CHAPTER 5

NMR SPECTROSCOPY

5.1 INTRODUCTION

As it is known, Nuclear Magnetic Resonance (NMR) spectroscopy can be incorporated and used in a wide variety of situations and for a wide variety of purposes. In terms of the study at hand there were three main purposes. Firstly, as a means of additional proof that the complexes did in fact form, secondly to study the stoichiometry of the complexes and hence prove that the thiourea and thallium were in a 4:1 ratio to each other, and
thirdly to note any shifting of peaks relative to the values reported in the literature which, would be an indication of hydrogen bonding.
With in the study both proton NMR and COSY NMR will be employed to ensure the correct assignments of the peaks.

5.2 GROUP 13 METALS AND NMR

Before discussing the NMR spectra of the complexes, of interest is how group 13, which of course includes thallium, and NMR can be related to biologically relevant aspects as well as those chemical aspects which include the results and interpretation to come.

The group 13 metals are not considered essential to life, however, they are NMR-active nuclides which can be used in the NMR analysis of biologically relevant systems [1]. For example, there are links between aluminium and Alzheimer's disease. Gallium, although possessing no real metabolic role, is present in human tissue. Thallium of course is of a poisonous nature due to the thallium ions having the ability to mimic alkali metal ions present in the body such as K^+. However, this ability also opens up the opportunity for Tl^+ to be used as a probe in biological system studies of K^+ and Na^+. Protein systems also take advantage of the ability of ^{205}Tl NMR to investigate metals in their particular binding sites [1].

In terms of these metals and their NMR properties, they are all quadropolar

(spin I > 1) except for thallium which has spin I = ½, which in general allows for good structure information to be obtained. Although they are not of a quadropolar nature, ^{203}Tl and ^{205}Tl do possess high receptivities [1].

5.3 ANALYSIS OF COMPLEXES

In all cases the crystalline material obtained (recrystallised) were used. The four complexes which produced single crystals as well as 2-hydroxy, 3-bromo and 4-chloro complexes (a representation of the remaining complexes) were run on the Bruker ARX 300 at 300.13 Mhz. The NMR spectra were recorded at 303 K unless stated otherwise and were referenced relative to the solvent used.

The solvent used for the four single crystal complexes was deuterated dimethyl sulphoxide (DMSO-d_6), while the 2-hydroxy, 3-bromo and 4-chloro complexes were dissolved in deuteriunoxide (D_2O), as these could not be dissolved satisfactorily in DMSO.

The complexes were analysed using proton NMR due to the significant number of different hydrogens in the complexes.

Before discussing the individual spectra, a reference diagram is shown below which has a labelling pattern which will be continually referred to in the following section.

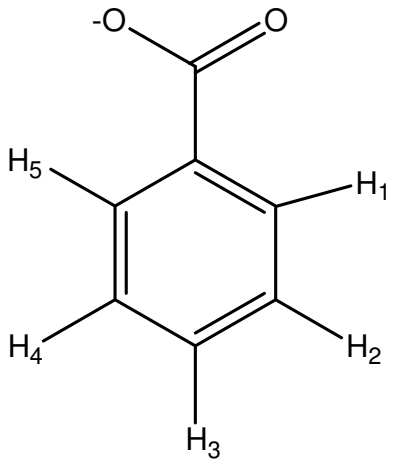

Fig. 5.1 Reference diagram showing the individual hydrogen numbers

It should also be noted that the discussion to follow is centred on the peaks relating to the hydrogen atoms attached to the benzene rings of the complexes, as the splitting patterns vary depending on the position of the substituent.

However, the spectra also show three other main peaks which are at constant positions in all the spectra, as could be expected. The first is the peak relating to the hydrogens of the thiourea molecules. It is a broad singlet peak (hydrogen bonded) at a chemical shift of $\delta = 7.04$ ppm. This is consistent with the literature value which is given at 7.05 ppm [2]. At a chemical shift of $\delta = 3.3$ ppm there is another peak which is present in all the spectra and is consistently at the same value. This broad peak relates to excess water from the synthesis which remained even after the drying of the complexes. Although it must be mentioned that every effort was made to dry the samples as full as possible under high vacuum to remove the majority of the water. Literature also gives a chemical shift value of $\delta = 3.3$ ppm [3].

The third and final consistently present peak is that of the solvent, deuterated DMSO-d_6 at chemical shift of $\delta = 2.49$ ppm [4].

5.4 ANALYSIS OF THE FOUR SINGLE CRYSTAL COMPLEXES

5.4.1 3-AMINO COMPLEX

In this particular spectrum the expected peaks are present, however, the benzoic hydrogens and the thiourea hydrogens resonance occur in the same region and so there is a definite overlap. In order to see the exact splitting pattern of the benzoic hydrogens a D_2O exchange was carried out. There is very little shifting of the peaks in the D_2O exchange spectrum compared to the DMSO spectrum. The chemical shift values given are from the D_2O exchange.

A multiplet with a chemical shift of $\delta = 7.07$ pm which represents H4 is visible, however, the splitting pattern is difficult to identify.

Slightly upfield is a doublet of triplets corresponding to H5 with a chemical shift of $\delta = 7.03$ pm. The coupling constants are as follows:

J (5, 1) 1.29 Hz J (5, 3) 1.29 Hz J (5, 4) 7.71 Hz

H1 is found at a chemical shift of δ = 6.95 ppm and is a triplet of doublets with the following coupling constants:

J (1, 3) 7.53 Hz J (1, 4) 0.39 Hz J (1, 5) 7.53 Hz

At a chemical shift of δ = 6.57 ppm H3 is the final multiplet which is a doublet of doublets of doublets and the 8 peaks are clearly visible. The coupling constants are as follows:

J (3,1) 2.37 Hz J (3, 4) 7.71 Hz J (3, 5) 1.29 Hz

When compared to the literature values of chemical shifts (H4, δ = 7.20 ppm, H5, δ = 7.17 ppm, H1, δ = 7.01 ppm, H3, δ = 6.74 ppm) [5] it is seen that the experimental values for the hydrogen atoms are slightly lower and could be an indication of hydrogen bonding.

A singlet at a chemical shift of 4.0 ppm is also seen which accounts for the hydrogens associated with the NH_2 substituent. It was not seen when the D_2O exchange was run as the hydrogens exchange.

An exact stoichiometric ratio between the thiourea hydrogens and the benzoic hydrogens can not be given as there is a certain amount of overlap, making the use of integration values meaningless.

A two-dimensional (1H-1H) homonuclear chemical shift correlation (COSY) experiment of 3-aminobenzoic acid was run to show the couplings.

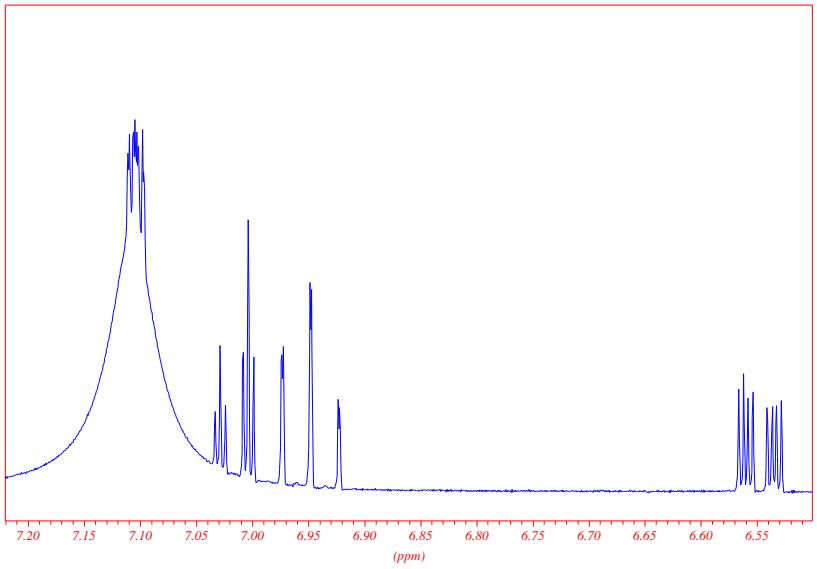

Fig. 5.2 NMR spectrum of the 3-amino complex dissolved in DMSO

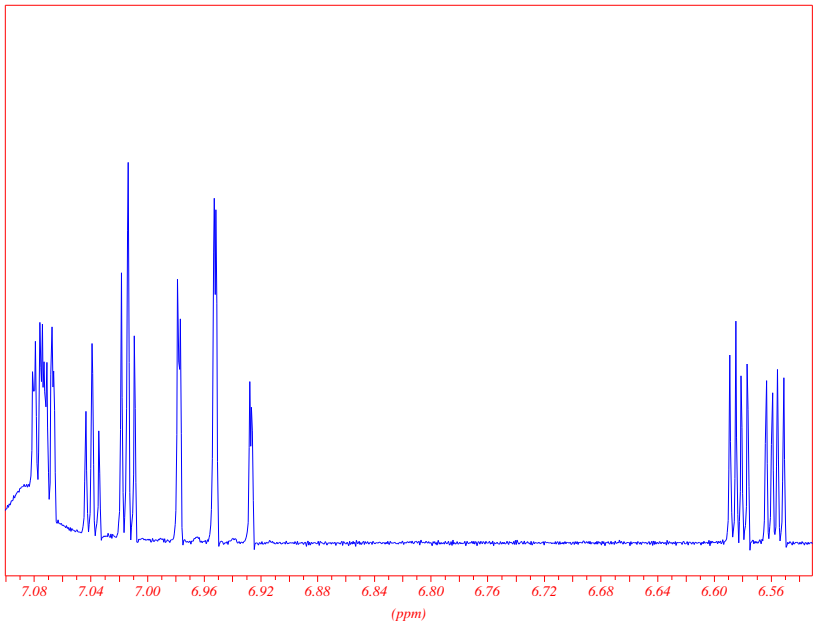

Fig. 5.3 NMR spectrum of the D_2O exchange carried out on the 3-amino complex

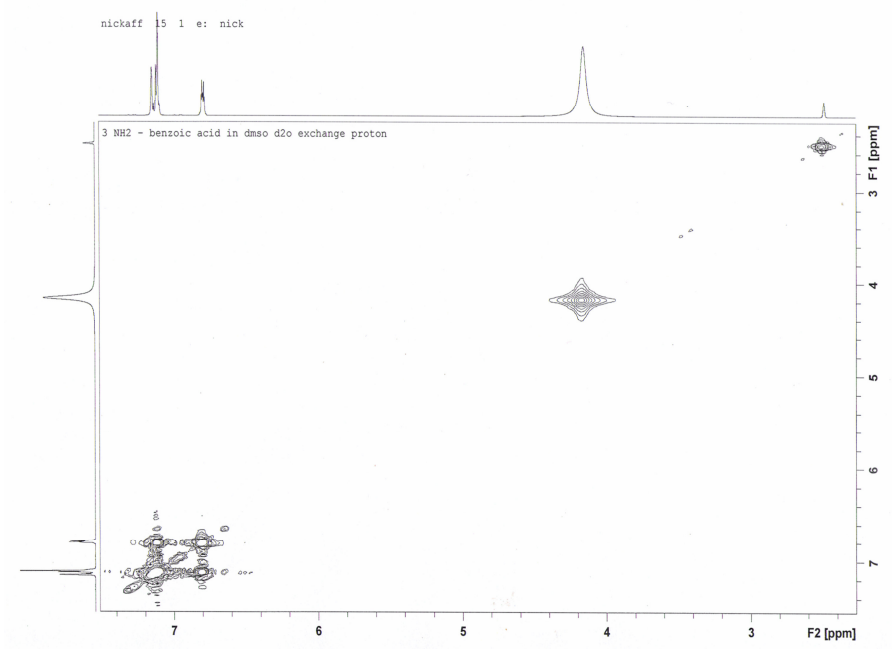

Fig. 5.4 COSY NMR of 3-aminobenzoic acid

5.4.2 2-FLUORO COMPLEX

As with the 3-amino complex, a D_2O exchange was necessary to identify the benzoic hydrogens. The spin = ½ of the fluorine can be seen to influence the spectrum to such an extent that the peak determination is not a simple matter. There is very little shifting of the peaks in the D_2O exchange spectrum compared to the DMSO spectrum. The chemical shift values given are from the D_2O exchange.

At a chemical shift of δ = 7.50 ppm H5 is the most deshielded hydrogen. It can be described as a triplet of doublets, however, coupling constants are difficult to assign.

The next multiplet at a chemical shift of δ = 7.28 ppm is H3. One would expect a doublet of doublets of doublets but the influence of the fluorine means that multiplet identification and coupling constants are not able to be determined.

Upfield to the H3 is a multiplet incorporating H4 and H2. The chemical shift of the multiplet is δ = 7.04 ppm. Unfortunately due to the influence of the fluorine it is not possible to determine exact chemical shift values for the respective hydrogens, nor obtain coupling constants.

The stoichiometric ratio is unable to be obtained as there is a large amount of overlap between the thiourea hydrogens and the benzoic hydrogens.

Note that a ^{19}F NMR was also run on the complex to prove the presence of fluorine as well as a COSY NMR of 2-fluorobenzoic acid to show the coupling.

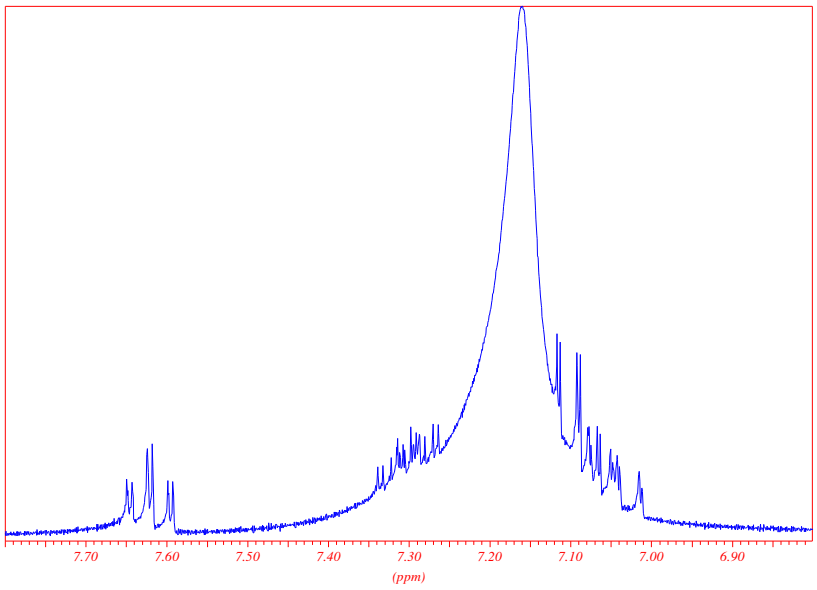

Fig. 5.5 NMR spectrum of the 2-fluoro complex dissolved in DMSO

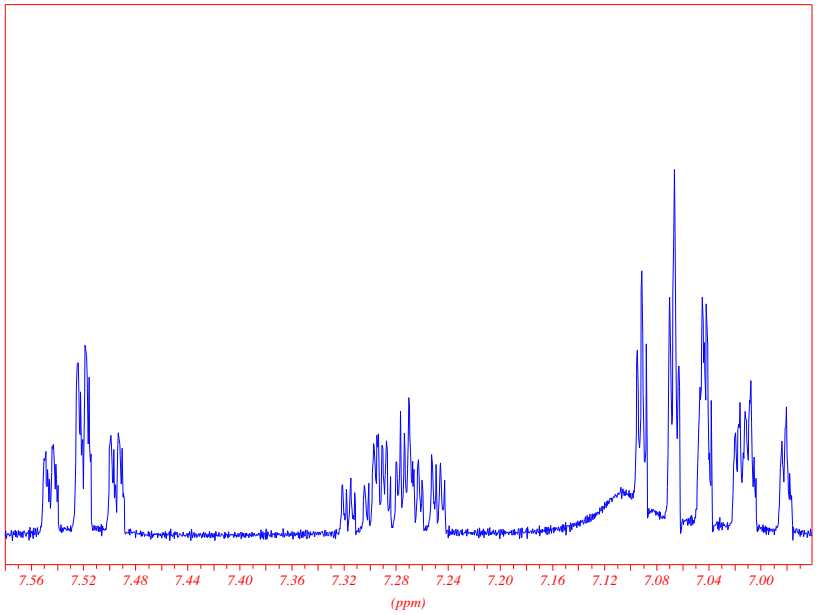

Fig. 5.6 **NMR spectrum of the D_2O exchange carried out on the 2-fluoro complex**

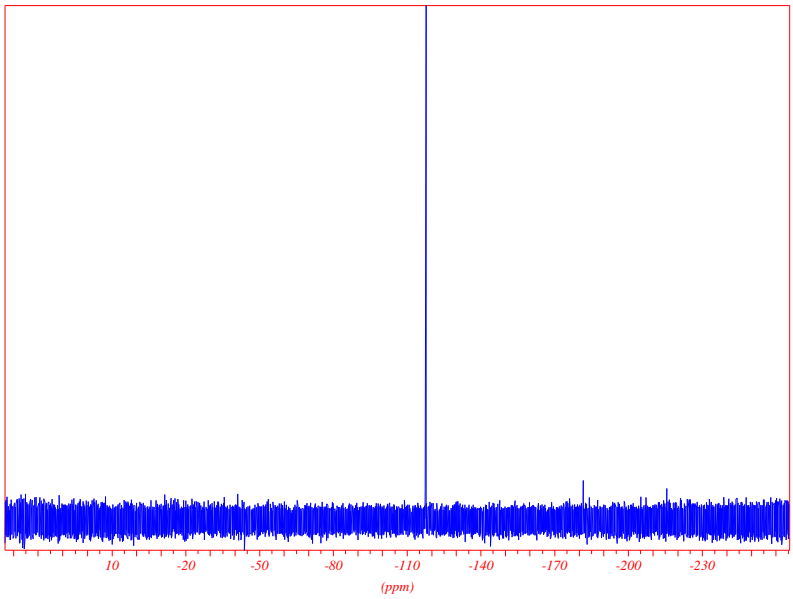

Fig. 5.7 **^{19}F NMR spectrum of the 2-fluoro complex**

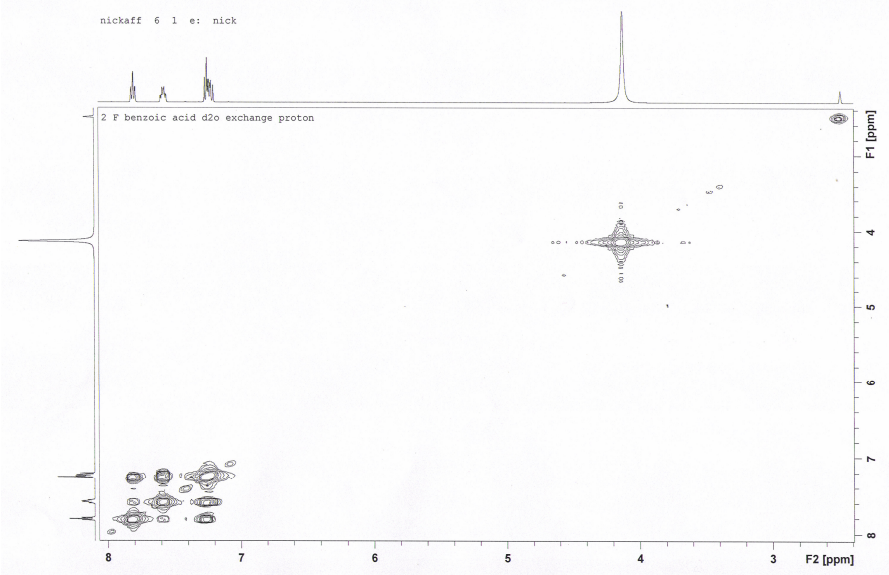

Fig. 5.8 COSY NMR of 2-fluorobenzoic acid

5.4.3 3-FLUORO COMPLEX

Once again the spin = ½ of the fluorine has an effect on the spectrum.

The H5 is represented by a doublet of triplets. The chemical shift is at a value of δ = 7.66 ppm. The fact that this hydrogen is represented by the peaks positioned the furthest downfield is to be expected as it is deshielded the most by the carboxylate group (COO^-).

The coupling constants are as follows,

J (5, 4) 4.59 Hz J (5, 3) 0.63 Hz J (5, 1) 0.66 Hz

At a chemical shift of 7.52 ppm is the second most deshielded hydrogen, H1. This hydrogen couples with the H5, the H3 as well as the fluorine atom. The eight expected peaks of the doublet of doublets of doublets are clearly identifiable.

Coupling constants are as follows,

J (1, 3) 1.74 Hz J (1, 4) 6.06 Hz J (1, 5) 0.84 Hz

The H4 is found at a chemical shift of δ = 7.36 ppm. The splitting pattern should be the same as that for the H1, doublet of doublets of doublets
(8 peaks), however, a certain amount of overlap has occurred and some of the peaks are not visible. This makes it difficult to determine coupling constants.

The fourth and final multiplet in this spectrum is that relating to the H3. In theory, due to its position on the ring, this hydrogen should couple with H5, H4, H1 as well as the fluorine. Therefore what should be seen is a doublet of doublets of doublets of doublets (12 peaks). Unfortunately no information can be obtained as this multiplet occurs in the same chemical shift region as the thiourea hydrogens and is involved in a large amount of overlapping. A D_2O exchange was carried out and the multiplet is indeed visible, however, the peaks are very broad and shifted upfield.

When compared to the literature values [5] of chemical shifts it is seen that the experimental values for the hydrogens are slightly lower. This may well be an indication of hydrogen bonding.

The stoichiometric ratio of the thiourea hydrogens to the benzoic hydrogens is 4:1, based on the integration values.

Note that a ^{19}F NMR was also run on the complex to prove the presence of fluorine as well as a COSY NMR of 3-fluorobenzoic acid to show the coupling.

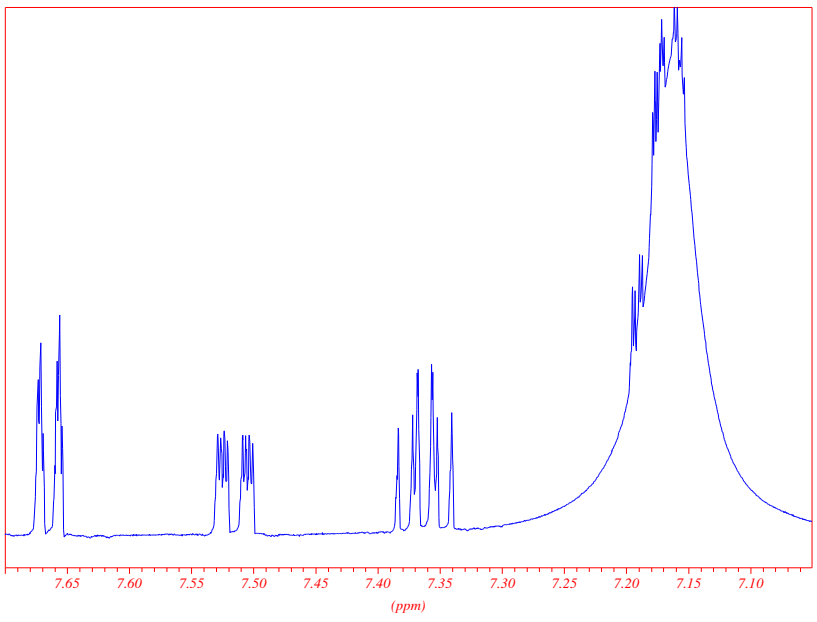

Fig. 5.9 NMR spectrum of the 3-fluoro complex dissolved in DMSO

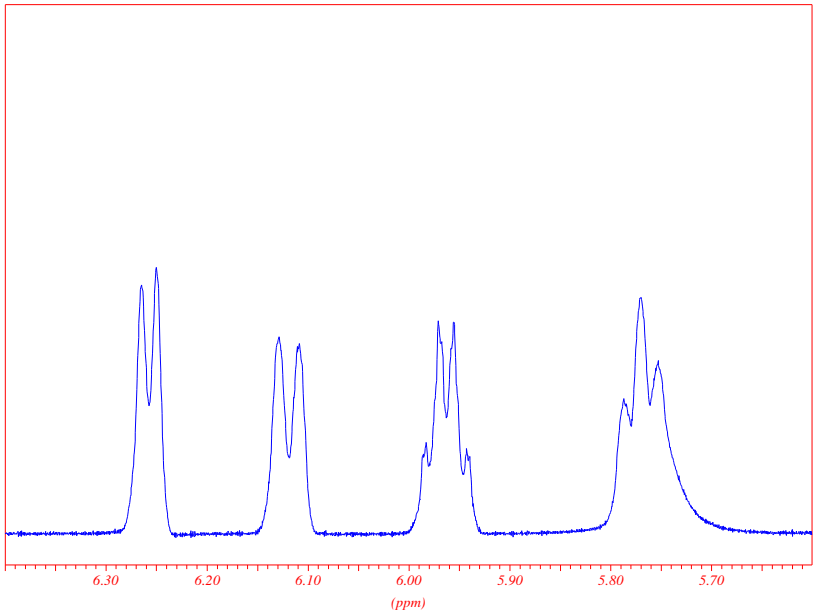

Fig. 5.10 **NMR spectrum of the D_2O exchange carried out on the 3-fluoro complex**

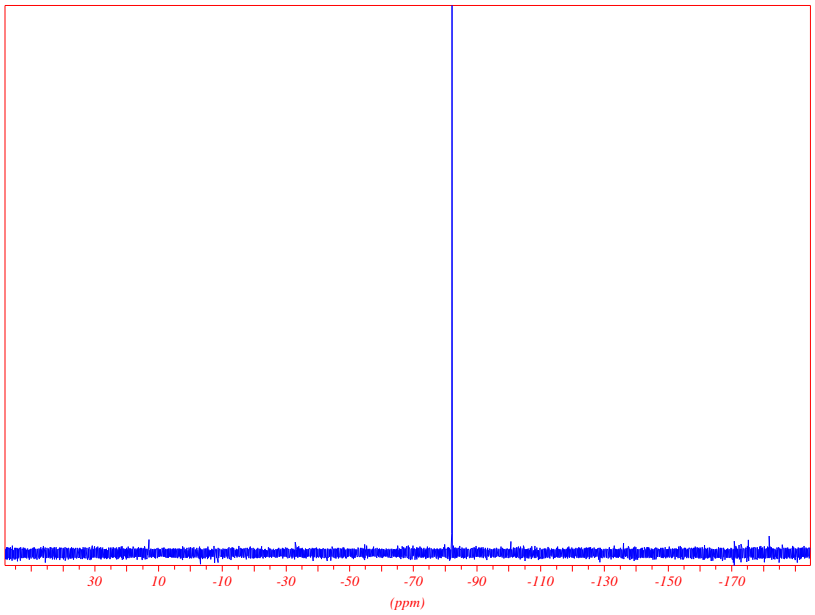

Fig. 5.11 **^{19}F NMR spectrum of the 3-fluoro complex**

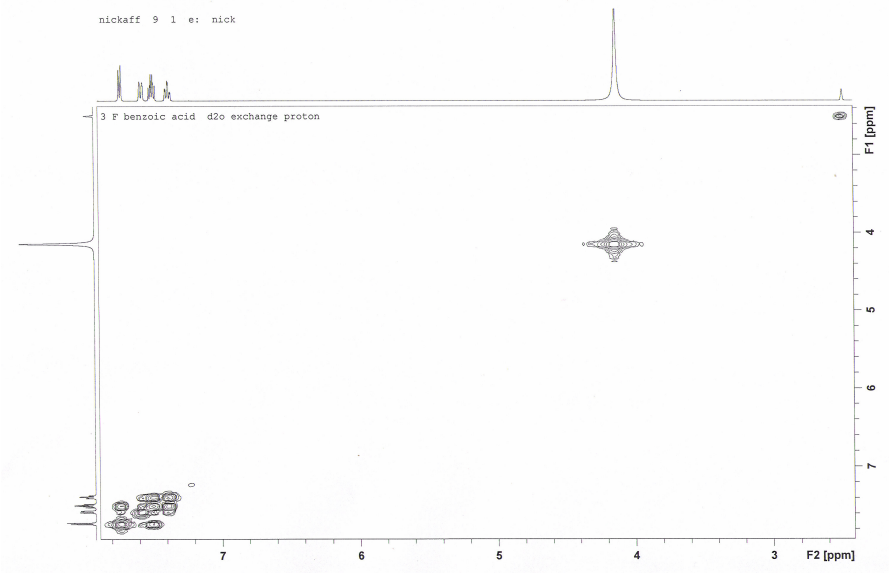

Fig. 5.12 COSY NMR of 3-fluorobenzoic acid

5.4.4 BENZOATE COMPLEX

Unlike the 3-amino and 2-fluoro complexes, D_2O exchange was not required to analyse the benzoic hydrogens.

As there is an element of symmetry to this complex two doublets should be seen representing H1, H5 and H2, H4.

The H1, H5 doublet can be seen at a chemical shift of δ = 7.82 ppm. However, further upfield the expected second doublet of H2, H4 at a chemical shift of δ = 7.28 ppm is not clearly identifiable due to overlap.

H3 has a chemical shift of δ = 7.32 ppm and should be a doublet of doublets but once again the effect of overlap has affected the ability to identify coupling constants.

Shifting of the H2, H3 and H4 indicate the possibility of hydrogen bonding.

The stoichiometric ratio of the thiourea hydrogens to the benzoic hydrogens is 4:1, based on the integration values.

A COSY NMR of benzoic acid was run to show the coupling.

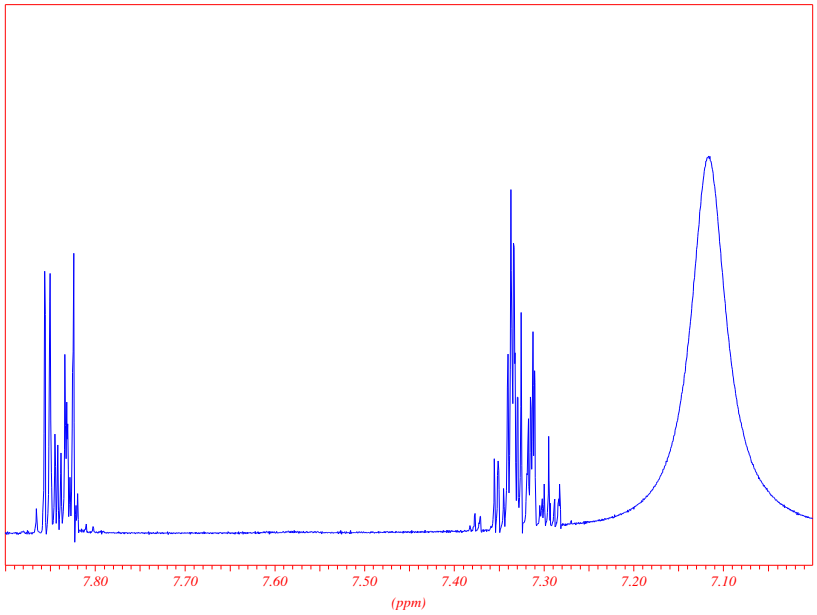

Fig. 5.13 **NMR spectrum of the benzoate complex dissolved in DMSO**

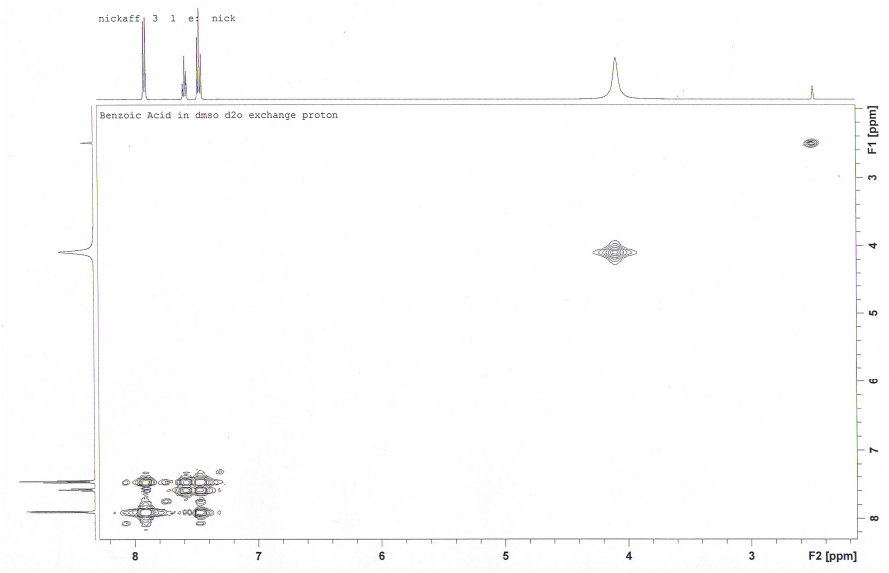

Fig. 5.14 **COSY NMR of benzoic acid**

5.5 ANALYSIS OF THE 2-HYDROXY, 3-BROMO, 4-CHLORO COMPLEXES

As has already been mentioned these three non single crystal quality complexes were analysed as a representation of all the other complexes to ensure the benzoic hydrogens were present.

The complexes were dissolved in D_2O but as they only remained soluble at 60˚C high temperature (60˚C) NMR was run. As a consequence the peaks in all three cases were very broad and therefore coupling constants were unable to be obtained. The chemical shifts were available but the increase in temperature shifted the values relative to the literature values [5].

COMPLEX	ACTUAL δ VALUES (ppm)	UNCOMPLEXED LITERATURE δ VALUES [5] (ppm)	Assignments
2-hydroxy	8.04	7.64	H5
	7.67	7.37	H3
	7.17 (merging)	7.01, 6.92	H4, H2
3-bromo	8.31	7.98	H1
	8.12	7.75	H5
	7.98	7.71	H3
	7.67	7.34	H4
4-chloro	8.13	7.75	H1, H5
	7.76	7.46	H2, H4

Table 5.1 **Table showing the actual and literature chemical shift values of the 2-hydroxy, 3-bromo and 4-chloro complexes**

The thiourea peak in each spectrum is not observed as the thiourea hydrogens exchange with the D_2O. This is also the reason why the hydroxy hydrogen is not seen.

To ensure it was in fact due to the temperature that the chemical shifts increased, the 3-bromo complex without thiourea was analysed as it dissolved readily in D_2O at room temperature. As expected the results showed chemical shift values similar to the literature [5].

COMPLEX	**ACTUAL δ VALUES**	**UNCOMPLEXED LITERATURE δ VALUES**	
	(ppm)	**(ppm)**	**Assignments**
3-bromo (no TU)	7.90	7.98	H1
	7.73	7.75	H5
	7.61	7.71	H3
	7.30	7.34	H4

Table 5.2 **Table showing the actual and literature chemical shift values for the 3-bromo complex without thiourea**

Although the peaks were more split than the 3-bromo complex with thiourea, there was still a large degree of merging which restricted the ability to obtain coupling constants.

5.6 CONCLUSION

The NMR results of all the complexes analysed show the presence of the benzoic hydrogens in the correct splitting patterns as well as the thiourea hydrogens and in the case of the 3-amino complex the NH_2 substituent hydrogens.

With respect to the four single crystal complexes the shifting of the peaks relative to the literature values was small, however, the fact that shifting did occur even in solution is further evidence of some hydrogen bonding, albeit in solution.

The stoichiometry of the benzoate and 3-fluoro complexes hydrogens relative to the thiourea hydrogens was 4:1 which was to be expected. The 2-fluoro and 3-amino complexes were assumed to have the same ratio but as was stated earlier in the chapter the ratios could not be identified due to overlapping of the thiourea hydrogens and the benzoic hydrogens, however, the correct 4:1 ratio was proved in the crystallographic study.

Overall, NMR provided this study with good results, results which further confirm that complexation of all the samples had taken place, as seen by the positioning and shifting of peaks.

5.7 REFERENCES

CHAPTER 5

1. J. P. Andre and H. R. Macke, Journal of Inorganic Biochemistry, 2003, **97**, Issue 4, 1, 2, 3, 4
2. S. Ahmad, A. A. Isab, W. Ashraf, Inorganic Chemistry Communications, 2002, **5**, Issue 10, 3
3. www.chem.ucla.edu/~webspectra/Notesonsolvents
4. www.eclipse.net/~numare/deutsolv
5. C. S. Chemdraw, Chemical Structure Drawing Standard, 1985-2000