acid.[10] The mixture was shaken in an atmosphere of nitrogen for 10 minutes at room temperature and then extracted with a total of 40 ml. of benzene. The benzene extracts were washed consecutively with dilute sodium thiosulfate, 5% sodium bicarbonate, and water, and then evaporated *in vacuo*. The viscous oil remaining was diluted to 24.3 ml. with benzene and introduced onto a column prepared from a slurry of 150 g. of Florisil in benzene. The results are presented in Table I. The simple procedure described resulted in a 70-fold purification with approximately 40% recovery of active material. Saponification[3] of 31.1 mg. of α-ester (233,000 units/mg.) prepared in this manner resulted in 16.4 mg. of crystalline α-lipoic acid (250,000 units/mg.), m.p. 47.5°. The recovery of active material was 56%. The X-ray powder data of this sample of α-lipoic acid were identical with those of other samples isolated from acid-hydrolyzed liver residue.[3]

TABLE I

REDUCTION OF CRUDE β-ESTER FRACTION AND CHROMATOGRAM ON FLORISIL

Fraction	Solvent	Total volume, ml.	Solids, mg.	Potency, units/mg.	Total units
Crude β-ester			2430	3050	7,400,000
Reduced β-ester				2200	5,350,000
1–5	Benzene	300		Inactive	
6	Benzene	50	8.8	192,000	1,690,000
7	Benzene	50	5.7	226,000	1,290,000
8	Benzene	50	1.4	140,000	196,000
9	Benzene	50	0.1	181,000	18,100
10–20	Benzene	500		Inactive	
21	EtAc	140	1780	67	120,000

Conversion of α- to β-Lipoic Acid. (a).—A concentrate of α-lipoic acid (11,600 units), obtained by countercurrent distribution (Figs. 2 and 3A), in 5 ml. of *M*/5 phosphate buffer, *p*H 6.5, was treated with 0.05 ml. of 0.001 *N* potassium permanganate at room temperature for three minutes. The residual permanganate color was discharged with sodium thioglycolate, the *p*H readjusted to 6.5 with acetic acid, and the sample subjected to a ten-tube countercurrent distribution. Active material (11,500 units) was recovered in tubes one and two, accounting for a 97% conversion of α- to β-lipoic acid.

(b).—A solution of 200 mg. of an α-ester fraction, obtained from an alumina chromatogram,[3] and 0.035 ml. of 30% hydrogen peroxide in 1.4 ml. of glacial acetic acid was allowed to stand at room temperature for 3 hours. The acetic acid was removed *in vacuo*, the oily residue dissolved in 2 ml. of benzene and introduced onto a column (1-cm. diameter) prepared from a slurry of 20 g. of Florisil in benzene. The results are presented in Table II.

TABLE II

OXIDATION OF CRUDE α-ESTER FRACTION AND CHROMATOGRAM ON FLORISIL

Fraction	Solvent	Total volume, ml.	Solids, mg.	Potency, units/mg.	Total units
Crude α-ester			200	15,000	3,000,000
Oxidized α-ester				14,900	2,980,000
1	Benzene	50			600,000
2–3	Benzene	100		Inactive	
4–7	20% EtAc[a]	80		Inactive	
8–11	20% EtAc	80	9	151,500	1,360,000
12–14	20% EtAc	60		Inactive	
15	EtAc	50		Inactive	

[a] In benzene.

Stability of Methyl β-Lipoate to Alkaline and Acid Hydrolysis.—A concentrate of methyl β-lipoate (22,500 units), prepared by treating β-lipoic acid (Figs. 2 and 3B) with diazomethane,[3] was shaken in a glass-stoppered test-tube under nitrogen with 2 ml. of 0.2 *N* potassium hydroxide for one hour at room temperature. The contents of the tube were adjusted to *p*H 6.5 with dilute phosphoric acid and subjected to a ten-tube countercurrent distribution analysis. Tubes 1 and 2 contained 1300 units and tubes 4 to 9 contained 900 units. Thus, 90% of the methyl β-lipoate was inactivated by treatment with alkali.

To a concentrate of methyl β-lipoate, 22,500 units, was added 2 ml. of 1 *N* hydrochloric acid and the mixture was autoclaved at 120° for 25 minutes. The reaction mixture contained approximately 20,000 units of which approximately 80% consisted of β-lipoic acid and 20% of α-lipoic acid, as revealed by countercurrent distribution analysis.

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(10) These reagents have been used for the conversion of cystine disulfoxide to cystine (G. Toennies and T. F. Lavine, *J. Biol. Chem.*, **113**, 571 (1936)).

[CONTRIBUTION FROM THE LABORATORIES OF BACTERIOLOGY, UNIVERSITY OF ILLINOIS; THE LILLY RESEARCH LABORATORIES, ELI LILLY AND COMPANY; AND THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TEXAS]

Synthesis of DL-α-Lipoic Acid

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Treatment of 4-(α-tetrahydrofuryl)-butyric acid with thiourea and hydrobromic acid, followed by hydrolysis, produces dimercaptoöctanoic acid. Iodine oxidation of the dimercapto acid produces a cyclic disulfide. The properties of this cyclic disulfide are those to be expected for DL-α-lipoic acid, *i.e.*, biological activity one-half that of α-lipoic acid from natural material, no optical activity, elemental and infrared analysis identical with the natural material. Treatment of 4-(α-tetrahydrofuryl)-butyric acid with hydrobromic acid cleaves the ether linkage and forms a bromolactone. Treatment of the unstable bromolactone with thiourea and acid, followed by hydrolysis, gives an improved yield of biologically active dimercaptooctanoic acid. An observed rearrangement of the unstable bromolactone indicates that one of the isothiouronium groups may become attached to a carbon other than in the 5-position of the octanoic acid due to carbonium ion migration. The method of synthesis suggests that α-lipoic acid is the cyclic disulfide derived from either 4,8-, 5,8- or 6,8-dimercaptoöctanoic acid.

α-Lipoic acid, a catalytic agent for the oxidative decarboxylation of pyruvic acid, has been isolated from natural material,[1,2] characterized by physical data,[2,3] and its structure partially elucidated.[2,4] These studies indicate that α-lipoic acid is a cyclic disulfide obtained from 4,8-, 5,8- or 6,8-dimercaptooctanoic acid.[2,4] In a preliminary communica-

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

(2) L. J. Reed, I. C. Gunsalus, G. H. F. Schnakenberg, Q. F. Soper, H. E. Boaz, S. F. Kern and T. V. Parke, THIS JOURNAL, **75**, 1267 (1953).

(3) L. J. Reed, Q. F. Soper, G. H. F. Schnakenberg, S. F. Kern, H. E. Boaz and I. C. Gunsalus, *ibid.*, **74**, 2383 (1952).

(4) L. J. Reed, B. G. DeBusk, C. S. Hornberger, Jr., and I. C. Gunsalus, *ibid.*, **75**, 1271 (1953).

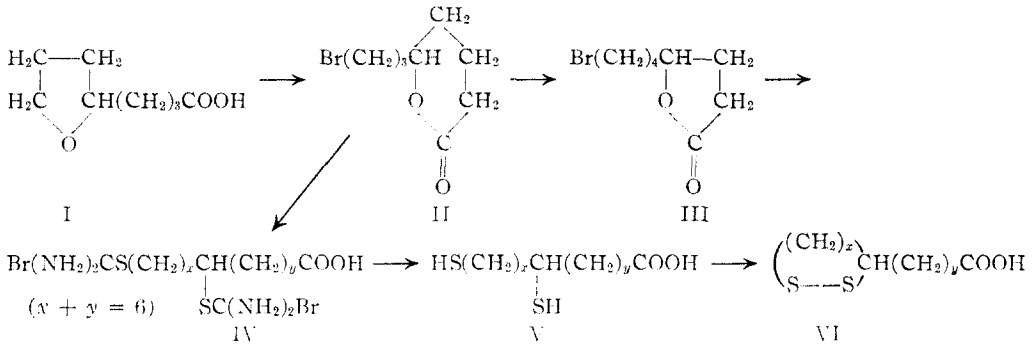

Fig. 1.

tion,[5] it was reported that treatment of 4-(α-tetrahydrofuryl)-butyric acid with thiourea and hydrobromic acid, followed by alkaline hydrolysis and oxidation, gave synthetic material with the biological properties of natural α-lipoic acid. The details of this method of producing crystalline material which we will call DL-α-lipoic acid (until the structure has been determined with certainty)[6] are reported herein.

DL-α-Lipoic acid is prepared by treating 4-(α-tetrahydrofuryl)-butyric acid with thiourea and acid to form a diisothiouronium salt. This reaction extends the preparation of S-alkylisothiouronium salts from alcohols by treatment with thiourea and acid.[7,8] Alkaline hydrolysis of the diisothiouronium salt gives the corresponding dimercaptoöctanoic acid. The dimercaptoöctanoic acid from 4-(α-tetrahydrofuryl)-butyric acid was found to replace natural α-lipoic acid in the pyruvic acid oxidation factor assay system.[9]

To improve the yield of DL-α-lipoic acid from 4-(α-tetrahydrofuryl)-butyric acid (I), attempts were made to open the ether ring prior to treatment with thiourea. Ring cleavage with hydrobromic and sulfuric acids, gave a small yield of dibromoöctanoic acid; the principal product was a bromolactone (II). An analogous cleavage of 3-(α-tetrahydrofuryl)-propionic acid with hydrogen bromide has been reported to yield 4,7-dibromoheptanoic acid which reacts further to give 7-bromo-4-hydroxyheptanoic acid-γ-lactone.[10]

The bromolactone (II), obtained as the principal product of ether cleavage, could be converted to biologically-active material in improved yield by treatment with thiourea and hydrobromic acid. It was noted, however, that the bromolactone after repeated distillation, or acid treatment, resulted in ring contraction to a five-membered lactone (III), as indicated by the shift in the carbonyl absorption band of the infrared spectrum from 1736 to 1760 cm.$^{-1}$.[11,12] Ring contraction of a similar type has been observed in seven- and eight-membered lactones.[13] Although, under the conditions used in the reaction with thiourea, the five-membered lactone (III) was not observed to form six-membered lactone (II), treatment of either with thiourea and hydrobromic acid, followed by hydrolysis and oxidation, gave lipoic acid activity in yields up to 10% and led to the isolation of a crystalline product possessing the biological activity and physical properties expected for DL-α-lipoic acid. These experiments strongly suggest that the route to lipoic acid is not directly from the six-membered lactone.

After hydrolysis of the diisothiouronium salt (IV), dimercaptoöctanoic acid (V) is treated with iodine to form a cyclic disulfide (VI). Air oxidation of the dimercaptoöctanoic acid (V) produces an insoluble gum, presumably a linear polymer. Oxidation of the dimercaptoöctanoic acid (V) to the cyclic disulfide (VI) is accompanied by an increased solubility in organic solvents as shown by a change in countercurrent distribution (Fig. 2). The routes to lipoic acid are summarized in Table I. The rearrangement as outlined in the flow sheet (Fig. 1) rationalizes the formation of a specific biologically-active compound from different precursors.

TABLE I

SUMMARY OF PREPARATION OF DL-α-LIPOIC ACID

Precursor	Reactants, thiourea +	Conditions t, °C.	Yield, units/μmole
4-(α-Tetrahydrofuryl)-butyronitrile	Hydrobromic acid	110	170
4-(α-Tetrahydrofuryl)-butyric acid	Hydrobromic acid	110	375
4-(α-Tetrahydrofuryl)-butyric acid	Hydrochloric acid	110	280
4-(α-Tetrahydrofuryl)-butyric acid	Sulfuric acid	110	60
Dibromoöctanoic acid	Thiourea	190	1100
Metal dibromoöctanoate	Thiourea	190	700
Bromolactone (six-membered)	Hydrobromic acid	Reflux	8200
Bromolactone (five-membered)	Hydrobromic acid	Reflux	8200

A comparison of this synthetic material with natural α-lipoic acid indicates that DL-α-lipoic acid has been prepared. Both substances correspond to the formula $C_8H_{14}O_2S_2$, as indicated by elemental analyses and molecular weight determination (Table II). The infrared spectra of the synthetic and natural substances are identical (Fig. 3), and both show the same migration characteristics in counter-

(5) C. S. Hornberger, Jr., R. F. Heitmiller, I. C. Gunsalus, G. H. F. Schnakenberg and L. J. Reed, THIS JOURNAL, **74**, 2382 (1952).

(6) M. W. Bullock, J. A. Brockman, Jr., E. L. Patterson, J. V. Pierce and E. L. R. Stokstad, *ibid.*, **74**, 3455 (1952), have recently reported that dithioöctanoic acid presumed to contain sulfur on carbons 6 and 8 is the most potent in lipoic acid activity.

(7) J. M. Sprague and T. B. Johnson, *ibid.*, **59**, 1837 (1937).

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

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

(10) R. Paul, *Compt. rend.*, **212**, 398 (1941).

(11) J. F. Grove and H. A. Willis, *J. Chem. Soc.*, 877 (1951).

(12) R. S. Rasmussen and R. R. Brattain, THIS JOURNAL, **71**, 1073 (1949).

(13) E. E. Blaise and A. Koehler, *Compt. rend.*, **148**, 1772 (1909).

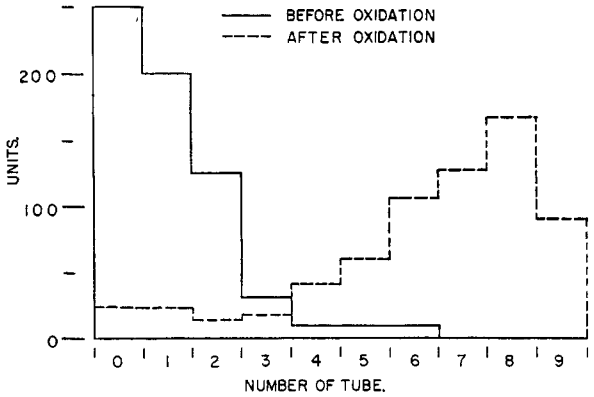

Fig. 2.—Effect of oxidizing dimercaptoöctanoic acid to the corresponding cyclic disulfide. Countercurrent distribution in a $M/5$ phosphate buffer at pH 6.5 *vs.* ether.

current distribution systems[14] and on paper chromatograms.[15] Whereas natural α-lipoic acid is optically active, the synthetic substance is optically inactive, and exhibits a different melting point than natural α-lipoic acid. The biological activity of the synthetic compound is one-half that of the natural substance. These characteristics are to be expected of a racemic mixture, one isomer of which is biologically active.

TABLE II

PROPERTIES OF α-LIPOIC ACID

	Natural α-lipoic	Synthetic DL-α-lipoic	Theory ($C_8H_{14}O_2S_2$)
Analysis, %			
Carbon	46.35	46.31	46.57
Hydrogen	6.79	6.97	6.84
Sulfur	31.21	31.31	31.08
Molecular wt.	217[a]	206.9	206.3
Optical rotation $[\alpha]^{25}D$	+96.7°	0°	
Melting point, °C.	47.5–48.5	59–60	

[a] By neutral equivalent.

Experimental

3-(α-Tetrahydrofuryl)-propanol-1.—Furfural and acetaldehyde were condensed in the presence of sodium hydroxide to give furylacrolein, which was reduced according to the method of Burdick and Adkins[16] to 3-(α-tetrahydrofuryl)-propanol-1. After removal of the solvent and a forerun, the reduced product boiled at 97° (12 mm.), $n^{20}D$ 1.4575. The yield was 82% of the theoretical amount.

This same alcohol was obtained from 3-(α-tetrahydrofuryl)-propionic acid in an 89% yield by reduction with lithium aluminum hydride. The 3-(α-tetrahydrofuryl)-propionic acid was prepared from furylacrylic acid in 40% yield.[17]

1-Bromo-3-(α-tetrahydrofuryl)-propane.—Thirty milliliters of distilled phosphorus tribromide and 500 ml. of dry ether were placed in a liter three-necked flask, equipped with an addition tube, a Hershberg type wire stirrer, a reflux condenser, and cooled to 0°. Atmospheric moisture was excluded with a calcium chloride tube. To the cold, rapidly stirred solution of phosphorus tribromide, 106.5 g. of 3-(α-tetrahydrofuryl)-propanol-1 in 200 ml. of dry ether were added slowly over a period of ¾ of an hour, and the reaction mixture stirred an additional hour in the cold. The ether solution was decanted into ice and the precipitate decomposed by shaking with water. The ether and water layers were separated, the ether layer washed with water, 5% sodium bicarbonate solution and water, and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo*. The yield of 1-bromo-3-(α-tetrahydrofuryl)-propane was 112 g. or 71%. This bromide was distilled through a 30-cm. Vigreux column, and boiled at 58–60° (1.0 mm.),[18] $n^{19}D$ 1.4850.

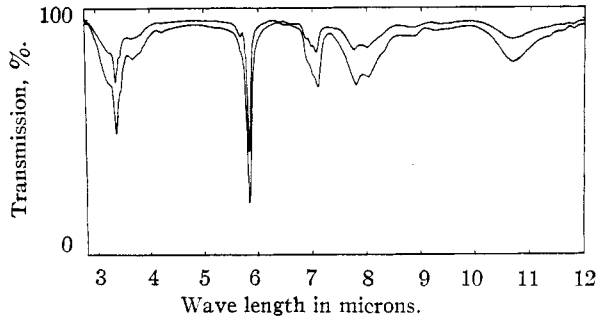

Fig. 3.—Infrared spectra of natural α-lipoic acid and synthetic DL-α-lipoic acid. Concentrations 2% and 4.4%, respectively, in carbon tetrachloride 0.093 mm. path.

4-(α-Tetrahydrofuryl)-butyronitrile.—Using a modification of a general procedure for nitrile formation,[19] a mixture of 112 g. of 1-bromo-3-(α-tetrahydrofuryl)-propane, 41 g. of sodium cyanide,[20] 115 ml. of alcohol and 52 ml. of water was heated under reflux for 19 hours. The reaction mixture was diluted with five volumes of water, and the nitrile extracted from the aqueous layer with three 200-ml. portions of ether. The combined ether layers were washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 72 g., 89.5% of the theoretical amount, of crude nitrile. The nitrile may be purified by distillation first or hydrolyzed directly. The nitrile boiled at 59–61° (0.5 mm.), $n^{20}D$ 1.4510, yield 55–65%.

Anal. Calcd. for $C_8H_{13}ON$: C, 69.03; H, 9.41. Found: C, 69.27; H, 9.57.

4-(α-Tetrahydrofuryl)-butyric Acid.—A mixture of 72 g. of 4-(α-tetrahydrofuryl)-butyronitrile, 72 g. of sodium hydroxide, 200 ml. of water and 110 ml. of ethanol was heated under reflux for 24 hours, or until the evolution of ammonia gas had ceased. The reaction mixture was cooled, poured into two volumes of water, and the basic solution extracted with two portions of ether. The water layer was cooled in an ice-bath, acidified carefully with concentrated hydrochloric acid, and extracted six times with ether. The combined ether extracts were dried over anhydrous sodium sulfate. After removal of the solvent the 4-(α-tetrahydrofuryl)-butyric acid was distilled using a claisen head; yield 45 g. of pure acid, b.p. 160–165° (1 mm.), $n^{25}D$ 1.457.[21] The over-all yield of acid based on the 1-bromo-3-(α-tetrahydrofuryl)-propane was 49% without purification of the intermediates.

Anal. Calcd. for $C_8H_{14}O_3$: C, 60.74; H, 8.92; neut. equiv., 158. Found: C, 60.55; H, 8.86; neut. equiv., 158.

4-(α-Tetrahydrofuryl)-butyric acid was prepared from 1-bromo-3-(α-tetrahydrofuryl)-propane in improved yield without isolation of the nitrile by the procedure of Lewis and Susi[22]: 0.45 mole of 1-bromo-3-(α-tetrahydrofuryl)-propane, and 0.50 mole of reagent-grade sodium cyanide were heated with stirring in 150 ml. of ethylene glycol. After about 20 minutes, a thermometer suspended in the vapors above the surface of the reaction showed no further rise in temperature. The reaction mixture was refluxed for an additional 20 minutes, and 0.9 mole of sodium hydroxide and 2.0 moles of water were added. The mixture was

(14) I. C. Gunsalus, L. Struglia and D. J. O'Kane, *J. Biol. Chem.*, **194**, 859 (1952).

(15) L. J. Reed, M. E. Getzendaner, B. G. DeBusk and P. M. Johnston, *ibid.*, **192**, 859 (1951).

(16) H. E. Burdick and H. Adkins, THIS JOURNAL, **56**, 438 (1934).

(17) E. Schwenk, D. Papa, H. Hankin and H. Ginsberg, *Org. Syntheses*, **27**, 68 (1947).

(18) B.p. 100–101° (16 mm.) according to G. Barger, R. Robinson and L. H. Smith, *J. Chem. Soc.*, 718 (1937).

(19) R. Adams and C. S. Marvel, THIS JOURNAL, **42**, 311 (1920).

(20) Substitution of C^{14}-labeled sodium cyanide in a modified procedure results in carboxyl-labeled α-lipoic acid which will be described in a future publication.

(21) Value of $n^{25}D$ 1.4572 and b.p. 145° (5 mm.) reported by H. Gilman and A. P. Hewlett, *Rec. trav. chim.*, **51**, 93 (1932).

(22) R. N. Lewis and P. V. Susi, THIS JOURNAL, **74**, 840 (1952).

refluxed for 36 hours, or until no more ammonia gas was evolved. The acid was isolated as above. The over-all yield of acid from the bromide was 67%.

Treatment of 4-(α-tetrahydrofuryl)-butyric acid with excess diazomethane forms the methyl ester, b.p. 121–122° (20 mm.), 62–63° (0.25 mm.), $n^{25}D$ 1.4440.

Anal. Calcd. for $C_9H_{16}O_3$: C, 62.77; H, 9.36; sapon. equiv., 172. Found: C, 62.60; H, 9.48; sapon. equiv., 173.

Treatment of the methyl ester with an excess of concentrated ammonium hydroxide gave, after recrystallization from a chloroform–heptane solution, an 85% yield of the amide as platelets, m.p. 66–67°.

Anal. Calcd. for $C_8H_{15}NO_2$: C, 61.12; H, 9.62. Found: C, 61.41; H, 9.62.

Ring Cleavage of Tetrahydrofurylbutyric Acid.[23] (Formation of Bromolactones).—With good stirring, 71.3 g. of 4-(α-tetrahydrofuryl)-butyric acid in 145 ml. of 40% hydrobromic acid was heated on a steam-cone. To this mixture was added, over a period of 20 minutes, 89 ml. of concentrated sulfuric acid. After heating for an additional hour, the mixture was cooled in an ice-bath and extracted twice with 100-ml. portions of chloroform. The organic layer was washed once with cold water and the solvent removed by a flash distillation, to give a dark oil which was distilled through a claisen head, b.p. 180–200° (15 mm.), to yield 92 g. (92%) of crude bromolactone. The distillate discolored rapidly on standing. This crude bromolactone was used to prepare lipoic acid.

In an attempt to purify the bromolactone, a portion of this crude material was subjected to the following treatment: One hundred grams was distilled from a short-path still *in vacuo* and about 80 g. recovered over the temperature range of 160–180° (20 mm.). The distillation was accompanied by the evolution of hydrogen bromide which was collected in a cold-trap. This distillate was redistilled through a claisen head and two fractions were collected, one at 60–63° (20 mm.) 10 g., the second at 157–160° (20 mm.), 60 g. Both fractions rapidly darkened on standing. A small amount of hydrogen bromide was evolved during this distillation.

The fraction boiling at 157–160° (20 mm.) was subjected to fractionation through a heated 50-cm. Vigreux column and three cuts collected over a temperature range of 130–175° (3 mm.). Although the fractions collected remained colorless, and approached a constant index of refraction, elemental analyses showed that they were mixtures. Infrared analysis indicated the carbonyl absorption at 1760 $cm.^{-1}$ due to a five-membered lactone.[11,12,24] This distilled bromolactone was divided into two portions: One 10-g. portion was converted to lipoic acid as described under (C) below. The yield was 150×10^6 units of α-lipoic acid activity. A second 10-g. portion was refluxed 10 hours with 10 ml. of 40% hydrobromic acid, the reaction mixture cooled, extracted with chloroform, and the chloroform removed *in vacuo* at room temperature. An infrared spectrum on the dark liquid residue again showed the carboxyl absorption attributed to a five-membered lactone. In neither case was there any absorption at 1736 $cm.^{-1}$ characteristic of a six-membered lactone. The remaining material was converted to lipoic acid by procedure (C) below to yield 150×10^6 units of α-lipoic acid activity.

Preparation of Dibromoöctanoic Acid.—Fifty-five grams of 4-(α-tetrahydrofuryl)-butyric acid and 103 ml. of 40% hydrobromic acid were heated to 100° on a steam-bath with stirring. To this hot, rapidly-stirred mixture was added 75 ml. of concentrated sulfuric acid over a period of 15 minutes. The mixture was heated for an additional half-hour, cooled, and mixed with 100 g. of ice. The crude dibromo acid was extracted from the cooled reaction mixture with three 100-ml. portions of chloroform. The dibromo acid was extracted from the chloroform with two 200-ml. portions of 5% sodium bicarbonate solution. The bicarbonate solution was cooled, acidified carefully with concentrated hydrochloric acid, and the dibromo acid was extracted with three 100-ml. portions of ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to yield 11.5 g. (11%) of the unstable dibromoöctanoic acid as an oil.

Anal. Calcd. for $C_8H_{14}Br_2O_2$: Br, 52.92; neut. equiv., 303. Found: Br, 49.49; neut. equiv., 302.

The unstable dibromoöctanoic acid could be converted in 89% yield to the more stable methyl ester by treatment with diazomethane.

Preparation of Lipoic Acid. (A) From 4-(α-Tetrahydrofuryl)-butyric Acid.—One-half gram of 4-(α-tetrahydrofuryl)-butyric acid, 0.3 g. of thiourea and 1.0 ml. of 40% hydrobromic acid were sealed in an ampoule and heated in a bath of refluxing acetic acid (110°) for 11.5 hours. After cooling, the ampoule was opened and the contents dissolved in 9 ml. of 5% sodium hydroxide solution. After standing at room temperature for two hours, the mixture was acidified, extracted with portions of benzene; yield 1,200,000 units of lipoic acid activity. Similar treatment with concentrated hydrochloric acid or 50% sulfuric acid instead of 40% hydrobromic acid also gave lipoic acid activity. These data are summarized in Table I.

(B) From Dibromoöctanoic Acid and Thiourea.—Crude dibromoöctanoic acid (1.98 g.), thiourea, 2.50 g., and absolute ethanol, 1.0 ml., were sealed in an ampoule and heated in a bath at 190° for 15 minutes. After about five minutes, the contents of the ampoule became a solid crystalline mass. The ampoule was cooled, opened, and the crystalline material dissolved in 50 ml. of concentrated ammoniun hydroxide and autoclaved at 120° for one-half hour; yield 7,500,000 units of lipoic acid activity.

This active material was diluted with 500 ml. of 5% bicarbonate solution and treated with a 0.1 *N* alcohol–iodine solution until the color of iodine persisted for one-half minute, then washed once with benzene, acidified to *p*H 1.0 with concentrated hydrochloric acid and extracted with three portions of benzene (total volume 435 ml.); yield (in benzene) 1,740,000 units. Countercurrent distribution in an ether–phosphate system at *p*H 6.5[14] showed that 95% of the activity was in the disulfide form.

(C) From Bromolactone.—A mixture of 10 g. of the crude bromolactone, 6 g. of thiourea and 10 ml. of 40% hydrobromic acid was refluxed for 10 hours. The mixture was diluted to a total volume of 500 ml. with distilled water, solid potassium carbonate added until the solution was neutral, then 5 g. of solid potassium hydroxide added with good stirring. The mixture was heated in an autoclave at 120° for 30 minutes, the solution cooled, and extracted with one 100-ml. portion of chloroform. The alkaline solution was acidified with 10 ml. of concentrated sulfuric acid, and extracted with 500 ml. of benzene in five portions. The yield was 370,000,000 units of lipoic acid activity.

Oxidation to Disulfide.—In a typical experiment, a benzene extract which contained 47.35 g. of solids and 214,500,000 units of crude α-lipoic acid activity, was evaporated to small volume and dissolved in 6 l. of chloroform and 1800 ml. of water. The two phase system was stirred vigorously in an atmosphere of nitrogen while 197 ml. of iodoform reagent[25] was added. The end-point was determined by the disappearance of the nitroprusside test[26] for sulfhydryl groups and the appearance of a slight excess of iodine. Addition of sodium cyanide to the nitroprusside test solution gave a very strong response indicating the presence of a disulfide. An analytical countercurrent showing the change in migration character is shown in Fig. 2. The aqueous phase was separated and discarded. The chloroform phase was washed twice with 1500-ml. portions of 1% sodium thiosulfate and twice with 1500-ml. portions of water, and the inactive washes discarded. The activity was extracted from the chloroform phase with ten 350-ml. portions of 5% sodium bicarbonate. After washing with 200 ml. of low-boiling petroleum ether, the bicarbonate solution was acidified to *p*H 1 by addition of 400 ml. of 6 *N* sulfuric acid and extracted with six 350-ml. portions of benzene. The benzene extract was washed with 150 ml. of water and concentrated *in vacuo* to a solids concentration of about 240 mg./ml. The oxidized product (37.48 g.) was obtained without significant loss in total activity.

Methyl Lipoate.—A cold benzene solution containing 59.64 g. of the crude disulfide prepared as above was added to 600 ml. of a cold benzene solution of diazomethane pre-

(23) N. J. Leonard and J. Figueras, Jr., THIS JOURNAL, **74**, 917 (1952).

(24) We are indebted to Miss H. P. Miklas for the infrared spectra measurements and interpretations.

(25) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," Third Edition, John Wiley and Sons, Inc., New York, N. Y., p. 138.

(26) Fritz Feigl, "Qualitative Analysis by Spot Tests," Elsevier Publishing Co., Inc., New York, N. Y., 1947, p. 226.

pared from 70 g. of nitrosomethylurea.[27] After standing one hour in an ice-bath and one hour at room temperature, the solution was concentrated *in vacuo* to about two-thirds volume. The benzene solution was washed successively with small amounts of water, 1% sodium bicarbonate, water, 1% acetic acid, and water, with no significant loss of activity. The benzene was removed *in vacuo* leaving 56.98 g. of a solvent-free oil containing essentially all the activity. Bioautographs[15] confirmed the presence of the ester and the absence of free acid.

This ester (60.7 g.) was slurried in a minimal amount of *n*-heptane and introduced onto a column (8-cm. diameter) prepared from a slurry of 2400 g. activated alumina[2] in *n*-heptane. The bulk of the solids was eluted with increasing amounts of benzene in heptane. At a concentration of 50% benzene in heptane the activity was eluted.

Crystallization of Synthetic DL-α-Lipoic Acid.—The most potent of the alumina column fractions were pooled, and the solvent removed *in vacuo*. The oil was suspended in 500 ml. of 0.1 *N* potassium hydroxide and shaken continuously in an atomosphere of nitrogen for 16 hours at 25°. The alkaline solution was washed with 150 ml. of low-boiling petroleum ether in two portions. No activity was removed. The alkaline solution was acidified to *p*H 1, by adding 10 ml. of 6 *N* sulfuric acid, and extracted with 400 ml. of benzene in five portions. After washing with 25 ml. of water, the benzene contained 236,000,000 units. Evaporation *in vacuo* gave 2.485 g. of solvent-free yellow oil. The oil was extracted by slurrying with several small (10-ml.) portions of hot low-boiling petroleum ether, leaving a residue of 204 mg. of insoluble oil. The extract (50 ml.) was allowed to cool slowly. The resulting small yellow platelets (1.400 g.) were collected and dried. The mother liquor contained 910 mg. of a non-crystallizable oil. After two recrystallizations from low-boiling petroleum ether the product melted at 59–60° (cor.) and possessed one-half the biological activity on a weight basis of α-lipoic acid from natural sources.

The usual over-all yield to crystalline DL-α-lipoic acid (based on the crude bromolactone) was about 4%.

(27) F. Arndt, *Org. Syntheses*, **2**, 461 (1948).

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroids. XLI.[1] Synthesis of 11α,17α-Dihydroxyprogesterone and of 11α,17α,21-Trihydroxyprogesterone, the 11-Epimer of Kendall's Compound F[2]

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Δ^{16}-Allopregnene-3β,11α-diol-20-one 3,11-diacetate (I) is converted by a nine-step synthesis to 11α,17α-dihydroxyprogesterone (Vb), and by a ten-step synthesis to 11α,17α,21-trihydroxyprogesterone (XII), the 11-epimer of Kendall's Compound F.

In view of the biological importance of 11β-hydroxylated hormones of the adrenal cortex, such as 11β,21-dihydroxyprogesterone (corticosterone) and 11β,17α,21-trihydroxyprogesterone (Kendall's compound F),[4] it was felt to be of interest to synthesize the 11α-hydroxy analogs of the various adrenal hormones in order to make them available for biological testing as hormone substitutes or antagonists. These compounds have very recently taken on special importance since they have been shown to be produced by the microbiological oxidation of the corresponding 11-desoxy derivatives.[5]

The chemical synthesis of 11α-hydroxyprogesterone, through the use of recently discovered methods[6] for the introduction of the 11α-hydroxy group into ring C unsubstituted steroids, has already been recorded.[7] We now wish to describe the synthesis of two more complex hormone analogs, that of 11α,17α-dihydroxyprogesterone (Vb), the 11α-hydroxy analog of 17α-hydroxyprogesterone,[8] and that of 11α,17α,21-trihydroxyprogesterone (XII),[9] the 11-epimer of Kendall's compound F.

The starting point for these syntheses was Δ^{16}-allopregnene-3β,11α-diol-20-one 3,11-diacetate (I), which has recently been obtained[10] by the side degradation of 22a-5α-spirostane-3β,11α-diol; the latter in turn had been derived from "diosgenin." Oxidation of I with alkaline hydrogen peroxide smoothly yielded the free 16α,17α-oxidoallopregnane-3β,11α-diol-20-one (IIb) as a low melting solid. It has already been shown[7] that a 3β,11α-diol of the 5α (allo) series may be preferentially oxidized at C-3 either with N-bromoacetamide or by the Oppenauer method, and the glycol IIb was oxidized by the latter procedure. The resulting 16α,17α-oxidoallopregnan-11α-ol-3,20-dione (IIIb), obtained in 75% yield, was acetylated at C-11 to furnish the acetate IIIa. Conversion of the latter to the bromohydrin with hydrogen bromide in

(1) Paper XL, F. Sondheimer and G. Rosenkranz, *Experientia*, in press.

(2) Presented in part at the Atlantic City Meeting of the American Chemical Society, Sept., 1952. A preliminary announcement of part of this work has appeared in *Chemistry and Industry*, 783, 834 (1952).

(3) Department of Chemistry, Wayne University, Detroit, Michigan.

(4) *Cf.* H. Reich, D. H. Nelson and A. Zaffaroni, *J. Biol. Chem.*, **187**, 411 (1950).

(5) (a) D. H. Peterson and H. C. Murray, THIS JOURNAL, **74**, 1871 (1952); (b) O. Mancera, A. Zaffaroni, B. A. Rubin, F. Sondheimer, G. Rosenkranz and C. Djerassi, *ibid.*, **74**, 3711 (1952); (c) J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, *ibid.*, **74**, 3962 (1952).

(6) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3546 (1951); C. Djerassi, E. Batres, M. Velasco and G. Rosenkranz, *ibid.*, **74**, 1712 (1952); F. Sondheimer, R. Yashin, G. Rosenkranz and C. Djerassi, *ibid.*, **74**, 2696 (1952); C. Djerassi, O. Mancera, M. Velasco, G. Stork and G. Rosenkranz, *ibid.*, **74**, 3321 (1952).

(7) O. Mancera, J. Romo, F. Sondheimer, G. Rosenkranz and C. Djerassi, *J. Org. Chem.*, **17**, 1066 (1952).

(8) J. J. Pfiffner and H. B. North, *J. Biol. Chem.*, **132**, 459 (1940); **139**, 855 (1941).

(9) After our work had been completed (*cf.* footnote 2), a preliminary communication by H. L. Herzog, E. P. Oliveto, M. A. Jevnik and E. B. Hershberg (THIS JOURNAL, **74**, 4471 (1952)) appeared, in which an independent and different synthesis of the 11,21-diacetate of this compound is described, proceeding *via* intermediates of the 5β (normal) series. Moreover A. Lardon and T. Reichstein at the Colloquium on the "Synthesis and Metabolism of Adrenal Cortical Steroids" sponsored by the Ciba Foundation, London (July 7–10, 1952) announced the conversion of sarmentogenin to the 11,21-diacetate of XII, a synthesis which also proceeded *via* 5β (normal) compounds. This conversion has now been published (*Pharm. Acta Helv.*, **27**, 287 (1952)).

(10) C. Djerassi, E. Batres, J. Romo and G. Rosenkranz, THIS JOURNAL, **74**, 3634 (1952).