Assignment of the Proton-association Constants for 3-(3,4-Dihydroxyphenyl)alanine (L-dopa)

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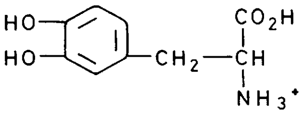
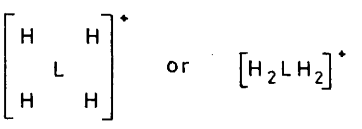
A re-examination based on linear free-energy relations and on kinetic evidence has been made of the assignment of the order of protonation of the L-dopa anion. This protonation order is reaffirmed as follows: first, one of the phenolato-groups; secondly, the second phenolato-group; this is followed closely, and overlapped, by the NH_2 group; and finally the carboxylato-group. The use of the following values of the microconstants is recommended for solutions of 0.100 mol dm^{-3} ionic strength ($K[NO_3]$): $\log K_1^H(OH)$ 13.55, $\log K_2^H(OH)$ 9.76, $\log K_3^H(NH_3^+)$ 8.93, and $\log K_4^H(CO_2H)$ 2.31.

THE assignment of proton-association constants to specific functional groups is always a problem in the study of polyprotic acids. The catecholamines such as L-dopa [3-(3,4-dihydroxyphenyl)alanine] are a particularly important class of these acids since both the biochemical and purely chemical reactivity depend markedly on the site involved (*viz.* the catechol or amino-acid site in this example). Boggess and Martin[1] recently questioned the usual assignment of the microconstants for these molecules. Presented here, however, is compelling evidence that, at least in the case of L-dopa, these workers are in error and that the order of protonation is as was previously always assumed, namely (*i*) one of the phenolato-groups, (*ii*) the second phenolato-group, (*iii*) the amine group, and (*iv*) the carboxylato-group, (*ii*) and (*iii*) being overlapping protonations.

If the fully protonated species is written as in (I) and L^{3-} represents the fully deprotonated anion, then the protonated species may be represented diagramatically by (II) and the anion by (III) where the phenolic protons are written to the left of L and the amino-acid ones to the right: the protonation positions on the anion are further distinguished by the numbering scheme included in (III). This notation is employed in the protonation scheme of Figure 1 in which the important contributions are represented by full lines (*i.e.* cognisance is taken of the fact that there is no ambiguity concerning the addition of the first and fourth protons, see below).

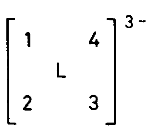


(I)



$$\left[\begin{array}{ccc} H & & H \\ & L & \\ H & & H \end{array}\right]^{+} \quad \text{or} \quad \left[H_2LH_2\right]^{+}$$

(II)



$$\left[\begin{array}{ccc} 1 & & 4 \\ & L & \\ 2 & & 3 \end{array}\right]^{3-}$$

(III)

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[1] R. K. Boggess and R. B. Martin, *J. Amer. Chem. Soc.*, 1975, **97**, 3076.

From Figure 1 it follows that in order to form $[H_2LH_2]^+$ we need consider only one form of H_3L, namely $\left[\begin{array}{ccc} H & & \\ & L & \\ H & & H \end{array}\right]$ and two forms of the doubly protonated species, that is $\left[\begin{array}{cc} H & \\ & L \\ H & \end{array}\right]^-$ and $\left[\begin{array}{ccc} H & & \\ & L & \\ & & H \end{array}\right]^-$ arising in turn from only one form of the monoprotonated species, written as $\left[\begin{array}{cc} H & \\ & L \\ & \end{array}\right]^{2-}$. (It is to be noted that the order of numbering of the phenolic positions is for the purposes of this paper completely arbitrary: no difference in the argument would result from interchange of the positions 1 and 2.) The important equilibria are thus seen to be (1)—(6).

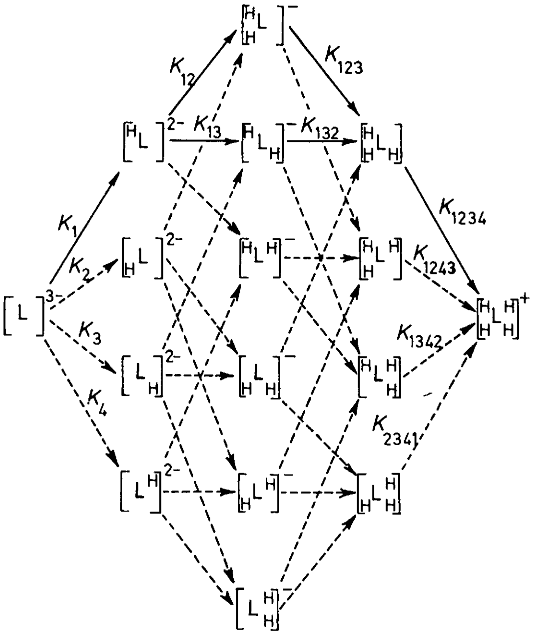

FIGURE 1 Protonation scheme for L-dopa. See text for explanation of symbols

$$L^{3-} + H^+ \overset{K_1}{\rightleftharpoons} [HL]^{2-} \qquad (1)$$

$$[HL]^{2-} + H^+ \overset{K_{12}}{\rightleftharpoons} [H_2L]^- \qquad (2)$$

$$[HL]^{2-} + H^+ \overset{K_{13}}{\rightleftharpoons} [HLH]^- \qquad (3)$$

$$[H_2L]^- + H^+ \overset{K_{123}}{\rightleftharpoons} [H_2LH] \qquad (4)$$

$$[HLH]^- + H^+ \overset{K_{132}}{\rightleftharpoons} H_2LH \qquad (5)$$

$$H_2LH + H^+ \overset{K_{1234}}{\rightleftharpoons} [H_2LH_2]^+ \qquad (6)$$

Now the macroscopic protonation constants obtained from titration data are definable only in terms of $[H_4\bar{L}]^+$ in which $\bar{L}^{3-}$ is the anion without identifiable sites for protonation, *i.e.* as in equations (7)—(10) where K_1^H is

$$\bar{L}^{3-} + H^+ \overset{K_1^H}{\rightleftharpoons} [H\bar{L}]^{2-} \qquad (7)$$

$$[H\bar{L}]^{2-} + H^+ \overset{K_2^H}{\rightleftharpoons} [H_2\bar{L}]^- \qquad (8)$$

$$[H_2\bar{L}]^- + H^+ \overset{K_3^H}{\rightleftharpoons} H_3\bar{L} \qquad (9)$$

$$H_3\bar{L} + H^+ \overset{K_4^H}{\rightleftharpoons} [H_4\bar{L}]^+ \qquad (10)$$

automatically identified with the microconstant K_1 and K_4^H with K_{1234} but K_2^H and K_3^H are ' mixed ' constants as in (11) and (12).

$$\begin{aligned} K_2^H &= [H_2\bar{L}^-]/[H\bar{L}^{2-}][H^+] \\ &= ([H_2L^-] + [HLH^-])/[HL^{2-}][H^+] \\ &= K_{12} + K_{13} \end{aligned} \qquad (11)$$

$$K_3^H = K_{123}K_{132}/(K_{123} + K_{132}) \qquad (12)$$

Further simplification is possible if it may be assumed that the protonation of site 2 is independent of the occupancy or otherwise of site 3 (this seems reasonable on the basis that the sites are well separated from each other) because then we have the equality (13) and (14)

$$K_{12} = K_{132} \qquad (13)$$

follows since the work of adding the two protons to

$$K_{13} = K_{123} \qquad (14)$$

$[HL]^{2-}$ is independent of the path. Therefore, from (12)—(14) we have (15). If K_2^H and K_3^H are determined

$$K_3^H = K_{12}K_{13}/(K_{12} + K_{13}) \qquad (15)$$

experimentally, then use of equations (11) and (15) enables two values for the required microconstants to be obtained, but *not* their assignment because the solution involves a quadratic equation. This assignment must be carried out on chemical grounds: here the problem is approached using kinetically obtained data and linear free-energy relations (l.f.e.r.s).

A recently completed study[2] of the vanadium(IV)-catalysed oxidation of L-dopa by molecular oxygen has led to the assignment by Boggess and Martin[1] being questioned. K_1^{ML} for the reaction of $[VO]^{2+}$ with the

$$[VO]^{2+} + [LH_n]^{(3-n)-} \rightleftharpoons [VO(LH_n)]^{(n-1)+} \; (n = 0, 1, \text{or } 2) \qquad (16)$$

catechol end of L-dopa has been determined kinetically to lie between 2.5×10^{17} and 3.0×10^{17} dm^3 mol^{-1} [equation (16)], this value relying on the values of the hydrolysis constants for $[VO]^{2+}$ quoted by Henry *et al.*[3] Similar reliance on the values quoted by Henry *et al.* also demands that the value of log β_2^H for the protonation of the catechol site must exceed 23 if the sum of the species present is to simplify to match the observed kinetics.[2]

Gorton and Jameson[4] determined accurate values of the protonation constants for L-dopa (at 25 °C, 0.100

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[2] R. F. Jameson and S. W. Thompson, unpublished work.

[3] R. P. Henry, P. C. H. Mitchell, and J. E. Prue, *J.C.S. Dalton*, 1973, 1157.

[4] J. E. Gorton and R. F. Jameson, *J. Chem. Soc.* (*A*), 1968, 2615.

mol dm^{-3} K[NO$_3$] as background electrolyte) as follows: $\log K_1^H$ 13.55, $\log K_2^H$ 9.82, $\log K_3^H$ 8.87, and $\log K_4^H$ 2.31. As stated above, there is no problem with K_1^H or with K_4^H and use of equations (11) and (15) gives $\log K$(micro) values of 9.76 and 8.93. The kinetic data are only consistent with the assignment of the higher of the two values to protonation of the second OH group. Figures 2—5 are constructed on the basis of this assignment, making use of standard l.f.e.r.s [5] and including points derived on the basis of the reverse assignment advocated by Boggess and Martin. They are self-explanatory and seem to clearly support the use of the following assignments and values of the protonation constants of L-dopa at 25 °C and at an ionic strength of 0.100 mol dm^{-3} K[NO$_3$]: $\log K_1^H$(OH) 13.55, $\log K_2^H$(OH) 9.76, $\log K_3^H$(NH$_3^+$) 8.93, and $\log K_4^H$(CO$_2$H) 2.31.

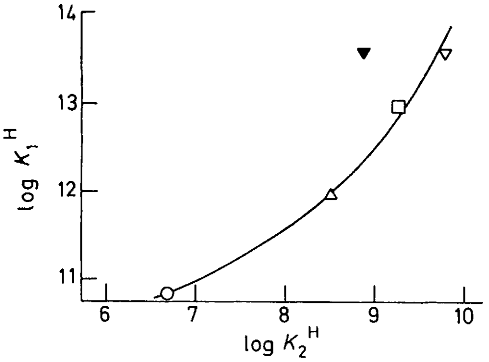

FIGURE 2 Correlation of log K_1^H with log K_2^H for some 4-substituted catechols: (○) 4-nitrocatechol, (△) 4-chlorocatechol (R. F. Jameson and M. F. Wilson, *J.C.S. Dalton*, 1972, 2617); (□) catechol (R. F. Jameson and M. F. Wilson, *J.C.S. Dalton*, 1972, 2610); (▽), (▼) L-dopa (recalculated,[4] the filled symbol being plotted using the assignment of microconstants proposed by Boggess and Martin [1])

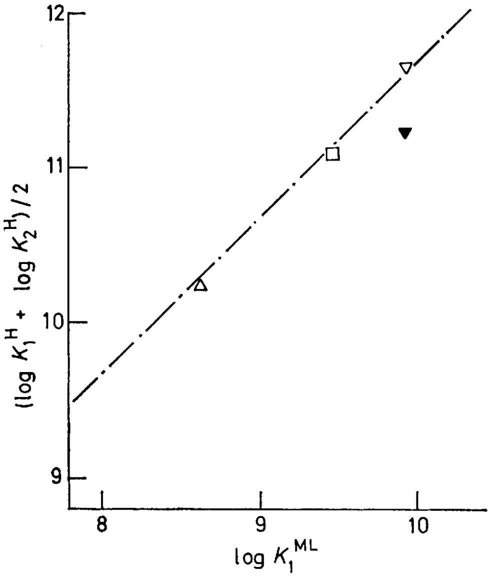

FIGURE 3 Correlation of the logarithms of the stability constants for proton and zinc complexes of some 4-substituted catechols: (△) as in Figure 2; (□) catechol (C. A. Tyson and A. E. Martell, *J. Amer. Chem. Soc.*, 1968, **90**, 3379); (▽), (▼) L-dopa (J. E. Gorton and R. F. Jameson, *J.C.S. Dalton*, 1972, 310) (filled symbol uses assignment proposed by Boggess and Martin [1]). The line is drawn with the theoretical gradient of unity [5]

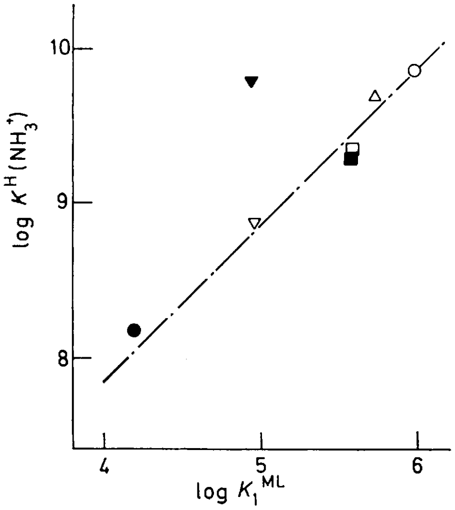

FIGURE 4 Correlation of the logarithms of the stability constants for proton and nickel(II) complexes of some amino-acids: (●) glycylglycine (N. C. Li and M. C. M. Chen, *J. Amer. Chem. Soc.*, 1958, **80**, 5678); (▽), (▼) as in Figure 3; (■) 3-phenylalanine (K. P. Anderson, W. O. Greenhalgh, and R. M. Izatt, *Inorg. Chem.*, 1966, **5**, 2106); (□) leucine (S. P. Datta, R. Lebermann, and B. R. Rabin, *Trans. Faraday Soc.*, 1959, **55**, 1982); (△) glycine (A. Gergely, I. Nagypal, and J. Mojzes, *Acta Chim. Acad. Sci. Hung.*, 1967, **51**, 381); (○) alanine (C. B. Monk, *Trans. Faraday Soc.*, 1951, **47**, 292). The line is drawn with the theoretical gradient of unity [5]

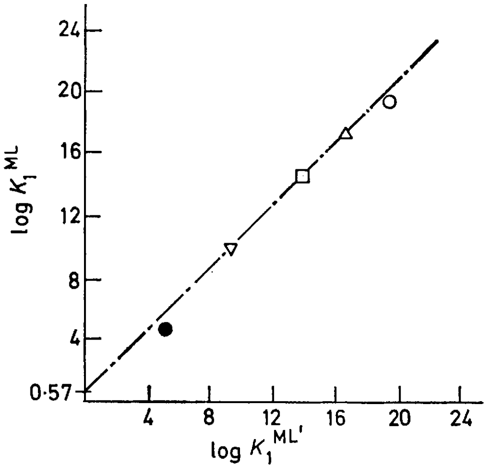

FIGURE 5 Correlation of log K_1^{ML} (L = dopa) with log $K_1^{ML'}$ (L′ = catechol) for various cations: (○) Fe^{3+} (W. F. S. Niellie, Ph. D. Thesis, University of St. Andrews, 1966; J. E. Gorton, Ph.D. Thesis, University of St. Andrews, 1968); (△) [VO]$^{2+}$; (□) Cu^{2+} (R. F. Jameson and M. F. Wilson, *J.C.S. Dalton*, 1972, 2614; ref. 4); (▽) Zn^{2+} (C. A. Tyson and A. E. Martell, *J. Amer. Chem. Soc.*, 1968, **90**, 3379; J. E. Gorton and R. F. Jameson, *J.C.S. Dalton*, 1972, 310); (●) Mg^{2+} (V. T. Athavale, L. H. Prebhv, and D. G. Vartak, *J. Inorg. Nuclear Chem.*, 1966, **28**, 1237; J. E. Gorton and R. F. Jameson, *J.C.S. Dalton*, 1972, 310). The line is drawn with the theoretical [5] gradient of unity and intercept given by 0.5 [log β_2^H (dopa) − log β_2^H (catechol)] = 0.57. The references are given in the order catechol then dopa

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[5] F. J. C. Rossotti in 'Modern Coordination Chemistry,' eds, J. Lewis and R. G. Wilkins, Interscience, London, 1960.