A Generalized Multistep Mechanism of Nucleophilic Substitution of Heterobenzylic Cations by Sulfite Ion

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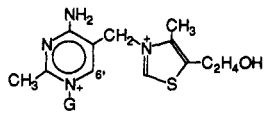
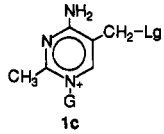
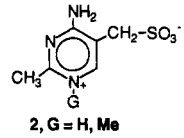
Thiamin (**1a**), its *N*-methyl derivative[1] **1b**, and their analogs **1c**[2,3] react with sulfite ion by an unusual multistep addition–elimination mechanism of nucleophilic substitution designated $S_N(AE)$. Instead of the sulfite nucleophile attacking the methylene side chain directly to displace the leaving group and give the observed sulfonate substitution product **2** in the normally expected S_N2



1a, G = H
1b, G = Me



1c
Lg = a pyridine
Lg = OAr
Lg = SAr



2, G = H, Me

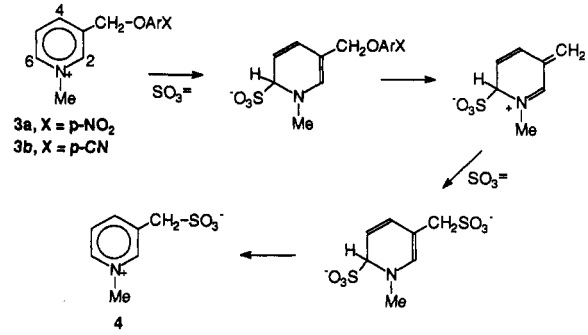
mechanism, it adds to the 6′ position of the pyrimidinium ring to give an adduct. Expulsion of the leaving group from the adduct provides a resonance-stabilized intermediate cation that then is trapped by a second sulfite to give the final substitution product following elimination of the first sulfite ion. Although two sulfite ions are involved in this complex pathway it is only under special conditions that the rates of substitution become second order in sulfite,[4–6] an important observation supporting the complex mechanism.

We now present kinetic results for a heterobenzylic ring system **3** reacting with sulfite ion. The simple pyridinium cations **3a** and **3b** react by the same kind of $S_N(AE)$ mechanism discovered for **1a–c**.

Heterobenzylic phenoxides **3a** and **3b** lack many of the substituents of the pyrimidine ring of **1**, but they do contain the two defining and characteristic structural features which seem to be necessary for this addition–elimination pathway to occur. (1) An aromatic ring is made electrophilic by a quaternized annular nitrogen atom to facilitate nucleophilic addition, and (2) a saturated carbon chain with a leaving group attached is located "meta" to the nitrogen atom. Because of its structural simplicity **3** becomes the archetypical substrate for this mechanism, illustrated in Scheme I.

The phenoxide ion nucleofuges were selected deliberately because they usually only depart with considerable difficulty from a saturated carbon of the resultant ether and therefore are uncommon leaving groups.[7] Our facile substitution highlights the special reactivity of sulfite ion

Scheme I



3a, X = p-NO_2
3b, X = p-CN

in the $S_N(AE)$ reaction with poor leaving groups. These nucleofuges when bonded to saturated carbon have been employed in E1cb elimination reactions[8,9] and in S_N2 reactions of an acetal,[10] for example.

Results

Rates. Substrates were synthesized from 3-(chloromethyl)pyridine and the phenoxide ion in methanol followed by quaternization of the product of this substitution reaction with methyl iodide.

Pseudo-first order rate constants, k_{obs}, were obtained by our usual spectrophotometric method using mostly phosphate and occasionally carbonate buffers to maintain constant pH in the presence of variable amounts of sulfite ion at an ionic strength made constant to 1.0 M with KCl.[11] The total concentration of sulfite ion was corrected for the fractional amount present in the reactive free base form and not as unreactive bisulfite by using the term $K_a/(K_a + [H^+])$ where the pK_a of bisulfite ion is 6.59 and $[H^+]$ is the value from the measured pH. The data were then subjected to a linear regression analysis of k_{obs} versus the concentration of free sulfite ion. The calculated intercept was negligible, indicating that no competing hydrolysis took place. For nitro **3a** the free sulfite ion concentration was varied by a factor of 11, and for cyano **3b** the factor was 3.6. The reactions clearly are first-order in sulfite ion. The derived second-order rate constants and their standard deviations are presented in Table I.

A control experiment served to establish the upper limit to the rate constant for the hydrolysis of nitro **3a**. At pH 10.76 in the absence of sulfite the long half-life of about 74 h (4.5×10^{-3} M^{-1} s^{-1}) confirms that hydrolysis does not compete with the much faster sulfite reaction.

Attempts to isolate pure betaine sulfonate product **4** were unsuccessful, and so the structure of the product was verified by NMR analysis of reaction mixtures. To separate samples of the two substrates was added some sulfite, and the NMR spectrum was recorded. In both cases the same pyridine product was observed and its spectrum was consistent with structure **4**. The methylene signal at 5.50 ppm of starting material moved to 4.40 ppm and is similar to that for sulfonate betaine **2** at 4.16 ppm[1] and unlike that at 4.85 ppm for the methylene position of authentic hydrolysis product, 3-(hydroxymethyl)-1-methylpyridinium ion.[12] The phenylene protons of **3** move upfield on being converted to the phenoxide ion.

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Table I. Conditions and Kinetic Results for the Reaction of Sulfite Ion with 3-([Aryloxy)methyl]-1-methylpyridinium Iodides 3 at 25 °C in Aqueous Buffers[a]

substituent	$10^2[SO_3^{2-}]$,[b] M	no. of runs	pH	10^3k_2, M^{-1} s^{-1}	λ,[c] nm
p-NO_2	3.89–11.0	9	6.44–9.71	14.8 (1.1[d])	395
p-CN	3.89–9.99	4	6.98–10.3	7.00 (0.18[d])	285

[a] Ionic strength 1.0 M maintained with KCl. [b] Total amount. [c] Analytical wavelength. [d] Standard deviation.

Trapping Reaction. In order to verify the expected presence of an intermediate a trapping reaction was employed using the thiolate ion of 4-nitro-3-carboxythiophenol conveniently generated from Ellman's reagent[13] in the presence of sulfite.[14] The cyano substrate **3b** was selected for study since the *p*-cyanophenoxide ion leaving group does not provide a chromophore that interfers with the absorbance of the nitrothiolate nucleophile. In related experiments with sulfite we have successfully used a thiolate ion to trap the intermediate generated from substrate **1c** having the same thiolate ion as a leaving group, an example of common ion retardation.[4]

The rate constant for the reaction of **3b** with 0.111 M sulfite in a carbonate buffer in the presence of 2×10^{-4} M nitrothiolate was obtained under pseudo-first-order conditions by following the decrease in absorbance at 412 nm associated with reductions in the concentration of the nitrothiolate ion. The value of the observed rate constant, 5.94×10^{-3} M^{-1} s^{-1}, compares favorably with that of 7.00×10^{-3} M^{-1} s^{-1}, Table I, obtained in the usual way by following the release of the cyanophenoxide ion leaving group at 285 nm. This crucial experiment reveals that an intermediate is present and that the thiolate ion reacts after the rate-limiting step to trap this material generated from the substrate and sulfite ion.

Control Experiments To Define the Limitations of the Subsitution Reaction. (1) The nonquaternized synthetic precursor of **3a** was exposed to a high concentration of sulfite ion (0.333 M, pH 10.68), and the absorption spectrum was scanned over 65 h at 25 °C without revealing a change. By comparison, the half-life of its quaternized form **3a** is 2.3 min under these conditions. Clearly, the nitrogen atom must be quaternized in order for the reaction with sulfite to take place with some facility.

(2) Quaternized nitro **3a** was exposed to a new nucleophile, 0.333 M thiosulfate ion, for 40 h at 25 °C; again, there was no change in the absorbance spectrum. Thiosulfate ion does not react under conditions where sulfite ion does.

Discussion

The following evidence supports the $S_N(AE)$ mechanism for the nucleophilic substitution reactions between sulfite ion and heterobenzylic **3a** and **3b** rather than the usual S_N2 mechanism found for carbocyclic benzylic compounds.[15] (1) The nonquaternized precursor of **3a** does not react with sulfite ion. By contrast, benzyl and *p*-nitrobenzyl bromides have a similar reactivity in authentic S_N2 reactions.[16] For example, the former bromide is a mere 1.5 times more reactive than the latter toward aqueous sulfite ion.[17] (2) Thiosulfate ion does not react with **3a**, and yet it is known to be a highly reactive nucleophile in S_N2 reactions.[18,19] For example, thiosulfate is only 5.8 times less reactive than sulfite towrd benzyl bromide.[17] (3) When the absorbance changes associated with a nitrobenzenethiolate ion are used to measure the kinetic effect of sulfite reacting with **3b** the same rate constant as that observed in the absence of the thiolate trapping agent is obtained. An intermediate must be generated from **3b** and sulfite that is trapped in a fast reaction by the thiolate ion. Moreover, the ability of the thiolate ion present at a much lower concentration to compete with the much higher concentration of sulfite ion (about 500 times) suggests that the trapping of this intermediate by sulfite is probably an activation-limited and not a diffusion-limited reaction. Diffusion-limited reactions have been observed for sulfite reacting with a simple ether[20] and for azide and thiolate nucleophiles trapping benzyl cations.[21] (4) The reactivity of **3a** relative to **3b**, a factor of 2.1, Table I, is similar to the value of 1.5 found for *N*-methylthiamin analogs **1c** with the same phenoxide leaving groups, all reacting with sulfite ion.[2,3] This small kinetic dependence on the nature of the phenoxide ion leaving group for both the pyridine and pyrimidine rings suggests a common reaction pathway. Interestingly, the reactivity of pyridines **3a** and **3b** toward sulfite ion is only 5.8 and 8.3 times less, respectively, than that of pyrimidines **1c** with the same leaving groups.[2,3]

Mechanism. The bimolecular mechanism of substitution for **3a** and **3b** reacting with sulfite ion is likely to be similar to the $S_N(AE)$ multistep addition–elimination we have discovered for sulfite and thiamins **1a** and **1b** and also their analogs **1c** having pyridine, phenoxide, and thiophenoxide leaving groups.[1-3]

According to this mechanism, Scheme I, the first step is the addition of sulfite to the quaternized electrophilic aromatic ring at a position either α or γ to the positively charged annular nitrogen atom so as to neutralize the charge. There is no information as to whether this reactive site is the 2, 4, or 6 position of the ring (or more than a single site), and so we arbitrarily add sulfite to position 6. Following this, the leaving group departs to give a resonance-stabilized cation. This cation may then be trapped by sulfite ion or by the nitrobenzenethiolate ion when present in competition with it. The resultant adduct then expels the sulfite ion to yield the observed substitution product **4**.

While it is unlikely that the first sulfite adduct is present in any significant amount,[22,23] it is the amino group generated in this adduct that provides the significant driving force for the expulsion of the poor leaving group.

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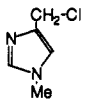
(23) Equilibrium constants, K_{assn}, for the association of sulfite ion with the 4-position of 1-methyl-3-substituted pyridinium ions have been correlated by a linear free energy relationship.[22] This correlation gives an approximate equilibrium constant for the formation of the corresponding adduct from the unsubstituted parent ion of 10^{-7} M^{-1}. To the extent that this equilibrium constant applies in our case, an amazingly large value of 10^5 s^{-1} may be estimated for the rate constant for the loss of the leaving group from a sulfite adduct of **3** using the expression k_2/K_{assn} and the values of k_2 in Table I.

The high reactivity of the adduct compensates for its low concentration thereby leading to a net favorable reactivity. Remarkably, nitro **3a** is only 33 times and cyano **3b** 70 times less reactive than benzyl bromide toward sulfite ion under the same conditions.[17] Benzyl bromide with its much superior leaving group reacts, of course, by the alternate S_N2 mechanism.[16,24] Our amino group activation is reminiscent of that provided by the imidazole ligand in the facile hydrolysis by an S_N1 route of 4-(chloromethyl)-1-methylimidazole (**5**) to give a resonance-stabilized cation (40 s^{-1}, 30 °C).[25]

Generalized Mechanism of Nucleophilic Substitution. The $S_N(AE)$ mechanism presented in Scheme I for pyridinium ions and a similar scheme for thiamin and thiamin-like substrates has considerable generality beyond that for these two ring systems. Already we have demonstrated that a wide variety of leaving groups may be tolerated in the substitution process for **1c**,[2,3] including bromide ion.[17] In addition to sulfite ion the $S_N(AE)$ multistep mechanism has been demonstrated to take place with the lyate ions hydroxide and methoxide.[11,26]

Sulfite ion has long been known to give adducts with pyridinium ions.[22,27-29] Hydroxide ion also adds to quaternized pyrimidones[30] and to azines such as pyridinium,[22] quinolinium, and isoquinolinium ions to form adducts called pseudobases.[31] Cyanide[22,32,33] and thiolate[30,34] ions among others also give similar adducts. Numerous anions also add to uncharged heteroaromatic rings to give anionic σ complexes.[35] These nucleophiles and these ring systems also would seem to be good candidates for the multistep pathway of substitution, perhaps even for synthetic purposes.

In related reactions *nonquaternized* substrates such as cytidine[36] and uracil[37] add nucleophiles including sulfite ion to their ring positions. Compound **6**, used in model



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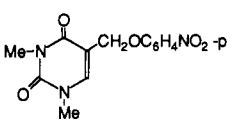

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studies of thymidylate synthetase,[38] undergoes side-chain hydrolysis by an addition–elimination route, further increasing the number of examples of the $S_N(AE)$ mechanism.

Now that the essential features have been delineated, the scope of the $S_N(AE)$ reaction remains to be established.

Experimental Section

3-(Hydroxymethyl)-1-methylpyridinium Iodide.[12] This highly hygroscopic salt was prepared from the corresponding carbinol and methyl iodide by mixing the two neat liquids together and cooling in ice following an induction period. The crystals were stored under ethyl acetate: ^1H NMR (D_2O, DSS) δ 8.80 (2-H), 8.72 (6-H, J = 5.7 Hz), 8.49 (4-H, J = 8.1 Hz) 8.04 (5-H), 4.85 (CH_2), 4.40 (CH_3).

3-[(4-Nitrophenoxy)methyl]pyridine. To 0.85 g (6.1 mmol) of 4-nitrophenol and 1.8 g (1.6 mmol) of K_2CO_3 was added 5 mL of methanol followed by 1.0 g (6.1 mmol) of 3-picolyl chloride hydrochloride. The yellow solution was allowed to stand overnight at room temperature. The brown mixture was added to 12 mL of water containing one pellet of NaOH and filtered to give 0.37 g (1.6 mmol, 26%) of product, mp 134–137 °C dec, which then was recrystallized from ethanol–water to give the analytical sample, mp 134–136 °C. Anal. Calcd for $C_{12}H_{10}N_2O_3$: C, 62.61; H, 4.38; N, 12,17. Found: C, 62.89; H, 4.18; N, 12.06.

1-Methyl-3-[(4-nitrophenoxy)methyl]pyridinium Iodide (3a). To a suspension of 0.37 g (1.6 mmol) of nitrophenoxy pyridine in 10 mL of methanol was added 1.4 g of methyl iodide. After the mixture was stirred overnight, it was filtered and washed with a little ethanol to give 0.32 g (0.86 mmol, 53%) of yellow solid, mp 175–177 °C dec. Recrystallization from ethanol gave the analytical sample, mp 175–177 °C, dec: ^1H NMR (DMSO-d_6–D_2O, DSS) δ 9.02 (H-2), 8.82 (H-6), 8.65 (H-4), 8.13 (H-5), 8.31, 7.92 (arom), 5.50 (CH_2), 4.42 (Me). Anal. Calcd for $C_{13}H_{13}N_2IO_3$: C, 41.96; H, 3.52; N, 7.53. Found: C, 41.93; H, 3.11; N, 7.45.

3-[(4-Cyanophenoxy)methyl]pyridine. To a solution of 1.33 g (11.2 mmol) of 4-cyanophenol and 1.8 g (13 mmol) of K_2CO_3 in 6 mL of methanol was added 1.0 g (6.1 mmol) of 3-picolyl chloride hydrochloride. The yellow solution was stirred at room temperature overnight, and then to the thick mixture was added 5 mL of methanol and 10 mL of water, followed by heating on the steam cone to achieve solution. On cooling pale needles formed; they were removed by filtration and washed with water to give 0.31 g (1.5 mmol, 25%) of product, mp 99–101 °C. The analytical sample was prepared by recrystallization from water with a little ethanol, mp 99–101 °C. Anal. Calcd for $C_{13}H_{10}N_2O$: C, 74.27; H, 4.79; N, 13.32. Found: C, 74.25; H, 4.48; N, 13.12.

3-[(4-Cyanophenoxy)methyl]-1-methylpyridinium Iodide 3b. To a solution of 0.21 g (1.0 mmol) of 3-[(cyanophenoxy)-methyl]pyridine in 2 mL of methanol was added 1 mL of methyl iodide. After the mixture stood overnight at room temperature, the crystals were removed and washed with ethyl acetate to give 0.052 g (0.76 mmol, 76%) of product, mp 178–182 °C. The compound was recrystallized from isopropyl alcohol with a little ethanol to increase solubility, mp 180–182 °C: ^1H NMR (DMSO-d_6, DSS) δ 9.22 (H-2), 9.05 (H-6), 8.70 (H-4), 8.23 (H-5), 7.90, 7.31 (arom), 5.50 (CH_2), 4.45 (Me). Anal. Calcd for $C_{14}H_{13}N_2IO{\cdot}H_2O$: C, 45.42; H, 4.08; N, 7.57. Found: C, 45.69; H, 3.99; N, 7.57. An infrared spectrum of the solid (DRIFT IR) did not show the presence of an amide carbonyl band but instead nitrile stretching at 2214 cm^{-1}, confirming that the material contains water of crystallization. It is not the amide hydrolysis product.

Kinetics. Aqueous stock solutions of 0.333 M Na_2SO_3 and of phosphate (carbonate) buffer usually containing >0.5 M KCl were mixed together in a cuvette to give a final ionic strength of 1 M and thermally equilibrated before a few microliters of substrate was added. Absorbance changes were submitted to a computerized linear regression analysis using the standard first-order method (log ($A_t - A_\infty$) vs time) and found to be linear for >4 half-lives, some as many as 9 half-lives.

Verification of the Formation of Betaine Product 4 by NMR Analysis of Reaction Mixtures. Approximately 10 mg (0.03 mmol) of **3a** or **3b** was added to an NMR tube containing about 12 mg (0.1 mmol) of Na_2SO_3 and a crystal of DSS followed by the addition of 0.4 mL of D_2O. The substrate slowly dissolved. About 3 h later the 300-MHz spectrum was recorded. The substitution reaction was clean and complete. Betaine product **4** has the following shifts: δ 8.87 (H-2), 8.78 (H-6), 8.55 (H-4), 8.06 (H-5), 4.40 (CH_2) and 4.42 (Me). Signals for the phenoxide ions appear upfield from the phenylene protons of starting material.

Control Experiments. (1) Hydrolysis. 1-Methyl-3-[(4-nitrophenoxy)methyl]pyridinium iodide in a carbonate buffer (0.30 M carbonate ion, ionic strength 1 M with KCl, pH 10.76) was kept at 25 °C for 190 min and the small absorbance increase

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measured at 395 nm. Some solid sodium sulfite then was added to produce rapidly a large asorbance increase and thereby simulate the "infinity" value. The slow hydrolysis represents 3.0% of the total absorbance change. This small change gives with the standard initial rate expression ($\Delta C/C_0 = kt$), an initial rate constant of 2.6×10^{-6} s^{-1}, and a second-order value of 4.5×10^{-3} M^{-1} s^{-1} based on the initial pH.

(2) Thiosulfate Ion. 1-Methyl-3-[(4-nitrophenoxy)methyl]-pyridinium iodide and 0.333 M sodium thiosulfate were kept at 25 °C for 40 h. No change in the ultraviolet absorption spectrum was found.

(3) Sulfite Ion. 3-[(4-Nitrophenoxy)methyl]pyridine stood for 65 h in 0.333 M Na_2SO_3 in a carbonate buffer (0.30 M carbonate ion, 1 M with KCl, pH 10.68) at 25 °C without showing an absorbance change.

Thiolate Ion Trapping of the Intermediate in the Reaction of 3-[(4-Cyanophenoxy)methyl]pyridinium Iodide with Sulfite Ion. The reaction mixture consisted of 1 mL of each of the following: 0.333 M sodium sulfite, a carbonate buffer made to an ionic strength of 1 M with KCl, and Ellman's reagent[13] (about 6 mg of the reagent in a phosphate buffer made to an ionic strength of 1 M with KCl). On mixing the three solutions the absorbance of the free thiolate ion (about 2×10^{-4} M) appeared immediately due to reaction with sulfite ion.[14] The reference cell of the spectrophotometer contained the same solution. On addition of the substrate there was a decrease in the absorance at 412 nm followed by a slow, linear increase, the first representing the desired change, the second a drift. Time-dependent absorbance changes were computed after back extrapolation of the linear region and comparison to this extrapolated line, a procedure amounting to a variable "infinity" value. The pseudo-first-order plot was linear over 4.5 half-lives with a first-order rate constant of 6.53×10^{-4} s^{-1} and a second-order value of 5.94×10^{-3} M^{-1} s^{-1}.