priately re-extracted. The combined organic layers were dried over sodium sulfate and evaporated to yield 0.19 g. of reduction product having an infrared spectrum similar to the corresponding reduction product of methyl ricinoleate; gas chromatographic analysis indicated 64.3% 9,15-octadecadiene-1-ol (X) accompanied by unidentified products.

Permanganate–Periodate Oxidation of X.—The oxidation of X was carried out essentially as described for oleyl alcohol. Gas chromatographic analyses of the isolated cleavage products were carried out both as free acids and as methyl esters (prepared by esterifying the acids with 1% methanolic sulfuric acid). Propionic acid (XI) was the only short-chain acid ($< C_9$) detected in appreciable amount among the free acids. The methyl esters of adipic acid (XII; 35–37%) and 9-hydroxynonanoic acid (XIII; 55–58%) were the main constituents detected in the ester preparation; the balance was comprised by trace amounts of various unidentified components.

Acknowledgment.—The authors thank K. L. Mikolajczak, J. W. Hagemann, and Karen O'Connor for gas chromatographic analyses; M. O. Bagby and R. O. Butterfield for assistance with countercurrent distribution; Mrs. Clara McGrew for microanalyses; D. H. Johnson, Department of Chemistry, University of Illinois, Urbana, Illinois, for n.m.r. spectra; and Dr. Quentin Jones, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, for making *Lesquerella densipila* seed available to us.

Zwitterion Structure and Acylative Ring-Opening Reactions of 2-Aminothiazoline-4-carboxylic Acid

OSCAR GAWRON, JOSEPH FERNANDO, JOHN KEIL, AND T. J. WEISMANN

Department of Chemistry, Duquesne University, and Gulf Research & Development Company, Pittsburgh 19, Pennsylvania

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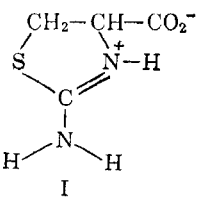
pK_A values (pK_1 2.03, pK_2 8.48), variation of optical rotation with pH and other physical properties indicate that 2-aminothiazoline-4-carboxylic acid is a zwitterion in solution and in the solid state and that pK_2 can be ascribed to ionization from the ring nitrogen. Acylation of 2-aminothiazoline-4-carboxylic acid in aqueous medium is accompanied by ring opening. With acetic anhydride N′,S-diacetyl-N-carbamylcysteine is obtained and with benzoyl chloride S-benzoyl-N-carbamylcysteine and 2-imino-3-benzoylthiazolidine-4-carboxylic acid were obtained. Structures of the acylated products were proven by chemical methods and by interpretation of n.m.r. data.

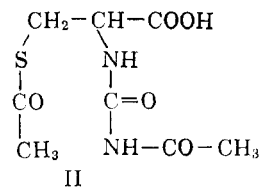
In connection with previous work on the kinetics of the cystine–cyanide reaction[1] and on the anti-acetylcholinesterase activity of thiazoline derivatives,[2] the zwitterion structure and acylative ring opening reactions of 2-aminothiazoline-4-carboxylic acid[3,4] have been investigated.

2-Aminothiazoline-4-carboxylic acid is a product of the reaction of cyanide with cystine[3,4] and is also a naturally occurring material.[5] As obtained from the reaction of cyanide with L-cystine, 2-aminothiazoline-4-carboxylic acid decomposes at 232–234° and the high decomposition temperature plus the presence of a basic ring system and a carboxyl group alpha to a ring nitrogen is indicative of a zwitterion structure. Evidence from titration studies, infrared spectra, optical rotation as a function of pH and n.m.r. studies corroborates a zwitterion structure which may be written as I.

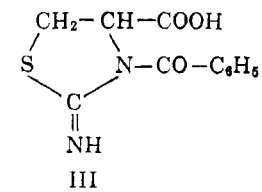


I

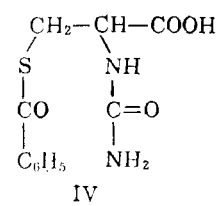
On acylation with acetic anhydride in aqueous medium, 2-aminothiazoline-4-carboxylic acid yields N′,S-diacetyl-N-carbamylcysteine (II) and on acylation with benzoyl chloride in aqueous medium, 3-benzoyl-2-iminothiazolidine-4-carboxylic acid (III) and S-benzoyl-N-carbamylcysteine (IV) are obtained. Acetylation of 2-aminothiazoline-4-carboxylic acid with acetic anhydride in aqueous solution has previously been reported[5,6] to yield 3-acetyl-2-iminothiazolidine-4-carboxylic acid, m.p. 179–180°. In our work the only compound that could be isolated from the aqueous acetylation was the diacetyl derivative II. Since this compound



II



III



IV

(1) O. Gawron and J. Fernando, *J. Am. Chem. Soc.*, **83**, 2906 (1961).
(2) O. Gawron and J. Keil, *Arch. Biochem. Biophys.*, **89**, 293 (1960).
(3) A. Schoberl and R. Hamm, *Ber.*, **81**, 210 (1948).
(4) A. Schoberl, M. Kawohl, and R. Hamm, *ibid.*, **84**, 571 (1951).
(5) J. L. Wood and S. L. Cooley, *J. Biol. Chem.*, **218**, 449 (1956).
(6) J. L. Wood, personal communication.

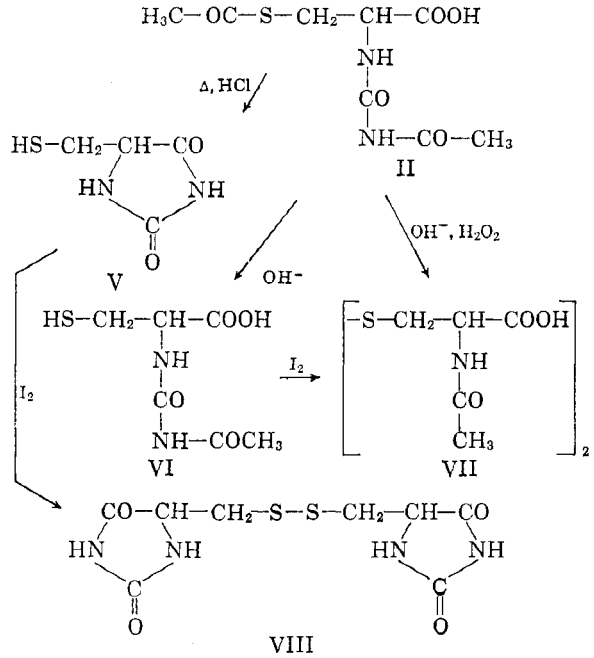


Fig. 1.—Reactions leading to assignment of structure to N′,S-diacetyl-N-carbamylcysteine.

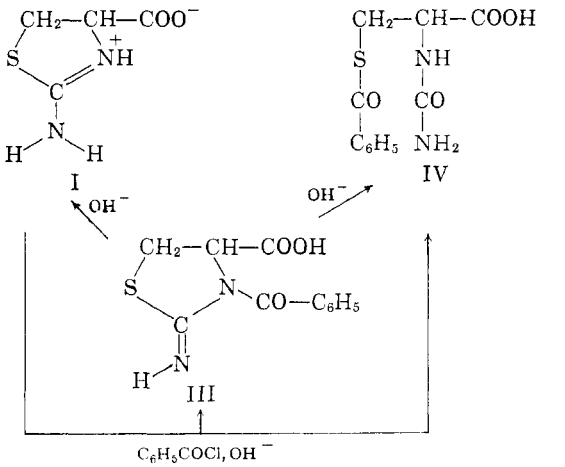


Fig. 2.—Alkaline conversion of 2-imino-3-benzoylthiazolidine-4-carboxylic acid to 2-aminothiazoline-4-carboxylic acid and S-benzoyl-N-carbamylcysteine.

(II) also melts at 179–180°, it is presumably identical with that obtained by Cooley and Wood[5,6] and the assignment of structure by these workers is problematical.[7] Compound II was identified by us as N′,S-diacetyl-N-carbamylcysteine by elemental analysis, mild alkaline saponification to a monoacetyl derivative (Fig. 1, VI) containing a free thiol group, conversion to cysteine hydantoin (Fig. 1, V) by simultaneous deacetylation and cyclization with hot hydrochloric acid and by n.m.r. assignment of the acetyl group attached to nitrogen to the carbamyl nitrogen atom.

The benzoylation product III was identified as 3-benzoyl-2-iminothiazolidine-4-carboxylic acid by elemental analysis, by conversion with dilute alkali to 2-aminothiazoline-4-carboxylic acid (Fig. 2, I) and by assignment of the benzoyl group to the ring nitrogen on the basis of chemical evidence. The other benzoylation product, S-benzoyl-N-carbamylcysteine (Fig. 2, IV) was identified by elemental analysis, by its chemical properties and by the fact it could be obtained from 3-benzoyl-2-iminothiazolidine-4-carboxylic acid (III) by treatment with dilute alkali.

Experimental

Titration Data.—A solution of 108.3 mg. (0.742 mmole) of 2-aminothiazoline-4-carboxylic acid in 25 ml. of carbon dioxide-free water and containing the equivalent amount of hydrochloric acid was titrated with standard 0.1 *N* sodium hydroxide. The titration was performed under nitrogen at 25° utilizing the Beckman Model G pH meter and appropriate glass and calomel electrodes. pK_2, 8.48, was obtained directly from the titration curve and pK_1, 2.03, was obtained from a plot of H^+ bound per millimole *vs.* pH.

Optical Rotation Studies.—Optical rotations of 1% solutions of varying pH of 2-aminothiazoline-4-carboxylic acid were measured at 25° in a conventional polarimeter. Adjustment of pH to a desired value was carried out with either hydrochloric acid or sodium hydroxide.

2-Aminothiazoline-4-carboxylic Acid.—The preparation of this compound was carried out by published procedures[3,5] and the product, decomposing at 232–234° (after some discoloration at 212–214°) and with $[\alpha]^{25}D$ −118°, has been described.[3] Both the decomposition point and the specific rotation are higher than that previously reported.[5]

Acetylation of 2-Aminothiazoline-4-carboxylic Acid.—Attempts to utilize the acetylation procedure of du Vigneaud and Irish[8] as employed by Cooley and Wood[5] failed to give any acetylation product and, accordingly, acetylation was carried out by a modified procedure. To an ice-cooled, stirred solution of 7.3 g. (50 mmoles) of 2-aminothiazoline-4-carboxylic acid in 50 ml. of 1 *N* sodium hydroxide, 9.6 ml. (100 mmoles) of acetic anhydride was added in one portion. Cooling and stirring were continued for 2 hr. and during this period unchanged 2-aminothiazoline-4-carboxylic acid separated from solution. After removal of unchanged starting material by filtration, the filtrate was concentrated *in vacuo* to approximately one fifth its volume and then 50 ml. of 1 *N* sulfuric acid was added. The white, crystalline substance which precipitated on acidification was removed by extraction with three 25-ml. portions of ethyl acetate. The combined ethyl acetate extracts were dried with anhydrous sodium sulfate and concentrated to a small volume. On cooling, the acetylation product crystallized out of solution. Recrystallization from water and drying over phosphorus pentoxide *in vacuo* gave 3.0 g. (25%) of material melting at 179–181°, subsequently demonstrated to be N′,S-diacetyl-N-carbamylcysteine (III).

Anal.[9] Calcd. for $C_8H_{12}N_2O_5S$: C, 38.71; H, 4.83; N, 11.29; S, 12.90. Found: C, 38.81; H, 4.90; N, 11.42; S, 12.68. Calcd. for $C_6H_8N_2O_3S$ (3-acetyl-2-iminothiazolidine-4-carboxylic acid): C, 38.29; H, 4.25; N, 14.89; S, 17.02.

Acetylations were also carried out with limited quantities of acetic anhydride, with excess acetic anhydride, with excess alkali and at room temperature. In each case the di-

(7) It is also of interest to note that the analytical carbon and hydrogen values obtained by C. W. Todd, J. H. Fletcher, and D. S. Tarbell, *J. Am. Chem. Soc.*, **65**, 350 (1943), for a presumed sulfoxide of an acetylated 2-aminothiazoline are in closer agreement with an S-acetyl-N-carbamylthioethanolamine structure for the product than the presumed sulfoxide and it is likely ring opening has occurred.

(8) V. du Vigneaud and O. J. Irish, *J. Biol. Chem.*, **122**, 349 (1938).

(9) Analyses performed by Dr. A. Bernhardt.

acetyl product was obtained as the sole product in 25–30% yields.[10]

N-(N′-Acetyl)carbamylcysteine (VI).—Five hundred and sixty milligrams (2.25 mmoles) of N′,S-diacetyl-N-carbamylcysteine was dissolved in 116 ml. of 0.077 *M* potassium hydroxide solution, and the solution under nitrogen was kept at 35° for 2 hr. At the end of this period 92.8 ml. of 0.097 *M* hydrochloric acid solution was added and the reaction mixture was then concentrated *in vacuo* to dryness. The residue was extracted with alcohol and the solid obtained on concentration of the extract *in vacuo* to dryness was recrystallized from water to give N-(N′-acetyl)carbamylcysteine (VI) melting at 212–213°.

Anal. Calcd. for $C_6H_{10}N_2O_4S$: C, 34.95; H, 4.85; N, 13.59; S, 15.53; neut. equiv., 206; —SH, 1.00. Found: C, 34.84; H, 4.59; N, 13.44; S, 15.31; neut. equiv., 205; —SH,[11] 0.99.

Bis-N-(N′-acetyl)carbamylcysteine (VII).—This compound was obtained by carrying out the above hydrolysis in peroxide-containing dioxane and by the iodine oxidation of N-(N′-acetyl)carbamylcysteine (VI) in aqueous solution. For the iodine oxidation, 50 mg. of the thiol (VI) was dissolved in water and 0.1 *N* iodine solution was added dropwise until the yellow color persisted. The disulfide precipitated during the course of the oxidation and after filtering and washing with water was recrystallized from hot water to give pure disulfide melting at 205–206°.

Anal. Calcd. for $C_{12}H_{18}N_4O_8S_2$: C, 35.12; H, 4.39; N, 13.65; S, 15.60. Found: C, 35.14; H, 4.54; N, 13.51; S, 15.58.

Cysteine Hydantoin (V) from N′,S-Diacetyl-N-carbamylcysteine (II).—Two hundred milligrams of the diacetyl compound (II) was refluxed for 30 min. with 10 ml. of 2 *N* hydrochloric acid. The solvent was then removed *in vacuo*, and the residue was recrystallized from ethanol to give cysteine hydantoin, melting at 143–144° and identified as such by mixed melting point determination and by comparison of its infrared spectrum with authentic cysteine hydantoin prepared according to the procedure of Karabinos and Szabo.[13] The cysteine hydantoin was also oxidized with aqueous iodine to cystine hydantoin (VIII), decomposing at 310° and identical with authentic cystine hydantoin.[14]

Benzoylation of 2-Aminothiazoline-4-carboxylic Acid.—To a solution of 3.0 g. (20 mmoles) of 2-aminothiazoline-4-carboxylic acid in 30 ml. of 2 *N* sodium hydroxide solution, 4.8 ml. (40 mmoles) of freshly distilled benzoyl chloride was added in one portion and the mixture was vigorously shaken. After shaking for 0.5 hr., 15 ml. of 2 *N* sodium hydroxide solution was added, and shaking was continued until all of the benzoyl chloride had disappeared. Acidification with hydrochloric acid yielded a white solid which was removed by filtration and from which the reaction products were isolated as follows:

S-Benzoyl-N-carbamylcysteine (IV).—The white solid obtained above was extracted with hot water, and the aqueous extract was concentrated *in vacuo* to give a solid crystalline mixture of benzoic acid and the product. The benzoic acid was removed by ether extraction and the product was recrystallized from 1:1 ethanol–water to give S-benzoyl-N-carbamylcysteine, m.p. 210–211°. The compound gave a negative thiol test with the phosphotungstate reagent in pH 5.0 acetate buffer but gave a positive thiol test with ammoniacal nitroprusside.

Anal. Calcd. for $C_{11}H_{12}N_2O_4S$: C, 49.25; H, 4.47; N, 10.47; S, 11.94; neut. equiv., 268. Found: C, 49.44; H, 4.64; N, 10.48; S, 11.69; neut. equiv., 267.4.

2-Imino-3-benzoylthiazolidine-4-carboxylic Acid (III).—After hot water removal of benzoic acid and S-benzoyl-N-carbamylcysteine from the mixed benzoylation product, the residue was dried *in vacuo* over phosphorus pentoxide and then recrystallized from 95% ethanol to give fine white flakes, m.p. 204–206°.

Anal. Calcd. for $C_{11}H_{10}N_2O_3S$: C, 52.59; H, 4.38; N, 11.15; S, 12.74; neut. equiv., 250. Found: C, 52.71; H, 4.11; N, 11.19; S, 12.62; neut. equiv., 250.

Yields under the above conditions of 2-imino-3-benzoylthiazolidine-4-carboxylic acid and of S-benzoyl-N-carbamylcysteine are 60–65% and 10–15%, respectively. When the amount of alkali used is increased to 50 mmoles the thiazolidine yield drops to 40–45% and the S-benzoyl derivative increases to 15–20%.

Alkaline Treatment of 2-Imino-3-benzoylthiazolidine-4-carbonyl Acid (III).—2-Aminothiazoline-4-carboxylic acid (I) and S-benzoyl-N-carbamylcysteine (IV) were obtained on treatment of 2-imino-3-benzoylthiazolidine-4-carboxylic acid with alkali. One hundred and forty milligrams of 2-imino-3-benzoylthiazolidine-4-carboxylic acid was dissolved in 5 ml. of 2 *N* potassium hydroxide solution, and the solution under nitrogen was incubated at 35° for 6 hr. At the end of the period the solution was acidified (pH 1) with hydrochloric acid and then evaporated *in vacuo* to a solid residue. The residue thus obtained was extracted with hot 95% ethanol and the alcohol extract in turn was concentrated *in vacuo* to an oily residue. From this residue 2-aminothiazoline-4-carboxylic acid (I) and S-benzoyl-N-carbamylcysteine (IV) were obtained in small amounts by fractional crystallization. The residue was treated with 1 ml. of water and the 2-aminothiazoline-4-carboxylic acid which crystallized was filtered off. The filtrate was then evaporated to 0.5 ml. and S-benzoyl-N-carbamylcysteine was obtained by crystallization. The 2-aminothiazoline-4-carboxylic acid was identified by a positive test with diazotized sulfanilic acid,[5] mixed melting point determination, and its infrared spectrum. The S-benzoyl-N-carbamylcysteine was identified by mixed melting point determination and by its infrared spectrum.

Nuclear Magnetic Resonance Spectra.—All the spectra studied here were obtained using a Varian Associates Model 4300B high resolution n.m.r. spectrometer operating at 40 Mc./sec. and equipped with a flux stabilizer. Calibrations were performed by the conventional sideband technique using a Hewlett-Packard oscillator and Hewlett-Packard Model 522-B frequency counter. Tetramethylsilane (TMS) was used as the internal standard in trifluoroacetic acid solutions. Chemical shifts from the TMS are reported on the Tiers[15] scale:

$$\tau \text{ (in p.p.m.)} = 10.000 - (J_{obs} - J_{Me_4Si})/J_0$$

Discussion

Zwitterion Structure of L-2-Aminothiazoline-4-carboxylic Acid (I).—The fact that an aqueous solution of 2-aminothiazoline-4-carboxylic acid is essentially neutral in reaction and the presence in the molecule of a carboxylic acid group, pK_1 2.03, and a basic group, pK_2 8.48, is proof of the zwitterion nature of the compound in solution. It is also, of course, definite from the high decomposition point and by analogy with the amino acids that the compound is a zwitterion in the solid state. While pK_1 is readily identifiable as that for the carboxyl

(10) Similar yields were reported by Wood, ref. 6.

(11) By amperometric titration with 0.002 *M* silver nitrate solution in pH 10 ammonia, ammonium buffer, the concentration of thiol being 10^{-4} *M*. Qualitatively, free thiol groups were identified by the blue color obtained with the Folin phosphotungstate reagent (ref. 12) in pH 5 acetate buffer and acylated thiol groups were identified by a negative test with the phosphotungstate reagent and a slowly developing positive test with nitroprusside in dilute ammonia.

(12) O. Folin and J. M. Looney, *J. Biol. Chem.*, **51**, 427 (1922).

(13) J. V. Karabinos and J. L. Szabo, *J. Am. Chem. Soc.*, **66**, 649 (1944).

(14) W. C. Hess, *ibid.*, **56**, 1421 (1934).

(15) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

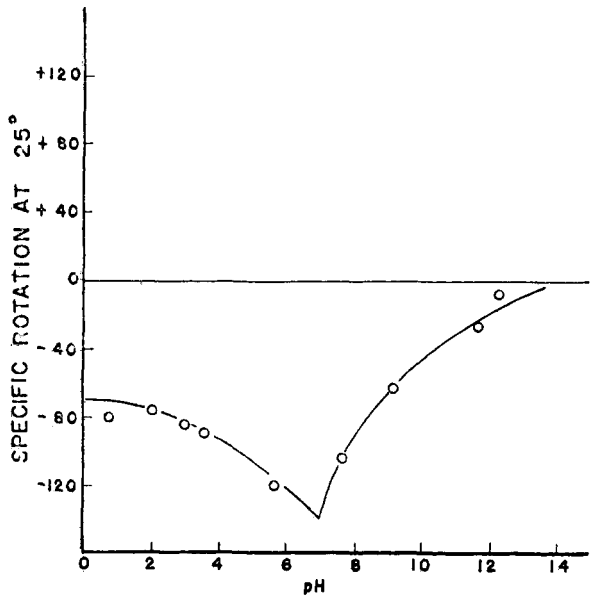


Fig. 3.—Specific rotation *vs.* pH for 2-aminothiazoline-4-carboxylic acid.

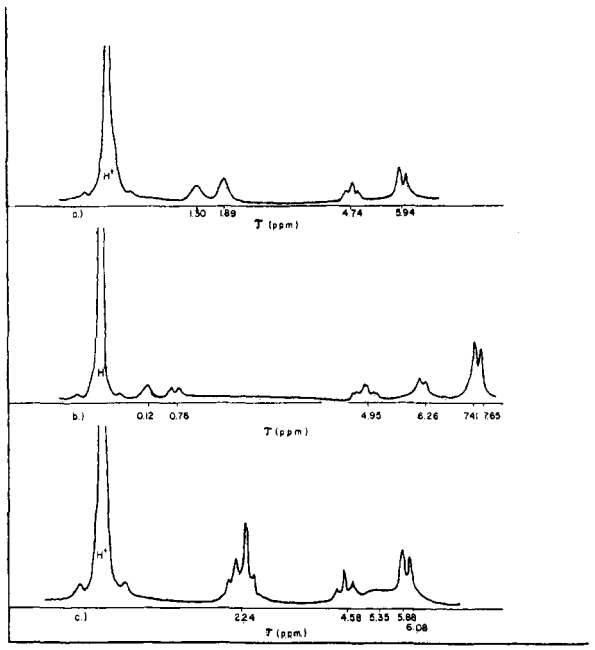
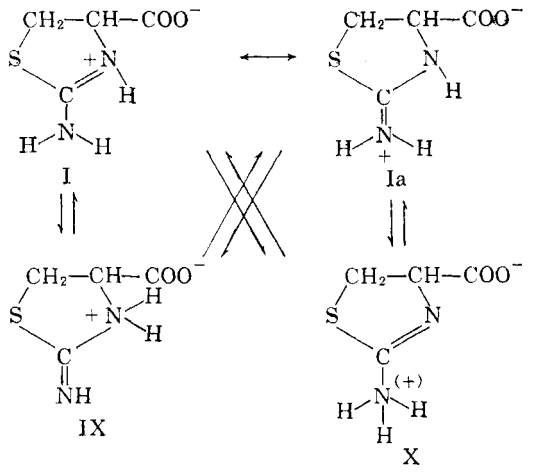


Fig. 4.—Proton magnetic resonance spectra in trifluoroacetic acid. (a) 2-Aminothiazoline-4-carboxylic acid. (b) N′,S-Diacetyl-N-carbamylcysteine. (c) 2-Imino-3-benzoylthiazolidine-4-carboxylic acid.

group and while pK_2 is to be ascribed to an ammonium group, its exact assignment is somewhat problematical by virtue of the possibility of ionization from several resonance forms (I, Ia) or from several tautomeric forms, IX and X.

From a comparison of the pK_A values of 2-aminothiazoline-4-carboxylic acid with pK_A values of related amino acids (Table I) the positively charged ammonium group is to be ascribed to the α-nitrogen atom since pK_1, 2.03, is close to those,



2.03 and 1.96, for the related amino acids, S-ethylcysteine and cysteine, respectively. A higher pK_1 would be expected if the ammonium group were on the exocyclic nitrogen atom as, for example, is the case with alanine and β-alanine, pK_1 2.34 and pK_1 3.60, respectively. It is also of interest to note that the basicity of 2-aminothiazoline, pK_A 8.70, is reduced by the presence of the 4-carboxylate ion, pK_2 of 2-aminothiazoline-4-carboxylic acid being 8.48. This reduction in basicity of ammonium groups by a negatively charged α-carboxyl group is, of course, common to the α-amino acids and can be seen from a comparison of the pK_2 values, 9.69 and 10.19, respectively, of alanine and β-alanine. Assignment of the positively charged ammonium group to the α-nitrogen is also corroborated by the optical rotation of the compound as a function of pH (Fig. 3). Both the acid limb and the alkaline limb of the plot are typical of those for L-α-amino acids.[16] It would thus appear that 2-aminothiazoline-4-carboxylic acid is best represented by structure I or by the tautomeric thiazolidine structure IX. This structure assignment is confirmed by the proton magnetic resonance spectrum of 2-aminothiazoline-4-carboxylic acid in trifluoroacetic acid (Fig. 4a). The resonances at 5.94 and 4.74 τ (relative to TMS) represent the methylene and methine protons, respectively, exhibiting the expected doublet–triplet hyperfine pattern with first-order splittings of 7 c.p.s. The N—H absorptions at 1.30 and 1.89 τ exhibit apparent half-height widths of 10–12 c.p.s. and give no evidence for strong coupling with other protons in the molecule. While an unambiguous assignment is impossible, the occurrence of the N—H absorption at the lowest field favors the assignment of the 1.30-τ peak to the positively charged α-nitrogen proton and that at 1.89 τ (with relative area twice that at 1.30 τ) to the exocyclic NH_2 group, and these assignments indicate that structure I is the best representation for 2-aminothiazoline-4-carboxylic acid.

(16) O. Lutz and B. Jirgensons, *Ber.*, **63**, 4481 (1930); *ibid.*, **64**, 1221 (1931); *ibid.*, **65**, 784 (1932).

TABLE I

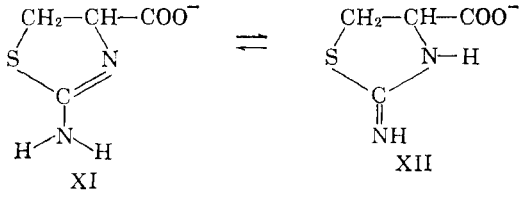
pK_A VALUES

Compound	pK_{COOH}	$pK_{NH_3^+}$
2-Aminothiazoline-4-carboxylic acid	2.03	8.48
Thiazolidine-4-carboxylic acid[a]	1.51	6.21
S-Ethylcysteine[b]	2.03	8.60
Cysteine	1.96[c]	8.86[d]
Alanine[c]	2.34	9.69
β-Alanine[c]	3.60	10.19
2-Aminothiazoline[e]		8.70

[a] S. Ratner and H. T. Clarke, *J. Am. Chem. Soc.*, **59**, 200 (1937). [b] L. R. Ryklan and C. L. A. Schmidt, *Arch. Biochem.*, **5**, 89 (1944). [c] J. T. Edsall in "Proteins, Amino Acids and Peptides as Ions and Dipolar Ions," Reinhold, New York, 1943, p. 75. [d] For the ionization of the ammonium group from that species, $HS—R—NH_3^+$, which contains an unionized thiol group; R. E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955). [e] Ref. 2.

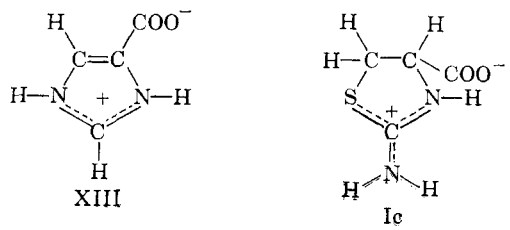
At temperature above 30°, the N—H protons of 2-aminothiazoline-4-carboxylic acid undergo rapid exchange in trifluoroacetic acid with the carboxyl protons averaging to a single broadened resonance peak for the acid proton at 70°. On cooling to about 50° the 1.89-τ resonance peak reappears. That at 1.30 τ returns gradually at temperatures in the region of 40°; the two N—H resonance peaks attain the original 1:2 area ratio at 30°.

The anion (XI) of 2-aminothiazoline-4-carboxylic acid also shows tautomeric possibilities and an n.m.r. spectrum of 2-aminothiazoline-4-carboxylic acid in sodium deuteroxide was obtained. The spectrum gave evidence of rapid exchange and no structural assignment could be made.



XI XII

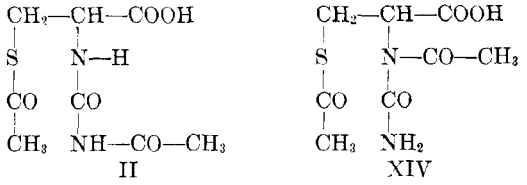
It is of interest to note that 4(or)-5-imidazolecarboxylic acid is also a zwitterion and its structure has been represented as XIII, a structure which takes into account the possible resonance forms.[17] A similar structure Ic, may be written for 2-aminothiazoline-4-carboxylic acid and *a priori* structure I with its greater number of resonance possibilities (Ic) is favored over the tautomeric form IX.



XIII Ic

(17) R. W. Cowgill and W. M. Clark, *J. Biol. Chem.*, **198**, 33 (1952).

Structure of N′,S-Diacetyl-N-carbamylcysteine (II).—The reactions outlined in Fig. 1 establish the diacetyl compound as an N-carbamyl derivative of cysteine with one of the acetyl groups assigned to sulfur and the other acetyl group to be assigned to one of the two nitrogen atoms. Figure 4b represents the proton magnetic resonance spectrum of the diacetyl compound in trifluoroacetic acid. Examination of the spectrum of this compound in dimethylformamide indicates that the carboxyl group is protonated, ruling out the existence of a zwitterion as demonstrated in the case of 2-aminothiazoline-4-carboxylic acid. Particularly pertinent in trifluoroacetic acid are the resonances at 0.12 and 0.76 τ, each being assigned to an amide proton. The latter proton resonance exhibits spin-spin coupling with the methine hydrogen, with a splitting of 7 c.p.s. The resonance at 0.12 τ represents the hydrogen attached to the nitrogen of the carbamyl group. The only structure compatible with two separate amide protons, one being on the α-nitrogen, is II, the alternative structure XIV being ruled out on both counts.



II XIV

Of further interest is the resonance at 4.95 τ, the methine proton resonance, with its major splitting corresponding to spin coupling with the methylene protons (6.26 τ) and further splitting by the protons of the α-nitrogen. The methylene proton resonance exhibits a splitting of 6 c.p.s. Non-equivalence of the methyl protons of the $CH_3CO—S—$ and $CH_3—CO—NH$ is indicated by the resonances at 7.41 and 7.65 τ.

Coupling of the proton on the α-nitrogen of acylated amino acids with the methine proton on the α-carbon has also been previously noted in cases where the electric field at the N^{14} nucleus has been made homogeneous by symmetric substitution at the N^{14} nucleus.[18,19]

Structure of Benzoylation Products.—The structure of S-benzoyl-N-carbamylcysteine (IV) is established by its empirical formula, the presence of an S-benzoyl group and by analogy with the acetylation product. The other benzoylation product, 2-imino-3-benzoylthiazolidine-4-carboxylic acid (III), is established by alkaline conversion (Fig. 2) to the parent thiazolidine[20] and to S-benzoyl-N-carbamylcysteine (IV). Conversion to the latter compound locates the benzoyl group on the ring nitrogen since the N-acyl to S-acyl shift

(18) G. V. D. Tiers and I. A. Bovey, *J. Phys. Chem.*, **63**, 302 (1959).

(19) A. Kowalski, 140th American Chemical Society Meeting, Division of Biological Chemistry, Chicago, Illinois, September, 1961.

(20) The benzoylated thiazolidine did not give an immediately positive test with diazotized sulfanilic acid in potassium carbonate solution.

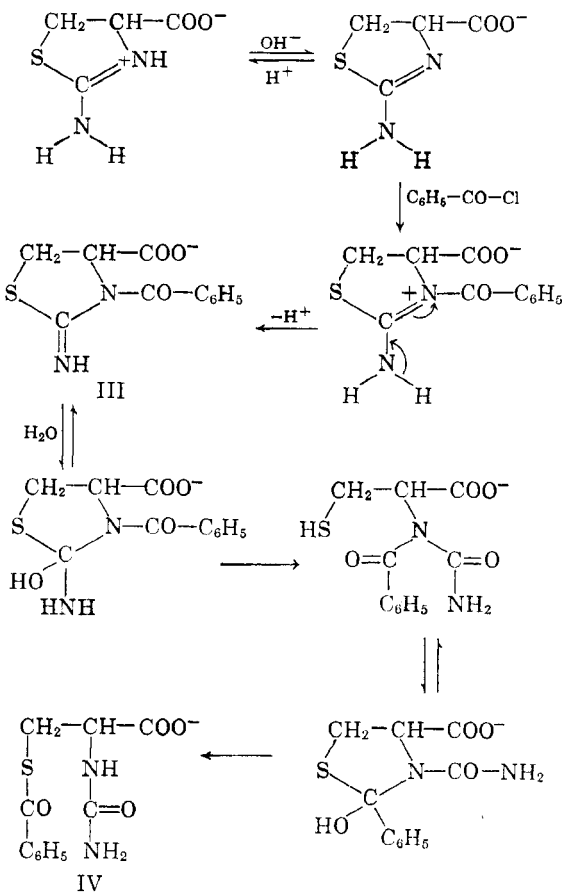
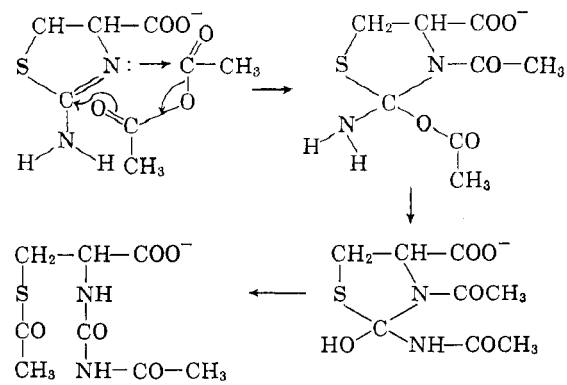


Fig. 5.—Mechanism of ring opening of 2-aminothiazoline-4-carboxylic acid on benzoylation in aqueous medium.

which occurs on ring opening (Fig. 5) would seemingly not take place were the benzoyl group located on the exocyclic nitrogen. The n.m.r. spectrum of 2-imino-3-benzoylthiazolidine-4-carboxylic acid in trifluoroacetic acid (Fig. 4C) is in keeping with the structural assignment. A low field resonance peak for a protonated positively charged nitrogen is not evident, the basicity of the amidine moiety having been reduced by benzoylation. The broad absorption in the region of 5.3 τ suggests quadrupole broadening of the protons attached to the amide nitrogen by the N^{14} nucleus.

Mechanism of Product Formation.—The benzoylation of 2-aminothiazoline-4-carboxylic acid providing two products, 2-imino-3-benzoylthiazolidine-4-carboxylic acid and S-benzoyl-N-carbamylcysteine, the former compound being the precursor of the latter compound, is a logical choice for discussion of mechanism. Figure 5 presents in schematic form a sequence of reactions leading to the observed products and this scheme may be taken as a basis for mechanistic discussion. The tartial reaction sequence leading to 2-imino-3-benzoylthiazolidine-4-carboxylic acid (III) would seem to be straightforward. Following deprotonation of 2-amino-3-thiazoline-4-carboxylic acid, benzoyl chloride is attacked by the more nucleophilic[21] α-nitrogen to yield an intermediate which is stabilized by loss of a proton and thus yields III. The next section of the sequence dealing with the conversion of 2-imino-3-benzoylthiazolidine-4-carboxylic acid (III) to S-benzoyl-N-carbamylcysteine (IV) has been tentatively formulated as proceeding *via* opening of the thiazolidine ring to give the intermediate N-benzoyl-N-carbamylcysteine which undergoes, through a cyclic thiazolidine intermediate, an N,S acyl shift in which the benzoyl moiety migrates from N to S. In formulating this sequence, the iminothiazolidine (III) has been considered to be hydrated to give a hydroxylated intermediate similar to those proposed[22-24] for the ring opening of 2-alkyl substituted thiazolines and oxazolines and for the conversion[25,26] of S,2-aminoethylisothiourea and related compounds to 2-aminothiazolines and to 2-mercaptoethylguanidines. The hydroxylated intermediate is then considered to undergo ring opening to give as the next intermediate N-benzoyl-N-carbamylcysteine[27] which, in turn through a hydroxylated cyclic intermediate, yields S-benzoyl-N-carbamylcysteine. It is of interest to note that the above cyclic intermediates, though similar, undergo ring opening in different directions. In the first case the C—S bond is broken and in the second case a C—N bond is broken. If these reactions are under thermodynamic control, then the more stable acyl derivative will be expected in each case. It is not unlikely that in the first case N-benzoyl-N-carbamylcysteine is more stable with respect to carbamyl transfer than the corresponding S-carbamyl-N-benzoylcysteine and in the second case that S-benzoyl-N-carbamylcysteine is more stable than



(21) Presumably because this nitrogen atom is the more basic of the two, it being preferentially protonated.

(22) R. B. Martin, S. Lowey, E. L. Elson, and J. T. Edsall, *J. Am. Chem. Soc.*, **81**, 5089 (1959).

(23) R. B. Martin and A. Parcell, *ibid.*, **83**, 4830 (1961).

(24) R. B. Martin and A. Parcell, *ibid.*, **83**, 4835 (1961).

(25) J. X. Khym, R. Shapira, and D. G. Doherty, *ibid.*, **79**, 5663 (1957).

(26) J. X. Khym, D. G. Doherty, and R. Shapira, *ibid.*, **80**, 3342 (1958).

(27) Albeit decomposition to 2-keto-3-benzoylthiazolidine-4-carboxylic acid and to S-carbamyl-N-benzoylcysteine is conceivable. No extensive search for these compounds was made.

N-benzoyl-N-carbamylcysteine with respect to benzoyl transfer. While the sequence of reactions from III to IV seems reasonable, kinetic data are not available for definitive conclusions. In connection with this mechanism it would also seem that the acetylation reaction proceeds by a similar sequence and that these reactions are followed by acetylation at the carbamyl nitrogen to give the isolated N′,S-diacetyl-N-carbamylcysteine. However, one would expect from this sequence the formation of, at least, S-acetyl-N-carbamylcysteine. This compound was not found even with less than stoichiometric amounts of acetic anhydride and the possibility of a concerted acetylation by the anhydride suggests itself, although no definitive conclusion can be reached.

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Acid-Catalyzed Interchange Reactions of Carboxylic Acids with Enol Esters[1b]

EDWARD S. ROTHMAN, SAMUEL SEROTA, THEODORE PERLSTEIN, AND DANIEL SWERN

Eastern Regional Research Laboratory,[1b] Philadelphia 18, Pennsylvania

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The reaction of vinyl acetate with carboxylic acids in the presence of sulfuric acid catalyst gives a mixture of the acylals ethylidene diacetate, 1-acetoxy-1-acyloxyethane, and 1,1-diacyloxyethane. By contrast, the homologous isopropenyl acetate gives the ester-acid interchange reaction as well as anhydride formation previously reported. Attempts to isolate the geminal diester, bromoisopropylidene distearate *via* the reaction of isopropenyl stearate, *N*-bromosuccinimide, and sodium stearate were not successful, although evidence for its formation includes spectral disappearance of isopropenyl unsaturation bands, hydrogenolysis to *n*-octadecyl alcohol and *n*-octadecyl stearate, ethanolysis to ethyl stearate, and hydrolytic rearrangement to hydroxyacetone stearate.

The interchange reaction of vinyl esters with carboxylic acids is described by Adelman[2] as distinctly different in nature from ordinary transesterifications or ester-acid interchanges. The former reaction is reported to be unaffected by acid[3] or basic catalysis and specifically catalyzed by metal salts, typically mercuric salts with or without added boron trifluoride,[4] capable of forming complexes with acetylene. Evidence was brought to bear that the reaction course actually involved primary formation of an acetylene–mercury salt complex which then underwent a second stage addition reaction with reagent carboxylic acid:

$$CH_3COOCH{=}CH_2 + HgSO_4 \rightleftharpoons CH_3COOH + HC{=}CH(HgSO_4) \quad (1)$$

$$RCOOH + CH{=}CH(HgSO_4) \rightleftharpoons RCOOCH{=}CH_2 + HgSO_4 \quad (2)$$

On the other hand, sulfuric acid–catalyzed reaction of vinyl acetate with carboxylic acids without mercury salts catalysis is reported by other workers as giving reaction products. Thus in a German patent,[5] the reaction of vinyl acetate with acetic acid catalyzed by sulfuric acid is stated to yield an acylal, namely, ethylidene diacetate.

$$CH_3COOCH{=}CH_2 + CH_3COOH \xrightarrow{H^+} CH_3{-}C(OCOCH_3)(H){-}OCOCH_3 \quad (3)$$

Vinyl acetate and the homologous enol ester, isopropenyl acetate, might be expected to show significant differences in reactivities. Nevertheless, Hagemeyer and Hull[6] report results paralleling those reported by Adelman[2] namely, that isopropenyl esters and carboxylic acid react in the presence of mercury salts to form isopropenyl esters of the reactant acids; that is, "vinyl interchange" is the predominant reaction. But Hagemeyer and Hull[6] also report that, using only sulfuric acid catalysis, acetic anhydride and the anhydride of the reagent carboxylic acid are formed.

In the case of sulfuric acid catalysis our own findings are at variance with this previous report and give evidence that a fairly complicated situation actually exists. We find, for example, that

(1)(a) To be presented at 142nd National Meeting of the American Chemical Society, September 9–14, 1962, Atlantic City, New Jersey; (b) One of the laboratories of the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

(2) R. L. Adelman, *J. Org. Chem.*, **14**, 1057 (1949).

(3) In reaction of vinyl acetate with carboxylic acids, sulfuric acid is reported (ref. 2) to have no catalytic activity whatsoever. See also T. Asahara and M. Tomita, *J. Oil Chemists' Soc., Japan*, **1**, 76 (1952).

(4) Based on analogous examples given in U. S. Patent 2,646,437, July 21, 1953 (J. B. Dickey and T. E. Stanin), the reaction of isopropenyl acetate and stearic acid, using mercuric acetate–boron trifluoride catalyst should have given isopropenyl stearate. Under these conditions we obtained only traces of the expected product but instead isolated stearic anhydride in at least 74% yield (see Experimental).

(5) Ger. Patent 313,696, July 19, 1919.

(6) H. J. Hagemeyer, Jr., and D. C. Hull, *Ind. Eng. Chem.*, **41**, 2920 (1949).