Nitrosyliron Complexes with Mercapto-purines and -pyrimidines studied by Nuclear Magnetic and Electron Spin Resonance Spectroscopy

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The formation of dinitrosyliron complexes with mercapto-purines and -pyrimidines has been studied by e.s.r. and n.m.r. spectroscopy. The binding sites were established by the aid of computer-simulated spectra and of isotopic substitution with ^{15}NO. The sulphur atom complexed with the iron only if ionized. Hindered tautomerism caused complexation *via* the imidazole nitrogen atoms in mercaptopurines. Two complexes were present at pH 7 in mercaptopyrimidines. A nine-line e.s.r. spectrum was attributed to a complex with two base molecules bonded to the iron atom, one *via* N-3 and the other *via* S^-. A quintet was attributed to a complex with two bases bonded *via* S^-.

THE study of nitrosyliron complexes has received increasing attention in the last few years, both from the inorganic and the biological points of view.[1-5] A possible relationship between Fe(NO) paramagnetic species and carcinogenesis has been examined [6] and the sulphur atom in proteins turned out to be the binding site for the iron atom.

In the presence of nitric oxide iron(II) forms mono- and di-nitrosyl complexes. The Fe(NO) have octahedral or pyramidal structures.[7-9] Their e.s.r. spectra display simple triplets with a_{NO} 15 G. The nuclear hyperfine structure of the ligand molecules is not detectable in these spectra. The $Fe(NO)_2$ complexes give rise to e.s.r. spectra with five lines (relative intensities 1 : 2 : 3 : 2 : 1) which convert to triplets (1 : 2 : 1) on isotopic substitution with ^{15}NO. The a_{NO} coupling constants are of the order 2—3 G.[10] The formula $Fe(NO)_2L_2$, where L is a unidentate ligand, has been proposed for these complexes on the basis of Mössbauer data,[3,11] low temperature structural e.s.r. studies,[12] and the nuclear hyperfine structure.[2,3,10] The $Fe(NO)_2L_2$ complexes show additional hyperfine structure, due to the ligand molecules, whenever the ligand nuclei directly bonded to the central iron atom have a nuclear spin $I \neq 0$. In these complexes S is $\frac{1}{2}$, and the unpaired electron spin density is for the most part on the iron atom which is in the low spin d^7 configuration (formal oxidation state $+1$ [10,12]).

The aim of this paper is to clarify the following points: (i) the types of complexes formed by thio-nucleic acid bases with $Fe^I(NO)_2$, *i.e.* the number of ligand molecules involved and the equilibria between different complexes, with particular attention devoted to the behaviour in the range of biologically interesting pH values; (ii) the binding sites of the nucleotide bases. The structural information was obtained from the e.s.r. nuclear hyperfine splitting, which was well resolved as a consequence of the long electron spin relaxation time: [2,13] the Fermi contact interaction between the *d* electron of the iron atom and the base nuclei gives direct evidence of a metal–base bond.

The selective broadening of the ligand n.m.r. peaks after the addition of the paramagnetic probe $Fe(NO)_2$ confirms the assignment of the binding sites. This broadening is interpreted in terms of the Solomon–Bloembergen equations; the proton relaxation times depend on the distance between the electron spin (iron atom), and the nuclear spin (nuclei in the base). This independent check by means of the n.m.r. technique gives direct information about the ligand in addition to the e.s.r. data on the paramagnetic centre.

EXPERIMENTAL

The best conditions for recording e.s.r. spectra are obtained for aqueous, alcoholic 0·1M-$Fe(ClO_4)_2,6H_2O$ at room temperature. NO Was bubbled into the solutions

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[5] J. C. Woolum, E. Tiezzi, and B. Commoner, *Biochim. Biophys. Acta*, 1968, **160**, 311.
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[7] P. T. Manoharan and H. B. Gray, *J. Amer. Chem. Soc.*, 1965, **87**, 3340.
[8] J. A. McCleverty, N. M. Atherton, J. Locke, E. J. Wharton, and C. J. Winscom, *J. Amer. Chem. Soc.*, 1967, **89**, 6082.
[9] G. R. Davies, R. H. B. Mais, and P. G. Owston, *Chem. Comm.*, 1968, 81.
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[11] J. P. Crow, W. R. Cullen, F. G. Herring, J. R. Sams, and R. L. Topping, *Inorg. Chem.*, 1971, **10**, 1616.
[12] D. S. Burbaev, A. F. Vanin, and L. A. Blyumenfeld, *Zhur. Strukt. Khim.*, 1971, **12**, 252.
[13] G. Martini and E. Tiezzi, *Trans. Faraday Soc.*, 1971, **67**, 2538.

to saturation under nitrogen according to Blanchard's method.[14] $Fe(^{15}NO)_2$ Was obtained *via* reduction of $Na^{15}NO_2$ (Irvine) with ascorbic acid. Most of the bases were gifts from Dr. C. Helene.

E.s.r. spectra were recorded with a Varian V-4502 X-band 100 kHz field modulation spectrometer. The klystron frequency was measured by a Hewlett–Packard model X532B frequency meter. The *g* factors, linewidths, and hyperfine coupling constants were measured by comparison with an external reference standard (Fremy's salt; *g* 2·0055), using a double cavity. In many cases comparison with computer-simulated spectra allowed the calculation of isotropic hyperfine constants and the clarification of some complicated patterns. Simulation was carried out assuming pure Lorentzian lineshapes and computing the spectra in the derivative form. The variables in the program were the peak-to-peak distances, the relative intensities of the lines, and the widths of each single line.

N.m.r. spectra were recorded with a Hitachi–Perkin-Elmer spectrometer operating at 60 MHz using tetramethylsilane and 3-trimethylsilylpropanesulphonic acid sodium salt as internal standards.

TABLE 1

Mercaptopurines

Base	*g* Value	pH	Number of lines (^{14}NO)	$a(^{14}NO)$ (G)	Number of lines (^{15}NO)	$a(^{15}NO)$ (G)	a(N) (G)	Complex type
8-Mercaptoadenine	2·027	5—7	5	1·7	3	2·4		(I)
2-Mercaptoadenine	2·027	5—7	5	1·8	3	2·5		(I)
6-Mercaptopurine	2·031	5—8	5	2·3	3	3·2		(I)
6-Hydroxy-2-mercaptopurine	2·027	5—8	5	2·3				(I)
2-Amino-6-mercaptopurine	2·029	5—8	5	2·2				(I)
6-Ethylthiopurine	2·028	8—10	9	2·1	7	2·9	2·7	(II)
6-Mercapto-1-methylpurine	2·028	8—10	9	2·2	7	3·1	2·8	(II)
6-Mercapto-7-methylpurine	2·024	5—7·5	9	2·7	5	3·8	4·4	(III)
	2·031	6·5—8	5	2·5	3	3·4		(I)
6-Mercapto-9-methylpurine	2·025	5—7						[(III)]
	2·031	6·5—8	5	2·3	3	3·3		(I)
2-Mercapto-*N*-methylimidazole	2·031	5—8	5	2·0				(I)

RESULTS AND DISCUSSION

The $Fe(NO)_2$ complexes display overlapping e.s.r. spectra, due to the existence of 'slow exchange conditions,' *i.e.* chemical exchange is not rapid enough to give rise to an average spectrum. Owing to the presence of solvent molecules the simultaneous presence of the $[Fe(NO)_2(ROH)_2]^+$ and $[Fe(NO)_2(RO)_2]^-$ complexes (R = H or Et) must be taken into account.[13] The former shows only one broad e.s.r. line at *g* 2·033 up to pH 5 whereas the latter is present at pH $\geqslant$10 displaying a quintet in aqueous solution and a thirteen-line spectrum in alcoholic solution at *g* 2·025.

Mercaptopurines.—The e.s.r. spectra obtained from dinitrosyliron complexes of several mercaptopurines are summarized in Table 1. It can be seen that almost all these bases give rise to five-line spectra (relative intensities 1 : 2 : 3 : 2 : 1) at *g* 2·027—2·031 in the pH range 5—8, and this is due to hyperfine interaction with two equivalent nitrogen nuclei ($I_{^{14}N}$ 1). When selective isotopic substitution with ^{15}NO ($I_{^{15}N}$ $\frac{1}{2}$) is performed, a three-line spectrum (1 : 2 : 1) is obtained. This indicates that two NO molecules must be bonded to the iron atom. The ratio between the coupling constants of ^{14}NO and ^{15}NO reflects the ratio between the two different nuclear magnetic moments. Similar e.s.r. results were obtained from dinitrosyl iron compounds of other thio-ligands.[10,15]

The nine-line e.s.r. spectrum due to the complex formed by 6-ethylthiopurine at pH 9 is shown in Figure 1. With ^{15}NO a seven-line spectrum is obtained under the same conditions. The nine-line pattern results from hyperfine interaction between the unpaired electron and two pairs of equivalent nitrogen nuclei while the seven-line spectrum indicates that two NO molecules and two equivalent base nitrogen atoms are bonded to the metal ion. The same results are obtained with

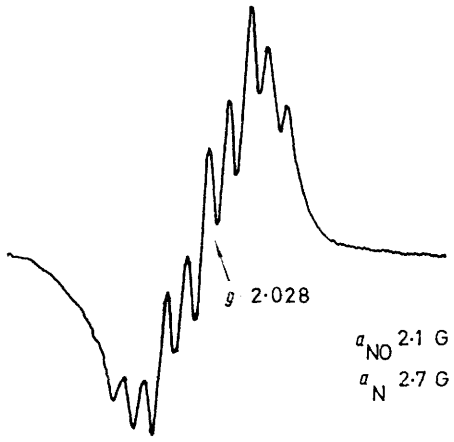


FIGURE 1 Experimental e.s.r. spectrum of $Fe(NO)_2$(6-ethylthiopurine)$_2$

6-mercapto-1-methylpurine. Figure 2 reports the n.m.r. spectrum of 6-mercapto-1-methylpurine in D_2O at pD 9 and the effect of adding $Fe^{I}(NO)_2$.

6-Mercapto-7-methylpurine gives rise to peculiar e.s.r. spectra. At pH 5·5—6·5 a nine-line spectrum (1 : 2 : 4 : 4 : 5 : 4 : 4 : 2 : 1) with *g* 2·024 is obtained; at pH 7—8 a quintet with *g* 2·031 (triplet with ^{15}NO) is present; in the intermediate range there is the simultaneous presence of two $Fe(NO)_2$ complexes under slow exchange conditions. The nine-line spectrum converts to a quintet (1 : 3 : 4 : 3 : 1) with ^{15}NO. Computer

[14] A. A. Blanchard, *Inorg. Synth.*, 1964, **2**, 126.

simulation of the signal at g 2·024 (both with ^{14}NO and ^{15}NO) shows that the hyperfine interaction results from one base nitrogen and two equivalent NO nitrogen

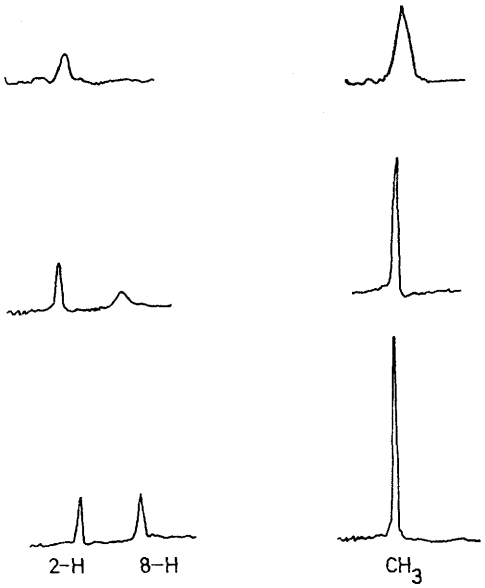
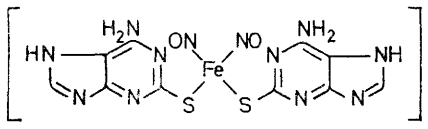


FIGURE 2 N.m.r. peaks of 6-mercapto-1-methylpurine in the presence of $Fe(NO)_2$

atoms. The same e.s.r. data are obtained for several mercaptopyrimidines (see later).

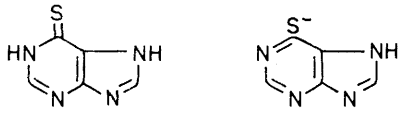
The e.s.r. results lead to an understanding of the binding behaviour of the bases with the dinitrosyliron probe. The general trend is the formation of a complex which gives rise to a five-line e.s.r. spectrum at pH 5—8 [type (I) complex]. On the basis of the e.s.r. data and of their agreement with previous results,[10,15] type (I) complexes have the iron atom bonded to two NO molecules and two base molecules *via* the sulphur atom (I 0), as shown for the case of 2-mercaptoadenine.



type (I)

It is not possible to mistake the five-line spectrum for that at g 2·025 of the $[Fe(NO)_2(RO)_2]^-$ species, which is not present in this pH range.[13]

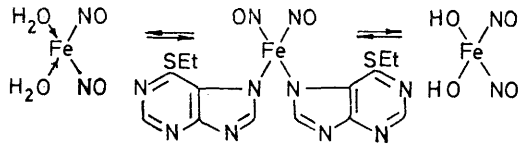
An important point to take into account is the fact that mercaptopurines exhibit thiol–thione tautomerism



(BH) (B)

and exist in neutral and anionic forms. Because almost all the bases display e.s.r. spectra derived from type (I) complexes when the pH allows ionization to S^-, it is reasonable to presume that this is the major species, unless the tautomeric equilibrium is perturbed or the deprotonation on the sulphur atom is hindered. Furthermore, as all the bases give rise to identical e.s.r. spectra, one can conclude not only that the position of the mercapto-group is not important in complex formation but also that the presence of other groups, such as NH_2, OH, *etc.*, does not interfere with the stronger ligand activity of S^-, in agreement with data obtained from organic ligands.[10,15]

As further proof of these statements, when the thiol–thione tautomerism is perturbed (as in the case of 6-mercapto-1-methylpurine) or the ionization of the mercapto-group is hindered (as in the case of 6-ethylthiopurine) the above e.s.r. results are not obtained. Both these ligands give rise to one single broad line e.s.r. spectrum up to pH 8 and to the nine-line spectrum at pH 9 (Figure 1); at higher pH values the nine-line spectrum and the quintet characteristic of the $[Fe(NO)_2(RO)_2]^-$ species are simultaneously present. The nine-line spectrum is due to a compound which we denote as a type (II) complex and it is similar to that obtained from dinitrosyliron complexes with non-mercaptopurines.[16] In this case a structure in which two base molecules are bonded to the iron atom through N-7 together with two NO molecules can be suggested.[16] The sulphur atom (in spite of the inductive effect of the ethyl group in 6-ethylthiopurine) gives rise to complexes only if ionized. These results are explained in terms of the pH-dependent equilibrium (1).



(1)

type (II)

The n.m.r. spectra give further proof of the involvement of an imidazole ring nitrogen in complex formation. Upon adding $Fe(NO)_2$ to 6-mercapto-1-methylpurine (Figure 2) and to 6-ethylthiopurine in D_2O at pD 9, the peak due to 8-H broadens to a greater extent than the 2-H peak.

Finally, there is the case of 6-mercapto-7-methylpurine whose e.s.r. behaviour strongly suggests the existence of a third type of complex. Before the quintet of type (I) complex becomes the predominant species, a nine-line spectrum (five lines with ^{15}NO) is obtained due to the hyperfine interaction with one nitrogen nucleus and two equivalent nitrogen nuclei. This last spectrum is due to a type (III) complex. Since at such pH values (5·5—6·5) the involvement of the imidazole ring is ruled out and the same e.s.r. data are obtained with mercaptopyrimidines (see later) a structure is suggested in which two NO molecules and two base molecules are bonded to the iron atom, one

[15] G. Martini and E. Tiezzi, *Z. Naturforsch.*, 1973, **28b**, 300.
[16] R. Basosi, E. Tiezzi, and G. Valensin, *Biophys. Chem.*, in the press.

through the deprotonated mercapto-group, the other through a pyrimidine nitrogen: a mixed complex is formed. This particular behaviour is explained by the high K_b value of 6-mercapto-7-methylpurine, which is of the order of magnitude of the mercaptopyrimidine K_b values.

The three different types of complexes are shown in the Scheme where B and BH are ligand molecules whose

$[Fe(NO)_2(B)_2]^-$	$[Fe(NO)_2(BH)_2]^+$	$[Fe(NO)_2(B)(BH)]$
type (I)	type (II)	type (III)
via S^-	*via* N-7	B *via* S^-
		BH *via* N-1 (or N-3)

SCHEME

binding sites are the deionized mercapto-group and a nitrogen atom respectively.

mercaptopyrimidines. Almost all the bases display two different e.s.r. spectra, often simultaneously present in solution, depending on the pH range studied. Between pH 4 and 5·5 a nine-line e.s.r. spectrum with relative intensities 1 : 2 : 4 : 4 : 5 : 4 : 4 : 2 : 1 at g 2·021—2·022 is obtained. Computer simulation suggests that the hyperfine pattern derives from the coupling of the unpaired electron with one nitrogen atom and two equivalent nitrogen nuclei. Using ^{15}NO instead of ^{14}NO a five-line e.s.r. spectrum (1 : 3 : 4 : 3 : 1) is obtained under the same conditions, and further simulation of this last spectrum fits the experimental quintet perfectly: two NO molecules and a pyrimidine nitrogen interact with the unpaired spin density on the iron atom.

At pH 7 however a five-line e.s.r. spectrum (triplet with ^{15}NO) with g 2·029—2·031 is obtained. As in the case of mercaptopurines and thio-ligands,[10,15] the hyperfine pattern and the isotopic substitution show that two equivalent nitrogen nuclei of two NO molecules are bonded to the iron atom. Furthermore, there is a pH range in which the two complexes are simultaneously

TABLE 2

Mercaptopyrimidines

Base	g Value	pH	Number of lines (^{14}NO)	$a(^{14}NO)$ (G)	Number of lines (^{15}NO)	$a(^{15}NO)$ (G)	a(N) (G)	Complex type
2-Mercaptouracil	2·021	4—7	9	2·4	5	3·4	4·0	(III)
	2·029	5·5—8	5	2·3	3	3·2		(I)
2-Mercapto-6-methyluracil	2·021	4—7	9	2·4	5	3·4	4·0	(III)
	2·031	5·5—8	5	2·0	3	2·8		(I)
4,6-Dihydroxy-2-mercapto-pyrimidine	2·022	4·5—7	9	2·4	5	3·4	4·0	(III)
	2·029	6—8	5	2·1	3			(I)
2-Methylthiouracil	2·025	7—9						
5-Mercaptouracil	2·027	4—9	5	2·2	3	3·1		(I)
2-Mercaptopyrimidine	2·022	4—6·5	9	2·5	9	3·5	2·0	(IV)
	2·029	5—8	5	2·2	3	3·1		(I)
2,4-Dimercaptopyrimidine	2·022	4—7	9	2·4	5	3·4	4·0	(III)
	2·030	6—8	5	2·2	3			(I)
2,4-Dimercapto-5-methyl-pyrimidine	2·022	4—7	9	2·4	5	3·4	4·0	(III)
	2·029	7—8·5	5	2·2	3			(I)
1-Cyclohexyl-2-hydroxy-4-mercaptopyrimidine	2·021	5—7	9	2·4	5	3·4	3·9	(III)
	2·029	6—8	5	2·2	3			(I)
2-Mercaptocytosine	2·028	7—9						

Some data from the literature on metal binding of mercaptopurines and thionucleosides, based on potentiometric titrations,[17] u.v. spectroscopy, and X-ray crystallographic studies [18] suggest the chelation on the metal ion of both sulphur and N-7 whereas an i.r. and conductometric study [19] points to the conclusion that only the anionic form of 6-mercaptopurine forms metal complexes. Our experimental findings suggest that in dinitrosyliron complexes the mercapto-group is the preferred binding site, provided the pH allows deprotonation; at lower pH values a pyrimidine nitrogen atom may be the preferred binding site. When deprotonation of the mercapto-group is hindered an imidazole nitrogen atom becomes the preferred binding site but only at pH $\geqslant$9.

The mercapto-group acts as the preferred binding site even in mercaptoimidazoles. 2-Mercapto-*N*-methylimidazole gives rise to a dinitrosyliron complex which displays a five-line e.s.r. spectrum in the pH range 5—8 [type (I) complex] whereas a type (II) complex (nine-line e.s.r. spectrum) is obtained with *N*-methylimidazole.

Mercaptopyrimidines.—Table 2 summarizes the e.s.r. results obtained from dinitrosyliron complexes of

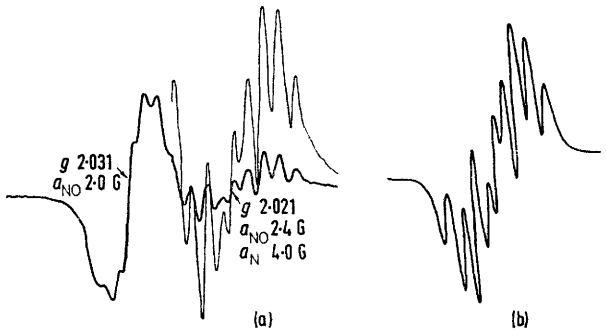


FIGURE 3 (a) Experimental e.s.r. spectrum of the $Fe(NO)_2$ complexes with 2-mercapto-6-methyluracil, (b) simulated e.s.r. spectrum

[17] M. M. Taqui Khan and C. R. Krishnamoorthy, *J. Inorg. Nuclear Chem.*, 1971, **33**, 1417.

[18] H. I. Heitner, S. J. Lippard, and H. R. Sunshine, *J. Amer. Chem. Soc.*, 1972, **94**, 8936.

[19] J. Brigando and D. Colaitis, *Bull. Soc. chim. France*, 1969, 3440.

present in solution under conditions of slow chemical exchange.

Figure 3(a) shows both the five-line and the nine-line e.s.r. spectra in the case of dinitrosyliron complexes of 2-mercapto-6-methyluracil. We have superimposed the enlarged nine-line spectrum in order to compare it with the simulated one in Figure 3(b). The calculated coupling constants are a' 4 G for the base nitrogen and a'' 2·4 G for the nitric oxide nitrogen atoms; the single peak linewidth ΔH is 1·5 G. Figure 4(a) shows the

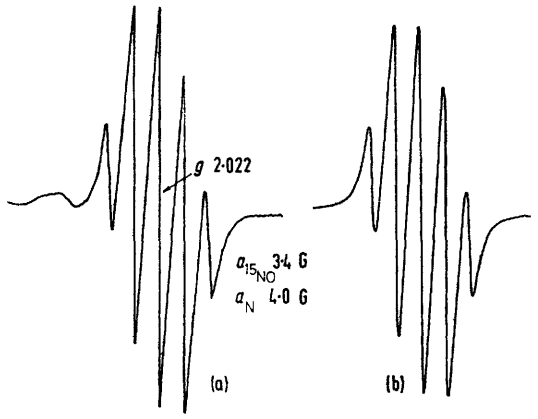


FIGURE 4 (a) Experimental e.s.r. spectrum of the $Fe(^{15}NO)_2$ complex with 4,6-dihydroxy-2-mercaptopyrimidine; (b) simulated e.s.r. spectrum

quintet obtained from the complex with 4,6-dihydroxy-2-mercaptopyrimidine using ^{15}NO; agreement with the simulated spectrum [Figure 4(b)] is fairly good assuming the following values for the nuclear hyperfine constants: a_N 4, $a_{^{15}NO}$ 3·4 G and for the linewidth: ΔH 1·5 G. Similar e.s.r. results are obtained with 2-mercaptouracil, 2,4-dimercapto-5-methylpyrimidine, and 2,4-dimercaptopyrimidine. The n.m.r. spectrum of this last base is shown in Figure 5 before and after the paramagnetic

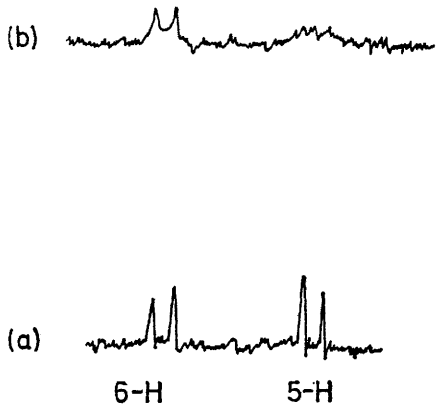


FIGURE 5 (a) N.m.r. spectrum of 2,4-dimercaptopyrimidine; (b) in the presence of $Fe(NO)_2$

probe is added. The e.s.r. spectra obtained with 1-cyclohexyl-2-hydroxy-4-mercaptopyrimidine at pH 6, both with ^{14}NO and ^{15}NO, are reported in Figures 6(a) and (b) respectively.

Dinitrosyliron complexes with 2-methylthiouracil do not display any e.s.r. spectrum under the above conditions and at very high pH values only a poor signal with some hyperfine structure is obtained.

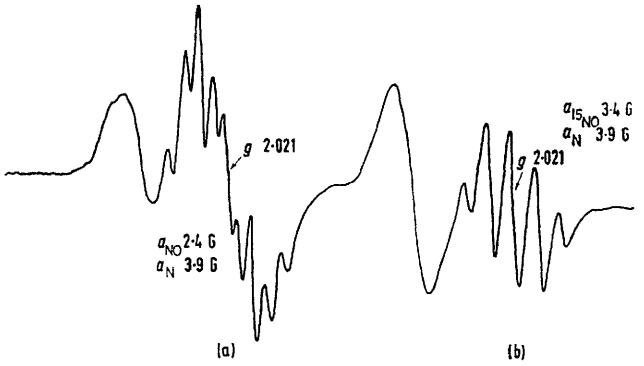


FIGURE 6 (a) Experimental e.s.r. spectrum of the $Fe(NO)_2$ complex with 1-cyclohexyl-2-hydroxy-4-mercaptopyrimidine; (b) the same complex with $Fe(^{15}NO)_2$

In the particular case of 2-mercaptopyrimidine, two e.s.r. spectra are obtained: in addition to the quintet with g 2·029 at pH 7, one can see a nine-line spectrum at pH 5 [Figure 7(a)]. Computer simulation [Figure 7(b)] of this last spectrum agrees with a hyperfine pattern due to two pairs of equivalent nitrogen nuclei and leads

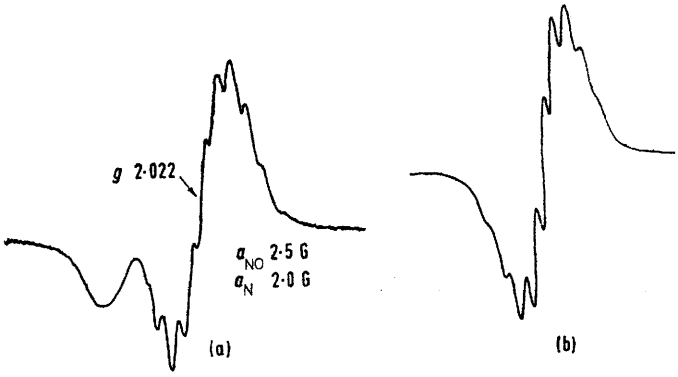


FIGURE 7 (a) Experimental e.s.r. spectrum of $Fe(NO)_2$ (2-mercaptopyrimidine)$_2$; (b) simulated e.s.r. spectrum

to the following values for the coupling constants: a'_N 2·5, a''_N 2·0 G. Using ^{15}NO the nine-line spectrum converts to another nine-line spectrum, whose computer simulation fits the experimental signal very well, giving the following values for the coupling constants: $a'_{^{15}N}$ 3·5, a''_N 2·0 G. On this basis it is inferred that two NO molecules and two equivalent base nitrogen atoms are bonded to the iron atom, such that $a'_N = a_{NO}$ and $a''_N = a_{N\text{-base}}$.

E.s.r. spectra with poor resolution are obtained from $Fe(NO)_2$ complexes with 2-mercaptocytosine whereas a well-resolved quintet with ^{14}NO and a triplet with ^{15}NO are displayed by 5-mercaptouracil in the pH range 4—9 [as shown in Figures 8(a) and (b) respectively].

The general trend for mercaptopyrimidines is the formation of two different dinitrosyliron complexes depending on the pH range under study. At smaller pH values the major complex is that in which two base

molecules are bonded to the $Fe(NO)_2$ probe, one *via* the mercapto-group, the other *via* a pyrimidine nitrogen whereas at higher pH values the predominant complex is

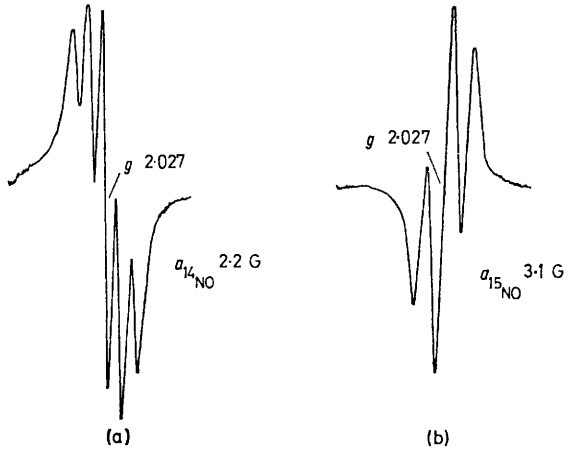
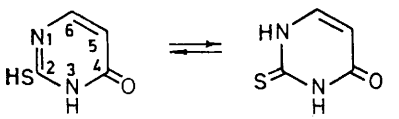


FIGURE 8 (a) Experimental e.s.r. spectrum of $Fe(NO)_2$(5-mercaptouracil)$_2$; (b) experimental e.s.r. spectrum of $Fe(^{15}NO)_2$(5-mercaptouracil)$_2$

that in which both the base molecules are bonded to the iron atom *via* the mercapto-group. The same types of compounds were obtained with some mercaptopurines [types (III) and (I) complexes respectively]. On the basis of the experimental e.s.r. results, it can be suggested that the deprotonated mercapto-group is the preferred binding site for the d^7 Fe^I ion, because whenever the pH allows mercapto-group ionization the characteristic five-line e.s.r. spectrum is present and becomes the predominant signal.

As in the case of mercaptopurines the thiol–thione tautomeric equilibrium of the base must be taken into account [equilibrium (2) for the case of 2-mercaptouracil]. When the base is in the thione form, the



(2)

pyrimidine nitrogen atom is the binding site whereas in the thiol form the deprotonated mercapto-group is preferred. Furthermore the pH determines whether the type (I) or (III) complex is the major species in solution. As further proof of this statement, when the sulphur atom is methylated (as in the case of 2-methylthiouracil) both type (I) and (III) complexes are absent and at basic pH values only a poor signal with some hyperfine structure is obtained.

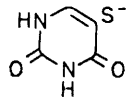
N.m.r. spectra support these experimental e.s.r. findings in the case of 2,4-dimercapto- and 2,4-dimercapto-5-methyl-pyrimidine. The preferential broadening of the 5-H signal strongly confirms the involvement of the 4-mercapto-group in complex formation.

As 1-cyclohexyl-2-hydroxy-4-mercaptopyrimidine forms dinitrosyliron complexes which display the same e.s.r. behaviour, the participation of N-3 as the nitrogen binding site can be inferred and similar binding behaviour may be expected for thio-nucleosides.

In addition to the above results which depend on the K_b value of the base, the pH range, and the ligand-forming ability of the mercapto-group, it is apparent that a further complex can be formed, in which two base molecules are bonded to the metal ion both through a pyrimidine nitrogen atom. The binding behaviour of 2-mercaptopyrimidine is proof of this statement. The K_b value [20] of this base is the highest for the ligands studied and thus this new complex (IV) is present at pH values in which the type (I) complex is not yet formed.

On the other hand, if the K_a value of the mercapto-group is not large enough (as in the case of 2-mercaptocytosine [20]), e.s.r. spectra with poor resolution due to the presence at basic pH values of $[Fe(NO)_2(RO)_2]^-$ species are obtained.

Finally when the mercapto-group is not engaged in thiol–thione tautomerism, completely different binding properties can be expected. In these cases the main tautomeric form has a conformation such that the base strength of the nitrogen site is lowered and the acidic properties of the mercapto-group are enhanced. For these reasons 5-mercapto-uracil behaves differently; only a type (I) complex is obtained over the whole pH range investigated. This is explained by invoking tautomeric form (V) as the main ligand species.



(V)

On the basis of this magnetic resonance study, it can be concluded that the mercapto-group in mercaptopyrimidines is the preferred binding site for the iron atom, provided the pH value allows its ionization, and also that its position is not relevant in complex formation. A pyrimidine nitrogen atom is the binding site only at lower pH values. The main characteristic of these bases is the equilibrium between type (I) and (III) dinitrosyl complexes at biologically relevant pH values.

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[20] D. J. Brown, 'Heterocyclic Compounds. Pyrimidines,' Wiley–Interscience, New York, 1962, p. 464.