valent anions or other inhibitors. This spectrum shows a single, broad (~300 Hz) line centered at about 228 ppm from aqueous Cd(II) at infinite dilution. Under no experimental condition at 25 °C have we observed a resonance more narrow than ~250 Hz for uninhibited $^{113}Cd^{II}HCAB$. This is at variance with a recent report[9] of a rather sharp (28 Hz) resonance centered at 146 ppm for $^{113}Cd^{II}HCAB$ at pH 9.6. Our studies on complexes of Cd(II) with heterocyclic nitrogen ligands result in ^{113}Cd resonances in the range 200 to 270 ppm.[10] In the pH* range 7.3-9.7 we have consistently observed a broad peak of ~300 Hz or greater line width, generally centered at about 200 ppm at lower pH* values and about 230 ppm at higher pH* values.

Figure 1B shows the effect of addition of 2 equiv of NaCl. The resonance sharpens to ~60 Hz, and the chemical shift value is 238.6 ppm. The addition of several more equivalents of NaCl has no discernible effect. The effect of a single equivalent of NaCl has not yet been determined. Assuming the presence of a single, tight Cl^- binding site with at least 90% occupancy, we calculate a Cl^- dissociation constant of 7×10^{-4} M or less. With the reported inhibition constant $K_i \geq 2 \times 10^{-2}$ M for $Cd^{II}HCAB$,[6] it is unlikely that Cl^- binds directly to Cd(II) under the conditions of Figure 1B. There is ample evidence for the existence of two strong anion binding sites[3b,11]—one which inhibits enzyme activity, presumably by direct metal binding, and an even tighter but noninhibitory binding site which is probably within ~4 Å of the metal ion. The presence of Cl^- bound near the metal has apparently affected whatever exchange process is responsible for the peak broadening in uninhibited $^{113}Cd^{II}HCAB$.

Any ^{113}Cd resonance of line width less than ~45 Hz in the proton-coupled $^{113}Cd^{II}HCAB$ spectrum must be viewed cautiously considering the presence of five C(2) and C(4) protons[2a] with vicinal Cd-N-C-H spin-coupling constants (10-13 Hz in analogous compounds[12]).

Figure 1C shows the $^{113}Cd^{II}HCAB$ spectrum after addition of 1 equiv of $K^{13}CN$ (≥90% isotopic enrichment, Merck). The resonance splits into a doublet centered at 410 ppm with a separation $J_{CdC} = 1{,}060$ Hz and line width ~50 Hz. This is the largest known cadmium coupling constant and indicates a Cd-C bond of lifetime $>10^{-2}$ s. Addition of a second equivalent of $K^{13}CN$ produced no further change.[13] There has been considerable speculation regarding the existence of stable pentacoordinate Zn(II) in HCA.[14] Considering the larger ionic radius of Cd(II), we conclude that there is probably only one available binding site for CN^- in $Zn^{II}HCAB$. A large excess of $^{13}CN^-$ has not yet been tried on $^{113}Cd^{II}HCAB$, although this was apparently not necessary to produce the pentacoordinate species in $Co^{II}HCAB$.[14b]

Our experiments to date indicate T_1 values of 2-3 s for ^{113}Cd in Cd(II)HCAB based on flip angle optimization. In agreement with a previous report,[9] we find that proton decoupling leads to a loss of the ^{113}Cd signal. These results and our dipolar T_1 and N.O.E. calculations based on five carbon-bound imidazole protons at 2.8 Å distance from $^{113}Cd(II)$ in a molecule having a rotational correlation time of 10^{-8} s (and a negative gyromagnetic ratio for ^{113}Cd) are consistent with a purely dipolar relaxation mechanism.

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Electron Spin Exchange in Rigid Biradicals

Sir:

We have prepared six nitroxyl biradicals in which the extent of conformational change is strongly limited by the rigidity of the structure connecting the radical groups. These biradicals

Table I. Exchange Energies for the Reported Biradicals in Various Solvents

		I		II		III		IV		V		VI		
Solvent	Z	J	a	J	a	J	a	J	a	J	a	J_1	J_2	a
Hexane	60	92.4	14.1	25.1	14.2	32.3	14.2	7.0	14.2	92.4	14.2	6.1	35.6	14.1
Xylene	63	113.2	14.3	27.4	14.3	36.0	14.5	5.7	14.5	103.1	14.2	6.5	33.7	14.4
Acetone	65.7	117.4	14.4	28.4	14.5	36.6	14.6	8.8	14.7	111.4	14.7	8.9	33.2	14.6
DMSO	71	133.2	14.6	30.1	14.6	39.6	14.8	8.0	14.7	126.3	14.8	9.3	34.0	14.8
Chloroform	63.2	155.6	14.8	22.0	14.8	43.9	14.9	7.2	15.0	136.9	15.0	15.4	25.0	14.9
Methanol	83.6	156.5	15.0	29.0	14.9	42.9	15.1	9.4	15.2	143.4	15.0	20.6	22.9	15.2

should prove very useful in understanding the effects of structure, solvent, and temperature on the spin exchange energy. Each biradical was prepared from the known corresponding steroid diketone[1] by the method of Keana[2] and purified by column and preparative TLC on silica gel.

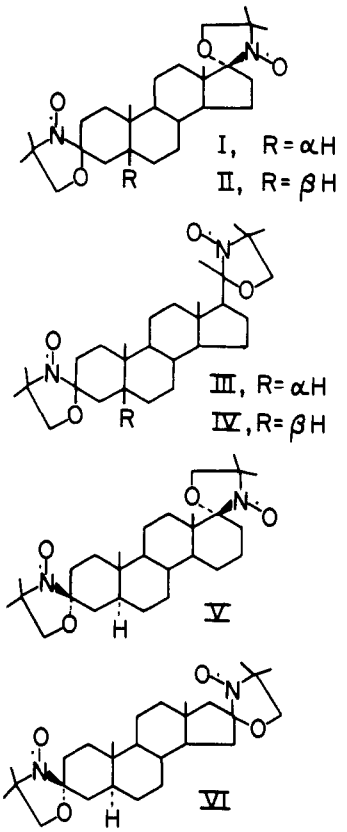
S resonances[3] were clearly observed for all reported biradicals[4] and were used to calculate the exchange energy (J) from the separation ΔH between the strongest (high or low field) S resonance and the center of the spectrum:

$$J = \Delta H - a^2/\Delta H \qquad (1)$$

where a is the hyperfine splitting due to the nitroxyl nitrogen. Values of J ranging from 6 to 172 G were determined using eq 1 and should be accurate to ±1 G.

Prior to crystallization the compounds exhibited multiple sets of S resonances with one clearly predominating.[5] This is expected since four diastereomers are possible for each compound. Upon crystallization the biradicals exhibited only one set of S resonances corresponding to the major set in the mixture with the exception of VI which showed both sets present before crystallization.[6] These are listed in Table I.

The recent studies of Michon and Rassat[7] and others have allowed us to assign structures to the major isomers of I-VI as being the isomers with equatorial N-O groups in the 3 and 17a positions. The stereochemistry at the 17 position of I and II is not known with certainty but is most likely the 17β isomer since the bulky amine group should prefer the less hindered β configuration during the oxazolidine ring formation.[8] Compound VI appears to be a mixture of isomers, differing in configuration at C-16, which cannot be separated by crystallization. Biradicals III and IV with the position-20 nitroxyl groups at first may not appear "rigid", but space-filling models indicate that rotation about the C_{17}-C_{20} bond is quite hindered. Support for this conclusion is given by the observation that the much less bulky acetyl side chain of 20-ketopregnane has a preferred conformation.[9]



It can be seen from Table I that changing from the 5α configuration as in the case of I and III to the 5β configuration in the case of II and IV results in a 4- to 5-fold decrease in J. Since the distance between N-O groups is less in the 5β isomers, this would seemingly imply that an indirect, through-bond, exchange mechanism may be important in these biradicals. It is interesting to note that the extent of W-plan arrangement[10] of the σ bonds is greater for the 5α isomers and, if the W-plan arrangement is important to indirect exchange as it is for spin coupling in NMR, the higher values of J for the 5α isomers are consistent with an indirect exchange mechanism.

By comparing biradicals I with III and IV it appears that increasing the number of σ bonds separating the N-O groups by one results in a 3- to 4-fold decrease in J. This also appears to be consistent with a through-bond exchange mechanism.[11]

The exchange energy of I and V are similar as would be expected since the connecting bridges are very much alike. However, by comparing biradical I with VI (either isomer), it appears that J is extremely sensitive to the position of the oxazolidine ring on the steroid bridge and that predictions of J based on structure must be approached with caution.

The effect of solvent on these rigid biradicals is similar to that reported for flexible biradicals.[12] The exchange energy and hyperfine interaction, a, tend to increase with increasing solvent polarity as measured by the Kosower Z value. One isomer of VI shows a decrease in J with increasing Z which complicates the discussion of the effects of solvent on J. However, the rigid nature of these biradicals makes it unlikely that conformational changes alone are responsible for the large solvent effects. One possible explanation is that the changes in J are directly related to changes in the distribution of the unpaired electrons as is reflected in the dependence of a on solvent polarity.[13] Indeed the exchange energy may be found to be useful as a sensitive solvent polarity probe.

The exchange energies of the biradicals were also studied as a function of temperature in hexane, xylene, and chloroform. Variation of J with temperature was essentially linear over the range examined.[14] Temperature coefficients were small and varied from −0.1 to +0.1 G/°C with the exception of I in chloroform which showed a coefficient of −0.27 G/°C. These small changes in J with temperature are possibly due to changes in solvation with temperature since conformational changes would be expected to result in modulation of J and consequently S-resonance broadening.[12]

For the most part the results have been considered to be more in line with the indirect, through-bond mechanism since many of the results appear inconsistent with the direct exchange mechanism. However, it is possible that both exchange mechanisms are of importance and together lead to the rather complex dependence of J on structure, solvent, and temperature.

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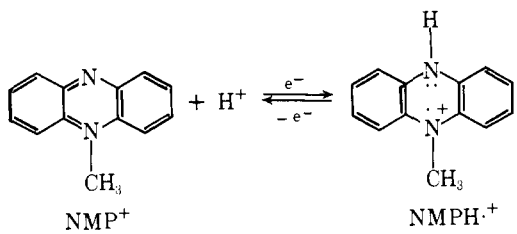
Nuclear Magnetic and Electron Spin Resonance Evidence for the Strength and Site of Attachment of *N*-Methylphenazonium Cation Radical to Sodium Dodecyl Sulfate Micelles[1]

Sir:

N-Methylphenazonium (NMP^+) cation salts[2] have been shown to be highly efficient promoters of cyclic photophosphorylation in photosynthetic systems.[3] Ample evidence exists to show that artificial cofactors, such as NMP^+, which are capable of stimulating the production of adenosine triphosphate (ATP) are lipophilic[4] and are able to translocate protons across a membrane.[5] The *N*-methylphenazonium cation radical ($NMPH^{+}\cdot$), obtained by one-electron reduction of NMP^+ (reaction 1), is in principle capable of carrying a proton across



(1)

a membrane since, on oxidation of $NMPH^{+}\cdot$, a proton is released. An a priori objection to involvement of $NMPH^{+}\cdot$ in photophosphorylation is that as a cation it would be expected to be hydrophilic and should therefore not readily interact with the hydrophobic part of the membrane. In an effort to understand the mechanism by which NMP^+ stimulates cyclic photophosphorylation we have now investigated the interaction of $NMPH^{+}\cdot$ with sodium dodecyl sulfate (NaLS) micelles. We have chosen micelles as perhaps the simplest model for the membrane-water interface. In this communication we report ESR and NMR results which bear on the strength of the interaction and the site of attachment of $NMPH^{+}\cdot$ to the NaLS micelle.

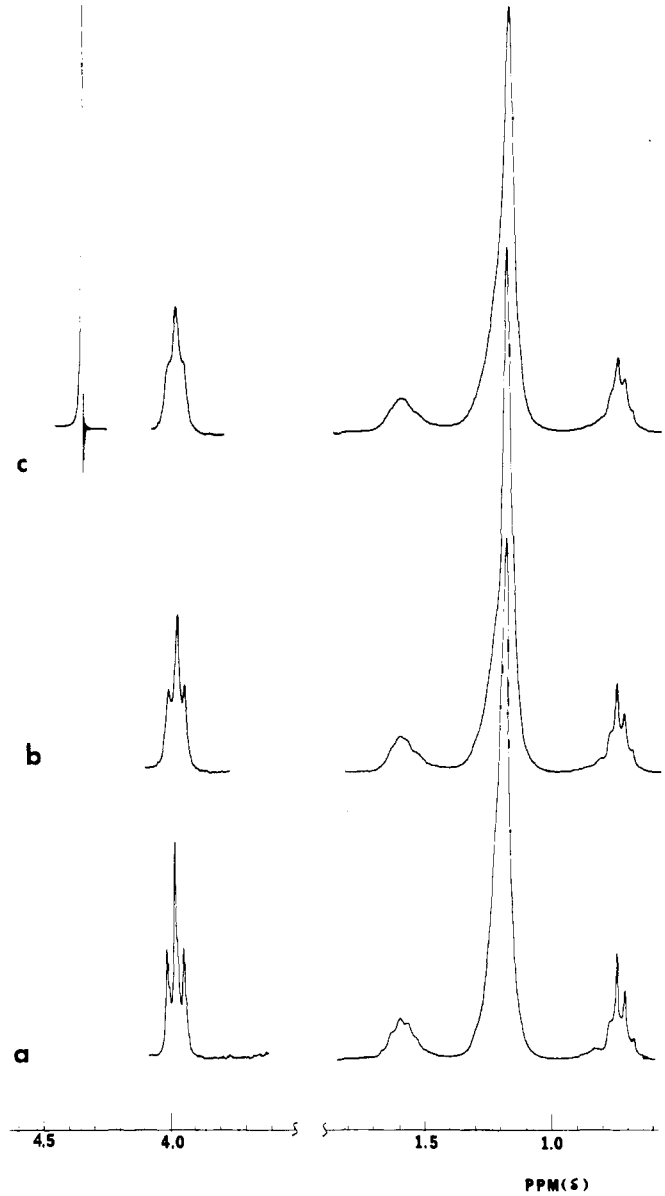


Figure 1. The 220-MHz NMR spectrum (Varian model HR220) of 0.1 M NaLS solutions in D_2O containing 10^{-2} M NMP^+ under conditions of varying photolysis time. There is no change in the HDO resonance during the course of these experiments and it is accordingly shown only once. The chemical shift indicated is in parts per million from TMS (external standard). NMR conditions for all spectra: sweep time 500 s, sweep width 1000 Hz, receiver gain 30 db, rf field level 20 db, signal amplitude 8.0 (for α-CH_2) and 3.2 (other resonances), frequency response 2 Hz. Photolysis time: (a) 0 s, (b) 5 s, (c) >3 min.

The ESR spectrum of $NMPH^{+}\cdot$ in water (produced either by visible light photolysis or sodium borohydride reduction of solutions of NMP^+ is rich in hyperfine structure, consisting of 5832 theoretical lines, over one hundred of which are resolved.[6-8] In contrast, the ESR spectrum of $NMPH^{+}\cdot$ in 0.1 M NaLS shows nine broad lines in which the small hyperfine