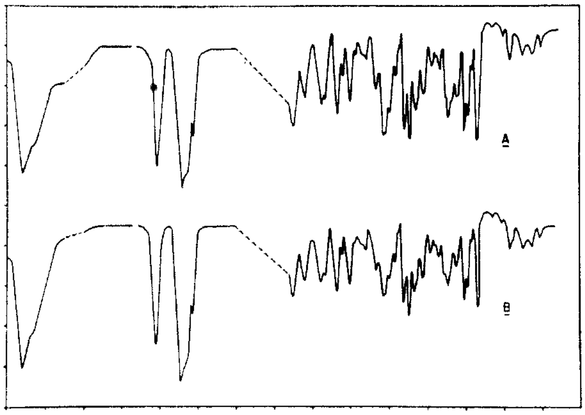

Fig. 1.—Infrared absorption spectra (Nujol mulls), Perkin Elmer spectrophotometer, model 12C: Curve A, authentic 17-hydroxycorticosterone; Curve B, crystalline product from *Streptomyces fradiae* conversion of 11-desoxy-17-hydroxycorticosterone.

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]

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(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) $[\alpha]^{20}\text{D}$ +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]

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(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).

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

(6) L. J. Reed, *et al.*, *J. Biol. Chem.*, **192**, 851 (1951).

(7) I. C. Gunsalus, L. Struglia and D. J. O'Kane, *ibid.*, **194**, 859 (1952).

(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

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

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