Potentiometric and Spectrophotometric Studies of the Copper(II) Complexes of Methyldopa, Methyltyrosine, and Catechol in Aqueous Solution

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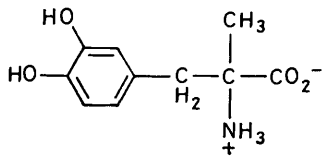
The system of copper(II) ions in aqueous solution in the presence of L-3-(3′,4′-dihydroxyphenyl)-2-methylalanine (methyldopa), (1), at 25 °C and I = 0.150 mol dm^{-3} (Na)[ClO_4] has been characterized using glass-electrode potentiometry. Both mononuclear and oligonuclear complexes are found to occur, the former including a series of successively deprotonated species in the titrations at higher ligand-to-metal ratios, the latter including a cyclic dimer in 1 : 1 ligand-to-metal solutions. Interpretation of the results was facilitated by (*i*) glass-electrode potentiometric measurements on DL-3-(3′-hydroxyphenyl)-2-methylalanine (methyltyrosine) and catechol in the presence of copper(II) ions, (*ii*) visible spectrophotometry of solutions containing copper(II), (1), or alanine or catechol at various pH, and (*iii*) ^{1}H n.m.r. measurements of (1) in ^2H_2O solutions. Formation constants are given for three proton and 11 copper complexes of (1), three proton and four copper complexes of methyltyrosine, and one proton and two copper complexes of catechol.

L-3-(3′,4′-DIHYDROXYPHENYL)-2-METHYLALANINE (methyldopa), (1), has become important as a therapeutical for treating hypertension.[1-4] Although the mechanism of its clinical action is not fully understood [5-13] there is reason to suspect that the interaction of this compound with metal ions is significant. This is based, for example, on reports that copper(II)-containing enzymes catalyse oxidative transformations of catecholamines [14,15] and that transport across the blood–brain barrier of the related compound 3-(3′,4′-dihydroxyphenyl)-L-alanine (dopa) is facilitated by chelation to copper(II) and zinc(II) ions.[16]

A current fundamental approach to investigating the role of metal ions in biological fluids involves computer simulation of the equilibria between these and low-molecular-weight ligands including those which occur naturally and those administered as medications.[17-19] The data required for such studies comprise the specific stoicheiometric identities of the metal complexes that co-exist in equilibrium together with their formation



(1)

constants. In order to advance these investigations, the work described in the present paper on aqueous solution equilibria of the methyldopa–copper(II) system was undertaken.

Although reports of earlier work on the metal complexes of methyldopa are sparse [13] there are several publications concerning dopa and dopa–copper(II) complexes.[16,20-23] One might expect methyldopa to form a set of copper(II) complexes with the same stoicheiometry as dopa–copper(II) but possibly with slightly different values of the corresponding formation constants. There are, however, differences of opinion expressed in the literature concerning the identities of the complexes formed. This controversy, no doubt, is partially ascribable to the complicated co-ordination chemical behaviour to be expected from the existence of two potential metal-chelating sites on the ligand. Gorton and Jameson [20] envisaged mononuclear complexes involving exclusively chelation at the alanine end of the dopa molecule (*O,N* co-ordination) at lower pH values and exclusively catechol-type *O,O* co-ordination in alkaline solutions. They postulated, in addition, the formation of polymeric species in which both *O,N*– and *O,O*–metal bonds are necessarily formed. By contrast, Kwik *et al.*[24] and Gergely and Kiss [23] found mononuclear mixed complexes with both types of chelation in the intermediate pH range, particularly at physiological pH values.

In order to establish an appropriate chemical model to describe aqueous solutions containing methyldopa and copper(II) ions we used potentiometry as our main experimental approach. In seeking postulated models to fit the data, not only did we make use of the above conclusions reported for the dopa–copper(II) system but we also investigated by potentiometry the complexation equilibria of copper(II) with the related compounds DL-3-(3′-hydroxyphenyl)-2-methylalanine (methyltyrosine) and catechol (benzene-1,2-diol). With the former *O,O* chelation is, of course, impossible. To facilitate, further, our model fitting we investigated methyldopa–, catechol–, and alanine–copper(II) solutions by visible spectrophotometry. N.m.r. measurements were made with a view to elucidating the sites of protonation on methyldopa anions.

EXPERIMENTAL

Chemicals.—Methyldopa (Fluka, Puriss) (Found: C, 50.3; H, 6.75; N, 6.0. Calc. for $C_{10}H_{13}NO_4 \cdot 1.5H_2O$: C, 50.4; H, 6.70; N, 5.9%) and methyltyrosine (Sigma Chemical Co.) (Found: C, 61.3; H, 6.70; N, 7.25. Calc. for $C_{10}H_{13}NO_3$: C, 61.5; H, 6.70; N, 7.20%) were dried and used without further purification. Catechol (Fluka, Puriss) was triply sublimed (Found: C, 65.35; H, 5.5. Calc. for $C_6H_6O_2$: C, 65.3; H, 5.50%). Alanine (Sigma) (Found: C, 40.4; H, 8.00; N, 15.75. Calc. for $C_3H_7NO_2$: C, 40.45; H, 7.90; N, 15.7%) was used directly for the

visible spectrophotometry. Carbonate-free solutions prepared from copper(II) perchlorate (G. F. Smith Chemical Co.) were analyzed for metal by titration against Na_2-[H_2edta](B.D.H.) * and for mineral acid by Gran titration.[25] The preparation and standardization of perchloric acid, sodium hydroxide, and sodium perchlorate solutions were as described in ref. 26. The sodium perchlorate solutions were assayed not only by flame emission spectrophotometry [26] but also gravimetrically after evaporation to dryness in a vacuum oven. Nitrogen (Afrox), obtained from a high-purity cylinder, was passed through concentrated potassium hydroxide, 15% alkaline pyrogallol, 1% alkaline sodium 1,2-naphthoquinone-4-sulphonate in which lead wool was immersed, acidic chromium(II)–chromium(III) sulphate in contact with an excess of zinc amalgam, an empty wash bottle, distilled water, and ionic background solution thermostatted at 25 °C. After it had passed through the titration vessel it was released to the atmosphere *via* a trap containing the ionic background solution to prevent back diffusion of oxygen and carbon dioxide.

Potentiometric Measurements.—Solutions containing ligand, copper(II) perchlorate (zero concentration for the protonation titrations), and perchloric acid or sodium hydroxide were titrated against either Na[OH] or $HClO_4$ in a cell as shown in the diagram. The ionic strength was maintained at 0.150 mol dm^{-3} $[ClO_4]^-$ by added sodium perchlorate. The 0.100 mol dm^{-3} sodium chloride and 0.150 mol dm^{-3} sodium perchlorate arms of the salt bridge terminated at sintered glass discs which dipped into the more concentrated and hence more dense 3 mol dm^{-3} NaCl. In consequence of the latter's being at a lower height than the two connecting solutions, diffusion potentials were kept to a minimum. All liquid junctions, being reproducible, were renewed in between titrations. E.m.f. readings were taken on a Radiometer PHM64 Research pH meter. The electrodes were calibrated against perchloric acid and acetic acid–sodium acetate solutions of accurately known hydrogen-ion concentration and which contained Na[ClO_4] to maintain the ionic strength at 0.150 mol dm^{-3}. Burettes (Metrohm, Cat. no. E274) and pipettes were calibrated. A purified nitrogen atmosphere was maintained in the titration vessel during titrations.

Thermostatted calomel electrode with 0.1 mol dm^{-3} NaCl	0.100 mol dm^{-3} NaCl	3 mol dm^{-3} NaCl	0.150 mol dm^{-3} Na[ClO_4]	Experimental solution in Metrohm thermostatted titration vessel (Cat.no. EA876-20)	Metrohm glass electrode (Cat.no.EA109)

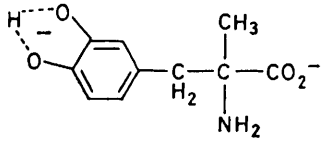
Computations.—Titration data were processed initially by ZPLOT [27] in order to obtain formation curves ($\bar{Z}_{obs.}$ against p*a*) and subsequently by MINIQUAD [28] in order to obtain best-fitting chemical models and refined formation constants (β_{pqr} †). By applying the formation constants to PSEUDOPLOT,[29] theoretical formation curves ($\bar{Z}_{calc.}$ against p*a*) were regenerated so as to facilitate hypothesis testing. The constants of the chemical model eventually obtained were processed by HALTAFALL [30] to give profiles of the distribution of each complex species *versus* pH applicable to various concentrations of ligand and metal ion. All the programs were run in FORTRAN V on the Univac 1106 computer at the University of Cape Town.

Visible Spectrophotometry.—Spectra of solutions containing ligand and copper(II) perchlorate prepared and maintained under purified nitrogen, at various pH values in the range 4—10 and with the ionic strength maintained at 0.150 mol dm^{-3} with respect to $[ClO_4]^-$, were run on a Beckman DK2A spectrophotometer using water as a reference.

N.M.R. Measurements.—Spectra were recorded at 90 MHz on a Bruker WH-90DS spectrometer operating in the Fourier-transform mode. Ligand solutions (0.05 mol dm^{-3}) in ^2H_2O were adjusted to the required pH with Na[O^2H] or ^2HCl. The pH of the solutions was calculated from pD = pH + 0.4. The sodium salt of 3-trimethylsilylpropanesulphonic acid was used as internal reference.

RESULTS AND DISCUSSION

Protonations.—Several titrations of each ligand, initially in the presence of perchloric acid, were carried out up to pH *ca.* 11.6, the total ligand concentration ranging from 0.008 to 0.037 mol dm^{-3} in the case of methyldopa and being fixed at *ca.* 0.01 mol dm^{-3} with both methyltyrosine and catechol. No attempt was made to determine the first protonation constant of either the trianion of methyldopa or the dianion of catechol (p*K* 13—14) because at pH >12 the ionic strength could not be maintained sensibly constant at 0.15 mol dm^{-3}. Accordingly, in the present paper, the ligand species, A, of methyldopa and catechol are taken, respectively, as the dianion L-3-(μ-H-3',4'-dioxyphenyl)-2-methylalaninate(2—) (2) and the monoanion *o*-hydroxyphenoxide ($Hcat^-$). Incidentally, the very strong basic nature of one of the catechol oxygens has been attributed to hydrogen bonding between the hydroxyl and phenoxide groups.[31] All the sets of



(2)

titration data yielded superimposable curves of $\bar{Z}_{obs.}$ against pH which show the presence solely of simple HA, H_2A, and H_3A complexes. The protonation constants found are shown in Table 1 together with literature values for the same or related ligands. Taking into account differences in experimental conditions and in the nature of the ligands, the agreement is satisfactory.

* edta = Ethylenediaminetetra-acetate.

† β_{pqr} refer to the general complex $A_pB_qH_r$ where A = ligand, B = metal ion, and H = proton. When $r = -1$ this refers to a proton removed to a water molecule or to a hydroxide ligand added.

TABLE 1

Logarithms of formation constants (β_{pqr}) for ligand protonation at 25 °C and I = 0.15 mol dm^{-3} (Na)[ClO_4]. s Denotes the site of protonation as determined by n.m.r. d = Standard deviation in log β, and n = number of experimental observations

Ligand	p q r	s	log β_{pqr}	d	n	Literature data (ligand, θ_c/°C, I/mol dm^{-3}, log β)	Ref.
(2)	1 0 1	NH_2	9.982	0.005	289	*a*, 25, 1.0 (K[NO_3]), log β_1 9.74, log β_2 18.45, log β_3 20.76	20
	1 0 2	O^-	18.866	0.006	439	*a*, 25, 1.0 (K[NO_3]), log β_1 9.78, log β_2 18.58, log β_3 20.98	16
	1 0 3	CO_2^-	21.104	0.014	288	*a*, 25, 0.37 (Na[NO_3]), log β_1 9.87, log β_2 18.68, log β_3 20.72	21
						a, 25, 0 (KCl), log β_1 10.629, log β_2 19.786, log β_3 22.004	22
						a, 25, 0.2 (KCl), log β_1 9.83, log β_2 18.63, log β_3 20.85	23
b	1 0 1		10.239	0.005	130	*c*, 25, 0.16 (K[NO_3]), log β_1 10.11, log β_2 19.20	*e*
	1 0 2		19.383	0.006	169		
	1 0 3		21.543	0.011	51		
d	1 0 1		9.248	0.004	68	*d*, 25, 0.1, log β_1 9.23	*f*
						25, 1.0, log β_1 9.23	*f*
						25, 0.0, log β_1 9.40	*f*

[a] L-3-(3′-Hydroxy-4′-oxyphenyl)alaninate(2−). [b] 3-(3′-Oxyphenyl)-2-methylalaninate(2−). [c] *m*-Tyrosinate(2−). [d] *o*-Hydroxyphenoxide. [e] J. E. Letter, jun., and J. E. Bauman, jun., *J. Amer. Chem. Soc.*, 1970, **92**, 443. [f] A. E. Martel and R. M. Smith, 'Critical Stability Constants,' Plenum Press, New York and London, 1977, vol. 3, p. 200.

The sites of protonation on methyldopa dianion (2) were determined by measuring the chemical shifts as functions of pH for the protons on $C^{2'}$, $C^{5'}$, $C^{6'}$, C^3, and $C^2(CH_3)$. The pH corresponding to the maximum rate of change of chemical shift was taken as approximately equal to the pK_a of the nearest group with a dissociable proton. For C^3 and $C^2(CH_3)$ the observed pK_a corresponding to the protonated amino-group was in the range 9.80—9.96. For the aromatic protons the apparent pK_a was in the range 9.1—9.2. By analogy with the phenol–phenoxide and also substituted-phenol systems,[32,33] for a unique site of deprotonation the protons in the *ortho* and *para* positions would be expected to show much larger upfield shifts than the *meta* protons. However, the proton shifts observed are: $C^{2'}$, 20; $C^{5'}$ 24; and $C^{6'}$, 26 Hz. The most probable explanation for this is that the remaining hydroxide proton on the catechol monoanion is in rapid exchange between two sites or held in a ' symmetric ' hydrogen-bonded bridged structure. Evidently the phenolic group and the protonated amino-group of methyltyrosine are less acidic than the corresponding groups on methyldopa.

Complexations.—Series of replicated forward and reverse titrations of ligand with copper(II) perchlorate were carried out up to respective pH values limited by the appearance of precipitates. Total concentrations of the reactants ranged from 0.004 to 0.037, 0.003 to 0.06, and 0.01 to 0.02 mol dm^{-3} for methyldopa, methyltyrosine, and catechol, respectively. Various ligand : metal ratios were employed with methyldopa and methyltyrosine, while the titrations involving catechol were restricted to a ratio of 1 : 1. The purpose of this plan was to facilitate the search for not only mononuclear binary complexes but also protonated, hydroxo-, and oligonuclear species particularly in the methyldopa–copper(II) system. Indeed, the computed $\bar{Z}_{obs.}$ and p*a* points for methyldopa–copper(II) and methyltyrosine–copper(II) fell on sets of non-overlapping curves which in the case of the former formed a ' fanning-back ' pattern as shown in Figure 1. Had only mononuclear binary complexes been formed, that is with $q = 1$ and $r = 0$ throughout, the individual formation curves corresponding to different ligand and metal concentrations would have overlapped each other exactly.[29] The non-overlapping patterns observed in Figure 1, and the ' fanning back ' feature indicate the presence of protonated, hydroxo-, and/or oligonuclear species, that is with $r \neq 0$ and/or $q > 1$. This is because $Z_{obs.}$ has been computed assuming $q = 1$ and $r = 0$ for all the complexes present. The resulting $\bar{Z}$ has been named ' pseudo $\bar{Z}$ ' by Williams.[29] These implications necessitated our including complexes with $q > 1$ and $r \neq 0$ when postulating models for describing the system.

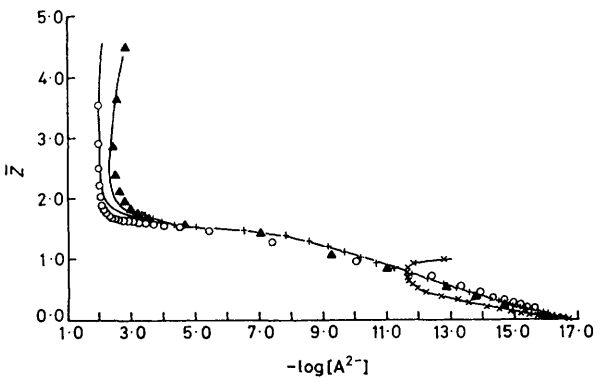


FIGURE 1 PSEUDOPLOT curves calculated from the methyldopa–proton and –copper(II) β values in Tables 1 and 2 plotted with experimental ZPLOT points. Concentrations (10^{-3} mol dm^{-3}) of A (ligand) and B (metal ion) are: 37.3 and 4.00 (○); 20.8 and 4.00 (▲); 37.2 and 15.0 (+); and 10.0 and 10.0 (×)

As a preliminary to the selection of postulated species for the methyldopa–copper(II) system, Gorton and Jameson's GEPOLYC program [20] was run on our 1 : 1 titration data. In order to apply this treatment we had to make an estimate of β_{10-1} through seeking a correlation, based on the Davies equation,[34] between our methyldopa and Gorton and Jameson's dopa protonation constants. Our best GEPOLYC fit conforms to the findings obtained by these workers for the dopa–copper(II) system, namely that under the conditions concerned the ligand and metal form an infinite set of open-chain linear polymers, $A_nB_nH_{2-n}$ ($n = 2-\infty$) and a species, $A_2B_2H_{-2}$, postulated by Gorton and Jameson to be a cyclic dimer. Our values of $10^{13.30}$ and $10^{15.55}$ for the Gorton and Jameson constants, K and K_c, are comparable with the values

found for dopa–copper(II), namely $10^{12.435}$ and $10^{14.605}$, respectively. The main value of the application of GEPOLYC lay in providing justification for inferring the formation of polymers in the 1 : 1 titrations and in the estimation of their relative concentrations. The linear polymers, $A_nB_nH_{2-n}$ ($n \geqslant 5$), were found to have negligible concentrations and therefore in subsequent treatment of the data by MINIQUAD only the linear dimer, trimer, and tetramer and the cyclic dimer were included amongst the oligonuclear species postulated.

Owing to the existence of two potential metal-chelating sites on (2) numerous different types of complex could be envisaged. When applying MINIQUAD, therefore, in order to obtain consistent chemical models to explain the entire set of potentiometric data, the latter were successively divided and grouped in various ways according to ligand : metal ratio and ranges of pH. Numerous combinations of β_{pqr} were tried ($p = 1$—5, $q = 1$—4, $r = -4$ to 2) and, moreover, the copper(II) hydrolysis reactions (1) and (2) [35] were incorporated into

$$Cu^{2+} + H_2O \rightleftharpoons [Cu(OH)]^+ + H^+; \quad \log K = -8.0 \quad (1)$$

$$2Cu^{2+} + 2H_2O \rightleftharpoons [Cu_2(OH)_2]^{2+} + 2H^+; \quad \log \beta = -10.6 \quad (2)$$

every trial. The most consistent set of complexes found, together with their respective formation constants, is shown in Table 2 which also includes data from the literature for comparison. Our chemical model for the methyldopa–copper(II) system is in substantial agreement with the conclusions reached by Gergely and Kiss [23] for the dopa–copper(II) system.

The validity of the model is illustrated by the excellent matching of the experimental $\bar{Z}_{obs.}$ and pa points in Figure 1 to corresponding theoretical formation curves computed by PSEUDOPLOT, using our formation constants. Distributions as calculated by HALTAFALL [30] of the postulated complexes in one each of our titrations at A : B = 10 : 1, 2.5 : 1, and 1 : 1 are shown in Figure 2. The 5 : 1 distributions are similar to the 10 : 1 and may be found in Supplementary Publication No. SUP 22602 (21 pp.).* The introduction of the minor complex ABH_2 at pH <3.5 greatly facilitated model fitting. ABH, A_2BH_2, and ABH_2 were found for all the A : B ratios studied in the lower pH range. A_2BH, A_2B, A_2BH_{-1}, and A_2BH_{-2} were found in the 10 : 1 and 5 : 1 ratios but could not be detected in the 1 : 1 titrations. The MINIQUAD refinement and PSEUDOPLOT verification procedures as applied to the 1 : 1 titrations were consistent with GEPOLYC in that the species A_2B_2, $A_3B_3H_{-1}$, $A_4B_4H_{-4}$, and $A_2B_2H_{-2}$ were found to be significant. Thus, the distributions of complexes in the 1 : 1 solutions are seen to be distinctly different from those with A : B ratios of 10 : 1 and 5 : 1. In the 1 : 1 titrations oligonuclear complexes feature strongly, whereas a series of successively deprotonated bis complexes characterizes the 10 : 1 and 5 : 1 ratios. The distinct character of the 1 : 1 solutions is also evident in Figure 1 where their formation curve is seen to reside in quite a different position from the family of curves formed by the 10 : 1, 5 : 1, and 2.5 : 1 ratios. It seems, therefore, as is reasonably expected, that the propensity to form oligonuclear species is promoted by the relatively high proportions of metal present in the 1 : 1 solutions. It is further interesting to note that oligonuclear complex formation becomes important in the range pH *ca.* 5—6 in which only extremely small concentrations of the ligand or protonated ligand occurred [see Figure 2(*f*)]. The 2.5 : 1 solutions appeared to be of partially inter-

TABLE 2

Logarithms of formation constants (β_{pqr}) for the copper complexes at 25 °C and I = 0.15 mol dm^{-3} (Na)[ClO$_4$]. d = Standard deviation in log β, n = number of experimental observations

Ligand	p	q	r	log β_{pqr}	d	n	Literature data (ligand, θ_c/°C, I/mol dm^{-3}, log β)	Ref.
(2)	1	1	1	17.613	0.005	167	*a*, 25, 1.0 (K[NO$_3$]), log β_{111} 16.86, log β_{212} 32.89, log β_{11-1} −0.41,	
	2	1	2	34.004	0.031	75	log β_{21-2} −1.86, log β_{22-2} 14.59	20
	1	1	2	19.568	0.085	167	*a*, 25, 1.0 (K[NO$_3$]), log β_{111} 17.38, log β_{11-1} 3.08	16
	2	1	1	28.234	0.011	66	*a*, 20, 0.37 (Na[NO$_3$]), log β_{111} 17.47, log β_{212} 34.25	21
	2	1	0	19.487	0.018	105	*a*, 25, 0.2 (KCl), log β_{111} 17.35, log β_{212} 33.81, log β_{211} 27.01,	
	2	1	−1	8.447	0.264	48	log β_{210} 18.53, log β_{21-1} 9.03, log β_{21-2} −1.33, log β_{220} 26.55,	
	2	1	−2	−1.454	0.094	44	log β_{22-2} 15.10	23
	2	2	0	27.290	0.035	107		
	3	3	−1	36.850	0.136	89		
	4	4	−2	46.625	0.129	82		
	2	2	−2	16.894	0.012	87		
b	1	1	1	18.050	0.013	131	*c*, 25, 0.16 (K[NO$_3$]), log β_{111} 17.93, log β_{212} 34.66, log β_{211} 25.92,	
	2	1	2	35.012	0.017	135	log β_{210} 15.42	*e*
	1	1	2	20.058	0.138	82		
	2	1	1	25.744	0.078	11		
d	1	1	−1	0.936	0.002	130	*d*, 25, 0.1, log β_{11-1} 0.85	*f*
	2	1	−2	−0.944	0.107	57	25, 1.0, log β_{11-1} 0.55	*f*
							25, 0.0, log β_{11-1} 1.30	*f*
							25, 0.1, log β_{21-2} −1.1	*f*
							25, 1.0, log β_{21-2} −1.06	*f*
							25, 0.0, log β_{21-2} −1.4	*f*

For footnotes see Table 1.

* For details see Notices to Authors No. 7, *J.C.S. Dalton*, 1978, Index issue.

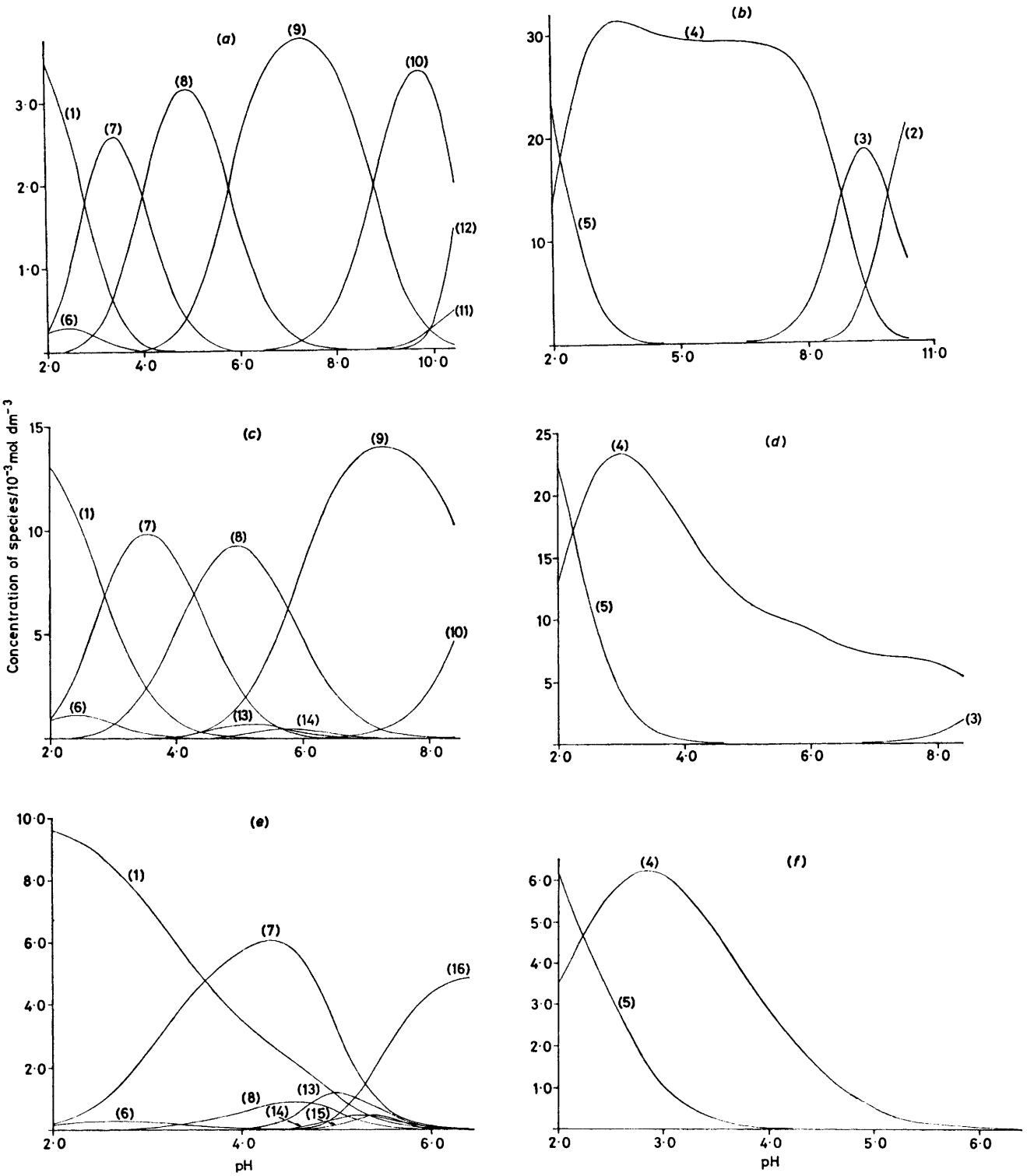


FIGURE 2 HALTAFALL plots of the concentrations of complexes present as a function of pH for the methyldopa–copper(II) system when the total concentrations (10^{-3} mol dm^{-3}) of A and B(Cu^{2+}) are: 37.3 and 4.00 [(*a*), (*b*)]; 37.2 and 15.0 [(*c*), (*d*)]; and 10.0 and 10.0 [(*e*), (*f*)]. Species: Cu^{2+} (1), A^{2-} (2), HA^{-} (3), H_2A (4), H_3A^{+} (5), $[Cu(H_2A)]^{2+}$ (6), $[Cu(HA)]^{+}$ (7), $[Cu(HA)_2]$ (8), $[CuA(HA)]^{-}$ (9), $[CuA_2]^{2-}$ (10), $[CuA(H_{-1}A)]^{3-}$ (11), $[Cu(H_{-1}A)_2]^{4-}$ (12), $[Cu_2A_2]$ (13), $[Cu_3A_3H_{-1}]^{-}$ (14), $[Cu_4A_4H_{-2}]^{2-}$ (15), $[Cu_2A_2H_{-2}]^{2-}$ (16)

mediate character between 10 : 1 and 5 : 1 on the one hand and 1 : 1 on the other hand. In the 2.5 : 1 solutions A_2BH and A_2B were found to be important, as in the case of 10 : 1 and 5 : 1, while the oligonuclear species A_2B_2 and $A_3B_3H_{-1}$, as found in the 1 : 1 titrations, were also significant.

Unlike the methyldopa–copper(II) data, the treatment of the methyltyrosine–copper(II) and catechol–copper(II)

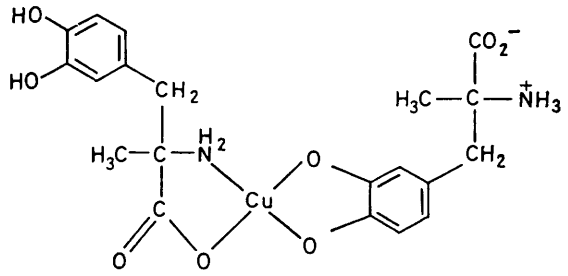
data was much more straightforward in that extensive division and grouping turned out to be unnecessary. Since the primary interest in the present paper lies in the methyldopa–copper(II) complexes, the investigations of the methyltyrosine– and catechol–copper(II) systems were more limited. Again, numerous combinations of β_{pqr} were tried on the data collected for the two subsidiary ligands and the best-fitting models found are presented in Table 2. HALTAFALL-computed distributions of the complexes in these two systems may be found in SUP 22602. The maximum pH values attainable before the onset of precipitation were appreciably lower than with methyldopa–copper(II).

The species found in the methyltyrosine–copper(II) system correspond, stoicheiometrically, with the first four listed in Table 2 for methyldopa–copper(II); the significantly greater values of log β for ABH and A_2BH_2 in the former system correlate with the higher basicity of the amino-group on 3-(3′-oxyphenyl)alaninate, *cf.* methyldopa dianion (2). The two log β_{112} values do not differ from each other significantly. One can infer, therefore, that the copper ion is co-ordinated to the alanine end of (2) in the ABH, A_2BH_2, and ABH_2 complexes of methyldopa–copper(II) because this is the only mode of binding likely with methyltyrosine.[36] In support of this inference, species corresponding to these three complexes could not, in fact, be found in the catechol–copper(II) titrations. That the metal is co-ordinated solely to one kind of site in the complexes ABH and A_2BH_2 of both the methyldopa–copper(II) and the methyltyrosine–copper(II) systems can be illustrated using a principle introduced by Sigel.[37] According to this, the difference in the logarithms of the stepwise formation constants for the mono and bis complexes formed from a bidentate ligand should have a value between 1 and 2. With K_{111} and K_{212} defined as [ABH]/[AH][B] and $[A_2BH_2]$/[AH][ABH], respectively, log K_{111} − log $K_{212} \approx 1.2$ for methyldopa–copper(II) and $\approx$1.1 for methyltyrosine–copper(II) thereby confirming a common site of co-ordination within each system. The proposed mode of co-ordination in ABH and A_2BH_2 is consistent with the conclusions of Gergely and Kiss [23] concerning copper complexes of dopa. Kwik *et al.* [24] confirm alanine-type co-ordination in the latter complex.

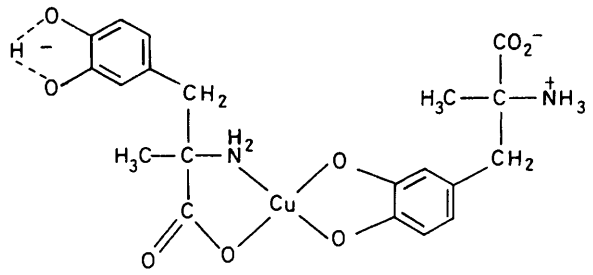
The minor species, ABH_2, which evidently has comparable structures in the methyldopa– and methyltyrosine–copper(II) systems, probably resembles monoacetatocopper(II) in that the ligand co-ordinates unidentately through the carboxylate while the amino-group remains protonated.[38]

If the metal in A_2BH is also tentatively assumed to be co-ordinated to the alanine ends of both ligand moieties and, furthermore, if the dissociation quotient $[A_2BH]$-[H]/$[A_2BH_2]$ is assumed to approximate to K_{102}^{-1} = [AH][H]/$[AH_2]$, it follows that log β_{211} should approximate to log β_{212} − log K_{102}. In the case of methyltyrosine–copper(II), log β_{212} − log K_{102} = 25.87 which is sufficiently close to the determined value of log β_{211} (25.744) for one to accept the above assumption concerning the co-ordination sites. On the other hand, for methyldopa–copper(II), log β_{212} − log K_{102} = 25.12 which differs markedly from the observed value of log β_{211}, namely 28.234. In the latter system, therefore, A_2BH appears to be anomalous. A reasonable explanation, which indeed is consistent with the observed enhanced stability, is that the methyldopa–copper(II) species, A_2BH, is a mixed-ligand complex with the copper co-ordinating to the alanine end of one ligand moiety and to the catechol end of the other. This structure accords with the corresponding proposal in the dopa–copper(II) studies of Gergely and Kiss.[23]

In the catechol–copper(II) titrations, the only complexes which could be found were $[Cu(Hcat)H_{-1}]$ and $[Cu(Hcat)_2H_{-2}]$ (see Table 2). An extensive search failed to reveal species such as [Cu(Hcat)] and [Cu(Hcat)H] in which either one or both phenoxides of the ligand are protonated. Accordingly it may be



(3)



(4)

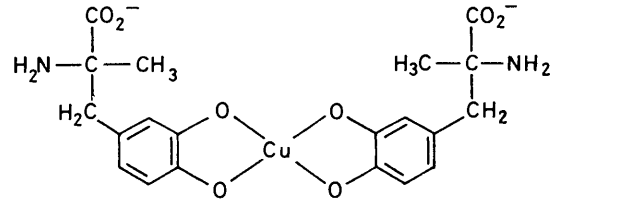
assumed, likewise, that in every species involving catechol-type binding in the methyldopa–copper(II) system neither phenoxide is protonated; that is the phenolic proton of methyldopa dianion (2) becomes displaced upon *O,O* co-ordination to copper(II). Thus, we propose the structure (3) for A_2BH.

By regarding the methyldopa–copper(II) complex, A_2B, as being derivable from (3) through the loss of a proton and by assuming the dissociation quotient $[A_2B][H]/[A_2BH]$ to approximate to K_{102}^{-1}, log β_{210} can be estimated as log β_{211} − log K_{102} = 19.35. The latter value, being in rough agreement with the observed value, 19.48, lends support to the proposed structure, (4), for A_2B. This conclusion is in contrast with Gergely and Kiss [23] and Kwik *et al.*[24] who propose *O,O* binding of

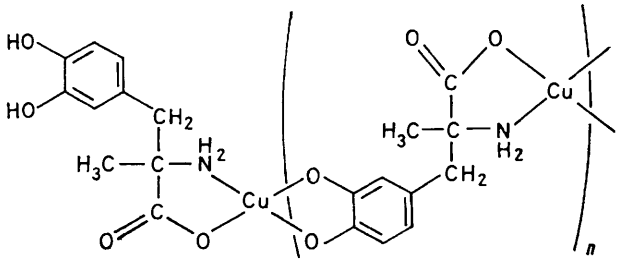
both ligand moieties to the metal in the complex corresponding to A_2B of the dopa–copper(II) system.

Taking into account the high value of pK for the phenolic proton on the methyldopa dianion (2), *i.e.* 13—14, and the fact that A_2BH_{-2} starts to form at pH <9 in the methyldopa–copper(II) system, the only feasible structure for the latter complex appears to be (5). This accords with the corresponding structure in Gergely and Kiss's [23] description of the dopa–copper(II) system.

In attempting to decide on the structure of the methyldopa–copper(II) complex, A_2BH_{-1}, one can consider the formation of this species to result from either the deprotonation of (4) or the protonation of (5). Comparisons of the corresponding estimated log β_{21-1} values with the observed value, however, are inconclusive. The oligonuclear species A_2B_2, $A_3B_3H_{-1}$, and $A_4B_4H_{-2}$ found in the methyldopa–copper(II) titrations are most likely to involve co-ordination of the copper to the alanine and catechol ends, respectively, of alternate ligand moieties. This expectation is consistent with



(5)



(6) n = 1 or 3

our failure to find species with corresponding stoicheiometries in the methyltyrosine– and catechol–copper(II) titrations. Assuming that the phenolic proton is displaced upon *O,O* co-ordination to copper(II), as rationalized above, therefore, the structures proposed for these three oligonuclear complexes are those implied by the displayed formula (6).

It may be seen from Figure 2 that the species corresponding to the structure implied by (6) are formed in significant concentrations at pH values as low as 4—5. Evidence that copper(II) can, in fact, co-ordinate to catechol-type sites at these low pH values has been presented earlier [23,24] and is confirmed both by our potentiometric investigation of the catechol–copper(II) system and by an extensive visible spectrophotometric study of the methyldopa–, alanine–, and catechol–copper(II) systems. By reference to the appropriate distribution diagrams in SUP 22602, it may be seen that the species $[Cu(Hcat)H_{-1}]$ is significant throughout the experimental pH range covered, namely 4.2—6.5, while the bis complex $[Cu(Hcat)_2H_{-2}]$ is observed at pH >5.4. The spectrophotometric investigation was carried out on series of solutions containing ligand and copper(II) with concentration, A : B ratio, and pH values selected from those used in the potentiometric titrations. Figure 3 shows typical absorption spectra obtained for solutions of methyldopa and copper(II) perchlorate at different pH values. A complete set of the spectra obtained is included with SUP 22602. Two types of absorption maximum may be distinguished. Type (1), in the wavelength range 600—700 nm, can be ascribed to *d–d* transition of copper(II) complexes; [23,24,39,40] the maximum shifts towards shorter wavelengths with increasing pH and the variation of absorbance with pH is complicated. With type (2), the absorption maximum at a wavelength of *ca.* 415 nm is almost independent of pH, metal, or ligand concentration, and the absorbance increases monotonically with increasing pH.

Alanine–copper(II) showed type (1) maxima only whereas catechol–copper(II) and methyldopa–copper(II) showed both types. The maxima of type (2) are probably charge-transfer bands and are evidently characteristic of *O,O*-copper(II) bonds.[40] Their appearance in the methyldopa–copper(II) system confirms our postulate above that binding of the metal to the catechol end of the molecule occurs in some of the complexes. The main conclusions to be drawn are that at low pH (<*ca.* 3.5) copper(II) is co-ordinated to the alanine end of the methyldopa dianion (2) whereas at pH >3.8—3.9 binding to both the alanine and the catechol ends is evident. Hence these spectrophotometric observations confirm our conclusions concerning the structures shown

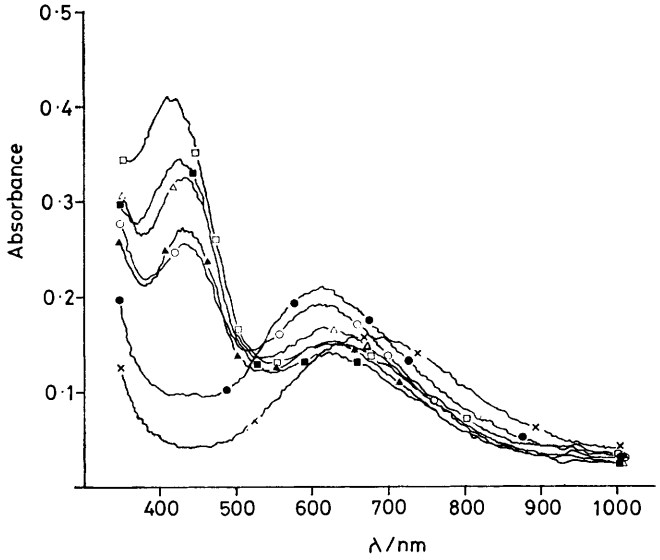
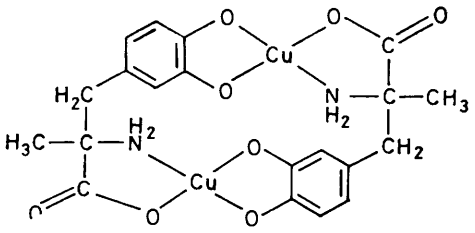


FIGURE 3 Spectra of methyldopa–copper(II) complexes. Concentrations (10^{-3} mol dm^{-3}) of A and B are 10.0 and 4.0. Reference, water. pH (4) (×), 5 (●), 6 (○), 7 (▲), 8 (△), 9 (■), and 10 (□)

in the displayed formulae (3)—(6) as well as the co-ordination of the metal solely to the alanine end of (2) in ABH, A_2BH_2, and ABH_2.

The species with stoicheiometry $A_2B_2H_{-2}$ found in our methyldopa–copper(II) 1 : 1 titrations has also been reported by earlier workers.[20,23] We found that an equally good fit could be obtained by substituting ABH_{-1} for $A_2B_2H_{-2}$. Species corresponding to the former, however, could not be detected in our methyltyrosine–copper(II) data, suggesting that $A_2B_2H_{-2}$ is the preferred choice for the methyldopa–copper(II) system. Gorton and Jameson [20] and Gergely and Kiss [23] propose a cyclic dimeric structure for $A_2B_2H_{-2}$ with each copper(II) ion chelated to an alanine and a catechol moiety. Whereas the methyl group in the 2 position of methyldopa would not interfere sterically with the cyclic structure, we saw, initially, another difficulty in that a Dreiding model suggested an apparently unacceptable short distance, namely *ca.* 300 pm, for the copper(II)–aromatic ring distance together with the implication that one apical aquo-ligand per copper(II) ion would be absent. This difficulty is, at least partially, resolved by the crystal structures of bis(L-tyrosinato)-copper(II) [41] and the copper(II) chelates of glycyl-L-leucyl-L-tyrosine [42] and glycyl-L-tryptophan [43] in which a



(7)

weak interaction (*ca.* 300 pm) is observed between each π-electron system of the phenolic ring and a copper(II) ion. Thus we propose, in conformity with refs. 20 and 23, the structure (7) for $A_2B_2H_{-2}$ in the methyldopa–copper(II) system.

Under human blood-plasma conditions (pH 7.2—7.6) the free copper(II)-ion concentration [17] lies between 10^{-11} and 10^{-19} mol dm^{-3} while the total methyldopa concentration [44] for a patient under treatment is typically 0.5×10^{-5}—1.9×10^{-5} mol dm^{-3}. In solutions containing methyldopa as the only ligand and copper(II) as the only metal ion, both at plasma concentrations, the distribution of complexes is likely to resemble that in Figure 2(*a*), that is with $[CuA(HA)]^-$ predominating at pH 7.2—7.6. The latter is confirmed by preliminary ECCLES [17] computations. In view of the electrical charge on $[CuA(HA)]^-$ it is unlikely that this species would readily undergo passive transport across biological membranes. In order to clarify the situation more extensive ECCLES computations are in hand, involving potential ternary complex formation with other low-molecular-weight ligands amongst the 40 already considered together with seven metal ions in the simulation of human blood plasma.[17]

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