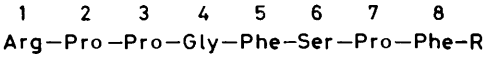
Amino-acids and Peptides. Part 46.[1] Synthesis of Bradykinin Analogues Modified in the Vicinity of the Carboxy-group

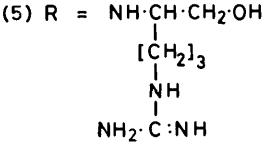
By **Jan Christiansen** and **Geoffrey T. Young,*** The Dyson Perrins Laboratory, Oxford University, Oxford OX1 3QY
Appendix by **Norman G. Bowery**, Department of Pharmacology, St. Thomas's Hospital Medical School, London SE1 7EH

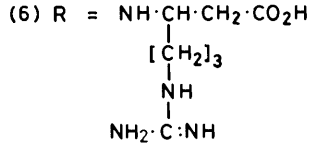
In order to investigate further the effect on biological activity of structural changes in the vicinity of the terminal carboxy-group, the following analogues of bradykinin have been synthesised by the picolyl ester method: bradykinyl-L-isoleucine (2), -L-alanine (3), and -β-alanine (4); [9-L-argininol]-bradykinin (5), [9-*O*-acetyl-L-argininol]-bradykinin (7), [9-β-homoarginine]-bradykinin (6), and bradykinin heptyl ester (8). The biological activities of these analogues on the isolated guinea-pig ileum and rat uterus are reported; the results confirm the marked reduction in activity caused by structural changes in this area.

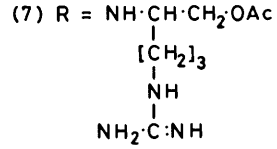
In earlier Parts of this series [2-5] we have reported investigations of the effect of modifications of the guanidino side-chains of the two arginine residues of the local tissue hormone bradykinin (1),† and of modifications of the terminal amino-group, on its activity on smooth muscle. We have also shown that it is unnecessary to



(1) R = Arg (2) R = Arg-Ile (3) R = Arg-Ala (4) R = Arg-β-Ala







(8) R = Arg-O[CH$_2$]$_6$CH$_3$

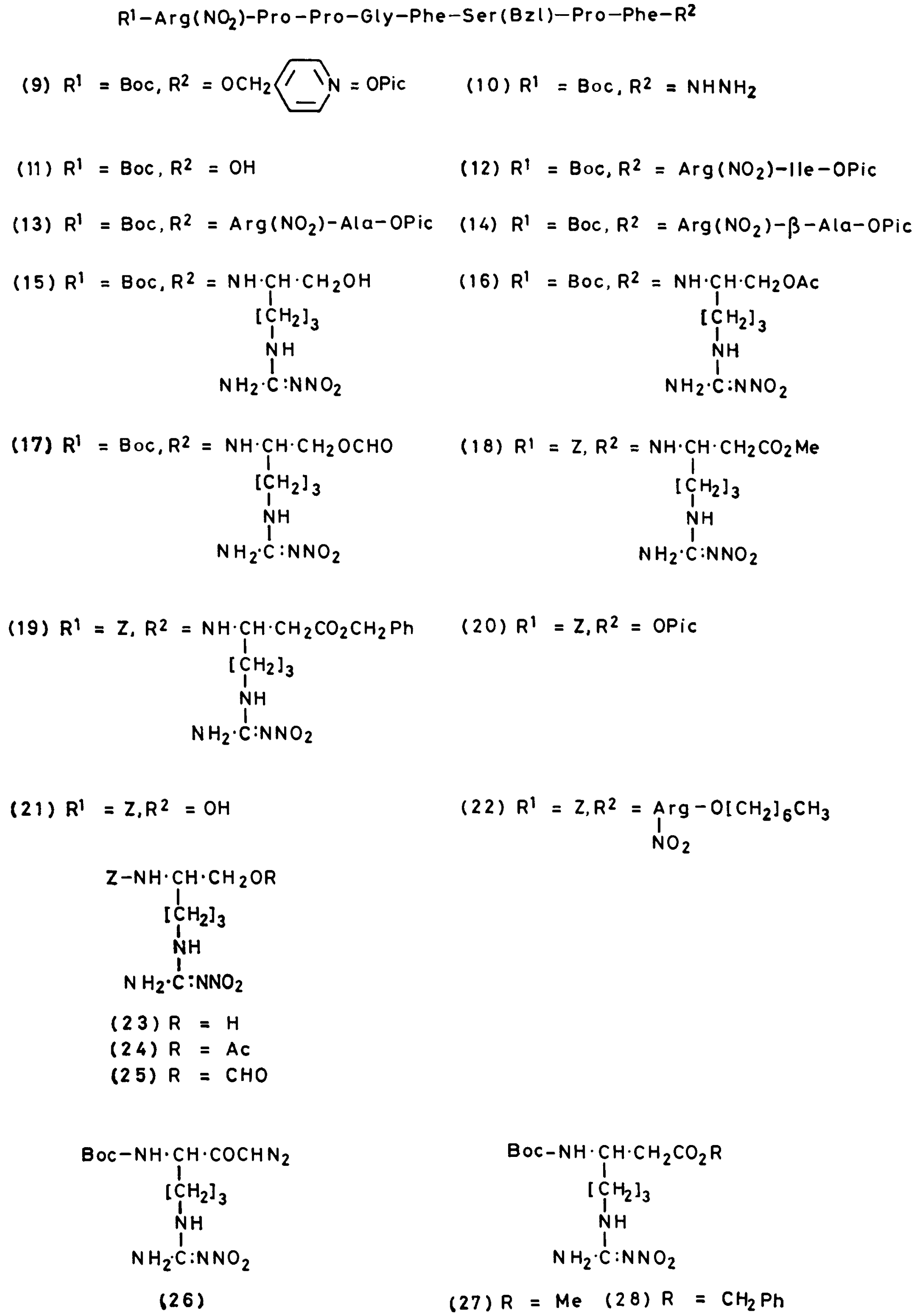
have phenyl groups in positions 5 and 8, since β-cyclohexylalanine can replace either or both of the phenylalanine residues without diminishing the activity. In brief, the very large volume of work reported in the literature (for a review see ref. 6) shows that, of the functional groups in bradykinin, the terminal amino-group [7] and the serine hydroxy [8] are not needed, but the two guanidino-groups are important [2,9] and so also is the terminal carboxy-group.[10] In the rat uterus test, bradykinin amide has only 1% of the activity of bradykinin itself,[11] the de-carboxy derivative has 0.25—0.5% [7] and bradykinyl-glycine and -L-arginine 0.7% and 4% respectively.[10] Bradykinyl-L-alanine has been reported previously [12] but without full chemical characterisation; the relative affinity compared with bradykinin on the cat ileum was 1.9%. However, phyllokinin (bradykinyl-L-isoleucyl-L-tyrosine-*O*-sulphate), isolated from the skin of the Brazilian amphibian *Phyllomedusa rohdei*, has 30—40% of the activity of bradykinin on the rat uterus and 25—40% on the guinea-pig ileum.[13] It should be noted that in this paper biological activity refers to the effect on the isolated rat uterus or guinea-pig ileum, as distinct from that on rabbit aorta strips described by Regoli, Barabé, and Park,[14] for which the structural requirements appear to be quite different.

We have therefore explored further the effect of structural changes in the vicinity of the terminal carboxy-group; a preliminary communication has been made.[15] Bradykinyl-L-isoleucine (2), -L-alanine (3) and -β-alanine (4) were synthesised *via* the protected octapeptide 4-picolyl ester (9), which was prepared in the usual stepwise fashion by the picolyl ester method; [4] it was converted into the hydrazide (10) and acid azide, which was then condensed with the 4-picolyl esters of nitro-L-

† Abbreviations follow the I.U.P.A.C.–I.U.B. rules, reprinted in the Chemical Society Specialist Periodical Report 'Amino-acids, Peptides, and Proteins,' ed. G. T. Young, The Chemical Society, 1972, vol. 4, p. 441. Pic = 4-picolyl. Chiral amino-acids are of the L-configuration.

arginyl-L-isoleucine, nitro-L-arginyl-L-alanine, and nitro-L-arginyl-β-alanine giving protected decapeptides (12), (13), and (14). Deprotection (trifluoroacetic acid and then hydrogenolysis) gave the required decapeptides (2), (3), and (4). Compounds (2) and (3) were completely hydrolysed by a mixture of microsomal leucine aminopeptidase and prolidase. In an alternative synthesis of analogue (2), the ester (9) was hydrolysed to the acid (11) and this was coupled to nitro-L-arginyl-L-isoleucine 4-picolyl ester by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole; the protected product (12) was not distinguishable from that prepared by the acid azide coupling.

None of these three analogues, (2), (3), and (4), had



R1–Arg(NO2)–Pro–Pro–Gly–Phe–Ser(Bzl)–Pro–Phe–R2
(9) R1 = Boc, R2 = OCH2 … N = OPic
(10) R1 = Boc, R2 = NHNH2
(11) R1 = Boc, R2 = OH
(12) R1 = Boc, R2 = Arg(NO2)–Ile–OPic
(13) R1 = Boc, R2 = Arg(NO2)–Ala–OPic
(14) R1 = Boc, R2 = Arg(NO2)–β–Ala–OPic
(15) R1 = Boc, R2 = NH·CH·CH2OH
(16) R1 = Boc, R2 = NH·CH·CH2OAc
(17) R1 = Boc, R2 = NH·CH·CH2OCHO
(18) R1 = Z, R2 = NH·CH·CH2CO2Me
(19) R1 = Z, R2 = NH·CH·CH2CO2CH2Ph
(20) R1 = Z, R2 = OPic
(21) R1 = Z, R2 = OH
(22) R1 = Z, R2 = Arg–O[CH2]6CH3
Z–NH·CH·CH2OR
(23) R = H
(24) R = Ac
(25) R = CHO
Boc–NH·CH·COCHN2
(26)
Boc–NH·CH·CH2CO2R
(27) R = Me (28) R = CH2Ph

significant activity (0.5, 0.2, and 0.1% of bradykinin respectively; see Appendix) in the isolated guinea-pig ileum test, confirming the restriction on extension at this end of the molecule. We therefore turned to modifications of the carboxy-group involving little or no increase in size, specifically the 9-argininol analogue (5) and the 9-β-homoarginine analogue (6); we also made the *O*-acetyl derivative (7) and, because of reports that arginine heptyl ester [16] and D-prolyl-L-phenylalanyl-L-arginine heptyl ester [17] are bradykinin antagonists, we made bradykinin heptyl ester (8).

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginine methyl ester was reduced by sodium borohydride to N^{2}-benzyloxycarbonyl-N^{ω}-nitro-L-argininol (23), and from this the *O*-acetyl and *O*-formyl derivatives (24) and (25) respectively were prepared. The α-amino-protection was removed from compounds (23) and (24) by hydrogen bromide in acetic acid and the products were coupled to the acid azide derived from the protected octapeptide hydrazide (10). Treatment with trifluoroacetic acid followed by hydrogenolysis gave bradykinin analogues (5) and (7). The formyl derivative (17) was prepared by direct formylation of the protected argininol nonapeptide (15). However, the formyl group proved to be labile during the deprotection steps and the free nonapeptide with *O*-formylargininol in position 9 was not prepared. The diazoketone (26) was prepared by the action of diazomethane on the carbonic mixed anhydride derived from N^{α}-t-butoxycarbonyl-N^{ω}-nitro-L-arginine.[18,19] The Wolff rearrangement in methanol gave the methyl ester (27) of the protected (3*S*)-β-homoarginine; rearrangement in the presence of benzyl alcohol gave the benzyl ester (28). The protected octapeptide 4-picolyl ester (20) was hydrolysed to the acid (21) and this was coupled as usual to the amino-components derived from the β-homoarginine methyl and benzyl esters (27) and (28), giving protected nonapeptides (18) and (19) respectively. Alkaline hydrolysis of the methyl ester (18) proved to be slow, but hydrogenolysis of the protected nonapeptide benzyl ester (19) proceeded smoothly,

TABLE 1

Synthesis of Boc-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-OPic: protected peptide intermediates

(29) Boc-Pro-Phe-OPic
(30) Boc-Ser(Bzl)-Pro-Phe-OPic
(31) Boc-Phe-Ser(Bzl)-Pro-Phe-OPic
(32) Boc-Gly-Phe-Ser(Bzl)-Pro-Phe-OPic
(33) Boc-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-OPic
(34) Boc-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-OPic
(9) Boc-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-OPic

Compound [a]	Amino-component [b] (mmol)	Acylating component [c] (mmol)	Isolation [d]	Yield [e] (%)	$[\alpha]_D^{20}$ (°) [f]	R_F (t.l.c.)
(29)	Boc-Phe-OPic (5.0)	Boc-Pro (7.5)	A	81 [g]	−39.5	0.76(G3); 0.56(P1)
(30)	Compound (29) (5.0)	Boc-Ser(Bzl) (6.5)	A	82	−38	0.61(G3); 0.64(P1)
(31)	Compound (30) (35.3)	Boc-Phe (46.4)	*h*	79 [i]	−36	0.65(G3); 0.47(P1)
(32)	Compound (31) (26.2)	Boc-Gly (34.1)	*j*	89 [k]	−32	0.66(G3); 0.59(P1)
(33)	Compound (32) (10.0)	Boc-Pro (15.0)	*l*	88 [m]	−45	0.47(G3); 0.59(P1)
(34)	Compound (33) (2.9)	Boc-Pro (4.5)	A	80 [n]	−52	0.62(G3); 0.52(P1)
(9)	Compound (34) (1.8)	Boc-Arg(NO_2) (3.0)	A [o]	69 [p]	−58	0.80(G3); 0.33(P1)

Compound	Found (%) C	H	N	Formula	Required (%) C	H	N
(29)	66.3	6.8	9.0	$C_{25}H_{31}N_3O_5$	(66.2) [q]	(6.9)	(9.3)
(30)	65.6	6.6	8.7	$C_{35}H_{42}N_4O_7,\frac{1}{2}H_2O$	65.7	6.8	8.75
(31)	67.7	6.7	9.3	$C_{44}H_{51}N_5O_8$	67.9	6.6	9.0
(32)	66.0	6.7	10.0	$C_{46}H_{54}N_6O_9$	66.2	6.5	10.1
(33)	65.0	6.5	10.65	$C_{51}H_{61}N_7O_{10},\frac{1}{2}H_2O$	65.1	6.6	10.4
(34)	64.2	6.5	10.7	$C_{56}H_{68}N_8O_{11},H_2O$	64.2	6.7	10.7
(9)	59.4	6.4	14.45	$C_{62}H_{79}N_{13}O_{14},1\frac{1}{2}H_2O$	59.2	6.6	14.5

[a] All compounds excepting compound (29) are new. [b] The amino-component was prepared by the action of trifluoroacetic acid on the stated t-butoxycarbonyl derivative (see 'General Procedures for the Synthesis of Protected Peptides'). [c] Coupling was by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole (see 'General Procedures for the Synthesis of Protected Peptides'). [d] A = Amberlyst method, using ethyl acetate as solvent for application of the product to the resin except when otherwise stated. [e] Yields are of product with the stated constants and analysis. [f] Optical rotations were measured in dimethylformamide (*c* 1). [g] M.p. 102—104 °C. R. Camble, R. Garner, and G. T. Young, *J. Chem. Soc. C*, 1969, 1911 reported m.p. 102—103 °C. [h] The product (m.p. 141—143 °C) crystallised from ethyl acetate solution, before application to the Amberlyst resin, and was therefore collected at that stage. [i] Found after acid hydrolysis: Ser, 0.82; Pro, 1.03; Phe, 1.96. [j] The product solidified after evaporation of the solution prior to application to the Amberlyst resin and was collected and recrystallised from ethyl acetate–light petroleum; m.p. 129—131 °C. [k] Found after acid hydrolysis: Ser, 0.80; Pro, 1.00; Gly, 1.02; Phe, 1.98. [l] The product solidified on trituration with ether prior to application to the Amberlyst resin and was collected at that stage. [m] Found after acid hydrolysis: Ser, 0.75; Pro, 1.98; Gly, 1.01; Phe, 2.00. [n] Found after acid hydrolysis: Ser, 0.79; Pro, 3.02; Gly, 1.03; Phe, 1.94. [o] The product was applied to the Amberlyst resin in dichloromethane. [p] Found after acid hydrolysis: Arg, 0.82; Orn, 0.21; Ser, 0.78; Pro, 3.00; Gly, 1.02; Phe, 1.97.

giving the free nonapeptide (6). Coupling of the protected octapeptide acid (21) to nitro-L-arginine heptyl ester gave protected nonapeptide heptyl ester (22), which on hydrogenolysis gave bradykinin heptyl ester (8).

The importance of the terminal carboxy-group is confirmed by low activity of the 9-argininol analogue (5) (0.5%) in the guinea-pig ileum test; its *O*-acetyl derivative (7) had scarcely detectable activity (0.02%). Surprisingly, bradykinin heptyl ester (8) had 13.6% (±6%) activity in this test and 12.7% (±7%) in the rat uterus test, but some at least of this activity may be due to enzymic hydrolysis in the tissues, giving bradykinin itself. This possibility is supported by experiments in which solutions of the heptyl ester (8) were incubated at 20 °C with samples of intestinal muscle immediately before the assay; the contractile activity was thereby increased, up to a maximum reached after 4.5—13.5 min incubation (see Appendix). The 9-β-homoarginine analogue (6) had significant activity (6%) on the guinea-pig ileum but markedly less (1%) on the rat uterus. Details of the pharmacological assays are given in the Appendix.

EXPERIMENTAL

T.l.c. was on Merck Kieselgel-60 F-254 plates, using the following solvents (proportions are by volume); (A5), n-butanol–acetic acid–water, 2 : 1 : 1; (E4), chloroform–methanol, 9 : 1; (G3), pyridine–acetic acid–water (20 : 6 : 11, 1 part) and ethyl acetate (3 parts); (G4), as for (G3) but 4 parts and 1 part of the components respectively; (H), η-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 12); (P1) chloroform–methanol–acetic acid (17 : 2 : 1); (P2) as for (P1) but in proportions 10 : 2 : 1. M.p.s were determined with a Kofler hot-stage apparatus. Evaporation was by rotary evaporator at reduced pressure below 35 °C. Optical rotations were measured on a Perkin-Elmer 141 automatic polarimeter (1 dm cell). Samples for amino-acid analysis (JEOL JLC-5AH analyser) were hydrolysed in 6M-hydrochloric acid containing 0.5% (w/v) phenol at 110 °C during 18—22 h. Electrophoresis was on a Camag apparatus at 600 V, on cellulose-coated glass plates. Column chromatography was monitored by the u.v. absorption at 254 nm and simultaneously by polarimetry (usually at 436 nm) using a 1 dm microcell in the Perkin-Elmer 141 polarimeter, coupled with a 2-pen recorder. Dimethylformamide was redistilled, and *N,N*-di-isopropylethylamine was dried over potassium hydroxide and distilled immediately before use.

General Procedures for the Synthesis of Protected Peptides.—(1) *Removal of the* N$^{\alpha}$*-t-butoxycarbonyl group and liberation of the amino-component.* The t-butoxycarbonyl-amino-acid or -peptide was dissolved in trifluoroacetic acid (2—5 ml per mmol) at 0 °C. 5 Min after dissolution was complete the solution was evaporated to dryness (t.l.c. to confirm complete deprotection). The residue was triturated with ether and the crude trifluoroacetate salt was then dissolved in the coupling solvent (dimethylformamide) and the free amino-component was liberated by the addition of dried, freshly distilled *N,N*-di-isopropylethylamine until an apparent pH 8.5—9.0 (moist indicator paper) was obtained; the apparent pH was then reduced to 7 by addition of 1-hydroxybenzotriazole hydrate.

(2) *Coupling.* This was normally by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole hydrate (both equimolar to the carboxy component),[20] with 'pre-activation' for 1 h at 0 °C and 1 h at room temperature; in general, the proportion of acylating mixture to amino-component was 1.3 : 1, increased to 1.5 : 1 for hindered couplings and when arginine was the acylating amino-acid. The coupling of fragments was by the 'eintopf' procedure.[20] The apparent pH during coupling was 4.5—5.0. The progress of coupling reactions was followed by t.l.c. (solvents E4, P1 and P2 were especially useful). The reaction mixture was usually left overnight at 0 °C.

(3) *Isolation of coupled product.* This was normally by the Amberlyst procedure,[3] as described in Part 42.[21] The reaction mixture (solvent, dimethylformamide) was diluted with an equal volume of ethyl acetate, stirred for 1 h at 0 °C, dicyclohexylurea was filtered off, and the filtrate was evaporated to dryness. The residue was taken up in ethyl acetate or (if necessary) dichloromethane and washed (5% sodium hydrogen carbonate, water, brine), and dried. The solution was concentrated by evaporation and applied to the Amberlyst-15 resin (saturated with 3-bromopyridine and the same solvent) with gentle shaking; the resin was washed as a column and eluted with cooled pyridine (25%) in dimethylformamide as described in Part 42.[21]

t-Butoxycarbonyl-L-phenylalanine 4-Picolyl Ester.—t-Butoxycarbonyl-L-phenylalanine was esterified with 4-picolyl alcohol by means of dicyclohexylcarbodi-imide by the method of Pinker *et al.*,[4] giving crystalline *ester* (yield, 81%) of m.p. 92—94 °C, $[\alpha]_D^{20}$ −28° (*c* 0.99 in Me_2NCHO); R_F 0.63 (E4), 0.79 (G3) (Found: C, 67.25; H, 6.6; N, 8.05. $C_{20}H_{24}N_2O_4$ requires C, 67.4; H, 6.8; N, 7.9%).

N$^{\alpha}$*-t-Butoxycarbonyl-*N$^{\omega}$*-nitro-L-arginyl-L-prolyl-L-prolyl-glycyl-L-phenylalanyl-*O*-benzyl-L-seryl-L-prolyl-L-phenylalanylhydrazide* (10).—The protected octapeptide 4-picolyl ester (9) (550 mg, 0.437 mmol) in dimethylformamide (11 ml) reacted with hydrazine hydrate (0.220 ml, 4.53 mmol) at 0 °C during 8 h. The solution was evaporated, the residue was triturated with water, and dried in a desiccator, and then taken up in dimethylformamide (3 ml) and applied to a column (120 × 3.5 cm) of Sephadex LH-20. The product was precipitated from methanol by dried ether, giving *protected octapeptide hydrazide* (10) (400 mg, 78%) of $[\alpha]_D^{20}$ −68° (*c* 0.5 in Me_2NCHO); R_F 0.25 (G3), 0.46 (P1) (Found: C, 57.1; H, 6.6; N, 16.5. $C_{56}H_{76}N_{14}O_{13},1\frac{1}{2}H_2O$ requires C, 57.0; H, 6.75; N, 16.6%. Found after acid hydrolysis: Arg, 0.80; Orn, 0.24; Ser, 0.80; Pro, 3.00; Gly, 1.01; Phe, 2.00).

N$^{\alpha}$*-t-Butoxycarbonyl-*N$^{\omega}$*-nitro-L-arginyl-L-prolyl-L-prolyl-glycyl-L-phenylalanyl-*O*-benzyl-L-seryl-L-prolyl-L-phenylalanine* (11).—Hydrolysis of the protected octapeptide 4-picolyl ester (9) (221 mg, 0.176 mmol) in tetrahydrofuran–water (9 : 1; 4.5 ml) was effected by 1.0M-sodium hydroxide (0.2 ml) during 30 min at room temperature. The solution (brought to pH 5) was evaporated and the residue was partitioned between dichloromethane and water at pH 2.5. The organic layers were washed (water), dried, and evaporated. The residue was applied in dimethylformamide (2 ml) solution to a column (83 × 3 cm) of Bio-Beads S-X8, and the product was precipitated from methanol by dried ether, giving *protected octapeptide acid* (11) (144 mg, 70%) of $[\alpha]_D^{20}$ −51° (*c* 0.5 in Me_2NCHO); R_F 0.16 (G3), 0.42 (P1) (Found: C, 57.1; H, 6.6; N, 14.2. $C_{56}H_{74}N_{12}O_{14},2H_2O$ requires C, 57.2; H, 6.7; N, 14.3%. Found after acid hydrolysis: Arg, 0.83; Orn, 0.19; Ser, 0.75; Pro, 3.02; Gly, 0.96; Phe, 2.01).

t-*Butoxycarbonyl-β-alanine 4-Picolyl Ester.*—This ester

was prepared from t-butoxycarbonyl-β-alanine,[22] 4-picolyl alcohol, and dicyclohexylcarbodi-imide in dichloromethane by the method of Pinker, Young, Elliott, and Wade.[4] The *ester* (yield, 83%), which could not be crystallised, had R_F 0.62 (G3), 0.50 (P1) (Found: C, 60.3; H, 7.0; N, 9.6. $C_{14}H_{20}N_2O_4$ requires C, 60.0; H, 7.2; N, 10.0%).

Nα-t-Butoxycarbonyl-Nω-nitro-L-arginyl-L-isoleucine 4-Picolyl Ester.—*Nα*-t-Butoxycarbonyl-*Nω*-nitro-L-arginine (3.00 g, 9.4 mmol) was coupled to L-isoleucine 4-picolyl ester [generated *in situ* by addition of *N,N*-di-isopropylethylamine to the dihydrobromide (2.50 g, 6.5 mmol)] in dimethylformamide (35 ml) by the dicyclohexylcarbodiimide/1-hydroxybenzotriazole method.[20] The product was isolated on Amberlyst-15 (3-bromopyridinium form) using dichloromethane as solvent, giving *protected dipeptide* (2.38 g, 68%) of $[\alpha]_D^{20}$ −9° (*c* 1.0 in Me_2NCHO); R_F 0.55 (G3), 0.43 (P1) (Found: C, 51.2; H, 7.2; N, 18.5. $C_{23}H_{37}N_7O_7,\frac{3}{4}H_2O$ requires, C, 51.4; H, 7.2; N, 18.25%.

The following protected nitroarginyl peptides were prepared similarly:

Nα-t-Butoxycarbonyl-Nω-nitro-L-arginyl-L-alanine 4-Picolyl Ester.—L-Alanine 4-picolyl ester was prepared from the t-butoxycarbonyl derivative [1] by the action of trifluoroacetic acid and addition of *N,N*-di-isopropylethylamine to the trifluoroacetate so obtained. The *protected dipeptide* (73% yield) had $[\alpha]_D^{20}$ −13° (*c* 1.0 in Me_2NCHO); R_F 0.43 (G3), 0.30 (P1) (Found: C, 48.9; H, 6.5; N, 19.5. $C_{20}H_{31}N_7O_7,\frac{3}{4}H_2O$ requires C, 48.5; H, 6.6; N, 19.8%).

Nα-t-Butoxycarbonyl-Nω-nitro-L-arginyl-β-alanine 4-Picolyl Ester.—The amino-component was prepared as usual from t-butoxycarbonyl-β-alanine 4-picolyl ester (described above). The *protected dipeptide* (71% yield) had $[\alpha]_D^{20}$ −2.4° (*c* 1.0 in Me_2NCHO); R_F 0.58 (G3), 0.27 (P1) (Found: C, 46.9; H, 6.5; N, 19.2. $C_{20}H_{31}N_7O_7,1\frac{1}{2}H_2O$ requires C, 47.2; H, 6.7; N, 19.3%).

Nα-t-Butoxycarbonyl-Nω-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-benzyl-L-seryl-L-prolyl-L-phenylalanyl-Nω-nitro-L-arginyl-L-isoleucine 4-Picolyl Ester (12).—*Method A.* The protected octapeptide hydrazide (10) (169 mg, 0.143 mmol) in dimethylformamide was converted into the acid azide by the method of Honzl and Rudinger [23] using 4.85M-hydrogen chloride in ethyl acetate and t-butyl nitrite. Excess of acid was neutralised by addition of *N,N*-di-isopropylethylamine to the azide solution, which was then coupled with *Nω*-nitro-L-arginyl-L-isoleucine 4-picolyl ester [prepared from the t-butoxycarbonyl derivative (79 mg, 0.15 mmol) by the action of trifluoroacetic acid and liberated from the trifluoroacetate by *N,N*-di-isopropylethylamine in dimethylformamide] during 18 h at 0 °C, the pH being adjusted to 7.5 by addition of the tertiary amine. The product was recovered by the Amberlyst procedure (solvent, dichloromethane) and then passed down a column (120 × 3.5 cm) of Sephadex LH-20 (solvent, dimethylformamide), giving *protected decapeptide* (12) (122 mg, 54%) of $[\alpha]_D^{20}$ −50° (*c* 0.5 in Me_2NCHO); R_F 0.27 (G3), 0.44 (P1) (Found: C, 55.8; H, 6.6; N, 16.8. $C_{74}H_{101}N_{19}O_{18},2\frac{1}{2}H_2O$ requires C, 55.9; H, 6.7; N, 16.7%. Found after acid hydrolysis: Arg, 1.59; Orn, 0.37; Ser, 0.76; Pro, 3.00; Gly, 1.03; Ile, 0.98; Phe, 2.00).

Method B. The protected octapeptide acid (11) (140 mg, 0.122 mmol) was coupled with *Nω*-nitro-L-arginyl-L-isoleucine 4-picolyl ester [prepared as in Method A from 68 mg (0.127 mmol) of the t-butoxycarbonyl derivative] in dimethylformamide by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole overnight at 0 °C. The product was isolated as in Method A, giving the *decapeptide* (12) (129 mg, 67%) of $[\alpha]_D^{20}$ −48° (*c* 0.5 in Me_2NCHO), R_F 0.27 (G3), 0.43 (P1) (Found: C, 56.0; H, 6.7; N, 16.6. Found after acid hydrolysis: Arg, 1.60; Orn, 0.42; Ser, 0.80; Pro, 3.01; Gly, 1.01; Ile, 1.01; Phe, 1.98).

The following protected decapeptides were prepared from the protected octapeptide hydrazide (10) by Method A.

Nα-t-Butoxycarbonyl-Nω-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-benzyl-L-seryl-L-prolyl-L-phenylalanyl-Nω-nitro-L-arginyl-L-alanine 4-Picolyl Ester (13).—*Nα*-t-Butoxycarbonyl-*Nω*-nitro-L-arginyl-L-alanine 4-picolyl ester (75 mg, 0.15 mmol) provided the amino-component and the product was isolated using Amberlyst-15 (solvent, dichloromethane) and Sephadex LH-20 (solvent, dimethylformamide), giving *protected decapeptide* (13) (129 mg, 54%) of $[\alpha]_D^{20}$ −56° (*c* 0.5 in Me_2NCHO); R_F 0.20 (G3), 0.39 (P1) (Found: C, 54.9; H, 6.5; N, 17.2. $C_{71}H_{95}N_{19}O_{18},2\frac{1}{2}H_2O$ requires C, 55.1; H, 6.5; N, 17.2%. Found after acid hydrolysis: Arg, 1.61; Orn, 0.41; Ser, 0.77; Pro, 2.97; Gly, 1.01; Ala, 0.98; Phe, 2.01).

Nα-t-Butoxycarbonyl-Nω-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-benzyl-L-seryl-L-prolyl-L-phenylalanyl-Nω-nitro-L-arginyl-β-alanine 4-Picolyl Ester (14).—*Nα*-t-Butoxycarbonyl-*Nω*-nitro-L-arginyl-β-alanine 4-picolyl ester (145 mg, 0.285 mmol) provided the amino-component and the product was isolated using Amberlyst-15 (solvent, dichloromethane) and Sephadex LH-20 (solvent, dimethylformamide), giving *protected decapeptide* (14) (235 mg, 50%) of $[\alpha]_D^{20}$ −49° (*c* 0.5 in Me_2NCHO); R_F 0.16 (G3), 0.33 (P1) (Found: C, 55.2; H, 6.4; N, 17.15. $C_{71}H_{95}N_{19}O_{18},2\frac{1}{2}H_2O$ requires C, 55.1; H, 6.5; N, 17.2%. Found after acid hydrolysis: Arg, 1.53; Orn, 0.48; Ser, 0.80; Pro, 3.03; Gly, 0.99; Phe, 1.99; β-Ala, 0.98).

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginyl-L-isoleucine (2) (*Bradykinyl-L-isoleucine*) *Diacetate.*—The corresponding protected decapeptide 4-picolyl ester described above (86 mg, 0.054 mmol) was dissolved in trifluoroacetic acid and after 30 min the solution was evaporated; the residue was dissolved in 80% acetic acid (10 ml) and hydrogenolysed over palladium–charcoal (10%, 32 mg) during 18 h. After removal of the catalyst, trifluoroacetate anion was removed by means of Amberlite IR-45 (acetate form, 10 ml). The product was purified on a column (28 × 0.9 cm) of carboxymethylcellulose ion-exchanger CM 32, using gradient elution with triethylammonium acetate buffer from 0.05M, pH 5.0 to 0.60M, pH 7.0. After evaporation of the appropriate fractions, residual salts were removed on a Sephadex G-25 column (75 × 2.5 cm) with 5% acetic acid as solvent, giving *bradykinyl-isoleucine* (2) *diacetate* (39 mg, 52%) of $[\alpha]_D^{20}$ −83.5° (*c* 0.5 in H_2O); R_F 0.79 (G4); 0.37 (H); $E_{Arg}^{6\cdot4}$ 0.83 (Found: C, 51.4; H, 7.1; N, 16.1. $C_{60}H_{92}N_{16}O_{16},6H_2O$ requires C, 51.4; H, 7.5; N, 16.0. Found after acid hydrolysis: Arg, 2.04; Ser, 0.84; Pro, 2.98; Gly, 0.99; Ile, 0.97; Phe, 2.00. Found after hydrolysis by microsomal leucine aminopeptidase (Sigma) and prolidase (Sigma) in Tris-hydrochloride buffer at pH 7.7 and 37 °C for 24 h: Arg, 1.98; Pro, 3.24; Gly, 1.00; Phe, 1.91; Ser, 0.80; Ile, 1.07).

The following were prepared analogously from the corresponding protected decapeptide 4-picolyl esters: *L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginyl-L-alanine* (3) (*bradykinyl-L-alanine*) *diacetate*, yield 60%, $[\alpha]_D^{20}$ −74° (*c* 0.5 in H_2O); R_F 0.74 (G4); 0.27 (H); $E_{Arg}^{6\cdot4}$ 0.85 (Found: C, 51.15; H, 7.2; N, 17.0. $C_{57}H_{86}N_{16}O_{16},4\frac{1}{2}H_2O$ requires C, 51.4; H, 7.2; N,

16.8%. Found after acid hydrolysis: Arg, 1.97; Ser, 0.84; Pro, 3.02; Gly, 1.02; Ala, 0.99; Phe, 2.00. Found after enzymic hydrolysis: Arg, 2.03; Pro, 3.19; Gly, 0.97; Phe, 1.91; Ser, 0.82; Ala, 1.08). Park *et al.*[12] give $E_{Arg}^{2\cdot1}$ 0.84 but give no analyses for their preparation.

L-*Arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-L-*arginyl*-β-*alanine* (4) (*Bradykinyl*-β-*alanine*) *Diacetate*.—Yield, 46%, $[\alpha]_D^{20}$ −84° (*c* 0.5 in H_2O); R_F 0.79 (G4), 0.27 (H); $E_{Arg}^{6\cdot4}$ 0.83 (Found: C, 51.3; H, 6.9; N, 17.1. $C_{57}H_{86}N_{16}O_{16},4\frac{1}{2}H_2O$ requires C, 51.4; H, 7.2; N, 16.8%. Found after acid hydrolysis: Arg, 1.97; Ser, 0.87; Pro, 2.98; Gly, 1.00; Phe, 2.05; β-Ala, 1.02).

N^2-*Benzyloxycarbonyl*-N^{ω}-*nitro*-L-*argininol* (23).—This was prepared by a modification of the procedure of Ito, Takahashi, and Baba.[24] N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginine methyl ester[24] (2.58 g, 7.02 mmol) in isopropyl alcohol–tetrahydrofuran (1 : 1; 56 ml) at 0 °C was reduced by sodium borohydride (0.53 g, 14 mmol) added in portions. After the addition the temperature was allowed to rise to room temperature; the following day the excess of borohydride was destroyed by methanol, acetic acid was added to bring the solution to pH 5, and the solution was evaporated. The residue was chromatographed on a column of Kieselgel-60, with chloroform–methanol (4 : 1, v/v) as solvent. The product crystallised from chloroform and was recrystallised from tetrahydrofuran–ether, giving alcohol (23) (1.80 g, 76%) of m.p. 134—136 °C, $[\alpha]_D^{20}$ −17° (*c* 1.0 in Me_2NCHO); R_F 0.56 (G3), 0.25 (P1) (Found: C, 49.3; H, 6.2; N, 20.7. Calc. for $C_{14}H_{21}N_5O_5$: C, 49.55; H, 6.2; N, 20.6%) [lit.,[24] m.p. 134—137 °C, $[\alpha]_D^{19}$ −17.8° (*c* 1.5 in Me_2NCHO)].

O-*Acetyl*-N^2-*benzyloxycarbonyl*-N^{ω}-*nitro*-L-*argininol* (24).—The above alcohol (23) (193 mg, 0.57 mmol) was acetylated by acetic anhydride (1.6 ml) in dimethylformamide–pyridine (3 : 1 v/v; 2 ml) at 0 °C, rising to room temperature. After addition of water the solution was evaporated and the residue crystallised on trituration with ether. Recrystallisation from tetrahydrofuran–ether gave *acetyl derivative* (24) (165 mg, 76%) of m.p. 88—90 °C, $[\alpha]_D^{20}$ −8° (*c* 1 in Me_2NCHO); R_F 0.74 (G3), 0.51 (P1) (Found: C, 50.2; H, 6.35; N, 18.3. $C_{16}H_{23}N_5O_6$ requires C, 50.4; H, 6.1; N, 18.4%).

N^2-*Benzyloxycarbonyl*-O-*formyl*-N^{ω}-*nitro*-L-*argininol* (25).—The above alcohol (23) (187 mg, 0.551 mmol) in dimethylformamide (2 ml) and formic acid (2 ml) at 0 °C reacted on dropwise addition of acetic anhydride (0.80 ml). The solution was allowed to rise to room temperature and after 5 h water was added and the solution was evaporated. The residue crystallised on trituration with ether and was recrystallised from tetrahydrofuran–ether giving the *formyl derivative* (25) (187 mg, 92%) of m.p. 123—125 °C, $[\alpha]_D^{20}$ −10° (*c* 1.0 in Me_2NCHO); R_F 0.74 (G3), 0.47 (P1) (Found: C, 48.8; H, 5.6; N, 19.3. $C_{15}H_{21}N_5O_6$ requires C, 49.0; H, 5.8; N, 19.1%). The ester hydrolysed slowly in 80% acetic acid and in 60% formic acid.

N^{α}-*t-Butoxycarbonyl*-N^{ω}-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-O-*acetyl*-N^{ω}-*nitro*-L-*argininol* (16).—*O*-Acetyl-N^2-benzyloxycarbonyl-N^{ω}-nitro-L-argininol (24) (64 mg, 0.17 mmol) reacted with a solution of hydrogen bromide in acetic acid (45%, 1 ml) during 1 h at 0 °C. The resulting hydrobromide was precipitated by dry ether and dissolved in dimethylformamide (1.5 ml). *N,N*-Di-isopropylethylamine (0.050 ml) was added to liberate the amino-component. This was then coupled to the azide prepared from protected octapeptide hydrazide (10) by Method A, described above for the coupling of that octapeptide to nitroarginylisoleucine 4-picolyl ester. After 24 h at 0 °C, the reaction mixture was evaporated to dryness and the residue was triturated with water; it was then dried, dissolved in dimethylformamide (2 ml) and purified on a Sephadex LH-20 column (110 × 3 cm), giving O-*acetyl protected nonapeptide* (16) (153 mg, 74%) of $[\alpha]_D^{20}$ −59° (*c* 0.55 in Me_2NCHO); R_F 0.34 (G3), 0.31 (P1) (Found: C, 55.1; H, 6.7; N, 16.9. $C_{64}H_{89}N_{17}O_{17},1\frac{1}{2}H_2O$ requires C, 55.1, H, 6.6; N, 17.1%. Found after acid hydrolysis: Arg, 0.85; Orn, 0.18; Ser, 0.77; Pro, 3.00; Gly, 1.00; Phe, 2.00).

N^{α}-*t-Butoxycarbonyl*-N^{ω}-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N^{ω}-*nitro*-L-*argininol* (15).—This compound was prepared analogously from N^2-benzyloxycarbonyl-N^{ω}-nitro-L-argininol (57 mg, 0.17 mmol) and protected octapeptide hydrazide (10) (175 mg, 0.148 mmol). Gel filtration on Sephadex LH-20 gave a product containing a contaminant of higher R_F in solvent P2, and this was removed by plate chromatography on Kieselgel-60, using chloroform–methanol–acetic acid (15 : 2 : 1, v/v), as mobile solvent, giving the *protected nonapeptide alcohol* (15) (81 mg, 40%) of $[\alpha]_D^{20}$ −57° (*c* 0.55 in Me_2NCHO); R_F 0.21 (G3), 0.16 (P1) (Found: C, 54.2; H, 6.6; N, 17.3. $C_{62}H_{87}N_{17}O_{16},2\frac{1}{2}H_2O$ requires C, 54.3; H, 6.8; N, 17.4%. Found after acid hydrolysis: Arg, 0.92; Orn, 0.08; Ser, 0.72; Pro, 3.00; Gly, 1.03; Phe, 1.96).

N^{α}-*t-Butoxycarbonyl*-N^{ω}-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-O-*formyl*-N^{ω}-*nitro*-L-*argininol* (17).—This compound was prepared by formylation of the above protected nonapeptide alcohol (15). Formic acid (1 ml) was added to acetic anhydride (0.4 ml) and after 3 h at room temperature the solution was added dropwise to a solution of the protected nonapeptide alcohol (15) (88 mg, 0.064 mmol) in pyridine (1.5 ml) at 0 °C. Next day water was added and the solution was evaporated; the residue was precipitated from dimethylformamide by dried ether, giving O-*formyl protected nonapeptide* (17) (84 mg, 93%) of $[\alpha]_D^{20}$ −60° (*c* 0.52 in Me_2NCHO); R_F 0.31 (G3), 0.29 (P1) (Found: C, 53.6; H, 6.4; N, 16.6. $C_{63}H_{87}N_{17}O_{17},3H_2O$ requires C, 53.7; H, 6.65; N, 16.9%. Found after acid hydrolysis: Arg, 0.82; Orn, 0.19; Ser, 0.77; Pro, 3.00; Gly, 1.00; Phe, 2.00).

L-*Arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-L-*argininol* (5) *Triacetate*.—The protected nonapeptide alcohol (15) (61 mg, 0.045 mmol) was deprotected by trifluoroacetic acid and then by hydrogenolysis as described for the preparation of bradykinyl-isoleucine [compound (2)], and the product was purified similarly on carboxymethyl-cellulose CM 32 and then on Sephadex G-15 columns. Precipitation from methanol by ether gave *nonapeptide alcohol* (5) *triacetate* (33 mg, 56%) of $[\alpha]_D^{20}$ −89.5° (*c* 0.77 in H_2O); R_F 0.12 (A5), 0.35 (H); $E_{Arg}^{6\cdot4}$ 0.86 (Found: C, 51.2; H, 7.0; N, 16.05. $C_{56}H_{87}N_{15}O_{16},5H_2O$ requires C, 51.1; H, 7.4; N, 16.0%. Found after acid hydrolysis: Arg, 0.99; Ser, 0.86; Pro, 2.97; Gly, 1.01; Phe, 2.02).

L-*Arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-O-*acetyl*-L-*argininol* (7) *Triacetate*.—This compound was prepared similarly from the protected nonapeptide ester (16), giving O-*acetyl nonapeptide alcohol* (7) (46 mg, 49%) of $[\alpha]_D^{20}$ −87° (*c* 0.52 in H_2O); R_F 0.17 (A5), 0.38 (H); $E_{Arg}^{6\cdot4}$ 0.88 (Found: C, 50.6; H, 7.1; N, 15.5. $C_{58}H_{89}N_{15}O_{17},6H_2O$ requires C, 50.6; H, 7.4; N, 15.3%. Found after acid hydrolysis: Arg, 1.01; Ser, 0.88; Pro, 2.95; Gly, 1.02; Phe, 2.02). Deprotection of the analogous

O-formyl derivative (17) in the same way gave a product which appeared to be mainly the argininol nonapeptide (5).

Nβ-t-Butoxycarbonyl-Nω-nitro-(3S)-β-homoarginine Methyl Ester (27).—*Nα*-t-Butoxycarbonyl-*Nω*-nitro-L-arginyldiazomethane was prepared by the action of diazomethane on the carbonic mixed anhydride of *Nα*-t-butoxycarbonyl-*Nω*-nitro-L-arginine by the method of Penke *et al.*;[18] after recrystallisation from ethyl acetate–light petroleum it had m.p. 146—148 °C, $[\alpha]_D^{20}$ −47° (*c* 0.57 in Me_2NCHO); Kettner and Shaw[19] reported m.p. 150—151 °C, $[\alpha]_D^{24}$ −57.2° for the L-isomer and $[\alpha]_D^{24}$ 50.7° for the D-isomer, in dimethylformamide (concentration not stated). Freshly prepared silver oxide (45 mg, 0.19 mmol) was added to a solution of the diazoketone (242 mg, 0.705 mmol) in refluxing methanol. When evolution of nitrogen ceased (5 min) the solution was allowed to cool, a small amount of charcoal was added, and the solution was filtered and then evaporated. The residue was chromatographed on Kieselgel 60 (solvent, chloroform–methanol, 9 : 1); the product was recrystallised from ethyl acetate–light petroleum, giving the *ester* (27) (186 mg, 76%) of m.p. 100—101 °C, $[\alpha]_D^{20}$ −10° (*c* 1.1 in Me_2NCHO); R_F 0.30 (E4), 0.47 (P1) (Found: C, 45.0; H, 7.2; N, 20.3. $C_{13}H_{25}N_5O_6$ requires C, 44.95; H, 7.25; N, 20.2%).

Nβ-t-Butoxycarbonyl-Nω-nitro-(3S)-β-homoarginine Benzyl Ester (28).—*Nα*-t-Butoxycarbonyl-*Nω*-nitro-L-arginyldiazomethane[18,19] (129 mg, 0.376 mmol) reacted with benzyl alcohol (1 ml) in dioxan (5 ml) in the presence of silver oxide as described above for the preparation of the methyl ester. Chromatography of the product on Kieselgel-60 (solvent, chloroform–methanol, 9 : 1) gave the *benzyl ester* (28) (yield, 52%) which could not be crystallised; it had $[\alpha]_D^{20}$ −10° (*c* 0.6 in Me_2NCHO); R_F 0.38 (E4), 0.53 (P1) (Found: C, 53.8; H, 7.1; N, 14.9. $C_{19}H_{29}N_5O_6,\frac{1}{2}CH_3CO_2Et$ requires C, 53.95; H, 7.1; N, 15.0. The presence of ethyl acetate, derived from its use in attempts to crystallise, was confirmed by ^1H n.m.r.

Nα-Benzyloxycarbonyl-Nω-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-benzyl-L-seryl-L-prolyl-L-phenylalanine 4-Picolyl Ester (20).—This was prepared by coupling *Nα*-benzyloxycarbonyl-*Nω*-nitro-L-arginine with the amino-component derived from Boc-Pro-Pro-Gly-Phe-Ser-(Bzl)-Pro-Phe-OPic [compound (34), Table 1], by the same procedure as that used for the preparation of the t-butoxycarbonyl analogue, compound (9) (Table 1). Isolation was by the Amberlyst procedure (solvent, dichloromethane), giving *protected octapeptide* (20) (63% yield) of $[\alpha]_D^{20}$ −50° (*c* 1.0 in Me_2NCHO); R_F 0.33 (G3), 0.28 (P1) (Found: C, 60.5; H, 6.2; N, 14.0. $C_{65}H_{77}N_{13}O_{14},1\frac{1}{2}H_2O$ requires C, 60.45; H, 6.2; N, 14.1%. Found after acid hydrolysis: Arg, 0.83; Orn, 0.18; Ser, 0.75; Pro, 3.00; Gly, 1.00; Phe, 2.00).

Nα-Benzyloxycarbonyl-Nω-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-benzyl-L-seryl-L-prolyl-L-phenylalanine (21).—The above 4-picolyl ester (20) (309 mg, 0.239 mmol) was hydrolysed by 1.0M-sodium hydroxide (0.29 ml) in tetrahydrofuran–water (9 : 1; 6 ml) during 20 min at room temperature. The solution was neutralised and then evaporated; water was added, the pH was brought to 2 and the product was extracted into dichloromethane. The solution was concentrated and the addition of ether gave *protected octapeptide acid* (21) (244 mg, 85%) of $[\alpha]_D^{20}$ −51° (*c* 0.55 in Me_2NCHO); R_F 0.21 (G3), 0.25 (P1) (Found: C, 59.1; H, 6.0; N, 13.7. $C_{59}H_{72}N_{12}O_{14},1\frac{1}{2}H_2O$ requires C, 59.0; H, 6.3; N, 14.0%. Found after acid hydrolysis: Arg 0.76; Orn, 0.23; Ser, 0.78; Pro, 2.98; Gly, 1.01; Phe, 2.01).

Nα-Benzyloxycarbonyl-Nω-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-benzyl-L-seryl-L-prolyl-L-phenylalanyl-Nω-nitro-(3S)-β-homoarginine Methyl Ester (18).—The protected octapeptide acid (21) (240 mg, 0.20 mmol) was coupled by means of dicyclohexylcarbodi-imide with 1-hydroxybenzotriazole to the amino-component obtained by the action of trifluoroacetic acid on *Nβ*-t-butoxycarbonyl-*Nω*-nitro-(3*S*)-β-homoarginine methyl ester (27) (72 mg, 0.21 mmol). The product was purified on Sephadex LH-20 (solvent, dimethylformamide); precipitation from methanol by ether gave *protected nonapeptide methyl ester* (18) (181 mg; 63%) of $[\alpha]_D^{20}$ −61° (*c* 0.54 in Me_2NCHO); R_F 0.33 (G3), 0.25 (P1) (Found: C, 55.55; H, 6.3; N, 16.35. $C_{67}H_{87}N_{17}O_{17},2\frac{1}{2}H_2O$ requires C, 55.6; H, 6.4; N, 16.45%. Found after acid hydrolysis: Arg, 0.80; Orn, 0.19; Ser, 0.80; Pro, 3.01; Gly, 1.00; Phe, 1.99). Saponification of the ester (126 mg, 0.087 mmol) by M-sodium hydroxide (0.105 ml) in tetrahydrofuran–water (9 : 1, 2.5 ml) at room temperature was incomplete after 2 h; after addition of more sodium hydroxide (0.03 ml) and a further 3 h the reaction was still incomplete.

An analogous preparation using *Nβ*-t-butoxycarbonyl-*Nω*-nitro-(3*S*)-β-homoarginine benzyl ester (28) gave *Nα-benzyloxycarbonyl-Nω-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-benzyl-L-seryl-L-prolyl-L-phenylalanyl-Nω-nitro-(3S)-β-homoarginine benzyl ester* (19) (66% yield, after precipitation from acetone by ether) of $[\alpha]_D^{20}$ −56° (*c* 0.5 in Me_2NCHO); R_F 0.42 (G3), 0.25 (P1) (Found: C, 57.8; H, 6.3; N, 15.5. $C_{73}H_{91}N_{17}O_{17},2H_2O$ requires C, 57.9; H, 6.3; N, 15.7%. Found after acid hydrolysis: Arg, 0.84; Orn, 0.16; Ser, 0.78; Pro, 3.02; Gly, 0.99; Phe, 1.99).

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-(3S)-β-homoarginine (6) *Diacetate*.—The protected nonapeptide benzyl ester (19) (54 mg, 0.036 mmol) was hydrogenolysed in 80% acetic acid over palladium–charcoal (10%, 20 mg) during 17 h. The product was purified on carboxymethylcellulose CM 32 in triethylammonium acetate buffer, with a gradient from 0.20M, pH 5.0 to 0.30M, pH 5.0. After evaporation of much of the buffer, the peptide was desalted on a column of Sephadex G-15 using 25% acetic acid as a solvent (this concentration of acetic acid gave a more effective separation than the 5% acetic acid used in earlier experiments). The product was reprecipitated from methanol by ether, giving [9-(3S)-β-*homoarginine*]-*bradykinin* (6) *diacetate* (26 mg, 55%) of $[\alpha]_D^{20}$ −87.5° (*c* 0.26 in H_2O); R_F 0.11 (A5), 0.29 (H); $E_{Arg}^{6\cdot4}$ 0.86 (Found: C, 50.4; H, 6.8; N, 15.9. $C_{55}H_{83}N_{15}O_{15},6H_2O$ requires C, 50.7; H, 7.35; N, 16.1%. Found after acid hydrolysis: Arg, 0.97; Ser, 0.81; Pro, 2.96; Gly, 1.01; Phe, 2.03).

Nα-t-Butoxycarbonyl-Nω-nitro-L-arginine Heptyl Ester.—*Nω*-Nitro-L-arginine suspended in toluene was esterified with heptan-1-ol by azeotropic distillation in the presence of toluene-*p*-sulphonic acid hydrate. The crude product obtained by precipitation with ether was chromatographed on Kieselgel-60 (solvent, n-butanol–acetic acid–water, 10 : 1 : 3, v/v) and converted into the t-butoxycarbonyl derivative directly by reaction with di-t-butyl dicarbonate[25] in dimethylformamide containing *N,N*-di-isopropylethylamine during 3 h at room temperature. Chromatography of the product on Kieselgel-60 (solvent, ethyl acetate–chloroform, 1 : 1, v/v) and recrystallisation from ethyl acetate–light petroleum gave *ester* (overall yield, 25%) of m.p. 95—96 °C, $[\alpha]_D^{20}$ −15° (*c* 1.1 in Me_2NCHO); R_F 0.32 (chloroform–ethyl acetate, 1 : 1, v/v), 0.56 (P1) (Found:

C, 51.9; H, 8.4; N, 16.9. $C_{18}H_{35}N_5O_6$ requires C, 51.8; H, 8.45; N, 16.8%).

N^{α}-*Benzyloxycarbonyl*-N^{ω}-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N^{ω}-*nitro*-L-*arginine Heptyl Ester* (22).—The protected octapeptide acid (21) (177 mg, 0.147 mmol) was coupled by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole to the amino-component obtained by the action of trifluoroacetic acid on N^{α}-t-butoxycarbonyl-N^{ω}-nitro-L-arginine heptyl ester (66 mg, 0.16 mmol). The product was purified on Sephadex LH-20 (solvent, dimethylformamide) and then precipitated from acetone by ether, giving *protected nonapeptide heptyl ester* (22) (174 mg, 79%), $[\alpha]_D^{20} -53°$ (*c* 0.57 in Me_2NCHO); R_F 0.48 (G3), 0.37 (P1) (Found: C, 57.8; H, 6.6; N, 16.2. $C_{72}H_{97}N_{17}O_{17},H_2O$ requires C, 58.0; H, 6.7; N, 16.0%. Found after acid hydrolysis: Arg, 1.66; Orn, 0.33; Ser, 0.78; Pro, 3.01; Gly, 1.01; Phe, 1.98).

L-*Arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-L-*arginine Heptyl Ester* (8) (*Bradykinin Heptyl Ester*) *Triacetate*.—The protected nonapeptide heptyl ester (22) (100 mg, 0.067 mmol) in 80% acetic acid (10 ml) was hydrogenolysed over palladium–charcoal (10%, 29 mg) during 17 h as usual. The product was chromatographed on carboxymethylcellulose CM 32 (triethylammonium acetate buffer from 0.25M, pH 5.0 to 0.5M, pH 5.0) and desalted on Sephadex G-10 and on Bio-Gel P-2 (solvent, 5% acetic acid in both cases); final reprecipitation from methanol by ether gave *bradykinin heptyl ester* (8) *triacetate* (32 mg, 33%) of $[\alpha]_D^{20} -81°$ (*c* 0.23 in H_2O); R_F 0.43 (A5), 0.45 (H); $E_{Ar}^{6.4}$ 0.75 (Found: C, 52.8; H, 7.1; N, 14.7. $C_{63}H_{99}N_{15}O_{17},5H_2O$ requires C, 53.0; H, 7.7; N, 14.7%. Found after acid hydrolysis: Arg, 1.98; Ser, 0.83; Pro, 2.97; Gly, 1.02; Phe, 2.02).

APPENDIX

Biological activity of the seven analogues was determined in isolated preparations of the guinea-pig ileum and rat uterus. Comparison was made in all cases with contractions produced by authentic bradykinin (Sigma Ltd). Assays were performed where possible using a Latin Square 4 × 4 point design. Each compound was applied for 1 min at 4.5 min intervals. The relative potency of each analogue relative to bradykinin is shown in Table 2 and each value is the mean of 2 or 3 determinations obtained in separate preparations. Clearly all of the analogues were much weaker agonists than bradykinin. The most active analogue was the heptyl ester but even this activity may have resulted, in part, from contact with the assay tissue causing hydrolysis to bradykinin. Incubation of solutions of the heptyl ester with samples of intestinal muscle at 20 °C (600—800 mg guinea-pig ileum in 3—4 ml solution) and assaying aliquots every 4.5 min for 60 min indicated an enhancement of the apparent activity of the solution. This enhancement was maximal between 4.5 and 13.5 min of incubation after which activity diminished to below the basal level and was finally absent after 60 min. This is illustrated in the Figure. The apparent activity increased 2.5 fold after 9 min of incubation. By contrast, incubating bradykinin with intestinal muscle in the same way resulted in a gradual diminution of activity with no enhancement. The decrease was apparent within the first 5 min when activity was reduced by 30%;

TABLE 2

The biological activities of the synthetic peptides

Compound	Relative potency (bradykinin = 100) Guinea-pig ileum	Rat uterus
Bradykinyl-L-isoleucine (2)	0.54 ± 0.1	0.29 ± 0.1
Bradykinyl-L-alanine (3)	0.2	0.18
Bradykinyl-β-alanine (4)	0.1 ± 0.02	0.15 ± 0.03
[9-L-Argininol]-bradykinin (5)	0.5	
[9-*O*-Acetyl-L-argininol]-bradykinin (7)	0.02	
[9-β-Homoarginine]-bradykinin (6)	6.0 ± 2	1.0 ± 0.1
Bradykinin heptyl ester (8)	13.6 ± 6	12.7 ± 7

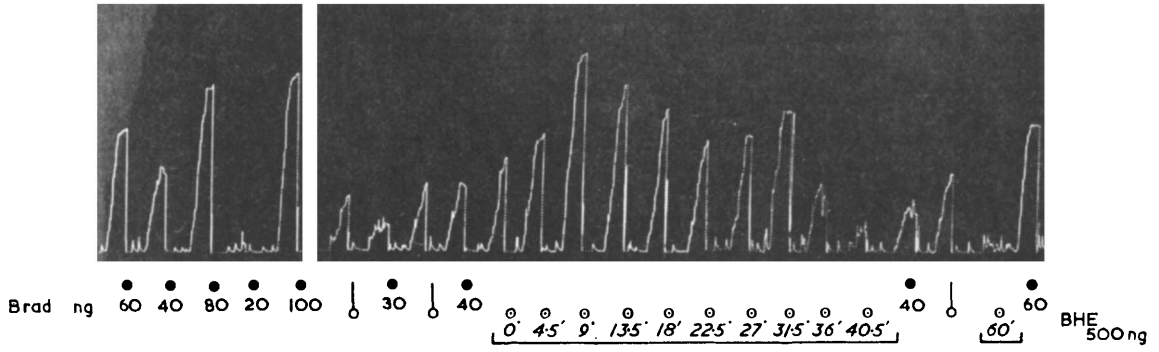


FIGURE Recording of the contractile activity of a guinea-pig isolated ileum preparation in response to bradykinin and bradykinin heptyl ester (BHE). The tissue was suspended in Tyrode's solution at 34 °C and the agonists were added as indicated below the record for periods of 1 min at 4.5 min intervals. At the end of each minute application the recorder was stopped and the tissue was rinsed and allowed to relax. Bradykinin (brad ●) was added in varying amounts (ng) as shown, whereas BHE (○) was added in a fixed amount (500 ng). The organ bath volume was 15 ml. During the period indicated by the horizontal line 3 ml of 5 μg/ml BHE in Tyrode's solution was incubated with 640 mg ileal muscle at 20 °C. 500 ng Aliquots of the incubate were removed at the times shown and applied to the test tissue for 1 min (○). Note the increase and subsequent decrease in bradykinin-like activity with time

activity was abolished after 15—20 min. None of the analogues exhibited any antagonism of responses to bradykinin when tested at doses at or below the level producing responses themselves.

We thank the Danish Natural Science Research Council and the British Council for financial support for J.C.

[1/1607 *Received*, 15th *October*, 1981]

REFERENCES

[1] Part 45: S. Coyle, A. Hallett, M. S. Munns, and G. T. Young, *J. Chem. Soc., Perkin Trans. 1*, 1981, 522.
[2] D. J. Schafer, G. T. Young, D. F. Elliott, and R. Wade, *J. Chem. Soc. C*, 1971, 46.
[3] G. A. Fletcher and G. T. Young, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1867.
[4] T. G. Pinker, G. T. Young, D. F. Elliott, and R. Wade, *J. Chem. Soc., Perkin Trans. 1*, 1976, 220.
[5] V. S. Chauhan and G. T. Young, *Bioorg. Chem.*, 1979, **8**, 333.
[6] J. M. Stewart, ' Handbook of Experimental Pharmacology,' ed. E. G. Erdös, vol. 25, suppl., 1979, p. 227—272.
[7] W. H. Johnson, H. D. Law, and R. O. Studer, *J. Chem. Soc. C*, 1971, 748.
[8] M. Bodanszky, J. T. Sheehan, M. A. Ondetti, and S. Lande, *J. Am. Chem. Soc.*, 1963, **85**, 991.
[9] E. Schröder, *Annalen*, 1964, **673**, 220.
[10] J. M. Stewart and D. M. Woolley, *Nature*, 1965, **207**, 1160.
[11] J. M. Stewart, *Fed. Proc.*, 1968, **27(1)**, 63.
[12] W. K. Park, S. A. St-Pierre, J. Barabé, and D. Regoli, *Canad. J. Biochem.*, 1978, **56**, 92.
[13] A. Anastasi, G. Bertaccini, and V. Erspamer, *Br. J. Pharmacol. Chemother.*, 1966, **27**, 479.
[14] D. Regoli, J. Barabé, and W. K. Park, *Can. J. Physiol. Pharmacol.*, 1977, **55**, 855.
[15] J. Christiansen, G. T. Young, and N. G. Bowery, in ' Peptides 1980,' ed. K. Brunfeldt, Scriptor, Copenhagen, 1981, p. 612.
[16] A. Gecse, E. Zsilinszky, and L. Szekeres, *Adv. Exp. Med. Biol.*, 1976, **70**, 5.
[17] G. Claeson, J. Fareed, C. Larsson, G. Kindel, S. Arielly, R. Simonsson, H. L. Messmore, and J. U. Balis, *Adv. Exp. Med. Biol.*, 1979, **120B**, 691.
[18] B. Penke, J. Czombos, L. Baláspiri, J. Petres, and K. Kovács, *Helv. Chim. Acta*, 1970, **53**, 1057.
[19] C. Kettner and E. Shaw, *Biochemistry*, 1978, **17**, 4778.
[20] W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.
[21] D. M. Bratby, S. Coyle, R. P. Gregson, G. W. Hardy, and G. T. Young, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1901.
[22] K. Inouye, A. Tanaka, and H. Otsuka, *Bull. Chem. Soc. Jpn.*, 1970, **43**, 1163.
[23] J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, 1961, **26**, 2333.
[24] A. Ito, R. Takahashi, and Y. Baba, *Chem. Pharm. Bull. Jpn.*, 1975, **23**, 3081.
[25] L. Moroder, A. Hallett, E. Wünsch, O. Keller, and G. Wersin, *Z. Physiol. Chem.*, 1976, **357**, 1651.