Anal. Calcd. for $C_9H_{11}SO_6Br$: C, 34.74; H, 3.54; Br, 25.69. Found: C, 34.95; H, 3.48; Br, 25.37.

The identical conditions with the other barium salt produced 5.4% yield of the identical acid, m.p. 263°, mixed m.p. 261–263°, as the sole product in the above reaction.

(2) **Furan.**—Barium *trans*-β-sulfoacrylate (1.04 g., 3.46 mmoles) was warmed into about 2 ml. of water, neutralized as above with 3.85 ml. of 0.91 *M* sulfuric acid, and the clear filtrate, made up to 10 ml., was shaken with 1.0 g. (15 mmoles) of furan for 36 hours. The aqueous layer was separated and brominated with 0.22 *M* aqueous bromine solution until it was no longer rapidly decolorized and the fine colorless needles which crystallized from solution filtered and dried. The identical experiment was performed on 1.04 g. of the other barium salt and a control on furan and water alone with the results as tabulated.

The mixed m.p. of the products was 224–228°. The product was recrystallized from water to long fine needles, m.p. 224–229°.

Barium β-sulfoacrylate	Bromine required[a] Ml.	%	Cryst. prod. Mg.	%	M.p., °C.
trans	8.8	55	174	16	226–230
cis	6.0	38	105	10	225–229

[a] Minus the control experiment quantity (2.4 ml.).

Anal. Calcd. for $C_7H_7SO_6Br$: C, 28.11; H, 2.33; Br, 26.72. Found: C, 27.90; H, 2.61; Br, 26.21.

When the reaction mixture was half-neutralized first to remove the strong acid of the sulfonic group or the time increased to 10 days or the amount of furan varied, the results were substantially the same. The methyl ester of the acid was obtained from ethereal diazomethane as brilliant needles, recrystallized from methanol, m.p. 184°.

Anal. Calcd. for $C_8H_9SO_6Br$: C, 30.68; H, 2.88; Br, 25.55. Found: C, 30.75; H, 3.07; Br, 25.55.

[CONTRIBUTION FROM THE CLAYTON FOUNDATION BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AUSTIN, TEXAS]

Synthesis and Properties of 2-Acetyl-3,4-dimethylthiazolium Iodide[1]

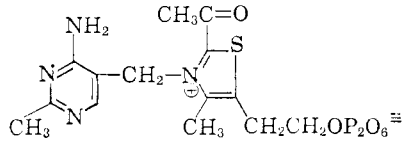
BY KOJI DAIGO[2] AND LESTER J. REED

RECEIVED JULY 17, 1961

2-Acetyl-3,4-dimethylthiazolium iodide (II) was synthesized by reaction of 2-acetyl-4-methylthiazole with methyl iodide. Evidence is presented that compound II undergoes nucleophilic attack by water to give acetic acid, by hydroxylamine to give acethydroxamic acid and by mercaptide ions to give thiolacetates. These data point up the kinetic and thermodynamic instability of the 2-acetylthiazolium salt and provide support for the mechanism previously proposed for thiamine pyrophosphate action in the oxidative decarboxylation of α-keto acids.

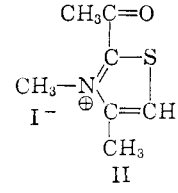
Implicit in the mechanism of thiamine action proposed by Breslow is the requirement that 2-acetylthiamine contain an "active" acetyl group.[3–5] Breslow and McNelis[5] reported in a preliminary communication the preparation in crude form of 2-acetyl-3,4-dimethylthiazolium nitrate and presented evidence that this compound is easily deacylated in the presence of water or methanol. White and Ingraham[6] reported similar observations indicating kinetic instability of a crude preparation of 2-benzoyl-3,4-dimethylthiazolium iodide.

Breslow has suggested[4] that 2-acetylthiamine

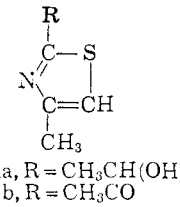


I

pyrophosphate (I) is an intermediate in the phosphoketolase catalyzed reactions. In a recent publication from this Laboratory[7] evidence was presented that an energy-rich acetyl compound, presumably compound I, is an intermediate in the ferricyanide-linked oxidation of pyruvate catalyzed by the pyruvic carboxylase component of the *Escherichia coli* pyruvate dehydrogenation complex. Compound I also was considered to be a possible intermediate in enzymatic oxidations of pyruvate involving electron acceptors other than ferricyanide, *e.g.*, protein-bound lipoic acid. Similar suggestions were made independently by Holzer and Crawford[8] and by Krampitz.[9] In view of the possible significance of 2-acetylthiamine pyrophosphate in these enzyme catalyzed reactions, it appeared highly desirable to obtain a pure sample of a 2-acetylthiazolium salt for model studies. This paper reports the synthesis of 2-acetyl-3,4-dimethylthiazolium iodide (II) and evidence indicating its kinetic and thermodynamic instability.



II



IIIa, R = $CH_3CH(OH)$
b, R = CH_3CO

2-(α-Hydroxyethyl)-4-methylthiazole (IIIa)[10] was oxidized with dichromic acid to obtain 2-acetyl-4-methylthiazole (IIIb). Quaternization of the latter compound was found to be rather difficult in agreement with the findings of Breslow and McNelis.[5] Some quaternization was accomplished

(1) This investigation was supported in part by a research grant (RG-6590(C1)) from the Division of General Medical Sciences, United States Public Health Service.

(2) Rosalie B. Hite Postdoctoral Fellow, 1959–1961.

(3) R. Breslow, *J. Am. Chem. Soc.*, **80**, 3719 (1958).

(4) R. Breslow, *J. Cellular Comp. Physiol.*, **54** (Suppl. 1), 100 (1959).

(5) R. Breslow and E. McNelis, *J. Am. Chem. Soc.*, **82**, 2394 (1960).

(6) F. G. White and L. L. Ingraham, *ibid.*, **82**, 414 (1960).

(7) M. L. Das, M. Koike and L. J. Reed, *Proc. Natl. Acad. Sci. U. S.*, **47**, 753 (1961).

(8) H. Holzer and R. M. M. Crawford, *Nature*, **188**, 410 (1960).

(9) Paper presented at the 45th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April, 1961.

(10) R. Breslow and E. McNelis, *J. Am. Chem. Soc.*, **81**, 3080 (1959).

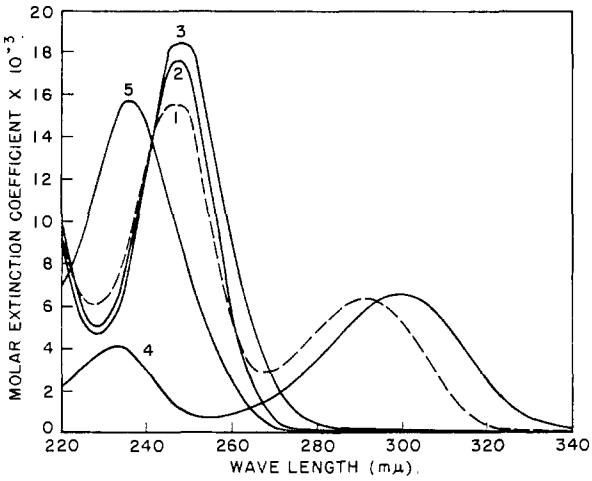


Fig. 1.—Absorption spectra of 2-acetyl-3,4-dimethylthiazolium iodide, 2-(α-hydroxyethyl)-3,4-dimethylthiazolium iodide, 3,4-dimethylthiazolium iodide and 2-acetyl-4-methylthiazole: (1) 2-acetyl-3,4-dimethylthiazolium iodide in acetonitrile; (2) 3,4-dimethylthiazolium iodide in acetonitrile; (3) 2-(α-hydroxyethyl)-3,4-dimethylthiazolium iodide in acetonitrile; (4) 2-acetyl-4-methylthiazole in acetonitrile; (5) 2-acetyl-3,4-dimethylthiazolium iodide (3.55×10^{-5} *M*) in 90% acetonitrile–water after 30 min. at 25°.

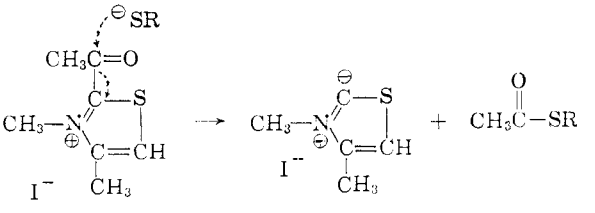
by heating compound IIIb with an excess of methyl iodide in a sealed tube for 64 hr. at 80°. Crude II was isolated from the reaction mixture by extraction with hot butanone. Recrystallization from butanone–ether gave a pure product.

The ultraviolet absorption spectrum of 2-acetyl-3,4-dimethylthiazolium iodide (in acetonitrile) shows maxima at 246 and 292 mμ (Fig. 1, curve 1). In agreement with the findings of Breslow and McNelis[5] the latter peak appears to be characteristic of the 2-acetyl substituted thiazole chromophore since it is not present in the spectrum of 3,4-dimethylthiazolium iodide (curve 2) or 2-(α-hydroxyethyl)-3,4-dimethylthiazolium iodide (curve 3), but a similar peak (at 300 mμ) is present in the spectrum of 2-acetyl-4-methylthiazole (curve 4). When water (10%) was added to the solution of the 2-acetylthiazolium salt II in acetonitrile, the 292 mμ peak gradually disappeared. The 246 mμ peak was shifted to 236 mμ (curve 5). The resulting spectrum was identical with that of an authentic sample of 3,4-dimethylthiazolium iodide under the same conditions. The 300 mμ peak of 2-acetyl-4-methylthiazole was not affected under these conditions.

A rapid deacylation of the 2-acetylthiazolium salt II occurred in methanol at 25°. The solid obtained by evaporating the solution was identified as 3,4-dimethylthiazolium iodide by comparison of its melting point and ultraviolet and infrared spectra with those of an authentic sample.

The 2-acetylthiazolium salt II was rapidly deacylated in aqueous solutions at *p*H 7–8 to give acetic acid. The half-life in 0.02 *M* imidazole buffers, *p*H 7–8, was less than 30 sec. In view of this rapid rate of hydrolysis, reactions of Compound II with potential acetyl acceptors were carried out initially in dimethoxyethane–water mixtures. With 0.1 *M* hydroxylamine, *p*H 8.0, in 88% dimethoxyethane, acethydroxamic acid was produced in 66% yield. 2-Acetyl-4-methylthiazole (IIIb) did not give acethydroxamic acid under these conditions. Since these results indicated that compound II reacts with hydroxylamine in preference to water, experiments were conducted with aqueous hydroxylamine. With 2 *M* hydroxylamine at *p*H 8.0, 7.5 and 7.0, the yields of acethydroxamic acid were, respectively, 67, 52 and 34%. These results suggested that the reaction involves nucleophilic attack by un-ionized hydroxylamine on the carbonyl carbon atom of compound II. However, some evidence was obtained that O-acetylhydroxylamine was the major initial product at *p*H 7.0 and that this product underwent further reaction with hydroxylamine to give acethydroxamic acid. Thus, whereas spectrophotometric measurement at 292 mμ indicated that disappearance of compound II in 2 *M* hydroxylamine, *p*H 7.0, was complete in less than 30 sec., the yield of acethydroxamic acid measured within 85 sec. was only 5%. After 10 min. contact of compound II with the neutralized hydroxylamine solution the yield of acethydroxamic acid was 34%. As shown previously by Jencks[11] a number of acylating agents react rapidly with hydroxylamine at neutral *p*H to give O-acylhydroxylamine as well as hydroxamic acid. This reaction is followed by a slow reaction of O-acylhydroxylamine with hydroxylamine to give hydroxamic acid.

Attempts to produce a thiolacetate by reaction of 2-mercaptoethanol and compound II in aqueous solution at *p*H 7–8 were unsuccessful. However, when *n*-butyl mercaptan and compound II were allowed to react in 98% dimethoxyethane–water in the presence of sodium acetate or sodium hydroxide, *n*-butyl thiolacetate was produced in 30–38% yield. A thiolester was also produced in 38% yield by reaction of DL-dihydrolipoamide (6,8-dithioloctanamide) with compound II under similar conditions. It has not been ascertained whether the primary and/or secondary thiol group in the dihydrolipoamide was acetylated. When the base (sodium acetate or sodium hydroxide) was omitted from the reaction mixtures no thiolacetate was formed. These results indicate that the reaction involves a nucleophilic attack of mercaptide ion on the carbonyl carbon atom of the 2-acetylthiazolium salt II. The results also provide evidence for the energy-rich nature of the 2-acetylthiazolium salt II and support the mechanism previously proposed[7] for thiamine pyrophosphate action in the lipoic acid-linked oxidative decarboxylation of α-keto acids.



Attempts to produce acetyl phosphate by reaction of the 2-acetylthiazolium salt II with aqueous

(11) W. P. Jencks, *J. Am. Chem. Soc.*, **80**, 4581, 4585 (1958).

phosphate buffers at *p*H 5–8 were unsuccessful. Preliminary studies with dimethoxyethane–water mixtures were also unsuccessful, due apparently in part to the limited solubility of phosphate buffers in such mixtures and to the fact that mono- and dihydrogen phosphate ions are poor nucleophiles compared to hydroxylamine and mercaptide ion.

Experimental[12]

Materials.—3,4-Dimethylthiazolium iodide[13] was prepared by reaction of 4-methylthiazole with excess methyl iodide. After 20 hr. at room temperature, the crystals were collected, washed with ether and recrystallized from ethanol; m.p. 119–120° (rept.[3] 119–120°), $\lambda_{max}^{CH_3CN}$ 247 mμ (ε 17,600).

2-(α-Hydroxyethyl)-4-methylthiazole (IIIa) was prepared by the method of Breslow and McNelis,[10] b.p. 105–107° (4.5 mm.) (reptd.[10] 91–94° (2.9 mm.)), $\lambda_{max}^{CH_3CN}$ 247 mμ (ε 3,900).

2-(α-Hydroxyethyl)-3,4-dimethylthiazolium iodide was synthesized by the method of Breslow and McNelis,[10] m.p. 148–149° (reptd.[10] 149–150°), $\lambda_{max}^{CH_3CN}$ 247 mμ (ε 18,700).

2-Acetyl-4-methylthiazole (IIIb).—To a solution of 10 g. of 2-(α-hydroxyethyl)-4-methylthiazole in 50 ml. of glacial acetic acid was added gradually a solution of 8 g. of sodium dichromate in 8 ml. of water. The temperature was controlled with an ice-bath. The mixture was allowed to stand for 1 hr. at room temperature. Then 2 g. of sodium dichromate in 2 ml. of water was added and the solution was allowed to stand at room temperature for 16 hr. The mixture was diluted with 100 ml. of water and extracted with four 150-ml. portions of ether. The combined ether extracts were washed with water and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the residue was distilled in a 50-ml. Erway distillation unit. The fraction boiling at 90–93° (12 mm.) was collected, (reptd.[14] 92–94° (12 mm.)); yield 4.72 g. (47%). The product solidified at room temperature; m.p. 34°; $\lambda_{max}^{CH_3CN}$ 233 mμ (ε 4,170), 300 mμ (ε 6,510).

Anal. Calcd. for C_6H_7NOS: C, 51.04; H, 5.00; N, 9.92. Found: C, 50.89; H, 4.77; N, 9.68.

2-Acetyl-3,4-dimethylthiazolium Iodide (II).—A mixture of 1.0 g. of 2-acetyl-4-methylthiazole and 2.0 g. of methyl iodide was heated in a sealed tube at 80° for 64 hr. A dark red oil separated and was washed with three 2-ml. portions of anhydrous ether. It gradually solidified on standing (1.1 g.). The solid was extracted with three 20-ml. portions of hot butanone. To the combined extracts was added 45 ml. of anhydrous ether. Orange colored needles separated, 0.25 g., m.p. 122–126°. This material was recrystallized twice from butanone–ether to obtain 0.10 g. of gold needles, m.p. 137–138°, $\lambda_{max}^{CH_3CN}$ 246 mμ (ε 16,500), 292 mμ (ε 6,400).

Anal. Calcd. for $C_7H_{10}NOSI$: C, 29.69; H, 3.56; N, 4.95. Found: C, 29.44; H, 3.61; N, 5.24.

Reaction of Compound II with Methanol.—The 2-acetylthiazolium salt II (5.67 mg.) was added to 3.0 ml. of methanol at 25°. Spectrophotometric measurement at 292 mμ indicated that disappearance of Compound II was complete in less than 80 sec. The solution was evaporated by means of a stream of nitrogen. The residue was triturated with ether to obtain a light tan solid, m.p. 119–120°, undepressed by authentic 3,4-dimethylthiazolium iodide. The ultraviolet spectrum (in acetonitrile) and the infrared spectrum of this solid were identical with those of the authentic sample. The infrared spectrum did not show the carbonyl band at 5.88 μ which was present in the spectrum of Compound II. A new band at 10.9 μ, due apparently to C–H bending vibration of thiazolium salts unsubstituted at C-2,[3] was present in the spectrum of the isolated material and the authentic sample.

Formation of Acetic Acid by Hydrolysis of Compound II.—The 2-acetylthiazolium salt II (0.85 mg., 3.0 μmoles) was added to 1 ml. of 0.05 *M* potassium phosphate buffer, 7.5. Spectrophotometric measurement at 292 mμ indicated disappearance of over 90% of compound II in approximately 2 min. The reaction mixture was assayed for acetic acid with acetokinase by the method of Rose, *et al.*[15] The yield of acetic acid was 90%. No acethydroxamic acid was produced in the absence of acetokinase.

Reaction of Compound II with Hydroxylamine.—To minimize hydrolysis of compound II the reaction with hydroxylamine was carried out in mixtures of dimethoxyethane–water. The highest yield of hydroxamic acid was obtained under the following conditions. To 0.1 mole of relatively salt-free hydroxylamine[16] in 0.12 ml. of water, *p*H 8.0, was added 0.88 ml. of dimethoxyethane. To the clear solution was added 0.56 mg. (1.98 μmoles) of compound II. After 10 min. at 25° the mixture was evaporated *in vacuo*, 2 ml. of water and 4 ml. of 10% ferric chloride in 0.7 *N* hydrochloric acid were added, and the absorbance was measured at 540 mμ[11,17] with a Bausch and Lomb Spectronic 20 colorimeter. Acethydroxamic acid, m.p. 89–91° (reptd.[11] 90–91°), was used as the standard. The yield of hydroxamic acid was 66%. The hydroxamic acid was identified as acethydroxamic acid by comparative paper chromatography[18] with an authentic sample in *n*-butyl alcohol–acetic acid–water (4:1:5), R_f 0.57, and in *n*-amyl alcohol–acetic acid–water (4:1:5), R_f 0.36.

When compound II (1.77 μmoles) was added to 1 ml. of 2 *M* aqueous hydroxylamine, *p*H 8.0, and the solution was allowed to stand at 25° for 10 min. before adding ferric chloride reagent, the yield of acethydroxamic acid was 67%. With 2 *M* hydroxylamine, *p*H 7.5 and 7.0, the yield of acethydroxamic acid was, respectively, 52 and 34%. Spectrophotometric measurement at 292 mμ indicated that disappearance of the 2-acetylthiazolium salt II at *p*H 7.0 was complete in less than 30 sec. When ferric chloride reagent was added within 85 sec. of the start of the reaction the yield of acethydroxamic acid was only 5%.

Reaction of Compound II with Thiols. (a) With *n*-Butyl Mercaptan.—To a solution of 100 μmoles of *n*-butyl mercaptan in 0.98 ml. of dimethoxyethane was added 2 μmoles of sodium acetate in 0.02 ml. of water. To the clear solution was added 1.98 μmoles of compound II. After 30 min. at 25° 1 ml. of water was added, and the mixture was extracted with two 1-ml. portions of ether. The combined ether extracts were washed with 0.5 ml. of water and evaporated to approximately 0.05 ml. by means of a stream of nitrogen. To the residue was added 1 ml. of 2 *M* aqueous hydroxylamine, *p*H 7.0. After 10 min at 25° 1 ml. of 3 *N* hydrochloric acid was added, and the mixture was extracted with two 2-ml. portions of ether to remove *n*-butyl mercaptan. To the aqueous phase were added 1 ml. of 5% ferric chloride in 0.1 *N* hydrochloric acid and water to give a final volume of 6 ml., and the absorbance was measured at 540 mμ. The yield of hydroxamic acid was 0.75 μmole (38%). The hydroxamic acid migrated at the same rate as authentic acethydroxamic acid on paper chromatograms with the solvent systems specified above.

In a parallel experiment the ether extract of the reaction mixture was evaporated and the residue was dissolved in 2 ml. of methanol. To 1 ml. of this solution was added 0.005 ml. of 2 *N* sodium hydroxide, and the mixture was heated in a sealed tube at 100° for 10 min. to hydrolyze the *n*-butyl thiolacetate. The solution was acidified with 0.01 ml. of 2 *N* hydrochloric acid. To the remaining 1 ml. of methanol solution was added 0.005 ml. of 2 *N* hydrochloric acid and 0.01 ml. of water. The difference spectrum of this latter solution *versus* the alkali-treated sample showed a maximum at 232 mμ, which is characteristic of *n*-butyl thiolacetate.[19] The yield of thiolacetate, calculated from the absorbance and the value, ε = 4,300[19], was 30%.

When sodium hydroxide (2 μmoles) was used in place of sodium acetate in the preceding experiments, the yield of *n*-butyl thiolacetate was 38% determined as acethydroxamic acid and 32% by spectrophotometric measurement at 232 mμ as specified above. When either the base (sodium acetate or sodium hydroxide) or the 2-acetylthiazolium salt

(12) Ultraviolet absorption spectra were determined with a Beckman model DK-2 recording spectrophotometer. Measurements at absorption maxima were made with a Beckman model DU spectrophotometer. Melting points and boiling points are uncorrected.

(13) H. Erlenmeyer, H. Baumann and E. Sorkin, *Helv. Chim. Acta*, **31**, 1978 (1948).

(14) J. Metzger and B. Koether, *Bull. soc. chim. France*, 702 (1953).

(15) I. A. Rose, M. Grunberg-Manago, S. R. Korey and S. Ochoa, *J. Biol. Chem.*, **211**, 737 (1954).

(16) S. Kaufman, *ibid.*, **216**, 153 (1955).

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

(18) E. R. Stadtman and H. A. Barker, *ibid.*, **184**, 769 (1950).

(19) L. H. Noda, S. A. Kuby and H. A. Lardy, *J. Am. Chem. Soc.*, **75**, 913 (1953).

II was omitted from the reaction mixture, no hydroxylamine-reactive material was detected by the hydroxamic acid assay.

(b) With DL-Dihydrolipoamide.—To a solution containing 4.0 mg. (19.3 μmoles) of DL-dihydrolipoamide,[20] 2 μmoles of sodium acetate in 0.02 ml. of water and 0.98 ml. of dimethoxyethane was added 2.1 μmoles of compound II. After 30 min. at 25° the mixture was evaporated by means of a stream of nitrogen. To the residue was added 1 ml. of water, and the mixture was extracted with a total of 3 ml. of benzene. The combined benzene extracts were washed with 0.5 ml. of water and evaporated with a stream of nitrogen. The residue was dissolved in 0.1 ml. of ethanol and 1 ml. of 2 *M* aqueous hydroxylamine, *p*H 7.0, was added. After 10 min. at 25° 1 ml. of 3 *N* hydrochloric acid was added and the mixture was extracted with two 2-ml. portions of benzene. To the aqueous phase were added 1 ml. of 5% ferric chlorice in 0.1 *N* hydrochloric acid and water to give a total volume of 6 ml., and the absorbance was measured at 540 mμ. The yield of acethydroxamic acid was 0.8 μmole (38%). When either sodium acetate or the 2-acetylthiazolium salt II was omitted from the reaction mixture, no hydroxylamine-reactive material was detected by the hydroxamic acid assay.

In a parallel experiment the benzene extract of the reaction mixture was evaporated *in vacuo* and the residue was dissolved in 2 ml. of methanol. One milliliter of this solution was treated with sodium hydroxide and then acidified as described above. The difference spectrum of this solution *versus* the remaining 1 ml. of methanol solution (acidified) showed a maximum at 236–238 mμ.[21] Assuming the value, ε = 4,400, for S-acetyldihydrolipoamide, the yield of thiolester calculated from the absorbance was 27%.

Acknowledgments.—We are indebted to Dr. C. G. Skinner and staff of the Clayton Foundation Biochemical Institute for the elemental analyses and to Mrs. Elizabeth Thompson for the acetokinase assays.

(20) L. J. Reed, M. Koike, M. E. Levitch and F. R. Leach, *J. Biol. Chem.*, **232**, 143 (1958).

(21) Crude ethyl 6,8-diacetylthioöctanoate, prepared from ethyl 6,8-dibromoöctanoate and potassium thiolacetate (ref. 22), showed $\lambda_{max}^{CH_3OH}$ 232 mμ (ε 8800). The difference spectrum of a mixture of this thiolester and a 10-fold amount of dihydrolipoamide *versus* dihydrolipoamide showed a maximum at 236–238 mμ.

(22) L. J. Reed and C-I. Niu, *J. Am. Chem. Soc.*, **77**, 416 (1955).

[CONTRIBUTION FROM THE CLAYTON FOUNDATION BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AUSTIN, TEXAS]

Synthesis of Some N-Lipoyl Amino Acids and Peptides[1]

BY KOJI DAIGO,[2] WILLIAM T. BRADY AND LESTER J. REED

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N-Lipoyl amino acids were prepared from DL-lipoic-isobutyl carbonic anhydride. The products were reduced to the dihydrolipoyl derivatives with sodium borohydride. The synthesis of N^{α}-(α-L-aspartyl)-N^{ϵ}-lipoyl-L-lysine[3] (I), N^{α}-(β-L-aspartyl)-N^{ϵ}-lipoyl-L-lysine (II) and N^{α}-(L-asparaginyl)-N^{ϵ}-dihydrolipoyl-L-lysine (III) is described.

During the course of studies on the nature of protein-bound lipoic acid a number of N-lipoyl and N-dihydrolipoyl (6,8-dithioloctanoyl) amino acids and peptides were synthesized. The N-lipoyl amino acids were prepared by the mixed carbonic–carboxylic anhydride method,[4] using DL-lipoic-isobutyl carbonic anhydride.[5] Some of the products were obtained as yellow oils, which polymerized readily. These oils were converted to benzhydrylammonium salts, which were obtained in crystalline form. The N-lipoyl amino acids were reduced with sodium borohydride to obtain the N-dihydrolipoyl amino acids.

Evidence has been presented in previous papers[6,7] which indicates that the sequence Asp.ε-Lipoyl-Lys is present in the *Escherichia coli* pyruvate and α-ketoglutarate dehydrogenation complexes. Three of the peptides isolated from partial hydrolysates of the performic acid-oxidized pyruvate dehydrogenation complex appeared to have the structure Asp. ε-6,8-disulfoöctanoyl-Lys.[7] To confirm the structures assigned to these peptides N^{α}-(α-L-aspartyl)-N^{ϵ}-lipoyl-L-lysine (I), N^{α}-(β-L-aspartyl)-N^{ϵ}-lipoyl-L-lysine (II) and N^{α}-(L-asparaginyl)-N^{ϵ}-dihydrolipoyl-L-lysine (III) were synthesized and then oxidized with performic acid to the corresponding disulfonic acids.[7]

Condensation of N^{ϵ}-(6,8-dibenzylthioöctanoyl)-L-lysine with N-trifluoroacetyl-L-aspartic anhydride, and then treatment with ammonium hydroxide to remove the trifluoroacetyl group,[8] gave a mixture of the α- and β-aspartyl peptides (IV and V), as indicated by paper chromatography. Two ninhydrin-positive spots were observed, one purple and the other blue. According to the findings of Bryant, *et al.*,[9] and other investigators (*cf.* ref. 9), α-aspartyl peptides give a purple color when heated with ninhydrin at 100–120°, whereas β-aspartyl peptides give a blue color. The α-aspartyl peptide IV was found to be more soluble than the β-isomer V in hot methanol, permitting isolation of the β-isomer in pure form. The benzyl groups were removed from the latter isomer by reduction with sodium in liquid ammonia and the dithiol obtained was oxidized to the disulfide II with oxygen in the presence of ferric ion. The α-aspartyl peptide IV could not be separated completely from the β-isomer V. However, after reduction of the mixture with sodium in liquid ammonia, the N^{α}-(α-L-aspartyl)-N^{ϵ}-dihydrolipoyl-L-lysine was separated from the corresponding β-isomer by chromatog-

(1) This investigation was supported in part by a research grant (RG-6590(C1)) from the Division of General Medical Sciences, United States Public Health Service. Abstracted in part from the doctoral dissertation of William T. Brady, The University of Texas, 1960.

(2) Rosalie B. Hite Postdoctoral Fellow, 1959–1961.

(3) In the present study no attempt was made to determine the proportion of the two diastereoisomers in the products prepared from DL-lipoic acid or DL-6,8-dibenzylthioöctanoic acid and L-amino acids.

(4) J. R. Vaughan, Jr., *J. Am. Chem. Soc.*, **74**, 6137 (1952).

(5) L. J. Reed, M. Koike, M. E. Levitch and F. R. Leach, *J. Biol. Chem.*, **232**, 143 (1958).

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