pairs in MTHF. As was observed for the previously discussed equilibria, both ΔH and ΔS become less negative as the cation radius increases. It is also gratifying that the results for NaTp and KTp, arrived at via different techniques, are so similar.

Finally, using the equilibrium constant K_2 at $+20°$ given in I, we have calculated the limiting frequency maximum for NaTp in MTHF. Figure 3 shows that this calculated frequency maximum satisfies rather well the linear relation of frequency vs. the cation radius, indicating the overall consistency of our treatment.

Conclusion

From examination of the absorption spectra of alkali metal reduced solutions of Tp at various temperatures,

we have been able to demonstrate the existence of four different species, the free ion and three ion pairs. Further we have measured the equilibria that connect one with the other. For two species the dependence of both the absorption spectra and the equilibria on the cation radius was clearly shown. From the temperature dependence of the equilibria we have calculated the thermodynamic constants for these processes. The values show that all solvation processes studied are accompanied by both a loss of enthalphy and entropy, the effect being strongest for the smallest cation.

Yields of Chemically Produced Excited States

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Abstract: The chemiluminescence quantum yields of ten substituted 2,3-dihydrophthalazine-1,4-diones and the fluorescence quantum yields of the corresponding phthalic acids were measured. From these data, the yields of excited state formation were calculated. The results show that the excited state yield is dependent on the substituents, and they indicate that an important factor in this dependence may be the energy of the excited state formed.

Chemically produced excited electronic states are involved in chemiluminescence[2] and bioluminescence,[3] and they have been used as sources of energy for "photochemical reactions."[4] The efficiency of production of these excited states is usually low, the overall efficiency of light production of most chemiluminescent reactions being less than 1%.[2a] A few reactions are notably more efficient. Fused ring hydrazides are up to 7% efficient in DMSO,[5] the oxalate-H_2O_2-fluorescer system can be up to 23% efficient,[6] and the bioluminescence of Cypridina[7] and firefly[8] luciferins are 28 ± 15 and 88 ± 25% efficient, respectively. In some cases the yield of excited states has been determined; the cleavage of dioxetanes leads to

the production of excited states with 4,[4b] 15,[9] or 20–100%[10] efficiency, depending on the system, and benzoperylenedicarboxylic hydrazide is about 50% efficient in the production of excited states.[5]

In situations where energy transfer is occurring to a common emitter, the efficiency of excited state production can be obtained readily as a function of some substituent, such as R, from measurement of the total light emitted (eq 1 and 2),[6] provided that conditions are such that the energy transfer step is of unit efficiency.

$$ROCCOR + H_2O_2 \longrightarrow X \text{ (a common intermediate)} \quad (1)$$

$$X + A \longrightarrow A^* \longrightarrow h\nu \quad (2)$$
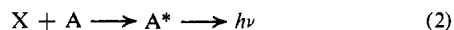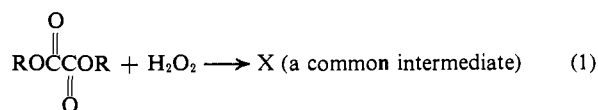
Many chemiluminescent reactions lead, however, to an excited state product which is also the light emitter (eq 3). Thus, the efficiency of fluorescence of C must

$$B \longrightarrow C^* \longrightarrow C + h\nu \quad (3)$$
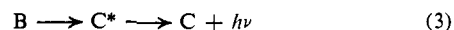
be factored out to determine the effect of substituents on the efficiency of excited state production from B. We now report on one of the first of such studies;[2c] we have examined the effect of substituents on the efficiency of chemiluminescence of the cyclic hydrazides.

The oxidation of cyclic hydrazides (1) in basic media leads to the formation of the corresponding carboxyl-

(1) (a) The Johns Hopkins University; (b) Loyola College.

(2) (a) K. D. Gundermann, "Chemilumineszenz Organischer Verbindungen," Springer-Verlag, Berlin, 1968; (b) E. H. White and D. F. Roswell, Accounts Chem. Res., 3, 54 (1970); (c) F. McCapra, Pure Appl. Chem., 24, 611 (1970).

(3) T. Goto, ibid., 17, 421 (1968); (b) J. W. Hastings, Annu. Rev. Biochem., 37, 597 (1968).

(4) (a) E. H. White, J. Wiecko, and D. F. Roswell, J. Amer. Chem. Soc., 91, 5194 (1969); (b) E. H. White, J. Wiecko, and C. C. Wei, ibid., 92, 2167 (1970); (c) E. H. White and C. C. Wei, Biochem. Biophys. Res. Commun., 39, 1219 (1970); (d) H. Gusten and E. F. Ullman, Chem. Commun., 28 (1970); (e) A. A. Lamola, Biochem. Biophys. Res. Commun., 43, 893 (1971).

(5) C. C. Wei and E. H. White, Tetrahedron Lett., 3559 (1971).

(6) M. M. Rauhut, L. J. Bollyky, B. G. Roberts, M. Loy, R. H. Whitman, A. V. Lanotta, A. M. Semsel, and R. A. Clark, J. Amer. Chem. Soc., 89, 6515 (1967).

(7) F. H. Johnson, O. Shimomura, Y. Saiga, L. G. Gershman, G. T. Reynolds, and J. R. Waters, J. Cell. Comp. Physiol., 60, 85 (1962).

(8) H. H. Seliger and W. D. McElroy, Arch. Biochem. Biophys., 88, 136 (1960).

(9) P. D. Wildes and E. H. White, J. Amer. Chem. Soc., 93, 6286 (1971).

(10) T. Wilson and A. P. Schaap, ibid., 93, 4126 (1971).
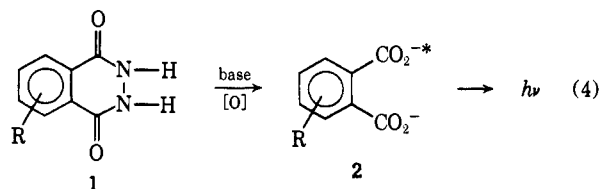
$$\Phi_{ch} = \Phi_r\Phi_{es}\Phi_{fl} \tag{5}$$

ates (2) in the first excited singlet state; light emission occurs from that state.[2b] The overall efficiency of chemiluminescence, Φ_{ch} (eq 5), is a product of the efficiencies

of the component steps Φ_r, Φ_{es}, and Φ_{fl}, where Φ_r is the fraction of molecules that follow the "correct" chemistry, Φ_{es} is the fraction of molecules that cross over to the singlet excited state (travelling the correct chemical path), and Φ_{fl} is the quantum efficiency of fluorescence of the emitter. We have measured Φ_{ch} and Φ_{fl} for a series of hydrazides and the corresponding carboxylates, and conclude that the effect of substituents occurs not only at the Φ_{fl} level but also at the $\Phi_r\Phi_{es}$ level. The factor Φ_r is unknown. However, since the chemiluminescent reactions lead to high yields of the corresponding carboxylates and since the reaction conditions used give nearly the maximum amount of light possible (eq 4), we have assumed for this study that Φ_r has a common value of 1.

Results

Hydrazides IIId, IIIe, and IIIf were synthesized by the alkylation of 4-amino-N-methylphthalimide with an alkyl tosylate followed by reaction of the resulting imide with hydrazine in ethanol (eq 6). The phthalic acids
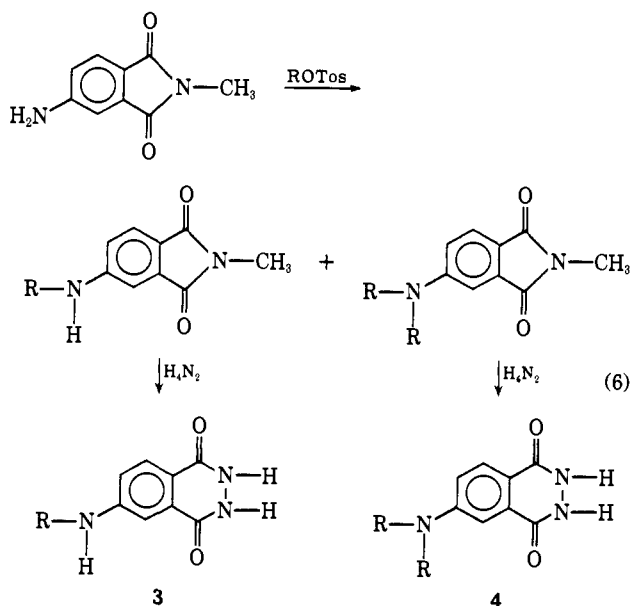

(as the anhydrides) VIIb, VIIc, VIId, VIIe, and VIIf (see Chart I) were synthesized from the corresponding hydrazides by oxidation in an aprotic solvent followed by sublimation (eq 7). Hydrazide IVb and the corresponding acid VIIIb were prepared as shown in eq 8. The nitration gave two isomers in a ratio of ~8:2 (by nmr). The major component was separated by crystallization, the minor component by preparative tlc (benzene on alumina). The corresponding aminoimides were obtained by hydrogenation. Both nitro-

Chart I
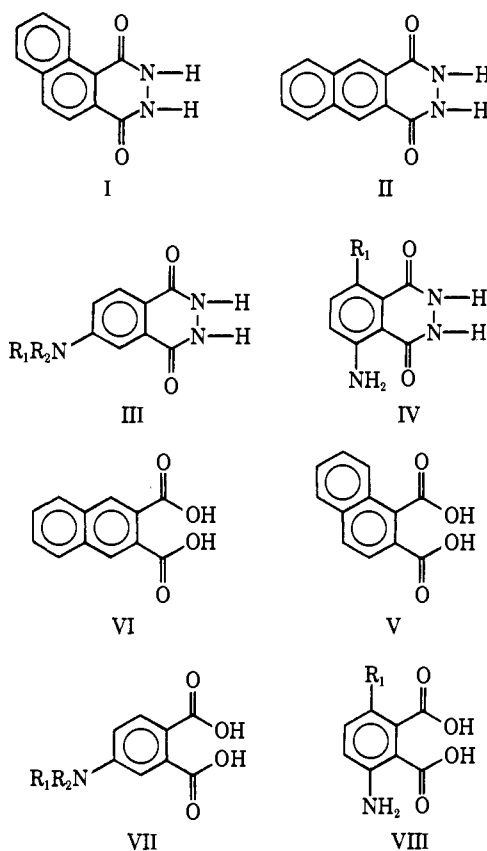
a, $R_1 = R_2 = H$
b, $R_1 = R_2 =$ methyl
c, $R_1 = R_2 =$ ethyl
d, $R_1 = R_2 = n$-butyl
e, $R_1 = R_2 = n$-heptyl
f, $R_1 = H$; $R_2 = n$-butyl
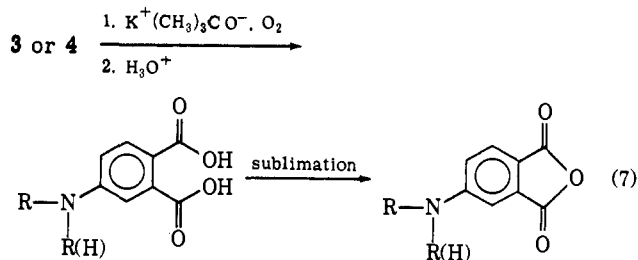
imides and both aminoimides showed ortho (8 Hz) coupling of the aromatic hydrogens in the nmr. The distinction between the isomers was made on the basis
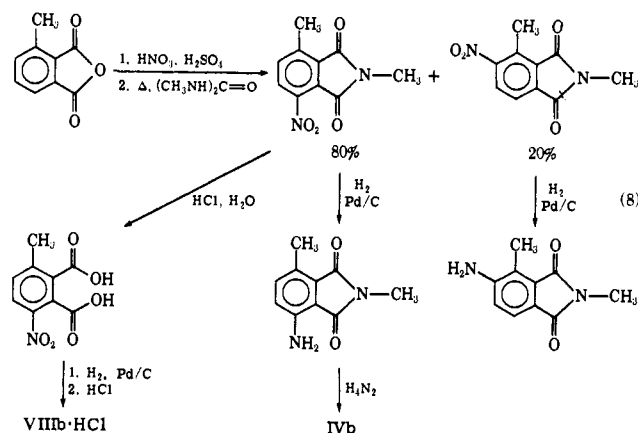
of uv spectra of the aminoimides. The uv spectrum of the aminoimide obtained from the major component (395 nm) was similar to that of 3-aminophthalimide (385 nm) while the uv of the other aminoimide (305 and 380 nm) was similar to that of 4-aminophthalimide (305 and 380 nm). Hydrazide IVb was obtained from the imide through refluxing in 95% hydrazine; the hydrazine–ethanol solution used in other cases gave a nonchemiluminescent material, probably the N-aminoimide.[2b] Phthalic acid VIIIb was obtained by acid hydrolysis of the nitroimide followed by hydrogenation to the amine. The preparation of acids by oxidation of the corresponding hydrazides does not work well if unalkylated amino groups are present, probably because of oxidation of the amino group.

The chemiluminescence of hydrazides I–IV was measured using the aqueous system with hemin–hydrogen peroxide as the oxidizing couple.[2b] The measured values of Φ_{ch} for the hydrazides, Φ_{fl} for the corresponding acids, and the values of Φ_{es} calculated from them are listed in Table I. The Φ_{ch} values of

better allow comparison of the results, a standard pH of $\sim$11.4 was used for all of the runs (0.1 M K_2CO_3). At this pH, compounds IIIa, IIIc, and IVa have quantum yields of emission of about 85% of their maximum values. Thus the values of Φ_{ch} used to calculate Φ_{es} (Table I) are in certain cases less than the maxima possible, but not different enough to significantly affect the results. The fluorescence intensities of the acids corresponding to hydrazides II, IIIa, IIIc, and IVa were decreased by less than 10% on saturation of the solution with oxygen, or in the presence of hemin and hydrogen peroxide at the concentrations used to measure Φ_{ch}. The lack of strong quenching of acid fluorescence by hemin, hydrogen peroxide, or oxygen, which are present in the chemiluminescence reaction but not in the solution used to measure Φ_{fl}, indicates that the measured values of Φ_{fl} are representative of the fluorescence efficiencies of the acids under the chemiluminescence reaction conditions.

The chemiluminescence emissions of the ten hydrazides in Table I and the fluorescence emissions of the corresponding acids are listed in Table II. In all cases

Table I. Quantum Yields

Compd	$\Phi_{ch}{}^{a,b}$	$\Phi_{fl}{}^{b,c}$ (acids)	Φ_{es}
I	0.000047[d]	0.029 (V)	0.0017
II	0.00052[d]	0.12 (VI)	0.0043
IIIa	0.0012[f]	0.13[e] (VIIa)	0.0092
IIIb	0.0075[f]	0.25 (VIIb)	0.030
IIIc	0.0094[f]	0.31 (VIIc)	0.030
IIId	0.0140	0.30 (VIId)	0.046
IIIe	0.0038	0.26 (VIIe)	0.015
IIIf	0.0058	0.28 (VIIf)	0.021
IVa	0.015	0.30[e] (VIIIa)	0.050
IVb	0.028	0.28 (VIIIb)	0.10

[a] Obtained in aqueous 0.1 M K_2CO_3 solution (pH 11.4) with hydrogen peroxide and hemin; the values are relative to luminol at 0.0125 (J. Lee and H. H. Seliger, *Photochem. Photobiol.*, 4, 1015 (1965)). [b] The precision of the measurements is estimated to be $\pm10\%$. [c] Obtained in aqueous 0.1 M K_2CO_3 solution; the values are relative to quinine bisulfate at 0.55 (W. H. Melhuish, *J. Phys. Chem.*, 65, 229 (1961)). [d] Corrected for photomultiplier sensitivity, factor = 0.9. [e] Values of 0.11 and 0.30 have been reported for VIIa and VIIIa, respectively (J. Lee and H. H. Seliger, *Photochem. Photobiol.*, 11, 247 (1970)). [f] Relative values of 0.10, 0.87, and 1.20 for IIIa, IIIb, and IIIc, respectively, at a higher pH (0.030 N NaOH) have been reported (K. D. Gundermann and M. Drawert, *Chem. Ber.*, 95, 2018 (1962)). Φ_{ch}, above the pH optimum, decreases for IIIa and IVa but is nearly pH independent for IIIc.

Table II. Chemiluminescence and Fluorescence Spectral Data[a]

| | ——Hydrazides—— | | ————Phthalic acid products———— | | |
| | Chemi-luminescence | | Fluorescence | Fluorescence | Fluorescence |
Compd	λ_{max}, nm[b]	Compd	λ_{max}, nm[b]	ν_{max}, cm^{-1} [c]	ν_{min}, cm^{-1} [c,d]
I	350	V	355	28,100	31,200
II	362	VI	370	26,100	30,500
IIIa	416	VIIa	419	23,400	28,200
IIIb	437	VIIb	438	22,200	25,700
IIIc	439	VIIc	439	22,200	25,700
IIId	442	VIId	442	21,900	25,500
IIIe	442	VIIe	442	21,900	25,500
IIIf	435	VIIf	435	22,400	26,400
IVa	423	VIIIa	425	23,200	26,600
IVb	405	VIIIb	405	24,100	27,200

[a] All in aqueous 0.1 M K_2CO_3 solution. [b] Determined from uncorrected spectra (error $\sim\pm3$ nm). [c] Determined from corrected spectra (error $\sim\pm200$ cm^{-1}). [d] Value is that obtained by extrapolating the straight portion of the high frequency side of the fluorescence curve to zero intensity.

four of the hydrazides, II, IIIa, IIId, and IVa, were shown to be nearly independent of the concentrations of hydrazide, hemin, and hydrogen peroxide.[11] Increasing the hydrazide concentration from 2 $\times$ 10^{-6} to 5 $\times$ 10^{-5} M caused decreases in Φ_{ch} of about 10–15%, increasing the hydrogen peroxide concentration from 0.01 to 0.1 M caused increases in Φ_{ch} of about 10–20%, and increasing the concentration of hemin from 10^{-6} to 10^{-5} M caused decreases in Φ_{ch} of 10–20%. The pH optimum of IVa is 11.0;[12] the optima for IIIa and IIIc (using NaHCO$_3$ and NaOH as bases) were found to be about 11.5 and 12.0, respectively. To

(11) The Φ_{ch} of luminol, IVa, has been reported to be essentially independent of the concentration of hemin and hydrogen peroxide in the ranges of 10^{-5}–10^{-7} M and 0.3–0.003 M, respectively: J. Lee, A. S. Wesley, J. F. Ferguson, and H. H. Seliger, "Bioluminescence in Progress," F. Johnson and Y. Haneda, Ed., Princeton University Press, Princeton, N. J., 1966, p 35.
(12) H. H. Seliger, "Light and Life," W. D. McElroy and B. Glass, Ed., The Johns Hopkins University Press, Baltimore, Md., 1961, p 204.

the chemiluminescence spectrum of the hydrazide gave a good match with the fluorescence spectrum of the corresponding acid. The corresponding acid was the only product detectable by tlc in the spent chemiluminescent reaction mixtures, and ultraviolet analysis of the spent mixtures showed greater than 85% yields of acids in all cases except IIIa; the latter compound led to a 60% yield of 4-aminophthalic acid, VIIa. These results and the lack of Φ_{ch} dependence on hydrazide concentration show that the acids are the emitters in the chemiluminescence reaction and that the mechanism does not involve an energy transfer step as in the case of phthalic hydrazide.[13]

Discussion

The results in Table I show that, in general, Φ_{ch} increases with electron donating substituents[14] and that

(13) E. H. White, D. F. Roswell, and O. C. Zafiriou, *J. Org. Chem.*, 34, 2462 (1969).
(14) The chemiluminescence intensities of substituted lophines (quantum yields not measured) give linear Hammett plots: G. E. Philbrook
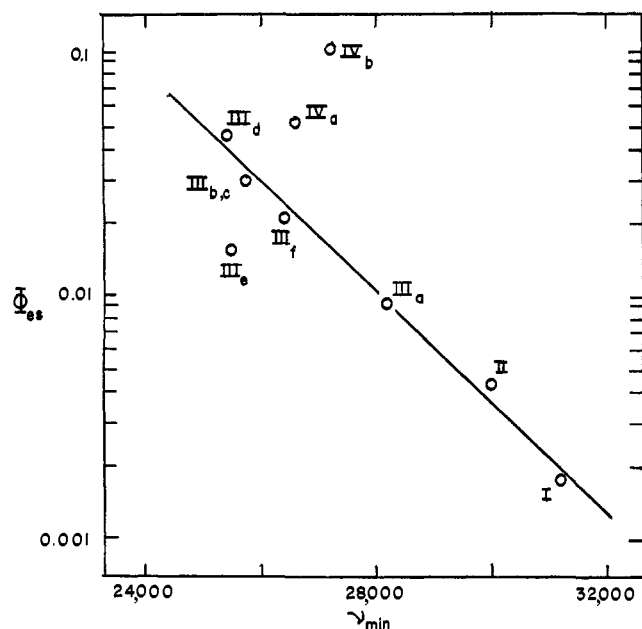
Figure 1. Variation of the efficiency of excited state production in hydrazide chemiluminescence, Φ_{es}, with the energy of the excited state of the corresponding carboxylate formed, ν_{min}.

Figure 2. Energy diagram for chemiluminescent reactions.

a substituent in the 3 position (IVa) has a greater effect than one in the 4 position (IIIa) (in agreement with the rules proposed by Drew[15]). Also the fluorescence yields of the acids, in general, increase with electron donating substituents.[16] However, our results show that the fluorescence yields of the acids are not the major factor governing chemiluminescent efficiency. The values of Φ_{ch} (Table I) from I to IVb differ by a factor of 600, while the values of Φ_{fl} of the corresponding acids, V and VIIIb, differ by only a factor of 10. Thus, the calculated yields of excited states (eq 5) increase by a factor of 60 from I to IVb.

Figure 1, which is a plot of log Φ_{es} (Table I) vs. ν_{min} (Table II), shows that Φ_{es} increases with decreasing frequency (energy) of fluorescence. The values of ν_{min} are most assuredly closer to the energy of the 0-0 transition than are the values of ν_{max}, although a similar result is obtained if Φ_{es} is plotted against ν_{max}. Three of the hydrazides deviate from this relationship. Compound IIIe forms a soapy solution and it may be chemiluminescing from micelles. The high efficiency of IVa and IVb may be due to a special ortho effect between the amino and methyl groups and the adjacent carbonyls; this effect is under further investigation. The increase in Φ_{es} with decreasing excited state energy is similar to the preliminary results of McCapra.[2c] He noted that, in the case of substituted indolenyl peroxides (eq 9), Φ_{es} increases as the fluorescence of the emitter shifts to longer wavelength.

$$X = N(CH_3)_2, \quad OCH_3. \quad H, \quad F, \quad Cl, or \quad Br$$

A possible explanation of the Φ_{es} dependence on the energy of fluorescence is shown in Figure 2, which is adapted from the paper of Marcus dealing with electron transfer chemiluminescence.[17] In the present case, the critical step in the chemiluminescence of the hydrazides may indeed be an electron transfer[18] (eq 10). The value of Φ_{es} results from the partitioning of the

$$RCO_2\cdot \ + \ 1e^- \longrightarrow RCO_2^{-*} \tag{10}$$

critical intermediate, CI, between ground state products, P, and excited state products, P*. This partitioning will depend on the relative heights of the energy barriers, E_{gs} leading to P and E_{es} leading to P*. If electron donating substituents stabilize the excited state (P*) more than the ground state (CI and P), and some evidence suggests that substituents do have a greater effect on excited states than on ground states,[19] then a stabilization of P* (reflected in a decrease of E_{fl}) will result in a greater decrease in E_{es} than in E_{gs}. Thus, stabilizing substituents (1 and II can be viewed as isomeric benzo-substituted compounds) should enhance the efficiency of crossover to the excited state surfaces. A 30-fold effect from I to IIId in this direction is seen for the hydrazides (Figure 1). Clearly other factors

and M. A. Maxwell, *Tetrahedron Lett.*, 1111 (1964). The total light emission of substituted indoles also gives linear Hammett plots: N. Sugiyama, H. Yamamoto, Y. Omote, and M. Akutagawa, *Bull. Chem. Soc. Jap.*, 41, 1977 (1968). In both cases, electron releasing substituents increase the light emission.

(15) (a) H. D. K. Drew and F. H. Pearman, *J. Chem. Soc.*, 586 (1937); (b) H. D. F. Drew and R. F. Garwood, *ibid.*, 836 (1939).

(16) It was earlier noted that the fluorescence yields of the hydrazides (the hydrazides were once thought to be the emitters) and of aromatic compounds in general are increased by electron donation, with the fluorescence yields of the hydrazides roughly paralleling the chemiluminescence yields of the hydrazides: A. Spruit-van der Burg, *Recl. Trav. Chim. Pays-Bas*, 69, 1536 (1950).
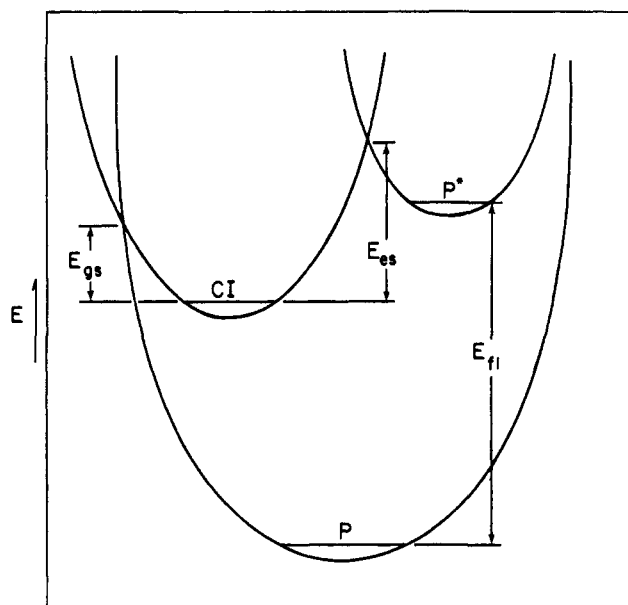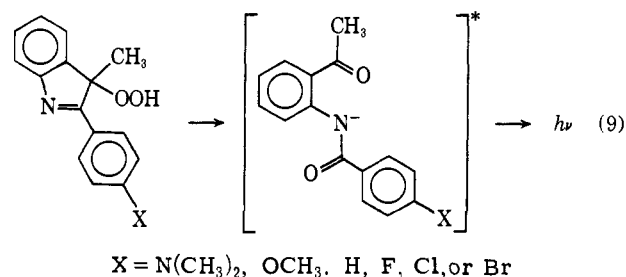
(17) R. A. Marcus, *J. Chem. Phys.*, 43, 2654 (1963).

(18) E. Rapaport, M. Cass, and E. H. White, *J. Amer. Chem. Soc.*, 94, 3153 (1972).

(19) The pK_a values of substituted anilines are more sensitive to substituent effects in the excited state than in the ground state: J. P. Idoux and C. K. Hancock, *J. Org. Chem.*, 32, 1935 (1967). The pK_a values of nitrophenols show a greater substituent effect in the excited singlet state than in the ground state: S. G. Schulman, L. B. Sanders, and J. D. Winefordnes, *Photochem. Photobiol.*, 13, 381 (1971).

are involved, however, since if the decrease in E_{fl} were manifested entirely in E_{es}, an increase in Φ_{es} of about 10^{10} would result ($E_{fl}[\nu_{min}]$ for V = 89 kcal and for VIId = 73 kcal; V and VIId are the emitting acids corresponding to hydrazides I and IIId, respectively).

Experimental Section[20]

Materials. All of the hydrazides and acids (or anhydrides) used were pure by tlc. 1,2-Naphthalenedicarboxylic anhydride and 2,3-naphthalenedicarboxylic anhydride were commercial materials purified by sublimation. 3-Aminophthalic acid hydrochloride, 4-aminophthalic acid, 5-amino-2,3-dihydrophthalazine-1,4-dione hydrobromide, and quinine bisulfate were commercial materials purified by crystallization from concentrated hydrochloric acid, dilute hydrochloric acid, 48% hydrobromic acid, and water, respectively. Benzo[f]2,3-dihydrophthalazine-1,4-dione, benzo[g]-2,3-dihydrophthalazine-1,4-dione,[15b] and 6-amino-2,3-dihydro-1,4-phthalazinedione[21] were prepared by the methods of Drew. 6-(Dimethylamino)-2,3-dihydrophthalazine-1,4-dione and 6-(diethyl-amino)-2,3-dihydrophthalazine-1,4-dione were prepared by the methods of Gundermann.[22]

4-(Dimethylamino)phthalic Anhydride. Oxygen was bubbled through a solution of 6-(dimethylamino)-2,3-dihydrophthalazine-1,4-dione (50 mg, 0.25 mmol) and potassium *tert*-butoxide (0.20 g) in DMSO for 12 hr. The resulting solution was evaporated to dryness, the residue was dissolved in water, and the aqueous solution was acidified with hydrochloric acid to pH ~6 and extracted repeatedly with ether. The yellow residue obtained from the evaporation of the ether was sublimed (130° (0.1 mm)), and the sublimate was purified by preparative tlc (cellulose–benzene). The mobile yellow band was cut out and extracted with methylene chloride, and the solvent removed under vacuum to give bright yellow crystals of product (21 mg, 0.10 mmol, 40%): mp 195–196°; ir (KBr) 1825 and 1750 cm^{-1}.

Anal. Calcd for $C_{10}H_9NO_3$: C, 62.82; H, 4.74; N, 7.39. Found: C, 62.60; H, 4.53; N, 7.17.

4-(Diethylamino)phthalic Anhydride. The procedure analogous to the synthesis of 4-(dimethylamino)phthalic anhydride gave a 57% yield of bright yellow crystals of product: mp 95–97°; ir (KBr) 1825 and 1740 cm^{-1}; nmr (CDCl$_3$) δ 7.8 (1 H, d, J_{13} = 8 Hz), 7.1 (1 H, d, J_{23} = 2 Hz), 6.9 (1 H, d of d, J_{13} = 8 Hz and J_{23} = 2 Hz), 3.5 (2 H, q), and 1.3 (3 H, t).

Anal. Calcd for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C, 66.05; H, 6.11; N, 6.53.

4-(*n*-Butylamino)-*N*-methylphthalimide. A mixture of 4-amino-*N*-methylphthalimide (0.5 g, 2.85 mmol) and *n*-butyl tosylate (4 g, 17.5 mmol) was heated at 165–170° for 3 hr. After cooling, 10% NaOH (10 ml) and ether (30 ml) were added and the mixture stirred until all solids had dissolved. The ether layer was separated and dried over K$_2$CO$_3$, and the solvent removed under vacuum. Chromatography (alumina–benzene) of the resulting green-yellow oil gave two yellow bands. The fraction containing the second yellow band was evaporated to dryness under vacuum to give a bright yellow powder (0.2 g, 0.86 mmol, 30%): mp 136–137°; ir (KBr) 3360, 1760, and 1700 cm^{-1}; nmr (CCl$_4$) δ 7.6 (1 H, d, J_{12} = 8 Hz), 7.0 (1 H, d, J_{23} = 2 Hz), 6.7 (1 H, d of d, J_{12} = 8 Hz and J_{23} = 2 Hz), 4.5 (1 H, s), 3.3 (1 H, t), 3.1 (3 H, s), 0.8–1.9 (7 H, m).

Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94. Found: C, 67.48; H, 7.22.

6-Monobutylamino-2,3-dihydrophthalazine-1,4-dione. The imide above (50 mg, 0.21 mmol) was heated on a steam bath under nitrogen for 4 hr with 95% hydrazine (0.2 ml) and ethanol (1.5 ml). The solvent was removed under vacuum, and the resulting solid was recrystallized from methanol–water and dried under vacuum

to yield a white solid (30 mg, 0.13 mmol, 60%): mp 203–205°; ir (KBr) 1650 and 1610 cm^{-1}.

Anal. Calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.01. Found: C, 61.61; H, 6.51; N, 17.91.

4-(*n*-Butylamino)phthalic Anhydride. The procedure analogous to the synthesis of 4-(dimethylamino)phthalic anhydride but with a reaction time of 48 hr and no purification by tlc gave a 40% yield of bright yellow crystals of product: mp 71–73°; ir (KBr) 3370, 1830, and 1760 cm^{-1}.

Anal. Calcd for a 60:40 mixture of anhydride and diacid: C, 62.75; H, 6.21; N, 6.10. Found: C, 62.73; H, 5.90; N, 5.80.

4-(Di-*n*-butylamino)-*N*-methylphthalimide. The fraction containing the first yellow band from the chromatography in the synthesis of 4-(*n*-butylamino)-*N*-methylphthalimide was evaporated to dryness and rechromatographed (silica gel-benzene). The yellow fraction, which came off after a fraction containing unreacted *n*-butyl tosylate, yielded a yellow-orange solid on evaporation of the solvent (0.1 g, 0.35 mmol, 12%): mp 55–57°; ir (neat) 1760 and 1700 cm^{-1}; nmr (CCl$_4$) δ 7.5 (1 H, d, J_{13} = 8 Hz), 6.9 (1 H, d, J_{23} = 2 Hz), 6.7 (1 H, d of d, J_{12} = 8 Hz and J_{23} = 2 Hz), 3.4 (4 H, t), 3.1 (3 H, s), 0.8–2 (14 H, m).

Anal. Calcd for $C_{17}H_{24}N_2O_2$: C, 70.80; H, 8.39. Found: C, 70.90; H, 8.18.

6-(Di-*n*-butylamino)-2,3-dihydrophthalazine-1,4-dione. The procedure was analogous to the synthesis of 6-(*n*-butylamino)-2,3-dihydrophthalazinedione. Recrystallization from ethanol-water and drying under vacuum gave a white powder (70%): mp 166–168°; ir (KBr) 1660 and 1620 cm^{-1}.

Anal. Calcd for $C_{16}H_{23}N_3O_2 \cdot 0.5H_2O$: C, 64.46; H, 8.10; N, 14.10. Found: C, 64.04; H, 7.83; N, 14.09.

4-(Di-*n*-butylamino)phthalic Anhydride. The procedure analogous to the synthesis of 4-(dimethylamino)phthalic anhydride gave a 70% yield of yellow crystals: mp 73–75°; ir (KBr) 1840 and 1770 cm^{-1}.

Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.74; H, 7.69; N, 5.09. Found: C, 70.00; H, 7.89; N, 4.82.

4-(Di-*n*-heptylamino)-*N*-methylphthalimide. The procedure was analogous to that used to prepare 4-(di-*n*-butyl)-*N*-methylphthalimide except that potassium carbonate (equivalent to the amount of tosylate used) was included in the reaction mixture which was heated for 5 hr. After chromatography on both alumina and silica gel, the product was obtained as a yellow-green oil (30%): ir (neat) 1760 and 1700 cm^{-1}; nmr (CCl$_4$) δ 7.5 (1 H, d, J_{13} = 8 Hz), 6.9 (1 H, d, J_{23} = 2 Hz), 6.7 (1 H, d of d, J_{13} = 8 Hz and J_{23} = 2 Hz), 3.4 (4 H, t), 3.1 (3 H, s), 0.8–2 (26 H, m).

Anal. Calcd for $C_{23}H_{36}N_2O_2$: C, 74.15; H, 9.74. Found: C, 73.89; H, 9.70.

6-(Di-*n*-heptylamino)-2,3-dihydrophthalazine-1,4-dione. The procedure was analogous to the preparation of 6-(*n*-butylamino)-2,3-dihydrophthalazine-1,4-dione. Two recrystallizations from ethyl acetate and drying under vacuum gave a 45% yield of white crystals: mp 119–121°; ir (KBr) 1660 and 1620 cm^{-1}.

Anal. Calcd for $C_{22}H_{35}N_3O_2$: C, 70.74; H, 9.44; N, 11.25 Found: C, 70.89; H, 9.47; N, 11.25.

4-(Di-*n*-heptylamino)phthalic Anhydride. The procedure was analogous to the synthesis of 4-(dimethylamino)phthalic acid but with no purification by tlc. The product was a yellow solid (83%): mp 41–43°; ir (KBr) 1845 and 1765 cm^{-1}.

Anal. Calcd for $C_{22}H_{33}NO_3$: C, 73.50; H, 9.25; N, 3.90. Found: C, 73.56; H, 9.07; N, 3.92.

N,3-Dimethyl-6-nitrophthalimide. Fuming nitric acid (2 ml, sp gr 1.59) was added dropwise to a stirred solution of 3-methyl-phthalic anhydride (5.5 g, 34 mmol) in concentrated sulfuric acid (6.5 ml) maintained at 80°. Then concentrated nitric acid (10 ml, sp gr 1.42) was added, and the mixture heated at 85–90° for 2 hr. Water (30 ml) was added, and the mixture was extracted with ether (150 ml). The ether layer was separated and dried, and the ether was removed under vacuum to yield a thick paste. The paste was heated slowly to 130° under vacuum (0.1 mm) to remove the nitric acid present. 1,3-Dimethylurea (2.4 g, 27 mmol) was added to the resulting solid, and the mixture was heated to 160–165° with stirring for 1 hr. An additional portion of dimethylurea (1.2 g) was then added and heating was continued for 15 min more. After cooling, the resulting solid was dissolved in a mixture of water and chloroform. The chloroform layer was separated and dried, and the solvent was removed under vacuum. Two crystallizations from carbon tetrachloride (100, 75 ml) gave light yellow-orange crystals of compound (4.0 g, 17 mmol, 50%): mp 108–110°; ir (KBr) 1780 and 1720 cm^{-1}; nmr (CDCl$_3$) δ 7.9 (1 H, d, J = 8 Hz), 7.6 (1 H, d, J = 8 Hz), 3.2 (3 H, s), 2.8 (3 H, s).

(20) Melting points were taken with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were taken on a Perkin-Elmer Model 337 and were calibrated with polystyrene film. Nuclear magnetic resonance spectra were taken on a Varian Associates A-60 instrument, and chemical shifts are reported in δ units relative to tetramethylsilane, external in D$_2$O, internal in all other solvents. Ultraviolet spectra were taken on a Cary 14 instrument. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Tlc was done on cellulose with an ethanol-water-concentrated ammonium hydroxide mixture (8:1:1). Solvents, unless otherwise stated, were reagent grade and used as received.

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Anal. Calcd for $C_{10}H_8N_2O_4$: C, 54.55; H, 3.66. Found: C, 54.30; H, 3.73.

3-Amino-*N*,6-dimethylphthalimide. *N*,3-Dimethyl-6-nitrophthalimide (1 g, 4.3 mmol) and 10% Pd/C (0.1 g) were stirred together in ethanol for 3 hr under hydrogen (1 atm). During this time 310 ml (14 mmol, 108%) of hydrogen was consumed. The mixture was then filtered through Celite, and the solvent removed under vacuum. The resulting solid was crystallized from ethanol to give yellow-orange crystals (0.54 g, 2.8 mmol, 66%): mp 160–161°; ir (KBr) 3460, 3350, 1740, and 1680 cm^{-1}; nmr (CDCl$_3$) δ 7.1 (1 H, d, *J* = 8 Hz), 6.85 (1 H, d, *J* = 8 Hz), 5.1 (2 H, s), 3.1 (3 H, s), 2.5 (3 H, s); uv (95% ethanol) 395 nm (log ε 3.75).

Anal. Calcd for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30. Found: C, 63.22; H, 5.30.

5-Amino-8-methyl-2,3-dihydrophthalazine-1,4-dione. 3-Amino-*N*,6-dimethylphthalimide (0.1 g, 0.52 mmol) was sublimed (150–160° (0.1 mm)), and then was refluxed under nitrogen for 4 hr with 95% hydrazine (2 ml). Then water (15 ml) was added, and the product was precipitated by acidification with glacial acetic acid. The solid was collected, washed with 5% acetic acid (10 ml), dissolved in 10% ammonium hydroxide (15 ml), and reprecipitated with acetic acid. The solid was collected washed with water (10 ml), and with boiling ethanol (10 ml). Drying under vacuum gave a white solid (0.075 g, 0.39 mmol, 75%): ir (KBr) 1650 and 1590 cm^{-1}.

Anal. Calcd for $C_9H_9N_3O_2$: C, 56.54; H, 4.74; N, 21.98. Found: C, 56.65; H, 4.74; N, 22.05.

3-Amino-6-methylphthalic Acid Hydrochloride. *N*,3-Dimethyl-6-nitrophthalimide (500 mg, 2.3 mmol) was refluxed in a mixture of hydrochloric acid (5 ml, concentrated) and ethanol (2 ml) for 6 days. The mixture was cooled and extracted with ether, the ether phase was dried, and the solvent was removed under vacuum. The resulting solid, 10% Pd/C (50 mg) and dry THF (50 ml) were stirred together under hydrogen for 1.5 hr. The mixture was filtered through Celite, and the solvent was removed under vacuum. The resulting yellow solid was dissolved in dilute hydrochloric acid, and the solution was saturated with gaseous hydrogen chloride and cooled to −10°. The desired product precipitated out overnight (230 mg, 1 mmol, 44%): ir (KBr) 1710 cm^{-1}; uv (0.1 *M* K$_3$CO$_3$) 306 nm (3.36);[23] nmr (1 *M* K$_2$CO$_3$ in D$_2$O) δ 7.5 (1 H, d, *J* = 8 Hz), 7.2 (1 H, d, *J* = 8 Hz), 2.8 (3 H, s).

Fluorescence and Chemiluminescence Emission Spectra. Emission spectra were measured on a Hitachi-Perkin-Elmer MPF-2A spectrophotofluorimeter with a stabilized Xenon arc source and R106 photomultiplier detector which was calibrated for nonlinearity of the source and detector as previously described.[24] Fluorescence spectra were obtained on an ~10^{-5} *M* solution of phthalates in 0.1 *M* aqueous potassium carbonate (anhydrides were first dissolved in a small amount of ethanol and diluted with the carbonate). Chemiluminescence spectra were determined by reacting solutions of hydrazide (~10^4 *M*) in 0.1 *M* carbonate with aqueous hydrogen peroxide (0.1 *M*) and hemin (10^{-5} *M*) while running the spectrofluorimeter with the source off. Because the intensity of chemiluminescence is decaying during the scan, the chemiluminescence spectra are shifted to shorter wavelengths.

Fluorescence Quantum Yields. The fluorescence yields were measured relative to quinine bisulfate in 0.1 *N* sulfuric acid. All solutions used had optical densities of 0.1 or less. The fluorescence spectra were tabulated for intervals of 5 nm. A computer program was used to correct the spectra and calculate the quantum yields as previously described.[24]

Chemiluminescence Quantum Yields. The chemiluminescence yields were determined relative to the luminol standard of Seliger[25] using a RCA 1P21 photomultiplier powered by a Fluke 4128 DC power supply. The signal from the photomultiplier was amplified and fed to a capacitor, the charge on the capacitor being proportional to the integrated light output. Aliquots (1 ml) of known hydrazide concentration (~10^{-5} *M*) in 0.1 *M* potassium carbonate were placed in a glass vial in a light-tight compartment attached to the photomultiplier. The reaction was initiated by injecting 0.1 ml of ~0.03 *M* hydrogen peroxide followed by 0.1 ml of ~3 × 10^{-6} *M* hemin solution. Additional peroxide and hemin were injected to ensure complete reaction.

(23) 3-Aminophthalic acid gives 304 nm (3.35) (J. Lee and H. H. Seliger, *Photochem. Photobiol.*, **4**, 1015 (1965).

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