

## Two-dimensional Intermolecular Heteronuclear $^{13}\text{C}$ , $^1\text{H}$ Overhauser Effect as a Probe for Hydration of the Amide Group

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Heteronuclear  $^{13}\text{C}$ ,  $^1\text{H}$  two-dimensional Overhauser effect spectroscopy (HOESY) furnishes a new method for studying intermolecular hydrogen bonding between water and the amide group as shown for 2-pyridones.

The two-dimensional nuclear Overhauser effect (NOE) between protons is now well recognized as a powerful tool for establishing the solution structure of proteins, oligosaccharides, oligonucleotides, and various other bio-organic or organo-metallic compounds.<sup>1</sup> Heteronuclear 2D Overhauser effect spectroscopy (HOESY) recently introduced by Rinaldi<sup>2</sup> and shortly afterwards by Yu and Levy<sup>3</sup> also appears to be a convenient and informative way of mapping the through-space interactions between protons and other nuclei; it has been extensively used for quantitative extraction of proton-carbon distances in small or medium-sized molecules.<sup>4</sup>

However, the full potential of HOESY for the investigation of intermolecular interactions, such as those between a solute and a solvent, has not yet been explored. Yu and Levy<sup>3</sup> have shown that the terminal phosphorus at ATP is the most accessible to water by using  $^{31}\text{P}$ ,  $^1\text{H}$  NOE (specific interactions). By detecting a large effect between  $^6\text{Li}$  and  $^1\text{H}$  nuclei, Schleyer *et al.*<sup>5</sup> were able to elucidate stereospecific lithiation and to demonstrate close lithium-hydrogen contacts ('agostic' interactions) in aromatic compounds. Finally, our group<sup>6</sup> has reported non-specific van der Waals interactions between the carbon atoms of  $\text{CS}_2$  or  $\text{CCl}_4$  as a solute in alkane solvents.

This communication describes the first application of an intermolecular carbon-13-proton HOESY 2D experiment to the study of the hydrogen bond between water and 2(1H)-pyridone (HP) or 1-methyl-2(1H)-pyridone (MP); these two compounds are known to be strongly solvated in hydroxylic solvents and are good models for pyrimidinic nucleic bases (a uracil-like moiety).<sup>7,8</sup>

It is crucial to choose the appropriate mixing time between the interacting spins to obtain HOESY cross peaks of significant intensity.<sup>4,6</sup> Optimisation of this time to enhance intermolecular cross peaks at the expense of intramolecular ones can be calculated according to the simplified two-spin AX theory of Macura and Ernst.<sup>9</sup> With the measured longitudinal relaxation times (Table 1), the following optimised  $t_{m,\text{opt}}$  mixing times are deduced: (a) intramolecular cross peaks between carbon atoms and their attached protons  $\approx 1.6$  s; (b) intermolecular cross peaks between all carbon atoms except those in carbonyl and methyl groups, and water protons  $\approx 3.8$  s; (c) intermolecular cross peaks between

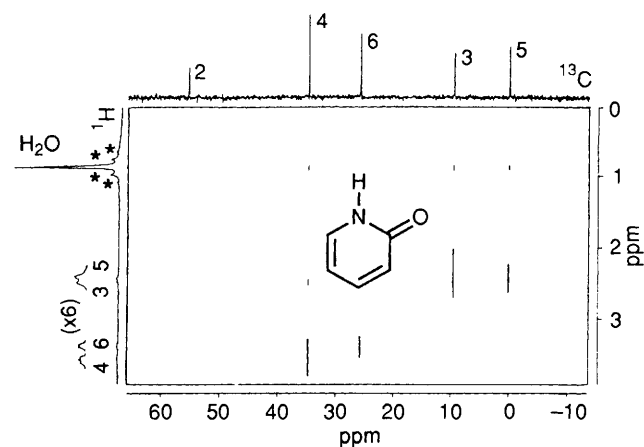
**Table 1.** Carbon-13 longitudinal relaxation times  $T_1$  for 2-pyridones ( $\sim 1$  M) in  $\text{H}_2\text{O}-\text{D}_2\text{O}$  (50 : 50) H at 300 K.<sup>a,b</sup>

	$T_1/\text{s}$					
	C-2	C-3	C-4	C-5	C-6	Me
HP	29.2	3.2	2.8	2.8	3.0	
MP	30.4	2.4	2.3	2.1	2.2	5.3

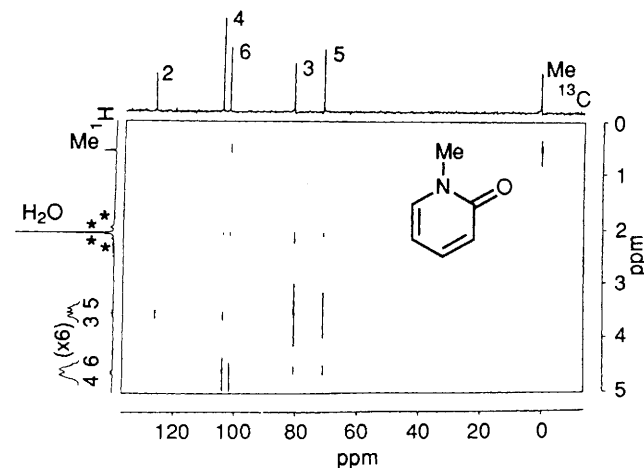
<sup>a</sup> Measured on non-degassed samples with a small amount of ethylenediaminetetra-acetic acid added to complex paramagnetic ions by the inversion-recovery method. <sup>b</sup> For the water protons of the solution, a common value  $T_1 \approx 4.0$  s has been measured.

carbonyl carbon atoms and water  $\approx 9.2$  s and between methyl carbon of MP and water 4.5 s.

Figure 1 shows a contour plot of the HOESY experiment for HP with a mixing time of 6.5 s [an average between the two optimised intermolecular mixing times (b) and (c)] while Figure 2 is the same experiment for MP with  $t_m = 4.2$  s (case c). Experimental recording conditions have been described elsewhere.<sup>6</sup> Although intramolecular one-bond and two-bond  $^{13}\text{C}$ ,  $^1\text{H}$  interactions are always present in the spectra, strong hydration of HP and MP is clearly demonstrated by the significant cross peaks between water protons and 2-pyridone



**Figure 1.** Contour plot (magnitude mode) of the 2D HOESY  $^{13}\text{C}$ ,  $^1\text{H}$  spectrum of 2(1H)-pyridone (HP) in water ( $\approx 1$  M) at 300 K (mixing time,  $t_m \approx 6.5$  s). Peaks marked with asterisks are spinning side bands on the  $\text{H}_2\text{O}$  resonance in the  $F_1$  ( $^1\text{H}$ ) dimension.



**Figure 2.** As Figure 1 for 1-methyl-2(1H)-pyridone (MP;  $\approx 1$  M;  $t_m$  4.5 s).

carbon atoms. Not only is hydrogen bonding (specific interactions) by two water molecules at the acceptor carbonyl group confirmed,<sup>7,8</sup> but also, above all, important non-specific 'agostic' interactions (close contact) at all the other carbon atoms, except HP C-6, are unambiguously revealed. Moreover, the important cross relaxation between water protons and methyl carbons (Figure 2) is also proof that the methyl group is not as hydrophobic as is sometimes stated. In contrast, the absence of a cross peak for the HP C-6 carbon may be explained by a very fast exchange on the correlation time scale between nearby water protons and the amide proton.

If a distance of 2.6 Å between the C-2 carbon atoms of HP or MP and the protons of the water hydrogen bonded to the carbonyl is assumed,<sup>10</sup> one can obtain a rough idea of the water organisation in the first hydration shell of 2-pyridones by comparing the magnitudes of the various cross peaks in Figures 1 and 2. This suggests that water slightly penetrates the van der Waals surface of 2-pyridones, so that a water proton is never more than about 2.5–2.7 Å from a carbon atom. It is likely that such a penetration results from an important electronic dispersion interaction between the negative  $\pi$ -cloud of the aromatic ring and the positively charged proton of water. In addition, the more intense cross peak between water and the MP C-3 carbon may be due to an easier

approach of water to the more negative carbonyl oxygen from the opposite side of the NMe group.

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