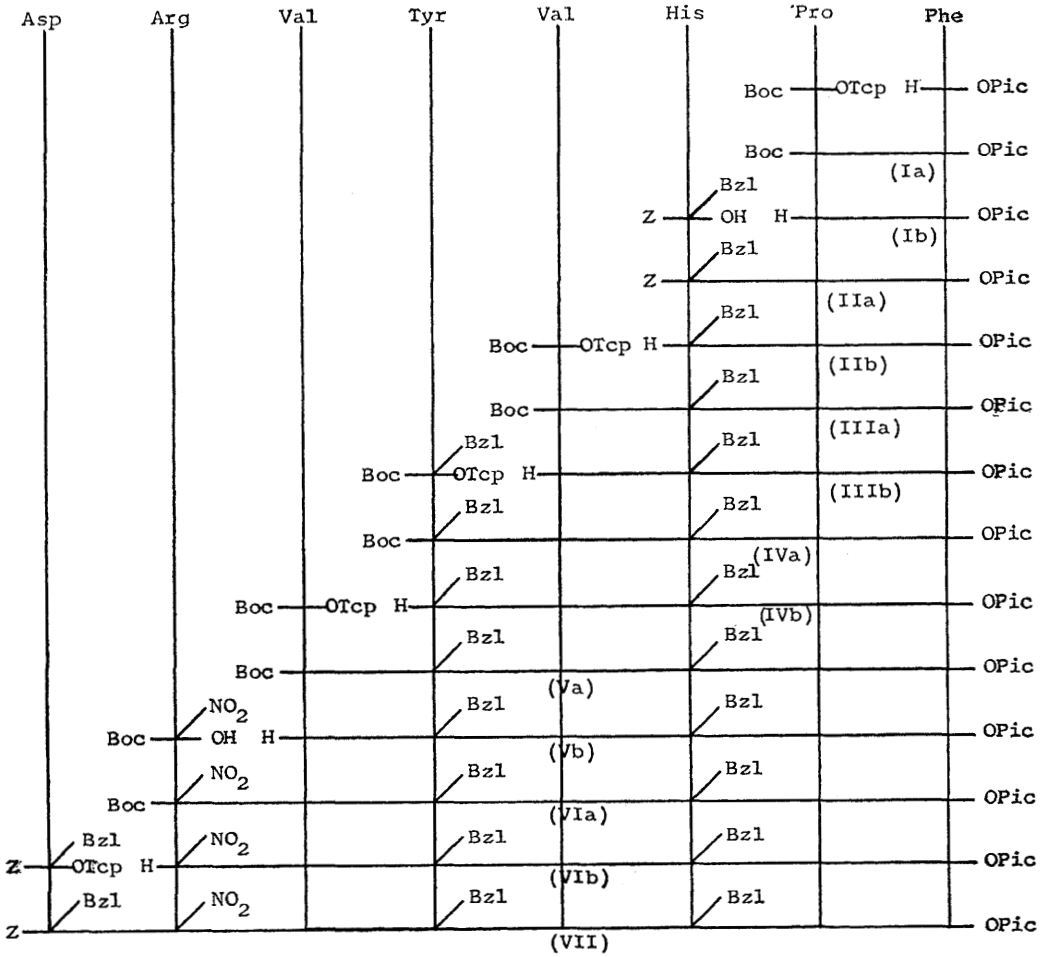
Amino-acids and Peptides. Part XXXIII.[1] Synthesis of Val[5]-Angiotensin-II by the Picolyl Ester Method

By R. Garner and G. T. Young,* The Dyson Perrins Laboratory, Oxford University

A further example of the use of 4-picolyl esters to facilitate peptide synthesis is provided in a synthesis of protected Val[5]-angiotensin-II (overall yield 38%). The side-chain 4-picolyl esters of benzyloxycarbonyl- and t-butoxycarbonyl-aspartic and -glutamic acids, and derivatives, are described, and in a further synthesis of protected Val[5]-angiotensin-II the aspartic acid was introduced as its β-4-picolyl ester, so facilitating the separation procedure. Hydrogenation of both protected octapeptides gave biologically active Val[5]-angiotensin-II.

THE use of the carboxy-terminal amino-acid as its 4-picolyl ester [2] provides in peptide synthesis a simple means by which the coupling product can, at every stage, be separated from the excess of acylating agents, co-products, and by-products. The synthesis of the local case of bradykinin,[1] the key step in the isolation procedure was extraction of the product into aqueous citric acid, and without fractionation or recrystallisation the protected intermediates so obtained had a satisfactory elemental analysis and were chromatographically pure



SCHEME Abbreviations here and in the tables follow the rules in 'Abbreviated Designation of Amino-Acid Derivatives and Polypeptides' (Information Bulletin No. 26, I.U.P.A.C.). Tcp = 2,4,5-Trichlorophenyl; Pic = 4-picolyl.

tissue hormone bradykinin by this procedure has been described;[1] the synthesis of Val[5]-angiotensin-II has been reported briefly [3] and is now described in detail.

The stepwise route is shown in the Scheme; the coupling conditions and the yields and constants of the protected intermediates are given in Table 1. As in the (by t.l.c. in several solvents). It should be noted that this separation will not be effective when, as with benzyloxycarbonyl-*N*-^{im}benzylhistidine, the acyl component has a basic grouping; at that step the minimal excess of acylating agents was used, and the dipeptide ester [(IIb)

[1] Part XXXII, D. J. Schafer, G. T. Young, D. F. Elliott, and R. Wade, preceding paper.

[2] R. Camble, R. Garner, and G. T. Young, *J. Chem. Soc.* (*C*), 1969, 1911.

[3] R. Garner and G. T. Young, *Nature*, 1969, **222**, 177.

in the Scheme] trihydrobromide obtained after removal of the benzyloxycarbonyl group was recrystallised before the synthesis was continued. The t-butoxycarbonyl protecting group was removed in trifluoroacetic acid, and the yields and analysis of the intermediate trifluoroacetates, and of the hydrobromide of (IIb), are shown in Table 2; again, each of these intermediates [excepting that from (IIb)] had a satisfactory elemental analysis without recrystallisation. It is of course essential in this procedure that each coupling should proceed to completion, and hence that the amino-component should showed minor contaminants on electrophoresis at pH 7·2 and 9·35. In the rat pressor test the biological activity was 688 ± 70 units/mg., when compared with the International Research Standard A (assumed to have 950 units/mg.).

It could clearly be advantageous to introduce during a synthesis additional 4-picolyl groups as side-chain substituents in order to facilitate the extraction of the product into an acidic phase. We have therefore prepared intermediates useful for the incorporation of aspartic and glutamic acids as the β- and γ-4-picolyl

TABLE 1

Protected peptide intermediates [a]

Compd.	Amino-component (mmole)	Acylating agents (mmole)	Solvent (ml.)	Reaction time	Yield (%)	Found (%) C	H	N	Formula	Required (%) C	H	N
(Ia)	Phe.OPic,2HBr (5·0)	Boc-Pro-OTcp (6·0)	EtOAc (15 ml.)	5 days	92·5 [b]	66·4	6·9	9·5	$C_{25}H_{31}N_3O_5$	66·2	6·9	9·3
(IIa)	(Ib),$2CF_3{\cdot}CO_2H$ (4·0)	Z-His(Bzl) (4·2) DCCI (4·2)	$Me_2N{\cdot}CHO$ (40 ml.)	20 hr.	78 [c]	66·7	6·1	10·9	$C_{41}H_{42}N_6O_6,1{\cdot}5H_2O$	66·4	6·1	11·3
(IIIa)	(IIb),3HBr (1·0)	Boc-Val-OTcp (1·5)	EtOAc (10 ml.)	48 hr.	96·5 [d]	64·4	7·0	12·8	$C_{43}H_{53}N_7O_7,H_2O$	64·7	6·95	12·3
(IVa)	(IIIb),$3CF_3{\cdot}CO_2H$ (0·77)	Boc-Tyr(Bzl)OTcp (1·2)	EtOAc (10 ml.)	4 days	95 [e]	67·4	6·6	10·45	$C_{59}H_{68}N_8O_9,H_2O$	67·4	6·7	10·3
(Va)	(IVb),$3CF_3{\cdot}CO_2H$ (0·39)	Boc-Val-OTcp (0·59)	EtOAc (10 ml.)	8 days	94 [f]	66·4	6·7	10·5	$C_{64}H_{77}N_9O_{10},H_2O$	66·8	6·9	11·0
(VIa)	(Vb),$3CF_3{\cdot}CO_2H$ (0·30)	Boc-Arg(NO_2) (0·89) DCCI (0·89)	EtOAc (4 ml.) $Me_2N{\cdot}CHO$ (1 ml.)	18 hr.	84 [g]	62·7	6·5	14·5	$C_{70}H_{88}N_{14}O_{13}$	63·0	6·65	14·7
(VII)	(VIb),$3CF_3{\cdot}CO_2H$ (0·11)	Z-Asp(Bzl)-OTcp (0·21	$Me_2N{\cdot}CHO$ (2·5 ml.)	18 hr.	90 [h]	64·3	6·0	13·5	$C_{84}H_{97}N_{15}O_{16}$	64·1	6·2	13·4
(VIII)	(Ib),$2CF_3{\cdot}CO_2H$ (1·69)	Boc-His(Bzl) (1·85) DCCI (1·77)	$Me_2N{\cdot}CHO$ (10 ml.)	16 hr.	85 [i]	67·0	6·6	11·9	$C_{38}H_{44}N_6O_6$	67·0	6·5	12·3
(IX)	(VIb),$3CF_3{\cdot}CO_2H$ (0·06)	Z-Asp(OPic)-OTcp (0·13)	$Me_2N{\cdot}CHO$ (2 ml.)	2 days	91 [j]	61·7	5·9	13·4	$C_{88}H_{96}N_{16}O_{16},2H_2O$	61·9	6·3	13·9

[a] The compounds are numbered as in the Scheme; (VIII) is Boc-His(Bzl)-Pro-Phe-OPic, (IX) is Z-Asp (OPic)-Arg(NO_2)-Val-Tyr(Bzl)-Val-His(Bzl)-Pro-Phe-OPic. Yields are of product with stated constants and analysis. [b] M.p. 101—103° (recryst. from aqueous ethanol, m.p. 102—103°); $[\alpha]_D^{20}$ −46° (*c* 1·0 in EtOH); R_F 0·59 (A), 0·72 (MeOH). [c] $[\alpha]_D^{20}$ −45° (*c* 1·05 in EtOH); R_F 0·42 (A), 0·73 (B1), 0·72 (E1). [d] M.p. 75°; $[\alpha]_D^{20}$ −62° (*c* 1·0 in EtOH); R_F 0·37 (A), 0·71 (B1), 0·67 (MeOH). In another preparation, Boc-Val (6·3 mmoles), (IIb) (4·2 mmoles), and DCCI (6·3 mmoles) in EtOAc (12 ml.), 18 hr. at 0°, gave an 81% yield of chromatographically identical product. [e] M.p. 89—92°; $[\alpha]_D^{20}$ −54° (*c* 1·0 in EtOH); R_F 0·43 (A), 0·68 (B1) 0·61 (MeOH). In another preparation, Boc-Tyr (Bzl) (1·2 mmole., (IIIb) (0·76 mmole), and DCCI (1·2 mmole) in EtOAc (7 ml.), 16 hr. at 20°, gave an 83% yield of chromatographically identical product. [f] M.p. 127—130°; $[\alpha]_D^{20}$ −49·5° (*c* 1·0 in EtOH); R_F 0·59 (A), 0·75 (B1), 0·68 (MeOH). In another preparation, Boc-Val (2·7 mmoles), (IVb) (0·88 mmole), and DCCI (1·8 mmole) gave a 98% yield of a chromatographically identical product. [g] M.p. 124—125°; R_F 0·60 (A), 0·80 (B1), 0·61 (MeOH), 0·72 (E1). [h] R_F 0·46 (A), 0·81 (B1), 0·72 (MeOH), 0·80 (E1). [i] M.p. 67—68°; $[\alpha]_D^{20}$ −46° (*c* 0·9 in EtOH); R_F 0·24 (A), 0·63 (B1), 0·54 (MeOH), 0·61 (E1). [j] M.p. 120—124°; R_F 0·34 (A), 0·67 (B1), 0·60 (MeOH), 0·77 (E1.).

TABLE 2

Trifluoroacetate and hydrobromide intermediates [a]

Compound	Yield (%)	Found % C	H	N	F (Br)	Formula	Required % C	H	N	F (Br)
(Ib),$2CF_3{\cdot}CO_2H$	99 [b]	49·5	4·5	7·5	20·0	$C_{24}H_{25}F_6N_3O_7$	49·6	4·3	7·2	19·6
(IIb),2HBr	92 [c]	46·2	5·2	9·8	(Br 27·7)	$C_{33}H_{39}Br_2N_6O_4,2H_2O$	46·1	5·0	9·8	(Br 27·9)
(IIIb),$3CF_3{\cdot}CO_2H$	97 [d]	49·8	4·9	9·9	16·6	$C_{44}H_{48}F_9N_7O_{11},2H_2O$	49·9	4·95	9·3	16·2
(IVb),$3CF_3{\cdot}CO_2H$	100 [e]	56·1	5·0	9·1	12·5	$C_{60}H_{63}F_9N_8O_{13}$	56·5	5·0	8·8	13·4
(Vb),$3CF_3{\cdot}CO_2H$	98 [f]	56·9	5·6	9·05	12·4	$C_{65}H_{72}F_9N_9O_{14}$	56·8	5·3	9·2	12·4
(VIb),$3CF_3{\cdot}CO_2H$	86 [g]	53·4	5·6	12·0		$C_{71}H_{83}F_9N_{14}O_{17},H_2O$	53·5	5·4	12·3	

[a] The compounds are numbered as in the Scheme. Yields are of product with the stated constants and analysis. [b] R_F 0·25 (A), 0·41 (MeOH). [c] Recrystallised from EtOH; m.p. 171—173°; $[\alpha]_D^{20}$ −40° (*c* 0·95 in H_2O); R_F 0·32 (A), 0·43 (B1), 0·23 (MeOH), 0·33 (E1). [d] M.p. 92—96°; $[\alpha]_D^{20}$ −37·5° (*c* 0·9 in H_2O); R_F 0·23 (A), 0·55 (B1), 0·43 (MeOH), 0·46 (E1). [e] M.p. 110—112°; R_F 0·31 (A), 0·67 (B1), 0·54 (MeOH), 0·63 (E1). [f] M.p. 157—160°; R_F 0·49 (A), 0·68 (B1), 0·61 (MeOH), 0·81 (E1). [g] M.p. 136—138°; R_F 0·23 (A), 0·63 (B1), 0·43 (MeOH), 0·58 (E1).

be freed completely from its trifluoroacetate (or hydrobromide); this was effected by the addition of an excess of tertiary amine (trimethylamine or triethylamine) to a solution or suspension of the salt, and removal of the excess by evaporation. The protected Val[5]-angiotensin-II [(VII) in the Scheme] was not soluble in aqueous citric acid and was separated on Sulphoethyl–Sephadex C-25 (saturated with 3-bromopyridine)[2], and the overall yield (from L-phenylalanine 4-picolyl ester dihydrobromide) of analytically pure product was 38%. Some alternative coupling steps, including the use of t-butoxycarbonyl-N^{im}-benzyl-L-histidine in place of the benzyloxycarbonyl analogue, are reported in Table 1. Hydrogenation of the crude protected octapeptide gave Val[5]-angiotensin-II monoacetate pentahydrate (86% yield), which without fractionation had a satisfactory elemental and amino-acid analysis and gave a single ninhydrin-positive spot on paper electrophoresis at pH 5·3 and 6·35, but it esters, respectively. t-Butoxycarbonyl-L-aspartic acid was converted into its di-4-picolyl ester (not crystallised) by treatment with 4-pyridylmethanol and dicyclohexylcarbodi-imide; partial hydrolysis with lithium hydroxide gave crystalline β-4-picolyl t-butoxycarbonyl-L-aspartate. A corresponding sequence gave the corresponding glutamic acid derivative, and the benzyloxycarbonyl analogues were also made. We report also di-4-picolyl L-aspartate and di-4-picolyl L-glutamate trihydrobromides, useful as starting materials for the synthesis of peptides having carboxy-terminal aspartic or glutamic acids (or their diamides).

As an example of this extension of the use of picolyl esters, α-2,4,5-trichlorophenyl β-4-picolyl benzyloxycarbonyl-L-aspartate was condensed with the heptapeptide 4-picolyl ester (VIb), giving the protected octapeptide ester (IX) (see Table 1). In contrast to the analogue (VII), which has a β-benzyl in place of a

β-4-picolyl group on the aspartic acid residue, this product was readily soluble in aqueous citric acid and it was isolated by this method in 91% yield; the crude product had a satisfactory elemental analysis and was chromatographically pure in five solvents. Hydrogenation again gave biologically active octapeptide.

EXPERIMENTAL

The general instructions and the designations of t.l.c. solvent systems given in Part XXXI apply, unless otherwise stated.

General Reaction Procedures.—The amino-component was liberated from its trifluoroacetate (or hydrobromide) by the addition of trimethylamine (or triethylamine; *ca.* twice the theoretical amount) to a solution or suspension of the salt in the solvent to be used for coupling; the excess of tertiary amine was then evaporated off at room temperature. Lost solvent was replaced and the active ester (or the carboxy-component together with dicyclohexylcarbodi-imide) was immediately added. Coupling reactions were continued at room temperature until t.l.c. (for useful solvents see footnotes to Tables 1 and 2) detected (by ninhydrin or by chlorine and starch–iodide) no amino-component; in typical cases, control experiments showed that 0·2% could be detected. When ethyl acetate was the solvent, the reaction solution was diluted with an equal volume of solvent and then washed with water, N-sodium hydrogen carbonate, and brine. When dimethylformamide was the solvent the reaction solution (filtered if necessary) was evaporated at 25°/0·1 mm., and the residue was dissolved in ethyl acetate and washed as before. In either case, the protected peptide was then extracted into chilled 0·7M-citric acid (3 × 0·5 vol.). The combined extracts were made alkaline with solid sodium hydrogen carbonate and the product (if not precipitated as a solid) was extracted into ethyl acetate (4 × 0·5 vol.). The extract was dried ($MgSO_4$) and evaporated. The t-butoxycarbonyl group was removed by dissolving the protected peptide 4-picolyl ester in trifluoroacetic acid (*ca.* 3 ml. for 0·5 g.); after 1 hr., the solution was evaporated to dryness at 25°/1 mm., final traces of acid being removed by the addition and evaporation of small amounts of water, and finally by dissolution in ethanol and precipitation with dried ether. Special cases in which these general procedures were substantially modified are described later.

*N-Benzyloxycarbonyl-β-benzyl-L-aspartyl-*N(ω)*-nitro-L-arginyl-L-valyl-*O*-benzyl-L-tyrosyl-L-valyl-*N^{im}*-benzyl-L-histidyl-L-prolyl-L-phenylalanine 4-Picolyl Ester* (VII).—The reaction mixture was stirred for 1 hr. with Sulphoethyl–Sephadex C-25 (5 g.) saturated with 3-bromopyridine. The resin was then washed (as a column) with dimethylformamide (200 ml.) and eluted with a mixture of triethylamine (0·4 ml.), water (0·4 ml.), and dimethylformamide (20 ml.). Evaporation of the eluate at 25°/0·1 mm gave a residue which on trituration with ethanol gave protected Val^5-angiotensin-II (VII) (see Table 1).

Val^5-Angiotensin-II.—The protected octapeptide (VII) (163 mg.) in 80% acetic acid (3 ml.) was hydrogenated during 66 hr. over palladium–charcoal (10%; 330 mg.). The mixture was then filtered (Celite) and the filtrate was evaporated at 25°/0·1 mm. The residue was triturated with ethanol and extracted with cold water; lyophilisation of the solution and drying (P_2O_5) gave Val^5-angiotensin-II monoacetate pentahydrate (105 mg., 86%) (Found: C, 51·7; H, 7·05; N, 15·05. Calc. for $C_{49}H_{69}N_{13}O_{12},C_2H_4O_2,5H_2O$: C, 51·8; H, 7·1; N, 15·4%). Amino-acid analysis (hydrolysis by 6N-HCl at 100° for 3 days): Asp, 1·06, Arg, 0·97; Val, 1·86; Tyr, 0·89; His, 0·91; Pro, 1·12; Phe, 0·99. High-voltage electrophoresis at pH 5·3 and pH 6·35 showed single spots (ninhydrin and Pauly tests); at pH 7·2 three minor components migrated towards the cathode and at pH 9·35 a minor compononent moved faster than the main product towards the anode. In the rat pressor assay, the product had 688 ± 70 units per mg. when compared with the International Research Standard A, assumed to have 950 units per mg.

N^{im}-Benzyl-L-histidyl-L-prolyl-L-phenylalanine 4-Picolyl Ester (IIb) *Trihydrobromide.*—The benzyloxycarbonyl derivative (IIa) (1·50 g.) was dissolved in 3N-hydrogen bromide in acetic acid (10 ml.). After 1 hr. at room temperature ether (70 ml.) was added, and the precipitate was collected and washed with ether. Crystallisation from ethanol gave (IIb) trihydrobromide as prisms (see Table 2).

β-4-Picolyl t-Butoxycarbonyl-L-aspartate.—Dicyclohexylcarbodi-imide (4·12 g., 20 mmoles) was added to a solution of t-butoxycarbonyl-L-aspartic acid (2·34 g., 10 mmoles) and 4-pyridylmethanol (2·18 g., 20 mmoles) in dichloromethane (20 ml.). After 18 hr. the solution was filtered and evaporated. The residue was dissolved in ethyl acetate; the solution was washed (sodium hydrogen carbonate, brine) and the product was extracted into 0·7M-citric acid; sodium hydrogen carbonate was added and the product was extracted into chloroform. The extract was dried ($MgSO_4$), treated with charcoal, and evaporated. The residual red syrup (2·9 g., 70%) was di-4-picolyl t-butoxycarbonyl-L-aspartate, sufficiently pure (by n.m.r. spectroscopy) for the next stage. To a solution of the syrup (2·75 g., 6·63 mmoles) in acetone (60 ml.) and water (15 ml.) a solution of lithium hydroxide hydrate (0·278 g., 6·63 mmoles) in water (6 ml.) was added dropwise during 45 min. The acetone was evaporated off and the aqueous residue was washed with chloroform (20 ml.). Citric acid (0·465 g., 2·21 mmoles) was added to the aqueous layer and the product was then extracted into chloroform (3 × 30 ml.). Evaporation gave a residue which solidified on trituration with ether. Crystallisation from ethyl acetate gave the *β-monoester* (1·1 g., 36% overall), as needles, m.p. 133—135°, $[\alpha]_D^{20}$ −23° (*c* 1·0 in Me_2N·CHO) (Found: C, 55·7; H, 6·2; N, 8·5. $C_{15}H_{20}N_2O_6$ requires C, 55·5; H, 6·2; N, 8·6%). The following were prepared analogously: *β-4-picolyl benzyloxycarbonyl-L-aspartate* (34% yield overall), m.p. 158—160° (from ethyl acetate), $[\alpha]_D^{20}$ −20° (*c* 0·8 in Me_2N·CHO) (Found: C, 60·0; H, 4·9; N, 7·7. $C_{18}H_{18}N_2O_6$ requires C, 60·3; H, 5·1; N, 7·8%); *γ-4-picolyl t-butoxycarbonyl-L-glutamate* (19% yield overall), m.p. 156—158° (from ethyl acetate), $[\alpha]_D^{20}$ −16° (*c* 1·0 in Me_2N·CHO) (Found: C, 56·8; H, 6·8; N, 8·3. $C_{16}H_{22}N_2O_6$ requires C, 56·8; H, 6·6; N, 8·3%); and *γ-4-picolyl benzyloxycarbonyl-L-glutamate* (27% yield overall), m.p. 138—139° (from ethyl acetate), $[\alpha]_D^{20}$ −12·3° (*c* 1·2 in Me_2N·CHO) (Found: C, 61·0; H, 5·6; N, 7·0. $C_{19}H_{20}N_2O_6$ requires C, 61·3; H, 5·4; N, 7·5%).

α-2,4,5-Trichlorophenyl β-4-Picolyl Benzyloxycarbonyl-L-aspartate.—Dicyclohexylcarbodi-imide (0·412 g., 2·0 mmoles) was added to a stirred solution of β-4-picolyl benzyloxycarbonyl-L-aspartate (0·736 g., 2·0 mmoles) in dichloromethane (10 ml.). After 16 hr. the solution was filtered, ethyl acetate (30 ml.) was added, and the solution was

washed (2N-sodium carbonate, brine), dried, and evaporated. The residue solidified under ether and was recrystallised from ethyl acetate–light petroleum (b.p. 40—60°), giving the *ester* (0·65 g., 60%), m.p. 106° $[\alpha]_D^{20}$ −27° (*c* 1·1 in $Me_2N{\cdot}CHO$) (Found: C, 53·8; H, 3·7; N, 5·3. $C_{24}H_{19}Cl_3N_2O_6$ requires C, 53·6; H, 3·6; N, 5·2%).

Di-4-picolyl L-*Aspartate Trihydrobromide*.—Crude di-4-picolyl benzyloxycarbonyl-L-aspartate (prepared as already described; 0·10 g.) was dissolved in acetic acid (0·5 ml.) and 5·6N-hydrogen bromide in acetic acid (0·75 ml.) was added. After 1 hr. the crystalline product was collected and washed repeatedly with ether. Recrystallisation from ethanol–water (*ca.* 10%) gave a product (0·124 g., 97%), m.p. 159°; further crystallisation gave the *salt*, m.p. 161—162°, $[\alpha]_D^{20}$ +1·2°, $[\alpha]_{365}^{20}$ +3·8° (*c* 1·0 in H_2O) (Found: C, 33·5; H, 3·9; Br, 42·2; N, 7·1; $C_{16}H_{20}Br_3N_3O_4,H_2O$ requires C, 33·4; H, 3·8; Br, 41·6; N, 7·3%).

Di-4-picolyl L-*Glutamate Trihydrobromide*.—This was prepared as described for the aspartic acid analogue; recrystallisation from ethanol gave the *salt* (92% yield), m.p. 140°, $[\alpha]_D^{20}$ +8·0° (*c* 1·1 in H_2O) (Found: C, 34·7; H, 4·2; Br, 39·5; N, 6·9. $C_{17}H_{22}Br_3N_3O_4,H_2O$ requires C, 34·6; H, 4·1; Br, 40·6; N, 7·1%).

*Benzyloxycarbonyl-β-4-picolyl-*L*-aspartyl-*N(ω)*-nitro-*L*-arginyl-*L*-valyl-*O*-benzyl-*L*-tyrosyl-*L*-valyl-*N^{im}*-benzyl-*L*-histidyl-*L*-prolyl-*L*-phenylalanine 4-Picolyl Ester* (IX).—α-2,4,5-Trichlorophenyl β-4-picolyl benzyloxycarbonyl-L-aspartate (68·3 mg., 0·127 mmoles) was added to a solution of *N*(ω)-nitro-L-arginyl-L-valyl-*O*-benzyl-L-tyrosyl-L-valyl-N^{im}-benzyl-L-histidyl-L-prolyl-L-phenylalanine 4-picolyl ester trifluoroacetate monohydrate (VIb) (100 mg., 0·063 mmoles) and triethylamine (0·028 ml., 0·2 mmoles) in dimethylformamide (2 ml.). After 2 days, t.l.c. (solvent A) detected no amino-component; the solvent was evaporated off and the residue was triturated with 2N-sodium hydrogen carbonate and then water. The white solid was extracted with 1·4M-citric acid; the solution was filtered and then made alkaline with sodium hydrogen carbonate. The *protected octapeptide ester* (IX) (92 mg., 91%) was precipitated as a white solid, which was washed with water and then ether and dried (see Table 1).

Val^5-Angiotensin-II.—Hydrogenation of the protected octapeptide (IX) as already described (but for 72 hr.) gave Val^5-angiotensin-II; amino-acid analysis (hydrolysis by 6N-HCl at 110° for 3 days): Asp, 1·1; Arg, 0·9; Val, 2·0; Tyr, 0·7; His, 1·0; Pro, 1·0; Phe 1·0. In the rat pressor assay (by Dr. W. F. Cook) the product had 694 ± 70 units per mg. (based on the nitrogen content, 13·3%), when compared with the International Research Standard A (assumed to have 950 units per mg.).

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