Amino-acids and Peptides. Part XVI.[1] Synthesis of Cyclo-[L-(α-amino)-β-alanylglycyl-D-(α-amino)-β-alanylglycyl] and Related Fourteen-membered Cyclotetrapeptides

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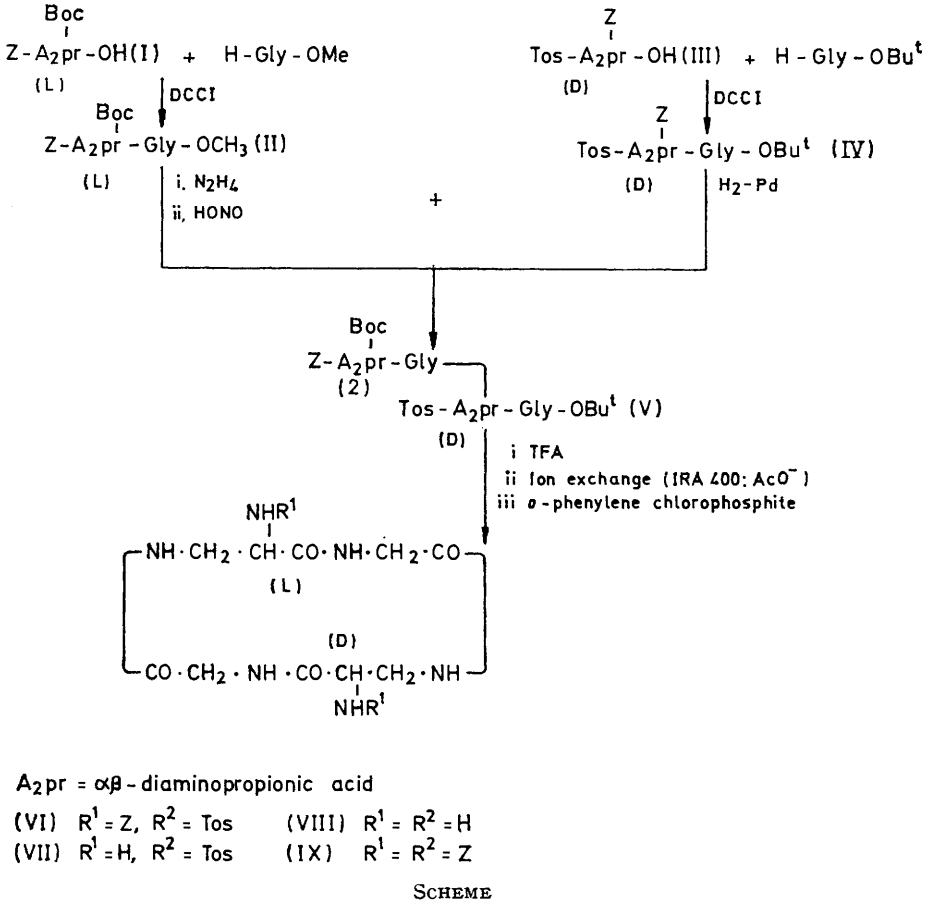
The title cyclotetrapeptide was synthesised through cyclisation of the corresponding linear *N*-tosyl-*N'*-benzyloxycarbonyltetrapeptide with imidazole and *o*-phenylene chlorophosphite. followed by deprotection. Cyclodi-[L-(α-benzyloxycarbonylamino)-β-alanylglycyl] has been prepared by cyclodimerisation.

EARLIER papers in this series[2,3] have described procedures for the preparation of fourteen-membered cyclotetradepsipeptides and cyclotetrapeptides through both cyclodimerisation and the cyclisation of linear systems. This investigation has been concerned with the synthesis of two related diaminocyclotetrapeptides, (VIII) and (XIV), which were designed as 'building blocks' for the synthesis of a cylindrical peptide.[4] Of the several alternatives, the linear tetrapeptide derivative (V) was chosen as an intermediate for the synthesis of the cyclopeptide (VIII) to reduce the likelihood, at the cyclisation stage, of racemisation or of interfering reactions due to the tosylamino-group. The procedure for the synthesis is outlined in the Scheme. When the preparation of the dipeptide (IV) was attempted by the acid chloride method,[5,6] a low yield was obtained, presumably owing to the formation of toluenesulphonamide by the action of base, as illustrated for compound (X). Subsequent attempts at coupling by a mixed-anhydride synthesis[7] involving pivalic acid[8] or dicyclo-



A_2pr = αβ-diaminopropionic acid

(VI) R^1 = Z, R^2 = Tos (VIII) $R^1 = R^2$ = H
(VII) R^1 = H, R^2 = Tos (IX) $R^1 = R^2$ = Z

SCHEME

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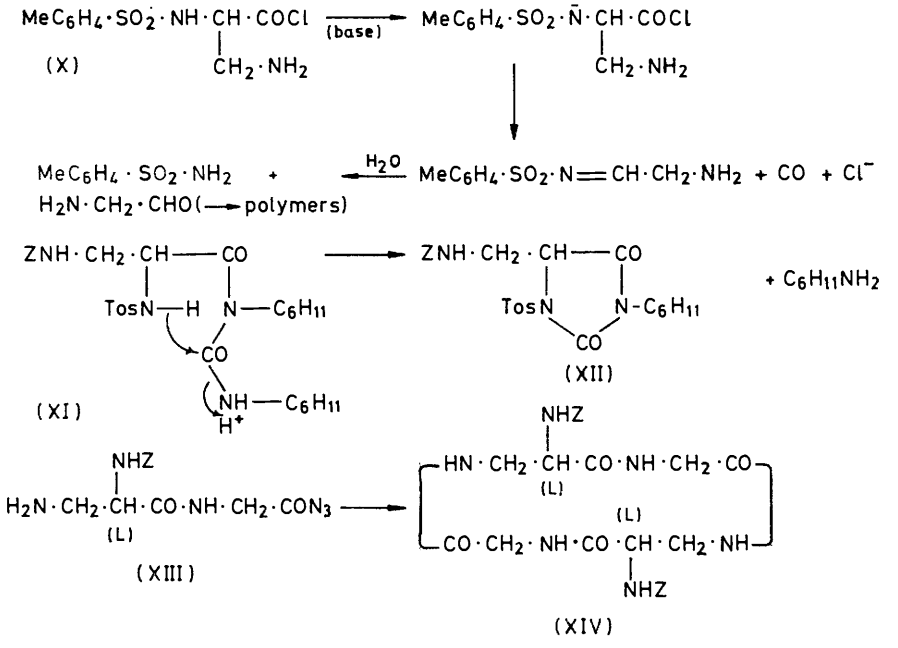
hexylcarbodi-imide (DCCI) in dimethylformamide did not give good yields. In the latter case, a substantial amount (30%) of the trisubstituted hydantoin (XII) was produced; it was identified by its ^{1}H n.m.r. spectrum. Presumably, it was formed by cyclisation of the *N*-acylurea (XI), a reaction reminiscent of the rearrangement of the peptide derivative *N*-tosylglycylglycylpyroglutamyl.[9] When the coupling was repeated with DCCI in methylene dichloride,[10,11] high yields (85%) of the dipeptide (IV) were obtained and none of the by-product (XII) was formed.

The remaining stages of the synthesis of the protected tetrapeptide (V) employed well established procedures. The identity and optical purity of the products were confirmed at each stage. The cyclisation with *o*-phenylene chlorophosphite and imidazole in diethyl phosphite [2] gave a yield of over 60% of the cyclic compound (VI); the deprotected diaminocyclopeptide (VIII) was obtained through hydrogenolysis followed by reduction with sodium in liquid ammonia. The ^{1}H n.m.r. and the mass spectra of the cyclic compounds (VI)—(VIII) were in accord with the structures proposed.

We undertook the preparation of cyclodi-[L-(α-benzyloxycarbonylamino)-β-alanylglycyl] (XIV) in order to investigate the conformations of related D,L- and L,L-systems, as for the case of the related cyclotetradepsipeptides.[3] There was some basis for expecting to achieve the synthesis of this peptide by cyclodimerisation. Similar cyclodepsipeptides have been prepared in this way [3] and cyclodi-(β-alanylglycyl) is formed from the azide of the monomer.[12] However, the formation of an L,L-isomer occurs less readily than of a D,L-form, presumably owing to the less favourable orientation for ring closure of the linear intermediate.[13,14] We have obtained the product (XIV) but in only 5% yield by cyclodimerisation of the azide (XIII) in aqueous alkali.



EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus Infra-red spectra were determined for potassium bromide discs, with a Perkin-Elmer 257 spectrophotometer. N.m.r. spectra were measured with a Varian HA100 (100 MHz) spectrometer (tetramethylsilane as internal standard). For optical rotation measurements we employed a Perkin-Elmer 141 polarimeter. Mass spectra were determined with an A.E.I. MS9 double-focusing spectrometer. Measurements at high resolution were performed by using a direct inlet system at a source temperature in the range 250—300 °C. For t.l.c. we employed plates coated with Kieselgel G (Merck); the following solvent systems: (A) ethanol–ammonia (s.g. 0.880) (5 : 1 v/v), (B) benzene–methanol–glacial acetic acid (10 : 2 : 1 v/v), (C) ethyl acetate, (D) ethyl acetate–benzene (4 : 1 v/v), (E) ethyl acetate–benzene (1 : 1 v/v), (F) benzene–ethyl acetate (2 : 1 v/v), (G) acetone–chloroform (1 : 1 v/v); and the following spray reagents: (P) a saturated solution of iodine in chloroform; (Q) a 1% (w/v) solution of ninhydrin in acetone (the plate was then heated at 110 °C for 2 min). Light petroleum had b.p. 60—80 °C unless otherwise specified.

L-α-*Benzyloxycarbonylamino*-β-t-*butoxycarbonylamino-propionylglycine Methyl Ester* (II).—DCCI (6.19 g, 30

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mmol) was added to a well stirred mixture of glycine methyl ester hydrochloride (3.77 g, 30 mmol), triethylamine (3.03 g, 30 mmol), and L-α-benzyloxycarbonylamino-β-t-butoxycarbonylaminopropionic acid (10.14 g, 30 mmol) [15] in dichloromethane (150 ml) at 0 °C. After 26 h *NN'*-dicyclohexylurea and triethylamine hydrochloride were removed by filtration, and the last traces of DCCI were removed by treating the solution with glacial acetic acid (0.2 ml) at 0 °C for 3 h and filtering. The filtrate was made up to 400 ml, washed with 0.5N-hydrochloric acid (150 ml), 0.5N-sodium hydrogen carbonate (150 ml), and water (2 × 150 ml), dried, and evaporated to yield a solid (12.6 g) which crystallised from ethyl acetate–light petroleum as needles. This *product* (10.3 g, 84%) had m.p. 129—130°, $[\alpha]_D^{19}$ −26.8° (*c* 2 in $CHCl_3$) (Found: C, 55.9; H, 6.7; N, 10.3. $C_{19}H_{27}N_3O_7$ requires C, 55.7; H, 6.7; N, 10.3%), R_F(E) 0.40, (F) 0.19, τ ($CDCl_3$) 2.70 (5 H, C_6H_5), 2.7—2.8br (1 H, NH·CH_2·CO), 3.73br (1 H, CH·N*H*Z), 4.65br (1 H, CH_2·N*H*Boc), 4.92 (2 H, s, PhCH_2·O), 5.68 (1 H, q, ZNH·C*H*·CO), 6.05 (2 H, d, NH·CH_2·CO_2), 6.32 (3 H, s, CO_2·CH_3), 6.54 (2 H, t, BocNH·CH_2), and 8.58 (9 H, s, Me_3C·O).

D-β-*Benzyloxycarbonylamino-α-tosylaminopropionic Acid* (III).—D-β-Amino-α-tosylaminopropionic acid (12.6 g, 0.05 mol) [16] was dissolved in N-sodium hydroxide (50 ml) and cooled to 0 °C in an ice-bath. Over 1 h, benzyl chloroformate (10 g, 0.059 mol) was added to the stirred solution and the pH was maintained at 9—10 by addition of N-sodium hydroxide (50 ml). The sodium salt of the product precipitated out during the reaction, and ice-cold water was added to aid dissolution. After the addition of the benzyl chloroformate, the suspension was stirred at 0 °C for a further 3 h. The resulting slurry was diluted to 500 ml and washed with ether (2 × 150 ml), and the aqueous phase was acidified at 20 °C to pH 3 with 3N-hydrochloric acid. The mixture was extracted with ethyl acetate (2 × 250 ml) and worked up in the usual way to yield the *product* (16.7 g, 85.2%) as a white, amorphous solid, m.p. 57—60°, $[\alpha]_D^{20}$ −26.5° (*c* 2 in CH_2Cl_2), $[\alpha]_D^{19}$ +77.7° (*c* 4 in N-NaOH) (Found: C, 54.6; H, 5.2; N, 7.2. $C_{18}H_{20}N_2O_6S$ requires C, 55.1; H, 5.1; N, 7.1%), R_F(A) 0.72, (B) 0.61, τ ($CDCl_3$) 0.20br (1 H, s, CO_2H), 2.3—3.0 (4 H, AA'BB' pattern, MeC_6H_4·SO_2), 2.78 (5 H, s, Ph), 3.90br (1 H, d, TosN*H*·CH), 4.40br (1 H, ZN*H*·CH_2), 5.08 (2 H, s, PhCH_2O), 6.05 (1 H, q, TosNH·C*H*), 6.57 (2 H, t, ZNH·CH_2), and 7.74 (3 H, s, CH_3·C_6H_4·SO_2).

Crystallisation from benzene–light petroleum yielded *plates*, m.p. 62—64° (Found: C, 61.1; H, 5.6; N, 6.1. $C_{18}H_{20}N_2O_6S,C_6H_6$ requires C, 61.3; H, 5.6; N, 6.0%), τ ($CDCl_3$) 2.70 (C_6H_6).

The *dicyclohexylammonium salt* crystallised from ethyl acetate as needles, m.p. 175—178°, $[\alpha]_D^{26}$ −50.9° (*c* 1 in EtOH). (Found: C, 62.7; H, 7.6; N, 7.2. $C_{30}H_{43}N_3O_6S$ requires C, 62.8; H, 7.6; N. 7.3%).

D-β-*Benzyloxycarbonylamino-α-tosylaminopropionylglycine t-Butyl Ester* (IV).—(*a*) DCCI (0.72 g, 3.5 mmol) was added to a mixture of the acid (III) (1.37 g, 3.5 mmol) and glycine t-butyl ester (0.46 g, 3.5 mmol) in dichloromethane (20 ml). After 23 h 0 °C the mixture was worked up in the usual way to give the *product* (1.5 g, 85%), m.p. 142°, $[\alpha]_D^{20}$ +25.7° (*c* 2 in $CHCl_3$) (Found: C, 57.1; H, 6.5; N, 8.4. $C_{24}H_{31}N_3O_7S$ requires C, 57.0; H, 6.2; N, 8.3%), R_F(F) 0.41, τ 2.22—2.82 (4 H, AA'BB' pattern, MeC_6-H_4·SO_2), 2.70 (5 H, s, Ph), 2.7—2.8br (1 H, CO·N*H*·CH_2), 3.48br (1 H, d, CH·N*H*·Tos), 4.47br (1 H, CH_2·N*HZ*), 4.96 (2 H, s, O·CH_2Ph), 6.1—6.4 (3 H, m, NH·CH_2·CO_2 and TosNH·C*H*·CO), 6.64 (2 H, t, ZNH·CH_2), 7.64 (3 H, s, CH_3·C_6H_4·SO_2), and 8.55 (9 H, s, Me_3CO_2C).

(*b*) When the reaction was carried out on a similar scale with dimethylformamide (12 ml) rather than dichloromethane as solvent, a mixture was obtained. The component which was less soluble in ethyl acetate was the product (IV), m.p. 142° (0.88 g, 50%), but the mother liquor gave, by recrystallisation, 5-(*benzyloxycarbonylaminomethyl*)-3-*cyclohexyl*-1-*tosylhydantoin* (0.51 g, 29.2%) as needles, m.p. 146—147° (Found: C, 60.5; H, 5.7; N, 8.5. $C_{25}H_{39}N_3O_6S$ requires C, 60.1; H, 5.8; N, 8.4%), R_F(F) 0.71, τ 2—2.8 (4 H, AA'BB', C_6H_4), 2.73 (5 H, s, Ph), 4.98 (2 H, s, $ArCH_2$), 5.0br (1 H, NH), 5.8—6.5 (4 H, m, N·C*H*·CH_2·N and N·C*H*·$[CH_2]_2$), 7.59 (3 H, s, $ArCH_3$), and 7.8—9.0 (10 H, m, C_6H_{10}).

L-α-*Benzyloxycarbonylamino-β-t-butoxycarbonylaminopropionylglycine Hydrazide*.—The methyl ester (II) (4.09 g, 10 mmol) in methanol (60 ml) was treated with an excess of hydrazine hydrate (1.7 ml, 44 mmol) during 6 h at 20 °C. Evaporation of solvent and the excess of hydrazine gave the *product*, which crystallised from water (3.17 g, 74%), m.p. 115—117°, $[\alpha]_D^{23}$ −10.1° (*c* 2 in EtOH) (Found: C, 50.5; H, 6.5; N, 16.5. $C_{18}H_{26}N_5O_6,H_2O$ requires C, 50.6; H, 6.8; N, 16.4%), R_F(A) 0.80 [red with reagent (Q)].

L-α-*Benzyloxycarbonylamino-β-t-butoxycarbonylaminopropionylglycyl*-D-β-*amino-α-tosylaminopropionylglycine t-Butyl Ester* (V).—The azide was prepared from the preceding hydrazide (1.03 g, 2.5 mmol) by treatment with nitrosyl chloride (0.28 g, 4.3 mmol) in dioxan (2.5 ml) and tetrahydrofuran (8 ml) at −20 °C during 20 min. The conditions were similar to those of Honzl and Rudinger [17] for other cases. The resulting solution was diluted with cold (−10 °C) ethyl acetate (50 ml) and worked with a precooled (−10 °C), saturated solution of sodium hydrogen carbonate in sodium chloride (20 ml). The ethyl acetate solution was dried and treated at 0 °C with a solution of β-amino-D-α-tosylaminopropionylglycine t-butyl ester (0.93 g, 2.5 mmol) in ethyl acetate (10 ml). The *product* (1.6 g) had separated after 48 h 0 °C, giving crystals (1.45 g, 76%) from acetone–water, m.p. 142—143°, $[\alpha]_D^{21}$ −5.3° (*c* 2 in Me_2N·CHO) (Found: C, 53.5; H, 6.3; N, 10.9. $C_{34}H_{48}$-$N_6O_{11}S,H_2O$ requires C, 53.5; H, 6.6; N, 11.0%), R_F(C) 0.49, (D) 0.24, (G) 0.44, τ ($CDCl_3$) 2.2—2.8 (4 H, AA'BB', MeC_6H_4·SO_2), 2.70 (5 H, s, Ph), 4.90 (2 H, O·CH_2Ph), 7.62 (3 H, s, CH_3·C_6H_4·SO_2), 8.58 (9 H, s, Me_3CO_2C), and 8.61 (9 H, s, Me_3C·O·CO·NH).

A lower yield of the tetrapeptide (71%) was obtained when the reaction was carried out in aqueous conditions by using procedures based on those developed by Boissonnas [18] and Schwyzer [19] for azide coupling.

L-β-*Amino-α-benzyloxycarbonylaminopropionylglycyl*-D-β-*amino-α-tosylaminopropionylglycine*.—The protected tetrapeptide (V) (0.16 g., 0.2 mmol) was dissolved in anhydrous trifluoroacetic acid (2 ml) and kept at 20 °C for 30 min. The residue obtained by evaporation was dissolved

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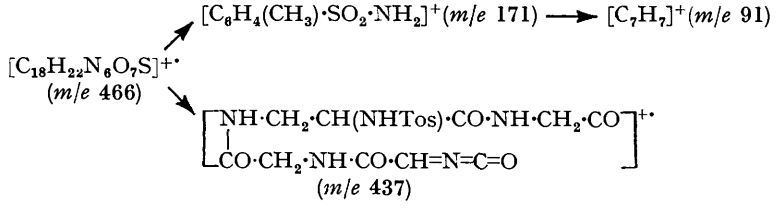
[19] B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, 1961, **44**, 169; R. Schwyzer and H. Kappeler, *ibid.*, p. 1991.

in ethanol (1 ml) and water (2 ml). The solvent was removed under reduced pressure. The gum which remained consisted of essentially one product [R_F(A) 0.67]. The solution, in ethanol (2 ml) and water (1 ml) was passed through a column (8.5 × 1 cm) of Amberlite IRA-400 (OAc^-) which was then washed with N-acetic acid. The eluate containing ninhydrin-positive material (first 25 ml) was evaporated to give the *product* (0.12 g, 92%), m.p. 143—146°, $[\alpha]_D^{20}$ −1.8° (*c* 2 in $Me_2N{\cdot}CHO$) (Found: C, 48.3; H, 5.6; N, 12.8. $C_{25}H_{32}N_6O_9S,C_2H_4O_2,H_2O$ requires C, 48.3; H, 5.7; N, 12.5%), R_F(A) 0.62 [violet with reagent (Q)].

Cyclo-[L-(α-*benzyloxycarbonylamino*)-β-*alanylglycyl*-D-(α-*tosylamino*)-β-*alanylglycyl*] (VI).—A solution of the preceding tetrapeptide (134 mg, 0.2 mmol), imidazole (15 mg, 0.22 mmol), and *o*-phenylene chlorophosphite (54.6 mg, 0.32 mmol) in diethyl phosphite (40 ml) was heated at 140 °C in nitrogen for 10 h with occasional stirring. After cooling, the mixture was kept at 20 °C for 12 h. The pale yellow solution was filtered and evaporated under reduced pressure to yield an amorphous white residue. This was treated with ethyl acetate (10 ml) at 0 °C for 12 h, filtered, washed thoroughly with warm ethyl acetate, and then dried to yield the *product* (72 mg, 60%) as a white amorphous solid. It chars without melting from 315 to 350 °C and shows $[\alpha]_D^{20}$ 0° (*c* 1 in Me_2SO) (Found: C, 51.0; H, 5.3; N, 14.2. $C_{25}H_{30}N_6O_8S,H_2O$ requires C, 50.7; H, 5.4; N, 14.2%), R_F(A—G) 0. An *anhydrous sample* was obtained by heating the monohydrate at 140 °C, under reduced pressure, for 24 h (Found: C, 51.9; H, 5.1; N, 14.5. $C_{25}H_{30}N_6O_8S$ requires C, 52.3; H, 5.3; N, 14.6%), τ 1.98br (3 H, CO·NH), 2.3—2.8 (12 H, m, ArH and CONH), 5.02 (2 H, s, $ArCH_2$), 5.9—7.1 (10 H, m, aliphatic CH), and 7.66 (3 H, s, $ArCH_3$). The mass spectrum did not include a peak for the molecular ion but there was one (*m/e* 466) for the isocyanate arising from replacement of the −NHZ function by −N=C=O. Two typical fragmentations of this ion *m/e* 466 are given below; other fragmentations observed could be explained similarly in terms of both side-chain and ring cleavages.

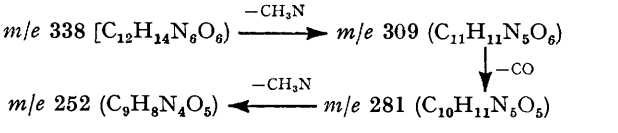


Cyclo-[L-(α-*amino*)-β-*alanylglycyl*-D-(α-*tosylamino*)-β-*alanylglycyl*] (VII).—A solution of compound (VI) (0.237 g, 0.4 mmol) in purified dimethylformamide (80 ml) was hydrogenated at room temperature and atmospheric pressure for 44 h over 10% palladium–charcoal (0.5 g). After work-up in the usual way, the residue was extracted into warm water (100 m); the extract was filtered and evaporated to yield the *product* (165 mg), crystals (110 mg, 60%) from water, m.p. 257—261° (decomp.), $[\alpha]_D^{20}$ +23.3° (*c* 1 in $Me_2N{\cdot}CHO$) (Found: C, 44.6; H, 5.6; N, 18.4. $C_{17}H_{24}N_6O_6S,H_2O$ requires: C, 44.5; H, 5.7; N, 18.3%). R_F(A) 0.51 [mauve with reagent (Q)], M^+ 440.

Cyclo-[L-(α-*amino*)-β-*alanylglycyl*-D-(α-*amino*)-β-*alanylglycyl*] (VIII).—A solution of compound (VII) (0.075 g, 0.17 mmol) in liquid ammonia (25 ml) was vigorously stirred at the b.p. of ammonia, and small pieces of freshly cut sodium were added until a blue colour was maintained for 5 min. The excess of sodium was destroyed by adding a mixture of resins [Amberlite IR 120 (NH_4^+) and Amberlite IRA 400 (OH^-) (1 : 1 ratio; 0.150 g)]. The mixture was vigorously stirred at 20 °C until the ammonia had evaporated (the last traces were removed under high vacuum). The residue was dissolved in water (5 ml) and the filtrate was neutralised with N-acetic acid (2.0 ml). On addition of ethanol (100 ml), the solution became milky. After 6 h at 20 °C, the white precipitate was filtered off. The volume of filtrate was reduced to 2 ml, more ethanol (100 ml) was added, and the mixture was left at 20 °C for 7 days to give small rods (0.011 g, 21%), charring slowly above 250 °C, $[\alpha]_D^{20}$ ±0° (*c* 1 in $Me_2N{\cdot}CHO$), R_F(A) 0.31, M^+ 286.

The bisbenzyloxycarbonyl derivative of (VIII), prepared by using benzyloxycarbonyl chloride and work-up in the usual way, had R_F(A—G) 0, and charred slowly above 310 °C. The mass spectrum was indistinguishable from that of the L,L-isomer (XIV).

Cyclodi-[L-(α-*benzyloxycarbonylamino*)-β-*alanylglycyl*] (XIV).—L-α-Benzyloxycarbonylamino-β-t-butoxycarbonylaminopropionylglycine hydrazide (0.818 g, 2 mmol) was dissolved in methanolic 2N-hydrogen chloride (35 ml) and kept for 2 h at 20 °C. The solvent was evaporated off under reduced pressure and the white residue was triturated with dry ether for 24 h at 0 °C, filtered off, and dried to give a white hygroscopic solid (0.76 g), R_F(A) 0.28 [red with reagent (Q)]. This was dissolved in 0.2N-hydrochloric acid (10 ml, 2 mmol), cooled to 0 °C in an ice-bath and mixed with a cold aqueous solution (2 ml) of sodium nitrite (0.133 g, 2 mmol). After stirring for 15 min at 0 °C. the solution was poured into ice-cold water (1 l) containing sodium hydrogen carbonate (3 g) and set aside for 72 h at 0 °C. The amorphous, pale brown solid (0.058 g) which slowly precipitated out was filtered off and washed with acetone and warm ethyl acetate to yield the *product* (0.027 g, 5.1%) as a white amorphous solid; it chars slowly above 290 °C with complete decomposition at 340 °C; $[\alpha]_D^{24}$ −12.5° (*c* 1 in Me_2SO) (Found: C, 54.2; H, 5.6; N, 14.8. $C_{26}H_{30}N_6O_8,H_2O$ requires C, 54.5; H, 5.6; N, 14.7%), R_F(A—G) 0 [no colour with reagent (Q)]. The mass spectrum did not include a peak for the molecular ion but there were peaks for monoisocyanate (*m/e* 446) and diisocyanate (*m/e* 338). The sequential fragmentation pattern involving stepwise losses of 29 and 28 mass units was explained in terms of decomposition to neutral imine and carbon monoxide. The molecular formulae were established by accurate mass measurements in each case:



[6/617 *Received*, 31*st March*, 1976]