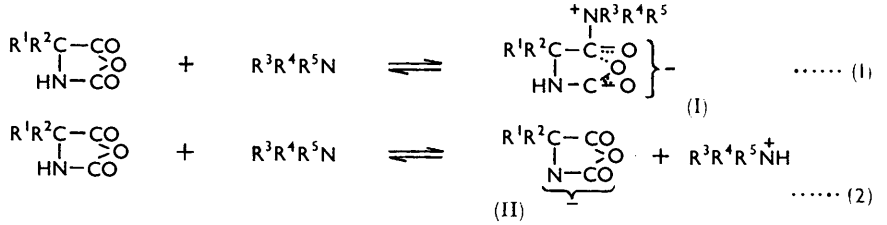
981. *The Initiation Step in the Polymerization of* N-*Carboxy-α-amino-acid Anhydrides. Part I. Catalysis by Tertiary Bases.*

By C. H. Bamford and H. Block.

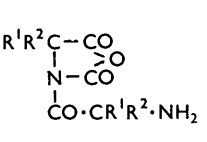
Observations on the polymerizations of *N*-carboxy-γ-ethyl-L-glutamate anhydride catalysed by pyridine, α-picoline, and 2,6-lutidine, and of *N*-carboxy-DL-phenylalanine anhydride catalysed by pyridine and 2,6-lutidine, show that the initial attack of the bases on the monomer molecule involves removal of the proton from the endocyclic NH group and not addition to a carbonyl group.

Investigations in these laboratories have shown that two mechanisms may be envisaged for the attack of a tertiary base on a *N*-carboxy-α-amino-acid anhydride molecule,[1,2,3] *viz.*, addition to the 5-carbonyl group (1) or removal of the proton from the endocyclic NH group (2). Either of the resulting intermediates (I) and (II) would be expected to react with a further molecule of monomer to give the bifunctional compound (III) which has been proposed as an intermediate in the polymerization of *N*-carboxy-α-amino-acid anhydrides initiated by tertiary and other aprotic bases.[1-4] Simple growth and cyclization involving (III) lead to the observed products, polypeptides, usually together with a small proportion of the corresponding 3-hydantoinylacetic acid.



(I) (II)

It is desirable to distinguish between these alternative mechanisms of initiation. A similar problem arose in the interpretation of the mechanism of hydrolysis of carboxylic anhydrides catalysed by tertiary bases, and in this instance the elegant investigations of Gold and Jefferson [5] have shown that association with a carbonyl group is involved. The diagnostic technique employed involves the use of a series of tertiary bases having different relative abilities to associate with Lewis acids and to act as Brönsted bases. Since these processes are paralleled by those in equations (1) and (2) the mechanism may be inferred from the relative rates of reaction. Three suitable bases are pyridine, α-picoline, and 2,6-lutidine; it is known that their base strengths towards Brönsted acids increase in the order given, while their base strengths towards Lewis acids decrease in the order given.[6] The latter effect is attributable to steric shielding of the nitrogen lone pair by the α-methyl groups. We have used these bases, highly purified, to initiate the polymerizations of the *N*-carboxy-anhydrides of γ-ethyl-L-glutamate and DL-phenylalanine, and have thus been able to show unambiguously that the primary step in the polymerization is represented by (2). These findings should not be taken to indicate that (1) does not occur, but rather that, if this equilibrium is set up, it does not lead to further reaction at a significant rate.



(III)

[1] Ballard, Bamford, and Weymouth, *Nature*, 1954, **174**, 173.
[2] Ballard and Bamford, *J.*, 1956, 381.
[3] Bamford, Block, and Pugh, *J.*, 1961, 2057.
[4] Ballard, Bamford, and Weymouth, *Proc. Roy. Soc.*, 1955, *A*, **227**, 155.
[5] Gold and Jefferson, *J.*, 1953, 1409, 1416.
[6] Brown, Schlesinger, and Cordon, *J. Amer. Chem. Soc.*, 1942, **64**, 325; Brown and Barbaras, *ibid.*, 1947, **69**, 1137.

Experimental

Materials.—*N*-Carboxy-γ-ethyl-L-glutamate anhydride was prepared from γ-ethyl-L-glutamate kindly supplied by Dr. J. Watson. The *N*-benzyloxycarbonyl-derivative of this ester, prepared as described by Hanby *et al.*,[7] was cyclized by the use of thionyl chloride under the following standardized conditions. *N*-Benzyloxycarbonyl-γ-ethyl-L-glutamate was refluxed with thionyl chloride (20 ml.) for 12 min. in sodium-dried benzene (250 ml.). To the cooled mixture carbon tetrachloride (250 ml.) was added, followed, after crystallization, by light petroleum (b. p. 60—80°) (500 ml.). After collection, the resulting anhydride was washed successively with carbon tetrachloride and light petroleum (b. p. 60—80°), then recrystallized from dry ethyl acetate until free from chloride. Finally the anhydride was molecularly distilled at 110°/$<10^{-4}$ mm.

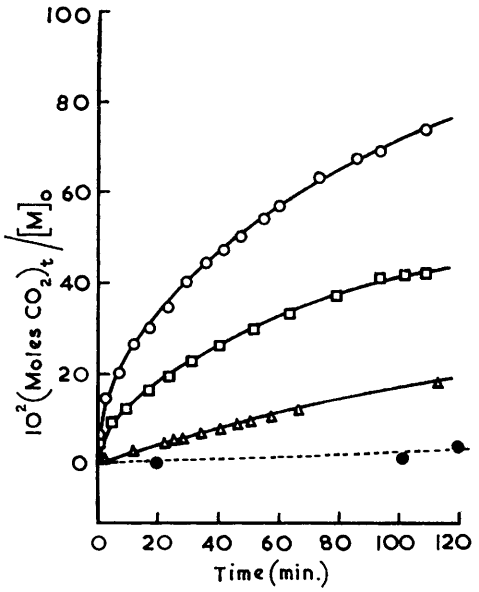


Fig. 1.

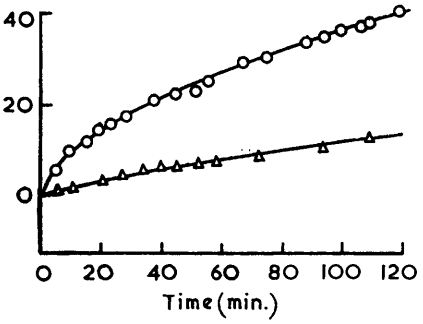


Fig. 2.

Fig. 1. Conversion–time curves for the polymerization of *N*-carboxy-γ-ethyl-L-glutamate anhydride (0·2 mole $l.^{-1}$) catalysed by pyridine and homologues (2·0 mole $l.^{-1}$) in *NN*-dimethylformamide at 25°:

△ pyridine, □ α-picoline, ○ 2,6-lutidine.

The broken curve shows a comparative experiment with *N*-carboxysarcosine anhydride (0·2 mole $l.^{-1}$) and 2,6-lutidine (2·0 mole $l.^{-1}$).

Fig. 2. Conversion–time curves for the polymerization of *N*-carboxy-DL-phenylalanine anhydride (0·2 mole $l.^{-1}$) catalysed by pyridine and 2,6-lutidine (2·0 mole $l.^{-1}$) in nitrobenzene at 25°:

△ pyridine, ○ 2,6-lutidine.

N-Carboxy-DL-phenylalanine anhydride[8] was purified by recrystallization from ethyl acetate–benzene until free from chloride. Immediately before use it was sublimed at $<10^{-4}$ mm.

N-Carboxysarcosine anhydride was prepared by the general method of Hanby *et al.*[8] and purified as described by Ballard *et al.*[4]

Pyridine, α-picoline, and 2,6-lutidine were purified by using vapour-phase chromatography.

[7] Hanby, Waley, and Watson, *J.*, 1950, 3239.
[8] Cf. *e.g.*, Hanby, Waley, and Watson, *J.*, 1950, 3009; Coleman, *J.*, 1950, 3222.

A 180 cm. column of " polyethylene glycol-400 " (Shell Chemical Co., Ltd.) (5%) on " Embacel " (May & Baker Ltd.) at 100° was used, argon being the carrier gas. The purity of individual cuts was confirmed by refractionation and in some cases by refractive-index measurements; *e.g.*, found for 2,6-lutidine n_D^{25} 1·4950 (lit.,[9] 1·4953).

Nitrobenzene and *NN*-dimethylformamide were purified as described in refs. 2 and 3, respectively.

Purified materials were handled exclusively in a dry box.

Method.—Polymerization is accompanied by the evolution of carbon dioxide, measurement of which provides a convenient way of following the reaction. For this purpose solutions of the monomer and base were placed in separate limbs of the reaction vessel described previously [3] connected to a constant-volume manometric system. Kinetic experiments were carried out in a thermostat at 25°.

Results and Discussion

Figs. 1 and 2 show typical conversion–time curves for the polymerizations at 25° of *N*-carboxy-γ-ethyl-L-glutamate anhydride in *NN*-dimethylformamide and *N*-carboxy-DL-phenylalanine anhydride in nitrobenzene. The bases show the same order of increasing reactivity towards both anhydrides, *viz.*, pyridine < α-picoline < 2,6-lutidine. Clearly the presence of traces of impurities could vitiate these observations. Normal purification techniques may not be adequate if the impurities include strong tertiary bases, which if present at a concentration of 0·015% (molar) in the catalysts could lead to initial rates of reaction of the order of 10% of those observed. The method of purification adopted ensures the absence of impurities at such concentrations. The absence of primary and secondary bases from the initiator solutions was confirmed by their inability to induce the polymerization of *N*-carboxysarcosine anhydride. This is illustrated for 2,6-lutidine in Fig. 1.

For reasons already given, the observations show that the major initial attack of the base on the anhydride involves ionization [equation (2)] since the rates are in the order of the base strengths [10] (pyridine $K_B = 1{\cdot}7 \times 10^{-9}$ mole l.$^{-1}$; α-picoline $K_B = 9{\cdot}1 \times 10^{-9}$ mole l.$^{-1}$; 2,6-lutidine $K_B = 4{\cdot}2 \times 10^{-8}$ mole l.$^{-1}$). Addition at a carbonyl group cannot be a rate-determining step, nor can an adduct such as (I) formed in a pre-equilibrium be involved, since both the rate of addition and the equilibrium concentration of (I) would be affected by the stereochemistry of the base. As a result the rates of reaction would be in the reverse order to that found. The possibilities that equilibrium (1) is established and even that (I) is responsible for a minor part of the reaction cannot be excluded.

These observations and conclusions contrast markedly with those appertaining to the catalysed hydrolysis of carboxylic anhydrides.[5]

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Maidenhead, Berks. [*Received, June 2nd,* 1961.]

[9] Brown, Johnson, and Podall, *J. Amer. Chem. Soc.*, 1954, **76**, 5556.
[10] Gero and Markham, *J. Org. Chem.*, 1951, **16**, 1835.