Hydrolysis and Alkylating Reactivity of Aromatic Nitrogen Mustards

Charmian J. O'Connor,*[a] William A. Denny,[b] Jun-Yao Fan,[a] G. Lance Gravatt,[b] Bruce A. Grigor[a] and Duncan J. McLennan[a]

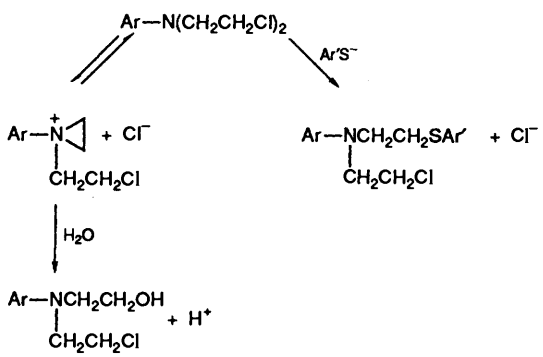
[a] *Department of Chemistry, University of Auckland, Private Bag, Auckland, New Zealand*

[b] *Cancer Research Laboratory, School of Medicine, University of Auckland, Private Bag, Auckland, New Zealand*

Rate constants for the hydrolysis of a series of aromatic nitrogen mustards Ar–X–C_6H_4–$N(CH_2CH_2Cl)_2$ (X = O, CH_2, CONH, S, CO and SO_2) in buffered aqueous acetone mixtures have been obtained using an HPLC technique which allows evaluation of the rate constants for hydrolytic displacement of both chlorines in consecutive reactions. Hydrolysis has a general-base-catalysed component and is accompanied by external chloride ion return. An aziridinium ion intermediate is implicated in at least one pathway. The mustards also alkylate the nucleophiles thiourea and 4-(4-nitrobenzyl)-pyridine (NBP) and again the aziridinium ion intermediate is involved since the kinetic behaviour rules out a direct S_N2 pathway.

Aromatic nitrogen mustards have been extensively studied because of their potential activity as antitumour chemotherapeutic agents.[1] However, the mechanism associated with their biological effectiveness remains contentious. We have recently published a study of the kinetics of alkylation of DNA by 4-anilinoquinolinium aniline mustards;[2] the present study comprises the mechanistic physical organic background to this work.

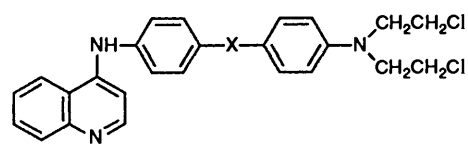
The question of the mechanism of hydrolysis of aliphatic nitrogen mustards has been reviewed.[3] There is little agreement in the research literature as to the mechanisms employed by aliphatic and aromatic nitrogen mustards. Claims of S_N1 mechanisms (some involving primary carbocations in solution), S_N2 mechanisms, and internal S_N2 processes wherein nucleophilic nitrogen participation affords an aziridinium ion intermediate have been made.[4–11] The most cogent evidence for the latter mechanism has been provided by Benn *et al.*,[12] who demonstrated that isotopic scrambling accompanied hydrolysis of mustards $ArN(CH_2CD_2Cl)_2$. However, alkylation of a thiolate ion was not accompanied by scrambling suggesting direct S_N2 displacement. A mechanism of minimum complexity is shown in Scheme 1, wherein displacement of only one chloride is considered. A review by Mandolini raises the question of small-ring formation in intramolecular S_N2 reactions.[13] Formation of three-membered rings is not always as unfavourable as ring-strain considerations would predict.



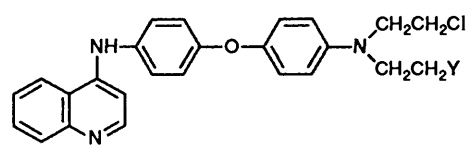
Scheme 1

Results and Discussion

Substrate Basicity.—The nitrogen mustards used in this study, compounds **1–6**, are dibasic. The 4-anilinoquinoline residue has a pK_a of *ca.* 8.4,[14] so that under the pH conditions used here the quinoline nitrogen will remain largely protonated and should thus not interfere as a nucleophilic species. Spectrophotometric measurements in KH_2PO_4–K_2HPO_4 buffer solutions at 20 °C established the following pK_a values for the nitrogen in the $-N(CH_2CH_2Cl)_2$ moiety: 6.15 (**1**), 6.05 (**2**), 6.50 (**3**), 5.95 (**4**), 5.85 (**5**) and 5.95 (**6**). These pK_a values correlate but poorly with the Hammett σ parameters for the CH_2X-groups[15] considered appropriate for comparison, principally because of the deviation of compound **3** (X = CONH), which is much more basic than expected. Interestingly, this compound is more toxic and exhibits greater antitumour activity than do other members of the series.[16]



1 X = O
2 X = CH_2
3 X = CONH
4 X = S
5 X = CO
6 X = SO_2



7 Y = H
8 Y = OH

Hydrolysis Kinetics.—Analysis by HPLC allowed the consecutive displacements of both chlorines of the mustards to be followed as a function of time. Typical traces of the eluents are shown in Fig. 1, and lead to the operational scheme proposed in Scheme 2. In subsequent discussion, species **9** will be called the half-mustard and species **10** the diol. Integration of peak areas for the mustard, half-mustard and diol gives a proportional measure of concentration changes as a function of time. For each substrate the molar absorption cofficients of mustard (A), half-mustard (B) and diol (C) were identical at the analytical wavelength, and thus all peak areas are direct measures of concentration. Plots of peak area against time are

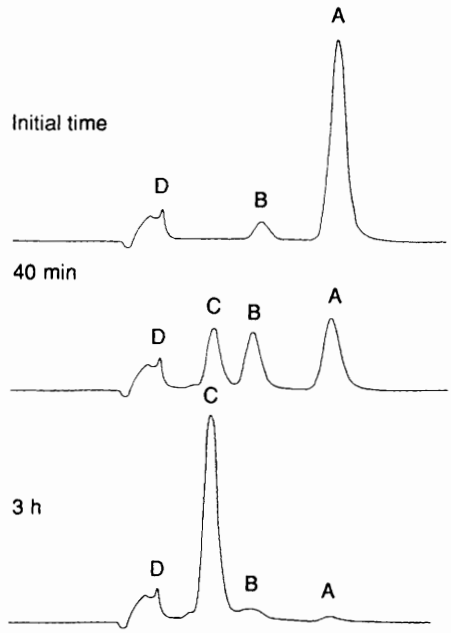


Fig. 1 Typical HPLC traces for mustard hydrolysis. Hydrolysis of 1 in 50% acetone–H_2O (v/v) at pH 6.50, 60 °C. Peaks A, B, C and D correspond to mustard 1, half-mustard **8**, diol, and solvent, respectively. Upper plot, zero time; middle plot, 40 min reaction time; lower plot, 3 h reaction time.

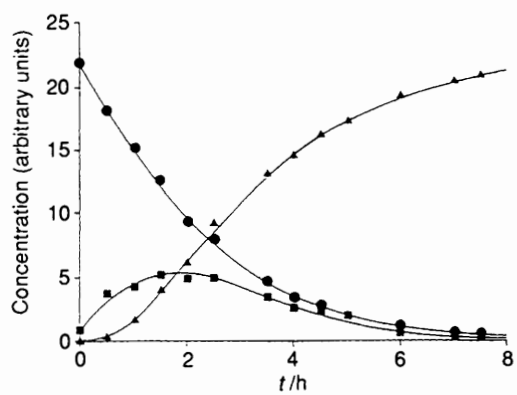
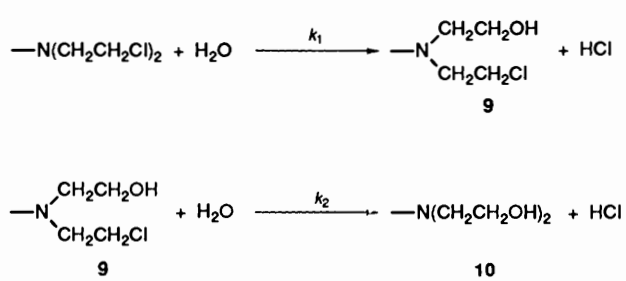


Fig. 2 Typical plots of change in concentration of mustard ●, half-mustard ■, and diol ▲ during the hydrolysis reaction. The curves are calculated from the best-fit rate constants for consecutive first-order reactions.



Scheme 2

exemplified by Fig. 2, which is symptomatic of consecutive first-order reactions.[17] First-order rate constants for the first (k_1) and second (k_2) steps were obtained from an iterative curve-fitting procedure, and the best-fit curves are also displayed in Fig. 2.

Table 1 shows the values of k_1 and k_2 for the mustards **1–6** over a pH range between 5.40 and 7.25 at 66 °C. In all cases the

Table 1 Pseudo-first-order rate constants of hydrolysis of 6.66 μmol dm^{-3} mustards at 66 °C and various pH values

Mustard	Rate constant	$10^5 k_{obs}/s^{-1}$ pH 5.40[a]	6.50[b]	6.95[a]	7.25[c]
1	k_1	24.5	31.5	42.4	13.1
	k_2	23.5	25.0	33.0	12.1
2	k_1	24.1	27.9	48.7	13.4
	k_2	35.1	27.9	85.3	16.7
3	k_1	18.9	32.6	38.6	16.8
	k_2	24.1	70.1	51.5	25.0
4	k_1	3.14	4.41	4.52	1.63
5	k_1	0.326	0.353	0.482	0.359
	k_2	0.506	0.694	0.994	14.4
6	k_1	0.116	0.148	0.221	0.184
	k_2	0.381	0.289	0.592	0.316

[a] 50% acetone–50% 0.02 mol dm^{-3} Tris/maleic acid buffer. [b] 50% acetone–50% water. [c] 50% acetone–50% 0.02 mol dm^{-3} Tris buffer.

k_1 and k_2 values for a given mustard are comparable. Overlapping peaks precluded the calculation of accurate k_2 values for **4**; these are omitted. As expected for a system where the basicities of the $-N(CH_2CH_2Cl)_2$ nitrogen vary over a 5.6-fold range, the overall reactivity order is dependent upon the pH. It will later be shown that general-base catalysis is a component of at least one step of the hydrolysis, and thus the complex dependence of rate constant for a given mustard on pH must be at least partly due to changes in chemical composition of the buffer.

Nevertheless, the solvolytic reactivity of the mustards is dependent on the identity of the X group separating the $-C_6H_4N(CH_2CH_2Cl)_2$ and anilinoquinoline moieties. Eqns. (1) and (2) show the dependence of k_1 and k_2 on the Hammett σ constant for CH_3X– at pH = 6.50.

$$\log k_1 = -(2.56 \pm 0.38)\sigma - (4.03 \pm 0.17) \quad (1)$$
$$n = 6,\ r = 0.92$$

$$\log k_2 = -(2.29 \pm 0.54)\sigma - (3.84 \pm 0.28) \quad (2)$$
$$n = 5,\ r = 0.86$$

These are far from perfect Hammett plots, but the substantial negative ρ values are of interest. Assistance by electron donation from group X is clearly evident, and the magnitude of ρ suggests that the assistance is reasonably strong. Inductive through-bond electron donation to C_α of the $-N(CH_2CH_2Cl)$ group is improbable in view of the presence of an insulating methylene group, and resonance plus field electronic effects can reasonably be expected to be transmitted no further than the nitrogen. Thus the nitrogen nucleophilicity is implicated in the reaction, which implies anchimeric assistance and possibly aziridinium ion intermediacy for the k_1 steps at least. The k_1 and k_2 rate orders for mustard variation are not the same at all pH values, and further intramolecular nucleophilic assistance by the –OH group of the half-mustard cannot be ruled out as a component of the k_2 step. Nevertheless there is no obligatory evidence for this.

Temperature Effects.—First-order rate constants have been obtained over a temperature range for some of the mustards at two different pH values (Table 2). Arrhenius plots of ln k_{obs} *vs.* $1/T$ were linear, and the associated Arrhenius parameters are also presented in Table 2. We ascribe no mechanistic significance to these, but one aspect of the temperature effects is

Table 2 Pseudo-first-order rate constants of hydrolysis of mustards (6.66 μmol dm $^{-3}$) at various temperatures and derived Arrhenius parameters

Mustard	pH	Rate constant	$10^5 k_{obs}/s^{-1}$ 37 °C	50 °C	66 °C	80 °C	E_a/ kJ mol^{-1}	ln A
1	5.40[a]	k_1	1.12	5.82	24.5	67.7	86	22
	5.40[a]	k_2	0.290	2.86	23.5	76.5	118	34
	6.50[b]	k_1	1.17	5.20	31.5	112	97	27
	6.50[b]	k_2	0.307	2.38	25.0	91.4	122	35
2	6.50[b]	k_1	1.81	6.22	27.9	105	86	22
	6.50[b]	k_2	1.40	4.32	27.9	119	96	26
5	6.50[b]	k_1	—	0.049	0.353	1.97	117	29
	6.50[b]	k_2	—	0.058	0.694	2.36	118	30
7	6.50[b]	k_1	4.62	29.4	134	—	96	28

[a] 50% acetone–H_2O buffered by 0.2 mol dm^{-3} Tris/maleic acid. [b] Unbuffered 50% acetone–H_2O.

Table 3 First-order rate constants of hydrolysis of mustard **2** in 50% acetone–H_2O (imidazole buffer) at 66 °C and I = 0.025[a]

Salt	[Salt]/ mol dm^{-3}	pH	$10^5 k_1/s^{-1}$	$10^5 k_2/s^{-1}$
$NaClO_4$	0.0183	6.52	36.9	—
NaCl	0.0183	6.52	4.18	6.73
$NaClO_4$	0.0150	6.25	35.9	—
NaCl	0.0150	6.25	5.27	8.03

[a] Total buffer concentration [Imidazole] = 0.020 mol dm^{-3}.

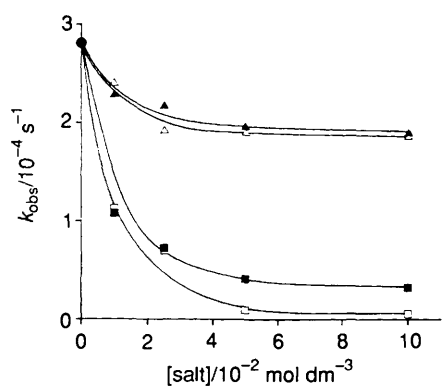


Fig. 3 Effect of increasing concentrations of NaCl or $NaClO_4$ on the first-order rate constants for hydrolysis of **2** in 50% acetone–H_2O at 66 °C, pH 6.50. ▲ k_1, and △ k_2 in presence of added $NaClO_4$; ■ k_1, and □ k_2 in presence of added NaCl.

worthy of note. Mustard **1** is 3.9 times more reactive than the corresponding half-mustard at 37 °C (pH = 5.40) but the latter is 1.13 times more reactive at 80 °C. This reversal of reactivity is not seen at pH = 6.50 (unbuffered media), but recurs for mustard **2** and its associated half-mustard at pH 6.50. These rare examples of isokinetic temperatures for two related reactions falling within the measured temperature range add further weight to the caution employed in interpreting the reactivity data in Table 1.

Salt Effects.—Results of a salt effect study on mustard **2** are shown in Table 3. All experimental factors are held constant save for the identity of the salt used to maintain constant ionic strength. At two different pH values, rate constants in media containing NaCl as added electrolyte are some 7–9 times lower than when $NaClO_4$ is present. In unbuffered media the results for the same mustard are shown in Fig. 3. Addition of $NaClO_4$ initially causes the hydrolysis rate constants k_1 and k_2 to diminish by a small amount, but the retardation when NaCl is added is more severe. This finding is typical of common-ion retardation of solvolysis rates, which in turn is symptomatic of external ion return. It is noteworthy that a return process (whether internal ion pair or external ion is not clear) is implicated in the scrambling studies reported by Benn *et al.*[12] An external return process is inconsistent with direct S_N2 attack by solvent on covalent substrate as the only reaction step.

Buffer Catalysis of Hydrolysis.—The limited set of results in Table 3 suggests at first sight that the hydrolysis reaction rate is pH independent when $NaClO_4$ is present, but is acid catalysed in the presence of NaCl. On the other hand the results in Table 1, obtained using different buffers, show that the dependence of rate on pH is not simple. It is not even clear, for instance, whether the hydrolyses are acid-catalysed or base-catalysed. To resolve this question we systematically investigated the hydrolysis (k_1 values only) of mustard **2** at constant ionic strength ($NaClO_4$) in 50% acetone–H_2O in the presence of imidazole buffers [pK_a (imidazole) = 6.95 at 25 °C in this solvent]. Since the pK_a of the $-N(CH_2CH_2Cl)_2$ nitrogen of this substrate is 6.05, it is clear that over the pH range of 6.35–7.25 used, variable proportions of substrate molecules will be protonated, and that corrections will need to be made for protonation.

If the system under study is characterised as eqn. (3), then the

$$\underset{\mathbf{11}}{-N(CH_2CH_2Cl)_2} + H_3O^+ \rightleftharpoons \underset{\mathbf{12}}{-\overset{+}{N}H(CH_2CH_2Cl)_2} + H_2O \quad (3)$$

fraction of substrate in the unprotonated form, f_{RX} = [**11**]/([**11**] + [**12**]), is given by eqn. (4) and the fraction of substrate

$$f_{RX} = \frac{K_a}{K_a + [H_3O^+]} \quad (4)$$

in the protonated form, f_{RHX} = [**12**]/([**11**] + [**12**]), is given by eqn. (5); where K_a is the acidity constant of **12** and $[H_3O^+]$ =

$$f_{RHX} = \frac{[H_3O^+]}{K_a + [H_3O^+]} \quad (5)$$

10^{-pH}. If k_{obs} is the measured first-order rate constant and k_ψ is the rate constant for hydrolysis of **12** then, symbolising **11** and **12** as RX and RHX^+, respectively, we obtain eqn. (6); where

$$\text{rate} = k_{obs}[RX]_t = k_{obs}\{[RX] + [RHX^+]\} = k_\psi[RHX^+] \quad (6)$$

$[RX]_t$ is the stoichiometric concentration of mustard substrate. Hence k_ψ, the specific rate coefficient for reaction of protonated substrate is given by eqn. (7).

Table 4 Rate constants for hydrolysis of mustard **2**[a] in 5% acetone–water containing imidazole buffer (I = 0.025)[b] at 66 °C

[Imid]/ mol dm^{-3}	[ImidH$^+$]/ mol dm^{-3}	pH	k_{obs}/ 10^{-4} s^{-1}	f_{RHX}	$k_\psi/10^{-3}$ s^{-1}
0.002	0.008	6.35	3.36	0.333	1.01
0.003	0.0067	6.65	3.46	0.201	1.72
0.005	0.005	6.95	3.47	0.112	3.10
0.0067	0.0033	7.25	3.35	0.059	5.68
0.015	0.015	6.95	3.85	0.112	3.43
0.004	0.016	6.35	3.62	0.333	1.09
0.0067	0.0133	6.65	3.68	0.201	1.83
0.010	0.010	6.95	3.59	0.112	3.21
0.0133	0.0067	7.25	3.69	0.059	6.25
0.020	0.010	7.25	3.82	0.059	6.47
0.0267	0.0133	7.25	4.10	0.059	6.95
0.020	0.020	6.95	4.02	0.112	3.59

[a] k_1 step only. [b] Maintained with $NaClO_4$.

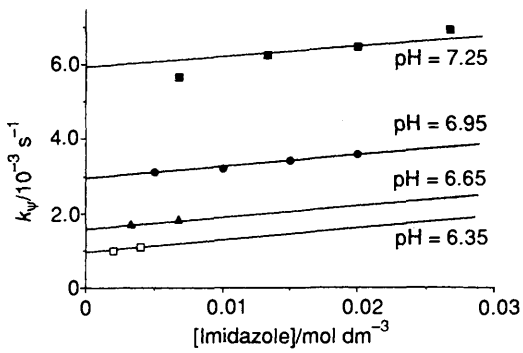


Fig. 4 Plots of k_ψ against imidazole concentration for hydrolysis of **2** in imidazole buffers of varying pH

$$k_\psi = k_{obs}/f_{RHX} \quad (7)$$

We now explore the dependence of k_ψ on concentrations of buffer components. In Table 4 are shown the experimental data together with derived values of f_{RHX} and k_ψ. These data are consistent with general-base catalysis, *i.e.* with eqn. (8); where K_w

$$k_\psi = k_B[\text{Imid}] + k_{OH}[OH^-] + k_w[H_2O] = k_B[\text{Imid}] + k_{OH}K_w10^{pH} + k_w[H_2O] \quad (8)$$

is the autopyrolysis constant of water in the solvent concerned. Eqn. (8) predicts that plots of k_ψ *vs.* [Imid] at constant pH should be linear, and parallel to similar plots at other values of pH. Furthermore, the intercept should increase with increasing pH.

Fig. 4 shows that these expectations are largely realised. The buffer plots at two pH values are two-point plots, and the slope of the pH = 7.25 plot is marginally greater than the others, but the overall result is as required for general-base catalysed hydrolysis of the protonated mustard. An unweighted value for k_B of 3.9 × 10^{-2} dm^3 mol^{-1} s^{-1} for imidazole catalysis can be calculated from the slopes.

Alternative modes of catalysis examined and rejected were: general-base catalysis of RX hydrolysis, and general-acid catalysis of hydrolysis of both RX and RHX^+. The data were inconsistent with the appropriate formulations.

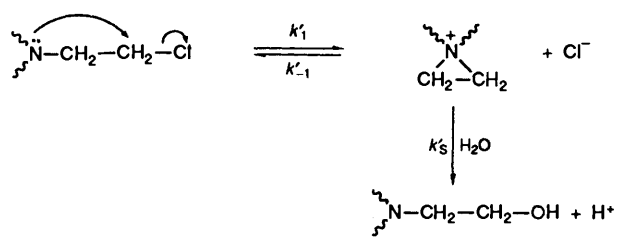
Fig. 4 reveals that the extent of base catalysis is weak, and that the major mode of hydrolysis of the protonated substrate, even at low pH is represented by the intercepts of these plots, which are the sum of the OH^- and water-catalysed components. Remembering that k_ψ represents the consumption of only 33% of the substrate at the most, we postulate a dual pathway for hydrolysis in which the remaining 67% (at least) of the substrate in the unprotonated form hydrolyses *via* a non-catalysed pathway. This is shown in operational (non-mechanistic) form in Scheme 3. Thus, if Rate = $k_{obs}[RX]_t = k_\psi[RHX^+] + k_S[RX]$, it is easily shown that eqn. (9) follows. This is of the

$$k_\psi = \frac{k_{obs}}{f_{RHX}} = k_B[B] + 10^{pH}(k_{OH}K_W + k_SK_a) + k_w[H_2O] \quad (9)$$

same form as eqn. (8), and hence the base catalysis results are not incompatible with accompanying normal solvolysis of the unprotonated mustard.

Scheme 3

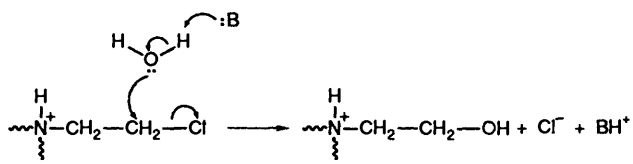
In tentatively assigning a mechanism to the hydrolysis we can thus separate the pathways for hydrolysis of protonated and unprotonated mustard. Dealing with the latter first, it is clear from the finding of common-ion retardation that external return of Cl^- to an intermediate is involved, and thus nucleophilic attack of water on covalent substrate is not a predominant pathway. On the basis of earlier work, and our observation that overall hydrolysis rates are a function of electron accessibility to the neighbouring nitrogen, we invoke an aziridinium ion mechanism in Scheme 4, where $k'_{-1} \approx k'_s$



Scheme 4

to allow for external ion return. The present results are insufficiently detailed to allow for inclusion of solvent-separated and/or contact ion pairs, either of the aziridinium chloride or carbocation chloride variety, as additional precursors of the half-mustard product shown, but neither can we exclude them. What is reasonably certain is that a free aziridinium ion, subject to competitive attack by H_2O and Cl^-, is at least one product precursor.

Our postulated mechanism for base-catalysed hydrolysis of the protonated substrate involves base-catalysed S_N2 attack by water molecules on the protonated substrate, Scheme 5.



Scheme 5

Deprotonation by buffer base is shown to be irreversible and concerted with release of Cl^- by the observation of general-base catalysis rather than specific hydroxide ion catalysis. Nucleophilic assistance to C–Cl heterolysis by ring closure is now impossible since the neighbouring nitrogen is non-nucleophilic. External nucleophilic attack by solvent water is thus strongly implicated. The base catalysis results suggest however that water is insufficiently nucleophilic to carry out this task

Table 5 Pseudo-first-order rate constants for alkylation of NBP by mustards in a variety of solvents and at various temperatures: [NBP] = 0.60 mmol dm^{-3}, [mustard] = 0.01 mmol dm^{-3}

	$k_{obs}/10^{-5}$ s^{-1}					
Mustard	37 °C[a,b]	50 °C[a,b]	66 °C[a,b]	66 °C[a,c]	66 °C[c,d]	80 °C[a,b]
1[f]	0.998	5.72	24.7	23.7	17.2	75.8
2[g]	0.971	5.09	22.7	19.9	12.1[e]	107
3	—	—	23.0	22.5	10.5	—
4	—	—	5.06	4.87	3.32	—
5[h]	0.0075	0.051	0.32[i]	—	0.729	1.45
6	—	—	0.068	—	0.312	—

[a] 50% acetone–H_2O. [b] HPLC. [c] Conductimetry. [d] 50% methanol–H_2O. [e] $k_{obs} = 13.4 \times 10^{-5}$ s^{-1} in 50% ethanol–H_2O. [f] $E_a = 91$ kJ mol^{-1}, ln $A = 24$. [g] $E_a = 98$ kJ mol^{-1}, ln $A = 26$. [h] $E_a = 106$ kJ mol^{-1}, ln $A = 25$. [i] Interpolated using data at other temperatures.

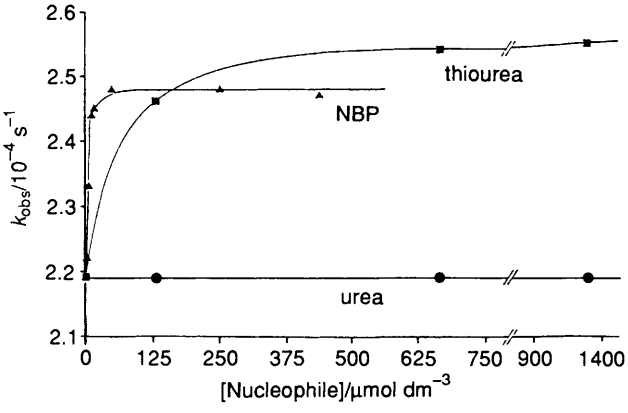
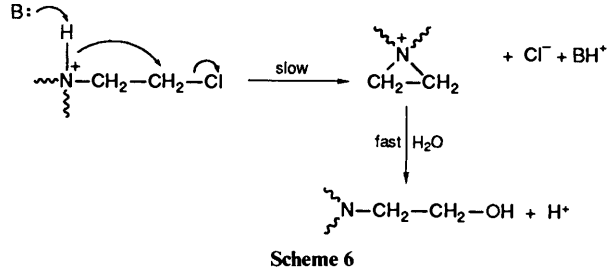


Fig. 5 Pseudo-first-order rate constants for reaction of **1** in the presence of NBP ▲, thiourea ■ and urea ●, in 50% acetone–H_2O at 66 °C, pH 6.50

unaided. Scheme 5 thus shows proton transfer from water concerted with nucleophilic attack. If the hydroxide ion in equilibrium with the base were the nucleophile, specific-base catalysis would be observed, but this is not the case.

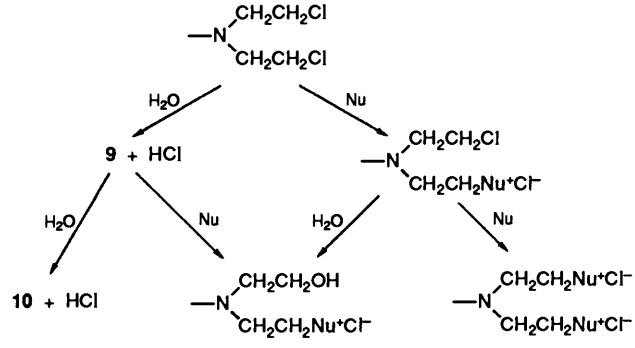
A superficially attractive alternative for the base-catalysed pathway is presented in Scheme 6, wherein the intermediacy of the aziridinium ion is preserved. It involves an E2 type mechanism. It is however not certain that this base-promoted ring closure could compete with simple base-induced equilibrium deprotonation, which should occur at a diffusion-controlled rate. Nevertheless our interest in aziridinium ion intermediates in these reactions makes the question of Scheme 6 worth pursuing, and we are planning theoretical studies of the relevant energetics.



Scheme 6

We emphasize that the preceding proposals properly apply only to hydrolysis of **2** in imidazole buffers. Detailed study of the other buffer media employed to generate the results in Table 1 has yet to be carried out. Thus the irregular pH profiles derivable from the Table 1 data await detailed interpretation, but the very irregularity implies some form or forms of buffer catalysis rather than specific hydronium or hydroxide ion catalysis, and this is at least consistent with the foregoing proposal.

Alkylation of Nucleophiles.—The neutral nucleophiles thiourea and NBP were chosen for study. Overlapping HPLC peaks precluded the use of this technique for kinetic work in some cases and hence the conductivity increase of reaction mixtures was followed as a function of time. The situation when a neutral nucleophile competes with solvent for the mustard is more complex, since there are three possible additional ionic products, as shown in Scheme 7. Evidence for more than one



Scheme 7

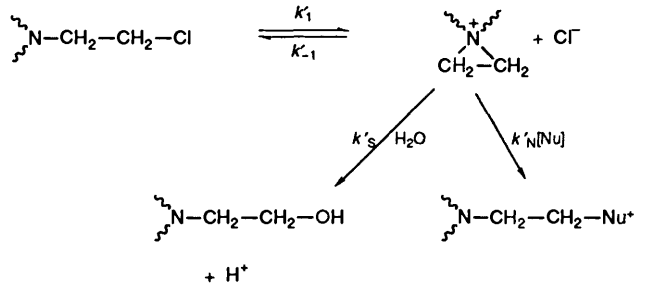
ionic salt as product was adduced (TLC) for NBP as nucleophile, while only one alkylated thiourea was detected (HPLC.) In addition, analysis for total cations formed and for [H_3O^+] when thiourea was the nucleophile showed that non-solvolytic products were formed. Nevertheless the conductivity kinetics were strictly first-order over reaction times corresponding to consumption of *ca.* 90% of the starting mustard with selected examples, so it is reasonable to take the view that the measured pseudo-first-order rate constant comprises the sum of pseudo-first-order rate constants for the first steps involving hydrolysis of and alkylation by the starting mustard. In cases where comparison could be made by both HPLC and conductivity techniques, pseudo-first-order rate constants based on consumption of mustard and increase in conductivity were equal within experimental error, which justifies the assumption.

These and other pseudo-first-order rate constants ([NBP]$_0$ ≫ [mustard]$_0$) are shown in Table 5, wherein data for a range of mustards, in a variety of aqueous–organic solvents at different temperatures, are also displayed. Solvent effects are small, as befits a study where solvent polarities were not widely varied. The reactivity order of the mustards is roughly the same as in the solvolytic reactions, Table 2, and the best-fit Hammett plot of k_{obs} *vs.* $\sigma(CH_3X)$ yields eqn. (10). Again the fit is not

$$\log k_{obs} = -(2.78 \pm 0.33)\sigma - (4.13 \pm 0.15) \quad n = 6 \quad r = 0.95 \qquad (10)$$

spectacular, but it is significant that ρ lies within the combined experimental uncertainties of the large ρ values for the solvolyses [eqns. (1) and (2)]. This strongly suggests that nucleophilic interaction of the –N(CH$_2$CH$_2$Cl)$_2$ nitrogen with the reaction centre is an important component of the activation step, which in turn suggests that aziridinium ions are involved.

A detailed study with mustard **1** yielded results consistent with this view. The use of varying concentrations of NBP or thiourea produces an initial small rate increase (*ca.* 15%), and thence the reaction is zero-order with respect to added nucleophile (Fig. 5). The curved rate profile which reaches a plateau is reminiscent of Sneen's 'unified' S_N1/S_N2 proposal, wherein a change in rate-limiting step, from nucleophilic attack



Scheme 8

on an intermediate to production of the intermediate, occurs as the nucleophile concentration is increased.[18] While this proposal has been criticised[19] in respect of the cases raised by Sneen, it has credence in the present case. Neglecting base-catalysed decomposition of protonated mustard, Scheme 8 accounts for the present results. This is merely an extension of Scheme 4 and allows for nucleophilic competition at the aziridinium ion stage. The possibility of direct S_N2 attack by the nucleophile on protonated mustard remains, and in view of the fact that stronger nucleophiles than water were employed, base catalysis would not be a necessity, as it is in Scheme 5. The possibility of a Scheme 6 E2-like process is ruled out by the fact that both nucleophiles are weak bases.

The steady-state expression for k_{obs} based on the pathway in Scheme 8 is given by eqn. (11) which in the limit of large [Nu]

$$k_{obs} = \frac{k'_1 k'_N[Nu]}{k'_{-1}[Cl^-] + k'_S + k'_N[Nu]} \qquad (11)$$

yields the observed zero-order dependence on nucleophile concentration, $k_{obs} = k'_1$. At the very low substrate concentrations used here, external ion return can be only a minor component of the reaction (and the fact that pseudo-first-order rate constants do not drift downwards as a function of time is evidence for this). Addition of an external nucleophile stronger than Cl^- thus further reduces the incidence of external return and induces a small rate acceleration.

That the acceleration is not simply a medium effect induced by the addition of a polar nucleophile is discounted by the urea result also shown in Fig. 5. Urea has no effect on solvolysis rate whatsoever, and is not nucleophilically involved in the reaction. There is thus no polar non-electrolyte effect on the rate with this model non-electrolyte, which implies that the modest acceleration induced by thiourea is indeed due to nucleophilic participation.[20] This contrasts with the reaction of benzoyl chloride with *ortho*-nitroaniline[21] where a curved rate profile similar to the one in Fig. 5 was claimed as evidence for nucleophilic attack on a pre-formed ion pair.[18] Bentley *et al.* later showed that this was merely a medium effect superimposed on a linear rate profile.[22] Our urea result discounts this possibility in the present case.

The rate acceleration should be a function of the product ratio resulting from the competition between solvent and added nucleophile for the intermediate.[19] Unfortunately, because of experimental difficulties and the very complexity of the system (Scheme 7) we were unable to evaluate this ratio.

It is of interest to note that a mustard chlorohydrin (sulfur mustard) fails to undergo accelerated solvolysis in the presence of added thiourea[23] and a mechanism that involves anchimeric assistance (k_Δ) as the sole rate-limiting step has been proposed. There is a point of similarity with respect to the present case in that both reaction pathways appear to involve anchimeric assistance to ionization* and a point of difference in that the present case is complicated by a small but vital return component. There is however no doubt that attack by external nucleophiles on the starting covalent mustard is precluded. Such a mechanism has been proposed for reaction of a thiolate with aromatic nitrogen mustards[12] but requires reactions which have a first-order rate dependence on the concentration of added nucleophile over the entire range of practical concentrations. The horizontal plateaux in Fig. 5 show that this is not the case in the reactions under study here.

Experimental

The mustards **1**–**7** were prepared as described elsewhere[16] and were used as a monohydrochloride salts. The corresponding diols **1**–**6** were formulated as the dihydrochloride salts[16] and were available as pure samples for standardising HPLC spectra for product analysis. The biological evaluation of these compounds has been described elsewhere.[16]

Determination of pK_a *Values.*—A portion of a stock solution of mustard (33.3 mm^3, 0.2 mmol dm^{-3} in MeOH) was injected into 3 cm^3 of KH_2PO_4/K_2HPO_4 buffer (0.01 mol dm^{-3}) whose pH had been adjusted within the pH range 5.00–8.44. The absorbance was measured at 260 nm and the value of pK_a calculated according to the method of Albert and Serjeant.[24]

Determination of Rate Constants of Hydrolysis of Mustards by HPLC.—A portion of a stock solution of mustard (100 mm^3, 0.2 mmol dm^{-3} in MeOH) was added to acetone–water or acetone–aqueous buffer (3 cm^3, all 50:50 v/v) held at the required temperature. The pH of these solutions had been measured using a combination electrode calibrated with standards made up in 50% acetone–50% aqueous solvent. At intervals 20 mm^3 of the reaction mixture was monitored for product formation by HPLC. Shimadzu LC-6A equipment was used with a C-18 10.0 μm reversed-phase 250 × 5.0 mm column. The eluting solvent was a mixture of solvent A [10% CH_3CN + 90% buffer (1 mol dm^{-3} NH_4OAc + 10 mmol dm^{-3} heptanesulfonate + 10 mmol dm^{-3} triethylamine, pH 5.00)] and solvent B (90% MeOH + 10% CH_3CN) in the ratio of 20:80 for compounds **1**, **2**, **4** and **7**, 30:70 for **3** and **5** and 35:65 for **6**. The flow rate was 0.5 cm^3 min^{-1} and the analytical wavelength was 340 nm.

Determination of Rate of Alkylation of NBP by Mustards.—Mustard (300 mm^3, 0.2 mmol dm^{-3} in MeOH) and 300 mm^3 of a stock solution of NBP (up to 10 mmol dm^{-3}) were injected into 5 cm^3 of 50% (v/v) aqueous acetone held at the required temperature in a conductimetric cell. The conductivity was measured during the course of the reaction, up to at least 70% completion, using a Philips PW9550/60 cell. Since we had confirmed the linearity of the calibration curve of conductivity against concentration of pyridinium chloride salt by using the literature[25] method it was then possible to substitute the conductivity data directly into the rate eqn. (12) for

$$\ln \frac{C_\infty - C_t}{C_\infty - C_0} = -k_{obs}t \qquad (12)$$

determination of the pseudo-first-order rate constant, k_{obs}, where C_0, C_∞ and C_t are the conductivities measured at time = zero, infinite time, and time t, respectively.

* Note that anchimeric assistance to ionization has not been unequivocally proved in the present case. The results yield no information as to nucleophilic participation or the lack of it in the ionization step. There is less doubt concerning anchimeric assistance to dissociation.

It was necessary to use a partially aqueous solvent for the alkylation reaction because in anhydrous organic solvents (*e.g.* MeOH, EtOH, Me_2SO, MeCN, $HCONMe_2$ or Me_2CO) the reaction was too slow to be measured. Hence the value of k_{obs} in eqn. (12) is the sum of the pseudo-first-order rate constants of hydrolysis and alkylation. HPLC was also used to follow the loss of mustard during the course of the reaction and the results obtained were generally very comparable. Unfortunately, the HPLC retention times of the pyridinium salts were identical with that of the solvent acetone and we were unable to monitor the formation of product by this method. However, confirmation of the presence of the pyridinium salt was obtained for each alkylation reaction by TLC (silica plate, moving phase $CH_3OH:CHCl_3 = 1:9$). The R_f values were NBP (0.84), mustards (0.53–0.63), salts (0.093–0.100).

Determination of Rate of Reactivity of Mustards in the Presence of Thiourea and Urea.—Mustard (60 mm^3 of 0.2 mmol dm^{-3} in MeOH) and 60 mm^3 of a stock solution of thiourea or urea (up to 20 mmol dm^{-3}) were injected into 1 cm^3 of 50% (v/v) aqueous acetone held at 66 °C. At intervals, 20 mm^3 of the reaction mixture was monitored by HPLC, for the loss of mustard and the formation of product. Under the experimental conditions outlined above, the formation of a product assumed to be the alkylated thiourea was observed with a retention time of 9.6 min. No evidence was obtained for formation of alkylated urea.

Stoichiometry of Reaction of Mustard **1** *in Presence of Thiourea.*—Samples of reaction mixture containing mustard (0.001 mol dm^{-3}) and thiourea (0.1 mol dm^{-3}) in 20 cm^3 50% (v/v) aqueous acetone solvent were made up. An initial blank and a 24 h sample were placed on a 'Zeo-Karb' 225 cation exchange column and H_3O^+ was eluted therefrom. The total cation concentration was determined by pH titration with standard NaOH solution. A second 24 h sample was subjected to direct conductimetric titration for H_3O^+ (end-point indistinct). The results indicated that at the most only about 64% of the cationic product was H_3O^+, the remainder being assumed to be isothiouronium salt(s).[20,26]

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