tions of 0.65 and 0.35 for Santomerse D and histone hydrochloride, respectively.

Discussion of Results

The ultracentrifuge behavior of tetrasodium nucleate shows no evidence of complex formation. The single peak shown in Fig. 1 (A, B, C) for the nucleate sedimenting in absence of Santomerse D is unchanged in its presence (D, E, F). The experiments with the quantity type rotor confirm the absence of complex formation. Under the conditions used no Santomerse D micelles sedimented. The intrinsic viscosity of the nucleate was unchanged in presence of Santomerse D, as shown in Table III. The molecular weight, which is of the same order of magnitude as reported values[8,9] was unchanged in Santomerse D solution. In the electrophoretic studies previously reported,[2] the mobilities of the nucleate were essentially unchanged by Santomerse D. However, area measurements of the electrophoretic patterns indicated that some complex formation had occurred. No explanation is offered at the present for this lack of agreement.

Histone hydrochloride sedimenting in absence of Santomerse D shows at least three fast moving components, two of which are reported in Table I, and a main slow sedimenting component. The fast moving components are probably histone aggregates. The presence of more than one component in dialyzed histone has been previously reported.[5] In Santomerse D, only one component was observed (Fig. 1, H, I, J), an indication that a single complex of Santomerse D and histone was sedimenting. The Santomerse D composition of the complex, as measured by the quantity type rotor experiment of Table II is in agreement with that obtained from area measurements of "schlieren" patterns of a similar system (Fig. 1). Further information concerning the composition of the complex was obtained from consideration of the molecular weight obtained in Santomerse D solution. The assumption of one histone molecule per molecule of complex is in agreement with the experimental data obtained in the present work.

A proposed structure for the histone hydrochloride–Santomerse D complex[10] assumes the binding of a monomolecular layer of detergent to the histone molecule by means of a primary ionic interaction, followed by binding of additional detergent molecules to the complex through attractive forces between the hydrocarbon chains of the detergent. The existence of a double layer of Santomerse D on the histone molecule would be consistent with the reversal of the charge by Santomerse D in electrophoresis,[2] the quantity type rotor experiments, and the results of area measurements of ultracentrifuge "schlieren" patterns.

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The Preparation and Properties of 4-Oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione[1]

BY A. H. SOLOWAY[2] AND D. K. FUKUSHIMA

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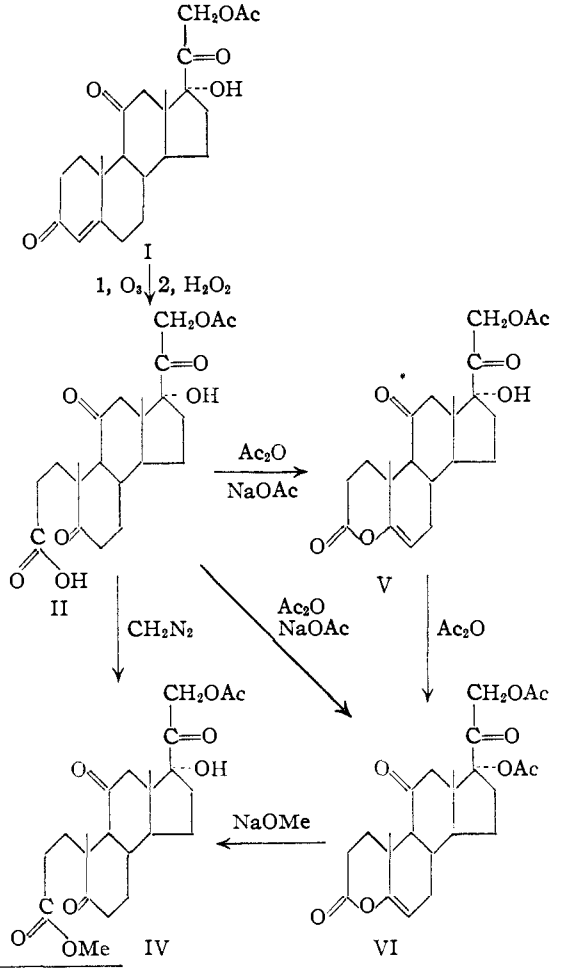
In the course of investigations designed to introduce carbon-14 into the ring system of cortisone, the preparation of 4-oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione (V) was undertaken. Ozonolysis of cortisone acetate (I) followed by oxidation with hydrogen peroxide[3] yielded an acid, 3,5-seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnane-3-oic acid (II). The acid II was converted to an enol lactone in refluxing acetic anhydride in the presence of a small amount of sodium acetate. The product obtained proved to be 4-oxa-17α,21-diacetoxy-Δ⁵-pregnene-3,11,20-trione (VI).[4] With less prolonged heating, the 17α-hydroxyl group was not acetylated and 4-oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione (V) was produced. By heating with acetic anhy-



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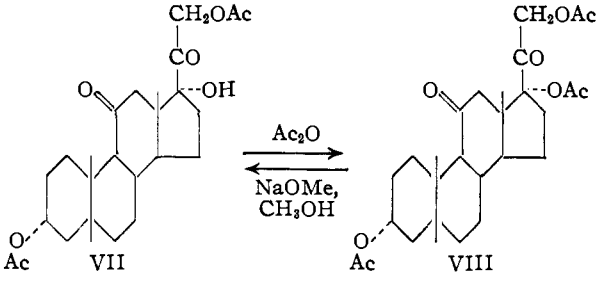
(4) We wish to express our gratitude to Dr. George I. Fujimoto, University of Utah, Salt Lake City, Utah, for a sample of this compound.

dride as described by Turner[5] and Huang-Minlon, *et al.*,[6] V was converted to the diacetate VI. That no fundamental alteration of the molecule occurred under these conditions was demonstrated by conversion of VI to the methyl ester IV, with one mole of sodium methoxide in methanol.

This selective removal of the 17α-acetoxy group is a general reaction as evidenced by the ready conversion of 3α,17α,21-triacetoxypregnane-11,20-dione (VII) to 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione (VIII) under the same conditions without any indication for the formation of either the 17α,21-dihydroxy or 3,17,21-trihydroxy compound.



Experimental[7]

3,5-Seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnane-3-oic Acid (II).—A stream of approximately 6% ozone was passed through a solution of 3.0 g. of cortisone acetate in 400 ml. of ethyl acetate cooled in a Dry Ice–methanol-bath. When the ozonolysis was complete, as evidenced by the oxidation of potassium iodide in a trap through which the effluent gases were passed, the reaction was discontinued and a solution of 4 ml. of 30% hydrogen peroxide and 4 ml. of methanol was added to the ethyl acetate solution. After standing at room temperature for 16 hours, the solvent was removed under reduced pressure. The oily solid was recrystallized from acetone–petroleum ether yielding 1.62 g. of rhombohedral crystals, m.p. 140–144°. Difficulty in recrystallization of the free acid prompted preparation of the hydrate which was obtained in long white needles from either aqueous methanol or aqueous acetone, m.p. 118–121°, $[\alpha]^{24}D$ +91.6° (ethanol). Both products had identical spectra in the region from 1150–850 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{30}O_8 \cdot H_2O$: C, 59.98; H, 7.31. Found: C, 59.98; H, 7.17.

The methyl ester of the acid was prepared with an excess of ethereal diazomethane and was recrystallized with difficulty from methanol. After recrystallization, long white needles, m.p. 154.5–156.5°, $[\alpha]^{23}D$ +99.1° (chloroform) were obtained. The hydrate likewise yielded this same methyl ester.

Anal. Calcd. for $C_{23}H_{32}O_8 \cdot {}^1/_2CH_3OH$: C, 62.37; H, 7.57. Found: C, 62.34; H, 7.43.

4-Oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione (VI).—A slurry of 209 mg. of II in 15 ml. of acetic anhydride containing 53 mg. of anhydrous sodium acetate was heated to boiling under reflux for 1 hour. The product went into solution during heating and at the end of the reaction the acetic anhydride was removed by distillation under diminished pressure. The oily solid was dissolved in ether–ethyl acetate and extracted several times with water followed by dilute sodium bicarbonate solution and again with water, dried over anhydrous sodium sulfate, and the solvent was removed. The residue, 214 mg. of yellow oil, was chromatographed upon silica gel, and 95 mg., m.p. 200–220°, was obtained. After successive recrystallizations from acetone–petroleum ether 4-oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione (VI) melted at 231–234°, $[\alpha]^{25}D$ −24° (chloroform).

Anal. Calcd. for $C_{24}H_{30}O_8$: C, 64.57; H, 6.77. Found: C, 64.97; H, 6.73.

4-Oxa-17α-hydroxy-21-acetoxy-Δ5-pregnene-3,11,20-trione (V).—A slurry of 2.01 g. of the keto acid II in 80 ml. of acetic anhydride containing 240 mg. of anhydrous sodium acetate was heated under reflux for 15 to 20 minutes. The acetic anhydride was removed under diminished pressure and the product was isolated as in the preceding section. After crystallization from acetone–petroleum ether 986 mg. of product, m.p. 200–220°, was obtained; recrystallization from the same solvents afforded prisms of 4-oxa-17α-hydroxy-21-acetoxy-Δ5-pregnene-3,11,20-trione (V), m.p. 247–253°, $[\alpha]^{23}D$ +30° (chloroform).

Anal. Calcd. for $C_{22}H_{28}O_7$: C, 65.32; H, 6.73. Found: C, 64.95; H, 6.77.

4-Oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione (VI) from V.—A solution of 107 mg. of 4-oxa-17α-hydroxy-21-acetoxy-Δ5-pregnene-3,11,20-trione (V) in 10 ml. of acetic anhydride was heated under reflux for 16 hours, and at the end of this time the acetic anhydride was removed under diminished pressure. The residual yellow oil after chromatography upon silica gel was recrystallized from acetone–petroleum ether to yield 99 mg. of VI, m.p. 231–234°, identical in all respects including infrared spectrum with the product prepared from the keto acid.

A solution of 25 mg. of the enol lactone V in 2 ml. of glacial acetic acid and 2 ml. of acetic anhydride containing 26 mg. of *p*-toluenesulfonic acid monohydrate was allowed to stand at room temperature for 60 hours. The solution was diluted with ethyl acetate, extracted with water, 10% sodium bicarbonate solution and again with water and dried over anhydrous sodium sulfate. The solvent was removed and the product crystallized from acetone–petroleum ether to yield 19 mg. of 4-oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione (VI) identical in all respects including infrared spectrum with the product obtained in the preceding reaction.

Methyl 3,5-Seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnanoate (IV) from VI.—A stream of nitrogen was passed through a solution of 30 mg. of 4-oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione in 3 ml. of methanol, and to this was added 1 ml. of 0.67 *M* sodium methoxide in methanol (1 equivalent). The solution was stirred for 5 minutes by means of the nitrogen stream and at that point, 1 ml. of water was added. After an additional 3 minutes, 1 ml. of glacial acetic acid was added and the solution was extracted with ether. The ether solution was washed with water, dilute sodium bicarbonate and water and dried over anhydrous sodium sulfate. The solvent was removed and after chromatography on silica gel followed by recrystallization from methanol, 21 mg. of methyl 3,5-seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnanoate (IV) was obtained, m.p. 154.5–156.5°. The product was identical in all respects with that obtained directly from the esterification of the keto acid II.

3α,21-Diacetoxy-17α-hydroxypregnane-11,20-dione (VII) from 3α,17α,21-Triacetoxypregnane-11,20-dione (VIII).—In the manner described in the preceding experiment, 49 mg. of 3α,17α,21-triacetoxypregnane-11,20-dione was converted with sodium methoxide to 16 mg. of 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione, m.p. 228–232°. The mother liquors contained the triacetate VIII as shown by the infrared spectra but there was no indication of any 3α-acetoxy-17α,21-dihydroxypregnane-11,20-dione.

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(7) All melting points are corrected.

The Use of the Schlieren Optical System for Sampling after Preparative Angle Ultracentrifugation[1]

BY SAM SOROF

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