The Synthesis and Properties of Analogues of Angiotensin modified in the 1- or the 4-Position †

By **M. A. Cresswell,** Miles Laboratories, Stoke Court, Stoke Poges, Bucks.
R. W. Hanson, Ipswich Civic College, Rope Walk, Ipswich
H. D. Law, Liverpool Regional College of Technology

The synthesis and properties of 1-L-(γ)glutamic acid-5-L-valine angiotensin II, 1-L-(α)glutamic acid-5-L-valine angiotensin II, 1-L-asparagine-4-L-(α)glutamic acid-5-L-valine angiotensin II, and 1-L-asparagine-4-L-*O*-methyltyrosine-5-L-valine angiotensin II are described. 1-L-Pyroglutamic acid-5-L-valine angiotensin II was prepared by heating the 1-(α)glutamic acid analogue in aqueous solution.

SINCE the elucidation of the structures of equine [1] (1a) and bovine [2] (1b) angiotensin II, many analogues of these compounds have been tested for biological activity.[3]

Reported in this Paper are syntheses and properties of two more analogues of bovine angiotensin: 1-L-(γ)glutamic acid-5-L-valine angiotensin II (1c) and 1-L-asparagine-4-L-(α)glutamic acid-5-L-valine angiotensin II (1d).

† The suggestions of the Committee on Nomenclature which reported at the Fifth European Peptide Symposium are adopted throughout this Paper (Peptides: Proc. Fifth European Peptide Symp., Oxford, September 1962, ed. G. T. Young, Pergamon Press, Oxford, 1963).

[1] K. Lentz, L. T. Skeggs, K. R. Woods, J. R. Kahn, and N. P. Shumway, *J. Exp. Med.*, 1956, **104**, 183; L. T. Skeggs, K. Lentz, J. R. Kahn, N. P. Shumway, and K. R. Woods, *ibid.*, p. 193.

[2] D. F. Elliott and W. S. Peart, *Nature*, 1956, **177**, 527; *Biochem. J.*, 1957, **65**, 246.

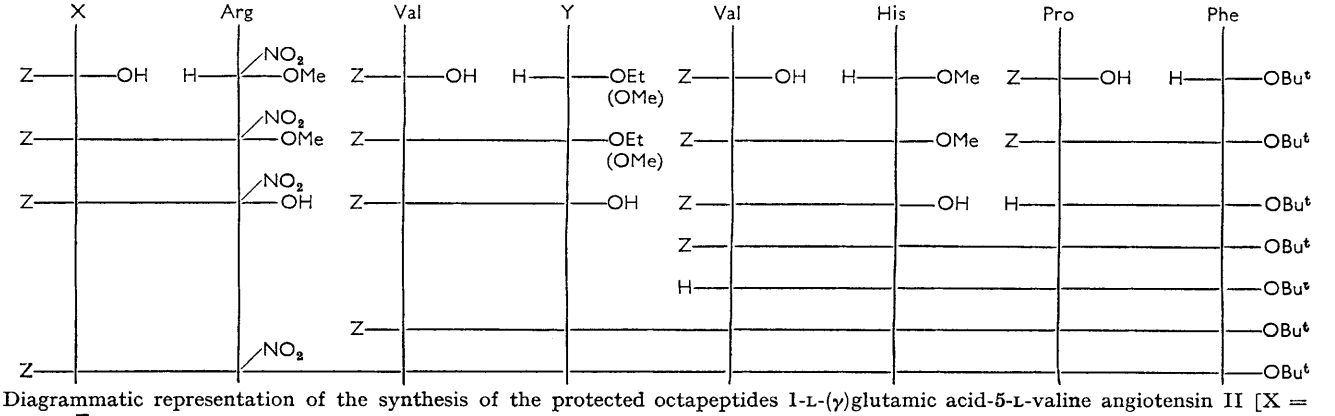
[3] For reviews see (*a*) R. Schwyzer and H. Turrian, *Vitamins and Hormones*, 1960, **18**, 237; (*b*) I. H. Page and F. M. Bumpus, *Physiol. Rev.*, 1961, **41**, 331; (*c*) Proceedings of the International Symposium on Angiotensin, Sodium, and Hypertension, *Canad. Med. Ass. J.*, 1964, **90**, 153; (*d*) H. D. Law, *Progr. in Medicin. Chem.*, 1965, **4**, 125; (*e*) W. S. Peart, *Pharm. Rev.*, 1965, **17**, 143.

Two other analogues, 1-L-(α)glutamic acid-5-L-valine angiotensin II (1e) and 1-L-asparagine-4-L-*O*-methyltyrosine-5-L-valine angiotensin II (1f), were prepared during this work but these have now been reported by other workers.[4,5]

(I) X.Arg.Val.Y.Z.His.Pro.Phe

	X	Y	Z
a;	Asp—	—Tyr—	—Ile—
b;	Asp—	—Tyr—	—Val—
c;	Glu	—Tyr—	—Val—
d;	Asp(NH_2)—	—Glu—	—Val—
e;	Glu—	—Tyr—	—Val—
f;	Asp(NH_2)—	—Tyr(Me)—	—Val—
g;	Asp(NH_2)—	—Tyr—	—Val—
h;	Glu—	—Tyr—	—Val—

The principal route employed for the synthesis of the analogues is summarised in the Figure. Except in detail it resembles the method since described by Schröder [6] in which the protected octapeptide is built up by the addition of dipeptide units to the t-butyl ester of the *C*-terminal dipeptide. The main difference is that the tyrosine residue, when required, was incorporated as the t-butyl ether. In each case, the protecting groups were finally removed by hydrogenolysis followed by treatment with anhydrous trifluoroacetic acid, and the analogues were purified by chromatography. 1-L-Asparagine-5-L-valine angiotensin II (1g) was prepared to confirm that the route was satisfactory.



Diagrammatic representation of the synthesis of the protected octapeptides 1-L-(γ)glutamic acid-5-L-valine angiotensin II [X = —Glu(OBu^t)—, Y = —Tyr(Bu^t)—], 1-L-asparagine-4-L-(α)glutamic acid-5-L-valine angiotensin II [X = —Asp(NH_2)—, Y = —Glu(OBu^t)—], 1-L-(α)glutamic acid-5-L-valine angiotensin II [X = —Glu(OBu^t)—, Y = —Tyr(Bu^t)—], and 1-L-asparagine-4-L-*O*-methyltyrosine-5-L-valine angiotensin II [X = —Asp(NH_2)—, Y = —Tyr(Me)—].

In the case of the 4-*O*-methyltyrosine and 4-L-glutamic acid analogues, the hexapeptides were also built in a stepwise manner from the tetrapeptide derivative, using the *N*-benzyloxycarbonyl amino-acid *p*-nitrophenyl esters. These variations seemed worthwhile in view of the known racemisation of the tyrosine residue during the coupling of *N*-benzyloxycarbonyl-L-valyl-L-tyrosine to the corresponding tetrapeptide methyl ester.[7] The protected *O*-methyl octapeptide was also prepared by coupling the *N*-terminal tripeptide derivative, *N*-L-asparaginyl-L-nitroarginyl-L-valine, with the pentapeptide derivative, but the yield was poor. Difficulties in coupling *C*-terminal valine and *N*-terminal tyrosine peptides have been reported previously in this series,[8] but in the present case, unlike the previous one, they cannot be accounted for by the reactivity of the phenolic group. In the event, no differences were detected between the peptides synthesised by the various approaches.

RESULTS AND DISCUSSION

The analogues were tested for their ability to raise the blood pressure of rats anaesthetised with urethane. 1-(γ)Glutamic acid angiotensin II (1c) possessed about the same activity as 1-asparagine-5-valine angiotensin II (1g) which is known to be equiactive with the parent compound. In agreement with Schröder, the 1-(α)glutamic acid analogue was found to be about 50% more active. No prolonged activity was detected with either compound.

The presence of the *N*-terminal dipeptide of angiotensin II is not essential for the compound to manifest pressor activity.[3] On the other hand, only in the octapeptide series have compounds as active as angiotensin been found, and outstanding amongst these are the L-(β)aspartic, D-(α)aspartic, and D-(β)aspartic acids analogues.[9] The enhanced activity of these analogues is attributed to resistance to enzymic decomposition. In the light of this, the greater activity of the 1-(α)glutamic acid analogue than the 1-(γ)glutamic acid analogue and the absence of attenuated effects are somewhat unexpected.

Aspartyl and, particularly, asparaginyl peptides are susceptible to a rearrangement involving imide formation

[4] E. Schröder, *Annalen*, 1966, **691**, 232.
[5] E. Schröder and R. Hempel, *Annalen*, 1965, **684**, 243.
[6] E. Schröder, *Annalen*, 1964, **680**, 132.
[7] B. Riniker and R. Schwyzer, *Helv. Chim. Acta*, 1961, **44**, 658.
[8] R. Paul and G. W. Anderson, *J. Org. Chem.*, 1962, **27**, 2094; R. Paul, *ibid.*, 1963, **28**, 236.
[9] B. Riniker, H. Brunner, and R. Schwyzer, *Angew. Chem.*, 1962, **74**, 469; H. Brunner and D. Regoli, *Experientia*, 1962, **18**, 504; D. Regoli, B. Riniker, and H. Brunner, *Biochem. Pharmacol.*, 1963, **12**, 637; B. Riniker and R. Schwyzer, *Helv. Chim. Acta*, 1964, **47**, 2357.

and transfer of the peptide chain from the α- to the β-carboxy-group of the aspartic acid residue; this rearrangement originally created difficulties in the synthesis of 1-L-asparagine angiotensin II.[9] In general, glutamyl peptides seem less likely to rearrange in this way than the corresponding aspartyl peptides, whereas the possibility of pyrrolidone formation affords a mode of decomposition which is not available for aspartyl derivatives. Marked chemical differences between glutamyl and aspartyl peptides have been found previously.[10]

Thin-layer chromatography was used to follow the decomposition of the 1-glutamic acid analogues which occurred when they were heated in neutral aqueous solution to 100°. 1-(α)Glutamic acid-5-L-valine angiotensin II (1e) was converted entirely to a single new compound. The reaction was virtually complete after 48 hours, and after 96 hours the new compound was isolated by counter-current distribution. It was ninhydrin-negative, did not give an *N*-terminal residue when treated by the FDNB method,[11] and had an amino-acid analysis identical to that of the starting material. It analysed as 1-pyroglutamic acid-5-valine angiotensin II (1h). In the pressor assay it was about 25% less active than angiotensin. Schröder prepared 1-L-pyroglutamic acid-5-L-valine angiotensin II, which was about 50% more active than angiotensin, by incorporating the pyroglutamic acid residue as the *N*-benzyloxycarbonyl *p*-nitrophenyl ester derivative.[4] In the present case, some racemisation may have occurred during the heating, but we have no evidence on this point. No trace of the 1-(γ)glutamic acid analogue was detected during the decomposition of the 1-(α)glutamic acid analogue, which makes it probable that no imide formation occurred.

The 1-(γ)glutamic acid analogue (1c) was more stable than the 1-(α)glutamic acid analogue, but was almost completely decomposed after 96 hours at 100° in aqueous solution (pH 6) to pyroglutamic acid and the *C*-terminal heptapeptide. The products were separated by chromatography on cellulose phosphate. The heptapeptide was identified by amino-acid analysis.

1-Asparagine-4-L-*O*-methyltyrosine-5-L-valine angiotensin II (1f) possessed of the order of 0·1% of the pressor activity of angiotensin and showed no inhibitory activity towards angiotensin.[3] Schröder reported 0·2% activity for the *O*-methyl compound.[5] This analogue therefore differs from *O*-methyltyrosine derivatives of other biologically active peptides, which have been shown in some cases to possess antagonist activity towards the parent compounds.[12] 1-L-Asparagine-4-L-glutamic acid-5-L-valine angiotensin II (1d) possessed less than 0·1% of the activity of angiotensin II.

EXPERIMENTAL

All evaporations were carried out under reduced pressure. The purity of intermediates was checked by thin-layer chromatography (t.l.c.) on Kieselgel-G in two solvent systems: (*a*) n-butanol–acetic acid–water (62 : 12 : 26 v/v); (*b*) s-butanol–aqueous ammonia (0·3%) (120 : 44 v/v). Free octapeptides were chromatographed on alumina plates in these solvent systems and in methanol–chloroform–aqueous ammonia (17%) (20 : 20 : 9 v/v). Spots were developed (i) by treatment with ninhydrin, and (ii) by chlorination followed by spraying with starch–iodide.

1-L-*Asparagine-5-L-valine Angiotensin II*

Methyl N-*Benzyloxycarbonyl-L-valyl-L-histidinate.*—*N*-Benzyloxycarbonyl-L-valine (7·53 g., 0·03 mole) was dissolved in dry *NN*-dimethylformamide (30 ml.) and di-*N*-imidazolyl ketone [5·5 g. (90%), 0·031 mole] was added to the stirred solution at −5°. After 20 min., a solution of methyl L-histidinate in *NN*-dimethylformamide (40 ml.) at 0° was slowly added [the free base was prepared from the methyl ester dihydrochloride (7·62 g., 0·032 mole) by the addition of sodium methoxide in methanol]. Stirring was continued at 0 to −5° for 30 min., and then at room temperature for 16 hr. Removal of the solvent gave a gel which was triturated with water, filtered at 0° and dried to give the protected dipeptide (10 g., 84%). After reprecipitation from aqueous methanol the product had m. p. 163—166°, $[\alpha]_D^{16}$ −19·4° (*c* 1·5 in ethanol) [lit.,[13] m. p. 165—166°, $[\alpha]_D^{23}$ −22 ± 2° (*c* 2 in ethanol)] (Found: C, 59·8; H, 6·3; N, 13·7. Calc. for $C_{20}H_{27}N_4O_5$: C, 59·7; H, 6·5; N, 13·9%).

N-*Benzyloxycarbonyl-L-valyl-L-histidine.*—The ester was saponified in aqueous methanol and the resulting sodium salt converted into the free acid with Amberlite IRC-50 as previously described for the synthesis of *N*-t-butoxycarbonyl-L-isoleucyl-L-histidine.[8] The product crystallised from aqueous methanol to give the N-*protected dipeptide* (28 g., 78·2%), m. p. 150—152° (from water), $[\alpha]_D^{15·5}$ +26·8° (*c* 1·5 in methanol) (Found: C, 54·1; H, 6·6; N, 13·4. $C_{19}H_{25}N_4O_5,2H_2O$ requires C, 53·8; H, 6·8; N, 13·2%).

Ethyl N-*Benzyloxycarbonyl-L-valyl-O-t-butyl-L-tyrosinate.*—Prepared from *N*-benzyloxycarbonyl-L-valine (17 g., 0·068 mole) and ethyl *O*-t-butyl-L-tyrosinate[14] (16·3 g., 0·062 mole) in tetrahydrofuran (100 ml.) solution by the *NN'*-dicyclohexylcarbodi-imide method, the *protected dipeptide* (23·6 g., 73%) had m. p. 103—105° (from ether–n-hexane), $[\alpha]_D^{17}$ +32·3° (*c* 1·0 in chloroform) (Found: C, 67·6; H, 7·8; N, 5·8. $C_{28}H_{38}N_2O_6$ requires C, 67·4; H, 7·7; N, 5·6%).

N-*Benzyloxycarbonyl-L-valyl-O-t-butyl-L-tyrosine.*—Saponification of the ethyl ester (4·95 g., 0·01 mole) with N-sodium hydroxide (1 equiv.) in acetone (100 ml.) gave the *dipeptide acid* (4·04 g., 86%), m. p. 140—141° (from ethyl acetate–light petroleum), $[\alpha]_D^{20}$ +20·8° (*c* 1 in methanol) (Found: C, 66·15; H, 7·5; N, 5·9. $C_{26}H_{34}N_2O_6$ requires C, 66·4; H, 7·3; N, 5·95%).

Methyl N-*Benzyloxycarbonyl-L-asparaginyl-L-nitroargininate.*—*N*-Benzyloxycarbonyl-L-asparagine (48·2 g., 0·18 mole) was dissolved in chloroform (350 ml.) containing pyridine (14·6 ml., 0·18 mole) and *N*-ethylpiperidine (24·8 ml.,

[10] A. P. Fosker, R. W. Hanson, and H. D. Law, *Chem. and Ind.*, 1963, 569.

[11] H. Fraenkel-Conrat, J. I. Harris, and A. L. Levy, 'Methods of Biochemical Analysis,' ed. Glick, Interscience, New York, 1955, vol. 2, p. 359.

[12] H. D. Law and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1960, **82**, 4579; K. Jöst, J. Rudinger, and F. Sörm, *Coll. Czech. Chem. Comm.*, 1961, **26**, 2496; 1963, **28**, 1706; M. Zaoral, E. Kasáfirek, J. Rudinger, and F. Sörm, *ibid.*, 1965, **30**, 1869; J. M. Stewart and D. W. Woolley, *Fed. Proc.*, 1965, **24**, 657.

[13] R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel, and H. Zuber, *Helv. Chim. Acta*, 1958, **41**, 1273.

[14] E. Schröder, *Annalen*, 1963, **670**, 127.

0·18 mole), and pivaloyl chloride (21·6 g., 0·18 mole) was added to the stirred solution at 0—5°.[15] The mixture was cooled to −10° and a solution of methyl L-nitroarginate [prepared from the hydrochloride (48·3 g., 0·179 mole) by the addition of sodium methoxide in methanol] in dioxan was added. A gel formed which, after 12 hr. was filtered and dissolved in hot water (900 ml.). The protected dipeptide (54·5 g., 63%) separated from the cooled solution, homogeneous (t.l.c.), m. p. 180—182° (from water), $[\alpha]_D^{16}$ +5·8° (*c* 3 in acetic acid) [lit.,[8] m. p. 180—183°, $[\alpha]_D$ +5·4° (*c* 2 in acetic acid); lit.,[16] m. p. 170—173°, $[\alpha]_D^{24}$ +5° (acetic acid); lit.,[6] m. p. 173—174·5°, $[\alpha]_D^{23}$ +5° (*c* 2 in acetic acid)].

N-Benzyloxycarbonyl-L-asparaginyl-L-nitroarginine.— The methyl ester (30 g., 0·062 mole) was saponified in batches (4—5 g.) with an equivalent of N-sodium hydroxide and Vibromix stirring for 30 min. at room temperature. Water was added to the mixture and insoluble material was filtered off and re-saponified. The combined filtrates were acidified with 5N-hydrochloric acid and extracted with ethyl acetate. Some crystals (1·5 g., 5·16%), m. p. 145—148°, separated from the dried ethyl acetate solution overnight. Evaporation of the rest of the solution gave a crystalline solid (19·6 g., 67·5%), m. p. 147—148° (from methanol), $[\alpha]_D^{15}$ +7·9° (*c* 1·1 in methanol) (Found: C, 44·3; H, 5·7; N, 19·2%. Calc. for $C_{18}H_{25}N_7O_8,H_2O$: C, 44·5; H, 5·6; N, 19·25%) [lit.,[16] m. p. 98—101°, $[\alpha]_D$ 10 ± 4° (*c* 2 in methanol); lit.,[8] m. p. 107—110° (dihydrate) or 109—113° (anhydrous), $[\alpha]_D$ +7·4° (*c* 2 in methanol); lit.,[6] m. p. 103—104·5°, $[\alpha]_D^{23}$ +8·4° (*c* 1 in acetic acid)].

t-Butyl N-Benzyloxycarbonyl-L-valyl-L-histidyl-L-prolyl-L-phenylalaninate.— Dry *N*-benzyloxycarbonyl-L-valyl-L-histidine (21 g., 0·54 mole) was dissolved in *NN*-dimethylformamide (250 ml.) and di-*N*-imidazolyl ketone [10·9 g. of (89%), 0·059 mole] was added to the stirred solution at −10°. After 1 hr. the temperature was allowed to rise to −5°, and distilled water (0·2 ml.) was added. After 10 min. the solution was cooled to −10° and a precooled solution of t-butyl L-prolyl-L-phenylalaninate [6] (17·2 g., 0·054 mole) in *NN*-dimethylformamide (100 ml.) was added. The mixture was stirred at −10° for 15—20 min. and overnight at room temperature. Evaporation of the solvent gave an oil which was heated with water (700 ml.). The solid which separated from the cooled solution was filtered off and taken up in ethyl acetate, and the solution was washed and dried. The foam obtained on evaporation was treated with light petroleum to give the required tetrapeptide (30·1 g., 78·5%). A sample was prepared for analysis by chromatography on alumina and reprecipitation from ethyl acetate–light petroleum, and was indistinguishable from the above material by t.l.c., m. p. 95—100°, $[\alpha]_D^{20}$ −62·5° (*c* 1·1 in methanol) (Found: C, 64·3; H, 6·8; N, 12·3. Calc. for $C_{37}H_{48}N_6O_7$: C, 64·5; H, 7·0; N, 12·2%) [lit.,[6] m. p. 75—80°, $[\alpha]_D^{23}$ −51·5° (*c* 1 in ethanol)].

t-Butyl L-Valyl-L-histidyl-L-prolyl-L-phenylalaninate.—The protected tetrapeptide (30 g., 0·044 mole), dissolved in absolute ethanol (250 ml.) containing acetic acid (20 ml.), was hydrogenolysed overnight over 10% palladium–charcoal (3·5 g.). Work-up in the usual way gave an oil containing acetic acid which could not be removed by evaporation. The oil was dissolved in the minimum of water, extracted with ethyl acetate to remove starting material, and basified with solid sodium carbonate, and the free base was extracted into ethyl acetate. Evaporation of the washed and dried solution gave the tetrapeptide ester free base (21·5 g., 89%), m. p. 65—72°, $[\alpha]_D^{17}$ −47·2° (*c* 2 in methanol) (Found: C, 62·6; H, 7·5; N, 14·8. Calc. for $C_{29}H_{42}N_6O_5$: C, 62·8; H, 7·6; N, 15·15%) [lit.,[6] $[\alpha]_D^{23}$ −48·1° (*c* 1 in ethanol)]. Some starting material (1·2 g.) was recovered from the ethyl acetate extract.

t-Butyl N-Benzyloxycarbonyl-L-valyl-O-t-butyl-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalaninate.— t-Butyl L-valyl-L-histidyl-L-prolyl-L-phenylalaninate (8·0 g., 0·0145 mole) and *N*-benzyloxycarbonyl-L-valyl-*O*-t-butyl-L-tyrosine (6·8 g., 0·0145 mole) were dissolved in tetrahydrofuran 50) ml.) and *NN'*-dicyclohexylcarbodi-imide (3·28 g., 0·016 mole) was added to the stirred solution at 0°. After 2·5 days at room temperature, the mixture was worked up in the usual manner to yield a solid (12·1 g., 90%), m. p. (gels) 124—128° (sinters) 140°.

This product (12·5 g.) was chromatographed on neutral alumina. The pure *protected hexapeptide* (6·6 g., 53%) had m. p. 178—180°, $[\alpha]_D^{20}$ −62·4° (*c* 1 in methanol) (Found: C, 65·7; H, 7·3; N, 10·9. $C_{55}H_{74}N_8O_{10}$ requires C, 65·6; H, 7·4; N, 11·1%). Amino-acid analysis: His, 0·98; Pro, 1·00; Val, 1·84; Tyr, 0·62; Phe, 1·00; after treatment of the hydrolysate with D-amino acid oxidase: His, 0·98; Pro, 1·00; Val, 1·72; Tyr, 0·59; Phe, 0·89.

t-Butyl N-Benzyloxycarbonyl-L-asparaginyl-L-nitroarginyl-L-valyl-O-t-butyl-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalaninate.—The protected hexapeptide was hydrogenolysed in dry methanol containing a little acetic acid over 10% palladium–charcoal; the free base was liberated with sodium carbonate and extracted into ethyl acetate.

N-Benzyloxycarbonyl-L-asparaginyl-L-nitroarginine (1·68 g., 0·0036 mole) was coupled to the hexapeptide ester (2·62 g., 0·003 mole) in *NN*-dimethylformamide–tetrahydrofuran solution by the mixed anhydride technique with ethyl chloroformate. The product, after trituration with aqueous sodium carbonate and repeated precipitation from *NN*-dimethylformamide with triethylamine–water, gave a product which was further purified by precipitation from aqueous methanol to give the *protected octapeptide* (1·75 g., 43%), m. p. 215° (decomp.), $[\alpha]_D^{15}$ −36° (*c* 1·1 in *NN*-dimethylformamide) (Found: C, 57·7; H, 6·6; N, 15·2. $C_{65}H_{91}N_{15}O_{15}$ requires C, 57·5; H, 7·0; N, 15·5%).

L-Asparaginyl-L-arginyl-L-valyl-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalanine (1-L-*Asparagine-5-L-valine Angiotensin II*).—The protected octapeptide (0·5 g., 0·00038 mole) dissolved in methanol–acetic acid–water, was hydrogenolysed over 10% palladium–charcoal (0·5 g.) for 48 hr. Work-up in the usual way gave 0·46 g. (95%) of product.

A sample of this material (0·24 g.) was dissolved in trifluoroacetic acid (2 ml.) and after 2 hr. at room temperature, most of the acid was removed under reduced pressure. The oil solidified when triturated with ether to give a crude product, which was purified by chromatography on a carboxymethylcellulose column by elution with 0·05—0·125M-ammonium acetate solution. The solutions comprising the main peak were removed and freeze-dried. The product was slightly impure (t.l.c.), and a chromatographically homogeneous sample (0·12 g., 57%) was obtained by countercurrent distribution in n-butanol—0·1M-ammonium acetate (1 : 1) (pH 7·5) over 200 transfers (K = 0·34). A sample was prepared for analysis by freeze-drying this product

[15] M. Zaoral, *Angew. Chem.*, 1959, **71**, 743; *Coll. Czech. Chem. Comm.*, 1962, **27**, 1273.

[16] W. Rittel, B. Iselin, H. Kappeler, B. Rinker, and R. Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 614.

three times and allowing it to come to equilibrium in the atmosphere (Found: C, 51·8; H, 6·9; N, 16·5; AcOH, 7·38. $C_{49}H_{70}N_{14}O_{11}$,1·5AcOH,5H_2O requires C, 51·6; H, 7·15; N, 16·2; AcOH, 7·45%). Amino-acid analysis: Asp, 1·03; Arg, 0·92; Val, 2·00; Tyr, 0·95; His, 0·91; Pro, 1·04; Phe, 1·02.

1-L-(γ)*Glutamic Acid*-5-L-*valine Angiotensin II*

Methyl N-*Benzylcarbonyl*-α-*t*-*butyl*-γ-L-*glutamyl*-L-*nitroargininate*.—α-t-Butyl *N*-benzyloxycarbonyl-L-glutamate [17] (2·9 g., 0·0086 mole) in *NN*-dimethylformamide–tetrahydrofuran solution was coupled to methyl L-nitroargininate [prepared from the hydrochloride (2·55 g., 0·0095 mole] by the mixed anhydride procedure with ethyl chloroformate. The *dipeptide derivative* was isolated and obtained, by evaporation of an ethyl acetate solution, as a solid foam (4·0 g., 84%), m. p. 50—55°, $[\alpha]_D^{17}$ −21·3° (*c* 1·3 in methanol) (Found: C, 51·9; H, 6·4; N, 15·0. $C_{24}H_{36}N_6O_9$ requires C, 52·2; H, 6·6; N, 15·2%).

N-*Benzyloxycarbonyl*-α-*t*-*butyl*-γ-L-*glutamyl*-L-*nitroarginine*.—The methyl ester (3·0 g., 0·0055 mole) was dissolved in acetone (25 ml.) and saponified at room temperature with 2N-sodium hydroxide (2·8 ml., 0·0056 mole). After 1 hr. the acetone was evaporated off and the aqueous solution was acidified with citric acid. The product was extracted into ethyl acetate (3 × 25 ml.) which was washed, dried, and evaporated to give the free *dipeptide acid* as a foam (2·5 g., 85%), m. p. 66—70°, $[\alpha]_D^{17}$ −12·4° (*c* 1 in methanol) (Found: C, 51·35; H, 6·3; N, 15·5. $C_{23}H_{34}N_6O_9$ requires C, 51·3; H, 6·4; N, 15·6%).

t-Butyl N-*Benzyloxycarbonyl*-α-*t*-*butyl*-γ-L-*glutamyl*-L-*nitroarginyl*-L-*valyl*-O-*t*-*butyl*-L-*tyrosyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalaninate*.—*N*-Benzyloxycarbonyl-α-t-butyl-γ-L-glutamyl-L-nitroarginine (0·845 g., 0·00157 mole) in tetrahydrofuran solution was coupled to the hexapeptide ester (1·14 g., 0·00131 mole) by the mixed anhydride technique with ethyl chloroformate. Work-up gave a solid foam which was reprecipitated from methanol containing a small amount of triethylamine by the addition of water. Further reprecipitation gave the chromatographically pure *octapeptide derivative* (1·35 g., 74%), m. p. 165°, $[\alpha]_D^{16}$ −26·8° (*c* 1·2 in *NN*-dimethyl formamide) (Found: C, 59·8 H, 7·2; N, 13·9. $C_{70}H_{101}N_{14}O_{16}$,0·5$H_2O$ requires C, 59·9; H, 7·3; N, 14·0%).

γ-L-*Glutamyl*-L-*arginyl*-L-*valyl*-L-*tyrosyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine* (1-L-(γ)-*Glutamic Acid*-5-L-*valine Angiotensin II*).—Removal of the protecting groups from the protected octapeptide (600 mg., 0·00042 mole) by hydrogenation and trifluoroacetic acid treatment gave the crude analogue (550 mg., 95%). Purification by gradient elution from cellulose phosphate with ammonium acetate buffer (0·025—0·2M; pH 5·2) gave 380 mg. (65%). Countercurrent distribution (140 mg.) over 100 transfers in the system n-butanol–0·1M-ammonium acetate (pH 7·5) (K = 0·087) gave the homogeneous *octapeptide* (90 mg.) (Found: C, 51·1; H, 7·2; N, 14·95; AcOH, 5·23. $C_{50}H_{71}N_{13}O_{14}$,AcOH,6H_2O requires C, 51·4; H, 7·4; N, 15·0; AcOH, 4·94%). Amino acid analysis: Glu, 1·08; Arg, 1·02; Val, 1·97; Tyr, 0·78; His, 0·96; Pro, 0·94; Phe, 1·03.

1-L-*Asparagine*-4-L-(α)*glutamic Acid*-5-L-*valine Angiotensin II*

Ethyl N-*Benzyloxycarbonyl*-L-*valyl*-γ-*t*-*butyl*-L-*glutamate*.—This compound, prepared by the method of Katsoyannis, Fukeda, and Tometsko,[18] had m. p. 75—77°, $[\alpha]_D^{17}$ −26·6° (*c* 2 in ethanol) [lit.,[18] m. p. 70—71°, $[\alpha]_D^{26}$ −26·7° (*c* 1 in ethanol)].

N-*Benzyloxycarbonyl*-L-*valyl*-γ-*t*-*butyl*-L-*glutamic Acid*.—Saponification of the ethyl ester (1·16 g., 0·0025 mole) with 2N-sodium hydroxide in the usual way gave the *dipeptide acid* (0·76 g., 70%), m. p. 114—115° (from ethyl acetate), $[\alpha]_D^{17.5}$ −18·3° (*c* 1 in methanol) (Found: C, 60·1; H, 7·2; N, 6·4. $C_{22}H_{32}N_2O_7$ requires C, 60·5; H, 7·4; N, 6·4%).

t-Butyl N-*Benzyloxycarbonyl*-γ-*t*-*butyl*-L-*glutamyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalaninate*.—The tetrapeptide ester (0·554 g., 0·001 mole) and *p*-nitrophenyl *N*-benzyloxycarbonyl-γ-t-butyl-L-glutamate [19] (0·457 g., 0·001 mole) were stirred in ethyl acetate solution for 2 days. A fine suspension of the product separated out (0·52 g., 60%). Evaporation of the washed solution gave a further batch of material (0·15 g., 17·4%). Reprecipitated from methanol–ether, the *pentapeptide derivative* had m.p. 133—135°, $[\alpha]_D^{20}$ −60·0° (*c* 0·5 in methanol) (Found: C, 62·8; H, 7·1; N, 11·0. $C_{46}H_{63}N_7O_{10}$ requires C, 63·2; H, 7·3; N, 11·2%).

t-Butyl N-*Benzyloxycarbonyl*-L-*valyl*-γ-*t*-*butyl*-L-*glutamyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalaninate*.—(*a*) t-Butyl-L-valyl-L-histidyl-L-prolyl-L-phenylalaninate (4·88 g., 0·0089 mole) and *N*-benzyloxycarbonyl-L-valyl-γ-t-butyl-L-glutamic acid (3·85 g., 0·0089 mole) in tetrahydrofuran solution (25 ml.) at 0° were coupled with *NN'*-dicyclohexylcarbodiimide. The product was chromatographed on neutral alumina to yield the *hexapeptide derivative* (5·18 g., 60%), m. p. 177° (gels 168—172°), $[\alpha]_D^{15}$ −61·5° (*c* 3 in methanol) (Found: C, 62·8; H, 7·4; N, 11·5. $C_{51}H_{72}N_8O_{11}$ requires C, 62·95; H, 7·5; N, 11·5%). Alternatively, the product was washed in ethyl acetate solution and obtained in homogenous form by evaporation of the solvent (73·5%), m. p. 170°, $[\alpha]_D^{17}$ −62·1° (*c* 2 in methanol).

(*b*) t-Butyl *N*-benzyloxycarbonyl-γ-t-butyl-L-glutamyl-L-valyl-L-histidyl-L-prolyl-L-phenylalaninate (0·4 g., 0·0005 mole) was dissolved in methanol (10 ml.) containing acetic acid (0·4 ml.) and hydrogenolysed over 10% palladium–charcoal (50 ml.) overnight. The crude product was treated with solid sodium carbonate and extracted into ethyl acetate. Evaporation of the solvent gave a solid foam (0·290 g., 88%).

p-Nitrophenyl *N*-benzyloxycarbonyl-L-valinate [20] (0·143 g., 0·0004 mole) reacted with the pentapeptide free base in ethyl acetate solution (25 ml.) for 60 hr. The product (0·32 g., 74%) had m. p. 171—173°, $[\alpha]_D^{16}$ −57·1° (*c* 1 in methanol).

t-Butyl N-*Benzyloxycarbonyl*-L-*asparaginyl*-L-*nitroarginyl*-L-*valyl*-γ-*t*-*butyl*-L-*glutamyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalaninate*.—Hydrogenolysis of the protected hexapeptide (4·5 g., 0·0047 mole) gave the free base (3·4 g., 85%). This was coupled with *N*-benzyloxycarbonyl-L-asparaginyl-L-nitroarginine (1·002 g., 0·00215 mole) by the mixed anhydride procedure described above. Reprecipitation of the crude product from *NN*-dimethylformamide–triethylamine–water and from methanol–water gave the *octapeptide*

[17] J. Kovacs, U. R. Ghatak, G. N. Schmit, F. Koide, J. W. Goodman, and D. E. Nitecki, International Symposium on the Chemistry of Natural Products, Kyotes, Japan, April 1964, p. 175.

[18] P. Katsoyannis, F. Fukeda, and A. Tometsko, *J. Amer. Chem. Soc.*, 1963, **85**, 1681.

[19] E. Schnabel, *Z. Naturforsch.*, 1964, **19***b*, 120.

[20] H. D. Law, *J. Chem. Soc.*, 1965, 3897.

derivative (1·375 g., 60%), m. p. 160—163°, $[\alpha]_D^{20}$ −28·8° (*c* 0·6 in *NN*-dimethylformamide) (Found: C, 55·7; H, 6·8; N, 16·0. $C_{61}H_{89}N_{15}O_{16},0{\cdot}5H_2O$ requires C, 55·7; H, 7·05; N, 16·0%).

L-*Asparaginyl*-L-*arginyl*-L-*valyl*-L-*glutamyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine* (*4-Glutamic Acid Angiotensin II Amide*).—The protected octapeptide (0·9 g.) was hydrogenolysed in methanol–acetic acid–water (8 : 1 : 1; 60 ml.) over 10% palladium–charcoal. After treatment with trifluoroacetic acid, the product was chromatographed on carboxymethylcellulose and eluted with 0·02—0·1M-ammonium acetate, to give the *analogue* (425 mg., 53%) (Found: C, 49·8; H, 6·6; N, 17·15; AcOH, 6·0. $C_{45}H_{68}N_{14}O_{12},5H_2O$,AcOH requires C, 49·6; H, 6·4; N, 17·3; AcOH, 5·25%). Amino acid analysis: Asp, 1·04; Arg, 0·90; Val, 1·96; Glu, 1·05; His, 0·85; Pro, 0·97; Phe, 1·01.

1-L-(α)*Glutamic Acid*-5-L-*valine Angiotensin II*

Methyl N-*Benzyloxycarbonyl*-γ-*t*-*butyl*-α-L-*glutamyl*-L-*nitroargininate*.—γ-t-Butyl *N*-benzyloxycarbonyl-L-glutamate [21] [from the dicyclohexylammonium salt (5·18 g., 0·01 mole)] and methyl L-nitroargininate [from the hydrochloride (2·7 g., 0·01 mole)] were coupled in tetrahydrofuran solution at 0° with *NN'*-dicyclohexylcarbodi-imide (2·26 g., 0·011 mole). After 20 hr. at room temperature the reaction was worked up to yield the crude protected dipeptide as a solid foam (4·84 g., 88%), m. p. *ca.* 43—53°, $[\alpha]_D^{19}$ −8·2° (*c* 3 in methanol) (Found: C, 52·3; H, 6·7; N, 14·8. Calc. for $C_{24}H_{36}N_6O_9$: C, 52·2; H, 6·6; N, 15·2%) [lit.,[4] m. p. 62—64°, $[\alpha]_D^{23}$ −13·0° (*c* 1 in methanol)].

N-*Benzyloxycarbonyl*-γ-*t*-*butyl*-L-*glutamyl*-L-*nitroarginine*.—The methyl ester (2·76 g., 0·0051 mole) in dioxan solution (25 ml.) was saponified with 2N-sodium hydroxide (2·63 ml.) at room temperature. Work-up gave the dipeptide acid as a solid foam (2·23 g., 83%), m. p. 70—72°, $[\alpha]_D^{20}$ −4·6° (*c* 2·9 in methanol) (Found: C, 50·95; H, 6·6; N, 15·1. Calc. for $C_{23}H_{34}N_6O_9$: C, 51·3; H, 6·4; N, 15·6%) [lit.,[4] m. p. 74—76°, $[\alpha]_D^{23}$ −5·0° (*c* 1 in methanol)].

t-Butyl N-*benzyloxycarbonyl*-γ-*t*-*butyl*-L-*glutamyl*-L-*nitroarginyl*-L-*valyl*-O-*t*-*butyl*-L-*tyrosyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalaninate*.—*N*-Benzyloxycarbonyl-γ-t-butyl-L-glutamyl-L-nitroarginine (1·94 g., 0·0036 mole) in *NN*-dimethylformamide solution was coupled to the hexapeptide derivative (2·62 g., 0·003 mole) by the mixed anhydride procedure with ethyl chloroformate. The product was precipitated from *NN*-dimethylformamide–water and from hot methanol to yield the pure *octapeptide derivative* (2·2 g., 41%), m. p. 200—203° (decomp.), $[\alpha]_D^{18}$ −31·7° (*c* 1 in *NN*-dimethylformamide) (Found: C, 59·7; H, 7·0; N, 13·9. $C_{70}H_{101}N_{14}O_{16},0{\cdot}5H_2O$ requires C, 59·9; H, 7·3; N, 14·0%).

α-L-*Glutamyl*-L-*arginyl*-L-*valyl*-L-*tyrosyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine* (1-L-(α)*Glutamic Acid*-5-L-*valine Angiotensin II*).—The protecting groups were removed from the octapeptide derivative to give the crude analogue (1·1 g., 95%). Chromatography by gradient elution with ammonium acetate (0·02—0·08M, pH 5·2) on carboxymethylcellulose gave the *product* (800 mg., 82%), which was chromatographed on cellulose phosphate with ammonium acetate eluent (0·05—0·2M, pH 5·6). Counter-current distribution, with 100 transfers in n-butanol–ammonium acetate (pH 7·5), was necessary to obtain a homogeneous sample ($K = 0{\cdot}075$) (Found: C, 52·3; H, 7·3; N, 15·5; AcOH, 4·7. $C_{50}H_{71}N_{13}O_{12}$,AcOH,$5H_2O$ requires C, 52·1; H, 7·2; N, 15·2; AcOH, 5·0%). Amino acid analysis: Glu, 0·96; Arg, 1·06; Val, 2·00; Tyr, 1·00; His, 0·96; Pro, 1·04; Phe, 0·99.

[21] H. Kappeler and R. Schwyzer, *Helv. Chim. Acta*, 1961, **44**, 1136.

1-L-*Asparagine*-4-L-O-*methyltyrosine*-5-L-*valine Angiotensin II*

Methyl N-*Benzyloxycarbonyl*-L-*valyl*-O-*methyl*-L-*tyrosinate*.—Prepared by the *NN'*-dicyclohexylcarbodi-imide method from methyl *O*-methyl-L-tyrosinate (2·01 g., 0·0096 mole) and *N*-benzyloxycarbonyl-L-valine (2·53 g., 0·0101 mole), the protected dipeptide was obtained as a gel (3·4 g., 80·2%), m. p. 150° (reprecipitated from acetone–ether and methanol–ether), $[\alpha]_D^{20}$ +52·8° (*c* 0·703 in chloroform), $[\alpha]_D^{16}$ −28·5° (*c* 0·7 in methanol) (Found: C, 65·3; H, 6·75; N, 6·4. Calc. for $C_{24}H_{30}N_2O_6$: C, 65·1; H, 6·8; N, 6·3%) [lit.,[5] m. p. 141—142°, $[\alpha]_D^{23}$ −20·2° (*c* 0·5 in methanol)].

N-*Benzyloxycarbonyl*-L-*valyl*-O-*methyl*-L-*tyrosine*.— The methyl ester (3·4 g., 0·0077 mole) was saponified with N-sodium hydroxide (1 equiv.) in aqueous methanol to give the *dipeptide acid* (2·9 g., 83%), m. p. 193° (from acetone–water), $[\alpha]_D^{15{\cdot}5}$ +25·9° (*c* 4 in pyridine) (Found: C, 64·65; H, 6·7; N, 6·8. $C_{23}H_{28}N_2O_6$ requires C, 64·5; H, 6·6; N, 6·5%).

t-Butyl N-*Benzyloxycarbonyl*-L-*asparaginyl*-L-*nitroarginyl*-L-*valinate*.—Di-*N*-imidazolyl ketone [7 g. (95%), 0·041 mole] was added to a stirred solution of *N*-benzyloxycarbonyl-L-asparaginyl-L-nitroarginine (16·41 g., 0·035 mole) in *NN*-dimethylformamide (200 ml.) at −10 to −15°. After 75 min. at this temperature the reaction mixture was allowed to warm to −5° and water (0·15 ml.) was added. The mixture was then recooled to −10° and a solution of t-butyl L-valinate in *NN*-dimethylformamide was slowly added [the free base was prepared from t-butyl L-valinate hydrochloride (10 g., 0·048 mole) by the addition of potassium carbonate]. After 15 min. at −15°, the mixture was stirred at room temperature for 40 hr. Evaporation of the solvent left an oil which was boiled with ether (350 ml.). The ether was poured off and the oil dried to give a solid foam (15·2 g.) which was extracted into ethyl acetate. Evaporation of the washed and dried ethyl acetate solution gave the *protected tripeptide* (13·5 g., 62%), m. p. 145—147° (from methanol–ether), $[\alpha]_D^{17}$ −22·5° (*c* 1·35 in methanol) (Found: C, 51·6; H, 6·5; N, 18·4. $C_{27}H_{42}N_8O_9$ requires C, 52·0; H, 6·8; N, 18·0%). [Attempts to prepare this compound by coupling *N*-benzyloxycarbonyl-L-asparagine and t-butyl L-nitroarginyl-L-valinate were unsuccessful].

N-*Benzyloxycarbonyl*-L-*asparaginyl*-L-*nitroarginyl*-L-*valine*.—The t-butyl ester (12·5 g., 0·002 mole) was dissolved in trifluoroacetic acid (20 ml.) and kept at 0° overnight. Evaporation of the trifluoroacetic acid left an oil which, when triturated with ether, gave the *tripeptide derivative* (10·4 g., 92%), m. p. 137—142° (from methanol–ether) (Found: C, 48·4; H, 6·0; N, 19·4. $C_{23}H_{35}N_8O_9$ requires C, 48·7; H, 6·0; N, 19·7%).

t-Butyl N-*Benzyloxycarbonyl*-O-*methyl*-L-*tyrosyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalaninate*.—t-Butyl-L-valyl-L-histidyl-L-prolyl-L-phenylalaninate (3·35 g., 0·006 mole) and *p*-nitrophenyl *N*-benzyloxycarbonyl-*O*-methyl-L-tyrosinate [20] (2·64 g., 0·006 mole) were dissolved in ethyl acetate (60 ml.). After 60 hr., the solution was washed with aqueous sodium carbonate, citric acid solution, and water. Evaporation of the dried solution gave the *protected pentapeptide* (4·2 g., 82%), m. p. 102—108° (from ethyl acetate–light

petroleum), $[\alpha]_D^{17}$ −59° (*c* 1 in methanol) (Found: C, 65·35; H, 6·9; N, 11·35. $C_{48}H_{59}N_7O_9$ requires C, 65·65; H, 6·8; N, 11·15%).

t-Butyl N-*Benzyloxycarbonyl*-L-*valyl*-O-*methyl*-L-*tyrosyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalaninate*.—(*a*) The tetrapeptide free base (3·88 g., 0·007 mole) and *N*-benzyloxycarbonyl-L-valyl-*O*-methyl-L-tyrosine (3·0 g., 0·007 mole) were coupled with *NN'*-dicyclohexylcarbodi-imide (1·58 g., 0·0073 mole) in a manner similar to that used for the preparation of the hexapeptide t-butyl ether. The crude protected hexapeptide (4·4 g., 50%) had m. p. 205—210° (decomp.). An analysis sample prepared by precipitation from ethyl acetate–ether, had m. p. 173—176°, $[\alpha]_D^{17}$ −36·8° (*c* 1 in *NN*-dimethylformamide) (Found: C, 64·5; H, 7·0; N, 11·45. Calc. for $C_{52}H_{68}N_8O_{10}$: C, 64·7; H, 7·1; N, 11·6%) [lit.,[5] m. p. 209—211°, $[\alpha]_D^{23}$ −38·4° (*c* 5 in *NN*-dimethylformamide)].

(*b*) The *N*-benzyloxycarbonyl-*O*-methyl-pentapeptide t-butyl ester (5·2 g., 0·006 mole), in methanol solution containing acetic acid (1·38 ml.), was hydrogenolysed over 10% palladium–charcoal (0·8 g.) for 16 hr. Work-up gave a foam which was converted into the free base with sodium carbonate and extracted into ethyl acetate. The washed and dried ethyl acetate solution gave on evaporation a homogeneous foam (3·9 g., 89%). This material was redissolved in ethyl acetate (50 ml.) and stirred for 2 days with *p*-nitrophenyl *N*-benzyloxycarbonyl-L-valinate (2·1 g., 0·0058 mole). Evaporation of the washed and dried solution gave the required hexapeptide derivative (4·1 g., 80%), m. p. 170—178°, $[\alpha]_D^{14}$ −59·5° (*c* 0·7 in methanol) (Found: C, 64·6; H, 6·8; N, 11·3. Calc. for $C_{52}H_{68}N_8O_{10}$: C, 64·7; H, 7·1; N, 11·6%).

t-Butyl N-*Benzyloxycarbonyl*-L-*asparaginyl*-L-*nitroarginyl*-L-*valyl*-O-*methyl*-L-*tyrosyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalaninate*.—(*a*) The *O*-methyl-hexapeptide t-butyl ester was prepared from the *N*-benzyloxycarbonyl compound (90%) by hydrogenolysis as described for the *O*-t-butyl derivative. *N*-Benzyloxycarbonyl-L-asparaginyl-L-nitroarginine (1·5 g., 0·0032 mole) was coupled to the hexapeptide ester free base (2·22 g., 0·0027 mole) in *NN*-dimethylformamide–tetrahydrofuran solution by the mixed anhydride technique with ethyl chloroformate. The crude product was precipitated from hot methanol to give the pure *protected octapeptide* (2·2 g., 60%), m. p. 215°, $[\alpha]_D^{15}$ −32·8° (*c* 1 in *NN*-dimethylformamide) (Found: C, 56·6; H, 6·7; N, 15·7. $C_{62}H_{85}N_{15}O_{15},2H_2O$ requires C, 56·6; H, 6·8; N, 16·0%).

(*b*) t-butyl *O*-methyl-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalaninate (2·5 g., 0·0034 mole) and *N*-benzyloxycarbonyl-L-asparaginyl-L-nitroarginyl-L-valine (2·3 g., 0·0041 mole) were coupled in *NN*-dimethylformamide solution with *NN'*-dicyclohexylcarbodi-imide (0·83 g., 0·0041 mole). After 2 days at room temperature the mixture was worked up to give the protected octapeptide (2·8 g., 64·5%), m. p. 190° (precipitated from hot methanol). A sample prepared by precipitation from *NN*-dimethylformamide–ethyl acetate, methanol–water, and methanol, had m. p. 210°, $[\alpha]_D^{16}$ −30·4° (*c* 1·1 in *NN*-dimethylformamide) (Found: C, 55·5; H, 6·35; N, 15·75. $C_{62}H_{85}N_{15}O_{15},3H_2O$ requires C, 55·8; H, 6·8; N, 15·75%) [lit.,[5] m. p. 205°, $[\alpha]_D^{23}$ −34·9° (*c* 0·5 in *NN*-dimethylformamide)].

L-*Asparaginyl*-L-*arginyl*-L-*valyl*-O-*methyl*-L-*tyrosyl*-L-*valyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine* (O-*Methyl Angiotensin II Amide*.—Removal of the protecting groups from the protected octapeptide (1·4 g., 0·00106 mole) by hydrogenation and trifluoroacetic acid treatment gave the crude *octapeptide*, which was chromatographed on two carboxymethylcellulose columns with ammonium acetate as eluent (0·02—0·15M; pH 5·2). The solutions comprising the main peak were removed and lyophilised (Found: C, 52·4; H, 7·05; N, 16·7; AcOH, 5·17. $C_{50}H_{72}N_{14}O_{11},AcOH,5H_2O$ requires C, 52·25; H, 7·25; N, 16·4; AcOH, 5·02%). Amino-acid analysis: Asp, 1·06; Arg, 1·03; Val, 2·00; Tyr, 0·74; His, 1·02; Pro, 1·05; Phe, 0·96.

Stability Studies

1-(α)*Glutamic Acid Analogue*.—In preliminary experiments samples (5 mg.) of the analogue were refluxed in aqueous solution at pH 6 for various times. Decomposition of the starting material was followed by t.l.c. on alumina. Very little starting material remained after 48 hr.

1-L-*Pyroglutamic Acid*-5-L-*valine Angiotensin II*.—The 1-α-glutamic acid analogue (110 mg.) was refluxed in aqueous solution at pH 6 for 96 hr. Lyophilisation of the resulting solution gave a *powder* which was distributed over 100 transfers in the system n-butanol–0·1M-ammonium acetate, pH 7·5 (1 : 1) ($K = 0·69$). Material isolated from the solutions comprising the main peak (40 mg.) was chromatographically homogeneous and ninhydrin-negative (Found: C, 51·4; H, 7·2; N, 14·9; AcOH, 4·4. $C_{50}H_{69}N_{13}O_{11},AcOH,7H_2O$ requires C, 51·4; H, 7·4; N, 15·0; AcOH, 4·9%). Amino-acid analysis: Glu, 0·995; Arg. 1·00; Val, 1·85; Tyr, 0·91; His, 1·00; Pro, 1·04; Phe, 1·00.

1-(γ)*Glutamic Acid Analogue*.—Preliminary experiments indicated that this substance was more stable than the 1-(α)glutamic acid analogue and, under the conditions described above, a considerable amount of starting material remained after 96 hr. In a preparative experiment the 1-(α)glutamic acid analogue (50 mg.) was refluxed in aqueous solution at pH 6·0 for 96 hr. Chromatography on cellulose phosphate, with 0·01—0·1M-ammonium acetate (pH 5·2) as eluent, gave the heptapeptide product (25 mg.). Amino-acid analysis: Arg, 1·00; Val, 2·09; Tyr, 0·99; His, 1·00; Pro, 0·99; Phe, 0·98.

The authors thank Dr. P. J. Mill of Miles Laboratories, Limited, for advice on gradient elution chromatography, Dr. R. C. Sheppard of The Robert Robinson Laboratories, University of Liverpool, for the amino-acid analyses, and Professor E. W. Horton and Dr. I. H. Main of the Department of Pharmacology, the School of Pharmacy, University of London, for the biological testing.

[7/799 *Received, June 29th,* 1967]