Keto–Enol Tautomerism of Phenacylpyrazine: Acid Catalysis with Protonation at Nitrogen

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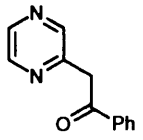
Kinetic and equilibrium measurements for ionisation and enolisation of 2-phenacylpyrazine in aqueous solution at 25 °C yield a tautomeric constant $pK_E = 2.05$ (where K_E = [enol]/[ketone]) and pK_as for loss of a methylene proton and for protonation at nitrogen of 11.90 and 0.40, respectively. In contrast to 2-phenacylpyridine the low basicity of the pyrazine nitrogens renders an enaminone tautomer less stable than the enol and a value of $pK_M = 4.4$ (K_M = [enaminone]/[ketone]) is estimated for this equilibrium. Evidence is presented that acid catalysis of keto–enol tautomerism occurs with protonation at the N-1 nitrogen atom rather than carbonyl group (or N-4 nitrogen) despite the proton being bound to oxygen in the enolic product. This preference reflects relative magnitudes of binding constants ($1/K_a$) and activating factors (PAF) for protonation at the different positions. Brønsted and Marcus equations are used to express catalytic efficiency in terms of equilibrium constants for binding the catalyst to the reactant and products of the uncatalysed reaction. The form of catalysis observed, which reflects the influence of proton binding on the activation energy of the reaction, is contrasted with that in intramolecular or enzymatic reactions, which normally derives from approximation of the reactants and is entropic in origin. The significance of optimum binding of the catalyst to the transition state in the two cases is briefly compared.

α-Heterocyclic ketones are subject to keto–enol and imine–enamine tautomerism.[1] In aqueous solutions the dominant tautomerism for 2- and 4-phenacyl-pyridines and -quinolines is imine–enamine with the prototropic hydrogen located at a ring nitrogen or α-carbon atom.[2–6] In this paper we show that for phenacylpyrazine (**1**, KH) by contrast the enol tautomer (**2**, EH) is favoured over the enamine (**3**, MH).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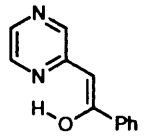
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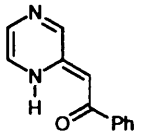
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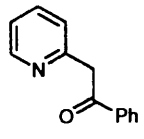
Pyrazine is a weakly basic heterocycle ($pK_a = 0.4$)[7] and phenacylpyrazine shows characteristics of a β-diketone, including a high enol content, low pK_a for enolate anion formation and susceptibility to metal-ion complexation and catalysis of tautomerisation.[8,9] The low pK_a assists kinetic measurements, since a solution of enolate anion in dilute sodium hydroxide may be quenched in acidic or buffer solutions to generate the enol, relaxation of which to the more stable ketone can be monitored spectrophotometrically.[5] Rate constants for ketonisation may then be combined with rate constants for enolisation, measured by iodine trapping, to yield a keto–enol equilibrium constant.[5,6] These measurements are summarised in this paper and form part of continuing studies of acid–base and metal-ion catalysed reactions of heterocyclic ketones.[4–6,9–12]

A question highlighted by the greater stability of the enol than enamine for phenacylpyrazine is whether acid-catalysis of tautomerisation occurs, as it does for simple ketones, with protonation on oxygen[13] or, as for phenacyl pyridines and quinolines, which tautomerise to enaminones with protonation on nitrogen.[5] Protonation on oxygen offers a more economical pathway for enolisation in so far as it avoids the enaminone as an intermediate but it may be energetically less favourable. This question has been addressed in a preliminary communication of part of this work,[14] and is dealt with more fully here.

Results

UV Spectra and Identity of Tautomers.—As discussed in detail by Levine and co-workers, the UV spectrum of phenacylpyrazine in water corresponds to a superposition of the spectra for pyrazine and acetophenone, indicating that it is present principally as its keto tautomer (**1**).[8] In chloroform or dioxane solutions however, a longer wavelength absorption is observed with λ_{max} = 362 nm. When a few microlitres of the dioxane solution are injected into water the same spectrum is seen initially, but in a short time it is transformed into the spectrum of the ketone.

Measurements of δ_C chemical shifts for the proacyl carbon atom indicates that the species present in dioxane and chloroform is the enol.[10] The identity of the UV spectrum in dioxane and transient spectrum in water indicates that the enol is the principal *minor* tautomer in water and is thus more stable than the enaminone. Had the enaminone been more stable it would have been formed rapidly from the enol by proton transfer between oxygen and nitrogen atoms. For phenacylpyridine the keto tautomer (**4**) also predominates in water and the enol in dioxane (λ_{max} = 344 nm), but in water the appearance of a weak long-wavelength absorption (λ_{max} = 400 nm) indicates that the principal minor tautomer is not the enol but the enaminone (**5**). This is confirmed by the similarity of the absorption to that of the corresponding *N*-methyl enaminone.[3]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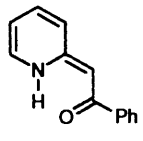
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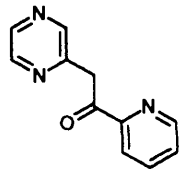
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For phenacylpyrazine, there is no sign of a longer wavelength absorption accompanying the transient enol spectrum in water implying that the ratio of enol to enaminone concentrations is > 10. We have not been able to prepare an *N*-methyl derivative

Table 1 Principal UV absorption maxima (λ_{max}/nm) for 2-phenacyl- and 2-pyridacyl-pyrazines (Pz) and -pyridines (Py) in aqueous solution[a] assigned to enol and enamine tautomers and enolate anions[b]

	$PzCH_2COPh$	$PzCH_2COPy$	$PyCH_2COPh$	$PyCH_2COPy$
Neutral	—	440	400	400
	362[c]	355[d]	344[e]	342[f]
Anion[g]	387	386	350	358

[a] Except as indicated. [b] From present work and refs. 3, 6 and 11. [c] H_2O; in $CHCl_3$, λ_{max} = 366 nm, ε = 18 200 $dm^3\ mol^{-1}\ cm^{-1}$. [d] H_2O; in $CHCl_3$, λ_{max} = 360 nm. [e] Cyclohexane. [f] $CHCl_3$. [g] Aqueous NaOH solutions.

Table 2 Slopes (k) and intercepts (k_o) of plots of first-order rate constants against buffer acid concentration ([AH]) at 25 °C and constant buffer ratio (R = $[A^-]/[AH]$), and rate constants for general-acid (k_{GA}) and general-base (k_{GB}) catalysis for ketonisation of 2-phenacylpyrazine enol in aqueous solution at ionic strength 0.1 mol dm^{-3} (NaCl)

Buffer acid	R[a]	pH	k/$dm^3\ mol^{-1}\ s^{-1}$[b]	$k_o/10^{-2}\ s^{-1}$	k_{GA}[c]	k_{GB}[c]
Hydronium ion			300		300	
Dichloroacetic	4.0	2.50	630		630	
Cyanoacetic	0.25	2.28	120		120	
Chloroacetic	0.25	2.40	118		118	
Glycolic	0.25	3.15	20.4	1.06	20.4	
Acetic	0.25	4.08	5.70	0.46	5.43	1.54
Acetic	1.0	4.61	7.44			
2,6-Lutidinium	4.0	5.20	10.70			
2,6-Lutidinium	0.25	6.25	4.70			19.0
2,6-Lutidinium	1.0	6.86	19.3			
Imidazolium	0.25	6.50	9.18	0.37		37.8
Imidazolium	4.0	7.60	38.6	0.71		
Phenol	0.33	9.70	1.29×10^4			4.30×10^3
H_2O		7.0		0.45[d]		4.20×10^4[e]

[a] Buffer ratio [base]/[acid]. [b] Slope of plot of k_{obs} *vs.* [acid]. [c] Second-order rate constants with units $dm^3\ mol^{-1}\ s^{-1}$. [d] Measurement in unbuffered aqueous solution. [e] From pH-profile (pH 7–10).

of 2-phenacylpyrazine to establish its UV spectrum. However, in the case of pyridacylpyrazine (**6**), which also exists predominantly in the enol form in dioxane and chloroform solutions, the transient spectrum observed following injection of a dioxane solution into water shows *two* peaks, at 355 nm and 440 nm, and we infer that the latter represents the enamine tautomer.[15] That the enamine tautomer of pyridacylpyrazine provides a satisfactory model for the enamine of phenacylpyrazine is confirmed by the similarity of measured enaminone spectra for phenacylpyridine and 2-(2-pyridacyl)pyridine for which short and long wavelength absorptions attributable to enol and enamine are also observable.[15] Values of λ_{max} for these compounds are compared in Table 1. This table also shows λ_{max} values for the enolate anions which show a consistent hypsochromic shift of *ca.* 20 nm with respect to the enols.

Equilibrium and Kinetic Measurements.—A pK_a for proton loss from phenacylpyrazine (pK_a^{KH}) was measured spectrophotometrically in dilute NaOH solutions as 11.77 at ionic strength 0.1 mol dm^{-3} (NaCl). This yields a thermodynamic value of 11.90, which compares with a value of 12 reported previously in 50/50 v/v water–dioxane.[8] A pK_a for protonation was measured in dilute $HClO_4$ solutions as 0.4, again spectrophotometrically. In this case the pK_a is an apparent value since the relative extents of protonation of the two ring nitrogen atoms is unknown.

Rates of ketonisation of phenacylpyrazine enol were measured spectrophotometrically by stopped flow. The reaction is subject to general-acid and general-base catalysis with the former dominant at pH below 5 and the latter above. For acetic acid buffers both acid and base catalysis were observed and first-order rate constants k_{obs} are described by eqn. (1) in which k_o includes contributions from catalysis by H^+ and HO^- and k_{GA} and k_{GB} are the rate constants for the buffer acid and buffer base.

$$k_{obs} = k_o + k_{GA}[AH] + k_{GB}[A^-] \quad (1)$$

Values of k_o and slopes k of plots of k_{obs} against buffer base concentrations at constant buffer ratio ($R = [AH]/[A^-]$) together with values of k_{GA} and k_{GB} are listed for carboxylic acid, phenol and imidazole buffers in Table 2. Where catalysis by both buffer species is significant values of k_{GA} and k_{GB} have been factorised from measurements of k at different buffer ratios. Also in Table 2 is a rate constant ($k_{obs} = 4.5 \times 10^{-3}\ s^{-1}$) for unbuffered ketonisation measured by injecting a few microlitres of the dioxane solution of the enol into pure water. Observed rate constants for reaction in dilute solutions of strong acids or sodium hydroxide, together with values from borate buffers corrected for very weak catalysis by the borate anion, are given in Table 3. The values in sodium hydroxide were measured from the reaction of the keto tautomer to form the enolate anion.

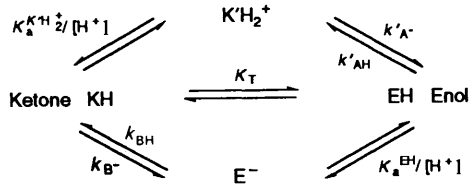
The observation of general acid–base catalysis of tautomerisation is consistent with the usual mechanistic interpretation of keto–enol tautomerisation shown in Scheme 1 where KH, EH, KH_2^+, and E^- denote the ketone and enol and their conjugate acid (*O*-protonated) and base, respectively, and the rate constants k_{AH}, k_{BH}, k_A^- and k_B^- refer, respectively, to attack of buffer acid on enol and enolate anion and of buffer base on protonated and unprotonated ketone. $K_E = [EH]/[KH]$ is the equilibrium constant for tautomerisation and $K'_a{}^{KH_2^+}$ and $K_a{}^{EH}$ are ionisation constants for protonation of the ketone and proton loss from the enol. Experimentally derived rate constants may be expressed in terms of the 'molecular' rate and equilibrium constants of Scheme 1 as $k'_{AH} = k_{GA}$, $k'_{A^-} = k_{GA}K_a/K_a^{KH_2^+}K_E$, $k_{BH} = k_{GB}K_a/K_a^{EH}$ and $k_B^- = k_{GB}K_E$.

For phenacylpyrazine, however, while it is safe to suppose that the base-catalysed pathway is correctly represented by Scheme 1, the acid-catalysed reaction probably occurs with protonation not on the keto oxygen but on a ring nitrogen atom, leading to reaction *via* an enaminone intermediate MH (**3**) as in

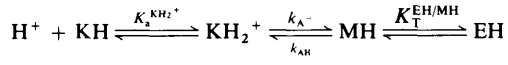
Table 3 First-order rate constants for ketonisation[a] of 2-phenacylpyrazine in aqueous solutions of HCl, NaOH and borate buffers (ionic strength 0.1 mol dm^{-3}) at 25 °C

$[H^+]$	k_{obs}/s^{-1}	$[OH^-]$/mol dm^{-3}	k_{obs}/s^{-1} [b]	pH[c]	k_{obs}/s^{-1} [c]
0.0025	0.923	0.010	6.53	8.85	0.286
0.005	1.95	0.005	4.63	8.97	0.378
0.010	4.24	0.0025	3.83	9.14	0.530
0.015	5.48			9.36	0.922
0.020	6.32			9.37	0.887
0.030	8.78			9.37	0.911
0.040	11.7			9.65	1.63
0.050	13.7			9.84	2.00
0.060	16.5			9.86	2.11
				9.99	2.11
				10.2	2.50
				11.00	4.29[d]

[a] Except as indicated in footnote *b*. [b] Reaction of keto tautomer to form enolate anion. [c] Borate buffers corrected for borate catalysis. [d] Bicarbonate buffer.



Scheme 1



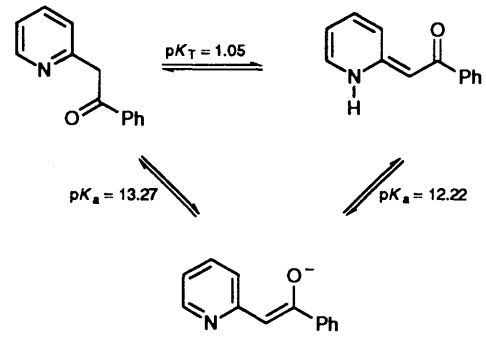
Scheme 2

Scheme 2.[14] In this case $k_{AH} = k_{GA}k_T^{EH/MH}$ and $k_A^- = k_{GA}K_EK_a^{KH_2^+}/K_a$ where $K_T^{EH/MH}$ is the tautomeric constant for conversion of enamine into enol and the molecular rate constant k_{AH} could not be obtained without knowing this value. Moreover, evaluation of molecular rate constants for the base reaction requires a knowledge of the ionisation constant of the unstable enol tautomer K_a^{EH} which, as implied by the lower of the thermodynamic cycles of Scheme 1, may be obtained by combining the directly measured ionisation constant of the stable keto tautomer K_a^{KH} with the tautomeric constant for conversion of ketone into enol, *i.e.*, $K_a^{EH} = K_a^{KH}K_E$.

The equilibrium constant for keto–enol tautomerism K_E was found by combining rate constants for ketonisation with rate constants for the reverse enolisation, measured by trapping enol (or enolate ion) with iodine under zero-order conditions. Iodination measurements in 1:1 acetic acid buffers gave $k = 6.67 \times 10^{-2}$ dm^3 mol^{-1} s^{-1} as the slope of a plot of first-order rate constants against buffer acid concentration in 1:1 acetic acid buffers. Combining this with the corresponding value of $k = 7.74$ for ketonisation from Table 2 gives from the ratio of values $K_E = 8.9 \times 10^{-3}$ and $pK_E = 2.05$ for the keto–enol tautomeric constant.

No measurement was made of the enamine–enol tautomeric constant, but a value for this could be estimated from a correlation of ionisation constants of correspondingly substituted pyrazines and pyridines described by eqn. (2).[16] Thus from measured equilibrium constants for ionisation of 2-phenacylpyridine in base ($pK_a = 13.27$) and tautomerisation to its enaminone tautomer ($pK_T^{MH} = 1.05$) the pK_a for proton loss from the enaminone may be obtained as $pK_a = 12.22$ making use of the thermodynamic cycle of Scheme 3. Substituting this value in eqn. (2) yields $pK_a = 7.5$ for ionisation of the corresponding enaminone of 2-phenacylpyrazine. Combining this value in turn with $pK_a = 11.90$ for ionisation of the keto form of phenacylpyrazine gives $pK_M = 4.4$ (11.90–7.5) for the keto–enamine tautomeric constant of phenacylpyrazine (K_M = [MH]/[KH]) based on a similar thermodynamic cycle to that of Scheme 3. Finally from $pK_E = 2.05$ for keto–enol

$$pK_a\,(\text{pyrazine}) = -3.6 + 0.9\,pK_a\,(\text{pyridine}) \quad (2)$$

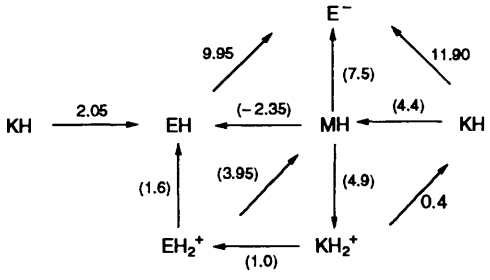


Scheme 3

tautomerism of phenacylpyrazine we obtain $pK_T^{EH/MH} = -2.3$ for the enol–enamine tautomerism; *i.e.*, the concentration of enaminone at equilibrium is less than 1% of that of the enol.

We are now in a position to evaluate the molecular rate constants from Schemes 2 and 3 in the manner described above. These rate constants are shown in Table 4 with values based on the estimate of $pK_T^{EH/MH} = -2.3$ given in parentheses. Molecular rate constants are shown for H^+, OH^- and H_2O as well as for buffer acids and bases.

The tautomeric and ionisation constants associated with phenacylpyrazine are summarised as pK values ($-\log K$) in Scheme 4. The scheme is similar to that used previously to



Scheme 4

represent the corresponding equilibrium constants for isomeric phenacyl pyridines.[11] Ionisation constants (pK_a) are written vertically and tautomeric constants (pK_T) horizontally. At the apex of the diagram is the enolate anion, which is the conjugate base common to the three phenacylpyrazine tautomers. Values of tautomeric and ionisation constants involving the pK_a for the enaminone tautomer estimated from eqn. (2) are again shown in parentheses. The ionisation constants for *N*-protonation to yield EH_2^+ and KH_2^+ are apparent values including protonation of both nitrogen atoms of the pyrazine ring. However, protonation of the nitrogen remote from the side chain is expected to predominate.

Brønsted Relationship.—Rate constants for general-acid and general-base catalysed ketonisation of phenacylpyrazine are plotted in Fig. 1 as $\log k$ *versus* pK_a. A best straight line is drawn through the points for catalysis by the carboxylic acids to give a Brønsted relationship with $\log k = 3.70 + 0.60 \log K_a$. The slope of this line corresponds to a Brønsted exponent $\alpha = 0.60$, which is similar to that for the previously studied 2-

Table 4 Rate constants[a] ($dm^3\ mol^{-1}\ s^{-1}$) for reaction of 2-phenacylpyrazine its enaminone and its conjugate acid and base with oxygen and nitrogen acids and bases in aqueous solution at 25 °C and ionic strength 0.1 mol dm^{-3}

Acid (AH or BH)[b]	pK_a	k_{AH}[c]	k_{A^-}[d]	k_{BH}[e]	k_{B^-}[f]
H_3O^+	−1.74	(6.72×10^4)	0.85/55.5		
$Cl_2CHCOOH$	1.29	(1.91×10^5)	43.6		
$NCCH_2COOH$	2.43	(2.69×10^4)	115		
$ClCH_2COOH$	2.86	(2.64×10^4)	303		
$HOCH_2COOH$	3.83	(4.57×10^4)	490		
CH_3COOH	4.76	(1.22×10^3)	1108	1.89×10^5	0.0137
2,6-Lutidine	6.77			2.88×10^4	0.169
Imidazolium ion	7.20			1.57×10^4	0.336
Phenol	9.95			3.42×10^3	38.3
H_2O	15.74			3.0	3.8×10^2

[a] Units $dm^3\ mol^{-1}\ s^{-1}$. [b] AH and BH denote the same acid and are used in subscripts to distinguish reaction with enaminone (AH) and enolate anion (BH); A^- and B^- represent the corresponding conjugate base in the reverse reactions. [c] For reaction of acid with enaminone the parentheses indicate that values are based on an estimate of the enol–enamine tautomeric constant (K_T = [EH]/[MH] = 225). [d] For reaction of the base with N-1 protonated ketones assuming pK_a = 0.4, the measured apparent pK_a for N-1 and N-3 protonation. [e] For reaction of acid with enolate anion. [f] For reaction of base with ketone.

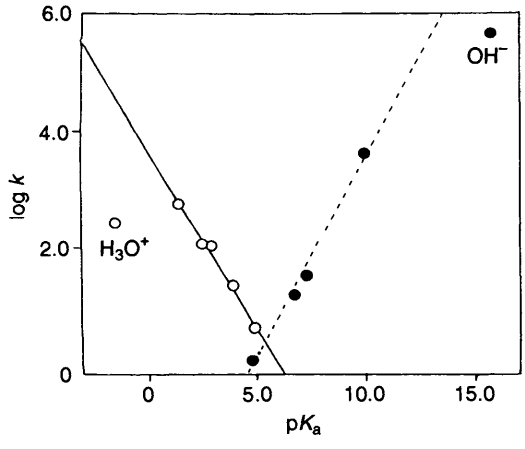


Fig. 1 Brønsted plot of log k *versus* pK_a for ketonisation of 2-phenacylpyrazine enol catalysed by acids (○) and bases (●)

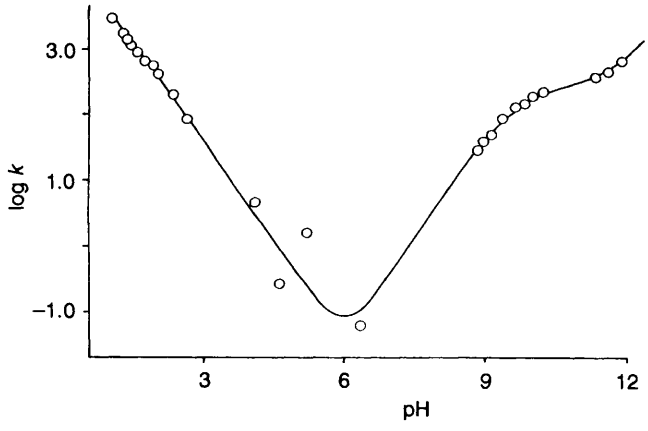


Fig. 2 log k–pH profile for ketonisation of 2-phenacylpyrazine enol

phenacylquinoline (α = 0.56). As usual α may be interpreted as the degree of proton transfer from the carboxylic acid to the carbon base at the transition state.[17]

A Brønsted plot for base catalysis is less well defined than for acid catalysis because of the wide variation in structure of the reacting bases. However, an approximate value of β = 0.7 is obtained from the slope of the dashed line (log k = −3.3 + 0.7 pK_a) shown in the figure. This value of β may be converted into a value of $\alpha = 1 - \beta = 0.3$ for protonation of the enolate anion. The lower value of α here than for protonation of the enamine in the acid reaction is consistent with the greater reactivity of the enolate ion.

Surprisingly β = 0.70 measured here is quite different from β = 0.44 found for ketonisation of the enamine of 2-phenacylquinoline. However, this is due at least in part to the use of a different family of bases, namely substituted trimethylamines (XCH_2NME_2) for the reaction of 2-phenacylquinoline. The negative deviations of H_3O^+ and HO^- in Fig. 1 are large but normal.

pH-Profile.—A pH-profile for the ketonisation reaction may be constructed by plotting values of k_o from Table 2 and rate constants for reaction in H^+ or OH^- solutions as log k against pH as shown in Fig. 2. Empirically, these rate constants correspond to relaxations of the 'quenched' enolate anion. Ionisation of the keto tautomer to this anion thus appears as an inflection at high pH (11.8) and a further inflection represents ionisation of the enol tautomer as the reactant changes from enol to enolate anion (at pH > 9.5). The line drawn through the points in the figure is based on eqn. (3) in which k_1^{OH} and k_1^H are rate constants for H^+- and OH^--catalysed ketonisation (corresponding formally to k_{GA} and k_{GB} for these ions) and k_{-1}^{OH} is the rate constant for the reverse hydroxide-ion catalysed enolisation reaction. Values of k_1^H = 300 and $k_1^{OH} = 4.2 \times 10^4$ were chosen to give a best fit to the plot, K_a^{KH} was taken as the independently measured equilibrium value (pK_a^{KH} = 11.90) and k_{-1}^{OH} was taken from $K_E = k_{-1}^{OH}/k_1^{OH}$. The satisfactory fit of the points to the inflections at high pH, which were measured from reaction of the keto tautomer to form enolate anion, confirms that agreement between directly measured and kinetically determined pK_as is good.

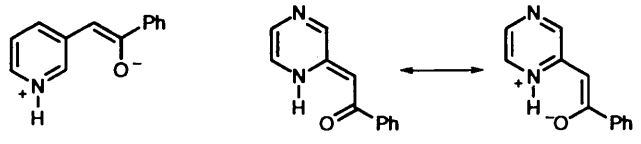
$$k_{obs} = \frac{k_1^{OH}}{1 + K_w/K_a^{KH}[OH]} + k_{-1}^{OH}[OH] + k_1^H[H^+] \qquad (3)$$

It is noteworthy that unlike ketonisation of phenacylpyridines[6] and phenacylquinolines[5] no inflection is observed at low pH reflecting *N*-protonation of the 2-phenacylpyrazine enol reactant. In practice, rate constants are high at low pH and the lowest pH that was studied was 1.4. The predicted value of the pK_a for protonation of the enol (at N-4) based on eqn. (1) and a value of pK_a = 5.84 for *N*-protonation of 3-phenacylpyridine enol is 1.6, so the rate at pH 1.4 is probably close to its limit. A possible reason for the lower than expected pK_a is an influence of hydrogen bonding between the OH of the enol and the nitrogen atom at the 1-position of the pyrazine ring, which is not subject to protonation.

Discussion

Enol and Enamine Tautomers.—Identification of the principal minor tautomer in equilibrium with the keto form of phenacylpyrazine in aqueous solution as the enol is consistent with the earlier conclusion reached by Levine and co-workers.[8] Indeed Levine isolated the enol by precipitation with acid from an aqueous solution of the enolate anion in sodium hydroxide and measured its m.p. as 82–83 °C. We felt, however, that more positive evidence excluding an enaminone structure was required and this is provided by the correlation of UV spectra for the enol in water and dioxane and measurement of δ_C for the enolic carbon atom in the latter solvent.[10] Levine indeed reported preparation of an enol ether of phenacylpyrazine[18] and, although its spectrum has not been published, presumably it is similar to that of the enol. As described above, an estimate of the tautomeric constant for conversion of ketone into enaminone as $pK_M = 4.4$ implies that the concentration of enaminone in aqueous solution is over 100 times less than that of the enol.

The greater stability of enol than enaminone is at first sight surprising because for 2- and 4-phenacylpyridines[3,6] and quinolines[2,4,5] the enaminone is consistently more stable than the enol and constitutes the principal minor tautomer (in the case of 2-phenacylquinoline indeed the major tautomer) in equilibrium with the keto form in aqueous solution. Only for 3-phenacylpyridine, in which the enaminone is replaced by a zwitterion (**7**) lacking stabilisation from resonance between

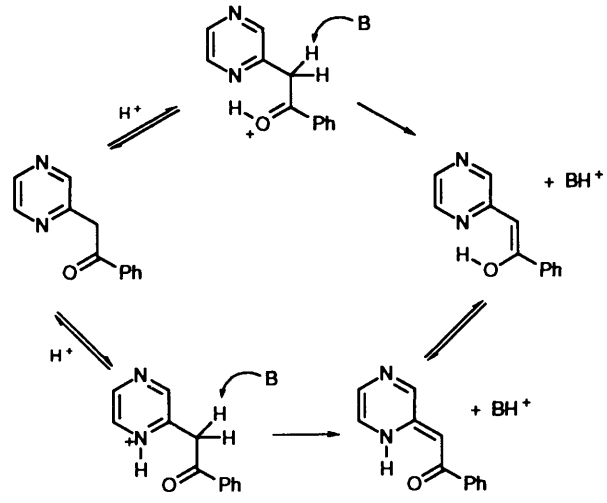


7 **3** **8**

the nitrogen and oxygen charge centres, is the enol more stable.[6] For 2-phenacylpyridine, for example, $pK_M = 1.05$ for the tautomerisation of ketomine to enaminone (K_M = [enaminone]/[ketone])[6] compared with the value of $pK_M = 4.4$ estimated for phenacylpyrazine.

The probable reason for the lower stability of the enaminone of 2-phenacylpyrazine is its dependence on the zwitterionic resonance interaction **3** ↔ **8**.[19] Since this resonance places a positive charge on the nitrogen atom it will be sensitive to the nearly 10^5-fold lower basicity of pyrazine than pyridine. As pointed out by Katritzky[19] and Greenhill[20] the stability of the enaminone relative to enol or ketoimine is also influenced by the loss of aromatic stabilisation of the heterocyclic ring in its principal resonance form **8**. Thus for 2-phenacylquinoline, for which aromatic stabilisation is less than for pyridine, the enaminone content is higher ($pK_M = -1.09$ compared with 1.05). The aromatic stabilisation of pyrazine may be less than that of pyridine[21] but, if so, apparently this is more than offset by the lower basicity of the nitrogen atom. It is also noteworthy that the enol is the more stable minor tautomer for other weakly basic phenacyl heterocycles such as benzoxazole or benzothiazole.[10] The relatively small values of pK_E for keto–enol tautomerism of 2-phenacyl-pyridine and -pyrazine ($pK_E = 2.0$ and 2.05, respectively) compared with 3- and 4-phenacylpyridines (4.86 and 4.4)[6] implies that the enol is also stabilised by intramolecular hydrogen bonding.

Catalysis by N-versus O-*Protonation.*—The pH-profile for ketonisation of 2-phenacylpyrazine enol shows acid- and base-catalysed reaction pathways (Fig. 2). As already pointed out, the mechanism for base catalysis may be interpreted as involving reaction *via* an enolate anion, as in Scheme 1. For the acid-catalysed pathway, however, alternative mechanisms are possible depending on whether reaction occurs with protonation on the oxygen atom of the keto group or on one of the nitrogen atoms of the heterocyclic ring. Two of these possibilities are set out in Scheme 5. It can be seen that *O*-protonation in the top



Scheme 5

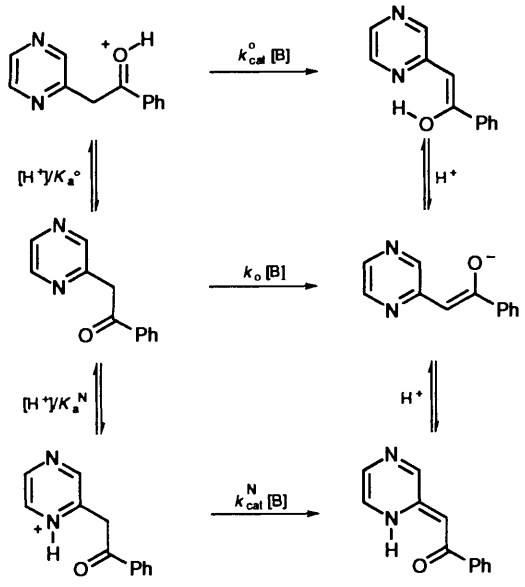
half of the scheme provides the more economical pathway in requiring only a single intermediate whereas *N*-protonation (at the bottom of the scheme) involves an enaminone or zwitterion (depending on which nitrogen is protonated) in addition to the *N*-protonated ketone.

Which pathway is followed can be determined experimentally by comparing the measured rate constant for enolisation with that predicted by extrapolation from measurements for reactions in which protonation on oxygen represents the only possible reaction path. If the measured value is significantly larger than predicted then protonation on nitrogen is indicated. In practice, many hydrogen-ion-catalysed enolisation reactions are quite insensitive to the structure of the ketone and have a rate constant close to $k_H = 10^{-5}$ dm^3 mol^{-1} s^{-1}.[15] In a more detailed analysis Keefe, Kresge and Schepp[22] have noted a mild dependence of k_H on enol content as shown in eqn. (4), in which case the value *ca.* 10^{-5} refers to ketones with pK_E in the 'normal' range of 6.5–8.5, *e.g.*, acetaldehyde, acetone and acetophenone. The likelihood that k_H also depends on the pK_a of the ketone is noted below.

$$\log k_H \simeq -3.6 - 0.17\, pK_E \qquad (4)$$

For phenacylpyrazine $pK_E = 2.05$ and Keefe, Kresge and Schepp's equation implies $k_H \simeq 10^{-4}$. However, k_H can also be derived by considering phenacylpyrazine as a deoxybenzoin containing electron-withdrawing substituents. Thus measurements of $k_H = 9 \times 10^{-6}$ dm^3 mol^{-1} s^{-1} for enolisation of deoxybenzoin itself[23] and the observation that deoxybenzoin and its derivatives containing *p*-nitro and *m*-nitro substituents in the benzyl ring undergo bromination at almost the same rate as the parent ketone (for H^+ catalysis in aqueous acetic acid)[24] suggest that k_H for *O*-protonation of phenacylpyrazine may be nearer 10^{-5} dm^3 mol^{-1} s^{-1}. The measured rate constant for enolisation of 2-phenacylpyrazine by hydrogen ions is 1.3 dm^3 mol^{-1} s^{-1}, which is more than 10^4 times faster even than the higher of the predicted constants for enolisation *via* *O*-protonation. There is little doubt therefore that phenacylpyrazine tautomerizes *via* *N*-protonation. For other heterocyclic ketones association of a high enolisation rate with *N*-protonation is confirmed by the similarity of rates for reaction of the *N*-protonated and *N*-methylated ketones.[14,25]

Interpretation of Acid Catalysis.—To understand the preference for *N*- over *O*-protonation it is helpful to consider the two possibilities in relation to the uncatalysed reaction, namely base-promoted carbon–hydrogen bond-breaking of phenacylpyrazine itself. Catalysed and uncatalysed reactions are shown in Scheme 6. Proton removal is effected by a base B



Scheme 6

which for reaction of H_3O^+ is a water molecule. In contrast with Scheme 4 the enol is no longer a reaction product but an intermediate on the *O*-protonation pathway on a par with the enamine intermediate for *N*-protonation. The 'product' of the reaction is the enolate anion, and the enamine and enol represent alternative positions for binding the catalyst (H^+) complementing the *N*- and *O*-protonation sites of the ketone reactant.

In the preliminary report of this work the *N*- and *O*-protonation pathways of Scheme 6 were compared directly.[14] In this paper we compare rather the catalysed and uncatalysed reactions. If rate constants for these are denoted k_H and k_o respectively, where k_H may refer to either *O*- or *N*-protonation, then the experimentally measured rate constants (k_{obs}) may be written as in eqns. (5) and (6).

$$k_{obs} = k_H[H^+][B] \quad \text{(catalysed)} \tag{5}$$

$$k_{obs} = k_o[B] \quad \text{(uncatalysed)} \tag{6}$$

As implied in Scheme 6 the rate constant k_H for the catalytic pathway may also be expressed as the ratio of a reaction rate constant (k_{cat}) and acid dissociation constant (K_a^R) for the protonated reactant [eqn. (7)]. Interpreting $1/K_a^R$ as a binding constant we see that k_H corresponds, as it should, to a product of an equilibrium constant for binding of the catalyst to the substrate and a rate constant for reaction of the (activated) catalyst-bound substrate. Now, taking the ratio of rate constants for catalysed and uncatalysed reactions k_H/k_o, we see in eqn. (8) that this corresponds to an equilibrium constant for catalyst binding ($1/K_a^R$ as before) and a ratio of rate constants for bound and unbound substrate k_{cat}/k_o.

$$k_H = k_{cat}/K_a^R \tag{7}$$

$$k_H/k_o = (k_{cat}/k_o)/K_a^R \tag{8}$$

In comparing *N*- and *O*-protonation then it is clear that the preferred pathway will depend upon the respective binding constants and activating effects of protonation at different sites. Stewart and Srinivasan have called the activating effect of protonation a 'proton activating factor', paf.[26] This is the ratio of rate constants for protonated and unprotonated substrates. Identifying k_{cat}/k_o with paf we may rewrite eqn. (8) as eqn. (9).

$$k_H/k_o = \text{paf}/K_a^R \tag{9}$$

For phenacylpyrazine, binding constants for protonation of the nitrogen atoms of the aromatic ring ($pK_a \simeq 0.4$) are probably at least 10^5 times greater than for the keto oxygen atom, if the pK_a for acetophenone (-3.9)[27] is considered as a generous upper limit for that of phenacylpyrazine. The activating effect of *O*-protonation is expected to be greater than that of *N*-protonation but apparently this does not compensate for the unfavourable difference in binding constants.

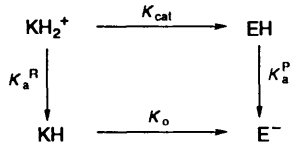
Can the relative catalytic effects of protonation at different sites by predicted? Since we are dealing with proton transfer reactions we can call on the Brønsted or Marcus equations to relate rate constants to equilibrium constants. Thus, if the protonated and unprotonated substrates in Scheme 6 are treated as Brønsted acids and the equilibrium constants for the steps to which k_{cat} and k_o refer are denoted K_{cat} and K_o we can write $k_{cat}/k_o = (K_{cat}/K_o)^\alpha$ where α is the exponent of the Brønsted relationship. The ratio K_{cat}/K_o may be regarded as an equilibrium proton activating factor which may be written in capitals (PAF) to distinguish it from the kinetic value (paf). Then, if the kinetic value in eqn. (9) is replaced by $(PAF)^\alpha$, eqns. (8) and (9) may be rewritten as (10) and (11). We note that the main difference between the Brønsted and Marcus equations is that whereas in the Brønsted equation α is constant, in Marcus' treatment α is an average of values for protonated and unprotonated substrates.

$$k_H/k_o = (K_{cat}/K_o)^\alpha/K_a^R \tag{10}$$

$$= (\text{PAF})^\alpha/K_a^R \tag{11}$$

A feature of eqns. (10) and (11) is that α refers, not as usual to reaction of oxygen and nitrogen acids, but to carbon acids. This presents no difficulty for the formalism but may lead to anomalously small or large values of α arising from imbalance of charge distribution in the transition state relative to reactants and products[28] or (in Marcus' analysis) variations in intrinsic barrier.[29] The interpretation of α as an average of values in the Marcus equation is appropriate only if the intrinsic barrier remains constant.

Eqn. (10) for k_H/k_O may be taken a step further by rewriting one of the catalytic cycles of Scheme 6, for example that



Scheme 7

involving *N*-protonation, in terms of the equilibrium constants relating reactants and products and their *N*-protonation equilibria as in Scheme 7. In this scheme, the symbols for species have their usual significance (*cf.*, Scheme 1) and K_a^R and K_a^P are acid dissociation constants for the *N*-protonated reactants and products, respectively. Single arrows rather than double arrows are again used for equilibria to indicate the direction to which the equilibrium constants refer.

It can be seen that the cycle of Scheme 7 implies a relationship

Table 5 Calculated values of log k_H/k_o for protonation of phenacylpyrazine at different oxygen and nitrogen atoms

Protonation site	pK_a^R	pK_a^P	$(pK_a^P - pK_a^R)/2$ log (paf)[a]	$(pK_a^P + pK_a^R)/2$ log (k_H/k_o)[b]
O	−5	9.5	7.3	2.25
N-1[c]	0.4	7.5	3.6	3.95
N-4	0.4	3.4	1.5	1.9

[a] Calculated value of log (paf). [b] Calculated values of log (k_H/k_o) compared with measured value of 4.23. [c] Number indicates position of N relative to the 2-phenacyl substituent.

between the equilibrium constants K_{cat} and K_o for reaction of bound and unbound substrates and the acid dissociation (or proton binding) constants of protonated reactants and products, *i.e.*, $K_{cat}/K_o = K_a^R/K_a^P$. This means that $(K_{cat}/K_o)^\alpha$ in eqn. (10) can be replaced by $(K_a^R/K_a^P)^\alpha$ to give eqn. (12) or, in its logarithmic form, eqn. (13).

$$k_{cat}/k_o = (K_a^R/K_a^P)^\alpha/K_a^R = (1/K_a^P)^\alpha(1/K_a^R)^{1-\alpha} \quad (12)$$

$$\log (k_{cat}/k_o) = \alpha pK_a^P + (1 - \alpha)pK_a^R \quad (13)$$

These equations have the simple interpretation that the effectiveness of the catalysis depends on a (weighted) product of the binding constants $(1/K_a)$ of the catalyst to the reactants and products of the catalysed step. Commonly the efficiency of catalysis is expressed in terms of the relative effectiveness of binding of the catalyst in the transition state and reactants. Use of the Brønsted or Marcus equations in eqns. (12) and (13) transforms the contribution from binding of the catalyst in the transition state into contributions from binding of the catalyst in reactants and products.

Eqns. (12) and (13) can be simplified by taking $\alpha = 0.5$. The contribution from reactants and products then become equal and the logarithmic expression of eqn. (13) reduces to an average of the pK_as in reactants and products. This approximation is a convenient one and has some justification in that slopes close to 0.5 for plots of kinetic *versus* equilibrium carbon acidity over wide reactivity ranges have been observed.[30] Its usefulness for predicting the site of protonation of phenacylpyrazine is easily tested because we know, or can interpolate, the required pK_as, *e.g.*, based on eqn. (2). Only the pK_a for *O*-protonation is lacking and we can adequately approximate this as −5. The results of these predictions are shown in Table 5 where the last column corresponds to the calculated value of log (k_H/k_o), *i.e.*, $(pK_a^R + pK_a^P)/2$.

From Table 5 it can be seen that protonation at N-1 is indeed predicted to be more effective than protonation on oxygen, by a factor of 50. Even though the binding constant in the product is more favourable for *O*- than *N*-protonation ($pK_a^P = 9.5$ rather than 7.5) this is more than offset by the unfavourable pK_a for *O*-protonation of the reactant.

An equivalent analysis of the preferred reaction path is provided by considering the proton activating factor and binding constant for the reactant in eqn. (9), $(k_H/k_o = paf/K_a^R)$. With $\alpha = 0.5$, log (paf) may be calculated as log $(PAF)^{0.5} = (pK_a^P - pK_a^R)/2$, and this is shown in column 3 of Table 5 [note that log k_H/k_o is obtained by adding pK_a^R to log (paf)]. It can be seen that log (paf) is much larger for *O*- than *N*-2-protonation but that this is not sufficient to offset the advantage of the large N-2 binding constant.

For protonation at N-4 by comparison the binding constant is similar to that at N-2 but the binding constant in the product (and the proton activating factor) is much less favourable, so protonation at N-1 should also be favoured over N-4.

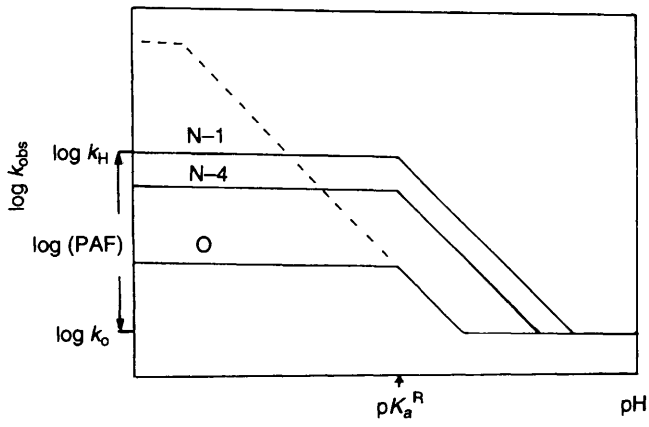


Fig. 3 Comparison of log *k*–pH profiles for H^+-catalysed enolisation of 2-phenacylpyrazine by *N*- and *O*-protonation pathways

Experimental accessibility of k_H/k_o (and proton activating factors) has been discussed by Stewart and Srinivasan.[26] For phenacylpyrazine k_H/k_o cannot be evaluated when the base [B in eqn. (6)] is water because the water reaction (k_o) fails to compete with catalysis by H^+ and OH^-. It can be measured for acetic acid buffers, however, for which catalysis by both acid and base components of the buffer are observable, and is then given by $k_H/k_o = (k_{GA}/k_{GB})\ K_a^{AcOH}$. In this case the base B of Scheme 6 is the acetate ion and the measured value of log $(k_H/k_o) = 4.23$, is in good agreement with the predicted value of 3.95 in Table 5.

pH-Dependence of Catalysis.—To complete the treatment of H^+ catalysis it is useful to show its dependence upon catalyst concentration. This can be done by combining eqns. (5) and (7) and making allowance for protonation of the substrate becoming complete at low pH. We then obtain eqn. (14) in which k_{obs} is the measured first-order rate constant, S is the substrate and K_a is the acid dissociation constant of the protonated substrate SH^+.

$$k_{obs}[S] = \frac{k_{cat}[S][H^+]}{[H^+] + K_a} \quad (14)$$

Eqn. (14) has the same form as the Michaelis–Menten equation and shows that catalysis is subject to kinetic saturation, with the difference that this arises from increasing the concentration of the catalyst (H^+) rather than the substrate. The dependence on concentration of H^+ is conveniently displayed on a pH-profile, and Fig. 2 shows plots of log k_{obs} against pH for each of the predicted catalytic pathways for 2-phenacylpyrazine (Table 5) as well as for the uncatalysed reaction.

Fig. 3 illustrates a number of features of the catalysis. Firstly there is only a limited range of catalyst concentration over which catalysis is observed. This range lies between a threshold concentration at which the rate of the catalysed reaction exceeds that of the uncatalysed reaction, and saturation of catalysis when the substrate becomes fully protonated. The threshold for catalysis is conveniently defined as the pH at which the rates of catalysed and uncatalysed reactions become equal, *i.e.*, pH = log (k_H/k_o) $[\simeq (pK_a^R + pK_a^P)/2]$. If the onset of saturation is considered to occur when the pH equals the pK_a of the substrate (pK_a^R) then the range over which catalysis is readily observable is $\Delta pH = \log (k_H/k_o) - pK_a^R$, which from eqn. (9) is equal to log (paf).

Saturation of catalysis applies equally to the dominant and minor pathways for reaction so that their relative importance

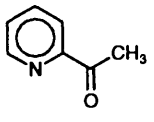
remains the same at all pHs. This is well illustrated by the solid lines labelled N-1, N-4 and O in Fig. 3. pK_a^R for saturation refers to the most basic protonation site and in practice, for 2-phenacylpyrazine, is probably an apparent value corresponding to partial protonation at both N-2 and N-4 nitrogen atoms. Protonation of an unreactive basic site does not affect the threshold pH or k_H but it does reduce the saturation rate and increase the apparent reactant pK_a to that of the unreactive protonation site.

The dashed line in Fig. 3 shows the pH-range of and acceleration by *O*-protonation that would occur in the absence of *N*-protonation. The acceleration exceeds that from *N*-protonation but the lower threshold of the *N*-protonation pathway determines that this is the form of catalysis observed.

Fig. 2 strictly refers to catalysis by H_3O^+ with H_2O as base. When the acid and base are buffer species the accessible range of pH is sensibly limited by the pK_a of the buffer ($\pm \simeq$ 1pH unit) and catalysed and uncatalysed reaction are observable when the pK_a of the buffer acid $\simeq \log k_H/k_o$, *i.e.*, close to the threshold pH for catalysis, although this value may show some dependence on the nature of the buffer.

Generality of N- versus O-*Protonation.*—Does the preference for *N*- over *O*-protonation in the enolisation of phenacylpyrazines extend to other heterocyclic ketones? For a considerable range of structures the answer appears to be yes. This is implied by the much greater rate constant for H^+-catalysed enolisation than expected for *O*-protonation ($k_H \leq 10^{-5}$ dm^3 mol^{-1} s^{-1}). Examples include 2- and 4-phenacylquinolines[5] and 2- and 4-acetyl-,[25] 2-, 3- and 4-phenacyl-[6] and -phenylacetyl-[16] pyridines. One factor favouring *N*-protonation is that for most of these ketones, for example phenacylpyridine (**4**), the enaminone (*e.g.*, **5**) is more stable than the enol. However, this is usually not an important factor. Thus the equilibrium constant for converting enol into enaminone for 2-phenacylpyridine is less than ten, whereas catalysis of enolisation by *N*-protonation is favoured over *O*-protonation by a factor of *ca.* 10^9, reflecting the much greater basicity of the pyridyl nitrogen (pK_a = 5.0) than the keto oxygen atom in the reactant.[6]

The advantage of *N*-protonation in the enolisation reaction is reduced to perhaps less than 100-fold for 2-acetylpyridine as a result of the lower basicity of the nitrogen atom (pK_a = 2.29) and its insulation from the site of reaction by the carbonyl group (**9**).[14,25] Where the basicity of the nitrogen is further reduced, as in *N*-methyl-2-acetylpyrrole (**10**)[31] and 2-acetylthiazole (**11**),[32] measurements of enolisation rates by de Maria indicate no preference for *N*-protonation.



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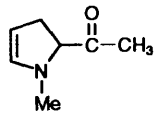

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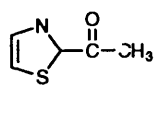

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'Transition-state Binding' and Enzymatic Catalysis.—Before concluding this discussion it may be appropriate to distinguish the sense in which the expression 'binding of the catalyst to the transition state', is used here from that in which it is normally used in connection with enzymatic catalysis.[33-35]

The catalytic behaviour discussed above involves binding of a catalyst at a reaction site, leading to an increase in rate relative to the uncatalysed reaction. This rate increase is a result of chemical activation. Without oversimplifying enzymatic catalysis, binding of the substrate to an enzyme characteristically involves groups removed from the reaction site and the rate of the uncatalysed reaction is enhanced principally through approximation of the substrate and functional groups responsible for catalysis. Enzymatic reactions are then favoured entropically over bimolecular or higher-order reactions between reagents of similar chemical reactivity. Approximation can thus lead to large rate accelerations with little or no chemical activation.[36]

Very often, the thermodynamically preferred site for binding a substrate (to form a Michaelis complex) does not provide the optimum geometry for interaction of the substrate with the catalytic groups of the enzyme. Binding may then also occur at a thermodynamically less favourable site for which a binding constant may not be accessible but which is more conducive to reaction. Thus it is well known that modifications of substrate structure away from the reaction site can affect enzymatic reactivity without affecting the measured binding constant.[35] For this reason a good substrate is said to bind efficiently to an enzyme 'at the transition state' rather than in a thermodynamically favoured but non-productive reactant configuration.*

Use of the term 'transition state binding' in this context, *i.e.*, in distinguishing competitive binding between a more favourable but less reactive and a less favourable but more reactive site (which translated to phenacylpyrazine corresponds to protonation at the more basic but less efficiently activating N-3 than N-1 nitrogen atom) differs from the sense in which we have used it in discussing catalysis of the tautomerisation of phenacylpyrazine. For phenacylpyrazine, to a first approximation, there is little or no entropic advantage associated with binding of the catalyst or the subsequent reaction with base. In contrast to the enzyme the catalyst is bound at the reaction site and its principal function is to provide chemical activation.

It is not implied that there is no binding at the reaction site or chemical activation in enzymatic reactions. Indeed, this is a principal function of cofactors such as pyridoxal or thiamine. Moreover in the absence of cofactors activation by electrostatic, hydrogen bond or other non-covalent interactions *at* the reaction site lead to significant rate enhancements. Nevertheless, remote binding and approximation of reacting functional groups is the principal characteristic of catalyses by simple proteins, for which the distinction between covalent catalysis and catalysis by approximation has long been recognised.[37]

In summary, the purpose of this addendum is to note that the rate–equilibrium relationship between binding of a catalyst at the transition state and in the reactants and products of the catalysed steps developed in this paper cannot be applied to 'transition state binding' in an enzymatic reaction: (*a*) because the measured (Michaelis) binding of the substrate to the enzyme may involve an unproductive complex and (*b*) because the catalytic influence of approximation, *e.g.*, upon acid–base catalysis, is commonly expressed exclusively at the transition state and not upon equilibria preceding and following the rate-determining step. The distinction is emphasised partly because the different elements of catalysis, *i.e.*, activation, approximation and binding of the catalyst (productively or unproductively, at or away from the reaction site), are brought into focus by the comparison.

Experimental

2-Phenacylpyrazine was prepared by condensation of methylpyrazine with methyl benzoate in liquid ammonia as described by Behan and Levine.[38] The product was purified by flash chromatography on silica using ether as the eluent. Details of ^{1}H and ^{13}C NMR spectra are given elsewhere.[10]

The pK_a for ionisation of phenacylpyrazine in aqueous

* Of course 'productive' binding may be achieved by a conformational change of the Michaelis complex (or induced fit) rather than in an independent equilibrium.

sodium hydroxide was measured spectrophotometrically with ionic strength maintained at 0.1 mol dm^{-3} by addition of NaCl. The value obtained was $pK_a = 11.77$ based on nine measurements at $\lambda_{max} = 385$ nm ($\varepsilon = 12\,200$ dm^3 mol^{-1} cm^{-1}) for the enolate anion. Applying the usual correction for ionic strength based on eqn. (15) in which pK_a and pK_a' are the thermodynamic and measured pK_as, respectively, yields a thermodynamic value of 11.90.

$$pK_a = pK_a' + 0.512\,[\sqrt{I}/\sqrt{(I + 1)}] - I/10 \quad (15)$$

The pK_a for protonation of phenacylpyrazine by H^+ was measured from the increase in absorbance accompanying protonation in the range 0.08–1.33 mol dm^{-3} $HClO_4$. Constant ionic strength was not maintained and measurements were based on an increase in absorbance at $\lambda = 280$ nm ($\varepsilon = 12\,000$ dm^3 mol^{-1} cm^{-1}). The value of $pK_a = 0.39 \pm 0.20$ was obtained by the method of Paul and Long[39] by plotting log ($C_{BH^+}/C_BC_{H^+}$) against acid concentration and deriving the pK_a as the intercept of a linear extrapolation to zero concentration. The measured pK_a is presumably an apparent value corresponding to partial protonation of both nitrogen atoms.

Rates of ketonisation of phenacylpyrazine enol were measured by stopped flow or conventional spectrophotometry at 25 ± 0.1°. For stopped flow measurements, normally the enolate anion of phenacylpyrazine was placed in one syringe as a solution in 0.04 mol dm^{-3} NaOH and a buffer or acid solution in the other. On mixing, the pH fell below the pK_a of the anion with consequent neutralisation, and initial formation of the enol. Kinetics were measured from the drop in absorption at 360 nm accompanying relaxation of the enol to the more stable ketone. For measurements at pH 7 in the absence of buffer a solution of phenacylpyrazine enol in dioxane (in which solvent the enol is the predominant tautomer) was injected by microlitre syringe into water in a prethermostatted 1 cm spectrophotometric cell (typically 20 μl in 2 cm^3), and kinetics were measured by recording the change in absorbance at a constant wavelength. This method was applicable only for reactions with half lives of a few seconds or longer and could not be used for the faster ketonisation reactions catalysed by acid, base or buffer.

Kinetics of iodination of phenacylpyrazine were measured in acetic acid buffers under zero- and first-order conditions in the manner described previously.[5] The zero-order measurements were made by stopped flow and first-order measurements by conventional spectrophotometry using the absorbance of I_3^- at 353 nm to monitor the reaction. Relatively low concentrations of iodide ion were used (*e.g.*, 0.02 mol dm^{-3}) to limit reversibility and corrections for dissociation of I_3^- to I_2 and I^- were made in the usual way.

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