromoestradiol 3,17-Diacetate (Va).—To a solution of 6-dehydroestradiol diacetate[29] (101 mg.) in pure peroxide-free dioxane (5 ml.) and 0.5 *N* perchloric acid (0.63 ml.), solid N-bromoacetamide (50 mg.) was added with stirring in the dark over a period of 2 min. After 40 min. 5% sodium sulfite solution (0.24 ml.) was added with stirring until the iodide starch reaction was negative. Water was added and the turbid mixture was extracted with chloroform (50 ml.). The residue (164 mg.) from the washed and dried chloroform solution crystallized from 90% methanol as rods (80 mg.; m.p. 186–187°); $[\alpha]^{20}D$ +9.9° (*c* 1.069); λ_{max}^{alc} 268 mμ (ε 585), 276 (570); λ_{max}^{Nujol} 2.87 (m), 5.61 (m), 5.82 (m), 7.84 (s) μ.

Anal. Calcd. for $C_{22}H_{27}O_5Br$ (451.4): C, 58.55; H, 6.03; Br, 17.71. Found: C, 58.44; H, 5.88; Br, 18.19.

The **triacetate Vb** obtained from the diacetate by acetylation in pyridine formed hairlike crystals from dilute methanol which melted at 164–165°; $[\alpha]^{20}D$ +83° (*c* 1.011); λ_{max}^{alc} 268 mμ (ε 720), 276 (656), 214 sh (11,720), 263 sh (562); λ_{max}^{Nujol} 5.69, 5.74, 5.78 (triplet, s), 8.10 (s) μ.

Anal. Calcd. for $C_{24}H_{29}O_6Br$ (493.4): C, 58.42; H, 5.92. Found: C, 58.57; H, 5.67.

6-Keto-7α-bromoestradiol Diacetate (VIa). A. From 6β-Hydroxy-7α-bromoestradiol 3,17-Diacetate (Va).—A stirred solution of 6β-hyroxy-7α-bromoestradiol diacetate (45 mg.) in reagent acetone (6 ml.) was treated dropwise with 1 ml. of 0.402 *N* chromic acid in dilute sulfuric acid and acetone.[3] After 10 min., the excess chromium trioxide was destroyed with 95% ethanol and the reaction mixture was diluted with water. The acetone and ethanol were removed *in vacuo* and the resulting precipitate was filtered off and washed well with water. Two crystallizations from methanol yielded small needles (27 mg.), m.p. 172–174°; $[\alpha]^{21}D$ −15° (*c* 0.798); λ_{max}^{alc} 212 mμ (ε 20,500), 258 (10,790), 307 (2283); λ_{max}^{Nujol} 5.67 (s), 5.76 (s), 5.90 (s), 8.02 (s) μ.

Anal. Calcd. for $C_{22}H_{25}O_5Br$ (449.3): C, 58.80; H, 5.61. Found: C, 59.06; H, 5.90.

B. From 6-Ketoestradiol Diacetate (Ia).—To a solution of 6-ketoestradiol 3,17-diacetate (248 mg.) in glacial acetic acid (15 ml.) 0.65 *M* bromine in acetic acid (2 drops) was added. The yellow color faded out on the addition of 1 drop of 32% hydrobromic acid. A total of 1.08 ml. (5% excess) of bromine solution was added dropwise. The reaction mixture was diluted with water and extracted twice with chloroform. The chloroform was then washed with water, sodium bicarbonate, and again with water. The residue from the sodium sulfate-dried chloroform after three crystallizations from methanol melted at 171–172; $[\alpha]^{19}D$ −16° (*c* 1.033). The spectral characteristics were identical with those of the specimen prepared from Va.

Anal. Calcd. for $C_{22}H_{25}BrO_5$ (449.3); C, 58.80; H, 5.61. Found: C, 58.70; H, 5.73.

The **17-monoacetate VIb** of the bromo ketone was obtained when a solution of the diacetate (102 mg.) and *p*-toluene sulfonic acid (204 mg.) in acetic acid (4 ml.) was heated under reflux in an atmosphere of nitrogen for 14 hr. After the addition of water, the product was isolated by extraction with ether–benzene. The residue from the extract (95 mg.) after two crystallizations from dilute methanol yielded needles (63 mg.), m.p. 158–160°; $[\alpha]^{20}D$ −21° (*c* 1.031); λ_{max}^{alc} 216 mμ (ε 16,890), 261 (8880), 336 (1700), 316 sh (1640); λ_{max}^{Nujol} 2.95 (m), 5.64 (m), 5.77 (s), 5.84 (s), 5.93 (s), 8.07 (s) μ.

Anal. Calcd. for $C_{20}H_{23}O_4Br$ (407.3): C, 58.97; H, 5.69. Found: C, 58.82; H, 5.98.

For **6-keto-7α-bromoestradiol diacetate**, O.R.D. in dioxane (*c* 0.05525) showed $[\alpha]_{700-525}$ −20, $[\alpha]_{450}$ −60, $[\alpha]_{373}$ +576, $[\alpha]_{333}$ −1257, $[\alpha]_{325}$ −494°.

For **6-ketoestradiol diacetate**, O.R.D. in dioxane (*c* 0.06075) showed $[\alpha]_{600}$ −30, $[\alpha]_{468}$ −60 (broad trough), $[\alpha]_{370}$ +488, $[\alpha]_{365}$ +430, $[\alpha]_{357}$ +553, $[\alpha]_{317.5}$ −1460, $[\alpha]_{310}$ −824°.

6α-Hydroxyestradiol 3,17-Diacetate (IIc) from 6β-Hydroxy-7α-bromoestradiol 3,17-Diacetate (Va).—A solution of 6β-hydroxy-7α-bromoestradiol diacetate (305 mg.) in 95% ethanol (19 ml.) was added to a suspension of hydrogenated 5% palladium on calcium carbonate (303 mg.) in 95% ethanol (11 ml.). The hydrogen uptake ceased after 6 hr. when the equivalent of 1 mole had been consumed. The catalyst was filtered off and the ethanol was removed *in vacuo*. The residue was dissolved in chloroform, washed twice with water, and dried over sodium sulfate. After the removal of the solvent *in vacuo*, the residue (241 mg.) was twice recrystallized from ethyl acetate–hexane, yielding 113 mg., m.p. 140–141°; $[\alpha]^{21}D$ +53° (*c* 0.754); λ_{max}^{alc} 268 mμ (ε 705), 276 (660), 261 sh (562); λ_{max}^{Nujol} 2.88 (s), 5.69 (s), 5.88 (s), 7.90 (m) μ.

Anal. Calcd. for $C_{22}H_{28}O_5$ (372.4): C, 70.94; H, 7.58. Found: C, 71.16; H, 7.73.

Acetylation of the diacetate IIc in anhydrous pyridine and acetic anhydride gave the triacetate IIb, identical with an authentic sample by mixture melting point, rotation, and infrared spectrum. Oxidation of IIc (33 mg.) in acetone with Jones reagent yielded 6-ketoestradiol diacetate (Ia, 21 mg.), m.p. 173–174°; λ_{max}^{alc} 248 mμ (ε 10,000), 300 (2045). Admixture with an authentic sample did not depress the melting point.

In another debromination experiment conducted under the same conditions on Va (153 mg.), the crude reaction product was chromatographed on a thin layer of Woelm neutral alumina, grade V. The chromatogram was developed with 24:1 chloroform–ethyl acetate. Short irradiation with an ultraviolet lamp served to locate the bands; elution was effected with 1:9 methanol–ethyl acetate. The material eluted in the first band just behind the front (29 mg.) was inhomogeneous and was, therefore, rechromatographed. The second band contained 6β-hydroxyestradiol diacetate (16 mg.) identified by its melting point, 158–160°, and its infrared spectrum; from the third band 56 mg. of the lower melting polymorphous modification of 6α-hydroxyestradiol diacetate, m.p. 128–130°, was obtained after purification. On rechromatography of the first band on Woelm neutral alumina in which chloroform alone was used for development, estradiol 3,17-diacetate was isolated as the main product. A small amount of estradiol 17-monoacetate[30] was also present. For identification the 17-monoacetate was prepared by treating the 3,17-diacetate with potassium carbonate in 90% methanol.

When the triacetate Vb of the *trans*-bromohydrin was hydrogenated in 95% EtOH with 5% palladium on calcium carbonate as the catalyst and then chromatographed, only estradiol 3,17-diacetate could be isolated.

6α-Hydroxy-7α-bromoestradiol 3,17-Diacetate (VIIa).—A solution of 6-keto-7α-bromoestradiol 3,17-diacetate (32 mg.) in methanol (15 ml.) was cooled to 0° and treated dropwise under anhydrous conditions with a chilled solution of sodium borohydride (33 mg.) in methanol (6 ml.). After 15 min., the reaction mixture was acidified to about pH 5 with 10% acetic acid and diluted with cold water (60 ml.). Extraction with ether in the usual way yielded 32 mg. of residue which after two crystallizations from ethyl acetate–hexane was obtained as long narrow plates (19 mg.) melting at 170–171°; $[\alpha]^{20}D$ +29° (*c* 0.771);

(29) The 6-dehydroestrone from which 6-dehydroestradiol diacetate was prepared was obtained from Dr. Earl Chamberlin of the Merck Sharp and Dohme Research Laboratories through the good offices of the Cancer Chemotherapy National Service Center. We wish to thank Dr. Chamberlin for the generous gift of 10 g. of this compound.

(30) K. Miescher and C. Scholz, *Helv. Chim. Acta*, **20**, 263 (1937).

λ_{max}^{alc} 267 mμ (ε 610), 275 (570); λ_{max}^{Nujol} 3.94 (m), 5.66 (s), 5.80 (s), 8.03 (s) μ.

Anal. Calcd. for $C_{22}H_{27}O_5Br$ (451.4): C, 58.55; H, 6.03. Found: C, 58.62; H, 5.92.

The triacetate VIIb prepared from VIIa by acetylation in pyridine formed small prisms from methanol melting at 228–230°; $[\alpha]^{20}D$ +68° (*c* 1.114); λ_{max}^{alc} 267 mμ (ε 580), 275 (560), 217 sh (10,840); λ_{max}^{Nujol} 5.69 (s), 5.74 (s), 5.78 (s), 8.10 (s) μ.

Anal. Calcd. for $C_{24}H_{29}O_6Br$ (493.4): C, 58.42; H, 5.92. Found: C, 58.30; H, 5.84.

Debromination of 6α-Hydroxy-7α-bromoestradiol 3,17-Diacetate (VIIa).—A solution of 6α-hydroxy-7α-bromoestradiol 3,17-diacetate (103 mg.) in 90% ethanol (10 ml.) was added to a suspension of hydrogenated 5% palladium on calcium carbonate (106 mg.) in 90% ethanol (2 ml.) and was shaken with hydrogen at atmospheric pressure. The hydrogen uptake ceased after 6 hr. when only 0.6 equiv. of 1 mole had been consumed. The catalyst was removed by filtration and the solution was evaporated to dryness *in vacuo*. A chloroform solution of the residue, after being washed and dried, yielded 95 mg. which was chromatographed on a thin layer of Woelm neutral alumina, grade V, and developed with 24:1 chloroform–ethyl acetate. The infrared spectrum and optical rotation of the main fraction (37 mg., m.p. 125–127°) were identical with those of 6α-hydroxyestradiol 3,17-diacetate (IIc), and in mixture with an authentic sample the melting was not depressed. The second crystalline compound obtained proved to be some unattacked starting material.

Conversion of 6α-Hydroxy-7α-bromoestradiol 3,17-Diacetate (VIIa) to 6-Ketoestradiol 17-Monoacetate (Ib).—A solution of 6α-hydroxy-7α-bromoestradiol 3,17-diacetate (58 mg.) in anhydrous *t*-butyl alcohol was treated with 0.45 *M* potassium *t*-butoxide in *t*-butyl alcohol (1.14 ml.). After 5 min. ether (40 ml.) was added and the reaction mixture was washed twice with water and dried over sodium sulfate. The residue (39 mg.) crystallized from 95% ethanol as diamond-shaped plates, m.p. 280–283°, which were identified as 6-ketoestradiol 17-monoacetate (Ib) by analysis and comparison (infrared, mixture melting point) with an authentic sample. The latter was obtained by treating 6-ketoestradiol 3,17-diacetate (Ia, 65 mg.) with 1% potassium bicarbonate in 90% aqueous methanol (5.5 ml.) for 1 hr. at room temperature and isolating the product by ether extraction. The ether residue (38 mg.) was thrice crystallized from 95% ethanol and then melted at 284–287°; $[\alpha]^{20}D$ −18° (*c* 0.997, dioxane); λ_{max}^{alc} 221 mμ (ε 20,920), 256 (8840), 327 (3090); λ_{max}^{Nujol} 2.96 (m), 5.76 (s), 6.01 (s), 6.21 (m), 8.01 (s) μ.

Anal. Calcd. for $C_{20}H_{24}O_4$ (328.4): C, 73.14; H, 7.37. Found: C, 73.34; H, 7.41.

6β,7β-Oxidoestradiol Diacetate (IXa).—6β-Hydroxy-7α-bromoestradiol 3,17-diacetate (200 mg.) was dissolved in dry *t*-butyl alcohol (5 ml.) and treated at room temperature with freshly prepared 0.31 *M* potassium *t*-butoxide in *t*-butyl alcohol (2.14 ml.). After 15 min. ether (200 ml.) was added and the mixture was washed twice with water and then dried over sodium sulfate. The glassy residue (167 mg.) crystallized from dilute methanol as rods which melted at 124–126°; $[\alpha]^{19}D$ −61° (*c* 0.858); λ_{max}^{alc} 272 mμ (ε 736), 278 (791), 215 sh (6510), 287 sh (354); λ_{max}^{Nujol} 5.64 (s), 5.78 (s), 7.97 (s), 8.06 (s) μ.

Anal. Calcd. for $C_{22}H_{26}O_5$ (370.4): C, 71.33; H, 7.08. Found: C, 71.23; H, 6.91.

The **17-monoacetate IXb** of the β-oxide was obtained in the above reaction when the potassium *t*-butoxide was not limited to 1.5 mole equiv. and also when in the work-up water was added before ether. This product crystallized from methanol as rectangular plates, m.p. 183–186; $[\alpha]^{20}D$ −37° (*c* 0.44); λ_{max}^{alc} 244 mμ (ε 6430), 286 (2265); λ_{max}^{Nujol} 2.97 (m), 5.83 (s), 7.83 (s) μ.

Anal. Calcd. for $C_{20}H_{24}O_4$ (328.4): C, 73.14; H, 7.37. Found: C, 73.26; H, 7.63.

When an attempt was made to acetylate the 17-monoacetate IXb in anhydrous pyridine and acetic anhydride, only a small amount of an amorphous product could be recovered by ether extraction, the remainder, probably 6-pyridinium salts, staying in the aqueous phase. However, the diacetate IXa was readily obtained when the monoacetate was allowed to stand in acetic anhydride overnight, and the mixture was worked up in the usual way by ether extraction. The residue from the washed and dried ether solution on crystallization from methanol melted at 124–125°, and its infrared spectrum was identical with that of the diacetate.

7β-Hydroxyestradiol (XIb). A. From 6β,7β-Oxidoestradiol Diacetate (IXa).—A solution of 6β,7β-oxidoestradiol diacetate (100 mg.) in dry tetrahydrofuran (6 ml.) was added slowly to a mixture of a saturated ether solution of lithium aluminum hydride (2 ml.) and tetrahydrofuran (20 ml.) which was then heated under reflux for 6 hr. Excess lithium aluminum hydride was destroyed by adding ethyl acetate dropwise and the reaction mixture was distributed between ethyl acetate (100 ml.) and water (100 ml.). Hydrochloric acid (2 *N*) was added to dissolve the flocculent precipitate in the water before the layers were separated. The residue (75 mg.) from the washed and dried ethyl acetate crystallized from a minimum amount of methanol and ethyl acetate yielding plates (44 mg.) melting at 237–238°; $[\alpha]^{21}D$ +38° (*c* 0.863, in absolute ethanol); λ_{max}^{alc} 281 mμ (ε 2050), 220 sh (6200); λ_{max}^{Nujol} 2.91, 2.99 (doublet, m) μ.

Anal. Calcd. for $C_{18}H_{24}O_3$ (288.4): C, 74.97; H, 8.39. Found: C, 74.92; H, 8.49.

The **triacetate XIc** obtained by acetylating the triol with anhydrous pyridine and acetic anhydride in the usual manner crystallized from acetone–hexane as small needles, m.p. 171–173°; $[\alpha]^{20}D$ +60°; λ_{max}^{KBr} 5.69 (s), 5.78 (s), 8.02 (s) μ.

B. From 7β-Hydroxyestrone.—A solution of sodium borohydride (60 mg.) in water (3 ml.) was added dropwise to a stirred solution of 7β-hydroxyestrone (30 mg.) in methanol (9 ml.). After 3 hr. at room temperature, the excess sodium borohydride was decomposed with 10% acetic acid, and water (15 ml.) was added. Two extractions with ethyl acetate (40 ml.), which was washed with water and dried, yielded the crude triol which after recrystallization from ethyl acetate (22 mg.) melted at 239–240°, and was found to be identical with the specimens obtained from the oxide IXa by comparison of the infrared spectra.

6β,7α-Dihydroxyestradiol 3,17-Diacetate (XIIa).—6α,7α-Oxidoestradiol diacetate[12] (X, 30 mg.) was dissolved in 50% aqueous acetone (20 ml.) containing a drop of 70% perchloric acid and was heated under reflux for 15 min. The residue obtained by evaporation *in vacuo* of a washed and dried ethyl acetate extract of the diluted reaction mixture was twice crystallized from ethyl acetate–hexane and yielded 17 mg., m.p. 170–171°; $[\alpha]^{20}D$ +16° (*c* 0.622, dioxane); λ_{max}^{alc} 267 mμ (ε 570), 275 (550), 215 sh (9430); λ_{max}^{Nujol} 2.92 (s), 5.65 (s), 5.86 (s), 7.93 (s) μ.

Anal. Calcd. for $C_{22}H_{28}O_6$ (338.4): C, 68.02; H. 7.27. Found: C, 68.02; H, 7.26.

The tetraacetate XIIb was prepared by the acetylation of the diacetate XIIa in anhydrous pyridine and acetic anhydride at room temperature overnight. The small rods from dilute ethanol melted at 134–136°; $[\alpha]^{21}D$ +68° (*c* 0.311), λ_{max}^{alc} 269 mμ (ε 737), 276 (690), 214 sh (12,300); λ_{max}^{Nujol} 5.68 (m), 5.77 (s), 8.05 (s) μ.

Anal. Calcd. for $C_{26}H_{32}O_8$ (472.5): C, 66.08; H, 6.83. Found: C, 66.29; H, 6.72.

6α,7β-Dihydroxyestradiol Tetraacetate (XIVb). A. From 6β,7β-Oxidoestradiol Diacetate.—A solution of 6β,7β-oxidoestradiol diacetate (21 mg.) in 50% aqueous ethanol (5 ml.) containing a drop of 70% perchloric acid was boiled under reflux for 15 min. After the addition of water the reaction mixture was extracted with ethyl acetate and subsequently washed with dilute sodium bicarbonate solution and water. The amorphous residue was acetylated in 0.1 ml. of anhydrous pyridine and 0.2 ml. of acetic anhyride at 90° for 1 hr. The reaction mixture, worked up in the usual manner, yielded 24 mg. which crystallized from ethyl acetate–hexane as small rods and melted at 215–216°; $[\alpha]D$ −1° (*c* 0.791). The ultraviolet and infrared spectra were identical with those of the 6α,7β-dihydroxyestradiol tetraacetate obtained from 6β-hydroxy-7α-bromoestradiol 3,17-diacetate as described below.

B. From 6β-Hydroxy-7α-bromoestradiol 3,17-Diacetate (Va) *via* 6α,7β-Dihydroxyestradiol 17-Monoacetate (XIVa).—A solution of 6β-hydroxy-7α-bromoestradiol diacetate (45 mg.) in methanol (2 ml.) was flushed with nitrogen and then was treated with 10% aqueous potassium carbonate (0.2 ml.) which had previously been flushed with nitrogen. After 1 hr. glacial acetic acid (2 drops) was added and the mixture was diluted with water. The noncrystallizable residue (33 mg.) obtained by evaporation *in vacuo* of a washed and dried ether extract consisted largely of the tetrol 17-monoacetate XIVa as shown by the following data: $[\alpha]^{22}D$ +30° (*c* 0.613); λ_{max}^{alc} 282 mμ (ε 2150); λ_{max}^{Nujol} 2.98 (m), 5.83 (s), 8.00 (s) μ.

Anal. Calcd. for $C_{26}H_{28}O_5$ (346.4): C, 69.34; H, 7.57. Found: C, 69.36; H, 7.58.

A solution of the above monoacetate (64 mg.) and *p*-toluenesulfonic acid hydrate (96 mg.) in acetic acid (3.6 ml.) and acetic anhydride (1.25 ml.) was allowed to stand at room temperature overnight. The reaction mixture was poured onto crushed ice

and dilute sodium carbonate solution and then extracted with ether. The residue (78 mg.) from the washed and dried ether was twice crystallized from ethyl acetate–hexane, yielding small rods, m.p. 216–217°; $[\alpha]^{21}D$ −2° (*c* 0.578, dioxane); λ_{max}^{alc} 267 mμ (ε 780), 276 (723), 260 sh (595); λ_{max}^{Nujol} 5.63, 5.68, 5.75 (triplet, s), 8.05 (s) μ.

Anal. Calcd. for $C_{26}H_{32}O_8$ (472.5): C, 66.08; H, 6.83. Found: C, 65.76; H, 6.78.

The monoacetate XIVa was also obtained as the sole product when in the preparation of 6β,7β-oxidoestradiol diacetate glacial acetic acid equivalent to the moles of potassium *t*-butoxide was added at the beginning of the work-up.

6β-Methoxy-7α-hydroxyestradiol 3,17-Diacetate(XVa).—6α,7α-Oxidoestradiol diacetate (32 mg.) was dissolved in reagent methanol (10 ml.) containing 70% perchloric acid (0.04 ml.). After about 10 min. at room temperature, the reaction mixture was neutralized with a saturated solution of sodium bicarbonate. The methanol was removed *in vacuo* and the residue from a washed and dried chloroform extract crystallized on standing in dilute methanol as small needles, m.p. 83–88°; $[\alpha]^{21}D$ +25° (*c* 0.777, dioxane); λ_{max}^{alc} 267 mμ (ε 620), 275 (580); λ_{max}^{Nujol} 2.95 (m), 5.67 (s), 5.77 (s), 8.07 (s) μ.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.5): OCH_3, 7.71. Found: OCH_3, 7.79.

When the methanolic mother liquor of 6α,7α-oxidoestradiol diacetate (X) was allowed to stand at room temperature for several days and the solid obtained by removal of the methanol was chromatographed, the main product proved to be 6β-methoxy-7α-hydroxyestradiol 3,17-diacetate. Its melting point, rotation, and spectral data were identical with those of the methanolysis product of the oxide.

Triacetate XVb.—6β-Methoxy-7α-hydroxyestradiol 3,17-diacetate (50 mg.) was acetylated in anhydrous pyridine and acetic anhydride in the usual manner. The reaction product was recrystallized twice from ethyl acetate–hexane yielding fine rods (41 mg.), m.p. 182–183°; $[\alpha]^{21}D$ −9° (*c* 0.867); λ_{max}^{alc} 267 mμ (ε 613), 275 (564), 263 sh (503); λ_{max}^{Nujol} 5.63 (s), 5.74 (s), 8.04 (s) μ.

Anal. Calcd. for $C_{25}N_{32}O_7$ (444.5): C, 67.55; H, 7.26. Found: C, 67.56; H, 7.37.

6α-Methoxy-7β-Hydroxyestradiol 3,17-Diacetate XVI.—6β,7β-Oxidoestradiol diacetate (43 mg.) was dissolved in reagent methanol (15 ml.) containing 70% perchloric acid (0.053 ml.). After about 10 min. at room temperature, the reaction mixture was neutralized with a saturated solution of sodium bicarbonate. The methanol was removed *in vacuo* and the residue (42 mg.) from the washed and dried chloroform extract was twice crystallized from ethyl acetate–hexane yielding small needles, m.p. 177–178°; $[\alpha]^{20}D$ −18° (*c* 0.775, dioxane); λ_{max}^{alc} 267 mμ (ε 563), 273 (527); λ_{max}^{KBr} 2.94 (m), 5.57 (s), 5.77 (s), 8.06 (s) μ.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.5): C, 68.63; H, 7.51; OCH_3, 7.71. Found: C, 68.82; H, 7.79; OCH_3, 7.35.

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Microbial Hydroxylation of Estrone and Estradiol in the 6β-, 7α-, and 15α-Positions

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Estrone and estradiol have been converted into their 7α- and 15α-hydroxy derivatives by microorganisms of the genera *Glomerella* and *Aspergillus*, and to 6β-estradiol by *Mortierella alpina*. 15α-Hydroxyestrone and 15α-hydroxyestradiol are new compounds of low estrogenic potency.

The stereospecific hydroxylation of steroidal substrates by microbial enzymes has during the past decade developed into one of the most important and widely employed reactions in steroid chemistry. It has been applied to a large variety of substrates particularly those possessing the pregnane and androstane skeleton, and with very few exceptions all available positions of the steroid nucleus have been hydroxylated by this elegant procedure.[2] There is one important class of steroids, however, that is only sparsely represented in the literature in connection with this reaction and these are the phenolic estrogens. Only two reports have appeared,[3,4] both presenting evidence for the 16α-hydroxylation of estrone and estradiol. This limited measure of accomplishment is surely not due to a lack of interest in the hydroxylation of the estrogenic hormones, but, to judge from our own experience, a reflection of the tendency of these phenolic steroids to suffer multiple enzymic attack thereby giving rise to difficultly separable mixtures. The present report, therefore, represents the result of considerable effort involving a large variety of organisms.

The organisms destined for further exploration were selected on the basis of paper chromatographic evidence for complete utilization of the substrate and the formation of substantial amounts of hydroxylated products. They belong to the genera *Glomerella*, *Aspergillus*, and *Mortierella*. The organisms were grown in flasks which were vigorously shaken to promote maximum growth, then transferred to a fresh growth medium, to which the steroid had been added, and allowed to grow under the same conditions. Samples were taken at intervals and analyzed for the degree of conversion by paper chromatography. The fermentations were terminated when all the substrate had disappeared. The steroid metabolites were then recovered from the filtered broth by extraction with chloroform and methyl isobutyl ketone, and the individual components were isolated after chromatography on alumina. It soon became evident that when the time of fermentation was extended from 2 to 7 days the organisms, in addition to hydroxylating estrone, also caused partial reduction of the 17-keto group, thus producing the corresponding hydroxylated estradiol derivatives. The reverse was true when estradiol was used as a substrate. This complicated the isolation process leading to twice as many metabolites as positions hydroxylated. Thus, *Glomerella fusarioides* (ATCC 9552) furnished at least

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(2) For a most recent review on this subject with citations of earlier review papers, *cf.* C. Tamm, *Angew. Chem.*, **74**, 225 (1962).

(3) K. A. Kita, J. L. Sardinas, and G. M. Shull, *Nature*, **190**, 627 (1961).

(4) B. F. Stimmel, T. E. Bucknell, and V. Notchev, *Federation Proc.*, 115 (1960).

(5) J. Iriarte, H. J. Ringold, and C. Djerassi, *J. Am. Chem. Soc.*, **80**, 6105 (1958).