similar values ($1.9 \pm 0.1 \times 10^{-4}$ M) for the LDL compound.

Lokshina, *et al.*,[4] have reported that, in the presence of 0.027 mM pepsin, acetyl-L-phenylalanyl-(β-phenyl)-L-lactic acid (2.7 mM) is cleaved to 30% (as measured by the disappearance of material reacting with NH_2OH to form a hydroxamate) in 48 hr at 37° and pH 2 (10–15% ethanol was present); no hydrolysis was observed at pH 4. When expressed as moles of substrate cleaved per minute per mole of enzyme, the rate measured by Lokshina, *et al.*, at pH 2 is approximately 0.01 min^{-1}; this may be compared to the hydrolysis of Z-His-Phe(NO_2)-Pla-OMe (0.25 mM) by 0.00014 mM pepsin at an initial rate of 20 min^{-1} at 37° and pH 4. The present work, in confirming the conclusion reached by Lokshina, *et al.*, thus places the esterase activity of pepsin on a sounder experimental basis and provides suitable substrates for the study of the mechanism of pepsin action at ester bonds.

In view of the current interest in the development of specific chemical reagents for pepsin,[5–8] it may be noted that the results reported here make it desirable to determine the effect of chemical modification of this enzyme not only with proteins and synthetic peptides as substrates but also with suitable synthetic esters. In the present work it was found that the specific reagent L-1-diazo-4-phenyl-3-tosylamidobutanone[8] inhibits both the esterase activity (substrate, Z-His-Phe(NO_2)-Pla-OMe) and the peptidase activity (substrate, Z-His-Phe(NO_2)-Phe-OMe) of pepsin.

Acknowledgment. This work was aided by grants from the U. S. Public Health Service (GM-06452) and from the National Science Foundation (GB-5212X).

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Received November 15, 1966

Pre-annihilation Electrochemiluminescence of Rubrene

Sir:

Several reports of electrochemically generated luminescence resulting from electrolysis of solutions of fluorescent aromatic molecules have recently been published.[1–6] In most cases, the luminescence has been attributed to a radical anion–radical cation annihilation reaction which leaves one of the resulting neutral molecules in an excited state.

Alternative explanations for light-emitting reactions seen under some conditions have involved luminescent oxidation of the anion by either cation decomposition products[2] or solvent oxidation products.[3] Although we have found that the luminescence is brightest and most generally seen under conditions where the annihilation process is most likely, we have also observed light under conditions such that none of the above explanations is pertinent.[2,6]

Specifically, with certain aromatic hydrocarbons light can be detected while oxidizing the anion or reducing the cation at potentials insufficiently anodic or cathodic, respectively, to generate the oppositely charged radical ion and at potentials where there is no appreciable background electrolysis. Rubrene is a highly electrochemiluminescent molecule which typifies this kind of behavior, and therefore we report a more detailed study of the pre-annihilation electrochemiluminescence of this molecule.

The light out putfrom a 1×10^{-3} M solution of rubene in DMF with 0.1 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte was observed with a photomultiplier in two series of double-potential-step experiments. In the first series, designed to study the light output obtained while oxidizing the anion (formed at -1.37 v *vs.* sce and moderately stable), the potential of the first step was held at -1.6 v for 5 sec, and the potential of the second step was increased incrementally in the positive direction. The light output was measured 0.2 sec after initiation of the second step. A similar series of experiments was used to study the light produced while reducing radical cation (formed at $+0.95$ v and considerably less stable). In this case, the potential of the first step was held at $+1.0$ v, and the potential of the second step was shifted incrementally in the negative direction.

Light was detected while oxidizing at the anion voltages positive of -0.2 v and while reducing the cation at voltages negative of -0.95 v. The intensity of this pre-annihilation light, observed while oxidizing the anion and reducing the cation, was about one and two orders of magnitude, respectively, less than that observed when the potential excursion was great enough to produce the maximum intensity of anion–cation annihilation light.

Both of these threshold voltages are sufficiently far removed from the potentials at which the oppositely charged ion is produced to rule out a simple annihilation reaction as the source of light. Also it is apparent that light is produced while oxidizing the anion and reducing the cation at overvoltages well below the energy of the rubrene 0–0 singlet transition (2.3 ev). However, only singlet emission is seen. This requires a mechanism more involved than direct excitation to the singlet. One possibility is that this pre-annihilation light-emitting process initially generates triplets by a direct heterogeneous electron transfer which is followed by a triplet–triplet annihilation reaction to produce singlets.[7] This has been held

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improbable on theoretical grounds,[8] but it is difficult to rule out low-level luminescence on this basis alone.

A second explanation involves oxidation or reduction of impurities in the system, followed by homogeneous chemiluminescent electron-transfer reactions. However, it is still necessary to invoke either triplets or some energy-doubling process in order to account for the energy discrepancy.

This type of impurity mechanism was shown to be responsible for a major fraction of the pre-annihilation luminescence of the rubrene anion by the following experiments. In a 1.7×10^{-3} M solution of rubrene in DMF, 0.1 M in TBAP, the radical anion ($R\cdot^-$) was generated at constant potential until a slight excess of $R\cdot^-$ was present in the bulk of the solution (as indicated by a persistent green color and by the voltammetry of the solution). Thus, impurities which react with $R\cdot^-$ were essentially purged by titration with $R\cdot^-$. The coulombs passed to reach the end point represent an impurity level of 10^{-4} to 10^{-3} equiv/l. When the voltammetry of a freshly titrated solution was examined, an oxidation process commencing at −0.2 v and extending to the cation formation region was clearly evident. Furthermore, when the potential of the electrode was maintained in this region, light could be detected continuously (solution stirred) with no switching of the voltage required. No such oxidation process or light emission could be detected prior to the purging titration. After the solutions aged (20 min), both the oxidation process and the light emission disappeared. Further generation of anion failed to revive either one.

Clearly the rubrene radical anion reacts with some impurity (perhaps H_2O) to produce a moderately unstable decomposition product which, when oxidized, produces a chemiluminescent reaction.[9] Whether this chemiluminescent reaction necessarily involves traces of $R\cdot^-$ is difficult to determine, but it is known that the decomposition of the rubrene cation yields low levels of light in the absence of $R\cdot^-$.[10] A similar purging experiment with the cation was impossible owing to the intrinsic instability of this species.

The purged and aged solutions exhibiting anion lifetimes of many minutes (at least 20) and exhibiting no continuous luminescence in the −0.2 to +0.9-v region were also examined by a double-potential-step experiment. Apparent luminescent oxidation of the anion was still detected. However, the threshold voltage was shifted from −0.2 to +0.5 v *vs.* the sce. The intensity of the luminescence was about two orders of magnitude below the intensity produced by the annihilation reaction. It resembles the pre-annihilation light observed for cation reduction both in intensity and in overvoltage required and is only observed in a double-pulse or ac experiment while substantial anion oxidation current is passing. Although the mechanism of this residual luminescent process remains unproven, it may be due to direct generation of the triplet in a heterogeneous electron-transfer step.

Acknowledgment. The authors gratefully acknowledge many helpful discussions with G. J. Hoijtink, University of Amsterdam, The Netherlands, and G. W. Kennerly, M. M. Rauhut, and A. Zweig of these laboratories.

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Received August 18, 1966

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Book Reviews

Thermoanalytical Methods of Investigation. By Paul D. Garn, Department of Chemistry, The University of Akron, Akron, Ohio. Academic Press Inc., 111 Fifth Ave., New York, N. Y. 1965. xvi + 606 pp. 16 × 23.5 cm. $19.50.

The chapters of the book are headed: "Changes in State on Heating"; "Differential Thermal Analysis"; "Evaluation of Differential Thermal Analysis Curves"; "Kinetics"; "Atmosphere Control"; "Special Techniques"; "Thermogravimetric Analysis"; "Thermogravimetric Apparatus"; "Simultaneous Measurements"; "Other Techniques"; "Miscellaneous Topics"; "Analysis of Gaseous Products"; "Recording, Control, and Power Equipment"; "Miscellaneous Apparatus and Information"; and "Apparatus Design". Appendices are added listing manufacturers of commercial equipment for differential thermal analysis, thermogravimetric analysis, and dilatometry; thermocouple emf's and enthalpic changes for a number of selected materials; and questions to be answered by prospective users to assist in equipment selection. An extensive bibliography is employed for documentation. As is evident from the chapter headings, the book essentially is devoted to differential thermal analysis and thermogravimetric analysis. However, as the author indicates, most of the parameters discussed under these two techniques are applicable to the other methods. Chapter 12, "Other Techniques," includes brief sections on dilatometry, electrical measurements, X-ray diffraction, thermomicroscopy, thermal conductivity, and calorimetry. Under "Miscellaneous Topics," the author presents brief coverage of polymers, mechanical effects in polymers "integral procedural decomposition temperature," dolomite and siderite decompositions, study of metathetical reactions at elevated temperatures, clay minerals as a continuous series, biological studies, fractional thermogravimetric analysis, detection of formation of compounds, and qualitative identifications. "Analysis of Gaseous Decomposition Products," Chapter 14, incorporates the author's personal experience in effluent gas analysis by the usual thermal conductivity technique, as well as by means of gas density measurement and gas chromatography. Mass spectrometry and the emanation method are also discussed.

The reviewer was disappointed in the arrangement of the text. This appears to be partly the fault of the publisher and partly that of the author. In any event, there appeared to be an excessive need to refer to other pages of the text for figures and for complete discussions of specific points.

A remarkable feature of the book is the interjection of the author's critical comments with respect to the significance of previous investigations in the field. These comments, based on the extensive experience of the author, are unique and add significantly to the value of the book. Typical of such comments is that recommending the utilization of other techniques for acquisition of kinetic data following 26 pages of discussion of the subject.

The sections on instrumentation are particularly well done, providing the reader with the advantages and limitations of system