the terms 20-iso-,[16] neo-,[9b] ana-[18] and cyclopseudo-[9a] to be used as prefixes to the trivial name of the parent sapogenin. As now seems apparent, the use of 20-iso does not cover all cases. With the structure of the 20β-sapogenins now on firm ground there no longer seems to be a need for the non-specific terms neo, ana, or cyclopseudo. We therefore propose 20-iso or 20,22-iso as shown above.

Experimental

Pseudomerization of Sapogenins. (a) Micro Procedure.—To a Pyrex tube (8 mm. × 12.5 cm.) sealed at one end was added 0.2 g. of sapogenin acetate followed by 0.5 ml. of acetic anhydride containing a trace of acetic acid.[19] The tube was sealed close to the open end, wrapped in a copper spiral,[20] immersed in a heating bath and removed after the desired time interval. The pseudomerization runs were conducted at temperatures of 100, 170, 180 and 188° using heating-baths of water, butyl Cellosolve, aniline and propylene glycol, respectively. Trial runs quickly indicated that for the acetic anhydride pseudomerization of natural sapogenins, a temperature of 170° was best for determination of pseudomerization rates, the reaction proceeding too slowly at lower and too rapidly at higher temperatures. For 20-iso- or 20,22-isosapogenins, a temperature of 100° was found most suitable. In experiments in which these last named sapogenins were heated without addition of acetic anhydride, a temperature of 188° was convenient with quantities of 0.10 g.

Determination of Pseudosapogenins.—After the appropriate heating periods, the tubes were opened, and the contents refluxed one-half hour with 20 ml. of methanol containing 1.0 g. of potassium hydroxide. Three volumes of water was added, and the precipitated product filtered, thoroughly washed with hot water and dried *in vacuo* at 80°. The ultraviolet absorption of a weighed sample (0.025 g. in 100 ml. of methanol, and further diluted if necessary) was determined at 215 mμ.[21] The percentage of conversion to pseudosapogenin was determined by dividing the specific absorption coefficient found experimentally by the corresponding value of the pure pseudosapogenin. In the case of the 188° pseudomerization of the 20-isosapogenins with omission of acetic anhydride, the product was, on completion of the heating period, dissolved directly in methanol and the ultraviolet absorption determined as above. The data for natural sapogenins are shown in Table I, for 20-iso and 20,22-isosapogenins in Table II. The results obtained by ultraviolet measurement were checked by paper chromatography. Whatman No. 4 paper, 12 by 18 inches, was impregnated with propylene glycol by dipping the sheets into a solution of 70% acetone–30% propylene glycol (v./v.). Approximately 200-microgram quantities of reaction products, pure pseudosapogenin or pure sapogenin were spotted on the paper. The mobile phase was a mixture of 25% benzene–75% cyclohexane (v./v.). Using a descending system with 12 × 18 inch circular tanks, the chromatograms were allowed to develop for 2 hours. The papers were dried and sprayed with 5% phosphomolybdic acid in ethanol.[22] The unreacted sapogenin moved much further than the pseudosapogenin so that mixtures were easily separated. The results qualitatively were in line with the data given in Tables I and II. In addition, there was no evidence to indicate that products other than pseudosapogenin or unreacted sapogenins were formed.

(b) Macro Procedure.—Sarsasapogenin acetate, 20.0 g., and 50.0 ml. of acetic anhydride containing a trace of glacial acetic acid were placed in a Pyrex, 250 ml. 24/40 ☨, round-bottom flask and the air flushed out with dry, oxygen-free nitrogen. The flask was then closed with a stopper previously ground with carborundum to make an air-tight seal. The stopper was held in place by a strong spring attached to a collar secured to the neck of the flask. The heating system consisted of a 2-liter resin flask containing 1 liter of a butyl Cellosolve fraction boiling at 170°. By means of a metal weight the reaction flask was completely immersed in the heating-bath. The butyl Cellosolve was then heated to boiling and the reaction flask heated 4 hr.[23] The reaction flask was then removed, cooled and the acetic anhydride distilled *in vacuo*. The residue was refluxed for one-half hour in methanol containing 5% potassium hydroxide. After addition of two volumes of water, the precipitated pseudosarsasapogenin was filtered, washed and dried to give 18.2 g., m.p. 140–170°. Crystallization from ethyl acetate gave 15.6 g., m.p. 168–171° (lit.[24] gives m.p. 168–171°) yield 85.5%. Papergram analysis of the mother liquors indicated that there was considerable pseudosarsasapogenin left in these residues. In a similar manner 20.0 g. of smilagenin acetate, heated 17 hr. at 170°, gave 16.0 g. of crystals from ethyl acetate, m.p. 153–161° (lit.[24] gives m.p. 153–161°) yield 88%. Tigogenin acetate, 17 hr. at 170° gave 16.6 g. of crystals from methanol, m.p. 170–180° (lit.[21] gives m.p. 179–189°), yield 91.5%; diosgenin acetate, 17 hr. at 180°, gave 11.1 g. of crystals from aqueous methanol, m.p. 160–170° (lit.[21] gives double m.p. 157–163°, 174–177°) yield 62%, and 3.6 g. of a less pure fraction, m.p. 150–165°, yield 20.0%; hecogenin acetate, 17 hr. at 180°, gave 16.0 g. of crystals from ether, m.p. 190–195° (lit.[21] gives m.p. 190–191°), yield 88.0%.

PHILADELPHIA 18, PENNA.

(18) D. H. W. Dickson *et al.*, *Chemistry & Industry*, 692 (1954).

(19) To obtain consistent results at 170° it was necessary to use reagent grade acetic anhydride, 454.5 g., to which was added 0.5 ml. of glacial acetic acid.

(20) This was both a safety precaution and a convenient means of removing the tube from the heating-bath by means of a length of copper wire attached to the spiral.

(21) A. F. B. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones and A. G. Long, *J. Chem. Soc.*, 2807 (1955).

(22) D. Kritchevsky and M. R. Kirk, *Arch. Biochem. and Biophys.*, **35**, 346 (1952).

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Synthesis of Racemic, Optically Active and Radioactive α-Lipoic Acids

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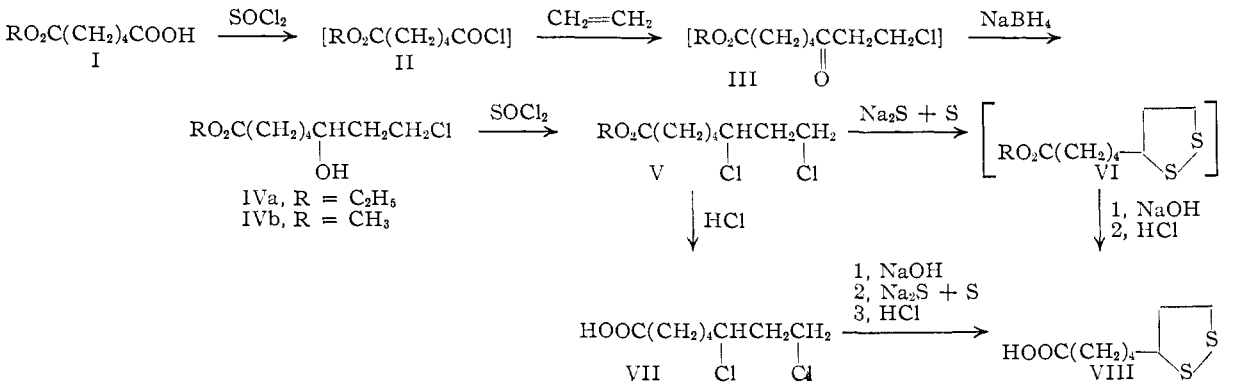
A convenient and versatile route to α-lipoic acids using the reaction of 6,8-dichloroöctanoic acid or its esters with sodium disulfide is described. The synthesis of DL-α-lipoic acid, (+)-α-lipoic acid and DL-α-lipoic acid-S_2^{35} is reported. The preparation of (+)-α-lipoic acid by resolution of DL-α-lipoic acid is described.

α-Lipoic acid, 1,2-dithiolane-3-valeric acid, has been recognized as a new cofactor involved in the biochemical decarboxylation of α-keto acids and as a growth factor for a variety of microörganisms.



Since the first isolation[1] and identification[2-6] of (+)-α-lipoic acid from natural sources, several routes for the synthesis of DL-α-lipoic acid by chemical methods have been developed.[4,6-13] Syntheses for (+)- and (−)-α-lipoic acids[9] and for DL-α-lipoic acid-S_2^{35} [14,15] also have been described.

We wish to report a convenient and versatile new synthetic route to α-lipoic acids. The alkylation of sodium disulfide to form the 1,2-dithiolane ring has been described.[16] We have treated 6,8-dichloroöctanoic acid and its esters[8] with solutions of sodium disulfide to give α-lipoic acid and its esters, respectively.[17] The sequence of reactions represented below permits the preparation of DL-α-lipoic acid, (+)-α-lipoic acid and DL-α-lipoic acid-S_2^{35}.

The methyl or ethyl ester of DL-6,8-dichloro-octanoic acid was prepared from the corresponding monoalkyl adipate (I) by a series of reactions previously described.[8] However, it was found convenient not to isolate the intermediate alkyl δ-chloroformylvalerate (II) and alkyl 8-chloro-6-ketoöctanoate (III). Reaction of chlorohydrin IVa or IVb with thionyl chloride yielded the corresponding alkyl DL-6,8-dichloroöctanoate (V). The dichloro ester V reacted smoothly with sodium disulfide in alcohol solution to form alkyl DL-α-lipoate (VI), which was not isolated but was saponified to yield, upon acidification, DL-α-lipoic acid (VIII) in as high as 68% yield. The sodium disulfide was prepared readily by heating equimolar quantities of hydrated sodium sulfide and sulfur in alcohol.[16] The alkyl DL-α-lipoates (VI) were not isolated because they polymerize very readily. The over-all yield of DL-α-lipoic acid (VIII) from monomethyl adipate by this route approached 40%.

An alternate route to DL-α-lipoic acid involved hydrolysis of methyl or ethyl DL-6,8-dichloroöctanoate (V) with refluxing concentrated hydrochloric acid to yield DL-6,8-dichloroöctanoic acid (VII). The dichloro acid VII readily was converted by reaction of its sodium salt with sodium disulfide to DL-α-lipoic acid (VIII) in 45% yield.

DL-6,8-Dichloroöctanoic acid (VII) was resolved through its (−)-ephedrine salt to permit preparation of (+)- and (−)-α-lipoic acids. The (+)-6,8-dichloroöctanoic acid (−)-ephedrine salt was the least soluble diastereoisomer and was obtained pure by recrystallization from ethyl acetate. Regeneration of (+)-6,8-dichloroöctanoic acid (VII) followed by its reaction with sodium disulfide yielded pure (+)-α-lipoic acid (VIII). The (−)-6,8-dichloroöctanoic acid (−)-ephedrine salt, obtained from the original salt solution after removal of the less soluble diastereoisomer, was difficult to purify by recrystallization from ethyl acetate and as a consequence the (−)-α-lipoic acid obtained was contaminated with 15–20% of the dextrorotatory enantiomorph. It should be possible to purify completely the (−)-6,8-dichloro-octanoic acid through its (+)-ephedrine salt.

The synthesis of pure (+)-α-lipoic acid outlined above affords a convenient route which is superior to the resolution of DL-α-lipoic acid itself. Resolution of DL-α-lipoic acid was accomplished by fractional crystallization of its cinchonidine salt, but only in yields of 0.5–4.4%. This route likewise did not yield pure (−)-α-lipoic acid.

The reaction of DL-6,8-dichloroöctanoic acid (VII) with sodium disulfide affords a very simple route to sulfur-35 labeled α-lipoic acid of high purity and almost any specific radioactivity desired. The solution of sodium disulfide is prepared from elemental sulfur containing the amount of sulfur-35 desired, and sodium sulfide. By this method 50 mc. of sulfur-35 was converted into 4 g. of DL-α-lipoic acid-S_2^{35} of specific activity 4 mc./g. in 45% yield. It should be possible to prepare

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(+)-α-lipoic acid labeled with sulfur-35 by starting with (+)-6,8-dichloroöctanoic acid.

The ease with which α-lipoic acid polymerizes has been noted.[18,19] The several α-lipoic acids described in this paper polymerized to varying extents during their distillation and recrystallization. The liquid esters of α-lipoic acid polymerized with extreme ease. If polymer is formed during the reaction of the dichloro ester V or the dichloro acid VII with sodium disulfide, it is depolymerized to α-lipoic acid by the subsequent treatment with alkali.[19] The DL-, (+)-, (−)- and sulfur-35 labeled α-lipoic acids prepared in this work all showed infrared absorption spectra identical to those reported for natural (+)-α-lipoic acid[3] and synthetic DL-α-lipoic acid.[4] The pyruvic acid oxidation factor (POF) activity[20] of DL-α-lipoic acid, including that labeled with sulfur-35, was about 100% of that of a known sample of DL-α-lipoic acid. The POF activity of (+)-α-lipoic acid was twice that of DL-α-lipoic acid, while the activity of (−)-α-lipoic acid was 26% of that of DL-α-lipoic acid or higher, depending upon how much of the dextrorotatory enantiomorph it contained.

Experimental

Ethyl DL-8-chloro-6-hydroxyoctanoate (IVa) was prepared by a method similar to that reported by Reed and Niu.[8] It was found convenient to prepare the ethyl δ-chloroformylvalerate in tetrachloroethane solution and to use this solution directly for the reaction with ethylene. After decomposing the Friedel–Crafts reaction mixture with ice and hydrochloric acid, the organic layer was treated with a solution of sodium borohydride in 95% ethyl alcohol. The excess reducing agent was decomposed with hydrochloric acid and the organic layer washed with water and dried over anhydrous sodium bicarbonate and magnesium sulfate. Distillation gave a 73% yield of ethyl DL-8-chloro-6-hydroxyoctanoate, b.p. 129–132° (0.35 mm.), $n^{25}D$ 1.4583.[17] Reed and Niu[8] reported b.p. 121° (0.5 mm.), $n^{25}D$ 1.4580 for this compound.

Methyl DL-8-Chloro-6-hydroxyoctanoate (IVb).—Monomethyl adipate served as the starting material for the preparation of methyl DL-8-chloro-6-hydroxyoctanoate, b.p. 123–125° (0.45 mm.), $n^{25}D$ 1.4607–1.4618, in yields of 71–83%.

Ethyl DL-6,8-dichloroöctanoate (V) was prepared by treating ethyl DL-8-chloro-6-hydroxyoctanoate with thionyl chloride by the procedure of Reed and Niu.[8] It was obtained in 77.5% yield as a clear, slightly yellow liquid with b.p. 82–94° (0.05–0.1 mm.), $n^{25}D$ 1.4596–1.4606. Reed and Niu[8] reported this compound to have b.p. 109° (0.7 mm.), $n^{25}D$ 1.4603.

Methyl DL-6,8-dichloroöctanoate (V) was prepared from methyl DL-8-chloro-6-hydroxyoctanoate and thionyl chloride in 79% yield, b.p. 89° (0.2 mm.), $n^{25}D$ 1.4638. *Anal.* Calcd. for $C_9H_{16}O_2Cl_2$: C, 47.59; H, 7.10; Cl, 31.22. Found: C, 47.78; H, 7.19; Cl, 31.45.

DL-6,8-Dichloroöctanoic Acid (VII).—A mixture of 170 g. (0.7 mole) of ethyl DL-6,8-dichloroöctanoate and 500 ml. of concd. hydrochloric acid was refluxed with vigorous stirring for 18 hours. The reaction mixture was cooled to room temperature, and the aqueous layer saturated with sodium chloride. The oil layer was separated by four extractions with ether (1 l. total), and the ether extract washed twice with cold saturated sodium chloride solution. The ether extract was then cooled with an ice-bath, and 600 ml. of ice-cold 20% potassium carbonate solution was slowly added to it with stirring during 15 minutes. The stirring and cooling were continued for another 15 minutes. The yellow aqueous layer (*p*H 8) was separated and washed once with ether to remove traces of unhydrolyzed ester. The aqueous layer, cooled with an ice-bath, was acidified with ice-cold 20% hydrochloric acid. It was then saturated with potassium chloride by addition of the solid salt and extracted four times with ether (1.5 l. total). The ether extract was washed five times with cold saturated sodium chloride solution (about 150 ml. total) and dried over anhydrous sodium sulfate at 0°. After removal of the drying agent and ether, the remaining yellow oil was distilled rapidly through a vapor bath still under reduced pressure (0.2 mm.) and a vapor temperature up to 157° to yield 118 g. of distillate. This distillate was redistilled through a 6-inch column to yield 112 g. (74%) of DL-6,8-dichloroöctanoic acid, b.p. 122–127° (0.2 mm.), $n^{25}D$ 1.4774–1.4781. A middle fraction with $n^{25}D$ 1.4780 was analyzed. *Anal.* Calcd. for $C_8H_{14}O_2Cl_2$: C, 45.10; H, 6.63; Cl, 33.22; neut. equiv., 213. Found: C, 44.94; H, 6.64; Cl, 32.93; neut. equiv., 209.

(+)-6,8-Dichloroöctanoic Acid (−)-Ephedrine Salt.—A 12.06-g. (0.073 mole) portion of (−)-ephedrine was dissolved in a solution of 15.57 g. (0.073 mole) of DL-6,8-dichloroöctanoic acid in 100 ml. of ethyl acetate. The solution, cooled to −18°, soon crystallized to a continuous mass. The crystals were filtered cold and pressed dry. A sample was dried *in vacuo*, m.p. 81.5–94.5°, $[\alpha]^{24.5}D$ −19.1° (*c* 1.2, ethyl alcohol). The pressed-dry crystals were redissolved in the minimum amount of ethyl acetate at 35°. Crystals formed at −18°. The crystals were recrystallized in this manner a total of six times to yield 3.85 g. of (+)-6,8-dichloroöctanoic acid (−)-ephedrine salt, m.p. 106.5–108°, $[\alpha]^{24.2}D$ −11.1° (*c* 1, ethyl alcohol). *Anal.* Calcd. for $C_{18}H_{29}O_3NCl_2$: N, 3.70; Cl, 18.72. Found: N, 3.69; Cl, 18.87.

The filtrates from the six recrystallizations were combined and concentrated under reduced pressure at 35° almost to dryness. The crystals which formed were recrystallized six times from ethyl acetate as described above to yield an additional 2.6 g. of salt, m.p. 105–107°. The filtrates obtained were treated by a like procedure to yield another 1 g. of salt, m.p. 105–106°. The total yield was thus 7.45 g. (54%) of (+)-6,8-dichloroöctanoic acid (−)-ephedrine salt.

(+)-6,8-Dichloroöctanoic Acid (VII).—A 7.43-g. (0.0196 mole) portion of (+)-6,8-dichloroöctanoic acid (−)-ephedrine salt was treated with 60 ml. of ice-cold 5% hydrochloric acid. The aqueous layer was saturated with potassium chloride, and the mixture extracted four times with a total of 400 ml. of ether. The ether extract was washed four times with a total of 120 ml. of ice-cold saturated potassium chloride solution and dried over anhydrous sodium sulfate in the cold. After removal of drying agent and ether, the remaining liquid was distilled to yield 4.07 g. (97.4%) of (+)-6,8-dichloroöctanoic acid, b.p. 121.5–123° (0.2 mm.), $n^{25}D$ 1.4768–1.4778. A middle fraction, $n^{25}D$ 1.4776, $[\alpha]^{23.8}D$ +30.5° (*c* 2, benzene), was analyzed. *Anal.* Calcd. for $C_8H_{14}O_2Cl_2$: C, 45.10; H, 6.63; Cl, 33.22; neut. equiv., 213. Found: C, 45.54; H, 6.79; Cl, 33.17; neut. equiv., 208.

(−)-6,8-Dichloroöctanoic Acid (−)-Ephedrine Salt.—The filtrate remaining after filtration at −18° of the crude (+)-6,8-dichloroöctanoic acid (−)-ephedrine salt from 15.57 g. of DL-6,8-dichloroöctanoic acid, as described above, was concentrated under reduced pressure at 35° to 25 ml. and stored at −18°. White crystals formed, m.p. 68.5–78°, $[\alpha]^{23.6}D$ −30.8° (*c* 0.8, ethyl alcohol). The crystals were recrystallized five times by dissolving them in the minimum amount of ethyl acetate at 35° and cooling to −18°. There was obtained 0.36 g. of (−)-6,8-dichloroöctanoic acid (−)-ephedrine salt, m.p. 78.5–82°, $[\alpha]^{24.2}D$ −33.4° (*c* 1, ethyl alcohol). *Anal.* Calcd. for $C_{18}H_{29}O_3NCl_2$: N, 3.70; Cl, 18.72. Found: N, 3.78; Cl, 18.76.

The filtrates from the five recrystallizations were combined and concentrated to yield crystals which were recrystallized three times. The operation then was repeated on these filtrates. There was thus obtained two additional portions of (−)-6,8-dichloroöctanoic acid (−)-ephedrine salt amounting to 2.29 g., m.p. 77–80°, and 1.93 g., m.p. 77.5–81°. The total yield was thus 4.58 g. (33%).

(−)-6,8-Dichloroöctanoic Acid (VII).—A 4.58-g. (0.0121 mole) portion of (−)-6,8-dichloroöctanoic acid (−)-ephedrine salt was treated with cold 5% hydrochloric acid and the (−)-6,8-dichloroöctanoic acid isolated as described above for (+)-6,8-dichloroöctanoic acid. Distillation of

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the product yielded 2.5 g. (97%) of (−)-6,8-dichloroöctanoic acid, b.p. 122–126° (0.3 mm.), $n^{25}D$ 1.4753–1.4779. A middle fraction, $n^{25}D$ 1.4776, $[\alpha]^{23.8}D$ −29.3° (*c* 2, benzene), was analyzed. *Anal.* Calcd. for $C_8H_{14}O_2Cl_2$: C, 45.10; H, 6.63; Cl, 33.22; neut. equiv., 213. Found: C, 45.51; H, 6.78; Cl, 33.17; neut. equiv., 208.

DL-α-**Lipoic Acid (VIII). A. From Methyl DL-6,8-Dichloroöctanoate.**—A mixture consisting of 300 g. (1.25 moles) of sodium sulfide ($Na_2S \cdot 9H_2O$) and 40 g. (1.25 gram atoms) of sulfur in 2500 ml. of 95% ethyl alcohol was heated under nitrogen on a steam-bath until the solids dissolved. A solution of 227.1 g. (1 mole) of methyl DL-6,8-dichloroöctanoate in 500 ml. of 95% ethyl alcohol was added to the refluxing solution over a 5-hour period. After the addition was completed, 1000 ml. of solvent was removed by distillation, and a solution of 80 g. of sodium hydroxide in 2500 ml. of water was added to the reaction mixture. The heating was continued overnight. The resulting solution was cooled to room temperature and acidified with concd. hydrochloric acid (*p*H 1–2). The oil layer was removed and the aqueous layer extracted with two 400-ml. portions of benzene. The organic layers were combined and extracted with 500 ml. of water. If benzene-insoluble material was present, it formed the bottom layer and was removed. The benzene layer was concentrated under reduced pressure, and the residue was distilled through a vapor bath still under reduced pressure (1–2 mm.) and a vapor temperature of 197° to yield 141 g. (68%) of DL-α-lipoic acid, which crystallized upon cooling to room temperature. When greater quantities were desired, it was convenient to combine the benzene-soluble material from several runs and distil using a spinning disk molecular still. Recrystallization from cyclohexane or methylcyclohexane gave high purity DL-α-lipoic acid, m.p. 58–59°.

B. From DL-6,8-Dichloroöctanoic Acid.—A mixture of 4.33 g. (0.018 mole) of sodium sulfide ($Na_2S \cdot 9H_2O$) and 0.577 g. (0.018 gram atom) of sulfur in 45 ml. of 95% ethyl alcohol was stirred and heated under nitrogen to reflux. The solids dissolved to form a deep yellow solution. A solution of sodium DL-6,8-dichloroöctanoate was prepared by adding 2.45 ml. of 5.84 *N* sodium hydroxide solution (0.0143 mole of NaOH) to a cold solution of 3.072 g. (0.0144 mole) of DL-6,8-dichloroöctanoic acid in 40 ml. of absolute ethyl alcohol. This cold solution was added to the refluxing reaction mixture during two hours. Stirring and refluxing were continued for another 30 minutes. The reaction mixture, consisting of a yellow solution and salt crystals, was cooled somewhat, and about one-half of the alcohol was distilled under reduced pressure. A solution of 0.58 g. (0.0144 mole) of sodium hydroxide in 36 ml. of water was then added to the reaction mixture, and the resulting solution was refluxed under nitrogen for 15 minutes to decompose complex sulfides. The reaction mixture then was cooled to room temperature, and 40 ml. of 5% hydrochloric acid was added to form a cloudy, strongly acid mixture. This mixture was extracted three times with a total of 200 ml. of benzene, and the benzene extract washed four times with a total of 100 ml. of water and dried over sodium sulfate in the cold. After removal of drying agent and benzene, the remaining yellow oil was distilled through a vapor bath still under reduced pressure (0.2 mm.) and a vapor temperature of 180°, to give a yellow oil which crystallized. The product was recrystallized from *n*-pentane to yield 1.36 g. (45.6%) of DL-α-lipoic acid as blunt yellow needles, m.p. 56–58°. The product was recrystallized again from *n*-pentane, m.p. 57–59°. Its infrared spectrum (4% in carbon tetrachloride, 0.127-mm. path) was identical with that of natural (+)-α-lipoic acid[3] and of synthetic DL-α-lipoic acid.[4] In enzymatic POF assays[20] its activity was 103% of that of DL-α-lipoic acid. *Anal.* Calcd. for $C_8H_{14}O_2S_2$: C, 46.55; H, 6.84; S, 31.05; neut. equiv., 206. Found: C, 46.75; H, 6.72; S, 31.24; neut. eqiv., 207.

(+)-α-Lipoic Acid (VIII). A. From (+)-6,8-Dichloroöctanoic Acid.—A mixture of 0.606 g. (0.018 gram atom) of sulfur and 4.54 g. (0.0189 mole) of sodium sulfide ($Na_2S \cdot 9H_2O$) in 45 ml. of 95% ethyl alcohol was heated under nitrogen to form a solution. To this refluxing solution was added a cold solution prepared by adding 2.5 ml. of 5.83 *N* sodium hydroxide solution (0.0146 mole of NaOH) to an ice-cold solution of 3.22 g. (0.0151 mole) of (+)-6,8-dichloroöctanoic acid in 50 ml. of absolute ethyl alcohol. The addition was made over a 2-hour period, and refluxing was continued for another 30 minutes. About one-half of the alcohol was distilled from the reaction mixture, and then a solution of 0.6 g. (0.0151 mole) of sodium hydroxide in 36 ml. of water was added. The solution was refluxed for 15 minutes under nitrogen. The reaction mixture was made acid with 40 ml. of 5% hydrochloric acid and extracted with benzene. The benzene extract was washed with water and dried over sodium sulfate in the cold. After removal of the drying agent and benzene, the remaining yellow oil was distilled through a vapor bath still under reduced pressure (0.1 mm.) and a vapor temperature of 180° to give a yellow oil which crystallized. The product was recrystallized from *n*-pentane to yield 1.89 g. (60%) of (+)-α-lipoic acid as yellow leaflets, m.p. 45–47°, $[\alpha]^{23.3}D$ +91° (*c* 2, benzene). The (+)-α-lipoic acid was recrystallized again from *n*-pentane, m.p. 46.5–48°. Its infrared spectrum (4% in carbon tetrachloride, 0.127-mm. path) was identical to that of natural (+)-α-lipoic acid,[3] and in enzymatic POF assays[20] its activity was about 200% of that of DL-α-lipoic acid. *Anal.* Calcd. for $C_8H_{14}O_2S_2$: C, 46.55; H, 6.84; S, 31.05; neut. equiv., 206. Found: C, 46.83; H, 6.83; S, 30.60; neut. equiv., 206.5.

B. From DL-α-Lipoic Acid.—A 10.00-g. (0.04845 mole) portion of DL-α-lipoic acid was dissolved in 100 ml. of ethyl acetate at room temperature. To this solution was added a solution of 14.28 g. (0.04845 mole) of cinchonidine in 4100 ml. of ethyl acetate. As an aid to crystallization, 1 ml. (1.1 moles/mole of salt) of water was added to the solution. The container was then scratched with a glass rod and the whole stored at −18°. Crystals developed over a period of several days. The crystals were filtered cold, pressed dry, and then redissolved in the minimum amount of ethyl acetate by stirring at room temperature. Crystals formed during storage at −18°. The crystals were recrystallized in this manner a total of five times. After the fifth recrystallization, there was obtained 0.35 g. (there was some loss due to spillage) of (+)-α-lipoic acid (−)-cinchonidine salt as light yellow crystals which melted over the range 99.5–156°, $[\alpha]^{24.1}D$ −50° (*c* 0.9, ethyl alcohol). *Anal.* Calcd. for $C_{27}H_{36}O_3N_2S_2$: N, 5.60; S, 12.80. Found: N, 5.57; S, 13.20.

All of the above-described operations were carried out in the dark or in red light and under nitrogen to minimize side reactions of lipoic acid such as oxidation and polymerization. A 0.35-g. portion of (+)-α-lipoic acid (−)-cinchonidine salt was treated with 50 ml. of 5% hydrochloric acid, and the mixture extracted three times with ether. The ether extract was washed five times with water and dried over sodium sulfate in the cold. Removal of drying agent and ether left a yellow solid which was taken up in 30 ml. of *n*-pentane. The solution crystallized to yield 0.026 g. (0.5%) of (+)-α-lipoic acid as yellow leaflets, m.p. 44.5–46.5°, $[\alpha]^{23.8}D$ +87.4° (*c* 0.18, benzene). Its activity in the enzymatic POF assay[20] was 196% of that of DL-α-lipoic acid.

Upon repeating the above preparation, the least soluble salt fraction after four recrystallizations consisted of 0.9 g. of (+)-α-lipoic acid (−)-cinchonidine salt as light yellow crystals which melted over the range 99.5–152°, $[\alpha]^{24.6}D$ −54.6° (*c* 0.6, ethyl alcohol). Treatment of the 0.9 g. of salt with 5% hydrochloric acid resulted in a yellow solid which was recrystallized from *n*-pentane to yield 0.22 g. (4.4%) of (+)-α-lipoic acid as yellow leaflets, m.p. 46–48°, $[\alpha]^{24.5}D$ +115.9° (*c* 1, benzene). Its activity in the enzymatic POF assay was 188% of that of DL-α-lipoic acid.

(−)-α-Lipoic Acid (VIII). A. From (−)-6,8-Dichloroöctanoic Acid.—A mixture of 0.317 g. (0.01 gram atom) of sulfur and 2.38 g. (0.01 mole) of sodium sulfide ($Na_2S \cdot 9H_2O$) in 25 ml. of 95% ethyl alcohol was heated under nitrogen to form a solution. To this refluxing solution was added during the course of one hour, a cold solution prepared by adding 1.36 ml. of 5.83 *N* sodium hydroxide solution (0.00793 mole of NaOH) to a cold solution of 1.7 g. (0.00793 mole) of (−)-6,8-dichloroöctanoic acid in 25 ml. of absolute ethyl alcohol. After another hour of refluxing, about one-half of the alcohol was distilled off, and a solution of 0.137 g. (0.00793 mole) of sodium hydroxide in 25 ml. of water was added. The mixture was refluxed under nitrogen for 15 minutes. The reaction mixture was then acidified with 25 ml. of 5% hydrochloric acid and extracted with benzene. The benzene extract was washed with water and dried over sodium sulfate in the cold. After removal of drying agent and benzene, the remaining oil was distilled in a vapor bath still under reduced pressure (0.2 mm.) and a vapor temperature of 180° to give a yellow oil which crystallized.

The product was recrystallized from *n*-pentane to yield 0.77 g. (47%) of (−)-α-lipoic acid as blunt yellow needles, m.p. 47–52°, $[\alpha]^{23.8}D$ −63° (*c* 1.6, benzene). The (−)-α-lipoic acid was recrystallized again from *n*-pentane, m.p. 50–53°. Its infrared spectrum (4% in carbon tetrachloride, 0.127-mm. path) was identical to that of (+)-α-lipoic acid[3] and of DL-α-lipoic acid.[4] Its activity in enzymatic POF assays was 39% of that of DL-α-lipoic acid.[21] *Anal.* Calcd. for $C_8H_{14}O_2S_2$: C, 46.55; H, 6.84; S, 31.05; neut. equiv., 206. Found: C, 46.37; H, 6.90; S, 30.63; neut. equiv., 200.

B. From DL-α-Lipoic Acid.—In the preparation of (+)-α-lipoic acid (−)-cinchonidine salt described above, the filtrate remaining after removal of the crystalline salt from the original reaction mixture was reduced in volume by stages by distillation under reduced pressure at 35°. At each stage, additional crystalline material which formed by cooling the solution to −18° was removed by filtration. There finally remained 4.5 g. of a yellow sirup which did not crystallize, $[\alpha]^{24.7}D$ −79.6° (*c* 1, ethyl alcohol). The sirup was treated with 50 ml. of 5% hydrochloric acid, and the mixture extracted three times with ether. The ether extract was washed five times with water and dried over sodium sulfate in the cold. Removal of drying agent and ether left a yellow solid. Recrystallization of the solid from *n*-pentane yielded 2.29 g. of (−)-α-lipoic acid as yellow leaflets, m.p. 57–59°, $[\alpha]^{24.4}D$ −50° (*c* 1, benzene). Its activity in the POF assay[20] was 26% of that of DL-α-lipoic acid.[22]

DL-α-Lipoic Acid-S_2^{35} (VIII).—A solution of 1.015 mg. (50 mc.) of elemental sulfur-35 in 2.9 ml. of benzene[23] was transferred from a sealed tube to a 500-ml. round-bottomed flask using 75 ml. of benzene for rinsing. A 1.75-g. (0.0545 gram atom) portion of elemental sulfur (USP precipitated) was added to the solution, which was then heated to dissolve the sulfur in the benzene. The benzene was then distilled to leave crystalline elemental sulfur containing 50 mc. of sulfur-35. A 13.1-g. (0.0545 mole) portion of sodium sulfide ($Na_2S \cdot 9H_2O$) and 125 ml. of 95% ethyl alcohol were added and the mixture stirred and refluxed under nitrogen to form a yellow-brown solution. To this refluxing solution was added during the course of three hours, a cold solution prepared by adding 7.3 ml. of 5.82 *N* sodium hydroxide solution (0.0425 mole of NaOH) to a cold solution of 9.29 g. (0.0436 mole) of DL-6,8-dichloroöctanoic acid in 140 ml. of absolute ethyl alcohol. After another 30 minutes of refluxing, about one-half of the alcohol was distilled, and a solution of 1.75 g. (0.0436 mole) of sodium hydroxide in 100 ml. of water was added. The mixture was refluxed under nitrogen for 15 minutes to decompose complex sulfides. The reaction mixture was then cooled, acidified with 140 ml. of 5% hydrochloric acid and extracted four times with a total of 500 ml. of benzene. The benzene was washed four times with saturated sodium chloride solution and dried over anhydrous sodium sulfate in the cold. After removal of drying agent and benzene, the remaining oil was distilled in a small vapor bath still under reduced pressure (0.2 mm.) and vapor temperature up to 180°, to give a yellow oil which crystallized. The crystals were recrystallized from *n*-pentane to yield 4.05 g. (45%) of DL-α-lipoic acid-S_2^{35} as yellow prisms, m.p. 59–60°. Its specific activity, determined by combustion to barium sulfate, was approximately 4 mc./g. (theoretical specific activity, 4.4 mc./g.). Its infrared spectrum (4% in carbon tetrachloride, 0.127-mm. path) was identical with that of DL-α-lipoic acid.[4] Its assay for POF activity was 109% of that of DL-α-lipoic acid.

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(21) The optical rotation and POF assay values for this sample of (−)-α-lipoic acid indicated that it contained 15–20% of (+)-α-lipoic acid.

(22) The optical rotation and POF assay values for this sample of (−)-α-lipoic acid were not in good agreement but indicated that the sample contained 13–22% of (+)-α-lipoic acid.

(23) Elemental sulfur-35 was obtained from Oak Ridge National Laboratory.

[CONTRIBUTION FROM THE PRODUCTS DEVELOPMENT LABORATORIES, PARKE, DAVIS & CO.]

Chloramphenicol. 2,4,8-Substituted-1-aza-3,7-dioxabicyclo[3.3.0]octanes

BY WILLIAM H. EDGERTON,[1] JAMES R. FISHER AND GEORGE W. MOERSCH

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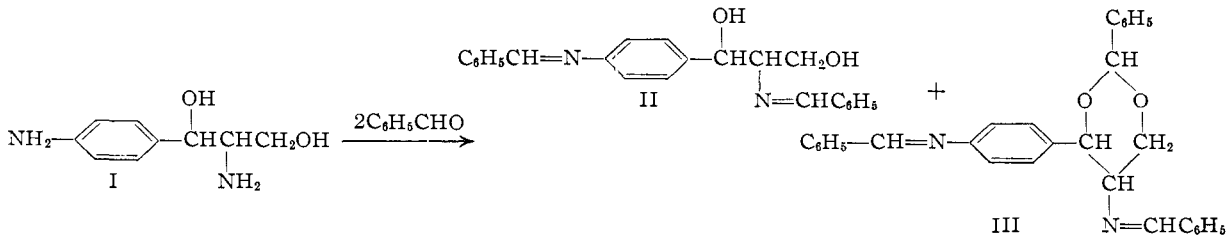
A series of 1-aza-3,7-dioxabicyclo[3.3.0]octanes derived from the chloramphenicol skeleton was prepared. A novel series of isomers, believed related by a *cis–trans* relationship, was isolated and characterized. The structure of a by-product previously reported from the reaction of benzaldehyde with L-*threo*-2-amino-1-(*p*-aminophenyl)-1,3-propanediol was demonstrated to be L-*threo*-4-(*p*-benzylideneaminophenyl)-2,8-diphenyl-1-aza-3,7-dioxabicyclo[3.3.0]octane.

During the preparation of L-*threo*-2-benzylideneamino-1-(*p*-benzylideneaminophenyl)-1,3-propanediol (II) by treating benzaldehyde with L-*threo*-2-amino-1-(*p*-aminophenyl)-1,3-propanediol (I) in refluxing ethanol, a by-product was obtained which was reported to be a cyclic tribenzylidene derivative with a 1,3-dioxane structure (III).[2] This assumption was made on the ease of preparation and the ready decomposition of the compound to the known dibenzylidene derivative II. Subsequent study of this by-product has proved it to be L-*threo*-4-(*p*-benzylideneamino-



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(2) W. H. Edgerton and J. R. Fisher, *J. Org. Chem.*, **19**, 593 (1954).