Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/11/14 For personal use only.

## Metal-nitroxyl interactions. 25. Exchange interactions via saturated linkages in spin-labeled porphyrins

KUNDALIKA M. MORE AND GARETH R. EATON<sup>1</sup>

Department of Chemistry, University of Denver, Denver, CO 80208 U.S.A.

and Sandra S. Eaton<sup>1</sup>

Department of Chemistry, University of Colorado at Denver, Denver, CO 80202 U.S.A.

Received July 15, 1981

KUNDALIKA M. MORE, GARETH R. EATON, and SANDRA S. EATON. Can. J. Chem. 60, 1392 (1982).

A series of spin-labeled copper porphyrins has been prepared in which nitroxyl radicals are attached to a porphyrin pyrrole ring by propionamide and propionate ester linkages. The solution epr spectra at room temperature indicate that the values of the electron spin – electron spin coupling constant, J, for these compounds are in the range 4 to 18 G, depending on the solvent and the size of the nitroxyl ring. Below  $-45^{\circ}$ C two isomers with different values of J were observed in the epr spectra of two of the compounds. Variable temperature epr spectra were also analyzed for analogous spin-labeled copper porphyrins which have *cis* and *trans* acrylic ester and acrylamide linkages between the porphyrin and the nitroxyl ring. Comparison of the values of J for the complexes with saturated and unsaturated linkages suggests that  $\sigma$ -bonding pathways dominate the copper–nitroxyl interaction in these compounds.

KUNDALIKA M. MORE, GARETH R. EATON et SANDRA S. EATON. Can. J. Chem. 60, 1392 (1982).

On a préparé une série de phorphyrines de cuivre, contenant un marqueur de spin, dans lesquelles les radicaux nitroxyles sont attachés à la portion porphyrine du cycle pyrrole par des amides et des esters propioniques. Les spectres de rpe, en solution et à la température ambiante, indique que les constantes de couplage spin électron – spin électron dans ces composés varient de 4 à 18 G selon le solvant et la taille du cycle nitroxyle. A des températures inférieures à  $-45^{\circ}$ C, on a observé, dans les spectres de rpe de deux de ces composés, la présence de deux isomères ayant des valeurs différentes de J. On a également analysé les spectres de rpe à température variable des porphyrines de cuivre analogues contenant un marqueur de spin dans lesquelles on a intercalé entre le valeurs de J de ces complexes avec celles des produits saturés indique que l'interaction cuivre–nitroxyle induite dans ces composés se transmet principalement par les liaisons  $\sigma$ .

[Traduit par le journal]

#### Introduction

Interactions between unpaired electrons may be observed via a variety of physical measurements. Of particular interest in biological systems are the interactions observable by electron paramagnetic resonance (epr) among various pairs or clusters of paramagnetic transition metals and/or organic free radicals (1). These paramagnetic centers may be a natural component of the biological system or they may be species purposely added as probes or labels (2). Interactions between these centers may affect the epr spectra enough to invalidate the usual interpretations. For example, a spin-labeled biomolecule may yield an epr spectrum which is not validly interpreted in terms of the motion of the label if a paramagnetic metal is present. If, however, the effect of the metal on the nitroxyl epr spectrum were understood in detail, additional information could be obtained from the epr spectrum. For example, an assessment was made several years ago of the effect of dipolar interactions between a metal and a nitroxyl radical on the epr lineshape of the nitroxyl in a rigid matrix (3). This approach has been used by many investigators to obtain metrical information on spin-labeled biomolecules (1). Others have studied the closely related problem of metal – organic radical interactions in vitamin  $B_{12}$ -dependent enzyme reactions also seeking metrical information (4, 5).

There is promise of achieving considerable insight into the metric and dynamic features of complicated systems, such as enzymes and membranes, by theoretical analysis and experimental calibration of the impact on their epr spectra and relaxation properties of interaction between unpaired electrons. Substantial confusion can result, however, if proper attention is not given to relevant features of the interaction regime. For example, one must consider relative motion of the two species, whether this motion is correlated or not, the nature of the interaction pathway, environment effects such as specific solvation, and potential nonstatistical effects such as specific ion pair or acid/base interactions. Consideration must also be given to relative values of the various characteristic times of the system, such as tumbling or collisional

0008-4042/82/121392-10\$01.00/0

©1982 National Research Council of Canada/Conseil national de recherches du Canada

<sup>&</sup>lt;sup>1</sup>To whom all correspondence should be addressed.

correlation times, electron spin relaxation times, nuclear spin relaxation times, reciprocal of the microwave frequency, and modulation frequency, etc. In some cases the electron-electron interaction can be observed in the lineshape of the normal continuous wave (CW) epr spectrum, and in other cases the interaction is observed only via a nonlinear technique (such as CW saturation studies) or time domain (pulsed) epr.

Most work on interacting spin systems has been conducted either in low viscosity fluid solutions or in essentially rigid matrices such as frozen solutions or very slowly tumbling biomolecules. Still to be explored are intermediate tumbling regimes,

Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/14/14 For personal use only.

liquid crystals, phase boundaries (e.g., membrane surfaces), and single crystals.

In this paper we briefly survey the range of phenomena which have been observed in low viscosity fluid solutions of paramagnetic transition metal complexes containing nitroxyl radicals, and mention some preliminary results from work in progress. Detailed description is given of a series of copper(II) porphyrin complexes I-IX in which a nitroxyl is observed to exhibit exchange interaction with the copper through a variety of metal-nitroxyl linkages, including a saturated --CH<sub>2</sub>--CH<sub>2</sub>--chain.



## **Experimental section**

Infrared spectra were obtained in Nujol mulls on a Perkin-Elmer 337 grating spectrometer. Electronic spectra were obtained in chloroform solution on a Beckman Acta V spectrometer. Data are given below with wavelengths in nm and log  $\varepsilon$  in parentheses. X-band epr spectra were obtained on a Varian E-9 spectrometer as previously described (6). Electron paramagnetic resonance spectra were run on degassed samples in dried purified solvents with concentrations about  $10^{-3}$  M. All spectra were obtained with power levels well below saturation. g-Values were measured relative to DPPH (g = 2.0036). Electron paramagnetic resonance spectra are shown in the figures with the magnetic field increasing to the right. All coupling constants are given in G. High resolution mass spectra were obtained at the Midwest Center for Mass Spectrometry. Elemental analyses were performed by Spang Microanalytical Laboratory.

## Preparation of compounds

Spin-labeled copper pophyrins I–VI were prepared as previously reported (7). *meso*-Tetraphenyl-1-[2-ethoxycarbonyl-ethyl]porphyrin, X, was prepared by the method of Momenteau *et al.* (8). The presence of the propionate ester was confirmed by a high resolution mass spectrum. M<sup>+</sup>/e observed: 714.3051  $\pm$  0.0126. Calcd. for C<sub>49</sub>H<sub>38</sub>N<sub>4</sub>O<sub>2</sub>: 714.2995.

#### Copper(II) meso-tetraphenyl-I-[2-ethoxycarbonylethyl]porphyrin, XI

Cupric chloride (0.2 g, 1.2 mmol) was refluxed with porphyrin X (0.72 g, 1 mmol) in DMF (100 mL) until the visible spectra indicated complete conversion to the metalloporphyrin. Water (100 mL) was added to the cooled reaction mixture and the precipitate was collected by filtration. The product was chromatographed on silica gel in CHCl<sub>3</sub> solution and the first red band was collected. The product was recrystallized from CHCl<sub>3</sub>/hep-tane. Yield: 90%, ir:  $v_{CO}$  1720 cm<sup>-1</sup>; vis: 570 (sh, 3.50), 539 (4.29), 416 (5.66).

# Copper(II) meso-tetraphenyl-1-[3-propionic acid]porphyrin, XII

KOH methanol in (7.5 mL, 2 N) was added to 100 mg of XI in 30 mL of pyridine. The solution was stirred for 24 h at room temperature. The solvent was removed and the residue was neutralized with aqueous HCl. The product was extracted into CHCl<sub>3</sub> and the solution was dried over Na<sub>2</sub>SO<sub>4</sub>. The volume of the solution was reduced to 30 mL before putting it on a silica gel column. Unreacted XI was eluted with CHCl<sub>3</sub>. The product was eluted with 2% ethanol in CHCl<sub>3</sub> and recrystallized from CHCl<sub>3</sub>/heptane. The acid appears to decompose when heated for prolonged periods in vacuum. Yield: 67%; ir: v<sub>c0</sub> 1685 cm<sup>-1</sup>; vis: 570 (sh, 3.62), 539 (4.42), 416 (5.81).

## Copper(II) meso-tetraphenyl-1-[3-(N-(2,2,6,6-tetramethylpiperidinyl-1-oxy))propionamido]porphyrin, VII

Oxalyl chloride (1.1 g, 10 mmol) was added to a solution of XII (0.15 g, 0.2 mmol) in 50 mL of dry benzene. The solution was stirred for 2 h at room temperature. The solvent was removed in vacuum and the residue was kept under vacuum for 2 h before dissolving it in 75 mL dry THF and 1 mL dry pyridine. 4-Amino-2,2,6,6-tetramethylpiperidinyl-1-oxy (0.034 g, 0.2 mmol) in 2 mL dry THF was added dropwise under nitrogen. After refluxing the solution for 6 h, the solvent was removed in vacuum and the residue was chromatographed on silica gel in CHCl<sub>3</sub> solution. The first red band was collected. The product was recrystallized from CHCl<sub>3</sub>/heptane. Yield: 61%; ir: v<sub>col</sub> 1655 cm<sup>-1</sup>, v<sub>NH</sub> 3300 cm<sup>-1</sup>; vis: 570 (sh, 3.65), 539 (4.48), 416 (5.86). *Anal.* calcd. for C<sub>56</sub>H<sub>49</sub>CuN<sub>6</sub>O<sub>2</sub>: C 74.60, H 5.48, N 9.32; found: C 74.81, H 5.59; N 9.47.

#### Copper(II) meso-tetraphenyl-I-[3-(O-(2,2,6,6-tetramethylpiperidinyl-1-oxy))propionic ester]porphyrin, VIII

The ester was prepared from copper porphyrin XII and 4-hydroxy-2,2,6,6-tetramethyl-piperidinyl-1-oxy by the same procedure as described for VII. Yield: 55%. ir:  $v_{c0}$  1725 cm<sup>-1</sup>; vis: 570 (sh, 3.64), 539 (4.51), 416 (5.89). Anal. calcd. for C<sub>56</sub>H<sub>48</sub>CuN<sub>5</sub>O<sub>3</sub>: C 74.52, H 5.36, N 7.76; found: C 74.24, H 5.23, N 7.57.

## Copper(II) meso-tetraphenyl-1-[3-(N-(2,2,5,5-tetramethylpyrrolidinyl-1-oxy))propionamido]porphyrin, IX

The amide was prepared from copper porphyrin XII and 3-amino-2,2,5,5-tetramethylpyrrolidinyl-1-oxy by the same procedure as described for VII. Yield: 67%; ir:  $v_{C=0}$  1650 cm<sup>-1</sup>,  $v_{NH}$  3320 cm<sup>-1</sup>; vis: 570 (sh, 3.47), 539 (4.28), 416 (5.66). *Anal*. calcd. for C<sub>55</sub>H<sub>47</sub>CuN<sub>6</sub>O<sub>2</sub>: C 74.43, H 5.34, N 9.47; found: C 74.27, H 5.21, N 9.28.

## **Computer simulations**

The interaction between two spins can be expressed in terms of dipolar and exchange components. The main distinguishing feature of dipolar interactions relative to exchange interactions is the angular and distance dependence of the dipolar interactions. It can be shown that expansion of the exchange and dipolar parts of the spin-spin interaction both lead to linear combinations of the same set of nine components of the interaction dyadic (5). The exchange and dipolar components differ only in the scalar coefficients of the dyads (which are bilinear in the spin operators). An epr experiment can yield the values of the coefficients, but their interpretation in terms of exchange and/or dipolar contribution is not unambiguous without additional information such as the geometry of the interaction. The exchange interaction can be written as the sum of three matrices: isotropic exchange, antisymmetric exchange, and symmetric anisotropic exchange. The symmetric anisotropic exchange matrix has precisely the same formal structure as the dipole-dipole interaction matrix, i.e., it is anisotropic but symmetrical about the main diagonal. In rigid media all of these terms have to be considered. However, in rapidly tumbling molecules with the normally encountered g-values and distances, it can be estimated that terms other than isotropic exchange average to nearly zero, i.e., less than the linewidths due to other effects. In this case, to which we shall restrict our attention in the rest of the discussion, the isotropic exchange can be treated as a scalar J.

It has sometimes been assumed that spin-spin exchange interactions are negligible for distances greater than about 6 Å (9, 10). However, protonproton spin-spin coupling attributable to exchange interactions has been observed through five bonds in saturated systems and through nine bonds in conjugated hydrocarbons (11). Coupling to distant protons has also been observed in ENDOR spectra (12). Since the electron magnetic moment is much larger than the proton magnetic moment, it is reasonable to expect even longer range spin-spin interactions between two electrons than between two protons or between a proton and an electron. Orbital interactions through six bonds have also been found to be sufficiently large to alter chemical reactivity (13).

A few years ago we reported the first examples of high resolution electron spin – electron spin splitting in epr spectra (14, 15). These spectra can be interpreted in terms of the Hamiltonian [1].

## 1394

Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/11/14 For personal use only.

$$[1] \quad \mathcal{H} = g_{1}\beta H \hat{S}_{1Z} + g_{2}\beta H \hat{S}_{2Z} + hJ \hat{S}_{1Z} \hat{S}_{2Z} + \frac{hJ}{2} (\hat{S}_{1+} \hat{S}_{2-} + \hat{S}_{1-} \hat{S}_{2+}) + hA_{M} \hat{S}_{1Z} \hat{I}_{1Z} + hA_{N} \hat{S}_{1Z} \hat{I}_{2Z} + hA_{N'} \hat{S}_{2Z} \hat{I}_{3Z} + \frac{hA_{M}}{2} \times (\hat{S}_{1+} \hat{I}_{1-} + \hat{S}_{1-} \hat{I}_{1+}) - g_{M} \beta_{N} H \hat{I}_{1Z} - g_{N} \beta_{N} H \hat{I}_{2Z} - g_{N'} \beta_{N} H \hat{I}_{3}$$

where  $g_1$  and  $g_2$  are the g values of the metal and nitroxyl electrons;  $\hat{S}_1$  and  $\hat{S}_2$  refer to the metal and nitoxyl electron spins, respectively; J is the electron-electron coupling constant in Hertz;  $I_1$ ,  $I_2$ , and  $I_3$  refer to the metal nuclear spin, the nuclear spin of the coordinated nitrogens, and the nuclear spin of the nitroxyl nitrogen, respectively;  $A_{\rm M}$  is the metal electron - metal nuclear coupling constant in Hertz;  $A_N$  is the coupling constant in Hertz between the metal electron and the nuclear spins of the coordinated nitrogens;  $A_{N'}$  is the coupling constant in Hertz between the nitroxyl electron and the nuclear spin of the nitroxyl nitrogen; and all other symbols are defined as in ref. 16. The first seven terms in the Hamiltonian were treated exactly and the last four were treated as a perturbation to second order for the transition energies and to first order for the transition probabilities. To facilitate visual comparison with the field-swept experimental spectra the values of J,  $A_M$ ,  $A_N$ , and  $A_{N'}$  are discussed below in units of G with the conversion between Hertz and Gauss given by eqs. [2]-[4]. The conversion factor for  $A_N$  is the same as for  $A_M$ . Only the absolute value of J can be determined from these calculations.

$$[2] \quad J(G) = J(Hz) \cdot \frac{h}{2\beta} \left( \frac{1}{g_1} + \frac{1}{g_2} \right)$$

[3] 
$$A_{\rm M}({\rm G}) = A_{\rm M}({\rm Hz}) \cdot \frac{h}{g_{\rm s}\beta}$$

Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/11/14 For personal use only.

[4] 
$$A_{N'}(G) = A_{N'}(Hz) \cdot \frac{h}{g_2\beta}$$

The simulation of epr spectra using Hamiltonian [1] has been reported in detail (16). The computer program CUNO described in ref. 16 has been extended to handle any nuclear spin and to allow more flexibility in simulating linewidths. The essential feature of the calculation can be seen with a simple "stick" diagram (Fig. 1). Each copper transition interacts with each nitroxyl transition producing an "AB quartet" of four lines, analogous to the well-known AB patterns of <sup>1</sup>H nmr. The



FIG. 1. Schematic representation of two of the AB patterns resulting from interaction of a copper electron  $(A_{cu} = 86 \text{ G})$  with a nitroxyl electron  $(A_N = 15 \text{ G})$  for a value of J of 200G. The stick spectrum labeled a arises from coupling between the  $m_1 = 3/2$  copper line (lowest field copper line) and the  $m_1 = 0$  nitroxyl line. The stick spectrum labeled b arises from coupling between the  $m_1 = -3/2$  copper line (highest field copper line) and the  $m_1 = 0$  nitroxyl line.

lines in the spectra are referred to as copper or nitroxyl depending on the nature of the transition as J approaches zero. The intensities of the inner and outer lines of the quartet are dependent on the magnetic field separation between the original copper and nitroxyl lines that are interacting and the magnitude of J. Figure 1 illustrates this for two different cases. The  $m_I = +3/2$  Cu line and the  $m_I$ = 0 line of the nitroxyl are sufficiently far apart that with a J of  $200\,\text{G}$ , a clear four-line pattern is produced in which the outer lines have significant intensity (Fig. 1, stick spectrum a). The Cu -3/2line and the nitroxyl 0 line are so close together, though, that with a J of 200 G the inner copper and nitroxyl lines are almost superimposed, and the outer lines have little intensity and may in practice not be observable if the linewidths are large (Fig. 1, stick spectrum b). Thus, even within a single compound a wide range of ratios of J to the metal-nitroxyl energy separations may occur.

Figure 2 shows observed and calculated room temperature epr spectra for spin-labeled copper porphyrins VIII, XIII (15, 17), and XIV (18). When J is small, as in Fig. 2A, the nitroxyl region of the spectrum is an overlapping doublet of triplets and the copper lines only appear to be broadened. In Fig. 2B the value of J (78G) is close to the copper nuclear hyperfine coupling (86G) so the copper signal is an apparent five-line pattern partially obscured by the sharper nitroxyl lines. The nitroxyl lines are a clearly resolved doublet of triplets. When J is 800G, as in Fig. 2C, the copper and nitroxyl inner lines are nearly coincident, the

1396

Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/11/14 For personal use only.

CAN. J. CHEM. VOL. 60, 1982



FIG. 2. X-band epr spectra of three spin-labeled copper porphyrins at room temperature and computer simulations to demonstrate the effect of the value of J on the appearance of the spectra. The three spectra are 800 G scans centered at 3025 G. A, The spectrum of VIII in toluene solution was obtained with 0.63 G modulation amplitude and 4.0 mW power. The computer simulated spectrum was obtained with J = 14.5 G. B, The spectrum of XIII in CHCl<sub>3</sub> solution was obtained with 0.8 G modulation amplitude and 20 mW power. The value of J in the simulated spectrum is 78 G. C, The spectrum of XIV in toluene solution was obtained with 2G modulation amplitude and 15 mW power. The value of J in the simulated spectrum is 800 G.

apparent copper hyperfine splitting is one-half  $A_{cu}$  (1/2 × 86G) and the outer lines of the AB pattern are not observed. Overall there is good agreement between the observed and calculated spectra. There is, however, some discrepancy for the lowest field copper lines due to incomplete motional averaging of g- and A-anisotropy which is not included in the computer simulations.

## **Results and discussion**

Details are given in the Experimental section for the preparation and characterization of the nitroxyl spin labeled copper porphyrin complexes VII–IX. In this section we describe the epr spectra of these compounds and compare them with the spectra of the closely related compounds I–VI for which the room temperature epr spectra have previously been reported (7). This series of compounds was designed to compare spin–spin interaction through saturated and unsaturated C–C linkages, keeping everything else as similar as is possible in a series of different compounds.

In a variety of solvents at room temperature the value of J for spin-labeled copper porphyrin VII





ranged from 9 to 17G (cf. Table 1). Similar values of J were observed for VIII which contains an ester linkage instead of an amide linkage. The epr spectra of VIII in CHCl<sub>3</sub> solution (Fig. 2A) are typical of the spectra observed for both VII and VIII at room temperature. The values of J for VII and VIII are smaller than those previously observed for IV and V (19-26G, cf. Table 1) which have a trans olefinic linkage between the porphyrin and the nitroxyl (7). For the analogous cis isomers I-III in noncoordinating solvents the values of Jare strongly dependent on solvent and temperature which was attributed to an interaction between the carbonyl oxygen and the porphyrin  $\pi$ -system or the copper (7). This interaction is blocked by pyridine coordination, so all comparisons in this paper involving the *cis* isomers will be based on spectra taken in the presence of coordinating solvents. Under these conditions the values of J for I and II are smaller than for VII and VIII, indicating that exchange interactions through the C-Clinkages at room temperature increase in the order

$$cis$$
 C=C < saturated <  $trans$  C=C

Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/11/14 For personal use only.

The values of J for III, VI, and IX which contain the pyrrolidine nitroxyl are so small for all three linkages that trends are difficult to ascertain.

We had previously observed that at room temperature in CHCl<sub>3</sub> solution two species were observed in the epr spectrum of V(7) with values of J of 15.5 and 26.0 G. When the solution is cooled, the lines in the spectrum sharpen and at  $-40^{\circ}$ C the values of J are 13 and 38G. At room temperature in 2:1 toluene: pyridine solution and in other solvents tested, a single value of J is observed for V. However, when a solution of V in 2:1 toluene: pyridine is cooled, two species are observed in the epr spectra at 10°C and below (cf. Fig. 3). The values of J for the two isomers at  $-60^{\circ}$ C are 11 and 38G. We previously reported that nmr studies indicated a specific interaction between halogenated solvents and spin-labeled copper porphyrin V (7). It appears that this interaction raises the barrier



to interconversion of the two isomers such that two species are observable at room temperature in halogenated solvents but reduced temperatures are required to observe separate signals for the two



FtG. 3. A, 100 G scan of the nitroxyl region of the X-band epr spectrum of V at  $-29^{\circ}$ C in 2:1 toluene:pyridine solution obtained with 0.4 G modulation amplitude and 20 mW power. B, Computer simulated spectrum with two isomers, J = 34 G (60%) and J = 10.3 (40%). C, Contribution to the simulated spectrum from isomer with J = 34 G. D, Contribution to the simulated spectrum from isomer with J = 10.3 G.

1398



FIG. 4. A, 100 G scan of the nitroxyl region of the X-band epr spectrum of VIII at  $-59^{\circ}$ C in 1:1 toluene:CH<sub>2</sub>Cl<sub>2</sub> solution obtained with 0.4 G modulation amplitude and 10 mW power. B, Computer simulated spectrum with two isomers J = 29 G (65%), and J = 6.3 G (35%). C, Contribution to the simulated spectrum from isomer with J = 29 G. D, Contribution to the simulated spectrum from isomer with J = 6.3 G.

isomers in other solvents. Only a single isomer was observed in the epr spectra of the amides with *trans* olefin linkages, **IV** and **VI**.

When solutions of VIII in 1:1 toluene: $CH_2Cl_2$  or 2:1 toluene:pyridine were cooled, two isomers were observed in the epr spectra at temperatures below  $-45^{\circ}C$ . As shown in Fig. 4 the spectra in 1:1 toluene: $CH_2Cl_2$  at  $-59^{\circ}C$  could be simulated with J = 6 and 30 G. Similarly at temperatures below  $-45^{\circ}C$  two isomers were observed in the epr spectra of VII. At  $-60^{\circ}C$  in 1:1 toluene: $CH_2Cl_2$  the values of J are 4 and 25 G. Low temperature spectra of I-III and IX were also examined, but the values of J are sufficiently small and the low temperature linewidths sufficiently broad that it is difficult to determine whether more than one isomer is present.

## Conformational equilibria

Wojcik and Witanowski have studied halogenated

CAN. J. CHEM. VOL. 60, 1982

acrylamides and concluded that for the *trans* olefin there is a single conformer **XV** (X = Cl, Br, I) (19) with a Z conformation (20) around the  $C_a-C_b$ bond. The barrier to rotation about the amide C--N bond in acrylamides has been observed to be 12-16 kcal/mol, depending on the substituents on the olefin (21) so the rotation about the C--N bond in **IV** would be expected to be slow on the epr time scale at room temperature. By analogy with polypeptide amide linkages it would be expected that the piperidine ring would be oriented *cis* to the carbonyl group (22). These analogies suggest that **XVI** (Y = NH) is the conformation of **IV**, that other



isomers are unlikely, and that the structure is rigid on the epr time scale. The observation of a single isomer of IV with relatively narrow lines over a wide temperature range is consistent with the prediction of structural rigidity. Conformation XVI (Y = NH) gives a zig-zag or "extended W plan" orientation of the bonds between the porphyrin and nitroxyl ring. This bond geometry has frequently been observed to give rise to large long-range couplings in  $\sigma$ -bonded systems in both nmr and epr studies (11, 23).

For the two isomers of the *trans* ester V at low temperature the values of J are 11–13 G and 38 G. The similarity of the larger value of J to that observed for amide IV suggests that the isomer with the larger value of J has conformation XVI (Y = O). The smaller value of J could then be due to an isomer which does not exhibit a zig-zag conformation. Most carboxylic esters have been observed to adopt conformation XVII (24, 25). Oki and Nakashi observed that formate esters exist exclusively in conformation XVII (R' = H)



unless R is sterically demanding (26). When R is *t*-butyl or 3-ethylpentyl 7–9% of the ester exists in conformation **XVIII** at temperatures below – 100°C (26). Since the two isomers of V have approximately equal populations and the piperidine ring is less sterically demanding than a *t*-butyl group, it appears that rotation around the C—O bond is not the source of the isomerism in V. Wennerbeck and

Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/11/14 For personal use only.



Sandstrom have observed that the barrier to rotation about the  $C_a$ — $C_b$  bond in XIX is 8.1 kcal/mol (27). In this "push-pull" ethylene the formal C=Cbond is weaker than a usual C-C double bond and the  $C_a$ — $C_b$  bond stronger than in a typical acrylic ester (27). Thus 8.1 kcal/mol should be substantially greater than the barrier to rotation around the analogous bond in V. Based on a coalescence temperature of 293 K and an energy separation of 20 G the  $\Delta G^{\pm}$  for interconversion of the two isomers of V is 6.3 kcal/mol (28). This would appear to be a reasonable barrier for rotation about the olefin to carbonyl bond in V and suggests that the Zand E conformers about this bond, XVI(Y=O) and XX, are the two isomers which are observable below room temperature.



Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/11/14 For personal use only.

In the spin-labeled complexes with CH<sub>2</sub> linkages between the porphyrin and the amide or ester linkage, VII and VIII, two isomers were observed in the epr spectra at temperatures below  $-45^{\circ}$ C. With a coalescence temperature of about 228 K and an energy separation of 25 G (cf. Table 1) the  $\Delta G^{\pm}$ for interconversion of the isomers is about 5 kcal/mol. This barrier to rotation is sufficiently low that it is difficult to determine which bonds are involved. However, the larger of the two values of J observed for both VII and VIII, 25 and 30G, respectively, is sufficiently similar to that observed for conformation XVI of IV and V to suggest that a zig-zag conformation may also occur for VII and VIII. In the cis isomers I and II the conformation around the olefin linkage precludes a zig-zag conformation consistent with the observation that J is small at all temperatures for I and II. The order cis < saturated < trans observed at room temperature is consistent with rapid rotation in the complexes with saturated linkages between zig-zag conformers like those of the *trans* isomers in which J can be relatively large and conformers like those of the cis isomers in which J is small. It appears that variations in the values of J for I, II, IV, V, VII, and

MORE ET AL.

VIII are consistent with explanations in terms of the stereochemistry around the bonds in the linkage between the porphyrin and nitroxyl rings. Although solvent effects shift the equilibria and the observed activation energies the similarity of behavior in a variety of solvents suggests that the differences in J are not due primarily to solvation effects. The similarity in the values of J for the saturated and unsaturated linkages indicates that in these compounds the metal-nitroxyl interaction is likely dominated by  $\sigma$ -bonding interactions.

## Comparison with other results

In the perspective of the epr background available at the time these studies were initiated, the observation of high resolution electron spin electron spin coupling over a distance of about 12 Å, including a saturated carbon pathway, could be considered surprising. However, such an observation is not out of line with nuclear spin coupling data when the differences between nuclear and electron properties are considered. Over the past five years our studies have shown that AB patterns in epr spectra can be observed due to interaction of a nitroxyl unpaired electron with Cu(II), Ag(II) (29), and vanadyl (30) unpaired electrons. Since the electron-electron interaction is so sensitive to the orbital overlaps in the intervening bonds, it is proving to be a useful technique for detection of isomers which cannot readily be studied by other physical methods (17, 30-34). It is also not surprising that in some cases the values of J have been found to be strongly dependent on both solvent and temperature (6, 17, 18).

The present study emphasizes the importance of  $\sigma$ -bonding pathways for metal-nitroxyl interactions. In a study of spin-labeled pyridine adducts of  $Cu(hfac)_2$ ,  $VO(tfac)_2$ , and  $VO(hfac)_2$  it was observed that spin-delocalization into the pyridine orbitals was dominated by o-bonding pathways for Cu(II) but  $\pi$ -bonding pathways were also important for the vanadyl complexes (30). In the same series of compounds it was also observed that interactions through ester, amide, and Schiff base linkages between the pyridine and nitroxyl rings appeared to correlate with the extent of  $\pi$  bonding (27). In a series of spin-labeled metalloporphyrins the magnitude of the metal-nitroxyl exchange interaction increases in the order VO(IV) < Cu(II)< Ag(II), which parallels other indications of the extent of delocalization of the metal unpaired electron into the porphyrin orbitals (29). It can be anticipated that long range exchange interactions through  $\sigma$  as well as  $\pi$  pathways may be a general feature affecting nitroxyl epr spectra. Unless the possibility of exchange interactions affecting the

#### CAN. J. CHEM. VOL. 60, 1982

		Linkage		
		trans <sup>b</sup>	Saturated	cis <sup>b</sup>
R	Solvent	C=C	CH2CH2	C=C
	CHCl <sub>3</sub> Toluene	IV 19.9	VII 9.3 17.5	<b>I</b> ∼0
	1:1 Toluene:CH <sub>2</sub> Cl <sub>2</sub>		$\begin{cases} \frac{4}{25}(-60^{\circ}C) \end{cases}$	
	2:1 Toluene:pyridine	25.6	14.0	3.5
	2:1 Toluene:pyridine	32 (-60°C)	$\begin{cases} \frac{3}{22} (-60^{\circ} \text{C}) \end{cases}$	6 (-60°C)
	Pyridine	25.6	13.5	3.5
-o-(-)×-o		V	VIII	II
	CHCl <sub>3</sub> {	15.5 26.0	15.5	~0
	CHCI <sub>3</sub>	$^{13}_{38}$ (-40°C)		
	Toluene	23.5	14.5	
	1:1 Toluene:CH <sub>2</sub> Cl <sub>2</sub>		$\begin{cases} \frac{6}{30} (-60^{\circ} \text{C}) \end{cases}$	
	2:1 Toluene:pyridine	20	13	~4
	2:1 Toluene:pyridine	$\frac{11}{38}$ (-60°C)	$\begin{cases} \frac{6}{26} (-55^{\circ}C) \end{cases}$	4 (-50°C)
	Pyridine	20.5	14	
	CU CI	VI	IX	III
	CHCl <sub>3</sub> Toluene	د	5 4 5	550°
	2:1 Toluene:pyridine	5	5.5	~8
	2:1 Toluene:pyridine	5 (-60°C)	4 (-50°C)	10 (-50°C)
aThe designations Direction in the		J.4	0.0	3.9

TABLE 1. Values of the spin-spin coupling constant, J, for spin-labeled copper porphyrins  $I-IX^a$ 

<sup>a</sup>The designations R and linkage refer to the structural diagram for I-IX. Values o (20-22°C) unless a different temperature is noted in parentheses after the value of J. <sup>b</sup>Some of the room temperature values in this column were taken from ref. 7. <sup>c</sup>The large value of J for III in noncoordinating solvents is discussed in ref. 7.

results is somehow excluded, caution should be exercised in applying calculations which assume only dipolar interactions.

The observation of metal-nitroxyl interactions is not limited to lineshape changes of "normal" epr spectra. The interaction also changes the nitroxyl relaxation times. For an accurate measure of this change in relaxation time, time domain (pulsed) epr spectroscopy is needed. However, significant relative measurements can be made using non-linear CW epr, such as a power saturation curve (signal amplitude vs. square root of power) and simulation of saturated spectra. In all of the AB spectra we have obtained, the relaxation times of the nitroxyl signals are substantially altered, such that it is often impossible to saturate the nitroxyl signals at room temperature whereas free nitroxyl spectra generally show effects of saturation above 1-5 mW at the same concentration. In work not yet complete we have also observed nitroxyl relaxation time changes in spin-labeled complexes of Ni(II), Mn(II), Mn(III), and Fe(III). In some cases the unsaturated epr spectrum is almost indistinguishable from a spectrum of the same nitroxyl in the absence of metal, but the power saturation curves clearly reveal the metal-nitroxyl interaction. In these studies it is critically important to distinguish between inter- and intra-molecular interactions.

Beyond the answers these studies are providing to the interpretation of epr in biological systems, and to fundamental magnetic interactions, they also are of importance as measures of the electronic structure and stereochemistry of molecules.

## Acknowledgments

This work was supported in part by the National

1400

Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/11/14 For personal use only.

Institutes of Health (GM21156). Microanalyses were performed by Spang Microanalytical Laboratory. We appreciate the use of the JEOL FX-90Q spectrometer at the Solar Energy Research Institute, Golden, CO, to obtain Fourier Transform 'H nmr spectra. The mass spectrometry results were obtained at the Midwest Center for Mass Spectrometry at the University of Nebraska, supported under the National Science Foundation Regional Instrumentation Facilities Program.

alas Taratar Taratar

Can. J. Chem. Downloaded from www.nrcresearchpress.com by NORTHEASTERN UNIVERSITY on 11/11/14 For personal use only.

- 1. S. S. EATON and G. R. EATON, Coord. Chem. Rev. 26, 207 (1978).
- J. S. HYDE, H. M. SWARTZ, and W. E. ANTHOLINE. In 2. Spin labeling II: theory and applications. Edited by L. J. Berliner. Academic Press, New York. 1979. p. 71.
- J. S. LEIGH, JR. J. Chem. Phys. 50, 2608 (1970).
- 4. J. F. BOAS, P. R. HICKS, J. R. PILBROW, and T. D. SMITH.
- J. Chem. Soc. Faraday II, 74, 417 (1978). G. R. BUETTNER and R. E. COFFMAN. Biochim. Biophys. Acta, 480, 495 (1977).
- K. M. MORE, G. R. EATON, and S. S. EATON. Inorg. Chem. 6 18, 2492 (1979).
- K. M. MORE, S. S. EATON, and G. R. EATON. Inorg. Chem. 7. 20, 2641 (1981).
- 8. M. MOMENTEAU, B. LOOCK, E. BISAGNI, and M. ROUGEE. Can. J. Chem. 57, 1804 (1979).
- V. T. WEE, B. K. SINHA, P. W. TAYLOR, and C. F. CHIGNELL. Mol. Pharm. 12, 667 (1976).
- H. H. RUF and W. NASTAINCZYK. Eur. J. Biochem. 66, 139 (1976).
- 11. M. BARFIELD and B. CHAKRABARTI. Chem. Rev. 69, 759 (1969).
- 12. L. KEVAN and L. D. KISPERT. Electron spin double resonance spectroscopy. Wiley-Interscience, New York. 1976. Chapt. 3.
- M. N. PADDON-ROW and R. HARTCHER. J. Am. Chem. 13 Soc. 102, 671 (1980).
- P. M. BOYMEL, J. R. CHANG, D. L. DUBOIS, D. J.

GREENSLADE, G. R. EATON, and S. S. EATON, J. Am. Chem. Soc. 99, 5500 (1977).

- 15. G. A. BRADEN, K. T. TREVOR, J. M. NERI, D. J. GREEN-SLADE, G. R. EATON, and S. S. EATON. J. Am. Chem. Soc. 99, 4854 (1977).
- S. S. EATON, D. L. DUBOIS, and G. R. EATON. J. Magn. 16. Reson. 32, 251 (1978).
  - B. M. SAWANT, G. A. BRADEN, R. E. SMITH, G. R. EATON, and S. S. EATON. Inorg. Chem. 20, 3349 (1981).
- K. M. MORE, B. M. SAWANT, G. R. EATON, and S. S. 18. EATON. Inorg. Chem. 20, 3354 (1981).
- 19. J. WOJCIK and M. WITANOWSKI. J. Mol. Struct. 49, 249 (1978).
- 20. J. MARCH. Advanced organic chemistry. 2nd ed. McGraw-Hill, New York. 1977, p. 113.
- 21. R. F. HOBSON and L. W. REEVES. J. Mag. Res. 10, 243 (1973).
- 22. R. H. HASCHEMEYER and A. E. V. HASCHEMEYER. Proteins: a guide to study by physical and chemical methods. Wiley, New York. 1973. p. 333. F. W. KING. Chem. Rev. **76**, 157 (1976).
- 23.
- G. I. L. JONES and N. L. OWEN. J. Mol. Struct. 18, 1 (1973).
- A. D. LOPATA and R. L. KUCZKOWSKI. J. Am. Chem. Soc. 103, 3304 (1981) and references therein.
- 26. M. OKI and H. NAKASHI. Bull. Chem. Soc. Jpn. 43, 2558 (1970).
- I. WENNERBECK and J. SANDSTROM. Org. Mag. Res. 4, 783 27. (1972).
- 28. F. A. L. ANET and R. ANET. In Dynamic nuclear magnetic resonance spectroscopy. Edited by L. M. Jackman and F. A. Cotton. Academic Press, New York. 1975. pp. 543-619.
- K. M. MORE, S. S. EATON, and G. R. EATON, J. Am. 29. Chem. Soc. 103, 1087 (1981).
- 30. B. M. SAWANT, A. L. W. SHROYER, G. R. EATON, and S. S. EATON. Inorg. Chem. In press.
- D. L. DUBOIS, G. R. EATON, and S. S. EATON. J. Am. 31 Chem. Soc. 101, 2624 (1979).
- D. L. DUBOIS, G. R. EATON, and S. S. EATON. Inorg. 32 Chem, 18, 75 (1979).
- S. S. EATON, K. M. MORE, D. L. DUBOIS, P. M. BOYMEL, and G. R. EATON. J. Mag. Res. 41, 150 (1980).
- P. M. BOYMEL, G. R. EATON, and S. S. EATON. Inorg. Chem. 19, 727 (1980); 19, 735 (1980).