Intramolecular Catalysis of Amide Hydrolysis by Two Carboxy-groups

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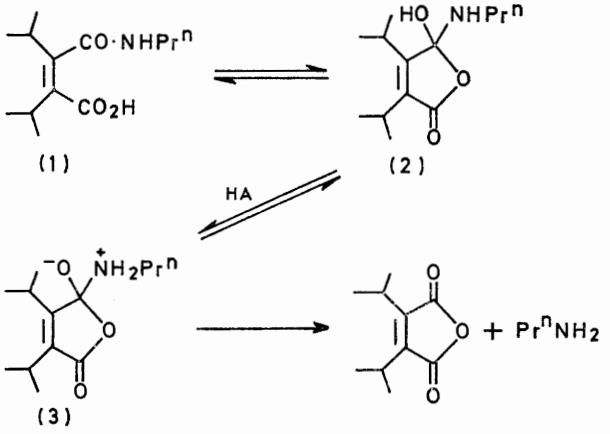
In di-isopropylmaleamic acids derived from β-amino-acids (i) the rate constant for hydrolysis of the unactivated amide group is comparable with k_{cat} for pepsin hydrolysing good synthetic substrates; (ii) a neighbouring carboxy-group participates as a nucleophilic catalyst, but requires a specific structural relationship with the amide group to reach high efficiency; (iii) there is a *requirement* for a second carboxy-group, also in a special structural relationship with the amide group, but this time in the ionised form, for full catalytic efficiency to be maintained. Remarkably, the ionised carboxy-group acts as a general base to catalyse the interconversion of neutral and zwitterionic forms of the tetrahedral carbinolamine intermediate, a reaction which is catalysed by external general *acids*. As a result we have developed a system showing a bell-shaped pH–rate profile for hydrolysis, of the sort shown by many enzyme-catalysed reactions, and for the same reasons.

We have shown [1] that the very fast hydrolysis of the amide group of *N*-n-propyldi-isopropylmaleamic acid (1) involves both intramolecular catalysis by the neighbouring carboxy-group and external general acid catalysis. We are interested in the mechanism of hydrolysis of the



maleamic acids because they are simple models for the hydrolysis of the peptide bond catalysed by enzymes with a carboxy-group at the active site. Appropriate structural variation in the series leads to very large increases in catalytic efficiency,[2] until a limit is reached with di-isopropylmaleamic derivatives. Intramolecular catalysis of amide hydrolysis by the carboxy-group of (1) is many times more efficient than in any other system yet studied, so we consider it the best available model for the corresponding enzymic reaction.

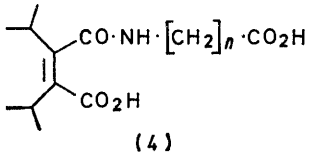
In the preceding paper we described the evidence that the general acid catalysed step in the hydrolysis of (1) is the proton transfer process (2) ⇌ (3). In an investigation of this type, where we have reached the maximum catalytic efficiency attainable by simple structural variation, the logical next step in our search to improve catalytic efficiency still further is to introduce a second catalytic group. In the present case the choice of catalytic group follows from what we know of the simple intramolecular reaction. This is catalysed [3] by general acids, and most efficiently by carboxylic acids.[1] So we set out to study the effect of introducing a second carboxy-group into (1). Synthetically it is a simple matter to introduce a carboxy-function into the leaving group, and we have shown [1] that the overall hydrolysis reaction

[1] M. F. Aldersley, A. J. Kirby, P. W. Lancaster, R. S. McDonald, and C. R. Smith, preceding paper.

[2] A. J. Kirby and P. W. Lancaster, *J.C.S. Perkin II*, 1972, 1206.

[3] W. P. Jencks, personal communication; see M. I. Page and W. P. Jencks, *J. Amer. Chem. Soc.*, 1972, **95**, 8829.

is not sensitive to the electronic effects of such substituents. Accordingly we have studied the hydrolysis of a number of maleamic acids with the general structure (4), made by treating the appropriate amino-acid with di-isopropylmaleic anhydride.



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EXPERIMENTAL

Materials and methods have been described previously in some detail.[1,2]

N-Ethoxycarbonylmethyldi-isopropylmaleamic acid (5) was prepared from the anhydride and glycine ethyl ester in ether, and obtained as the *amine salt*, m.p. 100—101° (Found: C, 55·6; H, 8·35; N, 7·1. $C_{18}H_{32}N_2O_7$ requires C, 55·65; H, 8·3; N, 7·2%). Derivatives of amino-acids were not isolated, but prepared in solution as follows. A solution of the amino-acid with the amino-group 50% neutralised was prepared by adding KOH to a solution of the amino-acid in 3 : 2 water–methanol, and the maleamic acid was prepared by dissolving solid di-isopropylmaleic anhydride in an excess of this buffer solution. Use of 5—7 mg per ml of anhydride gave a *ca.* 0·04M-solution of the dianion of the maleamic acid. For the kinetic experiments this stock solution (5 μl) was injected into aqueous buffer (3 ml). This had a negligible effect on the pH of the final solution, and the rate constants obtained did not vary when the concentration of the original amino-acid solution was increased tenfold.

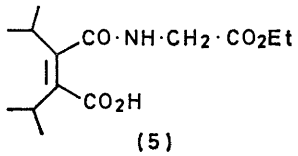
RESULTS

The hydrolysis of each of the amino-acid derivatives shows buffer catalysis with saturation kinetics, as observed for the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid (1). Our analysis of the kinetics of hydrolysis of (1) showed that only general acid catalysis is involved, and we have analysed the data for the amino-acid derivatives on the assumption that that is the case for their reactions also. This assumption is clearly sound for the hydrolysis of the undissociated forms; but general acid catalysis of the hydrolysis of the monoanions is kinetically indistinguishable from general base catalysis of the hydrolysis of the undissociated diacids. Here the assumption that only general acid catalysis is involved gives results that are internally consistent and quantitatively closely comparable to those obtained [1] for the *N*-n-propyl compound. The quantitative analysis depends also on the pK_a values taken for the two carboxy-groups. These cannot be measured directly, because of the very high reactivity of the compounds, but the best fit of the kinetic data is obtained by using values for the dissociation constants which are close to those expected. The pK_a of the maleic carboxy-group, for example, is 4·15 ± 0·1 in each case, the same as that found for the *N*-n-propyl derivative.

We discuss the data for each amide separately. In the case of the glycine compound we have studied the hydrolysis of the glycine ethyl ester derivative also, since the corresponding derivatives of maleic acid are hydrolysed at the same rate.[1] In the present case also the CO_2H and CO_2Et groups have almost identical effects on the rate of the hydrolysis reaction.

[4] V. Gold and D. C. A. Waterman, *J. Chem. Soc.* (*B*), 1968, 839.

Glycine Derivatives.—The hydrolysis of the ethyl ester (5) is qualitatively similar to that of the n-propylamide (1); but the plateau rate at low pH is three times greater, and the



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second order rate constants for buffer catalysis are an order of magnitude smaller. As a result the second-order plots for buffer catalysis are linear over a useful range of buffer concentration, and values of k_0 and the second-order rate constants can be obtained more accurately than for the hydrolysis of (1). For the same reason, k_∞ values are not readily accessible for this compound.

TABLE 1

Data for the hydrolysis of *N*-ethoxycarbonylmethyldi-isopropylmaleic acid (5) at 15·5° and ionic strength 1·0

pH	Conditions	No. of runs	k_0/min^{-1}
	1M-HCl	2	20·6 ± 0·1
	0·46M-HCl	2	15·6 ± 0·1
	0·21M-HCl	2	11·7 ± 0·5
1·18	0·10M-HCl	2	8·7 ± 0·2
1·51	0·46M-HCl	2	5·5 ± 0·2
1·73	H_3PO_4 buffer	3	3·2
1·81	0·021M-HCl	2	3·1 ± 0·1
1·99	$Cl_2HC{\cdot}CO_2H$ buffer	3	2·48
2·05	H_3PO_4 buffer	3	1·90
2·18	0·01M-HCl	3	1·45 ± 0·1
2·36	$ClH_2C{\cdot}CO_2H$ buffer	3	0·97
3·62	HCO_2H buffer	3	5·0 ± 10^{-2}
4·78	$MeCO_2H$ buffer	3	1·63 ± 10^{-2}

TABLE 2

Catalysis of the hydrolysis of *N*-ethoxycarbonylmethyldi-isopropylmaleamic acid (5) at 15·5° and ionic strength 1·0

pK_a	General acid	No. of runs	k_{AH} [a]/ l mol^{-1} min^{-1}
−1·7	H_3O^+	12	218 ± 5
1·29	$Cl_2{\cdot}CH{\cdot}CO_2H$	6	11 ± 3
2·12	H_3PO_4	9	15 ± 4
2·86	$ClCH_2{\cdot}CO_2H$	9	5·0 ± 0·3
3·77	HCO_2H	9	3·3 ± 0·2
4·76	$MeCO_2H$	9	0·98 ± 0·05
15·7	H_2O		3·78 ± 10^{-4}

[a] Calculated by using pK_a = 4·25 for the substrate.

The data for the hydrolysis reaction in the curved region (k_0 values) are given in Table 1, and the full pH–rate profile is plotted in Figure 1. The line drawn is calculated on the basis of a substrate pK_a of 4·25, a plateau rate constant, k_∞ (not quite reached in 1M-acid) of 22 min^{-1}, a spontaneous (uncatalysed or water-catalysed) rate constant of 0·02 min^{-1}, and k_H = 218 l mol^{-1} min^{-1}.

Second-order rate constants for general acid catalysis are given in Table 2. With the relatively simple buffer catalysis kinetics found for (5) it was feasible to work with the strongly acidic phosphoric acid and dichloroacetic acid buffers, making the usual [4] corrections as the pH varied at low buffer concentrations. We have used a value of 0·66 for the activity coefficient of the hydronium ion, which is the mean value found under our conditions ($-\log \gamma_H = 0{\cdot}18 \pm 0{\cdot}02$). We used these strongly acidic buffers because the Brønsted plot for general acid catalysis (not shown)—in contrast to that for the hydrolysis of the n-propylamide (1)—does not show well-defined regions of zero and unit slope. This is not unexpected: if the pK_a of the tetrahedral intermediate

(see below, and discussion in ref. 1) derived from glycine ethyl ester is lowered relative to the n-propylamide (1) by a factor comparable with the difference between the parent

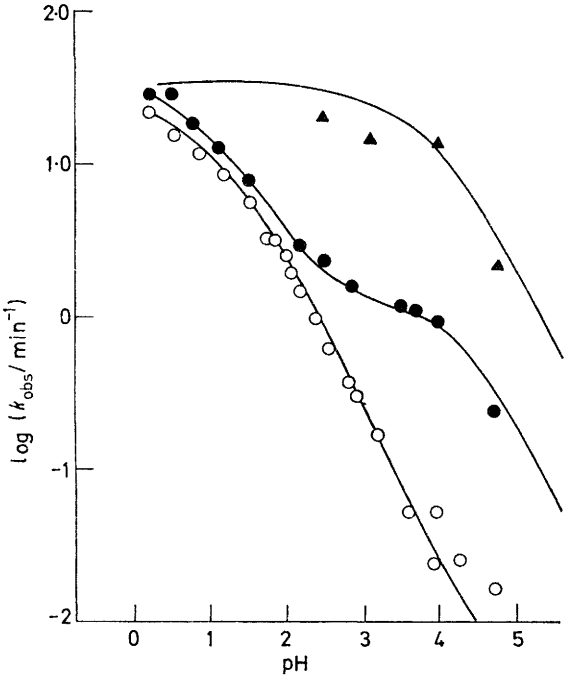


FIGURE 1 pH–Rate profiles for the hydrolysis of two di-isopropylmaleamic acids. The lowest curve (open circles) is for the hydrolysis of the glycine ethyl ester derivative, extrapolated to zero buffer concentration (k_0). The closed symbols refer to the derivative from glycine: circles for the k_0 curve, triangles for k_∞ (extrapolated to infinite buffer concentration)

amines, then the break in the Brønsted plot should occur 2—3 pK units below that in the Brønsted plot for (1). The break is observed just above pK 4 for (1). The data for (5) are consistent (Table 2) with a break near pK 2 and a limiting catalytic constant of 12—13 l mol^{-1} min^{-1} (with k_H greater by the expected factor of 20). (The best *straight line* drawn through the points on the Brønsted plot has a slope of −0·34, with a correlation coefficient of 0·989.)

The analysis of the data for the glycine derivative (4; $n = 1$) is more complicated, because two different ionic forms are reactive. The pH–rate profiles (k_0 and k_∞) are shown in Figure 1, and the data are given in Table 3. The data at low pH, where the undissociated diacid is the reactive form, give a plateau rate constant (again not quite reached in 1M-HCl) of 32 min^{-1} and a catalytic constant for the hydronium ion $k_H = 240$. This figure is similar to the corresponding value for the ethyl ester (5), and we have assumed that the catalytic constants for other general acids are the same for the two compounds. The uncorrected catalytic constant for chloroacetic acid is greater at pH 2·82 than at 2·48, because it contains a large contribution from the (larger) catalytic constant for monoanion hydrolysis at the higher pH, and the assumption described makes it possible to separate the reactions of the two ionic forms. The k_0 data can then be fitted (the line drawn in Figure 1 is the calculated curve) by using substrate pK_a values of 3·5 and 4·25, a plateau rate constant of 32 min^{-1} and an uncatalysed rate constant of 0·8 min^{-1} for the diacid, and an uncatalysed rate constant of 1·2 min^{-1} for the monoanion (the *dianion* is unreactive in this as in every other case).

There is a kinetic equivalence between the uncatalysed hydrolysis of the diacid and the acid catalysed hydrolysis of the monoanion. This we have resolved by assuming (for the monoanion) the same ratio of k_H to the catalytic constants for chloroacetic and formic acids as we found for the n-propylamide (1). This is reasonable, since the catalytic constants for the monoanion are quantitatively similar to those measured [1] for (1); and assigns about 2/3 of the relevant rate constant to the uncatalysed hydrolysis of the diacid. The catalytic constants obtained are given in Table 4. In summary, the diacid behaves like the corresponding ethyl ester (5), and the monoanion like the n-propylamide (1); except that the uncatalysed reaction is at least an order of magnitude faster in each case.

β-Alanine Derivative (4; $n = 2$).—The pH–rate profile for hydrolysis of this compound (Figure 2) differs from those already described in that the curves for k_0 and k_∞ merge at high pH as well as on the low-pH plateau. This is because buffer catalysis is not observed at high pH, which means that the C–N cleavage step [1] is slower even than the *water* catalysed proton-transfer step for the monoanion, which is the only reactive form present at high pH. Therefore the hydrolysis of the monoanion must be uncatalysed at all pH values, and all the observed catalysis must involve the diacid. This simplifies the analysis considerably.

Data for the hydrolysis reaction are given in Table 5. The best fit is obtained with substrate pK_a values of 3·9 and 4·6; the plateau rate constant, reached in 1M-HCl, is 12 min^{-1}, and the hydrolysis rate for the monoanion is 2·4 min^{-1}. Buffer catalysis constants are 725 l mol^{-1} min^{-1} for k_H, and 20 ± 5, 28 ± 10, and 35 ± 15 for chloroacetic, formic, and

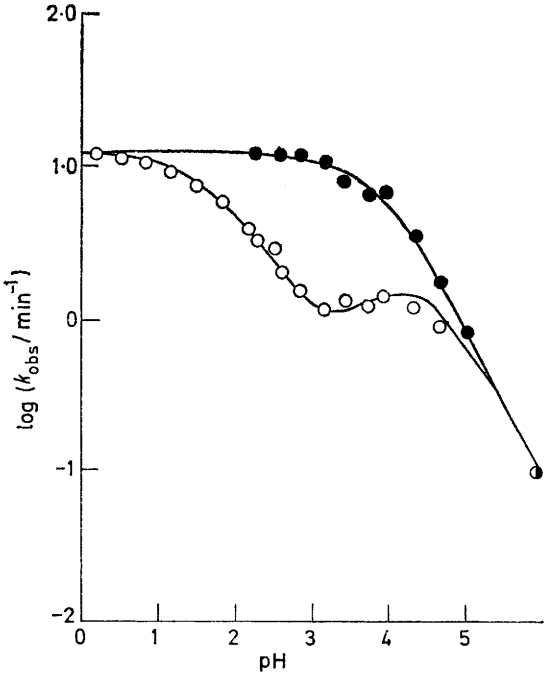


FIGURE 2 pH–Rate profile for the hydrolysis of the di-isopropylmaleamic acid derived from β-alanine; the two curves are for k_0 (open circles) and k_∞ (closed circles)

acetic acids, respectively. The accuracy of these constants falls as less diacid, and thus less catalysed reaction, is found at higher pH values; but they are identical within experimental error, as would be expected if the break in the Brønsted plot is slightly higher than for the n-propylamide (1).

The rate constant for the uncatalysed proton transfer for the neutral diacid is 0·4 min^{-1}, giving k_{H_2O} = 7·3 × 10^{-3} l mol^{-1} min^{-1} for this reaction.

TABLE 3

Data for the hydrolysis of *N*-carboxymethyldi-isopropylmaleamic acid (4; n = 1) at 15·5° and ionic strength 1·0

pH	Buffer (runs)		k_0/ min^{-1}	k_{HA}/ l mol^{-1} min^{-1}	k_∞/ min^{-1}
	1M-HCl	(4)	27·9		
	0·5M-HCl	(2)	28·8		
	0·25M-HCl	(1)	17·9		
1·18	0·1M-HCl	(4)	12·2		
1·48	0·05M-HCl	(2)	7·5		
2·20	0·01M-HCl	(3)	2·9		
2·48	$ClH_2C{\cdot}CO_2^-$	(9)	2·2	7·8	20
2·82	$ClH_2C{\cdot}CO_2^-$	(3)	1·56	15·4	
3·48	HCO_2^-	(9)	1·15	30	14
3·68	HCO_2^-	(3)	1·09	21	
3·98	HCO_2^-	(14)	0·9	16	13
4·72	$MeCO_2^-$	(6)	0·23	5·4	2·1

TABLE 4

General acid catalysis of the hydrolysis of *N*-carboxymethyldi-isopropylmaleamic acid at 15·5°

pK_a	General acid	k_{HA} (diacid)/ l mol^{-1} min^{-1}	k_{HA} (monoanion)/ l mol^{-1} min^{-1}
−1·7	H_3O^+	240	1200 [a]
2·86	$ClH_2C{\cdot}CO_2H$	5·0	50 ± 20
3·77	HCO_2H	3·3	40 ± 10
4·76	$MeCO_2H$	0·98	21 ± 5
15·7	H_2O	1·45 × 10^{-2}	2·22 × 10^{-2}

[a] Estimated value; see text.

TABLE 5

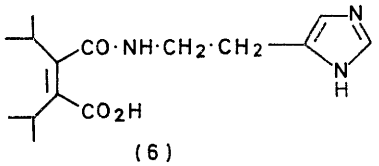
Hydrolysis data for *N*-2-carboxyethyldi-isopropylmaleamic acid (4; n = 2), at 15·5° and ionic strength 1·0

pH	Buffer	Runs	k_0/ min^{-1}	k_{HA} [a]/ l mol^{-1} min^{-1}	k_∞/ min^{-1}
	1M-HCl	4	12·4		
	0·47M-HCl	3	11·0		
	0·23M-HCl	2	10·9		
1·18	0·1M-HCl	4	9·3		
2·51	4·7 × 10^{-2}M-HCl	4	7·59		
2·84	2·3 × 10^{-2}M-HCl	4	5·88		
2·18	10^{-2}M-HCl	5	3·93		
2·30	$ClH_2C{\cdot}CO_2^-$	7	3·18	15	12·4
2·60	$ClH_2C{\cdot}CO_2^-$	12	2·0	15	12·2
2·85	$ClH_2C{\cdot}CO_2^-$	9	1·55	18	11·5
3·18	$ClH_2C{\cdot}CO_2^-$	21	1·2	20	7·2
3·45	HCO_2^-	6	1·3	14·5	8·0
3·76	HCO_2^-	17	1·2	19	6·5
3·97	HCO_2^-	6	1·42	16	6·6
4·37	$MeCO_2^-$	13	1·16	4·0	3·65
4·68	$MeCO_2^-$	10	0·87	3·4	1·64
5·04	$MeCO_2^-$	8	0·77	[b]	0·77
5·94	$MeCO_2^-$	3	0·096	[b]	0·096

[a] Uncorrected for substrate ionisation. [b] No detectable buffer catalysis.

γ-Aminobutyric Acid Derivative (4; n = 3).—We have collected only sufficient data to define the pH–rate (k_0) profile for hydrolysis of this compound. This is similar to that for the hydrolysis of the glycine derivative (Figure 1). The plateau rate constant at low pH is 7·8 min^{-1}, almost the same as for the *N*-n-propyl compound (1). The k_H value for the diacid is about 900 l mol^{-1} min^{-1} and the catalytic constant for formic acid no more than 30 l mol^{-1} min^{-1}. The rate constant for the uncatalysed proton transfer of the monoanion is 0·7 ± 0·3 min^{-1}.

Histamine Derivative (6).—Here also we measured the pH–rate (k_0) profile only, and have not collected enough data for a full analysis. The plateau rate constant at low pH is the same as for the *N*-n-propyl compound (1), though the second-order rate constants for general acid catalysis are about 3 times less than for (1). The reaction is too slow to be measured conveniently at 15·5° in the region of the pK_a



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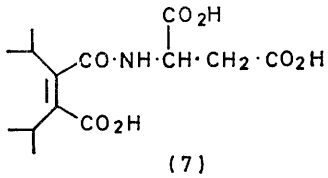


(7)

of the imidazole group, so we followed this part of the pH–rate profile at 39°, using the particularly effective [1] phosphate buffers to search for catalysis. The second-order plots obtained are not significantly curved, so that external general acid catalysis is observed for both cationic and neutral forms of (6). The full pH–k_0 profile (not shown) thus resembles those for the glycine and γ-aminobutyric acid derivatives (4; n = 1 or 3), with the shoulder representing hydrolysis of the half-neutralised form shifted to pH 6—7.

Aspartic Acid Derivative (7).—This compound combines the structural features of the glycine and β-alanine derivatives, and the pH–rate profile for hydrolysis (Figure 3) shows the influence of both α- and β-carboxy-groups. The reaction is buffer-catalysed at low pH but no catalysis can be detected at pH 4·94 in a 1 : 2 acetic acid–acetate buffer. The pH–rate profile is linear with a slope of −1 at low pH as the combined electronic effects of two carboxy-groups depress the rate of the hydronium ion catalysed proton transfer (to 11 l mol^{-1} min^{-1}), and increase the rate of the C–N cleavage step. As the pH rises the mono- and di-anions are seen to be hydrolysed more rapidly than the undissociated form. The dianion is presumably responsible for the uncatalysed hydrolysis observed at high pH so that the observed buffer catalysis must be largely of the hydrolysis of the monoanion. (If the ratio of k_H to the catalytic constants for other general acids is as large for the triacid as for the other derivatives we have measured, then catalysis by carboxylic acid buffers will be weak for this species.) The curvature of the second-order plots increases with increasing pH, as expected if the catalytic constants increase and the limiting rate decreases as the triacid ionises.

This information can be deduced from the measurements we have made at 7 different pH values (Table 6) and accounts satisfactorily for the emergence of the bell-shaped maximum at pH 4—5 (Figure 3). A full analysis of the kinetics of hydrolysis of (7) would require a much more detailed investigation.

Estimation of pK_a *Values.*—We have estimated pK_a values for the hydroxy- and amino-groups of tetrahedral intermediates (8) involved in this work using a method based on $\sigma_I\rho_I$ correlations, developed by Jencks.[3] The acidities of substituted alcohols and diols are satisfactorily correlated by the Taft equation, with ρ^* = −1·32,[5] corresponding [6] to

[5] S. Takahishi, L. A. Cohen, H. K. Miller, and E. G. Peake, *J. Org. Chem.*, 1971, **36**, 1205; J. Hine and E. F. Koser, *ibid.*, p. 1348.

[6] M. Charton, *J. Org. Chem.*, 1964, **29**, 1222.

TABLE 6

Hydrolysis data for the aspartic acid derivative (7), at 15·5° and ionic strength 1·0

pH	Buffer	No. of runs	k_0/ min^{-1}	k_{HA}/ l mol^{-1} min^{-1}	k_∞/ min^{-1}
	1M-HCl	1	7·25		
	0·1M-HCl	1	0·72		
2·18	10^{-2}M-HCl	1	0·30		
2·76	$ClH_2C{\cdot}CO_2^-$	6	0·27	0·75	*a*
3·25	HCO_2^-	5	0·44	2·8	3·6
3·98	HCO_2^-	7	1·0	5	2·0
4·94	$MeCO_2^-$	5	0·89	*b*	0·89

[a] This second-order plot shows little if any curvature. [b] No catalysis detectable.

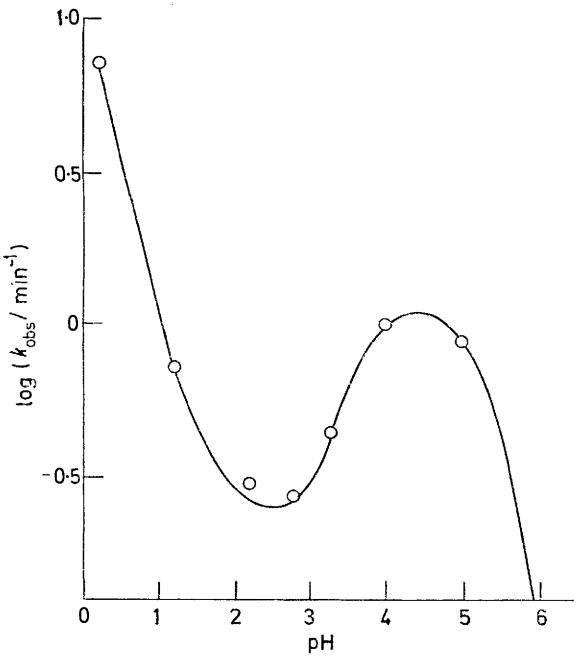
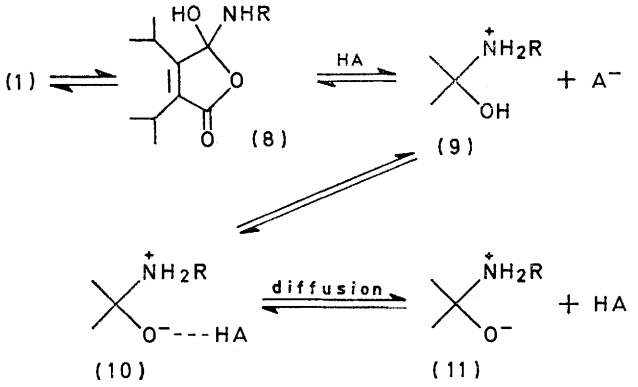


FIGURE 3 pH–Rate profile for the hydrolysis of the di-isopropylmaleamic acid derived from aspartic acid; data are extrapolated to zero buffer concentration (k_0)

$\rho_I = -8·2$. Correlations for aliphatic alkylammonium ions are less satisfactory, but ρ_I is estimated by Jencks [3] as $-8·3 \pm 1·0$. We use $\rho_I = -8·4$ for both groups, and σ_I values taken from the compilations of Charton [6] and Ritchie and Sager.[7] The values calculated for the amino-groups are based on the pK values of the free amines. Those for the OH groups are based on a value of 16·0 (corresponding to the pK_a of EtOH), which predicts the value (10·0) for the pK_a of $MeN\overset{+}{H}_2{\cdot}CH_2{\cdot}OH$ estimated by Hine.[8] We have not attempted to estimate the effects on the pK_a of the hydroxy-group of substituents on the N atom of the tetrahedral intermediate, apart from an indication of the direction of the expected effect. These estimates are summarised in Table 7.

DISCUSSION

The mechanism proposed in the preceding paper to account for general acid catalysis of the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid (1) involves a rate-determining diffusion-controlled proton transfer. In this process (Scheme 1) the neutral tetrahedral intermediate (8), which will break down exclusively to starting materials, is converted by two consecutive proton-transfer steps into the zwitterionic form (11). The zwitterion then breaks down to products by cleavage of the C–N bond.

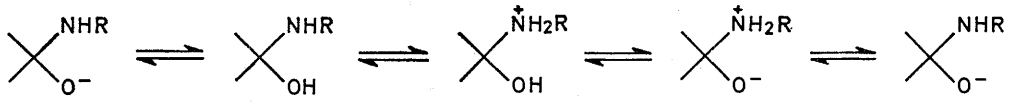


SCHEME 1

Since the conversion of (8) into (11) is thermodynamically unfavourable,[1] the rate-determining step of the overall process is the diffusion away [9] of the general acid HA from (11). This implies that the low equilibrium concentration of zwitterion formed in the encounter complex (10) does not itself break down to products at a significant rate (the mechanism designated by Schowen [10] as 'heavy atom reorganisation with spectator present').

TABLE 7

Estimated [a] pK_a values for tetrahedral intermediates (8) involved in this work



R (pK of RNH_2)					
Pr^n	(10·5)	9·3	2·5	5·0	5·5
$CH_2{\cdot}CO_2H$	(7·8)	<9·3	−0·2	<5·0	2·8
$CH_2{\cdot}CO_2^-$	(9·6)	9·3	1·6	5·0	4·6
$[CH_2]_2{\cdot}CO_2H$	(9·13)	<9·3	1·1	<5·0	4·1
$[CH_2]_2{\cdot}CO_2^-$	(10·2)	9·3	2·2	5·0	5·2

[a] Based on $\sigma_I\rho_I$ correlations using $\rho_I = -8·4$ for both amine and OH groups; see text.

[7] C. D. Ritchie and W. F. Sager, *Prog. Phys. Org. Chem.*, 1964, **2**, 323.

[8] J. Hine, J. C. Craig, J. G. Underwood, and F. A. Via, *J. Amer. Chem. Soc.*, 1970, **92**, 5194.

[9] W. P. Jencks, 'Catalysis in Chemistry and Enzymology, McGraw-Hill, New York, 1969, p. 208.

[10] L. D. Kershner and R. L. Schowen, *J. Amer. Chem. Soc.*, 1971, **93**, 2014.

This is reasonable since both the proton transfer step [back to (9)] and the diffusion step are expected to be faster reactions of (10) than C–N bond cleavage.

In many cases, especially with general acids of $pK_a > 4$, it is likely that both proton transfers take place during a single encounter[11] of catalyst molecule and intermediate (8). If a carboxy-group is already present in a suitable position in the molecule it should be able to catalyse both proton transfers, thus avoiding the rate-determining diffusion step and dispensing with the need for external general acid. We have studied four derivatives (4) with extra carboxy-groups in the leaving group, at least one of which would be expected to be suitably positioned. Yet in no case does the introduction of an extra carboxy-group remove the requirement for external general acid catalysis. There is evidence that weak intramolecular catalysis does occur, because the uncatalysed rate (extrapolated to zero hydrogen ion activity) is greater than for the n-propylamide in each case (Table 8). But the effective concentration of the carboxy-group is no greater than 0·2M even in the most favourable instance.

Hydrolysis of Undissociated Diacids.—The glycine derivative shows the largest effect. If k_H (Table 8) refers to the diffusion away of the solvated proton from the encounter complex (10), the rate constant for this particular step will not be expected to differ significantly over the series of compounds. The differences in the observed values of k_H must therefore be due to the different equilibrium constants for the formation of (10) from the starting materials, and thus reflect mainly the different concentrations of zwitterion (11) formed from the different substrates. For example, the value of k_0 for the derivative of ethyl glycinate (Table 8) is of the expected magnitude, based on the corresponding rate constant for the n-propylamide (1) ($k_0 = 0{\cdot}02$; estimate $= 0{\cdot}13 \times 218/1700 = 0{\cdot}017$). By the same calculation k_0 for the diacid form of the glycine derivative should be similar; in fact it is some 40 times greater. For the other dicarboxylic acids (4; $n = 2$ or 3) the corresponding rate enhancements are 7- and 9-fold, respectively (Table 8).

These effects probably represent weak intramolecular general acid catalysis by the carboxy-group. A possible alternative explanation is that the introduction of polar groups into the solvation shell surrounding the tetrahedral intermediate leads to changes in solvent structure which facilitate the proton transfer (8) ⇌ (11) through water. But the rate constants for external general acid catalysis, which are a measure of the rate of diffusion of the general acid through the same solvent structure, show no evidence of any such effect. The simplest explanation is that we are observing the expected intramolecular catalysis of the proton-transfer step, and that this catalysis is unexpectedly weak.

We discuss the detailed mechanism of this part of the reaction in terms of the simplified energy-profile diagram shown in Figure 4. The curves of Figure 4, which are semiquantitative only, are calculated for the undissociated glycine derivative (4; $n = 1$), but qualitatively similar diagrams can be obtained for the other derivatives. We have assumed an energy of activation of 3·5 kcal for the diffusion of the general acid through water,[12] and a figure of −8 cal deg^{-1} mol^{-1} (corresponding to a ΔF_0 value of 2·4 kcal mol^{-1} at 300 K) for the entropy change[13] involved in bringing the general acid and the tetrahedral intermediate together in the encounter complex.

TABLE 8

Summary of rate constants [a] for the general acid catalysed hydrolysis of *N*-alkyldi-isopropylmaleamic acids, RHN·CO·Pr^{i}C=CPr^i·CO$_2$H, at 15·5° and ionic strength 1·0

R	k_0/min^{-1}	k_∞/min^{-1}	k_H (1 mol^{-1} min^{-1})	k_{HA} [b] (1 mol^{-1} min^{-1})
Pr^n	0·13	7·7	1700	69 ± 12
CH_2·CO_2Et	0·02	22	220	15
CH_2·CO_2H	0·8	32	240	15
$[CH_2]_2$·CO_2H	0·4	11·6	720	30
$[CH_2]_3$·CO_2H	<0·8 [c]	7·8	1000 [a]	30
CH(CO_2H)·CH_2·CO_2H		>100	11	
CH_3·CH_2ImH$^+$ (6)	0·1	7·8	500	25
CH_2·CO_2^-	1·2	18	1200	50
CH_2·CH_2·CO_2^-	(>2·4)	2·4	*d*	*d*
$[CH_2]_3$·CO_2^-	0·7 ± 0·3			
CH(CO_2^-)·CH_2·CO_2H [e]	(>2—3)	2—3	*d*	*d*
$[CH_2]_2$Im (6)	2·5 × 10^{-3}			

[a] Estimated errors where not quoted are ±1 in last significant figure; except for 1000 (±100) and k_{HA} values (±5). [b] Limiting value of k_{HA} for strong general acids. [c] Not separated from kinetically equivalent acid-catalysed hydrolysis of the monoanion, which will account for a minor fraction of this rate constant. [d] No catalysis observed. [e] Other ionic forms (mono- and di-anion) also involved.

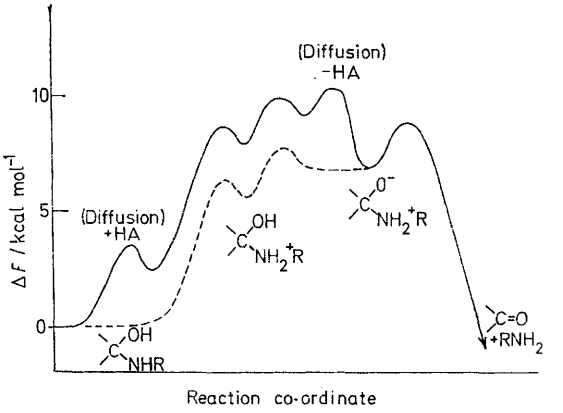


FIGURE 4 Energy profile diagram for the reaction in the region of the tetrahedral intermediate. The solid curve refers to the (external) general acid catalysed reaction; the broken curve is that expected for efficient intramolecular catalysis of the prototropic change. The curves are semi-quantitative only (see text), and are calculated on the basis of the data for the diacid form of the glycine derivative (4; $n = 1$)

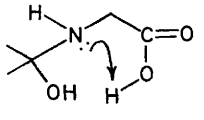
All but the final energy maximum for the (upper) external general acid catalysed path are lowered as the concentration of the general acid is increased, until the highest peak of the four falls below the final maximum, which represents the transition state for the C–N cleavage step. If the catalyst concentration is increased beyond

[11] W. P. Jencks, ref. 9, p. 211.
[12] D. N. Hague, ' Fast Reactions,' Wiley, London, 1971, p. 14.
[13] W. P. Jencks, ref. 9, p. 608.

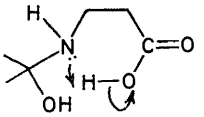
this point C–N cleavage becomes rate-determining and the dependence of the rate of hydrolysis on catalyst concentration disappears. This happens at concentrations of general acid of the order of 1M for most of our derivatives, so that $\Delta F^{\ddagger}$ for the C–N cleavage step of the zwitterion (11) is 2—3 kcal mol^{-1}. The energy profile expected for the intramolecular reaction (dashed line in Figure 4) shows energy barriers for the proton transfer only. These are known to be very low, so that if Figure 4 were a correct representation reaction should go almost exclusively by the intramolecular path, and external general acid catalysis should not be observed. In practice external catalysis is observed and it is the intramolecular catalysis that is weak. The most likely explanation is that we have neglected a necessary step on the intramolecular path, which costs 2—3 kcal mol^{-1} of free energy.

Energy barriers of this magnitude do not normally affect the observed rates of reactions significantly, but they are relatively important for diffusion-controlled processes. The most likely candidate for the step we have neglected is a conformational change involving the side-chain carrying the catalytic carboxy-group. Conformational barriers for rotation about carbon–carbon single bonds are of the order of 3—6 kcal mol^{-1} in butane,[14] and could have significant effects on the observed rates of our reactions.

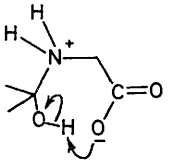
The glycine derivative is a special case. Super-fast proton transfer from the carboxy-group to the N atom (or *vice versa*) is not likely because the ring size of the cyclic transition state (12) is too small to accommodate a hydrogen bond. So the path to the tautomer (14) may



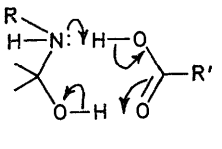
(12)



(13)



(14)



(15)

require external general acid, and the low energy path shown for intramolecular catalysis in Figure 4 may not be available. The carboxy-group of the β-alanine derivative, on the other hand, is suitably positioned to deliver a proton to the nitrogen (13) but only in a conformation close to the energetically unfavourable synperiplanar arrangement. The subsequent removal of the OH proton requires a conformational change, which could also account for the extra energy barrier.

These restrictions do not apply to the same reaction catalysed by an external general acid (or one held in the correct position in an enzyme–substrate complex). In particular it is possible for the carboxy-group to catalyse a concerted proton transfer (15) [15] when conformational restrictions are removed; this is geometrically impossible for our amino-acid derivatives.

Hydrolysis of Acid Anions.—Each of our amino-acid derivatives shows a significant anion reaction. In each case the fully ionised form is stable: the compounds are prepared in buffers of pH > 9, and can be stored for long periods under these conditions. So the reactive species is one containing both an undissociated carboxy- and a carboxylate group. In each case the partially ionised form is more reactive than the neutral di- or tri-acid. And in the two compounds where a β-carboxy-group is present [*e.g.* (4; $n = 2$)] the hydrolysis of the partially ionised form is particularly fast, and general acid catalysis is not observed.

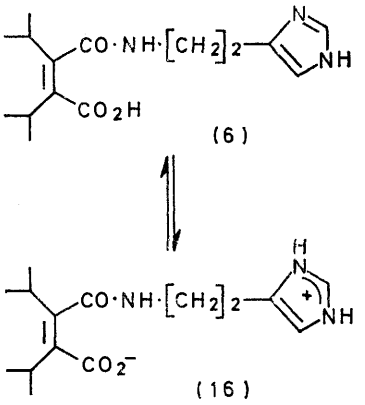
We discuss first the compounds which have no β-carboxy-group. The glycine derivative is typical. The pH–rate profile for hydrolysis (Figure 1) follows closely that for the hydrolysis of the ethyl ester (5) at low pH, but shows a distinct shoulder at pH 3—4 where the concentration of the monoanion is greatest. External general acid catalysis is observed over the whole pH range, and the rate of (uncatalysed) hydrolysis of the monoanion is some 12 times greater than expected from the comparison (above) with the n-propylamide. This could be an effect of the introduction of the charged group on the solvent structure around the tetrahedral intermediate, as described above. Or it could be a small catalytic effect, of the sort discussed below for compounds with a β-carboxy-group. Similar results are found for the γ-aminobutyric acid derivative (4; $n = 3$), and for the histamine derivative (6). In each case the pH–rate profile shows a shoulder representing the hydrolysis of the partially protonated form, and buffer catalysis is observed at all pH values. The rate enhancement is small for the γ-aminobutyric acid derivative: as the length of conformationally flexible carbon chain between catalytic group and substrate increases, competition with the diffusion-controlled external catalysis mechanism will rapidly become unfavourable.

The neutral form (6) of the histamine derivative is much less reactive than the carboxy-substituted compounds, because the predominant partially protonated form (16) is much less strongly acidic, and can support only a substantially reduced equilibrium concentration of the protonated amide form (the first intermediate on the reaction path [1]). This compound was studied at 39° in the pH 5—7 region, where it shows a shoulder in the pH–rate profile for hydrolysis (not shown) similar to that found for the glycine derivative (Figure 1). Calculations based on the pH–rate profile indicate that the effect of the β-imidazole group is comparable with that of a carboxy-group in the α-position. But buffer catalysis is still observed as high as pH 7·08, so the β-imidazole group does not fully satisfy the need for catalysis of the proton transfer step, as does the β-carboxylate group.

[14] E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, 'Conformational Analysis,' Interscience, New York, 1965, p. 9.

[15] B. A. Cunningham and G. L. Schmir, *J. Amer. Chem. Soc.*, 1966, **88**, 551.

The pH–rate (k_0) profile (Figure 2) for the hydrolysis of the β-alanine derivative (4; $n = 2$) differs from those for the other dicarboxylic acids in that the shoulder at pH 3—4 has become a maximum; and the separate



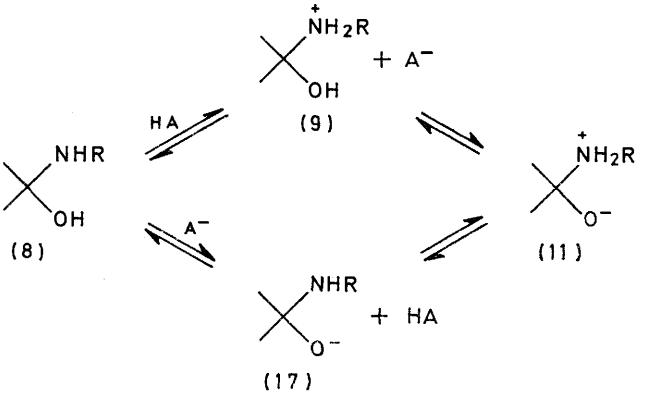
curves for k_0 and k_∞ merge at high pH, because the hydrolysis of the monoanion is not subject to buffer catalysis (see discussion in Experimental section). The appearance of the maximum in the pH–rate profile results from a combination of at least three factors: (i) The second pK_a is relatively high (4·6) so that higher concentrations of monoanion are present at higher pH values; (ii) the rate of the proton-transfer step is greater than for other di-isopropylmaleamic acids, high enough for this step no longer to be rate determining; and (iii) acid catalysis of the hydrolysis of the diacid form is weaker than for the n-propylamide (1), with the result that k_0 for the hydrolysis of the neutral diacid is lower (at pH values above the low-pH plateau region). We take this to result from the inductive effect of the β-carboxy-group, similar to the larger effect of the α-carboxy-group of the glycine derivative. Compared with the n-propylamide (1) the α-carboxy- (or ethoxycarbonyl) group reduces k_H by a factor of 8, while the figure for the β-carboxy-group is 2·4.

These results are discussed in more detail below. But the simple analysis presented here suggested that it should be possible to make the maximum in the pH–rate profile more dominant by introducing a third carboxy-group into the α-position of the β-alanine derivative. We therefore prepared the aspartic acid derivative (7).

The pH–rate profile (Figure 3, k_0 only) for the hydrolysis of (7) shows the expected features. The value of k_H for the neutral triacid is even lower than expected (11 l mol^{-1} min^{-1}; Table 8), while the partially ionised compound is hydrolysed at about the same rate as the monoanion of the β-alanine derivative; and the hydrolysis of the trianion (at least) is not buffer catalysed. Thus we have selectively depressed external general acid catalysis of the hydrolysis of the undissociated triacid, while retaining the catalytic effect of the β-carboxylate group. As a result the pH–rate profile for hydrolysis shows the bell-shaped curve typical of many enzyme-catalysed reactions—and for the same reasons.

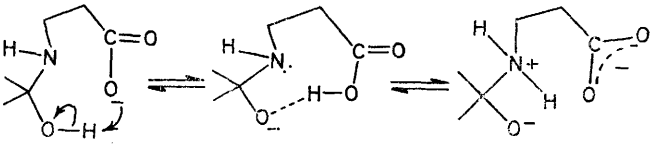
External catalysis of the hydrolysis of maleamic acids is exclusively general acid catalysis. We could find [1] no evidence for catalysis of the hydrolysis of the n-propylamide (1) or the glycine ethyl ester derivative (5) by the conjugate base form of any general acid. Yet we find that *intramolecular* catalysis of the same reaction is more efficient with the conjugate base. This is an unprecedented result. We have already established that there must be an additional potential energy barrier to intramolecular general acid catalysis: clearly there can be no such barrier to catalysis by the β-carboxylate group.

In the preceding paper we discussed our reasons for expecting the general acid catalysed path *via* the conjugate acid (9) of the tetrahedral intermediate (Scheme 2) to be preferred for the intermolecular reaction. The general base catalysed route, *via* the conjugate base (17) of the tetrahedral intermediate, is unfavourable because the hydroxy-group of the neutral tetrahedral intermediate (8) is only weakly acidic (estimated p$K_a > 9$). For weak general acids the equilibrium (8) ⇌ (9) also



SCHEME 2

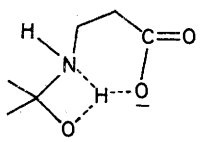
becomes strongly unfavourable: our estimates of pK_a values (Table 7) suggests that the equilibrium constants for the two equilibria (8) ⇌ (9) and (8) ⇌ (17) should be comparable when the pK_a of the catalyst is 5—6. The results suggest this estimate is at least one pK unit too low, but the calculation clearly suggests that the general base catalysed pathway is not a great deal less favourable for weakly acidic general species catalysts. Consequently it is not unreasonable that intramolecular general base catalysis by the carboxylate group should be favoured, if it is free from the conformational or other restriction that limits the efficiency of intramolecular general acid catalysis.



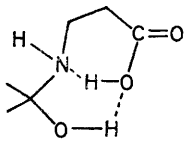
SCHEME 3

The simplest mechanism for intramolecular general base catalysis of the proton-transfer equilibrium is shown for the β-alanine derivative in Scheme 3. As for catalysis by the carboxy-group, the first proton transfer

is the energetically expensive one, but in this case the cyclic transition state is large enough to accommodate linear O–H–O geometry, with a *gauche* conformation of the ethylene chain. The subsequent proton transfer to nitrogen however should have the same geometry as the same step in intramolecular catalysis by the carboxy-group, and would therefore be subject to the same conformational restrictions. We can offer no simple explanation for the relatively high efficiency of intramolecular general base catalysis in this system. One possibility is that the geometrical requirements for super-fast proton transfer are less severe when the hydrogen bond concerned is between centres of closely similar basicity, as in the second stage of the general base catalysis mechanism: so that geometrical requirements elsewhere in the cyclic transition state can be relaxed. A logical extension of this line of reasoning suggests the four-centre transition state (18), in which the proton transfer is effected by one oxygen atom of the carboxylate group. Precedent for ground state structures resembling (18) is found in a



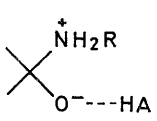
(18)



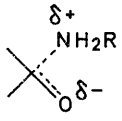
(19)

number of cases of bifurcated hydrogen bonds identified by crystal structure determinations.[16] It is at least conceivable that (18) provides a more favourable route for the proton transfer than the corresponding mechanism (19) involving the protonated carboxy-group.

Properties of the Tetrahedral Intermediate.—An unusual feature of this reaction is that it is possible to study the behaviour of a tetrahedral addition intermediate in two consecutive transition states; in the first of which (10) it is intact, as the zwitterion, while in the second (20) it breaks down to products.



(10)



(20)

The rate constants listed in Table 8 demonstrate clearly the opposing effects of changing the basicity of the leaving group on the two steps involved in the breakdown of the neutral tetrahedral intermediate (8). [Similar opposing electronic effects are involved in the two steps leading to the formation of (8), and it is this alternating effect of electron-donation or withdrawal throughout the reaction which explains the low dependence of overall reactivity on the basicity of the leaving group.[1]]

As the basicity of the nitrogen atom of the leaving group falls into the series R = Pr^n > $[CH_2]_3{\cdot}CO_2H$ > $[CH_2]_2{\cdot}CO_2H$ > $CH_2{\cdot}CO_2Et$, the equilibrium concentration of zwitterion formed from (8) in the encounter complex (10) will fall, while the rate of the C–N cleavage step will increase. The function k_∞ is a composite constant, which to a first approximation may be regarded as the product of an equilibrium constant [for the formation of the free zwitterion (11)] and the rate constant for the C–N cleavage step; and k_H is a measure of the magnitude of this equilibrium constant [as discussed above it is a measure of the equilibrium constant for the formation of (10), and the concentrations of (10) and (11) will be in a constant ratio]. So we can obtain relative rate constants for the C–N cleavage step by correcting the observed values of k_∞ for the changing equilibrium constants for zwitterion formation. For example, compared with the n-propylamide (1) (k_∞ 7·7 min^{-1}, k_H 1700 l mol^{-1} min^{-1}), the anion of the glycine derivative (4; $n = 1$) has a higher k_∞ (18 min^{-1}) and a lower k_H (1200 l mol^{-1} min^{-1}): if we correct k_∞ for the fall in k_H, the rate constant for C–N cleavage is therefore 18 × 1700/1200 = 25·5, 3·3 times greater than for the n-propylamide. Table 8 contains sufficient data for the comparison of four compounds, and we calculate relative rates for the C–N cleavage step of the zwitterion (11) of the tetrahedral intermediate of 7·7, 25·5, 27·4, and 240 for R = Pr^n, $CH_2{\cdot}CO_2^-$, $[CH_2]_2{\cdot}CO_2H$, and $CH_2{\cdot}CO_2H$, respectively. When these figures are plotted logarithmically against the pK_a of the conjugate acid of the amine leaving group they give a good straight line, with a Brønsted coefficient (β_N) of −0·55.

Jencks and Gilchrist [17] have measured β_N for the reverse reaction, the attack of amines on reactive carbonyl compounds. For primary amines with pK_a values between 7·8 and 10·5, corresponding to the leaving groups used in this work, their data give $\beta = 0{\cdot}35$ for attack on 2,4-dinitrophenyl acetate and the 1-acetoxy-4-methoxypyridinium cation. These figures allow a tentative dissection of the pathway for acyl transfer to nitrogen by way of the tetrahedral intermediate, and are consistent with other results for comparable systems. Adding the β-values of 0·55 and 0·35 for approach to the transition state (20) gives an estimate of $\beta = 0{\cdot}9$ for the addition of RNH_2 to the C=O group of a (leaving group) activated carbonyl compound. Formation of the addition intermediate is thought to be the rate-determining step in most reactions involving acyl transfer to nitrogen, and for reactions of weakly basic amines, where bond formation must be well advanced in the transition state, β approaches a limiting value of about 0·9.[17,18] Alternatively the reaction may be regarded as the quaternisation of the amine, which might reasonably be expected to have a β-value in the region of that (1·0) for protonation.

Conclusions.—Although we do not fully understand the proton-transfer mechanism concerned, we have achieved the immediate objective of this work. We have satisfied the requirement for an external catalyst in the original intramolecular reaction by introducing a second

[16] J. Donohue in 'Structural Chemistry and Molecular Biology,' eds. A. Rich and N. Davidson, W. H. Freeman, San Francisco, 1968, p. 450.

[17] W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, 1968, **90**, 2622.

[18] W. P. Jencks, B. Schaffhauser, K. Tornheim, and H. White, *J. Amer. Chem. Soc.*, 1971, **93**, 3917.

catalytic group into the molecule. Our understanding of the reaction indicated that this second group should be a carboxy-group: in fact it turned out that only a carboxylate group in one particular position would do. By adjusting electronic effects in the system thus developed, we have reproduced the bell-shaped curve characteristic of many enzyme-catalysed reactions, while retaining very high reactivity. [The rate constant for hydrolysis of the aspartic acid derivative (7) is 2—3 min^{-1} at 15·5°, comparable with k_{cat} for the hydrolysis of good synthetic substrates by pepsin,[19] an enzyme which uses carboxy-groups to hydrolyse peptides.] Some of the implications of the present results for the mechanism of the enzymic reaction are clear, while others require more detailed discussion, and further work on appropriate model systems. We defer a detailed discussion of this topic to a separate paper.

We thank the S.R.C. for support (to R. S. M.), and the National Research Council of Canada for a Fellowship (to C. R. S.).

[4/341 *Received*, 21*st February*, 1974]

[19] J. S. Fruton in ' The Enzymes,' ed. P. D. Boyer, Academic Press, New York, 3rd edn., vol. 3, p. 120.