in the x-ray isomorphous series.

The structure of polymeric $Cd(SCH_2CH_2OH)_2$ contains both tetrahedral and trigonal bipyramidal, CdS_4 and CdS_5 sites.[11] The CdS_4 site is distorted with a range in the S–Cd–S angles from 102 to 123°. The average Cd–S bond length of 2.56 Å is comparable with that observed in I. A distorted CdS_4 unit also is found in the tetrahedral site of the $[Cd_{10}(SCH_2CH_2OH)_{16}]^{4+}$ cluster[12] and shows a range in the S–Cd–S angles of 100–118°. The Cd–S bond lengths in this site are 2.50 and 2.53 Å.

The Zn–S bond lengths in II compare with the values reported for synthetic sphalerite[13] (2.340 Å) and the tetrahedral ZnS_4 units[14] in the dimeric bis(dimethyldithiocarbamato)-zinc(II) (2.312 (6) to 2.429 (6) Å). In this severely distorted tetrahedron, S–Zn–S angles range from 76.4 to 136.5°. A ZnS_4 geometry similar to that found in the present structure has been reported[15] for the bis(trithioperoxycumato)zinc(II) complex, where the Zn–S bond lengths are 2.316 (3) and 2.327 (2) Å, and the S–Zn–S angles range from 1І7.9 (1) to 96.7 (1)°. A less distorted ZnS_4 tetrahedron is found[16] in the structure of the polymeric bis(ethylxanthato)zinc(II) complex. In this molecule the Zn–S bond lengths range from 2.337 (10) to 2.369 (8) Å and the S–Zn–S angles range from 112.1 (3) to 102.6 (3)°.

The only reported four-coordinate nickel(II) complex with sulphur ligands that assumes a tetrahedral structure is the bis(imidotetramethyldithiodiphosphino-S,S)nickel(II) complex.[17] The structure of this compound has been determined.[18] The mean Ni–S distance of 2.282 (12) Å is very similar to that found in III, 2.287 (12) Å. Both distances are significantly longer than the corresponding distances 2.10–2.24 Å found in nickel(II) chelate complexes containing a square-planar center.[18]

The angular distortions in III are more pronounced than all of the other complexes, with a span in the S–Ni–S angles from 124.9 (2) to 92.0 (2)°. By comparison the S–Ni–S angles in the bis(imidotetramethyldithiophospino-S,S)nickel(II) complex range from 117.0 (1) to 106.0 (1)°.

The Mn–S bond lengths in V are similar to those reported in the MnS_4 tetrahedron in bis(tetraphenyldithiomidophosphinato)manganese(II), for which an average Mn–S bond length of 2.443 (12) Å was reported.[19] In the latter complex the MnS_4 unit is only slightly distorted and the deviations of the S–Mn–S angles from 109.5° range from +2.6 to −3.2°.

Ligand yield or charge-transfer absorptions in the electronic spectra of metalloenzymes and their metal substituted analogues have been used for the identification of both the environment and geometry at the active site. In Co–LADH absorptions maxima associated with ligand field transitions have been observed[8] at 655 nm (ϵ 1330), 730 (800), and between 1000 and 1800 (270–540). A charge-transfer absorption also is observed at 340 nm (ϵ 6500). In the electronic spectrum of the $Co(SC_6H_5)_4{}^{2-}$ in acetonitrile solution, similar absorptions are observed at 635 nm (sh, $\epsilon \simeq 500$), 690 (920), 730 (770), and 1450 (211). Two charge-transfer absorptions are observed at 285 nm (sh, ϵ 31 500) and 420 (3000). The position of the 420 nm absorption is lower in energy than bands of similar intensity found in bis(O-xylyl-α,α'-dithiolato)cobaltate(II)[20] (355 nm (ϵ 3450)), Co(II) substituted stellacyanin[9] (355 nm ($\epsilon \sim$1200)), and the 3,5-dimethyl-1-pyrazolyl borate-5-cysteinylcobalt(II) complex[21] (388 nm). In all of these complexes the absorptions near 350 nm are assigned to S → Co charge transfer.

The electronic spectrum of the $Cd(SC_6H_5)_4{}^{2-}$ complex shows a band at 340 nm (ϵ 4200) with a shoulder at 355 nm.

The absorptions attributed to the phenyl groups obscure the region between 260 and 280 nm. The unusually high intensity ($\epsilon \sim$58 000) and broad shape of the phenyl multiplet, compared

with those observed in the Co(II) complex ($\epsilon \sim$40 000) suggest the underlying presence of a charge-transfer band in this region. In Cd–LADH the Cd–S absorption is centered[8] at 245 nm (ϵ 10 200). In cadmium metallothionein the same absorption is observed[7] as a pronounced shoulder at 250 nm ($\epsilon \sim$14 000). The unusually intense (ϵ 48 000) and broad phenyl ring absorptions observed in the spectrum of the $Zn(SC_6H_5)_4{}^{2-}$ complex also suggest the presence of a charge-transfer absorption in that region. In Zn–LADH the Zn–S charge-transfer absorption occurs[8] at 275 nm.

Further studies are directed toward the synthesis and structural characterization of aliphatic mercaptide complexes of the type $M(SR)_4{}^{2-}$ with optically and sterically acceptable counterions. It is anticipated that such studies will assess the importance of packing forces in the structures of the $M(SR)_4{}^{2-}$ complexes and at the same time will allow for the unobstructed detection of charge-transfer electronic absorptions.

References and Notes

(1) D. G. Holah and D. Coucouvanis, *J. Am. Chem. Soc.*, **97**, 6917 (1975).
(2) A. Kostikas, V. Petrouleas, A. Simopoulos, D. Coucouvanis, and D. G. Holah, *Chem. Phys. Lett.*, **38**, 582 (1976).
(3) D. Coucouvanis, D. Swenson, N. C. Baenziger, D. G. Holah, A. Kostikas, A. Simopoulos, and V. Petrouleas, *J. Am. Chem. Soc.*, **98**, 5721 (1976).
(4) L. H. Jensen, *Annu. Rev. Biochem.*, **43**, 461 (1974).
(5) H. Eklund, B. Nordström, E. Zeppezauer, G. Söderlund, I. Ohlesson, T. Boiwe, and C. I. Bränden, *FEBS Lett.*, **44**, 200 (1974); H. Eklund, B. Nordström, E. Zeppezauer, E. Soderlund, I. Ohlsson, T. Boiwe, B. O. Soderberg, O. Tapia, and C. I. Branden, *J. Mol. Biol.*, **102**, 27 (1976).
(6) H. Rupp W. Voelter. and U. Weser, *FEBS Lett.*, **40**, 176 (1974).
(7) J. H. R. Kägi and B. L. Vallee, *J. Biol. Chem.*, **236**, 2435 (1961); J. H. R. Kägi, S. R. Himmelhoch, P. D. Whanger, J. L. Bethune, and B. L. Vallee, *ibid.*, **249**, 3537 (1974).
(8) D. E. Drum and B. L. Vallee, *Biochem. Biophys. Res. Commun.*, **41**, 33 (1970).
(9) D. R. McMillan, R. A. Holwerda, H. B. Gray, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1339 (1974).
(10) C. Scheringer, *Acta Crystallogr.*, **16**, 546 (1963).
(11) H. B. Bürgi, *Helv. Chim. Acta*, **57**, 513 (1974).
(12) P. Strickler, *Chem. Commun.*, 655 (1969).
(13) W. Swanson and J. Fugat, *Natl. Bur. Stand. (U.S.) Circ.*, **539**, 2, 16 (1953).
(14) H. P. Klug, *Acta Crystallogr.*, **21**, 536 (1966).
(15) J. P. Fackler, Jr., J. A. Fetchin, and D. C. Fries, *J. Am. Chem. Soc.*, **94**, 7323 (1972).
(16) T. Ikeda and H. Hagihara, *Acta Crystallogr.*, **21**, 919 (1966).
(17) A. Davison and E. S. Switkes, *Inorg. Chem.*, **10**, 837 (1971).
(18) M. R. Churchill, J. Cooke, J. P. Tennessey, and J. Wormald, *Inorg. Chem.*, **10**, 1031 (1971).
(19) O. Sliman and H. B. Gray, *Inorg. Chem.*, **13**, 1185 (1974).
(20) R. W. Lane, J. A. Ibers, R. B. Frankel, G. C. Papaefthymion, and R. H. Holm, *J. Am. Chem. Soc.*, **99**, 84 (1977).
(21) J. S. Thompson, T. J. Marks, and J. A. Ibers, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 3114 (1977).
(22) Alfred P. Sloan Fellow, 1972–1974.

D. Swenson, N. C. Baenziger, D. Coucouvanis[*22]

Department of Chemistry, University of Iowa
Iowa City, Iowa 52242
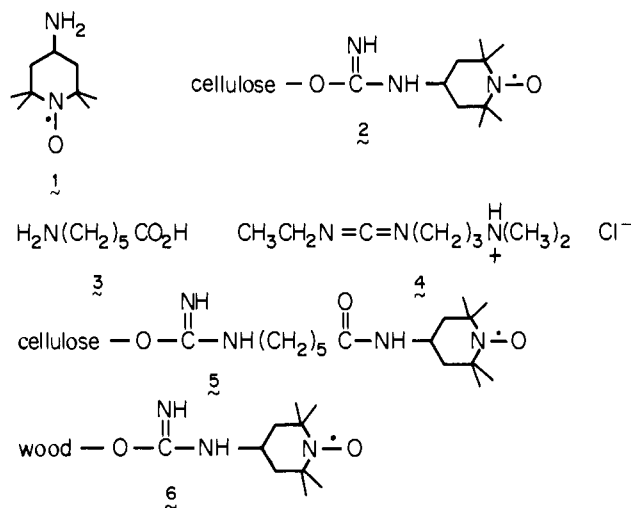*Received October 3, 1977*

Nitroxide Spin Labels as Surface and Structural Probes for Wood and Cellulose and Its Derivatives

Sir:

As a renewable source of carbon and a substance with numerous important industrial applications, cellulose has been widely investigated;[1] nevertheless, its properties and, in particular, its mode of interaction with solvents (including water)

and with other components of wood, of which it is the principal constituent, are not at all thoroughly understood. Following earlier studies of biological membranes and proteins,[2] we[3] and others[4] have found the spin labeling technique to be of considerable utility in the investigation of higher order structure in saccharidic systems, both soluble and insoluble, and we report herein preliminary results which indicate that this approach may both yield insights into the internal structure of cellulose aggregates and the pore model[5] for diffusion of ions and small molecules therein, and also provide a method for the study of other surfaces.[6]
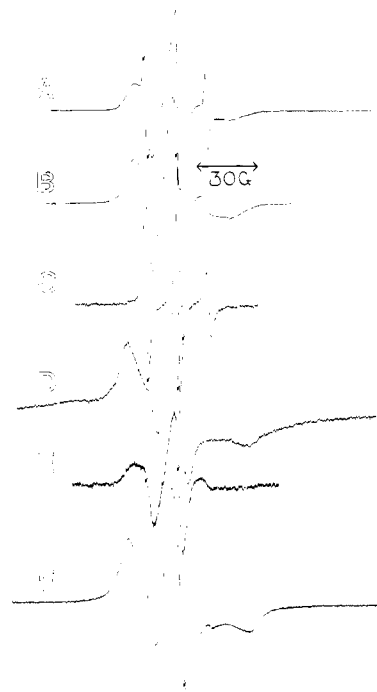
The EPR spectrum of cellulose powder (Whatman CF11), spin labeled at the hydroxyl functionalities (**2**), is shown in Figure 1B. Labeling was achieved by reaction with cyanogen bromide in aqueous sodium carbonate,[7] pH 11, followed by removal of excess reagent on a sintered-glass filter, and addition of an aqueous solution of 4-amino-2,2,6,6-tetramethyl-piperidine-1-oxyl (**1**) in 0.1 M bicarbonate buffer, pH 8, for

Figure 1. EPR spectrum of (A) compound **2** in the presence of unbound nitroxide (gain 0.6), (B) compound **2** (gain 1), (C) compound **5** (gain 16), (D) compound **2** in the presence of 2 M $Ni(H_2O)_6^{2+}$ (gain 16), (E) compound **2** after reduction using $Fe(H_2O)_6^{2+}$ (gain 13), (F) compound **6** (gain 2), in aqueous suspension recorded at 28 °C with a Varian E-3 spectrometer. Relative gains at 5-mW power, 1G modulation amplitude are given in parentheses.

12 h. The removal of unreacted spin label by washing with buffer solutions, or with urea, could be followed by EPR, since unbound label gave a readily recognizable sharp signal superimposed on the relatively broad spectrum due to bound label (Figure 1A). Repeated activations of this type did not significantly increase the extent of labeling. The broad "bound" signal in a typical sample of **2**, upon integration and comparison with standard solutions of **1**, indicated the presence of an *average* of one nitroxide molecule per 160 glucose residues, so that a gross disruption of the native cellulose structure was not to be expected.

Further experiments involved the interposition of a "spacer arm" between the cellulose matrix and the nitroxide label as follows. Cyanogen bromide activated cellulose was initially reacted with ε-aminocaproic acid (**3**) and, following the removal of unreacted amino acid as before, an amide linkage was formed at pH 5 between label **1** and the exposed carboxylate group with the aid of 1-ethyl-3-(*N,N*-dimethylaminopropyl)-carbodiimide hydrochloride (**4**) to give **5**, the spectrum of which is shown in Figure 1C.

Comparison of Figures 1B and 1C reveals two important features. First, as might be expected,[3a] the nitroxide moiety of **5** enjoys a substantially greater freedom of rotational reorientation than that of **2**. Second, there is some indication in the spectrum of **2** that two partially resolved spectral components may be present, indicating that populations of nitroxides may be present in different sites.[8] The existence of two types of surface in purified cellulose has previously been postulated on the basis of NMR relaxation of solvent resonances,[9] nitroxide adsorption,[10] and chemical labeling.[11] The apparent absence of two spectral components from Figure 1C may indicate either that the label in **5**, when extended to a distance

of ≤~12 Å from the surface (as compared with ≤~3.5 Å in **2**), is no longer sensitive to surface geometry or that the larger spacer molecule fails to penetrate to less accessible, more hindered sites.

We have tested this "two-site" model using two separate series of manipulations of the labeled matrices **2** and **5**. It has previously been shown that paramagnetic "probe" ions in solution cause exchange broadening of EPR lines both in solutions of spin labels[12] and in spin labeled macromolecules.[13] As the concentration of probe ion increases, the limit of detection of nitroxide signal is approached. The spectrum of **2** in the presence of 2 M $Ni(H_2O)_6^{2+}$, a concentration sufficient to broaden the same number of nitroxide spins in solution beyond the detection limit, is shown in Figure 1D; a population of radicals whose spectrum is rather sharper than the average (that is, whose rotational reorientation is more rapid) has been selectively relaxed and, hence, broadened, while a relatively immobile population of radicals remains, apparently inaccessible or almost inaccessible to the probe ion. The splitting (~30 G) between the center and high-field peaks of Figure 1D is only slightly greater than that for the broader component of Figure 1B. In contrast, identical treatment of **5** gave no evidence of differential relaxation, the spectrum disappearing in the presence of the same concentration of $Ni(H_2O)_6^{2+}$. When nickel ions were replaced by $Fe(CN)_6^{4-}$, no alteration in line shape was detectable in **2**, while the spectrum of **5** was again almost completely broadened.

In the second type of experiment, the accessibility of spin labels in **2** and **5** was tested using reducing agents. Both 0.1 M sodium ascorbate, pH 7, and 0.2 M sodium dithionite, when added either to **2** or to **5**, reduced all nitroxides immediately, causing disappearance of the EPR signal. However, placing 1.0 M $Fe(H_2O)_6^{2+}$ ions in solution in the presence of **2**, followed by washing to remove ferric ions (which themselves show a room temperature EPR signal), caused only partial reduction

of the nitroxide functionalities; the residual spectrum is shown in Figure 1E, and displays approximately the same line shape as Figure 1B at lower signal to noise. In this case the reaction took ~2 h to completion. When nickel ions in solution were added to this partially reduced material, the resultant EPR spectrum had a line shape similar to that of Figure 1D, again with reduced signal to noise ratio. Finally, addition of ferrous ions to **5** resulted in complete reduction of all spin labels.

The results of using divalent metal ions as both relaxing and reducing agents are consistent with the presence of the spin labels in **2** in more than one distinct site on the cellulose surface, as previously postulated. It would seem that, despite their similarity, ferrous and nickelous ions "select" nonequivalent populations of labels, the former a population of mobility similar to the average and the latter a population of mobility rather greater than average. The latter result, however, should be viewed with caution since the "residual" signal may still be partially broadened. Whether this difference simply reflects differences in the spatial and distance dependence of electron transfer (reduction) vs. electron exchange, or something about the cellulose surface, is not clear. The observation that a negligible proportion of the spins in **2** is accessible to ferricyanide ions suggests that most of the labels are present in pores that are small relative to the size of this ion. The fact that all nitroxides present in **5** are accessible to all of the reagents used in the study provides an additional pointer to the pore diameters involved, and further experiments will allow their quantitation.

The effective diameter of a pore may also be reduced by a surface layer of hydrogen-bonded water. It seems likely that participation of these cellulose surface water molecules in the first hydration sphere of $Ni(H_2O)_6{}^{2+}$ and $Fe(H_2O)_6{}^{2+}$, and even binding of these ions to cellulose hydroxyls or groups introduced during labeling, occurs during their penetration into the cellulose matrix. This cannot occur with the ferricyanide ion. A binding phenomenon of this kind may best explain the differences between the effects of metal ions and those of ascorbate and dithionite on **2**;[14] however, size, charge, hydrophilicity and even counterion variation are all important parameters which merit further investigation.

We are also investigating the application of these techniques to the study of soluble and insoluble cellulose derivatives, wood pulp, and native wood. The spectrum of the latter, provided in the form of a 50-μ vertical microtome section of an annual growth ring and labeled by the cyanogen bromide procedure (**6**), is shown in Figure 1F. It can be seen that the effect of lignin and other wood constituents has been to further immobilize the label compared with the purified cellulose shown in Figure 1B. Further decreases in mobility accompany drying of labeled samples and a detailed discussion of these systems will follow.

In conclusion, it is appropriate to remark upon the possible application of these methods to the study of other surfaces. In our hands, the combined use of variable-length "spacer arm" and chemical and physical manipulation of the labeled material has substantially facilitated the interpretation of EPR data, both in cellulose and in other systems.[3a] The enormous current interest in immobilized reagents both of a biological[15] and of a synthetic[16] nature fully justifies the development of techniques for the study of matrices, derivatized matrices, and their interaction in heterogeneous systems with species in solution.

References and Notes

(1) R. L. Whistler, Ed., *Methods Carboyhydr. Chem.* **3** (1963); J. C. Arthur, Jr., Ed., *ACS Symp. Ser.,* **No. 48** (1976).
(2) L. J. Berliner, Ed., "Spin Labelling, Theory and Applications", Academic Press, New York, N.Y., 1976.
(3) (a) J. D. Aplin and L. D. Hall, *J. Am. Chem. Soc.,* **99**, 4162 (1977); (b) J. D. Aplin and L. D. Hall, *Carbohydr. Res.,* in press.
(4) D. Gagnaire and L. Odier, *Bull. Soc. Chim. Fr.,* 2325 (1974); J. Martinie, J. Michon, and A. Rassat, *J. Am. Chem. Soc.,* **97**, 1818 (1975).
(5) S. P. Rowland, *ACS Symp. Ser.,* **No. 49**, 20 (1976).
(6) Spin labeling has already been successfully used in the study of low-density polyethylene (J. R. Rasmussen, D. C. Bergbreiter, and G. M. Whitesides, *J. Am. Chem. Soc.,* **99**, 4746 (1977)) and silica surfaces (N. Sistovaris, W. O. Riede, and H. Sillescu, *Ber. Burserges. Phys. Chem.,* **79**, 882 (1975); S. L. Regen, *J. Am. Chem. Soc.,* **97**, 3108 (1975)).
(7) S. C. March, I. Parikh, and P. Cuatrecasas, *Anal. Biochem.,* **60**, 149 (1974).
(8) Though similar line shapes can arise as a result of very anisotropic reorientation of nitroxides (R. P. Mason, C. F. Polnaszek and J. H. Freed, *J. Phys. Chem.,* **78**, 1324 (1974)).
(9) M. F. Froix and R. Nelson, *Macromolecules,* **8**, 726 (1975); Y. Ogiwara and H. Kubota, *J. Appl. Polym. Sci.,* **13**, 1989 (1969).
(10) R. M. Marupov, P. Ky. Bobojanov, N. V. Kostina, and A. B. Shapiro, *Biofizika,* **21**, 825 (1976).
(11) S. P. Rowland and E. J. Roberts, *J. Polym. Sci., Part A-1,* **10**, 2447 (1972).
(12) E. Odgaard, T. B. Melo, and T. Henriksen, *J. Magn. Reson.,* **18**, 436 (1975); K. M. Salikhov, A. B. Doctorov, Yu. N. Molin, and K. J. Zamaraev, *ibid.,* **5**, 189 (1971).
(13) A. V. Kulikov and G. I. Likhtenstein, *Adv. Mol. Relax. Int. Proc.,* **10**, 47 (1977); G. I. Likhtenstein, "Spin Labels in Molecular Biology," Nauka, Moscow, 1974; A. D. Keith and W. Snipes, *Science,* **183**, 666 (1974).
(14) In which case broadening of the nitroxide spectrum may no longer be mediated exclusively by an exchange mechanism (T. Sarna, J. S. Hyde, and H. M. Swartz, *Science,* **192**, 1132 (1976)).
(15) *Methods Enzymol.,* **44**, (1976).
(16) J. I. Crowley and H. Rapoport, *Acc. Chem. Res.,* **9**, 135 (1976).

Laurance D. Hall, J. D. Aplin*

Department of Chemistry
The University of British Columbia
Vancouver, British Columbia, Canada V6T 1W5
Received December 2, 1977

On the Role of Steric Effects in the Perturbational Molecular Orbital Method of Conformational Analysis[1]

Sir:

The perturbational molecular orbital (PMO) method of conformational analysis[2] proceeds in two stages. In the first stage, a polyatomic system A–B is disconnected conceptually into fragments, e.g., A and B. Then the nodal properties and relative energies of "important" orbitals of these fragments are somehow deduced, and two-orbital two-electron (stabilizing) and two-orbital four-electron (destabilizing) interactions between these "important" orbitals are estimated. When this process is performed for several relative orientations of the fragments (which correspond to different conformations of A–B), the eventual result is a prediction concerning the preferred conformation, and some insight concerning the factors responsible for this preference.

We have recently described[3] a quantitative procedure for the computation of orbital interaction energies, in which the required information concerning the fragment orbitals is generated from the ab initio wave function of the molecule A–B. The π-type orbital interaction energy differences calculated by this procedure parallel rather closely the calculated conformational energy differences. This has permitted a test of some of the usual assumptions of the PMO method. One of these is that the result of the analysis is independent of the fragmentation mode; i.e., in the case of a molecule A–B–C, the same result is expected for each of the choices A···B–C, A–B···C and A···B···C. A second assumption is that the PMO method should fail when "steric effects" become dominant.