The monopicrate melted at 176–178° (ethanol).

Anal. Calcd. for $C_{19}H_{21}N_5O_7$: N, 16.2. Found: N, 15.9.

p-Nitrobenzamide prepared by refluxing with *p*-nitrobenzoyl chloride in benzene was isolated as the *p*-nitrobenzamide hydrochloride, m.p. 188–189° (isopropyl alcohol–isopropyl ether).

Anal. Calcd. for $C_{20}H_{22}ClN_3O_3$: C, 61.9; H, 5.7; N, 10.8. Found: C, 61.9; H, 5.6; N, 10.6.

Method B: Sodium hydroxide (4.0 g., 0.1 mole) was dissolved in 25 ml. of water and added to 25 ml. of acetonitrile, and a solution of 9.3 g. (0.03 mole) of 2-amino-1-(2-chloro-2-phenyl)ethylpiperidine hydrochloride in 50 ml. of 50% aqueous acetonitrile was admitted over 1 hr. Stirring was continued for a total of 15 hr. after addition was complete. After addition of 50 ml. of water, the acetonitrile was removed and the residue extracted with five 20-ml. portions of ether. After drying and removal of solvent, the residue distilled to give 1.5 g. (25%) of product, b.p. 113–116° (0.2 mm.).

1-(2-Hydroxy-2-phenylethyl)-4-imino-1,4-dihydropyridine. A solution of 28.5 g. (0.3 mole) of 4-aminopyridine and 24.0 g. (0.2 mole) of styrene oxide in 110 ml. of ethanol was heated under reflux for 7.5 hr. When cool, the product of 20.0 g. (47%) was separated, m.p. 218–222°; recrystallized m.p. 232–234° (isopropyl ether–methanol). It was not obtained analytically pure.

The picrate melted at 150–152° (ethanol).

Anal. Calcd. for $C_{19}H_{17}N_5O_8$: C, 51.5; H, 3.9; N, 15.8. Found: C, 51.4; H, 4.0; N, 15.7.

In a similar reaction with 3-aminopyridine, 62% recovery of the reactant pyridine resulted and some intractable tar.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OKLAHOMA STATE UNIVERSITY]

2-Methyl-2-thiazoline-4-carboxylic Acid: Formation from *N*-Acetylcysteine and Hydrolysis

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Methyl 2-methyl-2-thiazoline-4-carboxylate has been synthesized and hydrolyzed to the corresponding acid. In mineral acid solutions this acid has a characteristic absorption spectrum with a maximum at 261 mμ. In strongly acid solution, *e.g.*, 7*M* hydrochloric acid, the carboxythiazoline is hydrolyzed very slowly, but the velocity of reaction increases with decreasing acid concentration to a maximum at about *p*H 1.7; the products are a mixture of *N*-acetyl- and *S*-acetylcysteine, as well as cysteine and acetic acid. At acid concentrations below 0.2*M* the last products are formed slowly, and a pseudo-equilibrium can be established between thiazoline, *N*-, and *S*-acetylcysteine. *N*-Acetylcysteine in 10*M* sulfuric acid is completely converted to 2-methyl-2-thiazoline-4-carboxylic acid. The amounts and the rates of formation of thiazoline in more dilute solutions of sulfuric or other strong acids have been determined.

The suggestion of Linderstrøm-Lang and Jacobsen[2] that the mercapto groups of proteins may become involved in thiazoline-ring formation has recently been receiving renewed attention, and evidence for the formation of thiazoline derivatives has been obtained in the case of glutathione,[3–6] 2-acetamidoethanethiol,[7] and *N*-formylcysteine.[8] Only in the last case, however, has the thiazoline derivative been isolated.

This paper reports the interconversion of *N*-acetylcysteine and 2-methyl-2-thiazoline-4-carboxylic acid (MTC). The methyl ester of this acid has been synthesized and converted to the acid by hydrolysis; the thiazoline derivatives exhibit a characteristic absorption maximum at 261 mμ by means of which their further reaction can be conveniently studied. The hydrolysis of 2-methyl-2-thiazoline-4-carboxylic acid in various acid concentrations has been investigated. *N*-Acetylcysteine, *S*-acetylcysteine, and the products of complete hydrolysis, acetic acid and cysteine, are formed.

Methyl 2-methyl-2-thiazoline-4-carboxylate hydrochloride was prepared by condensing cysteine methyl ester hydrochloride with ethyl acetimidate hydrochloride, a reaction previously employed to make other thiazoline derivatives.[9] Potentiometric titration of the ester with alkali showed the presence of one group, *p*K 3.05. The spectrum in acid solution, *e.g.*, 1*M* hydrochloric acid, showed a maximum at 261 mμ with a molar absorbancy coefficient of 5500.

Treatment of the ester with 0.1*M* sodium hydroxide for one hour at 40–50° resulted in a product

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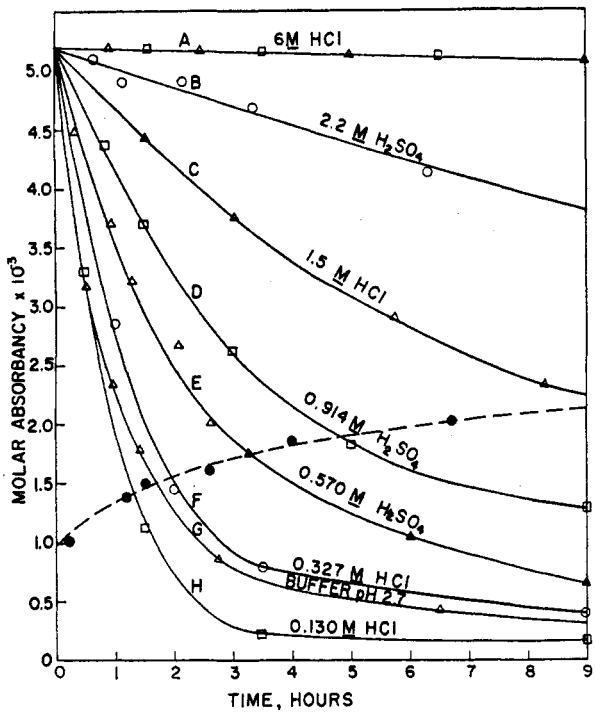

Fig. 1. Solid lines: Molar absorbancies at 261 mμ for MTC, *ca.* $10^{-4}M$. Curve A (triangles), MTC formed by acidic hydrolysis of its methyl ester; Curves A (squares), C, F, and H, MTC formed by basic hydrolysis of its methyl ester; Curves B, D, E, and G, MTC formed by cyclization of *N*-acetylcysteine in strongly acid media. Stippled line: Molar absorbancy at 228 mμ corresponding to Curve D

which still had the characteristic thiazoline absorption (maximum at 261 mμ, molar absorbancy coefficient 5200) but showed two titratable groups, *p*K 2.20 and 4.95. The hydrolysis product must be the corresponding acid, 2-methyl-2-thiazoline-4-carboxylic acid, which in acid solution exists as the thiazolinium cation, $MTCH^+$. The same product could be obtained by hydrolysis with 7*M* hydrochloric acid for ten minutes. When the ester was dissolved in the acid, the molar absorbancy at 261 mμ decreased from 5500 to 5200 within the time interval indicated, and thereafter decreased at a much slower rate. The thiazolinium cation is therefore comparatively stable in this medium.

In more dilute acid solutions, however, the absorption at 261 mμ waned more rapidly with time, as is illustrated for representative cases in Fig. 1. The maximum rate was observed at about *p*H 1.7.

Solutions of *N*-acetylcysteine in various concentrations of acid developed an absorption with a maximum at 261 mμ, identical with that of the thiazolinium cation. Furthermore, dilution of the solutions in which the absorption had developed resulted in diminution of the absorption with time, as observed in dilute acid solutions of 2-methyl-2-thiazoline-4-carboxylic acid. In Fig. 1 the data for these diluted solutions are interspersed with the data obtained for the thiazolinium cation.

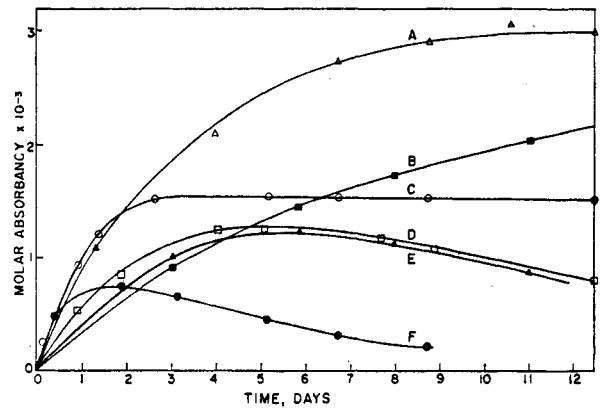

Fig. 2. Molar absorbancies at 261 mμ for *N*-acetylcysteine, *ca.* $10^{-4}M$: Curve A, in 5.53*M* H_2SO_4; B, 6.1*M* $HClO_4$; C, 11.4*M* HCl; D, 5.46*M* HCl; E, 3.8*M* $HClO_4$; F. 2.19*M* HCl

Clearly, cyclization of *N*-acetylcysteine takes place in strongly acid solutions. In 10*M* sulfuric acid the absorption increased to a maximum, reached in about 40 days, which corresponded to a molar absorbancy coefficient of 5150. Essentially complete conversion to thiazoline therefore occurs in this case.

Fig. 2 represents the results obtained in various other solutions of strong acids. In these cases, two consecutive reactions are taking place at comparable velocities, and the thiazoline is the intermediate product; consequently, its concentration rises to a maximum and then wanes. In concentrated acid, *e.g.*, 11.4*M* hydrochloric acid, thiazoline-ring formation is comparatively fast and hydrolysis slow, and hence a steady-state concentration is established, which remains virtually unchanged over a period of several days. At lower concentrations of hydrochloric acid, thiazoline formation is slowed and hydrolysis hastened; consequently, the maximum concentration of thiazoline attained is lower, and its gradual disappearance begins sooner.

When the hydrolysis of 2-methyl-2-thiazoline-4-carboxylic acid was effected at concentrations of hydrochloric acid between 2.23 and 0.043*M*, it was noted that an absorption peak at 228 mμ developed as the peak at 261 mμ disappeared (after making allowance for the absorbance of the thiazolinium cation at 228 mμ). Thiol esters are known to have absorption maxima at 228–231 mμ,[7,10,11] and it is therefore postulated that *S*-acetylcysteine is formed in these conditions. At acid concentrations above 0.5*M* the 228 mμ peak first grew and then waned to a negligible value; the maximal absorption attained was greater the lower the acid concentration. It may be deduced that *S*-acetylcysteine is an unstable intermediate, and, since thiolesters are susceptible to acid-

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

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catalyzed hydrolysis, that the thiolester is hydrolyzed to acetic acid and cysteine. The formation of cysteine was confirmed by application of the Sullivan reaction, which gives no test with either 2-methyl-2-thiazoline-4-carboxylic acid or *N*-acetylcysteine. In certain conditions, nearly complete conversion to cysteine (or cystine that might be formed by oxidation) could be demonstrated after sufficient intervals of time.

However, cysteine and acetic acid are not the only products of the hydrolysis. This is clearly shown by what happened when 2-methyl-2-thiazoline-4-carboxylic acid solutions, hydrolyzed in 1*M* hydrochloric acid until the 261 mμ peak had disappeared and the 228 mμ peak was at some intermediate stage of development, were made 6*M* in hydrochloric acid. The 228 mμ peak disappeared almost immediately, but the 261 mμ peak began to grow and finally attained about one fourth of the initial value. This indicates that *N*-acetylcysteine was one of the products of the hydrolysis.

Fig. 3 pictures the results obtained in a representative case, the hydrolysis of 2-methyl-2-thiazoline-4-carboxylic acid in 1*M* sulfuric acid. The

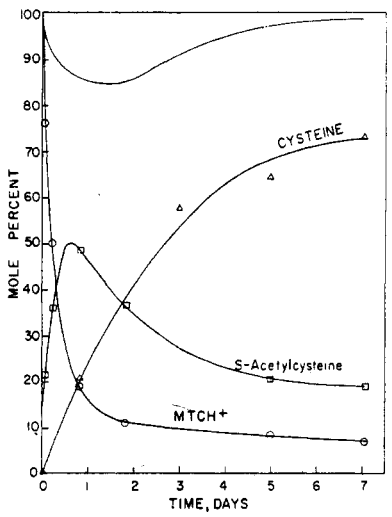

Fig. 3. Hydrolysis of 2-methyl-2-thiazoline-4-carboxylic acid in 1*M* sulfuric acid

amount of *S*-acetylcysteine was estimated assuming a molar absorbancy coefficient of 4350 (the same as that of 2-acetamidoethanethiol[7]; *N*,*S*-diacetylcysteine has a coefficient of 4300). The absolute values of the concentration may be somewhat uncertain, but the rapid accumulation and gradual disappearance of thiol ester can be clearly seen. The amount of *N*-acetylcysteine corresponds to the difference between the uppermost curve (which is the sum of the three lower ones) and 100%. Relatively more thiol ester was formed at higher acid concentrations, the initial ratio of *S*- to *N*-acetylcysteine being about 3:1

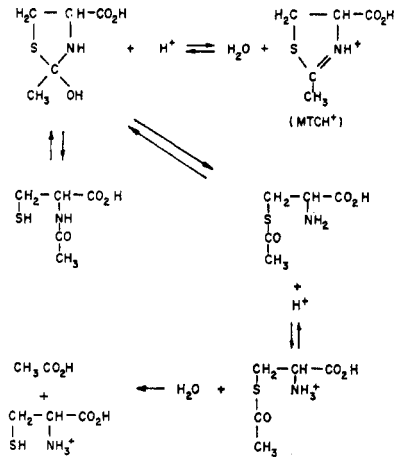

Figure 4

in 2.23*M* hydrochloric acid, and 1:2 in 0.043*M* acid.

At acid concentrations below 0.2*M* the absorptions at 261 and 228 mμ did not vanish, but attained values which then remained sensibly constant for long periods of time. In these conditions, the rate of the irreversible conversion to cysteine and acetic acid is very small, so that an equilibrium can be established between *N*-acetylcysteine, *S*-acetylcysteine, and the thiazolinium cation. Approximate equilibrium constants were calculated on the assumption that only these three substances were present:

$$K_1 = (\text{MTCH}^+)(H_2O)/(N\text{-acetylcysteine})(H^+) = 0.12 \pm .007$$

$$K_2 = (S\text{-acetylcysteine cation})/(N\text{-acetylcysteine})(H^+) = 0.61 \pm .04 \text{ molar}^{-1}$$

These results are qualitatively similar to those obtained by Edsall, *et al.*,[7] for 2-methyl-2-thiazoliline and 2-acetamidoethanethiol. Therefore, we postulate an analogous reaction scheme, shown in Fig. 4. However, the chemical characteristics of the present system differ quantitatively from the substances just mentioned, and from glutathione.

The equilibrium corresponding to K_1 is about 2.3 for 2-acetamidoethanethiol[7] and 3×10^{-3} for glutathione[5]; *N*-acetylcysteine is intermediate. When glutathione is dissolved in solutions of various acids, the rate of formation of the thiazoline derivative is faster, so that equilibrium can be established. In the case of 2-methyl-2-thiazoline-4-carboxylic acid, the rate of hydrolysis to other products is sufficiently fast with respect to that of its formation from *N*-acetylcysteine that a corresponding equilibrium cannot be established.

EXPERIMENTAL

Reagents. Triethylamine (Matheson, Coleman, and Bell) and methylene dichloride (Brothers Chemical Co.) were dried over calcium chloride and redistilled. 2-Methyl-2-thiazoline was obtained from K and K Laboratories, Long Island City, N. Y., and was also prepared according to Kuhn

and Drawert.[12] Sodium 1,2-naphthoquinone-4-sulfonate for use in the Sullivan procedure was an Eastman Organic Chemicals "White-label" product. All other reagents were of analytical reagent grade.

N-Acetylcysteine. Twenty grams of cystine was dissolved in 200 ml. of water containing 12 g. of sodium hydroxide, and 40 ml. of acetic anhydride was added dropwise with stirring and cooling in an ice bath over a period of 30 min. The solution was allowed to stand at room temperature 1 hr., then heated to 55° and zinc dust added. The solution was stirred for 15 min., cooled, and centrifuged to remove unchanged zinc. To a portion of the centrifugate 1*M* lead acetate was added. In some cases the lead mercaptide of *N*-acetylcysteine appeared as a yellow precipitate; in other cases, a yellow or yellow-green solution resulted, and precipitation of the mercaptide was accomplished by adding water. If a brown solution resulted, the remainder of the solution should be reduced further before adding lead acetate. The mercaptide was centrifuged, washed, and decomposed with hydrogen sulfide as described by Pirie and Hele.[13] The lead sulfide was removed by filtration and the filtrate was lyophillized; yield, 13 g. (48%). The crude product was purified by dissolving in hot isopropyl alcohol, adding dry ether, removing the white precipitate that formed by filtration, and evaporating the filtrate to dryness. After three such treatments a product was obtained, m.p. 107–107.5°, which did not depress the melting point of an authentic sample. The white precipitate removed by dilution with ether was deliquescent and showed in acid solution the characteristic ultraviolet spectrum of thiazolines.

Anal. Neut. equiv.: Calcd. for $C_5H_9O_2NS$, 163. Found, 167. SH content 0.98 groups per molecule.

N,S-Diacetylcysteine. Ten grams of cysteine hydrochloride monohydrate (0.057 mole) was dissolved in 40 ml. of water containing 7.1 g. of sodium hydroxide (0.177 mole) in an ice bath, and 11.63 g. (0.114 mole) of acetic anhydride was added dropwise with stirring over a 10-min. period. The *p*H was adjusted to 2 with hydrochloric acid, and the solution was quickly frozen and lyophilized. The residue was extracted several times with warm, dry ethyl acetate and the extracts evaporated to dryness *in vacuo;* yield, 8 g. (68%). Recrystallizations from ethyl acetate gave a product m.p. 120°; absorbancy maximum was at 231 mμ, molar absorbancy coefficient 4300.[14] Hydrolysis rates in dilute acid solutions accorded with those reported by Noda, Kuby, and Lardy.[10]

Anal. Calcd. for $C_7H_{11}O_4NS$: S, 15.62; N, 6.83; neut. equiv. 205.2. Found: S, 15.74; N, 6.92; neut. equiv. 205.

Methyl 2-methyl-2-thiazoline-4-carboxylate (hydrochloride). Cysteine methyl ester hydrochloride,[15] 17.16 g. (0.100 mole), 12.36 g. of ethyl acetimidate hydrochloride[16] (0.100 mole), and 10.12 g. of dry triethylamine (0.100 mole) were placed in a 250-ml. stoppered flask containing 100 ml. of dry methylene dichloride and stirred magnetically overnight. Methylene dichloride was removed *in vacuo* and the residue extracted four times with 50-ml. portions of dry ether. The insoluble ammonium chloride and triethylammonium chloride were removed by filtration. Dry hydrogen chloride gas was passed for a short time into the combined ether extracts, and the white hydrochloride was filtered off. By passing in further small portions of hydrogen chloride gas, seven precipitate fractions were collected; total yield 17.5 g. (90%). Fraction 6 melted at 143–144° dec. The product was soluble in water, alcohols, and chloroform; slightly soluble in acetone and *p*-dioxane. Recrystallization from several solvents was effected, but in every case the melting point was lowered.

Anal. Calcd. for $C_6H_{10}O_2NSCl$: S, 16.39; N, 7.16; neut. equiv., 196. Found for fraction 6: S, 16.35; N, 7.34; neut. equiv., 198.

Analysis for cysteine-cystine. Aliquot portions of acid solutions containing about 0.5 mg. cysteine-cystine were quickly neutralized with the calculated quantity of sodium hydroxide solution, with cooling if necessary. Five ml. of 5% sodium cyanide in 0.5*M* sodium hydroxide was added and the solution allowed to stand for 10 min. The procedure of Sullivan and Hess[17] for developing the color was followed. The solutions were then diluted to a known volume, and the absorbancy read at 500 mμ. Cysteine hydrochloride monohydrate and cystine were used as standards. A precision of about 10% was obtained.

Apparatus and methods. Measurements of *p*H and potentiometric titrations were done with a Beckman model G *p*H meter using a glass electrode.

Spectra were determined at room temperature in 1-cm. silica cells. A Beckman DK-1 spectrophotometer was used for scanning spectra, and a Beckman DU spectrophotometer for determining absorbance at a fixed wave length.

The solutions under study were maintained at 25°, except for short periods of time. For the hydrolysis studies of the thiazolinium cation, concentrated solutions of the cation were made up in concentrated acid and diluted with water to the desired acid concentration. External cooling was employed to maintain the temperature during dilution. For rate studies, fresh samples were withdrawn from glass-stoppered flasks for each reading.

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