# Multinuclear NMR studies of the fluoride-uracil complex

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Abstract—<sup>1</sup>H, <sup>19</sup>F and <sup>15</sup>N NMR spectroscopy has been carried out on the soluble hydrogen bonded complex  $(CH_3CH_2)_4N^+F^-$  (uracil). The studies suggest that deprotonation of the uracil does not occur and that in a medium dielectric constant solvent, hydrogen bonding occurs at the N(1)–H site. Spectroscopic monitoring of the controlled thermal decomposition of the complex shows that complex breakdown occurs via the uracil anion, HF<sub>2</sub> and neutral uracil.

### INTRODUCTION

We recently reported i.r. spectroscopic evidence for a strong  $(N \dots H \dots F)^-$  hydrogen bond in the uracil-fluoride system[1]. Subsequently EMSLEY, JONES and OVERILL have performed ab initio calculations on the uracil-fluoride complex which confirm the presence of a remarkably strong  $(N \dots H \dots F)^{-}$  hydrogen bond [2]. The theoretical studies indicate that for an isolated uracil-fluoride system, the most thermodynamically stable arrangement has F<sup>-</sup> bonded to the N(1) NH group (I) whereas for a hydrated system, bonding to the N(3) NH group (II) is preferred (see Scheme 1). In biological systems the uracil ring is covalently bonded to the ribose ring through N(1) whereas the N(3)-H is involved in hydrogen bonding to the nitrogen ring atom of uracil's base-pair, adenine. It is therefore, of considerable importance not only to seek further experimental evidence to support the existence of a stable uracil-fluoride complex but also to attempt to experimentally locate the site at which the anion is hydrogen bonded. We wish to report preliminary results from our <sup>1</sup>H, <sup>19</sup>F and <sup>15</sup>N NMR studies of a novel soluble uracilfluoride complex which we believe go some way towards achieving these goals.

#### EXPERIMENTAL

<sup>1</sup>H NMR spectra were recorded on a Varian EM360A (60 MHz) spectrometer. <sup>15</sup>N and <sup>19</sup>F NMR spectra were



recorded on a Jeol FX 90 spectrometer operating at 9.12 and 84.67 MHz, respectively. All spectra were recorded using  $d_6$ -DMSO solutions and internal TMS (<sup>1</sup>H NMR), internal C<sub>6</sub>F<sub>6</sub> (<sup>19</sup>F NMR) or external anhydrous ammonia (<sup>15</sup>N NMR) as references. Thermogravimetric analyses were carried out on a Stanton Redcroft 750/770 TGA-DTG instrument using platinum crucibles. An Orion 507A Ionanalyser with fluoride selective electrode was used to determine fluoride concentrations.

# Preparation of (CH<sub>3</sub>CH<sub>2</sub>)<sub>4</sub>N<sup>+</sup> (F... uracil)<sup>-</sup>

An aqueous solution of tetraethylammonium fluoride (TEAF) was prepared by titration of a 20% aqueous solution of the hydroxide with 1 M HF to pH 7-8. The resulting solution was reduced to a small volume on a rotary evaporator at ca. 35°C. For the preparation of approximately 5g of the fluoride, about 25 cm<sup>3</sup> of acetonitrile was then added and the resulting solution evaporated on the rotary evaporator at 30-40°C. This was repeated six times to produce a white solid which analysed as the dihydrate,  $(C_2H_5)_4N^+F^-2H_2O_1$ . The dihydrate (1.85 g, 0.01 moles) was added to a solution of uracil (1.12 g, 0.01 moles) in 40 cm<sup>3</sup> DMF. This solution was reduced to a small volume of viscous liquid by evaporation on a rotary evaporator at 60-70°C. On cooling overnight, an off-white crystalline deposit formed which was washed with ether and dried for 12 h on a vacuum line. Attempts at recrystallization were largely unsuccessful. Quantitative analysis of the solid for Fconfirmed that the product was the 1:1 complex, TEAF.uracil.

# Preparation of 1-cyclohexyluracil

1-Cyclohexyluracil was prepared by the method of CHENG and LEWIS [3] with some slight modifications. The method involves the cyclization of N-cyclohexyl- $N(\beta$ -cyanoethyl) urea (derived from addition of cyclohexyl-amine to acrylonitrile and treatment with potassium cyanate) with subsequent bromination and dehydrobomination. The overall yield was less than 10%. Recrystallization from water gave a white solid, m.p. 216°C (CHENG and LEWIS [3]: 217-218°C).

## **RESULTS AND DISCUSSION**

Apart from the normal multiplet resonances of the ethyl groups in the cation, the 'H NMR spectrum of the TEAF. uracil complex in  $d_6$ -DMSO show peaks due to the CH (two doublets) and NH (one broad singlet) protons of the uracil. The timeaveraged NH resonance gives us no information on the site of H-bonding or on the strength of the H-bonding as the chemical shift is very sensitive to concentration, temperature and solvent. The chemical shifts and coupling constants of the uracil CH protons have been shown to be sensitive to deprotonation at nitrogen and as there is a real possibility of  $F^-$  acting as a base[4] and removing a proton from the ring, it is of interest to compare these parameters for the complex, the possible uracil anions [5] and uracil itself (Table 1).

The lack of correlation between the values in Table 1 for the complex and for uracil monoanions is striking. All of the uracil monoanions show their CH protons at significantly lower field than those in the neutral molecule whereas the <sup>1</sup>H NMR of the complex shows that the C(6)-H proton occurs in the same position as that in the neutral molecule while the C(5)-H proton occurs at appreciably higher field. These observations clearly show that the complex does not contain a uracil monoanion. We are not able to offer an explanation for the effects of strong H-bonding on the uracil CH protons but the observations do serve to demonstrate the significant differences that may exist between a strongly H-bonded complex of a neutral molecule (where negative charge density builds up on the electron acceptor atom of the neutral molecule) and the "fully" deprotonated molecule.

<sup>1</sup>H NMR monitoring of controlled decompositions of the complex were carried out in an attempt to gain more information about the the nature of the complex. Representative spectra obtained at particular points on the weight-temperature plot are shown in Fig. 1. The early weight loss at *ca*. 70°C is assumed to be due to loss of loosely bound water and/or volatile solvent. The intense peak at *ca*. 250°C is certainly due to decomposition and the <sup>1</sup>H NMR spectra suggest that this decomposition produces the uracil anion and neutral uracil as might be expected for a decomposition initiated by the breakage of a strong H-bond to uracil. The following qualitative decomposition mechanism is suggested TGA studies were run on the more stable KF.uracil complex (KF.HUr)[1] and these revealed a relatively stable  $K^+Ur^-$  species which undergoes a complex multi-stage decomposition process possibly involving  $K_2^+Ur^{2-}$ . Clearly the thermally unstable onium cation forces the relatively early decomposition of the uracil ion.

The <sup>15</sup>N NMR spectrum of uracil in d<sub>6</sub>-DMSO shows singals at 162.5 and 135.0 ppm whereas that of 1-cyclohexyluracil shows signals at 161.5 and 152.1 ppm. Thus the lower field resonance can be attributed to the N(3) nitrogen. The <sup>15</sup> NMR spectrum of the 1:1 TEAF.uracil complex in d<sub>6</sub>-DMSO shows well-resolved resonances at 164.5 and 66.9 ppm. The higher field resonance is due to the onium cation so that the N-1 resonance in the complex is not readily observable. As the nitrogens in uracil have a directly bonded proton it seems likely that they experience a large nuclear Overhauser enhancement and it is possible that if it is the N(1)-H which is bonded to  $F^-$ , the hydrogen bond may offer an alternative route for relaxation which effectively competes with the <sup>15</sup>N-<sup>1</sup>H dipole-dipole relaxation. An alternative explanation is that the N(1)-H bond increases in length when it is H-bonded and that this reduces the efficiency of the <sup>15</sup>N-<sup>1</sup>H dipole-dipole relaxation route. The latter explanation is supported by the theoretical calculations on the uracil $-F^-$  complex anion which predict a  $N(1)-H \dots F NH$  bond length of 1.59 Å[2]. This may be regarded as indirect evidence for association at N(1) but more convincing evidence may be obtained by running the <sup>15</sup>N NMR of a solution of the complex and uracil in the mole ratio 1:4. Here we might expect to observe a time-averaged N(1) signal due to N(1)- $H \dots F^-$  and  $N(1)H \dots$  solvent. The observed spectrum shows three well-resolved resonances at 163.2, 144.4 and 66.9 ppm. Thus we can reasonably argue that the  $F^-$  is indeed H-bonded to the N(1)-H (structure I) and that the chemical shift of the <sup>15</sup>N in that environment is significantly downfield



The decomposition of free uracil can be observed to occur at *ca.* 350°C (the normal decomposition temperature for uracil). The presence of  $HF_2^-$  during the decomposition can easily be monitored by i.r. spectroscopy which shows the characteristic bifluoride bands at *ca.* 2040, 1830 and 1230 cm<sup>-1</sup> growing into the spectrum at *ca.* 240°C. Parallel from that in uracil itself (or more correctly, uracil associated to the solvent DMSO). The downfield shift observed is not consistent with a simple increase in the local diamagnetic screening of the nitrogen brought about by the predicted[6] increase in the negative charge density on N(1) when it is H-bonded  $F^-$ . We assume that the observed

downfield shift is due largely to changes in the local paramagnetic screening at N(1) brought about by a distortion from spherical symmetry of the electron distribution around the nitrogen on strong H-bond formation. This effect is presumably large enough to overcome the (opposite) diamagnetic effect. The <sup>15</sup>N NMR chemical shifts are summarized in Table 2.

Whereas contributions to <sup>15</sup>N chemical shifts due to changes in the local diamagnetic screening

are important, the local paramagnetic screening term dominates the total nuclear screening for <sup>19</sup>F chemical shifts. It has been shown[7] that Hbonding to F<sup>-</sup> results in an often large downfield shift of the <sup>19</sup>F resonance in line with a distortion of the anion from spherical symmetry. The TEAF.uracil complex shows a single <sup>19</sup>F resonance at 21.3 ppm. downfield from C<sub>6</sub>F<sub>6</sub> some 133 ppm downfield from "free F<sup>-</sup>" and inside the strong H-bonding chemical shift range[8]. As

Table 1. Chemical shifts and coupling constants for the CH protons in uracil, TEAF.uracil and monoanions of uracil recorded in  $d_6$ -DMSO

	δC(5)- <u>H</u> /ppm	δC(6)- <u>H</u> /ppm	J <sub>HC</sub> (5)C(6)H/Hz
TEAF.Uracil	5.10	7.29	6.70
Uracil	5.37	7.29	7.40
Uracil <sup>-</sup>	5.70	7.57	6.80
1-Methyluracil	5.71	7.44	7.32
3-Methyluracil <sup>~</sup>	5.74	7.66	6.26



Fig. 1. First derivative TGA plot of TEAF.uracil: (a) showing positions at which samples were removed for <sup>1</sup>H NMR analysis (b) and (c).

Table 2. <sup>15</sup>N chemical shifts for uracil, 1-cyclohexyluracil and TEAF.uracil recorded in d<sub>6</sub>-DMSO

	δ <sub>N(1)</sub> /ppm	δ <sub>N(3)</sub> /ppm	Et <sub>4</sub> N <sup>+</sup> /ppm
Uracil	135.0	162.5	-
l-Cyclohexyluracil	152.1	161.5	-
TEAF-Uracil	-	164.5	66.9
TEAF-Uracil + Uracil (l:4 mole rat:	144.4 10)	163.2	66.9

diamagnetic contributions to the <sup>19</sup>F chemical shift can be assumed to be negligible, the observed downfield shift for the complex is a measure of the distortion of the electron distribution that occurs on H-bond formation between  $F^-$  and NH and indicates appreciable covalent character to the Hbond.

#### CONCLUSIONS

Multinuclear NMR studies on the  $F^-$ ... uracil complex confirm the presence of a strong (F ... H ... N)<sup>Th</sup> H-bond. The <sup>1</sup>H NMR characteristics of this complex are unique to the Hbonded species and are quite different to those of the parent uracil or its anions. Thermal decomposition of the complex occurs via the uracil anion and the bifluoride anion,  $HF_2^-$  as well as the neutral uracil molecule.<sup>15</sup>N NMR strongly suggests that in DMSO (i.e. a medium dielectric constant solvent) the  $F^-$  is H-bonded at the N-1 position of the uracil.<sup>15</sup>N and <sup>19</sup>F NMR chemical shifts of the complex suggest that the H-bond has appreciable covalent-character. Acknowledgements—Some of this work was carried out as part of the University of York's M.Sc. course in Chemistry and Chemical Education. One of us (J.S.T.) gratefully acknowledges the support of St Anthony's– Leweston School, Dorset and the Salters Trust. We are indebted to Mr. M. S. ROBERTSON for assistance with the thermal analytical work, Mr. M. S. WHITE for preparing TEAF·2H<sub>2</sub>O and Dr. J. EMSLEY and R. E. OVERILL for revealing the results of their theoretical calculations.

### REFERENCES

- [1] J. H. CLARK and J. SHERWOOD TAYLOR, J. Chem. Soc., Chem. Commun. 466 (1981).
- [2] J. EMSLEY, D. J. JONES and R. E. OVERILL, J. Chem. Soc., Chem. Commun. 476 (1982).
- [3] C. C. CHENG and R. L. LEWIS, J. Het. Chem. 1, 260 (1964).
- [4] J. H. CLARK, Chem. Rev. 9, 91 (1980).
- [5] R. STOLARSKI, M. REMIN and D. SHUGAR, Z. Naturforsch, C. Biosci 32C, 894 (1977).
- [6] J. EMSLEY, D. J. JONES and R. E. OVERILL, personal communication.
- [7] J. M. MILLER, R. K. KANIPPAYOOR and J. H. CLARK, J. Chem. Soc. Dalton, in press.
- [8] J. EMSLEY, D. J. JONES, J. M. MILLER, R. E. OVERILL and R. WADDILOVE, J. Am. Chem. Soc. 103, 24 (1981);
  J. H. CLARK, Can. J. Chem. 57, 1481 (1979).