Equilibrium and Activation Thermodynamic Parameters of the Tautomerism of 6-Methoxy-2-pyridone in Water

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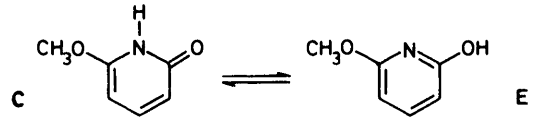
In neutral water, the interconversion of the two tautomers of the lactim–lactam equilibrium of 6-methoxy-2-pyridone occurs essentially *via* a pH-independent process. The influence of temperature on the position and on the dynamics of this tautomeric equilibrium has been studied by temperature-jump relaxation kinetics. The equilibrium and activation thermodynamic parameters obtained ($\Delta H°$ 26.8 kJ mol^{-1}, $\Delta S°$ 58 J K^{-1} mol^{-1}; lactam $\longrightarrow$ lactim, $\Delta H_C^‡$ 46 kJ mol^{-1}, $\Delta S_C^‡$ −40 J K^{-1} mol^{-1}) indicate a high value for the kinetic entropy term. These results indicate that the interconversion mechanism is ionic (rather than concerted) and involves the anionic form of the substrate in a cyclic transition state. In this transition state, at least two solvent molecules would temporarily ensure a hydrogen bond connection between the sites which undergo tautomerism.

We have previously shown that the tautomeric interconversion of 6-methoxy-2-pyridone in water proceeds according to two mechanisms, the relative importance of which varies with the pH. A first widely studied mechanism, said to be dissociative,[1a] involves protonation or deprotonation of the substrate, and thus tautomeric interconversion is subject to generalized acid–base catalysis. The second mechanism, said to be non-dissociative, is pH-independent, and involves catalysis by water in its neutral form; in neutral conditions, this mechanism contributes most to the tautomeric equilibrium.[1]

By studying the thermodynamic data of this interconversion, we have sought to specify the nature of the second mechanism. To this end we conducted a study on the influence of temperature on the equilibrium and on the interconversion kinetics by using a temperature-jump relaxation apparatus coupled to u.v. spectrophotometric detection. The latter technique makes possible direct observation of the equilibrium and to obtain simultaneously the relaxation time and the equilibrium constant of the system studied at a given temperature.



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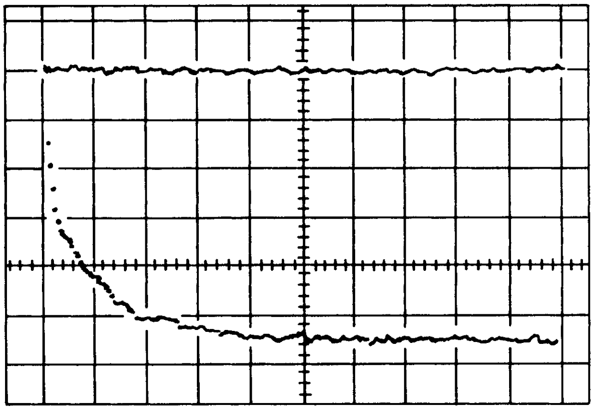


Figure 1. Relaxation spectrum of 6-methoxy-2-pyridone at 308 nm: T_i 2 °C; T_f 9 °C; pH 6.8; concentration 2.66 × 10^{-4}M. Scale: ordinate, 3.4 × 10^{-3} units of optical density per square; abscissa, 60 μs per square. The observed signal corresponds to a decrease in optical density

Experimental

6-Methoxy-2-pyridone was synthesized [2] from 2,6-dimethoxypyridine, then recrystallized from benzene–light petroleum and sublimed under vacuum, m.p. 104—105 °C (lit.,[2] 102—104 °C). Temperature-jump kinetic experiments were run according to a previously described procedure.[3] Commonly used experimental conditions were: a 6 mm optical path cell, an initial temperature varying between 2 and 23 °C, a 7° jump in temperature (produced by the discharge of a 0.05 μF condensor charged at 28 kV), a substrate concentration of 2.66 × 10^{-4}M, a 308 nm analysis wavelength in an area of the spectrum where only the lactam form absorbs. A Radiometer type 64 pH-meter, equipped with a G 202 C electrode and an NaCl reference electrode, was used to measure pH. Solutions were prepared by dissolving a small amount of 6-methoxy-2-pyridone (concentration identified by u.v.) in 0.2M solutions of $NaClO_4,2H_2O$ in distilled water. Solutions were degassed before use. Each datum related to the relaxation time or the amplitude was averaged from five values, each of which was obtained by treating nine accumulated signals. Uncertainties were estimated at 3% for the relaxation time, and at 5% for the amplitude.

Results

Description of the Phenomenon.—When a solution of 6-methoxy-2-pyridone is subjected to a sudden rise in temperature, the optical density at 308 nm displays two successive variations (Figure 1): a decrease as fast as the rise in temperature, followed by a time-dependent exponential decrease. A variation like the first has been observed with solutions of 6-methoxy-1-methyl-2-pyridone and with solutions of many pyrimidines and purines.[3b,4] In the case of 2-pyridones, it has been shown that neither tautomeric interconversion, nor ionization, nor self-association, nor conformational changes in the substrates can account for this rapid optical density change.[5] It presumably arises from a temperature-dependent modification in the solvation structure of the lactam tautomer. The second 'slow' variation, which is not observed

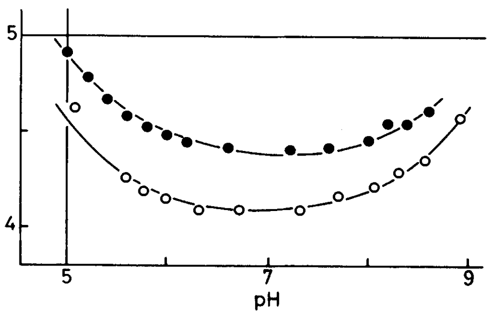


Figure 2. Variation in relaxation time of 6-methoxy-2-pyridone *versus* pH. Concentration 2.66 × 10^{-4}M; upper curve, *T* 32 °C; lower curve, *T* 9 °C. λ 308 nm

Table 1. Data for the evaluation of thermodynamic parameters

$10^3T^{-1}/K^{-1}$	10^3A_{308nm} *	$10^{-4}k_0/s^{-1}$ †	10^2K_T	$\log k_E/T$
3.546	4.88	1.19	1.07	3.73
3.509	5.61	1.32	1.26	3.82
3.465	6.35	1.47	1.46	3.92
3.407	7.32	1.59	1.74	3.97
3.356	8.06	1.82	1.97	4.09
3.295	9.77	2.20	2.48	4.27
3.252	10.99	2.78	2.87	4.47

* Estimated to within 5%. † Estimated to within 10%.

with 1-substituted derivatives, was previously ascribed to a shift in the tautomeric equilibrium.[1a] The present study deals only with the second relaxation.

Analysis as a Function of pH.—As shown in Figure 2, the relaxation time τ of the ' slow ' phenomenon is pH-dependent. Its variations are correctly described by equation (1).[1a] This

$$\tau^{-1} = k_0 + k_{H^+}[H^+] + k_{OH^-}[OH^-] \qquad (1)$$

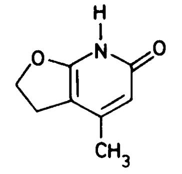
equation remains valid over a temperature range where the final temperature *T* varies between 9 and 32 °C: the correlation coefficient of a three-parameter multiple regression is always >0.994. It is seen that k_0, *i.e.* the non-dissociative contribution to the tautomeric interconversion, is the major term since a good fit of equation (1) for the extreme temperatures and pH 6.8 yields k_0 11 900 ± 1 000 s^{-1}, ($k_{H^+}[H^+] + k_{OH^-}[OH^-]$) 790 s^{-1} (*T* 9 °C); k_0 24 200 ± 3 000 s^{-1}, ($k_{H^+}[H^+] + k_{OH^-}[OH^-]$) 1 000 s^{-1} (*T* 32 °C). The intermediate values of k_0 given in Table 1 are obtained by the same treatment.

Standard Enthalpy and Entropy of Reaction from Relaxation Amplitudes.—The standard enthalpy and entropy of the reaction cannot be obtained from a standard thermodynamic study by u.v. spectrometry, because the result from the static measurement after a variation in the temperature would lead to the attribution of the total variation in amplitude to a shift in tautomeric equilibrium, taking into account the fact that the fast prephenomenon would blur these measurements with a very large error. In contrast, the variation of the slow relaxation amplitudes with temperature allows the enthalpy of the reaction and the equilibrium constant to be estimated. Its expression as a function of the various parameters is known.[3a] In our case, where the equilibrium constant is clearly smaller than unity the amplitude A_λ is expressed by equation (2) where $\Delta H°$ designates the enthalpy of the

$$A_\lambda = \bar{C}\,.\,l\,.\,K_T\,.\,\Delta\varepsilon_\lambda\,.\,\Delta T\,.\,\Delta H°/RT^2 \qquad (2)$$

Table 2. Equilibrium thermodynamic parameters of (I) and (II)

Solvent	(I) Water	(II) 90% water–10% EtOH	50% water–50% EtOH	25% water–75% EtOH	100% EtOH
$\Delta H°$/ kJ mol^{-1}	26.8 ± 4.2	15.2	8.6	6.7	2.4
$\Delta S°$/ J K^{-1} mol^{-1}	58 ± 12	36.3	23.1	21.9	13.7



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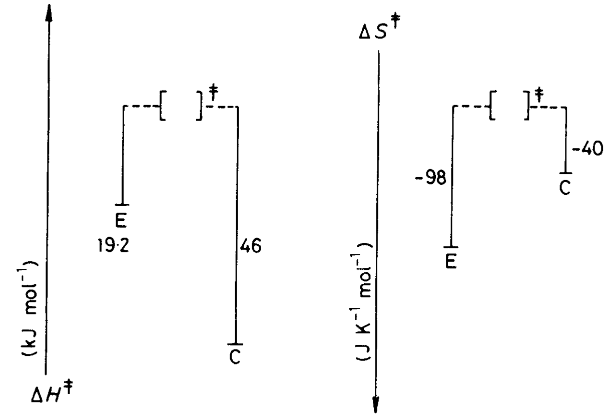

Figure 3. Diagram of the activation energies for the interconversion mechanism of 6-methoxy-2-pyridone in water. Maximum uncertainties in $\Delta H^\ddagger$ and $\Delta S^\ddagger$ are estimated to within 20%

reaction, K_T the constant of tautomerism [E]/[C] (ratio between the concentration of the minority and majority species), *l* the optical path, *T* and ΔT the final temperature and the temperature-jump amplitude, $\bar{C}$ the analytical concentration of substrate, and $\Delta\varepsilon_\lambda$ the difference between the molecular extinction coefficients of the two tautomers at wavelength λ.*

To a first approximation, we assume that $\Delta H°$ and $\Delta\varepsilon_\lambda$ are independent of the temperature in the range studied: AT^2 is proportional to K_T when the temperature varies. A plot of log (AT^2) against $1/T$ gives a direct measurement of the enthalpy of the reaction. By reintroducing this enthalpy into equation (2), the equilibrium constant can be obtained for each temperature.

Activation Parameters.—The simplest tautomeric interconversion is (3). So K_T is the ratio k_C/k_E. The measured rate

$$C \underset{k_E}{\overset{k_C}{\rightleftharpoons}} E \qquad (3)$$

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* An estimate of 10 000 l mol^{-1} cm^{-1} for $\Delta\varepsilon_{308}$, derived from the spectra of 6-methoxy-1-methyl-2-pyridone and 2,6-dimethoxypyridine, is used in this work.[1a]

constant k_0 is the sum of the forward and reverse rate constants. The combined values of the equilibrium constants (deduced from amplitude measurements) and of the measured relaxation times τ^{-1} make it possible to determine the elementary rate constants at each temperature. By tracing the graph of log k_C/T and log k_E/T against $1/T$, Eyring's law yields the activation thermodynamic parameters. Table 1 shows the results and their treatment.

The equilibrium and activation thermodynamic parameters are given in Table 2 and in Figure 3. The uncertainties derived from least-square procedures in the activation thermodynamic parameters are: $\Delta S_E^{\ddagger}$ − 98 ± 20 J K^{-1} mol^{-1}, $\Delta H_E^{\ddagger}$ 19.2 ± 4.2 kJ mol^{-1}; $\Delta S_C^{\ddagger}$ −40 ± 8 J K^{-1} mol^{-1}, $\Delta H_C^{\ddagger}$ 46 ± 8 kJ mol^{-1}.

Discussion

Equilibrium Thermodynamic Parameters.—A rise in temperature causes a shift in equilibrium from the lactam to the lactim.* The overall measured difference ΔS° is essentially the sum of $\Delta S^\circ_{int} + \Delta S^\circ_{solv}$. ΔS°_{int} designates the difference between the internal entropies of the tautomers, and ΔS°_{solv} the difference between the entropies of the solvation layers of these same tautomers. As the only difference between the internal entropies of the tautomers is the slight energy created by free rotation of the OH bond in the lactim tautomer,[7] it is very likely that they are of the same order of magnitude. This similarly implies that $\Delta S^\circ_{int} \ll \Delta S^\circ$. So the ΔS° value obtained essentially represents the difference between the solvation energies of the two tautomers. Its value, 58 J K^{-1} mol^{-1}, strongly suggests greater organization of the lactam solvation layer than that of the lactim. If one attempts to interpret this difference in terms of the freezing of a finite number of water molecules, the value deduced from the literature [8] is >1.†

A similar analysis of ΔH° is not easy. The difference between the internal energies cannot be estimated, because neither of them is easily calculated. Therefore, the observed results cannot be discussed in terms of the difference between solvation structure enthalpies.

Nevertheless, it should be noted that the smal $\Delta G^\circ_{25\,^\circ C}$ value of 9.2 kJ mol^{-1} results from compensation between two large contributions (at 25 °C: $-T\Delta S^\circ$ −17.5 kJ mol^{-1}, ΔH° +26.8 kJ mol^{-1}).

Activation Thermodynamic Parameters.—Activation enthalpies and entropies are high compared to the equilibrium values. They each indicate thereby that the transition state is structurally clearly different from the extreme lactam and lactim forms.‡

It has been assumed [1b,10] that the 'non-dissociative' tautomeric interconversion mechanism necessarily entails the formation of a ring from a bridge of water molecule(s) between the proton donor and withdrawing sites of the same molecule of 6-methoxy-2-pyridone. Up to now, the choice of mechanism assumed the stability of the ring formed in the interconversion; the existence, permanent or transient, of such a ring was not questioned. In water, the many possibilities of hydrogen bonding between substrate atoms involved in the tautomerism and the neighbouring solvent molecules leave one free to surmise that the bridge is not necessarily formed in the stable solvation states of each tautomer; if this is so, its formation would contribute highly to the measured activation entropy. This leads us to interpret the high entropies of 98 and 40 J K^{-1} mol^{-1} as the substantial loss in entropy, essentially that of translation, resulting from several water molecules being incorporated into a reaction complex; these high values indicate that, before considering the interconversion of the tautomers, ring formation must be taken into account as a major step.

Information about this step can be obtained by analogy with a reaction which can be compared directly with the lactim–lactam transformation, *i.e.* glucose mutarotation in water. Various studies [11] imply that this mutarotation occurs in water by the formation of a ring with two solvent molecules. The measured value of $\Delta S^{\ddagger}$ for this reaction has been estimated at −79 [10a] and −113 J K^{-1} mol^{-1}.[12] This is of the same order of magnitude as the value we obtained. The establishment of such a ring requires, for the organization of the molecules involved, a large variation in entropy, but entails relatively little variation in enthalpy since, in the overall outcome, there is no change in the number of hydrogen bonds. The high enthalpies measured stem from the subsequent proton transfer between the two sites.

Once the bridge *via* the hydrogen bonds is achieved, the interconversion can proceed through two extreme types of mechanisms: the first is a concerted mechanism characterized by a simultaneous proton transfer, the second is an ionic mechanism calling for a sequential proton transfer with charge localization. Until now, the concerted mechanism was preferred to explain the contribution of the rate term k_0 in the neighbourhood of neutrality. The argument advanced was that since the interconversion rate constants in water and methanol (τ^{-1}_{MeOH} 9 000 ± 1 000 s^{-1} at 10 °C) [13] are of the same order of magnitude, they do not reflect the difference between the dielectric constants of these solvents, a difference which could favour an ionic mechanism. Actually, the theory of Westheimer *et al.*[14] indicates that the true dielectric constant of the solvent in a small sphere including an organic molecule should not necessarily be related to its macroscopic dielectric constant. This leaves the occurrence of a concerted mechanism open to doubt.

In this study we made the following observations. An increase in the acidity of the substrate is associated with an increase in the rate constant (Table 3). This clear variation in rate strongly suggests that partial separations of charges exist in the transition state. Grunwald [15] has already observed similar behaviour for benzoic acids, and concluded that the mechanism must be ionic since the interconversion rate of a concerted system should be insensitive to p*K*.

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* A similar variation has been observed for the bicyclic compound in Table 2 which is structurally related to 6-methoxy-2-pyridone. The tautomeric thermodynamic parameters of this compound were determined in different water–alcohol mixtures.[6] The presence of alcohol which favours the shift in equilibrium towards the lactim (the minor form in water) made it possible to measure the variation in concentration of one tautomer and, thus, to determine the variation of the equilibrium constant *versus* temperature. By analysis of the results in Table 2, it is possible to deduce that our values constitute a good extrapolation in pure water of the results obtained for this model compound.

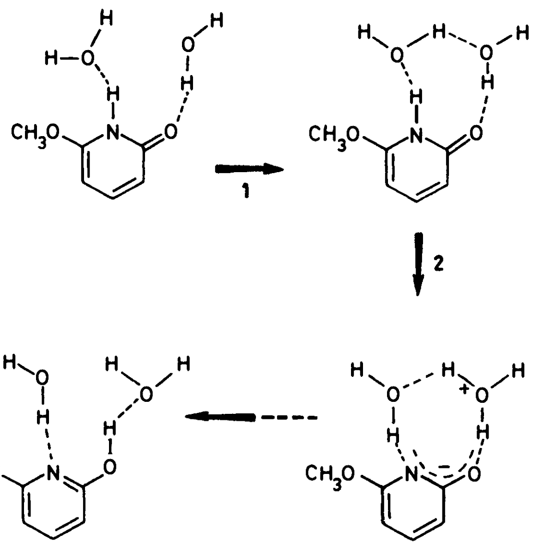
† If, like Kauzmann, one considers the freezing of a water molecule to correspond to a loss in entropy of *ca.* 20—25 J K^{-1} mol^{-1}, the number of water molecules involved in the studied interconversion would be 2 or 3.

‡ These high values contradict the interpretation of results obtained by CNDO/2 calculations [9] based on a transition state involving a single molecule of water: it had been suggested that the C $\longrightarrow$ E potential barrier disappeared when the reaction occurred in aqueous solution. This proposal disagrees with experimental results,[1a] because the absence of an energy barrier, if the latter truly reflects the variation in activation enthalpy, is formally incompatible with our experimental estimation of 46 kJ mol^{-1} for $\Delta H_C^{\ddagger}$. If this barrier could be reconciled to a variation in the free enthalpy of activation, it would yield an interconversion rate much faster than that observed.

Table 3. Variation of rate constant k_C *versus* p*K* for various 6-substituted 2-pyridones

	6-Methoxy-2-pyridone	6-Chloro-2-pyridone
p*K* [a]	9.82	7.84
k_C/s^{-1} [b]	120	6 200

[a] p*K* of deprotonation of lactam forms. [b] Measured at 10 °C.



Scheme. Water acts as a base. A minimum number of water molecules is represented for the sake of clarity. In stage 1, there is an entropy barrier, in stage 2, an enthalpy barrier

Lactam (I) is a weak acid and base. Its weak acid character makes it possible to assimilate its activation enthalpy with its ionization enthalpy; by measuring the variation in p*K* *versus* temperature, we determined the latter enthalpy to be 36.8 kJ mol^{-1}. Therefore, the activation enthalpy for deprotonation of the lactam form is *ca.* 36.8 kJ mol^{-1} and is of the same order of magnitude as the enthalpy of 46 kJ mol^{-1} of our direct reaction.*

These observations allow one to assume that the passage of the lactam form to the lactim form *via* an ionic mechanism involving the anionic structure † of the substrate is highly probable.

All the observations described herein lead us to propose, for the tautomeric interconversion *via* a non-dissociative process, an ionic mechanism in which a pair of water molecules forms the transient ring needed for proton transfer between the donor atom and the acceptor atom of the pyridone molecule. The low interconversion rate is therefore no longer explained by the constrained form of the ring, but has its own origin in the small probability of existence of this ring.

Acknowledgements

We are highly indebted to O. Bensaude for his constructive comments. O. Bruno's help is also gratefully acknowledged.

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Received 10*th September* 1982; *Paper* 2/1559

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* ~~Identical reasoning can be advanced for the E ⟶ C reaction,~~ but the enthalpy of deprotonation of the lactim form (rare in water) cannot be measured. However, one can note the similarity between the activation enthalpy measured here (19.2 kJ mol^{-1}) and the enthalpy of deprotonation of 3-methoxyphenol (22 kJ mol^{-1}).

† Interconversion *via* a zwitterionic mechanism can, theoretically, go through the anionic or cationic form of pyridone. Reaction by the cationic form of the substrate is not taken into account here because p*K* 1 for the protonation of 6-methoxy-2-pyridone renders passage through the cationic form very unlikely under the chosen experimental conditions.