THE PREPARATION OF COMPOUNDS RELATED TO *S*-2-AMINOETHYL-L-CYSTEINE

By H. LINDLEY*

[*Manuscript received October* 22, 1958]

Summary

The present paper describes the preparation of benzyloxycarbonyl-2-bromoethylamine, benzyloxycarbonyl-2-iodoethylamine, *S*-2-benzyloxycarbonylaminoethyl-L-cysteine, *S*-2-aminoethyl-L-cysteine, and polymers of the last two compounds. The preparation of carboxymethyl 2-aminoethyl sulphide is also described and it is reported that the latter compound does not act as an inhibitor for the hydrolysis of toluene-*p*-sulphonyl-L-arginine methyl ester by trypsin.

I. INTRODUCTION

The motive underlying the present work was to determine whether the structural similarity of the side chains of lysine and *S*-2-aminoethyl cysteine was sufficiently close for the enzyme trypsin to hydrolyse peptide bonds involving the latter amino acid. A preliminary account showing that the polymer of *S*-2-aminoethyl cysteine is an excellent substrate for trypsin has already been published (Lindley 1956), and the present paper gives details of the compounds prepared in connection with the study. *S*-2-Aminoethyl cysteine had already been prepared at the time this work was undertaken (Cavillini *et al.* 1955) by direct coupling of cysteine and 2-bromoethylamine. However, for the preparation of the polymer it was more desirable to use *S*-(benzyloxycarbonylaminoethyl) cysteine as the key compound and hence the present synthesis was via this intermediate.

It was proposed to make use of the trypsin susceptibility of peptide bonds involving *S*-aminoethyl cysteine residues by first reducing the cystine residues of proteins with mercaptoacetate (" thioglycollate ") followed by coupling the thiol groups of the reduced protein with bromoethylamine. It was therefore necessary to study the reaction between mercaptoacetic acid and bromoethylamine to see whether the product of this reaction, carboxymethyl 2-aminoethyl sulphide, could act as a trypsin inhibitor.

II. EXPERIMENTAL

All melting points are uncorrected and analyses are by the C.S.I.R.O. Microanalytical Laboratory.

(*a*) *Preparation of Benzyloxycarbonyl-2-bromoethylamine.*†—2-Bromoethylamine hydrobromide (10 g) and 4·2 g sodium bicarbonate were dissolved in 100 ml water and the solution cooled in an ice-bath. Benzyloxycarbonyl chloride (9 ml) was added followed by N sodium hydroxide (50 ml) in 10 ml portions with vigorous shaking after each addition. The product solidified after the

* Division of Protein Chemistry, C.S.I.R.O. Wool Research Laboratories, Melbourne.

† *Note added in Proof.*—It has now been realized that benzyloxycarbonyl-2-bromoethylamine and also the iodo derivative have been previously prepared (Katchalski and Ben Ishai 1950). These authors record melting points of 45 and 69° C respectively.

final portion of alkali had been added. (This procedure was followed rather than the conventional one of simultaneous addition of approximately equivalent amounts of the acid chloride and alkali because of the ease with which 2-bromoethylamine is converted to ethyleneimine hydrobromide under alkaline conditions. If the bromoethylamine was allowed to remain in alkaline solution before adding the benzyloxycarbonyl chloride, the sole product was a lachrymatory oil which was not further investigated but is presumably the *N*-benzyloxycarbonyl derivative of ethyleneimine.) After separation and washing by decantation the *product* was dissolved in ethyl acetate and the solution dried over calcium chloride. The product was recovered from solution by evaporation of the solvent in a vacuum and recrystallized as long needles from light petroleum (b.p. 40–60 °C), m.p. 47–49 °C (Found : C, 47·2 ; H, 4·8 ; N, 5·4 ; Br, 31·2%. Calc. for $C_{10}H_{12}O_2NBr$: C, 46·5 ; H, 4·7 ; N, 5·4 ; Br, 31·0%).

(*b*) *Benzyloxycarbonyl-2-iodoethylamine.*—One equiv. of benzyloxycarbonyl-2-bromoethylamine was refluxed for 1 hr in acetone with 1 equiv. of sodium iodide. After cooling the solution was filtered to remove sodium bromide and the acetone removed by vacuum distillation. The *product* crystallizes as long needles from light petroleum (b.p. 40–60 °C), m.p. 66–67 °C (Found : C, 39·6 ; H, 4·1 ; N, 4·2 ; I, 40·5%. Calc. for $C_{10}H_{12}O_2NI$: C, 39·3 ; H, 4·0 ; N, 4·6 ; I, 41·6%).

(*c*) S-*Benzyloxycarbonylaminoethyl*-L-*cysteine.*—(i) *By Coupling in Liquid Ammonia.* L-Cystine (4·6 g) was suspended in liquid ammonia (150 ml) and reduced to cysteine by metallic sodium. As soon as a permanent blue colour was obtained indicating excess sodium, this was discharged by the addition of the minimal quantity of ammonium chloride. The solution was then cooled in a solid CO_2–ethanol bath and benzyloxycarbonyl-2-bromoethylamine (10 g) added in small portions. After the final addition the ammonia was allowed to evaporate overnight. The solid product was dissolved in water and the solution filtered and the pH adjusted to 4 with hydrochloric acid. The product precipitated and was filtered and washed successively with water, ethanol, and ether. Recrystallization was carried out by dissolving the material in the minimal volume of hot N hydrochloric acid, diluting with 5 vol boiling water, and then adding a hot solution of sodium acetate (2 equiv. based on HCl used). The *product* crystallized as lustrous plates on cooling, m.p. 204–205 °C (decomp.). Yield (recrystallized product) 10·1 g (Found : C, 52·3 ; H, 6·1 ; O, 22·3 ; N, 8·9 ; S, 10·7%. Calc. for $C_{13}H_{18}O_4N_2S$: C, 52·3 ; H, 6·1 ; O, 21·4 ; N, 9·4 ; S, 10·7%).

(ii) *By Coupling in Aqueous Alcohol.* L-Cysteine hydrochloride (1·6 g) was dissolved in 50 ml water and a solution of benzyloxycarbonyl-2-iodoethylamine (3·25 g) in ethanol (100 ml) was added. A few drops of phenolphthalein were added and the solution titrated to a pink colour with 5N sodium hydroxide and left stirring for 30 min. The solution was then acidified to pH 4 and the precipitated product filtered and recrystallized as in Section II (*c*) (i). Yield 1·8 g.

The product could also be prepared using benzyloxycarbonyl-2-bromoethylamine instead of the iodo compound. Reaction in this case was slower and required 4 hr at 30 °C.

(*d*) S-*2-Aminoethyl*-L-*cysteine Hydrochloride.*—Treatment of the preceding compound with anhydrous hydrobromic acid (6N) in acetic acid caused quantitative removal of the benzyloxycarbonyl group in 30 min. Precipitation of the product was assisted by the addition of ether prior to filtration. The hygroscopic product was dissolved in water and converted to the free amino acid by passage through a column of " Amberlite 1R4 " in the basic form. The alkaline eluate (pH 9) was titrated to pH 4 with hydrochloric acid and evaporated to dryness in a vacuum. The *S*-2-aminoethyl cysteine hydrochloride was crystallized by dissolving in twice its weight of water and adding an equal volume of hot ethanol to the hot solution (Found : C, 29·8 ; H, 6·5 ; N, 13·5 ; S, 15·6 ; Cl, 17·8%. Calc. for $C_5H_{13}O_2N_2SCl$: C, 29·9 ; H, 6·5 ; N, 14·0 ; S, 16·0 ; Cl, 17·7%) ; m.p. 195 °C (decomp.), $[\alpha]_D^{16}$ —4·4° (*c*, 3% in H_2O). Cavallini *et al.* give m.p. 192–192·5 °C, $[\alpha]_D^{25}$ +7·2° (*c*, 1% in H_2O). No reason can be advanced for the discrepancy in optical rotation found in the present work and that reported by Cavillini *et al.* (1955). Dr. S. J. Leach kindly determined the cystine content of the sample of *S*-aminoethyl cysteine used for optical rotation measurements by amperometric titration and showed that it was less than 0·1%. Therefore, the discrepancy cannot be explained on the basis of contamination of the present sample with cystine.

A titration curve was carried out on the sample and gave 1·93 for the pK of the carboxyl group (25°). Calculated pK values for the amino groups were 8·8 and 9·2 but the curve showed little trace of any inflection.

(*e*) *Polymer of* S-*2-Aminoethyl-*L-*cysteine.*—The initial procedure outlined by Lindley (1956) is illustrated in protocol (i), but method (ii) used later is much to be preferred.

(i) *S*-Benzyloxycarbonylaminoethyl-L-cysteine (1·5 g) was dissolved in 1N sodium hydroxide (25 ml) at 0 °C, 1·7 ml benzyloxycarbonyl chloride added, and the reaction mixture shaken vigorously. After 10 min the solution was extracted once with ether to remove any excess benzyloxycarbonyl chloride, acidified with hydrochloric acid, and the product extracted into ether. Evaporation of the ether in a vacuum gave a colourless viscous oil. This is presumably the dibenzyloxycarbonyl derivative of *S*-aminoethyl cysteine, but was not further purified or characterized. The oil was heated with 1 ml thionyl chloride at 40 °C for 30 min. After evolution of SO_2 had ceased the excess thionyl chloride was removed by vacuum distillation (40 °C, 10^{-2} mm). On raising the temperature to 60 °C (10^{-2} mm) the residue decomposed with liberation of benzyl chloride. On raising the temperature still further to 85–95 °C for 2 hr it further decomposed, liberating CO_2, and leaving a brown glass. This was dissolved in acetic acid with warming and after cooling was treated with 6N anhydrous hydrobromic acid in acetic acid for 2 hr. At the end of this time ether was added to complete precipitation of the product, which was separated by decantation and further washed with ether by decantation and finally filtered. It was then dissolved in water, extracted once with ether, and then treated with decolourizing charcoal, filtered, and neutralized to pH 4. The solution was concentrated to a small volume and precipitated by the addition of ethanol, the product recovered by centrifugation, and dried in a vacuum over sulphuric acid. Yield 0·3 g.

(ii) *S*-2-Benzyloxycarbonylaminoethyl-L-cysteine (3 g) was suspended in dry dioxan, heated to 40 °C, and a stream of phosgene passed in for 3 hr using a calcium chloride tube to prevent ingress of moisture. The dioxan was removed by evaporation in a vacuum at 50 °C, leaving behind a colourless viscous oil. This was heated at 105 °C and 10^{-2} mm for 3 hr to form a brown glassy residue of the polymer of *S*-2-benzyloxycarbonylaminoethyl cysteine, which was then treated as in (i) above. Yield 1·3 g. On hydrolysis with 6N HCl at 105 °C for 48 hr both samples gave only one ninhydrin positive spot on a one-dimensional paper chromatogram using phenol–ammonia. This had an R_F of 0·82 agreeing with an authentic sample of *S*-aminoethyl cysteine and also gave a positive test with the iodoplatinate reagent (Consden, Gordon, and Martin 1946).

(*f*) *Carboxymethyl-2-aminoethyl Sulphide.*—Mercaptoacetic acid (4·6 g) was dissolved in water (50 ml) and bromoethylamine hydrobromide (10·5 g) added. 5N Sodium hydroxide (50 ml) was added and the solution let stand overnight. The solution was diluted with water to approx. 500 ml and passed through a column of " Zeo-Karb 225 " resin in the acid form, and washed well with water. The *product* was eluted with ammonia, the progress of the elution being followed by spot tests on filter paper using iodoplatinate solution. The eluate was concentrated in a vacuum to a thick gum which was finally induced to crystallize by leaving under dry ether. It was recrystallized by dissolving in twice its weight of water and adding 5 volumes of ethanol to the hot solution. Yield 5·5 g, m.p. 150 °C (Found: C, 35·7; H, 6·8; S, 23·8%. Calc. for $C_4H_9O_2NS$: C, 35·5; H, 6·7; S, 23·7%). The compound was tested for possible inhibition of trypsin by observing its effect on the rate of hydrolysis of toluene-*p*-sulphonyl-L-arginine methyl ester by trypsin, the reaction being followed in a pH-stat. With a substrate concentration of $0·7\times10^{-2}$M and a trypsin concentration of 0·0012 mg/ml at pH 8·0 and 30 °C, the rate of hydrolysis was not slowed down by the presence of $3·3\times10^{-2}$M carboxymethyl-2-aminoethyl sulphide.

III. References

Cavallini, D., de Marco, C., Mondovi, B., and Azzone, G. F. (1955).—*Experientia* **11**: 61.
Consden, R., Gordon, A. H., and Martin, A. J. P. (1946).—*Biochem. J.* **40**: 33.
Katchalski, E., and Ben Ishai, D. (1950).—*J. Org. Chem.* **15**: 1067.
Lindley, H. (1956).—*Nature* **178**: 647.