washed with ice-water and dried *in vacuo* over phosphorus pentoxide at room temperature; yield 1.3 g. (61%), m.p. 170° dec., $[\alpha]^{29}D$ −4.7° (*c* 1.1 in glacial acetic acid), R_f^{11} 0.82, R_f^{12} Phe+.

Anal. Calcd. for $C_{34}H_{43}O_{10}N_{11}(2H_2O)$: C, 50.9; H, 5.9; N, 19.2. Found: C, 51.2; H, 5.7; N, 19.0.

Carbobenzoxy-γ-benzyl-L-glutamyl-L-histidyl-L-phenylalanylnitro-L-arginine.—Carbobenzoxy-γ-benzyl-L-glutamate[20] (1.85 g.) was dissolved in dry dioxane (30 ml.), the solution was cooled until the dioxane partially solidified, when tri-*n*-butylamine (1.32 ml.) was added with stirring. Ethyl chloroformate (0.53 ml.) was then added and stirring was continued for 20 minutes. The mixture was completely frozen by cooling with an ice-salt-bath, and an ice-cold solution of L-histidyl-L-phenylalanylnitro-L-arginine (2.5 g.) in water (10 ml.) plus triethylamine (0.77 ml.) was gradually added with shaking and cooling. In order to maintain a clear solution, additional dioxane was added, and the mixture was stirred with cooling for 20 minutes and at room temperature for one hour. The solution was then acidified with glacial acetic acid (5 ml.) and evaporated to a small volume *in vacuo* (bath temp. 40–50°). The reaction product was precipitated by the addition of water, and the mixture was kept in the refrigerator for 12 hours. The product was collected, dried *in vacuo* over phosphorus pentoxide at room temperature, and purified by one precipitation from methanol with ether and by two precipitations from methanol with water. It was dried *in vacuo* over phosphorus pentoxide at 70° for 12 hours; yield 2.4 g. (57%), m.p. 185–187° dec., $[\alpha]^{29}D$ −2.4° (*c* 1.7 in glacial acetic acid), R_f^{11} 0.87, R_f^{12} Phe+.

Anal. Calcd. for $C_{41}H_{48}O_{11}N_{10}(H_2O)$: C, 56.3; H, 5.8; N, 16.0. Found: C, 56.3; H, 5.5; N, 16.1.

L-Glutamyl-L-histidyl-L-phenylalanyl-L-arginine.—Carbobenzoxy-γ-benzyl-L-glutamyl-L-histidyl-L-phenylalanyl-nitro-L-arginine (1.3 g.) was hydrogenolyzed over spongy palladium in glacial acetic acid (20 ml.) for 4.5 hours in a stream of hydrogen. Fresh catalyst was added at this point and the hydrogenation continued for an additional 4.5 hours. The catalyst was removed by filtration, the glacial acetic acid evaporated off (bath temp., 40–50°), and the product precipitated by addition of dry ethanol. The compound was dissolved in water (3 to 5 ml.), the solution filtered, concentrated to a volume of 1 ml. *in vacuo*, and absolute ethanol was added. The peptide was collected, washed with ethanol and dried *in vacuo* at room temperature; yield 810 mg. (93%), m.p. 200–205° dec., $[\alpha]^{26}D$ −5.0° (*c* 1.97 in water), R_f^{11} 0.32, R_f^{12} Arg+. A sample for analysis was dried at 60° *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_{26}H_{37}O_7N_9$: C, 53.2; H, 6.3; N, 21.4; NH_2–N, 2.4. Found: C, 53.0; H, 6.8; N, 21.5; NH_2–N, 2.6.

Analytical Procedures.—The paper chromatograms were prepared by the descending technique on Whatman No. 1 paper. Histidine and histidine peptides were localized on the chromatograms by the Pauly reaction.[21] For quantitative determination of amino acids, samples of the various peptides and peptide derivatives (2–5 mg.) were hydrolyzed with double-distilled (from glass) 6 *N* hydrochloric acid, in sealed tubes for 20 hours at 110°. The hydrolyzates were evaporated to dryness *in vacuo* at room temperature over phosphorus pentoxide and potassium hydroxide pellets, and the residues were dissolved in water. Aliquots were used for amino acid determination according to the method of Fowder,[22] using the Moore and Stein ninhydrin reagent.[23]

(20) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(21) R. J. Block, "Paper Chromatography," Academic Press, Inc., New York, N. Y., 1952, p. 64.

(22) L. Fowder, *Biochem. J.*, **48**, 327 (1951).

(23) S. Moore and W. H. Stein, *J. Biol. Chem.*, **211**, 907 (1954).

PITTSBURGH, PENNA.

[COMMUNICATION NO. 1853 FROM THE RESEARCH LABORATORIES, EASTMAN KODAK CO.]

The Reaction of Cystine and Lanthionine with Aqueous Calcium Hydroxide. The Identification of 2-Methylthiazolidine-2,4-dicarboxylic Acid

BY J. R. DANN, G. L. OLIVER AND J. W. GATES, JR.

RECEIVED OCTOBER 9, 1956

One of the products of the reaction between aqueous calcium hydroxide and cystine at room temperature has been identified as 2-methylthiazolidine-2,4-dicarboxylic acid, which was isolated as the diethyl ester. Proof of structure was carried out by comparison with synthetic 2-methylthiazolidine-2,4-dicarboxylic acid and by cleavage to a cysteine fragment and a pyruvic acid fragment. Lanthionine has also been found to form 2-methylthiazolidine-2,4-dicarboxylic acid in aqueous calcium hydroxide at room temperature.

Introduction

The instability of cystine in alkaline solutions is well known and has been studied and observed by many workers. Hoffman and Gortner[1] have surveyed the chemistry of cystine as known before 1922, and later work has included that of Andrews,[2a,b] Gortner and Sinclair,[3] Thor and Gortner,[4] Clarke and Inouye[5] and Lindstrom and Sandstrom.[6] Inorganic compounds including sulfides, sulfur, thiosulfate, sulfite, carbon dioxide and ammonia have been observed in the action of alkali upon cystine. Organic products which have been observed under various conditions are cysteine,[2a] alanine,[6] oxalic acid,[6] pyruvic acid,[5] uvitic acid,[6] uvitonic acid,[6] α-mercaptopropionic acid[6] and lanthionine.[7]

Discussion

In our laboratory, it had been observed that the action of aqueous calcium hydroxide upon L-cystine (I) at room temperature gave an acid II containing nitrogen and sulfur, which could be isolated in crude form by precipitating its calcium salt III in 80% ethanol. Attempts to purify this acid or to prepare derivatives of it for characterization had failed previously because of the great tendency of the crude acid or its salt to form intractable oils. This acid has now been identified as 2-methylthiazolidine-2,4-dicarboxylic acid (IV). This identification was made possible by the fact that the ethyl ester V of the unknown acid could be distilled

(1) W. F. Hoffman and R. A. Gortner, THIS JOURNAL, **44**, 341 (1922).

(2) (a) J. C. Andrews, *J. Biol. Chem.*, **80**, 191 (1928); (b) **87**, 681 (1930).

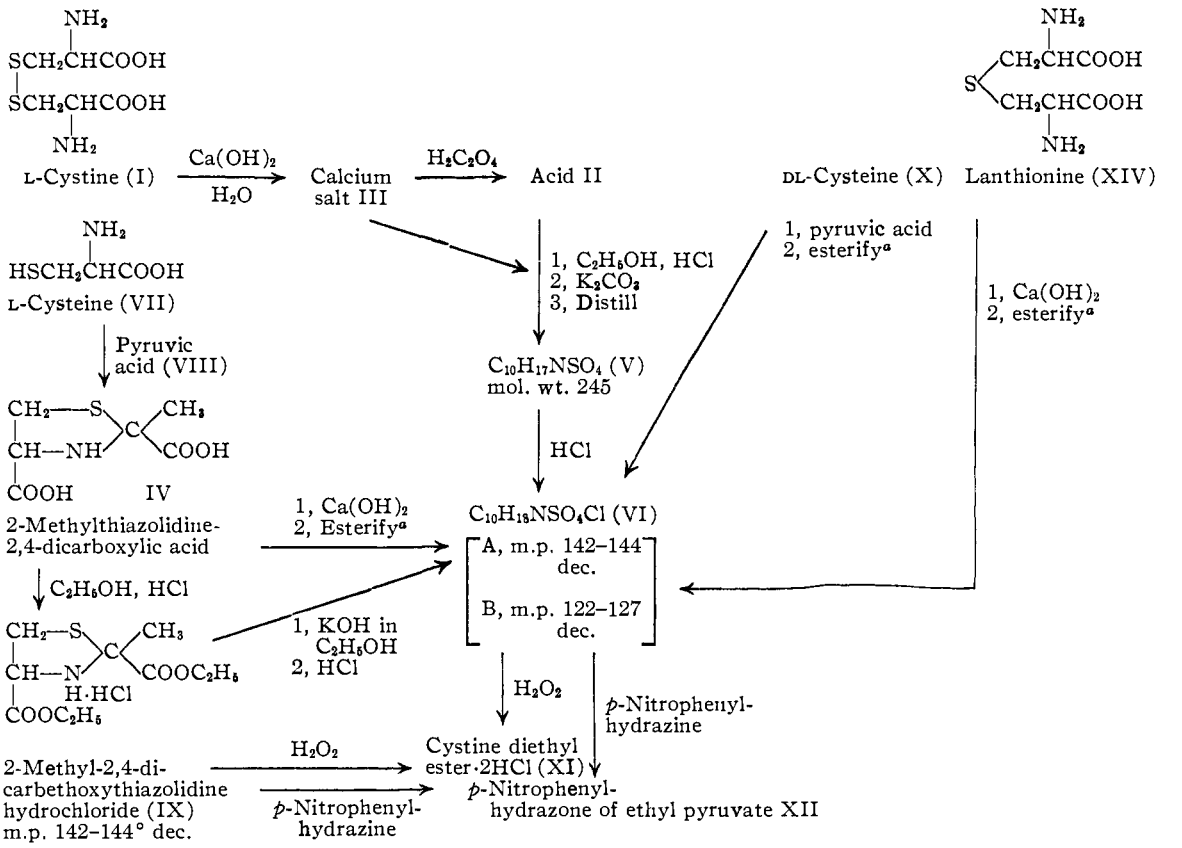
(3) R. A. Gortner and W. B. Sinclair, *ibid.*, **83**, 681 (1929).

(4) C. J. B. Thor and R. A. Gortner, *ibid.*, **99**, 383 (1932).

(5) H. T. Clarke and J. M. Inouye, *ibid.*, **89**, 399 (1930).

(6) H. V. Lindstrom and W. M. Sandstrom, *ibid.*, **138**, 445 (1941).

(7) M. J Horn, D. B. Jones and S. J. Ringel, *ibid.*, **138**, 141 (1941).



[a] Esterification procedure: 1, C_2H_5OH, HCl; 2, K_2CO_3; 3, distill; 4, HCl.

in vacuo and thus separated in a purified form for characterization. Elemental analysis of the purified ester V indicated the empirical formula, $C_{10}H_{17}NSO_4$, while the hydrochloride VI prepared from the ester was shown to have added only one mole of hydrogen chloride. This hydrochloride VI was separated by careful recrystallization into two forms, A, melting at 142–144° dec., and B, melting at 122–127° dec. Fraction A was present in only very small amounts, while fraction B made up the major portion of the crystals isolated. The same ester V was obtained also by esterification of either the filtered and dried-down aqueous calcium hydroxide–cystine reaction mixture or the crude acid II formed by acidifying the reaction mixture with oxalic acid, and filtering and evaporating it to dryness on the steam-bath at 3-mm. pressure. The yield of ester varied from 6.4–9.3% in several experiments. When synthetic 2-methylthiazolidine-2,4-dicarboxylic acid (IV) was prepared from L-cysteine (VII) and pyruvic acid (VIII) by the method of Schubert,[8] the hydrochloride of its diethyl ester IX was found to melt at 142–144° dec., corresponding to the minor fraction VIA obtained from the liming of cystine. The low-melting fraction VIB was found to be optically inactive, while the high-melting fraction IX or VIA was found to be optically active, having a specific rotation, $[\alpha]^{29}D$ of −56.6° in ethyl alcohol (*c* 0.05 g./cc.). It appeared that the low-melting fraction B might be a racemized form of the thiazolidine. This was confirmed by the following experiments.

In order to determine the stability of 2-methylthiazolidine-2,4-dicarboxylic acid in the aqueous calcium hydroxide solution, the synthetic acid IV was left in aqueous calcium hydroxide for 10 days and then worked up in the same fashion as was the cystine. It was found that both forms of the hydrochloride of the ester VI could now be isolated, the higher-melting form VIA making up about 40% of the total. The synthetic ester upon distillation *in vacuo* gave only the high-melting hydrochloride VIA, but upon being treated with catalytic amounts of potassium hydroxide in ethyl alcohol by the technique of McKenzie and Wren[9] both forms of the hydrochloride VI were isolated, the higher-melting form VIA now making up only 10% of the total. Infrared (Fig. 1) and ultraviolet (Fig. 2) absorption spectra of the synthetic ester hydrochloride IX were identical with both the high- and low-melting forms VIA and VIB, obtained from cystine, from 2-methylthiazolidine-2,4-dicarboxylic acid which had been treated with aqueous calcium hydroxide or from the synthetic ester which had been treated with potassium hydroxide in alcohol.[9] The infrared absorption spectrum (Fig. 1) of only the high melting synthetic hydrochloride of diethyl 2-methylthiazolidine-2,4-dicarboxylate (IX) is given since the spectra of the four compounds are practically identical. DL-Cysteine (X),[10,11] when treated with pyruvic acid and esterified, gave

(8) M. P. Schubert, THIS JOURNAL, **121**, 539 (1937).

(9) A. McKenzie and H. Wren, *J. Chem. Soc.*, **115**, 602 (1919).

(10) H. S. Loring and V. du Vigneaud, *J. Biol. Chem.*, **102**, 288 (1933).

(11) E. Baumann, *Z. physiol. Chem.*, **8**, 300 (1884).

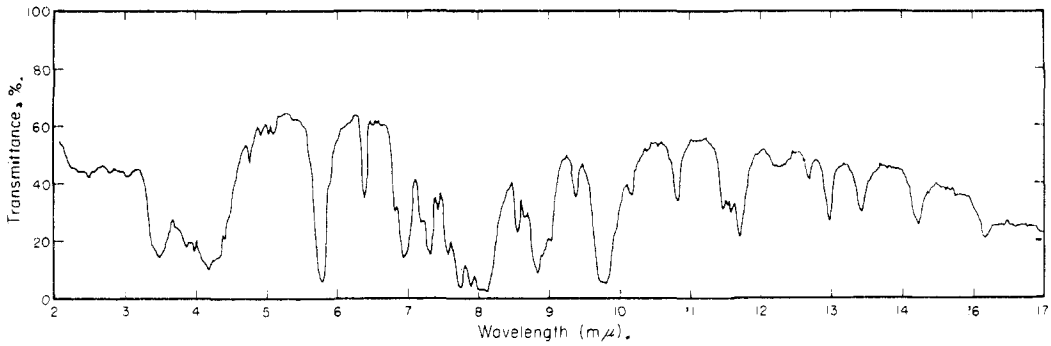


Fig. 1.—Infrared absorption spectrum of high-melting synthetic hydrochloride of diethyl 2-methylthiazolidine-2,4-dicarboxylate (IX).

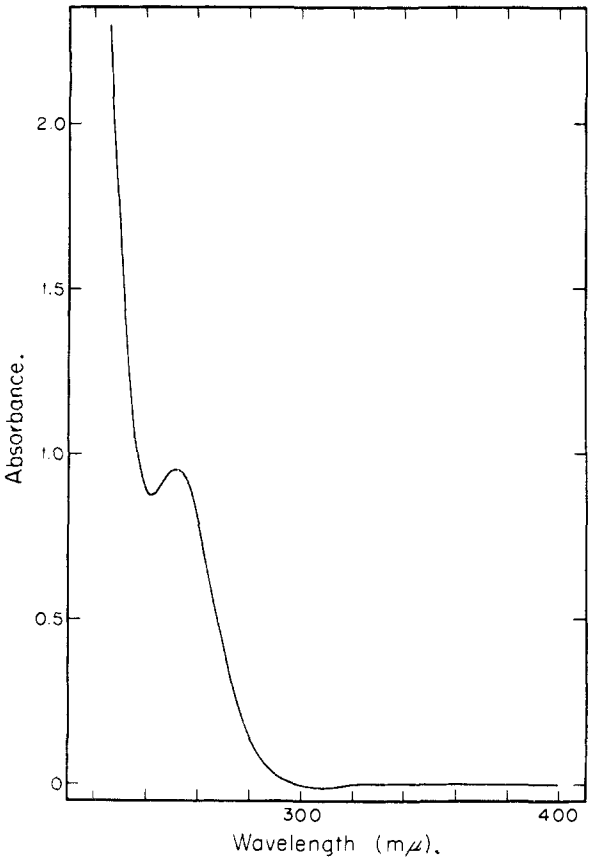


Fig. 2.—Superimposed ultraviolet absorption spectra of compounds VIA, VIB and IX (optical isomers of the hydrochloride of diethyl 2-methylthiazolidine-2,4-dicarboxylate).

both forms of the hydrochloride VIA and VIB, the lower-melting form B predominating.

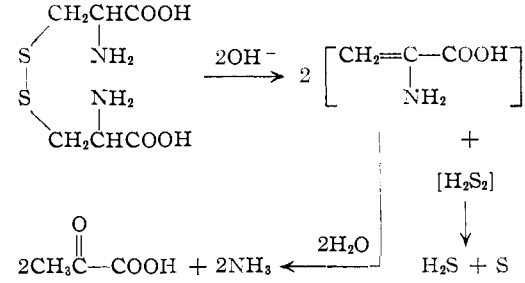
Further evidence of structure was obtained by treatment of both the synthetic ester hydrochloride IX and the low-melting ester hydrochloride VIB, with hydrogen peroxide by the method of Ratner and Clarke[12] to give cystine diethyl ester hydrochloride (XI) and also by reaction of both the synthetic ester IX and the ester VIB with *p*-nitrophenylhydrazine to give the *p*-nitrophenylhydrazone of ethyl pyruvate (XII).

When lanthionine (XIV) was treated with aqueous calcium hydroxide and the reaction mixture worked up and esterified in the same manner as was cystine, the same products VIA and VIB were obtained as had been obtained from cystine. The identity of the products was verified again by infrared absorption spectra.

(12) S. Ratner and H. T. Clarke, THIS JOURNAL, **59**, 200 (1937).

Discussion of Mechanism

Two schemes have been postulated to explain the decomposition of cystine and its derivatives by alkaline solutions. The first, originally suggested by Bergmann[13] in 1926 and amplified by the work of Clarke[5] and of Nicolet,[14] involved the removal of the sulfur atoms from cystine by what is now known as the bimolecular elimination mechanism.



Pyruvic acid has been isolated in yields as high as 60% from the alkaline decomposition of cystine.[5] Nicolet has compared this reaction with the known decomposition of β-ketosulfides and cited as evidence the fact that the rates of degradation and loss of optical activity of cystine are comparable.[14] Numerous cystine- and cysteine-containing peptides and other derivatives in which the amino group is protected have formed the corresponding α-aminoacrylic acid derivatives on treatment with alkali.[13,15] Since the reaction is reversible, thiols can be added to the α-aminoacrylic acid derivatives as well.[16]

Because he considered this scheme to account neither for the presence of cysteine nor for the ex-

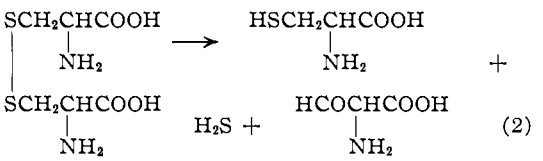
(13) M. Bergmann and F. Stather, *Z. physiol. Chem.*, **152**, 189 (1926).

(14) B. H. Nicolet, THIS JOURNAL, **53**, 3066 (1931).

(15) (a) J. C. Andrews and K. C. Andrews, *J. Biol. Chem.*, **105**, iv (1934); (b) M. Bergmann, J. C. Andrews and K. C. Andrews, *ibid.*, **92**, xxxvii (1931); (c) B. H. Nicolet, *ibid.*, **88**, 403 (1930).

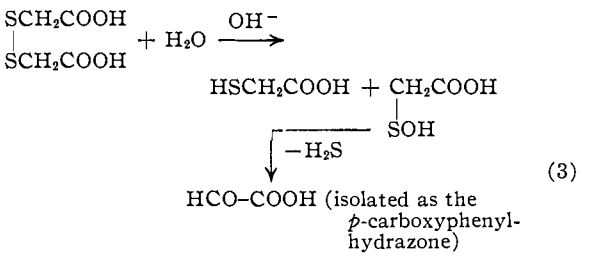
(16) (a) B. H. Nicolet, *ibid.*, **95** 389 (1932); (b) *J. Wash. Acad. Sci.*, **28**, 84 (1938); (c) B. H. Nicolet and L. A. Shinn, THIS JOURNAL, **63**, 2284 (1941); Abstracts of 103rd Meeting American Chemical Society, Biol. Chem. Sect., p. 11 (1942); (d) A. Schöberl and A. Wagner, *Chem. Ber.*, **80**, 379 (1947).

treme variation in the proportion of hydrogen sulfide and sulfur obtained in the decomposition of various cystine derivatives, Schöberl,[17] in 1938, postulated an alternative scheme which involved the cleavage of the cystine molecule between the two sulfur atoms, with the formation of hydrogen sulfide, cysteine and α-amino-α-formylacetic acid which was considered to be the precursor of pyruvic acid (equation 2).



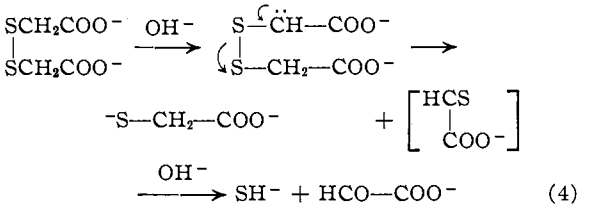
(2)

This proposal was an extension of a mechanism proposed for the decomposition of α,α'-dithiocarboxylic acids which are cleaved easily by alkali to form an aldo acid and a mercapto acid. Schöberl considered the reaction to be a hydrolysis of the disulfide linkage in the manner of the hydrolysis of aromatic disulfides to form a thiol and an unstable sulfenic acid which would give rise to hydrogen sulfide and an aldehyde (equation 3).



(3)

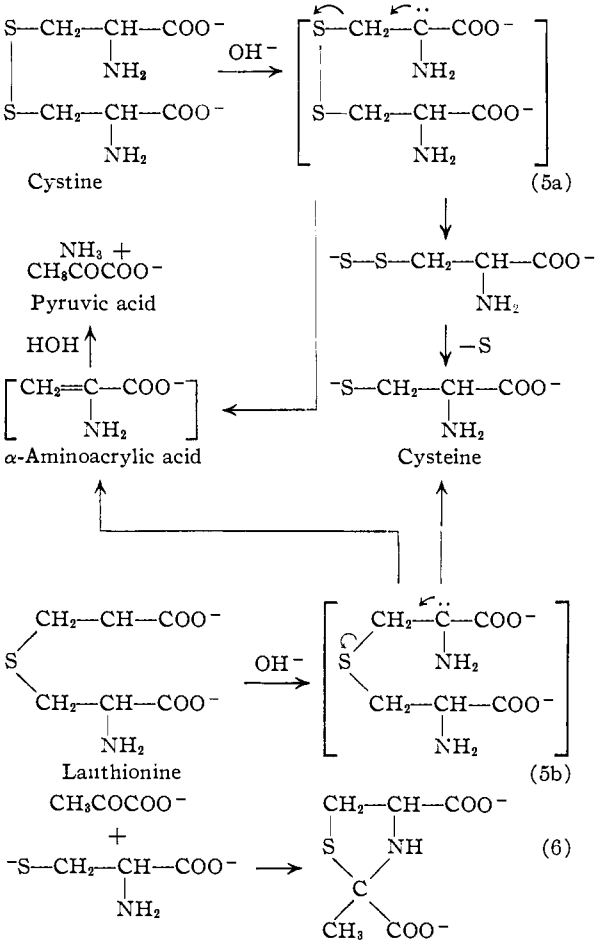
However, Rosenthal and Oster[18] have recently presented convincing evidence that the decomposition of the α,α'-dithiocarboxylic acids is actually a β-elimination of sulfide, forming a thioaldehyde which was subsequently hydrolyzed to an aldehyde (equation 4).



(4)

Schöberl's principal objections to the Bergmann scheme can be resolved by other means. Cysteine, as well as hydrogen sulfide, not only can be formed in the elimination reaction but can undoubtedly be formed also by the reduction of cystine by the sulfide or polysulfide ions present in the mixture. The erratic variation in the formation of elementary sulfur in the decomposition of various cystine derivatives could be attributed both to the variation in extent of disulfide reduction and to the complex behavior of sulfur in alkaline solution.[19] Furthermore, it is not likely that the mode of cleavage of the disulfide linkage in the α,α'-dithiocarboxylic acids (equation 4) can be extended to cystine, in which the hydrogen atom in the β-position is not labile. Indeed, Schöberl found that β,β'-dithiopropionic acid was quite unreactive. The Schöberl mechanism does not explain the complex transformation of the α-amino-α-formylacetic acid into pyruvic acid.

Thus, the alkaline decomposition of cystine is most favorably explained by the bimolecular elimination mechanism. A modification of this mechanism is proposed to explain the formation of 2-methylthiazolidine-2,4-dicarboxylic acid in the aqueous calcium hydroxide decomposition of both cystine and lanthionine (equations 5 and 6). Both



(5a)

(5b)

(6)

appear to decompose in the same manner, the extra sulfur of cystine separating as elementary sulfur in the formation of cysteine which is formed directly from lanthionine. A 63% yield of elementary sulfur, which separated during the esterification, was obtained from cystine, but there was no evidence of any free sulfur formed in the case of lanthionine. Whereas the calcium hydroxide solution of the cystine was dark yellow in color, the corresponding solution of the lanthionine was practically colorless; no precipitation or even cloudiness was observed during the esterification process of the latter.

The occurrence of hydrogen sulfide, evolved in considerable quantity on acidification of the cal-

(17) A. Schöberl and T. Hornung, *Ann.*, **534**, 210 (1938).

(18) N. A. Rosenthal and G. Oster, Abstracts of 126th Meeting American Chemical Society, 107-O (1954).

(19) J. W. Mellor, "Comprehensive Treatise on Inorganic Chemistry," Vol. II, Longmans, Green and Co., New York, N. Y., 1930, pp. 629-631; Vol. X, pp. 101-102.

cium hydroxide solution of cystine, can be explained as arising from the β-elimination of hydrosulfide ion from cysteine or of disulfide ion from cystine. Other products which have been reported in the alkaline decomposition of cystine, *e.g.*, α-mercaptopropionic acid, can be formed by combination of certain of the various components in the solution or by reduction with sulfide.

Experimental

Preparation of Crude Material from Cystine-Aqueous Calcium Hydroxide Mixtures. 1. Alcohol Precipitation of the Calcium Salt.—In a typical experiment, 120 g. of L(−)-cystine (Eastman Kodak Co.), 120 g. of calcium hydroxide and 3 liters of water were mixed together and left at about 25° for 4 months. The reaction mixture was then filtered and taken to *p*H 2.5 with oxalic acid, aerated 2 hours, filtered, treated with 50 g. of calcium hydroxide, filtered and poured into 12 liters of alcohol. The precipitate which formed in the alcohol was filtered off and dried; yield 106 g. of crude calcium salt III.

2. Direct Drying of the Crude Calcium Salt III.—A reaction mixture composed of 120 g. of L(−)-cystine, 120 g. of calcium hydroxide and 3 liters of water was allowed to stand at 25° for 4 months, then filtered, and the solution was evaporated to dryness *in vacuo* at room temperature; yield 154 g. of crude material.

3. Direct Drying of the Crude Acid II.—A reaction mixture composed of 120 g. of L(−)-cystine, 120 g. of calcium hydroxide and 3 liters of water was left at room temperature for one month, filtered and taken to *p*H 2.5 with oxalic acid. The solution was aerated overnight, filtered and the solution was evaporated to dryness *in vacuo* with gentle warming on the steam-bath to give 108 g. of crude product.

Esterification of the Calcium Salt in which the Reaction Mixture Was Dried Down Directly.—Fifty-eight grams of the crude calcium salt III (procedure 2) was suspended in 800 ml. of absolute ethanol, and dry hydrogen chloride gas was bubbled into the reaction mixture for 30 minutes. All of the solid material dissolved during this operation. The reaction mixture was refluxed for 30 minutes on the steam-bath and allowed to stand at room temperature for 2 days. A yellow precipitate was formed and was removed by filtration. This material weighed 3.8 g. and melted at 119–120°. It was found to be sulfur.

The filtered reaction mixture was evaporated *in vacuo* with gentle warming on a steam-bath until no more distillate could be collected. The residue was extracted with three 150-ml. portions of ether, which were combined, and dried over anhydrous calcium oxide. Evaporation of this ether left no residue.

The ether-insoluble residue was covered with 150 ml. of ether, and 80 g. of potassium carbonate in 150 ml. of water was added to make the aqueous layer alkaline. The ether was separated, and the aqueous layer was extracted with four more 150-ml. portions of ether, which were combined with the first and dried over anhydrous calcium oxide. Distillation of the ether left a yellow oil which was distilled *in vacuo*, in a semimicro distilling apparatus with a Claisen distilling head modified with a 5-cm. Vigreux column, giving 1.15 g. of distillate A, boiling at 35–47° at about 3 mm., and 4.35 g. of distillate B, boiling at 107–114° at about 3 mm. (9.3% yield of diethyl 2-methylthiazolidine-2,4-dicarboxylate).

Anal. Found: Distillate A: C, 50.6, 50.8; H, 7.8, 7.5. Distillate B: C, 48.6; H, 7.1; N, 6.0. Calcd. for diethyl 2-methylthiazolidine-2,4-dicarboxylate, $C_{10}H_{17}NSO_4$: C, 48.56; H, 6.93; N, 5.66.

One gram of distillate B in 5 ml. of dry ether was treated with dry hydrogen chloride gas for 30 seconds. After standing for about 10 minutes, a crude precipitate (0.85 g.) was formed which melted at 105–117°. Two recrystallizations from absolute alcohol gave large, block-like crystals from which was picked one single crystal, which melted at 142–143° dec. Mixed melting point with the hydrochloride of the diethyl ester of 2-methylthiazolidine-2,4-dicarboxylic acid (IX, m.p. 137° sinter; melt 142–144°) was 142–145° dec. The remaining crystals melted at 120°, sinter, 124–125° dec.

Esterification of the Alcohol-precipitated Calcium Salt.—Twenty grams of the alcohol-precipitated dry calcium salt III (procedure 1) was esterified and worked up in the same manner as described in the preceding section to give 1.5 g. of distillate, collected at 91–98° at 3-mm. pressure (6.45% yield of diethyl 2-methylthiazolidine-2,4-dicarboxylate).

Anal. Found: C, 48.5; H, 7.1; N, 5.30; S, 13.0; mol. wt. by boiling-point elevation, 245. Calcd. for diethyl 2-methylthiazolidine-2,4-dicarboxylate, $C_{10}H_{17}NSO_4$: mol. wt., 247.3.

Treatment of the distillate with dry hydrogen chloride in ether gave a crystalline derivative, m.p. 122–123° dec.

In a second esterification of the alcohol-precipitated calcium salt, 40 g. of the dry calcium salt (procedure 1) was treated as just described, except that after the esterified material was taken to dryness and extracted with ether the ether-insoluble residue was taken up in 60 ml. of water and then extracted with ether. Upon evaporation of the ether, 10.6 g. of light-brown oil was obtained, which upon distillation *in vacuo* gave two fractions, A and B. A distilled at 45° at 3 mm. and weighed 1.0 g., while B distilled at 107–111° at 3 mm. and weighed 2.0 g. (4.34% yield of diethyl 2-methylthiazolidine-2,4-dicarboxylate).

Anal. Found for A: C, 51.8; H, 7.3; $n^{24}D$ 1.4147. Found for B: C, 49.0; H, 7.0; N, 5.5; $n^{24}D$ 1.4798.

The aqueous solution from which A and B were separated was made alkaline with 60 g. of potassium carbonate and extracted with ether to give 5.9 g. of red oil which, upon distillation *in vacuo*, gave 1.45 g. of distillate boiling at 95–112° at 3-mm. pressure (3.1% yield of diethyl 2-methylthiazolidine-2,4-dicarboxylate).

Anal. Found: C, 48.2; H, 6.8; N, 5.7.

Treatment of this distillate with dry hydrogen chloride in ether gave a crystalline hydrochloride, m.p. 121–124° dec.

Anal. Found: C, 42.2; H, 6.5; N, 5.1; S, 11.1. Calcd. for the hydrochloride of diethyl 2-methylthiazolidine-2,4-dicarboxylate, $C_{10}H_{18}NSO_4Cl$: C, 42.32; H, 6.39; N, 4.94; S, 11.30.

This material gave no depression in melting point when mixed with the low-melting crystalline hydrochloride described.

Esterification of the Crude Acid.—Thirty-six grams of the crude acid II, from cystine treated with aqueous calcium hydroxide (procedure 3) was esterified, extracted and distilled as in the preceding experiment to give 0.80 g. of distillate A boiling at 43–45° at 3 mm. and 3.15 g. of distillate B boiling at 110–127° at 3 mm. (7.65% yield of diethyl 2-methylthiazolidine-2,4-dicarboxylate). Treatment of B with dry hydrogen chloride in ether gave 1.2 g. of crystals melting at 117–126° dec., and 0.1 g. melting at 125–146° dec.

Treatment of 2-Methylthiazolidine-2,4-dicarboxylic Acid with Aqueous Calcium Hydroxide and Esterification of the Product.—Thirty-eight and two-tenths grams (0.2 mole) of 2-methylthiazolidine-2,4-dicarboxylic acid (IV) was mixed with 48 g. of calcium hydroxide and 1200 ml. of distilled water and left at room temperature for 10 days. The reaction mixture was then filtered and poured into 4800 ml. of ethyl alcohol. The white precipitate was filtered off and dried to give 46 g. of solid material.

Twenty-three grams of this solid material was esterified and worked up in the same manner as was used for the material obtained from cystine to give 6.0 g. of oil, boiling at 96–108° at 3-mm. pressure. Treatment of this oil with dry hydrogen chloride gave a crude product melting at 127–141° dec., which upon slow recrystallization from absolute ethanol gave two fractions, A and B: (A) 1.338 g., m.p. 140–143° dec., mixed m.p. with the hydrochloride of diethyl 2-methylthiazolidinedicarboxylate (IX, m.p. 140–142° dec.), was 141–142° dec. (B) 2.15 g., m.p. 121° sinter, melt 124–129° dec.; mixed m.p. with the hydrochloride of the ethyl ester VIB (m.p. 122–124° dec.), was 120° sinter, melt 121–127° dec.

Preparation of 2-Methylthiazolidine-2,4-dicarboxylic Acid (IV).—Cysteine hydrochloride (Eastman Kodak Co.) was treated with pyruvic acid (Mathieson, Coleman and Bell) by the method of Schubert[8] to give 2-methylthiazolidine-2,4-dicarboxylic acid, m.p. 162° dec., $[\alpha]^{29}D$ −80.4° in water (*c* 0.05 g./cc.).

When cysteine hydrocholride obtained from the Mann Research Laboratory was used in the reaction, the product was found to have a specific rotation, $[\alpha]^{29}D$ −81.2°, in water (*c* 0.05 g./cc.).

Preparation of Diethyl 2-Methylthiazolidine-2,4-dicarboxylate and Its Hydrochloride IX.—Ten grams of 2-methylthiazolidine-2,4-dicarboxylic acid (IV)[8] (*Anal.* Calcd. for $C_6H_9NSO_4$: C, 37.69; H, 4.75; S, 16.77. Found: C, 37.8; H, 5.0; S, 16.5) was placed in 180 ml. of absolute ethanol and dry hydrogen chloride gas was bubbled into the solution for 30 minutes. The reaction mixture was left at room temperature for 2 days, and the ethyl alcohol was then distilled off *in vacuo* with gentle warming on the steam-bath leaving a white residue. One gram of this material was removed. The melting point of this crude material was 115–125° dec. The remaining residue was washed with ether and then dissolved in 50 ml. of water containing 8 g. of potassium carbonate. This aqueous mixture was extracted with four 150-ml. portions of ether, which were combined, and dried over calcium oxide. Upon removal of the ether on the steam-bath and distillation of the residue *in vacuo*, 6 g. of light-yellow oil, $n^{24}D$ 1.4782, boiling at 131–132° at about 3 mm., was obtained.

Anal. Found: C, 48.9; H, 7.3; N, 5.8.

This material and the 1 g. removed before distillation represent a 50% yield of the ester.

When the oil was dissolved in dry ether and dry hydrogen chloride gas was bubbled into the solution, a copious white precipitate formed immediately. This precipitate did not dissolve as the ether solution became warm. It melted at 139–142° dec. and, upon recrystallization from a solution of 1 part of absolute ethanol and 2 parts of dry ether, the melting point was raised to 142–143° dec., sintering taking place at about 138°.

Anal. Calcd. for the hydrochloride of diethyl 2-methylthiazolidine-2,4-dicarboxylate, $C_{10}H_{18}NSO_4Cl$: C, 42.32; H, 6.39; N, 4.94; S, 11.30. Found: C, 42.2; H, 6.5; N, 5.1; S, 11.1.

Preparation of Racemic Diethyl 2-Methylthiazolidine-2,4-dicarboxylate Hydrochloride.—Four and nine-tenths grams of diethyl 2-methylthiazolidine dicarboxylate was racemized in alcoholic potassium hydroxide.[9] This thiazolidine had been prepared from L-cysteine, and the hydrochloride prepared from this ester melted at 140–142° dec., $[\alpha]^{29}D$ −54.8 in ethyl alcohol (*c* 0.05 g./cc.). The racemization was carried out by allowing the ester (0.02 mole) to stand overnight at room temperature in 200 ml. of absolute ethanol containing 0.561 g. (0.01 mole) of potassium hydroxide. One-half of the reaction mixture was evaporated to dryness *in vacuo* and extracted with dry ether. Dry hydrogen chloride gas was bubbled into the ether for a few minutes and, upon standing, 1.126 g. of crystals was found, amounting to a 40% recovery of the ester. A few crystals (0.1128 g., 10% of the total) were found to be of the high-melting type (137° sinter; melt at 142° dec.), but the remaining 90% of the crystals melted at 123–127° dec. and gave no depression when mixed with the low-melting crystals obtained from the ester hydrochloride obtained from cystine. The low-melting crystals were found to be optically inactive. The mixed melting point of these crystals and cysteine ethyl ester hydrochloride (m.p. 126–127° dec.) was depressed (100–115°).

Preparation of Diethyl 2-Methylthiazolidine-2,4-dicarboxylate Hydrochloride from DL-Cysteine.—L-Cystine was racemized in boiling 20% hydrochloric acid by the method of Loring and Du Vigneaud.[10] The inactive cystine was reduced to DL-cysteine (X)[11] and treated with pyruvic acid[8] to give inactive 2-methylthiazolidine-2,4-dicarboxylic acid, m.p. 168° dec. This acid (10.5 g.) was esterified and worked up in the manner already described for active 2-methylthiazolidine-2,4-dicarboxylic acid (IV) to give 1.05 g. of distillate, b.p. 130° at 3 mm., $n^{24}D$ 1.4812. The hydrochloride obtained from this ester melted at 126–127° dec. and did not depress the melting point of VIB. A small amount of the material did not melt until 140°.

Treatment of Lanthionine (XIV) with Aqueous Calcium Hydroxide and Esterification of the Product.—Twenty-four grams of lanthionine[16d] was mixed with 24 g. of calcium hydroxide and 500 ml. of water and left at room temperature for 52 days. This material was worked up and esterified in the same manner as was the product from cystine (procedure 2) to give 2.56 g. of distillate boiling at 107–118° at 3 mm. (9.1% yield of diethyl 2-methylthiazolidine-2,4-dicarboxylate). The hydrochloride, m.p. range 121–143°, obtained in the usual way from this distillate, was shown by infrared spectra to be identical with VIA and VIB obtained from cystine.

Cleavage of the Ring in Diethyl 2-Methylthiazolidine-2,4-dicarboxylate and in the Ethyl Ester Obtained from Cystine Treated with Aqueous Calcium Hydroxide. 1. Oxidation[12] of Diethyl 2-Methylthiazolidine-2,4-dicarboxylate.—Seven-tenths of a gram (0.0025 mole) of the hydrochloride of diethyl 2-methylthiazolidine-2,4-dicarboxylate (IX) was placed in 30 ml. of water and 0.12 ml. of 30% hydrogen peroxide was added. After standing for 3 days at room temperature, the reaction mixture was extracted with three 50-ml. portions of ether, A, and then made alkaline with 1 g. of potassium carbonate. The aqueous solution was then extracted with three 50-ml. portions of ether, B, which were combined, and dried over anhydrous magnesium sulfate. The ether solution B was saturated with dry hydrogen chloride gas for 1 minute, and a white precipitate formed. This precipitate melted with decomposition at 180 or 185°, depending on the rate of heating,[20] and weighed 0.118 g.

Anal. Calcd. for cystine diethyl ester dihydrochloride (XI), $C_{10}H_{22}N_2S_2O_4Cl_2$: N, 7.59. Found: N, 7.8.

2. Oxidation[12] of the Ethyl Ester of Material from Liming of Cystine.—The hydrochloride of the ethyl ester VIB, m.p. 117–126° dec., 0.35 g., was oxidized in the same manner as was IX to give a precipitate which weighed 0.064 g. and melted at about 183°, with decomposition.

Anal. Calcd. for cystine diethyl ester dihydrochloride (XI), $C_{10}H_{22}N_2S_2O_4Cl_2$: N, 7.59. Found: N, 7.7.

When this material was mixed with that obtained from oxidation of the hydrochloride of diethyl 2-methylthiazolidine-2,4-dicarboxylate (IX), no depression in melting point was observed.

Cleavage of the Ring with *p*-Nitrophenylhydrazine. 1. Reaction with the Hydrochloride of Diethyl 2-Methylthiazolidine-2,4-dicarboxylate.—One-half gram of the hydrochloride of diethyl 2-methylthiazolidine-2,4-dicarboxylate (IX) and 0.5 g. of *p*-nitrophenylhydrazine in 15 ml. of absolute ethanol was heated to boiling, and one drop of glacial acetic acid was added. The reaction mixture was heated 15 minutes more and then left at room temperature for a month. Large, yellow crystals were formed, weighing 0.4227 g. (95%), m.p. 187–188°.[21]

Anal. Calcd. for the *p*-nitrophenylhydrazone of ethyl pyruvate, XII: $C_{11}H_{13}N_3O_4$: N, 16.74. Found: N, 17.1.

2. Reaction with the Hydrochloride of the Ethyl Ester of the Material Obtained from Cystine Treated with Aqueous Calcium Hydroxide.—One-half gram of the hydrochloride of the ethyl ester VIB, m.p. 117–126° dec., and 0.5 g. of *p*-nitrophenylhydrazine were allowed to react as described with IX. After standing at room temperature for a month, 0.3429 g. of yellow crystals was formed (77%). These melted at 186–186.5° and, when mixed with the *p*-nitrophenylhydrazone obtained from diethyl 2-methylthiazolidine-2,4-dicarboxylate (m.p. 187–188°), gave no depression in melting point.

Infrared and Ultraviolet Absorption Spectra.—Infrared absorption spectra were obtained on a Baird double-beam spectrophotometer using rock-salt optics. The samples were prepared as pressed plates in potassium bromide. Ultraviolet absorption spectra were obtained on a Cary double-beam recording spectrophotometer, model 11. Samples were run against the same thickness of solvent.

ROCHESTER, N. Y.

(20) R. A. Gortner and W. F. Hoffman, *J. Biol. Chem.*, **72**, 437 (1927), report that the dihydrochloride of L-cystine diethyl ester melts at 177–178° dec.; E. Friedmann, *Beitr. Chem. Physiol. u. Path.*, **3**, 16 (1903) (as quoted in Beilstein's "Handbuch Org. Chem.," Vol. IV, J. Springer, Berlin, 1922, p. 509), reports a melting point of 185° dec.

(21) I. M. Heilbron, "Dictionary of Organic Compounds," Vol. IV, Oxford Press, New York, N. Y., 1953, p. 288, gives the melting point of the *p*-nitrophenylhydrazone of ethyl pyruvate as 187°.