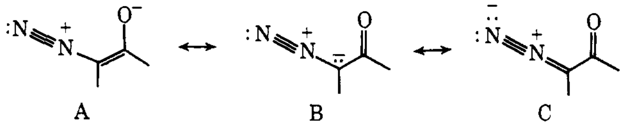
structural determinations of α-diazocarbonyl compounds by X-ray diffraction.

Compounds **1** and **2** represent a novel type of α-diazocarbonyl compound, for which three main resonance forms can be written: A, B, and C. The inter-



atomic bond lengths shown in the perspective drawings in Figures 1 and 2 indicate that all three forms contribute to the resonance hybrid in **1** and **2**. The C(4)–C(5) bond is intermediate in length between that of a single and a double bond, and is nearly the same as those found in uracil[9] and uridine,[10] while being significantly shorter than that in dihydrouracil (1.515 Å),[11] where no C(4)–C(5) resonance is possible. Similarly, the C(5)–N(5) bond length is intermediate between that of a double and a single bond, and is much shorter than the C–N bond found in aromatic diazonium compounds (1.40 ± 0.02 Å).[12]

It is of additional interest that the C–N and N–N bond distances reported[13] for diazomethane (H_2C^-—$N^+\equiv N$:) of 1.32 and 1.12 Å agree closely with those found in **1** and **2**, suggesting that these α-diazocarbonyl systems have a carbanion character similar to that of diazomethane. Similar C–N and N–N bond distances of 1.33 and 1.14 Å have also been found in the X-ray structure of 9-diazofluorene.[14]

Acknowledgment. We thank Dr. L. B. Townsend and T. C. Thurber for suggesting this problem and supplying crystals of both **1** and **2**. This work was supported by Contract No. PH-43-67-1186 with the Cancer Chemotherapy National Service Center, National Institutes of Health, and in part by NIH Grant No. GM-01728. We also wish to acknowledge the University of Pittsburgh Computing Center for generous amounts of computing time.

(8) (a) G. W. Cowell and A. Ledwith, *Quart. Rev., Chem. Soc.*, **24**, 119 (1970); (b) Q. P. Studzinskii and I. K. Korobitsyna, *Russ. Chem. Rev.*, 39, 834 (1970).

(9) R. F. Stewart and L. H. Jensen, *Acta Crystallogr.*, 23, 1102 (1967).

(10) E. A. Green, University of Pittsburgh, unpublished data.

(11) D. C. Rohrer and M. Sundaralingam, *Acta Crystallogr., Sect. B*, **26**, 546 (1970).

(12) (a) B. Greenbert and Y. Okaya, *ibid., Sect. B*, **25**, 2101 (1969); (b) C. Romming and T. Tjornhom, *Acta Chem. Scand.*, **22**, 2934 (1968); (c) C. Romming, *ibid.*, 17, 1444 (1963).

(13) (a) A. P. Cox, L. F. Thomas, and J. Sheriden, *Nature (London)*, **181**, 1000 (1958); (b) H. Boersch, *Monatsch. Chem.*, **65**, 331 (1935).

(14) P. W. R. Corfield, Ohio State University, personal communication.

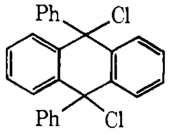
D. J. Abraham,* T. G. Cochran, R. D. Rosenstein
Departments of Medicinal Chemistry and Crystallography
University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Received May 27, 1971

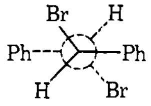
Novel Chemiluminescence Observed on Electroreduction of Aromatic Hydrocarbons in the Presence of Some Alkyl Halides[1]

Sir:

We would like to report a novel and unexpectedly intense chemiluminescent (cl) reaction that results from the electroreduction of solutions containing aromatic hydrocarbons and either 9,10-dichloro-9,10-dihydro-9,10-diphenylanthracene (DPACl$_2$) or 1,2-dibromo-1,2-diphenylethane (DBDPE) at platinum electrodes. In most previously reported examples of electrochemically generated chemiluminescence (ecl) of aromatic hydrocarbons, the excited state of the hydrocarbon is produced from the annihilation reaction of the radical anion, $R\cdot^-$, of the hydrocarbon with either the radical cation, $R\cdot^+$,[2,3] or another electrogenerated oxidizing agent.[4–6]



DPACl$_2$



meso-DBDPE

It was found that this cl, in a variety of nonaqueous solvents, is about two orders of magnitude greater in luminescence intensity than 9,10-diphenylanthracene annihilation ecl. This indicates that the mechanism by which the excited state is produced during the electrode reaction is different and probably more efficient than $R\cdot^-$ and $R\cdot^+$ annihilation under similar experimental conditions.[7]

All electrochemical and luminescence apparatus, experimental conditions, and solvent purification and organic syntheses procedures were essentially the same as reported previously.[5a,6a,8–11]

Chemiluminescence has previously been observed when DPACl$_2$ is reacted with either the 9,10-diphenylanthracene (DPA) or naphthalene radical anion (produced by sodium metal reduction).[11,12] The light in both cases was identified as DPA fluorescence.[11] The electrochemical reduction of fresh 1 m*M* DPACl$_2$ [0.1 *M* *tert*-butylammonium perchlorate (TBAP)] solutions in dimethylformamide (DMF), dimethyl sulfoxide (DMSO), acetonitrile (AN), and tetrahydrofuran (THF) exhibit intense ecl using a platinum electrode.[13]

---

(1) This research was supported in part by grants from the National Science Foundation, No. GP-9307 and GP-2716.

(2) (a) A. J. Bard, K. S. V. Santhanam, S. A. Cruser and L. R. Faulkner in "Fluorescence," G. G. Gilbault, Ed., Marcel Dekker, New York, N. Y., 1967, pp 627–651; (b) D. M. Hercules in "Physical Methods of Organic Chemistry," 4th ed, Part II, A. Weissberger and B. Rossiter, Ed., Academic Press, New York, N. Y., 1960; (c) A. Zweig, *Advan. Photochem.*, **6**, 425 (1968).

(3) L. R. Faulkner and A. J. Bard, *J. Amer. Chem. Soc.*, **91**, 209 (1969).

(4) (a) K. S. V. Santhanam and A. J. Bard, *ibid.*, **87**, 139 (1965); (b) J. Chang, D. M. Hercules, and D. K. Roe, *Electrochim. Acta*, **13**, 1197 (1968).

(5) (a) M. D. Malbin and H. B. Mark, Jr., *J. Phys. Chem.*, **73**, 2992 (1969); (b) D. L. Maricle, A. Zweig, A. H. Maurer, and J. S. Brinen, *Electrochim. Acta*, 13, 1209 (1968); (c) R. E. Visco and E. A. Chandross, *J. Phys. Chem.*, 72, 3781 (1968).

(6) (a) M. D. Malbin and H. B. Mark, Jr., *ibid.*, **73**, 2786 (1969); (b) T. Kuwana, *Electroanal. Chem.*, **1**, 228 (1966).

(7) J. T. Maloy and A. J. Bard, *J. Amer. Chem. Soc.*, **93**, 5968 (1971).

(8) M. E. Peover, *Electroanal. Chem.*, **2**, 1 (1967).

(9) C. K. Mann, *ibid.*, **3**, 57 (1969).

(10) E. Bergmann and O. Blum-Bergmann, *J. Amer. Chem. Soc.*, **59**, 1439 (1937).

(11) E. A. Chandross and F. I. Sonntag, *ibid.*, **88**, 1089 (1966).

(12) M. M. Rauhut, American Cyanamid Co., Stamford, Conn., U. S. Patent 3,391,068 (July 2, (1968)).

(13) It was found that the DPACl$_2$ decomposed at a significant rate at room temperature (fastest in DMF). DPACl$_2$ is known to decompose by several routes.[10,14] Both water (common to all of the above solvents)

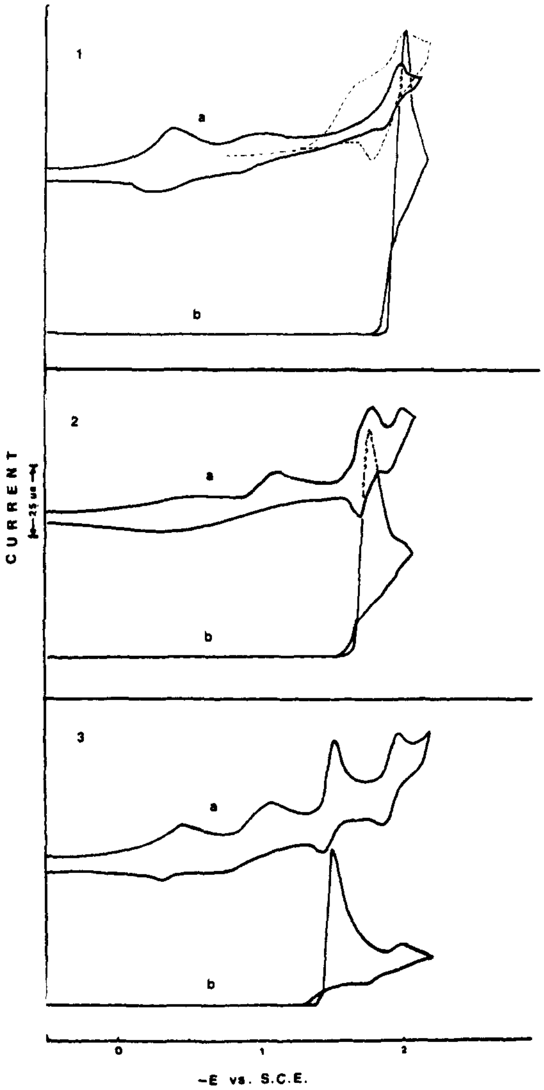


Figure 1. Electrochemical (a) and electrochemiluminescence (b) behavior of $DPACl_2$ in DMF (0.1 M TBAP supporting electrolyte, sweep rate = 0.5 V per sec): (1) $\sim 0.2 \times 10^{-3}$ M $DPACl_2$ only (----, THF solvent), (2) $\sim 0.2 \times 10^{-3}$ M $DPACl_2$–0.3×10^{-3} M perylene, and (3) $\sim 0.2 \times 10^{-3}$ M $DPACl_2$–0.3×10^{-3} M rubrene.

In DMF, DMSO, and AN, two irreversible waves at about −0.4 (the HCl wave) and −0.9 V *vs.* sce (all potentials are subsequently referenced to sce) and a well-defined wave with $E_p = -1.9$ V are observed (see curve 1a of Figure 1 for typical example). The reduction potential of this well-defined wave is identical with that of DPA in each solution. This reduction wave is concomitant (curve 1b, Figure 1) with an intense chemiluminescence whose spectrum is identical with the DPA fluorescence spectrum.[2-5] Steady-state reduction at −1.9 V gave a constant, high-level DPA fluorescence emission. In THF, the second irreversible wave is shifted to more negative potentials, as shown by the dashed line in curve 1a, and the ecl response remains the same.[15] Polarograms show one well-defined irreversible wave (apparent n value >1) at −0.2 V, followed by two one-electron waves corresponding the reduction of DPA to $R\cdot^-$ and R^{2-}. Again, at a mercury electrode, the ecl is concomitant only with the DPA radical-anion wave. Thus, the $DPACl_2$ reduction waves are strongly dependent on solvent and electrode nature. This is not understood at present. Extensive electrolysis at −1.1 V shows that the electroreduction product of $DPACl_2$ is DPA ($DPACl_2 + 2e^- \rightarrow DPA + 2Cl^-$).[16] Chandross and Sonntag[11] postulated that the chemiluminescence mechanism for $DPACl_2$ reacting with $DPA\cdot^-$ was

$$DPACl_2 + DPA\cdot^- \longrightarrow DPACl\cdot + DPA + Cl^- \qquad (1)$$

$$DPACl\cdot + DPA\cdot^- \longrightarrow DPACl^{-*} + DPA \qquad (2)$$

$$DPACl^{-*} \longrightarrow DPA^* + Cl^- \qquad (3)$$

$$DPA^* \longrightarrow DPA + h\nu \qquad (4)$$

In order to see if this mechanism is valid for the ecl of $DPACl_2$, the ecl was studied with either rubrene or perylene (1 mM) present in solution. Both of these hydrocarbons are more easily reduced than DPA.[2b,8,17] In both cases the cyclic voltammograms exhibited the characteristic rubrene or perylene reduction waves forming the radical anion[18] as shown in curves 2a and 3a, Figure 1. It is important to note that intense ecl is also concomitant with the rubrene or perylene wave and the subsequent DPA wave (curves 2b and 3b, Figure 1), indicating that a variety of hydrocarbon radical anions are capable of reducing $DPACl_2$ and of producing an excited state. It was found in the rubrene–$DPACl_2$ system (chosen because the rubrene and DPA fluorescence are distinctly different and easily identified) that the ecl at the rubrene wave is the characteristic yellow rubrene fluorescence, and when the DPA reduction wave is reached the light changes to a blue-yellow color which is *mixed* DPA–rubrene fluorescence. Under steady-state reduction conditions, it was found that for equal concentrations of rubrene and $DPACl_2$, only rubrene emission is observed at −1.4 (rubrene wave) and at −1.9 V (DPA wave). When $[DPACl_2] \gg [\text{rubrene}]$, the spectrum of the emitted light is a mixture of DPA and rubrene fluorescence only at the DPA wave. Similar results were obtained for DBDPE in the presence of either rubrene or DPA; in the presence of the rubrene–DPA mixture, only rubrene emission is observed at both the rubrene and DPA reduction waves. Dissimilarly, no emission is observed at the stilbene reduction wave.[19] These results seem to indicate that the Chandross–Sonntag mechanism for the cl process is not correct, as reaction 3

---

and amines (present as an impurity in DMF) replace the chloride in $DPACl_2$. Also, thermal rearrangement to 2-DPACl is known to occur at elevated temperatures ($\sim$180°). It is not known if this process occurs at lower temperature in solution. All of these decomposition routes liberate HCl, and characteristic HCl waves could be seen in the cyclic voltammograms of aged solutions which grew as a function of time. The hydrocarbon decomposition products, $DPA(OH)_2$ and $DPA(NR_2)_2$, are not reduced and 2-DPACl *does not* exhibit ecl under these conditions.

(14) C. K. Ingold and P. G. Marshall, *J. Chem. Soc.*, 3080 (1926); C. Dufraisse, A. Etienne, and J. Salmon, *Bull. Soc. Chim. Belg.*, **62**, 21 (1953).

(15) There are minor variations of intensity in the various solvents which could arise from various sources, such as differences in diffusion coefficients, radical ion stability, etc. This is under further investigation. No HCl wave is observed in THF, as $DPACl_2$ is stable in this solvent.

(16) (a) J. W. Sease, F. G. Burton, and S. C. Nikel, *J. Amer. Chem. Soc.*, **90**, 2595 (1968); P. Zuman and C. L. Perrin, "Organic Polarography," Interscience, New York, N. Y., 1969, pp 267–271; G. D. Sargent, J. N. Cron, and S. Bank, *J. Amer. Chem. Soc.*, **88**, 5363 (1966); (b) J. W. Haas, Jr., and J. E. Baird, *Nature (London)*, **214**, 1006 (1966).

(17) D. J. Freed and L. R. Faulkner, *J. Amer. Chem. Soc.*, **93**, 2097 (1971).

(18) The anodic scan peak current is smaller than usual. However, this is not unexpected, as $R\cdot^-$ is reacting with $DPACl_2$ diffusing toward the electrode.

(19) The product of the two-electron reduction of DBDPE is stilbene ($E_{1/2} = -2.11$ V[17]).

predicts only a DPA or stilbene excited state. The rubrene emission could possibly arise from some type of resonant transfer (such as Förster's transfer)[20] from the DPA or stilbene excited state. However, we have been unable to demonstrate Förster's transfer photochemically for these mixed systems. Also, Cruser and Bard found no evidence for Förster's transfer in mixed rubrene–DPA annihilation studies.[21] Furthermore, similar results are obtained for mixtures of fluoranthene and $DPACl_2$, where there is virtually no overlap between the fluoranthene absorption and the DPA emission spectrum. The fact that virtually all the luminescence osbserved for aromatic mixtures is that of the more easily reduced species cannot easily be explained on the basis of collisional electron or excited-state energy-transfer processes either.

As the direct (two-electron) electroreduction of $DPACl_2$ to DPA at -1.1 V does not yield ecl, it appears that the homogeneous reduction of $DPACl_2$ by electrogenerated $R\cdot^-$ goes by a distinctly different reaction path (yielding R*) than the electrochemical reduction. The reduction by $R\cdot^-$ probably involves two one-electron steps with intermediate chemical step(s).

As the high efficiency of this chemiluminescence reaction is of fundamental interest and appears to be general (a wide variety of aryl and alkyl halides[16b] exhibit similar behavior), and the "apparent" energy transfer phenomenon is totally unexpected, further study of this reaction is now in progress. This cl and the energy-transfer processes will be easier to study quantitatively as both transient and steady-state luminescence can be attained.

---

(20) F. Wilkinson, *Advan. Photochem.*, **3**, 241 (1964); Th. Förster, *Discuss. Faraday Soc.*, No. 27, 7 (1959); E. L. Wehry in "Fluorescence," G. G. Guilbault, Ed., Marcel Dekker, New York, N. Y., 1967, pp 114–115.

(21) S. A. Cruser and A. J. Bard, *J. Amer. Chem. Soc.*, **91**, 267 (1969).

Theodore M. Siegel, Harry B. Mark, Jr.*
Department of Chemistry, University of Cincinnati
Cincinnati, Ohio 45221

Received April 15, 1971

Cis Reduction of Δ^{24} of Lanosterol in the Biosynthesis of Cholesterol by Rat Liver Enzymes. A Revision

Sir:

For the definition of the overall (cis or trans) mechanism of reduction of Δ^{24} of lanosterol (**1**) in the biosynthesis of cholesterol (**2**) the prochirality at C-24 and C-25 of the produced cholesterol has to be determined. We have proven that in cholesterol biosynthesized in the S-10 fraction of rat livers a 24-pro-*S* proton is added.[1,2] The methyls at C-25 are derived from C-2 and from C-3′ of mevalonic acid (MVA).[3] To differentiate the two methyls we incubated cholesterol, biosynthesized from $[2\text{-}^{14}C]$MVA, with *M. smegmatis*,[4,5] isolated the 26-hydroxycholest-4-en-3-one[6] (**3**), and degraded it to 26-norcholestenone (**4**). The conversion of **3** to **4** involved the loss of *ca.* 0.8 atom of ^{14}C and revealed that the oxygen function resided on the methyl derived from C-2 of MVA.[6] To define the absolute configuration at C-25, comparison samples of 26-hydroxycholest-4-en-3-one (**3**) were prepared from kryptogenin and by various stereoselective procedures,[6] and we compared their specific rotations $[\alpha]$D with that of the microbial specimen.

We noticed that the $[\alpha]$D (measured on a Hilger MKIII instrument) of the microbially prepared sample of **3** was similar to that derived from kryptogenin and was more positive than that of the 25*RS* specimen (Table I, column A). The synthetic 25*R* and 25*S* specimens of **3** were more and less positive, respectively, than the 25*RS* samples (Table I, column A). Since kryptogenin has the 25*R* configuration,[7] we concluded that the microbial sample also has the 25*R* configuration. Because the geometry at Δ^{24} of lanosterol[8] is as in **1**, we inferred that the reduction of Δ^{24} entails a trans addition of two hydrogens.[6] We noted that our conclusions are at variance with the situation in tigogenin[6] and were in contrast with recent observations of Professors Ch. Tamm and D. Arigoni on the biosynthesis of sapogenins in *D. lanata*.[9] To clarify the discrepancy we first repeated the measurements of the specific rotations of the various samples of **3**, this time using a Rudolph and Sons photoelectric polarimeter (Table I, column B). Although the absolute $[\alpha]$D values differ somewhat from the results obtained with the Hilger MKIII polarimeter, clearly the relative situation remained unchanged. To solve conclusively the problem of the absolute configuration we carried out an X-ray diffraction analysis of the 26-*p*-bromobenzoate ester (mp 107–109°, *m*/*e* 582, 584; M^+) derived from the microbially prepared **3**.

Table I. Specific Rotation $[\alpha]$D (Degrees) of 26-Hydroxycholest-4-en-3-ones

Origin and method of preparation	$[\alpha]$D in chloroform A[a]	B[b]
Asymmetric hydroboration[c] with (+)-diisopinocampheylborane	+87.4; +86.0	+89.6
From kryptogenin	+84.45; +85.6	+92.7
Incubation of chesterol with *M. smegmatis*	+87.1; +86.1	+89.4
Symmetric hydroboration[c] with disiamylborane	+80.35	+85.6
Asymmetric hydroboration[c] with (−)-diisopinocampheylborane	+74.8	+78.8

[a] Measured using a Hilger MKIII polarimeter. [b] Measured using an O. C. Rudolph and Sons, Inc., photoelectric polarimeter with oscillating polarizer. [c] Hydroboration of cholest-5,26-dien-3β-ol.

The para bromobenzoate derivative crystallizes as flat plates in the monoclinic system, space group $P2_1$

---

(1) J. B. Greig, K. R. Varma, and E. Caspi, *J. Amer. Chem. Soc.*, **93**, 760 (1971).

(2) E. Caspi, K. R. Varma, and J. B. Greig, *Chem. Commun.*, 45 (1969).

(3) J. W. Cornforth, *Quart. Rev., Chem. Soc.*, **23**, 125 (1969).

(4) K. Schubert, G. Kaufmann, and C. Hörhold, *Biochem. Biophys. Acta*, **176**, 163, 170 (1969).

(5) I. I. Zaretskaya, L. M. Kogan, O. B. Tikhomirova, J. D. Sis, N. S. Wulfson, V. I. Zaretskii, V. G. Zaikin, G. R. Skryabin, and I. Torgov, *Tetrahedron* **24**, 1595 (1968).

(6) E. Caspi, M. Galli Kienle, K. R. Varma, and L. J. Mulheirn, *J. Amer. Chem. Soc.*, **92**, 2161 (1970)

(7) For pertinent references see L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, N. Y., 1959, p 838.

(8) A. J. Birch, M. Kocor, M. Sheppard, and J. Winter, *J. Chem. Soc.*, 1502 (1962); K. J. Stone, W. R. Roeske, R. B. Clayton, and E. E. van Tamelen, *Chem. Commun.*, 530 (1969).

(9) We are greatly indebted to Professor Ch. Tamm of the University of Basle, Switzerland, and Professor D. Arigoni of E. T. H., Zurich, for letting us know of their results prior to their publication.