Sequential Polypeptides. Part VI.[1] The Synthesis of Some Sequential Polypeptide Collagen Models containing Proline Analogues

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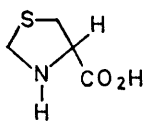
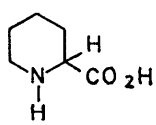
Syntheses of poly-(L-alanylglycyl-L-thiazolidine-4-carboxylic acid) and of poly-(L-piperidine-2-carbonyl-L-alanylglycine) *via* the corresponding tripeptide pentachlorophenyl esters and of poly-(glycyl-L-piperidine-2-carbonyl-L-alanine) and poly-(glycyl-L-azetidine-2-carbonyl-L-alanine) *via* the corresponding tripeptide 2-hydroxyphenyl esters are described. All of the polydisperse preparations were of low apparent molecular weight except the polymer containing thiazolidine-4-carboxylic acid, which on viscosity criteria had a weight average molecular weight in the region of 10,000.

POLYTRIPEPTIDES such as poly-(L-prolyl-L-alanylglycine) [2] and poly-(L-prolylglycyl-L-proline) [3] are able to adopt ordered structures in solution related to that of collagen. It is clear that the stereochemical role of proline is crucial, since its replacement by an alanine residue, as in poly-(L-alanyl-L-alanylglycine),[4] leads to ordered structures of a totally different nature. This paper describes the synthesis of some polytripeptide collagen models in which a cyclic imino-acid residue is retained, but in a modified form.

The starting point for our interest in sequential polypeptide collagen models was the observation [5] that poly-(glycyl-L-prolyl-L-alanine) was antigenic in guinea pigs and showed some cross-reactivity with collagen: similar experiments have been reported [6] for poly-(L-prolylglycyl-L-proline). We are now engaged in the synthesis of a series of polytripeptides closely related to poly-(glycyl-L-prolyl-L-alanine) for immunological investigations of the relationship between structure and immunological behaviour in these antigens. We have previously described [7] the synthesis of all the stereoisomers of poly(alanylglycylproline): we now report the preparation of some analogues in which the proline residue is replaced by L-thiazolidine-4-carboxylic acid (I), L-piperidine-2-carboxylic acid (pipecolic acid) (II), or L-azetidine-2-carboxylic acid (III).


(I)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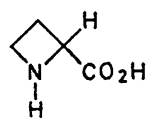
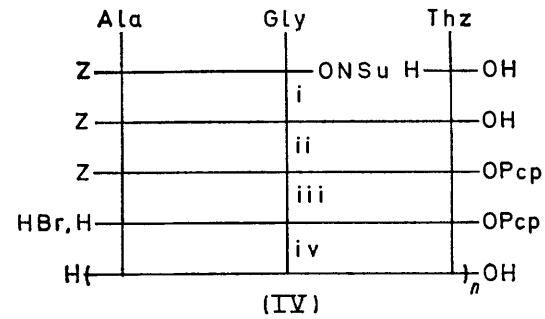

(III)

Although it is freely available by a simple reaction [8] from L-cysteine, L-thiazolidine-4-carboxylic acid (I) has not as far as we are aware been used as a proline analogue in oligopeptide synthesis; the only peptide containing it which has been made is homopoly-L-thiazolidine-4-carboxylic acid.[9] This homopolymer, in agreement with theoretical arguments,[10] has been shown to adopt a conformation like that of poly-L-proline form II, in which all the peptide bonds are *trans*. In contrast to poly-L-proline, however, no mutarotation to an all-*cis* form was detected under any conditions.[9] It was therefore of interest to prepare a sequential polytripeptide containing L-thiazolidine-4-carboxylic acid. The route shown in Scheme 1, which is identical in strategy to that



(IV)

SCHEME 1

Abbreviations for common amino-acids and their mode of use follow the relevant Tentative rules of the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature which are reprinted in Chem. Soc. 'Specialist Periodical Reports; Amino-acids, Peptides, and Proteins,' 1970, vol. 2, ch. 5. NSu = succinimido; Z = benzyloxycarbonyl; Pcp = pentachlorophenyl; Thz = thiazolidine-4-carboxylic acid. All residues are L throughout this paper. Conditions: i, Et_3N–DMF; ii, mixed carbonic anhydride method; iii, HBr–AcOH; iv, *N*-methylmorpholine–Me_2SO.

used [7] for the synthesis of poly-(L-alanylglycyl-L-proline), proved convenient for the synthesis of poly-(L-alanylglycyl-L-thiazolidine-4-carboxylic acid). The polymer (IV) was purified by exhaustive dialysis of a suspension and obtained as a white powder which was insoluble except in solvents such as dichloroacetic or trifluoroacetic acid. Inadequate solubility prevented molecular weight determination by our gel chromatography method,[11] but a rough estimate of the molecular weight could be made from viscosity measurements: the reduced specific viscosity of (IV) at 1% concentration in

[1] Part V, R. D. Cowell and J. H. Jones, *J.C.S. Perkin I*, 1972, 2236.
[2] F. R. Brown, tert., A. di Corato, G. P. Lorenzi, and E. R. Blout, *J. Mol. Biol.*, 1972, **63**, 85.
[3] J. Engel, J. Kurtz, E. Katchalski, and A. Berger, *J. Mol. Biol.*, 1966, **17**, 255.
[4] B. B. Doyle, W. Traub, G. P. Lorenzi, F. R. Brown, tert., and E. R. Blout, *J. Mol. Biol.*, 1970, **51**, 47.
[5] P. Brown and L. E. Glynn, unpublished results.
[6] F. Borek, J. Kurtz, and M. Sela, *Biochim. Biophys. Acta*, 1969, **188**, 314.
[7] Part IV, R. Fairweather and J. H. Jones, *J.C.S. Perkin I*, 1972, 1908.
[8] S. Ratner and H. T. Clarke, *J. Amer. Chem. Soc.*, 1937, **59**, 200.
[9] M. Goodman, K.-C. Su, and G. C.-C. Niu, *J. Amer. Chem. Soc.*, 1970, **92**, 5220.
[10] M. Goodman, G. C.-C. Niu, and K.-C. Su, *J. Amer. Chem. Soc.*, 1970, **92**, 5219.
[11] R. Fairweather, J. H. Jones, and J. K. Wilcox, *J. Chromatog.*, 1972, **67**, 157.

dichloroacetic acid was 0·32 dl g^{-1}, which in our experience [12] corresponds for sequential collagen models to a weight average molecular weight in the region of 10,000.

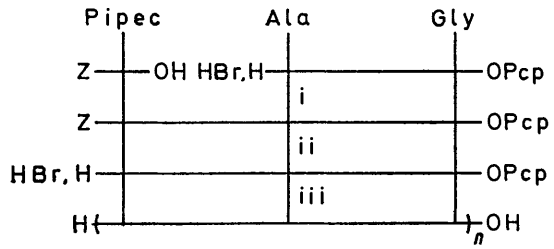
Angiotensin II,[13] bradykinin,[14] and oxytocin [15] analogues containing piperidine-2-carboxylic acid have been synthesised, but until recently [16] the difficulty of obtaining optically pure L-piperidine-2-carboxylic acid in quantity has restricted its use in oligopeptide synthesis. Homopoly-L-piperidine-2-carboxylic acid has been briefly described: [17] like poly-L-proline it can be obtained in two different ordered forms, but whereas the more stable form of poly-L-proline in acidic media has all its peptide bonds *trans*, it appears that the more stable form of poly-L-piperidine-2-carboxylic acid under these conditions has all its peptide bonds *cis*.

As explained in Part IV,[7] the synthesis of a polymer of sequence (Gly-imino-acid-Ala)$_n$ can in principle be approached *via* three kinds of tripeptide monomer in which the imino-acid is in the central, *N*-terminal, or *C*-terminal position (designated route 1, route 2, and route 3, respectively), the products of which are identical save for a few residues at each terminus. A synthesis of poly-(L-piperidine-2-carbonyl-L-alanylglycine) by a variant of route 2 as shown in Scheme 2 gave a low yield of undialysable material which, as judged by viscosity measurements,[12] was of low molecular weight: in concurrent work which has already been described [7] a precisely analogous route was almost equally unsatisfactory in a synthesis of poly-(L-prolyl-L-alanylglycine). An attempted synthesis of poly-(L-alanylglycyl-L-piperidine-2-carboxylic acid) by a route 3 approach was abortive, but we describe our experience to illustrate some of the difficulties associated with the use of piperidine-2-carboxylic acid in peptide synthesis, since a synthesis of poly-(L-alanylglycyl-L-proline) [7] by an identical approach was satisfactory. The protected tripeptide active ester (V) could not be obtained by 'backing off' reactions with L-piperidine-2-carboxylic acid pentachlorophenyl ester hydrobromide: both the pivalic and carbonic mixed anhydride methods gave complex mixtures. The alternative approach to the ester (V) through the acyltripeptide acid (VI) also met with difficulty: compound (VI) was prepared by the reaction of L-piperidine-2-carboxylic acid with benzyloxycarbonyl-L-alanylglycine succinimido ester and isolated as its dicyclohexylammonium salt in only 45% yield (under conditions which gave 80—90% yields [7] with D-proline as nucleophile), and attempted condensations of (VI) with pentachlorophenol by the dicyclohexylcarbodi-imide, pivalic, or carbonic mixed anhydride method gave mixtures from which the ester (V) could not be isolated. Only reaction with pentachlorophenyl trichloroacetate [18] gave a product from which (V) could be isolated, and in this case chromatography on Sephadex LH-20 was necessary and the yield was only 47%. Finally, treatment of the ester (V) with hydrogen bromide in acetic acid gave a pale brown powder from which the required peptide active ester hydrobromide could not be isolated, and attempted polymerisation of this highly impure material gave no undialysable polypeptide. We therefore turned to the variant of route 1 which is shown in Scheme 3 (X = piperidine-2-carboxylic acid), since an analogous route had earlier [19] given a satisfactory yield of poly-(glycyl-L-prolyl-L-alanine). The synthesis of the fully protected monomer (VII) was straightforward: both stages i and iii, which involved piperidine-2-carboxylic acid first as nucleophile and then in the *C*-terminal position of the carboxy-component, proceeded in high yield. Simultaneous deprotection and activation with hydrogen

Z—Ala—Gly—Pipec—OR

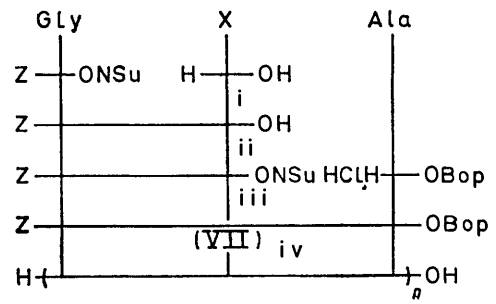
(V) R = C_6Cl_5

(VI) R = H



SCHEME 2

Pipec = piperidine-2-carboxylic acid. Conditions: i, pivalic mixed anhydride; ii, HBr–AcOH; iii, *N*-methylmorpholine–Me_2SO.



SCHEME 3

Bop = 2-benzyloxyphenyl. Conditions: i, Et_3N–$Me_2N{\cdot}CHO$; ii, dicyclohexylcarbodi-imide–HONSu; iii, Et_3N–$Me_2N{\cdot}CHO$; iv (X = piperidine-2-carboxylic acid), HBr–AcOH, then *N*-methylmorpholine–Me_2SO; iv (X = azetidine-2-carboxylic acid), H_2–Pd and then *N*-methylmorpholine–Me_2SO.

[12] See footnote, ref. 7.

[13] N. C. Chaturvedi, W. K. Park, R. R. Smeby, and F. M. Bumpus, *J. Medicin. Chem.*, 1970, **13**, 177.

[14] E. D. Nicolaides, H. A. Dewald, and M. K. Craft, *Ann. New York Acad. Sci.*, 1963, **104**, 15.

[15] Zh. D. Bespalova, I. A. Kaurov, V. F. Martynov, Yu. V. Natochin, M. I. Titov, and S. I. Shakhmatova, *Vestnik Leningrad, Univ.*, 1966, **21**(22), *Ser. Fiz. Khim.*, No. 4, 157 (*Chem. Abs.*, 1967, **67**, 108,999e); V. F. Martynov, Zh. D. Bespalova, M. I. Titov, and S. I. Shurukhina, *Zhur. obshchei Khim.*, 1967, **37**, 583.

[16] L. Balaspiri, B. Penke, J. Petres, and K. Kovacs, *Monatsh.*, 1970, **101**, 1177.

[17] E. Katchalski, A. Berger, and J. Kurtz, in 'Aspects of Protein Structure,' ed. G. N. Ramachandran, Academic Press, New York, 1963, p. 205.

[18] M. Fujino and C. Hatanaka, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 929.

[19] R. D. Cowell and J. H. Jones, *J. Chem. Soc.* (*C*), 1971, 1082.

bromide in acetic acid followed by polymerisation in the usual way gave after dialysis and lyophilisation a low yield of white fluffy polypeptide which had an apparent weight average molecular weight of *ca.* 2000 as shown by gel chromatography.

Azetidine-2-carboxylic acid (III) [20] is incorporated into collagen by both *in vitro* and *in vivo* collagen-synthesising systems. Small amounts do not affect the physicochemical properties of the resulting collagen beyond minor changes in the helix–coil melting behaviour, but there are marked effects on the rate at which collagen fibrils are deposited in the extracellular matrix.[21] As far as we are aware, no syntheses of peptides containing azetidine-2-carboxylic acid have been described, although VanEtten [22] has prepared some simple derivatives including the crystalline dipeptide benzyloxycarbonyl-L-prolyl-L-azetidine-2-carboxylic acid. The recent elaboration of a convenient synthesis and resolution [23] of this cyclic imino-acid (III) will no doubt facilitate the study of its peptides. We were fortunate in obtaining, through the generosity of Professor L. Fowden, a substantial quantity of slightly impure L-azetidine-2-carboxylic acid of natural origin, which was conveniently purified in high yield *via* the corresponding benzyloxycarbonylimino-acid dicyclohexylammonium salt. Exploratory attempts at polytripeptide syntheses with this material by routes 2 and 3 were confounded by frequent encounters with oily intermediate di- and tri-peptides which could not be purified, but the route 1 type synthesis of poly-(glycyl-L-azetidine-2-carbonyl-L-alanine) shown in Scheme 3 (X = azetidine-2-carboxylic acid) met no such problems. Exhaustive dialysis followed by lyophilisation gave the required polypeptide as a white fluffy powder which had the expected spectroscopic properties. Molecular weight determination by gel chromatography [11] showed a broad distribution of molecular weight, which was however heavily biased towards low molecular weight material with an apparent weight average of only *ca.* 2000.

Physicochemical and immunological studies of these materials are in hand.

EXPERIMENTAL

The general instructions given in Part II [24] apply. In calculating the concentrations of solutions of the polymers no correction was applied for residual solvents in the polymer samples.

Benzyloxycarbonyl-L-alanylglycyl-L-thiazolidine-4-carboxylic Acid.—Triethylamine (1·01 g, 10 mmol) was added to a stirred suspension of L-thiazolidine-4-carboxylic acid [8] (1·4 g, 10·5 mmol) in a solution of benzyloxycarbonyl-L-alanylglycine succinimido ester [7] (3·77 g, 10 mmol) in dimethylformamide (10 ml) at 20°. The resulting clear solution was stirred at room temperature overnight, then evaporated. A solution of the residue in water (20 ml) was acidified to pH 2 with 2N-hydrochloric acid and then extracted with ethyl acetate. The combined organic extracts were washed with water and brine, and dried. Evaporation and reprecipitation of the residue from ethyl acetate–light petroleum gave the *acyltripeptide* (3·80 g, 96%), m.p. 104—106°, $[\alpha]_D^{20}$ −89·8° (*c* 1 in MeOH), $\nu_{max.}$ (Nujol) 1720br cm^{-1}, τ [$(CD_3)_2SO$] 2·0 (1H, complex, N*H*·CH_2), 2·61 (5H, s, aromatic), 2·75—5·00 (3H, s at 4·94 superimposed on complex, PhCH_2 and urethane NH), 5·02—6·40 (6H, complex, all α-protons and N·CH_2·S), 6·50—7·00 (2H, complex, S·CH_2·CH), and 8·76 (3H, d, *J* 8 Hz, CH_3·CH) (Found: C, 51·8; H, 5·3; N, 10·65; S, 7·7. $C_{17}H_{21}N_3O_6S$ requires C, 51·6; H, 5·35; N, 10·6; S, 8·0%).

Benzyloxycarbonyl-L-alanylglycyl-L-thiazolidine-4-carboxylic Acid Pentachlorophenyl Ester.—Ethyl chloroformate (0·765 ml, 8 mmol) was added to a stirred solution of benzyloxycarbonyl-L-alanylglycyl-L-thiazolidine-4-carboxylic acid (3·16 g, 8 mmol) in chloroform (25 ml) at −15°. After 15 min a solution of pentachlorophenol (2·2 g, 8·25 mmol) and triethylamine (0·81 g, 8 mmol) in chloroform (10 ml) was added. The solution was stirred at −15° for 1 h, then at 20° for 4 h, and was then washed with water, 10% sodium carbonate, water, N-hydrochloric acid, and brine, and dried. Evaporation left an oil which crystallised on addition of ethyl acetate (5 ml). Recrystallisation from ethyl acetate gave white crystals of *acyltripeptide pentachlorophenyl ester* (3·09 g, 60%), m.p. 170—171°, $[\alpha]_D^{20}$ −66·5° (*c* 1·05 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1795, 1720, and 1670 cm^{-1}, τ ($CDCl_3$) 2·65 (5H, s, aromatic), 2·87br (1H, N*H*·CH_2), 4·2—4·7 (2H, complex, urethane NH and S·CH_2·*CH*·CO), 4·90 (2H, s, PhCH_2), 5·36 (2H, s, NH·CH_2), 5·5—6·0 (3H, complex, α-CH of alanine and S·CH_2·N), 6·3—6·6 (2H, complex, S·CH_2·CH), and 8·63 (3H, d, *J* 8 Hz, CH_3·CH) (Found: C, 42·9; H, 3·05; Cl, 27·9; N, 6·3; S, 5·0. $C_{23}H_{20}Cl_5N_3O_6S$ requires C, 42·9; H, 3·1; Cl, 27·5; N, 6·5; S, 5·0%).

L-Alanylglycyl-L-thiazolidine-4-carboxylic Acid Pentachlorophenyl Ester Hydrobromide.—5·6N-Hydrogen bromide (4 ml) was added to a solution of benzyloxycarbonyl-L-alanylglycyl-L-thiazolidine-4-carboxylic acid pentachlorophenyl ester (1·8 g, 2·8 mmol) in acetic acid (25 ml) at room temperature. After 45 min ether (200 ml) was added and the precipitate was collected, washed with ether, and reprecipitated from methanol–ether to give *tripeptide pentachlorophenyl ester hydrobromide* (1·08 g, 65%), $\nu_{max.}$ (Nujol) 1785 and 1660 cm^{-1}, τ (CF_3·CO_2H) 1·73 (1H, complex, NH), 2·57 (3H, complex, $\overset{+}{N}H_3$), 4·37 (1H, complex, S·CH_2·*CH*), 4·9—5·7 (5H, complex, α-CH of glycine and alanine, and S·CH_2·N), 6·0—6·5 (2H, complex, S·CH_2·CH), and 8·15br (3H, CH_3·CH). This compound was hygroscopic and was used without further characterisation.

Poly-(L-alanylglycyl-L-thiazolidine-4-carboxylic acid).—*N*-Methylmorpholine (0·375 ml, 3·4 mmol) was added to a stirred solution of L-alanylglycyl-L-thiazolidine-4-carboxylic acid pentachlorophenyl ester hydrobromide (1·00 g, 1·7 mmol) in dimethyl sulphoxide (1 ml). After 4 days the resulting solid mass was triturated with ethanol (50 ml) and the crude polymer was collected by centrifugation. It was washed with ethanol (3 × 50 ml) and ether (2 × 50 ml),

[20] For extensive references to the occurrence and biochemical effects of L-azetidine-2-carboxylic acid see L. Fowden, D. Lewis, and H. Tristram, *Adv. Enzymol.*, 1967, **29**, 89, and H. M. Berman, E. L. McGandy, J. W. Burgner, jun., and R. L. VanEtten, *J. Amer. Chem. Soc.*, 1969, **91**, 6177.

[21] J. M. Lane, L. J. Parkes, and D. J. Prockop, *Biochim. Biophys. Acta*, 1971, **236**, 528 and references there cited.

[22] R. L. VanEtten, personal communication.

[23] R. M. Rodebaugh and N. H. Cromwell, *J. Heterocyclic Chem.*, 1969, **6**, (*a*) 435; (*b*) 993.

[24] Part II, R. D. Cowell and J. H. Jones, *J.C.S. Perkin I*, 1972, 1809.

and dried to give a white powder (0·336 g). This was dissolved in dimethyl sulphoxide (35 ml) by warming, and the solution was dialysed against water (1 l) for 30 h; the water was changed every 10 h. During dialysis the polymer separated as a flocculent precipitate. Lyophilisation of the suspension gave a powder which was dried to constant weight at 100° and 0·1 mmHg affording *polymer* (0·254 g, 61·5%) of $[\alpha]_D^{20}$ −288° (*c* 0·43 in $CF_3{\cdot}CO_2H$), $\nu_{max.}$ (KBr) 1650br cm^{-1}, $\eta_{sp.}/c$ 0·32 dl g^{-1} (*c* 1 in $CHCl_2{\cdot}CO_2H$), τ ($CF_3{\cdot}CO_2H$) 1·6—2·6 (2H, both NH), 4·2—6·1 (6H, all α-CH and N·CH_2·S), 6·1—7·2 (2H after correction for peak due to residual Me_2SO, S·CH_2·CH), and 8·2—8·7 (3H, CH_3·CH). The n.m.r. spectrum contained a sharp peak at 7·00 due to Me_2SO superimposed on the broad polymer absorption: the intensity of this peak indicated the presence of *ca.* 1 molecule of dimethyl sulphoxide for every four repeating tripeptide units [Found: C, 39·7; H, 5·7; N, 14·5; S, 13·9%; C/N 2·74; C/S 2·86. $(C_9H_{13}N_3O_3S{,}0{\cdot}25Me_2SO{,}1{\cdot}5H_2O)_n$ requires C, 39·4; H, 6·0; N, 14·5; S, 13·8%; C/N 2·72; C/S 2·86].

*Benzyloxycarbonyl-*L*-piperidine-2-carboxylic Acid Dicyclohexylammonium Salt.*—The combined liquors remaining from several separations of the D-isomer from benzyloxycarbonyl-DL-piperidine-2-carboxylic acid by means of L-tyrosine hydrazide which had been performed [25] by another group in this laboratory were evaporated to give an oil (50 g) which was taken into ethyl acetate, washed with N-sulphuric acid, water, and brine, and dried. Dicyclohexylamine (45 g) was added, followed by light petroleum to the cloud point. The crystalline product (51 g) was recrystallised 4 times from the minimum amount of boiling ethanol, after which the specific rotation was unchanged by further recrystallisation. Benzyloxycarbonyl-L-piperidine-2-carboxylic acid dicyclohexylammonium salt (27·9 g) was obtained as needles, m.p. 162—172°, $[\alpha]_D^{20}$ −30·0° (*c* 0·53 in CCl_4) (Found: C, 69·9; H, 8·9; N, 6·3. Calc. for $C_{26}H_{40}N_2O_4$: C, 70·25; H, 9·1; N, 6·3%). After this part of the work was completed, Balaspiri *et al.*[16] reported the same compound with m.p. 156—158°.

On concentration of the combined liquors from the first two recrystallisations of the foregoing salt, feathery crystals separated: when left in their mother liquor these crystals changed to a cubic form of *benzyloxycarbonyl-*DL*-piperidine-2-carboxylic acid dicyclohexylammonium salt* (20·55 g), m.p. 155—167°, $[\alpha]_D^{20}$ 0·00° (*c* 0·51 in CCl_4), $\nu_{max.}$ ($CHCl_3$) 1687 and 1632 cm^{-1}, τ ($CDCl_3$) 0·64br (2H, s, $\overset{+}{N}H_2$), 2·66 (5H, s, aromatic), 4·85 (2H, s, PhCH_2), 5·2—5·4 (1H, complex, α-CH), and 5·7—9·2 (30H, complex, other protons) (Found: C, 70·4; H, 8·9; N, 6·2. $C_{26}H_{40}N_2O_4$ requires C, 70·25; H, 9·1; N, 6·3%).

*Benzyloxycarbonyl-*L*-piperidine-2-carboxylic Acid.*—A suspension of benzyloxycarbonyl-L-piperidine-2-carboxylic acid dicyclohexylammonium salt (27·65 g, 0·0625 mol) in ethyl acetate (100 ml) was shaken with N-sulphuric acid (125 ml) until dissolution was complete (15 min). The organic layer was then washed with water (3 × 100 ml) and brine, and dried. Evaporation left an oil which crystallised slowly. Recrystallisation from ethyl acetate–light petroleum gave benzyloxycarbonyl-L-piperidine-2-carboxylic acid (15·98 g, 97%), m.p. 110—112°, $[\alpha]_D^{20}$ −59·0° (*c* 2 in AcOH) {lit.,[16] m.p. 112—113°, $[\alpha]_D^{25}$ −57 ± 1° (*c* 5·3 in AcOH); for the D-isomer lit.,[25] m.p. 110—112°, $[\alpha]_D^{20}$ +59·2° (*c* 2 in AcOH), lit.,[16] m.p. 112—113°, $[\alpha]_D^{25}$ +57·2 ± 1° (*c* 5·3, AcOH)} (Found: C, 63·6; H, 6·3; N, 5·3. Calc. for $C_{14}H_{17}NO_4$: C, 63·9; H, 6·5; N, 5·3%).

*Benzyloxycarbonyl-*L*-piperidine-2-carbonyl-*L*-alanylglycine Pentachlorophenyl Ester.*—This was prepared on a 10 mmol scale *via* the pivalic mixed anhydride as described previously [17] for benzyloxycarbonyl-L-prolyl-L-alanylglycine pentachlorophenyl ester. Evaporation after drying gave an oil which afforded a white solid on addition of ether. Reprecipitation from chloroform–light petroleum gave *acyltripeptide active ester* as a flocculent white gel (3·40 g, 53%) of indefinite m.p., $[\alpha]_D^{20}$ −43·0° (*c* 0·5 in $CHCl_3$), $\nu_{max.}$ (Nujol) 1776, 1710, and 1645 cm^{-1}, τ ($CDCl_3$) 2·63 (5H, s, aromatic), 3·0—3·4 (2H, complex, both NH), 4·81 (2H, s, PhCH_2), 5·1—5·5 (2H, complex, α-CH of alanine and piperidinecarboxylic acid), 5·5—5·7 (2H, d, *J* 6 Hz, NH·CH_2·CO), and 5·7—8·8 (11H, complex, other protons) (Found: C, 46·6; H, 4·0; Cl, 27·45; N, 6·9. $C_{25}H_{24}Cl_5N_3O_6$ requires C, 46·9; H, 3·8; Cl, 27·7; N, 6·6%).

L-*Piperidine-2-carbonyl-*L*-alanylglycine Pentachlorophenyl Ester Hydrobromide.*—A solution of benzyloxycarbonyl-L-piperidine-2-carbonyl-L-alanylglycine pentachlorophenyl ester (2·8 g, 4·4 mmol) in 4N-hydrogen bromide in acetic acid (5 ml) was kept at 20° for 0·5 h, and ether (100 ml) was then added. The resulting oil was triturated with ether to give a hygroscopic white solid, which was recrystallised from methanol–ether to give *tripeptide active ester hydrobromide* (2·38 g, 92%), m.p. 170—172°, $[\alpha]_D^{20}$ −30·0° (*c* 1 in MeOH), $\nu_{max.}$ (Nujol) 1790 and 1660 cm^{-1}, τ ($CF_3{\cdot}CO_2H$) 1·8—2·9 (4H, complex, all NH), 5·0—5·9 (4H, complex, all α-protons), and 6·0—8·5 (11H, complex, other protons) (Found: C, 34·9; H, 3·15; Br, 13·9; Cl, 29·85; N, 7·3. $C_{17}H_{19}BrCl_5N_3O_4$ requires C, 34·8; H, 3·3; Br, 13·6; Cl, 30·2; N, 7·2%).

Poly-(L*-piperidine-2-carbonyl-*L*-alanylglycine).*— *N*-Methylmorpholine (0·5 ml, 4·5 mmol) was added to a stirred solution of L-piperidine-2-carbonyl-L-alanylglycine pentachlorophenyl ester hydrobromide (1·172 g, 2 mmol) in dimethyl sulphoxide (1 ml) at 20°. After 6 days the mixture was diluted with water (20 ml) and the precipitated pentachlorophenol was extracted with ether (2 × 5 ml). The yellow aqueous solution was dialysed against water (2 l) for 24 h; the water was changed every 8 h. Lyophilisation, followed by drying to constant weight at 100° and 0·1 mmHg gave *polymer* (0·077 g, 16%) as a pale yellow flocculent mass of $[\alpha]_D^{20}$ −111° (*c* 0·25 in H_2O), $\nu_{max.}$ (KBr) 1650br cm^{-1} $\eta_{sp.}/c$ 0·14 dl g^{-1} (*c* 0·85 in $CHCl_2{\cdot}CO_2H$), τ ($CF_3{\cdot}CO_2H$) 1·6—2·7 (2H, complex, both NH), 4·4—5·8 (4H, complex, all α-CH), and 6·0—8·7 (11H, complex, other protons) [Found: C, 47·6; H, 7·5; N, 15·3%; C/N 3·14. $(C_{11}H_{17}N_3O_3{,}2H_2O)_n$ requires C, 48·0; H, 7·6; N, 15·3%; C/N 3·17].

*Benzyloxycarbonyl-*L*-alanylglycyl-*L*-piperidine-2-carboxylic Acid Dicyclohexylammonium Salt.*—L-Piperidine-2-carboxylic acid [16] (1·68 g, 13 mmol) was added to a stirred solution of benzyloxycarbonyl-L-alanylglycine succinimido ester [7] (4·9 g, 13 mmol) and triethylamine (1·85 ml, 13 mmol) in ethanol (75 ml). After 16 h the solvent was removed, water (10 ml) was added, and the aqueous solution was acidified to pH 2 with 2N-hydrochloric acid. The precipitated oil was extracted into ethyl acetate and the organic solution was washed with water and brine, and dried. Evaporation gave an oil which could not be induced to crystallise. It was dissolved in ethyl acetate (10 ml) and dicyclohexylamine (2·4 g, 13·5 mmol) was added. Addition

[25] D. M. Brunwin, G. Lowe, and J. Parker, *J. Chem. Soc.* (*C*), 1971, 3756.

of ether caused deposition of *acyltripeptide dicyclohexylammonium salt* (3·35 g, 45%) as needles, m.p. 121—123°, $[\alpha]_D^{20}$ −42·4° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1720, 1675, and 1640 cm^{-1}, τ ($CDCl_3$) 1·30br (2H, $\overset{+}{N}H_2$), 2·50—2·85 (6H, s at 2·60 superimposed on a complex band, aromatic protons and CO·N*H*·CH_2), 4·30 (1H, complex, urethane NH), 4·87 (2H, s, PhCH_2), 5·4—6·1 (4H, complex, all α-CH), and 6·5—9·1 (33H, complex, other protons) (Found: C, 64·9; H, 8·3; N, 9·75. $C_{31}H_{48}N_4O_6$ requires C, 65·0; H, 8·45; N, 9·6%).

On concentration of the liquors, crystals of benzyloxycarbonyl-L-alanylglycine ethyl ester were deposited (0·68 g), m.p. 90—92°, $[\alpha]_D^{20}$ −22·8° (*c* 1·3 in EtOH) {lit.,[26] m.p. 97·5—98°, $[\alpha]_D^{20}$ −24·4° (*c* 1 in EtOH)}, $\nu_{max.}$ (Nujol) 1755, 1690, and 1650 cm^{-1}, τ ($CDCl_3$) 2·60 (5H, s, aromatic), 3·05br (1H, peptide NH), 4·15—4·40 (1H, complex, urethane NH), 4·85 (2H, s, PhCH_2), 5·44—5·88 (3H, complex, α-CH of alanine and O·CH_2·CH_3), 5·99 (2H, d, *J* 6 Hz, NH·CH_2·CO), and 8·4—9·0 (6H, complex, CH_3·CH and CH_3·CH_2·O).

Benzyloxycarbonyl-L-piperidine-2-carboxylic Acid Pentachlorophenyl Ester.—A solution of dicyclohexylcarbodi-imide (4·12 g, 20 mmol) in ethyl acetate (20 ml) was added during 5 min to a stirred solution of benzyloxycarbonyl-L-piperidine-2-carboxylic acid (5·26 g, 20 mmol) and pentachlorophenol (5·32 g, 20 mmol) at −10°. After 0·5 h at −10° and 5 h at 15° the mixture was filtered and the filtrate was evaporated to yield an oil which crystallised on trituration with ether. Recrystallisation from ethanol gave white needles of benzyloxycarbonyl-L-piperidine-2-carboxylic acid pentachlorophenyl ester (9·63 g, 94%), m.p. 110—111°, $[\alpha]_D^{20}$ −40·6° (*c* 1 in $CHCl_3$), $[\alpha]_D^{20}$ −38·1° (*c* 1 in Me_2N·CHO), $\nu_{max.}$ ($CHCl_3$) 1785 and 1700 cm^{-1}, τ ($CDCl_3$) 2·66 (5H, s, aromatic), 4·5—4·9 (3H, s at 4·83 superimposed on complex band, PhCH_2 and α-CH), and 5·6—8·8 (8H, complex, other protons) (Found: C, 47·2; H, 3·2; Cl, 35·0; N, 2·6. Calc. for $C_{20}H_{16}Cl_5NO_4$: C, 47·0; H, 3·2; Cl, 34·65; N, 2·7%). After the completion of this part of the work, Balaspiri *et al.*[16] reported the same compound with m.p. 110—111°, $[\alpha]_D^{25}$ −38·5° ± 1° (*c* 1 in Me_2N·CHO).

L-Piperidine-2-carboxylic Acid Pentachlorophenyl Ester Hydrobromide.—5·6N-Hydrogen bromide in acetic acid (10 ml) was added to a solution of benzyloxycarbonyl-L-piperidine-2-carboxylic acid pentachlorophenyl ester (7·67 g, 15 mmol) in acetic acid (15 ml) at room temperature. After 0·5 h ether (100 ml) was added and the solid was collected and washed with ether (500 ml). Recrystallisation from methanol–ether gave *imino-acid pentachlorophenyl ester hydrobromide* (6·15 g, 89·5%), m.p. 245—246° (decomp.), $[\alpha]_D^{20}$ +28·0° (*c* 1 in MeOH), $\nu_{max.}$ (Nujol) 1790 cm^{-1} (Found: C, 31·5; H, 2·4; Br, 17·75; Cl, 38·4; N, 2·9. $C_{12}H_{11}BrCl_5NO_2$ requires C, 31·4; H, 2·4; Br, 17·4; Cl, 38·7; N, 3·1%).

Benzyloxycarbonyl-L-alanylglycyl-L-piperidine-2-carboxylic Acid Pentachlorophenyl Ester.—Benzyloxycarbonyl-L-alanylglycyl-L-piperidine-2-carboxylic acid (2 mmol) was obtained by quantitative liberation from the corresponding dicyclohexylammonium salt (1·14 g, 2 mmol) in the usual way. The resulting oil was dissolved in ethyl acetate (10 ml) and triethylamine (0·28 ml, 2 mmol) was added, followed by a solution of *O*-trichloroacetylpentachlorophenol[18] (0·90 g, 2·18 mmol) in ethyl acetate (15 ml). The solution was stirred at 20° for 48 h and was then washed in the usual way. Removal of the solvent gave a chromatographically impure crisp white foam which was purified on a column of Sephadex LH-20 in dioxan (35 × 2 cm). The required active ester was obtained as a chromatographically pure foam (0·60 g, 47%) from which solvent could not be completely removed, $\nu_{max.}$ (Nujol) 1790, 1725, and 1660 cm^{-1}, τ ($CDCl_3$) 2·65 (5H, s, aromatic), 2·8—3·0 (1H, complex, N*H*·CH_2), 4·4—4·6 (1H, complex, urethane NH), 4·69 (2H, s, PhCH_2), 5·3—6·1 (4H, complex, all α-CH), and 6·2—8·9 (s at 6·3 on complex, other protons and residual dioxan protons) (Found: C, 48·4; H, 4·7; Cl, 24·85; N, 6·8. $C_{25}H_{24}Cl_5N_3O_6$ requires C, 46·9; H, 3·8; Cl, 27·7; N, 6·6%). Attempted preparations of this compound from the acyltripeptide by the dicyclohexylcarbodi-imide, pivalic mixed anhydride, and carbonic mixed anhydride methods all gave complex mixtures. Treatment of this compound with hydrogen bromide in acetic acid in the conventional manner consistently gave a brown powder which gave low chlorine analyses; attempted polymerisation of this material in the usual way gave no undialysable polypeptide.

Benzyloxycarbonylglycyl-L-piperidine-2-carboxylic Acid Di cyclohexylammonium Salt.—Triethylamine (1·12 ml, 8 mmol) was added to a stirred suspension of L-piperidine-2-carboxylic acid[16] (1·03 g, 8 mmol) in a solution of benzyloxycarbonylglycine succinimido ester[27] (2·5 g, 8 mmol) in dimethylformamide (12 ml). After 3 days 3-dimethylamino-1-propylamine[28] (2 drops) was added to the clear solution and the mixture was stirred for a further 1 h. It was then acidified to pH 2 with 2N-hydrochloric acid and extracted with ethyl acetate (3 × 25 ml). The combined organic extracts were washed with water and brine, and dried. Evaporation gave an oil which did not crystallise. It was dissolved in ethyl acetate (5 ml) and ether (20 ml), and dicyclohexylamine (1·6 ml) was added. On addition of light petroleum to the cloud point, crystals of *acyldipeptide dicyclohexylammonium salt* were deposited (3·87 g, 96·5%), m.p. 122—124°, $[\alpha]_D^{20}$ −38·6° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1710 and 1640 cm^{-1}, τ ($CDCl_3$) 1·15br (2H, singlet, $\overset{+}{N}H_2$), 2·64 (5H, s, aromatic), 3·9—4·1 (1H, complex, N*H*·CH_2), 4·89 (2H, s, PhCH_2), 5·4—6·1 (3H, complex, α-CH), and 6·5—9·4 (30H, complex, other protons) (Found: C, 67·4; H, 8·7; N, 8·3. $C_{28}H_{43}N_3O_5$ requires C, 67·05; H, 8·6; N, 8·4%).

Benzyloxycarbonylglycyl-L-piperidine-2-carboxylic Acid Succinimido Ester.—A suspension of benzyloxycarbonylglycyl-L-piperidine-2-carboxylic acid dicyclohexylammonium salt (2·5 g, 5 mmol) in ethyl acetate (30 ml) was shaken with N-sulphuric acid (2 × 10 ml). The organic solution was washed with water (2 × 20 ml) and brine, and dried. Evaporation gave an oil which was dissolved in ethyl acetate (10 ml) and combined with a solution of *N*-hydroxysuccinimide (0·6 g, 5 mmol) in dioxan (5 ml). A solution of dicyclohexylcarbodi-imide (1·1 g, 5 mmol) in ethyl acetate (10 ml) was added to the stirred solution at 0°. After 0·5 h at 0° and 3·5 h at 20° the mixture was filtered and the filtrate was evaporated to give an oil which formed a sticky amorphous solid on trituration with light petroleum. Precipitation from ethyl acetate–light petroleum gave *acyldipeptide active ester* (2·08 g, 100%) as an amorphous white solid of indefinite m.p. (40—60°), $[\alpha]_D^{20}$ −49·7° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1820, 1790, 1750, and 1720 cm^{-1}, τ ($CDCl_3$) 2·66 (5H, s, aromatic), 4·05—4·4 (2H, complex,

[26] L. Hastings and J. F. Arens, *Rec. Trav. chim.*, 1955, **74**, 769.
[27] G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, 1964, **86**, 1839.
[28] K. L. Agarwal, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc.* (*C*), 1969, 2218.

NH and α-CH of piperidinecarboxylic acid), 4·90 (2H, s, PhCH_2), 5·8—6·1 (2H, complex, NH·CH_2), and 6·25—8·9 (12H, complex, other protons) (Found: C, 57·7; H, 5·9; N, 10·2. $C_{20}H_{23}N_3O_7$ requires C, 57·55; H, 5·55; N, 10·1%).

Benzyloxycarbonylglycyl-L-piperidine-2-carbonyl-L-alanine 2-Benzyloxyphenyl Ester.—Triethylamine (0·58 ml, 4 mmol) was added to a stirred solution of benzyloxycarbonylglycyl-L-piperidine-2-carboxylic acid succinimido ester (1·67 g, 4 mmol) and L-alanine 2-benzyloxyphenyl ester hydrochloride [29] (1·25 g, 4 mmol) in dimethylformamide (10 ml) at 20°. After 6 h ethyl acetate (60 ml) was added and the solution was washed with water, 10% sodium carbonate, water, N-hydrochloric acid, and brine, and dried. Evaporation gave an oil which precipitated as a white solid from chloroform solution on addition of light petroleum to give *protected tripeptide* (1·96 g, 85%), m.p. 66—68°, $[\alpha]_D^{20}$ −71·7° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1765 and 1715 cm^{-1}, τ ($CDCl_3$) 2·66 (10H, practically superimposed singlets, both C_6H_5·CH_2), 2·85—3·20 (4H, complex, other aromatic protons), 3·47 (1H, d, *J* 7 Hz, N*H*·CHMe), 4·1—4·4 (1H, complex, urethane NH), 4·90 (2H, s, PhCH_2·O·CO), 5·00 (2H, s, PhCH_2·O·C_6H_4), 5·1—6·2 (4H, complex, α-protons), and 6·3—8·9 (11H, complex, other protons) (Found: C, 67·3; H, 6·4; N, 7·3. $C_{32}H_{35}N_3O_7$ requires C, 67·0; H, 6·15; N, 7·3%).

Poly-(glycyl-L-piperidine-2-carbonyl-L-alanine).— 5·6N-Hydrogen bromide in acetic acid (2 ml) was added to a solution of benzyloxycarbonylglycyl-L-piperidine-2-carbonyl-L-alanine 2-benzyloxyphenyl ester (1·15 g, 2 mmol) in acetic acid (1 ml). After 1 h ether (80 ml) was added and the mixture was triturated to give a white solid which was collected and washed with ether (500 ml). It was then dried (KOH) at 50° and 0·1 mmHg for 16 h. *N*-Methylmorpholine (0·66 ml, 6 mmol) was added to a stirred solution of this solid in dimethyl sulphoxide (1 ml) at 20°. After 4 days the resulting suspension was diluted with water (25 ml) and then extracted with ether (10 ml). The aqueous solution was dialysed against water (4 l) for 30 h; the water was changed every 10 h. Lyophilisation, followed by drying to constant weight at 70° and 0·1 mmHg, gave *polymer* (0·070 g, 15%) as a fluffy white solid, $[\alpha]_D^{20}$ −156°, $[\alpha]_{578}^{20}$ −165°, $[\alpha]_{546}^{20}$ −185°, $[\alpha]_{436}^{20}$ −325°, $[\alpha]_{365}^{20}$ −477° (*c* 0·06 in H_2O), $\nu_{max.}$ (KBr) 1650 cm^{-1}, $\bar{M}_w$ *ca.* 2000 (by gel chromatography [11]), τ (CF_3·CO_2H) 1·8—2·6 (2H, complex, both NH), 4·4—5·8 (4H, complex, all α-protons), and 6·0—8·7 (11H, complex, other protons) [Found: C, 49·5; H, 6·8; N, 15·6%; C/N 3·17. $(C_{11}H_{17}N_3O_3,1{\cdot}5H_2O)_n$ requires C, 49·1; H, 7·4; N, 15·6%; C/N 3·14].

Benzyloxycarbonyl-L-azetidine-2-carboxylic Acid Dicyclohexylammonium Salt.—Crude L-azetidine-2-carboxylic acid of natural origin (3·4 g) was converted into the benzyloxycarbonyl derivative.[30] The resulting syrup was dissolved in ethyl acetate (30 ml) and a solution of dicyclohexylamine (6·2 g) in ethyl acetate (25 ml) was added. The immediate white precipitate was collected and recrystallised from methanol–ether to give *acylimino-acid dicyclohexylammonium salt* (8·36 g, 60% based on 100% pure starting material), m.p. 162—174°, $[\alpha]_D^{20}$ −71·0° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1700 cm^{-1}, τ ($CDCl_3$) 0·95br (2H, $\overset{+}{N}H_2$), 2·67 (5H, s, aromatic), 4·90 (2H, s, PhCH_2), 5·43 (1H, complex, α-proton), 5·80—6·24 (2H, complex, N·CH_2), 6·70—7·76 (4H, complex, CH_2·CH·CO and CH·$\overset{+}{N}H_2$·CH), and 7·80—9·25 (20H, complex, other protons) (Found: C, 69·2; H, 8·5; N, 6·9. $C_{24}H_{36}N_2O_4$ requires C, 69·2; H, 8·7; N, 6·7%).

L-Azetidine-2-carboxylic Acid.—A suspension of benzyloxycarbonyl-L-azetidine-2-carboxylic acid dicyclohexylammonium salt (4·16 g, 10 mmol) in ethyl acetate (20 ml) was shaken with N-sulphuric acid (11 ml) until dissolution was complete. The organic layer was then washed with water (2 × 10 ml) and brine, and dried. Evaporation gave an oil which was dissolved in acetic acid (30 ml) and hydrogenated over 10% palladium–charcoal (0·5 g) at atmospheric pressure for 5 h. Filtration followed by evaporation gave an oil which was dissolved in boiling methanol (50 ml). Addition of ether caused white needles of L-azetidine-2-carboxylic acid to separate (1·00 g, quantitative), m.p. 210—211°, $[\alpha]_D^{20}$ −120·0° (*c* 0·5 in H_2O) {lit.,[30] $[\alpha]_D^{20}$ −108° (*c* 3·6 in H_2O); lit.,[31] $[\alpha]_D^{20}$ −118·4° (in H_2O); lit.,[23b] $[\alpha]_D^{20}$ −109° (*c* 3·6 in H_2O)} (Found: C, 47·5; H, 7·2; N, 13·7. Calc. for $C_4H_7NO_2$: C, 47·5; H, 7·0; N, 13·9%).

Benzyloxycarbonylglycyl-L-azetidine-2-carboxylic Acid Dicyclohexylammonium Salt.—L-Azetidine-2-carboxylic acid (0·404 g, 4 mmol) was added to a stirred solution of benzyloxycarbonylglycine succinimido ester [27] (1·225 g, 4 mmol) and triethylamine (0·56 ml, 4 mmol) in dimethylformamide (10 ml) at room temperature. After 0·5 h water (2 ml) was added dropwise, giving a clear solution. The solution was stirred overnight and was then acidified to pH 2 with 2N-hydrochloric acid, and extracted with ethyl acetate (3 × 20 ml). The combined organic extracts were washed with water and brine, dried, and evaporated to give an oil which did not crystallise. Dicyclohexylamine (0·8 ml) was added to a solution of this oil in ethyl acetate (5 ml) and ether (25 ml). On scratching, white needles of *acyldipeptide dicyclohexylammonium salt* were deposited (1·52 g, 80%), m.p. 144—149°, $[\alpha]_D^{20}$ −76·2° (*c* 1·1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1715 cm^{-1}, τ ($CDCl_3$) 1·20br (2H, $\overset{+}{N}H_2$), 2·70 (5H, s, aromatic), 4·22 (1H, complex, NH), 4·92 (2H, s, PhCH_2), 5·35 (1H, complex, N·CH·CO), 5·80—6·45 (4H, complex, NH·CH_2·CO and N·CH_2·CH_2), 6·70—7·60 (4H, complex, CH_2·CH·CO and CH·$\overset{+}{N}H_2$·CH), and 7·65—9·25 (20H, complex, other protons) (Found: C, 65·6; H, 8·5; N, 8·9. $C_{26}H_{39}N_3O_5$ requires C, 65·9; H, 8·3; N, 8·9%).

Benzyloxycarbonylglycyl-L-azetidine-2-carboxylic Acid Succinimido Ester.—A suspension of benzyloxycarbonylglycyl-L-azetidine-2-carboxylic acid dicyclohexylammonium salt (1·42 g, 3 mmol) in ethyl acetate (15 ml) was shaken with N-sulphuric acid (2 × 5 ml). The organic layer was washed with water and brine, and dried. The oil obtained on evaporation was dissolved in ethyl acetate (10 ml) together with *N*-hydroxysuccinimide (0·35 g, 3 mmol), and the solution was cooled to 0°. A solution of dicyclohexylcarbodi-imide (0·63 g, 3 mmol) in ethyl acetate (5 ml) was added and the mixture was stirred for 1 h at 0° and then overnight at 20°. After filtration and evaporation an oil was obtained which solidified slowly on trituration with light petroleum. Reprecipitation from ethyl acetate–light petroleum gave *acyldipeptide succinimido ester* (0·96 g, 82%) as an amorphous solid of indefinite m.p. (40—60°), $[\alpha]_D^{20}$ −86·6° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1820, 1790, 1745, and 1715 cm^{-1}, τ ($CDCl_3$) 2·67 (5H, s, aromatic), 4·35 (1H, complex, NH), 4·90 (2H, s, PhCH_2), 5·6—6·05 (3H, complex, all α-protons), 6·16 (2H, complex, N·CH_2), and 7·1—7·6

[29] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
[30] L. Fowden, *Biochem. J.*, 1956, **64**, 323.
[31] A. I. Virtanen, *Nature*, 1955, **176**, 984.

(6H, s at 7·20 superimposed on complex, CO·[CH_2]$_2$·CO and CH_2·CH) (Found: C, 55·8; H, 5·2; N, 10·6. $C_{18}H_{19}N_3O_7$ requires C, 55·5; H, 4·9; N, 10·8%).

*Benzyloxycarbonylglycyl-*L*-azetidine-2-carbonyl-*L*-alanine 2-Benzyloxyphenyl Ester*.—Triethylamine (0·32 ml, 2·3 mmol) was added to a stirred solution of benzyloxycarbonylglycyl-L-azetidine-2-carboxylic acid succinimido ester (0·9 g, 2·3 mmol) and L-alanine 2-benzyloxyphenyl ester hydrochloride [1] (0·73 g, 2·3 mmol) in dimethylformamide (5 ml) at 20°. After 6 h ethyl acetate (40 ml) was added and the solution was washed with water, 10% sodium carbonate, water, N-hydrochloric acid, water, and brine, and dried. Evaporation gave an oil which formed a sticky solid on trituration with light petroleum. Precipitation from chloroform–light petroleum gave *fully protected tripeptide* (1·1 g, 88%) as a white solid of m.p. 57—59°, $[\alpha]_D^{20}$ −104° (*c* 0·53 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1767, 1720, and 1685 cm^{-1}, τ ($CDCl_3$) 2·0—2·2 (1H, complex, peptide NH), 2·64 (10H, practically superimposed singlets, both C_6H_5·CH_2), 2·85—3·1 (4H, complex, other aromatic protons), 4·4—4·6 (1H, complex, urethane NH), 4·89 (2H, s, PhCH_2·O·CO), 4·94 (2H, s, PhCH_2·O·C_6H_4), 5·0—5·4 (2H, complex, NH·C*H*Me and N·CH), 5·8—6·3 (4H, complex, N·CH_2·CH_2 and NH·CH_2·CO), 7·1—7·8 (2H, complex, N·CH_2·CH_2), and 8·58 (3H, d, *J* 8 Hz, CH_3·CH) (Found: C, 65·9; H, 5·65; N, 7·7. $C_{30}H_{31}N_3O_7$ requires C, 66·0; H, 5·7; N, 7·7%).

*Poly(glycyl-*L*-azetidine-2-carbonyl-*L*-alanine)*.—A solution of benzyloxycarbonylglycyl-L-azetidine-2-carbonyl-L-alanine 2-benzyloxyphenyl ester (1·0 g, 1·84 mmol) in acetic acid (20 ml) was hydrogenated over 10% palladium–charcoal (0·20 g) at atmospheric pressure for 5 h. After filtration and evaporation a dark green oil was obtained which was dissolved in dimethyl sulphoxide (1 ml). *N*-Methylmorpholine (0·66 ml, 6 mmol) was added to this solution with stirring and after 5 days the solid grey mass was dissolved in water (40 ml). The aqueous solution was dialysed against water (4 l) for 24 h (the water was changed every 8 h) and was then lyophilised. The resulting fluffy white solid was dried to constant weight at 70° and 0·1 mmHg to give *polymer* (0·115 g, 27%), $[\alpha]_D^{20}$ −181°, $[\alpha]_{578}^{20}$ −187°, $[\alpha]_{546}^{20}$ −215°, $[\alpha]_{436}^{20}$ −372°, $[\alpha]_{365}^{20}$ −595° (*c* 0·071 in H_2O), $\nu_{max.}$ (KBr) 1650 and 1540 cm^{-1}, $\bar{M}_w$ *ca.* 2000 (by gel chromatography [11]), τ (CF_3·CO_2H) 1·8—2·4 (2H, complex, both NH), 4·4—5·3 (2H, complex, α-protons of alanine and azetidine-carboxylic acid), 5·3—6·2 (4H, complex, N·CH_2·CH_2 and NH·CH_2·CO), 6·7—7·7 (2H, complex, N·CH_2·CH_2), and 8·40 (3H, d, *J* 7 Hz, CH_3·CH) [Found: C, 45·5; H, 6·4; N, 16·9%; C/N 2·58. $(C_9H_{13}N_3O_3,2H_2O)_n$ requires C, 45·2; H, 7·1; N, 17·5%; C/N 2·58].

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