1176. *Polypeptides. Part I. The Synthesis of Peptides Related to Eledoisin.*

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A synthesis of the hexapeptide containing the *C*-terminal sequence of eledoisin is described which involves coupling of the tetrapeptide (V) with the dipeptide amide (VI). In the synthesis of the latter, the *o*-nitrophenylsulphenyl group was successfully used for *N*-protection of the leucyl residue.

Erspamer and his co-workers first isolated[1] the polypeptide eledoisin from the posterior salivary glands of two molluscan species (belonging to the octopod *Cephelopoda*) and demon-

[1] V. Erspamer and A. Anastasi, *Experientia*, 1962, **18**, 58; *Brit. J. Pharmacol.*, 1962, **19**, 326.

strated its powerful hypotensive effect in mammals and its effect on extravascular smooth muscle preparations at extremely low concentrations.[2–4] The endecapeptide structure (I) was deduced by degradation[1] and confirmed by synthesis.[5] Considerable interest has emerged in the relationship of structure and biological activity of eledoisin-like peptides. In particular, it has been found that many of the *N*-terminal amino-acids of eledoisin are not important for biological activity; the biological activity of the *C*-terminal hexapeptide (II) does in fact approach that of eledoisin itself.[6–9] Our own interest has been concerned with the effect of substitution at the *N*-terminal end of this hexapeptide and a number of acylhexapeptides, including heptapeptides, have been prepared and evaluated. The biological results will be published elsewhere, but meanwhile we report our synthesis of the hexapeptide (II), since it differs from the two syntheses which have recently been described.[10,11] These both involve the coupling of derivatives of the tripeptide (III; X = $PhCH_2$·O·CO or Bu^tO·CO) with the tripeptide amide (IV), whereas our own method involves the coupling of the *N*-t-butoxycarbonyl-tetrapeptide (V) with the dipeptide amide (VI).* We chose this procedure because activation involves only a *C*-terminal glycyl residue, thus eliminating the possibility of racemisation at this stage of the synthesis. The *N*-t-butoxycarbonyl protecting group was employed to avoid the complications that can arise in the removal of benzyloxycarbonyl groups from methionine-containing peptides.[12]

Our method of synthesis is shown diagramatically in the diagram (all amino-acids are of the L-configuration). Leucylmethionine amide (VI) was most conveniently prepared using the *o*-nitrophenylsulphenyl (NPS) group,[13] for *N*-protection of the leucine. In agreement with the general findings of Zervas,[13] the stable dicyclohexylammonium salt of *N*-(*o*-nitrophenylsulphenyl)leucine (VII) could be coupled cleanly with methionine methyl ester hydrochloride by means of *NN'*-dicyclohexylcarbodi-imide to give *N*-*o*-nitrophenylsulphenyl-leucylmethionine methyl ester in high yield. Ammonolysis of this ester proceeded

Pyr·Pro·Ser·Lys·Asp·Ala·Phe·Ile·Gly·Leu·Met–NH_2
(I)

X–Ala·Phe·Ile·Gly·Leu·Met–NH_2 (II) X–Ala·Phe·Ile–OH (III) Gly·Leu·Met–NH_2 (IV)

slowly but smoothly to give the protected dipeptide amide (VIII) and the protecting group was rapidly removed in the cold with two equivalents of hydrogen chloride in ethyl acetate, yielding highly crystalline leucylmethionine amide hydrochloride (VI, HCl) in quantitative yield. This method of synthesis was particularly attractive on the large scale, and an added advantage was that *o*-nitrophenylsulphenyl chloride could be readily recovered from the final cleavage and used again in the preparation of *N*-(*o*-nitrophenylsulphenyl)leucine (VII). The exclusion of methionine from the remaining sequence of the molecule enabled a simple stepwise synthesis of the tetrapeptide ester (XIII) starting from glycine methyl ester hydrochloride. This was extended from its amino-end by reaction with the 2,4,5-trichlorophenyl ester of *N*-benzyloxycarbonylisoleucine (IX) and subsequent hydrogenation (the

* Since the preparation of this paper, a similar method has been described by L. Bernardi, G. Bosisio, R. de Castiglione, O. Goffredo, and F. Chillemi, *Gazzetta*, 1964, **94**, 853.

[2] V. Erspamer and G. F. Erspamer, *Brit. J. Pharmacol.*, 1962, **19**, 337.
[3] V. Erspamer and A. Glaesser, *Brit. J. Pharmacol.*, 1963, **20**, 516.
[4] F. Sicuteri, M. Fanciullaci, G. Franchi, and S. Michelacci, *Experientia*, 1963, **19**, 44.
[5] Ed. Sandrin and R. A. Boissonnas, *Experientia*, 1962, **18**, 59.
[6] B. Camerino, G. de Caro, R. A. Boissonnas, Ed. Sandrin, and E. Stürmer, *Experientia*, 1963, **19**, 339.
[7] E. Stürmer, Ed. Sandrin, and R. A. Boissonnas, *Experientia*, 1964, **20**, 303.
[8] L. Bernardi, G. Bosisio, F. Chillemi, G. de Caro, R. de Castiglione, V. Erspamer, A. Glaesser, and O. Goffredo, *Experientia*, 1964, **20**, 306.
[9] E. Schroder and K. Lübke, *Experientia*, 1964, **20**, 19.
[10] F. Chillemi, *Gazzetta*, 1963, **93**, 1079.
[11] Ed. Sandrin and R. A. Boissonnas, *Helv. Chim. Acta*, 1964, **47**, 417.
[12] See references cited by B. Iselin, *Helv. Chim. Acta*, 1961, **44**, 61.
[13] L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.*, 1963, **85**, 3660.

TABLE 1.

Acylated tetra- and penta-peptide esters.[a] X–Ala•Phe•Ile•Gly–OMe.

				Found (%)				Required (%)		
X[b]	M. p.°	Yield (%)	Solvent[c]	C	H	N	Formula	C	H	N
Ac[d]	255—256	99	MeOH	59·3	7·4	12·0	$C_{23}H_{34}N_4O_6$	59·7	7·4	12·1
Bz[e]	246—246·5	76	MeOH	63·8	6·8	10·5	$C_{28}H_{36}N_4O_6$	64·2	6·9	10·7
BOC-Gly[f]	229—231	90	EtOH	58·7	7·5	12·3	$C_{28}H_{43}N_5O_8$	58·2	7·45	12·1
BOC-Ala[f]	251—252	100	MeOH	58·9	7·8	11·9	$C_{29}H_{45}N_5O_8$	58·8	7·7	11·8
BOC-β-Ala[f]	209—210	93	Aq. DMF	58·9	7·9	11·8	$C_{29}H_{45}N_5O_8$	58·8	7·7	11·8
BOC-Pro[f]	252—253	86	MeOH	59·7	7·5	11·2	$C_{31}H_{47}N_5O_8$	60·2	7·7	11·3
BOC-Try[f]	227—229	97	EtOH	62·4	7·1	11·8	$C_{37}H_{50}N_6O_8$	62·8	7·1	11·9
BOC-Met[f]	241—243	100	MeOH	56·9	7·4	S, 4·8	$C_{31}H_{49}N_5O_8S$	57·1	7·6	S, 4·9
Z-Pyr[f]	232—235	92	AcOH/EtOH	61·6	6·6	10·4	$C_{34}H_{43}N_5O_9$	61·2	6·5	10·5
H-Pyr[f]	253—254	95	MeOH	58·6	7·3	13·3	$C_{26}H_{37}N_5O_7$	58·7	7·0	13·2
EtHex-Gly[g]	267—268	91	2-Ethoxyethanol	61·9	8·4	11·5	$C_{31}H_{49}N_5O_7$	61·6	8·2	11·6

TABLE 2.

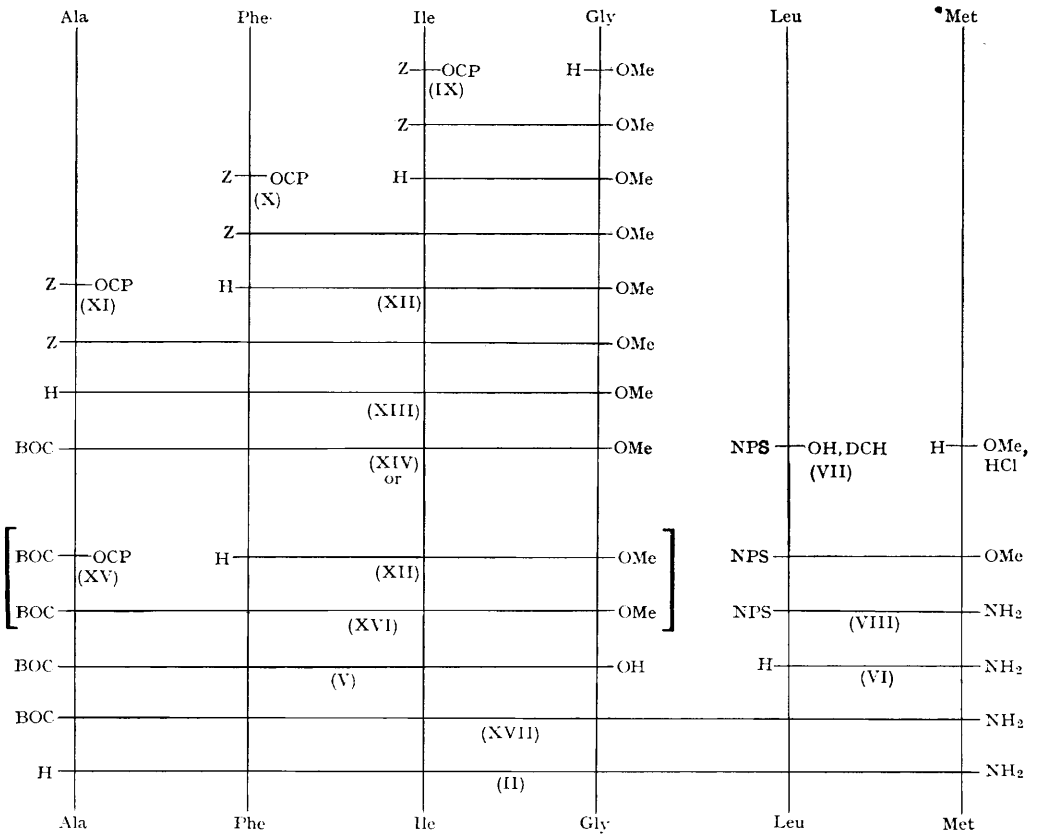
Acylated tetra- and penta-peptides.[a] X–Ala•Phe•Ile•Gly–OH.

				Found (%)				Required (%)		
X[b]	M. p.°	Yield (%)	Solvent[c]	C	H	N	Formula	C	N	N
Ac	265 (decomp.)	98	AcOH	58·9	7·3	12·4	$C_{22}H_{32}N_4O_6$	59·0	7·2	12·5
Bz	268—269	93	AcOH	63·6	6·7	10·9	$C_{27}H_{34}N_4O_6$	63·4	6·7	11·0
BOC-Gly	decomp. > 230	83	Aq. EtOH	57·3	7·3	12·5	$C_{27}H_{41}N_5O_8$	57·5	7·3	12·4
BOC-Ala	decomp. > 235	95	EtOH or THF	58·2	7·5	12·0	$C_{28}H_{43}N_5O_8$	58·2	7·45	12·1
BOC-β-Ala	248—250	78	Aq. DMF	57·8	7·5	12·1	$C_{28}H_{43}N_5O_8$	58·2	7·45	12·1
BOC-Pro	238	91	Aq. EtOH	59·6	7·8	11·6	$C_{30}H_{45}N_5O_8$	59·7	7·5	11·6
BOC-Try	decomp. > 222	93	Aq. EtOH	62·8	7·2	12·0	$C_{36}H_{48}N_6O_8$	62·5	7·0	12·1
BOC-Met	decomp. > 250	100	THF	56·5	7·6	S, 4·9	$C_{30}H_{47}N_5O_8S$	56·5	7·4	S, 5·0
H-Pyr	258—262	60	Aq. AcOH	57·8	6·9	13·5	$C_{25}H_{35}N_5O_7$	58·0	6·8	13·5
EtHex-Gly	259—260	100	MeOH	61·2	8·3	11·7	$C_{30}H_{47}N_5O_7$	61·0	8·05	11·8

Notes (Tables 1 and 2). [a] See Experimental section for method of preparation; [b] BOC = t-butoxycarbonyl, Z = benzyloxycarbonyl, EtHex = DL-2-ethylhexanoyl, Pyr = L-pyroglutamyl; [c] DMF = dimethylformamide, THF = tetrahydrofuran; [d] using *p*-nitrophenyl acetate (F. D. Chattaway, *J.*, 1931, 2495); [e] using *p*-nitrophenyl benzoate (M. O. Forster and H. E. Fierz, *J.*, 1907, **91**, 855); [f] using the corresponding 2,4,5-trichlorophenyl ester of X–OH (see Experimental section); [g] using the corresponding *p*-nitrophenyl ester of X–OH (see Experimental section).

use of 2,4,5-trichlorophenyl esters in peptide synthesis has been described by Pless and Boissonnas[14]). Repetition of the process with the 2,4,5-trichlorophenyl esters of *N*-benzyloxycarbonyl-phenylalanine (X) and -alanine (XI) afforded pure L-alanyl-L-phenylalanyl-L-isoleucylglycine methyl ester (XIII) as its acetate in 77% overall yield, based on glycine methyl ester hydrochloride. Reaction of the tetrapeptide ester with t-butoxycarbonyl azide in pyridine gave the *N*-t-butoxycarbonyl derivative (XIV) in 91% yield [also prepared directly by reaction of *N*-t-butoxycarbonylalanine 2,4,5-trichlorophenyl ester (XV) with the tripeptide ester (XII)], which was saponified to the acid (V), in aqueous 2-ethoxyethanol.

For the coupling of the *N*-t-butoxycarbonyl-tetrapeptide (V) with leucylmethionine amide (VI), neither the mixed anhydride procedure nor the use of condensing agents proved satisfactory. The former method gave only low yields of the *N*-t-butoxycarbonyl-hexapeptide (II) and the product from *NN'*-dicyclohexylcarbodi-imide coupling was difficult to purify. However, compound (V) readily gave a 2,4,5-trichlorophenyl ester (with 2,4,5-trichlorophenol and *NN'*-dicyclohexylcarbodi-imide) which could be easily purified, and which reacted with compound (VI) to give the *N*-t-butoxycarbonyl-hexapeptide (XVII) in 97% yield. Treatment of the latter with cold trifluoroacetic acid gave the free hexapeptide (II), as its trifluoroacetate, in quantitative yield.



(Z = benzyloxycarbonyl; BOC = t-Butoxycarbonyl; NPS = *o*-Nitrophenylsulphenyl; OCP = 2,4,5-Trichlorophenyl ester; DCH = Dicyclohexylamine.)

[14] J. Pless and R. A. Boissonnas, *Helv. Chim. Acta.* 1963, **46**, 160.

The required acyl-hexapeptides were usually prepared by direct acylation of the hexapeptide (II) trifluoroacetate. Alternatively, the tetrapeptide ester (XIII) was acylated and the product saponified and then coupled with leucylmethionine amide (VI). Details of the intermediate acylated tetrapeptide esters and acids used in the latter route are given in Tables 1 and 2.

Experimental

Ascending, thin-layer chromatograms were run on Kieselgel-G with butan-1-ol–pyridine–acetic acid–water (15:10:3:12 v/v) (R_{FA}), or butan-1-ol–acetic acid–water (12:3:5) (R_{FB}); descending chromatograms were run on Whatman No. 3 paper with butan-1-ol–acetic acid–water (4:1:5, top phase) (R_{FC}). Spots were revealed with ninhydrin. Amino-acid composition of the acid hydrolysates was determined with a Beckman–Spinco Amino Acid Analyser, Model 120, according to the method of Moore, Spackmann, and Stein.[15] Evaporations were carried out under reduced pressure. Melting points are uncorrected.

N-(*t-Butoxycarbonyl*)*amino-acids*.—L-Proline (57·6 g., 0·5 mole), magnesium oxide (40 g., 1·0 mole), t-butoxycarbonyl azide[16] (156 ml., 1·1 moles), water (650 ml.), and dioxan (1·5 l.) were stirred at 40—45° for 24 hr. Most of the dioxan and the excess of azide were then removed *in vacuo*, and water (2 l.) was added. The filtered solution was acidified below 10° to pH 3 with saturated aqueous citric acid, then extracted continuously for 24 hr. with ethyl acetate (2 l.). Evaporation of the dried ($MgSO_4$) extracts, and collection of the residue with light petroleum (b. p. 60—80°), gave *N*-t-butoxycarbonyl-L-proline (100 g., 93%), m. p. 131—135° (lit.,[17] m. p. 136—137°, 55% yield). In like manner (except that in this case the relevant partition coefficients permitted simple, non-continuous extraction of the product), β-alanine gave N-*t-butoxycarbonyl*-β-*alanine* (89% yield), m. p. 73—74°, from ethyl acetate–light petroleum (b. p. 60—80°) (Found: C, 50·6; H, 8·1; N, 7·6. $C_8H_{15}NO_4$ requires C, 50·9; H, 8·0; N, 7·4%). Applied to L-tryptophan, the method gave only a 40% yield of product, so the following procedure was adopted. A solution of L-tryptophan (10·2 g., 50 mmoles) in water (180 ml.) containing sodium hydroxide (4·4 g., 110 mmoles) was treated with a solution of t-butoxycarbonyl azide[16] (14 ml., 100 mmoles) in dioxan (100 ml.) and the mixture was stirred at 40—45° for 24 hr. *N*-t-Butoxycarbonyl-L-tryptophan (9·1 g., 60%), m. p. 143—144° (decomp.), isolated similarly, formed prismatic needles from ethyl acetate–light petroleum, (b. p. 60—80°) (lit.,[17] m. p. 136—140°, 36% yield).

N-(DL-2-*Ethylhexanoyl*)*glycine*.—(*a*) A solution of glycine (7·5 g., 0·1 mole) in 2N-sodium hydroxide (50 ml.) and acetone (20 ml.) was treated at 5—10° with 2N-sodium hydroxide (60 ml.) and DL-2-ethylhexanoyl chloride[18] (17 ml., 0·1 mole) for 45 min., the addition of the alkali being regulated to maintain a pH of 9·5—10·5. The mixture was kept for 1 hr. at 5—10°, then the acetone was removed. The solution was extracted with ether (2 × 50 ml.) then added to a mixture of concentrated hydrochloric acid (15 ml.) and ice (15 g.). N-(DL-2-*Ethylhexanoyl*)*glycine* (19·9 g., 99%), isolated by extraction with ethyl acetate, formed prismatic needles, m. p. 81—82°, from benzene–light petroleum (b. p. 70—80°) (Found: C, 59·2; H, 9·0; N, 7·0. $C_{10}H_{19}NO_3$ requires C, 59·7; H, 9·5; N, 7·0%).

(*b*) A stirred suspension of glycine methyl ester hydrochloride (25·1 g., 0·2 mole) in chloroform (300 ml.) and triethylamine (28 ml., 0·2 mole) was treated dropwise during 20 min. at 0—5° with DL-2-ethylhexanoyl chloride (17 ml., 0·1 mole). Triethylamine (28 ml.) was then added and more DL-2-ethylhexanoyl chloride (17 ml.) was dropped in during 15 min at 0—5°. The resulting solution was stirred at 20—23° for 1 hr., then washed successively with water, N-hydrochloric acid, water, sodium hydrogen carbonate solution, and water. After drying ($MgSO_4$) and evaporation, *N*-(DL-2-ethylhexanoyl)glycine methyl ester was obtained as an almost colourless oil (41·9 g., 97%). This was dissolved in cold methanol (400 ml.), N-sodium hydroxide (400 ml.) was added, and the solution was kept at 20—23° for 1 hr. The methanol was then removed and the aqueous solution was acidified to pH 1 with hydrochloric acid. The acid (33·8 g., 84%), m. p. 78—82°, so precipitated was isolated by extraction with ether.

Preparation of Active Esters.—These were prepared, using the appropriate *N*-acylamino-acid, 2,4,5-trichlorophenol or *p*-nitrophenol, and *NN'*-dicyclohexylcarbodi-imide, by the method (Method A) described by Pless and Boissonnas.[14] Details in parentheses refer to reaction solvent,

[15] S. Moore, D. H. Spackmann, and W. H. Stein, *Analyt. Chem.*, 1958, **30**, 1185.
[16] L. A. Carpino, *J. Org. Chem.*, 1963, **28**, 1909.
[17] G. W. Anderson and A. C. McGregor, *J. Amer. Chem. Soc.*, 1957, **79**, 6180.
[18] M. Sulzbacher and E. Bergmann, *J. Org. Chem.*, 1948, **13**, 303.

m. p., % yield of recrystallised product, and solvent for recrystallisation: *N*-benzyloxycarbonyl-L-alanine 2,4,5-trichlorophenyl ester (ethyl acetate, 101—102°, 81, benzene–light petroleum) (lit.,[19] m. p. 104°); *N*-t-butoxycarbonyl-L-alanine 2,4,5-trichlorophenyl ester (ethyl acetate, 80, 81—82°, isopropyl alcohol) (lit.,[19] m. p. 81—82°); N-*t-butoxycarbonyl-β-alanine* 2,4,5-*trichlorophenyl ester* (ethyl acetate, 70, 94—95°, benzene–light petroleum) (Found: C, 4·55; H, 4·6; N, 3·8. $C_{14}H_{16}Cl_3NO_4$ requires C, 45·6; H, 4·4; N, 3·8%); *N*-benzyloxycarbonyl-L-isoleucine 2,4,5-trichlorophenyl ester (ethyl acetate, 82—84°, 59, light petroleum) (lit.,[14] m. p. 79°); *N*-benzyloxycarbonyl-L-phenylalanine 2,4,5-trichlorophenyl ester (ethyl acetate, 141—142°, 79, ethanol) (lit.,[14] m. p. 142°); N-*t-butoxycarbonyl-L-methionine* 2,4,5-*trichlorophenyl ester* (ethyl acetate, 89—90°, 71, ethyl acetate–light petroleum), $[\alpha]_D^{23}$ −38·5° (*c* 2·0 in dimethylformamide) (Found: C, 45·0; H, 4·5; N, 3·3. $C_{16}H_{20}Cl_3NO_4S$ requires C, 44·8; H, 4·7; N, 3·3%); N-*t-butoxycarbonyl-L-proline* 2,4,5-*trichlorophenyl ester* (ethyl acetate, oil, 95, crude) (Found: C, 49·0; H, 4·8. $C_{16}H_{18}Cl_3NO_4$ requires C, 48·7; H, 4·6%); N-*t-butoxycarbonyl-L-tryptophan* 2,4,5-*trichlorophenyl ester* (ethyl acetate, 132—133°, 94, EtOH), $[\alpha]_D^{23}$ −30·5° (*c* 1·0 in dimethylformamide) (Found: C, 54·5; H, 4·3; N, 5·8. $C_{22}H_{21}Cl_3N_2O_4$ requires C, 54·6; H, 4·4; N, 5·8%); N-DL-2-*ethylhexanoylglycine* p-*nitrophenyl ester* (ethyl acetate, 135—136°, 93, ethanol) (Found: C, 60·0; H, 7·2; N, 8·7. $C_{16}H_{22}N_2O_5$ requires C, 59·6; H, 6·9; N, 8·7%); *N*-t-butoxycarbonylglycine 2,4,5-trichlorophenyl ester (ethyl acetate, 96, 107—108°, light petroleum) (lit.,[14] m. p. 106—107°); N-*benzyloxycarbonyl-L-pyroglutamic acid* 2,4,5-*trichlorophenyl ester* (tetrahydrofuran, 106—107°, 97, EtOH), $[\alpha]_D^{23}$ −44·1° (*c* 1·0 in dimethylformamide) (Found: C, 51·8; H, 2·8; N, 3·3. $C_{19}H_{14}Cl_3O_5N$ requires C, 51·5; H, 3·2; N, 3·2%). A solution of the last-named ester (10 mmoles) in dry tetrahydrofuran (50 ml.) was hydrogenolysed over 5% palladised charcoal (0·5 g.) at room temperature and pressure. Evaporation of the filtered solution and trituration of the residue with ether afforded L-*pyroglutamic acid* 2,4,5-*trichlorophenyl ester* (94%), m. p. 161—163° (unchanged after recrystallisation from methanol), $[\alpha]_D^{23}$ +14·8° (*c* 2·0 in dimethylformamide) (Found: C, 42·9; H, 2·6; N, 4·5. $C_{11}H_8Cl_3NO_3$ requires C, 42·8; H, 2·6; N, 4·5%).

N-(o-*Nitrophenylsulphenyl*)-L-*leucyl*-L-*methionine Amide* (VIII).—*N*-(*o*-Nitrophenylsulphenyl)-L-leucine dicyclohexylammonium salt[13] (233 g., 0·5 mole), L-methionine methyl ester hydrochloride (99·9 g., 0·5 mole), and AnalaR chloroform (1·2 l.) were stirred at 0° for 5 min., then a solution of *NN'*-dicyclohexylcarbodi-imide (103 g., 0·5 mole) in AnalaR chloroform (200 ml.) was added dropwise during 10 min. at −5 to 0° with vigorous stirring. The mixture was stirred at 0—2° for 18 hr. then filtered. The filtrate was evaporated and the residue was digested with warm ethyl acetate (600 ml.). The filtered digest was washed successively with 20% aqueous sodium chloride (100 ml.), saturated aqueous potassium hydrogen carbonate (100 ml.), and 20% aqueous sodium chloride (3 × 100 ml.), then dried ($MgSO_4$) and evaporated, yielding a yellow oil [this readily crystallised under di-isopropyl ether yielding the yellow ester, m. p. 87—88°, $[\alpha]_D^{23}$ −27·4° (*c* 2·0 in dimethylformamide)]. The oil was dissolved at 0° in dry methanol saturated with ammonia (2 l.) (stirring for ½ hr.), and the resulting solution was kept for 4 days at 0° and then for 2 days at room temperature. Evaporation followed by digestion of the residue with ether gave the pure *amide* (141 g., 68%), m. p. 157—158°, which separated from ethyl acetate (1·4 l.) in yellow needles, $[\alpha]_D^{23}$ −31·0° (*c* 1·0 in dimethylformamide) (Found: C, 49·0; H, 6·5; N, 13·4. $C_{17}H_{26}N_4O_4S_2$ requires C, 49·2; H, 6·3; N, 13·5%).

L-*Leucyl*-L-*methionine Amide* (VI).—A solution of *N*-(*o*-nitrophenylsulphenyl)-L-leucyl-L-methionine amide (82·9 g., 0·2 mole) in boiling ethyl acetate (2·5 l.) was rapidly cooled to 25°, and 5·4N-hydrogen chloride in ethyl acetate (81·4 ml., 0·44 mole) added dropwise during, 5 min. with stirring at 20—25°. Stirring was continued for 10 min. at 0—20°, after which the product (61·4 g., 100%) was collected and washed with ethyl acetate and ether. Recrystallisation from ethanol (200 ml.) gave long white needles of the *hydrochloride hemihydrate*, which underwent marked sintering at 90° followed by slow melting (clear melt at 195°) (Found: C, 42·9; H, 7·9; N, 13·6; loss in weight on drying at 80°/0·1 mm., 3·1. $C_{11}H_{23}N_3O_2S,HCl,0{\cdot}5H_2O$ requires C, 43·0; H, 8·2; N, 13·7; H_2O, 3·0%). A solution of the hydrochloride (29·8 g.) in water (50 ml.) was treated with 50% aqueous potassium carbonate (50 ml.) at 0—10°. The solid was collected, washed twice with ice–water, dried *in vacuo*, and recrystallised from ethyl acetate, yielding long, colourless needles of the *base* (86%), m. p. 130—131°, $[\alpha]_D^{23}$ −6·3° (*c* 2·0 in dimethylformamide), amino-acid ratios in acid hydrolysate, leu 1·00: met 0·96 (Found: C, 50·6; H, 9·0; N, 15·7. $C_{11}H_{23}N_3O_2S$ requires C, 50·6; H, 8·9; N, 16·1%).

N-*Benzyloxycarbonyl*-L-*isoleucylglycine Methyl Ester*.—(*a*) Triethylamine (74 ml., 0·5 mole)

[19] E. Sandrin and R. A. Boissonnas, *Helv. Chim. Acta*, 1963, **46**, 1637.

was added to a vigorously stirred suspension of glycine methyl ester hydrochloride (62·8 g., 0·5 mole) in methanol (125 ml.) at 0°, then a solution of *N*-benzyloxycarbonyl-L-isoleucine 2,4,5-trichlorophenyl ester (223 g., 0·5 mole) in dimethylformamide (250 ml.) was added. The mixture was stirred for 4 hr. at 0°, then left at 0° overnight. Water (700 ml.) was added and the solid was collected, washed with cold water, and then dissolved in hot ethanol (600 ml.). The colourless needles of the ester (151 g., 90%), m. p. 124—125°, $[\alpha]_D^{23}$ −10·0° (*c* 2·0 in dimethylformamide) (lit.,[20] m. p. 131°) which separated on cooling were collected and washed with ice-cold ether (Found: C, 60·2; H, 7·1; N, 8·5. Calc. for $C_{17}H_{24}N_2O_5$: C, 60·7; H, 7·15; N, 8·3%).

(*b*) A solution of *NN'*-dicyclohexylcarbodi-imide (41 g., 0·2 mole) in methylene chloride (200 ml.) was added to a mixture of *N*-benzyloxycarbonyl-L-isoleucine (53 g., 0·2 mole), glycine methyl ester hydrochloride (25 g., 0·2 mole), and triethylamine (28 ml., 0·2 mole) in methylene chloride (1·1 l.) at 5°. The mixture was stirred at 20° for 18 hr. then filtered from *NN'*-dicyclohexylurea. The filtrate and washings were evaporated and the residue was shaken with ethyl acetate (1·2 l.) and water (500 ml.). The organic phase was separated, washed successively with 0·5N-hydrochloric acid (150 ml.), saturated aqueous sodium hydrogen carbonate (150 ml.), and water (200 ml.), then dried ($MgSO_4$) and evaporated. Recrystallisation of the residue from ethyl acetate (600 ml.) and light petroleum (b. p. 60—80°) (300 ml.) afforded the ester (51·7 g., 77%), m. p. 121—122°.

L-*Isoleucylglycine Methyl Ester Hydrochloride*.—After hydrogenolysis of the *N*-benzyloxycarbonyl derivative as described below in acetic acid, or in methanol, an equivalent amount of hydrogen chloride in methanol was added before evaporation. The solid residue was the pure hydrochloride (100%), which separated from methanol–ether in needles, m. p. 175—177°, R_{FA} 0·64, R_{FC} 0·60, $[\alpha]_D^{23}$ +37·2° (*c* 1·0 in dimethylformamide) (lit.,[16] m. p. 175°).

N-*Benzyloxycarbonyl-L-phenylalanyl-L-isoleucylglycine Methyl Ester*.—*N*-Benzyloxycarbonyl-L-isoleucylglycine methyl ester (157 g., 0·467 mole) in glacial acetic acid (1 l.) was hydrogenated over 5% palladised charcoal (23·3 g.) at room temperature and pressure. The filtered (kieselguhr) solution was evaporated, and the residual oil was dissolved in dimethylformamide (300 ml.). Triethylamine (65·4 ml., 0·467 mole) and a solution of *N*-benzyloxycarbonyl-L-phenylalanine 2,4,5-trichlorophenyl ester (224 g., 0·467 mole) in dimethylformamide (450 ml.) were added at 0°, and the mixture was left at 0° for 16 hr. The solid, which separated on addition of ice–water (700 ml.), was collected and dissolved in hot 2-ethoxyethanol (1 l.); the pure *ester* (184 g., 81%), m. p. 186—187°, $[\alpha]_D^{24}$ −14·0 (*c* 2·0 in dimethylformamide), which separated on cooling, was collected, washed well with ether, and dried *in vacuo* at 50° (Found: C, 64·5; H, 7·0; N, 8·8. $C_{26}H_{33}N_3O_6$ requires C, 64·6; H, 6·85; N, 8·7%). A further 13·6 g. (6%) (total 87%) of the ester was recovered from the 2-ethoxyethanol mother liquors.

L-*Phenylalanyl-L-isoleucylglycine Methyl Ester Hydrochloride* (IX,HCl).—After hydrogenolysis of the *N*-benzyloxycarbonyl derivative as described below in acetic acid, an equivalent amount of hydrogen chloride in ethanol was added before evaporation. The solid residue was the pure *hydrochloride hemihydrate* (100%), which separated from methanol–ether in gelatinous needles, m. p. 213—215°, R_{FA} 0·70 ,R_{FC} 0·79, $[\alpha]_D^{23}$ −2·0° (*c* 1·0 in dimethylformamide) (Found: C, 55·1; H, 7·4; N, 10·5. $C_{18}H_{28}ClN_3O_4,0{\cdot}5H_2O$ requires C, 54·8; H, 7·35; N, 10·65%).

N-*Benzyloxycarbonyl-L-alanyl-L-phenylalanyl-L-isoleucylglycine Methyl Ester* (V; X = $PhCH_2{\cdot}O{\cdot}CO$, R = Me).—*N*-Benzyloxycarbonyl-L-phenylalanyl-L-isoleucylglycine methyl ester (156 g., 0·325 mole) in glacial acetic acid (2 l.) was hydrogenated over 5% palladised charcoal (16·2 g.) at room temperature and pressure. The filtered (kieselguhr) solution was evaporated, and the white, crystalline residue was dissolved in dimethylformamide (500 ml.) and treated with triethylamine (45·5 ml., 0·325 mole) and a solution of *N*-benzyloxycarbonyl-L-alanine 2,4,5-trichlorophenyl ester (131 g., 0·325 mole) in dimethylformamide (300 ml.) at 0°. The mixture was left at 0° for 16 hr. then stirred vigorously with water (3 l.) for 15 min. at 40—50°. The solid was collected, washed successively with water, ethanol, and ether, and dried *in vacuo* at 50°, yielding the *ester* (178 g., 99%), m. p. 228—229°, which separated from 2-ethoxyethanol (2 l.) in colourless needles (146 g., 82%), m. p. 229—230°, $[\alpha]_D^{23}$ −23·4° (*c* 1·0 in dimethylformamide) (Found: C, 62·8; H, 6·6; N, 10·3. $C_{29}H_{38}N_4O_7$ requires C, 62·8; H, 6·9; N, 10·1%).

L-*Alanyl-L-phenylalanyl-L-isoleucylglycine Methyl Ester Acetate and Hydrochloride*.—*N*-Benzyloxycarbonyl-L-alanyl-L-phenylalanyl-L-isoleucylglycine methyl ester (55·4 g., 0·1 mole) in glacial acetic acid (1 l.) was hydrogenated over 5% palladised charcoal (5 g.) at room temperature and pressure. The filtered (kieselguhr) solution was evaporated and the white crystalline residue

[20] H. Determann and T. Wieland, *Makromol. Chem.*, 1961, **44–46** 1637.

was collected with ether, yielding the acetate (48·1 g. 100%), m. p. 150—152°. In another experiment, an equivalent amount of hydrogen chloride in methanol was added before evaporation. The solid residue was the pure *hydrochloride hydrate* (100%), which separated from methanol–ether in gelatinous needles which slowly decomposed above 210°, R_{FB} 0·52, R_{FC} 0·76, $[\alpha]_D^{23}$ −5·7° (*c* 2·0 in dimethylformamide), amino-acid ratios in acid hydrolysate, ala 1·00: gly 1·00: ile 1·00: phe 1·03 (Found: C, 53·2; H, 7·4; N, 11·4. $C_{21}H_{23}ClN_4O_5,H_2O$ requires C, 53·2; H, 7·4; N, 11·8%).

N-*t*-*Butoxycarbonyl*-L-*alanyl*-L-*phenylalanyl*-L-*isoleucylglycine Methyl Ester* (V; X = $Bu^tO{\cdot}CO$, R = Me).—(*a*) L-Alanyl-L-phenylalanyl-L-isoleucylglycine methyl ester acetate (36·7 g., 70 mmoles), pure dry pyridine (200 ml.), and t-butoxycarbonyl azide[16] (19·6 ml., 140 mmoles) were stirred at 20—22° until all the suspended solid had dissolved (up to 18 hr.). The solution was kept at room temperature for 2 days then diluted with ice-water (800 ml.), yielding the ester (33·3 g., 91%), m. p. 207—208° (effervescence), which was collected, washed with water, and dried *in vacuo* at 50°. Recrystallisation from methanol gave colourless, silky needles, m. p. 208—210° (effervescence), $[\alpha]_D^{23}$ −23·5° (*c* 1·0 in dimethylformamide) (Found: C, 59·3; H, 7·8; N, 10·7. $C_{26}H_{40}N_4O_7$ requires C, 60·0; H, 7·7; N, 10·8%). Under otherwise identical conditions but using 77 mmoles of the azide the yield of product was 68%.

(*b*) L-Alanyl-L-phenylalanyl-L-isoleucylglycine methyl ester acetate (2·4 g., 5 mmoles), water (10 ml.), dioxan (20 ml.), magnesium oxide (0·4 g., 10 mmoles), and t-butoxycarbonyl azide[16] (1·4 ml., 10 mmoles) were stirred at 40—45° for 18 hr. The filtered mixture was diluted with ice-water (40 ml.), yielding the ester (2·23 g., 85%), m. p, 207—208° (effervescence).

(*c*) L-Phenylalanyl-L-isoleucylglycine methyl ester acetate (from hydrogenolysis of 0·1 mole of the corresponding *N*-benzyloxycarbonyl derivative) in dimethylformamide (200 ml.) was treated with triethylamine (14 ml., 0·1 mole) and a solution of *N*-t-butoxycarbonyl-L-alanine 2,4,5-trichlorophenyl ester (36·9 g., 0·1 mole) in dimethylformamide (100 ml.) at 0°. The solution was kept at 0° for 16 hr. then diluted with ice–water (1 l.). The solid was collected and crystallised from methanol, yielding the ester (44·3 g., 85%), m. p. 207—208° alone or when mixed with a sample prepared as described in (*a*) above.

N-*t*-*Butoxycarbonyl*-L-*alanyl*-L-*phenylalanyl*-L-*isoleucylglycine* (V; X = $Bu^tO{\cdot}CO$, R = OH).—The above methyl ester (32·3 g., 62 mmoles) in hot 2-ethoxyethanol (500 ml.) was added to cold water (250 ml.) with vigorous stirring, and the fine suspension was rapidly cooled to 20°. N-Sodium hydroxide (124 ml.) was then added and vigorous stirring was continued for 1 hr. at 20—22° (clear solution after 15 min.). The solution was added to ice-water (1 l.) containing conc. hydrochloric acid (136 mmoles) and acetic acid (1 ml.), and the crystalline *acid* (29·08 g., 93%), m. p. 310—312° (decomp.), which separated was collected and washed with water. After recrystallisation from aqueous ethanol (short needles), the m. p. fell to 186—187° (effervescence), $[\alpha]_D^{23}$ −22·3° (*c* 1·0 in dimethylformamide) (Found: C, 59·4; H, 7·5; N, 11·1. $C_{25}H_{38}N_4O_7$ requires C, 59·3; H, 7·6; N, 11·1%).

N-*t*-*Butoxycarbonyl*-L-*alanyl*-L-*phenylalanyl*-L-*isoleucylglycine* 2,4,5-*Trichlorophenyl Ester*.—A solution of *N*-t-butoxycarbonyl-L-alanyl-L-phenylalanyl-L-isoleucylglycine (10·14 g., 20 mmoles) in dioxan (200 ml.) was cooled to 15° and treated with a solution of *NN'*-dicyclohexylcarbodi-imide (4·12 g., 20 mmoles) in dioxan (50 ml.). The mixture was stirred at 13—18° for 4 hr., heated at 55—60° for 5 min., then filtered. Evaporation of the filtrate and recrystallisation of the residue from methanol (300 ml.) gave fine white needles of the *ester* (8·24 g., 60%), which slowly decomposed without melting above 260° (Found: C, 54·2; H, 5·8; N, 8·3. $C_{31}H_{39}Cl_3N_4O_7$ requires C, 54·3; H, 5·7; N, 8·2%).

N-*t*-*Butoxycarbonyl*-L-*alanyl*-L-*phenylalanyl*-L-*isoleucylglycyl*-L-*leucyl*-L-*methionine Amide* (II; X = $Bu^tO{\cdot}CO$).—(*a*) *N*-t-Butoxycarbonyl-L-alanyl-L-phenylalanyl-L-isoleucylglycine 2,4,5-trichlorophenyl ester (6·86 g., 10 mmoles) was dissolved in dimethylformamide (80 ml.) by a brief warming at 60—70°. The solution was immediately cooled to 5° and treated with L-leucyl-L-methionine amide (2·62 g., 10 mmoles) followed by acetic acid (2 drops). The mixture was kept at 0—5° for 18 hr., then water (80 ml.) was added. The solid was collected, washed thoroughly with water followed by ether, dried *in vacuo* at 50°, yielding the amide (7·27 g., 97%), which separated from methanol or aqueous acetic acid in fine needles, m. p. 261—262° (effervescence), $[\alpha]_D^{24}$ −31·2° (*c* 1·0 in dimethylformamide) {lit.,[11] m. p. 25° (decomp.), $[\alpha]_D^{24}$ −30·5° (*c* 1·0 in dimethylformamide[8]); m. p. 254—256° (decomp.), $[\alpha]_D^{22}$ −28° (in dimethylformamide)}.

(*b*) A solution of *N*-t-butoxycarbonyl-L-alanyl-L-phenylalanyl-L-isoleucylglycine (0·507 g., 1 mmole) and L-leucyl-L-methionine amide (0·262 g., 1 mmole) in dimethylformamide (10 ml.) was treated with *NN'*-dicyclohexylcarbodi-imide (0·206 g., 1 mmole) at 0°, and the mixture was kept

at 0—5° for 4 days. The solvent was removed *in vacuo* and the residue was triturated with 0·5N-sodium hydrogen carbonate. The solid was collected, washed with water, dried *in vacuo* at 40°, and then digested with hot chloroform (15 ml.). Two recrystallisations of the insoluble solid from methanol gave the pure amide (0·450 g., 60%), m. p. 261—262° (effervescence), identical with the sample prepared as described in (*a*).

(*c*) A finely divided supension of *N*-t-butoxycarbonyl-L-alanyl-L-phenylalanyl-L-isoleucylglycine (2·54 g., 5 mmoles) in chloroform (100 ml.) was stirred rapidly and treated with triethylamine (0·7 ml., 5 mmoles) at 20—22°. When all the solid had dissolved (~ 1 min.), the solution was cooled to −15° and treated with ethyl chloroformate (0·47 ml., 5 mmoles). Stirring was continued at −15° to −10° for 20 min. then a solution of L-leucyl-L-methionine amide (1·31 g., 5 mmoles) in chloroform (20 ml.) was added dropwise at −10°. The mixture was stirred for 18 hr. at 0° then the solvent was removed *in vacuo*. The residue was digested with 0·5N-sodium hydrogen carbonate (1·08 g. of the acid used as starting material was recovered from the digests) and the insoluble solid was collected and washed with water. Recrystallisation of the dried solid from methanol afforded the pure amide (1·50 g., 40%), m. p. 261—262° (effervescence), identical with the sample prepared as described in (*a*). When the mixed anhydride was prepared using pivaloyl chloride the yield of pure amide was 29%.

L-Alanyl-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methionine Amide (II; X=H) *and Trifluoroacetate*.—The above t-butoxycarbonyl derivative (5 g.,) was added to anhydrous trifluoroacetic acid (20 ml.) at 10°, and the resulting solution was kept for 1 hr. at 20—22°. Ether (200 ml.) was then added and the solid was collected, washed four times with ether, and dried *in vacuo* at 40°, yielding the pure trifluoroacetate (5·10 g., 100%), m. p. 265° (decomp.), unchanged after recrystallisation from acetic acid (50 ml.), $[\alpha]_D^{23}$ −21·9° (*c* 1 in 95% acetic acid), R_{FA} 0·82, amino-acid ratios in acid hydrolysate, ala 1·00: gly 1·00: ile 1·00: leu 1·01: met 0·96: phe 1·03 (Found: C, 51·8; H, 7·0; F, 7·5; N, 12·7. Calc. for $C_{31}H_{51}N_7O_6S{\cdot}C_2HF_3O_2$: C, 52·0; H, 6·9; F, 7·5; N, 12·8%) [lit.,[11] m. p. 260° (decomp.)], $[\alpha]_D^{22}$ −21·5° (*c* 1 in 95% acetic acid). A solution, prepared by warming the trifluoroacetate (0·76 g.) in dimethylformamide (10 ml.) at 40°, was rapidly cooled to 10° then added to a mixture of 2N-ammonium hydroxide (1 ml.) and ice–water (20 ml.). The gelatinous precipitate was collected and washed thoroughly with water by centrifugation, then dried at 80°/0·01 mm., yielding the base (0·638 g.), m. p. 235° (decomp.) (Found, immediately after drying: C, 56·9; H, 8·1; N, 14·9. Calc. for $C_{31}H_{51}N_7O_6S$: C, 57·3; H, 7·9; N, 15·1%) [lit.,[8] m. p. ~ 225° (decomp.);[9] + 1/3 H_2O, m. p. 233—234°,[10] m. p. 225° (decomp.)].

Preparation of Acylated Tetra- and Penta-peptide Methyl Esters (Table 1).—A solution of L-alanyl-L-phenylalanyl-L-isoleucylglycine methyl ester acetate (4·80 g., 10 mmoles) in dimethylformamide (50 ml.) (prepared by short shaking at 30°) was cooled to 0° and treated with triethylamine (1·40 ml., 10 mmoles) followed by the appropriate active ester (10 mmoles). The mixture was then left at 0° for 18 hr. Ice–water (50 ml.) was added, the solid was collected, washed exhaustively with water followed by ether, and then recrystallised from the solvent indicated in Table 1.

Preparation of Acylated Tetra- and Penta-peptides (Table 2).—A solution of the corresponding methyl ester (Table 1) (5 mmoles) in hot 2-ethoxyethanol (100 ml.) was added in one portion to cold water (25 ml.) with vigorous stirring. The resulting finely-divided suspension was rapidly cooled to 20° and treated with N-sodium hydroxide (10 ml.). Stirring was continued at 18—20° until all the solid had dissolved (usually 15—30 min.) and then for an additional ½ hr. The solution was then added to ice–water (100 ml.) containing N-hydrochloric acid (11 ml.) and acetic acid (5 drops) and the solid was collected, washed well with water, and dried *in vacuo* at 40—50°.

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