Electron Spin Resonance Studies. Part 64.[1] The Hydroxyl Radical-induced Decarboxylation of Methionine and Some Related Compounds

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Spin-trapping e.s.r. experiments employing both $MeNO_2$ (in conjunction with generation of $\cdot OH$ from the Ti^{III}–H_2O_2 couple in a flow system) and Bu^tNO (in conjunction with the photolytic decomposition of H_2O_2) confirm that reaction of $\cdot OH$ with methionine, S-methylcysteine, and some related compounds effects oxidative decarboxylation. It is proposed that the reaction proceeds *via* the sequential formation of an hydroxyl adduct at sulphur, a sulphur-centred radical-cation, and a (cyclic) sulphuranyl radical in which the carboxylate function becomes bonded to sulphur.

It has been shown that in some biological systems methionine [$MeSCH_2CH_2CH(CO_2^-)NH_3^+$] is degraded enzymatically to give ultimately ethene,[2] which is an important growth regulator. Labelling studies indicate that ethene is derived from carbon atoms C(3) and C(4) in the parent.[2] Evidence has been presented [3] that an oxygen species (possibly radical in nature) is responsible for at least the initial stages of the oxidation; one of the key steps is thought to be the conversion of methionine into methional ($MeSCH_2CH_2CHO$) and ammonia, followed by loss of the formyl group (as CO_2) and the methyl-thio-group.

Several *in vitro* studies of the oxidation of methionine with $\cdot OH$ have been undertaken, but no clear reaction mechanism has emerged and, to some extent at least, conflicting claims have been made. For example, flow-system e.s.r. studies, with $\cdot OH$ generated by reaction (1), have demonstrated that the sulphur-conjugated radicals $\cdot CH(SMe)CH_2CH(CO_2^-)NH_3^+$ and $\cdot CH_2SCH_2CH_2CH(CO_2^-)NH_3^+$ are formed.[4] On the other hand, spin-trapping experiments with 2-methyl-2-nitrosopropane (Bu^tNO) led to the detection of signals assigned [5] to adducts from $\cdot CH_2CH_2CH(CO_2^-)NH_3^+$ and either $\cdot CH(SMe)CH_2CH(CO_2^-)NH_3^+$ or $\cdot CH(CH_2SMe)CH(CO_2^-)NH_3^+$; although similar spectra were obtained in analogous γ-irradiation experiments, analysis with the aid of h.p.l.c. suggested that both $Me\cdot$ and the deaminated radical $\cdot CH(CO_2^-)CH_2CH_2SMe$ are formed.[6]

Pulse radiolysis experiments indicate [7] that initial attack by $\cdot OH$ is largely at sulphur (*ca.* 80%), with some direct reaction at the hydrogens on the carbon atoms adjacent to sulphur (*ca.* 20%). The $\cdot OH$-adduct (1) is thought to react to give a sulphur-centred radical-cation (2) which at low pH reacts to give the 'dimer' radical-cation (3) (*cf.* refs. 8 and 9); at pH > *ca.* 2, however, it is proposed that the radical-cation induces decarboxylation to give the α-aminoalkyl radical (5) *via* initial electron-transfer to give an aminium radical-cation (4), with subsequent decarboxylation (see Scheme 1).

The aims of the present work were several-fold: first, to employ e.s.r. spectroscopy (with spin traps, where appropriate) to identify the radicals involved in the reaction between $\cdot OH$ and methionine as a function of pH; secondly, to investigate the possibility that intramolecular electron-transfer from the sulphur radical-cation involves the carboxylate group rather than the amino-group (since the reaction is reported [7] to occur at a pH well below the pK_a of methionine,[10] 9.27); thirdly, to establish whether or not analogous *intermolecular* electron transfer involving sulphur radical-cations as oxidants with amino-acid substrates could be identified.

Results and Discussion

Most of the experiments were carried out with a three-way continuous flow system in which streams of Ti^{III}, H_2O_2, and

$$Ti^{III} + H_2O_2 \longrightarrow Ti^{IV} + \cdot OH + OH^- \quad (1)$$
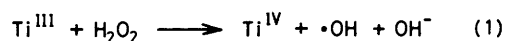
the substrate were mixed *ca.* 50 ms before passage through the cavity of an e.s.r. spectrometer. In experiments above pH *ca.* 2.5, edta was used to sequester the titanium; in some experiments above pH 8.5, nitromethane was also added (to the third stream) in order to generate the *aci*-anion $CH_2=NO_2^-$ which has proved a useful spin-trap for short-lived free radicals whose presence has been otherwise undetectable.[11,12] Spin-trapping studies of the reactions of $\cdot OH$ (generated photolytically) were also carried out by *in situ* photolysis over a range of pH values of aqueous solutions containing the substrate, hydrogen peroxide, and Bu^tNO.

(a) *Methionine.*—When methionine (*ca.* 0.02 mol dm^{-3}) was oxidised with $\cdot OH$ in the flow system at pH values in the range 1.5—9, only signals from the two sulphur-conjugated radicals $\cdot CH_2SCH_2CH_2CH(CO_2^-)NH_3^+$ and $\cdot CH(SMe)CH_2CH(CO_2^-)NH_3^+$ could be detected (see Table for the e.s.r. parameters). However, it is perhaps not surprising that there was no trace of either the dimer radical-cation (3) or the α-aminoalkyl radical (5) whose presence would have been anticipated at higher pH values on the basis of Scheme 1, for individual resonances of the former would probably be weak (in view of the expected [9] multiplicity of the lines and, not least, the possibility of its reduction by Ti^{III}), and, for the latter, electron-transfer (oxidation) processes could serve to lower the concentration of the radical to below the detectable limit (see later).

Reaction between methionine and $\cdot OH$ in the presence of nitromethane (and its *aci*-anion) at pH *ca.* 9.5 led to the detection of three radicals, in addition to traces of [11,12] $HOCH_2NO_2^-$, namely $MeNO_2^-$ [with a(N) 2.57, a(3 H) 1.20 mT, g 2.0051], present in high concentration, and weak signals from adducts identified as $MeSCH_2NO_2^-$ [with a(N) 2.42, a(2 H) 0.605, a(3 H) 0.075 mT, g 2.0056; see ref. 12] and $O_2NCH_2CH_2NO_2^-$. In contrast, only very weak signals from $MeNO_2^-$ were obtained in the absence of methionine or when certain other model substrates were included (see later), so that we interpret its formation in terms of the production of a precursor radical which is a good electron-donor. This we believe to be the α-aminoalkyl radical $\cdot CH(NH_2)CH_2CH_2SMe$ (5) (*cf.* the analogous behaviour [13] of α-aminoalkyl radicals generated from the reaction of N-alkylhydroxylamines with Ti^{III}), and further evidence for this view is provided by results described subsequently.
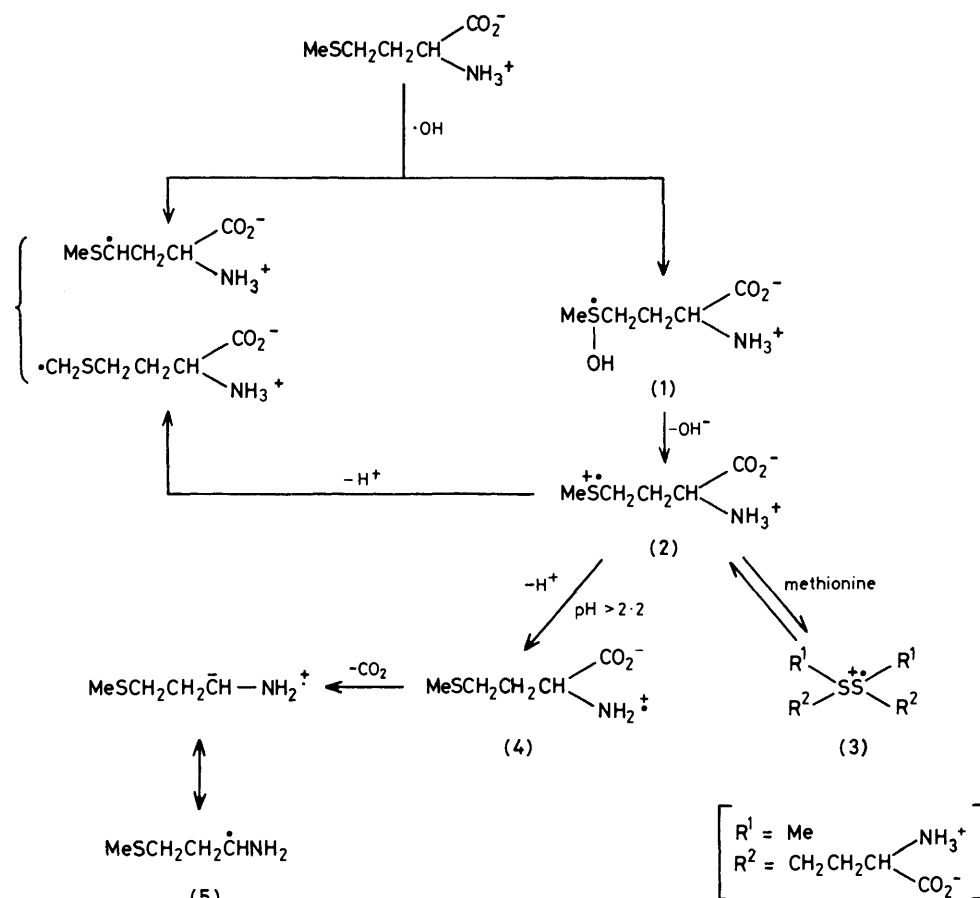
In experiments with $\cdot OH$ and either Me_2S or Et_2S in the presence of $MeNO_2$ at pH *ca.* 9.5, strong signals were observed from $O_2NCH_2CH_2NO_2^-$ and $HOCH_2NO_2^-$, to-

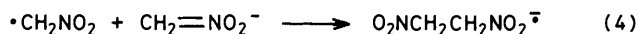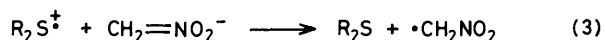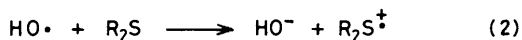E.s.r. parameters of radicals detected in the reactions of ·OH with methionine and related compounds

Substrate	Trap[a]	Radical	Hyperfine splittings (mT)[b]				g[c]
			a(N)	a(α-H)	a(β-H)	a(other)	
MeSCH₂CH₂CH(CO₂⁻)NH₃⁺	MeNO₂	·CH₂SCH₂CH₂CH(CO₂⁻)NH₃⁺				0.21(2, γ-H)	2.0048
		·CH(SMe)CH₂CH(CO₂⁻)NH₃⁺				0.29(3, γ-H)	2.0046
		MeNO₂⁻·	2.57(1)	1.64(2)	2.98[d]		2.0051
		MeSCH₂NO₂⁻·	2.42(1)	1.68(1)	1.20(3)	0.075(3, δ-H)	2.0056
		O₂NCH₂CH₂NO₂⁻·	2.55(1)		0.605(2) / 1.12(2)	0.05(2, γ-H)	2.0050
	Bu'NO pH 2.5—4.5	MeSCH₂CH₂CHNBu'(O·)(NH₃⁺) (6)	1.455(1)		0.145(1)	{0.290(β-N), 0.035(2, γ-H)}	2.0058
	Bu'NO pH >4.5	Bu'NHO·	1.445(1)	1.40(1)			2.0059
		MeSCH₂CH₂CHNBu'(O·)(NH₂) (7)	1.60(1)		0.14(1)	{0.14(β-N), 0.065(2, NH₂)}	2.0058
MeS(CH₂)₃NH₂	MeNO₂	·CH₂SCH₂CH₂CH₂NH₃⁺		1.63(2)	2.97[d]	0.21(2, γ-H)	2.0048
		·CH(SMe)CH₂CH₂NH₃⁺		1.68(1)	1.88(3)	0.28(3, γ-H)	2.0046
EtS(CH₂)₃CO₂H		·CHMeS(CH₂)₃CO₂⁻		1.65(1)	1.50(2)	0.17(2, γ-H)	2.0045
		·CH(SEt)CH₂CH₂CO₂⁻		1.71(1)		0.17(2, γ-H)	2.0044
MeSCH₂CH(CO₂⁻)NH₃⁺		·CH₂SCH₂CH(CO₂⁻)NH₃⁺		1.64(2)		0.19(2, γ-H)	2.0048
	MeNO₂	MeNO₂⁻·, HOCH₂NO₂⁻·	2.42(1)		0.605(2)	0.075(3, δ-H)	2.0056
		MeSCH₂NO₂⁻·					
		Bu'NHO·					
	Bu'NO pH 2.5—4.5	MeSCH₂CH₂CHNBu'(O·)(NH₃⁺) (9)	1.45(1)		0.21(1)	0.21(β-N)	2.0058
	Bu'NO pH >4.5	Bu'NHO·					
MeSCH₂CH₂CH(CO₂⁻)OH	MeNO₂	·CH₂SCH₂CH₂CH(CO₂⁻)OH				0.205(2, γ-H)	2.0048
		·CH(SMe)CH₂CH(CO₂⁻)OH				0.25(3, γ-H)	2.0046
		MeNO₂⁻·, HOCH₂NO₂⁻·	2.50(1)	1.665(2)	3.05[d]		2.0050
		MeSCH₂CHCH₂NO₂⁻· (12)		1.675(1)	{1.13(1), 0.965(1)} / 0.85(2)[e]	0.055(1, γ-H)	2.0050[e]
	Bu'NO	MeSCH₂CH₂CH(OH)—N(O·)Bu' (13)	{2.435(1)[e], 1.555(1)}		0.155(1)	0.025(1, OH)	2.0058

[a] Experiments with MeNO₂ were conducted in a flow system at pH ca. 9.5; Bu'NO was employed in conjunction with photolytic decomposition of H₂O₂ (see text). [b] ±0.005. [c] ±0.0001. [d] Sum of the two β-proton splittings. [e] See text.

$$MeSCH_2CH_2CH{\overset{CO_2^-}{\underset{NH_3^+}{\big\langle}}}$$

$\downarrow$ ·OH

$$Me\overset{\cdot}{S}CHCH_2CH{\overset{CO_2^-}{\underset{NH_3^+}{\big\langle}}}$$

$$\cdot CH_2SCH_2CH_2CH{\overset{CO_2^-}{\underset{NH_3^+}{\big\langle}}}$$

$$Me\overset{\cdot}{S}CH_2CH_2CH{\overset{CO_2^-}{\underset{NH_3^+}{\big\langle}}}$$
$$\underset{OH}{|}$$

(1)

$\downarrow$ -OH⁻

$$Me\overset{+\cdot}{S}CH_2CH_2CH{\overset{CO_2^-}{\underset{NH_3^+}{\big\langle}}}$$

(2)

$\xleftarrow{-H^+}$

$\overset{-H^+}{\underset{pH > 2\cdot 2}{\searrow}}$

methionine

$$MeSCH_2CH_2\overset{\cdot}{C}H{\overset{CO_2^-}{\underset{NH_2^+}{\big\langle}}}$$

(4)

$$R^1{\overset{+\cdot}{\underset{R^2}{S}}}{\overset{R^1}{\underset{R^2}{S}}}$$

(3)

$$MeSCH_2CH_2\overset{-}{C}H-NH_2^{+\cdot} \xleftarrow{-CO_2}$$

$\updownarrow$

$$MeSCH_2CH_2\overset{\cdot}{C}HNH_2$$

(5)

$$\begin{bmatrix} R^1 = Me \\ R^2 = CH_2CH_2CH{\overset{NH_3^+}{\underset{CO_2^-}{\big\langle}}} \end{bmatrix}$$

Scheme 1.

$$HO\cdot + R_2S \longrightarrow HO^- + R_2\overset{+\cdot}{S} \qquad (2)$$

$$R_2\overset{+\cdot}{S} + CH_2{=}NO_2^- \longrightarrow R_2S + \cdot CH_2NO_2 \qquad (3)$$

$$\cdot CH_2NO_2 + CH_2{=}NO_2^- \longrightarrow O_2NCH_2CH_2NO_2^{-\cdot} \qquad (4)$$

gether with very weak signals attributed to EtSCHMeCH₂-NO₂⁻· [with $a(N)$ 2.52, $\Sigma a(\beta\text{-H})$ 1.97, $a(\gamma\text{-H})$ 0.06 mT, g 2.0050] in the case of Et₂S; no signals from MeNO₂⁻· were observed. This both rules out the possibility that, in the oxidation of methionine, it is α-sulphur-substituted radicals (which can be directly detected in the absence of the trap) that are responsible for one-electron reduction of MeNO₂ and also suggests that sulphur-centred radical-cations are able to effect one-electron oxidation of the *aci*-anion [reactions (2)—(4); *cf.* reaction of CH₂=NO₂⁻ with SO₄⁻· which has established [14] reaction (4) as the source of O₂NCH₂CH₂NO₂⁻·].

Photolysis of freshly prepared, cooled mixtures of methionine, hydrogen peroxide, and BuᵗNO in the pH range *ca.* 2.5—4.5 led to the detection of a spectrum (Figure 1a) analysed in terms of a nitrogen splitting (1.455 mT) which is in the range characteristic of nitroxides, a further small (β) nitrogen splitting (0.290 mT), a hydrogen splitting (0.145 mT) typical of a β-methine proton, and a longer range coupling (0.035 mT) to two protons, detected under high-resolution conditions.*·† This is attributed to the radical-adduct (6) formed from the decarboxylated α-amino radical ·CH(NH₃⁺)-CH₂CH₂SMe (Scheme 2) (*cf.* similar adducts from α-amino-

alkyl radicals and BuᵗNO [13]). We believe that a previous assignment [5] of this signal to the adduct of ·C(NH₃⁺)(CO₂⁻)-CH₂CH₂SMe is incorrect since the pair of γ-proton splittings in the resulting nitroxide would be expected to be much smaller than the value of 0.145 mT attributed to one of them. In the analogous radiolysis work, the spectrum we attribute to (6) was observed but unassigned.[6]

When the pH was raised above *ca.* 4.5, this signal was replaced by signals from a mixture of two radicals, namely BuᵗNHO· (Table) and one with $a(N)$ 1.60, $a(1\ H)$ and $a(1\ N)$ 0.14, $a(2\ H)$ 0.065 mT, g 2.0058 (Figure 1b). These parameters are as expected for the adduct (7) from the decarboxylated α-aminoalkyl radical (5): thus, radical-adducts of similar type (with closely similar splitting values) arise together with BuᵗNHO· from reaction of α-aminoalkyl radicals from N-alkylhydroxylamines and Ti¹¹¹ in the presence of the same trap.[13] As shown in Scheme 2, we attribute the difference in behaviour as the pH is raised to the ionisation of the ⁻NH₃⁺ group in the *radical* ·CH(NH₃⁺)CH₂CH₂SMe (the pK_a value for which has been reported [7] as 3.8) and the consequent pro-

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* When solutions which had been standing for some time were photolysed, signals predominated from adducts formed by BuᵗNO with Me· and ·CH₂CH₂CH(CO₂⁻)NH₃⁺, which had evidently been derived *via* reaction of ·OH with the sulphoxide of methionine, formed *in situ* by peroxidation (see *e.g.* ref. 15). Other sulphides behaved similarly.

† Since in the absence of H₂O₂ no substrate-derived radicals were detected (though photolysis of the trap led to the detection of Buᵗ₂NO·), we attribute the radicals formed in its presence to reaction of the hydroxyl radical (*cf.* ref. 5).
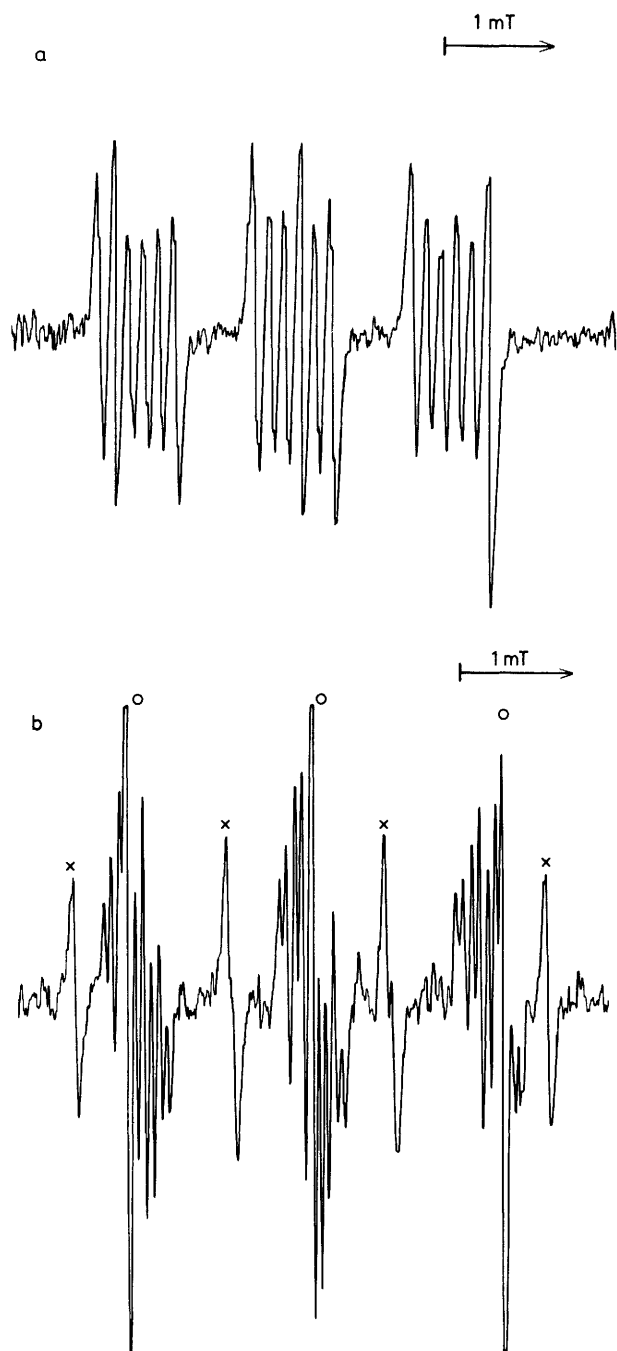
a, E.s.r. spectrum of (6), obtained from the reaction of ·OH with methionine in the presence of Bu^{t}NO at pH 3.5. b, E.s.r. spectrum of (7) and Bu^{t}NHO· (peaks marked ×) obtained from the reaction of ·OH with methionine in the presence of Bu^{t}NO at pH 8.0. Peaks marked O are from di-t-butyl nitroxide (from photolysis of the trap)

duction of the electron-donor (5) in significant quantities at pH > ca. 4.

(b) *Methionine Ethyl Ester.*—Our results yielded no evidence to suggest that an α-aminoalkyl radical is formed during reaction of ·OH with MeSCH$_2$CH$_2$CH(NH$_3$$^+$)CO$_2$Et (and its neutral counterpart) in the accessible pH range. For example, oxidation in the absence of a trap in the pH range 2—8 led to the detection solely of weak signals from the two

radicals generated by hydrogen abstraction from carbon atoms adjacent to sulphur (with parameters essentially the same as for those from methionine). Reaction with ·OH in the presence of MeNO$_2$ at pH ca. 10 (at which the amino-group will be unprotonated [10]) gave rise to signals from HOCH$_2$-NO$_2$$^-$· and O$_2NCH_2CH_2NO_2$$^-$· (as with other sulphides, see above) and, in contrast to methionine, only a trace of Me-NO$_2$$^-$·; with Bu^{t}NO, no signals were obtained which could be attributed to attack of ·OH on the ester.

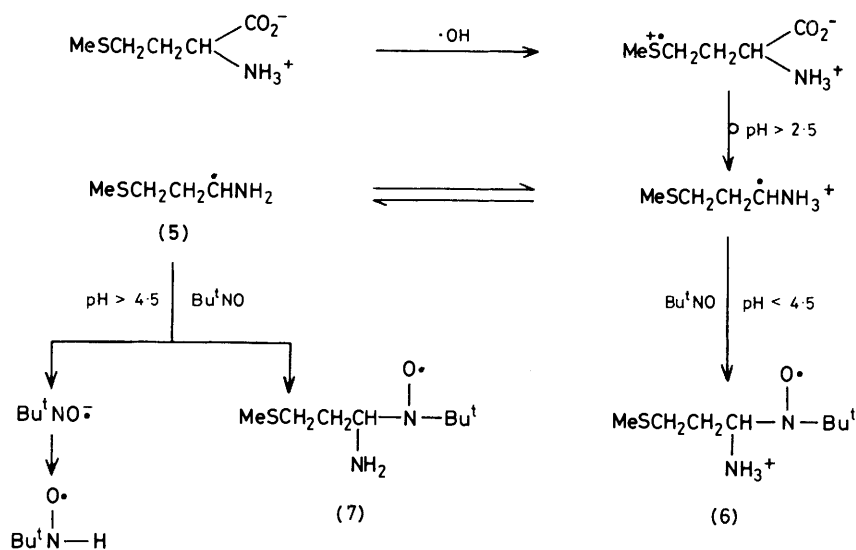(c) 3-(*Methylthio*)*propylamine.*—Reaction of MeS(CH$_2$)$_3$-NH$_2$ with ·OH in the flow system over a range of pH values led to the direct detection only of radicals resulting from overall removal of hydrogen from a carbon atom adjacent to sulphur (Table). As with the previous substrate, experiments with MeNO$_2$ and Bu^{t}NO provided no clear evidence for the formation of other radicals: the absence of signals from Me-NO$_2$$^-$· and Bu^{t}NHO· respectively, in contrast to the findings for methionine, suggests that α-aminoalkyl radicals are not formed.

Now it is known [13] that, under the conditions we have employed here, N-alkylaminium radical-cations (prepared by a different route) react rapidly by loss of a β-proton to give α-aminoalkyl radicals (which can both be trapped and also reduce MeNO$_2$ and Bu^{t}NO). It therefore follows that the production of an aminium radical (8), *e.g.* via electron transfer from the sulphur radical-cation [reaction (5)], does not occur with this substrate.
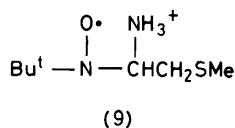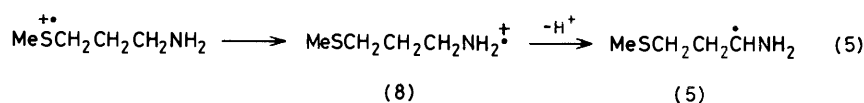
(d) 4-(*Ethylthio*)*butanoic Acid.*—This substrate was chosen in an attempt to discover whether direct electron-transfer to a sulphur-centred radical-cation from a carboxylate ion (*i.e.* not involving the amino-group) could take place.

Oxidation with ·OH led simply to the detection of ·CHMe-S(CH$_2$)$_3$CO$_2$H and ·CH(SEt)(CH$_2$)$_2$CO$_2$H, with no trace of the radical ·CH$_2$CH$_2$CH$_2$SEt which would have been anticipated above pH ca. 4.75 if oxidation of the carboxylate ion (either directly or via R^{1}R^{2}S$^+$·) had taken place. With Me-NO$_2$ at pH 9.5, signals from MeNO$_2$$^-$· (weak), HOCH$_2NO_2$$^-$·, and O$_2NCH_2CH_2NO_2$$^-$· were observed (the presence of the last-named being indicative of electron transfer to the sulphur radical-cation from the *aci*-anion). No substrate-derived radicals were trapped with Bu^{t}NO. It thus appears that, unlike the reaction of methionine, no decarboxylation has occurred.

(e) S-*Methylcysteine.*—Reaction of MeSCH$_2$CH(CO$_2$$^-$)-NH$_3$$^+$ with ·OH over the pH range 3—9 led to the direct detection solely of weak signals from ·CH$_2$SCH$_2$CH(CO$_2$$^-$)-NH$_3$$^+$ [·CH(SMe)CH(CO$_2$$^-$)NH$_3$$^+$ would also have been expected; possibly its rate of formation is too slow for its concentration to reach the detectable limit owing to the −*I* effect of the protonated amino-group]. In the presence of MeNO$_2$ at pH ca. 9.6, signals were detected from MeNO$_2$$^-$· (at a sufficiently high intensity to indicate the production of a good electron-donor precursor) together with a fairly strong signal from MeSCH$_2$NO$_2$$^-$· and the customary signal from HOCH$_2$NO$_2$$^-$·. Results with Bu^{t}NO proved particularly informative: thus, although at pH 2 no signals assignable to adducts of radicals from S-methylcysteine could be detected, above pH ca. 2.5 (*i.e.* above the pK_a of the carboxylic acid function) two such radicals were clearly characterized, namely Bu^{t}NHO· (weak) and (9) (for data see Table). As with methionine under similar circumstances, the trapping of the α-aminoalkyl radical proves that decarboxylation has occurred. As the pH was raised the signal from Bu^{t}NHO· increased in intensity up to pH ca. 4.5, by which point the signal from the adduct (9) had disappeared completely. As the pH

Scheme 2.

$$\overset{+\bullet}{MeSCH_2}CH_2CH_2NH_2 \longrightarrow MeSCH_2CH_2CH_2NH_2^{\overset{+}{\bullet}} \xrightarrow{-H^+} MeSCH_2CH_2\overset{\bullet}{C}HNH_2 \quad (5)$$

$$\qquad\qquad\qquad\qquad\qquad (8) \qquad\qquad\qquad\qquad\qquad (5)$$

(9)

was raised further, the signal from Bu^{t}NHO· remained dominant. The observations are interpreted in terms of the increase in pH through the pK_a of the amino-group in the α-aminoalkyl radical ·CH(NH$_3^+$)CH$_2$SMe (*ca.* 3.8),[7] after which the exceptionally good electron donor ·CH(NH$_2$)-CH$_2$SMe is the dominant form.
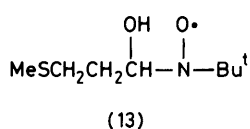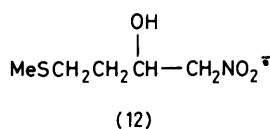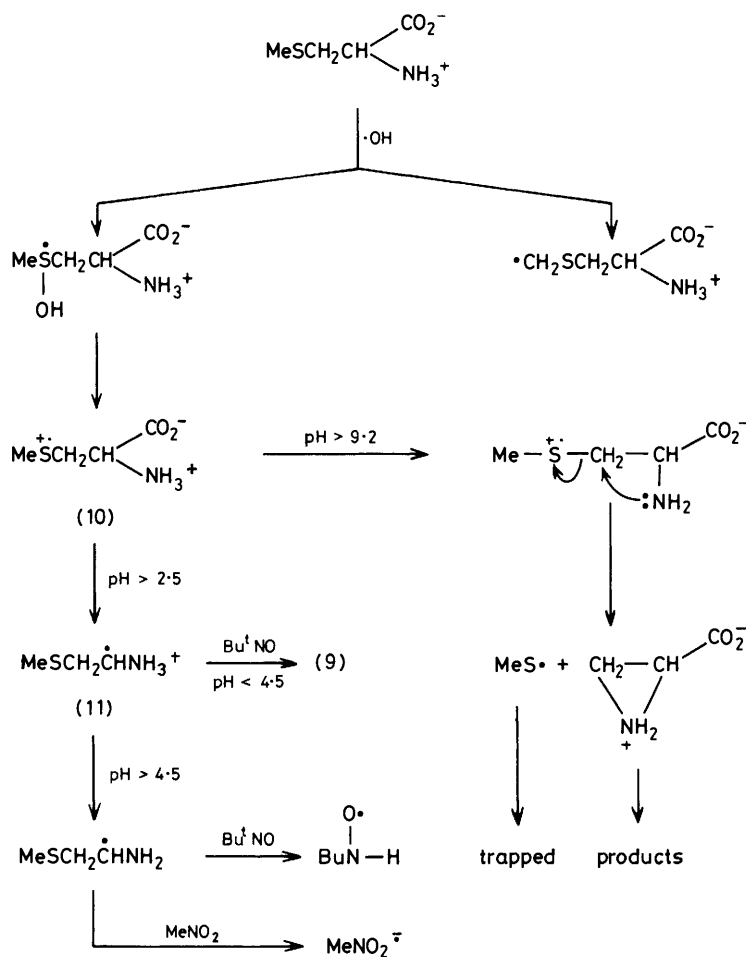
Scheme 3 outlines the overall features of the reaction as revealed by e.s.r. We envisage that the sulphur-centred radical-cation (10) undergoes decarboxylation above pH *ca.* 2 and that the subsequent radical (11) may be trapped (by Bu^{t}NO) or, above pH *ca.* 4.5, become deprotonated and thence reduce both Bu^{t}NO and MeNO$_2$. An alternative reaction attributed to (10) which becomes detectable with CH$_2$=NO$_2^-$ above pH *ca.* 9.2 (*i.e.* when the amino-group is no longer protonated) involves production of ·SMe. We suggest that this occurs *via* internal nucleophilic attack of the amino-group, as indicated in Scheme 3, and the observation that a somewhat lower concentration of MeS· is trapped from methionine (see earlier) is consistent with the known requirements for this type of process for it generally occurs more readily when a three- rather than a four-membered ring is formed. It is also likely that the more effective production of alkylthio radicals which has been noted for radical-cations from β-hydroxy-substituted dialkyl sulphides [*e.g.* S(CH$_2$CMe$_2$OH)$_2$] compared with those from substrates lacking this feature results from the β-oxygen atom's playing a similar (if less powerful) role.[4]

(f) *2-Hydroxy-4-(methylthio)butanoic Acid.*—Our finding that, in contrast to the results for EtS(CH$_2$)$_3$CO$_2$H, the presence of the amino-group in methionine apparently encourages decarboxylation prompted us to examine the reaction

with ·OH of this hydroxy-substituted analogue. In the absence of spin traps, only the two possible sulphur-conjugated radicals were detected (see Table). On the other hand, in the presence of MeNO$_2$ at pH *ca.* 9.5 signals were obtained from MeNO$_2^{-\bullet}$ (considerably more intense than the signal detected in the absence of this substrate), together with those from HOCH$_2$NO$_2^{-\bullet}$ and O$_2$NCH$_2$CH$_2$NO$_2^{-\bullet}$ (weak) as well as those attributed to (12) [with a(N) 2.50, a(1 H) 1.13, a(1 H) 0.965, a(1 H) 0.055 mT, g 2.0050; *cf.*[11,12] data for the adduct of CH$_2$=NO$_2^-$ with ·CH(OH)Et] and to the trapping of an oxygen-centred radical [with a(N) 2.435, a(2 H) 0.85 mT, g 2.0050] which may be the adduct from ·OCH(CO$_2^-$)CH$_2$CH$_2$-SMe, though further structural and mechanistic speculation is not justified.

With Bu^{t}NO, the only signal detected (Table) is attributed to (13). The formation of (12) and (13) and the detection of MeNO$_2^{-\bullet}$ are fully consistent with the generation from this substrate of the α-hydroxyalkyl radical ·CH(OH)CH$_2$CH$_2$-SMe (presumably *via* a decarboxylation pathway which is analogous to that which occurs for methionine); it is known that radicals of this type are readily trapped by Bu^{t}NO and that they add to CH$_2$=NO$_2^-$ and reduce MeNO$_2$ *via* one-electron transfer (although the propensity for the last reaction is presumably less than for α-aminoalkyl radicals). The importance of this finding is that it establishes that the presence of either an α-hydroxy- or an α-amino-group evidently promotes decarboxylation. Possible mechanisms of reaction and effects of OH and NH$_2$ are discussed later.

(g) *Oxidation of Mixtures of Dialkyl Sulphides and a Variety of Substrates.*—Evidence has so far been presented that an intermediate formed in the reaction between ·OH and sulphide derivatives (presumably a sulphur-centred radical-cation or hydroxy-adduct) can induce decarboxylation of certain hydroxy- and amino-acids. It remains to be established whether such reactions are confined to intramolecular processes or whether analogous intermolecular reactions can, and do, occur. Accordingly, we studied the reactions between ·OH

Scheme 3.

$$MeSCH_2CH_2\overset{OH}{\underset{|}{CH}}-CH_2NO_2^{\overline{\cdot}}$$

(12)

$$MeSCH_2CH_2\overset{OH}{\underset{|}{CH}}-\overset{O\cdot}{\underset{|}{N}}-Bu^t$$
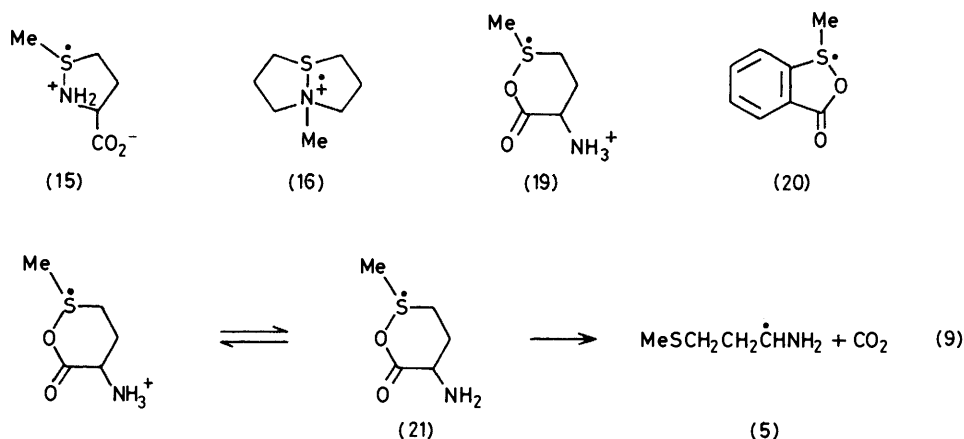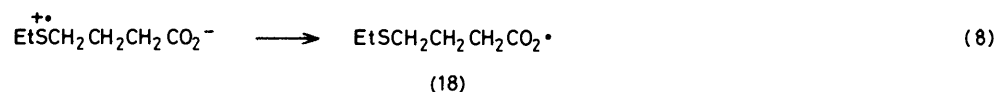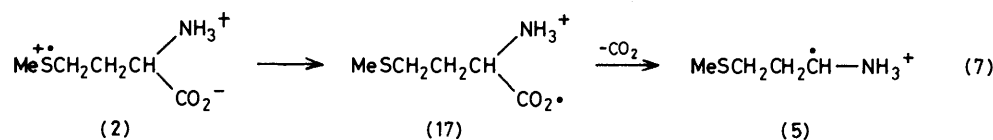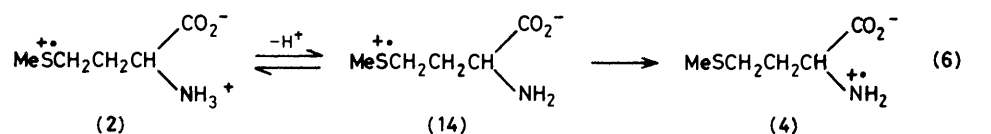
(13)

and some model compounds in the presence of dialkyl sulphides.

Reaction of ·OH with propionate ion at pH *ca.* 6 gave, essentially as described previously,[16] the radicals ·CHMeCO$_2^-$ and ·CH$_2$CH$_2$CO$_2^-$ (in a concentration ratio estimated by computer simulation as 2.4 : 1). In the presence of MeNO$_2$ at pH 9.8, signals were detected from both MeCH(CO$_2^-$)CH$_2$-NO$_2^{-\cdot}$ [with a(N) 2.50, a(1 H) 1.45, a(1 H) 0.66, a(1 H) 0.065 mT, g 2.0050] and CH$_2$(CO$_2^-$)CH$_2$CH$_2$NO$_2^{-\cdot}$ [with a(N) 2.55, a(2 H) 1.01, a(2 H) 0.06 mT, g 2.0050] (in the ratio *ca.* 3 : 1) as well as from HOCH$_2$NO$_2^{-\cdot}$ and MeNO$_2^{-\cdot}$ (in small quantities). When an equimolar mixture of EtCO$_2^-$ and Et$_2$S (*ca.* 0.25 mol dm^{-3}) was oxidized, there was a marked decrease in the intensity of the signals from ·CHMeCO$_2^-$ and ·CH$_2$CH$_2$CO$_2^-$ but no evidence for the formation of Et· which would result from decarboxylation. When the experiment was carried out in the presence of MeNO$_2$ at pH 9.5, the spectra were essentially identical to those in the absence of the sulphide except that O$_2$NCH$_2$CH$_2$NO$_2^{-\cdot}$ was also detected, and this is considered to arise from the direct oxidation of CH$_2$=NO$_2^-$ by Et$_2$S$^{+\cdot}$ (see earlier). In addition, since no signal from EtCH$_2$NO$_2^{-\cdot}$ was detected, we rule out any

significant one-electron oxidation of the carboxylate by the radical-cation.

Oxidation of butylamine with ·OH in the presence of MeNO$_2$ at pH values (*ca.* 10) just below the pK_a of the substrate (10.7 [10]) gave signals which characterize the trapping of (one or more) secondary radicals [a(N) 2.46, a(2 H) 1.025, a(1 H) 0.07 mT, g 2.0057]. These are likely to be ·CHMeCH$_2$-CH$_2$NH$_3^+$ and/or ·CHEtCH$_2$NH$_3^+$, formed by reaction of the electrophilic hydroxyl radical at methylene groups removed from the deactivating $^-$NH$_3^+$ substituent. At pH *ca.* 11, these signals were less intense and that from MeNO$_2^{-\cdot}$ was also present, presumably as a result of the generation of the reducing α-aminoalkyl radical ·CH(NH$_2$)Pr by reaction of hydroxyl radical with the unprotonated amine. When experiments were carried out in the presence of Me$_2$S (*ca.* 0.05 mol dm^{-3}) the results were essentially the same, the only difference being the detection of O$_2$NCH$_2$CH$_2$NO$_2^{-\cdot}$ (evidently formed from reaction of Me$_2$S$^{+\cdot}$ with the trap). There was no evidence for oxidation of the amine by Me$_2$S$^{+\cdot}$.

In contrast, addition of diethyl sulphide in experiments in which glycine was oxidised by ·OH in the presence of MeNO$_2$ at pH *ca.* 10 led to significant changes in the spectra. In the absence of the sulphide, HOCH$_2$NO$_2^{-\cdot}$ and a smaller concentration of MeNO$_2^{-\cdot}$ were detected, the latter presumably arising by reduction by ·CH(NH$_2$)CO$_2^-$ which has already been shown to be formed under these conditions [*cf.* ref. 17 for data on this and the related radical ·CH(NH$_2$)CO$_2$H]. In the presence of the sulphide (at an equimolar concentration of 0.05 mol dm^{-3}) the spectrum of MeNO$_2^{-\cdot}$ was dominant,

$$\overset{+\bullet}{Me}SCH_2CH_2CH\overset{CO_2^-}{\underset{NH_3^+}{}} \underset{}{\overset{-H^+}{\rightleftharpoons}} \overset{+\bullet}{Me}SCH_2CH_2CH\overset{CO_2^-}{\underset{NH_2}{}} \longrightarrow MeSCH_2CH_2CH\overset{CO_2^-}{\underset{\overset{+\bullet}{NH_2}}{}} \quad (6)$$

(2)   (14)   (4)

$$\overset{+\bullet}{Me}SCH_2CH_2CH\overset{NH_3^+}{\underset{CO_2^-}{}} \longrightarrow MeSCH_2CH_2CH\overset{NH_3^+}{\underset{CO_2\bullet}{}} \overset{-CO_2}{\longrightarrow} MeS CH_2CH_2\overset{\bullet}{CH}-NH_3^+ \quad (7)$$

(2)   (17)   (5)

$$\overset{+\bullet}{Et}SCH_2CH_2CH_2CO_2^- \longrightarrow EtSCH_2CH_2CH_2CO_2\bullet \quad (8)$$

(18)

(15)   (16)   (19)   (20)

$$MeSCH_2CH_2\overset{\bullet}{C}HNH_2 + CO_2 \quad (9)$$

(21)   (5)

with $[O_2NCH_2CH_2NO_2^{-\bullet}]$ considerably lower than when Et_2S itself was oxidized. That $[MeNO_2^{-\bullet}]$ was much greater when Et_2S was present suggests that intermolecular oxidation to give an α-aminoalkyl radical ($\cdot CH_2NH_2$) is occurring. However, the detection of the dimer radical $O_2NCH_2CH_2$ $NO_2^{-\bullet}$ in significant quantities, even when the ratio [glycine] : $[Et_2S]$ was 2 : 1, in contrast with the detection of weak signals from this radical during the oxidation of methionine, suggests that *intermolecular* oxidation of the amino-acid (rather than the *aci*-anion) by the sulphur-centred radical cation is much less effective than the intramolecular counterpart.

Conclusions.—Our e.s.r. study confirms the conclusion reached on the basis of pulse radiolysis experiments that methionine undergoes a decarboxylative oxidation when it interacts with $\cdot OH$ at pH > *ca.* 2.[7] It has shown, in addition, that the reaction occurs with *S*-methylcysteine and, from the results for glycine, that it has an acyclic analogue, albeit a seemingly less effective one; and that the presence of either an amino- or a hydroxy-group on the carboxy-bearing carbon is necessary, since $EtS(CH_2)_3CO_2H$ and propionic acid, in contrast to methionine, *S*-methylcysteine, 2-hydroxy-4-(methylthio)butanoic acid, and glycine did not give evidence for the reaction.

The first step is doubtless addition of $\cdot OH$ to sulphur, for this process is known, from studies of the sulphides, to occur at (close to) the diffusion-controlled limit [8] and therefore to be faster than reaction of $\cdot OH$ at, *e.g.* a carboxy- or amino-group. This is likely to be followed by loss of hydroxide to give the sulphur-centred radical cation (but see later the suggestion that this step may be assisted). We shall now discuss the possible subsequent steps with reference mainly to methionine.

The first possibility is that the radical-cation (2) abstracts an electron from the amino-group to yield the species (4) [reaction (6)]. This is unlikely, for two reasons. First, at a pH as low as 2, at which the oxidative decarboxylation is observed to occur, the proportion of the species (2) present in the free amino-form (14) is almost certainly $<10^{-4}\%$,* so that the electron-transfer process is unlikely to compete effectively with, *e.g.* proton loss from the carbons adjacent to sulphur in the radical-cation (2). Secondly, 3-(methylthio)propylamine would then have been expected to yield the amino radical-cation $MeS(CH_2)_3NH_2^{+\bullet}$ and thence [13] the α-amino-substituted radical $MeS(CH_2)_2\overset{\bullet}{C}HNH_2$, contrary to observation.

For the same reasons, we rule out the possibility that electron-transfer to amino occurs by way of the cyclic intermediate (15) [a precedent for which would be the formation of (16) from 5-methyl-1-thia-5-azacyclo-octane with $NOBF_4$ in $EtCN$ [18]]. A further reason may be adduced here: the observed oxidative decarboxylation of *S*-methylcysteine would require formation of a relatively strained four-membered ring. Moreover, it is notable that, at pH values (>9) at which a significant proportion of the radical-cation (10) is likely to be present in the free amino-form, an alternative reaction comes into operation (Scheme 3). (We cannot account for the apparent discrepancy between our results and those of the pulse-radiolysis study which suggested that *S*-methylcysteine is reluctant to undergo oxidative decarboxylation.[7])

However, the observations can be rationalised if, instead, the sulphur radical-cation interacts with the carboxylate ion,

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* The pK_a of the methionine amino-group is 9.27. That of this group in the radical-cation (2) is likely to be only a little lower.

and this is in keeping with the observation that oxidative decarboxylation is detected when the pH is raised to ca. 2, i.e. in the region of the likely pK_a of the carboxylic acid group in the radical-cation (2). We rule out the possibility that (2) yields (17) directly [reaction (7)], for in that case we should have expected the radical-cation from 4-(ethylthio)butanoic acid to give (18) and thence $EtSCH_2CH_2CH_2$· [reaction (8)]. Instead, we suggest that reaction occurs by way of the cyclic intermediate (19), precedent for which is the formation of (20) by the low-temperature photolysis of t-butylperoxy (2-methylsulphenyl)benzoate in CH_2Cl_2,[19] and that, in its free-amino form (21),* this cleaves in a concerted reaction to give (5) [reaction (9)]. Provided that (19) is in equilibrium with (21), the key role of the amino-group in promoting decarboxylation would then lie in its ability to delocalise the unpaired electron in radical (5) (delocalisation energy of ca. 40 kJ mol⁻¹ would be expected [21]). Likewise, the influence of the hydroxy-group in the hydroxy-analogue of methionine would be associated with the ca. 32 kJ mol⁻¹ delocalisation energy in the corresponding radical.[21,22]

Finally, it has already been noted that [23] the ionization potentials of sulphides are lowered by the presence of carboxylate groups capable of bonding to the incipient radical-cation. Thus, it may be that formation of the radical-cation (2) from the hydroxy-adduct (1) is actually bypassed, and that the cyclic intermediate (19) is formed directly from (1).

Experimental

A Varian E-104 e.s.r. spectrometer with 100 kHz modulation and an X-band Klystron was employed. Splitting constants were measured to within ±0.005 mT and g factors to within 0.0001 by comparison with Fremy's salt [a(N) 1.3091 mT,[24] g 2.0055 [25]]. Spectrum simulation with a program kindly supplied by Dr. M. F. Chiu was used to confirm splitting constants and to determine relative concentrations of radicals.

pH Measurements were made with a Pye-Unicam PW 9410 pH meter and electrode inserted into the effluent stream of the flow system, and the flow was maintained with a Watson–Marlow MHRE flow-inducer positioned on the inlet tubing. A mixing chamber which allowed the simultaneous mixing of three reactants was employed. All solutions were degassed by the passage of nitrogen. The first solution contained titanium-(III) chloride (0.008 mol dm⁻³), ethylenediaminetetra-acetic acid (3 g l⁻¹), and either concentrated sulphuric acid or ammonia to give the required pH, the second contained hydrogen peroxide (0.033 mol dm⁻³), and the substrate was included in the third stream (with a concentration typically in the range 0.01—0.1 mol dm⁻³). The spin-trapping experiments with the flow system were carried out by adding nitromethane (0.01 mol dm⁻³) to the third solution, both this and the first solution being treated with ammonia to give pH 9.5.

The photolytic spin-trapping experiments involved photolysis, with the unfiltered radiation from an Hanovia 100 W mercury lamp, of freshly prepared aqueous solutions containing 2-methyl-2-nitrosopropane (0.01 mol dm⁻³), hydrogen peroxide (0.03 mol dm⁻³), and the substrate (typically 0.05 mol dm⁻³).

Materials were commercially available, and used as sup-

plied, except for 4-(ethylthio)butanoic acid [26] which was prepared by the method of Truce and Abraham.[27]

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* The pK_a of the amino-group in (19) is likely to be substantially less than in methionine, owing to the influence of the electron-attracting carboxy-sulphur function. For comparison, MeO_2C–CH_2–NH_3^+ has pK_a 7.59.[20]