Synthesis of Some Dibasic Sulphur-containing Amino-acids related to L-Lysine

By D. B. Hope and K. C. Horncastle

S-2-Aminoethyl-L-cysteine (thialysine), its N^{ω}-methyl derivative, and *S*-2-aminoethyl-L-homocysteine have been prepared from the parent sulphur-containing α-amino-acids L-cysteine and L-homocysteine. The thiol amino-acids were condensed with *NO*-ditosylethanolamine and *NO*-ditosyl-*N*-methylethanolamine, to give N^{ω}-tosyl derivatives, from which the protective N^{ω}-tosyl group was removed by reduction with sodium in liquid ammonia.

S-2-AMINOETHYL-L-CYSTEINE, an amino-acid related to L-lysine by replacement of the C-4 methylene group by a sulphur atom, has been alternatively described in the literature as thiosine [1] and as thialysine.[2a] It has interesting biological properties as an antimetabolite of lysine,[1] and, in addition, four instances are known of enzymic action on *S*-2-aminoethyl-L-cysteine in peptide linkage.[3,4] The free amino-acid is decarboxylated by *Escherichia coli*,[5] and is oxidised by the L-amino-acid oxidase of *Mytilus edulis*.[6]

The total oxygen consumption when *S*-2-aminoethyl-L-cysteine is oxidised by the amino-acid oxidase is three times that observed with lysine. The compounds synthesised in this Paper were prepared in order to investigate this phenomenon further. The biochemical results will be published elsewhere. The oxidation of the *O*-analogue (*O*-2-aminoethyl-L-serine) by the enzyme proceeds similarly to that of *S*-2-aminoethyl-L-cysteine; the main product is ethanolamine.[7]

Eldjarn [8] prepared *S*-2-aminoethyl-L-cysteine as the hydrochloride by condensation of 2-bromoethylamine and L-cysteine in ethanolic solution. Cavallini, De Marco, Mondovì, and Azzone [9] carried out the same condensation in aqueous solution. The optical rotations of the products do not agree with those from preparations by Lindley [10] and Hermann and Grundig.[2a] These workers protected the amino group of 2-iodoethylamine with the toluene-*p*-sulphonyl (tosyl) [2] or benzyloxycarbonyl [10] group before condensation with L-cysteine in liquid ammonia, to give products from which the protective groups could be removed either by reduction with sodium in liquid ammonia (tosyl) or by hydrogen bromide in acetic acid (benzyloxycarbonyl).

The method described here is similar to that used by Hermann and Grundig.[2a] The procedure was simplified by replacing toluene-*p*-sulphonamido-2-iodoethylamine by di(toluene-*p*-sulphonyl)ethanolamine. The use of an *O*-toluene-*p*-sulphonyl ester for the preparation of a thioether was used by Sakami and Stevens [11] to prepare *S*-adenosyl-L-homocysteine. Di(toluene-*p*-sulphonyl)-ethanolamine was prepared from ethanolamine by treatment with toluene-*p*-sulphonyl chloride in pyridine.

[1] T. Shiota, J. Mauron, and J. E. Folk, *Biochim. Biophys. Acta*, 1961, **53**, 360.
[2] (*a*) P. Hermann and C. A. Gründig, Proceedings of the Fifth European Peptides Symposium, Oxford, September 1962, ed. G. T. Young, Pergamon Press, 1963; (*b*) K. Bláha, I. Frič, and P. Hermann, *Coll. Czech. Chem. Comm.*, 1965, **30**, 304.
[3] F. Tietze, J. A. Gladner, and J. E. Folk, *Biochim. Biophys. Acta*, 1957, **26**, 659.
[4] H. Lindley, *Nature*, 1956, **178**, 647.
[5] E. Work, *Biochim. Biophys. Acta*, 1962, **62**, 173.
[6] D. B. Hope, *Ciba Fdn. Study Grp.*, 1965, **19**, 88.
[7] K. C. Horncastle and D. B. Hope, *Biochem. J.*, 1965, **96**, 80P.
[8] L. Eldjarn, *Scand. J. Clin. Lab. Invest.*, 1956, **6**, Suppl. 13, 71.
[9] D. Cavallini, C. De Marco, B. Mondovì, and G. F. Azzone, *Experientia*, 1955, **11**, 61.
[10] H. Lindley, *Austral. J. Chem.*, 1959, **12**, 296.
[11] W. Sakami and A. Stevens, *Bull. Soc. Chim. biol.*, 1958, **40**, 1787.

The replacement of the *O*-toluene-*p*-sulphonyl by a chloro group can be avoided by keeping the reaction mixture cold, where pyridine hydrochloride is insoluble. This substance was one of a number isolated by Slotta and Behnish [12] from a similar reaction mixture at a higher temperature.

Di(toluene-*p*-sulphonyl)ethanolamine is a more convenient reagent than the corresponding iodo-compound used previously, because it can be made from ethanolamine in one step. Condensation with the disodium salt of either L-cysteine or L-homocysteine occurred rapidly in liquid ammonia at −33°; no trace of free thiol could be detected after 1 hr.

S-(2-*N*-Methylaminoethyl)-L-cysteine was prepared from di(toluene-*p*-sulphonyl)-*N*-methylethanolamine. The latter was prepared from *N*-methylethanolamine with toluene-*p*-sulphonyl chloride in pyridine. To facilitate the reduction of the tosyl *N*-methyl compound, dimethylformamide was added to the liquid ammonia in the proportion 1 : 16.

The tosyl group of the protected intermediates was removed by reduction with sodium in liquid ammonia following the well known procedure developed by du Vigneaud and Behrens.[13] Only slightly more than 2 atoms of sodium were consumed per molecule of the tosyl derivative, for complete removal of the tosyl group. This amount of sodium was consumed rapidly, although further sodium was consumed much more slowly. The end-point was easy to recognise, and in addition a yellow colour began to appear when more than 2 atoms of sodium were added. We have found, in accordance with the results of Rudinger and his co-workers,[14-16] that the tosyl group can be recovered as toluene-*p*-sulphinic acid in 80% yield. Since complete reduction occurred with 2 atoms of sodium, we conclude that the reduction reaction consumes 2 electrons and gives rise to the sulphinate ion and a substituted amide ion. The latter is neutralised by the proton of the carboxyl group. Thus, it appears that the reduction of the tosyl group takes precedence over that of the carboxyl group in reaction with the sodium. A similar phenomenon has already been observed in the reduction of *S*-benzyl-L-homocysteine (unpublished observations).

Purification of the detosylated compounds was performed by absorption on a column of Dowex AG 50 × 8 (200—400 mesh) (H^+ form). Prolonged washing of the column with water was necessary to remove the free toluene-*p*-sulphinic acid which was precipitated on the column. When the eluate from the column was neutral, the amino-acids were eluted with ammonia. The eluates were evaporated to dryness and converted either into the mono-hydrochloride by titration to pH 4 with 5N-hydrochloric acid or into the di-hydrochloride by addition of twice this amount of acid. The optical purity of the amino-acids was established enzymically.

EXPERIMENTAL

Melting points were determined on a Kofler block. Optical rotations were measured on the Ericson ETL-NPL Automatic Polarimeter calibrated with microanalytical grade sucrose {$[\alpha]_D^{20}$ +66·5° (*c* 3 in H_2O)}. Chromatographic data refer to descending chromatography on Whatman No. 4 paper with n-butanol–acetic acid–water.[17] The mobilities are recorded as R_A values, *i.e.*, relative to alanine. The elution volumes and colour yields were determined on an E.E.L. amino-acid analyser using the 50-cm. column at 30°.[18] The oxidation of the amino acids by the L-amino-acid oxidase of *Mytilus edulis* was followed by the conventional manometric techniques. The three substances and their N^{ω}-tosyl derivatives were substrates for the L-amino-acid oxidase of *Mytilus edulis*. All were completely oxidised, as shown by their absence from the oxidation mixture by paper chromatography or by analysis on the 50-cm. column of the E.E.L. amino-acid analyser run at 30°. The R_A values, elution volumes, and colour yields are recorded. The results of this work show that the amino-acids can be obtained, by the procedures described, free from racemic material.

Di-(toluene-p-*sulphonyl)ethanolamine.*— Ethanolamine (12·0 g.) was dissolved in anhydrous pyridine (15 ml.) and cooled to 0° in a bath of ice and salt. Toluene-*p*-sulphonyl chloride (80·0 g.), finely powdered, was suspended in pyridine (50 ml.) and cooled to −50°. The ethanolamine solution was added in five portions while the temperature was maintained below 10° for 30 min. The mixture was kept at room temperature for 6 hr. and then at +4° overnight. Water (2 ml.) was added to the semi-solid mass to remove excess of sulphonyl chloride. The mixture was poured, with stirring, on crushed ice (500 g.) and acidified with acetic acid (20 ml.), whereupon the oily material solidified. The solid was crushed to a slurry in water, collected, and washed with water. The product, dried over concentrated sulphuric acid, weighed 67·4 g. Recrystallisation of the product (67 g.) from hot chloroform (50 ml.) and addition of cold carbon tetrachloride (200 ml.) gave plates (50·0 g.), m. p. 84—86°. Recrystallisation of these (17·5 g.) from methanol (35 ml.) gave *material* (13·5 g.) of m. p. 86—87°, on cooling to 0° (Found: C, 52·2; H, 4·0; N, 4·9. $C_{16}H_{19}NO_5S_2$ requires C, 52·0; H, 3·8; N, 5·2%).

Toluene-p-*sulphonamido-2-chloroethylamine.*—The procedure was exactly the same as that in the previous experiment. Following the overnight period at +4° the temperature was raised to 65° for 4 hr. At first the crystals of pyridine hydrochloride dissolved, and after about 1 hr. a second crystalline solid, probably pyridine toluene-*p*-sulphonate, began to separate. The mixture was poured on crushed ice (300 g.) and acidified with acetic acid (20 ml.). The addition of the acid was followed immediately by solidification of an oily material. The solid was collected, washed with water, and dried over concentrated sulphuric acid, to give a product (34·0 g.) which was recrystallised from carbon tetrachloride (150 ml.), giving 32·7 g. of product, m. p. 96—98°. For analysis, the material (5·0 g.)

[12] K. H. Slotta and R. Behnish, *J. prakt. Chem.*, 1932, **135**, 225.
[13] V. du Vigneaud and O. K. Behrens, *J Biol. Chem.*, 1937, **117**, 27.
[14] Z. Pravda and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1955, **20**, 1.
[15] K. Jost and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1961, **26**, 2345.
[16] J. Rudinger, *Internat. Union of Pure and Appl. Chem.*, 1963, **7**, 335.
[17] S. M. Partridge, *Biochem. J.*, 1948, **42**, 238.
[18] D. H. Spackman, W. H. Stein, and S. Moore, *Analyt. Chem.*, 1958, **30**, 1190.

was recrystallised from light petroleum (250 ml.) (b. p. 100—120°), to give plates (3·9 g.), m. p. 97—98° (lit.,[12] 100—101°) (Found: Cl, 14·9; N, 6·1. Calc. for $C_9H_{12}ClNO_2S$: Cl, 15·2; N, 6·0%).

*Di(toluene-*p*-sulphonyl)*N*-methylethanolamine.*— *N*-Methylethanolamine (15·0 g.) was mixed with pyridine (25 ml.) and cooled to −5° in an ice–salt bath. Toluene-*p*-sulphonyl chloride (80 g.) was finely powdered, suspended in pyridine (50 ml.), and cooled to −5°. The *N*-methylethanolamine solution was added dropwise during 10 min. to the sulphonyl chloride. The temperature was kept below 0° for 15 min., and the mixture stored overnight at +4°. Water (1·0 ml.) was added to destroy the excess of sulphonyl chloride, and after ½ hr. the mixture was poured on crushed ice (500 g.). Acetic acid (15 ml.) was added to the mixture, which was stirred until the ice had melted. The yellow precipitate was collected, washed with water, and dried *in vacuo* over $CaCl_2$ and concentrated H_2SO_4. The dry solid (68·0 g.) was dissolved in hot ethanol (50 ml.). The *product* (60·2 g.), which crystallised on cooling, had m. p. 84·5—85·5°; for analysis it was recrystallised from ethanol with no change in m. p. (Found: C, 53·2; H, 5·5; N, 3·5. $C_{17}H_{21}NO_5S_2$ requires C, 53·2; H, 5·5; N, 3·6%).

General Method for the Condensation of a Thiol with a Tosylate in Liquid Ammonia.—The disulphide-containing amino-acid, either L-cystine or L-homocystine (1 mol.), was suspended in liquid ammonia and reduced to the thiol by metallic sodium. When a persistent blue colour was obtained, indicating an excess of sodium, the minimum quantity of ammonium chloride necessary to discharge the colour was added. The tosylate (2 mol.) was added during 15 min. The condensation occurred rapidly, as shown by the vigorous boiling of the solution. The ammonia was allowed to evaporate spontaneously, and the last traces were removed *in vacuo*. The residue was dissolved in water and, after the pH had been adjusted to 4·0, was cooled to 4°. The crystalline product was washed with water and dried over $CaCl_2$.

*S-(2-Toluene-*p*-sulphonamidoethyl)-L-cysteine.*—L-Cystine (10·0 g.) was reduced in liquid ammonia (250 ml.) and condensed with di(toluene-*p*-sulphonyl)ethanolamine (31·0 g.). The crude product 24·9 g. (90%) was recrystallised from water (20·0 g. from 420 ml.). The product (16·0 g.) showed m. p. 192—193° (decomp.) [lit.,[2a] 191—193° (decomp.)], $[\alpha]_D^{25}$ −9·9° (*c* 2 in N-HCl) (Found: C, 45·4; H, 5·6; N, 8·8. Calc. for $C_{13}H_{18}N_2O_4S_2$: C, 45·3; H, 5·7; N, 8·8%).

*S-(2-Toluene-*p*-sulphonamidoethyl)-L-homocysteine.*— L-Homocystine (8·2 g.), prepared from L-methionine,[19] was reduced in liquid ammonia (300 ml.) and condensed with di(toluene-*p*-sulphonyl)ethanolamine (23·0 g.). The crude *product* (18·0 g.), m. p. 198—200°, was recrystallised from water (250 ml.), to give 15·0 g., m. p. 198—200° (73%), $[\alpha]_D^{20}$ +12·0° (*c* 2 in N-HCl) (Found: C, 47·1; H, 6·0; N, 8·3. $C_{13}H_{20}N_2O_4S_2$ requires C, 47·0; H, 6·1; N, 8·4%).

*S-(N-Methyl-2-toluene-*p*-sulphonamidoethyl)-L-cysteine.*— L-Cystine (7·0 g.) was reduced in liquid ammonia (500 ml.). Dimethylformamide (50 ml.) and finely powdered di(toluene-*p*-sulphonyl)-*N*-methylethanolamine (23·5 g.) were added. The mixture was stirred until the ammonia had evaporated. The crude product (16·9 g.) had m. p. 184—188°. Following recrystallisation of 0·5 g. from water (380 ml.), the *product* had m. p. 184—188°, $[\alpha]_D^{20}$ +2·6° (*c* 2 in N-HCl) (Found: C, 47·3; H, 5·9; N, 8·6. $C_{13}H_{20}N_2O_4S_2$ requires C, 47·0; H, 6·1; N, 8·4%).

General Method for the Removal of the Tosyl Group by Reduction with Sodium in Liquid Ammonia; Isolation of the Dibasic Amino-acids as their Hydrochlorides.—The N^{ω}-tosylated intermediates were suspended in anhydrous liquid ammonia and reduced by metallic sodium added in small portions (0·2 g.). A blue colour appeared after the addition of about 2 atom equivalents of sodium. The solution remained colourless to this point, and if further sodium was allowed to be consumed the reaction mixture became yellow. The precipitate which accumulated was redissolved by addition of ammonium chloride or with glacial acetic acid. The ammonia evaporated, the residue was dissolved in water, and the amino-acid and cations were adsorbed on a column (2·5 × 40 cm.) of Dowex 50 × 8 (200—400 mesh) (H^+ form). The column was washed with water until the eluate (containing toluene-*p*-sulphinic acid) was neutral, and the amino-acid was eluted with 2N-ammonia solution until the eluate was strongly alkaline. The latter was concentrated almost to dryness to remove free ammonia, brought to pH 4·0 with hydrochloric acid, and evaporated to dryness; for the isolation of a dihydrochloride the quantity of hydrochloric acid was doubled.

S-2-Aminoethyl-L-cysteine.— *S*-(2-Toluene-*p*-sulphonamidoethyl)-L-cysteine (15·5 g.) was reduced with sodium in liquid ammonia (200 ml.). The *S*-2-aminoethyl-L-cysteine hydrochloride obtained crystallised from water (15 ml.) by addition of ethanol (80 ml.), to give 6·0 g., m. p. 193—195° (decomp.). The material (5 g.) was recrystallised from water–ethanol (1 : 2) cooled to 4°. The product (4·0 g.) consisted of needles, m. p. 194—195° (decomp.), $[\alpha]_D^{25}$ −7·0° (*c* 1 in N-HCl), $[\alpha]_D^{20}$ −9·0° (*c* 1 in N-HCl), $[\alpha]_D^{20}$ −8·1 (*c* 4 in H_2O), R_A 0·27 (L-lysine, R_A 0·43). On the amino-acid analyser the elution volume was 209—229 ml., and the colour yield was 20·795 (L-lysine; elution volume 259—282, colour yield 24·056) lit.,[2a] m. p. 194—195° (decomp.) $[\alpha]_D^{22}$ −4·4° ± 0·3° (*c* 4 in H_2O). Lit.,[2b] $[\alpha]_D^{20}$ −7·0 (*c* 1 in N-HCl) (Found: C, 29·9; H, 6·6; N, 14·0. Calc. for $C_5H_{13}ClN_2O_2S$: C, 29·9; H, 6·5; N, 14·0%).

S-2-Aminoethyl-L-homocysteine.— *S*-(2-Toluene-*p*-sulphonamidoethyl)-L-homocysteine (13·5 g.) was reduced with metallic sodium (2·15 g.) in liquid ammonia (400 ml.). Toluene-*p*-sulphinic acid (5·2 g.), m. p. 83·4°, was recovered from the acidic eluate from the column. An authentic sample of toluene-*p*-sulphic acid had m. p. 85·0°. The *S*-2-aminoethyl-L-homocysteine hydrochloride crystallised from methanol (80 ml.). The *product* (7·3 g., 84%) had m. p. 216—217° (decomp.), $[\alpha]_D^{20}$ +2·50° (*c* 1 in N-HCl), R_A 0·26. On the amino-acid analyser the elution volume was 483—530 ml., colour yield 22·620 (Found: C, 33·8; H, 7·0; N, 12·7. $C_6H_{15}ClN_2O_2S$ requires C, 33·6; H, 7·0; N, 13·1%).

This substance was also prepared without isolation of the intermediate tosylate. Following reduction of L-homocystine (11·0 g.) with metallic sodium in liquid ammonia (300 ml.), di(toluene-*p*-sulphonyl)ethanolamine (31·0 g.) was added. After 1 hr. the protective tosyl group was removed by reduction with sodium. Ammonium chloride (6·0 g.) was added before evaporation of the ammonia. The *S*-2-aminoethyl-L-homocysteine hydrochloride was crystallised first from water–methanol (1 : 2; 60 ml.). The product (13·8 g., 92%) had m. p. 215—217° (decomp.). For analysis the material (5·0 g.) was recrystallised from

[19] D. B. Hope and J. F. Humphries, *J. Chem. Soc.*, 1964, 869.

the same solvent mixture (45 ml.) to give *needles* (3·1 g.), m. p. 216—217° (decomp.), $[\alpha]_D^{20}$ +2·66° (*c* 1 in N-HCl) (Found: C, 33·8; H, 7·3; N, 13·3. $C_6H_{15}ClN_2O_2S$ requires C, 33·6; H, 7·0; N, 13·1%).

S-(N-*Methyl*-2-*aminoethyl*)-L-*cysteine Di-hydrochloride*.—*S*-(*N*-Methyl-2-toluene-*p*-sulphonamidoethyl)-L-cysteine (15·0 g.) was reduced with metallic sodium (2·4 g.) in liquid ammonia (500 ml.). Glacial acetic acid (2 ml.) was added before evaporation of the ammonia. The di-hydrochloride obtained as a syrup was crystallised by addition of ethanol (50 ml.). The *product* (4·7 g.) had m. p. 170—171° (decomp.), $[\alpha]_D^{20}$ −6·4 (*c* 1 in N-HCl), R_A 0·32. On the amino-acid analyser the elution volume was 224—245 ml., colour yield 23·275 (Found: C, 29·1; H, 6·7; N, 11·5. $C_6H_{16}Cl_2N_2O_2S$ requires C, 28·7; H, 6·4; N, 11·2%).

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University Department of Pharmacology,
South Parks Road, Oxford.
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