The Mechanisms of Polymerization of N-Unsubstituted N-Carboxyanhydrides[1]

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Abstract: Carbon-14-labeled compounds were used to study initiation, propagation, and termination steps for N-carboxyanhydride (NCA) polymerization. For primary amine initiated polymerizations, the use of C^{14}-labeled benzylamine and *n*-hexylamine showed that nucleophilic attack at the C-5 carbonyl of the NCA represents the major route to polymer. When the aprotic bases, C^{14}-labeled triethylamine and sodium methoxide, were used, the polymers contained little radioactivity. This shows that initiation proceeds *via* proton abstraction. Carbamate ions initiate NCA polymerizations as if they were strong aprotic bases. When C^{14}-labeled sodium N-benzyl carbamate is employed, essentially no radioactivity is found on the polymer. We conclude that strong base initiated polymerizations propagate *via* an "active monomer" mechanism where carbamate ion abstracts the proton from an NCA molecule. By using C^{14}-labeled diethylamine we showed that secondary amines are able to initiate by both mechanisms, with the "active monomer" route predominating. By the use of γ-benzyl-L-glutamate NCA, C^{14} labeled at the 2 position, we demonstrated that termination by attack at C-2 during strong or weak base initiated polymerization is negligible. Extremely low levels of radioactivity were found in the polymer. This is direct evidence of lack of C-2 attack since this step leads to C^{14} retention in the polymer.

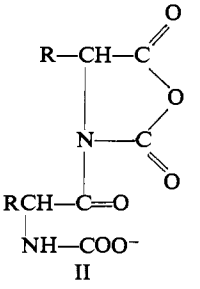
The widespread utilization of α-amino acid N-carboxyanhydrides (NCA) as monomers for the preparation of polypeptides has led to numerous studies of the polymerization mechanisms.[3,4]

Although many points have been explained in a recent review,[5] important questions remain to be answered. Among these are the generality of the primary amine polymerization mechanism, the nature of the propagation route for strong base initiated polymerization, and the extent of termination reactions in these polymerizations. In this paper we present data, based on radioactive compounds, which substantially clarify each of the points noted above.

The mechanism of primary amine initiated polymerization involves nucleophilic attack on the C-5 carbonyl of the NCA by the amine.[4] This is followed by ring opening, proton transfer, and decarboxylation to produce an amino acid amide which can continue the process with successive NCA molecules. Another mechanism becomes significant as the nucleophilicity of the amine is decreased and its basicity is increased.[5]

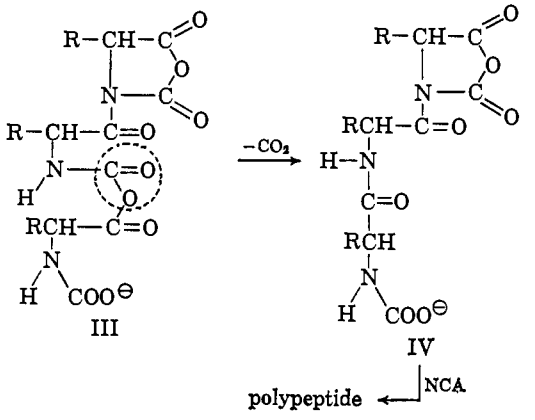
Strong aprotic bases initiate polymerization by proton abstraction from the nitrogen of the NCA.[4,6,7] The resulting NCA^- ion (I) attacks an additional NCA molecule leading to the following structure.

From this intermediate species two propagation mechanisms can be postulated. One involves nucleophilic attack by the carbamate ion on the C-5 carbonyl of an NCA molecule.[8]



II

II + NCA →



The other[5] postulates propagation *via* "active monomer" according to Scheme I.

Experimental Section

Preparation of N-Carboxyanhydrides. N-Carboxy-γ-benzyl-L-glutamate anhydride (NCA) was prepared by the method described by Blout and Karlson.[9] It was recrystallized just prior to use, from chloroform–hexane and ethyl acetate–hexane, a minimum of ten times. The specific rotation was $[\alpha]^{25}D$ −16.2° (*c* 1.2, ethyl acetate). Recrystallizations and other operations involving NCA were carried out in a drybox.

Radioactive N-carboxy-γ-benzyl-L-glutamate anhydride (C^{14} labeled at the 2 position) was specially prepared for us using radio-

(1) Independent of our work, Peggion and co-workers (E. Peggion, M. Terbojevich, A. Cosani, and C. Colombini, *J. Am. Chem. Soc.*, **88**, 3630 (1966)) have carried out radioactive initiator studies on NCA polymerizations. Their results complement ours and their conclusions are in agreement with our findings.

(2) Taken from the thesis submitted to the Faculty of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1966.

(3) E. Katchalski and M. Sela, *Advan. Protein Chem.*, **13**, 243 (1958).

(4) C. H. Bamford and H. Block, "Polyamino Acids, Polypeptides and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p 65.

(5) M. Szwarc, *Advan. Polymer Sci.*, **4**, 1 (1965).

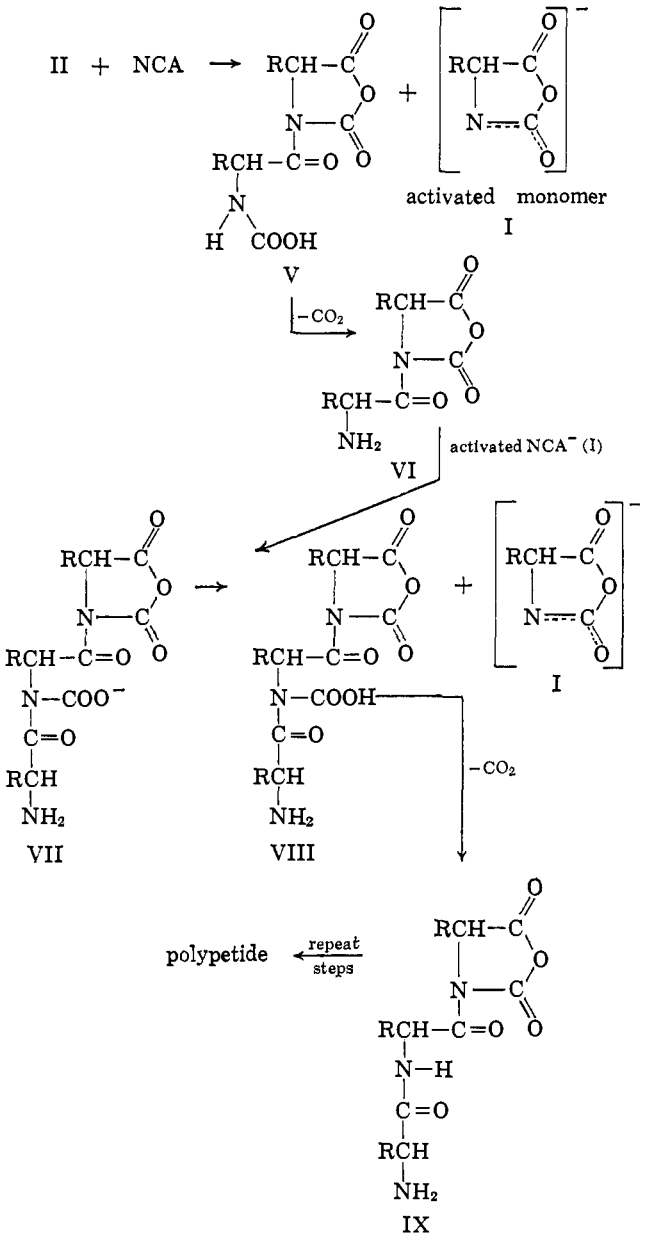
(6) M. Goodman and U. Arnon, *J. Am. Chem. Soc.*, **86**, 3384 (1964).

(7) M. Goodman and U. Arnon, *Biopolymers*, **1**, 500 (1963).

(8) M. Idelson and E. R. Blout, *J. Am. Chem. Soc.*, **78**, 941 (1956).

(9) E. R. Blout and R. H. Karlson, *ibid.*, **78**, 941 (1956).

Scheme I



active phosgene.[10] The product possessed an activity of 0.25 mcurie/g. It was purified as above.

Solvents. Dioxane was refluxed with molten sodium for a minimum of 2 days, distilled onto lithium aluminum hydride, and redistilled immediately before use. Tetrahydrofuran was stored over potassium hydroxide pellets and just prior to use it was distilled from lithium aluminum hydride. Ethyl acetate was purified by the method of Fieser[11] and stored over calcium hydride, from which it was distilled just before use. Hexane and dimethylformamide were refluxed over and distilled from phosphorus pentoxide. We employed reduced pressure for dimethylformamide distillation.

Initiators and Reagents. Sodium methoxide solution was prepared as described in a previous publication.[6]

Carbon-14-labeled sodium methoxide (methanol free) was prepared from cleaned, finely dispersed sodium (0.6 g) and C^{14}-labeled methanol[12] (3.0 ml, 1.0 mcurie) in 25 ml of anhydrous ethyl ether. The mixture was refluxed and stirred under a drying tube until all traces of sodium had dissolved (7 hr). All manipulations were carried out under a stream of nitrogen. The ether and excess methanol were removed under reduced pressure with gentle heating. This left a fine, white powder which was insoluble in all common inert organic solvents except methanol.

Carbon-14-labeled amine[13] initiator solutions of *n*-hexylamine, benzylamine, diethylamine, and triethylamine were prepared as follows. A known amount of radioactive amine (about 3 g, 0.5 mcurie) was dissolved in tetrahydrofuran in a volumetric flask, inside a drybox, to give solutions about 0.5 *N*. Concentrations, although known from the amounts used, were checked by titration with standard hydrochloric acid to the red end point of thymol blue.

Polymer Isolation. Poly-γ-benzyl-L-glutamate was isolated by decantation of the polymerization mixture into a 20-fold excess of purified methanol with vigorous stirring. The precipitate was washed with methanol and freeze dried twice from dioxane.

Radioactivity Measurements. To determine the relative amount of C^{14} in a sample, it was dissolved in 5.0 ml of inert solvent in a low-potassium glass counting vial.[14] To it was added 10.0 ml of a phosphor scintillation solution consisting of 0.5% 2,5-diphenyloxazole[14] in toluene. All liquids were added with volumetric pipets. In a second vial, 5.0 ml of the solvent and 10.0 ml of the scintillator were used for background determination. The measurements were made in an EKCO 612 well-type scintillation counter at −10°, using an EKCO N530 scaler at PM voltage 1150 and bias 12.5 v. To improve precision, duplicate or triplicate samples were used and the results were averaged. For statistical accuracy, from 30,000 to 90,000 counts were recorded for each sample.

Radioactivity determinations were carried out on polymer solutions in dimethylformamide, the work-up filtrate, the dioxane from the freeze drying, and a radioactive standard solution of the initiator in methanol. Setting the standard equal to 100% gave the C^{14}-content distribution reported in the table of polymerization data.

Measurement of carbon dioxide evolution and molecular weight determination have been previously described.[6]

Polymerization Initiated by Primary Amines. The Initiation Step. Since the "normal" mechanism for amine initiation of NCA compounds requires the initiator residue to be incorporated at the end of the polymer chain, the amount of initiator experimentally found in the polymer is a direct indication of the applicability of this scheme. Initiators labeled with C^{14} were chosen as the best means of locating the initiator fragments without affecting the nature of the polymerization.

Benzylamine was selected because of its low basicity (pK_a = 9.34 *vs.* 10.6 for aliphatic amines[15]). It was expected to produce the least deviation from the "normal" route. The results of the polymerization studies are shown in Table I. We obtained a propagation rate constant from the measured $\overline{DP}_w$ of the polymer and the slope of the first-order kinetics curve (based on measurement of carbon dioxide evolution[16]).

The degree of polymerization and propagation rate constant are typical of primary amine initiated polymerization. Approximately 72% of radioactivity from the benzylamine was found in the polymer. Thus at least 72% of benzylamine initiator molecules act as nucleophiles toward the NCA to produce polymer.

A similar experiment was performed with C^{14}-labeled *n*-hexylamine. The results are presented in Table I where it is shown that *n*-hexylamine deviates from the "normal" mechanism to a larger extent than benzylamine because of its greater basicity.

Scoffone, Peggion, and their associates[1] carried out experiments independently which indicate that the deviation may not be as large as our results show. These workers isolated polymers, after radioactive initiation, using petroleum ether to precipitate the polymers. They found that methanol (our precipitating solvent) dissolves low molecular weight poly-γ-benzyl-L-glutamate.[17] By employing petroleum ether (bp 40–60°) Scoffone and Peggion precipitate essentially all solutes and find a much higher C^{14} content in the polymer. This technique of isolation may give abnormally high values for the radioactivity content of the polymer because petroleum ether may also precipitate by-products or any impurities in the system. However, their observations may well explain the origin of much of the radioactivity we find in solution. This is

(10) We wish to thank Nuclear Research Chemicals Inc., Orlando, Fla., for their excellent synthetic work.

(11) L. F. Fieser, "Experiments in Organic Chemistry" D. C. Heath and Co., Boston, Mass., 1955, p 287.

(12) It was supplied by New England Nuclear Corp., Boston, Mass.

(13) They were supplied by Nuclear Research Chemicals, Inc., Orlando Fla.

(14) It was supplied by Amtradair, Long Island City, N. Y.

(15) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," John Wiley and Sons, Inc., New York, N. Y., 1962.

(16) A. Patchornik and Y. Shalitin, *Anal. Chem.*, **33**, 1887 (1961).

(17) A. Cosani, E. Peggion, E. Scoffone, and A. S. Verdini, *Makromol. Chem.*, in press.

Table I. Polymerization Results Using γ-Benzyl-L-glutamate NCA[a,b]

Initiator	A/I	$\overline{DP}_w$	kp, l./mole sec	% radioactivity In polymer	% radioactivity In work-up	% radioactivity In freeze dryings
Benzylamine-C^{14}	50	79	0.0097	72.2	17.4	0.1
n-Hexylamine-C^{14}	40	38	0.0017	50.6	44.2	...
Diethylamine-C^{14}	50	273	0.235	9.2	95.2	...
Triethylamine-C^{14}[c]	50	89	0.036	0.2	95.4	...
Sodium methoxide C^{14}[d]	53	244	...	1.3	89.4	0.4
Sodium N-benzyl-carbamate-C^{14}[e]	52	302	2.53	2.5	89.0	...

[a] Solvent for all polymerizations, unless otherwise noted, is tetrahydrofuran. [b] Polymer yield is high in all cases ≧90%. [c] Solvent, dimethylformamide. [d] Initiator added as a solid. [e] Initiator in methanol solution.

especially true in the *n*-hexylamine case where the polymer molecular weight is low and the yield (92%) is smaller than with the benzylamine initiation.

We conclude that while *n*-hexylamine may produce some deviation, the "normal" route is the major mechanism for primary amine initiated polymerization.

Polymerization Initiated by Aprotic Bases. The Initiation Step. Tertiary Amine. Our previous work with aprotic base initiation[7] has shown that it proceeds by proton abstraction from the NCA. We desired to extend this work to include tertiary amines which though not as basic as sodium methoxide or metal alkyls are similar in having no labile proton. They are thus excluded from the normal amine category.

We found a strong solvent dependence with triethylamine initiation of γ-benzyl-L-glutamate NCA. In tetrahydrofuran solution the rates were low and the yields were poor.

In dimethylformamide, the rate was moderate and the yield reasonable. Radioactive triethylamine was used as an initiator and the results are shown in Table I. It is clear that triethylamine initiation proceeds by proton abstraction from the NCA. The trace amount of radioactivity left on the polymer is probably due to adsorption.

The observed solvent effects may be partially explained by the nature of the initiation step. Tertiary amine initiation involves the conversion of two neutral compounds into two ionic species.

$$\text{NCA} + (C_2H_5)_3N \rightleftharpoons \text{NCA}^- + (C_2H_5)_3N^+H$$

The formation of such ionic species is enhanced in a polar solvent such as dimethylformamide while in nonpolar solvents, at best, tight ion pairs can form. In contrast, initiation by sodium methoxide involves no net change in the ionic balance of the reactants and thus can proceed easily in polar and nonpolar solvents.

The Initiation Step. Sodium Methoxide. We are including data on sodium methoxide initiation of γ-benzyl-L-glutamate NCA because we have been able to improve our experimental procedure.[18] We used methanol-free C^{14}-labeled sodium methoxide as a solid since it is insoluble in all inert solvents. Recording of kinetics was of course impossible, because the sodium methoxide did not dissolve instantly in the polymerization solution.

The results are shown in Table I and are quite typical of sodium methoxide initiation. A small amount of C^{14} was found in the polymer. This radioactivity appears to be chemically bonded to the polymer since it could not be removed with repeated purification steps. Its presence may be explained by a small amount of ester interchange with radioactive methoxide.

It has been suggested that the lack of initiator residues in the polymer may arise from hydrolysis of the C^{14} sodium methoxide by traces of water in the solvent. This possibility is eliminated by our later work with sodium methoxide initiation of N-substituted NCA's, such as those derived from sarcosine and proline, where a large percentage of the radioactivity is found in the polymer. Since exactly the same reaction conditions were employed, it is clear that appreciable hydrolysis of the sodium methoxide does not occur.

Our present results are in complete accord with the Bamford–Szwarc mechanism and our previous findings. Sodium methoxide initiation involves proton removal from the NCA.

The Propagation Step. In a preliminary report on these investigations,[19] we presented results from initiation by C^{14}-labeled sodium N-benzylcarbamate which showed that the carbamate ion acts as a base with N-unsubstituted NCA compounds. Only a small amount of radioactivity (2.5–3.0%) was found in the polymer (Table I). We obtained a polymer with a degree of polymerization significantly higher than the anhydride/initiator ratio (*A/I*). We also found a large rate of polymerization and observed a long induction period. These results are typical of strong base initiated polymers. The Bamford–Szwarc activated monomer mechanism[5] best fits the experimental facts.

Another example of an "activated monomer" polymerization mechanism was uncovered by Wichterle and his co-workers in dealing with the anionic polymerization of ε-caprolactam.[20]

Polymerization Initiated by Secondary Amines. When γ-benzyl-L-glutamate NCA was initated with C^{14}-labeled diethylamine, we noted significant differences from primary amine initiation. The rate and polymer $\overline{DP}_w$ were much higher (Table I). This behavior is similar to aprotic base initiation. The radioactivity measurements substantiate these observations. The percentage of C^{14} found in the polymer (9.2%) is much closer to the value with aprotic initiators than with primary amines. Thus the greater steric hindrance and basicity (pK_a = 10.93) of diethylamine have largely eliminated the "normal" amine mechanism. The total radioactivity recovered in the polymer and work-up in this case slightly exceeds 100%. However, this is within the range of experimental accuracy which is estimated at ±5%.

The nature of secondary amine initiation depends on the solvent. Shalitin and Katchalski[21] found that diethylamine initiation of γ-benzyl-L-glutamate NCA in dimethylformamide produced polymers where $\overline{DP} = A/I$. Such results are similar to those with primary amine initiation. However, Blout[9] using the same NCA and initiator in dioxane produced polymers with $\overline{DP} > A/I$ which agrees with our work in tetrahydrofuran. These solvent effects have been studied in more detail by Scoffone and co-workers.[22]

The Termination Step. It is well known that the C-5 carbonyl of the NCA is the most favorable site for nucleophilic attack since the C-2 carbonyl is less electropositive because of electron delocalization from the adjacent nitrogen atom. However, amine attack at C-2 can occur under some conditions.[23,24] If this were to happen during polymerization a termination step results because ring opening cannot produce a new propagating amine group.

In order to obtain quantitative data on the amount of C-2 attack in a typical polymerization, we used γ-benzyl-L-glutamate NCA which was C^{14} labeled at C-2. With this NCA, every reaction at C-5 will eliminate radioactivity from the polymer as radioactive carbon dioxide. Therefore, the mole per cent of radioactivity

(18) Previous measurements were carried out using methanol as a solvent for the C^{14}-labeled sodium methoxide. Proton exchange between the radioactive methoxide ions and the inactive methanol diluted radioactivity of the methoxide ion. Even if methoxide ion were to initiate by nucleophilic attack at the C-5 carbonyl there would be barely detectable levels of radioactivity at the end of the polymer chain. Therefore we repeated the experiment using methanol-free radioactive sodium methoxide as described above.

(19) M. Goodman and J. Hutchison, *J. Am. Chem. Soc.*, **87**, 3524 (1965).

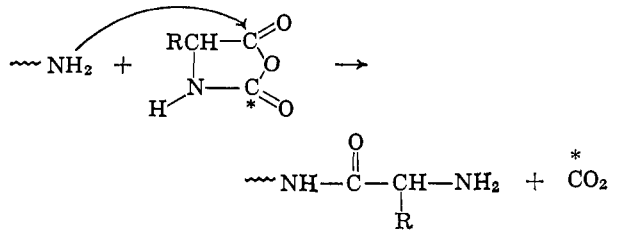
(20) O. Wichterle, J. Sebenda, and J. Kralicek, *Advan. Polymer Sci.*, **2**, 578 (1961).

(21) Y. Shalitin and E. Katchalski, *J. Am. Chem. Soc.*, **82**, 1630 (1960).

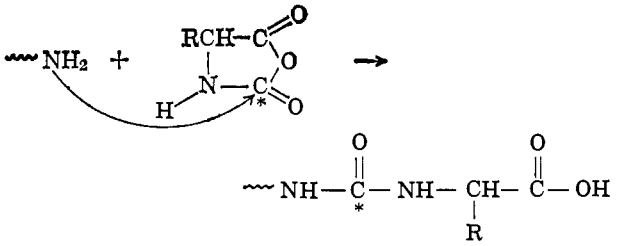
(22) A. Cosani, G. D'Este, E. Peggion, and E. Scoffone, *Biopolymers*, **4**, 595 (1966).

(23) K. D. Kopple, *J. Am. Chem. Soc.*, **79**, 662, 6442 (1957).

(24) M. Sela and A. Berger, *ibid.*, **77**, 1893 (1955).



Attack at C^{*2} retains the C^{14} in the polymer.



remaining in the polymer is directly equal to the per cent of C-2 attack.

Polymerization of the C^{14}-labeled NCA was initiated in tetrahydrofuran by *n*-hexylamine. Results are presented in Table II. Radioactivity measurements were carried out on polymer and NCA in dimethylformamide solutions. Only about 0.15% of the C^{14} content in the NCA remained in the polymer. In other words, only one in every 670 carbonyl attacks was at C-2. Since the $\overline{DP}_w$ is 59, this means that fewer than one in ten chains was terminated by C-2 attack.

Table II. Polymerization of C^{14}-Labeled (at 2 Position) γ-Benzyl-L-glutamate NCA[a,b]

Initiator	A/I	$\overline{DP}_w$	kp, l./mole sec	Polymer radioactivity, (counts/sec mole of amino acid residue)
n-Hexylamine	50	59	0.0022	1.0×10^6
Sodium methoxide[c]	50	255	1.75	1.7×10^5

[a] Solvent tetrahydrofuran. [b] Radioactivity of NCA, 7.0×10^8 counts/sec mole. [c] Initiator added in methanol solution.

With aprotic initiated polymerization as with the amine mechanism, attack at the C-2 carbonyl of the NCA results in termination. A similar, but more complex, analysis of the Bamford–Szwarc mechanism shows that C-2 attack produces retention of C^{14} in the polymer.

The labeled NCA was polymerized in tetrahydrofuran with sodium methoxide as an initiator (Table II). Radioactivity measurements, performed as before, showed only 0.025% of the NCA radioactivity content to be in the polymer. This indicates that only one in every 4000 carbonyl attacks was at C-2 and fewer than one in fifteen chains was thus terminated by C-2 attack.

General Conclusions

Our investigations lead us to conclude that the polymerization of N-unsubstituted NCA compounds can be described by two mechanisms. For initiators which are weakly basic but good nucleophiles (in practice only primary amines) the "normal" amine mechanism applies very well. Strong base initiated polymerization proceeds by the Bamford–Szwarc activated-monomer mechanism. Tertiary amines although not strong bases are included in this category because they cannot act as nucleophiles. Secondary amines lie in a region of basicity and nucleophilicity where the two mechanisms are both operative. The structure of the secondary amine and the nature of the solvent determine which predominates.

Termination appears to be negligible. Reaction at C-2 is minor in both mechanisms. A future report from our laboratories will be concerned with the polymerization mechanism of N-substituted NCA compounds.

Acknowledgment. We thank the Diamond Alkali Co. and the National Institutes of Health, Grant No. AM 03868, for their liberal support.

Mechanism of N-Carboxyanhydride (NCA) Polymerization in Dioxane. Initiation by Carbon-14-Labeled Amines[1]

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Abstract: The polymerization of γ-benzyl-L-glutamate N-carboxyanhydride (NCA) was studied in dioxane using C^{14}-labeled isopropyl-, diisopropyl-, and methyldiisopropylamine as initiators. All the radioactivity was found in the polymers only with isopropylamine initiation. Diisopropylamine leads to polymers containing only a few per cent of the initial activity, and methyldiisopropylamine leads to inactive polymers. These results support the hypothesis that the NCA polymerization in dioxane mainly proceeds *via* simple "primary amine" mechanism with isopropylamine, and *via* "strong base" mechanism with methyldiisopropylamine. Both of these mechanisms appear to operate simultaneously in the case of diisopropylamine.

According to the literature[3] the polymerization of NCA compounds initiated by amines or bases may proceed according two different mechanisms, *i.e.*, (1) normal "primary amine" polymerization and

(1) Independent work on the NCA's polymerization initiated by radioactive initiators was carried out by Goodman and Hutchison: *J. Am. Chem. Soc.*, 88, 3627 (1966). Their results complement ours and their conclusions are in agreement with our findings.

(2) General Chemistry Institute of the University of Padua, Padua, Italy.

(3) M. Szwarc, *Advan. Polymer Sci.*, **4**, 1 (1965).