277. *Syntheses of Poly-*S*-alkyl-*L*-cysteines.*

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Various *S*-alkylcysteine derivatives have been prepared and polymerised by various methods through their *N*-carboxy-anhydrides. Properties of the amino-acids and their polymers are reported.

SOME *S*-alkylcysteine derivatives have been found to occur in Nature. Stoll and Seebeck[1] isolated *S*-allyl-L-cysteine *S*-oxide (aliin) from garlic. *S*-Methylcysteine *S*-oxide has been found in various plants;[2] the deoxo-compound was enzymically synthesised from serine and methanethiol and has been suggested as an intermediate in the microbial biosynthesis of cysteine.[3] *S*-Ethylcysteine has antituberculosis activity.[4] *S*-Dichlorovinylcysteine produces fatal aplastic anæmia in young calves.[5] In an investigation of the biological mode of action of alkyl alkanesulphonates as alkylating agents,[6] *S*-alkyl derivatives of cysteine were isolated. Finally poly-*S*-benzyl-[7] and -*S*-allyl-cysteine[8] have been synthesised.

It seemed interesting to synthesise a series of *S*-alkylcysteines and their polypeptide polymers. General methods for the preparation of *S*-alkylcysteines, summarised by Armstrong and Lewis,[9] comprise Schotten–Baumann reaction of an alkyl halide with cysteine in liquid ammonia or in sodium hydroxide solution. In the latter procedure the reactants are dissolved in alcohol–water, and sufficient sodium hydroxide is added to neutralise the hydrogen halide formed and to enable salt formation with the carboxyl group of cysteine. We have found that on use of a smaller quantity of alkali, sufficient to remove only the hydrogen halide acid formed, the reaction proceeds relatively fast and the *S*-alkylcysteine is precipitated, as it is formed, provided it is insoluble in the alcohol–water mixture used; thus the equilibrium shifts in favour of the formation of additional alkylcysteine. In this way it was possible to prepare pure crystalline *S*-ethyl- or *S*-benzyl-cysteine in high yield in a comparatively short time. The use of a smaller amount of alkali is especially advisable in the preparation of *S*-alkylcysteine derivatives which are soluble in water and insoluble in ethanol, and thus are difficult to free from sodium halides. For the same reason triethylamine instead of sodium hydroxide is recommended.

The following *S*-alkyl-L-cysteines were prepared and polymerised: the known methyl, ethyl, propyl, isopropyl, butyl, and pentyl derivatives as well as the new hexyl-, dodecyl-, and 1-naphthylmethyl-L-cysteine.

Reaction of the reactive α-chloromethylnaphthalene and of benzyl chloride with cysteine was complete within a few to 30 min., while the reaction with dodecyl bromide required several days.

Generally, the lower *S*-alkylcysteines, such as methyl, ethyl, and allyl, are soluble in water and insoluble in ethanol. The higher homologues are less soluble in water and more soluble in ethanol. Recrystallisation of the former is effected from aqueous ethanol, and of the latter from a large volume of water, ethanol, or dilute acetic acid. Ascending paper chromatography with (A) 80% aqueous phenol, (B) butanol–acetic acid–water (40 : 10 : 10) (C) 65% aqueous pyridine, or (D) 60% aqueous acetone, showed that the R_F values of the

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[1] Stoll and Seebeck, *Adv. Enzymol.*, 1951, **11**, 377.

[2] Morris and Thompson, *Chem. and Ind.*, 1955, 951; *J. Amer. Chem. Soc.*, 1956, **78**, 1605.

[3] Wolff, Black, and Downey, *J. Amer. Chem. Soc.*, 1956, **78**, 5958.

[4] Brown, Matzuk, Becker, Conbere, Constantin, Solotorovsky, Winstein, Ironson, and Quastel, *J. Amer. Chem. Soc.*, 1954, **76**, 3860.

[5] McKenny, Weakley, Eldridge, Campbell, Cowan, Picker, jun., and Biester, *J. Amer. Chem. Soc.*, 1957, **79**, 3932.

[6] Roberts and Warwick, *Nature*, 1957, **179**, 1181; *Biochem. Pharmacol.*, 1958, **1**, 60.

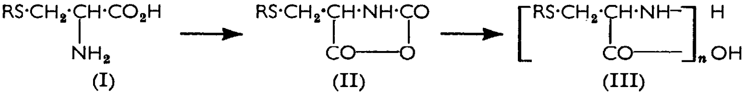
[7] Blackley, Sumner, and Spencer, *Canad. J. Technol.*, 1952, **30**, 258; Berger, Noguchi, and Katchalski, *J. Amer. Chem. Soc.*, 1956, **78**, 4483.

[8] Frankel and Zilkha, *Nature*, 1955, **175**, 1045.

[9] Armstrong and Lewis, *J. Org. Chem.*, 1951, **16**, 749.

S-alkylcysteines increase with lengthening of the *S*-alkyl chain. The *S*-alkylcysteines have high decomposition temperatures.

Polymerisation was carried out *via* the *N*-carboxy-anhydrides. Passing carbonyl chloride into a suspension of *S*-alkyl-L-cysteine (I) in dioxan at 60° or in tetrahydrofuran at room temperature yielded the *N*-carboxy-anhydrides (II) which were purified or recrystallised. Polymerisation of the anhydrides was carried out under various conditions. (*a*) The anhydride (about 1 g.) was dissolved in dry pyridine (about 20 ml.) and left at room temperature for about 2 days, then heated for several hours on a boiling-water bath. Polymerisation was fast, with precipitation of polymer, in some instances after a few minutes. In the few cases where the polymers were not precipitated, they were recovered on evaporation *in vacuo.* (*b*) In dry dioxan at room temperature polymerisation began only after the addition of a trace of water or triethylamine. It was allowed to proceed for several days at room temperature, then for several hours on boiling-water bath. The polymers were usually precipitated. Polymerisation was slower than in pyridine. (*c*) In a small quantity of boiling ethanol, polymerisation was finished within a few minutes, probably initiated by a trace of *S*-alkylcysteine ethyl ester formed. (*d*) The anhydrides polymerised when heated in a high vacuum above the melting points (up to 130—150°) for a few hours.



$$\underset{\text{NH}_2}{\text{RS·CH}_2\text{·CH·CO}_2\text{H}} \longrightarrow \text{RS·CH}_2\text{·CH·NH·CO} \;(\text{CO—O}) \longrightarrow \text{H}\left[\text{RS·CH}_2\text{·CH(CO)·NH}\right]_n\text{OH}$$

(I) (II) (III)

The polymers were insoluble in dilute acid or alkali, boiling water, or ethanol. They dissolved partly in hot glacial acetic acid, anhydrous formic acid, dichloroacetic acid, or dimethylformamide. They slowly gave positive biuret reactions in the presence of pyridine.

EXPERIMENTAL

M. p.s were determined on a Fisher–Jones apparatus.

S-*Benzyl*-L-*cysteine*.—(*a*) L-Cysteine hydrochloride (7·8 g., 0·05 mole) was dissolved in 2N-sodium hydroxide (50 ml.) and ethanol (60 ml.). Benzyl chloride (7 g., 0·055 mole) was added with stirring. In a few minutes an exothermic reaction started with precipitation of *S*-benzyl-L-cysteine. Stirring was continued for 30 min., the pH adjusted to 6—7, the mixture cooled in ice water, and the precipitate filtered off and washed with water, ethanol, and ether. The yield was 9·5 g. (90%). After recrystallisation from water, the m. p. was 211°.

This method of preparation is more convenient than that involving use of liquid ammonia.[10]

(*b*) L-Cysteine hydrochloride (7·8 g., 0·05 mole) was dissolved in water (30 ml.) and ethanol (50 ml.). Triethylamine (10 g., 0·1 mole) was added, followed by benzyl chloride (7 g., 0·055 mole). Stirring was started; the reaction proceeded as before, yielding 9 g. (85%) of *S*-benzyl-L-cysteine.

S-*Ethyl*-L-*cysteine*.—L-Cysteine hydrochloride (7·8 g., 0·05 mole) was dissolved in cold 2N-sodium hydroxide (50 ml.) under hydrogen. Ethanol (100 ml.) was added, followed by ethyl bromide (6 g., 0·055 mole). Stirring was started and after about 30 min. precipitation of *S*-ethylcysteine started. After 6 hr. the solution was acidified to pH 6—7 with concentrated hydrochloric acid, and the *S*-alkylcysteine filtered off and washed with alcohol–water (2 : 1) (yield, 4 g., 55%; m. p. 245°). Concentration of the mother-liquor gave a further 1·2 g. (15%). Recrystallisation from 50% ethanol raised the m. p. to 250° The substance was identical with that prepared in liquid ammonia.[9] R_F values were (A) 0·77, (B) 0·67, (C) 0·74, (D) 0·8.

N-*Carboxy-anhydride of* S-*Ethyl*-L-*cysteine*.—(*a*) Dry carbonyl chloride was passed with stirring into a suspension of *S*-ethyl-L-cysteine (2 g.) in dry dioxan (120 ml.) at 60° for 90 min. The solution was evaporated *in vacuo* at 40° and the residue left overnight in a vacuum-desiccator over phosphoric oxide. The N-*carboxy-anhydride* had m. p. 60°. Recrystallisation

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[10] Stevens, Johnson, and Watanabe, *J. Biol. Chem.*, 1955, **212**, 49; Goldschmidt and Jutz, *Chem. Ber.*, 1953, **86**, 1116.

from ethyl acetate–light petroleum gave 1·9 g. (80%), of m. p. 69°, resolidifying at about 120° (Found: N, 7·9. $C_6H_9O_3NS$ requires N, 8·0%).

(*b*) Carbonyl chloride was passed with stirring into a suspension of *S*-ethyl-L-cysteine (1 g.) in tetrahydrofuran (50 ml.; dried over sodium). After about 15 min. the substance dissolved and carbonyl chloride was passed in for an additional 30 min. The solution was filtered and evaporated *in vacuo* at room temperature. Purification of the anhydride was carried out as above.

Polymerisation of the N-*carboxy-anhydride of* S-*Ethyl*-L-*cysteine*.—(*a*) Dry, freshly recrystallised anhydride (1 g.) was dissolved in pyridine (20 ml.). After a few hours a gelatinous precipitate was formed. The whole was left for 2 days at room temperature, then heated on a water-bath for a few hours. Ether was added and the *polymer* filtered off and washed with ethanol and ether (yield, 0·52 g., 70%) [Found: C, 44·3; H, 6·7; N, 9·7; N (Van Slyke), 1·0; S, 23·1. $(C_5H_9ONS)_n + H_2O$ ($n = 11$) requires C, 45·2; H, 6·9; N, 10·5; N (Van Slyke), 1·0; S, 24·1%]. Analyses of polymers are often unsatisfactory.[11]

(*b*) The anhydride (0·5 g.) was dissolved in absolute ethanol (5 ml.) and immediately immersed in a boiling-water bath. The *polymer* was precipitated within a few minutes. Heating was continued for 30 min. The solution was cooled, and the precipitate then filtered off and washed with ethanol (yield, 0·18 g., 50%) [Found: C, 45·1; H, 7·0; N, 10·1; N (Van Slyke), 0·4; S, 23·1. $(C_5H_9ONS)_n + C_2H_5{\cdot}OH$ ($n = 25$) requires C, 45·9; H, 7·0; N, 10·5; N (Van Slyke), 0·42; S, 24·1%].

(*c*) The anhydride (0·5 g.) was heated at 10^{-3} mm. At about 70° the anhydride melted with a slight evolution of gas. The temperature was raised slowly to 130—140° in 30 min. and kept thereat for 2 hr. During this time there was much gas evolution, and the polymer solidified. The *polymer* was purified by trituration with hot water and ethanol, and washed with ethanol and ether, giving 0·26 g. (70%) [Found: C, 45·7; H, 6·9; N, 10·4; N (Van Slyke), 0·2; S, 23·7. $(C_5H_9ONS)_n + H_2O$ ($n = 53$) requires C, 45·9; H, 6·9; N, 10·7; N (Van Slyke), 0·2; S, 24·4%].

Poly-S-*methyl*-L-*cysteine*.—*S*-Methyl-L-cysteine was prepared from methyl iodide and cysteine in alkali;[9] recrystallisation from 50% ethanol gave m. p. 249—250°, R_F (A) 0·69, (B) 0·53, (C) 0·71, (D) 0·75. The N-*carboxy-anhydride*, prepared as described above and recrystallised from ethyl acetate–light petroleum, had m. p. 74° [Found: N, 8·6; N (Van Slyke), 8·8. $C_5H_7O_3NS$ requires N, 8·7%]. When the anhydride (2 g.) was dissolved in pyridine (50 ml.), *polymer* was precipitated almost immediately [Found: C, 41·2; H, 7·1; N, 11·7. $(C_4H_7ONS)_n$ requires C, 41·0; H, 7·0; N, 11·9%].

Poly-S-*propyl*-L-*cysteine*.—*S*-Propyl-L-cysteine, prepared from propyl bromide and cysteine[9] and recrystallised from 50% ethanol, had m. p. 248°, R_F (A) 0·79, (B) 0·77, (C) 0·78, (D) 0·84. The anhydride gave a *polymer* in dioxan or absolute ethanol. [Found for polymer from dioxan: C, 49·4; H, 7·6; N, 9·1; S, 21·8. Found for polymer from ethanol: C, 49·5; H, 7·8; N, 9·1; S, 21·5. $(C_6H_{11}ONS)_n$ requires C, 49·6; H, 7·6; N, 9·6; S, 22·1%].

Poly-S-*isopropyl*-L-*cysteine*.—*S*-Isopropyl-L-cysteine, prepared from isopropyl bromide and cysteine,[12] recrystallised from ethanol, had m. p. 224°,[13] R_F (A) 0·82, (B) 0·73, (C) 0·8, (D) 0·84. A *polymer* was prepared from the *N*-carboxy-anhydride in pyridine [Found: C, 48·8; H, 7·4; N, 9·2; S, 21·2. $(C_6H_{11}ONS)_n$ requires C, 49·6; H, 7·6; N, 9·6; S, 22·1%].

Poly-S-*butyl*-L-*cysteine*.—*S*-Butyl-L-cysteine, prepared from butyl bromide and cysteine[9] and recrystallised from water, had m. p. 247—248°,* R_F (A) 0·9, (B) 0·82, (C) 0·82, (D) 0·89. The N-*carboxy-anhydride*, recrystallised from ether or ethyl acetate–light petroleum, melted at 55—56° (Found: N, 6·7. $C_8H_{11}O_3NS$ requires N, 6·9%). *Polymers* were obtained in pyridine, dioxan, and nitrobenzene [Found for polymer from pyridine: C, 52·5; H, 8·2; N, 8·4; S, 19·5. Found for polymer from dioxan: C, 52·5; H, 8·2; N, 8·3; S, 19·6. Found for polymer from nitrobenzene: C, 52·3; H, 8·1; N, 8·4; S, 19·5. $(C_7H_{13}ONS)_n$ requires C, 52·8; H, 8·2; N, 8·8; S, 20·1%].

Poly-S-*pentyl*-L-*cysteine*.—*S*-Pentyl-L-cysteine, prepared from pentyl bromide and cysteine in alkali[9] and recrystallised from dilute acetic acid, had m. p. 248°, R_F (A) 0·91, (B) 0·88, (C)

* M. p. reported in ref. 9 was 242—244°; Izomiya, *J. Chem. Soc. Japan, Pure Chem. Sect.*, 1951, **72**, 1050, reported 247—249°.

[11] See for instance Marvel, Cooke, and Cowan, *J. Amer. Chem. Soc.*, 1940, **62**, 3497.

[12] Stoll and Seebeck, *Helv. Chim. Acta*, 1949, **32**, 866.

[13] Gawron and Lieb, *J. Amer. Chem. Soc.*, 1952, **74**, 834.

0·86, (D) 0·9. A *polymer* was obtained from the anhydride in pyridine and in a high vacuum [Found for polymer from pyridine: C, 54·8; H, 8·7; N, 7·5; S, 18·6. Found for polymer (*vacuo*): C, 54·9; H, 8·4; N, 7·5; S, 18·0. $(C_8H_{15}ONS)_n$ requires C, 55·5; H, 8·7; N, 8·1; S, 18·5%].

Poly-S-*hexyl*-L-*cysteine*.—S-*Hexyl*-L-*cysteine*, prepared (83%) from hexyl bromide (5·5 g.) and cysteine hydrochloride (5·2 g.) in 2N-sodium hydroxide (50 ml.) and ethanol (100 ml.), and recrystallised from 50% acetic acid, had m. p. 242° (Found: N, 6·6. $C_9H_{19}O_2NS$ requires N, 6·8%), R_F (A) 0·92, (B) 0·9, (C) 0·87, (D) 0·91. The N-*carboxy-anhydride*, recrystallised from ether–light petroleum, had m. p. 42—43° (Found: N, 5·9. $C_{10}H_{17}O_3NS$ requires N, 6·0%). *Polymer* was obtained from the anhydride in absolute ethanol and high vacuum [Found for polymer from ethanol: C, 57·1; H, 8·8; N, 7·3; S, 17·1. Found for polymer (*vacuo*): C, 57·1; H, 8·7; N, 7·2; S, 17·1. $(C_9H_{17}ONS)_n$ requires C, 57·8; H, 9·1; N, 7·5; S, 17·1%].

S-*Dodecyl*-L-*cysteine*.—To L-cysteine hydrochloride (4·7 g.) (0·03 mole), dissolved in 2N-sodium hydroxide (45 ml.) under hydrogen, ethanol (100 ml.) and dodecyl bromide (7·5 g., 0·03 mole) were added, followed by a few ml. of ether to dissolve the bromide. After 3 days with occasional shaking a negative reaction for free cysteine was obtained with nitroprusside. The solution was acidified with hydrochloric acid, the *dodecylcysteine* (7 g.; 80%) was filtered off, washed with water, and recrystallised from acetic acid; it had m. p. 216° and R_F (A) 0·92, (B) 0·95, (C) 0·9, (D) 0·92. It is slightly soluble in hot ethanol (Found: C, 62·3; H, 10·5; N, 4·8; S, 11·0. $C_{15}H_{31}O_2NS$ requires C, 62·3; H, 10·7; N, 4·8; S, 11·1%).

The N-*carboxy-anhydride*, prepared in tetrahydrofuran, melted at 65°; recrystallised from ethyl acetate–light petroleum, it had m. p. 66—67° (Found: N, 4·7. $C_{16}H_{29}O_3NS$ requires N, 4·9%). Polymerisation of the anhydride was carried out in pyridine and in a high vacuum [Found for *polymer* from pyridine: C, 65·7; H, 10·5; N, 4·9; S, 11·5. Found for polymer (*vacuo*): C, 66·2; H, 10·7; N, 5·2; S, 11·8. $(C_{15}H_{29}ONS)_n$ requires C, 66·4; H, 10·7; N, 5·2; S, 11·8%].

Poly-S-1-*naphthylmethyl*-L-*cysteine*.—S-1-*Methylnaphthyl*-L-*cysteine* (80%) was prepared from α-chloromethylnaphthalene (5·1 g.) and L-cysteine hydrochloride (4·7 g.) in 2N-sodium hydroxide (45 ml.) and ethanol (90 ml.). (On use of only 2 equivalents of alkali the naphthylmethyl-L-cysteine was precipitated immediately.) Yield 6·2 g. It is slightly soluble in hot water or hot ethanol; recrystallised from 60% aqueous pyridine or glacial acetic acid, it had m. p. 208° and R_F (A) 0·89, (B) 0·86, (C) 0·87, (D) 0·87 (Found: C, 60·4; H, 5·9; N, 4·9; S, 11·2. $C_{14}H_{15}NO_2S,H_2O$ requires C, 60·2; H, 6·1; N, 5·0; S, 11·4%). The N-*carboxy-anhydride*, prepared in tetrahydrofuran and recrystallised from ethyl acetate–light petroleum, melted at 105° (Found: N, 5·1. $C_{13}H_{15}O_3NS$ requires N, 5·3%). *Polymer* was obtained in pyridine (Found: C, 68·0; H, 5·1; N, 5·7; S, 12·8. $(C_{14}H_{13}ONS)_n$ requires C, 69·1; H, 5·4; N, 5·8; S, 13·2%). The anhydride also polymerised in hot glacial acetic acid, probably with initiation by the *N*-acetyl derivative of the *S*-alkylcysteine formed by reaction of acetic acid with the anhydride. After the anhydride (0·25 g.) had been heated in glacial acetic acid (3 ml.) on a boiling-water bath for 3 hr., the precipitated polymer was filtered off and washed with ether (Found: N, 5·7%).

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[*Received, September 22nd,* 1959.]