Reactivity of phenolic nucleophiles towards nitroso compounds

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The reactivities of a series of mono- and poly-phenolic ambident nucleophiles with the ambident electrophile *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (MNTS) were studied. Reaction with the MNTS sulfonyl group afforded the corresponding sulfonic esters, and reaction with the MNTS nitroso group afforded the *C*-nitrosophenol and/or NO. However, the rate of reaction with the nitroso group correlated well with the basicity of the phenolic oxygen atom, suggesting that both the sulfonyl and nitroso groups of MNTS react with phenolates exclusively through this single nucleophilic centre. Similar behaviour in the reaction between ascorbic acid and 2-ethoxyethyl nitrite suggests that *in vivo* generation of NO from alkyl nitrites may result from their reaction with biological reductones.

Introduction

Most studies of the mechanisms involved in the *C*-nitrosation of aromatic compounds have concentrated on the nitrosation of phenols and naphthols in acidic media by strong electrophiles derived from the protonation of nitrous acid (NO^+, NOCl, NOBr, *etc.*).[1-3] These reactions involve the formation of a neutral dienone, the loss of a proton from which is generally the rate-controlling step of the reaction; the resulting *C*-nitroso products undergo tautomerization to the more stable benzoquinone monooxime.

Phenolic compounds are known to act as natural antioxidants. The *C*-nitroso products of phenol, 4-methylphenol, 1,3-dihydroxybenzene (resorcinol) and 1,3,5-trihydroxybenzene (phloroglucinol) mediate catalysis of the *N*-nitrosation of amines by these phenols,[4] although other polyphenols [1,2-dihydroxybenzene (catechol), 1,4-dihydroxybenzene, ascorbic acid (vitamin C) and α-tocopherol (vitamin E)] are known to inhibit the formation of *N*-nitrosamines,[5] apparently by reducing the nitrosating agent to NO.

N-Methyl-*N*-nitroso-*p*-toluenesulfonamide (MNTS) has two electrophilic centres. It has been reported that nucleophiles reacting through N,[6,7] C,[8] S or I^- [9] react with its nitroso group, whereas nucleophiles reacting through O react mainly or exclusively with its sulfonyl group.[6,9] These findings can be explained by Klopman's theory of frontier orbitals[10] and by Pearson's theory of hard and soft acids,[11] which takes into account the specific affinities of the atoms involved in bond formation.

As a test of the above conclusions, and to follow up the initial finding that the reaction between ascorbic acid and nitrosating agents generates NO in both acidic and basic media,[12,13] in this work we studied reactions between the ambident electrophile MNTS and a series of phenolic nucleophiles, some of which are also ambident (as is illustrated by their reactions with nitroaromatic electrophiles).[14]

Experimental

Materials

N-Methyl-*N*-nitroso-*p*-toluenesulfonamide was supplied by Merck and used without further purification. Alkyl nitrites were prepared in an acid medium from sodium nitrite and the corresponding alcohol,[15] and solutions were stored in the dark over molecular sieves. Both MNTS and alkyl nitrite solutions were prepared in dioxane or acetonitrile; the percentage of organic solvent in final reaction mixtures is indicated in the Results section (Table 1).

Phenols (from Aldrich) were purified by recrystallization.[16] Sulfonic esters were prepared in pyridine from tosyl chloride and the corresponding phenol, followed by recrystallization from ethanol; their identities were confirmed from their ^1H NMR spectra. Other reagents were Aldrich or Merck products of the maximum available purity and were used without further purification.

Apparatus

The kinetics of most reactions were studied in Kontron Uvikon 930 or Milton Roy Spectronic 3000 diode array spectrometers equipped with thermostatted cell carriers. Fast reactions were studied in triplicate in a Beckman spectrophotometer equipped with a Hi-Tech rapid mixing accessory, and the three resulting rate constants were averaged. Reactions that for various reasons could not be studied spectrophotometrically were followed by HPLC using a Beckman System Gold apparatus with a Beckman Reverse Phase 5 μm Ultrasphere C_{18} column and UV detection.

pH was measured with a Radiometer pHM82 pH-meter equipped with a GK2401B combined glass electrode, after calibration with commercial buffers of pH 7.02 (from Crison) and pH 12.45 (from Beckman).

Kinetic measurements

All kinetic studies were carried out at 25 °C using pseudo-first order conditions. The nucleophile was always in great excess over MNTS or alkyl nitrite, except in the case of ascorbic acid, which because of its instability in basic media was used at a concentration at least 10 times less than that of the nitrosating agent.

Whenever possible, pH was controlled with buffers consisting of the substrate phenols plus NaOH. Reactions with catechol were studied in 0.6 mol dm^{-3} carbonate–bicarbonate buffers of pH 9–10.5. The reaction between ascorbic acid and ethoxyethyl nitrite was carried out in 10^{-2} mol dm^{-3} carbonate–bicarbonate buffer of pH 11 and in NaOH solutions of various concentrations and pH between 11.8 and 13.

The reactions with phenol, *p*-cresol, resorcinol and phloroglucinol were followed spectrophotometrically at wavelengths corresponding to absorption by the corresponding products (400 nm for phenol, 330 nm for *p*-cresol, 350 nm for resorcinol and 490 nm for phloroglucinol). All these reactions were affected by the susceptibility of phenols in basic media to oxidation in the presence of light. Thus the absorbance–time data initially obtained for phenol and *p*-cresol deviated slightly from the best-fitting first order integrated equation at the start of the reaction. For phenol, better fit was obtained by working

under argon with redistilled phenol bottled under nitrogen (from Aldrich); under these conditions, the reaction with the oxidation product was less than 3% of the total reaction. For *p*-cresol, the amount of oxidation product was reduced to a minimum by repeated purification prior to reaction. For resorcinol and phloroglucinol, complications due to the oxidation reaction were avoided by using conditions in which it is dominated by the nitrosation reaction.

Because of the instability of their products, the nitrosation reactions of 2,6-dimethylphenol and catechol were studied by following the disappearance of MNTS by HPLC with detection of absorption at 250 nm. This procedure was also used for 2,4,6-trimethylphenol, the UV–VIS spectrum of which showed no change upon reaction with the nitrosating agent. In all these cases, known volumes of a thermostatted reaction mixture containing MNTS and substrate were analysed periodically with a 2 cm^3 min^{-1} flow of methanol–water as mobile phase (50:50 *v*/*v* for catechol and 2,4,6-trimethylphenol, 40:60 *v*/*v* for 2,6-dimethylphenol). The areas of MNTS peaks were integrated electronically. In all cases the concentration–time data were fitted well by first order integrated equations for times greater than 4 reaction half-lives.

Since the reaction of 2-ethoxyethyl nitrite with ascorbic acid is too fast to be followed by conventional spectrophotometry, a rapid mixing device was used, with one of the syringes filled with alkyl nitrite solution in the presence of a low concentration of HCO_3^-–CO_3^{2-} buffer or NaOH (alkyl nitrites are reasonably stable under these conditions)[17] and the other with ascorbic acid solution.

Reaction products were identified by HPLC or UV–VIS spectroscopy, by comparison with authentic samples. *N*-Methyl-*p*-toluenesulfonamide and sulfonic esters were always identified by HPLC with acetonitrile–water as mobile phase, UV detection at 250 nm and electronic integration of peak areas. For *N*-methyl-*p*-toluenesulfonamide the mobile phase compositions (acetonitrile:water, *v*/*v*) were 30:70 for phenol and 2,4,6-trimethylphenol, 20:80 for resorcinol and phloroglucinol, and 10:90 for catechol and *p*-cresol, and the flow rate was 4 cm^3 min^{-1} for catechol and *p*-cresol and 2 cm^3 min^{-1} otherwise; for sulfonic esters the acetonitrile:water ratio (*v*/*v*) was 70:30 for 2,4,6-trimethylphenol and 50:50 otherwise, and the flow rate was always 2 cm^3 min^{-1}. For identification of 4-nitrosophenol by HPLC, a 2 cm^3 min^{-1} flow of 30:70 (*v*/*v*) acetonitrile–water was used and absorbance at 400 nm was recorded. The NO_2^- released in some of the reactions was determined by a modified form of Shinn's method, as described elsewhere.[7]

Results

Identification of products

The nitrosation of phenol by MNTS afforded a yellow product that had an absorbance peak at 400 nm (Fig. 1) and was identified, both from its spectral characteristics and by HPLC, as *p*-nitrosophenol (λ_{max} 400 nm).[18] However, quantification of this product, whether spectrophotometrically (using the published value = 27 100 dm^3 mol^{-1} cm^{-1})[18] or by HPLC (using standards made up from *p*-nitrosophenol prepared from phenol and sodium nitrite at acid pH),[1] showed that it accounted for no more than about 30% of the total reaction. Furthermore, analysis of the reaction mixtures by HPLC revealed that only 34 ± 5% of the MNTS was converted into *N*-methyl-*p*-toluenesulfonamide, the other expected product of reaction between phenol and the MNTS nitroso group. The suspicion that reaction was also occurring at the other electrophilic centre of MNTS, the S atom, was confirmed by identification of the precipitate formed in reaction mixtures with high MNTS concentrations, whose ^1H NMR spectrum in $(CD_3)_2SO$ [δ 2.40 (s, 3 H), 6.99 (d, 2 H), 7.33 (br, 3 H), 7.45 (d, 2 H), 7.71 (d, 2 H)] coincided with that of pure phenyl *p*-toluenesulfonate synthesized by standard methods (see Experimental) [δ 2.34 (s, 3 H), 6.95 (d, 2 H), 7.28 (br, 3 H), 7.40 (d, 2 H), 7.67 (d, 2 H)]; quantitative HPLC showed 70 ± 6% of MNTS to be converted into the sulfonic ester during reaction with phenol. Thus the phenolate ion exhibits ambident behaviour in its reaction with MNTS (Scheme 1), 70% of the reaction involving attack on the MNTS sulfonyl group by the phenolic oxygen atom, and 30% attack on the MNTS nitroso group (apparently by the *para* carbon, but see the Discussion). Also identified and quantified in the reaction mixture was a small amount of NO_2^- due to reaction of MNTS (< 5%) with the undesired oxidation product mentioned in the Experimental section.

Similar behaviour was exhibited in the reaction between *p*-cresol and MNTS (Scheme 2), except that in this case 50 ± 4% of the reaction involved the MNTS nitroso group (as was shown by HPLC determination of *N*-methyl-*p*-toluenesulfonamide) and 50 ± 6% the sulfonyl group (as was shown by HPLC determination of the sulfonic ester, which was isolated from reaction mixtures as a white solid whose ^1H NMR spectrum in $(CD_3)_2SO$ [δ 2.04 (s, 3 H), 2.20 (s, 3 H), 6.66 (d, 2 H), 6.95 (d, 2 H), 7.25 (d, 2 H), 7.50 (d, 2 H)] coincided with that of the authentic sulfonic ester [δ 2.21 (s, 3 H), 2.37 (s, 3 H), 6.84 (d, 2 H), 7.12 (d, 2 H), 7.42 (d, 2 H), 7.68 (d, 2 H)]. However, in this case 30 ± 2% of the MNTS produced NO_2^-, suggesting the formation of an *O*-nitrosated species during the reaction. The presence of *o*-nitroso-*p*-cresol in the final reaction mixture was also suggested by a band at 478 nm in the absorption spectrum

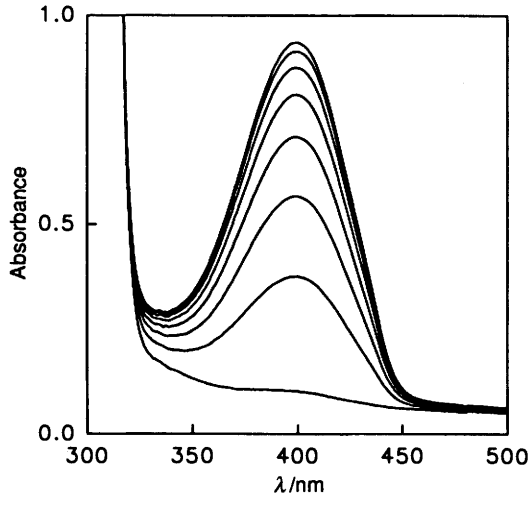
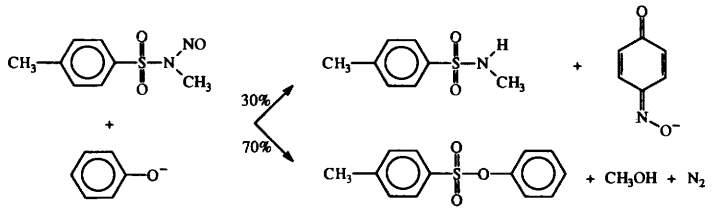
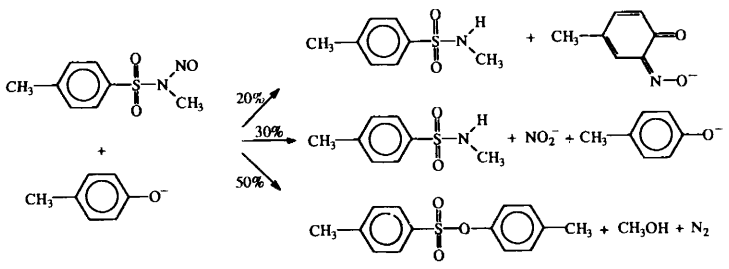


Fig. 1 Absorption spectra, taken at 10 min intervals, of the reaction between phenolate ion and MNTS in 10% dioxane at 25 °C and pH 9.95. $[Phenol]_t$ = 0.18 mol dm^{-3}, [MNTS] = 3 × 10^{-4} mol dm^{-3}.



Scheme 1



Scheme 2

of the latter, and was confirmed by extracting the final reaction mixture with *tert*-amyl alcohol at pH 6–7 (controlled by addition of a small amount of $H_2PO_4^-$–HPO_4^{2-} buffer) and pouring the organic phase over aqueous solutions of $Ni(NO_3)_2$ and $FeSO_4$, which produced the expected red and green colours, respectively.[19]

When the *para* and both *ortho* positions of the phenol are methylated, steric hindrance must prevent the MNTS sulfonyl group from being attacked to any significant extent. Quantitative HPLC found less than 2% of MNTS converted into *p*-toluenesulfonate at the end of its reaction with 2,4,6-trimethylphenol, which thus takes place almost exclusively at the MNTS nitroso group (quantitative HPLC showed about 94% conversion of MNTS into *N*-methyl-*p*-toluenesulfonamide). Although analysis of the reaction mixtures by Shinn's method showed about 90% formation of nitrite, initially thought to derive directly from the decomposition of an unstable *O*-nitroso intermediate, this figure fell to 4% when argon was bubbled through the reaction mixture, and we accordingly inferred that the nitrite in fact derived from a gaseous product whose conversion into NO_2^- was precluded by its removal by the stream of argon. This gas was identified as NO by bubbling it together with added O_2 through a solution of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) of pH 7, which produced the green colour typical of the oxidized form of this compound.[20]

Upon reaction between 2,6-dimethylphenol and either MNTS or certain alkyl nitrites, two bands appeared very close to each other in the UV–VIS spectrum of the reaction mixture. One gradually disappeared, leaving a band at 390 nm that was apparently due to the *C*-nitrosation product 2,6-dimethyl-4-nitrosophenol. This was the only band formed if the reaction was speeded up by the use of more reactive alkyl nitrites such as 2-chloroethyl or 2,2-dichloroethyl nitrites. When the nitrosating agent was MNTS, determination of NO_2^- by Shinn's method showed 18 or 64% yields, depending on whether argon was bubbled through the reaction mixture or not.

Reaction between MNTS and the polyphenols used (resorcinol, phloroglucinol and catechol) led to quantitative formation of *N*-methyl-*p*-toluenesulfonamide (as shown by HPLC), showing that reaction occurred only at the MNTS nitroso N atom. Resorcinol and phloroglucinol were totally converted into their *C*-nitroso derivatives (as was shown by comparison of the final UV–VIS spectra of the reaction mixtures with those of solutions of the authentic *C*-nitroso derivatives that had been prepared from nitrous acid in acid media and then brought to the working pH of the kinetic experiments), and in keeping with this the reactions with resorcinol and phloroglucinol produced no NO_2^-. The product of the reaction with catechol, however, underwent a complex decomposition process; probably, initial decomposition to *o*-quinone and NO was followed by oxidation of NO to NO_2 before formation of the NO_2^- detected in the final reaction mixture.

The reaction between 10^{-3} mol dm^{-3} ascorbic acid and 2×10^{-3} mol dm^{-3} 2-ethoxyethyl nitrite (EEN) in 10^{-2} mol dm^{-3} carbonate–bicarbonate buffer of pH 10.95 afforded an approximately 70% yield of NO_2^-, which rose to 85% if oxygen was bubbled through the reaction mixture and fell to less than 5% if it was argon that was bubbled through the reaction mixture. The formation of NO (oxidized to NO_2^- in the presence of O_2) was confirmed qualitatively by the ABTS test (this test is not quantitative because most NO is carried through the ABTS solution by the argon stream without reacting with ABTS); and measurements with an NO-selective electrode during the course of a reaction carried out with 0.1 mol dm^{-3} ascorbic acid and 9×10^{-6} mol dm^{-3} EEN at pH 12 indicated NO yields greater than 60%. These results imply that two EEN molecules react with each ascorbate ion. Similar results were obtained for the reaction between 2×10^{-3} mol dm^{-3} EEN and 1×10^{-3} mol dm^{-3} catechol at pH 13: nitrite yield was high in the absence of bubbled argon (*ca.* 80%) and low (< 4%) when argon was bubbled, and NO was identified by the ABTS test.

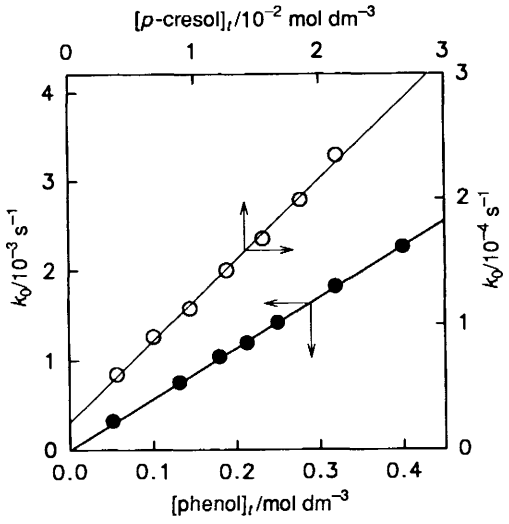


Fig. 2 Influence of total nucleophile concentration on the pseudo-first-order rate constants of the reactions between MNTS and (●) phenol, $[ArO^-]/[ArOH] = 1$, in 4% dioxane; (○) *p*-cresol, $[ArO^-]/[ArOH] = 1$, in 15% dioxane

Kinetic results

Since the rates of all the reactions studied were found in preliminary experiments to be independent of ionic strength (as in the reaction of other nucleophiles with MNTS and alkyl nitrites),[7–9] this parameter was not kept constant in subsequent work.

Reactions of monophenols with MNTS. The first order pseudoconstant k_o for the reaction between phenol and MNTS depended linearly on total phenol concentration at constant pH (Fig. 2). Its increasing with pH (Fig. 3) showed that the only reactive form of the substrate was the phenolate ion, and suggested eqn. (1) where k is the bimolecular rate constant

$$k_o = \frac{kK_a[\text{monophenol}]_t}{(K_a + [H^+])} \quad (1)$$

for reaction between phenolate and MNTS and K_a is the acidity constant of phenol. Fitting the linearized form of this equation

Table 1 Bimolecular rate constants for the reactions between phenols and MNTS and the reaction between EEN and ascorbic acid

Number	Nucleophile	pK_a	Electrophile	k/dm^3 mol^{-1} s^{-1}	Organic solvent (%)
1	Phenol	9.98[a]	MNTS	$k_S = 7.56 \times 10^{-3}$ $k_N = 3.24 \times 10^{-3}$	4[f]
2	4-Methylphenol (*p*-cresol)	10.27[a]	MNTS	$k_S = 9.7 \times 10^{-3}$ $k_N = 9.7 \times 10^{-3}$	15[f]
3	2,4,6-Trimethylphenol	10.89[a]	MNTS	0.264	2[f]
4	2,6-Dimethylphenol	10.59[a]	MNTS	5.23×10^{-2}	5[g]
5	1,3-Dihydroxybenzene (resorcinol)	$pK_1 = 9.44$[b] $pK_2 = 11.32$	MNTS	14.4[d]	2[f]
6	1,3,5-Trihydroxybenzene (phloroglucinol)	$pK_1 = 8.45$[b] $pK_2 = 8.88$	MNTS	0.25[d]	5[f]
7	1,2-Dihydroxybenzene (catechol)	$pK_1 = 9.40$[c] $pK_2 = 12.98$	MNTS	410 ± 40[d] 0.6 ± 0.08[e]	7[f]
8	Ascorbic acid	$pK_1 = 4.03$[a] $pK_2 = 11.34$	EEN	416[d]	10[g]

[a] Ref. 21. [b] Ref. 22. [c] Ref. 23. [d] Reaction with the dianion. [e] Reaction with the monoanion. [f] Dioxane. [g] Acetonitrile.

Table 2 Influence of dioxane concentration on k_o of the reaction between MNTS and phenol. $[Phenol]_t = 0.12$ mol dm^{-3} [ArO$^-$]/[ArOH] = 1

Dioxane (%)	$10^3 k_o$/s^{-1}
2	2.06
5	2.02
10	1.88
15	1.76
20	1.66

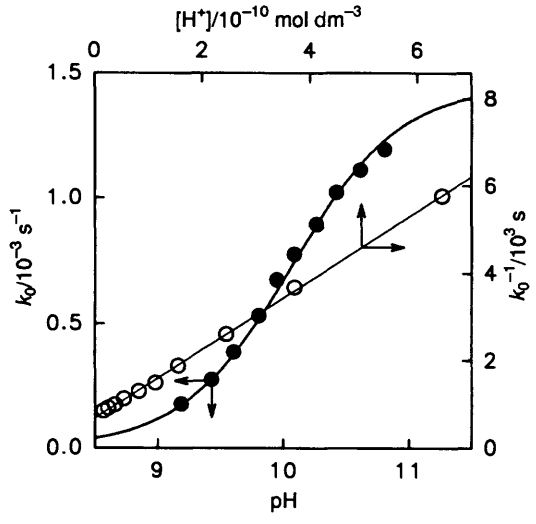


Fig. 3 Influence of acidity on the pseudo-first-order rate constant of the reaction between MNTS and phenol, $[phenol]_t = 0.12$ mol dm^{-3}, in 4% dioxane. (●) k_o plotted against pH; (○) $1/k_o$ plotted against [H$^+$].

to the experimental data (the plot of $1/k_o$ against [H$^+$] in Fig. 3) confirmed its validity, and afforded a value of 1.08×10^{-2} dm^3 mol^{-1} s^{-1} for k and a value of pK_a, 10.06, which agrees well with the published value,[21] 9.98. Since we have seen above (Identification of products) that 30% of the reaction occurs at the MNTS nitroso nitrogen and 70% at the sulfonyl S atom, the observed value of k (which is the sum of the rate constants for these two processes, k_N and k_S) implies the values of k_N and k_S listed in Table 1.

Because the inclusion of dioxane in the medium reduced its polarity, it also reduced k_o (by 19% on increasing the dioxane content of the medium from 2 to 20%). The observed dependence of the pseudo-first-order rate constant for the MNTS–phenol reaction on dioxane content (Table 2) was subsequently extrapolated to other reactions for conversion of observed values of k_o to values for a dioxane content of 5%. The rate-reducing effect of acetonitrile was much less than that of

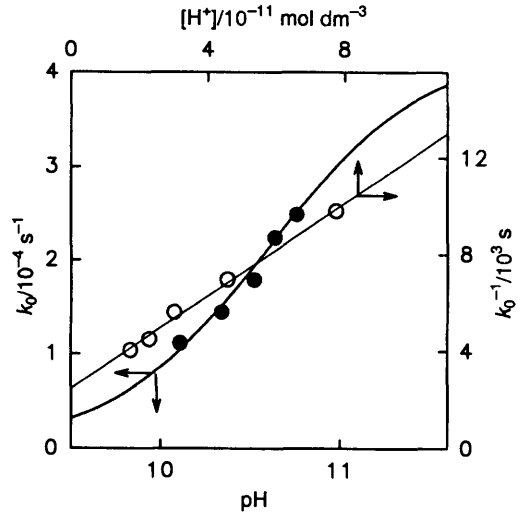


Fig. 4 Influence of acidity on the pseudo-first-order rate constant of the reaction between MNTS and *p*-cresol $[p\text{-cresol}]_t = 1.85 \times 10^{-2}$ mol dm^{-3}, in 15% dioxane. (●) k_o plotted against pH; (○) $1/k_o$ plotted against [H$^+$].

dioxane (because of the greater polarity of acetonitrile), and was not corrected for in either of the reactions in which acetonitrile was present.

The reaction between *p*-cresol and MNTS was also of first order with respect to the substrate (Fig. 2). The non-zero value of k_o at zero cresol concentration was due to basic hydrolysis of MNTS at the working pH, a process that is also first-order with respect to MNTS.[6] In order to take this process into account in studying the pH-dependence of k_o, the bimolecular rate constant of the hydrolysis reaction was determined in media containing 15% of dioxane ($k_{hyd} = 7.13 \times 10^{-2}$ dm^3 mol^{-1} s^{-1}). The increase in k_o with pH (Fig. 4) indicates, as in the case of phenol, that the anion is the reactive form of the substrate. Fitting the linearized form of eqn. (1) to the data (Fig. 4) confirmed its validity and afforded values of 1.94×10^{-2} dm^3 mol^{-1} s^{-1} for k and 10.6 ± 0.4 for pK_a; the latter agrees well with the published value,[21] 10.27, and the former, together with the value of unity deduced above for the ratio between the reactions at N and S (see Identification of products), implies the values of k_N and k_S listed in Table 1.

The concentration–time data obtained by HPLC for the reactions of MNTS with 2,6-dimethylphenol and 2,4,6-trimethylphenol were also fitted well by first order integrated equations, affording the bimolecular rate constants listed in Table 1.

Reactions of polyphenols and ascorbic acid. The kinetics of the nitrosation of resorcinol and phloroglucinol by MNTS were

Table 3 Pseudo-first-order rate constants k_o of the reaction between resorcinol and MNTS in 2% dioxane

pH	[Resorcinol]$_t$/mol dm^{-3}	$k_o/10^{-2}$ s^{-1}	k/dm^3 mol^{-1} s^{-1}
9.44	0.184	1.77	14.7
9.09	9.2×10^{-2}	0.246	14.7
9.44	9.2×10^{-2}	0.888	14.7
9.72	9.2×10^{-2}	2.11	14.1
9.34	4.1×10^{-2}	0.270	14.3
9.72	4.1×10^{-2}	0.940	14.1
10.18	4.1×10^{-2}	3.47	14.6

Table 4 Pseudo-first-order rate constants k_o of the reaction between phloroglucinol and MNTS in 5% dioxane

pH	[Phloroglucinol]$_t$/mol dm^{-3}	$k_o/10^{-2}$ s^{-1}	k/dm^3 mol^{-1} s^{-1}
9.26	8.1×10^{-2}	1.39	0.25
9.83	7.8×10^{-2}	1.82	0.26
9.49	7.8×10^{-2}	1.65	0.27
9.29	7.8×10^{-2}	1.33	0.24
9.10	7.8×10^{-2}	0.977	0.23
9.28	4.0×10^{-2}	0.696	0.25
9.94	2.7×10^{-2}	0.605	0.24
9.58	2.7×10^{-2}	0.561	0.25
9.31	2.7×10^{-2}	0.483	0.25
9.24	2.7×10^{-2}	0.420	0.23
9.32	1.8×10^{-2}	0.303	0.24

studied under conditions in which these polyphenols undergo no significant oxidation. Table 3 lists values of k_o for resorcinol measured at pH 9.1–10.2 using total substrate concentrations of (4.1–18.4) $\times 10^{-2}$ mol dm^{-3}; these data, in conjunction with the known acidity constants of resorcinol ($pK_1 = 9.44$, $pK_2 = 11.32$),[22] imply that the only reactive substrate species is the dianion, and that the bimolecular rate constant for its reaction with MNTS is 14.4 ± 0.7 dm^3 mol^{-1} s^{-1} (calculated as the average of the values listed in Table 3). Similarly, data obtained for phloroglucinol using substrate concentrations of (1.8–8.1) $\times 10^{-2}$ mol dm^{-3} at pH 9–10 (Table 4) indicate, together with the known values of its acidity constants ($pK_1 = 8.45$, $pK_2 = 8.88$),[22] that the dianion is again the only active species, with a bimolecular rate constant of 0.25 ± 0.02 dm^3 mol^{-1} s^{-1}.

In the case of catechol, concentration–time data for reactions carried out at pH 9–10.5 with 5×10^{-4} mol dm^{-3} MNTS and (5–20) $\times 10^{-3}$ mol dm^{-3} catechol afforded values of k_o (Table 5) which, in view of the known acidity constants of catechol ($pK_1 = 9.36$, $pK_2 = 12.98$),[23] were best explained assuming the involvement of both its monoanion and its dianion [eqn. (2)], and on this basis imply bimolecular rate constants of 0.6 ± 0.08 dm^3 mol^{-1} s^{-1} for the monoanion and 410 ± 40 dm^3 mol^{-1} s^{-1} for the dianion (Table 1).

$$k_o = k_m[AH^-] + k_d[A^{2-}] \quad (2)$$

The reaction of ascorbic acid with EEN was of first order with respect to EEN at pH 10.95 (Fig. 5), and its pH-dependence in the range pH 11–13 indicated that the only reactive form of the substrate was the dianion (Fig. 6). Fitting a straight line to a plot of $1/k_o$ against [H$^+$] (Fig. 6) afforded values of 12 for the second acidity constant of ascorbic acid (in good agreement with the published value of 11.34)[21] and 416 dm^3 mol^{-1} s^{-1} for the bimolecular rate constant of the reaction (Table 5).

Discussion

Earlier findings that MNTS reacts with I$^-$ [9] and with N,[6,7] C[8] and S[9] nucleophiles through its nitroso group, and with O nucleophiles mainly through its sulfonyl group,[6,9] were explained in terms of Klopman's theory[10] as due to the former

Table 5 Pseudo-first-order rate constants k_o of the reaction between catechol and MNTS in 7% dioxane

pH	[Catechol]$_t$/mol dm^{-3}	$k_o/10^{-4}$ s^{-1}
9.08	2×10^{-2}	3.47
9.32	1×10^{-2}	2.86
9.59	1×10^{-2}	3.77
9.90	5×10^{-3}	3.97
10.23	5×10^{-3}	6.41

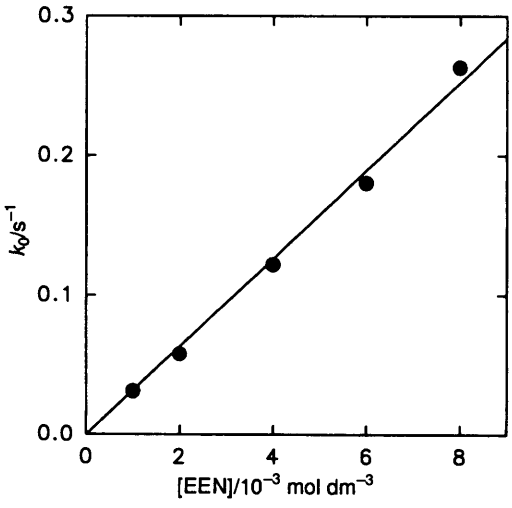


Fig. 5 Influence of EEN concentration on k_o of its reaction with ascorbic acid in 10^{-2} mol dm^{-3} Na_2CO_3–$NaHCO_3$ buffer (pH 10.95) containing 10% of acetonitrile

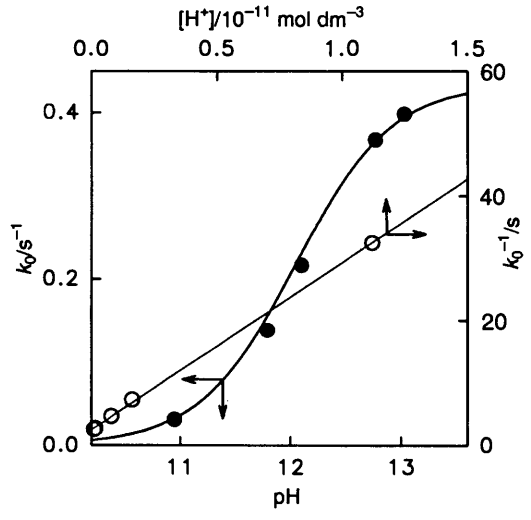


Fig. 6 Influence of acidity on k_o of the reaction between EEN and ascorbic acid ([EEN] = 1×10^{-3} mol dm^{-3}) in 10^{-2} mol dm^{-3} Na_2CO_3–$NaHCO_3$ buffer (pH 10.95) containing 10% of acetonitrile. (●) k_o plotted against pH; (○) $1/k_o$ plotted against [H$^+$].

reactions being controlled by the energies of the relevant frontier orbitals and the latter by electrostatic interactions. I$^-$ and N, C and S nucleophiles, which have high-energy HOMOs, low charge density on their nucleophilic centres and high polarizability, would (and do) react with the softer electrophilic centre of MNTS (the nitroso nitrogen), upon which the MNTS LUMO must be localized; while O nucleophiles, which have high charge density on their nucleophilic centre, would (and do) react with the harder of the MNTS electrophilic centres (the sulfonyl S atom).

A priori, reaction between MNTS and the ambident nucleophiles used in this work might occur between either of the electrophilic centres of MNTS (the nitroso N and the sulfonyl S) and either of the nucleophilic centres of the substrates (C or

O atoms). The above theory would suggest that two of these combinations should predominate, the reactions between C and –N=O and between O and S; and in keeping with this prediction, analysis of the reaction between the phenolate ion and MNTS appeared to reflect straightforward competition between these two reaction paths, 70% of the reaction appearing to occur between the phenolic oxygen atom and the sulfonyl group, and the remaining 30% between the nitroso group and the *para* carbon of phenol (Scheme 1). However, blocking the *para* carbon with a methyl group paradoxically increased the participation of the nitroso group to 50% while reducing *C*-nitrosation to 20%, the rest of the reaction with the nitroso group leading to the formation of NO_2^- (Scheme 2). 2,6-Dimethylphenol behaved similarly to *p*-cresol, except that in this case reaction of the phenolic O atom with the MNTS sulfonyl group was prevented by steric hindrance (which has likewise been considered responsible for the reactions of 2,6-dimethylphenol with the carbonyl groups of nitrophenyl acetates being about 40 times slower than the corresponding reactions of unsubstituted phenols[24,25]). 2,4,6-Trimethylphenol afforded NO_2^- quantitatively. For both 2,6-dimethylphenol and 2,4,6-trimethylphenol, the NO_2^- produced was found to derive from NO.

The behaviour of *p*-cresol, 2,6-dimethylphenol and 2,4,6-trimethylphenol suggests that these substrates can react with the MNTS nitroso group *via* their oxygen atoms. In fact, the satisfaction, by all the four monophenols studied, of a linear relationship between k_N and the pK_a of the phenolic oxygen atom (Fig. 7; $r = 0.99$), suggests that this is the *only* path in these reactions, since such uniformity would be unlikely if the reaction took place partly *via* the oxygen atom (totally in the case of 2,4,6-trimethylphenol) and partly at the carbon atom (totally in the case of phenol). A similar correlation for a wider range of pK_as (7.22–10.6) was observed for reaction of alkyl nitrites with phenols.[26] The differences among the four substrates as regards the final products of their reactions with the MNTS nitroso group may be attributed to the *O*-nitroso compound initially formed being able to undergo either internal rearrangement to the corresponding *C*-nitroso compound, or homolytic cleavage to afford phenolate and NO. This behaviour of phenols, acting only as oxygen nucleophiles towards MNTS, contrasts with the results of Buncel *et al.*,[14] who have published convincing evidence that the ambident phenoxide ion does react with nitroaromatic electrophiles *via* both its oxygen and carbon atoms.

The apparent discrepancy between Klopman's theory and the postulated reaction of the phenolic O atom with the MNTS nitroso group may be the result of overlooking the delocalization of charge by the phenyl ring, which must soften the O atom and so facilitate reaction with the nitroso group; similar effects have been pointed to as responsible for the acetohydroxamic acid anion being able to react with both the sulfonyl and nitroso groups of MNTS.[9]

The question arises whether *O*-nitrosation followed by internal rearrangement is not also the mechanism of the *C*-nitrosation of phenols by strong electrophiles in acid media, which has hitherto been assumed to involve direct attack on the C atom.[1,3] The mechanism involving rearrangement would parallel the known behaviour of aromatic secondary amines, whose initial *N*-nitrosation is followed by Fischer–Hepp rearrangement to the 4-nitroso compound, but for phenols this possibility would be difficult to substantiate experimentally.[27]

The polyphenols studied in this work reacted exclusively with the MNTS nitroso group. The linearity of a plot of $-\log k$ against pK_a for the anions shown by kinetic analysis to be involved in the reactions (the dianions of resorcinol and phloroglucinol and the mono- and di-anions of catechol) supports the hypothesis that, as in the case of the monophenols, reaction occurs exclusively through a phenolic oxygen atom (Fig. 8; $r = 0.99$). In the case of resorcinol and phloroglucinol,

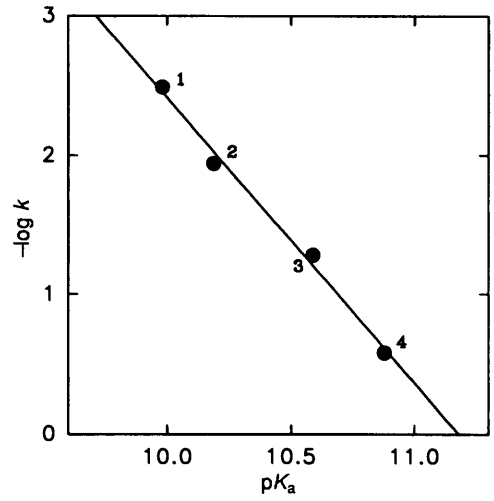


Fig. 7 Brønsted plot for the reactions of monophenolates with the MNTS nitroso group (individual substrates are indicated by the numbers assigned in Table 1)

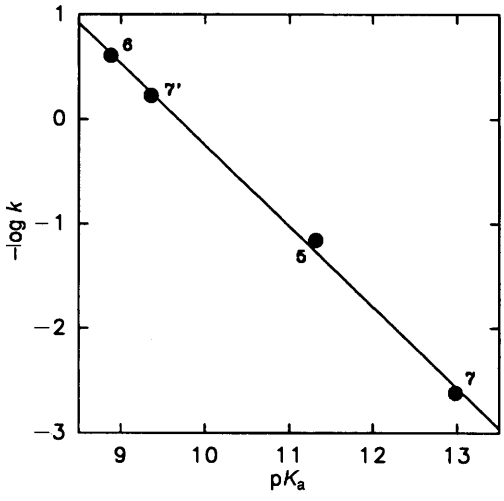


Fig. 8 Brønsted plot for the reactions of polyphenolates with the MNTS nitroso group (individual substrates are indicated by the numbers assigned in Table 1; **7** and **7'** correspond respectively to the dianion and monoanion of catechol)

the *O*-nitroso intermediate must undergo total rearrangement to the *C*-nitroso derivative, while in the case of catechol the *O*-nitroso intermediate is decomposed to afford NO.

As already mentioned (see Identification of products), the reactions between EEN and the ascorbate or catechol dianions exhibit 2:1 stoicheiometry. A possible mechanism for these reactions would consist in the initial nitrosation of the dianion being followed by the cleavage of the O–NO bond to release NO and form a radical anion susceptible to oxidation by the second EEN molecule. Thus reaction with these (or other) biological reductones[13,28] may constitute a path for *in vivo* generation of NO from alkyl nitrites and other vasodilators that are known to act *via* the production of this molecule;[29] hitherto, the action of these agents has been attributed to their reaction with thiols to afford unstable nitrosothiols whose decomposition would release NO.[30] Reaction with biological reductones may constitute an alternative mechanism of action of vasodilatory alkyl nitrites, and it might be possible to control the rate of NO release by varying the alkyl nitrite: for example, in view of the rate constant of the reaction with EEN, the reaction of similar concentrations of Cl_2CHCH_2ONO (which reacts with the 2,4-pentanedione carbanion about 1000 times faster than EEN) should take just a few seconds at lower pHs.

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