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Synthesis of (+)-α-Lipoic Acid and its Optical Antipode[1]

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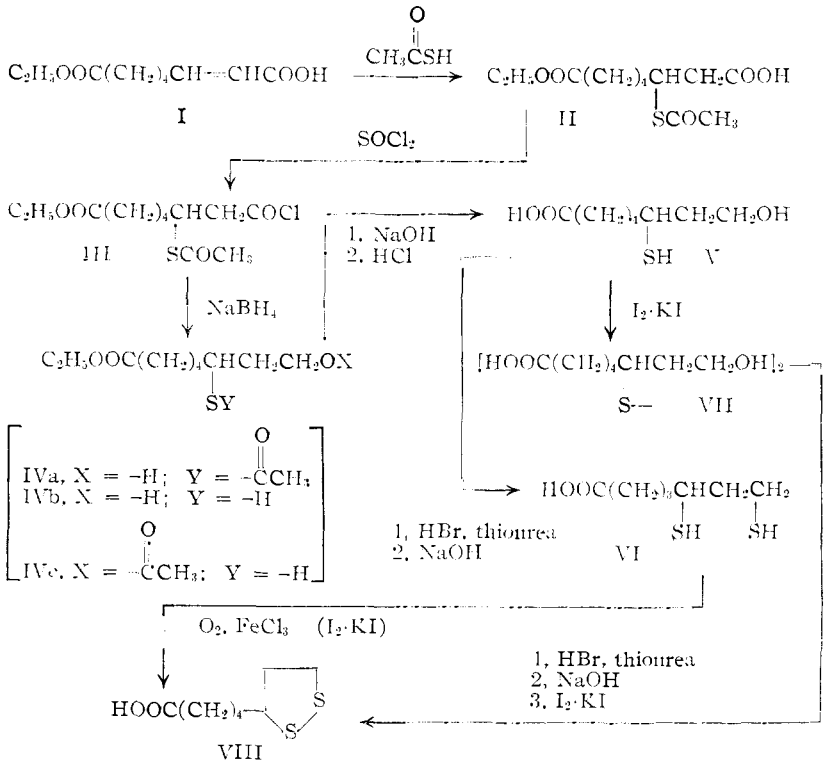
(+)-α-Lipoic acid has been synthesized. DL-3-Acetylthio-7-carbethoxyheptanoic acid was converted into its acid chloride which was reduced with sodium borohydride to yield a mixture of reduction products. Alkaline hydrolysis of this mixture produced 8-hydroxy-6-thiooctanoic acid. Replacement of the hydroxyl with sulfhydryl followed by oxidation gave DL-α-lipoic acid. Resolution of DL-3-acetylthio-7-carbethoxyheptanoic acid supplied the starting materials for the synthesis of the (+)- and (−)-α-lipoic acids.

α-Lipoic acid has been isolated from natural sources[2,3] and has been shown to be active as a coenzyme in the oxidative decarboxylation of pyruvate. On the basis of degradative[2,4] synthetic[5-8] and spectroscopic[9] evidence, α-lipoic acid is the cyclic disulfide, 5-[3-(1,2-dithiolanyl)]-pentanoic acid (VIII). The racemic form of this compound, designated[10] either DL-α-lipoic acid[5,7,8] or 6-thioctic acid,[6] has been synthesized. This paper describes the details of a synthesis of (+)-, (−)- and DL-α lipoid acid.[1]



Addition of thiolacetic acid to 7-carbethoxy-2-heptenoic acid (I)[11] yielded DL-3-acetylthio-7-carbethoxyheptanoic acid (II) which was converted into the acid chloride III. Reduction of the acid chloride III led to a mixture of esters (IVa, b and c). By alkaline hydrolysis, the mixture was converted to DL-8-hydroxy-6-thiooctanoic acid (V). Replacement of the 8-hydroxyl by sulfhydryl yielded DL-dihydro-α-lipoic acid (VI). The dithiol VI was oxidized to DL-α-lipoic acid. When the (+) and (−) isomers of 3-acetylthio-7-carbethoxyheptanoic acid (II) were used in the above sequence, (+)- and (−)-α-lipoic acids, respectively, were produced.

Thiolacetic acid was added to the α,β-unsaturated acid I to give DL-3-acetylthio-7-carbethoxyheptanoic acid (II). Thionyl chloride converted DL-3-acetylthio-7-carbethoxyheptanoic acid (II) into DL-3-acetylthio-7-carbethoxyheptanoyl chloride (III). This acid chloride was used immediately in the next step, since it decomposed on standing.

DL-3-Acetylthio-7-carbethoxyheptanoyl chloride (III) was reduced by sodium borohydride[12] suspended in dioxane. The reduction product was a mixture which contained varying amounts of ethyl DL-8-hydroxy-6-thiooctanoate (IVb) and ethyl DL-8-acetoxy-6-thiooctanoate (IVc) as well as the major product, ethyl DL-6-acetylthio-8-hydroxyoctanoate (IVa). The presence of the O-acetyl thiol IVc can best be explained by the migration of acetyl from sulfur to oxygen during the later part of the reaction. A six-membered cyclic orthoacetate has been proposed as the intermediate for this type of migration.[13] The hydroxy thiol IVb is produced by alkaline hydrolysis of the acetal group.

(1) A preliminary account of this work appeared in a Communication to the Editor, THIS JOURNAL, **76**, 4748 (1954).

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(3) E. L. Patterson, J. V. Pierce, E. L. R. Stokstad, C. E. Hoffmann, J. A. Brockman, Jr., F. P. Day, M. E. Macchi and T. H. Jukes, *ibid.*, **76**, 1823 (1954).

(4) J. A. Brockman, Jr., E. L. R. Stokstad, E. L. Patterson, J. V. Pierce and M. E. Macchi, *ibid.*, **76**, 1827 (1954)

(5) C. S. Hornberger, Jr., R. F. Heitmiller, I. C. Gunsalus, G. H. F. Schnakenberg and L. J. Reed, *ibid.*, **75**, 1273 (1953).

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(7) Q. F. Soper, W. E. Buting, J. E. Cochran, Jr., and A. Pohland, *ibid.*, **76**, 4109 (1954).

(8) L. J. Reed and Ching-I Niu, *ibid.*, **77**, 416 (1955).

(9) M. Calvin and J. A. Barltrop, *ibid.*, **74**, 6153 (1952).

(10) For a discussion concerning nomenclature see: G. W. Kidder, *Federation Proc.*, **13**, 695 (1954).

(11) G. B. Brown, M. D. Armstrong, A. W. Moyer, W. P. Anslow, Jr., B. R. Baker, M. V. Querry, S. Bernstein and S. R. Safir, *J. Org. Chem.*, **12**, 160 (1947).

(12) S. W. Chaiken and W. G. Brown, THIS JOURNAL, **71**, 122 (1949).

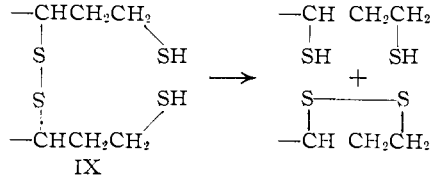
(13) J. S. Harding and L. N. Owen, *J. Chem. Soc.*, 1536 (1954).

The mixture of the three esters (IVa, b and c) was converted by alkaline hydrolysis into a single product, DL-8-hydroxy-6-thioloctanoic acid (V). This ω-hydroxy acid polymerized slowly even at room temperature.

Replacement of the 8-hydroxyl group of the acid V by sulfhydryl was done by reaction with thiourea in refluxing hydrobromic acid. The intermediate isothiouronium derivative was hydrolyzed to DL-6,8-dithioloctanoic acid (DL-dihydro-α-lipoic acid) (VI).

The oxidation of the dithiol VI to DL-α-lipoic acid was carried out by either of two procedures. A chloroform solution of the dithiol was shaken with aqueous iodine–potassium iodide solution, or an aqueous solution of the dithiol was air-oxidized using ferric chloride as a catalyst. In either case, polymeric by-products were formed. DL-α-Lipoic acid was separated by a cyclohexane extraction of the resultant oil. In parallel experiments, iodine oxidation gave better yields of the crystalline product than the air-oxidation procedure.

Alternatively, DL-8-hydroxy-6-thioloctanoic acid (V) was oxidized with iodine–potassium iodide solution to produce the disulfide, DL-6,6′-dithiobis-(8-hydroxyoctanoic acid) (VII). The hydroxyl groups were replaced by sulfhydryls by refluxing with thiourea and hydrobromic acid followed by alkaline hydrolysis. In earlier experiments the product was reduced using sodium borohydride and oxidized with iodine to yield DL-α-lipoic acid which was isolated as before. It was determined later that the sodium borohydride reduction was not necessary; direct oxidation gave similar yields of DL-α-lipoic acid. At least two causes of the disulfide cleavage necessary for obtaining DL-α-lipoic acid from these reactions can be considered. The disulfide group in the starting material may be cleaved during the reaction with thiourea. A second possibility is an intramolecular oxidation–reduction reaction of the intermediate dithia dithiol IX. In general, the yields of DL-α-lipoic acid obtained through the disulfide VII sequence were lower than those obtained when the hydroxy thiol V was used.



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DL-3-Acetylthio-7-carbethoxyheptanoic acid (II) was resolved for the preparation of (+)- and (−)-α-lipoic acids. Treatment of an ether solution of the DL-acetylthio acid with *l*-ephedrine yielded the crystalline salt of the levorotatory form. (+)-3-Acetylthio-7-carbethoxyheptanoic acid was regenerated from the non-crystalline *l*-ephedrine salt and was purified through its benzhydrylamine salt.

When the (+)- and (−)-acetylthio acids obtained from these salts were used in the above sequence of reactions, (+)- and (−)-α-lipoic acids, respectively, were produced. For convenience, a method of resolution leading to the direct precipitation of the precursor of (+)-α-lipoic acid was desirable. As would be predicted, (+)-3-acetylthio-7-carbethoxyheptanoic acid precipitated as the salt of *d*-ephedrine.

The preparation of these amine salts of the 3-acetylthio-7-carbethoxyheptanoic acids is accompanied by some acetylation of the amine by the anhydride-like acetylthio group. In one salt preparation, a considerable amount of N-acetylbenzhydrylamine was isolated as a by-product. This side reaction was minimized through rapid salt-precipitation at low temperatures. The 3-acetylthio acids were separated readily from the N-acetylamine impurity by extraction into cold bicarbonate solution and subsequent liberation of the acid by acidification.

In enzymatic POF assays,[14] which were carried out by Dr. George E. Boxer of these laboratories, the activity of synthetic (+)-α-lipoic acid was double that of DL-α-lipoic acid. The activity of (−)-α-lipoic acid was essentially zero.[15]

Equal amounts of (+)- and (−)-α-lipoic acids were mixed and recrystallized and the racemic compound, DL-α-lipoic acid, was obtained. The above data along with optical rotation, ultraviolet absorption spectrum, crystalline form, analysis and molecular weight determination substantiate the identity of our synthetic (+)-α-lipoic acid and the natural product isolated[2,3] and described[2,9] by other workers. The synthesis lends additional support to the structural conclusions advanced previously by these investigators.

Acknowledgments.—We are indebted to Dr. J. B. Conn for molecular weight determinations, to Mr. R. N. Boos and associates for microanalyses, and to Dr. N. R. Trenner and Mr. R. W. Walker for infrared spectral data.

Experimental

DL-3-Acetylthio-7-carbethoxyheptanoic Acid (II).—A solution of 294 g. (1.47 moles) of 7-carbethoxy-2-heptenoic acid[16] and 193 g. (2.53 moles, 180 ml.) of thiolacetic acid was kept at room temperature for 17 days.[17] The solution was diluted with 500 ml. of chloroform and washed with three 500-ml. portions of water. The chloroform layer was dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure to yield a residue of 380 g. (93%) of DL-3-acetylthio-7-carbethoxyheptanoic acid, $n^{26}D$ 1.4836.

Anal. Calcd. for $C_{12}H_{20}O_5S$: C, 52.15; H, 7.30; S, 11.60; neut. equiv., 276. Found: C, 51.63; H, 7.23; S, 11.48; neut. equiv., 276.

(−)-3-Acetylthio-7-carbethoxyheptanoic Acid *l*-Ephedrine Salt.—A solution of 5 g. (0.018 mole) of DL-3-acetylthio-7-carbethoxyheptanoic acid in 50 ml. of ether was mixed with a solution of 3 g. (0.018 mole) of *l*-ephedrine in 50 ml. of ether. After a short time, 4.4 g. of crystalline product, m.p. 125–128°, precipitated. The product was recrystallized once from 10 ml. of methanol to yield 3.5 g. of crystals, m.p. 129–131°. This material was recrystallized from 10 ml. of methanol to give 2.4 g. (30%) of (−)-3-acetylthio-7-carbethoxyheptanoic acid *l*-ephedrine salt, m.p. 129–131°.

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

(15) The activity of (−)-α-lipoic acid was approximately 0.5% that of (+)-α-lipoic acid. The presence of small amounts of (+)-α-lipoic acid in the product is a possibility.

(16) Prepared from monoethyl adipate in yields of 40–60% by the previously described method (ref. 11). The reaction product was an oil which was distilled (b.p. 133–137° (0.1 mm.)) to yield the analytically pure acid (I), a low melting (*ca.* 20°) solid, $n^{26}D$ 1.4650. *Anal.* Calcd. for $C_{10}H_{18}O_4$: C, 59.97; H, 8.06; neut. equiv., 200. Found: C, 60.16; H, 8.00; neut. equiv., 200.

(17) The acetylthio acid II formed in a 16-hour reaction was satisfactory for use in the preparation of DL-3-acetylthio-7-carbethoxyheptanoyl chloride (III).

Anal. Calcd. for $C_{22}H_{35}NO_6S$: C, 59.84; H, 7.99; N, 3.17; S, 7.26. Found: C, 60.14; H, 8.19; N, 3.49; S, 7.22.

(−)-3-Acetylthio-7-carbethoxyheptanoic Acid.—A 2.25-g. (0.0051 mole) portion of (−)-3-acetylthio-7-carbethoxyheptanoic acid *l*-ephedrine salt was suspended in about 50 ml. of water and 40 ml. of ether. The mixture was acidified and the ether extract was separated. The aqueous phase was extracted with a second 40-ml. portion of ether. The combined ether layers were dried and concentrated at reduced pressure to yield 1.2 g. (85%) of (−)-3-acetylthio-7-carbethoxyheptanoic acid, $n^{25}D$ 1.4840, $[\alpha]^{23}D$ −6.8° (*c* 8.5, methanol).

Anal. Calcd. for $C_{12}H_{20}O_5S$: C, 52.15; H, 7.30; S, 11.60; neut. equiv., 276. Found: C, 52.07; H, 7.39; S, 11.69; neut. equiv., 277.

(+)-3-Acetylthio-7-carbethoxyheptanoic Acid Benzhydrylamine Salt.—The non-crystalline (+)-3-acetylthio-7-carbethoxyheptanoic acid *l*-ephedrine salt (remaining after the crystallization of the (−)-3-acetylthio-7-carbethoxyheptanoic acid *l*-ephedrine salt from 153 g. (0.554 mole) of DL-3-acetylthio-7-carbethoxyheptanoic acid) was acidified in a mixture of 600 ml. of ether and 500 ml. of water. The ether layer was separated and the aqueous layer was extracted with another portion of ether. The combined ether extracts were dried and concentrated at reduced pressure to yield 87 g. of crude (+)-3-acetylthio-7-carbethoxyheptanoic acid.

A 35-g. (0.146 mole) portion of crude (+)-3-acetylthio-7-carbethoxyheptanoic acid in 200 ml. of isopropyl ether was treated with a solution of benzhydrylamine (from 32 g. (0.146 mole) of benzhydrylamine hydrochloride) in 200 ml. of isopropyl ether. Crystals (35 g., m.p. 74–91°) of the benzhydrylamine salt separated. The product was recrystallized twice from about 500 ml. of isopropyl ether to yield finally 15 g. (22%) of (+)-3-acetylthio-7-carbethoxyheptanoic acid benzhydrylamine salt, m.p. 92–96°, $[\alpha]^{23}D$ +1.3° (*c* 8.06, methanol).

Anal. Calcd. for $C_{25}H_{33}NO_5S$: C, 65.33; H, 7.24; N, 3.05; S, 6.98. Found: C, 65.33; H, 7.03; N, 2.90; S, 7.10.

(+)-3-Acetylthio-7-carbethoxyheptanoic Acid.—A 14.0-g. (0.030 mole) portion of (+)-3-acetylthio-7-carbethoxyheptanoic acid benzhydrylamine salt was dissolved in 100 ml. of chloroform and 100 ml. of water was added. The mixture was acidified with hydrochloric acid and the chloroform layer was separated. The chloroform layer was washed with water, with dilute hydrochloric acid and twice again with water. The chloroform layer was dried and concentrated at reduced pressure to yield 8.7 g. (97%) of (+)-3-acetylthio-7-carbethoxyheptanoic acid, $[\alpha]^{23}D$ +6.8° (*c* 8.65, methanol), neut. equiv. 283.

In another experiment, 54 g. (0.120 mole) of (+)-3-acetylthio-7-carbethoxyheptanoic acid benzhydrylamine salt was suspended in 300 ml. of chloroform and 200 ml. of water and 60 ml. of 2.5 *N* hydrochloric acid was added. The chloroform extract was separated and washed with 20 ml. of 2.5 *N* hydrochloric acid and with two 20-ml. portions of water. The chloroform layer was dried, filtered and concentrated at reduced pressure to yield 31.8 g. of (+)-3-acetylthio-7-carbethoxyheptanoic acid, $[\alpha]^{25}D$ +6.8° (*c* 5.93, methanol), neut. equiv. 285. The acid was dissolved in 200 ml. of ether and cooled to about 10° and washed with a cold solution of 20 g. of potassium bicarbonate in 200 ml. of water. The bicarbonate layer was acidified immediately and the product extracted into 200 ml. of ether. The ether layer was washed with water, dried and concentrated to yield 25 g. of (+)-3-acetylthio-7-carbethoxyheptanoic acid, $[\alpha]^{32}D$ +6.7° (*c* 8.9, methanol), $n^{25}D$ 1.4840.

Anal. Calcd. for $C_{12}H_{20}O_5S$: C, 52.15; H, 7.30; S, 11.60; neut. equiv., 276. Found: C, 52.47; H, 7.12; S, 11.06; neut. equiv., 276.

(+)-3-Acetylthio-7-carbethoxyheptanoic Acid *d*-Ephedrine Salt.—A solution of *d*-ephedrine (from 2.2 g. of *d*-ephedrine hydrochloride) in 10 ml. of ether was added to a solution of 2.8 g. (0.01 mole) of DL-3-acetylthio-7-carbethoxyheptanoic acid in 5 ml. of ether. An additional 15 ml. of ether was added. A precipitate of 2.3 g. of crystalline salt, m.p. 119–126°, was obtained. The product was recrystallized from methanol–ether (1:2) to yield 1.55 g. (35%) of (+)-3-acetylthio-7-carbethoxyheptanoic acid *d*-ephedrine salt, m.p. 129–131°.

Anal. Calcd. for $C_{22}H_{35}NO_6S$: C, 59.84; H, 7.99; N, 3.17; S, 7.26. Found: C, 59.86; H, 7.63; N, 3.05; S, 7.62.

The *d*-ephedrine salt was suspended in 6 ml. of chloroform and 6 ml. of 1.3 *N* hydrochloric acid was added. The chloroform extract was separated and concentrated at reduced pressure to yield 1.1 g. of oil. A 0.66-g. portion of the oil was dissolved in chloroform and extracted into cold aqueous sodium bicarbonate solution. The bicarbonate extract was acidified with dilute hydrochloric acid and the product was extracted into chloroform. The chloroform solution was washed with water, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated at reduced pressure to yield 0.3 g. of (+)-3-acetylthio-7-carbethoxyheptanoic acid, $[\alpha]^{25}D$ +7.5° (*c* 7.5, methanol), $n^{25}D$ 1.4836.

Anal. Calcd. for $C_{12}H_{20}O_5S$: C, 52.15; H, 7.30; S, 11.60. Found: C, 52.17; H, 7.29; S, 11.72.

DL-3-Acetylthio-7-carbethoxyheptanoyl Chloride (III).—A cold mixture of 125 g. (0.453 mole) of DL-3-acetylthio-7-carbethoxyheptanoic acid and 50 ml. of thionyl chloride was permitted to warm to room temperature over a period of 16 hours. The excess thionyl chloride was removed at reduced pressure. The acid chloride was freed of last traces of volatile materials by adding dry benzene and concentrating the solution at reduced pressure. The residual DL-3-acetylthio-7-carbethoxyheptanoyl chloride, $n^{25}D$ 1.4850, amounted to 132 g. (99%).

Anal.[18] Calcd. for $C_{12}H_{19}ClO_4S$: C, 48.89; H, 6.50; S, 10.88; Cl, 12.03; neut. equiv., 147. Found: C, 49.94; H, 7.32; S, 11.15; Cl, 12.28; neut. equiv., 148.

(+)-3-Acetylthio-7-carbethoxyheptanoyl Chloride (III).—(+)-3-Acetylthio-7-carbethoxyheptanoic acid (23 g., 0.084 mole) and 9.7 ml. of thionyl chloride reacted in the manner described for the DL-acid. The reaction yielded 24.4 g. (99%) of (+)-3-acetylthio-7-carbethoxyheptanoyl chloride, $[\alpha]^{29}D$ +29.3° (*c* 2.42, benzene), $n^{26}D$ 1.4855.

Anal. Calcd. for $C_{12}H_{19}ClO_4S$: C, 48.89; H, 6.50; S, 10.88; Cl, 12.03; neut. equiv., 147; sapn. equiv., 74. Found: C, 48.67; H, 6.37; S, 10.99; Cl, 11.20; neut. equiv., 147; sapn. equiv., 68.

(−)-3-Acetylthio-7-carbethoxyheptanoyl Chloride (III).—(−)-3-Acetylthio-7-carbethoxyheptanoic acid (36 g., 0.13 mole) and 14.5 ml. of thionyl chloride reacted in the manner described above to yield 38.7 g. (99%) of (−)-3-acetylthio-7-carbethoxyheptanoyl chloride, $[\alpha]^{26}D$ −30.1° (*c* 2.56, benzene), $n^{26}D$ 1.4852.

Anal. Calcd. for $C_{12}H_{19}ClO_4S$: C, 48.89; H, 6.50; S, 10.88; Cl, 12.03; neut. equiv., 147; sapn. equiv., 74. Found: C, 49.10; H, 6.41; S, 11.05; Cl, 10.37; neut. equiv., 147; sapn. equiv., 68.

Reduction of DL-3-Acetylthio-7-carbethoxyheptanoyl Chloride with Sodium Borohydride. A. In Dioxane.—A solution of 57 g. (0.193 mole) of the acid chloride in 50 ml. of dioxane was added dropwise to a stirred suspension of 46 g. (1.21 moles) of sodium borohydride in 500 ml. of dioxane. The addition was completed in 15 minutes and during the same time the temperature rose from 25 to 30°. About 100 ml. of ice-water was added while the temperature was maintained at <20°. The addition of water was followed by the addition of cold hydrochloric acid (100 ml. of concentrated hydrochloric acid plus 150 ml. of ice and water). The reaction mixture was diluted with 1500 ml. of water and extracted with three portions of chloroform. The combined chloroform extracts were washed with an aqueous solution of potassium bicarbonate and then with two portions of water. The chloroform layer was dried and concentrated under reduced pressure to yield 45.3 g. of neutral product. Distillation (b.p. 115–125° (40–70 μ)) yielded 36.5 g. of a mixture of reduction products.[19]

(18) Analytical data obtained on the products of several preparations were not entirely satisfactory. In general, analyses of freshly prepared acid chloride were acceptable, but after aging a few days poor analytical values were obtained. Further evidence of the unstable nature of the acid chloride was obtained when it was noted that samples kept at room temperature for a few weeks deposited a crystalline solid.

(19) The analytical data indicate about 50% free thiol. However, the acetyl value as well as mol. wt. and sapn. equiv. show that 70% of the original acetyl groups remain in the product. Hence 20% of the

Anal. Calcd. for $C_{12}H_{22}O_4S$: C, 54.93; H, 8.45; S, 12.22; –SH, 0; CH_3CO–, 16.3; mol. wt., 262.4; sapn. equiv., 131. Calcd. for $C_{10}H_{20}O_3S$: C, 54.51; H, 9.15; S, 14.55; –SH, 15.01; CH_3CO–, 0; mol. wt. 220; sapn. equiv., 220. Found: C, 54.91; H, 8.75; S, 13.55; –SH, 7.6; CH_3CO–, 11.36; mol. wt. (ebul.), 243 ± 3; sapn. equiv., 157.

B. In Ether.—A solution of 32.3 g. (0.11 mole) of DL-3-acetylthio-7-carbethoxyheptanoyl chloride in 150 ml. of ether was added in 30 minutes to a stirred suspension of 21 g. (0.55 mole) of sodium borohydride in 300 ml. of ether. During the addition the temperature did not exceed 28°. After being stirred for 2 hours, the reaction mixture was cooled to about 0° and 110 ml. of cold water was added slowly while the temperature was maintained at 0 ±5° with a Dry Ice-bath. The mixture was acidified with 90 ml. of cold 5 *N* hydrochloric acid and an additional 750 ml. of water was added. The layers were separated and the aqueous phase was extracted twice with ether. The combined ether layers were washed with aqueous sodium bicarbonate, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated to yield 23.1 g. of oil. The oil was distilled at reduced pressure and a 2.4-g. fraction of ethyl DL-8-acetoxy-6-thioloctanoate (b.p. 112–116° (3 μ), $n^{25}D$ 1.4660) was collected.

Anal. Calcd. for $C_{12}H_{22}O_4S$: C, 54.93; H, 8.45; S, 12.22; –SH, 12.60; mol. wt., 262; sapn. equiv., 131. Found: C, 55.03; H, 8.36; S, 12.04; –SH, 12.21; sapn. equiv., 131; mol. wt. (ebul.), 289 ± 4.

The infrared spectrum showed bands at 5.76 and 8.05 μ indicative of O-acetyl. No bands characteristic of hydroxyl or thioacetyl were evident.

The other distillation fractions were mixtures of reduction products. In addition an undistilled residue (13 g.) remained.

Anal. Found: C, 56.27; H, 7.88; S, 10.6; CH_3CO–, 15.6; C_2H_5O–, 17.3; mol. wt. (ebul.), 469 ± 14; sapn. equiv., 110.

The infrared spectrum showed bands at 5.77 and 5.90 μ of equal intensity, while no indication of hydroxyl function was noted.[20]

DL-8-Hydroxy-6-thioloctanoic Acid (V).—A mixture of 16.8 g. of the distilled reduction product (IVa, b and c), 28 ml. of 30% sodium hydroxide solution and 0.75 g. of zinc dust in 60 ml. of methanol was refluxed for 30 minutes. The reaction mixture was concentrated under reduced pressure to remove most of the methanol. The residue was diluted to about 100 ml. with water and filtered. The clear filtrate was washed with chloroform and acidified to about *p*H 3. The acidified aqueous phase was extracted with ether. The ether extract was dried and concentrated at reduced pressure. The residue was dissolved in ether and the slightly cloudy solution was filtered through a layer of ether-washed Super-cel. The filtrate was concentrated at reduced pressure to yield 10.6 g. (83%) of DL-8-hydroxy-6-thioloctanoic acid, $n^{23.5}D$ 1.4989.

Anal. Calcd. for $C_8H_{16}O_3S$: C, 49.97; H, 8.39; S, 16.67; –SH, 17.20; neut. equiv., 192; mol. wt., 192. Found: C, 50.09; H, 8.08; S, 17.17; –SH, 16.70; neut. equiv., 192; mol. wt. (ebul.); 210 ± 3

DL-6,6′-Dithiobis-(8-hydroxyoctanoic Acid) (VII).—One-half (14.2 g.) of the distilled product obtained from a sodium borohydride reduction of 54 g. (0.183 mole) of DL-3-acetylthio-7-carbethoxyheptanoyl chloride was dissolved in 60 ml. of methanol and 24 ml. of 30% aqueous sodium hydroxide. Following the addition of 0.20 g. of zinc dust, the reaction mixture was refluxed for one hour. Most of the methanol was removed at reduced pressure and the residue was dissolved in 60 ml. of water and filtered. The *p*H of the solution was adjusted to about 7 by adding concentrated hydrochloric acid. The product was oxidized by the addition of approximately 60 ml. of an iodine–potassium iodide solution (10% iodine) until the brown color persisted. The excess iodine was reduced by the addition of a solution of sodium bisulfite. The mixture was acidified to *p*H 3 and the oily product which separated was extracted into ethyl acetate. The acidic product was removed from the ethyl acetate by extraction with sodium bicarbonate solution. Acidification liberated the product which was re-extracted into ethyl acetate. The ethyl acetate extract was dried over anhydrous magnesium sulfate, filtered and concentrated at reduced pressure to yield 9.6 g. (55% from the acid chloride) of DL-6,6′-dithiobis-(8-hydroxyoctanoic acid).

Anal. Calcd. for $C_{16}H_{30}O_6S_2$: C, 50.24; H, 7.90; S, 16.76; –SS–, 8.38; neut. equiv., 192; mol. wt., 383. Found: C, 51.76; H, 8.10; S, 15.04; –SS–, 8.32; neut. equiv., 199; mol. wt. (ebul.), 412 ± 13.

(+)-8-Hydroxy-6-thioloctanoic Acid (V).—A solution of 23.7 g. (0.081 mole) of (+)-3-acetylthio-7-carbethoxyheptanoyl chloride in 30 ml. of dioxane was added dropwise to a stirred suspension of 19 g. (0.5 mole) of sodium borohydride. After being stirred for two hours, the reduction mixture was worked up in the manner described above (*cf.* reduction of DL-3-acetylthio-7-carbethoxyheptanoyl chloride in dioxane) to yield 16 g. of crude reduction product boiling at 115–125° (0.1 mm.).

A mixture of 14 g. of the reduction product, 24 ml. of 30% sodium hydroxide solution and 0.70 g. of zinc dust in 50 ml. of methanol was refluxed for 30 minutes. Most of the methanol was removed at reduced pressure and the residue was dissolved in water and filtered through a layer of Super-cel. A 99-ml. portiou of the filtrate (total vol. 110 ml.; 11 ml. was retained for the next experiment) was acidified to *p*H 3 and extiacted with three portions of ether.

The combined ether extracts were washed twice with water, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated at reduced pressure to yield 9.4 g. (70% from the acid chloride) of (+)-8-hydroxy-6-thioloctanoic acid, $n^{26}D$ 1.5000, $[\alpha]^{30}D$ +4.8 (*c* 7.7, methanol).

Anal. Calcd. for $C_8H_{16}O_3S$: C, 49.97; H, 8.39; S, 16.67; neut. equiv., 192. Found: C, 50.04; N, 8.29; S, 17.39; neut. equiv., 189.

(−)-6,6′-Dithiobis-(8-hydroxyoctanoic Acid) (VII).—The 11-ml. alkaline solution of (+)-8-hydroxy-6-thioloctanoic acid from the above experiment was adjusted to *p*H 7. The solution was oxidized with iodine potassium iodide solution (10% iodine) until a permanent brown color was obtained. The excess iodine was reduced with a solution of sodium bisulfite. The *p*H of the solution was adjusted to about 3 and the product was extracted into ethyl acetate which was washed with water, dried and concentrated at reduced pressure. A residue of 1.1 g. (75%) of (−)-6,6′-dithiobis-(8-hydroxyoctanoic acid), $[\alpha]^{27}D$ −47° (*c* 2.53, in methanol), was obtained.

(−)-8-Hydroxy-6-thioloctanoic Acid (V).—In a manner similar to that described for the corresponding (+)-isomer above, 38 g. (0.129 mole) of (−)-3-acetylthio-7-carbethoxyheptanoyl chloride was reduced with sodium borohydride to yield 23.3 g. of distilled reduction product. This product was hydrolyzed, using 95 ml. of methanol, 45 ml. of 30% sodium hydroxide and 1.2 g. of zinc dust. The mixture was concentrated, diluted with water and filtered. A 153-ml. portion of the filtrate (total 170 ml., 17 ml. being retained for the next experiment) was acidified and worked up to yield 13.6 g. (60%) of (−)-8-hydroxy-6-thioloctanoic acid, $n^{26}D$ 1.5000, $[\alpha]^{30}D$ −5.1° (*c* 7.96, methanol).

Anal. Calcd. for $C_8H_{16}O_3S$: C, 49.97; H, 8.39; S, 16.67; neut. equiv., 192. Found: C, 50.79; H, 8.26; S, 16.96; neut. equiv., 207.

(+)-6,6′-Dithiobis-(8-hydroxyoctanoic Acid) (VII).—The 17-ml. sample of alkaline hydrolysis mixture retained in the above experiment was acidified to *p*H 7 with hydrochloric acid. The product was oxidized with iodine–potassium iodide solution (10% iodine) until a permanent coloration was obtained; about 12 ml. of solution was used. The reaction was worked up as before (*cf.* (−)-isomer) to yield 1.7 g. (69%) of (+)-6,6′-dithiobis-(8-hydroxyoctanoic acid), $[\alpha]^{33}D$ +53.1° (*c* 3.2, methanol).

DL-α-Lipoic Acid (VIII).[5,6] **A. From DL-6,6′-Dithiobis-(8-hydroxyoctanoic Acid).**—A mixture of 46.7 g. (0.122 mole) of DL-6,6′-dithiobis-(8-hydroxyoctanoic acid), 135 g. of

acetyl should be accounted for as O-acetyl. These results indicate that the product is a mixture of 20% of ethyl DL-8-acetoxy-6-thioloctanoate, 50% ethyl DL-8-hydroxy-6-acetylthiooctanoate and 30% ethyl DL-8-hydroxy-6-thioloctanoate. Analytical data on other reduction products have shown that the proportion of these three products is variable.

(20) As this material was not purified, its structure cannot be stated with certainty. However, the data available suggest that diethyl DL-6,6′-dithiobis-(8-acetoxyoctanoate) may be the major component of this residue. *Anal.* Calcd. for $C_{24}H_{42}O_8S_2$: C, 55.11; H, 8.10; S, 12.26; CH_3CO–, 16.46; C_2H_5O–, 17.23; mol. wt., 523; sapn. equiv., 131.

thiourea and 360 ml. of hydrobromic acid (40%) was refluxed 17 hours. The acidic solution was neutralized with 30% sodium hydroxide solution. Enough sodium hydroxide solution then was added to make the solution 0.5 *N* and the mixture was refluxed for 30 minutes. The solution was acidified and the oil which separated was extracted into ethyl acetate. The ethyl acetate was removed at reduced pressure to yield a 46-g. residue. The residue was leached with three 100-ml. portions of chloroform which dissolved 17 g. of the product. The chloroform solution was shaken with an aqueous solution of iodine–potassium iodide until the color of iodine persisted. The excess iodine was reduced with a solution of sodium bisulfite. The chloroform layer was washed with water, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated at reduced pressure to yield 10.3 g. of residual oil. The oil was extracted with successive portions of boiling cyclohexane. The cyclohexane solutions were cooled and a total of 5.4 g. (10%) of crystalline DL-*α*-lipoic acid (m.p. 59–61°) deposited. An additional 0.66 g. of crystalline product (m.p. 47–55°) was obtained from the concentrated filtrates.

B. From DL-8-Hydroxy-6-thioloctanoic Acid.—A mixture of 40 g. (0.21 mole) of DL-8-hydroxy-6-thioloctanoic acid, 122 g. (1.6 moles) of thiourea and 320 ml. of 40% hydrobromic acid was refluxed for 16 hours. The reaction solution was cooled and 320 ml. of 30% sodium hydroxide solution was added. The alkaline mixture was refluxed for 1 hour, cooled and acidified with concentrated hydrochloric acid. The product was extracted into chloroform. The chloroform solution was washed with water, dried over anhydrous magnesium sulfate, filtered and divided into two equal parts.

One part of the chloroform solution was shaken with iodine–potassium iodide solution (10% iodine) until the color of iodine persisted; about 225 ml. of solution was used. The layers were separated and the chloroform solution was washed with aqueous sodium bisulfite to reduce excess iodine. The chloroform layer was washed with water, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated at reduced pressure to yield 18 g. of residual oil. The oil was leached with three 100-ml. portions of boiling cyclohexane which yielded a total of 7.81 g. (36%) of DL-*α*-lipoic acid, m.p. 57–60°.

The other half of the chloroform solution was concentrated at reduced pressure to give 19.6 g. of crude DL-dihydro-*α*-lipoic acid. This was dissolved in 200 ml. of water containing 12.6 g. of potassium carbonate. The milky solution obtained was filtered through a mat of Super-cel and the filtrate was adjusted to *p*H 7 with 2.5 *N* hydrochloric acid. After adding 4 ml. of 1% aqueous ferric chloride, a stream of air was bubbled through the solution for 2.5 hours. The dark color of the reaction solution changed to bright yellow. The solution was acidified with hydrochloric acid and the product was extracted into chloroform. The chloroform extract was dried over anhydrous magnesium sulfate, filtered and concentrated at reduced pressure to yield 6.5 g. of residual oil. The oil was extracted with three 50-ml. portions of boiling cyclohexane which on being cooled yielded a total of 4.96 g. (23%) of crystalline DL-*α*-lipoic acid, m.p. 57–61°.

(+)-*α*-Lipoic Acid VIII. A. From (−)-6,6′-Dithiobis-(8-hydroxyoctanoic Acid).—A mixture of 5 g. (0.018 mole) of (−)-6,6′-dithiobis-(8-hydroxyoctanoic acid), 8 g. of thiourea and 26 ml. of 40% hydrobromic acid was refluxed for 16 hours. The reaction mixture was cooled and neutralized with 30% aqueous sodium hydroxide. Enough 30% sodium hydroxide solution was added to make the solution 1 *N* and the mixture was refluxed for 15 minutes. The mixture was cooled and acidified to *p*H 3 with concentrated hydrochloric acid. The chloroform-soluble products were removed by two 100-ml. extractions. The combined chloroform layers were washed with water and the acidic product was removed by extraction with sodium bicarbonate solution. The alkaline extract was acidified. The product was extracted into chloroform and the extract was shaken with iodine–potassium iodide solution (10% iodine). The excess iodine was reduced with sodium bisulfite solution and the chloroform layer was washed with water. The chloroform solution was dried over anhydrous magnesium sulfate, filtered and the filtrate was concentrated at reduced pressure. The residual 1.12 g. was leached with 18 ml. of hot cyclohexane. The cooled cyclohexane extract yielded 530 mg. (8%) of (+)-*α*-lipoic acid, m.p. 45–48.5°.

A 95-mg. sample of (+)-*α*-lipoic acid was sublimed at 85–90° (25 *μ*) to yield 82 mg. of sublimate. This was recrystallized from 1.5 ml. of cyclohexane to give 41 mg. of purified product, m.p. 46–48° (micro-block), $[\alpha]^{23}D$ +104° (*c* 0.88, benzene), $\lambda_{max}^{CH_3OH}$ 333 mμ (ε 150).

Anal. Calcd. for $C_8H_{14}O_2S_2$: C, 46.60; H, 6.84; S, 31.05; neut. equiv., 206; mol. wt., 206. Found: C, 46.95; H, 6.85; S, 31.00; neut. equiv., 208 (pK_a 5.4); mol. wt. (ebul.), 194 ± 2.

B. From (+)-8-Hydroxy-6-thioloctanoic Acid.—A mixture of 7.6 g. (0.04 mole) of (+)-8-hydroxy-6-thioloctanoic acid, 27 g. (0.36 mole) of thiourea and 70 ml. of 40% hydrobromic acid was refluxed for 16 hours. The reaction mixture was neutralized with about 60 ml. of 30% aqueous sodium hydroxide and made 0.5 *N* with an additional 13 ml. of 30% aqueous sodium hydroxide. The alkaline mixture was refluxed 30 minutes. It was acidified and extracted with 3 portions of chloroform. The combined chloroform extracts were washed with water, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated at reduced pressure to yield 6.8 g. of crude (−)-6,8-dithioloctanoic acid, $n^{26}D$ 1.5238, $[\alpha]^{27}D$ −8.8° (*c* 6.92, methanol), neut. equiv. 203 (calcd. 208).

A solution of 5.8 g. of the (−)-dithiol in 75 ml. of water and 3.8 g. of potassium carbonate was adjusted to *p*H 7 by adding 2.5 *N* hydrochloric acid. The solution was treated with 1.5 ml. of ferric chloride (1%) which produced a dark coloration. Air was bubbled through the solution until the color changed to yellow. The reaction solution was acidified with hydrochloric acid and the liberated product was extracted into 3 portions of chloroform. The combined chloroform extracts were washed with water, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated at reduced pressure to leave a 3.5-g. residue. The residue was leached with two 25-ml. portions of boiling cyclohexane. The first cyclohexane extract yielded 1.47 g. (20% from (+)-8-hydroxy-6-thioloctanoic acid) of (+)-*α*-lipoic acid, m.p. 45–47°, $[\alpha]^{27}D$ +99° (*c* 1.07, benzene).

The filtrate and second cyclohexane extract were combined and concentrated to a 10-ml. volume. On cooling the concentrate, a second crop (0.65 g.) of product (m.p. 40–47°) was obtained.

(−)-*α*-Lipoic Acid. A. From (+)-6,6′-Dithiobis-(8-hydroxyoctanoic Acid).—A mixture of 11.9 g. (0.031 mole) of (+)-6,6′-dithiobis-(8-hydroxyoctanoic acid), 19.2 g. (0.25 mole) of thiourea and 63 ml. of 40% hydrobromic acid was refluxed for 16 hours. The products of the reaction were worked up in a manner similar to that described for the corresponding (+)-isomer above. The chloroform solution after iodine oxidation yielded 2.2 g. of product. This material was leached with 25 ml. of hot cyclohexane from which was obtained 800 mg. (6%) of (−)-*α*-lipoic acid, m.p. 45–47°.

A 300-mg. sample was recrystallized from 11 ml. of cyclohexane to give 200 mg. of purified (−)-*α*-lipoic acid, m.p. 45–47.5° (micro-block), $[\alpha]^{23}D$ −113° (*c* 1.88 benzene), $\lambda_{max}^{CH_3OH}$ 330 mμ (ε 140).

Anal. Calcd. for $C_8H_{14}O_2S_2$: C, 46.60; H, 6.84; S, 31.05; neut. equiv., 206; mol. wt., 206. Found: C, 46.65; H, 6.66; S, 31.32; neut. equiv., 208 (pK_a 5.4); mol. wt. (ebul.), 212 ± 2.

B. From (−)-8-Hydroxy-6-thioloctanoic Acid.—A mixture of 12.5 g. (0.065 mole) of (−)-8-hydroxy-6-thioloctanoic acid, 38 g. (0.50 mole) of thiourea and 100 ml. of 40% hydrobromic acid was refluxed for 16 hours. The reaction mixture was worked up in the manner described for the corresponding (+)-isomer above to give 11.6 g. of crude (+)-6,8-dithioloctanoic acid, $n^{26}D$ 1.5267, $[\alpha]^{30}D$ +11° (*c* 5.51, methanol), neut. equiv. 228 (calcd. 208).

A solution of 11.4 g. of (+)-6,8-dithioloctanoic acid in 150 ml. of water and 7.4 g. of potassium carbonate was adjusted to *p*H 7 and oxidized with air and ferric chloride (4 ml. of 1%). The reaction product was worked up as before to yield a chloroform-soluble fraction. This material was leached with one 100-ml. and two 50-ml. portions of hot cyclohexane. The 100-ml. extract yielded 3.65 g. (27%) of (−)-*α*-lipoic acid, m.p. 45–47°, $[\alpha]^{27}D$ −105° (*c* 0.9, benzene). The filtrate and two 50-ml. extracts were combined, concentrated and cooled to yield a second crop of 0.7 g. (5%) of product, m.p. 45–48°.

DL-α-Lipoic Acid from (+)- and (−)-α-Lipoic Acid.—Samples of 12.9 mg. of both (+)-α-lipoic acid and (−)-α-lipoic acid were mixed and ground together in a mortar. The mixture (m.p. 55–57°) was recrystallized from cyclohexane to give DL-α-lipoic acid, m.p. 60–61°.

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[CONTRIBUTION FROM THE DEPARTMENT OF ANESTHESIA, MERCY HOSPITAL AND SECTION ON ANESTHESIOLOGY, DEPARTMENT OF SURGERY, UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE]

Hydrolysis of Ester-type Local Anesthetics and their Halogenated Analogs by Purified Plasma Cholinesterase[1]

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The reaction rate constants (K) and the Michaelis constants (K_m) of three *p*-aminobenzoic acid esters and their 2-chloro substituted derivatives[2] were determined in fresh human plasma and a human plasma cholinesterase concentrate (Cholase[3]). The K and K_m values of the 2-fluoro[4] and 2-bromo[2] substituted procaine HCl were also determined. Halogen substitution in the 2-position caused a 4- to 6-fold increase in the K values of the compounds investigated. The influence of halogen substitution on the K_m values was less marked.

The influence of halogen substitution on the enzymatic hydrolysis rate of *p*-aminobenzoic acid esters in human plasma[5] and on the local anesthetic activity and systemic toxicity of these compounds[6,7] have been previously reported. In these studies the hydrolysis rates of the various compounds were observed in plasma samples obtained on different days from different individuals and were expressed as the time necessary for 50% hydrolysis. Despite precautions taken to obtain valid comparative values for the hydrolysis rates it was felt that more characteristic data could be obtained by using a uniform source of enzyme (Cholase), and by determining, instead of the 50% hydrolysis time, the K and K_m values of the different compounds.

Material and Methods

The K and K_m of procaine·HCl (I), tetracaine·HCl (V) and 2-*sec*-butylaminoethyl-4-aminobenzoate·HCl (VII) as well as their 2-chloro substituted analogs (III, VI and VIII, respectively) were measured using a purified human plasma cholinesterase concentrate (Cholase). The K and K_m of the 2-bromo (IV) and 2-fluoro (II) analogs of procaine·HCl were also determined.[8] The K and K_m of all compounds were also measured in freshly obtained, heparinized, pooled human plasma samples and occasionally also in individual plasmas.

To avoid extreme variations in the times necessary for the completion of the experiments and in order to obtain more accurate K_m values the quantity of the enzymes used was varied according to the hydrolysis rate of the compound investigated. Since too high plasma concentrations interfered with spectrophotometric readings, this principle could be followed more closely with Cholase than with plasma.

Cholase dilutions of 1 to 125, 1 to 375, 1 to 750 and 1 to 1500 were made with a phosphate buffer (containing 6.07 g. of Na_2HPO_4 and 2.00 g. of $NaH_2PO_4 \cdot H_2O$ in 1 liter of distilled water). 10^{-3} M solutions of all substrates were prepared in distilled water. The systems used for the hydrolysis studies were prepared by adding at zero time, 0.2 ml. of the substrate solution, to a mixture of 1.0 ml. of buffer, 0.8 ml. of distilled water and 2.0 ml. of the appropriate dilution of Cholase or plasma used. All solutions were warmed to 37° before mixing. The 0.8 ml. of distilled water was included in the systems to allow for the use of cholinesterase inhibitors in subsequent studies.

TABLE I

CHOLASE AND PLASMA DILUTIONS AND RELATED SUBSTRATE CONCENTRATIONS

Substrate	Cholase: Dilution used	Cholase: Substrate concn. in cholase, moles/ml.	Plasma: Dilution used	Plasma: Substrate concn. in plasma, moles/ml.
Procaine·HCl (I)	1–375	3.75×10^{-5}	1–5.0	5.0×10^{-7}
2-Fluoroprocaine·HCl (II)	1–1500	1.50×10^{-4}	1–10.0	1.0×10^{-6}
2-Chloroprocaine·HCl (III)	1–1500	1.50×10^{-4}	1–10.0	1.0×10^{-6}
2-Bromoprocaine·HCl (IV)	1–750	7.50×10^{-5}	1–10.0	1.0×10^{-6}
Tetracaine·HCl (V)	1–125	1.25×10^{-5}	1–2.5	2.5×10^{-7}
2-Chlorotetracaine·HCl (VI)	1–750	7.50×10^{-5}	1–5.0	5.0×10^{-7}
2-*sec*-Butylaminoethyl-3-aminobenzoate·HCl (VII)	1–125	1.25×10^{-5}	1–2.5	2.5×10^{-7}
2-*sec*-Butylaminoethyl-2-chloro-4-aminobenzoate·HCl (VIII)	1–750	7.50×10^{-6}	1–5.0	5.0×10^{-7}

The *p*H (7.4) and the Na^+ concentration (0.025 M) were identical with those present in the systems generally used for the measurement of the activities of the various enzyme sources with acetylcholine substrate in Warburg experiments. The volume of all systems was 4 ml. and their final substrate concentration was 5×10^{-5} M.

The only variables in the composition of the systems were the dilution of enzymes used and, consequently, the enzymes substrate ratio. These variables are summarized in Table I.

The hydrolysis rates of the various substrates were determined with a modification of the ultraviolet spectrophotometric method of Kalow.[9] The changes of the optical den-

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(2) These compounds were kindly supplied by Dr. Robert H. Hall of Wallace & Tiernan, Inc., Belleville, New Jersey.

(3) Cholase was kindly supplied by Dr. Edwin B. McLean, Cutter Laboratories, Berkeley, California.

(4) The 2-Fluoroprocaine HCl was placed at our disposal by Dr. A. C. Bratton, Jr., of Parke, Davis & Co., Detroit, Michigan.

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(6) F. F. Foldes and D. H. Rhodes, *Anesth. & Analg.*, **32**, 305 (1953).

(7) F. F. Foldes, D. L. Davis and O. J. Plekss, "Anesthesiology," in press.

(8) From here on, for the sake of brevity, the various compounds will be referred to by their serial number in Table II.

(9) W. Kalow, *J. Pharmacol. & Exper. Therap.*, **104**, 122 (1952).