Stepwise Proton Transfer in the Acid-catalysed Hydrolysis of 3,1-Benzoxazin-4-ones: Electrostatic or Hydrogen-bond Stabilisation of the Conjugate Acid

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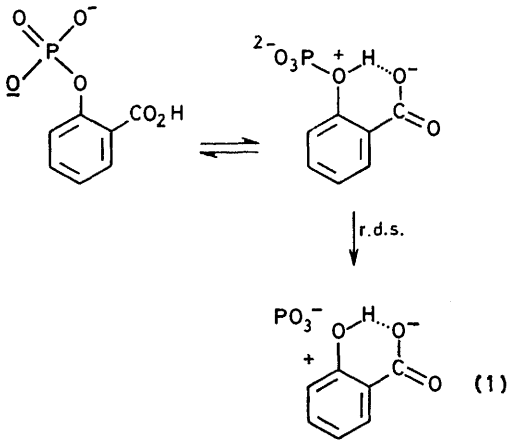
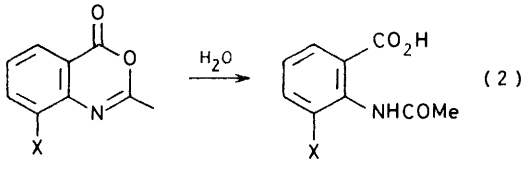
The hydrolysis of 3,1-benzoxazin-4-ones is catalysed by buffer acids and by intramolecular carboxylic acid groups suitably placed on the heterocyclic nucleus. Small or inverse deuterium oxide solvent isotope effects on the acid catalytic terms are consistent with both inter- and intra-molecular acid catalyses proceeding through a pre-equilibrium protonation mechanism. Proton transfer to the N-1 position concerted with attack at C-2 is not expected because the N-1 protonated 3,1-benzoxazin-4-one is a relatively stable species. The function of the 8-carboxy-anion in the catalysed ring hydrolysis of 8-carboxy-3,1-benzoxazin-4-one is to stabilise the protonated nitrogen (N-1) by electrostatic or hydrogen-bonding interaction.

INTRAMOLECULAR catalysis of nucleophilic displacement reactions by a carboxylic acid group may formally be of two types: [1a,b] proton transfer to an atom concerted with attack of nucleophile or pre-equilibrium protonation to



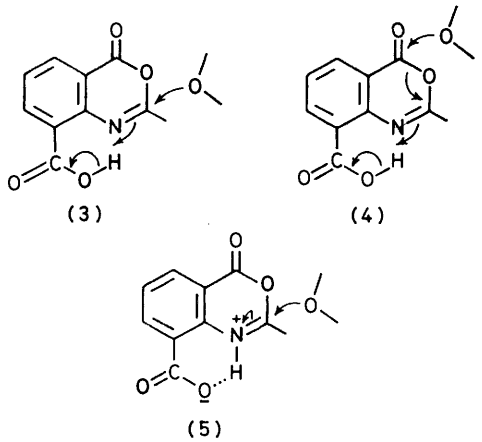
(1)

yield a cationic species which may be stabilised electrostatically or through internal hydrogen-bonding by the carboxylate anion. Bender and Lawlor [1a] discussed the latter mechanism for salicyl phosphate hydrolysis [equation (1)] where protonation of the phosphoryl oxygen was proposed to be complete in the transition-state of the rate-limiting step. Benkovic has discussed a similar mechanism for the hydrolysis of salicyl sulphate.[2] Recently, Hegarty and his co-workers [3] have



(1) (2)

shown that a carboxy-group is involved as a catalyst in the hydrolysis of 3,1-benzoxazin-4-ones [equation (2)]; the 8-carboxy-group is much more active than the 6-carboxy-group. The mechanism ascribed to this catalysis involves rate-limiting proton transfer from the 8-carboxy-group concerted with nucleophilic attack either on C-4 or -2 [structure (3 and (4)]. Some time ago we reported that the oxonium-ion-catalysed hydrolysis of 3,1-benzoxazin-4-ones involves protonation of the N-1 atom followed by rate-limiting water attack.[4] Consideration of the pK of the N-1 atom, which, although it has never been measured titrimetrically due to ring reactivity, must reside close to unity,[4] indicates that there is nothing to be gained by a concerted proton transfer where charge on N-1 is not fully expressed.[5] The present paper reports an investigation of the mechanism [equation (2; X = CO_2H and H)] of general acid-catalysed hydrolysis of benzoxazinones to establish an electrostatic or hydrogen-bond facilitation of the intramolecular



(3) (4) (5)

catalysis first proposed by Bender and Lawlor [1a] and as represented in structure (5).

EXPERIMENTAL

Materials.—2-Methyl-3,1-benzoxazin-4-one (1; X = H) was prepared from anthranilic acid by gentle reflux with acetic anhydride and had m.p. 77—78° (lit.,[6] 79—80°). 8-Carboxy-2-methyl-3,1-benzoxazin-4-one (1; X = CO_2H) was prepared from 2-aminoisophthalic acid by Hegarty's method [3] and had m.p. 227—228° (lit.,[3] 219—221°). 2-Aminoisophthalic acid, m.p. >330° (lit.,[7] >340°), was

obtained by reduction of the 2-nitro-species with tin and HCl. 2-Nitroisophthalic acid, m.p. 312—314° (lit.,[8] 310—312°), was obtained by $KMnO_4$ oxidation of 2-nitro-1,3-xylene (Aldrich).

Buffer components and other reagents used in the kinetics were of analytical reagent grade or were redistilled before use. Deuterium oxide (99.7% D) and DCl (35% w/w) in D_2O (99.0%) were obtained from Merck, Sharp and Dohme Ltd. Water was doubly distilled from glass apparatus.

Methods.—Kinetics of reaction were measured by adding an aliquot (*ca.* 25λ) of a stock solution of the substrate in acetonitrile (purified according to the method of Lewis and Smyth) [9] to 2.5 ml of the appropriate buffer in a silica cuvette. The cuvette was placed in the thermostatted cell holder in the spectrophotometer (Unicam SP 800 or Perkin-Elmer 124) and the substrate added on the flattened tip of a glass rod. Two or three strokes of the glass rod mixed substrate with buffer to effect a good zero time. The progress of the reaction was followed at a fixed wavelength (315 and 325 nm for parent and 8-carboxy-species respectively) using an external Servoscribe potentiometric recorder. Pseudo-first-order rate constants were obtained by plotting $A_t - A_\infty$ *versus* time on semi-logarithmic graph paper. The pH was measured before and after the kinetics using a Radiometer PHM 62 digital pH meter. Buffers were prepared at a standard fraction of base (FB) and made up to 1M ionic strength with KCl; serial dilutions were made in the cuvette with 1M-KCl. The experiments in deuterium oxide were carried out in a similar manner to those for water

$$pD = pH + 0.4 \qquad (3)$$

except that low-volume 1-cm path length cells were used; pD was estimated from the pH-meter reading using equation (3).

RESULTS

The degradation of the benzoxazinones followed excellent first-order kinetics over >90% of the total reaction. The products obtained from reaction of the benzoxazinones in aqueous buffer were 2-acetamidobenzoic acids as judged from the spectra after complete hydrolysis which correspond exactly with those of pure samples of the acids at the same concentrations.

The hydrolysis of the benzoxazinones is catalysed by buffers and the pH dependence for 8-carboxy-2-methyl-3,1-benzoxazin-4-one was studied with acetate buffers at 0.005M and taken to be that for zero buffer. Test experiments indicated that the difference between the rate constant at 0.005M and that of the intercept at zero buffer molarity was within the experimental error of the determinations. The pH(D) dependencies of the 8-carboxy-species are illustrated in Figure 1; the pH dependence (at 1M ionic strength) is similar to that obtained by Cremin and Hegarty [3] at 0.1M ionic strength. Cremin and Hegarty found that the rate law possessed oxonium ion, hydroxide ion, and neutral acid and carboxylic terms; we were only able to obtain the parameters relating to the term in free acid (k_2 and K_{a2} in Cremin and Hegarty's paper [3]) because we studied the hydrolysis over a much smaller pH range. The kinetics obeyed the theoretical rate law (4) and the

$$k_{obs.} = k^{max.}/(1 + K/a_H) \qquad (4)$$

related parameters are respectively $k_D^{max.}$ 0.155 s^{-1}, $k_H^{max.}$ 0.13 (0.22) s^{-1}, pK^D 4.00, and pK^H 3.80 (4.44) at 25° and 1M ionic strength. The values in parentheses refer to previously published figures [3] for 0.1M ionic strength.

The hydrolysis of benzoxazin-4-ones is catalysed by both acid and base forms of buffer. The analysis of the

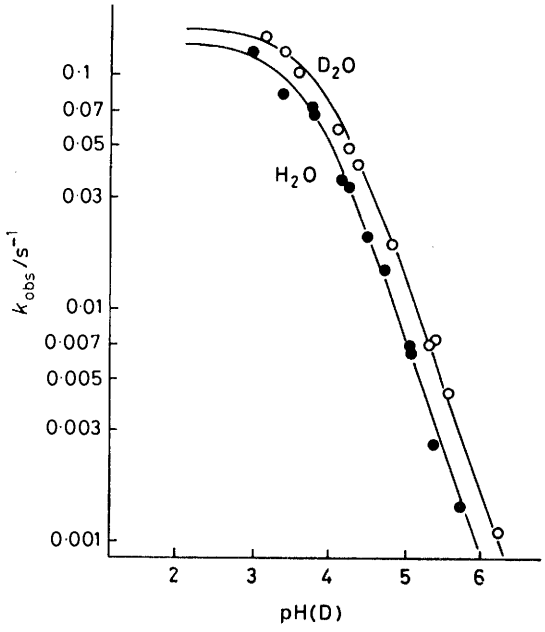


FIGURE 1 Dependence on pH(D) of the hydrolysis of 8-carboxy-2-methyl-3,1-benzoxazin-4-one at 1M ionic strength and 25°. The lines are theoretical according to equation (4) using parameters from the Results section; filled circles for water and open circles for deuterium oxide solutions

data for the 8-carboxy-derivative is complicated because of the ionisation of the substrate. Plots of $k_{obs.}$ *versus* buffer concentration are linear with intercepts at zero concentration fitting the rate law previously discussed [equation (4)]. The slopes of these lines (Figure 2) vary with pH according to a rate law [equation (5)] with two terms, one of which is kinetically ambiguous (k_B). The data are analysed (Table 1) by division of the slope of the rate

$$\text{Rate} = k_{HB}[HB][SH] + k_B[B][SH] \qquad (5)$$

constants against total buffer concentration by the fraction of the substrate acid species (FSH) present at the given pH; this quotient is plotted against the fraction of buffer present as base (FB) as indicated in Figure 3. The parameters k_{HB} and k_B for the acetate buffer (Table 1) are the intercepts on the ordinates at FB 0 and 1, respectively. A

TABLE 1

Hydrolysis of 8-carboxy-2-methyl-3,1-benzoxazin-4-one in acetic acid buffers at different pH values [a, d]

pH	FB	$10^2 \times k_M^{-1}$/ l mol^{-1} s^{-1} [c]	FSH [b]	$10^2 \times k_M^{-1}$ FSH^{-1}/ l mol^{-1} s^{-1}
3.42	0.1	3.3	0.706	4.67
4.00	0.235	2.0	0.388	5.15
4.13	0.3	1.63	0.319	5.11
4.50	0.48	0.9	0.167	5.39
4.73	0.6	0.55	0.105	5.24
4.77	0.64	0.5	0.097	5.15

[a] At 25°, 1M ionic strength made up with KCl. [b] Calculated from pK 3.80 and the pH. [c] Slope of the rate constant *versus* total buffer concentration. [d] The parameters k_{HOAc} and k_{AcO^-} are respectively 4.8×10^{-2} and 5.6×10^{-2} l mol^{-1} s^{-1} at 1M ionic strength and 25°.

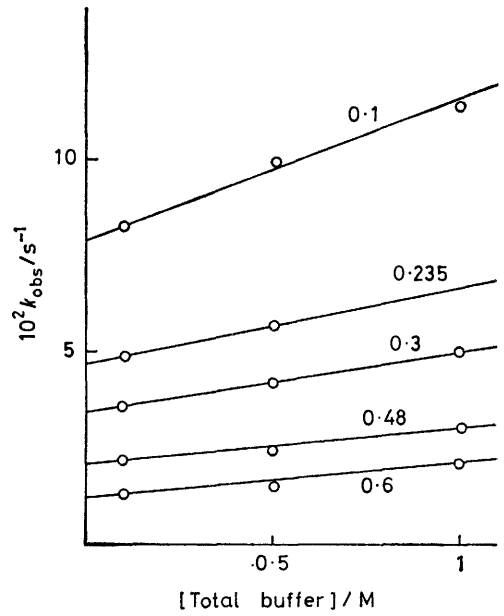


FIGURE 2 Dependence of the hydrolysis of 8-carboxy-2-methyl-3,1-benzoxazin-4-one on acetic acid buffer concentration at 1M ionic strength and 25°. The lines are theoretical calculated from equation (5) using parameters given in the Results section; numbers refer to FB the fraction of buffer present as the conjugate base

third term not shown in equation (5) involving base (B) and the ionised 8-carboxy species (S^-) would be expressed as a non-linear increase in rate constant as the fraction of base (FB) is increased in the plot illustrated in Figure 3; no such curvature is seen indicating that the terms k_{HB} and k_B take the major part of the reaction flux. The similarity between k_{HB} and k_B for the 8-carboxy species (Figure 3) is fortuitous; these parameters will increase and decrease, respectively, with increasing acidity of HB. Acetic acid

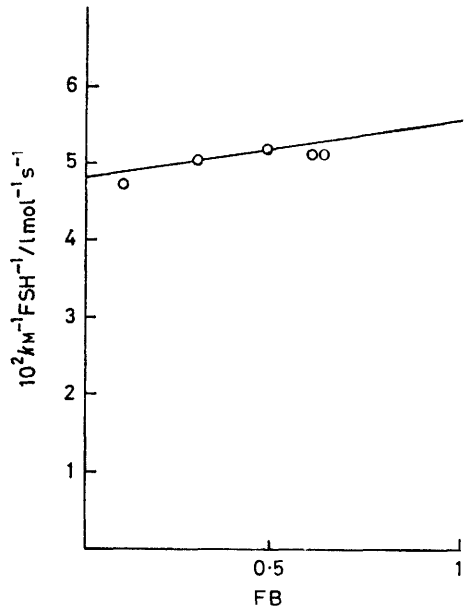


FIGURE 3 Plot against FB (for acetic acid buffer) of the slopes of the lines from Figure 2 divided by the fraction of substrate present in its acid form (FSH). Intercepts on FB 0 and 1 are, respectively, k_{HB} and k_B of equation (5)

presumably marks the pK where the terms have equal predominance. Acetic acid does not mark the changeover point for the parent heterocycle (Table 2) which occurs near pK 6.5.

Analysis of the buffer-catalysed hydrolysis of the parent 2-methyl-3,1-benzoxazin-4-one is straightforward and leads to an equation similar to that of equation (5); the parameters for different buffers are collected in Table 2. Cacodylic acid was employed to measure the effect of deuterium oxide solvent on buffer catalysis because this buffer gives rise to easily measured acid and base terms and is a uni-uni-electrolyte; the data are collected in Table 2 and the Bronsted plot for k_{HB} illustrated in Figure 4.

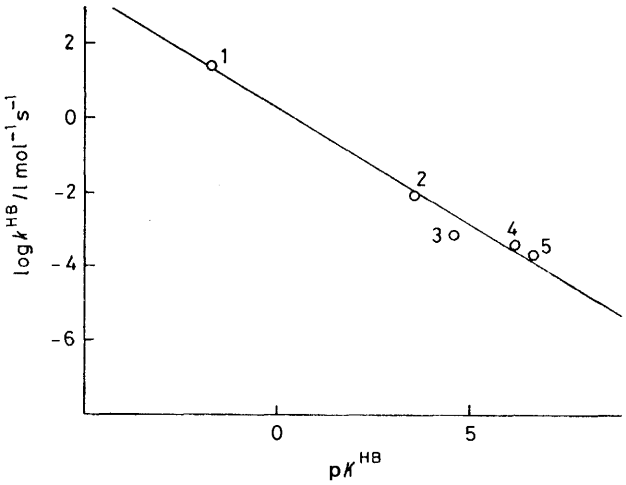


FIGURE 4 The Brønsted plot of k_{HB} *versus* pK of conjugate acid (HB) for acid-catalysed hydrolysis of 2-methyl-3,1-benzoxazin-4-one at 1M ionic strength and 25°. The data points are from Table 2: oxonium ion, 1; formic acid, 2; acetic acid, 3; cacodylic acid, 4; and phosphate, 5. The line obeys the equation $\log k_{HB} = -0.63\ pK^{HB} + 0.27$ (r 0.988)

TABLE 2

Hydrolysis of 2-methyl-3,1-benzoxazin-4-one in the presence of buffers [a]

Buffer	pK^{HB}	pH(D)	N [b]	k_{HB}/l mol^{-1} s^{-1} [c]	k_B/l mol^{-1} s^{-1} [e]
Acetate	4.5	3.9—5.03	13	8.1×10^{-4}	$< 10^{-5}$
Formate	3.49	2.8—4.1	15	8.9×10^{-3}	$< 10^{-4}$
Cacodylate (H)	6.05	5.2—6.6	17	4.1×10^{-4}	$1.8\ 10^{-3}$
Cacodylate (D)	6.69	5.66—7.57	18	3.4×10^{-4}	$2.0\ 10^{-3}$
Phosphate	6.55	6.23—6.55	7	2.7×10^{-4}	$5.3\ 10^{-4}$
Oxonium ion				31.6 [d]	
Hydroxide ion					56.2 [d]

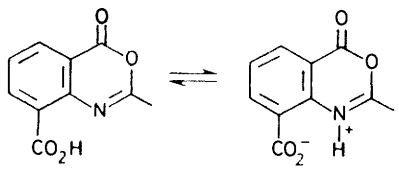
[a] At 25°, ionic strength made up to 1M with KCl. [b] Data points. [c] Maximum and minimum values of k_{HB} are shown in Figure 4. [d] From ref. 4. [e] There is insufficient data for the construction of a reliable Brønsted plot; a previous paper indicates β_{nuc} 0.67 at 50°.[4]

DISCUSSION

Stepwise Proton Transfer in Intramolecular Acid Catalysis.—The pK values determined kinetically from the hydrolysis of the 8-carboxy-derivative in water and deuterium oxide in the absence of buffer are close to those expected for benzoic acid derivatives. The ratio of ionisation constants for water and deuterium oxide (*ca.* 1.6) is greater than unity as predicted but a little less

than that of benzoic acid (3.13) of similar pK;[10] the interpretation of the isotope effect on ionisation as a function of structure is not clearly understood[11] and the difference may reside in greater hydrogen-bonding potential in the benzoxazinone derivative. The value of pK^{H} obtained in this study is lower than that of Cremin and Hegarty[3] probably due to the difference in ionic strength.

The value of the deuterium oxide solvent isotope effect on $k^{max.}$ [$k_D{}^{max.}/k_H{}^{max.}$ 1.19, equation (4)] is in the inverse order of that expected for a rate-limiting proton transfer. We should expect a normal deuterium oxide solvent isotope effect ($k_H/k_D > 2$)[12] for rate-limiting proton transfer concerted with nucleophilic attack of water at C-2 (or -4). The classical explanation of the inverse effect is the existence of a pre-equilibrium protonation of the substrate nitrogen (N-1) followed by a rate-limiting decomposition (A-2 mechanism) which does not involve proton transfer [equation (6)]. Both mechanisms proposed by Cremin and Hegarty [(3) and (4)][3] are not consistent with the observation of the inverse deuterium oxide solvent isotope effect.



(6)

Recent work on proton transfer between hetero-atoms[13,14] indicates that a maximum in k_H/k_D occurs when these atoms have similar pK values. The isotope effect maximum is quite narrow with a half-height width of only a few pK units compared with similar maxima for transfer to or from carbon where the width is much larger. A further complication is noted by Hibbert and Robbins[15] who observe no isotope effect in the transfer of a proton between the nitrogen atoms in 1,8-bis-(dimethylamino)-2,7-dimethoxynaphthalene. These complicating factors are for rate constants of proton transfer reactions; the isotope effects >2 recorded in Johnson's compilation[12a] are essentially for proton transfer *concerted* with a 'heavy atom' reorganisation. Although the theory of deuterium oxide solvent isotope effects is not yet clear it is becoming apparent that the mismatch of the pK values of donor and acceptor atoms is one contributor to the relatively low isotope effects observed.[12,16a-c] These considerations do not affect the conclusion that the present reaction has a pre-equilibrium protonation mechanism as rate-limiting *simple* proton transfer is not involved (see later).

The isotope evidence means that there is little change in zero-point energy of the transferred proton in the transition-state of the rate-limiting step. This could mean either that a concerted process is occurring with *almost* complete proton transfer or a stepwise process. Both mechanisms give a reaction co-ordinate passing through a structure close to that of the zwitterionic species in equation (6).

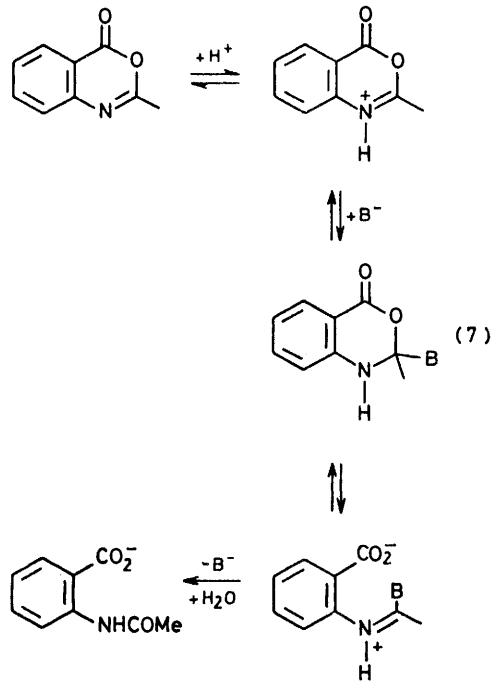
Intermolecular Acid–Base Catalysis.—The hydrolysis of 8-carboxy-2-methyl-3,1-benzoxazin-4-one and 2-methyl-3,1-benzoxazin-4-one is catalysed by both acids and bases. In the case of the 8-carboxy-species the basic term for acetate [k_B in equation (5)] buffer is kinetically equivalent to acetic acid catalysed hydrolysis of the conjugate base of the heterocycle. The ambiguity may be resolved by comparing k_B with that of the non-ambiguous acetate-ion catalysed hydrolysis of the parent heterocycle. The latter has a very low value which should be close to the observed upper limit of 10^{-5} l mol^{-1} s^{-1} (Table 2). The upper limit is *ca.* 5 000-fold less than the value for the 8-carboxy-derivative indicating that the major contributor to the reaction flux is the acetic-acid-catalysed hydrolysis of the conjugate base (S^-). The rate constant corresponding to this mechanism ($k_{OAc}K^{HOAc}/K^{SH} = 10^{-2}$ l mol^{-1} s^{-1}) is only 10-fold greater than the acetic acid term in the buffer-catalysed hydrolysis of parent benzoxazinone (Table 2).

In the case of acid-catalysed hydrolysis of the parent we may easily eliminate rate-determining proton transfer from the general acid to N-1 (followed by fast addition of water) because for example with cacodylic acid the ΔpK between donor and acceptor is *ca.* 6 units leading to a rate constant[16d] for proton transfer of *ca.* 10^4 l mol^{-1} s^{-1}; this value is some eight powers of ten larger than the observed (Table 2). Water attack concerted with proton transfer from cacodylic acid would be expected to be faster than the simple proton transfer because the build-up of charge on the nitrogen would be reduced. The absence of a deuterium oxide solvent isotope effect on the kinetic term for cacodylic acid (Table 2) also excludes a rate-limiting proton transfer (concerted or not with water attack). We attribute the acidic term (k_{HB}) to an A-2 mechanism involving pre-equilibrium protonation of N-1 followed by rate-limiting nucleophilic attack of the conjugate base of the buffer. It would be difficult to decide, using ^{18}O labelling, the site of attack of the conjugate base; by analogy with the results for the oxonium-ion catalysis where water attacks at C-2[4] we propose this as the site for base attack. Attack at C-2 would be favoured for the oxyanion bases owing to the electrostatic effect of the positively charged nitrogen. We propose a mechanism [equation (7)] involving the isoimide but we are unable to define the rate-limiting step which must be at or after the addition of the oxyanion base (B^-).

The involvement of the 8-carboxy-anion is different from that of the conjugate oxyanion in intermolecular acid catalysis. The stereochemistry requires that the 8-carboxy-group does not act as a nucleophile at C-2 (or C-4).

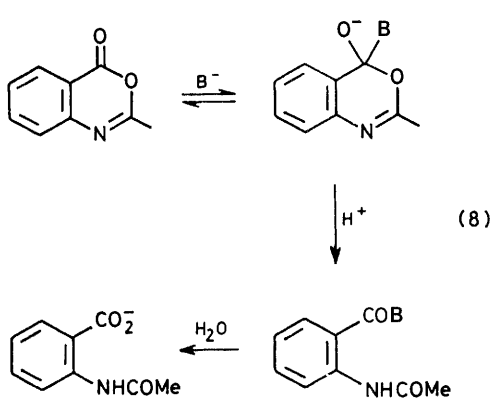
The intermolecular general base catalysis also possesses

no significant deuterium oxide solvent isotope effect; this rules out the participation of rate-limiting proton transfer (which occurs in the classical mechanism of base attacking water which in turn attacks the sub-



strate) and points to direct nucleophilic attack [12] [equation (8)]. The site of base attack is not easily proven but by analogy with the hydroxide ion reaction where ^{18}O-labelling experiments have been carried out [4] the C-4 position is favoured. Attack at C-2 by the conjugate oxyanion of the buffer would yield an amide anion which would be much more basic than the oxyanion produced from C-4 attack.

The preference for the *A*-2 mechanism over a mechanism involving proton transfer concerted with nucleophilic attack is most likely due to the relatively high



basicity of the N-1 nitrogen in benzoxazinones. Figure 5 illustrates a schematic free-energy surface for the acid-catalysed hydrolysis of a benzoxazinone; this assumes attack of the nucleophile is at C-2 and formation of the tetrahedral intermediate is rate limiting. The nitrogen anion (bottom right corner) section of the surface is expected to have a relatively high energy corresponding to the low acidity of aromatic NH groups; the energy of the top left corner (protonated nitrogen) is low due to a pK of *ca.* 0. An estimate of the latter pK may be obtained from the pK values of *N*-methylimino-ethers (*ca.* 6) [17] assuming the difference between aliphatic and aromatic species is the same as that between *N*-methylaniline (4.6) and methylamine (10.6).[18] Kinetic measurements at different low pH values indicate a pK value for

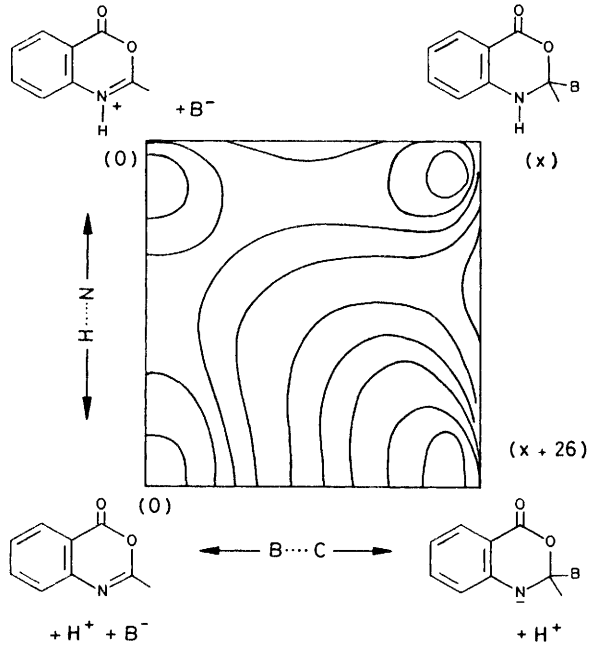
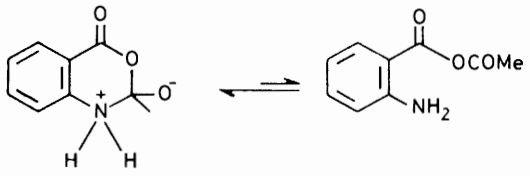


FIGURE 5 Free energy surface for the reaction of H^+ and B^- with benzoxazinone at C-2. The numbers at the corners represent the logarithm of the equilibrium constants in aqueous solution relative to the bottom left corner at zero and are essentially measures of free energy. We estimate the ionisation constant of the product NH from the data of R. Stewart and J. P. O'Donnell, *Can. J. Chem.*, **1964**, **42**, 1694, who find pK_{NH} 1.7 p$K_{anilinium}$ −30; we use as a model of the product 2-ethoxycarbonylaniline (p$K_{anilinium}$ 2.1; pK_{NH} *ca.* 26). There is no reliable estimate of the equilibrium constant for the addition of an oxyanion base but the reaction is likely to be thermodynamically unfavourable

benzoxazinones at *ca.* 0.[4] These considerations indicate that the free-energy surface is markedly skewed favouring the stepwise path over the symmetrical concerted mechanism.

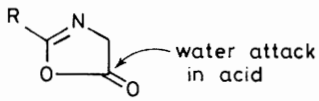
It is difficult to compare the reactivity of the hydrolysis of benzoxazinones with that of their acyclic analogues the imino-ethers [17,19] because the cleavage pattern is different. At low pH imino-ethers yield amine and ester due to the superior leaving ability of the amine from the initially formed *N*-protonated tetrahedral intermediate. It is very likely that in the present case C–N cleavage should also occur but the product anhydride [equation (9)] would rapidly revert to benzoxazinone rather than proceed to anthranilic acid. The only reaction leading away from reactants involves C–O bond

cleavage. Comparison of the hydrolysis with the oxazolinone analogue indicates that acid cleavage is at C-2 in the benzoxazinone but at the carbonyl in the oxazolinone (6). The more weakly basic nitrogen in the benzoxazinone species may account for the C-2 attack.



(9)

Kinetic Advantage of the Intramolecular Carboxylate in Benzoxazinone Hydrolysis.—Cremin and Hegarty [3] demonstrate that the enhanced reactivity of 8-carboxy-2-methyl-3,1-benzoxazin-4-one is not due to an electronic

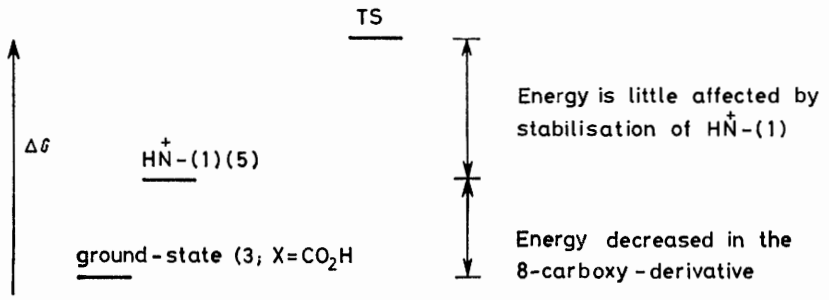


(6)

effect since the 6-carboxy-species has reactivity close to that of the parent heterocycle in the region of pH 3—7. that the energy between ground-state and zwitterion is reduced thus reducing the overall energy between ground- and transition-state. There is a difference of 2 pK units between quinoline-6- and -8-carboxylic acids [18] where we assume that a similar stabilisation process is occurring. This, together with the present catalysis, is in our opinion a hydrogen-bonding phenomenon but the possibility still exists of an electrostatic effect.*

Intermolecular general acid catalysis of the hydrolysis of the parent benzoxazinone is unlikely to involve stabilisation of the N-1 protonated species by the conjugate oxyanion of the acid; intermolecular hydrogen-bonding or electrostatic effects in water are too weak to be considered.

The mechanism proposed by Bender and Lawlor [1a] for the hydrolysis of the dianion of salicyl phosphate has now been discarded in favour of a transition-state with little proton transfer from the carboxy.[21] A similar timing of the proton transfer also exists in the hydrolysis of the acetal 2-methoxymethoxybenzoic acids [22] where electrostatic or hydrogen-bonding stabilisation of a protonated oxygen by an *o*-carboxylate group is possible. The absence of significant proton transfer in the transition state gives rise to a small deuterium oxide solvent isotope effect. This timing does not account for our



SCHEME

Possible explanations of this enhancement involve either electrostatic or hydrogen-bonding stabilisation of the N-1 protonated group by the adjacent 8-carboxy-anion; such stabilisation is not possible with the 6-isomer. The carboxylate oxygen in the 8-carboxy-species is 2.5 Å from N-1 according to the distance measured on a Dreiding model; this value is well within the range previously observed for hydrogen-bonds between O and N atoms.[20] It appears to us difficult to differentiate between these two explanations; * the hydrogen-bonding theory requires that the ground-state hydrogen-bonding capability between 8-carboxylic acid and N-1 is less efficient than that in the zwitterion (5) [HN^+–(1) (Scheme). We are not postulating an especially enhanced rate of reaction from zwitterion to product (Scheme) but observation of small or inverse effects because the Brønsted selectivity (Figure 4) would indicate a considerably advanced O–H bond cleavage.

* We mean by ' electrostatic stabilisation ' that there is solvent dielectric between the charges. We appreciate that hydrogen bonding has in any case a major electrostatic component and do not mean to imply a separation of canonical forms. Considering the distance between carboxylate and N-1 it is unlikely that solvent could separate the charges.

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