

temperature and allowing the solution to warm up to room temperature converts the dioxygen adduct back to $[\text{Cu}_2(\text{N4PY2})(\text{CO})_2]^{2+}$ (spectrum 3).^{9c} The process can be repeated, and as estimated from the change in absorption intensities, less than 10% decomposition occurs over the five cycles shown. The O_2 released from $[\text{Cu}_2(\text{N4PY2})(\text{O}_2)]^{2+}$ by reaction with CO is identified by sweeping the solution with CO and bubbling the gas into an aqueous alkaline pyrogallol test solution which forms an intense brown color in the presence of O_2 .¹⁰

$[\text{Cu}_2(\text{N4PY2})]^{2+}$ and $[\text{Cu}_2(\text{N4PY2})(\text{O}_2)]^{2+}$ can also be directly interconverted through several cycles (without appreciable decomposition) by alternate oxygenation and deoxygenation by warming under vacuum. These experiments can be monitored spectrophotometrically. The reversible binding of CO and O_2 by Cu(I) complexes of N3PY2 and N5PY2 has also been confirmed by similar cycling experiments.⁷

The extremely intense and multiple UV-vis absorptions observed for these O_2 complexes (Table I) have not, to our knowledge, been reported in any other Cu(I)/ O_2 synthetic systems.^{3,11,12} Also, the spectra of $[\text{Cu}_2(\text{NnPY2})(\text{O}_2)]^{2+}$ bear close similarities to the spectrum of the copper metalloprotein hemocyanin (Hc, an O_2 carrier); where oxy-Hc exhibits absorptions at 345 nm (ϵ 20 000 $\text{m}^{-1} \text{cm}^{-1}$), 570 nm (ϵ 1000 $\text{M}^{-1} \text{cm}^{-1}$), and a CD feature at 485 nm, all assigned to peroxo to Cu(II) LMCT transitions.¹³⁻¹⁶ The occurrence of strong charge-transfer transitions (suggestive of Cu(II)) in the spectra of $[\text{Cu}_2(\text{NnPY2})(\text{O}_2)]^{2+}$, the observed 2:1 Cu/ O_2 stoichiometry, and the reversibility of O_2 and CO binding all suggest that these dioxygen complexes are probably best described as peroxodicopper(II) compounds.

Since the ligands NnPY2 have no potential Cu...Cu bridging group, the present results indicate that a Cu...Cu bridging ligand (besides perhaps O_2^{2-} itself) is not a prerequisite for systems capable of binding CO and O_2 reversibly¹² and for exhibiting spectral features reminiscent of oxyhemocyanin.¹⁷ The implications of the present findings regarding the occurrence, spectroscopic effects, and functional role of the proposed "endogenous" bridge^{13,16} in oxy- or deoxy-Hc remain to be determined. While a recent X-ray structural determination of deoxy-Hc showed that each Cu(I) center appears to be coordinated by three imidazole ligands (Cu...Cu = 3.8 ± 0.4 Å), it did not, unfortunately, settle questions concerning an "endogenous" bridging ligand.¹⁹

Further physical and chemical characterization of these novel dioxygen-copper complexes is in progress.

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(19) (a) Gaykema, W. P. J.; Hol, W. G. J.; Vereijken, J. M.; Soeter, N. M.; Bak, H. J.; Beintema, J. J. *Nature (London)* **1984**, *309*, 23-29.

Indirect, Negative Heteronuclear Overhauser Effect Detected in a Steady-State, Selective $^{13}\text{C}\{^1\text{H}\}$ NOE Experiment at Natural Abundance[†]

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It has recently been shown that both selective $^{13}\text{C}\{^1\text{H}\}$ NOE measurements¹⁻¹⁰ and nonselective two-dimensional (2D) NOE¹¹⁻¹³ (cross-relaxation) spectroscopy are promising techniques for the determination of carbon-proton distances in organic molecules in solution.

In the present paper we show that the quasi-simultaneous saturation¹⁴ of all ^{13}C satellite lines of a proton in a ^{13}C isotopomer is an efficient new technique for selective $^{13}\text{C}\{^1\text{H}\}$ NOE measurements.¹⁵ More importantly, we have unambiguously detected a negative, indirect $^{13}\text{C}\{^1\text{H}\}$ NOE at a protonated carbon when the lines of a not directly bound proton were saturated.

As a model system, (-)- α -hydrastine (1) (Figure 1) has been investigated. Assignments of proton and carbon resonances are supported by the basic chemical shift correlation methods.¹⁶⁻¹⁸ Nonprotonated carbons have been assigned via long-range $^{13}\text{C}\{^1\text{H}\}$ spin-spin couplings in a 2D DEPT experiment.¹⁹⁻²² The preferred

[†] We dedicate this manuscript to the memory of Peter Kerekes.

(10) Gordon, A. J.; Ford, R. A. "The Chemist's Companion, A Handbook of Practical Data, Techniques and References"; Wiley: New York, 1972; p 440.

(11) A dicopper(II) complex containing a bridged water molecule and coordinated peroxo ligand was recently reported: Thompson, J. S. *J. Am. Chem. Soc.* **1984**, *106*, 8308-8309.

(12) Other instances of copper complex systems without apparent Cu...Cu bridging ligands and that are reported to bind O_2 reversibly are: (a) Thompson, J. S. *J. Am. Chem. Soc.* **1984**, *106*, 4057-4059. (b) Merrill, C. L.; Wilson, L. J.; Thamann, T. J.; Loehr, T. M.; Ferris, N. S.; Woodruff, W. H. *J. Chem. Soc. Dalton Trans.* **1984**, 2207-2221 and references therein. (c) Casella, L.; Silver, M. S.; Ibers, J. A. *Inorg. Chem.* **1984**, *23*, 1409-1418. (d) Nishida, Y.; Takahashi, K.; Kuramoto, H.; Kida, S. *Inorg. Chim. Acta* **1981**, *54*, L103-L104. (e) Bulkowski, J. E.; Burk, P. L.; Ludmann, M.-F.; Osborn, J. A. *J. Chem. Soc., Chem. Commun.* **1977**, 498-499. (f) Agnus, Y.; Louis, R.; Weiss, R. *J. Am. Chem. Soc.* **1979**, *101*, 3381-3384.

(13) (a) Solomon, E. I. In "Metal Ions in Biology"; Spiro, T. G., Ed.; Wiley-Interscience: New York, 1981; Vol. 3, pp 41-108. (b) Solomon, E. I.; Penfield, K. W.; Wilcox, D. E. *Struct. Bonding (Berlin)* **1983**, *53*, 1-57.

(14) (a) Lontie, R.; Witters, R. *Met. Ions Biol. Syst.* **1981**, *13*, 229-258. (b) Lerch, K. *Ibid.* **1981**, *13*, 143-186.

(15) "Copper Coordination Chemistry: Biochemical and Inorganic Perspectives"; Karlin, K. D.; Zubieta, J., Eds.; Adenine Press: Guilderland, NY, 1983.

(16) Wilcox, D. E.; Long, J. R.; Solomon, E. I. *J. Am. Chem. Soc.* **1984**, *106*, 2186-2194 and references cited therein.

(17) (a) Preliminary measurements on frozen CH_2Cl_2 solutions (77 K) of $[\text{Cu}_2(\text{N4PY2})(\text{O}_2)]^{2+}$ indicate that it is EPR "silent". (b) We speculate that a single peroxo bridging ligand also may be sufficient to provide the strong antiferromagnetic coupling (diamagnetism) observed for oxyhemocyanin^{13,14} since, for instance, monohydroxo-bridged dicopper(II) centers are known to possess this property.¹⁸

(18) (a) Coughlin, P. K.; Lippard, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 3228-3229. (b) Burk, P. L.; Osborn, J. A.; Youinou, M.-T.; Agnus, Y.; Louis, R.; Weiss, R. *Ibid.* **1981**, *103*, 1273-1274. (c) Haddad, M. S.; Wilson, S. R.; Hodgson, D. J.; Hendrickson, D. N. *Ibid.* **1981**, *103*, 384-391.

(1) Uzawa, J.; Takeuchi, S. *Org. Magn. Reson.* **1978**, *11*, 502.

(2) Seto, H.; Sasaki, T.; Yonehara, H.; Uzawa, J. *Tetrahedron Lett.* **1978**, 923.

(3) Kakinuma, K.; Imamura, N.; Ikekawa, N.; Tanaka, H.; Minami, S.; Ōmura, S. *J. Am. Chem. Soc.* **1980**, *102*, 7493.

(4) Ford, J. J.; Gibbons, W. A.; Niccolai, N. *J. Magn. Reson.* **1982**, *47*, 522.

(5) Aldersley, M. F.; Dean, F. M.; Mann, B. E. *J. Chem. Soc., Chem. Commun.* **1983**, 107.

(6) Leon, V.; Bolivar, R. A.; Tasayco, M. L.; Gonzalez, R.; Rivas, C. *Org. Magn. Reson.* **1983**, *21*, 470.

(7) Khaled, M. A.; Watkins, C. L. *J. Am. Chem. Soc.* **1983**, *105*, 3363.

(8) Niccolai, N.; Rossi, C.; Brizzi, V.; Gibbons, W. A. *J. Am. Chem. Soc.* **1984**, *106*, 5732.

(9) Niccolai, N.; Rossi, C.; Mascagni, P.; Neri, P.; Gibbons, W. A. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 739.

(10) Shapiro, M. J.; Kolpak, M. X.; Lemke, T. L. *J. Org. Chem.* **1984**, *49*, 187.

(11) Rinaldi, P. L. *J. Am. Chem. Soc.* **1983**, *105*, 5167.

(12) Yu, C.; Levy, G. C. *J. Am. Chem. Soc.* **1983**, *105*, 6994.

(13) Yu, C.; Levy, G. C. *J. Am. Chem. Soc.* **1984**, *106*, 6533.

(14) Kövér, K. E. *J. Magn. Reson.* **1984**, *59*, 485.

(15) In the transition-selective saturation technique, the total duration of the consecutive 90° pulses should be set to ca. 5-10 times the generally longer ^{13}C T_1 relaxation time. A heteronuclear NOE at a protonated carbon may be obtained by quasi-simultaneous irradiation of the one-bond satellites. In the case of nonprotonated carbons (if the long-range satellites cannot be resolved), effective saturation may be achieved by noncoherent irradiation of the parent lines. It is our experience that the saturation technique presented here offers a better saturation/selectivity ratio than the conventional methods; however, our technique is not a prerequisite for any measurements when the selectivity is not critical.

(16) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229. Bax, A.; Freeman, R.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 164. Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.

(17) Maudsley, A. A.; Müller, L.; Ernst, R. R. *J. Magn. Reson.* **1977**, *28*, 463.

(18) Bax, A. *J. Magn. Reson.* **1983**, *53*, 517.

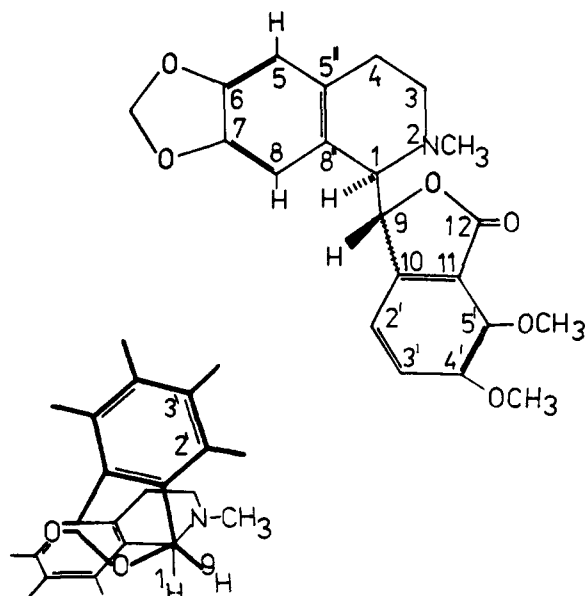


Figure 1.

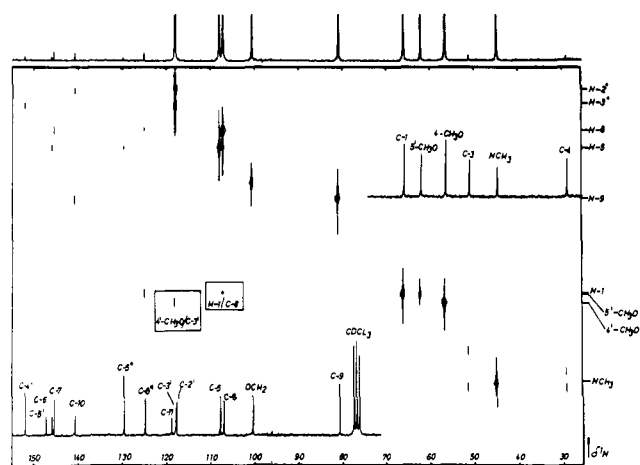


Figure 2. Contour plot of an absolute-value 50/200-MHz 2D heteronuclear NOE¹¹ spectrum (BRUKER WP-200 SY) of 0.5 M **1** in CDCl₃, $T = 298$ K. F2 projection of signals is shown on the top, while normal ¹³C spectrum is inside the frame. Indirect NOE effects at protonated carbons are indicated by small frames. The 2D map is composed of (128 × 8)K data points. A 2.5-s waiting time was allowed between each pulse sequence. A fixed mixing time of 1.5 s was used before the final ¹³C pulse and the 0.5-s acquisition time. Before the 2D Fourier transform data were multiplied with suitable Gaussian-type weighting functions.

conformation of **1** has been verified by ¹H{¹H} NOE experiments.²³ Finally, a heteronuclear 2D NOE (cross-relaxation)^{11,12} spectrum (Figure 2) has corroborated the carbon assignments. In Figure 2 two cross-peaks (indicated by small frames) may arise in theory from either direct or indirect effects. We demonstrate that they arise from the latter.

Indirect effects are well-known²⁴⁻²⁷ for spin systems containing more than two protons. In a spin system containing two protons

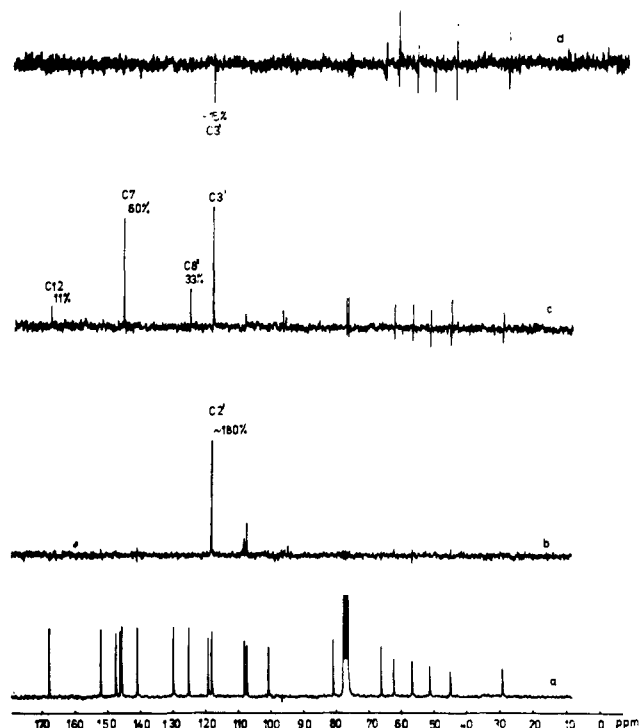


Figure 3. One-dimensional ¹³C{¹H} NOE difference spectra of **1** using the quasi-simultaneous transition-selective saturation technique: (a) Normal spectrum. (b) Selective measurement of heteronuclear NOE at protonated carbon C-2' after quasi-simultaneous saturation of the one-bond ¹³C satellites of H-2'. Residual positive signals below 110 ppm arise from accidental saturation of other satellites. (c) Heteronuclear NOE of nonprotonated carbons after saturation ($1000 \times 11/2$ (44 ms) pulses) of the H-8 parent proton lines. C-3' is also enhanced because the H-8 proton lines overlap with one of the one-bond H-3' satellites. (d) Measurement of an indirect, negative heteronuclear NOE at protonated carbon C-3', after saturation of the 4'-CH₃O singlet.

and one ¹³C nucleus, similar effects may occur. When the three spins are collinear, for example, and the ¹³C nucleus is situated at one end while the proton being irradiated is at the other end, the increased Boltzmann population at the proton in the middle decreases the population difference at the carbon. This is the negative, indirect effect, which may exceed the positive direct effect and results theoretically in an overall negative "enhancement" up to ca. -90% at the carbon nucleus.²⁸

Such an effect has been observed after selective irradiation of the 4'-CH₃O methyl singlet. Figure 3d shows the NOE difference spectrum, which results in a -15% "enhancement" at C-3'. The negative sign verifies that this is an indirect, three-spin effect, which can be explained by considering the strong dipole-dipole interaction between the 4'-CH₃O and H-3' protons ($f_{H_3}/(4'-CH_3O) = 30\%$) and the nearly complete ¹³C{¹H} NOE between C-3' and H-3'.

The use of the quasi-simultaneous line-selective preirradiation technique is demonstrated in Figure 3c,d for the measurement of ¹³C{¹H} NOE at nonprotonated and protonated carbons.

The unprecedented negative, heteronuclear NOE reported here may be thought as a ¹H → ¹H → ¹³C relay NOE, and it can be used for homo- and/or heteronuclear distance determination in accordance with theory.²⁴ Indirect effects are probably not restricted to protonated carbons and should be taken into account to improve the reliability of heteronuclear distances calculated from ¹³C{¹H} NOE data.

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(19) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.

(20) Bendall, M. R.; Pegg, D. T. *J. Magn. Reson.* **1983**, *53*, 144.

(21) Levitt, M. H.; Sorensen, O. W.; Ernst, R. R. *Chem. Phys. Lett.* **1983**, *94*, 504.

(22) Batta, Gy.; Lipták, A. *J. Chem. Soc., Chem. Commun.* **1985**, 368.

(23) Kövér, K. E.; Kerekes, P. *Magn. Reson. Chem.*, in press.

(24) Noggle, J. H.; Schirmer, R. E. "The Nuclear Overhauser Effect"; Academic Press: New York, 1971.

(25) Sanders, J. K. M.; Mersh, J. D. *Prog. Nucl. Magn. Reson. Spectrosc.* **1982**, *15*, 353.

(26) Mersh, J. D.; Sanders, J. K. M. *Org. Magn. Reson.* **1982**, *18*, 122.

(27) Mersh, J. D.; Sanders, J. K. M. *J. Chem. Soc., Chem. Commun.* **1983**, 306.

(28) All the NOE data mentioned in the text mean fractional enhancements; $(I_s - I_0)/I_0$.