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Properties and Derivatives of α-Lipoic Acid

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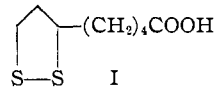
Several observations are recorded concerning the behavior of α-lipoic acid (I) in solution or in a fluid state. The preparation of the benzhydrylammonium salts of (+)-, (−)- and DL-α-lipoic acid is described, and the synthesis of DL-α-lipoamide (IV) and DL-α-lipol (V) from the intermediate acid chloride, DL-α-lipoyl chloride (III), is reported.

A study of the chemistry of α-lipoic acid (I) has revealed several interesting aspects of the behavior of the 1,2-dithiolane moiety. A study of the role



I

of α-lipoic acid in the photosynthetic cycle led to a systematic survey of five-, six- and seven-membered cyclic disulfides.[1] The results of this study pointed up the unique reactivity and instability of 1,2-dithiolane (II).

II

In the course of work in these laboratories on the synthesis[2] of racemic and optically active α-lipoic acid, several interesting observations were recorded on the stability and reactivity of these compounds. This paper presents these data and interprets them in the light of findings with 1,2-dithiolane. In addition, the preparation of several salts and derivatives of α-lipoic acid is described.

The instability of α-lipoic acid was first noted during the purification of synthetic material by fractional crystallization.[3] The extent of purification was determined from the ultraviolet absorption spectra[4] of the various fractions. Significant changes were noted when crude liquid samples of DL-α-lipoic acid were allowed to stand at room temperature for several days. These samples were soluble oils which on standing turned to viscous insoluble resins. This change was accompanied by a change in ultraviolet absorption characterized by decreasing absorption at 330 mμ and increasing absorption in the 250 mμ region.

Pure α-lipoic acid in solution or as a melt followed a similar pattern. When pure crystalline DL-α-lipoic acid was melted and allowed to stand at 65° for 5 minutes, the product was no longer completely soluble in cyclohexane. The cyclohexane-insoluble fraction was a sticky elastomer. Although α-lipoic acid polymerizes when heated, it has been purified by distillation.[5,6] This method of purification has been used to advantage in this Laboratory. In short-path distillations where the distillate solidifies rapidly, pure DL-α-lipoic acid is obtained. When the distillate is collected in such a manner that it remains liquid, little or no monomer is obtained. In a distillation where the distillate was collected in a relatively warm (*ca.* 70°) portion of the receiver, only an insoluble rubbery mass was obtained.

The behavior of pure α-lipoic acid in solution was studied by following the change in the ultraviolet absorption of the solution over a several-day period. The absorption spectrum of a cyclohexane solution[7] of (−)-α-lipoic exposed to ordinary light over a 54-hour period was compared with that of an identical solution protected from light. The absorption spectrum of the solution stored in the dark remained unchanged during this period. In the same interval the ultraviolet absorption spectrum of the solution exposed to ordinary light underwent a slow change[8] characterized by decreasing absorption at 330 mμ and increasing absorption in the 250 mμ region. In ether solution[9] the change in ultraviolet absorption on exposure to light was more pronounced. These results are shown in Fig. 1. In the 50-hour period, the solution protected from light showed no change in ultraviolet absorption. The solution exposed to light, however, shows the characteristic decreasing absorption at 330 mμ and increasing absorption in the 250 mμ region.

When either benzene or ether solutions[10] of pure α-lipoic acid were exposed to ordinary light and concentrated *in vacuo*, benzene-insoluble residues were obtained. The ultraviolet absorption spectra of methanol solutions of these waxy residues showed high end absorption below 300 mμ and little or no absorption in the 330 mμ region.

(1) M. Calvin and J. A. Barltrop, THIS JOURNAL, **74**, 6153 (1952); J. A. Barltrop, P. M. Hayes and M. Calvin, *ibid.*, **76**, 4348 (1954); M. Calvin, *Federation Proc.*, **13**, 697 (1954).

(2) E. Walton, A. F. Wagner, L. H. Peterson, F. W. Holly and K. Folkers, THIS JOURNAL, **76**, 4748 (1954); E. Walton, A. F. Wagner, F. W. Bachelor, L. H. Peterson, F. W. Holly and K. Folkers, *ibid.*, **77**, 5144 (1955).

(3) In the final step of the synthesis, 6,8-dithioloctanoic acid is oxidized to the cyclic disulfide. This oxidation also yields linear disulfides, but the majority of these by-products are left behind by extracting the α-lipoic acid with hot cyclohexane. The product is further purified by recrystallization from cyclohexane.

(4) Characteristic ultraviolet absorption maxima have been found (reference 1) for linear disulfides and the five-, six- and seven-membered cyclic disulfides. The absorption maximum for linear disulfides is located at 250 mμ; the maxima of the cyclic disulfides is progressively displaced toward longer wave lengths as the size of the ring decreases. The ultraviolet absorption spectrum of α-lipoic acid is characterized by a peak at 330 mμ.

(5) M. W. Bullock, J. A. Brockman, Jr., E. L. Patterson, J. V. Pierce, M. H. von Saltza, F. Sanders and E. L. R. Stokstad, THIS JOURNAL, **76**, 1828 (1954).

(6) The yields on distillation are good. Since we have noted extensive polymerization when α-lipoic acid is heated above the melting point, it appears that the polymer is thermally depolymerized during distillation with equilibrium being displaced by distillation of the monomer.

(7) Concentration 0.5 mg./ml.

(8) In the 54-hour period the $E^{1\%}_{1cm.}$ of the solution decreased from 7.6 to 7.0 at 330 mμ and increased from 3.8 to 5.3 at 250 mμ.

(9) Concentration 0.5 mg. of DL-α-lipoic acid/ml. of ether (Anhydrous Merck), sodium-dried and stored over sodium.

(10) Concentration 5–10 mg./ml.

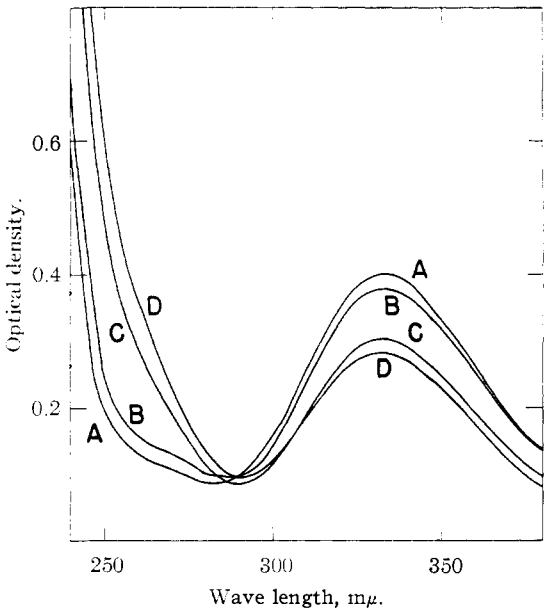


Fig. 1.—The influence of light on ether solutions of DL-α-lipoic acid: A, dark 0–50 hours; B, daylight 6 hours; C, daylight 14 hours; D, daylight 21 hours.

The thermal instability of pure α-lipoic acid in aqueous solutions at various *p*H's has also been observed. These conditions are of practical interest if optimal conditions for the extraction of α-lipoic acid from natural materials are to be established. Since in these experiments the heating was done by autoclaving, the influence of exposure of the solution to light was automatically minimized.

When solutions containing 1.7 mg. of pure (+)-α-lipoic acid per ml. of 2 *N* hydrochloric acid were autoclaved at 15 lb. pressure for 3 hr., all pyruvic acid oxidation factor activity[11] could be recovered within the error of the method. If, on the other hand, highly diluted solutions, containing 8 to 33 mγ of (+)-α-lipoic acid per ml. of 2 *N* hydrochloric acid, were similarly heated, less than 10% of the original activity was recovered. Apparently a constant small amount of α-lipoic acid was lost under these conditions. This amount was too small to be measurable when the sample was in the mg. range, but it was large enough to lead to the loss of most of the α-lipoic acid when the sample was in the mγ range. Albumin protects α-lipoic acid to some extent under these conditions since it was observed that 50–70% of the added α-lipoic acid was recovered when samples in the mγ range were autoclaved in the presence of 2% albumin.

Autoclaving in 0.1 or 2 *N* sodium hydroxide in open test-tubes at 15 lb. pressure for 3 hr. destroyed about 80% of the pyruvic acid oxidation factor activity in solutions containing (+)-α-lipoic acid either in the mg. or mγ concentration range. If, however, 0.1 *N* sodium hydroxide solutions in either concentration range were sealed in test-tubes under an atmosphere of nitrogen and then autoclaved, quantitative recoveries of pyruvic acid oxidation factor activity were obtained. The recoveries using 2 *N* sodium hydroxide under nitrogen were somewhat erratic, averaging about 80% of the added activity. From these data on the stability of (+)-α-lipoic acid in highly diluted aqueous solutions, it appears that autoclaving with 0.1 *N* alkali under nitrogen would be the safest condition for liberating pyruvic acid oxidation factor activity from biological material.

In the solid state α-lipoic acid appears to be stable. In the cases noted above the instability of the compound was apparent only when it was in a dissolved or fluid state. It appears, therefore, that once the crystal lattice of α-lipoic acid is broken the molecules tend to polymerize under certain conditions. These conditions must provide for opening the 1,2-dithiolane ring of α-lipoic acid. The necessary energy for opening or at least loosening the disulfide bond comes from the absorption of light in the visible or on the edge of the visible spectrum.[1] Once these bonds are activated, the molecules in a non-rigid state tend to polymerize.

Impure samples of α-lipoic acid were also purified by conversion to amine salts. The benzhydrylammonium salts of (+)-, (−)- and DL-α-lipoic acid were excellent in this respect. They precipitated from ether readily and were analytically pure after one recrystallization.[12] DL-α-lipoic acid could also be purified by precipitation with *l*-ephedrine. Several recrystallizations of the salt from *l*-ephedrine and DL-α-lipoic acid failed to effect even partial resolution.[13] The salt from *l*-ephedrine and DL-α-lipoic acid and the benzhydrylammonium salts of (+)- and DL-α-lipoic acid were fully active (calcd. for (+)-α-lipoate ion) in the enzymatic POF[11] assay. The regeneration of DL-α-lipoic acid from either of the two DL-salts was accomplished by acidifying either an aqueous solution or suspension of the salt followed by chloroform extraction. In these cases, however, the recovery of DL-α-lipoic acid was not quantitative. Approximately 20% of the recovered acidic fraction was cyclohexane insoluble.

During the course of work on synthesis, the reduction of DL-α-lipoic acid to DL-dihydro-α-lipoic acid was studied. This reduction was readily accomplished with sodium borohydride and DL-dihydro-α-lipoic acid was isolated and characterized as an oil. The 1,2-dithiolane ring of DL-α-lipoic acid was also reduced with zinc and hydrochloric acid in ethanol solution.[1] The ultraviolet absorption spectra of the products from both reductions were identical.[14]

DL-α-Lipoic acid was converted to DL-α-lipo-

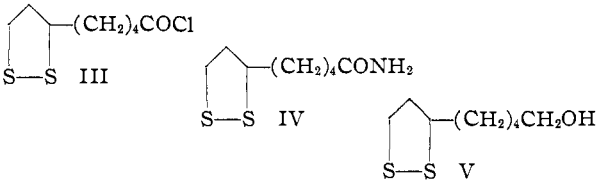
(11) I. C. Gunsalus, M. I. Dolin and L. Struglia, *J. Biol. Chem.*, **194**, 849 (1952).

(12) In a previous publication (reference 2) the following optical rotations were recorded: (+)-α-lipoic acid, $[\alpha]^{23}D$ +104° (*c* 0.88, benzene); (−)-α-lipoic acid, $[\alpha]^{23}D$ −113° (*c* 1.88, benzene). Several recrystallizations from cyclohexane failed to bring the absolute values of the optical rotations any closer. Precipitation of these acids with benzhydrylamine, however, yielded analytically pure salts with equal but opposite rotations: benzhydrylammonium (+)-α-lipoate, $[\alpha]^{25}D$ +50.0° (*c* 1.4, pyridine), and benzhydrylammonium (−)-α-lipoate, $[\alpha]^{25}D$ −49.3° (*c* 1.4, pyridine.)

(13) Even with a partially resolved (*ca.* 70%, $[\alpha]^{25}D$ −70°) sample of α-lipoic acid, precipitation with *l*-ephedrine failed to effect further resolution. With either pure DL-α-lipoic acid or partially resolved samples the combination of ephedrine salts behaves as a single compound.

(14) The zinc and acid reduction showed a slight tendency to eliminate sulfur from the molecule. *Anal.* Calcd.: S, 30.78. Found: S, 29.90.

amide (IV) and DL-α-lipol (V) through the intermediate acid chloride, DL-α-lipoyl chloride (III). The acid chloride III was best prepared from the



reaction of sodium DL-α-lipoate and oxalyl chloride.[15] The product was isolated as an oil and characterized by its neutralization equivalent. The instability of the acid chloride requires immediate conversion to the desired product.

When DL-α-lipoyl chloride (III) was added to a solution of ammonia in anhydrous dioxane, the corresponding amide, DL-α-lipoamide (IV), was obtained in 20% yield. This product had 25% the activity of DL-α-lipoic acid in the enzymatic POF assay.[11]

Sodium borohydride reduction of DL-α-lipoyl chloride (III) followed by iodine oxidation of the 6,8-dithiol yielded DL-α-lipol (V). This compound was isolated as an oil and purified by chromatography on alumina. The product, characterized as an oil, showed no activity in the enzymatic POF assay.[11]

Acknowledgment.—We are indebted to Mr. R. N. Boos and associates for the elemental analyses.

Experimental

Benzhydrylammonium Salt of DL-α-Lipoic Acid.—One gram (5×10^{-3} mole) of DL-α-lipoic acid was dissolved in 5 ml. of ether and a solution of 6×10^{-3} mole of benzhydrylamine in ether (30 ml.) was added. The product was collected by filtration to yield 1.66 g. (86%) of salt, m.p. 123.5–124.5°. The salt was recrystallized using methanol and isopropyl ether (1:10) to yield 1.2 g. of benzhydrylammonium DL-α-lipoate melting at 124.5–125.5°.

Anal. Calcd. for $C_{21}H_{27}NO_2S_2$ (389.56): C, 64.74; H, 6.99; N, 3.60; S, 16.46. Found: C, 65.06; H, 7.47; N, 3.50; S, 16.21.

Benzhydrylammonium Salt of (−)-α-Lipoic Acid.—A solution of 2.4×10^{-3} mole of benzhydrylamine in 10 ml. of ether was added to 390 mg. (1.9×10^{-3} mole) of (−)-α-lipoic acid dissolved in 3 ml. of ether. The salt precipitated immediately and was filtered to yield 460 mg. of product melting at 124°. The product was dissolved in 3.5 ml. of hot methanol and the solution was diluted with 18 ml. of isopropyl ether. The solution was cooled and then filtered to yield 200 mg. of benzhydrylammonium (−)-α-lipoate, m.p. 124–126°, $[\alpha]^{25}D$ −49.3° (*c* 1.4, pyridine).

Anal. Calcd. for $C_{21}H_{27}NO_2S_2$ (389.56): C, 64.74; H, 6.99; N, 3.60; S, 16.46. Found: C, 64.90; H, 7.54; N, 3.59; S, 16.79.

Benzhydrylammonium Salt of (+)-α-Lipoic Acid.—(+)-α-Lipoic acid (370 mg.) was converted to 500 mg. of benzhydrylammonium (+)-α-lipoate, m.p. 122–124°, in the manner described above. The product was dissolved in 3 ml. of hot methanol and crystallized after diluting the solution with 18 ml. of isopropyl ether to yield 240 mg. of benzhydrylammonium (+)-α-lipoate, m.p. 124–126°, $[\alpha]^{25}D$ +50.0° (*c* 1.4, pyridine).

Anal. Calcd. for $C_{21}H_{27}NO_2S_2$ (389.56): C, 64.74; H, 6.99; N, 3.60; S, 16.46. Found: C, 64.88; H, 6.79; N, 3.33; S, 16.48.

***l*-Ephedrine Salt of DL-α-Lipoic Acid.**—DL-α-Lipoic acid (15 mg.) was dissolved in 0.5 ml. of ether and a solution of 11.5 mg. of *l*-ephedrine in 1 ml. of ether was added. The product crystallized and was collected by filtration to yield 21 mg. of the *l*-ephedrine salt of DL-α-lipoic acid, m.p. 103–104°.

(15) R. Adams and L. H. Ulich, THIS JOURNAL, **42**, 599 (1920).

Anal. Calcd. for $C_{18}H_{29}NO_3S_2$ (371.54): C, 58.18; H, 7.87; N, 3.77; S, 17.27. Found: C, 58.03; H, 7.74; N, 3.74; S, 16.85.

DL-Dihydro-α-lipoic Acid.—One gram of DL-α-lipoic acid was dissolved in 10 ml. of ethanol. The alcohol solution was diluted with 10 ml. of water and the solution was cooled in an ice-bath. Two grams of sodium borohydride was added to the solution in small portions. After standing at room temperature for 4 hr., the reaction mixture was diluted with 50 ml. of water and acidified to *p*H 3 with concentrated hydrochloric acid. The product was isolated by chloroform extraction. The chloroform extract was washed with water, dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield 760 mg. of DL-dihydro-α-lipoic acid, $n^{25}D$ 1.5222; neut. equiv., 200 (calcd. 208); iodine equiv., 107 (calcd. 104).

Anal. Calcd. for $C_8H_{16}O_2S_2$ (208.33): C, 46.12; H, 7.74; S, 30.78. Found: C, 46.88; H, 7.38; S, 30.83.

Sodium DL-α-Lipoate.—DL-α-Lipoic acid (0.015 mole) was dissolved in 15 ml. of methanol and 150 ml. of 0.1 *N* aqueous sodium hydroxide was added. The mixture was warmed and filtered. The filtrate was frozen and lyophilized to yield 3.2 g. of yellow amorphous sodium DL-α-lipoate.

DL-α-Lipoyl Chloride.—A solution of 10 ml. of oxalyl chloride in 40 ml. of anhydrous benzene was cooled in an ice-bath and stirred while 3.2 g. of sodium DL-α-lipoate was added in four equal portions at 15-minute intervals. The reaction mixture was stirred for 2.5 hr. and was filtered and concentrated *in vacuo*. The residue was redissolved in benzene and concentrated *in vacuo* to remove the final traces of oxalyl chloride. DL-α-Lipoyl chloride was isolated as an oil in 80% yield; neut. equiv., 107 (calcd. 112).

DL-α-Lipoamide.—A solution of 1.35 g. (6×10^{-3} mole) of DL-α-lipoyl chloride in 25 ml. of anhydrous dioxane was added to an excess of ammonia in anhydrous dioxane solution. The dioxane solution was concentrated *in vacuo*, and the residue was triturated with warm chloroform. The chloroform solution was washed with water and dried over anhydrous magnesium sulfate. The anhydrous chloroform solution was concentrated *in vacuo* to yield 300 mg. of product melting at 123–126°. The product was recrystallized from 2 ml. of ethanol to yield 240 mg. of DL-α-lipoamide, m.p. 124–126°; $\lambda_{max}^{CH_3OH}$ 331 mμ (ε 146); $\lambda_{max}^{Nujol\ mull}$ 3.0, 3.18, 6.01 and 6.12 μ.

Anal. Calcd. for $C_8H_{15}NOS_2$ (205.33): C, 46.80; H, 7.37; N, 6.82; S, 31.23. Found: C, 46.74; H, 7.33; N, 6.88; S, 31.00.

DL-α-Lipol.—A solution of 1.35 g. of DL-α-lipoyl chloride in 25 ml. of anhydrous dioxane was stirred and cooled in an ice-bath. Three grams of sodium borohydride was added, and the mixture was stirred for 2 hr. at room temperature. The mixture was cooled and diluted slowly with an equal volume of water. The solution was acidified to *p*H 3 with concentrated hydrochloric acid, and the product was isolated by chloroform extraction. The product was oxidized by adding 10% iodine–potassium iodide solution to the chloroform solution until the iodine color was permanent. The excess iodine was reduced with dilute aqueous sodium bisulfite, and the chloroform solution was washed with dilute aqueous sodium bicarbonate and finally with water. The chloroform solution was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield 410 mg. of oil, $n^{25}D$ 1.5525; $\lambda_{max}^{CH_3OH}$ 330 mμ ($E_{1\ cm}^{1\%}$ 8.80); $\lambda_{max}^{oil\ film}$ 3 and 5.8 μ; sapon. equiv., 438.

Anal. Calcd. for $C_8H_{16}OS_2$ (192.3): C, 49.95; H, 8.39; S, 33.34. Found: C, 50.06; H, 8.29; S, 32.83.

The infrared spectrum and saponification equivalent show that the product contains an ester, possibly of DL-α-lipoic acid and DL-α-lipol, in addition to DL-α-lipol.

The product was purified by treating 350 mg. of the residual oil with 20 ml. of benzene. The benzene solution was decanted from the small amount of insoluble oil and was chromatographed on 50 g. of acid-washed alumina. Benzene elution was continued until the eluate was free of dissolved material. The product was then eluted with ethyl acetate to yield 137 mg. of oil. This oil was dissolved in 20 ml. of chloroform and the chloroform solution was filtered from a small amount of white insoluble material. Pure DL-α-lipol, $\lambda_{max}^{CH_3OH}$ 332 mμ (ε 165), $\lambda_{max}^{oil\ film}$ 3.02 μ, was isolated by concentrating the chloroform solution *in vacuo*.

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