ORGANIC AND BIOLOGICAL CHEMISTRY

[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY]

Synthesis and Reactions of Steroidal Quinols[1]

BY A. M. GOLD AND E. SCHWENK

RECEIVED JUNE 25, 1958

The *p*-quinols and *p*-quinol acetates derived from estrone and estradiol were prepared. 10ξ-Acetoxy-1,4-estradiene-3,17-dione (I) reacts with acetic anhydride and sulfuric acid to give the diacetate of 1,3-dihydroxy-1,3,5(10)-estratriene-17-one (XII). Rearrangement of 10ξ-hydroxy-1,4-estradiene-3,17-dione (II) with aqueous acid produces 1,4-dihydroxy-1,3,5(10)-estratriene-17-one (XV) and reaction with PBr_3 leads to 6-dehydroestrone (XXI). The steroidal quinones 3-hydroxy-2,5-(10)-estradiene-1,4,17-trione (XIII) and 2,5(10)-estradiene-1,4,17-trione (XIV) were produced by appropriate oxidation reactions.

Oxidation of alkylated phenols with lead tetraacetate leads to *p*- and *o*-quinol acetates and to *o*-quinone *gem*-diacetates.[2] The extraordinary reactivity of these substances makes them valuable intermediates in the synthesis of substituted phenols and polyphenols.[3,4] The corresponding free quinols were first synthesized by Bamberger[5] in 1900 by rearrangement of *p*-alkylphenylhydroxylamines, but the relative difficulty of preparing these substances has hindered extensive study of their properties. The possible biological importance of quinolic intermediates in the production of polyphenols recently has been suggested by Goodwin and Witkop,[4] although as early as 1908 a quinol was suspect in the biosynthesis of homogentisic acid from tyrosine.[6]

The isolation of oxidized derivatives of the natural estrogens, such as estriol,[7] 16α-hydroxyestrone[8] and 2-methoxyestrone[9] from human urine and 6-ketoestradiol and 6β-hydroxyestradiol[10] from experiments with mouse liver preparations emphasizes the metabolic importance of reactions which introduce oxygen atoms into the hormone molecule. The synthesis of quinols and quinol acetates related to the natural estrogenic hormones, estrone and estradiol therefore was undertaken and some of their transformations were studied.

Oxidation of estrone with lead tetraacetate produced the expected 10ξ-acetoxy-1,4-estradiene-3,17-dione (I) in yields of the order of 20%. The ultraviolet absorption maximum at 248 mμ (ε 13,000) in methanol and the appearance of maxima at 5.76, 6.02, 6.15 and 6.25 μ in the infrared spectrum of the substance strongly indicated the presence of the *p*-quinol acetate chromophore. Catalytic reduction of I resulted in consumption of one molar proportion of hydrogen and production of estrone in good yield.

In addition to I, two other substances were isolated in small yields from the oxidation mixture. The first crystallized in pale platelets of m.p. 207–208° dec. The infrared and ultraviolet spectra ($\lambda_{max}^{CH_3OH}$ 335 mμ, ε 3,400) indicated that the substance was an *o*-quinone *gem*-diacetate, one of the anticipated reaction products. Quantitative catalytic reduction confirmed the molecular weight and produced a crude product that appeared to be a catechol monoacetate derivative. The infrared spectrum of the latter substance contained a strong band at 12.3 μ, probably due to a pair of adjacent hydrogen atoms attached to a benzene ring.[11] Acetylation of the monoacetate gave a diacetate of m.p. 212.5–215.5° which also had a strong band in the infrared at 12.3 μ. The diacetate was identified as 3,4-diacetoxy-1,3,5(10)-estratriene-17-one (VI) by comparison with a sample prepared from authentic 4-hydroxyestrone.[12] The substances showed identical infrared spectra and the melting point of a mixture was not depressed. The monoacetate must have been 3-hydroxy-4-acetoxy-1,3,5(10)-estratriene-17-one (V) and the original oxidation product was 4,4-diacetoxy-1,5(10)-estradiene-3,17-dione (IV).

The other oxidation product (VII), m.p. 174–175°, remains unidentified. The infrared spectrum showed no hydroxyl absorption, a single sharp carbonyl band at 5.79 μ and absorption at 6.25 and 6.62 μ, apparently due to an aromatic ring. The ultraviolet absorption maximum at 281–286 mμ ($E_{1cm.}^{1\%}$ 126) suggests an aromatic ether. Attempted acidic and basic hydrolysis, as well as catalytic reduction in ethanol over Pd–charcoal catalyst, left the substance unchanged. Microanalysis indicated the empirical formula $C_{20}H_{26}O_3$.

Hydrolysis of the quinol acetate I was accomplished in high yield by gentle treatment with a slight excess of sodium methoxide in methanol. Reaction with a catalytic amount of sodium methoxide did not result in complete hydrolysis. The free 10ξ-hydroxy-1,4-estradiene-3,17-dione (II), m.p. 215–217°, appeared to be much more stable than the acetate I. The hydroxyl and characteristic dieneone bands in the infrared spectrum and the ultraviolet maximum at 235 mμ (ε 13,000) are in agreement with the *p*-quinol structure. Reduction of II with zinc in acetic acid resulted in

(1) This work was supported by a grant from the Schering Corp., Bloomfield, N. J. and by their generous gifts of estrone and estradiol.

(2) F. Wessely and F. Sinwel, *Monatsh.*, **81**, 1055 (1950).

(3) O. Polansky, E. Schinzel and F. Wessely, *ibid.*, **87**, 24 (1956); F. Langer and F. Wessely, *ibid.*, **88**, 298 (1957); W. Metlesics, F. Wessely and Budzikiewicz, *ibid.*, **89**, 102 (1958).

(4) S. Goodwin and B. Witkop, THIS JOURNAL, **79**, 179 (1957).

(5) E. Bamberger, *Ber.*, **33**, 3600 (1900).

(6) E. Friedmann, *Beitr. chem. Physiol. Pathol.*, **11**, 304 (1908); E. Mayer, *Deutsch. Arch. Klin. Med.*, **70**, 443 (1901).

(7) G. F. Marrian, *Biochem. J.*, **24**, 435 (1930).

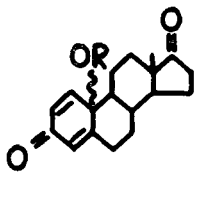
(8) G. F. Marrian, K. H. Loke, E. J. D. Watson and M. Panattoni, *ibid.*, **66**, 60 (1957).

(9) S. Kraychy and T. F. Gallagher, *J. Biol. Chem.*, **229**, 519 (1957).

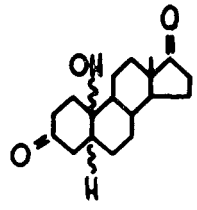
(10) G. C. Mueller and G. Rumney, THIS JOURNAL, **79**, 1004 (1957).

(11) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley & Sons, Inc., New York, N. Y., 1954, p. 67.

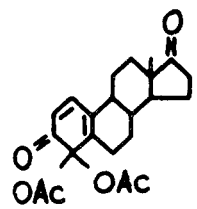
(12) Kindly provided by Drs. T. F. Gallagher and J. Fishman, Sloan–Kettering Institute, N. Y. C., prior to publication.



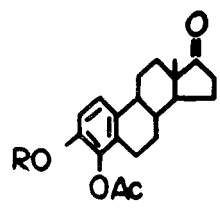
I, R=$COCH_3$
II, R=H



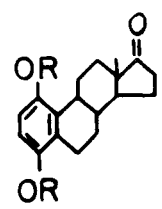
III



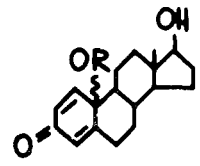
IV



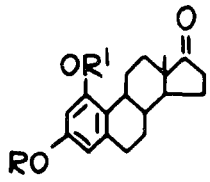
V, R=H
VI, R=$COCH_3$



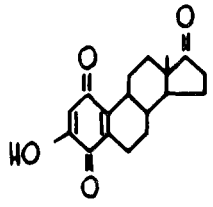
XV, R=H
XVI, R=$COCH_3$



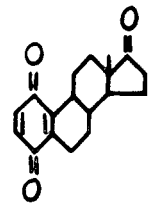
VIII, R=$COCH_3$
IX, R=H



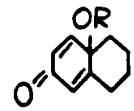
X, R=R'=$COCH_3$
XI, R=H, R'=$COCH_3$
XII, R=R'=H



XIII



XIV



XVII, R=$COCH_3$
XVIII, R=H

the formation of estrone, as expected. Catalytic reduction, however, took an unexpected course and produced the tetrahydro derivative of II, 10ξ-hydroxy-5ξ-estrane-3,17-dione (III).

Oxidation of estradiol-17β with lead tetraacetate resulted in a 10% yield of the corresponding *p*-quinol acetate, 10ξ-acetoxy-17β-hydroxy-1,4-estradiene-3-one (VIII). This substance could be catalytically reduced to estradiol under the same conditions used for I. Hydrolysis produced the free quinol 10ξ,17β-dihydroxy-1,4-estradiene-3-one (IX). No direct correlation of the configuration at C-10 was made between the 17-keto and 17β-hydroxy series, but the molecular rotation change on hydrolysis of I (+59°) compared to that on hydrolysis of VIII (+42°) indicates that the configurations are the same.

Rearrangement of I under the conditions of the Thiele reaction, acetic anhydride and sulfuric acid, resulted in isolation of a substance assigned the structure X, 1,3-diacetoxy-1,3,5(10)-estratriene-17-one. The analogous *p*-quinol acetate from 6-hydroxytetralin (XVII) gives 5,7-diacetoxytetralin under these conditions.[4] Mild acid hydrolysis of X produced a monoacetate assumed to be the 1-acetate XI. More vigorous acid hydrolysis gave the free 1,3-dihydroxy-1,3,5(10)-estratriene-17-one (XII). Both XI and XII could be reacetylated with pyridine and acetic anhydride to yield X.

Treatment of the free quinol II with acetic anhydride and sulfuric acid produced an oil from which traces of crystalline material could be isolated by careful chromatography. Reaction of II with aqueous acid gave a mixture which could not be easily purified, but oxidation of the crude material with chromic acid resulted in facile isolation of bright yellow crystals of 2,5(10)-estradiene-1,4,17-trione (XIV). This quinone showed ultraviolet absorption maxima at 250 mμ (ϵ 16,000) and 340 mμ (broad) (ϵ 1,200) in methanol and infrared maxima at 5.75, 6.03 and 6.25 μ.

The hydroquinone XV, 1,4-dihydroxy-1,3,5(10)-estratriene-17-one, was regenerated by chemical or catalytic reduction of the quinone. Chemical reduction, with zinc and acetic acid, appeared superior to catalytic reduction, possibly because of reoxidation of the material by atmospheric oxygen when the hydrogenation apparatus was dismantled. The hydroquinone had prominent absorption in the infrared at 12.4 μ due to the pair of hydrogen atoms at the 2- and 3-positions. A diacetate XVI prepared from XV showed the same infrared spectrum as the trace product from Thiele acetylation of the quinol II.

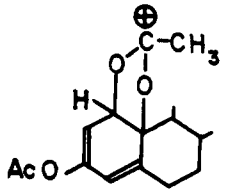
The *p*-quinol related to 6-hydroxytetralin (XVIII) has been shown to give the diacetate of 5,8-dihydroxytetralin in fair yield when treated with acetic anhydride and sulfuric acid.[13] The failure of II to give more than a trace of XVI under these conditions is difficult to explain.

Oxidation of the hydroxyestrone XII or its monoacetate XI with potassium nitrosodisulfonate (Fremy's salt)[14] produced 3-hydroxy-2,5(10)-estradiene-1,4,17-trione (XIII). This substance could be extracted from chloroform with aqueous bicarbonate, giving a deep red solution which deposited the yellow quinone when acidified. The ultraviolet spectrum in pure methanol showed λ_{max} 277 mμ (ϵ 14,000); in 0.2 *N* methanolic HCl, λ_{max} 275 mμ (ϵ 16,000); and in 0.1 *N* methanolic NaOH, λ_{max} 284 mμ (ϵ 11,000). The hydroquinone XV was also oxidized with Fremy's salt; the product was the quinone XIV.

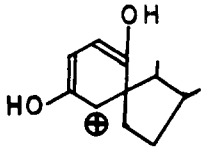
The theoretical aspects of these rearrangements have been discussed in some detail by Goodwin and Witkop.[4] Formation of a quasi-cyclic five membered ring (XIX) accounts for the ready migration of the acetoxyl group to the 1-position. In the free quinol, however, the hydroxyl group does not migrate with such facility and a hydroquinone is pro-

(13) Y. Asahina and T. Momose, *Ber.*, **71**, 1421 (1938).
(14) H. J. Teuber and G. Staiger, *ibid.*, **88**, 802 (1955).

duced through the *spiro* intermediate XX. The fact that it was found necessary to change the reaction conditions drastically in order to obtain acceptable yields of the hydroquinone XV suggests that this reaction is subject to some of the same uncertainties that cloud the rearrangement of 4,4-dialkylcyclohexadieneones.[15]

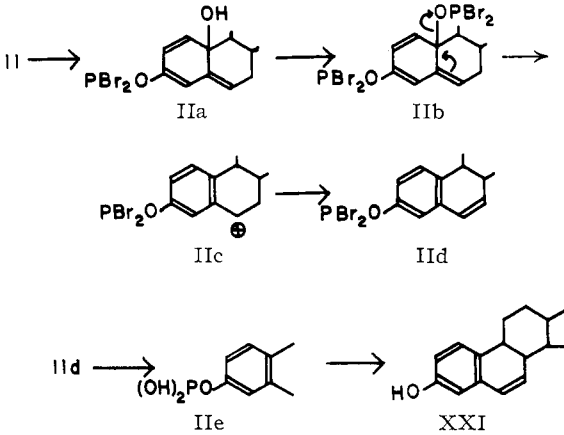


XIX



XX

When the quinol II was treated with phosphorus tribromide in methylene chloride, a small quantity of 6-dehydroestrone (XXI) was isolated. The identity of this substance was verified by mixture melting point and comparison of its characteristic infrared and ultraviolet spectra with those of an authentic sample.[16] This rather surprising result could be rationalized by assuming initial formation of the enol dibromophosphite ester of the steroid (IIa) under the influence of a Lewis acid. The enol ester may then react with more PBr_3 to give a diester IIb which is properly disposed to give IId by elimination through a benzylic carbonium ion (IIc). A concerted mechanism involving no cation can, of course, be written. Treatment with water converts the dibromophosphite ester IId to the acidic ester IIe which is extractable with aqueous bicarbonate solution. Finally, acid hydrolysis produces free 6-dehydroestrone. The necessity of postulating the intermediate IIb is indicated by the fact that the quinol acetate I gives only a trace of 6-dehydroestrone under the same conditions and is largely recovered.



IIa IIb IIc IId IIe XXI

Experimental[17]

10ξ-Acetoxy-1,4-estradiene-3,17-dione (I).—A suspension of powdered estrone (20.0 g.) in 600 ml. of glacial acetic acid was treated with 64 g. of lead tetraacetate and agitated until homogeneous. After standing at room temperature for 20 hours the solution was concentrated by distillation in vacuum, treated with 400 ml. of water and extracted with chloroform. The chloroform solution was washed with water and 10% $KHCO_3$, concentrated to a small volume, diluted with an equal volume of benzene and chromatographed on 500 g. of neutral alumina (Woelm, activity III). The column was eluted with benzene (1 l.), 1:9 chf.–bz. (500 ml.) and 1:1 chf.–bz. (500 ml.). The eluates were evaporated and the crystalline residues were freed from oils by washing with ether. The combined product was recrystallized from methanol, yielding 3.75 g. of large white crystals. Further elution with chf. yielded another 0.78 g. of crude I and a small amount of estrone. An analytical sample, prepared by repeated recrystallization, melted at 257–259° dec. (evacuated capillary, placed in bath at 255°), $[\alpha]^{22}D$ +33° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CH_3OH}$ 248 mμ (13,000); λ_{max}^{KBr} 5.76, 6.02, 6.15 and 6.25 μ.

Anal. Calcd. for $C_{20}H_{24}O_4$: C, 73.14; H, 7.37; $COCH_3$, 13.11. Found: C, 72.84; H, 7.34; $COCH_3$, 12.55.

Catalytic Reduction of Quinol Acetate I.—The quinol acetate I (47.5 mg.) was hydrogenated quantitatively in 5.0 ml. of absolute ethanol with 23 mg. of 5% Pd–charcoal catalyst. Hydrogen consumption ceased after 30 minutes when 3.17 ml. (S.T.P.) had been consumed (theoretical 3.24 ml.). After filtration and evaporation of the solvent the product was recrystallized from methanol. It was shown to be identical with estrone by comparison of infrared spectra, melting points and mixture melting point.

4,4-Diacetoxy-1,5(10)-estradiene-3,17-dione (IV).—The methanolic mother liquor from the preparation of the quinol acetate I was evaporated and the residue was triturated with a small volume of ether. The insoluble material was recrystallized from methanol and the product, which appeared to be a mixture, was extracted with two small portions of benzene, leaving crystals of I. Recrystallization of the benzene extract from methanol yielded 0.27 g. of pale platelets. Sublimation at 180° and 0.01 mm. followed by repeated crystallization gave material of m.p. 207–208° dec. (evacuated capillary), $[\alpha]^{21}D$ +135° (*c* 0.9, $CHCl_3$)$_2$, $\lambda_{max}^{CH_3OH}$ 335 mμ (3,400); λ_{max}^{KBr} 5.70, 5.75, 5.91, 6.11 and 6.39 μ.

Catalytic Reduction of IV.—The reduction was carried out similarly to the reduction of I. The steroid (29.6 mg.) consumed 1.74 ml. (S.T.P.) of H_2 (theoretical 1.72 ml.) in 17 minutes. Isolation was accomplished by filtration and evaporation. The crude product (V) showed absorption in the infrared at 3.0, 5.69, 5.81, 6.21, 6.31, 6.72 and 12.3 μ.

The material was acetylated directly with acetic anhydride in pyridine by the usual procedure. The diacetate VI was recrystallized from benzene–cyclohexane, sublimed in vacuum and repeatedly recrystallized. The product, m.p. 212.5–215.5°, $[\alpha]^{20}D$ +105° (*c* 1.0, $CHCl_3$), showed $\lambda_{max}^{CH_3OH}$ 263 mμ (310); λ_{max}^{KBr} 5.66, 5.80, 6.77 and 12.3 μ.

An authentic sample of 4-hydroxyestrone[12] was acetylated in the usual way and recrystallized from cyclohexane–benzene. The product, m.p. 212–213.5°, gave a mixture melting point of 211.5–215.0° with VI. The infrared spectra were identical.

Unknown Substance $C_{20}H_{26}O_3$ (VII).—The mother liquors from lead tetraacetate oxidation of a total of 32 g. of estrone were combined and rechromatographed. The appropriate crystalline fractions were washed with ether, combined and recrystallized from methanol to yield 1.51 g. of I. After concentration, the methanolic mother liquor gave 0.49 g. of slightly impure VII and a third crop consisted of 0.28 g. of nearly pure VII. Sublimation at 140° and 0.01 mm. and recrystallization from cyclohexane–benzene and methanol gave an analytical sample of m.p. 174–175°, $[\alpha]^{19}D$ +148° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CH_3OH}$ 281–286 mμ ($E_{1\,cm.}^{1\%}$ 126); λ_{max}^{KBr} 5.79, 6.25 and 6.62 μ.

Anal. Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.33. Found: C, 76.43; H, 8.23.

10ξ-Hydroxy-1,4-estradiene-3,17-dione (II).—A cold solution of the quinol acetate I (1.00 g.) in 200 ml. of absolute methanol (reagent grade, not specially dried) was treated with a solution of 110 mg. of sodium metal in 10 ml. of methanol and allowed to stand at 0° for 16 hr. The solution then was treated with 0.30 ml. of glacial acetic acid and evaporated to dryness. The solid residue was extracted with several small portions of cold water, dried and

(15) A. S. Dreiding, W. J. Pummer and A. J. Tomasewski, THIS JOURNAL, **76**, 3159 (1953).

(16) Kindly provided by Dr. O. Wintersteiner, Squibb Institute for Medical Research, New Brunswick, N. J.

(17) All melting points below 270° were taken on a Hershberg apparatus using Anschütz thermometers. The authors are indebted to Mr. E. Conner of the Analytical Department of the Schering Corp. for part of the microanalyses.

recrystallized from benzene to yield 0.69 g. of white crystals. A second crop of 0.09 g. brought the total yield to 90%. An analytical sample melted at 215–217° without decomposition, $[\alpha]^{22}D$ +58° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CH_3OH}$ 235 mμ (13,000); λ_{max}^{KBr} 2.95, 5.78, 6.02, 6.17 and 6.25 μ.

Anal. Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.74. Found: C, 75.44; H, 7.72.

Chemical Reduction of *p*-Quinol II.—A solution of 50 mg. of *p*-quinol II in 1.0 ml. of glacial acetic acid was treated with 0.20 g. of zinc dust and stirred for several minutes. The mixture was filtered and the solid was washed with several portions of acetic acid and ethyl acetate. The combined solvent was evaporated and the residue was taken up in ethyl acetate, washed with dilute acid and bicarbonate solution, dried and evaporated. The residue was recrystallized from methanol. The product was shown to be estrone by its melting point and infrared spectrum.

10ξ-Hydroxy-5ξ-estrane-3,17-dione (III).—Catalytic hydrogenation of 100 mg. of 10ξ-hydroxy-1,4-estradiene-3,-17-dione (II) in 10 ml. of absolute ethanol with 25 mg. of 5% Pd–charcoal catalyst resulted in consumption of two molar equivalents of H_2. The filtered solution yielded a white product which was purified by recrystallization from cyclohexane–benzene. Sublimation and several recrystallizations from different solvent systems gave an analytical sample of m.p. 203.0–204.4°, $[\alpha]^{19}D$ +92° (*c* 1.0, CH_3OH), λ_{max}^{KBr} 2.90 and 5.80 μ. No high intensity ultraviolet absorption was apparent above 220 mμ.

Anal. Calcd. for $C_{18}H_{26}O_3$: C, 74.44; H, 9.03. Found: C, 74.46; H, 9.26.

10ξ-Acetoxy-17β-hydroxy-1,4-estradiene-3-one (VIII).—The preparation was similar to that of I. A solution of estradiol-17β (5.00 g.) in 100 ml. of glacial acetic acid was oxidized with 16.6 g. of lead tetraacetate. The chromatographic fractions containing crystalline material were combined and recrystallized from benzene–ethyl acetate, yielding 0.63 g. of product. A purified sample melted at 197–199° dec., $[\alpha]^{23}D$ −39° (*c* 1.0, CH_3OH), $\lambda_{max}^{CH_3OH}$ 250 mμ (13,000); λ_{max}^{KBr} 2.74, 5.71, 6.01, 6.15 and 6.23 μ.

Anal. Calcd. for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.67; H, 8.38.

10ξ,17β-Dihydroxy-1,4-estradiene-3-one (IX).—The preparation was carried out similarly to that of II. The product was recrystallized from ethyl acetate–ethanol, methanol–acetone and methanol–acetonitrile to yield an analytical sample of m.p. 238–241°, $[\alpha]^{23}D$ −30° (*c* 1.0, CH_3OH), $\lambda_{max}^{CH_3OH}$ 241 mμ (12,000); λ_{max}^{KBr} 2.78–3.04, 6.02, 6.16 and 6.25 μ.

Anal. Calcd. for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 75.14; H, 8.56.

1,3-Diacetoxy-1,3,5(10)-estratriene-17-one (X).—To a mixture of 0.50 g. of quinol acetate I and 5.0 ml. of acetic anhydride was added 0.25 ml. of a solution of 0.10 ml. of H_2SO_4 in 1.0 ml. of acetic anhydride. The vessel was tightly stoppered and gently agitated until all the solid had dissolved. After standing at room temperature for 3 hr. the solution was poured into 50 ml. of water containing 1 g. of K_2CO_3 and agitated intermittently for one hour. The mixture then was extracted with two 20-ml. portions of $CHCl_3$ and the combined chloroform was washed with water and 10% $KHCO_3$ solution. The dried and filtered chloroform solution was evaporated and the oily residue was taken up in a very small volume of ether. Crystallization occurred presently and the product was recrystallized from cyclohexane–benzene to yield 0.32 g. of crystals. Sublimation and recrystallization gave analytically pure material of m.p. 185.0–185.5°, $[\alpha]^{23}D$ 220° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CH_3OH}$ 267 mμ (440).

Anal. Calcd. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.08. Found: C, 71.22; H, 7.06.

1,3-Dihydroxy-1,3,5(10)-estratriene-17-one (XII).—A mixture of 0.29 g. of the diacetate X, 12 ml. of 83% aqueous methanol and 2 ml. of concd. HCl was refluxed for 16 hours. The methanol was then evaporated and the residue was extracted with two 10-ml. portions of ethyl acetate. The combined ethyl acetate was washed with excess 10% $KHCO_3$ solution, dried over Na_2SO_4 and evaporated. The oily residue was taken up in a small volume of ether from which it rapidly crystallized yielding 0.21 g. of material, the infrared spectrum of which disclosed considerable impurity. Recrystallization from benzene–ethyl acetate produced 71 mg. of the desired product. Further recrystallization from methanol–water gave an analytical sample of m.p. 249–250° (evacuated capillary), $[\alpha]^{19}D$ +282° (*c* 1.0, $CHCl_3$) $\lambda_{max}^{CH_3OH}$ 280–287 mμ (2,060).

Anal. Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.74. Found: C, 75.27; H, 7.76.

Monoacetate of 1,3-Dihydroxy-1,3,5(10)-estratriene-17-one (XI).—A mixture of the crude diacetate X (0.37 g.), 32 ml. of 80% aqueous methanol and 0.30 g. of *p*-toluenesulfonic acid was refluxed for 5 hr. The methanol was evaporated and the precipitate was filtered, washed with water and dried. Recrystallization from ethyl acetate yielded 46 mg. of product. Sublimation and repeated recrystallization gave pure material of m.p. 265–268° (evacuated capillary), $\lambda_{max}^{CH_3OH}$ 282 mμ (1,700).

Anal. Calcd. for $C_{20}H_{24}O_4$: C, 73.14; H, 7.37. Found: C, 72.91; H, 7.50.

Acetylation of 1,3-Dihydroxy-1,3,5(10)-estratriene-17-one (XII).—The steroid (5 mg.) was dissolved in 1.0 ml. of pyridine and treated with 0.10 ml. of acetic anhydride. The solution was allowed to stand at room temperature for 24 hr. and was then worked up in the usual way. The product was recrystallized from benzene–cyclohexane and identified as X by its infrared spectrum.

3-Hydroxy-2,5(10)-estradiene-1,4,17-trione (XIII). A. From XII.—A solution of 1-hydroxyestrone (XII) (22 mg.) in 1.0 ml. of acetone was treated with a suspension of 0.20 g. of potassium nitrosodisulfonate in 1.5 ml. of 0.8% KH_2PO_4 solution. Water (5 ml.) was added and the mixture was agitated for one minute. The yellow product was extracted with chloroform and the chloroform was washed with 5% K_2CO_3 solution. The deep red carbonate solution was acidified with concd. HCl and extracted again with chloroform. The solution was dried and evaporated and the crude yellow product was recrystallized from benzene–cyclohexane, yielding 10 mg. of yellow crystals. Repeated recrystallization from benzene–ethyl acetate gave an analytical sample of m.p. 207–208° dec., $[\alpha]^{22}D$ +439° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CH_3OH}$ 277 mμ (14,000), $\lambda_{max}^{0.2\ N\ HCl-CH_3OH}$ 275 mμ (16,000), $\lambda_{max}^{0.1\ N\ NaOH-CH_3OH}$ 284 mμ (11,000); λ_{max}^{KBr} 3.14, 5.84, 6.10, 6.20 and 6.31 μ.

Anal. Calcd. for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.88; H, 6.69.

B. From XI.—The preparation was very similar to that from XII. The monoacetate (35 mg.) yielded 20 mg. of quinone XIII.

2,5(10)-Estradiene-1,4,17-trione (XIV).—A solution of 10ξ-hydroxy-1,4-estradiene-3,17-dione (II) (0.78 g.) in 24 ml. of glacial acetic acid, 3.2 ml. of water, and 8.0 ml. of concd. HCl was refluxed for 60 minutes. During this time the solution turned a deep green which slowly faded. Most of the solvent was evaporated and the residue was diluted with water and extracted with ethyl acetate. The organic extract was washed with excess $KHCO_3$ solution, dried over Na_2SO_4 and evaporated. The residue was thoroughly triturated with two 5-ml. portions of dry ether, leaving 0.41 g. of tan powder. The ether yielded 0.43 g. of oil.

The tan powder was suspended in 5 ml. of glacial acetic acid and treated with a solution of 0.20 g. of CrO_3 in 0.5 ml. of water and 5 ml. of acetic acid. After two minutes agitation the mixture was diluted with water and extracted with chloroform. The chloroform was washed with water and 10% $KHCO_3$ solution, dried and evaporated. The brown residue was extracted with portions of dry ether until no more yellow material could be removed. Evaporation of the ether left yellow crystals. Oxidation of the above 0.43 g. of oily material yielded a further small amount of crude product. The combined crude material was chromatographed on 20 g. of neutral alumina (Woelm, activity III) using benzene as eluent. The yellow quinone was finally crystallized from cyclohexane to yield 0.26 g. An analytical sample melted at 173.8–174.4°, $[\alpha]^{23}D$ +270° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{CH_3OH}$ 250 mμ (16,000), 340 mμ (1,200); λ_{max}^{KBr} 5.75, 6.03 and 6.25 μ.

Anal. Calcd. for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09. Found: C, 76.04; H, 7.24.

1,4-Dihydroxy-1,3,5(10)-estratriene-17-one (XV).—The quinone XIV (64 mg.) was treated with 1.0 ml. of glacial acetic acid and small portions of zinc dust were added, with agitation, until the yellow color had completely disappeared. Water was added and the mixture was extracted with ethyl

acetate. The organic extract was dried over Na_2SO_4 and evaporated to near dryness. The residual acetic acid was removed *in vacuo* over NaOH. The residue was recrystallized from ethyl acetate–benzene and sublimed at 240° and 0.01 mm. The product melted at 300–305° (evacuated capillary), $[\alpha]^{21}D$ +310° (c = 0.5, diox.), $\lambda_{max}^{CH_3OH}$ 295 mμ (3,600). Repeated recrystallization from methanol gave analytically pure material containing one mole of methanol of crystallization.

Anal. Calcd. for $C_{18}H_{22}O_3 \cdot CH_3OH$: C, 71.67; H, 8.23. Found: C, 71.55; H, 8.23.

1,4-Diacetoxy-1,3,5(10)-estratriene-17-one (XVI).—The crude hydroquinone XV from reduction of 60 mg. of quinone XIV was dissolved in 1.0 ml. of pyridine and treated with 0.20 ml. of acetic anhydride. After standing on the steam-bath 20 min. the solution was treated with 5 drops of water and allowed to stand at room temperature for 10 min. The reaction mixture was then worked up in the usual way. Sublimation and repeated crystallization from cyclohexane–benzene gave a product that melted at 163.0–163.6°, $[\alpha]^{22}D$ +273° (c 1.0, $CHCl_3$), $\lambda_{max}^{CH_3OH}$ 265 mμ (340).

Anal. Calcd. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.07. Found: C, 71.36; H, 7.06.

6-Dehydroestrone (XXI).—A solution of quinol II (50 mg.) in 10 ml. of methylene chloride was treated with 0.20 ml. of PBr_3 and allowed to stand at room temperature for 16 hours. The solution was shaken with 10 ml. of water for 10 minutes and the organic phase was extracted with 10 ml. of 10% $KHCO_3$ solution. The combined aqueous extracts were acidified with concd. HCl and heated on the steam-bath for one hour. Extraction of the cooled solution with chloroform produced a quantity of crystalline solid contaminated with a purple pigment. Recrystallization followed by sublimation in vacuum and recrystallization from methanol gave 5 mg. of colorless crystals, m.p. 259–261.5° (evacuated capillary). The identity of the product was verified by comparison of infrared and ultraviolet spectra with those of authentic 6-dehydroestrone.[16] A trace of impurity having $\lambda_{max}^{CH_3OH}$ 291 mμ appeared to be present. The melting point was not depressed by admixture of 6-dehydroestrone.

SHREWSBURY, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, GEORGETOWN UNIVERSITY, THE NATIONAL INSTITUTE FOR ARTHRITIS AND METABOLIC DISEASES, AND THE NAVAL MEDICAL RESEARCH INSTITUTE]

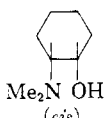
The Acetylcholinesterase Surface. IX. Dependence of Competitive Inhibition by Diaminocyclohexane Derivatives on Substrate Level[1a]

BY D. S. MASTERSON,[1b] S. L. FRIESS AND B. WITKOP

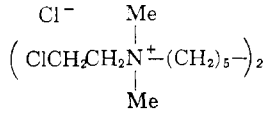
RECEIVED APRIL 16, 1958

Certain features of the inhibition of the system acetylcholinesterase–acetylcholine by the *cis* and *trans* isomers of 2-dimethylaminocyclohexyltrimethylammonium iodide (III and IV) have been shown to depend markedly on the relative substrate levels employed. At *p*H 7.4 and 25° with a protein concentration of the order of 2×10^{-4} mg. per ml., inhibition by each of these isomers is apparently competitive at substrate levels of 1.5×10^{-3} M and lower, but is found to deviate from the competitive relation at higher levels. A possible model to account for this behavior as well as the feature of inhibition of the system by excess substrate has been discussed. The amide V in the *trans* series of diamine derivatives has been found to be inert to the catalytic action of the enzyme in hydrolysis reaction.

Previous kinetic studies of the system acetylcholinesterase–acetylcholine (AChE–AC) have pointed to the competitive nature of the inhibition process characteristic of such reversible inhibitors as eserine[2] and certain substituted ethylenediamines.[3] However, for the tertiary and quaternary compounds I and II reversible inhibition in the

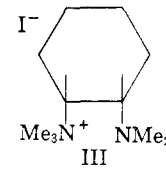


I

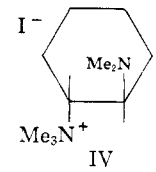


II

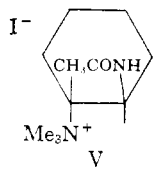
substrate concentration range up to 3×10^{-3} M was found to be clearly non-competitive,[4] with the intrinsic inhibitory power of either compound at *p*H 7.4 independent of the initial acetylcholine concentration employed. These observations and their implications with respect to surface equilibria, coupled with the marked ability of the surface to distinguish between stereochemical configurations of diamines[5] and aminoalcohols and acetates,[6] made it a matter of considerable interest to investigate the inhibitory properties of the *cis*- and *trans*-diamine derivatives III and IV, particularly



III



IV



V

in regard to the competitive or non-competitive character of their inhibition. These compounds possess both the quaternary ammonium function and the center of high electron density, with appropriate separation distances between the two, that might be expected[3a] to lead to significant inhibitory activity in the AChE–AC system. This study has been carried out at several substrate concentrations on the low branch of the $[substrate]_0$ *vs.* activity profile, and has been supplemented briefly by enzymatic hydrolysis experiments with the closely related amide V.

Results

Inhibition data from kinetic experiments involving III and IV were fitted to linear v/v_I *vs.* [I] plots

(1) (a) The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department. (b) Taken in part from the M.S. thesis of D. S. Masterson, Georgetown University, 1957.

(2) K. B. Augustinsson and D. Nachmansohn, *J. Biol. Chem.*, **179**, 543 (1949).

(3) (a) S. L. Friess and W. J. McCarville, THIS JOURNAL, **76**, 1363 (1954); (b) S. L. Friess and H. D. Baldridge, *ibid.*, **78**, 199, 966 (1956).

(4) S. L. Friess, *ibid.*, **79**, 3269 (1957).

(5) S. L. Friess, E. R. Whitcomb, Bart T. Hogan and P. A. French, *Arch. Biochem. and Biophys.*, **74**, 451 (1958).

(6) H. D. Baldridge, W. J. McCarville and S. L. Friess, THIS JOURNAL, **77**, 739 (1955).