(b) **For the Rearrangement of 2-Phenyl-2-(α-naphthyl)-ethanol-1-C^{14} (IV).**—Using 0.868 μc. as the average molar radioactivity of α-naphthoic-*carboxy*-C^{14} acid, and 1.66 μc. as the average molar radioactivity of IV (as determined for phenyl-(α-naphthyl)-acetonitrile-1-C^{11} and for 2-phenyl-2-(α-naphthyl)-acetic-1-C^{14}), (0.868 ÷ 1.66) × 100 = 52.3% α-naphthyl migration (and 47.7% phenyl migration).

Acknowledgment.—The authors are indebted to Drs. J. D. Roberts and W. M. Lauer for many helpful suggestions during the course of this research.

OAK RIDGE, TENNESSEE

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY]

Microbiological Transformations of Steroids. III.[1] Preparation of 11-Epi-corticosterone and of 6β-Hydroxy-11-desoxycorticosterone

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

RECEIVED SEPTEMBER 29, 1952

11-Desoxycorticosterone (or its acetate) is converted to 11-epicorticosterone by the mold *Rhizopus nigricans*. 11-Desoxycorticosterone (or its acetate) is converted to 6β-hydroxy-11-desoxycorticosterone by *Rhizopus arrhizus*.

Discussion

With our discovery that molds of the genus *Rhizopus* (order of *Mucorales*) can oxygenate progesterone to hydroxylated steroids and especially to 11α-hydroxyprogesterone,[2] a systematic study was made of the ability of these organisms to introduce oxygen into various steroids. This paper reports on the bioöxygenation of 11-desoxycorticosterone and its acetate by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b) and *Rhizopus arrhizus* Fischer (A.T.C.C. 11145).

Our general procedures and techniques have been reported in the preceding publications. Medium H at a *p*H of 4.5–5.0 was employed together with a growth to conversion cycle of usually 24 hr./24 hr. A concentration of 11-desoxycorticosterone acetate (I) of 0.25 g./liter of medium was optimal under the fermentation conditions used, whereas somewhat lower concentrations of 11-desoxycorticosterone, free alcohol, (II) were employed because of its greater inhibitory effect upon the course of the bioconversion. Since the fermentations with these two steroid substrates are otherwise identical, only the experiments on I with the two species of *Rhizopus* will be reported in detail. The major product of the bioöxygenation of I or II by *Rhizopus nigricans* can be isolated most conveniently from the steroid-containing solvent-free extractives in one of several ways—direct crystallization by addition of ether, crystallization from ethyl acetate after removal of extraneous oily material with *n*-hexane, or column chromatography over Florisil.[3] The major bioöxygenation product of I or II by *Rhizopus arrhizus* can be obtained by column chromatography over Florisil.

Under our conditions *Rhizopus nigricans* and *Rhizopus arrhizus* convert I or II completely in a 24-hour period. With *Rhizopus nigricans* one main component (III) is formed which by paper chromatography[2b,2c] has a mobility slower than that of corticosterone. A small amount of a material (IV) more polar than III is invariably formed (about 3% by paper chromatographic estimation). Its chemistry will be discussed at a future date.

By selective acetylation of III the C-21 acetate (V) was formed. Reaction with two equivalents of acetic anhydride yielded the diacetate (VII). Oxidation of V with chromic anhydride yielded a compound (VI) identical to 11-dehydrocorticosterone-21-acetate (compound A acetate) by the following physical criteria: melting point, mixed melting point, optical rotation, infrared, paper chromatography and elementary analysis. An authentic sample of corticosterone was carried through the same reactions. A comparison of the constants of the two preparations with those found in the literature for compound A acetate is given in Table I.

TABLE I

COMPARISON OF CONSTANTS OF COMPOUND A ACETATE DERIVED FROM EPICORTICOSTERONE AND CORTICOSTERONE

	VIa (from III)	VIb (from cortico-sterone)	VI (litera-ture)[4]
M.p., °C.	179–181	179–181	179–181
Mixed m.p., °C.	179–181		
$[\alpha]_D$ (dioxane)	239°	235°	234°

Infrared spectra identical for VIa and VIb.

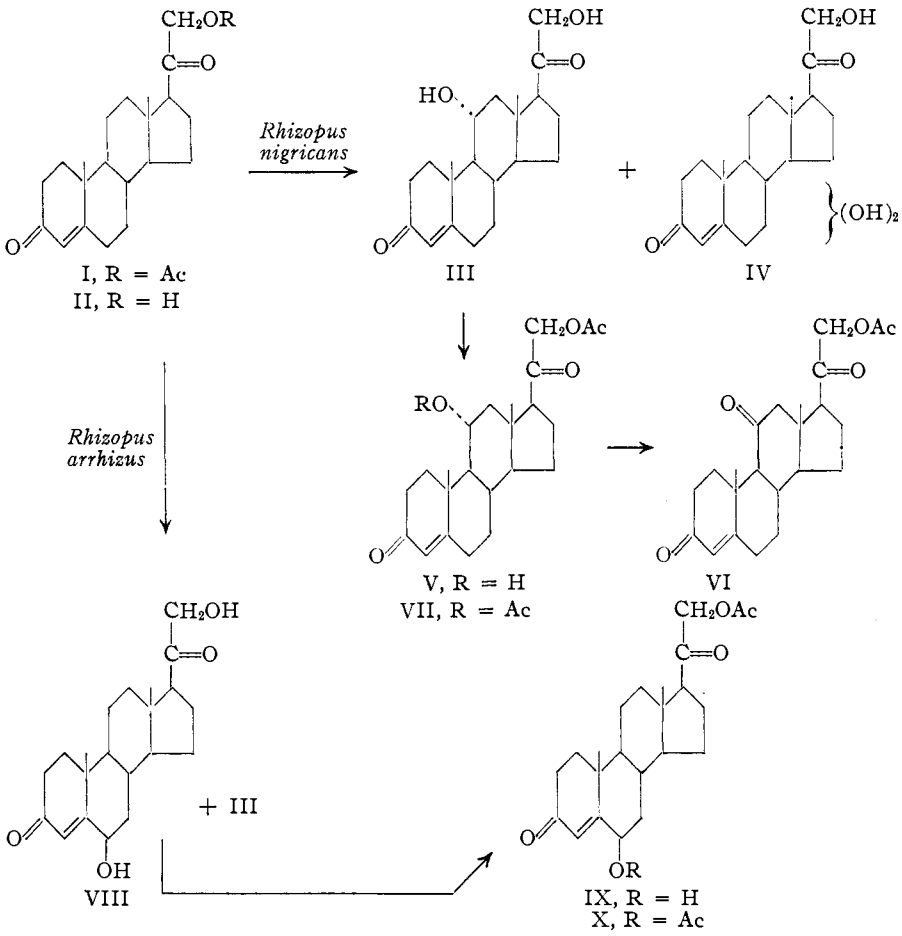
This sequence of reactions establishes III as 11-epicorticosterone or 11α,21-dihydroxy-4-pregnene-3,20-dione (since by paper chromatography and other physical properties as well as by its ability to form a diacetate readily, III is different from corticosterone).

(1) Paper II by P. D. Meister, D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub and H. Marian Leigh, THIS JOURNAL, **75**, 55 (1953).

(2) (a) D. H. Peterson and H. C. Murray, *ibid.*, **74**, 1871 (1952); (b) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. Marian Leigh, *ibid.*, **74**, 5933 (1952); (c) U. S. Patent 2,602,769, filed Feb. 23, 1952, issued July 8, 1952, based on an original application filed Aug. 19, 1950. The bioöxygenations described in detail in the present communication were disclosed in this patent. In THIS JOURNAL, **74**, 3962 (1952), J. Fried, *et al.*, *via* a Communication to the Editor describe the bioöxygenation of desoxycorticosterone to 11-epicorticosterone by *Aspergillus niger*.

(3) A synthetic magnesia-silica gel made by the Floridin Co., Warren, Pa.

(4) T. Reichstein and C. W. Shoppee, *Vitamins and Hormones*, **I**, 363 (1945).



When Shoppee and Reichstein[5] treated corticosterone acetate with hydrochloric–acetic acid (1:9) they recovered, beside dehydration products, an alcoholic substance which they considered to be 11-epicorticosterone acetate. This assignment of structure has been seriously questioned by Gallagher.[6] Our work shows that Shoppee and Reichstein were in error.

TABLE II

MOLECULAR ROTATION DIFFERENCES FOR 11-EPIMERS

	$[\alpha]_D$	M_D	ΔM_D
11α-Hydroxyprogesterone	+179° ($CHCl_3$)	+591°	+143°
11β-Hydroxyprogesterone	+222.5 ($CHCl_3$)	+734°	
11-Epi-F	+113 (MeOH)	+409°	+197°[a]
Compound F (Kendall)	+167 (EtOH)	+606°	
11-Epicorticosterone	+165 (EtOH)	+571°	+201°
Corticosterone	+223 (EtOH)	+772°	
11-Epicorticosterone acetate (This paper)	+159 (Acet)[b]	+618°	+139°
11-Epicorticosterone acetate (Shoppee and Reichstein)	+189 (Acet)[b]	+726°	
Corticosterone acetate	+195 (Acet)[b]	+757°	+ 31°

[a] The solvent effect of methanol was assumed to be negligible. [b] Acet = acetone.

The molecular rotation data (Table II) strengthen the contention of Gallagher[6] that the $\Delta M_D^{11\beta-11\alpha}$ of the substance of Shoppee and Reichstein was too small to allow the substance to be 11-epicorticosterone acetate. Our compound has a melting point of 165.5–166.5° in contrast to that of the disputed material of 122–125°. Indeed, if epimerization should occur under the Shoppee and Reichstein reaction conditions, it is doubtful that a free 11-hydroxy compound could be isolated. The well-known chemical reactivity of the 11α-hydroxyl group can be illustrated by the following experiment. On subjecting 11α-hydroxyprogesterone to the hydrochloric acid–acetic acid dehydration conditions employed by Shoppee and Reichstein we isolated 11α-*acetoxy*progesterone in good yield. Only about 20% of the 11α-hydroxyprogesterone had not reacted.[7] Inasmuch as Shoppee and Reichstein re-acetylated (acetic anhydride in pyridine) their reaction products, the isolation of the 11α-*hydroxy*steroid is virtually precluded.

Compound III is practically the sole steroid formed in this biological transformation with *Rhizopus nigricans*. Steroid yields of about 50 to 60% of crystalline product are obtained and the total steroid balance is 60–75% (by paper chromatography). If the developed chromatogram of the original crude fermentation extract is treated with 2,4-dinitrophenylhydrazine or with modified Tollens reagent,[2b] no components are detected other than those shown by ultraviolet absorption. Hence no appreciable amount of reduction in ring A or destruction of the ketol side chain occurs.

When the bioöxygenation of I or II is similarly carried out with *Rhizopus arrhizus*, one major transformation product (VIII) is formed which has a mobility, by paper chromatography, just slower than that of Reichstein's Compound S. Several minor transformation products are detectable by this technique, one of which has a mobility identical to that of III. The infrared spectrum of the isolated VIII was identical to that of 6β-hydroxy-11-desoxycorticosterone. The melting point and optical rotation of this material showed rather large discrepancies with the values reported by others.[8,9] Indeed the values in the literature are also at variance with each other. These differences are indicated below (Table III).

(5) C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **26**, 1316 (1943).

(6) L. F. Fieser and Mary Fieser, "Natural Products Related to Phenanthrene," 3rd Edition, Reinhold Publ. Corp., New York, N. Y., p. 409.

(7) Unpublished data.

(8) P. T. Herzig and M. Ehrenstein, *J. Org. Chem.*, **16**, 1050 (1951).

(9) W. J. Haines, "Recent Progress in Hormone Research," Vol. VII, Chap. III, 1952, p. 282.

TABLE III

PHYSICAL CONSTANTS FOR 6β-HYDROXY-11-DESOXYCORTICOSTERONE AND DERIVATIVES

	M.p., °C.	$[\alpha]_D$	E	Infrared
6β-Hydroxy-11-desoxycorticosterone (VIII)				
Herzig and Ehrenstein[8]	190–192	+ 62.6 ($CHCl_3$)	13,730	Identical[a]
Haines[9]	181–183	+110 (EtOH)	14,500	Identical
This paper	198–202	+101 ($CHCl_3$)	13,700	Identical
6β,21-Diacetoxy-4-pregnene-3,20-dione (X)				
Herzig and Ehrenstein[8]	125–127	+109.5 ($CHCl_3$)	15,950	Identical
This paper	127–129	+103 ($CHCl_3$)	13,150	
6β-Hydroxy-21-acetoxy-4-pregnene-3,20-dione (IX)				
This paper	196–198	+113 ($CHCl_3$)	13,900	

[a] Compared as the diacetates.[9]

Isolation of the minor transformation product having the mobility of III allowed the infrared identification of this transformation product as being, in fact, 11-epicorticosterone.

The specificity of the enzyme systems involved in these microbiological oxygenations and the profound effects of substituents in the steroid nucleus used as substrate are worthy of special notice. Thus with progesterone as a substrate *Rhizopus arrhizus* forms 11α-hydroxyprogesterone in fair yields (up to 50% as crystals) and in addition forms appreciable amounts of 6β-11α-dihydroxyprogesterone (up to 15% yield of crystals): *Rhizopus nigricans*, however, forms the 11α-hydroxyprogesterone in essentially quantitative yields.[2b] With 11-desoxycorticosterone (or its acetate) *Rhizopus arrhizus* forms predominantly 6β-hydroxy-11-desoxycorticosterone and only small amounts of 11-epicorticosterone, whereas *Rhizopus nigricans* produces essentially the latter compound; none of the 6β-hydroxy compound has been detected. Other cases of the influence of the steroid molecule on the course of the fermentation will be reported in future communications.[10] Perhaps the qualitative and quantitative differences herein described are the result of competition of different enzymes, one steroid structure being more suitable for a given enzyme. It is also possible that the substituents affect the spatial relations of the enzyme–substrate complex so that different positions of the steroid nucleus are activated. If this is the case, then one must postulate that considerable difference exists in the structures of the enzymes in question in these two species of *Rhizopus*.

Another characteristic of the *Rhizopus* molds is the presence of very active esterase. In general these organisms hydrolyze the 21-acetates with sufficient rapidity that only *non-esterified* steroids are found at the end of the fermentations. Presumably the oxygenating enzymes acted on the free steroid alcohol. Thus, if the fermentation with a 21-acetate is interrupted long before completion of the steroid oxygenation one finds non-acetylated oxygenated steroid, the free alcohol of the steroid substrate and traces of the original substrate (ester); never has oxygenated steroid *ester* been found.

(10) The interesting transformation of 16-dehydroprogesterone[1] should be recalled in this connection.

An error on the specific rotation of 11-ketoprogesterone appeared in our first paper in this series: the rotation of +227° as given was done in acetone, not chloroform, in which the rotation is 275°.

Experimental

A. Fermentation of 11-Desoxycorticosterone Acetate (21-Acetoxy-4-pregnene-3,20-dione) (I) by *Rhizopus nigricans* and Isolation of 11-Epicorticosterone (11α,21-Dihydroxy-4-pregnene-3,20-dione (III).—This fermentation was carried out in the stir bottle assembly.[2b] The concentration of I used was 3 g. in 12 liters of medium. The fermentation–conversion cycle was 24 hr./30 hr. Paper chromatography of crude extract showed a trace of II, a large amount of III and a very small amount of IV. The weight of the extract was 4.66 g. This was fractionated over 240 g. of Florisil with 390-ml. fractions of solvents as follows: ethylene dichloride (8 fractions); ethylene dichloride–acetone mixtures 25:1, 15:1, 12:1 and 10:1 (2 fractions in each instance); ethylene dichloride–acetone 8:1 (3 fractions); ethylene dichloride–acetone 5:1 (4 fractions); ethylene dichloride–acetone 2:1 (3 fractions); acetone (4 fractions). The fractions were analyzed by paper chromatography. Compound II appeared in a broad peak centering about fraction 8. Compound IV was present in fractions 21–23.

Fractions 14–20 (2.06 g.) were combined and crystallized from 15 ml. of ethyl acetate to give 1.46 g. of nicely crystalline product, m.p. 150–159°.[11] This is a 65% yield (taking into account the loss of the 21-acetate). Paper chromatography showed only III with a trace of IV. Recrystallization from ethyl acetate yielded pure III, m.p. 153–155°, $[\alpha]^{24}_D$ +166° (*c* 0.764 in chloroform) and +165° (*c* 0.711 in 95% ethanol).

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.88; H, 8.69.

The infrared spectrum was different from that of the then known steroids and indicated the addition of probably one new hydroxyl group. This was verified by the elementary analysis.

Fraction 21, mainly III with about 4% of IV, was crystallized from 2 ml. of ethyl acetate to yield 135 mg. of crystals, m.p. 140–155°, which by paper chromatographic analysis consisted of III with about 9% of IV. This brings the steroid balance of crystalline material to about 70% with some remaining in the mother liquors.

Formation of C-21 Monoacetate (11α-Hydroxy-21-acetoxy-4-pregnene-3,20-dione) (V) from III.—To 101.2 mg. of III in 2 ml. of dry pyridine was added 1 ml. of a solution of 0.304 ml. of redistilled acetic anhydride diluted to 10 ml. with pyridine. After 16 hours at room temperature, the reaction mixture was diluted with 50 ml. of water and, after an hour at room temperature, was refrigerated. Crystallization did not occur. The aqueous solution was five times extracted with 25-ml. portions of ether, the combined ether fractions washed with 2% hydrochloric acid until the washings were acid, then twice with 2% bicarbonate solution and once with water. The extract was dried with anhydrous sodium sulfate and the solvent allowed to evaporate at room temperature leaving 106.5 mg. of a colorless oil. This was fractionated over 5.3 g. of alumina (acid washed, activated at 120°) with 10-ml. portions of solvents in the following manner: ether–chloroform 1:1 (3 fractions); chloroform (3 fractions); chloroform–acetone 19:1 (2 fractions). Fraction 5, the second chloroform fraction, showed a very sharp peak representing a 62% yield of product V. Attempts to crystallize this material failed and so it was used as such for the subsequent oxidation with chromic anhydride.

Fraction 6 (6.4 mg.) was saved. This crystallized spontaneously during the course of 6 months. When it was recrystallized from 0.2 ml. of methanol at −5° and washed three times with 0.3 ml. of cold methanol (−5°) the product weighed 3 mg. and melted 159–163°. Infrared indicated the presence of the 21-acetoxy, the 11α-hydroxy and the 3-keto-Δ^4-configuration. Purified 21-acetoxy-11-epicorticosterone of identical infrared spectrum prepared in a subsequent experiment melted at 163–165°, and had an $[\alpha]^{23}_D$ of +159° (*c* 0.7324 in acetone), and of +168° (*c* 0.588 in chloroform).

(11) All melting points reported in this paper were taken on a Fisher-Johns block and are uncorrected.

Oxidation of V to Compound A Acetate (21-Acetoxy-4-pregnene-3,11,20-trione).—The 70.5 mg. of fraction 5 was dissolved in 1.4 ml. of chlorobenzene, cooled in an ice-bath and stirred for 2 hours with a solution of 53 mg. of sodium dichromate in 0.8 ml. of water and 0.135 ml. of sulfuric acid. Then 10 ml. of cold water was added and sufficient benzene to bring the organic phase to the top. This was separated and washed (twice) with cold water until colorless, twice with 5% sodium bicarbonate (5-ml. portions) and again with water. The benzene solution was dried with anhydrous sodium sulfate and the solvent allowed to evaporate spontaneously to give a white crystalline residue, weighing 50.2 mg. (71.4% yield), m.p. 177–180°. This was recrystallized from 1 ml. of methanol in the cold and washed twice with cold methanol (0.5-ml. portions) to give VIa, m.p. 179–181°, $[\alpha]^{23}D$ +239° (*c* 1.5 in dioxane).

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.47; H, 7.83. Found: C, 71.47, 71.60; H, 7.94, 7.90.

Fifty mg. of an authentic sample of corticosterone, m.p. 177.5–180° $[\alpha]D$ +220° (ethanol) (identity by infrared) was carried through the same series of reactions to give VIb of identical melting point, $[\alpha]^{24}D$ +235° (*c* 0.988 in dioxane).

Anal. Found: C, 71.28; H, 7.84.

The mixed melting point of the two preparations showed no depression, and the infrared spectra were identical. Since III was not corticosterone this sequence of reactions establishes it as 11α,21-dihydroxy-4-pregnene-3,20-dione or 11-epicorticosterone.

11α,21-Diacetoxy-4-pregnene-3,20-dione (VII) **from III.**—To 50 mg. of III, dissolved in 1 ml. of dry pyridine, was added 4 ml. of a solution of 0.304 ml. of acetic anhydride diluted to 10 ml. with pyridine (fourfold excess). The work-up was similar to that for the monoacetate (V). The non-crystalline product was partitioned over 3.5 g. of alumina (acid washed and activated at 120°) with 7-ml. portions of solvent as follows: ether (2 fractions); ether–chloroform 19:1 (2 fractions); ether–chloroform 9:1 (2 fractions); ether–chloroform 1:1 (2 fractions); chloroform (2 fractions). Fractions 7 and 8 (ether–chloroform 1:1) showed a sharp peak and were combined (39.7 mg.). First attempts to crystallize this material failed. As in the case of V it crystallized spontaneously during the course of six months standing. It was recrystallized from 0.3 ml. of methanol at −5° and washed with 0.5-ml. portions of cold methanol. The recrystallized product (long needles) weighed 21.3 mg., m.p. 145–149°, $[\alpha]^{23}D$ +152° (*c* 0.979 in chloroform).

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.46; H, 8.32.

Infrared indicated no hydroxyl and the presence of two acetoxy groups.

It is interesting to note that the acetates of 11-epicorticosterone crystallize with difficulty in contrast to the 21-acetate of corticosterone.[12]

B. Isolation of III by Direct Crystallization (*Rhizopus nigricans* Fermentation).—The oxygenation of 60 g. of I was carried out in a 100-gallon fermenter containing 240 liters of medium. The growth/conversion cycle was 24 hr./24 hr.

The tarry, steroid-containing, solvent-free extractives were dissolved in 180 ml. of methylene chloride and 900 ml. of ether added. The oily precipitate crystallized on refrigeration for 24 hours. The brown crystals were separated by decantation and washed with 200-ml. portions of ether until no more appreciable color was extracted. The yield of crude product was 24.4 g. This material was decolorized in methylene chloride solution with 20 g. of Magnesol,[2b,2c] evaporated to dryness and crystallized from 300 ml. of ethyl acetate to yield 15.32 g. of III, m.p. 156–162°. A second crop of crystals from the ethyl acetate mother liquor weighed 2.74 g., m.p. 156–161°. The total yield of purified III isolated by direct crystallization was 18.06 g. or 32.4% (based on the amount of II equivalent to the I used). The mother liquors of the direct ether precipitation contained some III (paper chromatography) which could only be purified by column chromatography.

C. Bioöxygenation of I by *Rhizopus arrhizus* with Isolation of 6β-Hydroxy-11-desoxycorticosterone and 11-Epicorticosterone.—A stir bottle assembly of 12 liters of medium was employed under fermentation conditions similar to those of section A, above. Three grams of I in 100 ml. of acetone was added in a 24 hr./24 hr. cycle. The solvent-free steroidal extractives weighed 5.727 g. Paper chromatography indicated the formation of a large amount of a new compound (VIII) with small amounts of several other compounds, one of which could not be distinguished from III by this technique.

The extractives were dissolved in 200 ml. of ethylene dichloride and chromatographed over 300 g. of Florisil with 600-ml. fractions of solvent as follows: ethylene dichloride, ethylene dichloride–acetone 25:1 and 15:1 (2 fractions in each instance); ethylene dichloride–acetone 12:1, 10:1, 8:1 and 5:1 (3 fractions in each instance); ethylene dichloride–acetone 3:1 (2 fractions); acetone (1 fraction). Fractions 10–16 (10.1 and 8:1 solvent mixtures) contained compound VIII. These fractions were individually triturated with 10 ml. of ether–acetone 3:1 and the solution decanted and discarded. The crystalline residues were dissolved in acetone–chloroform 1:1. The combined solutions were concentrated to a thin sirup and diluted with 10 volumes of ether to give 501 mg. of crystals, m.p. 198–205°. Recrystallization from 5 ml. of methanol yielded 396 mg. melting at 205–210°. An aliquot was again recrystallized from methanol to yield crystals melting 206–210°. Infrared spectroscopy confirmed that this material was 6β-hydroxy-11-desoxycorticosterone (VIII). The crystalline compound was solvated with one equivalent of methanol which was retained on drying at 60° (0.01 mm.). *Anal.* Calcd. for $C_{21}H_{30}O_4 \cdot CH_3OH$: C, 69.81; H, 9.05. Found: C, 70.26; H, 8.64; $[\alpha]^{23}D$ +97° (*c*, 0.859 chloroform). Drying for 15 hours over phosphorus pentoxide (0.01 mm.) yielded the anhydrous compound, m.p. 198–202°, $[\alpha]^{23}D$ +101° (*c* 0.9134 in chloroform).

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.96; H, 8.59.

6β-Hydroxy-11-desoxycorticosterone Acetate (6β-Hydroxy-21-acetoxy-4-pregnene-3,20-dione) (IX).—Two hundred fifty mg. of VIII was dissolved in 2.6 ml. of a pyridine–acetic anhydride mixture (1 ml. of this solution contained 30 mg. of acetic anhydride. One equivalent was calculated at 73.8 mg.; the amount used was 78 mg.). After 18 hours at room temperature the reaction mixture was cooled with ice and diluted with ice water to give a crystalline precipitate which was filtered off. The crystals (164 mg., m.p. 184–188°) were recrystallized from 2 ml. of acetone. After an additional acetone recrystallization the compound melted at 196–198°.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.12; H. 8.36; $\lambda_{max}^{alc.}$ 237 mμ (*E* 13,900), $[\alpha]^{23}D$ +113° (*c* 1.185 in chloroform).

The aqueous filtrate (above) was extracted twice with ether (50 ml.). The ether extracts were washed once with 10 ml. of 5% hydrochloric acid, twice with 10-ml. volumes of 5% sodium carbonate and four times with 10-ml. volumes of water. After drying over sodium sulfate and concentration, an additional crop of 103.5 mg. of crystals, m.p. 160–173°, was obtained.

6β-Hydroxy-11-desoxycorticosterone Diacetate (6β,21-Diacetoxy-4-pregnene-3,20-one) (X).—Forty-seven mg. of VIII was dissolved in 3 ml. of pyridine and 2 ml. of acetic anhydride. After standing at room temperature overnight the solution was diluted portionwise with ice-water. Some amorphous gray material which precipitated at the beginning of the dilution process was separated mechanically. On further dilution a milky amorphous suspension was obtained. This was extracted twice with 50-ml. portions of ether. The ether extracts were washed with hydrochloric acid, sodium carbonate solution and water and dried in the same manner as described for the monoacetate (above). Evaporation of the solvent yielded an oily residue of 61.2 mg. which could be crystallized from a few drops of ethyl acetate. The crystals were dissolved in a few drops of ethyl acetate, a like amount of ether was added and the mixture was refrigerated overnight. The resulting crystals melted at 125–127°. Recrystallization from ethyl acetate–ether once more yielded 19 mg. of X, m.p. 127–129°, $[\alpha]^{23}D$ +103° (*c* 0.610 in chloroform) $\lambda_{max}^{alc.}$ 236 mμ (*E* 13,150). The infrared spectrogram was identical to that of Ehrenstein's diacetate.

(12) T. F. Gallagher, "Recent Progress in Hormone Research, I," Chap. 4, p. 95, reported the chemical synthesis of the 11,21-diacetate but obtained only amorphous product.

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.