588. *Synthetic Polypeptides. Part I.*

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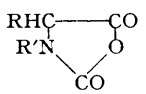
The preparation of several *N*-carboxyamino-acid anhydrides (I) is described. The methods that have been used for the polymerisation of these anhydrides are discussed and a new general method has been investigated. In this procedure the anhydrides are polymerised in nitrobenzene solution by amino-acid dimethylamides to give quantitative yields of polypeptides; co-polymers can readily be prepared by this method. The properties of the polymers and co-polymers are described.

The preparation and polymerisation of N-carboxyamino-acid anhydrides (I) was first described by Leuchs (*Ber.*, 1906, **39**, 857; 1907, **40**, 3235; 1908, **41**, 1721) and later studied by Curtius and Sieber (*ibid.*, 1922, **55**, 1543), Fuchs (*ibid.*, 1922, **55**, 2943), and by Wessely and his co-workers (*Z. physiol. Chem.*, 1925, **146**, 72; 1926, **157**, 91; 1926, **159**, 102; 1927, **170**, 167; *Monatsh.*, 1927, **48**, 1). These workers investigated the reaction of *N*-carboxyamino-acid anhydrides with water, alcohols, and amines and established that generally a mixture of products resulted.



(I.)

$H\cdot[NR'\cdot CHR\cdot CO]_n\cdot OEt$ (II.)

$H\cdot[NR'\cdot CHR\cdot CO]_n\cdot NMe_2$ (III.)

Thus decomposition with ethanol yielded the amino-ester (II; $n = 1$) and polymers (II; $n = 2, 3, 4 \ldots$). By varying the experimental conditions good yields either of the primary fission product or of polymers could be obtained. Some tertiary amines (*e.g.*, triethylamine) were found to affect the *N*-carboxyamino-acid anhydrides little, but pyridine readily afforded polymers. Even when precautions were taken to dehydrate the pyridine it still decomposed the anhydrides, and the nature of this reaction has not been elucidated.

During the last few years the synthesis of polypeptides by the polymerisation of *N*-carboxyamino-acid anhydrides has become a preparative reaction of some importance. Various methods of polymerisation have been employed. Thus pyridine has been used, either alone (Go and Tani, *Bull. Chem. Soc., Japan*, 1939, **14**, 510; Hanby, Waley, and Watson, *Nature*, 1948, **161**, 132) or in ethyl acetate solution (Astbury, Dalgliesh, Darmon, and Sutherland, *Nature*,

1948, **162**, 596). The last workers also conducted polymerisations in moist benzene, a method first used by Woodward and Schramm (*J. Amer. Chem. Soc.*, 1947, **69**, 1551). The anhydrides have also been polymersied by heat *in vacuo* (Frankel, Grossfeld, and Katchalski, *ibid.*, 1948, **70**, 2094; Frankel and Berger, *Nature*, 1949, **163**, 213; Katchalski and Spitnik, *Nature*, 1949, **164**, 1092). There seems thus to be little agreement about the best conditions for carrying out these polymerisations, and, until recently, no detailed study of the mechanism had been made.

The mechanism of the polymerisation of *N*-carboxysarcosine anhydride (I; R = H, R′ = Me) has now been elucidated (Waley and Watson, *Proc. Roy. Soc.*, *A*, 1949, **199**, 499). When polysarcosine dimethylamide (III; R′ = Me, R = H; $n \simeq 10$) was used to initiate the polymerisation of the anhydride in nitrobenzene solution the reaction took place smoothly and quantitatively at room temperature.

The progress of the reaction is readily followed by observing the evolution of carbon dioxide; the polymerisation is carried out in an evacuated flask with a manometer attached. The use of a low polymer to initiate the polymerisation has the advantage of eliminating the side reactions observed in some cases [formation of diketopiperazines (Wessely and Sigmund, *Z. physiol. Chem.*, 1926, **159**, 102) or of hydantoins (Wessely, *ibid.*, 1927, **170**, 167)]. Also the variation of velocity constants with length of chain is minimised. But for preparative purposes the use of the monomeric dimethylamide (III; $n = 1$) is usually satisfactory. This method seems more convenient than the others, and is especially satisfactory for co-polymerisations. In such reactions it is useful to have some idea of the overall rate of reaction of an anhydride (I) with the corresponding dimethylamide (III; $n = 1$). Some relative rates, referred to *N*-carboxysarcosine anhydride as unity, are set out in the table below; they are necessarily only a rough guide because the rates are governed by several velocity constants:

R.	R′.	Relative velocity.	R.	R′.	Relative velocity.
H	H	3	$C_6H_5{\cdot}CH_2$	H	10^{-1}
Me	H	1	H	$(CH_3)_2CH$	10^{-4}

These may be termed " straight reactions " in that the anhydride is reacting with the dimethylamide derived from the same amino-acid. But in co-polymerisations " cross-reactions " also occur, in which the anhydride reacts with the dimethylamide derived from a different amino-acid. Measurement of the initial rates of the reaction of *N*-carboxyglycine anhydride (I; R = R′ = H) with sarcosine dimethylamide (III; R = H, R′ = Me, $n = 1$), and of *N*-carboxysarcosine anhydride (I; R = H, R′ = Me) with glycine dimethylamide (III; R = R′ = H; $n = 1$), showed that the rates of the cross-reactions were of the same order as those of the corresponding straight reactions.

Our method of polymerisation can also be used to prepare polypeptides in which a number of residues of one amino-acid are followed by a number of residues of another amino-acid. Thus *N*-carboxyglycine anhydride has been decomposed by polysarcosine dimethylamide (III; R = H, R′ = Me, $n = 100$) to give a polymer in which, on the average, about a hundred glycine residues are followed by a hundred sarcosine residues. This novel method of preparing mixed polypeptides is very useful in certain cases. (The generic term " polypeptide " is used in this paper, the terminal dimethylamide group being neglected.)

N-Carboxyamino-acid anhydrides are usually prepared from the *N*-carbalkoxy-derivative (IV) by treatment with phosphorus pentachloride or thionyl chloride; the intermediate acid chlorides are not usually isolated. Although the early method of treating the

(IV.) $R''O_2C{\cdot}NHR'{\cdot}CHR{\cdot}CO_2H$ $Ph{\cdot}CH_2{\cdot}CH(CO_2H){\cdot}CO{\cdot}NH{\cdot}OR$ (V.)

carbomethoxy-derivative (IV; R″ = Me) with thionyl chloride is still the most common, the carbobenzyloxy-derivatives (IV; R″ = CH_2Ph) cyclise more readily and the use of phosphorus pentachloride frequently gives a cleaner product. Attempts to use the methoxybenzyl derivatives (IV; R = $CH_2{\cdot}C_6H_4{\cdot}OMe$) were unsuccessful, since methoxybenzyl chloroformate decomposed before it could be coupled with the amino-acid or characterised.

The anhydrides have also been prepared by the Curtius degradation (Curtius and Sieber, *loc. cit.*), and we attempted to use the Lossen degradation similarly. Ethyl benzylmalonate was hydrolysed with one equivalent of potassium hydroxide, and the half ester condensed with hydroxylamine to give α-carboxy-β-phenylpropionhydroxamic acid (V; R = H). Benzoylation afforded the *O*-benzoyl compound (V; R = Bz), but neither this nor its potassium salt yielded the desired *N*-carboxyphenylalanine anhydride (I; R = CH_2Ph, R′ = H) when heated.

Polymerisation of the appropriate anhydride, by the method described above, gave

polyglycine, poly-DL-alanine, polysarcosine, poly-L-leucine, poly-DL-valine, and poly-DL-phenylalanine dimethylamides. Of these, only poly-DL-alanine and polysarcosine dimethylamides were appreciably soluble in water, and only the latter was soluble in nitrobenzene and acetophenone. Most of these polypeptides were tough, horny solids, which dissolved in concentrated sulphuric acid and in dichloroacetic acid. Although they decomposed slowly in sulphuric acid, solutions in dichloroacetic were much more stable.

We hoped to be able to determine the molecular weights of these polypeptides by amino-nitrogen (van Slyke) analyses in dichloroacetic acid solution. This solvent, however, proved to be unsuitable for the determinations. By amino-nitrogen analysis a polymer of DL-alanine was found to have a degree of polymerisation of about 100; this agrees reasonably with the value (150) predicted from the amounts of *N*-carboxy-DL-alanine anhydride and initiator used.

By using two *N*-carboxyamino-acid anhydrides, co-polymers of the following were prepared: glycine–sarcosine, α-amino*iso*butyric acid–DL-phenylalanine, α-amino*iso*butyric acid–DL-valine, glycine–DL-phenylalanine, DL-valine–DL-phenylalanine, L-leucine–DL-phenyalanine. Co-polymers were also made from *N*-carboxy-γ-methyl-L-glutamate anhydride (I; R = $MeO_2C{\cdot}CH_2{\cdot}CH_2$, R′ = H) (whose preparation is described in Part II) with *N*-carboxyglycine anhydride and *N*-carboxy-DL-phenylalanine anhydride. In some cases, the solubilities of the co-polymers were intermediate between those of the simple polymers, but in other cases were greater than either. As with the simple polymers, the only satisfactory general solvent was dichloroacetic acid although in particular cases other solvents were found. Like other high polymers, these polypeptides often swell markedly in non-solvents.

In most cases evaporation of a solution of the polymer gave a coherent film. Extrusion of the solution into such non-solvents as water, alcohol, or ether afforded fibres. None of the polymers or co-polymers melted without decomposition.

Experimental.

N-Carbomethoxy-DL-valine.—A solution of DL-valine (23·5 g.) in water (250 c.c.) containing sodium hydroxide (16 g.) was cooled to 0° and methyl chloroformate (20 c.c.) added with stirring. After 1½ hours, the solution was extracted with ether, the aqueous layer acidified, and the oil isolated with ether. The *carbomethoxy*-compound, recrystallised from water, had m. p. 88—89° (23·7 g.) (Found: C, 48·0; H, 7·4; N, 8·0. $C_7H_{13}O_4N$ requires C, 48·0; H, 7·4; N, 8·0%).

N-Carboxy-DL-valine Anhydride.—Thionyl chloride (10 c.c.) and *N*-carbomethoxy-DL-valine (5 g.) were heated at 60° for 10 minutes. The *anhydride* was precipitated on the addition of light petroleum, and crystallised from ethyl acetate–light petroleum in colourless needles (3·8 g.), m. p. 78—79° (Found: C, 50·85; H, 6·5; N, 9·5. $C_6H_9O_3N$ requires C, 50·4; H, 6·3; N, 9·8%).

*α-N-Carbomethoxyamino*iso*butyric Acid.*—Methyl chloroformate (55 c.c.) was added to a stirred, cooled solution of α-amino*iso*butyric acid (50 g.) in 2N-sodium hydroxide (550 c.c.). After ¾ hour the solution was acidified, and the *amide* collected and recrystallised from water; it (29 g.) had m. p. 158—160° (Found: C, 45·1; H, 6·75; N, 8·8. $C_6H_{11}O_4N$ requires C, 44·7; H, 6·8; N, 8·7%).

*α-N-Carboxyamino*iso*butyric Anhydride.*—Thionyl chloride (20 c.c.) and *N*-carbomethoxy-α-amino-*iso*butyric acid (9 g.) were heated together for 10 minutes. The *anhydride*, precipitated by the addition of light petroleum, crystallised from amyl acetate–light petroleum in stout needles (6 g.), m. p. 100° (Found: C, 46·7; H, 5·55; N, 10·8. $C_5H_7O_3N$ requires C, 46·5; H, 5·4; N, 10·9%).

N-Carbomethoxy-DL-norleucine.—DL-Norleucine (13 g.) in N-sodium hydroxide (100 c.c.) was treated with methyl chloroformate (9 c.c.) and 6% sodium carbonate solution (100 c.c.) at 0°. After 3 hours, the *amide* (17 g.) was isolated as usual; recrystallised from carbon tetrachloride it had m. p. 64° (Found: C, 51·1; H, 8·0; N, 7·6. $C_8H_{15}O_4N$ requires C, 50·8; H, 7·95; N, 7·4%).

N-Carboxy-DL-norleucine Anhydride.—Phosphorus pentachloride (17·5 g.) was added to carbomethoxy-DL-norleucine (9·6 g.) in benzene (50 c.c.). After 3 hours at 60° the solvent was distilled off, and the residue extracted with light petroleum; the *anhydride* crystallised in low yield from the light petroleum extracts, and after purification by recrystallisation from carbon tetrachloride had m. p. 84—86° (decomp.) (Found: C, 53·9; H, 7·4; N, 8·9. $C_7H_{11}O_3N$ requires C, 53·5; H, 7·0; N, 8·9%).

N-Carboxy-N-Methyl-DL-alanine Anhydride.—Methyl chloroformate (25 c.c.) was added to a stirred, cooled solution of *N*-methyl-DL-alanine (28 g.) in 15% aqueous sodium hydrogen carbonate (380 c.c.). After 3 hours, the solution was acidified and evaporated. The oily amide was isolated with ether, treated with thionyl chloride (25 c.c.), and heated at 40° for 10 minutes. The *anhydride* was precipitated with light petroleum and recrystallised from benzene (yield, 13·7 g.). After sublimation *in vacuo* it had m. p. 75—76°, unchanged by recrystallisation from carbon tetrachloride (Found: C, 46·3; H, 5·2; N, 10·6. $C_5H_7O_3N$ requires C, 46·5; H, 5·4; N, 10·85%).

N-Carboxy-N-methyl-DL-phenylalanine Anhydride.—Methyl chloroformate (1·5 c.c.) was added to a cooled solution of *N*-methyl-DL-phenylalanine (3·3 g.) in 0·5N-sodium hydroxide (36 c.c.) containing sodium hydrogen carbonate (1·6 g.). The solution was then warmed, further sodium hydrogen carbonate (1·5 g.) and methyl chloroformate (1·4 c.c.) were added, and stirring was continued until the solution became clear. After acidification the oily amide (3·7 g.) was isolated with chloroform. This amide was treated with thionyl chloride as usual; the *N-carboxyamino-acid anhydride* crystallised from carbon

tetrachloride or benzene–*cyclo*hexane in feathery needles, m. p. 104—105° (Found : C, 64·3; H, 5·4; N, 6·9. $C_{11}H_{11}O_3N$ requires C, 64·4; H, 5·4; N, 6·8%).

N-*Benzyl*-N-*carboxy*-DL-*alanine Anhydride*.—Methyl chloroformate (7 c.c.) was added to a cooled solution of *N*-benzyl-DL-alanine (9 g.) in 6% sodium hydroxide solution (100 c.c.) and shaken for 5 minutes. The mixture was acidified and the oily amide (7·5 g.) isolated with ether, treated with thionyl chloride (8 c.c.), and heated at 50° for 20 minutes. After removal of excess of thionyl chloride the residue was triturated with light petroleum, and the *anhydride* twice recrystallised from ethyl acetate–*cyclo*hexane; it had m. p. 60—61° (4·5 g.) (Found : C, 64·0; H, 5·5; N, 7·0. $C_{11}H_{11}O_3N$ requires C, 64·4; H, 5·4; N, 6·8%).

N-*Carboxy*-DL-iso*leucine Anhydride*.—The crude oily *N*-carbomethoxy-DL-*iso*leucine (15 g.), prepared in the same way as *N*-carbomethoxy-DL-norleucine, was treated with thionyl chloride (15 c.c.) and boiled for 10 minutes. After removal of excess of thionyl chloride, the residue, triturated under light petroleum and then recrystallised from carbon tetrachloride, had 70—72° (8·5 g.) (Found : C, 53·8; H, 7·2; N, 8·8. $C_7H_{11}O_3N$ requires C, 53·5; H, 7·0; N, 8·9%).

Glycine Dimethylamide.—*N*-Carboxyglycine anhydride (Go and Tani, *Bull. Chem. Soc. Japan*, 1939, **14**, 510) was decomposed with excess of dimethylamine and, next morning, excess of dimethylamine was removed and the residue distilled. The *dimethylamide*, b. p. 60°/0·8 mm., had equivalent weight 103 (calc. 102) and was converted into the *picrate* which, after recrystallisation from ethanol, had m. p. 190° (Found : C, 36·3; H, 4·1. $C_{10}H_{13}O_8N_5$ requires C, 36·3; H, 3·9%).

DL-*Alanine Dimethylamide*.—*N*-Carboxy-DL-alanine anhydride (2 g.) was added to dimethylamine (25 c.c.) and, next morning, excess of dimethylamine was removed and the residue distilled at 1 mm. The distillate was converted into DL-alanine dimethylamide picrate, which after recrystallisation from ethanol had m. p. 204—206° (Freudenberg and Nikolai, *Annalen*, 1934, **510**, 223, give m. p. 203—204°) (Found : C, 38·6; H, 4·7; N, 19·8. Calc. for $C_{11}H_{15}O_8N_5$: C, 38·3; H, 4·35; N, 20·3%).

DL-*Phenylalanine Dimethylamide*.—*N*-Carboxy-DL-phenylalanine anhydride (1·5 g.) was added to dimethylamine (25 c.c.) and the excess of dimethylamine allowed to evaporate. The *dimethylamide* was distilled at 120°/0·1 mm. (Found : equiv., 190. Calc. for $C_{11}H_{16}ON_2$: equiv., 192). The *picrate*, after recrystallisation from ethanol, had m. p. 225° (Found : C, 48·45; H, 4·75; N, 16·7. $C_{17}H_{19}O_8N_5$ requires C, 48·5; H, 4·5; N, 16·6%).

Poly-DL-*alanine Dimethylamide*.—Freshly re-sublimed *N*-carboxy-DL-alanine anhydride (0·717 g.) was added to 0·011N-sarcosine dimethylamide in nitrobenzene (4 c.c.). After 3 days the *polymer* was precipitated with ether and dried at 70°; a quantitative yield of a waxy solid was obtained (Found : C, 49·7; H, 7·15. C_3H_5ON requires C, 50·8; H, 7·05%). The molecular weight was estimated by extracting the polymer (0·4 g.) with 50% aqueous lithium bromide (50 c.c.); the filtered solution had total N (Kjeldahl) 0·917 mg./c.c. and amino-N (van Slyke) 0·0091 mg./c.c. Thus the fraction (about half) of the polymer which dissolved had an average molecular weight of 7100. The quantities of anhydride and sarcosine dimethylamide used should give a polymer of average molecular weight 10,070.

The polymers described in the annexed table were prepared by the above general method. Elementary analyses of these polypeptides were frequently unsatisfactory.

		Found, %.			Calculated, %.			
Polymer.	$[M]_0/[X]_0$.*	C.	H.	N.	C.	H.	N.	Solubility.
L-Leucine	200	60·0	9·1	12·3	63·7	9·7	12·4	Trichloroacetic acid
DL-Valine	400	58·7	9·1	13·3	60·6	9·1	14·1	
DL-Phenylalanine	300	72·55	6·4	9·2	73·5	6·1	9·5	*m*-Cresol
α-Amino*iso*butyric acid–DL-phenylalanine, 1 : 1	400	66·3	7·0	11·9	67·0	7·3	12·0	*m*-Cresol
α-Amino*iso*butyric acid–DL-valine, 1 : 1	400	57·3	8·8	15·0	59·0	8·75	15·3	Formic acid, *m*-cresol
Glycine–DL-phenylalanine, 1 : 1	400	63·1	6·2	12·9	65·0	5·9	13·8	Trichloroacetic acid
DL-Valine–DL-phenylalanine, 1 : 1	400	66·7	7·5	11·1	68·4	7·3	11·4	
L-Leucine–DL-phenylalanine, 1 : 1	800	68·7	7·7	10·8	69·2	7·7	10·8	Benzene, *m*-cresol, trichloroacetic acid
γ-Methyl-L-glutamate–glycine, 4 : 1	200	48·8	6·5	10·7	49·6	6·2	11·1	
γ-Methyl-L-glutamate–DL-phenylalanine, 1 : 1	400	61·9	6·4	9·8	62·1	6·2	9·7	*m*-Cresol, formic acid, trichloroacetic acid

* $[M]_0/[X]_0$ is the ratio of the initial concentration of *N*-carboxy-anhydride to that of the dimethylamide.

α-*Carboxy*-β-*phenylpropionhydroxamic Acid*.—Ethyl benzylmalonate (60·4 g.) was added to 0·81N-ethanolic potassium hydroxide (300 c.c.) and the solution kept overnight. The solution of the potassium salt of the half ester was treated with ethanolic hydroxylamine [from hydroxylamine hydrochloride (14 g.)], and after several days at 0° the potassium salt (42·4 g.) was collected. This potassium salt (10 g.) in water (20 c.c.) was acidified and the solution evaporated to dryness. The residue was extracted with acetone–ether, the solution filtered, and the filtrate evaporated. On trituration under light petroleum, the material crystallised, and after being washed with chloroform the *hydroxamic acid* (1 g.) crystallised from ethyl acetate in needles, m. p. 150° (decomp.) (Found : C, 57·4; H, 5·4; N, 6·5. $C_{10}H_{11}O_4N$

requires C, 57·4; H, 5·3; N, 6·7%). This substance gave the usual deep red colour with ferric chloride solution.

O-*Benzoate.*—Benzoyl chloride (2·15 c.c.) was added to the hydroxamic acid (2·1 g.) in acetic acid (18 c.c.) and aqueous sodium acetate (18 c.c.). After being shaken for 4 hours the solution was diluted and acidified and the solid collected. After extraction with hot light petroleum, the ON-*diacylhydroxylamine* was recrystallised from toluene; it had m. p. 145—146° (decomp.) (0·6 g.) (Found: C, 64·7; H, 4·9; N, 4·5. $C_{17}H_{15}O_5N$ requires C, 65·2; H, 4·8; N, 4·5%).

Block Co-polymer from N-*Carboxysarcosine Anhydride and* N-*Carboxy*-DL-*phenylalanine Anhydride.*—*N*-Carboxysarcosine anhydride (2·3 g., 0·02 mol.) was added to a 0·01N-solution of sarcosine dimethylamide in nitrobenzene (20 c.c.) and the mixture left overnight in a sealed evacuated flask with a manometer attached. The carbon dioxide evolution having ceased, *N*-carboxy-DL-phenylalanine anhydride (0·191 g., 0·001 mol.) was added, and the apparatus evacuated again and kept until the carbon dioxide evolution had ceased. The polymer was precipitated with light petroleum and washed with hot ethyl acetate (yield, 1·55 g.). It was dissolved in water and extracted with light petroleum to remove any residual nitrobenzene; aliquots of this solution were analysed for total nitrogen (Found: 4·37 mg./c.c.), amino-nitrogen (Found: 0·0175 mg./c.c.), and dimethylamide-nitrogen (Found: 0·047 mg./c.c.). Thus the values of the ratios dimethylamide-nitrogen/total nitrogen and amino-nitrogen/total nitrogen are 0·0108 and 0·004 respectively. The low value of the latter ratio shows that *N*-carboxy-DL-phenylalanine anhydride reacts faster with amino-groups than with methylamino-groups.

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