radicals yields no intermediates before the stable product is formed. (Alternatively, if an intermediate is formed it is transferred into the stable products with a specific rate $k \geq 5 \times 10^5$ sec^{-1}.) The spectral properties of the product clearly indicate that it is the Fe(II)–hemin complex.

A Spectral and Thermodynamic Study of the 9-Cyclohexyladenine–Iodine Complex in Organic Solvents

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Contribution from the Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73069. Received October 12, 1973

Abstract: The conventional spectral method and the polyiodide spectral–solubility method have been employed to obtain thermodynamic data and spectral parameters for the complex of 9-cyclohexyladenine with iodine in CCl$_4$ and heptane solution. Results of the two methods are in reasonable agreement, and 9-cyclohexyladenine–I$_2$ is shown to exist primarily as a charge-transfer complex stabilized by donation of n electrons from a ring nitrogen. Evidence is also presented for the existence of a 1:2 cyclohexyladenine–I$_2$ complex in CCl$_4$ at iodine concentrations on the order of 0.001 M.

In spite of the effort that has been spent on studies of weak molecular complexes of biologically important compounds, there is little direct evidence that charge-transfer forces play an important role in biochemistry. It is commonly assumed that charge transfer is involved in the relatively long-range interactions between the bases of nucleic acids and that numerous biological control mechanisms probably rely on transfer of charge from donor to acceptor molecules. A large part of the work on model biological charge-transfer systems has involved the purines and pyrimidines. There have been reports that purines and pyrimidines form π complexes with aromatic donors and acceptors such as chloranil, steroids, and the flavins.[1,2] However, weak complexes of these types are undoubtedly stabilized in large part by interactions (such as dispersion forces and dipolar interactions) other than charge-transfer (CT) forces.[3,4] A recent report from this laboratory included initial structural, thermodynamic, and spectral information about the 1:1 charge-transfer complex of 9-cyclohexyladenine (CHA) with I$_2$, which is stabilized primarily by partial donation of an n electron from a ring nitrogen.[5] The present article presents detailed results of the solution studies, obtained with a conventional spectral method of the Benesi–Hildebrand type[6] and a polyiodide spectral-solubility method[7,8] developed in this laboratory.

Difficulties in applying the Benesi–Hildebrand technique are encountered because of the limited solubility of CHA in organic solvents; the solubility method is a convenient alternative technique for obtaining spectral and thermodynamic data for such a system.

Experimental Section

Chemicals. Heptane (Baker Chemical Co. Defined purity) was purified by standard techniques[9] and stored over drying agent (Linde 4A molecular sieve) in a 3-ft glass column. CCl$_4$ (Matheson Co. ACS analyzed reagent) was distilled from P$_2$O$_5$ and stored in the same way. Iodine (Malinckrodt, resublimed reagent grade) and tetramethylammonium iodide (Matheson, 99+%) were used without further purification. 9-Cyclohexyladenine (Cat. No. 2013), obtained from Cyclo Chemical Co., was used directly without further treatment.

Instrument. A single-beam Beckman DU-2 spectrophotometer equipped with a temperature-regulated cell holder was used in the spectral measurements. The temperature of the cell holder was controlled to within $\pm 0.2°$ by circulating water from an auxiliary thermostated circulator (Haake). A separate refrigerator unit provided cooling for the Haake circulator when needed for experimental temperatures near or below ambient.

Polyiodide Solubility Method. The method for preparing tetramethylammonium pentaiodide (TMAI$_5$)[10] and the experimental method[7] for obtaining K_{DA} and ϵ_{DA} have been reported previously. Application of this method requires the accurate determination of the wavelength of the isosbestic point prior to spectral measurements of equilibrated solutions. This point was located by an isothermal method in which the total concentration of iodine ([I$_2$]) is held constant while the total concentration of CHA ([CHA]) is varied and also by varying the temperature while holding both [I$_2$] and [CHA] constant. Typical spectra are shown in Figure 1; the isosbestic point is located at 473 nm. In the solubility experiments, the increase in absorbance at the isosbestic point which occurs in the presence of added donor is attributed solely to the formation of the complex.[11] Since the extinction coefficients of

(1) M. A. Slifkin, "Charge-Transfer Interaction of Biomolecules," Academic Press, New York, N. Y., 1971, pp 76–95.

(2) R. Foster, "Organic Charge-Transfer Complexes," Academic Press, New York, N. Y., 1969, pp 350–351.

(3) Reference 2, p 356.

(4) B. L. Van Duuren, *Nature (London)*, 210, 622 (1966); B. Pullman, P. Claverie, and J. Caillet, *Science*, 147, 1305 (1965).

(5) D. van der Helm, S. D. Christian, and L. N. Lin, *J. Amer. Chem. Soc.*, 95, 2409 (1973). Detailed structural results are given by D. van der Helm, *J. Crystal. Mol. Struct.*, 3, 249 (1973).

(6) H. A. Benesi and J. H. Hildebrand, *J. Amer. Chem. Soc.*, 71, 2703 (1949).

(7) J. D. Childs, S. D. Christian, and J. Grundnes, *J. Amer. Chem. Soc.*, 94, 5657 (1972); J. D. Childs, Ph.D. Dissertation, University of Oklahoma, Norman, Okla., 1971.

(8) J. D. Childs, S. D. Christian, J. Grundnes, and S. R. Roach, *Acta Chem. Scand.*, 25, 1679 (1971).

(9) A. E. Vogel, "A Textbook of Practical Organic Chemistry," Longmans, Green and Co., London, 1956, Chapter 2.

(10) F. D. Chattaway and G. J. Hoyle, *J. Chem. Soc.*, 123, 654 (1923).

(11) In the case of moderately strong complexes, such as CHA·I$_2$, corrections for variation of activity coefficients with donor concentra-
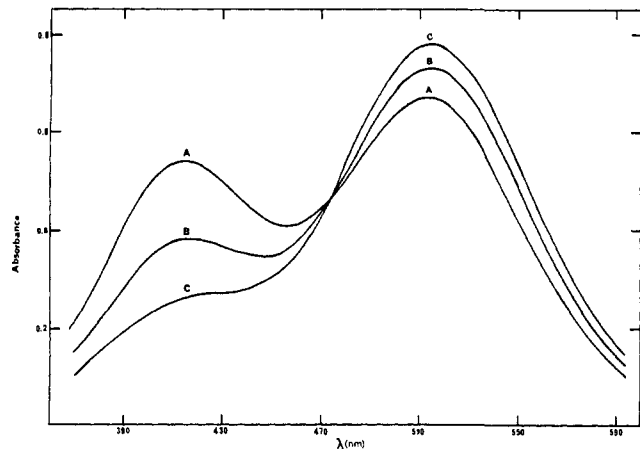
Figure 1. Spectra of a solution of CHA (at molar concentration 1.89 × 10⁻³) and iodine (at molar concentration 1.02 × 10⁻⁴) in carbon tetrachloride at different temperatures; curves A, B, and C correspond to experimental temperatures of 15, 25, and 35°, respectively, and to a cell path length of 10 cm.
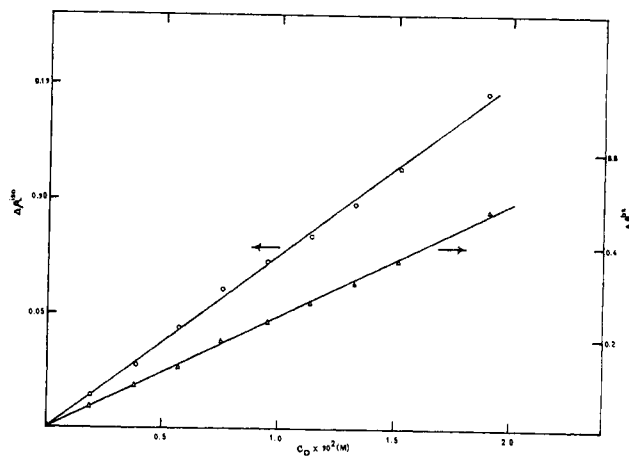

Figure 2. Change in absorbance at the isosbestic wavelength (473 nm) and at the blue-shifted band maximum (415 nm) of solutions of CHA (at molar concentration C_D) at constant iodine activity at 25°; curves represent least-squares fit of absorbance data for a path length of 2 cm.

the complexed and of the uncomplexed iodine are equal at this wavelength, the concentration of the complex and therefore K_{DA} can be calculated directly. In the dilute solution region, the absorbance increases linearly with increasing free donor concentration (Figure 2). Formation constants may be obtained from the slope of this line by the equation $K_{DA} = \text{slope}/A^0$, where A^0 is the absorbance of the blank (CCl₄ equilibrated with a mixture of solid TMAI₅ and TMAI₃ at 25°). The extinction coefficient (ϵ_{DA}^{bs}) at the wavelength of the blue-shifted band maximum (λ^{bs}) can be calculated using the relation $\epsilon_{DA}^{bs} = \Delta A^{bs}/lC_{DA}$, where ΔA^{bs} is the observed absorbance corrected for the absorbance of uncomplexed iodine, l is the path length of the cell, and C_{DA} is the total concentration of complex as determined at the isosbestic point; alternatively, ϵ_{DA}^{bs} may be evaluated from the slope of a plot of ΔA^{bs} vs. ΔA^{iso}, using the relation $\Delta A^{bs} = (\epsilon_{DA}^{bs}/\epsilon_A^{iso})\Delta A^{iso}$. Owing to the very limited solubility of CHA in heptane (3.0 × 10⁻⁵ M), it is difficult to obtain an accurate value of K_{DA} in heptane; therefore, thermodynamic results are reported here only for CHA·I₂ in CCl₄.

Conventional Spectral Method. A modified Benesi–Hildebrand technique was used to determine the equilibrium constant and extinction coefficient of the complex in CCl₄ from absorbance measurements (Table I) at the blue-shifted iodine band. The previously reported method for analysis of spectral data[12] was modified to

Table I. Conventional Spectral Data[a] for the CHA·I₂ System in CCl₄ at 25°

[CHA] × 10⁴, mol/l.	[A] × 10⁴, mol/l.	410	415	420	425
4.11	5.16	0.113	0.119	0.124	0.130
4.05	10.18	0.213	0.225	0.237	0.250
4.00	15.08	0.304	0.320	0.335	0.355
3.95	19.85	0.384	0.404	0.426	0.455
3.90	24.50	0.443	0.468	0.497	0.531
3.85	29.04	0.503	0.534	0.567	0.608
2.08	7.84	0.095	0.105	0.118	0.129
4.16	7.84	0.178	0.187	0.194	0.201
6.24	7.84	0.253	0.262	0.268	0.275
10.40	7.84	0.394	0.406	0.410	0.415
14.56	7.84	0.511	0.520	0.527	0.526
20.38	7.84	0.655	0.671	0.672	0.666

Absorbance at λ, nm

[a] Absorbance values represent raw spectral data (2-cm cell path length); data were fit using the method of Christian and Grundnes[12] which allows for the simultaneous absorbance of acceptor, donor, and complex at λ.

tion are unimportant; see S. D. Christian, J. D. Childs, and E. H. Lane, *J. Amer. Chem. Soc.*, **94**, 6861 (1972).

(12) J. Grundnes and S. D. Christian, *J. Amer. Chem. Soc.*, **90**, 2239 (1968).

include the possibility that a 1 : 2 complex (CHA·2I₂) formed in addition to the 1 : 1 complex. The analytical method employs a nonlinear search to fit data to the equations

$$[D] = C_D + K_{DA}C_DC_A + K_{DA_2}C_DC_A{}^2 \quad (1)$$

$$[A] = C_A + K_{DA}C_DC_A + 2K_{DA_2}C_DC_A{}^2 \quad (2)$$

$$\frac{\Delta A}{l} = A/l - \epsilon_A C_A - \epsilon_D C_D =$$

$$\epsilon_{AD}(K_{DA}C_DC_A + 2K_{DA_2}C_DC_A{}^2) \quad (3)$$

where C_A and C_D are the concentrations of uncomplexed acceptor (I₂) and donor (CHA). K_{DA} and K_{DA_2} are the formation constants of the 1 : 1 and 1 : 2 complexes, and ϵ_A and ϵ_D are the known extinction coefficients of iodine and CHA. A and l are the observed absorbance and the path length of the cell, ΔA is the absorbance due to the 1 :1 and 1 :2 complexes, and ϵ_{DA} is the extinction coefficient of CHA·I₂. To reduce the number of parameters required in fitting data, the reasonable assumption is made that $\epsilon_{DA_2} = 2\epsilon_{DA}$; that is, each iodine in the 1 : 2 complex is assumed to have the same blue-shifted spectrum as I₂ in the 1 : 1 complex.

The numerical technique involves the following steps. First, eq 1 and 2 are solved simultaneously (using assumed initial values of K_{DA} and K_{DA_2}) to yield trial values of C_A and C_D corresponding to each pair of [D], [A] values. Using these values of C_A and C_D, values of $\Delta A/l$ (eq 3) for each of the data points are calculated. The $\Delta A/l$ values are fit by ordinary least-squares analysis as a linear function of the variable $K_{DA}C_DC_A + 2K_{DA_2}C_DC_A{}^2$ to obtain the best value of ϵ_{DA} corresponding to the chosen values of K_{DA} and K_{DA_2}. By minimizing the value of

$$\Delta = \sum_i \left[\frac{\Delta A_i}{l} - \epsilon_{DA}K_{DA}C_{A,i}C_{D,i} - 2\epsilon_{DA}K_{DA_2}C_{A,i}{}^2C_{D,i} \right]^2$$

with respect to unrestricted variation of K_{DA} and K_{DA_2}, the ultimate best values of all three parameters are obtained. The optimum-seeking method used in this analysis is similar to one described previously;[12,13] both the least-squares constants and internal estimates of their standard deviations ($\sigma_{K_{DA}}$, $\sigma_{K_{DA_2}}$, $\sigma_{\epsilon_{DA}}$) are obtained.

Since the absorbance of CCl₄ becomes extremely large at 260–240 nm, it is difficult to obtain accurate spectral parameters for the CT band of the complex in that solvent; therefore, the ultraviolet spectra have been evaluated for solutions in heptane. In obtaining the CT spectrum, the same total CHA concentration in heptane was used in the reference beam in order to cancel part of the CHA absorption in the region of the CT band. The concentration of the CHA·I₂ complex was inferred from the absorbance of the blue-

(13) D. J. Wilde, "Optimum Seeking Methods," Prentice-Hall, Englewood Cliffs, N. J., 1964.

Table II. Thermodynamic and Spectral Results for Complexes of Iodine with CHA and Pyridine in Carbon Tetrachloride at 25°

Donor	Solubility method			Conventional method				
	λ_{max}^{bs}, nm	ϵ_{max}^{bs}, l. mol⁻¹ cm⁻¹	K, l./mol	λ_{max}^{bs}, nm	ϵ_{max}^{bs}, l. mol⁻¹ cm⁻¹	K_{DA}, l./mol	ΔH, kcal/mol	ΔS, eu/mol
CHA	415	1460 ± 40	180 ± 5	415	1630 ± 130[a,b]	185 ± 20[b]	−9.5 ± 0.5	21.6 ± 0.8
Pyridine				416[c]		105 ± 0.7[c]	−7.47 ± 0.06[c]	15.3 ± 0.2[c]
				418.5[d]	1389[d]	111[d]		

[a] Weighted average of extinction coefficients for 1:1 and 1:2 complexes at 415 nm. [b] Calculated from the analysis assuming the presence of both 1:1 and 1:2 complexes (see Table III). [c] W. T. McKinney and A. E. Popov, *J. Amer. Chem. Soc.*, **91**, 5218 (1969). [d] K. R. Bhaskar and S. Singh, *Spectrochim. Acta, Part A*, **23**, 1155 (1967).

shifted iodine band by assuming that the maximum extinction coefficient of the blue-shifted band of the complex in heptane is equal to the maximum extinction coefficient of the band in CCl₄. A reasonable CT spectrum of CHA·I₂ can then be obtained by correcting for the absorbance of free CHA and free I₂. The oscillator strength (f) and transition dipole moment (μ_{mn}) can be estimated from the equations[14]

$$f \simeq 4.32 \times 10^{-9} \epsilon_{max} \Delta \nu_{1/2}$$

$$\mu_{mn}, D \simeq 0.0958 [\epsilon_{max} \Delta \nu_{1/2} / \nu_{max}]^{1/2}$$

where ϵ_{max} is the extinction coefficient at λ_{max}^{ct}, $\Delta \nu_{1/2}$ is the half-width of the band in reciprocal centimeters, and ν_{max} is the frequency in reciprocal centimeters at the band maximum. From the frequency of the CT band, an ionization potential may be estimated for CHA.[15,16]

Results and Discussion

Solubility Methods. Figure 2 is a plot of ΔA^{iso} and ΔA^{bs} vs. molar concentration of the free CHA. The best value of the slope and intercept were calculated by the linear least-squares method; derived constants are listed in Table II. A well-defined isosbestic point is observed in this experiment.

Conventional Spectral Method. In the conventional spectral method, it was necessary to use maximum values of [I₂] on the order of 10^{-3} M in order to obtain reproducible results. If it is assumed that only the 1:1 complex is present, the apparent value of K_{DA} (obtained from the spectral data) increases as the range of [I₂] values increases. This suggests the possibility that a 1:2 complex (1 molecule CHA, 2 molecules I₂) is formed at I₂ concentrations greater than 10^{-3} M. Therefore, a three-parameter optimization scheme (described in the previous section) was employed to obtain K_{DA}, K_{DA_2}, and ϵ_{DA}. Table III shows how variation in the assumed value of K_{DA_2} affects the results; clearly, inclusion of the 1:2 constant improves the fit of data, although the uncertainties in the derived constants are relatively large. In spite of this, the K_{DA} values agree well with those of the solubility method. The best values of K_{DA} and ϵ_{DA} (obtained from the spectral data at 415 nm) are included in Table II along with values from the solubility method. The advantage of the solubility method is that spectral measurements on systems at very low total iodine concentration can be used directly to infer K_{DA} and ϵ_{DA}, whereas the interpretation of the conventional spectral results is complicated by higher order effects. The assumption made in the analysis (that $2\epsilon_{DA} = \epsilon_{DA_2}$) may not hold exactly, and this may explain the somewhat larger value of ϵ_{DA}

(14) R. S. Mulliken and W. B. Person, "Molecular Complexes: A Lecture and Reprint Volume," Wiley-Interscience, New York, N. Y., 1969, pp 23–26.

(15) H. Yada, J. Tanaka, and Nagakura, *Bull. Chem. Soc. Jap.*, **33**, 1660 (1960).

(16) R. S. Mulliken and W. B. Person, *Annu. Rev. Phys. Chem.*, **13**, 107 (1962).

Table III. Optimum Results of the Numerical Analysis of Conventional Spectral Data Assuming Presence of 1:1 and 1:2 Complexes[a]

λ, nm	K_{DA}, l./mol	K_{DA_2}, l.²/mol²	ϵ_{DA},[b] l. mol⁻¹ cm⁻¹	Rmsd[c]
410	146 ± 24	0	1970 ± 240	0.0091
	185 ± 17	3250 ± 600	1610 ± 110	0.0041
415	148 ± 21	0	1980 ± 210	0.0081
	185 ± 20	3000 ± 750	1630 ± 130	0.0038
420	147 ± 20	0	1960 ± 200	0.0076
	181 ± 19	2750 ± 600	1630 ± 120	0.0037
425	147 ± 20	0	1910 ± 200	0.0075
	181 ± 21	2750 ± 750	1590 ± 120	0.0044

[a] Data from absorbance measurements in CCl₄ at 25°. [b] ϵ_{DA_2} is assumed to equal $2\epsilon_{DA}$. [c] Rmsd is the root mean square deviation of the measured absorbances from the calculated values.

obtained from the conventional spectral method. The value of ϵ_{DA} obtained from the experiments at the higher iodine concentrations will of course represent some sort of weighted average of the extinction coefficients of DA and DA₂. It is assumed that the 1:1 complex in solution is primarily the form (as is found in the solid state)[5]
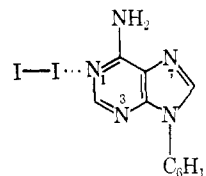
and that the second I₂ attacks at the 3 or 7 nitrogen. Of course various isomers of the 1:1 and 1:2 complexes would all contribute to values of K_{DA} and K_{DA_2}. The ratio of K_{DA_2} to K_{DA} (∼16 l. mol⁻¹) is the equilibrium constant for the reaction

$$CHA \cdot I_2 + I_2 = CHA \cdot 2I_2$$
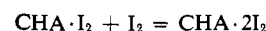
This value is about an order of magnitude smaller than the first constant ($K_{DA} = 185$ l. mol⁻¹), which may indicate that one of the basic nitrogens (probably N(1)) binds I₂ more strongly than any of the others. It is not believed that I₃⁻ forms in significant concentrations, since the spectral bands of I₃⁻ in the near-uv region are absent from the CCl₄ or heptane solutions.[7]

It is instructive to compare the spectral and thermodynamic properties of CHA·I₂ with those of pyridine–I₂ (Table II). Evidently CHA·I₂ is a somewhat stronger complex than pyridine–I₂, and the extinction coefficient of the CHA·I₂ blue-shifted band is also larger than that of pyridine–I₂. This agrees with Mulliken's theory,[17] which predicts that the extinction coefficient of the blue-
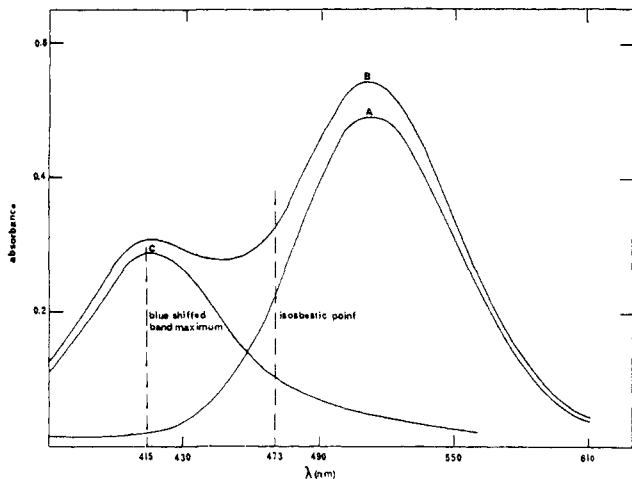
(17) Reference 14, Chapter 10.

Figure 3. Spectra obtained with the spectral–solubility method for a solution of CHA (at molar concentration 2.29 × 10⁻³) in carbon tetrachloride at constant iodine activity at 25°; curve A represents the system TMAI₅–TMAI₃–CCl₄ (uncomplexed iodine concentration of 5.30 × 10⁻⁴ M), curve B the corresponding system with CHA added to the carbon tetrachloride, and curve C the difference between curves A and B; spectra correspond to a cell path length of 1 cm.

shifted iodine band should increase with increasing stability of the complex through a series of similar donors. Figure 3 shows the visible spectra obtained with the spectral solubility method.

The charge-transfer band of $CHA \cdot I_2$ in heptane has a maximum at about 244 nm where the absorptivity is approximately 29,800 l. mol^{-1} cm^{-1}. It is estimated

that $\Delta\nu_{1/2}$ of the band is 6320 cm^{-1}, and from eq 4 and 5, f and μ_{mn} are estimated to be 0.814 and 6.49 D.

For pyridine-I_2 (λ_{max}^{ct} = 235 nm, ϵ_{max}^{ct} = 41400 l. mol^{-1} cm^{-1}), the extinction coefficient is somewhat greater than that for $CHA \cdot I_2$; however, it has been argued[18] that pyridine can function simultaneously as an n donor and a π donor in pyridine-I_2 and that the large value of ϵ_{max}^{ct} is attributable to a combination of n → σ_u and π_z → σ_u CT transitions. The trend of λ_{max}^{ct} from pyridine-I_2 to $CHA \cdot I_2$ is in the direction predicted by the order of complex stability.[17]

Figure 1 shows the spectra of the $CHA \cdot I_2$ in CCl_4 at several temperatures. Using the known extinction coefficient of the maximum of the blue-shifted band, the concentration of complex and formation constant can be determined; K_{DA} values for the complex in CCl_4 at 15, 25, and 35° are evaluated to be 307, 177, and 107 l. mol^{-1}, respectively. The K_{DA} value at 25° is in good agreement with that obtained from the solubility method. The values of $\Delta H°$, $\Delta G°$, and $\Delta S°$ are determined to be −9.5 kcal/mol, −3.07 kcal/mol, and 20.9 eu/mol for the 1 M, ideal dilute solution standard state in CCl_4. Again, comparison of the thermodynamic results with those for pyridine-I_2 in CCl_4 ($\Delta H°$ = −7.47 kcal/mol, $\Delta S°$ = 15.8 eu/mol) indicates that $CHA \cdot I_2$ is the stronger complex. From the frequency of CT band, an ionization potential of 9.1 eV for the nonbonding electrons localized on N(1) is estimated for CHA.

(18) R. S. Mulliken, *J. Amer. Chem. Soc.*, **91**, 1237 (1969).

Di-tert-butyl Nitroxide as a Convenient Probe for Excited Singlet States. A Study of Pyrene Luminescence

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Abstract: Di-tert-butyl nitroxide (DTBN) quenches both the pyrene monomer and excimer emissions in benzene solution at room temperature close to the diffusion limit: monomer, k_Q = 8.4 × 10⁹ M^{-1} sec^{-1}; excimer, $k_{Q'}$ = 4.1 × 10⁹ M^{-1} sec^{-1}. From steady-state fluorescence yield measurements and directly measured monomer and excimer lifetimes, values are derived for several important parameters of the pyrene monomer–excimer system.

Recently, we reported that the stable free radical di-tert-butyl nitroxide (DTBN) efficiently quenches the fluorescence of a series of aromatic hydrocarbons in solution at room temperature.[2] The viscosity dependence and rate constants for quenching k_Q suggested that the rate of the quenching reaction is very near the diffusion limit in solvents of moderate viscosity. As part of a program to test the generality of fluorescence quenching by DTBN, and to demonstrate further its capabilities and limitations as a mechanistic probe of

photochemical and photophysical processes, we have extended our study to the pyrene monomer–excimer[3-7] system.

We show that the DTBN fluorescence quenching data[2] can be employed to provide rate parameters for

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(4) Th. Förster and K. Kasper, *Z. Elektrochemie*, **59**, 977 (1955).

(5) E. Döller and Th. Förster, *Z. Phys. Chem. (Frankfurt am Main)*, **34**, 132 (1962).

(6) C. A. Parker and C. G. Hatchard, *Trans. Faraday Soc.*, **59**, 284 (1963).

(7) T. Medinger and F. Wilkinson, *Trans. Faraday Soc.*, **62**, 1785 (1966).