Amino-acids and Peptides. Part XXXIX.[1] Synthesis of Analogues of Bradykinin with Modifications in Positions 1, 6, and 9

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The following analogues of the local tissue hormone bradykinin (I) have been synthesised by the picolyl ester 'handle' procedure: [1-*N*(ω)-methyl-L-arginine]-, [9-*N*ω)-methyl-L-arginine]-, [1-*N*(δ)-acetimidoyl-L-ornithine]-, [9-*N*(δ)-acetimidoyl-L-ornithine]-, [1-*N*(α)-amidino-L-arginine]-, [1-*N*(α)-amidino-L-lysine]-, [1,9-L-ornithine]-, [6-L-ornithine]- and [6-*N*(δ)-(3-carboxypropionyl)-L-ornithine]-bradykinin. The first two analogues retained substantial biological activity, but the replacement of the guanidino-residues by acetamidino greatly reduced the activity, indicating a hitherto unsuspected specificity in this area. The introduction of the *N*(α)-amidino-groups did not greatly affect the activity. The three last-named analogues were inactive. A key step in the syntheses was the removal of the piperidino-oxycarbonyl amino-protecting group from the ornithine side-chain in the presence of t-butoxycarbonyl and nitro groups, by means of sodium dithionite.

EARLIER studies [2] on synthetic analogues of the local tissue hormone bradykinin (1) † have emphasised particularly the importance, for high biological activity, of the arginine residues in positions 1 and 9, the phenylalanine residues in 5 and 8, the proline residues in 2 and 7, and the terminal carboxy-group.[3] By synthesis of analogues in which the phenylalanine residues are replaced by β-cyclohexyl-L-alanine we have shown that aromatic side-chains are not essential,[4-6] and we have also shown that high activity is retained when the side-chain of arginine-1 is lengthened by one methylene group.[6] The

1 2 3 4 5 6 7 8 9
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
(1)

considerable reduction in activity observed when the arginine residues are replaced by lysine [7] or ornithine [8] prompted an investigation of the specific requirements in the area occupied by the guanidino-groups, and we have therefore synthesised analogues in which the guanidino-groups in positions 1 and 9 are replaced by acetamidino (NH:CMe·NH–) and by *N*-methylguanidino [MeNH·C(:NH)·NH–], both of which preserve the high basicity of this part of the molecule. We have also replaced the *N*-terminal amino-group of bradykinin and of [1-L-lysine]-bradykinin by guanidino, and in the course of this part of our work have prepared [1,9-L-ornithine]-bradykinin.

The syntheses have in each case used the picolyl ester method,[9] the coupling product being isolated by extraction into aqueous citric acid (in the early stages) or (more usually) by absorption on to Amberlyst-15 resin,[6,10] saturated with 3-bromopyridine to prevent cleavage of acid-sensitive groups. Coupling in every case was performed by means of dicyclohexylcarbodi-imide and l-hydroxybenzotriazole.[11] For the synthesis of bradykinins modified in position 9, we prepared the protected octapeptide (8) [formulae (2)—(8) appear above Table 1] by the stepwise route shown in Table 1. The ornithine side chain was protected by piperidino-oxycarbonyl,[12] which is stable to acid but rapidly removed by sodium dithionite in aqueous acetic acid at room temperature, conditions in which the t-butoxycarbonyl and nitro-groups are stable; the two first-named amino-protecting groups are therefore complementary, each being selectively removable in the presence of the other. *N*(α)-t-Butoxycarbonyl-*N*(δ)-piperidino-oxycarbonyl-L-ornithine was prepared by the reaction of 1-piperidyl 2,4,5-trichlorophenyl carbonate with *N*(α)-t-butoxycarbonyl-L-ornithine, and was converted into the 4-picolyl ester by reaction with 4-picolyl alcohol and dicyclohexylcarbodi-imide.[9] In the latter reaction, considerably improved yields were obtained by the gradual addition of the dicyclohexylcarbodi-imide to the solution of the acid and the alcohol at −3 °C and we recommend this modified procedure for the general preparation of 4-picolyl esters. *N*(α),*N*(δ)-Di-t-butoxycarbonyl-L-ornithine, *N*(α)-benzyloxycarbonyl-*N*(δ)-piperidino-oxycarbonyl-L-ornithine and its 4-picolyl ester, and *N*(δ)-piperidino-oxycarbonyl-L-ornithine 4-picolyl ester dihydrobromide are also reported here.

The t-butoxycarbonyl group was removed from the protected octapeptide (8) and the resulting amino-component was condensed with *N*(α)-benzyloxycarbonyl-*N*(ω)-nitro-L-arginine. The isolation of the protected nonapeptide (9) posed a problem which we have en-

† 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, London, 1972, vol. 4, p. 441, and in vol. 5 (ed. R. C. Sheppard) 1974, p. 476. Pic = 4-picolyl; Pipoc = piperidino-oxycarbonyl. Amino-acids are of the L-configuration.

[1] Part XXXVIII, R. Macrae and G. T. Young, *J.C.S. Perkin I*, 1975, 1185.
[2] For reviews, see E. Schröder and R. Hempel, *Experientia*, 1964, **20**, 529; J. M. Stewart, *Fed. Proc.*, 1968, **27**, 63.
[3] W. H. Johnson, H. D. Law, and R. O. Studer, *J. Chem. Soc.* (*C*), 1971, 748.
[4] D. J. Schafer, G. T. Young, D. F. Elliott, and R. Wade, *J. Chem. Soc.* (*C*), 1971, 46.
[5] D. F. Elliott, P. Moritz, and R. Wade, *J. Chem. Soc.* (*C*), 1972, 1862.
[6] G. A. Fletcher and G. T. Young, *J. Chem. Soc.* (*C*), 1972, 1867.
[7] E. Schröder, *Annalen*, 1964, **673**, 220.
[8] E. D. Nicolaides, H. A. De Wald, and M. K. Craft, *J. Medicin. Chem.*, 1963, **6**, 739; E. Schröder, H.-S. Petras, and E. Klieger, *Annalen*, 1964, **679**, 221.
[9] R. Camble, R. Garner, and G. T. Young, *J. Chem. Soc.* (*C*), 1969, 1911; G. T. Young in 'The Chemistry of Polypeptides,' ed. P. G. Katsoyannis, Plenum Press, New York, 1973, p. 43.
[10] J. Burton and G. T. Young, *Israel J. Chem.*, 1971, 201.
[11] W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.
[12] D. Stevenson and G. T. Young, *J. Chem. Soc.* (*C*), 1969, 2389.

countered in some (but not all) other cases in which nitroarginine and piperidino-oxycarbonyl groups are present in the same molecule. During absorption on the Amberlyst-15 resin (3-bromopyridinium salt) some decomposition can occur giving material which is not eluted by pyridine in dimethylformamide but is eluted by triethylamine in dimethylformamide; it appears that some piperidino-oxycarbonyl protection is removed, perhaps because of a favourable juxtaposition of the nitro- and piperidino-oxycarbonyl groups on the resin surface. In a model experiment, it was found that t-butoxycarbonyl-$N(\omega)$-nitro-L-arginyl-$N(\delta)$piperidino-oxycarbonyl-L-lysylglycine 4-picolyl ester can be isolated in high yield by the citric acid procedure but suffers loss on the Amberlyst resin.[13] On the the other hand good recoveries from Amberlyst have been obtained in other cases in which both groups occur; Table 2 gives four examples. It is advisable in such cases first to explore on a small scale the feasibility of the use of Amberlyst resin. The protected nonapeptide (9) was insufficiently soluble in aqueous citric acid to allow this alternative to be used, and the product was purified by means of Sephadex LH-20; the overall yield of protected nonapeptide [from $N(\alpha)$-t-butoxycarbonyl-$N(\delta)$piperidino-oxycarbonyl-ornithine 4-picolyl ester] was 52%. The 4-picolyl ester was saponified giving the acid (10), and the $N(\delta)$-piperidino-oxycarbonyl group was removed by means of sodium dithionite in 50% acetic acid, giving the monoacetate of (11) in 89% yield; the action of ethyl acetimidate then gave the $N(\delta)$-acetimidoylornithine derivative (12), which on hydrogenolysis yielded [9-$N(\delta)$-acetimidoyl-L-ornithine]-bradykinin (14) triacetate. The action of *N*-methyl-*S*-methylisothiouronium iodide on the amine (11) gave the protected nonapeptide (13) with $N(\omega)$-methylarginine in position 9, and hydrogenolysis gave [9-$N(\omega)$-methyl-L-arginine]-bradykinin (15) triacetate. The synthesis also afforded [1,9-L-ornithine]-bradykinin: $N(\alpha)$-benzyloxycarbonyl-$N(\delta)$-piperidino-oxycarbonyl-L-ornithine was coupled to the amino-component derived from the protected octapeptide (8) (Table 1), giving the protected nonapeptide (16), and hydrogenolysis gave [1,9-L-ornithine]-bradykinin triacetate.

TABLE 1

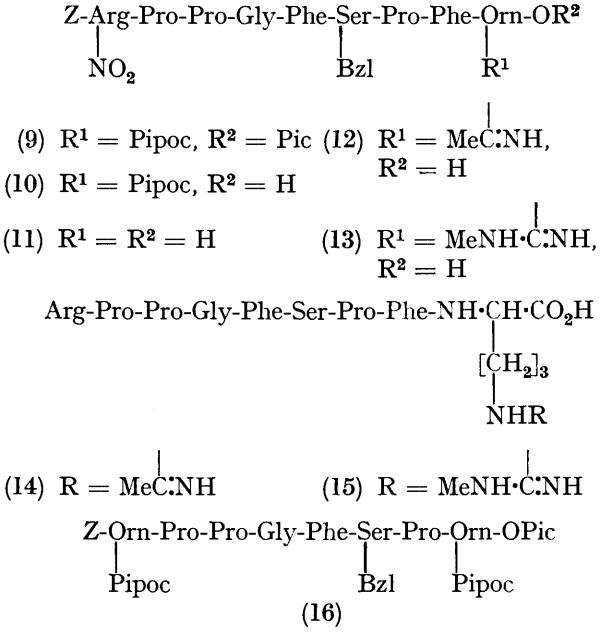
Synthesis of Boc-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-Orn-(Pipoc)-OPic: protected peptide intermediates [a]

(2) Boc-Phe-Orn(Pipoc)-OPic
(3) Boc-Pro-Phe-Orn(Pipoc)OPic
(4) Boc-Ser(Bzl)-Pro-Phe-Orn(Pipoc)-OPic
(5) Boc-Phe-Ser(Bzl)-Pro-Phe-Orn(Pipoc)-OPic
(6) Boc-Gly-Phe-Ser(Bzl)-Pro-Phe-Orn(Pipoc)-OPic
(7) Boc-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-Orn(Pipoc)-OPic
(8) Boc-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-Orn(Pipoc)-OPic

Compound [b]	Amino-component (mmol)	Acylating component (mmol)	$Me_2N{\cdot}CHO$ (ml)	Isolation [c]	Yield (%)	$[\alpha]_D^{20}$(c)[d]	R_F (t.l.c.)	Found (%) C	H	N	Formula	Required (%) C	H	N
(2)	17.8 [e]	Boc-Phe (23)	30	C	91	−6	E4, 0.59; G3, 0.70; H, 0.71	62.2	7.2	11.6	$C_{31}H_{43}N_5O_7$	62.3	7.25	11.7
(3)	15.0 [f]	Boc-Pro (20.0)	30	C	96	−31	E4, 0.55; G3, 0.57; A2, 0.56; G1, 0.77	61.0	7.5	12.0	$C_{36}H_{50}N_6O_8,H_2O$	60.7	7.3	11.8
(4)	6.7	Boc-Ser(Bzl) (8.7)	15	C	89 [g]	−32	E4, 0.52; G3, 0.74; A2, 0.63; H, 0.68	63.2	7.0	11.1	$C_{46}H_{61}N_7O_{10}$	63.4	7.0	11.25
(5)	5.75	Boc-Phe (7.47)	20	A	84 [h]	−32	E4, 0.65; G3, 0.77; A2, 0.59	64.7	6.6	11.2	$C_{55}H_{70}N_8O_{11}$	64.8	6.9	11.0
(6)	2.6	Boc-Gly (3.47)	16	A	91 [i]	−30	E4, 0.51; G3, 0.79; A2, 0.58; P, 0.70	63.3	6.5	11.6	$C_{57}H_{73}N_9O_{12}$	63.6	6.8	11.7
(7)	1.83	Boc-Pro (2.4)	10	A	92.5 [j]	−41	E4, 0.54; G3, 0.86; A2, 0.59; P, 0.87	63.2	6.95	11.6	$C_{62}H_{80}N_{10}O_{13}$	63.5	6.8	11.9
(8)	1.41	Boc-Pro (2.2)	11	A	96 [k]	−43.5	E4, 0.53; G3, 0.52; A2, 0.58; C, 0.79	63.5	6.9	12.1	$C_{67}H_{87}N_{11}O_{14}$	63.4	6.9	12.1

[a] The synthesis was performed stepwise from the carboxy-end. General procedures are described in the Experimental section. The amino-component for each coupling step was obtained from the appropriate t-butoxycarbonyl derivative, and the quantities given in the second column are those of the t-butoxycarbonyl derivative taken; yields are calculated based on this derivative and are for product with the stated constants and analysis. [b] All the compounds are new. [c] C = isolation by extraction into aqueous citric acid; A = isolation by means of Amberlyst-15 (saturated with 3-bromopyridine). [d] Optical rotations are for solutions in dimethylformamide (*c* 1). [e] The amino-component was derived from $N(\alpha)$-t-butoxycarbonyl-$N(\delta)$-piperidino-oxycarbonyl-L-ornithine 4-picolyl ester and the free amine was liberated by the addition of *NN*-di-isopropylethylamine (2.3 equiv.). In another preparation, the amino-component was $N(\delta)$-piperidino-oxycarbonyl-L-ornithine 4-picolyl ester, derived from the dihydrobromide by the addition of *NN*-di-isopropylethylamine (2.3 equiv.), and the yield (isolation by citric acid) was 86%. [f] The amino-component was liberated from the trifluoroacetate by careful addition of *NN*-di-isopropylethylamine until pH 8.5 (moist indicator paper). [g] Found: Ser, 0.86; Pro, 0.97; Phe, *1.00*; Orn, 0.99. [h] Found: Ser, 0.87; Pro, 1.02; Phe, *2.00*; Orn, 1.03. [i] Found: Gly, 0.95; Phe, *2.00*; Ser, 0.86; Pro, 1.05; Orn, 0.97. [j] Found: Pro, 1.95; Gly, 0.95; Phe, *2.00*; Ser, 0.86; Orn, 0.99. [k] Found: Pro, 2.88; Gly, 0.98; Phe, *2.00*; Ser, 0.86; Orn, 0.99.

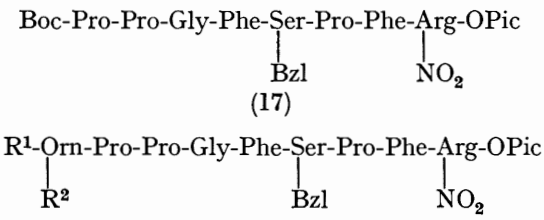
Similar modifications to the side-chains of position 1 were effected by coupling $N(\alpha)$-benzyloxycarbonyl-$N(\delta)$-t-butoxycarbonyl-L-ornithine to the amino-component



from the protected octapeptide (17); this was prepared [in 53% overall yield from $N(\omega)$-nitroarginine 4-picolyl ester dihydrobromide] by the route reported in Part XXXVI.[6] The protected nonapeptide (18) so obtained

[13] Unpublished work with Dr. D. M. Bratby.

was treated with trifluoroacetic acid to remove the $N(\delta)$-t-butoxycarbonyl group of the ornithine in position 1, and with ethyl acetimidate the product yielded the $N(\delta)$-acetimidoyl-ornithine derivative (19); hydrogenolysis then gave [1-$N(\delta)$-acetimidoyl-L-ornithine]-bradykinin (23) triacetate. Similarly, after removal of the $N(\delta)$-t-butoxycarbonyl group from the protected nonapeptide (18), and reaction with *N*-methyl-*S*-methylisothiouronium iodide the analogue (20), with $N(\omega)$-methylarginine

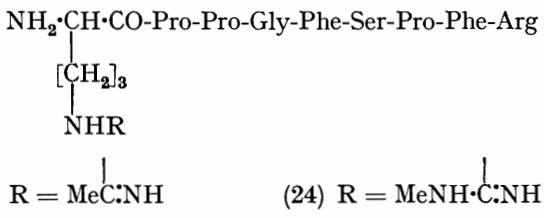


(17)

(18) R^1 = Z, R^2 = Boc (21) $R^1 = R^2$ = Boc

(19) R^1 = Z, R^2 = MeC(:NH)NH (22) $R^1 = R^2 = NH_2 \cdot C(:NH)NH$

(20) R^1 = Z, R^2 = $MeNH \cdot C(:NH)NH$

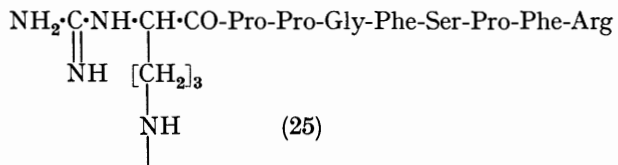


(23) R = MeC(:NH)NH (24) R = $MeNH \cdot C(:NH)NH$

in position 1, was obtained; hydrogenolysis then gave [1-$N(\omega)$-methyl-L-arginine]-bradykinin (24) triacetate.

As far as we are aware, the effect of the replacement in a biologically active peptide of the terminal α-amino-group by guanidino has not previously been examined. The condensation of $N(\alpha)$,$N(\delta)$-di-t-butoxycarbonyl-L-ornithine with the amino-component derived from the protected octapeptide (17) gave the protected nonapeptide (21), and removal of the $N(\alpha)$- and $N(\delta)$-t-butoxycarbonyl groups by trifluoroacetic acid followed by reaction with 1-amidino-3,5-dimethylpyrazole [14] amidinated both the α- and δ-amino-groups of the ornithine in position 1, giving the partly protected nonapeptide (22). Hydrogenolysis then gave [1-$N(\alpha)$-amidino-L-arginine]-bradykinin (25) triacetate. In order to examine the effect of an $N(\alpha)$-guanidino-group in the absence of such a group in the side-chain of position 1, $N(\alpha)$-t-butoxycarbonyl-$N(\varepsilon)$-benzyloxycarbonyl-L-lysine was condensed with the amino-component obtained from the protected octapeptide (17), giving protected nonapeptide (26). The $N(\alpha)$-t-butoxycarbonyl group was removed and the action of 1-amidino-3,5-dimethylpyrazole then afforded the guanidino-derivative (27); hydrogenolysis gave [1-$N(\alpha)$-amidino-L-lysine]-bradykinin (28) triacetate.

In an attempt to obtain a bradykinin analogue in which the conformation is restricted by cyclisation, we have synthesised protected [6-L-ornithine]-bradykinin and substituted the $N(\delta)$-ornithine amino-group by 3-carboxypropionyl, with the intention of condensing this



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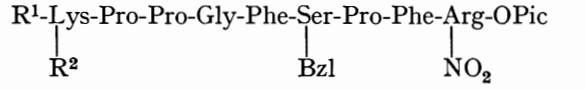
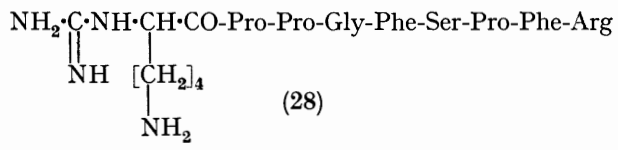
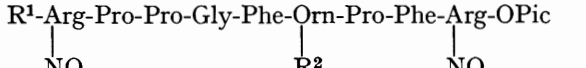


(26) R^1 = Boc, R^2 = Z (27) $R^1 = NH_2 \cdot C(:NH)NH$, R^2 = Z



(28)

carboxy-group with the *N*-terminal α-amino-group. For this synthesis we built up the protected nonapeptide (34) [formulae (29)—(34) appear above Table 2] in a stepwise fashion, as detailed in Table 2. The $N(\delta)$-piperidinooxycarbonyl group was removed by sodium dithionite, giving the diacetate of the partly protected nonapeptide (35) and leaving the t-butoxycarbonyl group intact. Reaction of the freed δ-amino-group with succinic anhydride gave the 3-carboxypropionyl derivative (36).



(35) R^1 = Boc, R^2 = H

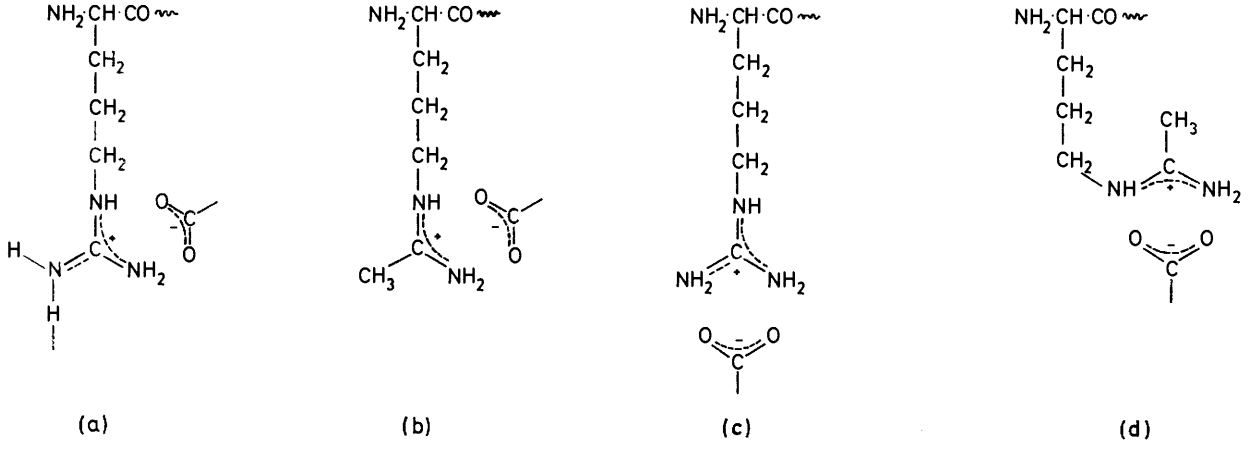
(36) R^1 = Boc, $R^2 = CO_2H \cdot CH_2 \cdot CH_2 \cdot CO$

Attempts to cyclise the product [after removal of the $N(\alpha)$-t-butoxycarbonyl group] have so far been unsuccessful. The protected nonapeptide (34) (Table 2) was hydrogenolysed in trifluoroacetic acid to yield [6-L-ornithine]-bradykinin triacetate, and similar treatment of the 3-carboxypropionyl derivative (36) gave [6-$N(\delta)$-(3-carboxypropionyl)-L-ornithine]-bradykinin diacetate.

[1-$N(\omega)$-Methyl-L-arginine]-bradykinin (24) and [9-$N(\omega)$-methyl-L-arginine]-bradykinin (15) had, in the isolated rat uterus test, about 23 and 60% of the activity of bradykinin itself, respectively, and the relatively large increase in the size of the guanidino-group has therefore little effect. [1-$N(\delta)$-Acetimidoyl-L-ornithine]-bradykinin (23) and [9-$N(\omega)$-acetimidoyl-L-ornithine]-bradykinin (14) each had about 1% activity in the same test, and since the acetamidino-group is still strongly basic this result indicates a hitherto unsuspected requirement in these positions. It may be that all three nitrogen atoms of the guanidino-group are required for contact with the receptor, as for example in the Scheme

[14] A. F. S. A. Habeeb, *Canad. J. Biochem. Physiol.*, 1960, **38**, 493.

(a); in that case, the methyl group of the acetamidine will prevent the third contact, as in (b). Another possibility is that in bradykinin the two unsubstituted nitrogen atoms make the necessary contact (perhaps with a carboxylate), as in (c), and in the acetamidino-derivatives the correct relative positioning of these two nitrogen atoms and of the side-chain is prevented, as indicated in (d). Whatever the explanation, these results show that from bradykinin; its activity was increased to the same extent by bradykinin-potentiating factor, it was metabolised at the same rate as bradykinin in lung, and it lowered blood pressure to the same extent. [1-(1-Deamino-L-arginine)]-bradykinin [3] and [1-$N(\alpha)$-acetyl-L-arginine]-bradykinin [16] are both active (22 and 50% respectively in the rat uterus test), but [1-$N(\alpha)$-t-butoxycarbonyl-L-arginine]-bradykinin is inactive:[6] the $N(\alpha)$-amino-group



SCHEME

TABLE 2

Synthesis of Boc-Arg(NO_2)-Pro-Pro-Gly-Phe-Orn(Pipoc)-Pro-Phe-Arg(NO_2)-OPic: protected peptide intermediates [a]

(29) Boc-Orn-(Pipoc)-Pro-Phe-Arg(NO_2)-OPic
(30) Boc-Phe-Orn(Pipoc)-Pro-Phe-Arg(NO_2)-OPic
(31) Boc-Gly-Phe-Orn(Pipoc)-Pro-Phe-Arg(NO_2)-OPic
(32) Boc-Pro-Gly-Phe-Orn(Pipoc)-Pro-Phe-Arg(NO_2)-OPic
(33) Boc-Pro-Pro-Gly-Phe-Orn(Pipoc)-Pro-Phe-Arg(NO_2)-OPic
(34) Boc-Arg(NO_2)-Pro-Pro-Gly-Phe-Orn(Pipoc)-Pro-Phe-Arg(NO_2)-OPic

Compound [b]	Amino-component (mmol)	Acylating component (mmol)	Me_2N·CHO (ml)	Isolation [c]	Yields (%)	$[\alpha]_D^{20}$(°) [d]	R_F (t.l.c.)	Found (%) C	H	N	Formula	Required (%) C	H	N
(29)	3.3 [e]	Boc-Orn(Pipoc) (4.3)	20	C	90	−40.5	E4, 0.36; M, 0.75; G4, 0.73; A2, 0.48	55.4	6.9	16.9	$C_{42}H_{61}N_{11}O_{11},H_2O$	55.2	6.9	16.9
(30)	2.0	Boc-Phe (2.6)	15	A	89 [f]	−41	E4, 0.51; G3, 0.63; B2, 0.65; A2, 0.50	57.5	6.7	15.7	$C_{51}H_{70}N_{12}O_{12},H_2O$	57.7	6.8	15.85
(31)	1.68	Boc-Gly (2.2)	12	A	79 [g]	−37	E4, 0.42; G3, 0.71; B2, 0.62; A2, 0.45	56.8	7.0	15.5	$C_{53}H_{73}N_{13}O_{13},2H_2O$	56.8	6.85	15.6
(32)	0.94	Boc-Pro (1.3) [h]	12	A [i]	90.5 [j]	−48	E4, 0.41; G3, 0.68; A2, 0.46; H, 0.71	58.4	6.6	16.2	$C_{58}H_{80}N_{14}O_{14}$	58.2	6.7	16.4
(33)	1.40	Boc-Pro (2.1)	15	A [i]	87 [k]	−46	E4, 0.51; G3, 0.75; P, 0.59; H, 0.76	56.6	6.7	15.9	$C_{63}H_{87}N_{15}O_{15},2H_2O$	56.9	6.85	15.8
(34)	0.22	Boc-Arg (NO_2) (0.36)	5	[l]	88 [m]	−49.5	E4, 0.23; G3, 0.33; A2, 0.28; H, 0.61	54.7	6.75	17.8	$C_{69}H_{93}N_{19}O_{18},2H_2O$	54.7	6.7	17.55

[a–d] As Table 1. [e] The amino-component was derived from t-butoxycarbonyl-L-prolyl-L-phenylalanyl-$N(\omega)$-nitro-L-arginine 4-picolyl ester [D. J. Schafer, G. T. Young, D. F. Elliott, and R. Wade, *J. Chem. Soc.* (*C*), 1971, 46]. [f] Found: Phe, *2.00*, Orn, 1.18; Pro, 0.99; Arg, 0.80. [g] Found: Gly, 1.04; Phe, *2.00*; Orn, 1.19; Pro, 0.99; Arg, 0.80; Arg(NO_2), 0.02. [h] After removal of the excess of triethylamine from the solution of the amino-component, the $N(\alpha)$-t-butoxycarbonyl-$N(\omega)$-nitroarginine and 1-hydroxybenzotriazole were added, followed by the dicyclohexylcarbodi-imide in portions over 1 h at 0 °C. The reaction was completed overnight at 0 °C. [i] The product was applied to Amberlyst in dichloromethane solution. [j] Found: Pro, 2.03; Gly, 1.00; Phe, *2.00*; Orn, 1.17; Arg, 0.79; Arg(NO_2), 0.01. [k] Found: Pro, 2.98; Gly, 1.01; Phe, *2.00*; Orn, 1.19; Arg, 0.80. [l] After removal of dicyclohexylurea, the filtrate was evaporated, the residue was taken up in dichloromethane, and the solution was washed and dried as usual and evaporated. The residue was purified on a column of Sephadex LH-20 (dimethylformamide as solvent). Evaporation of appropriate fractions and trituration of the residue with ether gave chromatographically pure product with the stated constants and analysis. [m] Found: Arg, 1.64; Pro, 3.08; Gly, 0.99; Phe, *2.00*; Orn, 1.32.

the requirements in this part of the molecule are much more demanding than has been suggested previously. The similarity to the requirements of the arginine in position 8 of corticotrophin-(1—24)-tetracosapeptide [15] is noteworthy. In both cases, replacement of the guanidinium group by ammonium (as in the lysine analogue) greatly decreases the biological activity, whereas lengthening of the side-chain (with homoarginine) has little effect.

[1-$N(\alpha)$-Amidino-L-arginine]-bradykinin (25) had twice the activity of bradykinin in the isolated rat uterus test, but against guinea-pig ileum it was indistinguishable is not essential but apparently a bulky lipophilic substituent interferes. The planar amidino-substituent can however be accommodated, and it may be that the positive charges assist binding in this position. Further probing of this area is in progress. [1-$N(\alpha)$-Amidino-L-lysine]-bradykinin (28) possessed 1—2% of the activity of bradykinin (rat uterus test), similar to that of [1-L-lysine]-bradykinin; [7] not surprisingly, the guanidino-residue in the $N(\alpha)$-position cannot replace that required

[15] G. I. Tesser, R. Maier, L. Schenkel-Hulliger, P. L. Barthe, B. Kamber, and W. Rittel, *Acta Endocrinol.*, 1973, **74**, 56.
[16] J. M. Stewart and D. W. Woolley, *Nature*, 1963, **207**, 1160.

in the side-chain in position 1. [1,9-L-Ornithine]-bradykinin, [6-L-ornithine]-bradykinin, and [6-$N(\delta)$-(3-carboxypropionyl)-L-ornithine]-bradykinin had an activity of less than 0.01% (rat uterus test); the first result is in accord with the inactivity of the 1- and 9- analogues [8] and of [1,9-L-lysine]-bradykinin,[7] and the inactivity of the two last new analogues is in accord with the effects of the introduction of lengthy side chains in position 6 (*e.g.* [6-L-leucine]-bradykinin).[17]

EXPERIMENTAL

The general instructions given in Part XXXVI [6] apply. Additional solvent systems for t.l.c. were (J) acetonitrile–water (3 : 1); (K) s-butyl alcohol–aqueous 3% ammonium hydroxide (3 : 1); (M) methanol–acetic acid–water (4 : 2 : 1); (O) chloroform–methanol–water (55 : 40 : 10); (P) chloroform–methanol–acetic acid (85 : 10 : 5). Samples for amino-acid analysis were hydrolysed by 6M-hydrochloric acid containing 1% phenol at 110 °C for 18—24 h. or at 115 °C for 16 h. $N(\alpha)$-Amidinoarginine was not detectable under the conditions of automated amino-acid analysis; $N(\alpha)$-amidinolysine and $N(\delta)$-acetimidoylornithine, although detectable, were not accurately determined. In the latter case partial decomposition occurred during acidic hydrolysis. Quantitative analysis of the $N(\omega)$-methyl analogue of arginine was also difficult because the elution curve it produced overlapped to some extent with that produced by arginine. Optical rotations were determined for solutions in dimethylformamide (*c* 1) unless otherwise stated.

General Synthetic Procedures.—(1) *Removal of the t-butoxycarbonyl group and liberation of the amino-component.* The t-butoxycarbonyl derivative of the amino-component was dissolved in trifluoroacetic acid (2—5 ml mmol^{-1}) at 0 °C, and 10—15 min after dissolution the solution was evaporated and the residue was triturated with ether. The trifluoroacetate salt so obtained was dissolved in dimethylformamide. In the synthesis of di- and tri-peptides, the acylating mixture was then added, followed by di-isopropylethylamine (this procedure was adopted in order to avoid dioxopiperazine formation from the free amino-component). For higher peptides, triethylamine in excess (*ca.* 5 equiv.) was added to the solution of the trifluoroacetate in dimethylformamide, and after 10 min the excess of triethylamine was removed on the water-pump; the absence of t-butoxycarbonyl derivative in the resulting solution was confirmed by t.l.c.

(2) *Coupling.* This was performed by use of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole,[11] in amounts equimolar to the carboxy-component; 'pre-activation' was carried out for 1 h at 0 °C and 1 h at room temperature, and the reaction normally proceeded overnight at 0 °C.

(3) *Isolation of coupled product.* (i) *By the citric acid procedure.* This followed the procedure described under 'General Synthetic Procedures' in Part XXXVI.[6]

(ii) *By use of Amberlyst-15.* This followed the procedure described under 'General Synthetic Procedures' in Part XXXVI,[6] with the following modifications. The resin (H^+ form) was saturated overnight with $2V$ ml (V = column volume) of a 10% solution of 3-bromopyridine in ethyl acetate. After removal of the dicyclohexylurea the filtrate was evaporated and the residue was taken up in ethyl acetate [for compounds (5)—(8), (16), and (29)], in chloroform [for compound (9)], or in dichloromethane [for compounds (17), (18), (21), (32), and (33)], and washed with water, aqueous sodium hydrogen carbonate, water, and brine, dried, and applied to the resin in ethyl acetate solution. For compounds (30) and (31) the residue (which was insoluble in water-immiscible solvents) was triturated with water and aqueous sodium hydrogen carbonate and then dried at 0.1 mmHg. The product was taken up in the minimum volume of freshly distilled dimethylformamide, and the solution was diluted with ethyl acetate (2—3 vol.) and applied to the Amberlyst resin. Transfer to the resin was effected in a vessel rotating slowly around a horizontal axis and fitted with ground glass stoppers at the top and bottom; to filter the solution, the lower stopper was replaced by a joint into which was sealed a porous glass filter, with a tap below. The time required for absorption (0.5—1 h in this work) was determined in each case by t.l.c. of a concentrated sample of the solution. The resin was then washed (in the same vessel) with ethyl acetate, and the product was eluted with pyridine (normally 30%) in dimethylformamide. The solvent was evaporated and the residue was triturated with dried ether (to remove 3-bromopyridine) and dried overnight at 0.1 mmHg and room temperature before analysis.

N(α)-*t-Butoxycarbonyl-N*(δ)-*piperidino-oxycarbonyl-*L-*ornithine Dicyclohexylammonium Salt.*—1,1,3,3-Tetramethylguanidine (5.75 g 50 mmol) was added to a stirred suspension of $N(\alpha)$-t-butoxycarbonyl-L-ornithine [18] [11.5 g, 50 mmol; prepared by hydrogenolysis of the $N(\delta)$-benzyloxycarbonyl derivative [19]] in dimethylformamide (30 ml). 1-Piperidyl 2,4,5-trichlorophenyl carbonate [12] (19.44 g, 60 mmol) was added in portions over 1 h and after a further 2 h the solution was evaporated. The residue was dissolved in chloroform (100 ml), washed with 0.7M-citric acid (3 × 100 ml) and water, and evaporated. The residue was dissolved in saturated aqueous sodium hydrogen carbonate, washed with ether, and acidified with solid citric acid; the liberated oil was extracted into ethyl acetate and the extract was washed with water and brine, dried, and evaporated. The crude product (which still contained trichlorophenol) was taken up in ether (150 ml) and dicyclohexylamine (50 mmol) was added. The *dicyclohexylammonium salt* (13.5 g, 50%) precipitated at 0—5 °C and was washed repeatedly with ether; it had m.p. 123—126°, $[\alpha]_D^{20}$ +12° (*c* 1 in EtOH); R_F 0.60 and 0.22 (dicyclohexylamine) (G3), 0.73 and 0.48 (A2) (Found: C, 62.1; H, 9.5; N, 10.15. $C_{28}H_{52}N_4O_6$ requires C, 62.2; H, 9.6; N, 10.4%).

N(α)-*Benzyloxycarbonyl-*N(δ)-*piperidino-oxycarbonyl-*L-*ornithine dicyclohexylammonium salt*, prepared analogously was recrystallised from isopropanol (yield 75%); it had m.p. 144—147°, $[\alpha]_D^{20}$ +9° (*c* 1 in EtOH); R_F 0.52 and 0.22 (dicyclohexylamine) (G3), 0.69 and 0.48 (A2) (Found: C, 64.8; H, 8.7; N, 9.5 $C_{31}H_{50}N_4O_6$ requires C, 64.8; H, 8.8; N, 9.7%).

N(α)-t-*Butoxycarbonyl-*N(δ)-*piperidino-oxycarbonyl-*L-*ornithine* 4-*Picolyl Ester.*—$N(\alpha)$-t-Butoxycarbonyl-$N(\delta)$-piperidino-oxycarbonyl-L-ornithine was liberated from its dicyclohexylammonium salt as usual by partitioning between ethyl acetate and 0.7M-citric acid, and the acid so obtained (9.0 g, 25 mmol) and 4-picolyl alcohol (2.73 g, 25 mmol) were dissolved in dichloromethane (10 ml). The

[17] K. Suzuki, M. Asaka, and T. Abiko, *Chem. and Pharm. Bull.* (*Japan*), 1966, **14**, 211.

[18] H. Arold and K. Haller, *J. prakt. Chem.*, 1969, **311**, 3.

[19] E. Schnabel, *Annalen*, 1967, **702**, 188.

solution was cooled to −3 °C and dicyclohexylcarbodiimide (5.15 g, 25 mmol) was added in portions over 2 h. Ethyl acetate was added to the solution at 0 °C and after 1 h the dicyclohexylurea was filtered off. The solution was evaporated and the residue was partitioned between ethyl acetate–ether (1 : 1; 50 ml) and citric acid (0.7M; 3 × 100 ml). The aqueous extracts were washed with ether and made alkaline (solid sodium hydrogen carbonate); the liberated oil was extracted into ethyl acetate, and the solution was dried and evaporated. The residue was recrystallised from ethyl acetate, giving the *ester* (8.4 g, 75%) m.p. 119—120.5°, $[\alpha]_D^{20}$ −16°; R_F 0.53 (E4), 0.52 (A2) (Found: C, 58.6; H, 7.55; N, 12.5. $C_{22}H_{34}N_4O_6$ requires C, 58.7; H, 7.6; N, 12.4%).

N(α)-*Benzyloxycarbonyl*-N(δ)-*piperidino-oxycarbonyl*-L-*ornithine* 4-*picolyl ester*, prepared analogously was a syrup (yield 87%), $[\alpha]_D^{20}$ +3° (*c* 1.2 in $CHCl_3$); R_F 0.65 (E4), 0.75 (Gl), 0.67 (M) (Found: C, 62.0: H, 6.8; N, 11.3. $C_{25}H_{32}N_4O_6$ requires C, 62.0; H, 6.6; N, 11.6%).

N(δ)-*Piperidino-oxycarbonyl*-L-*ornithine* 4-*Picolyl Ester Dihydrobromide*.—*N*(α)-Benzyloxycarbonyl-*N*(δ)-piperidino-L-ornithine 4-picolyl ester (3.87 g) was treated with hydrogen bromide in acetic acid (7.5%; 1 h) and the solution was poured into a large volume of ether; the precipitated solid was dissolved in water (4 ml) and the solution was poured into acetone (400 ml). The precipitated solid was hygroscopic; drying at room temperature and 0.1 mmHg overnight gave the *salt* (3.8 g, 84%), $[\alpha]_D^{20}$ −0.2°, $[\alpha]_{365}^{20}$ +4.3°, (*c* 0.9 in H_2O) (Found: C, 35.9; H, 5.85; Br, 28.2; N, 9.6. $C_{17}H_{28}N_4O_4Br_2,3H_2O$ requires C, 36.0; H, 6.1; Br 28.3; N, 9.9%).

N(α),N(δ)-*Di*-t-*butoxycarbonyl*-L-*ornithine Dicyclohexylammonium Salt*.—Prepared by the method of Schnabel,[19] the *salt* (92% yield) had m.p. 155—157°, $[\alpha]_D^{20}$ +10°; R_F 0.69 (G3), 0.30 (A2) (Found: C, 63.4; H, 9.65; N, 8.2. $C_{27}H_{51}N_3O_6$ requires C, 63.2; H, 9.9; N, 8.2%).

N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(δ)-*piperidino-oxycarbonyl*-L-*ornithine* 4-*Picolyl Ester* (9).—The t-butoxycarbonyl group was removed from the protected octapeptide (8) (Table 1) (600 mg, 0.47 mmol) and the free amino-component was liberated as usual. After removal of the excess of triethylamine from the dimethylformamide solution, *N*(α)-benzyloxycarbonyl-*N*(ω)-nitro-L-arginine (218 mg, 0.615 mmol) was added, followed by 1-hydroxybenzotriazole until the pH (moist paper) was 5.0. Dicyclohexylcarbodi-imide (127 mg, 0.615 mmol) was added in portions over 1 h at 0 °C. Next day the dicyclohexylurea was removed, the solution was evaporated to dryness, and the residue was taken up in chloroform (30 ml); the solution was washed and dried as usual. The crude product was dissolved in dimethylformamide (1 ml) and purified on a Sephadex LH-20 column, giving the *protected nonapeptide* (9) (700 mg, 98%), $[\alpha]_D^{20}$ −49°; R_F 0.16 (E4), 0.32 (G3) (Found: C, 60.7; H, 6.6; N, 15.0. $C_{76}H_{96}N_{16}O_{17}$ requires C, 60.6; H, 6.4; N, 14.9%. Found: Ser, 0.87; Pro, 2.98; Gly, 1.03; Orn, 1.18; Arg, 0.81; Phe *2.00*).

N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(δ)-*piperidino-oxycarbonyl*-L-*ornithine* (10).—The 4-picolyl ester (9) was saponified with 0.1M-sodium hydroxide (3 equiv.) in dimethylformamide at room temperature during 15 min, giving the *acid* (10) (89%), $[\alpha]_D^{20}$ −32°; R_F 0.48 (A2), 0.69 (H) (Found: C, 57.5; H, 6.7; N, 14.6. $C_{70}H_{91}N_{15}O_{17},3H_2O$ requires C, 57.2; H, 6.6; N, 14.3%).

N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-L-*ornithine* (11) *Monoacetate*.—Sodium dithionite monohydrate (150 mg, 1.3 mmol) was added to a solution of the peptide acid (10) (284 mg, 0.2 mmol) in 80% acetic acid (4 ml). T.l.c. (solvent A2) indicated that removal of the piperidino-oxycarbonyl group was complete after 45 min at room temperature, and the solution was evaporated to dryness. The residue was dissolved in dimethylformamide–water (1 : 9) and stirred for 1 h with Amberlite IR-45 resin (acetate form); the solution and washings were evaporated to dryness and the crude product was purified on Sephadex LH-20 in dimethylformamide, giving the partially *protected nonapeptide* (11) *monoacetate* (240 mg, 89%), $[\alpha]_D^{20}$ −46° (*c* 0.5 in $Me_2N{\cdot}CHO$); R_F 0.09 (A2), 0.88 (G4); $E_{Arg.}^{1.8}$ 0.20, $E_{Arg.}^{6.4}$ 0.0 (Found: C, 57.3; H, 6.7, N, 14.5. $C_{66}H_{86}N_{14}O_{17},2H_2O$ requires C, 57.5; H, 6.4; N, 14.2%).

N(α)-*Benzyloxycarbonyl*-*N*(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(δ)-*acetimidoyl*-L-*ornithine* (12) *Monoacetate*.—The partially protected peptide (11) (100 mg, 0.067 mmol) was dissolved in dimethylformamide (0.5 ml) and the pH (moist paper) was brought to 9.5 with *NN*-di-isopropylethylamine. Ethyl acetimidate hydrochloride[20] (41.5 mg, 0.335 mmol) was added. Next day the solution still gave a colouration with fluorescamine; a further similar amount of acetimidate was added and the pH was again brought to 9.5 After a further 24 h the solution gave no colour with fluorescamine and was then evaporated; the residue was taken up in water containing 10% dimethylformamide (10 ml) and the solution was stirred for 1 h with Amberlite IR-45 resin (acetate form; 10 ml). The product was washed from the resin by dimethylformamide–water (1 : 9, 200 ml); the eluate was evaporated and the residue was dissolved in 50% acetic acid (1 ml) and purified on a column (1.5 × 90 cm) of Bio-Gel P4 (200—400 mesh). Elution with 50% acetic acid gave the *acetimidoyl derivative* (12) (79.4 mg, 85.5%), $[\alpha]_D^{20}$ −44° (*c* 0.42 in $Me_2N{\cdot}CHO$); R_F 0.87 (G4), 0.08 (A2) [Found: C, 57.8; H, 6.5; N, 14.6. $C_{68}H_{89}N_{15}O_{17},H_2O$ requires C, 58.1; H, 6.5; N, 14.95%. Found: Orn, 0.65, Pro, 2.95; Gly, 0.98; Phe, *2.00*; Ser, 0.87; Arg, 0.82; *N*(δ)-acetimidoylornithine, 0.39]. The compound gave a deep orange colouration with nitroprusside reagent.

L-*Arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(δ)-*acetimidoyl*-L-*ornithine* (14) *Triacetate*.—The protected peptide (12) (65 mg, 0.47 mmol) in 80% acetic acid was hydrogenolysed over palladium–charcoal (10%; 10 mg) for 24 h. The product was purified on a column (1.2 × 38 cm) of carboxymethylcellulose CM-32, by using trimethylammonium acetate buffer (0.05M; pH 5.0), with gradient elution to 0.6M buffer of pH 7.0 (100 ml mixing vessel). Traces of buffer remaining in the peptide were removed by a column of Bio-Gel P4 (200—400 mesh), with 50% acetic acid as solvent, giving [9-N(δ)-*acetimidoyl*-L-*ornithine*]-*bradykinin* (14) *triacetate* (49 mg, 84.5%), $[\alpha]_D^{20}$ −84° (*c* 0.44 in H_2O); R_F 0.50 (H), 0.73 (G4); $E_{Arg.}^{1.8}$ 0.88, $E_{Arg.}^{6.44}$ 0.73 [Found: C, 48.5; H, 7.0; N, 13.8. $C_{57}H_{86}N_{14}O_{17},10H_2O$ requires C, 48.3; 7.3; N, 13.8. Found: Orn, 0.67; Pro, 2.98; Gly 1.04; Phe, *2.00*; Ser, 0.86; Arg, 0.98; *N*(δ)-acetimidoylornithine, 0.40].

[20] F. H. Suydam, W. E. Greth, and N. R. Langerman, *J. Org. Chem.*, 1969, **34**, 292.

N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*methyl*-L-*arginine* (13) *Monoacetate*.—To a solution of the partially protected peptide (11) (100 mg, 0.067 mmol) and *N*-methyl-*S*-methylisothiouronium iodide [21] (77.7 mg, 0.335 mmol) in dimethylformamide (0.5 ml) was added sufficient *NN*-di-isopropylethylamine to bring the pH (moist indicator paper) to 10.5. Next day the solution still gave a colouration with fluorescamine and a further similar quantity of thiouronium derivative was added. After a further 48 h the solution gave no colour with fluorescamine and was evaporated, and the product was isolated as described for the acetimidoyl derivative (12), giving the *protected nonapeptide* (13) *monoacetate* (90.6 mg, 96%), $[\alpha]_D^{20}$ −52° (*c* 0.57 in $Me_2N{\cdot}CHO$): R_F 0.89 (G4), 0.67 (H) [Found: C, 56.8; H, 6.7; N, 15.2. $C_{68}H_{90}N_{16}O_{17},2H_2O$ requires C, 56.75; H, 6.5; N, 15.6%. Found: Arg + *N*(ω)-methylarginine, 1.64; Orn, 0.40; Pro, 3.06; Gly, 1.01; Phe, 2.00; Ser, 0.88].

L-*Arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*methyl*-L-*arginine* (15) *Triacetate*. Compound (13) (70 mg) was hydrogenolysed and the product was isolated as described for compound (14), except that elution from the CM-32 column was performed with buffer of pH 10.2 (0.05—0.6M); traces of buffer were removed on Bio-Gel P4 in 50% acetic acid, giving [9-N(ω)-*methyl*-L-*arginine*]-*bradykinin* (15) *triacetate* (55 mg, 90%), $[\alpha]_D^{20}$ −81° (*c* 0.47 in H_2O), R_F 0.51 (H), 0.74 (G4); $E_{Arg.}^{1.8}$ 0.88, $E_{Arg.}^{6.44}$ 0.74 [Found: C, 48.4; H, 6.9; N, 15.2. $C_{57}H_{87}N_{15}O_{17},9H_2O$ requires C, 48.4; H, 7.4; N, 14.85%. Found: Arg, 1.05; Orn, 0.16; Pro, 2.99; Gly, 1.03; Phe, *2.00*; Ser, 0.86; *N*(ω)-methylarginine, 0.81].

N(α)-*Benzyloxycarbonyl*-N(δ)-*piperidino-oxycarbonyl*-L-*ornithyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(δ)-*piperidino-oxycarbonyl*-L-*ornithine* 4-*Picolyl Ester* (16).—*N*(α)-Benzyloxycarbonyl-*N*(δ)-piperidino-oxycarbonyl-L-ornithine was liberated from its dicyclohexylamine salt by partitioning between ethyl acetate and 0.7M-citric acid and coupled to the amino-component derived from protected octapeptide (8) (Table 1) (1.54 g, 1.21 mmol) in the usual way; the product was isolated by the Amberlyst procedure (solvent ethyl acetate), giving the *protected nonapeptide* (16) (1.60 g, 86%), $[\alpha]_D^{20}$ −46°, R_F 0.52 (E4), 0.56 (A2) (Found: C, 61.6; H, 6.9; N, 12.5. $C_{81}H_{104}N_{14}O_{17},2H_2O$ requires C, 61.5; H, 6.8; N, 12.4%. Found: Orn, 2.18; Pro, 3.03; Gly, 1.02; Phe, *2.00*; Ser, 0.88).

L-*Ornithyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-L-*ornithine Triacetate*.—Protected peptide (16) (154 mg, 0.1 mmol) was hydrogenolysed and the product was isolated as described for compound (14), giving [1,9-*ornithine*]-*bradykinin triacetate* (98 mg, 79%), $[\alpha]_D^{20}$ −76° (*c* 0.34 in H_2O); R_F 0.50 (H), 0.83 (G4); $E_{Arg.}^{1.8}$ 0.90, $E_{Arg.}^{6.44}$ 0.78 (Found: C, 52.3; H, 7.2; N, 12.1. $C_{54}H_{80}N_{11}O_{17},5H_2O$ requires C, 52.1; H, 7.2; N, 12.4%. Found: Orn, 2.09; Pro, 2.98; Gly, 1.04; Phe, *2.00*; Ser, 0.88).

N(α)-*Benzyloxycarbonyl*-N(δ)-t-*butoxycarbonyl*-L-*ornithyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (18).— *N*(α)-Benzyloxycarbonyl-*N*(δ)-t-butoxycarbonyl-L-ornithine was liberated from its dicyclohexylamine salt [22] (0.985 g, 1.8 mmol) by partition between ethyl acetate and aqueous citric acid as usual and dissolved in dimethylformamide (8 ml) at 0 °C. 1-Hydroxybenzotriazole (0.243 g, 1.8 mmol) and dicyclohexylcarbodi-imide (0.371 g, 1.8 mmol) were added. After 1 h at 0 °C and 1 h at room temperature, the solution was added to the solution of amino-component prepared from *N*-t-butoxycarbonyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-*O*-benzyl-L-seryl-L-prolyl-L-phenylalanyl-*N*(ω)-nitro-L-arginine 4-picolyl ester (17) [1.50 g, 1.2 mmol, synthesised in 53% overall yield as described in Part XXXVI] by removal of the t-butoxycarbonyl group with trifluoroacetic acid and liberation of the free amino-component by triethylamine in dimethylformamide as usual. The coupling was completed overnight at 0 °C. The product was isolated by the Amberlyst procedure, with dichloromethane as solvent; elution with 40% pyridine in dimethylformamide gave the *protected nonapeptide* (18) (1.58 g, 88%) of $[\alpha]_D^{20}$ −52°; R_F 0.47 (E4), 0.42 (A2) (Found: C, 61.0; H, 6.5; N, 14.5. $C_{75}H_{95}N_{15}O_{17}$ requires C, 60.9; H, 6.4; N, 14.2%. Found: Orn, 1.18; Gly, 0.98; Phe, *2.00*; Ser, 0.86; Pro, 2.97; Arg, 0.80).

N(α)-*Benzyloxycarbonyl*-N(δ)-*acetimidoyl*-L-*ornithyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (19) *Diacetate*.—The *N*(δ)-t-butoxycarbonyl group was removed from the protected nonapeptide (18) (200 mg, 0.133 mmol) with trifluoroacetic acid at 0 °C as usual; the product was acetimidoylated with ethyl acetimidate and the derivative was isolated as described above for the [Orn [9]]-analogue (12). The *acetimidoylated peptide* (19) (173 mg, 82%) had $[\alpha]_D^{20}$ −39°; R_F0.05 (A2), 0.64 (H), 0.87 (G4) (Found: C, 58.0; H, 6.3; N, 14.3. $C_{76}H_{98}N_{16}O_{19},2H_2O$ requires C, 57.9; H, 6.5; N, 14.2%).

N(δ)-*Acetimidoyl*-L-*ornithyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-L-*arginine* (23) *Triacetate*.—Compound (19) was hydrogenolysed and the product was isolated as described for compound (14), giving [N(δ)-*acetimidoyl*-L-*ornithine*]-*bradykinin* (23) *triacetate* (119 mg, 90.5%); $[\alpha]_D^{20}$ −82° (*c* 0.5 in H_2O); R_F 0.49 (H), 0.85 (G4), 0.06 (A2); $E_{Arg.}^{1.8}$ 0.87; $E_{Arg.}^{6.5}$ 0.76, $E_{Arg.}^{10.0}$ 0.60 [Found: C, 51.6; H, 6.8; N, 14.45. $C_{57}H_8{}^9N_{14}O_{17},5H_2O$ requires C, 51.5; H, 7.2; N, 14.8%. Found: *N*(δ)-acetimidoylornithine, 0.50; Orn, 0.47; Pro, 2.97; Gly, 1.04; Phe *2.00*; Ser, 0.88; Arg, 0.99].

N(ω)-*Methyl*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-L-*arginine* (24) *Triacetate*.—The t-butoxycarbonyl group was removed from compound (18) (200 mg, 0.134 mmol) as usual and the resulting trifluoroacetate was dissolved in dimethylformamide (0.5 ml) and allowed to react with *N*-methyl-*S*-methylisothiouronium iodide as described for compound (13). The same isolation procedure gave the protected nonapeptide (20) (174 mg, 89%) [R_F 0.62 (H), 0.05 (A2)], which was hydrolysed directly, and the product was isolated and purified as described for compound (14), except that the CM-32 column was eluted with buffer of pH 10.5 (0.05—0.6M); buffer was removed by chromatography on Bio-Gel P4 (200—400 mesh) in 50% acetic acid, giving [1-N(ω)-*methyl*-L-*arginine*]-*bradykinin* (24) *triacetate* [104 mg, 71% calculated on compound (20)], $[\alpha]_D^{20}$ −84° (*c* 0.48 in H_2O); R_F 0.47 (H), 0.82 (G4), 0.07 (A2); $E_{Arg.}^{1.8}$ 0.89, $E_{Arg.}^{6.44}$ 0.74 [Found: C, 51.6; H, 6.8; N, 15.8. $C_{57}H_{86}N_{15}O_{17},4H_2O$ requires C, 51.7; H, 7.1; N, 15.9%. Found: *N*(ω)-

[21] M. Schenck, *Arch. Pharm.*, 1911, **249**, 478; *Z. physiol. Chem.*, 1912, **77**, 349.

[22] G. I. Tesser and R. Schwyzer, *Helv. Chim. Acta*, 1966, **49**, 1013.

methylarginine 0.82; Pro, 3.04; Gly, 1.03; Phe, *2.00*; Arg, 1.01; Orn, 0.17].

N(α),N(δ)-*Di-t-butoxycarbonyl-*L*-ornithyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-*O*-benzyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-*N(ω)*-nitro-*L*-arginine 4-Picolyl Ester* (21).—*N*(α),*N*(δ)-Di-t-butoxycarbonyl-L-ornithine was liberated from its dicyclohexylammonium salt (133 mg, 0.26 mmol) by partitioning between ethyl acetate and aqueous citric acid as usual. The product was coupled to the protected octapeptide (17) (200 mg, 0.163 mmol) from which the t-butoxycarbonyl group had been removed, by the standard procedure (see ' General Synthetic Procedures '). The product was isolated by the Amberlyst method (solvent dichloromethane), giving the *protected nonapeptide* (21) (222 mg, 88%), $[\alpha]_D^{20}$ −56° (*c* 0.8 in Me_2N·CHO); R_F 0.52 (E4), 0.56 (A2), 0.55 (G3), 0.59 (N) (Found: C, 59.9; H, 6.5; N, 14.7. $C_{72}H_{97}N_{15}O_{17}$ requires C, 59.9; H, 6.7; N, 14.55%. Found: Orn, 1.20; Pro, 2.99; Gly, 1.01; Phe, *2.00*; Ser, 0.87; Arg, 0.78).

N(α)-*Amidino-*L*-arginyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-O-benzyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-N(ω)-nitro-*L*-arginine 4-Picolyl Ester* (22) *Triacetate.*—The t-butoxycarbonyl groups were removed from compound (21) (194 mg, 0.125 mmol) as usual; the trifluoroacetate was dissolved in aqueous dimethylformamide (50%; 3 ml) and triethylamine (76 mg, 0.75 mmol) was added. After 10 min the excess of triethylamine was removed at a water-pump and 1-amidino-3,5-dimethylpyrazole nitrate [14] (150 mg, 0.75 mmol) was added. After 2 days more pyrazole (150 mg) was added; after 4 more days, the solution gave no colour with fluorescamine, and the product was isolated and purified as described for compound (12), giving the *protected α-amidino-peptide* (22) (150 mg, 80%), $[\alpha]_D^{20}$ −42°; R_F 0.04 (A2), 0.59 (H), 0.77 (G4) (Found: C, 53.8; H, 6.1; N, 16.9. $C_{70}H_{93}N_{19}O_{19},3H_2O$ requires C, 53.95; H, 6.4; N, 17.1%. Found: Arg. 0.80; Pro, 2.98; Gly, 1.04; Phe, *2.00*; Ser, 0.87; Orn, 0.18).

N(α)-*Amidino-*L*-arginyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-*L*-arginine* (25) *Triacetate.*—Compound (22) (100 mg, 0.066 mmol) was hydrogenolysed and the product was isolated and purified as described for compound (14), giving [1-N(α)-*amidino-*L*-arginine]-bradykinin* (25) (85.4 mg, 93%), $[\alpha]_D^{20}$ −76.5° (*c* 0.48 in H_2O); R_F 0.07 (A2), 0.61 (H), 0.79 (G4); $E_{Arg.}^{1.8}$ 0.81, $E_{Arg}^{6.44}$ 0.76 (Found: C, 49.3; H, 7.2; N, 17.3. $C_{57}H_{88}N_{17}O_{17},6H_2O$ requires C, 49.2; H, 7.2; N, 17.1%. Found: Arg, 0.99; Pro, 2.97; Gly, 1.03; Phe, *2.00*; Ser, 0.88).

N(α)-t-*Butoxycarbonyl-*N(ε)*-benzyloxycarbonyl-*L*-lysyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-O-benzyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-*N(ω)*-nitro-*L*-arginine 4-Picolyl Ester* (26).— *N*(α)-t-Butoxycarbonyl-*N*(ε)-benzyloxycarbonyl-L-lysine was liberated from its dicyclohexylammonium salt [19] (146 mg, 0.26 mmol) by partitioning between ethyl acetate and aqueous citric acid as usual. The product was coupled as usual to the protected octapeptide (17) (200 mg, 0.163 mmol) from which the t-butoxycarbonyl group had been removed by trifluoroacetic acid. The product was isolated by the Amberlyst method (solvent dichloromethane), giving the *protected nonapeptide* (26) (216 mg, 87%), $[\alpha]_D^{20}$ −51°; R_F 0.26 (E4), 0.39 (A2), 0.37 (G3) (Found: C, 60.85; H, 6.8; N, 14.0. $C_{76}H_{97}N_{15}O_{17}$ requires C, 61.2; H, 6.5; N, 14.1%. Found: Lys + Orn, 1.18; Pro, 3.10; Gly, 0.99; Phe, *2.00*; Ser, 0.86; Arg, 0.79).

N(α)-*Amidino-*N(ε)*-benzyloxycarbonyl-*L*-lysyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-O-benzyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-*N(ω)*-nitro-*L*-arginine 4-Picolyl Ester* (27) *Diacetate.*—This was prepared from compound (26) (140 mg, 0.094 mmol) as described for the preparation of the arginine analogue (22), giving the *protected α-amidino-peptide* (27) (127 mg, 87%), $[\alpha]_D^{20}$ −47°, R_F 0.63 (H), 0.85 (G4), and 0.12 (A2) (Found: C, 59.0; H, 6.5; N, 15.4. $C_{76}H_{99}N_{17}O_{19}$ requires C, 58.7; H, 6.4; N, 15.3%. Found: Lys + Orn, 0.36; Pro, 3.07; Gly, 0.98; Phe, *2.00*; Ser, 0.86; Arg, 0.79).

N(α)-*Amidino-*L*-lysyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-*L*-arginine* (28) *Triacetate.*—Compound (27) (80 mg, 0.052 mmol) was hydrogenolysed and the product was isolated and purified as described for compound (14), giving [1-N(α)-*amidino-*L*-lysine]-bradykinin* (28) *triacetate* (63.6 mg, 91%), $[\alpha]_D^{20}$ −86.5° (*c* 0.49 in H_2O); R_F 0.66 (A2), 0.64 (H), 0.78 (G4); $E_{Arg.}^{1.8}$ 0.86, $E_{Arg.}^{6.44}$ 0.74 (Found: C, 50.8; H, 6.7; N, 15.8. $C_{57}H_{87}N_{15}O_{17},5H_2O$ requires C, 50.9; H, 7.2; N, 15.65%. Found: Pro, 3.00; Ser, 0.88; Gly, 1.01; Phe, *2.00*; Arg, 0.97).

N(α)-t-*Butoxycarbonyl-*N(ω)*-nitro-*L*-arginyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-*L*-ornithyl-*L*-prolyl-*L*-phenylalanyl-*N(ω)*-nitro-*L*-arginine 4-Picolyl Ester* (35) *Diacetate.*—The *N*(δ)-piperidino-oxycarbonyl group was removed from compound (34) (Table 2) (400 mg) by sodium dithionite (reaction time 1 h) and the product was isolated as described for compound (11), giving the *protected nonapeptide* (35) (344 mg, 87%), $[\alpha]_D^{20}$ −35°; R_F 0.10 (A2), 0.66 (H) (Found: C, 52.5; H, 6.75; N, 18.3. $C_{67}H_{97}N_{20}O_{20},2H_2O$ requires C, 52.3; H, 6.6; N, 18.2. Found: Arg, 1.61; Pro, 3.00; Gly, 0.97; Phe, *2.00*; Orn, 1.37).

N(α)-t-*Butoxycarbonyl-*N(ω)*-nitro-*L*-arginyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-*N(δ)*-3-carboxypropionyl-*L*-ornithyl-*L*-prolyl-*L*-phenylalanyl-*N(ω)*-nitro-*L*-arginine 4-Picolyl Ester* (36).—Succinic anhydride (84 mg, 0.84 mmol) was added to a solution of compound (35) (250 mg, 0.168 mmol) in dimethylformamide (3 ml) containing triethylamine (50.5 mg, 0.5 mmol) at 0 °C. After 1 h the solution was passed down a column of Sephadex LH-20 (135 × 2 cm) and the peptide was eluted with dimethylformamide, giving the *protected nonapeptide* (36) (258 mg, 97%), $[\alpha]_D^{20}$ −40°; R_F 0.37 (A2), 0.88 (G4), 0.09 (N) (Found: C, 54.6; H, 6.6; N, 18.0. $C_{67}H_{93}N_{19}O_{19}$ requires C, 54.8; H, 6.3; N, 18.1%. Found: Arg, 1.64; Pro, 2.98; Gly, 1.04; Phe, *2.00*; Orn, 1.35).

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-ornithyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate.— Compound (34) (Table 2) (138 mg) was hydrogenolysed as usual, but in solution in 80% trifluoroacetic acid; the product was isolated as for compound (14), giving [6-L-*ornithine*]-*bradykinin triacetate* (98 mg, 80%), $[\alpha]_D^{20}$ −85° (*c* 0.5 in H_2O); R_F 0.43 (H), 0.74 (G4); $E_{Arg.}^{1.8}$ 1.24, $E_{Arg.}^{6.44}$ 1.06 (Found: C, 52.3; H, 7.2; N, 15.8. $C_{60}H_{94}N_{16}O_{18},3H_2O$ requires C, 52.2; H, 7.25; N, 16.2%. Found: Arg, 1.98; Pro, 3.00; Gly, 0.97; Phe *2.00*; Orn, 1.04).

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-N(δ)-3-carboxypropionyl-L-ornithyl-L-prolyl-L-phenylalanyl-L-arginine Diacetate.—Compound (36) (100 mg) was hydrogenolysed as usual, but in solution in 80% trifluoroacetic acid; the product was isolated as for compound (14), giving [6-(3-*carboxypropionyl*)-L-*ornithine*]-*bradykinin diacetate* (76 mg, 80%), $[\alpha]_D^{20}$ −76° (*c* 0.52 in H_2O); R_F 0.76 (G4), 0.52 (H); $E_{Arg.}^{1.8}$ 0.88, $E_{Arg.}^{6.44}$ 0.45 (Found: C, 51.3; H, 7.0; N, 16.4. $C_{60}H_{90}N_{16}O_{17},5H_2O$ requires C, 51.6; H, 7.2; N, 61.1%. Found: Arg, 2.03; Pro, 2.99; Gly, 1.04; Phe, *2.00*; Orn, 1.02).

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