BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 42 1078—1082 (1969)

Catalysis in Peptide Synthesis with Active Esters. II. Effects of Concentrated Carboxylic Acids on the Aminolysis of Benzyloxycarbonyl-L-phenylalanine *p*-Nitrophenyl Ester in Dioxane

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(Received July 31, 1968)

The kinetics of the aminolysis reaction of benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester with glycine *t*-butyl ester in dioxane were studied in the presence of high concentrations of several carboxylic acids. Bell-shaped curves were obtained when the rate constants observed for the trimethylacetic acid-, acetic acid-, and monochloroacetic acid-catalyzed reactions were plotted against the logarithms of the reciprocals of the acid concentrations. Cyanoacetic acid and trichloroacetic acid did not accelerate, but, rather, retarded the reaction. In the mathematical derivation the catalyst apparently exhibits no accelerating effect, if $k_0K > k_{cat}$, where k_0 and k_{cat} are the uncatalyzed and the true catalyzed rate constants respectively, and where K is the acid-base equilibrium constant between the nucleophile and the catalyst in a given solvent. The true catalyzed rate constants, k_{cat}, from which the effect of the protonation of the nucleophile had been eliminated were estimated for the trimethylacetic acid- and acetic acid-catalyzed reactions. The fact that the k_{cat} value ($63\text{M}^{-2}\,\text{min}^{-1}$) for trimethylacetic acid, the weaker acid, was greater than that ($53\text{M}^{-2}\,\text{min}^{-1}$) for acetic acid strongly supports the bifunctional, concerted mechanism presented in the preceding paper.

In the preceding paper,[1] evidence was presented that some carboxylic acids act as bifunctional catalysts in the aminolysis of an active ester, benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (I), in anhydrous dioxane. The catalytic effect apparently decreased in the sequence: trimethylacetic acid>acetic acid>monochloroacetic acid. The apparent effects of these carboxylic acids must, however, be the sum of the accelerating effects due to the catalysis and the retarding effects due to the protonation of the amine component. In order to confirm this supposition, further investigations were undertaken in which the concentrations of the carboxylic acids were varied over wide ranges and the stronger carboxylic acids were used. If the supposition is correct, it is desirable to ascertain the "true" catalytic abilities of these acidic catalysts by substracting the effects due to the protonation of the nucleophile from the apparent effects. The present author has succeeded in estimating the "true" catalyzed constants for some carboxylic acids from the rate data. The present results support strongly the bifunctional, concerted mechanism which was presented in the preceding paper.[1]

Experimental

The cyanoacetic acid and trichloroacetic acid were recrystallized from ether and toluene respectively. The other materials and kinetic procedure were the same as have been described in the preceding paper. All the reactions were conducted at 30°C.

Results

Table 1 summarizes the observed second-order rate canstants in the aminolysis of I with glycine *t*-butyl ester (II) at 30°C in anhydrous dioxane in the presence of trimethylacetic, acetic, monochloroacetic, cyanoacetic, and trichloroacetic acid. The latter two acids did not accelerate, but, rather, retarded the reaction. The constants for trimethylacetic, acetic, and monochloroacetic acid are plotted against the logarithms of the reciprocals of the acid concentrations (p[acid]) in Fig. 1.

The possibilities of the mixed anhydride formation and the acyl exchange reaction between I and carboxylic acid can be ruled out, because benzyloxycarbonyl-L-phenylalanine and acylglycine *t*-butyl esters, which would be formed if these reactions took place, could not be detected by

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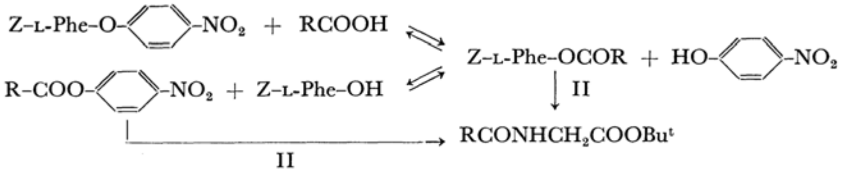
1) N. Nakamizo, This Bulletin, **42**, 1071 (1969).

TABLE 1. OBSERVED SECOND-ORDER RATE CONSTANTS FOR THE VARIOUS CARBOXYLIC ACID-CATALYZED REACTIONS AT 30°C

Concentration of acid M	k_{obs}, M^{-1} min^{-1}				
	CMe_3COOH	CH_3COOH	$CH_2ClCOOH$	$NCCH_2COOH$	CCl_3COOH
0.000	0.62	0.62	0.62	0.62	0.62
0.002	0.74	0.71	0.69	0.61	0.03
0.004	0.85	0.80	0.73	0.58	0.00
0.006		0.90	0.78		
0.007	1.05				
0.008		1.00	0.81	0.57	
0.010	1.21	1.09	0.85		
0.020	1.70	1.51	0.88	0.41	
0.030	2.16	1.88	0.91	0.34	
0.040	2.60	2.23			
0.050	2.98	2.53	0.84	0.26	
0.060	3.34	2.81			
0.080	3.98	3.30			
0.10	4.53	3.73			
0.25		5.09			
0.30	6.45	5.11			
0.50	6.50	4.57			
1.00	4.64	2.48			
pure acid		0.0			

[I] = [II] = 2×10^{-3} M



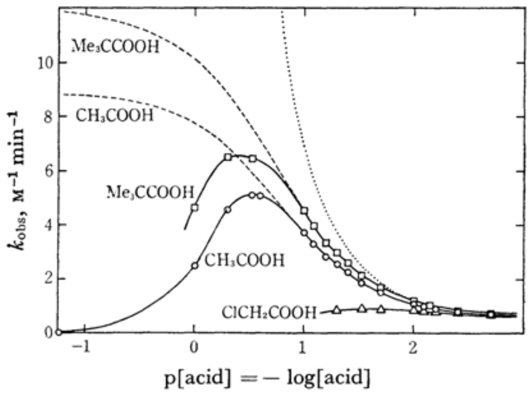


Fig. 1. Dependence of the observed rate constants on the acid concentrations at 30°C.
Solid lines: observed curves; dotted line: theoretical curve for trimethylacetic acid which was calculated from a linear relationship between the k_{obs} and the acid concentration; broken lines: theoretical curves which were derived from the assumption of the acid-base equilibrium.

thin-layer chromatography even at a high concentration of carboxylic acid.

Discussion

As has been shown in the preceding paper,[1] the observed second-order rate constants for the aminolysis reaction of I with II in the presence of trimethylacetic or acetic acid at concentrations lower than 10^{-2}M in dioxane were linearly proportional to the acid concentration. On the other hand, for the monochloroacetic acid-catalyzed reaction, plots of the rate constants against the catalyst concentration showed a negative deviation from the linear relationship. These facts appear to reflect the increase in the protonation of the amine component accompanying the increase in the acid strength and the concentration of the acidic catalyst. If this presumption is correct, similar negative deviations will also appear for the trimethylacetic acid- and acetic acid-catalyzed reactions in higher concentrations of the catalysts. Moreover, for stronger acids, the apparent catalytic effect may disappear.

The data in Table 1 and Fig. 1 show that this is indeed the case. For example the observed constants for trimethylacetic acid deviated from the calculated curve (dotted line) for which a linear relationship between the rate and the acid concentration was assumed. Furthermore, the values observed at concentrations of more than 0.1M of the acid exhibited a negative deviation even from the calculated curve (broken line) which was based on the assumption that the acidic catalyst partly protonates the amine component to convert it to inactive species, thus resulting in the characteristic bell-shaped curve. For the acetic acid- and monochloroacetic acid-catalyzed reactions also, similar phenomena were observed. The stronger the strength of the acid, the smaller becomes the maximum value of the curve, and the position of the maximum shifts toward a lower acid concentration.*2

In the case of cyanoacetic acid, no maximum was any longer detectable, and the values of k_{obs} for this acid decreased monotonically with an increase in the acid concentration (Table 1). Furthermore, trichloroacetic acid, which is the strongest acid among the catalysts used in the present investigation, inhibited the reaction almost completely when more than one equivalent was added to the amine component (Table 1).

If the apparent effects of the carboxylic acids are the sum of the accelerating effects by the catalysis and the retarding effects by the protonation of the nucleophile, it is necessary to obtain "true" rate constants which do not involve the effects of the protonation.

The acid-base equilibria between carboxylic acids and the amine in a given solvent were assumed to conform to the general equation:

$$\mathrm{RCOOH} + \mathrm{H_2N\text{-}R'} \overset{K}{\rightleftharpoons} \mathrm{RCOO^{-}\cdot H_3{}^{+}N^{-}R'} \qquad (1)$$

To simplify, the difference between the free carboxylic acid monomer and the monomer-solvent complex[5] is neglected. With relatively low concentrations (below 0.1M), the presence of the acid dimer in dioxane can be neglected.[6] Then, the ionization constant, K, is written:

$$K = \frac{\alpha}{(c-\alpha b)(1-\alpha)} \qquad (2)$$

where b and c are the total concentrations of II and carboxylic acid respectively, and where α is the degree of the ionization of II. When $c \gg \alpha b$, we may write:

$$\alpha = cK/(1+cK) \qquad (3)$$

If only free amine is a reactive species, the observed second-order rate constants are represented by:

$$k_{obs} = k_0(1-\alpha) + k_{cat}(1-\alpha)c \qquad (4)$$

where k_0 and k_{cat} are the uncatalyzed and the "true" catalyzed rate constant respectively. The substitution of $(1-\alpha)$ from Eq. (3) into Eq. (4) gives:

$$k_{obs} = \frac{k_0 + c\cdot k_{cat}}{1+cK} \qquad (5)$$

Differentiating this with respect to the concentration of the catalyst, we obtain:

$$\frac{dk_{obs}}{dc} = \frac{k_{cat} - k_0 K}{(1+cK)^2} \qquad (6)$$

Equation (6) reveals that when $k_{cat} > k_0 K$, dk_{obs}/dc is positive; that is, the catalyst exhibits a positive effect on the aminolysis reaction in a given solvent. On the other hand, when $k_{cat} < k_0 K$, the catalyst apparently shows a negative effect. The right limb of each profile shown in Fig. 1 corresponds to the former case, while the left side of each maximum, and the effects of cyanoacetic and trichloroacetic acid, correspond to the latter.

In order to determine the "true" rate constant, k_{cat}, it is necessary to know the value of K. This information is, however, not available at present. Therefore, the author tried conversely to derive the ionization constants, K, from the rate data obtained here.

Rearranging Eq. (5), we obtain:

$$k_{obs} = \frac{k_{cat}}{K} - \frac{k_{obs} - k_0}{cK} \qquad (7)$$

Equation (7) requires that a plot of k_{obs} against $(k_{obs}-k_0)/c$ be a straight line with a slope equal to $-(1/K)$ and an intercept at k_{cat}/K. The resulting plots (Fig. 2) for data below 0.1M of trimethylacetic and acetic acids were satisfactorily linear, with deviations only at the lower catalyst concentrations, where the proximity in the values of k_{obs} and k_0 brings about large uncertainties in the remainder $(k_{obs}-k_0)$. These good linearities may substantiate the validity of the assumptions on which Eq. (7) is based.

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*2 Litvinenko *et al.* found that for the acetylation of *p*-nitroaniline with acetic anhydride in mixture of benzene and acetic acid, the maximum of the rate constants appears at about 50%(v/v) of the acid,[2] while for the acylation of *m*-chloroaniline[3,4] or aniline[4] in mixtures of benzene and trichloroacetic acid, the rate constants have maxima at much lower concentrations (~0.01M) of the acid.

2) L. M. Litvinenko and D. M. Aleksandrova, *Ukr. Khim. Zh.*, **27**, 336 (1961).

3) L. M. Litvinenko, D. M. Aleksandrova and S. F. Prokopovich, *ibid.*, **27**, 494 (1961).

4) L. M. Litvinenko and D. M. Aleksandrova, *ibid.*, 634 (1961).

5) N. Muller and P. I. Rose, *J. Phys. Chem.*, **69**, 2564 (1965).

6) J. Lascombe, M. Haurie and M. L. Josien, *J. Chim. Phys.*, **59**, 1433 (1962).

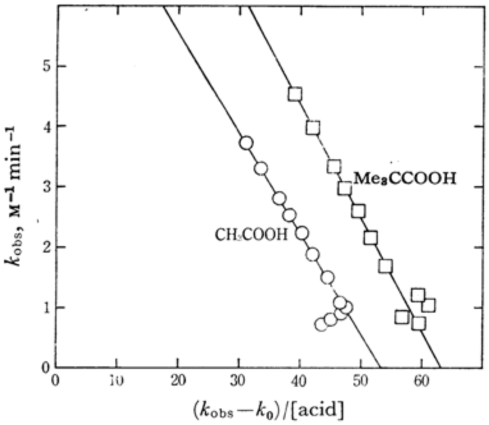

Fig. 2. Plots of Eq. (7), at 30°C.

TABLE 2. THE "TRUE" CATALYZED-RATE CONSTANTS AND THE IONIZATION CONSTANTS FOR TRIMETHYLACETIC ACID AND ACETIC ACID

	k_{cat}, M^{-2} min^{-1}	K, M^{-1}
Trimethylacetic acid	63 ± 2	5.3 ± 0.2
Acetic acid	53 ± 2	6.0 ± 0.2

From the least-squares calculations for the plots, k_{cat} and K values for these two acids were obtained (Table 2).

The theoretical curves calculated from these rate and equilibrium constants are shown as broken lines in Fig. 1.

Similar mathematical treatments were not applicable to other acids because of the larger uncertainties in the values of the remainder $k_{obs}-k_0$.

If the treatments mentioned above are correct, it follows that the weaker trimethylacetic acid (pk_a in H_2O 5.03) has truly, not apparently, a greater catalytic activity than the stronger acetic acid (pk_a 4.76), despite its larger steric requirement. This result, which it is impossible to interpret by a simple general acid catalysis, strongly supports the cyclic, concerted mechanism which was proposed in the preceding paper.[1] When carboxylic acids act bifunctionally, substituents in carboxylic acids will affect the proton-donating powers of the hydroxyl function and the proton-accepting powers of the carbonyl oxygen atom in opposite fashions. As a result, for example, the monomer-dimer equilibrium constants of carboxylic acids in the gas phase are almost independent of their acid strengths.[7]

When they act as bifunctional catalysts, whether the catalytic activities are directly proportional to the acid strengths or whether the reverse is true, will depend on the proportion of the contribution of both the acidic and basic functions to the lowering of the activation free energy. For the mutarotation of tetramethylglucose in a benzene solution, the order of the magnitude of the catalytic ability is as follows: $CCl_3COOH > C_6H_5COOH > \alpha$-pyridone $>$ 2-aminopyridine.[8] Similarly, for the acylation of aniline derivatives with acetic and benzoic anhydride or benzoyl chloride in benzene, the catalytic effect decreases in the order: $CCl_3COOH > CH_2ClCOOH > C_6H_5COOH > CH_3COOH$.[3,4] In these instances, although carboxylic acids act again as bifunctional catalysts, it seems that their role as acid catalysts rather than as base catalysts is of predominant importance. Bitter and Zollinger[9] found, on the contrary, that for the nucleophilic substitution reaction on cyanuric chloride by aniline in a benzene solution, the catalytic effect increases in the sequence: $CCl_3COOH < CH_2ClCOOH < CH_3COOH < \alpha$-pyridone. On the basis of this finding they considered that the proton-accepting property of a bifunctional catalyst is essential for the catalytic action.

In the present investigation, it may be premature to draw a definitive conclusion, for we still lack the "true" catalytic constants for any catalysts other than trimethylacetic acid and acetic acid. However, the data for the latter two acids seem to show that the proton-accepting ability of the catalyst in the transition complex plays a more important role in the catalysis. It should be noted that, in the transition complex, the proton of the catalyst is transferred to the substrate to a different extent depending on the kind of catalyst; therefore, the basicity of the proton-accepting site on the catalyst is not necessarily parallel to that of the initial catalyst molecule.

As for the bell-shaped, p[acid] - rate profiles illustrated in Fig. 1, a further comment is required. In many organic and enzymatic reactions bell-shaped profiles have been obtained.[10] The reason why such characteristic curves were obtained here, however, appears to be distinct from that for many of the hitherto-known instances in aqueous solutions. No change in the reaction mechanism in both sides of the maximum of the profile, such as those observed in imine formation,[11] nitrone formation,[12]

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[7] G. C. Pimentel and A. L. McClellan, "The Hydrogen Bonds," W. H. Freeman & Co., San Francisco (1960).

[8] C. G. Swain and J. F. Brown, Jr., *J. Am. Chem. Soc.*, **74**, 2538 (1952).

[9] a) B. Bitter and H. Zollinger, *Angew. Chem.*, **70**, 246 (1958); b) B. Bitter and H. Zollinger, *Helv. Chim. Acta*, **44**, 812 (1961).

[10] T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, New York, N. Y. (1966).

[11] G. E. Lienhard and W. P. Jencks, *J. Am. Chem. Soc.*, **87**, 3855 (1965), and the references cited therein.

[12] J. E. Reiman and W. P. Jencks, *ibid.*, **88**, 3973 (1966).

and the aminolysis of imido esters,[13] could be proved in the present case. Of course, the interpretation[10] proposed for the enzymatic reactions with bell-shaped pH-rate profiles are not applicable here either. The author prefers to explain this phenomenon by a change in the nature of the medium. As the concentration of carboxylic acid increases, the ionization "constant" will come not to be constant because of the change from pure dioxane to a mixed solvent of dioxane and carboxylic acid. At such a high acid concentration, the amine component is presumably protonated much more than would be expected from the ionization constant, K, determined above, and consequently the reaction rate decreases. In such a case the extent of the protonation of the amine component will have to be estimated, not by the concentration of the acid, but by the acidity function of the solution.[*3] Unfortunately, the acidity function for the dioxane-carboxylic acid system does not seem to have been established. Furthermore, the change in the medium effect on the aminolysis reaction itself, which accompanies the change in the solvent composition, can not be neglected. Therefore, it is difficult to predict theoretically the rate data on the left side of the maximum at the present time.

The author wishes to express his gratitude to Assistant Professor S. Sakakibara of Osaka University for his valuable advice and to Assistant Professor S. Nishida of Osaka University for his helpful discussions.

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[13] E. S. Hand and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 3505 (1962).

[*3] Such a situation may be analogous to that in hydrolytic reactions at low pH values (below pH 1.0), where maxima in acidity-rate profiles due to a decrease in the activity of water (the nucleophilic species) are sometimes observed.