Synthesis, Structure Elucidation and Determination of Acid Dissociation Constant, Tautomerism of Pyrido [1,2-a] benzimidazole-2,4-dione

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ABSTRACT
The first starting material, 1H-2-acetylbenzimidazole, 4, was synthesized by o-phenylenediamine, 1, and lactic acid solution, 2, in hydrochloric acid medium on the first step than, oxidation reaction was performed by CrO3 in acetic acid medium on the second step. The last starting material, ethyl 2-(2-acetylbenzimidazol-1-yl) acetate, 6, was synthesized by using first starting material, 4, ethyl 2-bromoacetate, 5, and K2CO3 in acetone medium. The target compound, pyrido[1,2-a] benzimidazole-2,4-dione, 7, was synthesized by using the last starting material, 5, in sodium ethoxide medium. The compound, 7, can be found in two forms which are keto and enol which would be evaluated in this study. For the evaluation, it was performed some spectroscopic studies. In addition, it was evaluated experimentally obtained acidity constant, pKₐ value and tautomeric equilibrium of the compound, 7, by using ultraviolet-visible (UV-Vis) spectrophotometer. All of these studies have shown that the valid form of the compound, 7, is keto form.

Keywords: Pyrido[1,2-a]benzimidazole, Acidity constant, pKa value, Tautomerism.

INTRODUCTION
The investigation of the proton tautomerism properties of heterocyclic compounds benefits the chemical and medicinal industry. The determination of acidity constants and tautomeric equilibrium are very important in understanding to predict reactions, ion transport behavior, binding to receptors and mechanisms of drug precursor compounds1-8.
It has been reported that pyrido[1,2-α]benzimidazole compounds have some biological activities which are antiviral, antimicrobial, analgesic, anti-inflammatory, anticancer and anti-HIV. Pharmacological properties of the heterocyclic compounds are related to the determination of their acidity and tautomerism. Because of this, in this study, target compound, pyrido[1,2-α]benzimidazole-2,4-dione, 7, was synthesized and investigated its identification, acidity and tautomerism based on its spectral data.

The compound, 7, has two carbonyl carbon on 2 and 4 positions such as 1,3-dione compounds. The 1,3-diones attracted the attention of researchers due to their two characteristic features. One of them is that 1,3-diones have synthetic potential due to the presence of β-dicarbonyl moiety. The other is that a wide range of physicochemical properties such as tautomerism, proton transfer, quantum mechanical calculation are associated with 1,3-diones.

The infra red (IR) and nuclear magnetic resonance (NMR) spectroscopic techniques were employed for identification of pyrido[1,2-α]benzimidazole-2,4-dione, 7, and experimentally obtained acidity constant, pK₀ value and tautomeric equilibrium of the compound, 7, were evaluated by using UV-Vis spectrophotometer in this pronounced study. We aimed to explain the tautomeric condition of the compound, 7, with all of the techniques. Previously, a research group published that some pyrido[1,2-α]benzimidazolone derivatives existed on enol form as dominated. Another group reported that diverse pyrido[1,2-α]benzimidazolone derivatives existed on keto forms on the other hand their enol forms could be dominated in different solvents.

**METHODOLOGY**

**Chemistry and Synthesis**

The melting points were determined using WRS-2A Microprocessor. Spectroscopic data were recorded on the following instruments: UV-Vis, Shimadzu 1800 UV; IR, Shimadzu 8400 FTIR spectrophotometer; NMR, Bruker 500 MHz NMR Spectrometer. Analyses for C, H, and N were within 0.4% of the theoretical values. The 1H-2-(1-hydroxyethyl)benzimidazole, 3, and 1H-2-acetylbenzimidazole, 4, compounds used as starting materials were prepared according to the methods in the literature. The reaction steps of syntheses in this study were shown in figure 1.

**The synthesis of 1H-2-(1-hydroxyethyl)benzimidazole, 3**

A mixture of o-phenylenediamine, 1, (185 mmol) and lactic acid solution, 2, (200 mmol) in 4N hydrochloric acid solution (75 mL) was refluxed for 120 h. The reaction medium was poured into cold water and kept for 24 h. The residue was fil-
tered and washed with water. The raw product was recrystallized from ethanol.

The synthesis of 1H-2-acetylbenzimidazole, 4

The 1H-2-(1-hydroxyethyl)benzimidazole, 3, (139 mmol) was completely dissolved in acetic acid (60 mL). The chromium trioxide solution, CrO₃ (104 mmol), was dissolved in water (70 mL) and gently and slowly dropped into the reaction medium while the 1H-2-(1-hydroxyethyl)benzimidazole, 3, solution was stirred in a hot water bath at 90°C. The mixture was refluxed for 2 h. The reaction medium was poured into water and chloroform in an extraction flask and kept for 15 min. The raw product in chloroform was extracted and evaporated by rotary evaporator, then recrystallized from toluene.

The synthesis of ethyl 2-(2-acetylbenzimidazol-1-yl)acetate, 6

A mixture of 1H-2-acetylbenzimidazole, 4, (30 mmol), ethyl 2-bromoacetate, 5, (30 mmol) and potassium carbonate (30 mmol) in acetone (40 mL) was stirred at room temperature for 4 h. The reaction medium was poured into cold water and kept for 24 h. The residue was filtered and washed with water. The raw product was recrystallized from ethanol.

The synthesis of pyrido[1,2-a]benzimidazole-2,4-dione, 7,

The sodium metal (18.3 mmol) was completely dissolved in ethanol (30 mL), then ethyl 2-(2-acetylbenzimidazol-1-yl)acetate, 6, (6.1 mmol) was added to this solution. The mixture was stirred in an ice bath for 1 h and then added to acetic acid (4 mL). The reaction medium was poured into cold water and kept for 24 h. The residue was filtered and washed with water. The raw product was recrystallized from chloroform-petroleum ether.

Determination of Acidity Constant and Tautomerism

Methanol, ethanol, glycine, KOH, H₂SO₄, HCl, CH₃COOH, CH₃COONa, NaOH, KH₂PO₄, Na₂CO₃, NaHCO₃, NaCl, methyl red indicator and standard buffer solutions were obtained from Merck and were not further purified for acidity studies. In addition, DMSO, ethanol, CHCl₃, C₆H₁₂, (C₂H₅)₃N, CF₃COOH were obtained from Merck and used for UV Studies. Spectroscopic data were recorded on the instrument: Unicam UV-2 UV-Vis spectrophotometer.

Determination of acidity constant

The acidity constant value was found by using the UV spectroscopic methods described in the literature¹⁵-¹⁸.

The general procedure applied was as follows: a stock solution of compound under investigation was prepared by dissolving about (10 to 20) mg of compound
in alcohol in a volumetric flask. A liquid (1 mL) of this solution was transferred into 10 mL volumetric flask and diluted to the mark with buffers of various pH. The pH was measured before and after addition of the new solution. The optical density of each solution was then measured in 1 cm cells, against solvent blanks, using a constant temperature cell holder UV-Vis spectrophotometer. The scanning spectrophotometer was thermostatted at 25°C (to within ±0.1°C). The wavelengths were chosen so that the fully protonated form of the substrate had a much greater or much smaller extinction coefficient than the neutral form. The analytical wavelengths, the half protonation values and the UV absorption maxima for substrate studied are given in Table 1.

Calculations of half protonation value was carried out as follows: the sigmoid curve of optical density or extinction coefficients at the analytical wavelengths (OD, I) was first obtained (figure 2).

The optical densities of the fully protonated molecule (ODca) and pure free base (ODfb) at acidity were then calculated by linear extrapolation of the arms of the curve. The following equation gives the ionization ratio where the optical density (OD) was converted into molar extinction (e) using Beer’s Law of eqs 1 and 2.

\[
OD = e . b . c
\]

\[\text{eq 1}\]

b: cell width, cm
c: concentration, mol/dm³

\[I = \frac{[BH^+]}{[B]} = \frac{(OD_{\text{obs}} - OD_{\text{fb}})}{(OD_{\text{ca}} - OD_{\text{obs}})} = \frac{( \varepsilon_{\text{obs}} - \varepsilon_{\text{fb}})}{(\varepsilon_{\text{ca}} - \varepsilon_{\text{obs}})}\]

\[\text{eq 2}\]

\(OD_{\text{ca}}\): optical density of conjugated acid

\(OD_{\text{fb}}\): optical density of free base

The linear plot of log I against pH, using the values -1 < log I< 1 had slope m, yielding the half protonation value as pH\(^{1/2}\) or more generally H\(^{1/2}\) at log I=0 (figure 3). The acidity constant gives as follows eq 3.

\[pK_a = m \ pH^{1/2}\]

\[\text{eq 3}\]

**Determination of tautomism**

The keto percentage of pyrido[1,2-α]benzimidazole-2,4-dione, 7, was defined by measuring UV spectra at room temperature (25°C ±2) in four solvents of increasing polarity (i.e. cyclohexane, chloroform, ethanol and dimethylsulfoxide) were given in Table 2. The molecule concentration is 10⁻⁵ mol/L. The UV–Vis spectra of molecule were studied in polar and nonpolar solvents in both acidic (CF₃COOH) and basic (Et₃N) medium. The tautomerism of the compound, 7, was given in figure 4.
The parameters of the spectra for the molecule in polar and nonpolar solvents in both acidic and basic medium were given in Table 2. The calculated keto-enol tautomeric equilibrium of molecule was given in Table 3.

RESULTS AND DISCUSSION

Chemistry and Synthesis

The synthesis of 1H-2-(1-hydroxyethyl)benzimidazole, 3

The 1H-2-(1-hydroxyethyl)benzimidazole, 3, was prepared in a yield of 75%. Its melting point was determined as 178.7-180.6 °C. The melting point in the literature was given as 180.0-181.0 °C14.

The synthesis of 1H-2-acetylbenzimidazole, 4

The 1H-2-acetylbenzimidazole, 4, was prepared in a yield of 59%. Its melting point was determined as 188.9-189.6 °C. The melting point in the literature was given as 189.0-191.0 °C14.

The synthesis of ethyl 2-(2-acetylbenzimidazol-1-yl)acetate, 6

The ethyl 2-(2-acetylbenzimidazol-1-yl)acetate, 6, was prepared in a yield of 92%. Its melting point was determined as 91.4-92.9 °C. The melting point in the literature was given as 91.0-93.0 °C9. IR (potassium bromide): 1742, 1687 (C=O), 1604-1455 (C=C, C=N) cm⁻¹; ¹H-NMR (DMSO-d₆) δ (ppm): 1.22 (t, J=7.0 Hz, 3H), 2.72 (s, 3H), 4.17 (q, J=7.0 Hz, 2H), 5.41 (s, 2H), 7.39 (td, J=7.5 Hz, j=1.0 Hz, 1H), 7.47 (td, J=7.5 Hz, j=1.0 Hz, 1H), 7.81 (d, J=8.5 Hz, 1H), 7.88 (d, J=8.0 Hz, 1H); ¹³C-NMR (125 MHz) δ (ppm): 14.61 (CH₃), 27.98 (CH₃), 47.39 (CH₂), 61.95 (CH₂), 112.63 (Ar-C), 122.51 (Ar-C), 124.94 (Ar-C), 127.20 (Ar-C), 137.82 (Ar-C), 142.16 (Ar-C), 147.17 (Ar-C), 169.65 (C=O), 194.30 (C=O).

The synthesis of pyrido[1,2-a]benzimidazole-2,4-dione, 7

The pyrido[1,2-a]benzimidazole-2,4-dione, 7, was prepared in a yield of 55%. Its melting point was determined as 142.2-145.1 °C. IR (potassium bromide): 1741, 1687 (C=O), 1582-1455 (C=C, C=N) cm⁻¹; ¹H-NMR (DMSO-d₆) δ (ppm): 2.72 (s, 2H), 5.33 (s, 2H), 7.40 (t, J=8.0 Hz, 1H), 7.46 (t, J=7.5 Hz, 1H), 7.79 (d, J=8.0 Hz, 1H), 7.87 (d, J=8.0 Hz, 1H); ¹³C-NMR (125 MHz) δ (ppm): 45.91 (CH₂), 54.65 (CH₂), 111.23 (Ar-C), 122.36 (Ar-C), 123.46 (Ar-C), 126.92 (Ar-C), 137.63 (Ar-C), 141.81 (Ar-C), 148.37 (Ar-C), 195.36 (C=O), 204.73 (C=O).
Determination of acidity constant

When the acidity constant value of 5.5620 for the protonation of benzimidazole is taken into account, it might be said that the molecule acidity constant value is close enough. Therefore the protonation pattern of this molecule should be similar and had to be aza protonation. In addition, hydrogen bond provides stability (figure 5). This is supported by ab initio calculation (HF/6-31G (d,p), CPCM method, Gaussian 03 program\textsuperscript{21}) which predict an interatomic bond distance between the protonated nitrogen atom and carbonyl of keto which was found as 2.70, sufficiently close for an intra molecular hydrogen bonding interaction. The UV spectra and protonation data for the molecule are given in Table 1.
Table 1: UV Spectral Data and Acidity constant values

| Molecule          | Spectral maximum \( l/\text{nm} \) | Acidity Measurements | \( \log \epsilon_{\text{max}} \)^a | \( \log \epsilon_{\text{max}} \)^b | \( \lambda_{/\text{nm}} \) | \( H_{1/2} \) | m  | \( pK_a \) | corrf |
|------------------|-----------------------------------|----------------------|-----------------|-----------------|----------------|----------|-----|----------|--------|
| Benzimidazole    | -                                 | -                    | -               | -               | -              | -        | -   | 5.5620   | -      |
| The compound, 7  | 282 (5.00)                        | 293 (6.19)           | 297             | -8.35           | -0.71          | 5.93±0.06 | 0.99 |

\( ^a \) Measured in pH=7 buffer solution for molecule 1.
\( ^b \) Measured in 1% H₂SO₄ for molecule 1.
\( ^c \) The analytical wavelength for \( pK_a \) determination.
\( ^d \) Half protonation value.
\( ^e \) Acidity constant value ± standard error.
\( ^f \) Correlation for log I as a function of pH graph.

Figure 2: \( \epsilon_{\text{max}} \) as a function of \( H_0 \) (at 297 nm) plot for the protonation of the compound, 7

Figure 3: pH as a function of log I (at 297 nm) plot for the protonation of the compound, 7
Determination of tautomerism

For the pure solvent, acidic and basic medium, increasing the solvent polarity shifts the absorption maxima to red. Comparison of the correlations in Table 2 with the data in Table 3 indicates that in nonpolar solvent (cyclohexane) and polar solvents (DMSO, ethanol, CHCl₃) K>>1. The results showed the keto form which was predominant.

The high intensity of absorptions in the 200-265 nm range showed that diketone forms were present²². In all three medium (solvent, acidic and basic) the keto-enol tautomers (%) were measured for the compound, 7. In pure solvent medium, chloroform, the percent ratio of the keto-enol tautomers of the compound, 7, was higher than in DMSO, ethanol and cyclohexane. In acidic and basic solutions of cyclohexane, the percent ratio of keto-enol tautomer was observed as higher than DMSO, chloroform and ethanol solutions (figure 6).

Table 2. The acid and base effects in different solvent for tautomerism (T=25°C ± 1)

| Solvent | λ max. (nm) (Absorbance) | Acidity | Basic |
|---------|--------------------------|---------|-------|
| Neutral |                          |         |       |
| DMSO    | 232(A=0.697)             | 228(A=0.850) | 223(A=1.978) |
|         | 240(A=0.651)             | 237(A=0.739) | 253(A=1.015) |
|         | 257(A=0.806)             | 244(A=0.874) | 262(A=0.703) |
|         | 278(A=0.640)             | 251(A=0.490) | 277(A=0.368) |
|         | 285(A=0.647)             | 256(A=0.731) | 315(A=0.430) |
|         | 302(A=0.557)             | 270(A=0.627) |       |
|         |                          | 279(A=0.600) |       |
|         |                          | 287(A=0.570) |       |
|         |                          | 301(A=0.578) |       |
| Solvent | Keto-enol tautomer, %a | Equilibrium constant, $K_{keto}$ d |
|---------|------------------------|-----------------------------------|
|         | Neutral    | Acidicb   | Basicc  | Neutral | Acidicb | Basicc  |
| DMSO    | 55.40      | 58.20     | 70.20   | 1.24    | 1.39    | 2.35    |
| EtOH    | 53.80      | 58.55     | 75.57   | 1.16    | 1.41    | 3.09    |
| CHCl₃   | 61.18      | 58.68     | 63.01   | 1.57    | 1.42    | 1.70    |
| C₆H₁₂   | 51.96      | 81.14     | 84.18   | 1.08    | 4.30    | 5.32    |

*aKeto isomer (%) = (A₂/A₁ + A₂) x100  
A₁ = the absorbance of enol form (π → π*)  
A₂ = the absorbance of keto form (π → π*)

bAcidic medium is attained by addition of CF₃COOH (~1 mL) to the given solution (molecule concentration 1x10⁻⁵ mol/L)

cBasic medium is attained by addition of Et₃N (~1 mL) to the given solution (molecule concentration 1x10⁻⁵ mol/L)

d$K_{keto} = keto%/1-keto%$  

$\text{eq } 4$  
$\text{eq } 5^{23}$
CONCLUSIONS
It has been seen that keto form is dominated when the spectral data from IR and NMR techniques for identification studies was taken into consideration. The results of IR spectrums showed two carbonyl peaks and no hydroxyl peaks in addition to the results of $^1$H-NMR spectrums explained that there are methylene peak between two carbonyls and the other methylene peak and no aromatic proton peaks for pyridine ring causing as a result of tautomerism. The results of $^{13}$C-NMR spectrums showed also two peaks of carbons from carbonyls and no aromatic carbons peaks from the pyridine ring as well.

The acidity constant study ended up using by UV spectrophotometer shows, the possible form as keto form. In this pronounced study, benzimidazole results were employed to compare the compound, 7. On the other hand, the results of Gaussian 03 program were utilized to support this situation. When the tautomerism tests that performed in there mediums which are acidic, basic and neutral were searched, the keto form was decided as predominant.

All of these results have proved that the compound, 7, recrystallized as keto form. It has been reported that some pyrido[1,2-α]benzimidazolone derivatives existed on keto forms$^6$. This situation has supported our study and results as well.

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