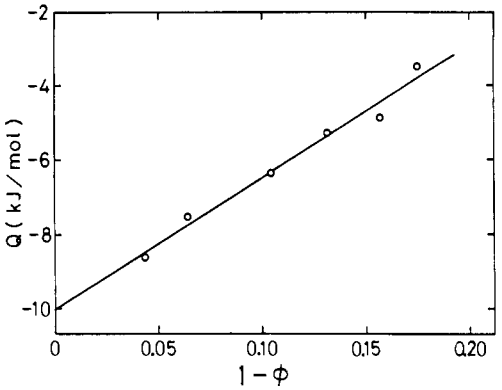


Figure 2. The molar heat of dilution (Q) as a function of $1 - \phi$. The solid line is the best fit of the data to eq 3.

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]

Acknowledgments. We wish to thank Dr. Y. Morita, the National Chemical Laboratory for Industry, Tsukuba, who generously made available a Hitachi-Corona 117 vapor pressure osmometer for the VPO measurements. We also thank Mr. T. Kuramochi for providing ϵAdo used in this study.

(20) Barrio, J. R.; Liu, F.-T.; Keyser, G. E.; VanDerLijn, P.; Leonard, N. J. *J. Am. Chem. Soc.* **1979**, *101*, 1564-1569.

(21) Stoesser, P. R.; Gill, S. J. *J. Phys. Chem.* **1967**, *71*, 564-567.

(22) Gill, S. J.; Downing, M.; Sheats, G. F. *Biochemistry* **1967**, *6*, 272-276.

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

Yasuo Inoue,* Takao Kuramochi, Michiharu Sakurai, and Ichiro Tazawa

Contribution from the Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo 113, Japan. *Received March 5, 1980*

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.

(2) Tolman, G. L.; Barrio, J. R.; Leonard, N. J. *Biochemistry* **1974**, *13*, 4869-4878.

(3) Lee, C.-H.; Tinoco, I., Jr. *Biochemistry* **1977**, *16*, 5403-5414.

(4) Baker, B. M.; Vanderkooi, J.; Kallenbach, N. R. *Biopolymers* **1978**, *17*, 1361-1372.

0002-7863/80/1502-5574$01.00/0

as that for ApA. More recently, Baker et al.[4] showed that the stacking in ϵApϵA was weaker than in ApA. This paper is thus concerned with the determination of the intramolecular stacking equilibrium quotients of this particular dimer by the two alternative methods (one the titration method[5] and the other the thermal denaturation method[6]) and these constants are compared with the corresponding values reported in the literature. Though the value estimated by the thermal denaturation method is somewhat lower than that estimated by the titration method, the values obtained by these two independent procedures agreed satisfactorily. Our values are in good agreement with that found by Tolman et al.[2] and are not in favor of the previous conclusion drawn by Baker et al.[4]

In the preceding paper[7] we have reported the intermolecular stacking association of ϵAdo in aqueous solution, and a comparison with the intra- and intermolecular stacking equilibrium quotients is also made here.

Experimental Section

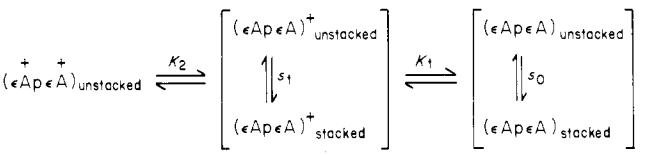
Materials. ApAp and distilled chloroacetaldehyde (50 equiv) at pH 4.5 were kept at room temperature for 6 days. After purification by a column of DEAE-Sephadex A-25 (HCO_3^- form) anion exchanger, ϵApϵAp was dephosphorylated with *E. coli* alkaline phosphatase to ϵApϵA. The purity of ϵApϵA thus obtained was checked by paper chromatography and paper electrophoresis and ascertained by the formation of ϵAdo and 3'-ϵAMP in 1:1 ratio when treated with calf spleen phosphodiesterase. The molar extinction coefficient of ϵApϵA was calculated from the hyperchromicity at 275 nm upon the above hydrolysis (hypochromicity at 275 nm is 18.5% at 25 °C and pH 7.0). The extinction coefficients of ϵAdo and 3'-ϵAMP were taken from the literature.[8] ApAp was prepared by the method as reported in the previous paper.[9]

Methods. A. p*K* Determinations of Nucleoside, Mononucleotides, and Dinucleoside Phosphate. All measurements were made by the spectrophotometric method at 25 °C and ionic strength 0.10 M. Changes in absorbance as a function of pH, in the pH range 1–7, were measured as difference spectra, $\Delta A = A(\text{pH}) - A(\text{pH } 7.0)$. Data analysis was performed by the iterative least-squares method used in this laboratory.[5] Actual computations were carried out at the Computer Center of the University of Tokyo.

B. Thermal Denaturation of ϵApϵA and Data Analysis. The unknown quantities $\Delta H°$, $\Delta S°$, ϵ_u, and ϵ_s defined by eq 5 are determined from the values of the difference in absorbance between sample (ϵApϵA) cell (l = 1.00 cm) and a reference cell of the same optical path containing ϵAdo at the same concentration (about 2.2×10^{-4} mol/L) as the sample cell with varying temperature from 4 to 81 °C. The solutions were thermostated to ±0.2 °C (Komatsu Electronics Inc.). Temperature was measured within the cells with a chromel–alumel thermocouple. A dry-nitrogen purge of the sample compartment avoided condensation of water vapor on the cell windows when the cell temperature was below room temperature. Changes of the difference in absorbance at selected wavelengths were recorded by applying the electronic output of the spectrophotometer, after amplification, to a recording milliammeter (Watanabe Sokki Co.). For each solution, more than 70 measurements were performed in the temperature range 4–80 °C. After the highest temperature was reached, reversibility was checked by cooling down to 4 °C. Both fast heating and cooling curves were reversibly obtained, and ϵApϵA could be carried out through repeated denaturation cycles without significant decomposition (The curve was retraced two to three times within experimental error.) At the concentrations used, the effect of intermolecular self-association is considered to be negligible. Based on the isodesmic self-association model, for a solution of, say, 2.24×10^{-4} mol L^{-1} $base^{-1}$ and K_{inter} = 20 L/mol, monomer, ϵApϵA, is calculated to account for as high as 99.11% of the initial ϵApϵA [ϵApϵA, 99.11%; $(\epsilon\text{Ap}\epsilon\text{A})_2$, 0.88%; $(\epsilon\text{Ap}\epsilon\text{A})_3$, <0.01%].

Data analysis was carried out with our computer program written in Fortran for use on a HITAC 8800/8700 computer at the Computer Center of the University of Tokyo as described previously.[6] The formalism and further detail are available in ref 6. The errors in s_0, $\Delta H°$, and $\Delta S°$ were calculated from the standard deviation of the mean.

Scheme I



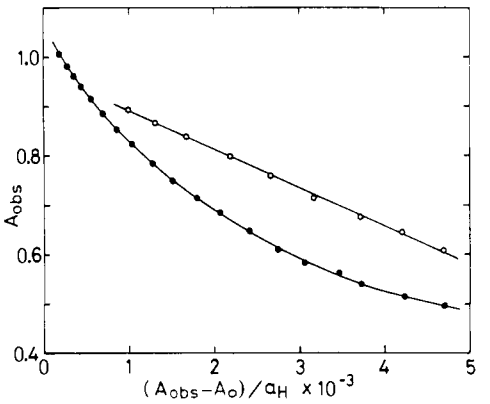


Figure 1. A_{obsd} vs. $(A_{obsd} - A_0)/a_H$ plots at 272.5 nm, showing a single-stage protonation for ϵAdo (-O-O-O-) and a non-single-stage protonation for ϵApϵA (-●-●-●-). A_0 and a_H are the absorbance of the molecular species (at pH 7.0) and the hydrogen ion activity, respectively.

Results and Discussion

In a series of previous papers,[5,6,10-12] we reported acid–base and intramolecular stacking equilibrium studies of some dinucleoside monophosphates. It is now interesting to consider the evaluation of the intramolecular stacking association constants of a particular dimer ϵApϵA by a couple of alternative methods because two laboratories have published rather conflicting reports concerning the degree of internal association.[2,4] Stacking is a facile reversible reaction involving relatively weak forces of interaction. Since it is not known a priori that the system studied yields a unique intramolecularly stacked conformation[13-15] the data are treated as if the simple situation existed, so that the system is considered to be at equilibrium between two sets of states or configurations of the molecules: stacked or unstacked, at any temperature or solution condition.[16-18] Whether or not this is the case with ϵApϵA, the stacking equilibrium quotient must be estimated by comparative methods.

Evaluation of Intramolecular Stacking Equilibrium Quotients from Titration Data. We have shown that the predominant site of protonation is N9 in ϵAdo.[19] Protonation may be expected to occur preferentially at the corresponding sites in the dimer, ϵApϵA, from analogy with the known case of GpG;[10] however, equilibria may exist involving nonprotonated, monoprotonated, and diprotonated forms. It has been suggested[5,10,11] that dinucleoside monophosphates undergo stepwise protonation, so that ϵApϵA is also subject to the equilibria illustrated in Scheme I.

Ultraviolet absorption spectral changes of ϵApϵA with pH did not exhibit clear isosbestic points between pH 1 and 7. The lack

(5) Ogasawara, N.; Inoue, Y. *J. Am. Chem. Soc.* **1976**, *98*, 7048–7053.
(6) Ogasawara, N.; Inoue, Y. *J. Am. Chem. Soc.* **1976**, *98*, 7054–7060.
(7) Sakurai, M.; Morimoto, S.; Inoue, Y. *J. Am. Chem. Soc.*, preceding paper in this issue.
(8) Secrist, J. A., III; Barrio, J. R.; Leonard, N. J.; Weber, G. *Biochemistry* **1972**, *11*, 3499–3506.
(9) Satoh, K.; Inoue, Y. *Biochem. J.* **1969**, *114*, 271–277.
(10) Ogasawara, N.; Watanabe, Y.; Inoue, Y. *J. Am. Chem. Soc.* **1975**, *97*, 6571–6576.
(11) Ogasawara, N.; Inoue, Y. *Chem. Lett.* **1975**, 547–5550.
(12) Sakurai, M.; Tazawa, I.; Inoue, Y. *Nucleic Acids Res. Spec. Publ.* **1976**, *No. 2*, s119–s122.
(13) Ezra, F. S.; Lee, C. H.; Kondo, N. S.; Danyluk, S. S.; Sarma, R. H. *Biochemistry* **1977**, *16*, 1977–1987.
(14) Kondo, N. S.; Danyluk, S. S. *Biochemistry* **1976**, *15*, 756–767.
(15) Pörschke, D. *Eur. J. Biochem.* **1973**, *39*, 117–126.
(16) Van Holde, K. E.; Brahms, J.; Michelson, A. M. *J. Mol. Biol.* **1965**, *12*, 726–739.
(17) Lowe, M. J.; Schellman, J. A. *J. Mol. Biol.* **1972**, *65*, 91–109.
(18) Powell, J. T.; Richards, E. G.; Gratzer, W. *Biopolymers* **1972**, *11*, 235–250.
(19) Inoue, Y.; Kuramochi, T.; Imakubo, K. *Biopolymers* **1979**, *18*, 2175–2194.

Table I. Ionization Constants of ϵAdo, 3'-ϵAMP, and 5'-ϵAMP and Intrinsic Ionization Constant (pK_{ii}) for ϵApϵA (25 °C, I = 0.1)

ϵAdo	3'-ϵAMP	5'-ϵAMP
4.10 ± 0.02	4.11 ± 0.01	4.30 ± 0.01
	pK_{ii} = 4.15 ± 0.01	

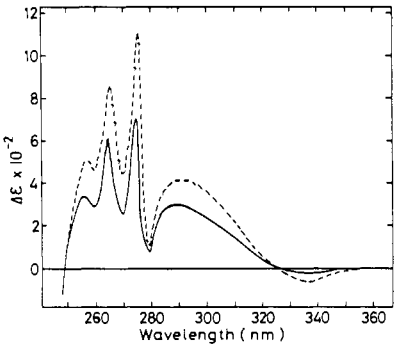


Figure 2. Thermal denaturation spectrum of ϵApϵA (—) at neutral pH: $\Delta\epsilon = \epsilon_{65°C} - \epsilon_{12°C}$ (correction for a change in concentration of ϵApϵA due to the thermal expansion of water was made). Change in extinction coefficient–wavelength profile at pH 7 for degradation to monomers (3'-ϵAMP + ϵAdo) of ϵApϵA is also shown (- - -).

of definite isosbestic points indicates stepwise protonation. The titration curve obtained at 272.5 nm where the absorptivities at pH 1 and 7 differ maximally does not fit a theoretical titration curve for a single pK (Figure 1). The iterative least-squares method has been used to extract the overlapping pK_1 and pK_2 values as described previously.[5] The average values (two runs) of the iterative least-squares best values of pK_1 and pK_2 for ϵApϵA are pK_1 = 4.40 ± 0.02; pK_2 = 3.41 ± 0.03. From the definitions of the equilibrium quotients, s_0 and s_1, involved in Scheme I, we have[5]

$$pK_1 = (pK_0 + \log 2) - \log (1 + s_0)/(1 + s_1) \quad (1)$$

$$pK_2 = (pK_0 - \log 2) - \log (1 + s_1) \quad (2)$$

where K_0 is the intrinsic ionization constant for each site of the two bases in the fully unstacked dimer, $(\epsilon Ap\epsilon A)_{unstacked}$. The values of p$K_1$ and pK_2, together with the intrinsic pK_0 value, enable estimates of the intramolecular stacking equilibrium quotients s_0 and s_1. We have shown[5] that the pK_0 value of the fully unstacked ϵApϵA can be approximated by $^1/_4$, [p$K_{3'\text{-}\epsilon AMP}$ + p$K_{5'\text{-}\epsilon AMP}$ + 2p$K_{\epsilon Ado}$]. A least-squares fit of the titration data for ϵAdo, 3'-ϵAMP, and 5'-ϵAMP at 25 °C is listed in Table I. By applying eq 2 and then eq 1 to ϵApϵA we find s_0 = 2.02 ± 0.27 (percent stacking 67 ± 3%) and s_1 = 1.75 ± 0.20 (percent stacking 64 ± 3%); approximately 67% of ϵApϵA molecules is present in the stacked conformation at neutral pH at 25 °C.

A prior fluorescence spectroscopic determination gave a value of 2.1 ± 0.4 for the stacking quotient (s_0) of ϵApϵA at 25 °C (percent stacking 68 ± 5%),[2,20] in agreement with the value found above. In the following section, we also present the results of the temperature study of the aqueous ϵApϵA solution which provide a further comparison of the intramolecular association constant with those estimated by the two alternative methods.

Analysis of Thermal Denaturation Data for ϵApϵA. The thermal denaturation spectrum of ϵApϵA is shown in Figure 2 as the difference in molar extinction coefficient per base residue at 12 and 65 °C. Ultraviolet difference spectra of ϵApϵA at a series of temperatures displayed a definite isosbestic point at 327 nm, and the shapes of the ϵApϵA thermal difference spectra are closely similar to the difference spectrum observed on hydrolyzing the dimer with calf spleen phosphodiesterase (Figure 2). All of these results are again considered as a good indication of the validity of the "two-state model" for the intramolecular stacking–destacking equilibrium system:[17,18,21] $(\epsilon Ap\epsilon A)_{unstacked} \rightleftarrows (\epsilon Ap\epsilon A)_{stacked}$. The essence of the two-state model is that the observed optical density or molar extinction coefficient, e_i, at the ith of n temperature readings can be expressed by a linear combination of the parameters ϵ_u and ϵ_s of the extreme conformers, $(\epsilon Ap\epsilon A)_{unstacked}$ and $(\epsilon Ap\epsilon A)_{stacked}$, with temperature coefficient:

$$\epsilon_i = f_{ui}\epsilon_u + f_{si}\epsilon_s = (1 - f_{si})\epsilon_u + f_{si}\epsilon_s \quad (3)$$

The intramolecular stacking equilibrium quotient at a given temperature may be written as

$$s_{0i} = \frac{[(\epsilon Ap\epsilon A)_{stacked}]}{[(\epsilon Ap\epsilon A)_{unstacked}]} = \frac{f_{si}}{1 - f_{si}} = \frac{\epsilon_u - \epsilon_i}{\epsilon_i - \epsilon_s} \quad (4)$$

or

$$\ln \frac{\epsilon_u - \epsilon_i}{\epsilon_i - \epsilon_s} = -\frac{\Delta H°}{RT_i} + \frac{\Delta S°}{R} \quad (5)$$

Assuming that ϵ_u, ϵ_s, $\Delta H°$, and $\Delta S°$ are all temperature independent, then eq 5 can be solved for these unknowns by the iterative least-squares computer method of the thermal denaturation data as described previously.[6] In order to minimize the errors involved in the present method of analysis, we took the ϵApϵA vs. component monomer thermal difference spectra at more than 70 temperatures in the range 4–81 °C. Also, in order to determine the effect of precision in the thermal denaturation data on the accuracy of the thermodynamic parameters extracted, synthetic ϵ_i values in a temperature range used were processed by the same computer program as reported previously. The results of the average best values of $\Delta H°$, $\Delta S°$, and stacking quotient at 25 °C, for more than three runs, are shown in Table II. The uncertainties are the standard deviations.

At each temperature, the fraction of molecules in the fully stacked state, f_{si}, can be calculated using the van't Hoff equation and the $\Delta H°$ and $\Delta S°$ values derived as described above. Figure 3 illustrates the variation of f_{si} of ϵApϵA with temperature. The s_0 value at 25 °C is now compared with that estimated from pK measurements. Though the s_0 value in Table II is somewhat

Table II. Thermodynamic Parameters and Intramolecular Stacking Equilibrium Quotient (s_{ii} at 25 °C) of ϵApϵA and ApA for Comparison

	$\Delta H°$, kJ/mol (σ)[a]	$\Delta S°$, J mol^{-1} deg^{-1} (σ)[a]	s_{ii} at 25 °C (σ)[a]	% stacking
ϵApϵA	−21.2 (1.7)	−67.4 (5.5)	1.6 (0.1)	61 ± 2
ApA[b]	−21.1 (0.4)	−70.1 (0.8)	1.1 (0.01)	52 ± 0

[a] The standard deviations (σ) were computed from the root mean square (rms) value between experimental and calculated melting profiles (rms is 1.59 × 10^{-3} for ϵApϵA when difference between the values of the optical parameters for the fully stacked and fully unstacked species is normalized to unity). [b] Data obtained most recently by I. Tazawa (to be reported elsewhere).

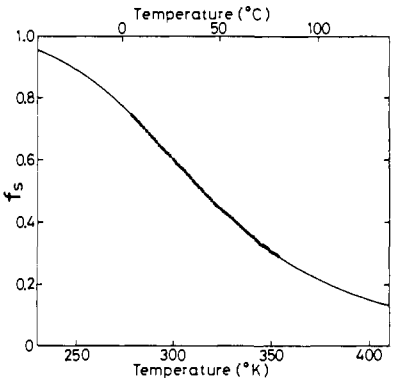


Figure 3. The fraction of stacked bases of ϵApϵA as a function of temperature. Solid curve represents the best fit to the experimental points, calculated by the iterative least-squares method.

(20) Leonard, N. J.; Tolman, G. L. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 43–58.

(21) Johnson, N. P.; Schleich, T. *Biochemistry* **1974**, *13*, 981–987.

smaller than that obtained by titration experiments, we consider the agreement sufficiently good in view of the differences in experimental approach and experimental errors.

The almost quantitative agreement among the values of the intramolecular stacking quotient or percent stacking at 25 °C obtained by the three independent procedures gives strong substantiation of the two-state model for describing the intramolecular stacking association of ϵApϵA. This is one of the principal conclusions from the present results.

Next, in connection with literature discrepancies[2-4] with regard to the degree of stacking, the present conclusion is contrary to the recent view of Baker et al.[4] and Lee and Tinoco,[3] and is in favor of the previous conclusion drawn by Tolman et al.[2,20] (Baker et al.[4] have obtained the thermodynamic parameters for ϵApϵA from the temperature-dependent circular dichroic data in *2 M NaCl*: $\Delta H° = -29$ kJ/mol and $\Delta S° = -113$ J mol^{-1} deg^{-1}. These values indicate that the stacking interaction is weaker than ApA.) It should be noted that such a tendency for the stacking interaction to be enhanced by the chloroacetaldehyde modification reaction has been observed in the intermolecular association of ϵAdo and the intramolecular association of ϵFAD before[22] (s_0 values at 20 °C of FAD and ϵFAD are 5 and 9, respectively.[22])

Finally, comparison between intermolecular and intramolecular stacking association constants shows that the former is greater than the latter. Such a tendency has also been observed in purine and other three-ring heterocyclic systems.[23-25] The ratio of the equilibrium quotients for the intramolecular and intermolecular associations is about 0.11 M. Here, we choose 1 *m* as a standard state for comparison simply because of convenience. If the comparison were based on a mole fraction standard state, it would be about 2.2×10^{-3} mole fraction ($\Delta S_{inter} - \Delta S_{intra} \simeq 0$ J (mol fraction)$^{-1}$ deg^{-1} on a unitary standard state, and $\Delta H_{inter} - \Delta H_{intra} = -14.4$ kJ/mol). This advantage from intermolecularity seems to result from a more extensive degree of overlapping of the bases in intermolecular association than in intramolecular association. In other words, the advantage could be gained if the intermolecular geometry of ϵAdo has more overlap than the stacking geometry in ϵApϵA in aqueous solution. Thus, the observed increase in the equilibrium quotient for the intermolecular association of ϵAdo may be mainly due to increased surface tension forces (enthalpy driven–entropy opposed) compared with the intramolecular stacking in ϵApϵA. It should, however, be noted that this situation is not necessarily true for every case, but different pairs of nucleoside and dinucleoside phosphate may behave differently. Nevertheless, it has generally been noticed that the stacking equilibrium quotients for intermolecular associations are greater than those for the corresponding intramolecular associations, presumably because the linkage of the two nucleosides through the phosphodiester bond introduces new steric constraints on the possible degree of base overlap.

Other intermolecular stacking associations that show a large increase in the unitary standard free energy changes relative to their intramolecular counterparts will be reviewed elsewhere.

(22) Barrio, J. R.; Tolman, G. L.; Leonard, N. J.; Spencer, R. D.; Weber, G. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 941–943.
(23) Ts'o, P. O. P. In "Basic Principles in Nucleic Acid Chemistry"; Ts'o, P. O. P., Ed.; Academic Press: New York, 1974; Vol. 1, pp 453–584.
(24) Kallenbach, N. R.; Berman, H. M. *Q. Rev. Biophys.* **1977**, *10*, 138–236.
(25) Barrio, J. R.; Liu, F.-T.; Keyser, G. E.; VanDerLijn, P.; Leonard, N. J. *J. Am. Chem. Soc.* **1979**, *101*, 1565–1569.

Photochemical Transformations. 28.[1] Comparisons of "Ionic" Intermediates Produced Photochemically with Corresponding Ground-State Intermediates. Further Studies in Some Chlorobenzobicyclooctadienyl Systems

Stanley J. Cristol* and Robert M. Strom

Contribution from the Department of Chemistry, University of Colorado, Boulder, Colorado 80309. *Received November 5, 1979*

Abstract: Irradiation of *exo*-2-deuterio-6,7-benzo-*endo*-2-bicyclo[3.2.1]octadienyl methanesulfonate (**8**-OMs) and its epimer, *endo*-2-deuterio-6,7-benzo-*exo*-2-bicyclo[3.2.1]octadienyl methanesulfonate (**10**-OMs), demonstrates that an unsymmetrical species intervenes in the photorearrangements observed, while the photosolvolyses proceed via intermediates similar to those of ground-state solvolyses. Plausible rationalizations are discussed briefly.

About a decade ago, members of this research group suggested[2] the intermediacy of carbocations in certain photochemical rearrangements, following the earlier reports[3] of carbocation intermediates in photosolvolysis of certain benzyl derivatives. Since that time there have been many reports[4] of carbocation formation in photochemically induced (or photosensitizer induced) rearrangements and solvolyses, so that it is now clear that bond heterolysis may be the result of photoexcitation or photosensitization in a variety of systems. Still to be determined are the kinds of mechanistic paths[6,7] leading to the carbocationic intermediates, which may, of course, differ with different substrates, different environments, and different excited-state multiplicities, and the question of whether the carbenium ions produced from the excited

(1) Part 27: Cristol, S. J.; Daussin, R.D. *J. Am. Chem. Soc.*, **1980**, *102*, 2866.
(2) Cristol, S. J.; Mayo, G. O.; Lee, G. A. *J. Am. Chem. Soc.* **1969**, *91*, 214.
(3) (a) Zimmerman, H. E.; Sandel, V. R. *J. Am. Chem. Soc.* **1963**, *85*, 915. (b) Zimmerman, H. E.; Somasakhara, S. *Ibid.* **1963**, *85*, 922.
(4) For leading references see ref 5 and 6.
(5) Cristol, S. J.; Stull, D. P.; McEntee, Jr., T. E. *J. Org. Chem.* **1978**, *43*, 1756.
(6) Cristol, S. J.; Stull, D. P.; Daussin, R. D. *J. Am. Chem. Soc.* **1978**, *100*, 6674.
(7) (a) Kropp, P. J.; Jones, T. H.; Poindexter, G. S. *J. Am. Chem. Soc.* **1973**, *95*, 5420. (b) Kropp, P. J.; Poindexter, G. S.; Pienta, N. J.; Hamilton, D. C. *Ibid.* **1976**, *98*, 8135.

0002-7863/80/1502-5577$01.00/0