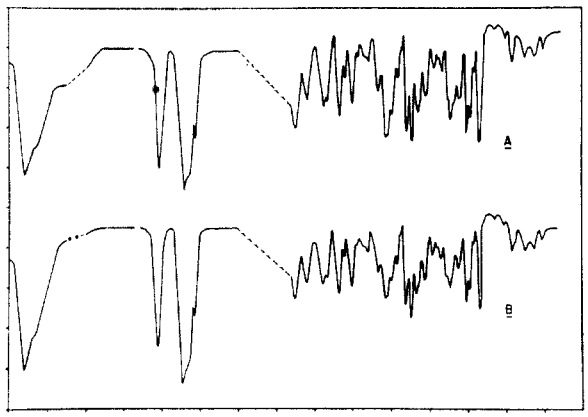

Fig. 1.—Infrared absorption spectra (Nujol mulls), Perkin Elmer spectrophotometer, model 12C: Curve A, authentic 17-hydroxycorticosterone; Curve B, crystalline product from *Streptomyces fradiae* conversion of 11-desoxy-17-hydroxycorticosterone.

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).

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

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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).

in the pharmacology of the veratrum alkaloids[3] and in the clinical use of the hypotensive activity of the tertiary ester alkaloids,[4] it appeared of importance to isolate and study the active principles of *Zygadenus venenosus*.

Heyl and co-workers[5] isolated the first crystalline alkaloid in this series, zygadenine. It appeared unlikely, however, that zygadenine is the most important toxic agent in *Zygadenus venenosus*.[5a]

The probable presence in *Zygadenus venenosus* of ester alkaloids similar to the tertiary alkamine ester alkaloids of the veratrum series has been indicated by the pharmacodynamic properties of the alkaloidal mixtures.[6] We have now isolated two of the ester alkaloids responsible for this activity.

The chloroform-extractable alkaloids of *Zygadenus venenosus* (WATS)[7] were subjected to simplified 8-plate countercurrent distribution patterned on the procedure of Fried, White and Wintersteiner.[4b] Benzene and phosphate buffer of *p*H 7.1 were used as solvents. Veratroylzygadenine separated from a solution of the crude plate 8-fraction in acetone; germine and zygadenine were obtained from the plate 0-fraction.

Veratroylzygadenine crystallized from absolute ethanol as rectangular prisms, m.p. 270–271° dec.; $[\alpha]^{20}D$ −27° (*c* 2.08, chf.); $\lambda_{max.}^{alc.}$ 262, 293 mμ (log ε 4.13; 3.85). *Anal.* Calcd. $C_{36}H_{51}O_{10}N$: C, 65.73; H, 7.82; N, 2.13. Found: C, 65.90; H, 7.86; N, 2.09. Alkaline hydrolysis of veratroylzygadenine yielded veratric acid and a base isomeric with zygadenine, pseudozygadenine. This base was also obtained by similar alkaline treatment of zygadenine. Pseudozygadenine crystallized from ethyl acetate–petroleum ether as needles, m.p. 169–171° dec.; $[\alpha]^{25}D$ −33° (*c* 2.00, chf.). *Anal.* Calcd. $C_{27}H_{43}O_7N$: C, 65.69; H, 8.78; N, 2.84. Found: C, 65.46, 65.79; H, 9.10, 8.69; N, 2.95. Acetylation with acetic anhydride and pyridine gave pseudozygadenine triacetate which crystallized from ether as rhomboids, m.p. 235–236° dec.; $[\alpha]^{23}D$ −33° (*c* 1.89, chf.). *Anal.* Calcd. for $C_{27}H_{40}O_7N(COCH_3)_3$: C, 63.95, H, 7.97; acetyl, 20.84. Found: C, 64.13; H, 8.11; acetyl, 20.69.

The filtrate obtained by removal of veratroylzygadenine from the plate 8-fraction was lyophilized and the residue was dissolved in chloroform and chromatographed on acid-washed alumina. Vanilloylzygadenine crystallized from an ethanol solution of the most difficultly eluted fractions as rods, m.p. 258–259° dec.; $[\alpha]^{20}D$ −27.5° (*c* 2.00, chf.); $\lambda_{max.}^{alc.}$ 264, 294 mμ (log ε 4.07, 3.83). *Anal.* Calcd. $C_{35}H_{49}O_{10}N$; C, 65.30; H, 7.67; N, 2.18; 1 OCH_3, 4.82. Found: C, 65.35; H, 7.93; N, 2.29; OCH_3, 4.34. Alkaline hydrolysis yielded vanillic acid and pseudozygadenine. Methylation of vanilloylzygadenine with diazomethane gave veratroylzygadenine, identical with an authentic sample by m.p., mixed m.p. and infrared spectrum.

The two ester alkaloids and zygadenine were examined by Professor O. Krayer at Harvard Medical School for their circulatory action in the anesthetized cat, their effect upon the failing heart in the heart-lung preparation of the dog, and their effect upon the amphibian skeletal muscle. In all three types of experiments, the actions of zygadenine were similar to those of cevine, and the actions of veratroylzygadenine and vanilloylzygadenine were similar, quantitatively and qualitatively, to the actions of the cevine ester, veratridine.

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(4) (a) E. Meilman and O. Krayer, *Circulation*, **1**, 204 (1950); (b) J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **72**, 4621 (1950).

(5) (a) F. W. Heyl, F. E. Hepner and S. K. Loy, *ibid.*, **35**, 258 (1913); (b) F. W. Heyl and M. E. Herr, *ibid.*, **71**, 1751 (1949).

(6) S. Yaffe and S. M. Kupchan, *Federation Proc.*, **9**, 326 (1950).

(7) Plant gathered in Washington in June, 1950. We are grateful to Dr. Reed Rollins, Grey Herbarium, Harvard University, for confirming the identity of the plant.

IDENTIFICATION OF THE CARBON SKELETON OF α-LIPOIC ACID

Sir:

It has been reported[1] recently that α-lipoic acid, a catalytic agent required for oxidative decarboxylation of pyruvic acid by certain lactic acid bacteria, is a monocarboxylic acid, *pKa* 4.7, containing a disulfide linkage and possessing the empirical formula $C_8H_{14}O_2S_2$. Infrared absorption spectrum failed to show the presence of any carbon–carbon double bonds. These data indicate that α-lipoic acid is a cyclic disulfide. The *pKa* value indicates that the sulfur atom is not attached to the carbon atoms α- or β- to the carboxyl group. Comparison of the polarography of α-lipoic acid with that of several dithiols and cyclic and linear disulfides also indicated its structure to be that of a cyclic disulfide. The presence of a six-membered ring in α-lipoic acid was suggested by the following observation. The catalysis of hydrogen ion reduction at the dropping mercury electrode by the reduced form of α-lipoic acid resembled that of 1,4-dithiols more than 1,3- or 1,5-dithiols.

Even with these limitations, the number of structures possessing the empirical formula of α-lipoic acid is considerable. It was therefore of paramount importance to determine the nature of the carbon skeleton. Assuming the cyclic disulfide nature of the molecule, much information could be obtained by the desulfurization of the substance. A 3-mg. sample was subjected to treatment with Raney nickel to remove the sulfur atoms.[2] The product was isolated as the crystalline silver salt. A comparison of the X-ray powder diagrams of this material with that of silver *n*-caprylate[3] revealed that the two samples were identical. The silver

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