65. *Stereochemical Aspects of the Reactions between α-N-Carboxyamino-acid Anhydrides and Primary and Secondary Bases.*

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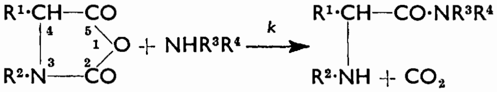
The bimolecular rate constants for the reaction between a number of substituted *N*-carboxyglycine anhydrides and three bases (glycine dimethylamide and derivatives of it) have been determined with a view to elucidating the steric course of the reactions. A substituent on the nitrogen atom in the anhydride ring causes steric interference which is particularly pronounced if the base carries an *iso*propyl group on the nitrogen atom. Substitution at position 4 reduces the rate of addition of a primary base at position 5 on the same side of the ring as the substituent; with a secondary base the effect is much greater. The configuration of the transition state is discussed; the observations are consistent with an approach to $C_{(5)}$ by the attacking base along a line passing over the ring-nitrogen atom slightly inclined to the plane of the ring. The fused rings in *N*-carboxy-L-proline anhydride confer a saucer-like shape on the molecule which results in a reduction of the steric interference mentioned above; this is reflected in higher rate constants.

POLYMERIZATION of the *N*-carboxy-anhydrides of α-amino-acids has been investigated in detail in recent years, and mechanisms have been advanced for the reactions initiated by amines and salts.[1] With primary and secondary bases the initiation and the propagation reactions are fundamentally similar, consisting of addition of a base molecule to the carboxyl group in position 5 followed by elimination of carbon dioxide:



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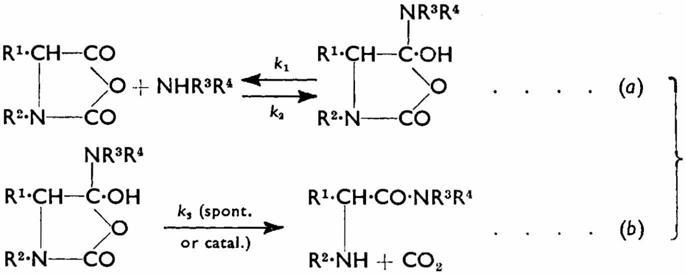
In at least one instance this type of reaction is strongly catalyzed by anions of weak organic acids, including carbamic acids, but when this complication is absent the reactions are of first order in anhydride and base.[2] The general mechanism may be written in the annexed form.



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[1] For a summary and references see Bamford, Hanby, and Elliott, "Synthetic Polypeptides," Academic Press, New York, 1956, Chap. III.

[2] Ballard and Bamford, unpublished work.

Two extreme cases are encountered; in one (exemplified by *N*-carboxysarcosine anhydride) the equilibrium in (2*a*) is effectively established and (2*b*) is rate-determining, while in the other (of which *N*-carboxy-DL-phenylalanine anhydride is an example) the reaction in (2*a*) appears to be rate-controlling.[3]

Different anhydrides polymerize at very different rates, and in particular those with substituents on the nitrogen and at position 4 frequently fail to polymerize when treated with any type of initiator. The *N*-carboxy-anhydride derived from α-methylalanine ("α-amino*iso*butyric" acid) is a good example; this does not polymerize at room temperatures when treated with amines, salts of weak organic acids, or sodium methoxide. As in this case, the substituents are usually saturated and comparatively non-polar and are therefore unlikely to produce pronounced changes in electron density, so it appears that their influence on reactivity is predominantly steric. We describe in this paper an investigation in which the substituents in the *N*-carboxy-anhydride and the attacking base were varied in a systematic manner, with the object of elucidating the steric features of the reactions. For this purpose concentrations of base at least equal to those of the anhydride were used; little polymerization then occurs if the initiating base and the base resulting from reaction (1) have comparable strengths, and the observed rates correspond closely to those of the initiation reaction. In all cases except proline the DL-anhydride was used. The observed differences in rate are discussed in terms of the dependence of the velocity coefficient k_1 on steric factors.

EXPERIMENTAL

Materials.—The *N*-carboxy-anhydrides were prepared from the α-amino-acids by standard methods which have been summarized elsewhere.[3] They were purified by recrystallization until the chloride content was less than 0·05%, then sublimed and used immediately. The sublimed materials contained no detectable chloride.

The bases used were glycine dimethylamide and substituted derivatives ($R^4 = CH_2\cdot CO\cdot NMe_2$, in all cases). They were made by two methods. In the first the appropriately substituted *N*-carboxy-glycine anhydride was treated with a large excess of liquid dimethylamine. Excess of the latter was distilled off and the residual oil fractionated in vacuum. In the second method chloroacetyl chloride with dimethylamine yielded α-chloro-*NN*-dimethylacetamide,[4] which was treated with a 33% w/v solution of the appropriate amine for 3 days at room temperature. The base was isolated by fractionation. The equivalent weights of the amines were determined and the b. p.s agreed with those recorded.[5] Elementary analysis gave results in satisfactory agreement with those calculated.

The purification of nitrobenzene, used as solvent, has been described elsewhere.[3]

Technique.—The reactions were followed by the evolution of carbon dioxide, essentially as described earlier.[3] The normal precautions were taken to exclude water; none of the reactants was allowed to come into contact with atmospheric moisture, and the reaction vessel containing the anhydride and the base solution in separate compartments was prepared in a dry-box.

The "initial" rates, corresponding to conversions of 5% or less, were measured. The rates were usually almost constant over this range, and it is unlikely that the pressure of carbon dioxide formed would be great enough to have a significant catalytic effect on the reaction rates. Many of the reactions were too rapid to be followed conveniently in the simple constant-pressure apparatus which was used in an earlier investigation of catalysis by carbon dioxide.[3]

The concentration of base was measured before and after reaction by potentiometric titration of the solution diluted with about ten times its volume of alcohol. In all cases the initial and the final concentration of base were the same within experimental error, except when reaction was very slow. This is discussed below.

RESULTS AND DISCUSSION

In all the systems examined the reactions were of first order in anhydride and base over the range studied. The Figure, a first-order plot for a typical run, shows that, while

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

[4] Freudenberg and Markert, *Ber.*, 1927, **60**, 2447.

[5] Waley and Watson, *Proc. Roy. Soc.*, 1949, *A*, **199**, 499.

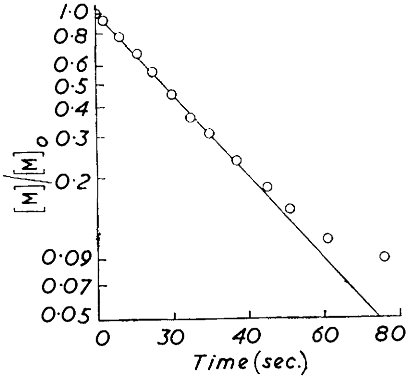
the rate constant for the initial reaction can be determined unambiguously, the reaction eventually departs from first-order dependence on anhydride concentration on account of the increasing importance of the propagation reaction.[3] Usually the anhydride and base concentrations were varied over an eight-fold and a five-fold range respectively; these represent the extremes which can be used with the present apparatus. Typical results are shown in Table 1 in which $[M]_0$, $[X]_0$ are the initial anhydride and base concentration and k the overall second-order constant. This constant will, in the limiting cases, be either k_1k_3/k_2 or k_1 [see equation (2)]. For present purposes distinction between the various cases is unnecessary since we are attributing the major differences observed to changes in k_1.

TABLE 1. *Reaction of* N-*carboxy*-N-*methyl*-DL-*alanine anhydride* ($R^1 = R^2 = Me$) *with glycine dimethylamide* ($R^4 = CH_2{\cdot}CO{\cdot}NMe_2$, $R^3 = H$) *in nitrobenzene at* 15°.

$10^2[M]_0$ (mole $l.^{-1}$)	9·00	5·46	4·92	2·66	2·29	1·08
$10^2[X]_0$ (mole $l.^{-1}$)	10·6	4·93	10·6	1·92	4·93	1·92
k (mole^{-1} l. sec.$^{-1}$)	0·43	0·45	0·44	0·45	0·45	0·48

Mean 0·45

The rate constants for the various anhydrides and bases are collected in Table 2; the extreme deviations from the mean values are shown; *N*-carboxyglycine anhydride is not included since its behaviour is in some ways abnormal and is insufficiently understood for kinetic analysis.



Reaction of N-*carboxy*-N-*methyl*-DL-*alanine anhydride with glycine dimethylamide in nitrobenzene at* 15°. $[M]_0 = 9{\cdot}0 \times 10^{-2}$ mole $l.^{-1}$; $[X]_0 = 10^{-2}$ mole $l.^{-1}$.

In discussing these results it is first necessary to consider the configuration of the *N*-carboxy-anhydride ring. In the absence of complicating factors the five-membered ring would be expected to be planar, with the $N\text{-}R^2$ bond and the carbonyl-oxygen atoms in the plane of the ring. The group R^1 would, of course, lie on one or other side of this plane, its position being determined by the asymmetry at the carbon atom $C_{(4)}$. This configuration would be expected to be the stable one if $R^1 = R^2 = H$. When $R^1 = R^2 =$ Me the situation is different. If the ring were planar some repulsion would be expected between R^2 and the neighbouring CO group, which would be in a completely eclipsed conformation, and also between R^1 and R^2. These repulsions would be relieved if the $N\text{-}R^2$ bond were to depart slightly from the mean plane of the ring; this might arise, for example, by a small amount of rotation about the $C_{(2)}\text{-}N$ and the $N\text{-}C_{(4)}$ bond. The centres of the two methyl groups would then lie on opposite sides of the mean plane of the ring. A greater deviation from planarity would be expected if R^1 and R^2 were groups more bulky than methyl. The molecule of *N*-carboxyproline anhydride consists of two fused five-membered rings, and models show that there is considerable strain in the structure. Both the $C_{(4)}\text{-}R^1$ and the $N\text{-}R^2$ bond deviate from the mean plane of the anhydride ring in the same sense, and the whole molecule has a saucer-like shape. If a pyramidal nitrogen atom is used it is possible to construct two configurations; one of

TABLE 2. *Reactions between* N-*carboxy-anhydrides and bases in nitrobenzene at* 15° ($R^3 = CH_2{\cdot}CO{\cdot}NMe_2$).

N-Carboxy-anhydride		Base	k	Rates * of addition on two sides of ring:	
R^1	R^2	R^3	(mole^{-1} l. sec.$^{-1}$)	same side as R^1	side opposite to R^1
Me	H	H	1·13 ± 0·10	m	n
		Me	0·48 ± 0·02	s	n
		Pr^i	0·10 ± 0·01	vs	m
H	Me	H	0·82 ± 0·03	n	
		Me	0·57 ± 0·03	n	
		Pr^i	$7{\cdot}4 \times 10^{-4}$	s	
Me	Me	H	0·45 ± 0·02	m	m
		Me	0·16 ± 0·03	s	m
		Pr^i	2×10^{-5}	vs	vs
Ph·CH$_2$	H	H	1·0 ± 0·2	m	n
		Me	0·40 ± 0·07	s	n
		Pr^i	0·30 ± 0·02	vs	m
H	Pr^i	H	0·83 ± 0·03	n	
		Me	0·48 ± 0·04	n	
		Pr^i	$< 10^{7}$	vs	
α-*N*-Carboxyamino-α-methylalanine anhydride		H	0·29 ± 0·05	m	
		Me	$8{\cdot}4 \times 10^{-4}$	s	
		Pr^i	1×10^{-6} approx.	vs	
N-Carboxy-L-proline		H	2·68 ± 0·08	vs	f
		Me	2·00 ± 0·07	vs	f
		Pr^i	0·12 ± 0·02	vs	m

* f = fast, n = normal, m = medium, s = slow, vs = very slow.

these is similar to that described above, while the other is so highly strained that it is unlikely to represent a possible configuration of the molecule. The saucer-like shape of the molecule has an important bearing on its reactivity, as will appear below.

We now consider the effect of changes in R^3 on the bimolecular rate constant. The strengths of the three bases used are not very different, the pK_b values at 20° being 5·67, 5·21, 5·32 respectively for R^3 = H, Me, and Pr^i. It is known that, other things being equal, an increase in the basic strength leads to an increase in the rate of reaction,[1] a change of 1 unit in pK_b corresponding to a factor of 3 approximately. The extreme difference in reactivity of the three bases attributable to changes in basic strength alone should therefore be less than a factor of 2. From Table 2 we see that if R^1 = Me and R^2 = H, the change of R^3 from H to Pr^i leads to a reduction in the rate constant by a factor of 10 approximately. This is in a direction opposite to that expected from considerations of basic strength, and indicates that steric interference plays a part when R^3 = Pr^i. Similar considerations apply to the reactions of *N*-carboxy-DL-phenylalanine anhydride (R^1 = Ph·CH$_2$, R^2 = H) with the three bases. The important point is that the presence of an *iso*propyl group in the base does not, by itself, cause a very large reduction in rate (*i.e.*, a reduction $\gg$ ten-fold). If now we consider the anhydride with $R^1 = R^2$ = Me, we see that if R^3 = H or Me the rates are somewhat lower than those already considered for R^1 = Me, R^2 = H—by a factor less than 4—but if R^3 = Pr^i the rates in the two cases differ by a factor of 5000. From the foregoing it will be clear that this cannot be an electronic effect, but can only be the result of steric interference involving the methyl group on the nitrogen atom in the ring and the *iso*propyl group in the base. A similar conclusion may be reached by comparing the rate constants for R^1 = H and R^2 = Me or Pr^i. Change of R^2 from Pr^i to Me produces no change in rate if R^3 = H, and it reduces the rate constant only slightly if R^3 = Me; however it decreases the rate by a factor of more than 1000 if R^3 = Pr^i. Here again there must be interference between bulky substituents on the nitrogen atom in the ring and in the base.

This interference must occur in the transition state and therefore implies that the base approaches $C_{(5)}$ along a path which is slightly inclined to the plane of the ring and passes more or less directly above (or below) the nitrogen atom in the ring. This is the path which is most nearly collinear with the bond that is being broken, *i.e.*, one of the carbonyl $C_{(5)}$–O bonds. It will be recalled that in simple bimolecular nucleophilic displacements the reagent approaches along a line which coincides in direction with the bond being broken; in the transition state the other three bonds of the carbon atom at which the displacement occurs are in a plane perpendicular to this direction, the attacking and the displaced group being on opposite sides of this plane. In the present case the direction of approach is therefore similar to that found in simple nucleophilic substitution, although the configuration of the transition state must necessarily be different since the bond being broken is part of a double bond, and the other bonds of the carbon atom concerned are part of a ring system. We therefore believe that in the transition state the nitrogen atom of the attacking base is located between $C_{(5)}$ and the nitrogen atom in the anhydride ring, with its centre above the plane of the ring. An examination of models reveals that with this arrangement considerable interference between R^2 and R^3 is possible if these are bulky groups. A further point to note is that the oxygen atoms of the carbonyl groups of the anhydride carry a negative charge, and therefore tend to resist the approach of the nitrogen of the attacking base, since this will be the negative pole of the C–N dipole. The nitrogen atom in the ring will, on the other hand, carry a positive charge, and the region of lowest electron density presumably lies between this atom and $C_{(5)}$. The suggested line of attack may therefore represent the most favourable direction of approach to $C_{(5)}$ on purely electrostatic considerations.

The suggested transition state will always involve a restriction on the rotation of alkyl groups attached to the basic nitrogen atom, and this will be at least partly responsible for the low frequency factors observed for these reactions.[1]

We now discuss the effects of substitution at $C_{(4)}$. A 4-methyl group necessarily gives rise to steric interference in the transition state which is particularly severe when the attacking base is secondary. In fact, addition of these bases to $C_{(5)}$ on the same side of the ring as the methyl group appears to be very difficult. The reaction of primary bases is affected to a much smaller extent. These points are illustrated by the behaviour of α-*N*-carboxy-α-methylalanine anhydride, in which $C_{(4)}$ carries two methyl groups and $R^2 = H$. This anhydride reacts with a primary base at a rate which is approximately one quarter of that obtained when $C_{(4)}$ carries only one methyl group and $R^2 = H$ (Table 2). A 4-methyl group therefore appears to reduce the rate of addition of a primary base to $C_{(5)}$ *on the same side of the ring* by a factor of about 7. The rate of addition of secondary bases is clearly reduced to a very much greater extent.

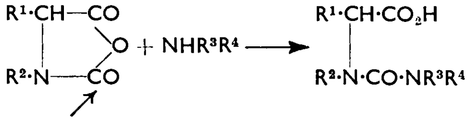
If addition on the side of the ring remote from R^1 were unaffected the maximum reduction in the overall rate constant which could be produced by a bulky substituent on $C_{(4)}$ would amount to a factor of 2. We have already seen, however, that if R^1 and R^2 are alkyl groups their centres will lie on opposite sides of the mean plane of the ring, and as a consequence addition to $C_{(5)}$ may be obstructed on both sides of the ring. It would be expected, therefore, that the introduction of a bulky group on $C_{(4)}$ might lead to a reduction in rate constant greater than that mentioned above if R^2 is other than H, particularly if the attacking base is secondary. These ideas are borne out of the results in Table 2. When $R^2 = Me$, $R^3 = H$, replacing $R^1 = H$ by $R^1 = Me$ reduces the rate constant by a factor of nearly 2. In this case the base is primary and the overall addition is not greatly impeded by R^1 or R^2. However, when $R^3 = Me$ or Pr^i the corresponding changes are greater, amounting to factors of 3·5 and 37 respectively. Here addition on the same side as R^1 is very greatly reduced, while addition on the other side is impeded by the projecting R^2 group.

It is interesting that, with $R^2 = Me$, the velocity constant falls off much more rapidly in passing from $R^3 = Me$ to $R^3 = Pr^i$ when $R^1 = Me$ than when $R^1 = H$. This is a further indication of the effect of a 4-methyl substituent, causing a departure of the N–R^2 bond from the plane of the ring. The resulting obstruction to addition on the side of the ring away from R^1 naturally increases with the size of R^3.

N-Carboxy-L-proline anhydride introduces some new features. The strain in the molecule may account in part for the relatively fast reaction of this anhydride with glycine and sarcosine dimethylamides ($R^3 = H$ or Me respectively), the reactions being 6—12 times faster than those with *N*-carboxy-*N*-methyl-DL-alanine anhydride ($R^1 = R^2 =$ Me). The last anhydride is structurally similar to the proline derivative except that no second ring is present. This conclusion will only hold if the kinetics are of the " sarcosine " type, *i.e.*, if the rate is proportional to the rate of decomposition of the complex formed between the anhydride and the base. In this case the strain in the ring might increase k_3 [equation (2)] and hence the overall rate constant. The molecule being saucer-shaped, attack at $C_{(5)}$ from the convex side of the saucer should occur comparatively readily since obstruction by the substituents on the nitrogen and $C_{(4)}$ is relatively small. Approach of the base along a path almost collinear with a $C_{(5)}$–O bond is in fact possible. This is probably the explanation of the relatively fast reaction of this anhydride with *N*-*iso*propylglycine dimethylamide ($R^3 = Pr^i$), whose rate constant is approximately 6000 times that of the corresponding reaction with the *N*-methyl-DL-alanine derivative.

The rates of addition of the bases on the two sides of the rings of the various anhydrides are summarized qualitatively in the right-hand portion of Table 2.

The decrease (see above), during the slow reactions, in the base content of the system may amount to as much as 20%. In these cases addition at $C_{(5)}$ is greatly hindered and addition at $C_{(2)}$ may take place, with formation of a ureido-derivative and a consequent reduction in the free base concentration:



. . . . (3)

This reaction may not be a carbonyl addition, and its steric course may therefore be different from that for attack at $C_{(5)}$. For example, the base may approach in the direction $C_{(2)}$–$O_{(1)}$ shown by the arrow. When there is no steric restriction on the addition at $C_{(5)}$ reaction (3) occurs only to a very small extent at room temperature since it has a higher activation energy than reaction (1). It is, however, detectable at high temperatures, and constitutes a termination reaction in the polymerization of *N*-carboxy-anhydrides under these conditions.[6]

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[6] Sela and Berger, *J. Amer. Chem. Soc.*, 1953, **75**, 6350; 1955, **77**, 1893.