Nitrosation by Alkyl Nitrites. Part 3.[1] Reactions with Cysteine in Water in the pH Range 6–13

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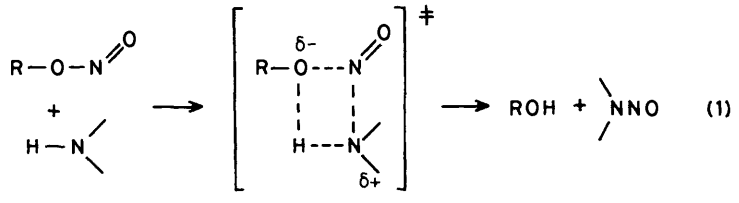
Cysteine reacts quite rapidly with isopentyl, isopropyl, and t-butyl nitrite in water at 25 °C in the pH range 6–13 to give the somewhat unstable *S*-nitrosocysteine. Rate measurements show clearly that reaction occurs exclusively *via* the RS^- species. Plots of the measured second-order rate constants against pH are in excellent agreement with the calculated curves, for all three alkyl nitrites, and the order of reactivity is $(CH_3)_2CHCH_2CH_2ONO:(CH_3)_2CHONO:(CH_3)_3CONO$ 16:6.7:1. The methyl ester of cysteine behaves similarly whereas *S*-methylcysteine does not form the thionitrite. The results suggest that the reaction involves a direct electrophilic nitrosation by the alkyl nitrite itself at the sulphur site in RS^-. The conclusions are discussed in terms of steric effects and of the other known reactions of alkyl nitrites.

In Part 2[1] we examined the nitrosation of a range of substrates including amines, thiols, thiourea, and hydrazoic acid, in water using alkyl nitrites under mildly acidic conditions. All the results were consistent with a mechanism in which reversible acid-catalysed hydrolysis of the alkyl nitrite occurs to give nitrous acid, which then in its protonated form effects nitrosation. With t-butyl nitrite the rate and extent of hydrolysis are such that in effect the solution behaves as one of nitrous acid itself. Generally either alkyl nitrite hydrolysis or nitrous acid nitrosation can be the rate-limiting step, depending on the structure of both the alkyl nitrite and the substrate and also upon the experimental concentrations. So in aqueous acid solution there is no evidence for a direct nitrosation reaction brought about by the alkyl nitrite molecule, other than the reaction of the protonated form of the alkyl nitrite with powerful nucleophiles such as bromide ion[1,2] and thiocyanate ion.[1]

Nitrosation by alkyl nitrites under alkaline conditions, usually in alcohol solvents, is a much more widely used reaction preparatively. Amines, alcohols, ketones, and nitro compounds and some hydrocarbons all undergo such reactions.[3] All the evidence[4] points to a mechanism involving a direction reaction between the alkyl nitrite and the substrate [see equation (1)], as



(1)

a synchronous process and not a two-stage addition–elimination. The reactions with amines are particularly favourable if the alkyl nitrite contains powerful electron-withdrawing groups.[5] The exchange of nitroso group between an alkyl nitrite and an alcohol also occurs readily in alkaline alcohol solution and probably involves the direct reaction with the alkoxide ion, since there is no reaction in neutral solution.[6] Thiols also react with alkyl nitrites to give thionitrites,[7,8] but nothing is known about the mechanism of these reactions. Such reactions are however of some interest, not only within the context of nitrosation chemistry, but also because it has been suggested[9] that the vasodilatory action of alkyl nitrites (and nitrates) arises in the first instance by nitrosation of tissue-bound thiol groups, to give thionitrites which then activate the enzyme guanylate cyclase, which in turn brings about smooth muscle relaxation. We have set out in this paper to establish the mechanistic features of the *S*-nitrosation of thiols by alkyl nitrites in aqueous solution in the pH range 6–13. We have chosen cysteine as a typical thiol which is also important *in vivo*, and have used three alkyl nitrites with a primary isopentyl (Pe^i), secondary isopropyl and tertiary t-butyl structure. We have previously shown[1] that in acid solution nitrosation of cysteine by alkyl nitrites involves prior hydrolysis of the alkyl nitrite and nitrosation *via* nitrous acid.

Experimental

The three alkyl nitrites were prepared by the standard method involving nitrosation of the corresponding alcohol by acidic solutions of nitrous acid.[10] All were fractionally distilled prior to use in the kinetic experiments. All other materials used were of the highest purity grade available. Most kinetic experiments were carried out in a stopped-flow spectrophotometer monitoring the increasing absorbance at 330 nm due to the product thionitrite. Some of the slower reactions at pH *ca.* 6 were followed in a conventional recording spectrophotometer. All experiments were carried out in aqueous solution at 25 °C using a number of buffer solutions to cover the pH range 6–13. Reactions were carried out under first-order conditions with $[RSH]_0 \gg [R'ONO]_0$ and good first-order behaviour was found throughout, following every run to at least 80% reaction. Each run was repeated five times and the average value of the first-order rate constant k_o, used in the subsequent analysis. Values of k_o were subject to a standard error of $\leqslant \pm 2\%$. A typical set of results is given in Table 1 for reaction of Pr^i nitrite with cysteine at pH 7.5. The plot of k_o *versus* [cysteine] is an excellent straight line passing through the origin, from which the second-order rate constant k_2 is obtained.

Table 1. Values of k_o/s^{-1} for the reaction of Pr^{i}ONO with cysteine at pH 7.5

10^2[Cysteine]/ mol dm^{-3}	Individual k_o values	Average k_o value
1.93	0.0359	0.0351 ± 0.0007
	0.0357	
	0.0353	
	0.0349	
	0.0346	
	0.0340	
3.87	0.0690	0.0697 ± 0.0007
	0.0695	
	0.0689	
	0.0707	
	0.0701	
	0.0699	
5.42	0.0980	0.0975 ± 0.0005
	0.0975	
	0.0966	
	0.0975	
	0.0982	
	0.0974	
7.74	0.143	0.142 ± 0.002
	0.142	
	0.140	
	0.139	
	0.143	
	0.142	

Table 2. Second-order rate constants $k_2/dm^3\ mol^{-1}\ s^{-1}$ for the reactions of three alkyl nitrites with cysteine as a function of the pH of the solution

$(CH_3)_2CHCH_2CH_2ONO$		$(CH_3)_2CHONO$		$(CH_3)_3CONO$	
pH	k_2	pH	k_2	pH	k_2
6.00	0.10	5.50	0.064	6.50	0.020
6.50	0.37	6.00	0.058	7.00	0.073
7.00	1.05	6.50	0.20	7.50	0.25
7.50	2.88	7.00	0.47	8.10	0.64
7.90	6.61	7.50	1.24	8.55	1.09
8.25	11.4	7.75	2.07	9.20	1.48
8.50	15.1	8.00	3.72	10.00	1.60
8.75	19.0	8.50	6.62	11.00	1.71
9.20	23.0	9.20	9.6	12.20	1.69
10.06	26.5	10.00	11.3		
11.10	27.0	11.10	11.3		
12.20	26.9	12.45	11.8		
		13.40	11.4		

Results and Discussion

All three alkyl nitrites reacted quite rapidly with cysteine at pH >6 to give initially the yellow *S*-nitrosocysteine.[11] With time the yellow colour faded and the disulphide cystine was formed. Initially we were surprised at the high rates of reaction, for example the reaction of isopentyl nitrite with cysteine (3.87×10^{-2} mol dm^{-3}, a 20-fold excess) at pH 7.5 has a half-life of only *ca.* 10 s. Table 2 gives the values of the second-order rate constants k_2 ($=k_o/[\text{Cysteine}]_o$) for the three alkyl nitrites as a function of pH over the range 6–13. At low pH reaction is very slow, but increases quite dramatically with pH until the rate constant levels off at pH >10. This suggests that the reactive species is an anion form of cysteine. The two possibilities are for

$$RSH \overset{K_a}{\rightleftharpoons} RS^- + H^+$$

$$RS^- + R'ONO \xrightarrow{k} RSNO + R'O^-$$

Scheme.

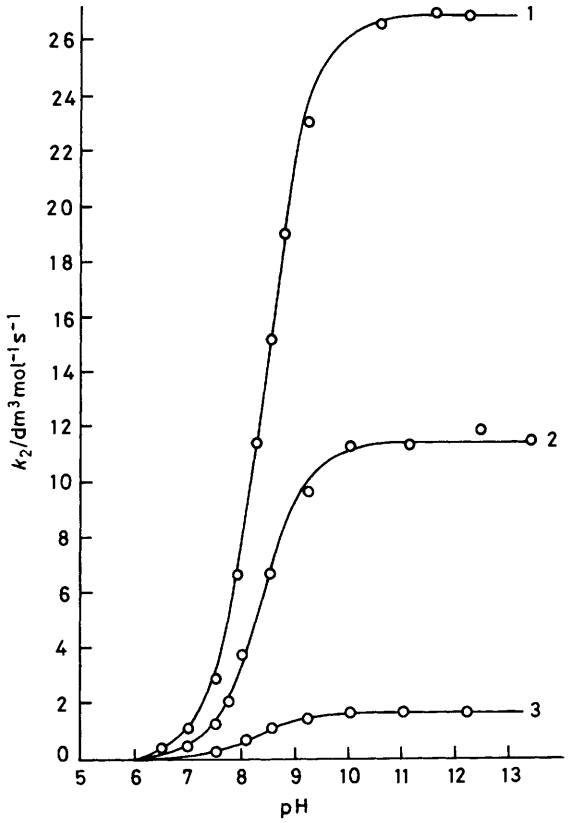


Figure. Second-order rate constants k_2 plotted against pH for the reaction of three alkyl nitrites with cysteine: 1, $(CH_3)_2CH(CH_2)_2ONO$; 2, $(CH_3)_2CHONO$; 3, $(CH_3)_3CONO$

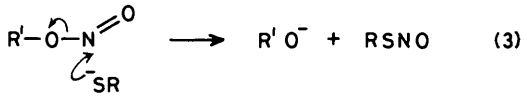
ionization of the carboxy group or of the thiol group. The pK_a value[12] for the former is 1.86 and the latter 8.37, which indicates that we are concerned here with the ionization of the thiol group. The proposed outline mechanism is given in the Scheme, where K_a is the dissociation of RSH and k is the bimolecular rate constant for reaction of RS^- with the alkyl nitrite. The expression for k_2, the measured second-order rate constant expected from the Scheme is that given in equation (2). This

$$k_2 = kK_a/(K_a + [H^+]) \qquad (2)$$

predicts the observed levelling off of k_2 at high pH. Using this limiting value $(k_2)_{lim} = k$, we constructed the pH–k_2 profiles for all three alkyl nitrites shown in the Figure. The solid line is the calculated curve and the points are the experimentally measured ones. The agreement is excellent in all cases, thus confirming reaction *via* the RS^- species. The curves all approach $k_2 = 0$ at pH <6 which shows that there is not a significant pathway for reaction *via* the RSH form. As expected the methyl ester of cysteine behaves in the same fashion, whereas *S*-methylcysteine gives no yellow thionitrite on treatment with alkyl nitrites.

The limiting values of k_2 at high pH (which are equal to k) are, respectively, 26.9, 11.4, and 1.7 dm^3 mol^{-1} s^{-1} for Pe^{i}ONO, Pr^{i}ONO, and Bu^{t}ONO. So we have the reactivity sequence, primary nitrite > secondary nitrite > tertiary nitrite. This can be attributed to the unfavourable increasing electron release from the methyl groups as we go to the tertiary structure. The same effect was noted by Challis and Shuker[5] where nitrosation of amines is particularly favoured by the presence of electron-withdrawing groups in the alkyl nitrite. However this may not be an electronic effect in our case since the methyl groups may be too far away. Further, the σ_I and σ_R values of

the alkyl groups are not very different,[13] so the effect is unlikely to arise from an electronic effect. The situation [equation (3)]



is analogous to that pertaining in the alkaline hydrolysis of alkyl acetate esters, where the reactivity trend Me > Et > Pr^i > Bu^t is more properly ascribed to a steric effect. The indications are that in these direct nitrosation reactions the reactivity differences have their origin in steric effects.

We have written the reaction in terms of a synchronous process and not as an addition–elimination which is the common mechanism in the alkaline hydrolysis of carboxylate. Our results are not discriminating on this point, but Oae and co-workers[4] have argued strongly that the reaction of an alkyl nitrite with amines is indeed a concerted one. One of the points in favour of such a process is that the alkyl nitrites react significantly faster with amines than they do with the more powerful hydroxide-ion nucleophile, where a two-step process is well established. Further, the reactivities of amines correlate with their vertical ionization potentials and not with their basicities, suggesting that the reactions are orbital controlled. We found no buffer catalysis (for measurements made at pH 7 and 8) using isopropyl nitrite. This result supports a synchronous mechanism in our reactions since general acid catalysis might be expected for the loss of the OR^- group from a tetrahedral intermediate.

This work shows that at least *in vitro* a direct and rapid reaction occurs between alkyl nitrites (particularly primary alkyl nitrites) at pH values likely to be encountered *in vivo*. This at least confirms the feasibility that such reactions could occur *in vivo* and could be an important feature in the mechanism of vasodilatory action of alkyl nitrites. Work is in progress on the corresponding reactions of alkyl nitrates.

Acknowledgements

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