Polypeptides. Part XXII.[1] The Synthesis of Peptides of α-Benzylphenylalanine by the Dicyclohexylcarbodi-imide Method

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Dicyclohexylcarbodi-imide appears to be generally useful for the synthesis of protected dipeptides of α-benzylphenylalanine and for adding α-benzylphenylalanyl residues to the *N*-terminus of larger peptides. It is not, however, generally suitable for adding further amino-acid residues to peptides with an *N*-terminal α-benzylphenylalanyl residue or for coupling reactions using peptides with a *C*-terminal α-benzylphenylalanyl residue. Suitable *N*-protecting groups for α-benzylphenylalanyl peptides are benzyloxycarbonyl and *o*-nitrophenylsulphenyl. Ethyl esters are not suitable for *C*-protection, since they are difficult to hydrolyse owing to steric hindrance; 2-methylthioethyl esters are not subject to this and can be used successfully.

Syntheses of two protected peptides corresponding to sequences 6—9 and 1—4 in bradykinin are described.

THE work described in this paper had as its ultimate objective the synthesis of analogues of bradykinin in which the phenylalanine residues in positions 5 and 8 were replaced, singly or together, by α-benzylphenylalanyl [$-NH\cdot C(CH_2Ph)_2\cdot CO-$] residues.* Although this objective was not realised, the presence of two benzyl side-chains gave rise to synthetic difficulties which seem worthy of record and comparison with earlier work on peptide synthesis with other αα-disubstituted amino-acids.

The αα-disubstituted amino-acid most fully studied as a peptide component is α-methylalanine, peptide syntheses with which were carried out by Kenner [2,3] and Faust [4,5] and their colleagues. Both groups found that the acylation of the amino-group in α-methylalanine and its derivatives is much more affected by steric hindrance than is acylation by the activated carboxy-group in such compounds, but this is not our experience with α-benzylphenylalanine. Of the commoner *N*-protecting groups, both benzyloxycarbonyl [2-4,6] and *p*-tolylsulphonyl [2] were used successfully. For carboxy-protection t-butyl esters were favoured,[3] although methyl [2,3] and benzyl [4] esters were also used. Of the commoner coupling methods, the dicyclohexylcarbodi-imide,[3,4] mixed pivalic anhydride,[2,7] *p*-nitrophenyl ester,[7] azide,[6,7] and acid chloride [2,4] procedures have all been used, with varying degrees of success. The present paper deals with our experience in applying the dicyclohexylcarbodi-imide method to the synthesis of peptides of α-benzylphenylalanine; the application of the oxazolinone procedure [2,3] will be reported later.

α-Benzylphenylalanine was prepared by the method of Felkin,[8] with modifications of the details of the Schmidt reaction on ethyl dibenzylacetoacetate which greatly improved the yield of the intermediate *N*-acetyl-α-

* The abbreviation Bphe is used for this residue. Other abbreviations are those recommended by I.U.P.A.C. (see *Biochem. J.*, 1972, **126**, 773). Nb = p-$NO_2\cdot C_6H_4\cdot CH_2$.

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[3] D. S. Jones, G. W. Kenner, J. Preston, and R. C. Sheppard, *J. Chem. Soc.*, 1965, 6227.

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benzylphenylalanine ethyl ester. No difficulty was experienced in the preparation of the *N*-*o*-nitrophenylsulphenyl derivative by reaction of α-benzylphenylalanine with *o*-nitrophenylsulphenyl chloride in alkaline solution;[9] this derivative was used extensively in coupling reactions and also as an intermediate in the preparation of the *N*-succinimidyl and 2-methylthioethyl esters of α-benzylphenylalanine.

N-*o*-Nitrophenylsulphenyl-α-benzylphenylalanine coupled smoothly with ω-nitroarginine *p*-nitrobenzyl ester in chloroform solution in the presence of dicyclohexylcarbodi-imide to give a 70% yield of the protected dipeptide (I). The similar coupling of *N*-*o*-nitrophenylsulphenyl-α-benzylphenylalanine with L-seryl-L-prolyl-L-phenylalanyl-ω-nitro-L-arginine *p*-nitrobenzyl ester (VI) in acetonitrile likewise gave a 70% yield of the protected

NO_2 | Nps·Bphe·Arg·ONb (I)

NO_2 | Nps·Bphe·Ser·Pro·Phe·Arg·ONb (II)

pentapeptide (II). These results indicate that the dicyclohexylcarbodi-imide method is likely to be generally useful for the introduction of α-benzylphenylalanine residues at the *N*-terminus of the growing peptide chain in stepwise peptide syntheses. However, attempts to couple the protected dipeptide, *N*-benzyloxycarbonylglycyl-α-benzylphenylalanine with L-seryl-L-prolyl-L-phenylalanine-ω-nitro-L-arginine *p*-nitrobenzyl ester (VI) using dicyclohexylcarbodi-imide failed, even in the presence of added 1,2,4-triazole[10] and *N*-hydroxysuccinimide;[11] the method is thus not generally applicable to the synthesis of α-benzylphenylalanine peptides by fragment condensation procedures involving peptides containing *C*-terminal α-benzylphenylalanyl residues.

The dicyclohexylcarbodi-imide method is likewise of only limited value in coupling reactions involving the amino-group of α-benzylphenylalanyl residues. Thus, although the coupling by this method of the appropriate carboxy-components with α-benzylphenylalanine esters gave the protected dipeptides (III; R = Et), (III; R = CH_2·CH_2·SMe), and (IV) in yields of 75, 90, and 75%, respectively, the corresponding reaction between *N*-*o*-nitrophenylsulphenyl-L-proline and α-benzylphenylalanyl-ω-nitro-L-arginine *p*-nitrobenzyl ester gave a very low yield of the protected tripeptide (V), the *N*-acylurea

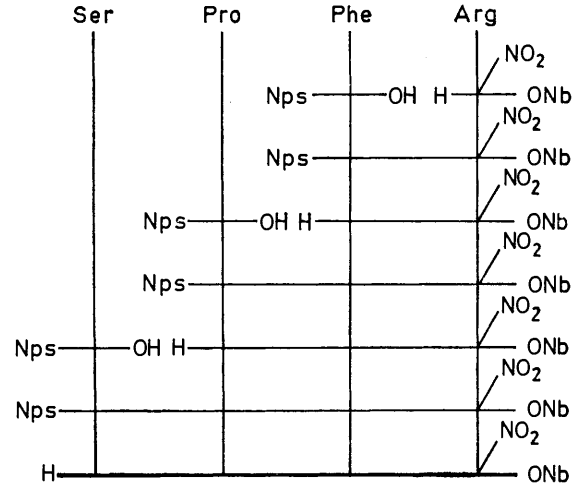
Z·Gly·Bphe·OR (III) Z·Pro·Bphe·OEt (IV)

Nps·Pro·Bphe·Arg(NO_2)·ONb (V)

derived from the carboxy-component being the main product, and attempts to couple *N*-benzyloxycarbonyl- and *N*-*o*-nitrophenylsulphenyl-glycine with α-benzylphenylalanyl-L-seryl-L-prolyl-ω-nitro-L-arginyl *p*-nitrobenzyl ester failed completely, even with added 1,2,4-triazole.

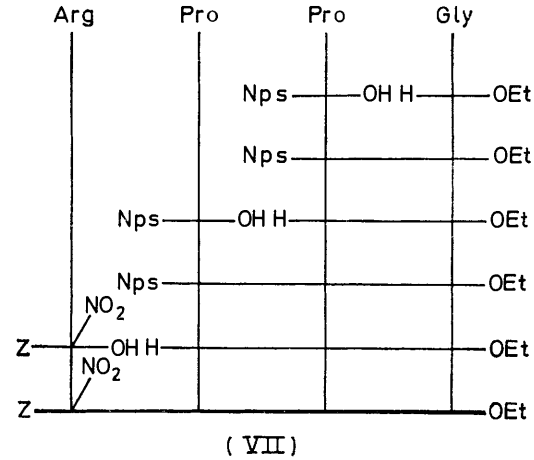
To summarise, our results indicate that although dicyclohexylcarbodi-imide is not generally applicable as a coupling reagent for the synthesis of peptides of α-benzylphenylalanine, it can be used successfully for the synthesis of dipeptides of α-benzylphenylalanine and of larger peptides containing *N*-terminal residues of this amino-acid.

As expected, *N*-deprotection of α-benzylphenylalanine peptides is less affected by steric hindrance than is



(VI)

SCHEME 1



(VII)

SCHEME 2

C-deprotection. The *o*-nitrophenylsulphenyl group was removed smoothly and in high yield from the peptides (I) and (II) by treatment with hydrogen chloride in dioxan and ethyl acetate, respectively, and the benzyloxycarbonyl group from (IV) by catalytic hydrogenolysis. The ethyl ester (III; R = Et) resisted all attempts at hydrolysis with either acid[12] or alkali,

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[10] H. C. Beyermann and W. Maassen-van der Brink, *Acta Chim. Acad. Sci. Hung.*, 1965, **44**, 187.

[11] F. Weygand, D. Hoffmann, and E. Wünsch, *Z. Naturforsch.*, 1966, **21b**, 426.

[12] J. R. Vaughan and J. A. Eichler, *J. Amer. Chem. Soc.*, 1954, **76**, 2474.

differing in this respect from the corresponding α-methylalanine derivative;[2,6] that this difficulty is indeed due to steric hindrance is suggested by the fact that the methiodide of (III; R = CH_2·CH_2·SMe) readily gives the free acid (III; R = H) in aqueous acetone at pH 10 at room temperature,[13] a reaction known to involve attack at a point distant from the α-carbon atom of the amino-acid residue.[14]

Details of syntheses of the protected tetrapeptides (VI) (Scheme 1) and (VII) (Scheme 2) required for the projected synthesis of bradykinin analogues are given in the Experimental section; dicyclohexylcarbodi-imide was used in all the coupling reactions.

EXPERIMENTAL

The purity of all peptides and intermediates was confirmed by t.l.c. on Kieselgel G. Compounds with free amino-groups were detected by means of 0·15% ninhydrin in n-butanol at 100°; peptides with *N*-terminal α-benzylphenylalanine residues gave very weak ninhydrin reactions[15] and were best detected, as were *N*-protected peptides, by the chlorine–starch–iodide method.[16]

Organic solutions were dried over magnesium sulphate and concentrated or evaporated under reduced pressure in a rotary evaporator. Light petroleum was the fraction b.p. 40—60° unless otherwise indicated. Optical rotations were measured with an ETL-NPL Polarimeter, model 143, (path length of 1 cm).

α-Benzylphenylalanine and Derivatives

The following procedure gave a much better yield than that (*ca.* 25%) obtained by the unmodified method of Felkin.[8] Concentrated sulphuric acid (36·4 ml, 0·68 mol) and sodium azide (34·3 g, 0·48 mol) were added alternately, in small portions, to a stirred solution of recrystallised ethyl dibenzylacetoacetate [8] (105 g, 0·34 mol) in redistilled trichloroacetic acid (700 g) at 75—85°. The mixture was kept overnight at room temperature and then poured into ice–water (1 l). The precipitated solid was dissolved in ether and the solution washed with 2M-sodium carbonate and water, dried, and concentrated. The product which crystallised from the solution (82 g in two crops) was recrystallised from cyclohexane–ether; *N*-acetyl α-benzylphenylalanine ethyl ester so obtained (78 g, 75%) had m.p. 121—123° (lit.,[8] 124—125°); the average yield from several preparations was 72%; the quality of the trichloroacetic acid is important. Hydrolysis by the method of Felkin [8] gave α-benzylphenylalanine, m.p. 307—308° (lit.,[17] 307—308°) (Found: N, 5·4. Calc. for $C_{16}H_{17}NO_2$: N, 5·5%) in 85—95% yield; the hydrochloride, obtained by rapid addition of 6M-hydrochloric acid to a hot solution of the acid in 2M-sodium hydroxide had m.p. 314° (lit.,[8] 314°) (Found: N, 4·6. Calc. for $C_{16}H_{18}ClNO_2$: N, 4·8%).

A suspension of the hydrochloride (15 g, 51 mmol) in ethanol (300 ml) was saturated with dry hydrogen chloride and then refluxed for 3 h. Evaporation and two repetitions of this procedure gave a solid residue, which was extracted with hot chloroform, leaving undissolved starting material (5·2 g, 35%). Addition of ether to the chloroform solution, followed by two recrystallisations of the precipitate from chloroform–ether, gave *α-benzylphenylalanine ethyl ester hydrochloride* (9·6 g, 58%), m.p. 174—175° (Found: C, 67·8; H, 6·9; N, 4·4. $C_{18}H_{22}ClNO_2$ requires C, 67·6; H, 6·9; N, 4·4%).

o-Nitrophenylsulphenyl chloride (10·5 g, 55 mmol) and 2M-sodium hydroxide were added alternately in small portions over 20 min to a stirred solution of α-benzylphenylalanine (12·75 g, 50 mmol) in 2M-sodium hydroxide (25 ml) and dioxan (62·5 ml), the pH being kept at about 8·5 during the reaction. The solution was diluted with water (500 ml) and filtered. The filtrate was acidified (Congo Red) with 0·5M-sulphuric acid and the gummy precipitate taken up in ethyl acetate, washed with water, and dried. The resulting solution was concentrated to 250 ml and treated with dicyclohexylamine (10 ml, 50 mmol). After 18 h at 0°, the yellow solid which had separated was filtered off and recrystallised from ethanol, giving N-o-*nitrophenylsulphenyl-α-benzylphenylalanine dicyclohexylammonium salt* (25·2 g, 86%), m.p. 198—199° (Found: C, 69·3; H, 7·4; N, 7·5. $C_{34}H_{43}N_3O_4S$ requires C, 69·2; H, 7·4; N, 7·1%).

The foregoing salt (5·9 g, 10 mmol) was shaken with ethyl acetate (30 ml) and 0·25M-sulphuric acid (50 ml). The ethyl acetate solution was separated, washed with water, dried, and evaporated. The residue, dried in a vacuum desiccator, was heated at 70° for 24 h with triethylamine (2·42 ml, 17·5 mmol) and 2-chloroethyl methyl sulphide (1·65 g, 15 mmol); triethylamine (1·38 ml, 10 mmol) and 2-chloroethyl methyl sulphide (1·1 g, 10 mmol) were then added and the mixture was heated for a further 36 h. More amine (0·69 ml, 5 mmol) and sulphide (0·55 g, 5 mmol) were then added. After being heated for a further 36 h, the product was dissolved in ethyl acetate; the solution was washed successively with water, 0·5M-sulphuric acid, saturated sodium hydrogen carbonate, and water, dried, and evaporated. The residue (4·0 g), dissolved in a mixture of anhydrous ethyl acetate (18 ml) and ether (18 ml), was treated with a freshly prepared solution of anhydrous hydrogen chloride (25 mmol) in ethyl acetate (7·5 ml), followed after 2 min by anhydrous ether (180 ml). Evaporation to dryness, trituration of the residue with ether, and recrystallisation from ethyl acetate gave *α-benzylphenylalanine 2-methylthioethyl ester hydrochloride* (2·4 g, 65%), m.p. 154—155° (Found: C, 62·1; H, 6·8; N, 3·9. $C_{19}H_{24}ClNO_2S$ requires C, 62·4; H, 6·6; N, 3·8%).

N-Hydroxysuccinimide (0·50 g, 4·35 mmol) was added to a solution of *N-o*-nitrophenylsulphenyl-α-benzylphenylalanine [from the dicyclohexylammonium salt (2·56 g, 4·35 mmol)] in dioxan (20 ml). A solution of dicyclohexylcarbodi-imide (0·90 g, 4·36 mmol) was added and the mixture shaken at room temperature for 18 h. Dicyclohexylurea was then filtered off, the filtrate evaporated, and the residue kept overnight at 0° in a little ethyl acetate. Filtration, evaporation of the filtrate, and recrystallisation of the residue from propan-2-ol–light petroleum gave N-o-*nitrophenylsulphenyl-α-benzylphenylalanine N-succinimidyl ester* (1·90 g, 87%), m.p. 73—75° (Found: C, 61·1; H, 4·8; N, 7·9. $C_{26}H_{23}N_3O_6S$ requires C, 61·8; H, 4·6; N, 8·3%); this ester is dimorphic, the more *stable form*, m.p. 187°, being obtained by recrystallisation from acetone (Found: C, 61·6; H, 4·6; N, 8·1%). This ester (1·67 g, 3·3 mmol) was kept for 30 s with hydrogen chloride (9·9 mmol) in

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[14] P. Mamalis and H. N. Rydon, *J. Chem. Soc.*, 1955, 1049.
[15] *Cf.* L. Tailleur and L. Berlinguet, *Canad. J. Chem.*, 1961, **39**, 1309; *J. Org. Chem.*, 1962, **27**, 653.
[16] H. N. Rydon and P. W. G. Smith, *Nature*, 1952, **169**, 922.
[17] L. H. Goodson, I. L. Honigberg, J. J. Lehman, and W. H. Burton, *J. Org. Chem.*, 1960, **25**, 1920.

dioxan (23 ml). Ether (100 ml) was then added and the precipitated solid collected and recrystallised from chloroform–ether, giving *α-benzylphenylalanine* N-*succinimidyl ester hydrochloride* (1·11 g, 87%), m.p. 151—152° (Found: C, 58·8; H, 5·3; N, 6·9. $C_{20}H_{21}ClN_2O_4,H_2O$ requires C, 59·0; H, 5·7; N, 6·9%).

Peptides of α-Benzylphenylalanine

ω-Nitro-L-arginine *p*-nitrobenzyl ester hydrobromide [18] (720 mg, 1·65 mmol) and *N-o*-nitrophenylsulphenyl-α-benzylphenylalanine dicyclohexylammonium salt (975 mg, 1·65 mmol) were shaken together in chloroform (60 ml) for 2·5 h. Dicyclohexylcarbodi-imide (375 mg, 1·82 mmol) in chloroform (10 ml) was then added and the mixture shaken overnight. A drop of acetic acid was added and the precipitated dicyclohexylurea was filtered off. The filtrate was washed successively with water, M-hydrochloric acid, water, saturated sodium hydrogen carbonate solution, and water, dried, and evaporated. The residue was taken up in a little acetone, kept overnight at 0°, and filtered from a little more urea. Evaporation of the filtrate and recrystallisation of the residue from ethanol gave N-o-*nitrophenylsulphenyl-α-benzylphenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester* (I) (870 mg, 71%), m.p. 99—102°, $[\alpha]_D^{19}$ −10·0° (*c* 1·0 in EtOAc) (Found: C, 56·3; H, 5·3; N, 14·9. $C_{35}H_{36}N_8O_9S$ requires C, 56·4; H, 4·9; N, 15·1%). This ester (1·4 g, 1·9 mmol) was kept for 1·5 min with hydrogen chloride (5·6 mmol) in dioxan (23 ml). Addition of ether (50 ml), filtration after 30 min at 0°, and trituration with ethyl acetate and ether, followed by recrystallisation from ethanol–ether gave *α-benzylphenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester hydrochloride* (760 mg, 64%), m.p. 213—215°, $[\alpha]_D^{19}$ −16·0° (*c* 1·0 in $HCO{\cdot}NMe_2$) (Found: C, 55·7; H, 5·5; N, 15·5. $C_{29}H_{34}ClN_7O_7$ requires C, 55·5; H, 5·5; N, 15·6%).

N-o-Nitrophenylsulphenyl-α-benzylphenylalanine dicyclohexylammonium salt (850 mg, 1·44 mmol) and L-seryl-L-prolyl-L-phenylalanyl-ω-nitro-L-arginine *p*-nitrobenzyl ester hydrochloride (p. 2638) (1·04 g, 1·44 mmol) were shaken for 2 h in acetonitrile (20 ml). Dicyclohexylcarbodi-imide (320 mg, 1·51 mmol) in acetonitrile (6 ml) was then added and shaking was continued overnight. Work-up as usual, with sodium carbonate instead of sodium hydrogen carbonate for the alkaline wash, followed by precipitation from ethyl acetate with ether, gave N-o-*nitrophenylsulphenyl-α-benzylphenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester* (II) (1·1 g, 71%), m.p. 124—126°, $[\alpha]_D^{25}$ −13·0° (*c* 1·0 in $CHCl_3$) (Found: C, 57·3; H, 5·5; N, 14·4. $C_{52}H_{57}N_{11}O_{13}S$ requires C, 58·0; H, 5·3; N, 14·3%). This ester (600 mg, 0·56 mmol) was kept for 30 s with hydrogen chloride (1·67 mmol) in ethyl acetate (15 ml). Addition of ether (80 ml) and reprecipitation from ethanol–ethyl acetate with ether gave *α-benzylphenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester hydrochloride* (470 mg, 87%), m.p. 155—157°, $[\alpha]_D^{25}$ −44·6° (*c* 0·6 in $HCO{\cdot}NMe_2$) (Found: C, 55·8; H, 5·7; N, 14·4. $C_{46}H_{55}ClN_{10}O_{11},2H_2O$ requires C, 55·5; H, 6·0; N, 14·1%).

Dicyclohexylcarbodi-imide (3·45 g, 16·5 mmol) was added in small quantities to a solution prepared from *N*-benzyloxycarbonylglycine [19] (3·135 g, 15 mmol), triethylamine (2·1 ml, 15 mmol), α-benzylphenylalanine ethyl ester hydrochloride (4·80 g, 15 mmol), and chloroform (105 ml). The mixture was shaken at room temperature for 24 h and then worked up in the usual manner. Recrystallisation from ethyl acetate–light petroleum gave N-*benzyloxycarbonylglycyl-α-benzylphenylalanine ethyl ester* (III; R = Et) (5·36 g, 75%), m.p. 81—82° (Found: C, 70·4; H, 6·5; N, 5·8. $C_{28}H_{30}N_2O_5$ requires C, 70·9; H, 6·4; N, 5·9%). *N*-Benzyloxycarbonyl-L-prolyl-α-benzylphenylalanine ethyl ester (IV), prepared similarly in 70% yield as an uncrystallisable gum, was hydrogenated for 4 h at room temperature over 5% palladised charcoal (500 mg) in 95% aqueous t-butyl alcohol (25 ml). Filtration, evaporation, treatment with ethereal hydrogen chloride, and recrystallisation from ethanol–ether–light petroleum gave L-*prolyl-α-benzylphenylalanine ethyl ester hydrochloride* (0·4 g, 50%), m.p. 176°, $[\alpha]_D^{19}$ +24·0° (*c* 1·0 in EtOH) (Found: C, 65·9; H, 6·9; N, 6·7. $C_{23}H_{29}ClN_2O_3$ requires C, 66·2; H, 7·0; N, 6·7%).

Dicyclohexylcarbodi-imide (650 mg, 3·15 mmol) in acetonitrile (5 ml) was added to a solution prepared from *N*-benzyloxycarbonylglycine (630 mg, 3·0 mmol), α-benzylphenylalanine 2-methylthioethyl ester hydrochloride (1100 mg, 3·0 mmol), and triethylamine (0·415 ml, 3·0 mmol) in acetonitrile (10 ml). After 18 h at room temperature the mixture was worked up as usual and the product, dissolved in acetone, was shaken with Zeo-Karb (H^+ form; 2 × 3·0 g) for 18 h. Evaporation of the filtrate gave the protected peptide (III; R = $CH_2{\cdot}CH_2{\cdot}SMe$) as a gum (1·41 g, 90%), which could not be induced to crystallise. This was refluxed with methyl iodide (2 ml, 32·5 mmol) for 3 h; more methyl iodide (2 ml) was then added, followed by a further 1 ml after 12 h. After being refluxed for another 3 h, the solution was evaporated and the residue triturated with ether. Recrystallisation from acetone gave N-*benzyloxycarbonylglycyl-α-benzylphenylalanine 2-methylthioethyl ester methiodide* (1·25 g, 70%), m.p. 104—105° (Found: N, 3·9. $C_{30}H_{35}IN_2O_5S$ requires N, 4·2%). The unrecrystallised methiodide (5·8 g, 11·1 mmol), dissolved in 25% aqueous acetone (20 ml), was titrated with 0·5M-sodium hydroxide at pH 10 for 45 min; consumption of alkali then ceased. Filtration, acidification with 0·25M-sulphuric acid, and recrystallisation of the gummy precipitate from aqueous ethanol gave N-*benzyloxycarbonylglycyl-α-benzylphenylalanine* (III; R = H) (2·73 g, 54% overall), m.p. 171—172° (Found: C, 70·1; H, 6·0; N, 6·2. $C_{26}H_{26}N_2O_5$ requires C, 69·9; H, 5·9; N, 6·3%).

N-o-Nitrophenylsulphenyl-L-proline dicyclohexylammonium salt [9] (1·0 g, 2·22 mmol) and α-benzylphenylalanyl-ω-nitro-L-arginine *p*-nitrobenzyl ester hydrochloride (1·4 g, 2·22 mmol) were shaken together in chloroform (30 ml) for 1 h. Dicyclohexylcarbodi-imide (0·51 g, 2·44 mmol) in chloroform (10 ml) was added and the mixture shaken for 60 h. The precipitated dicyclohexylurea was filtered off and the filtrate evaporated to dryness. The residue was taken up in ethyl acetate, washed as usual, and concentrated to 10 ml. After 12 h at 5°, the crystalline precipitate was filtered off and recrystallised from ethanol, affording NN′-*dicyclohexyl*-N-(N-o-*nitrophenylsulphenyl*)-L-*prolylurea* (0·46 g, 44%), m.p. 193—194° (Found: C, 60·8; H, 7·1; N, 11·8. $C_{24}H_{34}N_4O_4S$ requires C, 60·7; H, 7·2; N, 11·8%). Addition of ether to the ethyl acetate mother-liquor gave the protected dipeptide mixed with the original amino-component. Repeated washing with M-sulphuric acid, followed by aqueous 3% sodium carbonate, and recrystallisation from ethyl acetate–ether gave N-o-*nitrophenylsulphenyl-L-prolyl-α-benzylphenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester* (V), m.p. 114—116° (Found: C, 56·7; H, 5·4. $C_{40}H_{43}N_9O_{10}S$ requires C, 57·1; H, 5·2%) in low yield.

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Peptides related to Bradykinin

N-o-Nitrophenylsulphenyl-L-phenylalanine dicyclohexylammonium salt [9] (3·95 g, 7·9 mmol) and ω-nitro-L-arginine *p*-nitrobenzyl ester hydrobromide [18] (3·43 g, 7·9 mmol) were coupled in acetonitrile (110 ml) with the aid of dicyclohexylcarbodi-imide (1·78 g, 8·65 mmol). Work-up as usual gave N-o-*nitrophenylsulphenyl-L-phenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester* (4·4 g, 85%); a sample recrystallised from ethanol (70% recovery) had m.p. 94—96°, $[\alpha]_D^{25}$ −23·5° (*c* 1·0 in $CHCl_3$) (Found: C, 51·5; H, 4·9. $C_{28}H_{30}N_8O_9S$ requires C, 51·4; H, 4·6%). The crude ester (8·3 g, 12·7 mmol) was kept for 1 min with hydrogen chloride (38 mmol) in ethyl acetate (30 ml). Addition of ether (200 ml), reprecipitation from ethanol–ethyl acetate with ether, and recrystallisation from ethanol–ether gave L-*phenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester hydrochloride* (3·97 g, 58%), m.p. 197—198°, $[\alpha]_D^{25}$ −6·0° (*c* 1·0 in H_2O) (Found: C, 49·0; H, 5·2; N, 18·2. $C_{22}H_{28}ClN_7O_7$ requires C, 49·1; H, 5·3; N, 18·2%). Repetition of these procedures with the appropriate carboxy-components (Scheme 1) gave the following: N-o-*nitrophenylsulphenyl-L-prolyl-L-phenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester* (75%), m.p. 112—115° (from ethyl acetate–ether), $[\alpha]_D^{25}$ −47·0° (*c* 1·0 in $CHCl_3$) (Found: N, 16·8. $C_{33}H_{37}N_9O_{10}S$ requires M, 17·0%); L-*prolyl-L-phenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester hydrochloride* (70%), m.p. 137—139° (from ethanol–ethyl acetate), $[\alpha]_D^{25}$ −17·6° (*c* 1·0 in $HCO{\cdot}NMe_2$) (Found: C, 50·2; H, 5·4; N, 17·4. $C_{27}H_{35}ClN_8O_8$ requires C, 50·4; H, 5·6; N, 17·7%); N-o-*nitrophenylsulphenyl-L-seryl-L-prolyl-L-phenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester* (50%), m.p. 104—108° (from chloroform–ether), $[\alpha]_D^{25}$ −77·3° (*c* 1·0 in $CHCl_3$) (Found: C, 50·9; H, 4·9; N, 16·8. $C_{36}H_{42}N_{10}O_{12}S$ requires C, 51·5; H, 5·1; N, 16·7%); L-*seryl-L-prolyl-L-phenylalanyl-ω-nitro-L-arginine* p-*nitrobenzyl ester hydrochloride* (84%), m.p. 139—142° (from ethanol–ethyl acetate–ether), $[\alpha]_D^{25}$ −33·0° (*c* 1·0 in $HCO{\cdot}NMe_2$) (Found: C, 48·9; H, 5·6; N, 17·0. $C_{30}H_{40}ClN_9O_{10},H_2O$ requires C, 48·7; H, 5·7; N, 17·0%).

The following (Scheme 2) were prepared similarly: N-o-*nitrophenylsulphenyl-L-prolylglycine ethyl ester* (89%), m.p. 101—103° (from ethyl acetate–light petroleum), $[\alpha]_D^{19}$ −17·0° (*c* 1·0 in EtOAc) (Found: C, 51·2; H, 5·4; N, 11·9. $C_{15}H_{19}N_3O_5S$ requires C, 51·0; H, 5·4; N, 11·9%); L-prolylglycine ethyl ester hydrochloride (90%), m.p. 115° (from ethanol–ether), $[\alpha]_D^{19}$ −45·8° (*c* 2·4 in H_2O) (lit.,[20] m.p. 119—120°, $[\alpha]_D^{22}$ −39·5°); N-o-*nitrophenylsulphenyl-L-prolyl-L-prolylglycine ethyl ester* (90%), m.p. 48—51° (from benzene–light petroleum), $[\alpha]_D^{19}$ −140° (*c* 1·0 in EtOAc) (Found: C, 53·8; H, 5·8; N, 12·1. $C_{20}H_{26}N_4O_6S$ requires C, 53·3; H, 5·8; N, 12·4%); L-*prolyl-L-prolylglycine ethyl ester hydrochloride* (88%), m.p. *ca.* 85° (hygroscopic) (from ethanol–ether) (Found: N, 12·5. $C_{14}H_{24}ClN_3O_4$ requires N, 12·6%); N-*benzyloxycarbonyl-ω-nitro-L-arginyl-L-prolyl-L-prolylglycine ethyl ester* (VII) (45%), m.p. 84° (from ethyl acetate–ethyl–light petroleum), $[\alpha]_D^{19}$ −38·5° (*c* 1·0 in EtOAc) (Found: C, 53·6; H, 6·5; N, 17·5. $C_{28}H_{40}N_8O_9$ requires C, 53·1; H, 6·4; N, 17·7%).

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