982. *The Initiation Step in the Polymerization of* N-*Carboxy-α-amino-acid Anhydrides. Part II.*[1] *Effects Related to the Structure of Amine Initiators.*

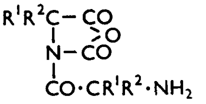
By C. H. Bamford and H. Block.

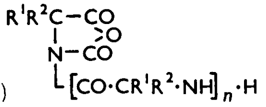
It is shown that the interaction of *N*-carboxy-α-amino-acid anhydrides with primary and secondary bases is not confined to the conventional mechanism involving addition to the 5-carbonyl group in the anhydride molecule. A reaction of the tertiary-base type may also occur, following removal of the proton of the endocyclic NH group; this reaction is fast and leads to polymers with molecular weights greater than expected; it is favoured by steric shielding of the lone pair in the initiator molecule and a high base strength.

In Part I[1] we have shown that the initiation of polymerization of *N*-carboxy-α-amino-acid anhydrides by tertiary bases involves the primary removal of the proton attached to the endocyclic nitrogen atom:

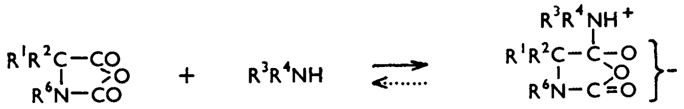

..... (1)

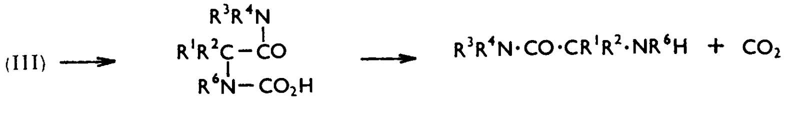
Previous studies[1-3] have indicated that this is followed by attack of the resulting ion (I) on the 5-carbonyl group of a second molecule of anhydride to form a bifunctional intermediate (IIa) which, by propagation and coupling reactions, may achieve the structure (IIb). Primary and secondary amines can initiate polymerization by direct addition to the 5-carbonyl group of the oxazolidine-2,5-dione ring,[4] as shown in schemes

(IIa) 

(IIb) 

(2a and b). If R^6 is not hydrogen, reactions (2a) and (2b) constitute the sole initiation processes with primary and secondary amines. (Addition to the 2-carbonyl group, which occurs much less readily, cannot be regarded as an initiation step.) Tertiary bases do not


(III)
.... (2a)


.... (2b)

react with such anhydrides[2,3] since reaction (2b) cannot occur, but this does not exclude the possibility of an equilibrium similar to (2a) being established.

We show in this paper that if the anhydride and initiator have the requisite protons

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[1] Part I, Bamford and Block, preceding paper.

[2] Ballard, Bamford, and Weymouth, *Nature*, 1954, **174**, 173; Ballard and Bamford, *J.*, 1956, 381.

[3] Bamford, Block, and Pugh, *J.*, 1961, 2057.

[4] See, *e.g.*, Bamford, Elliott, and Hanby, "Synthetic Polypeptides," Academic Press, Inc., New York, 1956; Katchalski and Sela, *Adv. Protein Chem.*, 1958, **13**, 243; Bamford and Block, Symposium on Poly-α-amino-acids, University of Wisconsin, 1961.

both reactions (1) and (2) can take place simultaneously, resulting in rates of reaction and polymer sizes inexplicable in terms of the mechanism (2) alone.

The extent to which these reactions deviate from the conventional scheme depends on the relative contributions of (1) and (2) to the polymerization, which, as discussed below, are determined by the strength and stereochemistry of the initiating base.

EXPERIMENTAL

Materials.—*N*-Carboxysarcosine anhydride, prepared by the general method of Hanby *et al.*,[5] was sublimed at 100°/10^{-4} mm.

N-Carboxy-γ-ethyl-L-glutamate anhydride was prepared and purified as described in Part I.[1]

Di-isopropylamine and n-hexylamine were kept over solid sodium hydroxide and then fractionated. This process was repeated twice.

NN-Dimethylformamide was purified as described in ref. 3.

Purified materials were handled exclusively in a dry-box.

Methods.—The rates of polymerization of *N*-carboxy-γ-ethyl-L-glutamate anhydride in *NN*-dimethylformamide were determined by measurement of the carbon dioxide evolved, using the constant volume technique described previously.[3] Since carbon dioxide catalyses [6] the polymerization of *N*-carboxysarcosine anhydride the rate of reaction of this anhydride with n-hexylamine was measured under effectively zero carbon dioxide pressure in an apparatus to be described later. The reaction with di-isopropylamine was followed in the constant-volume apparatus; it is so slow that for present purposes catalysis by carbon dioxide is irrelevant.

Reduced viscosities of the resulting poly-γ-ethyl-L-glutamate specimens at 0·5% concentration (w/v) were determined in dichloroacetic–trifluoroacetic acid (9 : 1 v/v) at 25°. For this purpose the polymers prepared by di-isopropylamine initiation were precipitated from the reaction mixture with ether. The polymers obtained from n-hexylamine initiation under the conditions used could not be precipitated satisfactorily. The solvent was therefore evaporated in a vacuum at room temperatures, and the polymer redissolved in chloroform and cast into a film.

RESULTS AND DISCUSSION

The Figure shows typical conversion–time curves for the polymerization at 25° of the two anhydrides initiated by di-isopropylamine. In the case of *N*-carboxysarcosine anhydride the reaction is slow initially but accelerates, the conversion–time curve becoming almost linear. The first step in this reaction is therefore slower than the subsequent propagation. This arises from the conversion of the slowly reacting di-isopropylamine into the more reactive base group of polysarcosine.[4] On the other hand, the reaction of *N*-carboxy-γ-ethyl-L-glutamate anhydride with di-isopropylamine is remarkably rapid, and the yield of carbon dioxide is less than 100%. However, these two anhydrides have approximately the same reactivity towards n-hexylamine, the conversion–time curves lying close together and between those for di-isopropylamine initiation.

Di-isopropylamine is a strong base [7] ($K_B = 1{\cdot}1 \times 10^{-3}$ mole l.$^{-1}$) and we believe that its low rate of reaction with *N*-carboxysarcosine anhydride is due to steric hindrance encountered during addition to the 5-carbonyl group of the anhydride [equation (2a)]. On this basis the high reactivity of di-isopropylamine towards *N*-carboxy-γ-ethyl-L-glutamate anhydride must indicate a different mode of initiation, which we identify with ionization according to equation (1), the primary step in the tertiary base-catalysed polymerization.

Support for this is provided by measurements of the reduced viscosity of the polymers.

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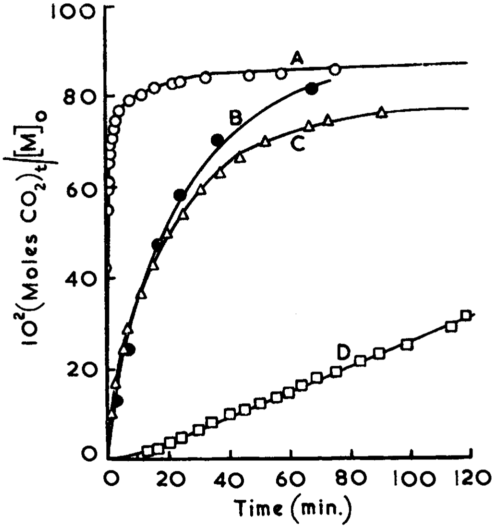
[5] Hanby, Waley, and Watson, *J.*, 1950, 3009.

[6] Ballard and Bamford, *Proc. Roy. Soc.*, 1954, *A*, **223**, 495.

[7] Hall and Sprinkle, *J. Amer. Chem. Soc.*, 1932, **54**, 3469.

The values obtained for poly-γ-ethyl-L-glutamate were 6·12 and 1·46 base mole^{-1} l. for initiation of the polymerization by di-isopropylamine and n-hexylamine respectively. In each case the initial ratio [anhydride] : [initiator] was 15 : 1. Comparison with the results of Doty *et al.*[8] on the molecular weights and intrinsic viscosities of poly-γ-benzyl-L-glutamate gives lower limits of the degrees of polymerization in our cases of 150 and 60 for di-isopropylamine and n-hexylamine, respectively. Similar observations have been reported by Blout and Karlson [9] for the polymerization of *N*-carboxy-γ-benzyl-L-glutamate anhydride initiated by diethylamine and n-hexylamine. The observation that one can obtain polymers with degrees of polymerization greatly in excess of the ratio [anhydride] : [initiator] must be interpreted in terms of a contribution to the reaction by the tertiary-base mechanism. It is known [2,4] that this type of reaction can readily lead to degrees of polymerization greater than the [anhydride] : [initiator] ratio.

If both modes of polymerization [initial steps (1) and (2)] occur together interaction between them will take place during propagation. The bifunctional intermediates (IIa and IIb), in addition to growing at both ends and coupling together as in the normal tertiary base mechanism,[1-3] can also react with the initiator or the terminal base group of a monofunctional chain which arises from initiation according to equation (2). It is thus conceivable that every polymer molecule should carry an initiator fragment as a terminal group, and that the number-average degree of polymerization would be given by the [anhydride] : [initiator] ratio. This is by no means necessary; some chains may be terminated as in the tertiary base-catalysed polymerization (*e.g.*, by cyclization) or there may be unchanged initiator at the end of the reaction. In all cases it is to be expected that the molecular distribution would be broader than that calculated for a polymerization based on (2) above (*i.e.*, a Poisson distribution [4]). The weight average and also the viscosity-average degrees of polymerization may therefore be considerably higher than expected, as is found in the above examples.



Conversion–time curves for polymerization of *N*-carboxy-α-amino-acid anhydrides (concn. 0·224 mole l.$^{-1}$) initiated by amines (concn. 0·015 mole l.$^{-1}$) at 25° in *NN*-dimethylformamide solution.

A, *N*-Carboxy-γ-ethyl-L-glutamate anhydride–di-isopropylamine; B, *N*-carboxysarcosine anhydride–n-hexylamine; C, *N*-carboxy-γ-ethyl-L-glutamate anhydride–n-hexylamine; D, *N*-carboxysarcosine anhydride–di-isopropylamine.

It is apparent that the stereochemistry of the initiating base has a marked effect on the relative importance of reactions 1 and 2, increasing hindrance favouring 1. We believe that the base strength of the initiator will also influence the course of reaction. The concentrations of the intermediate (I), with a series of amines, will be proportional to the square roots of the base strengths. Although in the absence of steric complications

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[8] Doty, Bradbury, and Holtzer, *J. Amer. Chem. Soc.*, 1956, **78**, 947.

[9] Blout and Karlson, *J. Amer. Chem. Soc.*, 1956, **78**, 941.

the association (2a) might appear to depend entirely on base strength, this is not so since the structure (III) really represents a series of tautomers in which the proton may be situated on any of the oxygen atoms. The energy decrease in the tautomerization would be expected to be an inverse function of the base strength. As a result the equilibrium (2a) is less dependent on base strength than is equilibrium (1). Thus, steric considerations apart, the stronger the initiating base, the greater the relative importance of the tertiary base type of initiation (1). These conclusions may be applied to the polymerizations of *N*-carboxy-α-amino-acid anhydrides initiated by preformed polymers, which are known experimentally to be of the first order in anhydride concentration.[4] In these circumstances the base is comparatively weak, so that initiation by (2) is favoured. The complete absence of initiation by (1) cannot be inferred since a minor portion of a tertiary-base type mechanism may not be detectable kinetically.

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