Structure and Efficiency in Intramolecular and Enzymic Catalysis. Catalysis of Amide Hydrolysis by the Carboxy-group of Substituted Maleamic Acids

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The efficiency of intramolecular catalysis of amide hydrolysis by the carboxy-group of a series of substituted *N*-methylmaleamic acids is remarkably sensitive to the pattern of substitution on the carbon–carbon double bond. A single alkyl group increases the rate of hydrolysis by a factor which increases with its size. A second alkyl substituent has a disproportionately larger effect, which is sharply reduced when the two groups are joined together in a ring. The rates of hydrolysis of a series of dialkyl-*N*-methylmaleamic acids range over more than ten powers of ten, and the ' effective concentration ' of the carboxy-group of the most reactive compound studied is greater than 10^{10}M. This amide, dimethyl-*N*-n-propylmaleamic acid, is converted into the more stable dimethylmaleic anhydride with a half-life of less than 1s at 39 °C below pH 3. The mechanism of catalysis, and the factors responsible for this extremely high reactivity, are discussed.

THE extraordinarily high efficiency of enzymic catalysis depends on a combination of a relatively small number of factors, most of which have been recognised but none of which is well understood.[1] Despite the growing attention being paid to the chemistry of enzyme action it is not impossible that some factor or factors of fundamental importance remain to be identified. This possibility has stimulated such recent suggestions as ' orbital steering ' [2] and ' stereopopulation control ',[3] which arose from attempts to interpret the changes in reactivity induced by structural variation in particular intramolecular reactions. These interpretations have been

[1] W. P. Jencks, ' Catalysis in Chemistry and Enzymology', McGraw-Hill, New York, 1969, ch. 1.

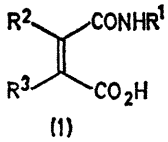
[2] D. R. Storm and D. E. Koshland, *Proc. Nat. Acad. Sci. U.S.A.*, 1970, **66**, 445; G. A. Daffon and D. E. Koshland, *ibid.*, 1971, **68**, 2463; *Bio-organic Chemistry*, 1971, **1**, 129.

[3] S. Milstien and L. A. Cohen, *Proc. Nat. Acad. Sci. U.S.A.*, 1970, **67**, 1143.

criticised [4-6] and their relevance to enzymic catalysis is questionable, but what does emerge very clearly is that the factors which affect reactivity even in intramolecular catalysis are not well understood. Although the validity of intramolecular reactions as models for reactions between the same groups in the enzyme–substrate complex must have limits, a proper understanding of the relevant processes in simple systems appears to be a prerequisite for a detailed understanding of enzymic catalysis.

Previous work on structure and reactivity in intramolecular catalysis, apart from studies of electronic effects by use of Hammett's equation, have been dominated by the problem of conformational isomerism. Bruice has shown how the rate of reaction between the carboxylate and ester groups of half-esters of dicarboxylic acids depends on the flexibility, length,[7] and pattern of substitution [8] of the intervening carbon chain. The more recent observations of Storm and Koshland [2] and Milstien and Cohen [3] are of the same general type. Invariably the largest rate enhancements are observed in systems where the molecule is more or less rigidly fixed in a favourable conformation. This is to be expected, since no increase in reactivity can result from raising the free energy of that conformation of the ground state most favourable for reaction if other conformations accessible by simple rotations about single bonds are unaffected. The result will simply be to increase the populations of the more stable conformations.

The object of our work is to identify those structural factors associated with high reactivity in intramolecular reactions, in the expectation that similar factors will be operative in enzymic catalysis also. The need for high reactivity itself dictates the choice of rigid systems, which have the further advantage that the variables associated with conformational mobility are eliminated at the outset. What could not have been predicted is the extraordinarily high sensitivity of reactivity to relatively minor structural changes attainable even in a rigid system. The initial indication came from the observation by Dixon and Perham [9] that the half amide formed by the reaction of arginine with dimethylmaleic anhydride is hydrolysed very much more rapidly than the unsubstituted maleic acid derivative. In this paper we present detailed structure–reactivity data for the hydrolysis of a wide range of substituted *N*-alkylmaleamic acids (1).



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[4] T. C. Bruice, A. Brown, and D. O. Harris, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 658.
[5] B. Capon, *J. Chem. Soc.* (*B*), 1971, 1207.
[6] M. I. Page and W. P. Jencks, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 1678; G. N. J. Port and W. G. Richards, *Nature*, 1971, **231**, 312.
[7] T. C. Bruice and U. K. Pandit, *J. Amer. Chem. Soc.*, 1960, **82**, 5858; *Proc. Nat. Acad. Sci. U.S.A.*, 1960, **46**, 402.

EXPERIMENTAL

Materials.—Inorganic salts were of analytical grade and were used without further purification. Organic buffer components were distilled or recrystallised. Distilled water was redistilled twice before use from all-glass apparatus.

Maleic, citraconic, dimethylmaleic, and cyclohex-1-ene-1,2-dicarboxylic acid anhydrides were commercial. Citraconic anhydride was distilled at reduced pressure. Ethylmaleic,[10] isopropylmaleic,[11] and t-butylmaleic [12] anhydrides were prepared by published procedures. Cyclopent-1-ene-1,2-dicarboxylic acid anhydride was prepared by a modification of a published method,[13] as follows. Ethyl 1,3-dibromo-2-oxocyclohexanecarboxylate (54 g) was dissolved in cold EtOH (80 ml) containing KOH (45 g). The mixture was stirred in the cold (0—5 °C) for 3 h before water was added to dissolve the KOH. The resulting alkaline solution was extracted with ether, then acidified to pH 0 with HCl. Ether extraction of the acid solution gave a yellow solid, which was recrystallised from boiling water (charcoal) to give large crystals, m.p. 177—178 °C (lit.,[13] 178 °C). The anhydride was formed when the acid was dissolved in acetic anhydride and heated for 30 min. Distillation gave the anhydride, b.p. 142—144 °C at 15 mmHg (lit.,[14] 130 °C at 5 mmHg).

Substituted maleamic acids (1). These were prepared as follows. To a solution of anhydride (0·10 mol) in sodium-dried ether (100 ml) was added the amine (0·11 mol) (methylamine as the commercial 33% ethanolic solution) in dry ether (25 ml). The mixture was cooled in ice–water and stirred vigorously for 2 h, by which time crystallisation always occurred. The product was filtered off, washed with dry ether, and dried. A number of these acid amides were hygroscopic, and all were stored in desiccators. Analyses and m.p.s are in Table 1.[15,16] In four cases (the most reactive compounds) the standard procedure gave the amine salts of the maleamic acids: otherwise the maleamic acids were obtained. In the case of dimethylmaleic anhydride the *N*-methylamide could not be obtained crystalline, and the *N*-n-propylamide was used.

The maleamic acids derived from unsymmetrically substituted maleic anhydrides appeared to be single isomers. Only in the case of the citraconyl compound was there any suggestion that a second isomer might be present. In this case a second spot appeared on t.l.c. in one solvent system only. Brooks *et al.*[15] had described the separation of two isomers of *N*-n-propylcitraconamic acid, but their only evidence for structure was different m.p.s. We could not repeat their result. We did obtain samples with various m.p.s, but all were homogeneous and identical on t.l.c. We made a determined effort to separate the isomers of *N*-methylcitraconamic acid using t.l.c., paper chromatography, electrophoresis, ion-exchange, and conventional column chromatography, and chromatography on a dry

[8] T. C. Bruice and W. C. Bradbury, *J. Amer. Chem. Soc.*, 1965, **87**, 4838, 4846, 4851.
[9] H. B. F. Dixon and R. N. Perham, *Biochem. J.*, 1968, **109**, 312.
[10] P. Walden, *Ber.*, 1891, **24**, 2025.
[11] W. R. Vaughan and K. S. Andersen, *J. Amer. Chem. Soc.*, 1955, **77**, 6702.
[12] S. R. Jensen and J. Munch-Petersen, *Acta Chem. Scand.*, 1967, **21**, 1963.
[13] *Chem. Abs.*, 1966, **65**, 20033*a* (patent application).
[14] S. C. Sen-Gupta, *J. Indian Chem. Soc.*, 1940, **17**, 183.
[15] N. B. Mehta, A. P. Phillips, F. Fu, and R. E. Brooks, *J. Org. Chem.*, 1960, **25**, 1012.
[16] A. Anschutz, *Ber.*, 1887, **20**, 3215.

silica column. An alternative preparative route from *N*-methylcitraconimide and hydroxide (designed to reverse the steric influences presumably responsible for the formation of a single isomer in the preparation from the anhydride) gave a sample which was chromatographically and kinetically identical. We concluded, therefore, that we were dealing with a single isomer (1; $R^2 = H$, $R^1 = R^2 = Me$) and this was subsequently confirmed by *X*-ray crystallography.[17] In no other case was there any hint of a second isomer formed from an unsymmetrical anhydride, and since steric effects would be more decisive for groups R^3 larger than Me, we have assumed that the other three unsymmetrical products have the structures given in Table 1.

TABLE 1

Analytical data for *N*-alkylmaleamic acids (1)

Substituents			M.p. (lit. m.p.)(°C)	Analysis					
				Calc. (%)			Found (%)		
R^2	R^3	R^1							
H	H	Me	154 (154 [15]) [a]	46·5	10·85	5·4	46·4	11·1	5·6
H	H	Ph	204—205 dec. (206—208 [16]) [b]	62·8	4·7	7·3	62·8	4·7	7·5
H	Me	Me	133—134 [a]	50·35	6·3	9·8	50·65	6·5	10·0
H	Me	Pr^n [h]	129·5—130·5 (140, 122 [15]) [b]	56·1	7·6	8·2	55·9	7·3	8·0
H	Et	Me [h]	70—71 [d]	53·5	7·0	8·9	53·4	7·0	8·85
H	Pr^i	Me [h]	119—121 [d]	56·15	7·6	8·2	56·15	7·4	8·4
H	Bu^t	Me [h]	163—164 dec. [d,e]	55·55	9·25	12·95	55·4	9·2	12·9
Cyclohex-1-ene-1,2-diyl		Me	123—124 [a,e]	56·1	8·4	13·1	56·0	8·2	12·75
Cyclopent-1-ene-1,2-diyl		Me	203—204 dec. [a]	56·8	6·5	8·3	56·8	6·45	8·1
Cyclobut-1-ene-1,2-diyl		Me	195—197 [g]	54·2	5·8	9·05	54·1	5·9	9·1
Me	Me	Pr^n	89—91 [c,f]	59·0	11·5	9·85	59·2	11·2	9·6

Recrystallised from [a] EtOH, [b] aqueous ethanol, [c] tetrahydrofuran. [d] Not recrystallised. [e] Isolated as methylammonium salt. [f] Isolated as n-propylammonium salt. [g] Prepared by a special route See text. [h] These unsymmetrical compounds are assumed, but not proved, to have the structures indicated, by analogy with *N*-n-propylcitraconamic acid (see text).

2-N-*Methylcarbamoylcyclobut*-1-*ene*-1-*carboxylic acid*. The anhydride cannot be made from cyclobut-1-ene-1,2-dicarboxylic acid, and the *N*-methylamide was made from the monomethyl ester. Dimethyl cyclobut-1-ene-1,2-dicarboxylate [18] (340 mg) and KOH (110 mg) in a little water were dissolved in methanol and kept at 40 °C with stirring for 5 h. The solution was diluted with water, acidified with HCl, and extracted 4 times with ether. The required ester, 2-methoxycarbonylcyclobut-1-ene-1-carboxylic acid crystallised from the dried, concentrated solution [130 mg (42%), m.p. 57—59 °C]. The ester was dissolved in methanol (5 ml) and treated with 33% ethanolic $MeNH_2$ (3 ml). After standing overnight the volatile components were evaporated off, and the residue crystallised. Recrystallised from acetone–CCl_4, the amide had m.p. 195—197 °C (see Table 1).

Kinetic Methods.—Reactions were followed for at least 3 half-lives, and end-points taken after at least 10, in the thermostatted cell-holder of a Zeiss PMQ II spectrophotometer, at the appropriate wavelength, determined as discussed below, for the compound concerned at the pH used. Reactions were run mostly at 39·0 ± 0·05 °C. Very high or very low rate constants were measured at 15·5 °C or 100 °C, respectively, and extrapolated to 39 °C by means of measurements at intermediate temperatures, by use of Arrhenius plots. Ionic strength was maintained at 1·0M with added KCl. Buffers used to maintain constant pH were HCl, formic and acetic acids, phosphate, TRIS, carbonate, and hydroxide, generally at 0·05M. For runs in D_2O at higher acid concentrations commercial 20% DCl in D_2O was used.

Fast reactions ($t_{\frac{1}{2}} < 10$ min) were started by adding 0·14 ml of stock solution of the maleamic acid (at pH 9—10) to 2·56 ml of buffer in the u.v. cell already in the cell compartment of the spectrophotometer. All solutions were incubated at the reaction temperature for at least 15 min before mixing. For slower reactions 0·5 ml of reagent was added to 9·5 ml of buffer in a tube kept in a thermostatted bath, and the u.v. cells filled from this. The very slowest reactions were followed by sealing the reaction mixture in a large number (15—20) of glass tubes, which were heated in an oil-bath and opened at intervals.

The pH of the solution was measured at the end of each run with a Vibron electrometer fitted with an E.I.L. C-33B pH-measuring attachment and a Pye–Ingold combined glass–reference electrode; at the temperature of the reaction concerned, except in the case of sealed-tube reactions, which were measured at 25 °C.

The maleamic acids are hydrolysed by way of the maleic anhydrides, and for the most reactive compound the rates of hydrolysis of the anhydrides are comparable with their rates of formation. Indeed dimethylmaleic anhydride is hydrolysed more slowly than *N*-n-propyldimethylmaleamic acid below pH 5·5. The initial reaction was then followed at the isosbestic point for the second step (hydrolysis of the anhydride) under the conditions of the experiment, and in some cases this could be established simply by repeated scanning of the u.v. spectrum of the anhydride as it hydrolysed in the appropriate buffer (95%, +5% of dioxan solution of the anhydride). But in the case of the cyclohexenyl and dimethylmaleic compounds the isosbestic points for the hydrolysis of the anhydride were uncomfortably close to those for its formation, and the spectral shifts caused by even 5% of dioxan were sufficient to make the technique unworkable. The precise wavelength of the isosbestic point then had to be found by trials involving a number of runs at slightly different wavelengths. Satisfactory kinetics were only obtained when the wavelength concerned was known to within ±0·1 nm, and this pro-

[17] In collaboration with Mrs. O. Kennard and her co-workers in this department.

[18] F. B. Kipping and J. J. Wren, *J. Chem. Soc.*, 1957, 1733.

cedure had to be repeated at each pH used. At a slightly different wavelength the isosbestic point for anhydride formation from the amide could be observed, and at this point the rate of the second step of the reaction could be measured in suitable cases, and was shown to be identical with the rate of anhydride hydrolysis under the same conditions. A selection of the plots of optical density against time obtained for one compound is shown in Figure 1. These experiments, designed to find precise wavelengths to follow the disappearance of the maleamic acids, also provide the first direct evidence that the anhydride is an intermediate in amide hydrolysis catalysed by a neighbouring carboxy-group. This proof will not be described in more detail, since it was repeated perhaps a

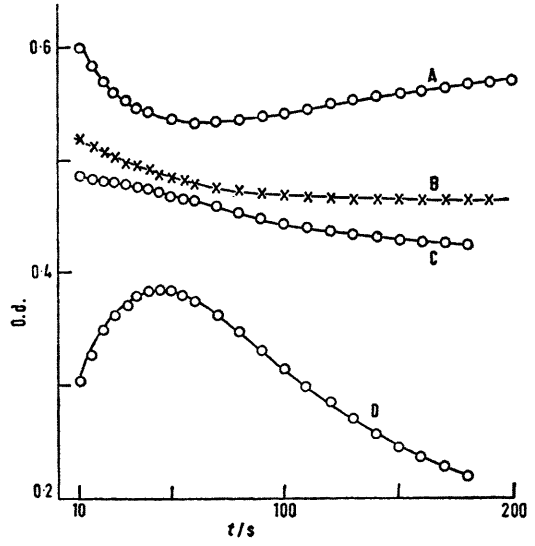
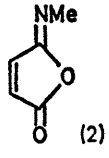


FIGURE 1 Time-course of the hydrolysis of 2-*N*-methylcarbamoylcyclohex-1-ene-1-carboxylic acid at 39 °C in 1N-HCl, measured at A, 243; B, 245·5; C, 247; and D, 260 nm. The two-step nature is readily apparent from the curves at 243 and 260 nm and isosbestic points for the first step (conversion of amide into cyclic anhydride) and for the second step (hydrolysis of the anhydride) are observed at 247 and 245·5 nm, respectively

hundred times during this work, under various conditions with the two most reactive maleamic acids. The situation was even clearer when the hydrolysis of the dimethylmaleamic acid was measured at pH values below 5: here the disappearance of the amide was too fast to detect, and the only reaction observed at 39 °C was the subsequent hydrolysis of the anhydride.

If it is assumed that the anhydride is formed from the maleamic acid by way of a tetrahedral intermediate, it is in principle possible to test whether the formation or breakdown of this intermediate is the rate-determining step by



(2)

generating it independently. The hydrolysis of *N*-methylisomaleimide (2) is expected to go by way of this same tetrahedral intermediate (see Discussion section) so we examined the hydrolysis products of (2) under the conditions of our experiments.

If the rate-determining step in the amide hydrolysis is the breakdown of the tetrahedral intermediate to the anhydride, the product from the hydrolysis of (2) should be the maleamic acid. (This technique has been used previously, notably by Cunningham and Schmir.[19]) When freshly sublimed *N*-methylisomaleimide (2) (11·1 mg; m.p. 84—85 °C), prepared by the method of Cotter *et al.*,[20] was dissolved in 1N-HCl (100 ml) the u.v. spectrum of the solution (and the rate of disappearance of the main peak) was identified as that of *N*-methylmaleamic acid. By quantitative comparison with the spectra of authentic samples of maleamic acid and maleic acid at 280 nm the yield of maleamic acid was calculated to be 97 ± 4%. Thus the rate-determining step in maleamic acid hydrolysis occurs after the tetrahedral intermediate.

RESULTS

We measured pH–rate profiles in the region pH 0—5 for the hydrolysis of all the maleamic acids listed in Table 1. For *N*-alkylmaleamic acids the pH–rate profile is of the form shown in Figure 2. The reactive species is the neutral maleamic acid (1), and the rate of hydrolysis is independent of pH from pH 0 to 2, falling at higher pH as the proportion of the unreactive anion rises. The data can thus be described fully by the rate constant for the hydrolysis of the acid form, and the apparent pK_a of the carboxy-group. In no case did the hydrolysis of the anion proceed at a detectable rate. The measurements are summarised in terms of these two parameters in Table 2. The rate

TABLE 2

Rate constants for the hydrolysis of *N*-alkylmaleamic acids (1) at 39 °C and ionic strength 1·0

Acid (1)					Followed
R^3	R^2	R^1	pK_{app}	k_{hyd}/min^{-1}	at (nm)
H	H	Me	4·0	3·90 ± 0·1 × 10^{-3}	260·0
H	H	Ph		2·4 ± 2 × 10^{-2} [a]	275·0
Me	H	Me	3·2	12·2 ± 0·4 × 10^{-2}	240·0
The same, in D_2O				16·7 × 10^{-2} [b]	240·0
The same, at 28·7 °C				3·2 ± 0·1 × 10^{-2}	240·0
The same, at 48·6 °C				3·5 ± 0·1 × 10^{-1}	240·0
Me	H	Pr^n		9·03 ± 0·02 × 10^{-2}	240·0
Et	H	Me	3·2	12·8 ± 0·5 × 10^{-2}	240·0
Pr^i	H	Me	3·6	17·4 ± 0·6 × 10^{-2}	240·0
Bu^t	H	Me	3·7	26·5 ± 0·5 × 10^{-2}	235·5
The same, at 28·7 °C				9·48 ± 0·08 × 10^{-2}	235·5
The same, at 48·3 °C				75 ± 1 × 10^{-2}	235·5
Cyclohex-1-ene-1,2-diyl		Me	3·5	2·10 ± 0·02	245·0—246·1
The same, in D_2O				1·99 [b]	245·2
Cyclopent-1-ene-1,2-diyl		Me	4·2	1·6 × 10^{-7} [c]	245·2
The same, at 80·0 °C				9·68 × 10^{-5} [d]	260·0
The same, at 90·0 °C				3·86 × 10^{-4} [d]	260·0
The same, at 100·0 °C				13·6 ± 0·2 × 10^{-4}	260·0
Cyclobut-1-ene-1,2-at 100·0 °C			3·65 [f]	13·4 ± 0·2 × 10^{-5} [e]	260·0
Me	Me	Pr^n [e]		68 ± 2	260·0
(*N*-Methylphthalmic acid				2·8 ± 0·1 × 10^{-3})	300·0

[a] pH-Independent reaction dominated by acid-catalysed hydrolysis (k_H = 0·27 l mol^{-1} min^{-1}) at low pH. [b] 1M-DCl in D_2O. Single measurement. [c] By extrapolation. [d] single points. [e] Complicated by acid-catalysed reaction (k_H = 5 × 10^{-4} l mol^{-1} min^{-1} at 100 °C). [f] pK_a measured spectrophotometrically.

[19] B. A. Cunningham and G. A. Schmir, *J. Amer. Chem. Soc.*, 1966, **88**, 555.

[20] R. J. Cotter, C. K. Sauers, and J. M. Whelan, *J. Org. Chem.*, 1961, **26**, 10.

constants are the means of at least three excellently reproducible values measured in the pH-independent region. The pK_a values are derived from the pH–rate curves and are only approximate.

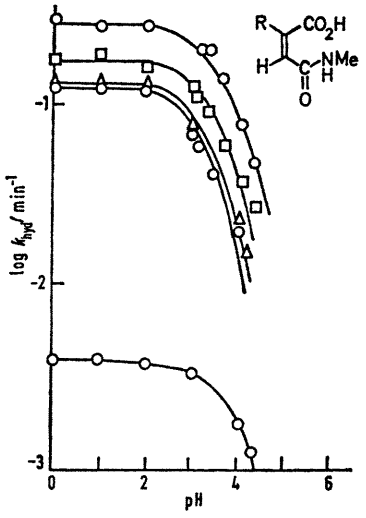


FIGURE 2 pH–Rate profiles for the hydrolysis of alkyl-*N*-methylmaleamic acids at 39 °C and ionic strength 1·0. In increasing order of reactivity R = H, Me, Et, Pr^i, and Bu^t

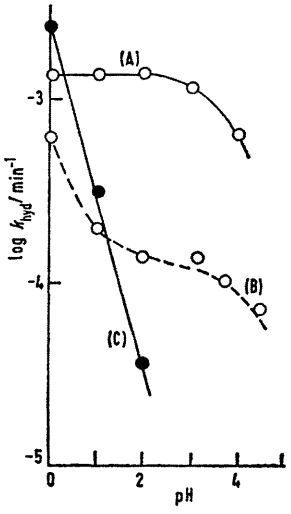


FIGURE 3 pH–Rate profiles for the hydrolysis of the two least reactive maleamic acids, the cyclopentenyl (A) and cyclobutenyl (B) compounds, compared with the hydrolysis of *N*-methylacrylamide (C) under the same conditions (100 °C, ionic strength 1·0)

The very slow hydrolysis of the cyclopentenyl and cyclobutenyl compounds is nevertheless subject to catalysis by the carboxy-group, as indicated by pH-independent regions down to below pH 0 for the former, and to pH 2 for the cyclobutenyl compound (Figure 3). External acid catalysis is significant only below these pH values. Comparison with an amide lacking the carboxy-group, *N*-methylacrylamide (Figure 3), shows that even the cyclobutenyl compound is hydrolysed some 30 times more rapidly than the acrylamide at pH 3.

Buffer catalysis was not significant even for the slowest hydrolyses measured. Nor could we find detectable catalysis by external general acid of the hydrolysis of *N*-methylacrylamide. This amide was hydrolysed at 100 °C and ionic strength 1·0 during 130 days (about one half-life) in 0·05 and 0·20M-formic acid–formate buffer (20% free base, pH 2·93 and 2·91, respectively). The two runs gave good pseudo-first-order plots with identical slopes. From these results we can estimate a maximum possible second-order rate constant for general acid catalysis by formic acid of the hydrolysis of *N*-methylacrylamide of 5×10^{-7} l mol^{-1} min^{-1} at 100 °C.

The rates of hydrolysis of the maleic anhydrides which are intermediates in the hydrolysis of the maleamic acids are not too high to measure by conventional techniques, and proved much less sensitive to structural variation than those of the maleamic acids. Data for five anhydrides are in Table 3. The rate of hydrolysis is pH-independent over

TABLE 3

Rate constants for the hydrolysis of substituted maleic anhydrides at 39 °C and ionic strength 1·0

Substituents	k_{hyd}/min^{-1}	pH Range	No. of runs	Measured at (nm)
H, Me	1·96 ± 0·06	0—2	3	260·0
H, Bu^t	0·378 ± 0·004	0—2	3	265·0
Cyclohex-1-ene-1,2-diyl	1·14 ± 0·02	0—5·31	8	260·0
Cyclopent-1-ene-1,2-diyl	3·2 ± 0·2	0—5·29	3	265·0
Me, Me [a]	0·67 ± 0·05	4·5—6·4	4	260·0
	4·2	2	1	260·0

[a] Probably two pH-independent regions. See text.

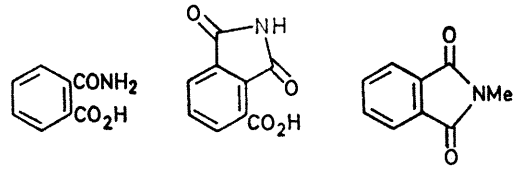
a considerable range above pH 0 in four cases, but acid catalysis is important in the hydrolysis of dimethylmaleic anhydride below pH 4 (although Eberson's data [21] indicate that hydrolysis is likely to be pH-independent below pH 2 also).

DISCUSSION

Intramolecular catalysis of amide hydrolysis by the carboxy-group has received much attention in recent years. Rapid hydrolysis has been observed both with conformationally mobile compounds such as aminoacylasparagines [22] and succinanilic acids,[23] and with the conformationally fixed phthalamic [24,25] and maleamic acids.[26,27] In the first systematic study Bender *et al.*[24] showed that phthalamic acid (3) is hydrolysed 10^5—10^6 times as fast as *p*-carboxybenzamide; and that nucleophilic attack by the carboxy-oxygen is almost certainly involved. Isotopic evidence is consistent with phthalic anhydride being an intermediate in the hydrolysis of phthalamic acid,[24] and catalysis is much less efficient, and mechanistically quite different, in the hydrolysis of

[21] L. Eberson, *Acta Chem. Scand.*, 1964, **18**, 1276.
[22] S. J. Leach and H. Lindley, *Trans. Faraday Soc.*, 1953, **49**, 921.
[23] T. Higuchi, L. Eberson, and A. K. Herd, *J. Amer. Chem. Soc.*, 1966, **88**, 3805.
[24] M. L. Bender, Y.-L. Chow, and F. Chloupek, *J. Amer. Chem. Soc.*, 1958, **80**, 5380.
[25] J. Brown, S. C. K. Su, and J. A. Schafer, *J. Amer. Chem. Soc.*, 1966, **88**, 4468.
[26] A. Bruylants and F. J. Kezdy, *Record Chem. Progr.*, 1960, **21**, 213.
[27] G. Dahlgren and N. L. Simmerman, *J. Phys. Chem.*, 1965, **69**, 3636.

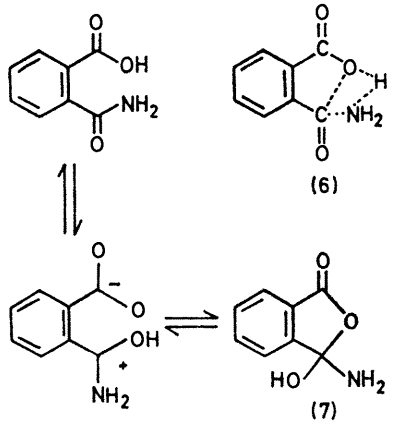
o-carboxyphthalimide (4), where attack by this mechanism is not possible.[28]



(3) (4) (5)

Schafer and his co-workers [25] found that cyclisation to *N*-methylphthalimide (5) is a significant side reaction in the hydrolysis of *N*-methylphthalamic acid under some conditions, but we find no evidence for the formation of *N*-alkylmaleimides during the hydrolysis of our *N*-alkylmaleamic acids. This negative conclusion is supported by our finding that *NN*-dimethylmaleamic acid is hydrolysed at almost the same rate ($4{\cdot}01 \pm 0{\cdot}05 \times 10^{-3}$ min^{-1}) as *N*-methylmaleamic acid ($3{\cdot}90 \pm 0{\cdot}01 \times 10^{-3}$ min^{-1}) under our conditions. Similar rates of hydrolysis for maleamic acid and its *N*-ethyl and *NN*-diethyl derivatives were also found by Dahlgren and Simmerman.[27]

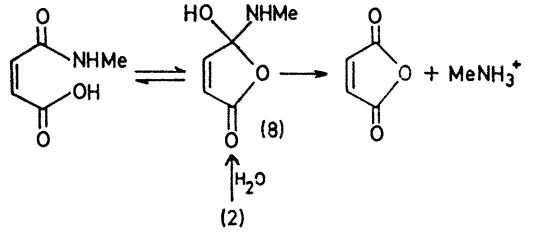
The mechanism of the hydrolysis is fairly well understood. Although Bender *et al.*[24] suggested an alternative one-step concerted electrophilic–nucleophilic catalysis mechanism (6) for the formation of the anhydride from phthalamic acid, there seems no good reason to prefer this to the simplest mechanism, in which an initial rapid transfer of the carboxy-proton to the amide group is followed by cyclisation to a neutral tetrahedral intermediate (7). We have tested this mechanism for the



(6) (7)

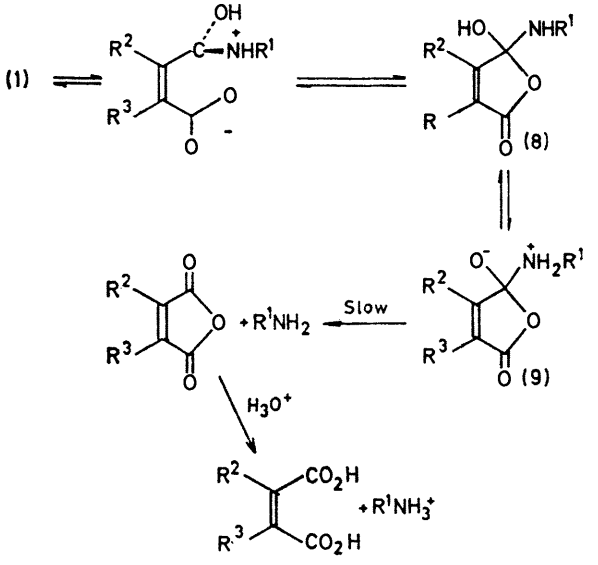
hydrolysis of *N*-methylmaleamic acid, generating the tetrahedral intermediate (8) independently, from *N*-methylisomaleimide (2). [We assume that the hydrolysis of (2) at low pH will involve the attack of water at the more electrophilic C=$^+$NHMe group, rather than at C=O. The disappearance of (2) in 1M-HCl is so fast that no change can be detected with conventional equipment. It is thus at least an order of magnitude faster than the hydrolysis of maleic anhydride, which has a half-life of 23 s between pH 0 and 2 at 39 °C.]

Under the conditions of our experiments the isomaleimide (2) is converted quantitatively into *N*-methylmaleamic acid, as it must be if the rate-determining step of the hydrolysis is the breakdown rather than



(8) (2)

the formation of the tetrahedral intermediate (8). So the detailed mechanism of hydrolysis of maleamic acids can be represented as in the Scheme. It is assumed that



SCHEME

the tetrahedral intermediate breaks down by way of a zwitterionic form (9). The proton transfers concerned are unlikely to be rate-determining,* so that the rate-determining step in the formation of the anhydride must be the elimination of the amine, as shown.

Structure and Reactivity.—It is well known that the equilibrium constant for the formation of the cyclic anhydride from an aliphatic dicarboxylic acid is profoundly affected by the pattern of substitution on the intervening carbon chain. Alkyl substituents favour the cyclic form, an example of a general phenomenon known as the Thorpe–Ingold effect.[30] The formation of

* Very recently we have found kinetic evidence for the existence of an intermediate in the conversion of a very reactive maleamic acid into the maleic anhydride, and conditions under which a proton transfer does appear to become rate-determining,[29] greatly strengthening the evidence for the mechanism shown in the Scheme.

[28] B. Zerner and M. L. Bender, *J. Amer. Chem. Soc.*, 1961, **83**, 2267.

[29] M. F. Aldersley, A. J. Kirby, and P. W. Lancaster, *J. C. S. Chem. Comm.*, 1972, 570.

[30] R. M. Beesley, C. K. Ingold, and J. F. Thorpe, *J. Chem. Soc.*, 1915, **107**, 1080; C. K. Ingold, *ibid.*, 1921, **119**, 205. For a general discussion see E. L. Eliel, ' Stereochemistry of Carbon Compounds,' McGraw-Hill, New York, 1962, p. 196.

anhydrides from half-esters and half-amides of dicarboxylic acids might be expected to be affected in the same way, and Higuchi and Eberson [23] have observed such effects in the hydrolysis of the various methylsuccinanilic acids. The rate of hydrolysis, and thus the efficiency of intramolecular catalysis by the carboxy-group, increases with the number of methyl substituents until tetramethylsuccinanilic acid is some 1200 times more reactive than the unsubstituted compound.

Similar effects have been observed by Bruice [8,31] and others [32] in intramolecular nucleophilic catalysis by the ionised carboxy-group of the hydrolysis of monoaryl glutarates and succinates. Bruice draws the general conclusion [4,8,33] that alkyl substitution decreases the populations of extended-chain conformations of these compounds, thus bringing the reacting groups into close proximity for more of the time. Consistent with this interpretation, the largest effects are observed in cases where the groups are held close together in conformationally rigid molecules, as in the maleate system; the increased rates result primarily from more favourable entropies of activation.

The effects that we observe are quite different. They come into play when the conformation is fixed, and they reflect exclusively changes in the enthalpy of activation. The activation entropies are actually less favourable for the more reactive maleamic acids, falling by some 20 cal mol^{-1} K^{-1} over the range of compounds measured and thus compensating in part for the enormous decrease of almost 19 kcal mol^{-1} in the enthalpy of activation (Table 4).

TABLE 4

Thermodynamic parameters for the hydrolysis of *N*-alkylmaleamic acids (1) at ionic strength 1·0

Compound (1)			Relative	$\Delta H^{\ddagger}$	$\Delta S_{39}^{\ddagger}$
R^3	R^2	R^1	rate (39 °C)	kcal mol^{-1}	cal mol^{-1} K^{-1}
H	H	Me	1·0	24·6 ± 0·2	2·9 ± 0·8
Me	H	Me	31·2	22·8 ± 0·2	2·1 ± 0·6
Et	H	Me	32·8		
Pr^i	H	Me	44·5		
Bu^t	H	Me	68·0	22·5 ± 0·2	2·7 ± 0·7
Cyclohex-1-ene-1,2-diyl		Me	540	20·5 ± 0·2	2·4 ± 0·7
Me	Me	Pr^n	23,500 [a]	14·8 ± 0·6	−8 ± 4
Cyclopent-1-ene-1,2-diyl		Me	$4·2 \times 10^{-5}$	33·9 ± 1·0	10·6 ± 3
Cyclobut-1-ene-1,2-diyl		Me	$4·2 \times 10^{-6}$ [b]		

[a] This figure includes a factor (1·35) to correct for the change from *N*-Me to *N*-Pr, based on the relative rates of hydrolysis of *N*-methyl- and *N*-n-propyl-citraconamic acid (Table 2). [b] Figure based on relative rates of hydrolysis of cyclopentene and cyclobutene compounds at 100 °C. This ratio would be greater at 39 °C.

The rates of hydrolysis of the monoalkylmaleamic acids increase with increasing size of substituent from Me to Bu^t, but there is no linear free-energy relationship between the rate and Taft's steric substituent constant.[34] The relationship fails because of the relatively small increase in reactivity caused by the introduction of the t-butyl group. There is however a good correlation with Taft's polar substituent constants [35] σ^*, with $\rho^* = -0·96$. This may be a genuine electronic effect, with the substituent acting almost exclusively on the adjacent carboxy-group, which is the nucleophilic centre in the reaction. Or the result may be coincidental: steric effects in this reaction are certainly qualitatively different from those on ester hydrolysis, and, with the exception of t-butyl, increasing size as measured by the steric substituent constant closely parallels the electron-donating ability of alkyl groups.

Whether the effect of a single alkyl group is steric or not, there is no doubt that the rate increase which results from the introduction of a second methyl group is far too large to be the result of an electronic effect. The dimethylmaleamic acid is hydrolysed some three orders of magnitude more rapidly than the monomethyl derivative (a small allowance being made for the change of leaving group from methylamine to n-propylamine) and the effect of the two methyl substituents together is to lower the enthalpy of activation for hydrolysis by some 10 kcal mol^{-1}. The simplest explanation of this result is that the steric compression which results from the accumulation of four coplanar substituents on a central double bond must raise the energy of the ground state relative to the transition state for cyclisation. In this picture the role of the alkyl groups of (1) is to act as buttresses, forcing the reacting groups into closer proximity. The effect would be expected to be reduced, therefore, if these groups were held back; as they would be, for example, as members of a small enough ring. So we were led to examine the series of cyclic maleamic acids listed in the Tables. For the cyclohexenyl compound the effect is in the expected direction, and the rate of hydrolysis shows a moderate decrease. But any further reduction in the size of the ring leads to a dramatic fall in the efficiency of catalysis: the cyclopentenyl compound is far less reactive than even the unsubstituted maleamic acid, and the effect of joining the two substituent alkyl groups directly, to form a four-membered ring, reduces the efficiency of catalysis by a factor of *ca.* 10^{10}. Presumably the reacting groups are actually pulled further apart in the smaller ring compounds, compared with the unsubstituted maleamic acid, so that the cyclic forms become destabilised relative to the ground states. This is in accord with Eberson's measurements [21] of equilibrium constants for the formation of substituted maleic anhydrides from the free acids. The equilibrium constant is *ca.* 5 for the formation of dimethylmaleic anhydride, but already too small to measure (0·1) for cyclohex-1-ene-1,2-dicarboxylic acid.[21] The anhydride from cyclobut-1-ene-1,2-

[31] T. C. Bruice and S. J. Benkovic, 'Bio-organic Mechanisms,' Benjamin, New York, 1966, vol. 1, p. 173; T. C. Bruice and W. C. Bradbury, *J. Amer. Chem. Soc.*, 1968, **90**, 3808.

[32] E. Gaetjens and H. Morawetz, *J. Amer. Chem. Soc.*, 1958, **80**, 2591.

[33] T. C. Bruice and U. K. Pandit, *J. Amer. Chem. Soc.*, 1960, **82**, 5858.

[34] R. W. Taft, jun., in M. S. Newman, 'Steric Effects in Organic Chemistry,' Wiley, New York, 1956, p. 598.

[35] Ref. 34, p. 591.

dicarboxylic acid has never been isolated and characterised. The effect is clearly principally on the free energies of the dicarboxylic acids, because the rates of hydrolysis of the anhydrides (Table 3 and ref. 21) are affected very little by ring formation or the nature of substituents on the double bond.

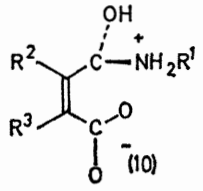
We have been at pains to measure the efficiency of catalysis by the neighbouring carboxy-group in the maleamic acid system. The usual comparison with the corresponding intermolecular reaction is not possible, because catalysis by carboxylic acids of simple amide hydrolysis cannot be detected. We followed the hydrolysis of *N*-methylacrylamide in 0·05 and 0·20M-formic acid buffers at 100 °C and pH 2·92 for over 4 months (about one half-life under these conditions), but could detect no difference in the rates of hydrolysis in the two cases. This allows an estimate of a maximum possible value (5×10^{-7} l mol^{-1} min^{-1} at 100 °C) for the second-order rate constant for catalysis by formic acid, but there is no way of knowing how close this figure is to the actual value. So we have to use the less meaningful comparison of rates of hydrolysis.

An ideal choice for a model compound suggested by our work would be a maleamic acid which showed no evidence of catalysis by the carboxy-group, but we could not find such a compound. Even the cyclobutenyl derivative shows a distinct pH-independent region near pH 2—3 (Figure 3), before external acid catalysis becomes significant, so we use as our standard compound *N*-methylacrylamide. Amide hydrolysis is very insensitive to the polar effects of substituents in dilute acid,[36] and although steric effects are generally important the diamides of maleic and fumaric acids are hydrolysed at very similar rates.[26] In fact, as can be seen from Figure 3, *N*-methylacrylamide is hydrolysed more rapidly than either the cyclobutenyl or cyclopentenyl acid amides at 100 °C in 1N-HCl. Its choice as a standard for comparison at least avoids the possibility of overestimating catalytic efficiency.

The rate constant for hydrolysis of *N*-methylacrylamide at 100 °C and ionic strength 1·0 is $4{\cdot}0 \times 10^{-6}$ min^{-1} at pH 3. Our cyclopentenyl compound is hydrolysed some 300 times more rapidly at 100 °C, and thus some two orders of magnitude more rapidly at 39 °C. We then estimate a rate enhancement, compared with *N*-methylacrylamide at 39 °C and pH 3, of 2×10^6 for maleamic acid itself (in the same region as the factor by which phthalamic acid hydrolysis is accelerated [24]). For our most reactive compound, dimethyl-*N*-n-propylmaleamic acid, the ratio is 4×10^{10}. The 'effective concentration' [37] of the carboxy-group is greater than 10^{10}M in this instance.

We are now engaged in a detailed examination of the structural factors associated with high reactivity in the maleamic acids. Since the system is conformationally rigid the structures of the ground states can be determined by static methods, and we are well advanced with *X*-ray crystallographic determination of three maleamic acids covering the complete range of reactivity.[17] Since relative reactivity in this system is determined principally by ground-state energies, as discussed above, the structural factors associated with high reactivity can in principle be identified by an examination of the three-dimensional structures of the ground states.

It seems clear that the step in the reaction most powerfully affected by structural changes is the (reversible) ring-closure of (10); since the rate-determining



step itself should be more sensitive to the nature of the leaving group, and because of the similarity of structural effects on the hydrolysis of (10) and on the equilibrium constants for the formation of the cyclic anhydrides from the corresponding maleic acids. The carbon skeleton of (10) should differ very little from that of the maleamic acid in the ground state, so it is a simple matter to calculate the structural features of principal interest. These appear to be the distance of the carboxylate oxygen from the central carbon atom of the amide group, to which it forms the new bond, and the most favourable angle of approach allowed by the geometry of the system. It is presumed that attack on the amide carbon takes place preferentially along a line perpendicular to the plane of the group,[24,28] which must therefore be rotated out of the plane of the central double bond of the maleamic acid, as shown in structure (10). A simple view would be that reaction should be fastest at any given favourable angle of approach when the attacking oxygen atom is closest to the amide carbon, as it is when the carboxylate group lies in the plane of the central double bond.

A preliminary examination of the structural problem by use of Dreiding models shows two significant trends. The closest distance of approach between the carboxy-oxygen and the amide carbon atom in the ground state is less for more reactive compounds, increasing from about 2·6 Å for the unsubstituted maleamic acid to 3·0 Å * for the cyclopentenyl and to 3·8 Å for the cyclobutenyl compound. The angle of approach of this oxygen can be within 10° of perpendicular for the maleamic acid, but widens to 15—20° * for the cyclopentenyl and to *ca.* 40° for the cyclobutenyl compound. Even crude measurements such as these are not possible for the alkylmaleamic acids, in which it seems likely that the reacting groups are forced together more closely still, and a detailed discussion must be deferred until three-dimensional structures are available for a representative

* These figures are close to those given by the preliminary *X*-ray diffraction results.

[36] P. D. Bolton, *Austral. J. Chem.*, 1966, **19**, 1013; P. D. Bolton and G. L. Jackson, *ibid.*, 1969, **22**, 527.

[37] Ref. 1, p. 10.

range of compounds. But from these preliminary results the chances seem good that we will be able to test at least two theories put forward to account for high reactivity in intramolecular and enzymic reactions. The orbital steering theory of Storm and Koshland [2] depends on a critical angle of approach, and one specific form of a strain theory involves the possibility [38] that some progress towards overcoming van der Waals repulsion may be made in the ground state. A simple calculation based on Hill's equation [39] suggests that van der Waals repulsion between the carboxylate oxygen and the amide carbon atom should increase rapidly as the internuclear distance falls below *ca.* 2·5 Å, a value which appears to be well within the bounds of possibility for our most reactive compound.

[1/2442 *Received*, 21*st December*, 1971]

[38] Ref. 1, p. 20.
[39] T. L. Hill, *J. Chem. Phys.*, 1948, **16**, 399.