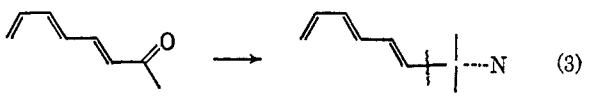
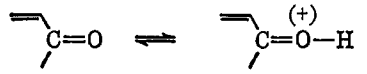
tion.[27] If, as seems likely from these results, the endoenergetic configurational change does occur, then any model of the active site of α-chymotrypsin must accommodate this requirement.

(27) External chemical modification of the ester linkage: none of the above-mentioned data excludes the possibility of chemical interaction somewhere in the proximity of the ester linkage. From the known properties of esters, aqueous solvent, and amino acid residues of the protein, the plausible loci of chemical interaction are at either of the two ester oxygens or at the carbonyl carbon. Nucleophilic interaction at the (electrophilic) carbonyl carbon can be rejected as a significant contribution, since such attack would effectively decrease the intensity and blue shifts. To a rough approximation, this type of interaction would transform the spectrum of a cinnamoyl derivative to that of a styrene derivative (eq 3). Such an acyl-enzyme derivative would be virtually



(3)

unobservable spectroscopically. Electrophilic interaction at oxygen is, however, a distinct possibility. The most obvious electrophilic agent is, of course, a proton (eq 4). Indeed, the spectra of the N-methylamides of cinnamic and furylacrylic acids in strongly acid solutions (approximately 1 *M* HCL)[28] are red shifted to precisely the same wavelengths as



(4)

the corresponding acryloyl enzymes. This result is not unexpected, since protonation of N-methylamides is known to occur at the carbonyl oxygen,[29] a process which would lead to electron delocalization at the C–N bond and hence to a spectrum essentially the same as that observed in the corresponding aldehydes and ketones. The spectra of the protonated furoyl and benzoyl-N-methylamides are similarly red shifted. No such large red shift is observed, however, with the corresponding furoyl and benzoyl enzymes.[2] Moreover, protonation of this type (eq 4) is seemingly unlikely at or near neutrality, where the spectra of the acyl enzymes have been measured. The possibility that hydrogen bonding at carbonyl oxygen, rather than complete proton transfer, is the origin of the red shift cannot be ruled out. No significant red shifts have been noted, however, in the spectra of cinnamoyl and furylacryloyl esters in the presence of very strong hydrogen-bond donors (relative to the corresponding spectra in pure H_2O).[3]

(28) S. A. Bernhard and S. J. Lau, unpublished results.

(29) A. Berger, A. Loewenstein, and S. Meiboom, *J. Am. Chem. Soc.*, 81, 62 (1959).

The Mechanism of Polymerization of N-Carboxyanhydrides in Dimethylformamide. Evidence of the Presence of Cyclic Terminals in Polymers Obtained by Strong Base Initiation

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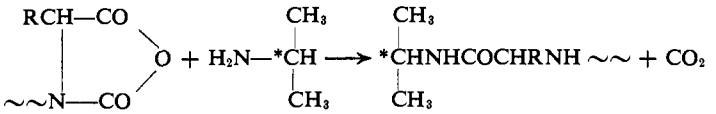
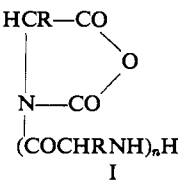
Contribution from the Institute of Organic Chemistry, University of Padua, Padua, Italy, and VIII Sez. Centro Nazionale di Chimica Macromolecolare, Padua, Italy. *Received December 28, 1966*

Abstract: γ-Benzyl-L-glutamate N-carboxyanhydride was polymerized in DMF using C^{14}-labeled amines as initiators. All the radioactivity was incorporated in the polymers obtained by C^{14}-isopropylamine initiation, indicating that normal primary amine polymerization is operative in this case. No radioactivity was found in the polymers when C^{14}-methyldiisopropylamine was used as the initiator. The Bamford mechanism is operative in this case. Using C^{14}-diisopropylamine both mechanisms are simultaneously operative. Polymers prepared by initiation with unlabeled diisopropyl- and methyldiisopropylamine and treated with an excess of C^{14}-labeled isopropylamine exhibit considerable radioactivity. This radioactivity must be due only to reaction between the labeled amine and cyclic terminal present in the polymers.



On the basis of extent of the radioactivity incorporated in polymers "killed" with C^{14}-labeled isopropylamine, the number of polymer molecules containing cyclic terminals, *i.e.*, formed *via* Bamford mechanism, was estimated. It was found that cyclic terminals deactivate *after* the end of the polymerization.

In previous papers[1,2] we presented evidence of the existence of bifunctional intermediates in the polymerization of N-carboxyanhydride (NCA) in dimethylformamide (DMF) initiated by strong bases. We found that when the polymerization mixture at 95% conversion is concentrated, a marked increase in molecular weight of the polymer is observed. We interpreted this fact assuming that coupling between bifunctional species occurs during concentration. These species are



I

formed if initiation and propagation occur *via* "active monomer" mechanisms, as suggested by Bamford[3] and Szwarc.[4]

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

(2) E. Peggion, E. Scoffone, A. Cosani, and A. Portolan, *ibid.*, 4, 695 (1966).

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

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

This paper presents definitive evidence for the Bamford–Szwarc mechanism in the case of NCA polymerization initiated by strong base type initiators in DMF.

Moreover, further support for the "normal primary amine" addition mechanism is presented when primary amines are used as the initiators. The work has been carried out using radioactive amines.

Experimental Section

Materials. Dimethylformamide (DMF) reagent grade was distilled under vacuum, over phosphorus pentoxide, immediately before use. DMF not freshly distilled was able to induce the polymerization of γ-benzyl-L-glutamate N-carboxyanhydride without addition of the initiator. Ethyl ether was refluxed over sodium metal and then distilled. Isopropylamine (IPA), diisopropylamine (DIPA), and methyldiisopropylamine (MDIPA) were of reagent grade. They were dried over potassium metal and then fractionally distilled. Monomer γ-benzyl-L-glutamate N-carboxyanhydride (NCA) was prepared according to the literature.[5]

Labeled Amines. C^{14} IPA, DIPA, and MDIPA hydrochlorides were prepared as previously described.[6] The free C^{14} amines were obtained by exchange between the labeled hydrochlorides and the corresponding unlabeled pure amines. Radioactive standardization was carried out as previously described.[7]

Radioactivity Measurements. Weighed samples of polymer were dissolved in a known volume of freshly distilled, anhydrous DMF. These solutions were mixed with the phosphor solution (5 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis[2-(5-phenyloxazolyl)benzene] in 1 l. of toluene). The radioactivity measurements were performed at −20° using a SELO scintillation counter with superscaler at 1020 v and bias at 5 v.

Initiation with Labeled Amines. i. **Physical Adsorption Checks.** Some preliminary checks were carried out in order to determine the physical adsorption of the labeled initiators on the polymers.

A typical experiment was as follows. To a solution of preformed polymer (500 mg) in DMF (25 ml) C^{14}-labeled IPA was added in an amount corresponding to that used to initiate polymerization. The mixture was stirred vigorously, and after 1 hr the solvent and the radioactive amine were evaporated under vacuum. The polymer was then dissolved in methylene chloride and precipitated by pouring the solution into ethyl ether. The polymer was isolated by filtration, redissolved in methylene chloride, and treated with a large excess of unlabeled IPA. The polymer was finally precipitated by pouring the solution into ethyl ether. Dissolution in methylene chloride, treatment with unlabeled IPA, and precipitation were repeated four times. The final polymer had only 0.5% (or less) of adsorbed radioactive amine. Analogous experiments were carried out with C^{14}-labeled DIPA and MDIPA with identical results.

ii. **Polymerization.** Poly-γ-benzyl-L-glutamate (PBLG) samples were prepared by NCA polymerization in DMF using proper A/I ratios (see Table II). The radioactive initiators were introduced by direct distillation into the reaction mixture cooled at −190°. The exact amounts of initiators used were determined by counting the total radioactivity of the polymerization mixtures. When the conversion reached at least 98% (checked by infrared) each polymerization mixture was divided in two parts. The first was treated according to procedure A and the second treated according to procedure B.

Procedure A. One-half of the reaction mixture was treated with a large excess of unlabeled IPA. Then the amine and solvent were distilled out, and the polymer was redissolved in methylene chloride and precipitated by pouring the solution into ethyl ether. Redissolution in methylene chloride, treatment with an excess of unlabeled initiating amine, and reprecipitation were repeated four times in the same way as described in Adsorption Checks.

Procedure B. One-half of the reaction mixture was concentrated to an oil in a rotating evaporator, diluted with methylene chloride, and precipitated by pouring into ethyl ether. The polymer was then redissolved, treated with an excess of unlabeled initiating amine, and reprecipitated; this procedure was repeated four times.

Killing Experiments with C^{14}-Labeled Isopropylamine. i. **Adsorption Test.** Polymer (230 mg) and 275 mg of monomer γ-benzyl-L-glutamate NCA were dissolved in 21 ml of DMF and treated with 2.5 ml of C^{14}-labeled IPA. After 1 hr the radioactive amine was distilled out and 2.5 ml of unlabeled IPA was added to the solution. After 1 hr the amine was again distilled out. The treatment with unlabeled IPA was repeated four times. Finally, all solvent was evaporated. The polymer was then redissolved in methylene chloride, treated with unlabeled IPA, and precipitated by pouring the solution into ethyl ether. Solution in CH_2Cl_2, treatment with unlabeled IPA, and precipitation into ethyl ether were repeated four times. The final polymer *exhibits no trace of radioactivity*. This means that the above procedure completely eliminates contamination of the polymer due to radioactive amine or to the product (ether soluble)

formed by reaction of the radioactive amine with the monomer.

ii. **Killing.** Two polymerization experiments were carried out in DMF using unlabeled DIPA and MDIPA as the initiators. In both cases the following polymerization conditions were used: monomer, 0.500 g; DMF, 25 ml; molar ratio of monomer to initiator, A/I, 20.

At various times of reaction, portions of the polymerization mixtures were taken away. Each portion was divided in two parts. The first was treated with 3 ml of C^{14}-labeled IPA. The polymer was then recovered according to the laborious procedure described in Absorption Test.

The second was concentrated to an oil in a rotating evaporator; the polymer was redissolved in methylene chloride and precipitated by pouring the solution into ethyl ether.

Molecular Weight Determinations. Molecular weights ($\bar{M}_w$) of the polymers were determined by viscometry in dichloroacetic acid solution (a Ubbelhode viscometer was used) using Doty's relation[8] $[\eta] = 2.78 \times 10^{-5} M^{0.87}$.

Results and Discussion

Initiation. Table I shows the results of polymerization experiments carried out in DMF using C^{14}-labeled initiators. These results are qualitatively similar to those obtained with the same monomer and initiators in dioxane.[7,9]

From these data it is evident that IPA mainly behaves like a "normal primary amine" initiator while MDIPA behaves like a strong base type initiator.

Both mechanisms appear to be operating simultaneously using C^{14}-DIPA. In this case we can roughly estimate the fraction of labeled molecules in the polymeric mixture. In fact, assuming $\overline{DP}_n = 0.5\overline{DP}_w$ (this assumption is justified by our previous work),[2] the number of polymer molecules per 100 mg of polymer can be calculated. The experimental radioactivity measured on the polymer is directly proportional to the number of polymer molecules containing labeled initiator at their ends. It is easy to verify that the ratio between the per cent of initial radioactivity present in the polymers and the quantity $T = (A/I)(100/\overline{DP}_n)$ gives directly the number fraction of labeled polymer molecules in the polymeric mixture. From Table II it can be seen that, by DIPA initiation, 5–10% of polymer molecules contain labeled initiator at their end, *i.e.*, have been formed *via* the "primary amine" mechanism. Of course these values must be considered as roughly approximate.

(5) E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, 78, 941 (1956).
(6) C. Colombini, M. Terbojevich, and E. Peggion, *J. Labelled Compds.*, 1, 195 (1965).
(7) E. Peggion, M. Terbojevich, A. Cosani, and C. Colombini, *J. Am. Chem. Soc.*, 88, 3631 (1966).
(8) P. Doty, J. H. Bradbury, and A. M. Holtzer, *ibid.*, 78, 947 (1956).
(9) M. Goodman and J. H. Hutchison, *ibid.*, 88, 3627 (1966).

Table I. Polymerization of γ-Benzyl-L-glutamate NCA in DMF; Initiation by C^{14}-Labeled IPA, DIPA, and MDIPA

Run code	Initiator	A/I	—Polymers obtained *via* procedure A[b]— $\bar{M}_w$[a]	Initial radioactivity in the polymers, %	—Polymers obtained *via* procedure B[b]— $\bar{M}_w$	Initial radioactivity in the polymers, %
Z1C14	IPA	20	11,000	100	11,000	100
AA1C14	DIPA	17	16,500	4.3	51,500	4.4
AA2C14	DIPA	20	15,000	2.8	39,500	2.4
AG1C14	DIPA	18	14,500	2.9	36,500	2.7
AA3C14	MDIPA	26	22,500	0.3	180,000	0
AG2C14	MDIPA	19	15,000	0.8	77,000	0.3

[a] $\bar{M}_w$ = weight-average molecular weight. [b] See Experimental Section.

Table II. Polymerization of γ-Benzyl-L-glutamate NCA in DMF; Initiation by C^{14}-DIPA in DMF; Polymers Have Been Obtained *via* Procedure A

Run code	A/I	$\bar{M}_w$	$\bar{M}_n$[a]	Labeled polymer molecules, %[b]
AA1C14	17	16,500	8250	10
AA2C14	20	15,000	7500	5
AG1C14	18	14,500	7250	5

[a] Number-average molecular weight. [b] Calculated by the ratio between the experimental radioactivities found on the polymers and the quantity $T = (A/I)(100/\overline{DP}_n)$ ($\overline{DP}_n$ being the number-average degree of polymerization, which is assumed to be equal to $0.5\overline{DP}_w$).

Presence of Cyclic Terminals in Polymers Obtained Using Strong Bases Type Initiators. The most serious objection to the Bamford–Szwarc mechanism was the lack of experimental evidence of the ring compound on the end of the polymer.[4]

The occurrence of coupling phenomena in polymers obtained in DMF using DIPA as the initiator was the first experimental evidence that bifunctional intermediates, I, do exist.[1,2] Also, from the data of Table I, large differences appear between $\bar{M}_w$ of polymers obtained by "killing" with IPA and polymers obtained by concentration of the reaction mixture before precipitation. We explained the higher $\bar{M}_w$ of the "concentrated" polymers by the extensive occurrence of coupling reactions between bifunctional intermediates. *Coupling does not occur for polymers initiated by IPA, indicating that, in this case, no cyclic terminals are generated.*

Further evidence of the presence of cyclic terminals on polymers obtained by DIPA or MDIPA initiation arises from the following experiments. We ran two polymerization experiments in DMF under standard conditions as indicated in Tables III and IV. Portions of the reaction mixture were removed at different reaction times. All portions were divided in two parts.

The first was treated with a large excess of C^{14}-labeled IPA. Then amine and solvent were evaporated. The polymer was then recovered as described in the Experimental Section.

The second was concentrated in a rotating evaporator, diluted with methylene chloride, and then poured into ethyl ether.

In Tables III and IV it is shown that all polymers "killed" with C^{14}-labeled IPA exhibit marked radioactivity. As described in the Experimental Section, the procedure we used in these experiments excludes the presence of radioactivity due to adsorption and to the presence of by-products of reaction between the residual unreacted monomer and radioactive amine or between the side chain of the polymer and radioactive amine.

Table III. Polymerization of γ-Benzyl-L-glutamate NCA in DMF; Initiation by Unlabeled DIPA; Killing Experiment with C^{14}-Labeled IPA

Reaction time, hr	Conversion, %	$\bar{M}_w$ Killed sample	$\bar{M}_w$ Concd sample	Radioactivity in killed samples, (mmole of labeled amine/100 mg of polymer) × 10^2	Estimated fraction of polymer molecules containing cyclic terminals[a]
0.2	70	7,000	80,000	1.74	0.61
1	98	9,000	42,000	0.93	0.42
3	100	10,000	33,000	0.66	0.33
24	100	11,700	13,500	0.11	0.07

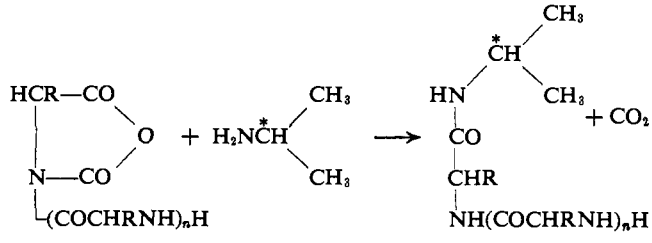
[a] Calculated by the extent of radioactivity incorporated in the killed samples and assuming $\overline{DP}_n = 0.5\overline{DP}_w$.

Table IV. Polymerization of γ-Benzyl-L-glutamate NCA in DMF; Initiation by Unlabeled MDIPA; Killing Experiment with C^{14}-Labeled IPA

Reaction time, hr	Conversion, %	$\bar{M}_w$ Killed sample	$\bar{M}_w$ Concd sample	Radioactivity in killed polymers, (mmole of labeled amine/100 mg of polymer) × 10^2	Estimated fraction of polymer molecules containing cyclic terminal[a]
0.5	60	10,000	108,000	1.42	0.71
24	100	28,000	43,000	0.24	0.34

[a] Calculated as in Table III.

Our results can be explained only by admitting the presence of cyclic terminals on the polymer chains



which react with the excess of radioactive amine leading to radioactive polymer.

An important fact emerges from the data of Tables III and IV. The extent of radioactivity incorporated in the "killed" polymers depends on the time at which the excess of radioactive amine has been added to the solution. For example, the first sample of Table III, killed at 70% conversion, contains 1.74×10^{-2} mmole of labeled amine per 100 mg of polymer. In the sample killed 24 hr after the beginning of the reaction, the amount of incorporated amine is 0.11×10^{-2} mmole per 100 mg of polymer. From the amounts of incorporated amine it is possible to calculate the percentage of polymer molecules containing cyclic ends. These calculations are reported in the last columns of Tables III and IV. From these data it appears clearly that the percentage of molecules with cyclic terminals decreases also *after* the practical end of the polymerization. Moreover, we must point out that the progressive deactivation of the cyclic terminals is not due to the coupling reaction between bifunctional intermediates. In fact, after the practical end of the polymerization, the molecular weight is substantially the same in all the samples killed at different times. The coupling reaction occurs only in the portions of reaction mixture concentrated in the rotating evaporator.

On the bases of all the above observations we can now explain the $\bar{M}_w$ data of killed and concentrated samples of Tables III and IV. In fact immediately after the end of the reaction, there is a large number of polymer molecules with cyclic terminals. Therefore, the concentration of the reaction mixture at this point induces a large increase in the $\bar{M}_w$ of the polymer due to the coupling reaction. About 24 hr after the beginning of the polymerization, a large fraction of cyclic terminals was deactivated. As a consequence, no important coupling reaction occurs by concentrating the polymerization mixture, and there are no substantial differences between $\bar{M}_w$ of "killed" and "concentrated" samples.

It is significant that if we treat with C^{14}-IPA the "concentrated" polymers, in which a large number of cyclic terminals disappeared because of coupling reactions, very little radioactivity goes into the polymers. The results of such an experiment are shown in Table V. These data clearly indicate that a very small number of cyclic terminals is still present in the "concentrated" polymers.

Table V. Polymerization of γ-Benzyl-L-glutamate in DMF; Initiation by DIPA; Treatment of Concentrated Samples with C^{14}-Labeled IPA

Reaction time, hr	Conversion, %	$\bar{M}_w$ of the concd sample	Radioactivity incorporated in the concd sample after treatment with C^{14}-labeled IPA, (mmole of IPA/100 mg of polymer) $\times 10^4$
0.5	80	60,000	4.6

Conclusions

The data presented in this work allow us to conclude that γ-benzyl-L-glutamate NCA polymerizes in DMF according to the Bamford mechanism when tertiary amines are used as initiators. In fact, we proved that bifunctional intermediates do exist and that cyclic terminals are present in polymer molecules. Moreover the normal primary amine mechanism is operative with IPA as the initiator. Finally, both mechanisms are simultaneously operating with DIPA amine initiation.

We pointed out that in the case of strong base type polymerization, cyclic terminals deactivate *after* the end of the reaction. Since DMF not freshly distilled is able to induce NCA polymerization without addition of initiator, we suggest that deactivation occurs by reaction of cyclic terminals with impurities originating from solvent decomposition.

It is remarkable to observe that by MDIPA initiation, we found only 70% of polymer molecules having cyclic terminals at 60% conversion. These findings can be explained by the fact that cyclic terminals also disappear during the polymerization by reaction with impurities. This termination reaction could be responsible for the presence of unlabeled polymer molecules in the killed polymers.

Also in the case of DIPA initiation, the per cent of polymer molecules with cyclic terminals is quite low. In this case, chain termination and the partial contribution of the "primary amine" mechanism can account for the low extent of polymer chains with cyclic terminals.