S-Nitrosation and the Reactions of *S*-Nitroso Compounds

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1 Introduction

Nitrosation reactions generally have been well known in chemistry for a long time. The reactions have been much used synthetically and many aspects have been examined mechanistically. By far the largest literature refers to *N*-nitrosation, which has been important synthetically, industrially, and biologically, as well as mechanistically. The nitrosation and diazotization of amines has been much studied [1] and it has been possible to identify a range of specific nitrosating agents, as well as to describe their reactivity quantitatively for a wide range of substrates. Standard texts in organic chemistry usually discuss the chemistry of *N*-nitrosation in some detail. Azo dyes result from the nitrosation of aromatic and heterocyclic amines, and there is much concern regarding the formation of carcinogenic nitrosamines from secondary (and tertiary) amines. In addition, *C*-nitrosation of both aliphatic and aromatic systems is well described, the synthesis of alkyl nitrites from alcohols represents an example of *O*-nitrosation, and there is a large literature referring to nitrosation at metal centres. Much less is known about *S*-nitrosation processes (although there is an obvious formal similarity to *O*-nitrosation) due at least in part to the relative instability of the initially formed *S*-nitroso species. It is to be expected that sulphur-containing compounds generally would be susceptible to electrophilic nitrosation since the more polarizable sulphur atom is known (in other reactions) to be more nucleophilic than a corresponding oxygen atom. In recent years however there has been a significant interest in the area of *S*-nitrosation, both from the synthetic and mechanistic viewpoints, derived from better handling techniques for the unstable compounds and the availability of fast reaction methods to measure rate constants of the rapid reactions involved. Further there is at least one important biological aspect involving the use of vasodilatory drugs such as alkyl nitrites, alkyl nitrates, and the pentacyanonitrosylferrate anion (nitroprusside) where it is believed that reactions with –SH sites are involved. It seems an appropriate time to review this area of *S*-nitrosation generally, to include both synthetic and mechanistic aspects, and to draw comparisons and contrasts, where appropriate, with the corresponding *O*- and *N*-nitrosation reactions.

2 Nitrosation of Thiols

The reaction of thiols, both aliphatic and aromatic, with nitrosating agents NOX (NOCl, RONO, N_2O_4, NO_2, N_2O_3, HNO_2 *etc.*) to form *S*-nitrosothiols or

[1] J. H. Ridd, *Quart. Rev.*, 1961, **15**, 418.

thionitrites (equation 1) probably represents the best-known example of a *S*-

$$RSH + NOX \longrightarrow RSNO + HX \quad (1)$$

nitrosation process. The products are coloured yellow or red or in some cases green, and have been known in solution for some time, indeed this reaction has been used as a quantitative test for nitrosyl sulphuric acid using thioglycolic acid as the reagent.[2] The reaction appears to be quite general from the point of view of the thiol and also from the range of conventional nitrosating agents, including the reaction of alkyl nitrites with thiols.[3] In contrast to the corresponding reaction of alcohols[4] the formation of nitrosothiols is essentially irreversible. There is a recent comprehensive review[5] of the chemistry of nitrosothiols written from the synthetic angle and in terms of the use of these compounds as synthetic reagents.

Nitrosothiols are mostly unstable (particularly when compared with alkyl nitrites), decomposing to give the disulphide and nitric oxide (equation 2),

$$2RSNO \longrightarrow RSSR + 2NO \quad (2)$$

presumably by a homolytic mechanism, although other non-radical pathways are possible. The most stable compounds appear to be those with bulky substituents at the carbon atom attached to the sulphur, *e.g.* t-butyl nitrosothiol,[6] triphenylmethyl nitrosothiol,[7] and the nitrosothiol derived from *N*-acetylpenicillamine[8] which (as a deep green solid with violet reflections) is indefinitely stable as the solid at room temperature, but which decomposes slowly in solution.

Until recently it was believed that the best yields of nitrosothiols were obtained by reaction of the thiol with dinitrogen tetroxide N_2O_4 in equimolar amounts at *ca.* -10 °C in an inert solvent such as hexane, ether, carbon tetrachloride, or acetonitrile.[9] Dinitrogen tetroxide can act as an excellent nitrosating agent (as well as being a source of NO_2 radicals) and can be thought of as $NO^+NO_3^-$ in a number of inorganic reactions[10] as well as organic reactions where, for example, alkyl nitrites can be made from alcohols,[11] nitrosamines from secondary amines,[12] nitrosamides from amides,[13] and the nitroso nitrate adduct from an alkene.[14]

Interestingly, a number of thiols can be converted into nitrosothiols directly

[2] M. Ziegler and O. Glemser, *Z. analyt. Chem.*, 1955, **144**, 187; G. Röbisch and E. Ludwig, *Z. Chem.*, 1974, **14**, 103.
[3] H. Lecher and W. Siefken, *Ber.*, 1926, **59**, 1314, 2594.
[4] P. A. S. Smith, 'The Chemistry of Open-Chain Organic Nitrogen Compounds', Benjamin, New York, 1966, Vol. II, p. 498.
[5] S. Oae and K. Shinhama, *Org. Prep. Proced. Int.*, 1983, **15**, 165.
[6] G. Kresze and U. Uhlich, *Chem. Ber.*, 1959, **92**, 1048.
[7] H. Rheinboldt, *Ber.*, 1926, **59**, 1311.
[8] L. Field, R. V. Dilts, R. Ramanthan, P. G. Lenhert, and G. E. Carnahan, *J. Chem. Soc., Chem. Commun.*, 1978, 249.
[9] S. Oae, Y. H. Kim, D. Fukushima, and K. Shinhama, *J. Chem. Soc., Perkin Trans. 1*, 1978, 913.
[10] C. C. Addison, *Angew. Chem.*, 1960, **72**, 193.
[11] P. Gray and A. D. Yoffe, *Chem. Rev.*, 1955, **55**, 1069.
[12] E. H. White and W. R. Feldman, *J. Am. Chem. Soc.*, 1957, **79**, 5832.
[13] E. H. White, *J. Am. Chem. Soc.*, 1955, **77**, 6008, 6011, 6014.
[14] L. Parts and J. T. Miller, *J. Phys. Chem.*, 1969, **73**, 3088.

using nitric oxide under conditions when oxygen is completely eliminated.[15,16] These include[15] cysteine, *N*-acetylcysteine, glutathione, β-D-thioglucose *etc.* On standing for 20 h however, the reaction mixture from cysteine gave the disulphide;[16] it is believed, by decomposition of the intermediate nitrosothiol. Earlier workers[17] had also reported that thiols reacted with nitric oxide in the presence of base to give either the nitrosothiols or the disulphides. Recent work[18] has suggested that nitrosation and subsequent decomposition to yield the disulphide occurs only by a base-catalysed reaction (see Scheme 1) and that reactions were much inhibited by reduction of the pH below 4.

$$RSH + B^- \rightleftharpoons RS^- + BH$$
$$RS^- + NO \longrightarrow RS\text{–}\dot{N}\text{–}O^- \overset{H^+}{\rightleftharpoons} RS\text{–}\dot{N}\text{–}OH$$
$$2RS\text{–}\dot{N}\text{–}OH \longrightarrow RSN(OH)\text{–}N(OH)SR$$
$$\downarrow$$
$$RSSR + H_2N_2O_2 \; (\longrightarrow N_2 + N_2O)$$

Scheme 1

If oxygen is not completely eliminated then a reaction *via* NO_2 is observed, again leading to the disulphide *via*, it is thought, the intermediate nitrosothiol.[18] This work suggests that NO and NO_2 could be potent inhibitors of thiol-dependent enzymes; such effects have been brought about by cigarette smoke,[19] which is known to contain nitrogen oxides.

A similar reaction scheme (see Scheme 2) has been proposed[20] for the reaction of thiolate anion with two simple nitrosamines, where minute quantities of a free radical, assigned as $R_2NN(O\cdot)SR'$ were detected by e.s.r. spectroscopy.

$$R'S^- + R_2NNO \rightleftharpoons R_2N\text{–}N(O^-)(SR')$$

$$R_2N\text{–}N(O^-)(SR') + R_2NNO + H^+ \longrightarrow R_2N\text{–}N(O^\bullet)(SR') + NO + R_2NH$$

$$R_2N\text{–}N(O^\bullet)(SR') \rightleftharpoons R_2NNO + \dot{S}R' \; (\longrightarrow R'SSR')$$

Scheme 2

[15] L. J. Ignarro, B. K. Barry, D. Y. Gruetter, J. C. Edwards, E. H. Ohlstein, C. A. Gruetter, and W. H. Baricos, *Biochem. Biophys. Res. Commun.*, 1980, **94**, 93.

[16] U. Schultz and D. R. McCalla, *Can. J. Chem.*, 1969, **47**, 2021.

[17] H. Reihlen, A. Friedolsheim, and W. Oswald, *Justus Liebigs Ann. Chem.*, 1928, **465**, 72; R. Longhi, R. O. Ragsdale, and R. S. Drago, *Inorg. Chem.*, 1962, **1**, 768.

[18] W. A. Pryor, D. F. Church, C. K. Govindan, and G. Crank, *J. Org. Chem.*, 1982, **47**, 156.

[19] M. L. Fenner and J. Braven, *Br. J. Cancer*, 1968, **22**, 474.

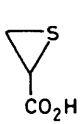
[20] W. A. Waters, *J. Chem. Soc., Chem. Commun.*, 1978, 741.

A similar situation with regard to possible reactions by nitric oxide exists for amines. There, in the complete absence of air and catalysts (such as metal halides and iodine) no reaction occurs either in organic solvents[21] or in the gas phase,[22] whereas rapid nitrosation occurs when air is admitted, no doubt *via* nitrogen dioxide. There is no report of nitrosation of an amine by nitric oxide in strongly basic media, though perhaps this is not a reaction one would expect, given that secondary amines are much less acidic than are the thiols.

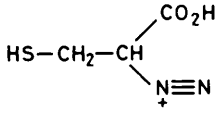
Cysteine is also converted almost quantitatively into cystine by a nitrosamide[16] in aqueous ethanol at pH 7 in an atmosphere of nitrogen. A mechanism was suggested based on the formation and subsequent decomposition of *S*-nitrosocysteine, though it was not established whether it was formed by direct attack of the nitrosamide or whether some free nitrosating species was first formed.

It has recently been suggested[23] that convenient syntheses of nitrosothiols can be achieved using an alkyl nitrite, specifically t-butyl nitrite, as the reagent in a solvent such as chloroform. Quantitative yields were observed from t-butyl thiol and benzyl thiol.

All the experiments so far described on the nitrosation of amino thiols (cysteine *etc.*) indicate that *S*- rather than *N*-nitrosation occurs. However it does seem that under certain conditions the product derived from *N*-nitrosation can be formed in reasonable yield. Thus[24] from cysteine itself 55% of the thiirancarboxylic acid (1) was recovered, probably by way of the diazonium intermediate (2) by nucleophilic



(1)



(2)

displacement of nitrogen by the thiol group. Similar results were obtained for the methyl ester of cysteine and (in rather lower yield) for penicillamine. It is possible that the *N*-nitroso intermediate derives from the initially formed *S*-nitroso species; an example of such a rearrangement will be discussed in Section 4.

Some rate measurements have been carried out on the nitrosation of thiols. Three groups of workers have independently established the rate law given in equation 3 for the reactions of t-butyl thiol (in 50% aqueous dioxan),[25] cysteine (in

$$\text{Rate} = k[\text{Thiol}][\text{H}^+][\text{HNO}_2] \qquad (3)$$

[21] B. C. Challis and S. A. Kyrtopoulos, *J. Chem. Soc., Perkin Trans. 1*, 1979, 299.
[22] G. B. Neurath, M. Dunger, and F. G. Pein in 'Environmental *N*-Nitroso Compounds, Analysis, and Formation', ed. E. A. Walker, P. Bogovski, and L. Griciute, IARC Scientific Publication No. 14, International Agency for Research on Cancer, Lyon, 1976, pp. 215—225; D. Spincer and D. T. Westcott, *ibid.*, pp. 133—139.
[23] M. P. Doyle, J. W. Terpstra, R. A. Pickering, and D. M. LePoire, *J. Org. Chem.*, 1983, **48**, 3379.
[24] C. D. Maycock and R. J. Stoodley, *J. Chem. Soc., Chem. Commun.*, 1976, 234.
[25] G. Kresze and J. Winkler, *Chem. Ber.*, 1963, **96**, 1203.

water)[26] and a number of mercapto-carboxylic acids (in water).[27] This is a familiar rate equation[28] which applies under certain experimental conditions to the nitrosation of a wide variety of substrates including primary and secondary amines (aromatic and aliphatic), alcohols, hydrazine, hydrazoic acid, ureas *etc.*, and is generally interpreted in terms of a mechanism involving rate-limiting attack by a positively charged species $H_2NO_2^+$ or NO^+. At the acidities employed the extent of protonation of the thiol is negligible so there is no complication here of the kind generally found for basic substrates. Values of the third-order rate constant k equation 3) for these thiols together with some unpublished results are included in Table 1, together with a few selected values for some other substrate for comparison

Table 1 *Values of k (equation 3) for acid-catalysed nitrosation in water at* 25 °C

Substrate	k/l^2 mol^{-2} s^{-1}	*Ref.*
$CO(NH_2)_2$	0.89	29
2,4-Dinitroaniline	2.5	29
HN_3	160	29
$\overset{+}{N}H_3NH_2$	620, 611	29, 30
MeOH	700	31
$(NH_2)_2CS$	6 960	26
Sulphanilic acid	7 300	29
Me_3CSH	47*	25
Cysteine methyl ester	213	32
Cysteine	456, 443	26, 27
N-Acetylpenicillamine	840†	31
Glutathione	1 080	32
Mercaptosuccinic acid	1 334	27
Thioglycolic acid	2 630	32
Mercaptopropanoic acid	4 764	27

* In 50% dioxan–water. † At 31 °C

purposes. It is clear that *S*-nitrosation is a very reactive process, and requires a fast reaction technique such as stopped-flow spectrophotometry to enable rate constants to be measured. Most of the thiols studied, for example are more reactive than HN_3, $^+NH_3NH_2$, and CH_3OH. It has been argued,[33] on the basis of the relative constancy of k values for very reactive species (*e.g.* thioureas and aniline derivatives), that these reactions occur at the encounter limit of the substrate with $H_2NO_2^+$ or NO^+. This limit appears to be around 7 000 l^2 mol^{-2} s^{-1} for neutral

[26] P. Collings, K. Al-Mallah, and G. Stedman, *J. Chem. Soc., Perkin Trans. 2*, 1975, 1734.
[27] L. R. Dix and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1984, 109.
[28] D. L. H. Williams, *Adv. Phys. Org. Chem.*, 1983, **19**, 381.
[29] J. Fitzpatrick, T. A. Meyer, M. E. O'Neill, and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1984, 927.
[30] J. R. Perrott, G. Stedman, and N. Uysal, *J. Chem. Soc., Dalton Trans.*, 1976, 2058.
[31] S. E. Aldred, D. L. H. Williams, and M. Garley, *J. Chem. Soc., Perkin Trans. 2*, 1982, 777.
[32] P. A. Morris and D. L. H. Williams to be published.
[33] J. H. Ridd, *Adv. Phys. Org. Chem.*, 1978, **16**, 1.

substrates. A number of the thiols studied (although containing deactivating substituents *e.g.* $-CO_2H$) have k values approaching this limit, which suggests that here also reaction occurs at or close to the diffusion limit. In one case[27] the measured experimental activation energy is 56 kJ mol^{-1} which is close to that expected for such a process because of the pre-equilibria involved and the temperature dependence of the viscosity of the solvent.

Nucleophilic catalysis of nitrosation of amines and other species is well known[1] and is believed to arise by reaction of an equilibrium concentration of the corresponding nitrosyl compound NOX (equation 4). The extent of catalysis is

$$HNO_2 + H^+ + X^- \rightleftharpoons NOX + H_2O \qquad (4)$$

principally governed[28] by the size of the equilibrium constant for NOX formation which increases along the series ONCl < ONBr < ONSCN < $ONS\overset{+}{C}(NH_2)_2$ as expected from the nucleophilicities of the anions concerned, even though the rate constants for attack by NOX decrease along the same series. Such catalysis does not occur for amides[34] nor for some amines containing electron-withdrawing groups,[29] and this has been attributed[35] to the importance of the reversibility of attack by the nitrosating species, in these systems. Thiols however all show catalysis by halide ion and thiocyanate ion and it is possible to extract the second-order rate constants k_2 for attack by the NOX species. The data are presented in Table 2 and

Table 2 *Second-order rate constants* k_2 *for the reactions of* NOCl, NOBr *and* NOSCN *with thiols in water at* 25 °C

	k_2 values/l mol^{-1} s^{-1}		
	NOCl	NOBr	NOSCN
Cysteine methyl ester	1.0×10^6	4.9×10^4	7.0×10^2
Cysteine	1.2×10^6	5.8×10^4	7.0×10^2
Glutathione	5.7×10^6	2.9×10^5	1.9×10^3
Mercaptosuccinic acid	—	2.6×10^4	—
Thioglycolic acid	1.4×10^7	1.1×10^6	2.5×10^4
Mercaptopropanoic acid	—	4.5×10^5	—

are taken from references 27 and 32. The by now well-established[28] trend of reactivity ONCl > ONBr > ONSCN is now extended to their reaction with thiols. Surprisingly the most reactive thiols towards ONCl have k_2 values which are 2—3 powers of ten less than the calculated value[33] expected for a diffusion-controlled process. It is not clear why this should be so.

The high reactivity of thiols in nitrosation suggests that in general they would make excellent scavengers for nitrous acid, particularly those with a high solubility in water, such as the mercaptocarboxylic acids. There are a number of situations

[34] C. N. Berry and B. C. Challis, *J. Chem. Soc., Perkin Trans. 2*, 1974, 1638; M. Yamamoto, T. Yamada, and A. Tanimura, *J. Food Hygiene Soc. Jpn.*, 1976, **17**, 363.
[35] G. Hallett and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1980, 1372.

which call for the removal of nitrite or nitrous acid from solution, *e.g.* in some nitration reactions in strong nitric acid and in some biologically related systems to prevent nitrosamine formation. The efficiency of mercaptopropanoic acid has been examined quantitatively[27] by noting its ability to suppress the reversibility of a denitrosation reaction of a nitrosamine. As expected, the thiol was very efficient in this respect and was significantly more so than azide, a well-known nitrous acid scavenger. Similarly it is to be expected that added thiols would suppress amine nitrosation by a direct competition reaction. This is indeed the case[36] for the nitrosation of an aniline derivative where the extent of *N*-nitrosation is markedly reduced by added cysteine or *N*-acetylpenicillamine, and it was possible in both cases to inhibit completely the *N*-nitrosation reaction when sufficient thiol was present.

A major difference between *O*- and *S*-nitrosation is that whilst the former is significantly reversible the latter is effectively not. For example,[31,37] equilibrium constants for the nitrosation of a number of alcohols (and carbohydrates) are in the approximate range 0.5–2.0 l mol^{-1}, whereas values are too large to measure reasonably for the corresponding sulphur case. The denitrosation of nitrosothiols can be effected at high acidity if steps are taken to remove the free nitrous acid as it is formed.[38] An explanation has been suggested[31] based on the differences between the nucleophilicity and basicity of the corresponding oxygen and sulphur sites. For the forward reaction in equation 5, the important factor in the comparison of the

$$RSH + NOX \rightleftharpoons RS\overset{+}{\underset{NO}{\overset{H}{}}} \xrightleftharpoons{H_2O} RSNO + H_3O^+ + X^- \qquad (5)$$

reactivities of ROH and RSH, is the nucleophilicity difference between the oxygen and sulphur atoms. There is much independent evidence which shows that the sulphur is the more nucleophilic. This is borne out in nitrosation studies, where it has been shown[31] that *N*-acetylpenicillamine (3) (a reasonably good model for t-butyl thiol) is several orders of magnitude more reactive than t-butyl alcohol (4). For the reverse reaction, however, an important factor (see equation 5) is the

$HSCMe_2CH(NHAc)CO_2H$ (3) $HOCMe_3$ (4)

relative basicities of the sulphur (in RSNO) and the corresponding oxygen (in RONO) atoms. Again there is much evidence to suggest that oxygen is the more basic by *ca.* 10^5. This corresponds quite well with the estimated[38] difference of 2×10^6 in the reactivity of nitrosothiols and corresponding alkyl nitrites, with the latter being the more reactive. This is an example of where the relative basicity and nucleophilicity of oxygen and sulphur operates in opposite directions for the forward and reverse reactions.

It is not possible to compare exactly the reactivities in *S*-, *O*-, and *N*-nitrosations,

[36] D. L. H. Williams and S. E. Aldred, *Fd. Chem. Tox.*, 1982, **20**, 79.
[37] J. Casado, F. M. Lorenzo, M. Mosquera, and M. F. R. Prieto, *Can. J. Chem.*, 1984, **62**, 136.
[38] S. S. Al-Kaabi, D. L. H. Williams, R. Bonnett, and S. L. Ooi, *J. Chem. Soc., Perkin Trans. 2*, 1982, 227.

because the relevant compounds have not been subject to a kinetic study under the same conditions, but it is clear that thiols are generally more reactive than corresponding alcohols, and are at least comparable in reactivity with amines. More results are needed in this area.

The reactivity of thiols towards nitrosamines has been measured quantitatively using the denitrosation reaction carried out in the presence of a sufficient excess of hydrazine to suppress the reverse reaction[39] (equation 6). Kinetic measurements

$$\mathrm{PhN(Me)NO + Y \xrightarrow{H^+} PhNH(Me) + \underset{\downarrow}{NOY^+}}_{\text{Removed}} \qquad (6)$$

with a range of nucleophiles Y gave the relative reactivity order shown in Table 3.

Table 3 *Relative reactivities of some nucleophiles towards* N-*nitroso*-N-*methylaniline in aqueous acid solution*

Nucleophile	*Relative Reactivity*
Chloride ion	1
Cysteine	2
Glutathione	3
S-Methylcysteine	35
Bromide ion	55
Methionine	65
Thiocyanate ion	5 500
Thiourea	13 000
Iodide ion	15 750

Clearly both cysteine and glutathione are about as reactive as chloride ion whereas, as expected, the two sulphides are somewhat more reactive whilst falling well short of that of thiourea.

Thiols are also oxidized to disulphides by reaction with the pentacyanonitrosylferrate ion in alkaline solution[40] as shown in equation 7. Reactions are

$$2[Fe(CN)_5NO]^{2-} + 2SR^- \longrightarrow RSSR + 2[Fe(CN)_5NO]^{3-} \qquad (7)$$

characterized by a very rapid formation of an intense colour, usually red, which then fades gradually. This has been interpreted as a rapid adduct formation (equation 8) which is the coloured species, and the rate of this process has been

$$[Fe(CN)_5NO]^{2-} + SR^- \rightleftharpoons [Fe(CN)_5NO(SR)]^{3-} \qquad (8)$$

measured by stopped-flow spectrophotometry.[41] The bonding in such adducts is not described, but since the nitroso group is believed [42] to act in the NO^+ sense in

[39] G. Hallett and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1980, 624.
[40] D. Mulvey and W. A. Waters, *J. Chem. Soc., Dalton Trans.*, 1975, 951; P. J. Morando, E. B. Borghi, L. M. de Schteingart, and M. A. Blesa, *ibid.*, 1981, 435.
[41] P. A. Rock and J. H. Swinehart, *Inorg. Chem.*, 1966, **5**, 1078.
[42] N. G. Connelly, *Inorg. Chim. Acta*, 1972, **6**, 47.

this iron complex, it could well be that the sulphur is bonded to the nitrogen of the nitroso group. An equivalent interaction has been proposed in the deamination of primary aliphatic amines by the pentacyanonitrosylferrate ion.[43] The decomposition of the adduct, in the case of the thiol reaction, has not been established unequivocally. Two suggestions have been made, equations 9 and 10, the first

$$[Fe(CN)_5NO(SR)]^{3-} + RS^- \longrightarrow [Fe(CN)_5NO]^{4-} + RSSR \quad (9)$$

$$[Fe(CN)_5NO(SR)]^{3-} \longrightarrow [Fe(CN)_5\dot{N}O]^{3-} + RS^{\bullet} \quad (10)$$

$$\downarrow$$

$$RSSR$$

involving attack by RS^- and the other a one-electron transfer process. An alternative might well be a unimolecular decomposition of the adduct, eliminating the nitrosothiol which then loses nitric oxide and forms the disulphide.

The physical properties of nitrosothiols are well documented in the review by Oae and Shinhama[5] and will not be reported in detail here. In general they are coloured red or green as the pure compounds but often are yellow in solution in common with many *S*-nitroso species. Dipole moments, infrared spectra, and u.v. and visible spectra have been reported and are much as expected, and compare reasonably with those of the more widely studied alkyl nitrites, given the expected changes consequent upon the greater electronegativity of oxygen than sulphur. ^{15}N-N.m.r. chemical shifts have been measured,[44] and have been used to identify *S*-compounds from model peptides such as *N*-acetylcysteine, *N*-acetylpenicillamine, and thioacetic acid, using triphenylmethylthionitrite as a reference. In addition ^{14}N-n.m.r. shifts have also been reported[45] for CF_3SNO and EtSNO. Finally the crystal structure of the stable nitrosothiol (3) derived from *N*-acetylpenicillamine has been determined.[8]

3 Reactions of *S*-Nitrosothiols (Thionitrites)

Nitrosothiols readily decompose thermally to give disulphides and nitric oxide (equation 11); the same products can be obtained photochemically. Both clearly

$$2RSNO \longrightarrow RSSR + 2NO \quad (11)$$

involve homolysis of the S–N bond. Oxidation can give the thionitrate, and reduction the thiol or the disulphide. Reaction with thiols gives the unsymmetrical disulphides, and with sulphinic acid the thiolsulphonates. Nitrosothiols have also been used to convert secondary amines into *N*-nitrosamines, to effect deamination of arylamines, and to convert alcohols into alkyl nitrites; these reactions are all described in reference 5 and in references therein. The remainder of this section will concentrate on the more recent work and in particular on the limited amount of mechanistic work undertaken.

[43] A. R. Butler, C. Glidewell, J. Reglinski, and A. Waddon, *J. Chem. Res.* (*S*), 1984, 279.
[44] R. Bonnett, R. Holleyhead, B. L. Johnson, and E. W. Randall, *J. Chem. Soc., Perkin Trans. 1*, 1975, 2261.
[45] L. O. Andersson, J. B. Mason, and W. van Bonswijk, *J. Chem. Soc.* (*A*), 1970, 296.

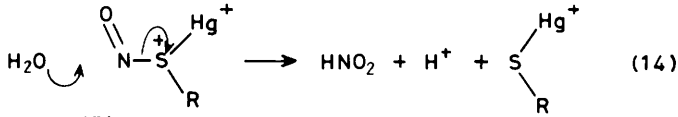
The acid-catalysed hydrolysis of nitrosothiols (equation 12) is many orders of

$$RSNO + H_2O \overset{H^+}{\rightleftharpoons} RSH + HNO_2 \qquad (12)$$

magnitude slower than that of alkyl nitrites in general; this has been attributed to the greater basicity of the oxygen atom.[38] The reaction has only been studied at relatively high acidities and in the presence of a sufficient excess of added sodium azide (or some other nitrous acid trap) to ensure irreversibility. The method is entirely analogous to that used to study the denitrosation of nitrosamines, where again the equilibrium greatly favours the formation of nitrosamines.[46] As for the nitrosamines the reaction of nitrosothiols is catalysed by halide ion and other nucleophiles,[38] in the sequence $Cl^- < Br^- < SCN^- \sim SC(NH_2)_2$ (equation 13).

$$\overset{+}{R}SH(NO) + Hal^- \longrightarrow RSH + \underset{\text{Removed}}{NOHal} \qquad (13)$$

It has been found[47] that the mercuric ion also catalyses the hydrolysis (again in the presence of a nitrous acid scavenger). This was used as the basis of an analytical procedure for the quantitative analysis of thiols. No kinetic data are available, but it has been suggested[47] that a *S*-bound mercury complex (5) is formed which undergoes hydrolysis as in equation 14.



(5) (14)

In a study relating to the possible *in vivo* formation of carcinogenic nitrosamines the *trans* nitrosation reaction (nitrosothiol + amine ⟶ nitrosamine) has been studied using *S*-nitrosocysteine, *S*-nitrosoglutathione, and a protein-bound nitrite model system.[48,49] All brought about nitrosation of *N*-methylaniline and other amines in acid and alkaline solution, thus reinforcing an earlier suggestion[50] that *S*-nitrosothiols can act as transfer-nitrosating agents (equation 15), although it is

$$RSNO + R'R''NH \longrightarrow R'R''NNO + RSH \qquad (15)$$

not yet established whether this is a direct one-stage process or not. In contrast there was no observable catalysis of nitrosation of morpholine by cysteine,[51] although *S*-methylcysteine did show a small catalytic effect.

[46] I. D. Biggs and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1975, 107.
[47] B. Saville, *Analyst*, 1958, **83**, 670.
[48] M. J. Dennis, R. Davies, and D. J. McWeeny, *J. Sci. Food Agric.*, 1979, **30**, 639.
[49] M. J. Dennis, R. C. Massey, and D. J. McWeeny, *J. Sci. Food Agric.*, 1980, **31**, 1195.
[50] A. Mirna and K. K. Hofmann, *Fleischwirtschaft*, 1969, **49**, 1361.
[51] T. A. Meyer and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1981, 361.

It has been known for some time that many potential nitrosating agents can act as vasodepressor agents and a number have been widely used in the treatment of angina, heart failure, and hypertensive emergencies. The substances used include organic nitrites (and nitrates), nitric oxide, sodium nitrite, and the pentacyanonitrosylferrate (nitroprusside) anion. Little is known about the mechanism of these compounds but it is thought [52] that the relaxation in vascular smooth muscle is dependent on the presence of tissue-bound SH groups. More recently it has been shown [53] that the vasodilator action of alkyl nitrites *etc.* can be attributed at least in part to the formation of unstable *S*-nitrosothiols as intermediates. The detailed mode of action is still not resolved but it is believed that *S*-nitrosothiols markedly activate the enzyme guanylate cyclase which brings about relaxation of vascular smooth muscle and so decreases the systemic arterial pressure. *S*-Nitrosothiols also bring about inhibition of human platelet aggregation.[54] More work remains to be done before a complete mechanistic picture emerges but it is clear that *S*-nitrosothiols play an important part.

4 Nitrosation of Sulphides

Although it is to be expected that the sulphur atom in a sulphide is at least as nucleophilic as that in a thiol, there is not such a convenient leaving goup (H^+) in the case of the sulphides so *S*-nitrosothiol formation is not to be expected, and indeed is not found from simple sulphides RSR. However there are reports of *S*-nitrosothiol formation from disulphides. Thus [55] the reactions of disulphides with N_2O_4 give products compatible with the intermediacy of RSNO and RSO^+ (equation 16). Similarly the photolysis of disulphides in the presence of nitric oxide

$$\mathrm{RSSR} + \mathrm{N_2O_4} \longrightarrow \underset{\text{Products}}{\mathrm{RSNO}} + \underset{\text{Products}}{\mathrm{RSO^+}} \qquad (16)$$

yields the *S*-nitrosothiol,[56] presumably according to equation 17. There are also

$$\mathrm{CH_3SSCH_3} \xrightarrow{h\nu} 2\mathrm{CH_3S^\cdot} \xrightarrow{\mathrm{NO}} 2\mathrm{CH_3SNO} \qquad (17)$$

reports of ring opening reactions of cyclic sulphides which are interpreted in terms of a *S*-nitrosation. It has also been known for a long time that simple alkyl sulphides react with alkyl nitrites (and tetranitromethane) to give coloured solutions which fade on standing.[57] As far as is known, intermediates and products of such reactions have not been identified positively but it does seem likely that the coloured intermediates are in fact *S*-nitroso ions.

[52] P. Needleman, B. Jakschik, and E. M. Johnson, *J. Pharmacol. Exp. Ther.*, 1973, **187**, 324.
[53] L. J. Ignarro, H. Lippton, J. C. Edwards, W. H. Baricos, A. L. Hyman, P. J. Kadowitz, and C. A. Gruetter, *J. Pharmacol. Exp. Ther.*, 1981, **218**, 739.
[54] B. T. Mellion, L. J. Ignarro, C. B. Myers, E. H. Ohlstein, B. A. Ballot, A. L. Hyman, and P. J. Kadowitz, *Mol. Pharmacol.*, 1983, **23**, 653.
[55] S. Oae, D. Fukushima, and Y. H. Kim, *Chem. Lett.*, 1978, 279.
[56] P. M. Rao, J. A. Copeck, and A. R. Knight, *Can. J. Chem.*, 1967, **45**, 1369.
[57] E. M. Harper and A. K. Macbeth, *Proc. Chem. Soc.*, 1914, **30**, 15; A. K. Macbeth and D. D. Pratt, *J. Chem. Soc.*, 1921, **119**, 354.

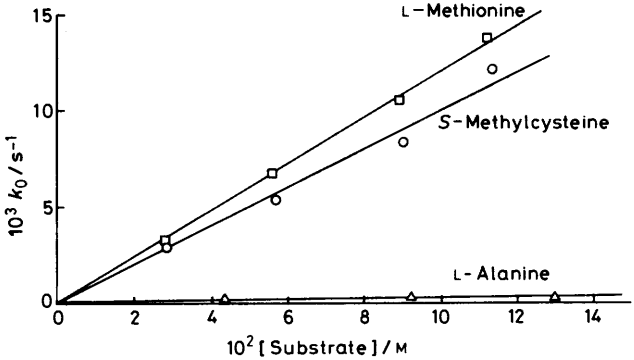
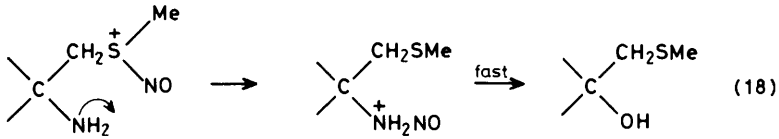


Figure 1 *Variation of the first-order rate constant with* [*Substrate*] *for the deamination of methionine* (1), S-*methylcysteine* (2) *and alanine* (3)

The deamination of both methionine and *S*-methylcysteine proceed normally to give the expected alcohol products,[58] but at a much enhanced rate (see Figure 1), compared with a similar amine without the –SR group.[59] This has been interpreted as first involving a *S*-nitroso intermediate which undergoes an intramolecular *S*- to *N*-rearrangement, leading to the alcohol product as shown in equation 18 for *S*-



(18)

(6)

methylcysteine. In this case a favourable five-membered ring transition-state would be involved, whereas for methionine a six-membered ring interaction is proposed. There are a number of examples in the literature where *S*- to *N*-rearrangement of this kind occurs, although it is not established whether it occurs intramolecularly or intermolecularly. For example,[60] acylation of thioamides and thioureas give initially the *S*-bonded isomer which rearranges on heating to the *N*-acylated product.

It is possible that *S*-nitroso ions of the type (6) could also act intermolecularly to effect nitrosation of a suitable species. In practice this would be observable kinetically as catalysis of nitrosation (or diazotization) by added RSR, in the same way as catalysis is brought about by added Cl^-, Br^-, SCN^- *etc.* Such experiments have not yet been described, although there is one report [51] of a measure of catalysis of diazotization of aniline by added *S*-methylcysteine, but this example is complicated by the possibility of an intramolecular reaction.

[58] G. A. Maw and C. M. Coyne, *Arch. Biochem. Biophys.*, 1966, **117**, 499.
[59] T. A. Meyer and D. L. H. Williams, *J. Chem. Soc., Chem. Commun.*, 1983, 1067.
[60] M. L. Moore and P. S. Crossley, *J. Am. Chem. Soc.*, 1940, **62**, 3273.

There is strong kinetic evidence for *S*-nitrosation of sulphides using nitrosamines on the source of the nitroso group.[39] The denitrosation of *N*-methyl-*N*-nitrosoaniline in the presence of a sufficient excess of hydrazine to ensure irreversibility is catalysed by both methionine and glutathione to approximately the same extent. Both are significantly more reactive than the thiol cysteine as expected and are approximately as reactive as bromide ion (see Table 3, Section 2). This reaction scheme (Scheme 3) implies also that the nitrososulphonium ion (7)

$$PhN(Me)NO + H^+ \underset{}{\overset{\text{fast}}{\rightleftharpoons}} Ph\overset{+}{N}H(Me)NO$$

$$Ph\overset{+}{N}H(Me)NO + RSR' \xrightarrow{\text{slow}} PhNHMe + R\overset{+}{S}R' \quad (\text{NO})$$

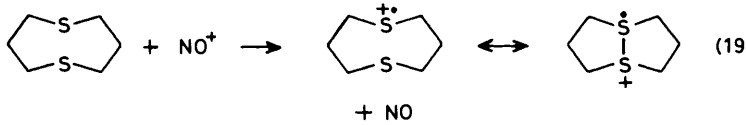
$$R\overset{+}{S}R' \quad (\text{NO}) + NH_2NH_2 \xrightarrow{\text{fast}} RSR' + \text{decomposition products}$$

(7)

Scheme 3

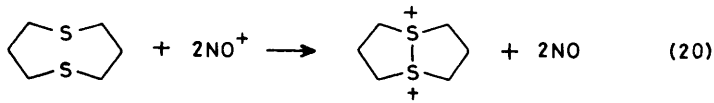
can itself nitrosate hydrazine. More work is needed to develop this idea further.

Sulphides can also undergo another kind of reaction with the nitrosonium ion in a one-electron transfer reaction leading to radical cation formation. This is not in the normal sense a *S*-nitrosation reaction but, since it is closely related, it is included in this section. The cyclic disulphides 1,5-dithiocyclo-octane and 1,5-dithiocyclononane are oxidized by one equivalent of NO^+ (added as the tetrafluoroborate) in acetonitrile or propionitrile to the long-lived cation radical[61] as shown in equation 19. The ion which can be isolated as the hexafluorophosphate



(19)

salt, shows sulphur–sulphur transannular interaction as evidenced by the e.s.r. spectrum. Similarly, two equivalents of NO^+ yield the dication (equation 20) again



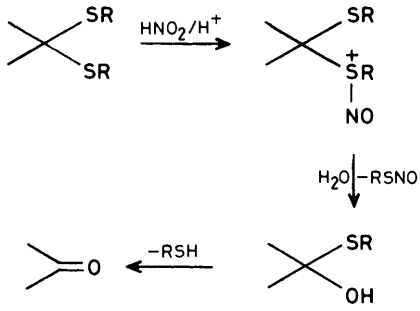
(20)

with the S–S transannular bridge, and an analogous N–S bond is formed when one of the sulphur atoms in the cyclo-octane is replaced by –NMe.[62]

S,S-Acetals (which can be thought of as sulphides) undergo reaction with nitrous

[61] W. K. Musker, T. L. Wolford, and P. B. Roush, *J. Am. Chem. Soc.*, 1978, **100**, 6416.
[62] W. K. Musker, A. S. Hirschon, and J. T. Doi, *J. Am. Chem. Soc.*, 1978, **100**, 7754.

acid (and with other electrophiles) to give the corresponding carbonyl compound.[63] Reaction is believed to involve *S*-nitrosation followed by loss of RSNO and subsequently loss of RSH as outlined in Scheme 4. This has a certain

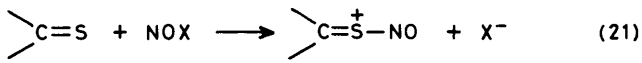


Scheme 4

similarity to the reaction discussed in Section 5 whereby thiocarbonyl compounds generally are converted into the carbonyl compounds by reaction with nitrous acid.

5 Nitrosation of Thiocarbonyl Compounds

The sulphur atom in thiocarbonyl compounds has a pronounced nucleophilic reactivity (*e.g.* thiourea is as reactive as iodide ion as measured by the Pearson nucleophilicity parameter[64]) so it is to be expected that electrophilic *S*-nitrosation occurs. This is indeed the case and all reactions studied in this area involve the formation of the *S*-nitrososulphonium ion as shown in equation 21. Most of the



(21)

work reported has been concerned with the nitrosation of thiourea and its alkyl derivatives no doubt because of the stability of these species compared with other thiocarbonyl compounds *e.g.* thioketones. It has been known for some time by the work of Werner[65] that thiourea can undergo two reactions with nitrous acid, one leading to nitrogen and thiocyanate ion (equation 22) and the other to a disulphide

$$HNO_2 + (NH_2)_2CS \longrightarrow H^+ + SCN^- + N_2 + 2H_2O \qquad (22)$$

cation (*C*,*C*-dithiodiformamidinium), equation 23. The former, which predominates

$$2HNO_2 + 2H^+ + 2(NH_2)_2CS \longrightarrow (NH_2)_2\overset{+}{C}SS\overset{+}{C}(NH_2)_2 + 2NO + 2H_2O \qquad (23)$$

at low acidities, can readily be rationalized in terms of *N*-nitrosation whilst the

[63] M. T. M. El-Wassimy, K. A. Jørgensen, and S. O. Lawesson, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2201.
[64] R. G. Pearson, H. Sobel, and J. Songstad, *J. Am. Chem. Soc.*, 1968, **90**, 319.
[65] A. E. Werner, *J. Chem. Soc.*, 1912, **101**, 2180; M. E. Coade and A. E. Werner, *J. Chem. Soc.*, 1913, **102**, 1221.

latter, which can be explained by *S*-nitrosation, takes over at higher acidity. Again, in common with all *S*-nitrosation reactions, a transient yellow or red colour is observed here and seems to be a property of the *S*-nitroso ion.

The structure of the dication has been established by crystal structure analysis of its salts.[66] The same product can be obtained using other oxidizing agents such as hydrogen peroxide, the halogens and peracids, and also using nitrosamines.[67] The reaction is very general and not only thioureas, but also thiocarbonates and thioketones (including heterocyclic systems) have been converted into stable dications containing the –S–S– bond by a variety of chemical and electrochemical oxidation procedures.[68]

The equilibrium constant for the formation of the *S*-nitrosothiouronium ion (equation 24) has been measured[69] as has the rate constant for *S*-nitrosation, by

$$H^+ + HNO_2 + (NH_2)_2CS \rightleftharpoons (NH_2)_2C\overset{+}{S}NO + H_2O \qquad (24)$$

noting the increasing absorbance due to the yellow *S*-nitroso ion in a stopped-flow spectrophotometer. The value of the equilibrium constant is 5 000 l^2 mol^{-2} at 25 °C, which means that at suitable concentrations of the reagents substantial conversion of the nitrous acid into the *S*-nitroso ion can occur. This contrasts with the corresponding situation for the reaction with halide ion where only very small equilibrium quantities of the nitrosyl halides are formed.

The rate constant k (equation 25) for the nitrosation of thiourea was found[26] to

$$\text{Rate} = k[H^+][HNO_2][(NH_2)_2CS] \qquad (25)$$

be 6 960 l^2 mol^{-2} s^{-1} at 25 °C which is only slightly greater than the values reported[70] for aniline, *o*-toluidine, and *o*-chloroaniline, and very close to that found for sulphanilic acid,[29] all of which suggests that in each case these reactions occur by diffusion-controlled reaction between the substrate and the positively charged nitrosating agent. Further the value of k is little changed by *N*-methyl substitution[26] and the activation energy of 65 kJ is close to that expected for such a diffusion-controlled process.

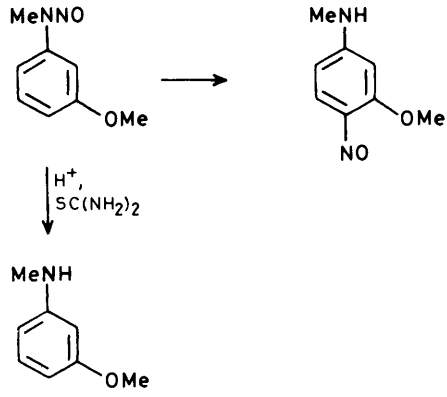
A similar reaction occurs with nitrosamines and thiourea in acid solution,[67] yielding initially the yellow colour characteristic of the *S*-nitroso species which then gives the disulphide salt as before. Quantitative kinetic measurements were carried out under slightly different conditions, in the presence of an excess of a suitable nitrous acid trap (hydrazine *etc.*) which destroyed the *S*-nitroso ion rapidly, so that the direct *S*-nitrosation of thiourea could then be examined without any reversibility problems. That the reaction is a direct one (equation 26) is shown by

[66] O. Foss, J. Johnsen, and O. Tvedten, *Acta Chem. Scand.*, 1958, **12**, 1782, and references therein.
[67] D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1977, 128.
[68] R. L. Blankespoor, M. P. Doyle, D. M. Hedstrand, W. H. Tamblyn, and D. A. Van Dyke, *J. Am. Chem. Soc.*, 1981, **103**, 7096.
[69] K. Al-Mallah, P. Collings, and G. Stedman, *J. Chem. Soc., Dalton Trans.*, 1974, 2469.
[70] H. Schmid and C. Essler, *Monatsh.*, 1960, **91**, 484.

$$RR'\overset{+}{N}(H)NO + SC(NH_2)_2 \longrightarrow ON\overset{+}{S}C(NH_2)_2 + RR'NH \qquad (26)$$

the fact that it occurs in the presence of nitrous acid traps so that hydrolysis to free nitrous acid can be ruled out. It was possible to establish the reactivity of thiourea (and alkyl thioureas) in this reaction for two nitrosamines,[67,71] along with that for a number of other nucleophiles. The data (in Section 2, Table 3 for R = Ph, R′ = Me) shows clearly that thiourea is indeed very reactive in this reaction and is comparable with iodide in this respect. The data for the nucleophiles fit well the Pearson nucleophilicity relationship[64] particularly for R = Ph, R′ = Me, but rather less well for R = R′ = Ph, in the case of the larger nucleophiles I^- and $SC(NH_2)_2$, where steric effects might operate.

The Fischer–Hepp rearrangement of aromatic nitrosamines occurs in parallel with a normally reversible denitrosation reaction[72] (Scheme 5). By the addition of powerful nucleophiles such as thiourea (and a nitrous acid trap) it is possible to divert the reaction pathway towards denitrosation at the expense of rearrangement.[73]



Scheme 5

Alkyl nitrites can also apparently directly bring about *S*-nitrosation of thiourea as deduced from the results of kinetic experiments using propyl nitrite in propanol solvent.[74] Reactions were first-order in halide ion, thiocyanate ion, and thiourea. *S*-Nitrosothiols (or thionitrites) behave in the same way.[38] It seems that the formation of the *S*-nitrosothiouronium ion from thiourea is a general one, occurring readily with any of the conventional nitrosating agents. An apparent exception is the reaction of thiourea with photolysed pentacyanonitrosylferrate ion,[75] where nitric oxide is generated and the iron complex product contains a bonded thiourea group.

[71] J. T. Thompson and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1977, 1932.
[72] D. L. H. Williams, *Tetrahedron*, 1975, 1343.
[73] D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1982, 801.
[74] S. E. Aldred and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1981, 1021.
[75] P. A. Stoeri and D. X. West, *J. Inorg. Nucl. Chem.*, 1974, **36**, 3883.

For reactions of thiourea with nitrous acid at lower acidities where products are derived from *N*-nitrosation, it had been suggested[69] that the initial attack might be at sulphur followed by a *S* to *N* rearrangement. The evidence for this is kinetic and derives from the absence of a term in $[H^+]$ in the rate equation for reaction leading to thiocyanic acid. However the results of ^{15}N-n.m.r. experiments[76] have been interpreted in terms of a direct *N*-nitrosation under these conditions. At low acidities it was possible to isolate the *N*-nitrosothiourea. The acid-catalysed hydrolysis of *N*-nitrosothioureas do appear to involve the reverse *N* to *S* migration.[77]

The final product of the nitrosation of thiourea has been described as the disulphide cation, but it appears that urea itself can also be formed (Scheme 6).

$$SC(NH_2)_2 + HNO_2 \xrightarrow{H^+} ONS\overset{+}{C}(NH_2)_2 \qquad \begin{matrix}(NH_2)_2\overset{+}{C}SS\overset{+}{C}(NH_2)_2 \\ OC(NH_2)_2\end{matrix}$$

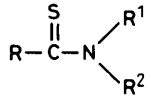
Scheme 6

Thus urea is claimed to be the major product from the *S*-nitrosation at higher acidity,[78] and is also formed in the hydrolysis of *N*-nitrosothiourea[77] (after *N* to *S* migration). It is believed that this occurs by nucleophilic attack of water or by the elimination of HSNO giving a carbodiimide which subsequently undergoes hydration. The transformation of thiocarbonyl compounds to carbonyl compounds (equation 27) can be achieved using a variety of other reagents

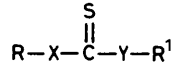
$$SC(NH_2)_2 \xrightarrow[\text{or RONO } etc.]{HNO_2,H^+} OC(NH_2)_2 \qquad (27)$$

including interestingly an alkyl nitrite,[79] a reaction which could also involve *S*-nitrosation. It is not clear under what conditions the two possible alternative products (the dication and the carbonyl compound) are formed. The kinetics of the carbonyl-forming reaction were studied[80] using *N*-methyl-2-thiopyrrolidone as the substrate. As expected the reaction is acid-catalysed and also catalysed by thiocyanate ion. On the synthetic side it has been shown[81] that a range of thiocarbonyl compounds (8) with R = Ph, Me and R^1 and R^2 Ph, Me and also various cyclic structures, can be converted smoothly and generally in good yield, into the corresponding amide structures, by treatment with excess nitrous acid in 4M-HCl. Similarly a range of thiono compounds (9) where X and Y = O, S *etc.*, are also converted into their carbonyl analogues by the same treatment. These results were discussed[81] in terms of the HSAB principle where the soft (borderline) acid NO^+ (or $H_2NO_2^+$) attacks the soft sulphur atom of the thiocarbonyl compound, but it is to be expected for the primary and secondary

[76] J. W. Lown and S. M. S. Chauhan, *J. Chem. Soc., Chem. Commun.*, 1981, 675.
[77] J. W. Lown and S. M. S. Chauhan, *J. Org. Chem.*, 1983, **48**, 3901.
[78] J. W. Lown and S. M. S. Chauhan, *J. Org. Chem.*, 1983, **48**, 507.
[79] K. A. Petrov and L. N. Andrew, *Russ. Chem. Rev.*, 1971, **40**, 505.
[80] K. A. Jørgensen and S. O. Lawesson, *Chem. Scr.*, 1982, **20**, 227.
[81] K. A. Jørgensen, A. B. A. G. Ghattas, and S. O. Lawesson, *Tetrahedron*, 1982, **38**, 1163.



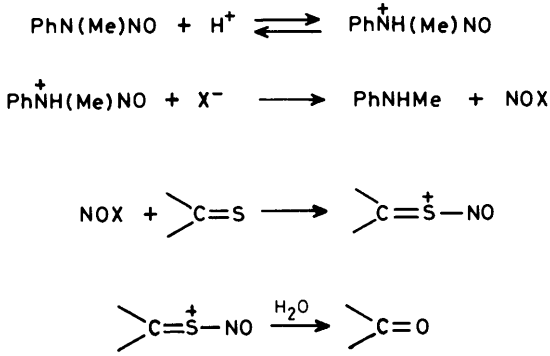
(8)



(9)

thioamides that the nitrogen atom is extensively protonated, and indeed no *N*-nitrosamides are recovered.

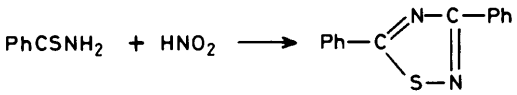
The thiocarbonyl–carbonyl transformation can also be brought about using nitrosamines as the source of NO^+.[82] A large range of thioamides (8) with R, R^1, and R^2 being various combinations of H, Ph, Me and other groups, yield the corresponding amides by treatment with *N*-methyl-*N*-nitrosoaniline or *N*-nitrosopiperidine in acid solution containing potassium iodide. In the presence of a nitrous acid trap (ascorbic acid) the reaction is extensively inhibited and so a likely mechanism involves the denitrosation of the nitrosamine (see *ref.* 46), followed by *S*-nitrosation of the thioamide by the free nitrosating species, as outlined in Scheme 7. The likely rate-limiting step is the denitrosation step with X^- as nucleophile



Scheme 7

which should then lead to a zero-order dependence upon [>C=S]. This was not examined experimentally however.

On the synthetic side, it has been shown[83] that nitrous acid (and alkyl nitrites) react with primary thiobenzamides to give the disubstituted thiodiazole (equation 28). It is not immediately clear whether this involves *S*-nitrosation.



(28)

[82] K. A. Jørgensen, M. T. M. El-Wassimy, and S. O. Lawesson, *Tetrahedron*, 1983, **39**, 469.
[83] M. W. Cronyn and T. W. Nakagawa, *J. Am. Chem. Soc.*, 1952, **74**, 3693.

6 Reactions of *S*-Nitrosothiouronium Ions

The further reactions of the *S*-nitrosothiouronium ions derived from thiourea and related compounds, have already been mentioned in Section 5, see Scheme 6. The reaction leading to the disulphide cation has been examined in more mechanistic detail.[84] The reaction was found to be inhibited by nitric oxide (in an oxygen-free atmosphere) and the rate equation given by equation 29 was established from

$$\text{Rate} = k_1[(NH_2)_2CS][(NH_2)_2CSNO^+] + k_2[(NH_2)_2CSNO^+]^2 \qquad (29)$$

initial rate measurements. The results were consistent with a mechanism involving two parallel pathways, the first a reversible formation of a radical intermediate (10) from thiourea and the *S*-nitrosothiouronium ion, and the second involving a bimolecular reaction between two molecules of the *S*-nitroso ion, as outlined in equations 30 and 31. The subsequent fate of (10) is open to speculation but could

$$(NH_2)_2CS + [(NH_2)_2CSNO]^+ \rightleftharpoons \underset{(10)}{[(NH_2)_2CSSC(NH_2)_2]^{+\cdot}} + NO \qquad (30)$$

$$2[(NH_2)_2CSNO]^+ \longrightarrow (NH_2)_2\overset{+}{C}SS\overset{+}{C}(NH_2)_2 + 2NO \qquad (31)$$

involve a bimolecular disproportionation, or a unimolecular radical cation breakdown, or possibly further oxidation of (10) by another molecule of the *S*-nitrosothiouronium ion. A similar rate equation was found for the decomposition of the tetramethylthiourea derivative, but an additional term now appears in the rate equation involving catalysis of the decomposition by nitrous acid, and this is not easy to interpret mechanistically.

The possibility arises that *S*-nitrosothiouronium ions generally might act as nitrosating agents in their own right. This was first suggested by some results of a kinetic study on the denitrosation of nitrosamines using thiourea as a nucleophilic catalyst.[67] The reaction was found to be reversed by added secondary amine product suggesting the outline mechanisn in Scheme 8. This was later substan-

$$PhN(Me)NO + H^+ \rightleftharpoons Ph\overset{+}{N}H(Me)NO$$

$$Ph\overset{+}{N}H(Me)NO + (NH_2)_2CS \rightleftharpoons PhNHMe + (NH_2)_2\overset{+}{C}SNO$$

$$(NH_2)_2\overset{+}{C}SNO + \text{Nitrite trap} \longrightarrow \text{Various products}$$

Scheme 8

tiated by the observation of marked catalysis of nitrosation and diazotization by added thiourea.[51,85] The well-known catalysis of nitrosation by added halide ion and thiocyanate ion has been interpreted in terms of intermediate formation of the corresponding nitrosyl halide or thiocyanate which effects nitrosation. It appears that the same is true for thiourea. The substantial catalytic effect of thiourea is shown in Figure 2 where catalysis of nitrosation of morpholine[51] by thiocyanate

[84] P. Collings, M. Garley, and G. Stedman, *J. Chem. Soc., Dalton Trans.*, 1981, 331; M. S. Garley, G. Stedman, and H. Miller, *J. Chem. Soc., Dalton Trans.*, 1984, 1959.

[85] M. Masui, C. Ueda, T. Yasuoka, and H. Ohmori, *Chem. Pharm. Bull.*, 1979, **27**, 1274.

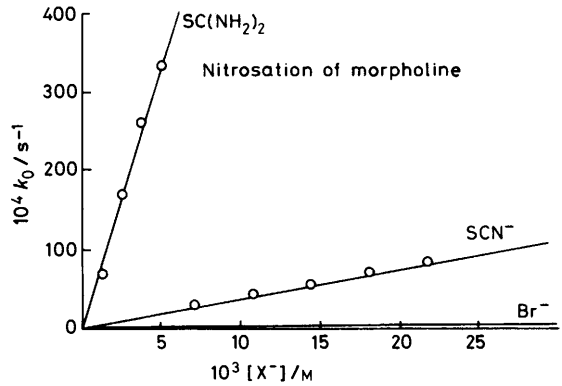


Figure 2 *Comparison of the catalytic efficiencies of* Br^-, SCN^-, *and* $SC(NH_2)_2$ *in the nitrosation of morpholine*

ion and bromide ion are also shown. The same effect is observed in diazotization of aniline. This makes thiourea one of the best catalysts known for nitrosation processes. The extent of catalysis by X^- depends on the magnitude of the equilibrium constant K_{NOX} for NOX formation and also upon the magnitude of its rate constant for reaction wth substrate S (see Scheme 9). K_{NOX} can be separately

$$HNO_2 + X^- \text{ (or X)} + H^+ \overset{K_{NOX}}{\rightleftharpoons} NOX \text{ (or } NOX^+) + H_2O$$
$$NOX \text{ (or } NOX^+) + S \xrightarrow{k} \text{product}$$

Scheme 9

measured, and is known for Cl^-, Br^-, SCN^-, and $SC(NH_2)_2$, so that k can readily be extracted. The extent of catalysis seems to depend more on the value of K_{NOX} than on the value of the bimolecular rate constant. Some data are collected in Table 4 for the diazotization of aniline and also 4-aminobenzoic acid. The rate constants

Table 4 *Values of* K_{NOX} *together with values of* k (*from Scheme* 9) *for the diazotization of aniline and* 4-*aminobenzoic acid at* 25 °C *in water*

NOX	K_{NOX}/l^2 mol^{-2}	k/l mol^{-1} s^{-1} (*Aniline*)	k/l mol^{-1} s^{-1} (4-*aminobenzoic acid*)
NOCl	1.1×10^{-3}	2.2×10^9	1.1×10^9
NOBr	5.1×10^{-2}	1.7×10^9	4.3×10^8
NOSCN	30	1.9×10^8	1.4×10^6
$NO\overset{+}{S}C(NH_2)_2$	5 000	1.3×10^6	1.8×10^4

are taken from references 86 and 87 and the equilibrium constants from references 88 (NOCl), 89 (NOBr), 90 (NOSCN), and 69 ($NO\overset{+}{S}C(NH_2)_2$). A reasonable Bronsted plot was obtained for both NOSCN and $NO\overset{+}{S}C(NH_2)_2$,[86] whereas k values tend towards the diffusion-controlled limit for both NOBr and NOCl[87]

as the pK_a of the aniline is increased. The reactivity trend NOCl > NOBr > NOSCN > $NO\overset{+}{S}C(NH_2)_2$ is now well-established for a range of substrates.

Catalysis of nitrosation of aliphatic amines by thiourea and alkyl thioureas also occurs,[85] and again the overall effect is greater than that of added thiocyanate ion.

7 Nitrosation of Thiocyanate Ion

Nitrosyl thiocyanate is known only as a blood-red species, stable only in solution,[91] which decomposes in high concentration at room temperature, and is readily synthesized from nitrous acid and thiocyanic acid, nitrosyl chloride and silver thiocyanate, or ethyl nitrite and thiocyanic acid.[92] Its structure has never been established, presumably as a result of its instability (it decomposes to give nitric oxide and thiocyanogen[93]), but it is generally believed that the nitroso group is bound to sulphur and not to nitrogen. It thus represents an example of *S*-nitrosation. Arguments based on Hard-Acid–Soft-Base theory favour bonding to sulphur and recent *ab initio* molecular orbital calculations[94] reveal that nitrosyl thiocyanate should be significantly more stable than the isomeric nitrosyl isothiocyanate.

There are now many examples of substantial degrees of catalysis by thiocyanate ion of nitrosation in the literature, and this is generally interpreted in terms of intermediate formation of nitrosyl thiocyanate which then effects nitrosation. Among substrates studied which show such catalysis are hydroxylamine and its methyl derivatives,[95] aniline derivatives,[96,86] morpholine,[51] hydrazoic acid,[97] alcohols,[31] thiols,[27] *etc.* For most cases the rate-limiting step is the attack of NOSCN with the substrate (Scheme 10). The equilibrium constant K_{NOSCN} has been

$$HNO_2 + H^+ \rightleftharpoons H_2NO_2^+$$

$$H_2NO_2^+ + SCN^- \rightleftharpoons NOSCN + H_2O$$

$$NOSCN + \text{Substrate} \longrightarrow \text{nitrosation product}$$

Scheme 10

determined separately[90] as 30 l^2 mol^{-2} at 25 °C and so the bimolecular rate constant for NOSCN attack can be determined. Such values are always less than for the corresponding NOCl and NOBr reactions, and this difference has been discussed theoretically by molecular orbital calculations[94] using the concept of

[86] L. R. Dix and D. L. H. Williams, *J. Chem. Res.* (*S*), 1984, 97.
[87] M. R. Crampton, J. T. Thompson, and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1979, 18.
[88] H. Schmid and E. Hallaba, *Monatsh. Chem.*, 1956, **87**, 560.
[89] H. Schmid and M. G. Fouad, *Monatsh. Chem.*, 1957, **88**, 631.
[90] G. Stedman and P. A. E. Whincup, *J. Chem. Soc.*, 1963, 5796.
[91] C. C. Addison and J. Lewis, *Quart. Rev.*, 1955, **9**, 115.
[92] E. Söderbäck, *Annalen*, 1919, **419**, 217; H. Lecher and F. Graf, *Ber.*, 1926, **59**, 2601.
[93] F. Seel and D. Wesemann, *Chem. Ber.*, 1953, **86**, 1107.
[94] K. A. Jørgensen and S. O. Lawesson, *J. Am. Chem. Soc.*, 1984, **106**, 4687.
[95] M. N. Hughes, G. Stedman, and T. D. B. Morgan, *J. Chem. Soc.* (*B*), 1968, 344.
[96] C. A. Bunton, D. R. Llewellyn, and G. Stedman, *J. Chem. Soc.*, 1959, 568.
[97] G. Stedman, *J. Chem. Soc.*, 1959, 2949.

charge- and frontier-controlled reactions. Such calculations predict that NOSCN should be less reactive than NOCl as is found experimentally.

For some very reactive substrates however (*e.g.* aniline[96] and azide ion[97]) it was found that the reaction became zero-order in substrate. This has been interpreted as a change in rate-limiting step to NOSCN formation. Such behaviour has also been found for other nitrosyl species. This enables the third-order rate constant k in equation 32 to be evaluated as 1 460[97] and 1 500[96] l^2 mol^{-2} s^{-1} at 0 °C. Values

$$\text{Rate} = k[H^+][HNO_2][SCN^-] \tag{32}$$

rather close to these have been observed for a whole range of anions which has prompted the explanation[1] that these reactions occur at the encounter-controlled limit. Reaction with very reactive neutral substrates produces the same effect except that the limit is somewhat lower than for the anions, which is to be expected by electrostatic considerations. More recently the same effect has been noted for reaction at 25 °C for both hydrazoic acid[29] and also thioglycolic acid,[32] where the values of k are 11 700 and 11 000 l^2 mol^{-2} s^{-1} respectively. This corresponds to an activation energy of *ca.* 56 kJ, which is in the region expected for an encounter-controlled reaction between a positively charged nitrosating species and an anion.

Catalysis of nitrosation by thiocyanate ion has implications in the *in vivo* formation of carcinogenic nitrosamines from naturally occurring secondary amines and sources of nitrous acid such as sodium nitrite (used widely as a food preservative particularly of cured meats) and nitrate ion in water supplies (which is readily reduced to nitrite in the saliva). Thiocyanate is secreted in the saliva and so will catalyse the formation of nitrosamines. This is particularly of concern for smokers, where the thiocyanate concentration is three or four times that of non-smokers.[98] Experiments with *N*-methylaniline[99] have shown that, in the presence of thiocyanate, reaction proceeds much more rapidly in acid conditions such as gastric juice (between pH 1 and 2), whereas the catalytic action is less at higher pH values.

Kinetic studies on the denitrosation of nitrosamines[46] strongly suggest that in the presence of thiocyanate ion a direct *S*-nitrosation occurs to give nitrosyl thiocyanate (equation 33). In this case it is necessary to include a nitrous acid trap

$$\overset{+}{\text{PhNH}}(\text{Me})\text{NO} + \text{SCN}^- \longrightarrow \text{PhNHMe} + \underset{\text{Removed}}{\underset{\downarrow}{\text{NOSCN}}} \tag{33}$$

to drive the reaction to the right. As expected thiocyanate is more reactive than both chloride ion and bromide ion. Similarly the observation of substantial thiocyanate catalysis in the denitrosation of alkyl nitrites[31] and also *S*-

[98] P. M. Densen, B. Davidow, H. E. Bass, and E. W. Jones, *Archs. Envir. Hlth.*, 1967, **14**, 865.
[99] E. Boyland and S. A. Walker, *Nature* (London), 1974, **248**, 601.

nitrosothiols[38] has been interpreted as intermediate formation of nitrosyl thiocyanate.

Use is made of thiocyanate catalysis in the *trans*-nitrosation reaction observed (equation 34) where a nitrosamine transfers its nitroso group to another amine.

$$R'R''NNO + R'''R''''NH \xrightarrow[SCN^-]{H^+} R'R''NH + R'''R''''NNO \quad (34)$$

This is believed to occur by a denitrosation reaction (thiocyanate ion catalysed) and subsequent nitrosation of the secondary amine.[100] Similarly in the nitrosation of amines by propyl nitrite in propanol (equation 35),[74] virtually no reaction occurs

$$PrONO + PhN(H)Me \xrightarrow[SCN^-]{H^+} PrOH + PhN(Me)NO \quad (35)$$

in the absence of a nucleophile such as halide ion or thiocyanate ion, but in their presence *N*-nitrosation occurs smoothly, again by way of nitrosyl thiocyanate for the thiocyanate ion catalysed reactions.

8 Nitrosation of Thiosulphate Ion

Yellow solutions are readily formed from nitrous acid and thiosulphate ion. Early kinetic measurements[101] yielded a rate equation which included a non-integral dependence upon the thiosulphate concentration and the interpretation included rate-determining formation of the nitrosonium ion NO^+ However a more recent kinetic investigation,[102] using the stopped-flow technique, resulted in the rate equation given in equation 36. These measurements were made in aqueous

$$\text{Rate} = k_1[H^+][HNO_2][S_2O_3^{2-}] + k_2[HNO_2]^2 \quad (36)$$

perchloric acid at 25 °C. The interpretation involves two pathways (*a*) rate-determining attack by NO^+ (or $H_2NO_2^+$) at the thiosulphate ion and (*b*) rate-determining formation of N_2O_3 which then effects the nitrosation of the thiosulphate ion more rapidly than its hydrolysis to nitrous acid. The second pathway has been observed on many occasions for many amine substrates whilst the first is also well-known. The value of k_1 is 18 000 l^2 mol^{-2} s^{-1}, which is taken to represent the diffusion-controlled limit. This is somewhat higher than that found for neutral (*ca.* 7 000 l^2 mol^{-2} s^{-1}) and singly negatively charged (*ca.* 11 000 l^2 mol^{-2} s^{-1}) substrates, but the trend is to be expected on consideration of the effect of coulombic interactions on the diffusion rate. The activation energy of 50 kJ mol^{-1} is close to that found for diffusion-controlled reactions involving singly charged anions.

The product (yellow in solution) is believed to be the *S*-nitrosated anion $[O_3SSNO]^-$ which decomposes fairly rapidly in solution. Its decomposition products have not been investigated. Nevertheless it was possible to measure the

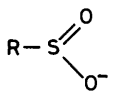
[100] S. S. Singer, *J. Org. Chem.*, 1978, **43**, 4612; S. S. Singer, G. M. Singer, and B. B. Cole, *J. Org. Chem.*, 1980, **45**, 4931; S. S. Singer and B. B. Cole, *J. Org. Chem.*, 1981, **46**, 3461.
[101] J. O. Edwards, *Science*, 1951, **113**, 392.
[102] M. S. Garley and G. Stedman, *J. Inorg. Nucl. Chem.*, 1981, **43**, 2863.

equilibrium constant for its formation at 1.66×10^7 l^2 mol^{-2} at 25 °C. The visible absorption spectrum is very similar to those of other *S*-nitroso species including *S*-nitrosothiols, with an extinction coefficient in the same region. The equilibrium constant is also constant over a range of concentrations of the starting materials, for measurements made in acetate buffers. This then appears to represent another example of a *S*-nitrosation.

The possibility arises that if the *S*-nitroso ion $[O_3SSNO]^-$ can itself act as a nitrosating agent, then it would represent an unusual example of a negatively charged electrophilic nitrosating species. Intermediates capable of nitrosation have been detected kinetically, by the observed catalysis of nitrosation by added nucleophiles, and include the nitrosyl halides, ONSCN, $ON\overset{+}{S}C(NH_2)_2$, ONSR, and possibly $ON\overset{+}{S}(RR')$. The extent of catalysis is governed principally by the size of the equilibrium constants for the formation of these intermediates, and not by the rate constants of their subsequent reactions. On these grounds it is perhaps reasonable to expect $[O_3SSNO]^-$ to behave similarly. We have looked, in a preliminary study,[103] for catalysis by thiosulphate ion in nitrosation. For the comparatively slow nitrosation of morpholine, no catalysis was detected, but the much more rapid nitrosation of *N*-methylaniline was markedly subject to catalysis. Further work in this area is necessary but the preliminary findings do support the suggestion that $[O_3SSNO]^-$ can act as a nitrosating agent.

9 Nitrosation of Sulphinic Acids

Sulphinic acids, particularly in their anion form (11) are well-known *S*-nucleophiles, reacting with a range of conventional systems including alkyl halides, carbonyl compounds, alkenes *etc.*[104] Reaction with alkyl halides invariably yields



(11)

(12)

the sulphone derivative. It has been suggested[105] that structure (12) contributes to the overall sulphinate ion, no doubt because of the clear indication of *S*- rather than *O*-bonded products formed. It is not surprising therefore that sulphinic acids undergo *S*-nitrosation, but the observed products, the alkane- or aryl-sulphonyl hydroxylamine derivatives (equation 37), are somewhat unexpected in the context

$$2RSO_2H + HNO_2 \rightleftharpoons (RSO_2)_2NOH + H_2O \qquad (37)$$

of nitrosation chemistry, in that two molecules of reactant are used up and apparently two nitrosation steps are involved. The reaction, which appears to be

[103] T. Bryant and D. L. H. Williams, unpublished work.
[104] C. J. M. Stirling, *Int. J. Sulfur Chem. B*, 1971, **6**, 280; S. Oae and N. Kunieda, 'Organic Chemistry of Sulfur', ed. S. Oae, Plenum, 1977, p. 603.
[105] E. N. Guryanova and Y. A. Syrkin, *Zh. Fiz. Khim.*, 1949, **23**, 105.

quite general, has been known for some time[106] and has been used in the characterization of a long chain aliphatic sulphinic acid.[107] The reaction has also been used[108] in the analytical determination of sulphinic acids by a simple titration with a solution of sodium nitrite in dilute acid. More recently a number of such hydroxylamine derivatives have been prepared using this reaction,[109] in an attempt to generate by oxidation of the hydroxylamines, neutral analogues of Fremy's radical ion.

The kinetics of the reaction of benzenesulphinic acid with nitrous acid have been determined very recently.[110] The reaction is very rapid and rate constants were determined by stopped-flow spectrophotometry. Reaction was first-order in nitrous acid and also in total stoicheiometric concentration of the sulphinic acid; the rate constants were the same, within experimental error, for the measurement of the appearance of the product, as for the disappearance of the reactant. Acid catalysis was observed but is not strictly first-order. A plot of the first-order rate constant *vs.* $[H^+]$ (when [sulphinic acid] $\gg$ $[HNO_2]$) is linear above *ca.* 0.06 M, but with a substantial positive intercept, and is curved at lower acidities. This type of behaviour is typical of a reaction which occurs *via* both the neutral substrate and its anionic form. Application of the protonation equilibrium (pK_a 1.84[111]) leads to the expression given in equation 38 for the first order rate constant, where k_1 is the

$$k_0 = \frac{(k_1[H^+]^2 + k_2K_a[H^+])[HA]_T}{K_a + [H^+]} \qquad (38)$$

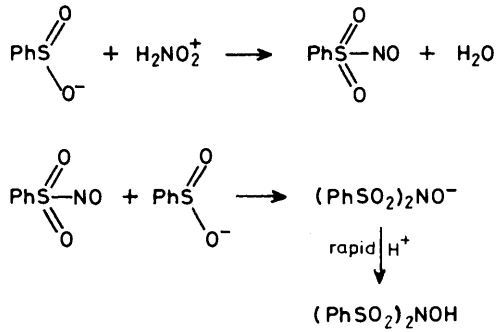
third order rate constant (Rate = $k_1[H^+][HNO_2][\text{Substrate}]$) for reaction of the neutral form and k_2 that for the anion reaction (Scheme 11). The total substrate

$$\begin{array}{ccc} RSO_2H & \rightleftharpoons & RSO_2^- + H^+ \\ {\scriptstyle HNO_2}\downarrow{\scriptstyle k_1} & & {\scriptstyle HNO_2}\downarrow{\scriptstyle k_2} \\ \text{Product} & & \text{Product} \end{array}$$

Scheme 11

concentration is $[HA]_T$ and K_a the acid dissociation constant for benzenesulphinic acid. This equation fits the experimental data well and values of k_1 and k_2 of 820 and 11 800 l^2 mol^{-2} s^{-1} are readily obtained. Thus both the acid and its anion are very reactive towards nitrosation, with the anion being significantly the more reactive. Its rate constant is very similar to that[29] of the reaction of SCN^- implying that this is the diffusion limit for an anion reaction generally with $H_2NO_2^+$ or

[106] R. Otto and H. Ostrop, *Annalen*, 1867, **141**, 370; W. Koenigs, *Ber.*, 1878, **11**, 615.
[107] C. S. Marvel and R. S. Johnson, *J. Org. Chem.*, 1948, **13**, 822.
[108] B. Lindberg, *Acta Chem. Scand.*, 1963, **17**, 383 and references therein; J. P. Danehy and V. J. Elia, *Anal. Chem.*, 1972, **44**, 1281.
[109] J. D. Birchall and C. Glidewell, *J. Chem. Soc., Dalton Trans.*, 1977, 10.
[110] T. Bryant and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1985 in the press.
[111] R. K. Burkhard, D. E. Sellers, F. De Cou, and J. L. Lambert, *J. Org. Chem.*, 1959, **24**, 767.



Scheme 12

NO^+. Since the reaction is clearly first-order in substrate, the mechanism is likely to involve rate-limiting attack by the nitrosating species (designated $H_2NO_2^+$ in Scheme 12), yielding the nitrososulphinate intermediate, which rapidly effects another *S*-nitrosation with a molecule of reactant (here written as the anion), finally forming the hydroxylamine derivative as product, by proton transfer from the solvent. Nitrososulphinates (or sulphonyl nitrites) have previously been suggested as intermediates in this reaction,[109] and have recently been isolated[112] as rather unstable brown crystals from the reaction of sulphinic acids with N_2O_4 at -20 °C in ether. They appear to be amongst the most powerful nitrosating agents known (although no quantitative comparison data are available), reacting with amines,[113] alcohols,[5] and thiols,[5] often yielding some of the hydroxylamine derivative as well, by nitrosation of the product sulphinic acid. The chemistry of nitrososulphinates is described fully in reference 5.

Sulphinic acids also react with alkyl nitrites[114] yielding the same hydroxylamine products as for the nitrous acid reaction, again it is believed by the intermediate formation of the reactive nitrososulphinates.

In common with many (but not all) nitrosation reactions in aqueous acid solution, the reaction of benzenesulphinic acid is also catalysed by added nucleophiles Cl^-, Br^-, SCN^-, and $SC(NH_2)_2$,[110] involving the appropriate nitrosyl halide *etc.* Again, reaction of both the neutral acid and the anion occurs, with the latter again being the more reactive. The usual sequence of reactivity of the various nitrosyl species was observed, with the reaction of nitrosyl chloride with the sulphinate ion being close to the calculated diffusion limit.

Acknowledgement. I would like to thank Dr. G. Stedman for many valuable discussions in the area of *S*-nitrosation, and for his comments on this manuscript.

[112] S. Oae, K. Shinhama, K. Fujimori, and Y. H. Kim, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 775.
[113] S. Oae, K. Shinhama, and Y. H. Kim, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 1065.
[114] G. Kresze and W. Kort, *Chem. Ber.*, 1961, **94**, 2624.