1161. *Peptides. Part XVIII.*[1] *Derivatives of* 1-*Aminocyclohexanecarboxylic Acid*

By G. W. Kenner, J. Preston, and R. C. Sheppard

1-Benzyloxycarbonylaminocyclohexanecarboxylic acid is converted exclusively into the symmetrical anhydride by pivaloyl chloride and triethylamine. Dipeptide derivatives of 1-aminocyclohexanecarboxylic acid were prepared from this anhydride, and tripeptide derivatives and the polypeptide were prepared by the oxazolone method. Cyclohexanespiro-4-oxazol-5-one is described.

Steric hindrance encountered in the synthesis of peptides of α-methylalanine may be overcome by use of the oxazolone and pivalic mixed anhydride methods.[2] These methods have also been successful with derivatives of other αα-dialkylglycines,[1] and the formally similar 1-aminocyclohexanecarboxylic acid has now been included in our work. Interest in the synthesis of peptides of this and related cycloalkane amino-acids [3] has been stimulated recently by the discovery that 1-aminocyclopentanecarboxylic acid inhibits tumour growth,[4]

[1] Part XVII, preceding Paper.
[2] M. T. Leplawy, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, 1960, **11**, 39.
[3] For a review, see J. Rudinger, in "Peptides, Proceedings of the 5th European Symposium, Oxford, 1962," ed. G. T. Young, Pergamon, Oxford, 1963, p. 133.
[4] T. A. Connors, L. A. Elson, and W. C. J. Ross, *Biochem. Pharm.*, 1958, **1**, 239.

and Connors and Ross,[5] and Shankman and his co-workers,[6] have described the preparation by conventional methods of several simple peptides containing the cyclopentane amino-acid. Tailleur and Berlinquet [7] have prepared some analogous compounds from 1-amino-cyclohexane carboxylic acid, and we now describe our own experiences with this readily accessible amino-acid.

In the α-methylalanine series, the crystalline mixed anhydride between benzyloxycarbonyl-α-methylalanine and pivalic acid formed quantitatively when the triethylammonium salt of the acylamino-acid was treated with pivaloyl chloride.[2] Rather surprisingly, the reaction took a different course in the cyclohexane series. Under the same conditions, the triethylammonium salt of 1-benzyloxycarbonylaminocyclohexanecarboxylic acid yielded a nicely crystalline anhydride ($\nu_{max.}$ 1812 and 1745 cm.$^{-1}$), but this proved to be the symmetrical anhydride (I). Structure (I) followed from elemental analysis and n.m.r. spectroscopy (which showed the complete absence of the t-butyl group of a pivaloyl residue), and from the reaction of (I) with methyl 1-aminocyclohexanecarboxylate. The reason for this unexpected difference between the two series is not clear, but may have its origin in a conformational effect. Molecular models of 1-benzyloxycarbonylaminocyclohexanecarboxylic acid indicate that the more stable conformer is likely to be that with the carboxyl group axial. The weaker acidity of axial carboxylic acids compared with their equatorial isomers (and with acyclic acids) is well known,[8] and correspondingly, 1-benzyloxycarbonylaminocyclohexanecarboxylic acid (pK^*_{MCS} 6·93) [9] proved to be distinctly weaker than the α-methylalanine analogue (pK^*_{MCS} 6·20). Presumably the preference for symmetrical anhydride formation is related to the smaller spread of acidities between the acylamino-acid and pivalic acid (pK^*_{MCS} 7·60), although the clear-cut difference in the two series is remarkable.* The mixed anhydride between benzyloxycarbonyl-α-methylalanine and pivalic acid showed no tendency to disproportionate, even when heated at 100°, although in the analogous α-phenylalanine series, disproportionation occurred more easily.[1]

Reaction of the anhydride (I) with methyl 1-aminocyclohexanecarboxylate afforded the dipeptide ester in 92% yield, and the expected amount of acylamino-acid was recovered. This procedure therefore provided an efficient route to dipeptide derivatives in this series, and was superior to the direct coupling of the benzyloxycarbonylamino-acid and amino-acid ester by, for example, dicyclohexylcarbodi-imide. This latter reagent afforded the benzyloxycarbonyl-dipeptide ester in only 24% yield.

Further extension of the peptide chain was possible by way of oxazolone intermediates, as in the α-methylalanine series. Acidic hydrolysis [10] of the benzyloxycarbonyl-dipeptide methyl ester afforded the free acid,† which was converted by brief warming with acetic anhydride into the oxazolone (IIa). Reaction of this oxazolone with methyl 1-aminocyclohexanecarboxylate yielded the expected tripeptide methyl ester derivative. In similar manner, reaction of (IIa) with the amino-acid t-butyl ester (prepared *via* the benzyloxycarbonyl derivative) yielded the corresponding benzyloxycarbonyl-tripeptide t-butyl ester.

Some experiments were also carried out in the *N*-formyl series. Dehydration of 1-formylaminocyclohexanecarboxylic acid with thionyl chloride and triethylamine, or more

* It would have been interesting to compare the corresponding reactions of 1-aminocyclohexanecarboxylic acid derivatives in which the conformations were fixed and Dr. L. Munday kindly provided small samples of the *cis*- and *trans*-4-t-butyl acids for this purpose. However these compounds proved to be completely insoluble in aqueous-organic media at all pH's between 7 and 13, and the benzyloxycarbonyl derivatives could not be prepared by the usual method.

† Alkaline hydrolysis could not be used because of hydantoin formation. A superior route to the dipeptide acid would be *via* acidic cleavage of the t-butyl ester.

[5] T. A. Connors and W. C. J. Ross, *J.*, 1960, 2119.

[6] S. Shankman, S. Higa, F. Lew, and M. E. Roberts, *J. Med. Pharm. Chem.*, 1962, **5**, 42.

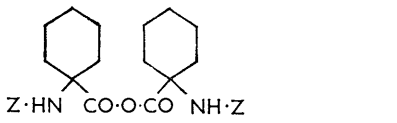
[7] P. Tailleur and L. Berlinguet, *Canad. J. Chem.*, 1961, **39**, 1309; *J. Org. Chem.*, 1962, **27**, 653.

[8] M. Tichy, J. Jones, and J. Sicher, *Coll. Czech. Chem. Comm.*, 1959, **24**, 3434; R. D. Stolow, *J. Amer. Chem. Soc.*, 1959, **81**, 5806.

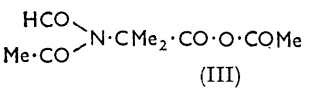
[9] W. Simon, E. Kovats, L. H. Chopard-dit-Jean, and E. Heilbronner, *Helv. Chim. Acta*, 1954, **37**, 1872.

[10] J. R. Vaughan and J. A. Eichler, *J. Amer. Chem. Soc.*, 1954, **76**, 2474.

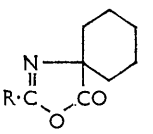
efficiently with warm acetic anhydride, readily yielded the oxazolone (IIc). This reaction with acetic anhydride was unexpected, because formyl-α-methylalanine yielded the *N*-formyl-*N*-acetyl mixed anhydride (III) under these conditions.[2] This difference between the α-methylalanine and 1-aminocyclohexanecarboxylic acid series may also have a conformational origin. Presumably formyl-α-methylalanine does not yield an oxazolone when

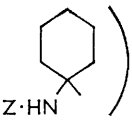


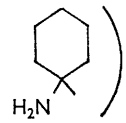
(I : Z = $C_6H_5 \cdot CH_2 \cdot OCO$)



(III)



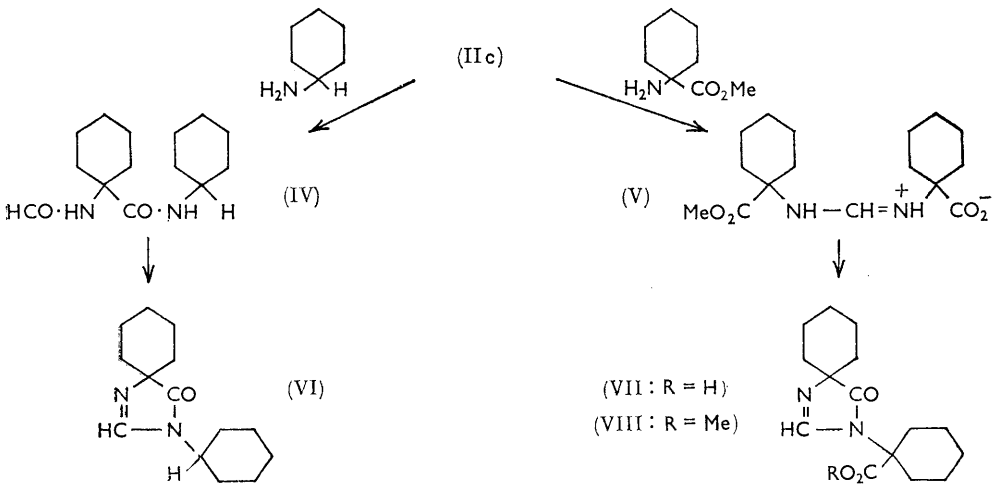
(IIa : R = )

(IIb : R = )

(IIc : R = H)

treated with acetic anhydride because *N*-acetylation precedes activation of the carboxyl group by formation of the mixed anhydride. In the cyclohexane series, this relationship could be reversed if the formylamino-group was in the more hindered axial conformation, and the carboxyl group equatorial. This contrasts with the previously suggested axial orientation of the carboxyl group in the benzyloxycarbonylamino-acid, but in that case the acylamino-group was very much more bulky. In support of the equatorial configuration of the carboxyl group, 1-formylaminocyclohexanecarboxylic acid (pK_a 3·98) was found to be an acid of comparable strength to formyl-α-methylalanine (pK_a 3·81). It is thus understandable that, in the cyclohexane series, activation of the carboxyl group and cyclisation to the oxazolone (IIc) is faster than *N*-acetylation, whereas with formyl-α-methylalanine the converse is true.

The crystalline oxazolone (IIc) proved to be distinctly more stable than 4,4-dimethyloxazolone,[2] but its reactions with amines closely paralleled those of the latter compound.



Thus reaction with cyclohexylamine yielded the normal product (IV) of attack at the oxazolone carbonyl group. Reaction with the more sterically hindered amine, methyl 1-aminocyclohexanecarboxylate, yielded the abnormal product (V) resulting from attack of the amine at the methine carbon atom of the oxazolone ring. The structures of (IV)

and (V) were established by their spectral properties (which were similar to those of the analogous compounds in the α-methylalanine series), by the formation of a stable hydrochloride of (V), and by their thermal cyclisation to the imidazolones (VI) and (VII). A small proportion of the corresponding methyl ester (VIII) accompanied (VII), and this was synthesised by reaction between ethyl orthoformate and methyl 1-aminocyclohexane carboxylate.

The utility of the dipeptide oxazolone derivative (IIa) for the preparation of polypeptide derivatives was investigated briefly. Hydrogenolysis of the benzyloxycarbonyl group yielded the oily amine (IIb), which was polymerised by heating, first in toluene solution and then in the solid state. A small amount of material sublimed during the latter process, and this was shown to be the dioxopiperazine (3%) by comparison with an authentic sample prepared by heating 1-formylaminocyclohexanecarboxylic acid. The involatile residue had the typical infrared spectrum of a polypeptide and was free from bands attributable to residual oxazolone. The weight average degree of polymerisation was estimated to be approximately 55 residues (mol. wt. *ca.* 6900) by viscosity measurement in dichloroacetic acid solution. Polymeric 1-aminocyclohexanecarboxylic acid has previously been prepared by the carboxyanhydride method.[11]

Experimental

1-Benzyloxycarbonylaminocyclohexanecarboxylic Anhydride (I).—Pivaloyl chloride (3·6 g.) was added slowly to a stirred and cooled (0°) solution of 1-benzyloxycarbonylaminocyclohexanecarboxylic acid [7,11] (8·31 g.) and triethylamine (3·03 g.) in dry benzene (40 ml.). After 2 hr. at 0° and 1 hr. at room temp. the solution was filtered and the filtrate evaporated. Light petroleum was added to the oily residue, and after standing overnight at 0°, the crystalline *anhydride* (7·95 g., 99%) was collected, m. p. 134—137°, raised to 138—139° after recrystallisation from cold benzene by the addition of light petroleum (Found: C, 67·4; H, 7·0; N, 5·2. $C_{30}H_{36}N_2O_7$ requires C, 67·1; H, 6·8; N, 5·2%).

Methyl 1-Aminocyclohexanecarboxylate.—Dry hydrogen chloride was passed into a boiling mixture of 1-aminocyclohexanecarboxylic acid hydrochloride (28 g.) and dry methanol (250 ml.) during 1 hr., and the clear solution heated for 8 hr. further. Next morning the solution was evaporated and the residual hydrochloride recrystallised from methanol, m. p. 340° (decomp.). The finely powdered product was stirred in suspension with chloroform (100 ml.) and liquid ammonia added dropwise until an excess was present. The mixture was stirred for several minutes, filtered, and the filtrate distilled. The *methyl ester* (14·2 g., 58%) had b. p. 84°/15 mm. (Found: C, 61·1; H, 9·4; N, 9·3. $C_8H_{15}NO_2$ requires C, 61·1; H, 9·6; N, 8·9%).

Methyl 1-(1′-Benzyloxycarbonylaminocyclohexanecarbonylamino)cyclohexanecarboxylate.—1-Benzyloxycarbonylaminocyclohexanecarboxylic anhydride (1·79 g.) was added to a solution of methyl 1-aminocyclohexanecarboxylate (0·52 g.) in dry benzene (25 ml.) at 0°, and after 30 min. was set aside at room temp. overnight. The solution was diluted with ethyl acetate, and the neutral product isolated by washing with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water. Evaporation of the organic phase yielded the *dipeptide-derivative* (1·28 g., 92%), m. p. 114—115°, raised by recrystallisation from ethyl acetate–light petroleum to 115° (Found: C, 66·15; H, 7·8; N, 7·0. $C_{23}H_{32}N_2O_5$ requires C, 66·3; H, 7·7; N, 6·7%). Acidification of the sodium hydrogen carbonate extracts yielded recovered 1-benzyloxycarbonylaminocyclohexanecarboxylic acid (0·76 g., 83%).

1-(1′-Benzyloxycarbonylaminocyclohexanecarbonylamino)cyclohexanecarboxylic Acid.—The foregoing ester (0·4 g.) was hydrolysed by boiling with a mixture of dioxan (5 ml.) and 2N-hydrochloric acid (1 ml.) during 6 hr. After evaporation of the solution, the residue was dissolved in ethyl acetate, extracted with aqueous sodium hydrogen carbonate, and the extracts acidified. The crystalline *benzyloxycarbonyldipeptide* (0·19 g., 50%) was collected, m. p. 166—167°, unchanged after recrystallisation from benzene–light petroleum (Found: C, 65·3; H, 7·7; N, 7·2. $C_{22}H_{30}N_2O_5$ requires C, 65·65; H, 7·5; N, 7·0%).

2-(1′-Benzyloxycarbonylaminocyclohexyl)cyclohexanespiro-4-oxazol-5-one (IIa).—The foregoing

[11] R. N. Macdonald, U.S.P. 2,572,843 (1951); *Chem. Abs.*, 1952, **46**, 778g.

benzyloxycarbonyldipeptide (0·40 g.) was heated at 110—120° (oil bath) with acetic anhydride (4 ml.) during 15 min. The excess acetic anhydride was distilled, and the last traces removed by repeated evaporation with toluene (2 × 4 ml.). The residual oxazolone (0·38 g.) ($\nu_{max.}$ 915, 1000, 1125, 1250, 1510, 1720, 1820, and 3320 $cm.^{-1}$) could not be crystallised.

Methyl 1-[1′-(1″-*Benzyloxycarbonylaminocyclohexanecarbonylamino*)*cyclohexanecarbonylamino*]*cyclohexanecarboxylate.*—The foregoing oxazolone (0·38 g.) in dry acetonitrile (8 ml.) was heated under reflux with methyl 1-aminocyclohexanecarboxylate (0·31 g.) during 6 hr. After evaporation of the solvent, the residual oil slowly crystallised on standing at 0°. The *tripeptide-derivative* (0·38 g.), collected and washed with benzene–light petroleum, had m. p. 162° (Found: C, 66·8; H, 8·2; N, 7·9. $C_{30}H_{43}N_3O_6$ requires C, 66·5; H, 8·0; N, 7·8%).

t-Butyl 1-*Benzyloxycarbonylaminocyclohexanecarboxylate.*—A solution of 1-benzyloxycarbonylaminocyclohexanecarboxylic acid (2·77 g.) in methylene dichloride (40 ml.) containing concentrated sulphuric acid (0·1 ml.) was saturated with isobutene at room temp. and then set aside for 3 days. The mixture was then added to N-sodium hydrogen carbonate solution (20 ml.), the methylene dichloride layer separated, dried (Na_2SO_4) and evaporated. The *ester* (3·06 g., 92%) had m. p. 59—61° (Found: C, 68·6; H, 8·1; N, 4·4. $C_{19}H_{27}NO_4$ requires C, 68·4; H, 8·2; N, 4·2%).

t-Butyl 1-*Aminocyclohexanecarboxylate.*—The foregoing benzyloxycarbonyl derivative (2·83 g.) in methanol (100 ml.) was hydrogenated over a 5% palladium–charcoal catalyst (0·3 g.) until the evolution of carbon dioxide ceased (10 hr.). The solution was filtered and evaporated, leaving the residual t-butyl ester as a colourless liquid (1·59 g.). The *picrate* had m. p. 146—147° (from benzene–light petroleum) (Found: C, 47·9; H, 5·6; N, 13·4. $C_{17}H_{24}N_4O_9$ requires C, 47·7; H, 5·6; N, 13·1%).

t-Butyl 1-[1′-(1″-*Benzyloxycarbonylaminocyclohexanecarbonylamino*)*cyclohexanecarbonylamino*]*cyclohexanecarboxylate.*—A solution of the foregoing t-butyl ester (0·54 g.) and the oxazolone (IIa) (0·585 g.) in dry acetonitrile (7·5 ml.) was heated under reflux during 8 hr. and then evaporated. The residue was dissolved in ethyl acetate which was washed successively with 1% aqueous citric acid, sodium hydrogen carbonate solution, and water, dried (Na_2SO_4), and evaporated. The *tripeptide-derivative* (0·35 g., 40%), m. p. 163—165°, recrystallised from ethyl acetate–light petroleum with unchanged m. p. (Found: C, 67·8; H, 8·3; N, 7·2. $C_{33}H_{49}N_3O_6$ requires C, 67·9; H, 8·5; N, 7·2%).

1-*Formylaminocyclohexanecarboxylic Acid.*—Acetic anhydride (13·3 ml.) was added during 15 min. to a solution of 1-aminocyclohexanecarboxylic acid (2·40 g.) in 98% formic acid (33 ml.) at 7—12°. The mixture was stirred at 15° for 30 min. and at room temp. for 2 hr. before being diluted with ice-cold water (14 ml.) and evaporated. Crystallisation of the product from water yielded the *formyl derivative* (2·87 g., 71%), m. p. 190—191° (Found: C, 55·9; H, 7·7; N, 8·15. $C_8H_{13}NO_3$ requires C, 56·1; H, 7·65; N, 8·2%).

Cyclohexanespiro-4-oxazol-5-one (IIc).—A solution of the foregoing formyl derivative (0·855 g.) in acetic anhydride (20 ml.) was heated at 110—120° (bath temp.) during 15 min. before being concentrated to 4 ml. The residual solution was transferred to a cold-finger sublimation apparatus, and the *oxazolone* (0·69 g., 92%) sublimed at 50°/0·05 mm. as large plates, m. p. 47—48° (Found: C, 63·5; H, 7·6; N, 8·6. $C_8H_{11}NO_2$ requires C, 62·7; H, 7·2; N, 9·1%). The *oxazolone hydrochloride* was prepared by reaction between 1-formylaminocyclohexanecarboxylic acid (0·86 g.) and thionyl chloride (10 ml.) during 15 min. at room temp. and dilution of the reaction mixture with light petroleum. Yield, 0·94 g. (99·5%). The hydrochloride decomposed above 130° without melting (Found: C, 50·8; H, 6·2; Cl, 18·5; N, 7·1. $C_8H_{12}ClNO_2$ requires C, 50·8; H, 6·35; Cl, 18·75; N, 7·4%). The hydrochloride was converted into the free oxazolone by treatment with triethylamine and sublimation of the product.

1-*Formylaminocyclohexane*(N-*cyclohexyl*)*carbonamide* (IV).—Cyclohexylamine (0·4 g.) was added to a cooled solution of cyclohexanespiro-4-oxazol-5-one (0·31 g.) in dry benzene (10 ml.). The *cyclohexylamide* (0·45 g.) separated almost immediately and after a few minutes was collected and recrystallised from aqueous methanol. Yield, 0·41 g. (82%), m. p. 214—215° (Found: C, 66·95; H, 9·6; N, 11·2. $C_{14}H_{24}N_2O_2$ requires C, 66·6; H, 9·6; N, 11·1%).

3-*Cyclohexyl*-5-*cyclohexanespiroimidazol*-4-*one* (VI).—The foregoing cyclohexylamide (0·25 g.) was heated at 260° during 10 min., whereupon the *imidazolone* (0·18 g., 77%) sublimed, m. p. 139—141° (Found: C, 71·6; H, 9·3; N, 12·2. $C_{14}H_{22}N_2O$ requires C, 71·75; H, 9·5; N, 12·0%).

NN′-*Bis*(1-*carboxycyclohexyl*)*formamidine Monomethyl Ester* (V).—Cyclohexanespiro-4-oxazol-5-one (0·40 g.) was added to a solution of methyl 1-aminocyclohexanecarboxylate (0·47 g.)

in dry benzene (5 ml.). After standing overnight, the crystalline *formamidine* (0·55 g.) was collected and washed with benzene, m. p. 163° (Found: C, 61·6; H, 8·1; N, 8·8%. $C_{16}H_{26}N_2O_4$ requires C, 61·9; H, 8·4; N, 9·0%). The *hydrochloride* (0·21 g.), prepared by saturating a chloroform solution (5 ml.) of the foregoing formamidine (0·2 g.) with hydrogen chloride and evaporation, had m. p. 191—193°, raised to 192—193° by recrystallisation from ethanol–benzene (Found: C, 55·5; H, 7·8; N, 7·8. $C_{16}H_{27}ClN_2O_4$ requires C, 55·4; H, 7·85; N, 8·1%).

3-(1′-*Carboxycyclohexyl*)-5-*cyclohexanespiroimidazol*-4-*one* (VII).—The foregoing formamidine (V) (0·05 g.) was heated in an evacuated sublimation tube at 140° during 5 hr. The sublimate (1·3 mg., 2·8%) was identified as the imidazolone methyl ester (VIII) by m. p. (86—87°) and mixed m. p. (86—87°) with material prepared as below. The involatile residue was recrystallised from water and yielded the *imidazolone acid monohydrate* (39 mg., 86%), m. p. 224—226° (Found: C, 61·3; H, 8·2; N, 9·7. $C_{15}H_{24}N_2O_4,H_2O$ requires C, 60·8; H, 8·2; N, 9·45%). The anhydrous *compound*, m. p. 224—226°, was obtained by drying at 100°/0·5 mm. (Found: C, 64·3; H, 8·0; N, 10·2. $C_{15}H_{24}N_2O_4$ requires C, 64·7; H, 8·0; N, 10·1%).

3-(1′-*Methoxycarbonylcyclohexyl*)-5-*cyclohexanespiroimidazol*-4-*one* (VIII).—(*a*) A mixture of methyl 1-aminocyclohexanecarboxylate (0·79 g.) and ethyl orthoformate (0·25 g.) was heated under reflux for 8 hr. and then set aside at room temp. overnight. Concentration *in vacuo* yielded colourless needles of the *imidazolone ester* which, recrystallised from ether–light petroleum (0·35 g., 72%), had m. p. 87° (Found: C, 65·6; H, 8·1; N, 9·7. $C_{16}H_{24}N_2O_3$ requires C, 65·7; H, 8·3; N, 9·6%).

(*b*) Methylation of the imidazolone acid (VII) with excess of ethereal diazomethane in the usual manner afforded the methyl ester (63%), m. p. and mixed m. p. 87°.

2,5-*Bis*(*cyclohexanespiro*)-3,6-*dioxopiperazine*.—1-Formylaminocyclohexanecarboxylic acid (1·0 g.) was heated rapidly to 140° and then slowly to 200—205° with stirring. At 195—200° the solid melted and there was rapid evolution of carbon monoxide; after a further 10 min. at 200—205° the melt solidified. The stiff paste was cooled and triturated with N-sodium hydroxide. Recrystallisation of the insoluble product from aqueous acetic acid yielded the dioxopiperazine (0·18 g., 21%) (Found: C, 66·9; H, 8·9; N, 11·2. $C_{14}H_{22}N_2O_2$ requires C, 67·2; H, 8·9; N, 11·2%).

Poly(1-*aminocyclohexanecarboxylic Acid*).—2-(1′-Benzyloxycarbonylaminocyclohexyl)-4-cyclohexanespiro-oxazol-5-one (0·77 g.) dissolved in anhydrous ethyl acetate (90 ml.) was hydrogenated over 5% palladium–charcoal catalyst (0·2 g.) until carbon dioxide evolution ceased (4·5 hr.). Filtration and evaporation of the filtrate yielded the oily oxazolone (IIb) (0·51 g.) ($\nu_{max.}$ 850, 915, 1000, 1060, 1350, 1450, 1670, 1820, 2950, and 3300 cm.$^{-1}$). This oxazolone was dissolved in anhydrous toluene (14 ml.) and heated under reflux for 24 hr. The toluene was evaporated and the residual polymer (0·39 g.) heated in a sublimation tube at 160°/0·05 mm. during 3 hr. The sublimed dioxopiperazine (11 mg., 3%) was removed and the involatile polymer collected ($\nu_{max.}$ 1175, 1290, 1380, 1460, 1530, 1670, and 3350 cm.$^{-1}$).

We are grateful to Dr. H. Block for the viscosity measurements.

THE ROBERT ROBINSON LABORATORIES,
UNIVERSITY OF LIVERPOOL.

[*Received, May 12th,* 1965.]