depress the melting point of an authentic sample (reported[19] m.p. 104–105°, $[\alpha]^{20}D$ −18.2° (*c* 3.81, chloroform)) prepared by acetylating the glucoside with acetic anhydride in pyridine. Section A-5 (20–45 mm. from the top) contained 0.05 g. of sirup which yielded 0.02 g. of crystals melting at 124–128°. Recrystallization from benzene–petroleum ether (b.p. 30–60°) raised the melting point to 129–130°, $[\alpha]^{25}D$ +18° (*c* 1.2, chloroform). The melting point of a mixture with an authentic sample of 2,3,4-tri-*O*-acetyl-6-*O*-benzoyl-β-D-glucoside, prepared by the method of Ohle and Spencker,[20] who report m.p. 127°, $[\alpha]^{19}D$ +15.15° (*c* 1.65, chloroform), was not depressed. Section A-6 (45–80 mm. from the top) contained 0.09 g. of sirup which crystallized easily giving 0.07 g. of methyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-glucoside (IV), m.p. 126–130°. Recrystallization from benzene–petroleum ether (b.p. 30–60°) increased the melting point to 130–132°, $[\alpha]^{28}D$ +76° (*c* 3.6, chloroform). The melting point was not depressed when a sample was admixed with authentic IV, prepared by the method of Levene and Raymond,[18] who report m.p. 131–132°, $[\alpha]^{20}D$ +79.8° (*c* 2, chloroform). Section A-7 (80–125 mm. from the top) contained 0.08 g. of a sirup which yielded 0.04 g. of crystals melting at 154–156°. Recrystallization from methanol increased the melting point to 156–157°. This compound was identical with that obtained from section A-2 of the crystalline mixture, a mixed melting point with an authentic sample of methyl 4-*O*-acetyl-2,3,6-tri-*O*-benzoyl-β-D-glucoside (VI) showing no depression. Section A-8 (125–160 mm. from the top) contained 0.05 g. of a sirup which yielded 0.025 g. of methyl tetra-*O*-benzoyl-β-D-glucoside (VII), m.p. 158–160°. Recrystallization from methanol gave a product with a melting point of 161–162°, $[\alpha]^{26}D$ +31° (*c* 2.6, chloroform). A mixed melting point with an authentic sample of VII (reported[21] m.p. 160–162°, $[\alpha]^{19}D$ +30.79° (*c* 10.5, chloroform)) prepared by treating the glucoside with benzoyl chloride in pyridine, showed no depression. The recovery of the various compounds for the several experiments are summarized in Table I.

Preparation and Benzoylation of the Borate Ester Complex of Methyl α-D-Glucoside.—Methyl α-D-glucoside (2.00 g., 1 mole) and metaboric acid-III (0.45 g., 1 mole) were mixed with 20 ml. of anhydrous reagent-grade acetone and processed in the same manner as described for the β-glucoside. The solid borate-ester complex was dissolved in 15 ml. of anhydrous pyridine to which was added slowly, with cooling, 6.0 ml. (5 moles) of reagent-grade benzoyl chloride. The reaction mixture was left at room temperature for 18 hours after which a few milliliters of water was added. After 30 minutes the reaction mixture was poured onto 100 g. of ice and water. The resulting suspension was extracted with 100 ml. of ether. The ether solution was washed twice each with 25-ml. portions of 2 *N* hydrochloric acid and 2 *N* sodium hydroxide, and once with 25 ml. of water. The solution was dried over anhydrous sodium sulfate and concentrated to a sirup on a steam-bath. The sirup was dissolved in 20 ml. of benzene, and petroleum ether (60–120°) was added to produce a slight turbidity at about 50°. Upon cooling slowly, flocculent crystals were deposited. Recrystallization from benzene–petroleum ether in a similar manner yielded 1.0 g. (24%) of methyl 2,6-di-*O*-benzoyl-α-D-glucoside, m.p. 130–136°. Several recrystallizations from the same solvent raised the melting point to 141–142°, $[\alpha]^{30}D$ +75° (*c* 3.1, chloroform). An admixture of this compound with an authentic sample prepared by the method of Lieser and Schweizer,[17] who report m.p. 144°, $[\alpha]^{23}D$ +80.37° (*c* 4.0, chloroform), showed no depression of melting point.

Acknowledgment.—J. C. P. gratefully acknowledges support from a University Research Committee Fellowship.

SALT LAKE CITY, UTAH

(19) C. S. Hudson and J. K. Dale, THIS JOURNAL, **37**, 1264 (1915).
(20) H. Ohle and K. Spencker, *Ber.*, **59B**, 1836 (1926).
(21) E. Fischer and B. Helferich, *Ann.*, **383**, 68 (1911).

[CONTRIBUTION FROM THE DEPARTMENT OF BACTERIOLOGY OF THE UNIVERSITY OF ILLINOIS]

Biosynthesis and Structure of Lipoic Acid Derivatives[1]

BY I. C. GUNSALUS, LOIS S. BARTON AND W. GRUBER

RECEIVED AUGUST 24, 1955

A thioester transacetylase, prepared from *E. coli*, transports one acetyl group from an acetyl donor (lithium acetyl phosphate) to reduced lipoic (dihydrolipoic[2]) acid in the presence of phosphotransacetylase and Coenzyme A. This transacetylation is stereospecific for (−)-dihydrolipoic acid[3] (II) obtained by $NaBH_4$ reduction of (+)-lipoic acid (I). By chemical acetylation a monoacetyl-dihydrolipoic acid has been prepared, and shown to be 8-S-acetyl-6,8-dithioloctanoic acid (IVb). The enzymatically prepared compound is different from the synthetic one and contains a free primary SH-group; therefore, it is 6-S-acetyl-6,8-dithioloctanoic acid (IIIa). The basic carbodiimide (VI) was used for the characterization of the acids producing crystalline and well defined ureas; N-propionyldiphenylketimine (VII) was found to be a useful reagent for the differentiation of primary and secondary thiol groups.

Lipoic acid, an essential cofactor for keto acid oxidation,[4] has been isolated by following its catalytic activity in a pyruvate oxidation assay employing cells of *Streptococcus faecalis* grown in a medium deficient in this substance.[5] Chemically, lipoic acid has been identified as the dextrorotatory isomer of 5-(dithiolane-3)-pentanoic acid.[6,7] Subsequent synthesis of (±)-lipoic acid yielded a racemate with biological activity only 50% of that shown by the dextrorotatory acid.[8,9] Synthesis of the (+)- and (−)-isomers[10] has further confirmed the optical specificity of the catalyst, *i.e.*, synthetic (+)-lipoic acid possesses activity equal to the isolated material whereas the (−)-isomer is inactive. Hypotheses of the mechanism of lipoic acid function in the keto acid oxidation have been summarized as[11] in Fig. 1.

As indicated, three derivatives of lipoic acid are visualized as playing catalytic roles. Two of the

(1) We wish to express our appreciation to the United States Public Health Service, Department of Health, Education, and Welfare, which have supported in part this research.

(2) The pyruvate oxidation factor (POF) first isolated as a yellow crystalline solid, m.p. 49–50°, $[\alpha]^{20}D$ +100°, was termed α-lipoic acid. This catalyst, identified as 5-(dithiolane-3)-pentanoic acid, has since been shown to react enzymatically in the disulfide and dithiol forms. For simplicity, the term lipoic is suggested for the disulfide and the term dihydrolipoic acid is used to designate the reduced or dithiol derivative (6,8-dithioloctanoic acid).

(3) All reference here is to the sign of rotation, the biologically active disulfide (+)-lipoic acid is reduced to a biologically active (−)-dihydrolipoic acid, and enzymatically acetylated to the (+)-6-S-acetyl-dihydrolipoic acid. Chemical and biological evidence indicates these acids to be of the same configuration.

(4) D. J. O'Kane and I. C. Gunsalus, *J. Bact.*, **56**, 499 (1948).
(5) L. J. Reed, *et al.*, *Science*, **114**, 93 (1951).
(6) L. J. Reed, *et al.*, THIS JOURNAL, **75**, 1267 (1953).
(7) J. A. Brockman, *et al.*, *ibid.*, **74**, 1868 (1952).
(8) C. S. Hornberger, Jr., *et al.*, *ibid.*, **75**, 1273 (1953).
(9) M. W. Bullock, *et al.*, *ibid.*, **74**, 1868 (1952).
(10) E. Walton, *et al.*, *ibid.*, **76**, 4748 (1954).
(11) I. C. Gunsalus, in "The Mechanism of Enzyme Action," The Johns Hopkins Press, Baltimore, Md., 1954, pp. 545.

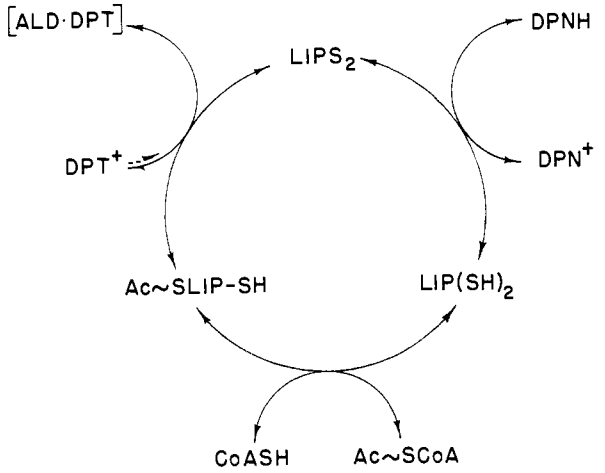


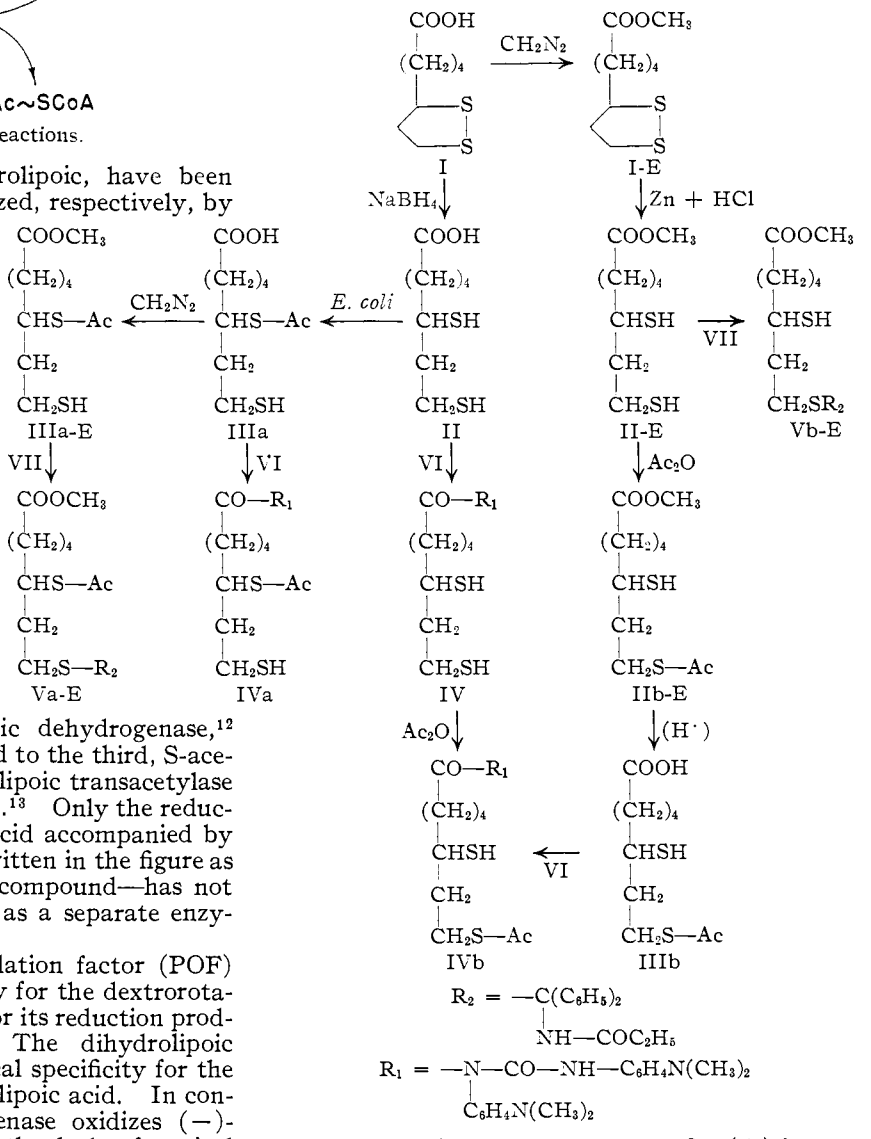
Fig. 1.—Lipoic acid reactions.

derivatives, lipoic and dihydrolipoic, have been shown to be reduced and oxidized, respectively, by a DPN-mediated dihydrolipoic dehydrogenase,[12] and the dihydrolipoate acylated to the third, S-acetyl-dihydrolipoate, by dihydrolipoic transacetylase with acetyl-CoA as acyl donor.[13] Only the reductive acetylation of the lipoic acid accompanied by oxidation of acetaldehyde—written in the figure as a diphosphothiamine-enzyme compound—has not been separated and measured as a separate enzymatic step.

The catalytic pyruvate oxidation factor (POF) assay[14] shows optical specificity for the dextrorotatory isomer of lipoic acid and for its reduction product (−)-dihydrolipoic acid. The dihydrolipoic transacetylase also shows optical specificity for the acetylation of the (−)-dihydrolipoic acid. In contrast, dihydrolipoic dehydrogenase oxidizes (−)- and (+)-dihydrolipoic acids—the lack of optical specificity holds for the *E. coli* enzyme in extracts[12] and the *S. faecalis* apoenzyme cells with substrate amounts of dihydrolipoic acid in place of pyruvate.[14]

Lynen, *et al.*,[15] have shown that the active form of Lipmann's[16] acetylating coenzyme is the S-acetyl derivative of the β-mercapto ethylamine end of Coenzyme A, *i.e.*, an acetyl ester of a primary mercapto group. In subsequent publications, chemical and biological properties of S-acyl-CoA were presented.[17] In this paper evidence for a thioester of a secondary thiol-group as an intermediate in pyruvate oxidation is given.

The present study has considered: (1) the structure of the biologically active S-acetyl-dihydrolipoic acid, and (2) the optical rotation of the disulfide, dihydro, and of the synthetic and enzymatically prepared acetyldihydro derivatives of lipoic acid.



Lipoic acid (racemate m.p. 59–60°; (+)-isomer m.p. 49°) reduced with sodium borohydride under carefully controlled conditions gave, respectively, (±)- and (−)-dihydrolipoic acids of the same biological (POF assay[14]) activity as the lipoic used, *i.e.*, racemate 0.5 μM activity/μM as compared to (+)-lipoic and (−)-dihydrolipoic 1 μM activity/μM.

(12) L. P. Hager and I. C. Gunsalus, THIS JOURNAL, **75**, 5767 (1953).
(13) L. P. Hager, Thesis, University of Illinois, 1953.
(14) I. C. Gunsalus and W. E. Razzell, "Methods of Enzymology," Vol. III, Academic Press, New York, N. Y., 1955, in press.
(15) F. Lynen, E. Reichert and L. Rueff, *Liebig's Ann. Chem.*, **574**, 1 (1951).
(16) F. Lipmann, *Bacteriol. Revs.*, **17**, 1 (1953).
(17) F. Lynen, *Ann. Rev. Biochem.*, **24**, 654 (1955).

The biologically active S-acetyl-dihydrolipoic acid was prepared with an extract of *Escherichia coli* as enzyme source by acylation of the (−)-dihydrolipoic acid as

(1) Dihydrolipoid transacetylase

(−)-dihydrolipoic acid + S-acetyl-CoA $\rightleftarrows$ (+)-S-acetyl-dihydrolipoic + CoASH . . . 1

(2) Phosphotransacetylase

Acetyl phosphate + CoASH $\rightleftarrows$ S-acetyl-Co A + iP . . . 2

With substrate amounts of acetylphosphate and (−)-dihydrolipoic acid, and catalytic amounts of CoA, the stoichiometric reaction is measured as

Acetyl phosphate + (−)-dihydrolipoic acid $\rightleftarrows$ (+)-S-acetyl-dihydrolipoic acid + iP . . . 3

Chemically synthesized S-acetyl-dihydrolipoic acid was prepared by acetylation of the methyl ester of (−)- and of (±)-dihydrolipoic acid.

These lipoic acid derivatives were characterized by the use of carbodiimides, well known reagents for the characterizations of organic acids, and with N-propionyldiphenylketimine (VII), which reacts with primary but not secondary hydroxyl and thiol groups.[18]

Certain carbodiimides (CDI) react with organic acids according to the reaction

$$R'\cdot COOH + R\cdot N{=}C{=}N\cdot R \longrightarrow R'\cdot CON(R)\cdot CO\cdot NH\cdot R$$

to yield stable acylureas.[19] Since the addition takes place in neutral and inert solvents one could expect that the labile S-acetyl group would not be attacked. Of all the CDI's tried, the "basic CDI" (VI)

$$(CH_3)_2N\cdot\langle\bigcirc\rangle\cdot N{=}C{=}N\cdot\langle\bigcirc\rangle\cdot N(CH_3)_2$$

yielded lipoic acid derivatives with suitable melting points.

N-Propionyldiphenylketimine (VII) is known to react preferentially with primary OH- and SH-groups according to

$$(C_6H_5)_2C{=}NCOC_2H_5 + R\cdot CH_2SH \longrightarrow (C_6H_5)_2C(SCH_2\cdot R)\cdot NHCOC_2H_5$$

(VII)

A comparative study with model compounds confirmed the specificity of this reagent.

For the chemical synthesis of mono-S-acetyl-dihydrolipoic acid (IIIb), lipoic acid was first converted into its methyl ester (diazomethane), the disulfide bond reduced with zinc and hydrochloric acid in methanol and acetylated with acetic anhydride in pyridine. A monoacetylmethyl ester was isolated from the reaction mixture by repeated fractionation *in vacuo*. Böhme and Schran[20] found that at *p*H 1 to 2 the O-ester is hydrolyzed preferentially to the S-ester. Thus, by acid hydrolysis at room temperature, S-acetyl-dihydrolipoic acid was obtained and characterized with VI yielding the urea IVb. The same derivative was synthesized in better yields by acetylating the dithioloctanoylurea (IV).

Evidence for formulation of compound IVb as the 8-S-acetyl derivative is the following: (1) even with a large excess of acetic anhydride only small amounts of the di-S-acetyl derivatives of dihydrolipoic acid methyl ester and of its acylurea (IV) are formed; (2) the methyl ester of the synthetic S-acetyl-dihydrolipoic acid does not react with N-propionyldiphenylketimine (VII).

In contrast the enzymatic acetylation gives the 6-S-acetyl derivative, as indicated by: (1) reaction with VI forms urea IVa different from the corresponding derivative IVb of chemically acetylated dihydrolipoic acid; (2) the methyl ester reacts smoothly with N-propionyldiphenylketimine (VII) to form the addition product (Va-E), thus indicating the presence of a free primary thiol-group.

The change in molecular rotation of CDI-adducts of the chemical and enzymatical acetylation products is in agreement with the rational assumption that addition of the acetyl to the asymmetric center (C6) would cause the greater change in optical properties.

TABLE I

MOLECULAR ROTATION

Compound	Free acid	CDI-add.	Ratio	Differences (Free acids)	Differences (CDI-add.)
6,8-Dithioloctanoic acid (DTO)	−3016	−1662	1.81	7041	3944
6-S-Acetyl-DTO (enz. acetylation)	+4025	+2282	1.76		
8-S-Acetyl-DTO (chem. acetylation)	(−2658)[a]	−1486		(358)	176

[a] Calculated: (−1486) × 1.785 (average of ratios).

Experimental[21]

(A) Lipoic Acid (5-(Dithiolane-3)-pentanoic Acid) (I). S-Benzyl-isothiuronium Salt.—(±)-α-Lipoic acid (206 mg.)[22] was exactly neutralized to phenolphthalein with 0.01 *N* NaOH, slightly acidified again by addition of one drop of 0.01 *N* hydrochloric acid, and a solution of 205 mg. of S-benzyl-isothiuronium chloride in methanol (5 ml.) added. The precipitate which formed was recrystallized twice from dilute methanol; m.p. 132–134°; yield 203 mg. (54%).

Anal. Calcd. for $C_{16}H_{24}N_2O_2S_3$ (372.57): C, 51.61; H, 6.45; S, 25.81. Found: C, 51.62; H, 6.21; S, 26.01.

Di-(*p*-dimethylaminophenyl)-urea.—(±)-α-Lipoic acid (200 mg.) and di-(*p*-dimethylaminophenyl)-carbodiimide (CDI) (280 mg.) were dissolved in 7 ml. of acetone; reaction and crystallization were complete after 12 hours. The compound was purified by recrystallization from acetone; m.p. 153–154° (short yellow prisms); yield 298 mg. (60%).

Anal. Calcd. for $C_{25}H_{34}N_4O_2S_2$ (486.66): C, 61.69; H, 7.04; N, 11.51; S, 13.18; act. H, 1.00. Found: C, 61.74; H, 7.14; N, 11.00; S, 12.85; act. H (100°), 0.68.

In the same way 80 mg. of (+)-lipoic acid[23] gave 115 mg. (61%) of the optically active derivative; m.p. 139–141°, $[\alpha]^{20}D$ +45.2 (*c* 1.233, benzene); found: N, 11.38; S, 13.02.

Methyl (±)-α-Lipoate (I-E).—To a chilled solution of 6.0 g. of the acid in ether (50 ml.) was added an ethereal solution of diazomethane (from 7 g. of nitrosomethylurea); the mixture was kept for 1 hour at 0°, then washed with dilute bicarbonate and finally with water. The ester was purified by distillation; b.p. 129–131° (0.7 mm.); yield 5.22 g. (82%); yellow oil unstable in light.

(B) Dihydrolipoic Acid (DTO) (6,8-Dithioloctanoic Acid) (II). (±)-DTO from (±)-α-Lipoic Acid.—To a solution of (±)-α-lipoic acid (6.00 g.) in 117 ml. of 0.25 *N* sodium bicarbonate, a total of 1.2 g. of sodium borohydride was added portionwise. The mixture was well stirred and kept below

(18) A. H. Redcliffe, *Roy. Aust. Chem. Inst. J. Proc.*, **17**, 205 (1950).

(19) F. Zetzsche, *et al.*, *Ber.*, **71B**, 1516 (1938).

(20) H. Böhme and H. Schran, *Chem. Ber.*, **82**, 453 (1949).

(21) All melting points are corrected (Kofler apparatus); microanalyses by Clark Analytical Laboratories.

(22) Kindly supplied by Chemical Department of E. I. du Pont de Nemours and Company.

(23) We are indebted to Dr. Karl Folkers of Merck and Company's Research Laboratories for the (+)-lipoic acid.

5°. After 30 minutes 100 ml. of benzene was added and the colorless reaction mixture was acidified to *p*H 1 with ice-cold 5 *N* HCl. The content of the benzene layer was distilled under reduced pressure; b.p. 169–172° (1 mm.); yield 5.50 g. (91%)

In the same way (+)-lipoic acid was reduced to (−)-DTO; the yield, without distillation, was almost quantitative; $[\alpha]^{20}D$ −14.5 (*c* 0.598, benzene). A 25-mg. sample of the pure (−)-isomer was distilled under 0.1 mm. yielding 23 mg. of a partly racemized substance ($[\alpha]^{20}D$ −8.9 (*c* 0.460, benzene)).

6,8-Dithioloctanoyl-di-(*p*-dimethylaminophenyl)-urea (IV). (DTO-CDI.)—128 mg. of the foregoing racemic acid and 180 mg. of the basic CDI were dissolved in abs. ether (6 ml.). Crystallization began after one hour and was completed the next day. Recrystallization from benzene–ether gave 174 mg. (58%); m.p. 122–124° (pale yellow needles).

Anal. Calcd. for $C_{25}H_{36}N_4O_2S_2$ (488.68): C, 61.44; H, 7.42; N, 11.47; S, 13.12; act. H, 3.00. Found: C, 61.60; H, 7.59; N, 11.83; S, 13.06; act. H (25°), 1.5, (100°), 2.3.

(−)-DTO was treated in the same way; m.p. 120–122° (plates), $[\alpha]^{20}D$ −3.4° (*c* 1.428, benzene); found, N, 11.54.

Methyl (±)-Dithioloctanoate (II-E).—A solution of freshly prepared methyl (±)-α-lipoate (5.00 g.) in methanol (150 ml.) was reduced with Zn-powder and concd. hydrochloric acid, with stirring at 0°. After 20 minutes, 150 ml. of benzene was added, the organic layer washed thoroughly with water, then with dilute bicarbonate, and finally with water. The solvent was removed under diminished pressure and the remainder distilled *in vacuo* through a short Widmer column; b.p. 125–128° (0.5 mm.); yield 4.87 g. (97%).

In the same way, omitting the distillation, methyl (−)-dithioloctanoate was prepared, but no attempt to determine the optical purity of this ester was made; $[\alpha]^{20}D$ −8.6 (*c* 4.134, benzene).

N-Propionyldiphenylketimine Derivatives (Vb-E).—A solution of methyl (±)-dithioloctanoate (220 mg.) and the reagent (237 mg.) in 20 ml. of petroleum ether was kept for 10 days at room temperature. The crystals were extracted with a large amount of the same solvent; m.p. 94–97°; yield 308 mg. (67%).

Anal. Calcd. for $C_{25}H_{34}NO_3S_2$ (460.65): C, 65.18; H, 7.44; S, 13.92. Found: C, 64.95; H, 7.32; S, 13.86.

With the (−)-isomer ($[\alpha]^{20}D$ −8.6° (*c* 4.134, benzene)) the same reagent gave a compound with the m.p. 116–119° and $[\alpha]^{20}D$ +20.0° (*c* 0.646, benzene); found, S 13.80.

(C) Synthetic S-Acetyl Compounds. Methyl S-Acetyldithioloctanoate (IIIb-E).—A mixture of methyl dithioloctanoate (b.p. 118–121° (0.2 mm.); 5.6 g. = 0.0252 mole)), pyridine (3.6 ml. = 0.03 mole) and acetic anhydride (3.6 ml. = 0.03 mole) was allowed to stand for 40 hours at room temperature. After dilution with benzene (50 ml.) the mixture was thoroughly washed with cold 1% HCl, water and dilute sodium bicarbonate and finally fractionated *in vacuo* through a short column. The fraction boiling within the range 132–137 (0.2 mm) and consisting mainly of the monoacetyl derivative redistilled; b.p. 134–135° (0.2 mm.); yield 1.9 g. (28.5%).

Anal. Calcd. for $C_9H_{17}O_2S_2(COCH_3)$ (264.13): $COCH_3$, 16.25; S, 24.35. Found: $COCH_3$, 16.02; S, 24.44.

No attempt was made to isolate the diacetyl compound. The highest boiling fraction (150–155° (0.3 mm.)) weighing 560 mg. was analyzed as crude methyl 6,8-acetyl-6,8-dithioloctanoate.

Anal. Calcd. for $C_9H_{16}O_2S_2(COCH_3)_2$ (306.16): $COCH_3$, 28.17; S, 20.90. Found: $COCH_3$, 23.80; S, 22.83.

S-Acetyldithioloctanoic Acid. (Ac-DTO) (IIIb) and Urea (IVb.)—A solution of 823 mg. of the foregoing methyl ester in 80 ml. of acetone:1 *N* hydrochloric acid (1:1) was kept at 0° for 12 hours. After dilution with benzene (100 ml.) the organic layer was washed repeatedly with water and finally the free acid removed from the benzene layer with 2% sodium bicarbonate solution. The aqueous layer was acidified and the Ac-DTO (IIIb) re-extracted with benzene to give a crude yield of 16 mg. (2%). Characterization with the basic CDI yielded a compound of m.p. 89–91°, which was found to be identical (mixed m.p., infrared spectra) with the substance prepared by acetylating the CDI-addition product of (±)-DTO (IV) in the following manner: a mixture of 488 mg. of IV, 2 ml. of pyridine and 0.1 ml. of acetic anhydride was kept for 100 hours (under nitrogen) at room temperature. The reaction mixture was diluted with benzene (25 ml.) and thoroughly washed with water; after recrystallization from benzene–petroleum ether m.p. 89–90°; yield 272 mg. (51%).

Anal. Calcd. for $C_{27}H_{38}N_4O_3S_2$ (530.72): N, 10.56; S, 12.08; $COCH_3$, 8.11. Found: N, 10.42; S, 11.89; $COCH_3$, 8.70.

Octanoyl-di-(*p*-dimethylaminophenyl)-urea (from caprylic acid and basic CDI); m.p. 139–141°, did not acetylate under the same conditions.

Acetylation of the (−)-DTO-CDI-derivative was carried out as above; m.p. 106–107° (plates), $[\alpha]^{20}D$ −2.8° (*c* 0.612, benzene); found, N 10.48. (IVb.)

(D) Enzymatically Acetylated Compounds. Enzyme Source and DTO Acetylation.—(−)-Dihydrolipoic acid was acetylated, according to reaction 3, with an enzyme fraction prepared from *Escherichia coli* by the procedure of Hager[13] as extended by Barton,[24] *i.e.*, sonic extracts of aerobically grown *E. coli*, Crookes strain, were fractionated with ammonium sulfate, nucleic acid removed by protamine treatment, and the protein precipitating between 0.4 and 0.5 saturation with neutral ammonium sulfate used—specific activity for both dihydrolipoic transacetylase and phosphotransacetylase 6 to 10. In a typical acetylation: 28 mg. (0.134 mmole) of (−)-dihydrolipoic acid was suspended in 2 ml. of 10^{-2} *M* ethylenediaminetetraacetic acid tetrasodium salt (Versene), and dissolved by neutralizing with normal KOH to *p*H 7.0. Lithium acetyl phosphate (150 μM) and Coenzyme A (5.8 μM) were added, the reaction volume brought to 6 ml., and 1 ml. of enzyme (160 μM units of dihydrolipoic transacetylase, 400 μM units of phosphotransacetylase)[25] added in *M*/50 phosphate *p*H 7.0. After 90 minutes at 30°, analysis of an aliquot for thioester (heat stable hydroxamate)[27] indicated 98% acetylation (132 μM acetyl).

Isolation.—The reaction mixture after nearly complete acetylation was chilled to 0° and covered with benzene (50 to 100 ml.) and with vigorous stirring brought to *p*H 1 with cold 2*N* hydrochloric acid. To destroy the emulsion the mixture was filtered with suction through a layer of rock salt, the organic layer was separated and the solvent removed. In a typical experiment 30.0 mg. of (−)-DTO acetylated to an extent of 98%, from which 18.2 mg. was recovered (50%); $[\alpha]^{20}D$ +16.1 (*c* 0.607, benzene (IIIa)). No racemization occurred during isolation. The CDI-derivative of this compound was prepared in the usual way using ether–petroleum ether as a solvent; m.p. 117–119° (prisms); $[\alpha]^{20}D$ +4.3 (*c* 1.190, benzene).

Anal. Calcd. for $C_{27}H_{38}N_4O_3S_2$ (530.72): N, 10.56; Ac, 8.11. Found: N, 10.43; Ac, 8.85.

14.7 mg. of this natural derivative was kept under nitrogen for three weeks at 0° in the dark; m.p. 102–107°; $[\alpha]^{20}D$ +9.5 (*c* 0.290, benzene).

In another experiment 194.5 mg. of the natural acetyl compound was methylated with diazomethane (from 2 g. of nitrosomethylurea) in benzene at 0° for an hour. After removal of polyoxymethylenes by filtration, the solvent was evaporated, the remainder dissolved in petroleum ether and added to a solution of 218 mg. of N-propionyldiphenylketimine in 15 ml. of the same solvent. After six days the precipitate was collected and recrystallized from benzene–petroleum ether; m.p. 107–109°; $[\alpha]^{20}D$ +41.8 (*c* 0.323, benzene); yield 56 mg. (13%); found, S 12.51, calcd. 12.78 (Va-E).

URBANA, ILLINOIS

(24) L. S. Barton, Thesis, University of Illinois, 1955.

(25) One unit of dihydrolipic transacetylase is that amount of enzyme required to produce 1 μM of S-acetyl-dihydrolipoic acid[24]; one unit of phosphotransacetylase is defined as that amount of enzyme which catalyzes the disappearance of 1 μM of acetyl phosphate.[26]

(26) E. R. Stadtman, *J. Biol. Chem.*, **196**, 527 (1952).

(27) F. Lipmann and L. C. Tuttle, *ibid.*, **159**, 21 (1945).