This value is derived using 94.5 kcal.[13] for a secondary C–H bond and 104.0 kcal.[14] for H–H.

$\Delta \bar{C}_p°$ for the reaction is calculated using the data for the specific heats listed in Table II. The heat capacity for the NO adduct radical is estimated, using 3-methyl-1,5-hexadiene as a model compound and correcting for the loss of eight C–H bending modes (each contributes 0.25 gibbs/mole at 300°K. and 0.8 gibbs/mole at 500°K.) and a decrease in rotation (~2.5 gibbs/mole) due to the generation of an allylic resonance in the radical. This leads to $\Delta \bar{C}_p°_{a,b} = 2.5$ gibbs/mole and

$\Delta H°_{a,b}(500°) = 11.4 + 0.5 = 11.9 \pm 0.7$ kcal. (standard error)

$\Delta S°_{a,b}(500°) = -28.2 \pm 1.0$ gibbs/mole is calculated using the entropy data given in Table II. 3-Methyl-1,5-hexadiene is used as a model compound for the radical, adding 1.4 gibbs/mole for the electronic degeneracy in the radical and taking into account a loss in entropy (2.3 gibbs/mole) owing to the stiffening effect of the allylic resonance.

An Electron Spin Resonance Study of Some Complexes of Iron, Nitric Oxide, and Anionic Ligands

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Contribution No. 1073 from the Central Research Department, Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, Delaware. Received March 20, 1965

Paramagnetic species are produced from reaction of nitric oxide with ferrous iron in aqueous solutions containing one or more of a wide variety of additional coordinating species (e.g., hydroxyl, phosphate, arsenate, and molybdate ions, phenol, benzoic acid, β-mercaptoethanol, cysteine). The paramagnetic species exhibit intense e.s.r. spectra with g values in the range from 2.02 to 2.04. The line widths of the resonances are sufficiently narrow in most instances to permit resolution of hyperfine splitting from a variety of magnetic nuclei in the complexes. For example, analysis of the Fe^{57}, N^{14}, N^{15}, and P^{31} hyperfine splittings of the complex formed from reaction of Fe(II), NO, and phosphate indicates that the complex consists of one iron atom, two NO groups, and two phosphate ions. A structure is proposed that involves a tetrahedral distribution of the four coordinating groups about iron. Similar structures are indicated from the e.s.r. spectra for the complexes formed with the other coordinating species mentioned above. Pyrophosphate and adenosine diphosphate appear to act as bidentate ligands in complexes involving iron and NO, while adenosine monophosphate behaves in the same fashion as phosphate ion. Dependences of e.s.r. intensities on complexing ligand concentrations suggest that many of the paramagnetic complexes exist in equilibrium with a diamagnetic complex involving two iron atoms which are antiferromagnetically coupled. Paramagnetic species formed in neutral aqueous solutions from Fe(II) (or Fe(III)), NO, and dithiols also exhibit strong e.s.r. spectra with g values from 2.02 to 2.04. The hyperfine structures of these spectra indicate coordination of one NO molecule and four sulfur atoms about one iron atom. The Fe^{57} and N^{14} splittings depend on the number of atoms in the chelate ring formed by the dithiol. The formal valence state of iron in all of the paramagnetic species reported here appears to be Fe(I).

Introduction

Complexes between iron and nitric oxide occupy a particularly honorable and ancient niche in the history and development of chemistry. Studies of such complexes extend back to Priestley,[1] and the complexes continue to furnish examples of new structures and modes of binding even to this date.[2]

Recently, complexes of iron and nitric oxide have also assumed some biological significance. Dobry-Duclaux has shown[3,4] that black Roussin's salt {[Fe$_4$S$_3$(NO)$_7$]K} effectively inhibits the enzyme alcohol dehydrogenase at very low concentrations (~1 × 10^{-7} M). Gordy and Rexroad[5] demonstrated that complexes of nitric oxide with hemoglobin and cytochrome-c exhibited electron spin resonance (e.s.r.) spectra that could be employed to elucidate the electronic structures of the iron atoms in these biologically important molecules.

Here, we present the results of an investigation by e.s.r. spectroscopy of complexes formed in aqueous solutions from Fe(II), NO, and a variety of inorganic and organic ligands. In most instances, these paramagnetic complexes exhibit e.s.r. spectra with well-resolved nucleus-electron isotropic hyperfine structure. Analyses of these spectra have provided considerable new information regarding the nature of iron–nitric oxide complexes.

(1) J. Priestley, "Experiments and Observations on Different Kinds of Air," Vol. I, Thomas Pearson, Birmingham, 1790, p. 328.
(2) W. P. Griffith, J. Lewis, and G. Wilkinson, *J. Chem. Soc.*, 3993 (1958).
(3) A. Dobry-Duclaux, *Biochim. Biophys. Acta*, **39**, 33 (1960).
(4) A. Dobry-Duclaux, *ibid.*, **39**, 44 (1960).
(5) W. Gordy and H. N. Rexroad in "Free Radicals in Biological Systems," M. S. Blois, Jr., H. W. Brown, R. M. Lemmon, R. O. Lindblom, and M. Weissbluth, Ed., Academic Press Inc., New York, N. Y., 1961, pp. 268–273.
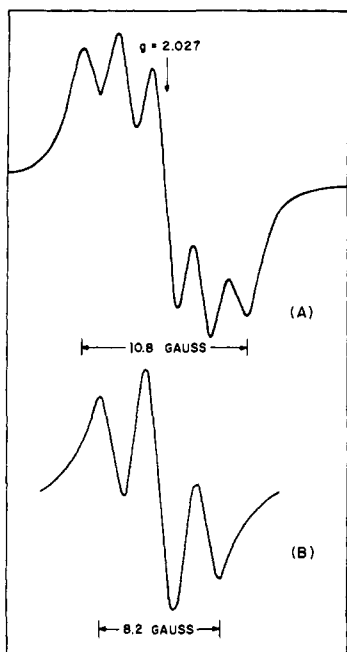
Figure 2. E.s.r. spectrum of $Fe^{57}-N^{15}O-OH^-$: Klystron frequency, 9.505×10^9 c.p.s., pH 11.

Figure 1. E.s.r. spectra of complexes prepared from Fe(II) and NO at pH 11: (A) $Fe-N^{14}O-OH^-$, (B) $Fe-N^{15}O-OH^-$.

Experimental

Aqueous solutions of typical iron–nitric oxide–anion complexes were prepared for investigation by e.s.r. spectroscopy in the following manner. Aqueous Fe-SO_4 (1×10^{-3} M), 4 ml., was freed of oxygen by stirring under a stream of nitrogen. During deoxygenation, the pH of the solution was adjusted to the desired value by the addition of small amounts of solutions of HCl or NaOH. In most experiments, salts of anionic ligands were also added. The solution was evacuated to the vapor pressure of water and stirred for 5 min. in contact with NO at 200 mm. pressure. Experiments employing longer periods of treatment with NO demonstrated that the reaction was complete in less than 5 min. The NO was then pumped off, and the reaction vessel was brought to atmospheric pressure with nitrogen. A portion of the solution was transferred under nitrogen into a flat quartz cell and was examined for e.s.r. at room temperature with a Varian Associates X-band spectrometer employing an electromagnet with 12-in. pole pieces and 100-kc./sec. field modulation. The Fe(II) solutions became colored during reaction with nitric oxide with the colors ranging from brown to dark green depending on the anion present as ligand. Most of the solutions were buffered either by excess concentrations of salts of ligands or by noncomplexing buffers such as sodium citrate. In the absence of buffering agents, the solutions become more acidic during treatment with nitric oxide.

The $N^{14}O$ and $N^{15}O$ (97% N^{15}) employed in this study were obtained from the Matheson Co. and Isomet, respectively, and were used without further purification. Some of the complexes were prepared with Fe^{57}(II) obtained by reduction of $Fe^{57}_2O_3$ (90% Fe^{57}) supplied by Oak Ridge National Laboratory. Samples of black Roussin's salt, red Roussin's salt, and Bahr's salt were prepared by Drs. D. C. Blomstrom, L. R. Melby, and N. J. Rose of this laboratory. Other
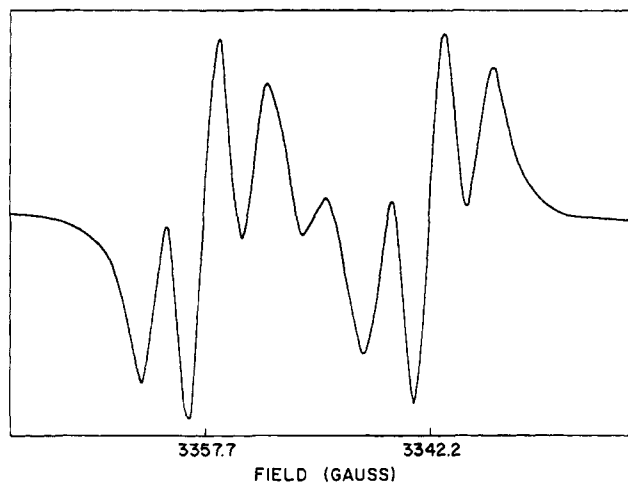
chemicals used were reagent grade or the best grades commercially available.

Results and Discussion

1. Complexes Prepared from Fe(II), NO, and Various Anionic Ligands. No e.s.r. absorption was detected for aqueous solutions containing only $FeSO_4$ or NO. Neutral solutions containing the reaction product of $FeSO_4$ and NO exhibited a single, weak resonance with a half-width of 9 gauss at a g value of 2.033. In more acidic solutions, this resonance was less intense and has not been detected at pH values less than 4. When the Fe–NO complex was prepared in basic solutions, the intensity of the e.s.r. spectrum increased very slowly with pH in the range from 7 to 10 but increased by about two orders of magnitude as the pH was increased from 10 to 11. Furthermore, at high pH values the g value shifted to 2.027 and the e.s.r. spectrum exhibited hyperfine structure, each hyperfine component having a half-width of about 1.5 gauss. The e.s.r. spectra of the complexes prepared from Fe(II) at pH 11 with $N^{14}O$ and with $N^{15}O$ are shown in Figures 1A and 1B, respectively. The five hyperfine lines of the $N^{14}O$ complex when analyzed in detail exhibit a 1:2:3:2:1 intensity relationship and a component separation of 2.2 gauss. The component separation is 3.1 gauss for the $N^{15}O$ complex with a 1:2:1 intensity relationship.

The number of hyperfine components and their relative intensities in these e.s.r. spectra, as well as the fact that the component separations increase by a factor of 1.4 when N^{15} ($I = \frac{1}{2}$) is substituted for N^{14} ($I = 1$),[6] clearly show that the hyperfine structure of the paramagnetic Fe–NO complex prepared at pH 11 arises from isotropic hyperfine contact interaction of the unpaired electron with two equivalent nitrogen nuclei. When the $N^{15}O$ complex was prepared at pH 11 with Fe^{57}(II) substituted for iron of natural isotope abundance,[7] the e.s.r. spectrum shown in Figure 2 was obtained. In this spectrum an additional doublet splitting of 15.5 gauss has been introduced by isotropic hyper-

(6) The ratio of magnetogyric ratios of the N^{15} and N^{14} nuclei is 1.403.

(7) The Fe^{57} isotope is present to the extent of only 2.2% in iron of natural isotopic composition.
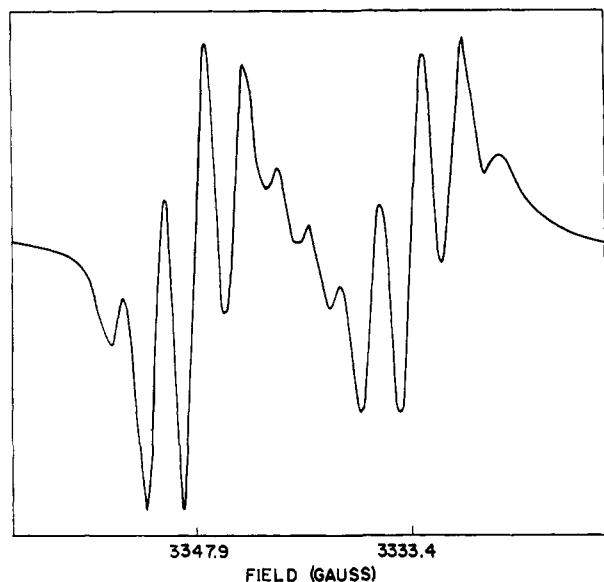
Figure 3. E.s.r. spectrum of $Fe^{57}-N^{15}O$-phosphate: Klystron frequency, 9.505×10^9 c.p.s., pH 7.

Figure 4. Dependence of e.s.r. intensity on phosphate concentration for an aqueous solution containing Fe–NO–phosphate (see text for details).

fine contact interaction of the unpaired electron with a single Fe^{57} nucleus ($I = {}^1/_2$). The profound changes in the intensities and hyperfine structures of the e.s.r. spectra of the Fe–NO complexes between pH 10 and 11 strongly suggest that hydroxyl ions are involved in the complexes formed at pH 11. No change was observed, however, in the e.s.r. spectrum of the Fe–NO complex when the complex was prepared at pH 11 in D_2O rather than H_2O. This result does not, of course, demonstrate the absence of bound water or hydroxyl ions in the complex but shows that any hyperfine contact interactions with protons or deuterons of these species are too small to affect the structure or line widths of the observed hyperfine pattern. In addition, it can be concluded with some certainty that the paramagnetic species is not a hydride.

When Fe(II) was allowed to react with $N^{15}O$ in a solution at pH 7 containing phosphate (0.20 M), the e.s.r. spectrum of the Fe–NO complex formed was again about two orders of magnitude more intense than that of the complex prepared at pH 7 in the absence of phosphate. The resonance occurred at a g value of 2.033 and consisted of five incompletely resolved hyperfine components. When this complex was made with $N^{14}O$, the e.s.r. spectrum exhibited seven hyperfine components. The number of and intensity relationships between hyperfine components of these spectra are compatible with hyperfine contact interaction involving two equivalent nitrogen nuclei and two equivalent P^{31} nuclei ($I = {}^1/_2$), with the splitting constants for P^{31} and N^{15} both being about 2.6 gauss. When this complex was prepared with $Fe^{57}(II)$ and $N^{15}O$, an additional hyperfine splitting of 14.5 gauss produced by a single Fe^{57} nucleus was observed (Figure 3).

The intensity of the e.s.r. spectrum of the Fe–NO–phosphate complex increased as the phosphate ion concentration of the reaction medium was increased to a concentration of 0.2 M and remained substantially constant at higher phosphate concentrations (Figure 4). The e.s.r. intensity of this complex also exhibited a dependence on pH, being undetectable at pH values less
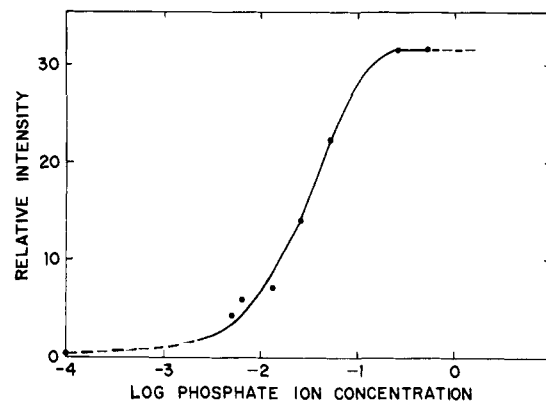
than 4, rising to a maximum at a pH of about 7 (where the predominant species of the free phosphate ligand is HPO_4^{-2}), and decreasing slowly at higher pH values. At a pH of about 10, the Fe–NO–phosphate complex appeared to convert to the high pH complex species described earlier for a phosphate-free solution. When the Fe(II) concentration was varied in the reaction medium (pH 7, phosphate 0.2 M), the intensity of the e.s.r. spectrum increased somewhat less than linearly without change in the hyperfine pattern for Fe(II) concentrations from 2×10^{-4} to 5×10^{-3} M. At higher concentrations, a precipitate formed during the reaction with NO, and a reduction of e.s.r. intensities was observed. The intensity of the e.s.r. spectrum of the Fe–NO–phosphate complex decreased rapidly if a solution containing the complex was exposed to air or to ultraviolet radiation.

Strong e.s.r. spectra with g values from 2.03 to 2.04 were also observed when neutral aqueous solutions of Fe(II) containing other anionic ligands were treated with NO. These ligands include pyrophosphate, other phosphate derivatives such as adenosine triphosphate (ATP) and creatine phosphate, arsenate, molybdate, carbonate, maleate, benzoate, mercaptans such as 2-mercaptoethanol and cysteine, and thiourea. E.s.r. spectra of Fe–NO complexes prepared with $N^{14}O$ in the presence of many of these anions (e.g., molybdate, maleate, benzoate, and thiourea) consisted of five hyperfine lines, thereby indicating hyperfine splitting by nitrogen nuclei from two equivalent NO groups. Spectra of complexes involving other anionic ligands of the group listed above exhibited more complex structure because of additional hyperfine splitting by magnetic nuclei of the anionic ligands. For example, the Fe–$N^{15}O$–arsenate complex produced an e.s.r. spectrum with nine hyperfine components, consistent with hyperfine splitting by two equivalent N^{15} nuclei and two equivalent As^{75} ($I = {}^3/_2$) nuclei with both N^{15} and As^{75} exhibiting coupling constants of about 3 gauss. Other examples of e.s.r. spectra of Fe–NO complexes with these various anionic ligands are discussed in later sections. The point we wish to make here is that the Fe–NO complexes involving all of these anion ligands exhibit similar g values. In addition, hyperfine structure, where it has been observed in the e.s.r. spectra, is compatible with hyperfine splitting by two equivalent nitrogen nuclei, one iron nucleus, and magnetic nuclei

of two equivalent, coordinately bound, anionic ligands. It would thus seem that the complexes formed from Fe(II), NO, and the above listed anions possess a common structure.

Weaker e.s.r. spectra with g values in the range from 2.02 to 2.04 were observed for Fe–NO complexes prepared in solutions containing metasilicate, tetraborate, acetate, and phenolate. Spectra of complexes involving the first three of these anions exhibited complex, poorly resolved, hyperfine structure. Complexes of iron and nitric oxide prepared in neutral solutions containing high concentrations (0.1 to 0.5 M) of sulfate, nitrate, nitrite, chloride, thiocyanate, or citrate produced only the single weak resonance described earlier for a neutral solution of 1×10^{-3} M FeSO$_4$ treated with NO.

We suggest that the paramagnetic species formed by Fe(II), NO, and anionic ligands such as hydroxyl, phosphate, and the others that have been mentioned are closely related structurally to the red Roussin's salt. Thomas, Robertson, and Cox[8] have shown by X-ray analysis that the structure of Roussin's red ethyl ester is

I
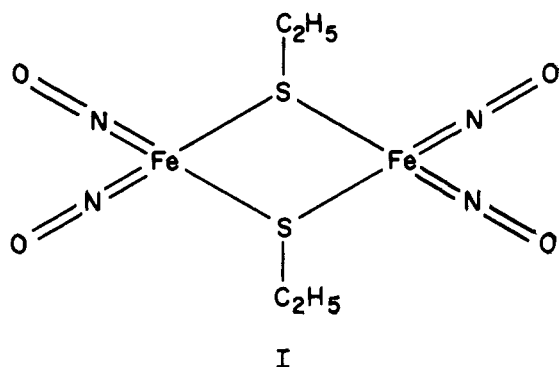
The C$_2$H$_5$S$^-$ and NO groups are distributed tetrahedrally about the iron atoms. Johansson and Lipscomb[9] have attributed the diamagnetism of the compound (the iron atoms being considered formally in the +1 state) to the short Fe–Fe distance (2.7 Å.) that permits strong Fe–Fe antiferromagnetic exchange interaction. The paramagnetic species in the present study, for example, the hydroxyl derivative, then is considered to be analogous to the dissociated form of the red Roussin's salt (III).

The above reasoning can be taken a step further by postulating the existence of an equilibrium between diamagnetic and paramagnetic forms of a series of analogs of the red Roussin salt formed in aqueous solutions from Fe(II), NO, and anions, A, such as hydroxyl, phosphate, etc. Species III is considered to possess a
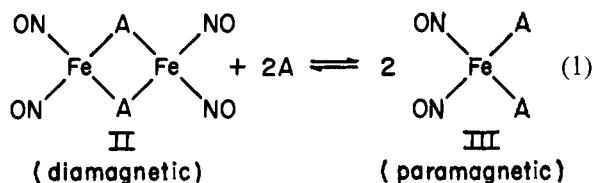
single unpaired electron, but II is diamagnetic because of Fe–Fe antiferromagnetic exchange interaction. Iron in the above species would be formally in the +1

state (d^7) and the two nominally unpaired electrons of the NO groups on each iron atom would be paired through occupancy of a molecular orbital extending over at least the ON–Fe–NO part of the molecule. The observations that we have presented would suggest that these diamagnetic and paramagnetic analogs of red Roussin's salt are formed fairly generally in aqueous solutions containing Fe(II), NO, and any one of a variety of anionic ligands. In support of the above model, we note particularly that the mercaptans such as β-mercaptoethanol and cysteine are functionally similar to ethylmercaptan which forms the Roussin's red ethyl ester of demonstrated stability and structure.[8]

In Figure 4, e.s.r. intensities of neutral aqueous solutions of Fe(II) (1 × 10^{-3} M) containing phosphate and treated with NO are plotted as a function of phosphate concentration. The e.s.r. intensity is low at phosphate concentrations comparable to the Fe(II) concentration, but approaches a much higher value asymptotically at phosphate–iron ratios in excess of 100. These observations are compatible with the existence of the equilibrium of eq. 1. To the extent that the equilibrium is displaced to the left, the e.s.r. signal is diminished in intensity. Increased concentrations of A would force the equilibrium to the right to give the paramagnetic species III.

The data of Figure 4 can be analyzed in terms of the equilibrium constant of eq. 1 defined as

$$K = \frac{(\text{III})^2}{(\text{II})(\text{A})^2} \qquad (2)$$

For the Fe–NO–phosphate system, a value for K (25°) of 1.4 fits the plot of e.s.r. intensity $vs.$ phosphate concentration of Figure 4 very well over the entire concentration range.

Further support for the notion of a dynamic equilibrium between diamagnetic and paramagnetic forms of the iron–nitric oxide complexes was provided by the following experiments. An Fe(II)–NO–phosphate complex was prepared in a neutral aqueous solution 0.01 M in phosphate ion. The nitric oxide was pumped off and phosphate ion concentration was increased to 0.2 M. The e.s.r. spectrum observed was as strong as for a solution 0.2 M in phosphate $before$ reaction with nitric oxide. Furthermore, if the ligand concentration was increased from 0.01 M phosphate by addition of arsenate (0.2 M) after the NO treatment, the e.s.r. spectrum changed from that characteristic of an Fe(II)–NO–phosphate complex to that of an Fe(II)–NO–arsenate complex. Similar displacement of phosphate from the prepared Fe–NO complex was accomplished with a number of ligands.

Iron–nitric oxide complexes prepared in the presence of other anionic ligands such as β-mercaptoethanol, cysteine, and adenosine monophosphate exhibited an e.s.r. spectrum of maximum intensity when the anionic ligand–iron ratio was from 5 to 10, implying much larger stability constants for complexes involving these ligands than for complexes involving phosphate. On the other hand, lesser stabilities are indicated for the Fe–NO–anion complexes discussed earlier that exhibit e.s.r. spectra with intensities considerably weaker than the Fe–NO–phosphate complex even when the reaction solutions contain high concentrations of the anionic ligands.

(8) J. T. Thomas, J. H. Robertson, and E. G. Cox, $Acta\ Cryst.$, **11**, 599 (1958).
(9) G. Johansson and W. N. Lipscomb, $ibid.$, **11**, 594 (1958).

It is not known what species is responsible for the single, weak resonance that is observed for a neutral solution containing only $FeSO_4$ (1×10^{-3} M) after treatment with NO. Presumably, it is a member of the group of species $Fe(NO)_2A_2$. If $A = OH^-$, the equilibrium of eq. 1 would be displaced to the right at pH 7 and a low concentration of the paramagnetic species III would be expected. However, A may be H_2O and the weak e.s.r. spectrum may result from a small K value for the equilibrium of eq. 1 or because only a small amount of all forms of the Fe–NO complex are formed.

The existence of multiple equilibria of the form of eq. 1 that involve more than one ligand A is clearly shown by the results from a reaction solution containing carbonate. At pH 6.7, spectra for two distinct species involving complexes of Fe(II), NO, and anionic ligands can be observed. These spectra appear to consist of two overlapping five-line spectra with different intensities at g values of 2.022 and 2.029. At pH 10.5, only one species is detected, suggesting that the equilibrium has been shifted in favor of one or the other of the two species. The nature of the anions involved in these complexes cannot be determined from the e.s.r. spectra alone, but plausible coordinating species are CO_3^{-2}, HCO_3^-, and OH^-. Evidence for more than one paramagnetic species has been observed in the e.s.r. spectra of several other Fe–NO–anionic ligand complexes, e.g., the Fe–NO complex involving penicillamine which is shown in Figure 6 and discussed later.

2. Complexes Formed from Fe(II), NO, and Phosphate Derivatives. The modes of binding of transition metals to adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) are of considerable biological interest and have been investigated by Cohn[10] and Shulman[11] and their collaborators using nuclear magnetic resonance techniques. In an attempt to further elucidate such binding, e.s.r. studies were performed on neutral, aqueous Fe(II)–NO solutions containing additionally the ligands AMP, ADP, ATP, or pyrophosphate.

Neutral aqueous solutions of Fe(II)–NO–phosphate, described in the previous section, exhibit e.s.r. spectra compatible with the existence of a paramagnetic complex consisting of one iron atom, two phosphate ions, and two NO groups. Isotropic hyperfine coupling constants for the magnetic nuclei of the $Fe(NO)_2$(phosphate)$_2$ complex are $A_{Fe^{57}} = 14.5$, $A_{P^{31}} \approx 2.7$, and $A_{N^{15}} \approx 2.7$ gauss. When the Fe–NO complex is prepared with pyrophosphate rather than phosphate, $A_{Fe^{57}}$ is reduced to 12.9 gauss and $A_{P^{31}}$ increases to 5.5 gauss. Hyperfine splitting from either N^{14} or N^{15} is not resolved for the pyrophosphate complex because of the widths of the resonances. Observation of only triplet P^{31} hyperfine splitting indicates (a) that only a single pyrophosphate group is bound to iron with the two phosphorus atoms occupying equivalent coordination sites or (b) that two pyrophosphate groups are bound but that each is bound in an asymmetric fashion such that only one of the two phosphorus atoms is capable of exhibiting resolvable isotropic hyperfine contact splitting. Since the P^{31} splitting in the pyrophosphate derivative (5.5. gauss) is about twice that in the

(10) M. Cohn and T. R. Hughes, *J. Biol. Chem.*, **237**, 176 (1962).
(11) R. G. Shulman, private communication.

phosphate derivative (2.7 gauss), the former alternative is preferred.

As discussed earlier, the probable structure of the $Fe(NO)_2$(phosphate)$_2$ derivative is that in which the NO and phosphate groups are arranged tetrahedrally about the iron atom (V). A probable structure for the $Fe(NO)_2$(pyrophosphate) species is that in which the two NO groups occupy two of the tetrahedral coordination sites about iron and the pyrophosphate group occupies the other two (IV).

IV   V
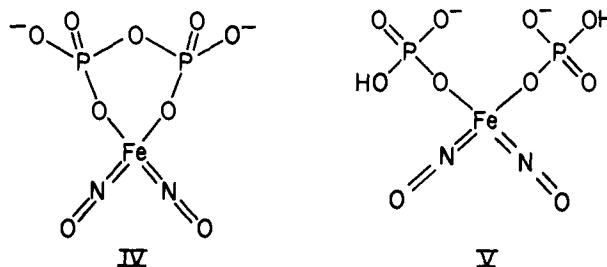
It is suggested that the P^{31} splitting is much greater in IV than V because the bidentate pyrophosphate ligand is more effective than phosphate in accommodating the unpaired electron centered principally on the iron atom. This conclusion seems to be borne out by the Fe^{57} splittings which are 14.5 gauss for V and only 12.9 gauss for IV. It is, of course, not expected that the increase in the P^{31} splitting should equal in absolute magnitude the decrease in the Fe^{57} splitting since the proportionality constants relating spin densities to coupling constants are likely to be quite different for P^{31} and Fe^{57}.

E.s.r. spectra of Fe–NO complexes prepared with AMP and ADP are very similar to those of the Fe–NO–phosphate and Fe–NO–pyrophosphate complexes, respectively, although resolution of the hyperfine structure is somewhat poorer in the former. These spectra are consistent then with the compositions $Fe(NO)_2$(AMP)$_2$ and $Fe(NO)_2$(ADP) and with structures analogous to V and IV, respectively. The Fe–NO–ATP complex exhibits a strong, broad e.s.r. spectrum at a g value (2.038) similar to that of the Fe–NO–pyrophosphate complex (2.040), but hyperfine structure is not resolved so that it is not possible to assign composition or structure to this complex on the basis of its e.s.r. spectrum. However, in light of the studies of Cohn[10] and Shulman,[11] the structure of the Fe–NO–ATP complex is expected to closely resemble those of the Fe–NO–ADP and Fe–NO–pyrophosphate complexes.

3. Complexes Formed from Fe(II), NO, and Mercaptans. Complexes of iron and $N^{14}O$ prepared in neutral aqueous solutions containing cysteine, cysteine ethyl ester, or β-mercaptoethanol all exhibited spectra similar to the one shown in Figure 5 for the Fe–$N^{14}O$–cysteine complex. These spectra are compatible with species possessing as ligands two NO groups and two sulfur-containing ligands. N^{15} splittings of NO in these complexes are about 3 gauss. Additional hyperfine splitting arises from the CH_2 protons adjacent to sulfur which presumably is directly bound to the iron The CH_2 splittings are about 1.5 gauss and undoubtedly arise through hyperconjugative interaction with spin density centered on sulfur. The structure for the complex involving cysteine then is believed to be that given as VI. Analogous structures are assigned to the com-
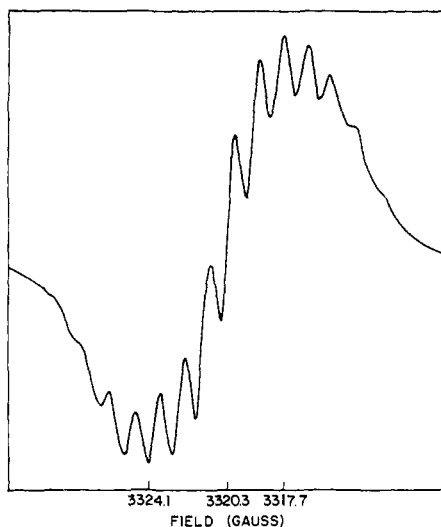
Figure 5. E.s.r. spectrum of Fe–N^{14}O–cysteine: Klystron frequency, 9.440×10^9 c.p.s.

Figure 6. E.s.r. spectrum of Fe–N^{14}O–penicillamine.

plexes containing cysteine ethyl ester and β-mercaptoethanol.
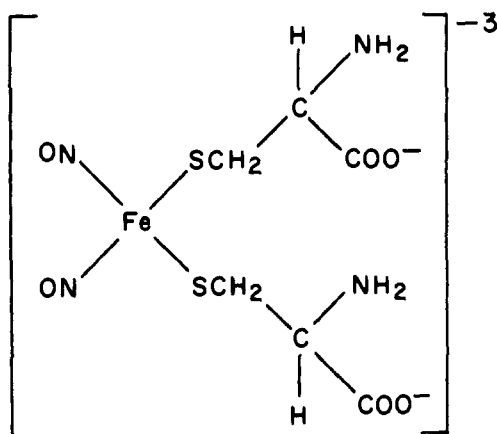
$\overline{\text{VI}}$

When penicillamine (VII) is employed as the anionic ligand, a well-resolved, five-line spectrum is observed as well as a broader, weaker spectrum of at least two lines
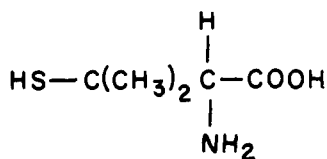
$\overline{\text{VII}}$

(Figure 6). The species giving rise to the five-line spectrum is believed to be Fe(NO)$_2$(penicillamine)$_2$ with a structure analogous to VI. The sharpness of the five-line spectrum is attributable to replacement of t^.. β-protons of cysteine by methyl groups which would h. expected to exhibit only very small and probably no.. resolvable hyperfine contact interaction with spin density centered on sulfur. The identity of the species giving rise to the weaker spectrum is not known but may be one in which both the carboxyl and mer.:. >to groups of penicillamine are involved in binding tc ..on and in which only one NO group is bound to iron as in other complexes to be discussed below.
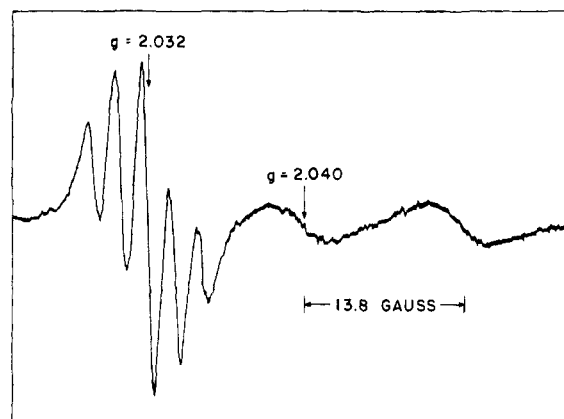
Interestingly, cystine, the disulfide of cysteine, when used as the added ligand in preparing the Fe–NO complex gives rise to a very broad, poorly resolved spectrum quite unlike the spectrum of Fe–NO–cysteine. Consequently, the Fe–NO complexes involving cysteine and cystine can be assumed to be quite different. This different behavior of the –SH group of cysteine and the –S–S– group of cystine with respect to bonding with the Fe–NO complex suggests that these reactions might form the basis of a study of active sites and tertiary structure of enzymes. In this regard, as mentioned earlier, the black Roussin's salt is a potent inhibitor of a number of enzymes, among them being alcohol dehydrogenase and ribonuclease.[3,4] In the case of ribonuclease, at least, disulfide linkages are present.

4. Complexes Formed from Fe(II), NO, and Dithiols or Sulfide. E.s.r. spectra of the paramagnetic species formed by maleonitriledithiol (VIII) in aqueous solutions containing Fe(II) or Fe(III) and treated with
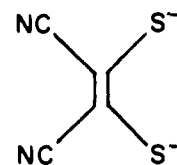
$\overline{\text{VIII}}$

N^{14}O or N^{15}O are shown in Figures 7A and 7B, respectively. The Fe–N^{14} O–VIII species gives rise to a triplet with component separations of 15.5 gauss while the Fe–N^{15}O–VIII species yields a doublet with a spacing of 21.7 gauss. The number of hyperfine lines in these spectra, their intensity relationships, and the ratio of component separations in the spectra of the two isotopic species are in accord with hyperfine splitting by one nitrogen nucleus. Thus, the Fe–NO–maleonitriledithiol complex contains but a single NO group that can be involved in hyperfine interaction with the unpaired electron. The presence of other NO groups in the complex that are unavailable for hyperfine interaction cannot be ruled out, but seems unlikely. Similar results were obtained when benzene-1,2-dithiol (IX) and 1,1-dicyanoethylene-2,2-dithiol (X) were substituted for maleonitriledithiol. For the Fe–NO–IX and Fe–NO–X complexes, the N^{14} hyperfine splitting constants were 15.1 and 12.8 gauss, respectively. Fe^{57} coupling constants were obtained for the Fe–NO

IX

X
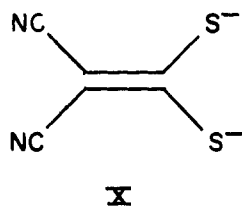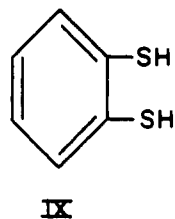
complexes of VIII, IX, and X and are collected, along with N^{14} coupling constants and average g values, in Table I.

Table I. E.s.r. Characteristics of Fe(NO)(dithiol)₂ Complexes

|	$A_{N^{14}}$, gauss	$A_{Fe^{57}}$, gauss	g_{av}
Fe(NO)[S₂C₂(CN)₂]₂	15.5	9.4	2.027
Fe(NO)[S₂C₆H₄]₂	15.1	9.3	2.028
Fe(NO)[S₂CC(CN)₂]₂	12.8	8.4	2.041
Fe(NO)[S₂CN(CH₃)₂]₂	12.7[a]	...	2.041[a]

[a] Reference 12.

Gibson[12] has studied the e.s.r. of Fe(NO)[S₂CN-(CH₃)₂]₂ which he reports to be isomorphous with Co(NO)[S₂CN(CH₃)₂][13] and therefore almost certainly possesses the structure XI. The molecule

XI
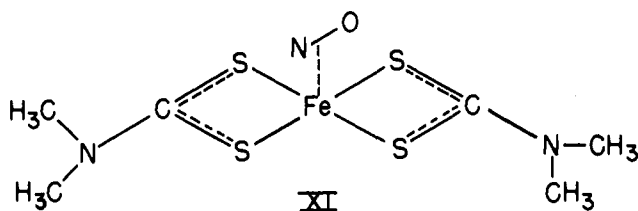
possesses but a single unpaired electron (μ_{eff} = 2.24 B.M.) and the e.s.r. of the $N^{14}O$ derivative consists of a triplet of spacing 12.7 gauss.

It is likely that the paramagnetic species formed in solution between Fe–NO and VIII, IX, and X are structurally analogous to XI. Indeed, the square-planar chelate XII has been isolated.[14] A major point of interest is the variation of the N^{14} and Fe^{57} isotropic

XII
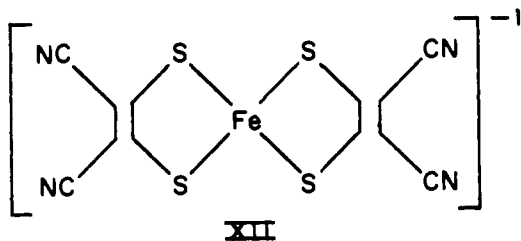
hyperfine coupling constants in the four compounds. VIII and IX which will form five-membered chelate rings in Fe–NO complexes exhibit almost identical N^{14} and Fe^{57} coupling constants (Table I). N^{14} coupling constants for the Fe–NO complexes prepared from X and XI, which will form four-memberd chelate rings, are the same but about 2.6 gauss less than for the complexes involving VIII and IX which form five-membered chelate rings. The Fe^{57} coupling constant for Fe-

(12) J. F. Gibson, *Nature*, **196**, 64 (1962).
(13) P. R. H. Alderman, P. G. Owston, and J. M. Rowe, *J. Chem. Soc.*, 668 (1962).
(14) J. F. Weiher, L. R. Melby, and R. E. Benson, *J. Am. Chem. Soc.*, **86**, 4329 (1964).
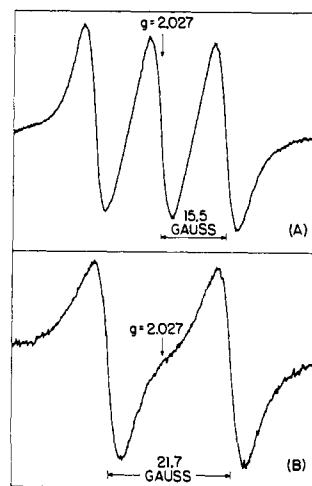

Figure 7. E.s.r. spectra of (A) Fe–N¹⁴O–maleonitriledithiol and (B) Fe–N¹⁵O–maleonitriledithiol.

(NO)(X)₂ is 8.4 gauss, 1.0 gauss less than for the Fe^{57} coupling constants for Fe(NO)(VIII)₂ and Fe(NO)-(IX)₂. Although the number of examples admittedly is limited, two trends appear to be discernible. Chelate ring size would seem to be the major determinant for g values and spin densities at the N^{14} and Fe^{57} nuclei in these complexes involving dithiol ligands. Additionally, a proportionality between N^{14} and Fe^{57} coupling constants would appear to be indicated. This latter correlation could reflect the distribution of the unpaired electron between the dithiol ligand (VIII, IX, X, S₂CN(CH₃)₂) and the Fe(NO) part of the complex. S^{33} hyperfine splittings would be useful in testing this hypothesis. Finally, in view of the above, one would predict with some confidence that the Fe^{57} hyperfine splitting in Fe(NO)[S₂CN(CH₃)₂]₂ would be close to 8.4 gauss.

In aqueous solutions containing sulfide ion, a paramagnetic species readily forms with Fe(II) and $N^{14}O$ that gives rise to a 1:1:1 e.s.r. triplet at a g value of 2.021 with 5.0 gauss component separations that must be attributed to hyperfine splitting by a single N^{14} nucleus. Again a complex containing only one NO group is implicated as the paramagnetic species. The black Roussin's salt in neutral aqueous solution is diamagnetic and does not exhibit an e.s.r. spectrum, but if the pH of the solution is increased to 11, a triplet e.s.r. pattern is observed that possesses a spacing of 4.7 gauss, essentially identical with the 5.0 gauss observed for the Fe–NO–sulfide complex. The e.s.r. behavior of the black Roussin's salt is reversible with pH, although it has not been determined whether the integrity of the black Roussin's salt structure is restored on reducing the pH from 11 to neutrality. In any event, it seems likely that the high pH form of the black Roussin's salt is related structurally to the Fe–NO–sulfide complex. Indeed, the latter complex may be a rather direct precursor to black Roussin's salt since the method of preparation of the salt[15] is not too different from that of preparation of the Fe–NO–sulfide complex.

5. Electronic Structures. Complete elucidation of the geometrical and electronic structures of the complexes discussed in this study must await chemical and

(15) O. Pawel, *Ber.*, **15**, 2600 (1882).

physical characterization on isolated, often single crystal forms. Only in this way will the necessary compositional, X-ray, spectroscopic, and magnetic susceptibility data be forthcoming. This isolation may not be easy inasmuch as NO complexes are notoriously unstable, and the paramagnetic species reported here may only exist in solution where the equilibrium of eq. 1 can be forced to the right.

The e.s.r. characteristics of the complexes reported in the present study point strongly to the presence of d^7 iron. E.s.r. characteristics with respect to g value and relaxation times as judged by line widths are very similar to those reported for $[Fe(CN)_5(NO)]^{-3}$ [16-18] and $Fe(NO)(S_2CNMe_2)_2$.[12] In fact, the similarities in N^{14} hyperfine splitting in the $Fe(NO)(S_2CNMe_2)_2$ complex and the Fe–NO ,complexes with maleonitriledithiol (VIII), benzene-1,2-dithiol (IX), and 1,1-dicyanoethylene-2,2-dithiol (X) indicate strongly that the geometrical

(16) I. Bernal and E. F. Hockings, *Proc. Chem. Soc.*, 361 (1962).
(17) J. B. Raynor, *Nature*, 201, 1216 (1964).
(18) D. A. C. McNeil, J. B. Raynor, and M. C. R. Symons, *Proc. Chem. Soc.*, 364 (1964).

and electronic structures of these four complexes must be very similar.

Structures of the complexes $Fe(NO)_2(A)_2$ are open to somewhat more question, and their only structural analogs would appear to be the dissociated form of the red Roussin's salt. It seems clear from the e.s.r. characteristics that iron in these complexes possesses the d^7 configuration. In the frozen state, e.s.r. signal intensities of the Fe–NO–phosphate complex exhibit Curie law behavior between -20 and $-155°$. It cannot, however, be unequivocally demonstrated from the e.s.r. results reported here whether the iron is tetrahedrally coordinated or, perhaps, pentahedrally or even octahedrally coordinated. Our results only demonstrate that the single unpaired electron of each of the complexes is in the isotropic hyperfine splitting environment of one iron atom, two nitric oxide molecules, and two anionic ligands such as phosphate, arsenate, cysteine, or β-mercaptoethanol. One or two water molecules could be involved as additional ligands about iron. However, the analogy with the red Roussin's salts is considered to be compelling, if not conclusive.

The Electron Spin Resonance Spectra of 2,1,3-Benzoxadiazole, -Benzothiadiazole, and -Benzoselenadiazole Radical Anions. Electron Withdrawal by Group VI Elements[1]

E. Thomas Strom[2] and G. A. Russell

Contribution from the Department of Chemistry, Iowa State University, Ames, Iowa, and the Pioneering Research Division, U. S. Army Natick Laboratories, Natick, Massachusetts. Received February 16, 1965

Radical anions of the title compounds have been prepared by electron transfer from carbanions to the parent aromatics. On the basis of hydrogen hyperfine splitting constants it is concluded that sulfur and selenium atoms in these heterocyclics accept the unpaired electron more readily than oxygen. LCAO–MO calculations have been performed for the benzoxadiazole and benzothiadiazole systems. Good agreement between experimental and calculated spin densities was obtained with a simple Hückel treatment. A p-orbital model for the sulfur atom gave results as good as those obtained using a d-orbital model.

The electron-withdrawing ability of group VI elements is a subject of considerable interest. From electronegativity considerations, oxygen should be electron withdrawing relative to carbon, whereas sulfur and selenium should have a slight electron-supplying ability.[3] In conjugated systems the inductive effect is

supplemented by resonance interactions involving the unshared p-electrons of the heteroatom
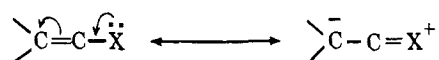
Thus, the *m*-methoxy group is electron withdrawing but the *p*-methoxy group is electron supplying on the basis of the ionization constants of substituted benzoic acids.[4]

The electronic situation is more complicated in the case of sulfur and selenium atoms because of the presence of vacant 3d- or 4d-orbitals. These orbitals may interact with a conjugated system to enhance the electron-attracting power of the atom. This can account for the fact that the *m*-thiomethyl group is strongly electron attracting.[4]

Electron spin resonance offers a direct method of determining the effect of an atom on the odd-electron distribution, *i.e.*, that of the highest occupied molecular orbital. The hyperfine splitting of hydrogen atoms in a benzenoid system is given by $a_{CH}{}^H = Q_{CH}{}^H \rho_C$ where a^H is the hyperfine splitting constant (h.f.s.c.), ρ_C is the spin density on the carbon bound to the hydrogen