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Metal Complexes of the Antibiotic Nalidixic Acid

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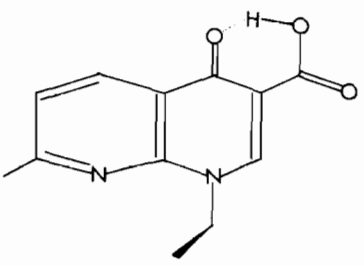
Abstract

The preparations and spectral properties are reported of a range of complexes of nalidixic acid (= HNal) with some metal ions in the series Cr–Zn and also Mg, Ca, Cd, Hg and Pd(II). Most of the compounds formed by the divalent metal ions had a 2:1 HNal:metal ratio, and some of them are polymeric. Complexes in which the carboxylate group of HNal functions as a chelate were isolated with Cu(II), Pd(II), Fe(III) and Cr(III).

Introduction

Metal ions play a vital role in a vast number of widely differing biological processes. The interaction of these ions with drugs administered for therapeutic reasons is a subject of considerable interest. It is known that some drugs act via chelation [1] or by inhibiting metalloenzymes [2] but for most of the drugs that could act as potential ligands little is known about how metal binding influences their activity.

In order to enhance understanding of drug–metal ion interactions, we have been studying the complexing ability of nalidixic acid (HNal) (**I**) and its analogues. These antibiotics are used in the clinical treatment of urinary tract infections caused by Gram-negative bacteria [3]



I

The antibacterial activity of nalidixic acid is apparently due to inhibition of DNA synthesis. Nalidixic acid is an inhibitor of the DNA–gyrase, a topoisomerase having a tetrameric structure formed by two subunits, A and B, and it is believed that subunit A is the direct target of the drug [4, 5] Unfortunately the composition of the target is not yet known in detail. but it has been suggested that it may be a metalloenzyme [6].

A recent study [7] of the mechanism of action of quinolone antibacterial agents (nalidixic acid being the first member of this family) suggested that they do not bind to gyrase subunit A, but rather to DNA. In this hypothesis, the proximal inhibitor of DNA gyrase-mediated breakage and reunion is the drug–DNA complex.

The results of computer simulation [8], based on formation constants of HNal complexing with a range of metal ions. suggested that HNal is mainly non-complexed in plasma, and so acts intracellularly. A mixed ligand complex between the drug, metal ion, and DNA (with the metal ion acting as a bridge) was proposed as a transition state. A study of the partition coefficients of nalidixic acid and of the complexes with Cu(II), Zn(II) and Mg(II) led to the conclusion that lipid solubility is not a major factor in the efficacy of either the parent drug or the 1:1 drug–metal complexes [9].

We report here the results of attempts to isolate some solid complexes of nalidixic acid with the aims of studying how HNal binds to various metal ions and of obtaining materials for biological testing.

Results and Discussion

Nalidixic acid is a monoprotic acid ($pK_a = 5.94$) which is absorbed gastrointestinally [10], so its use in the anionic form increases its solubility and absorp-

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TABLE I. Analytical Data for some Complexes of Nalidixic Acid (=HL)

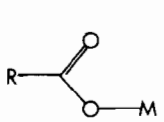
Complex	M:L[a]	Colour	Analysis (%)					
			Found			Calculated		
			C	H	N	C	H	N
$Mg(L)_2 \cdot H_2O$	1:2	white	56.64	4.67	10.87	57.11	4.79	11.10
Ca(L)(OH)	1:2	white	49.44	4.07	9.49	50.00	4.20	9.72
$Cr(L)_2(OH)$	1:3	green	53.69	4.49	9.97	54.24	4.36	10.54
$Mn(L)_2$	1:2	yellow	55.55	4.25	10.82	55.72	4.29	10.83
$Fe(L)_3 \cdot 2H_2O$	1:3	yellow	55.17	4.26	10.75	55.05	4.75	10.70
$Fe(L)_2(OH)$	1:3	red–brown	53.84	4.62	10.09	53.86	4.33	10.47
$Ni(L)(NO_3) \cdot H_2O$	1:2	green	40.01	3.51	11.17	38.96	3.54	11.36
$Ni(L)_2 \cdot 3H_2O$	1:2	blue–green	50.40	4.63	9.78	50.11	4.91	9.74
$Cu(L)_2 \cdot H_2O$	1:1	blue	52.84	4.11	10.14	52.98	4.45	10.30
$Zn(L)_2 \cdot H_2O$	1:1	white	52.14	4.33	10.09	52.81	4.43	10.26
$Pd(L) \cdot H_2O$	1:2	brown	48.07	3.77	9.30	49.12	4.12	9.55
$Cd(L)_2$	1:2	white	50.05	3.91	9.65	50.14	3.86	9.75
Cd(L)Br	1:1	white	33.83	2.57	6.44	34.03	2.62	6.61
Hg(L)Cl	1:2	white	31.56	2.65	6.68	30.84	2.37	6.00

[a]Ratio used in synthesis.

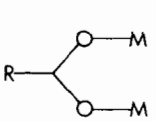
tion. For our study of the interaction of this antibiotic with metal ions we therefore chose to use the sodium salt as the starting ligand. The metal complexes we have prepared are listed in Table I. Unfortunately none of them was isolated in the form of crystals suitable for structure determination by X-ray diffraction methods, so information about their structures was obtained by spectral studies.

In the IR spectrum of HNal the ν(C=O) stretching mode of the carboxylic acid group is observed as a band at 1720 cm^{-1}. This disappears on deprotonation and in the sodium salt there are two new bands at 1590 and 1392 cm^{-1}, the carboxylate antisymmetric (ν_a) and symmetric (ν_s) vibrations respectively.

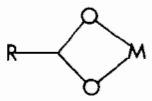
The carboxylate ion usually coordinates to metal ions in one of three main ways (**II**–**IV**). Such differences are reflected in the relative positions of the



II



III



IV

antisymmetric and symmetric stretching vibrations. The separations between the ν_a and ν_s, $\Delta\nu$, in unidentate complexes (structure **II**) are expected to be much larger than in the free ion. In a bidentate (chelate) complex (structure **IV**) $\Delta\nu$ will be significantly smaller than in the free ion whereas in the bridging complex (structure **III**) the $\Delta\nu$ value is closer to that of the free ion [11].

TABLE II. ν(OCO) Frequencies (cm^{-1}) for the Sodium Salt of Nalidixic Acid (Nal) and some of its Complexes

Compound	ν(OCO)			Coordination mode
	ν_a	ν_s	$\Delta\nu$	
NaNal	1590	1392	198	
Ca(Nal)OH	1583	1395	188	bridging
$Mg(Nal)_2 \cdot H_2O$	1572	1402	170	bridging
$Mn(Nal)_2$	1575	1388	187	bridging
$Ni(Nal)_2 \cdot 3H_2O$	1574	1398	176	bridging
$Ni(Nal)NO_3 \cdot H_2O$	1570	1400	170	bridging
$Zn(Nal)_2 \cdot H_2O$	1570	1390	180	bridging
$Cd(Nal)_2$	1572	1385	187	bridging
$Cr(Nal)_2OH$	1620	1495	125	chelate
$Fe(Nal)_3 \cdot 2H_2O$	1620	1483	137	chelate
$Cu(Nal)_2H_2O$	1610	1485	125	chelate
$Pd(Nal)_2H_2O$	1590	1485	105	chelate

The compounds obtained in the present work may be divided into two groups on the basis of their IR spectra (Table II): (a) those having bridging carboxylates and (b) those in which the carboxylate group chelates. In no case was evidence found for the keto group on C(4) coordinating to a metal ion as the band at 1625 cm^{-1} due to the ν(C=O) stretch of this group in NaNal remained invariant in the metal

complexes nor were any changes observed in the ketone deformation region (1200–1350 cm^{-1}).

Assignment of the type of carboxylate group coordination was based on both the position of the ν_s, band and the values of $\Delta\nu$ [12–14]. In the compounds in group (a), in which the carboxylate group functions as a bridging ligand, ν_s(OCO) was found at 1385–1402 cm^{-1} and $\Delta\nu$ was 170–190 cm^{-1}. Compounds in group (b), in which the carboxylate group acts as a chelate, have Δ_s(OCO) 1483–1495 cm^{-1} and $\Delta\nu$ 105–137 cm^{-1}.

Type (a) Complexes

This class, in which the carboxylate unit functions as a bridging group, comprises the compounds $M(Nal)_2 \cdot H_2O$ (M = Mg, and Zn), $M(Nal)_2$ (M = Mn, Cd), $Ni(Nal)_2 \cdot 3H_2O$, $Ni(Nal)(NO_3) \cdot H_2O$ and Ca(Nal)(OH). All the compounds were apparently air-stable. They were insoluble in water and common organic solvents, except for $Ni(Nal)(NO_3)H_2O$ which dissolved in ethanol.

The reflectance spectra of the solid nickel complexes were typical of the presence of an octahedral ligand field of oxygen donor ligands (e.g. for $Ni(Nal)_2 \cdot 3H_2O$: ν_1 at 8760 cm^{-1}, $\nu_2 + {}^3A_{2g} \rightarrow {}^1E_g$ at 14 050 and 14 780 cm^{-1}).

The X-band EPR spectrum of a polycrystalline sample of $Mn(Nal)_2$ consisted of a single band at $g = 2$ (15 mT width peak to peak). This is consistent with either a highly symmetrical monomeric ligand field or a polymeric structure with consequent dipolar interactions between the adjacent metal centres, resulting in the simple spectrum observed [15]. As the presence of carboxylate bridges may be inferred from the IR spectrum the latter, polymeric, structure seems more probable.

Cadmium forms a complex of the same stoichiometry, $Cd(Nal)_2$, and the two compounds $M(Nal)_2$ (M = Mn and Cd) have very similar ν_a and ν_s(OCO) values. The EPR spectrum of a sample of $Cd(Nal)_2$ doped with manganese (nominal 1 mol %) (Fig. 1) consisted of a band at $g = 2$ split into six hyperfine components (Mn $I = 5/2$). This result shows that the symmetry of the immediate environment of the Mn(II) ions in the $Cd(Nal)_2$ lattice is quite high, and an essentially tetrahedral geometry of oxygen atoms from bridging carboxylate groups is proposed. The slightly irregular form of the spectrum suggests the presence of a very small degree of zero-field splitting, reflecting a small departure from rigorously tetrahedral geometry. The spectrum of $Mn(Nal)_2$, is consistent with a similar structure, the absence of hyperfine structure being due to the dipolar interaction between adjacent Mn(II) ions in the polymer.

Type (b) Complexes

This class, for which the IR results suggest the presence of chelating carboxylate groups, comprises the compounds $Cu(Nal)_2 \cdot H_2O$, $Pd(Nal)_2 \cdot H_2O$, $Fe(Nal)_3 \cdot H_2O$, and $Cr(Nal)_2OH$.

The electronic spectrum of $Cu(Nal)_2H_2O$ had a strong band at 15 380 cm^{-1} with a shoulder at 16 700 cm^{-1}, and its X-band EPR spectrum was of the axial type (g_{xy} 2.067, g_z 2.173), indicative of a tetragonal environment.

The reaction of chromium(III) chloride with the sodium salt of nalidixic acid in water gave a green compound, which after drying at 110 °C, had the formula $Cr(Nal)_2(OH)$. The EPR spectrum of the solid compound showed a broad (44.5 mT peak-to-peak breadth) band centred at $g = 1.97$, indicative of the presence of strong dipolar coupling between the paramagnetic ions, as commonly found for polymeric complexes.

It is well known that Cr(III) in aqueous solution exhibits several hydrolysis equilibria [16]. Under the reaction conditions employed in the synthesis ([Cr] = 0.014 M, pH = 3.4) hydroxo-bridged species would be present and would favour the formation of a dinuclear structure for $Cr(Nal)_2(OH)$ as in **V**.

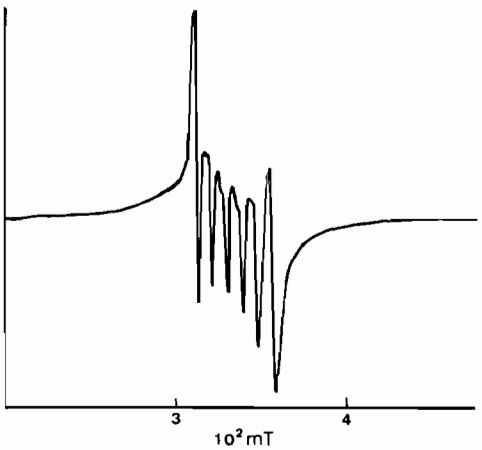
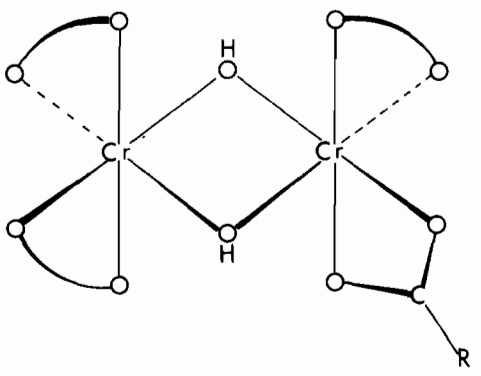


Fig. 1. X-Band EPR spectrum of $Cd(Mn)(Nal)_2$.



V

Two products were obtained from the reaction of iron(III) chloride and the sodium salt of nalidixic acid in water: yellow $Fe(Nal)_3 \cdot 2H_2O$ and red–brown $Fe(Nal)_2(OH)$. Under the reaction conditions employed ([Fe] = 0.025 m, pH = 2.1) both mononuclear $Fe(H_2O)_6^{3+}$ and dinuclear hydrolysed complexes are the principal species [16], so the formation of more than one nalidixic acid complex is not unexpected.

The major product was $Fe(Nal)_3 \cdot 2H_2O$ and this had an X-band EPR spectrum consisting of a set of at least eight bands in the 0–0.6T region (Fig. 2). Such spectra are expected [15] for high spin iron(III) species when the principal zero-field splitting parameter, D, is finite but small ($<$*ca.* 0.1 cm^{-1}), corresponding to only small deviations of the ligand field environment from regularly octahedral geometry.

This conclusion is supported by the Mössbauer spectrum of the complex (Fig. 3) which consisted of a single broad peak (unresolved doublet). The form of the spectrum and the chemical isomer shift value, δ, (Table III) are very similar to those of the trioxalatoferrate(III) anion, $Fe(C_2O_4)_3^{3-}$ [17].

The Mössbauer (Fig. 4) and EPR (Fig. 5) spectra of the minor product of the reaction, red–brown $Fe(Nal)_2(OH)$, differ appreciably from those of $Fe(Nal)_3 \cdot 2H_2O$. The quadrupole splitting observed

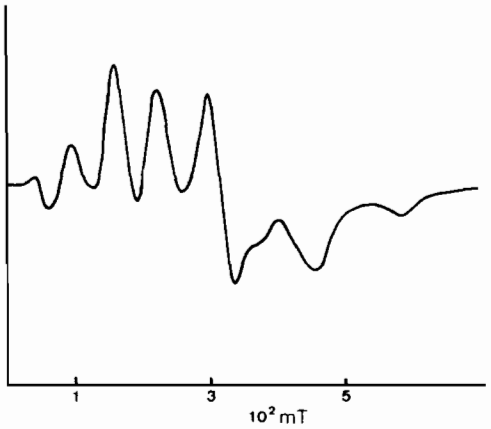


Fig. 2. X-band EPR spectrum of $Fe(Nal)_3 \cdot 2H_2O$.

in the Mössbauer spectrum and the form of the EPR spectrum, notably the presence of the major band in the $g \approx 4$ region, both indicate a more distorted environment about the iron atom than in $Fe(Nal)_3 2H_2O$. However, these results alone do not permit us reliably to assign a structure to $Fe(Nal)_2(OH)$.

In conclusion, it appears that, under the experimental conditions we have employed, a range of solid HNal metal complexes may be isolated. In these the

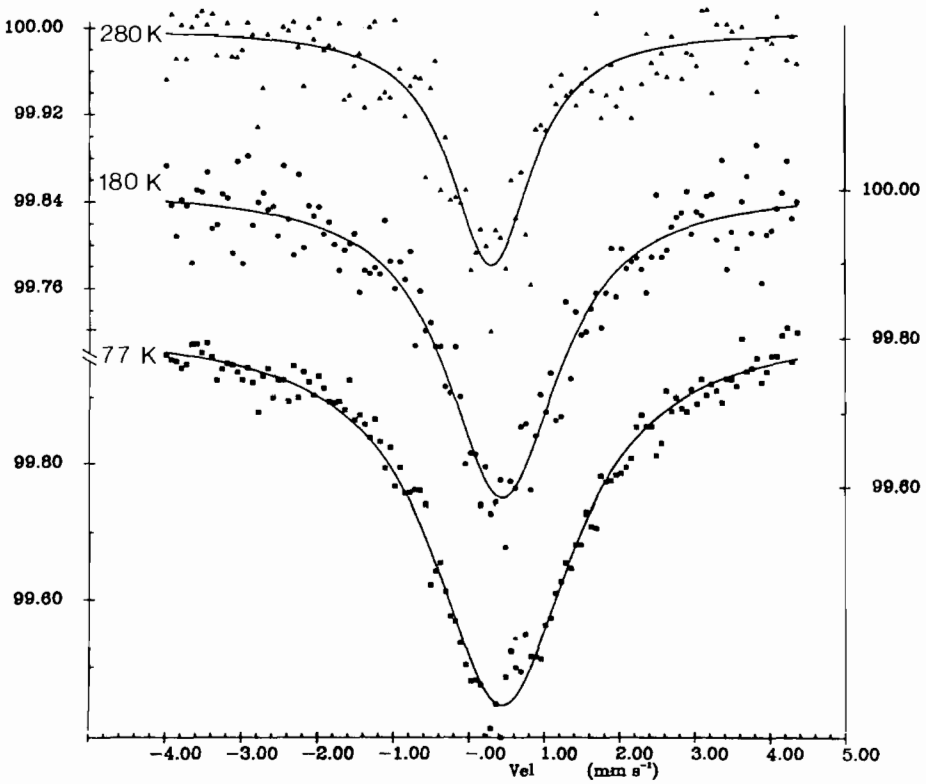


Fig. 3. Mössbauer spectrum of $Fe(Nal)_3 \cdot 2H_2O$.

TABLE III. Mössbauer Parameters (mm s^{-1}) of the Iron-(III) Complexes with Nalidixic Acid

Compound	δ^a	Δ^b	Γ^c	T (K)
$Fe(Nal)_3 \cdot 2H_2O$	0.44(2)		1.13(6)	80
	0.45(3)		0.91(9)	180
	0.29(4)		0.66(9)	280
$Fe(Nal)_2OH$	0.466(6)	0.80(1)	0.28(1)	80
	0.427(4)	0.787(7)	0.263(7)	180
	0.361(4)	0.767(7)	0.259(6)	280

[a]Chemical isomer shift (^{57}Fe as reference). [b]Quadrupole splitting. [c]Signal width at half maximum.

nalidixic acid anion binds through the carboxylate group either as a chelate or as a bridge to give a polymeric structure. Studies of the biological activity of some of these compounds are currently in progress.

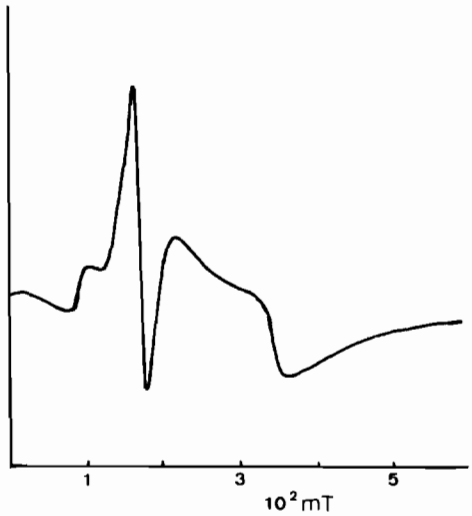


Fig. 5. X-band EPR spectrum of $Fe(Nal)_2OH$.

Experimental

Preparation

The sodium salt of nalidixic acid (NaNal) was prepared by mixing equimolar amounts of $NaHCO_3$ and HNal in aqueous solution. The reaction mixture was heated until clear and then used immediately. Except where stated otherwise, the complexes were prepared by the following general method. An aqueous solution of NaNal was added to one of the metal chloride in the stoichiometry ratios given in Table I. The complex usually precipitated immediately or within a short time after mixing the reactants. The products were washed with water and dried at 100 °C.

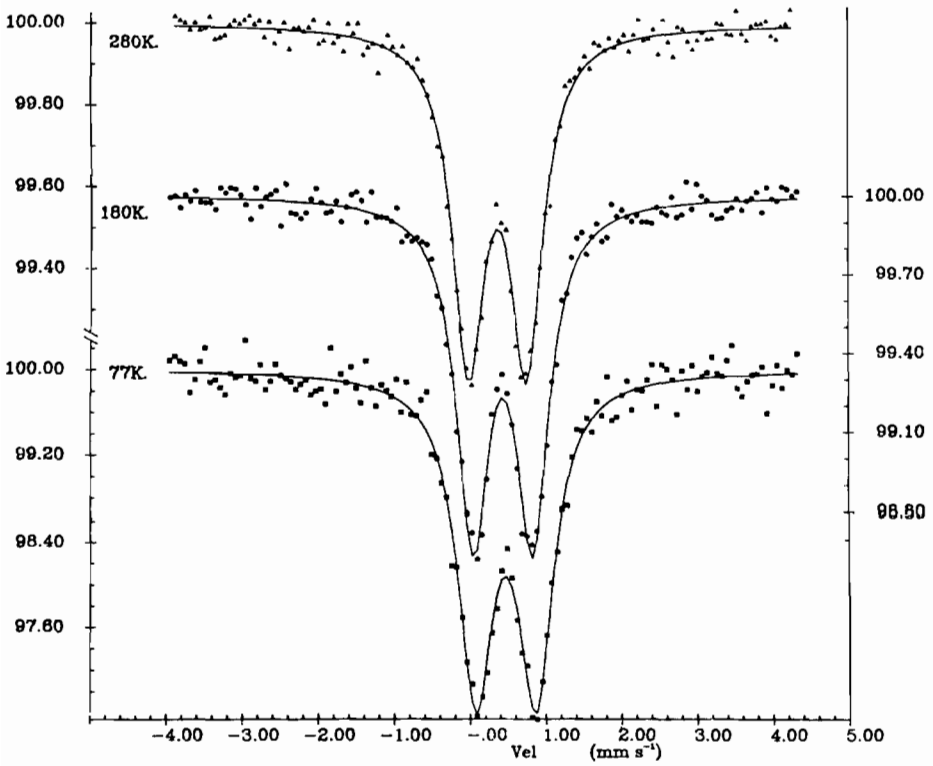


Fig. 4. Mössbauer spectrum of $Fe(Nal)_2OH$.

$Mn(Nal)_2$

The reaction was carried out in hot water.

$[Fe(Nal)_3]\cdot 2H_2O$ and $[Fe(Nal)_2(OH)]$

Both products were obtained from the same reaction. The reaction mixture was concentrated to small volume and the precipitate obtained was recrystallized from $CHCl_3$. The yellow solid which separated from the $CHCl_3$ solution was $Fe(Nal)_3\cdot 2H_2O$. The mother liquid subsequently precipitated red–brown $Fe(Nal)_2(OH)$.

$Ni(Nal)(NO_3)\cdot H_2O$

The salt employed was $Ni(NO_3)_2\cdot 6H_2O$ and the reaction was carried out in ethanol.

$Ni(Nal)_2\cdot 3H_2O$

The salt employed was $Ni(NO_3)_2\cdot 6H_2O$ and the reaction was carried out in water.

$Pd(Nal)_2\cdot H_2O$

The salt used was K_2PdCl_4.

$Cd(Nal)_2$

This was prepared from $Cd(NO_3)_2$ and NaNal by refluxing the reaction mixture with stirring for 6 h in ethanol.

$[Cd(Nal)Br_2]$

The starting metal salt employed was $CdBr_2\cdot 4H_2O$.

$Cd(Mn)(Nal)_2$

NaNal was added to an ethanolic solution of $Cd(NO_3)_2\cdot 4H_2O$ containing 1 mol% of $MnCl_2\cdot 4H_2O$, and the mixture was refluxed with stirring for 6 h.

Microanalyses (Table I) were by the Microanalytical Laboratory, Imperial College.

Physical Measurements

Infrared spectra were obtained using Perkin-Elmer 681-B and 599-B spectrometers. Electronic spectra were measured by the diffuse reflectance technique on a Cary 14 instrument. X-Band EPR spectra were measured on polycrystalline samples using a Varian E-12 spectrometer. The Mössbauer spectra were obtained on a standard spectrometer system by courtesy of Dr. L. V. C. Rees of the Physical Chemistry Section, Imperial College.

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