

A Spectrometric Study of the Chelating Properties of 6-Galactosyl-Rubrofusarin: Mg(II), Al(III), Fe(III), Ni(II) and Cu(II) Complexes

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Received: February 24, 1995; July 14, 1995

As propriedades quelantes do derivado galactosilado da rubrofusarina (2-metil-5,6-dihidroxi-6-O- β -D-galactosil-8-metoxi-nafto- γ -pirona, Rfg) foram estudadas em solução aquosa, através de medidas de espectros de absorção e de dicroísmo circular. Como se pode inferir pelos espectros de dicroísmo circular, dependendo da concentração o ligante em meio ácido pode existir sob a forma monomérica ou como um dímero de conformação direita. A auto-associação se desfaz em meio básico ou pela coordenação, sugerindo o envolvimento do próton fenólico em ligações de hidrogênio intermoleculares. Determinaram-se as constantes de acidez e de associação do ligante, bem como as constantes de formação dos complexos de Mg(II), Al(III), Fe(III), Ni(II) e Cu(II). O íon Mg(II) a pH 7,2, o íon Al(III) a pH 4,0 e o íon Fe(III) a pH 1,0 formam compostos do tipo ML, enquanto Cu(II) a pH 9,8 e Ni(II) a pH 11,4 formam espécies do tipo ML₂. Pela coordenação ao Fe(III) ou ao Cu(II) são formados complexos de conformação "gauche".

The chelating properties of the galactosyl derivative of rubrofusarin (2-methyl-5,6-dihydroxy-6-O- β -D-galactosyl-8-methoxy-naphto- γ -pyrone, Rfg) have been studied in aqueous solution using absorption and circular dichroism measurements. As shown by the CD spectra, depending on the concentration, the ligand exists either in the monomeric or in an associated form of right chirality in acidic media. The self association is disrupted in basic solution or upon coordination, suggesting the involvement of the phenolic proton in intermolecular hydrogen bonding. The acidity as well as the association constants of the ligand were determined. The formation constants of the Mg(II), Al(III), Fe(III), Ni(II) and Cu(II) complexes were calculated. Mg(II) at pH 7.2, Al(III) at pH 4.0, and Fe(III) at pH 1.0 form only one-to-one metal-to-ligand species, whereas one-to-two species were identified for Cu(II) at pH 9.8, and Ni(II) at pH 11.4. By coordination to either Fe(III) or Cu(II) a complex in the left-handed screw conformation is formed.

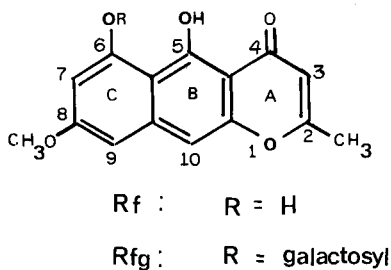
Keywords: rubrofusarin, metal complexes, molecular association, conformations

Introduction

Rubrofusarin (2-methyl-5,6-dihydroxy-8-methoxy-naphto- γ -pyrone), Rf hereafter, is a metabolic product of the fungus *Fusarium culmorum*¹, the structure of which was determined by Stout and Jensen². It was isolated for the first time from the seeds of the leguminosae, *Cassia tora* L, by Rangaswami³. More recently Oliveira *et al.* obtained the rubrofusarin glycoside (2-methyl-5,6-dihydroxy-6-O-

β -D-galactosyl-8-methoxy-naphto- γ -pyrone), Rfg hereafter, along with the aglycone, from the softwood of the Brazilian leguminosae, *Cassia macranthera* D.C⁴.

Rf and Rfg (see Scheme 1) are potential chelators, able to form six-membered ring complexes by coordination to metals through the carbonyl and phenolate oxygens at C4 and C5 respectively. In addition, Rf contains another potential phenolate binding site at C6. In a previous report⁵ the Ni(II) and Cu(II) complexes of Rf and Rfg were isolated and charac-



Scheme 1.

terized in the solid state. As we have shown⁵, Rf and Rfg coordinate to metals through the C4 and C5 oxygens.

In this report we present a spectroscopic investigation of the Mg(II), Al(III), Fe(III), Ni(II) and Cu(II) interactions with Rfg in aqueous solution using absorption and circular dichroism (CD) measurements.

Experimental

Apparatus

A Diode Array Hewlett Packard 8451 A spectrometer, equipped with a Masterline 2095 thermostat at 25 °C, and a Jobin Yvon Mark V dichrograph were used for UV visible absorption and CD measurements, respectively. The results are expressed in terms of the molar absorption coefficient and molar CD coefficient $\Delta\epsilon = \epsilon_l - \epsilon_r$. The values of both coefficients are related to the total concentration of the ligand. The pH measurements were obtained on a Methrom 633 titroprocessor equipped with a Methrom EA 147 combined glass electrode.

Chemicals

Rfg was kindly supplied by Professor Alaide Braga de Oliveira. Concentrations were determined spectroscopically using a molar absorption coefficient at 343 nm of $1794 \text{ M}^{-1} \text{ cm}^{-1}$. All other chemicals were of analytical quality.

Solutions and titration procedures

Doubly distilled water passed through a Milli Q reagent water system (Millipore Co.) was used throughout. The Mg(II), Ni(II) and Cu(II) solutions were prepared by dissolving $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Ni}(\text{ClO}_4)$ or $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ in water; the Fe(III) solution was obtained by dissolving $\text{Fe}(\text{ClO}_4)_3 \cdot 9\text{H}_2\text{O}$ in 0.1 M HClO_4 , and the Al(III) solution by dissolving $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ in water containing 0.1% HCl. All the metal ion concentrations were determined by titration with ethylenediaminetetracetic acid (EDTA).

The very low solubility of Rfg and its metal complexes in aqueous media precluded the determination of the formation constants by potentiometric methods. Therefore, the concentration stability constants $\beta_{pqr} = [\text{M}_p \text{A}_q \text{H}_r] / [\text{M}]^p [\text{A}]^q [\text{H}]^r$ were calculated from the absorption titration curves by means of the SQUAD⁶ program. At least three

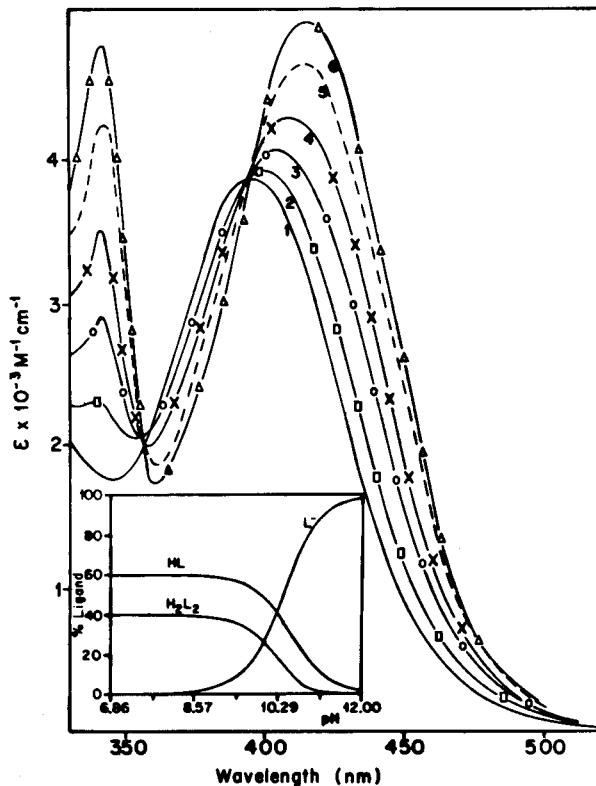


Figure 1. Absorption spectra of an aqueous solution of Rfg $1.5 \times 10^{-4} \text{ M}$, ionic strength $I = 0.1 \text{ M}$, at different pHs: 6.5(1), 9.4(2), 9.9(3), 10.3(4), 10.9(5) and 11.9(6). Inset: species distribution curves for Rfg: percentage of the H_2L_2 ; HL and L^- species against pH.

titration experiments were analyzed separately. In all cases fifty absorbances were measured per spectrum. The species distribution curves were calculated by the SCECS program⁷.

Results and Discussion

Proton binding

As we have previously reported⁵, and as shown in Fig. 1, the absorption spectrum of Rfg in aqueous solution at pH 9.4 exhibits one strong band at 400 nm and a very weak one at 340 nm which may be assigned, following Platt's notation⁸, to the ${}^1\text{A}_1 \rightarrow \text{L}_b$ and ${}^1\text{A}_1 \rightarrow \text{L}_a$ transitions of the condensed aromatic system, respectively. The CD spectrum in this region, illustrated in Fig. 2A, curve 1, displays two Cotton effects of opposite signs at 432 nm ($\Delta\epsilon = +0.28 \text{ M}^{-1} \text{ cm}^{-1}$) and at 378 nm ($\Delta\epsilon = -0.3 \text{ M}^{-1} \text{ cm}^{-1}$) centered at 400 nm. The presence of this couplet type signal reveals the existence of a molecular association of positive chirality⁹. In ethanol media this self association disappears and the CD spectrum in this region presents only one negative band at 394 nm ($\Delta\epsilon = -0.55 \text{ M}^{-1} \text{ cm}^{-1}$), corresponding to the absorption maximum at 398 nm (Fig. 2A, curve 2). This

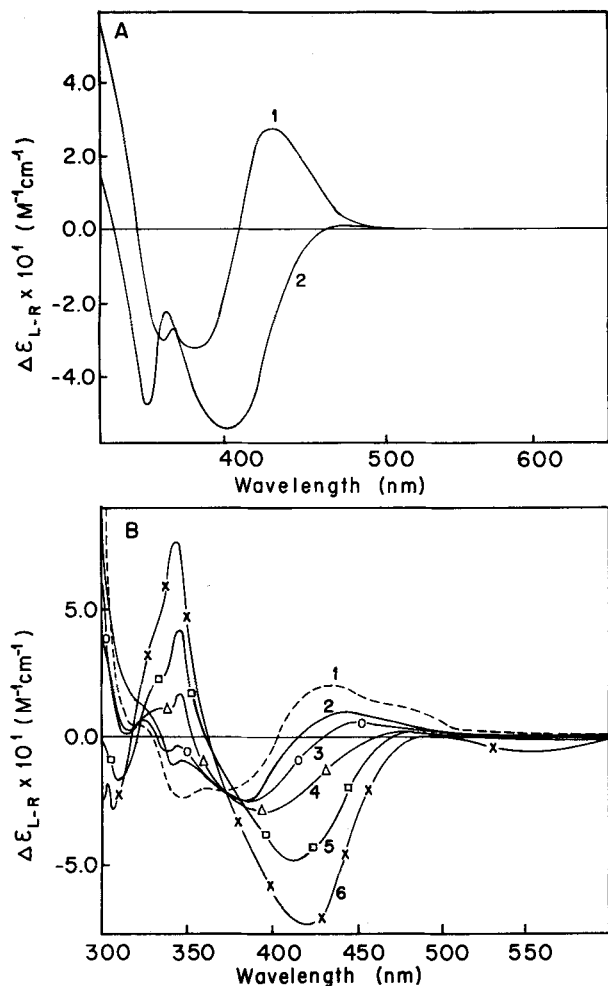


Figure 2. A) CD spectra of Rfg 5.0×10^{-4} M, pH 6.5 in aqueous solution, (curve 1), and in ethanol solution (curve 2); B) CD spectra of Rfg 4.4×10^{-4} M, $I = 0.1$ M at different pHs: 6.0(1), 9.7(2), 10.0(3), 10.4(4), 10.9(5), and 12.2(6).

spectrum is characteristic of the protonated monomeric form (HL, $pK_a = 11.30 \pm 0.08^5$).

Upon deprotonation the absorption band at 398 nm shifts to 420 nm, and a maximum at 340 nm appears (Fig. 1). At pH 12 the CD spectral pattern is characterized by a negative band at 418 nm ($\Delta\epsilon = -0.75 \text{ M}^{-1} \text{ cm}^{-1}$) and a positive one at 346 nm ($\Delta\epsilon = +0.77 \text{ M}^{-1} \text{ cm}^{-1}$, see Fig. 2B, curve 6). The couplet-type signal is no longer observed, indicating that the self association is disrupted at this pH. Since the stacking of monomers is disrupted as the pH increases and Rfg deprotonates, we may infer that the phenolic proton participates in intermolecular hydrogen bonding.

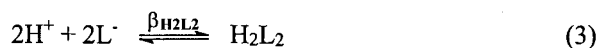
The formation constant of the dimer ($\log \beta_{H_2L_2}$) and the acidity constant of the monomer ($\log \beta_{HL}$) were calculated from spectrophotometric data (Fig. 1) using the SQUAD program⁶. We found $\log \beta_{H_2L_2} = 24.21 \pm 0.03$, and \log

Table 1. Formation constants of the proton and metal - Rfg systems.

System	Complex Stoichiometry	$\log \beta^{(1)}$
proton-Rfg	H_2L_2	24.21 ± 0.03
	HL	10.21 ± 0.01
Mg(II)-Rfg	ML^+	3.97 ± 0.03
Al(III)-Rfg	ML^{2+}	8.91 ± 0.01
Fe(III)-Rfg	$[M_2L_2]^{4+}$	29.38 ± 0.02
Ni(II)-Rfg	ML_2	12.08 ± 0.15
Cu(II)-Rfg	ML_2	12.95 ± 0.04

(1) Values obtained by analyzing at least three experiments separately.

$\beta_{HL} = 10.21 \pm 0.01$. Taking into consideration the following equilibria:



the association constant of HL ($\log K_{ass} = 3.79 \pm 0.04$) was estimated (Table 1). The species distribution curves against pH, calculated at the ligand concentration used in the spectral study (1.5×10^{-4} M), are shown in Fig. 1 (inset). At $pH \leq 7 \approx 60\%$ of the ligand exists as the protonated monomer (HL) and $\approx 40\%$ as the associated H_2L_2 form. For concentrations below 1×10^{-5} M, 95% of Rfg is present as the protonated monomeric form, HL.

Metal Binding

The Mg(II)-Rfg interaction in aqueous solution

In a 1×10^{-4} M aqueous solution at pH 7.2, 65% of the ligand exists as the protonated monomeric form, and 35% as the protonated dimer. By the addition of increasing concentrations of Mg(II) at molar ratios $[Mg(II)]:[Rfg]$ ranging from 0 to 2000, the absorption at 398 nm shifts to 420 nm and that at 340 nm it is enhanced, indicating coordination to the chromophore (see Fig. 3).

The absorption data in the visible range were used in the calculation of the complex formation constant by means of the SQUAD program⁶. The best fitted results were obtained by assuming the formation of only the $[ML]$ species. For the sake of clarity the charges were omitted in this text. We found $\log \beta_{ML} = 3.97 \pm 0.03$. The species distribution against the total metal concentration (not shown) indicates that even in the presence of a great excess of metal, only 73% of the ligand exists under the complexed form.

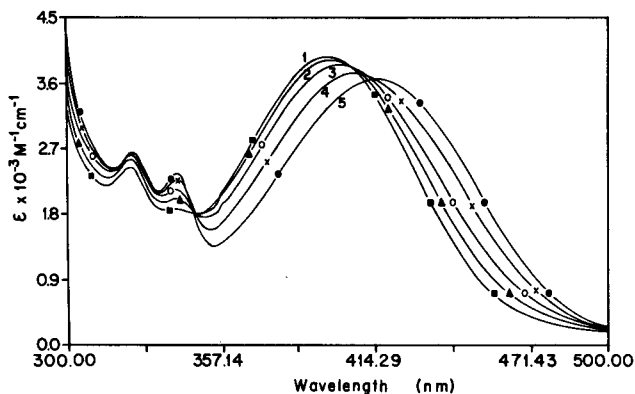


Figure 3. Absorption spectra of Rfg 9.9×10^{-5} M in HEPES buffer, pH = 7.25, I = 0.1 M (curve 1), and at increasing Mg(II) concentrations (curves 2 to 5): [Mg(II)]/[Rfg] = 150; 400; 950 and 2000).

The Al(III)-Rfg interaction in aqueous solution

The interaction of Rfg with Al(III) was studied in aqueous solution at pH 4.0 to minimize the formation of hydroxylated species.

In a 2×10^{-4} M aqueous solution at pH 4.0 almost 50% of the ligand is present as the monomer and $\approx 50\%$ as the associated form. By the addition of increasing concentrations of Al(III) at molar ratios [Al(III)]:[Rfg] ranging from 0 to 81 the absorption at 398 nm shifts to 438 nm, and that at 328 nm is enhanced, indicating complexation (Fig. 4).

In the CD spectrum the couplet-type CD signal disappears, suggesting that by coordination the molecular association of the ligand is disrupted (Fig. 4, inset).

As in the previous case, the absorption data were used in the calculation of the complex formation constant by means of the SQUAD program⁶. For the Al(III) complexes with acetic acid (HAc), the stability constants ($\log \beta$) assumed were: 1.505 for $[\text{Al}(\text{Ac})]^{2+}$, -1.569 for $[\text{Al}(\text{OH})(\text{Ac})]^+$ and 4.54 for HAc¹⁰. The best fitted results were obtained by assuming the formation of only the ML species. We found $\log \beta = 8.91 \pm 0.01$. The species distribution curves against the total metal concentration indicate that, even in the presence of a high quantity of metal ion, only 55% of the ligand is under the complexed form.

The Fe(III)-Rfg interaction in aqueous solution

This study was performed at pH 1.0 to avoid the formation of Fe(III) hydroxydes.

As stated earlier, at $\text{pH} \leq 7$ and 1×10^{-4} M, 60% of the ligand exists as the protonated monomeric form and 40% as the protonated dimer. By increasing the concentration of Fe(III) in molar ratios [Fe(III)]:[Rfg] ranging from 0 to 8.3 the intensity of the absorption at 398 nm decreases and a shoulder appears at 480 nm ($\epsilon = 1325 \text{ M}^{-1} \text{ cm}^{-1}$, Fig. 5) assigned to a ligand-to-metal charge transfer (LMCT) transition from the π phenolate orbital to the partially filled $d\pi^*$ orbital of Fe(III)¹¹⁻¹⁴. For higher excesses of Fe(III) no

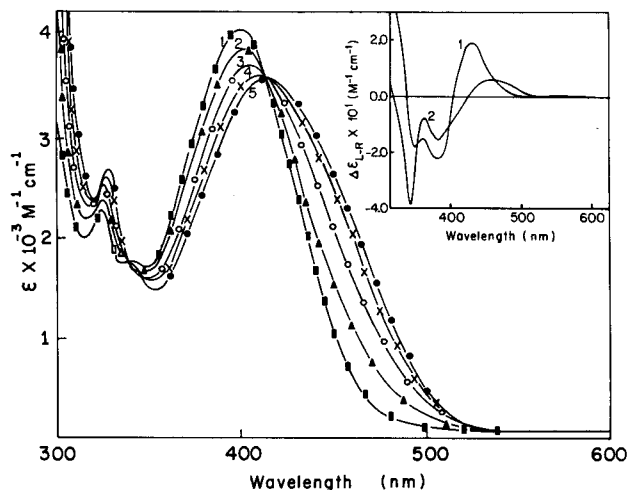


Figure 4. Absorption spectra of Rfg 2.1×10^{-4} M at pH 4.0 in acetate buffer, I = 0.1 M (curve 1), and at increasing Al(III) concentrations (curves 2 to 4): [Al(III)]/[Rfg] = 23, 29, 36, 81. Inset: CD spectra of Rfg 2.0×10^{-4} M in acetate buffer, I = 0.1 M (curve 1) and [Al(III)]/[Rfg] = 60 (curve 2).

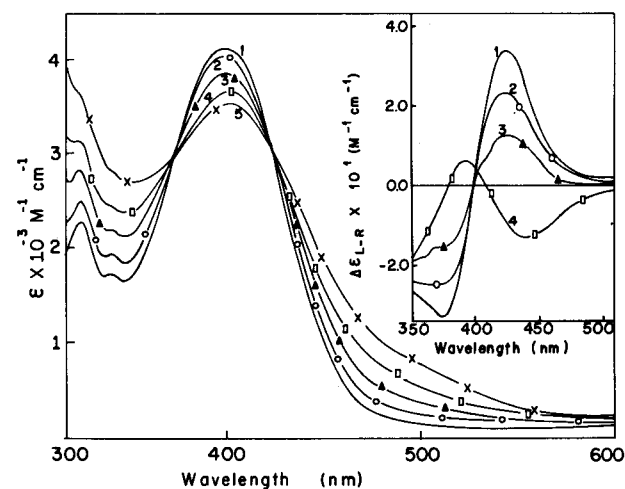


Figure 5. Absorption spectra of Rfg 1.0×10^{-4} M, pH 1.0, HClO₄ 0.1 M (curve 1) and at increasing Fe(III) concentrations (curves 2 to 5): [Fe(III)]/[Rfg] = 2.5, 3.8, 6.4, 8.3. Inset: CD spectra of Rfg 5.0×10^{-4} M, pH 1.0, HClO₄ 0.1 M (curve 1) and at increasing Fe(III) concentrations (curves 2 to 4): [Fe(III)]/[Rfg] = 4, 8 and 14.

variations of the absorption spectrum were observed. The best fit between the experimental and calculated spectra was obtained by assuming the formation of only the M_2L_2 species. We found $\log \beta = 29.38 \pm 0.02$.

Indeed, as shown in Fig. 5 (inset), by the addition of Fe(III) the intensity of the double Cotton effect of positive chirality centered at 400 nm in the CD spectrum decreases progressively, the chirality of the signal being reversed for high metal concentration ([Fe(III)]:[Rfg] = 14). The couplet-type signal of the complex suggests that Fe(III) coordinates to Rfg forming a dimer (M_2L_2) of negative chirality.

The Ni(II)-Rfg interaction

The interaction of Rfg with Ni(II) was studied in aqueous solution at pH 11.5 to avoid the precipitation of Ni(OH)₂. By addition of increasing concentrations of Ni(II) to a 1 × 10⁻⁴ M solution of Rfg in molar ratios [Ni(II)]/[Rfg] ranging from 0 to 0.2, the absorption maximum at 420 nm shifts to 430 nm with a decrease in intensity. For higher metal concentrations the formation of a precipitate is observed.

As in the previous instances the concentration stability constants were calculated from absorbance data by means of the SQUAD program⁶. For the hydroxo complexes of Ni(II) the stability constants (log β) assumed were -9.4 for [Ni(OH)]⁺, -24.0 for Ni(OH)₂, -31.8 for [Ni(OH)₃], and -43.0 for [Ni(OH)₄]²⁻¹⁵.

The best fit between the experimental and calculated spectra was obtained by assuming the formation of only the ML₂ species. We found log β = 12.08 ± 0.15.

The Cu(II)-Rfg interaction

The interaction of Cu(II) with Rfg was performed in aqueous solution at pH 9.8. At this pH, in a 1 × 10⁻⁴ M solution, 25% of the ligand exists as the protonated dimer, 20% as the protonated monomer, and 55% as the deprotonated monomeric form. By the addition of increasing concentrations of Cu(II) at molar ratios [Cu(II)]:[Rfg] ranging from 0 to 0.5, a decrease in the intensity of the maximum at 402 nm with a bathochromic shift to 450 nm is observed (Fig. 6). In the CD spectrum a positive Cotton effect (Δε = +1.6 M⁻¹ cm⁻¹) becomes noticeable at [Cu(II)]:[Rfg] = 0.3, indicating the formation of a first complex. For higher metal concentrations the spectral pattern changes greatly, an intense couplet-type CD signal with a negative peak at 497 nm (Δε = -2 M⁻¹ cm⁻¹) and a positive one at 447 nm (Δε = +5 M⁻¹ cm⁻¹) being observed for a molar ratio equal to 0.5. This spectral feature suggests the presence of an associated species of negative chirality (Fig. 6, inset).

As in the previous cases the absorbance data were used in the calculation of the complex formation constant by means of the SQUAD program⁶. The Cu(II) hydroxo stability constants (log β) assumed were -7.75 for [Cu(OH)]⁺ and -10.49 for [Cu₂(OH)₂]²⁺¹⁵. Convergence was only achieved by analyzing the spectral data for molar ratios ranging from 0 to 0.3. The best fit between the experimental and calculated curves was obtained by assuming the formation of the ML₂ species. We found log β = 12.95 ± 0.04. For higher molar ratios the absorption data could not be treated and the formation constant of the associated species could not be determined due to the very feeble spectral variations observed.

As expected on the basis of the lower ionic radius and higher charge, the Al(III) complex formation constant is greater than that of the Mg(II) complex. Furthermore, the

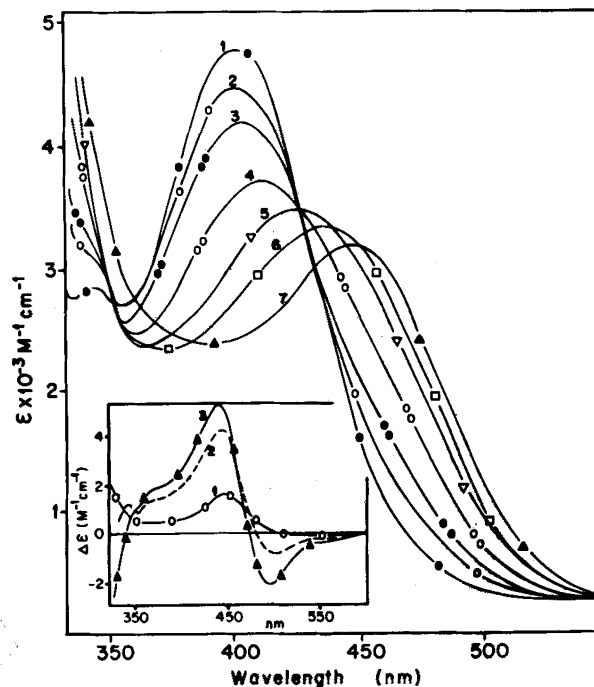


Figure 6. Absorption spectra of Rfg 1.8 × 10⁻⁴ M, borax buffer, I = 0.05 M, pH 9.8 (curve 1), and at increasing Cu(II) concentrations (curves 2 to 7): [Cu(II)]/[Rfg] = 0.05, 0.1, 0.2, 0.3, 0.5 and 1.0. Inset: CD spectra of the Cu(II) complexes of Rfg in borax buffer, I = 0.05 M, pH = 9.9. [Rfg] = 3.23 × 10⁻⁴ M. Curves 1 to 3: [Cu(II)]/[Rfg] = 0.3, 0.5 and 0.6.

Ni(II) complex formation constant is lower than that of the Cu(II) complex, in agreement with the Irving Williams stability series¹⁶.

As mentioned before, the appearance of a new band at 480 nm in the absorption spectrum of the Fe(III) complex was attributed to a LMCT transition. In fact, high spin Fe(III) is a totally symmetrical octahedral d⁵ ion of ground state A_{1g}. Although the d-d transitions are spin forbidden in this case, the ion is a good π acceptor. Therefore, not infrequently the absorption spectra of its complexes display bands arising from π - dπ* transitions. Moreover, phenolate complexes of transition metals exhibit very strong absorptions in their visible or near UV spectra (390-500 nm), which in the case of Fe(III) are assigned to ligand-to-metal charge transfer (LMCT) transitions from the pπ orbitals of the phenolate oxygen to the dπ* metal orbital¹¹⁻¹⁴. Indeed, the value of ε = 1325 M⁻¹ cm⁻¹ at 480 nm is too high to be attributed to a d-d transition and must be assigned to a LMCT transition from the pπ phenolate orbital to the partially filled dπ* orbital of Fe(III)¹¹.

A similar situation occurs in the case of either the Cu(II) or Ni(II) phenolate complexes in which LMCT transitions must arise from the pπ phenolate oxygen orbitals to the dσ* metal orbital. As such, in the spectra of Fig. 6 the band lying at 435 nm may result from the overlapping of the ligand transition at 402 nm (at pH 9.8) and the LMCT

transition. In the absorption spectrum of the Ni(II) complex (not shown) the band at 425 nm is approximately the envelope of the ligand and the LMCT transitions. Moreover, the LMCT energies of the Fe(III), Cu(II) and Ni(II) complexes follow the order of the reducing ability of those cations¹⁶.

In addition, the CD spectra provided very useful information on the presence and the chirality of the molecular associations. As stated before, Rfg can exist as a dimer in the right-handed screw conformation. By coordination to either Fe(III) or Cu(II) a complex in the left-handed screw conformation is formed.

Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We are indebted to Professor A. Garnier-Suillerot for CD facilities at the Laboratoire de Chimie Bioinorganique, Université Paris XIII, and to Professor E. Paniago for helpful suggestions and discussions. E.C. Pereira and C. Demicheli acknowledge fellowships from CNPq and CAPES.

References

1. J.N. Ashley, B.C. Hobbs and H. Raistrick, *Biochem. J.* **31**, 385 (1937).
2. G.H. Stout and L.H. Jensen, *Acta Cryst.* **15**, 451 (1962).
3. S. Rangaswami, *Proc. Indian. Acad. Sci.* **A57**, 88 (1963).
4. A.B. de Oliveira, M. de L.M. Fernandes, V.T. Shaat, I.A. de Vasconcelos and O.R. Gottlieb, *Rev. Latinoamer. Quim.* **8**, 82 (1977).
5. C.P. Demicheli, H. Beraldo and L. Tosi, *J. Braz. Chem. Soc.* **3**, 52 (1992).
6. D.J. Leggett, *Anal. Chem.* **47**, 1065 (1975).
7. H.A. Duarte, S. Carvalho, F.F. Campos, E.B. Paniago, *Química Nova* **17**, 397 (1994).
8. J.R. Platt, *J. Chem. Phys.* **17**, 484 (1949); *ibid.* **18**, 1168 (1950).
9. P.M. Bayley, *Progr. Biophys. Mol. Biol.* **27**, 3 (1973).
10. A.S. Kereichuk and L.M. II'icheva, *Russ. J. Inorg. Chem.* **21**(2), 205, 1976.
11. B.P. Gabor, V. Miskowski and T.G. Spiro, *J. Am. Chem. Soc.* **96**, 6868 (1974).
12. E.W. Ainscough, A.W. Brodie, J.E. Plowman, K.L. Brown, A.W. Addison and A.R. Gainsford, *Inorg. Chem.* **19**, 3655 (1980).
13. E.W. Ainscough, A.G. Bingman, A.M. Brodie, J.M. Husbands, and J.E. Plowman, *J. Chem. Soc., Dalton Trans.* 1701 (1981).
14. A. Garnier-Suillerot, J.P. Albertini, A. Collet, L. Faury, J.M. Pastor, and L. Tosi, *J. Chem. Soc., Dalton Trans.* 2544 (1981).
15. L.G. Sillén, *Stability Constants of Metal-Ion-Complexes* (The Chemical Society, London, 1964), Special Publication, vol. 17.
16. J.E. Huheey, E.A. Keiter, R.L. Keiter, *Inorganic Chemistry. Principles of Structure and Reactivity* Edition (Harper Collins College Publishers, 4th ed., 1993), p. 344, 596.