An NMR Study of Dioxygen Complexes of Bis(L-histidinato)cobalt(II)

DONALD R. EATON* and JOANNE D. WEEGAR

Department of Chemistry, McMaster University, Hamilton, Ont., L8S 4M1, Canada

Received April 20, 1984

Abstract

Nuclear magnetic resonance has been used to study the structures of the dimeric peroxy bis-(histidinato)-cobalt(III) complexes formed by the oxygenation of solutions containing L-histidine and Co(II) ions. ^{59}Co NMR only confirms the presence of peroxy complexes. Proton and ^{13}C NMR provides further details of their structures as a function of pH. At low pHs (4.0 to 5.8) a single isomer predominates which is identified as a complex containing one tridentate and one bidentate histidine for each Co ion. At higher pHs the number of isomers proliferates considerably. Above pH 5.8 the two carboxyl ligands are successively replaced by water molecules which ionize with pK_a s around 6.2 and 7.2 This process is accompanied by conformational changes in the histidine ligands. Ionization of the pyrrole protons on the imidazole rings commences at pH 9.2. This ionization is probably accompanied by the formation of a hydroxyl bridge between the two Co ions.

Introduction

The reversible reaction of molecular oxygen with solutions containing Histidine and Co(II) ions was first reported by Burk *et al.* [1]. It is now recognized that the compounds formed are correctly formulated as peroxy complexes of Co(III) and many analogous compounds have been discovered. The properties of peroxy complexes have been extensively reviewed [2–4].

The chemistry of metal histidine complexes has attracted a great deal of attention because of its relevance to biological problems. It was reviewed by Sundberg and Martin in 1974 [5] and there have been a number of subsequent publications. The impetus for study of the Co–oxygen complexes was initially based on their role as models for biological oxygen carriers. The initial studies demonstrated that the stoichiometry of the compounds is 2Co:4Hist:1O_2 and this has been confirmed in later publications [6]. The existence of a peroxy bridge was inferred from infrared spectroscopy [7]. Beyond this there is little firm information on the structures in solution. The electronic spectrum has been reported as a function of pH [8] and indicates that there are at least two different compounds, one stable at low pH and a second at high pH. It was initially suggested that isomerism about the peroxy linkage was responsible [8] but more recent publications favour a hydroxide bridged structure for the high pH compound [9]. The kinetics of the oxygenation reaction have been studied at both low [10] and high [11] pH and significant differences found. The purpose of the present study is to characterize the different complexes by means of NMR.

There have been previous NMR studies of the precursor Co(II) histidine complexes but not of the oxygenated solutions. McDonald and Phillips [12] studied the pH dependence of the proton spectrum and deduced the existence of labile carboxyl bonded complexes at low pHs, of 1:1 and 2:1 (hist:Co) compounds of octahedral symmetry at intermediate pHs and of tetrahedral complexes at high pHs. More recently Kitagawa *et al.* [13] have used ^{13}C NMR on the same systems. Their results agree with those of McDonald and Phillips and also provide additional kinetic information. The stoichiometries of the Co(II) complexes in solution at a given pH are therefore known and can be correlated with the structures of the product peroxy compounds.

Experimental

NMR spectra were obtained using a Bruker WP80 spectrometer operating at 80 MHz for proton and 20.115 MHz for ^{13}C. ^{59}Co spectra were obtained on a Bruker WH90 instrument. The probe temperature was 27 °C. A few spectra were run on a Bruker WM250 instrument to obtain better resolution. Solvent suppression of the water resonance was used throughout and the chemical shifts were measured relative to the sodium salt 3-(trimethylsilyl)-propionic acid (internal). Optical absorption spectra were obtained on a Pye Unicam model SP8100 spectrophotometer.

*Author to whom correspondence should be addressed.

0020-1693/84/$3.00

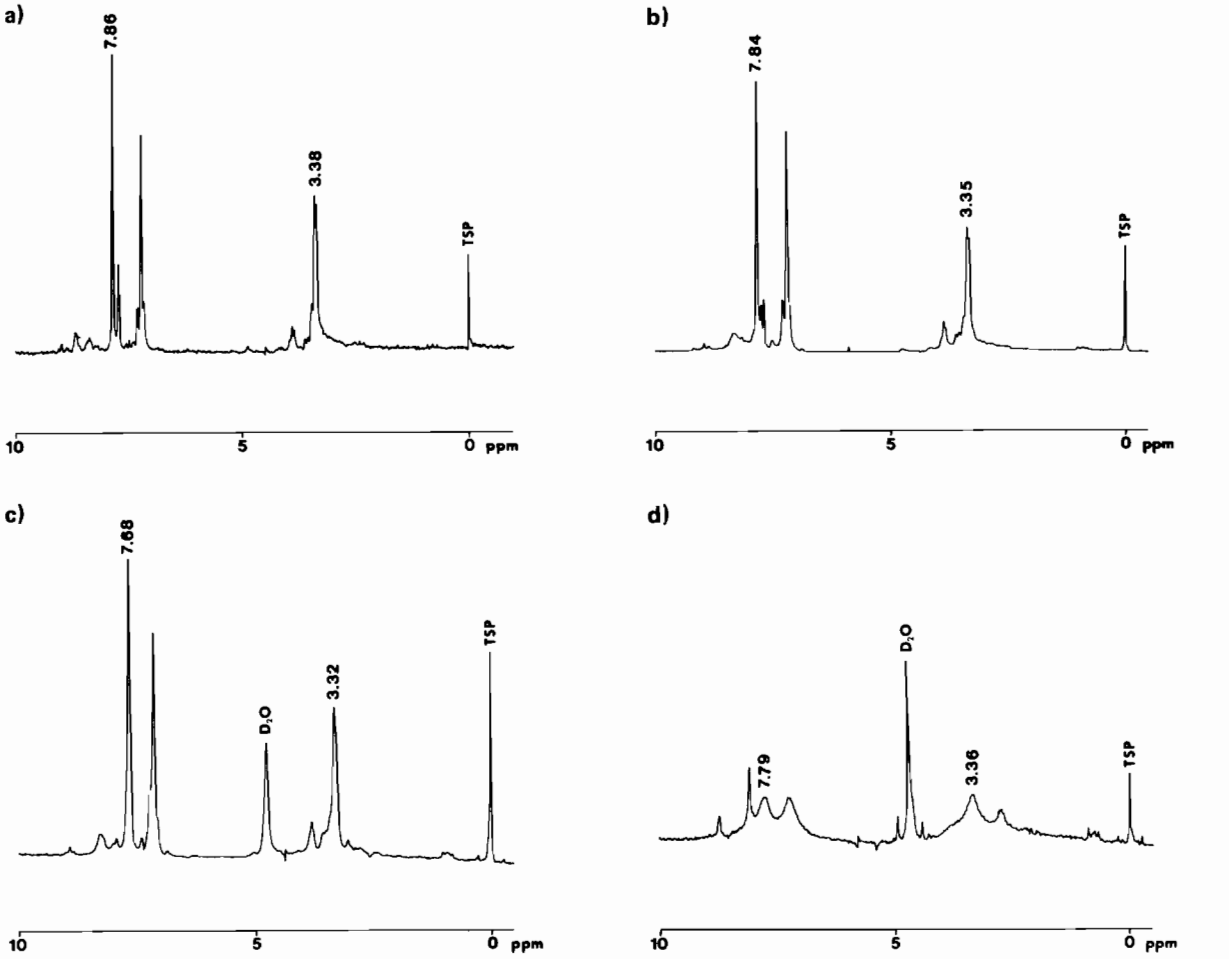

Fig. 1. 80 MHz ^{1}H NMR spectra of bis(histidinato)Co(III) complexes: a) pH 3.4, b) pH 5.0, c) pH 6.1, and d) pH 9.2.

Histidine L-(+)-monohydrochloride (reagent grade) and cobaltous chloride (ACS grade 99.58% purity) were both purchased from Fisher Scientific Co. Both were used without further purification. The solutions used were generally 0.50 M in histidine and 0.25 M in $CoCl_2 \cdot 6H_2O$ using D_2O as a solvent. The pH was adjusted by adding small amounts of HCl or NaOH and was measured with a Radiometer model PHM82 pH meter. The solutions were oxygenated by continuous bubbling of air for at least 30 minutes before obtaining spectra.

In order to be certain that NMR lines assigned to peroxy complexes do not arise from bis(histidinato)-Co(III) compounds we prepared samples containing mixtures of the Co(III) isomers. In our hands the procedure of Bagger *et al.* [20] does not lead to complete oxidation to the Co(III) complexes but results in a mixture of Co(II), Co(III), and peroxy complexes. Complete oxidation can be accomplished using hydrogen peroxide at 60 °C. Thus, 100 ml of an aqueous solution containing 3.57 gm of $CoCl_2 \cdot 6H_2O$, 6.92 gm of histidine monohydrochloride, 1.32 gm of NaOH, and 4 ml of 30% H_2O_2 was heated at 60 °C for 5 hours. Activated charcoal was present as a catalyst. The pH was raised to 5 and water removed by rotary evaporation. Elemental analysis, magnetic susceptibility measurements, and optical spectra all confirm that oxidation to Co(III) is complete and the NMR spectra are taken to be those of a mixture of bis(histidinato)Co(III) isomers. The proton chemical shifts obtained do not agree well with those reported by Bagger *et al.* [20]. The shifts of the imidazole protons can be matched moderately well with the shifts of the three individual isomers separated by Bagger, but there are discrepancies of around 0.2 ppm in the aliphatic shifts. We attribute these discrepancies to the use of t-Butyl alcohol as an internal NMR reference by Bagger *et al.* Hydrogen bonding of the alcohol to the carboxyl group of the histidine is very probable and invalidates its use as an internal reference. Comparison of the Co(III) NMR spectra with those obtained from the peroxy solutions shows that at most pH's they are completely different. With the exception

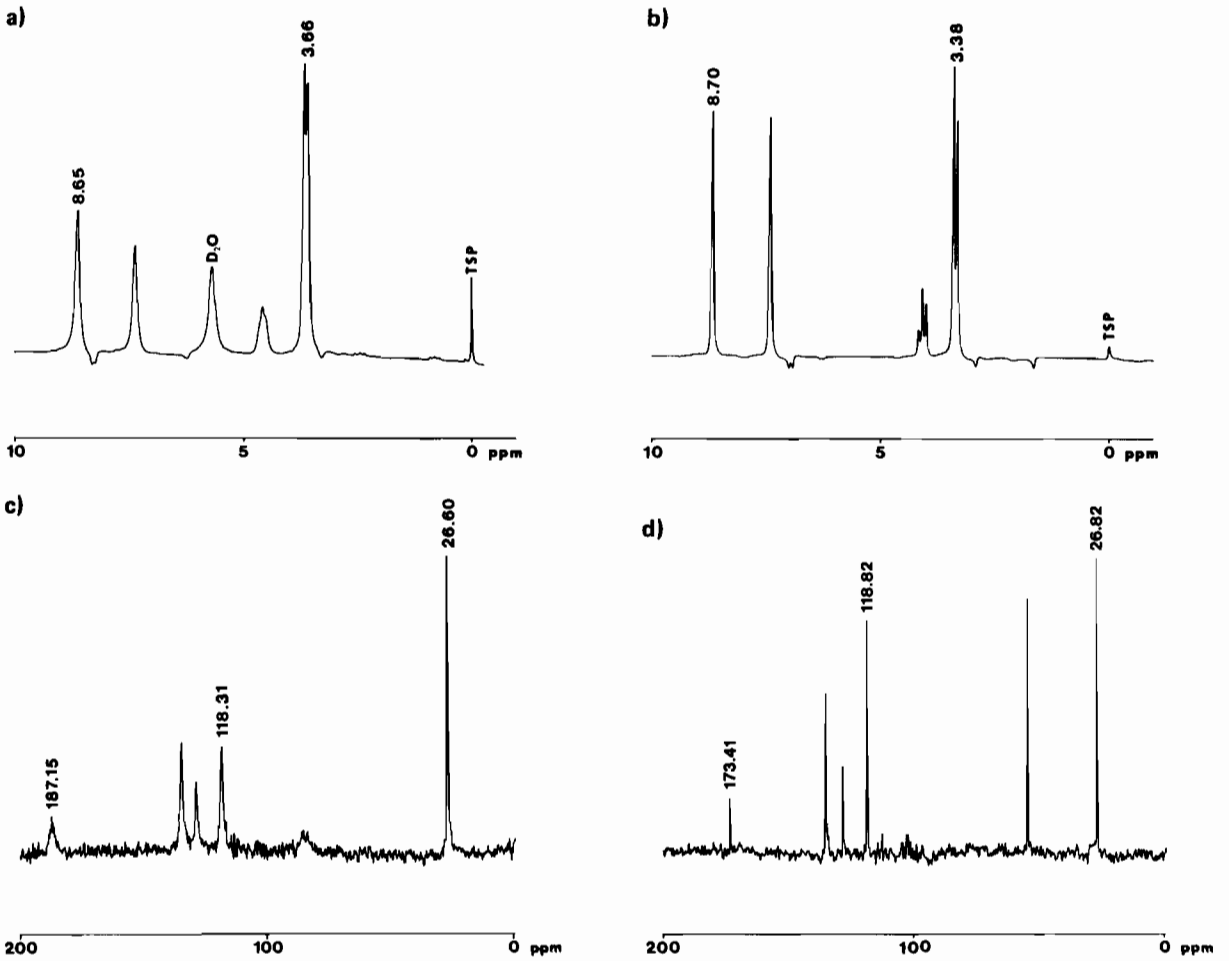

Fig. 2. NMR spectra at pH 3.0 of a) bis(histidinato)Co(III) peroxy complex, and b) L-histidine, both ^{1}H NMR at 80 MHz; c) bis-(histidinato)Co(III) peroxy complex and d) L-histidine both ^{13}C NMR at 20.115 MHz.

of some of the overnight ^{13}C spectral runs there is no evidence for significant Co(III) contamination of the peroxy solutions.

Results

The ^{59}Co, ^{1}H and ^{13}C NMR spectra of oxygenated solutions containing Co^{2+} and L-histidine in the mole ratio 1:2 have been examined. ^{59}Co N.M.R. has previously proved useful in identifying isomers of Co(III) peroxy complexes [14, 15]. However, in the present case only a single broad line at chemical shift 9036 ppm (linewidth 3170 Hz) was obtained at pH 5. The chemical shift is in the range expected for a peroxy complex [14, 15] but no further structural information is provided.

Proton and ^{13}C spectra have been obtained over the pH range 2.5 to 11.6. The pH dependence is quite complex with substantial changes occurring over narrow pH ranges. The proton spectra were therefore obtained at pH intervals of 0.2. ^{13}C NMR spectroscopy is more time consuming so spectra were obtained at pH intervals of approximately 2. Proton spectra were also run at a number of pHs with a sweep width of 25 KHz to obtain the spectra of any paramagnetic Co(II) complexes present. Isotropically shifted lines were found at all pHs. The spectra agree with those reported by McDonald and Phillips. It is difficult to make accurate intensity comparisons between the lines from the diamagnetic and paramagnetic compounds because of the very different instrumental power levels used to obtain the two sets of spectra but we estimate that between 5% and 15% of the Co(II) remains unoxygenated under the conditions of these experiments. The proton and ^{13}C spectra of L-histidine were also obtained as a function of pH to allow identification of any unreacted histidine. Both sets of spectra agree well with literature data [16–19]. The pH dependence of the proton spectrum of the Co(III) complexes has been measured. Chemical shifts are given in Table I and a set of representative spectra in Fig. 1. As pointed out in the experimental section, the chemical

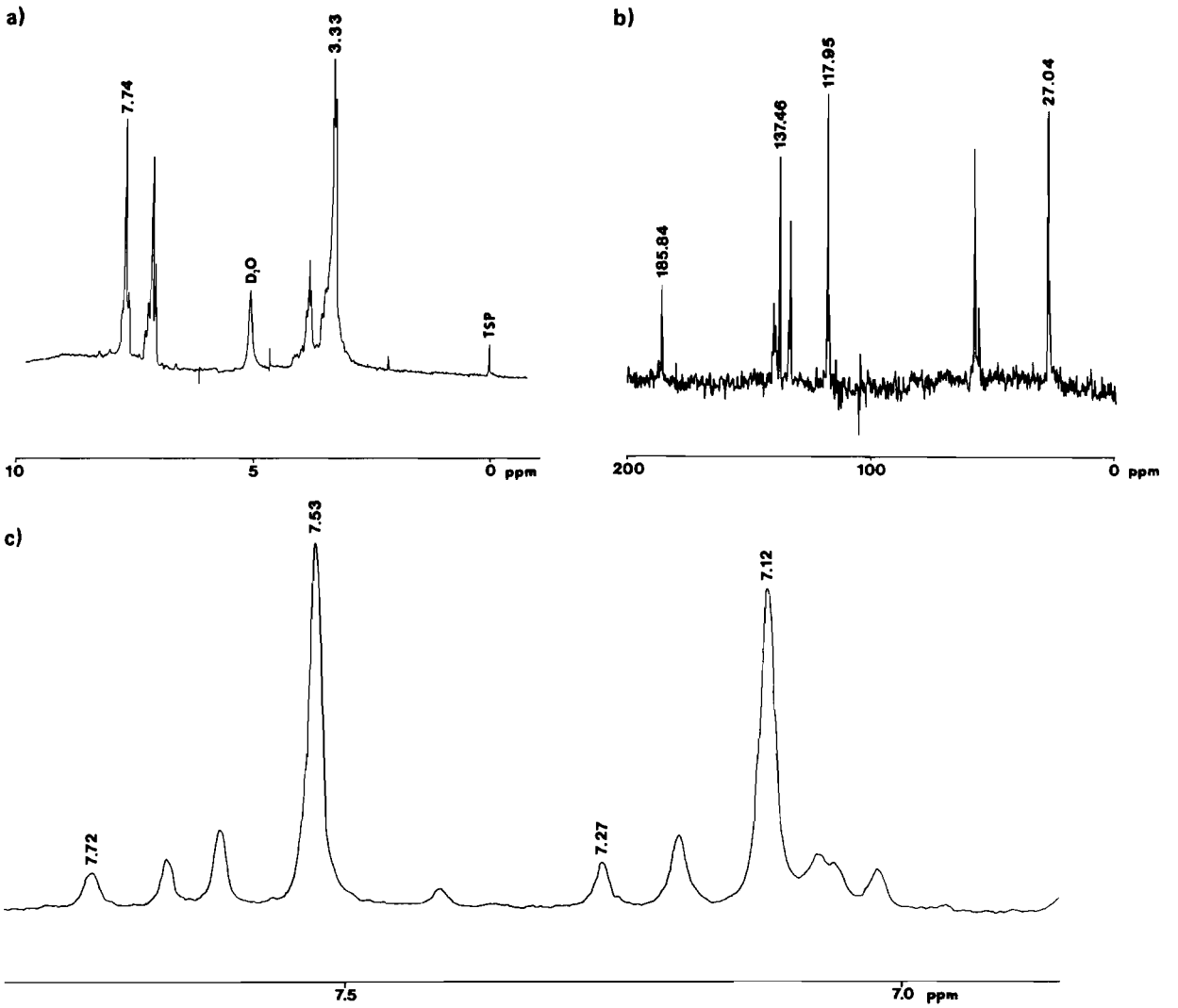

Fig. 3. NMR spectra of pH 5.0 bis(histidinato)Co(III) peroxy complex at: a) 80 MHz (^{1}H), b) 20.115 MHz (^{13}C), and c) 250 MHz (^{1}H), Imidazole region.

TABLE I. ^{1}H Chemical Shifts of Bis(histidinato)Co(III) Complexes.[a]

pH	H_2 peaks	H_4 peaks	H_α peaks	H_β peaks
3.4	7.86(di)	7.23(di)	3.91(t)	3.38(di)
4.0	7.84(di)	7.21(si)	3.88(t)	3.36(di)
5.0	7.84(si)	7.20(si)	3.87(t)	3.35(di)
6.1	7.68(s)	7.15(si)	3.81(b)	3.32(si)
7.0	7.66(s)	7.15(si)	3.79(b)	3.30(bi)
8.2	7.78(b)	7.20(b)		3.34(b)
9.2	7.79(b)	7.26(b)		3.36(b)

[a](s) = single, (d) = doublet, (t) = triplet, (b) = broad, (i) = lower intensity peaks assigned to less abundant isomers also observed.

shifts differ from those reported by Bagger *et al.* [20] due to the inert reference used in the present study. These spectra allow assignment of Co(III) impurities in the peroxy solutions. Changes in the optical spectrum of the oxygenated solutions as a function of pH have also been reexamined. Below pH 5 the spectra of the oxygenated and unoxygenated samples are virtually identical. Between pH 5 and pH 10.5 a new spectrum with peaks at 390 and 325 appears with the intensity increasing with pH. Above pH 10.5 this is replaced by a spectrum with only a single intensity maximum in the visible spectrum. These results agree with the literature reports [8, 9].

The NMR spectra demonstrate changes in structure corresponding to five different pH regions.

Region A, pH 2.8–4.0

Only 'free' ligand lines are observed. There is some shifting and broadening of these lines compared to spectra obtained in the absence of Co(II). In the ^{13}C spectra the CO and CH lines are preferentially broadened. The broadening increases

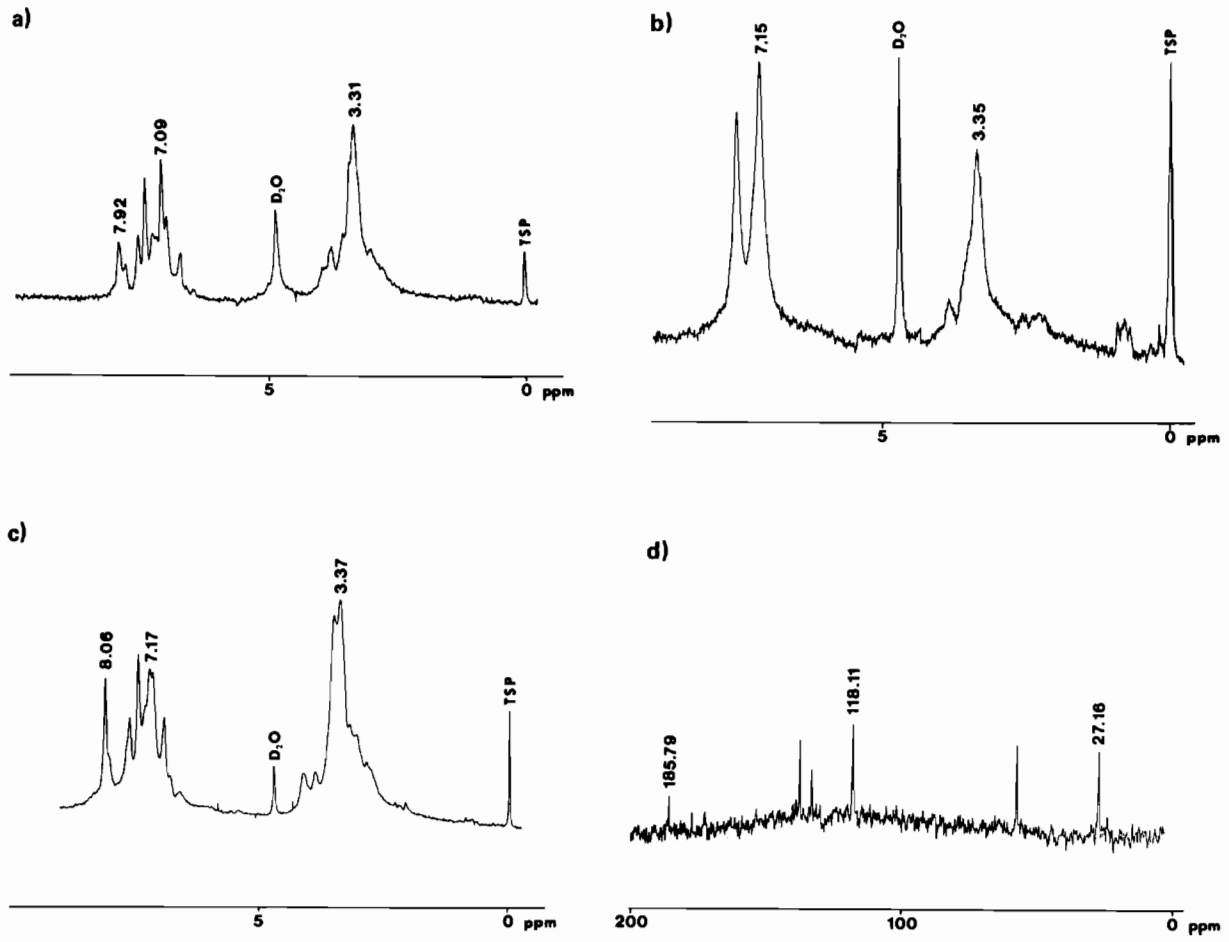

Fig. 4. Spectra of bis(histidinato)Co(III) peroxy complexes: a) pH 5.6, b) pH 6.2, and c) pH 6.8, all ^{1}H NMR at 80 MHz; and d) pH 5.8, ^{13}C NMR at 20.115 MHz.

smoothly with pH. Figure 2 shows spectra typical of this region.

Region B, pH 4.0–5.6

A new sharp line proton spectrum begins to appear at pH 4.0. It gradually increases in intensity without any shifting or broadening of the lines up to pH 5.6. At the same time the 'free' ligand resonances become progressively weaker and disappear entirely at pH 4.8. In this region the spectra of the Co(II) complexes reported by McDonald and Phillips can also be observed using 25 KHz sweep widths. Changes in the ^{13}C spectra parallel those in the proton spectra. At the high pH end of this region there is one principal series of ^{13}C lines accompanied by weaker lines. There is some variation of the relative intensities of the different lines from sample to sample. The principal lines differ in chemical shift from those of L-histidine at the same pH and also from those of the Co(III) complexes. We note in particular the difference in the carbonyl resonance (185.5 ppm compared with 174.0 for histidine). Figure 3 shows representative spectra obtained in this region. The spectrum Fig. 3c shows a 250 MHz expansion of the imidazole region of the proton spectrum.

Region C, pH 5.6–6.8

At pH 5.6 a new set of relatively sharp lines appears in the proton spectrum as shown in Fig. 4a. These lines rapidly broaden and coalesce to give the 'averaged' spectrum of Fig. 4b at pH 6.2. This process is then reversed and at pH 6.8 the spectrum is again fairly sharp as illustrated in Fig. 4c. We note that the spectra of Figs. 4a and 4c are quite different and demonstrate the presence of different mixtures of species at the different pHs. The ^{13}C spectra in this region are in general poorly resolved and complex showing only that a mixture of species is present. One feature is significant. As shown in Fig. 4d there are two groups of CO resonances, one centred around 186 ppm and one around 177 ppm. This feature is retained throughout the high pH regions.

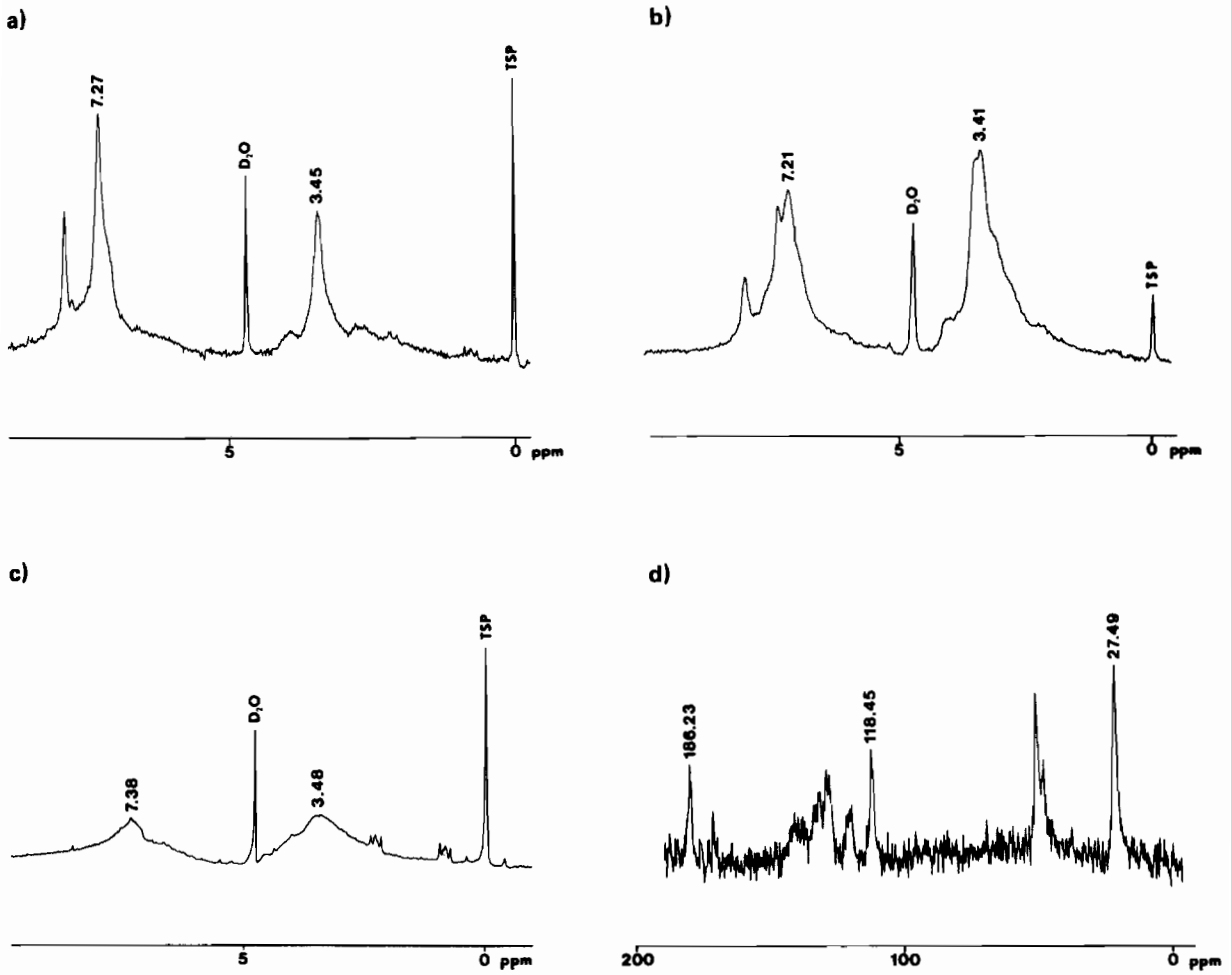

Fig. 5. Spectra of bis(histidinato)Co(III) peroxy complexes: a) pH 7.2, b) pH 9.2, and c) pH 11.6, all ^{1}H NMR at 80 MHz; and d) pH 9.6, ^{13}C NMR at 20.115 MHz.

Region D, pH 6.8–9.2

Above pH 6.8 coalescence and broadening again occurs leading to the broad spectrum of Fig. 5a at pH 7.2. This spectrum remains unchanged to pH 9. ^{13}C spectra in this region are poorly resolved and not particularly useful.

Region E, pH 9.2–11.6

Further changes become apparent around pH 9.2. New broad lines appear followed by still more extensive broadening. Finally at the highest pHs only two very broad lines corresponding to the aliphatic and aromatic regions of the spectrum can be distinguished. ^{13}C spectra are also broad but show more structure because of the larger chemical shifts. Representative spectra obtained in the high pH region are shown in Figs. 5b and 5c, and 5d. Tables II and III collect together chemical shift data obtained over the complete pH range.

Discussion

The pH dependence of the NMR spectra described above clearly demonstrates that a rather large number of Co histidine dioxygen complexes exist in solution. The results are not consistent with the implication of previous studies that a single species occurs below pH 10 and a second species above this pH.

The bis(histidinato)Co(III) spectra are also pH dependent. Broadening of the lines commences at about pH 6 and continues up to pH 9.2. Above this pH hydrolysis accompanied by precipitation of Co hydroxide occurs. The changes in the Co(III) spectrum are reversible below pH 9.2 and the chemistry involved could well be similar to that discussed below for the oxygen complexes.

The interpretation of the oxygen complex data in region A is straightforward. Below pH 4 no oxygenation occurs. A labile carboxyl bonded complex is formed between L-histidine and Co(II)

TABLE II. ^{1}H Chemical Shifts of Bis(histidinato)Co(III) Peroxy Complexes.[a]

	H_2 peaks		H_4 peaks		H_α peaks		H_β peaks	
Region A pH 2.8–4.0								
pH 3.0	8.65(b)		7.38(b)		4.59(b)		3.66(d)	
3.8	8.70(b)		7.47(b)		4.63(b)		3.62(b)	
Region B pH 4.0–5.6								
pH 4.0	8.74(vb)	7.80(b)	7.47(vb)	7.23(b)	4.66(vb)		3.58(b)	
4.4	8.80(vb)	7.76(si)	7.46(vb)	7.22(si)	4.62(vb)	3.83(b)	3.51(b)	3.35(b)
4.8		7.70(si)		7.15(si)		3.82(t)	3.47(b)	3.32(d)
5.0		7.74(di)		7.17(si)		3.79(t)		3.33(d)
5.2		7.92(bi)	7.56(bi)	7.12(bi)		3.79(b)		3.32
Region C pH 5.6–6.8								
pH 5.6	7.92(bi)	7.55(b)	7.40(b)	7.09(bi)	3.77(b)		3.31(b)	
6.2	7.55(b)		7.15(b)		3.85(b)		3.35(b)	
6.6	8.09(b)	7.75(b)	7.24(b)		3.92(b)		3.41(b)	
6.8	8.06(b)	7.58(b)	7.41(b)	7.17(bi)	4.12(b)	3.87(b)	3.51(b)	3.37(b)
Region D pH 6.8–9.2								
pH 7.2	7.88(b)		7.27(b)				3.45(b)	
8.0	7.87(b)		7.26(b)				3.43(b)	
9.0	7.87(bi)		7.27(b)		3.90(vb)		3.44(b)	
Region E pH 9.2–11.6								
pH 9.2	8.06(vb)		7.41(b)	7.21(b)			3.41(vb)	
9.8	7.83(b)		7.28(b)				3.44(b)	
10.6			7.30(b)				3.48(b)	
11.6			7.38(vb)				3.48(vb)	

[a](s) = singlet, (d) = doublet, (t) = triplet, (b) = broad, (vb) = very broad, (i) indicates satellites assigned to less abundant isomers.

TABLE III. ^{13}C Chemical Shifts of Bis(histidinato)Co(III) Peroxy Complexes.[a]

pH	C:O	Imidazole Ring Carbons	CH	CH_2
2.6	184.30(b)	134.48(s) 128.05(s) 118.31(s)		26.21(s)
3.0	187.15(s)	134.68(s) 128.54(s) 118.31(s)		26.60(s)
5.0	185.84(s)	139.87(s) 137.46(s) 133.00(s) 117.95(s)	57.44(s)	27.04(s)
5.8	185.79(s) 177.14(w) 172.71(w)	137.58(s) 133.21(s) 118.11(s)	57.26(s)	27.16(s)
8.4	185.79(sb) 177.74(w)	145.87(w) 137.90(m) 134.17(m) 126.78(w) 118.59(sb)	56.96(sb)	27.64(sb)
9.6	186.23(sb) 178.04(w)	147.21(w) 135.34(m) 125.91(m) 118.45(sb)	56.81(sb)	27.49(sb)
10.1	186.73(sb) 177.78(w)	147.33(w) 135.78(m) 127.44(sb) 118.52(sb)	56.81(sb)	27.93(sb)
11.5	186.45(sb) 177.67(sb)	146.30(sb) 135.63(sb) 126.71(sb) 118.67(sb)	56.30(m)	27.86(sb)

[a](s) = strong, (w) = weak, (b) = broad, (m) = multiplet.

as suggested by McDonald and Phillips [12]. The structure of this complex is supported by the preferential broadening of the CO and CH ^{13}C resonances.

Oxygenation commences at pH 4. The sharp spectrum at pH 5 shown in Fig. 3 is ascribed to a peroxy complex with a Co:histidine stoichiometry of 1:2. The chemical shifts do not agree with those of the bis(histidinato) Co(III) complexes at this pH. The sharpness of the lines and their 'normal' chemical shifts preclude assignment to a Co(II) complex. When the complex is fully formed (pH 4.8) no free histidine remains. This pH corresponds to the pH at which the formation of a peroxy complex is indicated by visible spectroscopy. The assignment to a 2:1 peroxy compound is therefore strongly indicated. This complex is not in fast chemical exchange on the NMR time scale with either free histidine or the precursor Co(II) compound since all three species can be observed in the same solution.

If the histidine ligand is tridentate in the Co(II) complex, as was demonstrated by McDonald and Phillips, it is necessary to detach one of the ligating groups to form the peroxy compound. It is usually assumed that one of the carboxyl groups becomes detached since this is the least strongly bound ligand. The resulting complex can exist in several isomeric forms. These may differ both in the arrangement of

the ligating atoms and in the conformation of the histidine molecule. Either or both effects could lead to different chemical shifts. Steric considerations dictate that the three coordinating groups of a tridentate histidine ligand adopt a facial arrangement about the Co ion. For two tridentate L-histidines three isomers are possible [20]. In each of these isomers both carboxyl groups are equivalent and it therefore makes no difference which is replaced by the peroxy ligand. A tridentate histidine is restricted to a single conformation. However, dissociation of the carbonyl to give a bidentate ligand allows two histidine conformations. An example of this possibility is provided by the study of Erickson *et al.* of the NMR spectra of Pt(L-histidine)$_2$ [21]. A total of six isomers is therefore possible and since the bidentate and tridentate histidines could show different chemical shifts, up to twelve different resonances are possible for each different proton or carbon. It is clear that not all the possible isomers are present.

The spectrum of Fig. 3c shows the region corresponding to the two imidazole protons attached to C2 and C4. The lower field lines are assigned to C2 and the higher field lines to C4 [16]. The two lines of equal intensity at 7.53 and 7.12 ppm contain 64% of the total intensity and therefore represent the most abundant isomer. Since there are two lines of equal intensity rather than four, it is clear that the chemical shift differences between the tridentate and bidentate histidines are not being resolved. This is not surprising in the light of the results of Sacks *et al.* [16] on the pH dependence of histidine ring protons. These authors found three pK_a values 2.0, 6.1 and 9.2 which they assigned to the titration of the carboxyl, the imidazole and the amino protons respectively. Subsequent C13 studies have confirmed these assignments [17, 18]. The important point is that the change in chemical shifts of the ring protons for carboxyl ionization is very small (less than 0.1 ppm) and is comparable to the line widths in the present spectra. Since the imidazole ring protons are little affected by ionization of the carboxyl group it is reasonable that they will be little affected by dissociation of the group from the Co ion. There is however reason to suppose that the chemical shifts will be considerably affected by changes in the conformation of the histidine ligand. Thus Blomberg *et al.* [22] have shown that the conformation of histidine depends on pH and that the change in conformation affects the interaction of the carboxyl group with the imidazole ring. Sagaguchi *et al.* [23] have demonstrated changes in conformation when histidine is complexed to metal ions. Erickson *et al.* [21] found that the conformations of Pt(Lhist)$_2$ complexes changed with pH and that the changes produced chemical shifts of around 0.6 ppm for the C2 proton and around 0.4 ppm for the C4 proton. On the basis of these considerations we would expect two lines in spectrum 3c for each isomer in which the two histidines have the same conformation and four lines for each isomer in which they have different conformations. Since the conformation of the tridentate ligand is fixed that of the bidentate ligand is determined . The major isomer clearly has the same conformation for both ligands. On the basis of an X-ray crystal structure of bis(L-histidinato)Co(II) [24] the structures of this type of compound have usually been drawn with the two imidazole residues *trans* to each other. Reference to the isomeric structures shown by Bagger *et al.* [20] shows that this places the peroxy group *cis* to the carboxyl. We would prefer a structure with the carboxyl *trans* to the peroxy on the basis of analogy with similar salicylaldimine compounds for which it is clear that stability is reduced by placing a π-bonding ligand *trans* to the peroxy group and enhanced with a non-π-bonding ligand in this position [25, 26]. However, the present NMR spectra provide no information on this point. The second most intense feature of Fig. 3a is a pair of lines of equal intensity at 7.61 and 7.20 ppm (representing 16% of the total intensity) which must be assigned to the second of the isomers discussed above. A set of four lines of equal intensity can also be found and these are most reasonably assigned to the isomer with the same geometric arrangement about the Co as the major isomer but the opposite conformation of the bidentate histidine. It is apparent that at least one more isomer is present in low abundance.

Changes in the aliphatic part of the proton spectrum also reflect the conformation of the histidine. The conformer which places the carboxyl group close to the imidazole ring gives rise to a simple 'first order' spectrum [16, 22] as shown in Fig. 6a. The other conformer gives a complex ABC type pattern of the type illustrated in Fig. 6b. The 250 MHz spectrum of Fig. 6c clearly belongs to the latter type. This is to be anticipated since the former conformation is not possible for a tridentate histidine.

The ^{13}C spectra are consistent with this interpretation. The spectrum of Fig. 2b shows a single set of intense lines, corresponding to the most abundant isomer, together with a number of weaker resonances. Only a single strong carbonyl resonance at 186 ppm is apparent. Quirt *et al.* [17] have examined the ^{13}C spectrum of histidine as a function of pH. The carbonyl shift is relatively insensitive to the carbonyl ionization (a shift of some 2 ppm) but changes by more than 9 ppm with the conformational change accompanying imidazole ionization. The observation of a single peak confirms that both histidines have the same conformation in the most abundant isomer. The second carbonyl resonance at 178 ppm, which appears at higher pHs, must be assigned to the other conformer. A model shows that a tridentate histidine must adopt the conformation assigned [17,

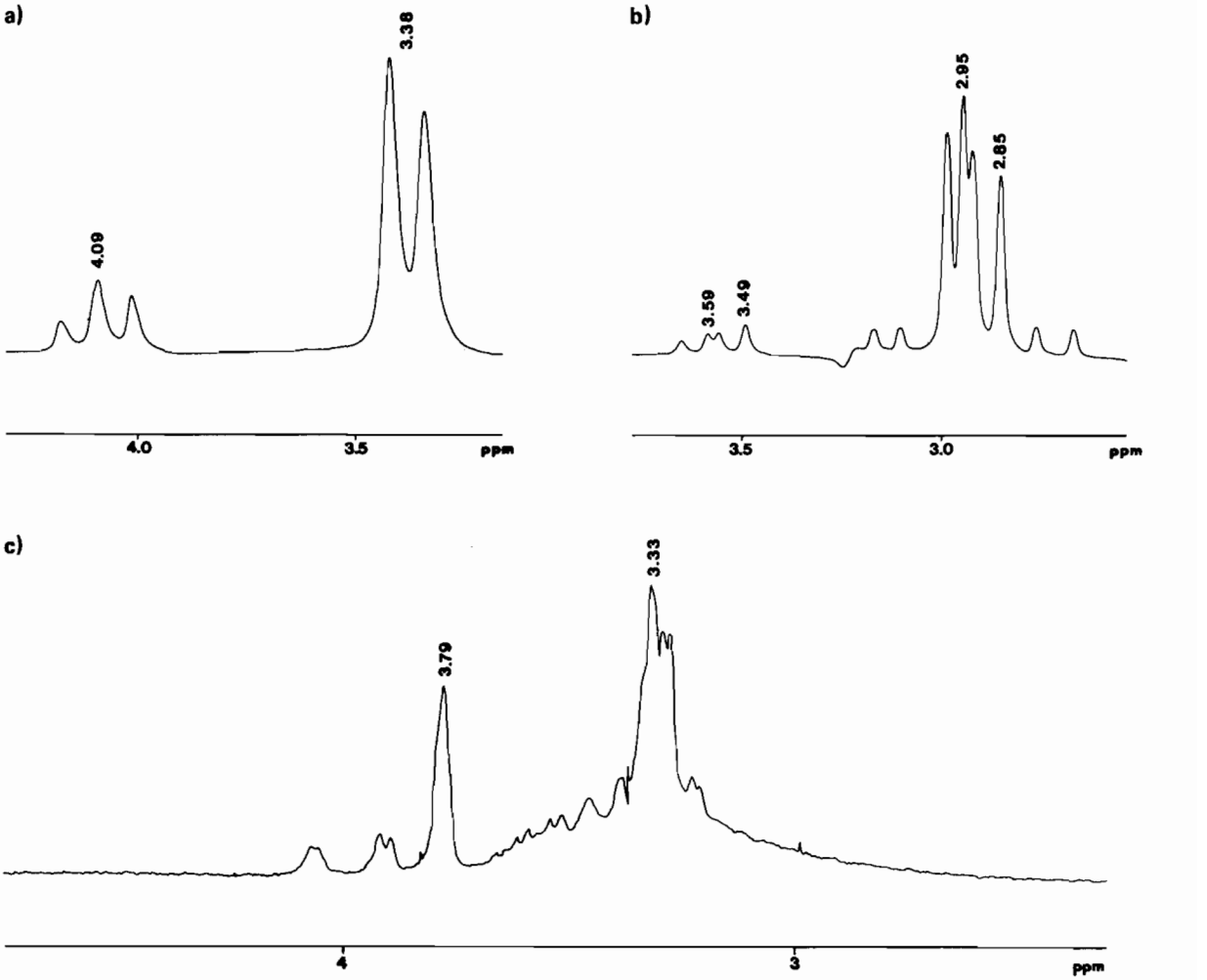

Fig. 6. ^{1}H NMR spectra of the aliphatic region of a) L-histidine, pH 3.0 and b) L-histidine, pH 10.0, both at 80 MHz; and c) bis(histidinato)Co(III) peroxy complex, pH 5.0, 250 MHz, Aliphatic region.

21] to the lower field ^{13}C chemical shift. The observed chemical shift in this pH region of 186 ppm is consistent with this requirement.

The interpretation of regions C and D of the pH profile presents an immediate problem. The only protons available for ionization in this pH range are the pyrrole protons on the imidazole rings. The pK_a for this proton on free histidine has been reported as 14.4 [5]. This pK_a is clearly lowered by complex formation and values as low as 4 have been suggested [27]. Such spectacular changes in pK_a wold be of substantial biological significance and the topic has therefore attracted considerable attention [28–31]. A pK_a of 12.5 has been reported for the Co(II) complex of L-histidine [32]. The consensus of recent publications seems to be that the very low values are unlikely. Thus a recent value [33] for pentammine imidazolatoCo(III) is 10.0. This complex should be a fairly good model for present compounds. Thus it is reasonable to assign changes in region E (pH 9.2–11.6) to pyrrole ionization but not changes at pH 5.6.

We suggest that ionization of an aquo complex is more likely. pK_a's of such complexes of Co(III) and Fe(III) are in the range 6 to 8 [34, 35]. The bis-(histidinato)Co(III) spectra show similar changes in this pH region and replacement of coordinated carboxyl by water is also probable. Aquo complexes can be formed by displacement of the remaining coordinated carboxyl groups. Two such groups are available for displacement and since ionization of the first water increases the negative charge on the molecule the pK_a of the second water will be rather higher than that of the first water. Our results suggest values of around 6.2 for the first water and around 7.2 for the second. Thus replacement of the first carboxyl commences around pH 5.8 and the additional lines appearing in the spectrum at this pH are assigned to a mono aquo compound. The appearance of sharp lines at this pH implies a ligand substitution rate

which is slow on the NMR time scale which is compatible with literature values for such processes [36]. This observation would not be compatible with a proton exchange reaction. The fraction of hydroxyl ligand gradually increases to pH 6.2 at which point the rapid proton exchange causes maximum broadening. The relatively sharp spectrum at pH 6.8 is the mono hydroxyl complex. Ionization of the second water commences above this pH and the spectra in the range pH 7.0 to 9.0 are assigned to the dihydroxy complex.

In the aquo and hydroxy compounds both histidines are bidentate. Four isomeric arrangements about the Co atom are possible and each of these can be combined with the four possible conformational arrangements of the two histidines giving 16 isomers. The appearance in the ^{13}C spectrum of two groups of carbonyl resonances around 178 and 186 ppm of similar intensities shows that both conformers occur to a similar extent. The lack of resolution in the proton spectra in the pH 7–9 region can probably be ascribed to extensive overlap of the many lines expected from this large number of isomers.

The notable feature of the spectra in region E (pH 9.2–11.6) is the lack of structure due to extensive line broadening. We ascribe this to rapid proton exchange arising from ionization of the pyrrole hydrogens. This is in accord with the literature explanations of the changes in the optical spectrum in this pH region [8, 9]. The suggestion [9] that this ionization is accompanied by hydroxy bridge formation seems very reasonable but the broadness of the spectra does not allow NMR investigation of this possibility.

Conclusions

This NMR study has demonstrated that the solution chemistry of the dioxygen complexes formed wih Co(II) and histidine is extensive and very pH dependent. The suggested structures are summarized below.

1. Dioxygen complex formation commences at pH 4. At pH 5 the formation of an octahedral complex containing a tridentate histidine, a bidentate histidine with a dangling carboxyl group and a bridging peroxy ligand is complete. One isomer of this complex is predominant (64%) and both histidine molecules have the same conformation in this isomer. It is suggested that the peroxy group is *trans* to the coordinated carboxyl in this isomer.

2. At pH 5.6 replacement of one coordinated carboxyl in the dimer by water commences. The ligand exchange is slow on the NMR time scale but there is rapid exchange between the aquo and the hydroxy complexes. At pH 6.8 a complex mixture of monohydroxy isomers predominates. Both conformers of the bidentate histidines are present in comparable percentages.

3. Above pH 6.8 the second carboxyl is replaced by water and the resulting compounds are in rapid exchange with the corresponding hydroxy complexes. In the pH region 7.2 to 9.2 a very complex mixture of dihydroxy isomers predominates leading to poor resolution of the NMR spectra.

4. Ionization of the pyrrole protons on the imidazole rings commences at pH 9.2 and continues up until the highest pH studied (11.6). There is rapid exchange between the protonated and deprotonated species. It is probable that this ionization is accompanied by hydroxy bridge formation between the two Co ions as has been inferred from spectroscopic studies but line broadening prevents NMR confirmation of this structural change.

Acknowledgement

We are indebted to the Natural Science and Engineering Research Council of Canada for financial support of this research.

References

1 D. Burk, J. Hearon, L. Caroline and A. L.Shade, *J. Biol. Chem., 165,* 723 (1946).
J. Z. Hearon, D. Burk and A. L. Shade, *J. Nat. Cancer Inst., 9,* 337 (1948).
2 G. McLendon and A. E. Martell, *Coord. Chem. Rev., 19,* 1 (1976).
3 R. D. Jones, D. A. Summerville and F. Basolo, *Chem. Rev., 79,* 139 (1979).
4 J. O. Smith and J. P. Pillon, *Coord. Chem. Rev., 39,* 295 (1981).
5 R. J. Sundberg and R. B. Martin, *Chem. Rev., 74,* 471 (1974).
6 L. J. Zompa, C. S. Sokol and C. H. Brubaker, *Chem. Comm.,* 701 (1967).
7 Y. Sano and H. Tanabe, *J. Inorg. Nucl. Chem., 25,* 11 (1963).
8 S. Bagger, *Acta Chem. Scand., 23,* 975 (1969).
9 M. S. Michaildidis and R. B. Martin, *J. Am. Chem. Soc., 91,* 4683 (1969).
L. G. Stadtherr, R. Prados and R. B. Martin, *Inorg. Chem., 12,* 1814 (1973).
10 F. Miller, J. Simplicio and R. G. Wilkins, *J. Am. Chem. Soc., 91,* 1962 (1969).
11 K. L. Watters and R. G. Wilkins, *Inorg. Chem., 13,* 752 (1974).
12 C. C. McDonald and W. D. Phillips, *J. Am. Chem. Soc., 85,* 3736 (1963).
13 S. Kitagawa, K. Yoshikawa and I. Morishima, *J. Phys. Chem., 82,* 89 (1978).
14 S. C. F. Au-Yeung and D. R. Eaton, *Inorg. Chim. Acta, 76,* L141 (1983).
15 S. C. F. Au-Yeung and D. R. Eaton, *Can. J. Chem.,* in press.
16 D. H. Sachs, A. N. Schechter and J. S. Cohen, *J. Biol. Chem., 246,* 6576 (1971).
17 A. R. Quirt, J. R. Lyerla, I. R. Peat, J. S. Cohen, W. F. Reynolds and M. H. Freedman, *J. Am. Chem. Soc., 96,* 570 (1974).

18 W. F. Reynolds, I. R. Peat, M. H. Freedman and J. R. Lyerla, *J. Am. Chem. Soc., 95,* 328 (1973).
19 M. Alei, L. O. Morgan, W. E. Wageman and T. W. Whaley, *J. Am. Chem. Soc., 102,* 2881 (1980).
20 S. Bagger, K. Gibson and C. S. Sorensen, *Acta Chem. Scand., 26,* 2503 (1972).
21 L. E. Erickson, J. W. McDonald, J. K. Howie and R. P. Clow, *J. Am. Chem. Soc., 90,* 6371 (1968).
22 F. Blomberg, W. Maurer and H. Ruterjans, *J. Am. Chem. Soc., 99,* 8149 (1977).
23 U. Sakaguchi, K. Morito and H. Yoneda, *Inorg. Chim. Acta, 37,* 209 (1979).
24 M. M. Harding and H. A. Long, *J. Chem. Soc. (A),* 2554 (1968).
25 C. Floriani and F. Calderazzo, *J. Chem. Soc. (A),* 946 (1969).
26 R. S. Gall, J. F. Rogers, W. P. Schaefer and G. G. Christoph, *J. Am. Chem. Soc., 98,* 5135 (1976).
27 M. L. Markley, *Accts. of Chem. Res., 8,* 70 (1975) and references therein.
28 R. B. Martin, *Proc. Nat. Acad. Sci., 71,* 4346 (1974).
29 D. Demoulin, A. Pullman and B. Sarkar, *J. Am. Chem. Soc., 99,* 8498 (1977).
30 M. Nappa, J. S. Valentine and P. A. Snyder, *J. Am. Chem. Soc., 99,* 5799 (1977).
31 W. W. Bachovchin and J.D. Roberts, *J. Am. Chem. Soc., 100,* 8041 (1978).
32 P. J. Morris and R. B. Martin, *J. Am. Chem. Soc., 92,* 1543 (1970).
33 N. S. Rowan, C. B. Storm and R. R. Rowan, *J. Inorg. Biochem., 14,* 59 (1981).
34 D. F. Martin and M. L. Tobe, *J. Chem. Soc.,* 1388 (1962).
35 Stability Constants. *Chem. Soc. Spec. Publ. 17 and 25,* (1964 and 1971).
36 Y. Sasaki, K. Z. Suzuki, A. Matsumoto and K. Saito, *Inorg. Chem., 21,* 1825 (1982).