Complex-forming Properties of Tyrosine Isomers with Transition Metal Ions

Tamás Kiss and Arthur Gergely *
Institute of Inorganic and Analytical Chemistry, Lajos Kossuth University, H-4010 Debrecen, Hungary

The thermodynamic quantities relating to the formation of the manganese(II), cobalt(II), nickel(II), copper(II), and zinc(II) complexes of tyrosine, *o*-tyrosine, and *m*-tyrosine have been determined pH-metrically and calorimetrically at 25 °C and at an ionic strength of 0.2 mol dm^{-3} (KCl). From the thermodynamic data and the spectral behaviour of the complexes formed, it was demonstrated that tyrosine and *m*-tyrosine do not interact directly *via* their phenolic hydroxy groups with the metal ions, even after deprotonation of these groups. However, this interaction occurs with *o*-tyrosine. The microconstants of the stepwise deprotonation processes taking place by various pathways for the tyrosine isomer (H_2A) complexes of type $[Zn(HA)_n]$ were also determined with a combined pH-spectrophotometric method. It was found that loss of proton from the complexes $[ZnHA]^+$, $[ZnH_2A_2]$, and $[ZnHA_2]^-$ occurs to practically the same extent from the phenolic hydroxy group and from the co-ordinated water molecule. However, the second phenolic hydroxy group deprotonates only to a slight extent in the complex $[ZnHA_2]^-$.

For a number of metalloproteins of biological importance it is assumed [1-3] that the phenolate oxygen in tyrosine acts as a metal ion binding site. Extensive investigations have therefore been made to establish the complex-forming properties of transition metal ions with tyrosine, with special regard to the participation of the phenolate group in complex formation. However, the reported thermodynamic data and spectral properties [4-10] and also the kinetic results [11] all point to the bidentate character of the ligand, in this way revealing considerable similarity with the results obtained for phenylalanine.[12] Co-ordination of phenolate has been concluded only in certain peptide complexes [13] and solid-phase complexes [14] of copper(II).

The phenolic hydroxy group in *o*-tyrosine is in a sterically more favourable position for co-ordination to the metal ion in its complexes. Letter and Bauman [9] made a comparative study of the copper(II) complexes of tyrosine, *m*-tyrosine, and *o*-tyrosine (4-, 3-, and 2-hydroxyphenylalanine respectively; H_2A). From the absorption band at 390 nm, indicative of the copper(II)–phenolate interaction for *o*-tyrosine, they assumed direct co-ordination of the phenolate oxygen at pH 10. A similar conclusion was reached by Bogges and Martin,[15] who examined the copper(II) complexes of various phenols and catechols, and ascribed the absorption at 370—460 nm to the phenolate→copper(II) electronic transition.

The phenolic hydroxy groups of the tyrosine isomers undergo dissociation at pH 9. At low pH the ligands form protonated complexes, which at higher pH deprotonate successively. Data on the equilibria of these processes have been published only by Pettit and Swash [10] (for tyrosine) and by Letter and Bauman.[9]

Because of the tendency of these metal ions, especially that of the zinc(II) ion, to form mixed hydroxo complexes, in the stepwise deprotonation processes of complexes $[M(HA)_n]$, one may assume not only the dissociation of the phenolic hydroxy groups but also the ionization of the water molecules in the co-ordination sphere. It means that in several cases species with the same stoicheiometric composition but with different bonding modes may be in equilibrium with each other. The equilibria concerning these processes can be characterized by microconstants.

Accordingly, we set out to make a thermodynamic study of the formation of the manganese(II), cobalt(II), nickel(II), copper(II), and zinc(II) complexes of the tyrosine isomers by means of pH-metric and calorimetric measurements over a wide pH range. From these data, and from the results of u.v. and visible spectral measurements in the cases of cobalt(II), nickel(II), copper(II), and zinc(II), conclusions can be drawn on the donor groups involved in the co-ordination in the various complexes.

Experimental

Chemicals and Experimental Conditions.—The DL-tyrosine used was a Reanal product of the highest analytical purity, while the DL-*o*-tyrosine and DL-*m*-tyrosine were Fluka products of 'Puriss' quality. Metal chloride stock solutions were prepared from compounds of the highest analytical purity; their concentrations were checked gravimetrically *via* the quinolin-8-olates.

The stability constants of the transition metal parent complexes of the ligands were determined by pH-metric titration of 25-cm^3 samples. The concentration of the ligands in the samples was 4×10^{-3} mol dm^{-3}, while the metal ion : ligand ratio was 1 : 1, 1 : 2, or 1 : 4. In every solution the ionic strength was adjusted to 0.2 mol dm^{-3} with KCl. The titrations were performed over the range pH 3—11 or up to the first appearance of a precipitate with carbonate-free KOH solution of known concentration (~0.2 mol dm^{-3}).

The enthalpy changes accompanying the complex-formation processes were determined calorimetrically under similar experimental conditions, with a continuous titration technique.[16]

The pH was measured with a Radiometer pHM 4 instrument, with G202B glass and K104 calomel electrodes. Calorimetric measurements were made with an LKB 8700-1 reaction and solution calorimeter. In all cases the temperature was 25 ± 0.1 °C.

In the spectrophotometric titrations in the u.v., visible, and near-i.r. range, the metal ion concentration was 2×10^{-3} mol dm^{-3}. The procedure used was reported previously.[17] A Beckman ACTA MIV double-beam recording spectrophotometer was used to measure light absorption.

Calculations.—The complexes formed can be characterized by the general equilibrium process (1). The stability constants of the species are given by equation (2).

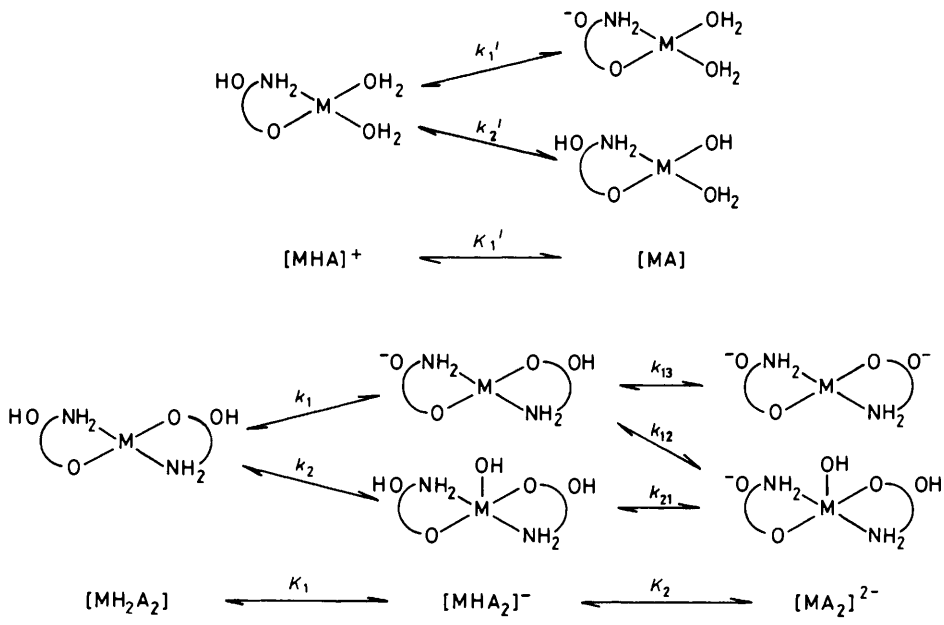

Figure 1. Micro-processes of deprotonation of species $[M(HA)_n]$ formed in the zinc(II)–tyrosine isomers systems

$$qM + pH + nA \rightleftharpoons M_qH_pA_n \quad (1)$$

$$\beta_{qpn} = [M_qH_pA_n]/[M]^q[H]^p[A]^n \quad (2)$$

The stability constants defined in this way were calculated from the titration curves as described earlier.[17,18]

As the first step in the determination of the metal complex formation microconstants, the molar absorbance of the complexes of given stoicheiometric composition (macro-species) were calculated at various wavelengths, by means of a non-linear least-squares curve-fitting method from the pH-spectrophotometric titration data and the stability constants. These absorbances arise as the sum of the molar absorbances (weighted according to mole ratios, α_i) of the complexes with the same stoicheiometric composition, but different bonding modes (micro-species): see Figure 1 and equation (3). By

$$\varepsilon^{macro} = \Sigma\alpha_i\varepsilon_i{}^{micro} \quad (3)$$

making various assumptions for the ε_i values, writing the equation for several wavelengths, and solving the resulting linear equation system with a least-squares method, the desired α_i values were obtained. The micro-constants could then be calculated from these by making use of the macro-constants.

The computations were made with a program written in the FORTRAN language.

Results and Discussion

The thermodynamic macro-quantities relating to the acid dissociation processes of the ligands, and the thermodynamic micro-quantities characterizing the overlapping deprotonation processes for the phenolic hydroxy group and the chain-terminal ammonium group, have been reported previously.[19]

The pH-metrically and calorimetrically determined thermodynamic data on the transition metal parent complexes of the three tyrosine isomers are listed in Tables 1 and 2.

Taking into account the differing ionic strengths, the tabulated data generally agree well with the earlier literature results.[4-10] However, we could not prove the existence of 1 : 3 complexes in the cobalt(II)–tyrosine systems, as was demonstrated by Pettit and Swash; [10] this can presumably be attributed to the fact that the highest metal ion : ligand ratio we used (1 : 4) was not sufficient for the formation of the low-stability 1 : 3 complexes in measurable concentration. In the manganese(II)–, cobalt(II)–, and zinc(II)–tyrosine systems, measurements could not be made at pH > 10, because of precipitate formation, even when the largest ligand excess was applied.

It is noteworthy that *o*-tyrosine in contrast to tyrosine and *m*-tyrosine forms only 1 : 1 and 1 : 2 complexes with nickel(II). The lack of the formation of 1 : 3 complexes can be considered as a result of the favourable steric arrangement of the three donor groups in *o*-tyrosine. This ligand is co-ordinated *via* all three donor groups at higher pH; the co-ordination sphere of the nickel(II) is therefore saturated in the 1 : 2 complex, and there is thus no possibility for co-ordination of a third ligand molecule.

For the three tyrosine isomers the complex formation begins on the amino acid side-chain. (Participation of the phenolate group in the co-ordination for *o*-tyrosine is to be expected only at pH > 8.[9]) Consequently, the ligand HA^- containing the protonated phenolic hydroxy group may be regarded as the complex-forming species in the parent complexes of type $[M(HA)_n]$ (M = Mn^{II}, Co^{II}, Ni^{II}, Cu^{II}, or Zn^{II}). The thermodynamic quantities were therefore calculated for complex formation corresponding to the processes $M + nHA \rightleftharpoons [M(HA)_n]$, and for deprotonation of the species $[M(HA)_n]$. These derived data were obtained in accordance with the actual conditions with the use of the micro-quantities pk_2 and Δh_2 for the dissociation of the ammonium group.[19] These values were calculated from the overall thermodynamic data in Tables 1 and 2, and are given in Tables 3 and 4.

The data in Tables 3 and 4 reveal that the thermodynamic quantities for the formation of the complexes of type $[M(HA)_n]$ for a given metal ion do not differ significantly for the three different ligands. When compared to the corresponding data for phenylalanine [4] the constants point to a slightly stronger interaction, in accordance with the electron-releasing properties of the phenolic hydroxy group. In the range of formation of the complexes $[M(HA)_n]$, the visible and near-i.r. spectra

Table 1. Thermodynamic data on metal complexes of the tyrosine isomers at 25 °C and I = 0.2 mol dm^{-3} (KCl) *

Species	Manganese(II) log β	Manganese(II) ΔH	Manganese(II) ΔS	Cobalt(II) log β	Cobalt(II) ΔH	Cobalt(II) ΔS	Copper(II) log β	Copper(II) ΔH	Copper(II) ΔS	Zinc(II) log β	Zinc(II) ΔH	Zinc(II) ΔS
(*a*) *o*-Tyrosine												
$[MHA]^+$	13.47	−26	170	14.73	−28	188	18.58	−43.8	208	14.59	−32.2	178
[MA]	—	—	—	—	—	—	—	—	—	6.49	2	131
$[MH_2A_2]$	—	—	—	29.0	−56	367	36.27	−98.2	364	29.99	−64.2	358
$[MHA_2]^-$	17.8	−29	243	20.4	−25	306	27.16	−75.3	267	21.33	−21	337
$[MA_2]^{2-}$	7.7	−5	130	10.5	−1	197	17.19	−50.5	159	11.78	12	266
(*b*) *m*-Tyrosine												
$[MHA]^+$	12.72	−26	156	13.89	−28	171	17.71	−45.6	186	14.10	−30.7	167
[MA]	—	—	—	—	—	—	—	—	—	5.76	7	135
$[MH_2A_2]$	24.6	−51	300	27.2	−57	329	34.53	−95.9	339	28.11	−64.6	321
$[MHA_2]^-$	15.7	−28	206	18.2	−33	237	25.32	−73.7	237	19.11	−35	248
$[MA_2]^{2-}$	5.6	13	151	8.2	4	170	15.42	−47.5	136	9.75	3	224
(*c*) Tyrosine												
$[MHA]^+$	13.08	−24	170	14.18	−27	181	17.79	−44.5	191	14.22	−31.4	167
[MA]	—	—	—	—	—	—	—	—	—	6.08	6	136
$[MH_2A_2]$	25.7	−48	331	28.1	−56	350	34.64	−97.7	335	28.15	−67.0	314
$[MHA_2]^-$	16.8	−25	237	19.1	−31	261	25.32	−74.4	235	19.26	−36	248
$[MA_2]^{2-}$	6.7	15	178	9.1	11	211	15.26	−50.0	124	9.97	4	204

* Uncertainty in ΔH ± 0.5—1.0 kJ mol^{-1} and in ΔS ± 1—4 J K^{-1} mol^{-1}.

Table 2. Thermodynamic data on the nickel(II) complexes of the tyrosine isomers at 25 °C and I = 0.2 mol dm^{-3} (KCl) *

Species	*o*-Tyrosine log β	*o*-Tyrosine ΔH	*o*-Tyrosine ΔS	*m*-Tyrosine log β	*m*-Tyrosine ΔH	*m*-Tyrosine ΔS	Tyrosine log β	Tyrosine ΔH	Tyrosine ΔS
$[MHA]^+$	15.69	−35.4	181	15.02	−37.4	162	15.00	−35.9	166
$[MH_2A_2]$	30.72	−74.7	337	29.38	−77.1	303	29.35	−74.8	310
$[MHA_2]^-$	21.81	−52.7	240	20.27	−55.1	203	20.03	−50.8	213
$[MA_2]^{2-}$	11.81	−28.9	129	10.53	−26.1	114	10.11	−24.9	110
$[MH_3A_3]^-$	—	—	—	42.54	−112.9	435	42.36	−114.0	428
$[MH_2A_3]^{2-}$	—	—	—	33.52	−94.3	325	33.32	−95.6	317
$[MHA_3]^{3-}$	—	—	—	24.00	−69.9	225	23.73	−71.8	213
$[MA_3]^{4-}$	—	—	—	13.83	−46.1	110	13.67	−46.5	105

* Uncertainty in ΔH ± 0.5 kJ mol^{-1} and in ΔS ± 1 J K^{-1} mol^{-1}.

of the cobalt(II)–, nickel(II)–, and copper(II)–tyrosine isomer systems agree with those of the corresponding metal complexes of alanine. In consequence a direct interaction between the phenolic hydroxy group and the metal ion can be excluded for all three tyrosine isomers.

Even in the pH range of deprotonation of the complexes of type $[M(HA)_n]$, tyrosine and *m*-tyrosine exhibit spectral behaviour that is not significantly different from that for the complexes of simple amino acids. Although there is no direct evidence of this for the manganese(II) and zinc(II) complexes, there is no reason why these metal ions should behave differently from the cobalt(II), nickel(II), and copper(II) ions, *i.e.* the deprotonated phenolate group does not participate in the co-ordination either in the case of manganese(II) or zinc(II).

In the cases of nickel(II) and copper(II), the thermodynamic quantities for the deprotonation of the complexes are also in agreement with the micro-quantities for the dissociation of the ligand (pk_1, Δh_1, and Δs_1).[19] It may be assumed that the acidity of the phenolic hydroxy group in tyrosine and *m*-tyrosine in the metal complexes is not different from that in the free ligand. If this condition is met, the statistical considerations hold for the ratio of the stepwise deprotonation constants in the molecule containing two or three donor groups of the same acidity (providing that there is no interaction at all between the donor groups): equations (4)—(7) (charges are omitted from subscripts for clarity).

$$K_{MH_2A_2} = 2k_1;\ K_{MHA_2} = \tfrac{1}{2}k_1 \quad (4)$$

$$K_{MH_2A_2}/K_{MHA_2} = 4 \quad (5)$$

$$K_{MH_3A_3} = 3k_1;\ K_{MH_3A_3}K_{MH_2A_3} = 3k_1^2;\ K_{MHA_3} = \tfrac{1}{3}k_1;\ K_{MH_2A_3} = k_1 \quad (6)$$

$$K_{MH_3A_3}/K_{MHA_3} = 9;\ K_{MH_3A_3}/K_{MH_2A_3} = 3;\ K_{MH_2A_3}/K_{MHA_3} = 3 \quad (7)$$

If the data in Tables 3 and 4 are compared with the pk_1 values for the tyrosine isomers (tyrosine, 9.54; *m*-tyrosine, 9.45),[19] it is found that equations (4)—(7) hold approximately for the tyrosine and *m*-tyrosine systems with nickel(II) and copper(II). The deprotonation enthalpy and entropy changes of the complexes also agree roughly with the micro-quantities for dissociation of the ligand (tyrosine, Δh_1 = 24.6 kJ mol^{-1}, Δs_1 = 99 J K^{-1} mol^{-1}; *m*-tyrosine, Δh_1 = 24.0 kJ mol^{-1}, Δs_1 = 100 J K^{-1} mol^{-1}).[19] At the same time, the thermodynamic data for the manganese(II), cobalt(II), and particularly the zinc(II) complexes exhibit differences, an indication

Table 3. Derived equilibrium constants of the metal complexes of the tyrosine isomers at 25 °C and $I = 0.2$ mol dm^{-3} (KCl)

Process	H_2A	Manganese(II) log K	Manganese(II) ΔH^a	Manganese(II) ΔS^b	Cobalt(II) log K	Cobalt(II) ΔH^a	Cobalt(II) ΔS^b	Copper(II) log K	Copper(II) ΔH^a	Copper(II) ΔS^b	Zinc(II) log K	Zinc(II) ΔH^a	Zinc(II) ΔS^b
$M^{2+} + HA^- \rightleftharpoons [MHA]^+$	*o*-tyrosine	2.73	−3	42	3.99	−5	60	7.84	−21.0	79	4.21	−9.3	49
	m-tyrosine	2.85	−4	42	4.02	−6	57	7.84	−23.5	71	4.23	−8.6	52
	tyrosine	3.14	−3	50	4.24	−5	64	7.85	−22.0	76	4.28	−8.9	55
$[MHA]^+ + HA^- \rightleftharpoons$ $[MH_2A_2]$	*o*-tyrosine	—	—	—	3.54	−6	48	6.95	−31.6	27	4.30	−9.1	52
	m-tyrosine	2.1	−2	30	3.45	−6	46	6.95	−28.2	38	4.14	−11.8	39
	tyrosine	2.7	−2	45	4.0	−6	56	6.91	−30.7	29	3.99	−13.0	33
$[MHA]^+ \rightleftharpoons [MA] + H^+$	*o*-tyrosine	—	—	—	—	—	—	—	—	—	−8.46	25.0	−78
	m-tyrosine	—	—	—	—	—	—	—	—	—	−8.34	38.1	−32
	tyrosine	—	—	—	—	—	—	—	—	—	−8.14	37.5	−30
$[MH_2A_2] \rightleftharpoons [MHA_2]^- +$ H^+	*o*-tyrosine	—	—	—	−8.6	31	−60	−9.11	22.9	−97	−8.66	43	−21
	m-tyrosine	−8.9	23	−94	−9.0	25	−88	−9.21	22.2	−100	±9.0	29.6	−73
	tyrosine	−8.9	23	−93	−9.0	25	−88	−9.32	23.3	−100	−8.89	31	−66
$[MHA_2]^- \rightleftharpoons [MA_2]^{2-} +$ H^+	*o*-tyrosine	−10.1	25	−109	−9.9	24	−109	−9.97	24.8	−107	−9.59	33	−73
	m-tyrosine	−10.2	41	−56	−10.1	36	−72	−9.90	26.2	−101	−9.36	38	−24
	tyrosine	−10.1	40	−59	−10.0	42	−50	−10.06	24.4	−111	−9.29	40	−43

[a] In kJ mol^{-1}. [b] In J K^{-1} mol^{-1}.

Table 4. Derived equilibrium constants of the nickel(II) complexes of the tyrosine isomers at 25 °C and $I = 0.2$ mol dm^{-3} (KCl)

Process	H_2A	log K	ΔH/ kJ mol^{-1}	ΔS/ J K^{-1} mol^{-1}
$M^{2+} + HA^- \rightleftharpoons [MHA]^+$	*o*-tyrosine	4.95	−12.6	52
	m-tyrosine	5.15	−15.3	47
	tyrosine	5.06	−13.4	52
$[MHA]^+ + HA^- \rightleftharpoons [MH_2A_2]$	*o*-tyrosine	4.29	−16.4	27
	m-tyrosine	4.49	−17.6	27
	tyrosine	4.44	−16.4	30
$[MH_2A_2] + HA^- \rightleftharpoons [MH_3A_3]^-$	*o*-tyrosine	—	—	—
	m-tyrosine	3.29	−13.7	17
	tyrosine	3.03	−16.6	2
$[MH_2A_2] \rightleftharpoons [MHA_2]^- + H^+$	*o*-tyrosine	−8.94	22.0	−97
	m-tyrosine	−9.11	22.0	−100
	tyrosine	−9.32	24.0	−98
$[MHA_2]^- \rightleftharpoons [MA_2]^{2-} + H^+$	*o*-tyrosine	−10.00	23.8	−111
	m-tyrosine	−9.74	29.0	−89
	tyrosine	−9.92	25.9	−103
$[MH_3A_3]^- \rightleftharpoons [MH_2A_3]^{2-} + H^+$	*o*-tyrosine	—	—	—
	m-tyrosine	−9.02	18.6	−110
	tyrosine	−9.04	18.3	−112
$[MH_2A_3]^{2-} \rightleftharpoons [MHA_3]^{3-} + H^+$	*o*-tyrosine	—	—	—
	m-tyrosine	−9.52	24.4	−100
	tyrosine	−9.59	23.8	−104
$[MHA_3]^{3-} \rightleftharpoons [MA_3]^{4-} + H^+$	*o*-tyrosine	—	—	—
	m-tyrosine	−10.17	23.8	−115
	tyrosine	−10.06	25.3	−108

that it is necessary to take into account the joint occurrence of dissociation of the phenolic hydroxy group and the co-ordinated water molecule, *i.e.* the formation of a mixed-hydroxo complex.

The deprotonation of the complexes $[M(HA)_n]$ of the tyrosine isomers with manganese(II), cobalt(II), and zinc(II) can occur by the different pathways shown in Figure 1. Hydroxo complexes with different stoicheiometries (presumably polynuclear) are formed at pH > 10.5; the equilibrium conditions of these have not yet been clarified. It was therefore necessary to omit the further steps of the micro-processes in Figure 1.

In the case of the complexes $[Zn(HA)_n]$, where the tendency of hydroxo complex formation is the most pronounced, the micro-constants for these part-processes were determined by following one of the processes *via* the u.v. band of the phenolate group. Evaluation of the resulting pH-spectrophotometric titration curves led to the molar absorbance values for the macro-species. From the general equation these can be described by equations (8)—(10).

$$\varepsilon_{MA} = \alpha_1'\varepsilon_1' + \alpha_2'\varepsilon_2' \quad (8)$$

$$\varepsilon_{MHA_2} = \alpha_1\varepsilon_1 + \alpha_2\varepsilon_2 \quad (9)$$

$$\varepsilon_{MA_2} = \alpha_3\varepsilon_3 + \alpha_4\varepsilon_4 \quad (10)$$

The following considerations may be given with respect to the ε_i values of the micro-species. (*a*) On evaluating the pH-spectrophotometric titration curves of the systems of copper(II) and nickel(II) with tyrosine and *m*-tyrosine, it was found that the molar absorbance values of the phenolic hydroxy and the phenolate groups of the ligand are the same, independently of whether the ligand is co-ordinated *via* its side-chain donor atoms to the metal ion or not. In the case of these metal ions, parallel hydroxo complex-formation

Table 5. Macro-and micro-constants for the deprotonation processes of the complexes $[Zn(HA)_n]$ of *m*-tyrosine and tyrosine at 25 °C and $I = 0.2$ mol dm^{-3} (KCl)

Complex	Constant *	*m*-Tyrosine	Tyrosine
	pK_1'	8.34	8.14
$[ZnHA]^+$	α_{O-}	0.40 ± 0.05	0.33 ± 0.05
	α_{OH-}	0.60 ± 0.05	0.62 ± 0.05
	pk_1'	8.74 ± 0.06	8.56 ± 0.06
	pk_2'	8.56 ± 0.04	8.35 ± 0.03
	pK_1	9.00	8.89
$[ZnH_2A_2]$	α_{O-}	0.55 ± 0.06	0.49 ± 0.06
	α_{OH-}	0.45 ± 0.06	0.51 ± 0.06
	pk_1	9.3 ± 0.1	9.2 ± 0.1
	pk_2	9.35 ± 0.07	9.18 ± 0.07
	pK_2	9.36	9.29
$[ZnHA_2]^-$	α_3	0.19 ± 0.04	0.13 ± 0.04
	α_4	0.81 ± 0.04	0.87 ± 0.04
	pk_{21}	9.20 ± 0.07	9.06 ± 0.05
	pk_{12}	9.3 ± 0.1	9.1 ± 0.1
	pk_{13}	9.9 ± 0.1	9.9 ± 0.1

* α_{O-} = mole ratio of the species containing phenolate group; α_{OH-} = mole ratio of the species containing hydroxy group.

processes need not be taken into account: equations (11) and (12). This agrees essentially with the general assumption made

$$\varepsilon_{MHA} = \varepsilon_{OH};\ \varepsilon_{MH_2A_2} = 2\varepsilon_{OH} \quad (11)$$

$$\varepsilon_1' = \varepsilon_{O-};\ \varepsilon_1 = \varepsilon_{O-} + \varepsilon_{OH};\ \varepsilon_3 = 2\varepsilon_{O-} \quad (12)$$

for proton complexes, that the value of a molar parameter of a given group is independent of the state of protonation of the other group.[20] For the complexes of *o*-tyrosine, where direct co-ordination of the phenolate group must also be considered, this finding no longer holds. Consequently, it was not possible to 'resolve' in this way the macro-constants obtained for the zinc(II)–*o*-tyrosine system.

(*b*) It may be assumed further that the value of the molar absorbance of the phenolic hydroxy or the phenolate group in the metal complexes is not influenced by the ionization of the water molecule bound in the co-ordination sphere of the metal ion. This appears permissible if the findings mentioned above are taken into consideration; this gives equations (13) and (14). Substitution of equations (11)—(14) into (8)—(10) leads to equations (15)—(17).

$$\varepsilon_2' = \varepsilon_{MHA} = \varepsilon_{OH} \quad (13)$$

$$\varepsilon_2 = \varepsilon_{MH_2A_2} = 2\varepsilon_{OH};\ \varepsilon_4 = \varepsilon_1 = \varepsilon_{O-} + \varepsilon_{OH} \quad (14)$$

$$\varepsilon_{MA} = \alpha_1'\varepsilon_{O-} + (1 - \alpha_1')\varepsilon_{OH} \quad (15)$$

$$\varepsilon_{MHA_2} = \alpha_1(\varepsilon_{O-} + \varepsilon_{OH}) + (1 - \alpha_1)2\varepsilon_{OH} \quad (16)$$

$$\varepsilon_{MA_2} = \alpha_3 \cdot 2\varepsilon_{O-} + (1 - \alpha_3)(\varepsilon_{O-} + \varepsilon_{OH}) \quad (17)$$

With the values of ε_{O-} and ε_{OH} being known, equations (15)—(17) were written for several wavelengths and solved by the least-squares method, and the α_i values were calculated. The micro-constants were obtained from the α values and the known values of K_1', K_1, and K_2, *via* equations (18)—(21).

$$k_1' = \alpha_1'K_1';\ k_2' = \alpha_2'K_1' \quad (18)$$

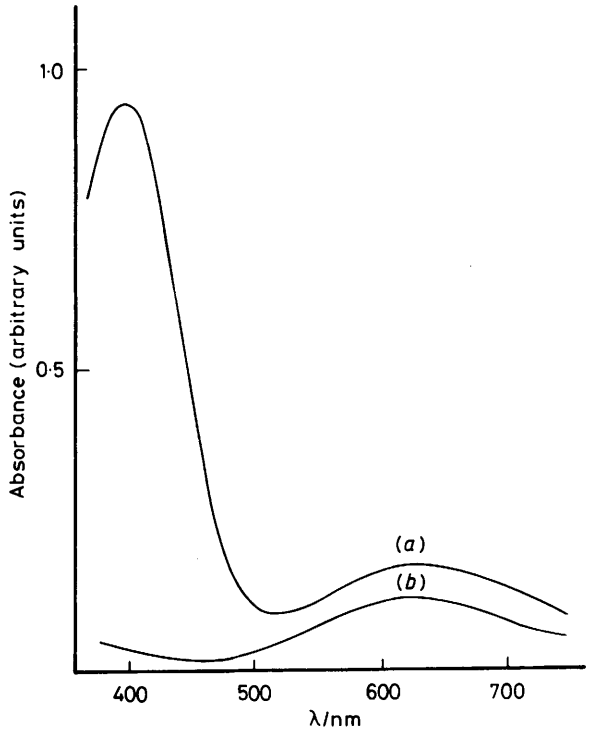

Figure 2. Visible spectra of the copper(II) complexes of *o*-tyrosine and tyrosine: (*a*) Cu^{II}–*o*-tyrosine (1 : 2), pH 11.0; (*b*) Cu^{II}–tyrosine (1 : 2), pH 11.0

$$k_1 = \alpha_1K_1;\ k_2 = \alpha_2K_1 \quad (19)$$

$$\alpha_3K_1K_2 = k_1k_{13} = \alpha_1K_1k_{13};\ k_{13} = \alpha_3/\alpha_1K_2 \quad (20)$$

$$\alpha_4K_1K_2 = k_2k_{21} = \alpha_2K_1k_{21};\ k_{21} = \alpha_4/\alpha_2K_2 \quad (21)$$

The α_i values and the calculated micro-constants for the deprotonation processes of the complexes $[ZnHA]^+$ and $[ZnH_2A_2]$ of tyrosine and *m*-tyrosine are listed in Table 5.

The data in Table 5 reveal that, in accordance with expectations, the micro-constants for tyrosine and *m*-tyrosine are approximately the same. For both ligands the loss of proton from the complexes $[ZnHA]^+$, $[ZnH_2A_2]$, and $[ZnHA_2]^-$ takes place in practically the same proportions from the phenolic hydroxy group and from the co-ordinated water molecule. However, the second phenolic hydroxy group deprotonated only to a slight extent in the complex $[ZnHA_2]^-$.

In the complex $[ZnHA]^+$ the acidity of the phenolic hydroxy group is slightly larger than in the free ligand (pk_{O-} = 9.45 for *m*-tyrosine and 9.54 for tyrosine).[19] This presumably results from the electron shift due to the co-ordination of the metal ion and extending to the aromatic ring too. At the same time, the stepwise deprotonation constants (pk_1 and pk_{13}) for the two phenolic hydroxy groups in the complex $[ZnH_2A_2]$ agree within 0.1—0.2 log unit with the corresponding data for the copper(II) and nickel(II) complexes (see Tables 3 and 4). It also holds for the pk_1 and pk_{13} values that their difference is *ca.* 0.6, which corresponds to the statistical case for the stepwise constants of donor groups with the same acidity.

The micro-constants relating to the same acid groups are practically independent of the protonation state of the other group ($pk_1 \sim pk_{21}$ and $pk_2 \sim pk_{12}$); *i.e.* the several atomic distances between them means that they can be regarded as groups well separated from each other.

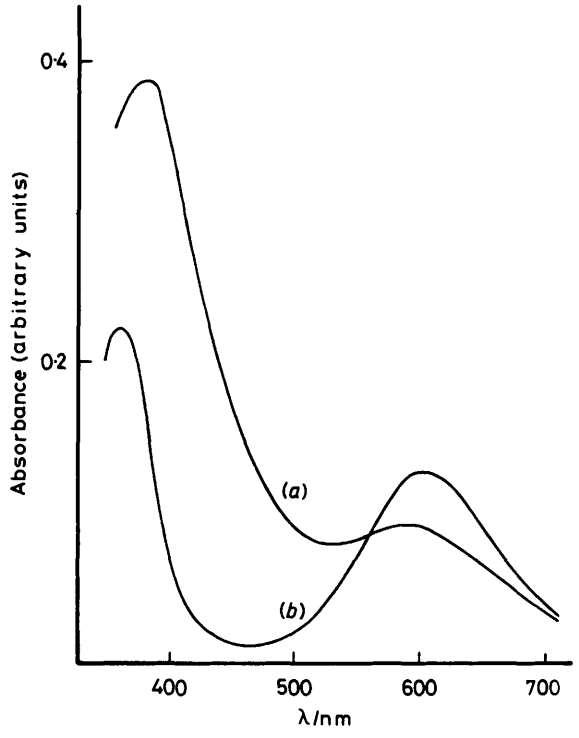

Figure 3. Visible spectra of the nickel(II) complexes of *o*-tyrosine and tyrosine: (*a*) Ni^{II}–*o*-tyrosine (1 : 3), pH 11.0; (*b*) Ni^{II}–tyrosine (1 : 3), pH 10.6

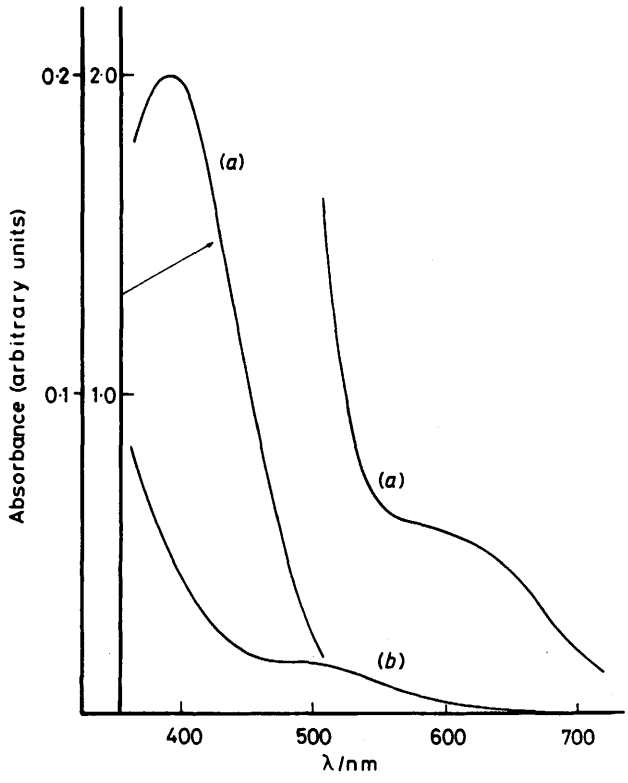

Figure 4. Visible spectra of the cobalt(II) complexes of *o*-tyrosine and tyrosine after standing in air: (*a*) Co^{II}–*o*-tyrosine (1 : 2), pH 10.5; (*b*) Co^{II}–tyrosine (1 : 5), pH 10.8

In contrast with tyrosine and *m*-tyrosine, for the systems of cobalt(II), nickel(II), and copper(II) with *o*-tyrosine the deprotonation of the complex $[M(HA)_n]$ is accompanied by an appreciable spectral change, as may be seen in Figures 2—4. That is, the direct participation of the phenolate oxygen in the co-ordination is probable.

The medium-intensity ($\varepsilon \sim 550$ dm^3 mol^{-1} cm^{-1}) band appearing in the copper(II)–*o*-tyrosine system at 400 nm at pH > 8 is clearly a charge-transfer band characteristic of the copper(II)–phenolate interaction.[9,15]

In the case of nickel(II)–*o*-tyrosine the spectral change is not so marked, but if O_h symmetry is assumed for the complexes, the Racah parameter calculated for the nickel(II)–*o*-tyrosine complex is substantially smaller than the values calculated for those of the tyrosine and *m*-tyrosine complexes; this can be interpreted by a more extensive electron transfer from the ligand to the metal ion, *i.e.* by the co-ordination of the phenolate group.[21]

When atmospheric oxygen was completely excluded, no essential spectral change was observed in response to an increase in pH for cobalt(II)–*o*-tyrosine. However, it was noteworthy that if the solution with pH ~ 10.5 was left to stand in air it became yellowish green, which resulted in the appearance of a high-intensity ($\varepsilon \sim 2\,900$ dm^3 mol^{-1} cm^{-1}) band at 390 nm in the spectrum. Spectral behaviour of this nature is also displayed by certain tyrosine-containing cobalt(III) proteins, and is ascribed to a phenolate→cobalt(III) charge transfer.[22] The cobalt(III) complexes of tyrosine and *m*-tyrosine do not exhibit such behaviour. Although no direct evidence is available, it may be assumed that at higher pH there is also a metal–phenolate interaction in the cobalt(II)–*o*-tyrosine complexes. This is supported by the experimental observation that precipitate formation in the cobalt(II)–*o*-tyrosine system begins only at a pH value one unit higher, at pH 10.5. This can be explained in that the co-ordination of all three of the *o*-tyrosine donor groups means that hydroxo complex formation and hydrolysis of the metal ion are less favoured than for the cobalt(II) complexes of the other tyrosine isomers, which involve only N,O-co-ordination.

Table 6. Thermodynamic quantities for the formation of complexes $[MA_2]^{2-}$ of the tyrosine isomers at 25 °C and I = 0.2 mol dm^{-3} (KCl)

Process	H_2A	log β	ΔH/ kJ mol^{-1}	ΔS/ J K^{-1} mol^{-1}
$Ni^{2+} + 2A^{2-} \rightleftharpoons [NiA_2]^{2-}$	*o*-tyrosine	11.81	−28.9	129
	m-tyrosine	10.53	−26.1	114
	tyrosine	10.11	−24.9	110
$Cu^{2+} + 2A^{2-} \rightleftharpoons [CuA_2]^{2-}$	*o*-tyrosine	17.19	−50.5	159
	m-tyrosine	15.42	−47.5	136
	tyrosine	15.26	−50.0	124

It is noteworthy that the probable metal–phenolate interaction for *o*-tyrosine is barely manifested in the enthalpy data. Table 6 gives the thermodynamic data for the process $M^{2+} + 2A^{2-} \rightleftharpoons [MA_2]^{2-}$ for the nickel(II) and copper(II) complexes of the tyrosine isomers. In these systems the disturbing hydroxo complex-formation processes need not be taken into consideration.

The literature data [12] suggest that the metal–phenolate interaction should mean a contribution of 10—20 kJ mol^{-1} for the metal ions studied. In the case of *o*-tyrosine the observed value is only 1—4 kJ mol^{-1}, which points to an essentially weaker interaction and a larger metal–oxygen distance. This can be explained by the co-ordination of the amino nitrogen and the phenolate oxygen giving rise to a seven-membered

chelate ring. In connection with the complex $[CuA_2]^{2-}$, it is also probable that, even following deprotonation, the amino nitrogen and the carboxylate oxygen occupy the equatorial sites around the copper(II), and the phenolate oxygen is co-ordinated only at the more distant axial sites. This is supported by the findings of Garnier and Tosi [3] in their interpretation of the spectral results relating to complexes of the type copper(II)–poly(L-tyrosine); on the basis of the position of the charge-transfer band (390 nm) and its medium intensity ($\varepsilon \sim 600$ dm^3 mol^{-1} cm^{-1}), they suggest a longer bond distance for the copper(II)–phenolate interaction. Accordingly, they assume the axial co-ordination of the phenolate. In accordance with the above, the larger entropy change accompanying the complex formation also points to the different degree of hydration of the species.

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