Determination of $pK_a$ of ammonium and phenolic groups: Evidence of intramolecular hydrogen bonding in aqueous solution

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The ionization constants of 2-hydroxyanilinium, 2-methoxyanilinium, 4-nitroanilinium ions, $N$-(2′-hydroxyphenyl)-phthalamic acid and $N$-(2′-methoxyphenyl) phthalamic acid, determined spectrophotometrically, have been rationalized in terms of internal hydrogen bonding and steric hindrance between $o$-substituent and reaction site.

Intramolecular hydrogen bonding, also known as internal hydrogen bonding (IHB) and induced intramolecular hydrogen bonding (IIHB) have been found to exert a crucial and highly effective influence on structural features of nonbio– and biomolecules as well as on the rates of various nonbiological and biological reactions. The structural representation of IIHB consists of a segment of a mixed normal micelle with two different monomers, i.e., \( \text{O}—\text{H} \) and \( \text{NH}_2 \). The presence of IIHB is considered to be one of the several essential features responsible for the double helical structure of DNA. The presence of extensive IHB in 1 was established by measuring the $pK_a$ of conjugate acids of 1 and 2 where $pK_a$ of conjugate acid of 1 was smaller by 0.38 $pK$ units than that of 2 (ref. 3). The $pK_a$ values of some compounds containing ammonium, phenolic and both ammonium and phenolic or ammonium and methoxy groups have been determined. The results and probable explanations are described herein.

**Experimental**

The compounds, 3H, 4H and 5H, were commercial products of highest available purity. All other chemicals used were also of reagent grade.

**Synthesis of N-(2′-hydroxyphenyl)phthalamic acid (6)**

Phthalic anhydride (5.00 g, 33.8 mmol) and $o$-aminophenol (3.69 g, 33.8 mmol) were dissolved in 5 ml of dichloromethane (CH$_2$Cl$_2$) in a separate flask before mixing together in a 50 ml round bottom flask containing 25.0 ml of CH$_2$Cl$_2$. The reaction mixture was stirred vigorously at room temperature for 35 minutes after which a pale yellow precipitate was observed. The resulting precipitates was filtered, washed quickly with $n$-hexane and dried to give a pale yellow solid (7.4 g, 86%). The solid was recrystallised in mixed acetone – diethyl ether and the crystal was filtered, washed with cold diethyl ether.
and dried under vacuum to give 6 (3.5 g, 57%) (m.pt. 220-222°C, from acetonite – diethyl ether; lit. value1, 224-225°C) as pale yellow crystals (Found: C, 65.2; H, 4.4; N, 5.4. C_{14}H_{10}NO_4 requires C, 65.4; H, 4.3; N, 5.2%); δ_f (400 MHz, DMSO-d_6): 6.80-6.84 (1H, m), 6.89 (1H, dt, J 8.0), 7.00-7.04 (1H, m), 7.56-7.60 (2H, m), 7.63-7.65 (1H, m), 7.65-7.67 (1H, m), 7.87 (1H, dt, J 8.0), 9.53 (1H, s), 9.62 (1H, s) and 13.12 (1H, s). δ_C (100 MHz, DMSO-d_6): 116.9, 120.2, 124.1, 126.4, 126.7, 128.4, 130.4, 130.6 (2 × s), 132.8, 138.6, 149.4, 168.7, 169.1. v_max (Nujol) (cm⁻¹): 3410 (OH), 3201 (NH), 1727 and 1648 (C=O).

**Synthesis of N-(2'-methoxyphenyl)phthalic acid (7)**

Phthalic anhydride (1.97 g, 13.3 mmol) and o-anisidine (1.50 ml, 13.3 mmol) were dissolved in 5 ml tetrahydrofurane (THF) in separate flasks before being mixed together in a 50 ml round bottom flask containing 10.0 ml of THF. The reaction mixture was stirred vigorously at room temperature for 30 min on which a brown precipitate was observed. The resulting precipitate was filtered, washed quickly with THF and dried to give a white solid (2.9 g, 81%). The solid was further purified by washing with diethyl ether and dried under vacuum to give a white solid 7 (m.pt. 172-175°C; lit. value1 187-188°C) (Found: C, 66.3; H, 4.9; N, 5.2. C_{14}H_{10}NO_4 requires C, 66.4; H, 4.8; N, 5.2%); δ_f (400 MHz, DMSO-d_6): 3.78 (3H, s), 6.93-6.96 (1H, m), 7.04 (1H, dt, J 8.0), 7.10-7.13 (1H, m), 7.52-7.57 (2H, m), 7.60-7.64 (1H, m), 7.83 (1H, dt, J 8.0), 7.97 (1H, dt, J 8.0), 9.33 (1H, s) and 12.97 (1H, s). δ_C (100 MHz, DMSO-d_6): 55.7, 111.3, 120.2, 122.7, 124.9, 127.4, 127.7, 129.4 (2 × s), 130.3, 131.5, 138.6, 150.3, 167.3, 167.7. v_max (Nujol) (cm⁻¹): 3406 (OH), 1716 and 1645 (C=O).

**Ionization constants (K_a) of organic acids**

UV spectrophotometric technique was used to determine the ionization constant (K_a) of 2-hydroxynilinilium ion (3H), 2-methoxyanilinium ion (4H), 4-nitroanilinium ion (5H), N-(2'-hydroxyphenyl)-phthalamic acid (6) and N-(2'-methoxyphenyl)phthalamic acid (7) at 35°C and 1 M ionic strength (maintained by NaCl). In a typical set of K_a determination, buffers of different desired pH at 1 M ionic strength were prepared in aqueous solution containing 0.6-3.0% v/v acetonitrile. These buffer solutions were temperature-equilibrated at 35°C in a thermostated water bath. An appropriate amount of the acid (3H, 4H, 5H and 6 dissolved in acetonitrile or 7 dissolved in 1,4-dioxan) was added to the temperature-equilibrated buffer solution. The absorbance of the buffered mixture was measured quickly at an appropriate wavelength (λ) where the difference in the molar extinction coefficient (ε) of nonionized acid (ε_{AH}) and ionized acid (ε_{A-}) was maximum. The observed initial absorbance (A_{obs}^0) (i.e., observed absorbance at reaction time t = 0) at different pH fits Eq. (1) reasonably well.

\[
A_{obs}^0 = \frac{(\varepsilon_{AH} a_H + \varepsilon_{A-} K_a)}{a_H + K_a} [X_0] \quad \ldots(1)
\]

where [X_0] represents initial concentration of acid (AH) and K_a is the ionization constant for the ionization process, AH → A^- + H^+. The nonlinear least squares technique was used to calculate unknown parameters, K_a, ε_{AH} and ε_{A-}, from Eq. (1) and the values of these calculated unknown parameters for 3H, 4H, 5H, 6 and 7 are summarized in Table 1. The calculated values of ε_{AH} and ε_{A-} for almost all acids were in good agreement with the corresponding values obtained experimentally under respective acidic pH (where [A^-] ≈ 0) and alkaline pH (where [AH] ≈ 0). A satisfactory and reliable fit of almost all observed data (A_{obs}^0 versus a_H/pH) is evident from the standard deviations associated with the values of K_a, ε_{AH} and ε_{A-} (Table 1) as well as from a few typical plots of Fig. 1 where solid lines are drawn through the least-squares calculated values of A_{calc}^0.

**Results and discussion**

The value of pK_a of 3H (pK_{a1} = 4.63) may be rationalized, at least qualitatively, in terms of probable IHB in the reactant and product of ionization processes for 3H and 4H. The presence of IHB in 3-1 increases the pK_{a} (due to predominant stabilization of ground state of forward step), while in 3-3 it decreases the pK_{a} (due to stabilization of ground state of backward step) of 3H. Reaction centre or site and o–substituent creates steric hindrance which causes change of certain degree in coplanarity between reaction site and benzene ring. Such a change in coplanarity decreases the electron donor ability of the ring as shown in 8 and consequently such steric hindrance between o–substituent and reaction site decreases the pK_a of o–substitued benzoic acid or anilinium ion. This fact is reflected from the reported
The values of \( pK_a \) of benzoic acid\(^5\), 2-methylbenzoic acid\(^5\), 2-methoxybenzoic acid\(^5\), anilinium ion\(^6\), 2-methylanilinium ion\(^6\) and 2-methoxyanilinium ion\(^6\) (Table 1). If the steric hindrance also causes change of coplanarity between benzene ring and substituent, then the resonance component of substituent constant (i.e. \( \sigma_R \)) should also be affected accordingly. The value of \( pK_a \) of 3H (\( pK_{a1} = 4.63 \)) is not significantly different from \( pK_a \) of anilinium ion (\( pK_a = 4.60 \), Table 1) which reveals that \( pK_a \) increasing effect of IHB in 3-1 and \( pK_a \) decreasing effects of IHB in 3-3 as well as effect of steric hindrance between 2–OH and ammonium groups of 3H, counterbalance each other leaving no effect of 2–OH substituent on \( pK_{a1} \) of 3H.

**Table 1—The values of ionization constants (\( K_a \)) for organic acids at 35°C**

<table>
<thead>
<tr>
<th>Acid (AH)</th>
<th>( K_a ) (M)</th>
<th>( pK_a )</th>
<th>( 10^{-2} s_{3H} ) (( M^1 \text{cm}^2 ))</th>
<th>( 10^{-2} s_{A-} ) (( M^{-1} \text{cm}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3H</td>
<td>((2.34 \pm 0.07) \times 10^{-2} ) (( K_{a1} ))</td>
<td>4.63</td>
<td>0.40 \pm 0.04(^a)</td>
<td>6.74 \pm 0.04(^a)</td>
</tr>
<tr>
<td>((\lambda = 300 \text{ nm and } [X_0] = 3.0 \times 10^{-4} \text{ M}))</td>
<td>((3.28 \pm 0.13) \times 10^{-10} ) (( K_{a2} ))</td>
<td>9.48</td>
<td>6.97 \pm 0.18</td>
<td>35.0 \pm 0.4</td>
</tr>
<tr>
<td>4H</td>
<td>((3.16 \pm 0.08) \times 10^{-5} )</td>
<td>4.50</td>
<td>1.7 \pm 0.1</td>
<td>28.4 \pm 0.1</td>
</tr>
<tr>
<td>((\lambda = 285 \text{ nm and } [X_0] = 2.0 \times 10^{-4} \text{ M}))</td>
<td>((7.80 \pm 1.00) \times 10^{-2} )</td>
<td>1.11</td>
<td>-0.2 \pm 0.1</td>
<td>15.7 \pm 2.4</td>
</tr>
<tr>
<td>5H</td>
<td>((4.55 \pm 0.41) \times 10^{-4} ) (( K_{a1} ))</td>
<td>3.34</td>
<td>20.0 \pm 0.1</td>
<td>16.2 \pm 0.1</td>
</tr>
<tr>
<td>((\lambda = 310 \text{ nm and } [X_0] = 6.0 \times 10^{-5} \text{ M}))</td>
<td>((3.01 \pm 0.13) \times 10^{-10} ) (( K_{a2} ))</td>
<td>9.52</td>
<td>16.5 \pm 0.5</td>
<td>58.4 \pm 0.5</td>
</tr>
<tr>
<td>6</td>
<td>((2.67 \pm 0.07) \times 10^{-4} )</td>
<td>3.57</td>
<td>48.1 \pm 0.5</td>
<td>59.6 \pm 0.2</td>
</tr>
<tr>
<td>((\lambda = 265 \text{ nm and } [X_0] = 2.0 \times 10^{-4} \text{ M}))</td>
<td>(C_6H_5NH_3^+)</td>
<td>4.60(^b,c)</td>
<td>4.46</td>
<td>9.53(^b,c)</td>
</tr>
<tr>
<td>(2–MeC_6H_4NH_3^+)</td>
<td>(C_6H_5OH)</td>
<td>9.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>((2.67 \pm 0.07) \times 10^{-4} )</td>
<td>3.85</td>
<td>3.91(^b,d)</td>
<td>3.70</td>
</tr>
<tr>
<td>((\lambda = 265 \text{ nm and } [X_0] = 2.0 \times 10^{-4} \text{ M}))</td>
<td>(2–MeC_6H_4CO_2H)</td>
<td>3.85</td>
<td>3.85</td>
<td>3.70</td>
</tr>
</tbody>
</table>

\(^a\)Error limits are standard deviations
\(^b\)\( pK_a \) values were estimated from the reported ionization constants at 25 °C
\(^c\)Ref. 6
\(^d\)Ref. 5

Fig. 1—The plots showing the dependence of \( A_{obs} \) on \( pH \) for 2-hydroxyaniline (3) (1, ▲); 2-methoxyaniline (4) (2, ■); and, \( pK_{a1} \) for N-(2’-hydroxyphenyl) phthalamic acid (6) (3, ●). [The solid lines are drawn through the calculated data points using Eq. (1) as described in the text].
The value of $pK_a$ (= 4.50) of 4H is influenced by IHB similar to that in 3-1 and change in coplanarity between reaction site (ammonium group) and benzene ring. The value of $pK_{a1}$ of 3H is $\sim 0.13$ pK units higher than that of 4H which could be due to larger steric hindrance between 2–OMe and –NH$_3^+$ groups in 4H than between 2–OH and –NH$_3^+$ groups in 3H because the size of –OMe is bulkier than that of OH. The value of $pK_{a2}$ (= 9.48) of 3H is $\sim 0.05$ pK units smaller than $pK_a$ (≈ 9.53) of phenol although overall effect of p–NH$_2$ is electron donor ($\sigma_{p,NH_2} = -0.66$). Consequently $pK_{a2}$ of 3H should be larger than $pK_a$ of phenol if polar effect of 2–NH$_2$ substituent is the only factor affecting the $pK_{a2}$. The value of $pK_{a2}$ of 3H is expected to decrease and increase due to IHB in 3-2 and 3-3, respectively. The effects of IHB in 3-2 and 3-3 on $pK_{a2}$ are primarily due to respective predominant stabilization of ground state of forward step as well as predominant stabilization of ground states of backward steps of the ionization processes. The presence of IHB in the product as shown in 3-4 is expected to decrease $pK_{a2}$ due to stabilization of product state. The steric hindrance between 2–OH and –NH$_2$ groups which is responsible for the change of coplanarity of –NH$_2$ group and benzene ring is expected to decrease $pK_{a2}$. Nearly 0.35 pK units smaller value of $pK_{a2}$ of 3H than $pK_a$ of phenol indicates that $pK_{a2}$ decreasing effects of 3H are stronger than its $pK_{a2}$ increasing effect. The value of $pK_{a}$ (= 1.11) of 5H is comparable with reported value of $pK_a$ = 1.12 (ref. 6).

The values of $pK_{a1}$ of 6 and $pK_a$ of 7 are 3.34 and 3.57, respectively, which are significantly smaller than $pK_a$ (= 3.91) of benzoic acid. At one glance, one might argue that these results may be explained in terms of polar and resonance effects of 2–CONHC$_6$H$_4$(2′–X) with X = OH, OMe. Since the value of Hammett substituent constant ($\sigma$) for 4–CONHMe is 0.36 (ref. 9), the value of $\sigma$ for 2–CONHR with $R = 2'$–OHC$_6$H$_4$, 2′–OMeC$_6$H$_4$ is expected to remain positive and the value of $\sigma$ for –CONHC$_6$H$_4$(2′–OH) should not be appreciably different from $\sigma$ for –CONHC$_6$H$_4$(2′–OMe) since $\sigma_{p,-OH} = -0.37$ and $\sigma_{p,-OMe} = -0.27$ (ref. 7). Under such circumstances, it is difficult to explain $\sim 0.23$ pK units higher $pK_a$ for 7 as compared to that of $pK_{a1}$ for 6 if polar and resonance components of substituents (2–OH, 2–OMe) are the only factors affecting the $pK_{a1}$ of 6 and $pK_a$ of 7. The alternative explanation for the values of $pK_{a1}$ for 6 and $pK_a$ for 7 may be in terms of IHB and steric hindrance between –COOH and –CONHC$_6$H$_4$(2–X) with X = OH, OMe. The presence of IHB in 6-1 and 7-1 should decrease the respective values of $pK_{a1}$ and $pK_a$ (compared to that of benzoic acid) due to IHB effect similar to that in 3-2. But such effects cannot explain $\sim 0.23$ pK units higher value of $pK_a$ of 7 than $pK_{a1}$ of 6.

It is worth mentioning that IHB, as in 6-1 and 7-1, is expected to decrease the value of rate constant ($k_{in}$) for intramolecular carboxylic group–assisted cleavage of 2-HO$_2$CC$_6$H$_4$CONHR compared to that of 2-HO$_2$CC$_6$H$_4$CONR$_2$ where IHB, similar to 6-1 and 7-1, is impossible. Nearly 62–fold larger value of $k_{in}$ for the aqueous cleavage of N,N-dimethylphthalamic acid ($10^3$ $k_{in} = 1.0$ s$^{-1}$) compared to that of N-methylphthalamic acid ($10^5$ $k_{in} = 1.6$ s$^{-1}$) may be the consequence of the absence of IHB, similar to 6-1 and 7-1, in N,N-dimethylphthalamic acid.

The presence of IHB in 6-2 and 6-3 decrease the value of $pK_{a1}$ due to IHB effect similar to that in 3-2 while IHB in 6-4 increases the $pK_{a1}$ of 6 due to IHB effect similar to that in 3-3. The steric hindrance between –COOH and –CONHC$_6$H$_4$(2–OH) groups is expected to change coplanarity between reaction site (COOH) and the benzene ring attached to it. Such a change in coplanarity should decrease $pK_{a1}$ of 6. The presence of IHB, similar to IHB in 6-2 and 6-3, is impossible for 7 and thus, the $pK_a$ value for 7 increases due to IHB in 7-2. This causes predominant stabilization of ground state of forward step of the ionization process and decreases due to steric hindrance between reaction site (–COOH) and 2–substituent. Nearly 0.23 pK units higher value of $pK_a$ of 7 than $pK_{a1}$ of 6 may be attributed to the presence of IHB as shown in 6-2 and 6-3 because the extent of steric hindrance in 6 is almost similar to that in 7.
Based on the experimentally observed fact that most amides exist in the predominant Z-configuration (i.e. planar s-trans configuration) for reasons not yet fully understood, and that a hydrogen bonded 10-membered ring network would be tenuous, one might argue that structures 6-2, 6-3, 6-4 and 6-5 are impossible. Although this assertion is apparently conceivable within the domain of a long-held view on the relative stability of n-membered ring systems with n ≥ 6, the exception to this general rule may exist. The IHB should be stronger than the intermolecular hydrogen bonding counterpart in aqueous solution for the same reasons which are responsible for generally much higher rate of an intramolecular reaction compared to that of an analogous intermolecular reaction. The strength of hydrogen bond is expected to increase in the order of intramolecular hydrogen bond > induced intramolecular hydrogen bond > intermolecular hydrogen bond.

The value of equilibrium constant for E/Z stereoisomerization (= [Z]/[E]) is expected to decrease with the presence and absence of IHB in E- and Z-configuration, respectively. The equilibrium concentrations of Z-6 and E-6 are expected to depend upon the delicate balance of the sum of n-membered ring strain energy due to IHB and energy of interaction that stabilizes Z compared to E as well as the strength of IHB as shown in 6-2 to 6-5. More than 6-membered IHB or IIHB ring systems are not unusual in various macromolecules, and the presence of 8-membered IIHB and 10-membered IIHB in respectively the double helical structure of DNA and β-pleated sheet secondary structure of proteins are well known. It is worth mentioning that the ball-and-stick molecular model of 6 suggests that both its COOH and 2-OH groups are within the hydrogen bonding distance in Z-6 form with almost complete coplanarity between N-C(O) and both benzene rings. But IHB in E-6 form requires a substantial change in this coplanarity and consequently the lone pair of electrons on nitrogen cannot fully interact with π-electrons of benzene ring through resonance.

The proposed IHB in 6-2 to 6-5 is based upon the following experimentally observed facts: (i) the value of pK_a of 6 is smaller than that of 7 by 0.23 pK units; (ii) first-order rate constant (k_obs) for intramolecular carboxylic group-assisted cleavage of amide bond of 7 is 2-fold larger than that of 6 in aqueous solution containing 4.9 × 10^-2 M HCl. Nearly 2-fold larger value of k_obs for cleavage of 7 as compared to that of 6 is concluded to be the consequence of stronger IHB in 6-4 than that in 7-2, and, (iii) the value of pK_a of N-(2-aminophenyl)-phthalamic acid is larger than that of N-phenylphthalamic acid by ~ 1.2-1.3 pK units and this significant pK_a difference is attributed to a significantly stronger IHB, similar to that shown in 6-4 (ref. 13).

The probable IHB in 6-6 may be ruled out for the reason that IHB could not be kinetically detected in mild acidic aqueous cleavage of 6 (ref. 13). The other possible IHB in 6-7, which should decrease pK_a, cannot be completely ruled out. The polar effect of substituent 2--NHCOC_6H_4(2--CO_2-) on pK_a of 6 should be ineffective for such polar effect could not explain pK_a of 3 and also σ_p-NHCOMe = 0.00 (ref. 9).
The above study shows that the nearly 0.23 $pK$ units larger value of $pK_a$ of 7 than the $pK_{a1}$ of 6 is attributed to the internal hydrogen bonding (IHB) as shown in 6-2 and 6-3. Although $pK_a$ change of $\sim 0.23$ $pK$ units is rather small, since some opposing interactions cancel out their effects leaving an overall small effect on $pK_a$, it may be compared with 0.38 $pK$ units change in $pK_a$ of 1 and 2, where IHB in 1 has also been established by kinetic measurements. The $pK_{a2}$ value of 3H is smaller than $pK_a$ of phenol by $\sim 0.05$ $pK$ units, which is ascribed to larger $pK_{a2}$ decreasing effects of steric hindrance and IHB in 3-2 than $pK_{a2}$ increasing effect of IHB in 3-3.

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