Crystal Structure and Carbon-13 Nuclear Magnetic Resonance Spectrum of $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$ † and Electron Spin Resonance Spectrum of $[Fe_4S_4(SCH_2CH_2OH)_4]^{3-}$ in Aqueous Solution

By **George Christou** and **C. David Garner,*** Department of Chemistry, Manchester University, Manchester M13 9PL
Michael G. B. Drew, Department of Chemistry, The University, Whiteknights, Reading RG6 2AD
Richard Cammack, Kings College, Department of Plant Sciences, 68, Half Moon Lane, London SE24 9JF

The compound $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$ is triclinic, space group $P\bar{1}$, with $a = 10.686(9)$, $b = 11.288(8)$, $c = 15.021(11)$ Å, $\alpha = 99.0(1)$, $\beta = 91.8(1)$, $\gamma = 108.4(1)°$, and $Z = 2$. 2 115 Independent reflections measured on a diffractometer have been refined to $R = 0.088$. The anion contains the Fe_4S_4 cubane-like cluster core distorted from T_d symmetry such that four approximately parallel Fe–S bonds are shorter (mean 2.239 Å) than the other eight (mean 2.306 Å). There are two strong intermolecular O · · · H–O hydrogen bonds (O · · · O 2.63, 2.70 Å) and there may be an intermolecular S · · · H–O hydrogen bond(S · · · O 3.16 Å). Carbon-13 n.m.r. (90 MHz) spectra have been recorded for the title compound in dimethyl sulphoxide solution between 317 and 363 K; the resonance position of thiolate carbon was observed between 103.3 and 106.4 p.p.m. downfield of $SiMe_4$. The anion $[Fe_4S_4(SCH_2CH_2OH)_4]^{3-}$, generated by dithionite treatment of the title compound in buffered aqueous solution containing excess of 2-hydroxyethanethiol and stabilised by rapid freezing, exhibits an axially symmetric e.s.r. spectrum with $g_{\parallel} = 2.045$ and $g_{\perp} = 1.929$ ($\bar{g} = 1.967$); similar treatment of the title compound with $K_3[Fe(CN)_6]$ also generated a species having an axially symmetric e.s.r. spectrum, with $g_{\parallel} = 1.963$ and $g_{\perp} = 2.006$.

COMPLEXES of the type $[Fe_4S_4(SR)_4]^{2-}$, the synthetic analogues of the four- and eight-iron ferredoxin proteins, continue to be studied in detail [1] and the variation in the redox potentials of [4Fe–4S] centres in proteins is of particular interest. The midpoint reduction potential of the (equivalent of the) 2–/3– couple has been observed to vary from –280 mV (*vs.* standard hydrogen electrode, s.h.e.) in *Bacillus stearothermophilus* ferredoxin [2] to –490 mV in *Chromatium vinosum* 2[4Fe–4S] ferredoxin.[3] The potential of this couple in *C. vinosum* high-potential iron–sulphur protein (HiPIP) is presumably even lower and has not been measured in aqueous solution.[4] *Clostridium pasteurianum* 2[4Fe–4S] protein had midpoint reduction potentials of *ca.* –405 mV, similar to those of the corresponding couples of *Peptococcus aerogenes* 2[4Fe–4S] ferredoxin,[5] and the former protein has been shown [6] to have very similar redox potentials to $[Fe_4S_4(SR)_4]^{2-}$ {R = CH_2CH_2OH or *R,S*)-$CH_2CH[NH(COMe)]CONHMe$} complexes in water–dmso (dmso = dimethyl sulphoxide) media. These observations together with the near structural congruency of the [4Fe–4S] centres of *P. aerogenes* ferredoxin [7] and $[Fe_4S_4(SR)_4]^{n-}$ ($n = 2$, R = Ph [8] or CH_2Ph; [9] $n = 6$, R = $CH_2CH_2CO_2$ [10]) complexes suggest that those ferredoxins employing the 2–/3– couple at a potential significantly different from –400 mV do so due to structural and/or electronic influences of the protein. One important influence could be the hydrogen bonding, detected in *P. aerogenes* ferredoxin [11] *C. vinosum* HiPIP,[12] between the S atoms of the $Fe_4S_4(Scys)_4$ core [cys = $CH_2CH(NH_2)CO_2H$] and nearby NH protons of the polypeptide amide linkages. Such bonding is unknown for the synthetic analogues, structural characterisations of which have not been reported for ligated thiols which contain acidic hydrogens. Therefore, we have determined the crystal structure of $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$, the hydroxyl groups of which could participate in intra- or inter-cluster S · · · H–O bonding. This compound is also of interest in that its aqueous solubility permits the possibility of generating the $[Fe_4S_4(SCH_2CH_2OH)_4]^{z-}$ (where $z = 1$ or 3) ions in aqueous solution, thus allowing a direct comparison of the e.s.r. properties of a ' free ' Fe_4S_4 centre in this medium with those observed for the corresponding centres within a protein. The ^{13}C n.m.r. spectrum of $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$ has also been recorded for comparison with the corresponding spectra obtained [13] for proteins containing Fe_4S_4 clusters.

EXPERIMENTAL

The compound $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$ was prepared as described elsewhere [14] and crystals suitable for *X*-ray diffraction studies were obtained by recrystallisation from $MeCN–MeCO_2Et$.

Crystal Data.—$C_{16}H_{44}Fe_4N_2O_4S_8$, $M = 808.2$, Triclinic, $a = 10.686(9)$, $b = 11.288(8)$, $c = 15.021(11)$ Å, $\alpha = 99.0(1)$, $\beta = 91.8(1)$, $\gamma = 108.4(1)°$, $U = 1\,691.2$ Å^3, $D_c = 1.59$ g cm^{-3}, $Z = 2$, $D_m = 1.58$ g cm^{-3}, $\mu = 22.2$ cm^{-1}, λ(Mo-K_α) radiation = 0.710 7 Å, $F(000) = 836$. Space group $P\bar{1}$ by the successful structure determination.

A crystal with dimensions *ca.* 0.3 × 0.4 × 0.3 mm was mounted with the normal to the (100) face parallel to the instrument axis of a General Electric XRD 5 diffractometer which was equipped with a manual goniostat, scintillation counter, and pulse-height discriminator. Zirconium-filtered Mo-K_α radiation was used. The stationary-crystal–stationary-counter method was used with a 4° take-off angle and a counting time of 10 s. Individual backgrounds were taken from plots of background against 2θ. 2 916 Reflections were measured with $2\theta < 35°$ of which 2 115 with $I > \sigma(I)$ were used in subsequent calculations. Neither an absorption nor an extinction correction was applied.

The structure was solved by statistical methods using SHELX 76.[15] The iron and sulphur atoms were refined

---

† Bis(tetramethylammonium) tetrakis[(2-hydroxyethanethio-ato)-μ_3-sulphido-ferrate].

anisotropically and the carbon, nitrogen, and oxygen atoms were refined isotropically. The hydrogens of methylene groups were fixed in tetrahedral positions, with thermal parameters that were refined successfully. The hydrogen atoms on the methyl groups of the cations were not located. Refinement by full-matrix least squares converged at $R = 0.088$. A weighting scheme was chosen so that values of $w\Delta^2$ were obtained over ranges of F_o and $(\sin\theta)/\lambda$; $w^{\frac{1}{2}} = 1$ for $F_o < 50$ and $w = 50/F_o$ for $F_o > 50$. Calculations were made on a CDC 7600 at the University of London Computer Centre. Scattering factors and dispersion corrections were taken from ref. 16. The final list of positions is given in Table 1, bond lengths and angles in Table 2.

TABLE 1

Fractional atomic co-ordinates ($\times 10^4$) for $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$, with estimated standard deviations in parentheses

Atom	x/a	y/b	z/c
Fe(1)	3 691(4)	1 717(4)	2 407(3)
Fe(2)	2 638(5)	1 433(4)	4 015(3)
Fe(3)	1 533(4)	2 408(4)	2 757(3)
Fe(4)	1 309(4)	−106(4)	2 440(3)
S(1)	5 599(9)	2 290(8)	1 770(7)
S(2)	3 163(13)	1 379(11)	5 472(7)
S(3)	692(9)	3 969(9)	2 570(6)
S(4)	−80(9)	−2 120(8)	1 975(6)
S(5)	421(8)	937(8)	3 538(6)
S(6)	1 791(9)	1 212(8)	1 440(5)
S(7)	3 350(8)	14(8)	3 070(6)
S(8)	3 610(8)	3 295(7)	3 566(5)
C(11)	5 912(33)	3 931(31)	1 676(25)
C(12)	7 247(33)	4 530(33)	1 349(24)
O(13)	7 362(23)	3 944(23)	466(17)
C(21)	3 050(52)	2 990(48)	6 139(38)
C(22)	1 696(47)	2 847(45)	5 984(34)
O(23)	1 381(27)	3 936(27)	6 452(19)
C(31)	2 050(46)	5 163(44)	2 147(33)
C(32)	2 576(43)	4 795(41)	1 439(31)
O(33)	3 600(25)	5 801(24)	1 153(17)
C(41)	648(34)	−2 717(32)	998(23)
C(42)	303(36)	−2 241(35)	185(25)
O(43)	950(24)	−2 697(23)	−577(17)
N(1)	3 665(25)	1 175(24)	−1 196(17)
C(1)	3 416(35)	2 382(34)	−809(25)
C(2)	3 627(42)	970(40)	−2 196(30)
C(3)	2 608(40)	66(39)	−979(28)
C(4)	4 994(40)	1 114(38)	−906(28)
N(2)	7 513(27)	2 866(26)	4 837(19)
C(5)	6 984(46)	3 932(45)	5 109(33)
C(6)	8 807(67)	3 400(62)	4 521(49)
C(7)	8 026(57)	2 459(55)	5 605(43)
C(8)	6 652(70)	1 824(69)	4 193(54)

The final difference-Fourier map was featureless. The zero-weighted reflections gave no large discrepancies. Lists of structure factors and thermal parameters are given in Supplementary Publication No. SUP 23061, 20 pp.*

N.M.R. Spectra.—Carbon-13 n.m.r. spectra of $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$, in $[^2H_6]$dmso solution at temperatures between 317 and 363 K, were recorded at 90 MHz using a Bruker Spectrospin spectrometer.

E.S.R. Spectra.—The compound $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$ was dissolved in buffered aqueous solutions containing additional 2-hydroxyethanethiol to suppress hydrolysis,[6] and reduced or oxidised with a solution of sodium dithionite or $K_3[Fe(CN)_6]$ respectively. After a reaction time of *ca.* 15 s, the solutions were rapidly frozen in liquid nitrogen and their e.s.r. spectra were recorded at temperatures between 5 and 120 K on a Varian E4 spectrometer with an Oxford Instruments ESR9 liquid helium flow cryostat.

TABLE 2

Molecular dimensions (distances Å, angles °) for $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$ with estimated standard deviations in parentheses

(*a*) Fe_4S_4 cube

Fe(1)–S(7)	2.236(11)
Fe(2)–S(8)	2.249(9)
Fe(3)–S(5)	2.235(10)
Fe(4)–S(6)	2.237(8)
mean	2.24(1)

Fe(1)–S(6)	2.319(10)
Fe(1)–S(8)	2.313(9)
Fe(2)–S(5)	2.316(10)
Fe(2)–S(7)	2.300(10)
Fe(3)–S(6)	2.291(9)
Fe(3)–S(8)	2.334(8)
Fe(4)–S(5)	2.282(10)
Fe(4)–S(7)	2.304(10)
mean	2.31(1)

Fe(1)–Fe(2)	2.719(6)
Fe(1)–Fe(3)	2.700(7)
Fe(1)–Fe(4)	2.729(5)
Fe(2)–Fe(3)	2.740(7)
Fe(2)–Fe(4)	2.752(6)
Fe(3)–Fe(4)	2.736(6)
mean	2.729(17)

Fe(2)–Fe(1)–Fe(3)	60.7(2)
Fe(2)–Fe(1)–Fe(4)	60.7(2)
Fe(3)–Fe(1)–Fe(4)	60.5(2)
Fe(1)–Fe(2)–Fe(3)	59.3(2)
Fe(1)–Fe(2)–Fe(4)	59.8(2)
Fe(3)–Fe(2)–Fe(4)	59.8(2)
Fe(1)–Fe(3)–Fe(2)	60.0(2)
Fe(2)–Fe(3)–Fe(4)	60.3(2)
Fe(1)–Fe(3)–Fe(4)	60.2(2)
Fe(1)–Fe(4)–Fe(2)	59.5(2)
Fe(1)–Fe(4)–Fe(3)	59.2(2)
Fe(2)–Fe(4)–Fe(3)	59.9(2)
mean	60.0(5)

S(5)–Fe(3)–S(6)	102.6(3)
S(5)–Fe(3)–S(8)	103.7(3)
S(6)–Fe(3)–S(8)	107.3(3)
S(5)–Fe(4)–S(6)	102.9(3)
S(5)–Fe(4)–S(7)	105.6(3)
S(6)–Fe(4)–S(7)	104.0(4)
S(6)–Fe(1)–S(7)	103.5(3)
S(6)–Fe(1)–S(8)	107.0(4)
S(7)–Fe(1)–S(8)	103.8(3)
S(5)–Fe(2)–S(7)	104.7(3)
S(5)–Fe(2)–S(8)	103.8(4)
S(7)–Fe(2)–S(8)	103.8(4)
mean	104.3(1.4)

S(5)–Fe(3)–S(3)	117.9(4)
S(6)–Fe(3)–S(3)	114.8(4)
S(8)–Fe(3)–S(3)	109.4(3)
S(5)–Fe(4)–S(4)	110.0(3)
S(6)–Fe(4)–S(4)	119.0(3)
S(7)–Fe(4)–S(4)	114.0(4)
S(6)–Fe(1)–S(1)	115.7(4)
S(7)–Fe(1)–S(1)	114.8(4)
S(8)–Fe(1)–S(1)	111.0(3)
S(5)–Fe(2)–S(2)	117.9(5)
S(7)–Fe(2)–S(2)	110.0(5)
S(8)–Fe(2)–S(2)	115.2(4)
mean	114.2(3.2)

Fe(2)–S(5)–Fe(3)	74.0(3)
Fe(2)–S(5)–Fe(4)	73.5(3)
Fe(3)–S(5)–Fe(4)	74.5(3)
Fe(1)–S(6)–Fe(3)	71.7(3)
Fe(1)–S(6)–Fe(4)	74.3(3)
Fe(3)–S(6)–Fe(4)	74.3(3)
Fe(1)–S(7)–Fe(2)	73.6(2)
Fe(1)–S(7)–Fe(4)	73.9(2)
Fe(2)–S(7)–Fe(4)	73.4(3)
Fe(1)–S(8)–Fe(2)	73.1(3)
Fe(1)–S(8)–Fe(3)	71.7(2)
Fe(2)–S(8)–Fe(3)	74.3(3)
mean	73.5(9)

(*b*) $-SCH_2CH_2OH$ groups

(*i*) Distances and angles

	$n = 1$	$n = 2$	$n = 3$	$n = 4$
Fe(*n*)–S(*n*)	2.231(10)	2.257(12)	2.265(12)	2.272(8)
S(*n*)–C(*n*1)	1.80(4)	1.97(6)	1.85(5)	1.82(4)
C(*n*1)–C(*n*2)	1.51(4)	1.41(8)	1.29(6)	1.49(6)
C(*n*2)–O(*n*3)	1.41(4)	1.46(4)	1.44(5)	1.47(5)
Fe(*n*)–S(*n*)–C(*n*1)	105.3(13)	103.8(19)	104.6(18)	103.9(10)
S(*n*)–C(*n*1)–C(*n*2)	113(3)	103(3)	118(3)	110(3)
C(*n*1)–C(*n*2)–O(*n*3)	112(2)	112(4)	114(4)	109(3)

(*ii*) Torsion angles

Fe(*n*)–S(*n*)–C(*n*1)–C(*n*2)	174	64	54	−80
S(*n*)–C(*n*1)–C(*n*2)–O(*n*3)	63	178	179	178

(*c*) Cation dimensions

N(1)–C(2)	1.50(5)	N(2)–C(6)	1.49(6)
N(1)–C(3)	1.48(5)	N(2)–C(7)	1.45(7)
N(1)–C(4)	1.49(5)	N(2)–C(8)	1.45(8)
N(1)–C(5)	1.50(5)	N(2)–C(9)	1.44(7)
C(2)–N(1)–C(3)	112(3)	C(6)–N(2)–C(7)	107(4)
C(2)–N(1)–C(4)	110(3)	C(6)–N(2)–C(8)	113(4)
C(2)–N(1)–C(5)	115(3)	C(6)–N(2)–C(9)	115(4)
C(3)–N(1)–C(4)	106(3)	C(7)–N(2)–C(8)	94(4)
C(3)–N(1)–C(5)	104(3)	C(7)–N(2)–C(9)	113(4)
C(4)–N(1)–C(5)	110(3)	C(8)–N(2)–C(9)	113(4)

---

* For details see Notices to Authors No. 7, *J. Chem. Soc., Dalton Trans.*, 1980, Index issue.

RESULTS AND DISCUSSION

Crystal Structure.—The asymmetric unit contains two NMe_4^+ cations and one $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ anion.

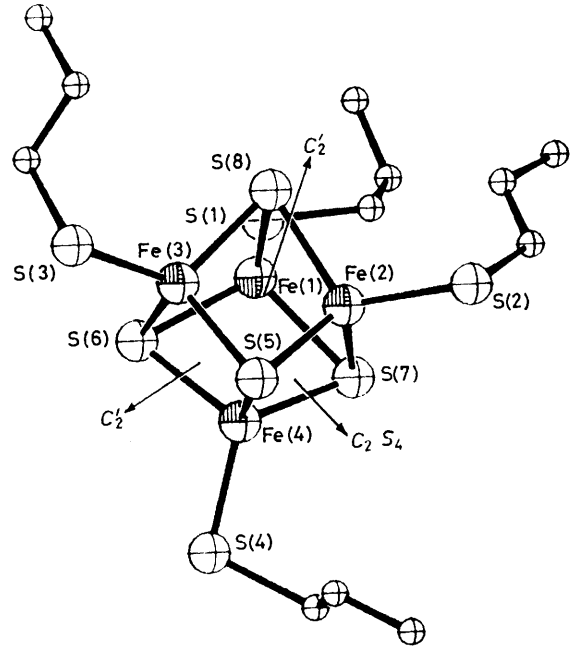

FIGURE 1 Structure of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$, including the directions of the principal axes relevant for D_{2d} symmetry (see text)

The atomic arrangement of the anion is shown in Figure 1 and the packing diagram of the cations and anions, in the c projection, is shown in Figure 2. The anion contains the now familiar distorted Fe_4S_4 cubane-like cluster,[8-10,17] with a SCH_2CH_2OH group co-ordinated to each of the iron atoms. The Fe_4S_4 core is distorted from T_d symmetry; the only regular feature of this distortion is that four approximately parallel Fe–S bonds [Fe(1)–S(7), Fe(2)–S(8), Fe(3)–S(5), and Fe(4)–S(6)] are shorter (mean bond length 2.239 Å) than the other eight (mean bond length 2.306 Å). This distortion, which leaves the cluster with approximately D_{2d} symmetry, is a consistent [18] feature of these Fe_4S_4 cores, as indicated by the data presented in Table 3. Thus the Fe_4S_4 cubane-like core, its dimensions, and the sense of the major distortion from T_d symmetry are independent of the nature of the co-ordinated thiolato-groups.

The structural details presented in Table 3 indicate that the cores of the $[Fe_4S_4(SR)_4]^{n-}$ ($n = 2$, R = Ph [8] or CH_2Ph[9]; $n = 6$, R = $CH_2CH_2CO_2$ [10]) clusters show, concomitantly with the squashing of the core, a variation in the Fe • • • Fe and S • • • S distances, such that the two distances of each related by the C_2' axes of the D_{2d} point group differ from the other four. For each of the four clusters described in Table 3, these two S • • • S distances are significantly longer than the other four; in the present case S(5) • • • S(7) and S(6) • • • S(8) are *ca.* 0.12 Å longer than the other four S_c • • • S_c distances. However, no simple pattern exists for the variation in the Fe • • • Fe distances. The two C_2' related Fe • • • Fe distances are shorter than the other four in $[Fe_4S_4(SPh)_4]^{2-}$,[8] but longer than the other four in $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ (ref. 9) and $[Fe_4S_4(SCH_2CH_2CO_2)_4]^{6-}$ (ref. 10); in $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$, the two Fe • • • Fe distances Fe(1) • • • Fe(3) and Fe(2) • • • Fe(4) are the

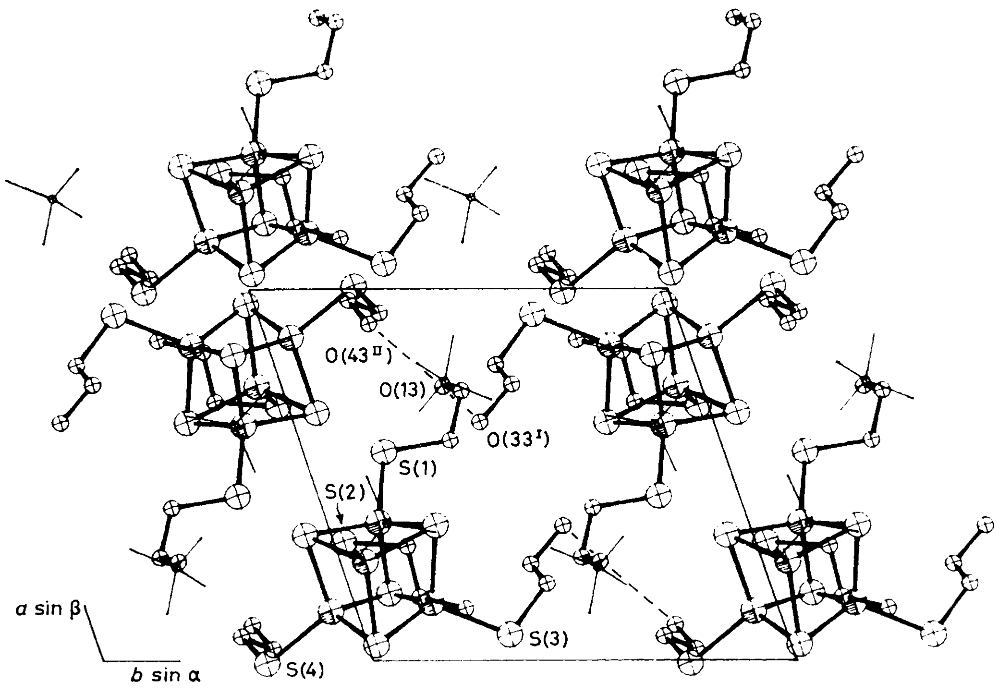

FIGURE 2 Packing of NMe_4^+ and $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ ions in crystals of $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$; as viewed in c projection, showing the important intermolecular contacts

TABLE 3

Average dimensions of $[Fe_4S_4(SR)_4]^{n-}$ cubane-like clusters [a]

	$[Fe_4S_4(SPh)_4]^{2-}$ [b]		$[Fe_4S_4(SCH_2Ph)_4]^{2-}$ [c]		$[Fe_4S_4(SCH_2CH_2CO_2)_4]^{6-}$ [d]		$[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ [e]	
(*a*) Distance/Å								
Fe · · · Fe(2,4)	2.730(2)	2.739(4)	2.776(2)	2.732(3)	2.778(3)	2.743(3)	2.729(6)	
S · · · S(2,4)	3.650(4)	3.592(6)	3.645(3)	3.586(4)	3.613(3)	3.596(3)	3.69(1)	3.57(1)
Fe–S_c(4,8)	2.267(5)	2.296(4)	2.239(4)	2.310(3)	2.261(3)	2.300(3)	2.24(1)	2.31(1)
Fe–S_t(4)	2.263(3)		2.251(3)		2.250(3)		2.26(1)	
(*b*) Angle/°								
Fe–S_c–Fe(12)	73.5(1)		73.8(1)		74.1(2)		73.4(3)	
S_c–Fe–S_c(12)	104.3(1)		104.1(1)		103.9(2)		102.6—107.3(3)	
S_c–Fe–S_t(12)	100.2—135.7(1)		110.2—117.3(2)		109.9—119.3(2)		109.4—117.9(4)	

[a] S_c = μ_3-sulphido-sulphur; S_t = thiolato-sulphur; number of distances averaged to give a particular value in parentheses. [b] Ref. 8. [c] Ref. 9. [d] Ref. 10. [e] This work.

shortest and the longest respectively, of these distances. Another feature observed for $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ (ref. 9) and $[Fe_4S_4(SCH_2CH_2CO_2)_4]^{6-}$ (ref. 10) is a grouping of the Fe–Fe–Fe angles consistent with the maintenance of D_{2d} symmetry; this was not observed for $[Fe_4S_4(SPh)_4]^{2-}$ (ref. 8) nor for the present structure.

Attached to each Fe atom of the Fe_4S_4 core is a 2-hydroxyethanethiolato-group, the mean Fe–S separation being 2.26(1) Å, in good agreement with the corresponding values obtained [8-10] for the other complexes of this type (Table 3). The remaining interatomic dimensions of the 2-hydroxyethanethiolato-group appear to be reasonable, given their relatively high standard deviations (Table 2).

An interesting aspect of the structure of $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$ is the nature of the hydrogen bonding involving the hydroxyl group of the ligands. The O · · · O and S · · · S contacts less than 3.75 Å are given in Table 4; the three shortest distances involve the O(13) atom and the angles subtended at this atom are listed in Table 5. It seems clear that O(13) participates in two strong intermolecular hydrogen bonds to $O(33^{I})$ and $O(43^{II})$ (see Figure 2), one of these presumably involves an O(13)–H · · · O and the other an O(13) · · · H–O arrangement. Despite the relatively short intramolecular S(1) · · · O(13) distance, neither atom is well placed for hydrogen bonding as the C(12)–O(13) · · · S(1) angle is 60.7° and the C(11)–S(1) · · · O(13) angle is only 49.6°. Therefore, it seems likely that this short S(1) · · · O(13) approach is a consequence of packing forces [*e.g.* O(13) · · · $O(33^{I})$ and O(13) · · · $O(43^{II})$ hydrogen bonding] and not indicative of a S · · · H–O hydrogen bond; the short distance is associated with an S(1)–C(11)–C(12)–O(13) torsion angle of 63°, whereas the other three S(*n*)–C(*n*1)–C(*n*2)–O(*n*3) (*n* = 2, 3, or 4) torsion angles are *ca.* 180° thus keeping the S(*n*) and O(*n*3) atoms well separated. [It is also relevant that of the Fe(*n*)–S(*n*)–C(*n*1)–C(*n*2) torsion angles, that for *n* = 1 is 174° whereas those for *n* = 2, 3, or 4 are 67 ± 13°.] There are no intermolecular distances, other than those given in Table 4, less than the sum of the constituent van der Waals radii. Therefore, although hydrogen bonding clearly occurs in the compound, there is no good evidence for intramolecular or intermolecular hydrogen bonding to any of the sulphur atoms of the $[Fe_4S_4(SR)_4]^{2-}$ cluster. Rather, intermolecular O–H · · · O hydrogen bonds are preferred, as was found for $[NEt_4]_3[Fe_6Mo_2S_8(SCH_2CH_2OH)_9]$.[19]

TABLE 4

Possible hydrogen-bonding interactions *

Atoms involved	Distance/Å
O(13) · · · $O(43^{II})$	2.63
O(13) · · · $O(33^{I})$	2.70
O(13) · · · S(1)	3.17
S(3) · · · $O(43^{IV})$	3.29
S(4) · · · $O(23^{III})$	3.41

* Roman numeral superscripts refer to equivalent positions with respect to the *x*, *y*, and *z* set in Table 1 as; I, 1 − *x*, 1 − *y*, −*z*; II, 1 − *x*, −*y*, −*z*; III, −*x*, −*y*, 1 − *z*; IV, −*x*, −*y*, −*z*.

TABLE 5

Angles (°) subtended at O(13) *

C(12)–O(13) · · · S(1)	60.7
C(12)–O(13) · · · $O(43^{II})$	108.2
C(12)–O(13) · · · $O(33^{I})$	130.0
S(1) · · · O(13) · · · $O(43^{II})$	88.9
S(1) · · · O(13) · · · $O(33^{I})$	123.2
$O(33^{I})$ · · · O(13) · · · $O(43^{II})$	121.2

* See footnote to Table 4.

Carbon-13 *N.M.R. Spectra.*—Packer *et al.*[13] have reported the ^{13}C n.m.r. spectra for some four-iron ferredoxins possessing Fe_4S_4 cores at the oxidation-state level equivalent to that of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$. These workers observed significant downfield shifts of the cysteinyl $^{13}C_\alpha$ and $^{13}C_\beta$ ($-S-C_\beta H_2-C_\alpha H=$) resonances attached to the Fe_4S_4 cores, as compared to these resonance positions in the corresponding apoprotein. In the case of oxidised *Clostridium acidi-urici* ferredoxin at 293 K, four of these C_β resonances were considered to occur in the range 117.8—101.5 p.p.m. (downfield of $SiMe_4$) and another at 87.6 p.p.m.; the remaining three C_β resonances were presumed to be broader and obscured by aromatic or protein carboxyl resonances. All eight C_α resonances were considered to be observed in the range 95.6—78.1 p.p.m. These assignments were aided by a study of the temperature dependences of the resonance positions, between 283 and 303 K; the C_β resonances moved downfield between 0.21 and 0.14 [average 0.17(3)] p.p.m. per degree increase in temperature, whereas the corresponding temperature dependence of the C_α resonance was found to vary between 0.14 and 0.06

[average 0.09(3)] p.p.m. The data obtained for $[NMe_4]_2$-$[Fe_4S_4(SCH_2CH_2OH)_4]$ are in reasonable accord with these results on the ferredoxins and their interpretation. In addition to features characteristic of the solvent, in $[^2H_6]$dmso solution at 317, 343, and 363 K, one resonance was clearly identified at 103.3, 104.6, and 106.5 ± 0.02 p.p.m. downfield of $SiMe_4$ at these temperatures respectively, together with a peak centred at 55.9, 56.0, and 57.1 ± 0.02 p.p.m. respectively, which showed some evidence of splitting into more than one component. From these and other [20] studies, we attribute the former set of resonances to the C(2) (or thiolate) carbon, shifted from its resonance at 25.7 p.p.m. downfield of $SiMe_4$ in 2-hydroxyethanethiol because of its proximity to the paramagnetic [1,6] Fe_4S_4 core. The C(1) (or hydroxyl-bound) carbon resonance occurs at 62.3 p.p.m. in the free ligand and we assign the latter set of resonances to this carbon atom plus that of the cation carbon atoms. The isotropic shift of these carbon resonances from their diamagnetic positions is presumed to occur because of spin delocalisation from the Fe_4S_4 centre, *via* a σ-contact shift mechanism, attenuating rapidly along the carbon chain, as proposed by Packer *et al.*[13] Also, the temperature dependence of the thiolate carbon atom (0.07 p.p.m. K^{-1}) is approximately twice that of the adjacent carbon atom (*ca.* 0.03 p.p.m. K^{-1}).

E.S.R. Spectra of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ *Derivatives.*—We have sought to improve the characterisation of the synthetic analogues of ferredoxin [4Fe–4S] centres by obtaining e.s.r. spectra for a reduced and oxidised analogue in a frozen *aqueous* medium, thus extending available comparisons between proteins and analogues in similar solvents.

The e.s.r. spectrum of $[Fe_4S_4(SCH_2CH_2OH)_4]^{3-}$ (Figure 3) in the aqueous medium is axially symmetric with principal apparent *g* values $g_{\parallel} = 2.045$ and $g_{\perp} = 1.929$ ($\bar{g} = 1.967$) and is very similar to the spectra obtained for other $[Fe_4S_4(SR)_4]^{3-}$ complexes in non-aqueous [21] or partially aqueous [22,23] solvents. Similar spectra are exhibited by the reduced [4Fe–4S] centres of ferredoxins in denaturing solvents.[24] However, in the normal protein environment (*e.g.* for a ferredoxin protein in an aqueous medium), the spectra are somewhat different. First, the linewidths are narrower, which probably reflects greater conformational flexibility in the analogue compounds. Secondly, the *g* values can vary. In ferredoxins of *Bacillus stearothermophilus*, the spectrum is rhombic, with *g* values at 1.88—1.89, 1.93—1.94, and 2.06—2.07,[2,25] while in *Rhodospirillum rubrum* ferredoxin III, the spectrum is axial, with $g_{\parallel} = 2.03$, $g_{\perp} = 1.94$.[26] Since the average *g* value is constant, these differences reflect subtle effects of the protein on the structural or electronic structure of the cluster. There is no obvious correlation between the shape of the e.s.r. signal and redox potential of the clusters.

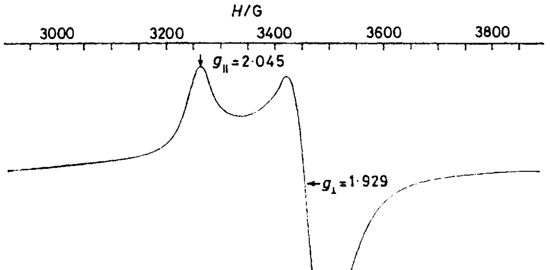

FIGURE 3 E.s.r. spectrum of $[Fe_4S_4(SCH_2CH_2OH)_4]^{3-}$, generated in aqueous solution by sodium dithionite reduction of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ and stabilised in a frozen glass; spectrum recorded at 22 K

Oxidized complexes of the type $[Fe_4S_4(SR)_4]^-$ have not been isolated because of their instability. They exist in the oxidized high-potential iron–sulphur proteins such as that from *C. vinosum*. These have e.s.r. spectra with $g_{av.} > 2$ and axial rhombic symmetry.[27,28] In dmso–water (80 : 20) the spectra are narrower and centred around $g = 2.01$.[29] In an attempt to generate this species, $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ was treated with a slightly less than stoicheiometric amount of $K_3[Fe(CN)_6]$ at 25 °C and frozen within a few seconds. The e.s.r. spectrum produced was axial, with $g_{\parallel} = 1.963$ and $g_{\perp} = 2.006$ (Figure 4). Similar spectra have been observed with oxidized peptide-bound $[Fe_4S_4(SR)_4]^-$ compounds.[23,30] This spectrum of Figure 4 resembles, in its shape, the spectra of certain oxidized iron–sulphur proteins, such as *C. vinosum* hydrogenase.[31] However, the electron-spin relaxation rate is slower than in the proteins, the spectra of which are normally detected at 20 K, and broaden out at 77 K. As can be seen in Figure 4, the spectrum of the oxidized compound is strongly saturated with microwave power (the power for half saturation at 23 K was 3 mW) and readily detected at 77 K. Spectra around $g = 2.01$ can also be obtained from certain oxidized proteins with three-iron clusters.[32,33] Therefore further work is needed to determine whether this spectrum is due to $[Fe_4S_4(SR)_4]^-$ or some other radical species.

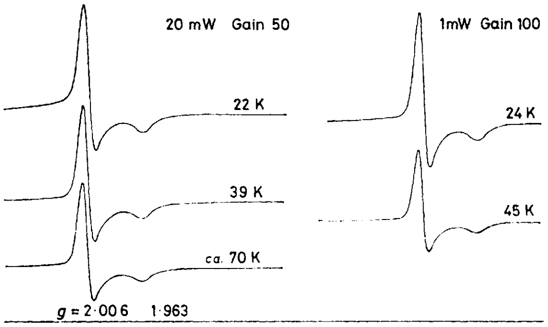

FIGURE 4 E.s.r. characteristics of the species obtained on oxidation of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ by $K_3[Fe(CN)_6]$ in aqueous solution and stabilised in a frozen glass

We thank the S.R.C. for financial support and Dr. B. N. Anand for assistance with the obtaining of the ^{13}C n.m.r. spectra.

[0/1703 *Received, 7th November,* 1980]

REFERENCES

[1] R. H. Holm and J. A. Ibers in ' Iron–Sulfur Proteins,' ed. W. Lovenberg, Academic Press, New York, 1977, vol. 3, ch. 7.

[2] R. N. Mullinger, R. Cammack, K. K. Rao, D. O. Hall, D. P. E. Dickson, C. E. Johnson, J. D. Rush, and A. Simopoulos, *Biochem. J.*, 1975, **151**, 75.

[3] R. Bachofen and D. I. Arnon, *Biochim. Biophys. Acta*, 1966, **120**, 259.

[4] R. Cammack, *Biochim. Biophys. Acta*, 1973, **54**, 548.

[5] N. A. Stombaugh, J. E. Sundquist, R. H. Burris, and W. H. Orme-Johnson, *Biochemistry*, 1976, **15**, 2633.

[6] C. L. Hill, J. Renaud, R. H. Holm, and L. E. Mortenson, *J. Am. Chem. Soc.*, 1977, **99**, 2549.

[7] E. T. Adman, L. C. Sieker, and L. H. Jensen, *J. Biol. Chem.*, 1973, **248**, 3987.

[8] L. Que, jun., M. A. Bobrik, J. A. Ibers, and R. H. Holm, *J. Am. Chem. Soc.*, 1974, **96**, 4168.

[9] B. A. Averill, T. Herskovitz, R. H. Holm, and J. A. Ibers, *J. Am. Chem. Soc.*, 1973, **95**, 3523.

[10] H. L. Carrell, J. P. Glusker, R. Job, and T. C. Bruice, *J. Am. Chem. Soc.*, 1977, **99**, 3683.

[11] E. T. Adman, L. C. Sieker, and L. H. Jensen, *J. Biol. Chem.*, 1976, **25**, 3801 and refs. therein.

[12] C. W. Carter, jun., *J. Biol. Chem.*, 1977, **252**, 7802 and refs. therein.

[13] E. L. Packer, J. C. Rabinowitz, and H. Sternlicht, *J. Biol. Chem.*, 1978, **253**, 7722.

[14] G. Christou and C. D. Garner, *J. Chem. Soc., Dalton Trans.*, 1979, 1093.

[15] G. M. Sheldrick, personal communication.

[16] ' International Tables for *X*-Ray Crystallography,' Kynoch Press, Birmingham, 1975, vol. 4.

[17] M. A. Bobrik, K. O. Hodgson, and R. H. Holm, *Inorg. Chem.*, 1977, **16**, 1851.

[18] E. J. Laskowski, J. G. Reynolds, R. B. Frankel, S. Foner, G. C. Papaefthymiou, and R. H. Holm, *J. Am. Chem. Soc.*, 1979, **101**, 6562.

[19] G. Christou, C. D. Garner, F. E. Mabbs, and M. G. B. Drew, *J. Chem. Soc., Chem. Commun.*, 1979, 91.

[20] B. N. Anand and C. D. Garner, unpublished work.

[21] E. J. Laskowski, R. B. Frankel, W. O. Gillum, G. C. Papaefthymiou, J. Renaud, J. A. Ibers, and R. H. Holm, *J. Am. Chem. Soc.*, 1978, **100**, 5322.

[22] M. W. W. Adams, S. G. Reeves, D. O. Hall, G. Christou, B. Ridge, and H. N. Rydon, *Biochem. Biophys. Res. Commun.*, 1977, **79**, 1184.

[23] R. Cammack, G. Christou, B. Ridge, and H. N. Rydon, unpublished work; G. Christou, Ph.D. Thesis, Exeter University, 1977.

[24] R. Cammack, *Biochem. Soc. Trans.*, 1975, **3**, 482.

[25] N. A. Stombaugh, R. H. Burris, and W. H. Orme-Johnson, *J. Biol. Chem.*, 1973, **248**, 7951.

[26] D. C. Yoch, R. P. Carithers, and D. I. Arnon, *J. Biol. Chem.*, 1977, **252**, 7453.

[27] G. Palmer, H. Brintzinger, R. W. Estabrook, and R. H. Sands, in ' Magnetic Resonance in Biological Systems,' eds. A. Ehrenberg, B. G. Malmström, and T. Vänngard, Pergamon Press Ltd., London, 1967, pp. 159—171.

[28] H. Blum, J. C. Salerno, and M. A. Cusanovich, *Biochem. Biophys. Res. Commun.*, 1978, **84**, 1125.

[29] R. Cammack, Proceedings of the 10th I.U.B. International Congress of Biochemistry, Toronto, 1980.

[30] R. Cammack and G. Christou, in ' Hydrogenases: Their Catalytic Activity, Structure, and Function,' eds. H. G. Schlegel and K. Schneider, E. Goltze Verlag, Göttingen, 1978, pp. 45—56.

[31] P. H. Gitlitz and A. I. Krasna, *Biochemistry*, 1975, **14**, 2561.

[32] M. H. Emptage, T. A. Kent, B. H. Huynh, J. Rawlings, W. H. Orme-Johnson, and E. Münck, *J. Biol. Chem.*, 1980, **255**, 1793.

[33] C. D. Stout, D. Ghosh, V. Pattabhi, and A. H. Robbins, *J. Biol. Chem.*, 1980, **255**, 1797.