Stock solutions (*ca.* 0.3 *M*) were prepared in dioxane diluted to twice its original volume with distilled water. A methyl iodide solution was similarly prepared and its concentration was determined from infinity titers. At zero time the proper volumes of the reactant solutions were mixed rapidly and the time was recorded. Zero time titers were determined by dilution of the stock hydroxide or cyanide solutions with water in the same manner as in the runs. Infinity titers were determined after at least ten half-lives and gave reproducible results.

The hydroxide ion runs were monitored titrimetrically using 0.145 *M* hydrochloric acid and brom thymol blue indicator. The runs using cyanide ion were followed by the argentimetric titration of cyanide ion in the presence of added ammonium hydroxide and potassium iodide, using an 0.0200 *N* silver nitrate solution.[10] Since cyanide ion is only slightly hydrolyzed in water at the concentrations employed the equilibrium

$$CN^- + H_2O \rightleftharpoons HCN + OH^- \qquad (5)$$

was considered for practical purposes to lie completely on the left.

All rate data were treated graphically by a plot of log $(a - x)/(b - x)$ against time and the results are shown in Table I. In each case the reaction was followed to at least 80% reaction and a smooth linear relationship obtained.

Acknowledgment.—The authors wish to thank the Rohm and Haas Co., Redstone Arsenal Research Division, and the Ordnance Corps of the United States Army for the financial support of this work.

(10) I. M. Kolthoff and E. B. Sandell, "Textbook of Quantitative Inorganic Analysis," The Macmillan Co., New York, N. Y., 1948, p. 574.

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9(11)-Dehydrotestosterone and Esters

BY F. W. HEYL AND M. E. HERR

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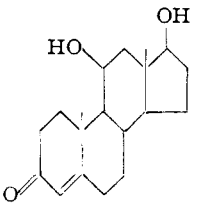
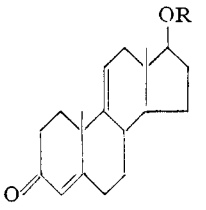
11β-Hydroxytestosterone (I) used for the work herein described was prepared from adrenosterone (V) by the method reported recently.[1] Dehydration of this compound (I) by *trans* elimination of the elements of water from carbon atoms 9 and 11 did not proceed smoothly according to published methods.[2] An efficient dehydration of this compound was effected, however, by using a two-phase system of benzene–ether and 18% hydrochloric acid; 9(11)-dehydrostestosterone (IIa) was obtained in 78% yield. This compound was esterified to form the propionate IIb, benzoate IIc and β-cyclopentylpropionate (IId).

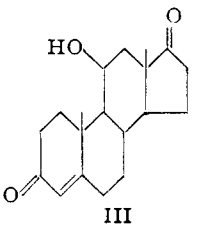
By a similar procedure, but using a higher concentration of acid, 11β-hydroxy-4-androstene-3,17-dione[1] (III) was dehydrated to 4,9(11)-androstadiene-3,17-dione (IV) in 87% yield.[3] This diene-dione IV was prepared also by the chromic acid oxidation of 9(11)-dehydrotestosterone (IIa).



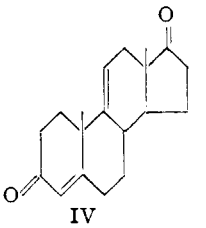
I



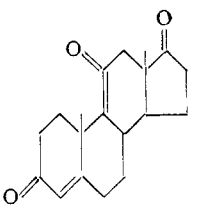
II a, R = H
b, R = COC_2H_5
c, R = COC_6H_5
d, R = $COC_2H_4CHC_4H_8$



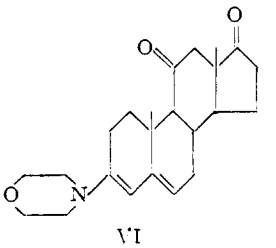
III



IV



V



VI

In studying the merits of the above two mentioned routes to IV it was determined that the method of dehydrating 11β-hydroxytestosterone (I) followed by chromic acid oxidation of IIa, although involving one more step from adrenosterone, was preferred over the procedure whereby III was dehydrated directly to IV. The reason for this becomes apparent when it is noted that the yield of hydroxy-dione III from adrenosterone was only 44% and involved a chromatographic purification.[1] On the other hand, the yield of I from adrenosterone was consistently better than 73% and the product was obtained by direct crystallization.[1] Moreover the total crude 11β-hydroxytestosterone (I) could be carried through the steps of dehydration and oxidation to give a better over-all yield of IV from adrenosterone (V). In the interest of brevity only the experiments conducted on the purified intermediates are described below.

During the course of this work we had occasion to study the reaction of morpholine with adrenosterone (V). It has been shown previously that the reaction of pyrrolidine in excess with V led to the facile isolation of the bis-enamine, 3,17-di-(N-pyrrolidyl)-3,5,16-androstatrien-11-one.[1] By comparison when morpholine was used, under the same conditions, the product isolated was 3-(N-morpholinyl)-3,5-androstadiene-11,17-dione (VI).

Experimental

9(11)-Dehydrotestosterone (IIa).—A two-phase mixture of 2.50 g. of 11β-hydroxytestosterone,[1] 250 ml. of benzene, 200 ml. of ether, 100 ml. of concentrated hydrochloric acid and 100 ml. of water was stirred vigorously and heated at gentle reflux for 18 hours. The aqueous acid layer was separated

(1) M. E. Herr and F. W. Heyl, THIS JOURNAL, **75**, 5928 (1953). Alternative methods of preparation have been reported by O. Mancera, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953); S. Bernstein, R. H. Lenhard and G. H. Williams, *J. Org. Chem.*, **18**, 1166 (1953).

(2) (a) C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **24**, 351 (1941); **26**, 1316 (1943); (b) H. Heymann and L. F. Fieser, THIS JOURNAL, **74**, 5939 (1952).

(3) S. Bernstein, R. H. Lenhard and J. H. Williams, *J. Org. Chem.*, **19**, 41 (1954), used 20% hydrochloric acid–glacial acetic acid to carry out this reaction according to the method of T. Reichstein, U. S. Patent 2,409,798; they report an inconsequential yield of IV by this procedure.

and extracted three times with 75-ml. volumes of ether. The combined ether–benzene solution was washed with dilute potassium carbonate solution, water and dried over sodium sulfate. Evaporation of the solvent yielded 2.10 g. of semi-crystalline residue which was crystallized from methylene chloride–Skellysolve B; yield 1.80 g. (77%), m.p. 151–154°. The analytical sample melted at 153–154°, $[\alpha]^{23}D$ +89° ($CHCl_3$).

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.93; H, 9.10.

9(11)-Dehydrotestosterone Propionate (IIb).—A solution of 0.3 g. of 9(11)-dehydrotestosterone in 2 ml. of dry pyridine was treated with 2 ml. of propionic anhydride and allowed to stand at 26° for 22 hours when it was poured into 25 ml. of water and the mixture stirred for 2 hours at room temperature. The crystalline product was recovered by filtration, washed with water and dried; yield 0.34 g., m.p. 112°. The analytical sample prepared by recrystallization from dilute methanol melted at 114°, $[\alpha]^{23}D$ +63° ($CHCl_3$).

Anal. Calcd. for $C_{22}H_{30}O_3$: C, 77.14; H, 8.83. Found: C, 77.22; H, 8.93.

9(11)-Dehydrotestosterone Benzoate (IIc).—A solution of 250 mg. of 9(11)-dehydrotestosterone in 30 ml. of benzene was distilled to remove 18 ml. of benzene and cooled to 26°. The dry benzene solution of steroid was treated with 0.32 ml. of dry pyridine and 0.32 ml. of benzoyl chloride and allowed to stand at 26° for 5 hours during which time pyridine hydrochloride separated from the solution. The mixture was stirred with 25 ml. of water and extracted with ether. After washing the extract with dilute hydrochloric acid, dilute sodium hydroxide solution and water, the solution was dried over sodium sulfate and the solvent evaporated *in vacuo*. The residue was recrystallized from absolute methanol to yield 270 mg. of benzoate, m.p. 175–177°, $[\alpha]^{23}D$ +113° ($CHCl_3$).

Anal. Calcd. for $C_{26}H_{30}O_3$: C, 79.96; H, 7.74. Found: C, 79.73; H, 7.63.

9(11)-Dehydrotestosterone β-cyclopentylpropionate (IId) was prepared from 250 mg. of 9(11)-dehydrotestosterone and β-cyclopentylpropionyl chloride by the procedure described above for the benzoate. The product was obtained by crystallizing from dilute methanol; m.p. 96°, yield 255 mg., $[\alpha]^{23}D$ +58° ($CHCl_3$).

Anal. Calcd. for $C_{27}H_{38}O_3$: C, 78.98; H, 9.33. Found: C, 78.94; H, 9.21.

4,9(11)-Androstadiene-3,17-dione (IV) by the *trans*-Elimination of the Elements of Water.—11β-Hydroxy-4-androstene-3,17-dione (III) (5.0 g.) suspended in 500 ml. of benzene and 200 ml. of ether was treated with 100 ml. of water and 200 ml. of concentrated hydrochloric acid. The two-phase mixture was stirred vigorously and heated at reflux for 18 hours, the layers separated, and the aqueous portion extracted three times with 100-ml. portions of a 50:50 mixture of ether–benzene. The combined ether–benzene solution was washed with water, dilute sodium carbonate, water and the solvent evaporated to yield 4.33 g. of crystalline residue, m.p. 190–200°. Recrystallization from methylene chloride–Skellysolve B mixture gave 4.06 g. (87%) of 4,9(11)-androstadiene-3,17-dione, m.p. 202–204°, $[\alpha]^{23}D$ +221° ($CHCl_3$), λ_{max}^{alc} 240 mμ (*E* 16,925).[4]

Anal. Calcd. for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.34; H, 8.41.

4,9(11)-Androstadiene-3,17-dione (IV) by Chromic Acid Oxidation of 9(11)-Dehydrotestosterone.—A solution of 280 mg. of 9(11)-dehydrotestosterone (IIa) in 10 ml. of glacial acetic acid was treated with a mixture of 0.14 g. of chromium trioxide, 0.3 ml. of water and 10 ml. of glacial acetic acid at room temperature for two hours. Excess oxidant was destroyed by the addition of 4 ml. of methanol after which the reaction mixture was poured into 50 ml. of water. The product was extracted several times with a 50–50 mixture of ether–benzene, the solution washed with water, dilute sodium hydroxide solution, water and dried over sodium sulfate. Evaporation of the solvent left a white solid residue of 250 mg. which was crystallized from methylene chloride–Skellysolve B to yield 180 mg. (64%) of 4,9(11)-androstadiene-3,17-dione, m.p. 203–205°, identical with the product prepared as described above.

3-(N-Morpholinyl)-3,5-androstadiene-11,17-dione (VI).—Three grams of adrenosterone (V), 40 ml. of Skellysolve C, 6.96 ml. (8 equivalents) of dry morpholine and 40 mg. of *p*-toluenesulfonic acid was stirred and heated at reflux for 3.5 hours. The water of reaction was removed continuously during this period by means of a water trap placed between the reaction flask and the condenser. The mixture was concentrated to dryness *in vacuo* and the residue crystallized from acetone in prisms; m.p. dec. above 230°, $[\alpha]D$ +26° ($CHCl_3$), λ_{max}^{ether} 269 mμ (*E* 21,300).

Anal. Calcd. for $C_{23}H_{31}NO_3$: C, 74.75; H, 8.46; N, 3.79. Found: C, 74.86; H, 8.50; N, 4.12.

The infrared spectrum in Nujol showed absorption for 17-ketone, 1730 cm.$^{-1}$; 11-ketone, 1692 cm.$^{-1}$.

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(4) ADDED IN PROOF.—These data are in close agreement with those reported by S. Bernstein, *et al.*, ref. 3. This compound has now been treated with pyrrolidine to yield 3-(N-pyrrolidyl)-3,5,9(11)-androstatrien-17-one, m.p. dec. above 157°, $[\alpha]D$ −139° ($CHCl_3$); calcd. for $C_{23}H_{31}NO$: C, 81.85; H, 9.26; N, 4.15. Found: C, 82.30; H, 9.36; N, 4.33. Treatment of this C_3-pyrrolidine derivative with lithium aluminum hydride, as previously described for the reduction of 3-(N-pyrrolidyl)-3,5-androstadiene-11,17-dione, ref. 1, and subsequent hydrolysis gave a 90% yield of 9(11)-dehydrotestosterone, m.p. 151–152°; admixture with the compound IIa prepared as described above gave no m.p. depressions.

Purification of Corticotropin

BY W. T. KOCH AND F. J. WOLF

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The use of cellulose for the purification of corticotropin was reported first by Payne, Raben and Astwood.[1] These workers achieved a tenfold concentration of a crude extract of hog anterior pituitary powder. Subsequently a cellulose derivative, oxycellulose, was found[2] to be superior to cellulose yielding a forty-fold purification by a batchwise adsorption technique. This adsorbent has been used subsequently by other workers[3–5] for the purification of hog and sheep pituitary extracts with similar results. In our laboratory experiments with other cellulose derivatives containing an acidic functional group such as cellulose acid citrate and sodium cellulose acid phosphate have shown that these cellulose derivatives also are effective adsorbents for beef corticotropin. Carboxymethyl cellulose appeared less effective. These findings support the view[2] that the cation-exchange properties are important in the fractionation of corticotropin by oxycellulose. However, the remarkable specificity of these substances for corticotropin when compared with common ion exchange materials as well as the use of exchange groups with markedly different dissociation constants indicates that other properties of the substituted cellulose derivatives are important for this fractionation. One possible explanation for this specificity may be the spatial arrangement of exchange groups of each substance such that the formation of a complex

(1) R. W. Payne, M. S. Raben and E. B. Astwood, *J. Biol. Chem.*, **187**, 719 (1950).

(2) E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951).

(3) N. G. Brink, F. A. Kuehl, Jr., J. W. Richter, A. W. Bazemore, M. A. Meisinger, D. E. Ayer and K. Folkers, *ibid.*, **74**, 2120 (1952).

(4) C. H. Li, *ibid.*, **74**, 2124 (1952).

(5) A. W. Bazemore, J. W. Richter, D. E. Ayer, G. Finnerty, N. G. Brink and K. Folkers, *ibid.*, **75**, 1949 (1953).