ylsilylated polyamino alcohol corresponding to Ser-Ile-Val-His was identified in this manner by mass chromatograms of sequence determining ions.

All together 27 peptide derivatives were identified (Figure 1), which can only be reassembled to two separate partial sequences indicating that one overlap was missing (Table I). However, identification of Trp as the N-terminal residue (as the trimethylsilylated phenylthiohydantoin by gas chromatography[14] and mass spectrometry[15]) permits reassembly of sequence A shown in Table I both manually and by a computer program[10] (Glx may be either glutamic acid or glutamine).

Due to the lability of the primary amide group of glutamine under the conditions of acid hydrolysis, the position of the single glutamine residue remained to be determined. The partial sequence -Lys^4-Glx^5-Glx^6- suggested a simple conventional experiment to solve this problem. A sample of the eicosapeptide was digested with trypsin which was expected to produce, among others, a peptide with the N-terminal sequence Glx-Glx-.... The digest was then subjected to a one-step Edman degradation.[16] The presence of Gln>PhNCS in the resulting mixture of phenylthiohydantoins as demonstrated by thin layer chromatography[16] and high resolution mass spectrometry places Gln at position 5. The latter technique was found to be an excellent method for the analysis of relatively complex mixtures of phenylthiohydantoins due to selectivity, high sensitivity and dynamic range.

Thus, the structure of this cyanogen bromide fragment of actin was unambiguously determined as

5 10
Trp-Ile-Thr-Lys-Gln-Glu-Tyr-Asp-Glu-Ala-Gly-Pro-Ser-Ile-
15 20
Val-His-Arg-Lys-AEtCys-Phe

The same structure has been deduced independently by conventional techniques.[17,18]

Acknowledgment. The authors are indebted to Dr. M. Elzinga, Boston Biomedical Research Institute, Boston, Mass., for the sample of the C-terminal cyanogen bromide peptide of actin and to the National Institutes of Health, Public Health Service, for support (Grants GM05472 and GM 01523).

(14) J. J. Pisano and T. J. Bronzert, *J. Biol. Chem.*, **244**, 5597 (1969).

(15) R. E. Harman, J. L. Patterson, and W. J. A. VandenHeuvel, *Anal. Biochem.*, **25**, 452 (1968).

(16) P. Edman in "Protein Sequence Determination," S. B. Needleman, Ed., Springer Verlag, New York, N. Y., 1972, pp 211–255.

(17) M. Elzinga and J. H. Collins, *Cold Spring Harbor Symp. Quant. Biol.*, 37, 1 (1973).

(18) Since it was one of the objectives of this investigation to test the reliability of this technique on a protein derived peptide of realistic size and amino acid composition, communication concerning the sequence was assiduously avoided between Dr. Elzinga's and our own laboratories before completion of the work described here.

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Quenching of Aromatic Hydrocarbon Excited Singlet States by Wurster's Blue Cation Radical

Sir:

The quenching of aromatic hydrocarbon excited singlet states ($^1R^*$) by doublet-state molecules such as nitric oxide,[1] nitroxyl radical,[2] and di-*tert*-butyl nitroxide (DTBN)[3] has been reported. Electron-exchange enhanced intersystem crossing

$$^1R^* + {}^2Q \longrightarrow {}^3R^* + {}^2Q \qquad (1)$$

has beeen implicated, both theoretically[4] and experimentally, as a possible mechanism for these singlet quenching reactions. In addition to the mechanistic and theoretical interest in the interaction of doublet-state species with excited singlet states, it is necessary to understand these processes in order to formulate a complete description of electrogenerated chemiluminescence (ECL). In ECL excited singlet states are generated by an anion radical–cation radical annihilation reaction in a region of space cooccupied by excess radical ions. The extent of this excess is controlled by the lifetime of the excited singlet state and the kinetics of the anion–cation radical annihilation reaction. Although it has been demonstrated that radical cations can act as triplet quenchers,[5] the extent to which they act as singlet quenchers has not been studied except in a preliminary way.[6] In this communication we report that the tetramethyl-*p*-phenylenediamine cation radical (Wurster's blue, ^{2}TMPD$\cdot^+$) is an extemely effective quencher of aromatic hydrocarbon excited singlets.

The bimolecular rate constants for fluorescence quenching, k_q, by ^{2}TMPD$\cdot^+$ were obtained from a Stern–Volmer analysis (eq 2) of aromatic hydrocarbon

$$\tau_F{}^0/\tau_F = 1 + k_q[{}^2\mathrm{TMPD}\cdot{}^+]\tau_F{}^0 \qquad (2)$$

fluorescence lifetimes, τ_F, measured by the time-correlated single photon timing technique.[7] The fluorescence lifetime approach for monitoring quenching was chosen because the lifetime is not dependent on complications such as competitive absorption by the quencher or radiative (trivial) energy transfer; both of which are present in the systems studied here. Fluorescence quenching samples were prepared by exhaustive electrooxidation at +0.20 V *vs.* sce of thoroughly deoxygenated acetonitrile solutions containing 10^{-4} *M* aromatic hydrocarbon, 0.1 *M* tetrabutylammonium perchlorate (TBAP), and the appropriate concentration of TMPD neutral. The free amine was prepared and purified as described by Bard, *et al.*[8] Resultant ^{2}TMPD$\cdot^+$ concentrations were determined both coulometrically and spectrophotometrically at 568 nm using ϵ_{568} 1.26 × 10^4 M^{-1} cm^{-1}. Uv-visible absorption spectra and fluorescence emission and excitation spectra were recorded for all samples. The absorption spectra could be reproduced exactly by summing the absorption spectra of the individual components. No

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(1) (a) G. Porter and M. W. Windsor, *Proc. Roy. Soc., Ser. A*, **245**, 238 (1958); (b) S. Siegel and H. S. Judeikis, *J. Chem. Phys.*, **48**, 1613 (1968); (c) B. Stevens, *Trans. Faraday Soc.*, **51**, 610 (1956); (d) J. T. Dubois, *J. Chem. Phys.*, **25**, 178 (1956); (e) J. B. Birks, *J. Lumin.*, 1, 154 (1970).

(2) A. L. Buchachenko, M. S. Khlopyankina, and S. N. Dobryakow, *Opt. Spectrosc.* (*USSR*), 22, 304 (1967).

(3) J. A. Green II, L. A. Singer, and J. H. Parks, *J. Chem. Phys.*, 58, 2690 (1973).

(4) G. J. Hoytink, *Accounts Chem. Res.*, 2, 114 (1969).

(5) L. R. Faulkner and A. J. Bard, *J. Amer. Chem. Soc.*, **91**, 6497 (1969).

(6) (a) L. R. Faulkner, unpublished results; (b) J. Bowman, Ph.D. Thesis, University of Texas, Austin, Texas, 1970.

(7) W. R. Ware in "Creation and Detection of the Excited State," A. A. Lamola, Ed., Marcel Dekker, New York, N. Y., 1971.

(8) L. R. Faulkner, H. Tachikawa, and A. J. Bard, *J. Amer. Chem. Soc.*, **94**, 691 (1972).

Table I. Aromatic Hydrocarbon Singlet Quenching Rate Constants

	Fluoranthene (358)[a]	Chrysene (316)	1,2-Benzanthracene (358)	Pyrene (357)	Naphthalene (316)
$\tau_F{}^0$ (nsec)	52.6	48.1	46.1	323.6	107.2
$\Delta E_{S_1-T_1}$ (cm^{-1})[b]	6800[c]	7700	9500	10,100	10,500
$-E_{1/2}(R/R\cdot^-)$[d]	1.81[e]	2.25	2.00	2.07	2.47
$E_{1/2}(R/R\cdot^+)$[d]	1.45	1.35	1.18	1.26	1.54
$k_q(^2TMPD\cdot^+) \times 10^{-10}$ (M^{-1} sec^{-1})[f]	2.60 ± 0.06[g]	2.10 ± 0.15	2.11 ± 0.15	2.15 ± 0.05	3.0 ± 0.30
$k_q(TMPD) \times 10^{-10}$ (M^{-1} sec^{-1})	1.99 ± 0.05		1.47[h]		
$k_q(DTBN) \times 10^{-10}$ (M^{-1} sec^{-1})[i]		0.85	0.80	0.78 1.14[j]	0.82

[a] Numbers in parentheses are wavelengths of excitation in nm. [b] C. S. Parmenter and J. D. Rau, *J. Chem. Phys.*, **51**, 2242 (1969). [c] Taken from J. B. Briks, "Photophysics of Aromatic Molecules," Wiley, New York, N. Y., 1970, pp 75 and 261. [d] Taken from C. K. Mann and K. K. Barnes, "Electrochemical Reaction in Nonaqueous Media," Marcel Dekker, New York, N. Y., 1970; in volts *vs.* sce. [e] K. F. Drake, unpublished results, Northwestern University, 1972. [f] In 0.1 *M* TBAP–CH_3CN solutions. [g] Standard deviation for least-squares fit to eq 2. [h] Reference 10. [i] Reference 3, in methylcyclohexane. [j] Reference 3, in acetonitrile.

aromatic hydrocarbon self-interactions were observed in the fluorescence spectra.

The results of fluorescence lifetime quenching studies on five aromatic hydrocarbon–$^2TMPD\cdot^+$ systems are presented in Table I. One can calculate an upper limit to the diffusion controlled rate constant, k_{diff}, for a bimolecular quenching process from the Smoluchowski relation[1e] equal to 2.4×10^{10} M^{-1} sec^{-1}. The results in Table I show that $^2TMPD\cdot^+$ is an effective singlet quencher with a quenching rate constant near to or somewhat greater than k_{diff}. Also included in Table I for comparison purposes are quenching rate constants for systems involving TMPD and DTBN as the quencher. In those cases where the available data permit a comparison, $^2TMPD\cdot^+$ is seen to react *faster* than TMPD. Since the diffusion coefficient of a radical ion is either essentially equal to[9a] or smaller than[9b] the diffusion coefficient of the corresponding neutral, this rate difference may be attributed to a larger interaction radius for the $^2TMPD\cdot^+$ quenching mechanism than the TMPD electron transfer quenching mechanism.[10] In addition $^2TMPD\cdot^+$ quenches pyrene fluorescence 1.9 times faster than DTBN in acetonitrile. Since this rate difference is also too large to be accounted for in terms of quencher diffusion coefficient differences, the interaction radius hypothesis may apply here also.

The data obtained in this preliminary investigation do not permit a definitive assignment of the $^2TMPD\cdot^+$ fluorescence quenching mechanism; however, we can rule out processes such as

$$^1R^* + {}^2TMPD^+ \longrightarrow {}^3R^* + {}^2TMPD\cdot^{+*} \qquad (3)$$

since $k_q(^2TMPD\cdot^+)$ is near k_{diff} for all five hydrocarbons even though $\Delta E_{S_1-T_1}$ is less than the energy of the lowest allowed doublet–doublet transition in $^2TMPD\cdot^+$ (16,350 cm^{-1}). The lack of correlation between $k_q(^2TMPD\cdot^+)$ and either $E_{1/2}(R/R\cdot^-)$ or $E_{1/2}(R/R\cdot^+)$ argues against an electron transfer mechanism analogous to that for quenching by TMPD. Collisional energy transfer,[11] electron-exchange induced intersystem crossing, and vibrational quenching[12] must all be considered possible mechanisms at this time. Further examination of the $k_q(^2TMPD\cdot^+)$ values shows that

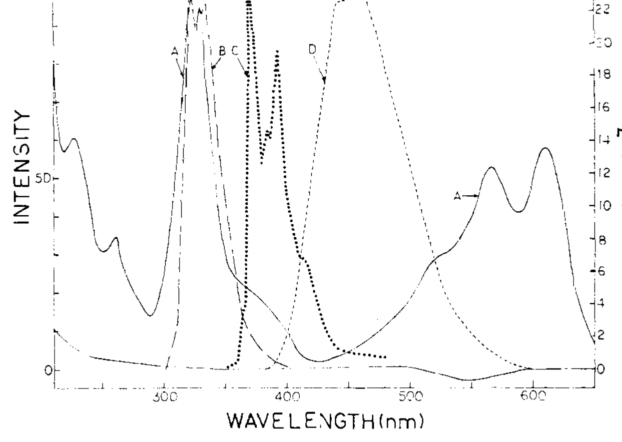

Figure 1. Spectral overlap characteristics of aromatic hydrocarbon fluorescence spectra and Wurster's blue cation radical absorption spectrum: A, Wurster's blue cation radical absorption spectrum in 0.1 *M* TBAP–acetonitrile solution; B, naphthalene fluorescence spectrum, 10^{-2} *N* in acetonitrile; C, pyrene fluorescence spectrum, 10^{-4} *M* in acetonitrile; D, fluoranthene fluorescence spectrum, 10^{-4} *M* in acetonitrile.

they fall into two groups: (i) chrysene, 1,2-benzanthracene, and pyrene with k_q near 2.1×10^{10} M^{-1} sec^{-1} and (ii) fluoranthene and naphthalene with $k_q > k_{diff}$. This grouping correlates with the extent of spectral overlap between the hydrocarbon donor fluorescence transition and the strongly allowed $^2TMPD\cdot^+$ acceptor absorption transitions. The spectral overlap characteristics are shown in Figure 1. The fluorescence spectra of chrysene and 1,2-benzanthracene are similar to that of pyrene and do not overlap appreciably with any $^2TMPD\cdot^+$ absorption bands. A tentative interpretation of this correlation is that, in the cases of fluoranthene and naphthalene, $^2TMPD\cdot^+$ quenching involves a long-range resonance energy transfer mechanism[13] as well as a collisional energy transfer mechanism; whereas, for the other hydrocarbons the collisional mechanism is the only reactive channel available since there is virtually no spectral overlap. The resonance mechanism apparently does not predominate in either the fluoranthene or naphthalene cases since the values of k_q are only slightly greater than k_{diff}. This may be qualitatively understood on the basis that the degree of spectral overlap between $^2TMPD\cdot^+$ absorption and fluoranthene emission is small and in the case of

(9) (a) R. V. Slates and M. Szwarc, *J. Phys. Chem.*, **69**, 4124 (1965); (b) G. Cauquis and D. Serve, *Anal. Chem.*, **44**, 2222 (1972).

(10) D. Rehm and A. Weller, *Isr. J. Chem.*, **8**, 259 (1970).

(11) D. L. Dexter, *J. Chem. Phys.*, **21**, 836 (1953).

(12) A. M. Braun, W. B. Hammond, and H. G. Cassidy, *J. Amer. Chem. Soc.*, **91**, 6196 (1969).

(13) Th. Forster, *Discuss. Faraday Soc.*, **27**, 7 (1959).

naphthalene although there is very strong spectral overlap the oscillator strength of the naphthalene transition is low.[14]

Acknowledgment. The assistance of Messrs. Dennis Rushforth, Charles Lombard, and Jeffrey K. McVey during various phases of this work is acknowledged. The author wishes to thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, The Research Corporation, and the National Science Foundation (GP-32953) for support of this research.

[14] I. B. Berlman, "Handbook of Fluorescence Spectra of Aromatic Molecules," Academic Press, New York, 1971, p 330.

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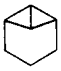
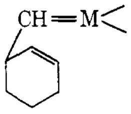
Received June 20, 1973

Transition Metal Catalyzed Rearrangements of Bicyclobutanes. Mechanism of Acid Production in Methanolysis[1]

Sir:

In our recent investigation of the transition metal catalyzed isomerization of tricyclo[4.1.0.0^{2,7}]heptane (**1**), the involvement of an organometallic carbene intermediate (**2**) was indicated.[2] It was also shown that the formation of 2-norcarane methyl ether (**3**) in the reaction of **1** with $[Rh(CO)_2Cl]_2$ in methanol resulted from a protic acid catalyzed reaction. These methanolic studies have been extended to substituted bicyclobutanes and, again, all reaction solutions became acidic. We now present evidence as to the nature of the acid-forming species.


1



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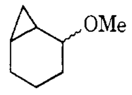
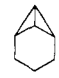
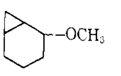
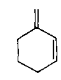
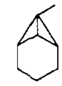
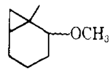
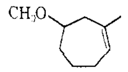
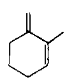
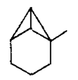
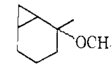
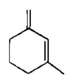
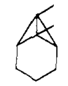
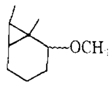
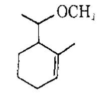
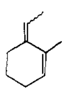


3

As in our initial studies, solid $[Rh(CO)_2Cl]_2$ was added to a methanol solution of the bicyclobutane, with and without solid $NaHCO_3$. Table I lists the results of the study. When $NaHCO_3$ was absent, the pH[3] rapidly dropped from about 8.0 for the bicyclobutane–methanol solution to about 2.5 when the Rh(I) catalyst was added, and the pH slowly increased.[4] With $NaHCO_3$ present, the pH only dropped to 5.0 and then rapidly increased.[5]

Table I. Reactions of $[Rh(CO)_2Cl]_2$ and Bicyclobutanes in Methanol

Reactant	Products and reaction conditions		
 1		 OCH3 3 100%[a] 10%[b]	 4 0%[a] 90%[b]
 5	 OCH3 6 84%[a] 72%[b]	 CH3O 7 16%[a] 13%[b]	 8 0%[a] 15%[b]
 9		 OCH3 10 100%[a] 90%[b]	 11 0%[a] 10%[b]
 12	 OCH3 13 75%[a] 75%[b]	 OCH3 14 25%[a] 25%[b]	 15 0%[a] 0%[b]

[a] Reactions were performed using 5 ml of methanol and a mole ratio of bicyclobutane to Rh(I) catalyst of approximately 20:1; 8 mg of Rh(I) catalyst was added to a methanolic solution of the bicyclobutane. Reaction times were approximately 10 min. Product distributions were analyzed by nmr and vpc and are normalized. In general as the degree of substitution on the bicyclobutane increased, product formation was not as quantitative as in the case of **1**. [b] Same reaction conditions as in footnote *a* with 50 mg of sodium bicarbonate present.

Recently, Gassman and Reitz[6] showed that the pH value obtained when a bicyclobutane solution was added to a methanolic solution of $[Rh(CO)_2Cl]_2$ depended upon the rate of addition of the hydrocarbon. The results of a similar study with substituted bicyclobutanes are reported in Table II. As the rate of addition of the bicyclobutane solution was decreased, larger amounts of diene were formed; the presence of $NaHCO_3$ also increased the amount of dienes formed.

Table II. Reaction of Bicyclobutanes and $[Rh(CO)_2Cl]_2$ in Methanol

	Products and reaction conditions (%)		
Reactants	I[a]	II[b]	III[c]
1	**3** (49)	**3** (35)	**3** (5)
	4 (51)	**4** (65)	**4** (95)
5	**6** (71)	**6** (70)	**6** (34)
	7 (13)	**7** (14)	**7** (13)
	8 (16)	**8** (16)	**8** (53)
9	**10** (83)	**10** (75)	**10** (18)
	11 (17)	**11** (25)	**11** (72)
12	**13** (75)	**13** (62)	**13** (2)
	14 (20)	**14** (18)	**14** (1)
	15 (0)	**15** (0)	**15** (0)
	12 (5)	**12** (20)	**12** (97)

[a] Reactions were carried out by dropwise addition (~1 drop every 12 sec) of a solution of bicyclobutane (~0.05 ml) and 2 ml of methanol to a solution of 8 mg of $[Rh(CO)_2Cl]_2$ and 3 ml of methanol. Products were analyzed by vpc. [b] Rate of addition of the bicyclobutane solution was decreased to about one drop every minute. [c] Rate of addition was the same as in footnote *b* with 20 mg of $NaHCO_3$ present.

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[1] This work was supported by National Science Foundation Grant No. GP-8700.

[2] (a) W. G. Dauben and A. J. Kielbania, Jr., *J. Amer. Chem. Soc.*, **94**, 3669 (1972); (b) see also S. Masamune, M. Sakai, and N. Darby, *J. Chem. Soc., Chem. Commun.*, 471 (1972).

[3] We monitored the pH of reaction solutions using a Sargent-Welch Model NX digital pH meter with a Sargent-Welch combination glass electrode S300 72-15. See R. G. Bates, "Determination of pH—Theory and Practices," Wiley, New York, N. Y., 1964.

[4] (a) The addition of $[Rh(CO)_2Cl]_2$ to methanol without bicyclobutanes present results in a rapid decrease in pH from about 8.0 to 4.0. This pH drop may be due to a bridge splitting reaction by methanol resulting in a rhodium complex in which methanol has been incorporated as a ligand. (b) See D. N. Lawson and G. Wilkinson, *J. Chem. Soc.*, 1900 (1965). (c) F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," 3rd ed, Wiley, New York, N. Y., 1972, p 1020.

[5] It was found that when **1** was allowed to react for 2 min in methanol of pH 3.4 only **3** was detected in the reaction solution. Using methanol of pH 4.4 after 2 min both **1** (66%) and **3** (34%) were identified.

[6] P. G. Gassman and R. R. Reitz, *J. Amer. Chem. Soc.*, **95**, 3057 (1973).