Amino-acids and Peptides. Part XVII.[1] Synthesis of Cyclo-[(*O*-t-butyl)-L-seryl-β-alanyl-glycyl-(*O*-methyl)-L-β-aspartyl], and Observations on the Rearrangement of β-Aspartyl Peptide Esters

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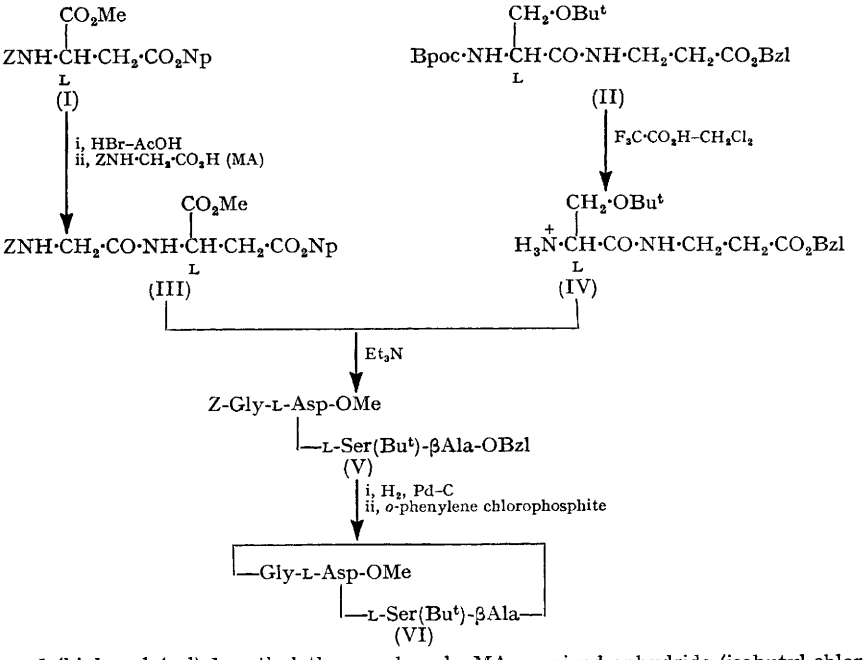
The title fourteen-membered ring system was synthesised by cyclisation of the corresponding linear tetrapeptide *p*-nitrophenyl ester. When ring closure of the related tetrapeptide with terminal β-alanine and glycine residues was attempted, no cyclic peptide was formed; the aspartyl residue rearranged remarkably readily from β- to α-. The synthesis of several peptides containing β-aspartyl residues established that the sequence *O*-methyl-L-β-aspartyl-(*O*-t-butyl)-L-seryl-β-alanyl particularly favoured this rearrangement.

CONSIDERATIONS which have been discussed briefly [2] and are outlined more fully in the following paper have led us to investigate the synthesis of particular substituted, cyclic fourteen-membered oligopeptides. This paper describes the preparation of the cyclopeptide (VI) incorporating one L-*O*-methyl-β-aspartyl and one protected L-seryl residue.

In the light of earlier experience,[1,3] the synthesis was attempted through cyclisation of the corresponding linear compound. It seemed likely that a compound with terminal glycyl and β-alanyl residues would undergo ring closure readily. The *N*- and *C*-protected tetrapeptide (V) was obtained in good yield as a pure crystalline solid through the preparation and coupling of suitably protected dipeptides, (III) and (IV) (Scheme 1). The synthesis involved, largely, conventional procedures but one stage deserves particular mention. The protecting Bpoc group could not be removed satisfactorily by the standard reagents, *viz.* acetic acid–formic acid–water [4] or dichloroacetic and acetic acids.[5] The former gave rise to substantial amounts of *N*-formyl contaminant, arising presumably from formylation at the coupling stage. The latter was unsuitable in view of the solubility of the product. However, treatment with 0.5% trifluoroacetic acid in dichloromethane [6] removed Bpoc without cleaving the t-butyl group.



SCHEME 1 Bpoc = 1-(biphenyl-4-yl)-1-methylethoxycarbonyl; MA = mixed anhydride (isobutyl chloroformate and *N*-methylmorpholine)

All attempts to obtain *C*- and *N*-terminal deprotected linear tetrapeptide from the pure, protected derivative (V) by hydrogenation gave complex mixtures. The spectra of these mixtures showed that the methoxycarbonyl function had virtually disappeared, presumably through rearrangement during the hydrogenation. Consequently, this route was abandoned and the rearrangement of the β-aspartyl residue was investigated

[1] Part XVI, C. H. Hassall, R. G. Tyson, and K. K. Chexal, preceding paper.

[2] C. H. Hassall, in 'Chemistry and Biology of Peptides: Proceedings of the Third American Peptide Symposium,' ed. J. Meienhofer, Ann Arbor Science, Ann Arbor, 1972, p. 153.

[3] C. H. Hassall, D. G. Sanger, and B. K. Handa, *J. Chem. Soc.* (*C*), 1971, 2814.

[4] P. Sieber and B. Iselin, *Helv. Chim. Acta*, 1968, **51**, 622.

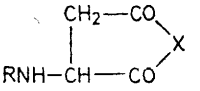
[5] P. Sieber and B. Iselin, *Helv. Chim. Acta*, 1968, **51**, 1528.

[6] S. S. Wong and R. B. Merrifield, *Internat. J. Protein Res.*, 1969, **1**, 235.

before the synthesis of an alternative linear tetrapeptide was attempted. When the product (V) was set aside in 50% aqueous pyridine, it was largely transformed, in 48 h, into several new products. Alternatively, treatment with methanol at room temperature gave a complex mixture from which a major component was separated and identified by ^{1}H n.m.r. as the succinimide derivative (VII). This was converted largely into the original peptide and a new isomer, presumably (VIII), on further treatment with methanol. The product of *C*- and *N*-terminal deprotection of the tetrapeptide (V) rearranged so readily that it would not serve as an intermediate for the formation of the cyclic compound (VI).

The rearrangement of α-aspartyl derivatives is well known but there is little direct information on the behaviour of β-aspartyl residues in peptides. When Sondheimer and Holley [7] attempted to saponify benzyloxycarbonyl-L-asparagine and benzyloxycarbonyl-L-isoasparagine methyl esters, they obtained benzyloxy-L-aminosuccinimide instead of the free acid. Battersby and Robinson [8] reported that alkaline hydrolyses of esters of α- and β-aspartyl peptides proceeded through such a succinimide intermediate to give a mixture of acids. More recently, Ondetti and his co-workers,[9] in studies relating to the synthesis of secretin, found that fragment condensation of small peptides with the sequence [*O*-benzyl (or t-butyl)]-α-aspartylglycyl gave mixtures through the formation of succinimido-derivatives. One peptide with this sequence rearranged on recrystallisation from boiling ethanol.[10] Iselin and Schwyzer [11] have made similar observations on the rearrangement of α-aspartyl esters in the sequence aspartylglycyl. Several studies [12,13] on model peptides with the combination (*O*-alkyl)-α-aspartylseryl show that this sequence particularly favours rearrangement. However, Ondetti *et al.*[10] point out that when seryl residues in the sequence aspartylseryl are *O*-protected, or glycine residues in the sequence aspartylglycyl are replaced by residues of amino-acids with side chains, the rearrangement is inhibited; they attribute this to steric hindrance.

In order to advance our understanding of the ready rearrangement of the tetrapeptide, we have prepared related peptides with β-aspartyl residues and studied their behaviour in aqueous pyridine (1 : 1) at room temperature. The results are summarised in the Table. Some comparison of the rates of rearrangement was made possible by observing at similar intervals, for each compound, the formation of new products. Studies of the extent of rearrangement were also carried out in methanol, with similar results. The dipeptide (IX) did not rearrange, whereas the dipeptide (X) did so slowly (*ca.* 20% in 14 days) to yield a compound identified by ^{1}H n.m.r. and mass spectrometry as the succinimide derivative (XV). The corresponding tripeptide (XII), with *C*-terminal glycine, rearranged more rapidly (*ca.* 50% in 14 days), whereas the tripeptide (XI) with *C*-terminal β-alanine and the tetrapeptide (V) both rearranged at similar but much greater rates (*ca.* 25% in 24 h). Evidently the presence of a third residue, in particular β-alanine, favoured the rearrangement.



L

(VII) R = Z-Gly, X = >L-Ser(Bu^t)-βAla-OBzl
(XV) R = Z X = >L-Ser(Bu^t)-OBu^t
(XVI) R = Z X = >L-Leu-βAla-OBzl

Z-Gly-L-Asp(OMe)-L-Ser(Bu^t)-β-Ala-OBzl
(VIII)

Rearrangement of β-aspartyl peptides in pyridine–water

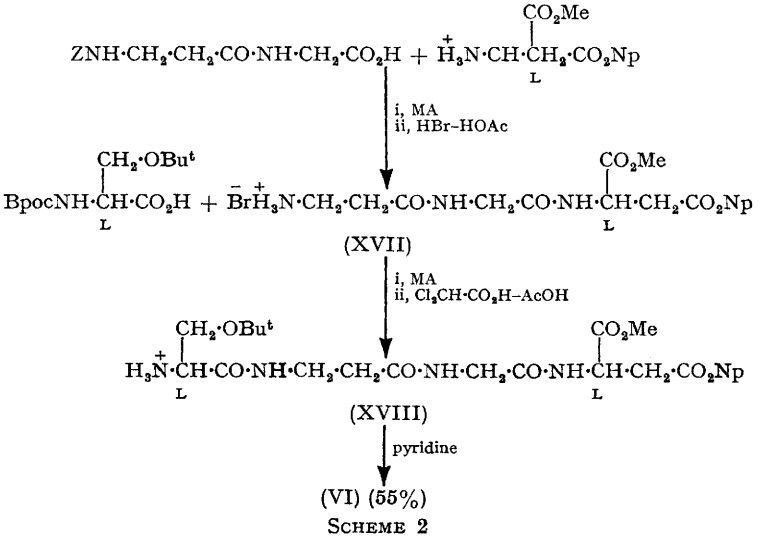
Peptide	Rearrangement rate
Z-Gly-L-Asp-OMe (V) —L-Ser(Bu^t)-βAla-OBzl	++++
Z-Gly-L-Asp-Me (IX) | ONp	−
Z-L-Asp(OMe)-L-Ser(Bu^t)-OBu^t (X)	+
Z-L-Asp-OMe (XI) —L-Ser(Bu^t)-βAla-OBzl	++++
Z-L-Asp-OMe (XII) —L-Ser(Bu^t)-Gly-OBzl	++
Z-L-Asp-OMe (XIII) —L-Leu-βAla-OBzl	++++
Z-Gly-L-Asp-OMe (XIV) —L-Leu-βAla-OBzl	++++

It was of interest to determine whether the neighbouring protected serine residue had a particular role in the rearrangement reaction. For this reason, the related tripeptide (XIII) with adjacent leucyl rather than protected seryl residue, was prepared. It rearranged readily (*ca.* 25% in 24 h) and a compound (XVI) with a succinimide residue was identified by spectroscopic evidence as one of the products. The rate of rearrangement was similar for the corresponding tetrapeptide (XIV) incorporating L-leucine. Evidently, the rearrangement of the β-aspartyl ester residue in an appropriate tripeptide sequence is affected in a similar way by adjacent leucyl or *O*-protected seryl residues.

With this evidence of the basis of the rearrangement we undertook the synthesis of the cyclic tetrapeptide (VI) by an alternative route (Scheme 2). The suitably protected tripeptide (XVII) was prepared, readily, from

[7] E. Sondheimer and R. W. Holley, *J. Amer. Chem. Soc.*, 1954, **76**, 2467.
[8] A. R. Battersby and J. C. Robinson, *J. Amer. Chem. Soc.*, 1955, **77**, 259.
[9] M. A. Ondetti, V. Narayanan, M. von Saltza, J. T. Sheehan, E. F. Sabo, and M. Bodansky, *J. Amer. Chem. Soc.*, 1968, **90**, 474.
[10] M. A. Ondetti, A. Deer, J. T. Sheehan, J. Pluščec, and O. Kocy, *Biochemistry*, 1968, **7**, 4069.
[11] B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, 1962, **45**, 1499.
[12] R. W. Hanson and H. N. Rydon, *J. Chem. Soc.*, 1964, 836.
[13] S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Schalatin, *J. Amer. Chem. Soc.*, 1962, **84**, 2421.

benzyloxycarbonyl-β-alanylglycine by coupling with α-methyl β-*p*-nitrophenyl L-aspartate (mixed anhydride procedure). Deprotection at the *N*-terminus, coupling with the Bpoc derivative of (*O*-t-butyl)-L-serine, and removal of the *N*-terminal protecting group gave an excellent yield of the active ester (XVIII). Cyclisation occurred to give a 55% yield of the crystalline protected cyclic tetrapeptide on treatment with base at room temperature and high dilution. The identity and purity of each of the new intermediates and the cyclic peptide were established by combustion analysis, amino-acid analysis, and ^{1}H n.m.r. and mass spectra. The



SCHEME 2

X-ray diffraction analysis of a crystal has provided evidence of the conformation of this cyclic peptide.[14]

EXPERIMENTAL

For instruments used, see preceding paper. For t.l.c. we employed plates coated with Kieselgel G. (Merck) and the following solvent systems: (a) butan-1-ol–pyridine–acetic acid–water (30 : 20 : 6 : 24 v/v), (b) ethyl acetate, (c) ethyl acetate–benzene (1 : 1 v/v), (d) benzene–methanol–acetic acid (10 : 2 : 1 v/v), (e) methanol–chloroform (1 : 4 v/v). Plates were treated as follows, in order to make the spots visible: (i) suspended in a chlorine chamber for 10 s and then sprayed with starch–potassium iodide solution; (ii) after spraying with 1% (w/v) solution of ninhydrin in acetone, the plate was heated at 110 °C for 2 min. Light petroleum had b.p. 60—80 °C.

1-*Methyl* 4-p-*Nitrophenyl Benzyloxycarbonyl*-L-*aspartate* (I).—1-Methyl hydrogen benzyloxycarbonyl-L-aspartate [3] (2.5 g, 7.1 mmol) and *p*-nitrophenol (1.5 g, 10 mmol) in ethyl acetate (5 ml) were treated with dicyclohexylcarbodiimide (DCCI) (2.5 g, 10 mmol), with stirring, at 0 °C for 2 h and then set aside overnight at room temperature. After removal of *NN'*-dicyclohexylurea by filtration, the last traces of DCCI were removed by treating the solution with glacial acetic acid (0.2 ml) at 0 °C for 3 h and filtering. The yellow oil obtained on evaporation crystallised from ethyl acetate–light petroleum to give the *ester* (I) (3.05 g, 85%), m.p. 100°, $[\alpha]_D^{20}$ −16.3° (*c* 1 in MeOH) (Found: C, 56.5; H, 4.6; N, 6.9. $C_{19}H_{18}N_2O_8$ requires C, 56.7; H, 4.5; N, 6.9%).

1-*Methyl* 4-p-*Nitrophenyl*-L-*aspartate Hydrobromide*.—The derivative (I) (0.5 g) was dissolved in hydrobromic acid–acetic acid (33%; 2 ml) at room temperature. After 25 min, when the effervescence had ceased, dry ether (100 ml) was added to precipitate the product, and the flask was stored at 0 °C for 15 h. The *solid* was filtered off and washed several times with ethyl acetate and ether, and dried; yield 0.44 g (100%), m.p. 145—146° (Found: C, 37.6; H, 3.5; N, 7.9. $C_{11}H_{13}BrN_2O_6$ requires C, 37.8; H, 3.7; N, 8.0%).

1-*Methyl* 4-p-*Nitrophenyl Benzyloxycarbonylglycyl*-L-*aspartate* (III).—A solution of benzyloxycarbonylglycine (0.32 g, 1.5 mmol) in dimethoxyethane (25 ml) was stirred at −20 °C with *N*-methylmorpholine (0.153 g, 1.5 mmol) and isobutyl chloroformate (0.21 g, 1.5 mmol) for 1 h. After this a suspension of 1-methyl 4-*p*-nitrophenyl-L-aspartate hydrobromide (0.54 g, 1.5 mmol) in dimethoxyethane (10 ml) and *N*-methylmorpholine (0.153 g, 1.5 mmol) was added, and the mixture was allowed to come to room temperature and then stirred for a further 24 h. After addition of a few drops of water, the solvent was removed at room temperature and the solid residue was extracted into ethyl acetate (3 × 50 ml). The gum obtained from this extract was crystallised from ethyl acetate–light petroleum to give the *product* (2.8 g, 96%), R_F (c) 0.6, m.p. 124—125°, $[\alpha]_D^{20}$ +4.5° (*c* 1 in MeOH), $\nu_{max.}$ 1 750 and 1 730 (carbonyls), 1 690 (benzyloxycarbonyl), and 1 650 and 1 545 cm^{-1} (amide I and II) (Found: C, 54.7; H, 4.6; N, 9.0. $C_{21}H_{21}N_3O_9$ requires C, 54.8; H, 4.6; N, 9.2%).

1-(*Biphenyl*-4-*yl*)-1-*methylethoxycarbonyl*-(O-*t*-*butyl*)-L-*seryl*-β-*alanine Benzyl Ester* (II).—(*O*-t-Butyl)-L-serine [15] was converted into the cyclohexylammonium salt of *N*-[1-(biphenyl-4-yl)-1-methylethoxycarbonyl]-*O*-t-butyl-L-serine as described by Sieber and Iselin.[4] This salt (1 g) was treated with 1 mol. equiv. of citric acid in ether (200 ml) at 0 °C and the product worked up in the usual way to give the free acid as a gum (0.72 g, 90%), which gave a single spot

[14] I. Karle, B. K. Handa, and C. H. Hassall, *Acta Cryst.*, 1975. **B31**, 555.

[15] C. H. Hassall and J. O. Thomas, *J. Chem. Soc.* (*C*), 1968, 1495.

on t.l.c. {R_F (e) 0.65; [ethyl acetate–methanol (19 : 1)] 0.7}. The ^{1}H n.m.r. spectrum indicated that the Bpoc group was not removed by this treatment. The gum was coupled with the toluene-*p*-sulphonic acid salt of β-alanine benzyl ester by the mixed anhydride procedure as described for the preparation of (III). The *dipeptide* (II) crystallised from ether–light petroleum (81%); m.p. 65°, $[\alpha]_D^{20}$ −9° (*c* 1 in MeOH), R_F (a) 0.85 (Found: C, 70.7; H, 7.2; N, 5.1. $C_{33}H_{40}N_2O_6$ requires C, 70.7; H, 7.1; N, 5.0%).

Benzyloxycarbonylglycyl-(O-*methyl*)-L-β-*aspartyl-*(O-*t-butyl*)-L-*seryl*-β-*alanine Benzyl Ester* (V).—(i) The dipeptide (II) (2 g, 3.5 mmol) was stirred with acetic acid–formic acid–water (7 : 1 : 2; 30 ml) for 2 h at room temperature. The gum obtained after the removal of most of the solvent under reduced pressure was triturated with water. The precipitate was removed by filtration and the aqueous filtrate was evaporated to give a gum (1.6 g), which was purified by chromatography on a silica gel column. The main product (1.1 g, 81%) was eluted with ethyl acetate–methanol (1 : 1). It gave a single spot on t.l.c., R_F (a) 0.66. This formate salt of (*O*-t-butyl)-L-seryl-β-alanine benzyl ester (2.4 g, 6.5 mmol) was treated in dimethylformamide (25 ml) with the ester (III) (2.9 g, 6.5 mmol) and triethylamine (0.63 g, 6.3 mmol) with stirring at room temperature for 3 days. The mixture was then treated with ethyl acetate (500 ml) and extracted with 0.5N-citric acid (1 × 150 ml), 0.5N-ammonia (until free from *p*-nitrophenol), 0.5N-citric acid (2 × 150 ml), and water, until neutral. The ethyl acetate solution was dried and evaporated to yield a gum which was a mixture of two products, R_F (b) 0.34 and 0.42, (a) 0.7 and 0.72.

This gum in dry benzene (5 ml) was passed through a silica gel column (50 g; 3 × 40 cm) prepared in light petroleum. The first product (0.65 g), R_F (b) 0.4, (a) 0.72, was eluted with chloroform–ethyl acetate (4 : 1). It could not be crystallised but was identified as *N*-formyl-(*O*-t-butyl)-L-seryl-β-alanine benzyl ester, M^+ 350, τ 1.7 (1 H, s, O=CH), 2.65 (5 H, s, Ph), 2.8—3.2br (2 H, NH), 4.8 (2 H, s, $ArCH_2$), 5.25—5.65 [1 H, m, α-CH(Ser)], 6—6.8 [4 H, m, CH_2(Ser,βAla)], 7.4 [2 H, t, α-CH_2(βAla)], and 8.9 (9 H, s, Bu^t).

The second product, obtained (2.5 g) by elution with chloroform–ethyl acetate (1 : 1), t.l.c. R_F (a) 0.7, (b) 0.34, crystallised from benzene–light petroleum with m.p. 89—90°, $[\alpha]_D^{20}$ −4.5° (*c* 1 in MeOH), $\nu_{max.}$ 1 650 (amide I), 1 545 (amide II), 1 740 (CO_2CH_3), and 2 900 cm^{-1} (Bu^t). It was characterised as *benzyloxycarbonylglycyl-*(O-*methyl*)-L-β-*aspartyl-*(O-*t-butyl*)-L-*seryl*-β-*alanine benzyl ester* on the basis of ^{1}H n.m.r. evidence (Found: C, 60.1; H, 6.5; N, 8.8. $C_{32}H_{42}N_4O_{10}$ requires C, 59.9; H, 6.5; N, 8.7%).

(ii) The dipeptide (II) (1.5 g, 2.68 mmol) was dissolved in 0.5% trifluoroacetic acid in dichloromethane (150 ml) and maintained at room temperature for 20 min; the solvent was then evaporated off and the solid obtained after trituration with water was filtered off. The filtrate was evaporated to yield a gum (1.26 g) which gave a single spot on t.l.c., R_F (d) 0.5. This was passed through a silica gel column (75 g; 2.5 × 50 cm) prepared in dry benzene. A pure product (1 g) was obtained on elution with benzene–chloroform (9 : 1). This product (2 g, 4.6 mmol) was coupled with the dipeptide *p*-nitrophenyl ester (III) (2.26 g, 4.8 mmol) in the presence of triethylamine (0.5 g, 4.8 mmol). The mixture was worked up for (V), as described above. The product, crystallised from benzene–petroleum (yield 2.4 g, 80%) had m.p. 89—90° and all the properties defined for (V) above. When this tetrapeptide derivative (0.25 g) in methanol (15 ml) was hydrogenated at room temperature for 36 h over palladium–charcoal, a complex mixture was obtained; t.l.c. indicated at least four compounds. The ^{1}H n.m.r. spectrum showed that the methoxycarbonyl group was absent.

*Benzyloxycarbonylglycyl-*L-2-*aminosuccinyl-*(O-*t-butyl*)-L-*seryl*-β-*alanine Benzyl Ester* (VII).—(i) The protected tetrapeptide (V) (100 mg), in purified methanol (15 ml), was stored in a stoppered flask at room temperature for 24 h. The gum obtained after removal of solvent under reduced pressure gave two spots on t.l.c., R_F (b) 0.4 and 0.34. The product (50 mg) of R_F 0.4 was obtained on eluting a silica gel column (50 g; 2.5 × 50 cm) with benzene–ethyl acetate (1 : 1); the second product (40 mg), R_F 0.34 was eluted with the solvent mixture in the ratio 1 : 3. The latter was identified as starting material (V). The ^{1}H n.m.r. spectrum of the first fraction indicated the absence of a methoxycarbonyl group and one amide proton. It was assigned the structure (VII). When this product was treated with methanol, t.l.c. of the resulting mixture showed three main spots, R_F (b) 0.4, 0.34, and 0.3. It appeared that the imide ring had opened to give the original tetrapeptide (V) together with a new tetrapeptide, presumably (VIII).

(ii) When the tetrapeptide (V) (200 mg) was dissolved in pyridine–water (1 : 1) (18 ml), it was transformed after 2 weeks into two products, R_F (b) 0.4 and 0.0. Silica gel column chromatography, as described above, established that the major product, R_F 0.4, was the compound mentioned above (the two preparations showed the same i.r. and n.m.r. spectra). The product with R_F 0.0 was not identified.

Benzyloxycarbonyl-(O-*methyl*)-L-β-*aspartyl-*(O-*t-butyl*)-L-*seryl*-β-*alanine Benzyl Ester* (XI).—(*O*-t-Butyl)-L-seryl-β-alanine benzyl ester trifluoroacetate (0.25 g, 0.57 mmol) was coupled with 1-methyl 4-*p*-nitrophenyl benzyloxycarbonyl-L-aspartate (0.23 g, 0.57 mmol) in the presence of triethylamine (0.06 g, 0.57 mmol) in dimethylformamide (5 ml) at room temperature. The usual work-up gave a gum which crystallised from ethyl acetate–light petroleum to give the *product* (0.28 g, 85%), m.p. 98°, R_F (b) 0.8, $[\alpha]_D^{20}$ +24.6° (*c* 1.26 in $CHCl_3$) (Found: C, 61.4; H, 6.8; N, 7.1. $C_{30}H_{39}N_3O_9$ requires C, 61.5; H, 6.7; N, 7.2%). This tripeptide was modified in methanol or pyridine–water (1 : 1) at room temperature. The results of t.l.c. of these solutions were similar to those for the protected tetrapeptide (V).

*Benzyloxycarbonyl-*L-(O-*methyl*)-β-*aspartyl-*L-(O-*t-butyl*)-*serine t-Butyl Ester* (X).—(*O*-t-Butyl)-L-serine butyl ester [16] (1.5 g, 6.9 mmol) in dimethylformamide (4 ml) was mixed with 1-methyl 4-*p*-nitrophenyl benzyloxycarbonyl-L-aspartate (2.8 g, 6.9 mmol). The mixture was worked up in the usual manner to give a gum (3.3 g, 100%), which was purified by chromatography on silica gel. The main *product* was eluted with benzene–ethyl acetate (1 : 1). It crystallised from n-pentane at 0 °C; yield 2.2 g (66%), m.p. 68°, R_F 0.6 [benzene–ethyl acetate (3 : 2)], $[\alpha]_D^{20}$ +34.65° (*c* 1 in $CHCl_3$) (Found: C, 59.7; H, 7.6; N, 5.5. $C_{24}H_{36}N_2O_8$ requires C, 60.0; H, 7.61; N, 5.8%).

*Isolation and Characterisation of Benzyloxycarbonyl-*L-2-*aminosuccinyl-*(O-*t-butyl*)-L-*serine t-Butyl Ester* (XV).—The dipeptide ester (200 mg, 0.4 mmol) obtained above was

[16] F. M. Callahan, G. W. Anderson, R. Paul, and J. E. Zimmerman, *J. Amer. Chem. Soc.*, 1963, **85**, 201.

kept in pyridine–water (1 : 1) (8 ml) at room temperature. The starting material [R_F 0.25 in ethyl acetate–benzene (1 : 4)] gradually disappeared and two new products (R_F 0.4 and 0.0) were observed. After 8 weeks, when the starting material had almost disappeared, the solvent was evaporated off at room temperature and the resulting gum in benzene was chromatographed on silica gel. The products of R_F 0.4 (10 mg) and 0.0 (100 mg) were separated. On the basis of the ^{1}H n.m.r. spectrum (in $CDCl_3$) the product of R_F 0.4 was identified as the rearranged cyclic intermediate (XV), τ 2.7 (5 H, s, Ph), 4.55 (1 H, d, NH), 4.85 (2 H, s, PhCH_2), 5.18 [1 H, q, CH(Ser)], 5.5 [1 H, m, CH(succinimide)], 6.2 [2 H, m, CH_2(Ser)], 6.8 [2 H, 8 signals, CH_2(succinimide)], 8.6 (9 H, s, Bu^t), and 8.9 (9 H, s, Bu^t).

The second product (100 mg) was a single compound, R_F (e) 0.45. The ^{1}H n.m.r. spectrum (60 MHz) indicated that this could be either of the two acids formed by opening the succinimide ring, τ 2.8 (5 H, s, Ph), 4.9 (2 H, s, CH_2Ph), 5.1—5.8br (2 H, m, α-CH of Ser and Asp), 6.2—6.6br (2 H, β-CH_2), 7.25br (2 H, β-CH_2), 2.9 (1 H, d, NH), 3.45br (1 H, d, NH), 8.65 (9 H, s, Bu^t), and 9.0 (9 H, s, Bu^t). The effect of methanol on the dipeptide butyl ester was also studied. There was only a slight change after 8 weeks.

Benzyloxycarbonyl-(O-*methyl*)-L-β-*aspartyl*-(O-*t-butyl*)-L-*serylglycine Benzyl Ester*.—*N*-[1-(Biphenyl-4-yl)-1-methylethyl]-(*O*-t-butyl)-L-serylglycine benzyl ester (0.5 g, 0.9 mmol), prepared from the corresponding amino-acid derivatives by the mixed anhydride procedure, was treated at room temperature for 1.5 h with acetic acid–dichloroacetic acid (6 : 1) (7 ml). After removal of the bulk of the acid, under reduced pressure, the residue was treated with n-pentane (50 ml) to give a gum which was washed with further quantities of n-pentane (3 × 50 ml). The filtrate obtained when the gum was dissolved in water (25 ml) was evaporated to give an amorphous product which was further dried over phosphorus pentaoxide. The product (0.25 g), which could not be crystallised, gave a single spot on t.l.c., R_F (e) 0.75. It was coupled with 1-methyl 4-*p*-nitrophenyl benzyloxycarbonyl-L-aspartate (0.24 g, 0.6 mmol) in anhydrous dimethylformamide (5 ml) containing triethylamine (0.06 g, 0.6 mmol), at room temperature. The mixture was worked up in the usual way after 24 h to give a gum which crystallised from ethyl acetate–light petroleum to yield the *product* (0.2 g, 60%), m.p. 110—111°, $[\alpha]_D^{20}$ +23.8° (*c* 1.05 in $CHCl_3$), R_F (b) 0.8 (Found: C, 60.6; H, 6.5; N, 7.1. $C_{29}H_{37}N_3O_9$ requires C, 60.9; H, 6.5; N, 7.3%).

Samples (25 mg) of this tripeptide were dissolved separately in methanol (2 ml) and pyridine–water (1 : 1) (2.5 ml). T.l.c. showed that this peptide behaved similarly to the peptides (V) and (XII), but the rate of decomposition was much less. Appreciable change was only apparent after 1 week.

Benzyloxycarbonyl-(O-*methyl*)-L-β-*aspartyl*-L-*leucyl*-β-*alanine Benzyl Ester* (XIII).—Benzyloxycarbonyl-L-leucyl-β-alanine benzyl ester {m.p. 71°, $[\alpha]_D^{20}$ −19.6° (*c* 1.02 in MeOH) (Found: C, 67.6; H, 7.2; N, 6.5. $C_{24}H_{30}N_2O_5$ requires C, 67.6; H, 7.1; N, 6.6%)} was prepared by the mixed anhydride procedure and converted into the corresponding hydrobromide by treatment with 5N-hydrobromic acid–acetic acid for 20 min at room temperature. The product was precipitated with ether. This hydrobromide, a gum, was coupled with 1-methyl 4-*p*-nitrophenyl benzyloxycarbonyl-L-aspartate in the presence of triethylamine as described in the preparation of (V). The *tripeptide derivative* (XIII) had m.p. 136°, R_F (b) 0.75, $[\alpha]_D^{20}$ −35.9° (*c* 0.98 in MeOH) (Found: C, 62.3; H, 6.75; N, 7.4. $C_{29}H_{37}N_3O_8$ requires C, 62.7; H, 6.7; N, 7.55%).

Benzyloxycarbonylglycyl-(O-*methyl*)-L-β-*aspartyl*-L-*leucyl*-β-*alanine Benzyl Ester* (XIV).—When the 1-methyl 4-*p*-nitrophenyl benzyloxycarbonylglycyl-L-aspartate (1.9 g, 4.3 mmol) was coupled with L-leucyl-β-alanine benzyl ester hydrobromide (1.6 g, 4.3 mmol), the tetrapeptide (XIV) was obtained in the usual way in 56% yield, as *crystals* (from ethyl acetate–light petroleum), m.p. 102°, $[\alpha]_D^{20}$ −18.5° (*c* 1.03 in MeOH), R_F (b) 0.5 (Found: C, 60.5; H, 6.7; N, 9.0. $C_{31}H_{40}N_4O_9$ requires C, 60.8; H, 6.6; N, 9.1%), ^{1}H n.m.r. spectrum as expected.

When 50 mg of each of the peptides (XIII) and (XIV) was kept in methanol (or aqueous pyridine) in a capped vial at room temperature, decomposition was observed by t.l.c., as for the serine derivatives (XI) and (V).

Benzyloxycarbonyl-L-2-*aminosuccinyl*-L-*leucyl*-β-*alanine Benzyl Ester* (XVI).—The tripeptide (XIII) (0.2 g, 0.36 mmol) in pyridine (4 ml) and water (8 ml) was kept in a stoppered flask at room temperature for 4 days. T.l.c. indicated the formation of two new products, R_F (b) 0.85 and 0.0. A trace of the starting material, R_F 0.75, was also in evidence. The solvent was removed at reduced pressure and, by storing over potassium hydroxide for 24 h under high vacuum. The gum which remained was dissolved in ethyl acetate (1 ml) and introduced onto a silica gel column (40 × 2 cm; 50 g) prepared in dry benzene. A pure product (100 mg) (R_F 0.85) was obtained by elution with benzene–ethyl acetate (1 : 1); m.p. 118° (from ethyl acetate–light petroleum), τ 2.7 (5 H, s, Ph), 3.1 (1 H, t, NH of βAla), 4.2 (1 H, d, NH of Asp), 4.9 (2 H, s, PhCH_2), 5.25 (1 H, q, α-CH of Leu), 5.65—5.95 [1 H, m, CH of succinimide (X)], 6.28—6.68 (2 H, m, β-CH_2 of βAla), 7.2 [2 H, 8 signals, β-CH_2 of succinimide (AB)], 7.45 (2 H, t, α-CH_2 of βAla), 7.68—8.22 (2 H, m, β-CH_2 of Leu), 8.4—8.7 (1 H, m, γ-CH of Leu), 9.05 (3 H, d, CH_3 of Leu), and 9.15 (3 H, d, CH_3 of Leu). Evidently this compound did not contain a methoxycarbonyl group and one amide proton present in the original tripeptide derivative. The AB part of the ABX pattern of the succinimide ring took the form of eight lines centred at τ 7.2 (J_{AB} 16 Hz). The large geminal coupling was in accord with a five-membered ring adjacent to a π system.

Two minor products (R_F 0) were eluted with methanol (30 mg); R_F (e) 0.5 and 0.6. No attempt was made to separate this mixture.

Benzyloxycarbonyl-β-*alanylglycyl*-(1-O-*methyl*)-L-*aspartic Acid* 4-p-*Nitrophenyl Ester*.—This was prepared by coupling benzyloxycarbonyl-β-alanylglycine [3] (2.9 g, 10 mmol) and 1-methyl 4-*p*-nitrophenyl L-aspartate hydrobromide (3.5 g, 10 mmol) in dimethoxyethane as described for the preparation of (III). The usual work-up gave a gum (4.5 g, 90%), which crystallised from ethyl acetate–light petroleum to give the *product*, m.p. 120°, R_F (b) 0.3, $[\alpha]_D^{20}$ +44.75° (*c* 1 in $CHCl_3$) (Found: C, 54.3; H, 5.0; N, 10.3. $C_{24}H_{26}N_4O_{10}$ requires C, 54.3; H, 4.95; N, 10.55%).

β-*Alanylglycyl*-(1-O-*methyl*)-L-*aspartic Acid* 4-p-*Nitrophenyl Ester Hydrobromide* (XVII).—The above tripeptide *p*-nitrophenyl ester (1 g, 1.9 mmol) in dry dichloromethane (10 ml) was treated with 45% hydrobromic acid–acetic acid (1.5 ml). The precipitation, which started after 0.5 h, was completed by addition of dry ether (150 ml). After 15 h at 0 °C, the solid was filtered off, washed repeatedly with ether, ethyl acetate, and dichloromethane, and dried to give

the *product* (0.8 g, 92%), m.p. 162°, $[\alpha]_D^{20}$ +8.3° (*c* 1.01 in MeOH); single ninhydrin positive spot (dull red), R_F (a) 0.7, (e) 0.2 (Found: C, 39.9; H, 4.4; N, 11.4. $C_{16}H_{21}BrN_4O_8$ requires C, 40.3; H, 4.4; N, 11.7%).

N-(1-*Biphenyl*-4-*yl*-1-*methylethoxycarbonyl*)-(O-*t*-*butyl*)-L-*seryl*-β-*alanylglycyl*-(1-O-*methyl*)-L-β-*aspartic Acid* 4-p-*Nitrophenyl Ester*.—Mixed anhydride coupling of the hydrobromide salt (XVII) (1.2 g, 2.5 mmol) and *N*-(1-biphenyl-4-yl-1-methylethoxycarbonyl)-(*O*-t-butyl)-L-serine (1 g, 2.5 mmol) in dimethoxyethane proceeded smoothly. A gum was obtained after the usual work up, which solidified but could not be crystallised. The solid was triturated with n-pentane, filtered off, washed with ether, and dried to give the *product* (1.6 g, 85%), single spot on t.l.c., R_F (e) 0.9 (ethyl acetate–methanol, 19 : 1) 0.55, m.p. 65°, $[\alpha]_D^{20}$ +53.8° (*c* 1 in $CHCl_3$) (Found: C, 59.35; H, 6.5; N, 8.1. $C_{43}H_{55}N_5O_{14}$ requires C, 59.6; H, 6.35; N, 8.1%). The i.r. and ^1H n.m.r. spectra were in agreement with the structure assigned.

(O-*t*-*Butyl*)-L-*seryl*-β-*alanylglycyl*-(1-O-*methyl*)-L-*aspartic Acid* 4-p-*Nitrophenyl Ester Dichloroacetate Salt* (XVIII).—The foregoing *p*-nitrophenyl ester (0.25 g, 0.3 mmol) and acetic acid–dichloroacetic acid (6 : 1; 7 ml) were stirred for 1.5 h at room temperature. The solid which separated on treatment with anhydrous ether (50 ml) was washed with ether (3 × 50 ml) to give the *product* (0.2 g, 90%), m.p. 59°, $[\alpha]_D^{20}$ +59.0° (*c* 1 in $CHCl_3$), R_F (e) 0.5 (Found: C, 44.2; H, 5.4; N, 10.25. $C_{25}H_{35}Cl_2N_5O_{12}$ requires C, 44.9; H, 5.3; N, 10.5%).

Cyclo-[(O-*t*-*butyl*)-L-*seryl*-β-*alanylglycyl*-(O-*methyl*)-L-β-*aspartyl*] (VI).—The dichloroacetate (XVIII) (0.6 g, 0.9 mmol) was dissolved in purified and dried tetrahydrofuran (8 ml) containing glacial acetic acid (0.4 ml). This solution was added dropwise, at room temperature, to purified and dried pyridine (800 ml) containing triethylamine (0.75 ml) over 3.5 h, with stirring. The mixture was then stirred for 3 days, and concentrated at reduced pressure and a temperature not exceeding 40 °C. The product was triturated with ethyl acetate, filtered off, and washed in turn with ethyl acetate (3 × 10 ml) and ether (3 × 10 ml). The solid residue crystallised from methanol to give the *product* (0.2 g, 55.5%), m.p. 269°, R_F (a) 0.8, $[\alpha]_D^{20}$ −23.5° (*c* 0.54 in $Me_2N{\cdot}CHO$) (Found: C, 49.8; H, 7.1; N, 13.6. $C_{17}H_{28}N_4O_7,\frac{1}{2}H_2O$ requires C, 49.9; H, 7.1; N, 13.7%), $\nu_{max.}$ 1 740 (CO_2CH_3), 2 900 (Bu^t), and 1 650 and 1 545 cm^{-1} (amide I and amide II), M^+ 400, τ [$(CD_3)_2SO$; 100 MHz] 1.35 [1 H, t, NH(Glu or βAla)], 1.82 [1 H, d, N*H*(Ser or Asp)], 3.0br (2 H, NH), 5.1—5.4 [1 H, m, CH (α of Asp)], 5.7—6.1 [1 H, m, CH (α of Ser)], 6.4 (3 H, s, CO_2Me), 6.2—7.4br (8 H, m, CH_2 α and β), 7.5—7.8br [2 H, CH_2 (α of βAla)], and 8.9 (9 H, s, Bu^t). Amino-acid analysis of the hydrolysate gave: Asp, 1.03; Ser, 0.98; Gly, 0.99.

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