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

Toshiki YAMAJI† and Takeshi SAITO

Introduction

Typical α-keto acids, such as pyruvic acid (PA) and phenylpyruvic acid (PPA), are recognized to be biologically important metabolic products. 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 tautomerism and conformation 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. 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. 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 keto-enol tautomerism of PPA.

Notes

† To whom correspondence should be addressed.
E-mail: toshiki-yamaji@aist.go.jp
T. Y. present address: Spintronics Research Center, Theory Team, National Institute of Advanced Industrial Science and Technology (AIST), Central 2, 1-1-1 Umehono, Tsukuba, Ibaraki 305-8568, Japan.

Scheme 1  keto-enol tautomerism of PPA.
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, 13C 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 ame 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 was DMSO. The concentration of PPA in DMSO was about 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.

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.6 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 C=O 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.14,15 while the enol form becomes predominant in the DMSO solvent.

\[ K_1 \approx \frac{[\text{enol}]}{[\text{keto}]} = \frac{\text{Peak intensity of CH}}{0.5 \times (\text{peak intensity of CH}_2)}. \]
Table 1 Summary of tautomer equilibrium constant $K_t$ and the mole ratio of the enol and keto form in the tautomerism of PPA

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$K_t$</th>
<th>Mole percent of enol</th>
<th>Mole percent of keto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature</td>
<td>14.3</td>
<td>93.5</td>
<td>6.5</td>
</tr>
<tr>
<td>313</td>
<td>11.8</td>
<td>92.2</td>
<td>7.8</td>
</tr>
<tr>
<td>323</td>
<td>8.7</td>
<td>89.7</td>
<td>10.3</td>
</tr>
<tr>
<td>333</td>
<td>6.9</td>
<td>87.3</td>
<td>12.7</td>
</tr>
<tr>
<td>343</td>
<td>5.6</td>
<td>84.7</td>
<td>15.3</td>
</tr>
</tbody>
</table>

The temperature dependence of $K_t$ experimentally obtained is summarized with the mole ratio of the enol and keto form in the tautomerism of PPA in Table 1. The enthalpy, $\Delta H^0$, and entropy, $\Delta S^0$, for the conversion of the keto to enol form were obtained by the van’t Hoff equation, as follows:\ref{11,16}

$$\ln K_t = \frac{\Delta H^0}{R} \left( \frac{1}{T} \right) + \frac{\Delta S^0}{R}$$

(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 $1/T$. $\Delta H^0$ and $\Delta S^0$ obtained from the regression line based on the least-squares method in Fig. 3 are $-22.2 \pm 0.85$ kJ/mol ($-5.3 \pm 0.2$ kcal/mol) and $-50.5 \pm 2.6$ kJ/(mol K) ($-12.1 \pm 0.62$ 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 $\Delta H^0$ 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).\ref{11}

Conclusions

It was revealed by $^1$H 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.

References