Molecular Properties of 1,N^6-Ethenoadenosine in Comparison with Adenosine: Self-Association, Protonation, Metal Ion Complexation, and Tryptophan-Adduct Formation. A Study on ϵ-Adenosine Using Proton Nuclear Magnetic Resonance, Ultraviolet Spectrophotometry, and Potentiometric pH Titration

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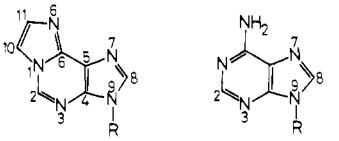
Abstract: The concentration dependence of the chemical shifts of the protons H-2, H-8, H-10, H-11, and H-1′ of 1,N^6-ethenoadenosine (ϵ-Ado) has been measured to elucidate the self-association. The results are consistent with the isodesmic model of indefinite noncooperative stacking; the association constant, $K = 9.4\ M^{-1}$, is smaller than the value for adenosine (Ado), $K = 15\ M^{-1}$. Protonation (which has been studied by NMR and UV spectroscopy and potentiometric pH titration) and complex formation inhibit the tendency for self-stacking of ϵ-adenosine considerably. The aromatic-ring stacking interactions between the indole moiety and the 1,N^6-ethenopurine residue of ϵ-adenosine ($K^{(\epsilon\text{-Ado})}_{(\epsilon\text{-Ado})(\text{Trp})} = 6.0\ M^{-1}$) are comparable to those of unaltered adenine derivatives. Hence, there are no indications for increased stacking tendencies of the three-ring ϵ-adenine moiety compared with those of the unmodified two-ring adenine residue. Under conditions where practically only the monomeric form of ϵ-adenosine exists, the stability constants of the 1:1 complexes with Mg^{2+}, Mn^{2+}, Cu^{2+}, and Zn^{2+} ($\log K^{Zn}_{Zn(\epsilon\text{-Ado})} = 1.5$) were determined by potentiometric pH titrations (for comparison: $\log K^{Zn}_{Zn(Ado)} = -0.3$); the results for the Cu^{2+} and Zn^{2+} complexes were confirmed by UV and ^1H NMR measurements, respectively. ϵ-Adenosine acts as chelating ligand via the N-6,N-7 site, and its metal ion complexes are about 100 times more stable than the corresponding complexes with adenosine. The ϵ-adenine moiety is also able to participate in the formation of mixed ligand complexes as demonstrated for the ϵ-adenosine/2,2′-bipyridyl (bpy)/Cu^{2+} system. The different structures (metal ion bridge between ϵ-adenosine and bipyridyl vs. stack between adenosine and $M(bpy)^{2+}$) of such ternary complexes are discussed. Some expected properties of ϵ-adenine nucleotide/metal ion systems are indicated, and the pH dependence of the different metal ion binding sites is exemplified.

Nucleotides are playing a key role in biology,[1-3] e.g., in the information storage via RNA and DNA or in energy-transfer processes. The use of structurally altered nucleotides as probes provides one way to study the involved enzymic reactions. Indeed, all three parts of nucleotides have been systematically modified: e.g., the ribose residue has been replaced by glucose;[4] in the phosphate moiety the bridging oxygen was substituted by a sulfur,[5] and imido,[6,7] a methylene,[7,8] or a peroxo bridge;[9] and the purine base was enlarged as in *lin*-benzoadenine nucleotides.[10] Other base modifications lead to 1,N^6-ethenoadenosine (ϵ-adenosine) and 3,N^4-ethenocytidine.[11]

Chart I



1,N^6-ethenoadenosine or ε-adenosine (ε-Ado) adenosine (Ado)

ϵ-Adenosine[11] and its nucleotides[12,13] were first synthesized 10 years ago, based on the previously described reaction of chloroacetaldehyde with 9-methyladenine.[14] In this reaction the N-6 and N-1 atoms are linked by the 1,N^6-etheno bridge to a five-membered ring.[15] As ϵ-adenosine and its derivatives exhibit fluorescence properties,[11-13] much energy has been spent to identify the species responsible for fluorescence.[16-18] These efforts are understandable, as the incorporation of the ϵ-adenosyl residue into biological macromolecules provides a powerful tool to gain

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structural information:[19] ε-adenosine derivatives have already been extensively used to probe active sites of adenine nucleotide dependent enzyme systems,[20] and the 1,N^6-etheno analogues of flavin adenine dinucleotides[21] and adenosylcobalamin, i.e., the ε analogue of coenzyme B_{12},[22] were also synthesized and studied.

The facts that all nucleotide-dependent enzyme systems need metal ions for their activation[1] and that metal ions alter the structure of nucleotides in solution[3,23] urge a detailed investigation of ε-adenine nucleotide–metal ion systems.[24] As a first step we studied metal ion complexes of ε-adenosine with the aim to compare their properties with those of adenosine. To facilitate comparisons between these two molecules, we adapted the conventional atom numbering for adenines, as shown in Chart I,[25] for ε-adenosine; a procedure that is common.[15,26]

In aqueous solution purine systems have a pronounced self-association tendency[23] and the extent of self-stacking of ε-adenosine had to be determined to learn under which conditions the monomeric species dominate; this clarification was achieved by ^{1}H NMR shift measurements. Potentiometric pH titrations were used to determine the stability constants of several monomeric metal ion complexes of ε-adenosine; some of the constants were confirmed by NMR and UV spectroscopy.

The interaction between an indole moiety of a tryptophan residue of a protein or enzyme and nucleic acid bases of nucleotides,[27] coenzymes,[28] or nucleic acids[29] are well-known, and tryptophan also quenches the fluorescence of ε-adenosine[30] and its derivatives.[31] In addition, due to intramolecular ligand–ligand interactions between the indole moiety of tryptophanate (Trp^-) and the purine residue of adenosine 5′-triphosphate (ATP^{4-}) in ternary $M(ATP)(Trp)^{3-}$ complexes, cooperative effects are observed.[32-34] Therefore we have also determined the stability of the ε-adenosine–tryptophan adduct by ^{1}H NMR to obtain a hint about the properties of this adduct within mixed ligand complexes.

Experimental Section

Materials. 1,N^6-Ethenoadenosine was purchased from Sigma Chemical Co., U.S.A. The nitrate salts of Na^+, Mg^{2+}, Mn^{2+}, Cu^{2+}, and Zn^{2+}, $NaClO_4$, $Mg(ClO_4)_2$, 2,2′-bipyridyl, L-tryptophan, NaOH (Titrisol), HNO_3 (all p.A.), DNO_3 and NaOD (both with >99 % D), and a 10% tetramethylammonium hydroxide solution (p.A.) (which was converted into the nitrate) were obtained from Merck AG, Darmstadt, F.R.G. D_2O (≥99.8%) was from CIBA-Geigy AG, Basel, Switzerland, and $Cu(ClO_4)_2$ from Fluka AG, Buchs, Switzerland.

The titer of the NaOH used for the titrations was determined with potassium hydrogen phthalate (Merck AG); the exact concentrations of the ε-adenosine solutions used in the titrations with metal ions (titrated in the presence of an excess of HNO_3, see below) were measured by titrations with NaOH. The concentrations of the stock solutions of the divalent metal ions were determined with EDTA.

^{1}H NMR and UV Spectroscopy. The ^{1}H NMR spectra were recorded with a Bruker WH-90 FT spectrometer (90.025 MHz) at 27 °C in D_2O as solvent with the center peak of the tetramethylammonium ion triplet as internal reference (for details and justification see ref 23). However, all chemical shifts were converted to a (trimethylsilyl)propanesulfonate reference by adding 3.188 ppm.

The ultraviolet absorbance spectra were recorded on a Cary 219 or on a Varian Techtron 635 spectrophotometer (the latter connected to a W + W recorder, Model 1100) in aqueous solutions at 25 °C and I = 0.1 M ($NaClO_4$) with 1-cm quartz cells.

The pH (or pD) of the solutions was measured with a Metrohm 605 digital pH meter using a Metrohm micro EA 125 glass electrode (Metrohm AG, Herisau, Switzerland). The desired pH (pD) of a solution was adjusted by dotting with a relatively concentrated HNO_3 (DNO_3) or NaOH (NaOD) on a thin glass rod. The pD of D_2O solutions was obtained by adding 0.40 to the pH meter reading.[35]

The experimental results of the ^{1}H NMR and UV spectroscopic measurements were analyzed with a Hewlett-Packard 9821A calculator connected to a 9862A calculator-plotter by using a Newton–Gauss nonlinear least-squares program.

Potentiometric pH Titrations. The pH titrations were carried out with a Metrohm potentiograph E536 and a Metrohm macro EA 121 glass electrode. The buffers (pH 4.64, 7.00, and 9.00) used for calibrations were also from Metrohm AG. The direct pH meter readings were used in the calculations for the acidity constants.

The acidity constant $K^H_{H(L)}$ of H(ε-adenosine)$^+$ was determined by titrating 50 mL aqueous 0.72 mM HNO_3 and $NaNO_3$ (I = 0.1, 25 °C) in the presence and absence of 0.48 (or 0.33) mM ε-adenosine under N_2 with 1 mL of 0.04 M NaOH. $K^H_{H(L)}$ was calculated from four inde-

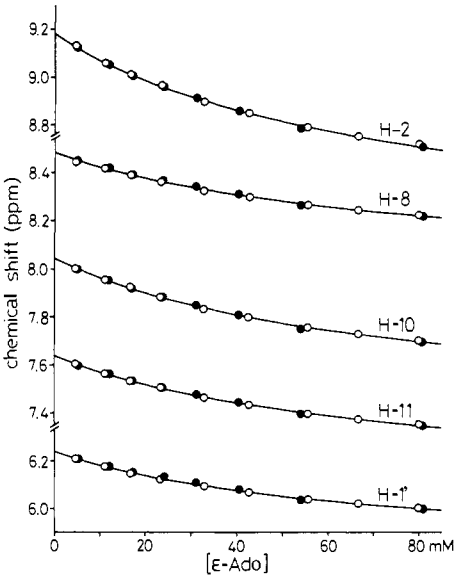

Figure 1. Variation of the chemical shift of H-2, H-8, H-10, H-11, and H-1′ with increasing concentrations of ε-adenosine (the ^{1}H NMR signals were assigned according to a deuterium labeling study).[13] The solid and the open circles refer to two independent series of experiments. The spectra were measured at 90.025 MHz (D_2O; pD = 7.0; 27 °C; I = 0.1, $NaNO_3$) relative to internal $(CH_3)_4N^+$ and converted to values relative to sodium (trimethylsilyl)propanesulfonate by adding 3.188 ppm. The curves shown are the computer-calculated best fit of the experimental data (calculated with K_{av} of Table I) using the indefinite noncooperative stacking model (eq 3). The shift data of the individual protons and the resulting association constants are listed in Table I.

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Table I. Chemical Shifts (ppm) of Some of the Protons of Monomeric (δ_0) and Self-Stacked (δ_∞) 1,N^6-Ethenoadenosine[a] and of Adenosine,[b] Together with the Corresponding Upfield Shifts ($\Delta\delta = \delta_0 - \delta_\infty$) and the Association Constants, K (M^{-1}), Calculated for the Individual Protons, Resulting in the Average Constant, K_{av} (M^{-1}) (D_2O; pD = 7.0, 27 °C; I = 0.1, $NaNO_3$).[c] Some Data of Related Systems Are Given for Comparison

nucleoside	H	δ_0	δ_∞	$\Delta\delta$	K, M^{-1}	K_{av}, M^{-1}
ε-Ado	H-2	9.182 ± 0.013	7.77 ± 0.11	1.41 ± 0.11	10.9 ± 1.8	
	H-8	8.487 ± 0.009	7.70 ± 0.07	0.79 ± 0.07	7.6 ± 2.2	
	H-10	8.044 ± 0.010	7.00 ± 0.08	1.04 ± 0.08	9.3 ± 2.0	9.4 ± 1.2
	H-11	7.639 ± 0.008	6.77 ± 0.07	0.87 ± 0.07	8.5 ± 2.0	
	H-1′	6.239 ± 0.008	5.52 ± 0.06	0.72 ± 0.06	9.1 ± 2.6	
Ado	H-2	8.278 ± 0.009	7.77 ± 0.08	0.51 ± 0.07	13.7 ± 3.4	
	H-8	8.350 ± 0.006	8.07 ± 0.04	0.28 ± 0.04	18.7 ± 5.4	15[d] ± 2
	H-1′	6.087 ± 0.005	5.86 ± 0.04	0.23 ± 0.03	15.1 ± 4.9	
inosine[e]				0.29–0.34		3.3 ± 0.3
uridine[e]				0.05–0.11		1.2 ± 0.5
bpy[f]				0.45–0.71		7.4 ± 1.0
phen[f]				0.86–2.35		23.6 ± 1.8

[a] Evaluation of the two experimental series shown in Figure 1. [b] From ref 23 and 38; the experimental conditions are identical with the present study. [c] The listed limiting shifts were calculated with K_{av}, which is the weighted mean (calculated by using log K) of the individual results. The ranges of error given are *twice* the standard deviation. [d] This average also includes the results of H-2′.[38] [e] In D_2O; pD = 6.9; 27 °C; I = 0.1, $NaNO_3$; from ref 23. [f] 2,2′-Bipyridyl (bpy) in H_2O and 1,10-phenanthroline (phen) in D_2O; from ref 45.

pendent titrations within the range of about 25% (lower values are not reached under the given conditions) to 99% neutralization.

The conditions for the determination of the stability constants $K^M_{M(L)}$ of the binary ε-adenosine complexes (I = 0.1, 25 °C) were the same as for the acidity constant, but $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$. With Mg^{2+} and Mn^{2+}, $M(NO_3)_2$ was 0.0333 M, i.e., the ratios of M^{2+}:ε-Ado were 70:1 and 100:1 ([ε-Ado] = 0.48 or 0.33 mM); $Zn(NO_3)_2$ was between 0.0167 and 0.0333 M; i.e., the ratios were between 35:1 and 100:1; and $Cu(NO_3)_2$ was between 1.67 mM and 33.3 mM, i.e., the ratios were between 3.5:1 and 100:1. Under these conditions practically only the 1:1 complexes form: i.e., the species $M(L)_m^{2+}$ with $m \geq 2$ can be neglected. The stability constants $K^M_{M(L)}$ were computed by taking into account the species H^+, $H(L)^+$, L, M^{2+}, and $M(L)^{2+}$,[36] but with Cu^{2+} the hydroxo complex $Cu(\epsilon\text{-Ado})(OH)^+$ was also considered.[37] The individually calculated values for log $K^M_{M(L)}$ showed no dependence on pH or on the excess of M^{2+}.

In the ternary Cu^{2+}/2,2′-bipyridyl/ε-adenosine system, complex formation between Cu^{2+} and bpy (at pH >2) is rather complete. Hence, as shown earlier,[36] this sytem could also be treated as a binary one by considering the species H^+, $H(L)^+$, L, $Cu(bpy)^{2+}$, and $Cu(bpy)(\epsilon\text{-Ado})^{2+}$. The concentration of Cu^{2+}/bpy was varied in the corresponding experiments between 6.67 and 33.3 mM: i.e., the ratios of [Cu^{2+}/bpy]:[ε-Ado] were between 14:1 and 100:1, and the properties of the system were as expected for a "binary" one.

Throughout, the data were collected from 5% or 10% complex formation to the beginning of the hydrolysis of M^{2+}_{aq}, which was evident from the titrations without ε-adenosine. With the exception of the Mg^{2+} system, which was titrated twice, for all systems three independent pairs of titration curves were recorded and the results averaged.

Results and Discussion

1. Comparison of the Self-Association of 1,N^6-Ethenoadenosine with Its Parent Compound. As the entire ε-adenine moiety is nearly planar with a maximum deviation of about 0.03 Å (= 3 pm) among the ring atoms,[15] self-association has to be expected in aqueous solution. Indeed, the ^1H NMR spectrum of ε-adenosine changes considerably as the concentration is increased from 5 to 81 mM. The variation of the upfield shifts for the protons of the 1,N^6-ethenopurine moiety and of H-1′ of the ribose residue are shown in Figure 1.

The upfield shifts observed for purine derivatives are much higher than would be expected for the shift due to a single adjacent molecule; i.e., these observations preclude the assumption of only dimer formation.[23,38] That polymers are formed must also be concluded from the large upfield shifts observed for ε-adenosine (vide infra); a conclusion in agreement with vapor-pressure osmometric data.[39] In fact, it is now generally agreed[23,38,40] that the self-association of purine derivatives[23,38–42] proceeds beyond the dimer stage, the distance between stacked molecules being in the order of 0.35 nm (= 3.5 Å).[15,43,44]

Indeed, the experimental results of Figure 1 are best interpreted by the isodesmic model for indefinite noncooperative self-stacking.[41] This model is based on the assumption that, e.g., for an adenine derivative (A), the equilibrium constants (eq 1) for the

$$K = [(A)_{n+1}]/[(A)_n][A] \quad (1)$$

$$(A)_n + A \rightleftharpoons (A)_{n+1} \quad (2)$$

equilibria (eq 2) are all equal. Adaption to ^1H NMR shift measurements leads to the relationship[23,38,45] given in equation 3 for the observed chemical shift (δ_{obsd}) and the total concentration

$$\delta_{obsd} = \delta_\infty + (\delta_\infty - \delta_0)[1 - (4K[A] + 1)^{1/2}]/2K[A] \quad (3)$$

[A]. In eq 3, δ_0 represents the shift at indefinite dilution (monomeric A) and δ_∞ the shift of a molecule in an infinitely long stack, while K is the association constant as defined in eq 1.

Application of this model to the experimental data results in the curves shown in Figure 1, which are the computer-calculated best fits according to eq 3. The corresponding limiting shifts, δ_0 and δ_∞, are listed in Table I, together with the association constants K for self-stacking calculated from the upfield shifts of the individual protons;[46] these values for K agree within experimental error. For comparison, the corresponding data of adenosine, together with the association constants of some related systems, are also shown in Table I.

Our value for the self-association constant of ε-adenosine, K = 9.4 M^{-1} (Table I), is only about half of that published recently by Inoue and co-workers[39] who arrived at K = 18.7 M^{-1} on the basis of vapor-pressure osmometry. Hence, in contrast to these authors, we have to conclude that the stacking tendency of ε-adenosine, despite its larger planar system, is somewhat smaller than that of adenosine, for which we recently determined[38] K = 15 M^{-1} under *exactly* the same experimental conditions. Our result agrees with the conclusion of Vanderkooi and co-workers[47] regarding the *intra*molecular association of the dinucleoside monophosphates 1,N^6-ethenoadenylyl-(3′→5′)-1,N^6-etheno-

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(46) For reasons of general comparisons it may be added that $K = 2K_D$, where K_D is the association constant if only dimer formation is assumed; for details, see ref 23 and 38.
(47) Baker, B. M.; Vanderkooi, J.; Kallenbach, N. R. *Biopolymers* **1978**, *17*, 1361–72.

adenosine (ϵApϵA) and adenylyl-(3′→5′)-adenosine (ApA); these authors found that stacking in ApA is more pronounced than in ϵApϵA (pH 7.0).[48]

Seemingly small alterations in a molecule influence the self-stacking drastically. A comparison with other systems listed in Table I shows that, e.g., replacement of the amino group at C-6 in adenosine by a hydroxy group and isomerization of the enol to the more stable keto isomer to give inosine, reduces the association constant to about one-fifth of that of adenosine (K = 3.3 M^{-1} vs. 15 M^{-1}). However, reduction of the two-ring system of inosine to a one-ring system by removing the imidazole ring to give a molecule structurally similar to uridine (K = 1.2 M^{-1}) reduces the constant to only about one-third of that of adenosine. Going from the strictly coplanar three-ring system 1,10-phenanthroline (K = 23.6 M^{-1}) to the two-ring system 2,2′-bipyridyl (K = 7.4 M^{-1}) again reduces the association constant by a factor of about 3. On the other hand, ϵ-adenosine, which has about the same size as phenanthroline, has a considerably lower tendency for self-association (K = 9.4 M^{-1}); i.e., one more similar to bipyridyl. This indicates that factors other than the size of the aromatic system also influence the self-stacking tendency. These factors are probably mainly steric effects introduced by substituents, thus altering the orientation of the stacks, and the addition (or removal) of heteroatoms or polar groups that will influence the bonding or charge transfer in the stack. It should also be pointed out that the formation of such stacks is connected with a negative enthalpy ($\Delta H°$)[39,52] contribution to $\Delta G°$.

Calculations[26] of the ring-current intensities show that the variation of these intensities is very sensitive to alterations in the molecular structure. Indeed, the ring-current intensities of the imidazole rings in the adenine and the ϵ-adenine moieties are quite similar, while they differ strongly in the six-membered pyrimidine rings; i.e., in the ϵ-adenine moiety they are reduced by about one-third. This is attributed[26] to the variation of the state of hybridization of N-1, which is pyridine-like in the adenine moiety and pyrrole-like in the ϵ-adenine residue. However, by far the largest ring-current intensity of the three rings of the ϵ-adenine moiety has the five-membered ring that includes the 1,N^6-etheno bridge.[26] As one would expect, the observed upfield shifts ($\Delta\delta$) are considerably larger for ϵ-adenosine than for adenosine, but they are significantly smaller than those of phenanthroline (Table I).

Finally it must be pointed out that experiments intended to characterize properties of the monomeric ϵ-adenosine should be carried out at low concentrations: e.g., in a 10^{-3} M aqueous solution, >98% of ϵ-adenosine exist in the monomeric form. This has been taken into account in the experiments presented in the following sections; it is especially important in section 4 where the stabilities of several metal ion complexes are described.

2. Inhibition of Self-Stacking by Protonation and Complex Formation. From an X-ray structural analysis of 10-ethyl-ϵ-adenosine hydrochloride,[15] it is known that in the solid state infinite stacks of the ϵ-adenine rings are formed. This has lead to the attempt to determine the association constant of D(ϵ-adenosine)$^+$ in D_2O at pD = 3.1, i.e. under conditions where the protonated species is formed to about 97% (see section 3). As expected under these conditions, the chemical shifts of all protons are more downfield compared with the shift positions in free monomeric ϵ-adenosine (section 3).

Plots of the chemical shifts vs. increasing concentration of D(ϵ-adenosine)$^+$ resulted in straight lines and the measured upfield shift differences were small: between a 5 and a 100 mM solution of H(ϵ-adenosine)$^+$ the shift differences were for the resonances of all protons ≤0.036 ppm. Hence, a curve-fitting procedure similar to those shown in Figure 1 was not possible, but by using the smallest $\Delta\delta$ value of the aromatic protons from the previous experiments with ϵ-adenosine (section 1), i.e., $\Delta\delta_{H-8}$ = 0.79 (Table I), the upper limit of the association constant of protonated ϵ-adenosine may be estimated: $K \leq 0.4$ M^{-1}.

Table II. Upper Limits of the Self-Association Constants, K (M^{-1}) (eq 1 and 2), of D(ϵ-adenosine)$^+$ and Zn(ϵ-adenosine)$^{2+}$ in Aqueous Solution, Together with Some Related Data

L	K, M^{-1}, for L^a	$H(L)^+$	$Zn(L)^{2+}$
ϵ-Ado	9.4 ± 1.2	≤0.4[b]	≤1.2[c]
bpy	7.4 ± 1.0		0.3 ± 0.1[e]
phen	23.6 ± 1.8	12.0 ± 2.0[d]	1.1 ± 0.2[e]

[a] See Table I. [b] Determined in D_2O at pD = 3.1; 27 °C; I = 0.1, $NaNO_3$. [c] Determined in D_2O at pD = 6.1 in the presence of 0.5 M $Zn(NO_3)_2$; 27 °C; I = 1.5. [d] From ref 53. [e] From ref 45.

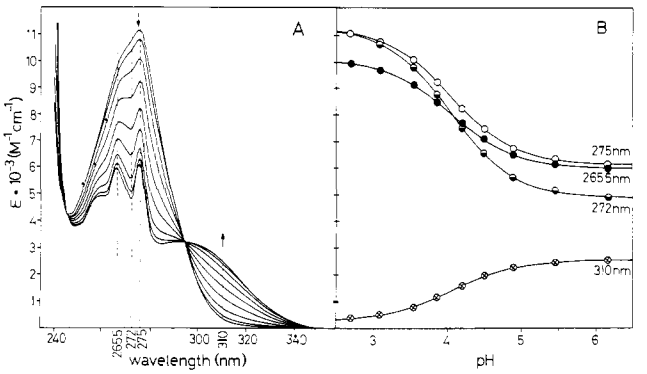

Figure 2. Dependence of the UV spectrum of ϵ-adenosine on pH in aqueous solution at I = 0.1 ($NaClO_4$) and 25 °C (measured in 1-cm cells with [ϵ-Ado] = 1.8 × 10^{-4} M): (A) change of the UV spectrum resulting from the increase of pH from 2.70 to 6.16 (via 3.05, 3.54, 3.86, 4.20, 4.50, 4.89, and 5.46), the direction of the spectral change is indicated by arrows; (B) evaluation of the spectra shown in A by plotting the extinction coefficient ϵ (M^{-1} cm^{-1}) vs. pH. The solid curves represent the computer-calculated best fits of the experimental data (see Table III).

The overall situation with the Zn^{2+}/ϵ-adenosine system is quite comparable. The coordination of Zn^{2+} to the base moiety leads, as expected, to downfield shifts (see section 4). Under conditions were Zn(ϵ-adenosine)$^{2+}$ is formed to at least 93% (calculated with the constants given in sections 3 and 4), the observed upfield shifts in dependence on increasing Zn(ϵ-adenosine)$^{2+}$ concentrations are small. The shift differences between a 5 and a 50 mM solution of the complex in D_2O are for all protons ≤0.044 ppm. With the procedure indicated in the preceding paragraph for H(ϵ-adenosine)$^+$, the upper limit of the self-association constant of Zn(ϵ-adenosine)$^{2+}$ was estimated: $K \leq 1.2$ M^{-1}.

The results of this section are in line with related data.[45,53] The association constants given in Table II clearly demonstrate that the repulsion of the positive charges resulting from protonation or complex formation at the aromatic system drastically inhibit self-stacking in solution.[54] In the solid state, forces other than stacking forces have a considerable influence: there is no base stacking in N,6-(2-chloroethyl)-substituted ϵ-(9-methyl)adenine hydroiodide,[55] i.e., the structure of this salt is very different from that of 10-ethyl-ϵ-adenosine hydrochloride,[15] and there is also no stacking in crystalline 1,10-phenanthroline hydrate,[56] despite the

(48) In contrast to this result, some authors[49] estimate that the intramolecular stacking interaction in the two dinucleoside monophosphates is roughly the same, while others[50,51] claim that it is larger in ϵApϵA than in the unmodified parent compound ApA. It appears that the experimental conditions and the evaluation methods influence the conclusions.

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(51) Inoue, Y.; Kuramochi, T.; Sakurai, M.; Tazawa, I. *J. Am. Chem. Soc.* **1980**, *102*, 5574–7.

(52) Sõvãgõ, I.; Martin, R. B. *FEBS Lett.* **1979**, *106*, 132–4.

(53) Mitchell, P. R. *J. Am. Chem. Soc.* **1980**, *102*, 1180–1.

(54) There are however also cases known[23] where such a coordination leads to bridging between different molecules as in the systems of $Zn(ATP)^{2-}$ or $Cd(ATP)^{2-}$, and then self-stacking is promoted.

(55) MacIntyre, W. M.; Zabrowsky, R. F. *Z. Kristallogr., Kristallgeom., Kristallphys., Kristallchem.* **1963**, *119*, 226–33. See also ref 15.

(56) Nishigaki, S.; Yoshioka, H.; Nakatsu, K. *Acta Crystallogr., Sect B* **1975**, *B31*, 1220, and personal communication of K. Nakatsu to P. R. Mitchell (see ref 30 in the present ref 45).

Table III. Extinction Coefficients, ϵ (M^{-1} cm^{-1}), of Free and Protonated ϵ-Adenosine at Several Wavelengths and Acidity Constant of H(ϵ-adenosine)$^+$ As Determined by UV Spectrophotometry in Water[a]

λ, nm	ϵ, M^{-1} cm^{-1} ϵ-Ado	ϵ, M^{-1} cm^{-1} H(ϵ-Ado)$^+$	$pK^H_{H(\epsilon\text{-Ado})}$
265.5	5990 ± 30[b]	10 080 ± 30	4.05 ± 0.02
272	4870 ± 40	11 290 ± 50	4.04 ± 0.02 } 4.05 ± 0.01
275	6110 ± 30[b]	11 290 ± 40	4.04 ± 0.02
310	2540 ± 30	270 ± 30	4.06 ± 0.03

[a] [ϵ-Ado] = 1.8×10^{-4}; I = 0.1, $NaClO_4$; 25 °C (see Figure 2). The given range of errors are *twice* the standard deviation. The average value of $pK^H_{H(\epsilon\text{-Ado})}$ is the weighted mean. [b] In ref 13, a value of ϵ = 6.0×10^3 M^{-1} cm^{-1} is given for both wavelengths.

large stacking tendency[45] of phenanthroline in solution (Table II).

3. Acidity Constant of H(ϵ-adenosine)$^+$ and Site of Protonation. The ultraviolet absorbance spectrum of 1,N^6-ethenoadenosine shows a pronounced dependence[13] on pH in the range from 2 to 6. As the absorbance is high,[13] UV spectrophotometry is ideal to determine the acidity constant, $pK^H_{H(\epsilon\text{-Ado})}$, of protonated ϵ-adenosine at a low concentration where no self-association occurs (section 1). Figure 2 depicts UV spectra of the ϵ-adenosine system in the pH range from 2.70 to 6.16 (Figure 2A), together with the evaluation for $pK^H_{H(\epsilon\text{-Ado})}$ at four different wavelengths by plotting the extinction coefficients vs. pH and calculating by computer the best fits (Figure 2B). The calculated extinction coefficients for free and protonated ϵ-adenosine and the acidity constant of H(ϵ-adenosine)$^+$ are given in Table III.

This acidity constant, $pK^H_{H(\epsilon\text{-Ado})} = 4.05 \pm 0.01$, as determined by UV spectrophotometry is identical with the result obtained from potentiometric pH titrations (see section 4 and Experimental Section), which were also carried out at 25 °C, I = 0.1, and under conditions where self-association is negligible. Published data are in reasonable accordance with the present result; they range from 3.9 to 4.10.[13,18,51] Evidently the introduction of the 1,N^6-etheno bridge into adenosine makes this molecule by about 0.4 log unit more basic; the acidity constant of H(adenosine)$^+$ in aqueous solution is only $pK^H_{H(Ado)} \simeq 3.6$.[3,57]

A similar difference in basicity between ϵ-adenosine and adenosine is also observed in D_2O. Due to the dependence of the chemical shifts of the aromatic protons on the extent of protonation, ^1H NMR is a suitable technique to determine acidity constants of nucleic bases, provided low concentrations are used to avoid excessive self-stacking. Figure 3 shows the dependence of the chemical shifts of H-2, H-8, H-10, H-11, and H-1′ on pD. The calculated limiting chemical shifts together with the $pK^D_{D(\epsilon\text{-Ado})}$ value for these protons appear in Table IV. The value $pK^D_{D(\epsilon\text{-Ado})}$ = 4.64 (I = 0.1, 27 °C) is 0.25 log unit higher than the corresponding value for adenosine, $pK^D_{D(Ado)} = 4.29$,[58] determined by the same method but under slightly different conditions (I = 0.5, 34 °C).

The remaining question is whether protonation occurs at N-6 or at N-7 (see Chart I). From the fact that the downfield shift upon protonation is largest for H-11 (see $\Delta\delta_H$* in Table IV), it cannot unambiguously be concluded that protonation occurs at the neighboring N-6, because in aromatic systems shifts induced by protonation do not necessarily decrease with increasing distance from the protonated site. An illustrative example is inosinate, where protonation at N-1 induces a larger downfield shift for H-8 (0.189 ppm) than for the closer H-2 (0.086 ppm).[58] However, a detailed study[17] comparing H(ϵ-adenosine)$^+$ with the corresponding compounds methylated at N-6 or N-7 shows that the predominant site of protonation is indeed N-6; a conclusion in agreement with the crystal structure analysis of 10-ethyl-ϵ-adenosine hydrochloride.[15] Chart II depicts the deprotonation

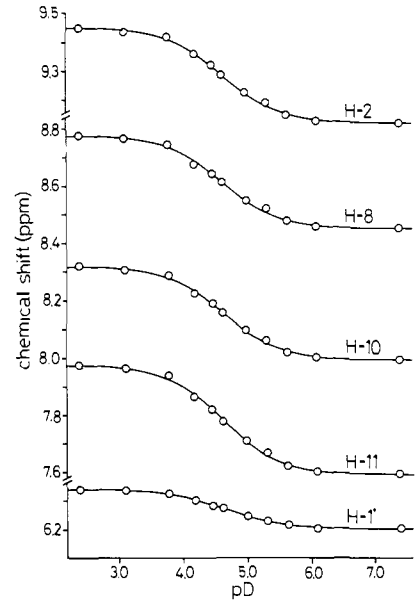
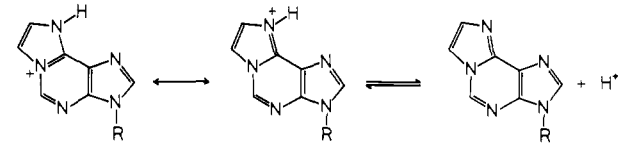


Figure 3. Variation of the chemical shift of H-2, H-8, H-10, H-11, and H-1′ of ϵ-adenosine in dependence on pD. The solid curves are the computer-calculated best fits of the experimental data using $pK^D_{D(\epsilon\text{-Ado})}$ = 4.64 (see Table IV). [ϵ-Ado] = 5×10^{-3} M; I = 0.1, $NaNO_3$; 27 °C; see also legend to Figure 1.

Chart II



equilibrium together with the two most likely resonance structures[15] of 1,N^6-ethenoadenosine. It may be added that protonation of adenosine (see Chart I) occurs at N-1.[3]

4. Stability and Structure of Some Binary Metal Ion Complexes. With the detailed knowledge about self-association and protonation of ϵ-adenosine, complexes of this ligand could now be studied. The stability constants of some binary metal ion complexes with ϵ-adenosine according to eq 4 were determined by potentiometric

$$M^{2+} + \epsilon\text{-Ado} \rightleftharpoons M(\epsilon\text{-Ado})^{2+}$$

$$K^M_{M(\epsilon\text{-Ado})} = [M(\epsilon\text{-Ado})^{2+}]/[M^{2+}][\epsilon\text{-Ado}] \qquad (4)$$

pH titrations under conditions ([ϵ-Ado] ≤ 4.8×10^{-4} M) where self-association is negligible. In the presence of Cu^{2+} or Zn^{2+} the buffer region of ϵ-adenosine ($pK^H_{H(\epsilon\text{-Ado})} = 4.05$) is significantly shifted toward lower pH. With Mn^{2+} this effect is small but still sufficient to determine the stability of Mn(ϵ-Ado)$^{2+}$, and the result is in excellent agreement with an earlier measurement by fluorescence quenching.[18] For the complex between ϵ-adenosine and Mg^{2+}, only an upper limit of the stability can be given. These results are summarized in Table V,[59] together with the stability constants of some related complexes.[61-66]

(57) (a) Martell, A. E.; Schwarzenbach, G. *Helv. Chim. Acta* **1956**, *39*, 653-61. (b) Wallenfels, K.; Sund, H. *Biochem. Z.* **1957**, *329*, 41-7. (c) Ogasawara, N.; Inoue, Y. *J. Am. Chem. Soc.* **1976**, *98*, 7048-53.

(58) Scheller, K. H.; Scheller-Krattiger, V.; Martin, R. B.; *J. Am. Chem. Soc.* **1981**, *103*, 6833-9.

(59) The Cu(ϵ-Ado)$^{2+}$ complex tends to release a further proton, which indicates the formation of hydroxo complexes; the constant for the formation of Cu(ϵ-Ado)(OH)$^+$ was estimated: $pK^H_{Cu(\epsilon\text{-Ado})(H_2O)} \simeq 4.3$. This estimate is valid only for the concentration range used in this study (see Experimental Section). There is a slight dependence of this "constant" on the total concentration, which indicates that also μ-hydroxo-bridged dimeric complexes are formed; these are probably of the type $[Cu(\epsilon\text{-Ado})(OH)]_2^{2+}$, a type well-known for 2,2′-bipyridyl as ligand.[60]

(60) Perrin, D. D.; Sharma, V. S. *J. Inorg. Nucl. Chem.* **1966**, *28*, 1271-8.

(61) Schneider, P. W.; Brintzinger, H.; Erlenmeyer, H. *Helv. Chim. Acta* **1964**, *47*, 992-1002.

(62) Taylor, R. S.; Diebler, H. *Bioinorg. Chem.* **1976**, *6*, 247-64.

(63) Mariam, Y. H.; Martin, R. B. *Inorg. Chim. Acta* **1979**, *35*, 23-8.

(64) Fiskin, A. M.; Beer, M. *Biochemistry* **1965**, *4*, 1289-94.

(65) Banerjea, D.; Kaden, T. A.; Sigel, H. *Inorg. Chem.* **1981**, *20*, 2586-90.

Table IV. Chemical Shifts (ppm) of Some of the Protons of Free and Protonated ε-Adenosine, Together with the Downfield Shift $\Delta\delta_D$* Resulting from Protonation, and Acidity Constant of D(ε-adenosine)$^+$ Determined by ^{1}H NMR Shift Measurements in D_2O[a]

	chemical shift,[b] ppm		downfield shift[c]			
H	ε-Ado	D(ε-Ado)$^+$	Δδ	$\Delta\delta_D$*	$pK^D_{D(\epsilon\text{-Ado})}$[d]	
H-2	9.119 ± 0.009	9.449 ± 0.010	0.330 ± 0.013	0.267	4.66 ± 0.05	
H-8	8.449 ± 0.011	8.775 ± 0.011	0.326 ± 0.017	0.288	4.62 ± 0.07	
H-10	7.993 ± 0.009	8.318 ± 0.010	0.325 ± 0.014	0.274	4.64 ± 0.06	4.64 ± 0.03
H-11	7.592 ± 0.011	7.974 ± 0.012	0.382 ± 0.017	0.335	4.61 ± 0.06	
H-1'	6.204 ± 0.004	6.340 ± 0.004	0.136 ± 0.006	0.101	4.65 ± 0.05	

[a] [ε-Ado] = 5 × 10^{-3} M; I = 0.1, $NaNO_3$; 27 °C (see Figure 3). The range of error is *twice* the standard deviation. The average value of $pK^D_{D(\epsilon\text{-Ado})}$ is the weighted mean. [b] Calculated with $pK^D_{D(\epsilon\text{-Ado})}$ = 4.64. The values for ε-Ado are somewhat smaller than the δ_0 values in Table I due to the small amount of self-association in 5 × 10^{-3} M solutions (section 1). However, under these conditions there is no self-association in the protonated state (section 2), so that the values given for D(ε-Ado)$^+$ hold for the monomeric species. [c] The downfield shifts, $\Delta\delta = \delta_{D(\epsilon\text{-Ado})} - \delta_{\epsilon\text{-Ado}}$, are somewhat influenced by $\delta_{\epsilon\text{-Ado}}$ as indicated in footnote *b*. Therefore the difference, $\Delta\delta_D$*, between $\delta_{D(\epsilon\text{-Ado})}$ and δ_0 of Table I was calculated as a better reflection of the effect of protonation on monomeric ε-adenosine. [d] To obtain the pD, 0.40 log unit was added to the pH meter reading.[35]

Table V. Logarithms of the Stability Constants (eq 4) of Several Metal Ion Complexes with ε-Adenosine (I = 0.1, $NaNO_3$; 25 °C)[a]

	log $K^M_{M(\epsilon\text{-Ado})}$		log $K^M_{M(Ado)}$		
M^{2+}	pH titration[g]	other method	UV spect[e]	other method	log $K^M_{M(Py)}$[i]
Mg^{2+}	≤0.3	<0.5[b]	≤−0.8		
Mn^{2+}	0.72 ± 0.10	0.72[c]	−0.82		0.14[j]
Co^{2+}		2.18[c]	−0.30		1.25 ± 0.02
Ni^{2+}		2.19[c]	−0.17	0.3[f]	1.87 ± 0.01
Cu^{2+}	2.81 ± 0.06	2.80 ± 0.08[b]	0.84	0.70[g]	2.49 ± 0.02
Zn^{2+}	1.51 ± 0.02	1.54 ± 0.09[d]	−0.28	−0.3 ± 0.2[h]	1.00 ± 0.03
Cd^{2+}		~1.8[k]		−0.11 ± 0.06[h]	1.28 ± 0.1[j]

[a] Corresponding data with adenosine (Ado) and pyridine (Py) are also given for comparison. Acidity constant $pK^H_{H(\epsilon\text{-Ado})}$ = 4.05 ± 0.01 by potentiometric pH titrations (see section 4 and Experimental Section). The range of errors given for all newly determined constants is *twice* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] By UV spectrophotometry see text; I = 0.1, $NaClO_4$; 25 °C. [c] From ref 18; by fluorescence quenching at ~22 °C. [d] By ^{1}H NMR in D_2O at I = 0.1–1.5, $NaNO_3$; 27 °C; see text, Figure 4, and Table VI. [e] From ref 61; natural ionic strength; 20 °C. [f] From ref 62 and 63; by spectrophotometry. [g] From ref 64. [h] From ref 23; by ^{1}H NMR in D_2O at I = 0.1–5 ($NaNO_3$) and 27 °C. [i] From ref 65 if nothing else is specified; $pK^H_{H(Py)}$ = 5.26 ± 0.01; by potentiometric pH titration; I = 0.1, $NaNO_3$; 25 °C. [j] From ref 66. [k] Estimate based on the other data listed in this table.

Table VI. Chemical Shifts (ppm) of Some of the Protons of Free and Zn^{2+}-Complexed ε-Adenosine, Together with the Downfield Shift $\Delta\delta_{Zn}$* Resulting from Zn^{2+} Complexation, and Apparent Stability Constant, log K_{app} (See Text), of Zn(ε-adenosine)$^{2+}$ at pD = 6.1 in D_2O As Determined by ^{1}H NMR Shift Measurements[a]

	chemical shift,[b] ppm		downfield shift[c]			
H	ε-Ado	Zn(ε-Ado)$^{2+}$	Δδ	$\Delta\delta_{Zn}$*	log K_{app}	
H-2	9.127 ± 0.009	9.273 ± 0.008	0.146 ± 0.013	0.091	1.63 ± 0.02	
H-8	8.454 ± 0.010	8.639 ± 0.010	0.185 ± 0.018	0.152	1.48 ± 0.04	
H-10	8.000 ± 0.007	8.124 ± 0.007	0.124 ± 0.011	0.080	1.51 ± 0.07	1.52 ± 0.09[d]
H-11	7.603 ± 0.009	7.747 ± 0.008	0.144 ± 0.013	0.108	1.56 ± 0.07	
H-1'	6.203 ± 0.009	6.292 ± 0.008	0.089 ± 0.015	0.053	1.33 ± 0.07	

[a] [ε Ado] = 5 × 10^{-3} M; I = 0.1–1.5, $NaNO_3$; 27 °C (see Figure 4). The range of error is *twice* the standard deviation. The average value of log K_{app} is the weighted mean. [b] Calculated with log K_{app} = 1.52. The values for ε-Ado are somewhat smaller than the δ_0 values of Table I due to the small amount of self-association in 5 × 10^{-3} M solutions (section 1). However, under these conditions there is no self-association of the Zn^{2+} complex (section 2), so that the values given for Zn(ε-Ado)$^{2+}$ hold for the monomeric species. [c] The downfield shifts, $\Delta\delta = \delta_{Zn(\epsilon\text{ Ado})} - \delta_{\epsilon\text{-Ado}}$, are somewhat influenced by $\delta_{\epsilon\text{-Ado}}$ as indicated in footnote *b*. Therefore the difference, $\Delta\delta_{Zn}$*, between $\delta_{Zn(\epsilon\text{-Ado})}$ and δ_0 of Table I was calculated as a better reflection of the effect of Zn^{2+} complexation on monomeric ε-adenosine. [d] This apparent constant at pD = 6.1 is transformed into the pD-independent stability constant with eq 5 by using $pK^D_{D(\epsilon\text{-Ado})}$ = 4.64 : log $K^{Zn}_{Zn(\epsilon\text{-Ado})}$ = 1.54 ± 0.09.

The stability constant of the Cu^{2+}/ε-adenosine system was also determined by UV spectrophotometry. The addition of increasing amounts of $Cu(ClO_4)_2$ at pH 3.50 alters the UV spectrum of ε-adenosine (Figure 2A).[67] This spectral change allows for determination[68] of the apparent stability constant, K_{app}, of the Cu(ε-Ado)$^{2+}$ complex at pH 3.50: log K_{app} = 2.14 ± 0.08. Under these conditions Cu^{2+} and H^+ compete for coordination to ε-adenosine; this competition is accounted for by eq 5.[69,70] The

$$\log K^M_{M(\epsilon\text{-Ado})} = \log K_{app} + \log (1 + [H^+]/K^H_{H(\epsilon\text{-Ado})}) \quad (5)$$

resulting constant (log $K^{Cu}_{Cu(\epsilon\text{-Ado})}$ = 2.80) is in excellent agreement with the value obtained from the potentiometric titrations (see Table V).

Attempts to determine the stability of Mg(ε-Ado)$^{2+}$ by spectrophotometric "competition experiments", i.e., by measuring the change in absorption of the Cu^{2+}/ε-adenosine system in dependence on added $Mg(ClO_4)_2$ (for details of the method see ref 68 and 71), again lead only to an upper limit for the stability constant (Table V).

^{1}H NMR shift measurements offer another independent way to determine the stability constants of complexes with fast exchanging diamagnetic metal ions. As Figure 4 shows, a marked downfield shift of the protons results upon addition of increasing

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(67) Three experiments were carried out with [ε-Ado] = 1.6 to 1.8 × 10^{-4} M and [$Cu(ClO_4)_2$] = 1.25 × 10^{-3} to 3 × 10^{-2} M; I = 0.1, $NaClO_4$; 25 °C.
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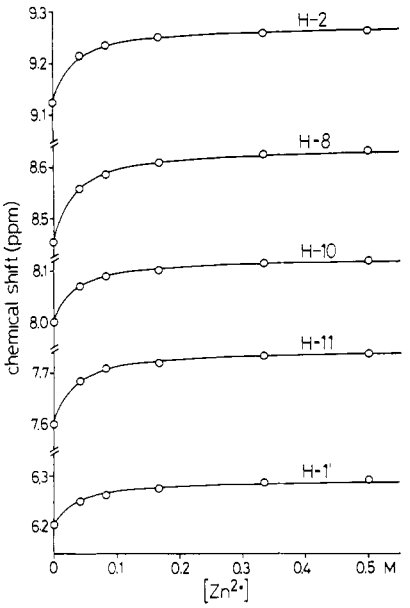

Figure 4. Variation of the chemical shift of H-2, H-8, H-10, H-11, and H-1′ of ε-adenosine with increasing concentrations of Zn^{2+} at pD 6.10. The solid curves shown are the computer-calculated best fits (with log K_{app} = 1.52, see text) of the experimental data, from eq 1–6 of ref 72 (cf. also ref 73); the results are summarized in Table VI. [ε-Ado] = 5×10^{-3} M; I = 0.1–1.5 M, $NaNO_3$; 27 °C; see also legend to Figure 1.

amounts of Zn^{2+} to a 5 mM solution of ε-adenosine at pD = 6.1, thus clearly indicating complex formation with the base moiety.[23,58] The experimental data could be perfectly fitted (Figure 4)[72,73] considering 1:1 complex formation. The limiting chemical shifts of the individual protons, together with the resulting values for the apparent stability constant, log K_{app}, at pD = 6.1 are listed in Table VI. The pD-independent stability constant of Zn(ε-adenosine)$^{2+}$ is obtained with eq 5 by using $pK^{D}_{D(\epsilon\text{-Ado})}$ = 4.64 (section 3): log $K^{Zn}_{Zn(\epsilon\text{-Ado})}$ = 1.54; this value perfectly agrees with the result from the potentiometric pH titrations (Table V).

The results of the ^1H NMR shift measurements (Table VI) give a first indication about the structure of the M(ε-adenosine)$^{2+}$ complexes. Though there is some uncertainty (see section 3), the downfield shifts, $\Delta\delta_{Zn}$*, observed for H-8 and H-11 point to a complexation of Zn^{2+} to N-6 and N-7 of ε-adenosine. That *chelate* formation occurs with ε-adenosine is confirmed by a comparison of the stability constants of the M(ε-adenosine)$^{2+}$ complexes with those of the corresponding M(adenosine)$^{2+}$ complexes, in which the metal ion is coordinated in a monodentate way preferably[74] to N-7;[3,23,43,63] the results listed in Table V show that the ε-adenosine complexes are more stable by about 2 orders of magnitude. The fact, that ε-adenosine is about three times more basic than adenosine (see section 3) can definitely not account for the high stability of the M(ε-adenosine)$^{2+}$ complexes. Therefore we conclude that ε-adenosine acts as a bidentate ligand, the donor atoms being N-6 and N-7.

This conclusion is confirmed by a comparison of the stability constants with those of the complexes of the monodentate pyridine (Table V). Pyridine is about 15 times more basic than ε-adenosine, but the pyridine complexes are by factors of about 3 *less* stable than the corresponding M(ε-adenosine)$^{2+}$ complexes. Hence, again a "phenanthroline-like" structure of the M(ε-adenosine)$^{2+}$ complexes is borne out.[75]

5. Stability and Structure of Ternary M(bpy)$^{2+}$ Complexes with Adenosine or 1,N^6-Ethenoadenosine. Mixed ligand complexes play an important role in biological systems.[79–81] To determine whether the steric restrictions resulting from the bidentate coordination of ε-adenosine still allow the formation of a stable ternary complex with another bidentate ligand, we measured the stability of the ternary Cu(bpy)(ε-Ado)$^{2+}$ complex (eq 6) by potentiometric pH

$$Cu(bpy)^{2+} + \epsilon\text{-Ado} \rightleftharpoons Cu(bpy)(\epsilon\text{-Ado})^{2+}$$

$$K^{Cu(bpy)}_{Cu(bpy)(\epsilon\text{-Ado})} = [Cu(bpy)(\epsilon\text{-Ado})^{2+}]/[Cu(bpy)^{2+}][\epsilon\text{-Ado}] \quad (6)$$

titration. The relative stability of a ternary complex is best quantified by a comparison with the stability of the binary complexes.[82–84] This is expressed by the stability difference, Δ log K (eq 7), which also quantifies the position of equilibrium 8.

$$\Delta \log K = \log K^{Cu(bpy)}_{Cu(bpy)(\epsilon\text{-Ado})} - \log K^{Cu}_{Cu(\epsilon\text{-Ado})} \quad (7)$$

$$Cu(\epsilon\text{-Ado})^{2+} + Cu(bpy)^{2+} \rightleftharpoons Cu(bpy)(\epsilon\text{-Ado})^{2+} + Cu^{2+}$$

$$10^{\Delta \log K} = [Cu(bpy)(\epsilon\text{-Ado})^{2+}][Cu^{2+}]/[Cu(\epsilon\text{-Ado})^{2+}][Cu(bpy)^{2+}] \quad (8)$$

The stability of the ternary Cu(bpy)(ε-Ado)$^{2+}$ complex is indeed quite significant; the stability constant being log $K^{Cu(bpy)}_{Cu(bpy)(\epsilon\text{-Ado})}$ = 2.46 ± 0.03 (I = 0.1, $NaNO_3$; 25 °C). This relatively high stability implies that even in the ternary complex ε-adenosine coordinates in a bidentate fashion. This conclusion agrees with Δ log K = −0.35, a slightly negative value as is expected on statistical considerations,[82,83] meaning that the position of equilibrium 8 is on the left side.

This result obtained with the ε-adenosine/Cu^{2+}/2,2′-bipyridyl system may be generalized in a twofold way: (i) It is to be expected that ε-adenosine is able to form mixed ligand complexes also with other metal ions and with ligands other than 2,2′-bipyridyl. (ii) On the basis of previous experience with mixed ligand complexes,[36,81,85] the stability of other ternary M(bpy)(ε-Ado)$^{2+}$ complexes may be estimated by subtracting 0.4 log unit from the stability constants of the binary complexes listed in Table V; the resulting estimate is expected to be correct within ±0.3 log unit.

The question now is how is the situation in ternary systems containing the monodentate adenosine, a metal ion, and 2,2′-bipyridyl? On the basis of results obtained with ternary systems containing the monodentate γ-picoline and the bidentate 2,2′-bipyridyl,[84] as well as on the general experience with mixed ligand complexes,[81–83] negative values for Δ log K (eq 7) are expected for the ternary M^{2+}/bpy/adenosine systems. Therefore, we may conclude for the metal ions listed in Table V that for the coordination of adenosine to M(2,2′-bipyridyl)$^{2+}$, log $K^{M(bpy)}_{M(bpy)(Ado)}$ < 0.8 (eq 6). However, this value is smaller than the stability constants of the stacking adducts between adenosine and 2,2′-bipyridyl (log $K^{(bpy)}_{(bpy)(Ado)}$ = 1.36 ± 0.06)[86] or M(2,2′-bipyridyl)$^{2+}$ (log $K^{[M(bpy)]}_{[M(bpy)](Ado)} \simeq$ 1.2 ± 0.2).[87,88] In other words, the stacking interaction between the aromatic ring systems is of a larger stability than the metal ion–adenosine interaction in these ternary systems. Consequently the ternary complexes have all about the

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(74) For exceptions, see ref 58.
(75) The fact that the M(ε-Ado)$^{2+}$ complexes (Table V) are not as stable as the corresponding M(phen)$^{2+}$ complexes[76,77] is most probably mainly due to the larger distance between N-6 and N-7 in ε-adenosine compared with N-1 and N-10 in 1,10-phenanthroline. This is evident from the angles N-6, C-6, C-5 and C-6, C-5, N-7 in ε-adenosine (see Chart I), which are 135.6° and 131.6°,[15] while the corresponding angles in phenanthroline are close to 120° (i.e., between 116.5° and 119.7°).[78]

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(87) The individual results (I = 0.1, $NaClO_4$; 25 °C)[88] for the stability of the stacking adducts between M(bpy)$^{2+}$ and adenosine are log $K^{[Co(bpy)]}_{[Co(bpy)](Ado)}$ = 1.33 ± 0.14, log $K^{[Ni(bpy)]}_{[Ni(bpy)](Ado)}$ = 1.25 ± 0.18, log $K^{[Cu(bpy)]}_{[Cu(bpy)](Ado)}$ = 1.20 ± 0.24, and log $K^{[Zn(bpy)]}_{[Zn(bpy)](Ado)}$ = 1.11 ± 0.27.
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Table VII. Chemical Shifts (ppm) of Some of the Protons of Free and L-Tryptophan-Stacked ε-Adenosine, Together with the Upfield Shift $\Delta\delta_{Trp}$* Resulting from Tryptophan-Adduct Formation, and Stability Constant, $K^{(\epsilon\text{-Ado})}_{(\epsilon\text{-Ado})(Trp)}$ (eq 9), of the Binary (ε-Adenosine)(L-tryptophan) Adducts in D_2O As Determined by ^1H NMR Shift Measurements[a]

H	chemical shift,[b] ppm		upfield shift[c]		
	ε-Ado	(ε-Ado)(Trp)	Δδ	$\Delta\delta_{Trp}$*	$K^{(\epsilon\text{-Ado})}_{(\epsilon\text{-Ado})(Trp)}$, M^{-1}
H-2	9.123 ± 0.005	7.88 ± 0.19	1.24 ± 0.19	1.30	5.64 ± 0.76
H-8	8.451 ± 0.001	8.11 ± 0.06	0.34 ± 0.06	0.38	7.51 ± 3.06
H-10	7.997 ± 0.002	7.45 ± 0.08	0.55 ± 0.08	0.59	5.45 ± 1.08
H-1'	6.204 ± 0.003	5.79 ± 0.07	0.44 ± 0.07	0.45	9.17 ± 2.88
					6.0 ± 1.1

[a] [ε-Ado] = 5×10^{-3} M; pD = 7.4; I = 0.1, $NaNO_3$; 27 °C (see Figure 5). The shift of H-11 could not be traced, as the H-11 signals are covered by Trp resonances. The range of error is *twice* the standard deviation. The average value of $K^{(\epsilon\text{-Ado})}_{(\epsilon\text{-Ado})(Trp)}$ is the weighted mean. [b] Calculated with $K^{(\epsilon\text{-Ado})}_{(\epsilon\text{-Ado})(Trp)}$ = 6.0 M^{-1}. The values of ε-Ado are somewhat smaller than the δ_0 values of Table I due to the small amount of self-association in 5×10^{-3} M solutions (section 1). [c] The upfield shifts, $\Delta\delta = \delta_{\epsilon\text{-Ado}} - \delta_{(\epsilon\text{-Ado})(Trp)}$, are somewhat influenced by $\delta_{\epsilon\text{-Ado}}$ as indicated in footnote *b*. Therefore the difference, $\Delta\delta_{Trp}$*, between δ_0 of Table I and the above $\delta_{(\epsilon\text{-Ado})(Trp)}$ was calculated as a better reflection of the effect of Trp adduct formation on monomeric ε-adenosine.

stability of the *binary* (bpy)(Ado) adduct,[86,88] the metal ion being *only* coordinated to the bipyridyl unit.

The stability of the stacking adduct between 1,N^6-etheno-adenosine and 2,2'-bipyridyl is expected (see section 6) to be of the same order as the corresponding adduct between adenosine and bipyridyl (i.e., log $K^{(bpy)}_{(bpy)(\epsilon\text{-Ado})} \simeq 1.4$). Hence, the structure of the ternary M(bpy)(ε-Ado)$^{2+}$ complexes with Co^{2+}, Ni^{2+}, or Cu^{2+} will be governed by the coordination tendency of these metal ions toward the N-6/N-7 unit of ε-adenosine, which is larger than the stacking tendency in the binary (bpy)(ε-Ado) adduct. This means that in these ternary complexes the metal ions are coordinated to ε-adenosine *and* to bipyridyl, thereby preventing a direct intramolecular ligand–ligand interaction within the ternary complex due to the geometry of their coordination spheres. This contrasts with the corresponding ternary systems containing Mg^{2+} or Mn^{2+}, where the structure will certainly be governed by the stacking interaction between the two ligands, while in the ternary system with Zn^{2+}, most probably an isomeric mixture of ternary complexes will exist: in some complexes Zn^{2+} will be bridging the two ligands, whereas in others a stack between the ligands will exist, with Zn^{2+} being coordinated only to bipyridyl.

6. Stability of the Stacking Adduct Formed between ε-Adenosine and L-Tryptophan. The structuring effects resulting from stacking interactions in mixed ligand metal ion systems as indicated in section 5, the occurrence of the indol moiety in many enzyme or enzyme-related systems,[27-29] and the fact that tryptophan quenches the fluorescence of ε-adenosine[30] and its derivatives[31] prompted us to study the association between ε-adenosine and L-tryptophan (Trp) by ^1H NMR shift measurements. The pD of the D_2O solutions was adjusted to 7.4; i.e., ε-adenosine is not protonated and tryptophan exists in its neutral zwitterionic form. Under these conditions no self-association of L-Trp was observed (up to a 70 mM solution);[33] a result in agreement with a study on tryptamine at pD = 7.9, where only insignificant self-association was found.[89] Hence, the stability of the ε-adenosine/tryptophan adduct may be defined as given in eq 9.

$$\epsilon\text{-Ado} + Trp \rightleftharpoons (\epsilon\text{-Ado})(Trp)$$

$$K^{(\epsilon\text{-Ado})}_{(\epsilon\text{-Ado})(Trp)} = [(\epsilon\text{-Ado})(Trp)]/[\epsilon\text{-Ado}][Trp] \qquad (9)$$

The experiments were carried out as described recently,[33,90] with [ε-Ado] = 5 mM, i.e., >91% of ε-adenosine existing in the monomeric unstacked form. The upfield shift of some of the proton resonances of ε-adenosine as a function of tryptophan is shown in Figure 5; the curves represent the computer-calculated best fit of the experimental data. The corresponding results are listed in Table VII.

The stability constant of the (ε-Ado)(Trp) adduct is given in Table VIII, together with the corresponding constants of some related stacks.[77,86,90,91] It is evident that an alteration in the overall charge of an adduct by replacing AMP^{2-} by ATP^{4-} (no. 2, 3) or adenosine by ATP^{4-} (no. 7, 8) does not significantly alter the stability of the stacks.[92] The stabilities of (Ado)(phen),

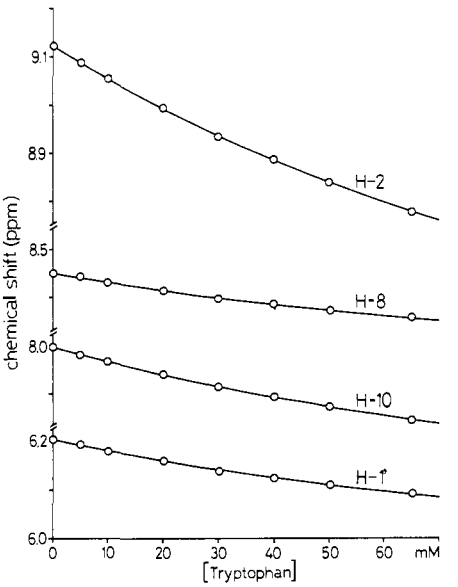

Figure 5. Variation of the chemical shift of H-2, H-8, H-10, and H-1' of ε-adenosine with increasing concentrations of L-tryptophan at pD = 7.40. The resonance of H-11 is covered by signals of tryptophan; therefore the H-11 shifts cannot be shown. The solid curves are the computer-calculated best fits of the experimental data using $K^{(\epsilon\text{-Ado})}_{(\epsilon\text{-Ado})(Trp)}$ = 6.0 M^{-1} (Table VII) and eq 3 of ref 73; the results are summarized in Table VII. [ε-Ado] = 5×10^{-3} M; I = 0.1, $NaNO_3$; 27 °C; see also legend to Figure 1.

Table VIII. Stability Constants, $K^{(A)}_{(A)(B)}$ (eq 9), of Some Binary Stacked Adducts Containing Nucleosides or Nucleotides[a]

no.	ref	(A)(B)	$K^{(A)}_{(A)(B)}$, M^{-1}	method/ionic strength
1	*b*	(ε-Ado)(Trp)	6.0 ± 1.1	NMR/0.1
2	90	(AMP^{2-})(Trp)	6.8 ± 1.6	NMR/0.1
3	33	(ATP^{4-})(Trp)	6.2 ± 1.2	NMR/0.1
4	90	(CMP^{2-})(Trp)	0.77 ± 0.35	NMR/0.1
5	86	(Ado)(bpy)	22.9 ± 2.1	UV/nat
	77		20	UV/0.1
6	77	(ATP^{4-})(bpy)	8.1 ± 1.8	NMR/0.1
	86		8.1 ± 2.8	UV/0.04–0.8
7	77	(Ado)(phen)	21.4	UV/0.1
8	77	(ATP^{4-})(phen)	28.2 ± 4.7	NMR/0.1
	77		15.5	UV/0.1
9	91	(uridine)(bpy)	~2	NMR/0.1
10	91	(UTP^{4-})(bpy)	~1	NMR/0.1

[a] The range of error is always *twice* the standard deviation. All ^1H NMR shift measurements were carried out in D_2O at 27 °C and the UV measurements in H_2O at 25 °C. The ionic strength was kept constant with $NaNO_3$, $NaClO_4$, or KNO_3. [b] This work.

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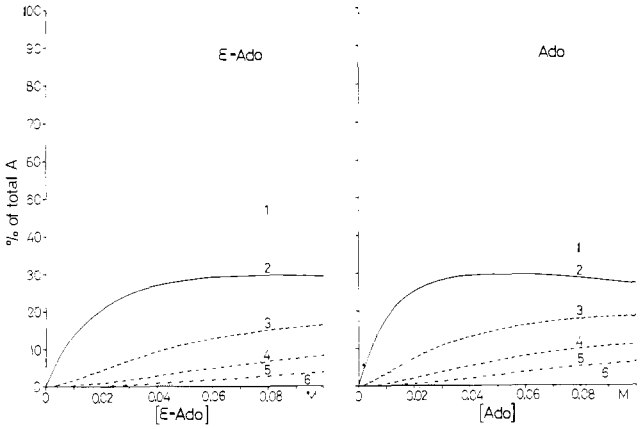

Figure 6. Variation of the proportions of ε-adenosine or adenosine (= A) present in the monomer (1), dimer (2), trimer (3), ..., and hexamer (6) in D_2O solutions in dependence on the total concentration of ε-Ado (K = 9.4 M^{-1}) or Ado (K = 15 M^{-1}) at 27 °C and I = 0.1 ($NaNO_3$).

(ATP^{4-})(phen), and (Ado)(bpy) (no. 5, 7, 8) are also rather similar, while (ATP^{4-})(bpy) is clearly less stable. As this observation can neither result from a difference in charge nor from the replacement of the three-ring system 1,10-phenanthroline by the two-ring system 2,2′-bipyridyl (cf. no. 5, 7, 8), it must result from a different orientation of the aromatic-ring systems in the stacks. This result corresponds with the fact that (ε-Ado)(Trp), (AMP^{2-})(Trp), and (ATP^{4-})(Trp) (no. 1–3) have the same stability, despite the presence of a third ring in ε-adenosine. However, replacement of the purine ring system (no. 1–3, 5–8) by a pyrimidine ring (no. 4, 9, 10) reduces the stability of all stacks drastically.

The overall result obtained now parallels that obtained for the self-association (section 1): the ε-adenine residue, despite its third ring, shows no increased stacking tendency, compared with that of the adenine residue. It is clear, however, that the ε-adenine residue in nucleotides will be able to participate in suitable ternary complexes in intramolecular ligand–ligand interactions, either by stacking with another aromatic-ring system or by hydrophobic interactions with an alkyl group, as it has been demonstrated for ternary adenine nucleotide complexes.[32-34,82]

General Conclusions

The results presented show that the tendency for self-stacking decreases from adenosine to ε-adenosine (section 1); it appears that this is mainly a result of the orientation within the stacks. Indeed, there are indications that in the corresponding nucleotides the stacking tendencies are much lower (due to the repulsion between the negatively charged phosphate residues)[23] but also more similar,[93] suggesting again that the orientation of the stacks plays a role. In any case, in experiments that are designed to quantify properties of the monomers, low concentrations must be used. With the association constants listed in Table I, it is possible to calculate the variation in the proportions of the various oligomers as the concentration is changed. The results of such calculations are summarized in Figure 6 for ε-adenosine and adenosine: in 10^{-2} M solutions only 85% of ε-Ado and 78% of Ado exist in the monomeric form; for 5×10^{-3} M solutions these values are 92% and 87%, while in 10^{-3} M solutions more than 98% of ε-adenosine is present in the monomeric form; to achieve the same degree of free monomer formation with adenosine, the concentration must be as low as 6×10^{-4} M. It may be noted that the self-association tendency of protonated or metal ion complexed ε-adenosine is much lower (section 2).

To a first approximation, one may conclude, despite the differences evident from Figure 6, that the self-stacking tendencies of ε-adenosine and adenosine are similar. This also holds for the formation of stacks with the indole moiety of tryptophan (section 6). The basicity of ε-adenosine and adenosine is also not very different (section 3). Hence, in systems where these properties are important, the ε-adenine moiety may well substitute for the adenine moiety, provided there are no steric restrictions regarding space and orientation.

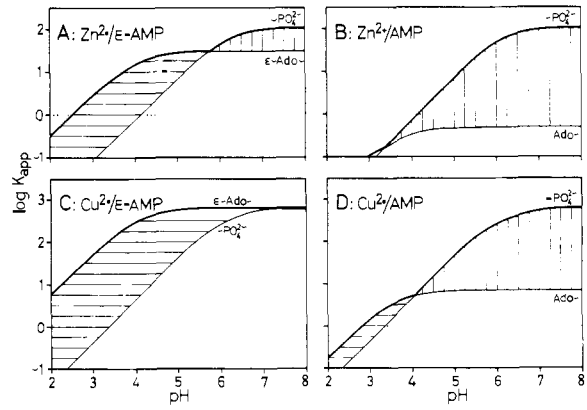

Figure 7. Comparison of the metal ion affinities of the 1,N^6-ethenoadenosine residue (ε-Ado-), the unmodified adenosine residue (Ado-), and the phosphate moiety ($-PO_4^{2-}$) occurring in 1,N^6-ethenoadenosine 5′-monophosphate (ε-AMP) or adenosine 5′-monophosphate (AMP) as quantified by the apparent stability constants, log K_{app}, in dependence on pH for Zn^{2+} and Cu^{2+}. Calculated according to eq 5 with the values of Table V, $pK^{H}_{H(Ado)}$ = 3.6 (section 3) and the following constants for uridine 5′-monophosphate (UMP) (which coordinates only through its phosphate group[3,23]): $pK^{H}_{H(UMP)}$ = 6.15, log $K^{Cu}_{Cu(UMP)}$ = 2.80, and log $K^{Zn}_{Zn(UMP)}$ = 2.03.[93]

These results contrast sharply with the metal ion coordinating properties of ε-adenosine and adenosine: M(ε-adenosine)$^{2+}$ complexes are more stable by a factor of about 100 (section 5, Table V). This result is certainly also reflected in the metal ion binding properties of the corresponding nucleotide derivatives. As the acidity constants of protonated base moieties ($pK_a \sim 4$) and phosphate groups ($pK_a \sim 6$) differ significantly, the coordination of a metal ion to the one or to the other binding site will be strongly pH dependent.[94]

This is illustrated in Figure 7 for the two nucleoside 5′-monophosphates, ε-AMP and AMP, by comparing independently their two sites with metal ion binding properties: the affinity of the ε-adenosine moiety for Zn^{2+} at low pH in ε-AMP is about 1.5 log units larger than that of the phosphate residue (Figure 7A), while in the upper pH range the affinity of the phosphate group dominates by about 0.5 log unit. Figure 7B shows that the phosphate group dominates the coordinating properties of AMP, allowing only a marginal influence for the adenosine residue. A similar situation exists with Co^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+} (Table V), where the coordinating properties of the ε-adenosine moiety in ε-AMP are so pronounced (for Cu^{2+} see Figure 7C) that they still equal those of the phosphate group even at pH ≥7, while the adenosine residue in AMP has again only a marginal influence (Figure 7D).

One has to expect that the metal ion coordinating properties of ε-AMP^{2-} are rather different from those of AMP^{2-}, and that protonated M(H·ε-AMP)$^+$ complexes are formed in appreciable amounts. The enhanced metal ion affinity of the modified base moiety will also considerably promote the simultaneous coordination of a metal ion to the base and phosphate groups (possibly with a bridging water molecule[3]); hence, the degree of macrochelate formation is expected to be quite large. The differences between the corresponding di- and triphosphates, i.e., ε-ADP and ε-ATP vs. ADP and ATP, are possibly less pronounced because the metal ion affinities of the di- and triphosphate groups are much larger, and a possibly formed macrochelate has more links in its ring; this means that the greater metal ion affinity of the ε-

(92) This conclusion holds only as long as one of the molecules in the stack is neutral; see ref 32 and 90.
(93) Sigel, H.; Scheller, K. H., results to be published.
(94) This was also shown for adenine nucleotide N^1-oxide complexes: Sigel, H. *Met. Ions Biol. Syst.* **1979**, *8*, 125–58.

adenosine moiety compared with that of the adenosine residue is expected to be overruled to some extent by the even greater affinities of the di- and triphosphate groups.

Predictions for mixed ligand complexes (section 5) are more difficult, as certain groups may be released from the coordination sphere of a metal ion due to the participation of an additional ligand. This could often mean that ternary complexes with ε-adenine nucleotides are of relatively low stability compared with their binary parent complexes (eq 7 and 8). However, in cases where an intramolecular ligand–ligand interaction is possible[32-34,82] between aromatic-ring systems, or a hydrophobic interaction with an alkyl residue, the ε-adenine moiety is expected to be about as effective as the adenine group itself.

Overall one may conclude that in systems which involve metal ions and ε-adenine nucleotides, the structural arrangements will often be altered, mainly due to the increased metal ion affinity of the modified base residue, so that conclusions reached with ε-adenosine derivatives should be transferred to the unaltered nucleotide system with reservations.

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Registry No. Mg, 7439-95-4; Mn, 7439-96-5; Co, 7440-48-4; Ni, 7440-02-0; Cu, 7440-50-8; Zn, 7440-66-6; Cd, 7440-43-9; 1,N^6-etheno-adenosine, 39007-51-7; ε-adenosine L-tryptophan adduct, 85048-85-7.

Preparation and Properties of Dinitrogen Trimethylphosphine Complexes of Molybdenum and Tungsten. 4. Synthesis, Chemical Properties, and X-ray Structure of *cis*-$[Mo(N_2)_2(PMe_3)_4]$. The Crystal and Molecular Structures of *trans*-$[Mo(C_2H_4)_2(PMe_3)_4]$ and *trans,mer*-$[Mo(C_2H_4)_2(CO)(PMe_3)_3]$

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Abstract: The complex *cis*-$[Mo(N_2)_2(PMe_3)_4]$ (**1**) has been prepared by reduction of $[MoCl_2(PMe_3)_4]$ with dispersed sodium under dinitrogen. Loss of ligating dinitrogen in **1** readily occurs by oxidation with alkyl halides, RX (X = Cl, R = Me_3SiCH_2; X = Br, R = Et; X = I, R = Me), to yield the monomeric Mo(II) halo derivatives *trans*-$[MoX_2(PMe_3)_4]$ (X = Cl, Br, and I) or by substitution with (i) carbon monoxide to give $[Mo(CO)_x(PMe_3)_{6-x}]$ complexes (x = 2,3), (ii) trimethylphosphine under argon to afford $[Mo(N_2)(PMe_3)_5]$ (**2**), or (iii) ethylene to yield *trans*-$[Mo(C_2H_4)_2(PMe_3)_4]$ (**3**). One of the phosphine ligands in **3** can be easily exchanged by CO to form *trans,mer*-$[Mo(C_2H_4)_2(CO)(PMe_3)_3]$ (**4**). The structures of complexes **1**, **3**, and **4** have been determined by X-ray crystallography. **1** crystallizes in the monoclinic space group $P2_1/c$ with unit cell parameters a = 9.371 (4) Å, b = 15.890 (6) Å, c = 16.692 (7) Å, β = 106.58 (4)°, and D_c = 1.27 g cm^{-3} for Z = 4. Least-squares refinement based on 1720 independent observed reflections led to a final R value of 0.038. Complex **3** belongs to the monoclinic space group $P2_1/n$ with a = 10.165 (3) Å, b = 13.683 (3) Å, c = 17.139 (4) Å, β = 98.84 (3)°, and D_c = 1.29 g cm^{-3} for Z = 4. The final R value based on 2715 observed reflections was 0.043. **4** is also monoclinic, crystallizing in the space group $P2_1/n$ with a = 10.637 (3) Å, b = 13.069 (3) Å, c = 15.201 (4) Å, β = 98.45 (2)°, and D_c = 1.30 g cm^{-3} for Z = 4. It was refined to a final R value of 0.037 on the basis of 2764 independent observed reflections. In **1** the cis N ligands are coordinated to the molybdenum atom at a Mo–N bond distance of 1.97 (1) Å. In **3** and **4** the planes formed by the two carbons of each of the trans ethylene ligands and the Mo atom are perpendicular and eclipse the trans P–Mo–P bonds. The average Mo–C(ethylene) distance is 2.270 (5) Å in **3** and 2.29 (3) Å in **4**.

Molecular dinitrogen complexes of molybdenum have received considerable attention in the past few years in the hope of finding model systems for the binding of N_2 and subsequent transformation into ammonia and amines.[1,2] The complexes studied generally contain tertiary phosphine as coligands, particular attention having been devoted to *trans*-$[Mo(N_2)_2(dppe)_2]$ (dppe = 1,2-bis(diphenylphosphine)ethane). Although up to four groups of dinitrogen complexes of molybdenum can be envisaged,[3] for zerovalent molybdenum they are basically of two types,[4] $[M(N_2)_2P_4]$ and $[M(arene)P_2]_nN_2$ (n = 1, 2) (P = phosphorus donor, either mono- or bidentate phosphine). The range of complexes

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