Hyperfine Coupling to ^{13}C-Cyanide in Electron Spin Resonance Spectra of Pentacyanocobaltate(II) and Cobalt(II) Carbonic Anhydrase

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Summary ^{13}C-Hyperfine coupling to the equatorial ligands of pentacyanocobaltate(II) was measured, leading to a structure for the low-spin cyanide complex of Co^{II} carbonic anhydrase.

ELECTRON SPIN RESONANCE studies of many tetragonal low-spin Co^{II} complexes reveal a $^2A_1$ ground state, with the unpaired electron largely localised in the d_{z^2} orbital.[1] Thus hyperfine coupling to axial nitrogen bases is well defined,[2] but no such interaction with equatorial ligands has been observed. Complexes of $^{13}CN^-$ were therefore investigated, since splittings should be larger than for ^{14}N [$g_N(^{13}C)/g_N(^{14}N) = 3{\cdot}49$].

Spectra of pentacyanocobaltate(II) containing $^{12}CN^-$ and $^{13}CN^-$ (87·43% enrichment) are presented in Figure 1, and demonstrate the extra splitting and line-broadening due to the ^{13}C nuclei. The $M_I = -\frac{1}{2}$ ^{59}Co hyperfine line in the g_{II} region was simulated by computer addition of spectra of the $^{12}CN^-$-complex suitably offset to represent the ^{13}C interactions.[3] Two coupling constants were used, for the single axial and 4 in-plane ligands. As the resulting line-shape was very sensitive to the precise numbers chosen, the parameters were obtainable with some precision (Figure 1 inset and Table). However, the complete spectrum could not be adequately reproduced by this technique, which demands isotropic A values. The relative magnitude of the ^{13}C coupling constants is noteworthy, since the probability function $\psi_N\psi^*$ for the axial lobes of d_{z^2} is 4 times that of the in-plane lobe. For an ideal square pyramid, the a_1^* (d_{z^2}) MO must include the following linear combination of ligand

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TABLE. E.s.r. parameters

Complex	$g_{\parallel}$	$\lvert A_{\parallel}(^{59}Co)\rvert \times 10^4/cm^{-1}$	$\lvert A_{\parallel}(^{14}N)\rvert \times 10^4/cm^{-1}$	$\lvert A_{\parallel}(^{13}C)\rvert \times 10^4/cm^{-1}$
$[Co(CN)_5]^{3-}$ [a]	2·004	82		39·5 ± 1·0[d] (ax) 9·0 ± 0·5[d] (eq)
Co(CN)cobalamin[b]	2·005	80		60 (ax)
$CoHCAB(CN)_2$[c]	2·004	89	13·2	10·3 ± 0·5[d] (eq)

[a] In methanol. [b] In 1:4 v/v ethylene glycol–water. Aquocobalamin in phosphate buffer pH 11·5 was reduced under vacuum with ascorbate, then mixed with KCN. [c] Aqueous solution. HCAB, human carbonic anhydrase B. [d] Parameters derived by simulation; consequent error limits are given.

σ orbitals: $\psi_{a1} = \sigma_1 - \frac{1}{2}(\sigma_2 + \sigma_3 + \sigma_4 + \sigma_5)$, where the axial ligand is labelled 1. The hyperfine coupling energy is related to the square of the MO coefficient, hence $A_{ax}/A_{eq} = 4$.

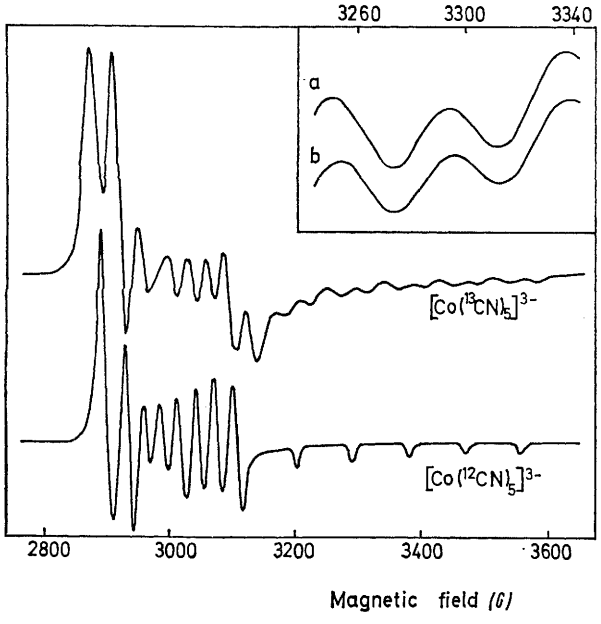


FIGURE 1. 9·10 GHz e.s.r. spectra of $[Co(CN)_5]^{3-}$ at 120 K. Samples were prepared under vacuum in methanol from anhydrous $CoCl_2$ 1 mM and KCN 6 mM. The inset shows ^{13}C splitting on the $M_I = -\frac{1}{2}$ Co hyperfine line in the $g_{\parallel}$ region; (a) experimental, (b) simulated, with $|A_{\parallel}(^{13}C_{ax})| = 39{\cdot}4 \times 10^{-4}$ cm^{-1} and $|A_{\parallel}(^{13}C_{eq})| = 8{\cdot}9 \times 10^{-4}$ cm^{-1}.

For cyano-Co^{II}cobalamin, the ^{13}C splitting was directly measurable, with $|A_{\parallel}(^{13}C_{ax})|$ 50% larger than in $[Co(CN)_5]^{3-}$. Since the benzimidazole base is detached [*cf.* cyano-Co^{II}cobinamide[2]], the increased delocalisation of unpaired electron density onto the axial ligand is attributed to greater donor character of the corrin group (a reverse *cis*-effect). Exactly parallel behaviour is shown by corresponding complexes with axial pyridine; $|A_{\parallel}(^{14}N_{ax})|$ values in $[PyCo(CN)_4]^{2-}$ and pyridine-Co^{II}cobinamide are 12 × 10^{-4} cm^{-1} (ref. 4) and 18 × 10^{-4} cm^{-1} (ref. 2) respectively. Moreover, for each CoL_4 system, $|A_{\parallel}(^{13}C_{ax})/A_{\parallel}(^{14}N_{ax})| = 3{\cdot}3$, *i.e.* close to the ratio of nuclear *g* factors.

The zinc enzyme carbonic anhydrase retains catalytic activity when reconstituted with cobalt(II).[5] However, the Co^{II} enzyme forms a unique low-spin cyanide complex thought to involve 2 CN^- ligands.[6] Figure 2 illustrates e.s.r. spectra of the $^{12}CN^-$- and $^{13}CN^-$-derivatives. The ^{14}N hyperfine triplets are assigned to an axial histidine ligand, one of 3 in the native enzyme.[7] The ^{13}C effect was

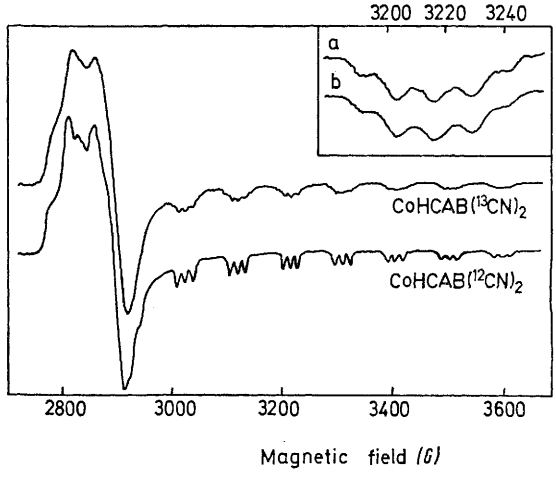
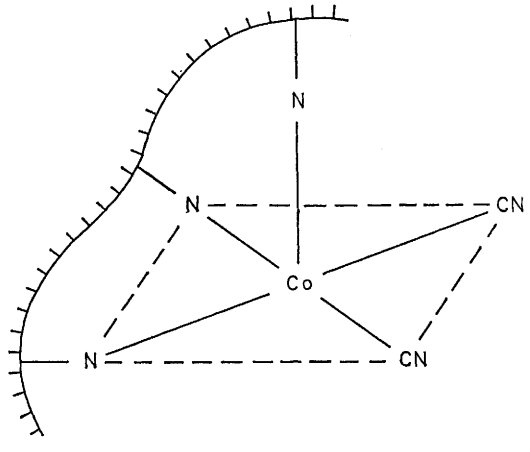


FIGURE 2. 9·15 GHz e.s.r. spectra of $CoHCAB(CN)_2$ at 77 K Samples were prepared under nitrogen from CoHCAB 0·4 mM and KCN 3 mM in 0·04 M tris-sulphate, pH 9·0. The inset shows ^{13}C and ^{14}N splitting on the $M_I = +\frac{1}{2}$ Co hyperfine line in the $g_{\parallel}$ region; (a) experimental, (b) simulated, with $|A_{\parallel}(^{13}C_{eq})| = 10{\cdot}3 \times 10^{-4}$ cm^{-1}.

reproduced as before with a spectrum accumulator, assuming 2 cyanides with equal coupling constant. The resulting value of $|A_{\parallel}(^{13}C)|$ in comparison with the previous results indicates 2 equatorial cyanides bound *via* carbon; an axial N-bonded cyanide is not possible since $C^{15}N^-$ has no effect



(I)

on the spectrum.[6] Titration experiments[8] confirm the presence of 2 CN^- ligands, which are probably in the *cis* configuration, as the metal ion lies at the base of the active-site crevice.[7] The e.s.r. spectrum at 35 GHz remains closely axial,[8] so H_2O is unlikely to occupy an equatorial position in place of a protein ligand. The square-pyramidal structure (I) is therefore proposed. The evident conformational flexibility at the metal binding site may be significant as regards enzymic activity.

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