BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 42 1071—1077 (1969)

Catalysis in Peptide Synthesis with Active Esters. I. Bifunctional Catalysis in the Aminolysis of Benzyloxycarbonyl-L-phenylalanine *p*-Nitrophenyl Ester in Dioxane

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(Received July 31, 1968)

The aminolysis reaction of benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (I) with glycine *t*-butyl ester (II) was kinetically investigated in anhydrous dioxane. The spontaneous reaction was confirmed to follow the equation:

$$d[p\text{-nitrophenol}]/dt = k_{obs}[\mathrm{I}] = (k_0 + k_{amine}[\mathrm{II}])[\mathrm{I}][\mathrm{II}]$$

The catalytic actions of various additives were studied at a 2×10^{-3}M concentration of I and II, where the amine-catalyzed term could be neglected. Of the many additives, only carboxylic acids and α-pyridone were effective as catalysts. The catalytic effect apparently decreased in the sequence: trimethylacetic acid$>$acetic acid$>\alpha$-pyridone$>$monochloroacetic acid. For these catalyses, a bifunctional, concerted mechanism was proposed which involves an 8-membered transition state.

In recent years, active ester procedures have been widely used in peptide synthesis.[1,2] It is also well-known that some carboxylic acids accelerate the aminolysis reactions of active esters.[3–5] On

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1) E. Schröder and K. Lübke, "The Peptides," Vol. I, Academic Press, New York, N. Y. (1966).

2) M. Bodanszky and M. A. Ondetti, "Peptide Synthesis," Interscience Publishers, New York, N. Y. (1966).

3) R. Schwyzer, M. Feurer and B. Iselin, *Helv. Chim. Acta*, **38**, 83 (1955); B. Iselin, M. Feurer and R. Schwyzer, *ibid.*, **38**, 1508 (1955).

4) a) E. Taschner, G. Błotny, B. Bator and C. Wasielewsky, *Bull. Acad. Polon. Sci., Sér. Sci. Chim.*, **12**, 755 (1964); b) E. Taschner, B. Rzeszotarska and Ł. Lubiewska, *Chem. Ind. (London)*, **1967**, 402.

5) a) S. M. Beumont, B. O. Handford and G. T. Young, *Acta Chim. Acad. Sci. Hung.*, **44**, 37 (1965); b) B. O. Handford, J. H. Jones, G. T. Young and T. F. N. Johnson, *J. Chem. Soc.*, **1965**, 6814.

the other hand, Beyerman *et al.*[6] proposed the use of bifunctional catalysts, such as α-pyridone, 1,2,4-triazole, and succinimide, for the syntheses of peptides *via* some active esters. Little information has, however, been available as to whether carboxylic acids act as simple acid catalysts or as bifunctional catalysts. The present author, therefore, undertook kinetic studies of the catalytic effects of various additives, including some carboxylic acids, on the aminolysis of benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (I) with glycine *t*-butyl ester (II) in anhydrous dioxane. These studies may also provide an important clue to the general mechanism[7] of the aminolysis of esters.

Experimental

Materials. Dioxane was purified by an ordinary method.[8] Reagent-grade acetic acid was distilled and was fractionally crystallized to a constant mp 16.5°C. Monochloroacetic acid and *p*-nitrophenol were recrystallized from toluene; 1,2,4-triazole, from ether; α-pyridone, from benzene, and tropolone,[*2] from cyclohexane. Pentachlorophenol was recrystallized from a mixture of methanol and water after treatment with active carbon; mp 185—188°C. 2,4-Dinitrophenol was recrystallized several times from water, and finally from carbon tetrachloride. Triethylamine was treated with benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester 2 days at room temperature to remove any primary and secondary amines, and then fractionally distilled; bp 89°C. (lit., 89.5°C). Trimethylacetic acid (an extra-pure reagent) and succinimide (a guaranteed reagent) were used without further purification.

Benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester was prepared from benzyloxycarbonyl-L-phenylalanine and *p*-nitrophenol by the dicyclohexylcarbodiimide method; mp 126—127°C (lit. 126—126.5°C)[9]; $[\alpha]_D^{23}$ −24.9 (*c* 2, DMF) (lit., −24.7, *c* 2, DMF).[9] Glycine *t*-butyl ester obtained by the hydrogenolysis of benzyloxycarbonylglycine *t*-butyl ester was fractionally distilled through a 35-cm Vigreaux column under reduced pressure; bp 51—54°C/15 mmHg (lit., 30°C/2 mmHg).[10] The purity of the ester was checked by an amino-group titration with standard hydrochloric acid (100.1% theoretical).

Kinetic Procedure. The reactions were carried out in 50-m*l* graduated flasks immersed in a constant-temperature bath which was controlled to 0.05°C. The reaction mixture was prepared in the following way. Known weights of I and an additive, if any, were placed in the reaction flask, and dioxane was added nearly to the mark. After a thermal equilibrium had been obtained, the mixture was made up with the solvent to exactly 50 m*l*. The reaction was initiated by adding an appropriate volume of a stock solution of II in dioxane. The quantities of both the reactants and the additive were predetermined in order to obtain the desired concentrations when the solution of II was added. Aliquots (0.1 m*l* or 0.2 m*l*) were withdrawn at suitable intervals, diluted to 5 m*l* with dioxane containing an excess anhydrous hydrogen chloride, and analyzed spectrophotometrically. The reactions were followed by the rate of the formation of *p*-nitrophenol. The molar extinction coefficients of I and *p*-nitrophenol were measured at 267 and 304 mμ in anhydrous dioxane; the following data were obtained:

	I	*p*-Nitrophenol
267 mμ	10600 (max)	3430
304 mμ	2250	12150 (max)

The concentrations of *p*-nitrophenol were determined by measuring the absorbance at the two wavelengths and by solving the simultaneous equations. The analysis of the known mixtures of I and *p*-nitrophenol by this method gave an agreement within ±1%. The absorptions of the benzyloxycarbonyl-L-phenylalanylglycine *t*-butyl ester and of the hydrochloride of II can be neglected at these wavelengths. The reference solution was dioxane containing amounts of the additive and hydrogen chloride equal to those in the sample solution. The reaction was generally followed to 60—90% completion with at least 10 readings.

The average deviation from the mean value of the rate constant in duplicate or triplicate experiments was ±3% or less.

Reaction Products and Stoichiometry. The kinetic experiments in dioxane suggested that the reaction of I with II is virtually quantitative. This was checked by the isolation of the benzyloxycarbonyl-L-phenylalanylglycine *t*-butyl ester from a reaction mixture after kinetic measurements. The protected dipeptide ester was recrystallized from ethyl acetate-petroleum ether in a yield of about 90%; mp 88.5—89.5°C. Found: C, 66.99; H, 6.87; N, 6.75%. Calcd for $C_{23}H_{28}O_5N_2$: C, 66.97; H, 6.84: N, 6.79%.

Results

Kinetic studies of the aminolysis reactions of I with II were carried out in anhydrous dioxane in the absence and in the presence of various additives.

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6) a) H. C. Beyerman and W. Maassen van den Brink, *Proc. Chem. Soc.*, **1963**, 266; b) H. C. Beyerman and W. Maassen van den Brink, Proc. 6th European Peptide Symposium in Athens, 1963, Pergamon Press, Oxford (1965), p. 85; c) H. C. Beyerman, W. Maassen van den Brink, F. Weygand, A. Prox, W. König, L. Schimdhammer and E. Nintz, *Rec. Trav. Chim.*, **84**, 213 (1965).

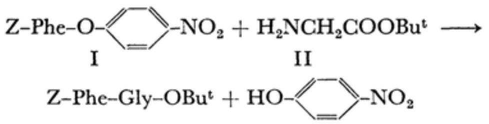
7) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, New York, N. Y. (1966).

8) L. F. Fieser, "Experiments in Organic Chemistry," 3rd ed., revised, D.C. Heath and Co., Boston (1957), p. 284.

[*2] This material was a gift from Professor Takase, Tohoku University, to whom my thanks are due.

9) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 6072 (1959).

10) G. W. Anderson and F. M. Callahan, *ibid.*, **82**, 3359 (1960).



In the presence of a large excess of II, good pseudo-first-order canstants, $k^{\mathrm{I}}_{\mathrm{obs}}$, were obtained. In all the kinetic experiments under these conditions the concentration of I was 1.0×10^{-3}M. The second-order rate constants, $k^{\mathrm{II}}_{\mathrm{obs}}$, derived from $k^{\mathrm{I}}_{\mathrm{obs}}$ were variable functions of the concentrations of II, as represented by the following equation:

$$\frac{k^{\mathrm{I}}_{\mathrm{obs}}}{[\mathrm{II}]} = k^{\mathrm{II}}_{\mathrm{obs}} = k_0 + k_{\mathrm{amine}}\,[\mathrm{II}] \qquad (1)$$

where k_0 and k_{amine} are the rate constants for the second- (uncatalyzed) and third-order (amine-catalyzed) processes respectively, and where the superscript I or II denotes the reaction order. This relationship is illustrated in Fig. 1, where plots of $k^{\mathrm{II}}_{\mathrm{obs}}$ against the concentration to II give a straight line at each temperature. The values of k_0 and k_{amine} which were calculated from, respectively, the intercepts and slopes of these straight lines by the method of least squares are summarized in Table 1. It follows from these values that the contribution of the amine-catalyzed term to the overall reaction rate is not more than 1% at the concentration of the amine component lower than 10^{-3}M. Thus, in order to simplify the kinetic analysis, all the kinetic runs with additives were carried out at low concentrations where the contribution of the amine-catalyzed term was negligible. Spectrophotometric studies showed that, in anhydrous dioxane, the *p*-nitrophenol formed did not detectably ionize even in the presence of a large excess of II or triethylamine,[*3] indicating that the amine component remained in its free form. Accordingly, the formation of *p*-nitrophenol does not disturb the second-order kinetics.

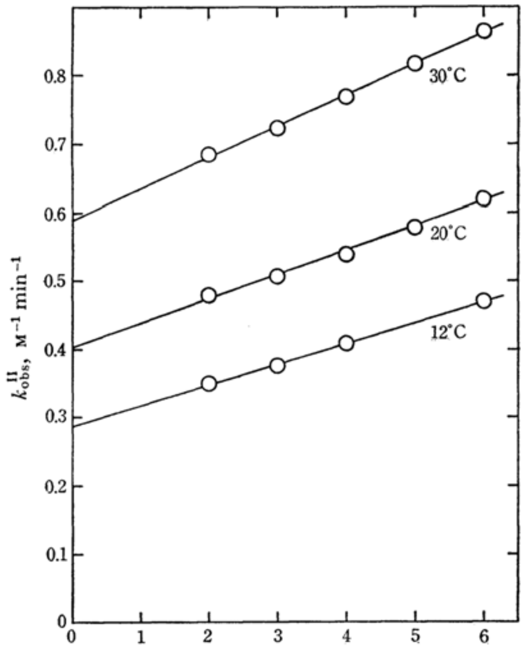


Fig. 1. Effects of the concentration of the amine component on the observed second-order rate constants at different temperatures.

Table 1. Uncatalyzed and amine-catalyzed rate constants at various temperatures

Temperature, °C	k_0, M^{-1} min^{-1}	k_{amine}, M^{-2} min^{-1}
30	0.59	4.6
20	0.40	3.6
12	0.29	3.1

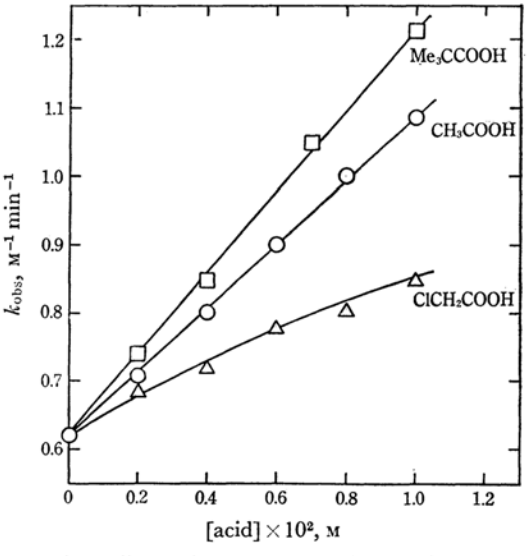


Fig. 2. Effects of the concentration of three carboxylic acids on the observed rate constants at 30°C.

As is shown in Fig. 2, carboxylic acids with a relatively weak acidity, such as trimethylacetic acid and acetic acid, powerfully catalyze the aminolysis of the active ester in dioxane. When

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[*3] Although it has often been assumed, without any experimental support, that in anhydrous dioxane *p*-nitrophenol ionizes to give the *p*-nitrophenolate anion in the presence of tertiary amine,11) this is incorrect.

11) a) M. Goodman and L. Levine, *J. Am. Chem. Soc.*, **86**, 2918 (1964); b) M. Goodman and W. J. McGahren, *ibid.*, **87**, 3028 (1965); c) M. Goodman and W. J. McGahren, *Tetrahedron*, **23**, 2031 (1967).

TABLE 2. APPARENT CATALYTIC CONSTANTS OF VARIOUS ADDITIVES AT 30°C

Additive			pK_a (in aq. soln.)	k_{obs}[a)] $\text{M}^{-1}\,\text{min}^{-1}$	k'_{cat} $\text{M}^{-2}\,\text{min}^{-1}$
Carboxylic acid	Phenol	Other			
				0.62	
		NEt_3			0
		$MeCONMe_2$			1.8
		H_2O			0
		1,2,4-Triazole	(2.55)	0.63	1.5
		α-Pyridone	11.62 (0.75)	0.71	41
		Succinimide	10.52	0.62	0
	p-$O_2NC_6H_4OH$		7.14	0.61	0
		Tropolone	6.92	0.62	0
		$CCl_3COOH \cdot NEt_3$	5.72	0.50	0
	C_6Cl_5OH		5.3	0.60	0
CMe_3COOH			5.03	0.74	59
CH_3COOH			4.76	0.71	47
	2,4-$(O_2N)_2C_6H_3OH$		4.11	0.57	0
$CH_2ClCOOH$			2.85	0.69	32
$[\text{I}] = [\text{II}] = 2 \times 10^{-3}\text{M}$					

a) The observed rate constants when the concentrations of additives are $2 \times 10^{-3}\text{M}$.

the acid concentrations were lower than 10^{-2}M, the following relationship was found to hold:

$$k_{obs} = k_0 + k'_{cat}\,[\text{catalyst}] \qquad (2)$$

where k'_{cat} was the (apparent)[*4] catalyzed constant (Fig. 2). The values of k'_{cat} for each catalyst can be calculated from the slopes of the lines shown in Fig. 2.

Monochloroacetic acid, a slightly stronger acid, also showed a catalytic effect, but a negative deviation[*5] from Eq. (2) was observed (Fig. 2).

Such phenols as p-nitro-, pentachloro-, and 2,4-dinitro-phenol did not accelerate, but, rather, came to retard the reaction as their acid strengths increased (*cf.* the 5th column in Table 2).

The trichloroacetate of triethylamine, an acidic salt, also retarded the reaction (Table 2).

α-Pyridone, a classical example of a bifunctional catalyst,[6,12] was almost as effective as acetic acid. Water, triethylamine, succinimide, and tropolone showed little or no catalytic activity. Dimethylacetamide (not as a solvent[*6] but as a catalyst) and 1,2,4-triazole showed only a small accelerating effect. Although Bayerman *et al.*[6] found that the catalytic effects of succinimide and 1,2,4-triazole were as large as that of α-pyridone for the syntheses of peptides *via* active esters in acetonitrile or others, the catalytic activities of the former compounds were much smaller than that of the latter under the present conditions. The results mentioned above are summarized in Table 2.

The second-order rate constants observed for the acetic acid-catalyzed reaction at three different temperatures are shown in Table 3.

Table 4 shows the Arrhenius parameters for the uncatalyzed, amine-catalyzed, and acetic acid-catalyzed reactions.

TABLE 3. OBSERVED SECOND-ORDER RATE CONSTANTS FOR THE ACETIC ACID-CATALYZED REACTION AT THE THREE DIFFERENT TEMPERATURES

Acetic acid, M	k_{obs}, $\text{M}^{-1}\,\text{min}^{-1}$		
	30°C	20°C	12°C
0.000	0.620		
0.002	0.707	0.486	0.360
0.004	0.800	0.565	0.421
0.006	0.900		
0.007		0.685	0.520
0.008	1.000		
0.010	1.085	0.800	0.613

$[\text{I}] = [\text{II}] = 2 \times 10^{-3}\ \text{M}$

TABLE 4. ARRHENIUS PARAMETERS FOR THE UNCATALYZED, AMINE-CATALYZED, AND ACETIC ACID-CATALYZED REACTIONS

	Apparent activation energy, kcal/mol	Frequency factor
Uncatalyzed	7.0	6.5×10^4
Amine-catalyzed	3.8	2.8×10^3
Acetic acid-catalyzed	3.4	2.9×10^3

[*4] On the meaning of "apparent," see the author's subsequent paper; This Bulletin, **42**, 1078 (1969).

[*5] The reason for the negative deviation will be discussed in a subsequent paper (*cf.* *4).

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

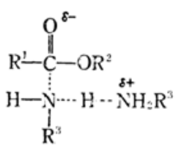
[*6] The results of the study on the effect of dimethylacetamide as a solvent will be published in a separate communication.

Discussion

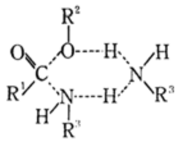
The general acid-base catalysis for the aminolysis of esters is a well-established phenomenon.[7] In the aminolysis (and related reactions) of esters[7] or thiolesters[7] in protic solvents, the kinetic orders of amines in rate expressions are often not unity, because amine components themselves can act as base catalysts. Recently some evidence has been presented for the catalytic actions of the amine components in the acylation of amines with ketenes or esters in aprotic solvents.[13–17] A similar catalysis was found for the present system also.

At a relatively higher concentration of reactants (10^{-2}M), the integrated second-order rate constant decreased with the progress of reaction, indicating the presence of a higher-order process. As has been described in the preceding section, when II was present in at least a twenty-fold excess over I, pseudo-first-order constants, $k^{\rm I}_{\rm obs}$, were obtained. The second-order rate constants, $k^{\rm II}_{\rm obs}$, were variable functions of the concentrations of II, as are represented by Eq. (1) and Fig. 1. This finding implies that the reaction consists of two independent processes involving one and two amine molecules. In the latter process the second amine molecule apparently acts as a catalyst.

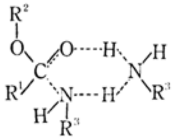
It is possible to write three transition states, III, IV, and V, for the catalytic process:



III



IV



V

In III, the second amine molecule makes the attacking amine more nucleophilic by accepting a proton partly. This process involves a charge creation. The transition state IV, first proposed by Bruice and Mayahi[18] in the ammonolysis of substituted phenyl acetates, is a cyclic, concerted process which involves no charge formation in the transition state. In this cyclic transition state, the catalytic molecule removes a proton from the one undergoing acylation, while there is a simultaneous proton transfer to the leaving group. The transition state V also represents a cyclic mechanism, but it differs from IV in that the carbonyl oxygen atom accepts the proton of the catalyst.

If the first mechanism is correct, a tertiary amine can be expected to exhibit a catalytic effect, because it too can remove a proton from the nucleophile. If a cyclic concerted mechanism, IV or V, is correct, then the tertiary amine should have little effect on the rate, because it cannot participate in the concerted process because of the absence of a labile proton. The author found that triethylamine has little or no catalytic activity in spite of its strong basicity. This fact favors the concerted mechanism. Similar arguments have recently been proposed for the aminolysis of *p*-nitrophenyl acetate in chlorobenzene[15] and for the the acylation of aniline derivatives by ketenes in ether.[13b,c]

It must be remembered, however, that the effectivenesss of a given amine as a general-base catalyst depends not only on its basicity but also on the steric factor. It has been pointed out that the aminolysis by sterically-hindered amines, especially secondary amines, is less susceptible to the general-base catalysis in spite of the availability of a proton.[14,19,20] Therefore, at present, care must be taken in drawing conclusions about the amine-catalyzed mechanism. The cyclic, concerted mechanism is nevertheless very attractive in that, in a nonpolar solvent such as dioxane, the charge creation (ionization) which is required for the mechanism III is not favored.

Even in an aqueous solution much experimental evidence suggestive of cyclic mechanism has been presented in analogous reactions, such as the hydrolysis[7] and ammonolysis[18] of esters.

The validity of this cyclic, concerted mechanism has been more unambiguously demonstrated in the case of carboxylic acid catalysis. As is shown in Table 2, with the single exception of α-pyridone, only carboxylic acids among the many acidic additives examined were more effective as catalysts. Phenols or acidic salts such as triethylammonium trichloroacetate exhibited no effect or a negative effect on the reaction rate, depending on the concentration and strength of the acid. If the reaction proceeds through the simple general-acid catalysis, these acids should possess a catalytic activity similar to that of carboxylic acids. This

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[13] a) J. M. Briody and D. P. N. Satchell, *Tetrahedron*, **22**, 2649 (1966); b) P. J. Lillford and D. P. N. Satchell, *J. Chem. Soc.*, (*B*), **1967**, 360; c) P. J. Lillford and D. P. N. Satchell, *ibid.*, **1968**, 54.

[14] M. G. Dmitrieva and Yu. I. Khurgin, *Izvest. Akad. Nauk SSSR, Ser. Khim.*, **1965**, 1174.

[15] F. M. Menger, *J. Am. Chem. Soc.*, **88**, 3081 (1966).

[16] A. S. A. S. Shawali and S. S. Biechler, *ibid.*, **89**, 3020 (1967).

[17] R. L. Snell, Wo-Kong Kwok and Y. Kim, *ibid.*, **89**, 6728 (1967).

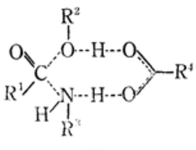
[18] T. C. Bruice and M. F. Mayahi, *ibid.*, **82**, 3067 (1960).

[19] W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 675 (1960).

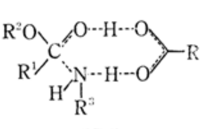
[20] L. do Amaral, K. Koehler, D. Bartenbach, T. Pletcher and E. H. Cordes, *ibid.*, **89**, 3537 (1967).

is, however, not the case. The possibility that the effect of carboxylic acids may be due to a nonspecific "medium effect" was also eliminated by the finding that water and dimethylacetamide, which have much larger dielectric constants, were far less effective in the acceleration of the reaction.

These facts suggest a specific mechanism, that is, one with a cyclic transition state, as is shown by VI or VII:[*7]



VI

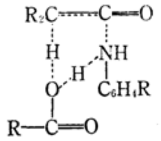


VII

A similar proposal has been made regarding the carboxylic acid-catalysis of the methoxyaminolysis of *p*-nitrophenyl acetate in an aqueous solution on the ground that the catalytic constants for the carboxylic acids are, within the limits of error of the experimental measurements, independent of the acid strength of the catalyst.[20] That Litvinenko and his co-workers established an analogous mechanism of the carboxylic acid catalysis in the acylation of aniline derivatives by carboxylic acid anhydrides or chlorides in a benzene or nitrobenzene solution is particularly noteworthy in this connection.[21–27]

Although the form of this transition state is an eight-membered ring, it should be noted that it perhaps has an atomic configuration analgous to that of the carboxylic acid cyclic dimer. The fact that carboxylic acid and α-pyridone are most effective as catalysts indicates that their atomic configurations are most suitable to the formation of the cyclic transition state. That the geometry of a catalyst (its ability to fit into the eight-membered ring involving two hydrogen atoms) could be an important factor is also supported by the fact that 1,2,4-triazole[6] and tropolone, which would form 7- and 9-membered rings respectively, exhibit little or no catalytic activity (Table 2). Tropolone will prefer forming an intramolecular hydrogen bond of a five-membered ring to acting as a bifunctional catalyst.

Lillford and Satchell,[13b] who recently established the carboxylic acid catalysis for the acylation of aniline derivatives by dimethylketene or isobutyric anhydride in an ether solution, proposed a cyclic transition state such as VIII for the catalysis.



VIII

However, such a formulation cannot explain our finding that phenols do not catalyze the aminolysis of the ester.

Now, there remains a qeutsion which states are correct, IV or V and VI or VIII. In order to answer this question it is necessary to understand the characteristics of the reactions of this type. There is a distinct difference in the characteristics of the reaction between the nucleophilic displacement on the carbonyl carbon and the bimolecular substitution on saturated carbon. In the former the negative charge brought into the reacting site by the attacking nucleophile is once "absorbed" into the carbonyl oxygen, whereas in the latter the charge is directly transmitted to the leaving group. In the former, consequenlty, the factors which assist the "absorption" of the negative charge by the carbonyl oxygen, such as the presence of acid species protonating the oxygen atom (acid catalysis), or the addition of protic solvent which forms a hydrogen bond with the oxygen atom,[28] will promote the formation of an intermediate. The charge "stored" in the carbonyl oxygen atom becomes a driving force to accelerate the decomposition of the intermediate in both forward and backward directions. In this way one cannot speak of the nucleophilic displacement reaction on the carbonyl carbon without considering the role of the carbonyl oxygen. If the formation of the intermediate is the rate-determining step in the presence of the catalyst, as is expected to be indeed

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[*7] In the aminolysis of the 1-hydroxypiperidine ester and the related compounds, Young *et al.*[5] attributed the accelerating effect of acetic acid to a specific acid catalysis, in which the nitrogen atom of the leaving group is protonated. The acid may, however, act as a bifunctional catalyst, as in the present case.

21) L. M. Litvinenko and D. M. Aleksandrova, *Dokl. Akad. Nauk.*, **118**, 321 (1958).

22) a) L. M. Litvinenko, D. M. Aleksandrova and N. I. Pilyuk, *Ukr. Khim., Zh.*, **25**, 81 (1959); b) L. M. Litvinenko and D. M. Aleksandrova, *ibid.*, **26**, 66 (1960); c) L. M. Litvinenko, and D. M. Aleksandrova and A. A. Zhilinskaya, *ibid.*, **26**, 476 (1960); d) L. M. Litvinenko and D. M. Aleksandrova, *ibid.*, **27**, 212 (1961); e) *ibid.*, **27**, 336 (1961); f) *ibid.*, **27**, 487 (1961); g) L. M. Litvinenko, D. M. Aleksandrova and S. F. Prokopovich, *ibid.*, **27**, 494 (1961); h) L. M. Litvinenko and D. M. Aleksandrova, *ibid.*, **27**, 634 (1961).

23) L. M. Litvinenko and N. M. Oleinik, *Zh. Obshch. Khim.*, **32**, 2290 (1962).

24) L. M. Litvinenko and N. M. Oleinik, *ibid.*, **33**, 2287 (1963).

25) L. M. Litvinenko, *Ukr. Khim. Zh.*, **30**, 317 (1964).

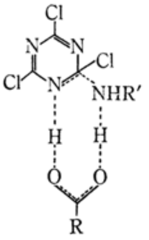
26) L. M. Litvinenko, *Reakt. Sposobnost. Organ. Soedin., Tartsusk. Gos. Univ.*, **2**, 57 (1965); *Chem. Abstr.*, **63**, 17860h (1965).

27) L. M. Litvinenko and N. M. Oleinik, *Ukr. Khim. Zh.*, **32**, 174 (1966).

28) S. L. Jung, J. G. Miller and A. R. Day, *J. Am. Chem. Soc.*, **75**, 4664 (1953), and their preceding papers.

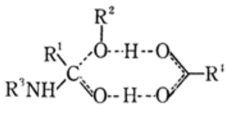
the case,[*8] the catalyst must give its proton, not to ether oxygen, but to the carbonyl oxygen atom of the substrate in the transition state. In brief, it follows that the mechanisms which include the transition states V and VII are probably correct. This conclusion is also in accord with the prediction by the "Anthropomorphic rule."[29]

This situation is very similar to that found in the carboxylic acid catalysis for some aromatic nucleophilic substitution reactions; that is, while carboxylic acids catalyzed the aminolysis of cyanuric chloride by aromatic amines,[30] they failed to catalyze the analogous substitution reactions on 2,4,6-trinitrochlorobenzene by amines.[21,30a,31] This fact has been explained as follows. In the former reaction the catalyst accelerates the reaction by protonating the nitrogen atom on the triazine ring, as is shown in IX, but in the latter the catalyst does not accelerate because of the absence of a nitrogen atom.[30a] If the proton of the carboxylic group is directly transferred to the leaving atom (Cl^-), the reaction of 2,4,6-trinitrochlorobenzene will be promoted by carboxylic acids in the same manner as the reaction of cyanuric chloride.



IX

Since the formation of the intermediate is probably a rate-determining step, the collapse rate of the intermediate will be of no importance in the kinetics. However, the carboxylic acid may participate in the collapse process also, as is formulated in X:



X

The assistance to the nucleophilic attack by catalysts should bring about a lowering of the potential energy barrier. On the other hand, since the catalytic processes represent the higher-order reactions, it may be anticipated that they will have low frequency factors compared with the uncatalyzed process. The Arrhenius parameters for the uncatalyzed, amine-catalyzed, and acetic acid-catalyzed reactions shown in Table 4 reveal that this is indeed the case.

The author would like to express his gratitude to Assistant Professor S. Sakakibara, Osaka University, for his guidance and valuable advice and to Assistant Professor S. Nishida, Osaka University, for his helpful discussions. The author wishes also to thank the Kyowa Hakko Kogyo, Co., Ltd., for the opportunity to conduct this work and publish his resutls.

[*8] Since the $-OC_6H_4NO_2$ group is a much better leaving group than the $-NHCH_2COOBu^t$ group, it is probable that the collapse rate of the intermediate to the product is larger than to the starting material, —that, in other words, the second step is of no importance in the kinetics.

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

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

31) L. M. Litvinenko and D. M. Aleksandrova, *Ukr. Khim. Zh.*, **26**, 621 (1960).