Investigación

Acid-base equilibrium studies of 2-(aminomethyl)benzimidazole in aqueous solution

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Abstract. The acid-base equilibrium constants of the 2-(amino-methyl)benzimidazole 2AMBZ in aqueous solutions have been determined at 25 °C by means of nuclear magnetic resonance spectroscopy (^{13}C NMR), and corroborated by potentiometric and spectrophotometric techniques. pKa values were obtained from experimental data using the computer programs HypNMR, SUPERQUAD and SQUAD respectively, finding $pKa_1 = 3.103 \pm 0.079$, $pKa_2 = 7.624 \pm 0.063$ and $pKa_3 = 12.540 \pm 0.038$ by NMR at variable ionic strength. Likewise, it was possible determine the deprotonation mechanism of 2AMBZ.
Keywords: 2-(aminomethyl)benzimidazole, pKa, HypNMR, SUPERQUAD, SQUAD.

Resumen. Las constantes de equilibrio ácido-base del 2-(aminometil) bencimidazol 2AMBZ en solución acuosa han sido determinadas a 25 °C mediante resonancia magnética nuclear (^{13}C RMN), y corroboradas por técnicas potenciométricas y espectrofotométricas. Los valores de pKa fueron obtenidos a partir de los datos experimentales, usando los programas computacionales HypNMR, SUPERQUAD y SQUAD respectivamente, encontrando $pKa_1 = 3.103 \pm 0.079$, $pKa_2 = 7.624 \pm 0.063$ y $pKa_3 = 12.540 \pm 0.038$ por RMN a fuerza iónica variable. Asimismo, fue posible determinar el mecanismo de desprotonación del 2AMBZ.
Palabras clave: 2-(aminometil)bencimidazol, pKa, HypNMR, SUPERQUAD, SQUAD.

Introduction

There is a considerable interest in benzimidazole due to its known biological activity. It has been used to develop many pharmaceutical utility drugs such as omeprazole, mebendazole, astemizole, etc. [1]. Thus, some metallic complexes of benzimidazoles have been proposed as low-molecular-weight mimetic models of the active sites in some metalloproteins [2,3].

Particularly, 2-(aminomethyl)benzimidazole (2AMBZ) (Fig. 1) has a special relevance. Recently it has been employed to develop new, interesting antimicrobial, antiparasite and AIDS antiviruses agents [4-12], as well as useful compounds for the treatment of cancer, diabetic retinopathy, osteoporosis, arthritis, etc. [13-17]. Most of these studies have been the subject of diverse patents.

Because of the biological and commercial importance of 2AMBZ, some studies have determined the acidity constants [18-20] and stability constants for complexes with Cu(II) and Ni(II) [19-20]. These thermodynamic parameters can be used to obtain a better understanding of their biological properties, as well as to optimize synthetic routes.

In these reports graphical methods have been used for equilibrium constant determination, and they do not describe clearly the methodology and experimental conditions. Likewise, in some cases comparisons with other techniques do not appear and it does not allow to evaluate the quality of the results, and the reported statistic are not well defined.

Previous works report the first and second pKa of 2AMBZ [19-20]. Only one reference reports the third [18].

In the present work the acidity constants of 2AMBZ were determined in aqueous solutions at 25 °C by potentiometry, spectrophotometry UV/Vis and for the first time by ^{13}C NMR techniques. Computational programs were used to process the information in order to obtain statistical parameters that allow evaluating in a better way the quality of the results. From the studies of ^{13}C NMR it was possible to establish clearly the deprotonation mechanism. Although the acid-base mechanism has been reported elsewhere, there are discrepancies between published mechanisms [18,19].

Experimental

Purification. 2-(Aminomethyl)benzimidazole dihydrochloride hydrate is commercially available with a 98 % of purity (Aldrich), therefore it was purified by recrystallization from absolute ethanol, as reported elsewhere [19-21], and obtained as a white crystalline solid. The melting point was determined using a Büchi B-545 (263 °C) [21]. Also, the IR absorption spectrum was obtained for the recrystallized solid in a TF-IR 2000 Perkin-Elmer spectrophotometer, matching with the one reported in the FT-IR spectra catalog [22]. Finally, the elemental analysis EA was obtained in a CHNS / O Analyzer 2400 Perkin Elmer found: C, 40.8 %; H, 5.7 %; N 17.3 %. Calc. for $C_8H_{11}N_3Cl_2,H_2O$: C, 40.3 %; H, 5.5 %; N, 17.7 %.

The recrystallized 2-(aminomethyl)benzimidazole dihydrochloride hydrate was then used in its purified form for all experiments.

NMR determinations. NMR spectra were recorded in a JEOL Eclipse 400 MHz spectrometer with an auto tune 5-mm probe (P-31 to N-15 NMR tunable probe VT) at a constant temperature of 25 ± 1 °C (corroborated with methanol [23]) using TMS as electronic reference. Experimental parameters for ^{13}C nucleus were as follows: spectral frequency = 100.53 MHz, spectral width = 25.19 KHz (250 ppm), 32 K data points in the frequency domain, pulse width = 3.17 µs (9.5 µs at 90o flip angle), number of scans = 3995. Spectra were acquired using single pulse proton decoupling and coupling experiments.

Spectra were recorded for solutions of 0.1M 2AMBZ at pH values between 1.1 and 13.2, with 10 % v/v of D$_2$O for lock. pH measurements were obtained using a Radiometer-Copenhagen PHM 250 pH-meter equipped with a ORION combined ROSS pH-electrode. Calibration of the electrode system was performed with Radiometer-Copenhagen IUPAC standard buffers of pH 4.005, 7.000 and 10.012 ± 0.010 obtained cell efficiency greater than 98 %. The temperature of the solutions was kept constant to 25 ± 0.01 °C with a thermostatable cell holder and a Techne Tempette TE-8D temperature controlled water bath.

The refinement of the equilibrium constants was done using the HypNMR program [24] from ^{13}C chemical shifts. The program refines equilibrium constant values by minimizing the sum of residual squares between calculated (δ_i^{calc}) and observed (δ_i^{obs}) chemical shifts U (eq 1). Wi are the weights assigned to each observation and ideally should be equal to the reciprocal of the variance of the measurement.

$$U = \sum_i W_i \left(d_i^{calc} - d_i^{obs} \right)^2 \qquad (1)$$

Spectrophotometric determination. *Absorbance versus time studies.* In order to demonstrate absorbance on time independence during measurements, absorption spectra for 48 h for 1 × 10^{-4} M 2AMBZ solutions at different pH values were recorded. In all cases a maximum variation of 1.5 % was observed over this period, indicating stability of the different acid-base species.

Spectrophotometric data. Two aqueous solutions of 2AMBZ of the same concentration were prepared, one at low pH adjusted with HCl, and other at high pH adjusted with NaOH. The experiments were carried out in two steps: the basic solution was added to the acid solution in order to obtain different pH's until reach neutral pH, and the acid solution was added to the basic solution in order to obtain different pHs until reach neutral pH. The study was carried out at concentrations of 1.5 × 10^{-4}, 1 × 10^{-4}, 5 × 10^{-5} and 2 × 10^{-5} M 2AMBZ solutions at variable and 1.0 M ionic strength adjusted with NaClO$_4$. All experiments were done by triplicate.
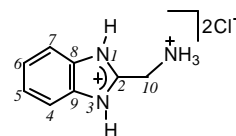

Fig. 1. 2-(aminomethyl)benzimidazole dihydrochloride.

Absorbance data were recorded approximately every 0.5 pH units in a UV-Vis Perkin-Elmer Lambda 40 spectrophotometer and pH values were monitored with the same equipment used in the NMR determinations. Constant temperature was kept at 25 ± 0.01 °C.

The determination of the equilibrium constants was done using SQUAD [25,26]. This program is analogous to Hyp NMR, and calculates the best values for the stability constants of the proposed equilibrium model from absorption spectra by employing a non-linear least square approach to minimize the sum of residual square for absorbance U. The program also calculates absorptivity coefficients for each one of the absorbing species in the range of wavelength and pH studied.

In order to determine the number of absorbing species in solution, the absorbance matrices were analyzed using TRI-ANG [27].

Potentiometric determination. pH titrations of 20 mL of 0.001, 0.005, 0.01 and 0.05 M of 2AMBZ solutions were performed under nitrogen atmosphere with carbonate-free solutions of NaOH at a constant temperature of 25 ± 0.01 °C. These titrations were done by triplicate at variable and 0.1 M ionic strength NaClO$_4$.

NaOH solutions were standardized with potassium hydrogen phthalate and these were obtained by dilution of a stock solution containing 50 % w/v of NaOH low in carbonates, filtered and was kept in a dessicator over ascarite [28].
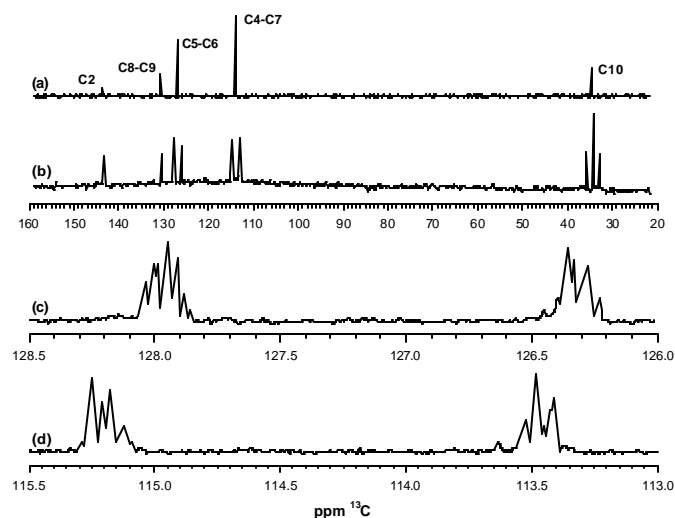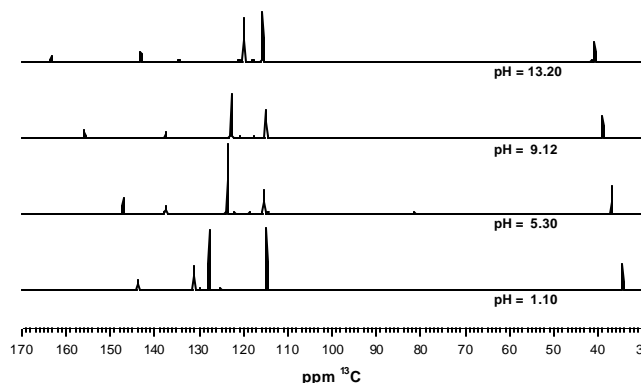

Fig. 2. ^{13}C NMR spectra of 0.1 M 2AMBZ aqueous solutions at 25 °C. (a) decoupled experiment, (b), (c) and (d) coupled ^{13}C-^{1}H experiment. Chemical shifts are reference to TMS frecuency.

Fig. 3. ^{13}C NMR spectra of 0.1M 2AMBZ aqueous solutions at 25 °C at different pH's.

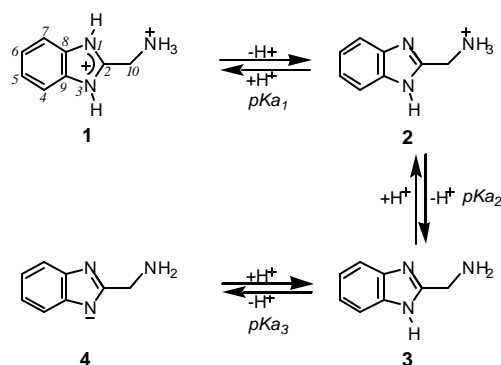**Fig. 4.** Schematic representation of deprotonation of 2-(aminomethyl)benzimidazole dihydrochloride.

The titrations were performed using an automatic DL53 Mettler titrator equipped with a combined Ag / AgCl glass electrode and a borosilicate glass burette of 10 mL.

Acid-base constants were calculated from the experimental data corresponding to the pH titration's with the aid of the SUPERQUAD program [26,29]. It refines equilibrium constant values by minimizing the weighting sum of residual squares for cell potentials U. The weighting factor W_i is assigned for each observation as the inverse of the variance which is calculate from uncertainties of potential and titrant volume measurements by a standard error propagation formula.

Results and discussion

NMR Study. The molecule of 2AMBZ (Fig. 1) has a set of three pairs of chemically equivalent carbons, besides C of methylene group and C-2, consequently only five distinct signal can be observed (Fig. 2a). The assignment of 2AMBZ was done with an experiment of ^{13}C NMR spectra coupled with [1]H (Fig. 2b).

The Figs. 2c and 2d show the coupling patterns of [13]C-^{1}H that allow to differentiate the signal of C4-C7 (δ = 114.5 ppm) respect to C5-C6 (δ = 127.37 ppm). Furthermore it was possible to discern the resonance frequencies of C2 (d = 143.51 ppm), C8-C9 (d = 131.01 ppm) and C10 (d = 34.543 ppm). Therefore, the equivalent nucleus of C4 and C7 are only coupled at three hydrogen atoms (H4, H5, H6 and H7, H6, H5

respectively), the greater multiplicity of C5 and C6 is due to coupling with four hydrogen atoms (H4, H5, H6 and H7) (Figs. 2c and 2d). By other hand, C2 is only coupled with hydrogen atoms of methylene; this signal has multiplicity of three and is easily differentiated of C8 and C9, which have couplings with three different hydrogen atoms. Finally, the methylene carbon C10 has a multiplicity of three. The assignement was corroborated with a HMBC experiment and is in accord with previous reports [30].

The number of signals in all spectra does not change during the procedure of pH variation (Fig. 3), because of symmetry and tautomerism phenomena.

In the diprotonated chemical species **1** (Fig. 4), there is a plane of symmetry perpendicular to annular system, causing the equivalence chemical of different aromatic carbons; C5 with C6, C4 with C7 and C8 with C9. On the other hand, in bencimidazolic species monoprotonated **2** and neutral **3**, the imidazolic ring carries a tautomeric process, the proton is transferred from N1 to N3 (Fig. 4).

From the chemical shifts of ^{13}C NMR (Fig. 5) it was possible to find the values of the acid-base constants. The results obtained with the HypNMR program (Tab. 1) have a good statistic and agree with previous reports (Tab. 2).

The ^{13}C NMR spectra also provide information about the deprotonation mechanism of **1**. Chemical shifts change significantly when the pH is varied and the identification the four molecular structures during this process was possible. It is known that the formation of ammonium salts causes protec-

Table 1. HypNMR, SQUAD and SUPERQUAD results for pKa values of 2-(aminomethyl)benzimidazole.

Method	I	[2AMBZ] / M	n	pKa1	pKa$_2$	pKa$_3$	σ	χ^2
NMR[1]	var	0.1	95	3.103 ± 0.079	7.624 ± 0.063	12.540 ± 0.038	4.1 × 10^{–2}	25.1
Spectrophotometric[2]	var	2-15 × 10^{–5}	1032	2.777 ± 0.111	7.481 ± 0.073	12.593 ± 0.010	1.5 × 10^{–2}	nd
Spectrophotometric[2]	1.0	2-15 × 10^{–5}	1032	2.982 ± 0.116	7.887 ± 0.082	12.487 ± 0.015	1.6 × 10^{–2}	nd
Potentiometric[3]	var	1-50 × 10^{–3}	420	2.996 ± 0.006	7.569 ± 0.003	nd	4.5	12.7
Potentiometric[3]	0.1	1-10 × 10^{–3}	101	3.044 ± 0.016	7.611 ± 0.008	nd	5.0	22.0

[1]HypNMR, [2]SQUAD, [3]SUPERQUAD. I = Ionic strength adjusted with NaClO$_4$; n = number of experimental data; $s = \sqrt{U/n}$; var = ionic strength variable; nd = not determinated. Uncertainties are the standard deviations. Equilibrium constant units mol L^{–1}.

Table 2. pKa values of 2-(aminomethyl)benzimidazole reported at literature.

| Method	T (°C)	Ionic strength / M	pKa$_1$	pKa$_2$	pKa$_3$	Reference
Spectrophotometric	25	0.3 (NaClO$_4$)	3.17 ± 0.03	7.83 ± 0.07	nr	[20]
Spectrophotometric	30	0.3 (NaClO$_4$)	3.14 ± 0.06	7.68 ± 0.05	nr	[20]
Spectrophotometric	27	var	3.1	8.1 ± 0.5	12.8	[18]
Fluorescence	27	var	1.8	8	12	[18]
Potentiometric	30	0.2 (NaClO$_4$)	3.45 ± 0.02	7.30 ± 0.02	nr	[19]
Potentiometric	30	0.5 (NaClO$_4$)	3.33 ± 0.02	7.02 ± 0.02	nr	[19]

nr = not reported; var = ionic strength variable. Meanings of uncertainties are not reported.

tion phenomena in the carbon atoms bonded at nitrogen atoms [31]. So, the chemical shifts of carbon nucleus in α position with respect to quaternary nitrogen are shifted to high field respect to free amine. In our case, we found that when the pH of **1** increases, the signals of C2, C8, C9 and C10 are shifted to low field. Although, in Fig. 5 it can be observed that only the positions C2 and C10 were affected by the successive lost of protons bonded at nitrogen atoms of the molecule. The chemical shifts of C8 and C9 are constants between pH 4 and 11. This can be explained if we assume that the first deprotonation site occurs at imidazolic nitrogen producing the mono-cationic species **2**, whereas the second deprotonation site

occurs in NH$_3$ group. On the other hand it has been reported that the resonance frequencies of C5 and C6 are affected by inductive effects produced by imidazolic nitrogens [31] , thus any deprotonation of these nitrogen atoms will shift the signals of C5 and C6 to low frequencies. The spectra show that these chemical shifts are constants between 4-11 pH confirming that the second deprotonation site is at NH$_3$. The proposed deprotonation mechanism is according to Dash [19], but does not with Sinha [18].

Spectrophotometric study. Absorption spectra of 2AMBZ show one isosbestic point at acid solutions and other one at basic pH range, indicating two chemical species in equilibrium corresponding to pKa$_1$ and pKa$_3$. At the range of neutral pH it is difficult observe the isosbestic point, because there are small changes on absorption spectra [18] due to the tautomeric process mentioned earlier. From pH-absorbance matrices TRIANG indicated the presence of at least 3 or 4 absorbing species over the pH interval studied, corresponding with a model of three pKa (four species).

The equilibrium constants refined by processing absorption spectra using SQUAD are given in Table 1. The values are comparable to NMR results obtained and previously reported information (Tables 1 and 2). Using spectrophotometric refined values and the absorptivity coefficients calculated with the program, it was possible the simulation of the spectra for each concentration and pH with a spreadsheet. Fig. 6
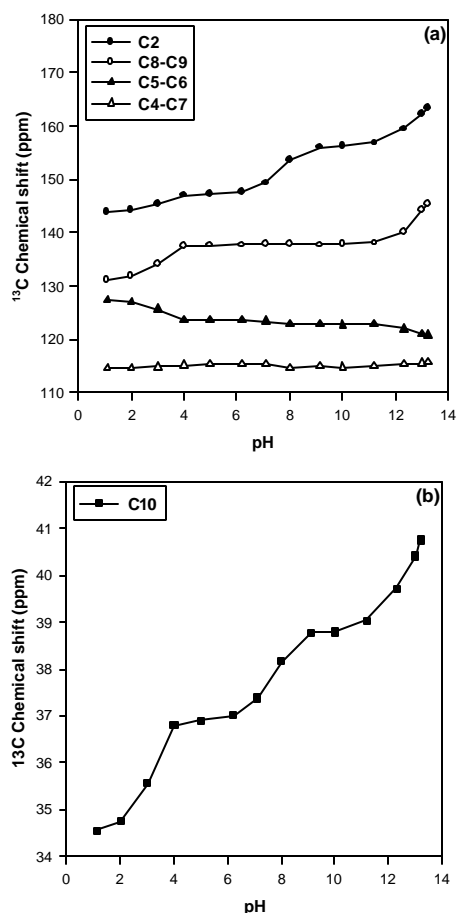
Fig. 5. Chemical shifts in 2AMBZ 0.1 M solutions at 25 °C against pH.
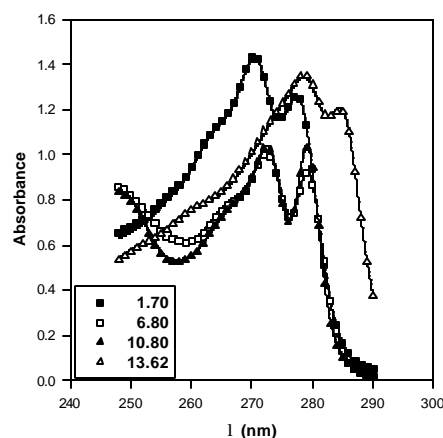
Fig. 6. Absorption spectra simulated (solid lines) and experimental (symbols) at different values of pH for a total concentration of 2AMBZ of 1.5×10^{-4} M I=var.
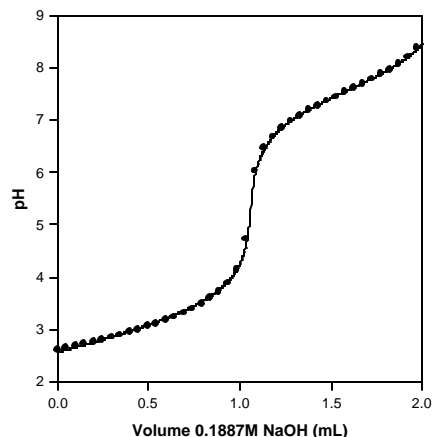
Fig. 7. Simulated (solid lines) and experimental (symbols) potentiometric titration curve for 20 mL of 2AMBZ 0.01M. I=var.

shows overlapped experimental and simulated spectra, as can be seen that the simulation agrees very well with the experimental results.

Potentiometric study. The Fig. 7 shows a typical titration curve of 2AMBZ with NaOH solution. By this method it was possible to determine two constants, because the low concentration of the NaOH solutions used in the titration do not allow to reach values of pH as high as the ones required to refine conveniently the third pKa, which is between 12 and 12.8 [18]. The values obtained are collected in Table 1 and show good statistical parameters, they are in agreement with the values found with the others techniques and the values published previously (Tab. 2).

Using the values refined with SUPERQUAD it was possible to simulate the data obtained with a spreadsheet. The simulated and experimental potentiometric curves are shown in the Fig. 7, the simulation agrees very well with the experimental results.

Conclusions

The pKa values determined for 2AMBZ for a model of three acid-base equilibriums are clearly explained by NMR, spectrophotometry and potentiometry.

It may be noted that values obtained here are consistent among the techniques used with a good statistical, and they are in agreement with values reported in the literature. Likewise, it was confirmed that the value of the third pKa is 12.540 ± 0.038 (NMR). This equilibrium constant has been reported previously, but without statistical information [18].

From ^{13}C NMR studies it was possible to determine the deprotonation mechanism of 2AMBZ. This work is the first report on the determination of acid-base constants of 2-(aminomethyl)benzimidazole by this technique.

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