The Reaction between Cyanide and the Mixed Disulphide of Cysteine and Penicillamine

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N.B. ββ dimethylcysteine = RSSC = "mixed disulphide"

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The Reaction between Cyanide and the Mixed Disulphide of Cysteine and Penicillamine

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Cyanide reacts with ββ-dimethylcystine, but much more slowly than with cystine. The products are penicillamine and 2-amino-2-thiazoline-4-carboxylic acid.

THE urine of individuals under treatment with penicillamine contains the mixed disulphide with cysteine (PeS·SCy);[1] Lotz, Potts, and Bartter[2] reported that this compound does not give a positive cyanide–nitroprusside test, but we have shown that a reaction does take place with cyanide, to yield penicillamine and 2-amino-2-thiazoline-4-carboxylic acid. No reaction could be detected between cyanide and penicillamine disulphide.[2,3]

Cyanide reacts with cystine and related compounds by nucleophilic attack on sulphur, to yield a thiol and a thiocyanate. The thiocyanate reacts further to yield a more stable cyclic isomer.[4,5] In unsymmetrical disulphides, there are three factors which may be considered to influence the direction of the reaction:[6] the relative stabilities of the anions of the possible thiol products, the relative stabilities of the possible thiazoline (or thiazine) products, and steric hindrance. Probably the last is crucial in the case of PeS·SCy. One cannot compare the possible thiazoline products, since 2-amino-5,5-dimethyl-2-thiazoline-4-carboxylic acid has not been prepared. Although the alkyl substituents in penicillamine might be supposed to reduce the thiolate anion stability relative to the anion from cysteine, the thiol dissociation constants are, in fact, approximately equal (pK 7·9).[7]

The change in u.v. absorbance on treatment with cyanide forms a useful assay for either cystine or PeS·SCy. The conditions described below are especially convenient. Related disulphides, *e.g.* homocystine and the homocysteine–cysteine mixed disulphide, can also be estimated in this way. However, a sample solution with a low background absorbance is necessary, and this may demand laborious pre-treatment of samples from biological sources; there are complications if a thiol or another disulphide is also present.

Evidence is given below that cysteine catalyses the reaction between PeS·SCy and cyanide, probably through the exchange reaction:

$$\mathrm{PeS{\cdot}SCy} + \mathrm{CyS^-} \rightleftharpoons \mathrm{PeS^-} + \mathrm{CyS{\cdot}SCy}$$
$$\mathrm{CyS{\cdot}SCy} + \mathrm{CN^-} \rightleftharpoons \mathrm{CyS^-} + \mathrm{CyS{\cdot}CN}\ (\rightarrow\text{thiazoline}),$$

Presumably this explains why cystine accelerates the reaction of PeS·SCy with cyanide, sufficient free cysteine being formed by reaction of the cystine with cyanide. Interference by cystine has made it difficult to apply the present results directly in a differential assay for PeS·SCy and cystine.

[1] J. C. Crawhall, E. F. Scowen, and R. W. E. Watts, *Brit. Med. J.*, 1963, **1**, 589.
[2] M. Lotz, J. T. Potts, and F. C. Bartter, *Brit. Med. J.*, 1965, **2**, 521.
[3] H. R. Crooks, in 'The Chemistry of Penicillin,' ed. H. T. Clarke, R. Robinson, and J. R. Johnson, Princeton University Press, Princeton, New Jersey, 1949, p. 455.
[4] A. Schöberl, M. Kawohl, and R. Hamm, *Chem. Ber.*, 1951, **84**, 571.
[5] A. Schöberl and M. Kawohl, *Chem. Ber.*, 1957, **90**, 2077.
[6] A. J. Parker and N. Kharasch, *Chem. Rev.*, 1959, **59**, 583.
[7] L. Eldjarn and L. Hambreus, *Scand. J. Clin. Lab. Invest.*, 1964, **16**, 153.

One must consider also whether the above mechanism could account for the whole of the observed reaction between PeS·SCy and cyanide—the required CyS^- ion could be formed in several ways. In fact, this possibility is excluded by the evidence given below on the relative rates in the presence and absence of added cysteine.

EXPERIMENTAL

L-2-amino-2-thiazoline-4-carboxylic acid was prepared according to method 2 of Schöberl and Hamm.[8] The material was finally recrystallized from 70% ethanol, which is known to yield the free acid;[9,10] m.p. 235° (decomp.) (lit.,[10] 234°).

Penicillamine disulphide was prepared by the method of Crooks[11] from D-penicillamine (Dista Products Ltd.). PeS·SCy was prepared by the method of Crawhall, Scowen, and Watts[12] from D-penicillamine and L-cysteine hydrochloride (Koch-Light). The product was homogeneous by t.l.c. in the phenol–formic acid system described below; m.p. 220° (decomp.); it was hygroscopic, but could be dried *in vacuo* (P_2O_5).

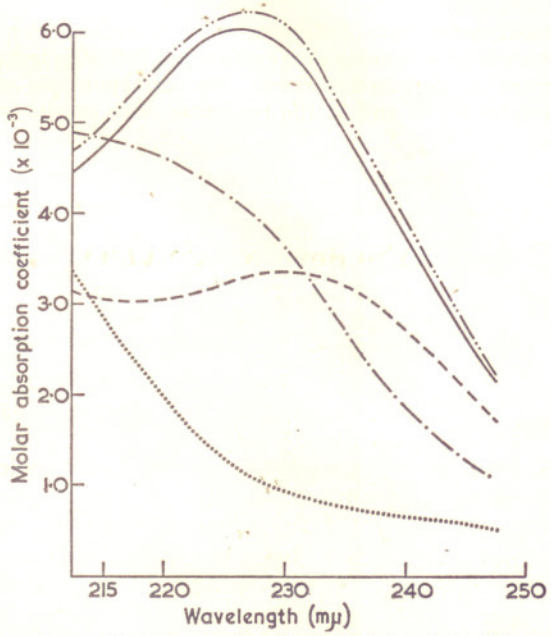
Reaction of ββ-Dimethylcystine with Cyanide.—(*a*) To ββ-dimethylcystine (70 mg.) in water (2 ml.) was added 10% potassium cyanide (1 ml.) and benzyl chloride (1 ml.). The mixture was shaken at room temperature for 8 hr. The benzyl chloride was separated and the aqueous layer was extracted with ether (2 × 30 ml.) to yield an almost clear yellow-brown solution. Acetic acid was added to bring the pH to 5 and the yellow-brown precipitate was filtered off and dried.

A sample of the precipitate in dilute hydrochloric acid was subjected to t.l.c. [0·25 mm. silica gel + 13% gypsum (Merck) in ethanol–water–ammonia (*d* 0·880) (18 : 1 : 1)] in comparison with *S*-benzylcysteine and *S*-benzylpenicillamine prepared in a similar manner from the amino-acids. The unknown gave a single ninhydrin-positive spot corresponding to *S*-benzylpenicillamine. (R_F values: *S*-benzyl penicillamine 0·87, unknown 0·88, *S*-benzylcysteine 0·70).

(*b*) ββ-Dimethylcystine (3 mg.) in water (10 ml.) was mixed with 10% potassium cyanide (1 ml.) and the pH was brought to 9 with dilute hydrochloric acid. The solution was heated at 56° (water-bath) for 2 hr. and on t.l.c. [support as above, eluant saturated aqueous phenol–formic acid (15%) (75 : 25)] gave a single ninhydrin-positive spot corresponding in colour and R_F value (cherry-red, 0·42—0·43) to penicillamine (cherry-red, 0·43) and distinct from cysteine (dull red, 0·28) and PeS·SCy (dull red, 0·35).

(*c*) ββ-Dimethylcystine (16·4 mg.) was shaken with water (0·4 ml.) and 10% aqueous potassium cyanide (0·1 ml.) The pH was adjusted to 8·9 with acetic acid (30 μl). The solution was set aside overnight and acetone (10 ml.) was added. The white precipitate was collected by centrifugation after 30 min. for flocculation. This material was reprecipitated twice from solution in water (0·2 ml.) with acetone (4 ml.); each time the precipitate was dried by resuspension in acetone, centrifugation, and decantation, and kept *in vacuo* for 1 hr. over silica gel. Finally, it was recrystallised from 70% aqueous ethanol (0·2 ml.), dried *in vacuo* (yield 3·6 mg.), and identified as L-2-amino-2-thiazoline-4-carboxylic acid by m.p. [234° (decomp.)] and mixed m.p. [233° (decomp.)] and comparison of i.r. spectra and chromatographic mobilities: $\nu_{max.}$ 688, 712, 730, 801, 906, 967, 1010, 1081, 1142, 1161, 1222, 1305, 1405 (diffuse), 1610br, 1700, 2360infl, 2960, 3130infl, and 3450infl $cm.^{-1}$. T.l.c. on silica gel in methanol–water–triethylamine (80 : 20 : 0·1) was employed; spots were detected by heating the plate for 5 min. at 250°. No other treatment was necessary, but this technique was successful after location of amino-acids by spraying with ninhydrin and heating at 100°. The thiazoline gave a spot, R_F 0·59, of characteristic shape and colour (yellow-brown).

(*d*) To a solution (3·0 ml.) containing ββ-dimethylcystine (0·110 mg.) and 0·050M-sodium tetraborate was added 10% aqueous potassium cyanide (0·1 ml.). The solution was rapidly mixed and its absorbance recorded between 260 and 215 mμ (Unicam SP 800 spectrophotometer, fast scan) within 1 min. of adding the cyanide and again 24 hr. later. The spectra are shown in the Figure, with the spectrum predicted from the presumed course of the reaction. The blank contained sodium tetraborate and cyanide, as above, and was treated in parallel with the test solution.



Change in u.v. absorbance when PeS·SCy is treated with cyanide: (· · · ·) PeS·SCy; (— · — · —) 2-amino-2-thiazoline-4-carboxylic acid; (– – – –) penicillamine (Sigma Chemical Co.); (— ·· — ·· —) predicted absorbance; (——) observed absorbance. All absorbances were determined for solutions in 48·5mM-sodium tetraborate containing 49·6mM-potassium cyanide, pH 9·3. The absorbance due to cyanide is negligible, so that the new absorbance at each wavelength can be calculated from ε(thiazoline) + ε (penicillamine) − ε(PeS·SCy)

Kinetic Experiments.—Sodium tetraborate (0·075M; 2·0 ml.) was pipetted into a spectrophotometer cuvette, and to this was added the sample solution and water to bring the volume to 3·0 ml. At zero time, 10% potassium cyanide (0·1 ml.) was added and the solution was stirred. Readings of absorbance at 226 mμ were taken at intervals of 1 min. for at least 5 min., and then at greater intervals for as long as the experiment demanded. The absorbance at zero time was determined by extrapolation. The pH after addition of

[8] A. Schöberl and R. Hamm, *Chem. Ber.*, 1948, **81**, 210.
[9] J. L. Wood and S. L. Cooley, *J. Biol. Chem.*, 1956, **218**, 449.
[10] O. Gawron and J. Fernando, *J. Amer. Chem. Soc.*, 1961, **83**, 2906.
[11] Ref. 3, p. 469.
[12] J. C. Crawhall, E. F. Scowen, and R. W. E. Watts, *Brit. Med. J.*, 1964, **1**, 1411.

cyanide was 9·3. Gawron *et al.*[13] found that the optimum pH for the cystine–cyanide reaction was 8·9.

For samples containing cystine or PeS·SCy, the absorbance increased progressively to a maximum; this was reached in about 90 min. for cystine and about 24 hr. for PeS·SCy (25°). After the maximum was reached, the absorbance of the PeS·SCy was stable, whereas that of the cystine samples decreased at about 3% per hr., presumably because of instability of the free cysteine produced.

The maximum absorbance increment in each case agreed well with that calculated from the molar absorbances of reactants and products (Figure). Reaction rates were therefore calculated directly from the observed rate of increase of absorbance and the appropriate molar absorbances.

Second-order rate constants were calculated for the reaction rates of cyanide with cystine and PeS·SCy, from the observed initial reaction rates. No correction was made for impurities in any of the reagents. The results were: cystine; k' 1·6 l. mole^{-1} min.$^{-1}$; PeS·SCy; k' 0·1 l. mole^{-1} min.$^{-1}$.

A sample containing penicillamine disulphide (123 μg.) was treated as described above. There was no detectable change of absorbance during 140 min.

If mixtures of cystine and PeS·SCy were used, the reaction rate was greater than would have been expected from the rates obtained with each alone at the same concentrations. A corresponding effect was obtained with cysteine. If cysteine was added to the PeS·SCy samples the rate of increase of absorbance was enhanced (cysteine alone showed no change of absorbance, as expected). The rate was approximately doubled in the presence of 0·065mM-cysteine and trebled in 0·13mM-cysteine. The concentration of PeS·SCy used was 0·07mM.

[8/596 *Received, March 15th,* 1968]

[13] O. Gawron, S. Mahboob, and J. Fernando, *J. Amer. Chem. Soc.*, 1964, **86**, 2283.