Studies on Phenylpyruvic Acid, II. Keto-Enol Equilibrium in Water-Dimethylsulphoxide Mixtures

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Phenylpyruvic Acid, Tautomerism and Analysis

A spectroscopic study of the keto-enol equilibrium of phenylpyruvic acid (PPA) was performed in water-dimethylsulphoxide (DMSO) mixtures, throughout the composition range and in an extended temperature range. The thermodynamic quantities of the tautomerism were also calculated. The high value of enolization entropy in pure DMSO is attributed to hydrogen bonding of PPA to this solvent. The experimental conditions and the optimum composition range of the H_2O-DMSO mixtures for PPA analytical determination were determined.

Introduction

The determination of PPA in urine is currently carried out by acidification and addition of $FeCl_3$. If PPA is present in urine, a green colour develops, increases to a maximum after about 2 – 3 min and then quite rapidly disappears [1]. The green colour is due to the formation of a complex between enol PPA and Fe^{3+} ions [2]. Moreover, its formation rate is controlled by the enolization rate of the keto form, which is far more stable in aqueous solution than the enol one. The disappearance of the colour is due to the instability of the complex in water.

On the other hand, a stability of this complex in H_2O-DMSO mixtures [2] would make it possible to carry out more accurate PPA determinations by adding an acidified solution of $FeCl_3$ in DMSO to urine.

Therefore, the keto-enol equilibrium of PPA was studied in H_2O-DMSO mixtures throughout the composition range and in an extended temperature range. Moreover, the optimum composition range of the mixtures which could be used for PPA analysis carried out directly on urine was determined.

Experimental Section

Instruments and techniques

^1H NMR spectra were recorded at 100 MHz with a Varian HA 100 spectrometer under the experimental conditions: expansion scale of 2 Hz mm^{-1} and sweep rate of 2 Hz s^{-1}. The measurements were carried out in the temperature range 28.5 – 105 °C. The concentrations of PPA in H_2O-DMSO mixtures and in pure DMSO ranged between 0.41 and 0.49 M.

The optical measurements were carried out using a Zeiss PMQ II spectrophotometer fitted up with a temperature-controlled cell holder. The tautomeric equilibrium in pure H_2O was studied by UV spectrophotometry in the temperature range 20 – 60 °C because of low PPA solubility in this solvent. The absorbance of the solution was measured at $\lambda =$ 287 nm assuming $\varepsilon = 25{,}000$ on the supposition that absorption of the keto form was neglegible compared with the enol one [3, 4]. The concentration realized was 0.385×10^{-2} M. The equilibrium constant was obtained from the expression:

$$K_T = C_c / C_E$$

where $C_c = C_T - C_E$, $C_T =$ PPA total concentration, $C_E = A_t/(\varepsilon \cdot l)$. The absorbance of solutions with different $FeCl_3$/PPA mole ratios in various H_2O-DMSO mixtures covering the range 20 – 80 vol.% DMSO was measured at $\lambda = 640$ nm and at 25 °C. The variation of $FeCl_3$/PPA mole ratio over the range 0.5 – 4 was realized keeping constant the PPA concentration at 0.4×10^{-3} M.

The different pH values in the H_2O-DMSO mixtures were realized by adding 3.0 N HCl by means of Chemetron micro-syringe. The pH measurements were performed with a digital Radiometer PHM63 pH meter provided with a U 9255 combined thalimid-glass electrode from Jenaer Glaswerk Schott and Gen., Mainz, Germany.

The mole fraction of the H_2O-DMSO mixtures was determined from the data of Cowrie and Toporowski [5] by measuring the refraction index at 25 °C on an Abbé refractometer using the sodium D line.

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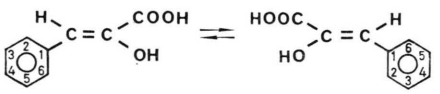
Abbreviations: DMSO, dimethylsulphoxide; PPA, phenylpyruvic acid.

The volume percentages in DMSO were calculated from the mole fraction data using the equation:

$$V_{\mathrm{DMSO}}\% = \frac{X_1(M_1/d_1)\,100}{X_1(M_1/d_1) + (1 - X_1(M_2/d_2)}$$

where X_1 is the mole fraction of DMSO, M_1 and M_2 the molecular weights of DMSO and H_2O, respectively, and d_1, d_2 [6] the densities of the two pure components at a given temperature.

In Fig. 1 the spectrum of PPA in DMSO is reported [2]. The signals at $\delta = 4.1$ and $\delta = 6.4$ originate from the CH_2 group of the keto form and from the CH group of the enol form, respectively. It seems reasonable to assume that, like styrene and 3.4 halostyrenes [7], there is a fast interconversion between:



which mediates the deshielding effect of the double bond on the two *ortho* protons which absorb between $\delta = 7.6$ and $\delta = 7.8$ [8]. Moreover, the signals derived from the three remaining aromatic protons of the enol form and from the five aromatic protons of the keto form range between $\delta = 7.1$ and $\delta = 7.4$ [2].

In pure DMSO the equilibrium constant was obtained by integration of the signals of the CH_2 keto protons and of the CH enol proton according to the equation:

$$K_T = (1/2\,CH_2)/CH\,. \qquad (1)$$

For the other solutions it was not possible to determine the equilibrium constant by means of formula (1) as the intense signal due to H_2O does not allow completely accurate integration of the signals originating from the CH_2 protons of the keto form. However, assuming equal to $2x$ the integral of the signals between $\delta = 7.6$ and $\delta = 7.8$ originated from the two *ortho* protons of the enol tautomer C(2)-H and C(6)-H, the contribution of the five aromatic protons of the keto form to the integral, A, of the signal between $\delta = 7.2$ and $\delta = 7.4$ is equal to $A - 3x$. Hence, the quantity $y = (A - 3x)/5$ represents the contribution of a single proton of the keto form to the integral. The ratio y/x gives the equilibrium constant K_T.

The values of K_T were obtained by averaging at least ten integrals.

Materials

Commercially available solvents were used. PPA was obtained from its sodium salt as described elsewhere [2]. Sodium phenylpyruvate was purchased from the British Drug Houses Ltd., Poole, England.

Experimental Results

Keto-enol equilibrium

The standard Gibbs free energy changes were calculated assuming ideal behaviour:

$$\Delta G^0 = -RT \ln K_T\,.$$

The difference of enthalpy ΔH and entropy ΔS between the two tautomers were computed by means of the relationship between the equilibrium constant and the temperature applying the linear least-squares method:

$$\lg K_T = -\Delta H/(2.303\,RT) + \Delta S(2.303\,RT)\,.$$

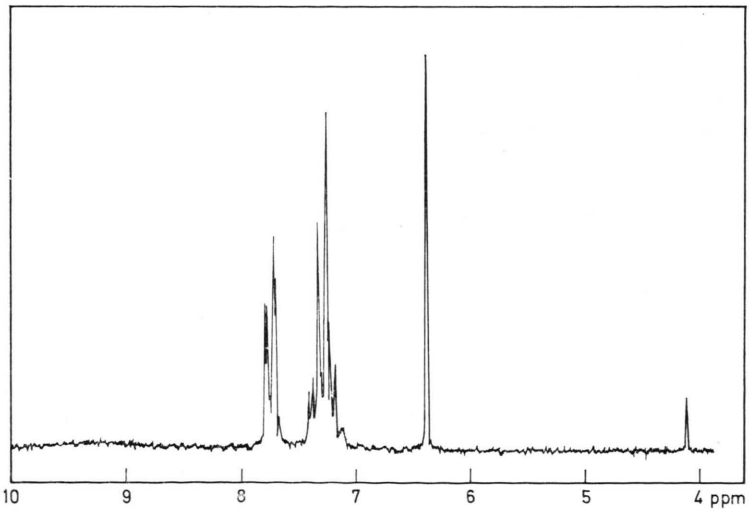


Fig. 1. ^{1}H NMR spectrum (100 MHz) of PPA in DMSO-d_6 (0.49 M).

On considering the influence of the medium on the tautomeric equilibrium in terms of the Onsager model [9], it is deduced that an increase of the polarity of the solution must favour the more polar tautomer [10]. In fact, going from DMSO to H_2O there is an increase in the keto fraction.

There is not a linear relationship between $\lg K_T$ or ΔG^0 and the solvent quantity $(\varepsilon_{12}-1)V_{12}/(2\varepsilon_{12}+1)$ where ε_{12} is the dielectric constant and V_{12} the means molar volume of the mixed solvent [11]. Indeed, the determination of interaction energies between each tautomer and its own neighbourhood is based on the supposition that the latter is a homogeneous and continuous medium and there are no specific associations, whereas, in the present case, both the keto and the enol molecules are able to form hydrogen bonds with the solvent molecules. Therefore, the enol fraction in H_2O-DMSO mixtures goes from 0.68 to 0.05 whereas ε_{12} from 73 to 78.

The thermodynamic quantities relative to the keto-enol equilibrium are expressed as a function of the mixture composition in Fig. 2. The values

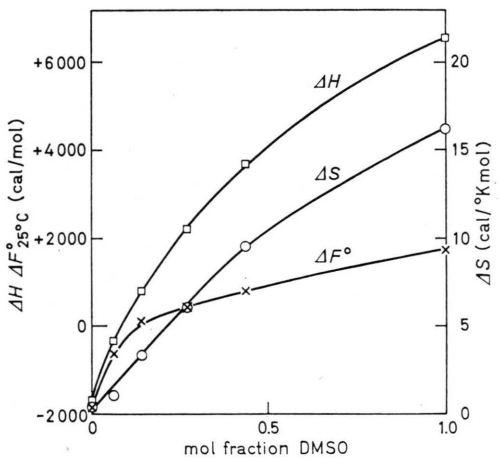
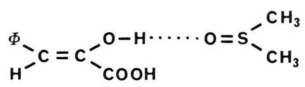


Fig. 2. Thermodynamic quantities of tautomerism *versus* the composition of the mixed solvent.

of ΔH and ΔS are independent from the temperature in the experimental range used.

The standard free energy of enolization changes with the mixed medium composition and points out that free energies of solvation play a more prominent role than free energies of formation on the stabilization of either tautomer.

It is noteworthy that, whereas the entropy of the two tautomers is pratically equal in H_2O, the enolization process in DMSO shows a remarkable decrease in the system entropy. This feature may be explained by the formation of a strong intermolecular hydrogen bond between the hydrogen of the enol hydroxyl group and a DMSO molecule.



Analytical topics

Knowledge of thermodynamic quantities of the keto-enol equilibrium allows the computation of the enol fraction as a function of the solvent composition and temperature.

Fig. 3 shows a plot of the enol fraction *versus* the volume percentage in DMSO in the 20 – 37 °C

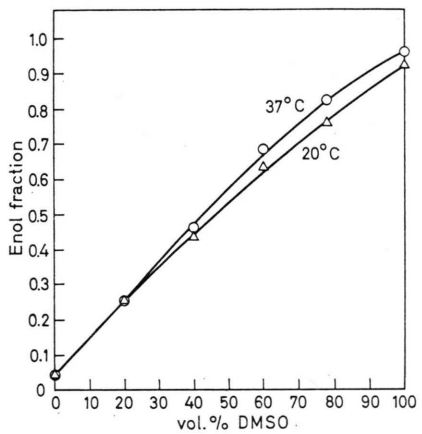


Fig. 3. Enol fraction *versus* volume percentage of DMSO in the 20–37 °C temperature range.

temperature range, in which clinical determinations are normally carried out. The volume percentage scale was preferred because it may be used more easily in clinical laboratories.

The trend of the plot is not linear and shows a regular increase in the enol fraction with the volume percentage in DMSO. Therefore, it appears clear that, in the analytical test of PPA, it would be worthwhile to operate with solutions containing at least 50 vol. per cent DMSO. Indeed, for such solutions the enol fraction increases to more than 0.55 making a rapid response of the colorimetric reaction reliable.

The absorbance values of solutions with different $FeCl_3$/PPA mole ratios, *i.e.* from 0.5 to 4, in

various H_2O-DMSO mixtures show that PPA is completely complexed even for a 1:1 mole ratio in mixtures containing at least 40 vol.% DMSO. For such solutions the green colour develops istantaneously and the absorbance values remain steady within 120 min at least. Moreover, a decrease of absorbance which is due to complex decomposition is observed for mixtures containing 20 vol.% DMSO and this phenomenon becomes faster in pure H_2O as pointed out by other authors [1], too. For this reason, it seemed not convenient to extend to this solvent the experiments carried out in mixed solvent. A plot of the absorbance values of solutions in which PPA in completely complexed *versus* volume percentage DMSO (Fig. 4) reveals that the maximum sensibility is for mixture compositions comprised between 40 – 80 vol.% DMSO. This result is very important to improve the sensibility of the colorimetric method used for quantitative determinations of PPA [1, 2].

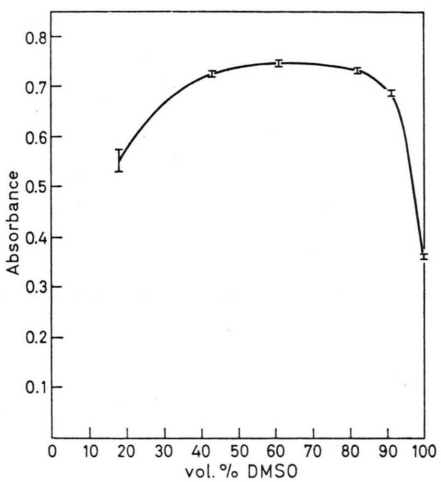


Fig. 4. Absorbance of solution with $FeCl_3$/PPA mole ratio of 4 to 1 in H_2O-DMSO mixtures *versus* vol.% DMSO: λ_{max} =640 nm, cell path 1 cm, temperature 25 °C, PPA conc. 0.4×10^{-3} M.

To detect PPA directly on urine by adding an acidified solution of $FeCl_3$ in DMSO, the limit of dilution of urine with DMSO might be due to phosphate precipitation. Tests carried out on urine samples of normal subjects show that phosphate precipitation does not occur in 50 vol.% DMSO in urine by acidification at pH = 3 – 4. An addition of acidified solutions of $FeCl_3$ in DMSO would cause the pH of urine samples to decrease to different values according to their alkaline reserve. Accordingly it was appropriate to investigate the influence of acidity on the absorbance of solutions with a $FeCl_3$/PPA mole ratio of 4 to 1 in mixed solvent over the composition range 20 – 80 vol.% DMSO.

Table I reveals that solutions with $FeCl_3$/PPA mole ratio of 4 to 1 in mixed solvent with a composition ranging from 40 to 60 vol.% DMSO do not present large changes in absorbance over a 0.8 pH unit range.

Although the pH values measured in the mixed solvent have no thermodynamic meaning, their differences prove to correspond to effective changes in pH units. For this reason, measurements of e.m.f. were carried out in mixtures having 61% DMSO at various HCl concentrations. A plot of millivolts *versus* logarithm of the acid concentration show the theoretical 59 mV slope within ± mV over a range of acid concentration from 10^{-4} to 10^{-2} M [12]. Indeed, the concentration dependence of the potential, both in the H_2O-DMSO mixtures and in pure H_2O, is Nerstian:

$$\Delta pH_{(61\%\ mixture)} = \Delta pH_{H_2O} = \frac{\Delta E}{0.0591}$$

Table I. Absorbance values in dependence of volume % DMSO (18–82%) and pH ($FeCl_3$/PPA mole ratio: 4, PPA conc. 0.4×10^{-3} M).

18%		43%		61%		82%	
pH	A_t	pH	A_t	pH	A_t	pH	A_t
3.4	0.553	3.8	0.726	4.2	0.744	4.4	0.730
3.1	0.520	3.5	0.710	3.8	0.741	4.1	0.715
2.8	0.515	3.2	0.712	3.6	0.743	3.8	0.680
2.5	0.492	2.9	0.690	3.4	0.736	3.4	0.458
2.1	0.440	2.5	0.605	3.1	0.703	3.2	0.265
—	—	2.1	0.350	2.9	0.606	—	—
—	—	—	—	2.6	0.440	—	—

In conclusion, by considering the sensibility of the test, the stability of the complex and the invariance of the absorbance in the pH range of interest, the optimum compositions which would be appropriate for a direct analysis of PPA are comprised between 40 and 60 vol.% DMSO.

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