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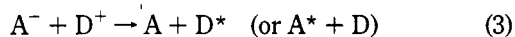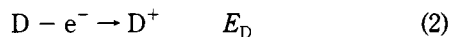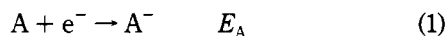- Copyright permission to reproduce figures and/or text from this article

Electrogenerated Chemiluminescence. 57. Emission from Sodium 9,10-Diphenylanthracene-2-sulfonate, Thianthrenecarboxylic Acids, and Chlorpromazine in Aqueous Media

Thomas C. Richards and Allen J. Bard*

Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712
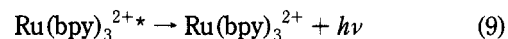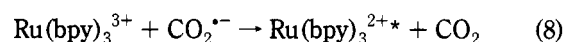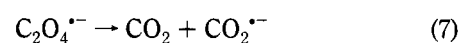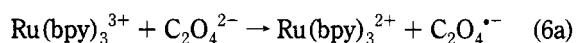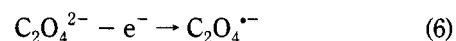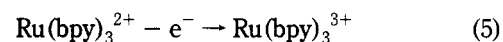
The anodic oxidation of aqueous sodium 9,10-diphenyl-anthracene-2-sulfonate (DPAS) and 1- and 2-thianthrene-carboxylic acid (1- and 2-THCOOH) in the presence of tri-n-propylamine as a coreactant in aqueous solution produces electrogenerated chemiluminescence (ECL). In addition, the cathodic reduction of DPAS in the presence of peroxydisulfate as a coreactant also produces ECL in an acetonitrile–water solution (1:1 v/v). For DPAS, both the oxidative and the reductive ECL spectra are characteristic of DPAS fluorescence, with peak intensities at 430 nm. In contrast, the ECL spectra for 1- and 2-THCOOH are significantly red-shifted from the fluorescence spectra, indicating that the emission arises from a product of THCOOH rather than the excited state of the intact acid. The oxidation of chlorpromazine produces an ECL emission in the absence of an added coreactant following an unprecedented "self-annihilation" mechanism. These new aqueous ECL reactions should be useful in the design of new labels for ECL analysis of biomolecules (i.e., immunoassays, DNA probes).

Electrogenerated chemiluminescence (ECL)[1] arises from an energetic electron-transfer reaction between electrogenerated redox species represented by A⁻ and D⁺, typically radical ions, to form an excited state (A* or D*) that emits in the visible region:

$$A + e^- \rightarrow A^- \qquad E_A \qquad (1)$$

$$D - e^- \rightarrow D^+ \qquad E_D \qquad (2)$$

$$A^- + D^+ \rightarrow A + D^* \quad (\text{or } A^* + D) \qquad (3)$$

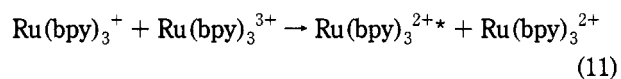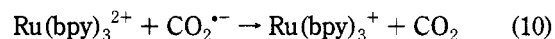$$D^* \rightarrow D + h\nu \qquad (4)$$

More recently, it was discovered that ECL precursors can also be generated in aqueous solution despite the limited potential range imposed by the oxidation and reduction of water.[2] Here, ECL is achieved by the simultaneous oxidation of tris(2,2′-bipyridinyl)ruthenium(II) [Ru(bpy)₃²⁺] and a coreactant (e.g.,

$C_2O_4^{2-}$ [3] or tri-n-propylamine (TPrA)[4]) capable of generating a suitable reductant upon oxidation (i.e., an oxidative-reduction mechanism):

$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+} \qquad (5)$$

$$C_2O_4^{2-} - e^- \rightarrow C_2O_4^{\bullet-} \qquad (6)$$

$$Ru(bpy)_3^{3+} + C_2O_4^{2-} \rightarrow Ru(bpy)_3^{2+} + C_2O_4^{\bullet-} \qquad (6a)$$

$$C_2O_4^{\bullet-} \rightarrow CO_2 + CO_2^{\bullet-} \qquad (7)$$

$$Ru(bpy)_3^{3+} + CO_2^{\bullet-} \rightarrow Ru(bpy)_3^{2+*} + CO_2 \qquad (8)$$

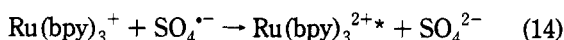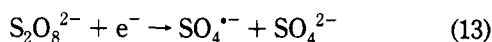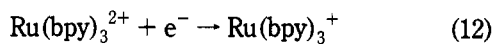$$Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + h\nu \qquad (9)$$

In these ECL schemes, the coreactant can be oxidized either at the electrode via eq 6 or in solution by the emitter via eq 6a.[3,4a] The excited state can also be generated by the sequence of eqs 10 and 11, as previously discussed for oxalate[3] and TPrA.[4a] In

$$Ru(bpy)_3^{2+} + CO_2^{\bullet-} \rightarrow Ru(bpy)_3^+ + CO_2 \qquad (10)$$

$$Ru(bpy)_3^+ + Ru(bpy)_3^{3+} \rightarrow Ru(bpy)_3^{2+*} + Ru(bpy)_3^{2+} \qquad (11)$$

the parallel reaction sequence for TPrA, the bond-breaking step (eq 7) involves the loss of a proton from the α-carbon of the radical cation. These ECL reactions have been used for the determination of both Ru(bpy)₃²⁺ [3c] and oxalate.[3b] To complement these oxidative-reduction examples, peroxydisulfate ($S_2O_8^{2-}$)[3c,5] and Ru(bpy)₃²⁺ undergo an analogous inversion of this scheme (a reductive-oxidation mechanism, eqs 12–14 followed by eq 9) when the initial reactants are reduced rather than oxidized. In this case, the reduction of $S_2O_8^{2-}$ results in the formation of the strong oxidizing agent $SO_4^{\bullet-}$.

(1) Faulkner, L. R.; Bard, A. J. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1977; Vol. 10, p 1.
(2) Knight, A. W.; Greenway, G. M. *Analyst* 1994, *119*, 879.

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$$Ru(bpy)_3^{2+} + e^- \rightarrow Ru(bpy)_3^+ \qquad (12)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{\bullet-} + SO_4^{2-} \qquad (13)$$

$$Ru(bpy)_3^+ + S_2O_8^{2-} \rightarrow Ru(bpy)_3^{2+} + SO_4^{\bullet-} + SO_4^{2-} \quad (13a)$$

$$Ru(bpy)_3^+ + SO_4^{\bullet-} \rightarrow Ru(bpy)_3^{2+*} + SO_4^{2-} \qquad (14)$$

By analogy with eqs 10 and 11 for oxalate and TPrA, an alternative to eq 14 for generation of the excited state with $S_2O_8^{2-}$ is eq 15 followed by eq 11.

$$Ru(bpy)_3^{2+} + SO_4^{\bullet-} \rightarrow Ru(bpy)_3^{3+} + SO_4^{2-} \qquad (15)$$
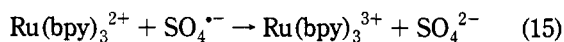
The sensitivity and selectivity of these coreactant analyses has led to the recent adaptation of the $Ru(bpy)_3^{2+}$/TPrA system for commercial analytical applications utilizing $Ru(bpy)_3^{2+}$ as a label (IGEN, Inc., Gaithersburg, MD, and Perkin-Elmer Corp., Norwalk, CT). Such applications and the need for novel labels to allow simultaneous analyses and internal referencing has prompted us to consider emitters other than $Ru(bpy)_3^{2+*}$ as ECL labels in aqueous solutions. The successful development of new labels is subject to several constraints, including the following: (1) the emitter must be soluble in aqueous solution, (2) the emission wavelength must be distinct from that of $Ru(bpy)_3^{2+*}$, (3) the oxidative or reductive electrochemistry must proceed within the relatively narrow potential range imposed by the oxidation and reduction of water, and (4) the oxidized or reduced intermediate must react with the electrogenerated coreactant intermediate, allowing formation of the excited state.

Sodium 9,10-diphenylanthracene-2-sulfonate (DPAS) has a strongly fluorescent excited state and is an energy acceptor from singlet ketones in aqueous media.[6]
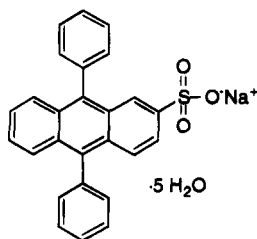
DPAS

DPAS was of particular interest for ECL studies, given its high fluorescence efficiency and short wavelength emission ($\Phi_F = 0.87$, peak emission at 428 nm[6]) compared to those of $Ru(bpy)_3^{2+}$ ($\Phi_F \approx 0.05$,[7] peak emission at 620 nm[3a]). We show here that DPAS does produce ECL, and we report the blue emission resulting from an oxidative-reduction scheme involving DPAS as the emitter and TPrA as the coreactant in aqueous media. We also report the ECL of DPAS in a reductive-oxidation scheme using $S_2O_8^{2-}$ as a coreactant in an MeCN−water solution (1:1 v/v).

With an interest in additional blue emitters, we have also studied the aqueous ECL of two carboxylic acid derivatives of thianthrene (TH), namely 1- and 2-thianthrenecarboxylic acid (1- and 2-THCOOH). We were attracted to the THCOOH derivatives

TH   **1-THCOOH**   **2-THCOOH**
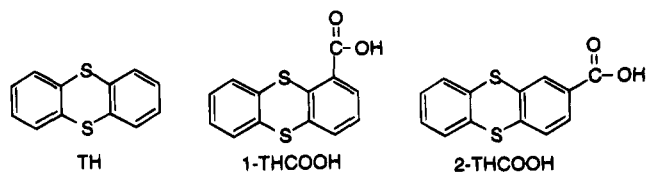
given the known blue ECL emission for TH (peak intensity at 434 nm[8]) in MeCN following the ion annihilation scheme of eqs 1−4. Derivatization of TH to its carboxylic acid derivative afforded the necessary solubility in aqueous solution, and these derivatives do produce ECL emissions in an oxidative-reduction scheme with TPrA as a coreactant. However, for these compounds, the ECL spectra are significantly red-shifted from the fluorescence spectra.

Finally, we have considered the aqueous ECL of the tranquilizer chlorpromazine (CPZ),[9] which is structurally related to TH.

CPZ
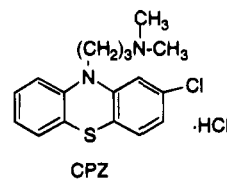
Although we are unaware of reports of ECL for CPZ, the fluorescence, chemiluminescence, and electrochemistry of this pharmaceutical are well documented, including analytical applications. McCreery et al. studied the oxidation of CPZ and various reactions of the cation radical[10] and, more recently, extended the work to consider the reaction of the CPZ cation radical with dopamine,[11,12] methoxypromazine,[12] and hydroquinone.[12] In addition, the fluorescence[13-16] (including analytical applications[17,18]) and chemiluminescence[19,20] of CPZ have been investigated. CPZ also emits when oxidized in the absence of an added coreactant, presumably because the tertiary amine on the CPZ side chain acts as an internal coreactant. Related work (without the covalent binding of the coreactant to the emitter) is cited in a recent paper by Holeman and Danielson[21] on the chemiluminescence reactions of thiazide compounds with $Ru(bpy)_3^{3+}$. Here they discuss the

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utilization of tertiary amines present in the structures of erythromycin[22] and clindomycin[23] to assay these antibiotics via reactions with $Ru(bpy)_3^{3+}$ using flow injection and liquid chromatographic analyses.

EXPERIMENTAL SECTION

Chemicals. MeCN (Burdick and Jackson, Baxter Health Care Corp., Muskegon, MI) was transferred unopened into a glovebox (Vacuum Atmospheres Corp., Los Angeles, CA) under helium atmosphere and was used as received. For experiments run in MeCN, a sealed cell was loaded in the glovebox prior to each experiment. Tetrabutylammonium tetrafluoroborate (TBABF$_4$, electrometric grade; SACHEM, Inc., Austin, TX) was purified by recrystallization from either ethyl acetate–pentane (2 times; 1:1 v/v) or ethyl acetate–diethyl ether (3 times; 9:1 v/v), followed by drying under vacuum at 100 °C. Tetraethylammonium perchlorate (TEAP, electrometric grade wet with 8% water; SACHEM, Inc.), tri-*n*-propylamine (TPrA, 98%; Aldrich, Milwaukee, WI), thianthrene (97%, Aldrich), chlorpromazine hydrochloride (98%, Aldrich), sodium phosphate, monobasic monohydrate (NaH$_2$-PO$_4$·H$_2$O, 99%; Baker, Phillipsburg, NJ), sodium phosphate, dibasic heptahydrate (Na$_2$HPO$_4$·7H$_2$O, 99%; Mallinckrodt, Paris, KY), and ammonium peroxydisulfate ((NH$_4$)$_2$S$_2$O$_8$, 99%; Fischer Scientific, Fair Lawn, NJ) were used as received.

Sodium phosphate buffer solutions, 0.15 M, prepared with Milli-Q water (Millipore Corp., Bedford, WA) were 0.10 M in Na$_2$-HPO$_4$·7H$_2$O and 0.05 M in NaH$_2$PO$_4$·H$_2$O. Buffer solutions containing TPrA were prepared similarly except that 0.15 M NaH$_2$-PO$_4$·H$_2$O was used to offset the basicity of TPrA. The pH of these buffer solutions was adjusted with either concentrated phosphoric acid (H$_3$PO$_4$) or 4 M NaOH.

DPAS was synthesized according to a variation on the procedure of Catalani and co-workers.[6] Thus, the initial reaction mixture consisted of 1 g of 9,10-diphenylanthracene (DPA, 98%; Aldrich) suspended in 7 mL of nitrobenzene (99%; Kodak), to which 0.25 mL of fuming sulfuric acid (27–33% free SO$_3$; Aldrich) was added. The final product was purified by ion-exchange column chromatography using Dowex 1 × 2–100 resin (50–100 dry mesh; Aldrich). Aqueous solutions of DPAS·5H$_2$O were prepared by dissolving the required amount of solid in a few drops of methanol prior to transfer to the aqueous solution.

1- and 2-THCOOH were synthesized according to procedures of Gilman and Swayampati[24] with the exception that 1.6 M *n*-butyllithium in hexane (Aldrich) was substituted for their ethereal solutions of *n*-butyllithium.

Instrumentation. Cyclic voltammetry experiments without photon detection utilized either a BAS 100A electrochemical analyzer (Bioanalytical Systems, West Lafayette, IN) or a Princeton Applied Research Model 173/175 potentiostat/universal programmer (PAR, Princeton, NJ) and an Omnigraphic 2000 $X-Y$ recorder (Bausch and Lomb–Houston Instrument Division, Austin, TX). Cyclic voltammograms with simultaneous photon detection were recorded using the PAR 173/175 in conjunction with a Hamamatsu C1230 photon counter (Bridgewater, NJ) equipped with a Hamamatsu R928-P photomultiplier tube. The photomultiplier tube was housed in a water-jacketed Products For Research, Inc. Model

TE308TSRF refrigerated chamber maintained at −10 °C. Primary cooling of the water jacket to 10 °C was accomplished using an MGW Lauda Model RMS6 refrigeration unit (Brinkmann Instruments Co., Westbury, NY). For plots of ECL intensity versus potential, the output from the photon counter was fed into the *y*-axis of the $X-Y$ recorder during potential scans.

Fluorescence and ECL spectra were recorded using a charge-coupled device (CCD) camera and spectrometer (1 mm entrance slit) interfaced to a Dell System 200 personal computer (Austin, TX) as previously described.[25] The same procedures (spectral calibration, etc.) were followed except that different pulse sequences were used and the CCD detector was maintained at −110 °C for the TPrA/THCOOH and TPrA/DPAS experiments and at −100 °C for the S$_2$O$_8^{2-}$/DPAS experiments. Excitation for the fluorescence spectra was achieved by holding a longwave (366 nm) UV lamp (Model UVGL-25; UVP, Inc., San Gabriel, CA; sold by Fisher Scientific, Pittsburgh, PA) next to the cell and at right angles to the entrance slit of the spectrometer.

Cells and Electrodes. Except where noted, all electrochemical and ECL experiments utilized Pt disk working electrodes (sealed in glass) in a conventional three-electrode cell configuration. These working electrodes were polished on a felt pad (Buehler, Ltd., Lake Bluff, IL) before each experiment with 0.05 μm alumina (Buehler, Ltd.) suspended in water.

For ECL experiments employing a Pt gauze working electrode, the electrode consisted of a 6 × 9 mm gauze flag (52 mesh; Aldrich). The electrode was cleaned prior to each experiment by immersion in concentrated nitric acid, followed by rinsing in distilled water and heating in a Bunsen burner flame. The CPZ experiments utilized a highly oriented pyrolytic graphite (HOPG; Union Carbide, Cleveland, OH) working electrode (with freshly exposed material for each experiment) in a recently described configuration.[26] This same arrangement was used with a Pt electrode for the 2-THCOOH ECL emission versus potential experiments.

Nonaqueous potential referencing to ferrocene was accomplished by adding the standard directly to the solutions. Potential referencing of aqueous solutions to the saturated calomel electrode (SCE) was accomplished by acquiring data versus a Ag quasi-reference electrode for which the potential versus SCE was known. For qualitative comparison purposes, the ferrocene $E^{\circ\prime}$ vs SCE varies (due primarily to differences in liquid junction potentials) from +0.307 to +0.56 V, depending upon the solvent/supporting electrolyte system.[27,28]

RESULTS AND DISCUSSION

Sodium 9,10-Diphenylanthracene-2-sulfonate. The maximum solubility of aqueous DPAS in 0.15 M sodium phosphate buffer (pH 7.5) is ≈1 × 10^{-5} M. At this low concentration, no anodic current is observed for DPAS above the background oxidation of water. However, if 1 × 10^{-5} M DPAS is oxidized in

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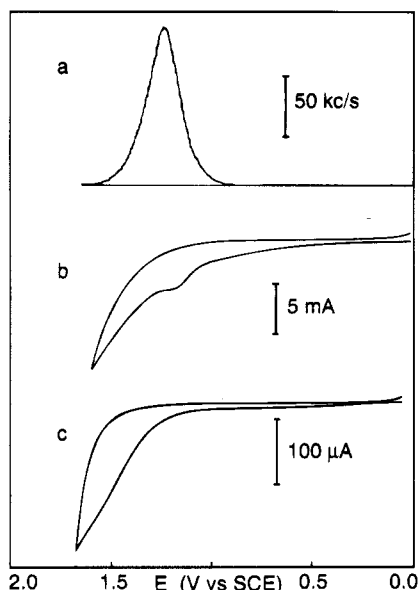(24) Gilman, H.; Swayampati, D. R. *J. Am. Chem. Soc.* **1957**, *79*, 208.

(25) The setup for spectral acquisition utilized a hybridization of schemes a and b shown in Figure 1 of ref 4b. Thus, instead of removing the 100 mm Pentax camera lens, the spectrometer was placed about 15 in. from the camera, with the lens focused on the spectrometer exit port. This allowed recording of broader spectra than was possible with the setup shown in Figure 1b.

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Figure 1. Emission (a) during cyclic voltammetry (b) of 1×10^{-5} M DPAS and 0.15 M TPrA in pH 7.5 sodium phosphate buffer at 6 × 9 mm Pt gauze (52 mesh) electrode. Scan rate, 100 mV/s. (c) Cyclic voltammogram of 0.05 M TPrA in pH 7.5 sodium phosphate buffer at a 1.5 mm diameter Pt electrode. Scan rate, 200 mV/s.
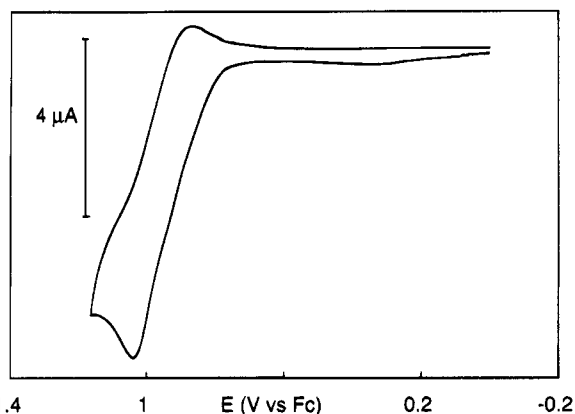

Figure 2. Cyclic voltammogram of 0.92 mM DPAS in MeCN at a 1.5 mm diameter Pt electrode. Supporting electrolyte, 0.1 M TBABF$_4$; scan rate, 200 mV/s.
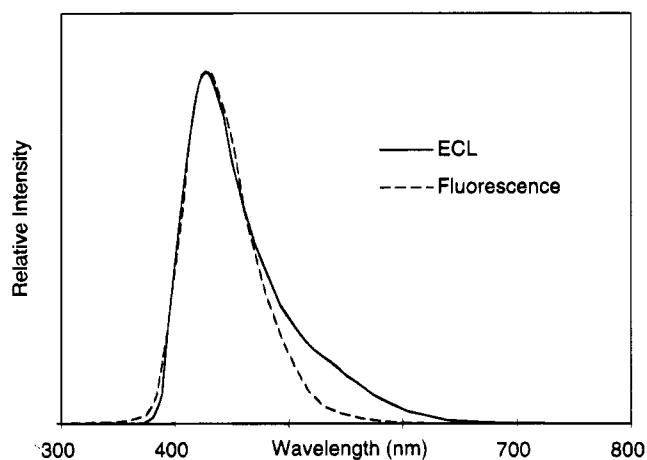

Figure 3. Scaled fluorescence and ECL spectra for a solution of 1 × 10^{-5} M DPAS and 0.15 M TPrA in pH 7.5 sodium phosphate buffer. The ECL emission was generated by repetitively pulsing a 6 × 9 mm Pt gauze (52 mesh) electrode from 0.0 V (vs SCE, 8 s) to +1.2 V (2 s) to −1.4 V (2 s) and back to 0.0 V. Light was generated on the positive pulse in this sequence (the negative pulse was for electrode cleaning purposes) and was integrated for 2 min to produce the spectrum. Both peak intensities are at 430 nm.

the presence of 0.15 M TPrA, an emission is observed that coincides with an oxidation wave that is superimposed on the TPrA oxidation and background oxidation of water, as shown in Figure 1, parts a and b. For comparison, the oxidation of 0.05 M TPrA in the absence of DPAS is shown in Figure 1c. The fact that an oxidation current is observed for 1×10^{-5} M DPAS only in the presence of TPrA suggests a catalytic current enhancement due to the regeneration of DPAS via reduction of DPAS$^+$ by TPrA or an intermediate. Presumably, ECL results from a sequence of reactions analogous to eqs 5−8 (or eqs 10 and 11) and eq 9, where Ru(bpy)$_3^{3+/2+/+}$ is replaced by DPAS$^{+/0/-}$, C$_2$O$_4^{2-}$ by TPrA, CO$_2^{\bullet-}$ by Pr$_2$NCHEt and CO$_2$ by H$^+$. Despite the low DPAS concentration (1×10^{-5} M), this emission is visible when viewed in a dark room, and no emission is observed in the absence of either TPrA or DPAS. For the DPAS/TPrA system, electrode passivation occurs after one scan and prevents observation of subsequent emissions without electrode cleaning. This behavior suggests that products of chemical reactions following the oxidation of DPAS passivate the electrode. The electrode can be cleaned mechanically or by pulsing into the background reduction of water (−1.4 V vs SCE) for a few seconds, after which ECL can again be observed upon oxidation. In an effort to understand the passivation process, we undertook cyclic voltammetric experiments in MeCN; a typical voltammogram is shown in Figure 2. Clearly, the oxidation of DPAS is complicated by subsequent chemical reactions, as evidenced by the lack of chemical reversibility. This behavior, which persists at scan rates as high as 10 V/s, could be due to reaction of DPAS$^+$ with water or OH$^-$ (supplied by the five waters of hydration) to form products. The addition of neutral alumina to these solutions in an attempt to remove water did not result in profound changes in the electrochemistry. Attempts to increase the ECL emission intensity by stabilization of DPAS$^+$ using surfactant or by polysulfonation of DPA were unsuccessful. For the DPAS/TPrA system, ECL emissions can be detected (using the photon counter) at a concentration as low as 2×10^{-8} M. For comparison, Ru(bpy)$_3^{2+}$ can be detected at concentrations

about 60 times more dilute at similar emission intensities. At higher concentrations of 5×10^{-6} M and using the CCD camera (and correcting for the detector sensitivity), the Ru(bpy)$_3^{2+}$ emission is ~100 times more intense than the DPAS emission. Figure 3 shows the ECL and fluorescence spectra of 1×10^{-5} M DPAS. The similarity between the fluorescence and ECL spectra (despite the discrepancy on the long wavelength side) suggests that the ECL emission is from DPAS*.

Details concerning the electrochemistry and ECL of the Ru-(bpy)$_3^{2+}$/S$_2$O$_8^{2-}$ system in MeCN−water have been considered previously[5] and the reductive cyclic voltammogram of S$_2$O$_8^{2-}$ is shown in Figure 4a. Here, the irreversibility is due to the process outlined in eq 13. Under these conditions, the onset of the background reduction of water (not shown) producing gaseous H$_2$ occurs at about −1.0 V. The reduction of DPAS in MeCN is shown in Figure 4b, and the reversibility of this couple (DPAS$^{0/-}$) compared to the oxidation of DPAS shown in Figure 2 indicates that DPAS$^-$ is significantly more stable in MeCN than DPAS$^+$. The small prewave in Figure 4b appears to be due to adsorbed DPAS, since the current for this wave increases linearly with scan rate (ν), whereas that for the more negative couple increases with
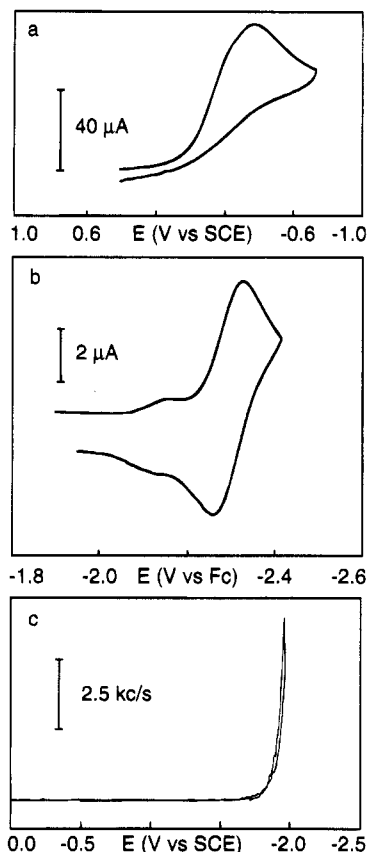
Figure 5. Fluorescence and ECL spectra for the same solution as in Figure 4c. The ECL emission was generated by pulsing a 2.0 mm diameter Pt electrode to −2.2 V (vs SCE) and integrating the emission for 5 s. Both peak intensities are at 430 nm.

Figure 4. (a) Cyclic voltammogram of 11 mM (NH₄)₂S₂O₈ in an MeCN−water solution (1:1 v/v) at a 1.5 mm diameter Pt electrode. Supporting electrolyte, 0.2 M TEAP; scan rate, 100 mV/s. (b) Cyclic voltammogram of 0.93 mM DPAS in MeCN at a 1.5 mm diameter Pt electrode. Supporting electrolyte, 0.1 M TBABF₄; scan rate, 200 mV/ s. (c) ECL emission for the reduction of 1.1 mM DPAS and 25 mM (NH₄)₂S₂O₈ in MeCN−water solution (1:1 v/v) at a 2.0 mm diameter Pt electrode. Supporting electrolyte, 0.2 M TEAP; scan rate, 100 mV/ s.

$v^{1/2}$ over a range of 0.1−10 V/s. Because this reduction occurs well into the background reduction of water, no cathodic current above background is evident in MeCN−water (1:1 v/v) solutions. However, if the potential is scanned into the background reduction of water in the presence of $S_2O_8^{2-}$, an emission is observed at a potential corresponding to the reduction of DPAS (after making the approximate conversion from ferrocene to SCE potentials using the values given in the Experimental Section), as shown in Figure 4c. No emission is observed in the absence of either $S_2O_8^{2-}$ or DPAS. This result is consistent with previous results with the $Ru(bpy)_3^{2+}/S_2O_8^{2-}$ system,[5] in which no emission is seen until the potential is scanned sufficiently negative to reduce $Ru(bpy)_3^{2+}$ and allow generation of the excited state via the sequence of reactions 12−15. This scheme can be modified to describe the ECL reactions for DPAS by substituting DPAS$^{+/0/-}$ respectively for $Ru(bpy)_3^{3+/2+/+}$. As shown in Figure 5, the blue ECL spectrum for the DPAS emission matches the fluorescence spectrum and also matches the ECL and fluorescence spectra for the oxidative-reduction of DPAS with TPrA shown in Figure 3.

In considering the energetics of an ECL system, one notes the standard potentials of the relevant half-reactions (yielding the free energy of the electron-transfer reaction) and the energy of the emitting state. For the oxidation of DPAS and TPrA, these
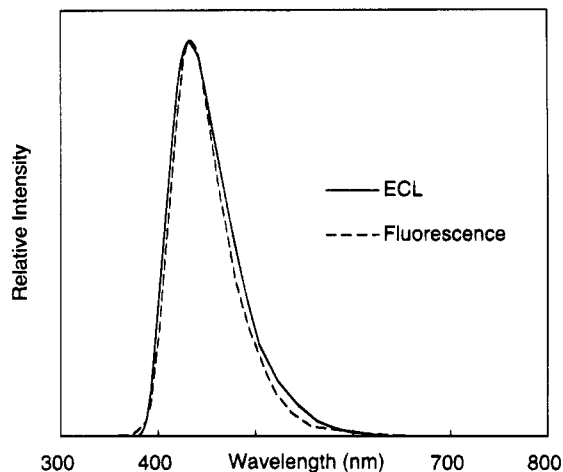
are

$$DPAS^{\cdot +} + E^- \rightarrow DPAS \qquad E^\circ \approx +1.3 \text{ V vs SCE}$$

$$Pr_2NC^+HEt + e^- \rightarrow Pr_2N\dot{C}HEt \qquad E^\circ \approx -1.1 \text{ V vs SCE}^{29}$$

$$DPAS^* \text{ (singlet)} \rightarrow DPAS + h\nu \qquad E_s = 2.9 \text{ eV}$$

The enthalpy of the electron-transfer reaction between DPAS$^{\cdot +}$ and Pr₂NĊHEt, correcting for an entropy of about 0.1 eV, is −2.3 eV, clearly well below that needed to produce singlet DPAS*, so that this would be classified as an "energy-deficient" reaction. As with other reactions of this type, the excited singlet probably forms via triplet-triplet annihilation (a T-route).[1] Although the oxidative DPAS/oxalate system would be "energy-sufficient", no emission was observed for experiments with oxalate as the coreactant.

The reductive path energetics corresponds to the following half-reactions:

$$DPAS + e^- \rightarrow DPAS^{\cdot -} \qquad E^\circ \approx -1.9 \text{ V vs SCE}$$

$$SO_4^{\cdot -} + e^- \rightarrow SO_4^{2-} \qquad E^\circ \approx +3.2 \text{ V vs SCE}^{30}$$

In this case, the electron-transfer reaction between DPAS$^{\cdot -}$ and SO₄$^{\cdot -}$ is sufficient to produce singlet DPAS* directly (an S-route). Despite these favorable energetics, the emission intensity for 1 × 10⁻⁵ M DPAS is about the same for the DPAS/TPrA oxidation as it is for the DPAS/$S_2O_8^{2-}$ reduction.

Thianthrenecarboxylic Acids. The oxidation of 2-THCOOH in aqueous solution occurs near +1.0 V, as shown in Figure 6a. The broad anodic process between 0.0 and +0.5 V and the more prominent cathodic process at −0.2 V are characteristic of oxide formation and reduction at a Pt electrode in aqueous solution at this pH and appear in scans in the absence of 2-THCOOH. These processes can be eliminated if the potential is cycled no further negative than +0.15 V, as shown in Figure 6b. The chemical

(29) The reduction potential for a similar species, Et₂NC⁻HMe, is reported as −1.12 V vs SCE: Wayner, D. D. M.; McPhee, D. J.; Griller, D. *J. Am. Chem. Soc.* **1988**, *110*, 132.

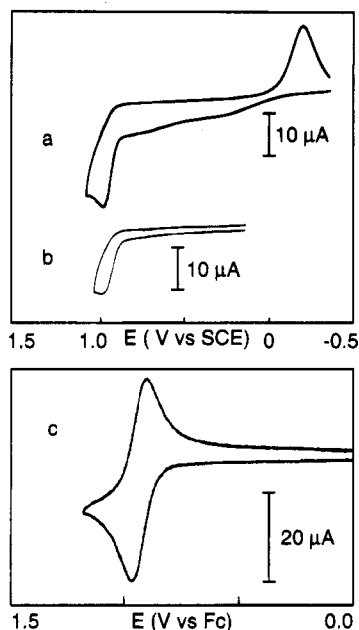(30) Memming, R. *J. Electrochem. Soc.* **1969**, *116*, 785.

Figure 8. Cyclic voltammogram (bottom) and simultaneous emission (top) of 0.26 mM 2-THCOOH and 0.15 M TPrA under the same conditions as in Figure 7.

Figure 6. (a) Cyclic voltammogram of 2.0 mM 2-THCOOH in pH 8.5 sodium phosphate buffer at a 1.5 mm diameter Pt electrode at a scan rate of 200 mV/s. (b) Cyclic voltammogram of 1.4 mM 2-TH-COOH under the same conditions as for part a, except that the scan cycled from +0.15 V instead of from −0.35 V. (c) Cyclic voltammogram of 2.0 mM 2-THCOOH at a 1.5 mm diameter Pt electrode in MeCN, 0.1 M TBABF$_4$ at a scan rate of 200 mV/s. Aqueous scans a and b are referenced to SCE. Nonaqueous scan c is referenced to ferrocene.
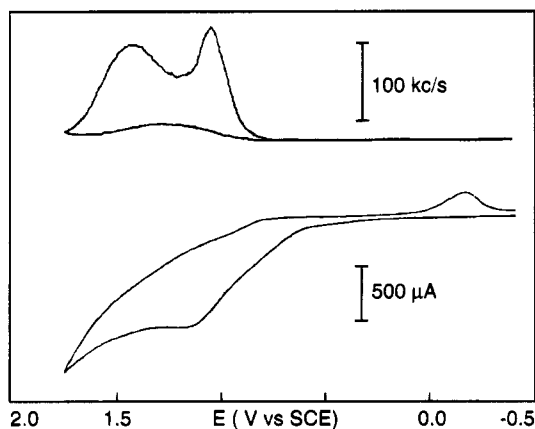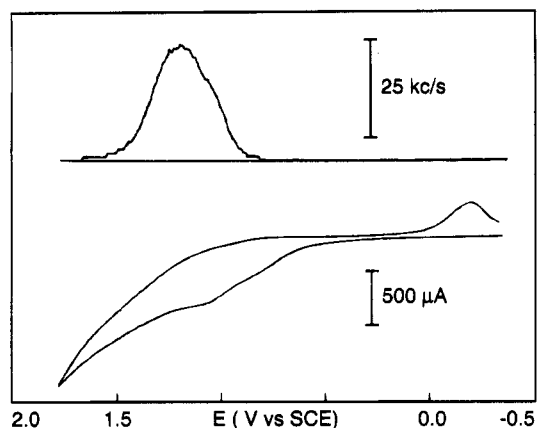

Figure 7. Cyclic voltammogram (bottom) and simultaneous emission (top) of 5.2 mM 2-THCOOH and 0.15 M TPrA in pH 7.5 sodium phosphate buffer at a 6 mm diameter Pt electrode at a scan rate of 100 mV/s.

irreversibility shown in parts a and b of Figure 6 (and observed in similar experiments scanning up to 50 V/s) indicates the instability of oxidized 2-THCOOH in aqueous solution and its rapid conversion to some product. In contrast to this, similar experiments run in dry MeCN (Figure 6c) show the oxidized product to be stable. This suggests that the decomposition of the oxidized acid is due to reaction with water as opposed to other possibilities, such as decarboxylation with loss of CO_2.

Both 1- and 2-THCOOH produce ECL emission when oxidized in aqueous sodium phosphate buffer solution in the presence of TPrA as a coreactant, as shown in Figures 7 and 8 for 2-THCOOH. (The behavior of 1-THCOOH is similar except that the peak emission intensity is 6−7 times less intense than that of 2-TH-COOH.) If these same experiments are run in the absence of

TPrA or the acid, no emission is observed. Although the anodic current is dominated by the oxidation of the deprotonated acid, the electrochemistry is complicated by the coincidence of this process with both the onset of the broad, irreversible oxidation wave for TPrA (Figure 1c) and the background oxidation of water. The two distinct emission peaks as a function of potential in Figure 7 suggest contributions from two different electrode processes. It is possible that the first peak is due to reactions associated with the homogeneous oxidation of TPrA following reaction 6a (with 2-TH$^+$COO$^-$ replacing Ru(bpy)$_3^{3+}$) and that the second peak results from the direct heterogeneous oxidation of TPrA at the electrode following reaction 6. This hypothesis is supported by the results of Figure 8 recorded at a lower 2-THCOOH concentration, where the second-order reaction 6a will be slower and less important. Under the conditions of Figure 8, the first emission appears as a small shoulder in the dominant second emission.

The fluorescence and ECL spectra of 1- and 2-THCOOH are shown in Figure 9. The fluorescence maxima for 1- and 2-TH-COOH are at 505 and 480 nm, respectively. For comparison, the emission maximum for thianthrene fluorescence in MeCN is at 434 nm.[8] The ECL maxima for each acid are at 570 nm, showing a strong shift toward longer wavelength compared to the respective fluorescence spectra. Under these optimized conditions, the 2-THCOOH emission is just visible to the dark-adapted eye. The differences between the fluorescence and ECL spectra for the THCOOH acids suggest that at least part of the ECL emission is from some species other than an excited state of the intact acid.

Chlorpromazine. McCreery and co-workers[10] previously investigated reactions associated with the oxidation of CPZ, and the voltammetric curves found in our laboratory (Figure 10) agree with their results. Under acidic aqueous conditions, the oxidation proceeds as two discrete processes (Figure 10a), the first of which is quasi-reversible and the second irreversible. The abnormally sharp peak on the first anodic wave seen in parts a and b of Figure 10 has been attributed[10] to adsorption of CPZ. Likewise, the shoulder on the cathodic return wave in Figure 10b can be attributed to an adsorption process. When the scan is reversed after the first oxidation, as shown in Figure 10b, the increased reversibility (compared to Figure 10a) suggests that a product from the second oxidation reacts with the oxidized form of CPZ.

If CPZ is oxidized at higher pH in sodium phosphate buffer solution, the electrochemistry changes to that shown in the bottom
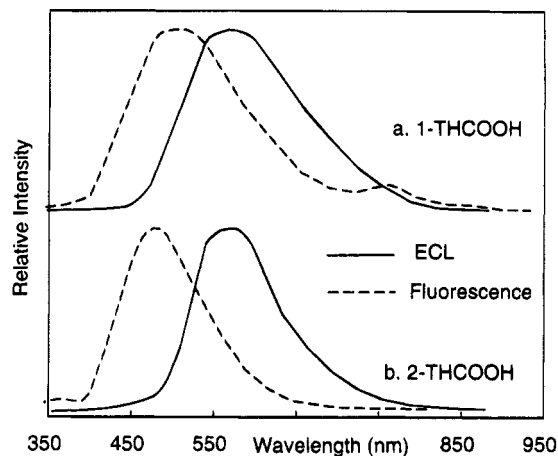
Figure 9. Fluorescence and ECL spectra of 9.1 mM 1-THCOOH (a) and 20 mM 2-THCOOH (b) taken in pH 8.5 sodium phosphate buffer containing 0.15 M TPrA. ECL emissions were generated by alternately pulsing a 6 × 9 mm Pt gauze (52 mesh) electrode between +1.4 (0.5 s) and −0.5 V (2 s) vs SCE for a duration of 40 min for 1-THCOOH and 20 min for 2-THCOOH with a stirred solution. The peak fluorescence intensity is at 480 nm for 2-THCOOH and 505 nm for 1-THCOOH. The peak ECL intensities are at 570 nm for both isomers. The intensities have been scaled for comparison purposes. At equal THCOOH concentrations (20 mM) and acquisition times, the intensities of the 2-THCOOH emissions are ~6−7 times those of 1-THCOOH.
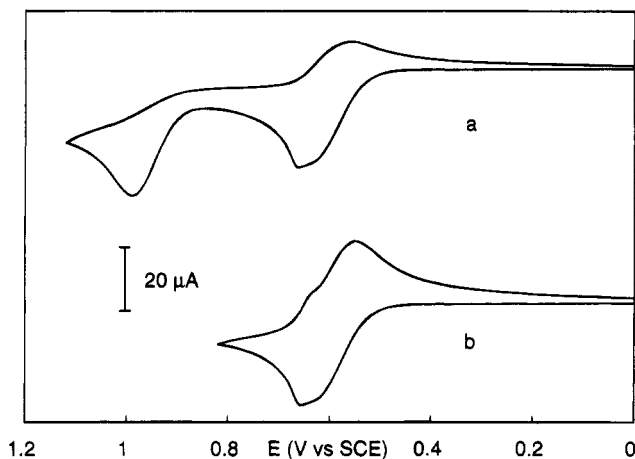

Figure 10. (a) Cyclic voltammogram of 0.98 mM CPZ in 0.01 M HCl, 0.2 M NaCl at a 6 mm diameter HOPG electrode at a scan rate of 50 mV/s. (b) Cyclic voltammogram for the same system and conditions as in part a with scan reversal after the first oxidation.

of Figure 11. The first oxidation is now irreversible, and the electrochemistry is in general more complex than that of Figure 10, as evidenced by a shoulder on the first oxidation wave, increased current, and a broadening of the second wave. As with the TPrA oxidation, analysis of the second oxidation process is complicated by its coincidence with the background oxidation of water. A more important observation under these conditions is ECL emission associated with the second oxidation process (in the absence of an added coreactant), as shown in Figure 11. Attempts to acquire the ECL spectrum for this process for comparison to the photoluminescence spectrum were unsuccessful due to low emission intensities and electrode fouling. Also unsuccessful were attempts to determine the wavelength of the peak emission using interference filters for specific, narrow wavelength ranges between 450 and 600 nm. No emission is
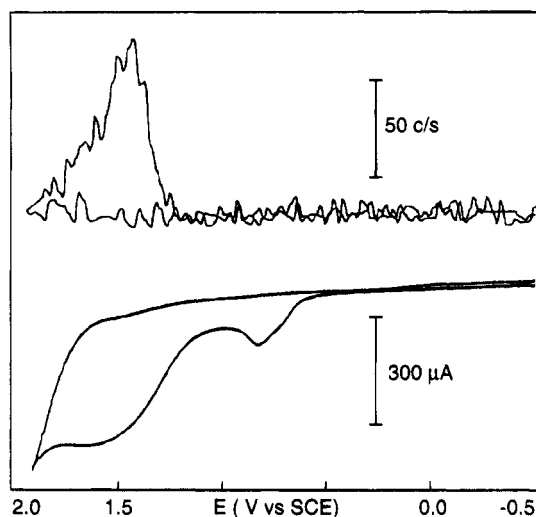

Figure 11. Cyclic voltammogram (bottom) and simultaneous emission (top) for 1.0 mM CPZ taken at a 6 mm diameter HOPG electrode in pH 7.5 sodium phosphate buffer at a scan rate of 200 mV/s.

observed under the conditions of Figure 10 (with or without 50 mM TPrA added), where the electrochemistry is less complex and electrode fouling does not occur.

Earlier mechanistic work by McCreery and co-workers[10] has shown that monooxidized forms of CPZ are converted to sulfoxides in aqueous solution via nucleophilic attack by water at the sulfur position. That work, in conjunction with our results, suggests that the first oxidation of CPZ is associated with the tricyclic ring system and that the second oxidation involves the tertiary amine on the side chain (analogous to the oxidation of THCOOH and TPrA). Presumably, the amine then undergoes reactions similar to those seen with TPrA,[4a] eqs 6 and 7, to produce a strong reducing agent that reacts with the ring system to generate a luminescent excited state. Thus, it is likely that this "self-annihilation" ECL process proceeds as shown in eqs 5−8, where the tricyclic portion of CPZ replaces $Ru(bpy)_3^{2+}$ and is covalently bonded to the coreactant X (the tertiary amine on the side chain). The self-annihilation could proceed via an intramolecular or an intermolecular process.

CONCLUSIONS

New ECL reactions of modified hydrocarbons and aromatic heterocyclic compounds that occur in aqueous solutions with the coreactants TPrA and $S_2O_8^{2-}$ have been found. These can be useful as ECL-active labels for analysis of biomolecules (immunoassay, DNA probes) and complement $Ru(bpy)_3^{2+}$ which is used in such studies.

When DPAS is oxidized in the presence of TPrA or reduced with $S_2O_8^{2-}$, a blue ECL emission results which is characteristic of DPAS fluorescence. The spectral separation between this emission and that for $Ru(bpy)_3^{2+}$ makes DPAS a good choice for use as a complementary label to $Ru(bpy)_3^{2+}$ in bioanalytical applications.

Oxidation of thianthrenecarboxylic acid in the presence of TPrA results in an ECL emission from a product that is different from the intact acid. Oxidation of chlorpromazine without added

coreactant (TPrA) produces an ECL emission via an unprecedented self-annihilation reaction.

ACKNOWLEDGMENT