[CONTRIBUTION FROM THE DIVISION OF STEROID RESEARCH, THE JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE, UNIVERSITY OF PENNSYLVANIA]

INVESTIGATIONS ON STEROIDS. XXIII. 6-HYDROXY DERIVATIVES OF 17α-HYDROXYPROGESTERONE AND REICHSTEIN'S COMPOUND S. STUDIES ON 6β,11α-DIHYDROXYPROGESTERONE*, [1]

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The recent recognition that mammalian as well as microbial organisms possess enzyme systems capable of hydroxylating 3-oxo-Δ^4-unsaturated steroids at carbon atom 6[3] greatly stimulated the interest in 6-hydroxy derivatives of steroid hormones, first prepared by partial synthesis in this laboratory.

The 6-acetoxy derivatives of such steroids as androstenedione, progesterone, and 11-desoxycorticosterone acetate were synthesized a number of years ago (2–4, *cf.* also 5). The correct assignment of steric configurations at carbon atom 6 of these compounds was made possible by a reinvestigation of the preparation of 6-acetoxy-11-desoxycorticosterone acetate (6). It was established that treatment of a 3-oxo-5α-hydroxy-6β-acetoxy steroid with dry hydrogen chloride in ethanol-free chloroform causes dehydration to the 3-oxo-6β-acetoxy-Δ^4-unsaturated compound. If the reaction is carried out in the presence of a small amount of ethanol, as it is used as a stabilizer in commerical chloroform, the corresponding 6α-acetoxy compound results. The epimerization at carbon atom 6 is not coupled with the dehydration mechanism because the 3-oxo-6β-acetoxy-Δ^4-unsaturated steroid can be epimerized under these experimental conditions. The 6β-acetoxy and 6α-acetoxy unsaturated steroids can be saponified to the 6β-hydroxy and 6α-hydroxy compounds respectively. These relationships, first observed in the 11-desoxycorticosterone series (6, *vide supra*), were confirmed by the preparation of the analogous derivatives of androstenedione and of progesterone (7). The synthetic 6-hydroxy derivatives have served for the identification of biosynthetic hydroxylation products obtained by various investigators (*cf.* 1).

The kind of epimerization discussed above has recently been realized in the cholestenone series (8, 9). The study of further examples appeared indicated,

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[1] A part of the findings of this paper (transformations leading to the 6β-acetoxy derivative of Reichstein's compound S) was presented on July 22, 1952, at the 2nd International Congress of Biochemistry in Paris (*cf.* M. R. Ehrenstein, *6-Hydroxy Derivatives of Steroid Hormones. Their Stereochemistry and Possible Biochemical Significance*, Symposium sur la Biochimie des Stéroides, p. 13, II[e] Congrès International de Biochimie. Société d'Edition d'Enseignement Supérieur, Paris, 5[e], 1952).

[2] Merck & Co. Predoctoral Fellow (1951–1952). This paper is based on a section of a thesis submitted by Klaus Georg Florey to the Graduate School of Arts and Sciences of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Physiological Chemistry (June 1954).

[3] *Cf. e.g.* the review article, *Hydroxylierungen von Steroiden durch biologische Methoden* (1).

not merely in order to demonstrate the scope of these reactions, but also to aid in the identification of steroids of biosynthetic origin. The 17α-hydroxypregnane type represents a group of 3-oxo-6-hydroxy-Δ^4-unsaturated steroids which had not yet been obtained by partial synthesis. Therefore, the preparation of the 6β- and 6α-hydroxy derivatives of 17α-hydroxyprogesterone (VIII and X) and of 17α-hydroxy-11-desoxycorticosterone, *i.e.* Reichstein's compound S, (XXII and XXIV) was undertaken, utilizing procedures developed in this laboratory (3, 4, 6, 7).

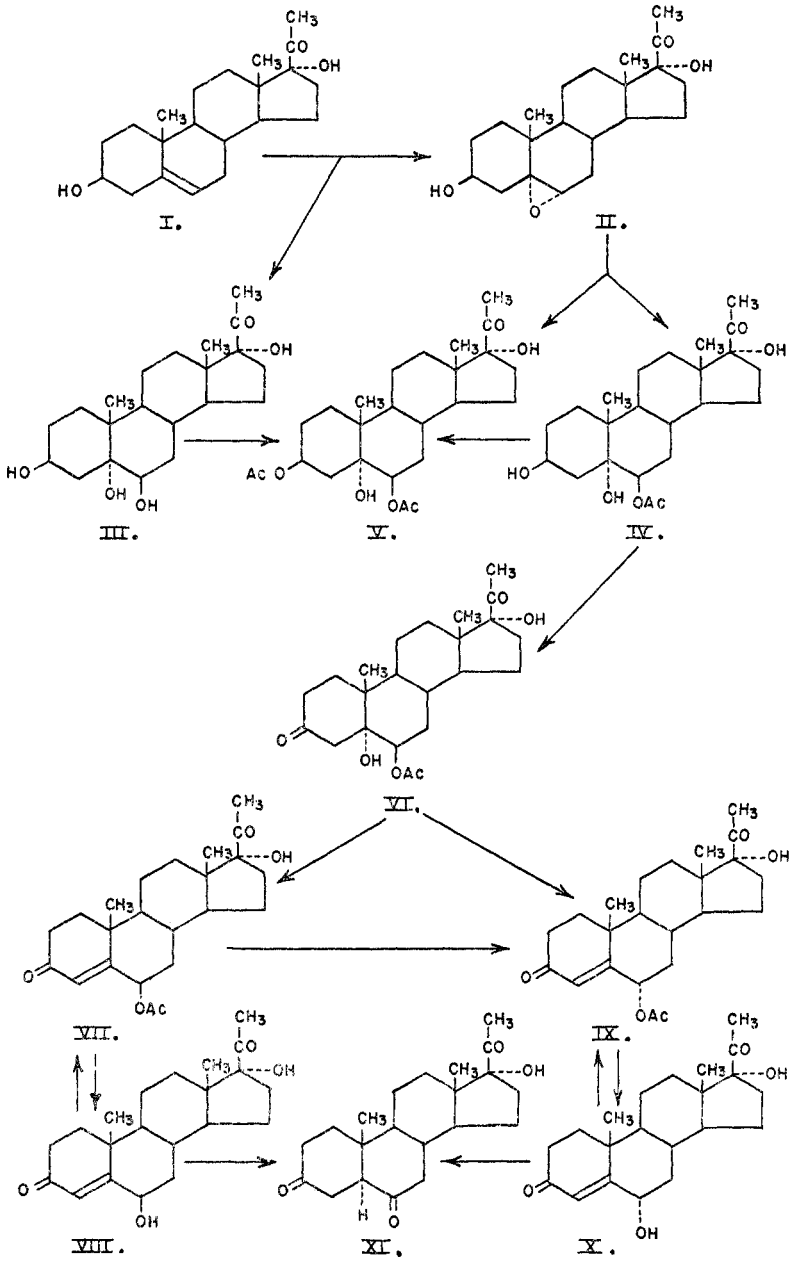
The starting material for the partial synthesis of the 6β- and 6α-hydroxy derivatives of 17α-hydroxyprogesterone (VIII and X) was Δ^5-pregnene-3β,17α-diol-20-one (I). This compound was first prepared in Reichstein's laboratory in 1941 (10, 11), and more recently by a different and more convenient route by Julian and co-workers (12). Hirschmann and Hirschmann (13) reported the isolation of I from the urine of a boy with adrenocortical carcinoma. I was converted into 5α,6α-oxidoallopregnane-3β,17α-diol-20-one (II) by the action of perbenzoic acid in chloroform solution in 85% yield. As a by-product of the reaction a small amount (1%) of allopregnane-3β,5α,6β,17α-tetrol-20-one (III) was isolated. Acetolysis of the epoxide II by refluxing with glacial acetic acid for 50 minutes, yielded 67% of 6β-acetoxyallopregnane-3β,5α,17α-triol-20-one (IV) and 10% of 3β,6β-diacetoxyallopregnane-5α,17α-diol-20-one (V). Both, III and IV, could be converted into V by acetylation. Oxidation of IV with chromium trioxide (1.03 equivalents) in 90% acetic acid in the cold overnight yielded 6β-acetoxyallopregnane-5α,17α-diol-3,20-dione (VI) as the major reaction product (25% yield). In addition to oxidizing the 3-hydroxyl group, the chromic acid also attacked the ketol side chain and consequently small amounts of 6β-acetoxyandrostan-5α-ol-3,17-dione (3) (6% yield) and 6β-acetoxyandrostane-3β,5α-diol-17-one (3) (1% yield) were obtained. Furthermore, a fair amount (22% yield) of starting material (IV) was isolated.

6β-Acetoxy-Δ^4-pregnen-17α-ol-3,20-dione (VII) resulted from VI by dehydration with hydrogen chloride in ethanol-free chloroform (50% yield) or by refluxing with glacial acetic acid (65% yield). VII was saponified to Δ^4-pregnene-6β,17α-diol-3,20-dione (6β,17α-dihydroxyprogesterone) (VIII) with ethanolic potassium hydroxide (1.1 equivalents) in 89% yield.

As has been shown by investigators of the Research Laboratories of the Upjohn Company (14), fermentation of 17α-hydroxyprogesterone by either of the microorganisms *Rhizopus nigricans* or *Rhizopus arrhizus* yields 11α,17α-dihydroxyprogesterone and a second compound to which the structure of 6β,17α-dihydroxyprogesterone (Δ^4-pregnene-6β,17α-diol-3,20-dione) (VIII) had been tentatively assigned. Comparison of the characteristics of the latter biosynthetic product and of its diacetate with those of the synthetic VIII[4] and VII (*cf.* expt'l part) established the identity of these compounds.

6α-Acetoxy-Δ^4-pregnen-17α-ol-3,20-dione (IX) was prepared by dehydration of VI with hydrogen chloride in ethanol-containing chloroform in 76% yield.

[4] 6β,17α-Dihydroxyprogesterone (VIII) has been obtained independently by a novel synthetic procedure (15).

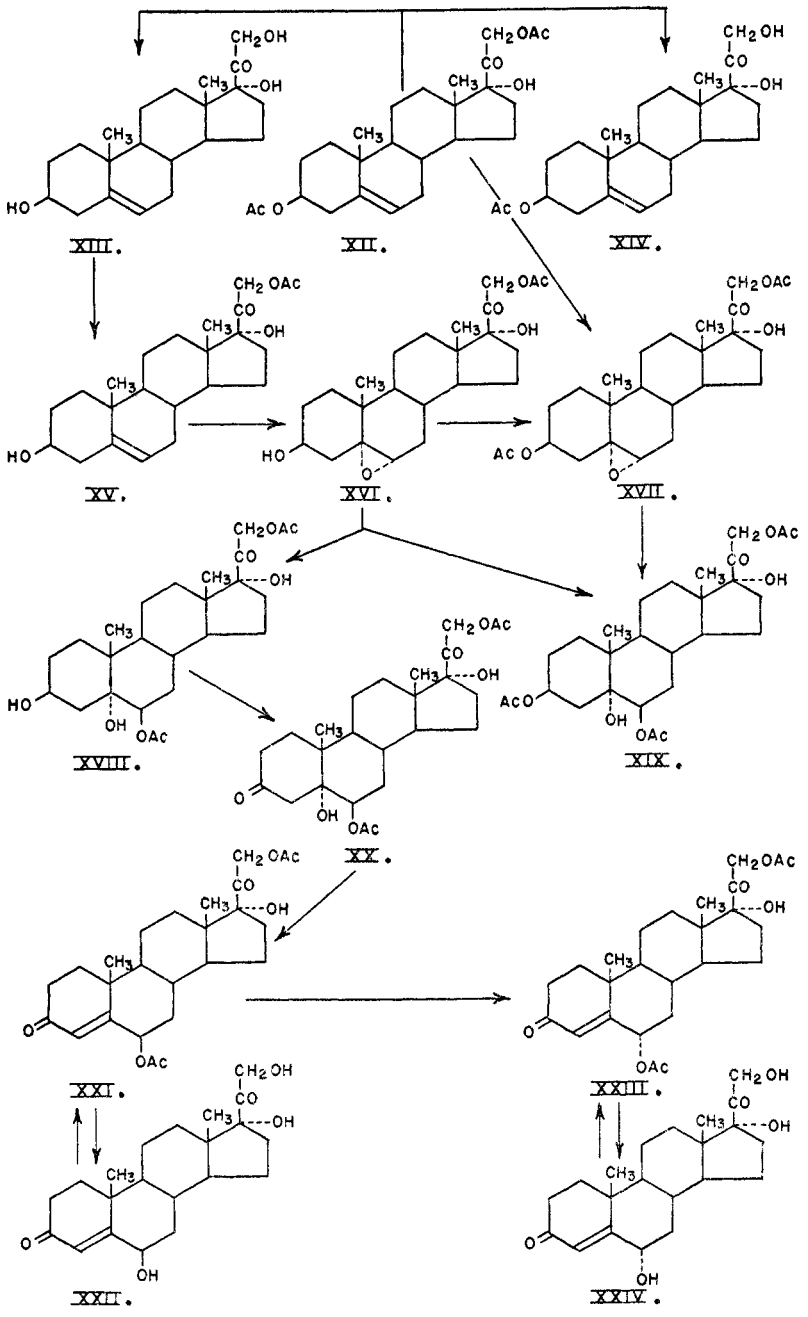


Chromatographic purification gave also a small amount (14 %) of the 6β-acetoxy compound (VII). It should be noted that VII was eluted from the chromatographic column after the 6α-acetoxy compound (IX) with a more polar solvent mixture. This is in agreement with previous observations on compounds of this type (6, 7) (*cf.*, however, 16, p. 1036). Rearrangement of VII, as obtained by

acetylation of a biosynthetic sample of VIII, with hydrogen chloride in ethanol-containing chloroform gave IX in 90% yield. IX was saponified to Δ^4-pregnene-6α,17α-diol-3,20-dione (6α,17α-dihydroxyprogesterone) (X) with sodium methoxide (1.15 equivalents) in methanol solution. In order to exclude the possibility of rearrangement of X under the influence of an adsorbing agent, chromatographic purification was avoided in this instance. Thus the reaction product was isolated by selectively extracting first with ether and then with ethyl acetate. Both extracts yielded X in an over-all yield of 76%. By the criteria of ultraviolet extinction coefficients and melting points the material obtained from the ethyl acetate extract was somewhat purer than the material resulting from the ether extract. The latter was probably contaminated by the rearrangement product XI (*vide infra*). The purity of the reference sample of X was confirmed by reacetylation to IX.

The 6β-hydroxy compound VIII was rearranged to allopregnane-17α-ol-3,6,20-trione (XI) by treatment with a trace of 3% sulfuric acid in glacial acetic acid at room temperature for 40 hours. However, even prolonged treatment of the 6α-hydroxy compound X under the same conditions produced only a small amount (8%) of XI together with unchanged X. A study of the exact conditions under which the 6β- and 6α-hydroxy derivatives of 3-oxo-Δ^4-unsaturated steroids rearrange to 3,6-dioxo-5-allo compounds appears indicated.

The starting material for the partial synthesis of the 6β- and 6α-hydroxy derivatives of Reichstein's compound S was 3β,21-diacetoxy-Δ^5-pregnen-17α-ol-20-one (XII). XII was partially synthesized first by Fuchs and Reichstein (10) in 1941 and more recently by Heer and Miescher (17). The latter investigators also prepared the 3-monoacetate (XIV) and the free compound (XIII) by saponification of XII with methanolic potassium bicarbonate and potassium carbonate respectively, in the presence of air. The 21-monoacetate (XV) was obtained by partial acetylation of XIII. No yields were reported. Since it is well known that the dihydroxyacetone side chain is unstable to alkali in the presence of air (oxygen) (*cf. e.g.* 18), saponification was performed in this laboratory in sodium methoxide solution (2.3 equivalents) under nitrogen at room temperature. The products obtained depended on the time of reaction. Thus saponification of XII for 30 minutes yielded the 3-monoacetate (XIV) as the major reaction product (50%) and only 8% of the free compound (XIII). In a 3 hour experiment 20% of XIV and 49% of XIII were obtained. In a 16 hour experiment the only saponification product, isolated in 85% yield, was Δ^5-pregnene-3β,17α,21-triol-20-one (XIII). XIII was partially acetylated to 21-acetoxy-Δ^5-pregnene-3β,17α-diol-20-one (XV) with 1.1 equivalents of acetic anhydride in pyridine in 67% yield. The melting point and rotation of XV are in good agreement with the values reported by Heer and Miescher (17). As expected, XII and unchanged XIII were isolated in minor quantities. XV was converted into 21-acetoxy-5α,6α-oxidoallopregnane-3β,17α-diol-20-one (XVI) with perbenzoic acid (1.1 equivalents) in chloroform solution in 75% yield. Acetolysis of the epoxide (XVI) by refluxing with glacial acetic acid for 50 minutes yielded 52% of 6β,21-diacetoxyallopregnane-3β,5α,17α-triol-20-one (XVIII) and 10% of 3β,6β,21-



triacetoxyallopregnane-5α,17α-diol-20-one (XIX). The latter was also obtained by acetolysis of 3β,21-diacetoxy-5α,6α-oxidoallopregnan-17α-ol-20-one (XVII) which in turn was prepared by two methods: (a) treatment of XII with perbenzoic acid in chloroform; this apparently produced also a certain amount of the

corresponding β-oxido compound; (b) acetylation of XVI. Oxidation of XVIII with chromium trioxide (1.05 equivalents) in 90% acetic acid at room temperature overnight gave 6β,21-diacetoxyallopregnane-5α,17α-diol-3,20-dione (XX) in 47% yield. In this instance no 6β-acetoxyandrostan-5α-ol-3,17-dione (3) was isolated after chromatography. However, small amounts of this compound must have been formed during the reaction, since in dehydration experiments with crude XX which are not reported in the experimental part, small quantities of 6β- and 6α-acetoxy-Δ^4-androstene-3,17-dione (7) were isolated.

6β,21-Diacetoxy-Δ^4-pregnen-17α-ol-3,20-dione (XXI) resulted from XX by treatment with hydrogen chloride in ethanol-free chloroform (25% yield) or by refluxing with glacial acetic acid (65% yield). The physical constants of XXI have been reported in a footnote of a previous publication from this laboratory (7, p. 1591). XXI was saponified to Δ^4-pregnene-6β,17α,21-triol-3,20-dione (XXII) with ethanolic potassium hydroxide (2.2 equivalents) under nitrogen in 65% yield.

Investigators of the Upjohn Company (19, 20) reported the conversion of Reichstein's compound S (17α-hydroxy-11-desoxycorticosterone) by *Rhizopus nigricans* or *Rhizopus arrhizus* to the 11-epimer of Kendall's compound F and a second product which has tentatively been described as the 6β-hydroxy derivative of Reichstein's compound S (XXII). Comparison of the characteristics of the latter substance and of its diacetate with those of the synthetic XXII and XXI (*cf.* expt. part) established the identities beyond doubt.

XXI, as obtained by acetylation of biosynthetic XXII, was rearranged to 6α,21-diacetoxy-Δ^4-pregnen-17α-ol-3,20-dione (XXIII) by treatment with hydrogen chloride in ethanol-containing chloroform in 72% yield. As in the 17α-hydroxyprogesterone series, a small amount (1.3%) of unchanged starting material (XXI) was eluted from the chromatographic column with a more polar solvent mixture. XXIII was saponified to Δ^4-pregnene-6α,17α,21-triol-3,20-dione (XXIV) with sodium methoxide (2.2 equivalents) in methanol under nitrogen. Chromatographic treatment of XXIV was avoided because of its apparent instability. The yield of pure XXIV was 49%. Reacetylation of this product gave XXIII.

In bioassays conducted in the laboratory of Dr. John A. Luetscher, Jr.,[5] Stanford University School of Medicine, the 6α-hydroxy derivative of Reichstein's compound S (XXIV) produced a modest sodium-retaining action (approx. 4% that of DOCA), whereas the 6β-hydroxy derivative (XXII) tended to cause an opposite effect. In a like fashion, 6α-hydroxy-11-desoxycorticosterone (6) caused moderate sodium retention, whereas in 6β-hydroxy-11-desoxycorticosterone (6) the sodium-retaining activity appeared abolished. Bioassays conducted with the latter two epimers somewhat earlier by Drs. Sylvia A. Simpson and J. F. Tait,[5] Middlesex Hospital (London), had yielded essentially the same results. The findings indicated weak sodium retention (2–6% that of DOCA) of 6α-hydroxy-11-desoxycorticosterone and no detectable activity of the 6β-compound.

[5] Private communication.

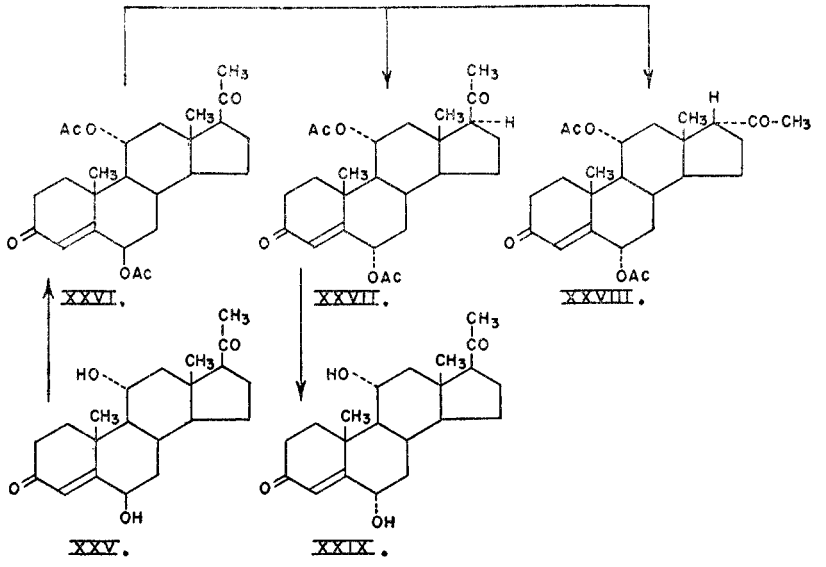
PHYSICAL CONSTANTS OF SYNTHETIC COMPOUNDS

Compound	M.P., °C.	$[\alpha]_D$	M_D	$M_D^\beta - M_D^\alpha$	λ_{max}^{alc}	ϵ_{max}
6β - Acetoxy - Δ⁴ - pregnen - 17α - ol - 3,20-dione (VII)	194–195	+15.6°	+63	−179	235	12,100
6α - Acetoxy - Δ⁴ - pregnen - 17α - ol - 3,20-dione (IX)	199–200	+62.2°	+242		237	16,400
Δ⁴ - Pregnene - 6β,17α - diol - 3,20 - dione (VIII)	243–245	+6.8°	+24	−309	236	14,000
Δ⁴-Pregnene-6α,17α-diol-3,20-dione (X)	273–275	+96.2°	+333		240	14,900
6β,21-Diacetoxy- Δ⁴-pregnen-17α-ol-3,20-dione (XXI).	191–192	+62.2°	+278	−193	234.5	13,300
6α,21 - Diacetoxy - Δ⁴ - pregnen - 17α - ol-3,20-dione (XXIII)	184–185	+105.5°	+471		236	16,800
Δ⁴ - Pregnene - 6β,17α,21 - triol - 3,20 - dione (XXII)	229–233	+43.7°	+158	−246	235	13,100
Δ⁴ - Pregnene - 6α,17α,21 - triol - 3, 20-dione (XXIV)	219–221	+111.3°	+404		240	14,500

In an exploratory experiment the epimerization of the diacetate (XXVI) of 6β,11α-dihydroxyprogesterone (XXV) to 6α,11α-diacetoxyprogesterone (XXVII) was studied according to the procedure developed in this laboratory (6). XXV has been obtained by fermentation of progesterone with *Rhizopus arrhizus* and *Rhizopus nigricans* (19, 21, 22) and with *Aspergillus niger* (23). The assignment of this structure for this compound was tentative and it was hoped that the now well established epimerization reaction would furnish supporting evidence.[6] When XXVI, obtained by acetylation of biosynthetic XXV, was treated with dry hydrogen chloride in chloroform, containing 0.8% of ethanol, in the usual manner, chromatography of the reaction product gave a crystalline substance (XXVIII) in about 20% yield and large amounts of a more polar amorphous product (XXVII) which even after rechromatography refused to crystallize. The analytical data of both transformation products are in agreement with a diacetoxyprogesterone. However, the optical rotations of the two substances differ markedly. The slight levorotation of XXVIII ($[\alpha]_D^{26}$ −9.6°; M_D −41) as compared with the high dextrorotation of XXVII ($[\alpha]_D^{28}$ +107.5°; M_D +466) and the molecular rotation difference between XXVII and XXVIII ($M_D^{XXVII} - M_D^{XXVIII}$: 507) suggested that in XXVII epimerization had occurred, as expected, at carbon atom 6, whereas in XXVIII epimerization had taken place both in positions 6 and 17. Molecular rotation differences for pairs of

[6] For further attempts to elucidate the structure of XXV, *cf.* (8).

20-ketosteroids epimeric at carbon atom 17 range from 448 to 730 depending on the functional groups and solvents (*cf.* 24, 25). Thus the structure of 6α,11α-diacetoxy-17α-progesterone was assigned to XXVIII. The difference in molecular rotation of XXVI and XXVII ($M_D^{\beta} - M_D^{\alpha}$: -166) seems to be in agreement with the molecular rotation differences reported for other 6β- and 6α-acetoxy epimers (see table, *cf.* also 6–8) although the figure appears somewhat low. Therefore the structure of 6α,11α-diacetoxyprogesterone was assigned to the amorphous XXVII.



The appreciable degree of epimerization at carbon atom 17 in the present instance raises a number of questions. Basically this could occur under the described experimental conditions with any 20-ketosteroid having a hydrogen atom in position 17. Thus small amounts of the 17-epimers may have been overlooked in previous investigations of this series (6, 7). On the other hand, it appears possible that in the present instance (XXVI) the 11α-acetoxy group was an influencing factor. At any rate, this aspect requires further investigation.

Careful saponification of the amorphous XXVII yielded the crystalline 6α, 11α-dihydroxyprogesterone (XXIX) which apparently was partly rearranged to and consequently contaminated with 11α-hydroxyallopregnane-3,6,20-trione.

The studies on diacetoxyprogesterone will be continued. Furthermore, attempts will be made to determine the factors influencing the epimerization at carbon atom 17.

EXPERIMENTAL

Melting points. Unless stated otherwise, the m.p.'s were determined with the Fisher-Johns melting point apparatus. The readings are sufficiently near the true melting points so that no corrections have been made.

Absorption spectra. Ultraviolet spectra were determined in absolute ethanol with a Beckman Model DU spectrophotometer.

Chromatography. The alumina (Alumina Adsorption, 80–200 mesh, Fisher Scientific Company) was washed with dilute acetic acid, methanol and water, dried at 180° for 48 hours and standardized according to Brockmann (26). The silica gel (100–200 mesh, The Davison Chemical Corporation, Baltimore, Md.) was washed with water and methanol and dried at 180° for 48 hours. The solvents used were reagent grade and were freshly distilled.

Analyses. Unless stated otherwise, the microanalyses were performed by Dr. E. W. D. Huffman, Wheatridge, Colorado, on samples which were dried to constant weight *in vacuo* over phosphorus pentoxide at 80–90° according to Milner and Sherman (27). The percentage loss of weight on drying and gain of weight on exposure of the sample to the atmosphere are recorded.

Optical rotations. No corrections for crystal solvent have been made. Unless stated otherwise, the sample was dissolved in chloroform to make a 2-cc. solution and the rotation was determined in a 2-dm. semi-micro tube.

Acknowledgement. The authors are indebted to The Upjohn Company in Kalamazoo, especially Drs. Robert H. Levin, Durey H. Peterson and Peter D. Meister, for the gift of generous supplies of 6β,17α-dihydroxyprogesterone (VIII), Δ⁴-pregnene-6β,17α,21-triol-3,20-dione (XXII), and 6β,11α-dihydroxyprogesterone (XXV).

I. 17α-HYDROXYPROGESTERONE SERIES

5α,6α-Oxidoallopregnane-3β,17α-diol-20-one (II) *from Δ⁵-pregnene-3β,17α-diol-20-one*[7] (I). To 1.0 g. of chromatographically uniform I, m.p. 260–265°, in 500 cc. of chloroform was added in two portions a total of 11 cc. of a chloroform solution containing 620 mg. of perbenzoic acid (1.5 equivalents) in the cold. The mixture was kept at room temperature for 72 hours and was then washed with *N* sodium carbonate and with water. After drying over sodium sulfate, evaporation of the solvent *in vacuo* left 1.0 g. of solid residue which was chromatographed on 30 g. of alumina (activity III). With various ether-ethyl acetate combinations several consecutive solid fractions, m.p. range 212–218°, totalling 889 mg. (85% yield) were eluted. Repeated recrystallization of a 129-mg. portion from ethyl acetate-methanol gave 72.2 mg. of glistening rods of II, m.p. 214–215° (slight discoloration). When a solution of this material in methanol-acetone was brought completely to dryness, a m.p. of 222–225° was observed: $[\alpha]_D^{27}$ −42.2° (absol. ethanol; 14.19 mg.; α −0.60°).

Anal. Calc'd for $C_{21}H_{32}O_4$ (348.47): C, 72.38; H, 9.26.

Found: C, 72.56; H, 9.38. No weight loss.

With ethyl acetate-methanol (19:1) 36.4 mg. of semi-solid material was eluted from the alumina. Recrystallization from methanol gave 12.7 mg. of crystals (1% yield), m.p. 252–254°. This product was identified as allopregnane-3β,5α,6β,17α-tetrol-20-one (III). By repeated recrystallization the m.p. was raised to 255–258°; $[\alpha]_D^{27}$ −21.9° (absol. ethanol; 3.29 mg.; α −0.07°).

Anal. Calc'd for $C_{21}H_{34}O_5$ (366.48): C, 68.82; H, 9.35.

Found: C, 69.14; H, 9.54. No weight loss.

6β-Acetoxyallopregnane-3β,5α,17α-triol-20-one (IV) *and 3β,6β-diacetoxyallopregnane-5α,17α-diol-20-one* (V) *by acetolysis of 5α,6α-oxidoallopregnane-3β,17α-diol-20-one* (II). A total of 1.072 g. of II, m.p. range 207–214°, was refluxed with 15 cc. of glacial acetic acid for 50 minutes. After taking to dryness *in vacuo*, the residue was dissolved in 100 cc. of ethyl acetate which was then washed with *N* sodium bicarbonate and water and dried over sodium sulfate. Evaporation yielded 1.335 g. of resin which was chromatographed on 110 g. of silica gel. Two major fractions were eluted. With chloroform-ethyl acetate (7:3 and 3:2) 211.4 mg. of resin was obtained which upon crystallization from ether gave 144.0 mg.

[7] Purchased from: The Glidden Company, Chicago 39, Illinois.

of V, m.p. 187–189°. By repeated recrystallization from ethyl acetate the m.p. was raised to 190–192°; $[\alpha]_D^{25}$ −73.2° (15.07 mg.; α −1.10°).

Anal. Calc'd for $C_{25}H_{38}O_7$ (450.55): C, 66.64; H, 8.50.

Found: C, 66.57; H, 8.58. No weight loss.

With chloroform-ethyl acetate (2:3, 3:7, and 1:4) 968 mg. of resin was eluted which upon crystallization from ethyl acetate-ether yielded 850 mg. of IV, m.p. 243–245°. By repeated recrystallization the m.p. was raised to 245–246°; $[\alpha]_D^{25}$ −69.4° (12.00 mg.; α −0.83°).

Anal. Calc'd for $C_{23}H_{36}O_6$ (408.52): C, 67.62; H, 8.88.

Found: C, 67.52; H, 8.89. No weight loss.

3β,6β-Diacetoxyallopregnane-5α,17α-diol-20-one (V). *A. By acetylation of 6β-acetoxyallopregnane-3β,5α,17α-triol-20-one* (IV). To a total of 23 mg. of IV, m.p. range 242–248°, in 0.5 cc. of pyridine was added 0.25 cc. of acetic anhydride. The solution was kept at room temperature overnight and was then poured into 10 cc. of 3% sulfuric acid. After extracting with ethyl acetate, the solvent was washed with *N* sodium bicarbonate and water and dried over sodium sulfate. Evaporation *in vacuo* left 26.7 mg. of resin which from ether yielded 15.8 mg. of crystals, m.p. 186–188°. The mixture m.p. with V, as obtained by acetolytic treatment of II (*vide* preceding expt.) gave no depression.

B. By acetylation of allopregnane-3β,5α,6β,17α-tetrol-20-one (III). To 23 mg. of III, m.p. 250–252°, as obtained as a by-product of the perbenzoic acid oxidation of I, in 0.5 cc. of pyridine was added 0.25 cc. of acetic anhydride. The solution was kept at room temperature overnight and worked up as described in the preceding experiment. Crystallization from ether yielded 20.4 mg. of crystals, m.p. 186–188°. The mixture m.p. with V as obtained by acetolytic treatment of II, showed no depression.

6β-Acetoxyallopregnane-5α,17α-diol-3,20-dione (VI) *from 6β-acetoxyallopregnane-3β,5α,17α-triol-20-one* (IV). To 1.189 g. of IV, m.p. 243–245°, in 12 cc. of glacial acetic acid, chilled to 7°, was added 3.5 cc. of 85% glacial acetic acid, containing 200 mg. (1.03 equivalents) of chromium trioxide. The solution was kept at 7° overnight and then was evaporated to dryness *in vacuo* at 40°. The residue was partitioned between ethyl acetate and water, the aqueous phase was extracted once more with ethyl acetate, and the combined extracts were washed with sodium bicarbonate and water and dried over sodium sulfate. Evaporation of the solvent left 1.080 g. of resin which was chromatographed on 100 g. of silica gel. A total of 36 solvent fractions was collected. In addition to the desired VI several by-products were obtained. The main product (VI) was eluted with fractions 17–24 (chloroform-ethyl acetate, 7:3 and 3:2) which yielded 424.8 mg. of crystalline residue; range of the m.p.'s: 140–182°. Recrystallization of the individual fractions from ethyl acetate-ether gave a total of 303.7 mg. of crystals, m.p. 190–194° (25% yield). By repeated recrystallization the m.p. was raised to 194–195°; $[\alpha]_D^{29}$ −50.5° (11.80 mg.; α −0.60°).

Anal. Calc'd for $C_{23}H_{34}O_6$ (406.50): C, 67.95; H, 8.43.

Found: C, 67.82; H, 8.78. No weight loss.

Fractions 9–15 (chloroform-ethyl acetate, 4:1) yielded 135.1 mg. of crystalline residue; m.p. range: 201–215° (13% yield). Repeated recrystallization from ethyl acetate-ether gave material of m.p. 218–219°. The mixture m.p. with a sample of 6β-acetoxyandrostan-5α-ol-3,17-dione (3) showed no depression.

Fractions 25–29 (chloroform-ethyl acetate, 3:2) gave 108 mg. of crystalline residue; m.p. range: 182–223°. Repeated recrystallization of the individual fractions from ethyl acetate-ether gave a total of 12.2 mg. (1% yield) of crystals, m.p. 286–288° (discoloration). The mixture m.p. with a sample of 6β-acetoxyandrostane-3β,5α-diol-17-one (3) showed no depression.

Fractions 31–34 (chloroform-ethyl acetate, 3:7 and 1:6) yielded 260 mg. of crystalline material, m.p. 228–237° (22% yield). Upon repeated recrystallization of the individual fractions, 56.5 mg. of crystals, m.p. 238–239°, was obtained. This was identified as starting material (IV) by mixture m.p.

6β-Acetoxy-Δ^4-pregnen-17α-ol-3,20-dione (VII) *from 6β-acetoxyallopregnane-5α,17α-diol-3,20-dione* (VI). *A. By the action of hydrogen chloride in ethanol-free chloroform.* Through

a solution of 156.2 mg. of VI, m.p. 192–196°, in 20 cc. of ethanol-free chloroform (*cf.* 6, p. 1056) was passed a moderate stream of hydrogen chloride at a temperature of −7° to −12° for 1½ hours. The solution was immediately neutralized with cold *N* sodium bicarbonate, washed with water, and dried over sodium sulfate. Evaporation of the solvent left 150 mg. of resin which crystallized from ether; 146.2 mg.; m.p. 178–182°. Chromatography of this material on 10 g. of alumina (activity III) and elution with benzene-ether mixtures yielded several crystalline fractions, m.p. range 162–182°, totalling 109.9 mg. Recrystallization of the individual fractions from ethyl acetate-ether yielded a total of 75.3 mg. (50% yield) of crystals, m.p. 188–193°. Repeated recrystallization from acetone-ether gave needles; first m.p. 95–100°, resolidification at 110–120°, final m.p. 194–195°. (The crushed needles did not exhibit the m.p. of 95–100°) λ_{max}^{alc} 235 mμ; ε 12,100. $[\alpha]_D^{27}$ +15.6° (9.60 mg.; α +0.15°). Acetylation product of biosynthetic VIII; Meister, *et al.* (14): m.p. 95–100° and 192-197°. λ_{max}^{alc} 236 mμ; ε 12,500. $[\alpha]_D^{23}$ +14°. The mixture m.p. with the product of biosynthetic derivation was not depressed.

Anal. Calc'd for $C_{23}H_{32}O_5$ (388.49): C, 71.10; H, 8.30.

Found: C, 71.06; H, 8.59. Weight loss, 3.90; weight gain, 0.00.

Elution with ethyl acetate-methanol (99:1) yielded 14.0 mg. of crystalline residue, m.p. 178–180°, which upon recrystallization from ethyl acetate-ether gave 5.6 mg. of crystals, m.p. 190–191°. The mixture m.p. with starting material (VI) was not depressed.

B. By refluxing with glacial acetic acid. A solution of 137.4 mg. of VI, m.p. 188–194°, in 8 cc. of glacial acetic acid was refluxed for 90 minutes and was then brought to dryness *in vacuo*. The residue was taken up in 100 cc. of ethyl acetate which was subsequently washed with *N* sodium bicarbonate and water and dried over sodium sulfate. Evaporation of the solvent yielded 131 mg. of resin which was chromatographed on 6 g. of alumina (activity III). With benzene-ether combinations a total of 109.9 mg. of crystalline residue, m.p. range 175-182°, was eluted. Recrystallization of the individual fractions from ethyl acetate-ether gave a total of 85.1 mg. of crystals (65% yield), m.p. 188–193°. By further recrystallization the m.p. was raised to 193–195°. The mixture m.p. with a sample of VII obtained by method A (*vide* preceding expt.) was not depressed.

Δ⁴-Pregnene-6β,17α-diol-3,20-dione (6β,17α-dihydroxyprogesterone) (VIII) *from 6β-acetoxy-Δ⁴-pregnen-17α-ol-3,20-dione* (VII). A total of 105.9 mg. of VII, m.p. 192–193°, was dissolved under nitrogen (*cf.* 6, p. 1058) in a solution of 17 mg. of potassium hydroxide (1.1 equivalents) in 8.5 cc. of absolute ethanol. The mixture was kept at room temperature under nitrogen for 35 minutes. Before admission of air the solution was acidified with 0.2 cc. of glacial acetic acid and was then concentrated to near dryness *in vacuo* at room temperature. After the addition of 100 cc. of water the aqueous mixture was extracted twice with 50 cc. of ethyl acetate which was then washed with *N* sodium bicarbonate and water and dried over sodium sulfate. Evaporation of the solvent gave 97.3 mg. of crystalline residue (89% yield), m.p. 230–232°. Recrystallization from acetone yielded 83.3 mg. of lustrous platelets of VIII, m.p. 239–240°. Another recrystallization raised the m.p. to 243–245°.

A 49 mg. portion of the crystalline material, m.p. 239–240°, was chromatographed on 4 g. of alumina (activity III). With ethyl acetate-ether combinations 40.9 mg. of crystalline product, m.p. range 230–234°, was eluted. Recrystallization of the separate fractions from acetone gave a total of 17.0 mg. of VIII, m.p. 243–245°. λ_{max}^{alc} 236 mμ; ε 14,000. $[\alpha]_D^{30}$ +6.8° (7.02 mg.; α +0.05°). Biosynthetic VIII; Meister, *et al.* (14): m.p. 244–246°. λ_{max}^{alc} 238 mμ; ε 12,600. $[\alpha]_D^{23}$ +6°. The mixture m.p. with biosynthetic VIII showed no depression.

Anal. Calc'd for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.73.

Found: C, 72.57; H, 9.38 (2.1-mg. sample!). Weight loss, 10.41; weight gain, 0.20.

6α-Acetoxy-Δ⁴-pregnen-17α-ol-3,20-dione (IX). *A. From 6β-acetoxyallopregnane-5α,17α-diol-3,20-dione* (VI) *by the action of hydrogen chloride in ethanol-containing chloroform.* Through a solution of 185.7 mg. of VI, m.p. 193–197°, in 25 cc. of ethanol-free chloroform (*cf.* 6, p. 1056) to which had been added 0.2 cc. (0.8%) of absolute ethanol, was passed a moderate stream of hydrogen chloride at a temperature of −10° for two hours. The solution was immediately neutralized with cold *N* sodium bicarbonate, washed with water, and

dried over sodium sulfate. The solvent was evaporated *in vacuo* and the resinous residue (181.9 mg.) was chromatographed on 11 g. of alumina (activity III). With benzene-ether (19:1) was eluted 132.4 mg. of crystalline material, m.p. range 174–176°, which upon recrystallization of the individual fractions from acetone-ether-petroleum ether and ethyl acetate-ether gave a total of 86.1 mg. of IX, m.p. 195–198° (49% yield). Renewed recrystallization raised the m.p. to 199–200°. λ_{max}^{alc} 237 mμ; ε 16,400. $[\alpha]_D^{27}$ +62.2° (8.48 mg.; α +0.53°).

Anal. Calc'd for $C_{23}H_{32}O_5$ (388.49): C, 71.10; H, 8.30.

Found: C, 71.01; H, 8.63. No weight loss.

With benzene-ether (1:1), ether, and ether-ethyl acetate several consecutive fractions were eluted which yielded 24.2 mg. of crystalline residue, m.p. 174–176°. Recrystallization from acetone-ether gave 14.2 mg. of crystals, m.p. 185–186°, (8% yield) identified as 6β-acetoxy-Δ⁴-pregnen-17α-ol-3,20-dione (VII) by mixture m.p.

B. By epimerization of 6β-acetoxy-Δ⁴-pregnen-17α-ol-3,20-dione (VII) *through the action of hydrogen chloride in ethanol-containing chloroform.* Through a solution of 385 mg. of VII, m.p. 192–194°, obtained by acetylation of a biosynthetic sample of VIII, in 30 cc. of ethanol-free chloroform (*cf.* 6, p. 1056) to which had been added 0.25 cc. (0.8%) of absolute ethanol, was passed a moderate stream of hydrogen chloride at a temperature of −10° for 2½ hours. The solution was immediately worked up as described in the preceding experiment. The resulting resinous residue (380 mg.) was chromatographed on 15 g. of alumina (activity III–IV). With petroleum ether-benzene, benzene, and benzene-ether there was eluted 344 mg. of crystalline material, m.p. 194–198° (90% yield). Recrystallization of the separate fractions from ethyl acetate-ether gave 160.9 mg. of crystals, m.p. 198–200° (42% yield). The mixture m.p. with IX, as obtained by method A, showed no depression.

C. By acetylation of Δ⁴-pregnene-6α,17α-diol-3,20-dione (X). To 7.8 mg. of X, m.p. 273–275° (*vide* subsequent expt.) in 0.3 cc. of pyridine was added 0.15 cc. of acetic anhydride. The solution was kept at room temperature overnight and was then poured into 30 cc. of 3% sulfuric acid. Working up yielded 8.1 mg. of resin which crystallized from ethyl acetate-ether; 5.2 mg.; m.p. 187–189°. Recrystallization gave 4.1 mg.; m.p. 194–196°. λ_{max}^{alc} 237 mμ; ε 15,500. The mixture m.p. with IX, as obtained by method B, was not depressed.

Δ⁴-Pregnene-6α,17α-diol-3,20-dione (6α,17α-dihydroxyprogesterone) (X) *from 6α-acetoxy-Δ⁴-pregnen-17α-ol-3,20-dione* (IX). To 141.1 mg. of IX, m.p. 198–200° (from preceding experiment; method B), in 3 cc. of nitrogen-saturated, absolute methanol was added a solution of 9.5 mg. of sodium (1.15 equivalents) in 9.5 cc. of nitrogen-saturated methanol (*cf.* 6, p. 1058). The reaction mixture was kept under nitrogen at room temperature for 1½ hours. Before admission of air, 0.1 cc. of glacial acetic acid was added. The solution was diluted with 50 cc. of water and extracted first with 60 cc. of ether and then with four portions of 60 cc. of ethyl acetate. Both extracts were washed with *N* sodium bicarbonate and water, dried over sodium sulfate and brought to dryness *in vacuo*. The crystalline residue of the ether extract (46.5 mg.) upon recrystallization from methanol gave 41.1 mg. of X, m.p. 261–264° (32% yield). Renewed recrystallization raised the m.p. to 264–268°. λ_{max}^{alc} 240 mμ; ε 14,500.

The crystalline residue of the ethyl acetate extract (71.5 mg.) gave from methanol 63.9 mg. of X, m.p. 268–270° (44% yield). λ_{max}^{alc} 240 mμ; ε 14,100. Several additional recrystallizations raised the m.p. to 273–275°. λ_{max}^{alc} 240 mμ; ε 14,900. $[\alpha]_D^{25}$ +96.2° (11.19 mg.; α +1.08°).

Anal. Calc'd for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.73.

Found: C, 72.79; H, 8.88. No weight loss.

Allopregnane-17α-ol-3,6,20-trione (XI). *A. By rearrangement of Δ⁴-pregnene-6β,17α-diol-3,20-dione* (VIII). To 28 mg. of a biosynthetic sample of VIII, m.p. 240–245°, in 2 cc. of glacial acetic acid was added 0.2 cc. of 3% sulfuric acid. After keeping the solution at room temperature for 40 hours, it was diluted with 100 cc. of ethyl acetate which was then washed acid-free with *N* sodium bicarbonate and with water. After drying over sodium sulfate, evaporation of the solvent left 25.3 mg. of solid residue which upon crystallization from methanol-acetone gave 13.4 mg. of crystals, m.p. 278–280° (48% yield). This material did not absorb ultraviolet light between 228 and 300 mμ. Chromatographic purification

(alumina, activity III; elution with benzene-chloroform, 4:1) did not change the m.p. $[\alpha]_D^{26}$ −43.5° (6.74 mg.; α −0.29°).

Anal. Calc'd for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.73.

Found: C, 72.12; H, 8.57; Residue, 0.17. No weight loss.

B. By rearrangement of Δ⁴-pregnene-6α,17α-diol-3,20-dione (X). To 20.3 mg. of X, m.p. 268–270°, in 2 cc. of glacial acetic acid was added 0.2 cc. of 3% sulfuric acid. The solution was kept at room temperature for 36 hours and was then worked up as described under A. The resulting solid residue (20.1 mg.) gave from methanol 15.6 mg. of crystals (m.p. 269–273°; λ_{max}^{alc} 240 mμ; ε 12,800), representing largely unchanged starting material (X). Therefore the total reaction product (20.1 mg.) was dissolved in 1 cc. of glacial acetic acid and 0.15 cc. of sulfuric acid. The solution was kept at room temperature for another 56 hours. After working up 19.7 mg. of solid material was obtained which from methanol-acetone gave 10.4 mg. of crystals (m.p. 270–273°; λ_{max}^{alc} 240 mμ; ε 8,700. The total reaction product (19.7 mg.) was chromatographed on 2 g. of alumina (activity III). With benzene-chloroform (4:1) 7.2 mg. of semi-solid material was eluted which from methanol gave 2.0 mg. of crystals, m.p. 275–277°. This material, representing XI, did not absorb ultraviolet light between 228 and 300 mμ. With benzene-chloroform (1:4) and chloroform 9.6 mg. of solid material was eluted which from methanol gave 7.0 mg. of crystals (m.p. 270–275°; λ_{max}^{alc} 240 mμ; ε 14,900) representing pure starting material (X).

II. 17α-HYDROXY-11-DESOXYCORTICOSTERONE (REICHSTEIN'S COMPOUND S) SERIES

3β-Acetoxy-Δ⁵-pregnene-17α,21-diol-20-one (XIV) *and Δ⁵-pregnene-3β,17α,21-triol-20-one* (XIII) *by saponification of 3β,21-diacetoxy-Δ⁵-pregnen-17α-ol-20-one*[7] (XII). The saponifications were carried out with sodium methoxide in methanol under nitrogen (*cf.* 6, p. 1058). XIV or XIII or both were obtained as saponification products, depending on the time of reaction.

A. Time of reaction: 30 minutes. A total of 100 mg. of XII, m.p. 194–196°, in 50 cc. of nitrogen-saturated, absolute methanol was mixed under nitrogen with a solution of 12 mg. of sodium (2.3 equivalents) in 10 cc. of nitrogen-saturated absolute methanol. The mixture was kept at room temperature for 30 minutes and was then acidified with 0.1 cc. of glacial acetic acid while still under nitrogen. The solution was concentrated to 10 cc. *in vacuo*, diluted with 50 cc. of water, and extracted four times with 40 cc. of chloroform-ethanol (3:1). The combined extracts were repeatedly washed with saturated sodium chloride solution, dried over sodium sulfate, and brought to dryness *in vacuo*. Yield: 72.8 mg. of crystals; m.p. 205–207°. A 57-mg. portion of this material was chromatographed over 7 g. of silica gel. With chloroform 35.4 mg. of crude XIV, m.p. 220–224°, was eluted (50% yield). By repeated recrystallization from ethyl acetate the m.p. of XIV was raised to 232–234°; $[\alpha]_D^{25}$ −35.0° (14.27 mg.; α −0.50°). Heer and Miescher (17): m.p. 225–230°.

Anal. Calc'd for $C_{23}H_{34}O_5$ (390.50): C, 70.74; H, 8.78.

Found: C, 70.69; H, 8.79. No weight loss.

With ethyl acetate a fraction of 5.3 mg. of crystals, m.p. 210–215°, was eluted, probably representing impure XIII (8% yield).

B. Time of reaction: 3 hours. A total of 100 mg. of XII was saponified as under *A* for 3 hours. The resulting crystalline material, 77 mg., m.p. 203–210°, was chromatographed over 8 g. of silica gel. With chloroform 18.3 mg. of crude XIV, m.p. 205–214°, was eluted (20% yield). With chloroform-ethyl acetate combinations 39.5 mg. of crude XIII, m.p. 215–221°, was obtained (49% yield). Repeated recrystallization of the latter from ethyl acetate and ethyl acetate-methanol raised the m.p. to 240–242°. $[\alpha]_D^{30}$ −15.7° (absol. ethanol; 15.67 mg.; α −0.25°). Heer and Miescher (17): m.p. 224–226°.

Anal. Calc'd for $C_{21}H_{32}O_4$ (348.47): C, 72.38; H, 9.26.

Found: C, 72.32; H, 9.20. No weight loss.

C. Time of reaction: 16 hours. A mixture of 300 mg. of XII in 500 cc. of absolute methanol and 48 mg. of sodium (3 equivalents) in 48 cc. of absolute methanol was prepared as described under *A*. After standing under nitrogen at room temperature for 16 hours and with

the addition of 0.2 cc. of glacial acetic acid, the solution was evaporated to dryness. The residue was dissolved in ethyl acetate which was washed with water and dried over sodium sulfate. Evaporation gave 244 mg. of crystalline material which was chromatographed on 20 g. of silica gel. By eluting with chloroform no XIV was obtained. With chloroform-ethyl acetate (1:1) 203.8 mg. of XIII, m.p. 228–230° (86% yield), was eluted. Recrystallization from methanol-ethyl acetate gave 166.1 mg. of crystals, m.p. 237–240°. The mixture m.p. with XIII, obtained by method B, was not depressed. In subsequent 16 hour saponification experiments no chromatographic purification was carried out. In a typical experiment 1.0 g. of XII gave 543 mg. of XIII, m.p. 237–239° (68% yield). From the mother liquor another crop of 113 mg. of XIII, m.p. 227–229°, was secured (82% total yield).

21-Acetoxy-Δ^5-pregnene-3β,17α-diol-20-one (XV) *by partial acetylation of Δ^5-pregnene-3β,17α,21-triol-20-one* (XIII). To 148.8 mg. of XIII, m.p. 237–240°, in 2 cc. of pyridine was added 0.94 cc. of an ether solution containing 0.047 cc. of acetic anhydride (1.1 equivalents). The mixture was kept at room temperature for 7 days and was then poured into 3% sulfuric acid. After chilling, the precipitate was collected, washed with water, and dried. The product (159 mg.) was dissolved in 50 cc. of benzene and chromatographed on 12 g. of silica gel. Elution with benzene-chloroform (1:9) gave 16 mg. of a crude product, m.p. 180–194°. After recrystallization from ethyl acetate the m.p. was 197–199°. The mixture m.p. with XII was not depressed.

Elution with chloroform-ethyl acetate (9:1) gave 87.2 mg. of XV, m.p. 214–216° (51% yield). After recrystallization from ethyl acetate the m.p. was 215–216°; $[\alpha]_D^{30}$ −10.2° (12.19 mg.; α −0.12°). Heer and Miescher (17): m.p. 211–213°; $[\alpha]_D^{21}$ −7°.

Anal. Calc'd for $C_{23}H_{34}O_5$ (390.50): C, 70.74; H, 8.78.

Found: C, 70.50, 70.27; H, 9.14, 9.25. Weight loss, 7.21, 7.10; weight gain, 0.13, 0.11.

From the late chloroform-ethyl acetate (9:1) eluates there resulted 15.5 mg. of additional, though slightly impure, XV (9.1% yield). Total yield of XV: 60.1%.

Elution with chloroform-ethyl acetate (3:2) yielded 18.7 mg. of crystals, m.p. 214–223°. After recrystallization from methanol the m.p. was 236–240°. The mixture m.p. with XIII showed no depression.

In another experiment (1.62 g. of XIII in 10 cc. of pyridine; 0.574 cc. of acetic anhydride; time of reaction: 48 hours), after chromatography the yield of XV, m.p. 212–216°, was 67%.

21-Acetoxy-5α,6α-oxidoallopregnane-3β,17α-diol-20-one (XVI) *from 21-acetoxy-Δ^5-pregnene-3β,17α-diol-20-one* (XV). To 98.4 mg. of XV, m.p. 215–217°, in 3 cc. of chloroform was added 0.6 cc. of a chloroform solution containing 37 mg. (1.1 equivalents) of perbenzoic acid. The mixture was kept in the cold for 20 hours and at room temperature for another 16 hours. After the addition of 20 cc. of chloroform the solution was washed in the cold repeatedly with *N* sodium bicarbonate and with water. After drying over sodium sulfate and evaporating to dryness, 90.7 mg. of white needles, m.p. 165–180°, resulted. Chromatography on 8 g. of silica gel and elution with chloroform-ethyl acetate combinations (3:2, 1:1) yielded 75.4 mg. of XVI, m.p. 235–238° (75% yield). Recrystallization from ethyl acetate gave fine needles, m.p. 238–239°; $[\alpha]_D^{24}$ −20.8° (16.80 mg.; α −0.35°).

Anal. Calc'd for $C_{23}H_{34}O_6$ (406.50): C, 67.95; H, 8.43.

Found: C, 67.77; H, 8.50; residue, 0.2. No weight loss.

In another typical experiment, 1.557 g. of XV yielded, after chromatography on alumina (activity V) and elution with ether-ethyl acetate combinations, 1.080 g. of XVI, m.p. 229–236° (67% yield).

3β,21-Diacetoxy-5α,6α-oxidoallopregnan-17α-ol-20-one (XVII). *A. From 3β,21-diacetoxy-Δ^5-pregnen-17α-ol-20-one*[7] (XII). To 99.3 mg. of chromatographically uniform XII, m.p. 194–196°, was added in the cold 0.69 cc. of a chloroform solution containing 35 mg. of perbenzoic acid (1.1 equivalents). The mixture was kept in the cold overnight and was then diluted with 20 cc. of chloroform. After washing with *N* sodium bicarbonate and water, drying over sodium sulfate, and evaporating the solvent, 107.5 mg. of crystalline material resulted. Chromatography on 5 g. of alumina (activity III–IV) and elution with benzene-ether (4:1) gave 48.5 mg. of crystalline product, m.p. 183–201°. By rechromatography and

recrystallization the m.p. was raised to 205–207°. The mixture melting points of this product with starting material (XII) and with the later eluates of this chromatogram (representing XVII) showed depressions. The substance, which was not further investigated, probably represented the β-oxido compound. Elution with ether-ethyl acetate (3:2) gave a total of 32 mg. of XVII, m.p. 204–207°. By repeated recrystallization from ethyl acetate the m.p. was raised to 210–211°.

Anal. Calc'd for $C_{25}H_{36}O_7$ (448.54): C, 66.94; H, 8.09.

Found: C, 66.60; H, 8.10. No weight loss.

B. From 21-acetoxy-5α,6α-oxidoallopregnane-3β,17α-diol-20-one (XVI). A solution of 35 mg. of XVI, m.p. 232–234°, in 1 cc. of pyridine and 0.5 cc. of acetic anhydride was kept at room temperature overnight and was then poured into 15 cc. of 3% sulfuric acid. After chilling for two hours, the precipitate was collected, washed with water, and dried. The material (35.0 mg.) was chromatographed on 5 g. of alumina (activity III–IV). With various ether-ethyl acetate combinations a total of 24.9 mg. of crystals, m.p. 209–212°, was eluted. Recrystallization from ethyl acetate gave 19.1 mg. of crystals, m.p. 211–212°. The mixture m.p. with XVII, as obtained under A, was not depressed. $[\alpha]_D^{26}$ −27.2° (14.83 mg.; α −0.40°).

6β,21-Diacetoxyallopregnane-3β,5α,17α-triol-20-one (XVIII) *and 3β,6β,21-triacetoxyallopregnane-5α,17α-diol-20-one* (XIX) *by acetolysis of 21-acetoxy-5α,6α-oxidoallopregnane-3β,17α-diol-20-one* (XVI). A solution of 1.08 g. of XVI, m.p. 229–236°, in 15 cc. of glacial acetic acid was refluxed for 50 minutes and was then evaporated to dryness *in vacuo* (45°). The residue was dissolved in ethyl acetate and the solution was washed with *N* sodium bicarbonate and water and dried over sodium sulfate. Evaporation of the solvent yielded 1.287 g. of brittle foam which was chromatographed on 180 g. of silica gel and eluted in 33 fractions of 200 cc. Fractions 16 to 19 (chloroform-ethyl acetate, 3:2) yielded 209 mg. of resin which upon crystallization from methanol-water gave 140 mg. of XIX, m.p. 150–152° (10% yield). Recrystallization yielded needles of m.p. 153–154°; $[\alpha]_D^{21}$ −7.3° (11.87 mg.; α −0.09°).

Anal. Calc'd for $C_{27}H_{40}O_9$ (508.59): C, 63.76; H, 7.93.

Found: C, 63.65; H, 7.94. No weight loss.

Fractions 25 to 29 of the chromatogram (chloroform-ethyl acetate, 1:4) yielded 732 mg. of resinous material which upon crystallization of the individual fractions from ethyl acetate-petroleum ether gave a total of 643 mg. of XVIII, m.p. 140–141° (52% yield). Further recrystallization did not raise the m.p. $[\alpha]_D^{24}$ −19.8° (10.88 mg.; α −0.22°).

Anal. Calc'd for $C_{25}H_{38}O_8$ (466.55): C, 64.36; H, 8.21.

Found: C, 63.90; H, 8.12. Weight loss, 2.07; weight gain, 0.35.

3β,6β,21-Triacetoxyallopregnane-5α,17α-diol-20-one (XIX) *by acetolysis of 3β,21-diacetoxy-5α,6α-oxidoallopregnan-17α-ol-20-one* (XVII). A solution of 32.4 mg. of XVII, m.p. 202–207°, in 2.5 cc. of glacial acetic acid was refluxed for 45 minutes and then brought to dryness *in vacuo*. The solution of the residue in ethyl acetate was washed with *N* sodium bicarbonate and water, and dried over sodium sulfate. Evaporation of the solvent yielded 33.7 mg. of resin which was chromatographed on 5 g. of alumina (activity III–IV). With ethyl acetate and ethyl acetate-methanol (300:1) a total of 20.6 mg. of resinous material was eluted. By repeated crystallization from methanol-water fine needles of m.p. 155–156° were obtained. No depression of the m.p., when mixed with a sample of XIX obtained in the preceding experiment.

6β,21-Diacetoxyallopregnane-5α,17α-diol-3,20-dione (XX) *from 6β,21-diacetoxyallopregnane-3β,5α,17α-triol-20-one* (XVIII). To 59.6 mg. of XVIII, m.p. 138–143°, in 1.5 cc. of glacial acetic acid was added 2.6 cc. of 90% acetic acid, containing 8.66 mg. (1.05 equivalents) of chromium trioxide. The solution was kept at room temperature overnight and was then evaporated to dryness *in vacuo* (25°). The residue was partitioned between ethyl acetate and water, and the water layer was again extracted with ethyl acetate. The combined ethyl acetate extracts were washed with cold *N* sodium bicarbonate and water and dried over sodium sulfate. Evaporation of the solvent gave 55.0 mg. of resin which was

chromatographed on 12 g. of alumina (activity V). With ethyl acetate-methanol (250:1) 23.0 mg. of resin was eluted which from ether yielded 18.9 mg. of clusters of needles, m.p. 195–196° (32% yield). The m.p. was not raised by further recrystallization. $[\alpha]_D^{26}$ −2.7° (13.48 mg.; α −0.04°).

Anal. Calc'd for $C_{25}H_{36}O_8$ (464.54): C, 64.63; H, 7.81.

Found: C, 64.53; H, 7.94. No weight loss.

In further oxidation experiments, XX, m.p. 196–197°, was obtained by direct crystallization without chromatography in 47% yield. This material, as evidenced in dehydration experiments not recorded here, contained small amounts of 6β-acetoxyandrostan-5α-ol-3,17-dione.

6β,21-Diacetoxy-Δ⁴-pregnen-17α-ol-3,20-dione (XXI) *from 6β,21-diacetoxyallopregnane-5α,17α-diol-3,20-dione* (XX). *A. By refluxing with glacial acetic acid.* Crude XX (113 mg.; m.p. 181–183°) was refluxed with 4 cc. of glacial acetic acid for one hour. After evaporating to dryness *in vacuo*, the residue was taken up in ethyl acetate, and the solution was washed with *N* sodium bicarbonate and water and dried over sodium sulfate. Evaporation of the solvent yielded 107.2 mg. of resin which from ether-ethyl acetate gave 70 mg. of crystalline XXI, m.p. 184–186° (65% yield). Repeated recrystallization from ethyl acetate-petroleum ether gave 37 mg. of crystals, m.p. 191–192°. λ_{max}^{alc} 234.5 mμ; ε 13,300. $[\alpha]_D^{26}$ +62.2° (15.15 m.g; α +0.94°). Acetylation product of biosynthetic XXII; Peterson, *et al.* (20): m.p. 192–195°; $[\alpha]_D^{23}$ +63°. The mixture m.p. with the product of biosynthetic derivation was not depressed.

Anal. Calc'd for $C_{25}H_{34}O_7$ (446.52): C, 67.24; H, 7.68.

Found: C, 67.31; H, 7.66. (Dried at 100° without special precautions).

B. By the action of hydrogen chloride in ethanol-free chloroform. Through a solution of 38.2 mg. of XX, m.p. 187–189°, in 10 cc. of ethanol-free chloroform (*cf.* 6, p. 1056) was passed a moderate stream of dry hydrogen chloride at a temperature of −12° for 1½ hours. The solution was diluted with 10 cc. of cold ethanol-free chloroform and washed to neutrality with cold *N* sodium bicarbonate and with water. After drying over sodium sulfate, evaporation of the solvent gave 31.4 mg. of resin which upon crystallization from ethyl acetate-ether-petroleum ether yielded 9 mg. of XXI, m.p. 185–187° (25% yield). By another recrystallization the m.p. was raised to 191–192°. The mixture m.p. with XXI, as obtained by method *A*, was not depressed. Chromatography of the material contained in the mother liquors did not raise the yield.

Δ⁴-Pregnene-6β,17α,21-triol-3,20-dione (XXII) *from 6β,21-diacetoxy-Δ⁴-pregnen-17α-ol-3,20-dione* (XXI). To 6.2 mg. of potassium hydroxide (2.2 equivalents) in 4 cc. of nitrogen-saturated absolute ethanol was added 22.5 mg. of XXI, m.p. 188–191°, as obtained by dehydration of XX. The solution was kept under nitrogen (*cf.* 6, p. 1058) at room temperature for one hour. Before admitting air, 1 cc. of 3% sulfuric acid was added. The solution was then diluted with 30 cc. of water and repeatedly was extracted with ethyl acetate. The combined extracts were washed with *N* sodium bicarbonate and water and dried over sodium sulfate. The solvent was removed *in vacuo*, leaving 19.9 mg. of semi-solid material which was chromatographed on 10 g. of silca gel. Chloroform-ethyl acetate (1:1) eluted several crystalline fractions, totalling 11.8 mg. (m.p. range 219–226°; 65% yield). Repeated recrystallization of the pooled material from acetone-ether raised the m.p. to 223–226°. In another experiment crystals with the m.p. 229–233° were obtained. λ_{max}^{alc} 235 mμ; ε 13,100. $[\alpha]_D^{27}$ +43.7° (chloroform, containing 3 drops of ethanol; 4.58 mg.; α +0.20°). Biosynthetic XXII; Peterson, *et al.* (20): m.p. 230–233°; $[\alpha]_D^{25}$ +58.5° (ethanol). The mixture m.p. with biosynthetic XXII was not depressed.

Anal. Calc'd for $C_{21}H_{30}O_5$ (362.45): C, 69.58; H, 8.34.

Found: C, 69.25; H, 8.52; residue, 0.46. (Dried without special precautions).

6α,21-Diacetoxy-Δ⁴-pregnen-17α-ol-3,20-dione (XXIII). *A. By epimerization of 6β,21-diacetoxy-Δ⁴-pregnen-17α-ol-3,20-dione* (XXI). Through a solution of 878 mg. of XXI, m.p. 191–192°, obtained by acetylation of a biosynthetic sample of XXII, in 50 cc. of ethanol-free chloroform (*cf.* 6, p. 1056) to which had been added 0.4 cc. (0.8%) of absolute ethanol, was passed a moderate stream of hydrogen chloride at a temperature of −10° for 2½ hours.

The solution was washed with cold *N* sodium bicarbonate and with water, dried over sodium sulfate, and freed from solvent *in vacuo*. The resulting resin (872 mg.) was chromatographed on 20 g. of alumina (activity III). Several consecutive resinous fractions, totalling 779.9 mg., were eluted with petroleum ether-benzene and benzene-ether combinations. Crystallization occurred slowly from ether, m.p. range 165–180°. Recrystallization of the individual fractions from ethyl acetate-ether yielded a total of 609.5 mg. of XXIII, m.p. 182–184° (72% yield). Renewed recrystallization of the pooled material raised the m.p. to 184–185°. λ_{max}^{alc} 236 mμ; ε 16,800. $[\alpha]_D^{27}$ +105.5° (8.16 mg.; α +0.86°).

Anal. Calc'd for $C_{25}H_{34}O_7$ (446.52): C, 67.24; H, 7.68.

Found: C, 67.49; H, 7.85; residue, 0.22. Weight loss, 1.33; weight gain, 0.29.

With ether-ethyl acetate (9:1) a small resinous fraction (11.7 mg.) was eluted from the alumina which crystallized from ethyl acetate-ether; m.p. 190–191°; no depression of m.p. with starting material (XXI).

B. By acetylation of Δ⁴*-pregnene-6α,17α,21-triol-3,20-dione* (XXIV). A solution of 7.7 mg. of XXIV, m.p. 210–212° (*vide* subsequent expt.), in 0.3 cc. of pyridine and 0.15 cc. of acetic anhydride was kept at room temperature for 48 hours and was then poured into 15 cc. of 3% sulfuric acid. Working up yielded 8.8 mg. of resin which crystallized from ether, m.p. 173–175°. Recrystallization from ethyl acetate gave 4.8 mg. of XXIII, m.p. 180–182°. λ_{max}^{alc} 236 mμ; ε 16,100. The mixture m.p. with the product obtained by method *A* was not depressed.

Δ⁴*-Pregnene-6α,17α,21-triol-3,20-dione* (XXIV) *from 6α,21-diacetoxy-*Δ⁴*-pregnen-17α-ol-3,20-dione* (XXIII). To 166 mg. of XXIII, m.p. 182–184°, in 3 cc. of nitrogen-saturated absolute methanol was added a solution of 20 mg. of sodium (2.2 equivalents) in 20 cc. of nitrogen-saturated absolute methanol. The mixture was kept under nitrogen (*cf.* 6, p. 1058) at room temperature for one hour. Before the admission of air, 0.1 cc. of acetic acid was added and the solution was subsequently diluted with 10 cc. of water and was extracted repeatedly with ether. The ether extracts were washed to neutrality and dried over sodium sulfate. Evaporation of the ether yielded 19.5 mg. of resinous residue which did not crystallize and was discarded. The aqueous washings of the ether extract were extracted repeatedly with ethyl acetate. These extracts yielded 16.3 mg. of resin which from acetone gave 7.8 mg. of impure XXIV; m.p. 200–202°. λ_{max}^{alc} 240 mμ; ε 11,900. The aqueous reaction mixture was then extracted six times with 50 cc. of ethyl acetate. The combined ethyl acetate extracts were washed with *N* sodium bicarbonate and with water. After drying over sodium sulfate and evaporation of the solvent, 84 mg. of solid material resulted which from acetone gave 66.0 mg. of XXIV, m.p. 204–206° (49% yield). Repeated recrystallization from acetone yielded 46.1 mg. of XXIV, m.p. 219–221°. λ_{max}^{alc} 240 mμ; ε 14,500. $[\alpha]_D^{25}$ +119.1° (ethanol; 9.54 mg.; α +1.14°). $[\alpha]_D^{25}$ +111.3° (chloroform, containing 0.05 cc. of ethanol; 6.72 mg.; α +0.75°).

Anal. Calc'd for $C_{21}H_{30}O_5$ (362.45): C, 69.58; H, 8.34.

Found: C, 69.46; H, 8.33; residue, 0.16. No weight loss.

III. STUDIES ON 6β,11α-DIHYDROXYPROGESTERONE (XXV)

6α,11α-Diacetoxyprogesterone (XXVII) *and 6α,11α-diacetoxy-17α-progesterone* (XXVIII) *by epimerization of 6β,11α-diacetoxyprogesterone* (XXVI). Through a solution of 505 mg. of chromatographically uniform XXVI, m.p. 154–156°, obtained by acetylation of a biosynthetic sample of 6β,11α-dihydroxyprogesterone (XXV) (m.p. 243–245°), in 60 cc. of chloroform containing 0.8% of ethanol was passed a moderate stream of dry hydrogen chloride at a temperature of −10° for 2¼ hours. The solution was immediately neutralized with cold *N* sodium bicarbonate, washed with water, and dried over sodium sulfate. The solvent was evaporated *in vacuo* and the resinous residue (502 mg.) was chromatographed on 25 g. of alumina (activity III). With petroleum ether-benzene (1:1) three crystalline fractions, m.p. 175–176°, totalling 61.6 mg., and three more fractions, m.p. range 120–150°, totalling 68.0 mg. were eluted. Recrystallization of the latter fractions from acetone-ether yielded 28.5 mg. of crystals, m.p. 176–177°. Total yield of material with m.p. 175–177°

(crude XXVIII): 90.1 mg. Repeated recrystallization from acetone-ether raised the m.p. of XXVIII to 186–188°. λ_{max}^{alc} 235 mμ; ε 14,800. $[\alpha]_D^{26}$ −9.6° (11.41 mg.; α −0.11°).

Anal. Calc'd for $C_{25}H_{34}O_6$ (430.52): C, 69.74; H, 7.96.

Found: C, 69.67; H, 7.75. No weight loss.

With petroleum ether-benzene (1:1) three additional, though resinous fractions totalling 55.8 mg. were eluted. This material did not crystallize, even after seeding with XXVIII. With petroleum ether-benzene (2:3 and 1:4) several resinous fractions, totalling 239 mg., were eluted. They were pooled and rechromatographed on 25 g. of alumina (activity III). The first two fractions, eluted with petroleum ether-benzene (3:7), totalling 17.0 mg., upon recrystallization from ether yielded only 2.5 mg. of impure XXVIII, m.p. 165–168°. Several fractions, eluted with petroleum ether-benzene (1:9), benzene and benzene-ether (9:1), again resisted all attempts at crystallization. These amorphous fractions, representing fairly pure XXVII, were combined (152.7 mg.) for saponification. Before pooling, the analytical data of XXVII were secured from the fraction representing the chromatographic peak. λ_{max}^{alc} 235 mμ; ε 14,000. $[\alpha]_D^{23}$ +107.5° (7.73 mg.; α +0.83°).

Anal. Calc'd for $C_{25}H_{34}O_6$ (430.52): C, 69.74; H, 7.96.

Found: C, 69.62; H, 7.84. (Dried at 60°). Weight loss, 0.66; weight gain, 0.0.

6α,11α-Dihydroxyprogesterone (XXIX) *from 6α,11α-diacetoxyprogesterone* (XXVII). To 130 mg. of amorphous XXVII (*vide* preceding expt.) was added under nitrogen a solution of 15.3 mg. (2.2 equivalents) of sodium in 15.3 cc. of methanol. The mixture was kept under nitrogen at room temperature overnight. Before admission of air 0.1 cc. of acetic acid was added. The solution was diluted with 120 cc. of water and extracted with 100 cc. of ether and then with 6 portions of 50 cc. of ethyl acetate. Both extracts were washed with *N* sodium bicarbonate and water, dried over sodium sulfate, and evaporated to dryness. The resinous residue of the ether extract (21.9 mg.) upon crystallization from acetone-methanol gave 5.4 mg. of impure crystalline XXIX, m.p. 208–210°. The crystalline residue of the ethyl acetate extract (65.7 mg.) upon recrystallization from acetone-methanol yielded 36.5 mg. of material, m.p. 208–210°. λ_{max}^{alc} 241 mμ; ε 8,400. Repeated recrystallization from acetone-methanol gave 22.2 mg. of XXIX, m.p. 241–242°. λ_{max}^{alc} 241 mμ; ε 13,200. $[\alpha]_D^{26}$ +127.3° (chloroform, containing 0.1 cc. of ethanol; 11.00 mg.; α +1.40°).

Anal. Calc'd for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.73.

Found: C, 72.39; H, 8.54; residue, 0.27. No weight loss.

The substance was subjected to a paper chromatographic study through the courtesy of Mr. Lester Reineke of the Research Division of the Upjohn Company. According to his analysis, the sample of XXIX contained 8% of a second component which seemed to be identical in its mobility with 11α-hydroxyallopregnane-3,6,20-trione (m.p. 224–228°; $[\alpha]_D^{23}$ +31° in $CHCl_3$) prepared in the Upjohn Laboratories. On this assumption the following corrected physical constants have been calculated for XXIX: $[\alpha]_D^{26}$ +135.7°; ϵ_{max} 14,400.

SUMMARY

1. Δ^5-Pregnene-3β,17α-diol-20-one (I) was converted, by way of 5α,6α-oxidoallopregnane-3β,17α-diol-20-one (II) and 6β-acetoxyallopregnane-3β,5α,17α triol-20-one (IV), into 6β-acetoxyallopregnane-5α,17α-diol-3,20-dione (VI). Depending on the conditions, VI was dehydrated to 6β-acetoxy-Δ^4-pregnen-17α-ol-3,20-dione (VII) or to the epimeric 6α-acetoxy-Δ^4-pregnen-17α-ol-3,20-dione (IX). The latter (IX) was also obtained by epimerization of VII. VII and IX were saponified to 6β,17α-dihydroxyprogesterone (VIII) and 6α,17α-dihydroxyprogesterone (X) respectively. By treatment with acetic acid in the presence of a catalytic amount of sulfuric acid, VIII and, with some difficulty, also X were rearranged to allopregnane-17α-ol-3,6,20-trione (XI). VIII was found to

be identical with a microbiological oxygenation product of 17α-hydroxyprogesterone.

2. 21-Acetoxy-Δ^5-pregnene-3β,17α-diol-20-one (XV), prepared by partial acetylation of Δ^5-pregnene-3β,17α,21-triol-20-one (XIII), was transformed, by way of 21-acetoxy-5α,6α-oxidoallopregnane-3β,17α-diol-20-one (XVI) and 6β,21-diacetoxyallopregnane-3β,5α,17α-triol-20-one (XVIII), into 6β,21-diacetoxyallopregnane-5α,17α-diol-3,20-dione (XX). XX was dehydrated to 6β,21-diacetoxy-Δ^4-pregnen-17α-ol-3,20-dione (XXI) which in turn was epimerized to 6α,21-diacetoxy-Δ^4-pregnen-17α-ol-3,20-dione (XXIII). XXI and XXIII were saponified to Δ^4-pregnene-6β,17α,21-triol-20-one (XXII) and Δ^4-pregnene-6α,17α,21-triol-20-one (XXIV) respectively, the latter two compounds being the 6-hydroxy derivatives of Reichstein's compound S. A number of additional products have been described in this series. XXII was found identical with a microbiological oxygenation product of Reichstein's compound S.

3. The sodium-retaining activity of some 6-hydroxy steroids has been discussed.

4. 6β,11α-Diacetoxyprogesterone (XXVI), prepared by acetylation of biosynthetic 6β,11α-dihydroxyprogesterone (XXV), was epimerized to the amorphous 6α,11α-diacetoxyprogesterone (XXVII) and the crystalline 6α,11α-diacetoxy-17α-progesterone (XXVIII). XXVII was saponified to the crystalline 6α,11α-dihydroxyprogesterone (XXIX).

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