tively.[7] On treatment with nitrous acid it gave a yellow-green oil which showed a positive Liebermann nitroso test.[8] The product gave no test for a primary amine after diazotization and treatment with β-naphthol.

The reaction of benzhydryl chloride with *n*-butylamine gave an amine which yielded a crystalline hydrochloride, m.p. 261–263°.

Anal. Calcd. for $C_{17}H_{22}NCl$ (N-benzhydryl-*n*-butylamine hydrochloride): C, 74.02; H, 8.04; N, 5.08; Cl, 12.86. Found: C, 73.92; H, 8.02; N, 5.70; Cl, 12.28.

(7) H. Gilman, J. E. Kirby and C. R. Kinney, THIS JOURNAL, **51**, 2206 (1929); A. Skita, *Ber.*, **48**, 1696 (1915); W. E. Bachmann, THIS JOURNAL, **53**, 2674 (1931); M. Busch, *Ber.*, **37**, 2693 (1904).

(8) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," Ed. 3, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 114.

DEPARTMENT OF CHEMISTRY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE 39, MASSACHUSETTS

Hydroxylation of Desoxycorticosterone with *Neurospora crassa*[1]

BY D. STONE,[2] M. HAYANO,[2] R. I. DORFMAN,[2] O. HECHTER,[2] C. R. ROBINSON[3] AND CARL DJERASSI[3]

RECEIVED MARCH 25, 1955

Since the original report[4] on the microbiological hydroxylation of progesterone to 11α-hydroxyprogesterone by means of *Rhizopus arrhizus*, a variety of microörganisms and substrates have been investigated.[5] In connection with studies under way in these laboratories to investigate the metabolism of steroids by *Neurospora crassa*, we have examined the action of this mold on a variety of steroids. It should be noted that with the exception of only a brief general statement[6] no reports have appeared concerning the steroid transforming capacity of this mold.

Pilot experiments with *Neurospora crassa*, indicated by paper chromatographic analysis that most of the steroids tested were transformed to a large extent into more polar products. These findings indicated that *Neurospora crassa* has versatile enzymatic capacities to effect modifications of the steroid molecule; the nature of the precise alterations produced awaits isolation and identification of the products obtained. In the case of desoxycorticosterone (I) it was possible to isolate one of the transformation products in crystalline form; the present note is concerned with a description of these experiments.

Incubation of 3 g. of I in 18 l. of medium by the procedure outlined in the experimental section, led to several crystalline fractions. The most abundant one was isolated in sufficient quantity (*ca.* 10–20%) so that its chemical constitution could be defined to a considerable extent. This product (m.p. 182–184°, $[\alpha]_D + 163°$) possessed the empirical formula $C_{21}H_{30}O_4$, thus indicating that an additional hydroxyl group had been introduced. The presence of the unaltered Δ^4-3-keto moiety and the ketol side chain was demonstrated by the ultraviolet and infrared spectral data and the typical red color produced with triphenyltetrazolium chloride.[7] Oxidation with sodium bismuthate[8] led to a crystalline etio acid, $C_{20}H_{28}O_4$, which represented additional chemical proof for the ketol side chain.

(1) Supported in part by a grant from the Commonwealth Fund and by Research Grant C-2207 from the Division of Research Grants and Fellowships, National Institutes of Health, United States Public Health Service.

(2) The Worcester Foundation for Experimental Biology.

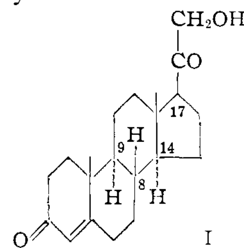
(3) Wayne University.

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

(5) *Cf.* K. Florey, *Chimia*, **8**, 81 (1954); O. Hanc and E. Riedl-Tumova, *Pharmazie*, **9**, 877 (1954); D. H. Peterson in "Perspectives and Horizons in Microbiology," edited by S. A. Waksman, Rutgers U. Press, New Brunswick, N. J., 1955, p. 121.

(6) H. C. Murray and D. H. Peterson, U. S. 2,602,769, p. 62.

The infrared spectrum (kindly determined by Mr. Paul Skogstrom, Worcester Foundation), differed from those of the following monohydroxylated derivatives of desoxycorticosterone; 2α, 6 α, 6 β, 11 α, 11 β, 14 α, 15 α, 16 α, 17 α and 19. The mobility of the hydroxylated desoxycorticosterone approximated that of corticosterone and a further similarity between the two substances was demonstrated by the fact that just as with corticosterone, the new product yielded only a monoacetate. This acetate still exhibited a free hydroxyl band in the infrared and possessed a mobility on paper which was essentially identical with that of corticosterone 21-acetate. In conjunction with our previous studies, the nature of the newly introduced hydroxyl group was indicated by its resistance toward chromium trioxide, which demonstrated that only three tertiary positions (8, 9 or 14 β) were open for the location of the hydroxyl group. If microbiological hydroxylations in the steroid series do not proceed *via* an intermediate double bond (as appears to be the case in the 11 β hydroxylation in adrenal tissue[9]) it is reasonable to assume tentatively (first suggested to us by Dr. B. M. Bloom) that a hydroxyl group enters only those spatial positions in the steroid nucleus where a hydrogen atom was present originally. On this basis positions 9 β, 8 α and 14 β are unlikely, leaving either the 8 β- or 9 α-positions as the location for the hydroxyl group in the presently described x-hydroxydesoxycorticosterone.[10]



Experimental[11]

Incubation of Desoxycorticosterone with *Neurospora crassa*.—A wild strain of *Neurospora crassa* (No. 74A) was

(7) *Cf.* A. Zaffaroni, *Recent Progr. Hormone Research*, **8**, 51 (1953).

(8) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

(9) M. Hayano and R. I. Dorfman, *J. Biol. Chem.*, **211**, 227 (1954).

(10) After the isolation of our hydroxylated derivative of desoxycorticosterone, Dr. D. H. Peterson of the Upjohn Co., kindly supplied us with a number of monohydroxylated derivatives of desoxycorticosterone for comparison purposes. One of these, tentatively designated as 8β-hydroxydesoxycorticosterone, isolated by Dr. P. D. Meister (Upjohn Co.) as a bioconversion product of 11-hydroxydesoxycorticosterone by *Mucor parasiticus*, has been found to have an identical infrared spectrum, m.p. 182–184°, and specific rotation $[\alpha]_D + 167°$, (*C*, 0.709 in chloroform) with the corresponding physical constants found for our compound. Clear-cut assignment of the hydroxyl group to position 8 or 9, has not yet been made.

(11) Melting points were determined on the Kofler block and rotations were measured in chloroform solution. The microanalyses were carried out by Geller Laboratories, Hackensack, New Jersey.

employed in these studies. Six 4-liter Pyrex bottles, each containing 3 liters of minimal medium[12] were prepared and sterilized by autoclaving. After cooling, the medium was inoculated and maintained at 30° with forced aeration for 4 days. At the end of this period, 500 mg. of desoxycorticosterone in 2.5 ml. of propylene glycol was added to the grown culture in each bottle, using aseptic technique. The propylene glycol solution had previously been sterilized by heating to 100° for 15 minutes. After the addition of the steroid the incubation with forced aeration was continued for a further 48 hours.

Following the incubation period, the medium was separated from the mold by straining through gauze, and extracted with ethyl acetate. The cells were extracted by homogenization in a Waring blendor with acetone. The acetone extract was combined with the ethyl acetate extract, dried over sodium sulfate, and taken down to a sirup *in vacuo*. The combined residue was taken up in benzene and chromatographed on silica gel. A crystalline compound was eluted using a benzene–ethyl acetate mixture (1:1). Several recrystallizations from acetone–petroleum ether furnished needles, m.p. 182–184°, $[\alpha]_D$ +163°, λ_{max}^{EtOH} 241 mμ, log ϵ 4.17. The infrared spectrum (kindly determined by Dr. T. F. Gallagher, Sloan-Kettering Institute for Cancer Research) in chloroform solution showed the presence of an associated hydroxyl group(s), 20-ketone (1706 cm.$^{-1}$) and the Δ^4-3-keto function (1668 and 1618 cm.$^{-1}$). In a comparative paper chromatogram with corticosterone with benzene on formamide-impregnated paper,[13] the product was found to be slightly more polar than corticosterone, a behavior which also conforms in the case of 14α-hydroxydesoxycorticosterone.[14]

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.50; H, 8.70.

Acetylation with acetic anhydride–pyridine (room temperature, 16 hours) followed by recrystallization from petroleum ether–acetone yielded the **21-monoacetate**, m.p. 200–205°, $[\alpha]_D$ + 185°, which in its paper chromatographic behavior in benzene or hexane–benzene on formamide-impregnated paper[12] was indistinguishable from corticosterone 21-acetate. The infrared spectrum in chloroform solution (courtesy of Dr. T. F. Gallagher) still showed the presence of a free hydroxyl group as well as the characteristic bands for the 20-keto-21-acetoxy (1748 and 1725 cm.$^{-1}$) and Δ^4-3-keto (1668 and 1617 cm.$^{-1}$) functions.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.33; H, 8.58.

The crystalline monoacetate was recovered unchanged after standing for 3 hours at 15° with chromium trioxide in 80% acetic acid.

x-Hydroxy-Δ^4-3-ketoetienic Acid.—A solution of 40 mg. of x-hydroxy-desoxycorticosterone in 10 cc. of 50% acetic acid was oxidized at room temperature for 30 minutes with 1.0 g. of sodium bismuthate. After processing in the conventional manner[8] and recrystallizing from petroleum ether–acetate, the etioacid, exhibited m.p. 254–260°, $[\alpha]_D$ +218° (pyridine).

Anal. Calcd. for $C_{20}H_{28}O_4$: C, 72.26; H, 8.49. Found: C, 72.14; H, 8.69.

(12) G. W. Beadle and E. L. Tatum, *Am. J. Bot.*, **32**, 678 (1945).

(13) *Cf.* A. Zaffaroni and R. B. Burton, *J. Biol. Chem.*, **193**, 749 (1951), and earlier papers.

(14) Private communication from Dr. A. Zaffaroni, Syntex, S. A., Mexico, D. F.

JOINT CONTRIBUTION FROM
THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
SHREWSBURY, MASS., AND
DEPARTMENT OF CHEMISTRY
WAYNE UNIVERSITY
DETROIT, MICHIGAN

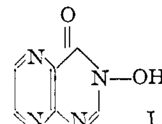
The Preparation of 3-Hydroxy-4-pteridinone

BY W. B. WRIGHT, JR., AND J. M. SMITH, JR.

RECEIVED MARCH 4, 1955

In recent years, a large number of hydroxy- and polyhydroxypteridines have been synthesized and characterized,[1] but no cyclic hydroxamic acid derivative has been described in the literature. In view of the antibacterial activity of cyclic hydroxamic acid derivatives of other heterocyclic systems[2] we have prepared such a derivative of pteridine.

The compound, 3-hydroxy-4-pteridinone (I), was



prepared by a modification of the method of Albert[3] for the preparation of 4-hydroxypteridine. A mixture of 3-aminopyrazinehydroxamic acid, acetic anhydride and ethyl orthoformate was heated at reflux and the acetate which was formed was hydrolyzed to I by heating with alkali.

3-Hydroxy-4-pteridinone decomposes at about 290°, the exact temperature depending somewhat upon the rate of heating. It can be recrystallized from hot water but is poorly soluble in cold water, acetone or hot ethanol. It dissolves easily in aqueous bicarbonate and is reprecipitated by the addition of acid. The *p*H of a saturated aqueous solution is 3, and the solution gives a green color with cupric salts and an amber color with ferric chloride. The ultraviolet spectra have been determined: in 0.1 *N* NaOH (λ_{max} 274 mμ, log ϵ 4.41) and in 0.1 *N* HCl (λ_{max} 240 mμ, log ϵ 4.12; λ_{max} 310 mμ, log ϵ 3.78).

Experimental

3-Aminopyrazinehydroxamic Acid.—A mixture of 15.3 g. of methyl 3-aminopyrazinoate, 10.4 g. of hydroxylamine hydrochloride and 250 ml. of 1 *N* sodium hydroxide was warmed for two hours at 35–50°. The clear solution was cooled to 30° and then was treated with 20 ml. of 5 *N* hydrochloric acid. The precipitate which separated was filtered and dried to yield 14.1 g. (92%). When this product was recrystallized from 35 parts of water, light yellow crystals which decomposed at 196° were obtained.

Anal. Calcd. for $C_5H_6N_4O_2$: C, 39.0; H, 3.92; N, 36.3. Found: C, 38.9; H, 4.07; N, 36.3.

3-Hydroxy-4-pteridinone (I).—A mixture of 11.0 g. of 3-aminopyrazinehydroxamic acid, 100 ml. of acetic anhydride and 100 ml. of ethyl orthoformate was heated under reflux for two hours. The clear solution was concentrated under reduced pressure to a brown residue and this was warmed at 70–75° for three minutes with 120 ml. of 1 *N* sodium hydroxide. After cooling in an ice-bath, the reaction mixture was treated with activated carbon and clarified. The filtrate was acidified to *p*H 2.5, and the precipitate was filtered, washed with a little water and then with acetone, and dried at 70°. The yield of 3-hydroxy-4-pteridinone, m.p. 287° dec., was 67%. Recrystallization from 50 parts of hot water raised the decomposition point to 290°.

Anal. Calcd. for $C_6H_4N_4O_2$: C, 43.9; H, 2.46; N, 34.1. Found: C, 44.1; H, 2.54; N, 34.0.

Acetate of 3-Hydroxy-4-pteridinone.—When the brown residue, obtained by concentrating the reaction mixture after the initial reflux period, was recrystallized twice from absolute alcohol, a crystalline product which melted at 172–

(1) A. Albert, *Fortsch. Chem. org. Naturstoffe*, **11**, 350 (1954).

(2) (a) J. D. Dutcher and O. Wintersteiner, *J. Biol. Chem.*, **155**, 359 (1944); (b) G. Dunn, *et al.*, *Nature*, **164**, 181 (1949); (c) S. R. Safir and J. H. Williams, *J. Org. Chem.*, **17**, 1298 (1952); (d) E. Shaw, THIS JOURNAL, **71**, 67 (1949); (e) A. Lott and E. Shaw, *ibid.*, **71**, 70 (1949); (f) G. T. Newbold and F. S. Spring, *J. Chem. Soc.*, 1864 (1948).

(3) A. Albert, D. J. Brown and G. Cheeseman, *ibid.*, 474 (1951).