# Oxidation of Thiols and Disulphides with the Ammoniumyl Radical-cation, $^{+\cdot}NH_3$: an E.S.R. Investigation

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E.s.r. spectroscopy has been employed to demonstrate that the reaction of $^{+\cdot}NH_3$ (from $Ti^{III}$ and $^{+}NH_3OH$) with thiols (RSH) proceeds *via* the initial formation of thiyl radicals (RS·); these react further to give disulphides as well as oxygen-containing radicals RSO· and $RSO_2\cdot$, formed by a series of one-electron oxidation reactions, and both $SO_2^{-\cdot}$ and $SO_3^{-\cdot}$ (which arise *via* sulphur dioxide formed in the decomposition of $RSO_2\cdot$). Reaction with disulphides, which leads to the detection of sulphinyl radicals and disulphide-conjugated radicals, is thought to involve both radical-cations ($RSSR^{+\cdot}$) and ammoniumyl adducts $[R\dot{S}(\overset{+}{N}H_3)SR]$ whose further reactions have been described.

THE action of thiols as radioprotectors[1] and disulphides as antioxidants[2] has prompted considerable interest in the reactions of radicals with these compounds and in the properties of the radicals derived from them. Techniques employed in studies of these systems include direct radiolysis and product studies,[3] pulse radiolysis in conjunction with conductometric and optical detection systems,[4] and e.s.r. spectroscopy.[5]

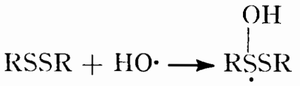
The usefulness of e.s.r. spectroscopy is demonstrated by the characterisation of a variety of radicals of different structure (*e.g.* RS·, by trapping, RSO·, and $RSO_2\cdot$) formed by a complex series of reactions during the oxidation of thiols and disulphides with ·OH (from the $Ti^{III}$–$H_2O_2$ couple). It was proposed[5] that alkanesulphinyl radicals (RSO·) are formed both *via* the decomposition of ·OH–disulphide adducts $[R\dot{S}(OH)SR]$ and by oxidation of sulphenic acids (RSOH) formed by reaction of RS· with $H_2O_2$; alkanesulphonyl radicals ($RSO_2\cdot$) are evidently derived from RSO· *via* oxidation with $H_2O_2$. Some of the important processes involved are shown in reactions (1)—(10).

$$RSH + HO\cdot \longrightarrow RS\cdot + H_2O \qquad (1)$$

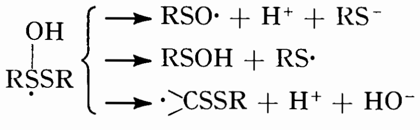
$$RS\cdot + RS\cdot \longrightarrow RSSR \qquad (2)$$

$$RS\cdot + H_2O_2 \longrightarrow RSOH + HO\cdot \qquad (3)$$

$$RSOH + HO\cdot \longrightarrow RSO\cdot + H_2O \qquad (4)$$



$$RSSR + HO\cdot \longrightarrow R\underset{\bullet}{\overset{\overset{OH}{|}}{S}}SR \qquad (5)$$



$$R\underset{\bullet}{\overset{\overset{OH}{|}}{S}}SR \left\{ \begin{array}{ll} \longrightarrow RSO\cdot + H^+ + RS^- & (6) \\ \longrightarrow RSOH + RS\cdot & (7) \\ \longrightarrow \cdot{>}CSSR + H^+ + HO^- & (8) \end{array} \right.$$

$$RSO\cdot + H_2O_2 \longrightarrow RSO_2H + HO\cdot \qquad (9)$$

$$RSO_2H + HO\cdot \longrightarrow RSO_2\cdot + H_2O \qquad (10)$$

We have previously described the use of e.s.r. spectroscopy in the study of the reactions of $^{+\cdot}NH_3$, generated from the $Ti^{III}$–$^{+}NH_3OH$ redox couple [reaction (11)];[6,7] this radical reacts *via* hydrogen abstraction from activated C–H bonds in, *e.g.* alcohols,[6] and *via* sulphur-centred radical-adducts with sulphides and sulphoxides.[7] We here describe the results of an investigation of the reaction of $^{+\cdot}NH_3$ with thiols and disulphides.

$$Ti^{III} + {}^{+}NH_3OH \longrightarrow Ti^{IV} + OH^- + {}^{+\cdot}NH_3 \qquad (11)$$

## RESULTS AND DISCUSSION

*Reactions of* $^{+\cdot}NH_3$ *with Thiols and Disulphides.*—The reactions of $^{+\cdot}NH_3$ with a selection of thiols and disulphides were studied at pH *ca.* 1.5 using a three-way flow-system of mixing time *ca.* 0.1 s. The three streams contained, respectively, titanium(III) chloride solution, hydroxylamine hydrochloride, and the substrate (for details, see the Experimental section); studies were carried out over a wide range of substrate concentrations and were also repeated using hydrogen peroxide, instead of hydroxylamine hydrochloride, in order to examine the effect of variations in substrate concentration of corresponding reactions of ·OH.

It was found that, depending on the structure and the concentration of the thiol or disulphide, a variety of different radicals could be detected. These included radicals of the type RSO· and $RSO_2\cdot$,[5] alkyl radicals, disulphide-conjugated radicals,[5] and also[8] $SO_2^{-\cdot}$ and $SO_3^{-\cdot}$. Table 1 lists the e.s.r. parameters of these radicals and indicates the substrate concentration ranges over which signals could be observed. The findings are described in more detail in the sequel.

(*a*) *Cysteine and Cystine.*—In the reaction of $^{+\cdot}NH_3$ with L-cysteine, $HO_2CCH(NH_3^+)CH_2SH$, the only radical detected for substrate concentrations $>10^{-3}$ mol dm$^{-3}$ was the sulphinyl radical[5] $HO_2CCH(NH_3^+)CH_2SO\cdot$. Reduction in the cysteine concentration below this resulted in the diminution of this signal and the detection of $SO_3^{-\cdot}$ and[9] $\cdot CH_2CH(NH_3^+)CO_2H$. The sulphinyl radical was also detected from the disulphide L-cystine, although the signal was generally weaker than that from the thiol under similar conditions; no extra signals appeared on dilution of this substrate and we conclude at this stage that the formation of $SO_3^{-\cdot}$ and $\cdot CH_2CH(NH_3^+)CO_2H$ from cysteine does not involve *in situ* disulphide formation.

(*b*) *2-Mercaptoethanoic Acid and 2,2′-Dithiodiethanoic Acid.*—In contrast to the results obtained for cysteine, the reaction of $^{+\cdot}NH_3$ with $HO_2CCH_2SH$ at concentrations greater than *ca.* $2 \times 10^{-3}$ mol dm$^{-3}$ led to the detection of $SO_2^{-\cdot}$ as well as a weak spectrum from $HO_2CCH_2SS\dot{C}HCO_2H$.[5] A weak, unassigned signal with $a$(1H) 1.55 mT, $g$ 2.0066 was also detected. At lower concentrations of the substrate, however, only $SO_3^{-\cdot}$ and [5,10] $\cdot CH_2SSCH_2CO_2H$ could be detected. The disulphide-conjugated radical $\cdot CH_2SSCH_2CO_2H$ was also detected over a wide concentration range from the reaction of the disulphide $HO_2CCH_2SSCH_2CO_2H$ (in addition to a weak signal from $SO_2^{-\cdot}$).

(*c*) 2-*Mercaptopropanoic Acid*.—The behaviour of $HO_2CCHMeSH$ was analogous to that of 2-mercaptoethanoic acid. Thus reaction at higher concentration ($>10^{-2}$ mol dm$^{-3}$) led to the detection of $SO_2^{-\cdot}$ and [5] $\cdot CMe(CO_2H)SSCHMeCO_2H$, and dilution led to the disappearance of these signals and the appearance of those from $SO_3^{-\cdot}$ and $\cdot CHMeSSCHMeCO_2H$. In addition, an unidentified singlet ($g$ 2.0108, $\Delta H$ 0.1 mT) was detected at intermediate thiol concentrations. The corresponding disulphide was not studied.

TABLE 1

Radicals characterized during the reaction of $\cdot^{+}NH_3$ with thiols and disulphides and their dependence upon substrate concentration

| | | | E.s.r. parameters [a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| R | Substrate (S) | Radical | $a(\alpha\text{-H})$/mT | $a(\beta\text{-H})$/mT | $a$(other)/mT | $g$ | $\Delta H$/mT | Range of $[S]_0$/mol dm$^{-3}$ for radical detection |
| $HO_2CCH(NH_3^+)$-$CH_2$ | RSH | $HO_2CCH(NH_3^+)CH_2SO\cdot$ | | 0.93(2H) | | 2.0107 | 0.22 | $>1.3 \times 10^{-4}$ [b] |
| | | $SO_3^{-\cdot}$ | | | | 2.0032 | 0.10 | $5.3 \times 10^{-4}$—$1.3 \times 10^{-4}$ |
| | | $HO_2CCH(NH_3^+)CH_2\cdot$ | 2.25(2H) | 2.63(1H) | 0.38(1N) | 2.0028 | 0.10 | $3 \times 10^{-4}$—$1.3 \times 10^{-4}$ |
| | RSSR | $HO_2CCH(NH_3^+)CH_2SO\cdot$ | | 0.93(2H) | | 2.0107 | 0.22 | *ca.* $3 \times 10^{-4}$—$8 \times 10^{-5}$ [b] |
| $HO_2CCH_2$ | RSH | $HO_2C\dot{C}HSSCH_2CO_2H$ | 1.55(1H) | | | 2.0056 | 0.10 | $>$*ca.* $2 \times 10^{-3}$ |
| | | $SO_2^{-\cdot}$ | | | | 2.0057 | 0.10 | $>$*ca.* $3 \times 10^{-3}$ |
| | | $SO_3^{-\cdot}$ | | | | 2.0032 | 0.10 | $1 \times 10^{-3}$—$6 \times 10^{-4}$ [b] |
| | | $HO_2CCH_2SSCH_2\cdot$ | 1.70(2H) | | | 2.0040 | 0.05 | $1 \times 10^{-3}$—$6 \times 10^{-4}$ [b] |
| | RSSR | $HO_2CCH_2SSCH_2\cdot$ | 1.70(2H) | | | 2.0040 | 0.05 | $>1.4 \times 10^{-4}$ |
| | | $SO_3^{-\cdot}$ | | | | 2.0032 | 0.10 | $>1.8 \times 10^{-2}$ and<br>$1.4 \times 10^{-4}$—$4 \times 10^{-5}$ |
| $HO_2CCH(Me)$ | RSH | $HO_2C\dot{C}(Me)SSCH(Me)CO_2H$ | | 1.78(3H) | | 2.0053 | 0.08 | $>$*ca.* $1 \times 10^{-2}$ |
| | | $SO_2^{-\cdot}$ | | | | 2.0057 | 0.10 | $>$*ca.* $4 \times 10^{-2}$ |
| | | $SO_3^{-\cdot}$ | | | | 2.0032 | 0.10 | $<5 \times 10^{-4}$ |
| | | $HO_2CH(Me)SS\dot{C}HMe$ | 1.80(1H) | 2.20(3H) | | 2.0037 | 0.10 | $<5 \times 10^{-4}$ |
| $HO_2CCH_2CH_2$ | RSH | $SO_2^{-\cdot}$ | | | | 2.0057 | 0.10 | *d* |
| | | $HO_2CCH_2CH_2SO_2\cdot$ | | 0.125(2H) | 0.25(2$\gamma$H) | 2.0050 | ·0.05 | $4 \times 10^{-3}$—$8 \times 10^{-5}$ |
| | RSSR | *c* | | | | | | $[1 \times 10^{-3}$—$1 \times 10^{-5}]$ [e] |
| $HOCH_2CH_2$ | RSH | $SO_2^{-\cdot}$ | | | | 2.0057 | 0.10 | *d* |
| | | $HOCH_2CH_2SO_2\cdot$ | | | 0.40(2$\gamma$H) | 2.0053 | 0.05 | $1.2 \times 10^{-2}$—$7 \times 10^{-5}$ |
| | RSSR | *c* | | | | | | $[1.1 \times 10^{-2}$—$2 \times 10^{-5}]$ [e] |
| $NH_3^+CH_2CH_2$ | RSH | $NH_3^+CH_2CH_2SO\cdot$ | | | 0.93(2H) | 2.0109 | 0.3 | $>4.5 \times 10^{-4}$ |

[a] Splittings $\pm 0.005$ mT; $g \pm 0.0001$; $\Delta H$ $\pm 0.02$ mT. [b] Lowest substrate concentration studied; radical still detected at this concentration. [c] No radicals detected. [d] $SO_2^{-\cdot}$ detected for [RSH] *ca.* 0.1M. [e] Range of concentrations studied.

(*d*) 3-*Mercaptopropanoic Acid and* 3,3′-*Dithiodipropanoic Acid*.—In contrast with the behaviour of the thiols mentioned previously, reaction of $HO_2CCH_2CH_2SH$ led to the detection of $SO_2^{-\cdot}$ (at high substrate concentrations) and the sulphonyl radical [5] $HO_2CCH_2CH_2SO_2\cdot$ for $[HO_2CCH_2CH_2SH] < 4 \times 10^{-3}$ mol dm$^{-3}$. Further dilution led to an increase in the intensity of this signal, giving a maximum concentration at $[HO_2CCH_2CH_2SH]$ *ca.* $4 \times 10^{-4}$ mol dm$^{-3}$. No signals were detected from the corresponding disulphides over a range of concentrations (see Table 1).

(*e*) 2-*Mercaptoethanol and* 2-*Mercaptoethylamine*.—Similar results to those described for 3-mercaptopropanoic acid were obtained for 2-mercaptoethanol; thus at high concentrations $SO_2^{-\cdot}$ and, at lower substrate concentrations, $HOCH_2CH_2SO_2\cdot$ were detected (with a maximum concentration of the latter for $[HOCH_2CH_2SH]$ $7 \times 10^{-4}$ mol dm$^{-3}$). There were no detectable signals from the corresponding disulphide. Reaction of $^{+\cdot}NH_3$ with $^{+}NH_3CH_2CH_2SH$ led simply to the detection of a signal assigned to [5] $^{+}NH_3CH_2CH_2SO\cdot$ over a wide range of substrate concentration; its intensity was essentially independent of substrate concentration down to $[^{+}NH_3CH_2CH_2SH]$ $5 \times 10^{-4}$ mol dm$^{-3}$, below which further reductions in thiol concentration simply resulted in a decrease of intensity.

*Trapping Experiments*.—The detection of disulphide-derived radicals in the reaction of thiols and $^{+\cdot}NH_3$ suggests that, as with $\cdot OH$, oxidation takes place to give thiyl radicals ($RS\cdot$) which then dimerize to give disulphides, which then undergo further reaction.* There are strong grounds for believing that alkylthiyl radicals (like alkoxyl radicals) will not be detectable by e.s.r. spectroscopy in fluid solution,[5,11] and we have hence carried out the thiol oxidation reactions in the presence of butenedioic acid as a radical trap. In each case a strong e.s.r. signal attributed to the radical adduct [5,12] $\cdot CH(CO_2H)CH(CO_2H)SR$ was detected (see Table 2) and all other signals were removed. We conclude that thiyl radicals are indeed formed in the first step and also that all radicals directly detected in the absence of the trap derive from further reactions of $RS\cdot$.

*The reactions of* $\cdot OH$ *with thiols and disulphides*. The results obtained for $^{+\cdot}NH_3$ and thiols and disulphides show a general similarity to those obtained [5] for $\cdot OH$ (where sulphinyl, sulphonyl, and disulphide-conjugated radicals could also be detected). Since data for the latter

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*An alternative route to disulphides involves the rapid reaction of $RS\cdot$ with RSH and oxidation of the resulting adduct $RS\dot{S}(H)R$.

system refer[5] to a restricted range of substrate concentrations, we have also extended this range in order to facilitate a direct comparison of the $\cdot OH$ and $^{+\cdot}NH_3$ systems.

The reaction of cysteine with $\cdot OH$ led to the detection of $HO_2CCH(NH_3^+)CH_2SO\cdot$ (for [thiol] $> 4 \times 10^{-4}$ mol $dm^{-3}$) and $\cdot CH_2CH(NH_3^+)CO_2H$ (in the concentration range $10^{-3} - 4 \times 10^{-4}$ mol $dm^{-3}$); unlike the $^{+\cdot}NH_3$ reaction, no $SO_3^{-\cdot}$ was detected. $S_1$ (the $Ti^{IV}$–$HO_2\cdot$ complex[13] with *g* 2.0134) was the only species detected for substrate concentrations below $4 \times 10^{-4}$ mol $dm^{-3}$.* Reaction of the corresponding disulphide also led to the detection of $HO_2CCH(NH_3^+)CH_2SO\cdot$ (as with $^{+\cdot}NH_3$) and $S_1$.

The reaction of $\cdot OH$ with 2-mercaptoethanoic acid showed significant differences compared with the reaction of $^{+\cdot}NH_3$. Thus although for [thiol] 0.1 mol $dm^{-3}$, for example, $\cdot CH(CO_2H)SSCH_2CO_2H$ was detected, a broad signal attributed to $HO_2CCH_2SO\cdot$ [*a*(2H) 0.95 mT, *g* 2.0104] was also observed; over the thiol concentration range $5 \times 10^{-2}$—$2.5 \times 10^{-3}$ mol $dm^{-3}$ this was the only detectable radical. Further reduction in the thiol concentration led to a spectrum analysed in terms of a mixture of signals from $S_1$, $\cdot CH_2SSCH_2CO_2H$, and also[9] $\cdot CH_2CO_2H$. Subsequent dilutions resulted in a decrease in the concentration of carbon-centred radicals so that these were undetectable by $[HO_2CCH_2SH]$ *ca.* $2 \times 10^{-4}$ mol $dm^{-3}$ (when $S_1$ remained). Signals from the sulphinyl radical $HO_2CCH_2SO\cdot$, but not $SO_3^{-\cdot}$, were clearly identified during the reaction of $\cdot OH$ with the corresponding disulphide. Signals from $S_1$ and $\cdot CH_2CO_2H$ were also detected at $[HO_2CCH_2SSCH_2CO_2H]$ *ca.* $10^{-3}$ mol $dm^{-3}$, while a further two-fold dilution led in addition to the detection of $\cdot CH_2SSCH_2CO_2H$.

TABLE 2

E.s.r. parameters of radicals of the type $RSCH(CO_2H)$-$\dot{C}HCO_2H$ formed during the reaction of thiols (RSH) with $NH_3^{+\cdot}$ in the presence of butenedioic acid

| R | Hyperfine splittings/mT[a] *a*(1H) | *a*(1H) | *a*(2H) | *g*[b] |
|---|---|---|---|---|
| $HO_2CCH(NH_3^+)CH_2$ | 2.00 | 0.84 | 0.11 | 2.0034 |
| $HO_2CCH_2$ | 2.00 | 0.91 | 0.08 | 2.0035 |
| $HO_2C(Me)CH$ | 2.01 | 0.89 | *c* | 2.0032 |
| $HO_2CCH_2CH_2$ | 2.00 | 0.90 | 0.11 | 2.0033 |
| $HOCH_2CH_2$ | 1.98 | 0.85 | 0.11 | 2.0034 |
| $NH_3^+CH_2CH_2$ | 1.98 | 0.85 | 0.10 | 2.0033 |

[a] ±0.005 mT. [b] ±0.0001. [c] No γ-proton splitting resolved.

2-Mercaptopropanoic acid behaved in a manner similar to 2-mercaptoethanoic acid in that at $[HO_2CCH(Me)SH]_0$ *ca.* $10^{-2}$ mol $dm^{-3}$ $HO_2CCH(Me)SO\cdot$[5] was detected (accompanied by an unidentified singlet of linewidth 0.15 mT and *g* 2.0104). A reduction in thiol concentration resulted in a decrease in the intensities of both spectra and in the detection of[5] $\cdot CHMeCO_2H$.

No radicals were detected from the reaction of $\cdot OH$ with 3-mercaptopropanoic acid at a thiol concentration of $5 \times 10^{-2}$ mol $dm^{-3}$; however a gradual dilution of the thiol (to $3 \times 10^{-3}$ mol $dm^{-3}$) resulted in the detection of both $S_1$ and $HO_2CCH_2CH_2SO_2\cdot$. The concentration of both species was increased by further dilution, that of the sulphonyl radical attaining a maximum value at $[HO_2CCH_2CH_2SH]_0$ *ca.* $5 \times 10^{-4}$ mol $dm^{-3}$. Reaction of a saturated solution of the corresponding disulphide led, in contrast to the $^{+\cdot}NH_3$ system (where no radicals were detected), to the detection of $S_1$, $HO_2CCH_2CH_2SO_2\cdot$, and[5] $HO_2CCH_2CH_2SO\cdot$. Dilution of the disulphide resulted in a reduction in the concentration of both sulphur-containing radicals.

Although no radicals were detected from the reaction of $\cdot OH$ with 2-mercaptoethanol in the range 0.1—0.02 mol $dm^{-3}$, a weak spectrum due to $HOCH_2CH_2SO_2\cdot$ was detected at $[HOCH_2CH_2SH]_0$ $10^{-2}$ mol $dm^{-3}$. Its intensity increased with reduction in thiol concentration, reaching a maximum at $[HOCH_2CH_2SH]_0$ *ca.* $7 \times 10^{-4}$ mol $dm^{-3}$. The reaction of $\cdot OH$ with the disulphide $HOCH_2CH_2SSCH_2CH_2OH$ (0.01 mol $dm^{-3}$) led to the detection of[5] $HOCH_2CH_2SO\cdot$; a reduction in the disulphide concentration led to its replacement by the signal of $HOCH_2CH_2SO_2\cdot$, the latter being formed exclusively at $[HOCH_2CH_2SSCH_2CH_2OH]_0$ $7 \times 10^{-4}$ mol $dm^{-3}$.

Finally, the reaction of $NH_3^+CH_2CH_2SH$ with $\cdot OH$ led to the detection of $NH_3^+CH_2CH_2SO\cdot$, as was also observed with $^{+\cdot}NH_3$. The spectrum's intensity was essentially independent of substrate concentration over the range 0.03—$4 \times 10^{-3}$ mol $dm^{-3}$, but decreased from $[NH_3^+CH_2CH_2SH]_0$ $<3 \times 10^{-3}$ mol $dm^{-3}$.

The reactions of the $\cdot OH$ and $^{+\cdot}NH_3$ systems clearly have features in common and in both cases there is evidence for the *in situ* formation of disulphides from thiols. However, we note that the two systems differ as follows.

(i) $SO_2^{-\cdot}$ and $SO_3^{-\cdot}$ (absent in the reactions of $\cdot OH$) are detected during the reactions of $NH_3^{+\cdot}$ with several thiols and with 2,2′-dithiodiethanoic acid.

(ii) No radicals could be detected from the reactions of $NH_3^{+\cdot}$ with 3,3′-dithiodipropanoic acid or 2,2′-dithiodiethanol, whereas sulphinyl and sulphonyl radicals were detected from the reaction of these substrates with $\cdot OH$.

(iii) The sulphinyl radicals detected during the reactions of $\cdot OH$ with 2-mercaptopropanoic acid and 2-mercaptoethanoic acid (and its corresponding disulphide) were absent during the analogous reactions of $NH_3^{+\cdot}$, as were the carbon-centred radicals $\cdot CHMeCO_2H$ and $\cdot CH_2CO_2H$.

(iv) The disulphide-conjugated radical $\cdot CH_2SSCH_2CO_2H$ was detected during the reaction of $NH_3^{+\cdot}$ with $HO_2CCH_2SSCH_2CO_2H$ over a wide range of substrate concentrations whereas this radical could only be detected from the analogous $\cdot OH$ system over a narrow concentration range ($5 \times 10^{-3}$—*ca.* $2 \times 10^{-4}$ mol $dm^{-3}$).

In the following section, mechanisms are proposed for the reaction of $^{+\cdot}NH_3$ with thiols and disulphides which account not only for the formation of the oxygenated radicals $RSO\cdot$, $RSO_2\cdot$, $SO_2^{-\cdot}$, and $SO_3^{-\cdot}$ but also for the

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* In general, for reactions of disulphides and thiols with $\cdot OH$, only $S_1$ was detected at substrate concentrations below *ca.* $2 \times 10^{-4}$ mol $dm^{-3}$.

similarities and differences in the behaviour of $^{+\cdot}NH_3$ and ·OH.

*Mechanisms of the Reactions*.—(a) *The reactions of $^{+\cdot}NH_3$ with thiols.* As indicated by the results of trapping experiments, the reactions of $^{+\cdot}NH_3$ with thiols result in the formation of thiyl radicals, presumably *via* S–H abstraction. The ready dimerization of the thiyl radicals will result in the *in situ* formation of disulphides[14] which can then react further to give, for example, disulphide-conjugated radicals (as discussed in the next section). Under experimental conditions where the ammoniumyl cation-radical is not completely scavenged by the thiol, the excess of $^{+\cdot}NH_3$ presumably reacts with the products of the $^{+\cdot}NH_3$-thiol reaction (*i.e.* RS· and RSSR). In particular, the detection of oxygenated radicals evidently not formed *via* the disulphide (*e.g.* the sulphonyl radicals from $HO_2CCH_2CH_2SH$ and $HOCH_2CH_2SH$, and $SO_2^{-\cdot}$ and $SO_3^{-\cdot}$) is indicative of a direct reaction between $^{+\cdot}NH_3$ and RS·.

The formation of $RSO_2\cdot$ ($R = HO_2CCH_2CH_2$ or $HOCH_2CH_2$) from the appropriate thiols with $^{+\cdot}NH_3$ has an interesting parallel in the reaction of $Ce^{IV}$ with, for example, $HOCH_2CH_2SH$;[5] the detection of $HOCH_2CH_2SO_2\cdot$ during the latter reaction was rationalized in terms of the oxidation of a first-formed thiyl radical by $Ce^{IV}$ to a sulphenic acid, and thence to the (detectable) sulphinyl radical RSO·, which is then further oxidized by $Ce^{IV}$ to a sulphinic acid and hence $RSO_2\cdot$ (we have previously shown[7] that $^{+\cdot}NH_3$ readily effects the last of these stages). A study of aliphatic sulphonyl radicals has shown that they exist in solution in equilibrium with the corresponding alkyl radical and $SO_2$ [reaction (12)], with the position of equilibrium markedly dependent upon the structure of the alkyl group; the equilibrium lies well over to the left for unsubstituted primary alkyl radicals but for $R = CH_2CO_2H$, for example, dissociation is favoured.[15] Thus if sulphonyl radicals are formed from $^{+\cdot}NH_3$ and the thiols, other than $HO_2CCH_2CH_2SH$ and $HOCH_2CH_2SH$, they would be expected to decompose readily to give $SO_2$ and alkyl radicals (which were detected from cysteine and in the corresponding reactions of HO· with certain substrates).

$$RSO_2\cdot \rightleftharpoons R\cdot + SO_2 \qquad (12)$$

Reaction (12) can also provide the source of $SO_2^{-\cdot}$ and $SO_3^{-\cdot}$. Thus, as has been noted,[8] these radicals are formed by reduction (with $Ti^{III}$) or oxidation (with ·OH, $\cdot NH_2$, or $Ce^{IV}$), respectively, of sulphite ions, which would be derived under our conditions if $SO_2$ is indeed formed [at pH *ca.* 1.5, $SO_2$ and $HOSO_2^-$ are the predominant forms of sulphurous acid[16] ($pK_a$ for '$H_2SO_3$' and $HSO_3^-$ 1.8 and 7.2, respectively[17])]. We confirmed that oxidation of sulphite ($10^{-2}$ mol $dm^{-3}$) under our conditions led to the formation of $SO_3^{-\cdot}$ (*g* 2.0032), *via* reaction (13), and when $Ti^{III}$ alone (9 mmol $dm^{-3}$) was flowed against sodium sulphite, $SO_2^{-\cdot}$, presumably formed *via* reaction (14), was detected.

The fact that $SO_2^{-\cdot}$ is only detected at high thiol concentrations (*i.e.* when $^{+\cdot}NH_3$ is effectively scavenged by the substrate) is consistent with the higher reactivity of sulphite ions with $^{+\cdot}NH_3$ compared with their reactivity towards $Ti^{III}$ (*cf.* ref. 8) while the absence of $SO_3^{-\cdot}$ during the oxidations with ·OH is consistent with a lower reactivity of the latter, compared with $^{+\cdot}NH_3$, towards $HOSO_2^{-\cdot}$. $SO_3^{-\cdot}$ is evidently not detected from

$$NH_3^{+\cdot} + HOSO_2^- \longrightarrow {}^+NH_4 + SO_3^{-\cdot} \qquad (13)$$

$$\begin{array}{ccc} Ti^{III} + SO_2 \longrightarrow & Ti^{IV} + SO_2^{-\cdot} & \qquad (14) \\ (\text{or } HOSO_2^-) & (\text{or } HO^- + SO_2^{-\cdot}) & \end{array}$$

$HO_2CCH_2CH_2SH$ and $HOCH_2CH_2SH$ because the relatively stable sulphonyl radicals from these substrates give rise to a low concentration of $SO_2$, in contrast to the significant desulphonylation of the sulphonyl radicals from $HO_2CCHRSH$ ($R = H$ or Me) and $HO_2CCH(NH_3^+)CH_2SH$ which give $SO_3^{-\cdot}$ (*via* $SO_2$) and, for the latter, a detectable concentration of the corresponding alkyl radical.

The similarity of the behaviour of $Ce^{IV}$ and $^{+\cdot}NH_3$ prompts the proposal of a mechanism for the latter involving one-electron oxidation and hydrogen-abstraction reactions [reactions (15)—(19)]. It is significant that for cysteine and $^+NH_3CH_2CH_2SH$ the *sulphinyl* radical itself is directly detectable [though these radicals could result to some extent *via* pathways involving the corresponding disulphides, since cysteine gives a signal (of reduced intensity) from the sulphinyl radical]. The detection of these radicals even at low thiol concentrations (when $^{+\cdot}NH_3$ is not completely scavenged by the thiol), suggests that these sulphinyl radicals are relatively resistant to further oxidation, possibly as a result of electrostatic repulsion between $^{+\cdot}NH_3$ and the positively charged amino-substituents. The overall scheme shown in reactions (15)—(19) resembles that for ·OH, but with one-electron oxidation of intermediate radicals by $^{+\cdot}NH_3$ rather than 'molecular' oxidation with hydrogen peroxide.*

$$RSH + {}^{+\cdot}NH_3 \longrightarrow RS\cdot + {}^+NH_4 \qquad (15)$$

$$RS\cdot + {}^{+\cdot}NH_3 \xrightarrow{H_2O} RSOH + {}^+NH_4 \qquad (16)$$

$$RSOH + {}^{+\cdot}NH_3 \longrightarrow RSO\cdot + {}^+NH_4 \qquad (17)$$

$$RSO\cdot + {}^{+\cdot}NH_3 \xrightarrow{H_2O} RSO_2H + {}^+NH_4 \qquad (18)$$

$$RSO_2H + {}^{+\cdot}NH_3 \longrightarrow RSO_2\cdot + {}^+NH_4 \qquad (19)$$

(b) *The reaction of $^{+\cdot}NH_3$ with disulphides.* By analogy with the mechanisms proposed for the reactions of $^{+\cdot}NH_3$ with sulphides[7] and for the reactions of ·OH with sulphides[7] and disulphides[4,5] it seems likely that the reaction of $^{+\cdot}NH_3$ with disulphides involves electron transfer (to give $RSSR^{+\cdot}$, which is not detectable by e.s.r. in fluid solution) or addition at sulphur [to give $R\dot{S}(\overset{+}{N}H_3)SR$], or both. The reaction with ·OH involves parallel electron-transfer[4] and addition,[5] to give $RSSR^{+\cdot}$ and $R\dot{S}(OH)SR$, respectively; formation of sulphinyl

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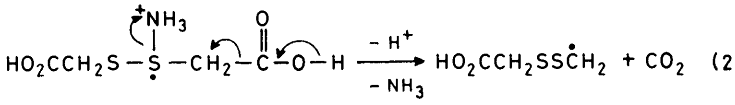
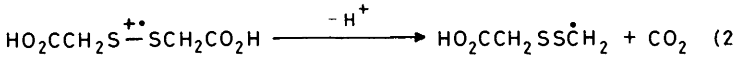
*A contribution from one-electron oxidation by $RSSR^{+\cdot}$ cannot be ruled out (see later).

radicals is thought mainly to involve direct fragmentation of the latter [5] (though fragmentation to give RS· is also likely). Pulse-radiolytic studies [4] of the kinetics of the decay of $RSSR^{+\cdot}$ (R = Me, Et, $Pr^i$, and $Bu^t$) show that this is second order in $[RSSR]^{+\cdot}$ (presumably *via* bimolecular disproportionation) and that hydration is relatively slow; this radical is also shown [4] to be an effective one-electron oxidising agent (*e.g.* with $Fe^{II}$, $k = 1.5 \times 10^{10}$ $dm^3$ $mol^{-1}$ $s^{-1}$). Disulphide-conjugated radicals, *e.g.* $\cdot CH_2SSCH_2CO_2H$ from $HO_2CCH_2SSCH_2CO_2H$, may derive in a variety of ways, including proton-loss from the corresponding radical-cation or concerted loss of $OH^-$, $CO_2$, and $H^+$ from the HO· adduct.[4]

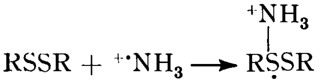


$$HO_2CCH_2S-\underset{\bullet}{\overset{{}^{+}NH_3}{S}}-CH_2-\overset{O}{\overset{\|}{C}}-O-H \xrightarrow[-\,NH_3]{-\,H^+} HO_2CCH_2SS\dot{C}H_2 + CO_2 \qquad (23)$$

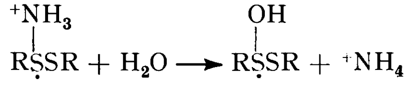


$$HO_2CCH_2\overset{+\bullet}{S}-SCH_2CO_2H \xrightarrow{-\,H^+} HO_2CCH_2SS\dot{C}H_2 + CO_2 \qquad (24)$$

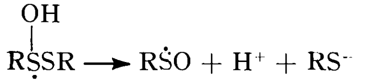
It seems likely that the formation of $HO_2CCH(NH_3^+)CH_2SO\cdot$ during the reaction of ${}^{+\cdot}NH_3$ with the disulphide cystine does not result from the hydration of a first-formed radical-cation (though the latter may be formed) but rather that it derives from hydrolysis of an amino-adduct [reactions (20) and (21)] to give the analogous hydroxy-adduct which then decomposes *via* reaction (22).[4,5]



$$RSSR + {}^{+\cdot}NH_3 \longrightarrow R\underset{\bullet}{\overset{{}^{+}NH_3}{S}}SR \qquad (20)$$



$$R\underset{\bullet}{\overset{{}^{+}NH_3}{S}}SR + H_2O \longrightarrow R\underset{\bullet}{\overset{OH}{S}}SR + {}^{+}NH_4 \qquad (21)$$

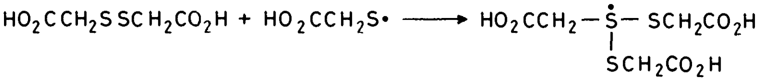


$$R\underset{\bullet}{\overset{OH}{S}}SR \longrightarrow R\dot{S}O + H^+ + RS^- \qquad (22)$$

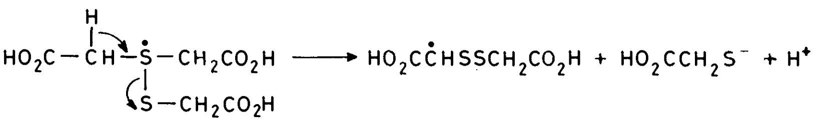
Although the formation of $\cdot CH_2SSCH_2CO_2H$ from reaction of ${}^{+\cdot}NH_3$ with 2,2′-dithiodiethanoic acid could also be rationalized in terms of the mediation of a similar ${}^{+\cdot}NH_3$ adduct [with subsequent fragmentation by loss of $NH_3$, $H^+$, and $CO_2$, reaction (23) (*cf.* the behaviour of the corresponding sulphide [7])], an alternative mechanism involving the radical-cation [reaction (24)] seems likely to contribute to a significant extent. Thus we have found that $Ce^{IV}$ oxidizes this substrate in a flow system under comparable conditions to give signals from $\cdot CH_2SSCH_2CO_2H$; this reaction involves one-electron transfer and indicates that the appropriate radical-cation does indeed decarboxylate (the detection of $SO_3^{-\cdot}$ in low concentrations also suggests that some thiyl radicals are formed, which lead to $SO_3^{-\cdot}$ *via* a series of one-electron oxidations). As in the reactions of 2,2′-dithioethanol and 3,3′-dithiopropanoic acid with ${}^{+\cdot}NH_3$, reaction of these substrates with $Ce^{IV}$ gave no detectable signals: this is thought to reflect, as with 2,2′-dithiodiethanoic acid, the formation of radical-cations which in these cases decay *via* routes which do not lead to detectable radicals (in contrast, oxygenated radicals are produced from ·OH with these substrates, which suggests that adduct-formation, rather than cation-radical formation, is the preferred mode for hydroxyl).

It is interesting to note that although $\cdot CH_2SSCH_2CO_2H$ was the only disulphide-conjugated radical detected from the reaction of ${}^{+\cdot}NH_3$ with the appropriate disulphide at substrate concentrations greater than *ca.* $10^{-4}$ mol $dm^{-3}$, it could not be detected from the corresponding thiol once the concentration exceeded $10^{-3}$ mol $dm^{-3}$; at high thiol concentrations ($>2 \times 10^{-3}$ mol $dm^{-3}$) the alternative disulphide-conjugated radical $\cdot CH(CO_2H)SSCH_2CO_2H$ was detected instead (*cf.* the analogous behaviour of $HSCHMeCO_2H$). We interpret these findings in terms of two different key intermediates in the ${}^{+\cdot}NH_3$ oxidation leading to $\cdot CH_2SSCH_2CO_2H$ and $\cdot CH(CO_2H)SSCH_2CO_2H$, respectively. We believe that the former radical, derived from the cation-radical of the disulphide, undergoes ready ‘ repair ’ in the presence of an excess of thiol [reaction (25)].* The formation of the latter, which involves high thiol concentrations, is believed to involve a thiyl radical adduct of disulphide formed *in situ* [reactions (26) and (27)]. A reversible reaction analogous to reaction (26) has previously been

$$HO_2CCH_2SS\dot{C}H_2 + HSCH_2CO_2H \longrightarrow HO_2CCH_2SSCH_3 + \cdot SCH_2CO_2H \qquad (25)$$



$$HO_2CCH_2SSCH_2CO_2H + HO_2CCH_2S\cdot \longrightarrow HO_2CCH_2-\underset{SCH_2CO_2H}{\underset{|}{\dot{S}}}-SCH_2CO_2H \qquad (26)$$



$$HO_2C-\overset{H}{\overset{|}{C}}H-\underset{S-CH_2CO_2H}{\underset{|}{\dot{S}}}-CH_2CO_2H \longrightarrow HO_2C\dot{C}HSSCH_2CO_2H + HO_2CCH_2S^- + H^+ \qquad (27)$$

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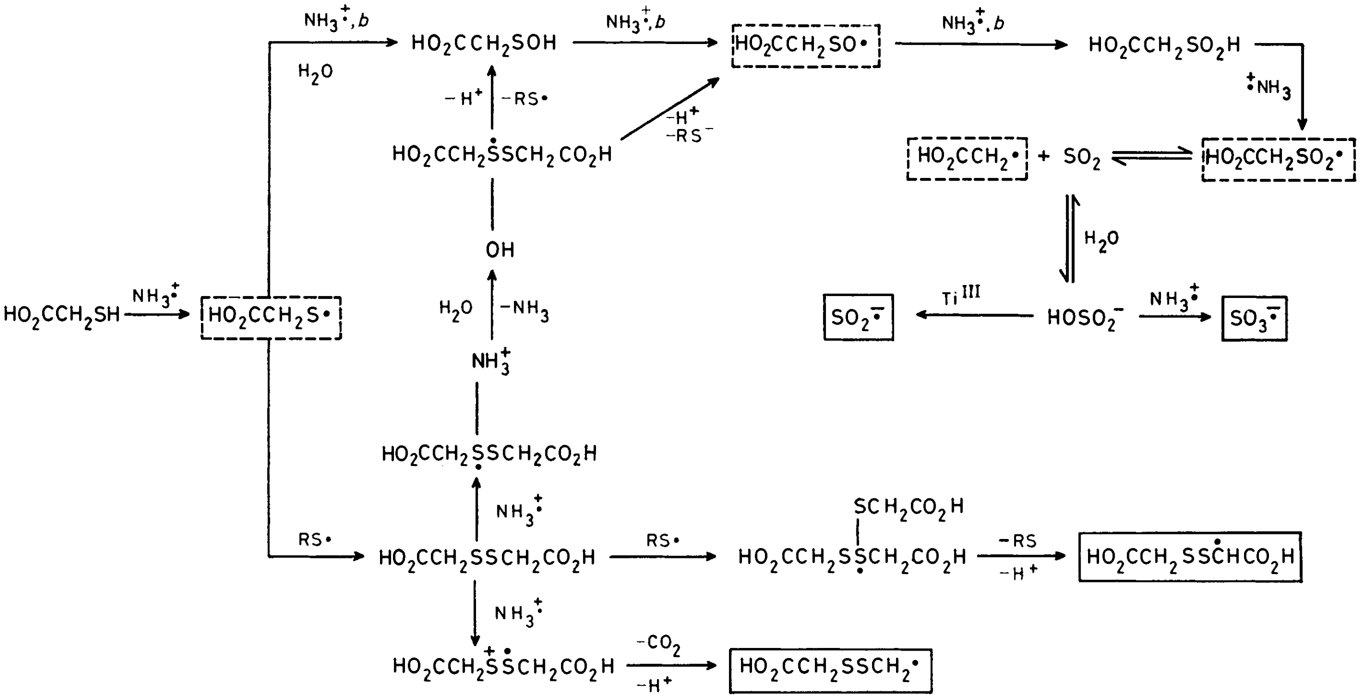
* The obervation that this radical is not detected at higher concentrations of $HO_2CCH_2SSCH_2CO_2H$ in the ·OH reaction may well reflect the scavenging effect (both for ·OH and $\cdot CH_2SSCH_2CO_2H$) of thiol produced by the decomposition of the ·OH adduct to the sulphinyl radical [reaction (22)].

put forward[3b] to account for the high yields of symmetrical disulphides isolated during the radiolysis of mixed disulphides. An analogous mechanism of reaction probably accounts for our observation that $\cdot CH(CO_2H)SSCH_2CO_2H$ is also detected from the reaction of $\cdot OH$ with the appropriate thiol, but not disulphide.

*Summary.*—As we have found for the oxidation of thiols with $^{+\cdot}NH_3$, the reactions of disulphides with $^{+\cdot}NH_3$ bear some resemblance to the reactions of these substrates with the hydroxyl radical. One-electron transfer reactions appear to play a more important role for $^{+\cdot}NH_3$: these include the oxidation of radicals (*e.g.* $RSO\cdot$ or $RS\cdot$) and of reaction products (*e.g.* $RSO_2H$ or $HOSO_2^-$) as well as of the parent disulphides (where adduct formation also plays an important part, to an extent depending on the structure of the attached alkyl groups). Radical-cations formed in reaction of $^{+\cdot}NH_3$ with disulphides may well themselves be instrumental in effecting one-electron oxidation of, *e.g.* $RS\cdot$, $RSO\cdot$, and $RSO_2H$, *etc.*



SCHEME Major reactions in the $^{+\cdot}NH_3$–RSH and $^{+\cdot}NH_3$–RSSR systems exemplified for $R = CH_2CO_2H$[a]

[a] □ Denotes radicals directly detected in this system ($R = CH_2CO_2H$); ⬚ Denotes radical trapped (by butenedioic acid) or radical-type detected for other alkyl groups R. [b] $RSSR^{+\cdot}$ may also be an oxidant in reactions thus labelled (see text).

Our findings are summarized in the Scheme, where the key processes have been illustrated for one specific thiol-disulphide pair, namely $HO_2CCH_2SH$ and $HO_2CCH_2SSCH_2CO_2H$.

EXPERIMENTAL

The e.s.r. spectrometer, details of spectrum measurement, simulation, and calibration, and the flow system have been described previously.[7] In the studies of the $^{+\cdot}NH_3$ system, stream (i) contained 12.5% titanium(III) chloride solution (8.3 cm$^3$ dm$^{-3}$, 6.7 mmol dm$^{-3}$), stream (ii) contained hydroxylamine hydrochloride (40 g dm$^{-3}$, *ca.* 0.6 mol dm$^{-3}$), and the organic substrate was contained in stream (iii) (for concentrations employed, see text). All solutions were prepared from deoxygenated water and purged with nitrogen during use; in addition streams (i) and (ii) contained sufficient sulphuric acid to ensure that the combined solution had pH *ca.* 1.5. The hydroxyl radical was generated in the same system, in which stream (i) contained titanium(III) chloride (6.7 mmol dm$^{-3}$) and concentrated sulphuric acid, stream (ii) contained 100-volume hydrogen peroxide (2.5 cm$^3$ dm$^{-3}$, 0.022 mol dm$^{-3}$) and sulphuric acid, with the substrate in stream (iii). Reactions of cerium(IV) were investigated using a two-way flow system in which stream (i) contained cerium(IV) ammonium nitrate (6.7 g dm$^{-3}$, 0.012 mol dm$^{-3}$) and stream (ii) contained the substrate; both streams were acidified with hydrochloric acid to give pH *ca.* 1.5.

Titanium(III) chloride (12.5% w/v) was obtained from Fisons Ltd. and both hydroxylamine hydrochloride and hydrogen peroxide (100-volume) were supplied by B.D.H. Ltd. All substrates were commercial samples which were used without further purification except for the following which were distilled before use: 2-mercaptoethanoic acid, b.p. 106—110 °C at *ca.* 20 mmHg (lit.,[18] 107—108 °C at 16 mmHg), 2-mercaptopropanoic acid, b.p. 103—105 °C at *ca.* 20 mmHg (lit.,[19] 95—100 °C at 16 mmHg), 3-mercaptopropanoic acid, b.p. 113—114 °C at *ca.* 14 mmHg (lit.,[19] 114—115.5 °C at 13 mmHg), and 2-mercaptoethanol, b.p. 56—58 °C at *ca.* 14 mmHg (lit.,[20] 55 °C at 13 mmHg).

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