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COORDINATION BEHAVIOUR OF ANTITHYROID DRUGS AGAINST THE $Fe(I)(NO)_2$ GROUP IN SOLUTION: ESR AND FT-NMR STUDY

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The binding sites of some types of antithyroid drugs in the presence of the $Fe(I)(NO)_2$ paramagnetic probe were investigated. Coordination behaviour in solution of different structured ligands was determined by means of ESR parameters and ^{13}C FT-NMR spectra. Selective isotopic substitution with ^{15}NO combined with computer simulation was used to elucidate overlapping ESR patterns. A correlation between chemical structure and antithyroid activity of the pharmacological bases is suggested.

1. Introduction

Magnetic resonance spectroscopies are a powerful aid to investigate coordination behaviour of ligand molecules in the presence of metal ions [1–2]. In fact these techniques provide structural information from the ESR nuclear hyperfine splitting and electronic and nuclear relaxation rates.

The $Fe(I)(NO)_2$ group is a very suitable paramagnetic probe in the investigation of the structural features and the dynamic properties of biological ligands in solution. The d^7 configuration with one unpaired electron, makes it very similar to a free radical giving favourable ESR parameters [3–5]. Furthermore some suggestions are possible about the involvement of metal complexes in which iron plays a prominent role [6].

Antithyroid drugs, which are effective in the inhibition of thyroid hormone, can be assorted in three general categories according to their chemical structure: thioamides, aniline derivatives and polyhydrophenols [7,8]. It is not easy to hypothesize only one mechanism for the action of such drugs but redox processes [9–13] and or metal ion involvement [14,15] are strongly suggested by many experimental evidences. This also efforted by the effective action of some metal ions [16] and of many thiol compounds.

The aim of our research is to analyze the Fe-NO complexes with these pharmacological molecules in order to clarify the following points: (i) the binding sites of the antithyroid drugs; (ii) the types of complexes formed with $Fe(I)(NO)_2$ and the equilibria between different species in solution.

1.1. ESR parameters

The analysis was based on the nuclear hyperfine coupling constants and on the change of nuclear hyperfine structure, in the presence of different ligands.

The interpretation of the ESR data was performed by utilization of the selective isotopic substitution with ^{15}N, combined with computer simulation of the ESR patterns.

1.2. Nuclear spin relaxation data

The selective broadening of the ligand NMR peaks after the addition of the paramagnetic $Fe(I)(NO)_2$ probe provides independent information about the assignment of the binding sites. This broadening was interpreted in terms of the Solomon–Bloembergen equations [17,18]. Nuclear relaxation rates of the ^1H and ^{13}C nuclei of the ligands in the presence of the $Fe(I)(NO)_2$ group allow inferences about dynamic and structural properties. In fact the dipolar and scalar $\boldsymbol{I} \cdot \boldsymbol{S}$ interactions became the most effective relaxation mechanisms whenever fast exchange conditions between bulk and bound ligand hold. As a consequence a paramagnetic contribution to the nuclear relaxation rates can be measured in the following way:

$$\frac{1}{T_{i\mathrm{p}}} = \frac{1}{T_{i(\mathrm{metal})}} - \frac{1}{T_{i(\mathrm{blank})}} = \frac{f}{T_{i\mathrm{M}}} \quad (i = 1, 2)$$

where $T_{i(\mathrm{metal})}$ and $T_{i(\mathrm{blank})}$ are the experimental relaxation times after and before the metal addition respectively. When the $\mathrm{Fe(I)(NO)_2}$ is the paramagnetic probe the following simplified Solomon–Bloembergen equations hold:

$$1/T_{2_\mathrm{M}} = KA^2\tau_\mathrm{c}\,, \qquad 1/T_{1_\mathrm{M}} = K'\tau_\mathrm{c}/r^6$$

that is to say the paramagnetic contribution to the transverse relaxation rate ($T_{2\mathrm{p}}^{-1}$) is determined only by the scalar interaction; whereas the paramagnetic contribution to the longitudinal relaxation rate ($T_{1\mathrm{p}}^{-1}$) rise from the dipolar interaction only. It is noteworthy that the ($T_{1\mathrm{p}}^{-1}$) contributions depend (as concerns nuclei in the same complex) upon the distance r between the ligand nuclei and the metal ion.

2. Materials and methods

ESR spectra were recorded with a Varian V-4502 ESR spectrometer, operating in the X band with a frequency modulation of 100 Kc. The apparatus was equipped with a double-cavity for the use of an external reference standard (Fremy salt) in order to measure g-factors and hyperfine coupling constants. The computer simulation of the ESR spectra was carried out assuming pure Lorentzian line-shape. The data given to the computer were the distances of the lines, the line-widths and the theoretical intensities of the hyperfine lines. NMR spectra were obtained using a Bruker WH-90 FT-NMR spectrometer operating at 90 MHz for the ^{1}H and 22.63 MHz for ^{13}C nucleus Transverse relaxation times were measured from the line-widths at half-height of the FT spectra and spin-lattice relaxation times were calculated from partially relaxed FT spectra by using the $(180°-\tau-90°-t)_n$ pulse sequence. The ^{13}C resonances were assigned by means of chemical shift values and off-resonance decoupled spectra.

The $\mathrm{Fe(I)(NO)_2}$ complexes were prepared in aqueous solution according to the Blanchard's method [19]. The source for ^{15}N was $\mathrm{Na^{15}NO_2}$ from I.C.N., Campus Drive, Irvin, California. All antithyroid drugs were obtained from Sigma Chemical and used without further purification.

3. Results and discussion

In a previous paper [20] it has been shown that it is possible to distinguish four types of Fe-NO paramagnetic complexes. They have octhaedral or pyramidal structures [21–23] and can be represented by the following general simplified formulas:

$\mathrm{Fe(NO)L_5}$	$\mathrm{Fe(NO)L_2}$	$\mathrm{Fe(NO)_2L_2}$	$\mathrm{Fe(NO)_2L}$
I	II	III	IV

where L may be either a ligand or a water molecule. The ligands are monodentate in structures I and III, bidentate in structures II and IV.

3.1. Thioamides

Thiourea is the simplest member of this class of antithyroid drugs which are the most effective in man. The thioureylene group is incorporated also in 1-methyl-2-thio-imidazole (or methimazole) and 2-thiouracil and their derivatives.

The ESR spectra obtained from dinitrosyl iron complexes with these organic ligands are summarized in table 1. It can be seen that all these bases give rise to a five-line spectrum (relative intensities 1 : 2 : 3 : 2 : 1) at g = 2.029 – 2.031 in the pH range 5 – 7 and this is due to hyperfine interaction with two equivalent nitrogen nuclei ($I_{^{14}\mathrm{N}}$ = 1). After isotopic substitution with ^{15}NO ($I_{^{15}\mathrm{N}}$ = 1/2) a three-line spectrum (relative intensities 1 : 2 : 1) is obtained. The experimental coupling constant values of ^{14}NO and ^{15}NO are in a good agreement with the theoretical ones. All these data have been interpreted in terms of a III type complex where two NO and two ligand molecules must be bonded to the iron atom via S$^-$. In the range of pH 4–7, thio-uracil compounds display overlapping ESR spectra. These can be explained by the existence of "slow exchange" conditions, i.e., the chemical exchange is not rapid enough to give an averaged spectrum. The nine-line ESR spectrum due to the complex formed by the 5-Iodo-2-thio-uracil, at pH lower than 7, is

Table 1
ESR parameters of the antithyroid drugs

Compound	pH	*g*-factor	Number of lines ^{14}NO	$a^{(G)}_{^{14}NO}$	Number of lines ^{15}NO	$a^{(G)}_{^{15}NO}$	$a^{(G)}_{N}$
Thioamides							
Thiourea	4–7	2.034	5	2.2	3	3.0	–
Methimazole	5–7	2.031	5	2.0	3	2.8	–
Carbimazole	5–7	2.031	5	2.0	3	2.8	–
2-thiouracil	5	2.021	9	2.4	5	3.5	4.0
	6.5–7	2.021–2.029	9+5	2.4–2.3	5+3	3.4–3.2	4.0
	7.5	2.029	5	2.3	3	3.2	–
6 propyl 2-thiouracil	5–7	2.021–2.030	9+5	2.4–2.3	5+3	3.4–3.2	4.0
5 butil 6CH_3 2-thiouracil	4–7	2.022–2.031	9+5	2.4–2.3	5+3	3.4–3.2	4.0
6 benzil 2-thiouracil	5–7	2.021–2.031	9+5	2.4–2.2	–	–	4.0
5 jodo-2-thiouracil	4–7	2.021–2.031	9+5	2.4–2.3	5+3	3.4–3.2	4.0
Aniline derivatives							
Sulphanilamide	7	2.027	9	2.2	7	3.0	2.8
Sulphathiazole	7–8	2.029	9	2.2	7	3.0	2.8
Polyhydric phenols							
Resorcinol	7	2.026	5	2.4	3	3.3	–
Phloroglucinol	7	2.026	5	2.4	3	3.3	–
	7.5	2.025	3	14.8	–	–	–

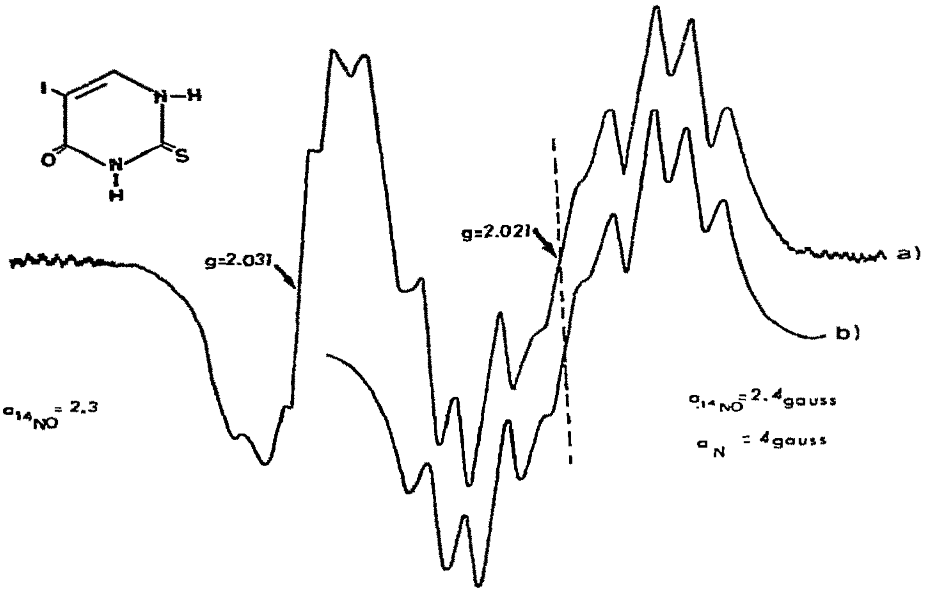


Fig. 1. (a) Experimental ESR spectrum of $Fe(NO)_2$ (5-iodo-2thio-uracil)$_2$. (b) Simulated ESR spectrum.

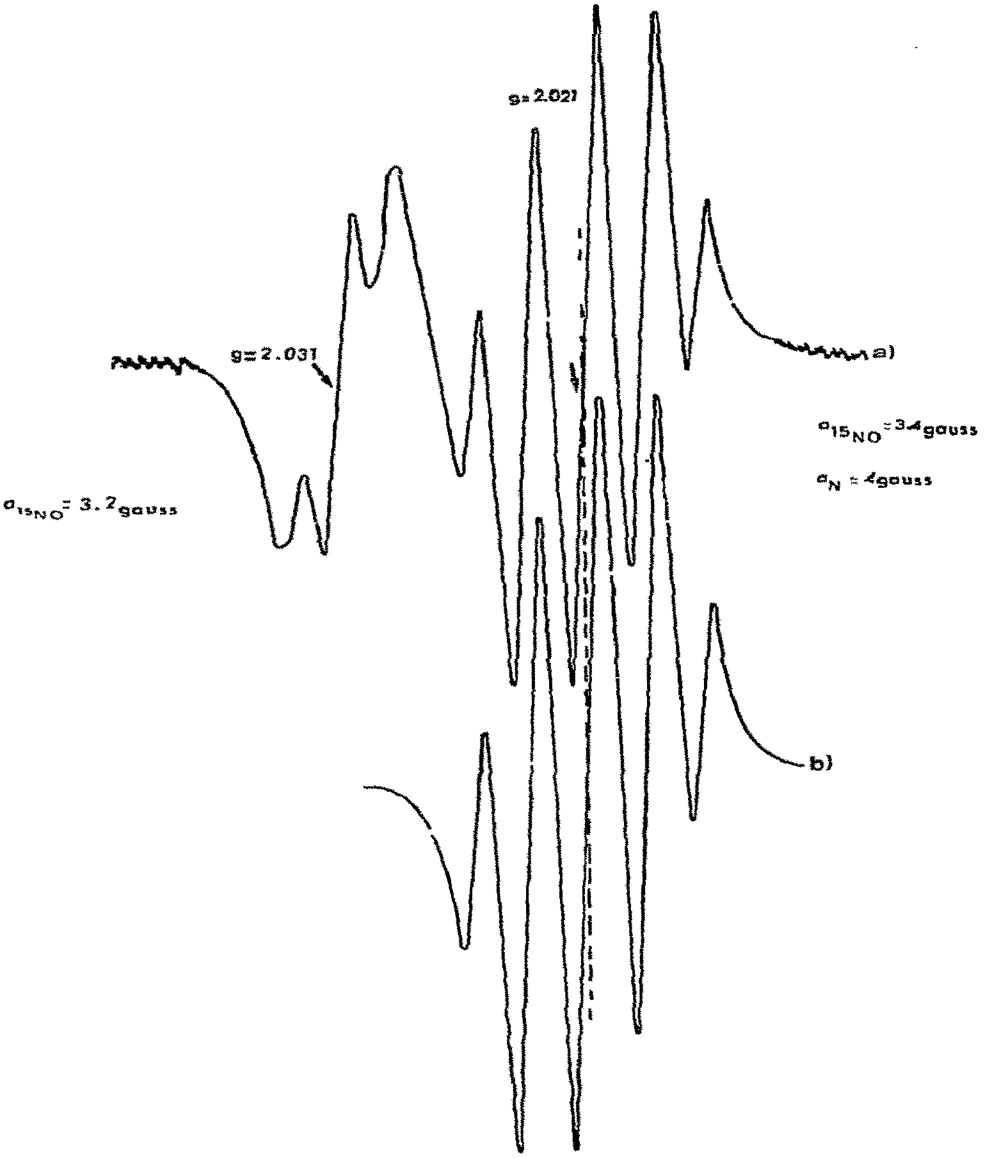
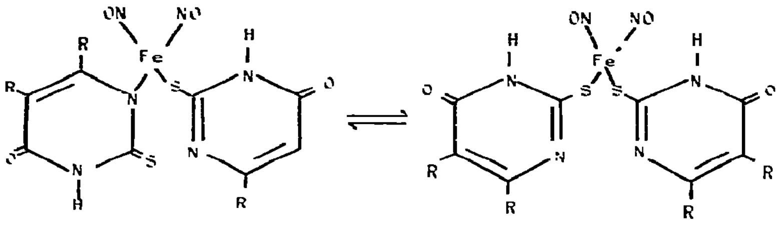


Fig. 2 (a) Experimental ESR spectrum of Fe(^{15}NO)$_2$(5 Iodo-2thio-uracil)$_2$. (b) Simulated ESR spectrum.

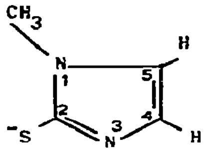
shown in fig. 1. The computer simulated spectrum shows a good fitting. With ^{15}NO, in the same condition, a five-line spectrum is obtained (fig. 2). The nine-line pattern results from hyperfine interaction between the unpaired electron, one base nitrogen and two equivalent NO nitrogens. Another base is linked via S$^-$, and does not affect the hyperfine structure. The pH dependent equilibrium as shown in scheme 1 may be proposed:



$-R = -H, -C_3H_7, -I$

Scheme 1.

The high resolution ^{1}H and proton noise decoupled ^{13}C FT-NMR spectra strongly effort the interpretation of the ESR data. In fact, a large broadening of the C(2) peak is observed after addition of the Fe(I)(NO)$_2$ paramagnetic probe to neutral aqueous solution of methimazole, and the measurements of the longitudinal and transverse relaxation times lead to identical paramagnetic contributions to H(4) and H(5) (scheme 2).



Scheme 2.

These findings, interpreted in terms of the Solomon—Bloembergen equations, point out the direct interaction of the paramagnetic probe with the mercapto-group.

The ^{13}C-{^{1}H}-FT-NMR spectrum (fig. 3) of 6-propil-thio-uracil displays a remarkable increase of the C(2) relaxation rate and a poor broadening of the C(6) peak, confirming the above mentioned molecular model.

3.2. Aniline derivatives

These compounds have been deeply investigated in the initial elucidation of the mechanism by which goitre is induced in rats, in spite of their poor antithyroid activity in man. The most important ones are sulphanilamide and sulphathiazole.

The ESR results with the Fe(NO)$_2^+$ group are summarized in table 1 and compared with the previous class of drugs. The ESR spectrum of the iron-dinitrosyl complex, with pure sulphanilamide in aqueous solution at pH 7—8, displays a nine-line hyperfine structure with relative intensities 1 : 4 : 10 : 16 : 19 : 16 : 10 : 4 : 1 (fig. 4) which are in agreement with the binding of two different pairs of nitrogen nuclei. The computer simulated spectrum fits the experimental one assuming a_{NO} = 2.2 gauss and $a_{\mathrm{NH_2}}$ = 2.8 gauss. The ESR spectra performed after isotopic substitution with ^{15}N show a septet (fig. 5) with relative intensities 1 : 4 : 8 : 10 : 8 : 4 : 1. The simulation shown a fine fitting assuming two ^{15}NO groups and two $-NH_2$ sites bonded to iron, with coupling constants $a_{^{15}\mathrm{NO}}$ = 3.0 gauss and $a_{\mathrm{NH_2}}$ = 2.8 gauss respectively: the first value is in agreement with the ratio between the ^{15}N and ^{14}N magnetic moments. Despite the presence of an heterocyclic ring in the molecule, sulphatiazole shows almost identical ESR parameters.

The ^{13}C-NMR data from sulphanilamide performed at the same conditions, display comparable selective broadening of both substituted carbons in the aromatic ring, leaving the remainder ones unchanged. The paramagnetic contribution to C(1) and C(4) is at least one order of magnitude greater than that to C(3,5) and C(2,6).

In the proton experiments the T_1 values are much more sensitive than T_2 values to selective paramagnetic relaxation: ^{1}H PR-FT spectra of sulphanilamide show a paramagnetic contribution to the longitudinal relaxation rate of H(2,6), T_{1p}^{-1} = 0.8 s^{-1} and of H(3,5), T_{1p}^{-1} = 0.44 s^{-1}.

The ESR results indicate the interactions of two equivalent sulphanilamide molecules with the iron atom

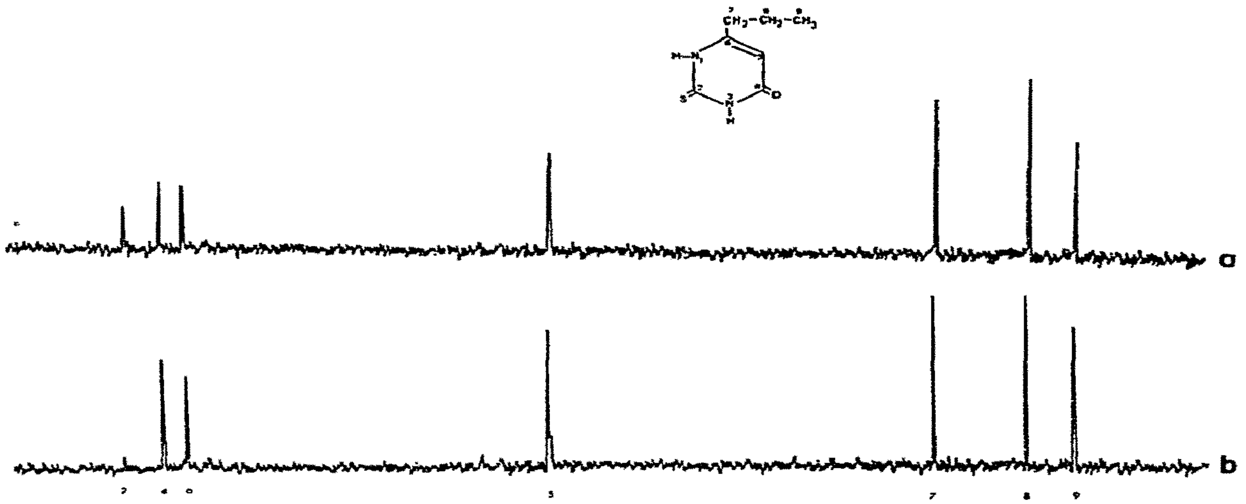


Fig. 3. (a) Experimental ^{13}C (^{1}H) FT-NMR spectrum of 6 Propil- 2 thio-uracil. (b) The same in the presence of the $Fe(NO)_2^+$ paramagnetic probe.

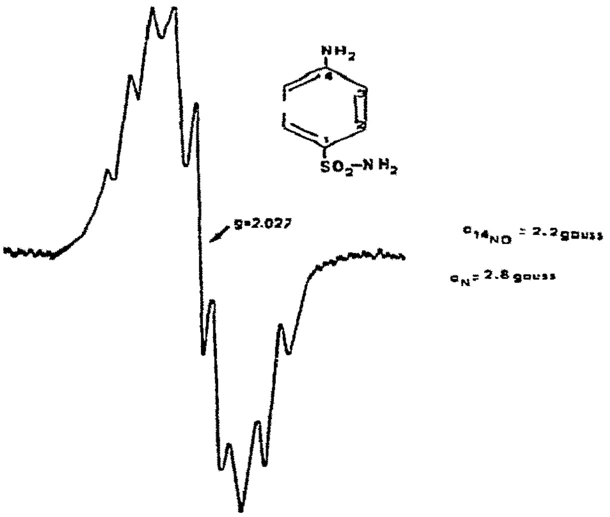


Fig. 4. Experimental ESR spectrum of $Fe(NO)_2$(sulphanilamide)$_2$.

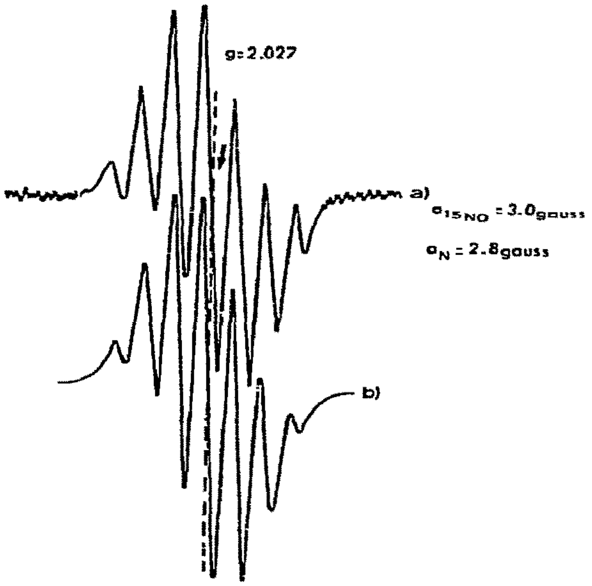
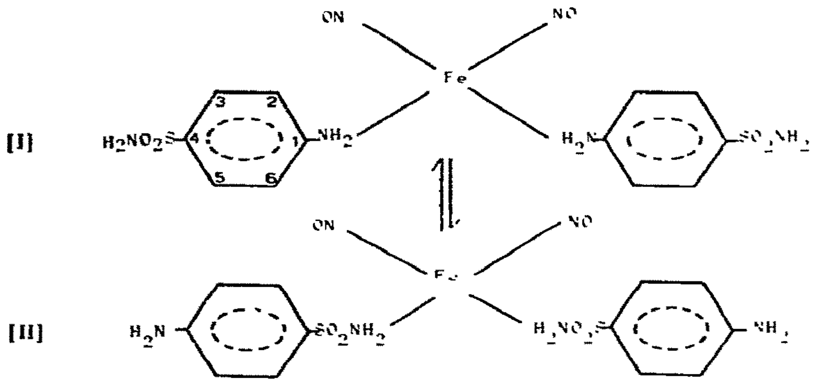


Fig. 5. (a) Experimental ESR spectrum of $Fe(^{15}NO)_2$(sulphanilamide)$_2$. (b) Simulated ESR spectrum.

The ^{13}C-NMR data point out the implication of both the (1) and (4) binding sites in the complexation, thus suggesting the existence of the equilibrium shown in scheme 3.



Scheme 3.

The proton relaxation data, although being of the same order of magnitude, seem to indicate that the equilibrium is slightly shifted towards species [I].

Fig. 6 shows the results obtained from sulphathiazole: similar paramagnetic contributions to relaxation, are apparent for the C(4), C(3') and C(4') nuclei*, confirming the presence of equilibria between different chemical species.

3.3. Poly-hydric phenols

Antithyroid activity of these drugs seems to be associated to the presence of two polar groups in meta position of the benzene ring. Because of the high mole-

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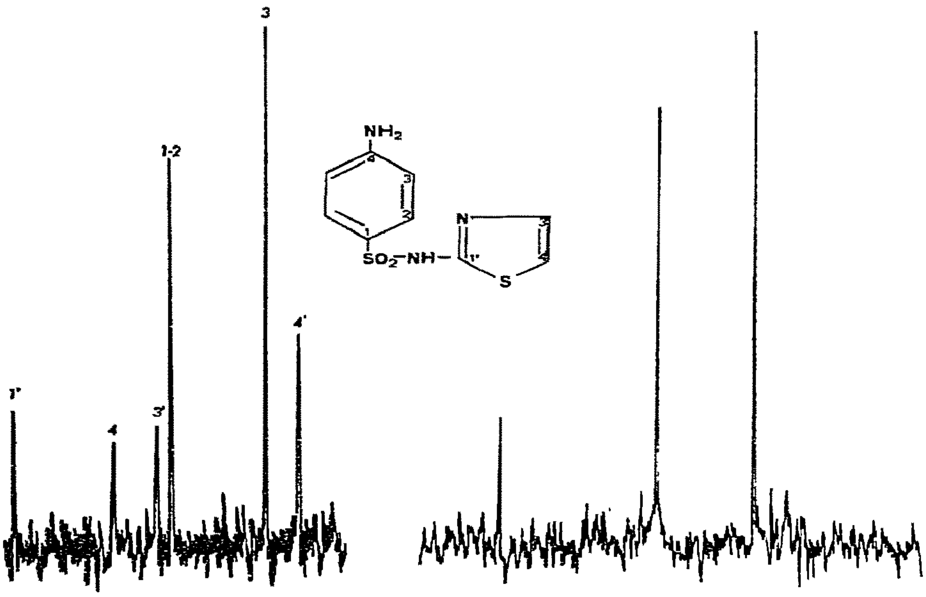
* For the assignment see ref. [24].



(a)

(b)

Fig. 6. (a) Experimental ^{13}C (^1H) FT-NMR spectrum of sulphathiazole. (b) The same in the presence of the paramagnetic probe.

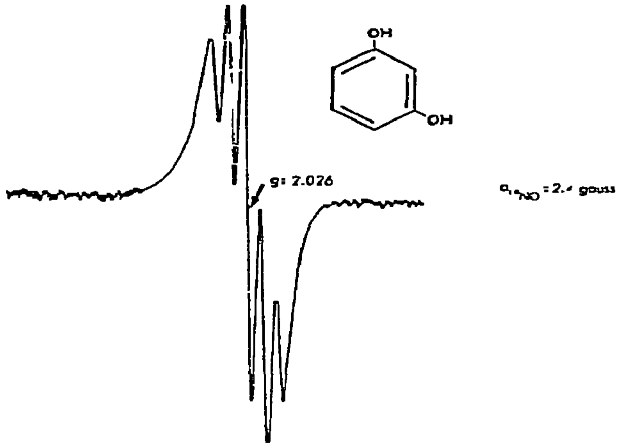


Fig. 7. Experimental ESR spectrum of $Fe(NO)_2(resorcinol)_2$.

cular symmetry of resorcinol and phloroglucinol. NMR data are not able to provide definitive information and only ESR spectra allow some inferences. Both these organic ligands give rise to a five-line ESR spectrum at $g = 2.026$ (fig. 7) in the physiological range of pH, attributable to the interaction of the unpaired electron only with two NO group. On the other hand, the existence under these pH conditions of a coordination by the Fe(NO) group has been demonstrated in literature [25].

It is noteworthy that phloroglucinol give rise also to a triplet with relative intensities 1 : 1 : 1 at $g = 2.025$, with a nitrogen coupling constant $a_{^{14}NO} \approx 14.8$ gauss, in the presence of the iron-nitrosyl group.

From these findings, the pH-dependent equilibrium as shown in scheme 4 may be suggested.

4. Conclusion

The independent check by means of the FT-NMR technique gives direct information of the ligand in most cases; whereas the ESR spectra give straightforward evidence of the metal-ligand bond by means of the Fermi contact interaction between the unpaired electron of the iron atom and the ligand nuclei.

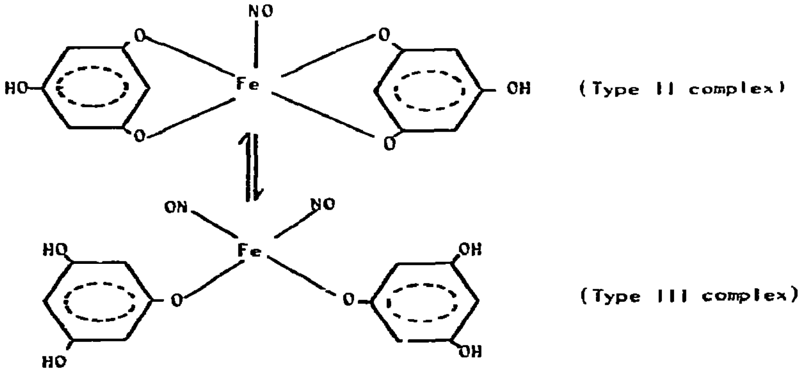
The following findings are worth noting:

(i) All the derivative of thiourea show a general trend in which the mercapto group is the preferred binding site for the Fe(I).

(ii) The thio-uracil derivatives give rise, at pH < 7, to iron-dinitrosyl complexes, in which two base molecules, one via the mercapto group and the other via a ring nitrogen, are bounded to the metal ion.

It is possible to suggest correlations of these structures with the antithyroid activity [26,27], compared with the strong activity of 2-thio-uracil, implies that the sulphur atom at (2)-position of the pyrimidine ring causes tiroxine inhibition [9].

Since the metal ion complexation capability (including iron in different oxidation states) and the increase of antithyroid activity have been shown to be possibly correlated [14–16], the study of model systems point out the features of the interactions between the metal ion and the binding sites may contribute to the elucidation of the biological function of these drugs. The $Fe(I)(NO)_2$ metal paramagnetic probe is a particularly suitable ion in studying the interactions with nucleobases [4] and thiobases [5]. Furthermore the $Fe(I)(NO)_2$ group has been shown to be involved in biological processes involving thiobinding sites in proteins [28].



Scheme 4.

The remainder two classes of antithyroid drugs show a weaker binding ability against metal ions. Our results about the coordination behaviour of sulphonamides via $-NH_2$ and of polyhydrophenol via $-OH$ against the paramagnetic probe, point out the weaker affinity of these binding sites with respect to the thio-group toward iron(I) ion.

Acknowledgement

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