Measuring the equilibrium constant of a keto-enol tautomerism using benchtop NMR

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Application benefits

The ethyl acetoacetate ^{1}H NMR spectrum demonstrates the utility of benchtop NMR in measuring an intramolecular equilibrium. NMR is ideal for studying reaction kinetics, as it is a nondestructive technique. An advantage of the Thermo Scientific™ picoSpin™ NMR spectrometer in monitoring reaction kinetics is that small quantities of reaction mixtures can be directly injected into the capillary micro probe without the need for dilution in a deuterated solvent.

Abstract

The ^{1}H NMR spectrum of a β-ketoester, ethyl acetoacetate, reveals the presence of tautomerism, a dynamic process of intramolecular hydrogen bonding. Proton exchange between the tautomeric species is slow, allowing for direct measurement of each of the enol and keto forms of ethyl acetoacetate. The signal areas for each species were then integrated. From which, the percent composition of each constitutional isomer was calculated and the equilibrium constant was derived. In addition to qualitative structure elucidation, ^{1}H NMR also provides quantitative information to evaluate solution dynamics.

Introduction

Ethyl acetoacetate (EAA), a β-ketoester, is a colorless liquid with a sweet, fruity aroma and flavor. Its primary application is as a starting material in the synthesis of α-substituted acetoacetic esters and cyclic compounds, as an intermediate in the synthesis of vitamins and pharmaceuticals, and in the manufacture of inks, plastics, dyes and lacquers. Ethyl acetoacetate undergoes self-enolization (Figure 1), where the keto form **1** exists in equilibrium with its enol isomer **2a** and **2b**. The process occurs because the α-hydrogen, adjacent to the carbonyl, is slightly acidic, with p*K*a values ranging from 9-13 in β-dicarbonyls, making ketones readily enolizable.[1] This intramolecular chemical equilibrium between keto and enol isomers is referred to as tautomerism. The chemical equilibrium is thermodynamically driven. Typically, the more stable keto form is favored at room temperature and above. However, in β-diketones, stabilization of the enol form arises from stabilization by π-system conjugation. Terminal groups and α-substituents also play a role in enol formation, where bulky R groups in the α position results in steric effects destabilizing the enol, while electron withdrawing groups in the α position help to stabilize it.

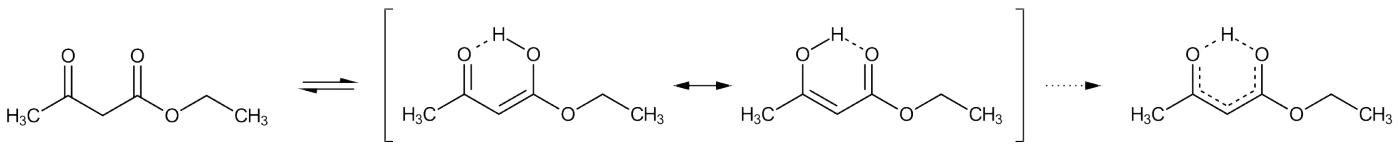

Figure 1: Keto-enol tautomerism of ethyl acetoacetate.

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Tautomers can be readily studied by proton NMR because the high energy barrier for the interconversion between enol-keto tautomers (Figure 1) makes it a slow process that's amenable to NMR analysis. Hence, keto and enol protons experience different local environments, resulting in easily identifiable proton chemical shifts.[2] For EAA, this is evident in the proton spectrum, where resonances due to the keto form dominate.[1-4] By integrating these signals the equilibrium constant, K_{eq}, and the %enol can be calculated for a given compound at a specific sample temperature.

Experimental

The ^{1}H NMR spectrum of EAA was measured from a neat sample at 82 MHz and 32 °C using the Thermo Scientific™ picoSpin™ 80 spectrometer. The spectrometer's capillary cartridge was fitted with micro-fluidic inlet and outlet connectors that allow liquid sample injection into the spectrometer's RF coil. The fluid path was Teflon/Quartz capillary tubing with a total flow-path volume of 40 microliters (µL). Liquid samples were introduced by manual injection using a disposable 1 mL syringe and a 22-gauge blunt tip needle.

Spectra were acquired using the following acquisition parameters: a 90° RF excitation pulse, a 1000 ms acquisition time, and a 12 second recycle delay. All spectra were acquired with signal averaging. Spectral data were stored in a JCAMP-DX file format and imported into MestrelLab Research's Mnova™ NMR analysis program for processing. Standardized data processing was applied across all spectra, specifically, by applying zero filling, applying phase correction, and filtering using exponential apodization. Resonance signals were fitted using generalized Lorentzian functions, and integrated using Mnova's Global Spectrum Deconvolution method.

Results and discussion

Figure 2 shows the ^{1}H NMR spectrum of ethyl acetoacetate. Based on chemical shifts, multiplicity and relative signal intensities, assignment of signals are straightforward and unambiguous. The enol and keto forms are indicated by 'e' and 'k', respectively, in Figure 2. The signals were internally referenced relative to the large singlet proton resonance at 2.21 ppm originating from the acetyl methyl (C_{18}) protons of the keto isomer.

The chemical shift of ethyl ester protons (-OCH_2CH_3) are unaffected by tautomerization and appear as classic spin-coupled signals. Centered at 1.23 ppm, the terminal methyl protons on C_{14} (e, C_8; $^3J_{HH}$ = 7.12 Hz) generates a triplet due to spin-spin coupling to the adjacent methylene protons on C_{13} (e, C_7). The methylene (CH_2) resonance due to the protons on C_{13} appears as a spin-coupled quartet centered at 4.14 ppm ($^3J_{HH}$ = 7.12 Hz). The enol tautomers **2a** and **2b** do not produce unique resonances separately because their conversion is a rapid exchange process. At the opposite terminus, the acetyl methyl protons are affected by tautomerization due to changes in electron density at the carbonyl carbon (e, C_4), resulting in an upfield shift of the enol signal (C_9) relative to those of the keto signal (C_{18}). The former appears as a weak singlet at 1.94 ppm, while the latter is at 2.21 ppm. The α-protons of the keto and enol (C_3) forms manifest as singlets at 3.48 ppm (C_{15}) and 5.03 ppm (C_3), respectively. The enol hydroxylic proton (C_{5a}) appears as an unsaturated alcohol, but intramolecular hydrogen bonding causes it to behave like a carboxylic acid, shifting its resonance far downfield to 12.14 ppm.

The enol-keto equilibrium constant, K_{eq} = [enol]/[keto], and %enol are functions of temperature. Their values can be determined by integrating the enol and keto signal areas. Table 1 summarizes the equilibrium constant and percent enol concentration values calculated for the different combinations of enol to keto ratio. Note that each peak integration area is weighted according to the number of protons that contribute to the respective signal. For example, the enol methyl contains three protons while the keto methylene has only two protons; the weighting factor, in this case, is 2/3.

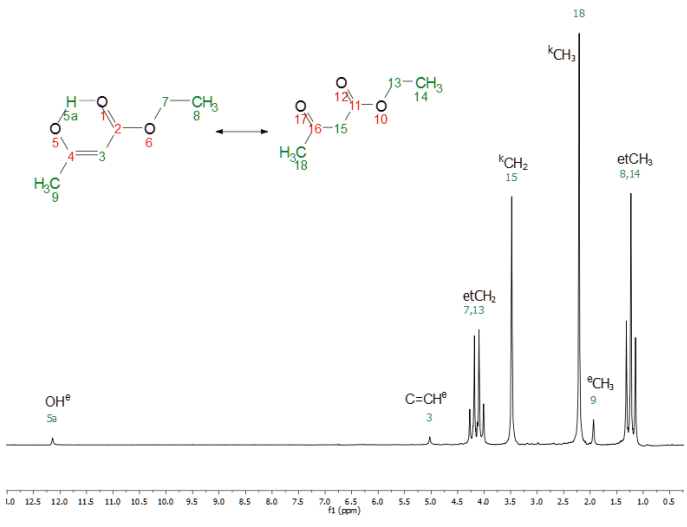

Figure 2: Full ^{1}H NMR spectrum of ethyl acetoacetate. Enol and keto forms are indicated by 'e' and 'k', respectively. The prefix "et" refers to ethyl. Inset shows the numbering system used to assign signals in the NMR spectrum of ethyl acetoacetate.

Signal	K_{eq}	%enol	Signal	K_{eq}	%enol	K_{eq}	%enol
$^{e}CH_3$			^{e}CH			0.09[a]	8.0%[a,b]
$^{k}CH_2$	0.094	9.4%	$^{k}CH_2$	0.103	10.3%		
$^{k}CH_3$	0.096	9.6%	$^{k}CH_3$	0.105	10.5%		

[a] Ref. 4. [b] Ref. 2.

Table 1. Equilibrium constant and percent tautomer composition measured at 32 °C.

From Figure 2, the calculated average K_{eq} is 0.0992 ±0.005 and %enol is 9.9%. Our values compare favorably to those reported by others,[1,4] where the calculated values for K_{eq} and %enol are 0.09 and 8.0%, respectively, at 32 °C. These results confirm that the keto tautomer is the more favorable structure, and that the ethyl ester group doesn't introduce additional steric interaction to destabilize the keto form.

Conclusions

The ^{1}H NMR spectrum of a β-ketoester, ethyl acetoacetate, reveals the presence of tautomerism, a dynamic process of intramolecular hydrogen bonding. Proton exchange between the tautomeric species is slow, allowing for direct measurement of each of the enol and keto forms of ethyl acetoacetate. Integrating signal areas for each species affords relative concentration information to calculate the equilibrium constant and percent composition of each constitutional isomer. In addition to qualitative structure elucidation, ^{1}H NMR also provides quantitative information used to evaluate solution dynamics.

References

1. Allen, G.; Dwek, R. A. *J. Chem. Soc. B,* **1966**, 161-163.
2. Cook, G.; Feltman, P. M. *J. Chem. Educ.* **2007**, *84*, 1827-1829.
3. Drexler, E. J.; Field, K. W. *J. Chem. Educ*. **1976**, *53*, 392-393.
4. Burdett, J. L.; Rogers, M. T. *J. Am. Chem. Soc.* **1964**, *86*, 2105-2109.

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