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THE IONIZATION OF 5-HYDROXYTRYPTAMINE AND RELATED COMPOUNDS AND AN APPRAISAL OF METHODS FOR THE ESTIMATION OF ZWITTERION CONSTANTS

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1 The dissociation constants of 5-hydroxytryptamine and 5-hydroxy-*N*,*N*-dimethyltryptamine have been measured and the zwitterion constants have been estimated from the pK_as of analogous methoxyamines and the phenolic quaternary ammonium salt.

2 Direct measurement of zwitterion constants has been made by a spectroscopic method which has been used also with several phenolic amines previously studied electrometrically. It makes fewer assumptions and so should be more reliable but an appraisal of the methods available indicates that none can be singled out as being best and it is desirable to obtain results with as many as possible.

3 The results consolidate previous observations on the relations between chemical structure and zwitterion constant; possibly zwitterions are stabilized by any factor which stabilizes water structure. Dimethylamino compounds have lower zwitterion constants than their methylamino or amino-analogues but there is no reason to doubt the previous finding that diethylamino compounds have higher zwitterion constants than their dimethylamino analogues.

4 The proportion of 5-hydroxytryptamine present as the neutral molecule or zwitterion in physiological conditions is small; however, with dopamine, the proportion present as the zwitterion could be as much as 10%.

Introduction

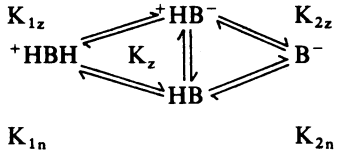
The ionization constants of 5-hydroxytryptamine are not known accurately. The values quoted by Perrin (1965; 1972) are confused by the inclusion of the pK_a of the creatinine in the complex used in the electrometric titration and the best values appear to be those of Vane (1959), 10.0 and 11.1, with concentrations of 0.02 M at an unspecified temperature. These values were assigned to the amino and phenolic groups, respectively, and from the studies of phenolic amines by Armstrong & Barlow (1976) there is reason to believe that this is incorrect and the compound exists to a considerable extent as a zwitterion.

Accurate measurements of the pK_as of 5-hydroxytryptamine have therefore been made, together with measurements on related compounds which should make it possible to assess the extent of zwitterion formation, as in work with phenolic amines and catecholamines (Sinistri & Villa, 1962; Armstrong & Barlow, 1976). Direct measurements of zwitterion constants at 25° and 37°C have also been made by a spectroscopic method. This was also used with tyramine and some other phenolic amines previously studied electrometrically and the results obtained by the various methods have been compared to try to assess which is most suitable.

Methods

Theory

If the dissociation of the 5-hydroxytryptamine ion, or any other phenolic ion, is represented



the equilibrium constants are:

$$K_{1z} = \frac{[^+HB^-]\,[H^+]}{[^+HBH]f}$$

$$K_{2z} = \frac{[B^-]\,f[H^+]}{[^+HB^-]}$$

$$K_z = \frac{[^+HB^-]}{[HB]}$$

$$K_{1n} = \frac{[HB]\,[H^+]}{[^+HBH]f} = \frac{K_{1z}}{K_z}$$

$$K_{2n} = \frac{[B^-]\,f[H^+]}{[HB]} = K_{2z}K_z$$

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where $[H^+]$ refers to activities, measured with the glass electrode, but the other species are in molar concentrations and f is the activity coefficient for a univalent ion. The activity coefficient of both the neutral species is assumed to be unity. For small zwitterions the effects of the opposite charges should cancel out. For larger zwitterions there will be an error; K_z and K_{1z} should be multiplied by the activity coefficient and K_{2z} should be divided by it (see discussion).

In the electrometric titration the dissociation into both $^+HB^-$ and HB is measured and the first dissociation constant,

$$K_1 = K_{1z} + K_{1n} = K_{1z}\left(1 + \frac{1}{K_z}\right)$$

For the second dissociation

$$\frac{1}{K_2} = \frac{1}{K_{2z}} + \frac{1}{K_{2n}} \quad \text{and} \quad K_2 = K_{2z}\left(\frac{K_z}{1 + K_z}\right)$$

The zwitterion constant and individual dissociation constants can be estimated in the following ways:

(A) *From the pK_as of analogues using any of the following assumptions*: (i) that the pK_a of the methoxy compound, $pK_{Me} = pK_{1n}$; (ii) that the pK_a of the quaternary ammonium salt, $pK_Q = pK_{1z}$; (iii) that the pK_a of a non-charged phenolic compound, such as 5-hydroxy-*N*-acetyltryptamine, $pK_{Ac} = pK_{2n}$. Accordingly

$$pK_Q - pK_1 = \log\left(1 + \frac{1}{K_z}\right)$$

$$pK_{Me} - pK_1 = \log(1 + K_z)$$

$$pK_2 - pK_1 = pK_{Ac} - pK_{Me} + 2\log(1 + K_z)$$

$$pK_{Me} - pK_Q = \log K_z.$$

These methods have all been used for phenolic amines (Armstrong & Barlow, 1976) and some for catecholamines (Sinistri & Villa, 1962) but the assumptions may not be justified and the methods do not give the same answer.

(B) *From a combination of electrometric and spectroscopic results.* Sinistri & Villa (1962) assumed that at a wavelength where the phenate group, $R\text{-}O^-$, absorbs strongly compared with the phenolic group, R-OH, the spectroscopic dissociation constant,

$$K_s = \frac{[^+HB^- + B^-][H^+]}{[^+HBH + HB]}$$

will approach K_{1z} at the more acid end of the region where dissociation occurs and the dissociation of the second group may be ignored. They then used this value to calculate K_z from K_1 (though the ionic strength was not the same in both types of experiment). K_s is not a constant, however, and the ratio of the concentration of phenate to phenolic forms

$$R = \frac{[^+HB^-] + [B^-]}{[^+HBH] + [HB]} = \frac{f\dfrac{K_{1z}}{[H^+]} + \dfrac{K_{1z}K_{2z}}{[H^+]^2}}{1 + \dfrac{fK_{1z}}{[H^+]K_z}}$$

so

$$R[H^+]^2 = \frac{fK_{1z}}{K_z}([H^+]K_z - R[H^+]) + K_{1z}K_{2z} \tag{1}$$

and R varies with $[H^+]$ in a complex way.

For the ionization of a phenolic quaternary ammonium salt, $K_z = \infty$ and $K_{2z} = 0$ and the equation becomes $R = fK_{1z}/[H^+]$, so the graph of log R against pH should be a straight line with a slope of unity and when $\log R = 0$, $pH = pK_{1z} - \log f$. If K_{1z} and K_{2z} are replaced by K_1 and K_2:

$$R[H^+]^2 = \frac{fK_1}{K_z}\left(\frac{K_z}{1 + K_z}\right) \times ([H^+]K_z - R[H^+]) + K_1K_2$$

and

$$K_z = \frac{C - BR - A}{A - B - C},$$

where

$$A = R[H^+]^2,$$

$$B = fK_1[H^+]$$

and

$$C = K_1K_2.$$

(C) *From spectroscopic results alone.* The equation (1) can be written:

$$R[H+]^2 = af[H^+] + bfR[H^+] + c$$

so from values of R and $[H^+]$, a least-squares fit of $R[H^+]^2$ on $f[H^+]$ and $fR[H^+]$ can be made by calculus and used to calculate a, b and c and hence K_{1z}, K_{2z} and K_z. Similarly a fit of $fR[H^+]$ can be made on $f[H^+]$ and $R[H^+]^2$; a fit can also be made of $f[H^+]$ on $fR[H^+]$ and $R[H^+]^2$. These transformations each introduce a different bias into the estimates. The first and the third usually gave similar values of the ionization constants but it is difficult to judge which should be the most reliable and the average value from all three was taken.

A least-squares fit of log R on pH should be more satisfactory because the errors are not compounded but it cannot be obtained by calculus. It can, however, be made by repeated calculations in which pK_{1z} and

$\log K_z$ are altered, provided these are set at starting values fairly close to those indicated by the other methods. Alterations were continued until changes in pK_{1z} or $\log K_z$ were less than 0.001 units. All line-fitting programs were written in Fortran with all small numbers in double-precision.

Electrometric titrations

These were made exactly as described by Armstrong & Barlow (1976). The only difference in the apparatus was the replacement of the pH meter by a more accurate digital instrument (Metrohm model E500). The only difference in the calculation of the results was the need to allow for the presence of oxalate in some of the salts; it was also necessary to include oxalate and sodium chloride in the ionic strength used to calculate the activity coefficients.

Armstrong & Barlow (1976) had observed that even with activity coefficients calculated from Debye-Hückel theory for the appropriate ionic strength and temperature, the estimates of ('thermodynamic') pK_a were not independent of concentration or ionic strength. Measurements were therefore made as before with each compound over a range of concentrations and fitted to the expression $pK = pK_0 + mC^{\frac{1}{2}}$. Measurements were also made in 0.1 M NaCl as well as in water. The temperatures were $25 \pm 0.1°$ and $37 \pm 0.1°C$.

Spectroscopic measurements

Solutions of 0.1 M NaOH and 0.1 M glycine were used to prepare a suitable range of buffers which were then diluted 1 to 10 with 0.1 M NaCl. Solutions of the phenolic amines in a concentration of 2×10^{-4} M were then prepared by diluting a 10^{-2} M stock solution in water 0.5 to 25 with each of the diluted buffers. The absorbance was measured with a Unicam SP 1800 spectrophotometer at 323 nm for 5-hydroxytryptamine and 5-hydroxy-*N*,*N*,dimethyltryptamine, at 295 nm for *p*-phenolic amines and at 290 nm for *m*-phenolic amines, and the pH was then measured. The solutions were kept stoppered and the pH measurement was made under nitrogen (as in the electrometric titrations). The wavelengths correspond to a maximum absorption by phenate, and from the absorbance in the presence of 0.1 M NaOH and the (very small) absorbance in 0.1 M HCl, the ratio, R, of phenate to phenolic species was calculated. Between 10 and 20 values of R and pH were obtained for each compound at each temperature. The ionic strength was assumed to be that of 0.1 M NaCl, because all other ions are present only in much lower concentrations, and the activity coefficient, f, for all univalent ions was taken as 0.79. The temperatures were $25 \pm 0.2°$ and $37 \pm 0.2°C$.

Compounds

The sources of materials or their melting-points and analyses are included in Table 1, unless the sample was the same as that used by Armstrong & Barlow (1976). The phenolic amines were selected so that from the previous results they were expected to cover a range of zwitterion constants from about 0.5 to about 5.0.

Microanalyses (C,H,N) are by Mr M. West, University of Bristol. Halide analyses are gravimetric with samples of 50 to 150 mg.

Results

The results of the electrometric titrations are shown in Table 1. They confirm the finding by Armstrong & Barlow (1976) that estimates of the thermodynamic pK_a are not truly constant but usually increase with concentration. The effects are more marked at lower concentrations and the empirical fit to the expression $pK_a = pK_0 + mM^{\frac{1}{2}}$ has been used as before to calculate the interpolated value at 10 mM and the extrapolated value, pK_0, which may not be very accurate. Measurements made in 0.1 M NaCl give values which are consistently higher than those in water so in attempts to estimate zwitterion constants it is important that the estimates of pK_a should all have been obtained in identical conditions.

In a few instances measurements were made which duplicated those of Armstrong & Barlow (1976). The agreement is within about 0.1 units for values at 10 mM. With the more accurate meter, the results in the present work are more likely to be correct. The statistical error associated with each titration is small (the standard error is usually less than 0.02 with from 9 to 15 measurements) but it seems that there are other greater sources of error, and that an accuracy of ± 0.05 units for values at 10 mM is the best that can be expected. Because of the need to use oxalate salts in some instances, a comparison was made of tryptamine hydrochloride and tryptamine oxalate in water at 25°C and the values at 10 mM were 9.80 and 9.88, respectively.

As a check on the value of spectroscopic information, estimates of the pK_as of phenolic quaternary ammonium salts were obtained and compared with those obtained electrometrically. When values of the logarithm of the ratio, R, of phenate to phenolic species were plotted against pH, satisfactory straight lines were obtained with slopes close to unity (Figure 1). A least-squares fit was used to calculate the pH when $\log R = 0$ and hence to estimate the pK_a: all measurements were made in 0.1 M NaCl and the activity coefficient, f, for singly charged ionic species was taken as 0.79, the most common value

Table 1 Estimates of pK_a

	pK_a			
	0 mM	*10 mM*	*m*	*n*
Tryptamine HCl, Sigma, recrystallized:				
in water, 25°C, 5–20 mM	9.43	9.80	0.117	11
in 0.1 M NaCl, 25°C, 5–20 mM	10.00	10.10	0.031	8
in water, 37°C, 5–15 mM	9.29	9.61	0.102	7
in 0.1 M NaCl, 37°C, 5–20 mM	9.79	9.81	0.005	6
Tryptamine oxalate, recryst. EtOH/H_2O, m.p. 141–143°C: found: C, 54.7; H, 5.75; N, 10.5; $C_{12}H_{14}N_2O_4$, H_2O requires: C, 53.7; H, 6.01; N, 10.4%				
in water, 25°C, 5–12 mM	9.48	9.88	0.129	6
5-Methoxytryptamine HCl, Aldrich:				
in water, 25°C, 5–20 mM	9.32	9.77	0.144	10
in 0.1 M NaCl, 25°C, 5–20 mM	10.08	10.11	0.010	7
in water, 37°C, 5–15 mM	9.34	9.67	0.104	6
in 0.1 M NaCl, 37°C, 5–15 mM	9.77	9.83	0.020	6
N,N-dimethyltryptamine oxalate, recryst. EtOH/H_2O, m.p. 149–150°: found: C, 60.0; H, 6.68; N, 9.96; $C_{14}H_{18}N_2O_4$ requires: C, 60.4; H, 6.52; N, 10.1%				
in water, 25°C, 5–12 mM	8.84	9.56	0.227	9
in 0.1 M NaCl, 25°C, 5–12 mM	9.50	9.65	0.047	3
in water, 37°C, 5–12 mM	8.99	9.33	0.108	8
in 0.1 M NaCl, 37°C, 5–12 mM	9.53	9.46	−0.022	6
5-Methoxy-N,N-*dimethyltryptamine oxalate*, recryst. EtOH/H_2O, m.p. 172.1–173.2°C (dec): found: C, 58.5; H, 6.53; N, 8.80; $C_{15}H_{20}N_2O_5$ requires: C, 58.4, H, 6.54; N, 9.09%				
in water, 25°C, 5–12 mM	9.18	9.51	0.104	6
in 0.1 M NaCl, 25°C, 5–12 mM	9.84	9.60	−0.076	4
in water, 37°C, 5–12 mM	8.90	9.25	0.111	8
in 0.1 M NaCl, 37°C, 5–12 mM	9.46	9.37	−0.028	8
5-Hydroxyindolylethyltrimethylammonium bromide (quaternary 5-HT), recryst. MeOH, m.p. 212.8–213.4°C (dec): found: C, 51.8; H, 6.23, N, 9.03; Br, 27.5; $C_{13}H_{19}N_2OBr$ requires: C, 52.2; H, 6.40; N, 9.36; Br, 26.7%				
in water, 25°C, 5–21 mM	9.68	10.07	0.123	8
in 0.1 M NaCl, 25°C, 5–20 mM	10.19	10.40	0.067	8
in water, 37°C, 5–20 mM	9.91	10.13	0.070	8
in 0.1 M NaCl, 37°C, 5–20 mM	10.23	10.27	0.015	8
5-Hydroxy-N-*acetyltryptamine*, Sigma:				
in water, 25°C, 5–15 mM	9.21	10.09	0.279	5
in 0.1 M NaCl, 25°C, 5–15 mM	10.44	10.63	0.061	3
in water, 37°C, 5–15 mM	9.40	10.06	0.207	3
in 0.1 M NaCl, 37°C, 10–15 mM	10.40	10.58	0.059	2
5-Hydroxytryptamine oxalate, Sigma:				
in water, 25°C, 5–15 mM	8.91	10.14	0.388	6
	10.06	11.05	0.313	
in 0.1 M NaCl, 25°C, 5–15 mM	9.69	9.89	0.064	6
	10.89	11.13	0.075	
in water, 37°C, 5–15 mM	9.17	9.51	0.108	6
	10.16	10.76	0.190	
in 0.1 M NaCl, 37°C, 5–15 mM	9.38	9.57	0.060	6
	10.73	10.93	0.065	
Vane (1959) recorded values of 10.0 and 11.1 (20 mM, temperature not stated)				
5-Hydroxy-N,N-*dimethyltryptamine oxalate*, recryst. EtOH/Et_2O, m.p. 88–91°C: found: C, 53.4; H, 6.73; N, 8.57; calc. for $C_{14}H_{18}O_5N_2$, H_2O; C, 53.8; H, 6.45; N, 8.97%				
in water, 25°C, 5–15 mM	9.57	9.45	−0.040	6
	10.38	10.83	0.143	
in 0.1 M NaCl, 37°C, 9.95 mM (one titration only), $pK_1 = 9.29 \pm 0.003$; $pK_2 = 10.92 \pm 0.006$ (s.e., 7 estimates)				
Vane (1959) recorded values of 9.8 and 11.2 (20 mM, temperature not stated)				
Tyramine HCl, Sigma:				
in 0.1 M NaCl, 25°C, 5–15 mM	9.36	9.50	0.046	6
	10.79	10.86	0.019	
in 0.1 M NaCl, 37°C, 5–15 mM	9.10	9.25	0.046	6
	10.57	10.62	0.024	

Table 1—*continued*

	pK_a 0 mM	10 mM	m	n
p-*Hydroxyphenethyldimethylamine HBr*:				
in 0.1 M NaCl, 25°C, 5–15 mM	9.03	9.10	0.023	6
	10.33	10.42	0.029	
in 0.1 M NaCl, 37°C, 5–16 mM	9.97	9.09	0.037	4
	10.35	10.48	0.042	
m-*Hydroxyphenethylmethylamine HBr*:				
in 0.1 M NaCl, 25°C, 5–16 mM	9.30	9.37	0.042	5
	11.06	11.02	−0.015	
in 0.1 M NaCl, 37°C, 5–15 mM	9.08	9.16	0.008	4
	10.90	10.73	−0.053	
m-*Hydroxyphenethyldimethylamine HCl*:				
in 0.1 M NaCl, 25°C, 5–15 mM	8.87	8.96	0.027	6
	10.17	10.27	0.032	
in 0.1 M NaCl, 37°C, 5–15 mM	8.80	8.94	0.043	4
	10.26	10.32	0.020	
Dopamine HCl, Sigma:				
in 0.1 M NaCl, 25°C, 5–15 mM	9.09	9.01	−0.024	11
	10.74	10.81	0.062	
in water, 37°C, 5–15 mM	8.04	8.47 (8.45)	0.135	6
	9.37	10.02 (9.74)	0.207	
in 0.1 M NaCl, 37°C, 5–15 mM	8.51	8.67	0.051	3
	10.28	10.40	0.038	
p-*Hydroxyphenethyltrimethylammonium bromide*, recryst. MeOH, m.p. 261.0–261.6°C: found: Br^-, 30.79; $C_{11}H_{18}ONBr$ requires Br^-, 30.71%				
in 0.1 M NaCl, 25°C, 5–19 mM	9.42	9.72	0.094	6
in 0.1 M NaCl, 37°C, 5–19 mM	9.41	9.42	0.004	4
m-*Hydroxyphenethyltrimethylammonium bromide*, recryst. MeOH, m.p. 191.5–192.0°C: found: Br^-, 30.77; $C_{11}H_{18}ONBr$ requires Br^-, 30.71%				
in 0.1 M NaCl, 25°C, 5–19 mM	9.70	9.61	−0.027	5
in 0.1 M NaCl, 37°C, 5–20 mM	9.37	9.30	−0.021	5
3,4-Dihydroxyphenethyltrimethylammonium bromide, recryst. MeOH, m.p. 237.0–238.0°C; (dec): found: Br^-, 29.28; $C_{11}H_{18}O_2NBr$ requires Br^-, 28.93%				
in 0.1 M NaCl, 25°C, 5–20 mM	8.82	8.88	0.020	4
in water, 37°C, 5–20 mM	8.13	8.49 (8.66*)	0.112	4
in 0.1 M NaCl, 37°C, 5–20 mM	8.75	8.71	−0.014	4
Adrenaline quaternary chloride:				
in 0.1 M NaCl, 25°C, 5–20 mM	8.66	8.69	0.011	4
in water, 37°C, 5–20 mM	7.93	8.31	0.120	7
in 0.1 M NaCl, 37°C, 5–20 mM	8.51	8.53	0.007	4
Phenethylamine HCl:				
in water, 25°C, 9–19 mM	9.38	9.67 (9.74)	0.090	9
in 0.1 M NaCl, 25°C, 5–21 mM	9.71	9.88	0.055	4
in water, 37°C, 5–20 mM	8.97	9.30 (9.46)	0.105	8
in 0.1 M NaCl, 37°C, 5–20 mM	9.47	9.57	0.032	8
p-*Methoxyphenethylamine HCl*:				
in 0.1 M NaCl, 25°C, 5–15 mM	10.04	10.04	0.001	4
in 0.1 M NaCl, 37°C, 5–20 mM	9.67	9.71	0.010	6
p-*Methoxyphenethyldimethylamine HCl*:				
in 0.1 M NaCl, 25°C, 5–20 mM	9.36	9.40	0.014	4
in 0.1 M NaCl, 37°C, 5–20 mM	9.13	9.21	0.024	4
m-*Methoxyphenethylmethylamine HCl*:				
in 0.1 M NaCl, 25°C, 5–15 mM	10.32	10.20	−0.038	5
in 0.1 M NaCl, 37°C, 5–15 mM	9.81	9.82	0.002	3

Table 1—*continued*

	pK_a 0 mM	pK_a 10 mM	m	n
m-*Methoxyphenethyldimethylamine HBr*, recryst. ethylmethylketone, m.p. 123.0–123.8°C: found: Br^-, 30.70, $C_{11}H_{18}ONBr$ requires Br^-, 30.71%				
in 0.1 M NaCl, 25°C, 5–15 mM	9.39	9.36	−0.012	5
in water, 37°C, 5–20 mM	8.68	9.92	0.076	6
in 0.1 M NaCl, 37°C, 5–20 mM	9.01	9.07	0.019	7
3,4-Dimethoxyphenethylamine HCl:				
in 0.1 M NaCl, 25°C, 5–20 mM	9.98	9.98	−0.001	6
in 0.1 M NaCl, 37°C, 5–20 mM	9.71	9.75	0.012	8

The range of concentrations is shown, the number of titrations (n), the slope (m) for the expression $pK = pK_0 + mC^{\frac{1}{2}}$ to which the values of pK_a were fitted by least-squares, weighted according to the reciprocal of the variance of each titration (which involves from 9 to 15 measurements of alkali added and pH). The values of pK at 0 mM and 10 mM calculated from the expression are shown and the values at 10 mM in parentheses were obtained by Armstrong & Barlow (1976) in what should be identical conditions, though the asterisk indicates that the bromide was used instead of the iodide.

used in the electrometric titrations in these conditions. There is moderate agreement between the two methods (Table 2); some of the discrepancies can be ascribed to the extrapolation necessary to estimate the electrometric pK_a at concentrations similar to those used spectroscopically.

This difference in concentration creates problems in the assessment of K_z by combining electrometric values of pK_1 and pK_2 with spectroscopic values of R and pH. The electrometric values should be for concentrations of 0.2 mM, which are not known as accurately as those at 10 mM.

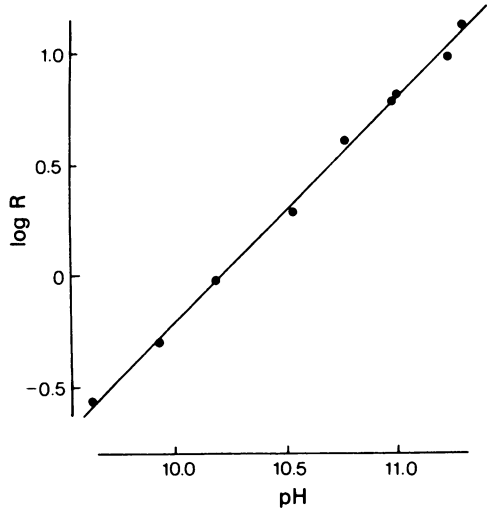

Figure 1 Ionization of 5-hydroxyindolylethyltrimethylammonium bromide ('5-HT quaternary'), 2×10^{-4} M in 0.1 M NaCl at 37°C. Values of the logarithm of the ratio, R, of phenate to phenolic, measured at 323 nm, are plotted against pH. The line is the least-squares fit and has a slope 1.00 ± 0.02 (s.e.). The $pK_a = 10.206 + \log(0.79) = 10.10$.

When only the spectroscopic values of R and pH were used to calculate K_z the difference in the estimates obtained by the three transformations was considerable. For 5-hydroxytryptamine at 25°C the estimates of pK_{1z} were 10.28, 10.06, 10.27; for pK_{2z} they were 11.29, 10.13, 10.99; for K_z they were 2.2, 0.2, 1.4. The average values of pK_{1z} and of pK_{2z} and the geometric mean of K_z were 10.20, 10.80 and 0.85, close to those obtained by the fit of log R on pH, which were 10.27, 10.82 and 1.0. The effects of pK_{1z}, pK_{2z} and K_z on fit are illustrated in Figure 2.

The mean of the estimates of K_z obtained by all the various methods is shown for each compound in Table 3, together with the number of times each method produced an estimate which differed by more than one standard deviation from the mean. This indicates the extent to which a method agrees with the general concensus but this does not necessarily indicate its accuracy. It is possible that many of the methods are similarly biassed, though there is no evidence for this.

Discussion

The results show the extent to which different methods give different estimates of zwitterion constants. The standard deviation of the mean values in Table 3 is usually at least 50% and the highest value can be as much as 5 times the lowest.

Because it makes fewer assumptions the spectroscopic method should give more reliable estimates;

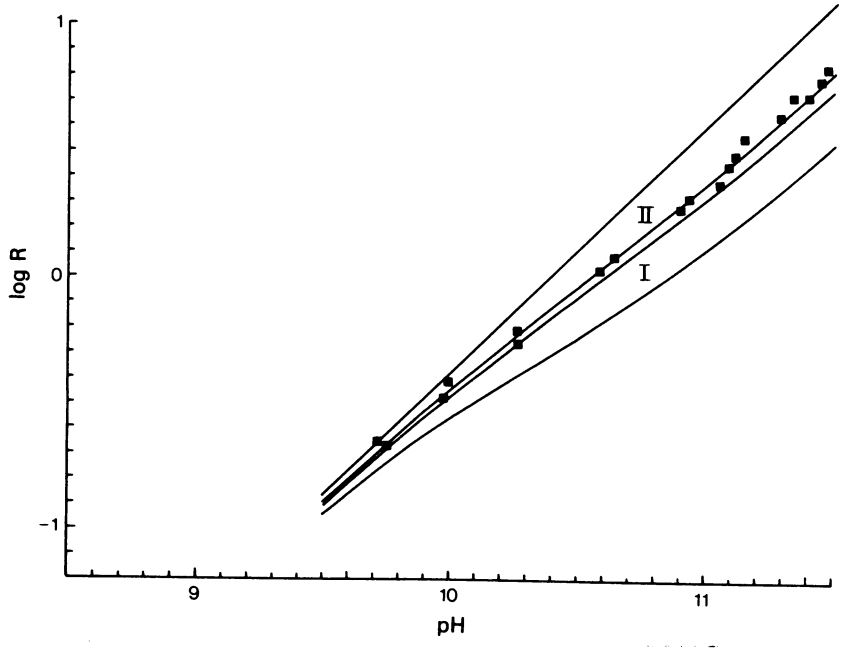

Figure 2 Log R plotted against pH for 5-hydroxytryptamine, 2×10^{-4} M in 0.1 M NaCl at 25°C. The line I shows the computed values with $pK_{1z} = 10.20$, $pK_{2z} = 10.80$ and $K_z = 0.79$; line II shows the computed values with $pK_{1z} = 10.27$, $pK_{2z} = 10.82$ and $K_z = 1.0$. The bottom line is obtained with the same values of pK_{1z} and pK_{2z} but with $K_z = 0.5$ and the top line with $K_z = 2.0$.

however, there is the practical difficulty that an error in the absorbance affects the estimates of phenate and phenolic in opposite ways and so has an exaggerated effect on the ratio R, especially when this is very small or very large. In this work, values have been restricted to the range from R = 0.1 to 10. The treatment of spectroscopic results by the fit of log R on pH should be better than by pooling values from the three transformations because the residual least squares (*es*) are appreciably lower (usually between one-half and one-tenth). In spite of this, the fit of log R on pH produces three discrepancies in Table 3 compared with two for the pooled transformations. Surprisingly the combination of spectroscopic and potentiometric results produces only one discrepancy but there is also another instance in which it fails to work and yet another in which shortage of material made it impossible to obtain enough potentiometric results to make the extrapolation.

With many compounds a spectroscopic method is

Table 2 pK_a values of quaternary ammonium salts measured spectrometrically (S) compared with values measured electrometrically (E), all in 0.1 M NaCl

	pK_a (S) 0.2 mM	pK_a (E) 0 mM	pK_a (E) 10 mM
5-Hydroxyindolylethyltrimethylammonium bromide			
25°C	10.34	10.19	10.40
37°C	10.10	10.23	10.27
m-*Hydroxyphenethyltrimethylammonium bromide*			
25°C	9.32	9.70	9.61
37°C	9.38	9.37	9.30
p-*Hydroxyphenethyltrimethylammonium bromide*			
25°C	9.50	9.42	9.72
37°C	9.41	9.41	9.42

Table 3 Estimates of zwitterion constants calculated from: (A) Electrometric values in 0.1 M NaCl at 10 mM of pK_1, pK_2, pK_{Me} (for the methoxy compounds) and pK_Q (for the trimethylammonium compounds) using the relations: *1* $pK_Q - pK_1 = \log(1 + 1/K_z)$; *2* $pK_{Me} - pK_1 = \log(1 + K_z)$; *3* $pK_2 - pK_1 = pK_{2n} - pK_{Me} + 2\log(1 + K_z)$; *4* $pK_{Me} - pK_Q = \log K_z$ where pK_{2n} was taken as the value of 5-hydroxy-*N*-acetyltryptamine, or for phenolic amines 9.80 (25°C) or 9.70 (37°C, Armstrong & Barlow, 1976), or for dopamine 9.50 (25°C) or 9.40 (37°C). (B) the combination of electrometric values of pK_1 and pK_2 extrapolated to 0 mM and spectroscopic values of R and pH. (C) spectroscopic values of R and pH alone: *1* shows the geometric mean of the values obtained by least-squares fit with the three transformations; *2* shows the least-squares fit of log R on pH. Values of pK_{1z} and pK_{2z} obtained by this last method are shown, together with the number (*n*) of measurements of R and pH; *es* is a measure of the fit, being $S(\log R_{obs} - \log R_{calc})^2/S(\log R_{obs})^2$.

	A				B	C		*mean*				
	1	*2*	*3*	*4*		*1*	*2*	± *s.d.*	pK_{1z}	pK_{2z}	*es*%	n
5-Hydroxytryptamine												
25°	0.4	0.7	1.3	0.5	0.4	0.8	1.0	0.73 ± 0.33	10.27	10.82	0.26	20
37°	0.2	0.8	1.0	0.4	0.2	0.5	0.8	0.56 ± 0.31	10.14	10.62	0.27	17
5-Hydroxy-N,N-*dimethyltryptamine*												
37°	0.1	0.2	0.6	0.1	—	0.4	0.4	0.30 ± 0.20	10.12	10.32	0.09	9
Tyramine												
25°	1.5	2.5	5.3	4.8	3.2	3.3	0.8	3.06 ± 1.63	9.48	9.82	0.19	24
37°	2.1	1.9	3.9	1.9	1.1	0.3	1.0	1.74 ± 1.15	9.43	10.12	0.25	12
p-*Hydroxyphenethyldimethylamine*												
25°	0.3	1.0	1.9	0.5	1.0	1.0	1.1	0.97 ± 0.51	9.40	10.03	1.06	16
37°	0.9	0.3	1.8	0.6	0.7	0.6	1.0	0.84 ± 0.48	9.43	10.10	0.22	11
m-*Hydroxyphenethylmethylamine*												
25°	1.4	5.8	9.6	3.9	nc	3.6	6.5	5.13 ± 2.83	9.26	10.54	0.02	10
37°	1.6	3.6	6.0	3.3	5.2	1.1	7.4	4.03 ± 2.30	9.18	10.99	0.13	12
m-*Hydroxyphenethyldimethylamine*												
25°	0.3	1.5	1.7	0.6	0.7	0.9	1.7	1.06 ± 0.57	9.33	10.30	0.94	20
37°	0.8	0.4	1.4	0.6	1.0	0.8	1.0	0.86 ± 0.32	9.19	9.91	0.11	12
ND	5	2	10	1	1	2	3					
Dopamine												
25°	nc	8.3	12.8	12.6				11.23 ± 2.54				
37°	10.4	11.0	10.0	11.0				10.60 ± 0.49				

The mean of all the estimates of K_z for each compound is shown with the standard deviation: the number of instances a method produced an estimate different from this mean by more than one standard deviation from the mean is indicated by ND.

In some instances (nc) the results gave values of K_z which were negative and in one instance (−) there were not enough electrometric results for extrapolation to low concentration.

not possible. With dopamine the rapid oxidation in alkaline solutions makes it difficult to measure the absorbance accurately (the products interfere far more than they do in the electrometric titrations). With other compounds there is no suitable absorbing group and estimates can only be made from the pK_as of analogues. Often, in fact, the choice of method is limited by what is possible with the analogues available, rather than what is most desirable, and it is important to know how estimates may be expected to differ from one method to another. The method with analogues with the fewest discrepancies (A4) actually uses no experimental results from the parent compound, whereas the method (A3) which uses both experimental values from the parent compound (pK_1 and pK_2) produces the greatest number of discrepancies. With this the estimates of K_z are always higher than with other methods, possibly indicating that the value of pK_{2n} used is too low. The values of pK_{2n} for 5-HT calculated from the values of pK_{1z}, pK_{2z} and K_z shown in Table 3 are 10.82 at 25°C and 10.72 at 37°C; the corresponding values for 5-hydroxy-*N*-acetyltryptamine are 10.44 and 10.40. With any method involving analogues, there is considerable uncertainty attached to the underlying assumptions and in this instance the analogue does not adequately indicate the supposed pK. As already observed by Armstrong & Barlow (1976), method A1 is likely to be unreliable when K_z is large (because $1/K_z \rightarrow 0$) and it failed to work with the present results for dopamine

at 25°C, which has the highest zwitterion constant of those studied.

A present, therefore, it is not possible to single out any one method as being the most suitable and it is desirable to obtain estimates by as many methods as possible. Consistent trends are seen, for instance, in the overall means in Table 3 which are not always apparent from individual results. In theory the spectroscopic method with a fit of log R on pH should be best but the method using the electrometric pK_as of the methoxy and quaternary compounds produces results which differ least from the mean.

The correction of estimates of K_z to allow for the activity coefficient of the zwitterion is largely a matter for conjecture. It is not clear how far apart the charges should be for them to be regarded as independent. A divalent ion has an activity coefficient of about 0.5 in 0.1 M NaCl, so the true value K_z should lie between the value shown in Table 3 and half this. The mean values for the present estimates of K_z (25°C) of compounds in 0.1 M NaCl, however, are close to the mean values of estimates obtained by Armstrong & Barlow (1976) in water, suggesting that the activity coefficient is actually close to unity. For tyramine the mean of the previous estimates of K_z is 3.35; for *p*-hydroxyphenethyldimethylamine it is 0.87; for *m*-hydroxyphenethylmethylamine it is 6.27; for *m*-hydroxyphenethyldimethylamine it is 0.86 and for dopamine it is 7.8. Even the greatest difference, that between the results for dopamine, is not statistically significant ($P = 0.05$).

The results consolidate some of the observations made in the previous paper on the relations between structure and zwitterion constants. Dimethylamino-compounds, including 5-hydroxy-*N*,*N*-dimethyltryptamine, have much lower zwitterion constants than their amino- or methylamino- analogues. This suggests that the zwitterion may be stabilized by hydrogen bonding to water with the water as a hydrogen acceptor and this is consistent with the reduction in zwitterion constant seen when a hydroxyl group (also a hydrogen donor) is placed β- to the amino- group. The mean value of K_z for the β-hydroxylated tyramine, for example is 0.6. However, it does not easily explain the previous finding that diethylamino compounds have substantially higher zwitterion constants than their dimethylamino analogues. Although these results were only obtained electrometrically, the present work indicates that there is no reason to doubt this finding. Possibly zwitterions are stabilized by any factor which stabilizes water structure, either by a hydrophilic interaction with groups containing hydrogen donors or by a hydrophobic effect with large alkyl groups. The resultant decrease in entropy would apparently operate against such an effect but could be offset by the change in enthalpy. Large enthalpies would therefore be expected for zwitterion formation but although in all the instances in Table 3 the estimate of the mean zwitterion constant at 37° is lower than that at 25°C, the temperature coefficient is not large. With tyramine it is 1.76 but with the other compounds it is around 1.3. More work over a wider range of temperature is needed.

Although it is not yet possible to present a comprehensive theory to account for relations between structure and zwitterion constant, the results in this and the previous paper should provide some of the information on which a satisfactory theory may ultimately be based. The original assignment by Vane (1959) of 10.0 to the amino group and 11.1 to the phenolic group in 5-hydroxytryptamine is clearly incorrect and the neutral form of the molecule is about 40% present as the zwitterion at 37° in 0.1 M NaCl. However, at pH 7.6 the compound is at least 98% in the form with the charged amino group and an intact phenolic group. It seems unlikely that either the zwitterion or the uncharged form contributes to the biological activity. This is very different from the situation with dopamine, for which the present results indicate that there should be about 10% in the neutral form in these conditions, mostly as the zwitterion.

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