Intramolecular Catalysis of Amide Hydrolysis by the Carboxy-group. Rate Determining Proton Transfer from External General Acids in the Hydrolysis of Substituted Maleamic Acids

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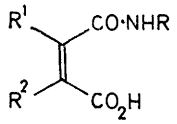
The highly efficient intramolecular catalysis by the carboxy-group of the hydrolysis of simple dialkylmaleamic acids is itself subject to external general acid catalysis. The kinetic characteristics of the general acid catalysed reaction are those expected for a diffusion-controlled proton transfer. At high concentrations of general acid, external catalysis disappears. This is shown to result from a change in rate-determining step, and is thus evidence for an intermediate on the reaction pathway. The intermediate can only reasonably be a tetrahedral addition intermediate. Kinetic evidence is now available for all the major steps on the reaction pathway, and the requirements for an enzyme catalyst carrying out the reaction can be specified in detail. The full mechanism specifically implicates the *O*-protonated amide as the reactive species in dilute acid.

THE heart of any enzymic reaction is a highly efficient multiple interaction between substrate and catalytic groups brought close together in the enzyme-substrate complex. We use the reactions between the same groups held close together on the same molecule as models for the enzymic reactions. In most known systems intramolecular catalysis is much less efficient than enzymic catalysis, but it is becoming clear that the rates of at

least some types of intramolecular reactions are very sensitive to structural variation.[1-3] An ideal model system for our purpose would match the efficiency of the enzymic process while remaining simple enough to be understood in detail. The work described in this series of papers [1,4,5] represents a systematic approach towards such an ideal system.

We have shown [1] that the efficiency of intramolecular catalysis of amide hydrolysis by the undissociated carboxy-group of the *N*-methylmaleamic system (1) (R = Me, R^1 = alkyl, R^2 = H) is very sensitive to the size of the alkyl groups R^1, and increases with increasing size of the substituent. The corresponding half-amide



(1)

from dimethylmaleic acid (R^1 = R^2 = Me) is more reactive still,[1] and the rate constant for its hydrolysis is comparable with k_{cat} for the reaction of the proteolytic enzyme pepsin with good synthetic substrates (*ca.* 1 s^{-1} at 39°). Our first objective was to increase catalytic efficiency in the simple system as far as possible, so we set out to study the hydrolysis of maleamic acids derived from di-isopropyl and di-t-butylmaleic anhydrides (1; R^1 = R^2 = Pr^i or Bu^t, respectively). In fact these compounds prove not to be significantly more reactive than the dimethylmaleamic acids; but the kinetic properties of the hydrolysis reaction are changed, in a way that allows a much deeper insight into the reaction mechanism. This in turn indicates how further increases in catalytic efficiency may be obtained. In this paper we describe a detailed investigation of the mechanism of hydrolysis of *N*-n-propyldi-isopropylmaleamic acid, and in the following paper we use the results to increase catalytic efficiency further.

EXPERIMENTAL

Materials and kinetic methods have been described.[1] Di-isopropylmaleic anhydride was prepared by the method of Eberson and Welinder,[6] and di-t-butylmaleic anhydride was a gift from Professor H.-G. Viehe.[7] Maleamic acids were prepared as described previously, from the anhydride and the amine in sodium-dried ether. Analytical data for a series of *N*-substituted derivatives are given in Supplementary Publication No. SUP 21073 (2 pp.).* *N-n-propyldi-isopropylmaleamic acid* was obtained as the n-propylammonium salt. Recrystallised from ether–chloroform, or from dioxan, this had m.p. 94—96° (decomp.) [109—110° (decomp.) in a sealed capillary tube] (Found: C, 63·8; H, 10·5; N, 9·35. $C_{16}H_{32}N_2O_3$ requires C, 64·0; H, 10·7; N, 9·3%).

The hydrolysis reaction has been described in some detail for a dimethylmaleamic acid,[1] and the di-isopropyl and di-t-butyl derivatives behaved similarly. The dialkylmaleic anhydride is an intermediate, and at pH values below 5 it is formed faster than it is hydrolysed. The anhydride is the stable form of the diacid for dialkylmaleic acids, and is the product of the reaction at low pH. All the rate constants measured in this work refer to the formation of the dialkylmaleic anhydride from the maleamic acid.

Buffer Catalysis.—The hydrolysis of *N*-n-propyldi-isopropylmaleamic acid is strongly catalysed by the constituents of the buffer solutions used to maintain pH control. We found no such catalysis in our work on the less reactive maleamic acids,[1] though we did not test the most reactive compound, the dimethylmaleamic acid derivative: we now find that catalysis is detectable for this amide also. The kinetics of the buffer-catalysed reaction are complex. At a given pH the catalysis shows saturation kinetics (Figure 1), the rate of hydrolysis becoming independent of buffer concentration when this is sufficiently high. This plateau can be reached at attainable concentrations of the more acidic buffers, but the observed curvature diminishes as the pK_a of the conjugate acid of the buffer rises, and catalysis by 2-morpholinoethane-1-sulphonate, the most weakly acidic buffer used, shows a simple first-order dependence on buffer concentration. It appears from Figure 1 that catalysis is more efficient with more strongly acidic buffers, but the effect is exaggerated by these uncorrected data because the maleamic acid ionises in the region of pH concerned, and only the undissociated form is reactive. Quantitative analysis of the kinetic results depends on the value taken for the dissociation constant of the maleamic acid. Since this compound has a half-life of less than 1 s at 39° the pK_a is not readily measured directly. We estimated it from the kinetic data, as follows.

The pK_a cannot be obtained accurately from the pH–rate profile for hydrolysis, because this is itself complex. Two curves can be plotted (Figure 2): one using rate constants (k_0) obtained by extrapolation to zero buffer concentration, and a second from rate constants (k_∞) obtained by extrapolation to infinite buffer concentration, where hydrolysis also becomes independent of buffer concentration. The first curve gives an apparent pK_a of about 2, clearly not the pK_a of the starting material, which is expected to be about 4. The points (k_∞) which define the second curve depend on a pK_a in the right region, but cannot be obtained with high accuracy, because of the nature of the extrapolation involved (see below). So we calculated the pK_a from the buffer catalysis data, as follows.

By making a large number of measurements at low buffer concentrations (at least five in the range 0·01—0·05M-buffer) we were able to measure approximate initial slopes for most of the curved buffer plots. These were consistent with catalysis by the acidic component of the buffer only (in no case is there any evidence for a contribution from the basic component). If the acidic component of the buffer is the catalytic species, then the decrease with the increasing pH of the observed second-order rate constant for a given buffer *acid* (*e.g.* formic acid, Table 1) must be due to the decrease in the concentration of the active form of the substrate.

* For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin II*, 1973, Index issue.

[1] A. J. Kirby and P. W. Lancaster, *J.C.S. Perkin II*, 1972, 1206.
[2] D. R. Storm and D. E. Koshland, *J. Amer. Chem. Soc.*, 1972, **94**, 5805, 5815.
[3] S. Milstien and L. A. Cohen, *Proc. Nat. Acad. Sci. U.S.A.*, 1970, **67**, 1143.
[4] M. F. Aldersley, A. J. Kirby, and P. W. Lancaster, *J.C.S. Chem. Comm.*, 1972, 570.
[5] M. F. Aldersley, A. J. Kirby, P. W. Lancaster, R. S. McDonald and C. R. Smith, following papers.
[6] L. Eberson and H. Welinder, *J. Amer. Chem. Soc.*, 1971, **93**, 5821.
[7] V. Jager and H. G. Viehe, *Angew. Chem. Internat. Edn.*, 1970, **9**, 795.

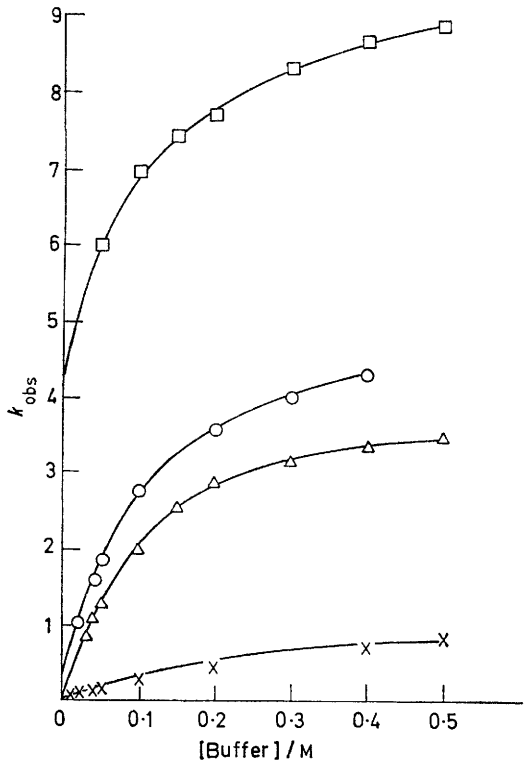


FIGURE 1 Buffer catalysis of the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid. Data, in order of decreasing apparent reactivity, are for chloroacetate, methoxyacetate, β-chloro-propionate, and acetate (all 50% free base), at 15·5° and ionic strength 1·0

Here too only the undissociated species reacts; so we could calculate a pK_a for the substrate from the observed variation with pH of the observed rate constants for general acid catalysis. The figure we obtained from the data for catalysis by formic acid (4·16) was consistent with the figure obtained in the same way from the data for acetate, and in the expected region.[1]

The buffer constants (k_{HA}) and k_∞ values were obtained most accurately from the curved plots as follows. The data at low buffer concentrations allowed a reproducible extrapolation to zero buffer concentration (giving k_0), and a measure of the initial slope of the curved plot, which was

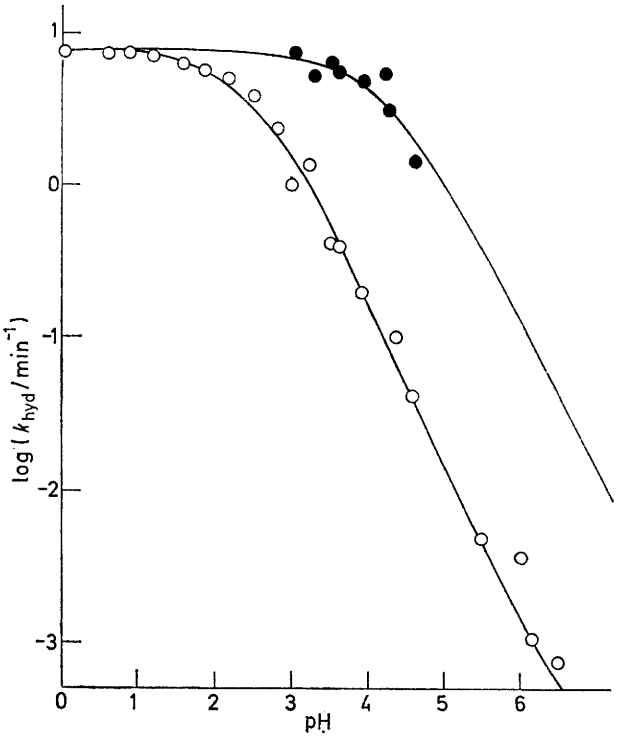


FIGURE 2 pH–Rate profiles for the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid at 15·5° and ionic strength 1·0. The open circles are points obtained by extrapolation to zero buffer concentration (k_0), and the closed symbols points (k_∞) obtained by extrapolation to infinite buffer concentration (see text)

most accurate (and agreed with the final value) for acids of pK_a higher than formic acid. (With 50% chloroacetate, and all dichloroacetate buffers tried, the pH varied so much at these low concentrations that the necessary corrections to constant pH were greater than the effects of changing catalyst concentration, so that accurate data for these more acidic buffers could not be obtained.) Double reciprocal

TABLE 1

Kinetic data for the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid at 15·5° and ionic strength 1·0

p*H* [a]	Conditions [b]	No. of runs	k_0/min^{-1}	k /min^{-1}	k_{HA}/l mol^{-1} min^{-1}
	0·22—1M-HCl	6	7·7 ± 0·2		
1·20	0·1M-HCl	2	7·02		
1·55	0·046M-HCl	3	6·34		
1·88	0·022M-HCl	2	5·49		
2·18	10^{-2}M-HCl	1	4·90		
2·51	$4·6 \times 10^{-3}$M-HCl	1	3·71		
2·84	$2·2 \times 10^{-3}$M-HCl	1	2·43		
3·02	0·01—0·5M-HCO_2^-, 80% HA	31	1·0 ± 0·2	6·9 ± 1	65 ± 10
3·22	10^{-3}M-HCl	1	1·35		
3·29	0·025—0·75M-$ClH_2C{\cdot}CO_2^-$, 25% HA	10	1·32	5·2 ± 1	50 ± 10
3·51	0·01—0·4M-$MeO{\cdot}H_2C{\cdot}CO_2^-$, 50% HA	14	0·43 ± 0·03	6·2 ± 0·3	66 ± 7
3·62	0·01—0·5M-HCO_2^-, 50% HA	18	0·42 ± 0·03	5·6 ± 0·3	46 ± 5
3·93	0·01—0·5M-$Cl[CH_2]_2{\cdot}CO_2^-$, 50% HA	17	0·20 ± 0·03	4·7 ± 0·2	47 ± 4
4·20	0·02—0·5M-HCO_2^-, 20% HA	11	0·10 ± 0·02	5·1 ± 0·4	29 ± 5
4·25	0·01—0·5M-$MeCO_2^-$, 70% HA	11	0·107 ± 0·002	3·0 ± 0·5	8·6 ± 0·2
4·60	0·01—0·5M-$MeCO_2^-$, 50% HA	18	0·042 ± 0·002	1·43 ± 0·03	5·75 ± 0·15
5·53	0·01—0·3M-HPO_4^{2-}, 90% HA	9	$4·8 \times 10^{-3}$		0·13 ± 0·01
6·03	0·1—0·3M-MES, 80% HA	3	$3·5 \times 10^{-3}$		$8·6 \times 10^{-3}$
6·18	0·1—0·3M-MES, 65% HA	3	$1·06 \times 10^{-3}$		$5·5 \times 10^{-3}$
6·55	0·1—0·3M-MES, 50% HA	3	$7·1 \times 10^{-4}$		$3·2 \times 10^{-3}$

[a] Measured pH. [b] HA = acid corresponding to buffer anion; MES = 2-morpholinoethane-1-sulphonate.

plots of ($k_{obs} - k_0$) against buffer concentration gave good straight lines in most cases, and the slopes and intercepts of these lines gave (as the reciprocals) k_{HA} and k_∞, respectively. The full set of data obtained in this way is given in Table 1, and second-order rate constants for general acid catalysis, corrected for substrate ionisation, appear in Table 2. The estimated errors are relatively large for k_{HA} and k_∞, but the results are internally consistent, and our interpretation is not affected by small changes in absolute magnitudes of these rate constants.

TABLE 2

Second-order constants for general acid catalysis of the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid [a]

General acid	pK_a	k_{HA}/l mol^{-1} min^{-1}
H_3O^+	(−1·7)	1700 ± 200 [b]
$ClCH_2{\cdot}CO_2H$	2·86	57 ± 10
$MeO{\cdot}CH_2{\cdot}CO_2H$	3·53	81 ± 10
HCO_2H	3·77	63 ± 5
$Cl[CH_2]_2{\cdot}CO_2H$	4·08	75 ± 7
$MeCO_2H$	4·76	21 ± 1
$H_2PO_4^-$	7·21	3·2 ± 0·2
MES [c]	6·15	0·65 ± 0·1
H_2O	(15·7)	2·4 ± 10^{-3}

[a] Calculated from the data given in Table 1, and based on an apparent pK_a of 4·16 for the substrate. [b] Based on hydronium ion activity. [c] See footnote *b* to Table 1.

The buffer catalysis constants for four carboxylic acids (chloroacetic, methoxyacetic, β-chloropropionic, and formic) all fall in the range 69 ± 12 l mol^{-1} min^{-1}, and we conclude that they are identical within experimental error. Acetic acid is 3—4 times less effective. This behaviour is characteristic of a rate-limiting diffusion-controlled proton transfer between the general acid and a base of pK_a about 4.[8,9] From these data we constructed the Brønsted plot shown (Figure 3), drawing a line of zero slope through the first four carboxylic acid points, and an intersecting line of unit slope through the point for acetic acid. Other data we have collected fall on this curve, or deviate from it predictably. Thus the catalytic constant for H_3O^+ is 25 times greater than the plateau rate established by the four carboxylic acids, as expected [8,9] for a diffusion-controlled proton transfer process. Catalysis by phosphate, which can act as a bifunctional acid–base catalyst for some proton transfers, is some two orders of magnitude more efficient than expected for a monofunctional catalyst; and catalysis by the tertiary amine 2-morpholinoethane-1-sulphonate is 100 times less efficient than catalysis by the four carboxylic acids, as predicted by the Brønsted plot described.

The set of data summarised by the Brønsted plot of Figure 3 is best interpreted by the curve shown, and we explain below why it is logical that the rate-determining step for the hydrolysis of a reactive maleamic acid should be a diffusion-controlled proton transfer. But the curve drawn (Figure 3) is not uniquely defined by the points we have measured, nor is it possible to add further points at low or high pK values. At low pH values catalysis disappears, and above pK 4 catalytic efficiency decreases 100-fold for every increase of one unit in the pK_a of the general acid catalyst. To strengthen the evidence that we are in fact dealing with a rate-determining diffusion-controlled proton transfer in this reaction at low concentrations of buffer, we have applied a separate and independent criterion.

[8] M. Eigen, *Angew. Chem. Internat. Edn.*, 1964, **3**, 1.
[9] R. E. Barnett and W. P. Jencks, *J. Amer. Chem. Soc.*, 1969, **91**, 2358.
[10] C. Cerjan and R. E. Barnett, *J. Phys. Chem.*, 1972, **76**, 1192.

Barnett [10] has suggested the use of glycerol–water mixtures of varying viscosity as a simple test for a diffusion-limited process. Such processes are expected to proceed at rates which are inversely proportional to the viscosity of the medium; other types of reaction should be unaffected. We have compared the rate constants (k_0 and k_{HA}) of the H_3O^+- and methoxyacetic acid-catalysed hydrolysis of *N*-n-propyldi-isopropylmaleamic acid in water and in 40 and 60% (w/w) glycerol–water mixtures, maintained at ionic strength 1·0 with KCl, and find substantial decreases in both rate constants as the solvent becomes more viscous (Table 3). Under these conditions there is no decrease in the rate of hydrolysis at pH 0, where the reaction is pH-independent because catalysis by H_3O^+ has levelled out.

The catalytic constants given in Table 3 should be directly comparable over the range of glycerol concentrations used, because the effects of the medium on the pK_a values of the

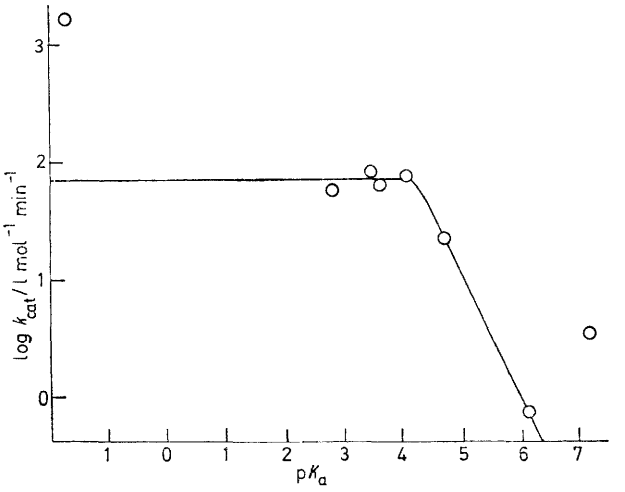


FIGURE 3 Brønsted plot for general acid catalysis of the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid; data from Table 2

TABLE 3

Rate constants for general acid catalysis of the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid in glycerol–water mixtures, at 15·5° and ionic strength 1·0

Glycerol % (w/w):	0	40	60
Viscosity (η/cP) [a]	1·11 ± 0·01	4·00 ± 0·02	11·8 ± 0·1
k_{obs} (1·0M-HCl)	7·6 ± 0·1	8·9 ± 0·2	7·9 ± 0·1
k_0 (pH 3·47 in water) [b]	0·6 ± 0·2	0·24 ± 0·02	0·12 ± 0·01
k_0 (pH 4·68 in water) [c]	0·043 ± 0·02		0·014 ± 0·0001
k_{HA} (for $MeO{\cdot}CH_2{\cdot}COOH$) [d]	66 ± 7	24 ± 2	11 ± 1

[a] Measured at 15·5° for 1M-KCl solutions using an Ostwald viscometer. [b] pH Values measured in 40 and 60% glycerol using the same buffer (1 : 1 formic acid–formate) were 3·61 and 3·76, respectively. [c] 1 : 1 Acetate–Acetic acid buffer, pH 4·92 in 60% glycerol. [d] Not corrected for substrate ionisation.

carboxylic acid groups of buffer and substrate should be similar (although the increase in the apparent pH as the glycerol concentration is increased may mean that part of the fall in k_0 is due to the lower hydrogen ion activity). We observe parallel falls in k_0 and k_{HA} with increasing glycerol concentration, both rate constants being 5—10-fold smaller in 60% glycerol. [The function k_0 contains both H_3O^+ and H_2O-catalysed (spontaneous) terms. The data at pH 4·68 in 50% acetate buffer, where no more than about 20% of the

H_3O^+-catalysed reaction remains, suggest that the fall in the rate constant for the spontaneous (H_2O-catalysed) reaction is only 2—3-fold. This means that $k_{H_3O^+}$ is depressed by more than the factor of 5 apparent from the data shown in Table 3 for pH 3·47.]

Because of the considerable difficulties involved in obtaining accurate rate constants for the various processes concerned even in water, we have not made the extensive range of measurements in glycerol–water mixtures that would be necessary to define the various rate constants accurately. So the data in Table 3 are not corrected for substrate ionisation, and in the case of k_0 are not separated into their components. But they show clearly that there is a strong inverse dependence on the viscosity of the medium of the rate constants for general acid catalysis, which are depressed 5—10-fold when the viscosity is increased by a factor of 10·6 (60% glycerol). This effect is consistent with a diffusion process being rate-determining for the hydrolysis reaction.

TABLE 4

Rate constants for the hydrolysis of *N*-substituted maleamic acids, $HO_2C{\cdot}CH{=}CH{\cdot}CO{\cdot}NRR'$, at 39° and ionic strength 1·0

R	R′	pK_a of $RR'NH_2^+$	k_{obs}/min^{-1}
H	H [a]	9·25	1·76 × 10^{-2}
H	Me	10·64	3·96 × 10^{-3}
Me	Me [a]	10·64	1·73 × 10^{-1}
H	$[CH_2]_2{\cdot}OMe$	9·20	5·1 × 10^{-3}
H	$CH_2{\cdot}CO_2Et$	7·75	1·13 × 10^{-2}
H	$CH_2{\cdot}CO_2H$		1·09 × 10^{-2}
H	$CH_2{\cdot}CF_3$	5·7	2·74 × 10^{-3}
H	Pr^i	10·63	2·76 × 10^{-3}
H	Bu^t	10·45	1·04 × 10^{-2}
H	Ph	4·58	1·81 × 10^{-2}

[a] Measured at 39·3°.

times faster still, so the most reactive compound studied in this series is the tertiary amide. This is also the case for hydrolysis of *N*-ethylmaleamic acids, studied by Dahlgren and Simmerman; [11] but the order of reactivity differs from that found for the *N*-methylbenzamides by Smith and Yates,[12] which is that found earlier for the corresponding acetamides,[13] primary > tertiary > secondary, over a wide range of temperature and acidity.

In Table 5 we compare the activation parameters we have measured for the hydrolysis of the *N*-methylmaleamic acids with the published figures for the corresponding *N*-ethylmaleamic acids [11] and *N*-methylbenzamides.[12] The Table also contains accurate activation parameters for the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid, measured at pH 1, where general acid catalysis is not observed, and thus directly comparable with the data for the unsubstituted maleamic acids. These data replace the figures we published previously [1] for *N*-propyldimethylmaleamic acid (Table 4 of ref. 1), which it is now clear contain a contribution from the general acid catalysed reaction. The enormous increase in reactivity over the series of maleamic acids we have studied [1] is entirely due to the decrease of nearly 15 kcal mol^{-1} in the enthalpy of activation: the entropy of activation does not change significantly over the whole series, except for a small increase for the least reactive compound.

The intramolecular reaction is characterised, as expected, by a more positive entropy of activation than the acid-catalysed hydrolysis of amides. The difference increases over the sequence primary < secondary < tertiary < (because $\Delta S^{\ddagger}$ falls considerably over this series for the benzamides, and increases slightly for the maleamic acids), from 13·5 for the primary amides to nearly 26 cal deg^{-1} mol^{-1} for the tertiary compounds. The small, irregular changes in $\Delta H^{\ddagger}$ over the series are similar for the two types of reaction (Table 5), so that the different sequence of rates is determined by the entropies of activation.

TABLE 5

Activation parameters for acid [a] and intramolecular general acid catalysis of amide hydrolysis

	NH_2		NHR		NR_2	
Benzamides [a] $\Delta H^{\ddagger}$/kcal mol^{-1}	20·3		21·2		18·7	
(R = Me) $\Delta S^{\ddagger}$/cal deg^{-1} mol^{-1}		−14·4		−17·5		−24·2
Maleamic acids [c] $\Delta H^{\ddagger}$ (±0·5)	23·1		24·2		22·5	
(R = Me) $\Delta S^{\ddagger}$ (±1·5)		−0·9		−0·3		1·6
Maleamic acids [d] $\Delta H^{\ddagger}$	21·9 ± 0·9		23·6		22·3	
(R = Et) $\Delta S^{\ddagger}$		−5·7 ± 3		−0·3		−3·3
cis-$Pr^nHN{\cdot}CO{\cdot}CPr^i{=}CPr^i{\cdot}CO_2H$ [e] $\Delta H^{\ddagger}$			19·2 ± 0·2			
$\Delta S^{\ddagger}$				3·7 ± 0·6		

[a] Data from ref. 12 for dilute acid. [b] This work. [c] Data from measurements at six temperatures between 39 and 64° (6 and 55° for *NN*-dimethyl compound). [d] Data from ref. 11. [e] Data from measurements at eight temperatures between 4·4 and 24·4°. Correlation coefficient for Arrhenius equation = 0·9998.

Effect of Leaving Group.—The rate-determining step in the hydrolysis of unsubstituted maleamic acids is the cleavage of the C–N bond of the tetrahedral intermediate (Scheme 1). To establish how reactivity depends on the structure of the leaving group we measured rate constants for the hydrolysis of a series of *N*-substituted maleamic acids in 0·1M-HCl. These data (Table 4) show that the electronic effects of substituents are negligible. Steric effects are also small. The *N*-t-butyl compound is hydrolysed a few times faster than *N*-methylmaleamic acid, but the reaction of the isopropylamide is slower. A factor of six covers the rates of all the secondary amides measured, over a wide range of basicity and size of leaving group. The most reactive secondary amide is the anilide, which is hydrolysed at the same rate as the primary amide. The dimethylamide is hydrolysed ten

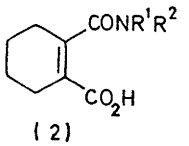
Buffer Catalysis of the Hydrolysis of Other Maleamic Acids.—We discuss below the evidence that general acid catalysis is observed for the hydrolysis of our most reactive maleamic acid because the rate of C–N cleavage is enhanced relative to earlier steps of the reaction, one of which then becomes rate-determining. We have examined several of the other more reactive maleamic acids for evidence of catalysis, and have found it in the hydrolysis of two half-amides (2) derived from cyclohexene-1,2-dicarboxylic acid. (We also found, but have not studied, catalysis of the hydrolysis of *N*-n-propyldimethylmaleamic acid, which would be expected to behave like the corresponding di-isopropyl derivative.)

[11] G. Dahlgren and N. L. Simmerman, *J. Phys. Chem.*, 1965, **69**, 3636.

[12] C. R. Smith and K. Yates, *J. Amer. Chem. Soc.*, 1971, **93**, 6578.

[13] P. D. Bolton, *Austral. J. Chem.*, 1966, **19**, 1013.

The results for the maleamic acids (2) are summarised in Table 6. There is no detectable catalysis of the hydrolysis



(2)

of the *N*-methyl compound (2; R^1 = H, R^2 = Me), for which C–N cleavage is still evidently rate-determining. So we examined compounds derived from amines which had proved particularly good leaving groups in the reaction with C–N cleavage rate-determining, that is, the most reactive compounds listed in Table 4. Under favourable conditions, working at relatively high pH to minimise the H_3O^+-catalysed reaction, we find catalysis of hydrolysis for both the glycine derivative (2; R^1 = H, R^2 = CH_2·CO_2H) and the dimethylamide (2; R^1 = R^2 = Me). For this latter compound catalysis by methoxyacetic acid at pH 3·0 is small but still detectable, but at pH 2·8 catalysis (by chloroacetic acid) can no longer be observed, because the proton-transfer step is catalysed sufficiently well by H_3O^+ for C–N cleavage to have become securely rate-determining once more. Under these conditions it proved possible to change the rate-determining step back again by working in 60% glycerol, where the diffusion process is selectively retarded and catalysis by chloroacetate can again be detected.

Either the rate-determining step has become insensitive to the structural change concerned; or it has changed to one which is insensitive. In this case we will demonstrate that both changes have occurred.

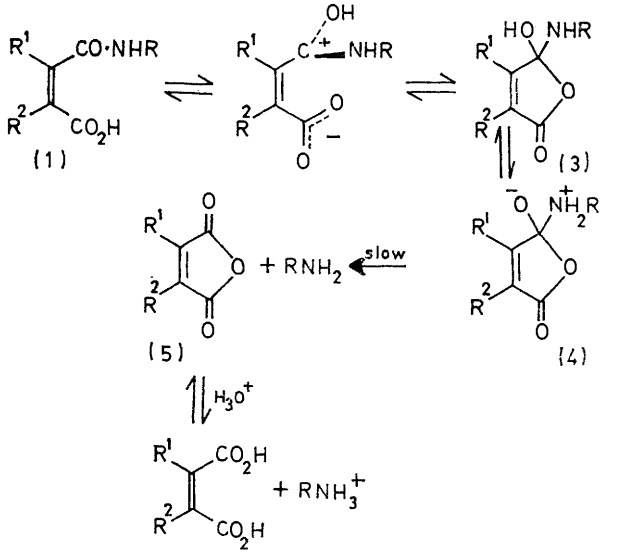
The evidence for the change of rate-determining step is that the hydrolysis of the *most reactive* compounds studied is subject to buffer catalysis: and that this catalysis shows saturation kinetics (Figure 1). At high buffer concentrations the reaction becomes independent of buffer concentration once more, and the reaction regains the characteristics of the simple unimolecular process, similar in all respects to the hydrolysis of the less reactive maleamic acids. This behaviour is expected for a complex reaction with two transition states of closely similar energies. If the slower step is subject to catalysis it is often possible to make this step faster than the second simply by increasing catalyst concentration so that the second step becomes rate-determining.

TABLE 6

Buffer catalysis of the hydrolysis of other maleamic acids, at 39° and ionic strength 1·0

Compound (2)					
R^1	R^2	Conditions	k_0/min^{-1}	k_∞/min^{-1}	k_{HA}/l mol^{-1} min^{-1}
H	Me	Phosphate, 0·1—0·6M, pH 5·92	$1·77 \times 10^{-2}$		
H	Bu^t	Phosphate, 0·1—0·6M, pH 5·92	$4·26 \times 10^{-2}$		
H	CH_2·CO_2H	Phosphate, 0·1—0·6M, pH 6·04	$8·8 \times 10^{-3}$	$2·1 \times 10^{-2}$	$2·9 \times 10^{-2}$
Me	Me	Phosphate, 0·1—0·6M, pH 5·92	0·167	0·53	0·30
Me	Me [a]	Methoxyacetate, 0·15—0·75M, pH 3·04	1·4	2·4	1·9
Me	Me [a]	Chloroacetate, 0·2—1M, pH 2·81	2·2		
		Formate, pH 3·97	1·3	200	90

[a] At 15·5°; data are not corrected for substrate ionisation.



SCHEME 1

DISCUSSION

Kinetics and Mechanism.—We have proposed the mechanism shown in Scheme 1 to account for the rapid hydrolysis of the maleamic acids. The reaction is a typical unimolecular process, with entropy of activation close to zero, and the rate-determining step is the cleavage of the C–N bond.[1] The very large effects of substituents R^1 and R^2 on the rate of hydrolysis are presumed to reflect their influence on the equilibrium constant for the formation of (3), and will be discussed in a separate paper of this series. We are concerned here specifically with the kinetics and mechanism of the reaction.

The rates of hydrolysis of monoalkylmaleamic acids (R^1 = H, R^2 = alkyl, R = Me) increase with increasing size of the alkyl group R^2, but we find no such effect for the dialkylmaleamic acids. The rates of hydrolysis of the dimethyl and di-isopropyl compounds are almost identical, and we have found the di-t-butyl compound no more reactive in another series. Two explanations can account for this type of observation if it is clear, as it is in this case, that the overall mechanism has not changed. This is the best explanation of our results for *N*-n-propyldi-isopropylmaleamic acid. The uncatalysed step is C–N cleavage [1] [(4) ⟶ (5) in Scheme 1]. This is rate-determining at saturating buffer concentrations, and the pH–rate profile for hydrolysis under these conditions (Figure 2, k_∞) is directly comparable with the pH–rate profiles obtained previously for the other maleamic acids.[1] The observed rate of hydrolysis on the plateau between pH 0 and 1, where the curves for k_0 and k_∞ have merged at high hydrogen-ion activity, is no greater for the di-

isopropylmaleamic acids than for the dimethylmaleamic acids. This must mean that the effects of increasing size of the alkyl group affect the ground and transition states equally in the dialkylmaleamic acids, as discussed above.

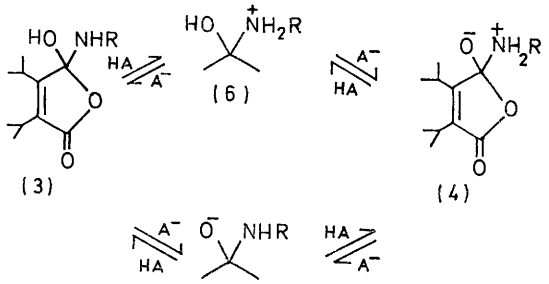
The rate-determining step at low buffer concentrations must be one of the other steps represented, explicitly or implicitly, in Scheme 1. All these other steps, with the exception of the ring closure to form (3), are either diffusion processes or proton transfers between O and N centres, which would be expected to have the characteristics of diffusion processes. Ring closure [(1) ⟶ (3)] is not a likely rate-determining step: this is the process we expect to be most affected by alkyl substituents R^1 and R^2, and this should be *fastest* for the dimethyl and di-isopropyl compounds; furthermore there is no simple mechanism for buffer catalysis of this step.

We therefore conclude that the new rate-determining step, whatever it is, is likely to be a diffusion process. Our analysis of the buffer catalysis results shows that the reaction is, kinetically, general acid catalysis of the hydrolysis of the undissociated maleamic acid. We can find no evidence for general base catalysis, nor any reaction of the maleamic acid anion. And this general acid catalysed reaction has the kinetic characteristics of a diffusion-controlled proton-transfer process.

These characteristics have been conveniently summarised by Barnett and Jencks,[9] and the general acid catalysed hydrolysis of the maleamic acids meets all their criteria. (i) The rate of the proton transfer process is independent of the pK_a of the general acid when this is below 4, as expected for a proton transfer between the general acid and a base of pK_a close to 4. (ii) The rate constant for proton transfer from H_3O^+ is 25 times greater than that for other acids of pK_a below 4. (iii) As the strength of the acid is decreased ($pK_a > 4$), and the proton transfer becomes thermodynamically unfavourable, the value of Brønsted's coefficient α approaches 1·0 (Figure 2). (iv) The rate constant for catalysis by phosphate, which can act as a bifunctional general acid–base catalyst, shows a marked positive deviation from the Brønsted plot (Figure 3), as does the point for catalysis by H_2O (Table 2, not shown in Figure 3). We find that $k_{H_3O^+}/k_{H_2O} = 7 \times 10^5$ for our reaction; values of 4×10^5 and 8×10^5 were found for general acid catalysis of the two acyl transfer reactions studied by Barnett and Jencks,[9] which involve similar proton transfer processes. A figure of this order of magnitude is likely to be characteristic of the process by which tetrahedral intermediates involved in amide hydrolysis or ester aminolysis are converted into the zwitterionic forms [*e.g.* (3) ⟶ (4) in Scheme 1]. (v) As a further test for a diffusion-controlled reaction we have measured the effect of increasing viscosity of the solvent, using the glycerol–water mixtures suggested by Barnett[10] for this purpose. As expected for a diffusion-controlled process the rate constants for general acid catalysis are sharply depressed in 60% glycerol, where the viscosity of the solvent is increased *ca.* 10-fold (Table 3); whereas the rate of hydrolysis in 1·0M-HCl, where C–N cleavage is rate-determining, is not significantly affected. These data are discussed in more detail in the Experimental section, but the important conclusion to emerge is that they too are consistent with a diffusion-controlled proton-transfer being rate-determining under conditions where general acid catalysis is observed. The effects of increasing viscosity on the catalytic constants for H_3O^+ and methoxyacetic acid are similar, while the water-catalysed process, which becomes the main pathway for the proton transfer in the absence of buffer above pH 4, is slowed to a lesser extent, as might be expected since solvent molecules are already present at the reaction site.

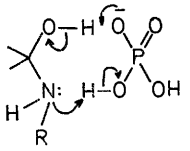
The Proton-transfer Step.—It seems certain that the proton-transfer step which is rate-determining at low catalyst concentrations must involve the tetrahedral intermediate (3) [or (4)]. Known rate constants for diffusion-controlled reactions[8] are so large that the observed rates of our reactions can only be accounted for if the species to which the general acid transfers the proton is present in very low concentration. This is true only of the high energy intermediates (3) and (4) of Scheme 1.

Two pathways are possible for the interconversion of (3) and (4), each involving two steps (Scheme 2). [All



SCHEME 2

these steps require the presence of a molecule of a general acid or base, because there is no low energy pathway available for a 1,3-proton shift between N and O in (3) or (4); and only a bifunctional reagent like phosphate can effect the interconversion in a concerted process (7).]



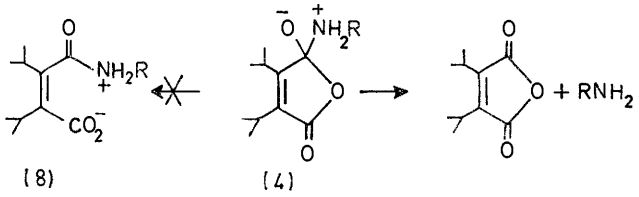
(7)

Under the conditions of our experiments the general acid catalysed pathway *via* the conjugate acid (6) is expected to be preferred. We estimate pK_a values of about 2·5 and 4·5 for the RNH_2^+ and OH groups of (6), and about 9 for the OH group of (3).* Under our con-

* These estimates use σ_I values, and $\rho_I = -8{\cdot}4$ for the ionisation of both substituted alcohols and amines. The value of ρ_I is calculated as −8·2 from the ρ^* value of −1·32 found for alcohols and *gem*-diols[14,15] corrected by a factor[16] of 6·23; and as −8·3 by a more complicated calculation for substituted ammonium ions, described by Jencks.[9,17]

[14] S. Takahashi, L. A. Cohen, H. K. Miller, and E. G. Peake, *J. Org. Chem.*, 1971, **36**, 1205.
[15] J. Hine and G. F. Koser, *J. Org. Chem.*, 1971, **36**, 1222.
[16] M. Charton, *J. Org. Chem.*, 1964, **29**, 1222.
[17] W. P. Jencks, personal communication.

ditions, therefore, a substantial amount of (3) should be present as its conjugate acid (6). The equilibrium for the conversion of (3) into (4) is expected to be unfavourable (*K ca.* 10^{-2}), so that the highest energy intermediate on the reaction pathway will be the zwitterion (4), and the rate-determining step therefore the diffusion away from (4) of the molecule of the general acid. [This is true whether the conversion (3) ⟶ (6) ⟶ (4) involves separate steps as shown in Scheme 2, or is a one-encounter process.] Barnett and Jencks [9] concluded, on the basis of similar arguments, that the corresponding step, in the reverse direction, is rate-limiting for the acetyl transfer reaction of 2-acetylthioethylamine above pH 2·3. Note that in our reaction (3) [and hence also (6), which is in equilibrium with (3)] will break down exclusively to starting material, because the carboxylate group is a far better leaving group than RNH^-. On the other hand (4) will break down almost exclusively to products, because RNH_2 is a viable leaving group and leaves a stable product, whereas the loss of carboxylate would generate the unfavourable *N*-protonated amide (8). Thus the tetrahedral intermediate (3) and its conjugate acid (6) normally revert rapidly to starting material, and only go on



(8) (4)

to products when the reverse reaction is inhibited by the removal of the OH proton of (6) by a general base. Kinetically, of course, general base catalysis of the removal of a proton from a conjugate acid is indistinguishable from true general acid catalysis.

The C–N *Cleavage Step.*—If the high reactivity of the dialkylmaleamic acids is accounted for mainly by the increased equilibrium constant for the formation of (3), then the rates of the proton-transfer and C–N cleavage steps must be affected equally. It follows that the change of rate-determining step we observe must result from a further effect specifically on the rate of C–N cleavage. It is reasonable that there might be some steric acceleration of the loss of RNH_2 from the highly substituted ring of (4), and we have found independent evidence consistent with this interpretation.

By studying a series of *N*-substituted maleamic acids (1; $R^1 = R^2 = H$) we could identify factors associated with high reactivity in this system. These must be factors increasing the rate of C–N cleavage, which is the rate-determining step in the hydrolysis of the unsubstituted compounds. Electronic effects are negligible and steric effects small, and the only structural change leading to a substantial increase in reactivity is the introduction of a second *N*-alkyl substituent (Table 4). Thus *NN*-dimethylmaleamic acid is hydrolysed 44 times faster than the corresponding secondary amide. Most of this factor of 44 represents an effect specifically on the C–N cleavage reaction, as is demonstrated by the data for *NN*-dimethyldi-isopropylmaleamic acid (Table 6). Under conditions where C–N cleavage is rate-determining this compound is hydrolysed 25—30 times faster than the n-propylamide, but the rate constant for general acid catalysis by formate is increased only 2—3-fold. Since we consider that the catalysed reaction is diffusion-controlled in both cases, the actual rate constant for the proton-transfer step will be the same for both compounds. The factor of 2—3 therefore represents the effects of the second alkyl group on the equilibrium constant for the formation of (3), and it appears that the C–N cleavage step is faster for the *NN*-dimethyl compound by a factor of 10—20.

We have therefore examined the dimethylamides of less reactive maleic acid systems for evidence of general acid catalysis. Although no catalysis can be detected in the hydrolysis of *NN*-dimethylmaleamic acid itself, catalysis is readily observed in the reaction of the cyclohexene derivative (2; $R^1 = R^2 = Me$) [the hydrolysis of the *N*-methyl compound (2; $R^1 = H$, $R^2 = Me$) is not catalysed]. A second example where catalysis is observed is the glycine derivative (2; $R^1 = H$, $R^2 = CH_2{\cdot}CO_2H$); the glycine derivative of the unsubstituted maleamic acid also shows relatively high reactivity (Table 4).

These results demonstrate satisfactorily that we can change the rate-determining step by selectively increasing the rate of C–N cleavage, and thus add further support to our proposed mechanism. The situation is not a simple one, however. It seems likely that *N*-substituents may affect predominantly either C–N cleavage or the pre-equilibrium, since we cannot produce the change in rate-determining step in other, apparently equally favourable cases. We have also tried to change the rate-determining step by increasing the viscosity of the solvent, as described above, which should slow down the proton-transfer step selectively. This failed for the methylamide (2; $R^1 = H$, $R^2 = Me$) under a range of conditions (phosphate, acetate, and methoxyacetate buffers), and we could achieve catalysis of 60% glycerol where none is observed in water, only under carefully controlled conditions for the dimethylamide (2; $R^1 = R^2 = Me$). At sufficiently low pH (50% chloroacetate buffer, pH 2·80) the proton-transfer step is catalysed so effectively by H_3O^+ that no catalysis by the buffer can be detected in water. Under the same conditions in 60% glycerol–water weak catalysis by chloroacetate was observed (k_{cat} 1—2 l mol^{-1} min^{-1} at 15·5°), indicating that catalysis of the proton transfer step by H_3O^+ has been slowed down relative to C–N bond cleavage until it is partially rate limiting.

Conclusions.—Although the kinetics of the hydrolysis of *N*-n-propyldi-isopropylmaleamic acid are complex, the mechanism of hydrolysis is not. Since our further work on these compounds (see following paper) is based on this mechanism it is useful to summarise our main conclusions here. The mechanism shown in Scheme 1 stands. For most maleamic acids C–N cleavage [(4) ⟶ (5)] is rate-determining, but for some dialkylmaleamic acids the

proton shift (3) ⟶ (4) becomes rate-determining under some conditions. This step is catalysed by general acids, including H_3O^+, and a spontaneous water-catalysed reaction is observed above pH 4. The second-order rate constants for general acid catalysis fall with increasing catalyst concentration until catalysis disappears at high buffer concentrations: under these conditions C–N bond cleavage is rate-determining for these compounds also. All this is evidence for an intermediate on the reaction pathway, which can only reasonably be the tetrahedral addition intermediate (3).

Finally, there is a more general implication of the existence of (3) as an intermediate. Several authors have proposed, or found it difficult to rule out, mechanisms for acid-catalysed amide hydrolysis involving the *N*-protonated amide. Our evidence specifically implicates an intermediate requiring a proton transfer, and is not consistent with the cyclisation of the *N*-protonated amide (8) to form either the zwitterion (4) or the anhydride directly. The irregular pattern of enthalpies of activation found for the acid-catalysed hydrolysis of the various *N*-methylated benzamides [12] is found also for the corresponding series of maleamic acids (Table 5), as might be expected if the mechanisms of hydrolysis were similar. Arguments based purely on kinetic parameters cannot be conclusive because it is possible to arrive at very similar transition states for hydrolysis by way of both protonated forms of the amide, and the question can only be settled by evidence relating directly to the existence and behaviour of the intermediates involved. The evidence from our reaction is only consistent with reaction by way of the *O*-protonated amide.

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