Temperature-variable NMR Study of the *keto-enol* Tautomerism of Phenylpyruvic Acid

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The *keto-enol* tautomerism of phenylpyruvic acid (PPA) has been studied by temperature-variable 9.4 T nuclear magnetic resonance (NMR) spectroscopy. It was found that PPA favored its existence in the *enol* form in dimethyl sulfoxide (DMSO) solvent, 93.5% of the *enol* and 6.5% of the *keto* tautomer was present in DMSO at room temperature. Increasing the temperature increased the percentage of the *keto* form. The experimental thermodynamic parameters for the tautomerism were successfully obtained from the spectral data.

Keywords Phenylpyruvic acid (PPA), *keto-enol* tautomerism, NMR, equilibrium constant, thermodynamic parameter

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Introduction

Typical α -*keto* acids, such as pyruvic acid (PA) and phenylpyruvic acid (PPA), are recognized to be biologically important metabolic products.^{1,2} PPA, which is the molecule studied in the present work, is an intermediate and catabolic byproduct of the phenylalanine metabolism in individuals afflicted with phenylketonuria (PKU). PKU is due to a lack of the enzyme phenylalanine hydroxylase (PAH), so that phenylalanine is converted not to tyrosine, but to PPA. PPA accumulates in the blood and tissues and is excreted in urine. The level of PPA is normally very low in blood or urine.

Many spectroscopic studies, ultra violet (UV), infrared (IR), Raman and nuclear magnetic resonance (NMR) studies, have been reported on the *keto-enol* tautomerism³⁻¹² and conformation^{6,9,10,12,13} of PA and PPA. These many investigations have revealed that these compounds exist as equilibrium mixtures consisting of the *keto* and *enol* forms. Here, the *keto-enol* tautomerism of PPA is shown in Scheme 1. Firstly, Larsen *et al.* found by ¹H NMR and UV spectroscopy that the *enol* isomer of PPA predominantly exists in dimethyl sulfoxide (DMSO), while PPA favors its existence in the *keto* form in 1 M HCl.³ Sciacovelli *et al.* also investigated the tautomerism of PPA by NMR, UV, and IR spectroscopy, and found that the *keto* isomer increases in a water-DMSO solvent, depending upon the water content.⁴⁻⁶ Furthermore, PPA was found to predominantly take the *keto* form in the solid state and in an aqueous solution. Ray *et al.* analyzed the complete IR and Raman spectra of liquid and crystalline PA, and found that PA takes the cyclic

hydrogen-bonded dimer structure in which the two carbonyl groups are in a *trans* configuration in the pure liquid and crystalline phase.⁷ Next, Hanai *et al.* investigated the IR, Raman, and NMR spectra of PPA in the solid state, organic solvents and those its solid salts.⁸⁻¹⁰ It was revealed that PPA takes the *enol* form in the solid state, and that the *keto* form is more stable in an aqueous solution. Carpy *et al.* assigned the structures of the two tautomeric forms of PPA in DMSO solvent by ¹H and ¹³C NMR spectroscopy, and found by X-ray crystallography of the single crystal that the crystalline form takes the *keto* isomer.^{11,12} Moreover, the substituent effect on the tautomeric equilibrium was studied by using *ab initio* calculations in the gas phase, and the thermochemical parameters were obtained. They confirmed a large preference for the *enol* isomer relative to the *keto* isomer for PPA and *o*-chloro PPA. It was also found that the strong electron-withdrawing nitro group in *o*-nitro PPA has a significant influence on the *keto-enol* tautomerism, resulting in a shifting of the equilibrium towards the *keto* formation. Recently, Hanai *et al.* reported a polymorphism study of PPA on the basis of IR, Raman and solid state ¹³C NMR spectroscopy, and revealed that two polymorph forms of PPA exist.¹³

NMR is perhaps the most important, powerful, and widely used form of spectroscopy in both academic and industrial chemical research. It provides the most detailed and unambiguous information on the chemical structure, functionality, and dynamics. We carried out 9.4 T solution NMR measurements as varying the temperature for the studying

Scheme 1 *keto-enol* tautomerism of PPA.

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Fig. 1 9.4 T ^1H NMR experimental spectrum of PPA at room temperature. The solvent is DMSO. The inserted figure is the expanded region between 7 and 8 ppm, showing the signals of the aromatic protons.

the *keto-enol* tautomerism of PPA. The ^1H NMR spectra clearly showed that PPA favored its existence in the *enol* form in DMSO solvent. The equilibrium constant, conversion enthalpy and entropy for the tautomerism were obtained by this experimental study.

Experimental

A powder sample available from Tokyo Chemical Industry Co., Ltd was used to conduct 9.4 T ^1H NMR measurements using a JEOL AL-400 NMR spectrometer. The acquisition time and the relaxation delay were 12.6 and 16.4 s for accurately estimating the intensity of the NMR signals, respectively. The scan times was 32. The chemical shift was measured with respect to the resonance frequency in tetramethylsilane (TMS). The solvent was DMSO. The concentration of PPA in DMSO was about 0.1 mM. For a structural analysis, except for the ^1H NMR spectrum reported in this paper, ^{13}C NMR, DEPT 135 (distorsionless enhancement by polarization transfer), HMQC (hetero-nuclear multiple quantum correlation spectroscopy), and HMBC (hetero-nuclear multiple bond correlation spectroscopy) measurements had also been conducted by the same NMR spectrometer. Those data are not shown since they are outside the main topic of this study.

Experimental Results

The experimental ^1H NMR spectrum at room temperature is shown in Fig. 1. The expanded region between 7 and 8 ppm is inserted in Fig. 1. An investigation of the ^1H NMR spectrum revealed the presence of a mixture of the *enol* and *keto* tautomers of PPA. The dissolution of PPA in a polar solvent, DMSO, can cause the tautomerization of the *keto* to the *enol* form. As can be seen in Fig. 1, the singlet peak at 4.14 ppm, the singlet peak at 6.41 ppm and the broad peak at 9.2 ppm can be assigned to the $-\text{CH}_2-$ proton of the *keto* form, the $-\text{CH}=$ proton of the *enol* form and the proton of the *enol* OH, respectively. It can be concluded from the expanded region shown in Fig. 1 that the triplet peaks at 7.23 ppm, the triplet peaks at 7.35 ppm and the doublet peaks at 7.76 ppm account for the *para*-, *meta*- and *ortho*-protons, respectively. These signals between 7 and 8 ppm consist of the peaks of the aromatic protons of both the *enol* and *keto* forms. It is known that the *enol* isomer with the *Z* configuration is dominantly present in the solution.⁴ The presence of the single peak due to the olefinic proton, even in

Fig. 2 Temperature dependence of the mole ratio of the *keto* form in the *keto-enol* tautomerism of PPA obtained from temperature-variable NMR measurements at above room temperature.

the expanded spectrum, points out the dominant formation of the *enol* form with the *Z* configuration.

The composition ratio of the *keto* and *enol* forms was precisely calculated from the relative peak-area integration of the $-\text{CH}_2-$ proton of the *keto* form and the $-\text{CH}=$ proton of the *enol* form, which were stable and observed at around the central region in the NMR spectrum. A peak-intensity calculation showed that this tautomeric mixture consisted of 93.5% of the *enol* form and 6.5% of the *keto* form. It can be considered that this *enol* form was remarkably stabilized by the intermolecular hydrogen bond between the hydrogen of the *enol* hydroxyl group and the DMSO molecule.⁵ This can also be seen from the fact that the OH proton of the *enol* form was more deshielded than an ordinary alcohol proton. It can be furthermore considered that PPA in DMSO, which is an aprotic solvent, is more stable in its *enol* form due to conjugation of the phenyl ring, the $\text{C}=\text{C}$ *enol* group and the carboxylic group. On the other hand, a protophilic solvent, such as water and HCl, would reduce the *enol* formation due to the ionization of PPA.^{3-6,10} Upon considering the influence of a solvent on the *keto-enol* tautomerism on the basis of the Onsager model,¹⁴ the tautomer should favor its existence in the more polar isomer, *i.e.*, the *keto* one as the polarity of a solvent increases.¹⁵ It can therefore be understood that the *keto* form is more stable than the *enol* one in another solvent, such as water and HCl,^{3-6,10} while the *enol* form becomes predominant in the DMSO solvent.

Discussion

It was found from ^1H NMR measurements that PPA exists as a tautomeric mixture of the *enol* and *keto* forms in a DMSO solution. Figure 2 shows the mole ratio of the *keto* form in the *keto-enol* tautomerism of PPA, obtained from temperature-variable NMR measurements controlled by a thermostat system above room temperature. As can be seen in Fig. 2, increasing the temperature of this mixture increases the percentage of the *keto* form. It can be considered that this is the result from cleavage of the intermolecular hydrogen bond between the hydrogen of the *enol* hydroxyl group and the DMSO molecule owing to more activated molecular motion in the solution at high temperature. The equilibrium constant of the *keto-enol* tautomerism, K_T , is defined as

$$K_T = \frac{[\textit{enol form}]}{[\textit{keto form}]} = \frac{\text{Peak intensity of CH}}{0.5 \times (\text{peak intensity of CH}_2)} \quad (1)$$

Table 1 Summary of tautomer equilibrium constant K_T , and the mole ratio of the *enol* and *keto* form in the tautomerism of PPA

T/K	K_T	Mole percent of <i>enol</i>	Mole percent of <i>keto</i>
Room temperature	14.3	93.5	6.5
313	11.8	92.2	7.8
323	8.7	89.7	10.3
333	6.9	87.3	12.7
343	5.6	84.7	15.3

Fig. 3 $\ln K_T$ as a function of $(1/T) \times 1000$. ΔH^0 and ΔS^0 were obtained from the slope and the y-intercept of the line fitted on the plots, respectively.

The temperature dependence of the K_T experimentally obtained is summarized with the mole ratio of the *keto* and the *enol* form in the tautomerism of PPA in Table 1. The enthalpy, ΔH^0 , and entropy, ΔS^0 , for the conversion of the *keto* to *enol* form were obtained by the van't Hoff equation, as follows:^{11,16}

$$\ln K_T = -\frac{\Delta H^0}{R} \left(\frac{1}{T} \right) + \frac{\Delta S^0}{R}, \quad (2)$$

where R and T are the molar gas constant and the temperature, respectively. Here, it was assumed that the values of the enthalpy and entropy for the tautomerism are independent from the temperature in the experimental range of this study. Figure 3 shows $\ln K_T$ as a function of $1/T$. ΔH^0 and ΔS^0 obtained from the regression line based on the least-squares method in Fig. 3 are -22.2 ± 0.85 kJ/mol (-5.3 ± 0.2 kcal/mol) and -50.5 ± 2.6 kJ/(mol K) (-12.1 ± 0.62 cal/(mol K)), respectively. It is noteworthy that the enolization process in DMSO showed a remarkable decrease in the system entropy and enthalpy. It may be considered that this feature is attributed to the hydrogen bonding of PPA to the solvent DMSO molecule because the degree of the flexibility of both PPA and DMSO molecules are reduced or restricted due to strong intermolecular hydrogen bonding. It is also well known that the formation, such as a hydrogen bonding and van der Waals interaction, is an exothermic reaction and ΔH^0 is negative. The results obtained

in this study are qualitatively consistent with those of an *ab initio* calculation study previously reported; $\Delta H^0 = -6.1$ kcal/mol and $\Delta S^0 = -7.7$ cal/(mol K).¹¹

Conclusions

It was revealed by ^1H NMR measurement that PPA predominantly exist as the *enol* form in DMSO at room temperature. Increasing the temperature of this mixture increased the percentage of the *keto* form. The thermodynamic parameters for the tautomerism of PPA were experimentally obtained by the temperature-variable NMR measurements of this study. It is well known that a molar volume of a *keto* form is smaller than that of an *enol* form. It can thus be expected that K_t decreases as the pressure increases. It is worth carrying out a pressure-variable NMR measurement of PPA for a more detailed thermodynamic characterization of PPA in the future. Furthermore, the state-of-the-art calculation studies as directly taking account of a solute-solvent interaction for a solvent effect, for instance, by a QM/MM method would be also expected.

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