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## Flavoquinone-Metal Complexes. I. Structure and Properties

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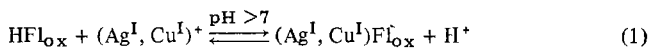
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Dark red flavoquinone-metal complexes have been detected by proton magnetic resonance and optical absorption in aprotic solvents. The stoichiometry and the formation constants of the complexes were measured by metal ion titration in acetone with the relative order of stability  $\text{Cu}^I > \text{Ni}^{II} > \text{Ag}^I, \text{Co}^{II} > \text{Cu}^{II} > \text{Zn}^{II} > \text{Cd}^{II} > \text{Fe}^{II} \gg \text{Mn}^{II}, \text{Mg}^{II}, \text{Fe}^{III}$  for the tris, bis, or mono complexes obtained in the presence of an excess of flavine. Stable bidentate chelates at O(4 $\alpha$ )-N(5) were observed, corresponding to octahedral tris complexes (Zn<sup>II</sup>, Cd<sup>II</sup>, Co<sup>II</sup>, Ni<sup>II</sup>, and Fe<sup>II</sup>) and tetrahedral (Ag<sup>I</sup> and Cu<sup>I</sup>) or square-planar (Cu<sup>II</sup>) bis complexes. Labile complexes corresponding to weak monodentate binding at the keto groups of the flavine were observed with Mg<sup>II</sup>, Mn<sup>II</sup>, and Fe<sup>III</sup>. They were also observed with the former ions as secondary sites of coordination. The nature—though not the kinetics—of the binding was found to be largely independent of the metal among the bidentate complexes, either with diamagnetic or with paramagnetic ions, excluding extensive charge transfer from the metal toward the electron-deficient flavoquinone ligand.

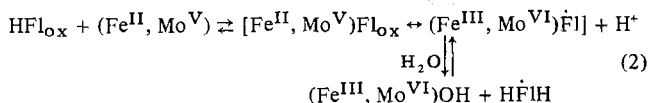
### Introduction

Previous studies of the interaction of flavines and flavo-coenzymes with metal ions,<sup>1-4</sup> as reviewed extensively by Hemmerich and Lauterwein,<sup>5</sup> have led to the distinction of three different types of flavine-metal complexes.

(1) Flavines in their oxidized state ("flavoquinone", HFl<sub>ox</sub>) do not form stable metal chelates in dilute aqueous solution except with the exceedingly "soft"<sup>6</sup> metal ions Ag<sup>+</sup> and Cu<sup>+</sup>.<sup>1,2</sup> These ions are the only ones capable of coordinating the flavoquinone anion (Fl<sub>ox</sub><sup>-</sup>) more strongly than OH<sup>-</sup>, according to eq 1. In the case of the biologically relevant donor-acceptor



ions of iron and molybdenum,<sup>3</sup> initial complex formation in the lower valence (donor) states Fe<sup>II</sup> and Mo<sup>V</sup> is followed by rapid hydrolysis in the upper metal valence states according to eq 2. These complexes of metal donor states with fla-

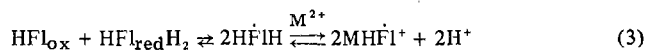


voquinone anion have been termed "charge transfer chelates". The more stable binary tetrahedral Ag<sup>I</sup> and Cu<sup>I</sup> chelates can only serve as models of distinct but limited relevance for the

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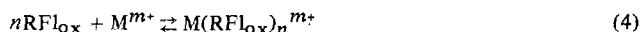
octahedral Fe<sup>II</sup> and Mo<sup>V</sup> chelates, whose stability is largely determined by ternary ligands (other than solvent). It should be emphasized that nothing can be inferred as to their electron and spin distribution at present.

(2) General flavine-metal affinity in dilute aqueous solution is only observed<sup>4</sup> for the radical state according to eq 3. In



all partly reduced flavine systems, therefore, semiquinone disproportionation is overcome at neutral to slightly alkaline pH by formation of "radical chelates" with the flavosemiquinone anion HFl<sup>-</sup>.

(3) Metal complexes made up from neutral flavoquinone and redox-inactive metal ions can, however, be observed in aprotic solvents for low water content. The present detailed study is devoted to those "flavoquinone chelates", which contain RFl<sub>ox</sub> as ligand (R = H or alkyl substituent at N(3)), in contrast to the first mentioned "charge-transfer chelates". The more polarizable monovalent or divalent ions, either diamagnetic such as Cu<sup>I</sup>, Ag<sup>I</sup>, Zn<sup>II</sup>, and Cd<sup>II</sup> or paramagnetic such as Fe<sup>II</sup>, Co<sup>II</sup>, Ni<sup>II</sup>, and Cu<sup>II</sup>, will be shown to chelate at the N(5)-O(4 $\alpha$ ) site according to eq 4. The less polarizable



ions such as Mg<sup>II</sup>, Mn<sup>II</sup>, or Fe<sup>III</sup> form only monodentate complexes very weakly bound at the carbonyl oxygen atoms of the flavine nucleus.

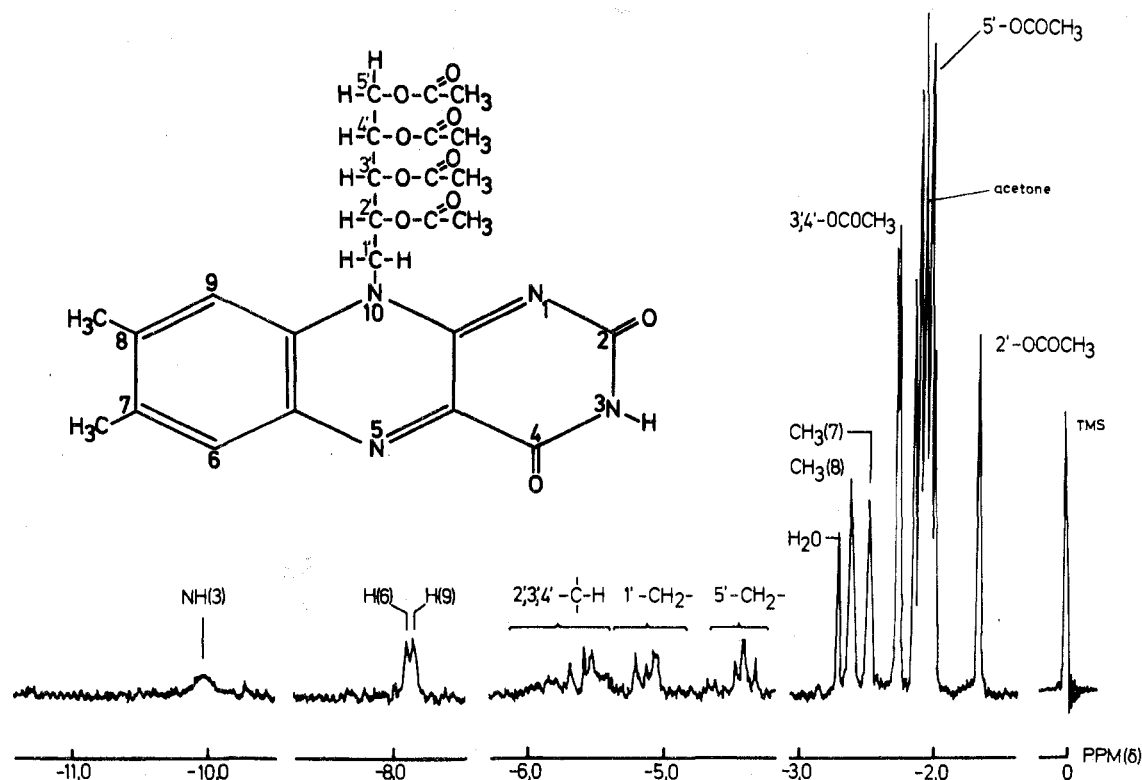


Figure 1. PMR spectrum of TADF,  $3 \times 10^{-2} M$ , in acetone- $d_6$ . The quintet at  $-2.15$  ppm is due to the presence of 0.5%  $CHD_2COCD_3$ .

The structures we were first to propose for these complexes<sup>7</sup> have also been confirmed in the solid state by recent X-ray crystallography of  $Ag^I$  and  $Zn^{II}$  complexes.<sup>8,9</sup> As shown below, proton magnetic resonance (PMR) reveals the details of the stoichiometry, stereochemistry, and stability of the complexes through the observation of the diamagnetic interactions. On the other hand, the paramagnetic perturbations introduced by open-shell ions provide an insight into the nature of the electronic binding within the complexes, which is described in the following paper.<sup>10</sup> Similar PMR studies have been independently undertaken on metal hydroxyquinolinates as models for flavine-metal interaction in biology.<sup>11</sup>

### Experimental Section

Most PMR spectra have been recorded at 60 MHz using a Varian A-60A spectrometer operated at 38°. Measurements at lower temperature were made using a variable-temperature probe accurate to  $\pm 1^\circ$ . Spin decoupling as well as internuclear double resonance (INDOR) measurements have been performed on a Varian XL-100 spectrometer. Chemical shifts are referenced to internal TMS and defined as negative for low-field shifts.

A compromise had to be found for the choice of the flavine and the solvents in order to get good solubility of both the metal ions and the flavine together with high equilibrium constants in favor of chelate formation. Hexadeuterated acetone (Merck) was found by PMR integration to contain approximately  $10^{-2} M$  water when freshly opened. Heavy water is also present at a concentration around  $10^{-1} M$  (Merck qualifications). These concentrations could be kept constant with time using 4-Å molecular sieves. Hydrated perchlorate salts of  $Mn^{II}$ ,  $Fe^{II}$ ,  $Fe^{III}$ ,  $Co^{II}$ ,  $Ni^{II}$ ,  $Cu^{II}$ ,  $Zn^{II}$ , and  $Cd^{II}$  (Fluka), used without further purification but dried under vacuum at 50–100°, contain six molecules of water of crystallization per metal ion. Thus, the total water contamination of the metal ion solutions in acetone- $d_6$  was  $[H_2O + D_2O] \approx 10^{-1} M + 6[M^{2+}]$ . Since the flavine concentrations used for PMR measurements were  $\sim 10^{-2} M$ , the constancy of the water concentration ( $10^{-1} M$ ) was only lost at high excess of metal ions over flavine.  $AgClO_4$  was obtained as monohydrate from Fluka.  $Cu^I(CH_3CN)_4ClO_4$  was prepared before use<sup>12</sup> and kept under argon once in solution.

Optical spectra were recorded using a Cary 14 spectrophotometer at room temperature by differential absorption between complexed and uncomplexed metal ion solutions.

Tetraacetylribosylflavine (TADF), tetraacetylisoribosylflavine (iTADF), and *N*<sup>3</sup>-ethyl-TADF were synthesized according to known procedures.<sup>13,14</sup> 10-(2'-Acetoxyethyl)flavine and 10-(5'-acetoxyethyl)flavine were obtained by acetylation of the corresponding hydroxyalkyl compounds.<sup>15</sup>

**Preparation of Crystalline Flavine-Metal Complexes.** To a warm solution of *N*<sup>3</sup>-Et-TADF,  $10^{-2} M$  in acetone, 0.5 molar equiv of metal perchlorate was added. When the reaction mixture was cooled, a reddish precipitate was formed which was collected by filtration, washed with acetone and ether, and dried in vacuo.

Anal. Calcd for  $(C_{27}H_{32}N_4O_{10}ClO_4H_2O)_2Zn$ : C, 44.9; H, 4.8; N, 7.7; H<sub>2</sub>O, 2.5. Found: C, 45.0; H, 5.1; N, 7.9; H<sub>2</sub>O, 2.1. Calcd for  $(C_{27}H_{32}N_4O_{10}ClO_4H_2O)_2Co$ : C, 45.1; H, 4.8; N, 7.8; H<sub>2</sub>O, 2.5. Found: C, 45.3; H, 4.7; N, 7.8; H<sub>2</sub>O, 2.8. Calcd for  $(C_{27}H_{32}N_4O_{10}ClO_4H_2O)_2Ni$ : C, 45.1; H, 4.8; N, 7.8; H<sub>2</sub>O, 2.5. Found: C, 45.2; H, 5.3; N, 7.8.

Slow cooling provided few crystals (ca. 1 mm) suitable for a preliminary X-ray analysis<sup>9</sup> in spite of extensive twinning.

### Results and Discussion

**PMR of the Free Flavoquinone.** In Figure 1 the PMR spectrum of tetraacetylribosylflavine (TADF) is shown. Assignments must be made of the methine protons H(6) and H(9), the aromatic methyl groups CH<sub>3</sub>(7) and CH<sub>3</sub>(8), and the N(10) side chain protons. By INDOR decoupling we find a very weak coupling between H(9) and the peri CH<sub>2</sub>(10 $\alpha$ ) protons. The broader line width of the H(9) signal relative to that of H(6) results from peri overcrowding.<sup>16</sup> As a consequence of this unambiguous assignment of H(6) and H(9), spin decoupling allows one, by the characteristic ortho coupling of  $<0.6$  Hz, to differentiate CH<sub>3</sub>(7) and CH<sub>3</sub>(8), thus confirming independently the earlier assignment made by Bullock and Jardetzky,<sup>17</sup> based on selective  $8\alpha$ -deuteration. It was, of course, chemically reasonable to assume that CH<sub>3</sub>(8) is deuterated in preference over CH<sub>3</sub>(7), but direct evidence was lacking up to date. Hence, CH<sub>3</sub>(8) appears always at lower field than CH<sub>3</sub>(7) in RFl<sub>ox</sub>, independent of the solvent, and we find the same for the cation RFl<sub>ox</sub>-1-H<sup>+</sup> in trifluoroacetic acid.

These assignments have turned out to be biochemically relevant in the elucidation of succinate dehydrogenase

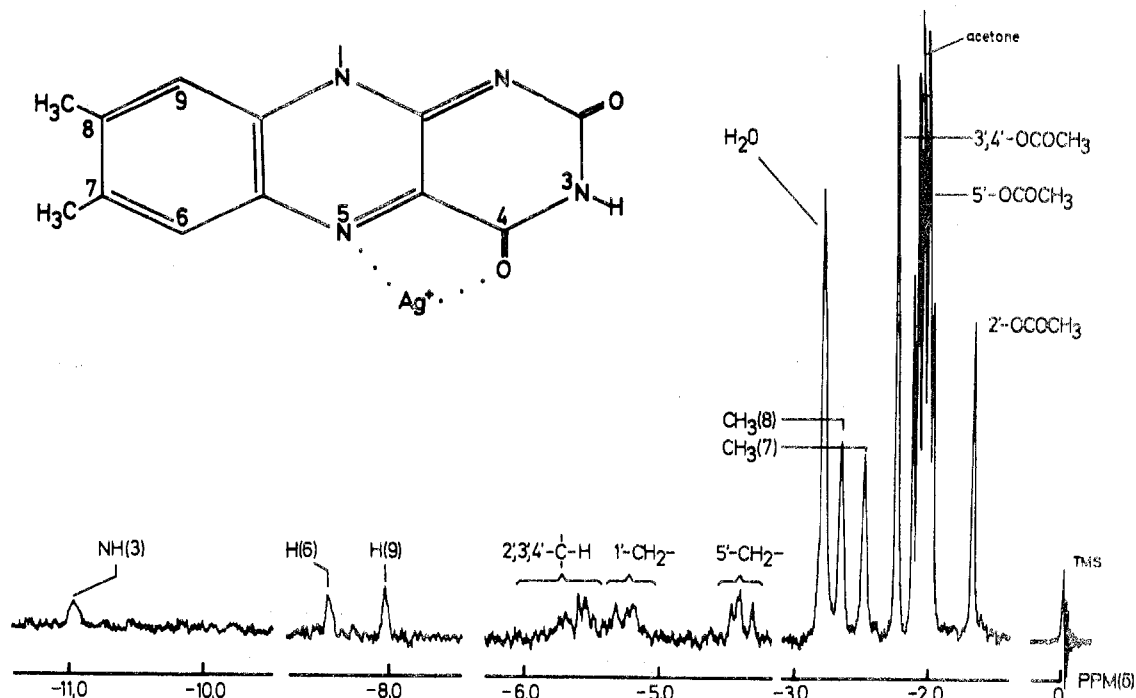


Figure 2. PMR spectrum of TARF,  $3 \times 10^{-2} M$ , in the presence of an equimolar amount of  $\text{AgClO}_4 \cdot \text{H}_2\text{O}$ ; solvent is acetone- $d_6$ . Notice the strong low-field shifts of the NH(3) and H(6) resonances.

flavocoenzyme<sup>18</sup> as well as hydroxyflavine coenzymes discovered recently.<sup>19</sup> They are also identical with those of Sarma et al.<sup>20</sup> in flavine adenine dinucleotide (FAD) which were based on early calculations of electron distribution.<sup>21</sup> In FAD, however, the sequence of H(6) and H(9) signals can be reversed as compared to flavine mononucleotide,<sup>22</sup> owing to the stacking interaction of flavine and adenine halves.

The N(10) side chain proton resonances have been assigned, as shown in Figure 1, by selective decoupling and reveal magnetic nonequivalence of both the 1'- and 5'-methylene protons, the former appearing at lower field values due to the ring current diamagnetic shielding effect of the isoalloxazine ring.

Three of the four resonance signals of the *O*-acetyl groups of TARF have shift values between -2.0 and -2.3 ppm (Figure 1). This is the normal range of absorption for *O*-acetyl groups as one can see by comparison with, e.g., triacetin (-2.1 ppm). The fourth signal, however, appears at abnormally high field (-1.65 ppm). This is due to an intramolecular (independent of flavine concentration) ring current effect associated with the back-folding of the side chain, in agreement with the calculation of Giessner-Prettre and Pullman<sup>23</sup> on the screening of a proton 3.4 Å above N(10) of the flavine plane (+0.6 ppm). The spectral comparison of a series of flavines having N(10) side chains of varying length, viz., 10-(2'-acetoxyethyl)flavine, 10-(5'-acetoxyethyl)flavine, indicates that the upfield signal in TARF must be assigned to the 2'-OCOCH<sub>3</sub> group. The screening effect is not as much pronounced in 10-(2'-acetoxyethyl)flavine (+0.2 ppm); hence, it must be the packing effect of the tetraacetylribityl substituent, which enforces close contact of 2'-OCOCH<sub>3</sub> with the heteroaromatic acceptor nucleus. The analogous behavior was found with trimethylsilylated<sup>19</sup> riboflavine.

No concentration dependence of the PMR spectra of RFl<sub>ox</sub> was observed up to  $10^{-2} M$  in slightly polar solvents like acetone or acetonitrile and up to  $10^{-1} M$  in dimethyl sulfoxide. If R = H, self-association through hydrogen bonding at the pyrimidine ring in chloroform is indicated by the concentration dependence of the NH(3) resonance. The conformational stability of the molecule and the absence of association

phenomena at low concentration are further confirmed in acetone by the lack of important changes in chemical shift and line shape in a large temperature range (-60 to +38°). The side chain resonances are more strongly affected by temperature due to changes in intramolecular mobility. At -60° the 2'-OCOCH<sub>3</sub> resonance appears broadened by a factor of 2 relative to the spectrum at 38°.

The formation of RFl<sub>ox</sub>-1-H<sup>+</sup> in trifluoroacetic acid results in a uniform low field shift of all isoalloxazine resonances by approximately -0.2 ppm for methyl groups and -0.4 ppm for methine protons. Thus, the added positive charge appears to be regularly spread around the molecule with no large difference in its distribution between either C(6) and C(9) ( $\Delta\delta = +0.07$  ppm) or C(7) and C(8) ( $\Delta\delta = -0.03$  ppm).

**PMR and Absorption Spectra of the Flaviquinone-Metal Complexes. Stoichiometry and Stability.** The PMR spectrum of TARF in the presence of an equimolar concentration of a diamagnetic metal ion, e.g., Ag<sup>I</sup> (Figure 2), exhibits large downfield shifts which vary in intensity from one nucleus to the other. The resonances are shifted progressively upon titration of a flavine solution with increasing concentrations of metal ions from the free-flavine value of the chemical shifts,  $\delta_f$ , up to a plateau value,  $\delta_c$ , corresponding to the saturation of the flavine with metal ions, i.e., to the complete formation of 1:1 complexes, e.g., AgHFl<sub>ox</sub><sup>+</sup> (Figure 3). These plateau values are reached at a metal to flavine ratio close to unity, suggesting that the complexes are relatively stable. A second rise in negative shifts is observed for metal concentrations 1 order of magnitude higher than the flavine concentration, indicating a weaker binding of a second metal ion to the 1:1 complexes. In Figure 3 a second plateau indicates the complete formation of Ag<sub>2</sub>HFl<sub>ox</sub><sup>2+</sup>.

The titration behavior of the chemical shifts, together with the absence of line broadening upon addition of metal, reflects the conditions of fast chemical exchange; i.e., the reciprocal of the average residence time of a given flavine molecule in the coordination sphere of a metal ion,  $1/\tau_{th}$ , is large as compared to the difference in resonance frequency of any proton in the uncomplexed and complexed flavines. Under such conditions the analysis of the PMR titration curves in

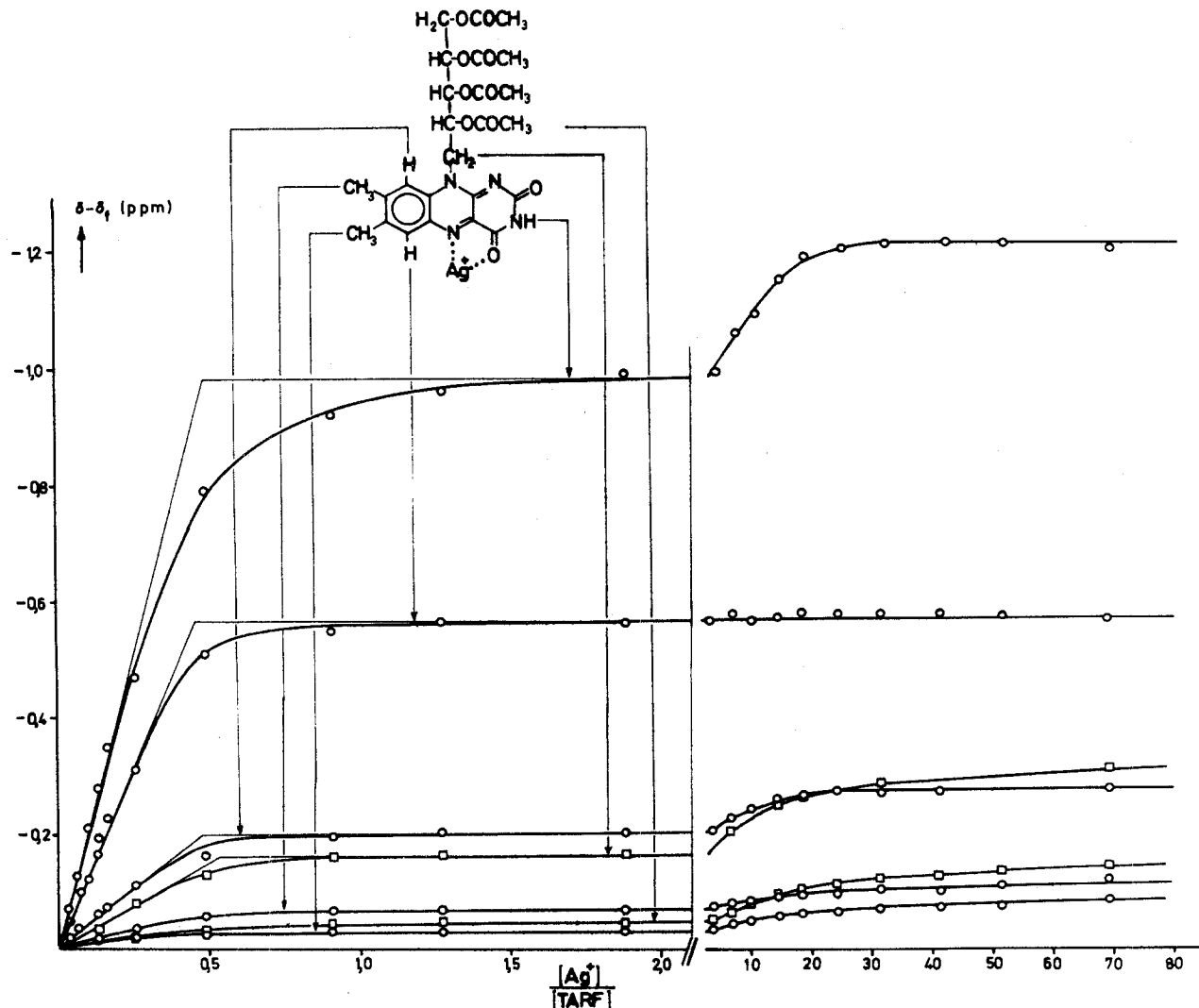
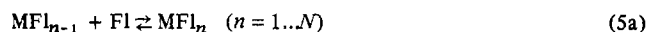


Figure 3. PMR titration curves for the  $\text{Ag}^{\text{I}}$  complexes with TARF: plot of the diamagnetic shifts,  $\delta - \delta_f$ , vs. the metal:flavine ratio ( $[\text{TARF}] = 3 \times 10^{-2} \text{ M}$ , solvent acetone- $d_6$ ). The sequence of shifts,  $\text{NH}(3) > \text{H}(6) > \text{H}(9) > \text{CH}_3(8) > \text{CH}_3(7)$ , confirms metal chelation at  $\text{O}(4\alpha)\text{-N}(5)$ .

terms of stability and stoichiometry of the complexes should take into account the multistep characteristics of the titration reaction which correspond to the successive formation of complexes having different stoichiometric ratios (to simplify the following equations we write Fl instead of  $\text{RFl}_x$  and omit all electric charges)



Each of these steps may be characterized by an equilibrium constant  $K_n$  and the overall reaction completed at low metal concentration



is characterized by a formation constant  $\beta_N = K_1 K_2 \dots K_N$ . If  $\beta_N$  is large enough, so that the saturated complex  $\text{MFl}_N$  is exclusively produced at the early stage of titration, the chemical shift observed for a given proton is

$$\begin{aligned} \delta &= \frac{[\text{Fl}_0] - N[\text{MFl}_N]}{[\text{Fl}_0]} \delta_f + \frac{N[\text{MFl}_N]}{[\text{Fl}_0]} \delta_c \\ &= \delta_f + (\delta_c - \delta_f) N[\text{MFl}_N] / [\text{Fl}_0] \end{aligned} \quad (6)$$

where  $[\text{Fl}_0]$  is the total flavine concentration and  $[\text{MFl}_N]$  the equilibrium concentration of the saturated complex. Wang and Li<sup>24</sup> have derived an analytical expression in order to calculate the value of  $\beta_N$  from the ratio of the linear slopes

$S_i/S_j$  of the titration curves at two different total concentrations of ligands  $[\text{Fl}_i]$  and  $[\text{Fl}_j]$

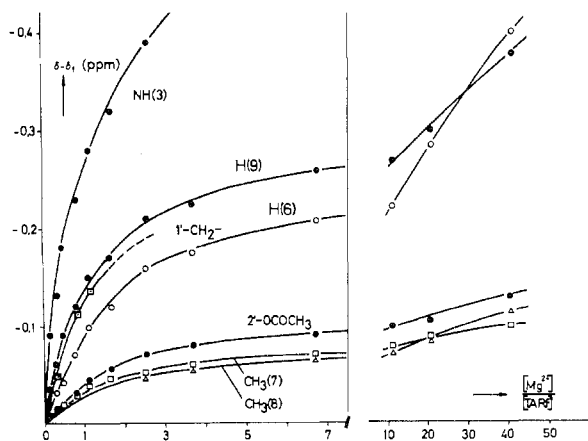
$$S_i/S_j = ([\text{Fl}_i]/[\text{Fl}_j])^{N-1} [(1 + [\text{Fl}_j]^N \beta_N)/(1 + [\text{Fl}_i]^N \beta_N)] \quad (7)$$

If the 1:1 complexes are sufficiently stable,<sup>25</sup> the stoichiometric number of the saturated complexes,  $N$ , is given directly by the abscissa of the intercept of the initial linear branch of the titration curve with the extrapolated plateau line corresponding to the completion of the 1:1 complex (Figure 3). For most metal ions the chemical shifts reach their stationary value  $\delta_c$  near this intercept point. This means that the nonsaturated complexes  $\text{MFl}_{N-1}$  or  $\text{MFl}_{N-2}$  must differ very little in the ligand proton chemical shifts from the values corresponding to the saturated complex  $\text{MFl}_N$ .

In the case of weak complex formation, e.g., with  $\text{Mg}^{\text{II}}$  (Figure 4) or with any metal ion using tetraacetylisoriboflavine (iTARF) as ligand (Figure 5), there are no plateau values observable for the shifts in the range of experimental concentrations. Assuming that only 1:1 complexes are formed under these conditions,  $K_1$  may be derived from the expression

$$\frac{\delta - \delta_f}{[\text{M}_0]} = \frac{(\delta_c - \delta_f)K_1}{1 + [\text{Fl}_0]K_1} - \frac{K_1}{1 + [\text{Fl}_0]K_1} (\delta - \delta_f) \quad (8)$$

by plotting the value of the first term, including the observed



**Figure 4.** PMR titration curves for the  $\text{Mg}^{\text{II}}$  complexes with TARF,  $3 \times 10^{-2} M$ ; solvent is acetone- $d_6$ . The sequence of shifts,  $\text{NH}(3) \gg \text{H}(9) > \text{H}(6) > \text{CH}_3(7) > \text{CH}_3(8)$ , as well as the relatively large shifts for the  $1'\text{-CH}_2$  and  $2'\text{-OCOCH}_3$  resonances, is in favor of monodentate complex formation at  $\text{O}(2\alpha)$ .

shift value  $\delta$  at total metal concentration  $[\text{M}_0]$ , as a function of  $(\delta - \delta_f)$ .

The  $N$  values and the complex formation constants derived following these procedures for various diamagnetic ions are given in Table I. The same procedure can also be applied to paramagnetic complexes. The paramagnetic perturbations introduced in the ligand resonances will be described in the following paper.<sup>10</sup> However, in spite of their diversity in sign and amplitude, the shifts of the various resonances behave upon titration in fast-exchange conditions in a manner similar to that observed for the diamagnetic ions allowing the determination of the  $K$  and  $N$  values (Table I).

The values of  $N$  may be confirmed by integration of the signals obtained in conditions of *slow*-exchange reactions. Then, each flavine proton exhibits two separate resonances at  $\delta_c$  and  $\delta_f$  corresponding to the complexed and free states, respectively. Such a behavior is observed even at  $38^\circ$  at stoichiometric concentration with  $\text{Ni}^{\text{II}}$ , but with other metal ions, e.g.,  $\text{Co}^{\text{II}}$  (Figure 6), this requires lowering of temperature. However, lines are usually broadened and the integration

**Table I.** Constants of Formation of the Complexes  $\text{M}(\text{HF}1_{\text{O}\alpha})_N^{\text{m}+}$  in Acetone

Metal ion	$N$	$\log \beta_N^a$	$\log K_1^b$
$\text{Cu}^+$	2	4.29	3.7
$\text{Ni}^{2+}$	3		3.3
$\text{Ag}^+$	2	4.21	3.1
$\text{Co}^{2+}$	3	5.68	3.1
$\text{Cu}^{2+}$	2	3.89	2.8
$\text{Zn}^{2+}$	3	5.72	2.7
$\text{Cd}^{2+}$	3	5.54	2.6
$\text{Fe}^{2+}$	3	5.83	2.0
$\text{Mn}^{2+}$	1		1.6
$\text{Mg}^{2+}$	1	1.74	1.1

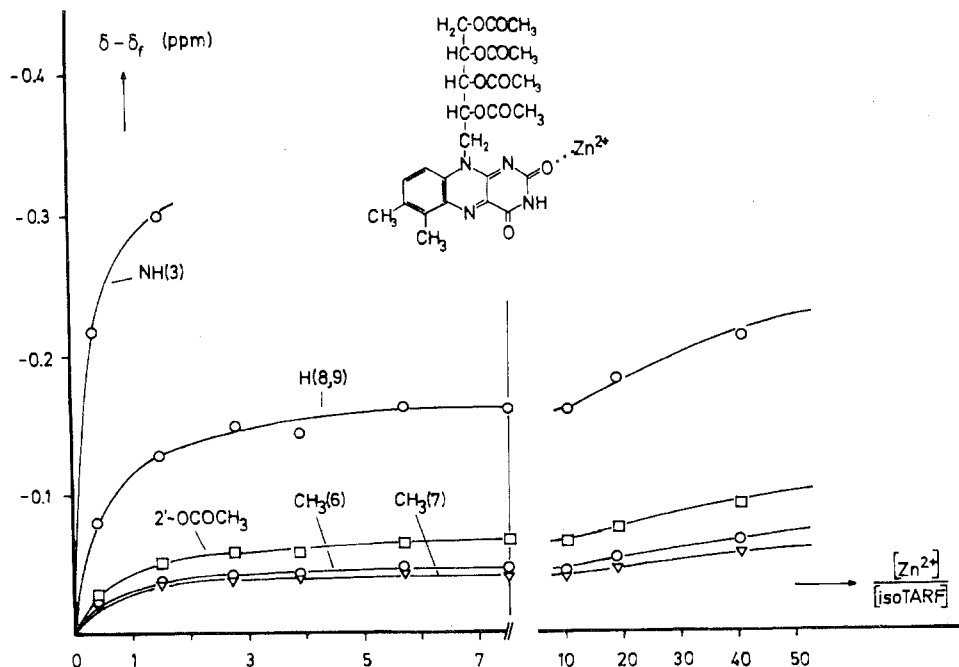
<sup>a</sup> Measured by PMR;  $[\text{TARF}] = 3 \times 10^{-2} M$ ,  $38^\circ$ . <sup>b</sup> Measured by optical absorption;  $[\text{TARF}] = 8 \times 10^{-4} M$ ,  $25^\circ$ .

procedure is rather imprecise. Incidentally, the larger broadening of the  $\text{Ni}^{\text{II}}$  complex lines relative to  $\text{Co}^{\text{II}}$  is in favor of an octahedral structure for the environment of the metal ion within the complex.<sup>26</sup> Line broadening in the  $\text{Mn}^{\text{II}}$  complex, together with very small shifts, does not allow a precise determination of  $\beta_N$ , but its value appears comparable to that measured for the  $\text{Mg}^{\text{II}}$  complex (Table I).

$N^3\text{-Et-TARF}$  was used in several experiments because of its higher solubility in acetone. Its complex formation constants are parallel but slightly higher than those for unsubstituted TARF. The only qualitative difference is the absence of a plateau for the chemical shifts of the ethyl protons upon titration. A secondary decrease of the magnitude of the negative shifts occurs for these protons once the other resonances remain fixed. This suggests that the perturbations induced by metal coordination in the ethyl group are somewhat smaller in the 1:1 and 2:1 complexes than in the saturated 3:1 complex although the perturbations at other nuclei remain constant.

In the case of iTARF only small shifts ( $<0.3$  ppm) are observed (Figure 5) with a relative magnitude comparable to the "secondary" shifts observed with TARF at the same high concentration of diamagnetic metal ions (Figure 3).

The formation constants  $K_1$  of the 1:1 complexes can also be measured by optical absorption. Since optical titration requires flavine concentrations 1 or 2 orders of magnitude lower than those used for the PMR experiments, the water content per flavine is such that an excess of metal ion is necessary for



**Figure 5.** PMR titration curves for the  $\text{Zn}^{\text{II}}$  complexes with iTARF,  $10^{-2} M$ ; solvent is acetone- $d_6$ . The sequence of shifts,  $\text{NH}(3) \gg \text{H}(8), \text{H}(9) > \text{CH}_3(6) > \text{CH}_3(7)$ , confirms complexation at  $\text{O}(2\alpha)$ .

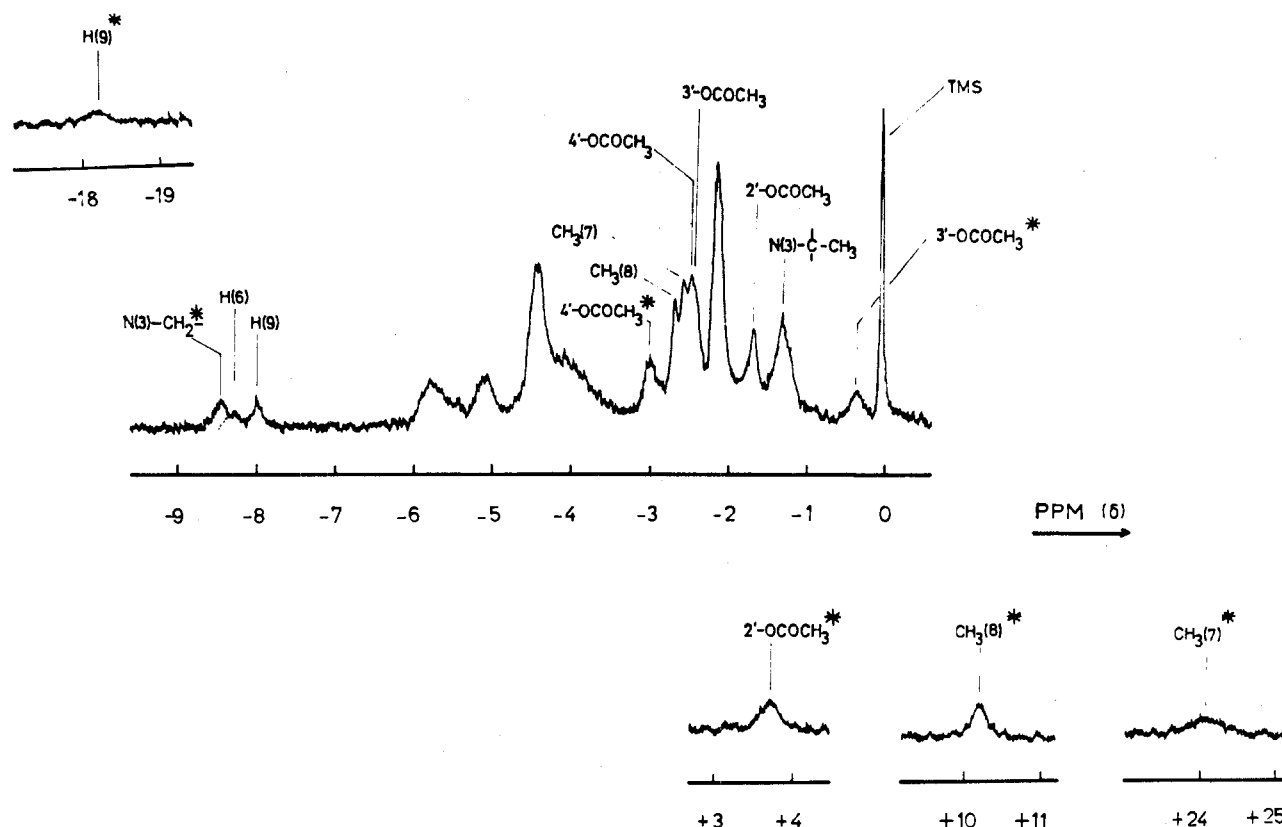


Figure 6. PMR spectrum of a solution of  $N^3$ -Et-TARF,  $9 \times 10^{-2} M$ , and  $\text{CoClO}_4 \cdot 6\text{H}_2\text{O}$ ,  $10^{-2} M$ , in acetone- $d_6$  at  $-60^\circ$ . The signals of the complexed flavines, as far as accessible for assignment,<sup>10</sup> are marked by an asterisk. From the intensity ratios, e.g.,  $\text{H}(9):\text{H}(9)^*$ , the stoichiometry of the complex was calculated as  $N = 2.9 \pm 0.3$ .

complex formation. Thus, the values of the formation constants measured under such different conditions cannot be compared in absolute terms but they exhibit the same relative order of stability among the various metal complexes (Table I).

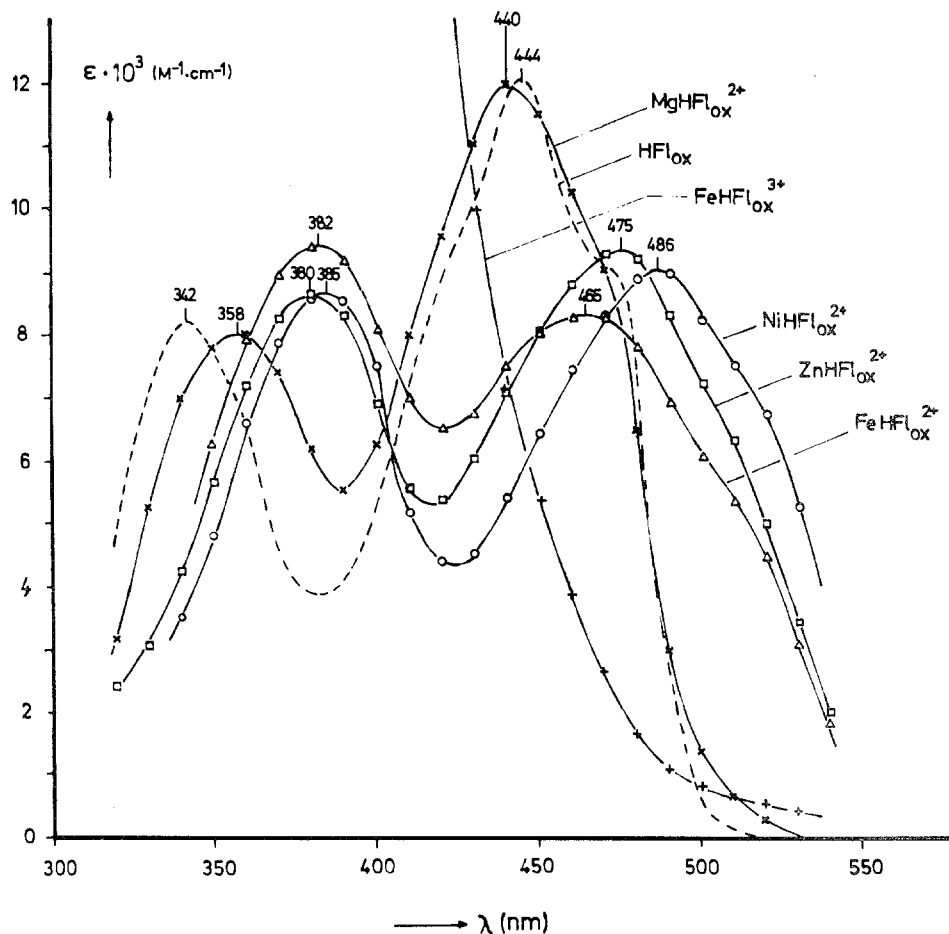
The line shape of the absorption spectra of the complexes (Figure 7) allows one to classify them within two groups corresponding to rather different values of the formation constants. In the more stable complexes the first two absorption bands are shifted to the red by around 40 nm relative to the  $\text{RFl}_{\text{ox}}$  spectrum. This group includes the diamagnetic complexes which are characterized by PMR spectra similar to those of  $\text{Zn}^{\text{II}}$  and  $\text{Ag}^{\text{I}}$  complexes, but it also contains most of the complexes with paramagnetic ions, except  $\text{Mn}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$ , suggesting that the perturbations which do *not* involve paramagnetism are rather similar within this whole series of complexes. On the other hand, the complexes characterized by weak metal interaction, as those obtained with  $\text{Mg}^{\text{II}}$  or  $\text{Mn}^{\text{II}}$ , exhibit an absorption spectrum in which only the second band in the near uv is red shifted; the first one is slightly blue shifted. A larger blue shift is observed with  $\text{Fe}^{\text{III}}$  resulting in an absorption spectrum somewhat similar to that of  $\text{RFl}_{\text{ox}}-1-\text{H}^+$ . This second type of absorption spectra characterizes also the weak complexes formed with iTARF. Finally, it should be noted that the addition of  $\text{Na}^+$  and  $\text{K}^+$  as well as the bulky benzyltrimethylammonium cation has practically no effect upon the spectra up to 0.5  $M$  concentration.

**Site of Coordination.** Diamagnetic metal ion coordination induces negative shifts of the isoalloxazine resonances which differ strongly in magnitude from one proton to the other (Figure 3), in contrast to the protonation effect  $\text{RFl}_{\text{ox}} \rightarrow \text{RFl}_{\text{ox}}-1-\text{H}^+$ . The various diamagnetic ions behave in a manner similar within each of the two classes defined from optical spectra and stability constants. This allows a straightforward identification of the metal ion coordination sites at the flavine nucleus. Because of the magnitude of the shifts of the  $\text{NH}(3)$

and  $\text{H}(6)$  protons relative to the other protons of  $\text{HFl}_{\text{ox}}$  (Figure 3), the more stable complexes must be considered as  $\text{O}(4\alpha)-\text{N}(5)$  bidentate chelates similar to those analyzed in the solid state by X-ray crystallography.<sup>8,9</sup> The small magnitude of the perturbations at the  $10\alpha$  protons clearly indicates that the neighboring  $\text{N}(1)$  atom, which is the site of protonation in the cation,<sup>13</sup> cannot be the preferential site of complexation in spite of its higher basicity. The  $\text{O}(2\alpha)-\text{N}(1)$  distance is too short for a strong bidentate chelation and this site is further hindered by the presence of  $\text{N}(10)$  substituents. The assignment of  $\text{O}(4\alpha)-\text{N}(5)$  as the main site of chelation is confirmed by the absence of corresponding large negative shifts in isoflavines (Figure 5), due to steric hindrance of the  $\text{C}(6)$ -substituting methyl group.

The properties which do not involve magnetic interactions in open-shell ion complexes, as the constant of formation or the optical characteristics, allow one to assign the same structure of chelation for the paramagnetic complexes of the first group defined above. PMR evidence for this arises from relaxation through dipole-dipole interaction<sup>27</sup> which is most effective to protons adjacent to the binding site of the paramagnetic ion. For most paramagnetic complexes, with the exception of  $\text{Mn}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$  complexes, one observes a severe broadening of the  $\text{H}(6)$  line at very low metal to flavine concentrations leaving all other resonances unchanged (Figure 8).

Indeed, the  $\text{Mn}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$  complexes which do not characteristically broaden the  $\text{H}(6)$  line belong to the second group of complexes represented by  $\text{Mg}^{\text{II}}$  in the diamagnetic case. These complexes exhibit monodentate coordination at one of the carbonyl groups of the flavine.  $\text{O}(2\alpha)$  appears to be the preferred site for that fixation as indicated by the relatively large shifts of the  $\text{N}(10)$  side chain protons (Figure 4) and the similarity in absorption spectra with  $\text{RFl}_{\text{ox}}-1-\text{H}^+$  or the flavoquinone 2-iminol esters ( $\lambda_{\text{max}}$  at 378 and 444 nm for the

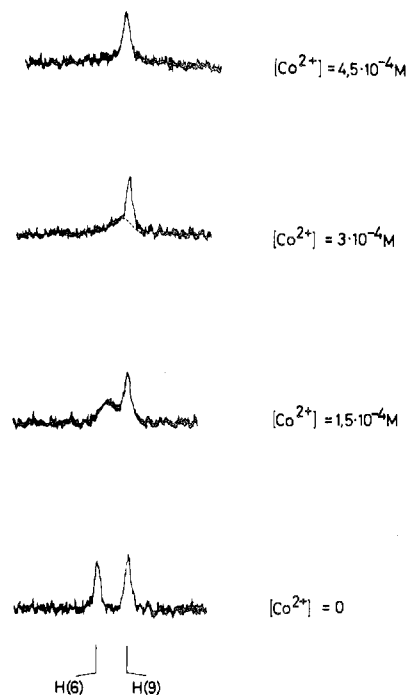


**Figure 7.** Optical absorption spectra of TARF,  $8 \times 10^{-4} M$  (---), in acetone and in the presence of  $Zn^{2+}$  ( $\square$ ),  $Ni^{2+}$  ( $\circ$ ), and  $Fe^{2+}$  ( $\triangle$ ), all  $5 \times 10^{-2} M$ , and  $Mg^{2+}$  ( $\times$ ) and  $Fe^{3+}$  ( $+$ ), both  $10^{-1} M$ . The solutions contain  $0.5 M$  benzyltrimethylammonium perchlorate for constant ionic strength.

ethyl ester in aqueous solution at pH 7).<sup>13</sup> This position must also be the only site of coordination of any metal ion with iTARF and the second site of coordination in the  $M_2HFl_{ox}^{2m+}$  complexes obtained with TARF in the presence of a large excess of metal ions. The H(6) resonance is not further shifted during the corresponding second step of the titration curves (Figure 3); the NH(3) resonance, however, is strongly shifted as well as the side chain resonances which were rather insensitive to the formation of  $MHFl_{ox}^{m+}$ . There remains some possibility of monodentate coordination at O(4 $\alpha$ ) as observed for  $Mg^{II}$  at high concentration (Figure 4) or as suggested by the paramagnetic broadening introduced by  $Mn^{II}$ . With the latter ions, large perturbations of the O-acetyl resonances suggest some additional direct interaction with the N(10) side chain. The weakly binding ions of the second class should be considered as truly coordinating the flavine when compared to monovalent ions such as  $Na^+$  or  $K^+$ . The shifts observed with these latter ions appear negligible as compared to the effect of divalent ions like  $Mg^{II}$  in a scale of ionic strength.

The above structural assignments agree entirely with the crystal structure of a 10-methylisalloxazine-silver(I) nitrate complex recently reported.<sup>8</sup> Each flavine in the crystal unit is bound to two distinct  $Ag^I$  ions, forming a centrosymmetric square-planar complex strongly bound at O(4 $\alpha$ ) and N(5) and a weaker complex through O(2 $\alpha$ ) in which the bonding with N(1) may be considered negligible.

**Nature of the Diamagnetic Interactions.** Metal coordination could decrease somewhat the ring current contribution to the PMR shifts by diminishing the electron conjugation through N(5). This effect, however, should give a positive contribution to the shifts which does not appear in any spectrum. Hence,



**Figure 8.** PMR spectrum of the two methine protons H(6) and H(9) of TARF,  $3 \times 10^{-2} M$ , at various concentrations of  $Co(ClO_4)_2$ ; solvent is acetonitrile- $d_3$ .

this should be a minor contribution, if any. Ring current effects may gain importance in the intermolecular interactions dis-

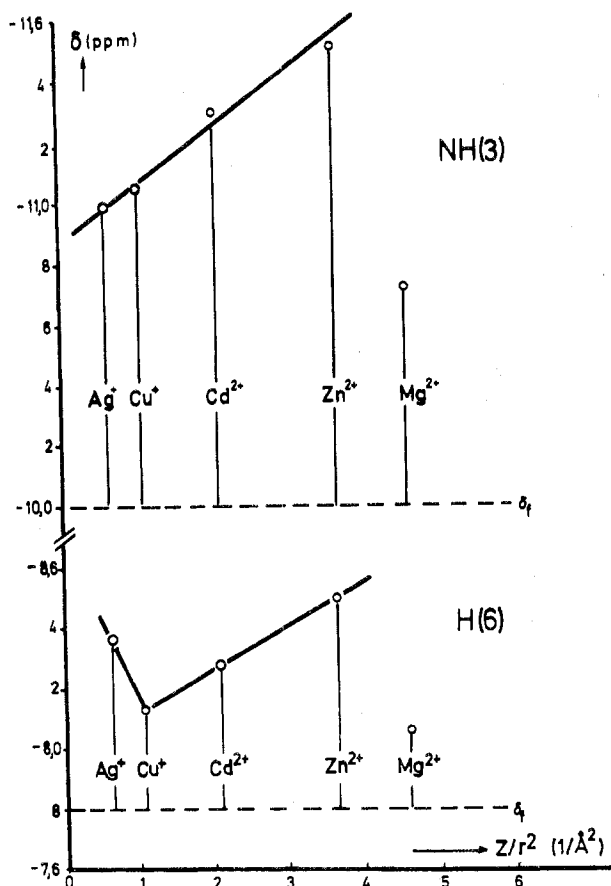


Figure 9. Proton chemical shifts of the diamagnetic complexes  $MHF_{lox}^{m+}$  plotted against the parameter  $Z/r^2$  characterizing the electron-withdrawing ability of the metal ions.

cussed in the following section. The negative shifts observed for the ligand proton resonances upon diamagnetic metal complexation appear to be directly related to a deshielding effect which parallels the electron-withdrawing ability of the metal ions. The effect of the electric field created by the positive ion at the coordination site and responsible for this polarization may be represented as the ratio of the electric charge  $Z$  to the square of the ionic radius of the ions,  $r^2$ . The large shifts observed in the 1:1 complexes for the NH(3) proton appear to be linearly correlated to this parameter (Figure 9). This correlation is still present, if not as linear, for other protons such as H(6). Indeed, the electron polarization under the influence of the metal ions, resulting in a decrease of the electron density in the 1s orbital surrounding the protons, may be due to a direct effect, e.g., at H(6), which should be very sensitive to the distance, but in conjugated molecules it corresponds also to long-distance indirect polarization through the  $\pi$  orbitals. Such indirect effects should also be very sensitive to the nature of the bonding. The large and regular shifts observed for the NH(3) proton (Figure 9) suggest that the contribution of the O(4 $\alpha$ ) binding in the bidentate chelation is essential and comparable within the series of diamagnetic metal ions examined. The relatively strong stabilization of the complexes with N<sup>3</sup>-Et-TARF also demonstrates the low degree of cyclic conjugation through the pyrimidine part of the isoalloxazine ring. Metal-induced indirect  $\pi$ -electron perturbation at longer distances should, therefore, occur mostly through the N(5) coordination and may be rather variable from one metal to the other, as it will be shown from the analysis of the paramagnetic perturbations.<sup>10</sup> The weak character of the N(5)-M bond is confirmed crystallographically in the bis(10-methylisoalloxazine)silver(I) complex in which the N(5)-Ag bond is 0.15 Å longer than in the strong bis(8-

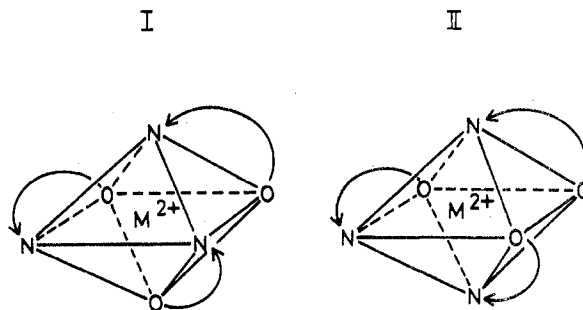


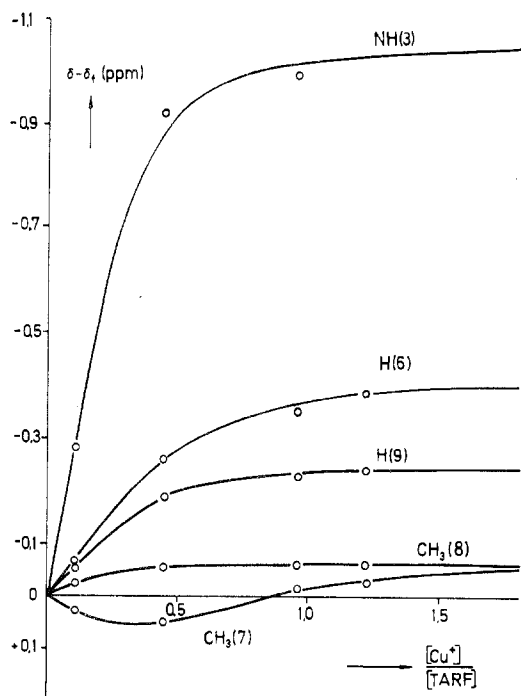
Figure 10. The two possible isomers for the octahedral complexes  $M(RF_{lox})_3^{2+}$ : the cis form, I, and the trans form, II.

hydroxyquinoline)silver(I) complex, in contrast to equal O-Ag bond lengths in both complexes.<sup>8</sup> A similar tendency was also observed in the Zn<sup>II</sup> complex in spite of lower resolution.<sup>9</sup> This may have its origin in the very low basicity of this nitrogen in RFl<sub>ox</sub> as well as in steric hindrance introduced by H(6).

**Geometry of the Complexes.** The analysis of the PMR titration curves with the O(4 $\alpha$ )-N(5) chelated ions showed that, depending upon the nature of the metal ion, either 3:1 or 2:1 complexes are obtained in the presence of a large excess of flavine. RFl<sub>ox</sub> being an asymmetrical bidentate ligand, the octahedral tris complexes observed with the divalent ions (with the exception of Cu<sup>II</sup>) may exist in two different kinds of geometrical isomers which are not magnetically equivalent. The cis isomers (Figure 10, I) have a threefold symmetry axis corresponding to a propeller type structure in which the three flavines have an identical environment. In the trans isomers (Figure 10, II) one cannot perform a true symmetry operation. Therefore, each of the three flavines is in a different environment and one should expect three separated PMR lines for each kind of protons. In case of a statistical formation of the stereoisomers one should expect a cis:trans ratio of 1:3 and a total splitting of every proton signal into four lines of equal intensity.<sup>29</sup> But even under conditions of slow chemical exchange the PMR lines of the tris complexes never split. Thus, either the electronic interactions are identical in the four isomers, a situation which is unacceptable at least for the paramagnetic interactions due to large differences in magnetic symmetry, or one predominant structure exists for the octahedral complexes for stereochemical reasons. Steric hindrance may favor the cis isomers since H(6) has to approach a neighbor flavine at rather a short distance in the octahedral complexes, as models based on crystallographic data<sup>30</sup> indicate. Molecular models also show that this strain can be more easily released in the cis isomer by a slight trigonal distortion of the octahedral structure. Such a distortion is indicated by magnetic susceptibility measurements<sup>10</sup> which indicate some quenching of the orbital contribution resulting from an important distortion of the octahedral environment of the ions. On the other hand, the presence of an electric dipole moment in the flavine plane should also favor the cis configuration of the octahedral complexes in solution. This will be definitely confirmed for the Co<sup>II</sup> ions by the analysis of the paramagnetic interactions.<sup>10</sup>

The bis complexes obtained with Cu<sup>II</sup>, Cu<sup>I</sup>, and Ag<sup>I</sup> must exhibit square-planar or tetrahedral configurations. Crystal field stabilization favors the square-planar configuration for the Cu<sup>II</sup> complex, as is confirmed by electron spin resonance.<sup>10</sup> Steric hindrance requires a centrosymmetric trans structure for this complex as observed crystallographically for the Ag<sup>I</sup> complex of 10-methylisoalloxazine<sup>8</sup> and for the Zn<sup>II</sup> complex of N<sup>3</sup>-Et-TARF.<sup>9</sup> In solution, when the packing forces vanish, the Ag<sup>I</sup> complex may prefer a tetrahedral structure similar to that expected for the Cu<sup>I</sup> complexes. Such a structure for the monovalent ion complexes in acetone is indicated (Figure 11) by a positive shift for the CH<sub>3</sub>(7) resonance induced by





**Figure 11.** PMR titration curves for the  $\text{Cu}^{\text{I}}$  complexes with TARF,  $3 \times 10^{-2} \text{ M}$ , in acetone- $d_6$ . Note the positive shift of  $\text{CH}_3(7)$  at low metal concentration where the bis complex is formed.

intermolecular ring current effects and observed only in the presence of an excess of flavine when the bis complex is exclusively formed. The models show that this intermolecular effect should also be present at H(6) but it is masked by the opposite intramolecular interactions.

### Conclusions

The constants of complex formation measured for the various metal ions have only relative value since they are strongly dependent upon the water content of the solutions and upon the polarity of the dry solvent. Nevertheless, it appears that the affinity of  $\text{RFl}_{\text{ox}}$  for most of these ions slightly exceeds the hydration energy except for monovalent  $\text{Ag}^{\text{I}}$  and  $\text{Cu}^{\text{I}}$  ions which can complex  $\text{RFl}_{\text{ox}}$  even in aqueous solution.<sup>1</sup> The present investigation in aprotic solvents has confirmed the conclusions drawn from preliminary chemical data and optical spectra<sup>2</sup> that the monovalent ions as well as most divalent ions form bidentate chelates at  $\text{O}(4\alpha)\text{-N}(5)$  of flavoquinone as does flavosemiquinone.<sup>4</sup> It has furthermore provided a precise stoichiometry of complexation and shown that the strongest interaction occurs at the  $\text{O}(4\alpha)$  carbonyl group in agreement with available crystallographic data.<sup>8,9</sup> The existence of a second site of coordination, also observed in the  $\text{Ag}^{\text{I}}$  crystals,<sup>8</sup> was found in solution for most ions and appeared to be the only site of coordination for the less polarizable ions  $\text{Mg}^{\text{II}}$ ,  $\text{Mn}^{\text{II}}$ , and  $\text{Fe}^{\text{III}}$  or in the case where the access at  $\text{N}(5)$  is sterically hindered.

It is significant from the biological point of view that the  $\text{Fe}^{\text{II}}$  complexes, for which the measured formation constants are perhaps somewhat underestimated due to partial oxidation, are stable bidentate complexes, in contrast to the  $\text{Fe}^{\text{III}}$  complexes which are labile and monodentate. The oxidation state of the flavines in the ferrous complex, as well as that in the  $\text{Ag}^{\text{I}}$  or  $\text{Cu}^{\text{I}}$  complexes, is clearly that of a flavoquinone since the PMR behavior of these complexes is qualitatively identical with that of the complexes formed with valence-stable ions. This rules out any significant charge transfer from the metal ion to the  $\text{RFl}_{\text{ox}}$  ligand. This is also true for  $\text{R} = \text{H}$  under all conditions of pH where the  $\text{NH}(3)$  proton is not released

as can be shown by diluting the acetone solution of  $\text{AgHFl}_{\text{ox}}^+$  with  $\text{D}_2\text{O}$ , which has no essential influence upon the PMR spectrum. However, severe broadening of the ligand proton resonances occurs upon deprotonation, without line shape alteration of the acetone or water resonances. This suggests that paramagnetic perturbations occur in the neutral complex  $\text{AgFl}_{\text{ox}}$  since the solubility of the complex is not altered. This could correspond to the charge-transfer structure proposed by Hemmerich et al.,<sup>1,2</sup>  $[\text{Ag}^{\text{I}}\text{Fl}_{\text{ox}} \leftrightarrow \text{Ag}^{\text{II}}\text{Fl}]$ . A first possibility is that the neutral complex has a diamagnetic ground state and a low-lying excited triplet state which could be populated thermally. On the other hand, the formation of some semi-quinone  $\text{Fl}^-$  at low concentration but involved in fast-exchange phenomena with the chelated flavines could also take into account the observed broadening.

The present study makes it clear, in any case, that metal-flavine electron transfer cannot involve the presently described complexes  $\text{M}(\text{RFl}_{\text{ox}})_{n^{m+}}$ . As a consequence for biological electron transfer, the following alternative is established by this fact: either N(3) is an "active site" of the flavine in flavine-mediated one-electron transfer, the acidobasic properties of which are being controlled by protein interactions within the redox-active enzymes, or the biological one-electron transfer does not concern oxidized flavine at all, but only the half-reduced and the fully reduced state. Since most known flavoproteins are not—or at least not totally—inactivated upon replacement of the natural  $\text{HFl}_{\text{ox}}$  by an artificial  $\text{RFl}_{\text{ox}}$ , the latter assumption becomes most probable.

The properties of the chelates presently described provide, however, a direct means toward the electronic and sterically fine interactions in the metal-coordinated flavines, in particular by allowing the evaluation of the electron-nuclear spin interactions in the paramagnetic chelates to be reported in the following paper.<sup>10</sup>

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**Registry No.**  $\text{Cu}(\text{TARF})_2^{2+}$ , 55700-25-9;  $\text{Ni}(\text{TARF})_2^{2+}$ , 55648-64-1;  $\text{Ag}(\text{TARF})_2^{2+}$ , 55648-70-9;  $\text{Co}(\text{TARF})_3^{2+}$ , 55648-62-9;  $\text{Cu}(\text{TARF})_2^{2+}$ , 55648-66-3;  $\text{Zn}(\text{TARF})_3^{2+}$ , 55648-68-5;  $\text{Cd}(\text{TARF})_3^{2+}$ , 55648-69-6;  $\text{Fe}(\text{TARF})_3^{2+}$ , 55648-60-7;  $\text{Mn}(\text{TARF})_2^{2+}(\text{H}_3\text{CC}(\text{O})\text{CH}_3)_5$ , 55638-28-3;  $\text{Mg}(\text{TARF})_2^{2+}(\text{H}_3\text{CC}(\text{O})\text{CH}_3)_5$ , 55648-71-0;  $\text{Fe}(\text{TARF})_3^{3+}(\text{H}_3\text{CC}(\text{O})\text{CH}_3)_5$ , 55648-72-1;  $\text{Ag}_2(\text{TARF})_2^{2+}$ , 55648-73-2; TARF, 752-13-6;  $\text{Co}(\text{N}^3\text{-Et-TARF})_3^{2+}$ , 55648-67-4;  $(\text{C}_{27}\text{H}_{32}\text{N}_4\text{O}_{10}\text{ClO}_4\text{H}_2\text{O})_2\text{Zn}$ , 40309-90-8;  $(\text{C}_{27}\text{H}_{32}\text{N}_4\text{O}_{10}\text{ClO}_4\text{H}_2\text{O})_2\text{Co}$ , 55648-75-4;  $(\text{C}_{27}\text{H}_{32}\text{N}_4\text{O}_{10}\text{ClO}_4\text{H}_2\text{O})_2\text{Ni}$ , 55648-77-6; iTARF, 27857-40-5; Zn, 7440-66-6.

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## Flavoquinone-Metal Complexes. II. Paramagnetic Interactions

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Proton magnetic resonance studies were carried out on flavoquinone complexes using divalent paramagnetic ions of the 3d series: Mn<sup>II</sup>, Fe<sup>II</sup>, Co<sup>II</sup>, Ni<sup>II</sup>, Cu<sup>II</sup>. Magnetic susceptibility and electron spin resonance measurements provided complementary information about the magnetic state of these complexes. The paramagnetic contribution to the line width of the ligand resonances was analyzed as a function of metal to flavine concentration and of temperature in order to estimate the value of the correlation times involved in the relaxation processes. The isotropic shifts were analyzed in terms of contact and pseudocontact interactions using the relaxation data and structural information derived earlier, together with the *g*-tensor anisotropy measured by electron spin resonance. The relative importance of the two kinds of interactions compares favorably with that derived from the usual factorization procedure. Pseudocontact shifts appear noticeable in Co<sup>II</sup> and Cu<sup>II</sup> complexes and negligible in Ni<sup>II</sup> and Fe<sup>II</sup> complexes. The contact shifts exhibit a distribution pattern for the various ligand resonances very similar among the stable bidentate chelates, which could be attributed to a  $\pi$ -electron spin negatively polarized in the ligand orbitals. The spin-transfer mechanism appears to result from indirect  $\sigma$ - $\pi$  spin polarization at the coordination site combined with a delocalization of negatively polarized spin in the ligand  $\pi$ -bonding orbitals. This mechanism for contact interaction indicates that the stability of the flavoquinone-metal complexes in aprotic solvents arises from  $\sigma$ -type bonding, with little, if any,  $\pi$  bonding.

### Introduction

The stability, stoichiometry, and geometry of flavoquinone-metal chelates  $M(\text{RFl}_{\text{ox}})_{n^{m+1}}$  have been outlined in the preceding paper.<sup>2</sup> In this paper we present the results of a proton magnetic resonance (PMR) study of such chelates containing paramagnetic ions of the first transition series: Mn<sup>II</sup>, Fe<sup>II</sup>, Co<sup>II</sup>, Ni<sup>II</sup>, Cu<sup>II</sup>. The isotropic shifts and line broadening observed in the PMR spectrum of RFl<sub>ox</sub> in solution upon addition of paramagnetic ions arise from contact Fermi and from pseudocontact through-space interaction of the electron and nuclear spin.<sup>3</sup> The analysis of the contact interactions can provide a direct insight into the nature of the organometallic bond if a quantitative estimation of the pseudocontact interactions can be carried out.<sup>4</sup> Such an estimation is facilitated for the flavoquinone chelates since their geometry has been derived from analysis of the diamagnetic interactions<sup>2</sup> and since the anisotropy of the magnetic susceptibility can be measured by electron spin resonance (ESR) spectroscopy. PMR of paramagnetic complexes in solution further depends upon the dynamic conditions of relaxation which should first be investigated by line width analysis.

### Experimental Section

The PMR spectra were recorded at 60 MHz using a Varian A-60A spectrometer equipped with the variable-temperature accessory V-6040. The faster nuclear relaxation in the paramagnetic complexes permitted use of intense radiofrequency fields without saturation effects in conditions of slow passage. All spectra were referenced to internal TMS. Magnetic susceptibility measurements were carried out by PMR methods in axial double-walled tubes following the method reported by Evans.<sup>5</sup> The applicability of the method was checked

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Table I. Magnetic Data of Flavoquinone-Metal Complexes

Compd	$S^a$	$\mu_{\text{eff}}^b$	$10^6 \chi_M^c$	$\mu_{\text{eff}}^d$
Fe(TARF) <sub>3</sub> (ClO <sub>4</sub> ) <sub>2</sub>	2	4.90	10,600	5.17
Fe(H <sub>2</sub> O) <sub>6</sub> (ClO <sub>4</sub> ) <sub>2</sub>			10,980	5.25
Co(TARF) <sub>3</sub> (ClO <sub>4</sub> ) <sub>2</sub>	3/2	3.87	8,250	4.57
Co(H <sub>2</sub> O) <sub>6</sub> (ClO <sub>4</sub> ) <sub>2</sub>			9,850	5.00
Ni(TARF) <sub>3</sub> (ClO <sub>4</sub> ) <sub>2</sub>	1	2.83	3,800	3.16
Ni(H <sub>2</sub> O) <sub>6</sub> (ClO <sub>4</sub> ) <sub>2</sub>			4,340	3.30

<sup>a</sup> Spin quantum number of the ground state. <sup>b</sup> Effective magnetic moments (BM) as calculated from  $2[S(S+1)]^{1/2}$ .

<sup>c</sup> Molar susceptibilities (cgs units) as determined by PMR at 38° in acetone-*d*<sub>6</sub>. <sup>d</sup> Effective magnetic moments (BM) calculated from the measured  $\chi_M$  values assuming that the Curie law is obeyed.

by measuring the susceptibility of the hexaquo complexes of the corresponding metal ions. A Varian Model V-4500 X-band spectrometer operating at temperatures down to 4°K was used for the ESR measurements.

The solvents and the solutes used throughout this investigation as well as the sampling procedures have been described previously.<sup>2</sup>

3-Benzylflumiflavine, 3-benzyl-8-norlumiflavine, and 3-benzyl-7,8-norlumiflavine were synthesized according to known procedures.<sup>6</sup>

A crystalline Cu<sup>II</sup> complex with N<sup>3</sup>-ethyltetraacetylriboflavine was obtained in the same manner as described earlier for the Co<sup>II</sup>, Ni<sup>II</sup>, and Zn<sup>II</sup> complexes.<sup>2</sup>

Anal. Calcd for (C<sub>27</sub>H<sub>32</sub>N<sub>4</sub>O<sub>10</sub>ClO<sub>4</sub>H<sub>2</sub>O)<sub>2</sub>Cu: C, 45.0; H, 4.8; N, 7.8. Found: C, 45.3; H, 4.8; N, 7.9.

### Results and Discussion

**Magnetic Susceptibility and Electron Spin Resonance.** The molar magnetic susceptibilities of dilute solutions of tetraacetylriboflavine (TARF, I) in acetone-*d*<sub>6</sub>, measured as a function of the metal to flavine ratio, are reported in Figure 1 for the Fe<sup>II</sup>, Co<sup>II</sup>, and Ni<sup>II</sup> complexes. The susceptibility