

## Electron Spin Resonance Studies of Radical Formation in Nucleophilic Addition Reactions. I. Radical Formation and Chemiluminescence in the Hydroxide Ion Addition to N,N'-Dimethyl-9,9'-biacridinium Dinitrate (Lucigenin)<sup>1</sup>

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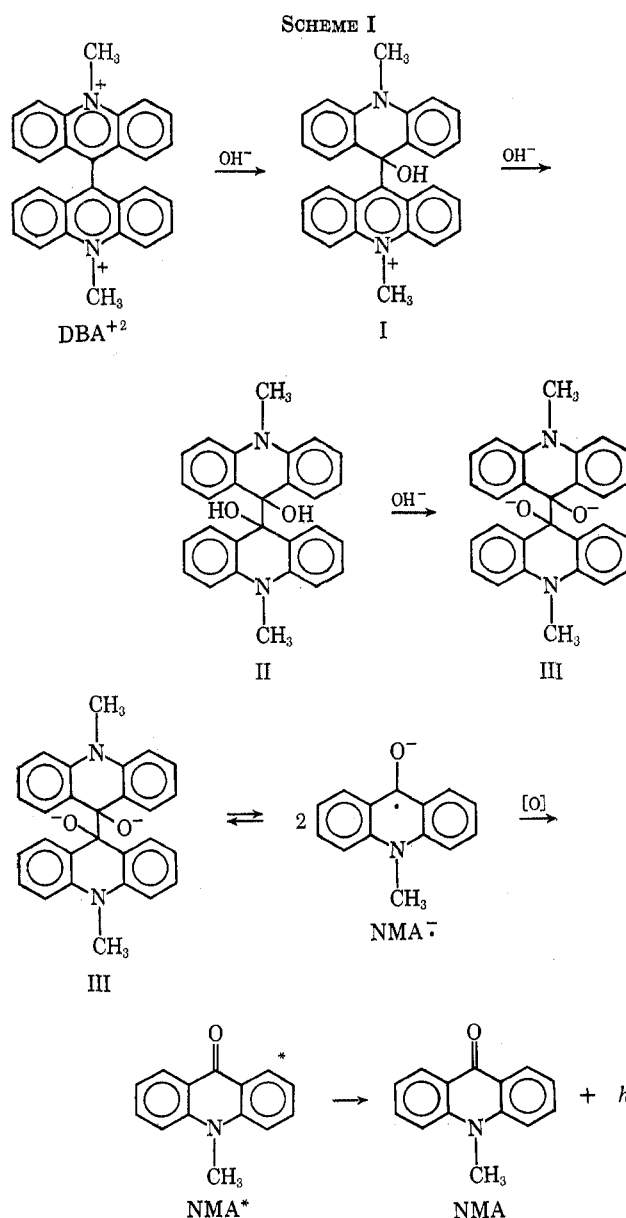
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An electron spin resonance study of the reaction of hydroxide ion addition to N,N'-dimethyl-9,9'-biacridinium dinitrate (DBA<sup>2+</sup>) in the absence of oxidizing agents has shown that substantial concentrations of stable radicals are produced readily in dimethyl sulfoxide-water solutions. Weak chemiluminescence is simultaneously observed. The radical cation DBA<sup>•+</sup>, produced by one-electron reduction of DBA<sup>2+</sup>, and the hydroxide ion addition product of DBA<sup>•+</sup> have been identified. A third unidentified free radical is produced at high base concentrations. The N-methylacridone ketyl spectrum has been obtained independently and analyzed. The coupling constants are  $a_H = 3.87$  (2 H), 3.71 (2 H), 1.31 (2 H), 0.50 (2 H), and 0.16 G (3 H) and  $a_N = 0.45$  G. These values are in agreement with coupling constants obtained from molecular-orbital calculations.

The reaction of N,N'-dimethyl-9,9'-biacridinium (DBA<sup>2+</sup>) salts with hydrogen peroxide in basic solution is known to be an intensely chemiluminescent reaction.<sup>2</sup> The main end product is N-methylacridone. It has been established that N-methylacridone is the light emitter.<sup>3</sup> Early work is reviewed in Acheson and Orgel.<sup>4a</sup> A more recent review<sup>4b</sup> is more critical of early interpretations of observed results. Because of the discovery<sup>5</sup> that many radical anions can be oxidized either chemically<sup>5b,d</sup> or electrolytically<sup>5a,c,d</sup> to the parent compound with light emission suggested that the chemiluminescence of DBA<sup>2+</sup> in basic solution might follow the course shown in Scheme I.

There is ample precedence for each step. Addition of nucleophiles to acridinium salts, such as hydroxide ion to DBA<sup>2+</sup>, is known to occur readily. The initial formation of the pinacol II (the "carbinol base") in the chemiluminescence of DBA<sup>2+</sup> is suggested by all authors.<sup>4</sup> Furthermore, it has been shown that aryl pinacols, *e.g.*, benzopinacol, fluorenone, and xanthenone pinacol,<sup>6</sup> dissociate in strongly basic solutions to produce the ketone radical anions (ketyls) in high yield.

Thus, III should dissociate substantially to NMA<sup>•-</sup> in strongly basic solutions. Since the oxidation of NMA<sup>•-</sup> is known to be chemiluminescent,<sup>5b</sup> the scheme would appear to fulfill all the requirements known for the system. It was thought that an electron spin resonance (esr) study of the addition of hydroxide ion to DBA<sup>2+</sup> in the absence of an oxidizing agent would substantiate this mechanism and would shed some light on the mechanism of DBA<sup>2+</sup> chemiluminescence.



(1) This work was supported by the Atomic Energy Commission, Contract AT-(40-L)-2851.

(2) (a) K. Gleu and W. Petsch, *Angew. Chem.*, **48**, 57 (1935); (b) H. Decker and W. Petsch, *J. Prakt. Chem.*, **143**, 211 (1935).

(3) J. R. Totter, *Photochem. Photobiol.*, **3**, 231 (1964).

(4) (a) R. M. Acheson and L. E. Orgel, "Acridines," Interscience Publishers, New York, N. Y., 1956, p 283; (b) F. McCapra, *Quart. Rev. (London)*, **20**, 485 (1966).

(5) (a) D. M. Hercules, *Science*, **145**, 808 (1964). (b) E. A. Chandross and F. I. Sonntag, *J. Amer. Chem. Soc.*, **86**, 3179 (1964); **88**, 1089 (1966). (c) R. E. Visco and E. A. Chandross, *ibid.*, **86**, 5350 (1964); E. A. Chandross, J. W. Longworth and R. E. Visco, *ibid.*, **87**, 3259 (1965); A. Zweig, G. Metzler, A. Maurer, and B. G. Roberts, *ibid.*, **88**, 2864 (1966); **89**, 4091 (1967). (d) This area has been reviewed in A. Zweig, *Advan. Photochem.*, **6**, 425 (1968). Other papers on this subject are L. R. Faulkner and A. J. Bard, *J. Amer. Chem. Soc.*, **90**, 6284 (1968); **91**, 209 (1969); S. A. Crusier and A. J. Bard, *ibid.*, **91**, 267 (1969).

(6) G. A. Russell, E. G. Janzen, and E. T. Strom, *J. Amer. Chem. Soc.*, **84**, 4155 (1962); E. G. Janzen, "Autoxidation of Carbanions. Occurrence of Electron Transfer Reactions," Thesis, Iowa State University, 1963.

### Experimental Section

Solutions of DBA<sup>2+</sup> were made up in various mixtures of dimethyl sulfoxide (DMSO) and water. Solutions of varying concentrations or saturated in KOH were added to DBA<sup>2+</sup> in a

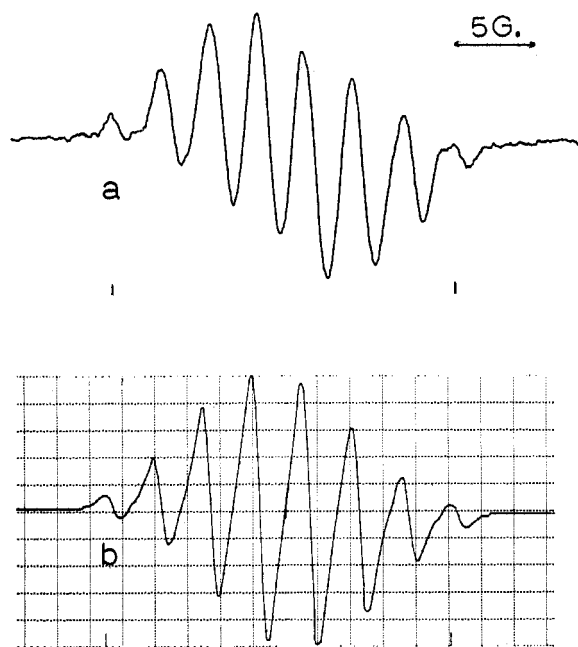


Figure 1.—(a) ESR spectrum obtained from  $3.8 \times 10^{-4} M$   $DBA^{2+}$  and  $0.078 M$   $KOH$  in 40:60 DMSO- $H_2O$  (40 vol. % DMSO and 60 vol. %  $H_2O$ ); (b) computer-simulated spectrum using the coupling constants given in text.

mixing vessel after the solutions were purged with prepurified nitrogen. The mixing vessel consists of an inverted U-shaped container wherein a solution of  $DBA^{2+}$  is placed in one arm and the basic solution in the other arm. A 5-in. hypodermic needle is introduced through a septum on top of each arm and used to pass nitrogen through the solution. Between the two septums, a flat esr cell is attached through a ground glass joint and serves as the exit for the nitrogen purge. After 15 min of gentle degassing, the bottom of the esr cell is closed, the nitrogen flow is stopped, the needles are removed, the solution is mixed, and the whole unit is inverted to allow the solution to run into the esr cell. The mixing vessel remains on the esr cell during the experiment. This vessel has been diagrammed elsewhere.<sup>7</sup>

A Varian 4502 spectrometer was used with a 41A X-band microwave bridge. The chemiluminescent emission was detected by the use of a "Firefly" photometer previously used in these laboratories.<sup>8</sup>  $N,N'$ -Dimethyl-9,9'-biacridinium dinitrate was obtained from K & K laboratories. Potassium *t*-butoxide was obtained from MSA Research Corp.

$N$ -Methylacridinium chloride was synthesized by treating acridine with dimethyl sulfate followed by ion exchange with  $NaCl$  to form the chloride salt according to the method of Albert,<sup>9a</sup> mp  $182-183^\circ$  dec (lit. mp  $183^\circ$  dec).<sup>9a</sup> Zinc reduction of  $N$ -methylacridone in acidic ethanol at  $70^\circ$  gave  $N,N'$ -dimethylbiacridylidene, mp  $381-382^\circ$  dec (lit. mp  $383-384^\circ$ ).<sup>9b</sup>  $N,N'$ -Dimethylbiacridan was prepared from the reaction of  $N$ -methylacridinium chloride with  $Na_2CO_3$  in ethylene glycol at  $100^\circ$ , mp  $269-270^\circ$  (lit. mp  $270-271^\circ$ ).<sup>9c</sup>

## Results

**Hydroxide Addition.**—The addition of hydroxide ion to  $DBA^{2+}$  in the absence of oxygen (and even in initially air-saturated solutions) produces free radicals at concentrations which are easily detectable by esr.

(7) G. A. Russell, E. G. Janzen, and E. T. Strom, *J. Amer. Chem. Soc.*, **86**, 1807 (1964); G. A. Russell, E. G. Janzen, A. G. Bemis, E. J. Geels, A. J. Moye, S. Mak, and E. T. Strom, *Advances in Chemistry Series*, No. 51, American Chemical Society, Washington, D. C., 1965, p 112.

(8) J. R. Totter, W. Stevenson, and G. E. Philbrook, *J. Phys. Chem.*, **68**, 752 (1964); G. E. Philbrook and M. A. Maxwell, *Tetrahedron Lett.*, 1111 (1964); G. E. Philbrook, J. B. Ayers, J. F. Garst, and J. R. Totter, *Photochem. Photobiol.*, **4**, 869 (1965).

(9) (a) A. Albert, "The Acridines," St. Martin's Press, New York, N. Y., 1966, pp 343-344; (b) ref 9a, p 399; (c) A. Albert and B. Catterall, *J. Chem. Soc.*, 4657 (1965).

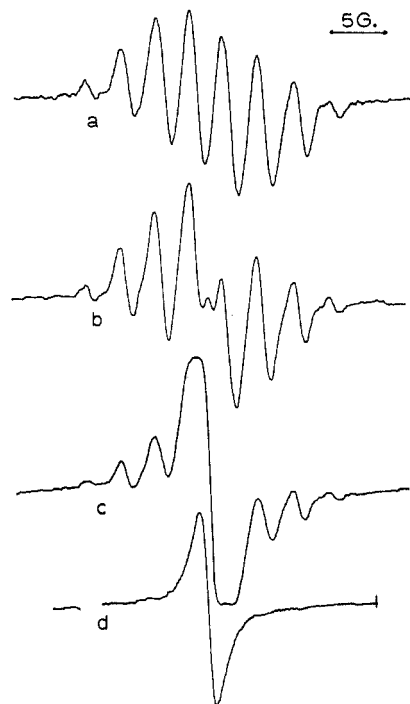


Figure 2.—(a) Same spectrum as Figure 1a; (b) superimposed spectra obtained from  $30 \times 10^{-4} M$   $DBA^{2+}$  and  $0.10 M$   $KOH$  in 50:50 DMSO- $H_2O$ ; (c) superimposed spectra obtained from  $30 \times 10^{-4} M$   $DBA^{2+}$  and  $0.16 M$   $KOH$  in 50:50 DMSO- $H_2O$ ; (d) signal obtained from  $49 \times 10^{-4} M$   $DBA^{2+}$  and  $0.16 M$   $KOH$  in 60:40 DMSO- $H_2O$ .

The spectra obtained depend upon the relative  $DBA^{2+}$  to base ratio and on the amount of water present in solution. The most frequently obtained spectrum in approximately 3:2 water to DMSO solution at moderate base concentrations is an eight-line spectrum (Figure 1), or a spectrum which is the superposition of the eight-line pattern and another signal. The spacing is 3.0 G. The  $g$  value is 2.00272. The lines are fairly broad and at no time has further resolution of the eight-line pattern been obtained, which indicates that a low concentration of another radical is probably present at all times when the spectrum is recorded. At very high base to  $DBA^{2+}$  concentrations, a very strong signal of a single narrow line which cannot be resolved is obtained (Figure 2d). The  $g$  value is 2.00276. The line width (from maximum to minimum) is 1.4 G. When more concentrated solutions of  $DBA^{2+}$  are used, a third spectrum of 13 lines has been obtained in a solution saturated with  $KOH$  (Figure 3). The spacing is 1.6 G. The  $g$  value is 2.00270. On further vigorous shaking with  $KOH$ , this spectrum changes to a spectrum of two radicals; one is the same as in Figure 1.

These observations can be summarized by the series of spectra shown in Figure 2. Although conditions have been found where the individual spectra are obtained alone (Figure 2a and 2d), more frequently spectra are obtained which appear to be superpositions of these individual spectra, as shown in Figure 2b and 2c. For example, a spectrum similar to Figure 2c is obtained in all experiments when the concentration of  $KOH$  is varied from 0.1 to 0.002  $M$  in DMSO-water solutions of  $DBA^{2+}$  (see Table I). On one occasion the spectrum in Figure 3 was seen first but changed into the spectrum in Figure 2c within 10 min after mixing. The intensity of the signal increases in general with an

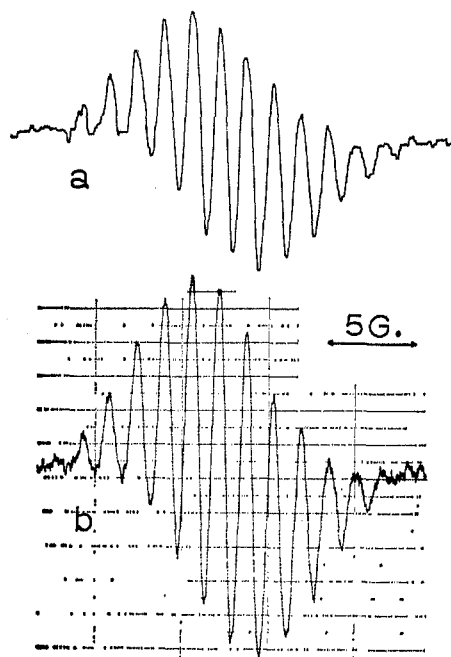


Figure 3.—(a) Spectrum obtained from  $10 \times 10^{-4} M$   $DBA^{2+}$  and  $0.004 M$   $KOH$  in 60:40  $DMSO-H_2O$ ; (b) spectrum of  $DBA^{2+}$  obtained by electrolytic reduction of  $DBA^{2+}$  in  $DMSO$  without supporting electrolyte.

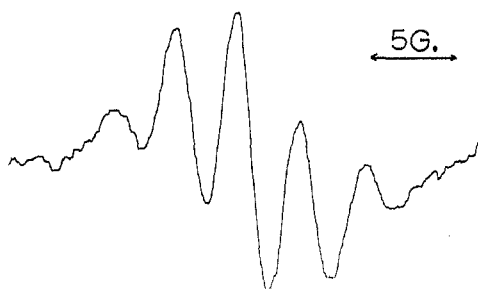


Figure 4.—Overmodulated spectrum of N-methylacridone ketyl obtained by electrolytic reduction of N-methylacridone in  $DMSO$  using tetraethylammonium perchlorate as supporting electrolyte.

increase in base strength. The signal also increases with time up to 60 min and then remains relatively constant and stable at least up to 3 hr after mixing.

TABLE I  
ESR SPECTRA PRODUCED IN THE ADDITION OF  
HYDROXIDE ION TO  $DBA^{2+}$

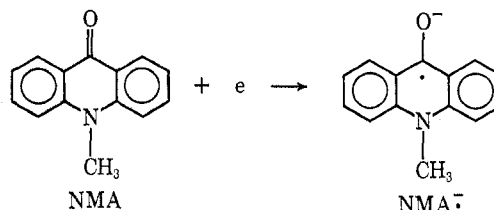
$[DBA^{2+}]$ , mol/l.	$[KOH]$ , mol/l.	$KOH/$ $DBA^{2+}$	$H_2O-$ $DMSO$ , vol. %	Figure
0.00038	0.078	205	60:40	1a, 2a
0.0030	0.10–0.16	33–53	50:50	2b, 2c
0.0010	0.002–0.040	2–40	57:43	2b, 2c
0.0020	0.10	50	50:50	2b, 2c
0.0049	0.16	33	40:60	2d
0.0010	0.0040	4	40:60	3a <sup>a</sup>
0.0070	0.025	4	20:80	3a <sup>b</sup>

<sup>a</sup> ESR signal stable for up to 10 min. <sup>b</sup> ESR signal stable for over 3 hr.

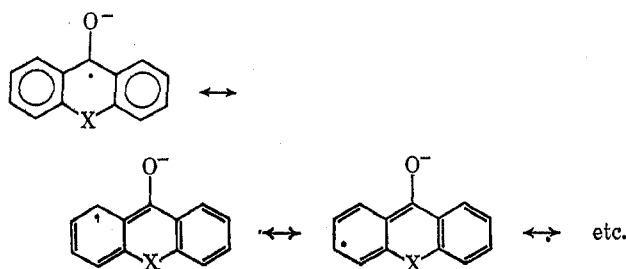
**N-Methylacridone Ketyl.**—The radical anticipated from the addition of hydroxide ion to  $DBA^{2+}$  was N-methylacridone ketyl ( $NMA^{\cdot-}$ ). Since the esr spectrum of this radical anion has not been published to

date, it was necessary to synthesize this radical by an independent method and obtain its esr spectrum. An analysis of the hyperfine pattern has also been made.

The esr spectrum of  $NMA^{\cdot-}$  was obtained by potassium reduction of NMA in dimethoxyethane, electrolytic reduction of NMA in  $DMSO$  using tetraethylammonium perchlorate as supporting electrolyte, and photolytic reduction in deoxygenated 70:30 ethanol-water containing  $NaOH$ .



Under conditions of moderate resolution, five broad lines are observed which is very characteristic of ketyls with this type of structure (Figure 4). Such spectra are obtained from xanthone,<sup>10</sup> thioxanthone,<sup>11</sup> and thioxanthone-5-dioxide.<sup>11,12</sup> In all cases, the main coupling is due to major interaction with four approximately equivalent "ortho" and "para" hydrogens. It is



interesting to note that the nitrogen coupling is relatively small in  $NMA^{\cdot-}$ , while in neutral radicals of similar structure the nitrogen coupling (and thus also the methyl hydrogen coupling) is much larger.<sup>13</sup> The analysis of the fully resolved spectrum of  $NMA^{\cdot-}$  (Figure 5a) gives  $a_H = 3.87$  (2 H),  $a_H = 3.71$  (2 H),  $a_H = 1.31$  (2 H),  $a_H = 0.50$  (2 H),  $a_N = 0.45$ , and  $a^{H_{CH_3}} = 0.16$  G (3 H). A simulated computer spectrum based on these coupling constants is shown in Figure 5b. This analysis compares favorably with coupling constants predicted from McLachlan-Hückel molecular orbital calculations:  $a_H = 4.05$  (2 H),  $a_H = 3.72$  (2 H),  $a_H = 1.22$  (2 H),  $a_H = 0.16$  (2 H), and  $a_N = 0.58$  G, using the heteroatom integral parameters<sup>14a</sup>  $\delta_0 = 1.6$ ,  $\gamma_{CO} = 1.63$ ,<sup>14b</sup>  $\delta_N = 1.5$ , and  $\gamma_{NC'} = 0.8$ .<sup>14c</sup> The value  $Q = 27$ <sup>14e</sup> in the McConnell equation,<sup>14d</sup>  $a_H =$

(10) E. G. Janzen and C. M. DuBose, Jr., *J. Phys. Chem.*, **70**, 3372 (1966).

(11) E. T. Kaiser and D. H. Eargle, Jr., *J. Amer. Chem. Soc.*, **85**, 1821 (1963); M. M. Urberg and E. T. Kaiser, *ibid.*, **89**, 5179 (1967); M. M. Urberg and E. T. Kaiser in "Radical Ions," E. T. Kaiser and L. Kevan, Ed., Interscience Publishers, New York, N. Y., 1968, p 301.

(12) G. Vincow, *J. Chem. Phys.*, **37**, 2484 (1962).

(13) J. W. Happ and E. G. Janzen, *J. Org. Chem.*, **35**, 96 (1970).

(14) (a) The relations  $\alpha_x = \alpha + \delta_x\beta$  and  $\beta_{ox} = \gamma_{ox}\beta$  define the heteroatom coulomb and resonance integral parameters,  $\delta$  and  $\gamma$ , as functions of the coulomb and resonance integrals,  $\alpha$  and  $\beta$ , for C-C bonds in benzene. (b) P. H. Rieger and G. K. Fraenkel, *J. Chem. Phys.*, **37**, 2811 (1962). (c)  $\delta_N$  and  $\gamma_{NC'}$  were varied ( $0.7 \leq \delta_N \leq 1.5$  and  $0.8 \leq \gamma_{NC'} \leq 1.0$ ) to obtain the best fit. (d) H. M. McConnell, *et al.*, *J. Chem. Phys.*, **28**, 51, 107 (1958).

(e) M. T. Jones, *J. Amer. Chem. Soc.*, **88**, 5060 (1966); E. G. Janzen and J. W. Happ, *J. Phys. Chem.*, **73**, 2335 (1969). (f) J. M. L'Hoste and F. Tornard, *J. Chim. Phys.*, **63**, 678 (1966); A. Carrington and J. Dos Santos-Veiga, *Mol. Phys.*, **5**, 21 (1962).

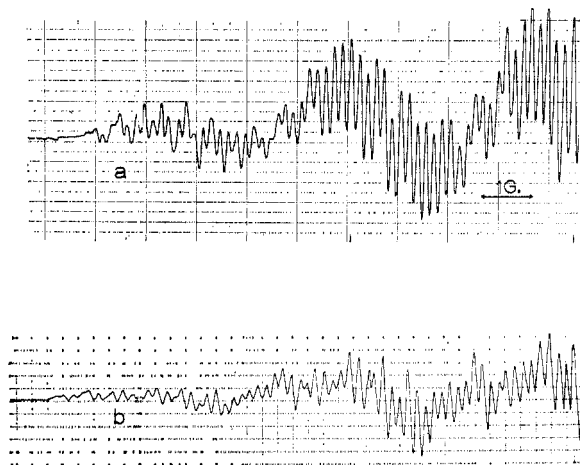
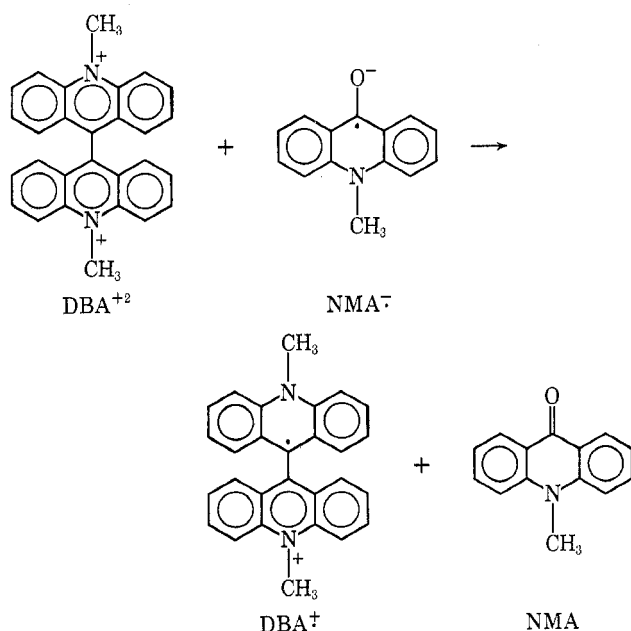


Figure 5.—(a) Resolved spectrum of N-methylacridone ketyl obtained by electrolytic reduction of N-methylacridone; (b) simulated spectrum using values given in text.

$Q_H \rho_c'$ , was used. Nitrogen coupling was obtained from a similar relationship,  $a_N = Q_N \rho_{N'}$  where  $Q_N = 28.8$ .<sup>14f</sup> The  $g$  value was found to be 2.00332. This data indicates that none of the spectra in Figures 1–3 is due to  $NMA\cdot^-$ .

**N,N'-Dimethyl-9,9'-biacridinium Radical Cation ( $DBA\cdot^+$ ).** Since the spectrum most readily obtained (Figure 1) could not be attributed to  $NMA\cdot^-$ , the production of a radical cation by one-electron reduction of  $DBA^{2+}$  was considered. The above electron-



transfer equilibrium should lie almost completely on the right-hand side, since the half-wave reduction potential for  $DBA^{2+}$  is much lower than for  $NMA$  ( $-0.25$  V vs.  $-1.7$  V vs. standard calomel electrode). Since the esr spectrum of  $DBA\cdot^+$  has not been previously reported, it was also necessary to make this radical by an independent route.

$DBA^{2+}$  was reduced electrolytically in DMSO without supporting electrolyte. The spectrum obtained is shown in Figures 3b and 6a. The  $g$  value is 2.00270. The reduction potentials obtained from polarographic determinations were  $-0.55$  V in DMSO and  $-0.25$  V in 3:1 DMSO–water vs. standard calomel

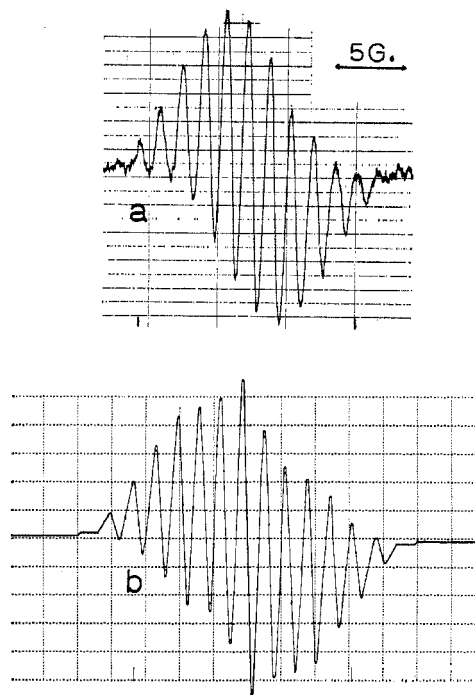


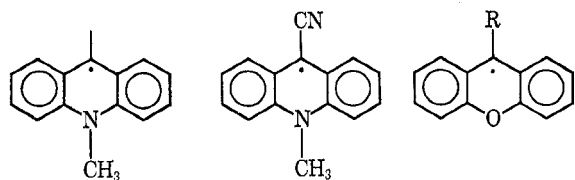
Figure 6.—(a) Same spectrum as Figure 3b; (b) simulated spectrum of  $DBA\cdot^+$  using values given in text.

electrode. The same spectrum was obtained by electrolytic reduction in acetonitrile solutions containing tetraethylammonium perchlorate and by zinc dust reduction in degassed absolute ethanol. The analysis of the spectrum assumes that the nitrogen coupling is 3.0 G and that the methyl hydrogen coupling is 1.8 G. The coupling constants predicted from molecular orbital calculations are  $a_N = 2.89$  (2 N),  $a^{H_{CH_3}} = 2.17$  (6 H),  $a_H = 0.76$  (4 H),  $a_H = 0.75$  (4 H),  $a_H = 0.48$  (4 H), and  $a_H = 0.46$  (4 H) G, using the parameters  $\delta_N = 1.2$ ,<sup>15a</sup>  $Q_H = 27$ ,<sup>14e</sup> and  $Q_N = 23.5$ .<sup>15b</sup> The methyl proton coupling can be predicted from the ratio  $a^{H_{CH_3}}/a_N = 0.85$ , where 0.85 is an average of the ratios 0.77, 0.87,<sup>13</sup> 0.94,<sup>15a</sup> 0.79, 0.88, and 0.89<sup>15c</sup> which have previously been observed for N-methylpyridyl and acridanyl radicals. This analysis predicts 15 lines if the coupling from the aromatic hydrogens is not resolved. The computer-simulated spectrum based on this analysis is shown in Figure 5b. It is clear that the 13-line spectrum shown in Figure 3a is due to  $DBA\cdot^+$ . The two outer lines (one on each side) are not resolved.

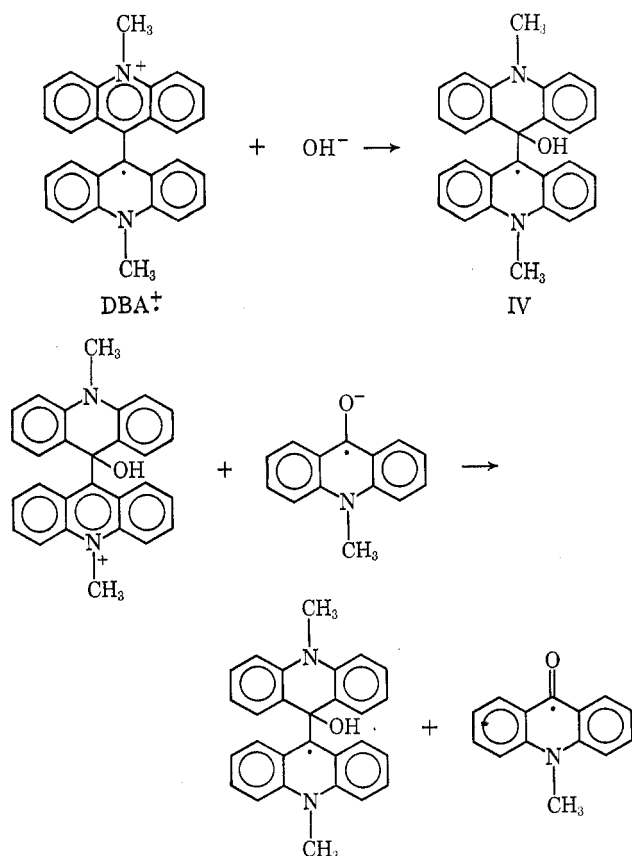
**Assignment of the Eight-Line Spectrum.**—By the preparation of  $NMA\cdot^-$  and  $DBA\cdot^+$  it has been shown that the spectra in Figure 1 and 2 are probably not due to either  $NMA\cdot^-$  or  $DBA\cdot^+$ . A comparison of the magnitudes of the hyperfine coupling constants of  $DBA\cdot^+$  and the spacing of the lines in Figure 1 suggests that in the latter case there is a *larger* interaction with *fewer* protons (approximately half the number) than present in  $DBA\cdot^+$ , since the total line widths of the spectra are about the same. This suggests a partial structure of a radical with the electron localized only in one ring of the DBA structure. If the hyperfine cou-

(15) (a) For an analysis of the methylviologen radical cation, see C. S. Johnson, Jr., and H. S. Gutowsky, *J. Chem. Phys.*, **39**, 58 (1963); (b) G. Vincow in "Radical Ions," E. T. Kaiser and L. Kevan, Ed., Interscience Publishers, New York, N. Y. p 184. (c) M. Itoh and S. Nagakura, *Bull. Chem. Soc. Jap.*, **39**, 369 (1966).

pling constants of the nitrogen atom, methyl group, and four aromatic hydrogens are all assumed to be ap-

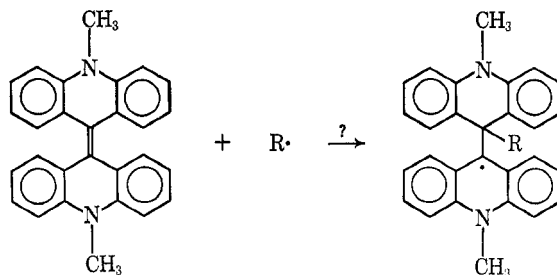


proximately equal, a ten-line spectrum with intensity sequence 1:8:29:63:91:91:63:29:8:1 is predicted. The two outer lines are 1% of the intensity of the most intense inner lines and might be difficult to detect. (On one occasion a spectrum of ten resolved lines was obtained.) The spacing in Figure 1 is 3.0 G. In 9-cyanoacridanyl radical,  $a_N = 3.55$ ,  $a^H_{CH_3} = 2.74$ ,  $a^H_{CH} = 2.18$  and  $2.74$  G,<sup>13</sup> and the  $g$  value is 2.00274. In 9-methylxanthyl radical, the hyperfine coupling constants for the aromatic hydrogens are  $a^H_{CH} = 3.84$ , 3.42, 0.88, and 0.71 G, and the  $g$  value is 2.0029.<sup>16</sup> It appears that the hyperfine coupling constants and  $g$  values of 9-cyanoacridanyl and 9-substituted xanthyl radicals support the (partial) structural assignment made for the radical observed in Figure 1. The coupling constants predicted by molecular orbital calculations agree only approximately for this partial structure:  $a_N = 2.82$ ,  $a^H_{CH_3} = 2.40$  (3 H),  $a_H = 3.88$  (2 H),  $a_H = 2.46$  (2 H),  $a_H = 0.69$  (2 H), and  $a_H = 0.57$  (2 H)G, using the parameters  $\delta_N = 1.0$ ,<sup>18</sup>  $Q_N = 28.8$ ,<sup>14f</sup>  $Q_H = 27$ ,<sup>14e</sup> and  $a^H_{CH_3}/a_N = 0.85$ .<sup>13,15a,c</sup> In view of the chemistry probably involved in the hydroxide addition to  $DBA^{2+}$ , the hydroxide addition product of  $DBA \cdot^+$  (IV) suggests itself as a probable



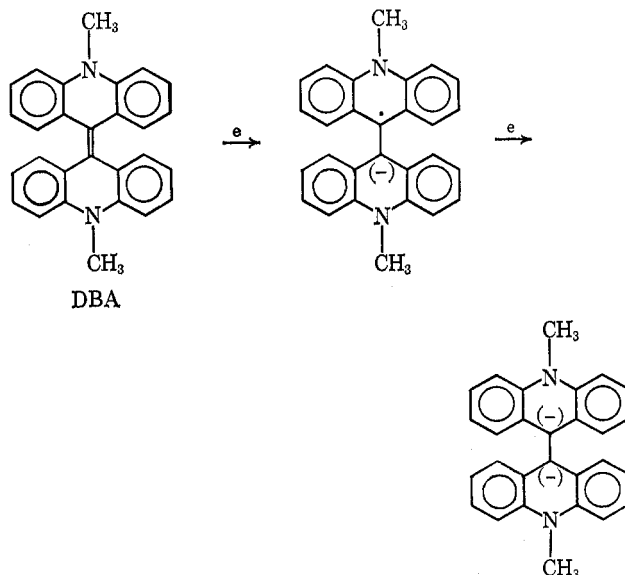
structure for the radical. This radical could be produced either by addition of hydroxide to  $DBA \cdot^+$  or by electron transfer from  $NMA \cdot^-$  to the monohydroxide adduct of  $DBA^{2+}$ . The relative reduction potentials of I and NMA would probably favor the electron transfer in the direction of the formation of IV. In a basic aqueous DMSO solution of sodium dithionite and  $DBA^{2+}$ , a high concentration of IV was detected. Unfortunately, this result does not distinguish between the two mechanisms of formation of IV.

Attempts to produce radicals similar to IV by *in situ* addition of reactive free radicals to  $N,N'$ -dimethyl-9,9'-biacridene (DBA) in benzene failed; for example,



photolysis of trifluoromethyl iodide or phenylazotriphenylmethane in benzene solutions of DBA directly in the esr cavity gave no signal in the first case and only a weak singlet in the latter case.

**$N,N'$ -Dimethyl-9,9'-biacridylidene Radical Anion ( $DBA \cdot^-$ ).**—The spectra in Figure 1 and 3 have been assigned to IV and  $DBA \cdot^+$ . The single narrow line in Figure 2d, however, remains unaccounted for. It was thought that possibly this spectrum was due to the radical anion of biacridylidene, which might have an unusually narrow total line width. The synthesis of this radical was attempted. Potassium *t*-butoxide in 80:20 DMSO-*t*-butyl alcohol containing equal amounts of  $N,N'$ -dimethylbiacridan and  $N,N'$ -dimethylbiacridylidene did not produce a change in color nor a detectable concentration of radical anions.<sup>6</sup> The air oxidation of biacridan in DMSO containing potassium *t*-butoxide was also unsuccessful.<sup>6</sup> The potassium reduction of biacridylidene in tetrahydrofuran under vacuum readily gave a dark red solution but no esr signal could be detected initially. After extensive

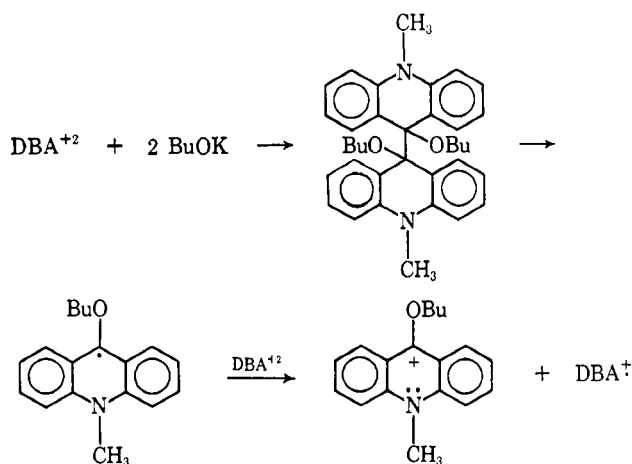


(16) M. D. Sevilla and G. Vincow, *J. Phys. Chem.*, **72**, 3647 (1968).

reduction a weak signal consisting of one broad unresolved line was obtained with 10.64-G line width (measured from maximum to minimum). This spectrum is thought to be  $\text{DBA}^{\cdot-}$ . However, the spectrum in Figure 2d cannot be  $\text{DBA}^{\cdot-}$  because of the large difference in the total line width of these spectra. It is interesting to note that the dark red solution obtained in the reduction may be due to the dianion, which, like tetraphenylethylene dianion, may be more "stable" than the radical anion in nonpolar solvents.<sup>17</sup>

The singlet in Figure 2d thus remains unexplained. Possibly, it is due to the 9,9'-biacridanyl radical anion produced by the demethylation of  $\text{DBA}^{2+}$ . Demethylation of methylviologen dichloride in high concentrations of sodium hydroxide has recently been reported.<sup>18</sup>

**Other Nucleophilic Additions to  $\text{DBA}^{2+}$ .**—A saturated solution of *t*-BuOK in degassed DMSO reacted with  $\text{DBA}^{2+}$  to produce a 13-peak spectrum, as in Figures 3 and 6, assigned to  $\text{DBA}^{\cdot+}$ . This result could be due to electron transfer from the dimethyl sulfoxide anion ( $\text{CH}_3\text{SOCH}_2^-$ ) to  $\text{DBA}^{2+}$  or from the reaction below. The latter possibility is supported by the



observation of a ten-line spectrum assigned to the 9-*t*-butoxyacridanyl radical when potassium *t*-butoxide in DMSO is treated with *N*-methylacridinium iodide in the presence of a trace amount of air (see following paper).

**Chemiluminescence.**—The proposed mechanism of chemiluminescence in Scheme I should produce *N*-methylacridone ketyl radicals, but no light, in the absence of oxidizing agents such as hydrogen peroxide or oxygen. However, in view of the observation that other radicals and not  $\text{NMA}^{\cdot-}$  are in fact produced, presumably *via* reduction by *N*-methylacridone ketyl, light emission would be expected to accompany the hydroxide ion addition to  $\text{DBA}^{2+}$  even in the absence of an externally added oxidizing agent. Small amounts of light are in fact observed, varying approximately from 1/1000 to 1/10 of readily eye-detectable light depending on the concentrations of KOH and  $\text{DBA}^{2+}$ . Typical results are shown in Table II. In general, the intensity of light increases with increase in the ratio of base to

$\text{DBA}^{2+}$ . The fact that an aqueous solution of DMSO also gives a readily detectable amount of light indicates that the water molecule can also function as a nucleophile in Scheme I, the basic nature of the amines subsequently produced probably serving to ionize the carbinol sufficiently to permit formation of a small amount of precursor to the emitter. The origin of the small chemiluminescence in pure DMSO is also of interest. The oxygen atom in dimethyl sulfoxide or a small amount of water in the solvent could serve as a nucleophile to initiate the reactions leading to chemiluminescence.

TABLE II  
CHEMILUMINESCENCE OF *N,N'*-DIMETHYLBIACRIDINIUM  
DINITRATE<sup>a</sup> IN OXYGEN-FREE BASIC AQUEOUS  
DIMETHYL SULFOXIDE<sup>b</sup>

$[\text{DBA}^{2+}] \times 10^4, M$	$[\text{KOH}] \times 10^4, M$	$[\text{KOH}]/[\text{DBA}^{2+}]$	Relative light intensity <sup>c</sup>
30	0		1
30 <sup>d</sup>	0		30
7	4	6	3
0.7	4	60	3
0.03	2	600	100
30 <sup>d</sup>	Saturated		300
0.02	2 <sup>e</sup>	1000	10 <sup>5</sup>

<sup>a</sup>  $\text{DBA}^{2+}$ . <sup>b</sup> 3:4 DMSO-water solutions. <sup>c</sup> A relative light intensity of  $3 \times 10^5$  is readily visible. <sup>d</sup> Pure DMSO, no water. <sup>e</sup> One drop of  $\text{H}_2\text{O}_2$  in 5 ml of solution.

The addition of potassium *t*-butoxide in *t*-butyl alcohol in the absence of air also gave a small amount of light. The maximum intensity appears within seconds after mixing.  $\text{DBA}^{\cdot+}$  radicals are produced in 5–10 min after mixing. The light production may be associated with a reaction which does not produce stable radicals.

## Discussion

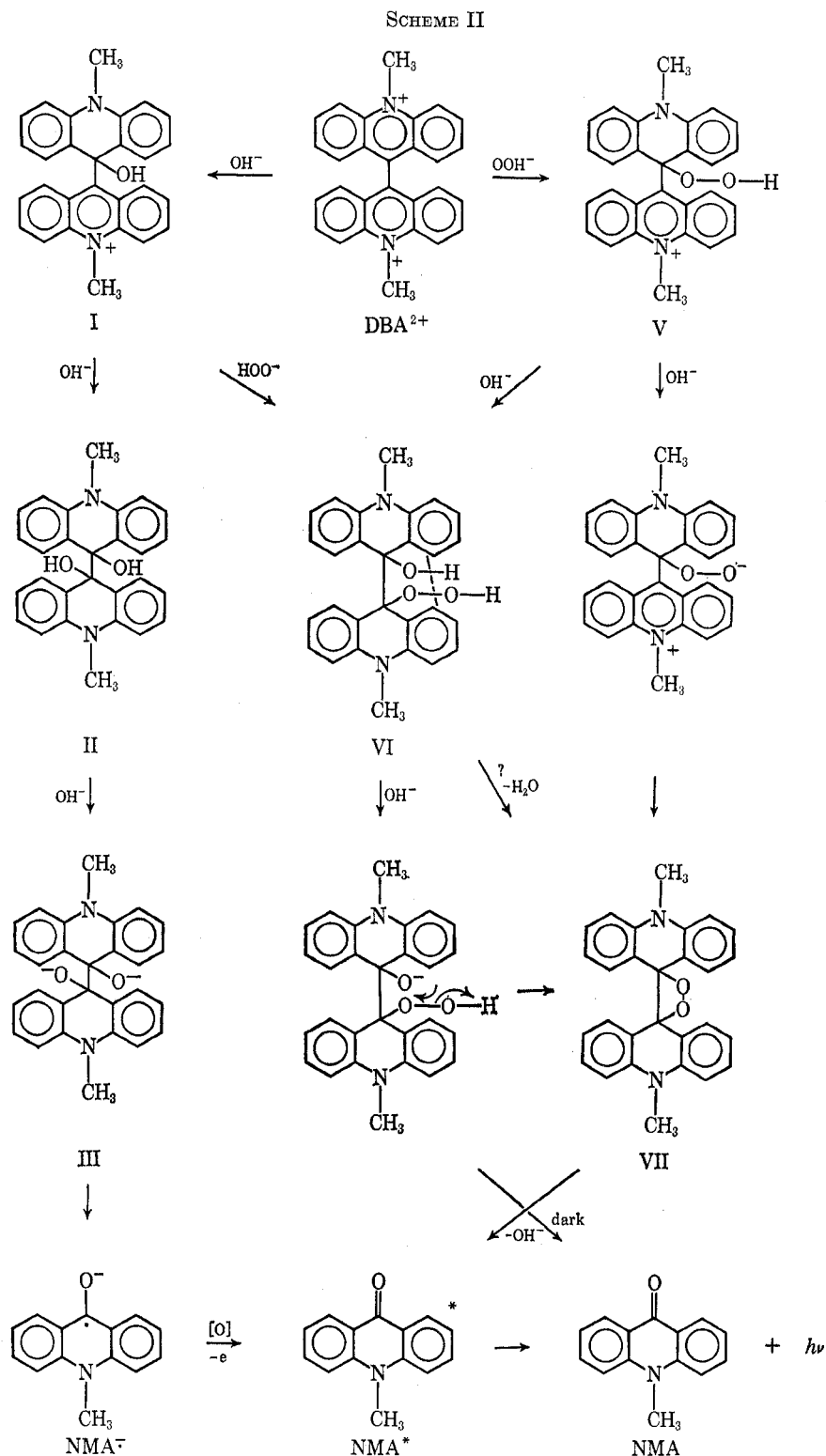
Although interest in the mechanisms of complex chemiluminescent reactions has accelerated in recent years,<sup>4b,5,19</sup> esr techniques have apparently not been extensively applied to this problem to date. In this report we have described the detection of free radicals in the addition of hydroxide ion to  $\text{DBA}^{2+}$ . It has been concluded with reasonable certainty that the radicals are the hydroxide ion addition product of  $\text{DBA}^{2+}$  and  $\text{DBA}^{\cdot+}$ . Until this date no radicals have been detected under similar conditions in the presence of basic hydrogen peroxide. Future esr experiments will be directed toward detection of radicals in a flow system.

Very little is known about the structure of the intermediates present in the chemiluminescence of  $\text{DBA}^{2+}$ . Early workers<sup>4</sup> implicated the pinacol (II) as the key intermediate in the chemiluminescence of  $\text{DBA}^{2+}$ . However, the reaction suggested is not feasible because it involved oxidation of the vicinal tertiary alcohol functions to the moloxide VII (see Scheme II). With present-day knowledge the suggestion that the pinacol could be the precursor to the key chemiluminescence intermediate is reasonable if the function of hydrogen peroxide is to oxidize  $\text{NMA}^{\cdot-}$  produced from

(17) J. F. Garst and E. R. Zabolotny, *J. Amer. Chem. Soc.*, **87**, 495 (1965); A. Cserhegyi, J. Chaudhuri, E. Franta, J. Jagur-Grodzinski, and M. Szwarc, *ibid.*, **89**, 7129 (1967); A. Cserhegyi, J. Jagur-Grodzinski, and M. Szwarc, *ibid.*, **91**, 1892 (1969).

(18) J. A. Farrington, A. Ledwith, and M. F. Stam, *Chem. Commun.*, 259 (1969); A. H. Corwin, R. R. Arellano, and A. B. Chivvis, *Biochem. Biophys. Acta*, **162**, 533 (1968).

(19) See, for example, *Photochem. Photobiol.*, **4**, 957–1247 (1965), proceedings of a symposium held at Durham, N. C., March 31–April 2, 1969; M. M. Rauhut, *Accounts Chem. Res.*, **2**, 80 (1969).

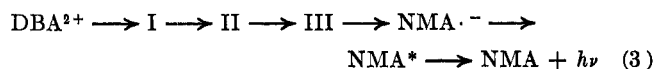
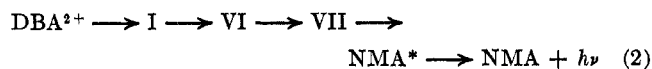
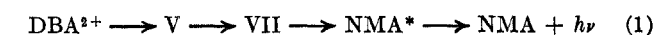


the cleavage of the pinacolate (Scheme I). However, it appears that this route cannot be the only chemiluminescence pathway, since the onset of light emission is found to come very quickly after mixing the appropriate solutions (Figure 7). The nucleophilic addition of two hydroxide ions, ionization of two protons, homolytic cleavage of a carbon-carbon bond, and oxidation of NMA<sup>-</sup> is expected to be a rather time-consuming process. The bursts of light observed immediately after mixing probably are due to the rapid formation of the moloxide VII *via* either V or VI.

In basic hydrogen peroxide solutions, formation of VII from V should be very rapid. The formation of VII *via* VI is also reasonable, because nucleophilic displacements on oxygen are recognized reactions. The decomposition of VI to NMA plus light has been cited as an example of a chemiluminescent concerted peroxide decomposition reaction by Rauhut and co-workers.<sup>20</sup> This pathway involves more steps and would probably have a slower over-all rate than the

(20) M. M. Rauhut, D. Sheehan, R. A. Clarke, and A. M. Semsel, *Photochem. Photobiol.*, **4**, 1097 (1965).

route involving V directly to VII. The suggested major routes to NMA\* thus are routes 1-3. The



hydroxide and hydroperoxide ion compete in the initial stages of the reaction, the immediate light emission probably coming from the fraction of  $\text{DBA}^{2+}$  going *via* route 1 and 2, and the delayed chemiluminescence is produced mainly *via* route 3.

In the above arguments the reasonable but unproven assumption is made that the moloxide VII is a high-energy intermediate which dissociates into excited singlet NMA molecules. Results of further experiments in our laboratory are consistent with this assumption.

The fact that NMA is not the only product of  $\text{DBA}^{2+}$  chemiluminescence points to the possibility of important reactions not included in Scheme II. The detection of reduced  $\text{DBA}^{2+}$  radicals as reported here and the suggestion of a mechanism involving two successive, equivalent reductions<sup>3,21</sup> has some bearing on this point. We attribute the formation of  $\text{DBA}^{\cdot+}$  and IV under basic conditions to reduction by  $\text{NMA}^{\cdot-}$ . In the presence of hydrogen peroxide it has been pointed out that, judging from redox potentials, basic hydrogen peroxide is capable of reducing  $\text{DBA}^{2+}$  to  $\text{DBA}^{\cdot+}$ . As noted earlier, we have been unable to detect radicals in basic hydrogen peroxide solutions of  $\text{DBA}^{2+}$ . The fate of the radicals produced by the reduction of  $\text{DBA}^{2+}$  under these conditions is not obvious. However, the reaction of  $\text{DBA}^{\cdot+}$  with hydroxide ion to produce IV is at least consistent with recent studies on the reaction of water with thianthrene cation radical.<sup>22</sup>

The detection of IV is relevant to a qualitative study of  $\text{DBA}^{2+}$  chemiluminescence in ammonium hydroxide reported by Totter and Philbrook.<sup>21</sup> At high concentrations of  $\text{NH}_4\text{OH}$  (14 M), relatively intense light emission was detected, which, however, was inhibited by introduction of air. At lower concentrations of  $\text{NH}_4\text{OH}$  (1.8 M), the initial chemiluminescence level was not significantly affected by air, although inhibition was observed by the introduction of air immediately after bubbling with nitrogen. Within the framework of the mechanistic pathways suggested here, the bright chemiluminescence at high base concentration would be consistent with Scheme I. The effect of air on the reaction is anomalous, however.  $\text{NMA}^{\cdot-}$  should be oxidized to NMA by oxygen, producing superoxide or peroxide, and IV should lead to the hydroperoxide *via* the peroxy radical. The diminished

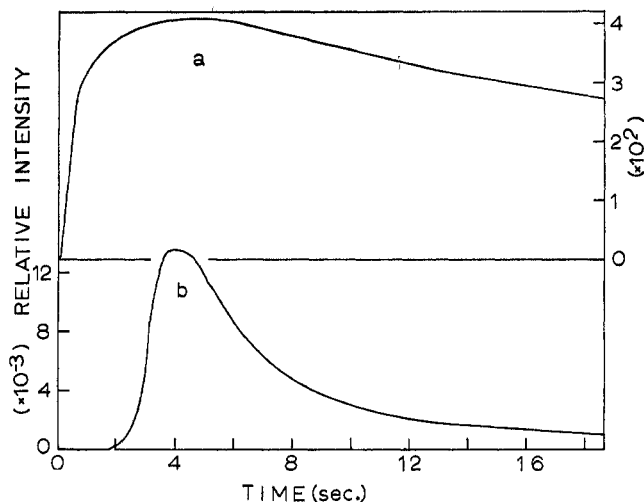
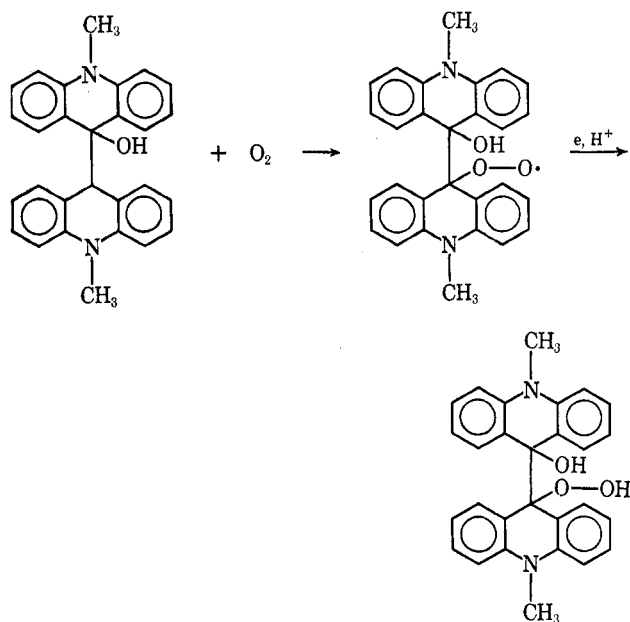


Figure 7.—(a) Relative intensity of chemiluminescence (right scale) of  $1.7 \times 10^{-3} M \text{DBA}^{2+}$ ,  $2.5 \times 10^{-3} M \text{KOH}$ , and  $6.2 \times 10^{-2} M \text{H}_2\text{O}_2$  vs. time; (b) relative intensity of chemiluminescence (left scale) of  $3.4 \times 10^{-3} M \text{DBA}^{2+}$  and  $2.5 \times 10^{-3} M \text{KOH}$  vs. time.

light intensity in the presence of air may be due to the strong quenching effect of oxygen. The involvement of ammonia as a possible nucleophile or reducing agent<sup>21</sup> leads to even greater mechanistic complexity.



### Conclusions

The radical cation of *N,N'*-dimethylbiacridinium dinitrate and the hydroxide ion addition product of this radical have been detected by esr in basic aqueous dimethyl sulfoxide solutions of *N,N'*-dimethylbiacridinium dinitrate. *N*-methylacridone ketyl is implicated as the reducing agent.

**Registry No.**— $\text{DBA}^{2+}$ , 22103-92-0;  $\text{DBA}^{\cdot+}$ , 22027-28-7;  $\text{NMA}^{\cdot-}$ , 22027-29-8; IV, 22027-30-1.

(21) J. R. Totter and G. E. Philbrook, *ibid.*, **5**, 177 (1966).

(22) H. J. Shine and Y. Murata, *J. Amer. Chem. Soc.*, **91**, 1872 (1969).