(7)G-N(3)C platinum chelation seems very unfavorable for GpC in the anti,anti conformation and that in this highly constrained structure one would expect the H(8)G to be upfield shifted by the ring current of the cytosine, which is not the case. On the contrary, N(7)G-N(3)C chelation is easily achieved with the guanine ring in the syn conformation, leading to a left-handed GpC[Pt] chelate. Such a chelate fits the ^{1}H NMR data (Figure 7b); however, the H(8) of the guanine appears to be only very slowly exchangeable with D_2O at pD 11.3. It is noteworthy that no or slight deuterium exchange of a guanine H(8) at pD 12 has also been reported in the case of *trans*-$[Pt(NH_3)_2(5'\text{-GMP})_2]^{2-}$, from Raman and ^{1}H NMR data.[26] If one compares these results to the exchange that we did observe for GpG[Pt], one can note that the CPK model of the left-handed GpC[Pt] complex shows that the H(8)G is in close proximity with the O(2) of the cytosine, a situation comparable to that of the two H(8)G of the *trans*-bis(5'-GMP) complex which are close to the O(6) of the other guanine, due to the "head-to-tail" arrangement of the bases. Such a proximity might contribute to the slowing down of the H(8)G deuterium exchange.

The analysis of the mixture from the ApC reaction reveals preferential binding of cytosine over adenine with competitive N(1) and N(7) binding for the latter. Owing to the complexity of the ^{1}H NMR spectrum of the mixture and to the nonconservative character of its CD signal, no conclusion can be drawn concerning the presence of a chelated platinum complex without separation of the components.

Conclusion

Among the five studied dinucleoside monophosphates, IpI, GpG, ApA, GpC, and ApC, reacting with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, the first three homodinucleotides have a geometry leading to N(7)-N(7) chelation of the metal. IpI and GpG give a single N(7)-N(7) chelated complex while ApA also gives other products due to competitive N(1) binding to the metal. GpC and ApC lead to mixtures of several complexes and in both cases cytosine appears to have more affinity for the platinum of the diaquo complex than do guanine and adenine.

The CD data in relation with the ^{1}H NMR data, particularly in the cases of the $[Pt(NH_3)_2(IpI)]^+$ and $[Pt(NH_3)_2(ApA)]^+$ complexes, show that a significant interaction exists between the two purines which are in a mutual orientation different from parallelism.

In the case of GpC one of the complexes appears as an N-(7)G-N(3)C platinum chelate, the CD of which suggests a left-handed helical arrangement of the bases.

As far as the perturbation of the DNA structure upon binding of the *cis*-$(NH_3)_2Pt^{II}$ moiety is concerned,[4,38,63] our results show that an efficient cross-linking of two adjacent guanines could be favored and contribute to the characteristic enhancement of CD ellipticity, at ca. 275 nm, observed at low Pt/DNA(P) ratios, bringing further support to the hypothesis of intrastrand cross-linking.[29,64] Because of the stereochemical constraints inherent in this type of interaction, such a chelation of two adjacent guanines could only occur after a local denaturation of DNA. This could be the result of a primary interaction of one base with the *cis*-diamminediaquoplatinum(II) complex,[12,13,29,36,63] or of a preexisting localized premelted region of kinked DNA.[65,66] Moreover, it seems that an N(7)G-N(3)C cross-linking of adjacent guanine and cytosine could occur for a left-handed segment of the polynucleotide.[62]

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Supplementary Material Available: ^{1}H NMR of GpG[Pt] (Figure 10); LC chromatograms of the ApA, GpC (including the separated a-c fractions), and ApC reaction mixtures (Figure 11); CD spectra of GpC[Pt] (fraction b) from pH 1.2 to 11.1 (Figure 12); Sephadex analysis, ^{1}H NMR (Figure 13), and CD data of the ApC mixture (6 pages). Ordering information is given on any current masthead page.

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Intermolecular Association of 1,N^6-Ethenoadenosine in Aqueous Solution. Vapor Pressure Osmometric and Heat of Dilution Studies

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Abstract: Self-association of 1,N^6-ethanoadenosine (εAdo) in aqueous solution was studied by vapor pressure osmometry (VPO) and heat of dilution measurements. The association process was found to be accounted by an isodesmic (indefinite) noncooperative self-association model and a simple monomer-dimer reaction cannot account for the data based on VPO experiments. The intermolecular stacking equilibrium quotient was determined to be 18.7 ± 0.8 M^{-1}. Thus, the self-complexing affinity is increased on going from adenosine (Ado) to εAdo. The enthalpy of self-association was also determined from a solution calorimetric dilution study: $\Delta H^\circ = -35.6 \pm 1.0$ kJ/mol.

εAdo[2] was prepared by Barrio et al.[3] in 1972, although the first derivative having the imidazo[2,1-*i*]purine skeletal structure was reported by Kochetkov et al.[4] a year earlier. The presence of a fused imidazole ring alters the properties of the adenine ring

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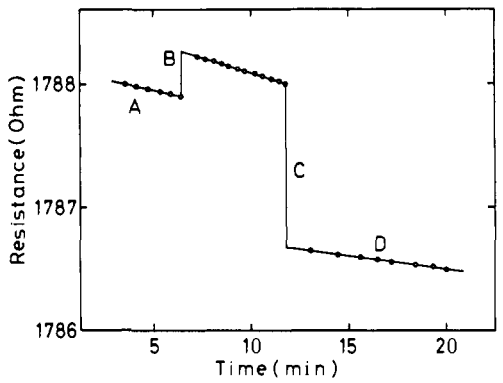

Figure 1. A typical example of thermistor resistance vs. time profile obtained during a heat of dilution determination for ϵAdo at 25 °C: (A) foreperiod; (B) resistance change upon the dilution (m_i = 0.078 21 M → m_f = 0.002 563 M; a change in resistance occurred upon ampule breakage and a correction of 24 mJ was made to account for the heat of ampule breakage in the data analysis); (C) electrical calibration; (D) afterperiod.

considerably. Unlike the parent compound, ϵAdo exhibits high fluorescence properties. During the initial period interest was focused on the fluorescence nature of the ϵAde moiety. In particular, Leonard and his co-workers[5,6] and Penzer[7] carried out work on identifying the species responsible for the fluorescence of this moiety. Although interest in this area continued[8,9] after their publication, the main aim was to use ϵAdo and its nucleotide derivatives such as ϵADP, ϵATP, ϵFAD, etc. as fluorescence probes in numerous investigations of biological systems. The considerable amount of work carried out in this direction is obvious from the recent review article by Stryer.[10]

Nucleic acid bases tend to associate in aqueous solution both intermolecularly and intramolecularly. This mode of association is referred to as "stacking" and a wide variety of the arsenal of spectroscopic and other physical methods has been utilized in order to study the stacking nature of nucleic acid bases and their analogues. Recently, for ϵApϵA the conformation and conformational stability have been studied by different physical methods.[11-13] In this connection it is our intention to present the results of the thermodynamic study on the intermolecular association of ϵAdo here and those on the intramolecular stacking of ϵApϵA in the following paper.[14]

Experimental Section

Materials. ϵAdo was prepared as described in the literature,[6] and it was found to be chromatographically and spectrophotometrically pure.[8] All other reagents were of analytical grade and used without further purification.

Methods. A. VPO Measurements. The method used to measure vapor-pressure lowering was similar to that of Broom et al.[15,16] All measurements were made using a Hitachi Corona 117 vapor pressure osmometer at 25 °C (we thank Dr. Y. Morita, the National Chemical Laboratory for Industry, for making the apparatus available to us and advising us on its operation). An NaCl solution was used as a reference:[17] NaCl (M) (ϕ) 0.010 735 (0.9685), 0.026 858 (0.9540), 0.049 346 (0.9440), 0.107 347 (0.9317), 0.155 923 (0.9272). Solubility limitations prevent us from obtaining data in a range of concentration higher than about 0.083 M in ϵAdo.

B. Heat of Dilution Measurements. Calorimetric measurements were carried out at 25 °C with a 8721-1 solution calorimeter of the precision calorimetry system (LKB Produkter AB) and by methods essentially the same as described previously.[18] The accuracy and precision of the calorimeter had been verified by determining the heat of solution of tris(hydroxymethyl)aminomethane (NBS standard reference material no. 724) in 0.100 M HCl, and found to be in good agreement with the literature value. Electrical calibrations were performed after each individual experiment (see Figure 1, for example). An initial solution was prepared by dissolving 0.135 59 g of ϵAdo in 5.954 65 g of pure water.

Table I. Molal Osmotic Coefficients of ϵAdo at 25 °C

molal concn	osmotic coefficient (ϕ)		std dev
0.0257	0.722	0.746	±0.03
	0.770		
	0.746		
0.0516	0.613	0.620	±0.007
	0.626		
	0.622		
0.0824	0.544	0.541	±0.004
	0.538		

Table II. Intermolecular Stacking Equilibrium Quotients for ϵAdo, Ado, and *lin*-Benzo-AMP at 25 °C[a]

	K, M^{-1}	ref
ϵAdo	18.7	
Ado	4.5	16
lin-benzo-AMP	42[b]	20

[a] 1,N^6-Ethenoadenosine data were determined in this study. Other values were taken from the literature. [b] Determined at 28 ± 1 °C.

Table III. Heat of Dilution of ϵAdo Solution with Water at 25 °C (Initial Concentration, m_i = 0.078 21 M)

expt	final concn, m_f, M	heat of dilution,[a] kJ/mol
1	0.002 563	−8.61
2	0.003 923	−7.54
3	0.006 981	−6.37
4	0.009 359	−5.27
5	0.011 871	−4.87
6	0.013 726	−3.51

[a] Minus sign indicates the heat effect upon dilution being endothermic.

Results and Discussion

Evaluation of the Intermolecular Association Quotient at 25 °C from the Data Based on VPO Measurements. The osmotic coefficients (ϕ) at 25 °C for the aqueous solutions of ϵAdo are shown in Table I. The data were analyzed in terms of the isodesmic self-association model. On this model the relationship between the osmotic coefficient and the stoichiometric molality of ϵAdo can be written as[15,19] $(1 - \phi) = Km\phi^2$, where K is the intrinsic molar association constant. The mean value of K obtained, using this relationship, is K = 18.7 ± 0.8 M^{-1} at 25 °C.

(1) (a) University of Tokyo. (b) Research Institute for Polymers and Textiles.
(2) Abbreviations following the IUPAC–IUB Commission on Biochemical Nomenclature 1971 recommendations (*J. Mol. Biol.* **1971**, *55*, 299–305) are used. The abbreviation "ϵ" denotes etheno, so that ϵAdo is 1,N^6-etheno-adenosine and ϵFAD is flavin 1,N^6-ethenoadenine dinucleotide.
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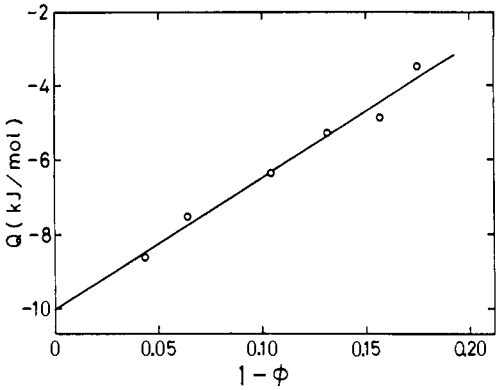

Figure 2. The molar heat of dilution (Q) as a function of $1 - \phi$. The solid line is the best fit of the data to eq 3.

Listed in Table II are the self-association constants for ϵAdo, Ado,[16] and *lin*-benzo-AMP[20] for comparison. A comparison of the intermolecular stacking association constant of ϵAdo with the value for Ado shows that the stacking interaction is at least four times stronger for ϵAdo. This seems to be again due to the strengthening of the stacking of π interactions by the additional ring in ϵAdo as is the case with the *lin*-benzoadenine nucleotide series,[20] though the effect of an additional ring on stacking is far more marked in going from the adenine to *lin*-benzoadenine nucleotide system. The same effect on intramolecular stacking association was also observed in the 1,N^6-ethenoadenosine dinucleoside phosphates.[11,14]

Determination of Thermodynamic Quantities for Intermolecular Stacking Equilibria of ϵAdo from Heat of Dilution Data at 25 °C. Now, it would be profitable to determine the enthalpy of self-association of ϵAdo in water by combining the above-estimated value of the equilibrium quotient, K, with the calorimetric data obtainable from heat of dilution measurements. A typical profile of the measured thermistor resistance of the dilution process for ϵAdo solution vs. time is reproduced in Figure 1. The results of heat of dilution measurements are shown in Table III.

The enthalpy of intermolecular stacking association can be related to the heat of infinite dilution per mole (Φ_L) from a given concentration and the osmotic coefficient (ϕ) by[21,22]

$$\Delta H^\circ = \Phi_L/(1 - \phi) \quad (1)$$

Letting the heat of dilution per mole from an initial concentration (m_i) to a final concentration (m_f) be Q, and letting the heats of infinite dilution per mole from m_i and m_f be ΔH_∞ and Φ_L, respectively, we may write

$$Q = \Delta H_\infty - \Phi_L \quad (2)$$

The combination of eq 1 and 2 yields the following form:

$$Q = \Delta H_\infty - \Delta H^\circ(1 - \phi) \quad (3)$$

With the equilibrium quotient, K, known, one can calculate $1 - \phi$ for each concentration using

$$\phi = \frac{(1 + 4Km)^{1/2} - 1}{2Km} \quad (4)$$

Then, from a plot of Q against $1 - \phi$ it is possible to determine the value of ΔH° from the slope (Figure 2). The value of ΔH° thus obtained is $\Delta H^\circ = -35.6 \pm 1.0$ kJ/mol. With the value of $K = 18.7 \pm 0.8$ M^{-1}, a value of $\Delta S^\circ = -95 \pm 3.5$ J mol^{-1} deg^{-1} is obtained. The relation to other work is to be discussed in the following paper.[14]

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Intramolecular Association of 1,N^6-Ethenoadenylyl-($3' \rightarrow 5'$)-1,N^6-ethenoadenosine (ϵApϵA). A Comparison of Intramolecular Stacking Equilibrium Quotients Estimated by Different Methods

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Abstract: Two approaches were taken to determine the intramolecular stacking equilibrium quotients in aqueous solution at 25 °C for 1,N^6-ethenoadenylyl-(3'→5')-1,N^6-ethenoadenosine (ϵApϵA), one involving measurements of the temperature dependence of the ultraviolet absorption spectrum and the other, measurements of the ionization constants of ϵApϵA and the component monomers. The values estimated by these two alternative methods are in agreement with the previous finding based on fluorescence techniques,[2] strongly indicating the validity of the so-called "two-state model" for the intramolecular stacking equilibrium system of ϵApϵA. These values of the equilibrium quotient for the intramolecular stacking association of ϵApϵA are also compared with that for the corresponding intermolecular association.

The 1,N^6-etheno derivative of ApA,[1] ϵApϵA, involves literature discrepancies with regard to the degree of intramolecular stacking:[2-4] Tolman et al.[2] first reported that in neutral aqueous solution the intramolecular stacking interactions in ϵApϵA were stronger than those in the parent unmodified dimer, ApA. Lee and Tinoco[3] recently estimated the percent stacking to be roughly the same

(1) Abbreviations following the IUPAC-IUB Commission on Biochemical Nomenclature 1971 recommendations are used. The abbreviation "ϵ" denotes etheno, so that ϵAdo is 1,N^6-ethenoadenosine and ϵFAD is flavin 1,N^6-ethenoadenine dinucleotide.

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