Complex Formation Between the Antiviral Nucleoside Ribavirin 
(1-β-D-Ribofuranosyl-1,2,4-Triazole-3-Carboxamide) 
and Bivalent Metal Ions: \(^1\)H and \(^{13}\)C NMR Studies, 
and Comparison with Inosine

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The interactions of metal chlorides (MeCl₂) of group IIA 
(Me = Mg, Ca, Sr, Ba) and group IIB (Me = Zn, Cd, Hg) with 
ribavirin, a potent broad spectrum antiviral nucleoside, and with 
its structural analogue inosine were studied in DMSO-d₆ using 
\(^1\)H and \(^{13}\)C NMR methods. Proton limiting shift analysis was 
made and \(^1\)H and \(^{13}\)C spin lattice relaxation times of ribavirin 
as a function of metal ion concentrations were determined. 
The nature of the interactions was determined on the basis of 
these data. The composition of complexes was determined using 
Job-plots as 1 : 1 and the formation constants of 1 : 1 Me\(^{2+}\)-ri­
бавирин and Me\(^{2+}\)-inosine complexes in equilibrium were 
evaluated either by concentration dependence or by a variable tem­
perature method.

INTRODUCTION

The in vivo role of metal ions in nucleic acid chemistry has long been 
recognized.\(^1\) Platinum(II) complexes with nucleosides or nucleotides have 
shown promising antitumour activity. The Pt(II) binding does not directly 
interfere at its site of coordination, namely, at N7 of guanine or adenine, 
with the hydrogen bonding sites of the bases, but markedly reduces hydrogen 
bonding between G and C, as well as affecting the selectivity for G and C 
base pairing.\(^2\) Since numerous nucleoside analogues have been synthesized 
as potential antiviral drugs in the hope that the drug may affect critical 
biochemical differences which exist in the replication of viruses and 
mammalian cells, we became interested in the role of metal ions in these 
systems.\(^3\) Shlomai\(^4\) et al. demonstrated that the synthesis of viral DNA is 
inhibited in the presence of 0.2 mM zinc sulfate in the infected cells, 
while DNA synthesis in uninfected cells is not affected. Zinc ions are 
required for the function of DNA and RNA polymerases as well. These facts

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prompted the study of the possible role of different metal ions on the inhibitory effect of ribavirin. The mode of action of this potent antiviral nucleoside with broad spectrum antiviral activity has been attributed to its inhibitory effect on the normal host cell enzyme IMP dehydrogenase (as 5'-phosphate), and consequently to its inhibition of RNA synthesis, rather than to a direct inhibition of protein synthesis itself. This competitive inhibition indicated the resemblance of ribavirin to its natural analogues guanosine and inosine. It is well known that at least three potential ligand-forming regions are possible on a nucleoside, one of them being a heterocyclic ring nitrogen atom and the other two functional groups.

Selectivity of coordination with a metal operates through the site at which the strongest bond is made. It depends primarily on the properties of the exocyclic group, which could either hinder coordination via the steric factor or stabilize coordination by the participation of favourable hydrogen bonding interactions.

The ribose moiety is one of these factors possessed by all derivatives but with different arrangements about the glycosidic bond and/or pseudorotational cycle.

Free rotation about the glycosidic bond is a crucial factor which allows the adoption of the necessary conformation during the interaction. When looking at the properties of ribavirin in solution, regarding its role in the virus replicative cycle, formation of a complex compound with the appropriate metal ion could differentiate between possible conformations necessary for distinct metabolic steps. Such an interaction may influence the hydrogen bonding capabilities of the carboxamide group. It is the objective of this study to find out if complexation really occurs, and an attempt was made to match the solution properties of ribavirin M2+ complexes to the same interactions of guanosine and inosine.

EXPERIMENTAL

Materials

A sample of ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) was obtained from Drs. R. K. Robins and J. T. Witkowski, ICN Pharmaceuticals; inosine was from Serva. The samples were dried at 70 °C/0.01–0.03 mmHg over P2O5 for more than four hours. Commercial grade HgCl2 was dried at room temperature (RT) over P2O5 at 0.03 mmHg. Commercial grade CaCl2, SrCl2 and BaCl2 were dried at 40 °C. Anhydrous MgCl2 was synthesized by thermally decomposing the magnesium-ammonium double salt, using MgCl2.6H2O as a starting material. Anhydrous ZnCl2 and CdCl2 were obtained by heating their hydrates in a quartz vessel filled with dry hydrochloric acid gas.

\(^1\text{H}\) and \(^{13}\text{C}\) Measurements

\(^1\text{H}\) NMR spectra in DMSO-d_6 (DMSO was dried over molecular sieves) at 21 ± 0.2 °C (RT) were obtained by a frequency sweep internal lock mode with a JEOL FS-100 spectrometer. The stated temperatures used in the variable temperature method are accurate to ± 1 °C. All samples were prepared in a dry box which was kept under a slight positive pressure of dry nitrogen. The frequencies were measured with respect to tetramethylsilane (TMS as internal standard to within ± 0.5 Hz. The concentration of nucleosides was 0.1 M in all samples.

\(^{13}\text{C}\) NMR spectra were determined with a JEOL 90 Q FX instrument using the internal deuterium lock mode. \(^{13}\text{C}\) chemical shifts were measured relative to the DMSO peak as internal standard. The data correspond to the \(^{13}\text{C}\) chemical
shifts measured with Me_4Si as an internal standard.

\(^1\)H_T_1_ and \(^1\)C_T_1_ relaxation time data were collected on the 90 Q-FX instrument at 24 ± 1 °C. T_1_ longitudinal relaxation times were obtained by a conventional 180°-90° pulse with an inversion-recovery sequence. The uncertainty in T_1_ measurement is about 10% (from several experiments) for both nuclei. Longer T_1_ relaxation times were determined by a saturation-recovery sequence (SR).

**Equilibrium Calculations**

### a. Concentration Dependence Method

The analysis of metal ion ligand equilibrium is based on 1:1 complex (MB) formation as confirmed by Job plot analysis. Then the equilibrium constant is given by the equation

\[
K^+ = \frac{(MB)}{(M_0 - (MB)) (B_0 - (MB))}
\]  

(1)

where M_0_ and B_0_ are the initial concentrations of metal ion and ligand respectively, and (MB) is the concentration of the complex.

Single resonances were observed in NMR spectra indicating rapid exchange between free and complexed ligand. Thus, the chemical shift \(\delta\) for the ligand is given by

\[
\delta = \frac{(MB)}{B_0} \delta_a + \frac{B_0 - (MB)}{B_0} \delta_c
\]  

(2)

where \(\delta_a\) and \(\delta_c\) are the chemical shifts of free and complexed ligand, respectively.

\[
(MB) = \frac{\delta - \delta_a}{\delta_a - \delta_c} B_0 = \frac{\Delta \delta}{\delta_c - \delta_a} B_0
\]  

(3)

Solving equation (3) and considering equation (1) we obtain

\[
\frac{M_0}{\Delta \delta} = \frac{1}{B_0 + M_0 - (MB)} + \frac{1}{K^+ \Delta \delta_c}
\]  

(4)

This equation can be solved iteratively for the values of \(K^+\) and \(\Delta \delta_c\) that give the best agreement between the observed and calculated shifts in the sense that they minimize the error square sum.\(^{13}\) These calculations were performed with locally written programs on a CDC-Cyber 172 computer.

### b. Variable Temperature Method

Raising the temperature can be thought of as the converse of adding an excess of one component as is done in the concentration dependence method, and in fact, we have observed chemical shift changes when raising the temperature of appropriate samples.

Eq. (1) is the governing equation, introducing \(p = \frac{\Delta \delta}{\Delta \delta_c}\), (5) where \(\Delta \delta\) represents the difference in chemical shift of a particular resonance in B at a given temperature in the presence of M and in the absence of M.

Therefore \((MB) = B\cdot P\), and from (1) and (3) and (5) we obtain

\[
K^* = \frac{P}{(1 - P) (M_0 - B_0 P)} = \frac{P}{(1 - P) B_0 \left(\frac{M_0}{B_0} - P\right)}
\]  

(6)

Since it is a real problem in using the correct value of \(\Delta \delta_c\) in this calculation, the effect of \(\delta \Delta \delta_c\) on the linearity of the log \(K^*\) vs \(T^{-1}\) plot with the temperature-shift data of H_5 from ligand-Me interaction was determined. The correlation coefficient (r) was used as the criterion and \(\Delta \delta\) used in the calculation is the one obtained at the maximum value of the correlation coefficient found by variation of \(\Delta \delta_c\) values. (The parabolic curve fitting was applied using a modified Ternai's computer program on a CDC Cyber 172 computer.\(^{17}\)
METHODS AND RESULTS

This study was undertaken in DMSO where the prospects of observing metal ion nucleoside complexes are enhanced since high concentrations of metal salts and nucleosides are achieved. The complexes between alkali earth metal species and nucleosides are expected to be weak as compared to nucleotides possessing a phosphate group. Nevertheless, the probability of observing such interactions with the base is increased when the phosphate group is missing. The structural features of ribavirin that appear to be necessary for antiviral activity are the carboxamide group, the 1, 2, 4-triazole ring and the ribose moiety. Preliminary line broadening results using CuCl₂ as a paramagnetic probe implicated nitrogen 4 (N⁺) as a possible binding site, which was later demonstrated as a coordination site in the solid state. These results encouraged further investigation of interactions with diamagnetic alkaline earth salts, namely, Mg, Ca, Sr, Ba chlorides of the IIa group and Zn, Cd, Hg chlorides of the IIb group.

The formation of a metal complex causes a decrease in magnetic shielding of the protons (NH, OH, NH₂) which are directly involved in coordination to the ions, and consequently a downfield shift. Smaller NMR effects are expected with aromatic ring proton resonances. These effect, expressed in downfield shifts are still detectable and have been observed by many authors and ascribed to extensive π-electron redistributions. H5 and amido protons of ribavirin, and H2 and H8 protons of inosine, show reasonable differences in chemical shifts with increasing concentrations of metal salts. The selectivity of the chemical shift differences was dependent on the nature of the metal salt added. This was not the case with H1', and/or changes of hydroxylic proton resonances attributed to anion binding to the ribose moiety. Minor differences in separate chemical shifts of amido protons were detected. We were aware that both cations and anions interact with nucleosides in a complicated way and that the observed limiting shifts are considered to be a superimposition of several effects of the two ions, especially when dealing with metal chlorides. Inosine is a case where weak hydrogen bonding between Cl⁻ and the NH proton at position 1 was postulated, and guanosine forms a strong hydrogen bonded interaction complex (I) with its NH and amino substituent.

The structure of I was suggested on the basis of proton chemical shifts (15s) and aromatic shortening of the 35Cl relaxation rate. Cytidine, on the other hand, does not possess a potent hydrogen binding site to Cl⁻. Disregarding the OH ribose sites, the carboxamide protons in ribavirin might have the potential to interact with Cl⁻. We have no reason to assign a charge reversed complex to this molecule.
Our explanation suggested that formation of such a chelate complex would certainly produce more selective shifts in individual NH resonances. Therefore the possibility existed that the method of $^1$H proton chemical shifts could be used to define the existence of the interaction. In addition, we were concerned with defining the possible positions of the individual binding sites and of obtaining insight into the relative stability of the complexes formed. Therefore the composition (molecularity) of the apparent complexes was determined and evaluation of the formation (stability) constants $K$ was attempted by limiting shifts analysis, and by the variable temperature method.

In addition, $^{13}$C chemical shifts and $^1$H, $^1$H$_T$, and $^{13}$CT, relaxation time data were collected to obtain sufficient information in support of proton chemical shift analysis. Self associations of ribavirin and inosine via hydrogen bonding or stacking interactions can be neglected under such conditions, since we used dilute solutions (0.05 M) of nucleosides. We were not able to observe characteristic $^{13}$C chemical shift dependence on the concentration of any carbon of our samples within experimental concentration limits.

The effects of the metal salts ZnCl$_2$, CdCl$_2$ and HgCl$_2$ on 0.1 M nucleoside chemical shifts with increasing concentration are given in Table I.

**TABLE I**

| The Limiting proton Shifts of Ribavirin, Inosine and Guanosine and the Equilibrium Constants for the Complexes of Ribavirin and Inosine with Cations, $K^+$, $K^*$, $K^*$, $M^{-1}$ |
|---|---|---|---|---|---|---|---|
| $u$, Hz | Mg | Ca | Sr | Ba | Zn | Cd | Hg |
| RIBAVIRIN | 5H | 12.6 | 14.6 | 18 | 15.6 | 10.6 | 13.6 | 14 |
| | NH$_a$ | 3.5 | 5.5 | 16 | 27.5 | 25.5 | 41.5 | 20 |
| | NH$_b$ | 7.9 | 9.1 | 16 | 31.9 | 19.9 | 34.9 | 17 |
| | l'H | 3.3 | 5.7 | 12 | 11.3 | 3.3 | 5.3 | 9 |
| $K^+$, M$_{-1}$ | 35.4 | 3.17 | 1.29 | 2.39 | 0.46 | 1.53 | 6.37 |
| $K^*$, M$_{-1}$ | 22.4 |
| INOSINE | 8H | 7.5 | 10 | 10 | 9 | 11 | 2 | 19 |
| | 2H | 1.3 | 6 | 8 | 10 | 3 | 1.5 | 13 |
| | l'H | 1.5 | 7 | 8 | 7 | 4.5 | 1 | 11 |
| $K^*$, M$_{-1}$ | 2.46* | 7.71 | 1.37 | 0.42* | 10.27 | 1.08 |
| $K^*$, M$_{-1}$ | 22.4 |

$^{*}$ Uncertain values.

The limiting shifts of ribavirin and inosine complexes with the appropriate metal ions are listed in Table I. It was noticed that the observed $^1$H chemical shift differences on complexation are smaller than those reported for cytidine, adenosine, guanosine etc., but the selectivity in $\Delta\delta$ between protons of the base (H$_5$ in s-triazole, H2 and H8 in inosine),

**LIMITING PROTON SHIFT ANALYSIS OF RIBAVIRIN AND INOSINE, AND THE NATURE OF THEIR INTERACTION WITH METAL IONS**
amino protons of the carboxamide group and sugar protons, suggested that the measured data could be further processed and analysed as metal-ion-nucleoside complexes in equilibrium.

A. Ribavirin-Metal Complexes

The changes in proton shielding caused by addition of various concentrations of metal salts in fact indicate the binding sites of the metals. The largest changes in the limiting shifts of H5, NHA (downfield) and NHB of two nonequivalent protons of the amide amino-groups and H1' of ribavirin upon interaction with the salts MgCl2, CaCl2, SrCl2 and BaCl2 show that two subgroups could be distinguished. Selectivity was found when one compared the amide proton resonances to the H5 and H1'. In fact the NHA proton shifts less than H5 (see Table I) and comparably to H1'. This data would suggest N4 as a binding site and a predominantly cationic interaction. 

The fact that NHB showed considerable downfield shift was attributed to the simultaneous coordination of Me2+ to N4 and an exocyclic oxygen atom of the carbonyl group in adduct II. Change of conformation with possible increase in the barrier height to rotation may occur at the same time. 

This fact was expressed and observed by the higher values of the coalescence temperature of amino signals, increasing by 5 °C from 55 °C in ribavirin to 60 °C in apparent Mg and Ca complexes. Simultaneous coordination to N4, O6 is preferred, even though theoretical studies have predicted priority to oxygen. Our data support simultaneous binding to both sites with different degrees of interaction, as suggested by Marzilli et al. BaCl2 and SrCl2 showed comparable shifts for all protons with the expected larger values for BaCl2. We consider that the absence of selectivity between amide protons confirms the weaker coordination to the exocyclic oxygen.

General agreement that HgCl2 binds strongly to endocyclic ring nitrogens of nucleosides in DMSO is expressed even in the case of ribavirin: the large shifts in H5 frequencies with respect to the small difference at H1 support this statement. Since large nonselective shifts are found for NH2 protons, a different model is plausible, namely, an N2 binding site with partial binding to the exocyclic nitrogen. This possibility was discarded on the basis of line broadening results and further investigations of 1H and 15N spin-lattice relaxation times. Therefore, N4 remains the only binding site, as suggested, and the large NH2-shifts are attributed to the different nature of the bonding of 2b group elements as
compared to the 2a group. Similar explanations could be applied to Cd$^{2+}$ and Zn$^{2+}$ interactions, ZnCl$_2$ showing a smaller influence on the ring proton. These results emphasize that a different type of binding is present according to whether complexation with a 2b or a 2a metal salt is involved. The formation (stability) constants are therefore comparable only within one series of ions.

B. Inosine-Metal Complexes

Unfortunately, we were not able to observe the resonance signal of the imino proton of inosine, and we consider the H$_5$ chemical shift to reflect the eventual interaction with the NH group. The results are listed in Table 1. Selectivity is observed with Mg and Ca at H$_8$ which shows larger shifts as compared to H$_4$ and H$_7$. No selectivity was observed with Sr and Ba. This means that Mg$^{2+}$ and Ca$^{2+}$ preferentially bind to N7 and/or exocyclic O$_5$. A more complicated situation is present with Sr and Ba. We cannot give a definite conclusion since a strong anionic (Cl$^-$) interaction is possible, as already reported.$^{15,16}$

An interesting situation is observed when comparing the soft Hg$^{2+}$ ion, with the Zn$^{2+}$ ion which has a known affinity for endocyclic nitrogens but is harder than Hg. The data for the Hg interaction show less selective differences between H$_4$ and H$_7$ frequencies than with Zn$^{2+}$. Thus the possible simultaneous binding of Zn$^{2+}$ to N$_7$ and O$_5$ agrees with its properties. Less selective differences do not exclude N$_7$ as an additional possible site of binding.

It was the aim of this study to compare the metal binding properties (binding sites, stability of complexes) of ribavirin as an antiviral synthetic nucleoside with its natural analogues, guanosine and inosine, and thus complete the picture regarding the stereochemistry of ribavirin metal adducts in solution, as compared with that of G and I. The first of the properties to be compared was the binding site, namely, at N4 and/or simultaneously to exocyclic nitrogen. These are the generally accepted binding sites for G and I.$^{13,15,16}$ The second property is the formation constant ($K$).$^{13,15,17}$ The order of stability and the values of $K$ have been reported elsewhere.$^{13,15,16}$ The order cytidine $>$ guanosine $>$ adenosine $>$ uridine was accepted, and our aim was to find the comparable position of ribavirin. Unfortunately, the values reported in the literature should be taken with reservations, especially when dealing with chlorides, since it is well established and accepted$^{16}$ that Cl$^-$ strongly interacts with guanosine. Nevertheless, ribavirin interactions were considered predominantly cationic and the calculations of $K$ were approached through the concentration dependance method$^{13}$ and the variable temperature method.$^{17}$

COMPOSITION OF THE COMPLEXES AND EVALUATION OF THEIR FORMATION (STABILITY) CONSTANTS

The values shown in Table 1 were calculated from H$_5$ frequencies for ribavirin and H$_8$ for inosine. 1:1 metal-ion-nucleoside complexation
was assumed by many authors. To ensure that our calculations were plausible and that we were dealing with 1:1 adducts, the Job method was applied to find the molecularity of association (Figure 1). The association was checked by HgCl₂, a soft metal ion able to bind to endocyclic nitrogens. The plots are symmetrical about the concentration ratio of 0.5, and thus the 1:1 ratio of ligands is proved.

The remarkable differences in values of $K^+$ comparing ribavirin —Mg++ ($K^+ = 35.4 \text{ M}^{-1}$) and ribavirin —Hg++ ($K^+ = 6.37 \text{ M}^{-1}$) with respect to inosine-Hg++ ($K^+ = 1.08 \text{ M}^{-1}$), (inosine-Mg++ values did not give reliable values of $K^+$) and similar values concerning Ca++ complexes, suggest selectivity in the strength of binding for different substrates and cations, the biological importance of which remains to be demonstrated.

Figure 1. Job plot for the interaction of HgCl₂ with ribavirin and inosine at 21°C.
One of the reasons for making the variable temperature measurements was to see if the changes in chemical shifts as a function of temperature were consistent with the proposed Me$^{2+}$-nucleoside models. Reallable data could even be used for the calculation of the formation constant $K$ and compared to the values already presented. We have mentioned before that the coalescence temperature of the amide protons NHA and NHB rose by $5\, ^\circ\text{C}$, suggesting formation of stable adducts. Selectivity of $\Delta T$ of various protons implied a different type of bonding of 2a and 2b metal ions. In support of these findings, $\Delta T$ — the difference in the appropriate frequency at a given temperature when the interaction is present and

![Figure 2. Variable temperature plot of the differences in chemical shifts of the mixtures of:](image)

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Graph</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) 0.1 M ribavirin 0.4 M HgCl$_2$ (x)</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td>(ii) 0.1 M ribavirin 0.4 M ZnCl$_2$ (o)</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td>(iii) 0.1 M inosine 0.4 M HgCl$_2$ (o)</td>
<td><img src="image" alt="Graph" /></td>
</tr>
</tbody>
</table>

that of the free nucleoside — is constant within the 2a series, while ZnCl$_2$ and HgCl$_2$ solutions (0.4 M Me$^{2+}$ — 0.1 M nucleoside) showed considerable differences, as displayed in Figure 2. Thus we could use the results of these last interactions for the calculation of formation constants by the temperature variation method. (This method was previously used for determination of $K$ for AMP-tryptophan interactions$^{15}$). We thought that this method could be generalized to our problem, and the values we obtained are as a rule higher than those using the concentration dependence method. The results obtained for ribavirin and inosine — HgCl$_2$ complexes are listed in Table I as $K^*$. 

![Diagram](image)
As already mentioned, we were not able to attribute unambiguously the interaction of MgCl₂ to N₄ only. We were aware that molecular motions and molecular structures of this kind have been investigated by spin-lattice relaxation rates (1/T₁) of ¹³C and ¹H of nucleosides, and more recently, by measuring ⁷Li on ³⁵Cl isotopes of the salt.¹⁴,¹⁵,¹⁶ Since diamagnetic ions generally produce much smaller NMR effects, except at NH and OH groups which are directly involved in ion coordination, a selective change of the shifts of amide protons with respect to other protons might be expected were any of the mentioned cations directly bonded to the exocyclic amido group. ¹³C and ¹H NMR relaxation data are listed in Tables II and III. The results of the measurement of ¹H and ¹³C, longitudinal relaxation times in ribavirin as a function of the concentration of bivalent metal ions showed that there were no considerable incremental changes in individual proton or carbon resonances. Longer relaxation times of carbons 3 and the exocyclic carbonyl correspond to the protonated form. The other carbons, ring carbon C₄ and sugar carbons C₁, C₂, C₃, and C₄′ have similar values within experimental error (±10%).

**TABLE II**

<table>
<thead>
<tr>
<th>M</th>
<th>¹H T₁ relaxation times in s ± 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (II)</td>
<td></td>
</tr>
<tr>
<td>Ca II/Ribavirin</td>
<td>⁵H</td>
</tr>
<tr>
<td>0.0</td>
<td>1.34</td>
</tr>
<tr>
<td>0.1</td>
<td>1.02</td>
</tr>
<tr>
<td>0.15</td>
<td>1.06</td>
</tr>
<tr>
<td>0.3</td>
<td>1.02</td>
</tr>
<tr>
<td>0.4</td>
<td>0.95</td>
</tr>
<tr>
<td>Mg (II)</td>
<td></td>
</tr>
<tr>
<td>Mg II/Ribavirin</td>
<td>⁴H</td>
</tr>
<tr>
<td>0.05</td>
<td>1.1</td>
</tr>
<tr>
<td>0.1</td>
<td>0.99</td>
</tr>
<tr>
<td>0.2</td>
<td>0.98</td>
</tr>
<tr>
<td>0.3</td>
<td>0.90</td>
</tr>
</tbody>
</table>

* Chemical shifts for carboxamide and hydroxy-protons as marked: a at lower field than b, and b — at lower than c.

The two protons at C₄′ relax this carbon to one half of the value of mono-protonated carbons, indicating dipole-dipole interactions as the predominant factors affecting the relaxation path. More information was expected from non-protonated carbons, where some other mechanism of relaxation with surrounding protons is weaker and consequently more sensitive to any conformational changes caused by complexation. Indeed, a certain influence on the relaxation rates of C₃ and C=O carbons was noticed (Table III) in the presence of Ca(II) and Hg(II) ions which corroborated the idea of simultaneous binding of metal ions on N₄ and O₇ or a possible conformational change of the carboxamide group due to the interaction. Selective incremental changes in relaxation rates re-
Regarding these two carbons were observed in the case of ribavirin interaction with Hg²⁺ ion. Unfortunately, the data were almost within experimental error limits (determined by the SR method) and we are not able to give a satisfactory explanation of this feature at present moment.

**TABLE III**

<table>
<thead>
<tr>
<th>Metal</th>
<th>T₁ Relaxation Times in s ± 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺</td>
<td>C₀, C₁, C₉, C₁₉, C₅’, C₆’</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>C₀, C₁, C₉, C₁₉, C₅’, C₆’</td>
</tr>
<tr>
<td>Hg²⁺</td>
<td>C₀, C₁, C₉, C₁₉, C₅’, C₆’</td>
</tr>
</tbody>
</table>

Nevertheless, assuming mainly dipolar relaxations, these minor changes could have an influence on proton relaxation. The binding of cations to the aminoprotons or N₂, if not otherwise affecting internal motions, would certainly hinder the rotation of the 5’CHOH substituent and thus influence ¹H relaxation rates of 5’protons. This is not the case even with HgCl₂, since the solution behaves in the same way as the others, supporting the conclusions made in the first section. Some minor selectivity observed in ¹H relaxation rates could be due to minor differences of preferential conformations about the glycosidic bond, and confirms that free rotation about the glycosidic bond is still present in these complexes. An additional argument is a small downfield shift (up to 0.1 ppm) of the H₅ proton signal of ribavirin and inosine on complexation with the metal ions used in this study.

Last but not least, the more covalent properties of Hg²⁺ and possibly Zn²⁺ adducts influenced the ¹³C NMR spectra by changing the carbon resonances close to coordination. We did not observe any reasonable change in carbon resonances of ribavirin with any of the interacting metal ions other than Hg²⁺, which caused a +0.70 ppm upfield shift of C₅, a +0.46 ppm shift of carbonyl frequency and a smaller downfield shift of -0.1 ppm at C₅.

**SUMMARY AND CONCLUSIONS**

binding sites inferred from ¹H, ¹³C chemical shift measurements, and ¹H and ¹³C T₁ spin-lattice relaxation times are given below.

f. Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺ bind simultaneously to the N₄ and exocyclic oxygen of ribavirin. A similar interaction is highly probable for inosine.
Hg$^{2+}$ shows more affinity for the endocyclic nitrogen N4 but its affinity for the exocyclic carboxamide group remains to be elucidated.

iii. $^1$H and $^{13}$CT, and variable temperature measurements support the above conclusions.

iv. The ribavirin Me$^{2+}$-complexes in DMSO appear to give stability values, as estimated by formation constants $K$, larger than inosine and comparable to guanosine.

v. Changes in the range of preferred conformations about the glycosidic bond and of the rigid carboxamide group caused by complexation were assumed.

The biological significance of these results remain to be elucidated. It the strong hydrogen bonding interaction of the postulated enzyme-ribavirin complex involving the carboxamide group is real, the solution studies with different metal ions in this study provide strong evidence that such interactions influence the stability of hydrogen bonding interactions and/or repulsive nonbonded interactions of ribavirin to the appropriate enzyme.

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REFERENCES

IZVLEČEK

Kompleksi antivirnega nukleozida ribavarina (1-β-D-ribofurazonil-1,2,4-triazol-3-karboksamid) z dvovalentnimi kovinskimi ioni: 1H in 13C NMR studija in primerjava z inozinom

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Študirali smo interakcijo kovinskih kloridov (MeCl2) skupine IIa (Me-Mg, Ca, Sr, Ba) in skupine III (Me — Zn, Cd, Hg) z ribavirinom, učinkovitim antivirnem, s programom DSSP in programom DAMMIF. Koncentracije ribavrina in kloridov so bili 10⁻⁴ M. Unikatna antivirna aktivnost, ki jo je imel ribavrin, je postal manj učinkovit pri dodavanju kloridov. Pri višjih koncentracijah kloridov je ribavrin postajal manj učinkovit.

Sestav komplexov smo došli z Jobovimi diagrami. Interpretacija rezultatov je bila učinkovita in je bila učinkovita za pravilno interpretacijo rezultatov.

Zadnjo metodo je bila koncentracije kloridov ali koncentracije ribavrina.