Copper(II) Chelation by Dopa, Epinephrine, and Other Catechols

Robert K. Boggess and R. Bruce Martin*

Contribution from the Chemistry Department, University of Virginia, Charlottesville, Virginia 22901. *Received July 26, 1974*

Abstract: Catechol and tiron form distinct mono- and bischelates with Cu(II) characterized by a ligand to metal ion charge-transfer transition near 440 nm in the mono- and 400 nm in the biscatecholate chelates. The absorption spectra, originally taken under nitrogen, change on exposure to air. The second ionization from unbound catechol is 3.0 log units less acidic than that from phenol. It is shown that intramolecular hydrogen bonding in catecholate monoanion contributes only 0.5 log units or 17% to the difference, while electrostatic effects contribute 2.2 log units or 73%. Stability constants calculated by potentiometric methods for binding of metal ions to L-3,4-dihydroxyphenylalanine (L-Dopa), epinephrine, and similar so-called catecholamines are incorrect because it has not been recognized that the first phenolic ionization precedes the ammonium deprotonation in these ammonium catecholates. In the initial stages of a titration, L-Dopa binds to Cu(II) as a substituted glycinate. Solutions containing Dopa, Cu(II), and OH^- in 1:1:3 or 2:1:3 molar ratios exhibit a charge-transfer absorption band at 436 nm and strong circular dichroism and yield a mixed dimeric complex where each Cu(II) is chelated by the glycinate site of one Dopa ligand and by the catecholate locus of another while each Dopa binds two Cu(II). Above pH 8 a second Dopa molecule begins to displace the bound glycinate site and by pH 11 the charge-transfer absorption band shifts to 405 nm and the circular dichroism weakens as two Dopa molecules are bound predominantly as catecholates. Thus, depending upon pH, Dopa forms three kinds of Cu(II) complexes, glycinate, mixed, and catecholate. Optical activity is induced in the charge-transfer band near 450 nm of optically inactive catecholate and Cu(II) by formation of mixed complexes with L-alanine or L-phenylalanine. Without the strong glycinate chelating site epinephrine forms primarily catecholate complexes with Cu(II). In equimolar solutions at high pH the amine group participates in formation of polymeric complexes. *o*-Tyrosine binds Cu(II) as a substituted glycinate to pH 7 with involvement of the hydroxy group in more basic solutions.

Stability constants calculated by potentiometric methods for metal ions and the ligands L-3,4-dihydroxyphenylalanine (L-Dopa), L-epinephrine (adrenalin), and similar so-called catecholamines require modification. The incorrect calculations are a result of misassignments of pK_a values as determined by titration to ammonium and phenolic deprotonations. The ligands in slightly acidic solutions contain three acid groups: one substituted ammonium group and the two phenolic groups of the catechol moiety. Successive acidity constants determined by titration yield pK_a values of about 8.8, 9.9, and 13. It is generally agreed that the last constant refers to the second phenolic group to ionize. Consistent with the designation catecholamine, the first pK_a value near 8.8 has most often been assigned to the ammonium deprotonation and the free ligand concentration calculated on this basis. However, the two acidity constants with pK_a values of about 8.8 and 9.9 represent competitive deprotonations and neither may be assigned exclusively to either the ammonium or first phenolic deprotonations.[1] Resolution of the contributions reveals that the most acid of the two groups is always the first phenolic group, with the ammonium group of lesser acidity so that the compounds are more appropriately termed ammonium catecholates. This designation is especially suitable for epinephrine where the ratio of ammonium catecholate to catecholamine is about 4, while for Dopa the ratio is less, about 1.6.[1] In order to calculate appropriate unbound ligand concentrations for stability constant calculations, it is necessary to know the acidity microconstants leading up to the ligand microspecies that is bound to a metal ion in a complex.

In the initial stages of a titration, Dopa chelates to transition metal ions as a substituted glycine. For this chelation mode the free ligand concentration should be calculated from the ligand microspecies with only the carboxylate and amine groups deprotonated. For Dopa the acidity constant with pK_a near 8.8 is composed of 61% phenolic and 39% ammonium deprotonations. The acidity microconstant leading to the microspecies with basic amine and protonated phenolic groups is 0.41 log units greater than the pK_a determined by titration.[1] This greater value has never been employed in stability constant calculations. In some investigations the error will only be of secondary quantitative significance but in others it may affect even qualitative conclusions. The conclusion that low values of temperature-jump complexation rate constants for Dopa are to be ascribed to an ion-pair complex due to hydrogen bonding of hydroxy groups with inner sphere water molecules of metal ions[2] needs to be reexamined with proper assignments of acidity constants to phenolic and ammonium groups with consequent revised stability and rate constants.

Quantitatively more serious is the calculation of stability constants when the ligand chelates the metal ion between the two catecholate oxygens with the ammonium group still protonated, as occurs throughout most of the pH range with epinephrine and Cu(II). Simply calculating the free ligand concentration from the first two acidity constants with pK_a values near 8.8 and 9.9 is patently wrong. Assumption of the free ligand concentration from the acidity constants of pK_a values near 9.9 and 13 is also incorrect. What is required is a knowledge of the acidity microconstants needed to calculate the free ligand concentration of the microspecies with a protonated ammonium group and two deprotonated phenolic groups. Since one of the requisite microconstants is unknown, the concentration of the microspecies cannot be calculated. It is possible to estimate the requisite microconstants by titration of both phenolic groups of the respective betaine, *N,N*-dimethylepinephrine. This approach has never been employed in any potentiometric stability constant determination of catecholamines.

In addition to appreciation of the complexity of identification of acidity constants with substituent groups, research on complexation of catecholates with transition metal ions has been plagued by oxidation of the ligand. A variety of color changes is reported and the intensity scales of absorption spectra have been reported in arbitrary units suggesting irreproducibility. Unless one wishes to study oxidation products, scrupulously oxygen free solutions are required.

In this research we characterize the complexes formed in solutions of Cu(II) and several catechols at a variety of pH conditions by a combination of potentiometric and optical

methods conducted under pure nitrogen. Both visible absorption and circular dichroism (CD) spectra are utilized. The ammonium catecholates possess two potential metal ion binding sites: at the amine nitrogen and chelation at two ionized phenolate oxygens. Chelation between an amine nitrogen and a 3- or 4-substituted phenolate oxygen on the same ligand is stereochemically impossible. Optical activity is generated in transitions involving Cu(II) when it is close to the asymmetric carbon of a ligand. Participation of the asymmetric carbon in a chelate ring such as occurs when L-Dopa serves as a substituted glycinate produces relatively strong CD. On the other hand chelation of Cu(II) at the catecholate locus remote from the asymmetric carbon is expected to yield little or no observable CD in transitions involving Cu(II). This expectation is borne out by the results reported, and thus CD provides a useful tool in addition to absorption spectra for delineating the site of Cu(II) binding to ammonium catecholates. In order to characterize the properties elicited on Cu(II) binding to the catecholate locus, preliminary experiments were conducted on Cu(II) binding to catechol and catechol-3,5-disulfonate (tiron). The interactions of Cu(II) with *o*-tyrosine are also investigated.

Experimental Section

All ligands were high grade commercial products, which in most cases were obtained from more than one source. Their purity was confirmed by potentiometric titration. Titrations were conducted under nitrogen with a Radiometer TTT2-SBR2 titrimeter-titrigraph equipped with a Sargent combination electrode that was standardized with standard buffer solutions. Prepurified nitrogen gas was passed over vanadous chloride scrubbers. Transfers of solutions from titrimeter to absorption cells were made directly under nitrogen. Absorption spectra were recorded on a Cary 14R spectrophotometer and circular dichroism spectra on a Jasco J-10 B instrument. All molar absorptivities are reported per mole of metal ion. All experiments were performed at room temperature, near 22°.

Results

Catechol and tiron (with ionized sulfonate groups) exhibit similar qualitative behavior on titration in the presence of Cu(II); the quantitative differences derive from the first phenolic pK_a values of the free ligands, 9.3 for catechol and 7.6 for tiron. Titration curves of these ligands performed under nitrogen in the presence of Cu(II), shown in Figure 1 for tiron, consist of three sections, not all of which appear at all ligand to metal ion ratios. The first section occurs in all solutions with a 1:1 or greater ligand to Cu(II) molar ratio and shows titration of 2.0 equiv of base per mole of Cu(II) by pH 6.5 for catechol and by pH 5.5 for tiron. The second section appears in solutions with a 2:1 or greater ratio and is completed at about pH 8 for catechol and pH 7 for tiron after addition of 2.0 more equiv of base per mole of Cu(II). The third section occurs only in solutions with 3:1 or greater ratios and corresponds to the titration of excess unbound ligand. The first section of the titration curves is identified with formation of a monochelate with both phenolate hydrogens having ionized. One additional equivalent of base is titrated from about pH 7.5 to 9.5 in the 1:1 solutions which evidently corresponds to formation of a monohydroxo complex of the chelate and to some precipitate of $Cu(OH)_2$. This additional equivalent does not appear in the 2:1 solutions due to the formation of a bischelate in the second section of the titration curves. Thus catechol and tiron form first in acid solutions monochelates with Cu(II) and then, in more neutral solutions, bischelates, which remain the predominant form of the Cu(II) complex in the presence of excess ligand. The similarity of the results for catechol and tiron precludes profound contribution of the sulfonate groups of the latter ligand in Cu(II) binding. Since the sulfonic acid groups of tiron undergo ionization in such acid solutions ($pK_a < 2$), it is difficult to detect directly their involvement by titration.

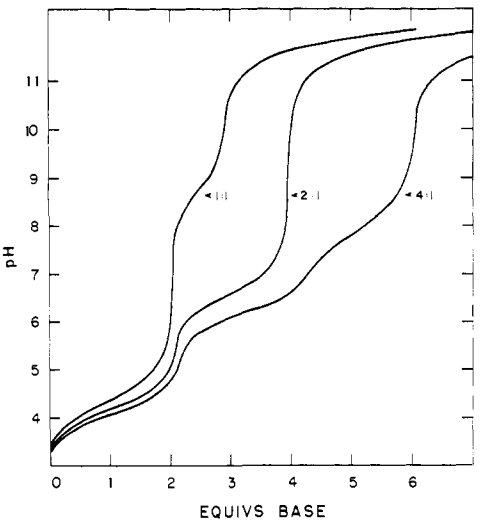


Figure 1. Titration of $(tiron)^{2-}$ in the presence of Cu(II). Equivalents of base are per mole of 4 m*M* Cu(II) at the ligand to metal ion ratios indicated in the figure.

Table I. Absorption Spectra of Cu(II) Complexes[a]

[L]:[Cu]: [OH⁻]	Catechol			Tiron		
	pH	nm	ε	pH	nm	ε
1:1:2	7.3	740	46	6.1	750	37
		440	68		440	62
2:1:2	6.5	740	44	5.5	750	38
		450	65		440	66
2:1:4	9.2	660	36	8.5	665	35
		400	220		375	280

[a] Under nitrogen atmosphere.

The preceding conclusions concerning the make up of the catechol and tiron complexes of Cu(II) are supported by visible absorption spectra shown in Table I. Catecholate involvement in binding to Cu(II) is signaled by appearance of an absorption band near 440 nm. Spectra for the first two complexes are nearly identical, supporting the identity of monocomplexes formed in 1:1:2 solutions and 2:1:2 solutions each after addition of 2 equiv of base. The expected blue shift occurs in the 740-nm ligand field band upon formation of a bis complex. When the catechol solutions of Table I are made up in the presence of air or are exposed to air, the intensity of the absorption bands increases, with some shifting in wavelength. The tiron solutions are more stable when exposed to air, with the high pH 8.5 solution stable for days.

A disconcerting aspect of the absorption spectra information is that solutions of catechol and tiron without added Cu(II) develop new absorption bands in the presence of air. Growth of absorption is more rapid in the more basic solutions. Under nitrogen at pH 11, an aqueous solution of catechol exhibits a single absorption band at 288 nm (ε 4100). Upon exposure to air over a period of hours, a new peak appears first as a shoulder near 325 nm with the main absorption band shifting to shorter wavelengths. As a result of these changes, appreciable absorption but no maximum occurs in the 440-nm region where the Cu(II) complexes absorb. In the case of free tiron at pH 10, a new peak appears at about 435 nm upon admission of air. Though the intensities of the catechol and tiron peaks induced by air in the absence of Cu(II) eventually exceed those of Table I, the lack

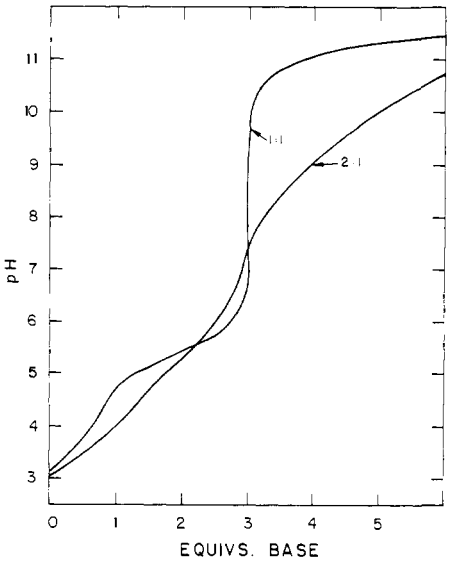


Figure 2. Titration of L-Dopa in the presence of Cu(II). Equivalents of base are per mole of 4 mM Cu(II) at the ligand to metal ion ratios indicated in the figure.

Table II. Optical Properties of L-Dopa Complexes of Cu(II)

[L]:[Cu]: [OH⁻]	pH	Absorption[a] nm	ϵ	CD[a] nm	$\Delta\epsilon$
1:1:1	4.7	700	30	630	−0.12
1:1:3	9	635	35	670	+0.29
		436	68	550 s	−0.35
		350 s	60	455	−1.07
2:1:1	4.1	680	34	625	−0.15
2:1:2	5.2	620	47	598	−0.35
				470 s	−0.10
2:1:3	7.6	635	34	675	+0.25
		436	68	550 s	−0.30
		350 s	60	455	−0.92
2:1:6	11.4	645	31	675	+0.04
		405	220	550 s	−0.05
				452	−0.16

[a] Key: s signifies shoulder.

of a precise correspondence suggests that the results in the table refer to peaks due to Cu(II) complexes.

L-3,4-Dihydroxyphenylalanine (L-Dopa) contains glycinate and catecholate loci for binding of metal ions. As shown in Figure 2, titration curves, under nitrogen, of solutions containing 1:1 or 2:1 mole ratios of L-Dopa and Cu(II) show a break at pH 7 after the addition of 3.0 equiv of base per mole of Cu(II). The titration curves of 1:1 solutions containing Cu(II) and either L-Dopa or D,L-Dopa are virtually superimposable, suggesting negligible stereoselectivity. In the 2:1 solution no further break occurs as 3 additional equiv is titrated from pH 7.5 to 10.7.

Absorption and circular dichroism spectra of solutions containing L-Dopa and Cu(II) are presented in Table II and the curves for 2:1 solutions shown in Figure 3. Upon addition of 1 equiv of base to a 1:1 solution and 1 and 2 equiv to the 2:1 solutions, the absorption and CD spectra are characteristic of Cu(II) binding at L-amino acids[3] so that L-Dopa serves as a substituted glycinate to these points in the titration curves. Addition of more base yields green solutions with absorption maxima near 635 and 436 nm. The latter band indicates that each Cu(II) is bound to a single catecholate moiety of a Dopa molecule. The CD results are nearly identical after addition of 3 equiv base to both the 1:1 and 2:1 solutions, strongly suggesting the presence of identical complexes.

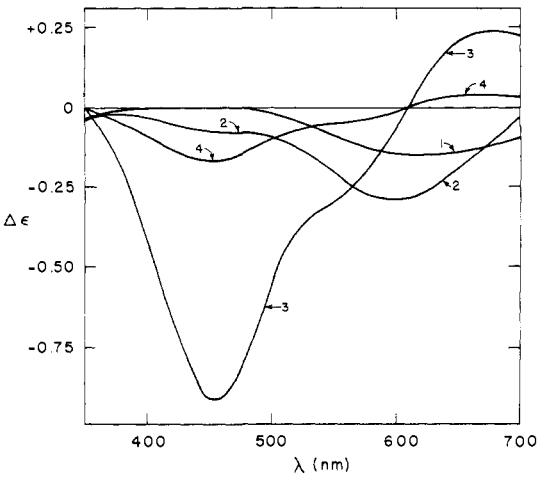


Figure 3. CD spectra of solutions containing a 2:1 molar ratio of L-Dopa to Cu(II). Equivalents of base per mole of 4 mM Cu(II) are indicated in the figure.

The polymeric composition of the complex present after addition of 3 equiv of base to solutions containing 1:1 and 2:1 molar ratios of L-Dopa to Cu(II) was investigated in a series of quantitative experiments performed wholly under nitrogen. Due to a tendency to precipitation in the 1:1 solutions, 2:1 mixtures were utilized. The analysis is similar to that developed to suggest formation of a tetramer in equimolar solutions of glycyl-L-histidine and tetragonal transition metal ions[4] and applied to show dimer formation in equimolar solutions of histidylglycine and Cu(II)[5] except that expulsion of excess ligand must be allowed for in the Dopa case. The reaction for development of n-mer absorbing near 435 nm upon addition of the third equivalent of base to 2:1 solutions of L-Dopa and Cu(II) is

$$n\mathrm{Cu(LH_2)_2^0} \rightleftharpoons (\mathrm{CuL^-})_n + n\mathrm{LH_3^0} + n\mathrm{H^+} \qquad (1)$$

We seek to find the polymeric composition of the species $(\mathrm{CuL^-})_n$ or the value of n. The total concentration of Cu(II), C_M, is half that of ligand, C_L, and since for each n-mer formed n molecules of LH_3^0 are expelled, we have

$$C_M = C_L/2 = [m] + n[d]$$

where m represents monomer and d polymer. The equilibrium constant for eq 1 may be written as

$$K^{1/n} = (H^+)[d]^{(n+1)/n}/[m]$$

The absorptivity in cells of 1 cm length is given by

$$A = 1\epsilon_m[m] + 1\epsilon_d[d]$$

For pure monomer the absorptivity becomes, $A_m = 1\epsilon_m C_M$, and for pure polymer, $A_d = 1\epsilon_d C_M/n$. Substituting these equations into the expression for the equilibrium constant yields

$$K^{1/n} = (H^+)\frac{(A - A_m)^{(n+1)/n}}{(A_d - A)}\frac{(1\epsilon_d - n1\epsilon_m)^{-1/n}}{n} \qquad (2)$$

If one kind of n-mer predominates, the last term on the right of eq 2 is a constant, and a plot of pH + log $(A_d - A)$ vs. log $(A - A_m)$ should give a straight line of slope $(n + 1)/n$ enabling calculation of n. Figure 4 shows such a plot for two solutions containing a 2:1 molar ratio of L-Dopa to Cu(II) from pH 6 to 7 in 0.2 M KCl. In the first solution C_M = 5.0 mM, and in the second solution the concentration is three times greater. For both cases the resulting straight line possesses a slope of 1.5, indicating that the predominant n-mer is a dimer. From the positions of the straight lines in Figure 4, substitution in eq 2 with n = 2 yields for the 5.0 and the 15.0 mM Cu(II) solutions values of log K =

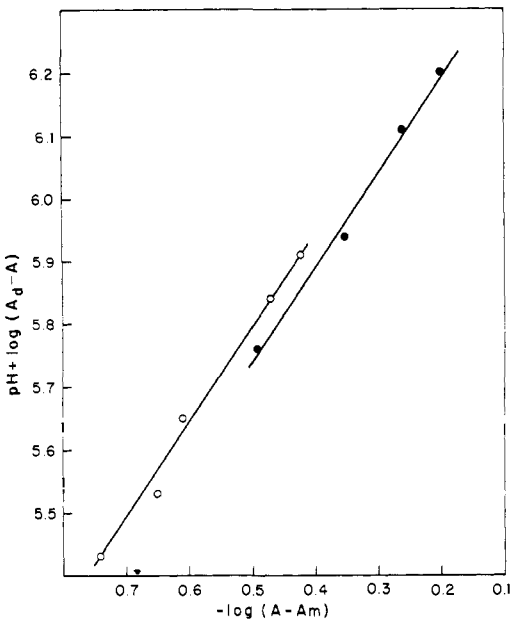


Figure 4. Plot for determination of the number of Cu(II) in *n*-mer for solutions containing a 2:1 ratio of L-Dopa to Cu(II) from pH 6 to 7. Open circles refer to 5.0 m*M* Cu(II) in 2.00-cm cells and closed circles to 15 m*M* Cu(II) in 1.00-cm cells. The slope of both straight lines is 1.5 indicating that $n = 2.0$.

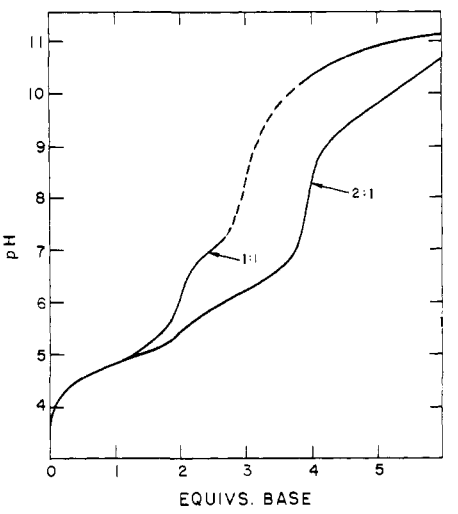


Figure 5. Titration of L-epinephrine in the presence of Cu(II). Equivalents of base are per mole of 4 m*M* Cu(II) at the ligand to metal ion ratios indicated in the figure. The dotted line indicates the region over which a precipitate appeared in solution.

-14.5 and -14.3, respectively. The average value of log $K = -14.4$ is identical with that calculated from the results of a potentiometric study.[6] The agreement between the two studies with disparate methods of analysis is remarkable. For the equilibrium of reaction 1 errors introduced into the wholly potentiometric results by considering macroconstants rather than microconstants are small and tend to offset each other. No assumptions involving microconstant equilibria are invoked in the combination spectrophotometric-pH study of reaction 1 in this paper.

In order to learn more of the structure of L-Dopa complexes, mixed Cu(II) complexes of fragments of the ligand were studied. A solution containing equimolar amounts of Cu(II), catechol, and either L-alanine or L-phenylalanine titrates 3 equiv of base to form a green solution by pH 8. The resulting solutions exhibit two absorption maxima at about 635 (ϵ 50) and 420 nm (ϵ 100). The CD spectra show two minima: for the L-alanine complex at 630 nm ($\Delta\epsilon = -0.07$) and 450 nm ($\Delta\epsilon = -0.02$), for the L-phenylalanine complex at 620 nm ($\Delta\epsilon = -0.19$) and 455 nm ($\Delta\epsilon = -0.20$). These CD spectra are not identical with those 1:1 or 2:1 complexes of the amino acids.[3] Mixed complexes of Cu(II), catecholate, and L-amino acid must form because optical activity appears in the 450-nm absorption band due to optically inactive catecholate and Cu(II). A solution containing L-phenylalanine, catechol, and Cu(II) in a 2:2:1 molar ratio titrates 6 equiv of base at pH 10.3. Since this value is much less than the greater $pK_a = 13$ of catechol, all the catechol must be chelated as catecholate. The nearly complete displacement of L-phenylalanine from chelation with Cu(II) is confirmed by the weak CD of a pH 11 solution.

As shown in Figure 5 a solution containing 2 mol of L-epinephrine (L-adrenaline) per mole of Cu(II) titrates 4 equiv of base by pH 8 where the curve displays a break. The absorption maximum near 400 nm listed in Table III and the weak CD suggest that these 4 equiv correspond to two pairs of catecholate hydrogens so that a biscatecholate complex is formed. The midpoint pH 9.7 for titration of two additional equivalents of base is in agreement with that expected for ammonium group deprotonation without complexation in a bis complex.[1] In the 1:1 solutions 2 equiv of base yields a break in the titration curve and an absorption maximum appears at 445 nm indicating catecholate binding to Cu(II). Due to precipitation 4 equiv of base was required for dissolution. The optical properties of the resulting solution are listed in Table III. The absorption band at 625 nm indicates amino group coordination, which is also supported by the relatively strong CD. The 2:1 solutions produce weaker CD intensities. L-Norepinephrine behaves similarly except that no negative CD extremum appears at 430 nm.

Table III. Optical Properties of L-Epinephrine Complexes of Cu(II)

[L]:[Cu]:[OH^-]	pH	Absorption[a] nm	ϵ	CD nm	$\Delta\epsilon$
1:1:2	6	750	39	Very	weak
		445	55		
1:1:4	11	625	45	675	−0.14
		370 s	320	520	+0.09
				430	−0.03
				340	+0.15
2:1:2	5.5	745	36	500	+0.005
		460	100	425	−0.01
				375	+0.01
				330	−0.05
2:1:4	8	650	34		Weak
		397	246		
2:1:6	11	645	36	700	−0.02
		400	264	535	+0.01
				435	−0.01
				350	+0.01

[a] Key: s signifies shoulder.

A solution containing a 2:1 molar ratio of DL-*o*-tyrosine and Cu(II) gives a titration curve that displays a break by pH 7 after addition of 2 equiv of base. The absorption spectrum shows a maximum at 610 nm (ϵ 58) typical of a bis(amino acid) complex bound to Cu(II) as a substituted glycinate.[3] Addition of 2 more equiv of base occurs from pH 8 to 10.5, at which point the spectrum shows two absorption maxima at 630 (ϵ 88) and 393 nm (ϵ 590). We were unable to find conditions to reproduce a puzzling strong absorption at 790 nm reported at pH 10.[7] The absorption band at 393 nm strongly suggests involvement of the hydroxy group in Cu(II) binding. Thus *o*-tyrosine serves as a substituted glycinate ligand to pH 7 with involvement of the hydroxy group in Cu(II) binding in basic solutions. The hydroxy group of *p*-tyrosine does not participate in binding to

Cu(II). These conclusions are in accord with those advanced mainly on the basis of thermochemical arguments.[7]

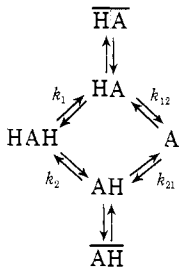
In order to inquire into the nature of the transition occurring in Cu(II) complexes of phenolate compounds from about 375 to 460 nm, the wavelength maxima of Eu(III), Cu(II), and Fe(III) complexes of tiron and *N,N'*-ethylenebis[2-(*o*-hydroxyphenyl)]glycine (EHPG)[8,9] were investigated. For tiron the respective maxima occur at 380 (shoulder), 440, and 560 nm and for EHPG at 345, 371, and 480 nm. All complexes are 1:1 except the tiron-Fe(III) complex which is 2:1. This ordering is precisely that expected for ligand to metal ion electron-transfer transitions.[10] This conclusion is identical with that reached by consideration of the nature of the substituent on the wavelength maxima of Cu(II) complexes of 4-substituted catechols.[11] Thus two independent arguments identify the transition occurring in Cu(II) complexes of phenols and catechols from about 370 to 460 nm as ligand to metal ion electron transfer. This transition also occurs in Fe(III) and Cu(II) complexes of conalbumin and transferrin, where a tyrosine phenolate group is thought to be involved in metal ion binding.[9]

Discussion

Formation of a strong intramolecular hydrogen bond in catecholate monoanion is often presented as the reason for a relatively low pK_1 and high pK_2 for catechol and derivatives. It is, however, easy to show that the main contributor to the high pK_2 value of catechol and derivatives is due to the electrostatic effect of the nearby negative charge and not to hydrogen bonding.

Scheme I outlines the ionization scheme for a catechol

Scheme I



via monoanions HA or AH which are not intramolecularly hydrogen bonded. The latter are in equilibrium with the hydrogen bonded species $\overline{HA}$ and $\overline{AH}$, which are in rapid tautomeric equilibrium. We define the hydrogen bonding equilibrium constant $k_H = [\overline{HA}]/[HA] = [\overline{AH}]/[AH]$ and the acidity microconstants $k_1 = (H^+)[HA]/[HAH]$, $k_2 = (H^+)[AH]/[HAH]$, $k_{12} = (H^+)[A]/[HA]$, and $k_{21} = (H^+)[A]/[AH]$.

The two macroscopic acidity constants determined by titration are given by

$$K_1 = (H^+)\{[HA] + [\overline{HA}] + [AH] + [\overline{AH}]\}/[HAH]$$

$$K_2 = (H^+)[A^-]/\{[HA] + [\overline{HA}] + [AH] + [\overline{AH}]\}$$

In a symmetrical system where $k_1 = k_2$ and $k_{12} = k_{21}$ the above equations lead to

$$K_1 = 2k_1(1 + k_H)$$

$$K_2 = k_{12}/2(1 + k_H)$$

Thus the acidity constants determined by titration are related to k_H, the equilibrium constant for hydrogen bond formation in the monoanion, and k_1 or k_{12}, acidity constants for one of which an estimate is required. Note that according to the last pair of equations, hydrogen bonding in the monoanion decreases pK_1 and increases pK_2 by identical amounts.

The acidity constant k_1 in Scheme I may be estimated from the K_a value for *o*-methoxyphenol, which cannot undergo hydrogen bonding in the monoanion. The slightly greater electron donating properties of the hydroxy as compared to the methoxy group do not alter the argument. Thermodynamic acidity constants at 25° are $pK_a = 9.99$ for *o*-methoxyphenol and $pK_1 = 9.19$ and $pK_2 = 12.98$ for catechol.[12] Substitution into the last equation for K_1 yields $k_H = 2.15$ and the augmentation due to hydrogen bonding $1 + k_H = 3.15$ or 0.50 log unit. Thus hydrogen bonding lowers pK_1 and raises pK_2 of catechol about 0.50 log units.

Compared to the pK_a of phenol = 10.0, the high value of $pK_2 = 13.0$ for the second ionization in catechol is due mainly to the inhibiting effect of the nearby negative charge. Calculation in Scheme I yields $pk_{12} = 12.2$ so that $pK_2' = 12.5$ is anticipated for ionization from catecholate monoanion in the absence of hydrogen bonding. To the high pK_2 of catechol electrostatic effects contribute 2.2 log units or 73%, intramolecular hydrogen bonding 0.5 log units or 17%, and statistics 0.3 log units or 10%.

It is also informative to consider the enthalpies of several equilibria in Scheme I. For enthalpies recorded for a variety of phenols,[12] that for *o*-methoxyphenol provides an estimate for the k_1 equilibrium in Scheme I, $\Delta H_3 = 24.0$ kJ mol^{-1}. For the two successive ionizations from catechol $\Delta H_1 = +34.5$ and $\Delta H_2 = +21$ kJ mol^{-1}. Thus the enthalpy change to be associated with intramolecular hydrogen-bonding equilibrium is +10.5 kJ mol^{-1}. The positive sign indicates that the enthalpy change hinders formation of a hydrogen bond in catecholate monoanion, which occurs only as a result of a high positive entropy change. It has been pointed out that that the enthalpy change ΔH_2 for the second catechol ionization is similar to that of phenol[12] (and other phenols as the ΔH_3 value above). However, the second catechol ionization occurs from an intramolecularly hydrogen bonded monoanion. For ionization from a non-hydrogen bonded monoanion in catechol, corresponding to the k_{12} equilibrium in Scheme I, $\Delta H = \Delta H_2 + 0.5 = +31$ kJ mol^{-1}, a value quite different from that of other phenols, where the proton is not removed from a species with a nearby negative charge. It is the entropy change for the k_{12} equilibrium which compares well with that of phenol and other phenols such as *o*-methoxyphenol.

In solutions containing at least a 2:1 molar ratio of catechol or tiron to Cu(II), mono and bis complexes are formed in two distinct steps. Additional ligand in excess of that required for a bis complex is not bound to Cu(II). That two distinct steps are involved in formation of the bis complexes is supported by the 2:1 titration curve of Figure 1, visible absorption spectra of Table I, and the successive stability constants for the catecholate complexes.[13] Binding to Cu(II) of a single catecholate is signaled by the appearance of a charge-transfer absorption maximum near 440 nm and of two catecholates by a maximum at shorter wavelength, near 400 nm.

Donor atoms in the L-Dopa complexes of Cu(II) are well characterized by the titration, absorption, and CD results. In solutions where the L-Dopa:Cu(II):OH^- molar ratio is 1:1:1 and 2:1:1 binding of a single Dopa molecule through the glycinate locus is indicated by absorption and CD results. Addition of a second equivalent of base to the latter solution to give a 2:1:2 mixture results in chelation by a second molecule of Dopa also bound as a substituted glycinate. The absorption at 620 nm and negative CD at shorter wavelength is typical of 2:1 complexes of ordinary amino acids with Cu(II).[3]

Further addition of base gives solutions containing 1:1:3 and 2:1:3 ratios that exhibit nearly identical absorption and CD spectra and contain identical complexes. The latter solution contains 1 equiv of unbound, neutral, excess ligand. The charge-transfer absorption peak at 436 nm signifies involvement of a single catecholate in binding to Cu(II). In the same absorption spectrum the ligand field band at 635 nm appears at shorter wavelength than in the pure catecholate complexes (Table I) and indicates three oxygen and one nitrogen donor atoms about Cu(II).[14] The relatively strong CD suggests binding near the asymmetric carbon consistent with involvement of the substituted glycinate locus. In order to accommodate both catecholate and glycinate chelation in an equimolar solution of L-Dopa and Cu(II), all four tetragonal coordination positions about each Cu(II) must be occupied. Examination of molecular models reveals that a dimer with mixed glycinate and catecholate chelation about each Cu(II) is the smallest permissible polymer. A satisfactory dimer may be built with either two L-Dopa molecules or one D- and one L-Dopa molecule accounting for the absence of stereoselectivity in the titration curves. The spectrophotometric-pH analysis described in the results section indicates that a dimer is the predominant polymer in a 2:1:3 [Dopa]:[Cu]:[OH^-] solution, as had previously been shown by a potentiometric study for a 1:1:3 mixture.[6] Though employing different methods of analysis on solutions of different mole ratios, the two studies obtained nearly identical equilibrium constants for dimer formation. In a 1:1:3 solution, the dimer is joined from two mononuclear, equimolar complexes with Dopa chelated as a glycinate ligand, while, in a 2:1:3 solution, the dimer is formed by ligand exchange of two Dopa molecules bound as substituted glycinates to each Cu(II). Because of expulsion of ligand on the right hand side of reaction 1, the dimer is more favored in dilute solutions containing a 2:1 ratio of Dopa to Cu(II). In a 2:1 solution at pH 7.6 containing 10^{-2} *M* dimer, the ratio of dimer to monomer is 25:1, while at 10^{-4} *M* dimer the ratio is 250:1. This dimer went undetected in electron spin resonance investigations.[15]

Due to stereochemical necessity the dimer is a mixed complex, with each Cu(II) bound to one molecule of Dopa as a catecholate and to a second molecule through the glycinate locus. Even if the polymer were of larger size so that stereochemical restraints did not occur, mixed ligand binding about each Cu(II) is still the expectation. It has been established that catechols exhibit a strong tendency to form mixed complexes with other ligands.[16] Similar mixed ligand binding to each Cu(II) is expected in the tetrameric complex of the related ligand 3,4-dihydroxyphenylglycine.[17]

Above pH 8 a second Dopa molecule begins to displace the glycinate locus bound to Cu(II) in the equimolar complex so that at pH ~11 in a 2:1:6 solution (Table II) both Dopa molecules are bound as catecholates and the glycinate locus is unbound in the predominant complex. This conclusion is supported by the shift of the catecholate-Cu(II) absorption band from 436 to 405 nm and the ligand field band from 635 to 645 nm. Only weak CD is expected for this bicatecholate complex where the Cu(II) is distant from the asymmetric carbon of the ligand. However, a weak CD of the 2:1:6 solution, of the same pattern as that of the 2:1:3 solution (Table II, Figure 3) suggests that about 15% of the Cu(II) is still bound through the glycinate locus in the equimolar type complex. These conclusions on ligand exchange are once again supported by the results obtained on the solutions containing the fragments of Dopa, where a second catechol molecule displaced phenylalanine from coordination to Cu(II) at pH 10. Thus depending upon pH, Dopa forms three kinds of Cu(II) complexes, with glycinate, mixed, or catecholate binding modes.

That the catecholate-Cu(II) charge-transfer absorption band at 436 nm in the Dopa dimer exhibits strong CD despite the distance of the catecholate oxygens from the asymmetric carbon on the same molecule suggests that optical activity is induced by an interaction through the Cu(II) orbitals from the other Dopa molecule bound in a substituted glycinate like manner. This conclusion is supported by the development of appreciable optical activity in the catecholate-Cu(II) charge-transfer absorption band at 455 nm of the complex of optically inactive catechol and L-phenylalanine.

Unlike L-Dopa, which presents a strong substituted glycinate chelating site, epinephrine is a substituted ethanolamine, and binding of metal ions at the amine locus is much weaker. Thus in contrast with the results and conclusions for Dopa, Cu(II) is chelated by the catecholate locus of epinephrine throughout most of the neutral and basic pH regions. Only in equimolar solutions where only water otherwise occupies coordination positions does appreciable amine binding occur to form polymeric complexes. From the CD spectra spectra some of this complex exists in 2:1 solutions at high pH. These conclusions derive from Table III and comments in the results section. It has been suggested that the high pH equimolar complex is a tetramer.[18] In accord with discussion above, it is anticipated that it is a mixed complex with each Cu(II) chelated by a catecholate grouping of one molecule and an ethanolamine function of another.

Acknowledgment. This research was supported by a research grant from the National Science Foundation.

References and Notes

(1) R. B. Martin, *J. Phys. Chem.*, **75**, 2657 (1971).
(2) R. L. Karpel, K. Kustin, A. Kowalak, and R. F. Pasternack, *J. Am. Chem. Soc.*, **93**, 1085 (1971); M. L. Barr, K. Kustin, and S-T. Liu, *Inorg. Chem.*, **12**, 1486 (1973).
(3) J. M. Tsangaris and R. B. Martin, *J. Am. Chem. Soc.*, **92**, 4255 (1970).
(4) P. J. Morris and R. B. Martin, *J. Inorg. Nucl. Chem.*, **33**, 2913 (1971).
(5) R. K. Boggess and R. B. Martin, *J. Inorg. Nucl. Chem.*, **37**, 1097 (1975).
(6) J. E. Gorton and R. F. Jameson, *J. Chem. Soc., Dalton Trans.*, 304 (1972); *J. Chem. Soc. A*, 2615 (1968).
(7) J. E. Letter, Jr., and J. E. Bauman, Jr., *J. Am. Chem. Soc.*, **92**, 443 (1970).
(8) A. E. Frost, H. H. Freedman, S. J. Westerback, and A. E. Martell, *J. Am. Chem. Soc.*, **80**, 530 (1958); G. Anderegg and F. L'Eplattenier, *Helv. Chim. Acta*, **47**, 1067 (1964).
(9) R. Prados, R. K. Boggess, R. B. Martin, and R. C. Woodworth, *Bioinorg. Chem.*, **4**, 135 (1975).
(10) R. B. Martin in "Metal Ions in Biological Systems", Vol. 1, H. Sigel, Ed., Marcel Dekker, New York, N.Y., 1974, Chapter 4, pp 135–137; J. C. Barnes and P. Day, *J. Chem. Soc.* 3886 (1964).
(11) R. F. Jameson and M. F. Wilson, *J. Chem. Soc., Dalton Trans.*, 2617 (1972).
(12) R. F. Jameson and M. F. Wilson, *J. Chem. Soc., Dalton Trans.*, 2610 (1972).
(13) R. F. Jameson and M. F. Wilson, *J. Chem. Soc., Dalton Trans.*, 2614 (1972).
(14) These conclusions differ from a claim that this complex contains four catecholate oxygen donors (W-L. Kwik, E. Purdy, and E. I. Stiefel, *J. Am. Chem. Soc.*, **96**, 1638 (1974)). They also propose that their complex II is kinetically inert, which is extremely unlikely for such a Cu(II) complex.
(15) J. R. Pilbrow, S. G. Carr, and T. D. Smith, *J. Chem. Soc. A*, 723 (1970); S. G. Carr, T. D. Smith, and J. R. Pilbrow, *ibid.*, 2569 (1971).
(16) H. Sigel, P. R. Huber, R. Griesser, and B. Prijs, *Inorg. Chem.*, **12**, 1198 (1973).
(17) J. E. Gorton and R. F. Jameson, *J. Chem. Soc., Dalton Trans.*, 307 (1972).
(18) R. F. Jameson and W. F. S. Neillie, *J. Inorg. Nucl. Chem.*, **27**, 2623 (1965).