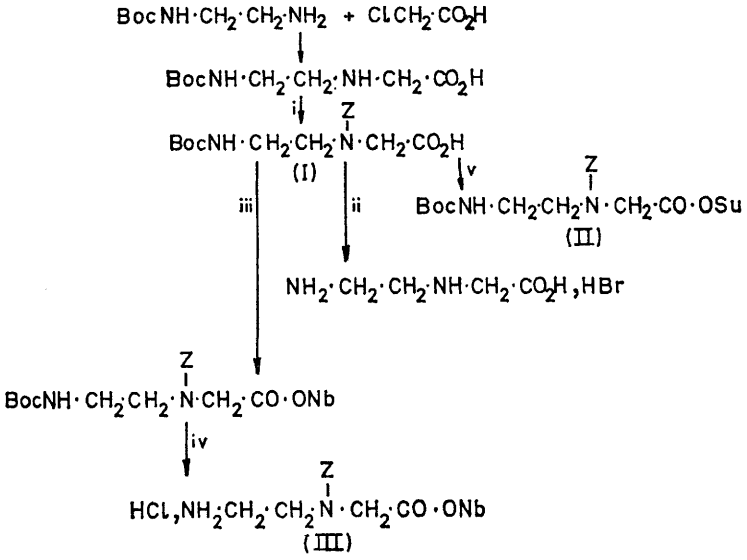
Synthesis of Peptides Containing *N*-2-Aminoethylglycine—' Reduction Analogues '

By **E. Atherton, H. D. Law,***† and **S. Moore,** Department of Chemistry, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF
D. F. Elliott and **R. Wade,** CIBA Laboratories, Horsham, Sussex

The synthesis and properties of peptides containing *N*-2-aminoethylglycine, the ' reduction analogue ' of glycylglycine, are described. The ' 3,4-reduction analogue ' of [3-glycine]-bradykinin was devoid of activity in the guinea-pig ileum assay.

SYNTHETIC analogues have been widely used for the study of structure–activity relationships of biologically active peptides.[1] For the most part, studies have involved modifications of the amino-acid sequences of the parent peptides. Apart from a few investigations in which amino-acid residues have been replaced by α-hydroxy-acid residues [2] or by aza-derivatives,[3] little attention has been paid to modifications of the peptide ' backbone ' itself. We felt that interesting compounds might be produced if a −CO·NH− group in a peptide were replaced, for example, by a secondary amino-group, a ketone group, or a reversed peptide bond. We now report the synthesis and properties of some peptides containing *N*-2-aminoethylglycine, which can be regarded as the ' reduction analogue ' of glycylglycine.

Suitably protected and activated forms of *N*-2-aminoethylglycine were obtained by standard methods. Thus, *N*-(benzyloxycarbonyl)-*N*-(2-t-butoxycarbonylaminoethyl)glycine (I), its *N*-hydroxysuccinimide ester (II) and the hydrochloride (III) of *N*-benzyloxycarbonyl-*N*-(2-aminoethyl)glycine *p*-nitrobenzyl ester were prepared according to Scheme 1.‡ No side reactions were observed when these compounds were converted into peptides; for example, *N*-[2-(L-phenylalanylamino)-ethyl]glycyl-L-phenylalanine (IV) was prepared from Boc-(Z)AEG (I) (Scheme 2).



SCHEME 1 Reagents: i, BocCl; ii, HBr–AcOH; iii, ClNb, NEt_3; iv, $CF_3·CO_2H$ followed by HCl; v, HOSu, DCC

We have used the *N*-hydroxysuccinimide ester (II) to synthesise [3,4-(*N*-2-aminoethylglycine)]-bradykinin by the route outlined in Scheme 3. The protected analogue (VII) was isolated by chromatography over silica gel. Hydrogenolysis gave the free peptide, which was purified by chromatography on carboxymethylcellulose in a gradient of ammonium acetate. The 3,4-reduction analogue of [3-glycine]-bradykinin was inactive in the

†*Present address:* Glasgow College of Techology, North Hanover Place, Glasgow C.4.

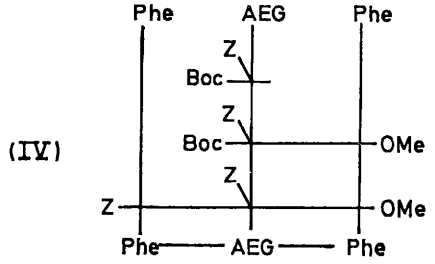
‡ Abbreviations throughout are in accord with the recommendations of the I.U.P.A.C.-I.U.B. Commission on Biochemical Nomenclature (I.U.P.A.C. Information Bulletin Nos. 26 and 27). In addition, ONb = O·CH_2·C_6H_4·NO_2-*p*; OSu = succinimidooxy; DCC = *NN'*-dicyclohexylcarbodi-imide; AEG = *N*-(2-aminoethyl)glycine; EDA = ethylenediamine.

[1] For reviews see *e.g.*, E. Schröder and K. Lübke, ' The Peptides,' Academic Press, London, vol. 2, 1966; J. P. Greenstein and M. Winitz, ' The Chemistry of the Amino Acids,' Wiley, New York, 1960—1961; H. D. Law, *Progr. Medicin. Chem.*, 1965, **4**, 86.

[2] L. A. Shchukina, S. A. Ravdel, M. P. Filatova, and A. L. Zhuze, *Acta Chim. Acad. Sci. Hung.*, 1965, **44**, 205; L. A. Shchukina, G. A. Ravdel, and M. P. Filatova, *Khim. prirod. Soedinenii*, 1966, **265**; M. M. Shemyakin, L. A. Shchukina, E. I. Vinogradova, S. A. Ravdel, and Yu. A. Ovchinnikov, *Experientia*, 1966, **22**, 535; C. Wasielewski, *Roczniki Chem.*, 1967, **41**, 1915.

[3] J. Gante, *Chem. Ber.*, 1965, **98**, 3334, 3340; 1966, **99**, 2521; H. Niedrich, *ibid.*, 1969, **102**, 1557.

guinea-pig ileum assay[4] and had no antagonistic properties towards bradykinin, but it is not possible to say whether this lack of activity should be ascribed to



SCHEME 2

the absence of the normal prolylglycyl peptide bond, or whether the absence of the pyrrolidine ring is more significant.

EXPERIMENTAL

The purity of all compounds was checked by t.l.c. on silica gel. R_F Values refer to the solvent systems as follows: R_F^a butan-1-ol–acetic acid–water (66 : 12 : 26 v/v); R_F^b butan-2-ol–3% aq. ammonia (120 : 44 v/v); R_F^c butan-1-ol–pyridine–acetic acid–water (90 : 18 : 60 : 72 v/v); R_F^d ethyl acetate–methanol (8 : 15 v/v). The plates were developed with ninhydrin and/or chlorine and starch–iodide.[6] Electrophoresis was carried out on Whatman 3MM paper (Locarte High Voltage Apparatus) with a potential gradient of 80 V cm^{-1}. Amino-acid analyses were obtained by use of Locarte or Beckman 120C analysers.

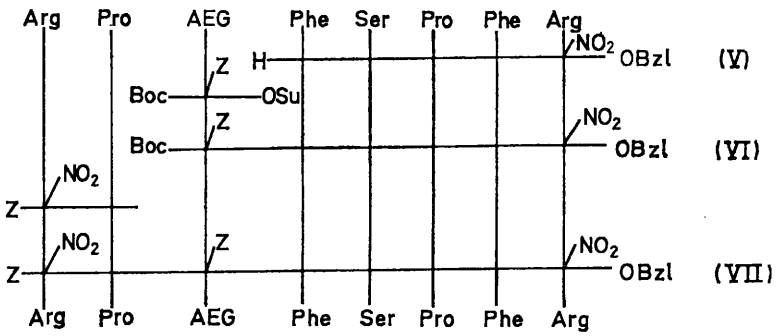
N-t-Butoxycarbonylethylenediamine.—A solution of t-butoxycarbonyl azide (12·0 g) in dioxan (100 ml) was added slowly to a stirred solution of ethylenediamine (30 ml) in dioxan (200 ml) and water (100 ml) in the presence of finely divided magnesium oxide (3 g). The mixture was stirred for 18 h at 40—50°, cooled, filtered, concentrated, and extracted with ether (4 × 200 ml). Boc-EDA (12·3 g, 91%) was obtained as a slightly impure oil (R_F^c 0·73) by evaporating the washed and dried extract. The impurity was shown by t.l.c. (R_F^c 0·87) and n.m.r. spectroscopy to be *NN'*-di-t-butoxycarbonylethylendiamine, which could be obtained in higher yield by increasing the proportion of azide in the reaction mixture; m.p. 137° (from ether) (Found: C, 55·4; H, 9·0; N, 10·5. $C_{12}H_{24}N_2O_4$ requires C, 55·4; H, 9·3; N, 10·8%).

N-(Benzyloxycarbonyl)-N-(2-t-butoxycarbonylaminoethyl)-glycine (I).—A solution of chloroacetic acid (11·54 g) in aqueous M-sodium hydroxide (122 ml) was added dropwise to a solution of Boc-EDA (12·31 g) in ethanol (95%; 30 ml), followed by more M-sodium hydroxide (61 ml). The mixture was stirred overnight at room temperature, the ethanol was evaporated off, and unchanged Boc-EDA (3 g) was extracted into ether. The remaining solution (75 ml) was cooled to 0°, and benzyloxycarbonyl chloride (9·5 g) was added during 1 h with the pH kept between 9 and 10 by addition of sodium hydroxide. After 4 h at room temperature the mixture was extracted with ether to remove excess of ZCl, cooled to 0°, acidified with cold citric acid solution (30%), and washed with ethyl acetate. Evaporation of the washed and dried ethyl acetate extract gave an oil which crystallised on trituration with petroleum. Recrystallisation from ether–petroleum gave the *protected amino-acid* (I) (8 g, 52%), m.p. 90—91°, R_F^c 0·77 (Found: C, 58·2; H, 6·7; N, 7·9. $C_{17}H_{24}N_2O_6$ requires C, 57·9; H, 6·9; N, 8·0%).

N-2-Aminoethylglycine Hydrobromide.—The protected amino-acid (I) (1·5 g) was dissolved in freshly distilled acetic acid (15 ml) and a solution of hydrogen bromide in acetic acid (5M; 15 ml) was added. After 1·25 h at room temperature the solution was poured into dry ether, and the product crystallised as the *monohydrobromide* (0·53 g), m.p. 188—190° (from aq. ethanol), R_F^a 0·11, R_F^b 0·08, R_F^c 0·53 (Found: C, 24·2; H, 5·4; N, 13·7. $C_4H_{11}BrN_2O_2$ requires C, 24·1; H, 5·6; N, 13·7%). The amino-acid emerges from the short column of the analyser after 35 min (pH 5·28 buffer) (lysine 38 min) (Locarte).

Arg—Pro—Pro—Gly—Phe—Ser—Pro—Phe—Arg

Bradykinin[5]



SCHEME 3

N-(Benzyloxycarbonyl)-N-(2-t-butoxycarbonylaminoethyl)-glycine N-Hydroxysuccinimide Ester (II).—Boc-(Z)AEG (I) (0·9 g) was dissolved in dimethoxyethane (10 ml), and *N*-hydroxysuccinimide (0·31 g) and DCC (0·6 g) were added.

[4] D. F. Elliott, E. W. Horton, and G. P. Lewis, *J. Physiol.*, 1960, **153**, 473.

[5] D. F. Elliott, G. P. Lewis, and E. W. Horton, (*a*) *Biochem. Biophys. Res. Comm.*, 1960, **3**, 87; (*b*) *Biochem. J.*, 1961, **78**, 60; R. A. Boissonas, St. Guttmann, and P. A. Jaquenoud, (*c*) *Helv. Chim. Acta*, 1960, **43**, 1349; (*d*) *Experientia*, 1960, **16**, 326.

[6] H. N. Rydon and P. G. Smith, *Nature*, 1952, **169**, 922.

After 48 h the urea was filtered off and the solvent evaporated to give the *active ester* (0·54 g, 49%), m.p. 109° (from ethyl acetate–petroleum), R_F[a] 0·75 (Found: C, 55·9; H, 6·15; N, 9·35. $C_{21}H_{27}N_3O_8$ requires C, 56·1; H, 6·05; N, 9·35%).

N-*Benzyloxycarbonyl*-N-(2-*aminoethyl*)*glycine* p-*Nitrobenzyl Ester Hydrochloride* (III).—*p*-Nitrobenzyl chloride (0·8 g) and triethylamine (0·65 ml) were added to a solution of Boc-(Z)AEG (I) (1·5 g) in ethyl acetate (10 ml), and the mixture was stirred at 60—70° for 24 h. Work-up in the usual manner gave an oil (1 g, 48%), R_F[c] 0·84, which could not be induced to crystallise. The oil was dissolved in trifluoroacetic acid at room temperature. After 1 h the acid was evaporated off and the resultant oil was dissolved in acetonitrile. Addition of excess of ethereal hydrogen chloride (1M) and ether caused the *hydrochloride* to crystallise (0·48 g), m.p. 155—160° (from acetonitrile), R_F[c] 0·76 (Found: C, 53·7; H, 5·2; N, 9·7. $C_{19}H_{22}ClN_3O_6$ requires C, 53·8; H, 5·2; N, 9·7%).

N-*Benzyloxycarbonyl*-N-(2-*t-butoxycarbonylaminoethyl*)-*glycyl*-L-*phenylalanine Methyl Ester*.—Triethylamine (1·4 ml), followed by DCC (2·1 g) and Boc-(Z)AEG (I) (3·1 g), was added to a solution of L-phenylalanine methyl ester hydrochloride (2·14 g) in the minimum amount of *NN*-dimethylformamide at −5°. The mixture was stirred overnight at 0—5°, filtered, poured into water, and extracted with ethyl acetate. Evaporation of the washed and dried extract gave an oil which crystallised on trituration with petroleum to give the *protected reduction analogue* (3·1 g, 67%), m.p. 115—116° (from ethyl acetate–petroleum), R_F[c] 0·80, $[\alpha]_D^{25}$ −3·6° (*c* 1 in Me_2N·CHO) (Found: C, 63·5; H, 6·7; N, 8·2. $C_{27}H_{35}N_3O_7$ requires C, 63·1; H, 6·9; N, 8·2%).

N-*Benzyloxycarbonyl*-N-[2-(N-*benzyloxycarbonyl*-L-*phenylalanylamino*)*ethyl*]*glycyl*-L-*phenylalanine Methyl Ester*.—(i) The foregoing t-butoxycarbonyl derivative (3·1 g) was treated in the usual way with trifluoroacetic acid followed by ethereal hydrogen chloride to give the hydrochloride salt (2·7 g, 100%), R_F[c] 0·69.

(ii) A solution of *N*-benzyloxycarbonyl-L-phenylalanine (1·8 g) in the minimum amount of *NN*-dimethylformamide was cooled to −15° prior to the addition of *N*-methylmorpholine (0·66 ml) and isobutyl chloroformate (0·87 ml). After 4 min the methyl ester hydrochloride (2·7 g), which had been preneutralised in *NN*-dimethylformamide solution with triethylamine (0·84 ml), was added to the resulting mixed anhydride. The mixture was stirred overnight, poured into water, and extracted with ethyl acetate. Evaporation of the washed and dried extract gave the *protected tetrapeptide reduction analogue* (3·8 g, 90%), m.p. 115—120° (from ethyl acetate–petroleum), $[\alpha]_D^{25}$ −10·4° (*c* 1 in Me_2N·CHO), R_F[c] 0·83 (Found: C, 67·1; H, 5·9; N, 8·3. $C_{39}H_{42}N_4O_8$ requires C, 67·4; H, 6·1; N, 8·1%).

N-*Benzyloxycarbonyl*-N-[2-(N-*benzyloxycarbonyl*-L-*phenylalanylamino*)*ethyl*]*glycyl*-L-*phenylalanine*.—The methyl ester (1 g) was stirred in solution in acetone (10 ml) and water (5 ml) containing sodium hydroxide (1M; 2 ml) at room temperature for 90 min, after which water was added and the mixture was extracted with ethyl acetate. Acidification of the aqueous layer with sulphuric acid (1M), followed by extraction with ethyl acetate and evaporation of the washed and dried extract, gave an oil which, upon trituration with ether, yielded the crystalline *tetrapeptide reduction analogue free acid* (0·46 g, 45%), m.p. 130—132° (from ethanol–petroleum), R_F[a] 0·69, R_F[c] 0·78, $[\alpha]_D^{25}$ −4·4° (*c* 1 in Me_2N·CHO), equiv. wt. (by titration) 678 (calc., 680) (Found: C, 66·6; H, 5·9; N, 8·1. $C_{38}H_{40}N_4O_8$ requires C, 67·0; H, 5·9; N, 8·2%). Evaporation of the ethereal washings gave a crystalline multicomponent mixture (0·25 g).

N-[2-(L-*Phenylalanylamino*)*ethyl*]*glycyl*-L-*phenylalanine* (IV).—The *N*-protected tetrapeptide analogue (0·4 g) was treated with a solution of hydrogen bromide in acetic acid in the usual way to yield the extremely hygroscopic *tetrapeptide reduction analogue salt* (0·2 g, 60%). This was homogeneous by t.l.c., R_F[a] 0·28, R_F[b] 0·28, R_F[c] 0·61, after reprecipitation from ethanol–ether. Amino-acid analysis: Phe 2·0; AEG 1·1.

N-*Benzyloxycarbonyl*-N-[2-(*t-butoxycarbonylamino*)*ethyl*]-*glycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanylnitro*-L-*arginine Benzyl Ester*.—The hydrochloride (2·01 g) of L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine benzyl ester [7] was dissolved in the minimum volume of *NN*-dimethylformamide, and triethylamine (0·34 ml) was added to the cooled solution followed by the *N*-hydroxysuccinimide ester (II) (1 g). After 24 h the mixture was poured into water and extracted into ethyl acetate. Evaporation of the washed and dried extract gave the *protected heptapeptide reduction analogue* (2·2 g), m.p. 100—110° (from acetone–ether), R_F[a] 0·77, R_F[d] 0·53 (Found: C, 58·9; H, 6·5; N, 13·9. $C_{56}H_{71}N_{11}O_{14},H_2O$ requires C, 59·0; H, 6·45; N, 13·5%).

N-*Benzyloxycarbonylnitro*-L-*arginyl*-L-*proline Methyl Ester*.—*N*-Benzyloxycarbonylnitro-L-arginine (6 g) was dissolved in the minimum amount of *NN*-dimethylformamide and the solution was cooled to −15° prior to the addition of *N*-methylmorpholine (1·9 ml) and isobutyl chloroformate (2·2 ml). After 4 min a preneutralised [triethylamine (2·8 ml)] solution of L-proline methyl ester (3·4 g of hydrochloride) in the minimum amount of *NN*-dimethylformamide was added. After 24 h at room temperature the solvent was evaporated off and the residue dissolved in ethyl acetate–water. Evaporation of the washed and dried organic phase gave the *protected dipeptide ester* (3·25 g, 45%), m.p. 155—156° (from propan-2-ol–petroleum), R_F[a] 0·64, $[\alpha]_D^{25}$ −21·5° (*c* 1 in Me_2N·CHO) (Found: C, 52·0; H, 6·4; N, 18·3. $C_{20}H_{28}N_6O_7$ requires C, 51·7; H, 6·1; N, 18·1%). Saponification of the ester and appropriate treatment gave the free acid (59%), m.p. 105—107°, $[\alpha]_D^{25}$ −28·9° (*c* 2 in Me_2N·CHO), R_F[a] 0·63 {lit.,[8] m.p. 108—110°, $[\alpha]_D^{20}$ −30° (*c* 2 in Me_2N·CHO); lit.,[5c] yield 51%, m.p. 119°, $[\alpha]_D^{22}$ −26·5° (*c* 2 in Me_2N·CHO)}.

N-*Benzyloxycarbonyl*-N-[2-(N-*benzyloxycarbonylnitro*-L-*arginyl*-L-*prolylamino*)*ethyl*]*glycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanylnitro*-L-*arginine Benzyl Ester*.—The mixed anhydride was generated by adding *N*-methylmorpholine (0·07 ml) and isobutyl chloroformate (0·099 ml) to a solution of the *NN'*-protected *N*-terminal dipeptide (0·34 g) in the minimum amount of *NN*-dimethylformamide at −15°. After 4 min this solution was added to a solution of the reduction analogue of the heptapeptide ester hydrochloride (0·73 g) (prepared from the t-butoxycarbonyl derivative with trifluoroacetic acid in the usual way) in the minimum amount of *NN*-dimethylformamide, followed by triethylamine (0·095 ml). After 24 h the mixture was worked up in the usual way. Evaporation of the ethyl acetate solution gave an amorphous solid (0·77 g). This

[7] W. H. Johnson, H. D. Law, and R. O. Studer, *J. Chem. Soc.* (*C*), 1971, 748.
[8] M. Ondetti, *J. Medicin. Chem.*, 1963, **6**, 10.

was subjected to column chromatography [silica gel (25 g); elution with increasing concentrations of methanol in ethyl acetate] to give the *protected nonapeptide reduction analogue* (0·43 g), m.p. 115—125° (from acetone–ether), R_F[d] 0·29 (Found: C, 56·7; H, 6·2; N, 15·9. $C_{69}H_{86}N_{17}O_{18},H_2O$ requires C, 56·8; H, 5·9; N, 16·3%).

N-[2-(L-*Arginyl*-L-*prolylamino*)*ethyl*]*glycyl*-L-*phenylalanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-L-*arginine*.—The protected nonapeptide analogue (0·376 g) was hydrogenolysed in acetic acid (8 ml), water (2 ml), and hydrochloric acid (3 drops conc.) over 5% palladium–charcoal for 24 h. Filtration and evaporation gave an oily residue which was essentially homogeneous by t.l.c. (R_F[c] 0·44, ninhydrin positive). The crude peptide (0·2 g) was dissolved in deionised water (1 ml) and chromatographed on carboxymethylcellulose (Whatman CM 32; 25 × 1·6 cm), with ammonium acetate buffer (0·01—0·5; pH 5·0) as eluant. The eluate was collected in 5 ml fractions and tested (Sakaguchi reagent [9]) for the presence of arginine-containing material. Fractions 102—130 were combined and lyophilised repeatedly from glacial acetic acid to give the free nonapeptide (80 mg). Electrophoresis: R_{Arg} 0·76 (pH 6·1; pyridine acetate buffer), R_{Arg} 0·78 (pH 1·85; formic acid–acetic acid buffer), R_F[c] 0·44. Amino-acid analysis: Ser 0·83; Pro 2·00; Phe 2·00; AEG 1·02; Arg 1·95.

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[9] El. Bujard and J. Mauron, *J. Chromatog.*, 1966, **21**, 19; S. Sakaguchi, *J. Biochem.* (*Japan*), 1925, **5**, 133.