ring, an effect paralleled in the case of cyclopentene and cyclohexene.[14] This agrees with the findings of Bladon, *et al.*,[10] who attribute this effect to weakening of the endocyclic bonds in a five-membered ring because of the "bending" of σ-bonds. The $\Delta^{5,7}$-conjugated diene system exhibited the characteristic intensification, splitting and shift to a longer wave length.[7]

C–H Bending.—All of the compounds studied showed the strong absorption at 6.8 to 6.9 μ attributed to the methylene deformation. Absorption by C–CH_3 deformation at 7.2 to 7.3 μ was also present in all cases.

C–O Stretching.—Rosenkrantz, *et al.*,[8] have suggested that the strong bands between 9 and 10 μ (1110–1000 cm.$^{-1}$) assigned to C–O stretching in alcohols[5] might serve in determining the steric configuration of the 3- and 5-positions. For the present series of compounds, however, all of which have 3β-hydroxyl groups and five of which have a *trans* C-5 configuration, enough variation existed with varying double bond position to confuse or invalidate other configurational assignment (Figs. 1 and 2). However, spectra of solutions appear to be somewhat less variable in this respect. Among the compounds having the same 3–5 configuration there was a migration of the C–O band to shorter wave lengths in the spectra of the solid films as the double bond approaches the A ring, but the occurrence of a trigonal carbon in the 5-position and the attendant elimination of the 5-hydrogen markedly disrupted this progression.

Spectra of Films *vs.* Solutions.—Although the spectra of solid and dissolved sterols bear sufficient resemblance to permit identification of most sterols by comparison of the fingerprint regions, the spectra often showed significant differences in band shape and location. The shift of bands associated with groups engaged in intermolecular association has already been noted in connection with O–H and C–O stretching. The greater effective concentration and orientation of the side chain in the solid state are manifested in the appearance of weak but distinct bands not resolved in the solution spectra. Also, absence of a solvent in the solid state permits observation of the entire spectral region scanned and eliminates the variable of solvent interaction.

MADISON, WISCONSIN

(14) American Petroleum Institute Research Project 44, Catalog of Infrared Spectral Data, No. 696 and 697 (1948).

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Microbiological Transformations of Steroids. II. The Preparation of 11α-Hydroxy-17α-progesterone

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

RECEIVED AUGUST 29, 1952

4,16-Pregnadien-3,20-dione is converted to 11α-hydroxy-17α-progesterone by *Rhizopus nigricans* Ehrb (A.T.C.C. 6227b).

Introduction

The microbiological introduction of an oxygen into position 11 of the steroid nucleus has been reported by us, in particular the formation of 11α-hydroxyprogesterone from progesterone.[1] This paper deals with the microbiological conversion of 4,16-pregnadien-3,20-dione by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b). This compound was first prepared by Butenandt and Schmidt-Thomé[2] from 3β-acetoxy-5-androstene-17-one. Today, however, it is readily available from steroidal sapogenins such as Diosgenin.[3]

Methods

The methods used in the microbiological conversion of 4,16-pregnadien-3,20-dione are in all details identical to those reported in the first paper of this series.[1c] For a preliminary orientation fermentations with *Rhizopus nigricans* were run on a small scale (25 mg. of substrate/100 ml.) in shake flasks. For purposes of isolation and identification 1–2 g. of substrate were incubated in a 24–24- or 24–48-hour cycle (24-hour growth and 24- or 48-hour transformation) with a culture of *Rhizopus nigricans*, which had been grown on our medium H at an average *p*H of 4.5 and an aeration of 1 l./hr. Chromatography over alumina was usually necessary to separate the bioconversion product from unchanged starting material.

Results

Paper chromatography of the small scale fermentations using the propylene glycol–toluene system, revealed that a new compound had been formed in 25–40% yield which showed a mobility similar to 11α-hydroxyprogesterone. Chromatography over alumina led to the isolation of a bioconversion product (II) $C_{21}H_{30}O_3$, in 25–30% yield. The comparison of its physical properties (Table I and II) to those of the starting material and 11α-hydroxyprogesterone led to the following interpretation: one hydroxyl group had been formed, as indicated by the microanalysis and the formation of a monoacetate.

(1) (a) D. H. Peterson and H. C. Murray, THIS JOURNAL, **74**, 1871 (1952); (b) First disclosed in U. S. Patent 2,602,769; filed Feb. 23, 1952, issued July 8, 1952, based on an original application filed Aug. 19, 1950. It is to be noted that the Australian and South African Counterparts of the original application were open for public inspection in Aug., 1951. (c) Paper I of this series: 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).

(2) A. Butenandt and J. Schmidt-Thomé, *Ber.*, **72**, 182 (1939).

(3) R. E. Marker, T. Tsukamoto and D. G. Turner, THIS JOURNAL, **62**, 2525 (1940).

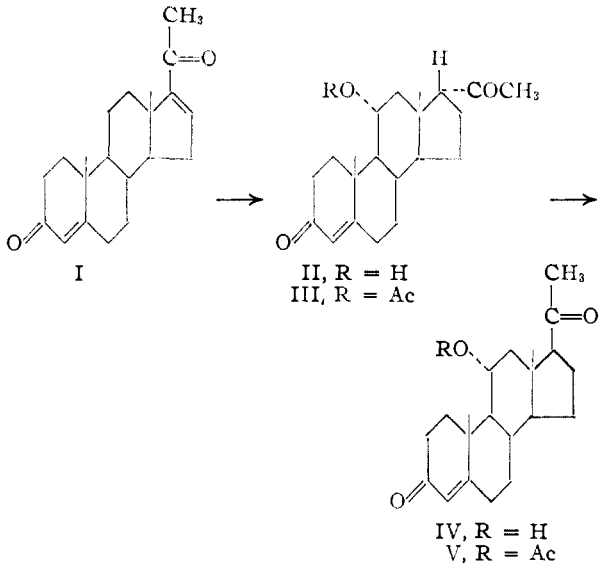


TABLE I

SPECTROSCOPIC DATA OF 4,16-PREGNADIEN-3,10-DIONE[4] (I), 11α-HYDROXY-17α-PROGESTERONE (II) AND 11α-HYDROXYPROGESTERONE (IV)

Spectra	Compound I	Compound II	Compound IV
Ultraviolet	E = 25,200	15,300	15,400
	240 mμ	242 mμ	242 mμ
Infrared, cm.$^{-1}$			
Hydroxyl	..	3486, 3354	3460
Non-conjugated ketone	..	1700	1695
Conjugated ketone	1663	1669	1671
	1682		
Δ^4-Double bond	1618	1608	1616
Δ^{16}-Double bond	1590	..	..

The ultraviolet spectrum showed the loss of one ultraviolet-absorbing chromophore. The infrared spectrum corroborated this and suggested the reduction of the 16–17 double bond. Since the compound and its spectroscopic data were closely related, but not identical to 11α-hydroxyprogesterone, these data were interpreted by assuming that 11α-hydroxy-17α-progesterone[5] was the product of the bioconversion. The validity of this assumption was further substantiated when the molecular rotation differences between II and IV and their respective acetates (III and V) were compared to values reported in the literature for stereoisomers epimeric at C_{17}.

The molecular rotation differences reported for pairs epimeric at C_{17} range from +448 to +730 depending on the functional groups and the solvents. The structure of 11α-hydroxy-17α-progesterone (II) was confirmed by an acidic isomerization to 11α-hydroxyprogesterone (IV).

Our earlier experience had shown that the enzymatic activity of *Rhizopus* led predominantly

(4) A. Butenandt and J. Schmidt-Thomé, ref. 2, give λ_{max} 234, E 26,100. The values here reported were determined in these laboratories.

(5) The designation "17α" replaces the term "17-iso" formerly used for steroids possessing the unnatural configuration of the side chain. See Report of the Subcommittee on Steroid Nomenclature of the National Research Council.

TABLE II

MOLECULAR ROTATION DIFFERENCES OF STEROIDS EPIMERIC AT C_{17}[6]

	$[\alpha]_D$	Solvent	M_D	$[M]_D^{17\alpha} - [M]_D^{17\beta}$	Ref
Progesterone	+193°	EtOH	+607	+607	7
17α-Progesterone	0	EtOH	0		7
11α-Hydroxyprogesterone (IV)	+176	$CHCl_3$	+581	+621	1
11α-Hydroxy-17α-progesterone (II)	− 12	$CHCl_3$	− 40		
11α-Acetoxyprogesterone (V)	+158	$CHCl_3$	+587	+687	1
11α-Acetoxy-17α-progesterone (III)	− 27		−100		

to oxygenation at various positions[8] and to a very slight degree to reduction of a double bond. 11α-Hydroxyallopregnane-3,20-dione is formed from progesterone in 1–4% of the total oxygenated material. Therefore, the high specificity of the enzyme systems for the 16–17 double bond and the ability to produce the thermodynamically labile stereoisomer were unexpected attributes of the *Rhizopus* organism.

Experimental

Fermentation of 4,16-Pregnadien-3,20-dione (I) by *Rhizopus nigricans* and Isolation of 11α-Hydroxy-17α-progesterone (II).—*Rhizopus nigricans* was grown on 2 l. of medium H^{1c} for 24 hours. Then 1 g. of substrate, dissolved in 20 ml. of ethanol, was added. The fermentation was continued for an additional 72 hours when the shake bottle procedure as described in paper I of this series was employed, and for 24–48 hours in case of a fermentation in the stir bottle assembly. Extraction with methylene dichloride gave brown, partially crystalline residues. Analysis by paper chromatogram showed a conversion to a compound with a mobility similar to 11α-hydroxyprogesterone (propylene glycol–toluene system) in 25–40% yield as well as the presence of some unchanged starting material (30%). The extract was dissolved in 50 ml. of benzene and chromatographed over 120 g. of alumina (acid-washed, reactivated at 120° for 4 hours). The column was eluted with 200-ml. portions of benzene, benzene + 50% ether, ether, ether + 5% chloroform, ether + 10% chloroform, ether + 50% chloroform, chloroform, chloroform + 50% acetone, acetone, methanol. Benzene–ether 1:1, ether and ether–chloroform mixtures eluted 421 mg. of crystalline material which on recrystallization from acetone gave 250.5 mg. of crystals, m.p. 184–189°[9]; mixed melting point with starting material 184–188°.

Chloroform and chloroform–acetone 1:1 fractions were individually dissolved in a few drops of methylene dichloride; after dilution with 10–20 ml. of ether the fractions were kept at room temperature until crystals were formed. After a short period of refrigeration the mother liquors were decanted and the crystals were recrystallized from methylene dichloride–ether. In this way 255 mg. of crystals, m.p. 209–211.5°, was obtained; $[\alpha]^{23}D$ −12° (c 0.995 in $CHCl_3$); λ_{max}^{EtOH}, 242 mμ (log E 4.18).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.07; H, 9.07.

The acetate was prepared with acetic anhydride–pyridine. It was recrystallized from methanol, m.p. 130–133°, $[\alpha]^{23}D$ −27° (c 0.95 in $CHCl_3$).

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.31; H, 8.54.

Rearrangement of 11α-Hydroxy-17α-progesterone to 11α-Hydroxyprogesterone.—Sixty mg. of 11α-hydroxy-17α-

(6) For a detailed compilation see: C. W. Marshall and T. F. Gallagher, *J. Biol. Chem.*, **179**, 1265 (1949).

(7) A. Butenandt and J. Schmidt-Thomé, *Ber.*, **72**, 1112 (1939).

(8) We will report on the oxygenation at positions other than 11 in a series of forthcoming papers.

(9) All melting points were taken on Fisher–Johns block and are uncorrected.

progesterone was dissolved in 3 ml. of alcohol and 0.3 ml. of hydrochloric acid was added. The solution was refluxed for one-half hour, then concentrated *in vacuo* to give an oily residue which was dissolved in a few drops of acetone. Refrigeration at −10° overnight gave crystals, m.p. 162–168°, which were recrystallized once from acetone–ether, m.p. 164–167°. Infrared spectrum and optical rotation, $[\alpha]^{23}D$ +170°[10] showed this compound to be identical with 11α-hydroxyprogesterone.

Acknowledgment.—We wish to express our thanks to the following people of the Upjohn Research Division for the advice and coöperation in connection with this problem: *viz.*, to Dr. J. L. Johnson, Mrs. G. S. Fonken and Mr. L. Scholten for the spectrographic data, to Mr. W. A. Struck and his associates for all rotations and microanalyses and to Misses Jennie I. Mejeur, Irene N. Pratt and Mr. Glenn Staffen for technical assistance. The authors are indebted to Drs. R. H. Levin and D. I. Weisblat for their helpful and stimulating interest.

(10) D. H. Peterson and H. C. Murray, ref. 1a report $[\alpha]^{23}D$ +179° for pure 11α-hydroxyprogesterone.

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE ABBOTT LABORATORIES]

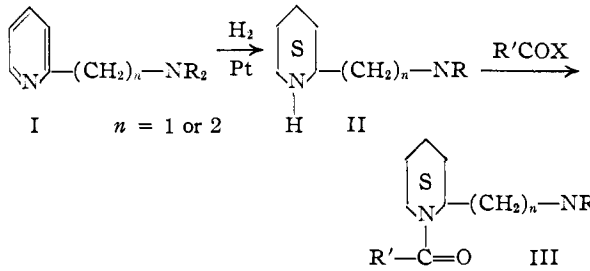
The Preparation of 1-Acyl-2-dialkylaminoalkylpiperidines[1]

BY ARMIGER H. SOMMERS, MORRIS FREIFELDER, HOWARD B. WRIGHT AND ARTHUR W. WESTON

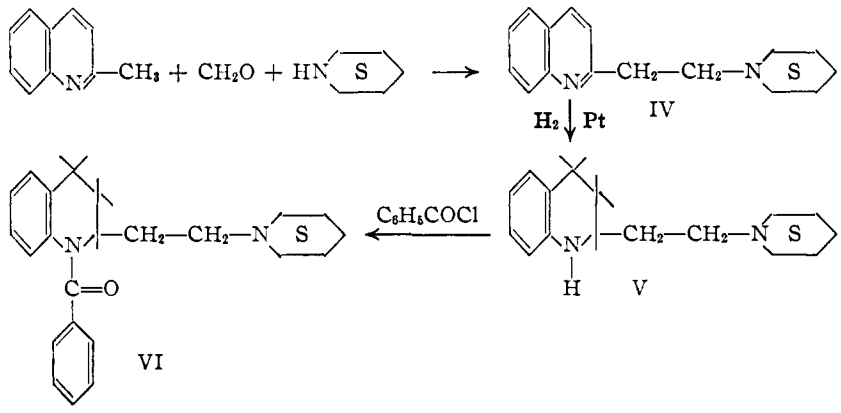
RECEIVED AUGUST 1, 1952

The synthesis of seventeen basic amides of structure III is described. The intermediate diamines were prepared by catalytic hydrogenation of the Mannich type 2-substituted pyridines (I), and by chemical and catalytic reduction of 1-(2′-picolinoyl)-piperidine (VII). The majority of the diamines were acylated by the Schotten–Baumann method.

The local anesthetic activity of 1-benzoyl-2-(β-N-piperidylethyl)-piperidine (III, n = 2, R′ = C_6H_5, NR_2 = N-piperidyl), which was prepared as an intermediate in another program, prompted the synthesis of a series of related basic amides by the reactions shown.

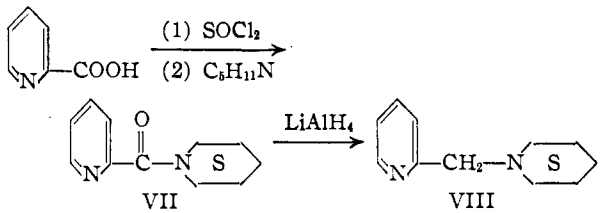


The addition of secondary cyclic amines to 2-vinylpyridine[2] provides, for certain compounds of structure I (n = 2), a synthesis more convenient than the Mannich reaction involving 2-picoline.



Good yields of the desired products were obtained by heating 2-vinylpyridine with piperidine,[2] morpholine and 1-methylpiperazine. Diethylamine, cyclohexylamine, dicyclohexylamine and 2-pipecoline, however, did not react. The Mannich procedure was employed with 2-picoline and quinaldine for the preparation of 2-(β-diethylaminoethyl)-pyridine[3] (I, n = 2, R = C_2H_5) and 2-(β-N-piperidylethyl)-quinoline (IV).[4]

The incorporation of the methylene side chain was accomplished by the scheme



Freshly prepared 2-picolinoyl chloride acted on piperidine to give 1-(2′-picolinoyl)-piperidine (VII) which was reduced to 2-N-piperidylmethylpyridine (VIII) using lithium aluminum hydride.

Catalytic hydrogenation of the pyridine ring in I and IV furnished the corresponding secondary amines (II, V) which on acylation gave the desired amides (III, VI). The basic amides having the β-N-piperidylethyl side chain are described in Table II. The majority were made by the Schotten–Baumann procedure, although compound 1, the formyl derivative, was obtained conveniently by the reaction of the amine with ethyl formate in ethanol,[5]

(1) Presented in part before the Medicinal Division of the American Chemical Society, Milwaukee, Wis., 1952.

(2) W. E. Doering and R. A. N. Weil, THIS JOURNAL, **69**, 2461 (1947).

(3) T. Heou-Feo, *Bull. soc. chim. France*, [5] **2**, 105 (1935).

(4) (a) W. O. Kermack and W. Muir, *J. Chem. Soc.*, 3089 (1931); (b) T. Heou-Feo, *Bull. soc. chim. France*, [5] **2**, 100 (1935).

(5) As other examples of this method, see: J. P. E. Human and J. A. Mills, *J. Chem. Soc.*, 1457 (1948), and A. E. Barkdoll, H. W. Gray and W. Kirk, Jr., THIS JOURNAL, **73**, 741 (1951).