Isolation of 11-Epicorticosterone (*Rhizopus arrhizus* Fermentation).—Fractions 18–21 from the Florisil column described above contained the material which had the mobility of 11-epicorticosterone by paper chromatography. These fractions (1 g.) were dissolved in 120 ml. of ethylene dichloride and rechromatographed over 65 g. of Florisil with 120-ml. portions of solvents as follows: ethylene dichloride (2 fractions); ethylene dichloride–acetone mixtures 10:1, 8:1, 5:1, 3:1, 2:1 and 1:1 (2 fractions in each instance); acetone (1 fraction). Fractions 11–14 (378 mg.) were combined and crystallized from 0.5 ml. of ethyl acetate to give 171.5 mg. of III, m.p. 154–158°, identified by infrared spectroscopy.

Acknowledgments.—The authentic corticosterone was furnished by Dr. W. J. Haines of the Endocrinology Department of the Upjohn Research Division. We are grateful to Dr. J. L. Johnson and Mrs. G. S. Fonken for the infrared analyses, to Mr. W. A. Struck and his associates for all optical rotations and microanalyses, and to Miss Jennie I. Mejeur, Miss Irene N. Pratt and Mr. Glenn Staffen for technical assistance. Again the authors wish to express their profound obligation and thanks to Drs. R. H. Levin and D. I. Weisblat for their helpful suggestions, discussions and interest in this project.

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY]

Microbiological Transformations of Steroids. IV. The 11-Epimer of Compound F and Other New Oxygenated Derivatives of Reichstein's Compound S. A New Route to Cortisone[1]

BY D. H. PETERSON, S. H. EPPSTEIN, P. D. MEISTER, B. J. MAGERLEIN, H. C. MURRAY, H. MARIAN LEIGH, A. WEINTRAUB AND L. M. REINEKE

RECEIVED SEPTEMBER 29, 1952

Incubation of *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b) with Reichstein's Compound S produced two new 11-oxygenated steroids; *viz.*, the 11-epimer of compound F which was easily converted to cortisone acetate in good yields; and a small amount of 11α,17α,21-trihydroxypregnane-3,20-dione. A third new, compound, 6β,17α,21-trihydroxy-4-pregnene-3,20-dione, was also obtained in good yield using *Rhizopus arrhizus* Fischer (A.T.C.C. 11145) on Reichstein's Compound S as the substrate. This new steroid was then also found as a minor transformation compound from the fermentation with *Rhizopus nigricans*.

Discussion

The introduction of oxygen into position 11 of the steroid nucleus by microörganisms in one step has been previously reported.[2] Earlier papers in this series have now recorded in detail the formation of 11α-hydroxyprogesterone, 11α-hydroxy-17α-progesterone and 11α,21-dihydroxy-4-pregnene-3,20-dione (11-epicorticosterone) from progesterone, 4,16-pregnadien-3,20-dione and 11-desoxycorticosterone, respectively. This paper reports the microbiological transformation of Reichstein's Compound S or its acetate by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b) and *Rhizopus arrhizus* Fischer (A.T.C.C. 11145).

Methods and conditions employed for the microbiological conversion have been described elsewhere.[3] For isolation and structure studies it was adequate to ferment 2.0 g. of Reichstein's Compound S (or the acetate) in 12 l. of medium H[3] at *p*H 4.5 to 5.0 for 48–72 hours (growth cycle 24 hours) with *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b) or *Rhizopus arrhizus* Fischer (A.T.C.C. 11145). Paper chromatography[4] (papergram studies) indicated the formation of one major steroid and two minor steroidal components on the concentrate obtained from *Rhizopus nigricans*. Based on isolation and structure studies it was found that 60–80% of the 11-epimer of compound F, epi F or 11α,17α,21-trihydroxy-4-pregnene-3,20-dione (II) could be isolated by direct crystallization or by chromatography over Florisil.

The crystalline concentrate contained 5–10% of 6β,17α,21-trihydroxy-4-pregnene-3,20-dione (III) which could be isolated by chromatography over Florisil.[1a] Traces of a saturated steroid 11α,17α,21-trihydroxypregnane-3,20-dione (IV) could also be detected by papergram analyses, and this compound was isolated and purified over Florisil. Compound III was detected by papergram analysis and then isolated by direct crystallizations in good yields from the fermentation of Reichstein's Compound S with *Rhizopus arrhizus*.

Microanalyses and conversion to a diacetate (VII) showed that the new compound II contained an additional hydroxy group, otherwise retaining the basic structure of the starting material (Reichstein's Compound S). Infrared studies and the absence of biological activity[5] showed that Compound II differed from Kendall's Compound F.

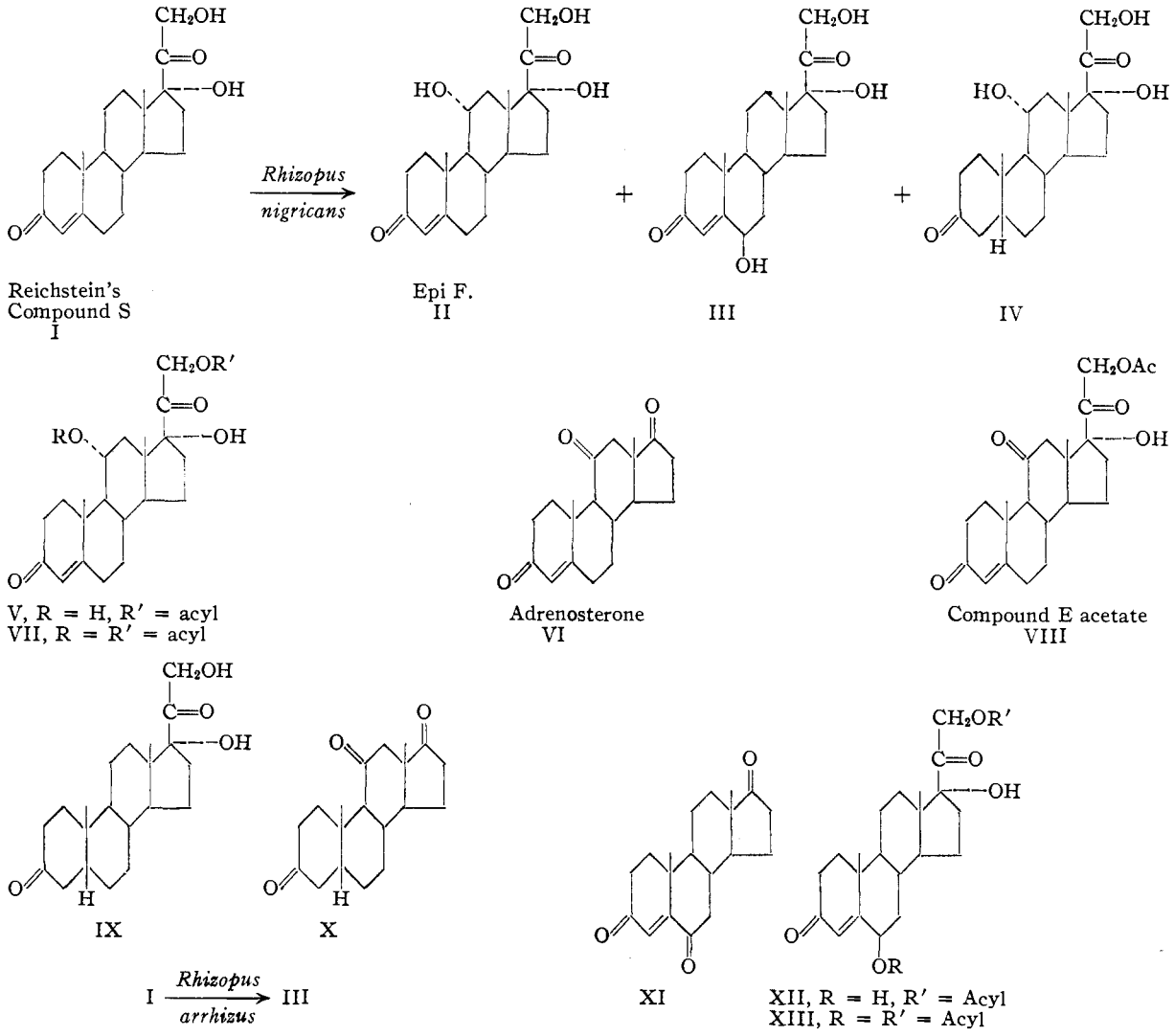
(1) (a) Paper III in this series: S. H. Eppstein, P. D. Meister, D. H. Peterson, H. C. Murray, H. Marian Leigh, D. A. Lyttle, L. M. Reineke and A. Weintraub, THIS JOURNAL, **75**, 408 (1953). (b) The transformations recorded in detail in this manuscript are contained in our U. S. Patent 2,602,769, issued July 8, 1952, based on an original application filed Aug. 19, 1950. (c) In THIS JOURNAL, **74**, 3962 (1952), Fried, *et al.*, reported the bioconversion of Reichstein's Compound S to the 11α-hydroxy Epimer of Compound F using *Aspergillus niger*. (d) In *Chemistry and Industry*, **32**, 783 (1952), J. Romo, A. Zaffaroni, J. Hendricks, G. Rosenkranz, C. Djerassi and F. Sondheimer have reported the chemical synthesis of the 11-epimer (epi F) as the diacetate. The biosynthesis of epi F by adrenal brei from 11α-hydroxyprogesterone on a micro scale was also accomplished. In the latter case the epi F was identified without isolation through the diacetate by paper chromatography and the ultraviolet absorption curve of the sulfuric acid-chromogen.

(2) D. H. Peterson and H. C. Murray, THIS JOURNAL, **74**, 1871 (1952).

(3) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. Marian Leigh, paper I, *ibid.*, **74**, 5933 (1952).

(4) A. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950).

(5) Tested by Dr. K. J. Olson of our laboratories by the rat liver glycogen assay (M. L. Pabst, R. Sheppard and M. H. Kuizenga, *Endocrinology*, **41**, 55 (1947)) in doses of 4 mg. per animal.



Selective acetylation of the 21-hydroxy group of II gave the 21-monoacetate (V) which was then oxidized to yield cortisone acetate (VIII) in 70% yield. Oxidation of II with chromic oxide gave 4-androstene-3,11,17-trione (VI) (adrenosterone).[6] This evidence thus presents unequivocal proof that the new compound II is the 11-epimer of Kendall's Compound F or 11α,17α,21-trihydroxy-4-pregnene-3,20-dione.

Microanalyses and infrared studies together with the formation of a diacetate provided evidence that one additional hydroxyl group was present in Compound III but otherwise possessed the basic structure of the starting material (Reichstein's Compound S). Selective acetylation produced a 21-monoacetate (XII). Oxidation of compound III with chromium trioxide yielded the known 4-androstene-3,6,17-trione (XI).[7] The infrared spectrum showed the Δ^4-double bond absorption at 1602 cm.$^{-1}$ as compared with 1618 cm.$^{-1}$ for the Δ^4-double bond of III. This shift confirmed the introduction of a keto group into position 6. Since it has been shown that *Rhizopus arrhizus* places a hydroxyl group in the 6β-position on desoxycorticosterone[1a] we have tentatively assigned the 6β-hydroxyl group to compound III.

Compound IV was originally detected in the methylene chloride extracts by spraying the paper strip with 2,4-dinitrophenylhydrazine and was also found to be saturated on the basis of ultraviolet and infrared studies. Hydrogenation of epi F (II) over palladium on charcoal yielded the allo and normal isomers and compound IV was shown to be identical with 11α,17α,21-trihydroxypregnane-3,20-dione. Oxidation of one of these reduction products to the known etiocholane-3,11,17-trione[8] confirmed the structure of IV. Conversion of 17α,21-dihydroxypregnane-3,20-dione (IX) to 11α,-17α,21-trihydroxypregnane-3,20-dione (IV) by *Rhizopus nigricans* provided further evidence of the structure of IV.

Experimental

A. Transformation of Reichstein's Compound S by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b). 1. Epi F-11α,17α,21-Trihydroxy-4-pregnene-3,20-dione (II) by Direct Crystallization.—To six liters of a 24-hour growth of *Rhizopus nigricans* was added 1.0 g. of Reichstein's Compound S in 200 ml. of ethanol. After a 48-hour transformation period the usual extraction was performed and the

(6) T. Reichstein, *Helv. Chim. Acta*, **20**, 953 (1937).

(7) A. Butenandt and Byron Riegel, *Ber.*, **69**, 1163 (1936).

(8) L. H. Sarett, THIS JOURNAL, **68**, 2478 (1946).

crude crystalline solids weighing 3.722 g. were washed three times with 4-ml. portions of ice-cold chloroform. The yield of II was 0.85 g. (81%), m.p. 190–200°. Recrystallization of 605 mg. in 3 ml. of methanol and 10 ml. of ether yielded 410 mg. of II, m.p. 209–212°,[9] $[\alpha]^{23}D$ +113° (chloroform), $\lambda_{max}^{alc.}$ 242 mμ (E 14,760 ± 250). Recrystallization did not change the physical constants. Compound II also melts at 217–219°. *Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.35. Found: C, 69.26; H, 8.34.

Adrenosterone (4-Androstene-3,11,17-trione) (VI).—Oxidation of 50 mg. of II in 5.0 ml. of glacial acetic acid was accomplished by the addition of 70 mg. of CrO_3 in one drop of water and 12.5 ml. of glacial acetic acid. To this solution, which was allowed to stand for 48 hours, was added 40 ml. of water. The mixture was extracted with four 25-ml. portions of ether. The combined extracts were washed four times with 11-ml. portions of a mixture of 10 ml. of 7% sodium bicarbonate and 1 ml. of 10% NaOH followed by three 15-ml. portions of H_2O. The ether was dried with 0.2 g. of anhydrous sodium sulfate and after evaporation 27 mg. of a crystalline compound VI resulted. Recrystallization from 25 ml. of ether and 25 ml. of Skellysolve B was accomplished in an ice-bath in one hour to give 12 mg. of VI,[6] m.p. 217–220°. Recrystallization from ether–Skellysolve B did not alter the melting point. Mixed melting point and infrared studies confirmed the structure of VI.

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.96; H, 8.05. Found: C, 75.61; H, 8.10.

Cortisone Acetate (VIII) from Compound Epi F (II).—Five hundred mg. of II was acetylated in the usual manner. The oily residue, obtained by methylene dichloride extraction of the reaction mixture, did not crystallize and was therefore dissolved in 25 ml. of ethylene dichloride and chromatographed over 80 g. of Florisil.[1a] Increasing amounts of acetone were added in the eluting solvents; *viz.*, ethylene dichloride, ethylene dichloride:acetone 25:1, then 15:1, 12:1, 10:1, 8:1, 5:1, 2:1 and finally pure acetone. The oily residue obtained from the ethylene dichloride:acetone (10:1, 8:1, and 5:1) eluates amounting to 355 mg. did not crystallize, but was shown to be highly purified compound epi F monoacetate (V) by infrared and papergram analyses. One hundred sixty-two mg. of the oily compound in 10 ml. of glacial acetic acid was oxidized by adding dropwise 30 mg. (calcd. 26.7 mg. for oxidation of one hydroxyl group) of chromium trioxide in 0.5 ml. of water and 2 ml. of glacial acetic acid. After 6 hours the green solution was diluted with 2 ml. of methanol and evaporated *in vacuo*. The crystalline residue was suspended in 25 ml. of 10% sodium bicarbonate solution and extracted with four 25-ml. portions of ether–chloroform 4:1. The extract was washed three times with 10-ml. portions of water and dried over sodium sulfate.

The crystalline oxidation product weighed 166 mg. Recrystallization from acetone yielded 138.5 mg. of compound E acetate (VIII), m.p. 243–245°, $\lambda_{max}^{alc.}$ 238 mμ (E 15,800), $[\alpha]^{25}D$ +172° (c 0.366 in acetone).

Anal. Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.52. Found: C, 68.52; H, 7.61.

Infrared confirmed the identity of the compound. From 37.5 mg. from the acetone eluate 25.0 mg. of compound epi F, m.p. 209–212°, was recovered.

Diacetate of Compound Epi F (VII).—The ethylene dichloride:acetone (12:1) eluates were combined and shown to be compound epi F diacetate by infrared and papergram studies. This residue was recrystallized from 3 ml. of acetone by adding dropwise petroleum ether. After three recrystallizations 35 mg. of a compound with a constant melting point was obtained. The infrared spectrum indicated that the compound is the 11,21-diacetate (VII) of compound epi F, m.p. 202–203°, $\lambda_{max}^{alc.}$ 240 mμ (E 14,900), $[\alpha]D$ +115° (c 1.145 in chloroform).

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.67. Found: C, 67.43; H, 7.94.

2. Epi F by Florisil Chromatography.—The mother liquor concentrates from an epi F run containing 31 g. of solids were chromatographed over 1500 g. of Florisil in the same manner as previously described for separation of the epi F monoacetate. The eluates from the 5:1 ethylene dichloride–acetone were pooled and the residue of 7.079 g. yield 1.08 g. of epi F, m.p. 216–218° when crystallized and recrystallized from methanol–ether as described previously It is interesting to note traces of compounds in the above eluates whose mobility, by papergram studies, were identical to that of compounds E and F.

It was also possible to isolate from the 8:1 ethylene dichloride–acetone eluates (6.96 g. solids) 912 mg., m.p. 230–234°, of pure 6β,17α,21-trihydroxy-4-pregnene (III) to be described later in this section.

11α,17α,21-Trihydroxypregnane-3,20-dione.—One gram of Reichstein's Compound S was added to a 30-hour growth of *Rhizopus nigricans* in 4.0 l. of medium H. After a 112-hour fermentation period, the medium (*p*H 7.9) was extracted in the usual manner. An aliquot of the extract examined by papergram studies showed that the substrate was well utilized but no other ultraviolet absorbing materials were detectable. Upon spraying the paper strip with 2,4-dinitrophenylhydrazine a substance was revealed whose mobility was slightly greater than that of epi F.

The main methylene chloride concentrate (1.908 g.) was partitioned over Florisil with ethylene dichloride–acetone solvent mixtures as developers, similar to the previously described Florisil chromatograph. The ethylene dichloride–acetone 2:1 eluates were combined (313 mg.) in 25 ml. of methylene chloride and decolorized with 0.5 g. of Magnesol.[3] The filtrate and washings of the Magnesol treatment were evaporated to dryness. The residue was washed with 5 ml. of hot ethyl acetate, then 5 ml. of methylene chloride. The insoluble material left was dissolved in 15 ml. of acetone, filtered and concentrated to dryness. This crystalline residue was washed with 5 ml. of ether to yield 86 mg. of III, m.p. 190–196°. Infrared studies revealed a spectrum whose interpretation was consistent with dihydro-epi F or 11α,17α,21-trihydroxypregnane-3,20-dione (IV).

11α,17α,21-Trihydroxypregnane-3,20-dione (IV) and 11α,-17α,21-Trihydroxyallopregnane-3,20-dione.—Eight hundred milligrams of epi F, m.p. 209–212° (II) dissolved in 50 ml. of 95% ethanol, was shaken (Parr shaker) with hydrogen at atmospheric pressure and 100 mg. of 5% palladium-on-charcoal catalyst. After 69 minutes at room temperature, 1 mole of hydrogen was consumed. The catalyst was removed by filtration and the alcohol evaporated in a current of air, leaving a colorless solid, m.p. 190–200°. Four recrystallizations from methanol–ether gave 90 mg. of a pure dihydro-epi F, m.p. 220–222°, and designated as sample A. The infrared spectrum showed this product to be identical with compound IV.

Anal. Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.07; H, 8.61.

Oxidation of sample A as described later, yielded the known normal compound etiocholane-3,11,17-trione (X).

A sample of the residues from the above crystallizations, 217 mg., m.p. 175–195°, was chromatographed over a mixture of Celite and Darco G-60, 2:1. Elution with a total of 800 ml. of acetone gave 138 mg. of (IV) m.p. 195–207°. Methylene chloride, 600 ml., eluted 56 mg. of crude allodihydro-epi F (11α,17α,21-trihydroxyallopregnane-3,20-dione), m.p. 220–225°. Recrystallization twice from acetone gave a product, m.p. 228–230°. Infrared curves showed this material to be different from the normal isomer above.

Etiocholane-3,11,17-trione (X).—Fifty-three milligrams of sample A, m.p. 220–222°, was dissolved in 3 ml. of glacial acetic acid and oxidized with 53 mg. of chromium trioxide in 0.2 ml. water and 2 ml. of glacial acetic acid. After standing overnight at room temperature, the solution was diluted with 5 ml. of methanol and dried *in vacuo*. The residue was extracted with two 50-ml. portions of ether. The ether was washed with two 10-ml. portions of 5% sodium carbonate and four 10-ml. portions of water and dried over 5 g. of anhydrous sodium sulfate. After removal of the solvent 40.5 mg. of an oily residue was crystallized from 0.5 ml. methanol, m.p. 135–137°, $[\alpha]^{23}D$ +148° (c 0.580 in chloroform). Infrared studies confirmed the structure of X.[8]

This evidence provides unambiguous proof that IV is 11α,-17α,21-trihydroxypregnane-3,20-dione.

C. Transformation of 17α,21-Dihydroxypregnane-3,20-dione (IX) by *Rhizopus Nigricans*.—Three hundred milligrams of IX was added in 50 ml. of ethanol to a 24-hour

(9) All melting points are uncorrected and were taken on a Fisher-Johns apparatus.

growth of *Rhizopus nigricans* in 2 l. of medium H.[3] After a transformation period of 39 hours, the steroid was extracted in the usual manner with methylene chloride. This extract, yielding 2.112 g. of solids, was triturated successively with 30-ml. and 10-ml. portions of ether. After decantation of the solvent, the crystalline residue (189.5 mg.) was dissolved in 5 ml. of methanol and stirred with about 50 mg. of charcoal (Nuchar). The charcoal was removed by filtration and the alcohol concentrated to about 2 ml. Upon the addition of a few drops of ether, crystals formed and 89.5 mg., m.p. 216–222°, was recovered. Recrystallization as before, yielded 36.5 mg., m.p. 218–222°, $[\alpha]^{23}D$ +57° (*c* 0.922 in chloroform).

Anal. Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.41, 69.87; H, 9.39, 8.86.

This product as well as compound IV isolated from the fermentation of Reichstein's Compound S with *Rhizopus nigricans* gave identical infrared spectra showing hydroxyl groups at 3575 and 3520 $cm.^{-1}$ and ketone at 1708 $cm.^{-1}$

B. Transformation of Reichstein's Compound S by *Rhizopus arrhizus*. 6β,17α,21-Trihydroxy-4-pregnene-3,20-dione (III) by Direct Crystallization.—To 6 l. of a 24-hour growth of *Rhizopus arrhizus* was added 1 g. of Reichstein's Compound S. The usual extraction with methylene dichloride yielded 1.834 g. of semicrystalline solids which were washed with three 5-ml. portions of ice-cold methylene chloride. The recovered crystals weighed 525 mg., m.p. 198–207°. Recrystallization from 13 ml. of methanol and 1 ml. of water produced 305 mg. of crystals, m.p. 228–232°. Recrystallization from methanol of 35 mg. yielded 26 mg. of crystals (III), m.p. 230–233°, $[\alpha]^{25}D$ +58.5° (*c* 0.894 in ethanol). Infrared, microcombustions and acetylation indicated the addition of one hydroxyl group to the substrate.

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.49; H, 8.31.

4-Androstene-3,6,17-trione (XI).—Two hundred mg. of III was dissolved in 10 ml. of glacial acetic acid. After cooling the solution to 5–10° a total of 185 mg. of chromium trioxide in 20 ml. of glacial acetic acid was added in 5-ml. portions every half hour with constant stirring. The solution was allowed to set for three days and then evaporated to dryness at room temperature. The oxidation mixture was transferred to a separatory funnel with three 10-ml. portions of ether and three 5-ml. portions of water. The ether layer was recovered and the aqueous layer extracted twice more with 25-ml. portions of ether. The combined ether extracts were washed twice successively with 25-ml. portions of 10% sulfuric acid, water, 7% sodium bicarbonate and water. The ether extract, after evaporation, yielded 98.6 mg. of long yellow needles. Recrystallization twice from 1.2 ml. of ethanol gave 24.4 mg. of XI, m.p. 220–225°, $[\alpha]^{25}D$ +32.3° (*c* 1.022 g. in acetone). The various physical constants for XI were identical with those of 4-androstene-3,6,17-trione.[7]

6β,17α-Dihydroxy-21-acetoxy-4-pregnene-3,20-dione (XII).—Acetylation to the 21-monoacetate (XII) was accomplished by dissolving 560 mg. of III in a mixture of 5.52 ml. of pyridine–acetic anhydride in which 1 ml. of pyridine contains 30 mg. of acetic anhydride (165.6 mg. of acetic anhydride; theoretical for one hydroxyl group 157.8 mg.). The suspension was warmed slightly to dissolve the crystals. This solution was maintained at room temperature for 18 hours and diluted with 50 ml. of water. A small amount of yellow amorphous material formed and was removed by filtration. Another 125 ml. of water was mixed with the filtrate and a colorless crystalline precipitate appeared. The crystals, 293 mg. of XII, m.p. 254–256°, were recovered by filtration. Recrystallization from 10 ml. of chloroform–acetone 1:1 did not raise the melting point above 258–260°, $[\alpha]^{23}D$ +74° (*c* 0.9675 in dioxane), $\lambda_{max}^{alc.}$ 237 mμ (*E* 14,100).

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.28; H, 8.19.

17α-Hydroxy-6β,21-diacetoxy-4-pregnene-3,20-dione (XIII).—Fifty-one mg. of III was dissolved in 4 ml. of acetic anhydride and 2 ml. of pyridine. The solution was warmed for a short time on the steam-bath in order to dissolve the crystals completely. After 15 hours at room temperature, the mixture was diluted with 30 ml. of ice-water and extracted with two 30-ml. portions of a 5:1 mixture of ether:methylene chloride. The combined extracts were washed with two 10-ml. portions of 2 *N* hydrochloric acid, one 10-ml. portion of water, three 10-ml. portions of 7% sodium bicarbonate, three 10-ml. portions of water and the solvents dried over anhydrous sodium sulfate. After removal of the solvents *in vacuo* 58.5 mg. of a crystalline residue remained. Recrystallization twice from 5 ml. of acetone by dropwise addition of ether yielded 34 mg. of XIII, m.p. 192–195°, $[\alpha]^{23}D$ +63° (*c* 0.9852 in chloroform).

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.67. Found: C, 67.30; H, 7.68.

Acknowledgment.—We are grateful to Dr. M. Ehrenstein, of the University of Pennsylvania, for supplying us with an authentic sample of 4-androstene-3,6,17-trione. We wish to thank various members of the Upjohn Company for their coöperation and assistance on various phases of our problems: *viz.*, Dr. J. L. Johnson, Mr. L. Scholten and Mrs. G. S. Fonken for ultraviolet and infrared analyses; Dr. W. P. Schneider for preparation of the allo and normal isomers of dihydro-epi F; Mr. W. A. Struck and his associates for rotations and microanalyses; and Misses Jennie I. Mejeur, Irene N. Pratt, Terrie Bovee and Mr. Glenn Staffen for technical assistance. We are also indebted to Drs. R. H. Levin and D. I. Weisblat for their stimulating interest in this work.

KALAMAZOO, MICHIGAN