# A $^1$H NMR Study of the Reaction of Adriamycin with Pd(II)

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The binding of metals to Adriamycin† has been studied in recent years because of the hope that the resulting complexes might be effective antitumour agents without Adriamycin's cardiotoxicity. Because of large perturbations in the visible spectrum it is known that Al(III), Mg(II) [1], Fe(II), Fe(III) [1, 2] and Cu(II) [3, 4] each bind to Adm in aqueous solution. The spectral changes indicate that these ions bind to the oxygen atoms at positions 11 and 12 or 5 and 6 or both. Recently a UV–Vis and $^1$H NMR spectroscopic study of the interaction of Adm with Yb(III) showed conclusively that this metal binds at the 11–12 site only, forming a 1:1 complex [5].

All of these metals are of the class referred to as 'hard' acceptors [6] and their preference for the oxygen sites on Adm is in keeping with their usual 'hard' behaviour. In this laboratory we have been exploring the possibility of preparing Adm complexes of 'soft' acceptors such as Ag(I), Hg(II), Pt(II), Pt(IV), Pd(II) etc. Although there is no truly soft donor site on Adm, it would be expected that these metals would interact less with the oxygen atoms and more with the 3′ amino group on the sugar ring. Binding at this site would go completely undetected by visible spectrophotometry because of its distance from the chromophore so we have been making extensive use of NMR in our work.

It was therefore with great interest that we read the report of Fiallo and Garnier-Suillerot [7] that Pd(II) reacts with Adm·HCl in a relatively simple way to form the complexes $(Adm)_2Pd$ and $(Adm–Pd)_2$. In each complex the 11–12 site as well as the 3′ amino group were postulated as binding sites because of the spectral changes and the measured release of protons.

NMR is arguably the best spectroscopic technique available today for the probing of chemical structures because of its ability to give information about individual atoms and their environments. One would expect that in the absence of any interferences that NMR would give unequivocal evidence of metal binding because of characteristic changes in the spectrum. We report herein a $^1$H NMR study of the reaction of Adm with $(NH_4)_2PdCl_4$ in both $H_2O$ and *N,N*-dimethylformamide (DMF) and give evidence that supports the finding of Fiallo and Garnier-Suillerot that Adm binds to Pd(II) at least at the 3′ amino site. We also show that the reaction may not be as simple as their interpretation of the visible spectrophotometric results might suggest.

## Experimental

Adriamycin was kindly supplied by Adria Laboratories as the clinical preparation containing 50 mg of lactose per 10 mg of Adm·HCl. It was purified using an extraction procedure developed in our laboratory§. Ammonium tetrachlorpaladate was purchased from Alfa Inorganics and used as received. Deuterated solvents ($D_2O$, 35% DCl in $D_2O$, DMF-$d_7$) were purchased from Merck, Sharpe and Dohme.

UV–Vis spectrophotometric measurements were made on a Perkin-Elmer Lambda 3 spectrophotometer. Measurements of the reaction mixture at $1.0 \times 10^{-2}$ M were made by diluting aliquots of the reaction mixture 100 fold with $H_2O$ and observing the spectrum. Those at $1.0 \times 10^{-3}$ M and $1.0 \times 10^{-4}$ M were made directly in 0.10 and 1.00 cm cells.

$^1$H NMR spectra were observed on a Bruker WH-400 Fourier transform NMR spectrometer operating at 400.13 MHz. The samples were contained in 5 mm tubes.

## Results and Discussion

### *Visible Spectrophotometry*

When Adm·HCl was reacted with $[PdCl_4]^{2-}$ in $H_2O$, each at a concentration of $1.0 \times 10^{-4}$ M, we obtained results identical with those reported by Fiallo and Garnier-Suillerot. The absorbance maximum at 530 nm due to the unperturbed chromophore of Adm decreased in intensity while new peaks at 520, 554 and 600 nm appeared. There was an isosbestic point at 545 nm.

The observation of $^1$H NMR spectra in $D_2O$ at such a low concentration is difficult because of interference from the large peak at 4.65 ppm due to residual HOD in the solvent. The reaction was repeated and followed by visible spectrophotometry

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†Adm is used to signify the free base specifically or Adriamycin generally. Adm·HCl signifies the hydrochloride.

§A manuscript describing this procedure is in preparation for publication. Details will be made available upon request to R.E.L.

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at concentrations of $1.0 \times 10^{-3}$ M and $1.0 \times 10^{-2}$ M in order to ensure that the reaction path did not depend upon the concentrations of the reagents. Identical results were obtained at each concentration except that the times required for completion of the reaction were significantly shorter than the reported 5 h at $10^{-4}$ M [7]. The times at $10^{-3}$ and $10^{-2}$ M were ~1½ h and 20 min, respectively.

*$^1H$ NMR*

The spectrum of Adm·HCl at $1.0 \times 10^{-2}$ M in $D_2O$ is shown in Fig. 1a. The addition of an equimolar amount of $[PdCl_4]^{2-}$ caused immediate broadening of the Adm resonances but little change was observed over the next 1/2 h although the solution went from orange to purple as expected. The line broadening appears to be due to chemical exchange and if so, indicates that the first reaction, probably coordination of the $NH_2$ group, is very fast. Identical results were obtained at $1.0 \times 10^{-3}$ M. Unfortunately, the region of the NMR spectrum of most interest, that containing the 3′ proton resonance, is very complex and is obscured by other resonances. This spectrum in $D_2O$ is therefore not very informative.

After several hours a purple precipitate formed in the $1.0 \times 10^{-2}$ solution which was filtered off and redissolved in DMSO-$d_6$. Its $^1H$ NMR spectrum is shown in Fig. 1c and indicates the presence of many species. It is likely that the glycosidic linkage is no longer intact because of the multitude of resonances in the 1′ and 5′-$CH_3$ regions. Whether this is the spectrum of the product that forms initially or is that of subsequent decomposition products is uncertain.

In order to help clarify this, attempts were made to release Adm from its proposed complex with Pd by the addition of DCl or KCN. If the reaction of Adm and $[PdCl_4]^{2-}$ is simply a complexation, then the addition of DCl would be expected to release free

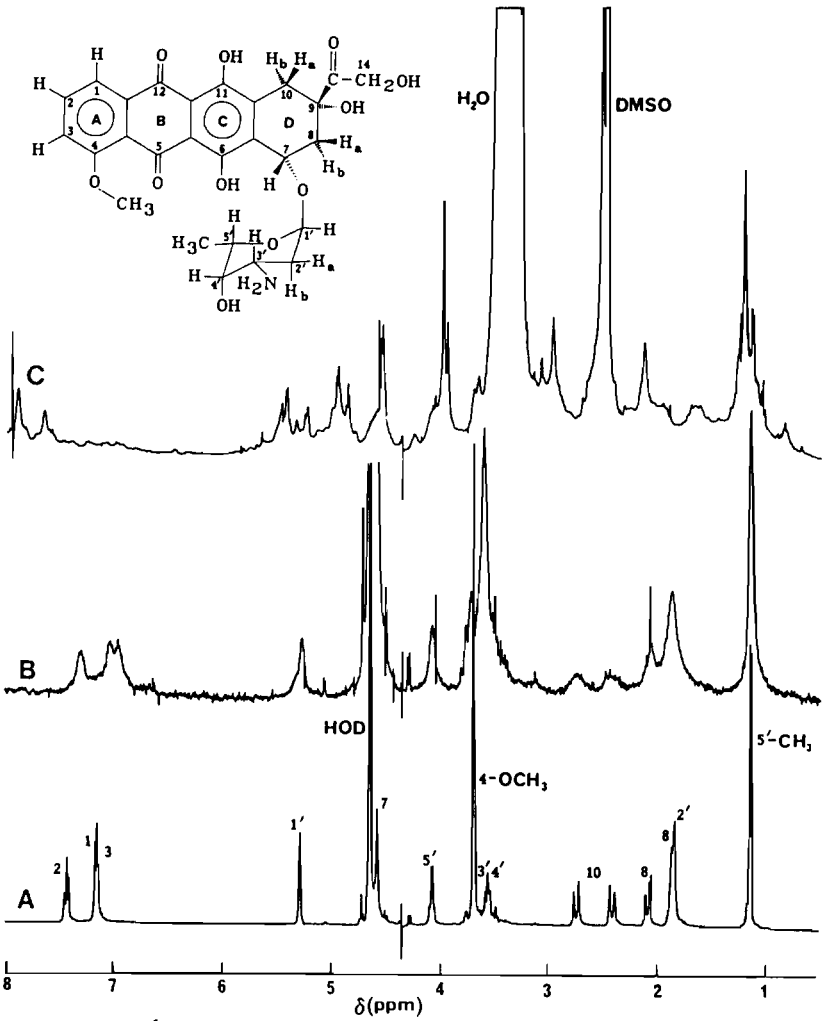


Fig. 1. (a) The $^1H$ NMR spectrum of Adm·HCl in $D_2O$ ($1.0 \times 10^{-2}$ M). (b) The same sample 2 min after the addition of 1 mol of $(NH_4)_2PdCl_4$. (c) The $^1H$ NMR spectrum of the precipitate formed by the reaction of Adm·HCl and $[PdCl_4]^{2-}$. The precipitate was redissolved in DMSO-$d_6$.

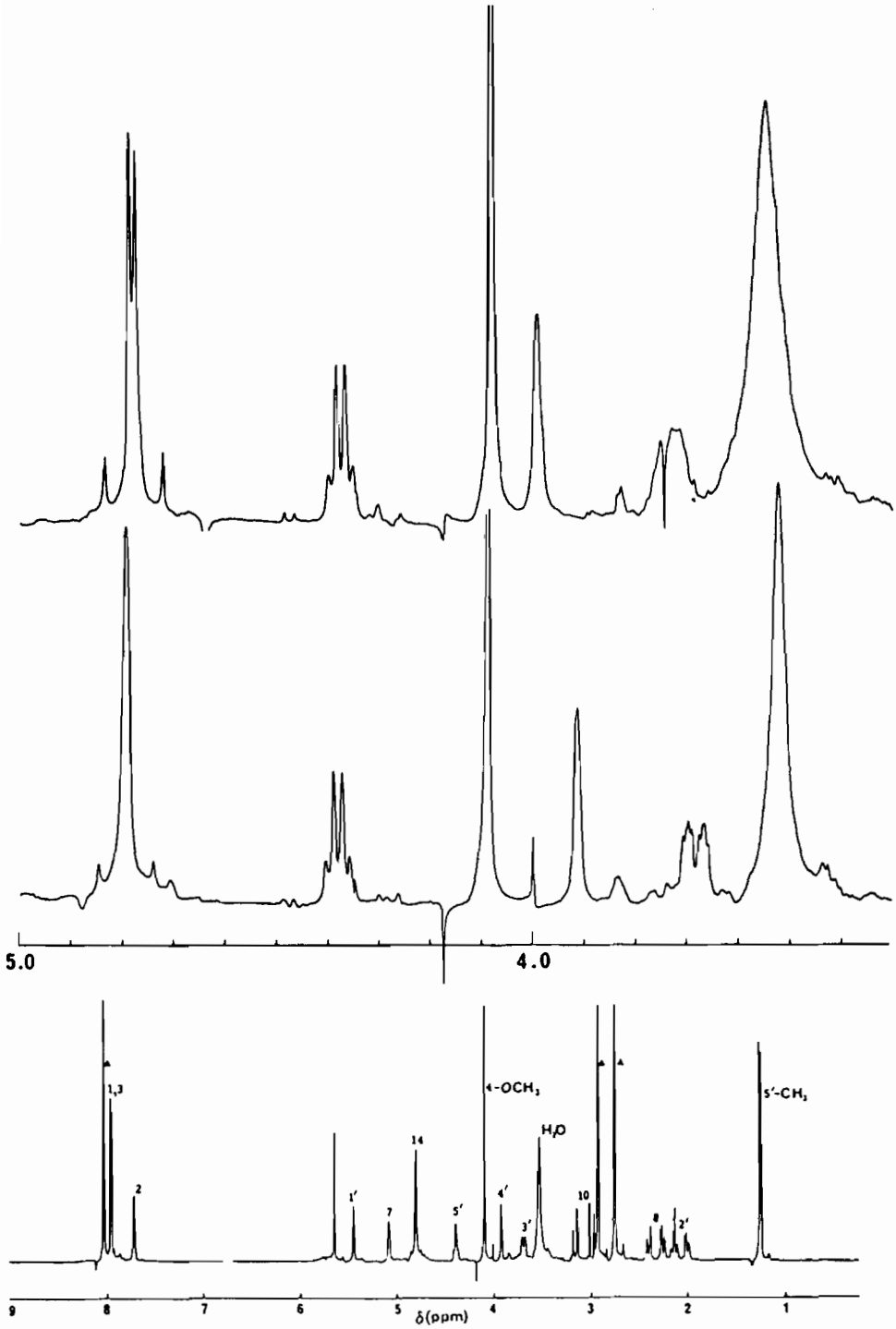


Fig. 2. Lower trace: The $^1H$ NMR spectrum of Adm·HCl in DMF-$d_7$. Centre trace: An expansion of the region containing the 3', 4' and 5' resonances. Upper trace: The same sample after the addition of 1 mol of $[PdCl_4]^{2-}$. Note the small downfield shifts of the 3' and 4' resonances. The resonances marked with triangles are due to residual $^1H$ in the solvent.

Adm by protonating the coordination sites. Fiallo and Garnier-Suillerot report a pH dependence for this reaction with virtually no complexation taking place below pH 2. A solution of Adm·HCl ($1.0 \times 10^{-2}$ M) in $D_2O$ was prepared and an equimolar amount of the Pd salt was added. After 1/2 h reaction time 10 $\mu$l of 35% DCl in $D_2O$ was added. The result was the immediate return of the orange colour but the $^1H$ NMR

spectrum indicated the presence of many species. Cyanide is a powerful ligand for Pd(II) and may be used to recover a ligand from its complex [8]. The addition of KCN to give a total concentration of $CN^-$ of 0.1 M caused some material to precipitate but the solution remained purple. Again the $^1H$ NMR spectrum showed that several species were present although the spectrum was not identical with the one observed after the addition of DCl.

Following this the reaction of Adm·HCl and $(NH_4)_2[PdCl_4]$ was repeated in DMF. DMF is a moderately basic solvent of high polarity and dielectric constant. It dissolves and solvolyses many inorganic salts and is a good solvent for Adm·HCl. When Adm·HCl and $[PdCl_4]^{2-}$ were reacted in DMF at $10^{-2}$ M there was no colour change nor was there any broadening of the Adm NMR spectrum. However, as shown in Fig. 2, there were small downfield shifts of the 3′ and 4′ protons but virtually no change in the spectrum otherwise. The most reasonable conclusion to be drawn is that there has been coordination of Adm to Pd at this site. Almost certainly the amino group has reacted but the shift of the 4′ proton suggests that chelation should not be ruled out.

Our experiments on the aqueous reaction point to its being more complex than suspected although they shed little light on its nature. The reaction involving the aromatic system is very much slower than one would expect if it were merely coordination of the 11–12 site to Pd [9]. The absence of this reaction in DMF, even though its more basic nature should promote deprotonation of the Adm, strongly suggests that simple coordination is not responsible for the colour change in $H_2O$. These observations, along with the inability of $D^+$ and $CN^-$ to release Adm from its 'complex' and the complexity of the spectrum of the precipitate lead us to believe that the colour change in aqueous solution is probably due to the decomposition of the Adriamycin rather than its further complexation to Pd(II) at the 11–12 site. Certainly the reaction is more complex than the report of Fiallo and Garnier-Suillerot suggests.


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