on the outside, degradation of the chymotrypsinogen into peptides and separation and measurement of their radioactivity should provide information about which peptides and, through further degradation, about which amino acid residues occupy the surface of the intact protein molecule in its natural conformation in aqueous solution. We further expect that a similar experiment performed in the presence of an enzyme substrate or inhibitor will give labeled protein molecules in which the radioactivity incorporated at and around the active site will be substantially reduced or eliminated, thus providing a general technique for pinpointing enzyme active sites. Such experiments are now in progress.

Tritium has previously been incorporated into solid ribonuclease using the Wilzbach procedure[2] (exposure to gaseous tritium) and recently by the production of radicals on ribonuclease through γ-ray irradiation followed by treatment with tritiated hydrogen sulfide.[3] It is possible that procedures similar to that proposed here might yield information about the structures of solid proteins.

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(2) D. Steinberg, M. Vaughan, C. B. Anfinsen, and J. Gorry, *Science*, **126**, 447 (1957).

(3) P. Riesz, F. H. White, Jr., and H. Kon, *J. Am. Chem. Soc.*, **88**, 872 (1966).

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Electrochemical Generation of the Phenanthrene Triplet

Sir:

The luminescence properties of phenanthrene have been thoroughly studied by many investigators.[1–5] Normal,[1] delayed,[2] and excimer[1] fluorescence emission have been detected. In addition, phosphorescence from this molecule has been observed in the crystal,[3] in rigid glasses,[4] and in fluid solution even at room temperature,[5] although the last of these conditions is not generally suitable for such detection.

By consecutive electrochemical oxidation and reduction or reduction and oxidation in double-potential-step experiments, rubrene[6] and other fluorescent organic molecules[7] can be raised to electronically excited singlet states. The potential range employed in these double-step experiments need not be large enough to encompass both the anion and cation of the fluorescer in order for fluorescence emission to be seen.[6,7] We wish to report here that phenanthrene, when subjected to this preannihilative treatment, produces a green emission which is not characteristic of phenanthrene fluorescence.

When the stable phenanthrene anion ($E_{1/2} = -2.47$ v *vs.* sce) is generated electrochemically in a 4 mM solution of purified phenanthrene[8] and 0.1 M tetra-*n*-butylammonium perchlorate in deaerated N,N-dimethylformamide at a 3×10 mm platinum sheet electrode, and then oxidized at sufficient overvoltage, a green emission is seen at the electrode. By going to more positive voltages, short of phenanthrene oxidation or of the dimethylformamide decomposition potential ($\sim+1.5$ v), the green emission becomes brighter.

If phenanthrene anion is generated for a sufficient time so that it diffuses through the entire solution, thus destroying possible traces of oxidants, this "purged" solution gives the same green emission on the oxidation cycle. The emission from this solution is first detectable on oxidation at $+0.15$ v, and the intensity also increases up to background. The minimum thermodynamic driving force in the purged solution for the emission measured from the half-wave reduction potential is 2.6 v (or $\sim$60 kcal/mole).

The emission spectrum was photographed by focusing a 1:1 image of the electrode on the slit of a fast grating Raman spectrograph,[9] using Eastman Kodak 103aF spectrographic plates. The plates were microphotometered on a Jarrell Ash Model 23-100 recording microphotometer and were corrected for the spectral sensitivity of the emulsion. The emission was found to be broad and structureless with a maximum between 4900 and 5000 A. This corresponds very closely with the room-temperature phenanthrene phosphorescence in ethanol reported by Parker and Hatchard.[5]

As shown in Table I, addition of various substances which are electroinactive between -2.5 and $+1.5$ *vs.* sce in DMF to the green-emitting phenanthrene system affected the emission in a manner which can be related to the triplet energies of the substances. The presence of 1,3,5-hexatriene does decrease the stability of the phenanthrene anion but not sufficiently to account for the total loss of emission. The other added substances have no detectable effect on the phenanthrene anion-radical stability.

Table I. Effect of Electroinactive Triplet Quenchers on Phenanthrene Electrochemiluminescence

	E_t, kcal/mole	% quenched
1,3,5-*trans*-Hexatriene	47[a]	100
2,3-Dimethylbutadiene	59[b]	87
Phenanthrene	62[c]	
Biphenyl	65[d]	0

[a] D. F. Evans, *J. Chem. Soc.*, 1735 (1960). [b] R. E. Kellogg and W. T. Simpson *J. Am. Chem. Soc.*, **87**, 4230 (1965). [c] Reference 1. [d] V. L. Ermolaev, *Usp. Fiz. Nauk*, **80**, 3 (1963).

These results, together with the observation that triplet quenchers had no effect on the *fluorescence* electrochemiluminescence of 1,3,4,7-tetraphenylisobenzofuran,[7] indicate that in the acceptor–phenanthrene systems irreversible triplet energy transfer is responsible

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(1) T. Azumi and S. P. McGlynn, *J. Chem. Phys.*, **41**, 3131 (1964).

(2) C. A. Parker and C. G. Hatchard, *Proc. Roy. Soc.* (London), **A269**, 574 (1962).

(3) R. M. Hochstrasser and G. J. Small, *J. Chem. Phys.*, **45**, 2270 (1966).

(4) E. C. Lim and J. D. Laposa, *ibid.*, **41**, 3257 (1964).

(5) C. A. Parker and C. G. Hatchard, *J. Phys. Chem.*, **66**, 2506 (1962).

(6) D. L. Maricle and A. H. Maurer, *J. Am. Chem. Soc.*, **89**, 188 (1967).

(7) A. Zweig, A. K. Hoffmann, D. L. Maricle, and A. H. Maurer, in press.

(8) The method of E. C. Kooyman and E. Farenhorst, *Trans. Faraday Soc.*, **49**, 58 (1943), was used. We thank Dr. W. A. Henderson, Jr., for making this material available.

(9) R. F. Stamm, *Ind. Eng. Chem.*, *Anal. Ed.*, **17**, 318 (1945).

for the light diminution and that phosphorescence is being seen! Undoubtedly, the fact that the solution is exceptionally well scavenged for oxygen by the ion radicals makes an important contribution to the ability of this solution to phosphoresce at room temperature.

In acetonitrile the green emission is detected on oxidation of the phenanthrene anion as long as the potential does not reach the point where phenanthrene cation is generated (+1.8 v).[10] The phenanthrene cation is exceedingly unstable; cyclic voltammetry employing rapid scan rates (60 cps) and displaying the current–voltage curve on an oscilloscope indicated that this cation has a lifetime of $<10^{-2}$ sec.

When the potential scan in acetonitrile is great enough to permit both oxidation and reduction of phenanthrene, the emission rapidly decreases in intensity, the solution yellows, and products are formed which are electroactive in the intermediate potential region. The spectrum of this emission, although too dim and variable for positive identification of the emitting species, appears to be the same as the preannihilative emission.

The dimness of this emission and the rapid darkening of the solution of phenanthrene in acetonitrile under such conditions raise questions as to the significance of the results previously reported for this system.[11]

Acknowledgment. We wish to thank A. K. Hoffmann and G. W. Kennerly for their interest and advise in this investigation.

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[10] E. S. Pysh and N. C. Yang, *J. Am. Chem. Soc.*, **85**, 2124 (1963).

[11] E. A. Chandross, J. W. Longworth, and R. E. Visco, *ibid.*, **87**, 3259 (1965).

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Book Reviews

Chemistry in Nonaqueous Ionizing Solvents. Volume I. Chemistry in Anhydrous Liquid Ammonia. Part I. Anorganische und Allgemeine Chemie in Flüssigem Ammoniak. By GERHART JANDER, Berlin, HANS SPANDAU, Braunschweig, and C. C. ADDISON, Nottingham. Interscience Publishers, John Wiley and Sons, Inc., 605 Third Ave., New York, N. Y. 1966. xxiv + 561 pp. 17 × 24.5 cm. $27.50.

For several years publishers and colleagues have urged this reviewer to write a comprehensive treatise on the physical and inorganic chemistry of liquid ammonia solutions. Fortunately, this is no longer to be considered since the present volume meets the admittedly existing needs admirably.

This is Part 1 of Volume 1 of a series of monographs concerned with "Chemistry in Nonaqueous Ionizing Solvents;" Part 2 of Volume 1 was published earlier and covers organic reactions in liquid ammonia. The original editors of this series were the late Professor Dr. Gerhart Jander and Professor Dr. Hans Spandau. Upon the death of Professor Jander, Dr. C. C. Addison became a co-editor.

After a brief introductory section (4 pages) dealing with the preparation, purification, and handling of liquid ammonia, about 30 pages are devoted to a discussion of physical properties of ammonia. Although this section could have been much expanded, the present treatment provides both adequate orientation and entree to the literature. The longest part of the book, some 430 pages, is concerned with essentially all of the important aspects of solutions of inorganic substances in liquid ammonia; examples include vapor pressure data, molecular weight determinations, solubility relationships, electrode potentials, polarography, and thermochemistry. Particularly noteworthy is the section dealing with solutions of metals in ammonia. This includes both a concise review of the relevant physiochemical measurements that have been made and a thorough review of the various models that have been proposed to account for these physical properties. Almost equally useful are the sections on acid–base relationships and oxidation–reduction processes. The third section covers the more recent literature, follows the same kind of subject matter organization, and is apparently an addendum.

Among the outstanding features of this monograph are: (1) the extent to which the authors have achieved conciseness without either loss of clarity or omission of important information; (2) the thoroughness with which the various sections are documented; (3) the extensive inclusion of equipment diagrams (albeit not always the best ones) and graphical representations of data; (4) the extensive recourse to data tabulation which makes the book especially useful for reference.

Seldom is one able to write about a book with so much enthusiasm; it is truly an outstanding contribution to the chemical literature. In its field, this volume will be *the* standard reference work for many years.

Errors are few, and they are of a nature (mostly typesetting) that does not lead to misunderstanding.

The price of the book ($27.50) is discouraging—even in Texas where everyone has money except the poor folks.

Finally, in the English language version of the Preface, this reviewer was successively surprised and concerned to learn that "reference must be made to the collections of WATT, which have survived for over a century."

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