Polypeptides. Part XXI.[1] Synthesis of Some Sequential Macromolecular Polypeptolides of L-Leucine and L-2-Hydroxy-4-methylpentanoic Acid

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Four protected oligopeptolides of L-leucine and L-2-hydroxy-4-methylpentanoic acid, with benzyloxycarbonyl as *N*-protecting and t-butyl as *C*-protecting group, have been synthesised; the depside linkage was formed by the action of dicyclohexylcarbodi-imide in ethereal pyridine. The action of triethylamine on the chloride hydrochlorides of the deprotected oligopeptolides gave the macromolecular sequential polypeptolides (I; x = 1, 2, or 3) in 40—45% yield with molecular weights, M_n, of 24,000, 32,000, and 46,000, respectively. An improved method of preparation of L-2-acetoxy-4-methylpentanoic acid from L-leucine is described.

HAYLOCK and RYDON [2] concluded, on the basis of ^{1}H n.m.r. studies of the helix–coil transition in solutions of poly-(L-methionine) in mixtures of deuteriochloroform and trifluoroacetic acid, that one in three or four peptide linkages was protonated before the α-helical conformation collapsed to the random coil. The effect of the protonation of a peptide group on intra-chain hydrogen bonding in a poly-amino-acid can be simulated by replacing the protonated peptide group by an ester group; the present paper is concerned with the synthesis of three polypeptolides (I; x = 1—3) in which one in two, three, and four peptide linkages of poly-(L-leucine) are modified in this way. The conformational studies,

$$H\text{-}\left[\left(\text{-NH}\cdot\text{CHBu}^{i}\cdot\text{CO}\right)_{x}\text{O}\cdot\text{CHBu}^{i}\cdot\text{CO}\right]_{n}\text{OH}$$

(I)

which support the conclusion of Haylock and Rydon,[2] will be reported elsewhere.

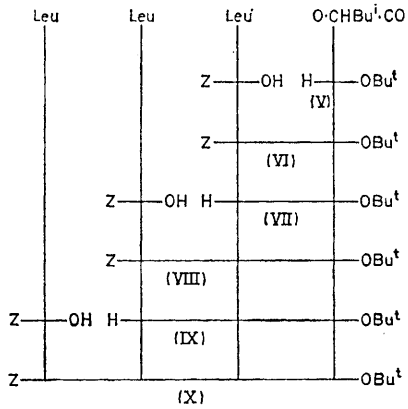
L-2-Hydroxy-4-methylpentanoic acid, the hydroxy-analogue of L-leucine, has been prepared previously from this amino-acid by deamination with nitrous acid,[3–6] but in our hands this method gave unacceptably low yields. Plattner *et al.*[7] obtained D-α-acetoxyisovaleric acid in 60% yield from D-valine by the action of isopentyl nitrite in anhydrous acetic acid; application of this method to L-leucine (II) gave only a 30% yield of L-2-acetoxy-4-methylpentanoic acid (III), but this was raised to 60—70% if the reaction mixture was heated to 60° for a short time after addition of the nitrite. The complete retention of configuration in such reactions is well established.[6,8] The acetoxy-acid (III) was converted into its t-butyl ester (IV) by acid-catalysed addition to isobutene;[9] alkaline hydrolysis of the ester (IV) gave t-butyl L-2-hydroxy-4-methylpentanoate (V), required for the synthesis of the peptolides. The properties of the L-2-hydroxy-4-methylpentanoic acid derivatives (III)—(V) were in agreement with those reported.[10]

$$\underset{\text{(II)}}{H_2N\cdot CHBu^{i}\cdot CO_2H} \longrightarrow \underset{\text{(III)}}{AcO\cdot CHBu^{i}\cdot CO_2H} \longrightarrow \underset{\text{(IV)}}{AcO\cdot CHBu^{i}\cdot CO_2Bu^{t}} \longrightarrow \underset{\text{(V)}}{HO\cdot CHBu^{i}\cdot CO_2Bu^{t}}$$

The synthesis of four fully protected peptolides is outlined in the Scheme. The hydroxy-acyl residue was placed at the *C*-terminus in order to avoid racemisation in the eventual polymerisation; oxazolinone formation, which is the major process leading to racemisation in peptide synthesis, is not possible with a *C*-terminal hydroxy-acyl residue, and Shemyakin and Ovchinnikov and their colleagues [11] have cyclised many such oligopeptolides without racemisation. We originally intended to use the *p*-nitrobenzyloxycarbonyl group [12] for

[1] Part XX, P. M. Hardy, H. N. Rydon, and H. T. Storey, *J.C.S. Perkin I*, 1972, 1523.
[2] J. C. Haylock and H. N. Rydon, *Peptides: Proc. IX European Peptide Symp.*, 1968, 19.
[3] H. Scheibler and A. S. Wheeler, *Ber.*, 1911, **44**, 2684.
[4] B. Iselin and E. A. Zeller, *Helv. Chim. Acta*, 1946, **29**, 1508.
[5] C. G. Baker and A. Meister, *J. Amer. Chem. Soc.*, 1951, **73**, 1336.
[6] M. Winitz, L. Bloch-Frankenthal, N. Izumiya, S. N. Birnbaum, C. G. Baker, and J. P. Greenstein, *J. Amer. Chem. Soc.*, 1956, **78**, 2423.
[7] P. A. Plattner, K. Vogler, R. O. Studer, P. Quitt, and W. Keller-Schierlein, *Helv. Chim. Acta*, 1963, **46**, 927.
[8] P. Brewster, F. Hiron, E. D. Hughes, C. K. Ingold, and P. A. D. S. Rao, *Nature*, 1950, **166**, 178; C. K. Ingold, 'Structure and Mechanism in Organic Chemistry,' Bell, London, 2nd edn., 1969, 538.
[9] *Cf.* R. W. Roeske, *Chem. and Ind.*, 1959, 1121; G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, 1960, **82**, 3359.
[10] Yu. A. Ovchinnikov, A. A. Kiryushkin, and M. M. Shemyakin, *J. Gen. Chem. U.S.S.R.*, 1966, **66**, 637.
[11] M. M. Shemyakin and Yu. A. Ovchinnikov, *Rec. Developments Chem. Natural Carbon Compounds*, 1967, **2**, 1.
[12] F. H. Carpenter and D. T. Gish, *J. Amer. Chem. Soc.*, 1952, **74**, 3818.

N-protection in order to avoid possible difficulties in the proposed establishment of the depside linkage by the benzenesulphonic mixed anhydride method.[13] The *p*-nitro-derivative of compound (VI) was prepared in high yield in this way, but the difficulty of completely



SCHEME Synthesis of fully protected oligopeptolides

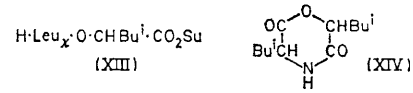
Here and elsewhere the abbreviations for amino-acid residues, *etc.*, are those recommended in I.U.P.A.C. Information Bulletin No. 28; Su = $-\overline{N{\cdot}CO{\cdot}[CH_2]_2{\cdot}CO}$.

removing *p*-toluidine from the hydrogenolysis product led us to abandon the method. The use of the unsubstituted benzyloxycarbonyl group for *N*-protection made it necessary to find another method for forming the depside group in (VI). The preparation of esters of *N*-protected amino-acids by means of dicyclohexylcarbodi-imide is less easy than that of peptides, and the addition of copper(I) chloride [14] or pyridine [15-17] has been recommended. Neither the method of Vowinkel [14] nor those of Morley [15] and Buzas *et al.*[16] were satisfactory for the preparation of compound (VI), but a procedure similar to that of Hassall *et al.*,[17] in which *N*-benzyloxycarbonyl-L-leucine was added to a mixture of the hydroxy-ester (V) and dicyclohexylcarbodi-imide in ethereal pyridine, gave a high yield. Hydrogenolysis of compound (VI) gave t-butyl *O*-L-leucyl-L-2-hydroxy-4-methylpentanoate (VII) as a liquid which could be purified by distillation; the ester slowly deposited 3,6-di-isobutyl-2,5-dioxopiperazine, formed by aminolysis of the depside ester group in (VII), as suggested, but not observed, by Schwyzer [18] and Stewart.[19] In view of this, the depside (VII) was best stored as its highly crystalline hydrogen oxalate. The remainder of the synthesis was uneventful; the coupling reactions were carried out by the ethyl carbonic mixed anhydride method. The fully protected peptolides (VIII) and (X) were crystalline solids, but the partially protected peptolide (IX) was an oil which, however, gave a highly crystalline hydrogen oxalate.

Preliminary polymerisation experiments (see later) showed that derivatives of *O*-L-leucyl-L-2-hydroxy-4-methylpentanoic acid cyclised so readily to the dioxomorpholine (XIV) as to make them useless for the preparation of the polypeptolide (I; $x = 1$). Accordingly, the t-butyl group was removed from the fully protected depside (VI) by treatment with trifluoroacetic acid and the resulting acid (XI) coupled with (VII) by means of dicyclohexylcarbodi-imide to give the fully protected tetrapeptolide (XII) in good yield.

Z·Leu·O·CHBu^i·CO_2H + (VII) ⟶
(XI)
Z·Leu·O·CHBu^i·CO·Leu·O·CHBu^i·CO_2Bu^t
(XII)

Earlier papers in this series [1,20,21] describe the successful preparation of macromolecular sequential polypeptides by the polymerisation of oligopeptide *N*-succinimidyl esters; we accordingly attempted to apply this method to the preparation of the synthetic polypeptolides (I). Treatment of the t-butyl esters (VI), (VIII), and (X) with trifluoroacetic acid gave the corresponding carboxylic acids as uncrystallisable oils which could, however, be purified through their dicyclohexylammonium salts. The acids regenerated from these were condensed with *N*-hydroxysuccinimide to give the *N*-succinimidyl esters. Treatment of the succinimidyl esters corresponding to (VI) and (VIII) with hydrogen bromide in acetic acid gave the hydro-



bromides of (XIII; $x = 1$ and 2). Attempted polymerisation of these compounds, after addition of 1 equiv. of triethylamine, in a wide variety of solvents at 2·5M concentration gave no polymeric products. Under these conditions compound (XIII; $x = 1$) gave only 2,5-di-isobutyl-3,6-dioxomorpholine (XIV), isolated by chromatography in chloroform–ethanol (4 : 1) on Sephadex LH20, and identified by comparison (i.r. and n.m.r. spectra) with an authentic specimen. The authentic specimen of (XIV) was obtained by the cyclisation of *O*-L-leucyl-L-2-hydroxy-4-methylpentanoic acid (XVI; $x = 1$), obtained from (VII) by treatment with trifluoroacetic acid, with dicyclohexylcarbodi-imide; the structure of the compound (in particular its monomeric nature) was established by mass spectrometry (M^+ 227) and by the absence of a *trans*-amide II band and the presence of a *cis*-amide II band (at 1460 cm^{-1}, shifted to 1224 cm^{-1} on deuteriation) in the i.r. spectrum.[22] T.l.c.

[13] E. Schröder and K. Lübke, 'The Peptides,' Academic Press, New York, 1965, vol. 1, p. 96.
[14] E. Vowinkel, *Chem. Ber.*, 1966, **99**, 1479.
[15] J. S. Morley, *Peptides: Proc. VI European Peptide Symp.*, 1966, 351.
[16] A. Buzas, C. Egnell, and P. Freon, *Compt. rend.*, 1962, **255**, 945.
[17] C. H. Hassall, J. G. Martin, J. A. Schofield, and J. O. Thomas, *J. Chem. Soc.* (*C*), 1967, 997.
[18] R. Schwyzer, B. Iselin, and M. Feurer, *Helv. Chim. Acta*, 1955, **38**, 69.
[19] F. H. C. Stewart, *Austral. J. Chem.*, 1969, **22**, 1291.
[20] P. M. Hardy, H. N. Rydon, and R. C. Thompson, *J.C.S. Perkin I*, 1972, 5.
[21] A. Ali, P. M. Hardy, and H. N. Rydon, *J.C.S. Perkin I*, 1972, 1070.
[22] K. Blahá, J. Smoliková, and A. Vitek, *Coll. Czech. Chem. Comm.*, 1966, **31**, 4296.

of the product from compound (XIII; $x = 2$) showed only one, ninhydrin-positive product, probably the unprotonated tripeptolide (XVI; $x = 2$).

Treatment of the fully protected tetrapeptolide (XII) with trifluoroacetic acid and hydrogenolysis of the product gave the free tetrapeptolide (XV), isolated as its highly crystalline hydrochloride; similar hydrogenolysis of the *N*-benzyloxycarbonyl acids from (VIII) and (X) gave the free tri- and tetra-peptolides (XVI; $x = 2$ and 3), which likewise gave highly crystalline hydrochlorides. Although polymers were produced from compounds (XVI; $x = 2$ and 3) by treatment with dicyclohexylcarbodi-imide in acetonitrile, the results were erratic and the products contaminated with *N*-acylureas, which were difficult to remove. Shemyakin *et al.*[23] used the acid chlorides of peptolides with *C*-terminal hydroxy-acyl residues in fragment condensation procedures for the synthesis of linear depsipeptides and showed the couplings to be racemisation-free. We successfully used the same procedure for the polymerisation of the peptolides (XV) and (XVI; $x = 2$ and 3); the method has the advantage of giving as by-product only triethylamine hydrochloride, which is easily removed by washing with water. The hydrochlorides of (XV) and (XVI; $x = 2$ and 3) were converted into the acid chloride hydrochlorides by the action of thionyl chloride at room temperature, the progress of the reaction being followed by i.r. spectroscopy; the products were polymerised in benzene (3·5M-solution) at room temperature for 24 h, after addition of 2 equiv. of triethylamine. The polypeptolides (I; $x = 1$, 2, or 3) were so obtained in 40—45% yield, after removal of material of low molecular weight by Soxhlet extraction with methanol.

H·Leu·O·CHBu^i·CO·Leu·O·CHBu^i·CO_2H (XV)

H·Leu$_x$·O·CHBu^i·CO_2H (XVI)

Insufficient material was available for the molecular weights of the polypeptolides to be determined by the osmotic pressure method and their insolubility in water ruled out the use of gel filtration on Sephadex. An end-group assay procedure was therefore used, the *N*-terminal amino-groups being determined fluorimetrically by dansylation.[24] The results, which give number average molecular weights, showed (I; $x = 1$) to have M_n 24,300 ($n = 107$), (I; $x = 2$) M_n 32,500 ($n = 96$), and (I; $x = 3$) M_n 46,000 ($n = 101$); although these are maximal values, it is clear that the polypeptolides have sufficiently high molecular weights for useful conformational studies. Our polypeptolides have much higher molecular weights than those obtained by Stewart [19] by the polymerisation of some oligopeptolide *p*-nitrophenyl esters.

EXPERIMENTAL

The purity of all compounds was confirmed by t.l.c. on Kieselgel GF 254, usually in two solvent systems. Compounds with free amino-groups were located by spraying with 0·3% ninhydrin in n-butanol and heating at 100° for 10 min, and *N*-acyl compounds by the chlorine–starch–iodide method.[25]

Organic solutions were dried over magnesium sulphate and evaporated or concentrated at 10—20 mmHg on a rotary evaporator. Light petroleum was the fraction b.p. 60—80°. Optical rotations were measured with a Bendix-NPL polarimeter, model 143 (1 or 2 cm cells). I.r. spectra were recorded on a Hilger–Watts Infrascan H900 spectrometer and n.m.r. spectra at 33·5° on a Perkin-Elmer R10 60 MHz spectrometer with tetramethylsilane as internal reference; for identification in the n.m.r. spectra NH and α-CH groups are numbered from the *N*-terminus.

Derivatives of 2-Hydroxy-4-methylpentanoic Acid

Isopentyl nitrite (6·66 g, 57 mmol) was added dropwise during 3 h to a slowly stirred suspension of L-leucine (6·5 g, 50 mmol) in a solution of anhydrous sodium acetate (4·1 g, 50 mmol) in glacial acetic acid (70 ml) at 15°. After the addition was complete, the mixture was heated to 60° for 30 min and then stirred at 15° for 48 h. The solution was evaporated and the residue partitioned between ether and water containing conc. hydrochloric acid (4·5 ml). The ether layer was washed five times with water and then exhaustively extracted with saturated potassium hydrogen carbonate solution. This extract was acidified (pH 2) with hydrochloric acid and thrice extracted with ether. The ethereal extract was washed with water, dried, and distilled, giving L-2-acetoxy-4-methylpentanoic acid (III) (5·5 g, 63%), b.p. 101—104° at 15 mmHg, $[\alpha]_D^{25}$ −42·3° (*c* 3·7 in C_6H_6), τ (CCl_4) −0·50 (1H, s, CO_2H), 4·93 (1H, t, *J* 6 Hz, 2-H), 7·93 (3H, s, MeCO·O), 8·1—8·5 (3H, complex, 3-H_2 and 4-H), and 9·04 (6H, d, *J* 5 Hz, Me_2 of Bu^i) (lit.,[10] b.p. 150—152° at 0·5 mmHg, $[\alpha]_D^{18}$ −40·5° in C_6H_6). In several runs the yields varied from 60 to 70%. Ovchinnikov *et al.*[10] carried out the reaction at a lower temperature and obtained a lower yield (46%). This acid was converted, essentially as described by Ovchinnikov *et al.*,[10] into its t-butyl ester (IV), b.p. 60—62° at 0·2 mmHg, $[\alpha]_D^{25}$ −43·6° (*c* 6·1 in C_6H_6), τ (CCl_4) 5·15 (1H, t, *J* 6 Hz, 2-H), 7·96 (1H, s, MeCO·O), 8·0—8·8 (3H, complex, 3-H_2 and 4-H), 8·49 (9H, s, Bu^t), and 8·98 (6H, dd, *J* 5 Hz, Me_2 of Bu^i), and thence into t-butyl L-2-hydroxy-4-methylpentanoate (V), b.p. 80—82° at 1 mmHg, $[\alpha]_D^{25}$ −7·2° (*c* 4·3 in C_6H_6), τ (CCl_4) 6·05 (1H, m, 2-H), 7·25 (1H, d, *J* 3 Hz, OH), 7·9—8·9 (3H, complex, 3-H_2 and 4-H), 8·51 (9H, s, Bu^t), and 9·05 (6H, d, *J* 6 Hz, Me_2 of Bu^i).

Synthesis of Oligopeptolides

Derivatives of Leucyl-hydroxymethylpentanoic Acid.—*N*-Benzyloxycarbonyl-L-leucine [26] (13·2 g, 50 mmol) was added at 0° to a stirred solution of compound (V) (9·4 g, 50 mmol) and dicyclohexylcarbodi-imide (11·2 g, 55 mmol) in ether (20 ml) containing pyridine (7·9 g, 100 mmol). The mixture was kept at 4° for 20 h, and a few drops of acetic acid were then added. After 10 min at room temp. the mixture was filtered and the filtrate washed with saturated sodium hydrogen carbonate solution and brine and then evaporated. The oily residue was taken up in a little acetone. After 4 h at −10°, the solution was filtered and evaporated.

[23] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, Yu. A. Ovchinnikov, and A. A. Kiryushkin, *J. Gen. Chem. U.S.S.R.*, 1964, **34**, 1796.

[24] C. Gross and B. Labouesse, *European J. Biochem.*, 1969, **7**, 463.

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

[26] M. Bergmann, L. Zervas, and J. S. Fruton, *J. Biol. Chem.*, 1936, **115**, 593.

Passage through a bed of silica gel (12 × 8 cm) and elution with ethyl acetate–benzene (1 : 3) gave a product (13·1 g, 60%), which was sufficiently pure for use in preparative work. Chromatography on acid alumina (Merck) (gradient elution with benzene–ethyl acetate) gave pure *t-butyl* O-(N-*benzyloxycarbonyl*-L-*leucyl*)-L-2-*hydroxy*-4-*methylpentanoate* (VI) as an uncrystallisable oil, $[\alpha]_D^{23}$ −48·1° (*c* 2·0 in MeOH), τ ($CDCl_3$) 2·71 (5H, s, Ph), 4·55 (1H, m, α_2-H), 4·95 (2H, s, benzyl CH_2), 5·1 (1H, m, NH), 6·65 (1H, t, *J* 6 Hz, α_1-H), 8·3 (6H, complex, side-chain CH_2 and CH), 8·57 (9H, s, Bu^t), and 9·05 (12H, dd, *J* 6 Hz, Me_2 of Bu^i) (Found: C, 65·8; H, 8·5; N, 3·1. $C_{24}H_{37}NO_6$ requires C, 66·2; H, 8·6; N, 3·2%).

The crude ester (VI) (12·0 g, 0·29 mmol) was hydrogenated over 5% palladised charcoal (2·5 g) in methanol (125 ml) containing acetic acid (3 ml) for 4 h. The catalyst was filtered off and the filtrate diluted with benzene, washed with saturated sodium hydrogen carbonate, dried, and distilled. *t-Butyl* O-L-*leucyl*-L-2-*hydroxy*-4-*methylpentanoate* (VII) (3·9 g, 52%), b.p. 118—120° at 0·2 mmHg, τ (CCl_4) 5·12 (1H, t, *J* 6 Hz, α_2-H), 6·6 (1H, m, α_1-H), 7·9—8·8 (8H, complex, NH_2 and side-chain CH_2 and CH), 8·53 (9H, s, Bu^t), and 9·03 (12H, dd, *J* 5 Hz, Me_2 of Bu^i), so obtained, in ether (50 ml) was added to oxalic acid (0·12 g) in ether (50 ml); recrystallisation of the precipitate from water gave the *hydrogen oxalate* (4·9 g, 50%), m.p. 129—130°, $[\alpha]_D^{23}$ −16·3° (*c* 1·1 in MeOH) (Found: C, 54·8; H, 8·5; N, 3·6. $C_{18}H_{33}NO_8$ requires C, 55·2; H, 8·5; N, 3·6%).

Unpurified ester (VI) (3·5 g, 8·05 mmol) was kept at room temp. for 2 h in trifluoroacetic acid (8 ml). After addition of toluene (25 ml) the solution was evaporated and the residue dissolved in light petroleum (40 ml). Addition of water (40 ml) precipitated the product at the interface as an oil. This was separated, washed thrice with water and light petroleum, and dissolved in ether; the solution was dried and evaporated to give *O*-(*N*-benzyloxycarbonyl-L-leucyl)-L-2-hydroxy-4-methylpentanoic acid (XI) (1·5 g, 49%), $[\alpha]_D^{23}$ +10·0° (*c* 2·1 in $CHCl_3$), which, although chromatographically pure, could not be induced to crystallise. This oil (3·15 g, 8·3 mmol) and dicyclohexylcarbodi-imide (1·8 g, 8·8 mmol) were stirred for 10 min at −5° in dichloromethane (5 ml). *N*-Hydroxysuccinimide (1·92 g, 16·6 mmol) was added and the mixture allowed to attain room temp. After 12 h a drop of acetic acid was added and the mixture stirred for 10 min and filtered. The filtrate was washed with saturated sodium hydrogen carbonate and evaporated. The residue was taken up in acetone and the solution kept for 4 h at −10°. Filtration, evaporation, and trituration with ether–light petroleum gave the *N*-succinimidyl ester (2·35 g, 60%) as an unstable solid. This was dissolved in acetic acid (10 ml) and treated with 45% hydrogen bromide in acetic acid (10 ml). After 45 min at room temp., the solution was evaporated at 1 mmHg. Trituration with ether and recrystallisation from dichloromethane gave N-*succinimidyl* O-L-*leucyl*-L-2-*hydroxy*-4-*methylpentanoate hydrobromide* (0·57 g, 33%), m.p. 152—156° (decomp.), $[\alpha]_D^{23}$ −18·2° (*c* 0·9 in CH_2Cl_2), τ ($CDCl_3$) 1·6br (3H, NH_3^+), 4·55 (1H, m, α_2-H), 5·7 (1H, m, α_1-H), 7·15 (4H, s, ester C_2H_4), 8·0 (6H, complex, side-chain CH_2 and CH), and 8·98 (12H, d, *J* 5 Hz, Me_2 of Bu^i) (Found: C, 45·2; H, 6·65; N, 6·8. $C_{16}H_{27}BrN_2O_6$ requires C, 45·4; H, 6·4; N, 6·6%). This hydrobromide (50 mg, 0·12 mmol) and triethylamine (0·016 ml, 0·12 mmol) were kept in chloroform (0·05 ml) for 4 days in a stoppered tube; the mixture was then diluted to 0·3 ml with more chloroform and kept for 2 more days. Evaporation and chromatography on Sephadex LH20 in chloroform–ethanol (4 : 1) gave only one peak. Evaporation of the eluate and recrystallisation from methanol–water gave 2,5-di-isobutyl-3,6-dioxomorpholine (XIV) (12 mg, 44%)(Found: C, 63·1; H, 9·5; N, 6·3%), with spectroscopic properties (i.r. and n.m.r.) identical with those of an authentic specimen (see later).

t-Butyl *O*-L-leucyl-L-2-hydroxy-4-methylpentanoate (VII) [from the hydrogen oxalate (1·96 g, 5·0 mmol)] was kept at room temp. for 2 h in trifluoroacetic acid (5 ml). The solution was evaporated and the residue re-evaporated thrice with toluene (5 ml). The oily product was dissolved in ethyl acetate (10 ml), cooled to 0°, and treated with triethylamine (0·70 ml, 5·0 mmol). Dimethylformamide (10 ml) was added and the ethyl acetate removed under reduced pressure. Dicyclohexylcarbodi-imide (1·03 g, 5·0 mmol) was added to the resulting suspension and the mixture was stirred at room temp. for 12 h. Dicyclohexylurea (0·84 g, 75%) was filtered off and washed with a little acetone. Acetic acid (0·1 ml) was added to the filtrate and washings; after 15 min the solution was evaporated and the residue dissolved in acetone (10 ml). After 2 h at −10°, more urea was filtered off and the filtrate evaporated. Two recrystallisations from cold aqueous methanol gave 2,3-*di-isobutyl*-3,6-*dioxomorpholine* (XIV) (0·42 g, 37%), m.p. 139·5—140·5°, $[\alpha]_D^{26}$ −79·0° (*c* 0·3 in MeOH); $\nu_{max.}$ (KBr) 1750 (lactone CO), 1691 (amide I), 1460 (*cis*-amide II) (moved to 1224 cm^{-1} on deuteration with $CF_3{\cdot}CO_2D$), and 1333 (*cis*-amide III) cm^{-1}; τ ($CDCl_3$) 2·33br (1H, NH), 5·22 (1H, m, α_2-H), 5·87 (1H, m, α_1-H), 7·8—8·6 (6H, complex, side-chain CH_2 and CH), and 9·0 (12H, d, *J* 4 Hz, Me_2 of Bu^i); *m/e* 227 (1%, M^+), 212 [4, $(M - CH_3)^+$], 171 [94, $(M - C_4H_8)^+$], 143 [28, $(M - 84)^+$], 115 [10, $(M - 2C_4H_8)^+$], 86 [100, $(M - 141)^+$]; metastable ions at 77·3 and 51·8 relate $(M - C_4H_8)^+$ and $(M - 2C_4H_8)^+$ and $(M - 84)^+$ and $(M - 141)^+$, respectively (Found: C, 63·6; H, 9·7; N, 6·5. $C_{12}H_{21}NO_3$ requires C, 63·4; H, 9·3; N, 6·2%).

Benzenesulphonyl chloride (1·28 ml, 100 mmol) was added dropwise at 0° to a solution of *p*-nitrobenzyloxycarbonyl-L-leucine [from the hydrate[12] (1·57 g, 48 mmol) by azeotroping with benzene] in pyridine (10 ml). After stirring for 10 min at 0° t-butyl L-2-hydroxy-4-methylpentanoate (0·94 g, 50 mmol) was added. The mixture was stirred for 30 min at 0° and 2·5 h at room temp. and then poured into ice–water. The product was extracted with ether and the extract washed successively with 0·2M-hydrochloric acid, 5% sodium hydrogen carbonate, and water, dried, and evaporated. Recrystallisation from aqueous methanol gave *t-butyl* O-(N-4-*nitrobenzyloxycarbonyl*-L-*leucyl*)-L-2-*hydroxy*-4-*methylpentanoate* (1·1 g, 50%), m.p. 60—62°, $[\alpha]_D^{23}$ −39·3° (*c* 0·7 in MeOH), τ (CCl_4) 1·75 (2H, (d, *J* 9 Hz, 3- and 5-protons of C_6H_4), 2·45 (2H, d, *J* 9 Hz, 2- and 6-protons of C_6H_4), 4·41 (1H, m, α_2-H), 4·80 (2H, s, $ArCH_2$), 5·05 (1H, m, NH), 5·61 (1H, m, α_1-H), 7·8—9·0 (6H, complex, side-chain CH_2 and CH), 8·55 (9H, s, Bu^t), and 9·01 (12H, d, *J* 5 Hz, Me_2 of Bu^i) (Found: C, 60·0; H, 7·5; N, 5·7. $C_{24}H_{36}NO_8$ requires C, 60·0; H, 7·5; N, 5·8%).

A solution of the *N*-protected dipeptolide (XI) (1·37 g, 3·6 mmol) and dicyclohexylcarbodi-imide (0·82 g, 4·0 mmol) in dichloromethane (20 ml) was stirred at −5° for 10 min. A solution of the ester (VII) (1·08 g, 3·6 mmol)

was then added and the mixture was kept at room temp. for 12 h. A few drops of glacial acetic acid were added and, after 10 min, the mixture was filtered and the filtrate washed with M-hydrochloric acid and saturated sodium hydrogen carbonate, dried, and evaporated. After being dried in a vacuum desiccator the resulting gum (XII) (1·86 g, 78%, 2·8 mmol) was kept at room temp. for 3 h in trifluoroacetic acid (1 ml). The solution was evaporated to dryness and the residue freed from trifluoroacetic acid by azeotroping thrice with toluene, and finally dried in a vacuum desiccator (KOH). Addition of dicyclohexylamine (0·18 g) to a solution in light petroleum (25 ml) and recrystallisation of the precipitate from ether–light petroleum gave *dicyclohexylammonium* O-[O-(N-*benzyloxycarbonyl*-L-*leucyl*)-L-2-*hydroxy*-4-*methylpentanoyl*-L-*leucyl*]-L-2-*hydroxy*-4-*methylpentanoate* (1·68 g, 76%), m.p. 84—85°, $[\alpha]_D^{23}$ −52·8° (*c* 0·4 in MeOH) (Found: C, 66·9; H, 9·2; N, 5·0. $C_{44}H_{73}N_3O_9$ requires C, 67·1; H, 9·3; N, 5·3%). The free acid (1·95 g, 3·2 mmol), obtained by washing a suspension of this salt in ether with M-hydrochloric acid and drying and evaporating the solution, was hydrogenated over 5% palladised charcoal (0·6 g) in methanol (25 ml) containing acetic acid (0·5 ml) for 4 h. The catalyst was filtered off and the filtrate evaporated. The residue was dissolved in ethyl acetate (15 ml) and 2M-hydrogen chloride in ethyl acetate (3 ml) was added. Addition of light petroleum and recrystallisation of the precipitate from ether–light petroleum gave the *hydrochloride* of O-(O-L-*leucyl*-L-2-*hydroxy*-4-*methylpentanoyl*-L-*leucyl*)-L-2-*hydroxy*-4-*methylpentanoic acid* (XV) (1·37 g, 84%), m.p. 102—105°, $[\alpha]_D^{24}$ −43·3° (*c* 0·6 in MeOH) (Found: C, 55·8; H, 8·7; N, 5·4. $C_{24}H_{44}ClN_2O_7,0·5H_2O$ requires C, 55·7; H, 8·8; N, 5·4%).

Derivatives of Di- and Tri-leucylhydroxymethylpentanoic Acid.—Ethyl chloroformate (1·8 g, 16·6 mmol) was added during 2 min to a stirred solution at −10° of *N*-benzyloxycarbonyl-L-leucine (4·41 g, 16·6 mmol) in anhydrous tetrahydrofuran (40 ml) containing triethylamine (1·62 g, 16·6 mmol). After stirring for a further 10 min at −10°, a solution of t-butyl *O*-L-leucyl-L-2-hydroxy-4-methylpentanoate (VII) [4·53 g, 15·1 mmol; made from the oxalate (5·9 g) by shaking an ethereal suspension with saturated sodium hydrogen carbonate] in tetrahydrofuran (25 ml) was added dropwise during 30 min. After 2 h at 0° and 30 min at room temp., the mixture was diluted with ether and the solution washed with M-hydrochloric acid and saturated sodium hydrogen carbonate, dried, and evaporated. The residue was taken up in methanol (50 ml); the solution was filtered from impurity and evaporated, and the residue was dried in a vacuum desiccator. The resulting *t-butyl* O-(N-*benzyloxycarbonyldi*-L-*leucyl*)-L-2-*hydroxy*-4-*methylpentanoate* (VIII) (6·3 g, 76%) was an oil which solidified after 9 months but could not be recrystallised; m.p. 47—48°, $[\alpha]_D^{23}$ −47·1° (*c* 1·9 in MeOH), τ ($CDCl_3$) 2·66 (5H, s, Ph), 3·2 (1H, m, $NH_{(2)}$), 3·91 (1H, d, *J* 8 Hz, $NH_{(1)}$), 4·87 (2H, s, benzyl CH_2), 4·7—6·0 (3H, complex, $\alpha_{1,2,3}$-H), 8·0—8·7 (9H, m, side-chain CH_2 and CH), 8·55 (9H, s, Bu^t), and 9·07 (18H, d, *J* 4 Hz, Me_2 of Bu^i) (Found: C, 65·6; H, 9·25; N, 5·4. $C_{30}H_{48}N_2O_7$ requires C, 65·7; H, 8·8; N, 5·1%). *t-Butyl* O-(N-*benzyloxycarbonyltri*-L-*leucyl*)-L-2-*hydroxy*-4-*methylpentanoate* (V), prepared similarly (77%), had m.p. 129—130° (from aqueous methanol or light petroleum), $[\alpha]_D^{23}$ −70·5° (*c* 1·4 in MeOH), τ ($CDCl_3$) 2·62 (5H, s, Ph), 3·15 (2H, m, $NH_{(1,2)}$), 4·35 (1H, d, *J* 8 Hz, $NH_{(3)}$), 4·86 (2H, s, benzyl CH_2), 4·6—6·0 (4H, complex, $\alpha_{1,2,3,4}$-H), 8·0—8·8 (12H, complex side-chain CH_2 and CH), 8·55 (9H, s, Bu^t), and 9·07 (24H, d, *J* 4 Hz, Me_2 of Bu^i) (Found: C, 65·8; H, 8·9; N, 6·35. $C_{36}H_{59}N_3O_8$ requires C, 65·2; H, 9·0; N, 6·35%).

Treatment of the foregoing t-butyl esters with trifluoroacetic acid as described for the monoleucyl derivative gave the corresponding acids in good yield as uncrystallisable gums; both were purified through their solid dicyclohexylammonium salts, but only *dicyclohexylammonium* O-(N-*benzyloxycarbonyltri*-L-*leucyl*)-L-2-*hydroxy*-4-*methylpentanoate* could be recrystallised; m.p. 109—111° (from ethyl acetate–light petroleum), $[\alpha]_D^{24}$ −56·2° (*c* 0·85 in MeOH) (Found: C, 65·5; H, 9·2; N, 6·9. $C_{44}H_{74}N_4O_8,H_2O$ requires C, 65·6; H, 9·3; N, 7·0%). Condensation with *N*-hydroxysuccinimide as described for the monoleucyl derivative gave the *N*-succinimidyl esters, of which only N-*succinimidyl* O-(N-*benzyloxycarbonyldi*-L-*leucyl*)-L-2-*hydroxy*-4-*methylpentanoate* could be recrystallised; m.p. 127—128° (from ether), $[\alpha]_D^{23}$ −46·6° (*c* 0·9 in dioxan), τ ($CDCl_3$) 2·62 (5H, s, Ph), 3·32 (1H, d, *J* 9 Hz, $NH_{(2)}$), 4·57 (2H, m, $NH_{(1)}$ and α_3-H), 4·85 (2H, s, benzyl CH_2), 5·25 (1H, t, *J* 6 Hz, α_2-H), 5·60 (1H, t, *J* 6 Hz, α_1-H), 7·20 (4H, s, ester C_2H_4), 8·10 (3H, m, side-chain CH), 8·30 (6H, m, side-chain CH_2), and 9·06 (18H, d, *J* 5 Hz, Me_2 of Bu^i) (Found: C, 61·5; H, 6·9; N, 7·1. $C_{30}H_{43}N_3O_9$ requires C, 61·1; H, 7·35; N, 7·1%).

The fully protected tripeptolide (VIII) was hydrogenolysed as described for the monoleucyl derivative and the oily product was treated with ethereal oxalic acid to give *t-butyl* O-*di*-L-*leucyl*-L-2-*hydroxy*-4-*methylpentanoate hydrogen oxalate* (72%), m.p. 150—151° (from water or ethyl acetate), $[\alpha]_D^{23}$ −36·3° (*c* 1·9 in MeOH) (Found: C, 57·2; H, 9·0; N, 5·8. $C_{24}H_{44}N_2O_9$ requires C, 57·1; H, 8·8; N, 5·6%).

O-(*N*-Benzyloxycarbonyldi-L-leucyl)-L-2-hydroxy-4-methylpentanoic acid (1·6 g) was hydrogenated for 4 h over 5% palladised charcoal (0·5 g) in methanol (30 ml) containing acetic acid (0·4 ml). The catalyst was filtered off and the filtrate evaporated. Addition of ether precipitated the acetate (1·2 g, 84%), which was dissolved in ethyl acetate (10 ml) and treated with 2M-hydrogen chloride in ethyl acetate (5 ml). Addition of light petroleum and recrystallisation of the precipitate from chloroform–light petroleum gave the *hydrochloride* of O-*di*-L-*leucyl*-L-2-*hydroxy*-4-*methylpentanoic acid* (XVI; *x* = 2) (1·05 g, 80%), m.p. 101—104°, $[\alpha]_D^{24}$ −25·7° (*c* 0·9 in MeOH) (Found: C, 52·5; H, 8·9; N, 6·9. $C_{18}H_{35}ClN_2O_5,H_2O$ requires C, 52·35; H, 9·0; N, 6·8%); the free peptolide, obtained by heating the acetate at 70° and 0·1 mmHg for 48 h over potassium hydroxide, had τ [$(CD_3)_2SO$] 1·25 (1H, d, *J* 8 Hz, removed by D_2O, NH), 2·18br (3H, s, removed by D_2O, NH_3^+), 5·11 (1H, t, *J* 6 Hz, α_3-H), 5·65 (1H, q, *J* 6 Hz, α_1-H), 6·30 (1H, t, *J* 6 Hz, α_2-H), 8·4 (9H, complex, side-chain CH_2 and CH), and 9·12 (12H, d, *J* 4 Hz, Me_2 of Bu^i). The *hydrochloride* of O-*tri*-L-*leucyl*-L-2-*hydroxy*-4-*methylpentanoic acid* (XVI; *x* = 3), similarly prepared (78% yield) and recrystallised from ethyl acetate, had m.p. 169—171°, $[\alpha]_D^{25}$ −49·2° (*c* 0·8 in MeOH) (Found: C, 55·5; H, 9·0; N, 8·0. $C_{24}H_{46}ClN_3O_6,0·5H_2O$ requires C, 55·7; H, 9·2; N, 8·1%).

Preparation and Properties of Polymers

The peptolide hydrochloride (0·75 mmol) was kept at room temp. for 1·5 h in purified thionyl chloride [27] (6 ml); i.r. spectroscopy showed that after 1 h the CO_2H carbonyl

[27] L. Friedmann and W. P. Wetter, *J. Chem. Soc.* (*A*), **1967**, 36.

band (1740 cm^{-1}) was completely replaced by the CO·Cl band (1795 cm^{-1}). The solution was evaporated to dryness at 1 mmHg and the residue re-evaporated several times with toluene to remove all traces of thionyl chloride. The final residue was treated with benzene (0·2 ml) and triethylamine (0·22 ml, 1·6 mmol). The paste was thoroughly mixed with a stirring rod and then kept for 24 h at room temp. in a closed vessel. Evaporation, followed by washing with water and Soxhlet extraction with methanol for 12 h, left the polypeptolide, which was dried in a vacuum desiccator. The following were prepared in this way: *poly*-(O-L-*leucyl*-L-2-*hydroxy*-4-*methylpentanoic acid*) (I; $x = 1$) (38%), $[\alpha]_D^{33.5}$ −118·6° (*c* 0·9 in $CHCl_3$), M_n 24,300 [Found: C, 63·6; H, 9·2; N, 6·5. $(C_{12}H_{21}NO_3)_n$ requires C, 63·4; H, 9·3; N, 6·2%]; *poly*-(O-*di*-L-*leucyl*-L-2-*hydroxy*-4-*methylpentanoic acid*) (I; $x = 2$) (45%), $[\alpha]_D^{33.5}$ −26·3° (*c* 0·8 in $CDCl_3$ containing 0·5% $CF_3{\cdot}CO_2H$), M_n 32,500 [Found: C, 62·7; H, 9·3; N, 8·4. $(C_{18}H_{32}N_2O_4)_n$ requires C, 63·5; H, 9·5; N, 8·2%]; *poly*-(O-*tri*-L-*leucyl*-L-2-*hydroxy*-4-*methylpentanoic acid*) (I; $x = 3$) (42%), $[\alpha]_D^{33.5}$ −41·6° (*c* 0·7 in $CHCl_3$), M_n 46,000 [Found: C, 64·1; H, 9·5; N, 9·3. $(C_{24}H_{43}N_3O_5)_n$ requires C, 63·6; H, 9·6; N, 9·3%].

Molecular Weights.—The polypeptolide (10 mg) was dissolved in deuteriochloroform (1 ml) and treated with triethylamine (10 μl), followed by dansyl chloride (20 mg). The mixture was stirred in subdued light for 14 h and then evaporated to dryness. The residue was washed repeatedly with methanol until the washings were free from dansyl derivatives (u.v.). The product was filtered off and dried. The fluorescence of a chloroform solution of known concentration was measured with an EIL filter fluorimeter (OX9A and OGr filters). The dansyl content was calculated from a calibration curve constructed from measurements on chloroform solutions of cyclohexylammonium dansyl-L-leucinate.

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