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THE IONIZATION OF PHENOLIC AMINES, INCLUDING APOMORPHINE, DOPAMINE AND CATECHOLAMINES AND AN ASSESSMENT OF ZWITTERION CONSTANTS

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1 The dissociation constants of many phenolic amines, including benzylamines, phenethylamines, phenylethanolamines, phenylpropylamines, catecholamines, and apomorphine have been measured by potentiometric titration at 25°C. Measurements have also been made with many of their methoxy derivatives and with series of phenolic quaternary ammonium salts. Some compounds were also studied at 37°C.

2 Usually at least five titrations were made with each compound and Debye–Hückel theory was applied to convert concentrations to activities but the estimates of pK_a were not constant and found to increase with increasing concentration. The range studied was usually 5–15 mM and a least-squares line-fit, based on the empirical assumption that pK_a varies with $(\text{concentration})^{1/2}$, has been used to calculate values for 10 mM solutions and to extrapolate to infinite dilution and to 100 mM. The dependence of pK_a on concentration was much less at 37°C than at 25°C.

3 At 37°C the pK_a values of many biologically interesting compounds in the group, dopamine, noradrenaline, adrenaline and isoprenaline, coryneine (the trimethylammonium derivative of dopamine) and apomorphine are within 1 log unit of physiological pH, indicating the presence of a significant proportion of either the zwitterion or of the uncharged phenolic amine.

4 Zwitterion constants have been estimated from the pK_a values of the phenolic amines and those of their methoxy and quaternary trimethylammonium analogues. Zwitterion formation does not appear to be associated with activity at α-adrenoceptors and probably not with activity at β-receptors. The active species seems likely to contain the unionised phenolic group but at dopamine receptors this may be in the uncharged phenolic amine rather than in the phenolic ammonium salt.

Introduction

Giesecke (1973) has pointed out that the dopamine 'molecule is hidden in the apomorphine skeleton' and suggested that this might account for the central effects of apomorphine at dopamine-sensitive receptors. The structural relationship is striking and easily seen from molecular models: *N,N*-dimethyldopamine, for instance, can theoretically be derived from apomorphine simply by breaking three bonds (Figure 1).

Results in the literature, however, suggest that the ionization of apomorphine is markedly different from that of dopamine. Kolthoff (1925) obtained pK values at 15°C of 7.0 and 8.92 from the titration of apomorphine with alkali (he used indicators to measure the pH), but he considered that the first value was the basic dissociation constant K_b of the amino group and the second value that of the acidic phenolic group. Phenolic amines with pK values as close to one another as this usually dissociate in the reverse order (see below) so his results indicate pK_a values at 15°C of 7.3 and 8.9 (Perrin, 1965). At physiological pH, therefore, a considerable proportion of the apomorphine should be in the form of the phenoxide ion. However, the pK_a values of dopamine obtained by Lewis (1954) were 8.9 and 10.6, so much less should be in the form of the phenoxide ion at physiological pH. It therefore seemed necessary to check the pK_a values of these substances and to measure those of related compounds, such as *N,N*-dimethyldopamine.

In the course of the work it became apparent that there were some uncertainties about estimates of the pK_as of phenolic and catecholic amines. In the work of Lewis (1954) both spectroscopic and potentiometric methods were used at 20°C; the concentrations were 0.2 mM and 10 mM, respectively but the ionic strength

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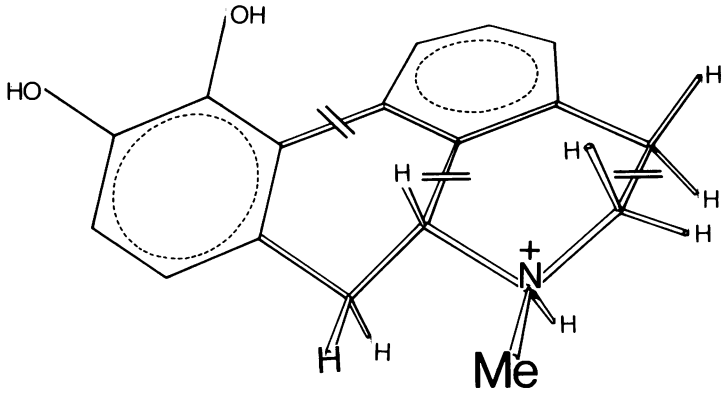


Figure 1 Structure of R-(–)-apomorphine; *N,N*-dimethyldopamine would be obtained by cutting the three bonds indicated.

was kept at 0.1 M by the addition of sodium chloride. Sinistri & Villa (1962) and Villa & Sinistri (1963), working at 25°C, used a spectroscopic method with concentrations of 0.2 mM and ionic strength of 0.1 M and a potentiometric method with concentrations of 5 mM and ionic strength between 5 and 9 mM. The glass electrode measures hydrogen ion activities and they corrected their pK_a values for activity coefficients based on Debye–Hückel theory and so obtained 'thermodynamic' values which should be independent of ionic strength (Güntelberg, 1926; Albert & Sergeant, 1962; 1971). Kappe & Armstrong (1965) used a spectroscopic method at 25°C with concentrations of 0.1 to 0.2 mM and ionic strength 0.1 M and a potentiometric method at 35°C with concentrations of 20 mM and similar ionic strength. The spectroscopic method should give the dissociation of the phenolic group; the potentiometric method should give the dissociation of all ionizable groups and to simplify the calculations some workers have used values from the spectroscopic method in the results of the potentiometric titrations so that these need to be solved for only one dissociation constant. This simplification may not be justified when the conditions in the two types of experiment are different and is certainly not justified if the zwitterion ($^-OC_xH_yNR_2H^+$) does not predominate over the form $HOC_xH_yNR_2$ (see below). It is also unnecessary if modern computing facilities are available.

The work has therefore been extended to include a systematic survey of the pK_as of phenolic and catecholic amines, analogous non-phenolic amines, and phenolic and catecholic quaternary ammonium salts (made because of their interesting nicotine-like properties; Barlow, Thompson & Scott, 1969; Barlow, Bowman, Ison & McQueen, 1974).

Methods

pK_a measurements

Accurately weighed amounts of material (10–100 mg) were dissolved in a known volume of deionized distilled water (boiled to remove dissolved gases) and the pH was measured with a Metrohm E 512 meter and EA 121 combination glass electrode following the addition of accurately measured amounts of standardized NaOH from a Metrohm E 412 Dosimat (fitted with a 4 ml burette). The titration vessel was surrounded by water circulated from a Haake model FE thermostat with a temperature control better than ±0.1°C. An interval of at least 10 min was allowed for thermal equilibration before the start of any titration and about 1 min was allowed after each addition of alkali for equilibration of the glass electrode. A slow stream of N_2 was blown through the solution, which was stirred continuously with a Teflon coated magnetic stirrer. The meter was calibrated with buffers of pH 6.99 and 9.15 at 25°C (6.97 and 9.07 at 37°C). The instrument remained stable for long periods but the calibration at pH 9 was usually checked before each titration. Only occasionally was there any detectable drift.

Most compounds were titrated in amounts of 0.1, 0.15, 0.2, 0.25 and 0.3 mmol, dissolved in 20 ml of water. The NaOH (BDH 0.1 molar 'AVS', free from carbonate) was consistently found to be 0.0995 to 0.0996 molar by titration with HCl (0.10 molar prepared from BDH 'CVS' solutions). With some compounds the concentration which could be titrated was limited by the low solubility of the product formed by loss of a proton. This applied particularly to apomorphine which could not be studied in solutions

much above 1 mM (compared with 5 to 15 mM for most other compounds); the volume in these experiments was 30 ml but even with this increase the weight of material was only 6–12 mg and there must be an appreciable error associated with its measurement. For the other compounds, however, it is the ability to calibrate the meter which is the main source of error provided the compounds tested are of adequate purity. Most experiments were performed by a single operator (JA). Practice in reading the meter should reduce errors (and it is noticeable that results are often more scattered when these include those of other operators) but it seems likely that there is a systematic error of at least ± 0.01 units in the pK_a values, associated with the calibration of the instrument.

Calculation of results

It is necessary to know the molecular weight of the compound, the amount taken, the initial volume in which it was dissolved (usually 20 ml), the strength of the NaOH (usually 0.0996 M), the volume added and the corresponding pH. It is also necessary to know the ionic product of water and the Debye–Hückel parameters *A* and *B* (respectively 13.997, 0.5115 and 0.3291 at 25°C and 13.590, 0.5242 and 0.3318 at 37°C; Robinson & Stokes, 1965) and to estimate the size parameter, *a*, in the Debye–Hückel expression (Albert & Sergeant, 1971, use a value of 5 Å but we have used 6.0 Å, see below).

For compounds with only one titratable proton the concentration of the substance, C, and of alkali, A, is calculated for each value of added alkali and observed pH, together with the hydrogen and hydroxyl ion concentrations. For the process

$$BH^+ \rightleftharpoons B + H^+$$

the ionization constant

$$K = \frac{[B][H^+]}{[BH^+]}$$

so

$$pK_a = pH + \log \frac{[BH^+]}{[B]}$$

For electrical neutrality of the solution when NaOH is added to a chloride salt,

$$[BH^+] + [Na^+] + [H^+] = [OH^-] + [Cl^-]$$

The concentration, C, of the salt $= [Cl^-] = [B] + [BH^+]$; the concentration of alkali $A = [Na^+]$. Hence

$$[B] = A + [H^+] - [OH^-]$$

and

$$[BH^+] = C - A - [H^+] + [OH^-]$$

The ratio $[BH^+]/[B]$ should therefore decrease as the titration proceeds and A increases and C decreases. As the glass electrode measures hydrogen ion activities, however, $[BH^+]$ should be multiplied by its activity coefficient, f, if the thermodynamic pK_a is to be obtained (the activity coefficient of the uncharged species, B, is taken as unity). From Debye–Hückel theory

$$-\log f = \frac{A[I]^{1/2}}{1 + Ba[I]^{1/2}}$$

so the sum of the concentration of ions (all univalent) was used to calculate the ionic strength, I, and hence estimate the activity coefficient. This was usually between 0.88 (for titrations with 15 mM initial concentrations) and 0.92 (with 5 mM).

Each addition of alkali and measurement of pH provides an estimate of pK_a and the mean estimate ($\pm$s.e.) was calculated for the number of additions made, excluding any values where the ratio $[BH^+]/[B]$ was >10 or <0.1.

For the phenolic quaternary ammonium salts the process is identical except that the loss of a proton leaves a zwitterion $R_3\overset{+}{N}(C_nH_m)O^-$, instead of the uncharged base, B. The activity coefficient of a zwitterion, however, is usually taken as unity so the same computer programme can be used to treat results with either type of compound.

For equilibria of the type $\overset{+}{H}BH = {}^+HB^- = B^-$ correction for activity coefficients, f, must be made to $[\overset{+}{H}BH]$ and $[B^-]$ so

$$pK_1 = pH + \log \frac{[\overset{+}{H}BH]\,f}{[{}^+HB^-]}$$

and

$$pK_2 = pH + \log \frac{[{}^+HB^-]}{[B^-]\,f}$$

The total concentration of salt, C, $= [\overset{+}{H}BH] + [{}^+HB^-] + [B^-]$ and for electrical neutrality $A + [H^+] + [\overset{+}{H}BH] = [OH^-] + [B^-] + C$ where A is the concentration of alkali.

The four equations can be reduced to

$$\frac{A + [H^+] - [OH^-]}{f[H^+] + 2K_2} = \frac{fK_1C}{[H^+]^2 + [H^+]K_1 + fK_1K_2}$$

where $[H^+]$ and $[OH^-]$ represent ion activities. To calculate K_1 and K_2 from the experimental values of A, C and H^+ (and OH^-), some workers have inserted a value of K_1 estimated spectroscopically but objections to this have been given above. Britton (1942; see Albert & Sergeant, 1962) took pairs of experimental values and described a procedure for calculating K_1 and K_2 from each pair. Speakman (1940; see Albert & Sergeant, 1962) has shown that the equation above can be written in the form

$$X = K_1Y + K_1K_2$$

where X and Y are expressions involving C, A, H^+, OH^- and activity coefficients, and a least squares fit for all the titration values can be used to obtain the best estimates of K_1 and K_2 (from K_1K_2).

We have used Britton's method with the results arranged in pairs about the half-neutralization point (addition of 1 equivalent of alkali). A starting estimate of the pK values was needed in order to calculate the ionic strength and hence the activity coefficient and the calculations were then repeated with the calculated values of pK_a; no further repetition was usually necessary as it could be seen that the activity coefficients were only slightly altered.

With this method the observations must be made with pairs symmetrically arranged about the half-neutralization point. If the titration has been poorly planned there are obvious discrepancies between the pK estimates for the various pairs. With the line-fitting procedure only a single estimate is obtained for each pK from all the results and an answer can be obtained with poorly designed titrations where the fit involves only a small part of the titration curve. However, with observations properly spread over the whole titration there is little difference between the two methods. With either procedure there is greater uncertainty about the larger pK_a; the value of K_2 will be much smaller than that of K_1 and the line-fitting procedure may attach more weight to the first part of the titration curve.

The calculations, and subsequent ones relating to the dependence of pK on concentration, were made using programmes written in Fortran II with a PDP81 Digital Computer. Additional calculations have been made with the ICL 4/75 machine at the University of Bristol Computer Centre.

Compounds

Dr G.M. Thompson, Dr K.J. Turnbull and Mr J. Duguid prepared the samples indicated by their initials in Table 1; this also indicates the sources of samples of commercial origin. Analyses are given except when the samples were identical with those for which results have already been published (Barlow *et al.*, 1974).

Analyses are for ionized halide estimated gravimetrically, as in previous work, and melting points were recorded with a Mettler FP1 instrument and a Vitatron pen-recorder, with a rate of heating of 0.2°/minute. All the compounds which contain an asymmetric carbon atom were tested as racemates, except for phenylephrine and apomorphine.

We are most grateful to Macfarlan Smith Ltd for the gift of samples of apomorphine HCl, to Minnesota 3M Laboratories Ltd for (±)-adrenaline HCl, to May & Baker Ltd and Professor H.L. Friedman, Medical College, Wisconsin, for samples of the quaternary derivative of adrenaline.

Results

These are shown in Table 1. It was clear from experiments with phenethylamine hydrochloride that

Table 1 Analytical results are given for all the samples used unless these have already been published or the samples are of commercial origin. 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, and the corresponding values of pK_a at 0, 10, and 100 mM. The symbol *S* indicates that the compound tended to come out of solution during the addition of alkali; the initials of Mr J. Duguid, Dr G.M. Thompson and Dr K.J. Turnbull have been used to identify samples supplied by them.

Phenethylamines

m.p.	Ionized halogen	pK_a 0	pK_a 10	pK_a 100 mM	m	n
$PhCH_2CH_2NH_2HCl$ (4.8–30.7 mM)						
218.1–219.8	22.52 (22.49)	9.43	9.74	10.41	0.098	18
at 37°C (5.0–15.1 mM)		9.32	9.46	9.77	0.045	10
(Leffler *et al.* (1951) recorded pK_a 9.83; Lewis (1954), 9.86; Tuckerman *et al.* (1959), 9.78; Kappe & Armstrong (1965), 9.88)						
$(MeO)_2C_6H_3CH_2CH_2NH_2HCl$ (4.1–15.0 mM)						
153.5–154.0	16.34 (16.29)	9.32	9.75	10.68	0.136	5
(Sinistri & Villa (1962) recorded pK_a 9.97)						
$PhCH_2CH_2NHMe$ HCl (4.7–15.1 mM)						
163.3–163.8	20.73 (20.65)	9.38	9.93	11.11	0.173	7
(Tuckerman *et al.* (1959) recorded pK_a 10.31)						

m.p.	Ionized halogen	O	pK_a 10	100 mM	m	n
m-$MeOC_6H_4CH_2CH_2NHMe$ HCl (5.0–15.0 mM)						
120.5–121.2	17.58 (17.58)	9.78	9.99	10.44	0.066	5
p-$MeOC_6H_4CH_2CH_2NHMe$ HCl (5.0–15.0 mM)						
180.8–181.7	17.64 (17.58)	9.72	10.04	10.72	0.099	5
$(MeO)_2C_6H_3CH_2CH_2NHMe$ HCl (4.4–15.1 mM) *KJT*						
138.2–138.7	15.20 (15.30)	9.38	9.87	10.92	0.154	5
$PhCH_2CH_2NHEt$ HCl (3.5–15.0 mM)						
184.5–185.2	19.17 (19.09)	9.63	10.04	10.92	0.129	7
$PhCH_2CH_2NH$*iso*Pr HCl (4.0–15.0 mM)						
168.5–169.2	17.75 (17.74)	9.52	10.04	11.16	0.164	6
$PhCH_2CH_2NMe_2$ HBr (5.0–15.0 mM) *GMT*						
149.3–150.0	34.78 (35.17)	8.80	9.19	10.02	0.122	5
m-$MeOC_6H_4CH_2CH_2NMe_2$ HCl (3.8–14.9 mM) *KJT*						
133.1–134.1	16.34 (16.44)	8.74	9.07	9.77	0.103	7
p-$MeOC_6H_4CH_2CH_2NMe_2$ HCl (5.0–15.0 mM) *KJT*						
173.5–175.6	16.41 (16.44)	8.93	9.24	9.90	0.097	4
$(MeO)_2C_6H_3CH_2CH_2NMe_2$ HCl (3.8–15.0 mM) *KJT*						
192.0–193.6	14.32 (14.43)	8.75	9.15	10.02	0.127	5
$PhCH_2CH_2NEt_2$ HBr (5.0–15.0 mM)						
107.3–107.7	31.03 (30.94)	9.35	9.79	10.74	0.139	5
Benzylamines						
$PhCH_2NMe_2$ HCl (5.0–15.1 mM)						
173.5–174.1	not analysed	8.40	8.80	9.68	0.128	6
$PhCH_2NMe_2$ HBr (5.0–15.1 mM)						
143.5–144.0	37.01 (36.97)	8.51	8.79	9.41	0.090	6
m-$MeOC_6H_4CH_2NMe_2$ HCl (5.0–15.0 mM)						
165.0–166.2	17.68 (17.58)	8.65	8.78	9.06	0.041	5
p-$MeOC_6H_4CH_2NMe_2$ HCl (5.0–15.0 mM)						
156.0–156.6	17.64 (17.58)	9.02	9.13	9.35	0.032	5
$PhCH_2NEt_2$ HBr (5.0–15.0 mM) *JD*						
160.8–161.4	32.78 (32.78)	8.91	9.41	10.50	0.160	5
o-$MeOC_6H_4CH_2NEt_2$ HBr (5.0–10.1 mM) *GMT*						
120.2–121.0	29.49 (29.14)	9.86	10.27	11.16	0.131	4
m-$MeOC_6H_4CH_2NEt_2$ HBr (5.0–15.0 mM) *GMT*						
145.3–146.0	29.38 (29.14)	8.95	9.26	9.94	0.099	4
p-$MeOC_6H_4CH_2NEt_2$ HBr (5.0–15.1 mM; *S*)						
106.5–107.2	29.17 (29.14)	9.39	9.70	10.38	0.099	5
Phenylpropylamines						
$PhCH_2CH_2CH_2NMe_2$ HBr (5.0–17.6 mM)						
112.5–113.2	32.78 (32.72)	9.18	9.52	10.24	0.106	9
m-$MeOC_6H_4CH_2CH_2CH_2NMe_2$ HCl (4.2–15.1 mM)						
131.0–131.5	15.51 (15.43)	9.23	9.54	10.21	0.098	7
$PhCH_2CH_2CH_2NEt_2$ HBr (3.3–8.6 mM; *S*) *GMT*						
142.0–142.7	29.44 (29.41)	9.67	10.34	11.79	0.212	5

m.p.	Ionized halogen	pK_a 0	10	100 mM	m	n
Phenylethanolamines						
$PhCHOHCH_2NH_2$ HCl (5.1–15.4 mM)						
sinters 137, 200–205	20.46 (20.42)	8.43	8.90	9.91	0.148	5
(Tuckerman *et al.* (1959) recorded pK_a 8.90; Villa & Sinistri (1963), 9.03)						
$PhCHOHCH_2NH_2$ HI (5.0–15.0 mM)						
116.7–118.5	47.77 (47.87)	8.45	8.85	9.72	0.127	6
$PhCHOHCH_2NHMe$ HCl (5.0–15.0 mM)						
104.3–104.9	18.92 (18.89)	8.90	9.29	10.14	0.123	5
(Tuckerman *et al.* (1959) recorded pK_a 9.31; Villa & Sinistri (1963), 9.44)						
$PhCHOHCH_2NMe_2$ HCl (5.1–15.1 mM)						
147.0–147.6	17.60 (17.58)	8.44	8.81	9.63	0.119	6
m-$MeOC_6H_4CHOHCH_2NMe_2$ HCl (5.0–15.1 mM)						
115.5–116.1	15.43 (15.30)	8.39	8.76	9.56	0.117	5
p-$MeOC_6H_4CHOHCH_2NMe_2$ HCl (5.1–15.1 mM)						
143.5–143.9	15.34 (15.30)	8.52	8.81	9.45	0.093	5
Ketonic phenethylamines						
$PhCOCH_2NH_2$ HCl (5.0–15.1 mM)						
187.4–187.5 dec	20.73 (20.65)	7.86	8.16	8.82	0.096	5
$PhCOCH_2NMe_2$ HCl (5.0–17.5 mM)						
172.4–173.3	17.82 (17.74)	7.88	8.04	8.38	0.050	5
Phenolic quaternary ammonium salts						
(Analytical details for all the samples have already been published: Barlow *et al.*, 1969; Barlow *et al.*, 1974)						
o-$HOC_6H_4CH_2\overset{+}{N}Me_3$ I^- (4.6–15.1 mM)		8.20	8.46	9.02	0.082	4
m-$HOC_6H_4CH_2\overset{+}{N}Me_3$ I^- (5.0–15.1 mM)		8.51	8.75	9.28	0.076	10
p-$HOC_6H_4CH_2\overset{+}{N}Me_3$ I^- (5.0–15.1 mM)		8.50	8.58	8.77	0.027	8
m-$HOC_6H_4CH_2\overset{+}{N}Et_3$ I^- (5.0–15.0 mM)		8.65	8.83	9.22	0.057	5
p-$HOC_6H_4CH_2\overset{+}{N}Et_3$ I^- (5.0–15.0 mM)		8.44	8.72	9.34	0.090	5
o-$HOC_6H_4CH_2CH_2\overset{+}{N}Me_3$ I^- (5.0–15.0 mM)		8.76	9.23	10.24	0.148	4
m-$HOC_6H_4CH_2CH_2\overset{+}{N}Me_3$ I^- (4.1–15.0 mM)		8.84	9.15	9.84	0.100	5
p-$HOC_6H_4CH_2CH_2\overset{+}{N}Me_3$ I^- (2.5–15.0 mM)		8.99	9.35	10.13	0.114	6
o-$HOC_6H_4CH_2CH_2CH_2\overset{+}{N}Me_3$ I^- (4.8–15.0 mM)		9.22	9.58	10.34	0.112	4
m-$HOC_6H_4CH_2CH_2CH_2\overset{+}{N}Me_3$ Br^- (5.0–15.0 mM)		9.18	9.47	10.09	0.091	3
m-$HOC_6H_4CH_2CH_2CH_2\overset{+}{N}Me_3$ I^- (5.0–14.9 mM)		9.21	9.51	10.15	0.093	4
at 37° (5.0–15.0 mM)		9.18	9.37	9.76	0.058	5

m.p.	Ionized halogen	O	pK_a 10	100 mM	m	n
p-$HOC_6H_4CH_2CH_2CH_2\overset{+}{N}Me_3$ I^- (3.0–15.1 mM)						
		9.26	9.52	10.08	0.082	6
$(HO)_2C_6H_3CH_2CH_2\overset{+}{N}Me_3$ I^- (2.2–25.0 mM)						
		8.42	8.75	9.47	0.105	7
		10.3	12.1	?	0.6	
at 37° (5.0–15.0 mM)		8.53	8.66	8.94	0.040	5
		10.9	12.2	?	0.4	
$(HO)_2C_6H_3CH_2CH_2CH_2\overset{+}{N}Me_3$ Br^- (4.2–15.1 mM)						
		8.77	9.06	9.67	0.089	5
		10.2	12.3	?	0.7	
Phenolic quaternary ammonium salts: alcohols						
m-$HOC_6H_4CHOHCH_2\overset{+}{N}Me_3$ Br^- (5.0–15.0 mM)						
162.3–164.2	28.86 (28.93)	9.02	9.18	9.51	0.049	6
p-$HOC_6H_4CHOHCH_2\overset{+}{N}Me_3$ Br^- (5.0–15.0 mM)						
230.6–231.1 dec	28.95 (28.93)	9.05	9.19	9.51	0.047	5
$(HO)_2C_6H_3CHOHCH_2\overset{+}{N}Me_3$ Cl^- (4.7–15.0 mM)						
161.3–164.8 dec	14.41 (14.31)	8.35	8.62	9.18	0.082	6
		10.8	12.2	?	0.5	
(Sinistri & Villa (1962) recorded pK_a 8.90 for the bromide)						
Ketones						
m-$HOC_6H_4COCH_2\overset{+}{N}Me_3$ Br^- (5.0–15.0 mM)						
230.2–230.4 dec	29.09 (29.14)	8.26	8.45	8.88	0.062	5
p-$HOC_6H_4COCH_2\overset{+}{N}Me_3$ Br^- (5.0–15.0 mM)						
230.4–230.7 dec	29.09 (29.14)	7.11	7.22	7.47	0.036	5
$(HO)_2C_6H_3COCH_2\overset{+}{N}Me_3$ Cl^- (5.0–15.0 mM)						
231.0–231.2 dec	14.52 (14.43)	6.84	6.95	7.21	0.037	7
		11.1	11.7	12.8	0.17	
Phenolic amines: benzylamines						
m-$HOC_6H_4CH_2NMe_2$ HBr (5.0–15.0 mM)		*GMT*				
134.4–134.5	34.23 (34.43)	8.15	8.42	9.00	0.086	3
		9.56	10.1	11.3	0.17	
p-$HOC_6H_4CH_2NMe_2$ HBr (5.0–15.0 mM)						
149.5–150.2	34.31 (34.43)	8.50	8.63	8.91	0.041	5
		10.1	10.4	11.0	0.10	
o-$HOC_6H_4CH_2NEt_2$ HBr (5.0–15.0 mM)		*GMT*				
255.0–257.0	31.03 (30.77)	7.88	8.13	8.65	0.077	3
		10.6	11.9	14?	0.4	
m-$HOC_6H_4CH_2NEt_2$ HBr (5.0–15.0 mM)		*GMT*				
131.1–131.9	30.92 (30.77)	8.30	8.62	9.32	0.102	3
		10.1	10.5	11.5	0.14	
p-$HOC_6H_4CH_2NEt_2$ HBr (5.0–15.0 mM)		*GMT*				
151.7–152.3	30.48 (30.77)	8.39	8.69	9.35	0.096	3
		10.2	10.8	12.2	0.20	

m.p.	Ionized halogen	O	pK_a 10	100 mM	m	n
Phenolic amines: phenethylamines						
m-$HOC_6H_4CH_2CH_2NH_2$ HBr (2.6–15.0 mM)						
115.5–116.5	36.90 (36.64)	8.58	9.07	10.12	0.153	6
		9.73	10.5	12.2	0.25	
(Kappe & Armstrong (1965) recorded pK_a 9.58, 10.5)						
p-$HOC_6H_4CH_2CH_2NH_2$ HCl (tyramine HCl, 5.0–15.0 mM)						
Koch-Light, recryst.		8.74	9.23	10.28	0.154	9
		9.91	10.6	12.0	0.20	
(Lewis (1954) recorded pK_a 9.5, 10.8; Kappe & Armstrong (1965), 9.74, 10.5)						
m-$HOC_6H_4CH_2CH_2NHMe$ HBr (5.0–15.0 mM)						
94.4–95.3	34.57 (34.43)	9.07	9.17	9.39	0.031	5
		10.4	10.7	11.5	0.11	
p-$HOC_6H_4CH_2CH_2NHMe$ HBr (5.0–15.0 mM)						
125.4–126.6	34.08 (34.43)	9.20	9.36	9.69	0.049	5
		10.5	10.9	11.6	0.11	
m-$HOC_6H_4CH_2CH_2NMe_2$ HCl (5.0–15.0 mM)						
161.6–162.1	17.66 (17.58)	8.50	8.83	9.54	0.103	5
		9.64	10.1	11.3	0.16	
p-$HOC_6H_4CH_2CH_2NMe_2$ HBr (5.0–15.0 mM)						
175.1–175.8	32.50 (32.46)	8.73	9.06	9.76	0.103	5
		9.95	10.3	11.1	0.12	
(Kappe & Armstrong (1965) recorded pK_a 9.78, 10.0)						
$(HO)_2C_6H_3CH_2CH_2NH_2$ HCl (dopamine HCl, 5.0–15.0 mM)						
242.7–243.5 dec	18.61 (18.69)	8.55	8.81	9.38	0.083	5
		9.84	10.5	12.0	0.22	
at 37°C (5.0–15.0 mM)		8.33	8.45	8.73	0.040	5
		9.61	9.74	10.0	0.042	
(Lewis (1959) recorded pK_a 8.87, 10.6 (20°C))						
6-Hydroxydopamine HCl (5.0–15.0 mM)						
Aldrich	not analysed	8.53	8.78	9.31	0.078	5
		9.88	10.3	11.2	0.14	
N-methyldopamine (Epinine) HBr (5.0–15.0 mM) *KJT*						
166.4–167.2	31.98 (32.21)	8.44	8.75	9.42	0.097	3
		10.1	10.7	12.0	0.20	
(Lewis (1954) recorded 8.90, 10.6 (20°C))						
N,N-dimethyldopamine HCl (5.0–15.2 mM) *KJT*						
123.2–125.0	16.16 (16.29)	8.51	8.71	9.14	0.063	5
		9.72	10.2	11.1	0.14	
at 37°C (5.0–15.0 mM)		8.54	8.54	8.55	0.001	5
		9.87	9.96	10.1	0.03	
Phenolic amines: phenylpropylamines						
m-$HOC_6H_4CH_2CH_2CH_2NMe_2$ HCl (2.3–14.9 mM)						
89.3–90.3	16.37 (16.44)	9.03	9.16	9.45	0.042	5
		9.87	10.3	11.4	0.15	
p-$HOC_6H_4CH_2CH_2CH_2NMe_2$ HBr (5.0–15.0 mM) *GMT*						
119.2–119.4	30.68 (30.76)	8.86	9.26	10.16	0.125	3
		9.73	10.4	11.7	0.20	
p-$HOC_6H_4CH_2CH_2CH_2NEt_2$ HBr (5.0–15.0 mM)						
152.9–153.1	28.03 (27.77)	9.08	9.47	10.29	0.121	6
		9.93	10.8	12.7	0.28	

m.p.	*Ionized halogen*	*O*	*pK_a 10*	100 mM	*m*	*n*
Phenolic amines: phenylethanolamines						
m-$HOC_6H_4CHOHCH_2NH_2$ HCl (Norphenylephrine, 5.1–15.1 mM)						
Aldrich	not analysed	8.34	8.65	9.31	0.097	5
		9.48	9.87	10.7	0.12	
p-$HOC_6H_4CHOHCH_2NH_2$ HCl (Octopamine, 5.0–15.0 mM)						
Sigma	not analysed	8.40	8.73	9.42	0.102	6
		9.41	9.89	10.9	0.15	
(Lewis (1954) recorded pK_a 9.53, 9.70; Kappe & Armstrong (1965), 8.57, 9.66)						
m-$HOC_6H_4CHOHCH_2NHMe$ HCl (Phenylephrine, 4.9–15.3 mM)						
Koch-Light	not analysed	8.53	8.84	9.50	0.096	5
		9.52	10.1	11.3	0.18	
p-$HOC_6H_4CHOHCH_2NHMe$ HCl (Synephrine, 5.1–15.1 mM)						
130.0–130.2 dec	17.61 (17.41)	8.61	8.83	9.30	0.069	5
		9.70	10.0	10.7	0.10	
(Lewis (1954) recorded pK_a 9.59, 9.71; Kappe & Armstrong (1965) 9.55, 9.79)						
Noradrenaline HCl (5.0–15.0 mM)						
Aldrich	not analysed	8.39	8.53	8.85	0.046	5
		9.46	9.75	10.4	0.09	
at 37°C (5.0–15.0 mM)		8.30	8.32	8.35	0.005	5
		9.56	9.58	9.61	0.004	
(Lewis (1954) recorded pK_a 8.73, 9.78; Sinistri & Villa (1962), 8.73, 9.83; Kappe & Armstrong (1965), 8.72, 9.72)						
Adrenaline HCl (5.0–10.1 mM; *S*)						
Minnesota	not analysed	8.31	8.57	9.14	0.083	6
3M Labs		9.56	10.01	11.0	0.14	
at 37°C (5.0–15.0 mM)		8.27	8.39	8.65	0.039	5
		9.72	9.85	10.1	0.04	
(Lewis (1954) recorded pK_a 8.71, 9.90; Sinistri & Villa (1962), 8.79, 10.09; Kappe & Armstrong (1965), 8.75, 9.89)						
Isoprenaline HCl (4.9–15.1 mM)						
Sigma	not analysed	8.49	8.55	8.68	0.019	5
		9.58	10.04	11.0	0.14	
at 37°C (5.0–15.0 mM)		8.42	8.43	8.46	0.004	7
		9.70	9.86	10.2	0.05	
(Lewis (1954) recorded pK_a 8.72, 9.87; Sinistri & Villa (1962), 8.83, 10.19)						
Phenolic amines: ketones						
m-$HOC_6H_4COCH_2NMe_2$ HBr (5.0–15.0 mM)						
190.8–191.5 dec	30.52 (30.72)	7.73	7.88	8.22	0.049	5
		9.20	9.36	9.70	0.050	
p-$HOC_6H_4COCH_2NMe_2$ HBr (5.0–15.0 mM)						
206.1–206.7 dec	30.61 (30.72)	6.82	7.15	7.85	0.103	5
		8.86	9.20	9.94	0.108	
$(HO)_2C_6H_3COCH_2NMe_2$ HCl (5.0–15.0 mM)						
210.7–211.2 dec	15.33 (15.30)	6.60	6.91	7.58	0.098	5
		8.78	9.07	9.71	0.093	
Apomorphine HCl, $\frac{1}{2}H_2O$ (0.6–0.8 mM; *S*)						
Macfarlan Smith	not analysed	7.08	8.68	12.2?	0.508	5
		8.39	9.76	12.7?	0.43	
at 37°C (0.8–1.2 mM)		7.45	7.36	7.15	–0.030	5
		8.93	8.62	7.95	–0.098	

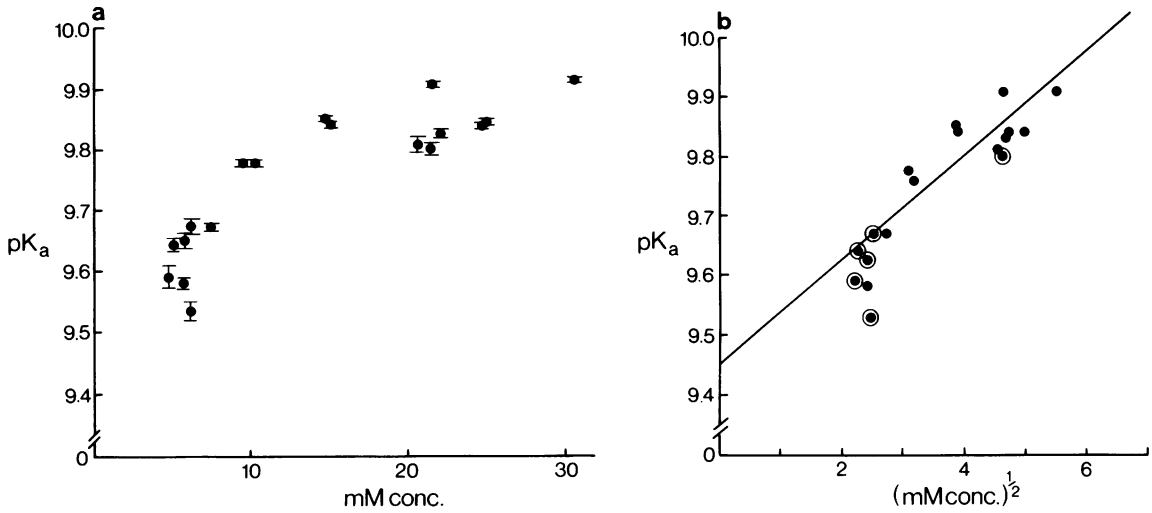


Figure 2(a) Estimates of the pK_a of phenethylamine hydrochloride at 25°C plotted against the initial concentration; the vertical lines indicate the standard error. (b) Estimates plotted against the square root of the initial concentration; the straight line indicates the best fit, using least squares weighted according to the reciprocal of the variance. The large circles indicate values with standard errors greater than 0.01 log units.

the pK_a values obtained were not constant but decreased with decreasing concentration (Figure 2). Other workers have not commented on any such concentration dependence, which seems to be much more marked at low concentrations. It seemed empirically that the dependence could adequately be represented by the expression $pK = pK_o + mC^{\frac{1}{2}}$; with this the dependence would be less at higher concentrations. The results for each compound have been fitted to this relationship by least-squares, weighted according to the reciprocal of the variance associated with the estimate of pK_a. The concentration of the compound is reduced by the volume of the alkali added during the titration to between 0.98 to 0.87 times the initial concentration and the line-fitting procedure has been applied to the concentration in mid-titration, except in Figure 2 where the initial concentrations were used. From the values of m and pK_o, the pK_a has been calculated for concentrations of 10 and 100 mM. The estimates at 10 mM should be the the most accurate, being in the middle of the range of concentrations tested (except for apomorphine). The extrapolated values for infinite dilution or at 100 mM are much more uncertain but the latter are of interest for comparison with some values already published.

Estimates obtained in this work are consistently lower than those previously reported but these usually lie within the range which might be expected from the observed dependence on concentration. The correction for converting 'mixed' pK_a values to thermodynamic pK_a values is small (log f is usually about log (0.9)=−0.05) and the use of a rather larger and more realistic size parameter (a=6.0 instead of 5.0 as used by Albert & Sergeant, 1971) makes a negligible difference to the estimate of the activity coefficient at this dilution. It is clear that corrections based on Debye–Hückel theory do not yield pK_a values which are actually constant but the results with low concentrations are more likely to be reliable than those with higher concentrations or at higher ionic strengths, because the corrections are smaller and the Debye–Hückel assumptions are more likely to be valid. It is unfortunate that it is values in a medium of higher ionic strength (blood) which are likely to be biologically important.

Estimates were made at 37°C with phenethylamine, dopamine, *N,N*-dimethyldopamine, noradrenaline, adrenaline, isoprenaline, *m*-hydroxyphenylpropyltrimethylammonium, coryneine (quaternary dopamine) and apomorphine and in every instance there was a reduction in pK_a (by usually 0.1–0.2 log units) and in the dependence of the estimate of pK_a on concentration (Table 2). This suggests that the dependence on concentration is not an experimental artefact.

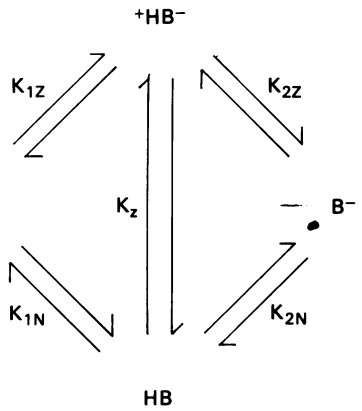
The results are in reasonable agreement with those

of Kolthoff (1925) for apomorphine (7.6 and 8.8 at 25° compared with 7.3 and 8.9 at 15°C) and of Lewis (1954) for dopamine (8.8 and 10.5 compared with 8.9 and 10.6). Two of the three bonds broken to convert apomorphine into dimethyldopamine (Figure 1) have important effects on pK_a; the second aromatic ring greatly enhances the acidity of the phenolic groups and the bond which makes the amino group a benzylamine derivative, rather than a phenethylamine, greatly reduces the pK_a of the amino group. Thus the striking structural resemblance between the two compounds does not really extend to their physicochemical properties (including the solubility of the species which has lost a proton).

Discussion

The results confirm the potential importance of ionization in the biological actions of compounds such as apomorphine, dopamine, and catecholamines. At receptors where there is appreciable stereospecificity there must clearly be other factors involved, because the pK_as of enantiomers are identical; it is, nevertheless, striking that all the biologically interesting compounds in Table 2 have pK_a values at 37°C which are within 1 unit of physiological pH.

The first pK_a will include the ionization which leads to the non-charged form with which the zwitterion is in equilibrium:



and it may be relevant to know the position of all the equilibria. If K_{1Z} and K_{1N} represent the dissociation constants into zwitterions and non-charged species,

Table 2 Effect of temperature on pK_a (10 mM)

	25°C		37°C		Δ
	pK_a	m	pK_a	m	
$PhCH_2CH_2NH_2$	9.74	0.094	9.46	0.045	0.28
Dopamine	8.81	0.083	8.45	0.040	0.36
	10.52	0.217	9.74	0.042	0.78
N,N-dimethyldopamine	8.71	0.063	8.54	0.001	0.17
	10.16	0.140	9.96	0.029	0.20
Noradrenaline	8.53	0.046	8.32	0.005	0.21
	9.75	0.093	9.58	0.004	0.17
Adrenaline	8.57	0.083	8.39	0.039	0.18
	10.01	0.141	9.85	0.040	0.16
Isoprenaline	8.55	0.019	8.43	0.004	0.12
	10.04	0.143	9.86	0.052	0.18
m-Hydroxyphenylpropyltrimethylammonium					
	9.51	0.093	9.37	0.058	0.14
Coryneine*	8.75	0.105	8.66	0.040	0.09
Apomorphine (at 1 mM)	7.59	0.508	7.42	−0.030	0.17
	8.82	0.433	8.83	−0.098	−0.01

* Trimethylammonium derivative of dopamine. Values for the dissociation of the second phenolic group have been omitted.

respectively, and K_Z represents the zwitterion equilibrium constant, $[^+HB^-]/[HB]$

$$K_1 = \frac{[^+HB^- + BH][H^+]}{[HBH^+]}$$
$$= K_{1Z} + K_{1N}$$
$$= K_{1Z}(1 + 1/K_Z)$$
$$= K_{1N}(1 + K_Z)$$

Spectroscopic methods which depend on the different u.v. absorption spectra of phenolic ($-C_6H_4OH$) and phenate ($-C_6H_4O^-$) groups should measure pK_{1Z} and can be used to estimate K_{1N} and K_Z when pK_1 has been measured by titration. Values of pK_{1Z} will be greater than pK_1 (this is apparent, for instance in the results obtained by Lewis, 1954) and the difference $(pK_{1Z} - pK_1)$ is log $(1 + 1/K_Z)$, so the bigger the difference, the smaller the extent of zwitterion formation. If K_Z is large, $(1 + 1/K_Z) \rightarrow 1$ and the difference in $pK \rightarrow 0$. Unfortunately it is difficult to estimate K_Z accurately if it is large. For example when $K_Z = 5$, $\Delta pK = \log 1.2 = 0.08$; for $K_Z = 20$, $\Delta pK = \log 1.05 = 0.02$.

Alternatively it may be supposed that the value of K_{1N} will be the same as that for the corresponding methoxy compound. The value of pK_1 will be less that of pK_{1N} by log $(1 + K_Z)$ so that the greater the extent of zwitterion formation, the greater will be the difference in pK_a. Sinistri & Villa (1962) used this method to calculate the four ionization constants (K_{1Z}, K_{1N}, K_{2Z}, K_{2N}) for noradrenaline, adrenaline, isoprenaline and some related compounds. They found that their estimates of K_{1Z} obtained in this way agreed well with values obtained spectroscopically (even though there is a difference in ionic strength) and moreover the value for the quaternary trimethylammonium derivative of noradrenaline, which can exist only in the ionized form, was the same to within 0.01 log units when measured by potentiometric titration or spectrophotometrically. Their results give values of K_Z of 1.8 for noradrenaline, 4.3 for adrenaline and 4.7 for isoprenaline. From the values of pK_1 obtained potentiometrically and spectroscopically by Lewis (1954; at constant ionic strength) the values of K_Z for noradrenaline, adrenaline and isoprenaline appear to be 2.1, 2.1 and 2.4 respectively. Clearly more information is needed to see what size of error may be attached to estimates of K_Z and whether there really is a difference between the extent of zwitterion formation by noradrenaline and adrenaline.

The zwitterion constant, K_Z, can also be calculated from pK_2 if it is assumed that pK_{2N} is the same as for a substituted phenol, such as hydroxymethyl-phenol, which contains a group electronically similar to amino but which cannot acquire a positive charge in the conditions of the titration. Sprengling & Lewis (1953) obtained pK_a values at 25°C and 0.02 M ionic strength of 9.83 for *m*-methylolphenol and 9.82 for the *p*-isomer and from the collection of pK_a values compiled by Kortüm, Vogel & Andrussov (1961) it seems likely that this should be close to the value of pK_{2N}, because changes in groups separated from the benzene ring by one or more methylene groups do not markedly alter the phenolic pK_a. As

$$K_2 = \frac{[B^-][H^+]}{[^+HB^- + HB]}$$
$$= \frac{[B^-][H^+]}{[HB](1 + K_Z)}$$
$$pK_2 = pK_{2N} + \log (1 + K_Z)$$

and the difference between pK_2 and 9.8 should be log $(1 + K_Z)$.

Instead of using information from only one experimental pK_a value, either pK_1 or pK_2, it is possible to use both values because the range

$$pK_2 - pK_1 = pK_{2N} - pK_{1N} + 2 \log (1 + K_Z)$$

and this might be more accurate than the other methods because the number actually calculated is bigger, so the experimental errors associated with the estimates of pK_a may be less important.

For many of the results obtained in this work it is possible to calculate K_Z by several of these methods and to compare the results obtained (Table 3). As the results for the quaternary compounds may indicate pK_{1Z}, and those for the methoxy compounds may indicate pK_{1N}, it is possible to predict the value of K_Z, for instance, for compounds which have not yet been prepared, and these values are also included.

The results suggest that the size of K_Z determines the most suitable method for estimating it. When it is large method 1 is unsuitable, as has already been indicated, and this will apply whether K_Q is used to estimate pK_{1Z} or if it is estimated spectrophotometrically. When the side chain contains strongly activating groups next to the benzene ring (carboxyl and to a lesser extent hydroxyl) method 3 is inadequate because the value of pK_{2N} is not the same as that of hydroxymethylphenol. In contrast the guessed values of pK_{Me} which it has been necessary to use in some instances are not likely to be seriously inaccurate. The effects of substituents, such as methoxyl, on pK_a are summarized in Table 4 and although they are by no means constant, they should be predictable to within about 0.1 log units.

Provided an unsuitable method has not been used, the estimates appear to fall within a 2-fold range and values of log K_Z would be consistent to within about 0.15 log units. In addition to values for noradrenaline, adrenaline and isoprenaline which can be calculated from the results of Lewis (1954) and of Sinistri & Villa

Table 3 Zwitterion constants (K_z)

These are calculated from pK_1, pK_2, pK_{Me} (for the methoxy compound) and pK_Q (for the trimethylammonium compound) using the relations:

1. $pK_Q - pK_1 = \log (1 + 1/K_Z)$
2. $pK_{Me} - pK_1 = \log (1 + K_Z)$
3. $pK_2 - pK_1 = 9.80 - pK_{Me}$
4. $\log K_Z = pK_{Me} - pK_Q$

In some instances the value of pK_{Me} has been guessed and is printed in italics. In other instances, marked 'nc', the difference between pK_Q and pK_1 is negative and a result cannot be calculated, and with some groups, notably the ketones, the values calculated by method 3 differ markedly from the rest (and sometimes cannot be calculated), indicating that the value assumed for the ionization of the substituted phenol (9.80) is incorrect. The asterisk indicates values of K_Q for the triethylammonium compound; all others are for trimethylammonium.

Compound		pK_1	pK_2	pK_{Me}	pK_Q	K_Z 1	2	3	4
$HOC_6H_4CH_2$–									
o-	NEt_2	8.13	11.90	10.27	8.46	0.8	140	130	65
m-	NMe_2	8.42	10.10	8.78	8.75	0.8	1.3	1.1	1.1
	NEt_2	8.62	10.51	9.26	8.83*	1.6	3.4	3.7	2.6
p-	NMe_2	8.63	10.38	9.13	8.58	nc	2.2	2.5	3.5
	NEt_2	8.69	10.82	9.70	8.72*	14	10	10	9.6
$HOC_6H_4CH_2CH_2$–									
m-	NH_2	9.07	10.52	*9.80*	9.15	5.0	4.4	4.4	4.5
	NHMe	9.17	10.72	9.99	9.15	nc	5.5	6.4	6.9
	NMe_2	8.83	10.15	9.07	9.15	0.92	0.74	0.95	0.83
p-	NH_2	9.23	10.56	*9.85*	9.35	3.1	3.2	3.9	3.2
	NHMe	9.36	10.88	10.04	9.35	nc	3.8	6.7	4.9
	NMe_2	9.06	10.32	9.24	9.35	1.0	0.51	1.2	0.78
3,4-dihydroxy									
	NH_2	8.81	10.52	9.75	8.75	nc	7.7	5.8	10
	NHMe	8.75	10.69	9.87	8.75	nc	12	9.1	13
	NMe_2	8.71	10.16	9.15	8.75	10	1.7	1.5	2.5
$HOC_6H_4CH_2CH_2CH_2$–									
m-	NMe_2	9.16	10.34	9.54	9.51	0.81	1.4	1.9	1.1
p-	NMe_2	9.26	10.36	*9.55*	9.52	1.2	1.0	1.3	1.1
	NEt_2	9.47	10.80	*10.38*	9.52	8.3	7.1	7.9	7.2
$HOC_6H_4CHOHCH_2$–									
m-	NH_2	8.65	9.87	*8.85*	9.18	0.42	0.58	0.35	0.47
	NHMe	8.84	10.08	*9.24*	9.18	0.84	1.5	1.2	1.2
p-	NH_2	8.73	9.89	*8.95*	9.19	0.53	0.66	0.44	0.58
	NHMe	8.83	10.03	*9.34*	9.19	0.78	2.2	1.3	1.4
3,4-dihydroxy									
	NH_2	8.53	9.75	*8.95*	8.62	4.3	1.6	0.55	2.1
	NHMe	8.57	10.01	*9.34*	8.62	8.3	4.9	2.1	5.2
	NH*iso*Pr	8.55	10.04	*9.41*	8.62	5.9	6.2	2.6	6.2
$HOC_6H_4COCH_2$–									
m-	NMe_2	7.88	9.36	*8.00*	8.45	0.37	0.32	nc	0.35
p-	NMe_2	7.15	9.20	*8.10*	7.22	5.9	7.9	0.5	7.6
3,4-dihydroxy									
	NMe_2	6.91	9.07	*8.04*	6.95	10	12	0.6	12

(1962), it is possible from the former to obtain values for dopamine (8.3) and tyramine (1.4), which are in the same range as those obtained in this work. Values of K_Z calculated by method 4 appear to be in reasonable agreement with values measured experimentally and this method seems likely to be useful for predicting the extent of zwitterion formation in new compounds.

The results indicate that there are differences between the extent of zwitterion formation in noradrenaline, adrenaline and isoprenaline and confirm that there is a trend to increasing zwitterion formation with increasing chain length, to be seen in the results of Sinistri & Villa (1962). There is considerable zwitterion formation in dopamine and epinine but it is markedly reduced by the insertion of a further methyl group, producing *N,N*-dimethyldopamine. In contrast the proportion is high for many diethylamino compounds. In the compounds with only one methylene group and in those with two methylene groups and an alcoholic or ketonic group adjacent to the benzene ring, the *p*-isomers have bigger zwitterion constants than the *m*-isomers: in the compounds with a simple ethylene side-chain the *m*-isomers have slightly larger zwitterion constants than the *p*-isomers.

The extent of zwitterion formation in apomorphine cannot be assessed directly from this work as there are

Table 4 Effects of changes in structure on pK_a (10 mM) in amines or phenolic quaternary ammonium salts with only one ionizable group

Compound	*Group*					
	m-MeO	*p*-MeO	3:4 $(MeO)_2$	$-CH_2-$	OH	C=O
$PhCH_2NMe_2$	−0.02	0.33		0.39		
NEt_2	−0.15	0.29		0.38		
$PhCH_2CH_2NH_2$			0.01		−0.87	−1.58
NHMe	0.06	0.11	−0.06		−0.64	
NMe_2	−0.12	0.05	−0.04	0.33	−0.38	−1.15
m-MeO					−0.31	
p-MeO					−0.43	
$PhCHOHCH_2NMe_2$	−0.05	0.00				
$PhCH_2CH_2NEt_2$				0.55		
$Ph(CH_2)_3NMe_2$	0.02					
$HOC_6H_4(CH_2)_n\overset{+}{N}Me_3$	*Effect of side-chain;* phenol = 9.90					
n=1						
o-		−1.44		0.77		
m-		−1.15		0.40		
p-		−1.32		0.77		
n=2						
o-		−0.67		0.35		
m-		−0.75		0.16	0.03	−0.70
p-		−0.55		0.17	−0.16	−1.93
3,4-dihydroxy		−1.15		0.31	−0.13	−1.80
n=3						
o-		−0.32				
m-		−0.39				
p-		−0.38				
3,4-dihydroxy		−0.84				
$HOC_6H_4CHOHCH_2\overset{+}{N}Me_3$						
m-		−0.72				
p-		−0.72				
3,4-dihydroxy-		−1.28				
$HOC_6H_4COCH_2\overset{+}{N}Me_3$						
m-		−1.45				
p-		−2.68				
3,4-dihydroxy		−2.95				

no values for the methoxy compound or for the quaternary compound. A rough idea may however, be obtained from the difference between pK_2 and pK_1; this seems to be about 1.1 log units at 10 mM. The pK_a of *o*-hydroxydiphenyl is about 10.0 (see Kortüm *et al.*, 1961) so for the dihydroxy compound the value is likely to be about 9.6. The pK_a of benzyldimethylamine obtained in this work is 8.8 at 10 mM so $2 \log (1+K_Z) = 1.1 - 0.8$ and $K_Z = 0.4$. At 100 mM the pK_a of benzyldimethylamine is calculated to be 9.4, so the value of K_Z might be as high as 1.8, but even so it seems unlikely that the zwitterion is a highly dominant species. In the circumstances it might well be worth investigating the matter spectroscopically.

It appears that there are significant concentrations of zwitterions present in solutions of apomorphine, dopamine and catecholamines, even allowing for effects of ionic strength on the estimates of 'thermodynamic' pK_a, and it might be thought that these species carrying both negative and positive charges, might be particularly effective in interacting with the receptor. However, the chemical features which favour zwitterion formation (alkylation of the nitrogen, *p*-substitution of the nitrogen atom rather than *m*-) are certainly not those which favour activity at α-adrenoceptors and there is no obvious correlation with activity at β-adrenoceptors. Activity appears therefore to be associated with the presence of a free hydroxyl group, either in the uncharged phenolic amine, with which the zwitterion is in equilibrium, or in the phenolic ammonium salt, $HOC_xH_y\overset{+}{N}R_2H$, which is the predominant species at physiological pH. The high activity of apomorphine at dopamine receptors with its relatively low zwitterion constant suggests that for these receptors the active species might be the uncharged phenolic amine. If this is so, *N,N*-dimethyldopamine might well be more active than dopamine as it has a lower zwitterion constant.

At the nicotine-sensitive acetylcholine receptor, where the quaternary derivative of dopamine is highly active, it is possible that the situation is quite different and the phenate ion is the active species. This could be investigated by observing the effects of pH on the activity of this compound.

The effects of substituents on pK_a (Table 4) show a variety of influences some obviously resonance in origin such as the change produced by converting methylene to carbonyl. The marked effect of the β-alcoholic group has been commented on by Tuckerman, Mayer & Nachod (1959) and by Sinistri & Villa (1962) and the decline in the effect with increasing methylation of the amino group supports the idea, suggested by the former, that in addition to the inductive effect of the hydroxyl group, there is hydrogen-bonding to the amino group. The effect of the trimethylammonium group on the ionization of the phenolic group is particularly interesting because it is greatly dependent on the distance separating the groups and is probably largely a field effect. This will be affected by the ionic strength of the solution in a way not allowed for by simple Debye–Hückel theory.

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