2-Thiazoline-4-carboxylic Acid

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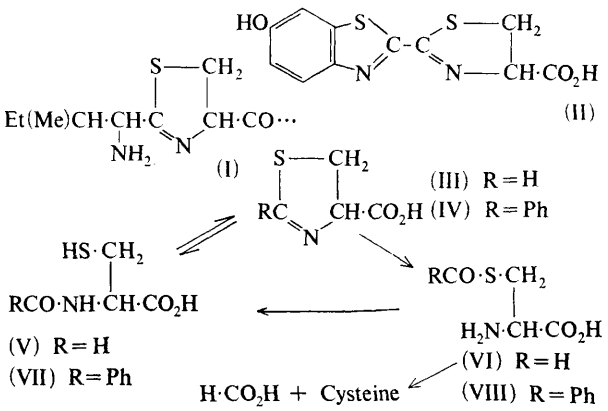
Ring-closure of *N*-formylcysteine in acid solution affords 2-thiazoline-4-carboxylic acid, the simplest thiazoline derivable from cysteine. The behaviour of this thiazoline in acid and in buffered aqueous solutions has been studied spectrophotometrically. Related observations on 2-phenyl-2-thiazoline-4-carboxylic acid are also recorded.

ALTHOUGH thiazolines derived from cysteine are implicit intermediates in the biosynthesis of the thiazoles found in the antibiotics micrococcin [1] and thiostrepton,[2] only two representatives have been encountered among natural products; these are the thiazoline moiety (I) found in the antibiotic bacitracin A (ref. 3) and the luciferin (II) of the firefly.[4] Nearly 30 years ago Linderstrøm-Lang and Jacobsen [5] proposed that the existence of cysteine residues in the form of thiazoline rings could account for masked thiol groups in proteins but no evidence has been obtained to substantiate this hypothesis. Calvin [6] and others,[7] however, have shown that glutathione in strongly acid solution develops an ultraviolet absorption spectrum attributable to the formation of a thiazoline ring. Similar observations have been made with pantetheine [8] and with *N*-formylcysteine,[9] and coenzyme A is believed to be capable of existence in a thiazoline form stable in neutral solution.[10] Model experiments with simple thiazolines have shown that opening of the thiazoline ring is inhibited in strongly acid solutions, and that the rate of reaction increases with decreasing acidity and then falls again as the pH is still further raised, giving typically bell-shaped rate–pH curves; [11] the region of maximum instability in relation to pH and relative rates of ring-opening are, of course, affected by substituents on the thiazoline ring.[12] Apart from brief reference to 2-thiazoline itself, which had a half-life of 80 sec. at pH 3 and 30°,[12] and a short note [9] on the behaviour of a specimen of 2-thiazoline-4-carboxylic acid ' of unknown purity ', no systematic examination of the behaviour in aqueous solution of a thiazoline unsubstituted in the 2-position has hitherto been reported. The present communication describes a spectrophotometric study of the behaviour of 2-thiazoline-4-carboxylic acid (III) in aqueous solution under a variety of conditions, together with some comparative observations on 2-phenyl-2-thiazoline-4-carboxylic acid (IV). The work was also undertaken in part to ascertain conditions that might be appropriate for the dehydrogenation of 2-thiazolines, derived from cysteine, to thiazoles in aqueous media.



N-Formylcysteine (V) undergoes rapid ring-closure to 2-thiazoline-4-carboxylic acid (III) in concentrated hydrochloric acid [9] and also in 10M-sulphuric acid [13] (Figure 1); this is associated with a rapid increase in absorbance at 265 nm. and fall in absorbance at 193 nm. In 5M-sulphuric acid the absorbance at 265 nm. rose rapidly to a maximum and then declined steadily indicating formation of a substantial proportion of 2-thiazoline-4-carboxylic acid followed by its destruction (Figures 2 and 3).

Pure 2-thiazoline-4-carboxylic acid hydrochloride was obtained by the ring-closure of *N*-formylcysteine in M-hydrogen chloride in dry glacial acetic acid and was used in subsequent experiments. In 5M-sulphuric acid the

[1] P. Brookes, A. T. Fuller, and J. Walker, *J. Chem. Soc.*, 1957, 689; G. E. Hall, N. Sheppard, and J. Walker, *J. Chem. Soc.* (*C*), 1966, 1371.

[2] G. W. Kenner, R. C. Sheppard, and C. E. Stehr, *Tetrahedron Letters*, 1960, no. 1, 23; D. F. W. Cross, G. W. Kenner, R. C. Sheppard, and C. E. Stehr, *J. Chem. Soc.*, 1963, 2143; M. Bodanszky, J. T. Sheehan, J. Fried, N. J. Williams, and C. A. Birkhimer, *J. Amer. Chem. Soc.*, 1960, **82**, 4747; M. Bodanszky, J. Fried, J. T. Sheehan, N. J. Williams, J. Alicino, A. I. Cohen, B. T. Keeler, and C. A. Birkhimer, *ibid.*, 1964, **86**, 2478.

[3] G. G. F. Newton and E. P. Abraham, *Biochem. J.*, 1953, **53**, 604; L. C. Craig, W. Hausmann, and J. R. Weisiger, *J. Amer. Chem. Soc.*, 1954, **76**, 2839.

[4] E. H. White, F. McCapra, G. F. Field, and W. D. McElroy, *J. Amer. Chem. Soc.*, 1961, **83**, 2402.

[5] K. Linderstrøm-Lang and C. F. Jacobsen, *Compt. rend. Trav. Lab. Carlsberg*, 1941, **23**, 289; *J. Biol. Chem.*, 1941, **137**, 443.

[6] M. Calvin, in ' Glutathione. A Symposium,' Academic Press, New York, 1954, pp. 21—26.

[7] G. Préaux and R. Lontie, *Biochem. J.*, 1957, **66**, 26P; ' Protides of the Biological Fluids,' Elsevier, Amsterdam, 1958, p. 217; D. Garfinkel, *J. Amer. Chem. Soc.*, 1958, **80**, 4833; I. Goodman and L. Salce, *Biochim. Biophys. Acta*, 1965, **107**, 129; P. C. Jocelyn, *Analyt. Biochem.*, 1967, **18**, 493.

[8] L. Salce and I. Goodman, *Biochim. Biophys. Acta*, 1965, **107**, 126.

[9] D. Cavallini, B. Mondovi, and C. DeMarco, *Experientia*, 1957, **13**, 436.

[10] R. E. Basford and F. M. Huennekens, *J. Amer. Chem. Soc.*, 1955, **77**, 3878.

[11] (*a*) R. B. Martin, S. Lowey, E. L. Elson, and J. T. Edsall, *J. Amer. Chem. Soc.*, 1959, **81**, 5089; (*b*) R. B. Martin, R. I. Hedrick, and A. Parcell, *J. Org. Chem.*, 1964, **29**, 3197.

[12] G. L. Schmir, *J. Amer. Chem. Soc.*, 1965, **87**, 2743.

[13] Cf. H. A. Smith and G. Gorin, *J. Org. Chem.*, 1961, **26**, 820 for N-acetylcysteine.

absorbance of 2-thiazoline-4-carboxylic acid at 265 nm. steadily declined but the resulting picture (Figure 4a) showed that the product of ring opening was essentially cysteine and not *N*-formylcysteine; hydrolysis of the

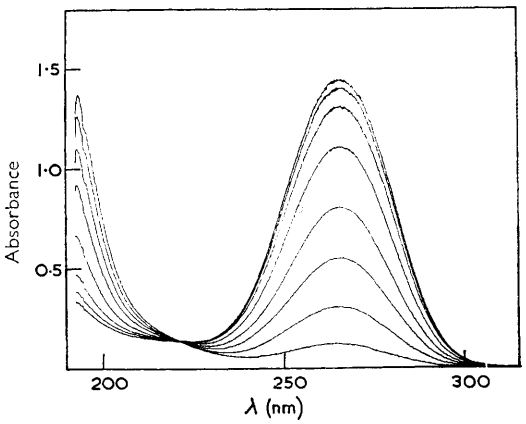


FIGURE 1 Ring-closure of N-formylcysteine (0·30 mM) in 10M-sulphuric acid at 33°; *t* 3, 6, 10 . . . 150 min. $k \times 10^4$/min. approximately 525

intermediate *S*-formylcysteine (VI) occurs too readily in 5M-sulphuric acid for the development of absorption at about 231 nm. to be obvious, although it may be noted that the absorbance after 10 min. exceeded the initial absorbance in this region. Thiol esters show a u.v. absorption at *ca.* 230 nm.,[11a,14] and Smith and Gorin [13] have shown in the case of 2-methyl-2-thiazoline-4-carboxylic acid that at acid concentrations above 0·5M a peak at 228 nm. first grew and then waned to a negligible value, the maximal absorption being greater the lower

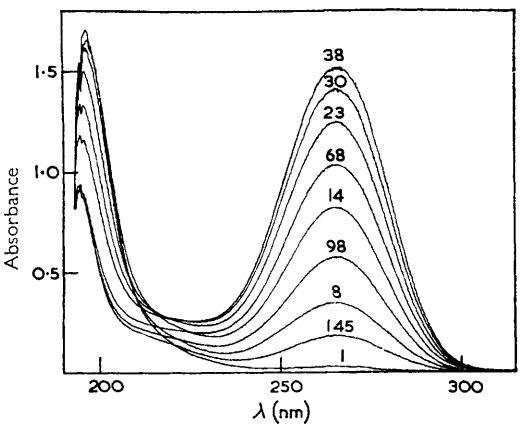


FIGURE 2 Ring-closure of *N*-formylcysteine (0·34 mM) and ring-opening of resulting 2-thiazoline-4-carboxylic acid in 5M-sulphuric acid at 32°. Spectra observed at *t* (min.) indicated

the acid concentration. The transient existence of *S*-formylcysteine became more obvious as the strength of the sulphuric acid was reduced to 3M (Figure 4b) and then to 2M (Figure 4c). At the same time the rate of destruction of the thiazoline ring increased *ca.* fivefold in passing from 5M- to 3M-sulphuric acid and again *ca.* fivefold in passing from 3M- to 2M-sulphuric acid. In M- (Figure 4d) and 0·5M-sulphuric acid (Figure 4e) the rate of decomposition of the thiazoline was too fast to be measured but the transient concentration of *S*-formylcysteine appeared to be about maximal in M-sulphuric acid. It should also be noted that as the strength of the sulphuric acid was reduced, the absorbance at short wavelength steadily increased indicating the formation of a steadily increasing proportion of *N*-formylcysteine (Figure 5). When dissolved in water, 2-thiazoline-4-carboxylic acid hydrochloride was completely destroyed in less than 2 min. to give well over 90 percent of *N*-formylcysteine with no trace of *S*-formylcysteine (Figure 4f). These observations are consonant with the observation [11a] that only *N*- and not *S*-acetyl-β-mercaptoethylamine is formed from 2-methylthiazoline at values of pH greater than 5, but in apparent contrast with the observation,[13] with 2-methyl-2-thiazoline-4-carboxylic acid, that at acid concentrations below 0·2M the absorptions at 261 and 228 nm. did not disappear but attained values which remained sensibly constant for long periods

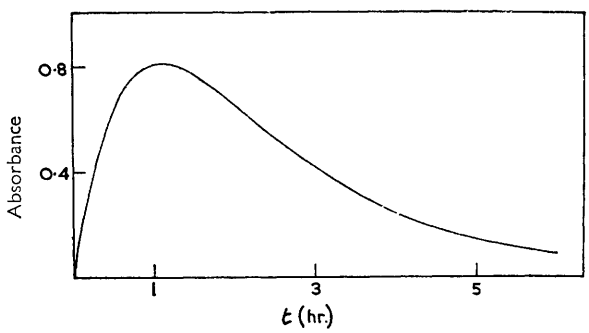


FIGURE 3 Change of absorbance at 265 nm. of a solution (0·16 mM) of *N*-formylcysteine in 5M-sulphuric acid at 25°

of time; the latter parallels the observation that the thiol ester produced by ring-opening of 2-(2-aminoethyl)-2-thiazoline, derived from pantetheine, shows considerable stability in 0·1M-hydrochloric acid.[8] For *S*-formylcysteine the rate of its further transformation to cysteine and formic acid by hydrolysis or to *N*-formylcysteine by intramolecular acyl-transfer [11a,15] is very much greater than that in the analogous case of either *S*-acetylcysteine [13] or 2-(*S*-β-alanylmercapto)-ethylamine.[8] There is no reason to assume that at high acidity *S*-formylcysteine is formed exclusively and breaks down to formic acid and cysteine, and that *N*-formylcysteine is formed directly and exclusively in water. *S*-Formylcysteine is probably the essential product under both sets of conditions and as the acid concentration is lowered S ⟶ N acyl-transfer occurs with progressively greater ease and takes place at hydrogen-ion concentrations where only an infinitesimally

[14] L. H. Noda, S. A. Kuby, and H. A. Lardy, *J. Amer. Chem. Soc.*, 1953, **75**, 913; T. Wieland and H. Koppe, *Annalen*, 1953, **581**, 1.

[15] T. Wieland and E. Bokelmann, *Annalen*, 1952, **576**, 20.

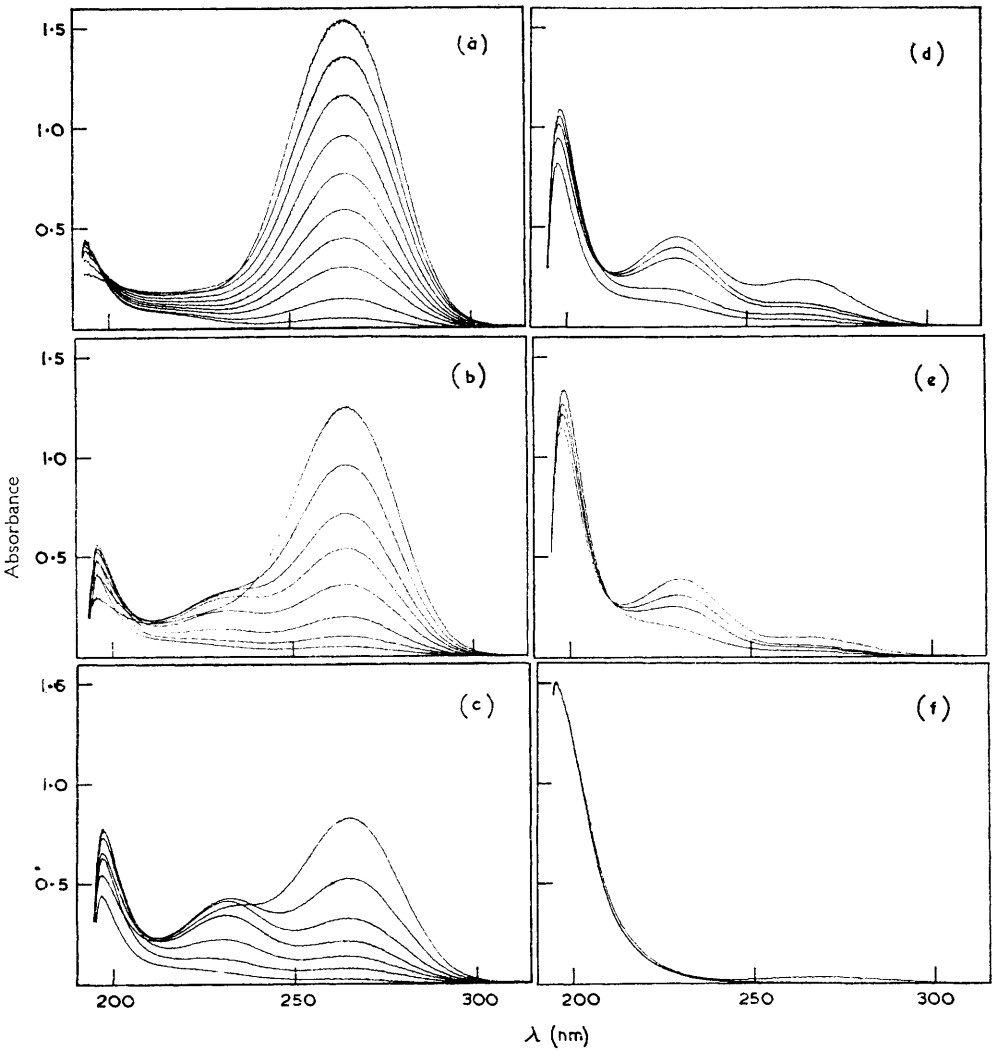


FIGURE 4 Decomposition of 2-thiazoline-4-carboxylic acid (hydrochloride)

(a) 0·30 mM-solution in 5M-sulphuric acid at 24°; t 2, 10, 18 . . . 140 min. $k \times 10^4$/min. approximately 193. (b) 0·27 mM-solution in 3M-sulphuric acid at 30°; t 1½, 4, 7 . . . 120 min. $k \times 10^4$/min. approximately 940. (c) 0·27 mM-solution in 2M-sulphuric acid at 30°; t 1½,3, 5 . . . 60 min. $k \times 10^4$/min. approximately 2600. (d) 0·27 mM-solution in M-sulphuric acid at 32°; t 1¼, 2½, 3¾, 15, and 60 min. (e) 0·27 mM-solution in 0·5M-sulphuric acid at 31°; t, 1, 2¼, 3½, and 10 min. (f) 0·18 mM-solution in water at 28°; t 1 and 2½ min.; pH 3·6 (glass electrode)

small proportion of amino-groups can be present in the uncharged nucleophilic form. This is in agreement with the experience of Wieland and his collaborators [16] that *S*-acylation of a mercaptan having a free adjacent amino-group must be effected at a pH below 3. *S*-Formylcysteine rearranges to *N*-formylcysteine below pH 4 although the pK_a of the amino-group is in the region of 10.

By pH 5·9 the stability of the thiazoline ring in 2-thiazoline-4-carboxylic acid had again become adequate for convenient measurement of its rate of destruction by observing the fall in the maximum at 252 nm. in buffered aqueous solution, and the stability progressively increased (Figure 6) with further increase of pH, showing

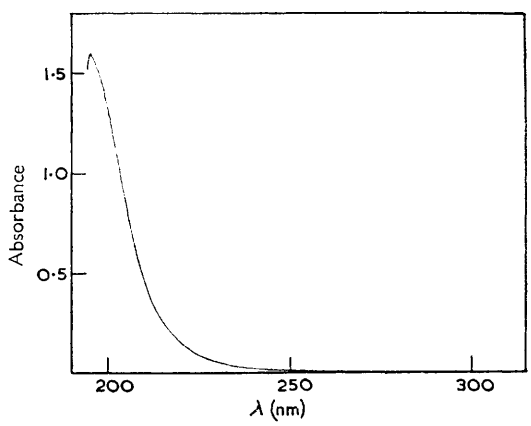


FIGURE 5 Absorption spectrum of 0·20 mM-solution of *N*-formylcysteine in water (log ε_{195} 3·90)

[16] T. Wieland, E. Bokelmann, L. Bauer, H. U. Lang, and H. Lau, *Annalen*, 1953, **583**, 129.

ca. a thousandfold increase between pH 5·9 and 10. This figure, however, requires downward correction for the absorption of ionised thiol in the more strongly alkaline solutions, since cysteine (thiol pK_a 8·33) shows a broad absorption [17] with a maximum in the 230—238 nm. region in alkaline solution. A typical decomposition experiment is shown in Figure 7.

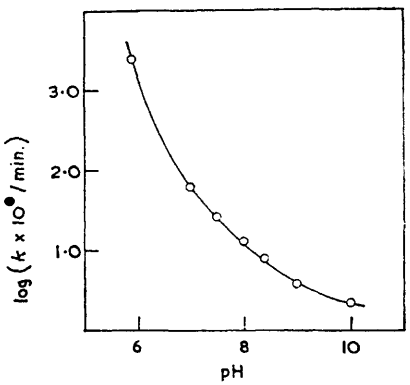


FIGURE 6 Rate of opening of thiazoline ring in 2-thiazoline-4-carboxylic acid (hydrochloride) as a function of pH at 25—26°

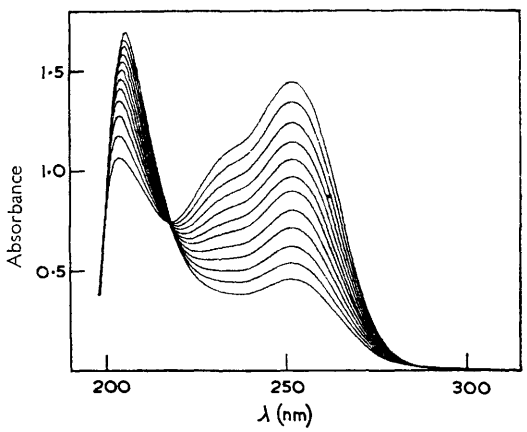


FIGURE 7 Decomposition of 0·56 mM-solution of 2-thiazoline-4-carboxylic acid (hydrochloride) in pH 7·5 phosphate buffer over a period of 6 hr. at 25—26°

In contrast with *N*-formylcysteine, *N*-benzoylcysteine (VII) showed little tendency to undergo ring-closure in strongly acid solution, although *N*-benzoylpenicillamine undergoes nearly quantitative conversion to 5,5-dimethyl-2-phenyl-2-thiazoline-4-carboxylic acid on being heated under reflux with *ca.* 0·23N-hydrochloric acid.[18]

2-Phenyl-2-thiazoline-4-carboxylic acid (IV) was stable in 10M-sulphuric acid (Figure 8), and decomposed only extremely slowly as the concentration of sulphuric acid was reduced, minimum stability being observed in 0·01M-acid (Figure 9). Even then the rate of ring-opening ($k \times 10^4$/min. at 30°, 14·2) was extremely slow and *ca.* 60% of that observed by Schmir for the amide.[12] In the ring-opening of 2-phenyl-2-thiazoline-4-carboxylic

[17] R. E. Benesch and R. Benesch, *J. Amer. Chem. Soc.*, 1955, **77**, 5877; G. Gorin, *ibid.*, 1956, **78**, 767.
[18] 'The Chemistry of Penicillin,' Princeton Univ. Press, Princeton, New Jersey, 1949, p. 471.

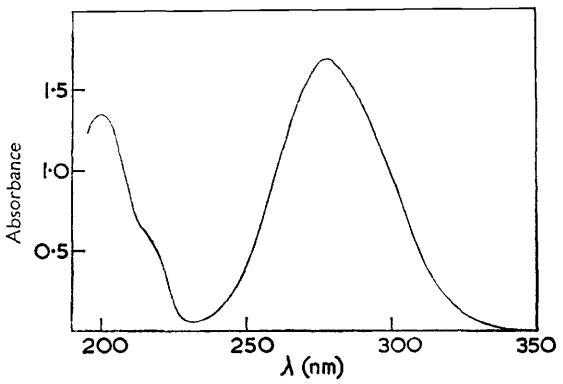


FIGURE 8 Absorption spectrum of 0·098 mM-solution of 2-phenyl-2-thiazoline-4-carboxylic acid in 10M-sulphuric acid; log ε_{278} 4·26, log ε_{200} 4·16

acid the formation of *S*-benzoylcysteine (VIII) was shown by the development of a maximum at 233—234 nm. during the first 12 hr. (Figure 10). Traces taken after 24 hr. no longer passed cleanly through the isosbestic points, indicating a secondary change in the nature of the reaction product, and the maximum at

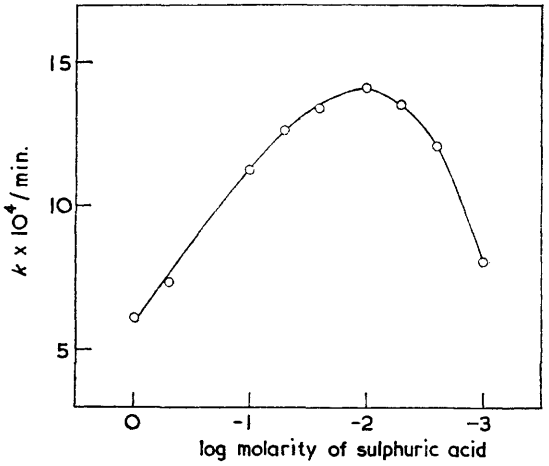


FIGURE 9 Rate of opening of thiazoline ring in 2-phenyl-2-thiazoline-4-carboxylic acid in 1·0—0·001M-sulphuric acid at 30°

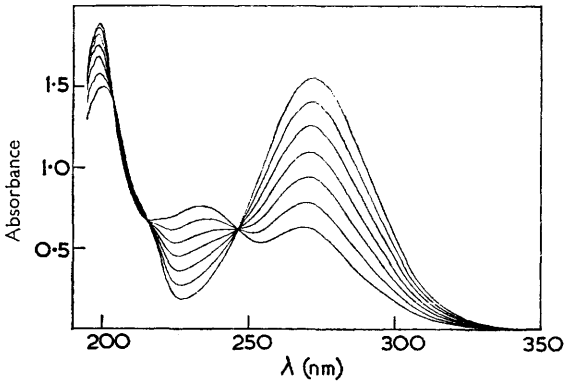


FIGURE 10 Decomposition of 2-phenyl-2-thiazoline-4-carboxylic acid (0·095 mM) in 0·01M-sulphuric acid at 30°; *t* 5 min.—12 hr.

233—234 nm. moved slowly towards the more intense and better defined maximum of *N*-benzoylcysteine at 228 nm.; conversion was complete after *ca.* 48 hr. at 36° or 12 hr. at 60°. In this respect the behaviour of 2-phenyl-2-thiazoline-4-carboxylic acid is comparable with that of 2-methyl-2-thiazoline-4-carboxylic acid [13] and of 2-(2-aminoethyl)-2-thiazoline [8] noted above. The pH of minimum stability of 2-phenyl-2-thiazoline-4-carboxylic acid (1·8) may be compared with that of the amide (*ca.* 1·5—1·6) [12] and that of 2-methyl-2-thiazoline-4-carboxylic acid (1·7).[13] It has been shown[15] that rearrangement of *S*-benzoyl-β-mercaptoethylamine to *N*-benzoyl-β-mercaptoethylamine takes place *ca.* ten times more slowly than the corresponding rearrangement of *S*-acetyl-β-mercaptoethylamine, but the present comparison of 2-thiazoline-4-carboxylic acid with its 2-phenyl analogue shows a much greater difference in stability. The lability of *S*-acyl-cysteines precludes the use of the acyl group for the protection of the thiol group in cysteine.[19]

EXPERIMENTAL

Materials (With N. A. Fuller).—*N*-Formylcysteine, m.p. 136° from water (lit.,[20] 131°, 137—138°), was prepared from diformylcystine [21] by reduction with zinc and acetic acid following the method used by Pirie and Hele for *N*-acetylcysteine.[22] Purity was assayed by thiol titration with M/60-potassium iodate [23] in N-hydrochloric acid.[24] 2-Phenyl-2-thiazoline-4-carboxylic acid and *N*-benzoyl-cysteine were prepared from cysteine methyl ester and ethyl benzimidate by the methods of Crawhall and Elliott.[25]

[19] R. G. Hiskey, T. Mizoguchi, and T. Inui, *J. Org. Chem.*, 1966, **31**, 1192.
[20] C. G. Mackenzie and J. Harris, *J. Biol. Chem.*, 1957, **227**, 393; W. O. Foye and M. Verderame, *J. Amer. Pharm. Assoc.*, 1957, **46**, 273.

2-*Thiazoline-4-carboxylic Acid Hydrochloride* (With N. A. Fuller).—*N*-Formylcysteine (2·0 g.) was dissolved under nitrogen in a solution of dry hydrogen chloride (4·75 g.) in dry glacial acetic acid (130 c.c.) at room temperature. Stout, colourless prisms separated in *ca.* 2 hr., and after 20 hr. the *hydrochloride* was collected (1·6 g., 71%), washed with a little M-hydrogen chloride in glacial acetic acid, and dried over sodium hydroxide pellets *in vacuo*, m.p. 181—183° (decomp.), $\lambda_{max.}$ 264—265 nm., log ε 3·76 in 10M-sulphuric acid (Found: C, 29·0; H, 3·8; Cl, 20·7; N, 8·1; S, 19·0. $C_4H_5O_2NS,HCl$ requires C, 28·7; H, 3·6; Cl, 21·2; N, 8·4; S, 19·1%). A further quantity was obtained on concentration of the mother-liquors under reduced pressure with exclusion of moisture.

Spectrophotometry.—Spectra were observed on a Unicam SP 800 instrument. Scans were started at the times (*t*) indicated, which relate to the first contact of solvent with solute. The buffers employed were: succinate (pH 5·5 and 5·9), phosphate (7·0, 7·5, and 10·0), phosphate–borate (8·0 and 8·4), and borate (9·0). Scan times were approximately 70, or 90 sec. for the wavelength ranges used (315—190, or 350—190 nm.). Pseudo-first-order specific reaction rates were calculated from the observed change with time in the long wavelength absorbance. Fixed wavelength experiments were made with a Unicam SP 500 instrument.

The writer thanks Mr. N. A. Fuller for participation in preliminary experiments and Mr. W. A. L. Marshment for preparative assistance.

[8/003 *Received, January* 1*st*, 1968]

[21] V. DuVigneaud, R. Dorfmann, and H. S. Loring, *J. Biol. Chem.*, 1932, **98**, 577.
[22] N. W. Pirie and T. S. Hele, *Biochem. J.*, 1933, **27**, 1716.
[23] K. J. Steel, *J. Pharm. Pharmacol.*, 1958, **10**, 574.
[24] Cf. C. C. Lucas and E. J. King, *Biochem. J.*, 1932, **26**, 2076.
[25] J. C. Crawhall and D. F. Elliott, *J. Chem. Soc.*, 1951, 2071; 1952, 3094.