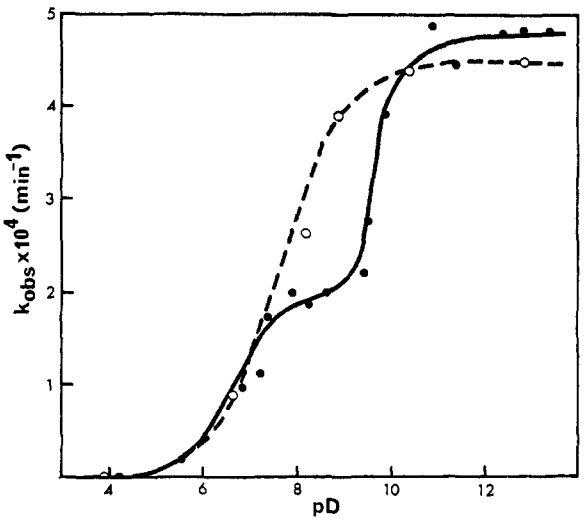

Figure 1. Graph of k_{obsd} (min^{-1}) *vs.* pD for *N*-acetyl-L-histidine (○) and L-histidine (●) at 37°.

rate of H–D exchange of 3–5% solutions of the compounds at various pD values (pD = pH meter reading + 0.4) was determined by following the decrease of the area (or height) of the C-2 proton resonances at 60 MHz as compared with the area (or height) of the C-4 proton resonances which remained constant.[5] A first-order rate constant k_{obsd} was determined from the gradient of a graph of log (corrected area or height of C-2 resonance) *vs.* time.[5,9] At pD <5 and 35° the rate of exchange is negligibly small; hence the reaction involving D_2O, which is appreciable at 65°,[5] can be neglected. Thus, for imidazole

$$\text{rate} = k_{obsd}[Im_t] = k_2[OD^-][Im^+] \quad (1)$$

where $[OD^-]$ is a constant in any particular run and $[Im_t]$ and $[Im^+]$ represent the total concentrations of imidazole and the charged form of imidazole, respectively. Substitution of the apparent dissociation constant of imidazole (K_2) and K_{D_2O}, the ionic product of D_2O, and rearrangement give

$$k_{obsd} = k_2K_{D_2O}/(K_2 + [D^+]) \quad (2)$$

This allows the determination of k_2 from measurements of k_{obsd} at different values of $[D^+]$.

For compounds which contain a separate nearby ionizable group with a pK of 6–12, the kinetics are complicated (see Figure 1) because of the different rate constants for the reaction of OD^- with the two forms of the compound. For example with histidine the two reactive forms are designated $N^+D_3Im^+DCOO^-$ (His^{2+}) and $ND_2Im^+DCOO^-$ (His^+), where the former structure represents the positively charged forms of the amino group and imidazole ring of histidine and the charged form of the carboxyl group. Thus

$$\text{rate} = k_{obsd}[His_t] = [OD^-](k_1[His^{2+}] + k_2[His^+]) \quad (3)$$

where $[His_t]$, $[His^{2+}]$, and $[His^+]$ represent the total concentrations of histidine and of the two reactive forms and k_1 and k_2 are second-order rate constants for the reactions of OD^- with His^{2+} and His^+, respectively. Substitution for $[His^{2+}]$ and $[His^+]$ in eq 3 in terms of K_1, K_2, and K_3 (defined in Table I) gives[10]

$$k_{obsd} = \frac{k_1K_{D_2O}}{K_1 + [D^+] + \frac{K_1K_3}{[D^+]} + \frac{K_1K_3}{K_2}} + \frac{k_2K_{D_2O}}{K_2 + [D^+] + \frac{K_2[D^+]}{K_3} + \frac{K_2[D^+]^2}{K_1K_3}} \quad (4)$$

By substitution of values for K_{D_2O}, K_1, K_2, K_3, and k_{obsd} at various values of $[D^+]$ a series of equations is obtained each with two unknowns, k_1 and k_2. Pairs of these equations are solved for k_1 and k_2 and the results (accuracy 5–10%) are given in Table I.

The S-shaped curve for *N*-acetyl-L-histidine shown in Figure 1 has been obtained hitherto[5] and the apparent pK of the imidazole can be determined from the center of the curve.[11,12] However, where there is a charged group nearby to the imidazole ring which titrates at pD >8, it is possible to obtain the pK of this group too, from the center of the second S-shaped curve, as shown for histidine in Figure 1.[13] This is useful for proteins such as ribonuclease A, in which there are charged amino groups nearby to histidines 12 and 119. Of greater importance for protein studies are conclusions obtained from examination of second-order rate constants. Firstly, the rate constant decreases greatly from the value of 14.4 in L-histidine, by moving the charged amino group progressively further away to a value of 5.0 in glycyl-L-histidine, 4.6 in β-alanyl-L-histidine, and finally 2.8 by removing the charge altogether as in L-histidine at high pD. Secondly, the rate constant increases greatly by eliminating a nearby charged carboxyl group as shown by comparing imidazole acetic acid with imidazole or L-histidine with histamine. Both effects are explained by a simple electrostatic mechanism in which the rate of attack of OD^- is increased by nearby positively charged groups and decreased by nearby negatively charged groups.

This study allows the determination of the pK of titratable groups (with pD >8) adjacent to imidazole rings and provides information on the proximity of nearby charged amino and carboxyl groups. The mapping of the environment of the histidine residues in ribonuclease A is in progress.

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(13) There is a further increase of k_{obsd} above pD 9, in spite of the much lower value of k_2 than k_1, because K_2 is much smaller than K_1; see Table I and eq 4.

J. H. Bradbury,* B. E. Chapman, F. A. Pellegrino
Chemistry Department, Australian National University
Canberra, A.C.T., Australia
Received June 11, 1973

Chemical and Physical Evidence for Anthracene–1,3-Diene Exciplexes. A Quencher-Sensitized Photodimerization

Sir:

The quenching of the fluorescence of aromatic hydrocarbons by 1,3-dienes has been interpreted in

Table I. Diene-Catalyzed Photodimerization of Anthracene[a]

[*trans,trans*-2,4-Hexadiene], *M*	Observed		Calculated	
	ϕ_{A_2}[b]	ϕ_{A_2}[c]	ϕ_{A_2}[d]	ϕ_{A_2}[e]
0	0.08_2	0.05_4	0.082	0.054
0.044	0.087	0.06_4	0.093	0.062
0.066	0.107	0.06_5	0.101	0.065
0.088	0.11_0	0.066	0.106	0.069
0.132	0.12_2	0.07_1	0.117	0.075
0.176	0.13_1	0.07_5	0.127	0.082
0.264	0.13_8	0.08_2	0.146	0.095
0.44	0.16_1	0.12_1	0.181	0.119
0.66	0.19_0	0.14_3	0.221	0.145
0.88	0.24_8	0.16_8	0.253	0.167
1.32	0.30_1	0.21_1	0.307	0.206
1.76	0.33_6	0.24_9	0.347	0.236

[a] The benzophenone-sensitized photoisomerization of *cis*-1,3-pentadiene was used for actinometry: A. A. Lamola and G. S. Hammond, *J. Chem. Phys.*, **43**, 2129 (1965). The initial anthracene concentration was 8.3×10^{-3} *M*. The range of anthracene loss was 8.4–14.8%. [b] Degassed samples. [c] Aerated samples, see text. [d] Using eq 11. [e] Using eq 15.

terms of exciplex formation between the lowest singlet excited state of the aromatic hydrocarbon and the 1,3-diene.[1-5] Strong evidence for this mechanism has been the observation of emission from 1-cyanonaphthalene–1,3-diene exciplexes.[6] More recently it has been shown that substantial fractions of the quenching interactions lead to ground-state adducts between the aromatic hydrocarbon and the 1,3-diene, and collapse to adducts has been suggested as a significant pathway for exciplex decay.[7-9] Since adduct formation could of itself account for the fluorescence quenching in many systems, independent evidence showing the involvement of exciplexes having finite lifetime is desirable. In this communication we report (i) evidence indicating that in the system anthracene–*trans,trans*-2,4-hexadiene the diene-catalyzed photodimerization of anthracene is a consequence of exciplex formation, and (ii) exciplex fluorescence in the system 9,10-dichloroanthracene–2,5-dimethyl-2,4-hexadiene.

Irradiation of benzene solutions of anthracene in the presence of *trans,trans*-2,4-hexadiene using the 366-nm Hg line leads to three competing photoreactions: anthracene dimerization, trans–cis diene photoisomerization, and anthracene–diene adduct formation.[10,11] Under our experimental conditions adduct formation is extremely inefficient and dimerization is the principal anthracene consuming reaction. Quantum yields for anthracene dimerization, ϕ_{A_2}, at several diene concentrations are listed in Table I.

The remarkable observation is that although anthracene singlets are intercepted by the diene, as evidenced by the quenching of anthracene fluorescence, the diene enhances anthracene photodimerization, a known singlet reaction.[14,15] Equations 1–10 give

$$A \xrightarrow{h\nu} {}^1A \quad (1)$$

$${}^1A \xrightarrow{k_2} A + h\nu \quad (2)$$

$${}^1A \xrightarrow{k_3} {}^3A \quad (3)$$

$${}^1A + A \xrightarrow{k_4} {}^1(AA) \quad (4)$$

$${}^1A + D \xrightarrow{k_5} {}^1(AD) \quad (5)$$

$${}^1(AA) \xrightarrow{k_6} 2A \quad (6)$$

$${}^1(AA) \xrightarrow{k_7} A_2 \quad (7)$$

$${}^1(AD) \xrightarrow{k_8} A + D \quad (8)$$

$${}^1(AD) \xrightarrow{k_9} AD \quad (9)$$

$${}^1(AD) + A \xrightarrow{k_{10}} A_2 + D \quad (10)$$

the simplest mechanism consistent with data obtained with degassed samples, where A, D, ^{1}(AA), ^{1}(AD), A_2, and AD represent anthracene, diene, anthracene excimer, anthracene–diene exciplex, dimer, and adduct, respectively. Application of the steady-state approximation to all excited species leads to eq 11 for

$$\phi_{A_2} = \left(k_4\tau_0[A]k_7/(k_6 + k_7) + \frac{k_5\tau_0[D](k_{10}/k_8)[A]}{1 + (k_{10}/k_8)[A]}\right) \times \frac{1}{1 + k_4\tau_0[A] + k_5\tau_0[D]} \quad (11)$$

the dimerization quantum yield, where τ_0 represents the lifetime of anthracene singlets, $(k_2 + k_3)^{-1}$, and $k_9 \ll k_8 + k_{10}[A]$ is assumed. Using the known values of $k_4 = 1.0 \times 10^{10}$ *M* sec^{-1} [14] and $\tau_0 = 4.9 \times 10^{-9}$ sec[16] gives $k_7/(k_6 + k_7) = 0.297$ in excellent agreement with the limiting quantum yield of photodimerization measured at high anthracene concentration.[14] The slope of the Stern–Volmer plot for anthracene fluorescence quenching by the diene gives $k_5\tau_0 = 0.60$.[17] Calculated ϕ_{A_2} values in excellent agreement with the observed values are obtained for $k_{10}/k_8 = 300$ M^{-1} using eq 11, Table I. Since diffusion control sets the maximum value of $k_{10} \leq 1 \times 10^{10}$ M^{-1} sec^{-1}, a minimum exciplex lifetime of 30 nsec is indicated (see below).

Dimerization quantum yields[18] measured in the

(1) L. M. Stephenson, D. G. Whitten, G. F. Vesley, and G. S. Hammond, *J. Amer. Chem. Soc.*, **88**, 3665, 3893 (1966).
(2) L. M. Stephenson and G. S. Hammond, *Pure Appl. Chem.*, **16**, 125 (1968); *Angew Chem., Int. Ed. Engl.*, **8**, 261 (1969).
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(5) G. N. Taylor and G. S. Hammond, *ibid.*, **94**, 3684, 3687 (1972).
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(9) N. C. Yang and J. Libman, *ibid.*, **94**, 9226 (1972).
(10) Irradiations were carried out in a merry-go-round apparatus at 30°.[12] Analyses for anthracene loss were by glpc and by uv. Adduct yields were determined by glpc. Adducts were observed only at very large anthracene conversions.
(11) The diene photoisomerization which is apparently caused by ^{3}T sensitization[13] will be described in a full paper.
(12) F. G. Moses, R. S. H. Liu, and B. M. Monroe, *Mol. Photochem.*, **1**, 245 (1969).
(13) R. S. H. Liu, *J. Amer. Chem. Soc.*, 90, 1899 (1968); R. S. H. Liu and J. Erdman, *ibid.*, 90 213 (1968); **91**, 1492 (1969).
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(15) For a recent review see B. Stevens, *ibid.*, **8**, 161 (1971).
(16) I. B. Berlman, "Handbook of Fluorescence Spectra of Aromatic Molecules," Academic Press, New York, N. Y., 1965.
(17) A Perkin-Elmer–Hitachi MPF-2A spectrofluorometer was employed.
(18) The anthracene disappearance quantum yield was converted to ϕ_{A_2} after correcting for anthracene peroxide formation using the data in ref 14. Since dienes react readily with singlet oxygen[19] all anthracene disappearance in the presence of excess diene is tentatively attributed to dimerization.
(19) K. Kondo and M. Matsumoto, *J. Chem. Soc., Chem. Commun.*, 1332 (1972).

presence of air, Table I, support the above mechanism. Oxygen has a pronounced quenching effect on the dimerization, far surpassing its effect on anthracene fluorescence. It follows that in addition to ^1A other dimer precursors are intercepted by oxygen. Inclusion of eq 12–14 in the mechanism leads to eq 15

$$^1A + O_2 \xrightarrow{k_{12}} \left.\begin{matrix} \\ \\ \end{matrix}\right\} \text{loss of singlet excitation} \quad (12)$$

$$^1(AA) + O_2 \xrightarrow{k_{13}} \quad (13)$$

$$^1(AD) + O_2 \xrightarrow{k_{14}} \quad (14)$$

$$\phi_{A_2} = \left(\frac{k_4\tau_0[A]k_7/(k_6 + k_7)}{1 + k_{13}[O_2]/(k_6 + k_7)} + \frac{k_5\tau_0[D](k_{10}/k_8)[A]}{1 + (k_{10}/k_8)[A] + (k_{14}/k_8)[O_2]} \right) \times \frac{1}{1 + k_4\tau_0[A] + k_5\tau_0[D] + k_{12}\tau_0[O_2]} \quad (15)$$

for ϕ_{A_2} in the presence of oxygen. The quenching of anthracene fluorescence in benzene by air gives $k_{12} \cdot [O_2] = 4.2 \times 10^7$ sec^{-1}.[16,17] Assuming that steps 12–14 are equally efficient, use of eq 15 gives $(k_6 + k_7)^{-1} = 8.0 \times 10^{-9}$ sec and $k_8^{-1} = 30 \times 10^{-9}$ sec as excimer and exciplex lifetimes, respectively.[20] As shown in Table I, calculated and observed ϕ_{A_2} values in the presence of air are in excellent agreement.

While no exciplex emission could be detected from degassed anthracene–*trans,trans*-2,4-hexadiene solutions in benzene, a broad, weak emission is observed from benzene solutions of 9,10-dichloroanthracene (DCA) and 2,5-dimethyl-2,4-hexadiene. As the diene concentration is increased the blue 9,10-dichloroanthracene emission shifts to a green exciplex emission, λ_{max} ~470 nm.[17] Apparently, the better donor–acceptor characteristics of the components of this exciplex give rise to emission in this case. No change in the absorption spectrum of DCA could be detected upon addition of diene, nor was there any loss of DCA detected following prolonged irradiation of benzene solutions of DCA and the diene.

Formally, the diene-catalyzed photodimerization of anthracene is a quencher-sensitized reaction. Electronic excitation is stored in a relatively long-lived intermediate giving rise to enhanced chemical reactivity for one of its components. It seems likely that the 1,3-pentadiene-catalyzed 9-phenylanthracene photodimerization can be similarly explained.[23,24]

It should be noted here that, while the mechanism in eq 1–10 adequately accounts for the observations, it may be incomplete. For example, excimer and exciplex[3] formation may be reversible, step 10 may not give dimer with unit efficiency, and there may be an additional dimer forming step[26] involving interaction of the excimer with the diene. Work is in progress on these aspects of the mechanism.[26a]

Acknowledgment. This research was supported by National Science Foundation Grant No. GP-24265.

(20) Rate constants for the quenching of the ^1S states of several aromatic hydrocarbons by oxygen in cyclohexane are diffusion controlled, being in the range of $2.5–3.1 \times 10^{10}$ M^{-1} sec^{-1}.[21,22] However, since it is not certain that the quenching of singlet excimers and exciplexes will be as efficient, the values of these lifetimes may have been underestimated.

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(22) C. S. Parmenter and J. D. Rau, *J. Chem. Phys.*, **51**, 2242 (1969).

(23) R. O. Campbell and R. S. H. Liu, *Chem. Commun.*, 1191 (1970).

(24) The *trans,trans*-2,4-hexadiene-catalyzed photodimerization of anthracene has been observed independently by Professor N. C. Yang's group,[25] and similar observations have been made by Professor D. O. Cowan's group[25] for the methyl ester of 9-anthroic acid in the presence of 1,3-pentadiene.

(25) Private communication of unpublished observations.

(26) R. S. H. Liu, private communication.

(26a) NOTE ADDED IN PROOF. A comparison of the efficiency of *trans,trans*-2,4-hexadiene quenching of anthracene fluorescence in the presence and in the absence of air suggests strongly that exciplex formation is not freely reversible in this system.

(27) Alfred P. Sloan Foundation Fellow.

Jack Saltiel,*[27] David E. Townsend
Department of Chemistry, Florida State University
Tallahassee, Florida 32306

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Cyclic Peptides. VI. Europium-Assisted Nuclear Magnetic Resonance Study of the Solution Conformations of *cyclo*(L-Pro-L-Pro) and *cyclo*(L-Pro-D-Pro)[1]

Sir:

Europium[2] has been found to bind the carbonyl oxygen of amides.[3] We have utilized this finding in conjunction with minimum energy calculations for detailed conformational analysis of cyclic dipeptides. The nmr analysis was possible even though europium bound at either oxygen affects all protons. The present study differs from previous nmr investigations of amino acids[4a,b] and linear peptides[4c] which have employed binding of lanthanides to the carboxylate group.

Nmr spectra of *cyclo*(L-Pro-L-Pro) (Figure 1) and *cyclo*(L-Pro-D-Pro) (Figure 2) were obtained by additions of a 0.2 *M* Eu(fod)$_3$-d_{27}–chloroform-*d* solution[5] in 25-μl aliquots to 0.5 ml of a 0.2 *M* chloroform-*d* solution of peptide. The shift of each proton was plotted (as ordinate) against the europium:peptide molar ratio up to an arbitrarily chosen limit of 1:4. All shifts were linear in this range,[6] and the least-squares slopes of these lines were employed as $\Delta\nu_i/\nu_0$ in the pseudocontact shift relation,[2,7] $\Delta\nu_i/\nu_0 = K(3\cos^2\theta_i - 1)r_i^{-3}$, where K is a proportionality constant, θ_i is the O–Eu–H$_i$ angle, and r_i is the Eu–H$_i$ distance. The y intercepts of these plots represent the initial chemical shifts.

The various resonances in the spectra of the europium-treated material were identified with α, β, γ, and δ protons by the use of chemical-shift and coupling data, and these identifications were confirmed by Fourier

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