When this mixture was washed with hexane (ca. 45 mL), all the D_{5h}-$H_{10}Si_{10}O_{15}$ was removed along with some O_h-$H_8Si_8O_{12}$. The residue was pure O_h-$H_8Si_8O_{12}$ (1.85 g; yield based on $HSiCl_3$ 17.5%).[16]

(16) Since the crystals were obtained from a filtered solution and were observed to dissolve completely in C_6D_6, it was assumed that they were not contaminated with SiO_2. The ^{29}Si satellites ($^1J_{Si-H}$ = ~170 Hz) were used to rule out any significant contamination by other $(HSiO_{1.5})_n$ species.

Acknowledgment. This work was supported by the Department of Chemistry at Virginia Polytechnic Institute and State University.

Department of Chemistry
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

Pradyot A. Agaskar[1]

Received December 28, 1990

Articles

Contribution from the Dipartimento Scienze Chimiche, Università di Catania, 95125 Catania, Italy, and Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico, CNR, 95125 Catania, Italy

Conformational Features and Coordination Properties of Functionalized Cyclodextrins. Formation, Stability, and Structure of Proton and Copper(II) Complexes of Histamine-Bearing β-Cyclodextrin in Aqueous Solution

Raffaele P. Bonomo,[1a] Vincenzo Cucinotta,[1a] Franca D'Alessandro,[1a] Giuseppe Impellizzeri,[1a] Giuseppe Maccarrone,[1a] Graziella Vecchio,[1b] and Enrico Rizzarelli*[,1a,b]

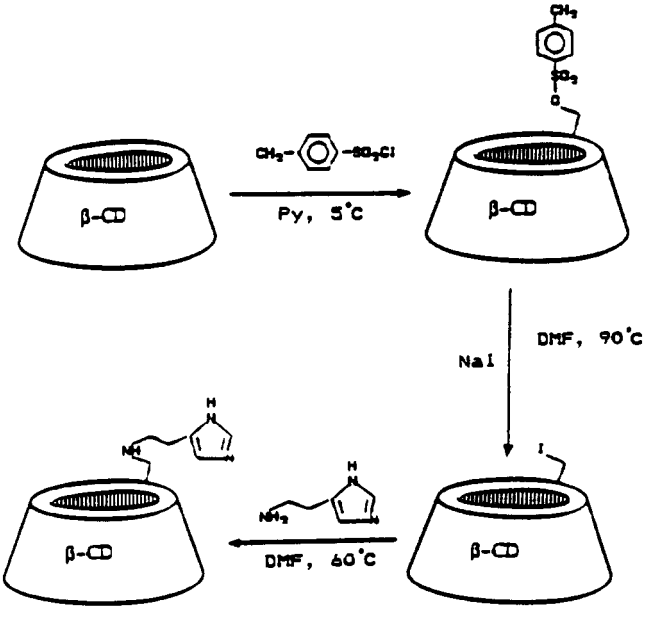
Received July 10, 1990

The functionalized cyclodextrin 6-deoxy-6-(*N*-histamino)-β-cyclodextrin was synthesized, and an NMR, EPR, pH-metric, and calorimetric investigation was carried out in aqueous solution in order to ascertain its behavior toward protonation and copper(II) complexation. Spectroscopic results as well as thermodynamic data give evidence of the influence of the β-cyclodextrin cavity. In particular, the NMR spectra data show a protonation-promoted inclusion of the imidazole ring, while the copper(II) coordination is markedly affected by the presence of the cyclodextrin cavity, as shown by the $A_{\parallel}$ value decrease, with respect to the analogous histamine complex.

Introduction

Cyclodextrins have been proposed as artificial enzymes due to their ability to form inclusion compounds and to show regiospecificity and stereospecificity with respect to the substrate and to the product during catalytic processing.[2-6] The introduction of functional groups has provided more efficient models of natural enzymes and receptors.[7-13] Furthermore, for more sophisticated molecular recognition, double (or multiple) recognition systems have been obtained by means of the metal complexes of functionalized cyclodextrins where the additivity of cavity and metal-ligand interaction has been demonstrated.[14-18]

Scheme I



Inclusion compounds have also been reported for the metal complexes of cobalt(III), iron(0), rhodium(I), ruthenium(0), and platinum(II), in which cyclodextrins behave like second-sphere ligands.[19-25]

(1) (a) Università di Catania. (b) Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico Farmaceutico, CNR.
(2) Griffiths, D. W.; Bender, M. L. *Adv. Catal.* **1973**, *23*, 209.
(3) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: New York, 1978.
(4) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344.
(5) Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akademiai Kiad: Budapest, 1982.
(6) Komiyama, M.; Bender, M. L. *The Chemistry of Enzyme Action*; Page, M. I., Ed.; Elsevier: Amsterdam, 1984; p 505.
(7) Breslow, R. *Acc. Chem. Res.* **1980**, *13*, 170.
(8) Breslow, R. *Science* **1982**, *218*, 532.
(9) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66.
(10) Anslyn, E.; Breslow, R. *J. Am. Chem. Soc.* **1989**, *111*, 5972.
(11) Anslyn, E.; Breslow, R. *J. Am. Chem. Soc.* **1989**, *111*, 8931.
(12) Willner, I.; Goren, Z. *J. Chem. Soc., Chem. Commun.* **1983**, 1469.
(13) Monzen, H.; Yoshida, N.; Fujimoto, M. *J. Coord. Chem.* **1988**, *18*, 177.
(14) Tabushi, I.; Kuroda, Y.; Mizutani, T. *Tetrahedron* **1984**, *40*, 269.
(15) Tabushi, I.; Kuroda, Y.; Mochizuki, A. *J. Am. Chem. Soc.* **1980**, *102*, 1152.
(16) Tabushi, I.; Shimizu, N.; Sugimoto, T.; Schiozuka, M.; Yamamura, K. *J. Am. Chem. Soc.* **1977**, *99*, 7100.
(17) Tabushi, I.; Kuroda, Y. *J. Am. Chem. Soc.* **1984**, *106*, 4580.
(18) Tabushi, I. *Pure Appl. Chem.* **1986**, *58*, 1529.
(19) Siegel, B.; Breslow, R. *J. Am. Chem. Soc.* **1975**, *97*, 6869.

0020-1669/91/1330-2708$02.50/0

Due to the poor coordination properties of their hydroxyl groups, the direct coordination of these cyclic oligosaccharides to metal ions was found only for copper(II)[26,27] and, recently, in complexes of cobalt(III).[28] However, the presence of potential coordinating groups attached to cyclodextrins, which promote the formation of stable metal complexes, has encouraged the use of these functionalized derivatives for metalloprotein models.[29-36] In particular, kinetic studies of the metal complexes with functionalized cyclodextrins have been carried out to obtain useful information about the ability of the systems to promote the hydrolysis[34,36] or the oxidation of biofunctional molecules[31] or to mimic carbonic anhydrase.[15,17] The remarkable acceleration rates observed have been ascribed to the presence of both the metal catalytic center and the hydrophobic binding cavity.

While it is easy to correlate the catalytic properties with a definite complex species when we are dealing with a cation that forms inert species, in the case of cations that form labile complexes a preliminary speciation study is required to ascertain what species are really present and their actual concentrations under the experimental conditions of the kinetic investigation. Notwithstanding the obvious need to carry out a quantitative speciation study, only qualitative or semiquantitative data have been reported in the literature. Actually, a large number of copper(II) compounds have been studied in order to determine their kinetic parameters in aqueous solution for reactions with O_2^{-}.[37-41] The catalytic activities toward O_2^{-} depend on the detailed arrangement of ligands in a way that is not yet well understood. However, no copper(II) complex with any cyclodextrin derivative has yet been tested.

We now report the synthesis of 6-deoxy-6-(*N*-histamino)-β-cyclodextrin (CDhm) (Scheme I), a detailed potentiometric and calorimetric investigation in aqueous solution (25 °C and I = 0.1 mol dm^{-3}, with addition of KNO_3) on CDhm, and the characterization of the different species in proton–CDhm and copper(II)–CDhm systems by a NMR and EPR investigation, respectively. This study is preliminary to the investigation of the "SOD-like" activity of copper(II)–CDhm complexes and the possible role played by the cavity.

Experimental Section

Synthesis. Materials and Apparatus. Commercially available reagents were used directly unless otherwise noted. β-Cyclodextrin (β-CD) (Sigma) was dried in vacuo (~10^{-2} mmHg) for 24 h at 80 °C by using a P_2O_5 trap. Pyridine was freshly distilled and collected over solid KOH. Dimethylformamide (DMF) was purified by refluxing over CaH_2 for about 10 h and then distilled under reduced pressure. It was collected over molecular sieves. Thin-layer chromatography (TLC) was carried out on precoated silica gel plates (60F-254, 0.25 mm, Merck), and the detection of cyclodextrin derivatives on SiO_2 thin-layer chromatography plates was achieved by UV irradiation (λ = 254 nm), by using the anisaldehyde test.[12] The Pauly test was used for the histamine derivative.[42] High-performance liquid chromatography (HPLC) was performed analytically on a Varian Model 5000 instrument.

Table I. Spin Hamiltonian Parameters for Copper(II) Complexes with CDhm in Water–Methanol (90:10) Solution at 150 K

complex	$g_\parallel$	$A_\parallel$, cm^{-1}	$g_\perp$	$A_\perp$, cm^{-1}
$[Cu(CDhm)]^{2+}$	2.304 (2)	0.0153 (2)	2.079 (5)	
$[Cu(CDhm)_2]^{2+}$	2.246 (2)	0.0190 (2)	2.061 (5)	0.0020 (5)
$[Cu(hm)]^{2+}$	2.305 (2)	0.0171 (2)	2.068 (5)	0.0015 (5)
$[Cu(hm)_2]^{2+}$	2.229 (2)	0.0200 (2)	2.049 (5)	0.0020 (5)

Preparation of 6-*O*-(*p*-Tosyl)-β-cyclodextrin (CDOTs). A solution of *p*-toluenesulfonyl chloride (1.5 g, 7.9 mmol) in anhydrous pyridine was added to a solution of dry β-CD (5 g, 4.4 mmol) dissolved in anhydrous pyridine, with stirring.[43] After 5 h at 5 °C, the pyridine was distilled off in vacuo at 40 °C and a syrup was obtained. After the addition of acetone to the residue a colorless solid precipitated and was collected by filtration and washed with acetone. TLC of the solid showed more than one spot (eluent 5:4:3 butanol–ethanol–water). In order to isolate CDOTs, a reversed-phase (Lichroprep Rp 8, 40–63 μm) column (35 × 360 mm) was used. The mixture (7 g) was dissolved in DMF, applied on the column, and eluted with 10% aqueous DMF to eliminate unreacted CD and pyridinium salts, followed by treatment with 20% aqueous DMF. Under these conditions only CDOTs was eluted. CDOTs was crystallized from water and dried under vacuum (1.8 g, 30% yield, mp 168–170 °C with decomposition, literature value 160–162 °C[16]).

The monosubstitution of CD was confirmed by the NMR spectra in DMSO. The ^{1}H spectrum of CDOTs showed, in the aromatic region, the characteristic pattern of an AA′BB′ tosyl system, and the integral spectrum confirmed the monosubstitution. The 6-hydroxyl substitution was confirmed by the decrease of the integral of the peak due to the hydroxyl protons in position 6, at about 4.5 ppm.

Preparation of 6-Deoxy-6-iodo-β-cyclodextrin (CDI). Dried CDOTs (1 g, 0.77 mmol) was dissolved in anhydrous DMF (40 mL), and NaI (10:1 NaI–CDOTs mole ratio) was added to this solution. The reaction was carried out at 90 °C for 5 h under stirring, followed by evaporating to dryness in vacuo at 40 °C. Acetone was added to the yellow residue, and the mixture was stirred. The solid was filtered out, and acetone was added again. This procedure was repeated three times to eliminate NaI, which is soluble in acetone. The resulting solid was dissolved in water and precipitated by addition of acetone. The product (yield 90%, based on CDOTs) was of sufficient purity on the basis of TLC (eluent 5:4:3 butanol–ethanol–water, R_f = 0.40). The ^{1}H spectrum of CDI in water showed the disappearance of aromatic protons, confirming the tosyl substitution, while, with respect to β-cyclodextrin, CDI shows two different peaks of the 1-protons in the integral ratio expected (6:1).

Preparation of 6-Deoxy-6-(*N*-histamino)-β-cyclodextrin (CDhm). Dried CDI (1 g, 0.8 mmol) was dissolved in DMF (40 mL), and histamine was added (1:10 CDI–histamine molar ratio). The reaction was carried out at 60 °C under nitrogen. After 24 h, the DMF was evaporated in vacuo at 40 °C. The yellow syrup obtained was washed with acetone until the acetone remained colorless. The solid obtained was dissolved in water and precipitated again with acetone. The precipitate collected by suction was dissolved in water, and the solution was applied to a column (45 × 500 mm) of CM-Sephadex C-25 resin (in NH_4^+ form). The column was eluted initially with water (800 mL) and then with a gradient from 0 to 0.2 M of aqueous ammonium hydrogen carbonate (4 L, total volume). The collected fractions were assayed by TLC. Fractions that gave only one spot with R_f = 0.52 (eluent 5:3:1 propanol–water–ammonia) were combined and evaporated to dryness at 40 °C in vacuo to decompose ammonium hydrogen carbonate. The residue was further precipitated from water by using acetone. The precipitated CDhm was a colorless powder (yield, 40% based on CDOTs). The purity was checked by HPLC using a Lichrosorb-NH_2 column (5 × 250 mm, 10 μm) and a mixture of CH_3CN and water (1:1) as eluent.

Anal. Calcd for $C_{47}H_{77}O_{34}N_3 \cdot 8H_2O$: C, 41.1; H, 6.78; N, 3.1. Found: C, 40.71; H, 6.2; N, 2.92.

Measurements. Equilibria Measurements. Stability constants for proton and copper(II) complexes were calculated from potentiometric titrations carried out at 25 °C by using total volumes of 2.5 cm^3. Alkali was added from a Hamilton buret equipped with 0.25- or 0.50-cm^3 syr-

(20) Breslow, R.; Czarniecki, M. F.; Emert, J.; Hamaguchi, H. *J. Am. Chem. Soc.* **1980**, *102*, 762.
(21) Trainor, G. L.; Breslow, R. *J. Am. Chem. Soc.* **1981**, *103*, 154.
(22) Yamanari, K.; Shimura, Y. *Chem. Lett.* **1982**, 1957.
(23) Harada, A.; Takahashi, S. *J. Chem. Soc., Chem. Commun.* **1984**, 645.
(24) Stoddart, J. F.; Zarzycki *Recl. Trav. Chim. Pays-Bas* **1988**, *107*, 515 and references therein reported.
(25) Alston, D. R.; Ashton, P. R.; Lilley, T. H.; Stoddart, J. F.; Zarzycki, R.; Slawin, A. M.; Williams, D. J. *Carbohydr. Res.* **1989**, *192*, 259.
(26) Matsui, Y.; Kurita, T.; Yagi, M.; Okayama, T.; Mochida, K.; Date, Y. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2187.
(27) Mochida, K.; Matsui, Y. *Chem. Lett.* **1976**, 963.
(28) Yamanari, K.; Nakamichi, M.; Shimura, Y. *Inorg. Chem.* **1989**, *28*, 248.
(29) Breslow, R.; Overman, L. E. *J. Am. Chem. Soc.* **1970**, *92*, 1075.
(30) Breslow, R. In *Evolution of Catalytic Function*; Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1987; Vol. 52, p 75.
(31) Matsui, Y.; Yokoi, T.; Mochida, K. *Chem. Lett.* **1976**, 1037.
(32) D'Souza, V. T.; Bender, M. L. *Acc. Chem. Res.* **1987**, *20*, 146.
(33) Tabushi, I. *Coord. Chem. Rev.* **1988**, *86*, 1.
(34) Komiyama, M.; Matsumoto, Y. *Chem. Lett.* **1989**, 719.
(35) Kuroda, V.; Sasaki, V.; Shiroiwa, Y.; Tabushi, I. *J. Am. Chem. Soc.* **1988**, *110*, 4049.
(36) Akkaya, E. U.; Czarnik, A. W. *J. Am. Chem. Soc.* **1988**, *110*, 8553.
(37) DeAlvare, L. R.; Goda, K.; Kimura, T. *Biochem. Biophys. Res. Commun.* **1976**, *69*, 135.
(38) Klug-Roth, D.; Rabani, J. *J. Phys. Chem.* **1976**, *80*, 588.
(39) Kubota, S.; Yang, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3283.
(40) Gartner, A.; Weser, U. In *Topics in Current Chemistry*; Vogtle, F., Weber, E., Eds.; Springer-Verlag: Berlin, 1986; p 1.
(41) Weinstein, J.; Bielski, B. H. *J. Am. Chem. Soc.* **1980**, *102*, 4916.
(42) Touchstone, J. C.; Dobbins, M. F. *Practice of thin layer chromatography*; Wiley: New York, 1983; pp 185 and 180.
(43) Takahashi, K.; Hattori, K.; Toda, F. *Tetrahedron Lett.* **1984**, *25*, 3331.

inges calibrated with mercury. Measurements of pH (−log [H$^+$]) were obtained with a glass electrode calibrated by titrating with HNO_3. The ionic strengths of all solutions were 0.1 mol dm^{-3} (KNO_3); β-cyclodextrin derivative (L) concentrations ranged from 0.002 to 0.006 mol dm^{-3}. Duplicate or triplicate titrations were carried out at 1:1 and 1:2 Cu/L ratios, with KOH as titrant. Other details were as previously described.[44,45]

$\Delta H°$ and $\Delta S°$ values were determined by calorimetric titrations using a Tronac Model 450 isoperibol calorimeter equipped with a 25-cm^3 reaction vessel. The calorimetric measurements were carried out by titrating the solution containing the metal and the ligand with HNO_3 (0.2–0.4 mol dm^{-3}). Other experimental details were as previously described.[44,45]

NMR Spectroscopy. ^{1}H NMR spectra were obtained on a Bruker AMX-600 spectrometer at 600 MHz. ^{13}C spectra were obtained on a Bruker AC-250 at 62.9 MHz. All the samples were prepared in D_2O, without reference, having verified that the most of references interact with the cyclodextrin cavity. Therefore, the ^{1}H spectra were referenced to HDO, verifying the constancy of its absolute frequency. The solutions of mono- and diprotonated CDhm were prepared by adding the stoichiometric amount of DCl in D_2O solution.

EPR Spectroscopy. Frozen-solution EPR spectra were recorded by a Bruker ER 200D X-band spectrometer, with a standard low-temperature apparatus. DPPH was used as a calibrant, and the magnetic field was measured by means of a gauss meter (ER 035 M), which leaves markers at preselected values. Spin Hamiltonian parameters were obtained directly from the experimental spectra and are reported in Table I. In the case of the mono complex, the solutions had 1:1 metal to ligand ratios, the pH value being about 6. To promote the formation of the bis species, a 4-fold excess of ligand was added to a 5×10^{-3} mol dm^{-3} solution of isotopically pure $^{63}Cu(NO_3)_2$). In this latter case the pH of the solution was about 7.5. After the pH was adjusted to the desired value, methanol up to 30% was added to these aqueous solutions to increase spectral resolution. All the measurements were carried out at 150 K.

Calculations. The calculations concerning the electron system were performed by the computer program ACBA,[46] which refines the parameters of an acid–base titration by using a nonlinear least-squares method minimizing the function $U = (v_{i,expt} - v_{i,calc})^2$, v being the volume of titrant added. All other potentiometric data were handled by the program SUPERQUAD,[47] which minimizes the error-square sum of the differences between measured and calculated electrode potentials. The heats of complex formation were calculated by the program DOEC.[48] Throughout the paper, the errors in the thermodynamic parameters are expressed as three times the standard deviations.

Results and Discussion

Conformational Analysis of Unprotonated and Protonated CDhm Species. Preliminarily, an NMR investigation has been used to ascertain the identity of our compound. Both ^{1}H and ^{13}C NMR spectra of CDhm clearly show that the histamine is bonded to atom 6C of β-CD through its amino group. In particular, both the presence of two signals, near each other, at 50 ppm in the ^{13}C NMR spectrum, and the value of their chemical shifts suggest the histamine substitution through the amino group. Furthermore, evidence of the amino group involvement in the bonding to β-CD is given by comparison between the ^{1}H NMR spectrum of CDhm and the ^{1}H NMR spectrum (unreported) of 6-deoxy-6-(*N*-imidazolyl)-β-CD, synthesized by us for comparison. In the imidazole derivative, the bond of the heterocyclic ring causes a downfield shift of the 6A protons, just opposite to the upfield shift observed for 6A protons in CDhm.

The ^{1}H NMR spectrum of CDhm, whose 2.7–4.1 ppm region is reported in Figure 1, was assigned by simple comparison with the β-cyclodextrin spectrum for the whole 8–3.5 ppm region; for the remaining peaks in the upfield region, the COSY spectrum allowed the assignment reported in Figure 1, in agreement with

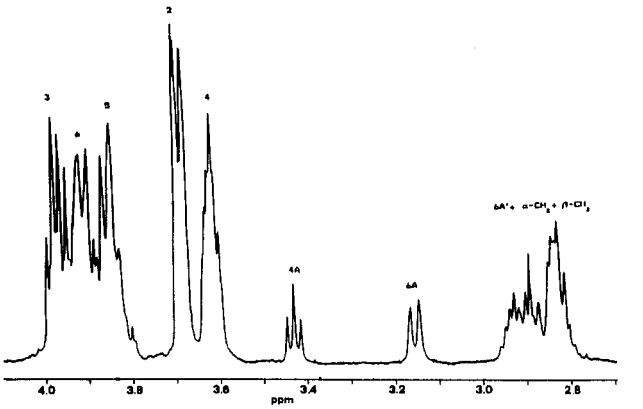


Figure 1. ^{1}H NMR spectrum of CDhm at 600 MHz: 2.7–4.1 ppm region.

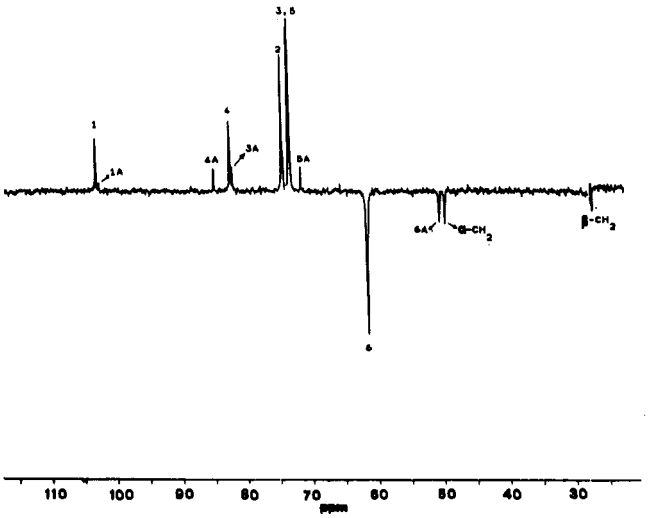


Figure 2. ^{13}C INEPT spectrum of CDhm.

the integral spectrum. The multiplet at 2.8–2.9 ppm appears to be very complex, suggesting a high multiplicity for each of the two histamine methylenes. Moreover, the histamine substitution causes significant shifts for only the two 6A, the 5A, and the 4A protons.

The ^{13}C INEPT spectrum, in Figure 2, was assigned by simple comparison with the β-cyclodextrin spectrum. For the assignment of the additional peaks of lower intensity, a ^{13}C–^{1}H correlation spectrum was run. The spectrum shows the correlation of 1A, 4A, 5A, and 6A carbons. Furthermore, a cross peak is observed in the H2 region, and thus, it should be assigned to the 2A carbon. Last, the small peak observed at 82 ppm, that correlates with the H3 region, may tentatively be assigned to the 3A carbon, which experiences a strong downfield shift.

The monoprotonation, involving the amino group, causes significant changes in the ^{1}H spectrum, namely (i) the downfield shift of all the peaks of the 2.7–3.5 ppm region, (ii) the appearance of a triplet at 4.15 ppm, and (iii) the appearance of other peaks, in the 3.75–3.85 ppm region. In particular, in the CDhmH$^+$ spectrum, whose 3.0–4.2 ppm region is reported in Figure 3, a clear separation of the multiplets of the two histamine methylenes is observed and more so at least one of the methylenes gives rise to a triplet. The COSY spectra allowed the assignment of the 6A, 5A, and 4A protons, thus showing that the triplet at 4.15 ppm, downfield of proton 3H, is due to the 5A proton. The peaks observed in the 3.8–3.9 ppm region, though not ascribable to specific protons, should be assigned to 5H and 6H of the unsubstituted rings. Also the ^{13}C INEPT spectrum of CDhmH$^+$ shows more peaks than the corresponding CDhm spectrum, confirming that, as a consequence of the protonation, a spread in the chemical shift values occurs.

NMR data suggest that in the unprotonated CDhm, the histamine chain interaction with cyclodextrin primarily concerns the

(44) Amico, P.; Arena, G.; Daniele, P. G.; Ostacoli, G.; Rizzarelli, E.; Sammartano, S. *Inorg. Chem.* **1981**, *20*, 772.
(45) Bonomo, R. P.; Cali, R.; Cucinotta, V.; Impellizzeri, G.; Rizzarelli, E. *Inorg. Chem.* **1986**, *25*, 1641.
(46) Arena, G.; Rizzarelli, E.; Sammartano, S.; Rigano, C. *Talanta* **1979**, *26*, 1.
(47) Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1985**, 1195.
(48) Rigano, C.; Rizzarelli, E.; Sammartano, S. *Thermochim. Acta* **1979**, *33*, 211.

Table II. Thermodynamic Parameters for Proton and Copper(II) Complexes of CDhm, hm, and *N*-CH_3hm[a] at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)

equilibrium	log K	$-\Delta G°$, kcal/mol	$-\Delta H°$, kcal/mol	$\Delta S°$, cal/mol deg	ref
CDhm + H^+ = $[(CDhm)H]^+$	8.01	10.92 (4)	9.9 (1)	3.3 (3)	*b*
$[(CDhm)H]^+ + H^+ = [(CDhm)H_2]^{2+}$	5.81	7.92 (6)	8.2 (1)	0.9 (4)	*b*
CDhm + Cu^{2+} = $[Cu(CDhm)]^{2+}$	7.26	9.90 (4)	9.85 (6)	0.2 (2)	*b*
$[(CDhm)H]^+ + Cu^{2+} = [Cu(CDhm)H]^{3+}$	2.94	4.01 (4)	4.4 (7)	−1 (2)	*b*
hm + H^+ = $[(hm)H]^+$	9.80	13.36	12.15	4.1	*c*
$[(hm)H]^+ + H^+ = [(hm)H_2]^{2+}$	6.08	8.28	7.52	2.5	*c*
hm + Cu^{2+} = $[Cu(hm)]^{2+}$	9.57	13.04	12.1	3.2	*c*
$[(hm)H]^+ + Cu^{2+} = [Cu(hm)H]^{3+}$	3.07	4.17	5.75	−5.1	*c*
N-CH_3hm + H^+ = $[(N\text{-}CH_3hm)H]^+$	9.90	13.49			*d*
$[(N\text{-}CH_3hm)H]^+ + H^+ = [(N\text{-}CH_3hm)H_2]^{2+}$	5.87	7.99			*d*
N-CH_3hm + Cu^{2+} = $[Cu(N\text{-}CH_3hm)]^{2+}$	8.35	11.62			*d*

[a] hm = histamine, *N*-CH_3hm = *N*-methylhistamine. 3σ in parentheses. [b] This work. [c] Arena, G.; Calì, R.; Cucinotta, V.; Musumeci, S.; Rizzarelli, E.; Sammartano, S. *J. Chem. Soc., Dalton Trans.* **1984**, 1651. [d] Braibanti, A.; Dallavalle, F.; Leporati, E.; Mori, G. *J. Chem. Soc., Dalton Trans.* **1973**, 2539.

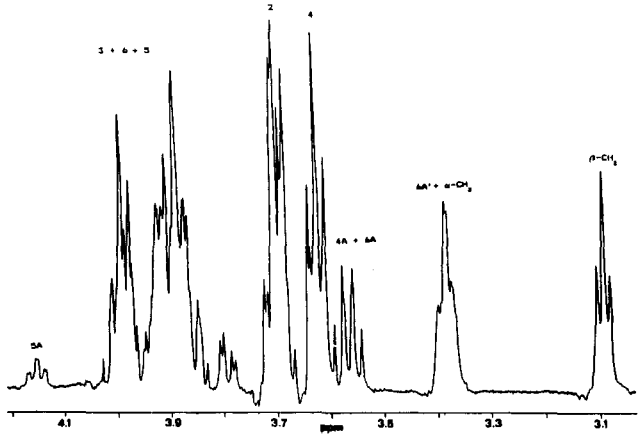


Figure 3. ^1H NMR spectrum of $CDhmH^+$ at 600 MHz: 3.0–4.2 ppm region.

A ring, which may be substantially rationalized on the basis of a through-the-chain electronic effect. The only exception in the ^1H NMR spectrum consists in the small 5A-proton downfield shift, which is opposite to the upfield shift expected for the 6-OH substitution, ascribable to a ring current effect, caused by the imidazole ring.

In the ^{13}C spectrum, the downfield shifts of the 4- and, probably, 3-carbons of the A ring, which is opposite to the 4H upfield shift and to the almost unshifted 3H, may be rationalized on the basis of increased strain, presumably due to the steric requirements of the histamine substitution, similarly to that hypothesized for the 4C chemical shift of the unfunctionalized cyclodextrins.[49] The consequent rigidity of the cyclodextrin system has a corresponding effect on the entire chain bonded to the 5A carbon, as shown by the strong diastereotopicity of the two 6A protons and by the multiplicity of the histamine methylenes. While, in most of the cases, two simple triplets are observed in AH_2CCH_2B ethanes, a more complex spectrum originates when one of the staggered rotamers is strongly "preferred". This preferred rotamer is usually the conformer having the substituting groups trans to each other. In such a case, an AA'BB' system is expected, due to the trans rotamer only. In our case, owing to the overlapping of three different multiplets, it is not possible to identify the type of spin system present. Thus, considering (i) that the histamine itself does not show an AA'BB' system and (ii) that the protonation changes this part of the spectrum (A_2B_2 spin system) and also considering (iii) the values of the thermodynamic parameters concerning the protonation (vide infra), the high multiplicity of the histamine methylenes may find a different explanation.

We can hypothesize that the imidazole at the end of the chain is in a fixed position, and considering the absence of selective effects on the atoms of the unsubstituted glucopyranosyl rings, the heterocyclic ring should not be inside the cavity. Though one expects that in a similar situation hydrogen bonds between the nitrogen atoms and one or more 6-OH groups should be possible, no evidence of such interaction is observed in NMR spectra. In particular, as regards the 6C atoms, we observe only two peaks for the carbon atoms, one for the 6A carbon (δ = 51 ppm) and the other for all the remaining 6C atoms (δ = 61 ppm). This suggests that an intrachain hydrogen bond between the amine and the imidazole N-1 atom exists. In this situation, a six-membered ring should be formed, and the histamine methylenes should give rise to an ABCD spin system, in agreement with the complexity of the ^1H NMR spectrum of the unprotonated CDhm. The imidazole ring should be placed perpendicular to the cavity, approximately in the same plane as 5A and as one of the 6A protons, in agreement with their downfield shifts (5A with respect to the other 5H, 6A with respect to the other proton α to the amino group).

The protonation of the amine nitrogen sharply changes the NMR spectra. The increased number of peaks, both in ^1H and ^{13}C spectra, shows that selective interactions arise between the histamine chain and some of the protons of unsubstituted glucopyranosyl rings. Furthermore, the multiplicity of one of the histamine methylenes at 3.11 ppm (triplet), observed in the $CDhmH^+$ ^1H NMR spectrum shows that the imidazole ring is no longer firmly bonded, though the diastereotopicity of the 6A protons (even in protonated species) suggests a favored orientation of the histamine chain.

The diprotonated species obtained by imidazole protonation shows no significant difference with respect to the monoprotonated species spectra, already described, except a general further downfield shift.

All the above suggests an increased interaction of the imidazole ring with the β-cyclodextrin cavity with the increase of the degree of protonation. The spread in the chemical shift values of other than the A-ring protons may be interpreted as due to the ring current effect, which causes upfield or downfield shifts, depending on the angle between the ring plane and the line connecting the atom to the ring center.[50] At the same time, it cannot be excluded that the protonated amino nitrogen forms a specific hydrogen bond with an oxygen atom of the cavity. In addition, since the ring current shift does not influence the 3H proton so much as both the 5- and 6H protons, one can conclude that in these protonated species a partial inclusion of the imidazole ring occurs.

Proton and Copper(II) Complexes: Formation and Bonding Details. The generalized formation reaction of ligand with protons and copper(II) ions is given in eq 1, where L is CDhm. Charges

$$mCu^{2+} + hH^+ + lL \overset{\beta_{mhl}}{\rightleftharpoons} Cu_mH_hL_l \qquad (1)$$

(49) Colson, P.; Jennings, H. J.; Smith, I. C. P. *J. Am. Chem. Soc.* **1974**, *96*, 8081.

(50) Jacjmann, L. M.; Sternhill, S. In *Applications of Nuclear Magnetic Resonance in Organic Chemistry*; Pergamon: Oxford, England, 1969; p 94.

on the copper(II) complexes are omitted for clarity. The stability constant β_{mhl} is defined by eq 2. Analysis of the titration data

$$\beta_{mhl} = \frac{[Cu_mH_hL_l]}{[Cu^{2+}]^m[H]^h[L]^l} \quad (2)$$

for CDhm in the absence of copper(II) gives the formation constants for amino and imidazole nitrogen protonation (eqs 3 and 4). The proton formation constants determined in this study are

$$H^+ + L \rightleftharpoons HL^+ \quad (3)$$

$$2H^+ + L \rightleftharpoons H_2L^{2+} \quad (4)$$

given in Table II together with the corresponding values for histamine and *N*-methylhistamine ligands.

The equilibria needed to fit the experimental titration curves for the solutions of copper(II) and CDhm under study are given by eqs 5 and 6. Under the experimental conditions, the major

$$Cu^{2+} + H^+ + L \rightleftharpoons Cu(HL) \quad (5)$$

$$Cu^{2+} + L \rightleftharpoons CuL \quad (6)$$

species was the [CuL] complex; below pH = 5, [CuHL] was also present. Another minor species [Cu(H_{-1}L)] was also determined, but excluding it resulted in no significant increase in the goodness of the fit; hence, there was no evidence for its existence. The [Cu($L)_2$] species (determined by EPR measurement) was not found in potentiometric measurement, owing to the low L/Cu ratios explored. The complex formation constant values are listed in Table II, as are those of histamine and *N*-methylhistamine; $\Delta G°$, $\Delta H°$, and $\Delta S°$ values for the proton and copper(II) complex formation are also given in Table II.

The log *K* for amine protonation of CDhm (8.01) is about 2 log units lower than that for hm and *N*-CH_3hm (9.80 and 9.90, respectively); the decrease is much less marked in the second protonation constant of CDhm with respect to both the hm and *N*-CH_3hm analogous constants. Furthermore, the unprotonated copper(II) complex formation of CDhm is drastically less favored with respect to analogous hm and *N*-CH_3hm complexes, the difference being negligible in the case of the monoprotonated species.

The protonation of amino and imidazole nitrogens is mainly favored on enthalpy grounds, as expected.[51] In comparison of the thermodynamic parameters accompanying the protonation of the CDhm amino group with those parameters associated with the corresponding group of hm, it is evident that the basicity decrease is almost completely due to a less favorable enthalpy term. Unfortunately, the $\Delta H°$ value for *N*-CH_3hm has not been determined, but the comparison above can be considered significant because the difference in enthalpy change of proton complex formation between the primary and secondary amine is smaller[52] than that here found between CDhm and hm. From the NMR study we know that a hydrogen bond is present in the unprotonated species. This bond is destroyed in the formation of the monoprotonated species. This structural detail, which deals with the initial state, CDhm, and the final state, $CDhmH^+$, allows one to interpret the $\Delta H°$ differences between hm and CDhm. The smaller enthalpy change of the species $CDhmH^+$ with respect to hmH^+ can be due to the cleavage of the hydrogen bond. On the contrary, the protonation of the imidazole group is slightly more enthalpy favored in the CDhm ligand than in hm. There is a basicity decrease that is due to a less favorable entropy contribution. Also in this case these enthalpy and entropy changes can be interpreted by starting from the NMR results: going from the monoprotonated $CDhmH^+$ to the diprotonated species $CDhmH_2^{2+}$, one sees that an increase of the interaction with cavity occurs with a partial inclusion of the imidazole. Solvophobic interactions are favored on enthalpy grounds and are unfavored on entropy grounds, as previously seen for proton and metal complex formation.[53,54] Furthermore, the hydrophobic feature of the cyclodextrin cavity has been claimed to explain a great number of inclusion phenomena and it has been reported that the basicity of uncharged N-sites usually decreases as the polarity of the solvent decreases[55] such as in a hydrophobic environment.

The $[Cu(CDhm)]^{2+}$ complex formation is significantly less favored than the $[Cu(hm)]^{2+}$ species due to both a smaller exothermic enthalpy contribution and a less positive entropy change. The difference in $\Delta H°$ values of copper(II) complexes parallels that found in the proton complex formation of amine and reflects the basicity decrease.

The comparison of the magnetic parameters of the $[Cu(CDhm)]^{2+}$ complex with those of the $[Cu(hm)]^{2+}$ (Table I) reveals a surprising decrease in the $A_\parallel$ value and a slight increase in $g_\perp$, $g_\parallel$ being practically equal. Lower $A_\parallel$ and higher $g_\parallel$ values have been generally observed when copper(II) complexes undergo a strong tetrahedral distortion or when five-coordinated adducts in square-pyramidal stereochemistry are formed.[56-59] In our case, the similarity between the two $g_\parallel$ values suggests that the two complexes, $[Cu(CDhm)]^{2+}$ and $[Cu(hm)]^{2+}$, should have the same coordinated atoms, probably in the same pseudooctahedral geometry. The slightly higher $g_\perp$ and lower $A_\parallel$ values are surely an indication of some kind of apical perturbation. This result may be explained by assuming that one of the two apical water molecules, as a consequence of the interaction with the cyclodextrin cavity, either is forced to be at a shorter or longer distance with respect to the distance present in the simple $[Cu(hm)]^{2+}$ complex or is replaced by one of the cavity hydroxymethyl oxygens, which is more firmly bound to copper. Anyway, the copper(II) complex with CDhm experiences a cavity interaction with a consequent "stiffening" effect, which probably explains the less positive entropy change with respect to $[Cu(hm)]^{2+}$. Interaction of the metal ion with the cavity has been suggested[36] to explain differences in hydrolysis activity between a cobalt(III) complex with primary side functionalized cyclodextrin and the analogous complex with secondary side functionalized cyclodextrin. As a consequence, the primary side derivative could assist the involvement of an adjacent hydroxymethyl group in the chelation of the metal ion. By analogy, we are inclined to believe that also the $[Cu(CDhm)]^{2+}$ complex contains such a bond.

The parameters of the copper(II) bis complex with CDhm are not very different from those obtained in the case of $[Cu(hm)_2]^{2+}$ (Table I). Slight increases in $g_\parallel$ and decreases in $A_\parallel$ can be ascribed to the bulkiness of the cyclodextrin cavity, which could be a real hindrance to the formation of a bis complex. Indeed, the presence of a second CDhm molecule in the coordination sphere of the copper(II) ion does not allow the unusual arrangement achieved in the case of the mono complex.

Concluding Remarks

Previous thermodynamic investigations[60] have been carried out on the inclusion complexes of cyclodextrins, generally unfunctionalized, to obtain information about the binding interactions of organic molecules[61,62] or of inert[25,63] complexes. The enthalpy and entropy changes here reported represent the first data on the

(51) Barbucci, R.; Barone, V. *J. Solution Chem.* **1979**, *8*, 427.
(52) Paoletti, P.; Barbucci, P.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1972**, 2010.
(53) Arena, G.; Rizzarelli, E. In *Metal Complexes in Solution*; Jenne, E. A., Rizzarelli, E., Romano, V., Sammartano, S., Eds.; Piccin: Padua, Italy, 1986; p 105.
(54) Amico, P.; Bonomo, R. P.; Calì, R.; Cucinotta, V.; Daniele, P. G.; Ostacoli, G.; Rizzarelli, E. *Inorg. Chem.* **1989**, *28*, 3555.
(55) Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201.
(56) Wayland, B. B.; Wisniewski, M. D. *J. Chem. Soc. D* **1971**, 1026.
(57) Wayland, B. B.; Kapur, V. K. *Inorg. Chem.* **1974**, *13*, 2517.
(58) Garito, A. F.; Wayland, B. B. *J. Chem. Soc.* **1969**, *91*, 866.
(59) Yokoy, H.; Addison, W. *Inorg. Chem.* **1977**, *16*, 1341.
(60) Harrison, J. C.; Eftink, M. R. *Biopolymers* **1982**, *21*, 1153.
(61) Barone, G.; Castronovo, G.; Di Ruocco, V.; Elia, V.; Giancola, C. *Carbohydr. Res.* **1989**, *192*, 331.
(62) Eftink, M. R.; Andy, M. L.; Bystrom, K.; Perlmutter, H. D.; Kristol, D. S. *J. Am. Chem. Soc.* **1989**, *111*, 6765.
(63) Alston, D. R.; Lilley, T. H.; Stoddart, J. F. *J. Chem. Soc., Chem. Commun.* **1985**, 1600.
(64) Bonomo, R. P.; Calì, R.; Riggi, F.; Rizzarelli, E.; Sammartano, S.; Siracusa, G. *Inorg. Chem.* **1979**, *18*, 3417.

proton and complex formation with functionalized cyclodextrins. These results confirm, as we have already reported,[45,54,65] that a combined spectroscopic and thermodynamic approach is very useful to infer structural information on the investigated species. In fact, through the NMR investigation it is possible to describe the conformational features of the initial and final states of the different species present in the system (these states determine thermodynamic parameters), and therefore, it is possible to know the role of the cavity in determining a peculiar structure of the complex species. The EPR results give evidence of the interaction with the cavity through the comparison between the magnetic parameters of the two mono complexes $[Cu(CDhm)]^{2+}$ and $[Cu(hm)]^{2+}$ and substantiate the smaller entropy value found for the former. The inclusion of the imidazole ring in the mono- and diprotonated species underlines the inclusion properties of the cyclodextrin cavity. It has been previously found that the protonation of a basic center removes the residue, as imidazole, from a hydrophobic center.[66] Thus, the unexpected promotion of inclusion due to the imidazole protonation is a finding that can contribute to the debate about the exact nature of binding interaction[67] and the description of the cyclodextrin cavity as a simply hydrophobic site.[60]

Acknowledgments. We thank the CNR (Rome) for partial support of this work (PF Chimica Fine II). We also thank Prof. I. Bertini (University of Florence—Servizio Nazionale di Risonanza Magnetica Nucleare) for the NMR facilities.

(65) Arena, G.; Bonomo, R. P.; Impellizzeri, G.; Izatt, R. M.; Lamb, J. D.; Rizzarelli, E. *Inorg. Chem.* **1987**, *26*, 785.

(66) Arena, G.; Impellizzeri, G.; Maccarrone, G.; Pappalardo, G.; Sciotto, D.; Rizzarelli, E. *Thermochim. Acta* **1989**, *154*, 97.

(67) Bergeron, R. J. In *Inclusion Compounds*; Davies, J. E. D., MacNicol, D. D., Eds.; Academic Press: London, 1984; Vol. 3, p 391.

Contribution from the Chemistry Departments, The Ohio State University, Columbus, Ohio 43210, The University of Kansas, Lawrence, Kansas 66045, and University of Warwick, Coventry CV4 7AL, England

Sterically Hindered Nickel(II) and Iron(II) Lacunar Cyclidene Complexes Containing *gem*-Dimethyl Groups on Their Saturated Chelate Rings

Peter A. Padolik, Alan J. Jircitano, Nathaniel W. Alcock, and Daryle H. Busch*

Received July 13, 1990

The structure of the cyclidene ligand in the corresponding iron(II)-centered dioxygen carrier has been altered by the addition of *gem*-dimethyl groups to increase the bulk on the saturated rings of the parent macrocycle. Dramatic responses are found in the dioxygen affinities of the resulting complexes. Placing the bulky group to the rear of the cavity, within which the O_2 binds, enhances the O_2 affinity, while placing the group at the entry greatly decreases the affinity. The presence of bulky groups at both positions essentially stops the binding of O_2. Interpretations are based on the crystal structure data and molecular modeling. X-ray structure data for $[DMNiDM(Me_2Me_2Mxyl[16]cyclidene)](PF_6)_2$: $NiC_{32}H_{48}N_6O_2F_{12}$, monoclinic, $P2_1/c$, a = 13.541 (4) Å, b = 15.408 (5) Å, c = 20.471 (7) Å, β = 106.10°, V = 4103 (2) Å^3, Z = 4, R = 0.083, and R_w = 0.091 for 3302 reflections with $I > 2.0\sigma$.

Introduction

Steric bulk is of major significance in the functioning of the dioxygen carriers of nature and the few well-characterized synthetic iron(II) dioxygen carriers. In the globular proteins of myoglobin and hemoglobin the mass of the protein prevents two iron(II) atoms from simultaneously binding to a single O_2 molecule. In the T-form of hemoglobin, a valine residue effectively blocks the O_2 binding site of the β-subunits.[1] The selection between O_2 and CO at the hemoglobin and myoglobin binding sites is affected by the nearest protein residues on the distal side of the porphyrin ring.[2] Many investigators,[3] beginning with Baldwin et al.[4] and Collman et al.,[5] have built superstructures on porphyrins to provide the same steric protection provided by the proteins of the natural products. Steric effects have been pursued at greater length by Traylor et al.[6] In these laboratories, the totally synthetic lacunar cyclidene ligands (Figure 1) utilize similar superstructures to stabilize their dioxygen adducts.[7] The cobalt(II) cyclidene complexes constitute a large family of highly efficacious dioxygen carriers whose O_2 affinities are readily fine-tuned by small incremental changes in the size of the O_2-accommodating cavity.[8] A relatively small group of the iron(II) cyclidene complexes comprise the only well-characterized non-porphyrin synthetic, hemeprotein-model dioxygen carriers based on iron(II).[9] In general a *m*-xylylene roof to the cyclidene cavity will provide the necessary shielding of the binding site to produce reversible O_2 binding. However, the *m*-xylylene-bridged complex is remarkably sensitive to the steric bulk of other substituents in the structure. For example, replacing the methyl groups at the R^2 and R^3 positions of Figure 1 with the bulky groups phenyl and benzyl, respectively, retards the rate of air oxidation of the oxygen carrier by about 4 orders of magnitude. In a very real sense this increased bulk is at the periphery of the structure but adjacent

* To whom correspondence should be addressed at The University of Kansas.

(1) (a) Perutz, M. F. *Nature (London)* **1970**, *228*, 726. (b) Perutz, M. F.; Fermi, G.; Luisi, B.; Shaanan, B.; Liddington, R. C. *Acc. Chem. Res.* **1987**, *20*, 309. (c) Brzozowski, A.; Derewenda, Z.; Dodson, E.; Dodson, G.; Glabowski, M.; Liddington, R.; Skarzynski, T.; Vallely, D. *Nature (London)* **1984**, *307*, 74.

(2) (a) Antonini, E.; Brunori, M. *Hemoglobin and Myoglobin and their Reaction with Ligands*; Elsevier: New York, 1971; p 93. (b) Collman, J. P.; Brauman, J. I.; Halbert, R. R.; Suslick, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3333. (c) Collman, J. P.; Brauman, J. I.; Doxsee, K. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 6035. (d) Geibel, J.; Cannon, J.; Campbell, D.; Traylor, T. G. *J. Am. Chem. Soc.* **1978**, *100*, 3575. (e) Traylor, T. G. *Acc. Chem. Res.* **1981**, *14*, 102.

(3) (a) Meade, T. J.; Busch, D. H. *Prog. Inorg. Chem.* **1985**, *33*, 59. (b) Baldwin, J. E.; Crossley, M. J.; Klose, T.; O'Rear, E. A.; Peters, M. K. *Tetrahedron* **1982**, *38*, 27. (c) Traylor, T. G.; Mitchell, M. J.; Tsuchiya, S.; Campbell, D. H.; Stunes, D. V.; Koga, N. J. *J. Am. Chem. Soc.* **1981**, *103*, 5234. (d) Traylor, T. G.; Koga, N.; Dearduff, L. A.; Swepston, P. N.; Ibers, J. A. *J. Am. Chem. Soc.* **1984**, *106*, 5132. (e) Chang, C. K. *J. Am. Chem. Soc.* **1977**, *99*, 2819. (f) Collman, J. P.; Brauman, J. I.; Collins, T. J.; Iverson, B. R.; Lang, G.; Pettman, R. G.; Sessler, J. L.; Walters, M. A. *J. Am. Chem. Soc.* **1983**, *105*, 3083. (g) Momenteau, M.; Lavalette, D. *J. Chem. Soc., Chem. Commun.* **1982**, 341.

(4) Almog, J.; Baldwin, J. E.; Huff, J. *J. Am. Chem. Soc.* **1975**, *97*, 227.

(5) (a) Collman, J. P.; Gagne, R. R.; Halbert, T. R.; Marchon, T. R.; Reed, J. C. *J. Am. Chem. Soc.* **1973**, *95*, 7868. (b) Collman, J. P. *Acc. Chem. Res.* **1977**, *10*, 265.

(6) (a) Traylor, T. G.; Taube, D. J.; Jongeward, K. A.; Magde, D. *J. Am. Chem. Soc.* **1990**, *112*, 6875. (b) Traylor, T. G.; Koga, N.; Deardurff, L. A. *J. Am. Chem. Soc.* **1985**, *107*, 6504.

(7) (a) Busch, D. H.; Stephenson, N. A. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1989**, *7*, 137. (b) Busch, D. H. *La Trasfusione del Sangue* **1988**, *1*, 57. (c) Busch, D. H. In *Oxygen Complexes and Oxygen Activation by Transition Metals*; Martell, A. E., Sawyer, D. T., Eds.; Plenum: New York, 1988; p 61. (d) Busch, D. H.; Cairns, C. In *Progress in Macrocycle Chemistry*; Izatt, R. M., Christensen, J. J., Eds.; Wiley-Interscience: New York, 1987; p 1. (e) Cairns, C. J.; Busch, D. H. *Inorg. Synth.* **1990**, *27*, 261.

0020-1669/91/1330-2713$02.50/0