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Disulfide Polymers of DL-α-Lipoic Acid

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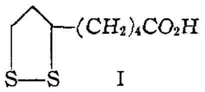
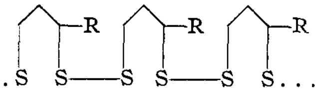
Evidence is presented for a linear disulfide structure of polymeric material produced when DL-α-lipoic acid is heated. The polymeric material is converted rapidly to DL-α-lipoic acid in the presence of dilute sodium hydroxide. The mechanism of this reaction is discussed.

It has been observed[1] that sticky, colorless polymers are produced as by-products in the oxidation of DL-6,8-dithioloctanoic acid to the cyclic disulfide, DL-α-lipoic acid (I). The product was separated from the polymeric material by extraction with benzene. During subsequent purification of the crude I by fractional crystallization, additional polymeric material was produced. Formation of polymeric materials under similar conditions has been noted also in the preparation of analogs of α-lipoic acid.[2] These analogs, as well as α-lipoic acid, contain the 1,2-dithiolane moiety, and their tendency to polymerize when in the fluid state is undoubtedly a reflection of the instability of this moiety.[3] Since the amount of polymeric material produced in these syntheses may be as much as that of the desired product, it was of practical interest to determine how the latter could be obtained from the polymer. For this purpose a polymeric material produced by heating pure I was employed. This paper presents data concerning the structure of this polymeric material, and describes a novel reaction by means of which the polymer is converted in high yield to DL-α-lipoic acid.


I

The ultraviolet absorption spectrum of the polymeric material resembled that of saturated organic disulfides,[4,5] characterized by a relatively weak and rather broad maximum near 250 mμ. The spectrum showed only low end absorption[6] in the 330 mμ region, where α-lipoic acid exhibits an absorption maximum.[1,3] The infrared spectrum of the polymer showed an absorption maximum at 5.84 μ, which is characteristic of a carboxyl group. The polymer was reduced rapidly in ethanol solution at 0° with zinc and hydrochloric acid. The product was not isolated, but it was converted to DL-α-lipoic acid in high yield by oxidation with iodine, suggesting that it was 6,8-dithioloctanoic acid. The polymer was converted slowly to DL-α-lipoic acid by heating it under conditions which permitted removal of the cyclic disulfide by distillation. These results are consistent with the interpretation that the polymer consists of linear disulfides (II).


II

R = $-(CH_2)_4CO_2H$

The polymer dissolved slowly in dilute sodium hydroxide at room temperature (28°) and rapidly at 100° to give a yellow solution which exhibited the characteristic ultraviolet absorption spectrum of α-lipoic acid. Crystalline DL-α-lipoic acid was isolated in 70% yield from the alkaline solution. Similar results were obtained with polymeric byproducts[1] from the oxidation of DL-6,8-dithioloctanoic acid to DL-α-lipoic acid. Also, as shown in the following papers,[2] polymeric materials obtained in the preparation of analogs of α-lipoic acid are converted to monomeric cyclic disulfides in the presence of dilute alkali. A survey of the literature revealed a previous report[7] that steam distillation of aqueous dispersions of certain polymeric disulfides produced monomeric cyclic disulfides at an extremely slow rate, which was significantly increased in the presence of small amounts of sodium hydroxide. However, no quantitative data were presented by the authors.

To study the mechanism of this reaction, ethanol solutions of the polymer were employed. At pH 12 and room temperature conversion of the polymer to DL-α-lipoic acid proceeded to the extent of 70–80% in five minutes and 80–90% in 30 minutes. The reaction rate was slower at less alkaline pH values and did not proceed to a measurable extent in the absence of alkali. Some variation in the reaction rates was observed with different batches of polymer, due probably to variation in the composition of the polymer. It was difficult to reproduce the experimental conditions for thermal polymerization of DL-α-lipoic acid, since a heterogeneous system, consisting of polymer and molten and solid monomer, is produced as the monomer melts.

In the presence of a small amount of DL-6,8-dithioloctanoic acid, conversion of the polymer to DL-α-lipoic acid at pH 12 proceeded to the extent of 80–90% in five minutes. That depolymerization under alkaline conditions is catalyzed by a trace of mercaptide ion was indicated further by the observation that a small amount of sodium p-chloromercuribenzoate completely inhibited the reaction and this inhibition was reversed by an amount of DL-6,8-dithioloctanoic acid in slight excess of the amount of sodium p-chloromercuribenzoate employed. The mechan ism of the reaction appears to be that of an anionic exchange or displacement

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[1] L. J. Reed and C-I. Niu, THIS JOURNAL, **77**, 416 (1955).

[2] R. C. Thomas and L. J. Reed, *ibid.*, **78**, 6150 (1956).

[3] J. A. Barltrop, P. M. Hayes and M. Calvin, *ibid.*, **76**, 4348 (1954).

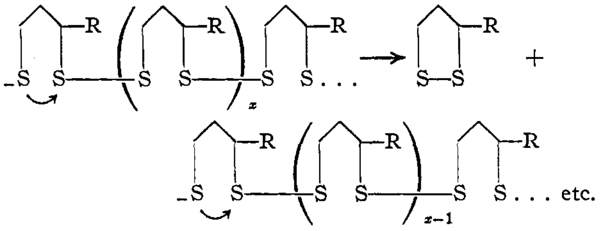
[4] H. P. Koch, *J. Chem. Soc.*, 394 (1949).

[5] J. E. Baer and M. Carmack, THIS JOURNAL, **71**, 1215 (1949).

[6] Approximately 6% of the maximum absorption of DL-α-lipoic acid.

[7] F. O. Davis and E. M. Fettes, THIS JOURNAL, **70**, 2611 (1948).

reaction of mercaptide ion with disulfide linkages



A similar mechanism has been suggested previously[8–10] to explain the reduction of disulfides with mercaptans and also disulfide interchange reactions. The trace of mercaptide ion necessary to catalyze the conversion of polymer to monomeric cyclic disulfide might have been present as thiol in the polymer[11] or might have been produced by hydrolysis.

Experimental[12]

Thermal Polymerization of DL-α-Lipoic Acid.—Crystalline DL-α-lipoic acid (5.0 g.) was melted at 65° and kept at this temperature for 15 minutes. The molten material was cooled and extracted repeatedly with benzene at room temperature until the extracts were colorless. Approximately 2.6 g. of a sticky, pale yellow polymeric material remained. To remove traces of DL-α-lipoic acid the polymer was stirred with a small amount of acetone and to this mixture benzene was added. A colorless, stringy material separated, which was dried *in vacuo*. The polymer was insoluble in most of the common organic solvents, and slightly soluble in ethanol. Prolonged stirring was necessary to dissolve the polymer.

Reduction of Polymer.—To a solution of 217 mg. of polymer in 21.7 ml. of 95% ethanol was added 1.0 g. of zinc dust and 2 ml. of concentrated hydrochloric acid. The mixture was stirred at 0° for 20 minutes, then filtered. The filtrate was titrated with a standard iodine solution; 1.87 meq. (89%) of iodine required. The oxidized solution was poured into 100 ml. of water and the mixture was extracted with two 15-ml. portions of chloroform. The yellow chloroform extracts were combined and dried over anhydrous sodium sulfate. The solvent was removed carefully *in vacuo* and the yellow semi-solid residue was leached with two 1-ml. portions of benzene. To the combined benzene extracts was added 15 ml. of Skellysolve B.[13] The resulting solution was seeded with a few crystals of DL-α-lipoic acid[1] and placed in a deep-freeze for several hours. Yellow crystals (116 mg.) separated; m.p. 60–61°. From the mother liquor there was obtained an additional 48 mg. of yellow crystals, m.p. 60–61°. The total yield of crystalline product was 164 mg. (75%). It possessed the characteristic ultraviolet absorption spectrum[1,3] of α-lipoic acid, $\lambda_{\min}^{95\%\ \text{ethanol}}$ 280 mμ, $\lambda_{\max}$ 332 mμ (ϵ 148). Mixed m.p. with an authentic sample of DL-α-lipoic acid[1] (m.p. 61–62°) was 60–61°.

Thermal Depolymerization of Polymer.—One milliliter of a 2% solution of polymer in absolute ethanol was evaporated carefully in a small sublimation apparatus, which was then heated at 120° (0.1 μ) for 1.5 hours. During this period 9.2 mg. (46%) of a yellow solid collected on the cold finger; m.p. 60–61°. Its ultraviolet absorption spectrum was identical with that of DL-α-lipoic acid,[1,3] $\lambda_{\min}^{95\%\ \text{ethanol}}$ 280, $\lambda_{\max}$ 332 mμ (ϵ 151). The colorless residue in the sublimation apparatus was warmed with 5 ml. of 0.5 *N* sodium hydroxide to convert it to DL-α-lipoic acid (see below). Spectrophotometric examination of the solution at 332 mμ showed that 7.8 mg. of DL-α-lipoic acid was present.

Conversion of Polymer to DL-α-Lipoic Acid with Dilute Alkali.—A mixture of 662 mg. of polymer and 15 ml. of 0.5 *N* sodium hydroxide was warmed on a steam-bath for 15 minutes. During this period the polymer gradually dissolved to give a bright yellow solution. From this solution was isolated, in a manner described previously,[1] 465 mg. (70%) of yellow crystals, m.p. 62–63°, mixed m.p. with an authentic sample of DL-α-lipoic acid[1] (m.p. 62–63°) 62–63°; $\lambda_{\min}^{95\%\ \text{ethanol}}$ 280 mμ, $\lambda_{\max}$ 332 mμ (ϵ 150).[1,3]

To 0.5 ml. of a 1% solution of polymer in 95% ethanol was added 0.5 ml. of 0.0984 *N* aqueous sodium hydroxide.[14] The clear solution became yellow immediately. At 5- and 30-minute intervals 0.3-ml. aliquots of the reaction mixture were diluted to 3.0 ml. with 50% ethanol and spectrophotometric readings taken at 332 mμ. In several control experiments the ultraviolet absorption spectra of the reaction mixtures were shown to be identical with that of DL-α-lipoic acid. To confirm the identity of the reaction product, it was isolated and characterized as follows. To a solution of 545 mg. of polymer in 54.5 ml. of 95% ethanol was added 54.5 ml. of 0.0984 *N* sodium hydroxide. The resulting yellow solution was allowed to stand at room temperature for 30 minutes and then was poured into 100 ml. of water. Two milliliters of 6 *N* hydrochloric acid was added and the mixture was extracted with two 15-ml. portions of chloroform. The yellow chloroform extracts were worked up as described in a preceding experiment to yield 422 mg. (77%) of yellow crystals, m.p. 61–62°; mixed m.p. with an authentic sample of DL-α-lipoic acid[1] (m.p. 61–62°) 61–62°; $\lambda_{\min}^{95\%\ \text{ethanol}}$ 280 mμ, $\lambda_{\max}$ 332 mμ (ϵ 150).

Inhibition of Alkaline Conversion with Sodium *p*-Chloromercuribenzoate and Reversal with DL-6,8-Dithioloctanoic Acid.—To 0.5 ml. of 0.0984 *N* aqueous sodium hydroxide containing 0.4×10^{-3} *M* sodium *p*-chloromercuribenzoate was added 0.5 ml. of a 1% solution of polymer in 95% ethanol. The resulting solution (A) remained colorless during a 10-minute period. At that time 0.5 ml. of the solution was treated with 0.1 ml. of a 1×10^{-3} *M* solution of DL-6,8-dithioloctanoic acid[1] in 95% ethanol. The resulting solution (B) became yellow immediately. After 10 minutes solution B was diluted to 3.0 ml. with 50% ethanol and its ultraviolet absorption spectrum determined against a control solution containing all of the chemicals employed except polymer. There was an absorption maximum at 332 mμ which showed 10.5 μ*M* (86%) of DL-α-lipoic acid was present. The spectrum of solution A, determined at 30 minutes against a control solution containing sodium hydroxide and sodium *p*-chloromercuribenzoate showed only low end absorption in the 332 mμ region.

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[8] M. Calvin, in "Glutathione," Academic Press, Inc., New York, N. Y., 1954, p. 16.

[9] C. Huggins, D. F. Tapley and E. V. Jensen, *Nature*, **167**, 592 (1951).

[10] F. Sanger, *ibid.*, **171**, 1025 (1953).

[11] Micro-titration of a solution of 20 mg. of polymer in absolute ethanol with a standard iodine solution revealed the presence of 4.9×10^{-5} milliequivalent of oxidizable material in the polymer.

[12] Ultraviolet absorption spectra were determined with a Beckman model DK-2 recording spectrophotometer. Measurements at 332 mμ were made with a Beckman model DU spectrophotometer. Melting points are uncorrected.

[13] A *n*-hexane fraction, b.p. 60–68°, obtained from the Skelly Oil Co., Kansas City, Missouri.

[14] The results were essentially the same when the order of addition was reversed. When the effect of DL-6,8-dithioloctanoic acid was studied, 0.03 ml. of 1×10^{-2} *M* solution of this substance in 95% ethanol was added to the 0.5-ml. aliquot of polymer solution prior to addition of alkali.