[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, COLUMBIA UNIVERSITY]

# The Action of Formaldehyde upon Cysteine

BY SARAH RATNER[1] AND H. T. CLARKE

The reaction of mercaptans with carbonyl compounds has received extensive study, but little attention has, until recently, been paid to the case of cysteine. In a study of the effect of formaldehyde on the titration curves of amino acids, Birch and Harris[2] observed, on the addition of formaldehyde to cysteine, the disappearance of the buffering action typical of the sulfhydryl group. Shinohara[3] in an investigation of the action of cysteine upon phosphotungstic acid, noted that the reducing power is inhibited completely by addition of formaldehyde. During the progress of the present investigation, Schubert[4] has described the formation of compounds of cysteine with various aldehydes, among them formaldehyde, and has produced evidence that the latter reacts with cysteine to form a thiazolidinecarboxylic acid. The present report covers a more extended investigation of this compound, its reactions, and its manner of formation.

The reaction between formaldehyde and cysteine can take place over a wide range of hydrogen ion activity. The product, which is remarkably stable toward both acid and alkali, possesses amphoteric properties, as is shown by the titration curve (Fig. 3). The empirical formula, $C_4H_7O_2NS$, and the conditions of formation indicate that the compound is formed from equimolecular quantities of cysteine and formaldehyde; its simple molecular character is indicated by the observed molecular weight of the methyl ester.

The nitrogen exists in the form of a secondary amino group, as is shown by the formation of an acetyl derivative and by the behavior with nitrous acid in the Van Slyke procedure.[5] The product, moreover, displays no tendency to racemize when its sodium salt is treated with acetic anhydride by the method of du Vigneaud and Meyer[6]—a result characteristic of secondary, in contrast to primary, $\alpha$-amino acids.

The sulfur is present as a thio ether, for the nitroprusside reaction is negative; oxidation of the acetyl derivative by hydrogen peroxide leads, according to the conditions, to a sulfoxide or a sulfone. The unsubstituted acid, on treatment with hydrogen peroxide or iodine, is converted, with loss of formaldehyde, into cystine; with bromine water, cysteic acid is produced.

Among the compounds described by Schubert[4] some are formed by addition and others by condensation. It therefore seems probable that the reaction of cysteine with formaldehyde occurs in two steps, firstly, addition of the aldehyde, and secondly, ring closure by removal of a molecule of water. The formaldehyde may, theoretically, react primarily with either the sulfhydryl or the amino group of cysteine. A decision between these alternative possibilities can be reached by noting the ease of reaction of formaldehyde with acetylcysteine on the one hand, and S-ethylcysteine on the other. Experiment shows that the rotation of S-ethylcysteine at *p*H 5.12[7] is not changed by the presence of formaldehyde, while that of acetylcysteine is. At this *p*H, the rate of change is extremely rapid, both with cysteine and with acetylcysteine, but can be measured at higher hydrogen ion concentrations (*p*H 1–4). With cysteine (Fig. 1) the rotation reaches the theoretical value; with acetylcysteine (Fig. 2), on the other hand, the levo-rotation is distinctly lower than that observed for pure acetylthiazolidinecarboxylic acid under the same conditions. This may be interpreted as indicating the formation of a less strongly levorotatory addition compound of the type $RSCH_2OH$. By analogy, the first stage in the reaction between cysteine and formaldehyde may be regarded as taking place at the sulfur atom.

In determining the rates of the reaction at different *p*H levels, advantage was taken of the small optical rotation of cysteine and the conveniently large one of thiazolidinecarboxylic acid by follow-

(1) This report is from a dissertation submitted by Sarah Ratner in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University.

(2) Birch and Harris, *Biochem. J.*, **24**, 1080 (1930).

(3) Shinohara, *J. Biol. Chem.*, **110**, 263 (1935).

(4) Schubert, *ibid.*, **111**, 671 (1935); **114**, 341 (1936).

(5) In this, 20 to 30%, according to the conditions, of the equivalent amount of nitrogen is set free, but this anomaly may be ascribed, as in the case of cystine [Laugh and Lewis, *ibid.*, **104**, 601 (1934)], to oxidative side reactions; under suitable conditions as much as 10% of the sulfur is converted into sulfuric acid by the reaction of nitrous acid.

(6) Du Vigneaud and Meyer, *ibid.*, **98**, 295 (1932); **99**, 143 (1932).

(7) Formaldehyde does not influence the titration curve at this point.

ing the change in rotation. Six buffered reaction mixtures between $p$H 1.5 and $p$H 12 were investigated at 25°. The velocity curves (Fig. 2) display a measurable rate of reaction which increases rapidly on raising the $p$H, and above 5 becomes too fast to follow.

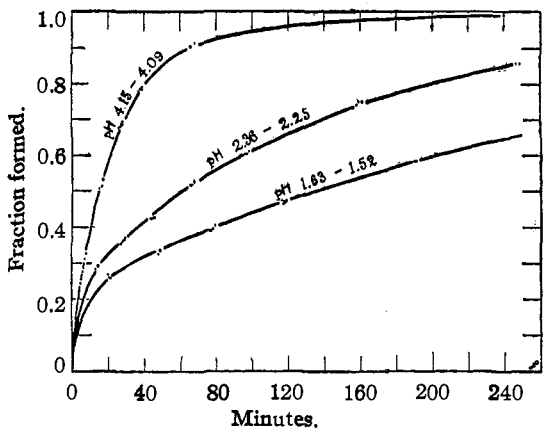



Fig. 1.—Rate of thiazolidinecarboxylic acid formation.

Thiazolidinecarboxylic acid behaves as an ampholyte (Fig. 3); the acidic dissociation ($pK_1$ 1.51) is strong, but the basic dissociation ($pK_2$ 6.21) extremely weak in comparison with those of $\alpha$-amino acids in general, including proline (Table I).

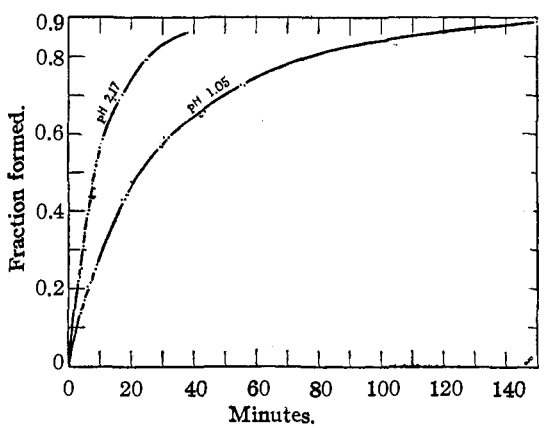



Fig. 2.—Rate of reaction of acetylcysteine and formaldehyde.

Replacement of the labile hydrogen atoms in thiazolidinecarboxylic acid produces changes in dissociation constants which are similar in direction to those which accompany similar substitutions in other amino acids.[8] The constants for the methyl ester and the acetyl derivative demonstrate the weakening effect, on both acidic and basic dissociation, of the inhibition of the ionizing function of one group (Figs. 5 and 6).

TABLE I

DISSOCIATION CONSTANTS OF AMINO ACIDS, THIAZOLIDINECARBOXYLIC ACID AND RELATED COMPOUNDS

| | $pK_1$ | $pK_2$ |
|---|---|---|
| Alanine[a] | 2.61 | 9.72 |
| Cysteine[b] | 1.96 | 8.18 |
| S-Ethylcysteine | 2.03 | 8.60 |
| Proline[c] | 1.99 | 10.60 |
| Thiazolidine-4-carboxylic acid | 1.51 | 6.21 |
| Acetylthiazolidine-4-carboxylic acid | 2.96 | .. |
| Methyl thiazolidine-4-carboxylate | .. | 4.00 |
| Thiazolidine | .. | 6.31 |

[a] Bjerrum, *Z. physik. Chem.*, **106**, 219 (1923). [b] Cannan and Knight, *Biochem. J.*, **21**, 1384 (1927). [c] McCay and Schmidt, *J. Gen. Physiol.*, **9**, 336 (1926).

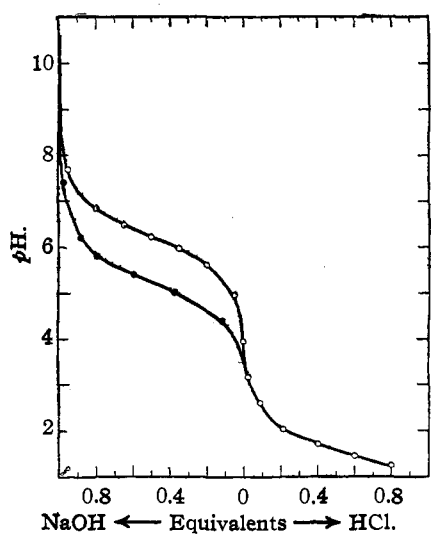



Fig. 3.—Titration of thiazolidinecarboxylic acid O–O in water and ●–● in 18% formaldehyde.

In the presence of a large amount of formaldehyde, the basic dissociation of thiazolidinecarboxylic acid is decreased (Fig. 3) but to a smaller extent than in proline and in S-ethylcysteine (Fig. 4). The presence of the $-CH_2S-$ group in the thiazolidine ring presumably exerts approximately the same effect on the basic dissociation constant as the introduction of a single hydroxymethyl group into a primary amino acid. The effect of formaldehyde upon the basic dissociation of thiazolidinecarboxylic acid, namely, depression by about one $pK$ unit, is probably much the same as that of the second hydroxymethyl group in a compound of two molecules of formaldehyde with one of a primary amino acid.[9]

(8) Cohn, *Ergebnisse Physiol.*, **33**, 781 (1931).

(9) Levy, *J. Biol. Chem.*, **79**, 767 (1933).

Acetylation or esterification of thiazolidinecarboxylic acid has no tendency to open the ring, and the corresponding derivatives are obtained in excellent yields.

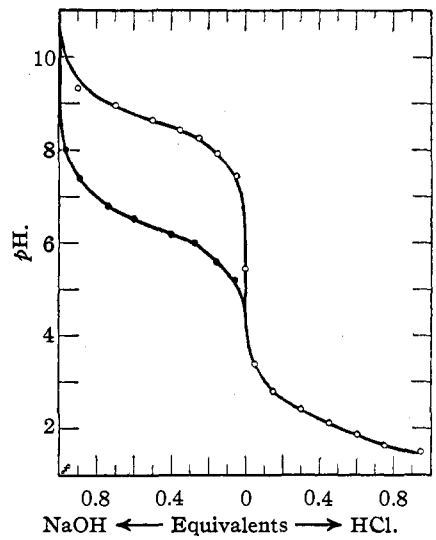



Fig. 4.—Titration of S-ethylcysteine O–O in water and ●–● in 18% formaldehyde.

Hydrolytic cleavage in *N* hydrochloric acid occurs only to a very small extent, and can be detected only when equilibrium is disturbed, as by removal of formaldehyde. A solution of thiazolidinecarboxylic acid in *N* hydrochloric acid was heated in a sealed tube at 100° for twenty-four hours; the rotation remained essentially unchanged. On the other hand, hydrolysis was detected by distilling the solution at constant volume. Formaldehyde was found in the distillate in amounts roughly equivalent to the cysteine produced.

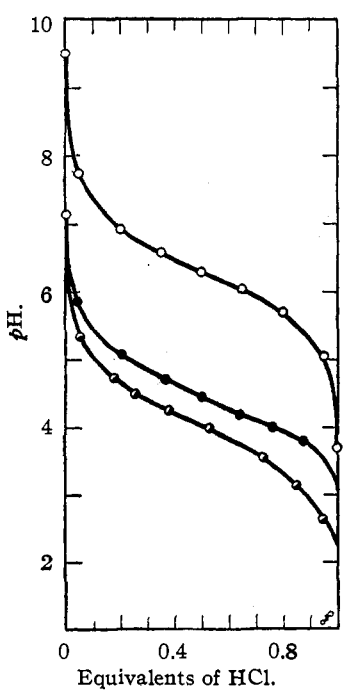



Fig. 5.—Titration of thiazolidine O–O in water, ●–● in 18% formaldehyde and ◑–◑ in methyl thiazolidinecarboxylate.

Oxidation of the sulfur atom exerts a progressive effect in increasing carboxyl dissociation. Acetylthiazolidinecarboxylic acid has a *pK* of 2.96, its sulfoxide 2.50 and its sulfone 2.23 (Fig. 6).

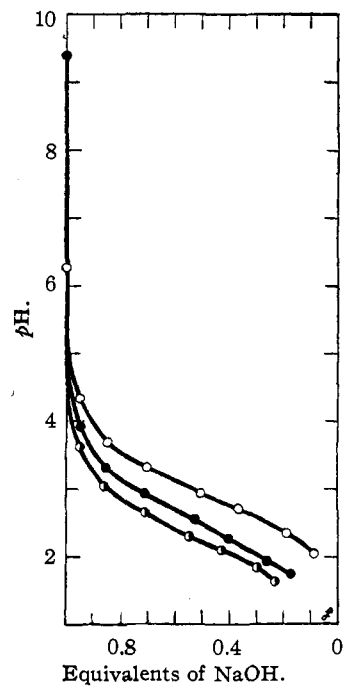



Fig. 6.—Titration of O–O acetylthiazolidinecarboxylic acid, ●–● acetylthiazolidinecarboxylic acid sulfoxide and ◑–◑ acetylthiazolidinecarboxylic acid sulfone.

With hot alkaline plumbite in an atmosphere of nitrogen, lead sulfide is formed from thiazolidinecarboxylic acid, but more slowly than from cysteine.[10] The first evidence of its formation was noticed after twenty-five minutes at 95°; after twenty-four hours 97.5% of the theoretical amount had precipitated.

With iodoacetic acid and benzyl chloride, which react with sulfhydryl groups,[11] the corresponding

(10) Fruton and Clarke, *J. Biol. Chem.*, **106**, 667 (1934); Blumenthal and Clarke, *ibid.*, **110**, 343 (1935).

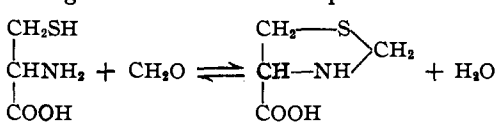
(11) Dickens, *Biochem. J.*, **27**, 1141 (1933); Michaelis and Schubert, *J. Biol. Chem.*, **106**, 331 (1934).

cysteine derivatives are formed from thiazolidinecarboxylic acid in alkaline solution (*p*H 10–11) at room temperature.

Ring opening may also be demonstrated at *p*H 10 by the action of air in the presence of a trace of ferric chloride; cystine is formed slowly. Other oxidizing agents bring about ring opening more readily: one equivalent of hydrogen peroxide or of iodine produces cystine and formaldehyde almost quantitatively. With bromine water, cysteic acid is formed, six equivalents of the halogen being consumed.

Thiazolidinecarboxylic acid reacts with sulfite apparently to form an equilibrium mixture. The progress of the reaction, which is rapid at *p*H 5 or above, can be measured at *p*H 4 by following the change in optical rotation. Sulfhydryl is formed in the process, for an increasing power to reduce phosphotungstic acid is observed. Thiazolidinecarboxylic acid alone does not reduce phosphotungstic acid at *p*H levels below 11.

All these observations may be interpreted as indicating the existence of an equilibrium



which lies so far to the right that sulfhydryl reactions are ordinarily imperceptible. Disturbance of this equilibrium by an irreversible process displaces it to the left.

Acetylation changes this behavior considerably. Iodine does not oxidize acetylthiazolidinecarboxylic acid, and hydrogen peroxide in suitable organic solvents yields the corresponding sulfoxide and sulfone, which are stable in aqueous solution. The sulfoxide is reduced by hydrogen iodide, with liberation of two equivalents of iodine; the sulfone is not affected in this way.

The parent thiazolidine, prepared for purposes of comparison by the condensation of β-aminoethyl mercaptan with formaldehyde, is a stable liquid of weakly basic character (Fig. 5 and Table I), and displays chemical properties analogous to those of the carboxylic acid. Its basic dissociation is depressed by formaldehyde (Fig. 5) to a greater extent than is the case with the carboxylic acid. With acetic anhydride it yields an acetyl derivative which is not oxidized by iodine but on treatment with two moles of hydrogen peroxide in glacial acetic acid yields a crystalline sulfone; the corresponding sulfoxide could not be obtained in crystalline form. The free base is oxidized by iodine to di-β-aminoethyl disulfide and by bromine to taurine. The boiling point, 164–165°, is notably higher than that of thiazole (117°); that of thiazoline (138–139°) occupies an intermediate position.

## Experimental

**Thiazolidine-4-carboxylic Acid.**—The cysteine hydrochloride from 30 g. of cystine was dissolved in 100 cc. of water; 22 cc. of commercial 40% formaldehyde (1.1 mole) was added, and the mixture allowed to stand overnight at room temperature. On the addition of 25 cc. of pyridine, crystals soon separated. The whole was diluted with 50 cc. of alcohol and filtered. The product (28.2 g.) was recrystallized from hot water. The resulting long, colorless needles, which melt with decomposition at 196–197°, are insoluble in alcohol, somewhat soluble in cold water, readily soluble in hot water, in acid and in alkali.

*Anal.* Calcd. for $C_4H_7O_2NS$: C, 36.06; H, 5.26; N, 10.52; S, 24.05. Found: C, 35.93; H, 5.27; N,[12] 10.38; S, 24.09. $[\alpha]^{20}D$ −141° in water; $[\alpha]^{22}D$ −100° in *N* hydrochloric acid; $[\alpha]^{22}D$ −203° in *N* sodium hydroxide.

The specific rotation was also determined at a series of *p*H levels in suitable buffered solutions, the *p*H values of which were determined potentiometrically.

TABLE II

| *p*H | 1.52 | 2.25 | 4.09 | 6.10 | 9.88 |
|---|---|---|---|---|---|
| $[\alpha]^{25}D$ | −120 | −135 | −142 | −173 | −214 |

A solution in 10–15% hydrochloric acid, when allowed to evaporate at room temperature *in vacuo*, deposited the hydrochloride as large quadrangular prisms, m. p. 184–185° with decomposition, readily soluble in alcohol.

*Anal.* Calcd. for $C_4H_7O_2NS \cdot HCl$: N, 8.26; Cl, 20.91. Found: N, 8.08; Cl, 21.00.

When a concentrated aqueous solution is diluted with water, the free acid crystallizes out.

**Thiazolidinecarboxylic Acid Methyl Ester Hydrochloride.**—A solution of 10 g. of thiazolidinecarboxylic acid in 100 cc. of methanol which had been saturated with dry hydrogen chloride was warmed under reflux on a steam-bath for one hour. The solution was evaporated to a small volume *in vacuo*, ether was added, and the crystalline product chilled, filtered off and dried *in vacuo* over solid alkali; yield 13 g. (94%). It was recrystallized by dissolving in methanol and precipitating with ether, and appears as shiny plates which decompose at 164–165°; very soluble in water and alcohol, insoluble in ether.

*Anal.* Calcd. for $C_5H_9O_2NS \cdot HCl$: Cl, 19.31; S, 17.46; N, 7.63; $CH_3O$, 16.89. Found: Cl, 19.39; S, 17.74; N, 7.60; $CH_3O$, 16.76.

**Methyl Thiazolidinecarboxylate.**—To a suspension of 13 g. of ester hydrochloride in 3–4 cc. of water, covered with about 30 cc. of ether, anhydrous potassium carbonate was added slowly in excess. The ether was decanted

(12) As most of the compounds described in this paper gave low values for nitrogen by the micro-Dumas method, the micro-Kjeldahl process was employed throughout. Grateful acknowledgment is made to Mr. William Saschek for the analytical results here reported.

after shaking. Extraction with ether was repeated several times. The combined ethereal solution was dried with barium oxide and the ether removed; the residual crude oil was distilled *in vacuo;* b. p. 75° (1.0 mm.); yield 7.85 g. (75%).

*Anal.* Calcd. for $C_6H_9O_2NS$: $CH_3O$, 21.08; S, 21.79; mol. wt., 147. Found: $CH_3O$, 21.14; S, 21.82; mol. wt., 149.2, 146.6 (cryoscopic in $C_6H_6$). $[\alpha]^{23}D$ −83.0 in water; $[\alpha]^{21}D$ −106.3 in benzene; $[\alpha]^{21}D$ −94.7° in $CH_3OH$; $d^{23}_4$ 1.324; $n^{25}D$ 1.527.

The ester showed no tendency to condense with itself even on long standing.

**Acetylthiazolidinecarboxylic Acid.**—To a suspension of 9.3 g. of thiazolidinecarboxylic acid in 30 cc. of water, 30 cc. of acetic anhydride was added slowly at 90° with constant stirring over a twenty-minute period. The clear solution was heated for forty minutes longer. Water and acetic acid were removed *in vacuo*, the residue dissolved in 30 cc. of hot water. On cooling, crystals appeared as six-sided prisms which were filtered off after chilling; yield 10.0 g. (82%), m. p. 143.5–144.5°; very soluble in water, somewhat less so in alcohol, acetone and ether.

*Anal.* Calcd. for $C_6H_9O_3NS$: C, 41.11; H, 5.18; N, 8.00. Found: C, 40.92; H, 5.07; N, 7.85. $[\alpha]^{20}D$ −133.5 in water (*p*H 2.1); $[\alpha]^{20}D$ −159.8° in an equivalent quantity of dilute sodium hydroxide.

**Acetylation of Sodium Salt of Thiazolidinecarboxylic Acid.**—Into a 10-cc. volumetric flask was weighed 0.875 g. of thiazolidinecarboxylic acid (5 mml.); 5 cc. of *N* sodium hydroxide and 3.0 cc. of acetic anhydride (30 mml.) was added and the volume made up to 10 cc. with water. The flask was kept at 37° and 2-cc. samples were withdrawn after 46, 92 and 168 hours. To each sample 12.7 cc. of *N* sodium hydroxide was added, followed after three hours by 6.85 cc. of 2 *N* sulfuric acid. The solution was taken to dryness *in vacuo;* the residue was extracted with 20 cc. of hot absolute ethyl alcohol, the alcohol was evaporated and the residue so obtained recrystallized from a small amount of water. $[\alpha]^{23}D$ −133.2° after 46 hours; −134.4° after ninety-two hours; −134.4° after one hundred and sixty-eight hours.

Phenylalanine when treated in exactly the same way was found to be completely racemized after twenty-four hours.

**Effect of *p*H on Velocity of Formation** (Figs. 1, 2).—All solutions were made up so that 25 cc. contained 1.70 mml. each of cysteine hydrochloride (or acetylcysteine) and formaldehyde, 15 cc. of the appropriate 0.1 *N* buffer (or sodium chloride solution of equivalent ionic strength) with appropriate amounts of acid or alkali. The initial rotation for each curve was determined by omitting formaldehyde from the solution, and the final values were determined from equimolar solutions of thiazolidinecarboxylic acid. The final value of the cysteine reaction mixture agreed with the rotation calculated for the end-product. The *p*H of all solutions were determined with the glass electrode. Despite buffering, a change in *p*H (about 0.1) occurs during the reaction owing to the large difference between the *pK'*s of cysteine and thiazolidinecarboxylic acid. The initial and final *p*H values are recorded on the curves. The concentrations of the acetylcysteine and cysteine hydrochloride stock solutions were calculated from Kjeldahl nitrogen determinations and the formaldehyde stock solution was analyzed by iodine titration.

The values plotted in the curves were those of $X$ calculated from the formula

$$\alpha_0 (1 - X) + \alpha_\infty X = \alpha_{obsd.}$$

In the experiments with acetylcysteine, the value for $\alpha_\infty$ was based upon the final steady value observed in the reaction mixture.

**Titration Curves** (Figs. 3–6).—Except in the formaldehyde titrations, 0.1 *M* solutions were titrated with *N* sodium hydroxide and hydrochloric acid. The *p*H measurements, made with a glass electrode,[13] are reliable to ±0.03. The values reported ($pK_1$ for the acid constants and $pK_2 = pK_b - pK_w$ for the basic constants) each represent the average of the values calculated from five points in the curve. In the formaldehyde titrations, 5 cc. quantities of 0.1 *M* solution were added to 100 cc. of 18% formaldehyde and titrated with 0.1 *N* sodium hydroxide. All curves were corrected for water blanks.

**Hydrolysis of Thiazolidinecarboxylic Acid.**—A solution of 0.3021 g. of the acid in 25 cc. of *N* hydrochloric acid was distilled slowly for nine hours with addition of water at the approximate rate of distillation. The distillate, which amounted to 100 cc., was collected under alcoholic dimedon solution; it yielded 65 mg. of the formaldehyde derivative, m. p. 187°.

The residual solution was made up to 25 cc.; it gave a strong reaction with nitroprusside. The observed rotation (2 dcm. tube) of the solution before distillation was −2.47°; after distillation −2.04°. The fall in rotation corresponds to a conversion of 15.3% of the thiazolidinecarboxylic acid into cysteine having[14] $[\alpha]D$ +7.6°.[15] The cysteine content, determined by the Lugg modification[16] of the method of Folin and Marenzi, was 11.0%.

**Reaction with Iodoacetic Acid.**—A solution of 0.66 g. of thiazolidinecarboxylic acid (5 mml.), 0.93 g. of iodoacetic acid (5 mml.) and 1.04 g. of potassium carbonate (15 m. eq.) in 10 cc. of water was allowed to stand overnight at room temperature. Upon the addition of 10 cc. of *N* hydrochloric acid crystals slowly appeared. The yield of S-carboxymethyl cysteine[11] was 0.77 g. (87%); thin trapezoidal plates from water, m. p. 200° (dec.).

*Anal.* Calcd. for $C_6H_9O_4NS$: H, 5.06; C, 33.51; N, 7.82. Found: H, 4.88; C, 33.33; N, 7.87 (Van Slyke amino N).

**Reaction with Benzyl Chloride.**—A mixture of 0.66 g. of thiazolidinecarboxylic acid, 0.63 g. of benzyl chloride and 0.69 g. of potassium carbonate in 10 cc. of water was stirred for twenty-four hours at room temperature. S-Benzylcysteine crystallized out during the reaction; m. p. 210° (dec.), unchanged by admixture with an authentic sample;[17] $[\alpha]D$ +23.8° in *N* sodium hydroxide.

**Action of Oxygen in Alkaline Solution.**—A solution containing 0.266 g. of thiazolidinecarboxylic acid (2 mml.), 2 cc. of *N* sodium hydroxide, 5 cc. of half-neutralized 0.2 *M*

(13) A modification by F. Rosebury of the apparatus described by him in *Ind. Eng. Chem., Anal. Ed.*, **4**, 398 (1932).

(14) Assuming $[\alpha]D$ +15.9° [Vickery and Leavenworth, *J. Biol. Chem.*, **86**, 129 (1930)] the conversion would be 14.3%.

(15) Toennies and Bennet, *J. Biol. Chem.*, **112**, 499 (1936).

(16) Lugg, *Biochem. J.*, **26**, 2160 (1932).

(17) Clarke and Inouye, *J. Biol. Chem.*, **94**, 549 (1932).

bicarbonate buffer and 0.1 cc. of 0.01 *M* ferric chloride was made up to a volume of 10 cc. Air was passed through the solution for twenty-four hours at room temperature. The *p*H (10.2) was then brought to 6.4 by the addition of 2 cc. of *N* hydrochloric acid. After several days hexagonal plates had separated. The product, weighing 8 mg. (3.3%), was identified as cystine.

**Oxidation with Hydrogen Peroxide.**—A solution of 0.266 g. of thiazolidinecarboxylic acid in 30 cc. of water and 0.12 cc. (1.05 equiv.) of 30% hydrogen peroxide was allowed to stand at room temperature for several days; regular hexagonal plates separated; yield 0.210 g. (87%) of cystine; $[\alpha]_D$ −214.5° in *N* hydrochloric acid. The filtrate was distilled into alcoholic dimedon; 0.408 g. (70%) of the formaldehyde derivative was secured.

**Oxidation with Iodine.**—To 10 cc. of 0.1 molar thiazolidinecarboxylic acid was added 10 cc. of 0.1 *N* iodine in 2.5% potassium iodide. The iodine was decolorized rapidly and regular hexagonal plates soon separated. Pyridine (0.2 cc.) was added and after two days 0.100 g. (83%) of cystine was obtained; $[\alpha]^{22}_D$ −212° in *N* hydrochloric acid.

The filtrate yielded 0.141 g. (50%) of the dimedon derivative of formaldehyde, m. p. 187°.

On titration with iodine, 1.6–1.7 equivalents were consumed by thiazolidinecarboxylic acid, 1.0–1.2 by its methyl ester. The consumption of iodine in excess of one equivalent is attributable to oxidation of cystine.[18]

**Oxidation with Bromine.**—A solution of 0.2896 g. (2.175 mml.) of thiazolidinecarboxylic acid in 10 cc. of water was titrated at 0° with *N* bromine in acetic acid. The end-point, taken when the yellow color persisted for thirty seconds, was reached with 6.45 mml. of bromine (5.94 equivalents). The resulting solution was evaporated to dryness *in vacuo*, and the crystalline residue purified by repeated precipitation from water by absolute alcohol. $[\alpha]^{24}_D$ +7.7° in water. The cysteic acid was identified as the copper salt.

*Anal.* Calcd. for $C_3H_7O_6NSCu$: N, 5.63; S, 12.89; Cu, 25.56. Found: N, 5.57; S, 13.22; Cu, 25.77.

**Action of Sulfite on Thiazolidinecarboxylic Acid.**—To a solution of 1.5 mml. was added 15 mml. of sodium sulfite, suitably buffered and the volume made up to 25 cc. The optical rotation of the resulting solution was determined at intervals:

OBSERVED ROTATIONS (2 DCM.) AFTER SPECIFIED TIMES

| *p*H | 0 | 5 min. | 15 min. | 20 min. |
|---|---|---|---|---|
| 4.03 | −2.26° | −2.02° | | −1.80° |
| 6.41 | −2.80° | | −0.96° | |

| *p*H | 30 min. | 95 min. | 8 hrs. |
|---|---|---|---|
| 4.03 | | −1.66° | −1.66° |
| 6.41 | −0.92° | | |

The zero values, determined in the absence of sulfite, were obtained by interpolation from the *p*H dependence data (Table II).

**Action of Phosphotungstic Acid and Sulfite on Thiazolidinecarboxylic Acid.**—To 2 cc. of 0.00832 molar thiazolidinecarboxylic acid was added 2 cc. of phosphotungstic acid solution, buffered to *p*H 5.7, according to the directions of Lugg.[16] No appreciable color appeared. When 1 cc. of molar sodium sulfite was incorporated in an otherwise similar mixture, a blue color appeared; this continued to increase in intensity at a rate considerably less than that observed with cystine. Comparison with cystine standards showed after thirteen hours a color intensity equivalent to 43% of that observed with an equimolar quantity of cystine.

In another experiment, 50 cc. of 0.00832 molar thiazolidinecarboxylic acid was mixed with 20 cc. of molar sulfite, and diluted to 100 cc. The color developed with phosphotungstic acid was determined at intervals under the conditions described by Lugg. The color intensities, expressed as percentages of that of the equimolar cystine standard, were

| Hours | 0.1 | 4 | 10 | 22 |
|---|---|---|---|---|
| Color | 11 | 27 | 28 | 28 |

With twice the amount of sulfite, the maximum color developed after six hours was 47%; after ninety-three hours, it had fallen to 30%. No attempt was made further to investigate the effect of variations in the proportion of sulfite.

**Sulfoxide of Acetylthiazolidinecarboxylic Acid.**—A solution of 0.876 g. (5 mml.) of acetylthiazolidinecarboxylic acid in 40 cc. of acetone was treated with 0.6 cc. (5.25 mml.) of 30% hydrogen peroxide. After three days the solvent was evaporated under reduced pressure. The residue was recrystallized by adding acetone to its solution in the minimum quantity of hot alcohol: 0.690 g. of octagonal prisms, m. p. 188–190° (dec.); $[\alpha]^{26}_D$ −118° in water.

*Anal.* Calcd. for $C_6H_9O_4NS$: C, 37.67; H, 4.75; N, 7.33; neut. eq., 191. Found: C, 37.57; H, 4.90; N, 7.32; neut. eq., 190.

The same product was obtained in substantially the same yield by the use of twice the above proportion of hydrogen peroxide in acetone.

A weighed amount (30.6 mg.) of the sulfoxide was added to a solution containing 1 cc. of 5 *N* KI and 0.5 cc. of 10 *N* HCl. After an hour 3 cc. of water was added and the solution quantitatively transferred to a separatory funnel with 5 cc. more water. The iodine extracted with peroxide-free ether and titrated with 0.1 *N* thiosulfate, required 3.23 cc. (2.02 equivalents).

**Sulfone of Acetylthiazolidinecarboxylic Acid.**—To a solution of 0.876 g. (5 mml.) of acetylthiazolidinecarboxylic acid in 20 cc. of glacial acetic acid (distilled over chromic anhydride) 1.5 cc. (11 mml.) of 30% hydrogen peroxide was added. After seven days the acetic acid was removed *in vacuo*. The crystalline residue (0.77 g.) was twice recrystallized from 10 cc. of hot alcohol; trilateral prisms, m. p. 190° (dec.); $[\alpha]^{25}_D$ −90.8° in water.

*Anal.* Calcd. for $C_6H_9O_5NS$: C, 34.76; H, 4.38; N, 6.76; neut. eq., 207. Found: C, 35.10; H, 4.48; N, 6.70; neut. eq., 205.

The compound liberated practically no iodine from acidified potassium iodide solution.

**Thiazolidine.**—Twelve grams of phthalimidoethyl mercaptan[19] was hydrolyzed by boiling for twelve hours under reflux with a mixture of 120 cc. of 20% hydrochloric acid

(18) Simonsen, *J. Biol. Chem.*, **101**, 35 (1933); Shinohara, *ibid.*, **96**, 285 (1932).

(19) Gabriel, *Ber.*, **24**, 1110 (1891).

and 20 cc. of glacial acetic acid. After cooling, phthalic acid was filtered off, and the filtrate was concentrated *in vacuo*, filtered again, and finally taken to dryness in a vacuum desiccator over potassium hydroxide. The crude aminoethyl mercaptan hydrochloride[20] was dissolved in 20 cc. of water, treated with 4.6 cc. of formalin, allowed to stand overnight, and taken to dryness *in vacuo*. The residue was crystallized from alcohol; yield, 6.63 g. (91%) of long needles; m. p. 180° (dec.).

*Anal.* Calcd. for $C_3H_7NS \cdot HCl$: C, 28.66; H, 6.42; S, 25.53; N, 11.15; Cl, 28.24. Found: C, 28.86; H, 6.76; S, 25.77; N, 10.88; Cl, 28.1.

The free base, liberated from the above hydrochloride by potassium carbonate, is readily volatile with steam, miscible in all proportions with water, and can be salted out readily with potassium carbonate. It is a colorless liquid, b. p. 164–165°, $d^{25}_4$ 1.131, $n^{30}D$ 1.551.

*Anal.* Calcd. for $C_3H_7NS$: N, 15.72; C, 40.39; H, 7.92. Found: N, 15.77; C, 40.40; H, 7.80.

**Acetylthiazolidine.**—An excess (2.5 cc.) of acetic anhydride was added to 1.51 g. of the free base; the acetic acid was removed *in vacuo*, and the residual liquid distilled under reduced pressure; b. p. 83–85° (0.7 mm.); yield 1.96 g. (88%).

*Anal.* Calcd. for $C_5H_9ONS$: C, 45.76; H, 6.91; N, 10.68. Found: C, 44.66; H, 6.74; N, 10.24.

Sulfone of **Acetylthiazolidine.**—To a solution of 0.712 g. of acetylthiazolidine in 10 cc. of glacial acetic acid, 1.5 cc. (2.3 mole) of hydrogen peroxide was added. After a week the solvent was removed *in vacuo* and the residue recrystallized from hot alcohol; stout hexagonal prisms or long plates, m. p. 122°.

*Anal.* Calcd. for $C_5H_9O_3NS$: C, 36.78; H, 5.56; N, 8.58. Found: C, 36.72; H, 5.33; N, 8.65.

**Oxidation of Thiazolidine with Bromine.**—*N* Bromine in acetic acid was added to 0.354 g. (4 mml.) of thiazolidine in 5 cc. of water to a faint permanent yellow (5.8 equivalents). The solution was taken to dryness *in vacuo*, and the residue recrystallized by adding alcohol to a concentrated solution in water. The product, taurine, formed fine needles, was neutral in reaction and did not melt below 260°; yield, 0.430 g. (86%).

*Anal.* Calcd. for $C_2H_7O_3NS$: N, 11.19. Found: N, 10.95.

**Oxidation of Thiazolidine with Iodine.**—To 0.3358 g. of free thiazolidine (3.77 mml.) in 5 cc. of water was added 37.7 cc. of 0.1 *N* iodine in 2.5% potassium iodide. A white solid appeared which redissolved slowly. After twenty hours the colorless solution was filtered to remove a small red precipitate, and 3.77 cc. of *N* sodium hydroxide was added. A colorless solid which precipitated was filtered off. Formaldehyde was identified in the filtrate. The colorless precipitate, apparently an aldehyde–ammonia, was dissolved in excess hydrochloric acid, when a strong odor of formaldehyde was evolved. The solution was taken to dryness *in vacuo* and the residue recrystallized from ethyl alcohol. The resulting needles, 0.250 g., m. p. 206°, were identified as di-β-aminoethyl disulfide hydrochloride, described by Gabriel.[21]

*Anal.* Calcd. for $C_4H_{12}N_2S_2 \cdot 2HCl$: N, 12.43. Found: N, 12.33.

## Summary

Formaldehyde reacts with cysteine over a wide range of *p*H to form thiazolidine-4-carboxylic acid, the mode of formation, constitution and properties of which are here discussed.

Thiazolidine, similarly prepared from formaldehyde and β-aminoethyl mercaptan, is also described.

(20) Gabriel, *Ber.*, **22**, 1137 (1889).

(21) Coblenz and Gabriel, *ibid.*, **24**, 1122 (1891).

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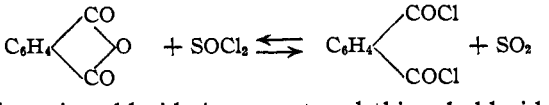
[CONTRIBUTION FROM THE RESEARCH LABORATORIES, MONSANTO CHEMICAL CO.]

# Phthalyl Chloride

BY L. P. KYRIDES

Phthalyl chloride has been prepared by the interaction of phthalic anhydride and phosphorus pentachloride.[1] Thionyl chloride converts phthalic acid into the anhydride, but is reported to have no action on the latter at refluxing temperatures even in presence of catalysts or pyridine.[2] We found that excellent yields of phthalyl chloride are readily obtained if the reaction is carried out at elevated temperatures in presence of very small amounts of anhydrous zinc chloride.[3] We also observed that the reaction is reversible, since thionyl chloride is obtained when sulfur dioxide and phthalyl chloride react at around 200°, also in presence of zinc chloride. The reaction is, therefore, expressed as



If no zinc chloride is present and thionyl chloride

(1) Bruehl, *Ann.*, **235**, 13 (1886).

(2) Meyer, *Monatsh.*, **22**, 437 (1901); McMaster and Ahmann, THIS JOURNAL, **50**, 145 (1928); Carré and Libermann, *Compt. rend.*, **199**, 1422 (1934).

(3) U. S. Patent 1,951,364.