

# AMINO ACIDS AND PEPTIDES\*

## IX. SOME UNSYMMETRICAL DISULPHIDES DERIVED FROM CYSTEINE

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Footner and Smiles (1925) first showed that disulphides are formed by the reaction of Bunte salts (*S*-alkyl or *S*-aryl thiosulphates) with thiols in alkaline solution (eqn. (1))



This reaction would seem to be convenient for the preparation of unsymmetrical disulphides, but in most cases where R and R' were different, Footner and Smiles obtained not the expected product, but an equimolar mixture of the two symmetrical disulphides. This result can be attributed to the strongly alkaline reaction media which provides conditions known to favour disulphide interchange reactions. Unsymmetrical protein disulphides have also been prepared by reaction of Bunte salts with reduced wool proteins (Haefele 1952; Haefele and Tucker 1952; Schöberl and Bauer 1957) and by reaction of thiols with wool proteins containing the  $-\text{SSO}_3^-$  group (Swan 1961). Schöberl and Bauer (1957, 1958) have prepared a number of unsymmetrical disulphides by reaction of cysteine with various Bunte salts, but no physical properties other than paper chromatographic  $R_F$  values were reported.

Independently, Swan (1957) reported that this reaction could be carried out under mild conditions to yield unsymmetrical disulphides, the reactants being mixed either in weakly alkaline solution containing strontium ions (the liberated sulphite being removed as insoluble strontium sulphite) or in weakly acid solution under a stream of nitrogen to remove sulphur dioxide.



Reaction of *S*-sulpho-L-cysteine (II) with L-cysteine gave optically pure L-cystine (I;  $\text{R} = -\text{CH}_2\text{CH}(\text{NH}_3^+)\text{COO}^-$ ); reaction with toluene- $\omega$ -thiol and with *N*-benzyloxycarbonyl-L-cysteine yielded respectively the unsymmetrical disulphides *S*-(benzylthio)-L-cysteine (I;  $\text{R} = -\text{CH}_2\text{Ph}$ ) and mono-*N*-benzyloxycarbonyl-L-cystine (I;  $\text{R} = -\text{CH}_2\text{CH}(\text{NH} \cdot \text{COOCH}_2\text{Ph})\text{COOH}$ ). The last two compounds were also obtained by the alternative reaction of L-cysteine with the appropriate Bunte salt. In the case of those Bunte salts which can be readily

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prepared from alkyl halides and sodium thiosulphate this alternative route is the more convenient. Thus *S*-(benzylthio)-L-cysteine was obtained in high yield by reaction of equimolar amounts of sodium *S*-benzyl thiosulphate and L-cysteine at pH 5–7. In this case the high yield can be attributed to the insolubility of the product in this pH range.

### Experimental

Microanalyses were performed in the C.S.I.R.O. and University of Melbourne microanalytical laboratory. Paper chromatography was carried out using Whatman No. 1 paper and downward flow development with the following solvent systems: Solvent A, phenol: water (3:1), solvent B, butan-1-ol: acetic acid: water (4:1:1); solvent C, butan-2-ol: acetic acid: water (5:1:4). The amino acid spots were detected with ninhydrin.

(a) *L*-Cystine.—L-Cysteine hydrochloride monohydrate (1.05 g) was dissolved in 10 ml of a solution containing an equivalent amount of sodium *S*-sulpho-L-cysteinate (Clarke 1932; for an improved procedure see Sorbo 1958), and acetic acid was added to bring the pH to 5. Nitrogen was bubbled through the solution, and after 18 hr, L-cystine (1.7 g) was filtered off and identified by colour reactions, paper chromatography, and optical rotation;  $[\alpha]_D^{27} -208^\circ$  (c, 1.0 in 1N HCl).

(b) *s*-Benzylthio-L-cysteine.—(i) A solution of cysteine hydrochloride monohydrate (17.6 g) in water (100 ml) was added to a solution of sodium acetate trihydrate (40 g) in 2N NaOH (30 ml), the final pH being 6.5–7.0. Sodium *S*-benzyl thiosulphate (23 g) (Purgotti 1890) in water (75 ml) was added and after 2–3 hr the product was filtered off and washed well with water and finally with ethanol. The yield was 22 g (92%), m.p. 196 °C (decomp.);  $R_F$  in solvent A, 0.86; solvent B, 0.42; solvent C, 0.84. Chromatography revealed the presence of a trace of cystine ( $R_F$  0.24 in solvent A, 0.12 in solvent B, 0.11 in solvent C).

The product was dissolved in 2N HCl (approx. 50 ml/g), the solution was filtered if necessary and the pH adjusted to c. 3 with NaOH or NaOAc. The unsymmetrical disulphide was thereby recovered in almost quantitative yield and was free of contaminating cystine (Found: C, 49.7; H, 5.6; O, 13.5%. Calc. for  $C_{10}H_{13}NO_2S_2$ : C, 49.4; H, 5.4; O, 13.2%),  $[\alpha]_D^{20} -108.3^\circ$  (c, 0.57 in 2N HCl). The yield was maximal (c. 90%) over the pH range 5.5 to 7.5 and decreased markedly to 21% at pH 3.5 with a significant increase in cystine content. Bretschneider and Klötzer (1950) have prepared *S*-benzylthio-L-cysteine hydrochloride by an alternative method.

The amino acid was recovered unchanged after being heated under reflux in N HCl for 3 hr or after being kept for 2 hr in 15%  $NH_4OH$  at room temperature. A solution in N NaOH (0.5 g in 10 ml) rapidly deposited dibenzyl disulphide (0.24 g, m.p. 71 °C) while acidification of the filtrate to pH 4 yielded cystine (0.22 g). These products were also isolated when the amino acid was heated under reflux in 6N HCl for 2 hr or when it was warmed to 50 °C in a saturated solution of HBr in acetic acid. A quantitative yield of dibenzyl disulphide was also formed when the amino acid was shaken in a solution of sodium sulphite for 24 hr.

(ii) To a solution of sodium *S*-sulphocysteinate (0.02 mole) and strontium chloride (0.02 mole) at pH 8 was added toluene- $\omega$ -thiol (0.02 mole). The heavy precipitate which formed immediately was filtered off after 30 min, digested with N HCl (20 ml) to remove strontium sulphite, and the insoluble amino acid filtered off and washed with water and ethanol. Yield 2.5 g (52%), m.p. 193–194 °C (decomp.). The compound was chromatographically identical with that obtained in (i) above.

(c) *Mono-N-benzyloxycarbonyl-L-cystine*.—(i) *NN'*-Bisbenzyloxycarbonyl-L-cystine (4 g) was dissolved in 0.1N  $KHCO_3$  (170 ml) containing sodium sulphite heptahydrate (6 g) and a trace of ferric chloride. Air was passed through the solution until the nitroprusside test was negative (c. 24 hr), the pH was adjusted to 4, and air was again passed to remove  $SO_2$ . To this solution, presumably containing *N*-benzyloxycarbonyl-*S*-sulpho-L-cysteine, was added L-cysteine hydrochloride (2.5 g) and sufficient alkali to maintain the pH at 4. Nitrogen was passed through the solution for 24 hr after which time mono-*N*-benzyloxycarbonyl-L-cystine, 1.6 g (54%), m.p. 190 °C, separated. Swan (1956) reports m.p. 193–194 °C (see also Marshall *et al.* 1957). The

product was chromatographically identical with an authentic specimen,  $R_F$  in solvent A, 0.60, in solvent B, 0.27. Chromatography following treatment on the paper with sodium sulphite showed the presence of a new intense spot due to *S*-sulphocysteine,  $R_F$  0.06 in solvent A.

(ii) *N*-Benzyloxycarbonyl-L-cysteine (5.1 g), prepared from *NN'*-bisbenzyloxycarbonyl-L-cysteine by reduction with Zn in HCl, was added to a solution containing equivalent amounts of *S*-sulpho-L-cysteine and strontium chloride at pH 9. The precipitate of strontium sulphite was removed, and the pH of the filtrate was adjusted to 4, when mono-*N*-benzyloxycarbonyl-L-cysteine separated. Yield 3.7 g (50%), m.p. 193 °C (decomp.).

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### CORRIGENDUM

#### VOLUME 14, NUMBER 2

Page 252, line 5: For (1 mole) read (half the theoretical quantity).