Recrystallization from alcohol containing a drop of pyridine gave 52 mg. (80% recovery) of yellow plates, m.p. 144.5–145.5°. The analytical sample of the **2,4-dinitrophenylhydrazone** of **3-(4′-keto-1-cyclohexenyl)-4-(4′-hydroxycyclohexyl)-hexane** melted at 145.3–146.5° (sint. 144°), $\lambda_{max}^{CHCl_3}$ 263.5 mμ (24,600).

Anal. Calcd. for $C_{24}H_{34}O_5N_4$: C, 62.9; H, 7.47. Found: C, 62.6; H, 7.45.

Similar reductions in ether gave less reduction (22–34% starting material present from ultraviolet spectrum). After hydrolysis of such a reduction product (550 mg.) in 30 ml. of methanol and 15 ml. of 3 *N* hydrochloric acid kept for three hours at 25°, chromatography on acid-washed alumina gave 18% of material micro m.p. 77–86°, in the first eluates. This proved to be reduced and demethoxylated material, probably **3-(1′-cyclohexenyl)-4-(4′-hydroxycyclohexyl)-hexane (XI)**, colorless needles from dilute acetone, m.p. 94–94.5° (sint. 93°), no selective absorption in the ultraviolet.

Anal. Calcd. for $C_{18}H_{32}O$: C, 81.7; H, 12.20. Found: C, 81.8; H, 11.95.

MADISON 6, WIS.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Microbiological Transformations of Steroids. VIII. Preparation of 17α-Hydroxycorticosterone

BY FREDERICK R. HANSON, KINGSLEY M. MANN, ELDON D. NIELSON, HUGH V. ANDERSON, MARI P. BRUNNER, JOHN N. KARNEMAAT, DONALD R. COLINGSWORTH AND WILLIAM J. HAINES

RECEIVED JULY 13, 1953

Incubation of Reichstein's Compound S with *Cunninghamella blakesleeana* strain H-334 yielded 17α-hydroxycorticosterone.

Discussion

Previous papers in this series have reported the microbiological oxygenation of steroids by fungi of the order *Mucorales*.[1] In an earlier paper[2] details were given for the conversion of Reichstein's Compound S (11-desoxy-17α-hydroxycorticosterone) by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b) to 11α,-17α,21-trihydroxy-4-pregnene-3,20-dione (epi-Compound F). We have isolated a fungus, identified as *Cunninghamella blakesleeana* of the order *Mucorales* and designated strain H-334, which converts Compound S directly to the biologically active steroid, 17α-hydroxycorticosterone (Kendall's Compound F, hydrocortisone, Reichstein's Compound M). The description of this one-step fermentation is the subject of the present paper. A similar conversion with an Actinomycete, *Streptomyces fradiae* Waksman 3535, has been reported previously.[3]

Experimental[4]

Fermentation.—Ten liters of a soybean–dextrose medium[5] was added to a 5-gallon glass bottle fitted with a revolving stainless steel paddle and an aluminum tube which delivered sterile air to the bottom of the vessel. After autoclaving at 120° for 45 minutes, the medium was inoculated with 500 ml. of vegetative mycelium[6] of *C. blakesleeana* H-334 and the mixture was incubated at constant temperature (26°), aeration (1 l. air/min.), and agitation (84 r.p.m.) for 24 hours.

At the end of this period of growth, 500 mg. of Reichstein's Compound S was added as a sterile ethanolic solution. The resulting concentration of ethanol in the fermentation mixture was 6%. The incubation was allowed to proceed for 16 hours.

Extraction.—After the mycelium was removed by filtration through a Celite[7] pad, the filtrate was extracted three times with 2-l. portions of ethylene chloride. Small amounts of steroids remaining in the mycelium were removed by further extractions in which the filter cake was stirred twice with 1-l. portions of ethylene chloride. All extracts were combined, and the solvent was removed[8] to give a crude steroid residue.

The presence of 17α-hydroxycorticosterone, 11-dehydro-17α-hydroxycorticosterone (cortisone) and unreacted Compound S in the crude steroid residue was indicated by the paper chromatographic techniques of Zaffaroni, *et al.*[9] The positions of the steroids on the papergram were determined by means of their absorption of ultraviolet light.[10] A quantitative determination of the 17α-hydroxycorticosterone present was made by eluting the steroid from the paper and measuring the absorption at 242 mμ. It was found that 35.2% of the Compound S had been converted to 17α-hydroxycorticosterone.

Chromatography.—The crude steroid residue from the fermentation was chromatographed first on a Florisil[11] adsorption column to effect the removal of extraneous lipids and secondly on a silica partition column to fractionate the steroidal components.

After the Florisil column (50 g. of adsorbent), bearing the crude steroid residue (about 3.0 g.), was washed with ethylene chloride (500 ml.) for removal of the extraneous lipids, the steroids of medium polarity were recovered by elution with 900 ml. of a mixture of ethylene chloride and acetone (2:1). This latter fraction (428.6 mg.), containing the 17α-hydroxycorticosterone, was separated into its constituent steroids by use of a partition column and an automatic chromatographic fraction cutter.[12,13] This column

(1) (a) Paper VII in this series: S. H. Eppstein, D. H. Peterson, H. Marian Leigh, H. C. Murray, A. Weintraub, L. M. Reineke and P. D. Meister, THIS JOURNAL, **75**, 421 (1953); (b) D. H. Peterson and H. C. Murray, *ibid.*, **74**, 1871 (1952); (c) 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); (d) H. C. Murray and D. H. Peterson, U. S. Patent 2,602,769 (July 8, 1952), based on an original application filed August 19, 1950.

(2) D. H. Peterson, S. H. Eppstein, P. D. Meister, B. J. Magerlein, H. C. Murray, H. Marian Leigh, A. Weintraub and L. M. Reineke, THIS JOURNAL, **75**, 412 (1953).

(3) (a) D. R. Colingsworth, M. P. Brunner and W. J. Haines, *ibid.*, **74**, 2381 (1952); (b) D. R. Colingsworth, J. N. Karnemaat, F. R. Hanson, M. P. Brunner, K. M. Mann and W. J. Haines, *J. Biol. Chem.*, **203**, 807 (1953).

(4) Melting points were taken on a Kofler micro hot stage.

(5) Soybean meal, 5 g.; dextrose, 20 g.; NaCl, 5 g.; K_2HPO_4, 5 g.; Brewers' yeast, 5 g.; tap H_2O, 1 l.; *p*H adjusted to 6.4.

(6) *C. blakesleeana* grown in soybean–dextrose medium on a reciprocating shaker at 26° for 48 hours.

(7) Diatomaceous earth produced by Johns-Manville, 22 East 40th Street, New York 16, New York.

(8) Solvents were always removed at 10 mm. pressure, under nitrogen, at temperatures less than 50°.

(9) A. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950).

(10) W. J. Haines and N. A. Drake, *Federation Proc.*, **9**, 180 (1950).

(11) Florisil is an activated magnesium silicate produced by the Floridin Company, 220 Liberty Street, Warren, Pennsylvania.

(12) W. J. Haines, N. A. Drake, C. D. Alway and M. P. Brunner, Abstracts of Papers, 118th Meeting Am. Chem. Soc., Chicago, Illinois, Sept. 1950, p. 11-M.

(13) W. J. Haines, "Recent Progress in Hormone Research," Vol. VII, Academic Press, Inc., New York, N. Y., 1952, p. 255.

composed of 30 g. of silica[14] impregnated with 21 ml. of ethylene glycol, was loaded with one-third (143 mg.) of the steroid mixture from the Florisil column and developed successively with mixtures of cyclohexane and methylene chloride in the following proportions: 10:1 (360 ml.)[15]; 2:1 (1,020 ml.) and 1:2 (1,620 ml.). A paper chromatographic examination of the ultraviolet absorbing bands recorded by the fraction cutter indicated the 17α-hydroxycorticosterone was eluted by the 1:2 cyclohexane–methylene chloride mixture. The remaining two-thirds of the steroid residue was similarly chromatographed and the 1:2 cyclohexane–methylene chloride effluents from the three columns were combined and concentrated to an ethylene glycol residue containing the 17α-hydroxycorticosterone. The residue was dissolved in 300 ml. of water and the steroid extracted with three 200-ml. portions of ethylene chloride. Following removal of the solvent, the residue was dissolved in acetone from which 17α-hydroxycorticosterone crystallized (112.2 mg., m.p. 193–195°). Recrystallization from methanol gave 98.8 mg. of a product which melted at 208–210°; $[\alpha]^{25}D$ +160° (*c* 0.324 in acetone).[16] The characterization of the compound as 17α-hydroxycorticosterone was confirmed by mixed melting point, infrared absorption data and paper chromatographic behavior.

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.54; H, 8.80.

The acetate of the above-mentioned 17α-hydroxycorticosterone was prepared in the usual manner with an acetic anhydride–pyridine mixture. This derivative melted at 212–215°; $[\alpha]^{24.5}D$ +159° (*c* 0.486 in dioxane). As judged by infrared absorption data, paper chromatographic behavior and the sulfuric acid chromogen test,[17] the 17α-hydroxycorticosterone 21-acetate was pure.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.98. Found: C, 68.21; H, 7.72.

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(14) Silica for chromatographic columns, manufactured by the G. Fredrick Smith Chemical Company, Columbus, Ohio.

(15) Ratios expressed in volumes of solvent before mixing. The solvent mixtures were saturated with ethylene glycol.

(16) T. Reichstein, *Helv. Chim. Acta*, **20**, 953 (1937).

(17) A. Zaffaroni, THIS JOURNAL, **72**, 3828 (1950).

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF DUKE UNIVERSITY]

Brominative Decarboxylation of Silver Salt of Apocamphane-1-carboxylic Acid[1]

BY PELHAM WILDER, JR., AND ANTHONY WINSTON[2]

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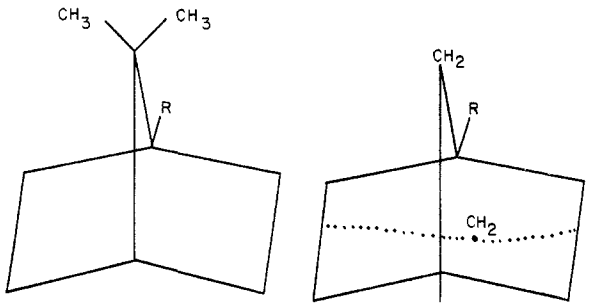
When silver apocamphane-1-carboxylate is brominated in hydrocarbon solution, 1-bromoapocamphane is obtained. When bromination is effected in carbon tetrachloride, a product containing both bromine and chlorine is obtained. The mechanism of the reaction is discussed in terms of these results.

When the silver salt of apocamphane-1-carboxylic acid (I) was treated with bromine in hydrocarbon solution, a neutral substance with a mildly camphoraceous odor was obtained. This substance which contained bromine was inert to alcoholic silver nitrate[3] and was easily reduced by sodium in alcohol to apocamphane (II).[4] It seems certain, then, that the product of the bromination of silver apocamphane-1-carboxylate is 1-bromoapocamphane (III).

Recently Arcus, Campbell and Kenyon reported that active silver α-phenylpropionate gave on reaction in carbon tetrachloride solution α-phenylethyl bromide with inversion of configuration accompanied by some racemization.[5,6] Arcus and his co-workers concluded that inversion of configuration was to be expected and that the reaction was the first established example of a class of replacement reactions in which a bimolecular substitution reaction occurs "with inversion of configuration effected by the electrophilic reagent positive bromine."[7]



I, R = -COOH
II, R = -H
III, R = -Br
IV, R = -Br
V, R = -COOH

These conclusions of Arcus, Campbell and Kenyon seem difficult to defend in light of the isolation of 1,3-dibromoadamantane (IV) by the smooth brominative decarboxylation of the silver salt of adamantane-1,3-dicarboxylic acid (V).[8] This dicarboxylic acid is of such structure that the approach to the rear of the carbon atom bearing the carboxyl group is blocked by the cage formed

(1) Presented in part at the Southeastern Regional Meeting of the American Chemical Society in Auburn, Alabama, October 25, 1952.

(2) Taken from a thesis submitted by Anthony Winston to the Graduate School of Duke University in partial fulfillment of the requirements for the degree of Master of Arts, May, 1952.

(3) (a) P. D. Bartlett and L. H. Knox, THIS JOURNAL, **61**, 3184 (1939); (b) W. von E. Doering and E. F. Schoenewaldt, *ibid.*, **73**, 2333 (1951).

(4) G. Komppa and T. Hasselstrom, *Ann.*, **496**, 164 (1932).

(5) C. L. Arcus, A. Campbell and J. Kenyon, *J. Chem. Soc.*, 1510 (1949).

(6) Arnold and Morgan have reported that the product of the reaction of bromine with (+)-α-ethylhexanoic acid was optically inactive [THIS JOURNAL, **70**, 4248 (1948)]. They did not determine whether an optically pure sample of the bromide would racemize under the conditions of the reaction. See also the case of bicyclo-[2,2,2]-octane-2-carboxylic acid of Doering and Farber [*ibid.*, **71**, 1514 (1949)].

(7) Reference 5, p. 1511.

(8) V. Prelog and R. Seiweith, *Ber.*, **74B**, 1769 (1941).