Peptides. Part XXX.[1] Synthesis of the Decapeptide Leu-Ala-Ala-Gly-Lys-Val-Glu-Asp-Ser-Asp

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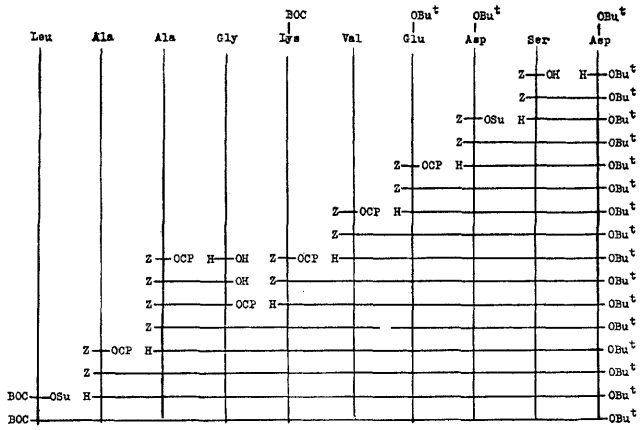
The synthesis of the decapeptide named in the title is described.

HEATHCOTE and WASHINGTON [2,3] have recently reported the presence in human gastric juice of a dialysable decapeptide, to which they assigned structure (I) on the basis of successive Edman degradations. This peptide was present in several pooled samples of normal, fasting, gastric juice, but not in gastric juice from any one of three patients suffering from pernicious anaemia. Dr. Heathcote communicated the proposed structure of this peptide to us prior to publication, and we now give details of the successful synthesis of the decapeptide (I) with this amino-acid sequence.

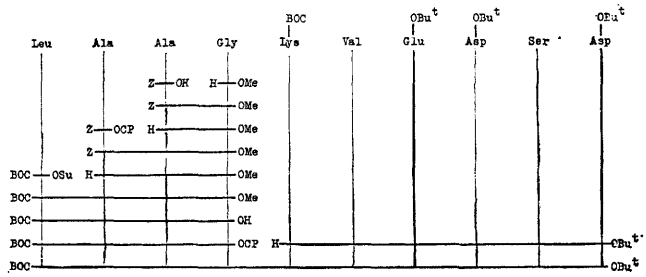
Leu-Ala-Ala-Gly-Lys-Val-Glu-Asp-Ser-Asp
(I)

The amino-acid sequence of (I) was such as to present no special problems in peptide synthesis, and we elected to use the well authenticated stepwise active ester method for the formation of the majority of the peptide bonds. Isolation of neutral peptide derivatives was in several instances much facilitated by brief treatment of the crude products with 3-dimethylaminopropylamine.[4] This amine reacts rapidly with any residual neutral acylamino-acid active ester, forming the corresponding dimethylaminopropylamide, readily eliminated by virtue of its solubility in dilute aqueous acid. This procedure was specially advantageous during the present synthesis where several of the intermediates were exceptionally soluble in organic solvents and thus difficult to separate from unchanged active ester. α-Amino-groups were protected by formation of benzyloxycarbonyl derivatives throughout, except for the *N*-terminal t-butoxycarbonyl residue, and the *C*-terminal carboxy-group was protected by formation of its t-butyl ester. Functional side chains were protected by formation of t-butoxycarbonyl (lysine) or t-butyl esters (glutamic and aspartic acids), but the hydroxy-group of the serine residue was left free without any consequent difficulty. This combination of protecting groups led to a final protected decapeptide derivative from which all the protecting groups could be removed by a single treatment with trifluoroacetic acid.

The glycine residue at position 4 provided an obvious point for chain division, and the synthesis was therefore carried through both in a nearly fully stepwise manner (Scheme 1) and by condensation of protected tetra- and hexa-peptide fragments (Scheme 2). The latter procedure required a saponification step which proceeded in only low yield on the single occasion it was carried out, but no difficulty was experienced in obtaining a



SCHEME 1



SCHEME 2

chromatographically and electrophoretically homogeneous final product by either method.

The comparison of the natural and synthetic decapeptides has been described by Heathcote and Washington.[3]

EXPERIMENTAL

The general instructions given in Parts XXVIII,[5] XXVII,[6] and XXV [7] apply. The additional solvent system for t.l.c. (TLC-16) was chloroform–acetone (5 : 1).

Di-t-butyl Benzyloxycarbonyl-L-seryl-L-aspartate.—A solution of dicyclohexylcarbodi-imide (10·3 g., 50 mmoles) in tetrahydrofuran (25 ml.) was added to a cooled (0°) and stirred solution of benzyloxycarbonyl-L-serine (10·0 g., 42 mmoles) and di-t-butyl L-aspartate (10·25 g., 42 mmoles) in

[1] Part XXIX, preceding paper.
[2] J. G. Heathcote and R. J. Washington, *Biochem. J.*, 1965, **95**, 24P.
[3] J. G. Heathcote and R. J. Washington, to be submitted for publication; reported at the Chemical Society Protein Group Meeting, Oxford, March 19th, 1969.
[4] *c.f.* M. Löw and L. Kisfaludy, *Acta Chim. Acad. Sci. Hung.*, 1965, **44**, 61.
[5] K. L. Agarwal, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc.* (*C*), 1969, 954.
[6] K. L. Agarwal, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc.* (*C*), 1968, 1384.
[7] J. Beacham, P. H. Bentley, G. W. Kenner, J. K. MacLeod, J. J. Mendive, and R. C. Sheppard, *J. Chem. Soc.* (*C*), 1967, 2520.

tetrahydrofuran (75 ml.). The mixture was stirred at 0° overnight and then at room temp. for 3 hr. before being filtered and evaporated. A solution of the residue in ethyl acetate (100 ml.) was washed with aq. sodium hydrogen carbonate (10%; 50 ml.), hydrochloric acid (5%; 100 ml.), and water (2 × 50 ml.). The dried ($MgSO_4$) solution was evaporated and the product crystallised from ethyl acetate–petroleum (b.p. 60—80°) to yield the *dipeptide ester* (17·0 g., 85%), m.p. 132—133°, $[\alpha]_D^{25}$ — 12·4° (*c* 1·0 in Me_2NCHO) (Found: C, 59·5; H, 7·4; N, 6·2. $C_{23}H_{34}N_2O_8$ requires C, 59·2; H, 7·3; N, 6·0%).

Di-t-butyl Benzyloxycarbonyl-β-t-butyl-L-aspartyl-L-seryl-L-aspartate.—The preceding dipeptide derivative (466 mg., 1 mmole) was hydrogenated in methanol (20 ml.) over palladium–charcoal (5%; 100 mg.) for 2 hr. at room temp. The combined filtrate and washings were evaporated to dryness, and the oily residue was dissolved in dimethylformamide (10 ml.) and treated with β-t-butyl benzyloxycarbonyl-L-aspartate hydroxysuccinimide ester (420 mg., 1 mmole) at room temp. for 12 hr. The solution was evaporated and the ethyl acetate (40 ml.) solution of the residue was washed as in the previous experiment. The dried (Na_2SO_4) solution was evaporated and the residue crystallised from ethyl acetate–petroleum (b.p. 60—80°) to give the *tripeptide derivative* (500 mg., 78%), m.p. 65—67°, $[\alpha]_D^{25}$ —19·4° (*c* 2·0 in MeOH), TLC-5 R_F 0·42, $Asp_{2\cdot00}$ $Ser_{0\cdot86}$ (Found: C, 58·4; H, 7·5; N, 6·6. $C_{31}H_{47}N_3O_{11}$ requires C, 58·4; H, 7·4; N, 6·6%). After removal of the protecting groups, by hydrogen and palladium–charcoal followed by trifluoracetic acid, and treatment with leucine aminopeptidase, the amino-acid analysis was $Asp_{2\cdot04}$ $Ser_{1\cdot00}$.

Di-t-butyl Benzyloxycarbonyl-γ-t-butyl-L-glutamyl-β-t-butyl-L-aspartyl-L-seryl-L-aspartate.—The preceding tripeptide ester (3·82 g., 6 mmoles) was hydrogenated in acetic acid (80%; 50 ml.) over palladium–charcoal at room temp. The combined filtrate and washings were evaporated and the residue was dried by repeated addition of benzene and evaporation. The residue was then coupled with γ-t-butyl α-2,4,5-trichlorophenyl benzyloxycarbonyl-L-glutamate (3·40 g., 6·6 mmoles) in dimethylformamide (15 ml.) containing triethylamine (0·60 ml., 4·43 mmoles) at 37° for 12 hr. The mixture was evaporated and the ethyl acetate (100 ml.) solution of the residue was treated with 3-dimethylaminopropylamine (0·2 ml.) at room temp. for 10 min. and then washed as already described. The *tetrapeptide derivative* obtained by evaporation of the ethyl acetate was crystallised from ethyl acetate–petroleum (b.p. 60—80°) (yield 3·7 g., 76%), m.p. 79—80°, $[\alpha]_D^{25}$ —28·05° (*c* 0·98 in MeOH), TLC-5 R_F 0·65, TLC-16 R_F 0·30, $Asp_{2\cdot20}$ $Ser_{2\cdot08}$ $Glu_{1\cdot00}$ (Found: C, 58·2; H, 7·6; N, 6·8. $C_{40}H_{62}N_4O_{14}$ requires C, 58·3; H, 7·65; N, 6·8%).

Di-t-butyl Benzyloxycarbonyl-L-valyl-α-t-butyl-L-glutamyl-β-t-butyl-L-aspartyl-L-seryl-L-aspartate.—The preceding tetrapeptide tetrabutyl ester (823 mg., 1 mmole) was hydrogenated in acetic acid (80%; 50 ml.) over palladium–charcoal (5%; 200 mg.) for 3 hr. at room temp. The product was treated with benzyloxycarbonyl-L-valine 2,4,5-trichlorophenyl ester (470 mg., 1·1 mmole) in dimethylformamide at 37° for 12 hr. in the presence of triethylamine (0·09 ml., 0·65 mmole). The neutral product isolated as described in the foregoing experiment was crystallised from ether–petroleum (b.p. 40—60°) to give the *pentapeptide derivative* (800 mg., 87%), m.p. 140—142°, $[\alpha]_D^{25}$ —36·4° (*c* 2·0 in MeOH), TLC-5 R_F 0·67, $Asp_{2\cdot20}$ $Ser_{0\cdot80}$ $Glu_{1\cdot00}$ $Val_{0\cdot97}$ (Found: C, 58·8; H, 7·9; N, 7·6. $C_{45}H_{71}N_5O_{15}$ requires C, 58·6; H, 7·7; N, 7·6%).

Di-t-butyl N(α)-*Benzyloxycarbonyl*-N(ε)-*t-butoxycarbonyl-L-lysyl-L-valyl-α-t-butyl-L-glutamyl-β-t-butyl-L-aspartyl-L-seryl-L-aspartate.*—The preceding pentapeptide derivative (460 mg., 0·5 mmole) was hydrogenated in acetic acid (80%; 60 ml.) for 4 hr. at room temp. The product was dissolved in dimethylformamide (10 ml.) and treated with *N*(α)-benzyloxycarbonyl-*N*(ε)-t-butoxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester (309 mg., 0·55 mmole) and triethylamine (0·04 ml., 0·3 mmole) at 37° for 24 hr. The *hexapeptide derivative* was isolated as in the foregoing experiment and crystallised from ethyl acetate–petroleum (b.p. 40—60°) (yield 500 mg., 8·7%); m.p. 160—164° (shrinks at 149°), $[\alpha]_D^{25}$ —30·8° (*c* 1·2 in MeOH), TLC-5 R_F 0·63, TLC-16 R_F 0·21, $Asp_{2\cdot20}$ $Ser_{0\cdot95}$ $Glu_{1\cdot07}$ $Val_{1\cdot00}$ $Lys_{0\cdot96}$ (Found: C, 57·7; H, 7·9; N, 8·65. $C_{56}H_{93}N_7O_{19}$ requires C, 57·6; H, 8·0; N, 8·4%).

*Di-t-butyl Benzyloxycarbonyl-L-alanylglycyl-*N(ε)-*t-butoxycarbonyl-L-lysyl-L-valyl-α-t-butyl-L-glutamyl-β-t-butyl-L-aspartyl-L-seryl-L-aspartate.*—The preceding hexapeptide derivative (1·15 g., 1 mmole) was hydrogenated in glacial acetic acid (80%, 50 ml.) for 4 hr. at room temp. over palladium–charcoal (300 mg.). The resulting acetate was coupled with benzyloxycarbonyl-L-alanylglycine 2,4,5-trichlorophenyl ester (505 mg., 1·1 mmole) in dimethylformamide (5·0 ml.) containing triethylamine (0·1 ml., 0·7 mmole) at 37° for 40 hr. Isolation as already described and precipitation from ethyl acetate–petroleum (b.p. 40—60°) gave the amorphous *octapeptide derivative* (1·23 g., 98%), m.p. 213—214°, $[\alpha]_D^{25}$ —26·9° (*c* 0·96 in MeOH), TLC-14 R_F 0·54, TLC-5 R_F 0·44, $Lys_{1\cdot00}$ $Asp_{2\cdot00}$ $Ser_{0\cdot90}$ $Glu_{1\cdot00}$ $Ala_{1\cdot04}$ $Gly_{1\cdot04}$ $Val_{1\cdot00}$ (Found: C, 57·25; H, 7·9; N, 9·9. $C_{61}H_{99}N_9O_{20}$ requires C, 57·3; H, 7·75; N, 9·9%).

*Di-t-butyl Benzyloxycarbonyl-L-alanyl-L-alanylglycyl-*N(ε)-*t-butoxycarbonyl-L-lysyl-L-valyl-α-t-butyl-L-glutamyl-β-t-butyl-L-aspartyl-L-seryl-L-aspartate.*—The preceding octapeptide derivative (1·08 g., 0·85 mmole) was hydrogenated over palladium–charcoal (5%; 300 mg.) in glacial acetic acid (80%; 50 ml.) for 4 hr. at room temp. and pressure. The product was treated with benzyloxycarbonyl-L-alanine 2,4,5-trichlorophenyl ester (446 mg., 1·1 mmole) in dimethylformamide (15·0 ml.) containing triethylamine (0·1 ml., 0·7 mmole) at 37° for 24 hr. Isolation of the neutral product as already described and precipitation from ethyl acetate–petroleum gave the amorphous *nonapeptide derivative* (950 mg., 90%), m.p. 220—223° (decomp.), $[\alpha]_D^{25}$ —22·2° (*c* 1·0 in Me_2NCHO), TLC-14 R_F 0·58, TLC-5 R_F 0·40, $Asp_{2\cdot00}$ $Ser_{1\cdot01}$ $Glu_{0\cdot97}$ $Ala_{2\cdot08}$ $Gly_{1\cdot00}$ $Val_{1\cdot01}$ $Lys_{1\cdot05}$ (Found: C, 57·6; H, 7·9. $C_{64}H_{104}N_{10}O_{21}$ requires C, 56·8; H, 7·7%).

*Di-t-butyl t-Butoxycarbonyl-L-leucyl-L-alanyl-L-alanylglycyl-*N(ε)-*t-butoxycarbonyl-L-lysyl-L-valyl-α-t-butyl-L-glutamyl-β-t-butyl-L-aspartyl-L-seryl-L-aspartate.*—(*a*) The foregoing nonapeptide derivative (900 mg., 0·7 mmole) was hydrogenated in glacial acetic acid (80%; 20 ml.) over palladium–charcoal (5%; 200 mg.) for 4 hr. at room temp. and pressure. The resulting acetate was coupled with t-butoxycarbonyl-L-leucine hydroxysuccinimide ester (263 mg., 0·8 mmole) in dimethylformamide (5·0 ml.) containing triethylamine (0·098 ml., 0·7 mmole), at 37° for 12 hr. The solution was evaporated and the residue was dissolved in warm ethyl acetate (400 ml.) and treated with 3-dimethylaminopropylamine (0·05 ml.). After 5 min. the neutral

fraction was isolated in the usual way. Concentration of the dried ($MgSO_4$) ethyl acetate solution and addition of petroleum (b.p. 40—60°) gave the *decapeptide derivative* which was further purified from ethyl acetate (yield 600 mg., 63%); m.p. 170—175° (decomp.), $[\alpha]_D^{25}$ −23·7° (*c* 1·0 in Me_2NCHO), TLC-14 R_F 0·34, TLC-3 R_F 0·12, $Lys_{1\cdot07}$ $Asp_{2\cdot04}$ $Ser_{0\cdot90}$ $Glu_{1\cdot00}$ $Gly_{1\cdot00}$ $Ala_{2\cdot04}$ $Val_{1\cdot04}$ $Leu_{1\cdot04}$ (Found: C, 57·0; H, 8·2; N, 10·8. $C_{67}H_{117}N_{11}O_{22}$ requires C, 56·4; H, 8·1; N, 10·8%).

(*b*) A solution of t-butoxycarboxyl-L-leucyl-L-alanyl-L-alanylglycine (see later) (56 mg.) in tetrahydrofuran (10 ml.) was treated with dicyclohexylcarbodi-imide (50 mg.) and 2,4,5-trichlorophenol (50 mg.) at room temp. for 3 days. The solution was evaporated and the residue was extracted with ethyl acetate. The extract was evaporated and the residual active ester was treated with di-t-butyl *N*(ε)-t-butoxycarboxyl-L-lysyl-L-valyl-α-t-butyl-L-glutamyl-β-t-butyl-L-aspartyl-L-seryl-L-aspartate [from hydrogenation in 80% acetic acid of the benzyloxycarbonyl derivative (100 mg.)] in dimethylformamide (2·0 ml.) containing triethylamine (0·01 ml.) at 37° for 24 hr. The solution was evaporated and the neutral product was isolated as in the foregoing experiment. Concentration of the dried ethyl acetate solution and addition of ether gave the decapeptide derivative (80 mg., 64%), m.p. 170—173° (decomp.). This product was chromatographically identical with that obtained in (*a*).

L-Leucyl-L-alanyl-L-alanylglycyl-L-lysyl-L-valyl-L-glutamyl-L-aspartyl-L-seryl-L-aspartyl Trifluoroacetate.—The preceding decapeptide derivative (25 mg.) was treated with anhydrous trifluoroacetic acid (2·0 ml.) under nitrogen at room temp. for 30 min. The solution was evaporated to dryness and the residue was washed several times with ether. The *decapeptide trifluoroacetate* crystallised from methanol containing a few drops of petroleum (b.p. 40—60°) (yield 20 mg.), m.p. 225—228°, $[\alpha]_D^{25}$ −56·3° (*c* 0·2 in 50% aq. Me_2NCHO), TLC-6 R_F 0·82, TLC-9 R_F 0·12, $E_{Glu}^{6\cdot5}$ 0·48, $Lys_{1\cdot02}$ $Asp_{1\cdot95}$ $Ser_{0\cdot82}$ $Glu_{1\cdot00}$ $Gly_{1\cdot00}$ $Ala_{2\cdot00}$ $Val_{0\cdot96}$ $Leu_{1\cdot03}$.

t-Butoxycarbonyl-L-leucyl-L-alanyl-L-alanylglycine Methyl Ester.—Benzyloxycarbonyl-L-alanyl-L-alanylglycine methyl ester (3·65 g., 10 mmoles) was hydrogenated in methanol (100 ml.) and acetic acid (20 ml.) over palladium–charcoal (400 mg.) for 3 hr. at room temp. and pressure. The combined filtrate and washings were evaporated and the residue was dried by repeated azeotropic distillation of benzene. The residue was treated with t-butoxycarbonyl-L-leucine hydroxysuccinimide ester (3·28 g., 10 mmoles) in dimethylformamide (15 ml.) containing triethylamine (1·4 ml., 10 mmoles) at room temp. for 12 hr. Isolation in the usual way gave the *tetrapeptide derivative* which crystallised from ethyl acetate (yield 2·0 g., 50%); m.p. 211—213°, $[\alpha]_D^{25}$ −73·8° (*c* 1·0 in MeOH), TLC-3 R_F 0·41, TLC-5 R_F 0·48, $Gly_{1\cdot00}$ $Ala_{2\cdot08}$ $Leu_{1\cdot00}$ (Found: C, 54·1; H, 7·95; N, 12·7. $C_{20}H_{36}N_4O_7$ requires C, 54·05; H, 8·1; N, 12·6%).

t-Butoxycarbonyl-L-leucyl-L-alanyl-L-alanylglycine.—The preceding tetrapeptide derivative (444 mg., 1 mmole) was treated with N-aq. sodium hydroxide (4 ml.) in acetone (10 ml.) for 1 hr. at room temp. The acetone was evaporated off and the residual solution was acidified with citric acid (10%; 25 ml.) and extracted with ethyl acetate. The extract was washed several times with water, dried (Na_2SO_4), and evaporated, to give the tetrapeptide acid (100 mg.), TLC-3 R_F 0·02, TLC-4 R_F 0·10.

[9/912 *Received, May 29th*, 1969]