82. *Syntheses of Some Polymeric Polypeptides of Lanthionine.*

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Polymers of lanthionine having defined structure were obtained on polymerisation of suitable protected monomers, *e.g.*, the *N*-carboxy-anhydride of mono-*N*-benzyloxycarbonyl-lanthionine monobenzyl ester.

LANTHIONINE, a tetrafunctional diamino-dicarboxylic acid, containing a sulphur bridge between two alanine residues, was obtained from various proteins,[1] and in hydrolysates of wool or hair treated with alkali.[2] Its formation in the degradation of cystine has been discussed by Schoeberl and others.[3]

Work on polymeric peptides obtained from tetrafunctional amino-acids has been scanty. A polymeric polypeptide from cystine was obtained by Jones and Lundgren [4] from its *NN'*-dicarboxy-anhydride, but as no precautions were taken to assure linear polymerisation it might have been three-dimensional.

Our preliminary experiments were on polymerisation of suitable protected monomeric derivatives of the more accessible cystine; the known conversion of cystine by alkali into lanthionine was tried to arrive at polylanthionine derivatives.

Monobenzyloxycarbonyl-L-cystine [5] in suspension in dioxan was treated with carbonyl chloride, giving the corresponding mono-*N*-carboxy-anhydride which polymerised when heated *in vacuo*. The structure of this polymer containing the free carboxyl group may

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[1] Stein, *Chem. and Ind.*, 1955, 774; Berridge, Newton, and Abraham, *Biochem. J.*, 1952, **52**, 529; Alderton and Fevold, *J. Amer. Chem. Soc.*, 1951, **73**, 463.

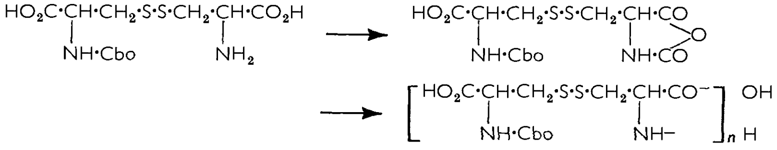
[2] Horn, Jones, and Ringel, *J. Biol. Chem.*, 1941, **138**, 141; 1942, **144**, 93.

[3] Schoeberl and Wagner, *Z. physiol. Chem.*, 1956, **304**, 97.

[4] Jones and Lundgren, *J. Amer. Chem. Soc.*, 1951, **73**, 5465.

[5] Marshall, Winitz, Birnbaum, and Greenstein, *J. Amer. Chem. Soc.*, 1957, **79**, 4538.

be assumed to be more defined than that obtained from the *NN'*-dicarboxy-anhydride by Jones and Lundgren.[4] Free polycystine was obtained by removal of the *N*-benzyloxycarbonyl group by hydrobromic acid in acetic acid.



Cbo = $-CO{\cdot}O{\cdot}CH_2Ph$

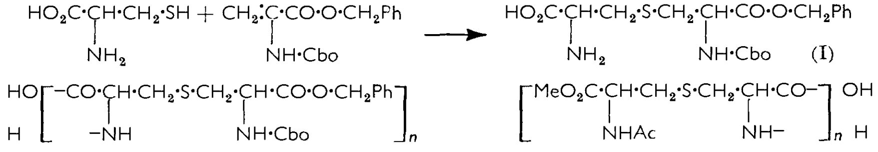
Attempts were made to obtain polylanthionine by treating the above polymer and the free polycystine with alkali, but the conversion was unsatisfactory. Consequently we tried to prepare polylanthionine from *meso*-lanthionine itself. Unlike cystine, *meso*-lanthionine did not give the mono-, but exclusively, the *NN'*-di-(benzyloxycarbonyl) derivative. As lanthionine did not yield the dicarboxyanhydride in dioxan this compound was synthesised from the *NN'*-di(benzyloxycarbonyl)-*meso*-lanthionine by reaction with phosphorus pentachloride in ether; it was polymerised in bulk by heating it *in vacuo*, and on its hydrolysis lanthionine was recovered. This polymer too is not satisfactorily defined in structure.

A structurally defined polylanthionine was finally prepared by using mono-*N*-benzyloxycarbonyl-lanthionine monobenzyl ester, which was obtained as follows:

Benzyloxycarbonylaminoacrylic acid[6] was esterified with benzyl alcohol, and L-cysteine was added to the double bond in aqueous alcohol at pH 7—8. One carboxyl and one adjacent amino-group of the lanthionine molecule thus formed were protected, so that only two functional groups remained free. Passing carbonyl chloride into a suspension of this product (I) in dioxan gave its *N*-carboxyanhydride which was polymerised in pyridine solution to a product (II) with a chain length of 19 units.

Similarly, the " adjacent " monomethyl ester of *N*-acetyl-lanthionine was synthesised from methyl acetamidoacrylate and L-cysteine and converted into the *N*-carboxyanhydride and thence into a polymer (III).

In the present work we also synthesised and polymerised *S*-2-methoxycarbonylethyl-L-cysteine, from methyl acrylate and L-cysteine. The product has structure (III) with the acetamido-group replaced by hydrogen; it has a high decomposition temperature and is insoluble in the ordinary organic solvents.



(I)

(II) (III)

EXPERIMENTAL

M. p.s were determined in a Fisher–Johns apparatus.

*Polymerisation of Mono-*N*-benzyloxycarbonyl-*L*-cystine.*—Dry carbonyl chloride was passed with stirring for 1 hr. through a suspension of dried mono-*N*-benzyloxycarbonyl-L-cystine[5] (0·75 g.; recrystallised from 25% aqueous acetic acid) in dry dioxan (100 ml.) at 50°. The solution was filtered, then evaporated *in vacuo* at 30°, and the residue was washed with light petroleum several times. It crystallised during several hours at 0° under light petroleum.

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[6] Kildisheva, Rasteikene, and Knunyants, *Bull. Acad. Sci. U.S.S.R.*, 1955, 231.

Recrystallisation from ether–light petroleum yielded the N-*carboxyanhydride*, m. p. 30—32° (Found: N, 6·8. $C_{15}H_{16}N_2O_7S_2$ requires N, 7·0%).

Polymerisation was carried out in bulk at 110°/10^{-3} mm. for 30 min., then at 130°/10^{-3} mm. for 2 hr. The *polymer* obtained was purified from acetic acid–water [Found: C, 46·9; H, 4·6; N, 7·4; amino-N (Van Slyke), 0·3. $(C_{14}H_{16}N_2O_5S_2)_{12},H_2O$ requires C, 47·0; H, 4·5; N, 7·8; amino-N, 0·3%].

*Poly-*L*-cystine.*—Poly-(*N*-benzyloxycarbonylcystine) (0·1 g.) was heated in a saturated solution of hydrobromic acid in acetic acid (20 ml.) for 15 min. at 30°, then kept at room temperature for 2 hr. and concentrated in the vacuum first of a water-pump, then of an oil-pump. The residue was extracted with ether, dissolved in a few ml. of water, neutralised with dilute aqueous ammonia, and immediately acidified with glacial acetic acid. The *polycystine* was filtered off and washed with water [Found: C, 31·9; H, 4·7; N, 12·3; S, 28·0. $(C_6H_{10}N_2O_3S_2)_{12},H_2O$ requires C, 32·2; H, 4·5; N, 12·5; S, 28·6%].

NN′*-Dibenzyloxycarbonyl-*L*-cystine Dibenzyl Ester.*—*NN′*-Di(benzyloxycarbonyl)-L-cystine [7] (5·1 g.), freshly distilled benzyl alcohol (4 ml.), and toluene-*p*-sulphonic acid (0·1 g.) in dry benzene (200 ml.) were refluxed for 8 hr. in an azeotropic-distillation apparatus. After extraction with 2% aqueous sodium hydrogen carbonate the solution was washed with water, dried and evaporated *in vacuo*. The NN′*-dibenzyloxycarbonyl-*L*-cystine dibenzyl ester* crystallised slowly (4·2 g.) and had m. p. 79° (from ethyl acetate–light petroleum) (Found: C, 63·0; H, 5·2; N, 3·9; S, 9·3. $C_{36}H_{36}N_2O_8S_2$ requires C, 62·8; H, 5·3; N, 4·1; S, 9·3%).

NN′*-Dibenzyloxycarbonyl-*meso*-lanthionine Dibenzyl Ester.*—*NN′*-Dibenzyloxycarbonyl-*meso*-lanthionine (2·4 g.), freshly distilled benzyl alcohol (2 ml.), and toluene-*p*-sulphonic acid (0·1 g.) in toluene (150 ml.) were refluxed for 8 hr. in an azeotropic distillation apparatus. Magnesium oxide was added (0·5 g.) and the whole shaken and filtered. The toluene was removed at the water-pump, then at the oil-pump. The *dibenzyl ester*, dissolved in acetone and precipitated with water, crystallised gradually (1·4 g., 40%); recrystallised from aqueous ethanol, it had m. p. 90° (Found: C, 65·5; H, 5·7; N, 4·3; S, 5·2. $C_{36}H_{36}O_8N_2S$ requires C, 65·8; H, 5·5; N, 4·3; S, 4·9%).

NN′*-Dibenzyloxycarbonyl-*meso*-lanthionine Diethyl Ester.*—A solution of *meso*-lanthionine (10 g.) in absolute ethanol (200 ml.) was saturated with hydrogen chloride and refluxed (10 hr.). Chromatography in aqueous phenol (80%) showed the presence of only one compound (R_F 0·95), and the absence of free lanthionine. The solution was evaporated at the water-pump, ethanol (50 ml.) added and removed again, and the residue was taken up in water (125 ml.), and treated with, first, magnesium oxide (5 g.) and then an 80% solution of benzyl chloroformate (25 g.) in toluene during 1 hr. with stirring and cooling (ice-bath). Stirring was continued for 2 hr., ether (2 × 50 ml.) being added. The solution was filtered, more ether (50 ml.) added, and the ether layer washed with water, dried, and evaporated. Trituration of the residue with light petroleum gave the *diethyl ester* (19 g., 70%), m. p. 72° (from ether–light petroleum) (Found: N, 5·2; EtO, 16·6. $C_{26}H_{32}N_2O_8S$ requires N, 5·3; EtO, 16·9%).

Di(acid Chloride) of NN′*-Dibenzyloxycarbonyl-*meso*-lanthionine.*—*NN′*-Dibenzyloxycarbonyl-*meso*-lanthionine (2·4 g.) in dry ether (50 ml.) was treated at 0° with phosphorus pentachloride (2 g.) with shaking. The precipitated *dichloride*, when washed with ether and recrystallised from dioxan–ether, had m. p. 75—76° (decomp.), resolidified 90—95° (1·6 g., 60%) (Found: N, 5·4; Cl, 13·4. $C_{22}H_{22}Cl_2O_6N_2S$ requires N, 5·4; Cl, 13·8%).

Polylanthionine.—The preceding dichloride (1 g.) was polymerised by heating in bulk at 10^{-3} mm. for 30 min. at 100° and 1 hr. at 120°. The *polylanthionine* formed a very hard polymer, insoluble in acetic acid, dimethylformamide, and other organic solvents [Found: C, 40·9; H, 5·2; N, 14·5. $(C_6H_8O_2N_2S)_n$ requires C, 41·9; H, 4·7; N, 16·3%].

N*-Benzyloxycarbonyl-lanthionine Monobenzyl Ester.*—Benzyloxycarbonylaminoacrylic acid [6] (12 g.), dry benzene (300 ml.), quinol (0·1 g.), toluene-*p*-sulphonic acid (0·1 g.), and freshly distilled benzyl alcohol (11 g.) were refluxed for 5 hr. in an azeotropic distillation apparatus, then shaken with magnesium oxide, filtered, and evaporated *in vacuo* at room temperature. The residue was taken up in ethanol (50 ml.), added to a solution of cysteine hydrochloride (8 g.) in water (40 ml.) that had been neutralised with N-sodium hydroxide, and stirred for 3 hr. at room temperature under hydrogen, then on a water-bath until it gave a negative reaction with sodium nitroprusside, while the pH was kept at 7—8 by addition of sodium hydroxide solution.

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[7] "Biochemical Preparations," Vol. II, p. 75, John Wiley, N.Y., 1952.

Chromatography showed a compound of R_F 0·9 besides little cystine. The solution was filtered, concentrated *in vacuo* to half its volume, and extracted with ether. On further concentration, an oil separated which solidified to a gelatinous precipitate of N-*benzyloxycarbonyl-lanthionine monobenzyl ester*; this was filtered off and washed with water. It had m. p. 175° after precipitation from pyridine by ether (14 g.) and was soluble in acetic acid, dioxan, and ethanol [Found: C, 58·5; H, 5·6; N, 6·6; amino-N (Van Slyke), 3·2; S, 7·5. $C_{21}H_{24}N_2O_6S$ requires C, 58·3; H, 5·5; N, 6·5; amino-N, 3·2; S, 7·4%].

Poly-(N-*benzyloxycarbonyl-lanthionine Monobenzyl Ester*).—The preceding monobenzyl ester (1·5 g.) in dioxan (50 ml.) was stirred while carbonyl chloride was passed through it for 30 min. at room temperature, then at 50° for 30 min.; after filtration and evaporation *in vacuo*, dioxan was added (25 ml.) and the whole was evaporated again. The residue was washed several times with light petroleum. The *N*-carboxyanhydride obtained was polymerised in dry pyridine (25 ml.) for 24 hr. at room temperature and 8 hr. on the water-bath. The pyridine was driven off *in vacuo* and the *polymer* washed with boiling water and dried. It was soluble in organic solvents and was purified from aqueous ethanol [Found: C, 60·6; H, 5·2; N, 6·5; amino-N (Van Slyke), 0·18; S, 7·4. $(C_{21}H_{22}N_2O_5S)_{19},H_2O$ requires C, 60·7; H, 5·3; N, 6·7; amino-N, 0·18; S, 7·7%].

N-*Acetyl-lanthionine Monomethyl Ester*.—Methyl acetamidoacrylate [8] (5 g.) was added to a solution of L-cysteine (3·2 g.) in water (25 ml.). Carbon dioxide was passed with stirring and N-sodium hydroxide added to give pH 7. After 30 minutes' stirring the temperature was raised to 60° and stirring continued for 2 hr. After extraction with ether the solution was evaporated *in vacuo*, and the residue dissolved in water, filtered, and precipitated with acetone. The precipitate was extracted with ethyl alcohol which on evaporation gave N-*acetyl-lanthionine monomethyl ester* (3 g.), m. p. 80—85°, R_F 0·84—0·86 [Found: C, 41·2; H, 6·4; N, 10·4; amino-N (Van Slyke), 5·2. $C_9H_{16}N_2O_5S$ requires C, 40·9; H, 6·1; N, 10·6; amino-N, 5·3%].

Poly-(N-*acetyl-lanthionine Monomethyl Ester*).—*N*-Acetyl-lanthionine monomethyl ester (0·5 g.) in dioxan (50 ml.) was stirred while carbonyl chloride was passed through it for 90 min. at 50°. The solution was filtered and evaporated *in vacuo*, dioxan (25 ml.) added, and the whole evaporated again. The *N*-carboxyanhydride was washed several times with light petroleum. It was polymerised in bulk at 10^{-3} mm. at 90° for 1 hr. and at 120° for 2 hr. The polymer gave a positive biuret reaction [Found: N, 11·1; amino-N (Van Slyke), 0·4. $(C_9H_{14}N_2O_4S)_{15},H_2O$ requires N, 11·3; amino-N, 0·4%].

S-2-*Methoxycarbonylethyl*-L-*cysteine*.—Methyl acrylate (5·7 g.) was added to a solution of L-cysteine hydrochloride (4·7 g.) in water (10 ml.), hydrogen was passed through it, and N-sodium hydroxide was dropped in with stirring to give pH 7. The solution became hot and crystals separated. Stirring was continued for another hour (to absence of SH). Recrystallisation from 70% ethanol gave S-2-*methoxycarbonylethyl*-L-*cysteine*, m. p. 217° (60%), R_F (80% phenol) 0·82—0·84 (Found: C, 40·8; H, 6·4; N, 6·7; S, 15·2. $C_7H_{13}NO_4S$ requires C, 40·6; H, 6·3; N, 6·8; S, 15·4%).

N-*Benzyloxycarbonyl*-S-2-*methoxycarbonylethyl*-L-*cysteine*.—The preceding acid (1 g.) in water (50 ml.) and ether (25 ml.) was stirred with magnesium oxide (0·5 g.) at 0° and benzyl chloroformate (1 g.) was dropped in. After 30 min. the solution was left at room temperature for 1 hr., then filtered, extracted with ether, and acidified. The oil was removed in ethyl acetate, dried, and recovered *in vacuo*. Triturated with light petroleum it gave N-*benzyloxycarbonyl*-S-2-*methoxycarbonylethyl*-L-*cysteine*, m. p. 63—64° (from ethyl acetate–light petroleum) (1·5 g.) (Found: N, 3·9. $C_{15}H_{19}NO_6S$ requires N, 4·1%).

Poly-(S-2-*methoxycarbonylethyl*-L-*cysteine*).—*S*-2-Methoxycarbonylethyl-L-cysteine (1 g.) in dioxan (50 ml.) was heated at 50° and carbonyl chloride passed in for 1 hr. The solution was filtered and evaporated *in vacuo*. The residue was taken up in dioxan (25 ml.), which was removed again. The *N*-carboxyanhydride was washed with light petroleum and left in a vacuum-desiccator over phosphorus pentoxide. Polymerisation was carried out in bulk at 10^{-3} mm. at 110° for 1 hr. and at 130° for 2 hr. The hard *polymer* was digested with acetic acid and the residue filtered off and dried [Found: C, 44·0; H, 5·5; N, 7·6; S, 16·3. $(C_7H_{11}NO_3S)_n,H_2O$ requires C, 44·4; H, 5·8; N, 7·4; S, 16·9%].

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[*Received, June 30th*, 1960.]

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[8] Rothstein, *J.*, 1949, 1968.