Acknowledgments.—The 6-dehydroprogesterone was kindly furnished by Dr. R. A. Donia of our laboratories. We wish to thank Dr. J. L. Johnson, Mr. L. Scholten and Mrs. G. S. Fonken for infrared and ultraviolet analyses, Mr. W. A. Struck and his associates for all optical rotations and microanalyses, Miss Jennie Mejeur, Miss Irene N. Pratt, and Mr. Glenn Staffen for technical assistance. We are also grateful for the helpful suggestions and interest of Drs. R. H. Levin and D. I. Weisblat.

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY]

Microbiological Transformations of Steroids. VII. Preparation of 11α-Hydroxypregnane-3,20-dione and 11α-Hydroxyallopregnane-3,20-dione[1]

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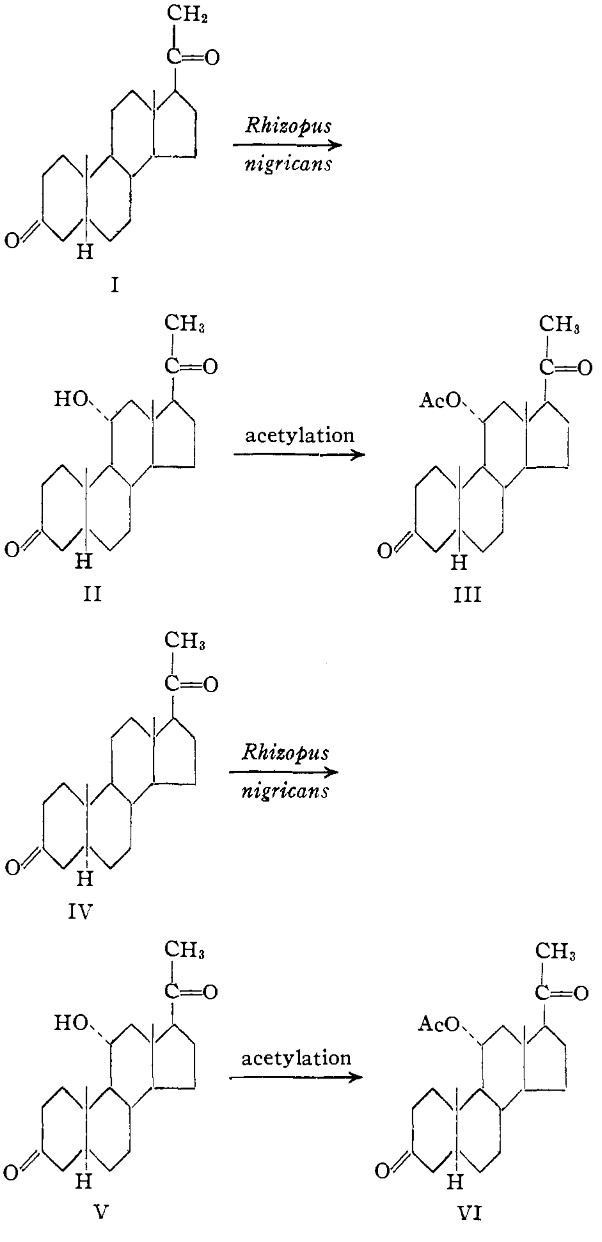
A one-step process is described for the microbiological conversion of pregnane-3,20-dione and allopregnane-3,20-dione to the corresponding 11α-hydroxy derivatives by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b).

Discussion

Previous papers of this series have described the microbiological transformation of C-21 3-keto-Δ^4-steroids to C-11 and C-6 oxygenated products by fungi of the genus *Rhizopus*. It then became of interest to expand our studies to include C-21 steroidal substrates where ring A is saturated. This paper therefore reports the successful bioconversion of pregnane-3,20-dione (I) and allopregnane-3,20-dione (IV), to the respective 11α-hydroxy derivatives. Methods of fermentation and extraction have been previously described.[2] Compounds I and IV were fermented with 24-hour growths of *Rhizopus nigricans* for 24 hours. At the end of this period the methylene chloride extracts were examined by paper chromatography. It was found that I had been completely transformed to one different compound and IV was also converted to a component in 40% yield and a minor amount of a more highly polar substance. In the latter case approximately 60% of the substrate was not utilized under the conditions employed. Isolation using alumina chromatography and crystallization procedures yielded 40% of pure 11α-hydroxypregnane-3,20-dione (II) and about 25% of pure 11α-hydroxyallopregnane-3,20-dione (V). Comparison of II and V with authentic samples prepared by us previously,[1a] established the structures.

Experimental

11α-Hydroxypregnane-3,20-dione (II).—To a 24-hour growth of *Rhizopus nigricans* was added 1.0 g. of pregnane-3,20-dione (I). After a 24-hour transformation period the methylene chloride extract was concentrated and shown to contain one main component whose mobility was identical to II. The semi-crystalline solids, amounting to 1.99 g., were chromatographed over 50 g. of alumina.[2] The main transformation product was located by means of papergram


I II III IV V VI

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[1] (a) Paper VI in this series: D. H. Peterson, A. H. Nathan, P. D. Meister, S. H. Eppstein, H. C. Murray, A. Weintraub, L. M. Reineke and H. Marian Leigh, THIS JOURNAL, **75**, 419 (1953); (b) D. H. Peterson and H. C. Murray, *ibid.*, **74**, 1871 (1952); (c) the bioconversions reported in detail in this paper were disclosed in our U. S. Patent 2,602,769 issued July 8, 1952, based on an original application filed August 19, 1950.

[2] D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. Marian Leigh, THIS JOURNAL, **74**, 5933 (1952).

studies in the ether–chloroform eluates. Evaporation of the solvent yielded 1.00 g. of a fraction which was recrystallized from 1 ml. of ethyl acetate and 10 ml. of methylcyclohexane to give 423 mg. of II, m.p. 102–106°, $[\alpha]^{26}\text{D}$ +83° (*c* 1.213 in chloroform).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.99; H, 9.90.

These physical constants together with infrared studies showed II to be identical with authentic 11α-hydroxypregnane-3,20-dione.[1a]

11α-Acetoxypregnane-3,20-dione (III).—Compound II (70.5 mg.) in pyridine was acetylated at room temperature with acetic anhydride and gave III, m.p. 152–153° after several recrystallizations from ether–Skellysolve B, $[\alpha]^{23}\text{D}$ +63° (*c* 0.800 in chloroform).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15; acetyl, 11.50. Found: C, 73.93; H, 9.32; acetyl, 11.60.

11α-Hydroxyallopregnane-3,20-dione (V).—To a 24-hour growth of *Rhizopus nigricans*, 0.5 g. of allopregnane-3,20-dione (IV) was added. Following a 24-hour conversion period, the steroids were extracted in the usual manner.[2] An aliquot, examined by papergram studies, indicated that approximately 40% of a compound was produced, whose mobility was identical to V and a small amount of a more highly polar compound. In addition about 60% of the substrate was unconverted.

The extract, 1.45 g. was dissolved in 50 ml. of benzene and chromatographed over 25 g. of alumina as previously described.[2] The components were located in the various eluates by means of papergram studies. The benzene–ether, ether, ether–chloroform, chloroform, chloroform–acetone and acetone fractions were combined and dissolved in 3 ml. of hot ethyl acetate. After refrigeration, 270 mg. of crystals obtained were decolorized with Magnesol[2] in methylene chloride solution. After solvent removal, crystallization from ethyl acetate was repeated to yield a product, m.p. 140–160°. Infrared analyses showed this material to be a mixture of 60% 11α-hydroxyallopregnane-3,20-dione (V) and 40% L-leucyl-L-proline anhydride[3] separable by fractional sublimation or chromatography over carbon.

A 99-mg. sample of crystals, as obtained from the above preceding chromatographic separation, was dissolved in 8 ml. of methanol and chromatographed over a mixture of 9 g. of carbon (Darco G-60) and 9 g. of diatomaceous earth (Celite #545). The column was developed with five 72-ml. portions of methanol and seven 72-ml. portions of methylene chloride. The first four eluates yielded 29 mg. of L-leucyl-L-proline anhydride analyzed by infrared. The methylene chloride eluates gave a fraction weighing 82.8 mg. which upon recrystallization from 1 ml. of ethyl acetate, resulted in 50.9 mg. of V, m.p. 197–200°, $[\alpha]^{23}\text{D}$ +82° (*c* 1.316 in chloroform).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.00; H, 9.48.

These physical constants together with infrared analyses demonstrated that II was identical to authentic 11α-hydroxyallopregnane-3,20-dione.[1a]

11α-Acetoxyallopregnane-3,20-dione (VI).—Compound V (24 mg.) in pyridine was acetylated at room temperature with acetic anhydride and yielded VI, m.p. 181–182° after several recrystallizations from ethyl acetate–Skellysolve B, $[\alpha]^{23}\text{D}$ +67° (*c* 0.480 in chloroform).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 74.09; H, 9.34.

Acknowledgment.—We wish to thank Dr. A. H. Nathan of our laboratories for assistance in preparing the 11α-acetoxy derivatives of pregnane-3,20-dione and allopregnane-3,20-dione, and Mr. Byron A. Johnson for supplying the pregnane-3,20-dione. We are grateful to the following members of the Upjohn Research Division for their coöperation and assistance on various aspects of our problems: Dr. J. L. Johnson, Mr. L. Scholten and Mrs. G. S. Fonken for ultraviolet and infrared analyses; Mr. W. A. Struck and his associates for rotations and microanalyses; and the Misses Jennie I. Mejeur, Irene N. Pratt and Mr. Glenn Staffen for technical assistance. The helpful suggestions and stimulating interest of Drs. R. H Levin and D. I. Weisblat have been greatly appreciated.

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[3] J. L. Johnson, W. G. Jackson and T. E. Eble, This Journal, **73**, 2947 (1951).

[Contribution from the Research Laboratories of Merck & Co., Inc.]

Approaches to the Total Synthesis of Adrenal Steroids.[1] V. 4b-Methyl-7-ethylenedioxy-1,2,3,4,4aα,4b,5,6,7,8,10,10aβ-dodecahydrophenanthrene-4β-ol-1-one and Related Tricyclic Derivatives

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The addition of methyl vinyl ketone to 5-methylperhydro-(4aα,8aα)-naphthalene-1β,4β-diol-6-one (I) proceeds stereospecifically to give 4b-methyl-1,2,3,4,4aα,4b,5,6,7,9,10,10aα-dodecahydrophenanthrene-1β,4β-diol-7-one (II). The steric relationship of C4a and C4b, which is established in this reaction, is shown to be *anti* from consideration of steric hindrance in the *cis*-decalin molecule. This tricyclic intermediate (II) and its 7-ethylenedioxy derivative (III) can be selectively oxidized to the corresponding 1-keto derivatives. Inversion at C10a with base affords the desired BC *trans*-keto alcohols IX and X. This reaction series provides a stereospecific and rational approach to the natural (*anti-trans*) series of tricyclic steroidal intermediates. The preparation and properties of some related tricyclic compounds are described. It is noted that, in contrast to the 11-keto function in the steroids, the corresponding 4-keto function in the *anti-trans*-polyhydrophenanthrenes is reduced to the 4α-hydroxy derivative by $LiAlH_4$. Also described is the use of chromium trioxide–pyridine, an oxidizer which smoothly converts hydroxyl to carbonyl groups without attack on double bonds, acid-sensitive groups and the like.

A single isomer,[2] 4b-methyl-1,2,3,4,4aα,4b,5,6,7,-9,10,10aα-dodecahydrophenanthrene-1β,4β-diol-7-one (II) resulted from the Triton B-catalyzed addition of methyl vinyl ketone to 5-methylperhydro-(4aα,8aα)-naphthalene-1β,4β-diol-6-one (I).[3a,b] The phenanthrene skeleton present in this condensa-

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[1] The recent total syntheses of non-aromatic steroids by R. B. Woodward, F. Sondheimer and D. Taub (This Journal, **73**, 3547, 4057 (1951)) and by H. M. E. Cardwell, J. W. Cornforth, S. R. Duff, H. Holtermann and R. Robinson (*Chemistry and Industry*, **20**, 389 (1951)) represent outstanding synthetic advances. There remains a distinct need, however, for direct synthetic routes to the adrenal hormones themselves.

[2] The nomenclatural convention used herein has been described in part I of this series: This Journal, **74**, 1393 (1952).

[3] (a) R. E. Beyler and L. H. Sarett, *ibid.*, **74**, 1406 (1952); (b) this method of adding a six-membered ring to β-decalones and β-tetralones is based upon the classical investigations of R. Robinson and his associates: E. C. DuFeu, F. J. McQuillin and R. Robinson, *J. Chem. Soc.*, 53 (1937); F. J. McQuillin and R. Robinson, *ibid.* 1097 (1938); R. Robinson and F. Weygand: *ibid.*, 386 (1941); Martin and R. Robinson, *ibid.*, 491 (1943).