

Reactivity of Pyrrole Pigments, XXI [1]: Structure and Reactivity of Cu(II) and Zn(II) Bilindione Chelates

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Summary. Several Mn, Cu, and Zn chelates of etiobiliverdin-IV- γ , mesobiliverdin-IX α , and its dimethyl ester are studied. The results show that the chemical constitution of the Cu biliverdin chelate corresponds to a formal metal oxidation state of (II) coordinated to a neutral radical of the NH trideprotonated biliverdin. The reactivity of the Cu(II) bilindione chelates in nucleophilic solvents agrees with that expected for a π neutral radical structure of the ligand; in CH₃OH, they undergo oxidation towards dimethoxybilipurpurins. The magnetic behaviour of Cu(II) etiobiliverdinat-IV- γ in the solid state shows an intramolecular weak antiferromagnetic coupling d⁹Cu-to- π -radical ($J = -23 \text{ cm}^{-1}$) and an intermolecular weak antiferromagnetic coupling π -radical-to- π -radical ($J = -45 \text{ cm}^{-1}$). The analogy of this magnetic behaviour to that of the π cation radical of metalloporphyrins is discussed.

Keywords. Bile pigments; Transition metal bilindione chelates; d- π Orbitals interaction; Metalloporphyrin π cation radical; Bilindione π neutral radical.

Reaktivität von Pyrrolpigmenten, 21. Mitt. [1]: Struktur und Reaktivität von Cu(II)- und Zn(II)-Chelaten von Bilindionen

Zusammenfassung. Einige Mn, Cu and Zn Chelate von Etiobiliverdin-IV- γ , Mesobiliverdin-IX α und seinem Dimethylester werden untersucht. Im Komplex koordiniert ein Metallatom (mit der Formalladung II) mit einem Neutralradikal des dreifach NH-deprotonierten Bilindions. Die Reaktivität des Komplexes gegenüber nukleophilen Lösungsmitteln entspricht erwartungsgemäß dem eines neutralen π -Radikals; in CH₃OH wird er zu Dimethoxybilipurpurin oxidiert. Bei Cu(II)-Etiobiliverdinat-IV γ beobachtet man im festen Zustand neben schwacher, antiferromagnetischer Kupplung zwischen d⁹Cu und π -Radikal ($J = -23 \text{ cm}^{-1}$) auch eine schwache, intermolekulare, antiferromagnetische Wechselwirkung zwischen zwei π -Radikalen ($J = -45 \text{ cm}^{-1}$). Dieses magnetische Verhalten wird dem des π -Kationradikals von Metalloporphyrinen gegenübergestellt.

Introduction

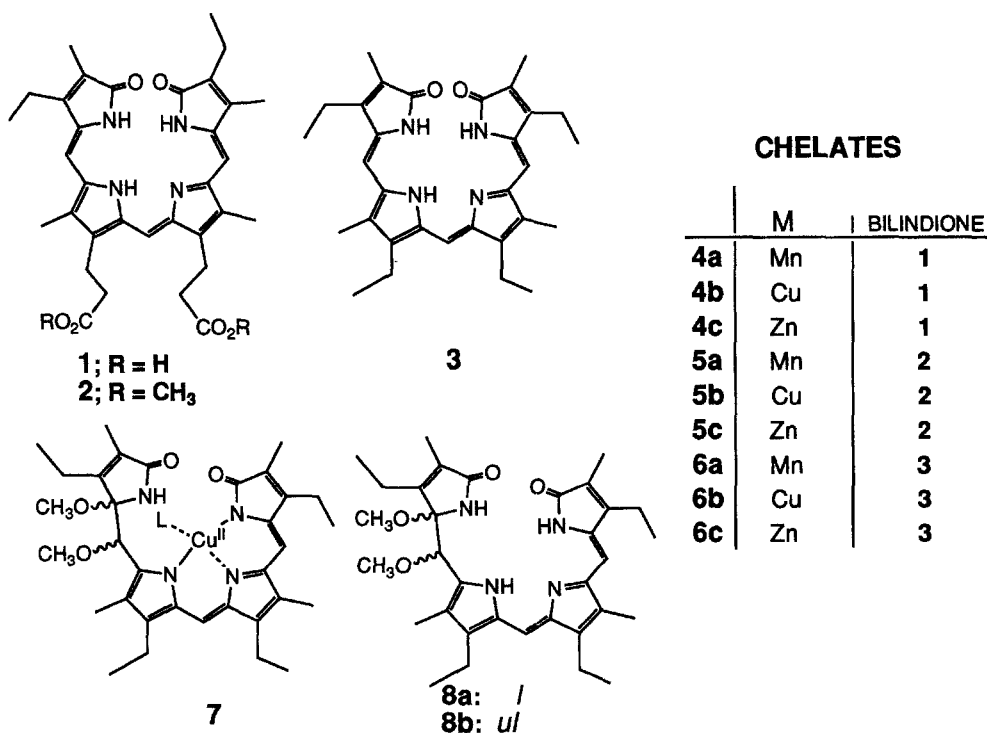
The first relevant studies on metal complexes of bile pigments have been performed on linear tetrapyrrolic systems unrelated to natural biliverdins [2] (*e.g.* 19-formyl-bilin-1-ones [3]). However, there are several reports on the structure of the com-

plexes of biliverdins with transition metal cations [4, 5]. The results most closely related to our study are *Fuhrhop's* study on the solution chemistry of the complexes of biliverdins of the natural series (*bis*-substituted at positions 8 and 12 with propionic acid substituents) [6], *Falk's* structural assignment for Zn(II) bilindiones [7] and his work on the role of bile pigments as ion carriers through liquid membranes [8], *Bonnett's* structural assignment to Ni octaethylbiliverdin chelate [9], and the recent work of *Balch* on several metal biliverdin complexes whose structures were determined by X-ray analysis [10].

For the metal series Mn–Zn, the X-ray structures of the octaethylbilindione chelates have been determined with the exception of the Zn complex: Mn [10c], Fe [10a], Co [10c], Ni [9], and Cu [10b]. All these complexes show a flat helicoidal structure of the bilindione ligand around the metal cation. In fact, this helicoidal geometry (*Z,Z,Z,syn,syn,syn*) is similar to that of the free biliverdins and bilindiones in solution [5].

The chelates of trivalent metals (Mn, Fe) have been isolated and identified as monomers and as dimers; their chemical constitution is that of a M(III) coordinated to an NH trideprotonated bilindione ligand [10c]. In the dimeric form, the flat helicoidal structure is maintained, and one of the two terminal oxygens acts as axial ligand of the metal of the other half of the molecule. However, there is a discrepancy with a previous study on the chemistry of Mn chelates in solution which had assigned Mn(II) to the Mn chelate of mesobiliverdin-IX- α dimethylester [6].

The structure of Zn biliverdin chelates in solution is well established; Zn etiobiliverdin-IV- γ shows the obvious Zn(II) oxidation state coordinated to a NH



dideprotonated ligand with the non deprotonated terminal ring tautomerized to the hydroximido form [7]. The geometry *Z,Z,Z,syn,syn,syn* is similar to that of the Mn, Fe, Co, Ni, and Cu bilindionates.

Although Co, Ni, and Cu bilindionate structures have been solved by X-ray crystallography and there are many data available concerning their chemistry in solution, the formal oxidation state of the metal is not yet fully established [10]. The Cu and Co chelates show constitution formulae corresponding to an NH trideprotonated bilindiones [10]. For Ni octaethylbilindion, the published results do not allow to distinguish between *bis-* or *tris-*deprotonated ligands, but probably it corresponds to a trideprotonated one [9]. However, the chemical characterization of these Cu, Ni, and Co chelates does not agree with the expected properties for M(III) or M(II).

Here, we present results for the Mn, Zn, and Cu chelates of bilindiones (mesobiliverdin-IX- α (**1**), mesobiliverdin-IX- α dimethyl ester (**2**), and 3,8,12,17-tetraethyl-2,7,13,18-tetramethyl-21*H*,24*H*-bilin-1,19-dione (etiobiliverdin-IV- γ , **3**)) in solution. Our goal is to clarify some of the aspects of constitution and reactivity of the transition metal biliverdin chelates. The Zn biliverdin chelates are well described, but the experiments reported here were performed in order to compare them under the same experimental conditions and in parallel tests with those of the Cu and Mn chelates.

Results and Discussion

The metal chelates **4–6** were obtained by extraction of the corresponding metal cation from an aqueous solution with an organic solution of the bilindione as previously described [1], or by mixing alcoholic solutions of the bilindione and the metal acetate (see Experimental).

Mn(III) chelates

The UV/Vis spectra of the Mn complexes (Table 1) agree with the data for Mn biliverdinates [6, 10c]. Thus, Mn(III) chelates show a bilindione like absorption with a strong bathochromic shift of the low energy absorption band ($\lambda_{\max} \approx 900$ nm; see Table 1). However, the differentiation between monomer and dimer forms is not possible due to the small differences in the UV/Vis spectra of both Mn(III) bilindionates [10c].

The solutions of **4a** and **5a** show no EPR signals and a magnetic susceptibility of $4.9 \pm 0.1 \mu_B$, these results point to Mn(III) [11]. In fact, the presence of alcohols in the solution of Mn(AcO)₂ used to obtain the Mn chelates rules out the presence of Mn(II) because it is oxidized to Mn(III) in these media. The experiments performed with fresh CH₃OH or EtOH solutions of Mn(AcO)₂ in a ratio of 1:1 to the bilindione show a slow formation of the complex which can be followed by UV/Vis and EPR spectroscopy. $2 \cdot 10^{-5}$ M solutions reach completion of the formation of the Mn(III) complex in about 30 min displaying isosbestic points. The mixing of CH₃OH or EtOH solutions of Mn(III) and bilindiones immediately afford the complex. Its formation seems to be controlled by diffusion as is the case for the Zn chelates. These results show that a Mn(II) chelates is not formed in significant

Table 1. UV/Vis spectra of Mn(III), Cu(II), and Zn(II) bilindione chelates (see Formula Scheme)

	Solvent	λ_{\max} (nm)	Intensity ratios
4a	CH ₃ OH	900, 389 sh, 365	28:96:100
5a	EtOH	888, 389	32:100
6a	EtOH	878, 800 sh, 386 sh, 344	33:22:79:100
4b	EtOH ^a	799, 733 sh, 665 sh, 417	29:24:22:100
5b	EtOH ^a	801, 665 sh, 725 sh, 420	36:19:13:100
6b	CH ₃ OH ^a	783, 403	34:100
	CH ₂ Cl ₂	795, 720 sh, 413	36:19:100
4c	EtOH	704, 655 sh, 388	53:36:100
5c	CH ₃ OH	707, 650 sh, 386	64:37:100
6c	EtOH	687, 630 sh, 370	81:45:100

^a The spectra were measured on degassed solutions (Ar) immediately after their preparation

amounts. The EtOH, CH₃OH, CH₂Cl₂, and CHCl₃ solutions of the Mn(III) biliverdinates are stable in air (no significant variations after 24 h).

Cu(II) chelates compared to Zn(II) and Mn(III) chelates

The spectrophotometric titration of biliverdins with Zn(AcO)₂ yields isosbestic points and a 1:1 stoichiometry (Fig. 1a). The *Job* plots corroborate the same stoichiometry. However, for Cu(II) the *Job* plots cannot be performed due to oxidation of the bile pigment in the presence of ionic Cu²⁺ species. Nevertheless, the spectrophotometric titration points to an approximate 1:1 stoichiometry. In some cases, *e.g.* for methanolic solutions of **2**, the titrations with Cu(II) show isosbestic points corresponding to the change from the bilindione spectrum to that of the Cu chelate. However, in most of the experiments isosbestic points are not observed (Fig. 1b). Nevertheless, the final spectrum was always the same, irrespective of the presence of isosbestic points. In the case of absence of isosbestic points, the first spectrum change shows a species with its two principal absorptions bathochromically shifted with respect the biliindione but at smaller values than the bilindionate of the final spectrum. These results can be explained assuming the easy formation of the Cu(II) bilindionate *via* simple acid-base exchange processes. This would involve a second Cu bilindionate which under most of the experimental conditions would be rate limiting.

The final Cu complex is stable in aprotic solutions (a dilute solution in CH₂Cl₂ in air does not show any change during 4 h). However, it decomposes in the presence of nucleophiles (*e.g.* traces of alcohols or water). This decomposition is accelerated by contact with air and by the presence of ionic Cu²⁺ in the solution. This points to an O₂ oxidation process catalyzed by the Cu²⁺–Cu⁺ system which requires the presence of a nucleophile. In alcoholic solutions, this oxidative decomposition of the

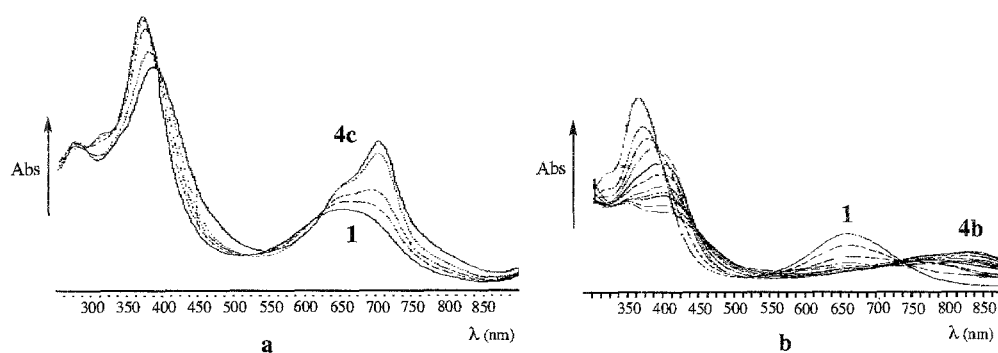


Fig. 1. Spectrophotometric titration of **1** ($2 \cdot 10^{-5}$ M) with $M(\text{AcO})_2$; a) Zn chelate; **1–4c** in ethanol solution; b) Cu chelate; **1–4b** in *Tris* solution at $\text{pH} = 7.0$

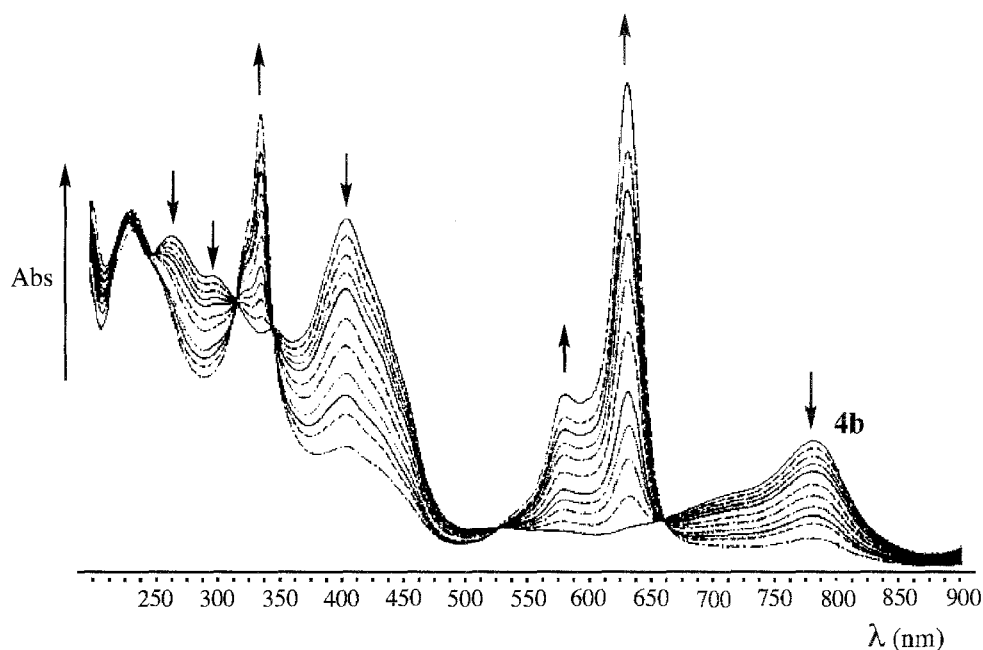


Fig. 2. UV/Vis spectra (10 min intervals) monitoring the air oxidation of **4b** (2.5 M in ethanol)

Cu complexes starts simultaneously with the complex formation. This is in contrast with the stability shown by the Mn(III) and Zn(II) biliverdin chelates in solution and also with the stability of the Cu(II)tripyrin-1-one chelates [1].

A characteristic of the Cu chelate electronic absorption spectra is a non zero absorption between the two principal bands of the spectrum (420 and 800 nm). This broad band, or bands, belongs to the Cu complex and must not be mistaken for the absorptions of the decomposition products of the complex in the same region.

The oxidation of a Cu chelate follows a pattern with well defined isosbestic points (see Fig. 2) towards a final spectrum, suggesting formation of bilipurpurins or biliviols [12, 13]. The reaction product **7** shows a UV/Vis spectrum similar to that of the tripyrin-1-one Cu(II) chelates [1]. The EPR spectrum corresponding to **7**, in

contrast to the absence of EPR signals for the Cu biliverdin chelate (see below), is very similar to that of the Cu(II) tripyrrin-1-one chelates [1]. Decomplexation of **7** affords dimethoxybilipurpurin (**8**) in 80% yield based upon the initial Cu bilindionate.

Dimethoxybilipurpurin could be isolated as its two diastereomers **8a** and **8b**. The *l*-diastereomer has been described by several authors and has been isolated as the main reaction product of the controlled oxidation of biliverdins in CH₃OH [12–15]. In these oxidations, the *ul*-diastereomer seems to be obtained in much lower proportion. In fact, this type of diastereomer has only recently been isolated [14]. Small amounts of the corresponding 14-formyltripyrin-1-one can also be detected. This compound probably originates from the dihydroxylated analog of the dimethoxybilipurpurin by a *retro*-aldol type fragmentation [13].

Other examples of the different reactivity of Cu and Zn bilindione chelates are obtained from electrospray mass spectrometry. The electrospray mode (CH₃OH, H₂O) in negative ion analysis shows an *m/z* peak at M-1 for the Zn chelate **6c** which can be attributed to the presence of an ionizable hydrogen in the chelate (hydroxylic proton of the hydroximino group). Furthermore, the Zn chelate results in an *m/z* peak at M + 1 in positive ion analysis which accounts for the presence of a C = O group able to be protonated. For the Cu chelate **6b**, only the molecular peak corresponding to the trideprotonated ligand plus Cu (*m/z* = 560–558) appears in positive and at negative ion analysis. These results indicate that for **6b** it is easier to suffer a one-electron loss or a one-electron capture than to yield the proton exchange expected for the electrospray experimental conditions.

Other structural data on **4b**, **5b**, and **6b** agree with those reported for the Cu octaethylbilindione chelate [10b]. Thus, the FT-IR spectrum of a solution of the etiobiliverdin chelate **6b** does not show the presence of NH or OH stretching absorptions. In conclusion, our results agree with those previously reported giving the constitution of a NH trideprotonated biliverdin for the ligand.

The constitution formula of a Cu atom and a trideprotonated biliverdin ligand of closed-shell electronic configuration corresponds to the formal oxidation state Cu(III). This rather exotic oxidation state cannot be excluded *a priori* because of the nearly square-planar geometry of the complex and an expected N–Cu distance of about 2.2 Å (see *e.g.* Ref. [16]). Nevertheless, the Cu bilindione chelates are paramagnetic in solution [10b] and, according to our results, also in the solid state, which rules out Cu(III). However, the absence of EPR signals and the magnetic properties of **6b** also exclude a normal coordinated Cu(II), as is the case with dipyrins and tripyrrinones [1, 17]. For the Cu(II) octaethylbilindione chelate, the constitution of Cu(II) coordinated by a neutral radical of the trideprotonated bilindione has been proposed by Balch [10b]. The above results on the reactivity of the Cu(II) bilindione chelates agree with those corresponding to the neutral bilindione π radical in which the unpaired electron resides in the HOMO of the bilindione [13].

Magnetic properties of the copper chelate 6b

The absence of EPR signals for Cu(II) bilindionates could be explained by a) the antiferromagnetic coupling between Cu atoms, b) the presence of an unpaired

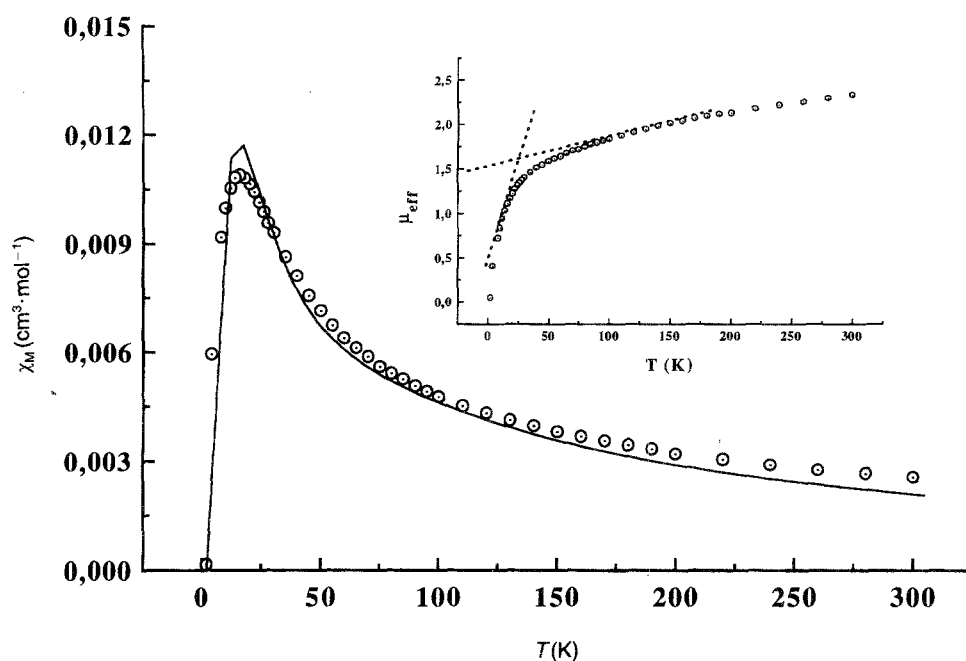


Fig. 3. Experimental values (points) of χ_M vs. T and μ_{eff} vs. T plots (*Squid* magnetometer) and best fit (continuous line) of χ_M vs. T for a four $S = 1/2$ system (see text) for **6b**

electron in the radical, giving a triplet state with large zero splitting, and c) a very efficient spin-spin relaxation between two non-interacting electrons.

For the Cu octaethylbilindione chelate, the magnetic susceptibility (χ) in solution is $2.7 \mu_B$ [10b] which points to an $S = 1$ ferromagnetic state (expected value: $2.8 \mu_B$). Our measurements on **6b** as a solid at room temperature show $X = 2.2 \pm 0.2 \mu_B$ for several samples (pendulum susceptometer and *Squid* magnetometer). For non coupled pairs of spins, the expected value is $2.45 \mu_B$.

The solid state magnetic behaviour of **6b** is studied in the 300–2 K range. The decay of μ_{eff} upon decreasing temperature (Fig. 3) indicates a moderate antiferromagnetic character which is confirmed by a maximum of χ_M at 16 K. Attempts to fit the experimental data to a single two-spin interacting system fail. The attempt to fit the experimental data to the isotropic expression for a $S = 1/2$ pair derived from $H = -JS_1S_2$ also fails because of the low g values and the poor quality of the curve adjustment obtained. Analysis of the μ_{eff} vs. T plot shows two well defined slopes that indicate the presence of two coupling parameters and, consequently, some kind of interaction between monomeric units for which there are two reasonable possibilities: a Cu–Cu interaction (two $d^9\text{Cu}$) through coordination of the terminal oxygen atoms in apical position (similar geometry to the dimers of Mn(III) or Fe(III) bilindionates [10c]), or a $\pi - \pi$ interaction between two unpaired radical ligands. The two cases allow for a four $S = 1/2$ system which can be analyzed in terms of the corresponding Hamiltonian. The best fit is obtained for the exchange coupling parameters $J = -44.6(6.3) \text{ cm}^{-1}$ for π -radical- π -radical exchange and $J = -22.5(1.6) \text{ cm}^{-1}$ for Cu(II)- π -radical exchange.

This type of d- π and π - π interaction is well known in the case of the π cation radicals of metalloporphyrins in the solid state [18].

According to Ref. [19], the Hamiltonian can be written as

$$H = -2J_{12}(S_1S_2) - 2J_{13}(S_1S_3 + S_2S_4) - 2J_{14}(S_1S_4 + S_2S_3) - 2J_{34}(S_3S_4)$$

Numbering of the exchange integrals J_{ij} and spin vector refers to the 3-1-2-4 arrangement of the 3-1 and 2-4 spin pairs of the d⁹Cu- π radical of each monomer (Cu(II) bilindionate). The explicit analytical expression derived from the above Hamiltonian, assuming reasonably that J_{14} and J_{34} are equal to zero due to the long distance between the unpaired spins, can be written as

$$\chi_M = Ng^2\beta^2/kT \cdot (10 \exp^A + 2 \exp^B + 2 \exp^C + 2 \exp^D) / (5 \exp^A + 3 \exp^B + 3 \exp^C + 3 \exp^D + 3 \exp^E + \exp^F)$$

with $A = (J_{12}/2 + J_{13})/kT$, $B = (J_{12}/2 - J_{13})/kT$, $C = (-J_{12}/2 + (J_{12}^2 + J_{13}^2)^{1/2})/kT$, $D = (-J_{12}/2 - (J_{12}^2 + J_{13}^2)^{1/2})/kT$, $E = (-J_{12}/2 - J_{13} - ((J_{12} - J_{13})^2 + 3J_{13}^2)^{1/2})/kT$, and $F = E = (-J_{12}/2 - J_{13} - ((J_{12} - J_{13})^2 + 3J_{13}^2)^{1/2})/kT$.

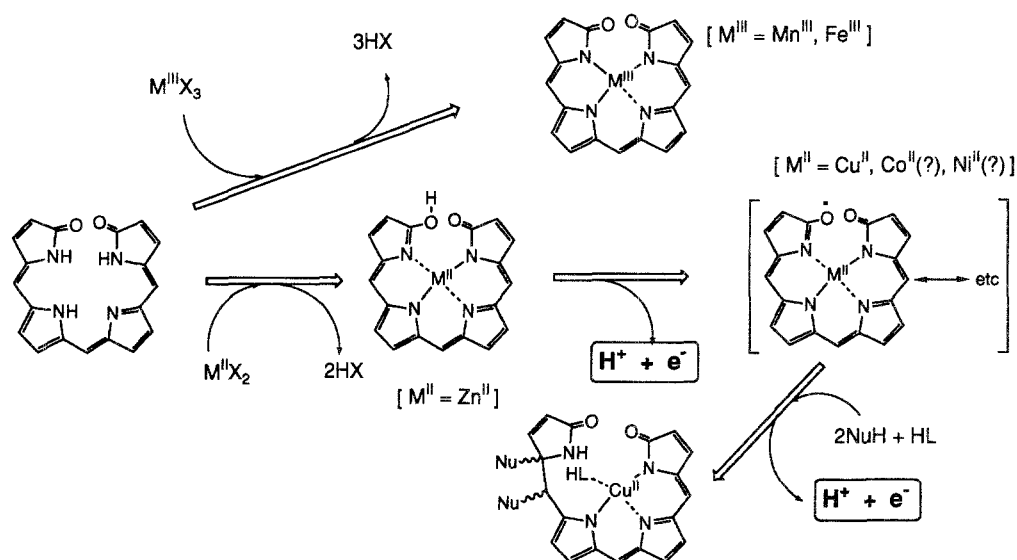
The best fit was obtained for the exchange coupling parameters $J_{12} = -44.6(6.3)$ and $J_{13} = -22.5(1.6) \text{ cm}^{-1}$, and for a Landé factor of 2.0 (Fig. 3). Assignment to an axial Cu-Cu interaction of a singlet-triplet splitting of $2J = 45.0$ or 89.2 cm^{-1} is extremely improbable due to the low mixing of unpaired electron density between the $d_{x^2-y^2}$ and d_z orbital of the Cu atom in a roughly square planar environment. As a consequence, the experimental results should be assigned to an intramolecular weak antiferromagnetic Cu- π radical coupling and a moderately weak π -radical- π -radical interaction.

The stabilization of the Cu(II) bilindione chelate by formation of a neutral π radical can only be explained from d- π interactions. Cu bilindione chelates would show a similar behaviour to Cu porphyrinato cation radicals. In planar Cu(II) porphyrin π cation radicals, the magnetic orbitals are orthogonal and there is intramolecular ferromagnetic coupling [18] in solution, but radical dimerization in the solid state. Core ruffling in sterically crowded porphyrins determines the loss of orthogonality and hindrance of dimerization. For the most non-planar Cu porphyrin π radical cations, paramagnetism occurs in the solid state, and the solutions show antiferromagnetic coupling. The cation radical of the crowded Cu(II) octaethyltetraphenylporphyrin, for example is paramagnetic in the solid state and diamagnetic in solution [18e]. With this respect, the helicoidal structure of the Cu biliverdin chelates seems to be more related to a *quasi*-planar porphyrin system than to a highly deformed porphyrin. However, in contrast to the porphyrinato systems, the magnetic behaviour of Cu(II) bilindionates in solution is not clearly different from that in the solid state, which could be attributed to a lower ligand flexibility of the bilindionates compared to the sterically crowded porphyrinates.

The study of metalloporphyrin π radicals has been proposed as a model for understanding magnetic coupling phenomena [18c]. With this respect, transition metal bilindione chelates are very promising; a more similar magnetic behaviour between solid and solutions and a lower tendency to dimerization as solids could be expected than for metalloporphyrin π cation radicals.

Structure and formation of metal transition cation bilindione chelates

Cu(II) and Zn(II) tripyrrin-1-one chelates, non-substituted at position 14 with electron donor groups, have the same constitutional formula [1], *i.e.* a bide-



Scheme 1

protonated ligand coordinating to the metal cation. In bilindione Zn chelates, the type of metal binding is similar to that of tripyrrin-1-ones: the fourth terminal ring of the biliverdin ligand tautomerizes into the hydroximino form [7] which avoids the steric hindrance exerted by the NH bond on the *Z,syn* geometry (see Scheme 1). Probably, Cu(II) coordinates to the bilindione, first through simple acid-base processes, which would lead to a complex similar to that of $d^{10}Zn$. The first species detected in the titration of the bilindione with $Cu(AcO)_2$ could correspond to a Cu(II) bilindione chelate with a constitution similar to the Zn(II) chelates. The formation of the final Cu(II) bilindione chelate then involves a one-electron oxidation allowed by the low oxidation potential of the coordinated ligand and the presence of an unpaired electron (d^9) in Cu(II).

With respect to the isostructural systems of Co and Ni, all published results point to a similar behaviour to that of the Cu(II) compounds. For the Co complex, an anomalous magnetic behaviour has been detected [10c]. However, it would be necessary to test for differences between species in solution and in the solid state, and in the presence of equilibria between different Co oxidation states. In the case of the Ni biliverdin chelate there is probably an equilibrium between Ni(II) and Ni(III) species [9].

UV/Vis absorption spectra of the bilindione chelates

We have suggested [1] that the changes in the UV/Vis spectra of tripyrrin-1-ones originating from the chelation of a transition metal cation could be mainly attributed to d - π orbital interactions. As suggested for the tripyrrin-1-one chelates, the bathochromic shifts of the biliverdin chelates as compared to the free ligand could be due to symmetry-allowed perturbations between the d_{xy} and d_{xz} occupied metal orbitals and the π (HOMO and other π orbitals near to the HOMO) system of the ligand (see Table 1).

Biological differentiation between rubinoid and verdinoid bile pigments

The fact that the biliverdin chelates of some metals of biological significance are reactive free organic radicals gives a new argument for speculations about the biological evolution of the porphyrin – biliverdin – bilirubin metabolic routes in the animal kingdom. Accepting the assumption that bile pigments are natural biological antioxidants, the results reported here point to a differentiation of the possible roles of bilirubin and biliverdin. All experimental results *in vitro* show that the antioxidant capabilities of biliverdin are the same as or higher than those of bilirubin (see *e.g.* Ref. [20]). However, when biliverdin coordinates to a metal ion such as Cu(II), it would act as a potential initiator for one-electron reactions (see Scheme 1). In this sense, it is perhaps significant that biliverdin has been identified as a hepatic tumor promoter [21].

Experimental

Melting points were determined on a Kofler-Reichert micro hot-stage apparatus. UV/Vis spectra: Perkin-Elmer Lambda 5 instruments; FT-IR spectra: Nicolet 510 spectrometer; mass spectra: for FAB analysis, Hewlett-Packard 5988A instrument with a Capillartron Frasca Cs gun (10 kV); for electro-spray analysis, VG-Quattro Fisons instrument (80 °C, 3.5 kV, focus 45–125 V); NMR spectra: Varian Unity 300 and a Varian Gemini (200 MHz) spectrometers; EPR spectra: Bruker EST 300 E instrument, X band; magnetic susceptibility measurements: pendulum susceptometer (Manics DSM8) at 15000 G and magnetometer SQUID (Institut de Chimie de la Coordination de Toulouse) at 1000 G. Magnetic susceptibility measurements on solutions were performed according the *Evans* method [22] using a ¹H NMR Perkin-Elmer R24B (60 MHz) instrument. *Job* plots were performed as described in Ref. [17].

The preparation and properties of the following compounds are described in the literature: **1** [23], **2** [23], **3** [24]. The following metal complexes were obtained in solution as described in the literature: **4b**, **4c**, **5b**, **5c** [6].

Mesobiliverdin-IX α -Mn(III) (**4a**; C₃₃H₃₁N₄MnO₆)

4a was obtained following the general method described in Ref. [3b]. 2.47 mg (6.3·10⁻³ mmol) of Mn(AcO)₂·4H₂O were added to a solution of 5.50 mg (6.3·10⁻³ mmol) **1** in 4 ml CH₃OH:CHCl₃ = 1:1. Evaporation of this solution gives **4a** as a solid. The formation of **4a** (\approx 30 min) was followed by UV/Vis and EPR spectroscopy (1·10⁻² mol·l⁻¹ in CH₃OH:CHCl₃). UV/Vis (CH₃OH): $\lambda_{\max}(\epsilon) = 900, 389$ sh, 365 nm (28:96:100); magnetic susceptibility (*Evans* method, DMSO-d₆:CH₂Cl₂ = 100:0.6, 1.5·10⁻² M): 5.05 μ_B .

Mesobiliverdin-IX α -Mn(III), dimethyl ester (**5a**; C₃₅H₃₅N₄MnO₆)

5a was obtained following the procedure described above for **4a**; the formation of **5a** (\approx 30 min) was followed by UV/Vis and EPR spectroscopy (5·10⁻² mol·l⁻¹ in CH₃OH:CHCl₃). **5a** has been described previously in Ref. [6]. UV/Vis (CH₃OH:CHCl₃): $\lambda_{\max}(\epsilon) = 888, 389$ sh, 365 nm (32:100); magnetic susceptibility (*Evans* method, CD₃OD:CHCl₃ = 100:0.6, 1.5·10⁻² M): 4.78 μ_B .

Etiobiliverdin-IV γ -Mn(III) (**6a**; C₃₁H₃₅N₄MnO₂)

6a was obtained following the procedure described above for **4a**. UV/Vis (CH₃OH): $\lambda_{\max}(\epsilon) = 878, 345, 285$ nm (35:100:90); (CH₂Cl₂): $\lambda_{\max}(\epsilon) = 874, 347, 286$ nm (33:100:96); magnetic susceptibility (*Evans* method): no EPR signals.

Etiobiliverdin-IV γ -Cu(II) (**6b**; C₃₁H₃₅CuN₄O₂)

For some experiments (for example its oxidation in CH₃OH), **6b** was obtained mixing stoichiometric amounts of Cu(AcO)₂·H₂O and **3**. **6b** was obtained as a solid following the general extraction procedure described above, but working with air degassed solutions (Ar). Ms(-)-ES (CH₃OH:H₂O = 1:1): (*m/z*) = 560–558 (M); MS(+)-ES (CH₃OH:H₂O = 1:1): (*m/z*) = 560–558 (M); FT-IR (KBr): 1673, 1544, 1109 cm⁻¹; UV/Vis(CH₃OH): $\lambda_{\max}(\epsilon)$ = 783 (10300), 403 (30500), shoulders at 850, 695, 637, 353, 317, and 273 nm; EPR: no signals as a solid and in several solvent systems at room temperature and at 77K; magnetic susceptibility χ : pendulum susceptometer, 2.03 μ_B (293 K) and 0.8 μ_B (6 K); *Squid* magnetometer, 0.6 μ_B (2 K).

Etiobiliverdin-IV γ -Zn(II) (**6c**; C₃₁H₃₆N₄O₂Zn)

To 11.1 mg (0.051 mmol) Zn(AcO)₂·2H₂O in 10 ml CH₃OH, 5.8 mg (0.012 mmol) **3** dissolved in 4 ml CH₂Cl₂ were added. The precipitate was filtered and the filtrate was evaporated under vacuum. The residue was dissolved in CH₂Cl₂ and filtered through a nylon filter (0.22 μ m pore). MS(-)-ES (CH₃OH:H₂O = 1:1): (*m/z*) = 563–561–559 (M – 1); FT-IR (KBr): 1684, 1600–1590, 1445, 1270 cm⁻¹; UV/Vis (CH₃OH:CH₂Cl₂ = 1:1): $\lambda_{\max}(\epsilon)$ = 687, 630 sh, 370, 272 nm (81:45:100:33); ¹H NMR (CDCl₃, 300 MHz): δ = 7.96 (s, 1H, N = OH), 6.81 (s, 1H, HC =, C10), 5.56 (s, 2H, HC =, C5 and C15), 2.56 (q, 4H, *J* = 8 Hz, 2 × CH₂CH₃), 2.10 (q, 4H, *J* = 8 Hz, 2 × CH₂CH₃), 1.97 (s, 6H, 2 × CH₃), 1.75 (s, 6H, 2 × CH₃), 1.15 (t, 6H, *J* = 8 Hz, 2 × CH₂CH₃), 0.84 (t, 6H, *J* = 8 Hz, 2 × CH₂CH₃) ppm; ¹³C NMR (CDCl₃, 75 MHz, DEPT): δ = 171.9 (C1, C19), 153.6 (C6, C14) 146.0 (C3, C17), 145.0, 139.6, 138.6, 127.9, 125.9, 120.9, (C10), 100.4 (C5, C15), 18.1 (CH₂), 17.6 (CH₂), 16.1 (CH₂CH₃), 14.4 (CH₂CH₃), 10.4 (C7, C13), 8.3 (C2, C18) ppm.

Reaction products obtained from the oxidation of 6b in CH₃OH (7, 8a, and 8b)

A solution of 0.024 mmol **6b** formed by mixture of stoichiometric amounts of **6** and Cu(AcO)₂·H₂O is left standing in contact with air. After 6 h, the disappearance of the absorption spectrum of **6b** is complete. A residue was obtained by evaporation under vacuum showing the following data: UV/Vis (CH₃OH): $\lambda_{\max}(\epsilon)$ = 632 (50500), 580 (17800), 338 (42600) nm; EPR (CH₂Cl₂:CH₃OH = 1:1, 77 K): *g* = 2.33. These data in combination with the products obtained by its demetallation (**8a** and **8b**) prove structure **7**.

7 was dissolved in CH₂Cl₂ and extracted with 5 M HCl. The organic layer was washed with water (NaHCO₃) and dried over Na₂SO₄. Evaporation under vacuum results in a mixture of compounds which is separated by preparative TLC (CH₂Cl₂:CH₃OH = 30:1). The two principal products are identified as *l*-4,5-dimethoxy-3,8,12,17-tetraethyl-2,7,13,18-tetramethyl-4,5-dihydro-21*H*,24*H*-bilin-1,19-dione (**8a**; 35% yield) and *ul*-4,5-dimethoxy-3,8,12,17-tetraethyl-2,7,13,18-tetramethyl-4,5-dihydro-21*H*,24*H*-bilin-1,19-dione (**8b**; 31% yield). The more mobile **8a** has been already described in the literature [12, 15].

ul-4,5-Dimethoxy-3,8,12,17-tetraethyl-2,7,13,18-tetramethyl-4,5-dihydro-21H,24H-bilin-1,19-dione (8b, C₃₃H₄₄N₄O₄)

MS(+)-ES (CH₃CN:H₂O = 1:1): (*m/z*) = 561 (M + 1), 529 (M + H – 32), 497 (M + H – 64); UV/Vis (CH₂Cl₂): $\lambda_{\max}(\epsilon)$ = 553, 520 sh, 323, 227 nm; ¹H NMR (CDCl₃, 200 MHz): δ = 8.52 (broad s, NH), 6.63 (s, HC =, C10), 5.94 (s, HC =, C15), 4.63 (s, HC =, C5), 3.33 (s, CH₃O), 3.17 (s, CH₃O), 2.57 (q, *J* = 7.5 Hz, CH₂CH₃), 2.53 (q, *J* = 7.5 Hz, 2 × CH₂CH₃), 2.19 (q, *J* = 7.5 Hz, CH₂CH₃), 2.03 (s, 2 × CH₃), 1.97 (s, CH₃), 1.61 (s, CH₃), 1.20 (t, *J* = 7.5 Hz, CH₂CH₃), 1.16 (t, *J* = 7.5 Hz, CH₂CH₃), 1.11 (t, *J* = 7.5 Hz, CH₂CH₃), 1.09 (t, *J* = 7.5 Hz, CH₂CH₃) ppm.

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