Polypeptides. Part X.[1] Variations of the Tryptophyl Position in the *C*-Terminal Tetrapeptide Amide Sequence of the Gastrins

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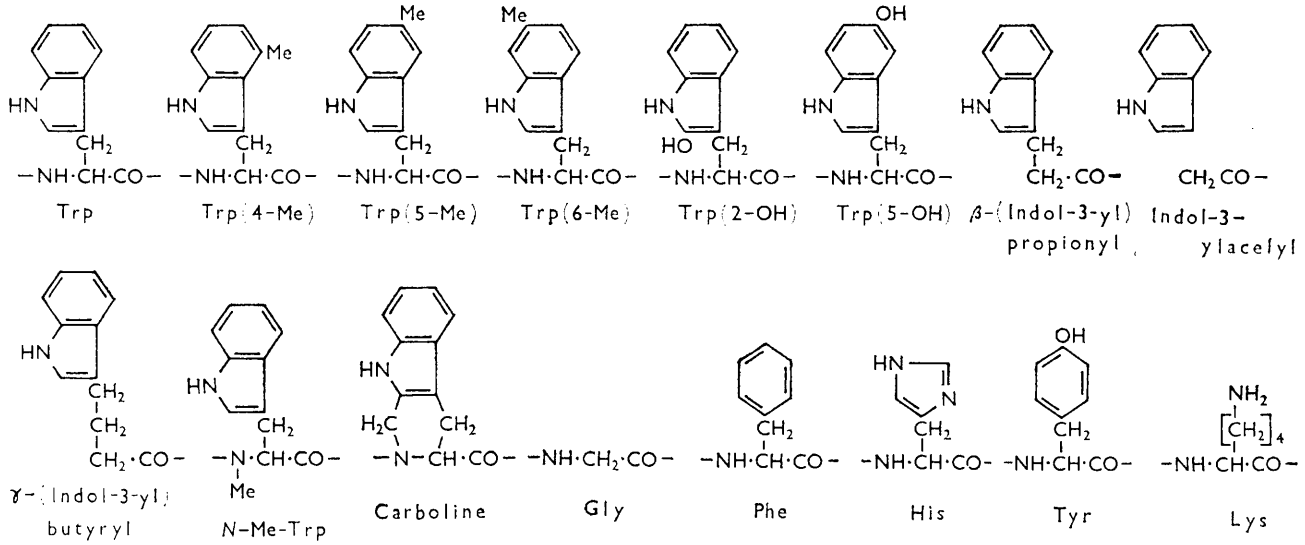
The synthesis is described of analogues of L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide (the *C*-terminal sequence of the gastrins), and its *N*-benzyloxycarbonyl or *N*-t-butoxycarbonyl derivatives, wherein the tryptophyl residue has undergone replacement by glycyl, histidyl, lysyl, phenylalanyl, tyrosyl, 4-, 5- or 6-methyltryptophyl, N^{α}-methyltryptophyl, or 2- or 5-hydroxytryptophyl residues, or has been modified in various other ways.

In Parts VI,[2] VII,[3] VIII,[4] and IX [1] of this series we gave details of the synthesis of analogues of the *C*-terminal tetrapeptide amide sequence of the gastrins, Trp-Met-Asp-Phe-NH_2,† wherein the terminal amide, phenylalanine, aspartic acid, or methionine residues have undergone change. The present paper describes analogues resulting from change of the tryptophan residue. If the variant of the tryptophan residue is described as X, then the analogues are acylated tripeptide amides of the type X-Met-Asp-Phe-NH_2, or, where X is an amino-acyl radical, tetrapeptide amide derivatives of the type H-, Z-, or BOC-X-Met-Asp-Phe-NH_2, or pentapeptide amide derivatives of the type BOC-Gly-X-Met-Asp-Phe-NH_2. All four types of analogue were prepared in one of the cases of variation by an amino-acyl radical (X = Phe). No appreciable differences in biological activity were found in changing from one type to another. In general therefore, only one type of analogue was prepared and used to follow the effect of structural change on biological activity.

The analogues involved in the work arise from modification of the tryptophan residue in the following ways: substitution in the indole nucleus by 4-, 5-, or 6-methyl- [X = Trp(4-Me), Trp(5-Me), or Trp(6-Me)], 2- or 5-hydroxy- [X = Trp(2-OH) or Trp(5-OH)], or 5-benzyloxycarbonyloxy- [X = Trp(5-OZ)] groups; removal of the α-amino-group [X = β-(indol-3-yl)propionyl]; removal of the α-amino-group accompanied by contraction (X = indol-3-ylacetyl) or extension [X = γ-(indol-3-yl)-butyryl] of the chain between the indole nucleus and the carbonyl group; methylation of the N^{α}-imino-group (X = *N*-Me-Trp); methylene bridging of the N^{α}-imino-groups with the indole nucleus (X = carboline); replacement by other amino-acyl radicals, *i.e.* glycyl (X = Gly), L-phenylalanyl (X = Phe), L-histidyl (X = His), L-tyrosyl (X = Tyr), N^{ϵ}-benzyloxycarbonyl-L-lysyl [X = Lys(Z)], N^{ϵ}-t-butoxycarbonyl-L-lysyl [X = Lys(BOC)], or L-lysyl (X = Lys) residues. *N*-Phenylacetyl (X = $PhCH_2{\cdot}CO$), *N*-phenoxyacetyl (X = $Ph{\cdot}O{\cdot}CH_2{\cdot}CO$), and *N*-phenylpropionyl [X = $Ph{\cdot}[CH_2]_2{\cdot}CO$] derivatives of L-methionyl-L-aspartyl-L-phenylalanine amide are also described. The relationship of these structural changes is shown below.

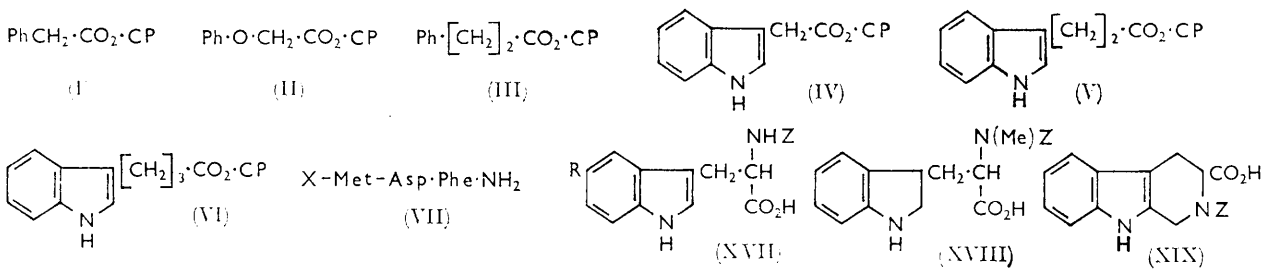
† The abbreviation for amino-acid residues and protecting groups and their mode of use are in accordance with the suggestions of the Committee on Nomenclature which reported at the Fifth European Peptide Symposium [G. T. Young (ed.), 'Peptides. Proc. Fifth European Peptide Symp.,' 1963, Pergamon Press, Lond., p. 261] with the modifications adopted by I.U.P.A.C. (Inform. Bull., I.U.P.A.C., 1966, No. 25, p. 32).

[1] Part IX, J. S. Morley and J. M. Smith, *J. Chem. Soc.* (*C*), 1968, 726.
[2] H. Gregory, A. H. Laird, J. S. Morley, and J. M. Smith, *J. Chem. Soc.* (*C*), 1968, 522.
[3] H. Gregory, D. S. Jones, and J. S. Morley, *J. Chem. Soc.* (*C*), 1968, 531.
[4] H. Gregory, J. S. Morley, J. M. Smith, and M. J. Smithers, *J. Chem. Soc.* (*C*), 1968, 715.



All except one of the analogues were prepared from L-methionyl-L-aspartyl-L-phenylalanine amide [5] (VII; X = H) [also (IX) in Scheme 1].

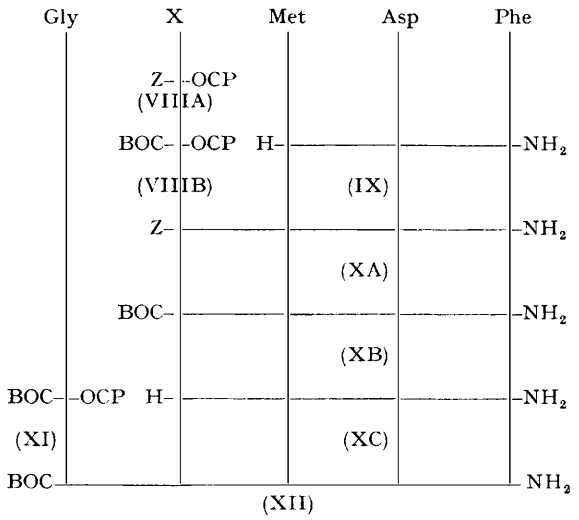
Condensation of the triethylammonium salt of this tripeptide amide with the 2,4,5-trichlorophenyl esters of phenylacetic acid (I), phenoxyacetic acid (II), β-phenylpropionic acid (III), indol-3-ylacetic acid (IV), β-(indol-3-yl)propionic acid (V), or γ-(indol-3-yl)butyric



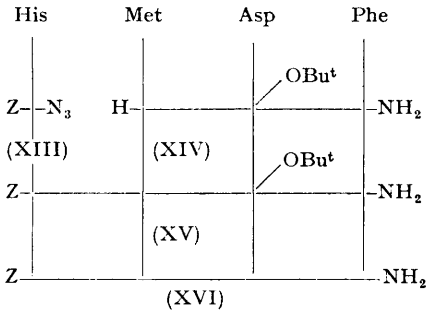
acid (VI) gave the acylated tripeptide amides [VII; X = $PhCH_2$·CO, Ph·O·CH_2·CO, Ph·$[CH_2]_2$·CO, indol-3-ylacetyl, β-(indol-3-yl)propionyl, and γ-(indol-3-yl)-butyryl].

Similarly (Scheme 1), the 2,4,5-trichlorophenyl esters of *N*-benzyloxycarbonyl-L-phenylalanine (VIIIA; X = Phe), *N*-t-butoxycarbonyl-L-phenylalanine (VIIIB; X = Phe), N^{α}-benzyloxycarbonyl-N^{ϵ}-t-butoxycarbonyl-L-lysine [VIIIA; X = Lys(BOC)], N^{α}-t-butoxycarbonyl-N^{ϵ}-benzyloxycarbonyl-L-lysine [VIIIB; X = Lys(Z)], N^{α}-t-butoxycarbonyl-4-, -5-, or -6-methyl-DL-tryptophan [VIIIB; X = Trp(4-, 5-, or 6-Me)], N^{α}-benzyloxycarbonyl-(5-benzyloxycarbonyloxy)-DL-tryptophan [VIIIA; X = Trp(5-OZ)], N^{α}-benzyloxycarbonyl-2-hydroxy-L-tryptophan [VIIIA; X = Trp(2-OH)], and *N*-t-butoxycarbonylglycine (VIIIB; X = Gly) were first prepared and condensed with the triethylammonium salt of the tripeptide amide [5] (IX) to give the *N*-benzyloxycarbonyltetrapeptide amides [XA; X = Phe, Lys(BOC), Trp(5-OZ), or Trp(2-OH)] or *N*-t-butoxycarbonyltetrapeptide amides [XB; X = Phe, Lys(Z), Trp(4-Me), Trp(5-Me), Trp(6-Me), or Gly]. Brief treatment of two of the t-butoxycarbonyltetrapeptide amides (XB; X = Phe or Gly) with hydrogen chloride in acetic acid gave the tetrapeptide amide hydrochlorides (XC,HCl; X = Phe or Gly). One of these (XC,HCl; X = Phe) gave the *N*-t-butoxycarbonylpentapeptide amide (XII; X = Phe) after treatment with *N*-t-butoxycarbonylglycine 2,4,5-trichlorophenyl ester (XI) in the presence of two equivalents of triethylamine. The t-butoxycarbonyl groups of the two lysine analogues [XA; X = Lys(BOC), and XB; X = Lys(Z)] were cleaved with trifluoroacetic acid to give N^{α}-benzyloxycarbonyl-L-lysyl-L-methionyl-L-aspartyl-L-phenylalanine amide (XA; X = Lys) and N^{ϵ}-benzyloxycarbonyl-L-lysyl-L-methionyl-L-aspartyl-L-phenylalanine amide [XC; X = Lys(Z)], and the triethylammonium salt of the latter product, after reaction with *N*-t-butoxycarbonylglycine 2,4,5-trichlorophenyl ester (XI), gave the *N*-t-butoxycarbonylpentapeptide amide [XII; X = Lys(Z)]. The bisbenzyloxycarbonyltetrapeptide derivative (XA; X = Trp(5-OZ)] was converted into the tetrapeptide amide [XC; X = Trp(5-OH)] by hydrogenolysis; as expected, the hydrogenolysis was severely hindered by the presence of methionine in the molecule, but the rate of reaction was considerably

[5] J. M. Davey, A. H. Laird, and J. S. Morley, *J. Chem. Soc.* (*C*), 1966, 555.



SCHEME 1



SCHEME 2

(Z = Benzyloxycarbonyl; BOC = t-Butoxycarbonyl; CP = 2,4,5-Trichlorophenyl).

increased by the addition of cyclohexylamine to the reaction mixture (this is in agreement with the general findings of Medzihradszky and Medzihradszky-Schweiger [6]).

The scheme of synthesis was modified slightly in the preparation of the histidine analogue (XVI) (see Scheme 2). N^{α}-Benzyloxycarbonyl-L-histidine azide (XIII) was coupled with the t-butyl ester of L-methionyl-L-aspartyl-L-phenylalanine amide [5] (XIV), and the resulting benzyloxycarbonyltetrapeptide amide t-butyl ester (XV) was converted into the required acid (XVI) by means of trifluoroacetic acid.

The individual 2,4,5-trichlorophenyl esters used in these syntheses were all prepared from the corresponding acid, 2,4,5-trichlorophenol, and *NN'*-dicyclohexylcarbodi-imide, following the general method of Pless and Boissonnas.[7] N^{α}-Benzyloxcarbonyl-(5-benzyloxycarbonyloxy)-DL-tryptophan (XVII; R = OZ) was prepared by carbobenzyloxylation of 5-hydroxy-DL-tryptophan (XVII; R = OH) at pH 9·5. N^{α}-Benzyloxycarbonyl-N^{α}-methyl-L-tryptophan (XVIII) and N^{2}-benzyloxycarbonyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (XIX) were best prepared from N^{α}-methyl-L-tryptophan or 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid by carbobenzoxylation at pH 10—11 at 50—60°. Details of the preparation of these and other intermediates will be found in the Experimental section.

All the tri-, tetra-, and penta-peptide analogues prepared are described in the Table. It should be noted that DL-amino-acids (4-, 5-, and 6-methyl- and 5-hydr-

[6] K. Medzihradszky and H. Medzihradszky-Schweiger, *Acta Chim. Acad. Sci. Hung.*, 1965, **44**, 15.

[7] J. Pless and R. A. Boissonnas, *Helv. Chim. Acta*, 1963, **46**, 1609.

Tri-, tetra-, and penta-peptide derivatives of the type X-Met-Asp-Phe-NH_2

X [a]	Method [b]	Yield (%)	M.p. [c]	Solvent [d]	$[\alpha]_D$ [f]	R_F [e] A	B	C	D	Analysis [g] Found (%) C	H	N	Formula	Required (%) C	H	N
$PhCH_2CO$	C1	80	236—238°	ME	−46·4		0·71	0·36		58·8	6·0	10·6	$C_{26}H_{32}N_4O_5S$	59·0	6·1	10·6
$PhOCH_2CO$	C1	80	212—213 d.	EtOH	−47·2	0·73	0·78			56·9	5·9	10·2	$C_{26}H_{32}N_4O_7S$	57·3	5·9	10·3
$Ph(CH_2)_2CO$	C1	82	226—228	Aq. AcOH	−46·0		0·71	0·37		58·5	6·1	10·0	$C_{27}H_{34}N_4O_5S,0·5H_2O$	58·8	6·4	10·2
Indol-3-ylacetyl	C2	75	218—220	EtOH	−29·8	0·73		0·44		59·2	6·0	12·1	$C_{28}H_{32}N_4O_5S$	59·2	5·9	12·3
β-(Indol-3-yl)propionyl	C2	83	207—209	EtOH	−35·9	0·74		0·47		59·9	6·2	12·1	$C_{29}H_{34}N_4O_5S$	60·0	6·1	12·0
γ-(Indol-3-yl)butyryl...	C2	78	216—218	EtOH	−39·8	0·77		0·51		60·3	6·1	11·5	$C_{30}H_{34}N_4O_5S$	60·4	6·3	11·7
Z-His, t-butyl ester [i]	A	49	154—156 d.	MeOH–EtAc	−20·5	0·56		0·77		58·2	6·3	13·7	$C_{34}H_{47}N_7O_8S$	58·6	6·4	13·3
Z-His	B1	100	209 d. (HCl)	MeOH–Et_2O	−16·5	0·54		0·36		51·8	5·5	12·9	$C_{35}H_{39}N_7O_8S,HCl,H_2O$	52·2	5·7	13·3
Z-Lys(BOC)	C3	73	191—193	Aq. DMF	−33·1	R_{FO} 0·84				56·9	6·7	10·5	$C_{37}H_{53}N_6O_{10}S$	57·1	6·6	10·9
Z-Lys	E	88	186—188 (TFA)	Et_2O [j]	−26·4	0·46		0·23	0·42							
BOC-Lys(Z)	C3	79	190—192	Aq. DMF						57·1	7·0	10·8	$C_{27}H_{53}N_6O_{10}S$	57·1	6·6	10·9
H-Lys(Z)	E	86	d. 160 (TFA)	Et_2O [j]	−9·3	R_{FM} 0·70				51·4	6·0	11·3	$C_{32}H_{44}N_6O_8S,C_2HF_3O_2$	51·9	5·8	10·7
BOC-Gly-Lys(Z)	C4	42	184—187 d.	Aq. DMF		R_{FO} 0·83				55·7	6·9	11·7	$C_{39}H_{55}N_7O_{11}S,0·5H_2O$	55·8	6·7	11·7
Z-Phe	C3	50	109—111	ME	−33·8	0·79		0·41	0·58	60·6	5·9	10·3	$C_{35}H_{41}N_3O_8S$	60·8	5·9	10·0
BOC-Phe	C3	69	187—189	ME	−28·9	0·99		0·47		57·9	6·3	10·2	$C_{28}H_{45}N_3O_8S$	58·3	6·6	10·6
H-Phe	B2	98	230—232 (HCl)	Et_2O [j]		0·49		0·33		met 0·96; asp 1·0; phe 2·01 [k]						
BOC-Gly-Phe	C4	49	90—95 d.	Aq. DMF	−21·6	0·90		0·37	0·59	56·5	6·7	11·5	$C_{34}H_{46}N_6O_9S,0·5H_2O$	56·4	6·6	11·6
BOC-Trp(4-Me) [h]	C3	57	214—219 d.	Aq. EtOH	−29·3	0·76		0·44		59·0	6·6	11·5				
BOC-Trp(5-Me) [h]	C3	67	184—187 d.	Aq. EtOH	−24·2	0·75		0·45		58·6	6·8	11·5	$C_{35}H_{46}N_6O_8S$	59·0	6·5	11·8
BOC-Trp(6-Me) [h]	C3	62	224—228 d.	Aq. EtOH	−35·0	0·71		0·48		58·8	6·7	11·6				
Z-Trp(5-OZ) [h]	C3	69	165—170 d.	Et_2O [j]	−18·7	0·80		0·42		59·0	5·5	8·9	$C_{45}H_{46}N_6O_{11}S,2H_2O$ [m]	59·0	5·7	9·2
H-Trp(5-OH) [h]	D	46	115 d.	MeOH–Et_2O	−25·9	0·51		0·34		trp(5-OH) 0·92; met 0·91; asp 1·07; phe 1·10 [l]						
Z-Trp(2-OH)	C3	44	192—193	EtAc [j]	−32·8	0·79	0·81	0·38		59·2	5·8	11·0	$C_{37}H_{41}N_6O_9S$	59·4	5·7	11·2
Z-N^{α}-Me-Trp	C3	49	130—135 d.	EtAc–Et_2O	−46·7	0·60		0·85		61·0	6·1	11·1	$C_{36}H_{44}N_6O_8S$	61·3	6·0	11·3
Z-Carboline	C3	61	146—148	Aq. EtOH	+5·8	0·86				S, 4·8			$C_{38}H_{42}N_6O_8S$	S, 4·3		
BOC-Gly	C3	75	202—203 d.	MeOH–EtAc	−32·5	0·79			0·60	52·3	6·6	12·3	$C_{25}H_{37}N_3O_8S$	52·8	6·6	12·3
H-Gly	B2	92	196—197	Et_2O [j]	−52·3	0·38		0·26	0·25							
Z-Tyr	C3	62	195—197 d.	Aq. ME	−22·7	0·85		0·41 [n]	0·60	59·3	5·8	9·3	$C_{35}H_{41}N_3O_9S$	59·4	5·8	9·9

[a] The abbreviations for amino-acid residues and protecting groups and their mode of use are in accordance with the suggestions given in footnote †; Trp(4-Me) = 4-methyltryptophan, Z-carboline = N^{2}-benzyloxycarbonyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl. [b] See Experimental section for description of methods. [c] HCl = hydrochloride, TFA = trifluoroacetate, d. = with decomposition or effervescence. [d] ME = 2-methoxyethanol, DMF = dimethylformamide, aq. = aqueous. [e] The methods of chromatography and solvent systems are explained in the introduction to the Experimental section; unless otherwise stated, the quoting of an R_F value implies the detection of one spot only by at least two of the visualisation methods described. [f] *c* 1·0 in dimethylformamide at 24—26°. [g] Samples for micro-analysis were usually dried at 60°/18 hr./0·1 mm. over phosphorus pentoxide. [h] Mixture of DLLL- and LLLL-isomers. [i] This refers to the compound Z-His-Met-Asp(OBut)-Phe-NH_2. [j] Triturated. [k] Amino-acid ratios found in acid hydrolysate. [l] Amino-acid ratios found in alkali hydrolysate. [m] Amino-acid ratios in 16 hr. acid hydrolysate: asp 0·94, met 1·00, phe 1·08, trp(5-OH) 0·94. [n] R_{FO} 0·69.

oxy-DL-tryptophan) were used as starting materials in four of the syntheses. In these cases the tetrapeptide analogues are mixtures of the DLLL- and LLLL-isomers. Otherwise, all optically active amino-acid residues are of the L-configuration.

Many of the analogues described in this paper are potent stimulators of gastric acid secretion in the rat. The results have been reported and discussed elsewhere.[8]

EXPERIMENTAL

Ascending thin-layer chromatograms were run on Kieselgel G with butan-1-ol–acetic acid–water (4 : 1 : 5 v/v) (R_{FA}), butan-1-ol–acetic acid–water–pyridine (15 : 3 : 12 : 10) (R_{FB}), butan-2-ol–ammonium hydroxide (3%) (3 : 1) (R_{FC}), acetonitrile–water (3 : 1) (R_{FD}), acetone–chloroform (1 : 1) (R_{FE}), ethanol–chloroform (4 : 1) (R_{FF}), cyclohexane–ethyl acetate (1 : 1) (R_{FG}), cyclohexane–ethyl acetate–methanol (1 : 1 : 1) (R_{FH}), or ethyl acetate (R_{FJ}). Descending chromatograms were run on Whatman no. 3 paper with butan-1-ol–acetic acid–water (4 : 1 : 5) (R_{FM}), or butan-2-ol–ammonium hydroxide (3%) (3 : 1) (R_{FO}). Spots were revealed with ninhydrin, sodium hypochlorite–potassium iodide–tolidine,[9] or Ehrlich's reagent, and, in the case of t.l.c., by incorporating a fluorescent indicator in the thin layer (Kieselgel GF 254, Merck) or by acid potassium permanganate [reagent prepared by dissolving potassium permanganate (100 mg.) in concentrated sulphuric acid (1 ml.) and diluting the solution to 100 ml. with water). Acid or alkali hydrolysates of peptide derivatives were prepared using 6N-hydrochloric acid (110°/16 hr.) or 2N-baryta (110°/72 hr.), and the amino-acid composition of the hydrolysates was determined with a Beckman-Spinco Amino Acid Analyser, model 120B. Optical rotations were determined with a Bendix NPL Automatic Polarimeter, model 143C, with Digital Converter, model 154C. Organic extracts were dried with anhydrous magnesium sulphate, and evaporations were carried out under reduced pressure in a rotary evaporator. M.p.s (uncorrected) were determined in capillary tubes with the Tottoli melting-point apparatus (manufactured by W. Buchi).

Starting Materials.—The following were prepared by literature methods: N^{α}-benzyloxycarbonyl-N^{ϵ}-t-butoxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester [Z-Lys(BOC)-OCP],[10] N^{α}-t-butoxycarbonyl-N^{ϵ}-benzyloxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester [BOC-Lys(Z)-OCP],[10] N^{α}-benzyloxycarbonyl-L-histidine hydrazide (Z-His-NH·NH_2),[11] *N*-benzyloxycarbonyl- and *N*-t-butoxycarbonyl-L-phenylalanine 2,4,5-trichlorophenyl ester (Z-Phe-OCP and BOC-Phe-OCP),[7,10] *N*-benzyloxycarbonyl-L-tyrosine 2,4,5-trichlorophenyl ester (Z-Tyr-OCP),[7] and N^{α}-t-butoxycarbonyl-4-, -5-, and -6-methyl-DL-tryptophan 2,4,5-trichlorophenyl ester [BOC-Trp(4-, 5-, and 6-Me)-OCP].[10]

N^{α}-*Benzyloxycarbonyl-(5-benzyloxycarbonyl)-*DL-*tryptophan* (XVII; R = ZO). A solution of 5-hydroxy-DL-tryptophan * (220 mg., 1 mmole) in 2N-sodium hydroxide (0·375 ml., 0·75 mmole) was diluted to 5 ml. with water and the pH was brought accurately to 9·5 with N-sodium hydroxide. Benzyloxycarbonyl chloride (0·36 ml., 2·3 mmoles) in acetone (3 ml.) was then added dropwise whilst maintaining a pH of 9·5 by the addition of N-sodium hydroxide (Radiometer pH-meter, type TTT 1B). The turbidity which developed was discharged by the addition of water (2 ml.). When the pH was steady (2·3 ml. of N-sodium hydroxide required), the solution was extracted with ether and the acqueous phase was added to N-hydrochloric acid (5 ml.) at 0°. The resulting oil was extracted into ethyl acetate, and the extracts were washed with water, dried, and evaporated, to yield the crude bisbenzyloxycarbonyl derivative as an oil (525 mg.) which did not crystallise. The *dicyclohexylammonium salt*, prepared in ether, was crystallised twice from ethyl acetate–light petroleum (b.p. 60—80°), to give needles (380 mg., 58%), m.p. 105—106°, R_{FA} 0·70, R_{FO} 0·64 (Found: C, 69·8; H, 6·8; N, 6·4. $C_{39}H_{47}N_3O_7$ requires C, 69·8; H, 7·1; N, 6·3%).

N^{α}-*Benzyloxycarbonyl-(5-benzyloxycarbonyloxy)-*DL-*tryptophan 2,4,5-trichlorophenyl ester* [VIIIA; X = Trp(5-OZ)]. The dicyclohexylammonium salt described above (380 mg., 0·58 mmole) in ethyl acetate (10 ml.) was treated at 0° with a solution of hydrogen chloride (0·63 mmole) in acetic acid (125 µl.). The mixture was stirred at 0° for 30 min. and then filtered, washed with water, dried, and evaporated. A solution of the residue and 2,4,5-trichlorophenol (115 mg., 0·58 mmole) in ethyl acetate (13 ml.) was treated at −10° with *NN'*-dicyclohexylcarbodi-imide (124 mg., 0·61 mmole) in ethyl acetate (5 ml.). The mixture was kept at −10° for 2 hr. and then at 2° for 2 days. Dicyclohexylurea (120 mg.) was filtered off and the filtrate was evaporated to give the trichlorophenyl ester as an oil (395 mg.), R_{FG} 0·62.

N^{α}-*Methyl-*L-*tryptophan* (*N*-Me-Trp). N^{α}-Methyl-L-tryptophan methyl ester hydrochloride, m. p. 172—173°, was prepared from N^{α}-(4-chlorobutyryl)-L-tryptophan methyl ester by the method of Peter, Brugger, Schreiber, and Eschenmoser.[12] A stirred solution of the ester hydrochloride (2·135 g., 7·95 mmoles) in methanol (9·5 ml.) was treated dropwise during 75 min. at 20—23° with N-sodium hydroxide (16·7 ml.). The solution was stirred at 20—23° for a further 5 min., and then acidified with N-hydrochloric acid (8·74 ml.). The resulting mixture was kept at 4° for 18 hr. The solid was collected and washed twice with water (30 ml. and 20 ml.) at 60°, to yield N^{α}-methyl-L-tryptophan (0·65 g., $[\alpha]_D^{22}$ +46·7° (*c* 2·06 in 0·5N-hydrochloric acid) (lit.,[12] +47·2°). T.l.c. of the product on Kieselgel G, in butan-1-ol–acetic acid–water (6 : 2 : 2), followed by spraying with ninhydrin–acetic acid–collidine–ethanol–cupric nitrate reagent, showed a single pink spot, R_F 0·64; under identical conditions, L-tryptophan gave a violet spot, R_F 0·69.

N^{α}-*Benzyloxycarbonyl-*N^{α}-*methyl-*L-*tryptophan 2,4,5-trichlorophenyl ester* (Z-*N*-Me-Trp-OCP) (VIIIA; X = *N*-Me-Trp). A stirred solution of N^{α}-methyl-L-tryptophan (0·7 g., 3·21 mmoles) in 0·5N-sodium hydroxide (6·42 ml.) and acetone (0·5 ml.) was treated dropwise during 15 min. at 50—60° with a solution of benzyloxycarbonyl chloride (0·501 ml., 3·53 mmoles) in acetone (0·5 ml.). The resulting mixture was stirred at 50—60° for 10 min. and then at ambient temperature for 40 min. During the addition and the subsequent stirring, the pH of the mixture was kept at

* Obtained commercially.

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10—11 by dropwise addition of 2N-sodium hydroxide (1·76 ml. required). After extraction with ether (2 × 5 ml.) (backwashing with water), the mixture was added to ice-cold 0·3N-hydrochloric acid (15 ml.) and the resulting oil (1·008 g., 87% as N^{α}-benzyloxycarbonyl-N^{α}-methyl-L-tryptophan) (XVIII) was isolated by ether extraction. A solution of the oil (2·86 mmoles) and 2,4,5-trichlorophenol (0·634 g., 3·21 mmole) in ethyl acetate (5 ml.) was treated at 0° with *NN'*-dicyclohexylcarbodi-imide (0·662 g., 3·21 mmoles). The mixture was kept at 0° for 4 hr., then acetic acid (4 drops) was added. The resulting mixture was kept at 20—25° for 20 min. then filtered and evaporated. The residue was dissolved in ether (20 ml.) and the solution was filtered and evaporated, to yield the 2,4,5-trichlorophenyl ester (1·457 g., 96%) as a syrup, R_F 0·41 in benzene–ethyl acetate (9 : 1), R_F 0·87 in benzene–ethyl acetate (1 : 1), R_F 0·09 in benzene.

N^{2}-*Benzyloxycarbonyl*-1,2,3,4-*tetrahydro*-β-*carboline*-3-*carboxylic acid* 2,4,5-*trichlorophenyl ester* (VIIIA; X = carboline). 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid [13] [R_{FA} 0·48, R_{FC} 0·40, $[\alpha]_D^{22}$ −131° (*c* 0·19 in 0·1N-sodium hydroxide) (lit.,[14] −133·2°) (Found: C, 66·4; H, 6·1; N, 12·9. Calc. for $C_{12}H_{12}N_2O_2$: C, 66·6; H, 6·0; N, 13·0%)] (1·08 g., 5 mmoles) was treated with benzyloxycarbonyl chloride under conditions similar to those described above for N^{α}-methyl-L-tryptophan, to yield N^{2}-*benzyloxycarbonyl*-1,2,3,4-*tetrahydro*-β-*carboline*-3-*carboxylic acid* (XIX) (45%), m. p. 183—184° (from ethyl acetate–light petroleum), R_{FA} 0·90, R_{FC} 0·54 (Found: C, 68·6; H, 5·3; N, 8·1. $C_{20}H_{18}N_2O_4$ requires C, 68·6; H, 5·2; N, 8·0%). A solution of the acid (200 mg., 0·571 mmole) and 2,4,5-trichlorophenol (119 mg., 0·602 mmole) in ethyl acetate (5 ml.) was treated at 0° with *NN'*-dicyclohexylcarbodi-imide (118 mg., 0·571 mmole). The mixture was kept at 4° overnight, then acetic acid (1 drop) was added. The resulting mixture was kept at 20—25° for 30 min. then filtered, washed with ice-cold 0·1N-sodium hydrogen carbonate and water, dried, and evaporated. Recrystallisation of the residue from isopropyl ether–light petroleum (b.p. 60—80°) gave the *trichlorophenyl ester* (233 mg., 77%), m.p. 123—125° after softening and effervescence at 70—82°, R_F 0·29 in benzene, R_F 0·77 in ethyl acetate (Found: C, 58·6; H, 3·7; N, 5·1. $C_{26}H_{19}Cl_3N_2O_4$ requires C, 58·9; H, 3·6; N, 5·3%).

N^{α}-*Benzyloxycarbonyl*-2-*hydroxy*-L-*tryptophan* 2,4,5-*trichlorophenyl ester* [VIIIA; X = Trp(2-OH)]. 2-Hydroxy-L-tryptophan [15] (176 mg., 0·8 mmole), sodium hydrogen carbonate (176 mg., 2 mmoles), benzyloxycarbonyl chloride (0·15 ml.), water (1·5 ml.), and acetone (0·1 ml.) were stirred together at 22—24° for 2 hr. The mixture was extracted twice with ether and the aqueous phase was then added to N-hydrochloric acid (4 ml.). The resulting mixture was extracted with ethyl acetate (3 × 30 ml.), and the extracts were washed with N-hydrochloric acid (15 ml.) and water (4 × 20 ml.) dried, and evaporated, to give N^{α}-*benzyloxycarbonyl*-2-*hydroxy*-L-*tryptophan* (219 mg., 77%), m. p. 86—88°, R_{FA} 0·81, R_{FB} 0·70, R_{FC} 0·39, R_{FE} 0·0 (2-hydroxy-L-tryptophan had R_{FA} 0·45, R_{FB} 0·61) (Found: C, 62·8; H, 5·3; N, 7·6. $C_{19}H_{18}N_2O_5,0·5H_2O$ requires C, 62·8; H, 5·3; N, 7·7%). A solution of this acid (124 mg., 0·35 mmole) in ethyl acetate (2 ml.) was treated at 5 — 10° with 2,4,5-trichlorophenol (69 mg., 0·35 mmole) followed by a solution of *NN'*-dicyclohexylcarbodi-imide (76 mg., 0·37 mmole) in ethyl acetate (2 ml.). The mixture was stirred at room temperature overnight and then filtered. The filtrate was evaporated and the residue was crystallised from light petroleum, to give the 2,4,5-*trichlorophenyl ester* (141 mg., 74%), m. p. >70° (effervescence), R_{FD} 0·84, R_{FE} 0·68, R_{FF} 0·78 (Found: C, 56·0; H, 3·4; N, 5·0. $C_{25}H_{19}Cl_3N_2O_5$ requires C, 56·3; H, 3·6; N, 5·25%).

Other active esters. A stirred solution of phenylacetic acid (5·456 g., 40 mmoles) and 2,4,5-triclorophenol (7·90 g., 40 mmoles) in ethyl acetate (10 ml.) was treated during 5 min. at 0—5° with a solution of *NN'*-dicyclohexylcarbodi-imide (8·24 g., 40 mmoles) in ethyl acetate (20 ml.). The mixture was stirred overnight at room temperature and then filtered from dicyclohexylurea. The filtrate was evaporated and the residue was crystallised from light petroleum (b.p. 60—80°), to give 2,4,5-*trichlorophenyl phenylacetate* (I) (12·4 g., 98%), m. p. 78—79° (Found: C, 53·6; H, 2·9; Cl, 33·4. $C_{14}H_9Cl_3O_2$ requires C, 53·3; H, 2·9; Cl, 33·7). Similarly, by use of the appropriate acid, there were obtained the 2,4,5-*trichlorophenyl esters* of the following (details in parenthesis refer to m.p., % yield of recrystallised product, and solvent for recrystallisation): *phenoxyacetic acid* (II) (83—84°, 85, light petroleum) (Found: C, 50·9; H, 2·4. $C_{14}H_9Cl_3O_3$ requires C, 50·6; H, 2·7%); β-*phenylpropionic acid* (III) (63—64°, 80, light petroleum) (Found: C, 54·8; H, 3·3; Cl, 32·4. $C_{15}H_{11}Cl_3O_2$ requires C, 54·6; H, 3·3; Cl, 32·4%); *indol*-3-*ylacetic acid* (IV) (151—152°, 72, ethanol) (Found: C, 54·1; H, 2·8; N, 4·0. $C_{16}H_{10}Cl_3NO_2$ requires C, 54·2; H, 2·85; N, 4·0%); β-(*indol*-3-*yl*)*propionic acid* (V) (108—109°, 67, ethanol) (Found: C, 55·3; H, 3·4; N, 3·8. $C_{17}H_{12}Cl_3NO_2$ requires C, 55·5; H, 3·3; N, 3·8%); and γ-(*indol*-3-*yl*)*butyric acid* (VI) (82—83°, 59, ethanol) (Found: C, 56·6; H, 3·7; N, 3·5. $C_{18}H_{14}Cl_3NO_2$ requires C, 56·5; H, 3·7; N, 3·65%).

Description of Methods (see Table).—A. *Azide coupling.* A solution of N^{α}-benzyloxycarbonyl-L-histidine hydrazide (272 mg., 0·9 mmole) in N-hydrochloric acid (2·7 ml.) was treated dropwise at 0° with a solution of sodium nitrite (63 mg., 0·9 mmole) in water (0·3 ml.). After 2 min., precooled 50% aqueous potassium carbonate (1·2 ml.) was added and the mixture was extracted with ethyl acetate (2 × 4 ml.). The extracts were dried and then added at 0° to a stirred solution of L-methionyl-(β-t-butyl)-L-aspartyl-L-phenylalanine amide hydrochloride [5] (233 mg., 0·5 mmole) and triethylamine (0·07 ml., 0·5 mmole) in dimethylformamide (5 ml.). The mixture was kept at 4° for 3 days; after 1 day, more of the azide solution [freshly prepared as described above from N^{α}-benzyloxycarbonyl-L-histidine hydrazide (0·5 mmole)] was added. The resulting mixture was diluted with water (10 ml.) and extracted with ethyl acetate. The extracts were washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated. Recrystallisation of the residue thrice from methanol–ethyl acetate gave N^{α}-*benzyloxycarbonyl*-L-*histidyl*-L-*methionyl*-(β-*t*-*butyl*)-L-*aspartyl*-L-*phenylalanine amide* (180 mg., 49%), m. p. 154—156° (decomp.).

B. *Cleavages with hydrogen chloride.* B1; The t-butyl ester prepared as described in A (75 mg., 0·1 mmole) and hydrogen chloride (0·5 mmole) in glacial acetic acid (1·8 ml.) were stirred together at 18—22° for 1 hr. The solution was evaporated below 35°, and the residue was triturated

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with ethyl acetate, to give N$^{\alpha}$-*benzyloxycarbonyl*-L-*histidyl*-L-*methionyl*-L-*aspartyl*-L-*phenylalanine amide hydrochloride* (75 mg., 100%), m. p. 209° (decomp.) (unchanged after crystallisation from methanol–ether).

B2; *N*-t-Butoxycarbonyl-L-phenylalanyl- or -glycyl-L-methionyl-L-aspartyl-L-phenylalanine amide (1 mmole) and hydrogen chloride (5 mmoles) in glacial acetic acid (3 ml.) were stirred together at 18—22° for 30 min. The solution was evaporated and the residue was triturated with ether and collected, to yield L-*phenylalanyl*- or -*glycyl*-L-*methionyl*-L-*aspartyl*-L-*phenylalanine amide hydrochloride* (279 mg., 98%).

C. *Active ester reactions*. C1. The appropriate 2,4,5-trichlorophenyl ester (X-OCP) (0·5 mmole) was added at 15—20° to a stirred solution of L-methionyl-L-aspartyl-L-phenylalanine amide hydrochloride [5] (224 mg., 0·5 mmole) and triethylamine (0·14 ml., 1 mmole) in dimethylformamide (7·5 ml.) amd water (2 ml.). The mixture was kept at room temperature for 18 hr., the pH was then adjusted to 3 with citric acid, and the mixture was diluted with ice–water (50 ml.). The solid was collected, washed with water and light petroleum (b. p. 60—80°), and crystallised from the solvent indicated in the Table.

C2. The appropriate indolyl acid 2,4,5-trichlorophenyl ester (0·5 mmole), L-methionyl-L-aspartyl-L-phenylalanine amide hydrochloride [5] (224 mg., 0·5 mmole), triethylamine (0·14 ml., 1 mmole), dimethylformamide (5 ml.), and water (1 ml.) were stirred together at 20—22° for 4 days. The solution was acidified at 0° with N-hydrochloric acid (1·1 ml.) and then added to a mixture of ice–water (40 ml.) and ether (20 ml.), to yield the product, which was collected, washed with water and ether, and crystallised from ethanol.

C3. A solution of the appropriate *N*-benzyloxycarbonyl- or *N*-t-butoxycarbonyl-amino-acid 2,4,5-trichlorophenyl ester (0·5 mmole) in dimethylformamide (1—3 ml.) was added to a solution of L-methionyl-L-aspartyl-L-phenylalanine amide hydrochloride [5] (244 mg., 0·5 mmole) and triethylamine (0·14 ml., 1 mmole) in dimethylformamide (5 ml.) and water (1 ml.). The mixture was stirred at 4° for 18 hr. and then at 20—22° for 1 day. The resulting solution was acidified to pH 3 at 0° with aqueous citric acid and then added immediately to a mixture of ice–water (50 ml.) and ether (20 ml.). The solid was collected, washed thrice with water and thrice with ether, and crystallised from the solvent indicated in the Table.

C4. *N*-t-Butoxycarbonylglycine 2,4,5-trichlorophenyl ester (71 mg., 0·2 mmole), L-phenylalanyl-L-methionyl-L-aspartyl-L-phenylalanine amide hydrochloride (115 mg., 0·2 mmole) or N$^{\epsilon}$-benzyloxycarbonyl-L-lysyl-L-methionyl-L-aspartyl-L-phenylalanine amide trifluoroacetate (0·2 mmole), triethylamine (64 µl., 0·4 mmole), dimethylformamide (4 ml.), and water (0·8 ml.) were stirred together at 18—22° for 3 days. The solution was acidified to pH 3 at 0° with citric acid and diluted with ice–water (50 ml.), to yield the pentapeptide derivative, which was collected, washed with water and ether, and crystallised from aqueous dimethylformamide.

D. *Hydrogenolysis*. A solution of N$^{\alpha}$-benzyloxycarbonyl-(5-benzyloxycarbonyloxy)-D- and -L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide (126 mg., 0·14 mmole) and cyclohexylamine (112 µl., 1·4 mmole) in methanol (50 ml.) was hydrogenated at room temperature and pressure for 24 hr. over 5% palladised charcoal (120 mg.). The filtered solution was evaporated, and the residue was collected in ether and precipitated from aqueous acetic acid, to give (5-*hydroxy*)-D- and -L-*tryptophyl*-L-*methionyl*-L-*aspartyl*-L-*phenylalanine amide* (40 mg., 46%) [no benzyloxycarbonyl absorption in i.r. spectrum (Nujol)].

E. *Cleavages with trifluoroacetic acid*. N$^{\alpha}$-Benzyloxycarbonyl-N$^{\epsilon}$-t-butoxycarbonyl-L-lysyl- [Table; X = Z-Lys(BOC)] or N$^{\alpha}$-t-butoxycarbonyl-N$^{\epsilon}$-benzyloxycarbonyl-L-lysyl- [Table; X = BOC-Lys(Z)] L-methionyl-L-aspartyl-L-phenylalanine amide (305 mg., 0·38 mmole) was dissolved in anhydrous trifluoroacetic acid (3 ml.) at 10°. The solution was kept at 18—22° for 45 min., and then evaporated below 35°, to give the trifluoroacetates (collected in ether).

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