[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE RICE INSTITUTE]

Acylation of 17-Hydroxy-20-ketosteroids[1]

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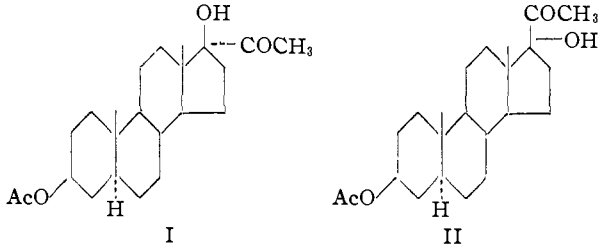
Acylation of the tertiary hydroxyl group of 17-hydroxy-20-ketosteroids is readily accomplished by treatment of the ketols with acid chlorides or anhydrides in the presence of various acidic reagents. 17α-Hydroxy-20-ketosteroids, previously considered incapable of acylation owing to steric hindrance, react smoothly under these conditions and furnish esterified products in good yield. Propionylation, as well as acetylation, has been accomplished by use of the present method. A number of substances including compounds L, iso-L, and S, cortisone, 17α-hydroxyprogesterone and 17α-hydroxypregnenolone has been investigated. The reaction provides a method for protecting sensitive ketol side chains from attack by reagents (aluminum alkoxides, chromic acid, etc.) that might otherwise promote rearrangement or degradation.

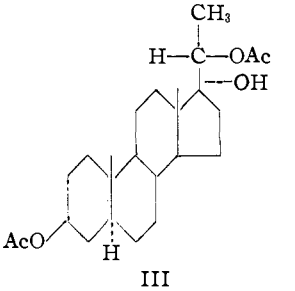
Acetylation of the tertiary hydroxyl group of 17α- and of 17β-hydroxy-20-ketosteroids by acetic anhydride in the presence of various acidic reagents was encountered in the course of work described in the preceding paper.[2] The esterification reaction was in a sense unexpected, for although acetylation of the 17-hydroxyl group of 17β-hydroxy-20-ketosteroids (*cf.* I) can be effected by vigorous treatment with acetic anhydride and pyridine,[3] acetylation of the epimeric 17α-hydroxy derivatives (*cf.* II) had not previously been accomplished.[4] Moreover, rearrangement of the epimeric ketols into D-homo derivatives under both acidic and basic conditions has been observed to proceed with considerable facility.[5] Details of the original observations and of the further investigation of this reaction are now reported.

When 3β,17α-dihydroxyallopregnan-20-one 3-monoacetate (L-monoacetate) (II)[6] is treated with *p*-toluenesulfonic acid in a mixture of acetic anhydride and acetic acid, there is obtained in virtually quantitative yield, a diacetyl derivative melting at 197.5–198.5°. Under the conditions employed the reaction is essentially complete after a period of one hour at room temperature. The compound on *mild* alkaline hydrolysis,[7] followed by partial acetylation with acetic anhydride and pyridine, is reconverted into the starting material II. Lithium aluminum hydride reduction furnishes Reichstein's compound O, isolated as the 3,20-diacetate III, which is also the major product of lithium aluminum hydride reduction of II. The possibility that the product can possess an enol–acetate structure IV is excluded by the infrared absorption spectrum, in which absorption bands appear at 1715 and 1735 cm.$^{-1}$ (carbonyl region) and at 1245 cm.$^{-1}$ ("acetate" region) in carbon disulfide solution. The strong bands at 1749–1754 cm.$^{-1}$ and at 1660 cm.$^{-1}$, characteristic of enol–acetates possessing a terminal double bond,[8] are not present in the spectrum of the new diacetate. On the basis of this evidence the product is formulated as 3β,17α-diacetoxyallopregnan-20-one (L diacetate) (V).

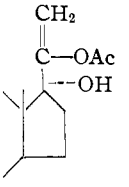


I

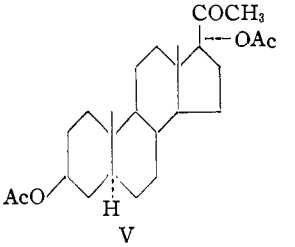
II



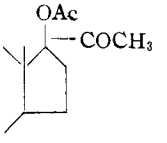
III



IV



V



VI

Treatment of 3β,17β-dihydroxyallopregnan-20-one 3-monoacetate (iso-L monoacetate) (I) with *p*-toluenesulfonic acid in acetic anhydride in like manner affords iso-L diacetate (VI), identical in all respects with a known sample.[3] The yield obtained in the acid-catalyzed reaction represents a considerable improvement over that resulting when acetic anhydride and pyridine are employed, presumably owing to the occurrence of partial rearrangement under the vigorous conditions required for base-catalyzed acetylation. Further support for the structure assigned to V is derived from a comparison of the infrared absorption of this substance (1715 and 1735 cm.$^{-1}$) with that of VI,[9] which exhibits characteristic absorption in the

(1) A preliminary communication describing a part of the material presented in this paper appeared in THIS JOURNAL, **74**, 4220 (1952). It is a pleasure to acknowledge the independent work of Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, *ibid.*, **74**, 5394 (1952), dealing with certain aspects of this subject.

(2) R. B. Turner, *ibid.*, **75**, 3484 (1953).

(3) C. W. Shoppee and D. A. Prins, *Helv. Chim. Acta*, **26**, 185 (1943).

(4) J. von Euw and T. Reichstein, *ibid.*, **30**, 205 (1947).

(5) References to earlier work will be found in the preceding paper (ref. 2).

(6) 17α-Hydroxy-20-ketosteroids are readily accessible through the elegant procedure of T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **73**, 184 (1951).

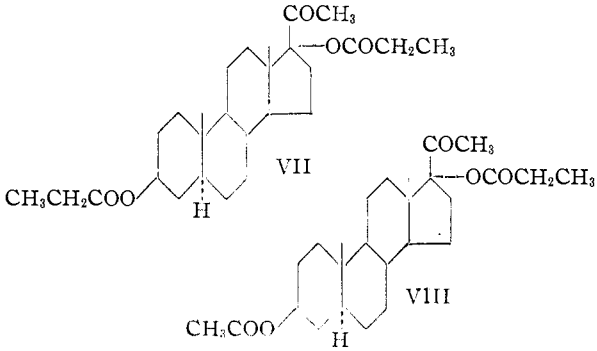
(7) Vigorous treatment with base results in homoannulation of ring D.; J. von Euw and T. Reichstein, *Helv. Chim. Acta*, **24**, 879 (1941).

(8) H. Vanderhaeghe, E. R. Katzenellenbogen, K. Dobriner and T. F. Gallagher, THIS JOURNAL, **74**, 2810 (1952).

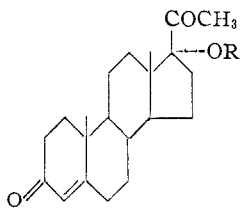
(9) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, *ibid.*, **74**, 2820 (1952).

carbonyl region at 1716 and at 1736 cm.$^{-1}$. Shift of the low frequency band (1716 cm.$^{-1}$) associated with the C-20 carbonyl group from its normal position (1706–1710 cm.$^{-1}$) has been attributed to interaction between the ketone and ester carbonyl groups.[9]

Acylation of L monoacetate (II) has been explored under a variety of conditions. Acetyl chloride–acetic acid or acetic anhydride alone give essentially the same results as acetic anhydride–acetic acid. Acetyl chloride in the absence of a diluent, however, is less satisfactory. The starting material is recovered unchanged when pure acetic acid is employed as the solvent. *p*-Toluenesulfonic acid may be replaced by hydrogen chloride, or by aluminum chloride, zinc chloride, or stannic chloride as noted previously.[2] That the reaction is not limited to acetylation is demonstrated by conversion of compound L (free diol) into L dipropionate (VII), m.p. 124–125°, by the action of *p*-toluenesulfonic acid in a mixture of propionyl chloride and propionic acid. It is of interest that L monoacetate (II) with the same reagents gives 3β-acetoxy-17α-propionoxyallopregnan-20-one (VIII), m.p. 172.5–173.5°, without appreciable acid interchange at C-3. Preparation of mixed esters can thus be accomplished.



Considerable reduction in yield attends reaction of those substances that possess an α,β-unsaturated ketonic grouping in ring A. 17α-Hydroxyprogesterone (IXa) on treatment with *p*-toluenesulfonic acid–acetic anhydride–acetic acid furnishes only about 50% of the corresponding 17-acetate (IXb), in contrast to the nearly quantitative yields

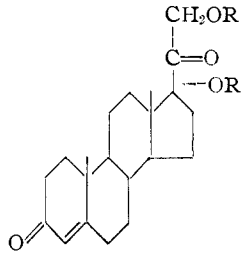


IXa, R = H
IXb, R = $COCH_3$

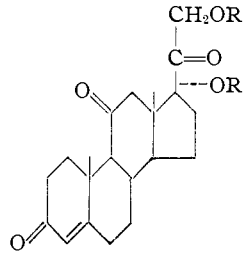
obtained in previous instances. Partial enol-acetylation of the ring A system is an obvious possibility, but could not be demonstrated in the present case. The infrared absorption spectrum of the total crude reaction product (1677, 1714, 1736 cm.$^{-1}$) lacks the high frequency band at 1754 cm.$^{-1}$ characteristic of the enol-acetate structure.[10]

(10) R. N. Jones and K. Dobriner, "Vitamins and Hormones," R. S. Harris and K. V. Thimann, Vol. VII, Academic Press, Inc., New York, N. Y., 1949, p. 323.

In view of current interest in adrenal cortical hormones possessing a dihydroxyacetone side chain at C-17, the behavior of these substances was also investigated. Both compound S (Xa) and cortisone (XIa) are transformed into the corresponding diacetyl derivatives (Xb and XIb, respectively) in yields approximating 50% when treated with acetic anhydride–acetic acid in the presence of *p*-toluenesulfonic acid. Slightly better yields of cleaner products are obtained when the 21-monoacetates are employed as starting materials. Mattox[11] has recently reported that 17α,21-di-

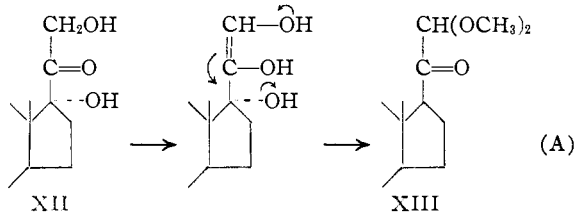


Xa, R = H
Xb, R = $COCH_3$

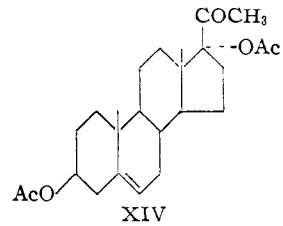


XIa, R = H
XIb, R = $COCH_3$

hydroxy-20-ketosteroids (XII) are smoothly converted by *methanolic* hydrogen chloride into glyoxal derivatives (XIII) with loss of the 17α-hydroxyl group according to scheme (A). This reaction is suppressed in appropriate solvents by acetylation of the 21-hydroxyl function.



Acylation of the tertiary hydroxyl group of 17α-hydroxy-20-ketosteroids provides a method for protecting sensitive ketol side chains from attack by reagents that might otherwise promote undesirable side reactions. Thus 3β,17α-diacetoxy-5-pregnen-20-one (XIV), on catalytic hydrogenation, followed by reoxidation of the resulting 20-hydroxyl group with chromic acid, is transformed in good yield into L diacetate (V), and thence by hydrolysis into Compound L. Direct oxidation of the 17,20-glycol or of the ketol grouping results in large measure in cleavage of the side chain and the production of 17-keto derivatives.[12] The 17-acetates are likewise stable to the action of boron

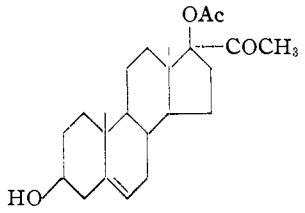


XIV

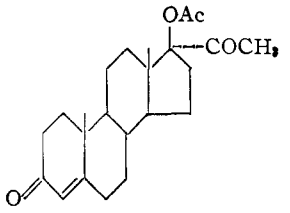
trifluoride and of aluminum alkoxides, reagents which effect rearrangement of the free ketols into derivatives of the D homo series.[2] Ruzicka and

(11) V. R. Mattox, THIS JOURNAL, **74**, 4340 (1952).
(12) L. H. Sarett, *J. Biol. Chem.*, **162**, 601 (1946).

Meldahl[13] have accomplished successful conversion of 3β,17β-dihydroxy-5-pregnen-20-one 17-monoacetate (XV), prepared by partial hydrolysis of the corresponding 3β,17β-diacetate, into 17β-acetoxyprogesterone (XVI) by use of the Oppenauer



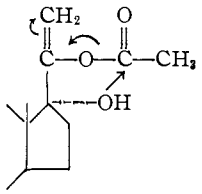
XV



XVI

procedure. The similar oxidation of 3β,17α-dihydroxy-5-pregnen-20-one 17-monoacetate (17α-acetoxypregnenolone), obtainable from XIV, to 17α-acetoxyprogesterone (IXb) was carried out in connection with the present work.

The mechanism of the acetylation reaction has not been examined in detail. The possibility of acyl migration in an enol intermediate XVII is deemed unlikely in view of the observation that 3β-acetoxyallopregnan-20-one is unaffected by



XVII

treatment with *p*-toluenesulfonic acid, acetic anhydride and acetic acid at room temperature. Compound O diacetate (III) under these conditions yields an intractable oil, from which no crystalline material can be isolated. A number of paths for dehydration and rearrangement of this substance can be visualized that are not available for the 17-hydroxy-20-keto derivatives. On the basis of present evidence there is little reason to doubt that acetylation proceeds normally with attack by the reactive fragment $CH_3\overset{+}{C}{=}O$. Such a mechanism would adequately account for the lack of hindrance in the acid-catalyzed acetylation as compared with that in the corresponding base-catalyzed reaction.

The generosity of Drs. T. P. Carney and J. Rowe, Eli Lilly and Co., and of Drs. G. Rosenkranz and F. Sondheimer, Syntex S. A., in supplying materials for this investigation is gratefully acknowledged.

Experimental[14]

Preparation of 3β,17α-Diacetoxyallopregnan-20-one (L Diacetate) (V).—A solution of 100 mg. of 3β,17α-dihydroxyallopregnan-20-one 3-monoacetate (L monoacetate) (II) and 100 mg. of *p*-toluenesulfonic acid monohydrate in a mixture of 5 ml. of acetic acid and 1 ml. of acetic anhydride was allowed to stand at room temperature for 3 hours. The reaction mixture was then diluted with water and extracted with ether. The ether extract was washed successively with water, dilute sodium hydroxide, water, and saturated sodium chloride solution, filtered through anhydrous sodium sulfate, and concentrated to dryness. The residue was crystallized from acetone–petroleum ether and furnished 103 mg. of product melting at 197–198°. Recrystallization from the same solvent mixture gave the analytical sample, m.p. 197.5–198.5°, $[\alpha]_D$ −9.3° (*c* 1.59, dioxane), infrared absorption 1735, 1715, 1245 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.72; H, 9.08.

When the preceding experiment was repeated with a one hour reaction time, the results were only slightly less satisfactory. Under these conditions 100 mg. of L monoacetate furnished L diacetate, isolated in 2 crops, 87 mg., m.p. 195–196.5°; 11 mg., m.p. 191–194°.

The following results were obtained from other modifications of the standard procedure. Acetylation of 200 mg. of L monoacetate in 10 ml. of acetic acid and 2 ml. of acetyl chloride (200 mg. of *p*-toluenesulfonic acid, overnight at room temperature) yielded 202 mg. of L-diacetate, m.p. 197–198°. Repetition of this experiment, with the exception that 5 ml. of acetic anhydride was employed in place of acetyl chloride–acetic acid, gave 206 mg. of L-diacetate, m.p. 198–199°. The use of acetyl chloride alone (5 ml.) afforded 80 mg. of product, m.p. 195–197°, together with some oily material. Diacetate (142 mg.) melting at 190–194° was obtained when 10 ml. of ether and 2 ml. of acetyl chloride was employed. The reaction of 100 mg. of L monoacetate with 5 ml. of acetic acid and 1 ml. of acetic anhydride, saturated at room temperature with dry hydrogen chloride, on standing overnight gave 105 mg. of L diacetate, m.p. 196–198°. Reactions catalyzed by metal halides are described in the preceding paper.[2]

Saponification of 3β,17α-Diacetoxyallopregnan-20-one (V).—A solution of 130 mg. of L diacetate (V) in 10 ml. of 80% aqueous methanol containing 1% of sodium hydroxide was allowed to stand overnight at room temperature. The reaction mixture was diluted with ether and methylene chloride, and was washed thoroughly with water. After drying over anhydrous sodium sulfate, the solvent was removed by evaporation, and the residue was reacetylated with acetic anhydride and pyridine at room temperature. Crystallization of the product from acetone–petroleum ether yielded 120 mg. of L monoacetate (II) melting at 188–189.5°. The melting point of a mixture with an authentic sample was not depressed.

Lithium Aluminum Hydride Reduction of 3β,17α-Diacetoxyallopregnan-20-one (V).—L diacetate (V) (130 mg.) was added to an ethereal solution containing an excess of lithium aluminum hydride. The mixture was stirred at room temperature for 4 hours, at the end of which time the excess hydride was decomposed by the addition of small amounts of alcohol. The ether solution was then washed with dilute hydrochloric acid, water, saturated sodium chloride, filtered through anhydrous sodium sulfate and concentrated to dryness. The crude product was re-acetylated (acetic anhydride and pyridine, room temperature) and on crystallization from dilute methanol yielded Reichstein's compound O diacetate (III), m.p. 246–248°, identical with an authentic sample.

Lithium Aluminum Hydride Reduction of L Monoacetate (II).—A solution of 2.0 g. of L monoacetate in anhydrous ether was added to an ethereal solution of excess lithium aluminum hydride. After stirring for 5 hours at room temperature, the reaction mixture was worked up in the manner described above. Crystallization of the crude diacetate from methanol furnished 1.2 g. of material, m.p. 241–243°, that on recrystallization from the same solvent gave pure O-diacetate, m.p. 247–248°. Chromatography of the mother liquors yielded 400 mg. of crude J diacetate, m.p. 152–156°. The melting point was raised to 158–159° by one recrystallization.

This procedure is much more satisfactory for the preparation of Compound O than catalytic hydrogenation,[15] which yields relatively larger amounts of compound J.

Preparation of 3β,17β-Diacetoxyallopregnan-20-one (Iso-L Diacetate) (VI).—A solution of 52.3 mg. of iso-L monoacetate (I) and 50 mg. of *p*-toluenesulfonic acid in 2 ml. of acetic anhydride was allowed to stand at room temperature

(13) L. Ruzicka and H. F. Meldahl, *Helv. Chim. Acta*, **21**, 1760 (1938).

(14) All melting points are corrected. Microanalyses were performed by S. M. Nagy, Department of Chemistry, M. I. T.

(15) T. Reichstein and K. Gätzi, *Helv. Chim. Acta*, **21**, 1497 (1938); P. Hegner and T. Reichstein, *ibid.*, **24**, 828 (1941).

overnight. The product, isolated by ordinary procedures, was crystallized from acetone–petroleum ether, and gave iso-L diacetate in 2 crops, 43.8 mg., m.p. 226–227°; 8.9 mg., m.p. 225–226.5°. The substance did not depress the melting point of a sample prepared for comparison by acetylation of iso-L monoacetate with acetic anhydride and pyridine at 100°.[3]

Preparation of 3β,17α-Dipropionoxyallopregnan-20-one (L Dipropionate) (VII).—A solution of 200 mg. of compound L (free diol) and 200 mg. of *p*-toluenesulfonic acid in 10 ml. of propionic acid and 2 ml. of propionyl chloride was allowed to stand overnight at room temperature. The product was isolated in the usual way and crystallized from acetone–petroleum ether; yield 200 mg., m.p. 123–124°. Two recrystallizations from acetone–petroleum ether gave the analytical sample, m.p. 124–125°, $[\alpha]_D$ −10.0° (*c* 1.22, dioxane).

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.69; H, 9.28.

Preparation of 3β,17α-Dihydroxyallopregnan-20-one 3-Acetate-17-propionate (VIII).—L monoacetate (II), 300 mg., was treated with propionyl chloride and propionic acid in the manner described in the preceding experiment. The crude product, 290 mg., m.p. 165–168°, after two recrystallizations from acetone–petroleum ether gave a pure sample, m.p. 172.5–173.5°, $[\alpha]_D$ −10.5° (*c* 1.31, dioxane).

Anal. Calcd. for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 72.27; H, 9.45.

Preparation of 17α-Acetoxyprogesterone (IXb).—A solution of 200 mg. of 17α-hydroxyprogesterone (IXa) and 200 mg. of *p*-toluenesulfonic acid in a mixture of 10 ml. of acetic acid and 2 ml. of acetic anhydride was allowed to stand at room temperature overnight. Crude material, 110 mg., m.p. 233–237°, was obtained by crystallization of the reaction product from methylene chloride–methanol. Several recrystallizations from benzene furnished the sample for analysis, m.p. 243–244.5°, $[\alpha]_D$ +56.0° (*c* 1.28, dioxane).

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.14; H, 8.56.

Preparation of Compound S Diacetate (Xb).—A solution of 300 mg. of compound S 21-monoacetate and 300 mg. of *p*-toluenesulfonic acid in 10 ml. of acetic acid and 2 ml. of acetic anhydride was allowed to stand overnight at room temperature (nitrogen atmosphere). Crystallization of the reaction product from acetone–petroleum ether gave 210 mg. of impure material melting at 205–211°. A pure sample of S diacetate, m.p. 220–221°, $[\alpha]_D$ +49.5° (*c* 1.21, dioxane), was obtained by several recrystallizations from acetone–petroleum ether.

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.91; H, 8.09.

Hydrolysis of S Diacetate (1% methanolic sodium hydroxide, room temperature, 15 min.) followed by partial acetylation with acetic anhydride and pyridine gave a sample of compound S 21-monoacetate, m.p. 236–238.5°, that did not depress the melting point of an authentic specimen.

Preparation of Cortisone Diacetate (IXb).—Cortisone 21-monoacetate (250 mg.) was treated as described above with 200 mg. of *p*-toluenesulfonic acid, 10 ml. of acetic acid and 2 ml. of acetic anhydride in an atmosphere of nitrogen. The crude product (130 mg.) melted at 214–217°. The sample for analysis, m.p. 223.5–224.5°, $[\alpha]_D$ +113° (*c* 1.15, dioxane), was obtained by recrystallization from methanol, followed by careful drying under high vacuum at 100°.

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 67.55; H, 7.26. Found: C, 67.36; H, 7.42.

Preparation of 17α-Hydroxypregnenolone Diacetate (XIV).—17α-Hydroxypregnenolone 3-monoacetate (500 mg.) was treated with 500 mg. of *p*-toluenesulfonic acid, 20 ml. of acetic acid and 4 ml. of acetic anhydride in the usual manner. Crystallization of the reaction product from acetone–petroleum ether furnished 2 crops, 420 mg., m.p. 180–182°; 127 mg., m.p. 177–180°. A sample of crop I after several recrystallizations from acetone–petroleum ether melted at 180–181°, $[\alpha]_D$ −64.9° (*c* 1.19, dioxane).

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 71.78; H, 8.73.

Preparation of 17α-Acetoxypregnenolone.—A solution of 100 mg. of 17α-hydroxypregnenolone diacetate from the preceding experiment was dissolved in 5 ml. of methanol, to which 0.2 ml. of acetyl chloride (for generation of hydrogen chloride) was added. After standing at room temperature for 9 hours, the solution was diluted with ether, washed with water, saturated sodium chloride, filtered through anhydrous sodium sulfate, and concentrated to dryness. The product was isolated in 2 crops, 48.2 mg., m.p. 223–226°; 28.0 mg., m.p. 228–230° by crystallization from acetone–petroleum ether. Several recrystallizations from dilute methanol and from acetone–petroleum ether gave pure material, m.p. 230–231.5°, $[\alpha]_D$ −68.0° (*c* 1.12, dioxane).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.86; H, 9.14.

Oppenauer oxidation of the 17-monoacetate prepared above (100 mg.) was carried out by refluxing for 7 hours with 100 mg. of aluminum *t*-butoxide in a mixture of 10 ml. of anhydrous benzene and 3 ml. of cyclohexanone. The product (77.3 mg., m.p. 238–240°) was identical with 17α-acetoxyprogesterone (IXb).

Conversion of 17α-Hydroxypregnenolone Diacetate (XIV) into L Diacetate (V).—17α-Hydroxypregnenolone diacetate (470 mg.) was hydrogenated (2 molar equivalents) over 100 mg. of platinum oxide catalyst in 10 ml. of acetic acid. The catalyst was filtered off, and 120 mg. of chromic acid in dilute acetic acid was added. After standing overnight at room temperature, the oxidation mixture was diluted with water and extracted with ether. The ethereal solution was washed successively with dilute hydrochloric acid, water, dilute sodium hydroxide, filtered through anhydrous sodium sulfate, and concentrated to dryness. Crystallization of the residual material from acetone–petroleum ether gave 446 mg. of L diacetate (V), m.p. 195–197°.

HOUSTON, TEXAS