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Determination of pK_a and Hydration Constants for a Series of α-Keto-Carboxylic Acids Using Nuclear Magnetic Resonance Spectrometry

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ABSTRACT

The determination of the acid-base dissociation constants, and thus the pK_a values, of α-keto acids such as pyruvic acid is complex because of the existence of these acids in their hydrated and nonhydrated or oxo state. Equilibria involved in the hydration and dehydration of the α-keto group of pyruvic acid and three other α-keto acids, 3-methyl-2-oxobutanoic acid, 4-methyl-2-oxopentanoic acid, and 2-oxo-2-phenylacetic acid, were investigated by proton and carbon nuclear magnetic resonance spectrometry, at constant ionic strength, 0.15, and 25°C. Dissociation constants for the oxo (pK_a^{oxo}) and hydrated (pK_a^{hyd}) acids of each compound were estimated from the change in the degree of hydration with changes in pH and directly from the changes in chemical shifts of various hydrogen and carbons nuclei with pH. α-Keto acids showed greater hydration in their acidic forms than their carboxylate forms. The degree of hydration was sensitive to steric and electronic/resonance factors. As expected, the oxo forms of the acids were stronger acids compared with their hydrated analogs, and their dissociation constants were also sensitive to steric and electronic factors.

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Introduction

The objective of this study was to determine the acid-base dissociation constants of the oxo, or nonhydrated, and hydrated forms, and thus their pK_a^{oxo} and pK_a^{hyd} values, respectively, and the hydration equilibrium constants of a series of α-keto acids 1-4 (Scheme 1) using both proton and carbon, nuclear magnetic resonance (NMR) spectrometry to follow the integration and shifts of various atoms in the molecules affected by changes in pH values.

Pyruvic acid (1) readily reacts with hydrogen peroxide to form acetic acid and carbon dioxide, and α-keto acids are of major importance in intermediary metabolism and as components of the Krebs cycle.[1] Pyruvic acid plays an important role *in vivo* metabolic pathways, such as gluconeogenesis, transamination, and fermentation. It can be involved in enzyme-catalyzed intracellular phenomena[2] and converted into fatty acids or energy through acetyl-CoA. α-Keto acids are used as model substrates of enzymes and in the development of enzyme inhibitors.[3] They have been used to treat some pathological conditions,[4-6] and are of interest as biosynthetic precursors[7] and intermediates in the syntheses.[8]

For many drugs and biological molecules,[9] including α-keto acids, the apparent acid–base dissociation constant, K_a, and thus their pK_a value, is an important physicochemical characteristic thought to be associated with biological activity and chemical reactivity and stability. It is known that most α-keto acids can exist in an equilibrium between their oxo form and as their hydrated, gem-diol (Scheme 1) form, depending on the electron-withdrawing or -donating properties and steric effects of the groups adjacent to the α-keto group, the center of the nucleophilic water addition reaction.[10] Several values have been reported for the pK_a of pyruvic acid (2.4 ± 0.2) and some other α-keto acids. The values represent composite values and reflect the acidities and the relative concentrations of the hydrated and oxo forms of the acids.[11]

The pK_a of the nonhydrated acid, oxo or keto form, is not readily measured directly but can be determined from knowledge of the equilibrium of hydration of the protonated and deprotonated forms and the apparent macroscopic pK_a or pK_a^{obs} values.[12,13] A number of

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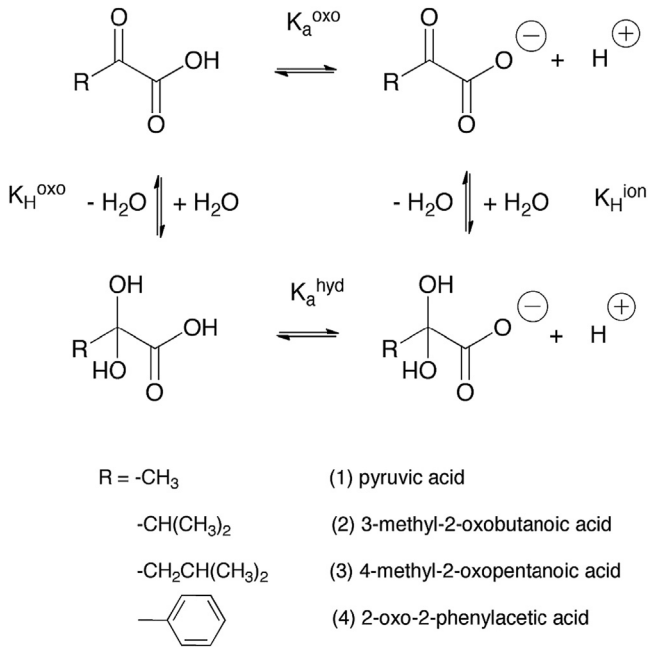

Scheme 1. Ionization and hydration equilibria of various α-keto acids, 1-4, in aqueous solution.

techniques have been used for the determination of the degree of hydration and the measurement of pK_a values of α-keto acids. NMR spectrometry represents the most powerful and unique of these techniques. Here, NMR was used to investigate and determine the equilibrium constants for hydration, and the pK_a^{oxo} and pK_a^{hyd} values of α-keto acids 1-4 (Scheme 1).[10] By following the shifts of selected protons and carbons[14] as a function of pH for both the acids, hydrated and oxo forms, the pK_a^{oxo} and pK_a^{hyd} values could be determined directly.

Materials and Methods

Materials

All the keto acids (Scheme 1) were purchased as their sodium salt, except 2-oxo-2-phenyl acetic acid (4), from Sigma-Aldrich (Milwaukee, WI), as was deuterium oxide (99.96%). Tetramethylsilylpropionate (TMSP-d4) was purchased from Cambridge Isotopes Laboratories (Tewksbury, MA), and methanol HPLC grade from Sigma-Aldrich were used as an internal and external standard for the NMR studies, respectively. Deionized water was used to prepare all the NMR samples. HCl 37% A.C.S. reagent was purchased from Sigma-Aldrich and NaOH 10 N was purchased from Fisher Scientific (Pittsburgh, PA).

Methods

The initial concentration of the acids used in the NMR samples was 150 mM in a volume of 0.5 mL of solvent (9:1, v/v, H_2O-D_2O). All spectra were acquired using 5 mm NMR tubes. The α-keto acid solutions were titrated to the desired pH using concentrated hydrochloric acid and/or sodium hydroxide such that the final ionic strength was 0.15 with sodium chloride. The pH of each sample was measured directly in the NMR tube using a 5-mm pH electrode purchased from Wilmad Labglass (Vineland, NJ). No correction was made for the deuterium isotope effect. The samples were stable during the analysis and no variation in spectra and pH values was observed when the runs were repeated.

One-dimensional ^{1}H and ^{13}C NMR spectra for compounds 1-4 were acquired on a 400 or 500 MHz Bruker (Rheinstetten, Germany) spectrometer equipped with a X-channel observe quadruple nuclei probe or carbon-enabled cryoprobe, respectively. Sample temperature was set to 25°C.

Quantitative ^{13}C NMR spectra[15] for compounds 4 were acquired using the inverse gated ^{1}H decoupling pulse sequence.[16] To insure the integrals are quantitative, the interscan delay is set to 75 s, which is greater than 5*T_1 as determined using the inversion recovery experiment.[16] Data were processed with the software MestreNova (MestreLab Researcher, S. L., Santiago de Compostela, Spain).

Chemical shifts were referenced to the internal standard, TMSP-d4, or to the external standard methanol. The relative amount of the hydrated and nonhydrated keto acids was determined using the relative peak area measured by the global spectral deconvolution algorithm implemented in MestreNova software.[17]

Data Fitting

Data fitting to estimate limiting hydration constants and K_a values was performed using GraphPad/Prism version 6.0 (GraphPad Software, La Jolla, CA). Nonlinear least squares regression analysis, choosing relative weighting (in order to minimize the weighted sum of squares), was used to obtain best-fit values of the parameters described later in Eqs. (7) and (14).

Results and Discussion

The reversible hydration of a series of α-keto acids 1-4 and their equilibria involved in ionization protonation of the oxo and hydrated forms (Scheme 1) in aqueous solution were investigated using ^{1}H and ^{13}C-NMR techniques. The hydration and dehydration kinetics, although fast, are considered in “slow exchange” on the NMR time scale, meaning that the lifetime of each species is longer than the amount of time required to distinguish them, which equals the inverse of their chemical shift difference in Hz (on the order of milliseconds on a 400 or 500 MHz spectrometer). Thus, one is able to see peaks in the NMR spectrum corresponding to both the hydrated and nonhydrated species.

The ^{1}H NMR spectra of pyruvic acid in aqueous solutions at 25°C are shown in Figure 1 at pH values 0.9, 2.7, and 4.6.

Each spectrum shows two peaks at around 1.5 and 2.4 ppm, which are the expected signals for the methyl protons of pyruvic acid corresponding to the hydrated and oxo forms, respectively. The relative positions of the peaks are in agreement with this assignment in as much as the shielding of the methyl protons of the oxo would be predicted to be smaller than that for the corresponding methyl protons in the hydrated form. At pH 0.9, a higher fraction of the hydrated form exists than at pH 2.7 and 4.6. That is, the degree or fraction hydrated significantly decreased following the overall ionization of the α-keto acids to their deprotonated or carboxylate form. The ^{1}H NMR spectrum at different pH values also shows that the observed chemical shift (δ_{obs}) values of the peaks associated with both forms decreased following the ionization of the two forms of the acid. That is, the protonated and nonprotonated forms are in fast exchange on the NMR time scale, meaning that one sees a weighted average, rather than individual peaks. The kinetics of this reaction must be much faster than hydration reaction.

The ^{13}C NMR spectra of pyruvic acid in aqueous solutions at 25°C are shown in Figure 2 at pH values 0.9, 2.3, and 5.4.

Each spectrum shows six peaks, which are the signals for the CH_3 carbons (28 and 29 ppm) of the hydrated and oxo forms, respectively: the carbon in position 2 (96 ppm) for the hydrated form; the carbon in position 1 (170 ppm) of the oxo form; the

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