Polypeptides. Part XXV.[1] Synthesis of Isariin

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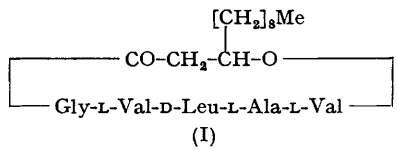
O-(Glycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-D- and L-3-hydroxydodecanoic acids (D- and L-isoisariic acids) have been synthesised and their cyclisation has been studied. D-Isoisariic acid was converted through its acid chloride into a cyclopeptolide indistinguishable from isariin, a metabolite of *Isaria cretacea*. High pressure liquid chromatography was necessary for the isolation of the product from the reaction mixture and facilitated comparison with the natural material. Attempts to cyclise D-isoisariic acid by other methods were unsuccessful.

THE preceding paper [1] described the synthesis of D-3-hydroxydodecanoylglycyl-L-valyl-D-leucyl-L-alanyl-L-valine and the establishment of its identity with isariic acid. Attempts to cyclise this compound to isariin (I), a cyclopeptolide from *Isaria cretacea*,[2] were unsuccessful; this was attributed to steric hindrance at the *C*-terminal valine residue. It was therefore apparent that a



(I)

successful synthesis of isariin would require closure of the ring between a different pair of residues.

There are two positions in the sequence of isariin which have particular merit in this respect; ring formation by activation of the carboxy-group of either the glycine or the 3-hydroxydodecanoic acid residue should proceed without racemisation. In the former residue there is no asymmetric centre, and in the latter the centre of chirality is in the position β to the carboxy-group and in general unaffected by carboxamide formation. We chose to investigate ring closure with the 3-hydroxydodecanoic acid in the *C*-terminal position. This route avoids the involvement of valine, albeit as *N*-terminal, in the cyclisation step, and is rather more economical in material as the most difficult step, synthesis of the peptolide bond, is accomplished before the peptide links are formed.

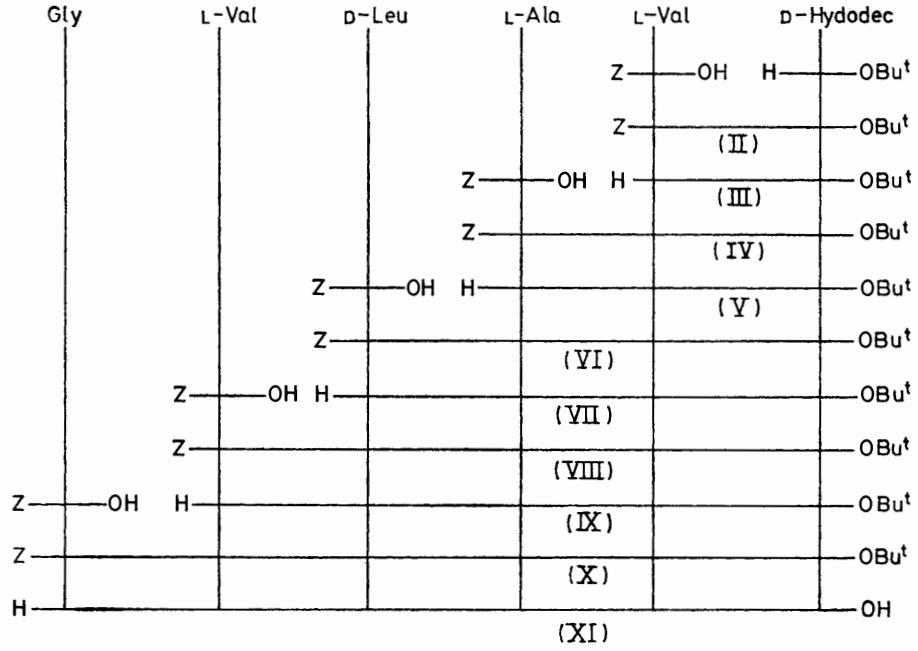
[1] Part XXIV, P. M. Hardy, R. A. Prout, and H. N. Rydon, preceding paper.

[2] L. C. Vining and W. A. Taber, *Canad. J. Chem.*, 1962, **40**, 1579.

Initially we attempted to make use of the pentapeptide *N*-benzyloxycarbonylglycyl-L-valyl-D-leucyl-L-valine t-butyl ester which was the basis of our synthesis of isariic acid.[1] The t-butyl ester was cleaved with anhydrous trifluoroacetic acid,[3] and the coupling of the resultant peptide acid with t-butyl or diphenylmethyl D-3-hydroxydodecanoate (the latter prepared by using diphenyldiazomethane[4]) was investigated. Since once again this reaction involves the carboxy-group of a *C*-terminal valine residue, yields were likely to be poor at best. The use of *NN'*-carbonyldi-imidazole[5] or benzenesulphonyl chloride[6] to effect ester formation gave little of the desired product; use of dicyclohexylcarbodi-imide[7] was more successful, but the yield (8·7% after chromatography on silica gel), in view of the amount of peptide available, made this route of little value.

Material for our successful cyclisation was therefore prepared by the route outlined in the Scheme. The t-butyl ester group was chosen for *C*-protection because of its proven compatibility with the *N*-benzyloxycarbonyl group.[1] t-Butyl D-3-hydroxydodecanoate was prepared from the hydroxy-acid by a three-step route involving *O*-acetylation, esterification with isobutene in the presence of sulphuric acid,[8] and selective saponification. Care was necessary in the last reaction to ensure that the temperature during the addition of alkali was kept below 10° or substantial β-elimination occurred. The overall yield in this synthesis (67%) was much superior to that obtained directly by the action of t-butyl iodide on the silver salt of the acid (18%). Formation of t-butyl *O*-(*N*-benzyloxycarbonyl-L-valyl)-D-3-hydroxydodecanoate (II) or its LL-diastereoisomer was examined by using the four methods of esterification hitherto found the most useful in forming peptolide bonds. *NN'*-Carbonyldi-imidazole[5] proved the most successful coupling reagent (59% yield); isobutyl chloroformate[9] (18% yield) was less satisfactory, giving principally the isobutyl ester of *N*-benzyloxycarbonyl-L-valine. Dicyclohexylcarbodi-imide[7] (11% yield) and benzenesulphonyl chloride[6] (6% yield) were even less useful in inducing the desired esterification. *NN'*-Carbonyldi-imidazole was therefore used for our larger-scale preparations.

The protected monodepsipentapeptide (X) was synthesised from the dipeptolide (II) by alternate catalytic hydrogenation and coupling with *N*-benzyloxycarbonyl-amino-acids by the mixed anhydride method.[9] Three of the intermediate protected peptolides [(II), (IV), and (VI)] could not be obtained in crystalline form. Although t-butyl (*O*-L-valyl)-D-3-hydroxydodecanoate (III) formed a crystalline hydrogen oxalate, the higher amino-esters of the series [(V), (VII), and (IX)] did not. Over five steps in the synthesis (III) ⟶ (VIII), then, we had no crystalline intermediates, although the compounds were chromatographically homogeneous. The



SCHEME Here and elsewhere abbreviations for amino-acid residues, *etc.*, are those recommended by I.U.P.A.C.–I.U.B. (*Biochem. J.*, 1972, **126**, 773)

[3] R. Schwyzer, W. Rittel, H. Kappeler, and B. Iselin, *Angew. Chem.*, 1960, **72**, 915.
[4] J. B. Miller, *J. Org. Chem.*, 1959, **24**, 560.
[5] H. A. Staab, *Chem. Ber.*, 1956, **89**, 1927.
[6] M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin, *Tetrahedron*, 1963, **19**, 581.
[7] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[8] R. Roeske, *J. Org. Chem.*, 1963, **281**, 1251.
[9] J. R. Vaughan and R. L. Osato, *J. Amer. Chem. Soc.*, 1951, **73**, 5553.

high yields obtained of two of the protected peptolides on coupling [(VI) 98·8% and (IV) 97·5%] undoubtedly reflect some solvent contamination of the oily products. However, this is unlikely to be more than 5% as the crystalline higher peptolide (X) was prepared in a yield of 91·5% under identical conditions. The mixed anhydride method of coupling [9] certainly proved to be very efficient in this series of compounds. Unlike our experience in the synthesis of isariic acid,[1] catalytic hydrogenolysis was as rapid for the higher members of the series as it was for the lower ones. The presence of acetic acid, absent in the earlier work, was no doubt a factor in this.

Treatment of the protected monodepsipentapeptide (X) with hydrogen bromide in acetic acid [10] stripped off both protecting groups to yield *O*-(glycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-D-3-hydroxydodecanoic acid (XI), which we have named D-isoisariic acid, as its crystalline hydrobromide. This linear peptolide was converted into its acid chloride with thionyl chloride; [11] i.r. spectroscopy showed the reaction to be complete after 3·5 h. This activated intermediate was treated with triethylamine in benzene at high dilution, and the neutral material subsequently isolated by solvent extraction. The crude product (26·8% calculated as monocyclic material) could not be adequately purified by simple low-pressure chromatography on a column of silica. However, reverse-phase high-pressure liquid chromatography (h.p.l.c.) on Bondapak-C_{18} Porasil B with acetonitrile–water (3 : 1 v/v) as eluant satisfactorily resolved the components. The major fraction had properties corresponding to those expected for *cyclo*-(*O*-glycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-D-3-hydroxydodecanoyl, and represented a yield of 19·2%.

A direct comparison of the properties of our synthetic *cyclo*-(D-isoisariic acid) with those of natural isariin was made possible by a generous gift of a sample of the latter by Professor L. C. Vining. This sample had not been rigorously purified, and was accordingly subjected to preparative h.p.l.c. The retention time of isariin was identical with that of our synthetic cyclopeptolide. Moreover, the two compounds showed no trace of resolution even on prolonged recycle when loaded as an equimolar mixture. For chromatographic and other comparison purposes an isomer of *cyclo*-(D-isoisariic acid) containing the L-3-hydroxydodecanoyl residue was synthesised by the route developed for the D-isomer. In this instance an 8·8% yield of cyclic material was obtained; the lower yield accords with previous work on the effects of D-residues on cyclisation.[12] This epimeric material proved to be readily separated from either our natural or synthetic isariin samples by h.p.l.c. Further evidence for the identity of our synthetic cyclopeptolide with isariin came from a comparison of their specific rotations, i.r. spectra, mass spectra, and *X*-ray powder photographs, all of which were closely similar.

Since our work began, a preliminary communication outlining a synthesis of isariin has been published.[13] This synthesis is complementary to ours in that an alternative point for cyclisation was chosen. Ring closure of a linear peptolide containing a *C*-terminal glycine *p*-nitrophenyl ester gave 23% of cyclopeptolide. In so far as comparisons are possible on the basis of the physical properties given in this communication, the two synthetic products are identical except for the specific rotations. Okada *et al.*[13] report $[\alpha]_D^{20}$ −2·7° (*c* 0·5 in $CHCl_3$), and state that natural isariin has an identical specific rotation. In their original paper on isariin Vining and Taber [2] do not record its optical rotation. We have found for natural isariin and our synthetic product, both purified by h.p.l.c., $[\alpha]_D^{24}$ −12·8° (*c* 0·5 in $CHCl_3$) and $[\alpha]_D^{22}$ −13·2° (*c* 0·3 in $CHCl_3$), respectively. The reason for the discrepancy between our rotations and that of Okada *et al.* is not entirely clear. Our sample of synthetic isariin after recrystallisation from aqueous ethanol but before h.p.l.c. had $[\alpha]_D^{24}$ −3·0° (*c* 0·5 in $CHCl_3$). This is similar to that reported by the Japanese workers; [13] it is possible to ascribe the lower rotation of their synthetic material to the presence of impurities, but since the crude natural sample we used had $[\alpha]_D^{23}$ −16·75° (*c* 0·4 in $CHCl_3$), this is unlikely to be the explanation for the low rotation recorded by them for the natural material, which, like ours, was obtained from Professor Vining.

Although we have explored the application of two other methods of cyclisation to D-isoisariic acid, neither of these proved a satisfactory alternative to the acid chloride method. Indeed, chromatography of the neutral products from both the mixed anhydride [9,14] and the *NN'*-carbonyldi-imidazole [5] method showed no material with properties comparable to those of isariin.

The success of the acid chloride method in our synthesis of isariin suggests that it should be a generally useful method for cyclising peptolides containing a *C*-terminal long straight-chain 3-hydroxyalkanoic acid residue.

EXPERIMENTAL

For general remarks, see preceding paper.[1]

Hydroxy-acid Derivatives.—Diphenylmethyl D-3-*hydroxydodecanoate*. A solution of D-3-hydroxydodecanoic acid [1] (1·00 g, 4·62 mmol) in ethyl acetate (20 ml) was warmed to 50° and a solution of diphenyldiazomethane [4] (1·15 g, 5·93 mmol) in ethyl acetate (10 ml) added in portions until the red colour persisted. The mixture was refluxed for 2 h and the solvent then evaporated off. The residue was triturated with light petroleum (10 ml; b.p. 60—80°), and the solvent decanted from the crystalline solid. Recrystallisation from light petroleum (b.p. 60—80°) gave the pure *ester* (1·42 g, 80%), m.p. 32—34°, $[\alpha]_D^{24}$ +3·4° (*c* 1·2 in

[10] D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 1952, **17**, 1564.
[11] M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov, *Tetrahedron Letters*, 1962, 301.
[12] P. M. Hardy, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, 1963, **19**, 95.
[13] K. Okada, Y. Kurosawa, and M. Hiramoto, *Tetrahedron Letters*, 1972, 2693.
[14] T. Wieland, J. Faesel, and W. Konz, *Annalen*, 1969, **722**, 197; F. Fahrenholz, H. Faulstich, and T. Wieland, *ibid.*, 1971, **743**, 83.

$CHCl_3$) (Found: C, 78·9; H, 9·4. $C_{25}H_{34}O_3$ requires C, 78·5; H, 9·0%).

t-Butyl D-3-*hydroxydodecanoate*. (a) via *the silver salt*. D-3-Hydroxydodecanoic acid (2·0 g, 9·25 mmol) was dissolved in an excess of concentrated ammonium hydroxide and the solution was then evaporated to dryness. The residue was dissolved in propan-2-ol and the solution evaporated nearly to dryness. The residue was then dissolved in water (20 ml) and silver nitrate (1·57 g) in water (10 ml) was added with stirring and the exclusion of light. The resulting suspension was stirred for a further 30 min. The precipitate was then filtered off, washed with water, and dried *in vacuo* overnight at 50° to give the silver salt (2·52 g, 84·5%), m.p. 145—153° (decomp.). The bulk of this material (2·00 g, 6·18 mmol) was suspended in dry ether (25 ml) and t-butyl iodide (0·81 ml, 6·79 mmol) was added dropwise in the dark. The mixture was stirred at 20° for 3 h, then the precipitate of silver iodide was filtered off and washed with ether, and the combined filtrates were evaporated. The crude product (0·60 g) was purified by chromatography on a column of silica (Merck Kieselgel G; 5·0 g; 15 × 2 cm) with benzene–ethyl acetate (9 : 1 v/v) as eluant. Evaporation of the first fraction to be eluted gave the oily t-butyl D-3-hydroxydodecanoate (0·30 g, 18%), which could not be redistilled without decomposition.

(b) via *the acetoxy-derivative*. To a stirred solution of D-3-hydroxydodecanoic acid (5·65 g, 0·026 mol) in dry pyridine (50 ml) at 4°, acetic anhydride (4·93 ml, 0·052 mol) was added slowly. After addition was complete the mixture was stirred at 0° for 30 min, and then at 20° for 24 h. The solvent was evaporated off and the residue triturated with light petroleum (25 ml; b.p. 60—80°), cooled to 0°, and filtered. Evaporation of the filtrate gave oily D-3-*acetoxydodecanoic acid* (5·98 g, 89%), which decomposed on attempted distillation; τ (60 MHz; $CDCl_3$) 1·4 (1H, s, D_2O-exchangeable, CO_2H), 4·67 (1H, complex β-CH), 7·25 (2H, d, *J* 6 Hz, α-CH_2), 7·80 (3H, s, acetoxy Me), 8·53br (16H, s, aliphatic CH_2), and 9·0 (3H, t, Me); $\nu_{max.}$ (film) 1740, 1715, 1230, and 1020 cm^{-1}.

A solution of D-3-acetoxydodecanoic acid (22·62 g, 0·088 mol) in dichloromethane (120 ml) containing sulphuric acid (0·82 ml) was cooled to −10°, and dry isobutene (250 ml) was added. The mixture was left in a sealed pressure bottle at 20° for 48 h, then cooled to −10°, and the bottle was opened. The excess of isobutene was allowed to evaporate, and the organic solution was washed with M-sodium hydrogen carbonate, water, and brine. The solution was then dried and evaporation gave *t-butyl* D-3-*acetoxydodecanoate* (25·62 g, 93%) as an undistillable syrup; τ (60 MHz; $CDCl_3$) 4·86 (1H, complex, β-CH), 7·60 (2H, d, *J* 6 Hz, α-CH_2), 8·00 (3H, s, acetoxy Me), 8·60 (9H, s, Bu^t), 8·70 (16H, s, aliphatic CH_2), and 9·14 (3H, t, Me); $\nu_{max.}$ (film) 1750, 1245, and 1160 cm^{-1}. Part of this material (14·67 g, 0·047 mol) was dissolved in methanol (30 ml) and cooled to 10°; to this stirred solution was added 2M-sodium hydroxide (25 ml) over 1 h at 10°. The mixture was stirred for a further 4 h at 20° before extraction of the resulting emulsion with ether (3 × 20 ml). The combined extracts were washed successively with water, M-sodium hydrogen carbonate, and brine, and then dried and evaporated. The residue was chromatographed on silica gel (Merck Kieselgel G; 200 g; 100 × 3·5 cm) with benzene–ethyl acetate (95·5 v/v) as eluant. Evaporation of the major peak gave oily *t-butyl* D-3-*hydroxydodecanoate* (10·3 g, 81%); τ (60 MHz; $CDCl_3$) 6·10 (1H, complex, β-CH), 6·90 (1H, s, D_2O-exchangeable, OH), 7·70 (2H, d, *J* 6 Hz, α-CH_2), 8·55 (9H, s, Bu^t), 8·75br (16H, s, aliphatic CH_2), and 9·15 (3H, d, Me); $\nu_{max.}$ (film) 3440, 1725, and 1150 cm^{-1}.

t-Butyl L-3-*hydroxydodecanoate*. L-3-Hydroxydodecanoic acid was treated in a similar way to its enantiomer to give the desired ester in an overall yield of 60%.

Couplings to Hydroxy-acids.—t-Butyl O-(N-*benzyloxycarbonyl*-L-*valyl*)-L- *and* D-3-*hydroxydodecanoates*. In many of the succeeding experiments the neutral products were isolated by solvent extraction. In this procedure a solution in an organic solvent (usually ethyl acetate) was washed in succession with 2M-hydrochloric acid, water, M-sodium hydrogen carbonate, water, and brine, then dried and evaporated.

(i) *Using dicyclohexylcarbodi-imide*.[7] *N*-Benzyloxycarbonyl-L-valine (0·92 g, 3·67 mmol) in ether (2 ml) was added dropwise to a stirred solution of t-butyl D-3-hydroxydodecanoate (1·0 g, 3·67 mmol), dicyclohexylcarbodi-imide (0·83 g, 4·04 mmol), and pyridine (0·59 ml, 7·34 mmol) in ether (3 ml) at 0°. The mixture was stirred for 30 h at 4°, and a few drops of acetic acid were added. After 10 min the mixture was filtered and the neutral products were isolated by solvent extraction (the initial 2M-hydrochloric acid wash was omitted). The oily residue was taken up in a little acetone; the solution was kept for 4 h at −10°, then filtered and evaporated. The syrup remaining was purified by chromatography on a column of silica (Merck Kieselgel G; 20 g; 30 × 3 cm) with benzene–ethyl acetate (9 : 1; v/v) as eluant to give the oily LD-*peptolide* (II) (0·20 g, 11%), identified by its n.m.r. and i.r. spectra (details given in the *NN'*-carbonyldi-imidazole method below). A second major fraction was N-*benzyloxycarbonyl*-L-*valyl*-NN'-*dicyclohexylurea* (0·67 g, 36%), m.p. 130—132°; τ [100 MHz; $(CD_3)_2SO$] 1·86 (1H, d, D_2O-exchangeable, *J* 8 Hz, NH), 2·70 (5H, s, Ph), 5·02 (2H, s, benzyl CH_2), 5·78 (1H, complex, D_2O addition gives d, *J* 8 Hz, α-CH), 6·06 (1H, complex, cyclohexyl-CH), 6·58 (1H, complex, cyclohexyl CH), 8·34br (11H, s, side-chain CH, cyclohexyl CH_2), 8·82br (10H, s, cyclohexyl CH_2), and 9·22 (6H, d, Me_2 of Pr^i); $\nu_{max.}$ (KBr) 3295, 1738, 1695, 1670, 1642, and 732 cm^{-1}.

(ii) *Using isobutyl chloroformate*.[8] *N*-Benzyloxycarbonyl-L-valine (0·92 g, 3·67 mmol) was dissolved in dry tetrahydrofuran (10 ml) containing triethylamine (0·52 ml, 3·67 mmol) and the solution cooled to −10° before addition of isobutyl chloroformate (0·48 ml, 3·67 mmol). This mixed anhydride solution was kept at −10° for 10 min and a solution of t-butyl L-3-hydroxydodecanoate (1·0 g, 3·67 mmol) in dry tetrahydrofuran (3 ml) was then added dropwise over 20 min. The mixture was left overnight at 20° and filtered, and the filtrate was evaporated. Work-up by solvent extraction gave a crude neutral product (2·42 g) which was purified by chromatography on silica gel (Merck Kieselgel G; 50 g; 60 × 3 cm). Elution with benzene–ethyl acetate (95 : 5 v/v) gave as a minor fraction the oily LL-*peptolide* (0·49 g, 18%); τ (100 MHz; $CDCl_3$) 2·64 (6H, s, Ph, NH), 4·88 (3H, s, benzyl CH_2, β-CH of hydroxy-acid), 5·70 (1H, complex, α-CH), 7·60 (2H, d, *J* 6 Hz, α-CH_2 of hydroxy-acid), 7·86 (1H, complex, side-chain CH), 8·54 (9H, s, Bu^t), 8·74 (16H, s, aliphatic CH_2s), and 9·08 (9H, q, Me_2 of Pr^i); $\nu_{max.}$ (film) 1735, 840, and 695 cm^{-1}. The oily major fraction (1·71 g) was dissolved in methanol (10 ml) containing acetic acid (0·1 ml) and hydrogenated over palladised charcoal (5%; 0·5 g) for 2 h. The catalyst was filtered off and the solution evaporated. A portion of

the residue dissolved in diethyl ether was treated with anhydrous oxalic acid in diethyl ether. The precipitated product was filtered off and dried to give the *hydrogen oxalate* of L-*valine isobutyl ester*, m.p. 128—130°; τ [100 MHz; $(CD_3)_2SO$] 2·40 (4H, s, D_2O-exchangeable, CO_2H, $\overset{+}{N}H_3$), 6·26 (2H, d, ester CH_2), 6·32 (1H, d, Val α-CH), 7·94 (2H, complex, 2β-CHs), and 9·06 (12H, q, 4 × CH_3). Another portion of the product of hydrogenolysis was dissolved in dry pyridine (1 ml) and treated with acetic anhydride (1 ml). After 3 h at 20° the solution was evaporated to give an oily product (Found *m/e*, 215. *N*-Acetyl-L-valine isobutyl ester, $C_{11}H_{21}NO_3$, requires *M*, 215). The major fraction is therefore confirmed as being the isobutyl ester.

(iii) *Using benzenesulphonyl chloride.*[6] Benzenesulphonyl chloride (0·52 ml, 4·04 mmol) was added dropwise to a solution of *N*-benzyloxycarbonyl-L-valine (1·11 g, 4·40 mmol) in pyridine (10 ml) at 0°. The solution was stirred at 0° for 10 min before adding to its t-butyl L-3-hydroxydodecanoate (1·0 g, 3·67 mmol). The mixture was kept at 0° for 2 h and then at 20° for 24 h before evaporation. The neutral products were isolated by solvent extraction and purified by column chromatography as in method (ii) to give the oily LL-peptolide (0·12 g, 6·4%), whose properties were similar to those of that prepared by method (ii).

(iv) *Using* NN′-*carbonyldi-imidazole.*[5] A solution of *N*-benzyloxycarbonyl-L-valine (1·33 g, 5·31 mmol) and *NN′*-carbonyldi-imidazole (0·90 g, 5·58 mmol) in dry tetrahydrofuran (3 ml) was stirred under anhydrous conditions at 20° for 30 min. Sodium (0·2 g) and imidazole (2·0 g) were dissolved in tetrahydrofuran (25 ml) and a portion of this solution (1·3 ml) was added, together with t-butyl D-3-hydroxydodecanoate (1·45 g, 5·31 mmol). The mixture was stirred overnight at 20° and evaporated, and the neutral products were isolated by solvent extraction. Column chromatography on silica gel was carried out as in method (ii) to give the oily LD-*peptolide* (II) (1·58 g, 58·8%); τ [100 MHz; $(CD_3)_2SO$] 2·40 (1H, d, D_2O-exchangeable, *J* 8 Hz, NH), 2·64 (5H, s, Ph), 4·96 (3H, s, benzyl CH_2, β-CH of hydroxy-acid), 6·04 (1H, complex, α-CH), 8·0 (1H, complex, side-chain CH), 8·62 (9H, s, Bu^t), 8·76 (16H, s, aliphatic CH_2), and 9·12 (9H, d, side-chain Me); $\nu_{max.}$ (film) 3410 and 1745 cm^{-1}. t-Butyl L-3-hydroxydodecanoate was treated in a similar way to give the LL-*peptolide* (68·9%) as an oil with properties similar to those described for the product from method (ii).

Oligopeptolide Synthesis.—*t-Butyl* O-L-*valyl*-D-3-*hydroxydodecanoate* (III). A solution of t-butyl *O*-(*N*-benzyloxycarbonyl-L-valyl)-D-3-hydroxydodecanoate (1·58 g, 3·13 mmol) in methanol (15 ml) containing acetic acid (0·18 ml) was hydrogenated (22°; 760 mmHg) over palladium–charcoal (10%; 0·2 g) for 4 h. The catalyst was then filtered off and the filtrate evaporated. The residue was dissolved in ethyl acetate and the solution washed with M-sodium hydrogen carbonate, dried, and evaporated to give the oily *product* (1·02 g, 87·8%). This was dissolved in diethyl ether (5 ml) and mixed with a solution of anhydrous oxalic acid (0·25 g, 2·75 mmol) in diethyl ether (5 ml). The precipitate was recrystallised from ethereal ethanol to give the *hydrogen oxalate* of (III) (1·22 g, 96·3%), m.p. 135—138°, $[\alpha]_D^{23}$ −5·0° (*c* 1·20 in MeOH); $\nu_{max.}$ (KBr) 1735 cm^{-1} (Found: C, 60·0; H, 9·7; N, 3·2. $C_{23}H_{43}NO_8$ requires C, 59·85; H, 9·4; N, 3·0%).

t-Butyl O-L-*valyl*-L-3-*hydroxydodecanoate.* This compound was prepared from the *N*-benzyloxycarbonyl derivative (2·21 g, 5·94 mmol) in the same way as its diastereoisomer and converted into the *hydrogen oxalate* (1·33 g, 95·5% overall yield), m.p. 145—148°, $[\alpha]_D^{23}$ +7·8° (*c* 1·15 in MeOH) (Found: C, 60·0; H, 9·7; N, 3·2. $C_{23}H_{43}NO_8$ requires C, 59·85; H, 9·4; N, 3·0%).

t-Butyl O-(N-*benzyloxycarbonyl*-L-*alanyl*-L-*valyl*)-D-3-*hydroxydodecanoate* (IV). Isobutyl chloroformate (2·28 ml, 17·4 mmol) was added to a stirred solution of *N*-benzyloxycarbonyl-L-alanine (3·88 g, 17·4 mmol) and triethylamine (2·42 ml, 17·4 mmol) in anhydrous tetrahydrofuran (20 ml) at −10°. The mixture was stirred at −10° for 10 min before a solution of t-butyl *O*-L-valyl-D-3-hydroxydodecanoate (6·45 g, 17·4 mmol; obtained by washing an ethereal solution of the hydrogen oxalate with M-sodium hydrogen carbonate) in tetrahydrofuran (10 ml) was added over 20 min. The mixture was kept overnight at 20°, then evaporated, and the neutral product was isolated by solvent extraction to yield the chromatographically pure oily *monodepsidipeptide* (IV) (9·76 g, 97·5%); τ (100 MHz; $CDCl_3$) 2·62 (5H, s, Ph), 3·46 [1H, d, D_2O-exchangeable, $NH_{(2)}$], 4·50 [1H, d, D_2O-exchangeable, *J* 8 Hz, $NH_{(1)}$], 4·80 (1H, complex, β-CH of hydroxy-acid), 4·88 (2H, s, benzyl CH_2), 5·50 (1H, complex, α-CH), 5·72 (1H, complex α-CH), 7·52 (2H, d, *J* 6 Hz, α-CH_2 of hydroxy-acid), 8·40 (1H, complex, side-chain CH), 8·60 (12H, s, Bu^t, Ala-Me), 8·74 (16H, s, aliphatic CH_2), and 9·10 (9H, t, side-chain Me); $\nu_{max.}$ (film) 3380, 1735, 1670, and 1535 cm^{-1}.

t-Butyl O-(N-*benzyloxycarbonyl*-L-*alanyl*-L-*valyl*)-L-3-*hydroxydodecanoate.* t-Butyl *O*-L-valyl-L-2-hydroxydodecanoate (6·90 g, 18·6 mmol) was treated exactly as its diastereoisomer to yield the oily LLL-*monodepsidipeptide* (10·55 g, 98·5%), with spectroscopic properties similar to those of the LLD-compound.

t-Butyl O-(N-*benzyloxycarbonyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-D-3-*hydroxydodecanoate* (V). t-Butyl *O*-(*N*-Benzyloxycarbonyl-L-alanyl-L-valyl)-D-3-hydroxydodecanoate (9·95 g, 17·3 mmol) was hydrogenated under the conditions described for t-butyl *O*-(*N*-benzyloxycarbonyl-L-valyl)-D-3-hydroxydodecanoate. The catalyst was then filtered off and the solution evaporated. A solution of the residue in ethyl acetate was washed with M-sodium hydrogen carbonate, dried, and evaporated to yield the oily *amino-ester* (IV) (6·67 g, 87·1%) (amino-acid ratio Ala, 1·00; Val, 1·08). Attempts to form a crystalline hydrogen oxalate of this compound were unsuccessful. The bulk of this product (V) (6·27 g, 14·2 mmol) was coupled with *N*-benzyloxycarbonyl-D-leucine (3·76 g, 14·2 mmol) by the mixed anhydride method as described for the monodepsidipeptide (IV). Work-up by solvent extraction gave the oily *monodepsitripeptide* (VI) (9·90 g, 99%) (amino-acid ratio Ala, 0·92; Val, 1·00; Leu, 1·04).

t-Butyl O-(N-*benzyloxycarbonyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-L-3-*hydroxydodecanoate.* Hydrogenation of t-butyl *O*-(*N*-benzyloxycarbonyl-L-alanyl-L-valyl)-L-3-hydroxydodecanoate (10·55 g, 18·29 mmol) gave the corresponding *amino-ester* (7·58 g, 93·7%) as an oil (amino-acid ratio Ala, 1·00; Val, 1·08). The bulk of this material (7·00 g, 15·8 mmol) was coupled with *N*-benzyloxycarbonyl-D-leucine as described for its epimer (V) to give the required *monodepsitripeptide* (10·83 g, 97%) as an oil (amino-acid ratio Ala, 0·97; Val, 1·00; Leu, 1·09).

t-Butyl O-(N-*benzyloxycarbonyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-D-3-*hydroxydodecanoate* (VIII). t-Butyl *O*-(*N*-benzyloxycarbonyl-D-leucyl-L-alanyl-L-valyl)-D-3-hydroxy-

dodecanoate (V) (9·40 g, 13·3 mmol) was hydrogenated as described for its peptolide precursors. Evaporation afforded the oily amino-ester (VII) (6·51 g, 85·5%). A sample of this product (0·5 g) was converted into the hydrogen oxalate (0·5 g, 0·87 mmol), but this was a sticky solid of indefinite m.p. (59—72°). The bulk of this product (5·1 g, 8·7 mmol) was therefore coupled directly with *N*-benzyloxycarbonyl-L-valine (2·24 g, 8·9 mmol) by the mixed anhydride method as described earlier in this section. Solvent extraction and crystallisation from aqueous methanol gave the *monodepsitetrapeptide* (VIII) (5·04 g, 72%), m.p. 128—130°, $[\alpha]_D^{25}$ +10·7° (*c* 1·0 in $CHCl_3$) (Found: C, 65·6; H, 9·9; N, 7·1. $C_{43}H_{72}N_4O_9$ requires C, 65·45; H, 9·2; N, 7·1%) (amino-acid ratio Ala, 1·00; Val, 1·99; Leu, 1·08).

t-Butyl O-(N-*benzyloxycarbonyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-L-3-*hydroxydodecanoate*. This compound was prepared in an analogous manner to its epimer (VIII) *via* the oily t-butyl *O*-(D-leucyl-L-alanyl-L-valyl)-L-3-hydroxydodecanoate (93·5%), which formed a hydrogen oxalate salt of indefinite m.p. (49—63°). Crystallisation from aqueous methanol gave the desired pure *protected monodepsitetrapeptide* (6·74 g, 87·7%), m.p. 133—136°, $[\alpha]_D^{25}$ +14·1° (*c* 1·0 in $CHCl_3$) (Found: C, 65·25; H, 9·4; N, 7·0. $C_{43}H_{72}N_4O_9$ requires C, 65·45; H, 9·2; N, 7·1%) (amino-acid ratio Ala, 0·94; Val, 2·10; Leu, 1·00).

t-Butyl O-(N-*benzyloxycarbonylglycyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-D-3-*hydroxydodecanoate* (X). t-Butyl *O*-(*N*-benzyloxycarbonyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-D-3-hydroxydodecanoate (VIII) (4·60, 5·83 mmol) was hydrogenated in the usual way to give the oily amino-ester (IX) (3·43 g, 90%). The bulk of this material (3·03 g, 4·63 mmol) was coupled with *N*-benzyloxycarbonylglycine (0·97 g, 4·63 mmol) by using isobutyl chloroformate in the manner previously described. The usual work-up gave material which crystallised from aqueous methanol to give the pure *monodepsipentapeptide* (X) (3·58 g, 91·5%), m.p. 182—185°, $[\alpha]_D^{25}$ +8·9° (*c* 1·0 in $CHCl_3$) (Found: C, 62·5; H, 9·2; N, 8·0. $C_{45}H_{75}N_5O_{10},H_2O$ requires C, 62·55; H, 9·0; N, 8·1%) (amino-acid ratio Gly, 1·00; Ala, 0·96; Val, 2·10; Leu, 1·08).

t-Butyl O-(N-*benzyloxycarbonylglycyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-L-3-*hydroxydodecanoate*. t-Butyl *O*-(*N*-benzyloxycarbonyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-L-3-hydroxydodecanoate (6·15 g, 7·8 mmol) was hydrogenated in the same way as its epimer (VIII) to give the corresponding amino-ester (4·75 g, 93·2%) as an oil. The bulk of this material (4·35 g, 6·64 mmol) was coupled with *N*-benzyloxycarbonylglycine in the usual manner. Crystallisation from aqueous methanol gave the desired *monodepsipentapeptide* (5·12 g, 91%), m.p. 184—186°, $[\alpha]_D^{25}$ +10·0° (*c* 1·0 in $CHCl_3$) (Found: C, 63·6; H, 9·5; N, 7·9. $C_{45}H_{75}N_5O_{10}$ requires C, 63·9; H, 8·9; N, 8·3%) (amino-acid ratio Gly, 0·94; Ala, 0·97; Val, 2·01; Leu, 1·00).

O-(*Glycyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-D-3-*hydroxydodecanoic acid* (XI). t-Butyl *O*-(*N*-benzyloxycarbonylglycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-D-3-hydroxydodecanoate (X) (0·846 g, 1 mmol) was dissolved in hydrogen bromide in acetic acid (45%; 10 ml) and the solution was kept at 20° for 1 h. The solvent was evaporated off, the residue was shaken with light petroleum (b.p. 60—80°), and the solvent was again removed. The residue was kept over sodium hydroxide pellets at 0·1 mmHg for 12 h. Crystallisation from chloroform–light petroleum (b.p. 60—80°) gave the crystalline *hydrobromide* of (XI) (0·69 g, 95%), m.p. 112—115°, $[\alpha]_D^{23}$ −17·7° (*c* 1·0 in $CHCl_3$); τ [100 MHz; $(CD_3)_2SO$] 1·34—2·04 [7H, complex, D_2O-exchangeable, NH_3^+, NH_{1-4}], 4·86 (1H, complex, β-CH of hydroxy-acid), 5·56 (3H, complex, α-CH), 5·80 (1H, complex, α-CH), 6·34 (2H, d, Gly-CH_2), 8·0 (3H, complex, side-chain *CH*), 8·50 (2H, complex side-chain CH_2), 8·76br (19H, s, aliphatic CH_2, Ala-Me), and 9·16 (21H, d, side-chain Me), $\nu_{max.}$ (KBr) 1735, 1655, and 1535 cm^{-1} (Found: C, 53·9; H, 8·95; N, 9·5. $C_{33}H_{62}BrN_5O_8$ requires C, 53·8; H, 8·5; N, 9·5%) (amino-acid ratio Gly, 1·00; Ala, 1·11; Val, 1·88; Leu, 1·04).

O-(*Glycyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-L-3-*hydroxydodecanoic acid hydrobromide*. t-Butyl *O*-(*N*-benzyloxycarbonylglycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-L-3-hydroxydodecanoate (0·846 g, 1 mmol) was treated exactly as the D-isomer (X) to give the crystalline *hydrobromide* (0·69 g, 85%), m.p. 125—130°, $[\alpha]_D^{21}$ +10·1° (*c* 1·2 in $CHCl_3$) (Found: C, 52·5; H, 8·55; N, 9·9. $C_{33}H_{62}BrN_5O_8,H_2O$ requires C, 52·5; H, 8·55; N, 9·3%) (amino-acid ratio Gly, 0·94; Ala, 0·94; Val, 2·01; Leu, 1·00). The n.m.r. and i.r. spectra resembled those of the D-isomer.

O-(N-*Benzyloxycarbonylglycyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-L-3-*hydroxydodecanoic acid*. t-Butyl *O*-(*N*-benzyloxycarbonylglycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-L-3-hydroxydodecanoate (0·85 g, 1 mmol) was dissolved in anhydrous trifluoroacetic acid (1·5 ml) and kept for 3 h at 20°. The solution was then evaporated, a little toluene added, and the solvent again removed. The residual material was kept over potassium hydroxide pellets for 6 h at 0·1 mmHg and then triturated with light petroleum (b.p. 60—80°). Filtration gave the N-*benzyloxycarbonylpeptolide* (0·76 g, 96·2%), m.p. 155—157°, $[\alpha]_D^{25}$ +9·5° (*c* 1·05 in $CHCl_3$) (Found: C, 62·7; H, 8·9; N, 8·6. $C_{41}H_{67}N_5O_{10}$ requires C, 62·3; H, 8·55; N, 8·9%).

Cyclisation of D- *and* L-*Isoisariic Acid*.—cyclo-O-(*Glycyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-D-3-*hydroxydodecanoyl*. *O*-(Glycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-D-3-hydroxydodecanoic acid hydrobromide (XI) (0·5 g, 0·68 mmol) was dissolved in thionyl chloride [10] (6·8 ml). I.r. spectroscopy showed reaction to be complete after 3·5 h at 20°. After 4 h at 20° the solution was evaporated and the residue triturated with a little toluene. The toluene was then evaporated off, and this solvent flushing was repeated twice more. The residual slightly yellow powder was dissolved in dry benzene (68 ml) and this solution added simultaneously with a solution of triethylamine (0·14 ml, 1·43 mmol) in dry benzene (68 ml) to dry benzene (1·35 l) with stirring over 5 h at 20°. The mixture was kept overnight at 20° and then evaporated. The residue was dissolved in chloroform and the neutral material isolated by solvent extraction. The oil obtained on evaporation was triturated with light petroleum (b.p. 40—60°), the solvent was decanted, and the solid product was crystallised from ethyl acetate. Chromatography of this crude material on silica gel (Merck Kieselgel G; 20 g; 30 × 3 cm) with benzene–ethyl acetate (9 : 1 v/v) as eluant and crystallisation from aqueous ethanol gave the *cyclopeptolide* (0·12 g, 26·8%), m.p. 229—232°, $[\alpha]_D^{24}$ −3·0° (*c* 0·5 in $CHCl_3$). Further fractionation by h.p.l.c. (system described later in this Experimental section) gave, as the third peak eluted, pure cyclo-O-(*glycyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-D-3-*hydroxydodecanoyl* (83 mg, 19·2%), m.p. 249·5—250·5°, $[\alpha]_D^{22}$ −13·2° (*c* 0·3 in $CHCl_3$); $\nu_{max.}$ (KBr) 3320br, 3060, 2960sh, 2928, 2870, 2855, 1735, 1650br, 1530br, 1467, 1445sh, 1338br, 1310, 1275, 1235, and 1190 cm^{-1} (Found:

C, 62·2; H, 9·5; N, 10·7%; *m/e* 637. $C_{33}H_{59}N_5O_7$ requires C, 62·1; H, 9·3; N, 11·0%; *M*, 637) (amino-acid ratio Gly, 1·01; Ala, 1·02; Val, 1·99; Leu, 1·00).

cyclo-O-(*Glycyl*-L-*valyl*-D-*leucyl*-L-*alanyl*-L-*valyl*)-L-3-*hydroxydodecanoyl*. *O*-(Glycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-L-3-hydroxydodecanoic acid hydrobromide (0·50 g, 0·68 mmol) was treated exactly as the D-isomer to give after h.p.l.c. the desired *cyclopeptolide* (19·1 mg, 8·82%), m.p. 120—125°, $[\alpha]_D^{22}$ −5·8° (*c* 0·2 in $CHCl_3$) (Found: C, 62·0; H, 9·8; N, 10·7%; *m/e*, 637. $C_{33}H_{59}N_5O_7$ requires C, 62·1; H, 9·3; N, 11·0%; *M*, 637) (amino-acid ratio Gly, 1·09; Ala, 1·00; Val, 1·94; Leu, 1·10). The i.r. spectrum was closely similar to that of the D-isomer.

Purification and Physical Properties of Natural Isariin.—Impure natural isariin (60 mg), $[\alpha]_D^{23}$ −16·75° (*c* 0·4 in $CHCl_3$), a sample generously made available by Professor L. C. Vining, was purified by h.p.l.c. to yield, as the fourth peak eluted, pure isariin (26 mg), m.p. 249—250°, $[\alpha]_D^{24}$ −12·8° (*c* 0·5 in $CHCl_3$) (Found: C, 62·0; H, 9·7; N, 10·7%; *m/e*, 637. $C_{33}H_{59}N_5O_7$ requires C, 62·1; H, 9·3; N, 11·0%; *M*, 637) (amino-acid ratio Gly, 1·08; Ala, 1·02; Val, 1·99; Leu, 1·00). The i.r. spectrum was identical with that of *cyclo-O*-(glycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-D-3-hydroxydodecanoyl.

Other Attempted Cyclisations of D-*Isoisariic Acid*.—(i) *Mixed anhydride method*.[14] A solution of *O*-(glycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-D-3-hydroxydodecanoic acid (XI) hydrobromide (100 mg, 0·136 mmol) in dry tetrahydrofuran (120 ml) was cooled to −15° and ethyl chloroformate (0·065 ml, 0·68 mmol) was added. The solution was stirred for 15 min at −15°, triethylamine (0·12 ml, 0·84 mmol) was added, and the mixture was stirred for 4 h at −15° and subsequently overnight at 20°. The solvent was evaporated off and the neutral product (50 mg), isolated by solvent extraction, was triturated with methanol. The resulting suspension was filtered and the filtrate examined by h.p.l.c. No peak with an elution volume corresponding to isariin was obtained.

(ii) NN′-*Carbonyldi-imidazole method*.[5] *NN*′-Carbonyldiimidazole (66 mg, 0·41 mmol) was added to a solution of *O*-(glycyl-L-valyl-D-leucyl-L-alanyl-L-valyl)-D-3-hydroxydodecanoic acid (100 mg, 0·136 mmol) in dry tetrahydrofuran (120 ml) at 20°. This solution was kept for 30 min at 20°, triethylamine (0·019 ml, 0·136 mmol) was added, and the mixture was stirred overnight at 20°. The solvent was then evaporated off and the neutral material (72 mg) isolated by solvent extraction. This crude product was triturated with methanol, the resulting suspension was filtered, and the filtrate was examined by h.p.l.c. The elution volumes of the components clearly distinguished them from isariin.

High-pressure Liquid Chromatograph.—The system previously described [1] was used in all cases. The following elution volumes (in ml) were observed for preparative column purification of products obtained from D-isoisariic acid:

Acid chloride method	60, 172, and 196
Mixed anhydride method	76 and 128
NN′-Carbonyldi-imidazole method	36, 67, and 110
[Isariin (pure)	196]
[Isariin (crude)	104, 124, 140, and 196]

X-*Ray Powder Photographs*.—Samples of natural and synthetic isariin were mounted on glass fibres with the aid of collodion. These fibres were exposed to Cu-K_α (Ni-K_β filtered) radiation in a Debye–Scherrer camera (diam. 114·6 mm) with continuous rotation of the specimen. Exposures of 24 h were necessary to obtain adequate intensities. *cyclo*-(L-Isoisariic acid) gave a very diffuse pattern which was not comparable to the other photographs. Debye–Scherrer reflexes (λ 1·542 Å) for isariin samples were as follows: natural isariin 22·45, 17·05w, 14·41, 12·90w, 11·95w, 8·98s, 8·50w, and 7·64w Å; synthetic isariin 22·52, 16·95w, 14·32, 13·05w, 12·13w, 8·90s, 8·45w, and 7·53w Å.

Mass Spectra.—The mass spectra of natural isariin, synthetic isariin, and *cyclo*-(L-isoisariic acid) were virtually identical, and closely resembled the published mass spectrum of natural isariin.[1]

We thank Mr. B. Sheldon of the Physics Department for taking the powder photographs and Dr. D. H. G. Crout for assistance with high-pressure liquid chromatography. We are greatly indebted to Professor L. C. Vining for a generous gift of isariin.

[3/2356 *Received*, 16*th November*, 1973]