

Electron Spin Resonance Studies. Part XLVI.¹ Oxidation of Thiols and Disulphides in Aqueous Solution: Formation of $RS\cdot$, $RSO\cdot$, $RSO_2\cdot$, $RSSR\cdot$, and Carbon Radicals

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Radicals which mediate in the oxidation of thiols with the $Ti^{III}-H_2O_2$ couple and Ce^{IV} and of disulphides with the former reagent, in aqueous solution at room temperature, have been studied by e.s.r. spectroscopy. The e.s.r. evidence establishes that signals with g ca. 2.01 and 2.005 are those of sulphinyl ($RSO\cdot$) and sulphonyl ($RSO_2\cdot$) radicals, respectively. Radicals of both these types are detected in almost all instances; thiyl radicals ($RS\cdot$) cannot be detected directly but their involvement is demonstrated by spin-trapping methods; and two dithiols yield spectra with g ca. 2.013 which are attributed to cyclic disulphide radical-anions. Reaction mechanisms consistent with the e.s.r. data and earlier product studies are suggested.

THE effectiveness of thiols and disulphides as radio-protectors² and of these compounds and their derivatives as anti-oxidants³ has prompted us to investigate their free-radical chemistries by e.s.r. spectroscopy.

The oxidation of both groups of compounds in aqueous solution has previously been studied by the e.s.r. technique with two types of oxidant. Armstrong and Humphreys, employing the titanium(III)-hydrogen peroxide rapid-flow system, reported broad-line spectra during the reactions of cysteine, cystine, homocystine, and penicillamine which they attributed to thiyl ($RS\cdot$) radicals,⁴ and similar results have been obtained with cysteamine and cystamine.⁵ Wolf and his co-workers, using cerium(IV) as oxidant, have reported the spectra of a variety of radicals from thiols; they have assigned some to thiyl radicals and others (from mercaptoalcohols) to alkoxy radicals.⁶⁻⁹ However, these assignments have been questioned;^{10,11} thus, there are strong grounds¹² for believing that $RO\cdot$ and $RS\cdot$ radicals will not be detectable by e.s.r. spectroscopy in fluid solution, owing to line-broadening arising from a marked anisotropy of the g -tensor, and the g -factors quoted for the supposed $RS\cdot$ radicals,⁶ 2.0106, do not accord with the results of other studies.¹¹ There is firmer evidence for two other types of radical, $>\dot{C}-S^-$ and $>\dot{C}-SSR$, from the oxidation of some sulphur-containing compounds at high pH.¹³

We have investigated the oxidation of thiols and disulphides with the titanium(III)-peroxide couple, and of thiols with cerium(IV), in order to obtain more clear cut evidence about the structure of the radicals which mediate in the oxidations and to elucidate some of the primary steps in the course of oxidations initiated by the hydroxyl radical. Reactions were carried out by mixing aqueous solutions of the thiol or disulphide and either titanium(III) chloride and hydrogen peroxide or cerium(IV) ammonium nitrate ca. 0.02 s before the combined

solution flowed through the cavity of the e.s.r. spectrometer.

RESULTS AND DISCUSSION

Radical Identification.—The e.s.r. parameters of radicals detected during the oxidation of thiols by the titanium(III)-peroxide couple and by cerium(IV) are in Tables 1 and 2, respectively, and those of radicals detected during the oxidation of disulphides with the former system are in Table 3. The assignments of spectra to specific structures are justified in the sequel.

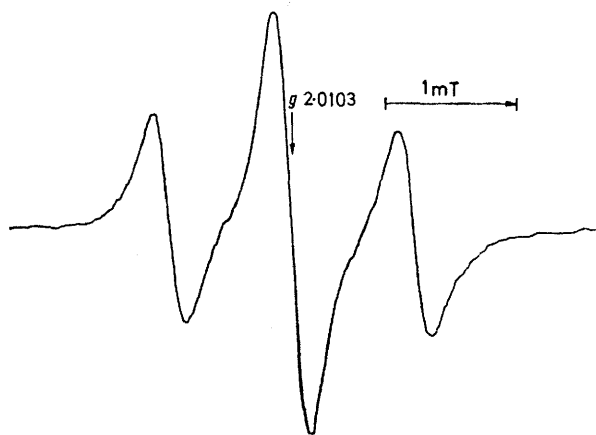


FIGURE 1 E.s.r. spectrum of the radical $HO_2C-CH(NH_3^+)\cdot CH_2SO\cdot$ from cysteine

Alkanesulphinyl Radicals $RSO\cdot$.—One group of radicals in Tables 1—3 is characterised by spectra with g -factors in the range ca. 2.010—2.011, proton splittings of ca. 1 mT, and broad lines (linewidth ca. 0.35 mT); an example is in Figure 1. Some of these spectra have been observed before and ascribed to thiyl radicals;⁴⁻⁹ however, we believe, for the following reasons, that they are correctly attributed to radicals of the type $RSO\cdot$.

⁷ J. C. Kertesz, W. Wolf, and H. Hayase, *J. Magnetic Resonance*, 1973, **10**, 22.

⁸ W. Wolf and J. C. Kertesz, *Intra-Sci. Chem. Reports*, 1969, **3**, 273.

⁹ J. C. Kertesz and W. Wolf, *Intra-Sci. Chem. Reports*, 1971, **5**, 371.

¹⁰ B. C. Gilbert and R. C. Sealy, in 'Electron Spin Resonance,' Chem. Soc. Special Publ., 1974, vol. 2, 260.

¹¹ A. J. Elliot and F. C. Adam, *Canad. J. Chem.*, 1974, **52**, 102.

¹² M. C. R. Symons, *J. Amer. Chem. Soc.*, 1969, **91**, 5924.

¹³ P. Neta and R. W. Fessenden, *J. Phys. Chem.*, 1971, **75**, 2277.

¹ Part XLV, B. C. Gilbert, R. O. C. Norman, G. Placucci, and R. C. Sealy, *J.C.S. Perkin II*, preceding paper.

² Z. M. Bacq, 'Chemical Protection against Ionizing Radiation,' Thomas, Springfield, 1965; A. Hollaender and D. G. Doherty, 'Radiation Damage and Sulphydryl Compounds,' International Atomic Energy Agency, Vienna, 1969.

³ K. U. Ingold, *Chem. Rev.*, 1961, **61**, 563.

⁴ W. A. Armstrong and W. G. Humphreys, *Canad. J. Chem.*, 1967, **45**, 2589.

⁵ C. Nicolau and H. Dertinger, *Radiation Res.*, 1970, **42**, 62.

⁶ W. Wolf, J. C. Kertesz, and W. C. Landgraf, *J. Magnetic Resonance*, 1969, **1**, 618.

TABLE 1

E.s.r. parameters for radicals detected during the oxidation of thiols with the $Ti^{III}-H_2O_2$ couple at pH 1-2

Thiol	Radical	Splitting constants (mT) ^a		g
		α -H	β -H	
EtSH	MeCH ₂ SO ₂ ·	0.095 (2H)	0.195 (3H)	2.0050
Bu ^t SH	Me ₃ CSO ₂ ·		0.26 (9H) ^b	2.0053
HOCH ₂ CH ₂ SH	HOCH ₂ CH ₂ SO ₂ ·		0.39 (2H)	2.0050
HO ₂ CCH ₂ SH	HO ₂ CCH ₂ SO·	1.07 (2H)		2.0108
	HO ₂ CCH ₂ SSCH ₂ CO ₂ H	1.55 (1H)		2.0054
	HO ₂ CCH ₂ ·	2.14 (2H)		2.0032
HO ₂ CCH ₂ CH ₂ SH	HO ₂ CCH ₂ CH ₂ SO·	1.07 (2H)		2.0107
	HO ₂ CCH ₂ CH ₂ SO ₂ ·	0.125 (2H)	0.25 (2H)	2.0047
	MeCH(CO ₂ H)SO·	0.78 (1H)		2.0103
MeCH(CO ₂ H)SH	MeC(CO ₂ H)SSCHMeCO ₂ H		1.72 (3H) ^b	2.0053
	MeCHCO ₂ H	2.01 (1H)	2.49 (3H)	2.0033
	HO ₂ CCH ₂ CH(CO ₂ H)SO·	0.86 (1H)		2.0100
HO ₂ CCH ₂ CH(CO ₂ H)SH	HO ₂ CCH ₂ CH(CO ₂ H)SO ₂ ·		0.3 (2H) ^c	2.0050
	HO ₂ CCH ₂ CHCO ₂ H	2.09 (1H)	2.22 (2H)	2.0034
	HO ₂ CCH(NH ₃ ⁺)CH ₂ SO·	0.93 (2H)		2.0103
HO ₂ CCH(NH ₃ ⁺)CH ₂ SH	HO ₂ CCH(NH ₃ ⁺)CH ₂ SO ₂ ·		0.21 (2H)	2.0048
	EtOCH ₂ CH ₂ SH	EtOCH ₂ CH ₂ SO·	0.91 (2H)	2.0109
MeO ₂ CCH ₂ SH	EtOCH ₂ CH ₂ SO ₂ ·		0.38 (2H)	2.0050
	MeO ₂ CCH ₂ SO·			2.0099
HSCH ₂ CH ₂ SH	MeO ₂ CCH ₂ ·	2.15 (2H)		2.0032
	HSCH ₂ CH ₂ SO ₂ ·		0.155 (3H) ^e	2.0032
HS(CH ₂) ₃ SH			0.26 (2H)	2.0052
	SCH ₂ CH ₂ CH ₂ S· ^f	0.68 (4H)	0.10 (2H)	2.0132
HS(CH ₂) ₄ SH	HS(CH ₂) ₄ SO ₂ ·	0.075 (2H)	0.23 (2H)	2.0050
	S(CH ₂) ₄ S· ^f	0.63 (4H)		2.0133
H ₃ N ⁺ CH ₂ CH ₂ SH	H ₃ N ⁺ CH ₂ CH ₂ SO·	0.96 (2H)		2.0106
	H ₃ N ⁺ CH ₂ CH ₂ SO ₂ ·		0.26 (2H)	2.0049

α and β refer to protons on the trivalent carbon, or the carbon adjacent to sulphur in sulphur-centred radicals, and the next carbon atom, respectively. ^b The outermost lines were lost in the noise. ^c Very weak spectrum; assignment tentative. ^d Splittings not measurable owing to overlap with signals from the other radical. ^e Splitting from methyl protons. ^f pH 7.

TABLE 2

E.s.r. parameters for radicals detected during the oxidation of thiols with Ce^{IV} at pH 1.5

Thiol	Radical	Splitting constants (mT) *		g
		α -H	β -H	
HOCH ₂ CH ₂ SH	HOCH ₂ CH ₂ SO·	0.95 (2H)		2.0109
HO ₂ CCH ₂ SH	HOCH ₂ CH ₂ SO ₂ ·		0.39 (2H)	2.0050
	HO ₂ CCH ₂ SO·	1.07 (2H)		2.0108

* See footnote *a* in Table 1.

TABLE 3

E.s.r. parameters for radicals detected during the oxidation of disulphides with the $Ti^{III}-H_2O_2$ couple at pH 1-2

Disulphide	Radical	Splitting constants (mT) ^a		g
		α -H	β -H	
(MeS) ₂	MeSO ₂ ·	0.08 (3H)		2.0049
(EtS) ₂	MeCH ₂ SO ₂ ·	0.095 (2H)	0.195 (3H)	2.0050
	HOCH ₂ CH ₂ SO·	0.95 (2H)		2.0109
(HOCH ₂ CH ₂ S) ₂	HOCH ₂ CH ₂ SO ₂ ·		0.39 (2H)	2.0050
	HO ₂ CCH ₂ SO·	1.07 (2H)		2.0109 ^c
(HO ₂ CCH ₂ S) ₂	HO ₂ CCH ₂ SSCH ₂ CO ₂ H	1.55 (1H)		2.0054
	HO ₂ CCH ₂ ·	2.14 (2H)		2.0032
	-O ₂ CCH ₂ SO· ^b	0.95 (2H)		2.0110 ^c
	-O ₂ CCH ₂ SSCH ₂ CO ₂ · ^b	1.61 (1H)		2.0056
(HO ₂ CCH ₂ CH ₂ S) ₂	HO ₂ CCH ₂ CH ₂ SO·	1.07 (2H)		2.0107
[HO ₂ CCH(NH ₃ ⁺)CH ₂ S] ₂	HO ₂ CCH(NH ₃ ⁺)CH ₂ SO·	0.93 (2H)		2.0103
HO ₂ CCH(NH ₃ ⁺)CH ₂ SSMe	HO ₂ CCH(NH ₃ ⁺)CH ₂ SO·	0.93 (2H)		2.0103
	MeSO ₂ ·	0.08 (3H)		2.0049

^a See footnote *a* in Table 1. ^b pH 7. ^c ± 0.0003 .

The *g*-factors are in the region previously reported for alkanesulphinyl radicals in solution ^{14,15} and in the solid state,¹⁶ and the broad lines are consistent with data for such radicals in solution at low temperature ^{14,15} and with

¹⁴ T. Kawamura, P. J. Krusic, and J. K. Kochi, *Tetrahedron Letters*, 1972, 4075.

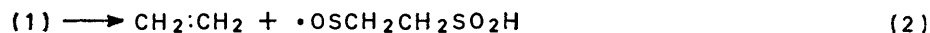
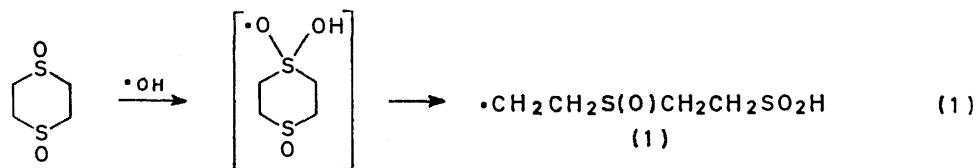
the *g*-factor anisotropy reported for MeSO· in solids.¹⁶ (Hyperfine splittings, which evidently arise from the protons on the carbon adjacent to sulphur, are considerably

¹⁵ J. A. Howard and E. Furimsky, *Canad. J. Chem.*, 1974, **52**, 555.

¹⁶ K. Nishikida and F. Williams, *J. Amer. Chem. Soc.*, 1974, **96**, 4781.

greater than those for some members of the series in cyclopropane at low temperature,¹⁴ but this is not unexpected since the splittings are known to increase with increasing temperature.¹⁵

Further evidence in support of our view that radicals with these characteristics are of the alkanesulphonyl type was obtained from the reaction of 1,4-dithian 1,4-dioxide with the $Ti^{III}-H_2O_2$ couple. This gave a spectrum with $a(2H)$ 0.95 mT, g 2.0105, which we assign to the radical $HO_2S(CH_2)_2SO\cdot$, the formation of which is consistent with the characteristic reaction of sulphoxides with the hydroxyl radical,^{17,18} which would give the radical (1) in this instance, followed by the fragmentation (2). The radical $\cdot CH_2CH_2OH$, evidently formed by reaction of hydroxyl with the ethylene formed in reaction (2), was also detected.



We were unable to detect spectra attributable to sulphonyl radicals during the oxidation of the disulphides $(MeS)_2$ and $(EtS)_2$ or the thiols $EtSH$ and Bu^tSH , although such species would have been expected by analogy with the behaviour of almost all the other thiols and disulphides which were studied. However, we believe, for two reasons, that this does not necessarily imply a low or zero rate of formation of these radicals; rather, they may be significant intermediates but undetectable by esr spectroscopy in aqueous solution at ambient temperature. First, oxidation of the disulphoxide $MeS(O)CH_2CH_2S(O)Me$ with the $Ti^{III}-H_2O_2$ couple gave the spectrum of the radical $\cdot CH_2CH_2OH$ but not one attributable to $MeSO\cdot$, although its formation was anticipated by analogy with reactions (1) and (2) and from the detection of the radical $\cdot CH_2CH_2OH$.¹⁹ Secondly, the spectra of the sulphonyl radicals which have been observed have unusually large linewidths which may reflect the operation of efficient relaxation through spin-rotation interactions (*cf.*, *e.g.*, the structurally related $Bu^tO_2\cdot$ ²⁰); if so, then the sulphonyl radicals we have been unable to detect may have been present but, because of their small size and lack of hydrogen-bonding capacity for solvent molecules, may have had excessively broad lines and correspondingly negligible amplitudes on account of their small rotational correlation times.

It should be noted that the methylene protons in the radical $HO_2C\cdot CH(NH_3^+)CH_2SO\cdot$, being bonded to a car-

¹⁷ B. C. Gilbert, R. O. C. Norman, and R. C. Sealy, *J.C.S. Perkin II*, 1975, 308.

¹⁸ B. C. Gilbert, R. O. C. Norman, and R. C. Sealy, *J.C.S. Perkin II*, 1975, 303.

¹⁹ P. M. Carton, B. C. Gilbert, H. A. H. Laue, R. O. C. Norman, and R. C. Sealy, to be published.

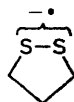
²⁰ J. R. Thomas, *J. Amer. Chem. Soc.*, 1966, **88**, 2064.

bon which is adjacent to chiral carbon, are magnetically inequivalent and might be expected to give different splittings.²¹ We were not able to resolve individual proton splittings, although this has been achieved for several radicals^{7,9} which are now known to be members of this class.

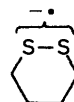
Alkanesulphonyl Radicals $RSO_2\cdot$.—Spectra with g ca. 2.005 detected during the oxidation of 2-mercaptoalcohols have been previously ascribed to alkoxy radicals (*e.g.* $HS\cdot CH_2CH_2O\cdot$ from 2-mercaptoethanol),⁷ on the basis, at least in part, of the occurrence of larger splittings from protons attached to the carbon atom adjacent to oxygen than from those on the carbon adjacent to sulphur. However, we believe that the signals are properly assigned to alkanesulphonyl radicals, $RSO_2\cdot$. Thus, not only have we detected species of this type from thiols and

disulphides which do not possess a hydroxy-substituent, but also they have the g -factors (2.0045—2.0055) and hyperfine splittings [note especially that $a(\beta-H) > a(\alpha-H)$] which are characteristic of alkanesulphonyl radicals prepared independently, by addition of substituted alkyl radicals to sulphur dioxide¹⁷ or *via* sulphinic acids;^{17,18,22} moreover, where comparison can be made, our data are in agreement, within experimental error, with those reported for radicals prepared by these methods in aqueous solution.¹⁷

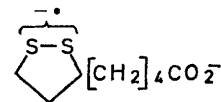
Disulphide Radical-anions.—Two dithiols, on oxidation with the $Ti^{III}-H_2O_2$ couple at $pH > ca. 5$, gave spectra with g -factors and hyperfine splittings similar to those reported for some disulphide radical anions in the solid state.^{23,24} The fact that such spectra were obtained from dithiols where the probability of intramolecular reaction is high, and not from either monothiols or from ethane-1,2-dithiol (which would yield a strained four-membered ring) leads us to ascribe the spectra to the cyclic radical-anions (2) and (3) from propane-1,3- and butane-1,4-dithiol, respectively.



(2)



(3)



(4)

²¹ B. C. Gilbert, J. P. Larkin, and R. O. C. Norman, *J.C.S. Perkin II*, 1972, 1272.

²² A. G. Davies, B. P. Roberts, and B. R. Sanderson, *J.C.S. Perkin II*, 1973, 626.

²³ H. C. Box, H. G. Freund, and G. W. Frank, *J. Chem. Phys.*, 1968, **48**, 3825.

²⁴ K. Akasaka, S.-I. Ohnishi, T. Suita, and I. Nitta, *J. Chem. Phys.*, 1964, **40**, 3110.

Support for these assignments was obtained as follows. The optical spectrum of a transient with λ_{max} 410 nm has been observed during a pulse radiolysis study in which the radical-anion $\cdot\text{CO}_2^-$ (an efficient one-electron reducing agent) was generated in the presence of the anion of lipoic acid; the absorption has been assigned to species (4).²⁵ When we generated $\cdot\text{CO}_2^-$ in the flow system from the $\text{Ti}^{\text{III}}-\text{H}_2\text{O}_2$ couple with formic acid in the presence of lipoic acid at pH 7, we obtained an e.s.r. spectrum (Figure 2) with $a(1\text{H})$ 0.78, $a(2\text{H})$ 0.435, $a(2\text{H})$ 0.145 mT, g 2.0129, which we ascribe to (4). The g -factor is similar to those of the species believed to be (2) and (3) and the hyperfine splitting is of the same order of magnitude; differences in the splitting constants between (2) and (4) doubtless arise from the perturbing effect of the substituent in the latter on the charge- and spin-distribution between the two sulphur atoms, and on the preferred conformation.

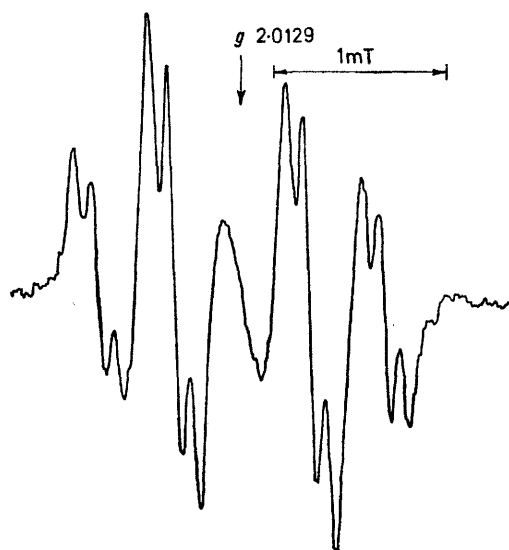


FIGURE 2 E.s.r. spectrum of the radical-anion (4) from lipoate

Carbon-centred radicals.—The following radicals were identified from literature data for their hyperfine splittings and g -factors: $\cdot\text{CH}_2\text{CO}_2\text{H}$, $\cdot\text{CHMeCO}_2\text{H}$, $\cdot\text{CH}(\text{CO}_2\text{H})\text{CH}_2\text{CO}_2\text{H}$,²⁶ and $\cdot\text{CH}_2\text{CO}_2\text{Me}$.²⁷ Assignments of spectra to the radicals $\cdot\text{CH}(\text{CO}_2\text{H})\text{SSCH}_2\text{CO}_2\text{H}$, $\cdot\text{CH}(\text{CO}_2^-)\text{SSCH}_2\text{CO}_2^-$, and $\cdot\text{CMe}(\text{CO}_2\text{H})\text{SSCHMeCO}_2\text{H}$ were based on the relationship of their hyperfine splittings and g -factors to those for radicals containing a substituent $-\text{SSR}$ at the tervalent carbon,²⁸ taking into account the spin-delocalising²⁹ and g -raising³⁰ effect of a carboxy-group attached to that carbon. However, our

²⁵ R. L. Willson, *Chem. Comm.*, 1970, 1425.

²⁶ A. L. J. Beckwith and R. O. C. Norman, *J. Chem. Soc. (B)*, 1969, 400.

²⁷ P. Smith, J. T. Pearson, P. B. Wood, and T. C. Smith, *J. Chem. Phys.*, 1965, **43**, 1535.

²⁸ J. Q. Adams, *J. Amer. Chem. Soc.*, 1970, **92**, 4535.

²⁹ A. J. Dobbs, B. C. Gilbert, and R. O. C. Norman, *J.C.S. Perkin II*, 1972, 786.

³⁰ A. J. Dobbs, in ref. 10, p. 281.

³¹ F. g. M. Z. Hoffman and E. Hayon, *J. Phys. Chem.*, 1973, **77**, 990.

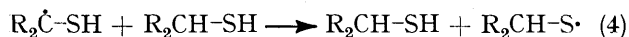
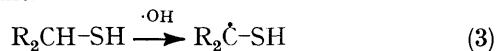
data for the radical $\cdot\text{CH}(\text{CO}_2^-)\text{SSCH}_2\text{CO}_2^-$ are in poor agreement with those previously reported, although it should be noted that the spectrum assignment in that case was tentative.¹³

Mode of Oxidation of Thiols.—(a) *With the titanium(III)-peroxide couple.* The first problem in the elucidation of the course of oxidation of thiols initiated by the $\text{Ti}^{\text{III}}-\text{H}_2\text{O}_2$ couple is to determine whether or not the chemistry is at least partly that of disulphides (formed from thiols by a molecular reaction with hydrogen peroxide) rather than that of thiols themselves.

To test for this possibility, we treated several thiols with hydrogen peroxide for up to 30 min at room temperature before admixture with titanium(III). In no case were the relative radical concentrations detected by e.s.r. spectroscopy significantly different from those observed under the conditions of Table I, whereas the corresponding disulphides give markedly different relative concentrations. We infer that no significant oxidation of the thiol to the disulphide by hydrogen peroxide takes place between mixing and observation under the conditions of Table I.

The major reaction of the hydroxyl radical with thiols is the formation of thiyl radicals.³¹ As we indicated earlier, we do not expect to be able to detect such radicals by e.s.r. spectroscopy in fluid solution owing to the excessively broad lines anticipated in their spectra. However, oxidation of thioglycolic acid, in the presence of maleic acid in acid solution, gave, apart from the spectrum of the hydroxyl radical adduct $[\cdot\text{CH}(\text{CO}_2\text{H})\cdot\text{CH}(\text{OH})\text{CO}_2\text{H}]$,^{32,33} only the spectrum of the radical³³ $\cdot\text{CH}(\text{CO}_2\text{H})\cdot\text{CH}(\text{CO}_2\text{H})\cdot\text{SCH}_2\text{CO}_2\text{H}$ [$a(1\text{H})$ 1.99, $a(1\text{H})$ 0.89, $a(2\text{H})$ 0.105 mT, g 2.0031], thus implying mediation of the thiyl radical $\text{HO}_2\text{C}\cdot\text{CH}_2\text{S}\cdot$.

A minor route in the oxidation of thiols by hydroxyl is the abstraction (3).^{13,34} By analogy with radicals of the type $\cdot\text{CR}_2\text{SR}'$, we should have expected to detect the species $\cdot\text{CR}_2\text{SH}$ by e.s.r. spectroscopy and infer that they did not achieve a detectable steady-state concentration in the e.s.r. cavity. This could be associated with the rapidity of the 'repair' reaction (4); such reactions generally have k ca. 10^8 l mol⁻¹ s⁻¹. If so, the detection of radicals of the type $\cdot\text{CR}_2\text{S}^-$ at high pH¹³ may be due at least in part to a much lower rate constant for the corresponding repair reaction; this would be reasonable given the possession of negative charge by each reactant.



The radicals detected from monothiols were principally

³² H. Fischer, *Z. Naturforsch.*, 1964, **19a**, 866.

³³ T. Kawamura, M. Ushio, T. Fujimoto, and T. Yonezawa, *J. Amer. Chem. Soc.*, 1971, **93**, 908.

³⁴ W. Karmann, A. Granzow, G. Meissner, and A. Henglein, *Internat. J. Radiation Phys. Chem.*, 1969, **1**, 395.

³⁵ B. C. Gilbert, J. P. Larkin, and R. O. C. Norman, *J.C.S. Perkin II*, 1973, 272.

³⁶ G. E. Adams, M. S. McNaughton, and B. D. Michael, *Trans. Faraday Soc.*, 1968, **64**, 902; G. Nucifora, B. Smaller, R. Remko, and E. C. Avery, *Radiation Res.*, 1972, **49**, 96.

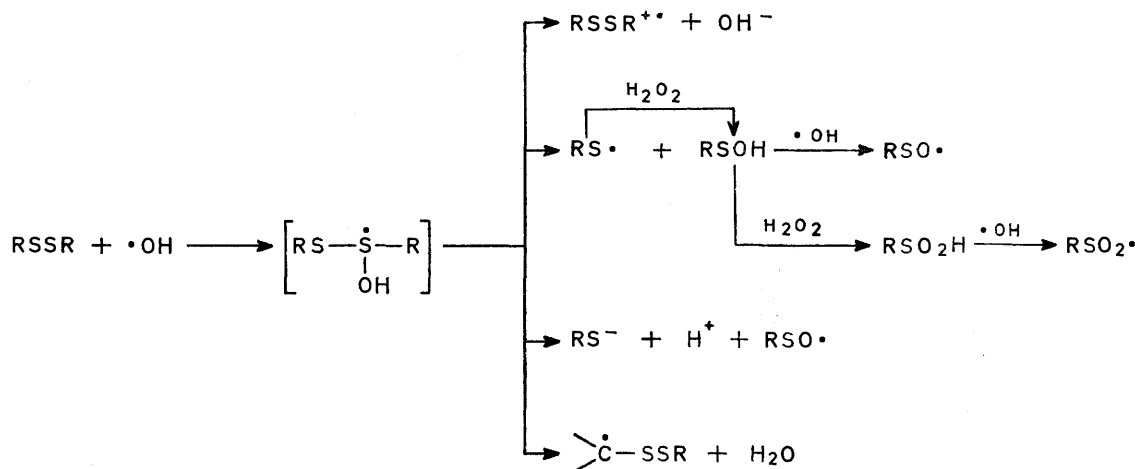
of two types, namely $\text{RSO}\cdot$ and $\text{RSO}_2\cdot$. In only two instances were radicals detected which clearly owe their origin to disulphides, namely the sulphur-conjugated carbon-centred radicals from thioglycolic acid and 2-thiopropionic acid; these are likely to be formed *via* dimerisation of thiyl radicals (formed by radical oxidation) and further oxidation as discussed later.

In three cases, the absence of a sulphonyl radical $\text{RSO}_2\cdot$ from RSH was accompanied by the appearance of a spectrum of the radical $\text{R}\cdot$ (thioglycolic acid and its methyl ester, and 2-thiopropionic acid). We have previously presented evidence that the radical $\text{HO}_2\text{C}\cdot\text{CH}_2\cdot\text{SO}_2\cdot$ readily loses sulphur dioxide at room temperature,¹⁷ and we infer that under our conditions the sulphonyl radicals are indeed formed but fragment as in reaction (5; $\text{R}, \text{R}' = \text{H}$ or Me).



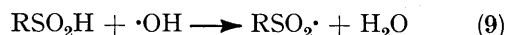
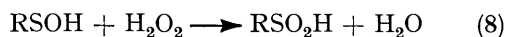
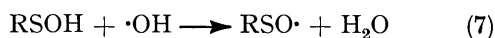
The formation of sulphinyl and sulphonyl radicals in detectable concentration implies that a number of oxidative steps must occur rapidly following the fast generation

of thiyl radicals; moreover, the first such step must be sufficiently fast to compete efficiently with the essentially diffusion-controlled dimerisation³¹ of thiyl radicals. Since titanium(IV) is a relatively poor oxidant, hydrogen peroxide is the likely molecular oxidant for some of these steps. Thus, reaction (6) followed by (7) would yield sulphinyl radicals, and, followed by reactions (8) and (9), sulphonyl radicals.



SCHEME 1

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Although reaction (6) seems not to have been previously documented, there is evidence for the corresponding displacement on di-*t*-butyl peroxide; *t*-butyl alkanesulphen-

³⁷ N. Kharasch, S. J. Potempa, and H. L. Wehrmeister, *Chem. Rev.*, 1946, **39**, 269.

³⁸ P. Koelewijn and H. Berger, *Rec. Trav. chim.*, 1972, **91**, 1275.

ates are apparently formed as intermediates during the photolysis of mixtures of this peroxide either with an alkanethiol or with a dialkyl disulphide and propan-2-ol¹⁴ [*e.g.* $\text{Bu}^t\text{O}\cdot + \text{RSH} \longrightarrow \text{RS}\cdot$; $\text{RS}\cdot + (\text{Bu}^t\text{O})_2 \longrightarrow \text{RS}\cdot\text{O}(\text{Bu}^t) + \text{Bu}^t\text{O}\cdot$]. To compete effectively with the dimerisation of thiyl radicals, reaction (6) must have a rate constant greater than *ca.* $10^5 \text{ l mol}^{-1} \text{ s}^{-1}$ $\{[\text{H}_2\text{O}_2]\}$ at the mixing point = 70mm, rate of radical initiation (in the cavity) *ca.* $10^{-3} \text{ mol l}^{-1} \text{ s}^{-1}$, $2k[\text{RS}\cdot + \text{RS}\cdot]$ *ca.*³¹ $10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$].

The oxidation of sulphenic acids is known to be a rapid process,³⁷ and possibly hydrogen peroxide acts as an oxidant as in reaction (8). Sulphenic acids are reported to be particularly efficient radical scavengers,³⁸ so that reaction (7) is to be expected. It is also known that sulphinic acids yield sulphonyl radicals readily^{17,18} [reaction (9)], and in both this instance and reaction (7) any carbon-centred radicals present could presumably also effect hydrogen-atom abstraction.

Our suggested mechanism for the oxidation of thiols by the $\text{Ti}^{\text{III}}\text{-H}_2\text{O}_2$ couple is summarised in Scheme 1. It is

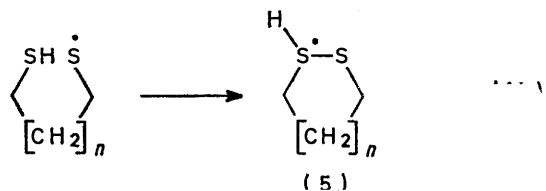
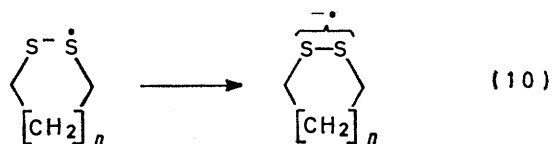
consistent with the following additional observations. First, sulphonyl radicals were not detected above *ca.* pH 3, consistent with their formation by abstraction of a hydrogen atom from RSO_2H and the reduced ease of abstraction of an electron from RSO_2^- (sulphinic acids have ³⁹ $\text{p}K_a$ *ca.* 2.5). Secondly, whereas thioglycolic acid gave a spectrum of the sulphinyl radical $\text{HO}_2\text{C}\cdot\text{CH}_2\cdot\text{SO}\cdot$ at pH up to *ca.* 5 but not above, the corresponding disulphide, under otherwise identical conditions, gave the same sulphinyl radical over the pH range 1–7. This suggests that the major proportion of the sulphinyl radicals detected during oxidation of the thiol does not arise by way of the disulphide (their possible origin when the disulphide is substrate is discussed later); it is likely that they are formed mainly from the hydroxyl radical and RSOH and that this is a faster reaction than that of hydroxyl with RSO^- ($\text{p}K_a$ values for sulphenic acids have not been measured; they are presumably weaker

³⁹ F. Wudl, D. A. Lightner, and D. J. Cram, *J. Amer. Chem. Soc.*, 1967, **89**, 4099.

than sulphinic acids, and it has been suggested⁴⁰ that they have $pK_a > ca. 6$). Thirdly, for a given thiol, the observed concentrations of both the sulphinyl and sulphonyl radicals increased with increase in $[H_2O_2]$ but the latter the more so, consistent with the extra requirement for peroxide as oxidant in this case.

Dithiols.—The fact that butane-1,4-dithiol yields the cyclic disulphide radical-anion (3) in basic solution but the sulphonyl radical $HS[CH_2]_4SO_2\cdot$ in acid suggests that reaction (10; $n = 2$) is considerably faster than (11; $n = 2$), allowing more effective competition for the thiyl radical from hydrogen peroxide in the latter case. This would be consistent with data for related intermolecular reactions; thus, the reactions $RS\cdot + RS^- \rightarrow RSSR^-$ have large rate constants,^{34,41,42} whereas $RS\cdot + RSH \rightarrow RSS(H)R\cdot$ is, for penicillamine, much slower.³¹

In contrast, propane-1,3-dithiol, although yielding the radical-anion (2) in neutral or basic medium, gave no detectable spectrum at $pH < 4$. We believe that this is because reaction (11; $n = 1$) is fast enough in this case



essentially to prevent reaction of thiyl radical with hydrogen peroxide (which leads ultimately to the sulphonyl radical), and that the radical (5; $n = 1$) is not detectable because its e.s.r. resonances are excessively broad; this would be expected owing to an anticipated marked anisotropy in its g -factor.⁴³ Evidence in accord with this view was obtained by monitoring the concentration of the radical-anion (2) as a function of pH (Figure 3); this varied in a manner consistent with the radical-anion being in equilibrium with its (undetectable) conjugate acid, the latter having $pK_a 6.05$ (*cf.* $pK_a 5.85$ for the related species from lipoic acid⁴¹), with an essentially constant rate of formation of the precursor thiyl radical in this pH-range, and with proton-transfer being, as we should expect (*cf.* ref. 1), faster than radical-termination processes.

(b) *With cerium(IV).* There is strong evidence that cerium(IV) reacts rapidly with thiols (k ca. $10^3 \text{ l mol}^{-1} \text{ s}^{-1}$) to form thiyl radicals which, at least in the absence of a high concentration of the oxidant, dimerise. The resultant disulphides can be further oxidised if an excess of cerium(IV) is present, but this is a much slower reaction (half-life, several hours).⁴⁴

⁴⁰ T. C. Bruice and B. T. Markiw, *J. Amer. Chem. Soc.*, 1957, **79**, 3150.

⁴¹ M. Z. Hoffman and E. Hayon, *J. Amer. Chem. Soc.*, 1972, **94**, 7950.

We re-examined the oxidation of 2-mercaptoethanol and thioglycolic acid with cerium(IV) ammonium nitrate and obtained essentially the same spectra as those

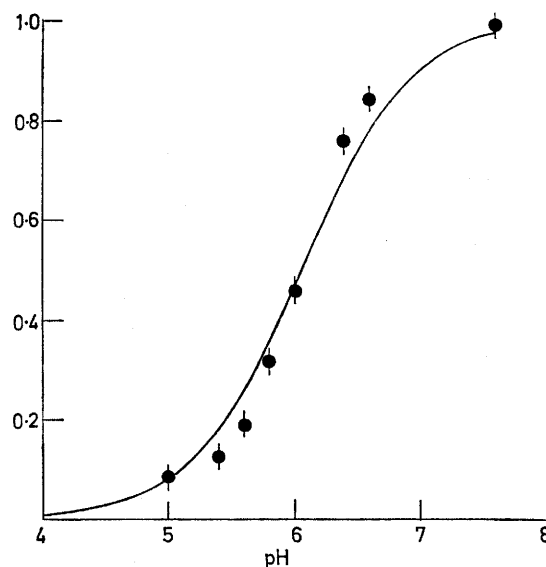
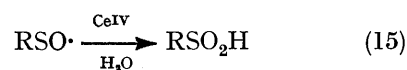
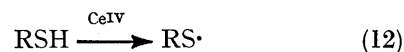


FIGURE 3 Concentration (●) of the radical-anion (2) (arbitrary units) as a function of pH. The continuous curve represents the expected dependence if the conjugate acid has $pK_a 6.05$

reported previously.^{6,7} However, as we have outlined above, the spectra originally attributed to $RS\cdot$ and $RO\cdot$ radicals are correctly assigned to $RSO\cdot$ and $RSO_2\cdot$ radicals, respectively (Table 2).

When the corresponding disulphides were oxidised under the same conditions, no radicals could be detected, from which we infer that both the $RSO\cdot$ and $RSO_2\cdot$ radicals formed from thiols with cerium(IV) are derived from thiyl radicals. In accord with this, when 2-mercaptoethanol was oxidised with cerium(IV) in the presence of maleic acid, the spectra of the sulphinyl and sulphonyl radicals were quenched and the spectrum observed [$a(1H) 1.98$, $a(1H) 0.85$, $a(2H) 0.14$ mT, $g 2.0031$] was attributable to the adduct $\cdot CH(CO_2H)CH(CO_2H)\cdot S(CH_2)_2OH$ formed from the thiyl radical $HO(CH_2)_2S\cdot$. We therefore suggest that reactions (12)—(16) are significant in the cerium(IV) oxidation of thiols.



⁴² G. E. Adams, M. S. McNaughton, and B. D. Michael, in 'The Chemistry of Ionization and Excitation,' eds. G. R. A. Johnson and G. Scholes, Taylor and Francis, London, 1967, p. 281.

⁴³ M. C. R. Symons, *J.C.S. Perkin II*, 1974, 1618.

⁴⁴ J. Hill and A. McAuley, *J. Chem. Soc. (A)*, 1968, 156.

It was notable that, compared with oxidation by the $Ti^{III}-H_2O_2$ couple, cerium(IV) gave higher concentrations of the sulphinyl radical relative to the corresponding sulphonyl radical; for example, 2-mercaptoethanol gave the sulphinyl as well as the sulphonyl radical with cerium(IV) but only the latter with the redox couple, and thioglycolic acid gave only the sulphinyl radical with cerium(IV) but both radicals with the redox couple. This is compatible with the suggested reactions (12)–(16) if, for example, the formation of RSO_2H from $RSO\cdot$ and cerium(IV) is relatively slow compared with its formation from $RSOH$ and hydrogen peroxide in a molecular reaction.

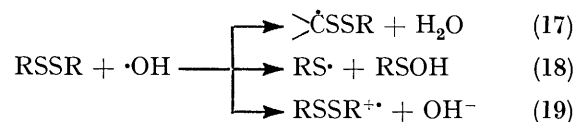
Mode of Oxidation of Disulphides with the $Ti^{III}-H_2O_2$ Couple.—Since the formation of the majority of radicals detected during the oxidation of disulphides involves S–S bond fission, the possibility was considered that thiols are formed by reduction of the disulphides by titanium(III) before the advent of radical reactions. However, when disulphides and titanium(III) were allowed to stand for up to 30 min before admixture with hydrogen peroxide, no changes in the e.s.r. spectra were detected; for example, 3,3'-dithiodipropionic acid still gave only the sulphinyl radical $HO_2C[CH_2]_2SO\cdot$, whereas the corresponding thiol gives both this and the sulphonyl radical. We infer that, in the time between mixing and observation for the experiments reported in Table 3, there is no significant reduction of disulphides by titanium(III).

Likewise, the pre-mixing of a disulphide with hydrogen peroxide before admixture with titanium(III) did not result in changes in the e.s.r. spectra observed, from which we infer that the radicals reported in Table 3 do not originate from products of the molecular oxidation of disulphides by hydrogen peroxide. (However, more drastic pre-mixing treatment, in which the disulphide was heated at 60° with 30% hydrogen peroxide, resulted in significant changes in the e.s.r. spectra; possibly these are associated with the formation of thiol-sulphinates and -sulphonates.)

Three types of interaction between disulphides and the hydroxyl radical have been suggested [reactions (17)–(19)].⁴⁵ Of the three types of radical which are thought to result, we detected, directly, carbon-centred radicals in low concentration from dithiodiglycolic acid and its anion and, indirectly, the thiyl radical $\cdot SCH_2CO_2^-$ from the latter, by trapping it with the nitromethane *aci*-anion³⁵ to give the radical-anion $^-O_2C\cdot CH_2SCH_2NO_2^-$ [$a(N) 2.41$, $a(2H) 0.595$, $a(2H) 0.08$ mT, $g 2.0056$]. However, the inclusion of maleic acid in the oxidation of dithiodiglycolic acid [under conditions in which, in the absence of maleic acid, $HO_2C\cdot CH_2\cdot SO\cdot$ and $\cdot CH(CO_2H)SSCH_2CO_2H$ were observed] did not give the spectrum of the adduct from the corresponding thiyl radical, whereas the same concentration of maleic acid in the oxidation of thioglycolic acid gave a strong spectrum of this adduct; we infer that the proportion of hydroxyl radicals which undergo reaction (18) is small. This attempted trapping experiment yielded a further result in that the spectrum

⁴⁵ H. Möckel, M. Bonifačić, and K.-D. Asmus, *J. Phys. Chem.*, 1974, **78**, 282.

of the sulphinyl radical, as well as that of the carbon-centred radical, was not quenched; this not only suggests that $RSO\cdot$ radicals are too stable to add to unsaturated carbon (*cf.* our earlier evidence that radicals of the type $RSO-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}$ fragment to $RSO\cdot$) but also shows that $RSO\cdot$ radicals from the oxidation of disulphides are not formed from thiyl radicals (*cf.* the quenching of the spectrum of $HO_2C\cdot CH_2SO\cdot$ when thioglycolic acid is oxidised in the presence of maleic acid as a trap for the thiyl radical).



We were not able to detect the spectrum of a radical-cation $RSSR^{+\cdot}$ in our experiments, although the formation of such species has been clearly demonstrated by pulse radiolysis-conductivity studies;⁴⁵ for example, there is good evidence for the formation of $MeSSMe^{+\cdot}$ from dimethyl disulphide and the hydroxyl radical with *ca.* 40% efficiency based on hydroxyl. There are two possible explanations for our failure to detect such a species. One is that *g*-factor anisotropy causes excessive broadening in the resonances so that their amplitudes are too low for detection; a simple calculation based on *g*-anisotropies of radicals detected in solids which are thought to be disulphide radical-cations^{23,46,47} is consistent with this. Secondly, under our conditions, these species, if formed, may not reach a detectable concentration owing to a very rapid one-electron reduction by titanium(III) to give the parent compound [*cf.* the evidence for their rapid reduction by hexacyanoferrate(II)⁴⁵].

It is unlikely that the formation of sulphinyl radicals in relatively high concentrations can be attributed to the oxidation of the sulphenic acids formed as in reaction (18), for the extent of formation of these acids between mixing of the reagents and e.s.r. observation is probably very small and in any case the oxidation of sulphenic to sulphenic acids by hydrogen peroxide is, we have inferred, sufficiently rapid for the observed concentration of the sulphinyl radical to be greater than that of the sulphinyl radical. Further, as we showed earlier, the sulphinyl radical is formed from dithiodiglycolic acid at a pH at which it is not detectable from the corresponding thiol, consistent with its formation from other than a sulphenic acid in the former case. We suggest the occurrence of reaction (20).

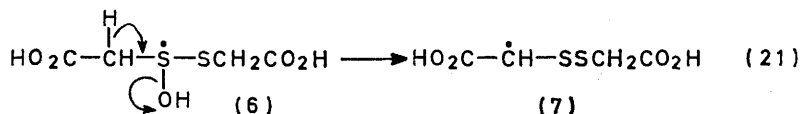


Given the formation of RSH and $RSOH$, it is likely that the sulphonyl radicals are formed by the reactions we have suggested in the case of thiol oxidation. As there, the radical $\cdot CH_2CO_2H$ from dithiodiglycolic acid is doubtless formed by fragmentation of the radical $HO_2C\cdot CH_2-$

⁴⁶ H. C. Box and H. G. Freund, *J. Chem. Phys.*, 1964, **41**, 2571.

⁴⁷ K. Akasaka, S. Kominami, and H. Hatano, *J. Phys. Chem.*, 1971, **75**, 3746.

SO₂•. Finally, the disulphide-conjugated, carbon-centred radicals from dithiodiglycolic acid or its conjugate base may be formed from the hydroxyl radical adduct, *e.g.* (6), by the elimination of water; the absence of analogous species from other disulphides could reflect the effect of the carboxy-group, which is conjugated to the radical centre, either in increasing the rate of formation of the species [*cf.* reaction (21)] or in reducing its rate of termination.

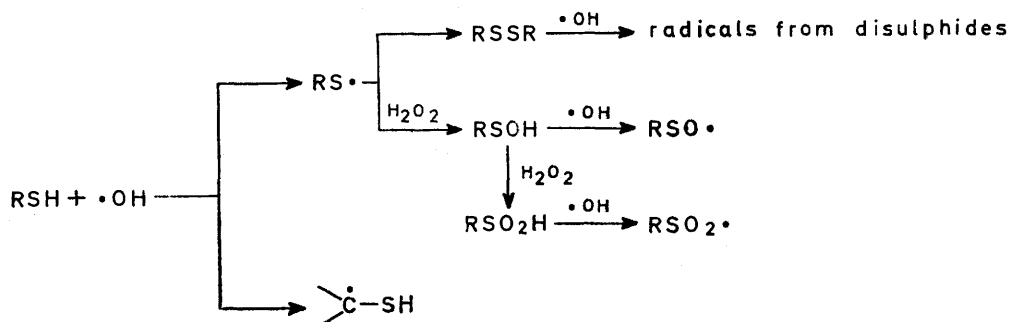


Our suggested mechanisms are summarised in Scheme 2. In summary, our interpretation of the e.s.r. data from the oxidation of thiols and disulphides, studied here and previously, resolves apparent inconsistencies both in

for detection, owing to *g*-factor anisotropy;¹¹ they could be formed as in reaction (24), which has an analogy in the generation of RSS• from the phenyl radical and di-*t*-butyl disulphide⁵³ and would be consistent with the formation of the appropriate unsaturated compound, R(-H), from penicillamine disulphide;⁵⁴ and their expected coupling with RS• or RSO• [reaction (25)] could lead to trisulphides, in the latter case by reactions analogous to those proposed for thiosulphonates.

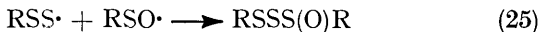
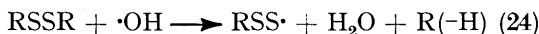
EXPERIMENTAL

Materials.—Diethyl disulphide was prepared from ethane-thiol by the general procedure of McAllan *et al.*⁵⁵ 3-Alanyl methyl disulphide was prepared by⁵² treating aqueous



SCHEME 2

earlier radical assignments and in mechanisms which were based on them. Further, our conclusions can be correlated with the products of radiolysis of deaerated neutral aqueous solutions of disulphides, namely,^{48,49} sulphinic acids, thiols, trisulphides, and traces of sulphonic acids. Thus, the radicals RSO• should dimerise, by way of RS(O)OSR, to give⁵⁰ thiosulphonates, RS(O₂)SR; a product of this type has been detected from the γ -radiolysis of cystine at low pH,⁵¹ but hydrolysis, especially above *ca.* pH 5, is known to yield sulphinic acids⁴⁸ [reaction (22)]. The formation of thiols could be accounted for by reaction (20); these also could lead to sulphinic acids by reaction (23) with thiosulphonates.⁵²



The formation of trisulphides may involve perthiyl radicals, RSS•, which probably have e.s.r. lines too broad

⁴⁸ J. W. Purdie, *J. Amer. Chem. Soc.*, 1967, **89**, 226.

⁴⁹ J. W. Purdie, *Canad. J. Chem.*, 1969, **47**, 1029, 1037.

⁵⁰ J. L. Kice, in 'Free Radicals,' vol. 2, ed. J. K. Kochi, Wiley, New York, 1973, p. 728.

⁵¹ D. W. Grant, S. N. Mason, and M. A. Link, *Nature*, 1961, **193**, 352.

⁵² F. Ostermayer and D. S. Tarbell, *J. Amer. Chem. Soc.*, 1960, **82**, 3752.

dimethyl thiosulphonate⁵⁶ with aqueous cysteine hydrochloride at pH 6. The disulphoxides of 1,4-dithian and 1,2-(bismethylthio)ethane were obtained following the method of Bell and Bennett;⁵⁷ the former was used without separation of the *cis*- and *trans*-isomers. All other materials were obtained commercially.

Methods.—E.s.r. spectra were measured on a Varian E-3 spectrometer with an X-band klystron and 100 kHz modulation. Splitting constants were determined to within ± 0.01 mT, *g*-factors (except where specified) to within ± 0.0001 , by comparison with Fremy's salt [*a*(N) 1.3091 mT,⁵⁸ *g* 2.0055⁵⁹]; in some cases *g*-factors were measured relative to one of the singlets (*g* 2.0132⁶⁰) from the Ti^{III}-H₂O₂ couple. pH Measurements were made to within ± 0.1 unit with a Beckman model 72 pH meter. Mixing chambers which allowed simultaneous mixing of either two or three reactant solutions *ca.* 0.02 s before passage into the spectrometer cavity were employed.

⁵³ G. W. Byers, H. Gruen, H. G. Giles, H. N. Schott, and J. A. Kampmeier, *J. Amer. Chem. Soc.*, 1972, **94**, 1016.

⁵⁴ J. W. Purdie, H. A. Gillis, and N. V. Klassen, *Canad. J. Chem.*, 1973, **51**, 3132.

⁵⁵ D. T. McAllan, T. V. Cullum, R. A. Dean, and F. A. Fidler, *J. Amer. Chem. Soc.*, 1951, **73**, 3627.

⁵⁶ H. J. Backer, *Rec. Trav. chim.*, 1948, **67**, 894.

⁵⁷ E. V. Bell and G. M. Bennett, *J. Chem. Soc.*, 1927, 1798.

⁵⁸ R. J. Faber and G. K. Fraenkel, *J. Chem. Phys.*, 1967, **47**, 2462.

⁵⁹ J. Q. Adams, S. W. Nicksic, and J. R. Thomas, *J. Chem. Phys.*, 1966, **45**, 654.

⁶⁰ R. O. C. Norman and B. C. Gilbert, *Adv. Phys. Org. Chem.*, 1967, **5**, 53.

Reactions with the $\text{Ti}^{\text{III}}\text{-H}_2\text{O}_2$ couple were carried out with the three-stream mixing chamber. One stream contained titanium(III) chloride (0.01—0.025M), the second contained hydrogen peroxide (0.05—0.25M), and the third contained the organic substrate (0.01—0.04M, or saturated if the solubility was low). For reactions at pH 1—2, each solution was acidified with concentrated sulphuric acid (usually 5 ml in 2.5 l); for those at pH > 2.5, EDTA (2—6 g l⁻¹) was added to the titanium(III) solution and the pH of this solution was adjusted with ammonia so that the pH after mixing had the required value. When more accurate control of the pH was required, a citric acid—disodium hydrogen orthophosphate buffer⁶¹ was used as solvent for the titanium(III) chloride. For the spin-trapping experiments with the *aci*-anion of nitromethane, 0.02M-titanium(III) chloride and the solution

containing the organic substrate and 0.04M-nitromethane were each adjusted to pH 9 with ammonia.

Reactions with cerium(IV) were carried out in the two-stream mixing chamber. One solution contained cerium(IV) ammonium nitrate (0.02—0.05M) and the second contained the organic substrate (0.02M); each was adjusted to *ca.* pH 1.5 with concentrated sulphuric acid.

We thank the S.R.C. for their support; the award of an I.C.I. Fellowship (to R. C. S.) is gratefully acknowledged.

[4/2605 Received, 13th December, 1974]

⁶¹ 'Data for Biochemical Research,' eds. R. M. C. Dawson, D. C. Elliott, W. H. Elliott, and K. M. Jones, Oxford University Press, Oxford, 1969.