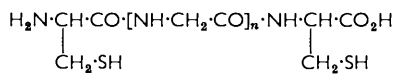
783. *Polypeptides. Part XII.*[1] *The Synthesis and Oxidation of* L-*Cysteinyl-6-aminohexanoyl-*L-*cysteine and* L-*Cysteinyl-11-aminoundecanoyl-*L-*cysteine*

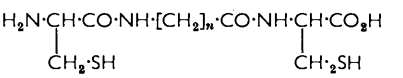
By H. N. Rydon and J. Šavrda

S-Benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-6-aminohexanoyl-*S*-benzyl-L-cysteine benzyl ester (VI; $n = 5$) and the corresponding 11-aminoundecanoyl compound (VI; $n = 10$) have been synthesised. Oxidation of L-cysteinyl-6-aminohexanoyl- and -11-undecanoyl-L-cysteine (II; $n = 5$ and 10), in dilute aqueous solution at pH 8·5, has been shown to produce as major products (*ca* 60%) the cyclic disulphides, *SS'*-dehydro-L-cysteinyl-6-aminohexanoyl- and -11-aminoundecanoyl-L-cysteine (IX; $n = 5$ and 10). The differences between these results and those previously obtained with L-cysteinyl-polyglycyl-L-cysteines of similar chain lengths are discussed briefly.

Earlier Papers in this Series[2-5] have dealt with the synthesis of a series of L-cysteinyl-polyglycyl-L-cysteines (I; $n = 0$—6) and the elucidation of the structures of the products of their oxidation by air in aqueous solution at pH 8·5. In order to ascertain to what extent the nature of the oxidation products was influenced by hydrogen bonding[3,6] in the peptides undergoing oxidation, it seemed desirable to carry out similar studies with peptides in which the number of peptide linkages was reduced by replacing glycine by trimethylene residues. The present Paper describes such studies with two peptides (II; $n = 5$ and 10) of this kind in which the distances between the two thiol groups are approximately the same as in the diglycyl (I; $n = 2$) and tetraglycyl (I; $n = 4$) compounds, respectively.



(I)

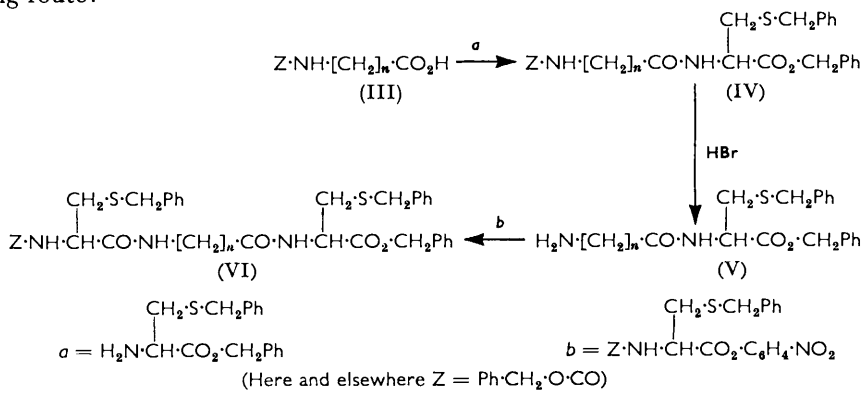


(II)

Very few peptides of 6-aminohexanoic and 11-aminoundecanoic acids are known. Rothe and Kunitz[7] synthesised a number of homogeneous peptides of 6-aminohexanoic acid, while glycyl-11-aminoundecanoic acid and 11-aminoundecanoylglycine were prepared by Champetier and Guinot.[8]

[1] Part XI, H. N. Rydon and F. O. dos S. P. Serrão, *J.*, 1964, 3638.
[2] K. C. Hooper, H. N. Rydon, J. A. Schofield, and G. S. Heaton, *J.*, 1956, 3148.
[3] G. S. Heaton, H. N. Rydon, and J. A. Schofield, *J.*, 1956, 3157.
[4] D. G. Large, H. N. Rydon, and J. A. Schofield, *J.*, 1961, 1749.
[5] D. Jarvis, H. N. Rydon, and J. A. Schofield, *J.*, 1961, 1752.
[6] Cf. R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, 1958, **41**, 2186; R. Schwyzer, J. P. Carrión, B. Gorup, H. Nolting, and T.-K. Aung, *ibid.*, 1964, **47**, 441.
[7] M. Rothe and F.-W. Kunitz, *Annalen*, 1957, **609**, 88.
[8] G. Champetier and M. Guinot, *Compt. rend.*, 1959, **248**, 1822; M. Guinot, *Ann. Chim.* (*France*), 1960, **5**, 1105.

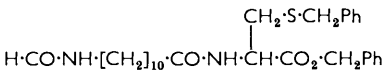
The required fully protected peptides (VI; $n = 5$ and 10) were best synthesised by the following route:



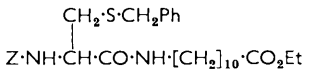
(Here and elsewhere Z = Ph·CH₂·O·CO)

The appropriate ω-*N*-benzyloxycarbonylamino-acid (III) was coupled with *S*-benzyl-L-cysteine benzyl ester, using either bis-*o*-phenylene pyrophosphite [9] or dicyclohexylcarbodi-imide,[10] the former reagent giving the better results for the aminohexanoyl and the latter for the aminoundecanoyl peptide. Treatment of the resulting fully protected dipeptide (IV) with hydrogen bromide in acetic acid [11] removed the *N*-benzyloxycarbonyl group. The partially protected dipeptides (V) so obtained were then coupled with *S*-benzyl-*N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine *p*-nitrophenyl ester to give the fully protected tripeptides (VI) in good yield.

A number of other routes to the aminodecanoic acid peptide (VI; $n = 10$) were investigated, but none proved satisfactory. *N*-11-Formamidoundecanoyl-*S*-benzyl-L-cysteine benzyl ester (VII) was satisfactorily prepared by the dicyclohexylcarbodi-imide coupling of 11-formamidoundecanoic acid and *S*-benzyl-L-cysteine benzyl ester, but attempted deformylation with hydrogen chloride in methanol or benzyl alcohol [12] failed.



(VII)



(VIII)

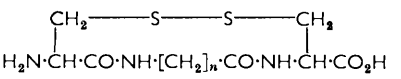
Ethyl *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-11-aminoundecanoate (VIII) was prepared in good yield by the dicyclohexylcarbodi-imide coupling of *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine and ethyl 11-aminoundecanoate, but the ester grouping proved unexpectedly resistant to alkaline hydrolysis, which could not be brought about without simultaneous elimination of benzyl thiol. Treatment of (VIII) with hydrazine hydrate gave the corresponding hydrazide, but the azide resulting from the action of nitrous acid on this compound proved unreactive towards *S*-benzyl-L-cysteine benzyl ester. The failure of this standard reaction sequence is remarkable and illustrates the difference in reactivity between derivatives of 11-aminoundecanoic acid and those of α-amino-acids.

The protected aminohexanoic peptide (VI; $n = 5$) was reduced with sodium in liquid ammonia and the resulting free dithiol (II; $n = 5$) oxidised, without isolation, by passing oxygen through a 1% aqueous solution at pH 8·5 in the presence of a trace of cupric ion. Chromatography and paper electrophoresis of the product showed the presence of five components, of which that with the highest R_F value preponderated, making up about two-thirds of the whole. This mixture, de-salted with ion-exchange resin, was treated, in water

[9] P. C. Crofts, J. H. H. Marks, and H. N. Rydon, *J.*, 1958, 4250; 1959, 3610.
[10] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[11] D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 1952, **17**, 1564; 1954, **19**, 62.
[12] S. G. Waley, *Chem. and Ind.*, 1953, 107.

with Ecteola, following the procedure of Yanari *et al.*;[13] the only component not retained by the Ecteola was the major oxidation product which was obtained in this way chromatographically homogeneous. This compound could not be obtained sufficiently free from inorganic contaminants for direct determination of its molecular weight by conventional methods. However, Dr. F. Serrão, working in these laboratories, has recently determined its moleular weight by gel-filtration on Sephadex;[14] the value obtained (385) confirms the assignment of the monomeric structure (IX) for which the calculated molecular weight is 335.

The crude, salt-containing, major oxidation product was very prone to disulphide interchange, especially in alkaline solution when it gave rise to a mixture of products similar to that obtained in the original oxidation of (II; $n = 5$). However, this was not the case after most of the salts had been removed and it was possible to convert the purified product into its *N*-2,4-dinitrophenyl derivative by reaction with 1-fluoro-2,4-dinitrobenzene in aqueous ethanolic sodium hydrogen carbonate.[15] The molecular weight of this derivative, determined by the Rast method in the lactam of 4-aminocyclohexanecarboxylic acid,[16] was 488, showing clearly that the major oxidation product was the monomeric cyclic disulphide (IX; $n = 5$). This conclusion was confirmed



(IX)

by following, by chromatography, the *N*-acylation of the oxidation product with deficiencies of 1-fluoro-2,4-dinitrobenzene and 2,4,5-trinitrotoluene.[17] In both cases only one derivative, *viz.* that of (IX; $n = 5$), was present throughout the reaction, there being no indication of the formation of a second derivative, as would be expected to be the case if the product had a dimeric structure containing, necessarily, two amino-groups.[5,18] Similarly, treatment of the major oxidation product with nitrous acid gave only a single deamination product.

In the 11-aminodecanoic acid series, the protected tripeptide (VI; $n = 10$) was similarly reduced with sodium in liquid ammonia and the resulting dithiol (II; $n = 10$) oxidised with oxygen in 1% aqueous solution at pH 8·5, in this case without added cupric ion. Chromatography and paper electrophoresis showed the resulting solution to contain four products; again the chromatographically fastest moving was the major product (60% of the whole). This could be purified by recrystallisation from pyridine–ether; although not entirely free from firmly held inorganic material the product was sufficiently so for a direct molecular weight determination. The molecular weight, determined cryoscopically in acetic acid, was 353, in fair agreement with the calculated value (405) for the cyclic disulphide (IX; $n = 10$). This was confirmed by the finding that only one derivative was detectable when the major oxidation product was treated with a deficiency of 2,4,5-trinitrotoluene in aqueous ethanol. In this case a parallel experiment with 1-fluoro-2,4-dinitrobenzene could not be carried out owing to the great ease with which the cyclic disulphide (X; $n = 10$) underwent disulphide interchange in slightly alkaline solution.

In the following Table are set out the yields of cyclic monomer obtained by the oxidation in 1% solution at pH 8·5 of a series of peptides containing two cysteine residues, the distance

[13] M. A. Mitz and S. S. Yanari, *J. Amer. Chem. Soc.*, 1956, **78**, 2649; S. S. Yanari, M. Volini, and M. A. Mitz, *Biochim. Biophys. Acta*, 1960, **45**, 595.
[14] P. Andrews, *Biochem. J.*, 1964, **91**, 222.
[15] A. L. Levy and D. Chung, *J. Amer. Chem. Soc.*, 1955, **77**, 2899.
[16] G. Wendt, *Chem. Ber.*, 1942, **75**, 425; I. Rothe and M. Rothe, *ibid.*, 1955, **88**, 282.
[17] G. Barger and F. Tutin, *Biochem. J.*, 1918, **12**, 402; J. H. Quastel, C. P. Stewart, and H. E. Tunnicliffe, *ibid.*, 1923, **17**, 586.
[18] Cf. A. R. Battersby and L. C. Craig, *J. Amer. Chem. Soc.*, 1951, **73**, 1887.

between the two sulphur atoms in the fully extended chains and the yields of cyclic monomer calculated by the, undoubtedly oversimplified, statistical treatment of Rydon:[19]

Peptide	S–S distance (Å)	Calc. yield of monomer (%)	Yield of monomer (%)	Ref.
(I; $n = 1$)	12·7	84	0	3
(I; $n = 2$)	16·3	77	15	3
(II; $n = 5$)	17·7	73	55	Present work
(I; $n = 3$)	19·9	66	40	3
(I; $n = 4$)	23·5	58	90	3
(II; $n = 10$)	24·0	57	60	Present work
(I; $n = 5$)	27·1	50	95	5
(I; $n = 6$)	30·7	44	95	5

The difference between the two groups of peptides is striking, the aminohexanoyl and aminodecanoyl peptides (II; $n = 5$ and 10) conforming much more closely to expectation than the glycyl peptides (I; $n = 1$—6). The molecular congestion which has previously been invoked [3] to explain the low yield of cyclic monomer formed on oxidation of the smaller glycyl peptides (I; $n = 1$—3) arises in large measure from the preferred *trans*-conformation of the peptide linkages, of which there are fewer in the aminohexanoyl compound (IX; $n = 5$) than in the corresponding glycyl peptides. It also seems likely that the high yields of cyclic monomer which are obtained on oxidising the larger glycyl peptides (I; $n = 4$—6) are due to intramolecular hydrogen bonding, both in the peptide undergoing oxidation and in the oxidised product; again, the smaller number of peptide linkages in the aminoundecanoyl compounds (II; $n = 10$) and (X; $n = 10$) would minimise the effect in this case. It may also be that the absence of such stabilisation is the cause of the marked susceptibility of the cyclic disulphides (IX; $n = 5$ and 10) to disulphide interchange. Clearly a more quantitative study of the problem is required and this is now in progress.

Experimental

Chromatograms (ascending) were run on either Whatman No. 1 filter paper with butan-1-ol–acetic acid–water (4 : 1 : 5) (R_{FA}) or on silica-gel G using butan-1-ol–acetic acid–water (3 : 1 : 1) (R_{FB}). Paper electrophoresis was carried out on Whatman No. 1 filter paper in a horizontal Durrum-type apparatus in 0·5N-acetic acid at 600—800v. Spots were revealed with ninhydrin, chlorine–starch–iodide,[20] iodine,[21] or nitroprusside, with or without added cyanide.[22]

All evaporations were carried out under reduced pressure.

S-Benzyl-L-cysteine, m. p. 214—215°, $[\alpha]_D^{20}$ +29·0° (*c* 1·0 in N-NaOH) was prepared by the method of Wood and du Vigneaud [23] and converted into its *N*-benzyloxycarbonyl derivative,[24] m. p. 92—94°, $[\alpha]_D^{20}$ −46·3° (*c* 3·9 in acetic acid), and benzyl ester toluene-*p*-sulphonate,[2] m. p. 158—160°, $[\alpha]_D^{18}$ −21·8° (*c* 1·1 in methanol).

Synthesis of Protected L-*Cysteinyl-6-aminocaproyl-*L-*cysteine.*—6-N-*Benzyloxycarbonylamino-hexan*-S-*benzyl-*L-*cysteine benzyl ester* (IV; $n = 5$). (*a*) Bis-*o*-phenylene pyrophosphite [9] (12·6 g.) was added to a solution of 6-*N*-benzyloxycarbonylaminohexanoic acid [7] (10·4 g.) and *S*-benzyl-L-cysteine benzyl ester toluene-*p*-sulphonate (18·5 g.) in anhydrous pyridine (80 ml.), and the mixture heated on a boiling-water bath for 30 min. The cooled solution was poured into ice–water (400 ml.), kept for some hours at 0°, and the product which separated collected by filtration and washed successively with 2N-hydrochloric acid, 2N-sodium hydroxide, and water; crystallisation from aqueous acetone gave the *protected dipeptide* (17·5 g.; 82%), m. p. 102—103°, $[\alpha]_D^{20}$ −32·2° (*c* 2·0 in acetone) (Found: C, 67·0; H, 6·2; N, 4·7. $C_{31}H_{36}N_2O_5S$ requires C, 67·8; H, 6·6; N, 5·1%).

(*b*) *NN'*-Dicyclohexylcarbodi-imide (2·1 g.) was added, at 0—5°, to a solution of 6-*N*-benzyloxycarbonylaminohexanoic acid (2·6 g.) and *S*-benzyl-L-cysteine benzyl ester toluene-*p*-sulphonate (4·7 g.) in anhydrous pyridine (25 ml.). After 1 hr. at 0—5°, the mixture was kept

[19] H. N. Rydon, Ciba Foundation Symposium on Amino-acids and Peptides with Antimetabolic Activity, 1958, p. 200; cf. R. A. Boissonnas and I. Schumann, *Helv. Chim. Acta*, 1952, **35**, 2229.
[20] H. N. Rydon and P. W. G. Smith, *Nature*, 1952, **169**, 922.
[21] G. C. Barrett, *Nature*, 1962, **194**, 1171.
[22] G. Toennies and J. J. Kolb, *Analyt. Chem.*, 1951, **23**, 823.
[23] J. L. Wood and V. du Vigneaud, *J. Biol. Chem.*, 1939, **130**, 109.
[24] C. R. Harington and T. H. Mead, *Biochem. J.*, 1936, **30**, 1598.

overnight at room temperature. A few drops of acetic acid were then added and the precipitated *NN'*-dicyclohexylurea removed by filtration; the filtrate was evaporated and the residual oil dissolved in a little acetone. After 12 hr. at 0°, a little more urea was removed by filtration, the filtrate evaporated, and the residue dissolved in ethyl acetate. The solution was washed with 2N-hydrochloric acid, 2N-sodium carbonate, and water, dried (Na_2SO_4), and filtered. Addition of light petroleum (b. p. 40—60°) precipitated the protected dipeptide, which was crystallised from aqueous acetone; yield 2·6 g. (48%), m. p. 100°, $[\alpha]_D^{19}$ −31·5°.

6-*Aminohexanoyl*-S-*benzyl*-L-*cysteine benzyl ester* (V; $n = 5$). The above benzyloxycarbonyl derivative (5·5 g.) in glacial acetic acid (10 ml.) was treated, at 6—8°, with a 33% solution of hydrogen bromide in acetic acid (8 ml.). After 45 min., anhydrous ether was added and the gummy hydrobromide which separated was collected and dried in a vacuum desiccator over sodium hydroxide. This product (5·0 g.), in chloroform (50 ml.), was treated with triethylamine (1·4 ml.), and the resulting solution washed with 2N-sodium hydroxide and water, dried (Na_2SO_4), and filtered. Addition of toluene-*p*-sulphonic acid monohydrate (1·9 g.) in 1 : 1 chloroform–ether, followed by more ether, precipitated the *ester toluene*-p-*sulphonate* which was crystallised from water; the yield was 5·1 g. (87%), m. p. 135—137°, $[\alpha]_D^{23}$ −21·5° (*c* 1·9 in ethanol) (Found: C, 61·0; H, 6·9; N, 4·6. $C_{30}H_{28}N_2O_6S_2$ requires C, 61·4; H, 6·5; N, 4·8%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-6-*aminohexanoyl*-S-*benzyl*-L-*cysteine benzyl ester* (VI; $n = 5$). Triethylamine (4·5 ml.) and *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine *p*-nitrophenyl ester [25] (15·4 g.) was added to the above ester toluene-*p*-sulphonate (19·3 g.) in tetrahydrofuran (250 ml.), and the mixture kept at room temperature for 3 days, during which time a few drops of acetic acid and one more equivalent of triethylamine (two portions of 2·25 ml.) were added. Addition of water precipitated the *protected tripeptide*, which was washed with 2N-sodium hydroxide, 2N-hydrochloric acid, and water, and crystallised from ethyl acetate; the yield was 23·1 g. (95%), m. p. 137—139°, $[\alpha]_D^{20}$ −37·0° (*c* 2·0 in acetic acid) (Found: C, 65·6; H, 6·5; N, 5·6. $C_{41}H_{47}N_3O_6S_2$ requires C, 66·4; H, 6·4; N, 5·7%). Complete acid hydrolysis showed the compound to be 98% optically pure.

Synthesis of Protected L-*Cysteinyl*-11-*aminoundecanoyl*-L-*cysteine*.—N-*Benzyloxycarbonyl*-11-*aminoundecanoic acid*, (III; $n = 10$). Benzyl chloroformate (17·8 g.) was added dropwise during 45 min. to a stirred solution of 11-aminoundecanoic acid (20·1 g.) in 2N-sodium hydroxide (100 ml.) and tetrahydrofuran (100 ml.). After the mixture had been stirred for a further 2 hr., the tetrahydrofuran was removed by evaporation; the sodium salt which separated was washed with ether and crystallised from aqueous acetic acid, giving the *derivative* (29·2 g.; 87%), m. p. 97° (Found: C, 67·7; H, 8·5; N, 4·5. $C_{19}H_{29}NO_4$ requires C, 68·0; H, 8·7; N, 4·2%).

N-*Benzyloxycarbonyl*-11-*aminoundecanoyl*-S-*benzyl*-L-*cysteine benzyl ester*, (IV; $n = 10$). (*a*) *NN'*-Dicyclohexylcarbodi-imide (15·9 g.) was added to an ice-cooled solution of *N*-benzyloxycarbonyl-11-aminoundecanoic acid (25·0 g.) and *S*-benzyl-L-cysteine benzyl ester toluene-*p*-sulphonate (35·4 g.) in pyridine (200 ml.). The mixture was kept at 0° for 1 hr. and then at room temperature overnight; a few drops of acetic acid were then added and dicyclohexylurea removed by filtration. The filtrate was evaporated and the residue treated with acetone (100 ml.). The precipitate which separated during some hours at 0° was collected, washed with 2N-hydrochloric acid, water, and cold ethanol; crystallisation from aqueous acetone gave the *protected dipeptide* (29·0 g.; 63%), m. p. 103°, $[\alpha]_D^{20}$ −24·9° (*c* 1·0 in acetone) (Found: C, 69·6; H, 7·8; N, 4·2. $C_{36}H_{46}N_2O_5S$ requires C, 69·9; H, 7·5; N, 4·5%).

(*b*) Bis-*o*-phenylene pyrophosphite (1·7 g.) was added to a solution of *N*-benzyloxycarbonyl-11-aminoundecanoic acid (1·7 g.) and *S*-benzyl-L-cysteine benzyl ester toluene-*p*-sulphonate (2·4 g.) in pyridine (10 ml.), and the mixture heated at 100° for 30 min. The cooled product was poured into ice–water (100 ml.). Working up as in (*a*) gave the protected dipeptide (1·3 g.; 42%), m. p. 103°, $[\alpha]_D^{20}$ −25·5° (*c* 1·0 in acetone).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-11-*aminoundecanoyl*-S-*benzyl*-L-*cysteine benzyl ester*, (VI; $n = 10$). The above protected dipeptide (21·5 g.) was kept for 30 min., with occasional shaking, with a 33% solution of hydrogen bromide in acetic acid (28 ml.). The solution was evaporated and the residue taken up in methanol and re-evaporated; this process was repeated four times and the final residue triturated with anhydrous ether. The resulting hygroscopic hydrobromide (19·1 g.) was dissolved in tetrahydrofuran (100 ml.); triethylamine (4·7 ml.) was added, followed by *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine *p*-nitrophenyl ester (15·7 g.)

[25] B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 373; M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1959, **81**, 5688.

and a few drops of acetic acid. The mixture was stirred at room temperature for 3 days, during which time more triethylamine (4·7 ml.) was added, in three equal portions. The mixture was then filtered and the filtrate evaporated to dryness. The residue was dissolved in ethyl acetate, and the solution washed with saturated aqueous sodium hydrogen carbonate, 2N-hydrochloric acid, and water and dried (Na_2SO_4). Evaporation, trituration of the residue with light petroleum (b. p. 40—60°), and crystallisation of the resulting solid from aqueous acetone gave the *protected tripeptide* (19·5 g.; 69%), m. p. 86—87°, $[\alpha]_D^{20}$ −28·9° (*c* 0·7 in acetic acid) (Found: C, 67·4; H, 6·9; N, 5·0. $C_{46}H_{57}N_3O_6S_2$ requires C, 68·0; H, 7·1; N, 5·2%).

S-*Benzyl*-L-*cysteinyl*-11-*aminodecanoyl*-S-*benzyl*-L-*cysteine*. The above protected tripeptide (3·0 g.) was dissolved in a 33% solution of hydrogen bromide in acetic acid (20 ml.). Dry hydrogen bromide was bubbled through the solution while it was heated at 60° for 5 hr. and then at 70—80° for 1 hr. The solution was then evaporated and the residue triturated with anhydrous ether. Precipitation from pyridine with water, followed by crystallisation from aqueous ethanol gave the *tripeptide* (1·65 g.; 74%), m. p. 150° (Found: C, 61·8; H, 7·6; N, 6·9. $C_{31}H_{45}N_3O_4S_2,H_2O$ requires C, 61·5; H, 7·8; N, 7·0%).

11-*Formamidoundecanoic acid*. Acetic anhydride (40 ml.) was added dropwise over 90 min. to a stirred solution of 11-aminoundecanoic acid (10·0 g.) in 98% formic acid (100 ml.) at 50—60°. After a further 2 hours' stirring at room temperature, ice–water (40 ml.) was added, and the solution evaporated to dryness. Two re-evaporations with aqueous ethanol, followed by crystallisation from aqueous ethanol gave the *acid* (9·6 g.; 84%), m. p. 106—108° (Found: C, 62·4; H, 10·1; N, 6·4. $C_{12}H_{23}NO_3$ requires C, 62·9; H, 10·1; N, 6·1%).

N-11-*Formamidoundecanoyl*-S-*benzyl*-L-*cysteine benzyl ester*, (VII). NN'-Dicyclohexylcarbodi-imide (6·4 g.) was added at 0—5° to a solution of the above formamido-acid (6·9 g.) and *S*-benzyl-L-cysteine benzyl ester toluene-*p*-sulphonate (14·2 g.) in pyridine (170 ml.). After 1 hr. at 0—5° and 18 hr. at room temperature, a few drops of acetic acid were added and the precipitated dicyclohexylurea removed by filtration. The filtrate was poured into ice–water (1·3 l.) and the precipitate washed with 2N-hydrochloric acid, 2N-sodium hydroxide, and water, decolourised by boiling with charcoal in ethyl acetate solution, and reprecipitated with light petroleum (b. p. 40—60°). Crystallisation from aqueous acetone gave the *protected dipeptide* (10·0 g.; 65%), m. p. 83—85°, $[\alpha]_D^{23}$ −16·3° (*c* 2·3 in dimethylformamide) (Found: C, 67·8; 7·8; N, 5·4. $C_{29}H_{40}N_2O_4S$ requires C, 67·9; H, 7·9; N, 5·5%).

Ethyl 11-*Aminoundecanoate*.—A stirred suspension of 11-aminoundecanoic acid (10·0 g.) in ethanol (150 ml.) was saturated at 0° with dry hydrogen chloride and then refluxed for 30 min. On cooling, some ester hydrochloride crystallised and was collected by filtration. The filtrate was evaporated to dryness and thrice re-evaporated with ethanol. The final residue was dissolved in a little warm ethanol and the ester hydrochloride precipitated by adding anhydrous ether and cooling. Crystallisation of the combined products from ethyl acetate gave the *ester hydrochloride* (11·1 g.; 84%), m. p. 143—144° (Found: C, 58·7; H, 10·3; N, 5·1. $C_{13}H_{28}ClNO_2$ requires C, 58·7; H, 10·6; N, 5·3%).

Derivatives of S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-11-*aminoundecanoic Acid*.—The above ester hydrochloride (13·3 g.) and *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine (17·2 g.) were coupled, in pyridine (200 ml.), as usual, with the aid of dicyclohexylcarbodi-imide (11·0 g.). Crystallisation of the product from ethyl acetate gave the *ethyl ester* (VIII) (21·4 g.; 77%), m. p. 94—95°, $[\alpha]_D^{23}$ −9·1° (*c* 2·4 in ethanol) (Found: C, 67·1; H, 7·5; N, 5·5. $C_{31}H_{44}N_2O_5S$ requires C, 66·9; H, 8·0; N, 5·0%).

This ester (5·5 g.), in ethanol (10 ml.), was refluxed for 45 min. with 99% hydrazine hydrate (5 ml.). More ethanol (20 ml.) was added and the solid, which crystallised on cooling, was collected, triturated with chloroform, and crystallised from ethanol, giving the *hydrazide* (2·9 g.; 54%), m. p. 121—123°, $[\alpha]_D^{23}$ −8·8° (*c* 1·3 in acetic acid) (Found: C, 63·6; H, 7·7; N, 9·9. $C_{29}H_{42}N_4O_4S$ requires C, 64·2; H, 7·8; N, 10·3%).

Oxidation of L-*Cysteinyl*-6-*aminohexanoyl*-L-*cysteine*.—(*a*) *S*-Benzyl-*N*-benzyloxycarbonyl L-cysteinyl-6-aminohexanoyl-*S*-benzyl-L-cysteine benzyl ester (2·2 g.), in liquid ammonia (100 ml.), was reduced with sodium in a reaction vessel similar to that described previously.[5] When the reduction was complete, the excess of sodium was destroyed by adding Zeo-Karb 225; the ammonia was then allowed to evaporate and the residue pumped out at 0·1 mm. for several hours. The residue was dissolved in water (100 ml.), the pH adjusted to 8·5 by the addition of more Zeo-Karb, and the resin was removed by filtration. A little cupric sulphate (*ca* 0·5 mg.) was added to the filtrate and a stream of oxygen (500 ml./min.) passed through

until the nitroprusside reaction was negative (25 min.). The pH was then brought to 5·0 with Zeo-Karb, the resin removed by filtration, and the filtrate lyophilised. Thin-layer chromatography and paper electrophoresis showed the presence of five components, the chromatographically fastest-moving (R_{FB} 0·35) being present in largest amount; photometric estimation of a ninhydrin-treated paper electropherogram showed this major product to make up about 55% of the whole.

A solution of this mixture (20 mg.) in water (10 ml.) was added to a suspension of Ecteola (400 mg.) in water (10 ml.). The mixture was stirred for 30 min. and then centrifuged; the procedure was repeated with the supernatant liquid. Lyophilisation of the second supernatant phase gave SS′-*dehydro*-L-*cysteinyl*-6-*aminohexanoyl*-L-*cysteine* (IX; $n = 5$) (7·5 mg.; 38%), m. p. 183—188° (decomp.), R_{FB} 0·35 (homogeneous) (Found, corrected for 4·4% inorganic residue: C, 43·3; H, 6·4; N, 11·7; S, 18·5. $C_{12}H_{21}N_3O_4S_2$ requires C, 43·0; H, 6·3; N, 12·5; S, 19·1%).

(*b*) The cyclic disulphide (80 mg.) was shaken at room temperature for 3 hr. with 1-fluoro-2,4-dinitrobenzene (800 mg.) and sodium hydrogen carbonate (800 mg.) in 67% ethanol (24 ml.). Water was then added and the solution extracted with ether to remove unchanged reagent. The aqueous residue was then acidified and freed from 2,4-dinitrophenol by extraction with ether; the residual aqueous suspension was then thoroughly extracted with ethyl acetate (800 ml.) and the extract washed with water, dried ($MgSO_4$), and evaporated. Trituration with acetone and crystallisation from aqueous tetrahydrofuran gave the N-2,4-*dinitrophenyl derivative* of (IX; $n = 5$) (72 mg.; 60%), m. p. 220—224° (decomp.), $[\alpha]_D^{23}$ −21·8° (*c* 1·1 in dimethylformamide), R_{FB} 0·76 (homogeneous) (Found: C, 43·2; H, 4·8; N, 13·6; S, 12·6. $C_{18}H_{23}N_5O_8S_2$ requires C, 43·1; H, 4·6; N, 14·0; S, 12·8%). In another experiment, with purified disulphide, a deficiency of 1-fluoro-2,4-dinitrobenzene was used and the progress of the reaction followed, for 5 hr., by thin-layer chromatography; the only compounds detectable were the starting material (R_{FB} 0·35; colourless, ninhydrin positive) and its dinitrophenyl derivative (R_{FB} 0·76; yellow, ninhydrin negative).

(*c*) The cyclic disulphide (10 mg.) was shaken for 24 hr. with 2,4,5-trinitrotoluene [26] (27 mg.) in 50% aqueous acetone (3 ml.); portions of the mixture were removed from time to time for thin-layer chromatography. Throughout the reaction the only detectable product, in addition to the starting material (R_{FB} 0·36; colourless, ninhydrin positive) and trinitrotoluene (R_{FB} 0·96; pale yellow, ninhydrin negative), was a yellow, ninhydrin negative substance (R_{FB} 0·82); the latter was identified as the 2,4-dinitrotolyl derivative of (IX; $n = 5$) by chromatographic comparison with material obtained by heating the crude oxidation product with an excess of 2,4,5-trinitrotoluene in boiling 75% aqueous ethanol, followed by isolation by thin-layer chromatography.

(*d*) 0·1N-Acetic acid (0·33 ml.; 1 equiv.) was added in small portions to a solution of the cyclic disulphide (11 mg.) and sodium nitrite (2·3 mg.; 1 equiv.) in water (2 ml.). Thin-layer chromatography of the solution after each addition of acid showed, in addition to starting material (R_{FB} 0·35), only a single deamination product (R_{FB} 0·62; ninhydrin negative, cyanide–nitroprusside positive); the same deamination product was formed when an excess of nitrous acid was used, but was then accompanied by a small amount of a second, possibly diastereoisomeric, product (R_{FB} 0·65; ninhydrin negative, cyanide–nitroprusside positive).

Oxidation of L-*Cysteinyl*-11-*aminoundecanoyl*-L-*cysteine*. (*a*) *S*-Benzyl-L-cysteinyl-11-aminoundecanoyl-*S*-benzyl-L-cysteine (1·5 g.) was debenzylated and oxidised as described above for the aminohexanoyl compound. Paper chromatography, thin-layer chromatography, and paper electrophoresis of the oxidised solution all showed the presence of four products; photometric estimation on a ninhydrin-treated paper chromatogram showed the proportions of these to be R_{FA} 0·60 (62%), R_{FA} 0·24 (33%), R_{FA} 0·09 (5%), R_{FA} 0·00 (trace).

The solution was then brought to pH 3·0 with hydrochloric acid, insoluble material removed by filtration, and the pH of the filtrate adjusted to 5·5 with aqueous ammonia. This solution, which now contained only two components (R_{FA} 0·60, 88%; R_{FA} 0·24, 12%) was evaporated to dryness and the residue extracted with hot anhydrous pyridine. Concentration of the extract and addition of ether, followed by two crystallisations of the precipitate from pyridine–ether gave SS′-*dehydro*-L-*cysteinyl*-11-*aminoundecanoyl*-L-*cysteine* (IX; $n = 10$) (67 mg.; 7%), m. p. 172—174° (decomp.), R_{FA} 0·60 (homogeneous), $[\alpha]_D^{20}$ −15·9° (*c* 1·1 in N-NaOH) (Found, corrected for 5·0% inorganic residue: C, 49·0; H, 7·5; N, 10·1. $C_{17}H_{31}N_3O_2S_2,H_2O$

[26] W. A. Gey, E. R. Dalbey, and R. W. Van Dolah, *J. Amer. Chem. Soc.*, 1956, **78**, 1803.

requires C, 48·3; H, 7·9; N, 9·9%). The same product was obtained similarly from the fully protected tripeptide (VI; $n = 10$).

(*b*) This cyclic disulphide (60 mg.) was refluxed for 2 hr. with 2,4,5-trinitrotoluene (65 mg; 2 equiv.) in 50% aqueous ethanol; the reaction was followed by thin-layer chromatography of small samples of the reaction mixture withdrawn at intervals; during the first 30 min., the starting material (R_{FB} 0·62; colourless, ninhydrin positive) disappeared and was replaced by the 2,4-dinitrotolyl derivative (R_{FB} 0·83; yellow, ninhydrin negative); at no time was any other product detectable.

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