

phore.^{8,9} More detailed accounts of the above structural considerations will appear in subsequent publications.

(8) F. Bohlmann, *Chem. Ber.*, **84**, 735 (1951).

(9) E. R. H. Jones, personal communication.

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RECEIVED FEBRUARY 28, 1952

HYPOTENSIVE ALKALOIDS OF VERATRUM ESCHSCHOLTZII

Sirs:

In view of the present interest in the Veratrum Alkaloids as hypotensive agents, we wish to report the isolation from *Veratrum eschscholtzii* Gray of neogermitrine,¹ and a new alkaloid, for which we propose the name escholerine.

Preliminary fractionation of a crude chloroform extract which was based on the selective solubilities of the alkaloids and their salts, in conjunction with assays² for hypotensive activity in anesthetized dogs, yielded an amorphous fraction that accounted for the major part of the hypotensive activity in the crude extract. Further fractionation by two 8-plate Craig countercurrent distributions³ yielded two fractions, A and B, each with a high hypotensive activity. Fraction A was resolved on a 24-plate distribution using 2 *M* acetate buffer at *pH* 5.5 and benzene as the solvent system. Neogermitrine was obtained from the material recovered from tubes 8 to 13 by crystallizing from acetone-water (m.p. 234–234.8 (cor.)); $[\alpha]^{25}_D -79 \pm 2^\circ$, (*c* 0.9 in pyridine); the sample was further identified by comparison of its infrared spectrum, and by a mixed melting point with an authentic sample of neogermitrine from *Veratrum viride* Aiton kindly provided by Dr. J. Fried.

Fraction B was distributed on a 24-plate Craig apparatus, using 0.5 *M* acetate buffer *pH* 5.0 and benzene-cyclohexane 25:75 as the immiscible phases. The material recovered from tubes 8 to 13, when crystallized from acetone-water, yielded escholerine (m.p. 235–235.3 with dec. (cor.)); $[\alpha]^{25}_D -30 \pm 2^\circ$ (*c* 1.0 in py.); $+7 \pm 2^\circ$ (*c* 1.0 in CHCl_3). The analytical data indicate the empirical formula $\text{C}_{41}\text{H}_{61}\text{O}_{13}\text{N}$; (calcd. C, 63.46; H, 7.92; N, 1.80; eq. wt., 775.9; found: C, 63.42, 63.59; H, 8.00, 7.97; N, 2.04; eq. wt., 782, 772; picrate, m.p. 259.5° (dec.), $(\text{C}_{41}\text{H}_{61}\text{O}_{13}\text{N} \cdot \text{HOC}_6\text{H}_4(\text{NO}_2)_3$: C, 56.17; H, 6.42; found: C, 56.41; H, 6.38); aurichloride, m.p. 191.4° (frothing), $(\text{C}_{41}\text{H}_{61}\text{O}_{13}\text{N} \cdot \text{HAuCl}_4$: C, 44.13; H, 5.60; Au, 17.67; found: C, 44.53; H, 5.61; Au, 17.21). Volatile acid determination, found: 3.7 equivalents of acid.

Hydrolysis of escholerine with 0.1 *N* methanolic potassium hydroxide afforded acetic acid, α -methylbutyric acid and a base that has so far

resisted all attempts at crystallization. A mixture of the *p*-phenylphenacyl esters of the acids after chromatography on a silicic acid⁴ column, yielded *p*-phenylphenacyl acetate, m.p. 110.8–111.2° (calcd. C, 75.58; H, 5.55; found, C, 75.38; H, 5.66) and *p*-phenylphenacyl α -methylbutyrate, m.p. 70–71° cor. (calcd. C, 77.01; H, 6.80; Found: C, 76.61; H, 6.80).

The hypotensive activity⁵ of neogermitrine and escholerine in anesthetized dogs was found to be 0.13 μg . [0.12–0.15] and 0.30 μg . [0.26–0.36], respectively.

The isolation procedure has, in addition, yielded the alkaloids isorubijervine, jervine, rubijervine, pseudojervine and veratramine, already known as constituents of *Veratrum viride*, and small amounts of four apparently new crystalline alkalamines and a new ester alkaloid which will be described more fully in a subsequent publication.

(4) J. G. Kurchner, Arthur H. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **18**, 31 (1946).

(5) Expressed as micrograms per kilogram of dog per minute required for a ten minute intravenous infusion to lower the mean arterial blood pressure 30% when administered according to the method of G. L. Maison and J. W. Stutzman.¹ The bracketed numbers express the 95% confidence limits.

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RECEIVED JUNE 18, 1951

MICROBIOLOGICAL OXYGENATION OF STEROIDS AT CARBON 11

Sir:

It is generally acknowledged that the most difficult series of steps in the partial synthesis of cortisone is that concerned with the introduction of oxygen at carbon 11 of the steroid nucleus.^{1,2,3,4} We wish to report the oxygenation of steroids, *e.g.*, progesterone, at carbon 11 in a single step by means of common molds of the order *Mucorales* after a transformation period of 24–48 hours, in a lactalbumin digest-dextrose-cornsteep medium. Thus, from progesterone, a new 11-oxygenated steroid intermediate is made available for conversion to the cortical hormones. In these studies we have made use of the procedure of Zaffaroni, *et al.*,⁵ for characterization of the transformation products.

The ability of several micro-organisms to oxidize a hydroxyl group or reduce a ketone group in a steroid is well recognized,⁶ but heretofore the only microbiological oxygenation of a steroid carbon atom was reported by Krámlí and Horváth⁷ in the

(1) B. F. McKenzie, V. R. Mattox, L. L. Engel and E. C. Kendall, *J. Biol. Chem.*, **173**, 271 (1948).

(2) E. M. Chamberlain, W. V. Ruyle, A. E. Erickson, J. M. Chermada, L. M. Aliminos, R. L. Erickson, G. E. Sita and Max Tishler, *THIS JOURNAL*, **73**, 2396 (1951).

(3) L. F. Fieser, J. E. Herz and Wei-Yuan Huang, *ibid.*, **73**, 2397 (1951).

(4) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3546 (1951).

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

(6) M. Welsch and C. Heughelem, *Compt. rend. soc. biol.*, **142**, 1074–1076 (1948).

(7) A. Krámlí and J. Horváth, *Nature*, **160**, 639 (1947); **163**, 219 (1949).

(1) J. Fried and P. Numerof, Abst. 119th meeting A.C.S., Cleveland, Ohio, April, 1951, 12L.

(2) Assays were run according to the method of G. L. Maison and J. W. Stutzman, *Arch. Int. Pharmacodyn.*, **85**, 357 (1951). Evaluations were made at Boston University School of Medicine, Boston, Mass.

(3) J. Fried, H. White and O. Wintersteiner, *THIS JOURNAL*, **72**, 4621–4630 (1950).

Af 319-DHD-108; Bf 11-Ketoprogesterone.

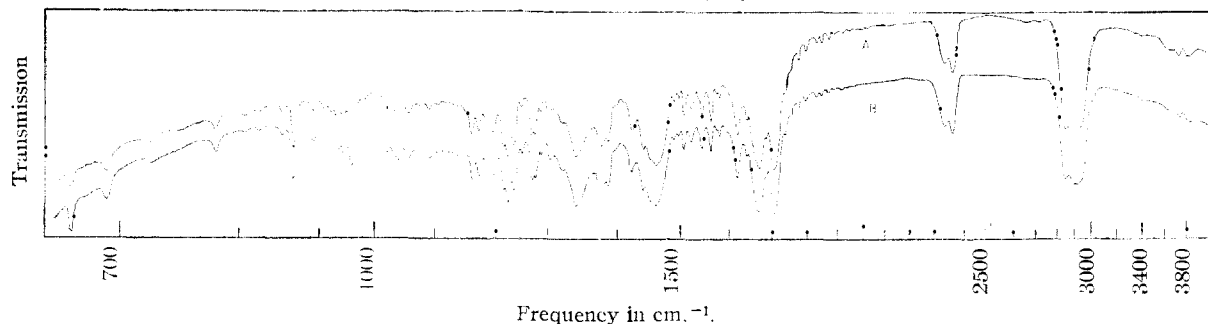
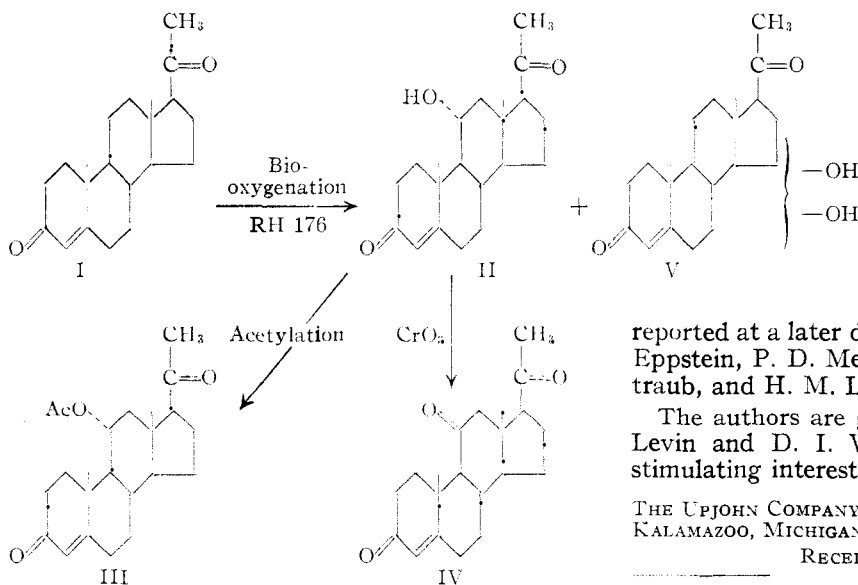


Fig. 1.—Infrared absorption spectra, liquid hydrocarbon mull, Perkin-Elmer Model 12C spectrophotometer: A, oxidation product of II; B, authentic 11-ketoprogesterone.

formation of a 7-hydroxycholesterol from cholesterol by *Proactinomyces roseus* or an *Azotobacter*.

After 1.0 g. of progesterone (I) had been subjected to the action of a species of *Rhizopus*, e.g., *Rhizopus arrhizus* (our strain RH 176), it was possible to isolate from the methylene chloride extract two new compounds. The first of these was a dihydroxyprogesterone (V), m.p. 245–248° (all melting points are uncorrected), $[\alpha]^{25}_D +144^\circ$ (pyridine)⁸; (Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.74. Found: C, 72.83; H, 8.67) in 2.3% yield. Acetylation of V yielded a diacetate (VI), m.p. 145–148° and 153–154°; $[\alpha]^{25}_D +71^\circ$ (absolute ethanol); (Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.80; H, 7.82). Infrared studies confirmed that V was a dihydroxyprogesterone and VI the diacetate of V. Further characterization of this compound will be reported at a later date.



Alumina chromatography of the concentrate from the mother liquors from V gave an additional 5% of V and a fraction which yielded a monohydroxy-

(8) We are grateful to W. A. Struck and his group for all rotations and microanalyses.

progesterone (II), m.p. 166–167°, $[\alpha]^{25}_D +179^\circ$ (chloroform); λ_{max}^{EtOH} 242 m μ , (log *E* 4.19); (Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.40; H, 9.15. Found: C, 76.77; H, 8.92) in 10% yield. From II was formed a monoacetate (III), m.p. 175–177°, $[\alpha]^{25}_D +143^\circ$ (acetone); (Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.33; H, 8.78).

Oxidation of the monohydroxyprogesterone (II) gave 11-ketoprogesterone (IV), m.p. 174–176°; $[\alpha]^{25}_D +227^\circ$ (chloroform); (Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.59; H, 8.55) identical in all respects with an authentic sample of 11-ketoprogesterone.⁹

Figure 1 shows a comparison of the infrared curves of the two 11-ketoprogesterones.¹⁰

Since Compound II has different physical properties than 11 β -hydroxyprogesterone¹¹ and readily forms an acetate, it is concluded that Compound II is 11 α -hydroxyprogesterone.

Similar microbiological oxygenations at carbon 11 using molds of the *Mucorales* order have been achieved on other steroid substrates, including androstenedione, 11-desoxy-17-hydroxycorticosterone (Reichstein's substance S), and 11-desoxycorticosterone. Complete details of this work will be reported at a later date in collaboration with S. H. Eppstein, P. D. Meister, L. M. Reineke, A. Weintraub, and H. M. Leigh of our Laboratories.

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(9) Kindly supplied by Dr. T. F. Gallagher, of the Sloan-Kettering Institute for Cancer Research, New York, N. Y.

(10) We are indebted to Dr. and Mrs. J. L. Johnson, Mrs. G. S. Fonken and L. Scholten for ultraviolet, infrared and X-ray diffraction studies.

(11) T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, **23**, 684 (1940).