

## Small Molecule Analogues for the Specific Metal-binding Site of Lactoferrin. Part 2.<sup>1</sup> Phenolato-complexes of Copper(II) and the Nature of the Charge-transfer Transition in the Visible Region

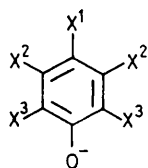
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The preparation of the following compounds is described: *cis*-[CuL<sub>2</sub>L<sup>1</sup>] [L = pentafluorophenolate (pfp), L<sup>1</sup> = ethylenediamine (en), *NNN'*-tetramethylethylenediamine (tmen), 2,9-dimethyl-1,10-phenanthroline (dmphen), or 2,2'-bipyridyl (bipy); L = pentachlorophenolate (pcp), L<sup>1</sup> = en or dmphen; L = 2,4,6-tribromophenolate (tbp), L<sup>1</sup> = en, tmen, or dmphen; L = 2,4,6-tri-iodophenolate (tip), L<sup>1</sup> = en, tmen, or dmphen; L = 2,6-di-iodo-4-nitrophenolate (dinp), L<sup>1</sup> = en, dmphen, or bipy], *trans*-[CuL<sub>2</sub>L<sup>2</sup><sub>2</sub>] [L = pfp, L<sup>2</sup> = pyridine (py), imidazole (imH), or 4-dimethylaminopyridine (dmap); L = pcp, pentabromophenolate (pbp), or dinp, L<sup>2</sup> = py; L = 2,4,6-trichlorophenolate (tcp), L<sup>2</sup> = dmap; L = tcp; L<sup>2</sup> = py or imH; L = tip, L<sup>2</sup> = py or dmap], and [CuL<sub>2</sub>L<sup>3</sup>] [L = pfp, L<sup>3</sup> = collidine (2,4,6-trimethylpyridine) (coll); L = pcp, L<sup>3</sup> = coll or *NN*-dimethylformamide (dmf); L = dinp, L<sup>3</sup> = coll or (dmf)<sub>2</sub>]. Spectroscopic studies show most *cis*-[CuL<sub>2</sub>L<sup>1</sup>] and *trans*-[CuL<sub>2</sub>L<sup>2</sup><sub>2</sub>] have tetragonal structures with axial bonding (sometimes weak) by the appropriate *ortho*-halogeno-groups or, if condensed, by bridging phenolato-groups. For *cis*-[CuL<sub>2</sub>(dmphen)] an out-of-plane arrangement towards distorted tetrahedral exists in solution. The complexes [Cu(pcp)<sub>2</sub>(coll)] and [Cu(pcp)<sub>2</sub>(dmf)] have low magnetic moments at room temperature, hence binuclear or polymeric structures with bridging phenolato-groups are suggested. All the compounds show a strong charge-transfer (c.t.) absorption in the range 400–550 nm, with an intensity about 1 200 dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup>, and weaker *d-d* bands at ca. 750 nm. By studying the variation in the c.t. absorption energy for this extensive series of compounds, where (a) the phenolate is kept constant and the coligands are varied and (b) the phenolate is varied and the coligands are kept constant, it is concluded that the assignment of the c.t. transition is consistent with a partial transfer of an electron from the *p<sub>π</sub>* orbital on the phenolate oxygen to a *d<sub>σ</sub>\** copper orbital. The c.t. band energies of the complexes are dependent on the nature of the solvent (solvents with low *E<sub>T</sub>* values lowering the c.t. energy), the basicity and number of coligands and phenolato-groups, and the chelate effect (which raises the energy). From these considerations, and e.s.r. data, it is concluded that the copper sites in copper(II) lactoferrin have a CuN<sub>2</sub>O<sub>2</sub>O' donor set containing two *cis* tyrosines, one histidine, and one water in the equatorial plane and possibly one histidine and an oxygen-donor ligand (non-ionized phenol) along the axial direction.

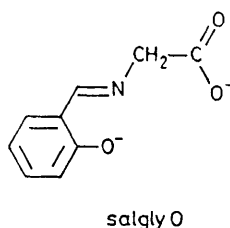
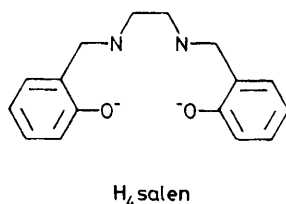
LACTOFERRIN is an iron-binding protein which occurs in high concentration in human milk and has been found in other bodily secretions and in intracellular components.<sup>2-4</sup> The extreme affinity of this protein for iron has led to the postulation that it has a bacteriostatic function in depriving micro-organisms of essential iron required for their growth.<sup>5</sup> The discovery that lactoferrin can yield iron to intestinal tissue<sup>6</sup> has given credence to the suggestion<sup>7</sup> that it also has a nutritional role in supplying iron to the newborn. It binds specifically two high-spin iron(III) ions<sup>8</sup> and it has also been shown to bind two chromium(III), manganese(III), cobalt(III),<sup>9,10</sup> and copper(II)<sup>8</sup> ions in a similar fashion. Our recent studies<sup>9,10</sup> have established lactoferrin to be an iron-tyrosinate protein, with the specific metal-binding sites very similar to those in serum transferrin and ovotransferrin (from egg whites). Proton release studies and u.v. difference spectral studies on the related serum transferrin and ovotransferrin proteins are consistent with, but do not prove that two tyrosine phenolate ligands are bound to each copper(II) ion,<sup>11</sup> while e.s.r. studies on all three proteins have implicated one bound histidine imidazole per copper.<sup>10,12-14</sup> These metallo-proteins require the presence of one bicarbonate ion per metal ion before their characteristic colours are developed,<sup>3,8,9</sup> and in the case of copper(II), which is the subject of this report, this colour is yellow as the result of a charge-transfer absorption in the visible region. Considering that two or three tyrosines<sup>11,15-17</sup> and two

histidines<sup>18</sup> appear to be involved when iron(III) is bound to these proteins, there are still uncertainties as to the exact nature and number of groups bound to copper(II), as well as the stereochemistry.

To complement our studies on lactoferrin itself<sup>9,10</sup> and in an attempt to resolve some of these uncertainties, the approach we have taken has been to examine some small molecule compounds as models for the copper-binding site. Hence we have studied complexes having the donor types CuN<sub>2</sub>O<sub>2</sub>, CuNOO'<sub>2</sub>, and CuN<sub>2</sub>OO'<sub>2</sub> (where O is a phenolato-ligand, O' a carboxylato-ligand, and N an aromatic or aliphatic nitrogen-donor ligand) in order to obtain the best spectroscopic match with dicopper(II) lactoferrin. To achieve this we have used deactivated ions<sup>19</sup> of the type 2,4,6-trichlorophenolate (tcp), 2,4,6-tribromophenolate (tbp), 2,4,6-tri-iodophenolate (tip), 2,6-di-iodo-4-nitrophenolate (dinp), pentafluorophenolate (pfp), pentachlorophenolate (pcp), and pentabromophenolate (pbp) (see below), rather than activated phenolates which react with copper to produce various oxidatively coupled phenols.<sup>20</sup> An attempt to model the copper site of transferrin has already been published;<sup>21</sup> our aim was to more fully investigate with the above mentioned compounds the factors that determine the energy of the charge-transfer (c.t.) band observed in the visible spectrum and hence, along with e.s.r. data, to suggest a possible structure for the copper site of lactoferrin as well as comment on the assignment of the c.t. band which has been the subject of debate.<sup>19,22-25</sup> A



tcp;  $X^1 = X^3 = \text{Cl}$ ,  $X^2 = \text{H}$   
 tbp;  $X^1 = X^3 = \text{Br}$ ,  $X^2 = \text{H}$   
 tip;  $X^1 = X^3 = \text{I}$ ,  $X^2 = \text{H}$   
 dinp;  $X^1 = \text{NO}_2$ ,  $X^2 = \text{H}$ ,  $X^3 = \text{I}$   
 pfp;  $X^1 = X^2 = X^3 = \text{F}$   
 pcp;  $X^1 = X^2 = X^3 = \text{Cl}$   
 pbp;  $X^1 = X^2 = X^3 = \text{Br}$



brief study of small molecule model chromium complexes for dichromium(III) lactoferrin is also presented.

## EXPERIMENTAL

Infrared spectra were recorded on a Beckman IR 20 (250–4 000 cm<sup>-1</sup>) spectrophotometer. Magnetic moments were measured by the Faraday method as well as by the Gouy method. Electronic spectra were recorded with a Shimadzu MPS 5000 spectrophotometer and e.s.r. measurements with a Varian model E-104A spectrometer at 77 K, the spectral values being calibrated with a diphenylpicrylhydrazyl standard. Conductivity measurements were made at 20 °C using a Philips PR 9500 conductivity meter and PW 9510 cell. Microanalyses were by Professor A. D. Campbell, University of Otago. Analytical data are summarised in Table 1. Molecular weights were determined on a Hitachi-Perkin-Elmer 115 vapour-pressure osmometer. Copper(II) acetate hydrate was obtained from May and Baker Ltd. All the phenols used, as well as 2,9-dimethyl-1,10-phenanthroline (dmphen), pyridine (py), imidazole (imH), and ethylenediamine (en), were from Aldrich Chemical Co. The compound [Cu(salglyO)]·H<sub>2</sub>O [salglyO = *N*-salicylidene-glycinate(2-)] was prepared according to Nakahara<sup>26</sup> and [Cu(H<sub>4</sub>salen)] [H<sub>4</sub>salen = *NN'*-ethylenebis(salicylamine)] was prepared according to a literature method.<sup>22</sup>

**Preparation of Copper Phenolate Complexes.**—Complexes of the type *cis*-[CuL<sub>2</sub>L<sup>1</sup>] (L = appropriate phenolato-ligand; L<sup>1</sup> = appropriate bidentate ligand) and *trans*-[CuL<sub>2</sub>L<sup>2</sup>]<sub>2</sub> (L<sup>2</sup> = appropriate unidentate ligand) were prepared by similar methods of which the following is typical.<sup>19</sup>

The appropriate phenol (2 mmol) was dissolved in water containing Na[OH] (2 mmol). The solution was heated gently and filtered if necessary. To this solution was added slowly an aqueous solution containing copper(II) acetate (2 mmol) and either the appropriate base L<sup>1</sup> (1 mmol), or

TABLE 1  
Analytical data, conductivities, and magnetic moments for the copper phenolate complexes

Compound	Colour	Analysis (%) <sup>a</sup>			Λ <sup>b</sup> /S cm <sup>2</sup> mol <sup>-1</sup>	Solvent	μ <sub>eff</sub> <sup>c</sup> /B.M.
		C	H	N			
[Cu(pfp) <sub>2</sub> (en)]	Olive green	34.25 (34.3)	1.7 (1.65)	5.7 (5.7)	13	Ethanol	1.67
[Cu(pfp) <sub>2</sub> (tmen)]	Dark brown	40.0 (39.6)	2.5 (2.95)	5.1 (5.1)	2	Ethanol	1.88
[Cu(pfp) <sub>2</sub> (dmphen)]	Rusty brown	48.9 (48.95)	2.0 (1.9)	4.4 (4.4)	10	Acetone	1.42
[Cu(pfp) <sub>2</sub> (py) <sub>2</sub> ]·0.5H <sub>2</sub> O	Dark green	44.4 (44.3)	1.6 (1.9)	4.7 (4.6)	1	Ethanol	2.10
[Cu(pfp) <sub>2</sub> (imH) <sub>2</sub> ]	Dark brown	38.4 (38.2)	1.3 (1.4)	9.9 (9.9)	4	Acetone	1.73
[Cu(pfp) <sub>2</sub> (coll)]	Black	44.2 (43.6)	2.6 (2.0)	2.95 (2.5)	8	Acetone	
[Cu(pfp) <sub>2</sub> (bipy)]	Brown	45.3 (45.1)	1.4 (1.4)	4.7 (4.8)	7	Ethanol	2.16
[Cu(pfp) <sub>2</sub> (dmap) <sub>2</sub> ]·H <sub>2</sub> O	Rusty brown	44.9 (45.1)	3.0 (3.2)	7.0 (8.1)			2.04
[Cu(pcp) <sub>2</sub> (en)]	Brown	25.6 (25.6)	1.4 (1.2)	4.0 (4.3)	6	Ethanol	1.71
[Cu(pcp) <sub>2</sub> (dmphen)]	Purple	40.2 (39.2)	1.7 (1.5)	3.5 (3.5)	23	Ethanol	1.76
[Cu(pcp) <sub>2</sub> (py) <sub>2</sub> ]	Brown	35.5 (35.0)	1.4 (1.3)	3.5 (3.7)	1	Ethanol	1.91
[Cu(pcp) <sub>2</sub> (coll)]	Brown	35.5 (35.3)	2.0 (2.0)	2.3 (1.9)	3	Ethanol	1.11
[Cu(pcp) <sub>2</sub> (dmf)]	Brown	27.9 (26.9)	1.1 (1.1)	1.8 (2.1)			1.30
[Cu(tcp) <sub>2</sub> (dmap) <sub>2</sub> ]	Mustard	44.4 (44.6)	3.9 (3.45)	8.0 (8.0)			
[Cu(tbp) <sub>2</sub> (en)]	Brown	21.9 (21.5)	1.9 (1.5)	3.0 (3.6)	5	Ethanol	1.76
[Cu(tbp) <sub>2</sub> (tmen)]	Red	26.1 (25.8)	2.5 (2.4)	3.0 (3.3)	1	Acetone	1.70
[Cu(tbp) <sub>2</sub> (dmphen)]·0.5H <sub>2</sub> O	Purple	34.5 (33.1)	1.8 (1.8)	2.8 (3.0)			1.65
[Cu(tbp) <sub>2</sub> (py) <sub>2</sub> ]	Brown	29.4 (30.0)	1.7 (1.6)	3.0 (3.2)	1	Ethanol	1.80
[Cu(tbp) <sub>2</sub> (imH) <sub>2</sub> ]	Brown	25.6 (25.2)	1.9 (1.4)	5.5 (6.5)			1.35
[Cu(pbp) <sub>2</sub> (py) <sub>2</sub> ]·H <sub>2</sub> O	Orange-red	21.5 (21.7)	1.3 (1.0)	2.3 (2.3)			
[Cu(dinp) <sub>2</sub> (en)]	Yellow	19.1 (18.6)	2.5 (1.3)	7.1 (6.2)			
[Cu(dinp) <sub>2</sub> (dmphen)]	Green	32.2 (29.7)	2.1 (1.5)	5.4 (5.3)			
[Cu(dinp) <sub>2</sub> (py) <sub>2</sub> ]	Orange-red	27.2 (26.4)	2.0 (2.5)	5.5 (5.6)			
[Cu(dinp) <sub>2</sub> (coll)]·4H <sub>2</sub> O	Brown-yellow	22.1 (23.2)	1.9 (2.2)	3.8 (4.1)			
[Cu(dinp) <sub>2</sub> (dmf) <sub>2</sub> ]	Dark red	22.1 (21.9)	1.8 (1.8)	5.8 (5.7)			
[Cu(dinp) <sub>2</sub> (bipy)]	Dark brown	27.5 (26.4)	1.3 (1.2)	5.7 (5.6)			
[Cu(tip) <sub>2</sub> (en)]	Brown	16.0 (15.8)	1.4 (1.1)	2.9 (2.6)			
[Cu(tip) <sub>2</sub> (tmen)]	Purple	20.25 (19.3)	1.9 (1.8)	2.4 (2.5)	2	Acetone	
[Cu(tip) <sub>2</sub> (dmphen)]	Dark blue	27.1 (25.7)	1.5 (1.3)	2.45 (2.3)			
[Cu(tip) <sub>2</sub> (py) <sub>2</sub> ]	Mauve-red	21.8 (22.7)	1.3 (1.2)	2.2 (2.4)			
[Cu(tip) <sub>2</sub> (dmap) <sub>2</sub> ]	Orange-red	25.8 (25.0)	2.7 (1.9)	4.4 (4.5)			

<sup>a</sup> Calculated values are given in parentheses. <sup>b</sup> 10<sup>-3</sup> mol dm<sup>-3</sup> solution. <sup>c</sup> Measured at 20 °C. 1 B.M. ≈ 9.274 × 10<sup>-24</sup> A m<sup>2</sup>.

$L^2$  (2 mmol). The resulting precipitate was filtered off, washed thoroughly with water, and vacuum dried. Complexes of the type  $[CuL_2(coll)] \cdot xH_2O$  [ $coll = collidine$  (2,4,6-trimethylpyridine)] ( $L = dinp$ ,  $x = 4$ ;  $L = pcp$  or  $pfp$ ,  $x = 0$ ) and  $[CuL_2(dmap)_2] \cdot xH_2O$  ( $L = tcp$ ,  $x = 0$  and  $L = pfp$ ,  $x = 1$ ;  $dmap = 4$ -dimethylaminopyridine) were prepared in similar ways to that above except that methanol was used as solvent. For the former set, the methanol solution was reduced to below half volume before  $[Cu(dinp)_2(coll)] \cdot 4H_2O$  precipitated and  $[CuL_2(coll)]$  ( $L = pcp$  or  $pfp$ ) was isolated upon the addition of water. For these cases collidine (1.5 mmol) was used.

The complexes  $[Cu(dinp)_2(dmf)_2]$  and  $[Cu(pcp)_2(dmf)_2]$  were prepared by dissolving  $[Cu(dinp)_2]$  and  $[Cu(pcp)_2]$  in a minimum volume of dimethylformamide (dmf) whereupon the products precipitated after *ca.* 30 min. These were filtered off, washed with diethyl ether, and vacuum dried.

The molecular weights determined in acetone solution (followed by the theoretical values) are:  $[Cu(tbp)_2(py)_2]$  794 (818);  $[Cu(pfp)_2(tmen)]$  ( $tmen = N,N,N',N'$ -tetramethylethylenediamine) 508 (546);  $[Cu(pfp)_2(imH)_2]$  491 (566);  $[Cu(pfp)_2(en)]$  495 (490);  $[Cu(pfp)_2(bipy)]$  ( $bipy = 2,2'$ -bipyridyl) 545 (586), and  $[Cu(pfp)_2(dmphen)]$  738 (638).

**Preparation of  $[Cu(salglyO)(py)] \cdot 1.5H_2O$ .**—The compound  $[Cu(salglyO)] \cdot H_2O$  was suspended in water and excess of pyridine added. The suspension completely dissolved and the solution turned dark green. After 10 min a blue-green solid precipitated which was washed thoroughly with water {Found: C, 48.6; H, 4.2; N, 8.0. Calc. for  $[Cu(salglyO)(py)] \cdot 1.5H_2O$ : C, 48.5; H, 4.3; N, 8.1%}.

**Preparation of  $[Cr(H_4salen)(py)(HOME)][PF_6]$ .**—The compound  $CrCl_3 \cdot 6H_2O$  (2 mmol) in water (20 cm<sup>3</sup>) was passed through a Jones' reduction column into a solution of  $H_4salen$  (2 mmol) in acetone (30 cm<sup>3</sup>) through which nitrogen was passed. An insoluble impurity was filtered off, and the solution reduced to below half volume. An excess of  $[NH_4][PF_6]$  (dissolved in water) followed by pyridine (1.5 cm<sup>3</sup>) were added and a grey-purple compound precipitated which was collected and recrystallised from  $CH_3OH$  to give purple crystals of the title compound in 15% yield {Found: C, 45.0; H, 4.8; N, 6.9. Calc. for  $[Cr(H_4salen)(py)(HOME)][PF_6]$ : C, 45.2; H, 5.1; N, 6.9%}.

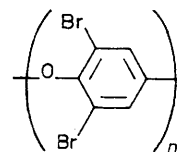
## RESULTS AND DISCUSSION

**Preparation, Structure, and Stability of the Copper Complexes.**—Complexes of the type *cis*- $[CuL_2L^1]$  ( $L = pfp$ ,  $pcp$ ,  $tbp$ ,  $tip$ , or  $dinp$ , and  $L^1 = en$ ,  $tmen$ ,  $dmphen$ , or  $bipy$ ), *trans*- $[CuL_2L^2]$  ( $L = pfp$ ,  $pcp$ ,  $pfp$ ,  $tcp$ ,  $tbp$ ,  $tip$ , or  $dinp$ ,  $L^2 = py$ ,  $dmap$ , or  $imH$ ),  $[CuL_2L^3]$  ( $L = pcp$ ,  $L^3 = coll$  or  $dmf$ ;  $L = pfp$ ,  $L^3 = coll$ ), and  $[Cu(dinp)_2(dmf)_2]$  have been prepared as described in the Experimental section. The analytical data are given in Table 1. For the type  $[CuL_2(coll)]$ , only one collidine can be attached and this may be attributed to steric problems associated with the formation of  $[CuL_2(coll)_2]$ . The  $pfp$  compounds were most soluble in ethanol and acetone while the  $tip$  compounds were the most insoluble. On ageing,  $[Cu(pcp)_2(py)_2]$  became less soluble in ethanol and this suggested dimerisation or polymerisation. Other  $pcp$  and  $tbp$  complexes also became less soluble with time. The magnetic moments of  $[Cu(tbp)_2(imH)_2]$ ,  $[Cu(pcp)_2(dmf)]$ ,  $[Cu(pcp)_2(coll)]$ ,  $[Cu(pfp)_2(en)]$ , and  $[Cu(pfp)_2(dmphen)]$  are lower than generally found for

copper(II) (Table 1) suggesting structures containing, at least, binuclear copper(II) with bridging phenolato-ligands. The molecular-weight determinations in acetone solution (see Experimental section) for  $[Cu(tbp)_2(py)_2]$ ,  $[Cu(pfp)_2(tmen)]$ ,  $[Cu(pfp)_2(imH)_2]$ ,  $[Cu(pfp)_2(en)]$ , and  $[Cu(pfp)_2(bipy)]$  indicate monomeric structures, while  $[Cu(pfp)_2(dmphen)]$  gives a value which is between a monomer and dimer but with the equilibrium lying closer to a monomer. In the solid state this complex may be a dimer.

Single-crystal X-ray structural analyses of  $[Cu(tcp)_2(imH)_2]$ ,<sup>27</sup>  $[Cu(fomp)_2(py)_2]$  ( $fomp = 4$ -formyl-2-methoxyphenolate),<sup>28</sup> and  $[Cu(mnop)_2(py)_2]$  ( $mnp = 2$ -methoxy-4-nitrophenolate)<sup>23</sup> all show the copper, the two phenolates, and the two tertiary nitrogens to be almost planar with a *trans* configuration, a tetragonal stereochemistry being completed by long axial Cu-Cl bonds and Cu-O (methoxy) bonds respectively. The chelated ligand complexes  $[Cu(mnop)_2(tmen)]$ <sup>29</sup> and  $[Cu(tcp)_2(tmen)]$ <sup>23,30</sup> both have a *cis* arrangement of oxygen and nitrogen ligands with a weak axial interaction between the methoxy-oxygens or chlorine atoms and the copper, giving tetragonal structures. For this latter compound the Cu-Cl bonds are longer than in  $[Cu(tcp)_2(imH)_2]$ . By comparison, the compounds in the present study which are monomeric in acetone may be considered to have the appropriate *trans* or *cis* configurations. However the recently prepared unsubstituted phenolate complex  $[Cu(OC_6H_5)_2(en)]_2 \cdot 2C_6H_5OH$ <sup>31</sup> is dimeric containing both bridging and terminal phenoxo-groups and this structural type may also be present in some of our complexes.

In an attempt to prepare  $[Cu(tbp)_2(coll)]$  a white precipitate with a melting point of 205 °C was isolated. It was not sufficiently volatile for mass spectral studies but by analogy with a similar  $pcp$  reaction<sup>32</sup> the product may be a dibromo-substituted phenylene ether as shown below. Many of the  $pcp$  and  $tbp$  complexes are not



stable in solution with colour changes and/or precipitates appearing with time.

**Electronic Spectra.**—The electronic spectra are characterised by an intense absorption around 410–545 nm. That this band is charge-transfer (c.t.) in origin is supported<sup>33</sup> by its movement to lower energy in a solvent with a low  $E_T$  value\* (*e.g.* benzene) and to higher energy in a solvent with a high  $E_T$  value (*e.g.* ethanol) (Table 2). There has been debate over the assignment of this band. Some have assigned it to a phenolate-to-copper charge transition (*i.e.* charge transfer presumably from the  $p_\pi$  orbital on the phenolate oxygen to the  $d_{\sigma^*}$  orbitals on the copper)<sup>23–25</sup> while others<sup>19,22</sup> to a copper-

\* A measure of polarity.

TABLE 2  
Electronic spectral data for the copper phenolate complexes <sup>a</sup>

Compound	$\lambda_{\max.}/\text{nm}$ (in benzene)		$\lambda_{\max.}/\text{nm}$ (in ethanol)		$\lambda_{\max.}/\text{nm}$ (in acetone)		$\lambda_{\max.}/\text{nm}$ (reflectance)	
	O→Cu c.t.	d-d	O→Cu c.t.	d-d	O→Cu c.t.	d-d	O→Cu c.t.	d-d
[Cu(pfp) <sub>2</sub> (py) <sub>2</sub> ].0.5H <sub>2</sub> O	432		393 (442)		402		450 (br)	693 (vbr)
[Cu(pcp) <sub>2</sub> (py) <sub>2</sub> ]	458	765 (br)	440 (1 230)	760 (130)(br)	455		448	654 (sh), 769 <sup>b</sup>
[Cu(tcp) <sub>2</sub> (py) <sub>2</sub> ]	450 <sup>b</sup> 444 <sup>c</sup>	763 <sup>b</sup>					467	600 (sh), 756 <sup>b</sup>
[Cu(pbp) <sub>2</sub> (py) <sub>2</sub> ].H <sub>2</sub> O	473		461		470		492	
[Cu(tbp) <sub>2</sub> (py) <sub>2</sub> ]	462	770 (br)	423 (1 370)	755 (185)(br)			490 (vbr)	640 (sh), 760 (sh)
[Cu(dinp) <sub>2</sub> (py) <sub>2</sub> ]	490	760	ca. 520 (sh)		ca. 490 (ca. 1 200)(sh)	753 (ca. 250)	524	
[Cu(tip) <sub>2</sub> (py) <sub>2</sub> ]	487	762 (br)	453		482		518	
[Cu(pfp) <sub>2</sub> (dmap) <sub>2</sub> ].H <sub>2</sub> O	415		383		412		415	
[Cu(tcp) <sub>2</sub> (dmap) <sub>2</sub> ]	442	751			438	745 (br)	442	738
[Cu(tip) <sub>2</sub> (dmap) <sub>2</sub> ]	472				465		488	
[Cu(pfp) <sub>2</sub> (tmen)]	453		438 (981)	570 (165)(sh)	432		443 (br) 580 (sh)	680 (sh)
[Cu(pcp) <sub>2</sub> (tmen)]	490 <sup>c</sup>							
[Cu(tcp) <sub>2</sub> (tmen)]	493 <sup>b</sup> 487 <sup>c</sup>						481	746 (sh) <sup>c</sup>
[Cu(tbp) <sub>2</sub> (tmen)]	507		492		484 (2 610)	ca. 750	510 (br)	ca. 720 (sh)
[Cu(tip) <sub>2</sub> (tmen)]	545		525		525 (1 660)		545	
[Cu(pfp) <sub>2</sub> (en)]	408		392 (1 440)	595 (144)(br)	412		ca. 405 (br)	760
[Cu(pcp) <sub>2</sub> (en)]	445		442 (1 270)	640 (sh)	444		470	705
[Cu(tbp) <sub>2</sub> (en)]	447	680 (sh)	437	640 (sh)			475 (br)	
[Cu(tip) <sub>2</sub> (en)]	480		472		470 (ca. 1 500)		478 (br)	
[Cu(pfp) <sub>2</sub> (dmphen)]	480 (3 390) <sup>d</sup>	ca. 950 <sup>d,e</sup>			455 (ca. 2 550)	775 (170) <sup>e</sup>	495 (vbr)	800 <sup>e</sup>
[Cu(pcp) <sub>2</sub> (dmphen)]	567 <sup>d</sup>	800 (sh) <sup>d</sup> 1 000 (sh)					590 (vbr)	ca. 800, ca. 1 000 ca. 800 <sup>d</sup>
[Cu(tbp) <sub>2</sub> (dmphen)].0.5H <sub>2</sub> O							567 (br)	
[Cu(dinp) <sub>2</sub> (dmphen)]					530 (sh)	765 (br)	592	
[Cu(tip) <sub>2</sub> (dmphen)]							600 (br)	
[Cu(pfp) <sub>2</sub> (bipy)]	468		440 (1 750)		441		505 (br)	700 (sh)
[Cu(tcp) <sub>2</sub> (bipy)]	491							
[Cu(dinp) <sub>2</sub> (bipy)]					524 (ca. 950)		547	
[Cu(pfp) <sub>2</sub> (coll)]			388		378 (1 361)(sh)		445 (br)	770
[Cu(pcp) <sub>2</sub> (coll)]			434 (1 210)	725 (sh)			485	790
[Cu(tbp) <sub>2</sub> (coll)]						725	475	740
[Cu(dinp) <sub>2</sub> (coll)]			ca. 520 (sh)		ca. 515 (sh)		ca. 540 (sh)	
[Cu(pfp) <sub>2</sub> (imH) <sub>2</sub> ]					415 (951)(br)		440 (br)	745 (br)
[Cu(tcp) <sub>2</sub> (imH) <sub>2</sub> ]					430 (1 200)	710 (150) <sup>f</sup>		
[Cu(tbp) <sub>2</sub> (imH) <sub>2</sub> ]					447	743 (br)		

<sup>a</sup> Absorption coefficients ( $\epsilon/\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ ) are given in parentheses. sh = Shoulder, br = broad, vbr = very broad. <sup>b</sup> From ref. 23. <sup>c</sup> From ref. 19. <sup>d</sup> In CH<sub>2</sub>Cl<sub>2</sub>. <sup>e</sup> Band tails into near i.r. <sup>f</sup> From ref. 21.

to-phenolate transition (*i.e.* a promotion from the  $d_{x^2-y^2}$  or  $d_{z^2}$  orbitals of the metal to antibonding orbitals of the phenolic residue). Results from our study would support the former view. To rationalise this assignment we have used the arguments of electron-withdrawing character of the halogen substituents on the phenolate and secondly, base strength of the coligand. Hence for a series of complexes of the type *trans*-[CuL<sub>2</sub>(py)<sub>2</sub>] where the coligand (pyridine) is kept constant and the phenolate is varied, it is found that the c.t. band moves to higher energy as the halogeno-substituents become more electron withdrawing; hence when L = tip,  $\lambda_{\max.} = 487$

nm, when L = tbp,  $\lambda_{\max.} = 462$  nm, when L = tcp,  $\lambda_{\max.} = 450$  nm, and when L = pfp,  $\lambda_{\max.} = 432$  nm (all in benzene, Table 2). In general this trend is also observed for ethanol and acetone solutions as well as the reflectance spectra. Ideally, for this comparison, the structures are hoped to be similar. Similar trends are also seen in the following series: [CuL<sub>2</sub>(dmap)<sub>2</sub>], [CuL<sub>2</sub>(tmen)], and [CuL<sub>2</sub>(en)]. Hence the weaker the conjugate base derived from the phenol the higher the c.t. band energy and this is consistent with an oxygen-to-copper c.t. transition.<sup>34,35</sup> Also for the series *trans*-[CuL<sub>2</sub>L'<sub>2</sub>] (L<sup>2</sup> = py or dmap) where the phenolate is kept



constant and the coligand  $L^2$  is varied, the charge-transfer band moves to higher energy as the base strength of the coligand increases. For example, dmap is a stronger base than py and hence for  $L^2 = \text{dmap}$  and  $L = \text{pfp}$ ,  $\lambda_{\text{max.}} = 415 \text{ nm}$  while for  $L^2 = \text{py}$  and  $L = \text{pfp}$ ,  $\lambda_{\text{max.}} = 432 \text{ nm}$  (in benzene, Table 2). A more extensive series of compounds is formed with *cis*- $[\text{CuL}_2\text{L}^1]$  ( $L^1 = \text{en}$  or *tmen*) e.g. *cis*- $[\text{Cu}(\text{pfp})_2(\text{en})]\cdot\text{H}_2\text{O}$  has  $\lambda_{\text{max.}}$  at 408 nm while *cis*- $[\text{Cu}(\text{pfp})_2(\text{tmen})]$  has  $\lambda_{\text{max.}}$  at 453 nm in benzene (Table 2) (*en* is a stronger base than *tmen*). Similar trends are observed in ethanol and acetone solutions as well as in the solid state. Hence increased electron build up on the metal for a series of complexes whose structures are similar results in an increase in the c.t. band energy and is consistent with a oxygen-to-metal type. A similar assignment has been given to the c.t. bands of the copper(II) and iron(III) complexes of the tetra-anion of *NN'*-ethylenebis[2-(*o*-hydroxyphenyl)glycine] which are at 371 and 480 nm respectively, this order being that expected on consideration of the charge of the metal ions.<sup>36</sup> The  $\sigma\text{S} \rightarrow \text{Cu}$  c.t. at ca. 600 nm in models for 'type 1' copper proteins is also displaced to higher energy as the mercaptide becomes more electron withdrawing<sup>37</sup> but as the optical electronegativity of oxygen is greater than sulphur the c.t. bands of phenolate derivatives are at higher energies. Even so, the c.t. bands of some phenoxo-complexes do approach 500 nm in polar solvents (Table 2). The relative optical electronegativities for the phenolate oxygens can be calculated by an empirical approach<sup>37</sup> using equation (1) where the c.t. frequency is proportional

$$\nu_{\text{c.t.}}(\text{cm}^{-1}) = 30\,000(\chi_{\text{ligand}} - \chi_{\text{metal}}) \quad (1)$$

to the difference in optical electronegativities of the ligand ( $\chi_{\text{ligand}}$ ) and the metal ion ( $\chi_{\text{metal}}$ ), taken as 2.4 for copper. Hence for the series *trans*- $[\text{CuL}_2(\text{py})_2]$  in acetone, when  $L = \text{pfp}$ ,  $\chi_{\text{ligand}} = 3.23$ , when  $L = \text{pcp}$ ,  $\chi_{\text{ligand}} = 3.13$ , when  $L = \text{pbp}$ ,  $\chi_{\text{ligand}} = 3.11$ , and when  $L = \text{tip}$ ,  $\chi_{\text{ligand}} = 3.09$ . For *p*-nitrobenzenethiolate,  $\chi_{\text{ligand}}$  is 2.87<sup>37</sup> consistent with the lower electronegativity of sulphur.

Another influencing factor is the chelate effect. For  $[\text{Cu}(\text{H}_4\text{salen})]$ , the c.t. band is at 394 nm in methanol<sup>22</sup> and 400 nm in pyridine. Unfortunately spectral data for  $[\text{Cu}(\text{OC}_6\text{H}_5)_2(\text{en})_2]\cdot 2\text{C}_6\text{H}_5\text{OH}$  or  $[\text{Cu}(\text{OC}_6\text{H}_4\text{Me-}p)_2(\text{en})]$  are not available for comparison, but the position of this band for  $[\text{Cu}(\text{H}_4\text{salen})]$  relative to other *en* complexes in Table 2 suggests that involving the phenolate in a chelate system increases the c.t. energy.

The complexes discussed so far are of the donor type  $\text{CuN}_2\text{O}_2$ . In view of the uncertainty as to whether one or two tyrosyl residues are bound to each copper in di-copper(II) lactoferrin we have investigated the spectral characteristics of  $[\text{Cu}(\text{salglyO})]\cdot\text{H}_2\text{O}$ , first prepared by Nakahara.<sup>26</sup> Our e.s.r. studies have detected a  $g = 4$  signal indicating a binuclear structure, and by analogy with the single-crystal *X*-ray structure for bis[2-hydroxy-*N*-3-hydroxypropyl-5-nitrobenzylideneamino-( $\mu$ -O)-copper(II)],<sup>38,39</sup> it is considered that each copper is co-ordinated by one terminal phenolato-group, two bridging

carboxylato-groups, one imine nitrogen, and an aquo-ligand, giving a  $\text{CuNOO}'_2\text{O}''$  type ( $\text{O}' = \text{carboxylato-oxygen}$  and  $\text{O}'' = \text{water}$ ). The complex  $[\text{Cu}(\text{salglyO})]\cdot\text{H}_2\text{O}$  shows an intense band at 357 nm in water. Its pyridine adduct has been isolated (see Experimental section), and this also shows a  $g = 4$  e.s.r. signal, hence indicating that the binuclear structure still persists. There is no change in the position of the charge-transfer transition, thus axial co-ordination by pyridine does not affect this energy, although the *d-d* transition moves from 690 to 670 nm. Recently, other groups<sup>37,40</sup> have also either recorded or predicted c.t. bands at ca. 330–370 nm for systems with one phenolato-ligand (the complexes even having different stereochemistries), however in all cases the complexes have three strong base coligands or are chelate systems. If the coligands were all weakly bound water molecules, it would be predicted that the energy of the  $p_\pi \rightarrow d_{\sigma^*}$  c.t. band would move to lower energies on decreasing the number of bound phenolates from two to one. As discussed earlier the energy of the  $\text{O} \rightarrow \text{Cu}$  c.t. band is very sensitive to the nature of the coligands (Table 2).

Many of the complexes have detectable *d-d* bands in the 650–800 nm region while others do not because of the intense c.t. band nearby (Table 2). Related compounds of known tetragonal symmetry such as *trans*- $[\text{Cu}(\text{tcp})_2(\text{imH})_2]$ <sup>21,27</sup> and *trans*- $[\text{Cu}(\text{mnop})_2(\text{py})_2]$ <sup>23</sup> have a single *d-d* band near 770 nm and sometimes a poorly resolved band at higher energy. For a complex where the mean axial distance is shorter such as *trans*- $[\text{Cu}(\text{fomp})_2(\text{py})_2]\cdot\text{H}_2\text{O}$ <sup>23,28</sup> two *d-d* transitions (ca. 800 and 610 nm) are observed. For the tetragonal complex *cis*- $[\text{Cu}(\text{mnop})_2(\text{tmen})]\cdot\text{H}_2\text{O}$ <sup>23,29</sup> two bands at 541 and 719 nm (shoulder) are found, while for the distorted square-planar *cis*- $[\text{Cu}(\text{tcp})_2(\text{tmen})]$ <sup>23,30</sup> one band is at 746 nm with the other considered to be obscured by the c.t. band at 481 nm. The complexes in this study which are similar to those above also show evidence of tetragonal symmetry from their *d-d* spectra (Table 2) having the axial positions occupied (sometimes weakly) by F, Cl, Br, or I groups. The dmphen complexes *cis*- $[\text{CuL}_2(\text{dmphen})]$ , however, absorb at lower energies than those discussed above. For  $[\text{Cu}(\text{pfp})_2(\text{dmphen})]$ , this may be attributed to an out-of-plane arrangement of ligands about copper, and as the magnetic moment is low, a possibility exists that it is dimeric in the solid state with each copper five-co-ordinated. The structures of other dmphen complexes may be similar in the solid state, however in solution where monomeric species appear dominant, a distorted tetrahedral structure seems to exist<sup>41,42</sup> and this is supported by e.s.r. data (see below).

**E.S.R. Spectra.**—The spectra (Table 3) are in general consistent with copper(II) in a tetragonal environment with the parameters being in line with a  $\text{CuN}_2\text{O}_2$  planar donor type. Tetrahedral distortions of tetragonal complexes increase  $g_{\parallel}$  and decrease  $A_{\parallel}$ . The ratio of  $g_{\parallel} : A_{\parallel}$  appears to be a convenient empirical index of this distortion, values ranging from 105 to 135 cm for tetragonal structures and above this for out-of-plane distortions

towards tetrahedral symmetry.<sup>43</sup> The latter type appear in solution with complexes of the type  $[\text{CuL}_2(\text{dmphen})] \cdot x\text{H}_2\text{O}$ . Some of the complexes show the presence of two species in polar solvents (Table 3) *e.g.*  $[\text{Cu}(\text{tbp})_2(\text{dmf})]$  in dmf has one species which can be attributed to tetragonal copper(II) while a second species shows distortion towards an out-of-plane structure. On the other hand *cis*- $[\text{Cu}(\text{pfp})_2(\text{en})]$  dissociates to give  $[\text{Cu}(\text{pfp})_2(\text{en})_2]$  as a minor product, although the corresponding *tbp* and *tip* precursors do not. A measure of the metal-ligand covalency ( $\alpha^2$ ) can be obtained by using an approximate expression developed by Kivelson and Neiman.<sup>44</sup> Al-

trolyte in  $\text{SMe}_2\text{O}$  and has *d-d* bands at 561 and 427 nm in water [*cf.* dichromium(III) lactoferrin, 612 and 442 nm]. The low energy of the first *d-d* band for dichromium(III) lactoferrin<sup>9</sup> has been interpreted as resulting from the presence of a chloro-ligand on each Cr and possibly weaker nitrogen binding. The c.t. transition occurs in the u.v. region for both chromium complexes. The e.s.r. spectrum in methanol shows  $g_{\text{eff.}}$  values at 4.62, 3.54, 2.5, and 2.19 while dichromium(III) lactoferrin has  $g_{\text{eff.}} = 5.58, 5.40, 5.11, 2.27$ , and 2.05. The e.s.r. spectrum of  $[\text{Cr}(\text{salen})(\text{py})\text{Cl}]$  [*salen* = *NN'*-ethylenedis-(salicylideneimine)]<sup>48</sup> shows a closer match with

TABLE 3  
E.s.r. spectral data for some copper phenolate complexes

Compound	$g_{\parallel}$	$A_{\parallel}$ <sup>a</sup>	$g_{\perp}$	$\alpha^2$ <sup>b</sup>	$g_{\parallel}/A_{\parallel}$ <sup>c</sup>	Solvent
Dicopper(II) lactoferrin	2.314	155	2.057	0.81	149	Phosphate buffer
$[\text{Cu}(\text{tbp})_2(\text{en})]$	2.260	185	2.054	0.83	122	Ethanol
$[\text{Cu}(\text{tbp})_2(\text{tmen})]$	2.240	183	2.083	0.82	122	Ethanol
$[\text{Cu}(\text{tbp})_2(\text{dmphen})] \cdot 0.5\text{H}_2\text{O}$	2.326 <sup>d</sup>	138	2.073	0.78	168	Acetone
	2.316	129			179	
$[\text{Cu}(\text{tbp})_2(\text{py})_2]$	2.265	179	2.063	0.82	126	Benzene
	2.266	180	2.062		125	Ethanol
$[\text{Cu}(\text{tbp})_2(\text{imH})_2]$	2.267	182	2.044	0.83	124	Ethanol
$[\text{Cu}(\text{tbp})_2(\text{coll})]$	2.331 <sup>d</sup>	152	2.075	0.82	153	Acetone
	2.303	182			126	
$[\text{Cu}(\text{pcp})_2(\text{en})]$	2.216	194	2.102	0.83	115	Benzene
	2.280 <sup>d</sup>	164	2.066	0.80	133	Ethanol
	2.276	103			220	
$[\text{Cu}(\text{pcp})_2(\text{dmphen})]$	2.304	142	2.126	0.79	162	Dichloromethane
$[\text{Cu}(\text{pcp})_2(\text{py})_2]$	2.239	187	<i>ca.</i> 2.08	0.81	119	Benzene
	2.301	163	2.056		141	Ethanol
$[\text{Cu}(\text{pcp})_2(\text{coll})]$	2.295	171	2.067	0.83	134	Nitromethane
	2.292 <sup>d</sup>	170	2.080	0.83	134	Ethanol
	2.394	140			171	
$[\text{Cu}(\text{pcp})_2(\text{dmf})]$	2.374	129	2.074	0.80	184	<i>NN</i> -Dimethyl- formamide
	2.311	164			140	
$[\text{Cu}(\text{tip})_2(\text{en})]$	2.262	178	2.101	0.84	127	Acetone
$[\text{Cu}(\text{tip})_2(\text{tmen})]$	2.236	185	2.108	0.83	120	Acetone
$[\text{Cu}(\text{tip})_2(\text{py})_2]$	2.261	180	2.086	0.83	126	Acetone
$[\text{Cu}(\text{tip})_2(\text{dmap})_2]$	2.267	182	2.065	0.83	125	Chloroform
$[\text{Cu}(\text{dinp})_2(\text{py})_2]$	2.272	184	2.078	0.85	123	Acetone
$[\text{Cu}(\text{dinp})_2(\text{dmf})_2]$	2.334	153	2.077	0.83	152	Acetone
$[\text{Cu}(\text{pfp})_2(\text{en})]$	2.278 <sup>d</sup>	171	2.067	0.82	133	Ethanol
	2.215 <sup>e</sup>	201	<i>ca.</i> 1.99	<i>ca.</i> 0.81	110	
$[\text{Cu}(\text{pfp})_2(\text{tmen})]$	2.279	163	2.061	0.80	140	Ethanol
$[\text{Cu}(\text{pfp})_2(\text{py})_2] \cdot 0.5\text{H}_2\text{O}$	2.297	163	2.069	0.81	141	Ethanol
$[\text{Cu}(\text{pfp})_2(\text{bipy})]^\text{f}$	2.280	159	2.066	0.79	143	Ethanol
$[\text{Cu}(\text{pfp})_2(\text{imH})_2]$			2.136 <sup>g</sup>			Acetone

<sup>a</sup> In units of  $10^{-4} \text{ cm}^{-1}$ . <sup>b</sup> A measure of the metal-ligand covalency calculated from the expression  $\alpha^2 = (A_{\parallel}/0.036) + (g_{\parallel} - 2.0023) + \frac{2}{3}(g_{\perp} - 2.0023) + 0.04$  (ref. 44). <sup>c</sup>  $g_{\parallel}/A_{\parallel}$  in cm. <sup>d</sup> Major species. <sup>e</sup> Species may be  $[\text{Cu}(\text{pfp})_2(\text{en})_2] \cdot \text{H}_2\text{O}$ . <sup>f</sup>  $A_{\perp}(\text{N}) = 12.9 \times 10^{-4} \text{ cm}^{-1}$ . <sup>g</sup> Isotropic value.

though useful for comparing various complexes, the expression has been subject to criticism.<sup>45</sup> Nevertheless, the  $\alpha^2$  values shown in Table 3 for various complexes fall in the range 0.81–0.84. This lies in between  $\alpha^2$  values for four-nitrogen-donor ligands (0.63–0.84)<sup>46</sup> and for four-oxygen-donor ligands (0.84–0.94),<sup>47</sup> hence supporting a covalent planar  $\text{CuN}_2\text{O}_2$  donor set. The lower  $\alpha^2$  values for the dmphen complexes (0.78–0.79) are related to structural deviations from square-planar geometry towards distorted tetrahedral geometry (as supported by electronic absorption spectra), rather than increased metal-ligand covalency.

**A Chromium Phenolate Complex.**—The compound  $[\text{Cr}(\text{H}_4\text{salen})(\text{py})(\text{HOMe})][\text{PF}_6]$  was prepared as a possible model for dichromium(III) lactoferrin. It is a 1 : 1 elec-

$g_{\text{eff.}} = 5.38, 5.02, 4.66, 3.57, 2.62$ , and 2.04 in methanol. The  $g_{\text{eff.}}$  values of 4–5.5 appear when distortions from regular octahedral symmetry exist.

**Conclusions.**—The compound dicopper(II) lactoferrin has a c.t. absorption band at 438 nm ( $\epsilon = 4800 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) in phosphate buffer solution,<sup>9</sup> which from this study is concluded to be a tyrosine-to-metal type. Although the  $\lambda_{\text{max.}}$  is coincidentally similar to some of the compounds listed in Table 2, the intensity per copper(II) ion is similar to the tmen complexes only. Variations in the phenolate-to-Cu c.t. transition energies arise from the nature of the coligands, the nature of the number of phenolates, and the nature of the solvent. As halogeno-substituted phenolates have been used [compared with *para*-substituted tyrosine type phenolates in dicopper(II)

lactoferrin] this would result in an increase of the c.t. transition energy for the former (assuming the same coligands and number of phenolates). The complex *cis*-[Cu(H<sub>4</sub>salen)] contains *ortho*-substituted phenolates and absorbs at 394 nm, however as the phenolato-ligands are in a quadridentate system, the effect of chelation would be to increase the energy by an unknown amount, but for purposes of this study, say at least 10 nm. Also replacement of the ethylenediamine component of H<sub>4</sub>salen by two imidazoles would be expected to shift  $\lambda_{\text{max}}$  by ca. 20 nm to lower energy (Table 2). On the basis of these approximate values a CuN<sub>2</sub>O<sub>2</sub> system (O = OC<sub>6</sub>H<sub>4</sub>Me-*o* and N = imidazole) would be predicted to absorb at ca. 400–410 nm. Replacement of an imidazole by a less strongly bound water molecule to give a CuNO<sub>2</sub>O' (O = phenolate, O' = water) system would lower the c.t. band energy to nearer 438 nm. Our studies have shown that axial ligands (*e.g.* pyridine or water) do not markedly alter this energy. Analysis of the *d-d* band of dicopper(II) lactoferrin favours only two (at most) bound imidazoles for each Cu<sup>II</sup><sup>9</sup> and the e.s.r. data for this and related proteins suggest only one is equatorially bound.<sup>10,12–14</sup> Hence the other, if present, may be axial. The e.s.r. spectral characteristics of dicopper(II) lactoferrin are essentially those for a tetragonal environment about copper.<sup>10</sup> The *g*<sub>||</sub> and *A*<sub>||</sub> values best fit the Peisach–Blumberg plots<sup>49</sup> for a CuNO<sub>3</sub> donor set with charge close to zero. Weak axial ligands alter the e.s.r. parameters very little. Of the complexes in Table 3 it is of interest that [Cu(tbp)<sub>2</sub>(coll)] and [Cu(pcp)<sub>2</sub>(coll)] have similar e.s.r. parameters to dicopper(II) lactoferrin, and these may be of the CuNO<sub>3</sub> donor type.

A proposed structure for the copper(II) sites in dicopper(II) lactoferrin can thus be given from the above considerations. Two *cis* tyrosine phenolates (although two *trans* cannot be excluded), one water, and a histidine imidazole in the equatorial plane, with a histidine and an oxygen donor (non-ionized tyrosine) along the axial direction are suggested as ligands. The bicarbonate ion may be hydrogen bonded to the bound in-plane water molecule or it may displace it. The in-plane donor set is thus suggested to be CuNO<sub>3</sub>. Recently we have also proposed a structure for the iron sites in di-iron(III) lactoferrin based on similar considerations.<sup>1</sup>

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