Acid Dissociation Constants of Phenylalkanolamines

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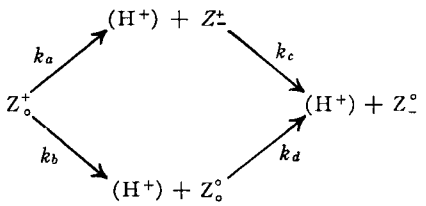
The acid dissociation constants of phenylephrine, synephrine, and tyramine were investigated by the new spectrophotometric method of Edsall. Epinephrine was studied by a titration and complexation method. It is concluded from analysis of the spectral data that the phenolic function is a stronger acid than the alkylammonium group. This is contrary to the accepted assignment of acid strengths to these acid functions. The spectrophotometric data were analyzed for the individual microscopic dissociation constants and the degree of dipolar character is reported.

THE ACCURATE evaluation of the dissociation constants of phenylalkanolamines is important to an understanding of their association and chelation reactions, their stability, and their comparative biological activity. Phenylalkanolamines may be considered initially as substituted phenols and compared to the acid strength of *o*-, *m*-, and *p*-cresol, whose dissociation constants are 10.32, 10.09, and 10.27, respectively (1). In contrast, the acidic dissociation constants of primary and secondary phenylalkylammonium groups vary from 9.8 to 10.8. Alkanolamines are somewhat stronger acids, i. e., ethanolamine has a pKa of 9.44 (2).

By analogy to these compounds, one would consider the first ionization step during the titration of a phenylalkanolamine salt to be related to the amine while the overlapping second dissociation step would be assigned to the phenol. However, evidence will be reported in this paper which will prove that the phenol group in these compounds is a stronger acid than is the alkanolammonium group.

It must be noted also that the ratio of the two successive dissociation constants obtained by pH titration may be as small as tenfold. A conventional titration, in which pH is determined as a function of the mean number of protons removed from the compound by the addition of hydroxyl ions, is not easily analyzed when the dissociation constants overlap. It is particularly difficult when the ratio of the constants for the two successive dissociation steps is 100 or less. Even when the ratio of the first dissociation constant to the second is as high as 500, one of the constants cannot be evaluated without correcting for the effect of the other, inasmuch as some simultaneous dissociation occurs. Bates (3) has stated that, under these conditions, accurate values may be unobtainable by the usual experimental procedures and has made use of the Speakman method (4) to analyze the data.

When both the phenolic and the alkylamine groups are found in the same molecule, they will not coexist without a degree of internal neutralization. Therefore, the compounds will be partly dipolar in character, depending on the relative strengths of the individual groups. At varying acidities, the molecules will exist in four different species. These may be represented as shown below



where Z^+_o = the phenylalkanolammonium ion, Z^- = the phenolate ion, and Z^+_- and Z°_o = the dipolar ion and uncharged molecule, respectively. The individual stages in the ionization result in individual or microscopic dissociation constants. These are noted as k_a, k_b, k_c, and k_d in the above diagram. The macroscopic or titration constants K_1 and K_2 bear a definite relation to the microscopic constants[1]

$$K_1 = k_a + k_b \qquad \text{(Eq. 1)}$$

$$K_2^{-1} = k_c^{-1} + k_d^{-1} \qquad \text{(Eq. 2)}$$

$$K_1K_2 = k_ak_c = k_bk_d \qquad \text{(Eq. 3)}$$

The assignment of K_1 or K_2 as determined from a titration of a compound to their corresponding dissociation function may be difficult on the basis of this information alone.

Spectrophotometry offers an alternative approach to this problem. It requires that one of the acidic groups in the molecule shall have an

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[1] All the constants listed in the foregoing equations are acid dissociation constants. The existing literature citations are by no means uniform in the assignment of symbols for the microscopic acid dissociation constants. We have chosen to use the subscripts *a* to *d* rather than Arabic or Roman numerals to denote the different constants. It should be pointed out, however, that k_b represents an acid dissociation constant for the reaction shown in the diagram given above.

absorption spectrum distinguishable from its conjugate base. This is the case with the phenylalkanolamines. The phenol peak of the three phenylalkanolamines studied has an adsorption maximum in the region 2,720 to 2,750 Å. and shifts to 2,900 to 2,930 Å. upon complete conversion to the phenolate form. The molar extinction coefficient at 2,940 to 3,000 Å. is negligible for the forms in which the OH group is unionized, whereas it is large in the forms in which $-OH$ has been converted to the anion ($-O^-$). See Fig. 1. At pH values below 12.0, there are two forms in which the $-O^-$ exists, i. e., Z^+_- and Z°_-.

Edsall has shown that it is possible to obtain the individual constants for tyrosine by appropriate analysis of the ultraviolet absorption spectra over a wide range of pH values (5). He defined a function α_{OH}, which represents the fraction of all the phenolic hydroxyl groups in the solution which are ionized

$$\alpha_{OH} = \frac{\text{concn. of all molecules with ionized OH}}{\text{concn. of all molecular species}} = [Z^+_-] + [Z^\circ_-]/[Z^+_-] + [Z^\circ_-] + [Z^\circ_\circ] + [Z^+_\circ] = \frac{k_a(H^+) + k_ak_c(H^+)^2}{1 + (k_a + k_b)/(H^+) + k_ak_c/(H^+)^2} \quad \text{(Eq. 4)}$$

Edsall further defined M_{OH}

$$M_{OH} = \frac{(H^+)\alpha_{OH}}{(1 - \alpha_{OH})} = \frac{k_a(H^+) + k_ak_c}{(H^+) + k_b} \quad \text{(Eq. 5)}$$

or from Eq. 3

$$M_{OH} = \frac{k_a(H^+) + k_bk_d}{(H^+) + k_b} \quad \text{(Eq. 6)}$$

or in the logarithmic form

$$pM_{OH} = pH + \log(\alpha_{OH}/1 - \alpha_{OH}) \quad \text{(Eq. 7)}$$

$$= -\log \frac{k_a(H^+) + k_bk_d}{(H^+) + k_b} \quad \text{(Eq. 8)}$$

It is apparent from Eqs. 7 and 8 that a plot of pM_{OH} *vs.* α_{OH} will extrapolate to pk_a when $\alpha_{OH} = 0$ ($[H^+] \rightarrow \infty$) and to pk_d when $\alpha_{OH} = 1$ ($[H^+] \rightarrow 0$).

Once pk_a and pk_d are known, it is possible to obtain pk_b from the best fit to the experimental data. From Eq. 7, one will note that at $\alpha_{OH} = 0.5$, $pM_{OH} = pH$. Substituting this value for pM_{OH} in Eq. 8 and solving for k_b gives

$$k_b = \frac{(H^+)[k_a - (H^+)]}{(H^+) - k_d}, \text{ at } \alpha_{OH} = 0.5 \quad \text{(Eq. 9)}$$

Alternatively, a second approach leads to the value of k_b. Considering the value of α_{OH} corresponding to the point at which $pM_{OH} = (pka + pkd)/2$, then for this particular value of $M = (k_ak_d)^{1/2}$ we obtain from Eq. 6

$$pk_b = \log \frac{\alpha_{OH}}{1 - \alpha_{OH}} + pk_a \quad \text{(Eq. 10)}$$

The above calculation is exact if the molar extinction coefficient of the two phenolate forms Z^+_- and Z°_- are identical at the wavelengths chosen for the analysis. At the worst, it is a close approximation for the phenylalkanolamines. The same applies to the free phenol forms Z°_+ and $Z^\circ_\circ$, but is less significant since they make a very small contribution to the total results. If either assumption were incorrect, repeating the analysis at several different wavelengths should result in different values for the derived constants. The data obtained for the three phenylalkanolamines herein reported met this critical test.

EXPERIMENTAL

Apparatus.—Melting points were determined on a Kofler melting point apparatus. Polarimetric measurements were done on a Rudolph research polarimeter. The pH measurements were made on a Beckman model G pH meter. The ultraviolet absorption spectra were measured on a Cary model 11 recording spectrophotometer.

Materials.—l-Phenylephrine hydrochloride U. S. P. (Gane and Ingram Co., N. Y.) was used; m. p. 134–144°; specific rotation $[\alpha]^{25}_D$ −43°. Tyramine monohydrochloride (Nutritional Biochemicals Corp.), m. p. 269°. Synephrine tartrate was furnished by Dr. M. L. Tainter of Sterling Winthrop Research Institute. In several instances, the free bases were recovered, recrystallized, and used for the studies. There appeared to be no difference in extinction coefficients relative to the original salts. Epinephrine base (Desmo Chemical Corp.) meeting U. S. P. specifications was used as received.

Procedure.—Stock solutions were freshly prepared and diluted to approximately 3×10^{-3} to $4 \times 10^{-4} M$. All solutions were adjusted to a constant ionic strength of 0.1 *M* by the addition of potassium chloride. The recording spectrophotometer was carefully adjusted to zero absorbance prior to the determination and verified regularly between determinations. The instrument was carefully adjusted to wavelength and to zero absorbance at 3,500 Å. where the solutions were optically transparent.

The solutions were neutralized directly in the cell by the addition of a 5 *M* solution of acid or base using a micropipet. A range of pH values from less than 4.0 to greater than 12.5 was covered. The Beckman model G pH meter with a type E-2 electrode was checked for calibration, using the appropriate primary or secondary buffer standard recommended by the National Bureau of Standards, immediately before and after each pH reading. Complete neutralization of the sample modified the volume less than 0.5%. In all instances, the pH of the solution being studied was determined directly in the cell before and after analysis of its U. V. ab-

sorption. The values rarely differed more than 0.02 pH unit. It was found necessary to add a buffer to the synephrine solutions in order to stabilize the pH values in the intermediate range. Tris-(hydroxymethyl)-aminomethane was used as the buffer at a concentration of approximately tenfold of the drug concentration. The buffer is optically transparent in the region of study. In the acidic range, a more concentrated solution of the phenylalkanolamine was used when necessary in order to increase the accuracy of the determination of the absorbance of the solutions predominately containing the free phenol forms. However, in order to calculate the fraction in the phenolate ion form, the solution had to be brought to the pH >12.5. A carefully calibrated spacer was used to reduce the path length in order to obtain the absorbance of the highly absorbing phenolate ion form. In order to reduce the effect of oxidative changes, all solutions were freshly prepared just prior to the study. The solutions remained colorless throughout the study. The absence of oxidative changes was proved by checking whether or not the original spectrum was recovered by conversion of the alkaline solution back into the free phenol form.

Calculations of pM_{OH} values were made at three wavelength values in the region of 2,920 to 3,000 Å. where the extinction coefficient of the free phenol form is minimal, but the phenolate forms result in a high extinction. Within experimental error, the results reported are independent of the wavelength selected.

The titrations of epinephrine in the presence and absence of boric acid were run in a four-neck, round-bottom flask of 500-ml. capacity. The epinephrine base was dissolved with a slight excess of hydrochloric acid adjusted to 0.1 *M* with potassium chloride, and titrated with carbon dioxide-free potassium hydroxide. The titrating solution and the flask were flushed free of dissolved oxygen by passing oxygen-free nitrogen through the solution. The nitrogen flushing was continued during the run. Only an insignificant discoloration appeared at the end of the titration. The nitrogen was freed of residual oxygen by passing the gas through a gas purification train containing vanadous sulfate (6) and 2,6-anthroquinone disulfonate as oxygen getters.

RESULTS AND DISCUSSIONS

Figure 1 illustrates a typical determination of ultraviolet absorption data of phenylephrine. It is generally recognized that binary mixtures of compounds will possess isosbestics at points of intersection when only the mole fraction is varied. Examination of the graphs indicate that the solutions do not possess perfect isosbestic points; they have slightly varying points of intersection. These results were demonstrated not to arise from errors in the measurement procedure. This indicates that there are three or more absorbing species present in the majority of the solutions studied. Imperfect isosbestic points may be seen in the published data of Edsall, *et al.*, on glycyl-L-tyrosine at various pH values (7); however, they did not comment on the significance of this phenomenon.

The ultraviolet absorption data were converted to the appropriate form according to Eq. 7. The results are plotted in Fig. 2 in the form of pM_{OH}

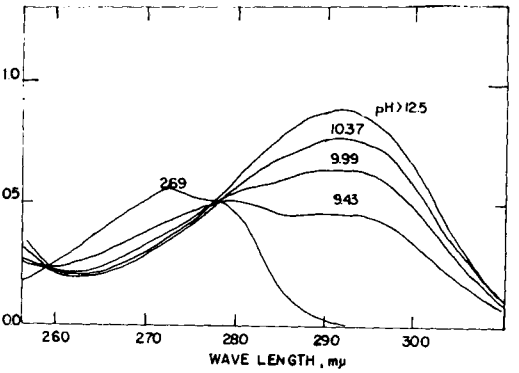

Fig. 1.—Ultraviolet absorption spectrum of phenylephrine hydrochloride at various pH values; temperature 22–23°; ionic strength 0.10.

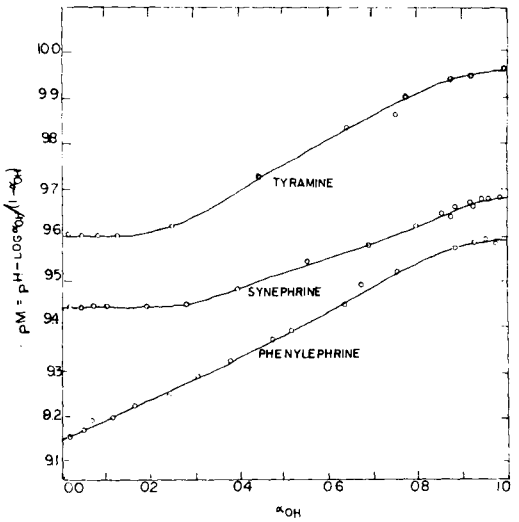

Fig. 2.—Values of pM_{OH} as a function of α_{OH} for phenylephrine, synephrine, and tyramine, respectively (see Eqs. 7 and 8).

vs. α_{OH}. As discussed above, the extrapolation to $\alpha_{OH} = 0$ and $\alpha_{OH} = 1$ results in the determination of pk_a and pk_d, respectively. These assignments are explicit from the equations listed earlier. Equations 9 and 10 were both used for the estimation of pk_b. The values were in good agreement. The solid line drawn through the points is the result of the substitution of the three microscopic constants into Eq. 8. Modification of the experimental microscopic constants by ±0.02 results in theoretical curve which deviates markedly in the sensitive portion of the graph.

Table I summarizes the data on the macroscopic and microscopic acid dissociation constants for the compounds studied. Both Fig. 2 and Table I indicate that the *meta*-substituted compound possesses ionization properties which differ from those of the *para*-substituted compounds. It is well recognized that several resonant forms of phenols exist with centers of high electron density at the *ortho* and *para* positions relative to the phenol group. Thus, phenylephrine with its *meta* substitution possesses carbons of increased negative charge density on

TABLE I.—NEGATIVE LOGARITHMS OF IONIZATION CONSTANTS

Constant[a]	Compound: Phenylephrine	Synephrine	Tyramine
pK_1	8.77	9.29	9.37
pK_2	9.84	10.24	10.70
pk_a	9.14	9.44	9.59
pk_b	9.02	9.84	9.66
pk_c	9.47	10.09	10.03
pk_d	9.59	9.69	9.97
$Z_-^+/Z_o^{\circ b}$	0.76	2.51	1.18

[a] Estimated error = ±0.02. $k_a/k_b = k_c/k_d = Z_-^+/Z_o^\circ$.

either side of the alkanolamine chain, while synephrine does not. An atomic model of the compounds indicates that the secondary amine group can make contact with these carbon atoms. The values listed for the pk_a, pk_b, and pk_c for phenylephrine show increased acidity relative to the analogous *para*-substituted compounds. In contrast the values for the pk_d of phenylephrine and synephrine show a smaller difference, more analogous to that shown by *m*-cresol when compared with *p*-cresol.

Table I includes the macroscopic acid dissociation constants as calculated by Eqs. 1 and 2. A similar spectrophotometric study was conducted in 1954 by Lewis (8) who determined the ionization constants of sympathomimetics including the drugs used in this study. He calculated the phenolic dissociation constants, pK_1, on the basis of an assumption that only one species contributed to the phenolate spectrum or to the free phenol spectrum. Our data, however, show the failure to produce unequivocal

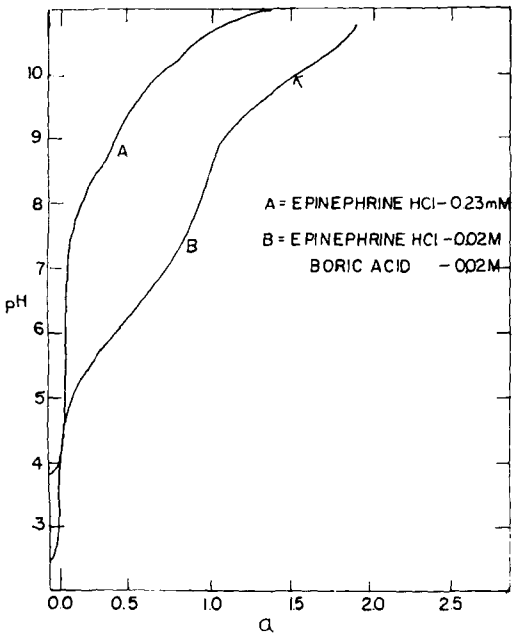

Fig. 3.—Titration of epinephrine hydrochloride with KOH, in the absence (*A*) and presence (*B*) of equimolar boric acid; ionic strength = 0.1; "a" denotes moles of base used per mole of epinephrine added.

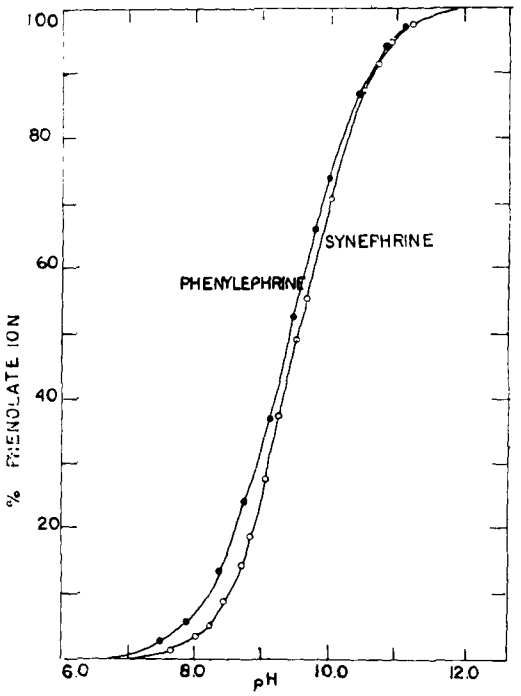

Fig. 4.—The degree of ionization of the phenol group of phenylephrine and synephrine (α_{OH}) as a function of pH.

isosbestic points in these systems. Therefore, the assumption of Lewis is unwarranted for these compounds and probably for the other dibasic compounds so calculated. Lewis then used the value obtained to estimate the pK_2 of the amino group from titration studies. Although both pK's are quantitatively inexact, they do confirm our conclusion that the conjugate acid of the amine is a weak acid relative to the phenol group. For experimental and theoretical reasons discussed in this paper, the acid dissociation constants (titration constants) determined by Tuckerman, *et al.* (9), using the approximation method of Parke and Davis (10) are invalid relative to their assignment of the particular dissociation constant to the amino group as well as to the relative accuracy of calculated constants.

The complication ensuing in the assignment of the titration acidity constants is illustrated in Fig. 3, the plot of our titration data of epinephrine in the absence (*A*) and presence (*B*) of equimolar concentration of boric acid. Boric acid is known to form chelates with adjacent or *cis* oriented hydroxy groups. In this manner, it is possible to block the normal ionization characteristics of phenolic groups. The first dissociation step in curve *B* of Fig. 3 is believed to be due to the second reaction represented below

$$H\,B + E \rightleftarrows H\,B\,E$$

$$H\,B\,E \rightleftarrows H^+ + B\,E^-$$

where HB = boric acid and E = epinephrine. Since the hydroxyl groups are blocked and no excess of boric acid is present, the second equivalent of base

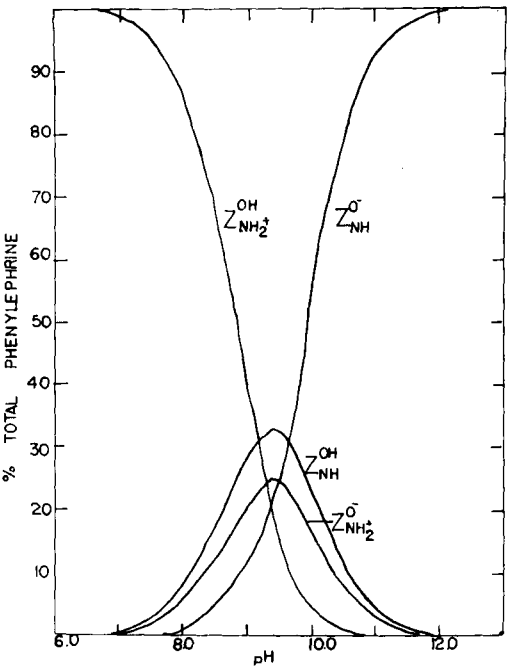

Fig. 5.—Relative concentrations of different microscopic forms of phenylephrine at different pH values.

which is utilized in the reaction must be assigned to the ionization of the secondary alkylammonium group of epinephrine. This leads to a pK_- of approximately 9.9. Tuckerman, *et al.*, incorrectly assigned a value of 8.55 to this functional group in epinephrine. Our titration value for epinephrine is in good agreement with the value of Lewis (7). It should be noted also that the pK's obtained by Albert (11) for tyramine of $pK_1 = 9.3$, $pK_2 = 10.9$ are in fair agreement with our results as shown in Table I considering the ionic strength effect and the differences in the method of calculation.

Table I also includes the ratio of the number of molecules in the dipolar ion form to the number in the uncharged form. This ratio gives no indication of the absolute amount of any of the species. However, these may be derived by further analysis of the absorption spectra data according to the procedures used by Benesch and Benesch (12). Figure 4 represents the degree of ionization of the phenol group (α_{OH}) of phenylephrine and synephrine as a function of pH, which is the sum of $Z^{\pm}_{-}$ and Z°_{-}. The data in Fig. 4, along with the fact that the ratio of the dipolar to the uncharged molecule is constant at all pH values, provide two simultaneous equations and to solve them for the relative concentrations of the different microscopic forms at different pH values. Figure 5 illustrates such an analysis of the data found for phenylephrine. It should be noted that the compound possesses significant concentrations of all species in the pH range from 8 to 10.5. At pH 9.35 where the dipolar ion form reaches its maximum concentration, the solution contains approximately 25% $Z^{\pm}_{-}$, 33% $Z^{\circ}_{\circ}$, 21% Z°_{-}, and 21% $Z^{+}_{\circ}$. It has been assumed, on occasion, that the evaluation of the ratio of $Z^{\pm}_{-}/Z^{\circ}_{\circ}$ was identical with a complete calculation of the per cent of the dipolar ion form. Such as assumption would lead to a conclusion that phenylephrine was 43% in the dipolar form. The more definitive analysis as given above shows that phenylephrine possesses a maximum of 25% in the dipolar ion form and this only at a pH of 9.35.

Postulation as to the significance of the individual microscopic dissociation constants on the stability and on the association or chelate formation properties of phenylalkanolamines will be left to a later publication.

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