[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY]

Microbiological Transformations of Steroids. VI. Preparation of 11α-Hydroxy-6-dehydroprogesterone

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

RECEIVED SEPTEMBER 29, 1952

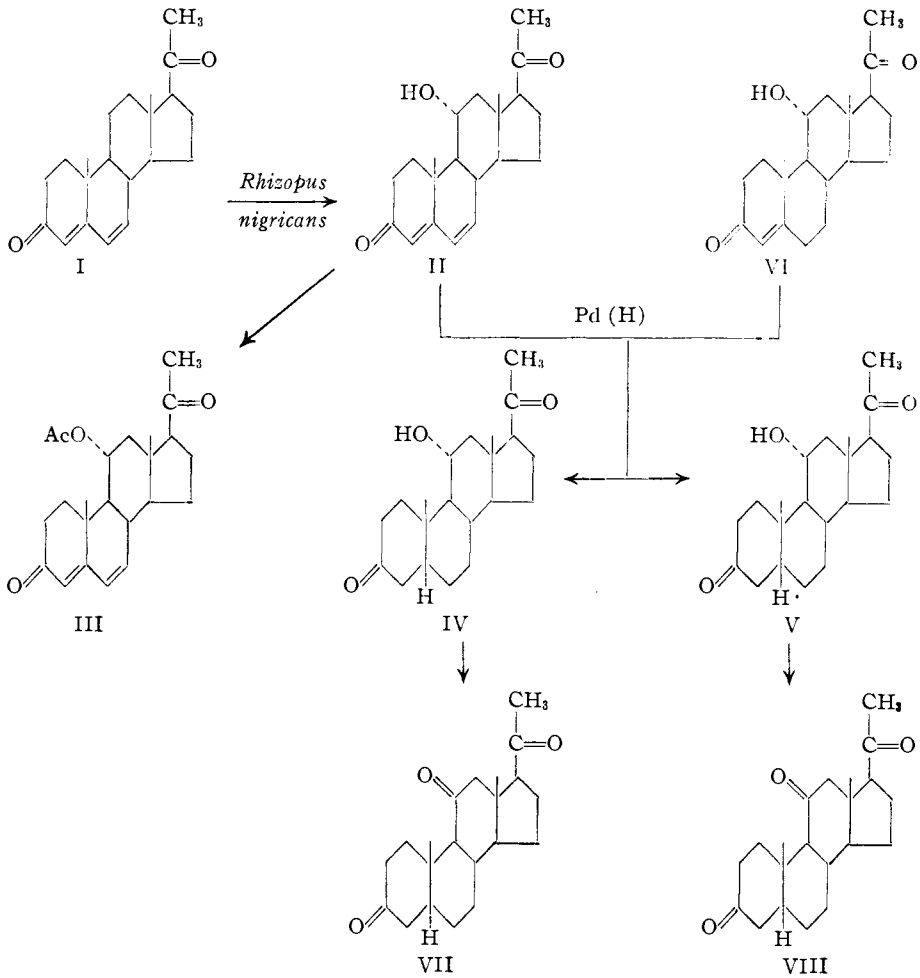
Rhizopus nigricans Ehrb. (A.T.C.C. 6227b) transforms 6-dehydroprogesterone in one step to a new compound 11α-hydroxy-6-dehydroprogesterone in good yield.

Discussion

Continuing our studies on the steroids[1] using fungi of the *Mucorales* order this manuscript reports the microbiological conversion of 6-dehydroprogesterone (I)[2] to a new compound 11α-hydroxy-6-dehydroprogesterone (II) using *Rhizopus nigricans* in 50–60% yield.

The fermentation and extraction was carried out as previously described.[3] Microbiological conversion was complete in 24 hours and papergram (paper chromatography) studies indicated the formation of one major component with a mobility in the same region as 11α-hydroxyprogesterone. Isolation studies were based on alumina chromatography of the crude methylene chloride extracts. Certain eluates were combined on the basis of papergram studies and a crystalline product (II) in 60% yield was recovered.

Infrared and ultraviolet studies indicated II to be a new compound with hydroxyl present. Acetylation was easily accomplished in the usual manner. Hydrogenation with palladium-on-carbon produced 11α-hydroxyallopregnane-3,20-dione (IV) and 11α-hydroxypregnane-3,20-dione (V).[1c,4] The structures of the authentic samples of IV and V were established by reduction of known 11α-hydroxyprogesterone (VI) with palladium. The two reduction products thus obtained could be readily oxidized to allopregnane-3,11,20-trione (VII)[5] and pregnane-3,11,-20-trione (VIII).[6]



Experimental

The methods of fermentation and extraction used in this work have been adequately described elsewhere.[3]

Fermentation of 6-Dehydroprogesterone (I) with *Rhizopus nigricans*. 11α-Hydroxy-6-dehydroprogesterone (II).—To a 24-hour growth of *Rhizopus nigricans* 2.0 g. of 6-dehydroprogesterone (I), m.p. 143–146°,[7] $\lambda_{max}^{alc.}$ 286 mμ (*E* 26,000), $[\alpha]^{26}D$ +149.5° (*c* 1.010 in ethanol), was added. After a

(1) (a) Paper V in this series, P. D. Meister, D. H. Peterson, H. C. Murray, G. B. Spero, S. H. Eppstein, A. Weintraub, L. M. Reineke and H. Marian Leigh, THIS JOURNAL, **75**, 416 (1953). (b) D. H. Peterson and H. C. Murray, *ibid.*, **74**, 1871 (1952). (c) The transformations recorded in this paper in detail are contained in U. S. Patent 2,602,769, issued July 8, 1952, based on an original application filed Aug. 19, 1950.

(2) This compound was first prepared by A. Wettstein, *Helv. Chim. Acta*, **23**, 388 (1940).

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

(4) This compound was also reported by O. Mancera, A. Zaffaroni, B. A. Rubin, F. Sondheimer, G. Rosenkranz and C. Djerassi, *ibid.*, **74**, 3711 (1952).

(5) M. Steiger and T. Reichstein, *Helv. Chim. Acta*, **21**, 161 (1938).

(6) P. Hegner and T. Reichstein, *ibid.*, **26**, 721 (1943).

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

24-hour transformation period the steroid was extracted with methylene chloride and concentrated. The concentrate, 2.913 g. in 200 ml. of benzene, was chromatographed over 100 g. of alumina as previously described[3] using 200-ml. portions of solvents yielding a fraction, from the ether–chloroform eluates, containing 2.181 g. of crystals. This fraction was dissolved in 10 ml. of acetone, filtered and concentrated on a steam-bath to a volume of 6 ml. After remaining at room temperature for one hour, 415 mg. of crystals, m.p. 143–157°, were recovered by filtration. The mother liquors were refrigerated to yield an additional 281 mg. of crystals, m.p. 143–155°. The solids from this latter mother liquor, obtained by evaporation of the solvent on a steam-bath, were dissolved in 2 ml. of acetone. Upon the addition of 2 ml. of Skellysolve B (petroleum ether) crystallization ensued and 480 mg. of crystals, m.p. 152–157°, was recovered. Refrigeration of the mother liquors resulted in an additional lot of crystals, 76 mg., m.p. 143–158°. All of the recovered crystals, 1.852 g. melting at 143–158° were combined and dissolved in 4 ml. of methylene chloride. This solution was stirred with 0.1 g. of Magnesol[3] (magnesium silicate) and filtered. The residue was washed three times with 1-ml. portions of methylene chloride and the washings were added to the main filtrate. The combined methylene chloride solution was evaporated on a steam-bath to 2 ml., and 4 ml. of ether was added. Crystallization began and after one hour at room temperature, 1.074 g. of crystals, m.p. 155–158°, was recovered by filtration. Upon recrystallization from 5 ml. of boiling methanol and refrigeration for two days, 456 mg. of crystals, m.p. 160–162°, was obtained by filtration. Recrystallization from 3 ml. of hot methanol yielded 213 mg. of 11α-hydroxy-6-dehydroprogesterone crystals (II), m.p. 160–162°, $\lambda_{max}^{alc.}$ 286 mμ (*E* 20,300), $[\alpha]^{24}D$ +111° (*c* 1.063 in chloroform).

Infrared studies showed 11α-hydroxy-6-dehydroprogesterone (II) to be a new compound and indicated the presence of one hydroxyl group which was confirmed by microcombustion data.

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.78; H, 8.59. Found: C, 76.78; H, 8.56.

11α-Acetoxy-6-dehydroprogesterone.—A 47.5-mg. sample of 11α-hydroxy-6-dehydroprogesterone was dissolved in 3 ml. of acetic anhydride and 2 ml. of pyridine added. After 48 hours at room temperature the solution was diluted with 100 ml. of water and extracted with ether. The combined ether extracts were washed with water, hydrochloric acid, sodium bicarbonate and water in the usual manner. The ether solution was dried over anhydrous sodium sulfate and evaporated by a stream of air. The oily residue crystallized from 0.2 ml. of methanol and 57 mg. was obtained. This material was twice recrystallized from acetone by the addition of Skellysolve B drop by drop until crystals appeared. Crystals of III recovered weighed 31.8 mg., m.p. 142–144°, $[\alpha]^{25}D$ +108° (*c* 1.150 in chloroform), $\lambda_{max}^{alc.}$ 284 mμ (*E* 23,000).

Anal. Calcd. for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.25; H, 8.17.

Catalytic Reduction of 11α-Hydroxy-6-dehydroprogesterone (II) to 11α-Hydroxyallopregnane-3,20-dione (IV) and 11α-Hydroxypregnane-3,20-dione (V).—A 100-mg. sample of (II) was dissolved in 100 ml. of methanol containing 100 mg. of 5% palladium–charcoal prereduced catalyst. This mixture was shaken in an atmosphere of hydrogen (10 p.s.i.) at room temperature in a modified Parr apparatus. The uptake of hydrogen for the two double bonds was exactly the same as that calculated. After removal of the catalyst by filtration, the solids weighed 101 mg. Chromatography of these solids was accomplished over 8 g. of Florisil (synthetic magnesium silicate) by a method previously described.[1c] A main fraction was obtained by combining the eluates from the ethylene chloride:acetone of 25:1, 15:1, 12:1 and 10:1. This fraction (90.7 mg.) dissolved in 30 ml. of acetone was then chromatographed over 5 g. of a mixture of Darco G-60 activated carbon and Celite 545 diatomaceous earth in 1:2 proportion using 30-ml. portions of acetone and methylene chloride. The first six acetone eluates A were combined after papergram analyses had shown they contained only V and the crystalline solids weighed 40.5 mg. The methylene chloride eluates (fractions 10–13) or fraction B, on the other hand, contained only IV and amounted to 40.3 mg. of crystalline solids. When twice recrystallized from a mixture of 0.15 ml. of ethyl acetate and 0.5 ml. of methylcyclohexane, fraction A yielded 22 mg. of pure crystalline V,[8] m.p. 106–110°, $[\alpha]^{23}D$ +86° (*c* 0.865 in chloroform) with a characteristic infrared spectrum.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.60; H, 9.78.

When twice recrystallized from 0.5 ml. of ethyl acetate, fraction B yielded 23 mg. of pure crystalline IV, m.p. 197–200°, $[\alpha]^{23}D$ +83° (*c* 0.982 in chloroform), and having a characteristic infrared spectrum.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.51; H, 9.71.

Structure Proof for 11α-Hydroxyallopregnane-3,20-dione (IV) and 11α-Hydroxypregnane-3,20-dione (V). Reduction of Known 11α-Hydroxyprogesterone (VI) to IV and V. Identification of IV and V by Subsequent Oxidation to the Known Triketones Allopregnane-3,11,20-trione (VII) and Pregnane-3,11,20-trione (VIII).—To 250 mg. of 11α-hydroxyprogesterone dissolved in 100 ml. of ethanol (3A denatured) containing 3 drops of triethylamine, 45 mg. of 30% palladium-on-charcoal was added. Hydrogen at a pressure of 8 lb. was passed through the mixture and in 17 minutes the theoretical amount of hydrogen was consumed. Evaporation of the solvent left 265 mg. of crystals, m.p. 145–185°. Extraction of the crystals with a mixture of ethyl ether and Skellysolve B (petroleum ether) in a one to nine proportion, respectively, yielded 146 mg. of crystals, m.p. 165–185°. Recrystallization twice from ethyl acetate gave crystals of authentic 11α-hydroxyallopregnane-3,20-dione (IV), m.p. 198–201°, $[\alpha]^{24}D$ +81° (*c* 1.052 in chloroform) as shown by the oxidation experiment which follows.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.92; H, 9.62.

To 0.5 g. of IV dissolved in 5.9 ml. of hot chlorobenzene which was then cooled, 450 mg. of sodium dichromate in 3.5 ml. of water containing 0.6 ml. of concentrated sulfuric acid was added at 0–5° with stirring for two hours. The phases were separated and the aqueous layer extracted twice with a total of 100 ml. of benzene. The combined benzene and chlorobenzene solutions were washed with sodium bicarbonate saturated water and then water. The organic solvent extract was dried with anhydrous sodium sulfate, filtered and concentrated in vacuum to yield 460 mg. of crystals. Recrystallization from ethyl acetate by the addition of Skellysolve B gave 450 mg. of known allopregnane-3,11,20-trione (VII),[5] m.p. 211–215°, $[\alpha]^{24}D$ +135° (*c* 0.756 in chloroform).

The crystals remaining, after extraction of the reduction products with ether:Skelly B (1:9), were recrystallized twice to yield 60 mg. of a constant melting compound V, m.p. 85–90°, $[\alpha]^{25}D$ +82° (*c* 0.538 g. in chloroform).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.13; H, 9.76.

This compound (V) was found to be 11α-hydroxypregnanedione by oxidation to known pregnane-3,11,20-trione as described below.

A sample of 1.00 g. of V in 30 ml. of glacial acetic acid was oxidized with 242 mg. of chromium trioxide in 50 ml. of glacial acetic acid for 1.75 hours. At the end of this time 1 ml. of methanol and 250 ml. of water were added. This solution was extracted with benzene four times and the latter solution washed with sodium bicarbonate and water. The benzene solution was then evaporated leaving 0.96 g. of crystals, m.p. 137–142°. Recrystallization from ether:Skelly B gave 0.68 g., m.p. 150–153.5°. Another sample from the same run as above was chromatographed over Florisil to yield, after two recrystallizations, a constant melting compound (VIII), m.p. 158–160°, $[\alpha]^{25}D$ +121° (*c* 1.235 in chloroform).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.45; H, 9.10.

Comparison of compound VIII with an authentic sample of pregnane-3,11,20-trione showed these compounds to be identical by mixed melting point, infrared, specific rotation and microanalyses.

(8) This compound had a peculiarly wide range of melting points, varying from 80–130° even when all other criteria such as specific rotation, infrared and oxidation studies showed it to be a pure compound.

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]

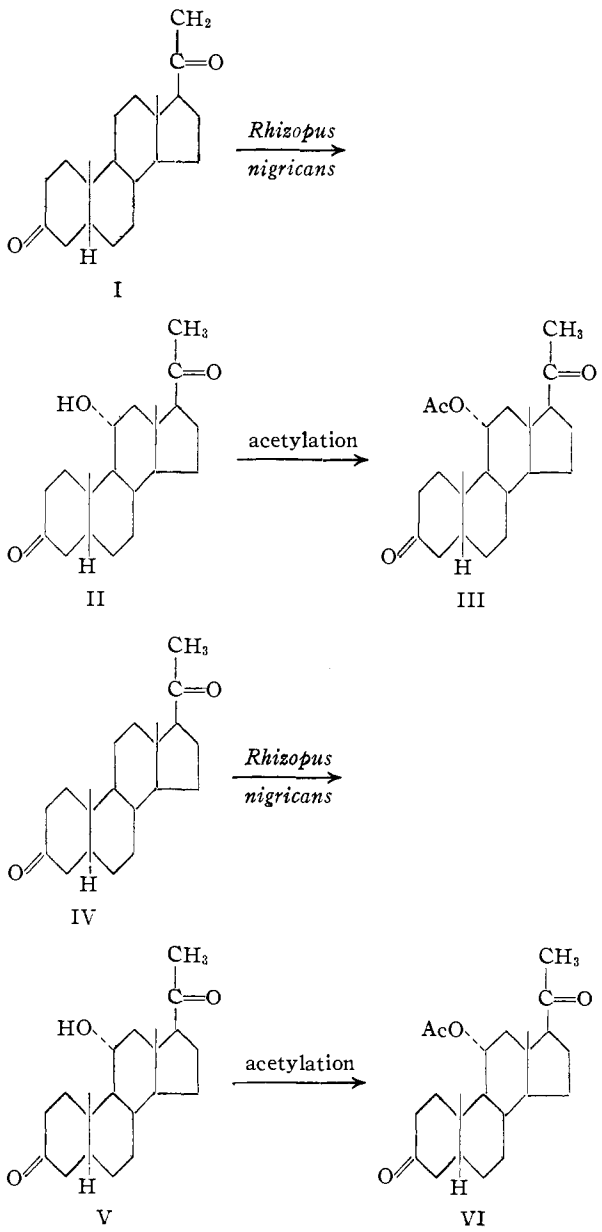
BY S. H. EPPSTEIN, D. H. PETERSON, H. MARIAN LEIGH, H. C. MURRAY, A. WEINTRAUB, L. M. REINEKE AND P. D. MEISTER

RECEIVED OCTOBER 2, 1952

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

(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).