Electron Spin Resonance Studies. Part 66.[1] Characterization of Copper(II) Complexes in the Oxidation of D-Penicillamine, L-Cysteine, and Related Sulphur-containing Compounds

Frederick J. Davis, Bruce C. Gilbert,* and Richard O. C. Norman
Department of Chemistry, University of York, Heslington, York YO1 5DD
Martyn C. R. Symons
Department of Physical Chemistry, University of Leicester, Leicester LE1 7RH

E.s.r. spectroscopy has been employed to characterize a series of copper(II)–dithiolate complexes formed in aqueous solution at pH *ca.* 10. Some examples, *e.g.* that from D-penicillamine [Cu(D-pen)$_2$], are long-lived whereas rapid-flow and fast-freezing methods are required for the study of more transient species (*e.g.* the analogous complex from L-cysteine). Spectra have been described for related copper complexes of S_2N_2, S_2O_2, and S_4 type and both isotropic and anisotropic parameters are interpreted in terms of significant spin-delocalization onto S (especially) and N ligands. Some transient ternary complexes [*e.g.* from L-cysteine and mercaptoethanoic acid)] have also been identified. Stopped-flow experiments on the kinetics of the (second-order) decay of the transient complexes reveal the importance of steric and electronic effects in governing their lifetimes.

The considerable recent interest in the reaction between copper(II) and thiols [2,3] reflects, at least in part, the possible biological significance of copper–sulphur interactions.[4] For example, the thiol D-penicillamine appears to be effective in removing excess of copper from patients suffering from Wilson's disease [5] and it has been suggested that its use in the treatment of rheumatoid arthritis may be related to its affinity for copper ions [6] (which is consistent with the inhibition of some *in vitro* reactions of superoxide ion by its red-violet copper complex [7]). It is also well established that the intense blue colour of certain copper-containing proteins such as stellacyanin and plastocyanin arises as a result of charge-transfer between copper(II) and the thiolate group in a cysteine residue in the proteins [8] (a methionine sulphur atom may also be involved in the bonding [9]).

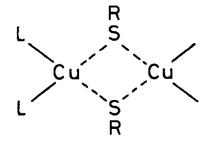
The reaction of copper(II) with an excess of thiol is known to produce the appropriate disulphide [10] and it has been suggested [11] on the basis of an investigation employing polarography and electronic absorption spectroscopy that the stoicheiometry is as in reaction (1) [the nature of the copper(I) complex formed has not been extensively investigated but there is some evidence that, for penicillamine at least, a 1 : 1 chelate is formed in which both sulphur and nitrogen are bound to copper [12]].

$$Cu^{II} + 2RSH \longrightarrow Cu^{I}(SR) + \tfrac{1}{2}RSSR + 2H^+ \quad (1)$$

There is controversy about the nature of the bonding and the oxidation state of copper involved in this type of reaction (whose occurrence has proved to be a considerable complication in studies [2,8] of the Cu–RS$^-$ ' chromophore ') and in the complexes formed when there is insufficient thiol available for complete reduction to copper(I) (*e.g.* the intensely coloured complexes formed from Cu^{II} and thiols, including D-penicillamine [13,14] and 2-mercaptosuccinic acid [11,15]). For example, Blumberg and Peisach have suggested [2] that the reaction between copper(II) and thiols results in the formation of a copper-stabilized sulphur radical (1) whereas the failure to detect a significant concentration of Cu^{II} in such systems by e.s.r. has been suggested to be the result of the formation of sulphur–sulphur bridged dimers (2) (L = RS$^-$).[16,17]

$(RS\cdot)\,Cu^{I}$

(1)



(2)

The application of different physical techniques leads to the conclusion that, depending upon the precise conditions employed, a number of different species may be formed during the reaction between copper(II) and thiols. For example both polarographic [11] and *X*-ray diffraction [14] results suggest that at low thiol concentrations a polymeric mixed-valence copper complex may be formed and there is evidence [15] for a 1 : 1 Cu^{II}–2-mercaptosuccinic acid complex in very acidic solution and at low [RSH]. An intermediate copper(II) complex with cysteine has been characterized [2] by e.s.r. following freezing of rapidly mixed solutions (and spectrophotometrically in stopped-flow experiments [18]) and a similar species from penicillamine and copper(II) appears to be identical to that obtained [10] in frozen and liquid solution at physiological pH. These results suggest a structure with a CuN_2S_2 chromophore. Certain other thiols (*e.g.* 2-mercaptoethylamine) appear [19] to give similar (but shorter-lived) species, and in one case a flow system has been employed [20] with e.s.r. spectroscopy to characterize a transient ternary complex from copper(II), glycylglycine, and cysteine.

We have previously employed [21] e.s.r. spectroscopy in conjunction with a rapid-flow system to characterize radicals (RS·, RSO·, RSO$_2$·, and some carbon-centred species) formed in the reaction between thiols (and disulphides) and ·OH; similar radicals were also detected during the oxidation of thiols with Ce^{IV}. We here describe the results of a corresponding study of the reaction with Cu^{II} of a variety of thiols (including D-penicillamine, L-cysteine, and related compounds) in which we employed e.s.r. spectroscopy and a flow system in an attempt to characterize any transient organic radicals or copper complexes formed during the oxidation.

Results and Discussion

(a) *Initial Experiments with D-Penicillamine and L-Cysteine.*—It was decided first to employ e.s.r. to monitor the reaction when increasing amounts of thiol (D-penicillamine or L-cysteine) were added to a copper(II) solution (typically 2×10^{-3} mol dm^{-3}) (up to a [thiol] : [copper] ratio of *ca.* 5 : 1) at different pH values. Spectra were recorded for thoroughly

Figure 1. E.s.r. spectrum from the stable complex [^{63}Cu(D-pen)$_2$] formed from the reaction of copper(II) (*ca.* 10^{-3} mol dm^{-3}) and D-penicillamine (*ca.* 5×10^{-3} mol dm^{-3}) at pH *ca.* 10: the peak marked O is attributed to part of the corresponding signal from ^{65}Cu and that marked × is from a geometric isomer (see text)

deoxygenated static samples, several minutes after mixing. Measured quantities of thiol were added to portions of a standard solution of copper(II) chloride dissolved in hydrochloric acid, ammonia solution, or suitable buffer solutions; copper(II) concentrations were estimated from the variation in signal height or, where different species were obtained, by double integration (see Experimental section).

In acid solution (pH < 4), addition of either thiol to copper(II) reduced the intensity of the broad signal (g 2.2) attributed [22] to hydrated copper(II) but this signal could still be detected (at considerably reduced intensity) at thiol concentrations greater than twice that of the copper. A transient brown colour was formed when the solutions were first mixed but flow experiments yielded only a rather broad and uninformative signal (the signals obtained on rapid freezing of these solutions are described subsequently).

In the pH range 4—7, addition of D-penicillamine to copper(II) under otherwise similar conditions produced an intensely red-violet, long-lived species; with L-cysteine a similar, but transient, colour was noted. With the former substrate, e.s.r. spectra indicated the disappearance of the signal from hydrated Cu^{II} and the appearance of a broad signal (presumably associated with the red-violet species) with isotropic parameters a(Cu) 7.5 mT, g 2.11; on further increase in [RSH], signals from this species were also reduced (as was the colour) and for a ratio of [RSH] : [Cu^{II}] > 2 : 1 no signals could be discerned.

Addition of penicillamine to a solution of copper(II) in aqueous ammonia at pH 10 gave an intensely blue-black solution and caused a reduction of the intensity of the signal from Cu^{II}. Increasing the concentration of thiol resulted in the formation of a brown colouration and the detection by e.s.r. of a different copper(II) species (Figure 1) showing coupling to copper and superhyperfine coupling attributed to two nitrogen atoms (a detailed analysis is presented later). The intensity of this signal increased up to a ratio of [RSH] : [Cu^{II}] of *ca.* 2 : 1, but further addition of thiol had no effect on the signal or its intensity; under these conditions effectively all the copper is present as this complex. In contrast, for cysteine, long-lived signals from copper(II) were not detected at high thiol concentrations, although transient, weak spectra similar to that shown in Figure 1 were detected directly after mixing. This suggests that with cysteine the initially produced copper complex is unstable; rapid mixing techniques were therefore employed to investigate the behaviour of this and a variety of related substrates.

(b) *Flow-system Studies of the Copper–Thiol Reaction at High pH*.—(i) *The identification of copper*(II)*-thiolate complexes*. To investigate the possible formation of transient paramagnetic intermediates in the reaction between copper(II) and some substituted thiols at high pH, a solution of CuCl$_2$ (*ca.* 10^{-3} mol dm^{-3}) in aqueous ammonia (typically 1 mol dm^{-3}) was rapidly mixed with an aqueous solution of the appropriate substrate (*ca.* 5×10^{-3} mol dm^{-3}) using a standard two-way flow system [21] positioned in the cavity of an e.s.r. spectrometer. Investigations were generally carried out at pH *ca.* 10 and with a time between mixing and observation of *ca.* 30 ms. All solutions were thoroughly deoxygenated prior to mixing.

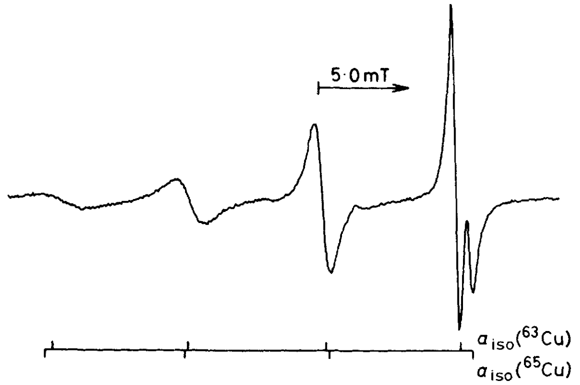


Figure 2. E.s.r. spectrum obtained *ca.* 30 ms after mixing aqueous solutions of copper(II)–ammonia (*ca.* 10^{-3} mol dm^{-3} in copper) and 2-mercaptosuccinic acid (*ca.* 5×10^{-3} mol dm^{-3}) at pH *ca.* 10: signals from ^{63}Cu and ^{65}Cu complexes are indicated

No organic radicals were detected, either for D-penicillamine or related substrates, even when the reactions were carried out in the presence of the *aci*-anion of nitromethane ($CH_2{:}NO_2^-$) as a spin trap [21] (under conditions under which thiyl radicals, for example, may be readily trapped). However, the disappearance of the signal from the Cu^{II}-ammonia complex [a(Cu) (−) 7.5 mT, g 2.12] was generally accompanied by the immediate appearance of signals from a different species which must be formed extremely rapidly. With D-penicillamine the spectrum was identical to that shown in Figure 1 and a closely similar spectrum was obtained for L-cysteine; the spectrum from 2-mercaptosuccinic acid is shown in Figure 2. Double integration of these signals showed that these complexes account for all the copper initially present.* Similar, but generally less intense, spectra were obtained for related substrates.

The compounds listed in Table 1 are those from which spectra of this type were recorded. For D-penicillamine itself and 2-mercapto-2-methylpropylamine the spectra did not decay significantly when the flow was stopped, but for most other examples the signal disappeared rapidly (generally over a period of between 1 and 60 s; kinetic results are presented later). For ethane-1,2-dithiol and propane-1,2-dithiol only weak signals of the appropriate complexes were detected on rapid mixing (with no trace of the Cu^{II}-ammonia complex) but, when the flow was stopped, the signal intensity of the Cu^{II}–thiol complex built-up over a period of several seconds. In the case of the ethane-1,2-dithiol complex a slow decay then set in. No signals could be detected from other substrates including benzenethiol, 2-mercaptoethanol, and 3-mercaptopropylamine, and it seems likely that this indicates a particularly rapid reduction of copper(II).

In a series of experiments with D-penicillamine, L-cysteine, and 2-mercaptobenzoic acid, the copper complexes described here were found to be detectable down to pH *ca.* 8.

---

* Although we have not studied the optical absorption spectra of the complexes it may be noted here that the absorption at 330 nm for Cu^{II}–cysteine solutions at high pH apparently characterizes the same species [18] (with identical decay behaviour, see later).

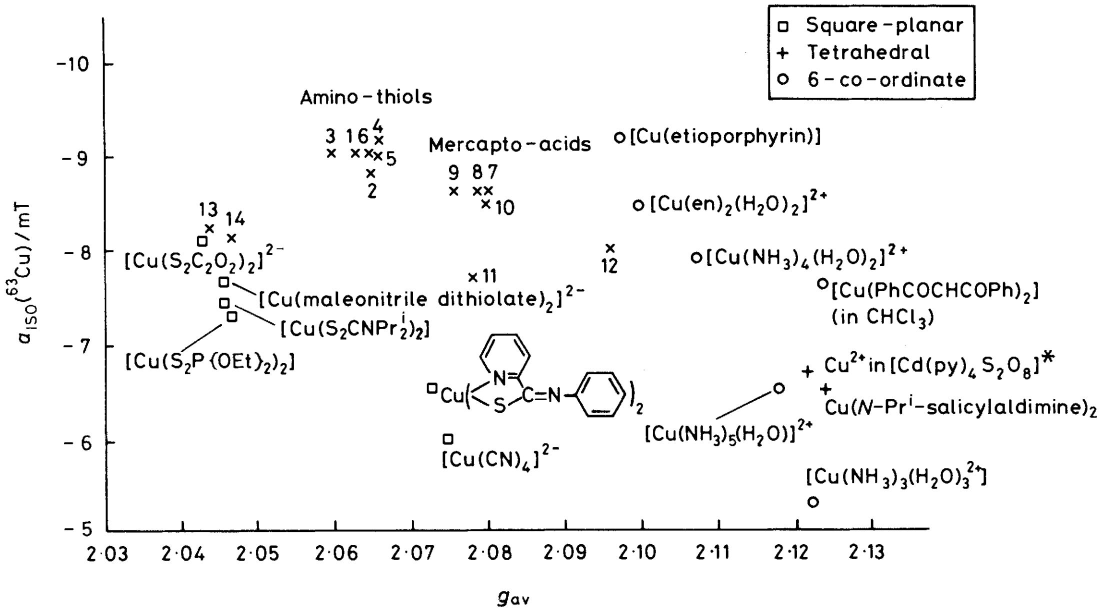


Figure 3. Values of a_{iso}(Cu) and g_{av} for a range of copper(II) complexes. The numbered points refer to the complexes studied here and listed in Table 1. * Calculated from anisotropic data

The spectra of the (mostly) short-lived transients characterize the formation of complexes which exhibit features typical of copper(II) complexes.[23] The basic four-line pattern arises as a result of coupling between the unpaired electron and the copper nuclei (^{63}Cu, ^{65}Cu, both of which have $I = 3/2$), and both measured splittings and g values are collected in Table 1. In some cases (*e.g.* Figure 2) which lack extra splittings, the small differences between the isotropic splittings for the two isotopes can be resolved in the narrow high-field line (and the observed separation and relative magnitudes of the lines are as expected on the basis of the gyromagnetic ratios and relative abundances of the two isotopes [24]).

One of the most notable features of the spectra is the relative narrowness of the lines compared with those of the Cu^{II}–ammonia complex (see above) or copper chelated by amino-acids lacking the thiolate substituent [25] (although, as with other copper complexes, the normal pattern [26] of reduction of line-widths across the spectrum from low to high field occurs *). In particular, the narrowness of the high-field ^{63}Cu line enables superhyperfine splittings to be resolved for the nitrogen-containing substrates (see *e.g.* Figure 1). We believe that the pattern is essentially a 1 : 2 : 3 : 2 : 1 quintet, from interaction of the unpaired electron with two equivalent nitrogen atoms in the ligands, though two extra small features indicated on the spectrum need to be explained. The small peak at the high-field end of the well resolved peak (marked ○) is assigned to the extreme high-field peak of the resonances associated with the ^{65}Cu isotope, and both its position and intensity are in accord with this. The small peak (marked ×) at the low-field extreme of the high-field group of lines is, as revealed by careful measurement of line-separations, not part of the splitting pattern associated with the main signal detected or with the ^{65}Cu analogue, and we suggest that it is part of a minor signal associated with an isomer of the major complex detected (see later).

The parameters for the complexes are characterized by relatively low values of g and high values of a(Cu) when compared with the range of parameters established for other copper complexes (see *e.g.* ref. 23 and references therein); such a combination of features is generally taken to indicate the possession of square-planar geometry (see *e.g.* refs. 27 and 28), though weak interaction with additional ligands, *e.g.* the axial ligands in octahedral complexes, cannot be excluded. The relatively low g values are a result of strong covalent interaction with equatorial ligands (which tends to quench the mixing of orbital angular momentum and the electron spin). The large (negative) values of a(Cu) can be explained in terms of spin-polarisation of the inner electrons: [28] the general tendency for a(Cu) to increase with decrease in g for complexes of this type (see *e.g.* Figure 3, in which our results are incorporated with those for a range of known copper complexes) is interpreted in terms of two effects, namely the increase in spin-polarisation involving metal–ligand bonds with increased covalency and the reduction in (positive) orbital contributions to a(Cu) as Δg (*i.e.* g — 2.0023) increases.

As the results in Table 1 and Figure 3 show, the parameters are correlated with the nature of the ligands bonded to copper, namely nitrogen and sulphur for L-cysteine, D-penicillamine, and related compounds † (to give complexes referred to as of CuN$_2$S$_2$ type, as previously described for penicillamine in ref. 2) and, by analogy, CuO$_2$S$_2$ and CuS$_4$ types for mercapto-acids and 1,2-dithiols. The decrease in g from CuO$_2$S$_2$ to CuN$_2$S$_2$ and then CuS$_4$ types is presumably indicative of an increase in the extent of covalent bonding in the order O < N < S, as would be expected; the somewhat low values of a(Cu) for HSCH$_2$CH$_2$SH and HSCHMeCH$_2$-SH may indicate some distortion from planarity (*cf. e.g.* the *N*-isopropylsalicylaldimine complex [28]).

Our results suggest that the formation of relatively long-lived thiolato-complexes at high pH requires the presence of a functional group in addition to $^-$S$^-$ in order to enable the

---

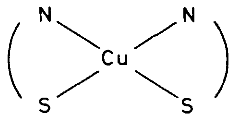
* The motional modulation of g- and A-tensors for copper complexes [26] will not be discussed further here, though it should perhaps be noted that the relatively small line-widths associated with these complexes indicate that the degree of anisotropy is low (see also later).

† The nitrogen splitting pattern confirms the presence of two nitrogens; the remaining sites must be occupied by sulphur, rather than carboxylate oxygen (*cf.* results for the corresponding de-carboxylated thiols).

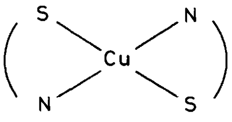
Table 1. Isotropic e.s.r. parameters for copper(II) complexes formed from D-penicillamine, L-cysteine, and related species at pH *ca.* 10

Thiol (RSH)	$a(^{63}Cu)$/mT [a]	a(N)/mT [b]	g_{iso} [c]	ΔH/mT [d]
1 D-$H_2NCH(CO_2^-)CMe_2SH$	-9.0	1.04	2.063	0.53
2 $H_2NCH_2CMe_2SH$	-8.8	1.05	2.065	0.53
3 L-$H_2NCH(CO_2^-)CH_2SH$	-9.0	1.05	2.060	0.56
4 $H_2NCH_2CH_2SH$	-9.1	1.13	2.066	0.64
5 $H_2NCH_2CHMeSH$	-9.0	1.12	2.066	0.55
6 $H_2NCHEtCH_2SH$	-9.0	1.08	2.065	0.58
7 $^-O_2CCH_2SH$	-8.6		2.080	0.7
8 $^-O_2CCHMeSH$	-8.6		2.079	0.7
9 $^-O_2CCH_2CH(CO_2^-)SH$	-8.6		2.076	0.7
10 $^-O_2CCH_2CH_2SH$	-8.5		2.080	0.7
11 $MeCONHCH(CO_2^-)CMe_2SH$	-7.7		2.078	0.7
12 o-$HSC_6H_4CO_2^-$	-8.0		2.096	0.7
13 $HSCHMeCH_2SH$	-8.2		2.044	0.5
14 $HSCH_2CH_2SH$	-8.1		2.047	0.5

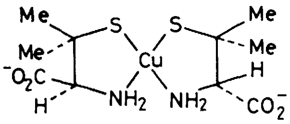
[a] ±0.1 mT. [b] ±0.03 mT. [c] Experimental values, ±0.001. [d] Linewidth of high-field line, typically ±0.03 mT for amino-thiol complexes, ±0.1 mT for other complexes.



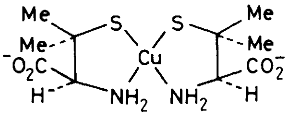
(3)



(4)



(5)



(6)

molecule to function as a bidentate ligand. Of the substrates studied which failed to give detectable signals from copper(II) complexes, benzenethiol possesses no such functional group, the hydroxy-substituent in 2-mercaptoethanol evidently cannot provide the necessary interaction (unlike the carboxylate function), and for 3-mercaptopropylamine it seems likely that the incipient six-membered ring is less favourable than the corresponding five-membered ring in the analogous complexes from $NH_2CH_2CH_2SH$ and related species. As inspection of Table 1 shows, however, complexes are formed from CuO_2S_2 systems with both five- and six-membered rings.

Close inspection of the high-field copper resonances from complexes containing the CuN_2S_2 chromophore [see *e.g.* Figure 1, and similar spectra from other amino acids including L-cysteine and from the (decarboxylated) substrate 2-mercapto-2-methylpropylamine] indicates an extra line at the low-field end of the nitrogen superhyperfine splittings. Similar additional lines have been detected in spectra of isotopically enriched (^{63}Cu) copper(II)–bis(aminoacid) complexes [25] and attributed to the occurrence of *cis*- and *trans*-isomers of the complexes. We propose by analogy that two forms of the CuN_2S_2 complexes exist, namely the *cis*- and *trans*-forms represented as (3) and (4), respectively.*

The major radical in each case has the smaller g value and/or a larger isotropic copper splitting [together with a slightly lower a(N)] which implies that in this isomer there is a slightly greater degree of covalency in the Cu–S bonds. We suggest that this characterizes the *cis*-structure (3) since, as a result of the '*trans*'-effect and the greater *trans*-directing influence of S^- compared with $-NH_2$,[29,30] the Cu–S bond should be shorter in (3) (*cf.* also ref. 25). A predominance of the *cis*-isomer is not unexpected in view of the structure of the red-violet mixed valence copper–penicillamine structure [in which the copper(II) is bound to two *cis*-chelating thiol ligands [14]]. It may also be relevant that the *cis*-structure (5) (illustrated for D-penicillamine itself) has the two carboxylate groups located on opposite sides of the molecule (but see also below). In contrast, for analogous CuN_2O_2 complexes the *trans*-form is evidently preferred.[25]

Although the copper(II) complexes of D- and L-penicillamine were found (as would be expected) to possess identical spectra, the spectra obtained from Cu^{II} in the presence of mixtures of the enantiomers showed small but significant changes. Although the proportion of *cis*- to *trans*-isomers remained unaltered, the position of the high-field copper resonance in the major signal was observed to shift: the maximum shift, 0.15 mT downfield (monitored with reference to the high-field nitrogen line in the e.s.r. spectrum of Fremy's salt [31]) was obtained when a racemic mixture of D- and L-penicillamine was used. It seems likely that the spectra observed are composite and overlapping signals (with slightly different parameters) from the Cu^{II}–(D-penicillamine)$_2$ [or Cu^{II}–(L-penicillamine)$_2$] complex and the Cu^{II}–(D-penicillamine) (L-penicillamine) complex (6). If this is the correct interpretation it implies that the extra stability of the *cis*- rather than the *trans*-complex (see above) is associated with the *cis*-arrangement of the two Cu–S bonds rather than the spatial relationships of carboxylate groups.

(ii) *Decay of copper*(II)*–thiolate complexes.* For some of the longer lived complexes formed at high pH, e.s.r. spectroscopy was employed to monitor their decay when the flow was stopped. Experiments were carried out with two solutions containing, typically, copper(II) (2×10^{-3} mol dm^{-3}) in aqueous ammonia and the appropriate thiol (in excess, in the range 1—5 $\times 10^{-2}$ mol dm^{-3}), the pH after mixing being adjusted to *ca.* 11.5.

Initial experiments in which [complex] was monitored as a function of t and for different [RSH] showed that the decays are essentially second order in complex (at least for the early stages of the reaction) and that when these relatively high concentrations of thiol are employed, the rate of decay is independent of [RSH]. The second-order dependence of the removal of the complexes was confirmed by a series of experiments in which initial rates of decay were obtained. The result-

---

* It is possible that similar isomerism is also exhibited by the complexes with CuO_2S_2 structure, but no separate lines could be resolved.

ing second-order rate constants are listed in Table 2 [the value for cysteine is in excellent agreement with that measured from decay of the absorption at 330 nm for copper(II)–cysteine mixtures in stopped-flow experiments].[18] Also included are values for the removal of the short-lived 2-mercaptopropanoic acid complex (measured *via* stop-flow experiments with spectrophotometric detection: see Experimental section) and an estimate of $2k_t$ for the particularly transient 2-mercaptoethanoic acid complex (this being derived by rapid-flow experiments from the measured concentration of complex, *ca.* 30 ms after mixing, on the assumption that the complex-forming reaction goes to completion at a time much shorter than this). The results suggest that, in general, CuN_2S_2 complexes are longer lived than CuO_2S_2 analogues (CuS_4 complexes are also longer lived, but the much slower rates of their formation preclude kinetic studies of their decay) and highlight the important retarding effect of a methyl group in the carbon skeleton. The latter effect, which is presumably steric in origin, probably accounts for the particular longevity under these conditions of the appropriate pencillamine complex.

(iii) *The formation of mixed* (*ternary*) *complexes with copper*(II). In a series of competitive experiments, three separate solutions containing copper(II)–ammonia (*ca.* 2 × 10^{-3} mol dm^{-3}), a mercapto-acid (*e.g.* 2-mercaptoethanoic acid, *ca.* 10^{-2} mol dm^{-3}), and an amino-thiol or amino acid such as L-cysteine, respectively, were simultaneously mixed prior to passage through the cavity of the e.s.r. spectrometer (other conditions being as described above). When the concentration of the amino-thiol was *ca.* 10^{-2} mol dm^{-3} the e.s.r. spectrum detected showed that only the copper complex of this substrate had been formed; it appears that complexes with a CuN_2S_2 chromophore are formed more readily than those with the CuO_2S_2 structure. However, as the concentration of the amino-thiol stream was reduced (so that an insufficient quantity for complete formation of the appropriate bis-chelate was present) the spectrum obtained showed that a mixture of copper complexes was present (at very low amino-thiol concentrations, only the mercapto acid complex could be detected). By studying spectra in the intermediate region and employing spectrum storage and substraction techniques, it was possible to obtain spectra characteristic of *mixed* (ternary) complexes of copper with two different thiols [*cf.* the use [20] of e.s.r. to characterize a ternary complex of copper(II) with glycylglycine and cysteine]. For example, Figure 4a shows the spectrum of the mixture of copper complexes detected in experiments with copper(II), cysteine, and mercaptoethanoic acid, Figure 4b shows the spectrum from cysteine and copper(II) alone, and Figure 4c shows the result of subtracting 4a from 4b: it shows interaction with a single nitrogen atom and is attributed to the complex (7). Results for other ternary complexes characterized in this way (including details on the glycylglycine–cysteine complex) are collected in Table 3; as might be expected, the values of the parameters *a* and *g* for the mixed complexes generally lie between those obtained for the bis-complexes of the component thiols.

Table 2. Second-order rate constants for the decay of bisthiolato-copper(II) complexes [a]

	Ligand	10^{-2} × $2k$/ mol^{-1} dm^3 s^{-1}
S_2N_2 type	$HSCHMeCH_2NH_2$	0.43 ± 0.05
	$HSCH_2CH(NH_2)CO_2H$	0.50 ± 0.05
	$HSCH_2CHEtNH_2$	2.5 ± 0.4
	$HSCH_2CH_2NH_2$	7.3 ± 0.8
S_2O_2 type	$HSCH(CO_2H)CH_2CO_2H$	2.0 ± 0.5
	$HSCHMeCO_2H$	110 ± 10 [b]
	$HSCH_2CO_2H$	700 ± 100 [c]

[a] Unless otherwise stated, obtained from stopped-flow e.s.r. experiments with, typically [Cu^{II}] 2 × 10^{-3} mol dm^{-3}, [thiol] 1—5 × 10^{-2} mol dm^{-3} at pH *ca.* 11.5. [b] Determined from stopped-flow spectrophotometric experiments. [c] Estimated from continuous-flow experiments in which [complex] was measured *ca.* 30 ms after mixing.

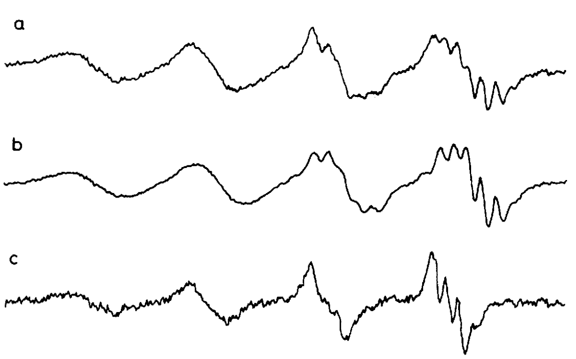
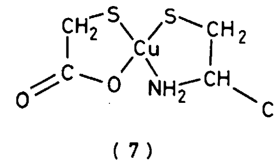


Figure 4. E.s.r. spectra from rapid mixing experiments (at pH 10) involving: a, copper(II) (2 × 10^{-3} mol dm^{-3}), L-cysteine (3.3 × 10^{-3} mol dm^{-3}), and mercaptoethanoic acid (3 × 10^{-2} mol dm^{-3}) and b, copper(II) (2 × 10^{-3} mol dm^{-3}) and L-cysteine (6.6 × 10^{-3} mol dm^{-3}). Figure 4c was obtained by subtraction of 4b (at half the intensity) from 4a and is assigned to the ternary complex Cu^{II}[L-cysteine][mercaptoethanoate] (see text)



(7)

(c) *Anisotropic Spectra from* Cu^{II}*–Thiolate Complexes.*— In order to obtain further structural information from anisotropic spectra for copper(II)–thiol complexes formed at

Table 3. Isotropic e.s.r. parameters for mixed complexes of copper(II) formed at pH *ca.* 10

Complex	$a(^{63}Cu)$/mT [a]	a(N)/mT [b]	g [c]
$Cu^{II}[CH(SH)(CO_2H)CH_2CO_2H]$ [d]$[L\text{-}H_2NCH(CO_2H)CH_2SH]$	(—) 8.65	0.85 (1N)	2.070
$Cu^{II}[HO_2CCH_2SH][L\text{-}H_2NCH(CO_2H)CH_2SH]$	(—) 8.30	0.92 (1N)	2.074
$Cu^{II}[HSCH_2CH_2SH][L\text{-}H_2NCH(CO_2H)CH_2SH]$	(—) 8.40	1.05 (1N)	2.056
$Cu^{II}[HO_2CCH_2SH][HSCH_2CH_2SH]$	(—) 8.16		2.066
Cu^{II}[glygly] [e]$[L\text{-}H_2NCH(CO_2H)CH_2SH]$	(—) 8.75	1.17 (3N)	2.074
$Cu^{II}[CH(SH)(CO_2H)CH_2CO_2H]$ [d]$[D\text{-}H_2NCH(CO_2H)CMe_2SH]$	(—) 8.40	1.00 (1N)	2.068

[a] ±0.2. [b] ±0.1. [c] ±0.001. [d] For the 2-mercaptosuccinic acid ligand, co-ordination may be *via* a five- or six-membered ring. The former structure appears more likely (see text). [e] Glycylglycine.

pH > 7 (and to investigate the possibility that $Cu^{II} \cdots Cu^{II}$ ' dimers ' might be trapped in the solid state), we froze mixtures of copper(II) and thiols. For the longer lived complexes (those from propane-1,2-dithiol, 2-mercapto-2-methylpropylamine, and D-penicillamine) the anisotropic spectra were readily obtained by placing standard cylindrical e.s.r. tubes containing a freshly prepared solution of the complex in a Dewar insert filled with liquid nitrogen. These spectra (*e.g.* Figure 5) show well resolved perpendicular and parallel features (with copper splittings); for the amino-substituted thiols, the spectra exhibit nitrogen splittings on the perpendicular features.

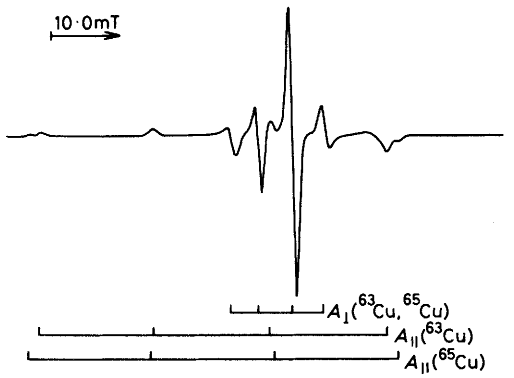


Figure 5. E.s.r. spectrum obtained following immediate freezing of rapidly mixed solutions of copper(II)–ammonia and propane-1,2-dithiol at pH *ca.* 10 (recorded at 77 K)

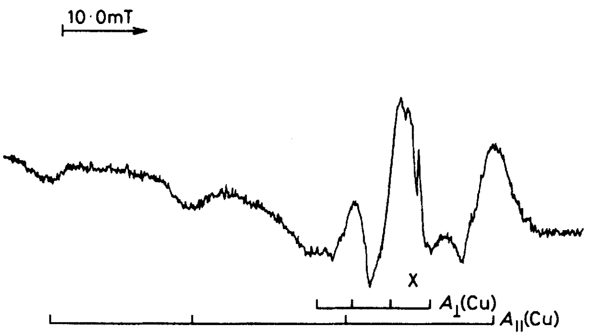


Figure 6. E.s.r. spectrum obtained following immediate freezing of rapidly mixed solutions of copper(II)–ammonia and 2-mercapto-ethylamine at pH *ca.* 10 (the signal marked × arises from a paramagnetic impurity in the glass cell)

For less long lived analogues, spectra could nevertheless be obtained by a technique which involved the rapid mixing of solutions of copper(II) and thiol (with concentrations as described above) with a two-way mixing chamber arranged such that the effluent solution could be injected directly into a flask containing liquid nitrogen; the ice so obtained was crushed and placed in a cylindrical e.s.r. tube under liquid nitrogen (we estimate that intermediates may be trapped in this way *ca.* 0.1 s after mixing). Spectra were readily obtained for a variety of amino-thiols (*e.g.* Figure 6) and, although these are less well defined than those of the longer lived analogues (*e.g.* Figure 5), the parameters indicate that analogous types of complex have been formed (Table 4). For the mercapto-acids, spectra were less readily obtained: optimum conditions for their detection appear to require a stoicheiometry of somewhat less than 2 : 1 (thiol to copper), although under these circumstances a broad extra signal (possibly from a polymeric species, see later) was also obtained. Even under these conditions no signals from 2-mercapto-ethanoate and 3-mercaptopropanoate complexes could be obtained.

The anisotropic parameters for the copper complexes (Table 4) are distinguished from those generally observed [28] for copper(II) complexes by relatively low values for g (both $g_{\perp}$ and $g_{\parallel}$) and $|A_{\parallel}|$. The results presented in Table 4 have also been corrected (to take into account the orbital contribution to $A_{\parallel}$ and $A_{\perp}$), using equations (2) and (3), in order to obtain values for A_{iso} and $2B$.[32] They show that whereas the values for A are generally close to those expected for A_{iso} for square-planar complexes, the corresponding values of $2B$ are very low (values of *ca.* -10.5 and -17.0 mT characterize square-planar complexes; for tetrahedral geometry typical values are -3.75 and -15.25 mT, respectively).

$$A_{\parallel} = A + 2B\left(1 - \frac{7}{4}\Delta g_{\parallel} - \frac{3}{4}\Delta g_{\perp}\right) \qquad (2)$$

$$A_{\perp} = \mathrm{A} - B\left(1 + \frac{11}{4}\Delta g_{\perp}\right) \qquad (3)$$

The low value for $2B$ indicates that the spin-density in the $d_{x^2-y^2}$ orbital on copper is significantly lowered, presumably because of effective delocalisation of the unpaired electron onto the ligands. Taking $2B°$ as -23.5 mT [33] we calculate ρ(Cu) as *ca.* 44% in these complexes (with somewhat lower

Table 4. Anisotropic parameters obtained from frozen solutions of Cu(II)–thiolate complexes (pH *ca.* 10)

Thiol	$A(Cu)_{\parallel}$ [a]/ mT	$A(Cu)_{\perp}$ [a]/ mT	$A(Cu)_{iso}$ [b]/ mT	$2B$ [c]/ mT	$A(N)$ [d]/ mT	$g_{\parallel}$ [e]	$g_{\perp}$ [e]	g_{av} [e]
D-$H_2NCH(CO_2H)CMe_2SH$	−18.5	−5.3	−11.0	−10.4	1.2	2.116	2.038	2.06
$H_2NCH_2CMe_2SH$	−18.5	−5.3	−11.1	−10.4	1.2	2.116	2.035	2.06
L-$H_2NCH(CO_2H)CH_2SH$	−18.5	−5.5	−11.1	−10.3	1.2	2.118	2.037	2.06
$H_2NCH_2CH_2SH$	−18.5	−5.5	−11.2	−10.3	— [f]	2.117	2.037	2.06
$H_2NCH_2CHMeSH$	−18.3	−5.3	−11.0	−10.2	1.2	2.117	2.039	2.07
$H_2NCHEtCH_2SH$	−18.3	−5.3	−10.9	−10.2	— [f]	2.115	2.033	2.06
o-$H_2NC_6H_4SH$	−14.8	−3.7	−8.7	−9.1		2.145	2.042	2.08
$CHMe(SH)(CO_2H)$	−19.3	−4.8	−11.4	−11.9		2.145	2.048	2.08
$CH(SH)(CO_2H)CH_2CO_2H$	— [g]	−3.0	—	—		— [g]	2.036	
o-$H_2NC_6H_4CO_2^-$	−16.0	— [h]	−10.0 [i]	−10.6 [i]		2.181	2.040	2.09
$CHMe(SH)CH_2SH$	−17.5	−4.8	−9.9	−9.6		2.085	2.025	2.05

[a] ±0.5 mT (taken as one-third of the separation between m_I −3/2 and m_I 3/2 lines). [b] Corrected value, see text. [c] ±0.7 mT. [d] ±0.1 mT. [e] ±0.005. [f] Splitting from nitrogen not resolved. [g] Weak signals: $A_{\parallel}$ not obtained here but previously reported [15] as 12.5 mT. [h] Splitting not resolved in parallel region. [i] Estimated (± *ca.* 2 mT) taking $A_{\perp}$ 4 mT.

values for the 1,2-dithio- and 2-mercapto-aminobenzene complexes).

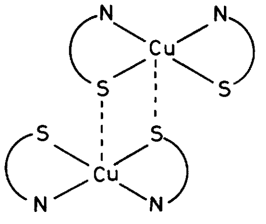
This adds further weight to our conclusion from the isotropic data that the complexes possess a significant degree of covalent character. The isotropic nitrogen splittings of *ca.* 1.1 mT in the amino-complexes suggest 2% *s* character giving *ca.* 8% spin density for sp^3-hybridized nitrogen. Thus we can make the crude estimate of *ca.* 16% spin density on nitrogen, *ca.* 44% spin density on copper, and hence *ca.* 20% spin density on each sulphur in the disulphur derivatives. For the tetrasulphur-liganded complexes, we suggest that there is *ca.* 40% of the unpaired electron on the copper and *ca.* 15% associated with each sulphur.

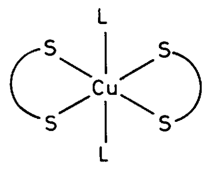
We searched unsuccessfully for e.s.r. evidence for the formation of dimeric copper(II) complexes of the type (8). Related dimer species, extensively studied by e.s.r. spectroscopy (particularly by Smith and Pilbrow[34,35]), are characterized by solid-state spectra containing both $\Delta m = \pm 1$ transitions (at *g ca.* 2) and ' forbidden ' $\Delta m = \pm 2$ transitions (at *g ca.* 4) which also often exhibit coupling to two copper nuclei.[36] However, we were unable to detect any such spectra in a variety of experiments in which rapidly mixed solutions of copper(II) and various representative substrates were frozen as explained above (with times between mixing and freezing of between 0.1 and 60 s).

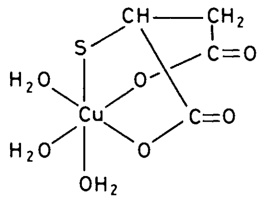
We also searched for evidence for ligand interaction in the axial sites [as represented by (9)] for the relatively long lived bisthiolatocopper(II) complexes [*cf. e.g.* the variation in the e.s.r. parameters of the bis(pentane-2,4-dionato)copper(II) complex in frozen solutions containing different nitrogen-containing heterocycles[37]]. Anisotropic spectra were obtained from the copper(II)–D-penicillamine complex by freezing dimethyl sulphoxide solutions containing either water, ammonia, pyridine, or aniline, but only when KOH (*ca.* 10^{-2} mol dm^{-3}) was also added; since the spectra were identical with each other [though slightly better resolved than when water was employed as solvent (see earlier)] it appears that different axially substituted complexes are not obtained with these substrates. Likewise, with the more basic ligands piperidine and 1,2-diaminoethane, though the bisthiolato-complex was detected in the absence of extra base, the spectra showed no significant differences.

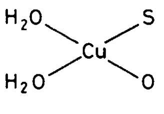
(d) *Complexes formed at High pH and Low Thiol Concentrations.*—When insufficient thiol was available for complete reduction of copper(II) to copper(I), it was found that the isotropic spectra detected depended upon the relative concentrations of Cu^{II} and thiol. For example, on admixture of an ammoniacal solution of $CuCl_2$ (*ca.* 10^{-3} mol dm^{-3}) and cysteine in a continuous flow system, it was found that for $[Cu^{II}] : [RSH] > 1 : 1$ only signals from the copper(II)–ammonia complex were detected. The intensity of the signals decreased with increasing thiol concentrations, but not with time after the flow was stopped; any copper(II) complexes formed must be either too broad for detection or very short lived. At higher thiol concentrations the signal from bis(cysteinato)copper(II) was detected, its intensity increasing up to a maximum for [Cu] : [RSH] 1 : 2.

Rapid freezing of mixtures of copper(II) in ammonia with relatively low concentrations of thiols (including 2-mercaptobenzoic acid and 2-mercaptopropanoic acid) gave spectra from undefined copper complexes which showed a very broad line at *g* 2.06 (usually together with signals from either the Cu^{II}-ammonia or bisthiolato complexes).


(8)


(9)


(10)


(11)

(e) *Complexes Detected at Low pH.*—Below pH 6, spectra from the bisthiolato complexes could not be detected, either in fluid or frozen solution. Reaction of copper(II) with various thiols which also possess a carboxylic acid substituent (such as L-cysteine and D-penicillamine) at pH *ca.* 2.5 gave a brown-coloured transient; there was no detectable e.s.r. signal from fluid solution but rapid freezing led to the characterization of anisotropic spectra attributed to the appropriate Cu^{II} complexes. (The spectra were also often accompanied by a broad signal at *g ca.* 2, which may well be from polymeric mixed valence species of the type described previously.[11,14]) No spectra could be detected for amino-thiol substrates.

The anisotropic spectra from cysteine and three related thiols (Table 5) have parameters typical for copper(II) in complexes with axial symmetry and with the unpaired electron in the $d_{x_2-y_2}$ orbital. These parameters are distinguished from those for the appropriate high pH bisthiolato analogues principally by the higher values of $g_{\parallel}$ and $g_{\perp}$ ($A_{\parallel}$ being somewhat lower, *cf.* Table 4). ' Corrected ' values of $2B$ and A_{iso} are included in Table 5; as with the high pH results, the values calculated for $2B$ are unusually low (though somewhat higher than for high pH), indicating that these complexes also exhibit extensive delocalization of the unpaired electron. Our results, which suggest that a thiolate sulphur and carboxylic acid function are required for complex formation, are analogous to those reported by Lappin and McAuley[15] for the transient intermediate formed between copper(II) and 2-mercaptosuccinic acid in the pH range 1—4 and for which a 1 : 1 complex structure (10) has been suggested. However, our analysis and, in particular, the observation of low values of $g_{\parallel}$, $A_{\parallel}$, and $2B$ suggest that the sulphur probably occupies an equatorial site [as indicated in (11)].

(f) *Conclusions.*—Our results establish the existence of a series of copper(II) complexes [CuL_2] of thiolate derivatives of CuS_4, CuS_2N_2, and CuS_2O_2 type at high pH. Evidence has been presented that these possess square-planar geometry, with considerable covalent character and delocalisation of the unpaired electron onto the ligands. Some, notably that from D-penicillamine, are long lived under these conditions; others undergo second-order decay and it seems likely that the formation and dimerization of such transients play a key role in the oxidation of such thiols by Cu^{II}. An attractive possibility is that dimerization involves the co-ordination of a sulphur atom in one complex with the copper in a second molecule [*cf.* structure (8), though direct evidence for such a $Cu^{II} \cdots Cu^{II}$ interaction was not obtained], followed by reorganisation of the bonds to give the appropriate disulphide and the

Table 5. Anisotropic parameters obtained from frozen solutions for the transient species formed from copper(II) and thiols in acid solution

Thiol	$A(\mathrm{Cu})_{\parallel}$/mT [a]	$A(\mathrm{Cu})_{\perp}$/mT [a]	$A(\mathrm{Cu})_{\mathrm{iso}}$/mT [b]	$2B$/mT [b]	$g_{\parallel}$ [c]	$g_{\perp}$ [c]	g_{av} [c]
$HO_2CCH_2\overset{SH}{\overset{\vert}{C}}HCO_2H$	−17.5	−4.1	−10.3	−11.0	2.143	2.045	2.08
HO_2CCH_2SH	−17.9	−3.7	−10.1	−11.6	2.138	2.036	2.07
$HO_2C\overset{NH_2}{\overset{\vert}{C}}HCH_2SH$	−17.0	−4.0	−10.3	−10.5	2.140	2.050	2.08
$HO_2C\overset{NH_2}{\overset{\vert}{C}}HCMe_2SH$	−17.8	−4.0	−10.4	−11.3	2.147	2.045	2.08

[a] ±0.5 mT. [b] ' Corrected ' value (see text). [c] ±0.005.

Cu^I complex of the ligand. There is no evidence for free-radical formation and dimerization.

Copper-thiol complexes of related type may exist (transiently) at lower pH and, in particular, it seems likely that the intensely coloured $Cu^I \cdots Cu^{II}$ complexes formed at relatively low thiol concentrations (*e.g.* the red-violet complex formed from penicillamine) are derived by subsequent reaction of CuL_2 complexes similar to those described here.

Experimental

The e.s.r. spectrometer, flow-system, and pH measurement have been described previously (see *e.g.* ref. 38 and references therein). A Datalab DL 4000 microcomputer was used for spectrum subtraction and for double integration (for the determination of absolute radical concentrations, although in some cases a numerical method[23] was employed): concentrations of thiol-bound copper complexes were determined by comparison with signals from standard solutions of (unchanged) copper(II). Isotropic coupling constants and *g* values were generally determined by comparison with those of Fremy's salt[31] [a(N) 1.3091 mT, g 2.0055]; for frozen solutions the absorption from a paramagnetic impurity trapped in the glass (g 2.0005) was generally used as a marker. Electronic absorption spectra were recorded on a Pye Unicam SP500 spectrometer which had been adapted to take the continuous flow system. The stopped-flow experiments were undertaken on an instrument supplied by Applied Photophysics Ltd. All solutions to be mixed were purged with nitrogen before use and all kinetic experiments were carried out at ambient temperatures (16—18 °C).

For experiments using D-penicillamine a standard aqueous solution ' flattened ' cell, rather than a flow cell, was employed. For experiments with ethane-1,2-dithiol, propane-1,2-dithiol, and benzenethiol, a small quantity of ethanol was generally added to enhance solubility; in addition, with these and other noxious substrates, the effluent stream was run into a solution of potassium permanganate to destroy unchanged thiol. Experiments with the *aci*-anion of nitromethane as a spin trap were carried out as described previously.[21,38]

The materials used were obtained commercially (and used without further purification) except for D-, DL-, and L-penicillamine (which were kindly provided by Roche Products Ltd.), and 2-mercapto-2-methylpropylamine which was synthesised by the method of Carroll *et al.*[39]

Acknowledgements

We thank the S.E.R.C. and Roche Products Ltd. for a CASE Award (to F. J. D.), and Dr. B. P. Tong for helpful discussions.

References

1 Part 65, B. Ashworth, M. J. Davies, B. C. Gilbert, and R. O. C. Norman, preceding paper.
2 W. E. Blumberg and J. Peisach, *J. Chem. Phys.*, 1968, **49**, 1793.
3 E. W. Wilson, Jr. and R. B. Martin, *Arch. Biochem. Biophys.*, 1971, **142**, 445.
4 I. M. Klotz, J. M. Urquhart, and H. A. Fiess, *J. Am. Chem. Soc.*, 1952, **74**, 5537; J. A. Fee, *Struct. Bonding*, 1975, **23**, 1.
5 See *e.g. Brit. Med. J.*, 1971, **2**, 270.
6 D. A. Gerber, *Biochem. Pharmacol.*, 1978, **27**, 469; J. R. Sorensen, *J. Med. Chem.*, 1976, **19**, 135.
7 M. Younes and U. Weser, *Biochem. Biophys. Res. Commun.*, 1977, **78**, 1247.
8 A. R. Amundsen, J. Whelan, and B. Bosnich, *J. Am. Chem. Soc.*, 1977, **99**, 6730.
9 P. M. Colman, H. C. Freeman, J. M. Guss, M. Murata, V. A. Norris, J. A. M. Ramsham, and M. P. Venkatappa, *Nature*, 1978, **272**, 319.
10 S. H. Laurie, T. Lund, and J. B. Raynor, *J. Chem. Soc., Dalton Trans.*, 1975, 1389.
11 I. M. Klotz, G. H. Czerlinski, and H. A. Fiess, *J. Am. Chem. Soc.*, 1958, **80**, 2920.
12 J. J. Vallon and A. Badinand, *Anal. Chim. Acta*, 1968, **42**, 445.
13 H. J. Schugar, C. Ou, J. A. Thich, J. A. Potenza, R. A. Lalancette, and W. Furey, Jr., *J. Am. Chem. Soc.*, 1976, **98**, 3047.
14 P. J. M. W. L. Birker and H. C. Freeman, *J. Am. Chem. Soc.*, 1977, **99**, 6890.
15 A. G. Lappin and A. McAuley, *J. Chem. Soc., Dalton Trans.*, 1978, 1606.
16 P. Kroneck, C. Naumann, and P. Hemmerich, *Inorg. Nucl. Chem. Lett.*, 1971, **7**, 659.
17 P. Hemmerich, H. Beinert, and T. Vänngard, *Angew. Chem., Int. Ed. Engl.*, 1966, **5**, 422.
18 D. Cavallini, C. de Marco, S. Dupré, and G. Rotilio, *Arch. Biochem. Biophys.*, 1969, **130**, 354.
19 G. Rotilo, C. de Marco, and S. Dupré, *Magn. Resonance Biol. Res. Rep. Int. 3rd Conf.*, 1969, 155.
20 A. Hanaki, *Chem. Lett.*, 1976, 1225.
21 B. C. Gilbert, H. A. H. Laue, R. O. C. Norman, and R. C. Sealy, *J. Chem. Soc., Perkin Trans. 2*, 1975, 892.
22 W. B. Lewis, M. Alei, and L. O. Morgan, *J. Chem. Phys.*, 1966, **45**, 4003.
23 P. B. Ayscough, ' Electron Spin Resonance in Chemistry,' Methuen, London, 1967.
24 H. M. Assenheim, ' Introduction to Electron Spin Resonance,' Hilger and Watts–Plenum Press, New York, 1967, p. 185.
25 B. A. Goodman, D. B. McPhail, and H. K. J. Powell, *J. Chem. Soc., Dalton Trans.*, 1981, 822.
26 B. R. McGarvey, *J. Phys. Chem.*, 1957, **61**, 1232.
27 H. A. Kuska and M. T. Rogers, *J. Chem. Phys.*, 1965, **43**, 1744.
28 B. A. Goodman and J. B. Raynor, *Adv. Inorg. Chem. Radiochem.*, 1970, **13**, 135.
29 J. N. Cooper, J. D. McCoy, M. G. Katz, and E. Deutsh, *Inorg. Chem.*, 1980, **19**, 2265.

30 R. C. Elder, L. R. Florian, R. E. Lake, and A. M. Yacynych, *Inorg. Chem.*, 1973, **12**, 2690.
31 R. J. Faber and G. K. Fraenkel, *J. Chem. Phys.*, 1967, **47**, 2462; J. Q. Adams, S. W. Nicksic, and J. R. Thomas, *ibid.*, 1966, **45**, 654.
32 M. C. R. Symons, D. X. West, and J. G. Wilkinson, *J. Chem. Soc., Dalton Trans.*, 1975, 1696.
33 M. C. R. Symons, 'Chemical and Biochemical Aspects of Electron Spin Resonance Spectroscopy,' Van Nostrand Reinhold, Wokingham, 1978, p. 176.
34 J. F. Boas, J. R. Pilbrow, C. R. Hartzell, and T. D. Smith, *J. Chem. Soc. A*, 1969, 572.
35 J. R. Pilbrow, A. D. Toy, and T. D. Smith, *J. Chem. Soc. A*, 1969, 1029.
36 J. F. Boas, J. R. Pilbrow, and T. D. Smith, in 'Biological Magnetic Resonance,' eds. L. J. Berliner and J. Reuben, Plenum Press, New York, 1978, vol. 1, p. 277.
37 T. Kogane, R. Hirota, K. Abe, and M. Hirota, *J. Chem. Soc., Perkin Trans. 2*, 1981, 652.
38 B. C. Gilbert, P. D. R. Marshall, R. O. C. Norman, N. Pineda, and P. S. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1981, 1392.
39 F. I. Carroll, J. D. White, and M. E. Wall, *J. Org. Chem.*, 1963, 1240.

Received 14*th March* 1983; *Paper* 3/392