Intramolecular Nucleophilic Catalysis of Anilide Hydrolysis by Pyrimidine Nitrogen

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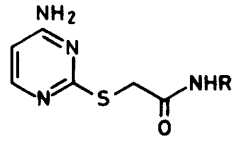
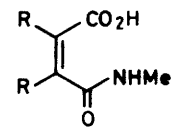
The neighbouring pyrimidine group of the conjugate acid of 2-(4-aminopyrimidin-2-ylthio)-2-methyl-*p*-nitropropananilide (**3**) is an effective nucleophilic catalyst for the hydrolysis of the *p*-nitroanilide function. The reactivity of pyrimidine nitrogen parallels that of the carboxy group, and the mechanisms of the reactions with neighbouring amide functions are similar in detail.

The amide (**1**; R = H) is hydrolysed extraordinarily rapidly at low pH (half-life *ca.* 1 h at 25 °C and pH < 3).[1] In a preliminary communication we showed[2] that this reaction involves intramolecular nucleophilic catalysis by pyrimidine nitrogen, and we were struck by the close similarities to reactions in which amides are hydrolysed with catalysis by neighbouring carboxy groups.[3] Specifically, the reaction of (**1**; R = Me) involves the protonated form of the catalytic group, the pK_a value apparent from the pH–rate profile differs from the true pK_a, and the reaction shows marked catalysis by formic acid–formate buffers: all properties observed also in the hydrolysis of *N*-methylmaleamic acids (**2**).[3]

The neighbouring carboxy group is a highly efficient nucleophilic catalyst not only for the hydrolysis of carboxylic esters and amides, but also for phosphate[4] and phosphonate[5] esters. The observation that pyrimidine nitrogen shows similar reactivity towards amides thus raises the intriguing possibility that it may be an efficient catalyst for the hydrolysis of phosphate esters also. We have therefore set out to characterise the nucleophilic reactivity of neighbouring pyrimidine nitrogen towards carboxylic acyl and phosphoryl centres. In this paper we describe the hydrolysis of the *p*-nitroanilide (**3**). A detailed study of buffer catalysis of the hydrolysis of (**1**; R = Me) presented problems, because the reaction could only be followed conveniently by n.m.r. We therefore turned to the *p*-nitroanilide (**1**; R = 4-nitrophenyl), since a detailed study of the hydrolysis of *p*-nitroanilides catalysed by the neighbouring carboxy group[6,7] is available for comparison. The hydrolysis of (**1**; R = 4-nitrophenyl) could be followed by changes in the u.v. spectrum in a region not masked by the strong pyrimidine chromophore, but proved inconveniently slow (half-life *ca.* 2 days at pH 3.5 and 39 °C). As for the carboxy-catalysed reaction,[6,7] the effect of increasing electron withdrawal in the departing amine is to slow the intramolecular reaction. The reaction of our chosen substrate (**3**) shows the same u.v. changes, but is up to 35 times faster, depending on the pH. This factor is a measure of the *gem*-dimethyl (Thorpe–Ingold) effect[8] on the cyclisation step.



(**1**)



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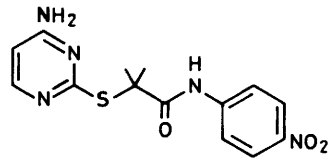


(**3**)

Experimental

Materials.—Inorganic reagents and buffer components were of analytical grade, and used without further purification. D_2O (Aldrich) was ≥99.6 atom % D. Amides (**1**) were prepared by the reaction of the anion of 2-thiocytosine with 2-halogenoacetamides.

2-(4-*Aminopyrimidin*-2-*ylthio*)-2-*methyl*-p-*nitropropananilide* (**3**).—Thiocytosine (508 mg, 4 mmol) was dissolved in boiling absolute ethanol (17 ml) containing an equivalent amount of sodium ethoxide. 2-Bromo-2-methyl-*p*-nitropropananilide (1.16 g, 4 mmol) in absolute ethanol (15 ml) was added, and the mixture refluxed for 15 min and set aside at room temperature overnight. Crystals (600 mg) separated, of the product mixed with unchanged 2-thiocytosine. Three recrystallisations from aqueous ethanol gave the p-*nitroanilide*, m.p. 209—209.5 °C (Found: C, 50.4; H, 5.2; N, 20.5. $C_{14}H_{15}N_5O_3S$ requires C, 50.4; H, 4.5; N, 21.0%).

Kinetic Measurements.—Rate constants were measured at 39.0 ± 0.1 °C, under pseudo-first-order conditions, in the thermostatted cell compartment of a Carl Zeiss PMQ3 or Gilford 2600 spectrophotometer. Reactions were initiated by injecting a solution of the anilide (**3**) in dimethyl sulphoxide into preheated aqueous buffer solutions (4 μl of 10^{-2}M-solution per ml), and followed by monitoring the increase of absorbance due to the release of *p*-nitroaniline at 380 nm or the decrease in absorbance at 320 nm (pH < 2). When both wavelengths were used for a given run, identical rate constants were obtained. After reaction was complete the spectra were as expected for an equimolar mixture of *p*-nitroaniline and a 2-alkylthiocytosine.

Ionic strength was made up to 1.0M with KCl, and the pH was measured at the end of each run in buffered solution, using an EIL 7050 pH meter equipped with a combination electrode, standardised at pH 4.04 and 6.97.

End-points were taken after at least ten half-lives, and the observed rate constants used for the buffer plots were corrected for the small changes in pH observed when buffer concentration is varied at a given buffer ratio. The pD of solutions in D_2O was obtained by adding 0.40 to the observed pH meter readings.

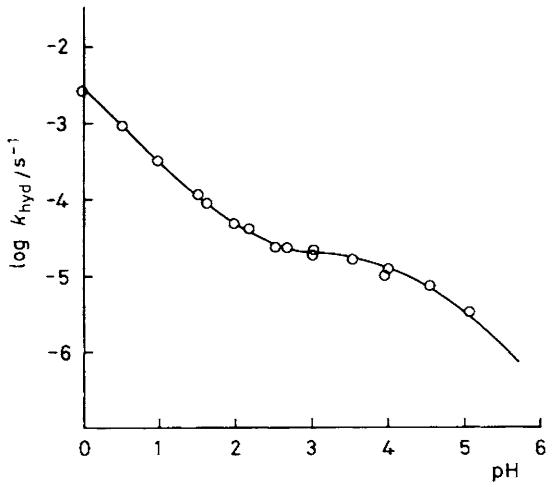


Figure 1. pH–Rate profile for the hydrolysis of the anilide (**3**) in water, at 39 °C and ionic strength 1.0M. The points are experimental, and above pH 2 mostly represent extrapolations to zero buffer concentration. The curve is calculated from equation (1)

Rate constants, and derived rate constants, were calculated by the least-squares method, and were reproducible to within ±3%.

pK_a *Measurements.*— The dissociation constant for the protonated anilide (**3**) was determined spectrophotometrically[9] in H_2O and D_2O under the conditions of the kinetic measurements [39 °C; ionic strength 1.0M (KCl)], using an analytical wavelength of 290 nm. The absorbances of the fully protonated and neutral compounds were measured in 3 × 10^{-3}M-HCl and 0.05M-Tris buffer (pH 8.60), respectively, and measurements were also made in the region of the pK_a in 0.1M-acetate, using six different buffer ratios. Under these conditions there was no significant change in the absorbance of the solutions during the first minute after mixing.

Results

The measured pK_a values of the anilide (**3**) were 4.37 ± 0.06 in H_2O and 4.99 ± 0.07 in D_2O.

Data for the hydrolysis of the anilide (**3**) are presented graphically in Figure 1. Rate constants for pH > 3 were obtained by extrapolation to zero buffer concentration, as described later. The pH–rate profile for hydrolysis is described by equation (1), with k_0 1.7 × 10^{-5} s^{-1}, k_H 3.0 × 10^{-3} dm^3 mol^{-1} s^{-1}, and pK_a 4.37 (measured directly).

$$k_{obs} = (k_0 + k_H[H^+])a_H/(a_H + k_a) \quad (1)$$

Rate constants measured in buffered solutions were normalised to a single pH value for each buffer ratio, and calculated in terms of the protonated form of the substrate, since the neutral species is unreactive (hydrolysis is too slow to be measured at pH > 6). When these derived rate constants were plotted against buffer concentration, points at high concentrations showed in most cases significant negative deviations (Figure 2). This appears to be a medium effect on the activity of the reactive form of the substrate, caused by the increasing concentration of organic acid in the solvent. It is clear that it is not due to an incipient change in rate-determining step, because the effect is not observed for catalysis by H_3O^+, nor phosphoric nor formic acid, and thus depends on the structure of the general acid concerned, rather than its reactivity as a catalyst.

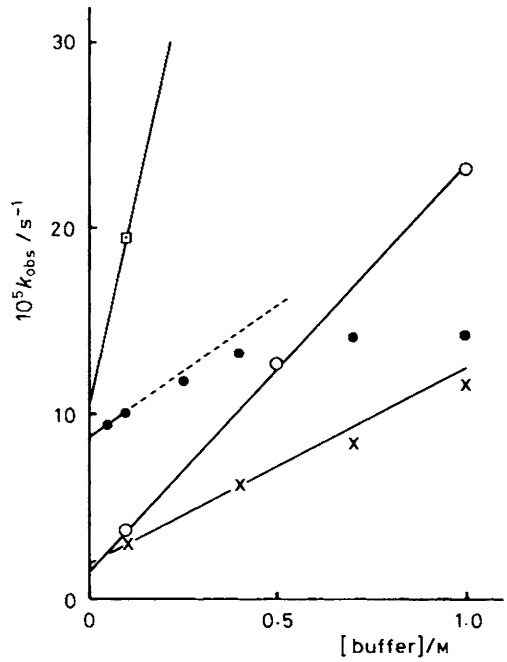


Figure 2. Buffer catalysis of the hydrolysis of the anilide (**3**) by phosphoric acid/phosphate (⊡), dichloroacetate (●), formate (⊙), and monochloroacetate (×) buffers, all at 50% free base. Increasing concentrations of dichloroacetic acid inhibit the reaction strongly in dilute HCl

This analysis was confirmed by measuring the effect of increasing concentrations of the buffer acids on the rate of hydrolysis of the anilide (**3**) in 0.05M-HCl. All the organic acids involved, except formic acid, caused inhibition, measured as k_i, the negative slope of the plot of k_{obs} *vs.* [HA]. These inhibition constants were used (as described previously[10]) to correct the buffer catalysis data for the inhibitory effect of the HA present, according to equation (2). This treatment reduced, and in some cases eliminated, the curvature. Where curvature remained, the second-order rate constants were obtained from the initial slopes of these plots. In a few cases, for the strongest acids at high % HA (*e.g.* 80% dichloroacetic acid), catalysis was negligible, or inhibition was observed. Such data were not used.

$$k_{corr} = k_{obs}/(1 + k_i[HA]/k_0) \quad (2)$$

The full set of data for buffer catalysis is given in the Table. In most cases catalysis by both the acid and the basic component of the buffer is significant, with the second-order rate constants for general acid and general base catalysis decreasing and increasing, respectively, with increasing pK_a of the acid form. The rate constants are correlated acceptably by the Brønsted equation, with coefficients $\alpha = 0.31 \pm 0.05$ for general acid catalysis, and $\beta = 0.54 \pm 0.03$ for general base catalysis (Figure 3). The points for $H_2PO_4^-$ showed significant positive deviations from the Brønsted lines, as frequently observed for catalysis of acyl-transfer reactions, but those for H_3O^+ and H_2O fall on lines through the data points for other general acids and bases.

Discussion

The main outlines of the mechanism of hydrolysis of the anilide (**3**) are clear. Hydrolysis is rapid as compared with the reaction of a simple *p*-nitroanilide (*p*-nitroacetanilide,[11] with an unhindered carbonyl group, is hydrolysed at least 10^4 times more

Table. Buffer catalysis data for the hydrolysis of the anilide (**3**) at 39 °C and ionic strength 1.0M

Buffer acid	pK_a[a]	Concn. range (M)	Runs	%BH^+	pH(pD)	10^5k_2/dm^3 mol^{-1} s^{-1}[b]	10^5k_B/dm^3 mol^{-1} s^{-1}	$10^5k_{BH^+}$/dm^3 mol^{-1} s^{-1}[b]	k_i/k_0
H_3O^+	−1.74						0.031[c]	301[d]	
H_3PO_4	2.17	0.1—1.0	3	70	1.88	101 ± 5	117 ± 25	95.8 ± 2.7	
		0.1—1.0	3	50	2.17	109 ± 6			
		0.1—1.0	3	25	2.53	111 ± 3			
Cl_2CHCO_2H	1.65	0.1—0.7	3	80	1.34	*e*	(0.35 ± 0.41)	34.9 ± 1.1	−0.62
		0.5—1.0	6	50	1.65	17.4			
		0.1—0.7	3	25	1.95	9.6			
		0.05—1.0	3	10	2.43	3.4			
$ClCH_2CO_2H$	2.86	0.1—0.7	3	80	2.20	9.4	7.83	14.6	−0.24
		0.1—1.0	4	50	2.68	10.4			
		0.1—0.7	3	30	3.08	9.4			
HCO_2H	3.77	0.1—1.0	3	75	3.02	13.8	47.4 ± 0.7	3.03 ± 0.67	
		0.1—1.0	3	50	3.55	25.9			
		0.1—1.0	3	25	4.02	36.0			
CH_3CO_2H	4.76	0.1—1.0	3	80	3.97	23.1	96.2 ± 1.5	3.93 ± 1.38	−0.28
		0.1—1.0	3	50	4.57	48.5			
		0.1—1.0	3	25	5.07	74.0			
CH_3CO_2H in D_2O		0.1—1.0	4	75	(4.70)	15.7	55.3 ± 0.4	2.59 ± 0.34	−0.28
		0.1—1.0	4	50	(5.16)	29.2			
		0.1—1.0	4	41	(5.35)	33.5			
KH_2PO_4	7.21	0.08—0.80	3	90	5.56	31 ± 8	1 780	156	
		0.06—0.60	3	70	6.21	643 ± 14			

[a] From W. P. Jencks and J. Regenstein, 'Handbook of Biochemistry and Molecular Biology,' ed. G. D. Fasman, CRC Press, Cleveland, Ohio, 3rd edn., 1976, vol. 1, p. 305. [b] Corrected values, calculated in terms of substrate conjugate acid, as described in the text. [c] $k_0/55.5$. [d] Value of k_H used in calculating pH–rate profile (Figure 1). [e] Inhibition observed.

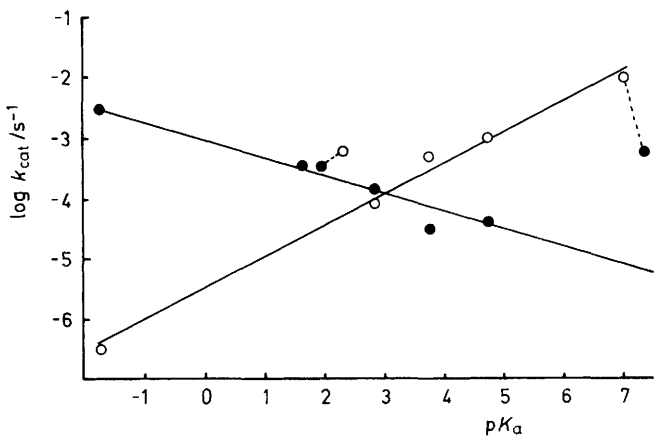
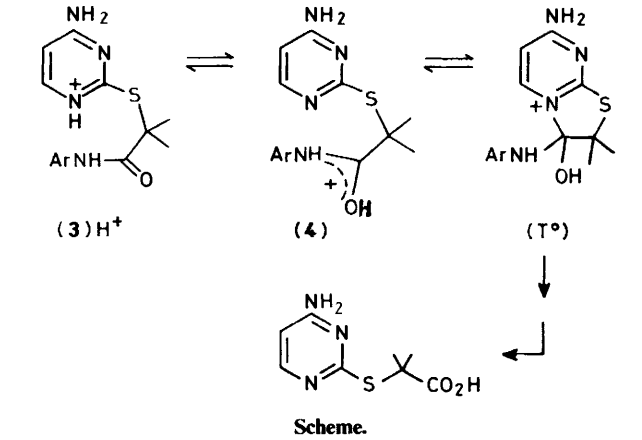


Figure 3. Brønsted plots of the data for general acid (●) and general base (○) catalysis (Table). Data for phosphoric acid–phosphate buffers (points linked by dashed lines) have been statistically corrected according to R. P. Bell, 'The Proton in Chemistry,' Chapman and Hall, London, 2nd edn., 1973, p. 198. Note the positive deviations for general base, and especially general acid catalysis by $H_2PO_4^-$. This is a common observation, explained in terms of bifunctional catalysis by the ambient ion of the breakdown of species such as (T°). This requires $H_2PO_4^-$ to act simultaneously as a general acid and a general base, and the statistically corrected rate constants for its general acid- and general base-catalysed reactions are indeed identical, within experimental error

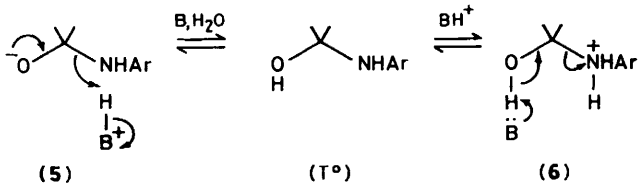
slowly at pH 4) and depends on the acid form of a group of pK_a 4.37, the same as that measured for the protonation of the pyrimidine ring. The efficiency of catalysis is lower than for the *N*-methylamide (**1**; R = Me), because the introduction of the *p*-nitrophenyl group reduces the rate of the intramolecular reaction, whereas it *increases* the rate of the acid-catalysed reaction of an acetamide or acetanilide;[11] but it is still too large to account for the reaction in terms of intramolecular general species catalysis.[8b] Such mechanisms are also incompatible with the observation of catalysis by external general acids and bases. So a nucleophilic mechanism must be involved.

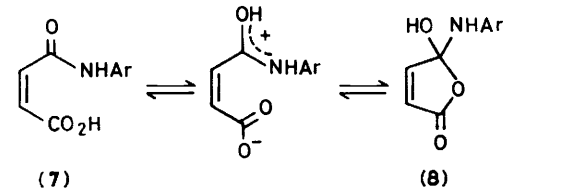
The likely mechanism is outlined in the Scheme. The equilibrium constant for the pre-equilibrium formation of (**4**) will be in the region of 10^{-6} [the pK_a of the conjugate acid of *p*-nitroacetanilide is about −2 (ref. 6)], and the cyclisation of (**4**) to (T°) will be rapid. The observation of general acid and general base catalysis is consistent with the breakdown of (T°), rather than its formation, being rate-determining.

The non-integral values observed for the Brønsted exponents for general acid and general base catalysis ($\alpha = 0.31$ and $\beta = 0.54$, respectively) indicate that the proton transfers are concerted with the breaking of bonds to heavy-atom centres in both cases. Neither this evidence, nor the solvent deuterium isotope effects [$k(H_2O)/k(D_2O) = 1.16$ and 1.21 in 1M- and 0.03M-HCl, respectively, 1.5 ± 0.7 for general acid catalysis by acetic acid, and 1.74 ± 0.04 for general base catalysis by acetate] allow an unequivocal assignment of mechanism for the breakdown of (T°). But the evidence that electron-withdrawing substituents on nitrogen *reduce* the rate of the spontaneous (water-catalysed) reaction of the anilide (**3**) shows that *N*-protonation is further advanced than C–N bond-breaking in the transition state, and is thus inconsistent with classical general base catalysis of the breakdown of (T°). The kinetically equivalent specific base–general acid mechanism [see (**5**)] is thus preferred. This in turn allows a choice of mechanism for general acid catalysis, since the observed Brønsted α (0.31) is *smaller* than the value (1 − 0.54 = 0.46) calculated for the mechanism shown in (**5**), thus ruling out simple general acid catalysis of the breakdown of (T°), which would require proton transfer to



Scheme.





nitrogen to be further advanced (higher α) than for the reaction (**5**) of the conjugate base. The preferred mechanism for general acid catalysis is thus as shown in (**6**).

The reactivity of neighbouring pyrimidine in the anilide (**3**), and the mechanism by which it acts, have remarkably close parallels in reactions catalysed by the neighbouring carboxy group. The pH–rate profile for the hydrolysis of 4-nitromaleanilic acid (**7**), for example, is qualitatively closely similar[6] to that observed for (**3**), and the rate constants for the water-catalysed reactions of the free acids differ by a factor of less than two. Both reactions are subject to general acid catalysis by external general acids, and Kluger and Lam[6] draw a tentative Brønsted line of slope *ca.* 0.4, from which the point for phosphoric acid shows a marked positive deviation. General base catalysis is not well defined for this substrate, but becomes more important than general acid catalysis for the otherwise similar reaction of 4-nitronorbornenylanilic acid,[7] where the carboxy group has a pK_a (4.8) closer to that of the pyridine ring of (**3**).

Thus the similarity we noted previously[2] between the reactivities of neighbouring pyrimidine and carboxy groups towards CONHMe is confirmed in detail for the hydrolysis of *p*-nitroanilides. In both cases the nucleophilic centre of the catalytic group is weakly basic enough to coexist with significant concentrations of the form [(**4**), (**8**)] with the amide group protonated, thus giving access to addition intermediates (T°) on the nucleophilic pathway. The subsequent breakdown of the tetrahedral intermediates also appears to follow similar pathways. There is thus a strong presumption that neighbouring pyrimidine nitrogen will be an efficient intramolecular nucleophilic catalyst for the hydrolysis of other systems known to be susceptible to catalysis by the neighbouring carboxy group, and we are currently investigating such reactions.

Acknowledgements

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