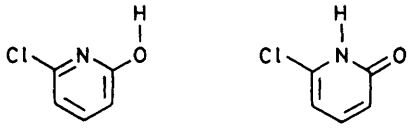
# Influence of Carboxylic Acid Association upon the Lactim–Lactam Tautomeric Equilibrium of 2-Hydroxypyridines

By **Jean Guillerez, Olivier Bensaude,** and **Jacques Emile Dubois,*** Institut de Topologie et de Dynamique des Systèmes, Université Paris VII, 1 rue Guy de la Brosse, 75005 Paris, France

I.r. and u.v. absorption spectroscopy in $CCl_4$ at room temperature provides evidence for lactim–acid and lactam–acid heterodimer formation in 6-chloro-2-hydroxypyridine–acetic acid mixtures. Measurements of association constants for two types of 1 : 1 hydrogen-bonded complexes reveal preferential association with the lactam tautomer, leading to a shift in the apparent tautomeric equilibrium constant. These results suggest that specific interactions are of great importance in understanding solvent effects on protomeric equilibria.

CONSIDERABLE attention has been devoted to studies on heteroaromatic protomeric equilibria.[1] The solvent is known to influence markedly the position of the equilibrium.[2-5] Specific molecule–molecule associations appear to be predominant under certain conditions. In the case of 2-pyridones, the lactam tautomers are favoured by dimerization [2b] and specific association with water molecules [6] and metal cations.[7] As i.r. and n.m.r. experiments indicate specific association of carboxylic acids with amides [8] and nucleic acid bases,[9] our interest



(E) (C)

in seeing how such associations could affect the biologically important [10] lactim–lactam tautomeric equilibria was aroused.

This paper deals with an investigation of the interactions of acetic acid with the lactim and lactam tautomers of 6-chloro-2-pyridone, referred to henceforth as (E) and (C), respectively. The lactim–lactam equilibrium of 6-chloro-2-pyridone was chosen because numerous spectroscopic studies of the tautomerism have been performed in many different phase conditions.[1,2b,11] However, experiments performed with other tautomeric 2-pyridones (6-methoxy, 6-bromo) afforded similar results. Carbon tetrachloride was chosen as a solvent because of the weak solute–solvent interactions therein.

## RESULTS

*U.v. Spectral Data.*—The u.v. spectrum of 6-chloro-2-pyridone in carbon tetrachloride displays two absorption bands, a major one at 278 nm and a minor one at *ca.* 310 nm. These are attributed [11] to the lactim (E) and the lactam (C), respectively. Upon addition of acetic acid, the lactim band shifts to 285 nm, the absorbance at 310 nm increases, and there is an isosbestic point at 283 nm. Since the spectrum of 2-pyridone (lactam tautomer mainly †) is rather insensitive to the presence of acetic acid, the increased absorbance at 310 nm for 6-chloro-2-pyridone is associated with a shift in the tautomeric equilibrium towards the lactam form.

6-Chloro-2-pyridone is known to dimerize in apolar aprotic solvents.[12] Upon dimerization the lactim band shifts to 285 nm and its molar extinction coefficient increases, but the lactam band appears insensitive to concentration. The spectral modifications induced by acetic acid are very similar and suggest the formation of pyridone–acetic acid cyclic heterodimers (2-pyridones and carboxylic acids are known to form cyclic homodimers in carbon tetrachloride solutions [13]).

*I.r. Spectral Data.*—The existence of quinolone–carboxylic acid heterodimers was demonstrated by i.r. spectra.[8] Therefore, in order to check independently the conclusions derived from the u.v. spectra, attention was directed to the i.r. spectra of the 6-chloro-2-pyridone–acetic acid system. It was found that pure 6-chloro-2-pyridone in carbon tetrachloride displays (a) two narrow bands at 3 558 and 3 397

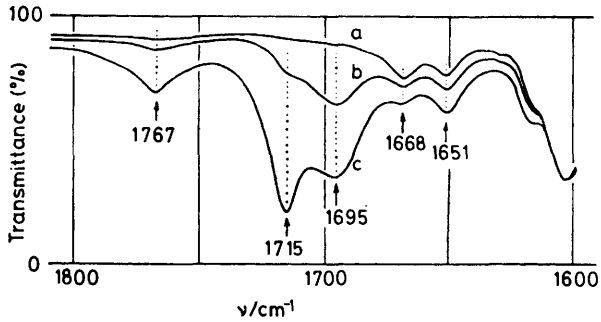


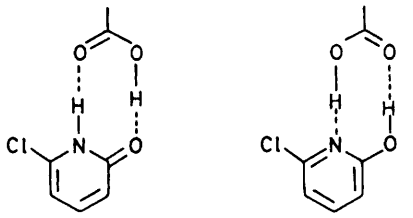

FIGURE 1 Evidence for acetic acid–6-chloro-2-pyridone mixed dimer formation. The 1 668 and 1 651 $cm^{-1}$ 6-chloro-2-pyridone bands are attributed to the carbonyl $\nu_{C=O}$ of the lactam in the monomeric and dimeric (homodimer and heterodimer) species, respectively. The 1 767 and 1 715 $cm^{-1}$ acetic acid bands are attributed to $\nu_{C=O}$ of the monomer and the cyclic homodimer species, respectively, whereas the 1 695 $cm^{-1}$ band corresponds to $\nu_{C=O}$ of acetic acid in heterodimers. [6-Chloro-2-pyridone] $1.9 \times 10^{-2}$M; [acetic acid] (a) 0M, (b) $1.4 \times 10^{-2}$M, (c) $3.5 \times 10^{-2}$M

$cm^{-1}$ ascribable [12b,14a] to the $\nu_{OH}$ and $\nu_{NH}$ of the free lactim and the free lactam tautomers, respectively, (b) a strong broad band between 3 200 and 2 500 $cm^{-1}$ characteristic of the cyclic dimers, and (c) two concentration-dependent bands at 1 668 and 1 651 $cm^{-1}$ (Figure 1) which are therefore attributed to $\nu_{C=O}$ in the lactam monomer and the lactam dimer, respectively.[14b] The low intensity of these bands (with respect to the 1 610 $cm^{-1}$ band) is due to the low proportion of lactam. Similarly, pure acetic acid in carbon tetrachloride presents: (a) a narrow band at 3 538 $cm^{-1}$ for

† Checked by i.r. spectroscopy in the 3 600—3 400 $cm^{-1}$ range. A (C) : (E) ratio of 10 was measured for the monomers.

the free $\nu_{OH}$ in the monomer, (b) a broad band between 3 200 and 2 500 $cm^{-1}$, characteristic of the cyclic dimer, and (c) two bands at 1 767 and 1 715 $cm^{-1}$ attributed [14c] to $\nu_{C=O}$ of the monomeric and the cyclic dimeric species, respectively.

Upon addition of acetic acid to the 6-chloro-2-pyridone solution, the intensity of the 3 558 $cm^{-1}$ free $\nu_{OH}$ band of the lactim monomer, as well as the 3 397 $cm^{-1}$ free $\nu_{NH}$ band of the lactam monomer decreases. No new bands ascribable to free $\nu_{OH}$ and $\nu_{NH}$ in open chain polymers are detectable.



(CA) (EA)

The 1 668 $cm^{-1}$ $\nu_{C=O}$ lactam monomer decreases, whereas the 1 651 $cm^{-1}$ lactam dimer (Figure 1) increases. We therefore conclude that, in the presence of acetic acid, the proportions of monomeric pyridone species diminish. The new 1 695 $cm^{-1}$ band is ascribable to $\nu_{C=O}$ of acetic acid in heterodimers.[8] This band appears rather broad and may consist of two bands resulting from acid associated with either the lactim or the lactam tautomer. From these data, it is reasonable to assume that two 1 : 1 cyclic complexes (CA) and (EA) are formed. Our quantitative interpretation of the u.v. spectral data in terms of equilibrium displacements is that preferential binding of acetic acid to one tautomer would be responsible for a shift in the tautomeric equilibrium.

The ' apparent ' tautomeric ratio $K_{ap}$ determined from the u.v. spectra, given as a function of total pyridone concentration $[S]_0$ and total acetic acid concentration $[A]_0$

| $[S]_0$/M | $10^5[A]_0$/M | $K_{ap}$ ($\pm 0.005$) [a] |
|---|---|---|
| $3.41 \times 10^{-4}$ | 0 | 0.077 |
| | 5.9 | 0.096 |
| | 11.8 | 0.113 |
| | 17.66 | 0.128 |
| | 29.44 | 0.152 |
| | 41.2 | 0.167 |
| | 64.3 | 0.197 |
| | 130.2 | 0.238 [b] |
| | 326.5 | 0.278 [b] |
| | 915 | 0.319 [b] |
| | 2 093 | 0.331 [b] |
| $3.2 \times 10^{-5}$ | 0 | 0.071 |
| | 2.5 | 0.086 |
| | 10.2 | 0.124 |
| | 24.8 | 0.174 |
| | 54.0 | 0.209 [b] |
| | 134.2 | 0.247 [b] |
| | 280 | 0.279 [b] |
| | 717 | 0.304 [b] |
| | 1 590 | 0.324 [b] |
| $8.4 \times 10^{-6}$ | 0 | 0.065 |
| | 34 | 0.201 |
| | 136 | 0.260 |
| | 544 | 0.305 [b] |
| | 1 563 | 0.329 [b] |
| | 3 601 | 0.345 [b] |

[a] Standard experimental error. [b] Results from experiments carried out to estimate $K_E$ and $K_T'$ from equation (9).

*Quantitative Treatment of the U.v. Spectral Data.*—The Table reports the apparent lactam–lactim tautomeric equilibrium constant $K_{ap}$ measured from the u.v. spectra at 310 nm by the usual methods [1] as a function of the total concentration $[S]_0$ and of the total acetic acid concentration $[A]_0$. The small differences observed in the absence of acid result from the dimerization of the pyridone; nevertheless, we shall assume that the concentrations of the dimeric species of pyridone are negligible in the pyridone–acetic acid mixtures studied. Assuming now that the spectrum of the lactam tautomer remains unaffected by association, the apparent tautomeric ratio $K_{ap}$ is expressed by equation (1). Various components can be accounted for by equilibria (2)—(5). The total acid concentration $[A]_0$ and the total pyridone concentration $[S]_0$ are expressed by equations (6) and (6'). Since the concentrations in pyridone and acetic acid remain rather low, ' physical ' solvent effects are neglected; consequently we shall assume that the equilibrium constants $K_C$, $K_E$, $K_A$, $K_T$, and $K_T'$ are independent of solvent composition and that activities are equal to concentrations. Then, expression (7) is readily derived.

$$K_{ap} = \frac{[C] + [CA]}{[E] + [EA]} \quad (1)$$

$$C + A \rightleftharpoons CA;\ K_c = \frac{[CA]}{[C][A]} \quad \text{formation of the heterodimers} \quad (2)$$

$$E + A \rightleftharpoons EA;\ K_E = \frac{[EA]}{[E][A]}$$

$$2A \rightleftharpoons A_2;\ K_A = \frac{[A_2]}{[A]^2} \quad \text{dimerization of the acid} \quad (3)$$

$$E \rightleftharpoons C;\ K_T = \frac{[C]}{[E]} \quad \text{'true' tautomeric equilibrium constant} \quad (4)$$

$$EA \rightleftharpoons CA;\ K_T' = \frac{[CA]}{[EA]} \quad (5)$$

$$[A]_0 = 2[A_2] + [A] + [EA] + [CA] \quad (6)$$

$$[S]_0 = [C] + [E] + [EA] + [CA] \quad (6')$$

$$\frac{K_{ap} - K_T}{K_T' - K_{ap}} = K_E[A] \quad (7)$$

The concentration of the acid monomeric species [A] can be calculated from equation (8) where $K_E$ and $K_C$ are unknown. In order to estimate these constants we shall, *at first*, consider only the data in which $[A]_0 \gg [S]_0$ and $[A]_0 \gg K_A^{-1}$. Then, the expression for acetic acid monomer simplifies to $[A] = \sqrt{[A]_0/2K_A}$, which gives equation (9)

$$[A]_0 = [A]\left(1 + \frac{[S]_0\,(K_E + K_C K_T)}{[(1 + K_E[A]) + K_T(1 + K_C[A])]}\right) + 2K_A[A]^2 \quad (8)$$

$$\frac{K_{ap} - K_T}{\sqrt{[A]_0}} = \frac{K_E}{\sqrt{2K_A}}\,(K_T' - K_{ap}) \quad (9)$$

from (7). Using an average tautomeric ratio $K_T = 0.07$ * and the previously measured [15] acetic acid dimerization constant $K_A = 4\,470$ l $mol^{-1}$, a plot of the quantity

* In the absence of acid, the tautomeric equilibrium constant slightly varies (Table) with substrate concentration. These variations, in order of magnitude of the experimental error, have little influence upon the forward correlations.

$(K_{ap} - K_T)/\sqrt{[A]_0}$ against $K_{ap}$ is linear. From a correlation of twelve experiments in the $10^{-4}$—$10^{-2}$M acid concentration range (correlation coefficient $r$ 0.994) $K_E$ and $K_T'$ can be estimated: $K_E$ 3 270 ± 400 l mol$^{-1}$, $K_T'$ = 0.388 ± 0.010. From equations (2), (4), and (5) we derive $K_C = K_T'K_E/K_T$, which leads to $K_C$ 17 900 ± 2 000 l mol$^{-1}$.

Returning to equation (8), it is now possible to calculate the acetic acid monomer concentration [A] exactly, even when the acid concentration is of the same magnitude as that of the pyridone. Then all the u.v. spectral data may be correlated by plotting $\log[(K_{ap} - K_T)/(K_T' - K_{ap})]$ against log ([A]) over the $10^{-6}$—$10^{-2}$M acid concentration range, using the previously determined $K_T'$ value.

Again a linear plot is obtained (Figure 2). If it is

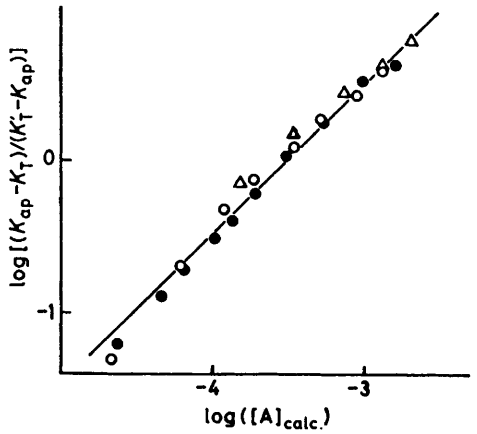



FIGURE 2 The stoicheiometric acetic acid–6-chloro-2-pyridone association is confirmed by the logarithmic plot of $(K_{ap} - K_T)/(K_T' - K_{ap})$ *versus* the ' true ' acid monomer concentration [A]. The tautomeric ratio $K_T'$ = [CA]/[EA] in the heterodimers and the ' true ' acid monomer concentration [A] were taken from other data. The solid line has a slope of unity as expected for 1 : 1 stoicheiometry. All data given in the Table are included. [6-Chloro-2-pyridone]: ●, 3.41 × $10^{-4}$M; ○, 3.2 × $10^{-5}$M; △, 8.42 × $10^{-6}$M; [acetic acid] 3 × $10^{-5}$—4 × $10^{-2}$M

assumed that the slope is unity, the intercept gives a new estimate for $K_E$ (3 420 ± 500 l mol$^{-1}$) in agreement with the previous one.

We therefore conclude that the effect of adding acetic acid to a solution of 6-chloro-2-pyridone in $CCl_4$ can be explained satisfactorily by specific association of this acid with the lactam and lactim tautomers.

## DISCUSSION

2-Quinolone was previously found to bind carboxylic acids [8] with an association constant of 19 000 l mol$^{-1}$ in $CCl_4$ at 297 K. This value is in good agreement with our $K_C$ estimations. Indeed, 2-quinolone is expected to be mainly in the lactam form. The association constant for the lactam is five times larger than for the lactim, thereby increasing the apparent lactam proportion. Entropy factors (rotation of the OH group of the lactim) or energy factors (relative strength of OH · · · N and O · · · HN hydrogen bonds) may account for the lower association constant of acid with the lactim tautomer.

In pure acetic acid,* the apparent tautomeric ratio is found to be much greater than $K_T'$. Such an observation, which cannot be explained by polarity effects, might result from a new type of pyridone–acid association, possibly in the form of open-chain polymers similar to those formed by pure acetic acid molecules.[14c]

*Conclusions.*—Since the heterodimers are cyclic, carboxylic acids should catalyse the tautomeric interconversion bifunctionally,[6a,7] thereby lowering the lifetime of the lactim forms. Such a result might be interesting for molecular biology, since double hydrogen-bond formation is probably one of the interactions involved in nucleic acid base recognition by proteins. 2-Hydroxypyridines are model compounds for hydroxypyrimidines (uracils and cytosines) and hydroxypurines (guanine, xanthine, and hypoxanthine) of biological importance. And indeed, 2-dimethylamino-6-hydroxy-9-methylpurine was found to associated with butyric acid with an equilibrium constant 660 l mol$^{-1}$ in $CHCl_3$ at 303 K.[9] This apparently low value is explained by the temperature effects and competition for hydrogen-bonding by solvent molecules.[16] Similarly, 1-cyclohexyluracil associates with butyric acid but with an equilibrium constant too low (80 l mol$^{-1}$) [9] for such an explanation. The lower acidity of the NH protons may account for this result and forthcoming investigation will try to elucidate this problem. Anyhow, these nucleic acid base–carboxylic acid interactions are expected to shift the tautomeric equilibria in favour of the lactam forms, the correct ones for Watson–Crick base pairing.[10]

## EXPERIMENTAL

2-Pyridone (Merck) was recrystallized from benzene, then sublimed in a vacuum, m.p. 108 °C. 6-Chloro-2-pyridone (Aldrich) was recrystallized from aqueous ethanol, m.p. 128 °C. Glacial acetic acid (Merck) and carbon tetrachloride (Baker Instra-Analysed) were used as such.

U.v. absorption spectra were recorded on a Cary 118 spectrophotometer, in 0.1, 1, or 10 cm path-length cells thermostatted at 293 K. The ratio between the volume of acid added and the total initial volume in the sample cell was always $< 0.005$ so that substrate dilution was negligible. I.r. absorption spectra were recorded at 313 K on a Perkin-Elmer 225 spectrophotometer fitted with 1 cm Infrasil cells (Hellma) for the hydroxy-stretching region. In the double bond region the optical path length was 0.5 mm ($CaF_2$ windows).

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* In glacial acetic acid the u.v. spectrum of 6-chloro-2-pyridone displays two bands at 287 and 305 nm with the ratio $A_{287} : A_{305}$ = 1.22.

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