784. *Synthesis of Cystinol.**

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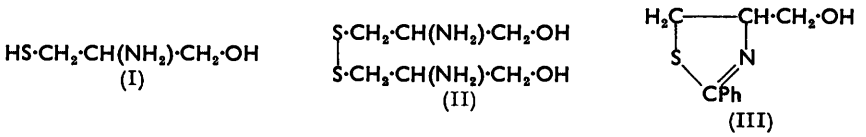
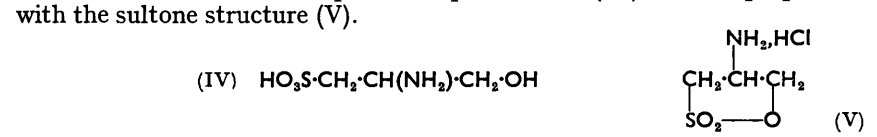
Cystinol (II) and some derivatives of cysteinol (I) have been prepared and their properties examined with a view to their use in the characterisation of *C*-terminal cysteine residues in proteins and peptides.

In connection with a study of the reduction of the *C*-terminal residues of peptides and proteins with metal hydrides [1] it was necessary to prepare 2-amino-3-hydroxypropane-1-thiol (cysteinol) (I) or its disulphide derivative (cystinol) (II).† Reduction of cysteine methyl ester with lithium aluminium hydride yielded a mixture containing some cysteinol, but the pure compound could not be isolated. A similar reduction of the thiazolidine derivative from cysteine methyl ester and benzaldehyde was also unsuccessful. The desired reduction took place when 4-alkoxycarbonyl-2-phenylthiazolines were treated with lithium aluminium hydride. The thiazoline alcohol (III) thereby obtained yielded on hydrolysis a solution giving a positive nitroprusside reaction and presumably containing cysteinol, but this substance could not be crystallised nor could crystalline derivatives be obtained. Mild oxidation of the solution, however, afforded cystinol as a crystalline dihydrochloride.



It was necessary to consider the conditions under which cysteinol or cystinol might be isolated from the mixture obtained when a peptide containing *C*-terminal cysteine had been reduced by a metal hydride and hydrolysed. In practice it is very difficult to avoid oxidation of thiol groups during hydrolysis and the product would certainly contain cystinol and, owing to the disulphide interchange reaction,[2] a mixed disulphide of cysteinol and cysteine might also be present. This complication might be overcome by conversion of the mixed disulphides into thiols by reduction, or into sulphonic acids by oxidation. Accordingly, the chromatographic properties of cysteinol and its derivative with *N*-ethylmaleimide [3] were examined in several solvent systems. *S*-Benzylcysteinol, a compound of possible value for the isolation of cysteinol from hydrolysates, was prepared from *S*-benzylcysteine methyl ester and isolated as the oxalate.

Oxidation of cysteinol hydrochloride with performic acid [4] yielded cysteic acid, whereas bromine water oxidation yielded variable results. In some experiments with bromine only cysteic acid was produced, but occasionally a neutral substance was obtained; this was prepared more easily by the action of bromine water on cystinol hydrochloride. Unlike an amino-alcohol, it was not attacked by periodate ions, and the absence of a disulphide grouping was demonstrated by a negative result with alkaline potassium cyanide–sodium nitroprusside solution. A potentiometric titration showed that a sulphonic acid grouping was not present; the titration curve was typical of an amine hydrochloride. The substance could not have been the expected sulphonic acid (IV), but its properties were consistent with the sultone structure (V).



* Submitted in honour of the seventieth birthday of Sir Ian Heilbron, D.S.O., F.R.S.

† In view of the trivial names adopted for amino-alcohols derived from naturally occurring amino-acids, it seems justifiable to name these two substances as cysteinol and cystinol, respectively.

[1] Crawhall and Elliott, *Biochem. J.*, 1955, **61**, 264.
[2] Ryle and Sanger, *ibid.*, 1955, **60**, 535.
[3] Hanes, Hird, and Isherwood, *Nature*, 1950, **166**, 288.
[4] Sanger, *Biochem. J.*, 1949, **44**, 126.

The R_F values of the various cysteinol derivatives, compared with the corresponding derivatives of cysteine, are given in the Table; these cysteinol derivatives all gave blue spots when their chromatograms were sprayed with the ninhydrin reagent used for detecting amino-acids.

Experimental

L-4-*Ethoxycarbonyl-2-phenylthiazoline.*—L-Cystine {$[\alpha]_D^{22\cdot0}$ −177° (*c* 1·003 in N-HCl); 25 g.} was reduced with a slight excess of sodium in liquid ammonia, the excess of sodium was decomposed with ammonium chloride, and the ammonia evaporated. The product was suspended in ethanol (250 ml.), and a rapid stream of dry hydrogen chloride passed in until the solvent boiled. Ammonium chloride was removed by filtration, and the filtrate saturated with hydrogen chloride at 0°. After boiling under reflux for 30 min. the solvent was evaporated and the crystalline residue of L-cysteine ethyl ester hydrochloride recrystallised from ethanol–ether. The yield was 28 g. (72%), and the m. p. 120—121°.

	Solvent system no.			
	1	2	3	4
Cystinol	0·95	0·60	0·05	0·05
Sultone (IV)	0·56	0·34	0·18	0·14
Cysteic acid	0·08	0·04	0·04	0·00
Cystine	0·3	0·04	0·03	0·00

	Solvent system no.			
	1	2	3	4
N-Ethylmaleimide derivative of:				
Cysteinol	0·95	0·88	0·50	0·31
Cysteine	0·88	0·34	0·39	0·18

Solvent systems: 1, phenol–ammonia;[a] 2, *sec.*-butyl alcohol–ammonia;[b] 3, *sec.*-butyl alcohol–formic acid;[b] 4, *n*-butyl alcohol–acetic acid.[c]

[a] Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224. [b] Hausmann, *J. Amer. Chem. Soc.*, 1952, **74**, 3181. [c] Fromageot, Jutisz, Meyer, and Penasse, *Biochem. Biophys. Acta*, 1950, **6**, 283.

The ester hydrochloride (37·5 g.) was dissolved in ethanol (100 ml.) by warming. Ethyl benzimidate[5] (30 g.) was then added and the mixture kept at room temperature for 1 hr. A mild exothermic reaction occurred after a few minutes and ammonium chloride was deposited. The L-4-*ethoxycarbonyl-2-phenylthiazoline* (38·2 g., 80·5%) remaining after filtration of the ammonium chloride and evaporation of the solvent had b. p. 162°/0·5 mm., $[\alpha]_D^{23\cdot8}$ +14·6° (*c* 1·098 in EtOH) (Found: C, 61·5; H, 5·2; N, 5·7. $C_{12}H_{13}O_2NS$ requires C, 61·3; H, 5·5; N, 5·9%).

DL- *and* L-4-*Hydroxymethyl-2-phenylthiazoline.*—DL-4-Methoxycarbonyl-2-phenylthiazoline (Crawhall and Elliott;[6] 3·7 g.) was dissolved in anhydrous ether (50 ml.) and added to a sludge of lithium aluminium hydride (0·6 g.) in anhydrous ether (50 ml.) at such a rate as to maintain gentle boiling. The solution became bright yellow. After 10 min. water (5 ml.) was added, the solution was filtered, and the ethereal layer was washed with water until colourless, dried (Na_2SO_4), and evaporated. The residual *thiazoline alcohol* crystallised from light petroleum (b. p. 60—80°). The yield was 1·7 g. (53%) and the m. p. 75—76° (Found: C, 61·9; H, 5·4. $C_{10}H_{11}ONS$ requires C, 62·2; H, 5·7%).

In a similar way L-4-ethoxycarbonyl-2-phenylthiazoline (10 g.) gave L-4-*hydroxymethyl-2-phenylthiazoline* (4·6 g., 56%), m. p. 75—76° (Found: C, 62·4; H, 5·7; N, 7·2; S, 16·8. $C_{10}H_{11}ONS$ requires C, 62·2; H, 5·7; N, 7·2; S, 16·6%). The compound had no optical rotation in EtOH at 23·5° (*c* 0·545) but yielded an optically active sultone hydrochloride (see below).

DL-*Cystinol Dihydrochloride.*—DL-4-Hydroxymethyl-2-phenylthiazoline (1 g.) was refluxed in concentrated hydrochloric acid (20 ml.) for 6 hr. in a stream of nitrogen. The cooled solution was extracted with ether and then evaporated to dryness. Cysteinol hydrochloride remained as an oil which gave a positive reaction with sodium nitroprusside and with Hopkins reagent yielded a green precipitate which rapidly darkened with liberation of hydrogen sulphide. When an aliquot part was titrated in N-hydrochloric acid at 0° with 0·1N-iodine, 90% of the theoretical amount for one thiol group was consumed.

The crude hydrochloride (0·27 g.) was dissolved in water (10 ml.) and the solution made alkaline with ammonia. A small crystal of ferrous sulphate was added and a rapid stream of air passed through the solution until the mauve colour had disappeared (1·5 hr.). The solution was kept overnight, the precipitate of iron hydroxide was removed by centrifugation, and the solvent evaporated. The *dihydrochloride* was crystallised by dissolving it in hot concentrated

[5] Elliott, *Biochem. J.*, 1949, **44**, 429.
[6] Crawhall and Elliott, *J.*, 1951, 2071.

hydrochloric acid and adding alcohol. The yield was 0·2 g. (74%), and the m. p. 183° (Found: C, 25·2; H, 6·0; N, 9·8. $C_6H_{18}O_2N_2S_2Cl_2$ requires C, 25·3; H, 6·3; N, 9·8%). The compound did not react with sodium nitroprusside solution, but gave a positive thiol test when potassium cyanide was added to the mixture.

L- *and* DL-2-*Amino*-1-*hydroxypropane*-3-*sulphonic Sultone Hydrochloride*.—L-Cysteinol hydrochloride, obtained by hydrolysis of L-4-hydroxymethyl-2-phenylthiazoline (3 g.) in the usual way, was dissolved in water (10 ml.) and a slight excess of bromine water added. The solution was evaporated, leaving a white solid which was crystallised from aqueous acetic acid. The L-*sultone hydrochloride* (0·43 g.) had m. p. 164—166°, $[\alpha]_D^{22}$ −5·6° (*c* 1·25 in H_2O). It gave no reaction with sodium periodate solution, sodium hydrogen carbonate solution, or alkaline potassium cyanide–sodium nitroprusside solution (Found: C, 20·6; H, 5·0; N, 7·7. $C_3H_8O_3NSCl$ requires C, 20·8; H, 4·6; N, 8·1%).

In some instances, this oxidation could not be controlled and only cysteic acid was obtained. The latter was the sole product when performic acid was used for the oxidation of cysteinol. The cysteic acid produced in these oxidations was identified by analysis (Found: C, 21·5; H, 3·8. Calc. for $C_3H_7O_5NS$: C, 21·3; H, 4·1%) and by its infrared absorption spectrum, which was identified with that of an authentic sample.

In the preparation of DL-*sultone hydrochloride*, it was preferable to use DL-cystinol dihydrochloride. The dihydrochloride (0·15 g.) when dissolved in water (2 ml.) and oxidised with bromine water gave the DL-sultone hydrochloride (0·08 g.) which, crystallised from aqueous acetic acid, had m. p. 251—252° (decomp.). Difficulty was experienced in obtaining accurate analyses for this compound (Found: C, 19·9; H, 4·5; Cl, 18·2. Calc. for $C_3H_8O_3NSCl$: C, 20·8; H, 4·6; Cl, 20·4%), but its structure followed from its chemical properties which were identical with those of the L-sultone. On potentiometric titration in aqueous solution, the compound combined with one equivalent of alkali between pH 6 and pH 10. This is considered to be due to the conversion of the NH_3^+ group into the un-ionised form.

L-S-*Benzylcysteinol Oxalate*.—*S*-Benzylcysteine ethyl ester hydrochloride (10 g.) was treated with triethylamine (3·7 g.), and the free ester extracted into ether. Reduction was carried out with lithium aluminium hydride (2 g.) in the usual way. The crude product was an oil (2·6 g.) to which was added a saturated solution of oxalic acid in ethanol. L-S-*Benzylcysteinol oxalate* (2·65 g.) crystallised and after seven recrystallisations from ethanol had m. p. 181—183°, $[\alpha]_D^{19\cdot5}$ −61·2° (*c* 0·196 in MeOH) (Found: C, 54·6; H, 6·8; N, 5·8. $C_{22}H_{32}O_6N_2S_2$ requires C, 54·6; H, 6·6; N, 5·8%).

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