Nature of the Specific Interaction of Rhodium Acetate Dimer with Adenosine

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Rhodium acetate dimer $[Rh_2(Ac)_4(H_2O)_2]$ reacts specifically with adenosine and not with guanosine, cytidine, or uridine. NMR studies have been conducted in DMSO solution to elucidate the structural basis for this specificity. By the use of various analogues, it was shown that neither N-7 nor N-1 binding can account for the formation of the complex between the rhodium atoms and adenosine in DMSO solution [although the former has been demonstrated in crystals of 1-methyl adenosine with the dimer (from water and methanol)]. NMR line broadening of C-2, C-6, and C-8, but not of C-4 and C-5, is in line with π-bonding. A Hückel calculation demonstrates that the former atoms exhibit electron density maxima. The effects of various analogues of adenosine are also in line with π-bonding. Since guanosine is a poorer π-acceptor, its inability to react in the manner of adenosine can be explained. The rhodium acetate dimer, unlike the previously studied copper acetate dimer, does not react with the ribose hydroxyl groups of nucleosides. It therefore does not differentiate between ribo- and deoxynucleosides, but is a potential probe of nucleic acid structure by virtue of its base specificity.

Introduction

Reagents that are specific for one of the nucleoside bases are of great interest for their potential use in probing nucleic acid structure. The rhodium(II) acetate dimer $[Rh_2Ac_4(H_2O)_2]$, which we abbreviate RAD, appears to react specifically with adenosine and not with the other nucleosides.[1,2] In fact guanosine, cytidine, and uridine exhibit very little activity with RAD.[2] It had been shown previously that RAD reacts with poly dA, but not with poly dG.[1] The present work was carried out to elucidate the structure of the reaction product between the rhodium acetate dimer (RAD) and adenosine in solution and to understand the reason for the specificity of this reaction.

We had initiated the study of the interaction of nucleosides with RAD, because we had previously shown that copper(II) acetate dimer can differentiate between ribonucleosides and deoxynucleosides by binding selectively to ribonucleosides at their 2′ and 3′ OH groups, which are the same distance apart as the two Cu(II) atoms of the copper dimer.[3] The copper compound exists only in non-aqueous solution, and it was felt that the rhodium compound, being water-soluble, would prove more useful in differentiating ribo- and deoxynucleosides. However, RAD reacts with 2′-deoxyadenosine as well as with adenosine. Thus the reaction with Rh(II) is not with ribose hydroxyl groups. The structural studies indicate specificity for adenosine base, rather than ribose hydroxyls, and the nature of the bonding inferred from these studies represents a novel example of metal specificity through π-bonding. This π-bonding seems to require special conditions, since other types of bonding occur under other circumstances, as will be pointed out below.

Experimental Section

Rhodium acetate dimer (RAD) was purchased from Aldrich Chemical Co. Adenosine, guanosine, uridine, cytidine, 2′-deoxyadenosine, and tubercidin were from Sigma; 1-methyladenosine, N_6-methyladenosine, N_6,N_6-dimethyladenosine, nebularine, N_6-benzyl-adenosine, and 8-bromoadenosine were from Vega. Deuterated DMSO was from Diaprep, Inc.

All solutions for NMR studies were prepared by dissolving the nucleoside in deuterated DMSO, followed by addition of RAD with stirring. DMSO was used to increase the solubility of the complexes. ^{13}C NMR measurements were carried out in 10-mm-o. d. tubes on a Varian XL 200 NMR instrument locked on the deuterated DMSO. The ambient temperature was 20 °C. Proton broad-band coupling was used generally. The broadening data were obtained by subtracting the peak width of nucleoside solutions from that of nucleoside–RAD solutions. All spectra in a given figure were recorded on the same vertical scale.

For spectrophotometric measurements, we obtained difference spectra using a Cary 219 instrument, with a 1-cm optical path.

Results

Visible Absorption Studies and Equilibrium Considerations. The addition of rhodium(II) acetate dimer (RAD) to a DMSO solution of an adenosine compound results in the appearance of a pink color which darkens on increasing RAD concentration. There is a significant increase in the solubility of RAD in DMSO containing the adenosine moiety (from 5×10^{-3} M up to 0.1 M). The position of λ_{max} of the absorption peak in the visible region is dependent on the relative concentrations of the adenosine and RAD, indicating the presence of more than one complex. The complexes are presumed to be those containing one or two molecules of adenosine bound to the rhodium *dimer*. These will be referred to as 1:1 and 1:2 complexes, respectively; thus a 1:1 complex contains 1 adenosine and 2 rhodium atoms, a 1:2 complex 2 adenosines and 2 rhodiums.

Visible absorption spectra were obtained for solutions of adenosine and RAD, so that absorbances at different wavelengths could be used to determine stoichiometry and stability constants from the total adenosine and RAD concentrations.[4] The measured absorbance of a solution 0.2 M in adenosine and 0.02 M in RAD was fitted by a nonlinear least squares method with stability constants K_1 and K_2 as the free fitted parameters, for the binding of the first and second adenosine, forming the 1:1 and 1:2

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complexes. The constants K_1 and K_2 were evaluated to be 95 and 24 M^{-1}, respectively.

From these constants the percentage of 1:2 and 1:1 complexes in any solution can of course be calculated. For example, a solution with 0.2 M base and 0.025 M RAD contains 98.5% of the RAD molecules bound to adenosine, of which 21% are in a 1:1 complex and 79% in a 1:2 complex. For a solution of 4.60×10^{-3} M adenosine and RAD, 27% of the RAD molecules are bound to adenosine, with 92.6% in a 1:1 complex and 7.4% in a 1:2 complex. That the same NMR broadening pattern is obtained in both cases indicates that adenosine interacts with RAD in the same way in both 1:1 and 1:2 complexes.

NMR Studies. Evidence for the structure of these complexes comes from ^{13}C NMR. The resonance peaks in the spectra of these complexes are broadened as a function of the relative concentrations of the base and RAD. To characterize the nature of the complexes, the reaction of adenosine and RAD was compared by NMR to those of RAD with the following other nucleosides: 2′-deoxyadenosine, guanosine, uridine, cytidine, tubercidin (7-deazaadenosine); 1-methyladenosine, N_6-methyladenosine, N_6,N_6-dimethyladenosine, nebularine (purine riboside), N_6-benzyladenosine, and 8-bromoadenosine.

Specificity of the Reaction. ^{13}C NMR spectra of adenosine in the presence of various amounts of RAD are given in Figure 1. (^1H spectra were also obtained under similar conditions, but the lack of protons at critical positions in the molecule led only to generalized broadening, with no consequence for structural interpretations.) Peak assignments are marked on the spectra and are taken from refs 5–9. Clearly the resonances are broadened as a result of an exchange between adenosine and RAD molecules and the complex formed between them. The origin of the broadening will be discussed later, but we now note a pattern of the broadening in the ^{13}C NMR spectra that is common to all the compounds that interact with RAD. It is apparent from Figure 1 that the C_8, C_2, and C_6 resonances are the most broadened (14, 10, and 8 Hz respectively), while C_5 (2 Hz) and C_4 (1 Hz) are relatively undisturbed. For the ribose, the $C_{1'}$ resonance (closest to adenine) is most broadened (3 Hz). $C_{4'}$ and $C_{2'}$ are broadened to a lesser extent (2–1.5 Hz) and $C_{3'}$ and $C_{5'}$ (furthest from adenine) are practically undisturbed (0.5–0.1 Hz). An identical broadening pattern is observed in a benzylated derivative (Figures 2 and 3). Small changes in chemical shifts closely paralleled the broadening patterns, as shown in Table 1. Nebularine, which is a deaminated adenosine, shows similar chemical shifts, and can be made more concentrated, so that the observed chemical shifts are amplified, as also shown in Table 1.

Binding of the rhodium dimer to the 2′- and 3′-hydroxyl groups, in the manner of the copper dimer, should result in specific broadening of the 2′- and 3′-carbon resonances. Our results indicate that this type of complex is not produced with rhodium. Further evidence against such a complex comes from the fact that 2′-deoxyadenosine experienced specific broadening of the same peaks as adenosine, indicating that the same type of complex is produced in the presence or absence of the 2′-OH group.

To further examine the specificity of the interaction, the ^{13}C NMR spectrum of adenosine was also compared with those of guanosine, cytidine, and uridine. No broadening occurred with any of these nucleosides, in line with the failure of these compounds to produce a pink color when added to RAD. Thus we conclude that, in the concentration range we have studied, the reaction

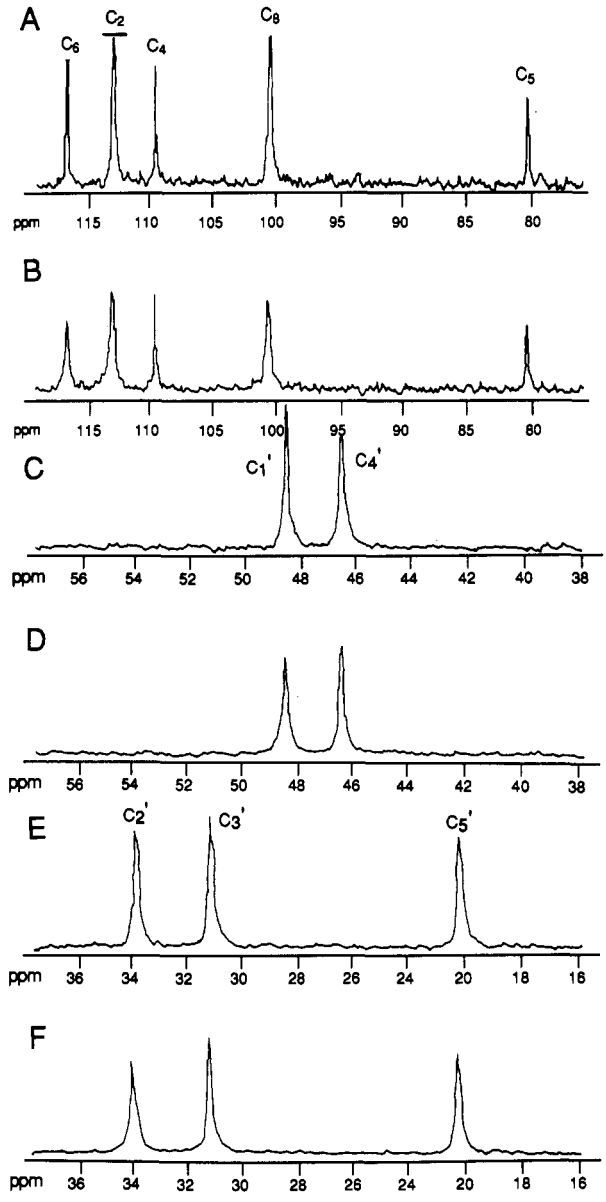

Figure 1. ^{13}C NMR of 0.2 M adenosine (A, C, E) and 0.2 M adenosine and 0.025 M RAD (B, D, F) in DMSO solution.

differentiated adenine from other nucleoside bases, i.e., guanosine, uridine, and cytidine, but does not differentiate between riboside and deoxyriboside. This conclusion is in line with other studies.[1,2] The results for the various compounds are summarized in Table II.

Structure of Complex. We now ask what are the binding sites for RAD on the adenine moiety and why is there no interaction with guanosine which has at least two possible binding sites (N_7 and N_1) similar to that of adenosine. An X-ray study of the 1-methyladenosine complex of rhodium dimer crystallized from methanol–water has shown that a σ-bond exists between rhodium and N_7.[10] Both N_1 and N_7 have been previously suggested as binding sites in solution, with somewhat meager evidence. N_1 binding was based on the observation[11] that reducing the pH of a RAD–5′-AMP aqueous solution to below 4 changes its color from pink (RAD–5′-AMP complex) to blue ($RAD(H_2O)_2$ species); N_1 has a pK_a of about 4. N_7 was suggested[11] as the binding site of adenosine in the RAD–AMP complex, with additional stabilization by a hydrogen bond between 6-NH_2 and the acetate oxygens, by analogy to the structure of bis-

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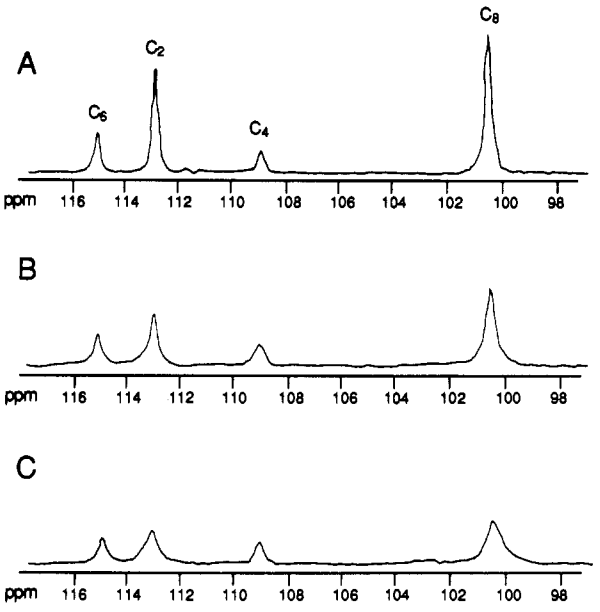

Figure 2. ^{13}C NMR of the purine moiety of 0.2 M N_6-benzyladenosine in the presence of (A) 0, (B) 0.025, and (C) 0.050 M RAD in DMSO solution. C_5 resonance not shown, but unaffected.

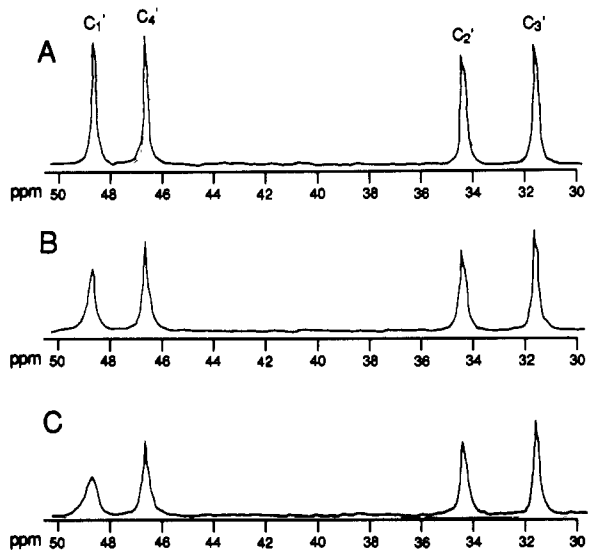

Figure 3. ^{13}C NMR of the ribose moiety of 0.2 M N_6-benzyladenosine in the presence of (A) 0, (B) 0.025, and (C) 0.050 M RAD in DMSO solution. $C_{5'}$ resonance not shown but unaffected.

(acetylacetonato)(nitrodeoxyadenosine) cobalt(II).[12] Both N_1 and N_7 are known binding sites of adenosine with metals.

The following results indicated that DMSO solution produces a different structure from that determined crystallographically. To test whether N_7 is in fact the binding site in this solution, tubercidin (7-deazaadenosine, i.e., adenosine with N_7 replaced by carbon), was interacted with RAD in DMSO solution. A pink color appeared, and the ^{13}C NMR exhibited the same pattern of broadening as adenosine, with C_8, C_2, and C_6 resonances most extensively broadened and then C_7, C_5, and C_4 in that order. Thus, in this case, N_7 binding appears ruled out; in fact, the adenosine–RAD spectrum should exhibit pronounced broadening of C_5, if N_7 were a binding site. We now tested N_1 as a possible binding site by the reaction of RAD with 1-*N*-methyladenosine, in which N_1 is blocked by a methyl group. The pink color was again produced, and the ^{13}C NMR spectrum showed the now familiar broadening pattern. Thus N_1 binding appears ruled out also. Of course, one could assume that blocking N_1 makes N_7 available, and eliminating N_7 makes N_1 available. However, such changes in the nature of the binding should lead to changes in the nature of the broadening effects, and such changes do not occur (Table II). Rather, effects previously noted in the unsubstituted adenosine are maintained. It would be expected that, if either N_1 or N_7 were bound in adenosine, the line broadening mode would change if the ligand changed. Besides, the specific broadening effects are not in line with either N_1 or N_7 binding.

Some additional, though less significant, evidence against σ binding was provided by experiments in which RAD was treated with N_6-methyladenosine, N_6-benzyladenosine, and N_6,N_6-dimethyladenosine. Solutions of RAD and the adenosines monosubstituted at the amino group gave a pink color, and the ^{13}C NMR demonstrated the characteristic broadening pattern of C_8, C_2, and C_6, shown for the benzylated derivative in Figure 2, and of $C_{1'}$, etc., shown for the benzylated derivative in Figure 3. No reaction took place with the disubstituted molecules, as was shown by a lack of broadening of the ^{13}C resonances and the lack of a color change.

A space-filled model indicates that the 6-amino substituent may tend to favor a conformation where it will be distal to N_7 because of steric hindrance. There is experimental evidence for this conformation.[13-20] In such a conformation, N_1 would be blocked as a possible coordination site and the interaction of RAD with N_6-methyl- and N_6-benzyladenosine would indicate that N_1 is not the binding site.

The fact that there is no reaction with (dimethylamino)-adenosine could be explained by blocking of the σ-binding sites. Evidence that the methyl groups in (dimethylamino)adenosine are out of the plane of the adenosine but not perpendicular to it[21] neither proves nor disproves this possibility, which is however clearly not in accord with our other results. We attribute the failure of this molecule to react with RAD to steric hindrance between the CH_3 groups on N_6 and the acetate groups on RAD.

The NMR studies discussed so far have involved the perturbation by RAD of NMR peaks on the purine as well as the ribose moieties. The N_6-benzyladenosine contains additional C atoms observable in the NMR spectrum, and these are shown in Figure 4. As can be seen, the resonance of the carbons ortho to the methylene group is unaffected by RAD while those of the carbons meta and para to the methylene group are significantly broadened. This broadening of benzyl carbons is accompanied by an upfield shift of 0.2–0.3 ppm in the peaks due to the methyl groups of the acetates of RAD, relative to the shift of these groups in other RAD–adenosine complexes. This mutual effect of the benzyl and acetate groups upon each other indicates a close proximity of these groups due to a positioning of the para- end of the phenyl ring adjacent to the acetate.

Chelation involving the NH_2 group and N_7 is very unlikely, since such chelation is not generally found in metal–adenosine complexes. Evidence against such chelation is provided by the results of the interaction of purine riboside, nebularine, which has no NH_2 group bound to C_6. The pink color as well as the characteristic broadening of the NMR carbon peaks indicates

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Table I. Chemical Shifts[a] for Purine–RAD Complexes in DMSO

	C_2	C_4	C_5	C_6	C_8	C_1'	C_2'	C_3'	C_4'	C_5'
adenosine (10:1)[b]	0.12	0	0.002	0.049	0.17	0.067	0.049	0	0.043	0
nebularine (10:1)[c]	0.12	0.07	0.03	0.06	0.23	0.12	0.07	0.01	0.03	–0.01
nebularine (2:1)[c]	0.54	0.24	0.13	0.45	0.93	0.51	0.27	0.02	0.18	–0.09

[a] The net chemical shifts, in ppm, for adenosine- and adenosine-like–RAD complexes. The appropriate free nucleoside was taken as reference. [b] The values are the results of six measurements done on different samples. Accuracy is ±0.005. [c] These values were measured for only one sample. Accuracy is ±0.01.

Table II. Summary of the Results of RAD Interacting with Purine Complexes in DMSO

		broadening of									
purine	pink color	C_2	C_6	C_8	C_4	C_5	C_1'	C_4'	C_2'	C_3'	C_5'
adenosine	+	+	+	+	–	–	+	+	+	–	–
tubercidin[a]	+	+	+	+	–	–	+	+	+	–	–
1-methyladenosine	+	+	+	+	–	–	+	+	+	–	–
N_6,N_6-dimethyladenosine	–	–	–	–	–	–	–	–	–	–	–
N_6-benzyladenosine	+	+	+	+	–	–	+	+	+	–	–
guanosine	–	–	–	–	–	–	–	–	–	–	–
uridine	–	–	–	–	–	–	–	–	–	–	–
nebularine	+	+	+	+	–	–	+	+	+	–	–
cytidine	–	–	–	–	–	–	–	–	–	–	–
8-bromoadenosine	+	+	+	+	–	–	+	+	+	–	–
2′-deoxyadenosine	+	+	+	+	–	–	+	+	+	–	–
N_6-methyladenosine	+	+	+	+	–	–	+	+	+	–	–

[a] Broadening of C_7 was also observed in this case.

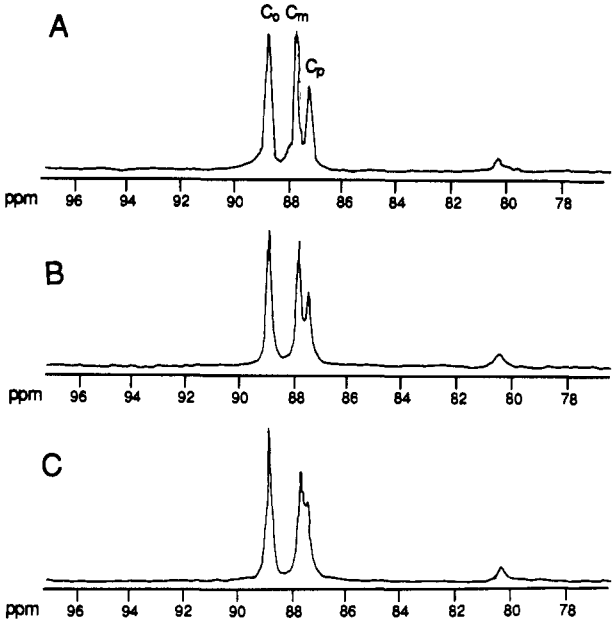

Figure 4. ^{13}C NMR of the benzyl moiety of 0.2 M N_6-benzyladenosine in the presence of (A) 0, (B) 0.025, and (C) 0.050 M RAD in DMSO solution. C_o, C_m, and C_p are ortho, meta, and para carbons on the benzyl.

that the NH_2 group is not necessary for the binding of RAD to adenosine compounds. The lack of broadening of the C_5 resonance comparable to that of the C_6 resonance provides additional evidence against the chelation. Dipolar interaction between carbons and their neighboring protons could broaden C_8 and C_2. Yet it could not account for C_6 broadening.

Nevertheless, we interacted 8-bromoadenosine with RAD and the result was the same pattern of broadening, C_8, C_6, and C_2, of the adenine moiety.

Anisotropic overall motion of the nucleoside–RAD complex cannot account for the specific broadening pattern of the nucleoside carbon atoms. Symmetry considerations of the complex would suggest a different pattern of broadening. Depending on the relative position of the Rh–acetate dimer and the nucleoside, a preferred broadening of C_4 and C_5 in one case and of C_2, C_4, C_5, and C_6 in the other case could be expected, but in no way can this account for the experimental broadening pattern.

A π-Bonded Structure. The σ-binding modes hitherto discussed cannot explain these results. They can, however, be explained by π-bonding of RAD to the adenosine moiety. Monomeric rhodium, molybdenum(II), and ruthenium(II) complexes have been shown to exhibit π-bonding with organic molecules that can act as donors.[22-24] The possibility that a rhodium dimer can act as a π donor to compounds acting as π acceptors was suggested some time ago[25] to explain thermodynamic measurements of adducts formed by rhodium butyrate dimer and Lewis bases. It was then suggested that the extensive overlap of d orbitals on the two metal centers gives rise to enhanced π back-donation from the rhodium π centers to the axial ligands. The d_{xz} or d_{yz} orbitals give rise to antibonding orbital lobes that project out toward an incoming donor ligand on the Z axis. Since the butyrate ligands in ref 25 and the acetate ligands in the present study are essentially σ donors, electron density concentrates in the antibonding π^* orbitals of the rhodium, making it a very effective π back-bonding donor. π-bonding has been previously suggested for a 5′-AMP–RAD complex as well as the RAD complexes with pyridine, imidazole, and histidine, because the stability of these complexes is of the same order as the ability of the ligands to act as π-acceptors.[26]

We have constructed a model for the π-bonding of RAD with adenosine in Figure 5. The interaction between rhodium and nucleoside base and rhodium and ribose are the same for all the other ligands that form complexes with the rhodium dimer. Figure 6 shows a similar model for the N_6-benzyl derivative, showing that the phenyl group can in fact come close to the methyl of the acetate, as indicated by the NMR broadening in Figure 4. The models describe 1:2 complexes, with an adenosine molecule bound to each of the two rhodium atoms.

Theoretical Basis for π-Bonding. The structure involving π^* back-donation illustrated in Figure 5 can explain the fact that

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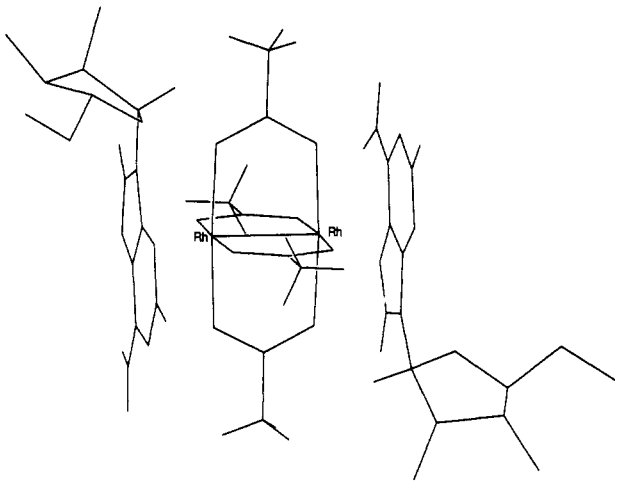

Figure 5. Model of the π-bonded structure of the 1:2 RAD–adenosine complex. The RAD is in the center of the diagram, with two acetate groups in the plane of the paper and the other two acetate groups perpendicular to that plane. The two adenosines are on either side of the RAD, with the adenine bases perpendicular to the plane of the paper. The ribose moieties are in the upper left and lower right. The π bonds from the rhodium atoms to the bases are not shown, but can be imagined as extensions of the Rh–Rh bond.

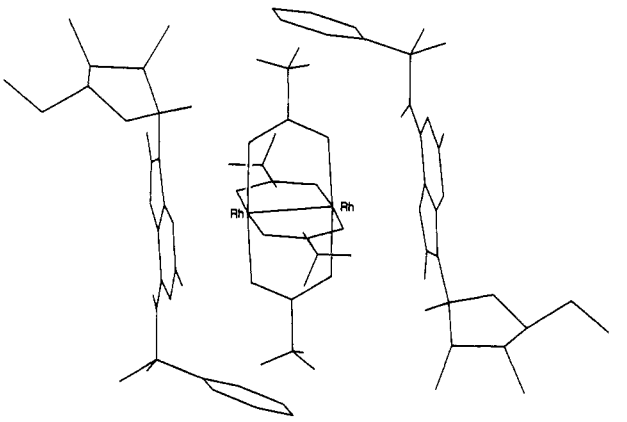

Figure 6. Model of the structure of the complex of RAD with N_6-benzyladenosine, showing the close approach of the phenyl group to the methyl groups on the benzyls of the RAD acetate.

guanosine, which is a better π donor[27-30] and has a higher stacking tendency than adenosine, does not interact with RAD. Guanosine is a poorer π *acceptor*, as indicated by the fact that it is not reduced under conditions that lead to the reduction of adenosine.[31] Since the interactions with RAD are through π^* back-donation, adenosine will interact with RAD while guanosine, a poorer π acceptor, will not.

This type of binding can explain the selective broadening by RAD of C_8, C_2, and C_6 carbon resonances in the ^{13}C NMR spectra of nucleoside bases. The carbon resonances of the nucleosides in the absence of RAD have practically the same width. It is expected that the postulated π^* back-bonding will contribute to the relaxation because of the exchange between the nucleoside and RAD. The case of an uncoupled two-site exchange has been extensively treated.[32] The nucleoside exchanges between two sites: the nucleoside–RAD complex and the bulk pool. From the band shape of the exchanging system, one may deduce the expression for the transverse relaxation time. For a fast exchange case, the broadening of the weighted line observed for each carbon nucleus depends on the differences in the chemical shifts, on the transverse relaxations, on the rate of exchange and on the population of nuclei in both sites.[33]

It is through the chemical shift that the π^* back-bonding contributions to the ^{13}C transverse relaxation time are observed. The chemical shift for each ^{13}C atom has a paramagnetic contribution which is the result of field-induced mixing of the electronic ground state with the excited electronic states.[34,35] This contribution may be visualized as being due to anisotropic, i.e., nonspherical, local electron circulations around the nucleus. The derived expression[35] for this paramagnetic contribution involves charge density (Q_{cc}) and bond order terms ($\sum Q_{ci}$), as well as a mean electronic energy (ΔE) and an expectation value of the inverse cube of the distance between a 2p electron and the nucleus ($\langle r^{-3}\rangle_{2p}$). Any change in the local electronic structure affects all the above parameters. In our case, complex formation by π bonding increases Q_{cc} (with $\sum Q_{ci} = 0$), decreases ΔE and increases $\langle r^{-3}\rangle_{2p}$ with the net effect of increasing the paramagnetic shielding term, resulting in a larger downfield shift (Table I) and in a different increase in the broadening of the observed line for each carbon atom.

Such a differentiation in the chemical shifts is to be expected in the postulated π^* back-bonding complex of Figure 5, since the electron density contribution of RAD to various atoms in the nucleoside molecule will be different, depending on the distribution of the electron density among the individual carbon atoms, i.e., on the square of the coefficients of each carbon wave function in the π molecular wave function (in the LCAO MO model). In order to estimate the distribution of the electron density, we carried out a Hückel type calculation[36] of the energy levels and the associated wave functions for the adenine moiety of the nucleoside molecules, assuming that the results are valid for the electron distribution in the nucleoside–RAD complex. The values of the Coulomb integrals for the different types of nitrogen atoms differ from those of carbons and were taken as $0.5\beta_N$ and $1.5\beta_N$. The resonance integral was taken as $0.8\beta_N$.[36] The value of β_N, the resonance integral for the nitrogen atom, was taken as –3.33 eV, from spectra of nucleotide bases.[37] The value of α_c, the Coulomb integral for carbon atoms, was calculated as –4.30 eV, from the ratio of $\alpha/\beta = 1.80$[35] and the value of $\beta_c = -2.389$ eV, obtained for benzene.[38] We determined the eigenvalues and eigenvectors of the secular equations. The molecular wave function of the first unoccupied π energy level has the following ratio of the square of coefficients, with the square of the C_4 coefficient taken as unity (Table III):

Table III

C_2^2	C_4^2	C_5^2	C_6^2	C_8^2
82	1	17	105	140

Because of the approximate character of the calculation, which involves even more assumptions than the regular Hückel calculation (e.g., adopting values of α and β instead of a direct solution of the secular determinant), these results cannot be used in a quantitative manner. Their importance lies in the fact that they correlate perfectly (in a qualitative manner) with the pattern of the experimental broadening. Very clearly C_8, C_6, and C_2 exhibit higher electron density, and therefore experience a more efficient relaxation mechanism than C_5 and C_4, as observed experimentally.

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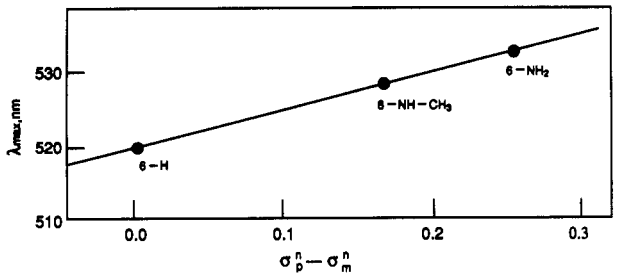

Figure 7. Dependence of λ_{max}, the maximum of the absorption peaks of nucleoside–RAD complexes, on σ_n, the Hammet modified constant.

The conclusion that σ-bonding is ruled out experimentally, and therefore π-bonding must occur, is further strengthened by this correlation.

Substituent Effects in Line with π-Bonding. The various substituents on the nucleoside will induce changes in the electron density of the nucleoside orbitals and thus influence the stability of the complex formed with RAD. Hammet constants provide a pseudoquantitative measure of the amount of electron-withdrawing or electron-donating power of the substituents. The original Hammet σ constants and the modified $\sigma\pm$ constants were derived and are mostly applicable to benzoic acid derivatives. Since the six-membered ring is a part of a more complicated ring system in adenosine, we thought that the values of σ normal (σ^n), calculated from data on reactions in which no extra resonance seemed probable,[39] will be the most appropriate to use. In Figure 7, it is seen that $\Delta\sigma^n$, i.e., $\sigma_p^n - \sigma_m^n$, which is an approximate measure of the resonance effect of the substituent, linearly correlates with the absorbance maxima of the various complexes for which σ^n values are available from the literature.[39] This is consistent with the concept that these maxima depend on the electron donor properties of the substituent. The greater the electron-releasing power of the substituent, the more the electron density in the π orbitals will be increased and electron donation from the rhodium antibonding orbitals reduced. Thus the π bond is weakened, the complex becomes less stable and λ_{max} is red-shifted. This correlation, which is predicted for a π-bonded complex, is in line with the structures shown in Figures 5 and 6.

Reactivity of π-Bonded Complex. RAD binds to single-stranded DNA, poly(A), and RNA but not to double-stranded DNA,[1] presumably as 1:1 complexes. This phenomenon can be explained by the π-bonding. The double stranded structure sterically prevents RAD from forming a π bond with adenosine, while RAD can approach the adenosine plane of a single strand and form the π bond.

Comparison with σ-Bonded Structures Obtained by X-ray Crystallography. The crystallographic studies clearly show that N_7 bonding of adenosine to rhodium is a preferred structure in the solid state, at least after crystallization from water–methanol.[10] Our results, such as the ability of tubercidin, which does not contain N_7, to produce a structure with the same NMR characertistics as adenosine, preclude the σ-structure but support the π-structure. The different structures may result from differences in crystal and solution states and from differences in solvent. The π-structure gives an excellent explanation for the specificity of RAD for adenosine but not guanosine, cytidine, and uridine.

Another recently obtained X-ray structure of a ligand bound to RAD is the bipyridyl complex of RAD.[40] This complex differs from both the X-ray structure and our solution structure of the RAD–adenosine complex, having the bipyridyl bound to one rhodium through two nitrogen atoms, while one of the acetates is bound to the other rhodium, producing an asymmetric structure with only three acetates bound to both rhodium atoms. Clearly the rhodium dimer structure is capable of a variety of coordination modes that depend on a variety of conditions.

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