A STUDY OF THE BINDING OF MANGANESE (II) WITH THE SODIUM SALTS OF NUCLEOSIDES IN AQUEOUS SOLUTIONS BY THE ^{13}C NMR METHOD

É. L. Kupche and I. P. Sekatsis

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The binding of manganese(II) with nucleosides — adenosine (A), guanosine (G), cytidine (C), and uridine (U) — in an alkaline D_2O solution has been investigated by the ^{13}C NMR method. It has been established that the structure of the paramagnetic Mn(II)—nucleoside complexes differs substantially in neutral and in alkaline media. The broadening of the resonance lines (C-2', C-3' > C-1', C-4' > C-5') shows the localization of the Mn(II) in the C-2' and C-3' hydroxyls of the ribose in an alkaline medium. It has been shown for the case of U that the degree of complex-formation depends on the pH of the solution. It is assumed that the nucleoside forms intramolecular complexes (I) with $Mn(OH)_2$.

Manganese(II) ions, acting as catalysts in some enzymatic reactions with the participation of DNA [1-3], considerably affect the biological functions of living organisms. It is assumed [4] that the substrate in such reactions is a metal-nucleotide complex. NMR spectroscopy is being used successfully in the study of the structures of the complexes mentioned because of the high sensitivity and specificity of the broadening of the signals in dependence on the position of paramagnetic ions [5, 6].

The investigation of complexes of nucleotides and nucleosides in neutral and acid media by the PMR method [7-10] has shown that paramagnetic ions (Mn^{2+}, Ni^{2+}, Cu^{2+}) are bound to the bases of the nucleosides. The formation of complexes has been observed at metal ion concentrations of the order of 10^{-4}-10^{-3} M under conditions of rapid exchange. The phosphate group and the corresponding base take part in the formation of the nucleotide complexes. The use of ^{13}C NMR has made it possible to determine the positions of the paramagnetic ions in the nucleoside bases more accurately [11-14]. The disappearance of the broadening of the resonance signals of the bases in the PMR spectra of the Cu^{2+} complexes in an alkaline medium [8] is explained by the formation of copper hydroxide. The broadening of the ribose signals was not considered since the latter are masked by the signal of water.

In the present paper we give the results of investigations by the ^{13}C NMR method of the formation of complexes of manganese(II) with nucleosides — adenosine (A), guanosine (G), uridine (U), and cytidine (C) in an alkaline medium.

Figures 1 and 2 show the change in the spectra of G and U* with increasing concentration of Mn(II). The dependence of the broadening of the resonance lines of the nucleosides studied on the concentration of Mn(II) is shown in Fig. 3. The change in the half-width of the signals caused by interaction with Mn(II) was determined by the expression [12]:

$$\Delta\nu_{1/2}(\mathrm{Mn}) = \Delta\nu_{1/2}(\mathrm{obs.}) - \Delta\nu_{1/2}(0),$$

where $\Delta\nu_{1/2}(\mathrm{Mn})$ is the half-width of the signal caused by interaction with Mn(II); $\Delta\nu_{1/2}(\mathrm{obs.})$, observed half-width of the signal; and $\Delta\nu_{1/2}(0)$, half-width of the signal in the absence of Mn(II).

As can be seen from Fig. 3, for all nucleosides in an alkaline medium a broadening of the ribose signals is observed which begins at a concentration of Mn(II) of the order of 10^{-4} M. At the same time, the width of the signals of the bases scarcely changes even at

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*The investigations were carried out initially with the sodium salts of the nucleosides, and therefore the pH values of the solutions corresoond to the pH values of solutions of these salts.

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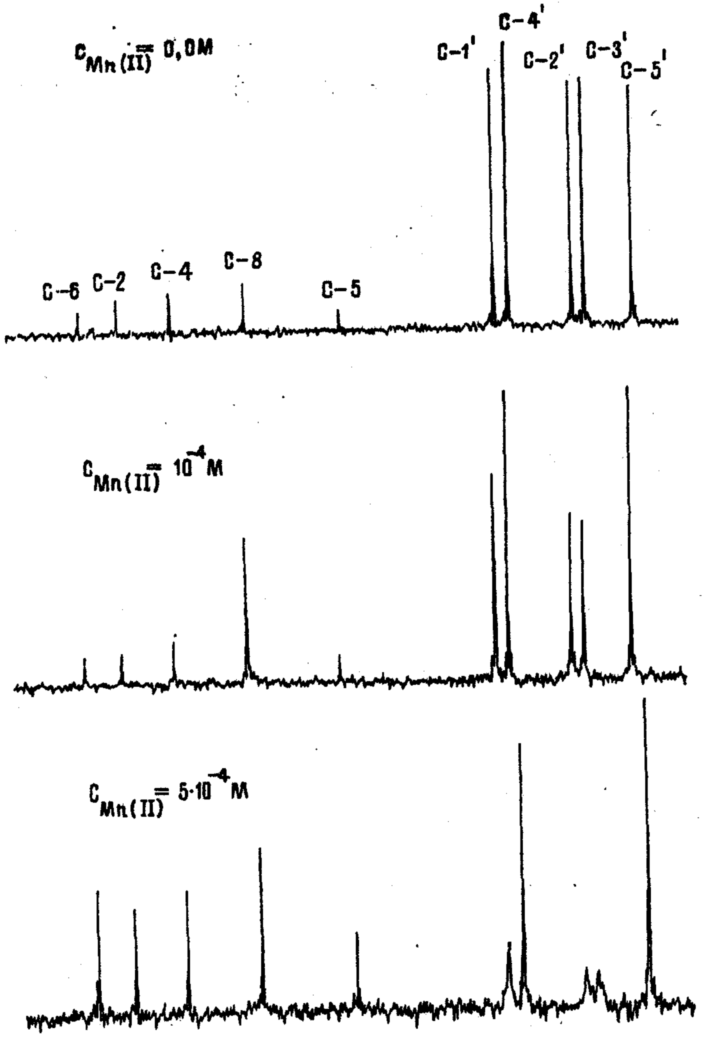


Fig. 1. Change in the ^{13}C spectrum of G with increasing concentration of Mn(II), pH 11.6.

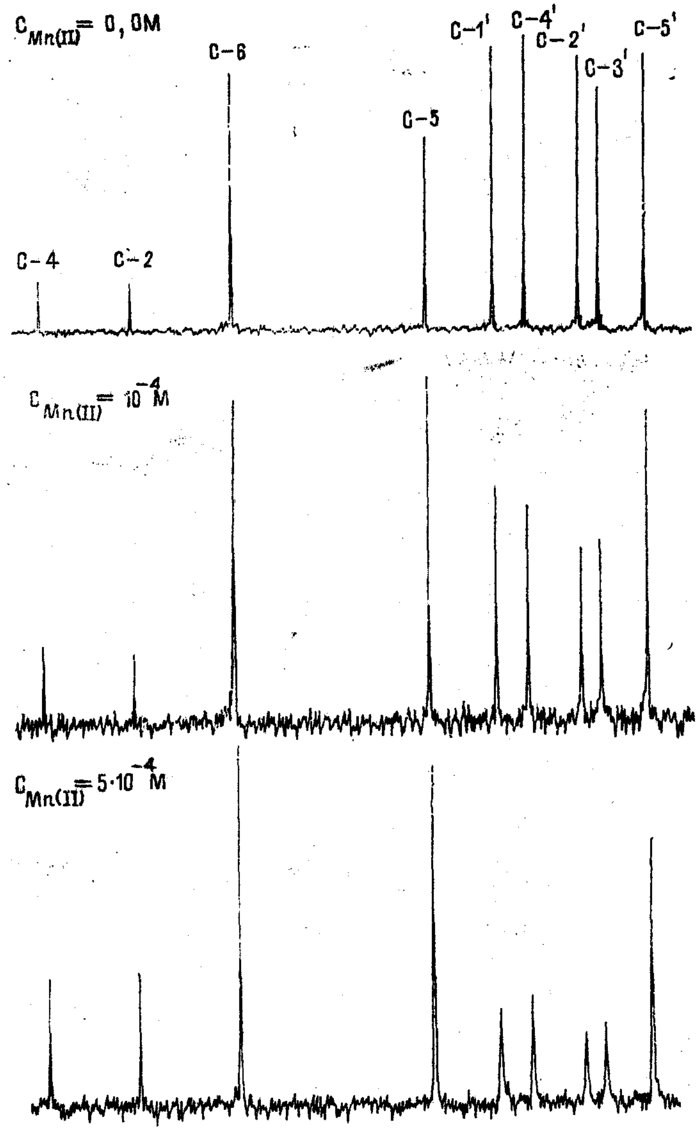


Fig. 2. Change in the ^{13}C spectrum of U with increasing concentration of Mn(II), pH 10.6.

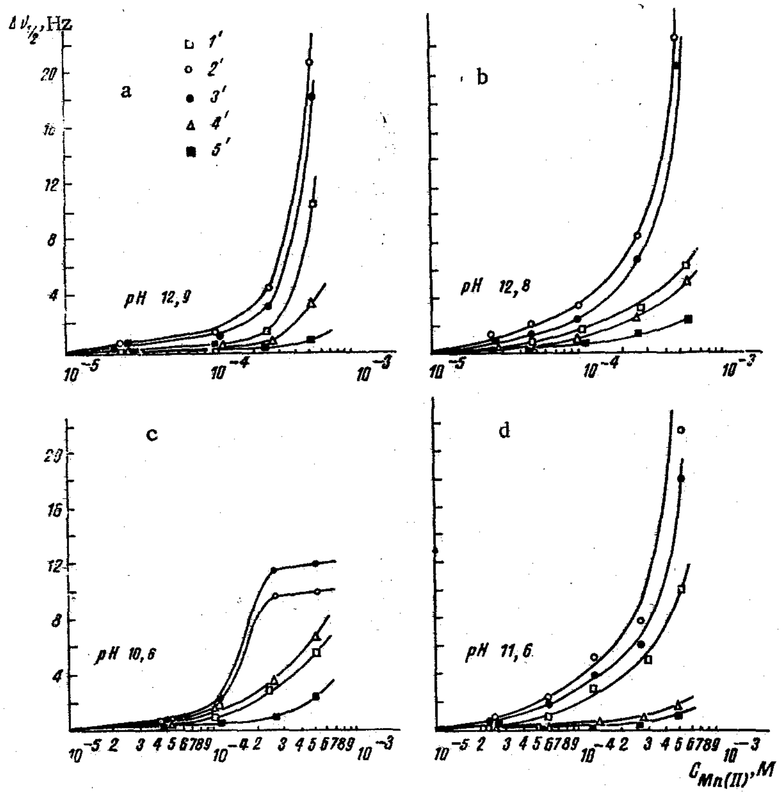


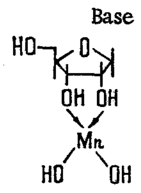
Fig. 3. Dependence of the width of the ^{13}C signals of A (a), C (b), U (c), and G (d) on the concentration of Mn(II).

concentrations of Mn(II) of 10^{-3} M and above. This shows the binding of Mn(II) with the ribose moiety of the nucleosides.

For A, G, C, and U the sequence of broadening of the spectral lines is as follows: C-2', C-3' > C-1', C-4' > C-5'. No change in the chemical shifts of the corresponding signals was observed. The results obtained correspond to the localization of Mn(II) in the C-2' and C-3' OH groups.

In the case of U, a broadening of the spectral lines is observed up to an Mn(II) concentration of $3 \cdot 10^{-4}$ M (Fig. 2). A subsequent increase in the concentration of Mn(II) to $5 \cdot 10^{-4}$ M and above caused no appreciable broadening of the signals. However, when the pH of a solution of U was raised, a further broadening of the signals of A, G, and C was observed.

The formation of the complexes apparently depends on the state of the Mn(II) in the system studied. Starting from $SP_{Mn(OH)_2}$ (SP = 10^{-14} g-ions^3/liter^3 [15]), an increase in the pH of the solution is accompanied by a sharp decrease in the concentration of Mn^{2+} ions. Since at pH > 9 no broadening of the ^{13}C signals of the base of the nucleosides is observed, we assume that the complexes with the nucleosides are formed by $Mn(OH)_2$, the solubility of which increases through complex-formation. The structure of these compounds can be represented in the form of the following complexes (I), in which the $Mn(OH)_2$ is bound coordinatively with the C-2' and C-3' hydroxy groups.



I

If the broadening of the ^{13}C NMR signals depends on the distance of the paramagnetic ions from the carbon nucleus, the half-width of the signal also depends on the conformational equilibrium of the ribose ring. This makes it possible to use these complexes in the conformational analysis of nucleosides.

EXPERIMENTAL

The ^{13}C NMR spectra were taken on a Bruker WH-90 instrument with a working frequency of 22.63 MHz. Water (D_2O) was used as the solvent, and tert-butanol as the internal standard, at a temperature of 30°C with the concentration of the nucleosides $\sim$0.5 M. The accuracy of the measurement of $\Delta\nu_{1/2}$ was ±1 Hz at $\Delta\nu_{1/2} \leqslant 10$ Hz and ±3 Hz at $\nu_{1/2} > 10$ Hz. The nucleoside salts were obtained by the method of Yoshihisa et al. [16].

SUMMARY

It has been established by the ^{13}C NMR method that in an alkaline medium the structure of Mn(II)—nucleoside complexes differs substantially from that of similar complexes in neutral and acid media. A hypothesis has been put forward concerning the structure of the complexes and the nature of the binding of the Mn(II) with the nucleosides under these conditions.

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