anol was heated under reflux for one hour. Three milliliters of glacial acetic acid was added to the warm, yellow reaction solution, followed by sufficient hot water to induce crystallization. After cooling, the solution was filtered and the light yellow crystalline precipitate recrystallized from absolute ethanol. 3-Benzyl-6,7-diphenyl-4(3*H*)-pteridinone (I) (0.26 g., 25%) separated as colorless platelets from the warm ethanol; m.p. 248°. A mixed melting point with an authentic sample of I[1] showed no depression. Addition of a small amount of water to the ethanol filtrate and further cooling caused the separation of 0.19 g. of 3-amino-N-benzyl-5,6-diphenylpyrazinamide (III); m.p. 187°.

The mother liquor from the original reaction mixture above was diluted with an equal volume of water. A heavy, tacky yellow solid separated which was collected by filtration and extracted with 20 ml. of hot 1 *N* sodium hydroxide. Acidification of the filtrate precipitated 0.195 g. of unreacted 6,7-diphenyl-4(3*H*)-pteridinone (II), while repeated recrystallizations of the base-insoluble solid yielded an additional 0.11 g. (total yield 29%) of pure III.

In a second experiment, a mixture of 40 ml. of freshly prepared, anhydrous methanol, 0.793 g. (0.00264 mole) of 6,7-diphenyl-4(3*H*)-pteridinone, 0.301 ml. (0.00262 mole) of benzyl chloride and 0.148 g. (0.00264 mole) of potassium hydroxide was heated under reflux for 24 hours. By the end of this time, the reaction mixture was only faintly basic. Addition of a few drops of acetic acid to acidity followed by water caused the crystallization of light yellow crystals; yield 0.530 g.; m.p. 230–238°. Repeated recrystallizations from methanol gave 0.21 g. (20%) of pure I melting sharply at 248°.

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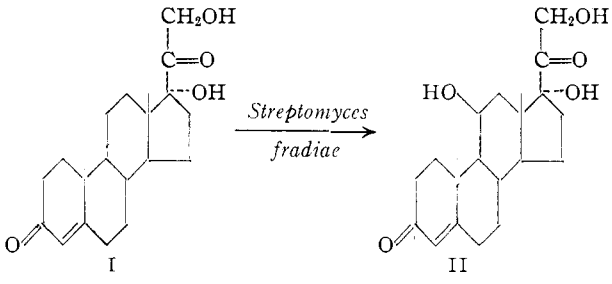
COMMUNICATIONS TO THE EDITOR

A PARTIAL MICROBIOLOGICAL SYNTHESIS OF ADRENAL CORTEX HORMONES

Sir:

It appears that either a hydroxyl group, having the *beta* configuration, or a ketone at the 11-position of the steroid nucleus is an obligatory structural requirement for the so-called carbohydrate-regulating hormone activity of the adrenal steroids, corticosterone, 11-dehydrocorticosterone, 17-hydroxycorticosterone and 11-dehydro-17-hydroxycorticosterone. Since both 11-desoxycorticosterone and 11-desoxy-17-hydroxycorticosterone are essentially devoid of this type of bioactivity, as measured by the Rat Liver Glycogen Deposition Assay,[1] it is possible to detect the introduction of an 11-β-hydroxyl group or an 11-keto group into these compounds by means of this assay.

We wish to report evidence for the microbiological oxygenation of these latter two steroids, with particular emphasis on the conversion of 11-desoxy-17-hydroxycorticosterone (Reichstein's compound S) (I) to 17-hydroxycorticosterone (Kendall's compound F, hydrocortisone) (II) by *Streptomyces fradiae*, Waksman's strain 3535.



Several species of *Streptomyces* were incubated with 100-mg. quantities of 11-desoxycorticosterone and 11-desoxy-17-hydroxycorticosterone. The quantitative measurement of glycogen deposition activity in the resulting beers[2] and calculation of this bioactivity in terms of a theoretical conversion to corticosterone and 17-hydroxycorticosterone gave values which varied from 1.4 to 5.8%.

In an experiment of somewhat larger scale 5.0 g. of I was incubated with *Streptomyces fradiae*, strain 3535, for 7 hours at 24° in rotary shaker flasks, using a medium containing dextrose, soybean meal and distillers' solubles. The total volume of the beer was 15 liters. A neutral hormone concentrate which was obtained from the beer by a standard procedure,[3,4] weighed 4.86 g. and possessed total bioactivity equivalent to 140 mg. of 17-hydroxycorticosterone. Evaluation of this material by paper chromatography[5,6,7] indicated the presence of II, a trace of 11-dehydro-17-hydroxycorticosterone and unreacted I.

One-half of the neutral hormone concentrate (2.43 g.) was subjected to automatic partition column chromatography.[8] The three adrenal steroids mentioned above were found in individual bands in the resulting chromatogram. The "17-hydroxycorticosterone band" weighed 110 mg. First crop crystals from acetone (22.8 mg.) were identified as II. Evidence for this characterization was afforded by long-term paper chromatography in which fermentation product moved at a rate identical with authentic II. In addition, a mixture of the fermentation product and authentic II could not be resolved under any of several conditions. Furthermore, the data from infrared spectroscopy,[9] as shown in Fig. 1, provided additional evidence for the identification of the crystalline product from

(1) M. L. Pabst, R. Sheppard and M. H. Kuizenga, *Endocrinology*, **41**, 55 (1947).

(2) We are indebted to Dr. K. J. Olson and his staff for the bioassays reported herein.

(3) M. H. Kuizenga, A. N. Wick, D. J. Ingle, J. W. Nelson and G. F. Cartland, *J. Biol. Chem.*, **147**, 561 (1943).

(4) W. J. Haines, R. H. Johnson, M. P. Goodwin and M. H. Kuizenga, *ibid.*, **174**, 925 (1948).

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

(6) R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951).

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

(8) 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.

(9) We are indebted to Dr. J. L. Johnson and his staff for the infrared data reported herein.

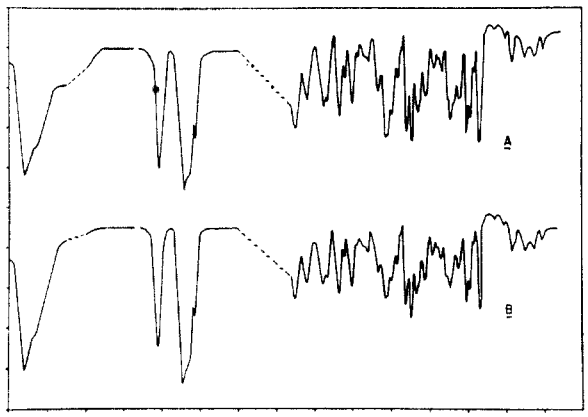


Fig. 1.—Infrared absorption spectra (Nujol mulls), Perkin Elmer spectrophotometer, model 12C: Curve A, authentic 17-hydroxycorticosterone; Curve B, crystalline product from *Streptomyces fradiae* conversion of 11-desoxy-17-hydroxycorticosterone.

the *Streptomyces fradiae* conversion as 17-hydroxycorticosterone.

The authors wish to acknowledge the helpful interest shown in this work by Dr. J. S. Evans.[10]

(10) Attention is directed to the microbiological oxidation of steroids at carbon 11, using fungi of the order *Mucorales*, as reported by Peterson and Murray, THIS JOURNAL, **74**, 1871 (1952).

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SYNTHETIC PREPARATION OF LIPOIC ACID

Sir:

Alpha lipoic acid, a catalytic agent, possessing pyruvate oxidation factor activity[1] has been obtained in crystalline form, and identified as a cyclic disulfide containing an *n*-octanoic acid carbon chain.[2,3] Physical data have been reported[3] which may be interpreted as follows: (a) *pKa* 4.7; no sulfur atom attached to carbon α or β to the carboxyl group, (b) lack of resolved methyl at 3.4μ; carbon 8 of the octanoic acid chain is probably substituted, (c) polarographic half-wave potential and hydrogen ion reduction potentials more nearly correspond to the values for 6-membered than to 5- or 7-membered disulfide rings, (d) $[\alpha]^{20}D$ +96.7; at least 1 center of asymmetry is indicated.

The following synthetic approach was used to confirm the presence of an 8-membered carbon chain in lipoic acid and to gain further insight into the location of the sulfur atoms. The 4-(α-tetrahydrofuryl)-butyric, 3-α-(α'-methyltetrahydrofuryl)-propionic, and 3-(α-tetrahydropyranyl)-propionic acids were prepared. These ether-acids were treated with hydrobromic acid and thiourea[4] to give thiouranium salts which were hydrolyzed without isolation to unstable dithioloctanoic acids, presumably 5,8-, 4,7- and 4,8-dithioloctanoic acids, respectively.

After spontaneous air oxidation in dilute solution, the preparations were assayed for biological activity in the pyruvate oxidation factor assay.[5] In one experiment, a 1-g. sample of each ether-acid was treated with one gram of thiourea and one milliliter of 40 per cent. hydrobromic acid in a sealed tube at 120° for ninety minutes, followed by hydrolysis with twenty-five milliliters of concentrated ammonium hydroxide at 120° for forty-five minutes in the presence of a trace of ferrous sulfate. The yields of pyruvate oxidation factor activity, "lipoic acid," were as follows

4-(α-Tetrahydrofuryl)-butyric acid	20,000 units
3-α-(α'-Methyltetrahydrofuryl)-propionic acid	500 units
3-(α-Tetrahydropyranyl)-propionic acid	250

Under similar conditions with a twelve-hour heating period, 0.5 g. of 4-(α-tetrahydrofuryl)-butyric acid gave 1,200,000 units of activity. These observations favor one of the optical isomers of the cyclic disulfide derived from 5,8-dithioloctanoic acid as the structure of α-lipoic acid.

The active material, obtained from 4-(α-tetrahydrofuryl)-butyric acid, in these and similar preparations, showed a behavior in the bioautographic[6] and counter-current[7] procedures characteristic of α-lipoic acid; including the formation of a more polar material[2] referred to as "β-lipoic acid." In the pyruvate oxidation factor assay, an excess (5 units) of the synthetic preparations and of crystalline α-lipoic acid obtained from liver each activated the assay maximally. Increasing levels of crystalline α-lipoic acid and of the synthetic preparations gave similar activity-concentration curves characterized by a K_m approximating 10^{-8} mole/liter by the dried cell assay method.[8]

(1) L. J. Reed, B. G. DeBusk, I. C. Gunsalus and C. S. Hornberger, Jr., *Science*, **114**, 93 (1951).

(2) L. J. Reed, B. G. DeBusk, I. C. Gunsalus and G. H. F. Schnakenberg, THIS JOURNAL, **73**, 5920 (1951).

(3) L. J. Reed, Q. F. Soper, G. H. F. Schnakenberg, S. F. Kern, H. Boaz and I. C. Gunsalus, *ibid.*, **74**, 2383 (1950).

(4) R. L. Frank and P. V. Smith, *ibid.*, **68**, 2103 (1946).

(5) I. C. Gunsalus, M. I. Dolin and L. Struglia, *J. Biol. Chem.*, **194**, 849 (1952).

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(8) I. C. Gunsalus and G. H. F. Schnakenberg, unpublished work.

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ZYGADENUS ALKALOIDS. I. VERATROYLZYGADENINE AND VANILLOYLZYGADENINE, TWO NEW HYPOTENSIVE ESTER ALKALOIDS FROM ZYGADENUS VENENOSUS

Sir:

The plant species *Zygadenus venenosus* has long been known to possess principles which are poisonous to livestock.[1] Some fifty years ago, the observation was made that these active principles possess pharmacological activity resembling that of the veratrum alkaloids.[2] In view of the recent interest

(1) U. S. Dep. Agr. Bull. 125 (1915); 1210 (1924); 1376 (1926).

(2) R. Hunt, *Am. J. Physiol.*, **6**, XIX (1902).