A plot of log ($k_f\epsilon$) *vs.* $1/T$ gives as slope, $\Delta H^{\ddagger} = (2.8 \pm 0.5)$ kcal/mol, experimentally indistinguishable from the one calculated from viscosity data for the solvent. Taking into account change in V with temperature in eq 17 has a negligible effect on the determined value of $\Delta H^{\ddagger}$.

Conclusions

The observed relaxation for $M^{II}BDS$ in methanol is interpreted as a diffusion-controlled process of formation of an outer-sphere ion pair with the simultaneous elimination of a molecule of methanol. The same barrier of energy of viscous flow is observed. The agreement with theories based on a continuum for this step of the association is even more remarkable if one considers the numerical approximations introduced by our ignorance of the values of the ionic mean activity coefficients and their concentration dependence.

Zwitterion Formation upon Deprotonation in L-3,4-Dihydroxyphenylalanine and Other Phenolic Amines

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In weakly basic solutions L-3,4-dihydroxyphenylalanine (DOPA) exhibits two overlapping acidity constants of $pK_1 = 8.76$ and $pK_2 = 9.84$ as determined by potentiometric titration at 0.16 ionic strength and 25.0°. A new, simple method for evaluating acidity constants of overlapping deprotonations is described. By analyzing the increase in absorption near 295 nm due to the first phenolic ionization it is shown that the K_1 acidity constant is composed of 61% phenolic ionization and 39% ammonium deprotonation corresponding to a ratio of 1.6 zwitterionic to neutral forms of these two groups. From an analysis of both model compound and spectrophotometric results appearing in the literature it is concluded that the corresponding ratio for norepinephrine is about 3 and that for epinephrine about 4. Of the two bidentate metal ion binding loci in DOPA, Cu(II) appears to bind predominantly at the glycinate site in neutral solutions and in the catecholate mode in basic ones, while Mn(II) should chelate in the latter mode under both conditions.

Various compounds containing both amine and phenolic groups are found in biological systems. Examples are tyrosine, 3,4-dihydroxyphenylalanine (DOPA), epinephrine (adrenaline), and related compounds. The acidities of the phenolic and substituted ammonium groups are comparable so that their deprotonations often occur in the same pH region. As a result a special analysis is required to assess their relative contributions to the acidity (acid ionization) constants determined by titration. A complete resolution of the phenolic and ammonium deprotonations has been performed for tyrosine,[1] while no analysis appears to have been tried for DOPA, and most of the attempts on the catechol amines are incorrectly formulated. This paper describes a new method for evaluation of overlapping acidity constants, reports the first resolution of phenolic and ammonium deprotonations for DOPA, and presents a new analysis of data appearing in the literature for several phenolic amines. Preliminary studies of metal ion binding sites in DOPA are mentioned.

Results and Discussion

Evaluation of Macroconstants. Overlapping deprotonations require special methods for evaluation of acidity constants, several of which have been described.[2-4] A new method, especially suitable for cases to be discussed below, is here introduced for evaluation of macroconstants of two overlapping deprotonations. Concentrations of hydrogen and hydroxide ions are assumed negligible compared with the concentration of other acidic and basic species.

For two acidic groups let $\bar{h}$ be the mean number of protons removed per molecule. The relationship between $\bar{h}$ and the macroconstants is given by[2]

(1) R. B. Martin, J. T. Edsall, D. B. Wetlaufer, and B. R. Hollingworth, *J. Biol. Chem.*, **233**, 1429 (1958).

(2) J. T. Edsall, R. B. Martin, and B. R. Hollingworth, *Proc. Nat. Acad. Sci. U. S.*, **44**, 505 (1958).

(3) J. C. Speakman, *J. Chem. Soc.*, 855 (1940).

(4) F. J. C. Rossotti and H. S. Rossotti, "The Determination of Stability Constants," McGraw-Hill, New York, N. Y., 1961, pp 99–101 for projection strip method.

$$\bar{h} = \frac{(H^+)K_1 + 2K_1K_2}{(H^+)^2 + (H^+)K_1 + K_1K_2} \quad (1)$$

where $\bar{h}$ may take values from 0 to 2.

The titration curve for two acidic groups is symmetrical about the midpoint ($\bar{h} = 1.0$). We take two symmetrically related points on the titration curve, $\bar{h} = a$ and $\bar{h} = 2 - a$. Substitution into eq 1 and elimination of K_1 yields

$$(H^+)_a(H^+)_{2-a} = K_1K_2 \quad (2)$$

At the midpoint in the titration curve where $\bar{h} = 1.0$ eq 2 becomes

$$(H^+)_1^2 = K_1K_2 \quad (3)$$

On the other hand, if the product K_1K_2 is eliminated upon substitution into eq 1 we obtain

$$K_1 = \frac{a(H^+)_a - (2 - a)(H^+)_{2-a}}{1 - a} \quad (4)$$

Equation 4 permits a determination of K_1 without the necessity of approximating K_2. After determining K_1 from eq 4, K_2 may be found from eq 2 or 3. Equation 4 takes on the especially simple form when $a = {}^1/_2$

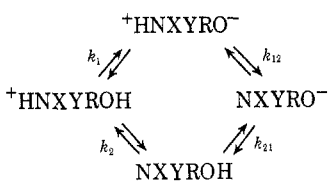
$$K_1 = (H^+)_{1/2} - 3(H^+)_{3/2} \quad (5)$$

Thus K_1 and K_2 may be evaluated from eq 3 and 5 by considering three well defined points on the titration curve at $\bar{h} = 0.5$, 1.0, and 1.5. Other points along the curve afford an opportunity for checks. Equation 4 is most useful when $\bar{h} < 1$. For $\bar{h} > 1$ it is more convenient to use the analogous equation for K_2 found by eliminating K_1 between eq 2 and 4.

Application of the above equations to the potentiometric titration curve obtained upon the addition of standard base to a solution containing DOPA yields $pK_1 = 8.76$ and $pK_2 = 9.84$ for a combination of the ammonium and first phenolic deprotonations at 25.0° and 0.16 ionic strength. These values agree closely with those of 8.71 and 9.74 obtained at the same temperature and 1.0 M ionic strength.[5] Under the latter conditions, the second phenolic ionization occurs with $pK_a = 13.4$ (due mainly to electrostatic effects) and the carboxylic acid ionization with $pK_a = 2.31$, so that they do not interfere with deprotonations occurring in the pH 6–11.5 region. Similar considerations apply to the other catecholamines discussed in this paper.

Resolution of Microconstants. Deprotonations from the phenolic and ammonium groups of phenolic amines may be described by equilibria shown in Chart I.

Chart I



The letters X and Y designate hydrogen or other substituent on nitrogen. The acidity (acid ionization) constants defined by the scheme are termed microconstants. Their subscripts 1 and 2 refer to the phenolic and ammonium deprotonations, respectively. The four microconstants are related to the two acidity constants determined by titration, called macroconstants, by

$$\frac{(H^+)([^+HNXYRO^-] + [NXYROH])}{[^+HNXYROH]} = K_1 = k_1 + k_2 \quad (6)$$

$$\frac{[^+HNXYRO^-] + [NXYROH]}{(H^+)[NXYRO^-]} = K_2^{-1} = k_{12}^{-1} + k_{21}^{-1} \quad (7)$$

Due to the properties of a cyclic system, we also have

$$K_1K_2 = k_1k_{12} = k_2k_{21} \quad (8)$$

Only three equations relate the four microconstants and at least one item of additional information is required if the relative contributions of the phenolic and ammonium deprotonations are to be assessed.[2]

Two different approaches are possible for resolution of the microconstants shown in Chart I or similar systems. If the two acidic groups are associated with characteristic spectral properties, deprotonation of one group may be followed independently of the other as illustrated below for ultraviolet absorption. In the other method one of the two deprotonation modes in Chart I is blocked by substitution, often of a methyl group for hydrogen. The competing phenolic and ammonium deprotonations in tyrosine were resolved by a study of the ether *O*-methyltyrosine where only the ammonium or k_2 process in Chart I occurs with $pk_2 = 9.28$ as listed in Table I.[1,2] N methylation to yield the betaine *N*-methyltyrosine gave a pk_1 value that was about 0.15 log unit too high when compared with other, internally consistent results.[1] Thus model compound substitution studies must be employed with some caution.

Methyl blocked derivatives were employed by an Italian school to resolve a correctly formulated microconstant scheme similar to Chart I for norepinephrine and epinephrine (*N*-methylnorepinephrine).[6] The dimethyl ether was employed to estimate pk_2, and in conjunction with titration results for pK_1 and pK_2, pk_1 was calculated by use of eq 6–8. Their results are quoted in Table I. These same authors have also determined that for *N*,*N*-dimethylnorepinephrine $pk_1 = 8.88$ so that a comparison is possible with a betaine model. The results of our calculations for the betaine are shown in Table I, where the ether and betaine give

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

(6) C. Sinistri and L. Villa, *Farmaco, Ed. Sci.*, **17**, 949 (1962), quoted by P. Pratesi and E. Grana, *Advan. Drug Res.*, **2**, 127 (1965).

Table I: Acidity Constants for Phenolic Amines

	pK_1	pK_2	pk_1	pk_2	$R = k_1/k_2$	$pk_{21} - pk_1$	T, °C	μ	Method	Ref
p-Tyramine	9.61	10.65	9.70	10.32	4.2	0.24	25	0.10	Spectra	*a*
	9.53	10.78	9.66	10.11	2.8	0.54	20	0.10	Spectra	*b*
Tyrosine ethyl ester	7.33	9.80	9.42	7.33	0.008	0.38	25	0.16	Titration	*c*
Tyrosine	9.12	10.20	9.63	9.28	0.44	0.41	25	0.16	Spectra and ether	*c*
DOPA	8.76	9.84	8.97	9.17	1.6	0.46	25	0.16	Spectra	*d*
Dopamine	8.87	10.63	8.90	10.06	15	0.54	20	0.10	Spectra	*b*
Norepinephrine	8.73	9.84	8.92	9.18	1.8	0.47	25	~0	Ether	*e*
	8.73	9.84	8.88	9.27	2.5	0.43	25	~0	Betaine	*e*
	8.64	9.70	8.70	9.54	6.9	0.10	25	0.10	Spectra	*f*
	8.73	9.78	8.84	9.37	3.4	0.30	20	0.10	Spectra	*b*
Epinephrine	8.79	10.10	8.88	9.51	4.3	0.50	25	~0	Ether and betaine	*e*
	8.66	9.95	8.72	9.57	7.1	0.32	25	0.10	Spectra	*f*
	8.71	9.90	8.81	9.39	3.8	0.41	20	0.10	Spectra	*b*
Isopropylnorepinephrine	8.82	10.20	8.91	9.58	4.7	0.53	25	~0	Ether	*e*
	8.72	9.87	8.81	9.43	4.1	0.35	20	0.10	Spectra	*b*

[a] Calculated from ref 8 as described in text. [b] Calculated from ref 7 as described in text. [c] References 1 and 2. [d] This work. [e] Reference 6. [f] Calculated from ref 8 and 9 as described in text.

identical conclusions for epinephrine and somewhat different though similar answers for norepinephrine.

Phenolic group ionizations give rise to a marked increase in absorption at 295 nm. This absorption is perturbed little if at all by the ammonium group deprotonation in tyrosine,[1,2] and the same assumption is made for the molecules of this study. The phenolic ionization may be followed separately by ultraviolet absorption spectra and the results utilized to determine all micro- and macroconstants on the molecule of interest, independently of separate titration experiments, which may be employed as a check. The method, originally developed for application to tyrosine,[2] is described briefly for new results obtained with DOPA.

The fraction of all molecules with ionized phenolic groups is defined by

$$\alpha = \frac{[^{+}\mathrm{HNXYRO}^{-}] + [\mathrm{NXYRO}^{-}]}{[\text{all species in chart I}]}$$

We may also define a function M such that

$$M = \frac{(\mathrm{H}^{+})\alpha}{1 - \alpha} = \frac{k_1(\mathrm{H}^{+}) + k_2k_{21}}{(\mathrm{H}^{+}) + k_2} \qquad (9)$$

The last equality, following from the definitions of the microconstants, has been derived previously.[2] The function M would represent a simple acidity constant over a range of (H^+) if the phenolic ionization occurs separately either in solutions more acid (k_1) or more basic (k_{21}) than the ammonium deprotonation. In cases where the two ionizations are competitive M is not constant but varies with (H^+) according to eq 9.

Equation 9 may be written in logarithmic form to give

$$\mathrm{p}M = \mathrm{pH} - \log\frac{\alpha}{1 - \alpha} = -\log\frac{k_1(\mathrm{H}^{+}) + k_2k_{21}}{(\mathrm{H}^{+}) + k_2} \qquad (10)$$

A plot of pM *vs.* α would yield a horizonatal straight line if the phenolic ionization occurs without competition from any other. Figure 1 shows the results for DOPA; the curvature indicates that the phenolic and ammonium deprotonations occur in the same pH region. From eq 10 it is evident that the left-hand intercept of Figure 1 at high (H^+) yields pk_1 and the right-hand intercept at low (H^+) gives pk_{21}. From the intercepts of Figure 1 for DOPA, we obtain pk_1 = 8.97 and pk_{21} = 9.42. The shape of the curve in Figure 1 is determined by k_2 as indicated in eq 10 and several methods have been described for determining

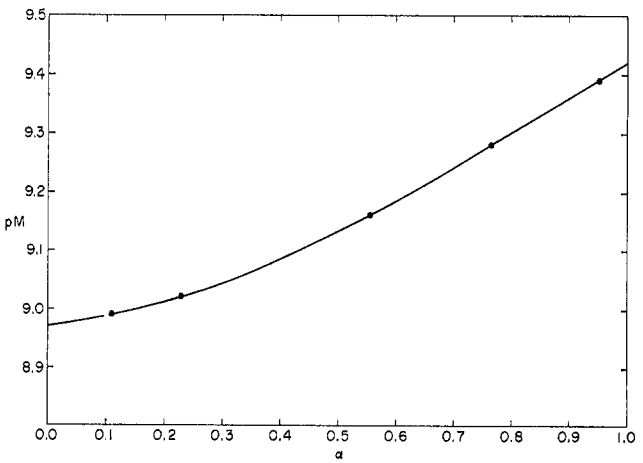


Figure 1. Plot of pM *vs.* fraction of molecules with ionized phenolic groups according to eq 5 for L-3,4-dihydroxyphenylalanine (DOPA). The data were obtained at 0.16 ionic strength and 25.0°.

the value of this constant.[1,2] For DOPA we obtain $pk_2 = 9.17$ and from eq 8, $pk_{12} = 9.62$. These four microconstant values determined by spectrophotometric titration may be combined according to eq 6 and 7 to yield $pK_1 = 8.76$ and $pK_2 = 9.83$. These calculated values for the macroconstants are in excellent agreement with the values determined directly by potentiometric titration at 0.16 ionic strength as described above in the section on macroconstants.

Table I includes results for the two macroconstants and two microconstants quoted as pk_1 and pk_2. The values of the two remaining microconstants, pk_{21} and pk_{12}, are easily calculated by eq 8 from the other values given and so are not listed in Table I. The ratio of molar concentration of zwitterionic to neutral forms, $[^+HNXYRO^-]/[NXYROH] = k_1/k_2 = R$, is tabulated in Table I for each method employed for every compound.

Two sets of workers measured the increase in absorption due to ionization of a phenolic hydrogen in a number of phenolic amines.[7,8] They did not formulate a microconstant scheme nor appreciate the variation in their calculated acidity constants with pH as a consequence of eq 10 as illustrated for DOPA in Figure 1. Both groups incorrectly assigned the acidity constant determined by standard methods for the phenolic absorption as K_1, but qualitatively consistent with the tendency of the phenolic group to ionize early in the titration curve of the two acidic groups for the compounds studied. Utilizing their data, we offer a reinterpretation of their results. We consider only those papers where two deprotonations are recognized in the pH 6–11 region of phenolic amines.

Let us assume that the spectrophotometrically determined acidity constants incorrectly assigned as K_1 represent an average of several determinations such that they correspond to a value of M in eq 9 where $\alpha = 0.5$, at the midpoint of phenolic ionization. We label as K_p this value of M at $\alpha = 0.5$ and note that K_p is some weighted average of k_1 and k_{21}. The acidity constant symmetrically related to K_p on the titration curve we label K_N. From eq 2 it is evident that $K_pK_N = K_1K_2$. By substitution of $M = K_p = (H^+)$ in eq 9 we obtain

$$K_p - K_N = k_1 - k_2 \qquad (11)$$

This equation when combined with eq 6 yields a solution to k_1 and k_2. Thus the results from the spectrophotometric studies can be reinterpreted to resolve the microconstant equilibria if we can assume that K_p corresponds to the point where $\alpha = 0.5$ in eq 9 and a value of K_1 is presented.

In his paper on ionization of sympathomimetic amines, Lewis remarks that pK_a values determined spectrophotometrically were higher than those determined by potentiometric titration.[7] This result is in accord with the equations presented in this paper which indicate that $K_1 > K_p$. Only for a phenolic ionization occurring unencumbered at more acid pH than ammonium deprotonation may K_p approach K_1. For five compounds for which Lewis gives pK_1 as well as pK_2 and pK_p values, the microconstants calculated with the aid of eq 6 and 11 are listed in Table I. Unfortunately in five monohydroxyphenylethanolamines, where the difference $pK_p - pK_1$ is of the order of 0.5, Lewis considered the equation he employed for overlapping macroconstants inapplicable and did not report pK_1 values. These five compounds, including synephrine (*p*-sympatol), are those in which the modes of deprotonation are most competitive. Since the five compounds exhibit similar values, we take averages of $pK_p = 9.57$ and $pK_N = 9.73$. Assuming $pK_1 = 9.07$ yields $pk_1 = 9.33$, $pk_2 = 9.42$, and $R = 1.2$. Though approximate, the value of R near unity provides a quantitative expression of the nearly equal proclivities of the phenolic and ammonium groups to deprotonate in these five monophenols.

In their paper on acidity of phenolic amines Kappe and Armstrong[8] do not consider the microconstant scheme and with one incidental exception report only pK_p and pK_N values (they incorrectly assign them as pK_1 and pK_2). In order to determine the microconstants from their paper, pK_1 values need be known and evidently are not generally available. Their description of experimental procedure mentions that $\bar{h} = 0.67$ at $pK_p = 9.74$ for *p*-tyramine. This information along with $pK_N = 10.52$ permits calculation of pK_1 by eq 4. This result and the microconstants evaluated are listed at the top of Table I. For norepinephrine and epinephrine the pK_p and pK_N values of these authors are combined with pK_1 and pK_2 values reported elsewhere in the literature[9] and obtained under identical conditions and with a similar sum to yield another set of values recorded in Table I for these compounds. The relatively high values of $R \sim 7$ probably indicate that the pK_p value is low.

The ratio R of zwitterionic to neutrally charged forms of the phenolic amines should be less dependent upon conditions than the microconstants from which the ratio is derived. Excluding the high values mentioned in the previous paragraph, there is excellent agreement among R values determined by totally different methods for the same compound. Even for *p*-tyramine (4-hydroxyphenylethylamine) the different values of 2.8 and 4.2 appear to exaggerate the difference between 74 and 81% zwitterionic form. Except for the two tyrosine compounds, the results of Table I indicate that the phenolic amines exist predominantly in the zwitterionic rather than the neutrally charged form of the two groups. In the pH 9–10 region these phenolic amines

(7) G. P. Lewis, *Brit. J. Pharmacol. Chemother.*, 9, 488 (1954).
(8) T. Kappe and M. D. Armstrong, *J. Med. Chem.*, 8, 368 (1965).
(9) R. F. Jameson and W. F. S. Nellie, *J. Chem. Soc.*, 2391 (1965).

are more aptly described as ammonium catecholates (or phenolates). Since both zwitterionic and neutral species bear identical net charges the R values are valid at all pH even when neither form predominates, as is the case at pH 7 for all compounds in Table I.

The reciprocal effects of a charged and uncharged ammonium group on the phenolic ionization and of an uncharged and charged phenolic group on the ammonium deprotonation are measured by the difference $pk_{21} - pk_1 = pk_{12} - pk_2$. Since the distance between these charge centers is nearly the same for all compounds listed in Table I, the pk differences should be similar and a further check is provided on the validity of the microconstant values.[1] As expected the pk differences tend to increase with decreasing ionic strength. Except for a low value in a result already mentioned as abnormal for norepinephrine, the close agreement among the pk differences indicates that the microconstant values listed present an accurate description of the equilibria involved.

Since the pk difference or the ratio $k_1/k_{21} = k_2/k_{12} \equiv S$ depends upon the structure of the compound, values may be estimated for a related series of compounds such as appear in Table I.[1,2] Unfortunately a knowledge of the ratio S does not provide the full fourth item of information to go with eq 6–8 required to resolve the four microconstant values. The relation among the two macroconstants and the ratios R and S is given by $(R + 1)^2/R = K_1/K_2S$. If the three values on the right-hand side are known, an ambiguity remains in a given case as to whether the solution of the equation yields R or $1/R$.

Experimental Section

L-3,4-Dihydroxyphenylalanine from Sigma Chemical Co. was 96% pure as determined by titration. Potentiometric titrations were performed at 5×10^{-3} M concentration with standard base at 25.0° in a thermostated reaction vessel under nitrogen passed through vanadous chloride scrubbers. Titration curves were recorded on a Radiometer TTT1a-SBR2b Titrator-Titrigraph combination. Spectrophotometric titrations were also performed under nitrogen. Absorbances were read on a Cary 11 spectrophotometer at 295 and 300 nm where, as illustrated for epinephrine,[7] the difference between ionized and un-ionized phenolic absorbances is nearly maximal and that of the acid form is off a steep slope. For the species of DOPA with one phenolic group completely ionized, an absorption maximum appears at 293 nm with ϵ 4400 near pH 11. The value of pk_1 obtained in Figure 1 was checked at each experimental point by the last equality of eq 8 in ref 2. Tris(hydroxymethyl)aminoethane and glycine buffers at about 0.01 M and DOPA at 1.6×10^{-4} M were employed to obtain the results plotted in Figure 1. In all experiments the ionic strength was controlled at 0.16 M with KCl, and the temperature was 25.0°.

In addition to the problem of proton binding sites in DOPA, there are two potentially chelating metal ion binding loci. On the basis of spectral comparisons with the alanine complex at pH 5 and the catechol complex at pH 9 it has been claimed that copper(II) chelates to DOPA in a glycinate mode at the lower pH and at the ortho phenolate groups at the higher pH.[5] The absorption scale in this reference is presented in arbitrary units so that a quantitative analysis is impossible. Nevertheless the absorption at 418 to 450 nm in the copper(II) complexes of catechol and DOPA at pH 9 seemed puzzling. The wavelength is much too short to be a ligand field band due to any oxygen donor atoms to copper(II). As a charge-transfer absorption band was a possibility, we undertook experiments to determine the molar absorptivities of the complexes. We find that solutions at pH >9 containing 2:1 molar ratios of either catechol or DOPA and Cu(II) exhibit absorption peaks at 400 and 418 nm, respectively, and that absorption in this region increases with time in the presence of air. Since intense absorption in the 400–450-nm region is typical of ortho-quinones in aqueous solutions and pyrocatechols are known to autoxidize easily to quinones and other products, the absorption may be accounted for. Some kind of Cu(II) complexes may also be involved, but we suggest that use of absorption spectra in the 400–450-nm region as a criterion for mode of metal ion binding in catechols be reinvestigated. An analysis of alternative bidentate binding modes based on estimated formation constants similar to that performed for histidine[10] suggests that for DOPA, Cu(II) binds predominantly at the substituted glycine locus in acid and neutral solutions with the catechol mode becoming dominant by pH 8 and increasingly so at higher pH. Though the overall binding is weaker, Mn(II) should bind predominantly in the catechol mode even in neutral solutions.

Acknowledgment. I thank Dr. Patsy Bostick Reed for performing the experimental measurements. This research was supported by a grant from the National Science Foundation awarded for other projects.

(10) E. W. Wilson, Jr., M. H. Kasperian, and R. B. Martin, *J. Amer. Chem. Soc.*, **92**, 5365 (1970).