BIOORGANIC CHEMISTRY 8, 333–338 (1979)

Amino Acids and Peptides

Part XLIII[1]. Structure–Activity Studies in the Bradykinin Series: Synthesis of Analogs Modified in Position[2]

VIRANDER S. CHAUHAN AND GEOFFREY T. YOUNG[3]

The Dyson Perrins Laboratory, Oxford University, Oxford, U.K.

Appendix by NORMAN G. BOWERY

Department of Pharmacology, St. Thomas' Hospital Medical School, London, U.K.

Received February 5, 1979

β-Alanyl-, acetimidoyl-, and carbamoyl-bradykinin [(**IV**), (**V**), and (**VI**), respectively] have been synthesized by the picolyl ester method. The first two analogs have very low smooth muscle contracting activity, but the carbamoyl derivative is as fully active as bradykinin itself.

The structural requirements in the neighborhood of the terminal amino group of the local tissue hormone bradykinin (**I**) are not yet clear. 1-Deamino-bradykinin (*1*) and $N(\alpha)$-acetyl-bradykinin (*2*) are both biologically active; but we found recently (*3*) that the $N(\alpha)$-*t*-butoxycarbonyl derivative has no significant activity, suggesting steric restrictions. Further, we have shown (*4*) that $N(\alpha)$-amidino-bradykinin has marked smooth muscle-contracting activity, approximately twice that of the natural hormone on the isolated rat uterus (but indistinguishable from it on the guinea pig ileum). We have therefore examined the effect of further changes in this area, substituting the amino-group with β-alanyl, acetimidoyl, and carbamoyl residues.

The protected nonapeptide (**II**), which has been described in earlier parts of this work (*3*, *5*), was again prepared stepwise by the picolyl ester method (*6*) with certain improvements in procedure; it was obtained in an overall yield of 45% (calculated on nitroarginine 4-picolyl ester dihydrobromide). Removal of the *t*-butoxycarbonyl group followed by reaction with benzyloxycarbonyl-β-alanine 2,4,5-trichlorophenyl ester, ethyl acetimidate, and potassium cyanate, respectively, effected the required substitution on the terminal amino group while other functional groups remained protected, and hydrogenolysis then provided the bradykinin derivatives (**IV**), (**V**), and (**VI**). Attempts to prepare $N(\alpha)$-methylamidino-bradykinin by reaction of the terminal amino group with 1-*N*-methylamidinium-3,5-dimethylpyrazole iodide failed; the preparations of this reagent, and also 1-*N*,*N'*-dimethylamidinium-3,5-dimethylpyrazole iodide, are described.

[1] Part XLII, D. M. Bratby, S. Coyle, R. P. Gregson, G. W. Hardy, and G. T. Young, *J. C. S. Perkin I*, in press.

[2] This paper is dedicated to the memory of George W. Kenner, in admiration of his achievements in peptide chemistry, and in affection for a valued friend.

[3] To whom correspondence should be addressed.

0045–2068/79/030333–06$02.00/0

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

(I)

R-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-Arg(NO_2)-O Pic

(II) R = Boc-Arg(NO_2); (III) R = Boc

R-NH · CH · CO-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
|
$(CH_2)_3$
|
NH
|
$NH_2 \cdot C : NH_2^+$

(IV) R = $NH_2 \cdot CH_2 \cdot CH_2 \cdot CO$
(V) R = $CH_3 \cdot C(:NH_2^+)$
(VI) R = $NH_2 \cdot CO$

Abbreviations follow the I.U.P.A.C–I.U.B. rules. Amino acids are of the L-configuration. Pic = 4-picolyl.

SCHEME 1

The results of biological tests on these three analogs are reported in the appendix. It will be seen that the insertion of a carbamoyl group into the terminal amino group did not appreciably affect the contractile activity (the effect on blood pressure was complicated by a secondary pressor response); this contrasts with the very low activity of oxytocin after *N*-carbamoylation (*7*). *β*-Alanyl- and acetimidoyl-bradykinin were almost inactive. It seems that, although no amino group is necessary here, there is more than a restriction on the size of substituents in this area (as assumed in the case of *t*-butoxycarbonyl) but also a restriction on the nature of the substituent; amidino, carbamoyl, and acetyl are acceptable, but *β*-alanyl and acetimidoyl are not. None of the analogs antagonized responses to bradykinin.

EXPERIMENTAL

The general instructions in Refs. (*3*) and (*4*) apply except that tlc was on Merck silica 60, F-254 plates using the following solvent systems (proportions by volume): (A2) *n*-butanol, acetic acid, water (10 : 1 : 3); (E4) methanol, chloroform (1 : 10); (G2) ethyl acetate (40 vol), pyridine, acetic acid, water (20 : 6 : 11, 60 vol); (G3) and (G4), as (G2) but in proportions 120 to 40 vol and 20 to 80 vol, respectively; (H) *n*-butanol, pyridine, acetic acid, water (15 : 10 : 3 : 12).

N(*α*)-*t-Butoxycarbonyl-N*(*ω*)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl-O-benzyl*-L-*seryl*-L-*phenylalanyl-N*(*ω*)-*nitro*-L-*arginine 4-Picolyl Ester* (II). This was prepared by a stepwise route from nitro-L-arginine 4-picolyl ester dihydrobromide (*6*). Some improvements of the earlier synthesis (*3*, *5*) were made. *t*-Butoxycarbonyl groups were removed by trifluoroacetic acid; all the coupling reactions used dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole in dimethylformamide solution;

the general synthetic procedures were as described in Ref. (*4*). Isolation of the coupling product up to and including the tetrapeptide stage was by extraction into aqueous citric acid and in subsequent stages was by absorption on Amberlyst-15 (3-bromopyridinium form), from ethyl acetate or dichloromethane solution; in each case the product so obtained was pure as shown by tlc (solvents A2, E4, G3) and elemental analysis. The constants of the protected intermediates agreed satisfactorily with those reported earlier (*5*) except that for the protected octapeptide (**III**) we found $[\alpha]_D^{20}$ −47° (*c* 1 in $Me_2N \cdot CHO$) instead of −63°. In view of the observed rotations of closely related compounds, particularly analogs in which each and both of the phenylalanine residues are replaced by β-cyclohexylalanine [$[\alpha]_D^{20}$ −48°, −50°, −50°, (*3*)] we believe the figure reported here to be correct. For the protected nonapeptide (**II**) itself we found $[\alpha]_D^{20}$ −48° (*c* 1 in $Me_2N \cdot CHO$) [*cf* $[\alpha]_D^{20}$ −51° for the *N*(α)-benzyloxycarbonyl analog (*5*)]. [Found for the protected nonapeptide (**II**): C, 55.8; H, 6.6; N, 16.9. Calc. for $C_{68}H_{90}N_{18}O_{17}$, $2H_2O$: C, 55.7; H, 6.4; N, 17.2%. Found after acid hydrolysis: Arg, 1.90; Orn, 0.06; Pro, 3.01; Gly, 0.97; Phe, 1.97; Ser, 0.92.] The yields at successive stages for the introduction of one amino acid residue were 86, 86, 85 89, 94, 92, 86, and 91%, giving an overall yield (calculated on nitroarginine 4-picolyl ester dihydrobromide) of 45%.

N-Benzyloxylcarbonyl-β-alanine 2,4,5-Trichlorophenyl Ester. Dicyclohexylcarbodiimide (0.94 g, 4.8 mmol) was added to a solution of benzyloxycarbonyl-β-alanine (*8*) (1.0 g, 4.5 mmol) and 2,4,5-trichlorophenol (0.947 g, 4.8 mmol) in ethyl acetate (20 ml) at 0°C. The temperature was allowed to rise and after 4 hr at room temperature the solution was filtered and evaporated; the residue was recrystallized from ethyl acetate–light petroleum, giving *ester* (needles; 1.65 g, 92%) of mp 78–79°C, R_f 0.95 (G3), 0.95 (E4). (Found: C, 50.8; H, 3.7; Cl, 26.7; N, 3.5; $C_{17}H_{14}Cl_3NO_4$ requires C, 50.7; H, 3.5; Cl, 26.4; N, 3.5%.)

*N(α)-Benzyloxycarbonyl-β-alanyl-N(ω)-nitro-*L*-arginyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-O-benzyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-N(ω)-nitro-*L*-arginine 4-Picolyl Ester.* The protected nonapeptide (**II**) (146 mg, 0.1 mmol) was dissolved in trifluoroacetic acid at 0°C and after 10 min the solution was evaporated; the residue was taken up in dimethylformamide (2 ml), triethylamine (0.07 ml) was added, and the excess was then evaporated. Benzyloxycarbonyl-β-alanine 2,4,5-trichlorophenyl ester (45 mg, 0.11 mmol) was added and after 12 hr at 0°C the reaction mixture was evaporated. The residue was triturated with ether, giving *protected decapeptide* (140 mg; 86%), $[\alpha]_D^{20}$ − 39° (*c* 0.6 in $Me_2N \cdot (CHO)$; R_f 0.45 (A2), 0.20 (G3), 0.39 (H). (Found: C, 54.9, H, 6.2; N, 15.9. $C_{74}H_{93}N_{19}O_{17}$, $6H_2O$ requires C, 54.5; H, 6.4; N, 16.3%. Found after acid hydrolysis: Arg, 1.80; Orn, 0.10; Pro, 3.01; Gly, 1.01; Ser, 0.90; Phe, 1.95; β-Ala, 0.95.)

*β-Alanyl-*L*-arginyl-*L*-prolyl-*L*-prolyglycyl-*L*-phenylalanyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-*L*-arginine* (**IV**) *Triacetate* (*β-Alanyl-bradykinin*). The fully protected decapeptide described above (120 mg, 0.073 mmol) in 80% acetic acid (3 ml) was hydrogenolyzed over palladium–charcoal (10%, 20 mg) as usual for 30 hr. The product was purified on a carboxymethyl-cellulose CM-32 column in trimethylammonium acetate buffer (0.05 *M*, pH 5.0) with gradient elution to 0.6 *M* buffer, pH 7.0. Much of the buffer was removed by evaporation from the fractions containing peptide, and the remainder was removed on a column of Bio-Gel P2 (200–400 mesh) with 5% acetic acid as solvent,

giving *β-alanyl-bradykinin* (**IV**) *triacetate* (85 mg, 86%); $[\alpha]_D^{20}$ −75° (*c* 0.89 in H_2O); R_f 0.42 (H); 0.30 (G2); 0.95 (G4); 0.09 (A2); $E_{Arg}^{1.8}$ 0.77. [Found: C, 52.2; H, 7.1; N, 15.65. $C_{59}H_{90}N_{16}O_{18}$, $4H_2O$ requires C, 51.9; H, 7.1; N, 16.4%. Found after acid hydrolysis: Pro, 2.96; Ser, 0.89; Gly, 1.01; Phe, 2.01; Arg, 1.82; Orn, 0.08; β-Ala, 0.94.]

*N(α)-Acetimidoyl-*L*-arginyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-*L*-arginine* (**V**) *Triacetate.* The *N(α)-t*-butoxycarbonyl group was removed from the protected nonapeptide (**II**) (150 mg, 0.10 mmol) by means of trifluoroacetic acid in the usual way; the trifluoroacetate salt was dissolved in dimethylformamide (2 ml) and the apparent pH of the solution was brought to 10.0 (moist indicator paper) by the addition of triethylamine. Ethyl acetimidate hydrochloride (*9*) (55 mg, 0.515 mmol) was added and the solution stirred at rt. Next day a further similar amount of acetimidate was added and the pH was adjusted to 10, and after 12 hr fluorescamine showed amino groups to be absent. The solution was evaporated, the residue was dissolved in aqueous dimethylformamide (10%, 10 ml), and chloride ion was removed by means of Amberlite IR-45 resin (acetate form, 15 ml). The product was washed from the resin by aqueous dimethylformamide (10%), the solvent was evaporated, and the residual product was hydrogenolyzed in 80% acetic acid with palladium charcoal (10%, 25 mg). The product was purified on carboxymethyl-cellulose CM-32 with trimethylammonium acetate buffer (0.05 *M*, pH 5.0) with gradient elution to 0.6 *M* buffer at pH 7.0. After evaporation the peptide-containing fractions were dissolved in 5% acetic acid and desalted on Bio-Gel P2 (200–400 mesh), giving *N(α)-acetimidoyl-bradykinin triacetate* (90 mg, 61% overall); $[\alpha]_D^{20}$ −65.6° (*c* 0.4 in H_2O); R_f 0.85 (G4), 0.05 (A2); $E_{Arg}^{1.8}$ 0.85, E_{Arg}^{10} 0.60. [Found C, 51.1; H, 7.0; N, 15.9. $C_{58}H_{88}N_{16}O_{17}$, $4H_2O$ requires C, 51.5; H, 7.15; N, 16.5%. Found after acid hydrolysis: Arg, 1.41; Orn, 0.42; Pro, 2.9; Gly, 1.02; Ser, 0.92; Phe 2.0.]

*N(α)-Carbamoyl-N(ω)-nitro-*L*-arginyl-*L*-prolylglycyl-*L*-phenylalanyl-O-benzyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-N(ω)-nitro-*L*-arginine 4-Picolyl Ester.* The *N(α)-t*-butoxycarbonyl group was removed from protected nonapeptide (**II**) (250 mg, 0.17 mmol) by means of trifluoroacetic acid as usual; the resulting trifluoroacetate was dissolved in dimethylformamide (1 ml), triethylamine was added to bring the apparent pH (moist indicator paper) to 7.5, and potassium cyanate (20.6 mg) was added, together with a few drops of water to clear the solution. After 5 hr a further 21 mg of cyanate was added and the pH was adjusted to 7.5; after a further 3 hr tlc of a sample of the solution showed no ninhydrin-positive material and the solution was therefore evaporated; the residue was extracted with water and dried over phosphoric oxide, leaving the *N(α)-carbamoyl derivative* as a chromatographically pure solid (220 mg, 83%); $[\alpha]_D^{20}$ −46° (*c* 1 in $Me_2N \cdot CHO$); R_f 0.12 (E4); 0.25 (G3), 0.54 (A2). [Found: C, 53.7; H, 6.3; N, 18.4. $C_{64}H_{83}N_{19}O_{18}$, $3H_2O$ requires C, 53.9; H, 6.2; N, 18.7%. Found: Pro, 2.97; Gly, 0.94; Phe, 1.98; Ser, 0.84; Arg, 1.76; Orn, 0.08.]

*N(α)-Carbamoyl-*L*-arginyl-*L*-prolyl-*L*-prolylglycyl-*L*-phenylalanyl-*L*-seryl-*L*-prolyl-*L*-phenylalanyl-*L*-arginine* (**VI**) *Diacetate* (*N(α)-Carbamoyl-bradykinin*). The protected *N(α)*-carbamoyl-nonapeptide (150 mg, 0.10 mmol) was hydrogenolyzed in 80% acetic acid over palladium charcoal (10%, 30 mg) as usual and the product was purified on carboxymethyl-cellulose CM-32 in triethylammonium acetate buffer as described above for β-alanyl-bradykinin; the buffer was removed similarly by evaporation and finally by

Bio-Gel P4, giving *N(α)-carbamoyl-bradykinin* (**VI**) *diacetate* (93 mg, 71%); $[\alpha]_D^{20}$ −84° (*c* 0.42 in H_2O); R_f 0.40 (H); 0.87 (G4); 0.15 (A2); $E_{Arg}^{1.8}$ 0.65; $E_{Arg}^{6.4}$ 0.60. [Found: C, 51.1; H, 6.6; N, 17.0. $C_{55}H_{82}N_{16}O_{16}$, $4H_2O$ required C, 51.0; H, 6.9; N, 17.2%. Found: Pro, 2.98; Ser, 0.89; Gly, 1.02; Phe, 1.98; Arg, 1.72; Orn, 0.08.]

1-Amino-2-methylguanidinium Iodide. Hydrazide hydrate (64%, 1.25 ml) reacted with *N*-methyl-*S*-methyl-isothiouronium iodide (*10*) (1.16 g, 5.0 mmol) in water (2 ml) during 2 hr at 10°C and 1 hr at room temperature. The solution was evaporated to dryness; the residue was triturated with ether and the product was recrystallized from ethanol–ether, giving *iodide* (0.8 g, 74%) of mp 119–120°C (decomp.). (Found: C, 10.95; H, 3.9; N, 26.5; I, 58.5. $C_2H_9N_4I$ requires C, 11.1; H, 4.1; N, 25.9; I, 58.7%.)

1-Amino-2,3-dimethylguanidinium Iodide. This was prepared similarly from *N,N'*-dimethyl-*S*-methyl-isothiouronium iodide (*10*) (70% yield); the *iodide* had mp 282–284°C. (Found: C, 15.3; H, 5.0; N, 24.0; I, 55·3. $C_3H_{11}N_4I$ requires C, 15.6; H, 4.8; N, 24.35; I, 55.2%.)

1-N-Methylamidinium-3,5-dimethylpyrazole Iodide. A solution of 1-amino-2-methylguanidinium iodide (500 mg, 2.3 mmol) in ethanol–water (4:1, 2 ml) and acetylacetone (230 mg, 2.3 mmol) was refluxed for 3 hr; the solution was evaporated, the residue was triturated with dried ether and recrystallized from ethanol–ether, giving *iodide* (400 mg, 65%) of mp 195°C (decomp.). (Found: C, 30.3; H, 4.7; N, 20.2; I, 45.0. $C_7H_{13}N_4I$ requires C, 30.0; H, 4.7; N, 20.0; I, 45.3%.)

1-N,N'-Dimethylamidinium-3,5-dimethylpyrazole Iodide. This was prepared similarly from 1-amino-2,3-dimethylguanidinium iodide giving *product* of mp 149–150°C. (Found: C, 32.6; H, 5.45; N, 19.3; I, 42.75. $C_8H_{15}N_4I$ requires C, 33.0; H, 5.2; N, 19.3; I, 42.4%.)

APPENDIX

The three bradykinin analogs were assayed for biological activity on two isolated preparations, the rat uterus and guinea pig ileum, and *in vivo* on the blood pressure of the anaesthetized rat (urethane 1.4 g/kg intraperitoneally). The contractile activity in the isolated preparations and the depressor activity *in vivo* were obtained by comparing with authentic bradykinin (Sigma Ltd). 4 × 4-Point assays incorporating a Latin Square design were performed wherever possible but the low potency of the acetimidoyl derivative in all preparations and the β-alanyl derivative in the rat blood pressure test prevented such detailed analyses; instead, "bracket" assays were obtained in those cases. The results are summarized in Table 1; values in the table refer to separate experiments:

TABLE 1[a]

Analog	Rat uterus	Guinea pig ileum	Rat bp.
β-Alanyl-bradykinin	0.012	0.001	<0.001
	0.006	0.004	
Carbamoyl-bradykinin	0.94	1.00	0.1–0.2
		1.20	(see below)
Acetimidoyl-bradykinin	0.003	0.001	<0.001
		<0.001	

[a] Potency (bradykinin = 1).

The carbamoyl derivative produced a marked pressor effect following the initial depression. Bradykinin also produced a secondary pressor response but this was less than that produced by the same dose of the carbamoyl derivative. This pressor action probably resulted from stimulation of the adrenal medulla to release adrenaline [W. Feldberg and G. P. Lewis, *J. Physiol.* **171**, 98 (1964)]. In the presence of phenoxybenzamine (0.25 mg/kg) the pressor action of bradykinin and of the carbamoyl derivative were abolished and the potency of the latter compound as a depressant was then equivalent to that of bradykinin. None of the analogs produced any antagonism of responses to bradykinin in doses at or below the level producing responses themselves.

ACKNOWLEDGMENT

We thank the Rhodes Trustees for a Scholarship held by V.S.C., and Dr. D. F. Elliott for helpful discussions.

REFERENCES

1. W. H. JOHNSON, H. D. LAW, AND R. C. STUDER, *J. Chem. Soc.* (*C*) 748 (1971).
2. J. M. STEWART, *Fed. Proc.* **27**, 63 (1968).
3. G. A. FLETCHER AND G. T. YOUNG, *J. C. S. Perkin I* 1867 (1972).
4. T. G. PINKER, G. T. YOUNG, D. F. ELLIOTT, AND R. WADE, *J. C. S. Perkin I* 220 (1976).
5. D. J. SCHAFER, G. T. YOUNG, D. F. ELLIOTT, AND R. WADE, *J. Chem. Soc.* (*C*) 46 (1971).
6. R. CAMBLE, R. GARNER, AND G. T. YOUNG, *J. Chem. Soc.* (*C*) 1911 (1969); G. T. YOUNG, "The Chemistry of Polypeptides" (P. G. Katsoyannis, Ed.), p. 43. Plenum Press, New York, 1973.
7. D. G. SMYTH, *J. Biol. Chem.* **242**, 1579 (1967).
8. R. H. SIFFERD AND V. DU VIGNEAUD, *J. Biol. Chem.* **108**, 753 (1935).
9. F. H. SUYDAM, W. E. GRETH, AND N. R. LANGERMAN, *J. Org. Chem.* **34**, 292 (1969).
10. M. SCHENCK, *Arch. Pharm.* **249**, 463 (1911); *Z. physiol. Chem.* **77**, 328 (1912).