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A Mechanism for Sulphite Ion Reacting With Vitamin B_1 and Its Analogues*

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ABSTRACT

Sulphite ion has been shown to react by a multi-step mechanism with thiamine, 1′-methylthiaminium ion and various thiamine analogues. Sulphite ion replaces the thiazole-leaving group of a thiamine and a pyridine or a phenoxy group of an analogue. Sulphite ion first adds to the pyrimidinium ring, and this is followed by expulsion of the leaving group. The resultant cationic intermediate then reacts with a second sulphite ion; the observed pyrimidine substitution product forms after expulsion of the first sulphite ion. Although substitution is usually first order in the sulphite ion, the required second-order kinetics in sulphite ions have been demonstrated under special conditions. When the leaving group is a pyridine, the rate-limiting step is expulsion of the pyridine, but when the leaving group is a phenoxide ion, addition of the first sulphite ion is rate limiting. Hydroxide ion reacts with thiamine analogues by a similar multi-step mechanism. Second-order rate constants for hydroxide ion are slightly greater than those for sulphite ion. Sulphite ion is shown to be 'special' because it is a weak base, a strong nucleophile and a poor leaving group.

INTRODUCTION

Sulphite salts and sulphur dioxide are widely employed in the food industry, usually for the purpose of preventing some unwanted chemical process from taking place (McWeeny, 1982). Unfortunately, the effect of

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* Dedicated to Professor Edward C. Taylor, Jr. on the occasion of his sixtieth birthday.

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sulphite ion, either added as a salt or formed by a reaction between sulphur dioxide and water, is not always beneficial. Sulphite ion can serve both as a powerful nucleophile and as a reagent in a redox reaction that brings about undesirable changes in the quality and character of food. One of the oldest and most notable harmful reactions of sulphite ion is the destruction of thiamine (vitamin B_1) (I).

EXPERIMENTAL

Substrates were prepared from 1′-methylthiaminium diperchlorate by the general method given by Zoltewicz (1980). Melting points of substrates not given in this reference include those with the following leaving groups: pyridine (262–263·5 ° dec), *m*-chlorophenoxy (298–299 ° dec), phenoxy (255–257·5 ° dec), *p*-methylphenoxy (245·5–247 ° dec) and *m*-methoxyphenoxy (289–290·5 ° dec).

Details of the spectrophotometric kinetic studies are given by Zoltewicz *et al.* (1981). Wavelengths employed (leaving group) include: 280 nm (pyridine, 4-methylpyridine, 3,4-dimethylpyridine), 285 nm (nicotinamide, *p*-cyanophenoxide and phenoxide ions), 310 nm (*p*-nitrophenoxide ion), 290 nm (*m*-chlorophenoxide ion) and 300 nm (*p*-methylphenoxide and *m*-methoxyphenoxide ions).

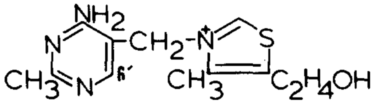
The pK_a of bisulphite ion at 25 ° was determined by the method of Albert & Serjeant (1971), except that the ionic strength was varied using KCl. Values (ionic strength) for H_2O solutions are: 6·59 (1·0M), 6·69 (0·50M), 6·85 (0·15M) and 6·99 (0·039M). A plot of pK_a versus $I^{1/2}/(1 + I^{1/2})$ (where I is the ionic strength) was linear with slope, 1·200 and intercept, 7·193. Values for other ionic strengths were calculated from this line.

Although the nicotinium ion is known to form an addition complex with sulphite ion (Pfleiderer *et al.*, 1960), under our conditions such a reaction is not significant. However, substrate having nicotinamide as a leaving group caused the most difficulties. Approximately half the runs were rejected due to changes in pH and/or sulphite ion concentration, all with pH < 5 where substitution is slow.

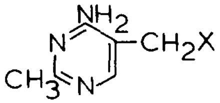
In order to determine whether the substitution product, 5-(4-amino-1,2-dimethylpyrimidinio)methane-sulphonic acid, is stable, a solution in pH 10·9 carbonate buffer was kept at 25 °C for 1360 min. No degradation was detected spectrophotometrically.

DESTRUCTION OF THIAMINE

Williams (Williams, 1935; Williams *et al.*, 1935*a*, *b*) reported that I is rapidly destroyed by aqueous solutions of sulphite ion at room temperature. This fortuitous, accidental discovery enabled him to determine the structure of the vitamin, because it gave rise in quantitative yield to two simple products—the highly insoluble pyrimidinylmethylsulphonic acid (II) and the soluble substituted thiazole (III). This remains to this day as the best known, most facile, substitution reaction of I.

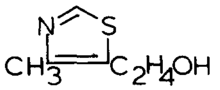


I

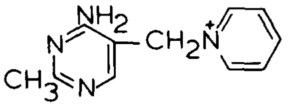


II, X = SO_3H

IV, X = N_3



III



V

When our interest in this nucleophilic substitution reaction commenced in 1976, there were a number of reports attempting to establish a mechanism. No satisfactory explanation of how the sulphite ion reaction takes place had been advanced. But these attempts indicated to us that the substitution reaction was complex and multi-step. Our initial success was achieved by using a pair of competing nucleophiles: sulphite and azide ions. The latter was selected because it traditionally serves as a trapping agent for carbocations.

The single step S_N2 mechanism was immediately eliminated by a few simple experiments, proton magnetic resonance serving as the means of analysing reaction mixtures. When azide ion was substituted for sulphite ion, no destruction of thiamine was observed under conditions that lead to substitution with sulphite ion. But when a mixture of azide and sulphite ions was employed, thiamine was destroyed. However, the substitution product, along with the usual thiazole, was not sulphonic acid (II), but the pyrimidinylmethylazide (IV). The organic azide did react with sulphite ion, on standing, to give sulphonic acid, but clearly the azide product preceded the sulphonic acid. The reverse was not observed; sulphonic acid (II) did not react with azide ion to give IV.

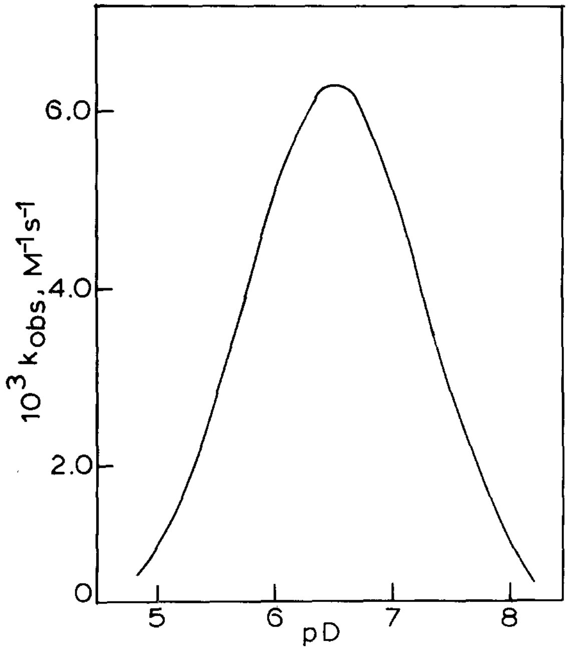


Fig. 1. pD–rate constant profile for the cleavage of thiamine by sulphite ion at 25·0 °C and 1·0M ionic strength in D_2O (Zoltewicz & Kauffman, 1977).

The implications of these simple experiments are clear: one or more intermediates must be present. Sulphite ion serves as a catalyst for the formation of azide (IV). In all these experiments sulphite ion is present in excess; thiamine is the limiting reagent.

Kinetic studies (Leichter & Joslyn, 1969; Zoltewicz & Kauffman, 1977) showed that the rates of reaction of thiamine and sulphite ion are highly dependent on pH. The pD-rate profile has the shape of a 'bell' (Fig. 1); that is, there is a maximum rate, and the rate rapidly falls as the pH is either raised or lowered from this optimum value: pH 5·7 at 25 °C (Leichter & Joslyn, 1969) or pD 6·5 at 25 ° ionic strength 1·0M (Zoltewicz & Kauffman, 1977). These data require a stoichiometry consisting of thiamine, a proton and a single sulphite ion.

Our kinetic studies show that protonated thiamine must react with sulphite ion. The kinetically equivalent process whereby thiamine-free base reacts with bisulphite ion nucleophile seems unlikely because bisulphite is expected to be a weaker nucleophile than sulphite ion.

The rate expression for substitution of I is given by eqn (1) where $[DB_I^+]$ is the concentration of I converted to its conjugate acid in D_2O. At low pD, a term equivalent to a reaction involving bisulphite ion becomes important, but it is not shown. Equation (2) describes how the observed rate constant, k_{obs}, depends on acidity and the dissociation constants for bisulphite ion (Ka) and the conjugate acid of I (Ka^B). The solid line in Fig. 1 is calculated using eqn (2) and $k_2 = 0{\cdot}192\,\text{M}^{-1}\text{s}^{-1}$, $Ka = 7{\cdot}24 \times 10^{-8}$ and $Ka^B = 1{\cdot}48 \times 10^{-6}$ at 25 °C and 1·0M ionic strength:

$$\text{Rate} = k_2[DB_1^+][SO_3^{2-}] \tag{1}$$

$$k_{obs} = k_2 \times \frac{[D^+]}{[D^+] + Ka^B} \times \frac{Ka}{[D^+] + Ka} \tag{2}$$

Chemistry is filled with numerous examples in which sulphite ion adds to an organic entity to give a covalent sigma complex, e.g. aldehydes and many heteroaromatic compounds, including those with a pyrimidine ring (Pitman & Sternson, 1976). An adduct therefore seems likely in the thiamine reaction.

In attempting to decide whether sulphite ion adds to the pyrimidine or to the thiazole ring of thiamine, the reactivity of a thiamine analogue (V) was examined. This analogue was synthesised from I; the thiaxole ring of I was replaced by pyridine in a reaction (Shimahara *et al.*, 1974) which again emphasises that I must react with sulphite ion by a multi-step route. The pyridine was substituted for the thiazole only in the presence of aqueous sulphite ion. Again, there must be a competition between two nucleophiles; in this synthesis they are sulphite ion and pyridine.

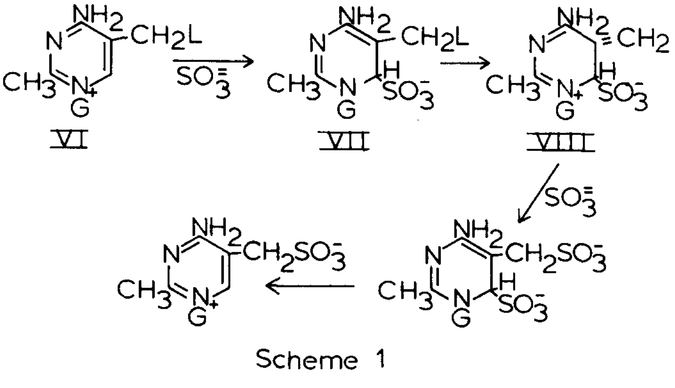
As with thiamine, the pyridine analogue did not react with azide alone under conditions where it underwent substitution with sulphite ion to give sulphonic acid (II) and pyridine. But again, when azide and sulphite ions compete, it is possible to observe the formation of azide substitution product (IV). Moreover, the pyridine analogue shows a 'bell' acidity-rate profile with a rate maximum at essentially the same acidity as thiamine. Comparison of acidity-independent second-order rate constants reveals that the pyridine substrate is only 2·0 times as reactive as thiamine. Clearly, the replacement of the thiazole ring by a pyridine ring gives a substrate that reacts with sulphite ion with no significant change in reactivity. Therefore, sulphite ion does not form a kinetically important adduct with either the thiazole or the pyridine ring. Rather, sulphite ion must add to the pyrimidine portion that is common to substrates I and V.

Addition of sulphite ion is expected to be facilitated when the pyrimidine ring is made more electrophilic by protonation. Protonation of N-1′, the thermodynamically favoured site (Cain *et al.*, 1977), gives VI (G = H and L denotes a leaving group such as thiazole (III)). Sulphite ion nucleophile is expected to add to those positions that will lead to neutralisation of the positive charge produced by protonation. Of the several sites, C-6′ seems to be the most likely because it is unsubstituted and therefore the least sterically hindered. Sigma complex VII (G = H) is therefore suggested to be an intermediate in the substitution reaction.

Adduct VII is not likely to be present in high concentrations; its presence has not been detected either by proton magnetic resonance or by spectrophotometry. We make a steady-state assumption regarding its presence in kinetic derivatives. The resultant kinetic expressions accurately describe observed rates.

More steps must be added to explain the substitution of thiamine. We suggest that adduct VII then eliminates the thiazole ring to give resonance stabilised cation VIII. A second sulphite ion must become involved now; this ion appears in the final product. Thus, addition of a second sulphite ion to VIII gives the precursor of the observed sulphonic acid product. Loss of the first sulphite ion regenerates the aromatic pyrimidine ring. The entire multi-step sequence of nucleophilic substitution of thiamine, and also of its analogues, is summarised in Scheme 1.

Important confirmation of this Scheme and the presence of two sulphite ions came from studies that employ low concentrations of sulphite ion (Doerge & Ingraham, 1980). In these experiments, the limiting reagent is not thiamine, but rather sulphite ion. Under these conditions, the rate-limiting step of the reaction changes to that in which intermediate VIII is trapped by the second sulphite ion. Kinetically,

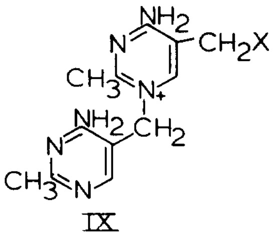


Scheme 1

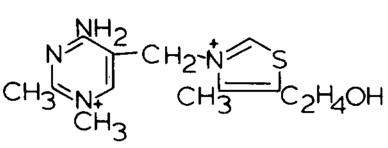
however, these experiments are quite complex because, under the conditions where sulphite ion becomes the limiting reagent, thiamine itself competes with sulphite ion for cationic intermediate VIII. That thiamine can trap VIII was demonstrated by isolation of an appropriate product, bispyrimidine (IX) (Zoltewicz & Uray, 1981*b*).

The isolated bispyrimidine (IX) does not contain a thiazole ring as in thiamine, but, instead, has a sulphonic acid group in its place, i.e. $X = SO_3H$. Trapping intermediate VIII by thiamine nucleophile gives thiamine derivative IX where group X is thiazole (III). But this is more reactive toward sulphite ion than thiamine itself (Zoltewicz *et al.*, 1981). Hence, our isolated bispyrimidine sulphonic acid is an expected product from a series of reactions in which intermediate VIII is trapped by thiamine to give a compound that, in turn, reacts with sulphite ion. Significantly, the presence of two pyrimidine rings does confirm that thiamine itself acts as a nucleophile.

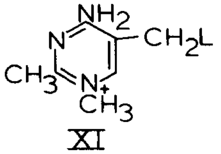
In order further to support the proposed mechanism, and especially to learn more about the early steps in the substitution scheme, kinetic studies with 1′-methylthiaminium ion X (Zoltewicz & Baugh, 1980) and its analogues (XI) (Zoltewicz, 1980) were undertaken. In the analogues leaving group, L is either a pyridine ring or a phenoxide ion. The *N*-methyl group was added to the pyrimidine ring in order to make it more electrophilic. Elaborate pH studies were not necessary for the *N*-methyl compounds, as was the case with substrates I and V having a free pyrimidine ring that requires protonation for activation. These activated substrates underwent facile substitution with sulphite ion, conveniently studied by spectrophotometry.



IX



X



XI

TABLE 1

Summary of Kinetic Results for the Cleavage of 1′-Methylthiaminium Ion and Its Analogues by Aqueous Buffered Sulphite Ion at 25·0 °C

Leaving group (*L*)	*Ionic strength* (M)	*Total sulphite ion* (M)	*pH*[a]	*Number of runs*	k_2 M^{-1} s^{-1}[b]	pK$_a$(*HL*)[c]
'Thiazole'[d]	1·0	0·025–0·049	6·27–7·57	9	0·041 0 ± 0·002 1[e]	3·5[f]
	0·50	0·048–0·050	6·95–6·98	3	0·104 ± 0·003	
	0·15	0·019 0	7·10	2	0·363 ± 0·008	
	0·071	0·014 6	6·55	2	0·528 ± 0·006	
	0·039	0·007 50	7·36	1	0·628	
Nicotinamide	1·0	0·008 4–0·050	5·08–6·32[a,g]	6	2·37 ± 0·17[h]	3·55[i]
Pyridine	1·0	0·024–0·049	7·33–7·52	4	0·087 ± 0·003[h]	5·52[i]
	0·50	0·048 0	6·95	2	0·232 + 0·013	
	0·15	0·019 8	7·10	1	0·833	
	0·071	0·014 3	6·55	2	1·19 ± 0·09	
	0·039	0·007 40	7·37	1	1·45	
	0·031	0·007 00	6·57	1	1·94	
4-Methylpyridine	1·0	0·049 6	6·83–7·34	2	0·011 0 ± 0·000 1[h]	6·33[i]
3,4-Dimethylpyridine	1·0	0·050–0·098	6·50–9·05[a,j]	3	0·003 49 ± 0·000 3[h]	6·79[k]
	0·50	0·048 0	6·95	1	0·009 70	
	0·039	0·007 70	7·36	1	0·063 7	
p-Nitrophenoxide ion	1·0	0·010–0·049	6·26–7·54	6	0·086 4 ± 0·014	7·15[l]
	0·50	0·049 0	6·95	1	0·153	
	0·15	0·020 0	7·08	1	0·284	
	0·071	0·014 1	6·48	1	0·392	
	0·031	0·007 20	6·46	1	0·480	
p-Cyanophenoxide ion	1·0	0·048–0·049	6·83–7·35	4	0·058 3 ± 0·002 0	7·95[l]
m-Chlorophenoxide ion	1·0	0·049 0	9·05[j]	2	0·038 0 ± 0·000 3	9·02[l]
m-Methoxyphenoxide ion	1·0	0·020–0·049	10·05[m]	2	0·012 9 ± 0·001 3	9·65[l]
Phenoxide ion	1·0	0·025–0·049	9·74–9·76[m]	3	0·006 36 ± 0·000 20	10·00[l]
p-Methylphenoxide ion	1·0	0·049–0·092	9·01–10·15[j,m]	4	0·005 5 ± 0·000 5	10·26[l]

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[a] Phosphate buffer except as noted. [b] Including average deviation. [c] Conjugate acid of leaving group. [d] 4-Methyl-5-(2-hydroxyethyl) thiazole. [e] Zoltewicz *et al.* (1981). [f] Lienhard (1970). [g] Acetate buffer. [h] Zoltewicz *et al.* (1982). [i] Jencks & Gilchrist (1968). [j] Borate buffer. [k] Fersht & Jencks (1970). [l] Albert & Serjeant (1971). [m] Carbonate buffer.

N-METHYLATED THIAMINE AND ITS ANALOGUES

The substitution reaction of X and sulphite ion gives thiazole (III) and sulphonate betaine (XI) ($L = SO_3^-$). Rates are independent of pH unless the acidity is high enough to change sulphite to bisulphite ion. Rates are first order in each of the two reactants. The *N*-methylated thiamine in H_2O is only 4·8 times less reactive than the conjugate acid of thiamine in D_2O (Zoltewicz *et al.*, 1981). Perhaps the lower reactivity of the *N*-methyl substrate reflects some inhibiting steric interaction involving the adjacent methyl group and the nucleophile, as well as retardation due to electron donation by the methyl substituent.

N-methylated analogues (XI) (where L is a family of pyridine-leaving groups) demonstrate rates that are first order in each of the two reactants. Second-order rate constants are highly dependent on the identity of the pyridine leaving group. Some substrates are more reactive than the *N*-methylated thiamine, others less. For example, when the leaving group is 3-carbamoylpyridine (nicotinamide), the substrate is 58 times more reactive than X, and when it is 3,4-dimethylpyridine it is 12 times less reactive (Table 1) (Zoltewicz *et al.*, 1982). An electron withdrawing substituent on the pyrimidine ring increases reactivity, whilst a donor group has the opposite effect.

By contrast, those analogues having a phenoxide ion as a leaving group show a much smaller kinetic sensitivity to structural changes in the leaving group. Again, rates are first order in each of the two reactants. The most reactive substrate in this class, that having a *p*-nitrophenoxide ion as a leaving group, is 2·1 times more reactive than X, whilst the least reactive, that having a *p*-methylphenoxide departing ion, is only 7·4 times less reactive than X. Again, electron-attracting groups promote reactivity, whilst donor substituents decrease rates of substitution.

Reactivity data for the previous pyridine analogues may be correlated using various linear free energy relationships (LFER), including Hammett and Brønsted equations. The linear four-point Hammett plot for compounds reacting at 1·0M ionic strength makes use of σ values (Exner, 1972) and has $\rho = 5{\cdot}88$ (correlation coefficient $r = 0{\cdot}998$). The Brønsted plot that employs the pK_a of the conjugate acid (HL) of a leaving group has slope (β) equal to $-0{\cdot}86$ ($r = 0{\cdot}994$). The large values of ρ and β indicate that electronic effects operating in the departing group are very significant and demonstrate that the rate-limiting step in the

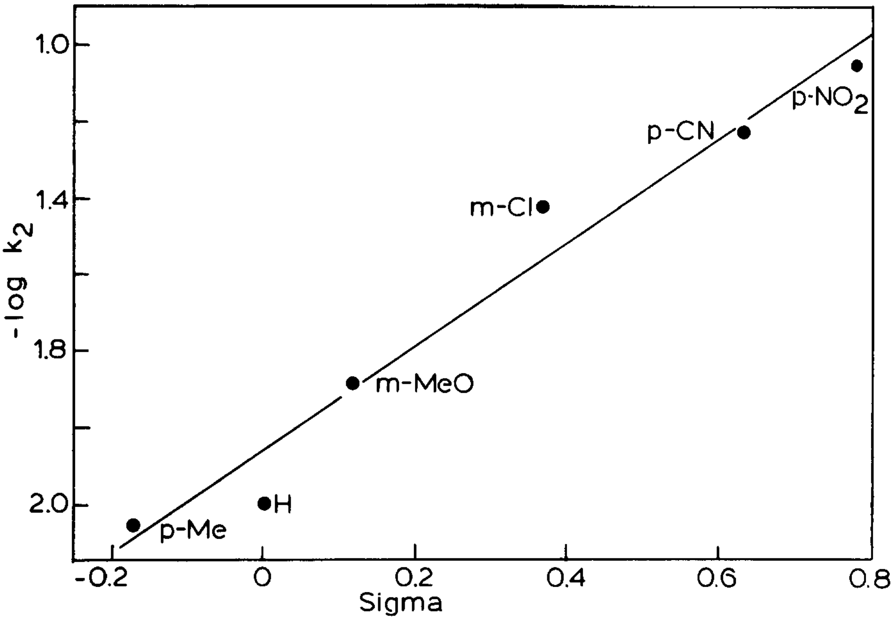


Fig. 2. Hammett plot for the cleavage of 4-amino-5-aryloxymethyl-1,2-dimethylpyrimidinium ions (XI) by sulphite ion at 25·0 °C and 1·0M ionic strength in H_2O.

multi-step sequence is loss of the pyridine leaving group. Addition of sulphite ion to the pyrimidinium ring is reversible.

Interestingly, the point for X does not fall on the Brønsted plot established by the pyridines. Judging from the pK_a of the conjugate acid of the thiazole leaving group, this substrate is about 75 times less reactive than a pyridinium ion with the same pK_a. Groups in which charge is highly stabilised by resonance are notoriously slow in leaving in the rate-limiting step (Gresser & Jencks, 1977).

Similarly, a Hammett plot (Fig. 2) for substrates having phenoxide ion leaving groups is also linear. But the point for phenoxide ion curiously lies below the line (40 % deviation) and that for *m*-chlorophenoxide ion is above (35 %); these deviations fall outside the range of experimental error. We have no explanation for this scatter. However, the overall correlation is satisfactory, ρ being 1·35 ($r = 0{\cdot}983$). A similar plot using σ^0 rather than σ values is of similar quality ($\rho^0 = 1{\cdot}28$, $r = 0{\cdot}975$). The σ^0 values are used when conjugation with a reaction site is not possible. For our substituents both sets of sigma values are similar and so no significant change is expected in the correlation.

By contrast, a similar Brønsted plot (not shown) for those analogues having phenoxide ion leaving groups is curved. There is a well-defined linear region having the *p*-nitro, *p*-cyano and *m*-chloro substrates with $\beta = -0{\cdot}19$. The less reactive substrates lie below this line. The slope of this second region is $-0{\cdot}71$ and includes the *m*-chloro value. It is tempting to conclude from this interesting observation that the change in slope shows there is a change in the identity of the rate-limiting step. Thus, for those substrates having the less negative Brønsted slope, it would seem that the leaving group does not depart in the rate-limiting step. Instead, addition of sulphite ion to the heterocycle ring is the critical step. But where the slope is more negative, loss of the leaving group becomes rate limiting. In other terms, the more basic phenoxide ions are poorer leaving groups and, when these groups become basic enough, their departure becomes critical—a reasonable and appealing interpretation. However, we believe this view is faulty.

The Hammett and Brønsted correlations do not suggest consistent interpetations for the phenol substrates. Both LFER indicate that the most reactive phenols undergo substitution by a mechanism in which addition of the nucleophile to the ring is the critical step. However, they point to divergent interpretations for those substrates having the least activating phenol substituents. For these, the Hammett plot remains linear while the Brønsted correlation becomes curved.

We believe the curved Brønsted plot is misleading. It is an artefact of the way substituents influence the pK_a values for phenols. Special σ^- constants are required to correlate acidities for a few selected groups such as *p*-nitro and *p*-cyano, where conjugation is possible between the oxide centre and these groups bonded to the phenoxide ion. Other substituents give pK_a values that correlate with σ. Supporting our conclusion that the Brønsted plot is not an accurate reflection of the rate-limiting step is a plot of σ versus σ^-. This plot generates the same biphasic shape and curvature occurs with the same substituents as is observed with the Brønsted plot. Therefore, the curved plot found with our substrates reflects not a change in rate-limiting step but a dual substituent scale required by pK_a.

The small Hammett ρ value of $1{\cdot}35$ suggests that a phenoxide ion is not departing in the rate-limiting step. The rate-limiting step occurs earlier in the mechanism and is the addition of sulphite ion to the pyrimidinium ring. According to this, the leaving group influences reactivity by means of a field (inductive) effect. Inappropriate, then, is an LFER that includes a parameter (pK_a or σ^-) reflecting conjugation between an oxide centre and a *para* electron withdrawing substituent.

Although the phenol and pyridine substrates have different rate-limiting steps—addition of sulphite ion for the former and loss of the leaving group for the latter—rate constants for the two classes can be similar. For example, the substrate having pyridine as a leaving group is only 14 times more reactive at ionic strength 1·0M than that having phenoxide ion as the departing entity. However, since Hammett ρ values for the two classes are so different, large differences in reactivity can also be encountered.

SALT EFFECTS

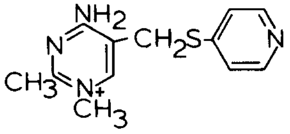
The data in Table 1 show that the rate constants for all substrates increase as the ionic strength of the solvent is reduced. For example, for X a reduction in the ionic strength from 1·0 to 0·039M gives rise to a 15·3-fold acceleration and for the pyridine substrate a decrease from 1·0 to 0·031M leads to a 22·3-fold acceleration. But, for the *p*-nitrophenoxide compound, lowering the ionic strength from 1·0 to 0·031M produces only a 5·6-fold rate increase.

Not surprisingly, reactivities of the pyridine substrates are much more responsive to variations in the polarity of the reaction medium than are the phenols. In the former case a di-cation reacts with a di-anion to give an overall neutral transition state whilst, in the latter, a mono-cation reacts with a di-anion to give a transition state which is anionic. The larger change in charge with the pyridine compounds gives rise to a wider rate constant variation. The pyridinium substrates must have considerable ground state electrostatic destabilisation associated with the presence of two positively charged rings bonded together by a single saturated carbon atom. The magnitude of this repulsive interaction—and consequently the energy of the ground state—is dependent on the polarity of the reaction medium.

COMMON ION RETARDATION

N-Methylated thiamine analogue (XII), having the anion of 4-thiopyridone as a leaving group, has special important properties. It can be made to undergo substitution under conditions where the kinetics become second order in sulphite ion. Moreover, in contrast with I, where it is necessary to make sulphite ion the limiting reagent, this analogue shows the second-order term in the presence of excess sulphite ion. The reason for this special property is that the leaving group. a thiolate ion, has a very high reactivity toward cationic intermediate VIII. The ability of the thiolate ion to capture cation VIII is even greater than that of sulphite ion.

In a series of experiments, rates of substitution of XII by sulphite ion were determined. Present at the start of a kinetic experiment was some of the leaving thiolate ion; this ion was prepared by adding 4-thiopyridone to the alkaline reaction medium. This ion, common to that in the substrate, serves to retard the rate of substitution by sulphite ion by capturing intermediate VIII and returning it to starting material, thereby preventing the forward reaction with sulphite ion. (A control showed that the sulphonic acid substitution product XI ($L = SO_3^-$) was unreactive toward the thiolate ion and did not give XII.) By increasing the amount of common ion present at the start of the reaction it is possible to make the kinetics second order in sulphite ion; addition of sulphite ion to starting compound and loss of the leaving group now become pre-equilibrium steps. Appropriate treatment of the kinetic data shows that the anion of 4-thiopyridone is about 250 times more reactive toward cationic intermediate VIII than is sulphite ion (Zoltewicz *et al.*, 1980). The complex mechanism of substitution given in Scheme 1 is made even more secure by these observations.



XII

SULPHITE VERSUS HYDROXIDE ION

In order to achieve some perspective on the nucleophilicity of sulphite ion, we made some reactivity comparisons with another nucleophile, hydroxide ion, reacting with some of the same substrates. However, neither I (Chahine & Dubois, 1983) nor X (Zoltewicz & Uray, 1980) undergoes nucleophilic substitution with hydroxide ion, because a faster reaction takes place—addition of hydroxide ion to the thiazolium ring to give a ring-opened hydrolysis product. But thiamine analogues do undergo facile nucleophilic substitution in alkaline solution.

Thiamine analogues, having *p*-cyanophenoxide ion or pyridine, underwent substitution in phosphate (pH 11) buffers at 25°. Proton magnetic resonance analysis of reaction mixtures confirmed the presence of the expected leaving groups and demonstrated that the pyrimidine product contained a $CH_2OPO_3^{2-}$ side-chain (Zoltewicz & Uray, 1981*a*). Since the kinetics are first order in substrate and in hydroxide ion, and since the product contains the buffer anion instead of hydroxide ion, an intermediate must again be present. That is, hydroxide ion controls the rate, but it does not appear in the final product. Therefore, product cannot arise by a single step S_N2 mechanism.

Further support for a multi-step hydrolytic substitution pathway is found in the results of competition experiments in which hydroxide ion competes with a thiolate ion. Present at the start of a kinetic run, in addition to substrate and buffer, was *p*-nitrobenzenethiolate ion. Although the rates of hydrolysis are not influenced by the presence of the thiolate ion, pyrimidine substitution product contains this nucleophile. The resultant sulphide is the major product containing the pyrimidine ring. Clearly, hydroxide ion, like sulphite ion, serves as a catalyst to allow the attachment onto the pyrimidine side-chain of a second nucleophile. A multi-step mechanism of substitution must operate (Zoltewicz & Uray, 1981*a*).

Minor modifications to Scheme 1 are required in order to express the hydrolysis pathway. As suggested by the kinetics, hydroxide, rather than sulphite, ion must add to the pyrimidine ring; the resultant adduct then eliminates the leaving group to give an intermediate that may be trapped by one or more different nucleophiles present in the reaction medium. Loss of hydroxide ion then regenerates the aromatic pyridine ring.

Revealing is a comparison of second-order rate constants for sulphite and hydroxide ions, both reacting by the common mechanism of Scheme 1 with the same substrates. Thus, when pyrimidine is the leaving group, hydroxide is 21 times more reactive than sulphite ion. For a substrate having *p*-cyanophenoxide ion, this same ratio is 5·3. Hydroxide is a little more reactive than sulphite ion.

From the standpoint of rates of reaction toward thiamine-like compounds, the reactivity of sulphite ion is not special. What distinguishes sulphite ion from hydroxide ion is its much lower basicity (pK_a 6·6, HSO_3^- versus 15·7, H_2O). Hence, sulphite ion can exist in high concentrations even in weakly acidic solutions, whereas hydroxide ion cannot.

SPECIAL PROPERTIES OF SULPHITE ION

Throughout our investigation there are numerous examples of interesting competitions between pairs of nucleophiles. For example, sulphite ion serves as a useful catalyst for the decomposition of thiamine, while azide ion does not. Yet azide ion can successfully compete with sulphite ion for intermediate VIII. Clearly, both sulphite and azide ions are powerful nucleophiles. What distinguishes sulphite from azide ion is the poor leaving ability of the former ion. Addition of a nucleophile to the pyrimidine ring (Scheme 1) does not give rise to substitution, if this nucleophile comes off the adduct to give back starting materials before the leaving group departs from the side-chain. Insufficient for catalysis is a good nucleophile which also is a good leaving group. Sulphite ion is useful not only because it is a good nucleophile which rapidly adds to the pyrimidine ring, but also because it is a poor leaving group, remaining on the adduct long enough so that departure of the leaving group from the side-chain takes place.

The combination of high nucleophilic reactivity, poor leaving group ability and weak basicity makes sulphite ion so effective in the substitution reactions of thiamine and its analogues.

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