Amino-acids and Peptides. Part VI.[1] The Synthesis, by Twinning, of Cyclodepsipeptides Related to Serratamolide

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A fourteen-membered cyclodepsipeptide has been synthesised by the interaction of two molecules of β-L-leucyloxypropionyl chloride. A similar synthesis utilising β-(*O*-t-butyl-DL-seryloxy)propionyl chloride gave two isomers. The relationship of these isomers has been discussed.

THE antibiotic serratamolide, a metabolite of *Serratia marcescens*, is the simplest of the naturally-occurring cyclodepsipeptides. The structure (IV) has been established both by degradation [2] and by an unambiguous synthesis utilising an interesting hydroxy-acyl incorporation reaction.[3] This involved acylation of *OO'*-diacetyl-L-seryl-L-seryl-lactam (II) with D-β-benzyloxydecanoyl chloride, hydrogenolysis to give the diacyl-derivative (III) followed by hydroxyl-amide interaction and rearrangement to give *OO'*-diacetylserratamolide which could be hydrolysed to the antibiotic.[4]

Shemyakin has proposed [5] that the biosynthesis of serratamolide may follow a similar course, starting from L-seryl-L-seryl-lactam (I). However, another biosynthetic route is an attractive possibility. The fourteen-membered ring could be derived through the condensation of two identical fragments, either the linear ester (IX) or, more likely, the amide (XV). The latter alternative is favoured by the observation that this linear amide, serratamic acid,[6] occurs with serratamolide in cultures of *Serratia marcescens*. Other symmetrical cyclic peptides and depsipeptides, such as fungisporin,[7]

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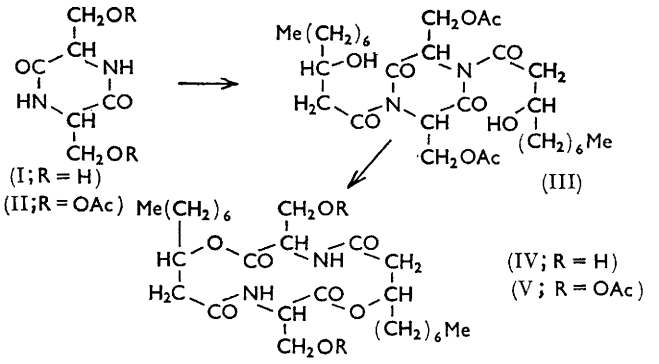
[2] H. H. Wasserman, J. J. Keggi, and J. E. McKeon, *J. Amer. Chem. Soc.*, 1962, **84**, 2978.
[3] M. M. Shemyakin, Yu. A. Ovchinnikov, V. K. Antonov, A. A. Kiryushkin, V. T. Ivanov, V. I. Shchelokov, and A. M. Shkrob, *Tetrahedron Letters*, 1964, 47.
[4] V. K. Antonov, V. I. Shchelokov, M. M. Shemyakin, I. I. Tovarova, and O. A. Kiseleva, *Antibiotiki*, 1965, 387; M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, V. I. Shchelokov, and Z. E. Agadzhanyan, *Tetrahedron*, 1965, **21**, 3537.
[5] V. K. Antonov, A. M. Shkrob, V. I. Shchelokov, and M. M. Shemyakin, *Tetrahedron Letters*, 1963, 1353.
[6] N. J. Cartwright, *Biochem. J.*, 1957, **67**, 663.
[7] K. Miyao, *Bull. Agr. Chem. Soc. Japan*, 1960, **24**, 23.

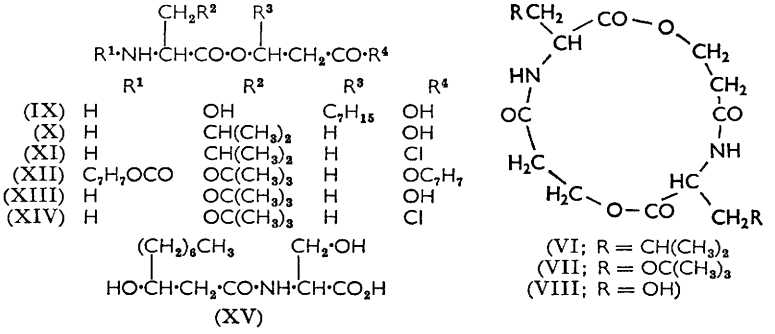
echinomycin,[8] and amidomycin,[9] could also be synthesised, in nature, by twinning but there is, as yet, no direct evidence of this.



The formation of a cyclic peptide by dimerisation was first observed in 1955 [10] when attempts to cyclise linear tripeptides gave rise to cyclohexapeptides. Following this, Schwyzer found that whereas esters of tetraglycine cyclised normally, but in small yield, to give cyclotetraglycyl, triglycine esters gave cyclohexaglycyl.[11]

process, it was proposed that initially two molecules of the linear peptide associated through hydrogen-bonding to give intermediates related in structure to the Pauling–Corey antiparallel pleated sheets; [17] this was followed by reaction of the suitably oriented terminal groupings to give the cyclic product. Later, the observation that simple linear depsipeptides could be dimerised, and that twinning of peptides occurred in polar media which did not favour the formation of intermolecular hydrogen bonds,[18] indicated that the role of hydrogen-bonding had been overemphasised. It seems likely that the conformation of the transition state and of the cyclic dimeric product are important, perhaps determining, in the formation of the cyclic dimer. Consequently, we have attempted the synthesis, by twinning, of cyclodepsipeptides related to serratamolide.

Fourteen-membered cyclodepsipeptides have been prepared in good yield by self-condensation of the acid chlorides (XI) and (XIV). The first of these intermediates was obtained from the linear ester, β-L-leucyloxypropionic acid (X) which was prepared by the interaction of *N*-benzyloxycarbonyl-L-leucine and benzyl



Subsequently, it was shown that cyclohexa-, cycloocta-, cyclodeca-, and cyclododeca-peptides could arise by means of this twinning process using a variety of procedures.[12–14] Likewise, formation of eighteen and twenty-four membered cyclodepsipeptides has been observed as a result of cyclodimerisation.[12b,15] In the first attempts by Schwyzer [16] to explain the twinning

β-diazopropionate, or through condensation of a mixture of *N*-benzyloxycarbonyl-L-leucine and benzyl β-hydroxypropionate using *NN'*-dicyclohexylcarbodiimide (DCCI), followed by removal of protecting groups. The cyclodepsipeptide (VI) was formed in good yield when triethylamine was added to a dilute solution of the chloride (XI) in benzene.* The crystalline product, $C_{18}H_{30}N_2O_6$, exhibited amide I, amide II and ester bands in the infrared spectrum. The mass spectrum, which has been discussed in a recent Communication,[1] confirmed the structure (VI). Two cyclodepsipeptides (VII) and (VIII) incorporating seryl residues have been synthesised

* A preliminary account of this work has already been published.[19]

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[9] L. C. Vining and W. A. Taber, *Canad. J. Chem.*, 1957, **35**, 1109.

[10] J. C. Sheehan, M. Goodman, and W. L. Richardson, *J. Amer. Chem. Soc.*, 1955, **77**, 6391; C. H. Bamford and F. J. Weymouth, *ibid.*, p. 6368.

[11] R. Schwyzer, B. Iselin, W. Rittel, and P. Sieber, *Helv. Chim. Acta*, 1956, **39**, 872.

[12] (*a*) R. Schwyzer, *Chimia* (*Switz.*), 1958, **12**, 53; R. Schwyzer, P. Sieber, and B. Gorup, *ibid.*, p. 90; (*b*) R. Schwyzer, J. P. Carrión, B. Gorup, H. Nolting, and Aung Tun-Kyi, *Helv. Chim. Acta*, 1964, **47**, 441, and references cited therein.

[13] J. C. Sheehan and D. N. McGregor, *J. Amer. Chem. Soc.*, 1962, **84**, 3000; M. Rothe, K.-D. Steffen, and I. Rothe, *Angew. Chem., Internat. Edn.*, 1964, **3**, 64; A. T. Moore and H. N. Rydon, *Acta. Chim. Acad. Sci. Hung.*, 1965, **44**, 103; J. A. Reader and P. W. G. Smith, *J. Chem. Soc.*, 1965, 3479; S. Rajappa and A. S. Akerkar, *Tetrahedron Letters*, 1966, 2893.

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[15] Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, and M. M. Shemyakin, *Doklady Akad. Nauk. S.S.S.R.*, 1963, **153**, 122; M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, and N. A. Aldanova, *Tetrahedron Letters*, 1963, 351.

[16] R. Schwyzer, in "CIBA-Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity," Churchill, London, 1958, p. 171.

[17] L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci. U.S.A.*, 1953, **39**, 253.

[18] I. M. Klotz and J. S. Franzen, *J. Amer. Chem. Soc.*, 1960, **82**, 5241; 1962, **84**, 3461.

[19] C. H. Hassall, T. G. Martin, and J. A. Schofield, *Tetrahedron Letters*, 1964, 3741.

in a similar manner. Hydroxyl-, carboxyl-, and amino-protecting groups for (XII) were chosen so that the latter pair could be removed, simultaneously, under conditions which would not affect the internal ester linkage or the hydroxyl-protecting group. The t-butyl group [20] was chosen for hydroxyl protection since it could be removed later under acidic conditions that did not cleave the cyclodepsipeptide ring. The use of alkali-labile protecting groups was precluded since it had been shown that the lactone functions of serratamolide were exceptionally susceptible to base-catalysed hydrolysis.[2]

The protected linear ester (XII) was obtained in high yield (90%) by the condensation of benzyl β-hydroxypropionate with *N*-benzyloxycarbonyl-*O*-t-butyl-DL-serine in the presence of DCCI and pyridine in ether. These conditions were selected after trial experiments had established that there was considerable variation in the nature and yield of the products under different conditions. Thus, using ether in the absence of pyridine, some ester was formed but the carboxylic anhydride of *N*-benzyloxycarbonyl-*O*-t-butyl-DL-serine was the major product (70%) whereas, using dioxan as solvent, *N*-(*N*-benzyloxycarbonyl-*O*-t-butyl-DL-seryl)-*NN'*-dicyclohexylurea was formed in substantial yield (49%) together with ester (33%). When the acid chloride (XIV) derived from the ester (XIII) was treated in benzene with triethylamine, self-condensation occurred in 60% yield, but two cyclodepsipeptides were isolated from the reaction mixture. These compounds, which had m. p.'s 231 and 256°, respectively, showed differences in mobility on thin-layer chromatograms but analyses confirmed that they were isomeric and had the formula $C_{20}H_{34}N_2O_8$. They had identical mass spectra [1] which were consistent with the structure (VII) but there were some differences in the region 1050—1450 cm.$^{-1}$ of the infrared spectra determined in potassium bromide discs. Two compounds, both melting at 241°, were obtained when the *O*-t-butyl groups were removed from the products, m. p.'s 231 and 256° using trifluoroacetic acid.[20] Like their precursors, these two isomers, $C_{12}H_{18}N_2O_8$ (VIII), had identical mass spectra [1] but differed in their mobility on thin-layer chromatograms and in some features of their infrared spectra.

The evidence as yet available does not define the relationship of the individual isomers. It is possible that the differences in properties are the result of different configurations. There is some indication, in previous work, of differences in the ease of cyclisation of diastereoisomeric linear peptides. For example, Kenner and his co-workers [21] have shown that the D-L(*meso*) form of the pentapeptide gly-leu-leu-gly-gly gave 39% cyclic pentapeptide in contrast to only 12% in the case of the L-L isomer. Similar differences in the ease of cyclisation of linear diastereoisomeric tetradepsipeptides have been observed.[22] These differences have been related to the conformations of the linear chains which are, in turn, determined by the configurations of the constituent amino-acids. If the twinning reaction, in this study, is visualised as proceeding through a linear dimer, it would be expected that the formation of the cyclic *meso* form, arising from the combination of one molecule of D-ester with one of L-ester, would be favoured. The other isomer would be the D-D, L-L racemate resulting from the twinning of two molecules (XIII) of similar configuration. In this connection, it has been reported [14,23] that cyclodimerisation of tripeptides derived from racemic amino-acids leads preferentially to the formation of the *meso*-cyclohexapeptide. We have undertaken the synthesis of the diastereoisomeric fourteen-membered cyclodepsipeptides (VII) by unambiguous routes to elucidate the nature of the two isomers obtained in the twinning process.[24]

It is also instructive to consider possible conformational isomers of the cyclodepsipeptide (VII). In 1963, Dale [25] postulated the strain-free conformation (Figure 1) for the hydrocarbon cyclotetradecane and extended this to include rings in which methylene groups had been replaced by ·NH·, ·O·, and ·CO·. Confirmation of the shape of the fourteen-membered ring has been provided recently by *X*-ray crystallographic studies of 1,8-diazacyclotetradecane derivatives.[26] Using Celmer's treatment, as applied to macrolide rings,[27] and Dale's assumptions concerning the conformation of cyclotetradecane (Figure 1), fourteen possible modifications of the conformation of the compound (VII) can be formulated. The results, which are summarised in Table 1 are the consequence of shifting functional groups clockwise, in single steps, around the ring formulated in the Dale conformation (Figure 1). Centrosymmetry reduces the number of different forms to seven (Figure 1, Table). If, in addition, it is taken into consideration that non-bonded interactions are minimised when side chains are assigned to those carbon atoms which, in the parent hydrocarbon, bear no intra-annular hydrogen atoms, the number of favoured conformations is reduced to two, (XVI) and (XVII) (Figure 2).

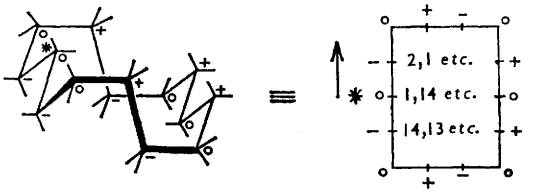


FIGURE 1 Cyclotetradecane, $(CH_2)_{14}$, (Dale [25])

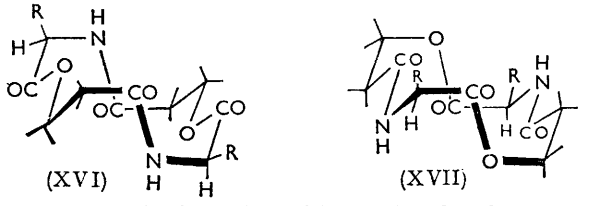
[20] H. C. Beyerman and J. S. Bontekoe, *Proc. Chem. Soc.*, 1961, 249.
[21] P. M. Hardy, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, 1963, **19**, 95.
[22] Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, and M. M. Shemyakin, *Doklady Akad. Nauk. S.S.S.R.*, 1963, **153**, 1342.
[23] H. Brockmann and M. Springorum, *Tetrahedron Letters*, 1965, 837.
[24] C. H. Hassall and Jean O. Thomas, to be published.
[25] J. Dale, *J. Chem. Soc.*, 1963, 93.
[26] J. D. Dunitz and E. F. Meyer, *Helv. Chim. Acta*, 1965, **48**, 1441; C. J. Brown, *J. Chem. Soc.* (*C*), 1966, 1108.
[27] W. D. Celmer, in "Biogenesis of Antibiotic Substances," Czechoslovak Academy of Sciences, Prague, 1965, p. 99.

TABLE

Theoretical modifications [27] of the substitution pattern of centrosymmetrical 14-membered cyclodepsipeptides in Dale's preferred conformation [25]

Ring member	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	—CO—	—CHR—	—NH—	—CO—	—CH_2—	—CH_2—	—O—	—CO—	—CHR—	—NH—	—CO—	—CH_2—	—CH_2—	—O—
I	0	−	0	+	−	0	+	0	+	0	−	+	0	−
II	−	0	+	−	0	+	0	+	0	−	+	0	−	0
III	0	+	−	0	+	0	+	0	−	+	0	−	0	−
IV	+	−	0	+	0	+	0	−	+	0	−	0	−	0
V	−	0	+	0	+	0	−	+	0	−	0	−	0	+
VI	0	+	0	+	0	−	+	0	−	0	−	0	+	−
VII	+	0	+	0	−	+	0	−	0	−	0	+	−	0

The positions of amide and ester groups, respectively, in (XVI) and (XVII), coincide with those suggested by Dale [25] for stable forms of the ring system. Moreover, these groups have the favoured *trans*-planar configuration in both conformations. This is in accord with the



(XVI) (XVII)

FIGURE 2 Conformations of 14-membered cyclotetradepsipeptides(L—L)

observation that amide I, II bands occur in the infrared spectra of serratamolide and the related synthetic compounds. Molecular models show that the ring systems in (XVI) and (XVII) are strain-free but of rigid conformation. The possibility that the two forms of the cyclodepsipeptides (VII) and (VIII) are conformational isomers cannot, as yet, be excluded but it is made less likely by the observation that the two isomers of (VII) sublime unchanged; we have obtained no indication that the two compounds can be interconverted.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. Infrared spectra were determined for potassium bromide discs, unless otherwise stated, on Perkin-Elmer model 237 or 257 spectrophotometers. Mass spectra were obtained for small samples (<0·5 mg.) using an Associated Electrical Industries Ltd. model MS-9 double-focussing mass spectrometer, at the University College of Swansea, Liverpool University, and at Imperial Chemical Industries Ltd. (Dyestuffs Division). Accurate mass measurements at high resolution were performed using a direct inlet system at a source temperature in the range 200—250°. R_f values refer to thin-layer chromatograms on Kieselgel G (Merck) unless otherwise stated. The following solvent systems were employed: *A*, ethyl acetate–benzene (1 : 1 v/v); *B*, benzene–methanol–acetic acid (10 : 2 : 1 v/v); *C*, ether–benzene (1 : 4 v/v); *D*, butan-2-ol–water–ammonia (*d* 0·880), (99 : 32 : 1 v/v); *E*, ethyl acetate; *F*, methyl ethyl ketone–pyridine–water (14 : 3 : 3 v/v). The chromoplates were routinely sprayed with reagent *G*, a solution of iodine in chloroform. Other spray reagents used were *H* [28] (hydroxylamine–ferric chloride), and *I*, a 1% (w/v) solution of ninhydrin in acetone. Thin-layer chromatography (t.l.c.) was used to check the purity of all synthetic intermediates. In general, organic extracts were dried over anhydrous sodium sulphate and evaporated under reduced pressure.

Benzyl β-Hydroxypropionate.—β-Propiolactone (60·0 g., 0·833 mole) was added slowly during 1 hr. to a well-stirred solution of sodium methoxide (2·25 g., 0·042 mole) in benzyl alcohol (540 g., 5·0 moles), cooled in an ice bath. Stirring was continued for a further 2 hr. at 0°, after which the temperature was allowed to rise to 20°. After standing for 7 hr. the reaction mixture was washed with water, dried and distilled to remove most of the benzyl alcohol in the fraction, b. p. 58—80°/1·2 mm. The residue was fractionally distilled to give *benzyl β-hydroxypropionate* (103·4 g., 69%) as a clear, colourless, mobile oil, b. p. 100—106°/0·15 mm., R_f 0·58 (system *A*). A portion of the product was redistilled. The middle fraction had b. p. 102°/0·2 mm. (Found: C, 66·4; H, 6·8. $C_{10}H_{12}O_3$ requires C, 66·65; H, 6·7%). The *3,5-dinitrobenzoate* crystallised from aqueous acetone as plates, m. p. 113—115° (Found: C, 54·6; H, 4·2; N, 7·1. $C_{17}H_{14}N_2O_8$ requires C, 54·55; H, 3·8; N, 7·5%).

N-Ethoxycarbonyl-β-alanine Benzyl Ester.[29]—*N*-Ethoxycarbonyl-β-alanine [29] (8·05 g., 50 mmoles), toluene-*p*-sulphonic acid (0·75 g., 5 mmoles), benzyl alcohol (8 g.), and benzene (75 ml.) were heated under reflux using a Dean and Stark receiver to remove the water formed in the reaction. When distillation of water ceased, the reaction mixture was washed with potassium hydrogen carbonate solution (5%). Benzene was removed from the dried solution and the residual clear oil was fractionally distilled to give *N*-ethoxycarbonyl-β-alanine benzyl ester (7·2 g., 58%) as a clear, colourless, mobile oil, b. p. 160—180°/1·1 mm. (Found: C, 62·5; H, 6·9; N, 5·4. Calc. for $C_{13}H_{17}NO_4$: C, 62·1; H, 6·8; N, 5·6%).

Benzyl β-(N-Benzyloxycarbonyl-L-leucyloxy)propionate.—(a) *From benzyl β-diazopropionate.* A solution of *N*-benzyloxycarbonyl-L-leucine [30] (3 g., 11·3 mmoles) in ether (15 ml.) was added dropwise to a stirred methanol–ether solution [29] of benzyl β-diazopropionate (derived from 5 g. of *N*-ethoxycarbonyl-β-alanine benzyl ester); effervescence was followed by disappearance of the deep yellow colour. The residue obtained by removal of solvent was dissolved in ethyl acetate (100 ml.) and the solution was washed with potassium carbonate solution (5%) and water. Removal of solvent

[28] M. Kelly, *J. Chromatog.*, 1964, **15**, 264.

[29] L. L. Braun and J. H. Looker, *J. Amer. Chem. Soc.*, 1958, **80**, 359.

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

from the dried solution gave the ester as a pale yellow oil (4·1 g., 48%) which was subjected directly to hydrogenolysis.

(b) *From benzyl β-hydroxypropionate.* *NN'*-Dicyclohexylcarbodi-imide (DCCI) (2·06 g., 10 mmoles) was added at 0° to a solution of *N*-benzyloxycarbonyl-L-leucine [30] (2·65 g., 10 mmoles) and benzyl β-hydroxypropionate (1·80 g., 10 mmoles) in ether (20 ml.). After the reaction had been left to stand at 0° for 18 hr., *NN'*-dicyclohexylurea was filtered off. The filtrate, obtained after excess carbodiimide reagent had been removed by treating the mixture with a few drops of glacial acetic acid at 0° for 2 hr. and filtering, was washed with sodium hydrogen carbonate solution (5%) and water. The ester was obtained as a straw-coloured oil (3·7 g., 87%) by evaporation of the dried solution. T.l.c. using system *A* gave a single spot. The compound was identical with the major product prepared from benzyl β-diazopropionate.

β-L-*Leucyloxypropionic Acid* (X).—Benzyl β-(*N*-benzyloxycarbonyl-L-leucyloxy)propionate (4·1 g., 9·6 mmoles) in methanol (40 ml.) was hydrogenolysed for 16 hr. at 1 atmosphere in the presence of 10% Pd–C as catalyst (1 g.). Removal of the solvent gave β-L-*leucyloxypropionic acid* as a white amorphous solid (1·5 g., 77%) which was purified by repeated reprecipitation from methanol by the addition of ether. T.l.c. showed a single yellow spot, using system *D* and spray *I*. The material had a double m. p. 188°, 215° (decomp.), $[\alpha]_D$ +6·0° (*c* 5·0 in 5N-HCl) (Found: C, 53·6; H, 8·2; N, 6·6. $C_9H_{17}NO_4$ requires C, 53·2; H, 8·4; N, 6·9%), $\nu_{max.}$ 1732 cm.$^{-1}$ (ester C=O).

Cyclodi-(β-L-*leucyloxypropionyl*) (VI).—Cyclisation of the acid chloride (XI) was performed under conditions of high dilution (0·31 mmole/l.). β-L-Leucyloxypropionic acid (126 mg., 0·62 mmole) was shaken with purified thionyl chloride (1 ml.) in pure benzene (10 ml.) for 1 hr. at 20°. The residue obtained by evaporation of the solution was extracted into anhydrous benzene (1·5 l.); this solution was stirred well during dropwise addition of a solution of triethylamine (0·19 ml., 1·3 mmoles) in anhydrous benzene (500 ml.) during 30 min. After standing at 20° for 16 hr. the solution was evaporated and the residue was dissolved in chloroform (100 ml.). This solution was washed with dilute hydrochloric acid (5%) and water, dried, and concentrated to precipitate the *cyclic depsipeptide* (VI) as needles (72 mg., 62%), m. p. 220—224°; R_f 0·78 (system *F*); $[\alpha]_D$ −108·0° (*c* 1·0 in DMF) [Found: C, 58·6; H, 8·5; N, 7·5%; *M* (mass spectrometry), 370. $C_{18}H_{30}N_2O_6$ requires C, 58·4; H, 8·2; N, 7·6%; *M*, 370]; the i.r. spectrum included peaks at 3342, 3307 (amide N–H), 1730 (ester C=O), 1646 (amide C=O), 1534 cm.$^{-1}$ (amide II).

N-*Benzyloxycarbonyl-*DL-*serine Methyl Ester.*—This compound has been prepared as a syrup,[31] but was not characterised. We have obtained it as a pure crystalline solid by the following procedure.

Benzyloxycarbonyl chloride [32] (60 g., 0·35 mole) was added dropwise over 30 min. at 20° to a vigorously stirred solution in saturated sodium hydrogen carbonate solution (840 ml.) of DL-serine methyl ester hydrochloride (48·0 g., 0·31 mole), which was prepared by the thionyl chloride procedure of Brenner and Huber.[33] Stirring was continued for a further 2 hr. The oil which formed was extracted with ethyl acetate and unchanged benzyloxycarbonyl chloride was destroyed by addition of a few drops of pyridine. The solution was washed with dilute hydrochloric acid (5%) and water, dried, and evaporated to give a colourless mobile oil; this was washed with pentane three times by decantation. The oil crystallised from pentane–ether as needles (69·4 g., 89%). Recrystallisation gave N-*benzyloxycarbonyl-*DL-*serine methyl ester* (65·4 g., 84%), m. p. 36—38° (Found: C, 57·1; H, 6·2; N, 5·3. $C_{12}H_{15}NO_5$ requires C, 56·9; H, 6·0; N, 5·5%).

N-*Benzyloxycarbonyl*-O-*t-butyl-*DL-*serine.*— *N*-Benzyloxycarbonyl-*O*-t-butyl-DL-serine methyl ester (24·8 g., 80 mmoles) was prepared as needles, m. p. 77°, by acid-catalysed addition of isobutene [34] to the foregoing ester (Found: C, 61·8; H, 7·8; N, 4·9. Calc. for $C_{16}H_{23}NO_5$: C, 62·1; H, 7·5; N, 4·5%). It was saponified as described by Callahan and his co-workers.[34] The m. p.s of our products from several experiments differed consistently from the highest value, m. p. 58—60°, reported by these workers.[34] We obtained the crude acid as a gum which crystallised readily under pentane. It was, then, recrystallised from isopropyl alcohol–water as a white solid (18·0 g., 76%), m. p. 75—77° (Found: C, 60·9; H, 7·5; N, 4·6. Calc. for $C_{15}H_{21}NO_5$: C, 61·0; H, 7·2; N, 4·7%).

Synthesis of Benzyl β-(N-*Benzyloxycarbonyl*-O-*t-butyl-*DL-*seryloxy*)*propionate* (XII).—(a) *Using DCCI.* Exploratory experiments showed that variation of conditions led to considerable differences in the yields of the desired product. The following typical experiments illustrate these results: (i) A solution of *N*-benzyloxycarbonyl-*O*-t-butyl-DL-serine (3·54 g., 12 mmoles) in anhydrous ether (7·5 ml.) was added dropwise over 30 min., at 0°, to a vigorously stirred solution in ether (12 ml.) of benzyl β-hydroxypropionate (2·16 g., 12 mmoles), DCCI (2·72 g., 13·2 mmoles), and absolute pyridine (1·02 ml., 12 mmoles) as catalyst. After standing for 20 hr. at 0°, the reaction mixture was freed of *NN'*-dicyclohexylurea (*ca.*, 2·5 g., 93%) and the filtrate worked up as described above for the leucyl analogue. The solid product (5·4 g., 98%) was washed with pentane containing about 1% ether, and recrystallised from ether–light petroleum (b. p. 60—80°) giving *benzyl* β-(N-*benzyloxycarbonyl*-O-*t-butyl-*DL-*seryloxy*)*propionate* (4·7 g., 85·5%) as needles, m. p. 44—45°; R_f 0·65 (system *C*) (Found: C, 65·9; H, 7·0; N, 2·9. $C_{25}H_{31}NO_7$ requires C, 65·6; H, 6·8; N, 3·1%).

(ii) A solution of *N*-benzyloxycarbonyl-*O*-t-butyl-DL-serine (2·95 g., 10 mmoles) in peroxide-free dioxan (12 ml.) was added dropwise during 25 min. to a well-stirred solution of benzyl β-hydroxypropionate (1·80 g., 10 mmoles) and DCCI (2·06 g., 10 mmoles) in dioxan (4 ml.) at *ca.*, 18°. The reaction mixture, after standing in a refrigerator for 10 min., and at 20° for 20 hr., was then worked up. The product was an oil (5·36 g.) which was shown by t.l.c. in system *C* to contain unchanged benzyl β-hydroxypropionate, and the required ester (R_f 0·65) which gave a purple colour with spray *H*, together with a compound (R_f 0·61) which gave a negative result with spray *H*. Crystals of this component were deposited from an ethereal solution of the oil at 0° and were recrystallised from benzene, giving N-(N-*benzyloxycarbonyl*-O-*t-butyl-*DL-*seryl*)-NN'-*dicyclohexylurea* (2·4 g., 48·5%), m. p. 122—124° (Found: C, 67·15; H, 8·7; N, 8·6. $C_{28}H_{43}N_3O_5$ requires C, 67·0; H, 8·6; N, 8·4%).

The oil (2·9 g.) remaining after removal of the *N*-acylurea was purified by column chromatography on silica gel (290 g.,

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B.D.H.) by gradient elution using ether–benzene mixtures. Elution with 2 and 5% ether in benzene gave an oil which was shown by its i.r. spectrum in chloroform, and by t.l.c., to be identical with the required ester (XII), but which resisted crystallisation until it was seeded at 0°. It was recrystallised from ether–light petroleum (b. p. 60—80°) as needles (1·5 g., 33%), m. p. 44—45°.

(iii) DCCI (0·26 g., 1·26 mmoles) was added to a solution of *N*-benzyloxycarbonyl-*O*-t-butyl-DL-serine (0·37 g., 1·25 mmoles) and benzyl β-hydroxypropionate (0·28 g., 1·55 mmoles) in anhydrous ether (25 ml.) at 20°. The reaction mixture, after standing at 0° for 20 hr., was worked up. *N-Benzyloxycarbonyl-O-t-butyl-DL-serine anhydride* (0·25 g., 70%) crystallised from the residual gum under pentane at 0°. The crystals, after washing thoroughly with pentane, had m. p. 89—91° (Found: C, 63·4; H, 7·4; N, 4·6. $C_{30}H_{40}N_2O_9$ requires C, 62·9; H, 7·0; N, 4·9%). The i.r. spectrum in chloroform included peaks at 3430 (N–H), 1835, 1755 (C=O of –CO–O–CO), 1725 cm.$^{-1}$ (C=O of –O–CO–NH). The same product (0·12 g., 42%), m. p. 88°, was obtained when *N*-benzyloxycarbonyl-*O*-t-butyl-DL-serine (0·295 g., 1 mmole) and DCCI (0·206 g., 1 mmole) underwent reaction in anhydrous ether (10 ml.) at 0°. Further, on standing overnight in methanol, both compounds gave *N*-benzyloxycarbonyl-*O*-t-butyl-DL-serine methyl ester, identified by comparison of its R_f with that of authentic material using system *A*.

The filtrate, from which the anhydride had been removed, was shown by t.l.c. to contain some of the required ester (XII) and the *N*-acylurea, together with an appreciable amount of unchanged benzyl β-hydroxypropionate.

(b) *Using ethyl chloroformate.* The reaction was carried out according to the procedure used for the synthesis of esters of benzyl penicillin.[35] A solution of triethylamine (0·255 g., 2·5 mmoles) in purified dichloromethane (1 ml.) was added to a stirred solution of *N*-benzyloxycarbonyl-*O*-t-butyl-DL-serine (0·738 g., 2·5 mmoles) in dichloromethane (10 ml.), and the solution left at 20° for 25 min. Ethyl chloroformate (0·27 g., 2·5 mmoles) was added dropwise to the stirred solution, which had been cooled to 0°, and the reaction mixture was kept at this temperature for 30 min. A solution of triethylamine (0·255 g., 2·5 mmoles) and benzyl β-hydroxypropionate (0·451 g., 2·5 mmoles) was added, after which the temperature was allowed to rise to 20°. After standing overnight at this temperature the reaction mixture was washed with acetic acid (5%), sodium hydrogen carbonate solution (2%), and water, dried, and concentrated to give a mobile oil (0·85 g.). T.l.c. in system *C* indicated that this contained starting material as well as the required ester. Column chromatography on silica gel (B.D.H., 100 : 1 adsorbent : compound) and elution with 1% ether in benzene gave an oil (0·186 g., 16%) which was shown by t.l.c. in several systems, and by its i.r. spectrum, to be the required ester (XII).

(c) *Using anhydrous oxalic acid.* *N*-Benzyloxycarbonyl-*O*-t-butyl-DL-serine (0·267 g., 0·9 mmole), benzyl β-hydroxypropionate (0·160 g., 0·9 mmole) and anhydrous oxalic acid (0·160 g., 1·8 mmoles) were heated under reflux in anhydrous benzene (200 ml.) for 6 days. Evaporation of benzene gave an oily solid to which ether was added. The filtrate was concentrated and chloroform added to the residue. The filtered solution was washed with sodium hydrogen carbonate solution (10%), and water, dried, and evaporated, yielding a colourless oil (0·099 g.). Development of chromatograms run in system *C* with sprays *G* and *H* indicated the presence of the required ester (XII) together with some benzyl β-hydroxypropionate.

β-(O-t-Butyl-DL-seryloxy)propionic Acid (XIII).—Benzyl β-(*N*-benzyloxycarbonyl-*O*-t-butyl-DL-seryloxy)propionate (5·056 g., 11·05 mmoles) was hydrogenolysed during 10 hr. in absolute methanol (200 ml.) using 10% Pd–C (1·105 g.) as catalyst. The residue obtained after removal of catalyst, and filtration, was reprecipitated from absolute methanol by addition of dry ether to yield *β-(O-t-butyl-DL-seryloxy)-propionic acid* (1·637 g., 63·5%); m. p. 143—144°. T.l.c. showed a single yellow spot, using system *D* and spray *I* (Found: C, 51·2; H, 8·5; N, 5·8. $C_{10}H_{19}NO_5$ requires C, 51·5; H, 8·2; N, 6·0%), $\nu_{max.}$ 1737 cm.$^{-1}$ (ester C=O).

Cyclodi-[β-(O-t-butylseryloxy)propionyl] (VII).— Cyclisation of the acid chloride (XIV) was carried out under conditions of high dilution (2·48 mmoles/l.). Purified thionyl chloride (10 ml.) was added slowly to a stirred suspension of the acid (XIII) (1·445 g., 6·2 mmoles) in benzene (100 ml.). The clear solution was kept at 20° for 25 min. in a stoppered flask and then concentrated to give a pale-yellow solid. This was immediately extracted with anhydrous benzene (2 l.) and the solution was stirred vigorously while triethylamine (1·8 ml., 13 mmoles) in anhydrous benzene (500 ml.) was added dropwise during 1·25 hr. The reaction mixture was stirred for a further 1 hr. and then left at 20° for 21 hr. The residue obtained on evaporation of benzene was dissolved in chloroform (200 ml.), washed with dilute hydrochloric acid (5%) and water, dried, and evaporated to give a faintly yellow solid (0·8 g., 60%) which was reprecipitated with ether from a saturated solution in chloroform. T.l.c. indicated that the product (0·67 g.) contained two compounds A and B, R_f values 0·28, 0·56, respectively (system *E*). Further fractional precipitation with ether from solutions in chloroform gave first the less soluble compound B (0·2 g.); m. p. 252—255°, with a transition to plates at *ca.* 220°. The m. p. was raised to 256—258° after sublimation as plates at 180—185°/0·04 mm. (Found: C, 55·8; H, 8·0; N, 6·3. $C_{20}H_{34}N_2O_8$ requires C, 55·8; H, 8·0; N, 6·5%). The ion of highest *m/e* in the mass spectrum had mass 431·2391 ± 0·0008 (mass standard, 413·9775); this cannot be the parent ion since the molecule contains an even number of nitrogen atoms.[36] There was no ion at *m/e* 430 in the spectrum. $C_{20}H_{34}N_2O_8$ requires *M*, 430; *M* + 1, 431·2391. The occurrence of (*P* + 1) peaks in the mass spectra of esters has been frequently observed.[37] Moreover, a detailed analysis of the fragmentation pattern [1] completely supports the proposed structure (VII). The i.r. spectrum included, in KBr, 3272 (N–H), 1744 (ester C=O), 1650 (amide C=O), 1555 cm.$^{-1}$ (amide II); in $CHCl_3$, 3438 (N–H), 1735 (ester C=O), 1672 (amide C=O), 1514 cm.$^{-1}$ (amide II). This evidence established that B was the required cyclic dimer, *cyclodi-[β-(O-t-butylseryloxy)propionyl]*.

Further separation of A and B was effected by column chromatography:

(a) *Using silica gel.* A mixture of A and B (100 mg.) in

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chloroform (*ca.* 1 ml.) was applied to a column of silica gel (20 g., B.D.H.). Elution with chloroform–benzene (1 : 3 v/v) gave, first, compound B (36 mg.) and then a mixture of A and B (20 mg.). Chloroform–benzene (1 : 1 v/v) eluted compound A (12 mg.) alone. This *isomer of cyclodi-*[β-(O-*t-butylseryloxy*)*propionyl*] was further purified by sublimation (140—145°/0·01 mm.) which gave feathery needles, m. p. 231—233° [Found: C, 55·6; H, 8·3; N, 6·6%; $M + 1$ (mass spectrometry), 431. $C_{20}H_{34}N_2O_8$ requires C, 55·8; H, 8·0; N, 6·5%; M, 430]. The mass spectra of A and B were identical.[1] The i.r. spectra of A included, in KBr, 3270 (N–H), 1731 (ester C=O), 1649 (amide C=O), 1547 cm.$^{-1}$ (amide II); in $CHCl_3$, 3442 (N–H), 1734 (ester C=O), 1672 (amide C=O), 1510 cm.$^{-1}$ (amide II). Attempts to repeat this separation using different batches of the same brand of silica gel failed. It became necessary to use neutral alumina in subsequent separation procedures although recovery in this case was in lower yield.

(b) *Using neutral alumina.* A mixture (65 mg.) of A and B in chloroform was applied to a column of neutral alumina (Woelm, activity I) (13 g.). After washing the column with benzene (100 ml.) elution with chloroform–benzene (1 : 9 v/v) gave isomer B (7 mg.). Further elution with chloroform–benzene (3 : 17 v/v) gave a mixture of the isomers (3 mg.). Then isomer A (10·8 mg.) was obtained by elution with chloroform–benzene (1 : 1 v/v). A and B were purified further by vacuum sublimation.

Cyclodi-(β-*seryloxypropionyl*) (VIII).—A solution of isomer B (50 mg., 0·116 mmole) in freshly distilled trifluoroacetic acid (5 ml.) was left at room temperature for 40 min. Evaporation of the solution at 30—40° yielded a gum which solidified readily on addition of ether. The product (47 mg.), after washing with ether, was recrystallised from aqueous ethanol as needles (30 mg., 81%) of *cyclodi-*(β-*seryloxypropionyl*), m. p. 241—243°; R_f 0·43 (Kieselgel G), 0·52 (alumina) (system *D*) [Found: C, 45·0; H, 6·0; N, 9·2%; M (mass spectrometry), 318·1063 ± 0·0006 (mass standard 313·9839). $C_{12}H_{18}N_2O_8$ requires C, 45·3; H, 5·7; N, 8·8%; M, 318·1063]. The i.r. spectrum included peaks at 3406 (O–H), 3309 (N–H), 1735 (ester C=O), 1649 (amide C=O), 1542 cm.$^{-1}$ (amide II), but there was no absorption due to the $C(CH_3)_3$ grouping (1365, 1390 cm.$^{-1}$, 2 : 1).

Treatment of isomer A (19·7 mg., 0·046 mmole) with trifluoroacetic acid (1·5 ml.), as in the case of B, gave a gum which solidified readily under ether. Recrystallisation from aqueous ethanol gave an *isomer of cyclodi-*(β-*seryloxypropionyl*), (10·4 mg., 71%); m. p. 241—243°; R_f 0·31 (Kieselgel G), 0·44 (alumina), (system *D*) [Found: (mass spectrometry), M 318·1063 ± 0·0006; $M + 1$, 319·1141 ± 0·0006, (mass standard, 313·9839); $C_{12}H_{18}N_2O_8$ requires M, 318·1063; $M + 1$, 319·1141]. The mass spectra of the two isomers of $C_{12}H_{18}N_2O_8$ were identical [1] and confirm the structure (VIII). The i.r. spectrum contained peaks at 3410 (O–H), 3262 (N–H), 1737 (ester C=O), 1651 (amide C=O), 1546 cm.$^{-1}$ (amide II); there was no absorption due to $C(CH_3)_3$ at 1365 cm.$^{-1}$

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