lowing values of c: dibenzofulvene, $c = 10^{-3}$; 6-methyldibenzofulvene, $c = 10^{-3}$; 6-cyclohexyldibenzofulvene, any value less than 10^{-1}; 6-phenyldibenzofulvene, any value less than 10^{-1}.

The data for the individual runs are shown in Table II. The ϕ values given refer in all cases to the initial rates observed. With dibenzofulvene itself, which was used only in low concentrations, the rate naturally soon begins to increase fairly rapidly due to consumption of the compound. However, even in this case the initial rates were measurable, albeit with somewhat less accuracy than in the other cases.

COLUMBIA, SOUTH CAROLINA

[CONTRIBUTION FROM THE RESEARCH AND ENGINEERING DIVISION, MONSANTO CHEMICAL CO.]

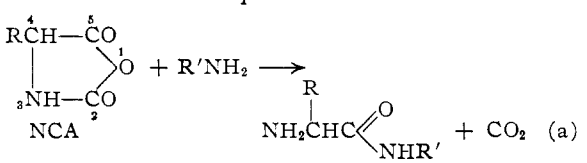
Kinetics and Mechanisms of the Polymerization of N-Carboxy-α-amino Acid Anhydrides[1]

BY HAROLD WEINGARTEN

RECEIVED JULY 15, 1957

The rates of amine-initiated polymerization for a series of N-carboxy-α-amino acid anhydrides have been measured in dioxane at 35°. The most probable form of the over-all rate law was found to be $-d[NCA]/dt = a[NCA][R'NH_2] + b[NCA][R'NH_2]^2$. L- and DL-isomers were observed to differ in both rate and mechanism. Possible mechanisms are discussed.

Although the polymerization of N-carboxy-α-amino acid anhydrides,[2] NCA's, is used extensively in the preparation of peptides, few mechanistic studies have been reported.[3-5]



The purpose of this work was to establish in some detail the relative reactivity of a cross section of NCA's and in so doing to provide insight into the mechanism of polymerization.

Specifically studied were the rates of polymerization of NCA's initiated by *n*-hexylamine[6] in dioxane at 35°.

Experimental

Melting points are uncorrected. The volumetric equipment was calibrated. The disappearance of NCA was followed by the technique described by Berger, Sela and Katchalski.[7] The dioxane was purified by the method of Fieser.[8] End-group determinations were performed by the Analytical Department of Monsanto Chemical Co., Dayton, Ohio.

Kinetic Method.—Between 0.005 and 0.015 mole of NCA was placed in a 100-ml. volumetric flask and 75 ml. of dioxane was added. When the NCA was dissolved completely an appropriate quantity of initiator solution was pipetted into the flask. The solution was brought to volume with dioxane and the flask immediately immersed in a 35 ± 0.1° constant temperature bath. The reaction flask was open to the atmosphere through a drying tube filled with Drierite. Five minutes was allowed for temperature equilibration and then aliquot 1 was removed with an automatic pipet. The aliquot (5 ml.) was emptied into a 25-ml. erlenmeyer equipped with a gas inlet tube. Five ml. of dioxane was added and dry nitrogen passed through the solution for five minutes to remove the carbon dioxide formed in the reaction. The remaining NCA was titrated with sodium methoxide solution to a thymol blue end-point. Aliquots were taken at 7- to 50-minute intervals as required. Figure 1 shows a typical rate plot.

N-Carboxy-α-amino Acid Anhydrides.—The NCA's were prepared by standard syntheses[9] and recrystallized to constant melting points, given in Table I.

TABLE I

N-CARBOXY-α-AMINO ACID ANHYDRIDE MELTING POINTS

NCA	M.p., °C.
Glycine	[a]
ε-N-Carbobenzoxy-L-lysine	99–100 (d.)
γ-Benzyl-L-glutamate	93–94
DL-Alanine	47–48
DL-α-Amino-*n*-butyric	112 (d.)
ε-N-Carbobenzoxy-DL-lysine	109.8–110.4 (d.)
DL-Leucine	48.6–49.2
DL-Valine	79.8–80.4
α-Aminoisobutyric	95.5–96.3

[a] Dec. above 100 without melting.

***n*-Hexylamine Initiator Solution.**—*n*-Hexylamine was distilled from solid sodium hydroxide, b.p. 128–129°. An appropriate amount of amine was weighed into a 100-ml. volumetric flask and dry dioxane was added. The solutions were standardized by titration against standard perchloric acid solution in glacial acetic acid, using crystal violet as indicator.

Sodium Methoxide Solution.—The sodium methoxide titrating solution was prepared by dissolving 1.4 g. of sodium metal in 200 ml. of absolute methanol and adding 800 ml. of dry benzene. This solution was standardized against benzoic acid, using thymol blue as indicator.

Preparation of Peptides for Van Slyke[10] Analysis.—The dioxane solution of peptide remaining in the volumetric flask after a particular "run" was allowed to stand through more than five half-lives. The solution was then poured into excess *n*-pentane and the precipitate collected, dried at 40° *in vacuo* and subjected to a Van Slyke analysis.

(1) Presented at the 131st Meeting of the American Chemical Society at Miami, Fla., April, 1957, Abstr., p. 17-O.

(2) Also referred to as 2,5-oxazolidinediones and Leuch's anhydrides.

(3) S. G. Waley and J. Watson, *Proc. Roy. Soc.* (*London*), **199A**, 499 (1949).

(4) D. H. G. Ballard and C. H. Bamford, *ibid.*, **223A**, 495 (1954).

(5) P. Doty and R. D. Lundberg, THIS JOURNAL, **78**, 4810 (1956).

(6) Although most other amines used as initiators give results similar to *n*-hexylamine, this does not appear to be entirely general. Diethylamine, for example, gives rates about twice those of *n*-hexylamine.

(7) A. Berger, M. Sela and E. Katchalski, *Anal. Chem.*, **25**, 1554 (1953).

(8) L. F. Fieser, "Experiments in Organic Chemistry," 2nd Ed., D. C. Heath and Co., Boston, Mass., 1941, p. 369.

(9) C. H. Bamford, A. Elliot and W. E. Hanby, "Synthetic Polypeptides," Academic Press, Inc., New York, N. Y., 1956, pp. 29–32 and pp. 53–58.

(10) D. G. Doherty and C. L. Ogg, *Ind. Eng. Chem., Anal. Ed.*, **15**, 751 (1943).

Results

As can be seen from Fig. 1 the polymerization occurs in two[11] stages. The first, a slow stage, probably involves amino amide, then dipeptide and tripeptide. Usually between tripeptide and octapeptide the second and faster stage begins. It is from this second stage that the principal rate data are derived.

All of the NCA's studied polymerize with rates which are first order in [NCA]. The amino groups,[12] $R'NH_2$, also appear in the rate law, but their concentration remains constant throughout the reaction since as each molecule of amine disappears another is produced immediately. The reaction is, therefore, pseudo-first-order.

Table II summarizes the principal rate data which are the pseudo-first-order rate constants, k_{obs}, for polymerization at various amino group concentrations, $[R'NH_2]$. Included in Table II is a relative rate sequence determined at $[R'NH_2] = 10^{-2}$ *M*. Although the rate difference from one end of this sequence to the other is not large, increased steric requirements in the R groups appear to be correlated with a systematic decrease in rate. The pseudo-first-order rate constants in Table II indicate that the first four NCA's listed polymerize with rates which are not only first order in [NCA] but also first order in $[R'NH_2]$. The remaining compounds, however, polymerize in reactions higher order in $[R'NH_2]$. The order of $[R'NH_2]$ appearance lies between one and two. Therefore, a change in mechanism as well as a change in rate occurs as R increases in size. Also apparent from Table II is a difference in rate and mechanism between L- and DL-ε-N-carbobenzoxylysine, similar to that observed by Doty and Lundberg.[5]

TABLE II

PRINCIPAL RATE DATA

NCA	Rel. rate at $10^{-2}M$-$[R'NH_2]$	10^2 $[R'NH_2]$, *M*	10^2k_{obs}, $min.^{-1}$
Glycine[a]	30	0.50	10.2
R = -H		0.05	1.20
ε-N-Carbobenzoxy-L-lysine	3.6	1.00	2.6 ± 0.3
R = $-(CH_2)_4NHCO_2CH_2C_6H_5$		0.50	1.2 ± .1
γ-Benzyl-L-glutamate[b]	3.5	0.75	1.74 ± .03
R = $-(CH_2)_2CO_2CH_2C_6H_5$			
DL-Alanine	3.4	1.00	2.3 ± .2
R = $-CH_3$		0.50	1.3 ± .1
		0.25	0.75 ± .03
DL-α-Amino-*n*-butyric	1.5	1.00	1.05 ± .07
R = $-CH_2CH_3$		0.25	0.12 ± .01
ε-N-Carbobenzoxy-DL-lysine	1.00	1.50	1.34 ± .02
R = $-(CH_2)_4NHCO_2CH_2C_6H_5$		1.00	0.70 ± .01
		0.75	.46 ± .02
		0.50	.25 ± .01
DL-Leucine	0.5	1.50	.74 ± .05
R = $-CH_2CH(CH_3)_2$		1.00	.37 ± .02
DL-Valine	0.14	2.25	.44 ± .03
R = $-CH(CH_3)_2$		1.50	.21 ± .01
α-Aminoisobutyric	?	Too slow to measure	
R and H = $-CH_3$			

[a] Data from first 15 min. of reaction, precipitation ends reaction within an hr. [b] See reference 5 for data at 25°.

(11) Actually three stages, a rapid reaction of NCA and initiating amine precedes those pictured in Fig. 1.

(12) By amino groups is meant the end amino groups of the growing peptides. Their concentration is assumed to be equal to that of initiating amine.

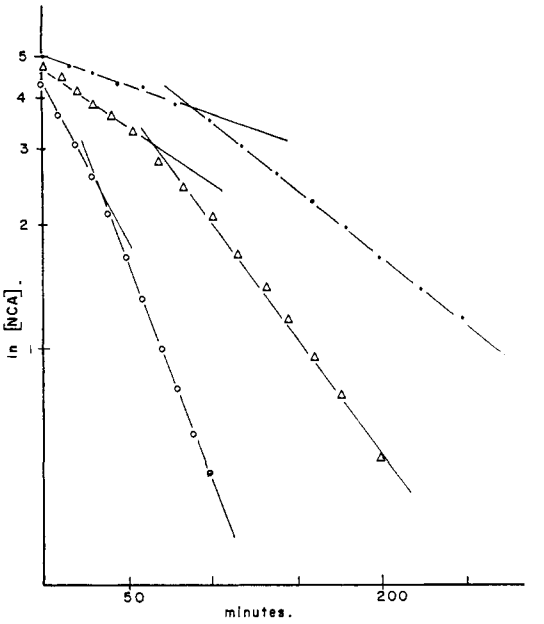


Fig. 1.—Typical rate plot (DL-alanine NCA): ●, $[R'NH_2] = 2.5 \times 10^{-3}$ *M*; △, $[R'NH_2] = 5.0 \times 10^{-3}$ *M*; ○, $[R'NH_2] = 10^{-2}$ *M*; $[NCA]_0 = 5.0 \times 10^{-2}$ *M*.

Table III describes the agreement between the mole ratio of NCA to initiating amine (A/I) and the observed degree of polymerization (DP) of the peptide products. The DP was determined by a Van Slyke analysis which measures the quantity of α-amino nitrogen. The close agreement between the two values allows the tentative conclusion that in the range of these experiments the polymerization proceeds as described in equation a and reaction at the number 2 carbonyl position is extremely limited or entirely lacking. Reaction at the number 2 carbonyl position would remove an amino group yielding ureido acid which does not continue to polymerize.[13]

TABLE III

COMPARISON OF A/I TO DP

Peptide	A/I	DP
Poly-ε-N-carbobenzoxy-L-lysine	7.5	7.8
Poly-γ-benzyl-L-glutamate	10.0	11.2
Poly-DL-alanine	10.0	10.6
	20.0	20.0
Poly-DL-α-amino-*n*-butyric	9.0	9.5
Poly-ε-N-carbobenzoxy-DL-lysine	7.5	6.8
Poly-DL-leucine	7.5	7.45

Discussion

Since the reaction is pseudo first order it can be described by equation b in which k_{obs} is some function of $[R'NH_2]$. Equation c, expressing k_{obs}

$$-d[NCA]/dt = k_{obs}[NCA] \quad (b)$$

as a function of $[R'NH_2]$, has several forms which

$$k_{obs} = f[R'NH_2] \quad (c)$$

will accommodate the observed mixed-order of $[R'NH_2]$ appearance. One form, equation d, rep-

$$k_{obs} = a[R'NH_2] + b[R'NH_2]^2 \quad (d)$$

resents two parallel reactions, one first order and

(13) M. Sela and A. Berger, THIS JOURNAL, 77, 1893 (1955).

another second order in $[R'NH_2]$. Alternatively, (c) can take the form of (e), which is derived from

$$k_{obs} = \frac{a[R'NH_2]^2}{b + c[R'NH_2]} \qquad (e)$$

a steady state approximation.

If (d) and (e) are converted into equations of straight lines, (d') and (e'), and appropriate variables are plotted over a range of $[R'NH_2]$ values,

$$k_{obs}/[R'NH_2] = a + b[R'NH_2] \qquad (d')$$
$$[R'NH_2]/k_{obs} = b/a[R'NH_2] + c/a \qquad (e')$$

the equation representative of the reaction should yield a straight line. ϵ-N-Carbobenzoxy-DL-lysine NCA was polymerized at several $[R'NH_2]$ values and the k_{obs}'s are recorded in Table II. The plots of $k_{obs}/[R'NH_2]$ *vs.* $[R'NH_2]$ for (d') and $[R'NH_2]/k_{obs}$ *vs.* $1/[R'NH_2]$ for (e') are reproduced in Fig. 2. While (d') yields a satisfying straight line,

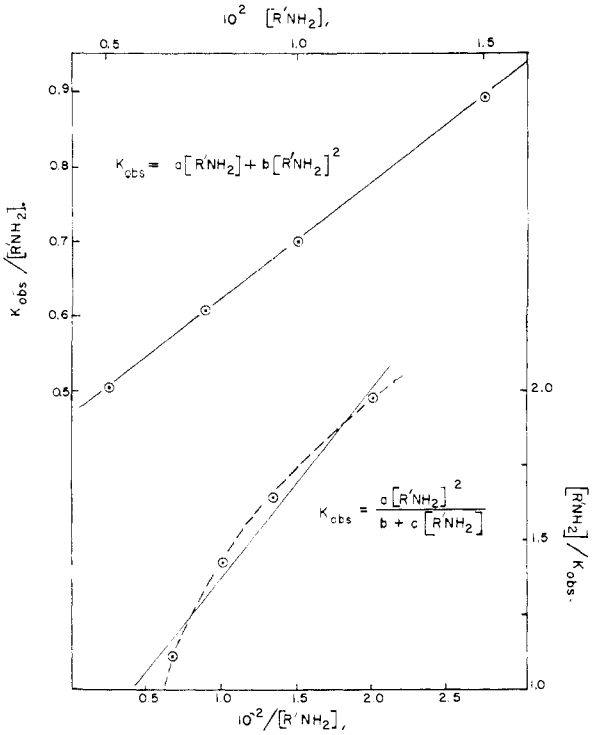


Fig. 2.—Comparison of equations d and e.

(e') gives a rather doubtful fit. On the basis of this analysis we must conclude that equation d is the most probable form of the rate law. Equation b then becomes (b').

$$-d[NCA]/dt = a[NCA][R'NH_2] + b[NCA][R'NH_2]^2 \qquad (b')$$

Figure 3, representing the polymerization of ϵ-N-carbobenzoxy-L- and DL-lysine NCA's performed under identical conditions, provides an interesting clue to the mechanisms of polymerization. The rate of reaction of both stages of the DL-isomer polymerization and of the first stage of the L-isomer polymerization do not differ significantly. Furthermore, all three areas are described by both terms of the over-all rate law. From these observations one is led to conclude that the same mechanisms are operative and that the growing peptide is the same in all three areas. It is reasonable to assume that the peptide structure is the β or randomly coiled type.

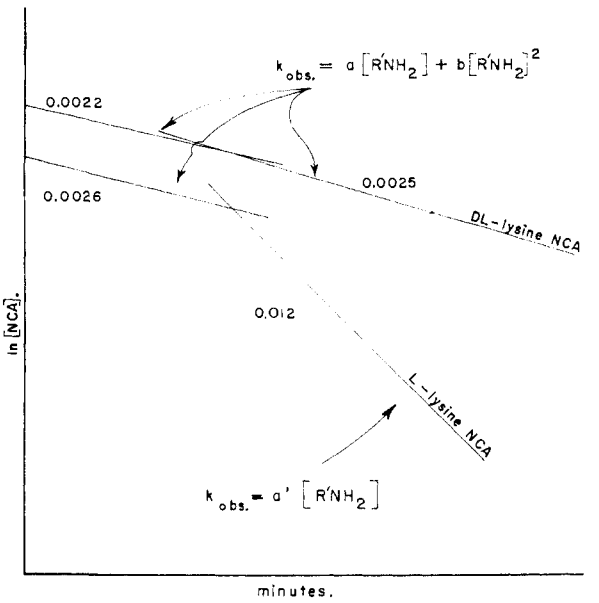
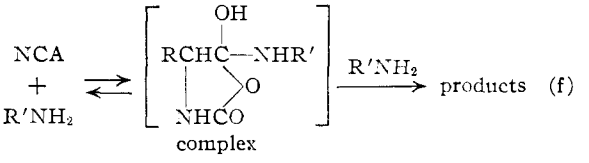


Fig. 3.—Comparison of ϵ-N-carbobenzoxy-L- and DL-lysine NCA polymerizations; $[R'NH_2] = 5.0 \times 10^{-3}$ *M*, $[NCA]_0 = 5.0 \times 10^{-2}$ *M*.

The second stage of the L-isomer polymerization is significantly more rapid than the first and is described by the first term of the over-all rate law only, with, of course, an appropriate constant change. It should be pointed out here that Doty and Lundberg[5] have suggested the second stage of the γ-benzyl-L-glutamate NCA polymerization involves an α or internally hydrogen-bonded form of the peptide.

It appears reasonable that not only two but three parallel reactions can occur: (1) a reaction, second order in $[R'NH_2]$, occurring throughout the DL-isomer polymerization and in the early stage of the L-isomer polymerization; (2) a reaction, first order in $[R'NH_2]$, occurring in the same areas; and (3) a reaction, first order in $[R'NH_2]$, occurring in the second stage of the L-isomer polymerization.

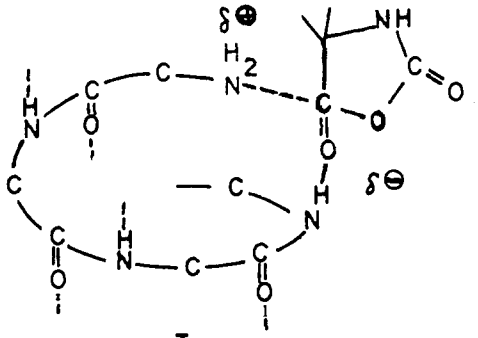
A mechanism for the reaction second order in $[R'NH_2]$, reaction 1, was proposed by Waley and Watson[3] during their studies of the polymerization of sarcosine NCA. This mechanism assumes NCA



and $R'NH_2$ combine in an equilibrium step to form a complex followed by a rate-determining reaction of this complex with another $R'NH_2$.

The slow reaction which is first order in $[R'NH_2]$, reaction 2, can be described by several mechanisms. For example, the complex in (f) might decompose without the second mole of $R'NH_2$. It is also possible that NCA and $R'NH_2$ combine to

give products without the formation of an intermediate complex. Several other mechanisms can be envisioned such as the one discussed below; however none can be selected or rejected on the basis of our present knowledge.



I

The faster reaction which is first order in [R'-NH_2], reaction 3, is a somewhat more interesting source of speculation. If we accept the suggestion of Doty and Lundberg[5] that this reaction involves an α-peptide and add the suggestion that NCA's can hydrogen-bond to the peptide,[14] a reaction proceeding through I becomes immediately attractive. In I, a transition state or intermediate, the negative charge generated on the carbonyl oxygen is distributed into the peptide by resonance. When I decomposes by rearranging electrons, the next unit added to the peptide is already hydrogen-bonded into the chain.

If we re-examine Table II in light of the preceding discussion, a consistent rationale begins to appear. Glycine NCA with only a hydrogen in the R position should readily form α-peptide and polymerize by the fast first-order reaction. A fast first-order rate is observed. L-Isomers should also form α-peptides since they can arrange their side chains in such a way that they do not sterically interact with each other. They would be expected to polymerize by the fast first-order reaction. A fast first-order rate is again observed. DL-Alanine NCA with a small R group, CH_3-group, could conceivably form an α-peptide in spite of its racemic properties. Again a fast first-order rate is observed. The mixed-order reaction begins to appear with DL-α-amino-iso-butyric NCA where R is CH_3CH_2–, and continues throughout the rest of the series. The anhydrides proceeding by a mixed-order reaction, (1) and (2) above, are presumably producing β-peptide.

DAYTON, OHIO

(14) D. G. H. Ballard and C. H. Bamford, *Nature*, **177**, 477 (1956).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Amine Oxides. V. Olefins from N,N-Dimethylmenthylamine and N,N-Dimethylneomenthylamine Oxides[1,2]

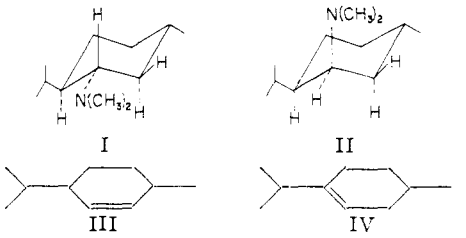
BY ARTHUR C. COPE AND EDWARD M. ACTON[3]

RECEIVED JULY 17, 1957

N,N-Dimethylneomenthylamine oxide forms pure 2-menthene on thermal decomposition. This observation provides additional evidence that the amine oxide pyrolysis proceeds by *cis* elimination of a β-hydrogen atom and the amine oxide function through a cyclic transition state. N,N-Dimethylmenthylamine oxide under similar conditions forms an olefin mixture (85%) containing 64.8% of 2-menthene and 35.2% of 3-menthene, both of which can be formed by *cis* elimination from this amine oxide.

This paper describes an additional test of the steric course of the conversion of amine oxides to olefins by study of the thermal decomposition of N,N-dimethylneomenthylamine oxide and N,N-dimethylmenthylamine oxide. Previous evidence that this type of reaction is a *cis* elimination process was obtained by pyrolysis of the oxides of *threo*- and *erythro*-2-(N,N-dimethylamino)-3-phenylbutane, which formed almost exclusively the conjugated olefins produced by *cis* elimination.[4]

N,N-Dimethylmenthylamine (I) could give either 2-menthene (III) or 3-menthene (IV) by *cis* elimination from the amine oxide, whereas application of the Hofmann method could form only 2-menthene by *trans* elimination. On the other hand, *cis* elimination from the oxide of N,N-dimethylneomenthylamine (II) could give only 2-menthene, while formation of either 2- or 3-menthene would be possible from the quaternary base.



I II

III IV

By using the optically active amines, the olefinic products could be analyzed by preferential acid-catalyzed racemization of 3-menthene.[5-7]

l-Menthylamine was prepared by reduction of *l*-menthone oxime with sodium in alcohol and characterized as *l*-menthylamine hydrochloride.[8] *d*-Neomenthylamine was prepared from *l*-menthone

(1) Sponsored by the Office of Ordnance Research, U. S. Army, under contract No. DA-19-020-ORD-3226, Project TB 2-0001(1112).

(2) Paper IV in this series, THIS JOURNAL, **79**, 4729 (1957).

(3) National Science Foundation Fellow, 1954–1955; National Institutes of Health Fellow, 1955–1956.

(4) D. J. Cram and J. E. McCarty, THIS JOURNAL, **76**, 5740 (1954).

(5) W. Hückel, W. Tappe and G. Legutke, *Ann.*, **543**, 191 (1940).

(6) N. L. McNiven and J. Read, *J. Chem. Soc.*, 153 (1952).

(7) N. L. McNiven and J. Read, *ibid.*, 2067 (1952); J. P. Wibaut, H. C. Beyerman and H. B. van Leeuwen, *Rec. trav. chim.*, **71**, 1027 (1952).

(8) E. S. Rothman and A. R. Day, THIS JOURNAL, **76**, 111 (1954).