Carbon-13 Nuclear Magnetic Resonance Studies of Synthetic $[Fe_4S_4(SR)_4]^{2-}$ Iron–Sulphur Clusters

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Carbon-13 n.m.r. spectra of several synthetic $[Fe_4S_4(SR)_4]^{2-}$ clusters have been recorded (R = CH_2CH_3, CH_2CH_2OH, Ph, C_6H_4Me-4, $C_6H_4NH_2$-3, or $C_6H_4NH_2$-4). Examination of the signs of the paramagnetic isotropic shifts leads to the conclusion that contact interactions dominate the shift mechanism. For alkanethiolates, a σ-spin delocalisation operates, and for arenethiolates both σ- and π-spin delocalisation pathways are observed. This behaviour parallels that observed in the corresponding ^1H n.m.r. spectra.

Proton n.m.r. spectroscopy has been extensively applied in studies of iron–sulphur proteins,[1,2] and synthetic models of iron–sulphur centres have also been thoroughly characterised by the same technique.[3,4] In addition, ^{19}F n.m.r. spectroscopy has proved to be a valuable tool for identifying iron–sulphur clusters extruded from proteins.[5] However, the use of more weakly magnetic nuclei such as ^{13}C for structural studies has been confined to a study by Packer *et al.*[6] on several [4Fe-4S] ferredoxins. These workers observed significant downfield shifts (*i.e.* shifts to high frequency) of the ^{13}C resonances of the α- and β-cysteinyl carbon atoms ($-SC_\alpha H_2C_\beta H=$, although Packer *et al.* used the opposite notation) attached to the $[4Fe\text{-}4S]^{2+}$ cores in the oxidised proteins, compared with these resonance positions for the corresponding apoprotein. Subsequently, Christou *et al.*[7] claimed reasonable agreement between these data and ^{13}C n.m.r. data obtained for the synthetic model cluster $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$.

We report herein the results of a more extensive study of the ^{13}C n.m.r. properties of several $[Fe_4S_4(SR)_4]^{2-}$ clusters co-ordinated by ethanethiolate, 2-hydroxyethanethiolate, or substituted benzenethiolate ligands.

Experimental

Analytically and spectroscopically (^1H n.m.r.) pure samples of the compounds detailed below were prepared by the method of Christou and Garner.[8] Broad-band proton-decoupled ^{13}C n.m.r. spectra were recorded on either a Varian XL300 spectrometer (75.4 MHz) for the $[4Fe\text{-}4S]^{2+}$ clusters or a Bruker WP80 spectrometer (20.1 MHz) for the free thiols (diamagnetic references) by the pulse Fourier-transform technique. Spectra of the $[4Fe\text{-}4S]^{2+}$ clusters as saturated solutions in $(CD_3)_2SO$ and contained in tubes of outside diameter 10 mm were assigned by means of selective and off-resonance proton decoupling. Chemical shifts are reported in p.p.m. downfield of $SiMe_4$. Isotropic shifts were calculated using the formula (1). Thus, chemical shifts downfield of

$$\left(\frac{\Delta H}{H_0}\right)_{\text{iso.}} = \left(\frac{\Delta H}{H_0}\right)_{\text{dia.}} - \left(\frac{\Delta H}{H_0}\right)_{\text{obs.}} \qquad (1)$$

$SiMe_4$ are positive whilst isotropic shifts downfield of $SiMe_4$ are negative.

Results and Discussion

The ^{13}C n.m.r. chemical shifts of the free thiols used in this study are given in Table 1, as diamagnetic references for the calculation of the isotropic shifts. The ^{13}C n.m.r. chemical and isotropic shifts calculated for the complexes $[Fe_4S_4(SR)_4]^{2-}$ (R = CH_2CH_3, CH_2CH_2OH, Ph, C_6H_4Me-4, $C_6H_4NH_2$-4, or $C_6H_4NH_2$-3) are given in Table 2.

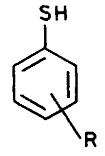
The α-carbon (thiolate carbon) resonances of the two alkanethiolate clusters occur as broad peaks at *ca.* 100 p.p.m., shifted considerably downfield from the corresponding free thiol resonances. The isotropic shifts are −73.8 and −84.2 p.p.m. for the mercaptoethanol and ethanethiol clusters, respectively. The β-carbon resonances are also shifted downfield to 102.2 p.p.m. for $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ and 53.6 p.p.m. for $[Fe_4S_4(SCH_2CH_3)_4]^{2-}$; the isotropic shifts are −39.1 and −35.4 p.p.m., respectively. Thus, the observed ^{13}C isotropic shifts for $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ and $[Fe_4S_4(SCH_2CH_3)_4]^{2-}$ parallel the observed proton shifts, in that the shifts are downfield and attenuate rapidly along the carbon chain. These data correct an error of the earlier assignment[7] of the ^{13}C n.m.r. spectrum of $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$. The resonance at 55.9 p.p.m. originally assigned to the C_β carbon of the ligands is in fact due to the $[NMe_4]^+$ cation (resonance position 55.7 p.p.m. in this study).

Packer *et al.*[6] reported that the cysteinyl α- and β-carbon resonances of oxidised *Clostridium acidi-urici* ferredoxin at 20 °C (core oxidation level $[4Fe\text{-}4S]^{2+}$) were shifted downfield from their resonance positions in the apoferredoxin by 63—93 and 22—40 p.p.m., respectively. These downfield shifts correspond to negative isotropic shifts, and good agreement is observed between these shifts and isotropic shifts for the model compound $[NMe_4]_2[Fe_4S_4(SCH_2CH_2OH)_4]$ (core oxidation level $[4Fe\text{-}4S]^{2+}$) which, at 20 °C, are −73.8 p.p.m. for C_α and −39.1 p.p.m. for C_β.

The isotropic shift pattern for each of the four arenethiolate clusters is very similar. The *ipso* (C^1) carbon atom resonance is broadened and shifted considerably downfield (isotropic shifts *ca.* −70 to −75 p.p.m.). These shifts are very similar to those noted for the C_α (thiolate carbon atom) resonances of $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ and $[Fe_4S_4(SCH_2CH_3)_4]^{2-}$ (−73.8 and −84.2 p.p.m., respectively). The other ^{13}C isotropic shifts of the benzene ring alternate in sign [*ortho* (C^2) negative, *meta* (C^3) positive, and *para* (C^4) negative]. The sense of this pattern is *opposite* to that observed for the corresponding proton isotropic

Table 1. 20.1-MHz ^{13}C N.m.r. spectral data for several thiols in solution in $(CD_3)_2SO$ at 20 °C

Thiol	Chemical shift/p.p.m. C_α	C_β
$HSC_\alpha H_2C_\beta H_3$	19.1	18.2
$HSC_\alpha H_2C_\beta H_2OH$	26.5	63.1



R	*i*	*o*	*m*	*p*
H	131.2	129.7	128.1	125.9
CH_3-4*	129.8	129.8	129.8	135.0
NH_2-4	121.0	132.8	114.3	149.3
NH_2-3	131.9	C^2 114.8 C^6 117.9	C^5 130.1 C^3 148.2	112.2

* Only the C^4 resonance could be assigned; the midpoint of the other unresolved resonances was 129.8 p.p.m. The CH_3 resonance is at 21.3 p.p.m.

Table 2. 75.4-MHz ^{13}C Chemical and isotropic shifts of the resonances of the thiolate carbon atoms of the complexes $[Fe_4S_4(SR)_4]^{2-}$ in solution in $(CD_3)_2SO$ at 20 °C

	Chemical shift (isotropic shift)/p.p.m.					
R	C_α	C_β	*i*	*o*	*m*	*p*
$C_\alpha H_2C_\beta H_3$	103.3 (−84.2)	53.6 (−35.4)	—	—	—	—
$C_\alpha H_2C_\beta H_2OH$	100.3 (−73.8)	102.2 (−39.1)	—	—	—	—
Ph	—	—	203.9 (−72.7)	160.0 (−30.3)	123.1 (+5.0)	134.2 (−8.3)
C_6H_4Me-4[a]	—	—	201.8 (−72.0)	160.4 (−30.6)	123.9 (+5.9)	143.0 (−8.0)
$C_6H_4NH_2$-4	—	—	196.1 (−75.1)	162.4 (−29.6)	110.1 (+4.2)	154.0 (−4.7)
$C_6H_4NH_2$-3[b]	—	—	203.4 (−71.5)	149.3 (−31.4) 146.1 (−31.3)	145.9(C^3) (+2.3) 122.3(C^5) (+7.8)	120.0 (−7.8)

[a] 4-Me resonance at 16.2 p.p.m., isotropic shift +5.1 p.p.m. [b] It was not possible to assign the *ortho*-carbons using selective proton decoupling. Isotropic shifts have been calculated assuming the 149.3 p.p.m. resonance is due to C^6 and the 146.1 p.p.m. resonance to C^2, which gives approximately equal isotropic shifts.

Table 3. Isotropic shifts of the thiolate carbon atoms of $[NBu^n_4]_2[Fe_4S_4(SPh)_4]$ in $(CD_3)_2SO$ solution at 20, 50, and 100 °C

	Isotropic shift/p.p.m.			
θ_c/°C	*i*	*o*	*m*	*p*
20	−72.7	−30.3	+5.0	−8.3
50	−76.9	−32.4	+5.2	−8.7
100	−83.4	−35.7	+5.4	−9.2

shifts (H^2 and H^4 positive, H^3 negative). The relative magnitudes of the particular shifts for the four clusters are similar, with the magnitude of the *ipso*-carbon atom shift being considerably larger than that of the *ortho* shift, which in turn is larger than the shifts of the *meta*- and *para*-carbon atoms. The carbon atom of the 4-CH_3 substituent has a positive isotropic shift.

The variation of the ^{13}C chemical shifts of $[NBu^n_4]_2[Fe_4S_4(SPh)_4]$ with temperature was investigated by recording the proton-decoupled ^{13}C n.m.r. spectrum in $(CD_3)_2SO$ solution at 20, 50, and 100 °C. The isotropic shifts of the thiolate carbon atoms at these temperatures are given in Table 3. The magnitudes of the observed shifts all increase with temperature, *i.e. ipso*, *ortho*, and *para* shifts become more negative and *meta* more positive, as the temperature increases. Calculated temperature dependencies (p.p.m. K^{-1}) are 0.13(C^1), 0.07(C^2), 0.005(C^3), and 0.01(C^4). Packer *et al.*[6] reported temperature dependencies of 0.14—0.21 (average 0.17) p.p.m. K^{-1} for the C_α resonances of *Clostridium acidi-urici* ferredoxin, which are similar to the value determined for the *ipso*-carbon atom of $[NBu^n_4]_2[Fe_4S_4(SPh)_4]$.

The observations noted above are consistent[9] with hyperfine contact interactions being the dominant factor in the observed paramagnetic isotropic shifts of the benzenethiolate ^{13}C resonances, as is found to be the case for the ^1H resonances of $[Fe_4S_4(SR)_4]^{2-}$ clusters.[3] For saturated ligands (R = CH_2CH_2OH or CH_2CH_3), spin is transferred by means of a σ-delocalisation mechanism, characteristic of which are: (*i*) the observed rapid attenuation of isotropic shifts along the carbon chain and (*ii*) non-alternation of the signs of the shifts. For benzenethiolate ligands, π delocalisation is also a mechanism for spin transfer, as shown by the alternation of the signs of the observed isotropic shifts around the ring. The signs of the observed ^{13}C isotropic shifts are opposite to the signs of the ^1H isotropic shifts at the *ortho*, *meta*, and *para* ring positions. This behaviour may be rationalised by a consideration of the McConnell relation $A = Q\rho_c$, where A is the electron–nuclear hyperfine coupling constant, ρ_c is the electron spin density at a particular carbon atom, and Q is a proportionality constant. For a π-delocalisation mechanism, the unpaired electron spin density at the proton of an aromatic C–H unit tends to be antiparallel to the average spin of the π electron, and Q_{CH} is negative.[10] For the carbon atom, unpaired spin density is provided by the π electron, and so Q_C is positive. The isotropic shift is proportional to $-A$,[3] and so it is expected that opposite signs will be observed for ^1H and ^{13}C isotropic shifts at a particular ring position. For a CH_3 substituent Q_{CC} is negative (as is Q_{CH}), hence the ^{13}C resonance of the CH_3 group will have an isotropic shift of opposite sign to that of the carbon atom to which it is attached.

The observed signs and magnitudes of the ^{13}C shifts for benzenethiolate clusters can be rationalised in terms of ligand–metal antiparallel spin transfer, which results in positive spin on sulphur,[3] delocalised *via* a σ mechanism which provides rapidly attenuating positive spin density, and a non-attenuating π mechanism which provides positive spin density at *ortho*- and *para*-carbon atoms and negative spin density at *ipso*- and *meta*-carbon atoms (through correlation effects). The very large negative shift for the *ipso*-carbon atom is attributed primarily to σ delocalisation, as is the case for the α-carbon atom (thiolate carbon) of $[Fe_4S_4(SCH_2CH_2X)_4]^{2-}$ clusters. The σ-delocalisation pathway is also important at *ortho*-carbon atoms and augments the π delocalisation to produce a large negative shift. At the *meta*- and *para*-carbon atoms, the observed positive and negative shifts, respectively, are considered to arise essentially from π delocalisation alone.

Conclusions

The isotropic shifts of the ^{13}C resonances of the thiolate carbon atoms of $[Fe_4S_4(SR)_4]^{2-}$ clusters are consistent with contact interactions dominating the shift mechanism. The pattern of the observed shifts can be rationalised in terms of σ-spin delocalisation for $[Fe_4S_4(SCH_2CH_2X)_4]^{2-}$ clusters and in terms of σ- and π-spin delocalisation for $[Fe_4S_4(SC_6H_4X)_4]^{2-}$ clusters. The ^{13}C n.m.r. spectrum of Christou *et al.*[7] for $[NMe_4]_2$-$[Fe_4S_4(SCH_2CH_2OH)_4]$ has been re-assigned, in the light of higher-frequency data and a comparison with the spectrum of $[AsPh_4]_2[Fe_4S_4(SCH_2CH_3)_4]$. The isotropic shifts of the α- and β-carbon atoms of these two compounds are very similar to those observed for the corresponding cysteinyl carbon atoms of *Clostridium acidi-urici* ferredoxin.[6] Also, the temperature dependence of the α-carbon atom resonance of this ferredoxin is very similar to that observed for the *ipso*-carbon atom resonance of $[NBu^n_4]_2[Fe_4S_4(SPh)_4]$. Thus, these model compound studies confirm the assignments made by Packer *et al.* and provide an improved basis for the use of ^{13}C n.m.r. spectroscopy in characterising systems containing $[4Fe\text{-}4S]^{2+}$ clusters.

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