Nucleophilic Catalysis in the Nitrosation of Sarcosine and Proline

Thomas A. Meyer and D. Lyn H. Williams*
Department of Chemistry, University Science Laboratories, South Road, Durham DH1 3LE

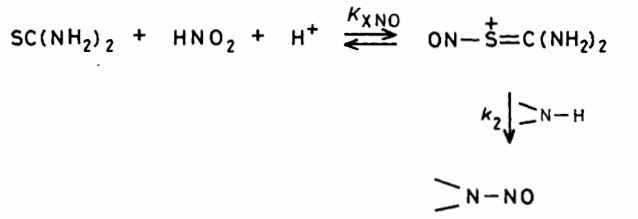
The formation of nitrosoamino acids from both sarcosine (Sar) and proline (Pro) and nitrous acid is very strongly accelerated by thiourea (TU) and tetramethylthiourea (TMTU), and to a lesser extent by thiocyanate ion. The effects of TU and TMTU are very similar and are greater than that of SCN^- by a factor of 17 for Sar and 30 for Pro. Thus these amino acids behave like amines and not like amides (where there is no nucleophilic catalysis). The second-order rate constants (k_2) for XNO [ONSCN or $ON\overset{+}{S}C(NR_2)_2$] attack were obtained (*a*) from the variation of the observed rate constant (k_o) with $[X^-]$ or [X] and (*b*) from the variation with [substrate]. The agreement is excellent. This analysis assumes that reaction occurs as *N*-nitrosation of the free –NH– function. The variation of k_o with acidity, however, shows that there is another (small) component to the reaction, and the results are consistent with an earlier idea involving initial *O*-nitrosation at the $-CO_2^-$ function. The k_2 values are discussed with reference to the reaction of other substrates with these (and other) nitrosating reagents, particularly with reference to encounter-controlled processes. The extent of the alternative pathway for $ON\overset{+}{S}C(NH_2)_2$ decomposition is assessed in relation to the ability of this species to act as a nitrosating agent.

In a previous paper,[1] we reported that the rate of *N*-nitrosation of morpholine in dilute aqueous acid solution was markedly enhanced by the addition of thiourea. The effect was much greater than that found for two other well known catalysts, SCN^- and Br^-. The reactivity ratios $SC(NH_2)_2:SCN^-:Br^-$ were measured as 4 200:240:1, making thiourea the most effective catalyst for nitrosation yet reported. The results are readily interpreted in terms of rate-controlling reaction of the free amine with the *S*-nitroso ion formed in a rapid pre-equilibrium step (Scheme 1). The observed order of catalysis arises essentially from the larger value of the equilibrium constant K_{XNO} for the formation of $ON\overset{+}{S}C(NH_2)_2$ (5 000 dm^6 mol^{-2}),[2] in comparison with ONSCN (32 dm^6 mol^{-2})[3] and BrNO (5.1×10^{-2} dm^6 mol^{-2});[4] all values refer to reaction in water at 25 °C. The nitrosation of thiourea has been studied mechanistically[2,5] and it is likely that *S*-nitrosation is the first step, even at low acidities, where the final products are those derived from *N*-nitroso intermediates. It is believed that *S*- to *N*-nitroso group rearrangement occurs; other workers have argued[6] that at low acidities *N*-nitrosation occurs directly. We assume that the important intermediate in thiourea-catalysed nitrosation is the *S*-nitroso species, which is characterized by a yellow colour in solution.

Masui *et al.*[7] have also shown (using concentration–time curves) that thiourea (TU) and tetramethylthiourea (TMTU) enormously accelerate the nitrosation of dimethylamine at pH 4. These workers found TMTU noticeably more efficient than TU. The *S*-nitroso ions derived from thioureas are not particularly stable species and their decomposition (to a variety of products) has been studied kinetically.[8] The competition between nitrosation and decomposition has been invoked by Masui *et al.*[9] to explain the difference in behaviour between TU and TMTU.

More recently other nitrosation reactions have been found to be catalysed by thiourea, *e.g.* the diazotization of aniline[10] and naphthylamine[11] derivatives. Indeed it appears that catalysis by thiourea is a general feature in nitrosation.

This paper describes the kinetic effects of SCN^-, TU, and TMTU on the nitrosation of two typical amino acids, sarcosine (Sar) and proline (Pro). Amides behave quite differently from amines in that there is a different rate-limiting step (proton transfer), and no nucleophilic catalysis occurs.[12] It is of interest to establish whether α-amino acids (where the powerfully electron-withdrawing carbonyl group is one carbon atom removed from the amino nitrogen atom) behave as typical amines or as typical amides. Further we have investigated whether these *S*-nitroso intermediates react at the encounter limit, and also have examined the significance of the decomposition pathway over a range of pH values.



Scheme 1.

The *in vivo* formation of carcinogenic nitrosamines is now a much publicised problem. It is likely that the catalytic features also operate *in vivo*. It is important therefore to quantify such effects, in this instance with regard to naturally occurring species such as thiocyanate ion and possibly thioamides, for which thioureas are model compounds.

Experimental

Sarcosine, proline, thiourea, tetramethylthiourea, and all other reagents used were of the highest purity grade available. All reactions, unless otherwise stated, were carried out in a citric acid–disodium phosphate buffer.[13] Because of the high basicity of the amino acids, the pH values of reaction solutions were adjusted with perchloric acid.

Rate measurements were conducted spectrophotometrically at 25 °C in the cell holder of a conventional u.v. spectrophotometer. All reactions were carried out in water. Generally reaction was followed by noting the appearance of the absorbance at 340 nm due to the product nitrosoamino acid. Some experiments were carried out at 260 nm; identical results were obtained. The amino acid was in a large excess over the nitrous acid. Good first-order behaviour was always found over

Table 1. A typical run for the reaction of nitrous acid (7.7×10^{-3}M) with Sar (0.132M) in the presence of thiourea (3.6×10^{-3}M) at pH 2.58

t/s	Absorbance	10^2k_o/s^{-1}
0	0.372	
10	0.460	1.81
20	0.531	1.78
30	0.591	1.78
40	0.642	1.78
50	0.685	1.79
60	0.722	1.80
70	0.752	1.80
80	0.777	1.81
90	0.794	1.77
100	0.811	1.76
∞	0.902	

Mean $k_o = (1.79 \pm 0.02) \times 10^{-2}$ s^{-1}

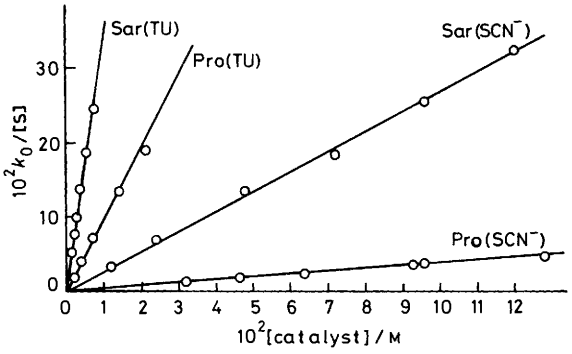


Figure 1. Catalysis by SCN^- and TU in the nitrosation of Sar and Pro

at least two and a half half-lives. A typical run is quoted in Table 1. After more than ten half-lives the product yields were estimated at 343 nm using published values[14] of the absorptivity constants (ε) of 91 and 86 for nitrosoproline and nitrososarcosine respectively. Over the range of pH used in this work ε does not vary significantly at 343 nm.

Results and Discussion

The catalytic effects of SCN^- and TU on the nitrosation of Sar and Pro are shown in Figure 1. The results for TMTU are very similar to those for TU and are not shown in the Figure. The results are presented as plots of the second-order rate constant (*i.e.* k_o/[S], where k_o is the measured first-order rate constant and [S] the total stoicheiometric concentration of the substrate), against [SCN^-] or [TU]. Catalysis is very marked. The reactions under these conditions in the absence of catalyst are negligibly slow. Overall Sar is *ca.* five times more reactive than is Pro. Throughout, nitrosamine formation is quantitative. Earlier Mirvish *et al.*[15] have noted the catalysis of Sar nitrosation by SCN^-.

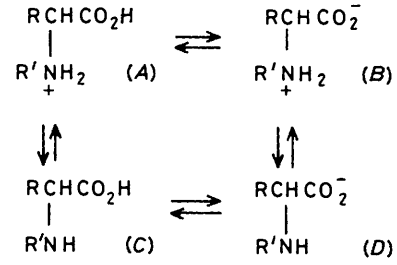
As expected, reaction is first-order in S throughout. The experimental results are given in Table 2 and 3. Amino acids can exist in various forms as depicted generally in Scheme 2. Inspection of the various pK values for Sar and Pro reveals that a negligible concentration of species (*D*) is present. *N*-Nitrosation can then only take place *via* species (*C*). If we assume that this is the only reaction pathway, then it is possible to deduce the expression for k_o given in equation (i). In this expression k_2 is the second-order rate constant for reaction of XNO with (*C*), K_N is the dissociation constant for nitrous acid dissociation (pK_a 3.30), [S] is the total stoicheiometric

Table 2. Variation of k_o with [Sar] at constant [catalyst] and at pH 2.58; $[HNO_2] = 7 \times 10^{-3}$M

10^2[Sar]/M	[Catalyst]/M	10^4k_o/s^{-1}
2.0	4.03×10^{-2} SCN^-	22
4.0	4.03×10^{-2} SCN^-	35
6.0	4.03×10^{-2} SCN^-	61
8.1	4.03×10^{-2} SCN^-	76
12.1	4.03×10^{-2} SCN^-	116
7.9	2.19×10^{-3} TU	63
15.8	2.19×10^{-3} TU	132
23.7	2.19×10^{-3} TU	188
7.9	2.05×10^{-3} TMTU	53
15.8	2.05×10^{-3} TMTU	110
23.7	2.05×10^{-3} TMTU	168

Table 3. Variation of k_o with [Pro] at constant [catalyst] and at pH 2.58; $[HNO_2] = 7 \times 10^{-3}$M

10^2[Pro]/M	[Catalyst]/M	10^4k_o/s^{-1}
8.1	6.97×10^{-2} SCN^-	19
16.6	6.97×10^{-2} SCN^-	38
24.9	6.97×10^{-2} SCN^-	57
33.2	6.97×10^{-2} SCN^-	79
8.1	7.05×10^{-3} TU	61
16.2	7.05×10^{-3} TU	114
24.2	7.05×10^{-3} TU	152
32.3	7.05×10^{-3} TU	198
14.1	1.19×10^{-3} TMTU	17
28.2	1.19×10^{-3} TMTU	33
42.3	1.19×10^{-3} TMTU	47



Scheme 2.

concentration of the amino acid, and K_1 is the macroscopic dissociation constant for the loss of a proton from (*A*). The individual dissociation constants for the formation of (*B*) and (*C*) have been obtained assuming that the constant for the formation of (*C*) is the same as that for the corresponding ester of the amino acid, designated K_e in equation (i). A further

$$k_o = \frac{k_2K_e[S][X][H^+]^2K_{XNO}}{(K_1 + [H^+])(K_N + [H^+])} \quad \text{(i)}$$

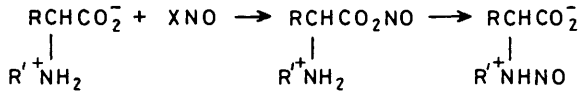
assumption is that [(*B*)] ≫ [(*C*)]. We have taken values of K_1 to be[16] 1.1×10^{-2} and 6.2×10^{-3} mol dm^{-3} for Pro and Sar, respectively, and also values of K_e to be[15] 5×10^{-9} and 1×10^{-8} mol dm^{-3} for Pro and Sar, respectively. This treatment assumes also that no substantial conversion of nitrous acid into XNO occurs, *i.e.* that $1 \gg K_{XNO}[H^+][X]$. This inequality holds for the conditions used in our experiments, except at the very highest [TU] and [TMTU], when the plot of k_o *vs.* [TU] or [TMTU] begins to curve, as expected.

We can then obtain k_2 values from (1) the dependence of k_o upon [X] and constant [H^+] and [S], and also (2) the dependence of k_o upon [S] at constant [X] and [H^+]. Our

Table 4. k_2 Values ($dm^3\ mol^{-1}\ s^{-1}$)

Reactant	Catalyst	*a*	*b*	*c*
Sar	SCN^-	3.3×10^7	3.0×10^7	
Pro	SCN^-	1.5×10^7	1.3×10^7	
Sar	TU	3.0×10^6	2.9×10^6	2.5×10^6
Pro	TU	2.4×10^6	2.3×10^6	1.8×10^6
Sar	TMTU	1.6×10^6	1.7×10^6	1.5×10^6
Pro	TMTU	1.6×10^6	1.5×10^6	1.1×10^6

[a] Obtained from the variation of k_o with [catalyst]. [b] Obtained from the variation of k_o with [reactant]. [c] Obtained from the variation of k_o with acidity.



Scheme 3.

values are given in Table 4; there is good agreement between methods (1) and (2).

When this work was complete a publication[17] reported kinetic results of the much slower nitrosation of Sar and Pro in the absence of added nucleophiles. One interesting feature of this work was the identification of another pathway involving *O*-nitrosation at the $-CO_2^-$ group in (*B*) followed by an internal *O*-to-*N* rearrangement. Previous papers[18] have shown that carboxylic acids can be nitrosated in this way and that the nitrosyl carboxylate species can then act as nitrosating agents. More recently it has also been suggested[19] that the nitrosation of amides involves initial *O*-nitrosation followed by an internal rearrangement. The alternative pathway in the amino acid nitrosation was detected experimentally from the variation of the rate constant with acidity. If we allow this additional pathway in the present work (Scheme 3), then an additional kinetic term features in the expression for k_o. This additional term is given in equation (ii) where k'_2 is the rate constant for

$$\text{additional kinetic term} = \frac{k'_2K_1[S][X][H^+]K_{XNO}}{(K_1 + [H^+])(K_N + [H^+])} \quad \text{(ii)}$$

XNO attack at the carboxylate group. This term is also first order in S and X but the hydrogen ion concentration dependence is different from that in equation (i). Accordingly a plot of $k_o(K_1 + [H^+])(K_N + [H^+])/[H^+]$ *vs.* $[H^+]$ should be linear, with a positive intercept if the pathway *via* *O*-nitrosation is significant. We have used the same treatment as was used for the uncatalysed reactions.[17] The variation of k_o with pH is given in Tables 5 and 6 for the reactions of Sar and Pro, respectively, in the presence of both TU and TMTU. For both amino acids and both catalysts the plots of $k_o(K_1 + [H^+])(K_N + [H^+])/[H^+]$ *vs.* $[H^+]$ are linear with small positive intercepts. Two such plots are shown in Figure 2, for the reactions of both Sar and Pro in the presence of TMTU. These results are consistent with the earlier suggestion that a component of the reaction does involve *O*-nitrosation at the carboxylate group. However in the case of the catalysed reactions, this component is very minor, as shown by the small intercepts. Further the k_2 values determined from the slopes of such plots (given in column 3 of Table 4) are reduced only slightly as compared with the values obtained if we consider reaction to involve only direct *N*-nitrosation. The pathway *via* the *O*-nitrosated intermediate represents only a very small component of the overall reaction, at least for the nucleophile-catalysed nitrosations.

Table 5. Effect of pH on the nitrosation of Sar; $[HNO_2] = 7 \times 10^{-3}$M

pH	[Catalyst]/M	$10^4k_o/s^{-1}$
4.07	1.80×10^{-3} TU	1.00[a]
3.56	1.80×10^{-3} TU	6.00[a]
3.08	1.80×10^{-3} TU	24.0[a]
2.58	1.80×10^{-3} TU	68.0[a]
1.76	1.80×10^{-3} TU	155[a]
3.58	1.83×10^{-3} TMTU	10.0[b]
3.10	1.83×10^{-3} TMTU	32.0[b]
2.73	1.83×10^{-3} TMTU	69.0[b]
2.43	1.83×10^{-3} TMTU	114[b]

[a] [Sar] = 9.48×10^{-2}M. [b] [Sar] = 0.142M.

Table 6. Effect of pH on the nitrosation of Pro; $[HNO_2] = 7 \times 10^{-3}$M

pH	[Catalyst]/M	$10^4k_o/s^{-1}$
3.06	2.47×10^{-3} TU	16[a]
2.80	2.47×10^{-3} TU	29[a]
2.71	2.47×10^{-3} TU	35[a]
2.51	2.47×10^{-3} TU	51[a]
2.24	2.47×10^{-3} TU	71[a]
3.02	1.00×10^{-3} TMTU	6.0[b]
2.61	1.00×10^{-3} TMTU	14[b]
2.42	1.00×10^{-3} TMTU	19[b]
2.26	1.00×10^{-3} TMTU	25[b]
2.08	1.00×10^{-3} TMTU	30[b]
1.80	1.00×10^{-3} TMTU	44[b]

[a] [Pro] = 0.225M. [b] [Pro] = 0.145M.

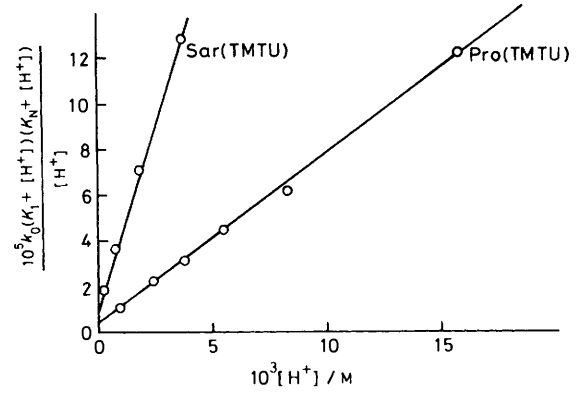


Figure 2. Plot of $k_o(K_1 + [H^+])(K_N + [H^+])/[H^+]$ *vs.* $[H^+]$ for the nitrosation of Sar and Pro in the presence of TMTU

The results as a whole confirm the earlier finding for morpholine,[1] that TU (and now also TMTU) is an excellent catalyst for nitrosation, and all the evidence suggests that the effective intermediate is $ONSC(NR_2)_2^+$. Our results and those obtained earlier show that ions of this type are somewhat less reactive than ONSCN, which in turn is less reactive than BrNO. The extent of the catalysis is governed by the size of K_{XNO}, which is particularly large for the thioureas.

The reactivities of Sar and Pro are very similar, as is to be expected for amino acids with similar basicities of the amino nitrogen atom. It is however worth noting the k_2 values with reference to the corresponding values for BrNO and ClNO reactions. For aniline derivatives with $pK_a > \sim 3$, the k_2 values for BrNO and ClNO reactions (*a*) are very close together, (*b*) do not increase significantly with pK_a, and (*c*) agree very well with the value calculated[20] for encounter-controlled reactions (*ca.* 7×10^9 $dm^3\ mol^{-1}\ s^{-1}$ at 25 °C for reactions in water).

Table 7. The importance of the decomposition pathway for nitrosation of Pro and Sar in the presence of TU at pH 2.58; for Pro, $[HNO_2] = 7.00 \times 10^{-3}$M; for Sar, $[HNO_2] = 7.62 \times 10^{-3}$M

10^3[TU]/M	[Substrate]/M	10^4[$FeSCN^{2+}$]/M	% TU decomp.	% Product nitrosamine
7.05	0.0808 Pro	9.19	13.0	85
7.05	0.1616 Pro	5.06	7.2	94
7.05	0.2424 Pro	1.75	2.5	96
7.05	0.3232 Pro	1.38	2.0	99
1.98	0.1426 Pro	5.19	26.2	93
3.95	0.1426 Pro	5.55	14.1	91
7.90	0.1426 Pro	5.85	7.4	92
7.05	0.1616 Pro	4.95	7.0	94
14.1	0.1616 Pro	5.23	3.7	95
21.1	0.1616 Pro	5.06	2.4	94
2.05	0.0792 Sar	<0.21	<1	99
2.05	0.158 Sar	<0.21	<1	100
2.05	0.237 Sar	<0.21	<1	100

Reactions with ONSCN and $ONSC(NH_2)_2^+$ are much less rapid and all the evidence suggests that for these reagents reaction does not occur at the encounter rate;[10] indeed there is quite a good Brønsted correlation with pK_a for anilines in the pK_a range 2.3—5.3. Similar behaviour has been noted for all these reagents in their reactions with 1-naphthylamine.[11] However for aliphatic amines, including those with pK_a up to *ca.* 11, the situation is a little different. Although there is no significant increasing trend in k_2 values in the pK_a range 8.0—11.2, the actual values for reaction with BrNO change very little within the range $2—3 \times 10^7$ dm^3 mol^{-1} s^{-1}.[21] The values now obtained for ONSCN reactions with both Sar and Pro also lie close to this range, as do those for the reactions of morpholine[1] and dimethylamine.[22] So the suggestion is that ONSCN reactions with these basic aliphatic amines and amino acids are also encounter-controlled. The measured overall activation energy of *ca.* 40 kJ for the morpholine reaction[23] supports this suggestion.

Turning now to the TU- and TMTU-catalysed reactions, we find that the k_2 values for both Sar (pK_a 10.2) and Pro (pK_a 10.6) are in the range $1—3 \times 10^6$ dm^3 mol^{-1} s^{-1}. There are very few literature data for TU- and TMTU-catalysed reactions for comparison, but it is interesting that the less basic morpholine (pK_a 8.6) is probably a little more reactive (k_2 7.0×10^6 dm^3 mol^{-1} s^{-1} at 31 °C).[1] This fact, coupled with a measured activation energy of *ca.* 40 kJ mol^{-1}, suggests again that even the reactions of $ONSC(NR_2)_2^+$ with basic aliphatic amines occur at the encounter limit or close to it. More results are needed for the nitrosothioureas, over a larger range of amine nucleophilicity, to confirm this suggestion. Evidence is now building up which argues that the value calculated for the encounter reaction rate constant (from the Schmoluchowski equation; see ref. 20) may not be the true limit, which may be significantly substrate dependent.

Importance of $ONSC(NR_2)_2^+$ *Decomposition.*—At low acidities the final products of the nitrosation of thiourea are thiocyanate ion and nitrogen [reaction (1)], whereas at higher acidities the disulphide cation and nitric oxide are formed [reaction (2)]. We have worked throughout at low acidities in

$$HNO_2 + SC(NH_2)_2 \rightleftharpoons SCN^- + H^+ + N_2 + 2H_2O \quad (1)$$

$$2HNO_2 + 2H^+ + 2SC(NH_2)_2 \rightleftharpoons (NH_2)_2\overset{+}{C}SS\overset{+}{C}(NH_2)_2 + 2NO + 2H_2O \quad (2)$$

the present study and have examined the concurrent formation of thiocyanate ion during nitrosation of Sar and Pro in the presence of TU and TMTU. This was achieved by adding a sample of the reaction solution (after at least ten half-lives) to an acidified solution containing an excess of Fe^{3+}. The absorbance at 460 nm due to $Fe(SCN)^{2+}$ was measured and hence $[SCN^-]$ determined, after calibration of the procedure with standard SCN^- solutions. The results of studies with (*a*) $[TU]_o = [HNO_2]_o$, (*b*) $[TU]_o < [HNO_2]_o$, and (*c*) $[TU]_o > [HNO_2]_o$ are shown in Table 7, for reactions of Pro, together with a few experiments using Sar. Except for low values of [Pro] the product nitrosamine (as measured by its u.v. absorbance) is formed almost quantitatively, and the thiourea decomposition (as measured by SCN^- formed) is relatively small. Indeed the decomposition can be effectively eliminated by working at high [Pro]. For the more reactive Sar, decomposition is less of a problem, as expected. At higher pH values, when nitrosation is slower, the decomposition reaction is more pronounced, as expected. All the kinetic experiments were carried out under conditions where the decomposition reaction does not compete significantly. However, under other experimental conditions and with less reactive substrates the decomposition of $ONSC(NH_2)_2^+$ is likely to become a serious factor in quantitative kinetic work, and should be treated as a reaction which is in competition with substrate nitrosation.

We believe that even at the low acidities used in this work the reactive intermediates in TU-catalysed nitrosations are these *S*-nitroso species, and that decomposition to SCN^- involves an *S*-to-*N* rearrangement. There is no evidence that the *N*-nitrosated intermediates derived from urea have any significant reactivity as nitrosating reagents, since no catalysis of nitrosation by urea takes place.

Acknowledgements

We thank the North of England Cancer Research Campaign for financial support of this work.

References

1 T. A. Meyer and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1981, 361.
2 K. Al-Mallah, P. Collings, and G. Stedman, *J. Chem. Soc., Dalton Trans.*, 1974, 2469.
3 G. Stedman and P. A. E. Whincup, *J. Chem. Soc.*, 1963, 5796.
4 H. Schmid and M. G. Fouad, *Monatsh. Chem.*, 1957, **88**, 631.
5 P. Collings, K. Al-Mallah, and G. Stedman, *J. Chem. Soc., Perkin Trans. 2*, 1975, 1734.
6 J. W. Lown and S. M. S. Chauhan, *J. Chem. Soc., Chem. Commun.*, 1981, 675.
7 M. Masui, C. Ueda, T. Yasuoka, and H. Ohmori, *Chem. Pharm. Bull.*, 1979, **27**, 1274.
8 P. Collings, M. Garley, and G. Stedman, *J. Chem. Soc., Dalton Trans.*, 1981, 331; M. S. Garley, G. Stedman, and H. Miller, *ibid.*, 1984, 1959.
9 M. Masui, H. Fijisawa, and H. Ohmori, *Chem. Pharm. Bull.*, 1982, **30**, 593.
10 L. R. Dix and D. L. H. Williams, *J. Chem. Res. (S)*, 1984, 96.
11 A. Castro, E. Iglesias, J. R. Leis, and M. E. Peña, *Ber. Bunsenges. Phys. Chem.*, 1986, **90**, 891.
12 G. Hallett and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1372.
13 'C. R. C. Handbook of Biochemistry,' 3rd edn., Cleveland Rubber Co., Cleveland, Ohio, 1976, p. 362.
14 W. Lijinsky, L. Keefer, and J. Loo, *Tetrahedron*, 1970, **26**, 5137.
15 S. S. Mirvish, J. Sams, T. Y. Fan, and S. R. Tannenbaum, *J. Natl. Cancer Inst.*, 1973, **51**, 1833.
16 D. D. Perrin, 'Dissociation Constants of Organic Bases in Aqueous Solution,' Butterworths, London, 1965.
17 J. Casado, A. Castro, J. R. Leis, M. Mosquera, and M. E. Peña, *J. Chem. Soc., Perkin Trans. 2*, 1985, 1859.

18 J. Casado, A. Castro, M. A. Lopez Quintela, M. Mosquera, and M. F. R. Prieto, *Monatsh. Chem.*, 1983, **114**, 647; J. Casado, A. Castro, M. Mosquera, M. F. R. Prieto, and J. V. Tato, *ibid.*, 1984, **115**, 669.
19 A. Castro, E. Iglesias, J. R. Leis, M. E. Peña, and J. V. Tato, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1725.
20 J. H. Ridd, *Adv. Phys. Org. Chem.*, 1978, **16**, 1.
21 A. Castro, J. R. Leis, and M. E. Peña, *J. Chem. Res. (S)*, 1986, 216.
22 T. Bryant and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1988, 97.
23 T. Y. Fan and S. R. Tannenbaum, *J. Agric. Food Chem.*, 1973, **21**, 237.

Received 11*th June* 1987; *Paper* 7/1032