NMR STUDIES OF CATECHOLAMINES. ACID DISSOCIATION EQUILIBRIA IN AQUEOUS SOLUTIONS

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1. Introduction

The three acid dissociation constants of catecholamines (pK_as approx. 9, 10, 13) are associated with the ionization of the two phenolic groups of the catechol ring, and the deprotonation of the ammonium group of the side chain. The last constant ($pK_a \sim 13$) is commonly assigned to the ionization of the second phenolic group. There are however differences of opinion regarding the relative acidity of the first phenolic group and the ammonium group, hence opposed assignments of the first and second constants have been presented. Since these two dissociation constants have close values, it is impossible to resolve them by potentiometric titration methods alone. A similar problem is also present with other derivatives of mono- and diphenol amines. In early studies, using potentiometric as well as u.v. spectrometry methods, Lewis [1], Reigelman et al. [2] and Kappe and Armstrong [3] assigned the first acid dissociation constant to the ionization of the phenolic group and the second to ammonium deprotonation. In more recent studies however, Jameson and Neillie [4] and Rajan et al. [5,6] introduced an opposite assignment, whereas Martin [7] and Antikainen and Witikainen [8] again assigned the phenolic group as the more acidic. It should also be noted that in studies of complexation of phenolic amines it has commonly been assumed (erroneously, as will be shown below) that the ammonium group is the stronger acid (e.g. [5,6,9–12] and references therein).

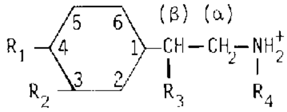
In this work, a proton magnetic resonance (p.m.r.) study of the acid ionization of β-phenylethylamine (PEA), tyramine (TA), dopamine (DA), L-norepinephrine (NE) and L-epinephrine (E) is presented. N.m.r. undoubtedly is a most suitable tool for the purpose of distinguishing between the relative acidity of the first phenolic and ammonium groups, since by examining the ring and the side-chain proton resonances, the effect of both deprotonations can be separately studied. Indeed just by inspection of the p.m.r. spectra it became unequivocally apparent that the phenolic group is more acidic than the ammonium group. A quantitative analysis of the experimental data on the basis of the ionization equilibria conditions yielded the acid dissociation constants characterizing the studied compounds in aqueous (D_2O) solutions.

2. Materials and methods

The materials, PEA, TA, DA and NE as hydrochlorides, and E (acid-free) of the highest purity available were obtained from Sigma Chemical Co. and were used without further purification. Aqueous solutions (0.12–0.18 M) were made up by dissolving the materials in D_2O (99.7%). The pD values, taken as the pH meter reading + 0.4 [13], were adjusted by the addition of concentrated base or acid solutions in D_2O. The error in the pH reading was ± 0.05. In order to prevent oxidation at basic pD, sodium sulfite was added to the NE and E solutions.

The proton magnetic resonance spectra were recorded on a Bruker HFX-10 spectrometer operating at 90 MHz, with an internal deuterium lock. A trace of p-dioxane in the experimental solutions served as internal reference for the shift measurements. All

Table 1
The structure of the studied compounds



	R_1	R_2	R_3	R_4
β-Phenylethylamine (PEA)	H	H	H	H
Tyramine (TA)	OH	H	H	H
Dopamine (DA)	OH	OH	H	H
Norepinephrine (NE)	OH	OH	OH	H
Epinephrine (E)	OH	OH	OH	CH_3

measurements were performed at an ambient probe temperature of 27°C. The experimental uncertainty in the chemical shifts is estimated as ± 1 Hz.

3. Results

The proton chemical shifts of PEA, TA, DA, NE and E were measured as a function of pD. The peaks of the ring moiety were assigned according to Lambert et al. [14]. In the case of PEA, NE, and E the ring proton peaks could not be accurately distinguished, hence only one line (assigned as H_5) was measured. The α and β protons were identified by taking the p.m.r. spectrum of the compounds in very acidic H_2O solution (pH ~ 1.5). The α protons lines were subjected to additional splitting due to spin coupling with the protonated amino group. The structure of the compounds and the proton numbering are given in table 1.

Representative titration curves of PEA and DA are shown in fig.1. As mentioned above, it is clear that the side-chain protons are being affected at higher pD than the aromatic protons, indicating that the ammonium group is the less acidic. The chemical shift data of the studied compounds are given in table 2. δ_+ is the chemical shift of the protonated term, measured at low pD and $\Delta\delta = \delta_- - \delta_+$, where δ_- is the chemical shift of the deprotonated form, measured at high pD. Not that while the aromatic and side-chain hydroxyl substitutions induce relatively large downfield shifts on the resonances of their adjacent protons (and have little effect on further removed protons), the effect on the $\Delta\delta$ values is minor (an obvious exception is $\Delta\delta(H_5)$ of TA relative to PEA). These findings indicate that the hydroxyl groups which produce appreciable effects on the electronic shielding in the protonated forms, do not affect the charge redistribution caused by deprotonation.

The equilibrium equation for PEA may be written as,

$$AD^+ \overset{K}{\rightleftharpoons} A + D^+$$

where AD^+ and A denote the protonated and

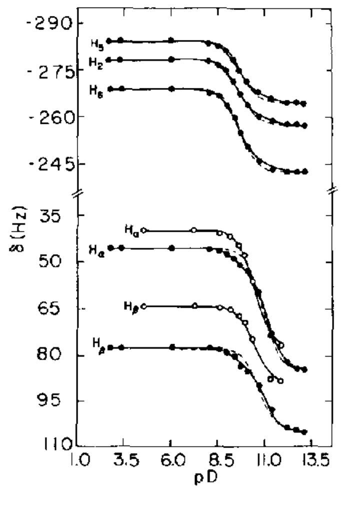


Fig.1. The pD dependence of the chemical shift (δ) of the proton resonances of DA (●) and PEA (○). The lines through the experimental points are the result of a least squares analysis. The shifts are measured from p-dioxane. Negative shifts are to low-field.

Table 2
Chemical shift data. The shifts (in Hz) are measured relative to a p-dioxane internal standard. Negative shifts are to low-field

	H_2		H_5		H_6		H_α		H_β	
	δ^+	$\Delta\delta$	δ^+	$\Delta\delta$	δ^+	$\Delta\delta$	δ^+	$\Delta\delta$	δ^+	$\Delta\delta$
PEA			−326	5			40	37	64.5	24
TA	−309	18	−280	17	−309	18	45.5	46	74	30
DA	−278	22	−284	21	−268.5	27	45.5	41	77.5	28
NE			−283.5	24			44	40	−102	36
E^a			−283.5	24			42.5	44	−105	30

[a] For the α-methyl: $\delta_+ = 86.5$ Hz, $\Delta\delta = 43$ Hz.

deprotonated forms respectively. The observed proton chemical shifts are thus given by,

$$\delta = (1-f)\delta_+ + f\delta_-$$

$$f = [A]/A_0 = P/(1+P) \qquad (1)$$

$$P = 10^{pD-pK}$$

f in the fraction of the deprotonated molecules, and A_0 is the total concentration of PEA. Application of eqn. (1) to the experimental data for the α and β protons of PEA, yields an average p*K* of 10.4, by a least squares fitting method. The data for all of the other compounds were also analyzed with eqn. (1) (ring and side-chain resonances separately). As expected the following sequence was obtained p*K* (ring) < p*K* (chain) ~ p*K* (PEA). However the fit of the data to this model was not very good (e.g. the dashed line in fig.1), implying that a model in which it was assumed that the first phenolic and the ammonium groups ionizations are independent does not describe well the dissociation of catecholamines (and the mono-phenylamines).

A more appropriate equilibrium model which takes into account the possibility of competition between the ring and the chain deprotonations is given by [15]

$$\begin{tikzcd}
& \mathrm{DOA} \arrow[dr, harpoon, shift left, "k_2"] \arrow[dl, harpoon, shift left] & \\
\mathrm{DOAD^+} \arrow[ur, harpoon, shift left, "k_1"] \arrow[dr, harpoon, shift left] & & {}^-\mathrm{OA} \arrow[ul, harpoon, shift left] \arrow[dl, harpoon, shift left] \\
& {}^-\mathrm{OAD^+} \arrow[ul, harpoon, shift left, "k_3"] \arrow[ur, harpoon, shift left, "k_4"'] &
\end{tikzcd}$$

$DOAD^+$, $^-OAD^+$, DOA and ^-OA denote the cationic, zwitterionic, neutral and anionic states of the compounds. The $k_i (i = 1,4)$ are the acidity microconstants characterizing the various isonization processes. Let $[DOAD^+] = C$, $[^-OAD^+] = Z$, $[DOA] = N$, $[^-OA] = A$, $[D^+] = D$, and A_0 be the total concentration, then

$$k_1 = ND/C, k_2 = AD/N, k_3 = ZD/C, k_4 = AD/Z \qquad (2)$$

Clearly $k_1k_2 = k_3k_4$, hence only three constants are to be determined independently. Using the relations (2), the total concentration my be written as,

$$A_0 = C + N + A + Z \equiv C\cdot R \qquad (3)$$

$$R = 1 + k_1/D + k_1k_2/D^2 + k_3/D \qquad (4)$$

Substituting Eq. (3) into Eq. (2) and rearranging, the following relations for the fractional concentrations of the various spices are obtained

$$f_C = C/A_0 = 1/R$$

$$f_N = N/A_0 = k_1/RD \qquad (5)$$

$$f_A = A/A_0 = k_1k_2/RD^2$$

$$f_Z = Z/A_0 = k_3/RD$$

For every proton in the compound, each one of the four ionization states is associated with a characteristic chemical shift. Since two of these shifts (δ_+ and δ_-) can be directly measured, only the other two remain to be determined. However, in order to avoid ambiguity by fitting the experimental data with five parameters (three acidity microconstants + two chemical shifts) the following assumption was introduced:

For the ring protons: $\delta_N = \delta_+, \delta_Z = \delta_-$

For the chain protons: $\delta_Z = \delta_+, \delta_N = \delta_-$

where δ_N and δ_Z denote the chemical shifts in the neutral and zwitterionic states respectively. Under this assumption eqn. (1) can also be applied for the analysis of the shift data of the other compounds, but substituting for f, $f_R = f_Z + f_A$ and $f_S = f_N + f_A$ for the fitting of the ring and the side-chain data respectively. Once the microconstants were derived, the macroscopic acid dissociation constants, usually obtained by potentiometric titration, can be calculated by the relations

$$K_1 = D(Z + N)/C = k_1 + k_2 \qquad (6)$$
$$K_2^{-1} = (Z + N)/AD = k_3^{-1} + k_4^{-1}$$

The ring and the side chain proton shift of each compound were analyzed simultaneously following the procedure described above, with the same three microconstants as fitting parameters. The values obtained for the acidity micro- and macroconstants thus obtained are summarized in table 3.

4. Discussion

The present p.m.r. results show that the dissociation of the catecholamines in aqueous solutions is well described by an ionization scheme in which the apparent acidic macroconstants are determined by competitive ionizations of the first phenolic and the ammonium groups, with the former being the more acidic, in accord with previous results [7]. Since neither of these two constants can be exclusively assigned to either of the two ionizing groups, any application of such assignments in the calculation of concentrations may lead to erroneous results. Proper equilibrium concentrations can be obtained using the relations [5] with the acidic microconstants. Shown in fig.2 are the calculated fractional concentration of the various ionized species present in the D_2O solutions of TA and E, as a function of pD. Note the shift to lower pD of the deprotonation processes of E relative to TA. It has been suggested [3] that this difference is the cause of the enhanced biological activity of catecholamines relative to that of the mono-phenolic amines.

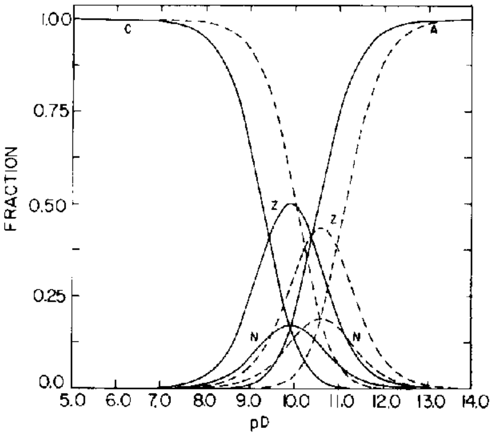


Fig.2. The pD dependence of the fractional concentrations of the cationic (C), zwitterionic (Z), neutral (N) and anionic (A) forms of TA (dashed lines) and E. (solid lines) in D_2O solutions at 27°C.

Table 3
Acid dissociation constants (± 0.1) of catecholamines and tyramine in D_2O solutions at 27°C

	pk_1	pk_2	pk_3	pk_4	pK_1	pK_2
TA	10.6	10.6	10.2	10.9	10.1	11.1
DA	10.2	10.4	9.6	11.0	9.5	11.1
NE	9.7	9.9	9.5	10.1	9.3	10.3
E	9.9	9.9	9.4	10.4	9.3	10.5

The effect of functional group substitution (cf. table 3) may be summarized as follows:

(a) A hydroxyl group in the para position of the benzene ring causes increase in the ammonium group basicity, as reflected in higher values of pk_1 and pk_4 for TA relative to pK(PEA).

(b) The second phenolic group causes an increase in the acidity of the cationic form (lower pk_1 and pk_3) but does not affect the basicity of the anionic form (pk_2 and pk_4 are almost unchanged).

(c) A β-hydroxyl group causes an overall increase in the acidity, especially that of the anionic form.

(d) The effect of an α-methyl group is to increase slightly the basicity of the ammonium group (higher pk_1 and pk_2).

The acidic constants given in table 3 are higher than those obtained in H_2O solutions [6,7,20]. This is due to the isotope effect on the acidity [16], resulting in higher values of acidic constants by about 0.5–0.6 log units for both the phenolic and ammonium deprotonations [17–19]. Taking this contribution into account, the results in table 3 are found to be in good agreement with those obtained in H_2O solutions.

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