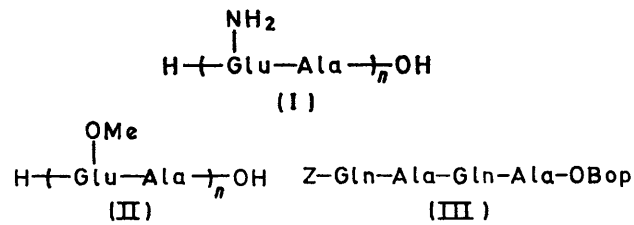
Sequential Polypeptides. Part VII.[1] The Synthesis of Poly-(L-glutaminyl-L-alanine), a Model of the Silks produced by Sawflies of the Family *Argidae*

By J. H. Jones * and J. Walker, The Dyson Perrins Laboratory, Oxford University, Oxford OX1 3QY

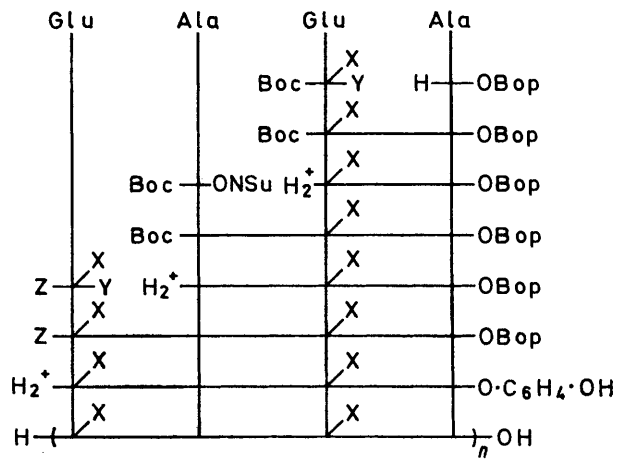
The synthesis of poly-(L-glutaminyl-L-alanine), which has the repeating sequence proposed for the silks produced by members of the sawfly family *Argidae*, is described. The *o*-hydroxyphenyl ester method was used, the synthesis being performed *via* the fully protected tetrapeptide derivative benzyloxycarbonyl-L-glutaminyl-L-alanyl-L-glutaminyl-L-alanine 2-benzyloxyphenyl ester. Exhaustive dialysis of the product gave the required polymer of weight average molecular weight 5000—10,000 judged on viscosity criteria. Poly-(γ-methyl-L-glutamyl-L-alanine) was similarly prepared. An *N*-t-butyl substituent was shown to be unsuitable for side-chain amide protection, since this group survives an hour's exposure to liquid hydrogen fluoride in the presence of anisole.

THE arthropod silk fibroins are diverse in structure and composition.[2-4] The fibroin of the commercially exploited silkworm *Bombyx mori*, which is the most thoroughly studied, contains large amounts of glycine, alanine, and serine. The proposal that much of the amino-acid sequence could be written (Ser-Gly-Ala-Gly-Ala-Gly)$_n$ has been corroborated by synthesis[5] of a sequential polypeptide with this sequence and the demonstration[6] of close similarity between the *X*-ray diffraction patterns of the natural and synthetic materials. The circumstantial evidence of amino-acid contents comprising large proportions of very few different residues implies that the silks of other species also have repeating sequences. One group of silks which has been of recent interest[2-4] is produced by the sawfly family *Argidae*. These are remarkable for their high content of alanine and glutamine, which are present in close to equimolecular amounts sufficient to account for 70—80% of the residues. The amino-acid content suggests that long segments of alternating sequence alanylglutaminyl may be present, and there is some *X*-ray support for this. The *X*-ray diffraction patterns of the three silks examined provided evidence of a predominant β-structure similar to that of *B. mori* fibroin (although the silks of *Argidae* contain practically no glycine, which constitutes nearly 50% of all residues in *B. mori* fibroin) with superimposed indications of an α-structure in one case. Lucas and Rudall suggested[3] that *X*-ray studies of a synthetic polypeptide consisting of alternating alanine and glutamine residues [as in (I)] would be a useful aid to interpretation: this paper is concerned with the preparation of such a material for this purpose.

The synthetic objective (I) could in principle be approached through an appropriate dipeptide monomer. The literature contains numerous examples of dipeptide active ester polymerisations, sometimes[7] in good yield, but experience with the synthesis of the closely related polymer (II) prompted us to prefer a route to (I) *via* a tetrapeptide derivative. Since we had in previous work[1,8,9] found a general approach to sequential polypeptides involving peptide *o*-hydroxyphenyl esters as the



activated monomers to be convenient, we again employed this strategy (see Scheme). Since *o*-hydroxyphenyl



SCHEME Synthesis of (I): X = NH_2, Y = OTcp; Synthesis of (II): X = OMe, Y = ONSu

Here and throughout this paper all amino-acid residues are L and abbreviated nomenclature follows the relevant Tentative Rules of the IUPAC–IUB Commission on Biochemical Nomenclature (*Specialist Periodical Reports on Amino-acids, Peptides and Proteins*, 1970, **2**, ch. 5): Z = benzyloxycarbonyl; Boc = t-butoxycarbonyl; NSu = succinimido; Tcp = 2,4,5-trichlorophenyl; Bop = 2-benzyloxyphenyl.

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[3] F. Lucas and K. M. Rudall in 'Comprehensive Biochemistry,' eds. M. Florkin and E. H. Stotz, Elsevier, Amsterdam, 1968, **26***B*, p. 475.
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[7] *E.g.*, P. M. Hardy, H. N. Rydon, and R. C. Thompson, *J.C.S. Perkin I*, 1972, 5.
[8] R. D. Cowell and J. H. Jones, *J. Chem. Soc.* (*C*), 1971, 1082.
[9] Part V, R. D. Cowell and J. H. Jones, *J.C.S. Perkin I*, 1972, 2236.

esters are not subject to detectable racemisation in model systems [8,10,11] the optical integrity of the polymer can be assumed with reasonable confidence. The synthesis of the protected monomer (III) was straightforward, all the fully protected derivatives in the Scheme being crystalline. Simultaneous deprotection and activation of compound (III) was performed by acidolysis or hydrogenolysis and the resulting peptide *o*-hydroxyphenyl ester was polymerised by treatment with tertiary base in concentrated dimethyl sulphoxide solution. Both procedures gave crude polymer in *ca.* 50% yield, which was reduced to *ca.* 10% after exhaustive dialysis of a solution made by dissolving the crude material in 98% formic acid and diluting with water. As dialysis progressed the polymer separated from solution: lyophilisation gave a fluffy white powder which had the expected spectroscopic properties and gave satisfactory analytical results. The purified polymer was freely soluble only in solvents such as dichloroacetic and trifluoroacetic acids; this frustrated attempts at molecular weight determination by conventional methods. Comparison of reduced specific viscosity measurements in dichloroacetic acid with literature values [12] for comparable sequential polypeptides suggests a weight average molecular weight in the range 5000—10,000.

We also examined the possibility of obtaining the polymer (I) by ammonolysis of a polymer (II) with side-chain ester groups.[13] The intermediate polymer (II) was prepared *via* a tetrapeptide *o*-hydroxyphenyl ester (X = OMe, Y = ONSu in the Scheme) after an attempt *via* a dipeptide monomer had given a very low yield. The polymer (II), however, proved intractable, being insoluble in solvents suitable for attempting ammonolysis of the side-chains, and this approach was abandoned.

We also considered the possibility of synthesising the polymer (I) *via* intermediates with *N*-protected glutamine side-chains (*i.e.* in the Scheme X = NHR, where R = alkyl) since these might have had advantages in solubility. For various reasons none of the published methods of amide protection seemed suitable. There is no report of any investigation of the possibility of using *N*-t-butyl groups for this purpose (although *N*-t-butylglutamine and asparagine derivatives have been encountered [14] as by-products from t-butylation reactions). An *N*-t-butylamide side-chain might be expected to confer desirable solubility properties. Furthermore it seemed reasonable to expect that an *N*-t-butylamide would be deprotected by liquid hydrogen fluoride since cleavage of *N*-*p*-methoxybenzylamides by this reagent in the presence of anisole had been reported,[15] and with protective groups for other functionalities, corresponding t-butyl and *p*-methoxybenzyl derivatives are comparable in acid-lability. We were therefore disappointed to find that treatment of *N*(α)-benzyloxycarbonyl-*N*(ω)-t-butyl-L-glutamine benzyl ester with liquid hydrogen fluoride in the presence of anisole for an hour at room temperature gave in high yield not glutamine but *N*(ω)-t-butylglutamine.

EXPERIMENTAL

The general instructions given in Part II apply. Liquid hydrogen fluoride was manipulated in apparatus supplied by Toho Kasei Co. Ltd., Osaka, Japan.

t-Butoxycarbonyl-L-glutaminyl-L-alanine 2-Benzyloxyphenyl Ester.—Triethylamine (0·28 ml, 2 mmol) and t-butoxycarbonyl-L-glutamine 2,4,5-trichlorophenyl ester [16] (0·90 g, 2 mmol) were added to a stirred solution of L-alanine 2-benzyloxyphenyl ester hydrochloride [9] (0·62 g, 2 mmol) in dimethylformamide (1 ml). After 18 h, the solution was evaporated at 30° and 0·5 mmHg and the residual oil was dissolved in ethyl acetate (40 ml), washed with 10% citric acid (4 ml), saturated sodium hydrogen carbonate (4 ml), and water (2 × 5 ml), and dried. Evaporation gave a white solid which on recrystallisation from chloroform–light petroleum gave the *protected dipeptide* as white needles (0·44 g, 81%), m.p. 159—160°, $[\alpha]_D^{20}$ −45·7° (*c* 1 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1765, 1675br, and 1610 cm^{-1}; τ ($CDCl_3$) 2·2 (1H, d, *J* 7 Hz, NH of alanine), 2·6—3·1 (9H, complex, aromatic), 3·7 and 4·1 (2H, equally intense singlets, *cis*- and *trans*- [17] protons, respectively of $CO{\cdot}NH_2$), 4·41 (1H, d, *J* 8 Hz, urethane NH), 4·95 (2H, s, $PhCH_2$), 5·2 (1H, complex, α-proton of alanine), 5·75 (1H, complex, α-proton of glutamine), 7·6—8·2 (4H, complex, $CH_2{\cdot}CH_2$), 8·6 (9H, s, Bu^t), and 8·62 (3H, d, *J* 6 Hz, $CH{\cdot}CH_3$) (Found: C, 62·4; H, 6·6; N, 8·3. $C_{26}H_{33}N_3O_7$ requires C, 62·5; H, 6·6; N, 8·4%).

t-Butoxycarbonyl-L-alanyl-L-glutaminyl-L-alanine 2-Benzyloxyphenyl Ester.—t-Butoxycarbonyl-L-glutaminyl-L-alanine 2-benzyloxyphenyl ester (1·0 g, 2 mmol) was dissolved in 90% trifluoroacetic acid (0·5 ml). After 1 h, the solution was evaporated to dryness at 40° and 10 mmHg, final traces of acid being removed by the addition and evaporation of small amounts of water. The resulting oil was dried by the repeated addition and distillation of benzene. Triethylamine (0·45 ml, 3 mmol) and t-butoxycarbonyl-L-alanine succinimido ester [18] (0·72 g, 2 mmol) were added together to a stirred solution of the oil in dimethylformamide (0·5 ml) After 18 h, the solution was evaporated at 30° and 0·5 mmHg, and the residual oil was dissolved in chloroform (50 ml), washed with 10% citric acid (3 ml), saturated sodium hydrogen carbonate (5 ml), and water (15 ml), and dried. Evaporation gave a gelatinous substance which on reprecipitation from chloroform–light petroleum gave the *protected tripeptide* (0·85 g, 75%), m.p. 147—149°, $[\alpha]_D^{20}$ −51·1 (*c* 1 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1760, 1650, and 1610 cm^{-1}; τ ($CDCl_3$) 2·0 (1H, d, *J* 8 Hz, peptide NH of alanine), 2·4—3·2 (10H, complex, aromatic and NH of glutamine), 3·6 and 4·1 (2H, equally intense singlets, *cis*- and *trans*-protons, respectively, of $CO{\cdot}NH_2$), 4·4—4·7 (1H, complex, urethane NH of alanine), 4·97 (2H, s, $PhCH_2$), 5·1—5·6 (2H, complex, α-protons of

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alanine), 5·6—6·0 (1H, complex, α-proton of glutamine), 7·6—8·2 (4H, complex, CH_2·CH_2), and 8·5—8·9 (15H, complex, Bu^t and CH·CH_3) (Found: C, 61·6; H, 6·7; N, 9·5. $C_{29}H_{38}N_4O_8$ requires C, 61·05; H, 6·7; N, 9·8%).

Benzyloxycarbonyl-L-glutaminyl-L-alanyl-L-glutaminyl-L-alanine 2-Benzyloxyphenyl Ester.—t-Butoxycarbonyl-L-alanyl-L-glutaminyl-L-alanine 2-benzyloxyphenyl ester (0·235 g, 0·5 mmol) was treated with 90% trifluoroacetic acid as in the preceding preparation. Triethylamine (0·14 ml, 1 mmol) and benzyloxycarbonyl-L-glutamine 2,4,5-trichlorophenyl ester [19] (0·23 g, 0·5 mmol) were added to a solution in dimethylformamide (1 ml) of the oil remaining after azeotropic drying. A precipitate was formed and stirring became impossible after 20 h. Trituration with water (15 ml) and isolation by centrifugation gave a white powder which was washed with water (2 × 10 ml), methanol (2 × 10 ml), and ether (2 × 10 ml) to give the *protected tetrapeptide* (0·29 g, 79%), m.p. 258—260°, $[\alpha]_D^{20}$ −27·5° (*c* 1 in Me_2SO); $\nu_{max.}$ (KBr) 1765, 1660, and 1630 cm^{-1}; amino-acid analysis: Ala 1·00; Glu 0·97; NH_3 1·08 (Found: C, 61·6; H, 6·1; N, 9·9. $C_{37}H_{44}N_6O_{10}$ requires C, 61·7; H, 6·1; N, 10·0%).

Poly-(L-glutaminyl-L-alanine).—*Method* (*A*). Benzyloxycarbonyl-L-glutaminyl-L-alanyl-L-glutaminyl-L-alanine 2-benzyloxyphenyl ester (1·0 g, 1·37 mmol) was dissolved in a mixture of glacial acetic acid (0·8 ml) and hydrogen bromide in acetic acid (5·6N; 2 ml) with warming. After 1·5 h, trituration with ether (100 ml) gave a white powder which was washed by decantation with three further portions (50 ml) of ether and dried for 1 h at 20° and 0·1 mmHg to give crude peptide active ester hydrobromide as a hygroscopic white powder. Triethylamine (0·45 ml, 3 mmol) was added to a stirred solution of this powder in dimethyl sulphoxide (2 ml). The mixture gradually darkened and thickened, and stirring became impossible after 21 h. After 6 days, trituration with methanol (40 ml) gave a brown suspension. The solid obtained by centrifugation was washed with methanol (40 ml) and ether (20 ml) and dried for 24 h at 20° and 0·1 mmHg to give crude polymer (0·267 g, 49%) as a pale brown powder. A solution of this material in 98% formic acid (5 ml) was filtered, diluted with water (15 ml), and dialysed against water (4 l) for 30 h, changing the water after 6, 10, 22, and 27 h. Lyophilisation and drying to constant weight at 50° and 0·5 mmHg gave the *polymer* as a fluffy white powder (0·050 g, 9%), $[\alpha]_D^{20}$ −64·6° (*c* 0·38 in $CHCl_2$·CO_2H, corrected for residual water); $\nu_{max.}$ (KBr) 3400vbr, 1670br, and 1635br cm^{-1}; τ (CF_3·CO_2H) 2·1br (4H, NH and NH_2), 5·25br (2H, α-protons), 6·5—8·5br (4H, CH_2·CH_2), and 8·4br (3H, CH_3); η_{sp}/c 0·27 dl g^{-1} (*c* 0·38 in $CHCl_2$·CO_2H, corrected for residual water); amino-acid analysis: Ala 1·00; Glu 1·02; NH_3 1·06 [Found: C, 40·0; H, 7·6; N, 16·9%; C/N ratio 2·36. $(C_8H_{13}N_3O_3,3H_2O)_n$ requires C, 39·2; H, 7·8; N, 17·3%; C/N ratio 2·26].

Method (*B*). Benzyloxycarbonyl-L-glutaminyl-L-alanyl-L-glutaminyl-L-alanine 2-benzyloxyphenyl ester (1·0 g, 1·37 mmol) was dissolved in glacial acetic acid (150 ml) and hydrogenated over 10% palladium–charcoal (0·6 g) at room temperature and atmospheric pressure for 3·5 h. Filtration through Celite and evaporation gave a foam which was dissolved in dimethyl sulphoxide (2 ml), and triethylamine (0·45 ml, 3·0 mmol) was added. The mixture gradually darkened and thickened on stirring and eventually set solid after 24 h. After 6 days, isolation as in method (A) gave crude polymer (0·275 g, 50%), which was dialysed and lyophilised to give the *polymer* as a fluffy off-white powder (0·057 g, 10%), $[\alpha]_D^{25}$ −67·7 (*c* 0·296 in $CHCl_2$·CO_2H, corrected for residual water); $\nu_{max.}$ (KBr) 3400vbr, 1660br, and 1630br cm^{-1}, η_{sp}/c 0·265 dl g^{-1} (*c* 0·296 in $CHCl_2$·CO_2H at 25°, corrected for residual water) (Found: C, 39·9; H, 5·8; N, 16·8%; C/N ratio 2·37).

t-Butoxycarbonyl-α-succinimido-γ-methyl-L-glutamate.— t-Butoxycarbonyl-γ-methyl-L-glutamic acid dicyclohexylammonium salt [20] (0·88 g, 2 mmol) was stirred with ethyl acetate (15 ml) and 10% citric acid (5 ml) was added. The organic phase was separated, washed with water (3 × 5 ml), and dried. Evaporation gave an oil which was dissolved in dioxan (10 ml) and cooled to 0°. *N*-Hydroxysuccinimide (0·23 g, 2 mmol) and dicyclohexylcarbodi-imide (0·45 g, 2·2 mmol) were added and the solution was maintained at this temperature overnight. The filtered solution was evaporated to give a yellow oil which crystallised on trituration with propan-2-ol. Recrystallisation from propan-2-ol gave the *succinimido ester* (0·52 g, 72%), m.p. 113—115°, $[\alpha]_D^{20}$ −20·8° (*c* 1 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1823, 1783, and 1744 cm^{-1}; τ ($CHCl_3$) 4·7 (1H, d, *J* 9 Hz, NH), 5·3 (1H, complex, α-proton), 6·25 (3H, s, O·CH_3), 7·12 (4H, s, CO·CH_2·, CH_2·CO), 7·3—7·8 (4H, complex, CH·CH_2·CH_2·CO), and 8·5 (9H, s, Bu^t) (Found: C, 55·4; H, 6·8; N, 8·7. $C_{15}H_{22}N_2O_8$ requires C, 55·2; H, 6·75; N, 8·6%).

t-Butoxycarbonyl-γ-methyl-L-glutamyl-L-alanine 2-Benzyloxyphenyl Ester.—Triethylamine (0·3 ml, 2·1 mmol) and t-butoxycarbonyl-α-succinimido-γ-methyl-L-glutamate (0·72 g, 2 mmol) were added to a stirred solution of L-alanine 2-benzyloxyphenyl ester hydrochloride [9] (0·62 g, 2 mmol) in dimethylformamide (2 ml). After 18 h, the solution was partially evaporated at 30° and 0·5 mmHg, and the residue was dissolved in ethyl acetate (30 ml), washed with 10% citric acid (5 ml), saturated sodium hydrogen carbonate (5 ml), and water (2 × 5 ml), and dried. Removal of solvent gave a white solid which on recrystallisation from ethyl acetate–light petroleum gave the *protected dipeptide* as needles (0·79 g, 78%), m.p. 138—140°, $[\alpha]_D^{20}$ −37·4° (*c* 1 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1730, 1710, and 1680 cm^{-1}; τ ($CDCl_3$) 2·6 (5H, s, Ph), 2·7—3·2 (5H, complex, O·C_6H_4·O·CH_2 and peptide NH), 4·6 (1H, d, *J* 8 Hz, urethane NH), 4·95 (2H, s, CH_2Ph), 5·05—5·45 (1H, complex, α-proton of alanine), 5·55—6·05 (1H, complex, α-proton of glutamic acid), 6·35 (3H, s, O·CH_3), 7·4—8·1 (4H, complex, CH_2·CH_2), 8·5 (9H, s, Bu^t), and 9·1 (3H, d, *J* 6 Hz, CH·CH_3) (Found: C, 63·1; H, 6·6; N, 5·7. $C_{27}H_{34}N_2O_8$ requires C, 63·0; H, 6·6; N, 5·45%).

t-Butoxycarbonyl-L-alanyl-γ-methyl-L-glutamyl-L-alanine 2-Benzyloxyphenyl Ester.—t-Butoxycarbonyl-γ-methyl-L-glutamyl-L-alanine 2-benzyloxyphenyl ester (1·38 g, 2·7 mmol) was treated with 90% trifluoroacetic acid as in the cases already described. Triethylamine (0·4 ml, 4 mmol) and t-butoxycarbonyl-L-alanine succinimido ester [18] (0·77 g, 2·7 mmol) were added to a solution in dimethylformamide (2 ml) of the oil remaining after azeotropic drying. After 18 h the solvent was evaporated off and the residue was dissolved in ethyl acetate (50 ml); the solution was washed with 10% citric acid (5 ml), saturated sodium hydrogen carbonate (10 ml), and water (2 × 10 ml), and dried. Removal of the solvent and recrystallisation of the gelatinous residue from chloroform–light petroleum gave the *protected tripeptide* (1·44 g, 8·1%), m.p. 125—127°, $[\alpha]_D^{20}$

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$-45{\cdot}1°$ (*c* 1 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1768, 1750—1680br, and 1673 cm^{-1}; τ ($CDCl_3$) 2·4—3·1 (11H, complex, aromatic and peptide NH), 4·8 (1H, d, *J* 7 Hz, urethane NH), 4·95 (2H, s, CH_2Ph), 5·05—5·95 (3H, complex, α-protons), 6·36 (3H, s, $O{\cdot}CH_3$), 7·5—8·05 (4H, complex, $CH_2{\cdot}CH_2$), 8·55 (9H, s, Bu^t), and 8·63—8·7 (6H, d, *J* 6 Hz, $CH{\cdot}CH_3$) (Found: C, 60·7; H, 6·7; N, 7·3. $C_{30}H_{39}N_3O_9$ requires C, 61·4; H, 6·7; N, 7·1%).

*Benzyloxycarbonyl-γ-methyl-*L*-glutamyl-*L*-alanyl-γ-methyl-*L*-glutamyl-*L*-alanine 2-Benzyloxyphenyl Ester.*—t-Butoxycarbonyl-L-alanyl-γ-methyl-L-glutamyl-L-alanine 2-benzyloxyphenyl ester (0·292 g, 0·5 mmol) was treated with 90% trifluoroacetic acid as in the cases already described. Triethylamine (0·14 ml, 1·0 mmol) and benzyloxycarbonyl-α-succinimido-γ-methyl-L-glutamate [18] (0·195 g, 0·50 mmol) were added to a solution in dimethylformamide (1 ml) of the oil remaining after azeotropic drying. Isolation of the product after 18 h as in the previous preparation gave the *protected tetrapeptide* (0·250 g, 66%), m.p. 157—159°, $[\alpha]_D^{20}$ $-32{\cdot}7°$ (*c* 1 in $CHCl_3$); $\nu_{max.}$ (KBr) 1770, 1740, 1705, and 1675 cm^{-1}; τ ($CF_3{\cdot}CO_2H$) 2·0—2·35 (3H, complex, peptide NH), 2·6—3·0 (14H, complex, aromatic), 3·2—3·5br (1H, urethane NH), 4·75 and 4·88 (4H, two s, both $PhCH_2$), 5·0—5·5 (4H, complex, α-protons), 6·2 (6H, singlet, $O{\cdot}CH_3$), 7·2—7·8 (8H, complex, $CH_2{\cdot}CH_2$), and 8·35 and 8·65 (6H, complex, $CH{\cdot}CH_3$) (Found: C, 61·1; H, 6·0; N, 7·4. $C_{39}H_{46}N_4O_{12}$ requires C, 61·4; H, 6·0; N, 7·35%).

*Poly-(γ-methyl-*L*-glutamyl-*L*-alanine).*—Benzyloxycarbonyl-γ-methyl-L-glutamyl-L-alanyl-γ-methyl-L-glutamyl-L-alanine 2-benzyloxyphenyl ester (1·0 g, 1·31 mmol) was dissolved in a mixture of glacial acetic acid (0·6 ml) and hydrogen bromide in acetic acid (5·6 N; 2 ml) by warming. After 1·25 h, trituration with ether (50 ml) gave a white solid which was washed by decantation with three further portions (50 ml) of ether and dried for 1 h at 20° and 0·1 mmHg to give crude peptide active ester hydrobromide as a hygroscopic white powder. Triethylamine (0·4 ml, 3 mmol) was added to a stirred solution of this powder in dimethyl sulphoxide (1 ml). The solution gradually darkened and thickened and after 48 h stirring became impossible. After 2 weeks, methanol (20 ml) was added and the mixture was stirred overnight. The resulting suspension was centrifuged and the brown powder so obtained was washed with methanol (3 × 50 ml) and ether (2 × 50 ml) and dried for 24 h at 40° and 0·5 mmHg to give a light brown powder (0·388 g). Soxhlet extraction for 5 h with methanol and drying to constant weight at 40° and 0·5 mmHg gave the *polymer* (0·380 g, 69·1%), $[\alpha]_{589}^{25}$ $-34{\cdot}5°$ (*c* 0·9 in $CHCl_2{\cdot}CO_2H$); $\nu_{max.}$ (KBr) 1745br, 1700br, and 1635 cm^{-1}; τ ($CF_3{\cdot}CO_2H$) 1·9—2·3br (2H, NH), 5·0—5·4br (2H, α- protons), 5·9—6·5br (*ca.* 5H, $O{\cdot}CH_3$ and residual $CH_3{\cdot}OH$ traces), 7·1—7·8br (4H, $CH_2{\cdot}CH_2$), and 8·35br (3H, d, *J* 6 Hz, $CH{\cdot}CH_3$); η_{sp}/c 0·20 dl g^{-1} (*c* 1·16 in $CHCl_2{\cdot}CO_2H$) (Found: C, 48·6; H, 6·4; N, 12·15%; C/N ratio 4·00. $C_9H_{14}N_2O_4{\cdot}0{\cdot}65CH_3OH$ requires: C, 49·2; H, 7·0; N, 11·8%; C/N ratio 4·13).

Preparation of the same sequential polypeptide by the same procedure but starting with the dipeptide derivative *benzyloxycarbonyl-*L*-glutaminyl-*L*-alanine 2-benzyloxyphenyl ester* {prepared in the same way as the corresponding t-butoxycarbonyldipeptide derivative; m.p. 125—127°, $[\alpha]_D^{20}$ $-32{\cdot}4°$ (*c* 1 in $CHCl_3$) (Found: C, 65·7; H, 5·9; N, 5·1. $C_{30}H_{32}N_2O_8$ requires C, 65·7; H, 5·8; N, 5·1%)} gave a 5% yield of polypeptide with properties similar to that obtained *via* the tetrapeptide intermediate.

*N(α)-Benzyloxycarbonyl-N(ω)-t-butyl-*L*-glutamine Benzyl Ester.*—t-Butylamine (0·15 g, 2 mmol) was added to a solution of benzyloxycarbonyl-α-benzyl-γ-succinimido-L-glutamate [21] (0·96 g, 2 mmol) in dimethylformamide (1 ml). After 18 h the solution was evaporated at 30° and 0·5 mmHg, and a solution of the residual oil in ethyl acetate (15 ml) was washed with 10% citric acid (5 ml), saturated sodium hydrogen carbonate (5 ml), and water (2 × 5 ml). Drying and evaporation gave an oil which crystallised on trituration with light petroleum. Recrystallisation from ether–light petroleum gave the protected glutamine derivative (0·78 g, 88%), m.p. 60—62°, $[\alpha]_D^{20}$ $-6{\cdot}2°$ (*c* 1 in $CHCl_3$); $\nu_{max.}$ (CCl_4) 1728 and 1680 cm^{-1}; τ (CCl_4) 2·78 (10H, two practically superimposed singlet Ph groups); 3·94 (1H, d, *J* 8 Hz, $NH{\cdot}CH$), 4·1 (1H, s, $NHBu^t$), 4·94 (2H, s, $PhCH_2$), 5·01 (2H, s, the other $PhCH_2$), 5·6—6·0 (1H, complex, α-proton), 7·7—8·2 (4H, complex, $CH_2{\cdot}CH_2$), and 8·75 (9H, s, Bu^t) (Found: C, 67·4; H, 7·0; N, 6·5. $C_{24}H_{30}N_2O_5$ requires C, 67·6; H, 7·0; N, 6·6%).

*N(ω)-t-Butyl-*L*-glutamine.—Method* (*A*). *N*(α)-Benzyloxycarbonyl-*N*(ω)-t-butyl-L-glutamine benzyl ester (0·426 g, 1 mmol) was dissolved in glacial acetic acid (15 ml) and hydrogenated over 10% palladium–charcoal at room temperature and atmospheric pressure for 3 h. Filtration through Celite and evaporation gave a white solid which on recrystallisation from methanol gave the *amino-acid* as white crystals (0·17 g, 86%), m.p. 216—218°, $[\alpha]_D^{20}$ $+27{\cdot}0$ (*c* 1 in 2N-HCl), $\nu_{max.}$ (Nujol) 1650 and 1580 cm^{-1}; τ ($CF_3{\cdot}CO_2H$) 1·8—2·8br (4H, NH_3^+ and NH), 5·3—5·7 (1H, complex, α-proton), 6·9—7·6 (4H, complex, $CH_2{\cdot}CH_2$), and 8·51 (9H, s, Bu^t) (Found: C, 53·1; H, 8·7; N, 13·8, $C_9H_{18}N_2O_3$ requires C, 53·4; H, 8·9; N, 13·9%).

Method (*B*). *N*(α)-Benzyloxycarbonyl-*N*(ω)-t-butyl-L-glutamine benzyl ester (0·518 g, 1·2 mmol) was dissolved in anisole (1 ml) and liquid hydrogen fluoride (*ca.* 7 ml). After 1 h at room temperature the hydrogen fluoride was removed by evaporation at 0·1—1 mmHg for 5 h, and the residue was removed from the reaction vessel by dissolution in glacial acetic acid (5 ml). Removal of acetic acid and trituration with ether (2 × 50 ml) gave a buff powder which was reprecipitated from methanol–ether to give the amino-acid (0·162 g, 64%), identical with that obtained by method (A).

[21] P. M. Hardy, J. C. Haylock, and H. N. Rydon, *J.C.S. Perkin I*, 1972, 605.

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