# Synthesis and Chemiluminescence of Derivatives of Luminol and Isoluminol

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Abstract: Five alkyl derivatives of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and two alkyl derivatives of isoluminol (6-amino-2,3-dihydrophthalazine-1,4-dione) were prepared and their chemiluminescence quantum yields were measured. Alkyl groups in the 6-position of luminol double the efficiency of excited state formation, whereas alkyl groups in the 5-position have little effect. A steric interaction of the alkyl groups with the adjacent carbonyl group is proposed to account for these effects.

Since the discovery of luminol[1] (I) by Albrecht[2] in 1928, much effort has been expended in an attempt to understand the mechanism of hydrazide chemiluminescence and to uncover the factors governing the efficiency of light production.[3] The early work of Drew revealed two substituent ef-

$$\text{I} \xrightarrow[\text{[O]}]{\text{base}} \text{II} \longrightarrow h\nu \quad (1)$$
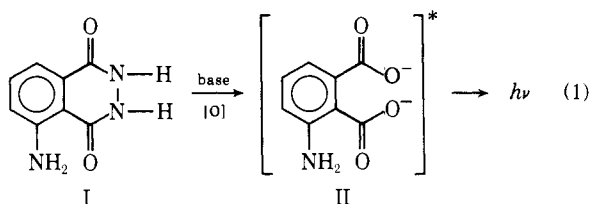
fects on the light yield: (1) electron donating substituents increase the light yield, substituents at C-3 and C-6 having a greater effect than those at C-4 and C-5,[4] and (2) substituents on the heterocyclic ring as in III and IV prevent light emission.[5]

III   IV
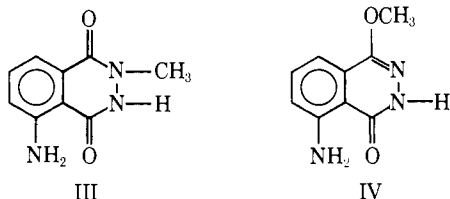
It was the application of the first rule which led to the synthesis of hydrazides that surpass the efficiency of luminol.[6] The most efficient cyclic hydrazide reported to date, benzo[*ghi*]perylene-1,2-dicarboxylic acid hydrazide, gives a 50% yield of excited carboxylate ions and a 7% yield of light.[7]

The chemiluminescent reaction of luminol has been shown to yield 3-aminophthalate ion (II) as the major organic product; the light emission results from aminophthalate fluorescence (eq 1).[8] With the exception of phthalic hydrazide and its alkyl derivatives,[9] the light emitter in the chemiluminescence of the cyclic hydrazides is the product phthalate ion, which is produced in the excited singlet state.
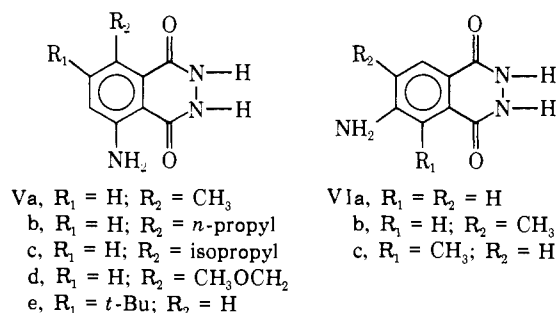
By factoring out the fluorescence quantum yields (Φ_{fl}) of the emitting phthalates from the overall chemiluminescence quantum yields (Φ_{ch}) of the hydrazides (eq 2), it has been

$$\Phi_{ch} = \Phi_r \Phi_{es} \Phi_{fl} \quad (2)$$

shown that substituents have an effect on the fraction of reacting molecules which end up in the excited state ($\Phi_r\Phi_{es}$).[10] Φ_r is the fraction of hydrazide molecules which follow the correct chemical path to give the critical intermediate, and Φ_{es} is the fraction of molecules of this intermediate which cross over to the excited state of the product. We assume that $\Phi_r = 1$ because nearly quantitative yields of the

expected phthalate products are produced and because the chemiluminescence quantum yields are measured under near optimum conditions and are not very sensitive to the concentrations of reactants (hydrazide, hydrogen peroxide, hemin).[10]

It has been shown that, in general, Φ_{es} and Φ_{ch} increase as the frequency of the emitted light decreases.[10] However, luminol is about twice as efficient as would be predicted from this relationship. Even more anomalous is 6-methylluminol (Va), which is nearly twice as efficient as luminol but

Va, R₁ = H; R₂ = CH₃   VIa, R₁ = R₂ = H
b, R₁ = H; R₂ = *n*-propyl   b, R₁ = H; R₂ = CH₃
c, R₁ = H; R₂ = isopropyl   c, R₁ = CH₃; R₂ = H
d, R₁ = H; R₂ = CH₃OCH₂
e, R₁ = *t*-Bu; R₂ = H

which emits at a higher frequency.[10] This paper covers the effect of alkyl substitution on the chemiluminescence yields of luminol and isoluminol derivatives in an attempt to understand this anomaly.

Syntheses

The hydrazides were all prepared by the action of hydrazine on an amino substituted *N*-methylphthalimide and the latter compounds were prepared from the corresponding *N*-methylnitrophthalimides by catalytic reduction (eq 3).

$$\quad (3)$$
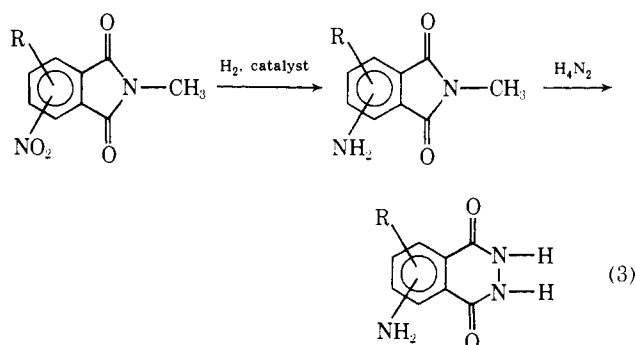
The *N*-methylnitrophthalimides were conveniently prepared from the corresponding phthalic acids, the phthalic anhydrides, or the diesters by heating them with 1,3-dimethylurea (DMU). The *N*-methylphthalimides were used because they are highly crystalline, they are quite stable to acid and base (unlike the phthalic anhydrides or esters), and they are readily converted to the hydrazides.
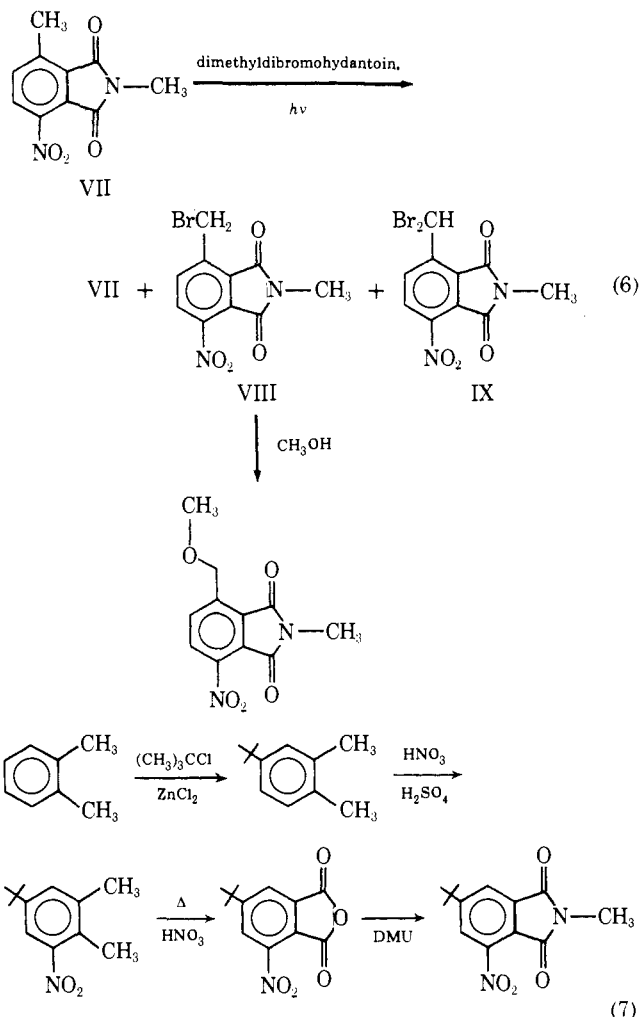
The nitro-N-methylphthalimide to be converted to 6-propylluminol (Vb) was prepared by a Diels–Alder reaction of 1,3-heptadiene with dimethyl acetylenedicarboxylate, aromatization by dehydrogenation with Pd/C, nitration with a nitric–sulfuric acid mixture, and heating with DMU (eq 4).

$$(4)$$

$$(5)$$
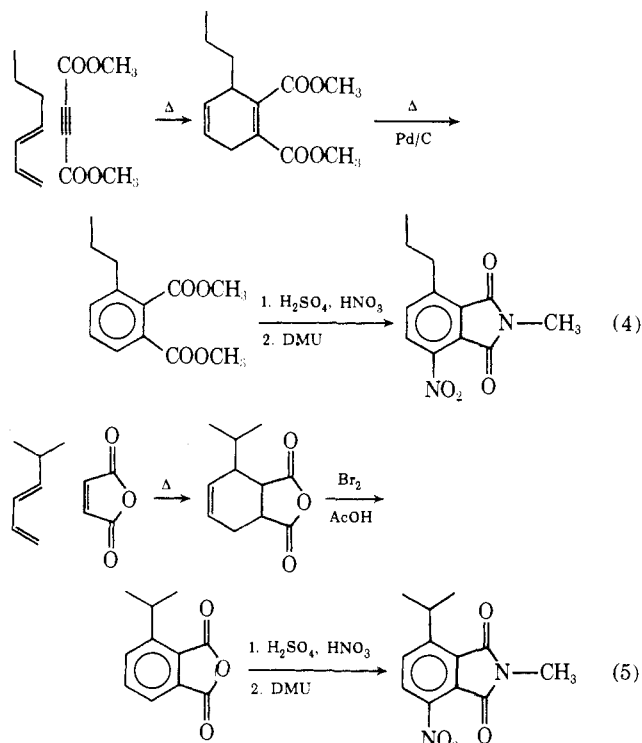
The imide to be converted to 6-isopropylluminol (Vc) was prepared by a Diels–Alder reaction of 5-methyl-1,3-hexadiene with maleic anhydride, aromatization by bromination and dehydrobromination, nitration, and reaction of the product with DMU. Of the two methods of aromatization used above, the dehydrogenation with Pd/C (eq 4) is by far the easiest to use; however, it does not give good results with the cyclohexene resulting from the Diels–Alder reaction of maleic anhydride. The nitrations of the 3-n-propyl and 3-isopropyl phthalates appear to give, almost exclusively, substitution in the C-6 position, unlike the nitration of 3-methylphthalic anhydride which gives ~20% of the 4-nitrated product[10] in addition to the C-6 substituted product.

The nitro-N-methylphthalimide to be converted to 6-(methoxymethyl)luminol (Vd) was obtained (eq 6) by refluxing 3-(bromomethyl)-N-methyl-6-nitrophthalimide (VIII) in methanol for several days. It was found that when VIII was treated with sodium methoxide, it was rapidly destroyed (the solution became bright red indicating a possible radical anion reaction) and none of the desired substitution product was formed. The bromination of N,3-dimethyl-6-nitrophthalimide yields (by nmr) a mixture of about equal amounts of starting material (VII), monobrominated product (VIII), and what is probably the dibrominated product (IX) from which the desired monobrominated product was separated by tlc.

The nitration of 4-$tert$-butyl-o-xylene, obtained by Friedel–Crafts alkylation of o-xylene, gave 3-nitro-5-$tert$-butyl-o-xylene (eq 7). The position of nitration was established by the meta coupling found for the aromatic protons (2 Hz). This compound was converted to the known 5-$tert$-butyl-3-nitrophthalic anhydride[11] by oxidation with nitric acid. This anhydride was converted to the N-methylphthalimide and on to 5-$tert$-butylluminol (Ve) in the usual way (eq 3).

$$(6)$$

$$(7)$$

N,3-Dimethyl-6-nitrophthalimide[10] had been previously synthesized by nitrating 3-methylphthalic anhydride, converting the product to the N-methylphthalimide, and purifying the imide by crystallization from carbon tetrachloride.[10] The carbon tetrachloride mother liquors contain about equal amounts of the 6- and the 4-nitro-N,3-dimethylphthalimides which can be separated by tlc, but it was easier in this synthesis to reduce the mixture to the corresponding aminophthalimides. Since 4-aminophthalimides are, in general, less soluble than the 3- (or 6-) aminophthalimides, the desired 4-amino-N,3-dimethylphthalimide could be readily separated by crystallization. Treatment of the imide with hydrazine yielded hydrazide VIc.

3-Amino-6-isopropylphthalic anhydride was prepared from 3-isopropyl-6-nitro-N-methylphthalimide by: (1) hy-

$$(8)$$
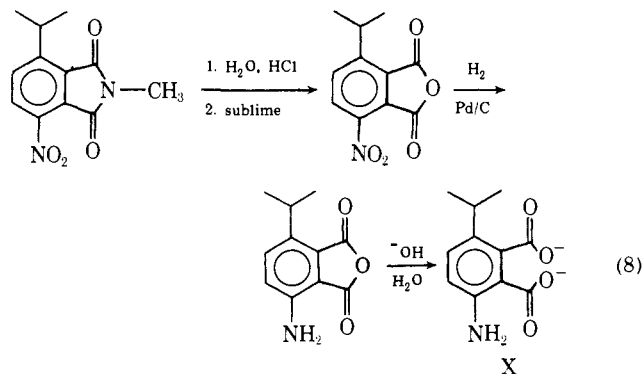
drolysis with aqueous hydrochloric acid and sublimation to give 3-isopropyl-6-nitrophthalic anhydride, and (2) catalyt-

Table I. Luminescence of Substituted Phthalic Hydrazides and the Corresponding Phthalic Acids

Compound	$\Phi_{ch}{}^{a,b}$ (eq 2)	Chemiluminescence λ_{max} (nm)^{a,c}	Fluorescence λ_{max} of corr phthalates	$\Phi_{fl}{}^{d}$ of corr phthalates	$\Phi_{es} = \Phi_{ch}/\Phi_{fl}$	Φ_{es} (value expected on basis of λ_{max})^{e}
I^{f}	0.015	423	425	0.30	0.05	0.023
Va^{f}	0.028	405	405	0.28	0.10	0.019
Vb	0.017	408				
Vc	0.012	403	403	0.13	0.092	0.018
Vd	0.006	405				
Ve	0.012	425				
VIa^{f}	0.0012	416	419	0.13	0.0092	0.01
VIb	0.0011	408				
VIc	0.0006	410				

^{a} In aqueous 0.1 M K_2CO_3 solution initiated by 0.03 M hydrogen peroxide and 3×10^{-6} M hemin. ^{b} Values were measured with respect to luminol at 0.0125 (J. Lee and H. H. Seliger, *Photochem. Photobiol.*, **4**, 1015 (1965)); precision is estimated to be $\pm 10\%$. ^{c} Uncorrected value; actual value is slightly (≤ 5 mm) to longer wavelength due to decay of light intensity during measurement. ^{d} Obtained in aqueous 0.1 M K_2CO_3 solution; values are relative to quinine bisulfate at 0.55 (W. H. Melhuish, *J. Phys. Chem.*, **65**, 229 (1961)). ^{e} Value interpolated from plot of Φ_{es} *vs.* ν obtained for a series of "normal hydrazides" (Figure 1 of ref 10). ^{f} Reference 10.

ic reduction of the nitro anhydride to the amino anhydride (in the presence of a drying agent to remove the water generated).

Results

The chemiluminescence quantum yields and the emission maxima of the hydrazides and the fluorescence quantum yields and emission maxima of several of the corresponding phthalates are given in Table I. The chemiluminescence data were all measured under the same conditions using the hemin catalyzed reaction with hydrogen peroxide in 0.1 M aqueous bicarbonate solution.[3a] The fluorescence data were obtained in 0.1 M aqueous bicarbonate solution.

Discussion

The introduction of a methyl group into the C-6 position of luminol (to yield compound Va) doubles the chemiluminescence efficiency (Φ_{ch}, Table I). Larger groups in the same position (Vb-d) lead to lower efficiencies, however. The substituent effect does not appear to be electronic in origin in view of the trends in Φ_{ch} observed for compounds Va-d and VIa-c (Table I). We believe that the effects at C-6 are principally steric in origin (following discussion). A *tert*-butyl group was introduced into the C-5 position of luminol (giving compound Ve) in the hope that this bulky group would reduce quenching of the excited states by partially blocking collisions involving interactions with the π-electrons. A similar blocking role has been proposed for the phenyl groups in 9,10-diphenylanthracene.[12] In the present example no appreciable effect was noted, however (Table I); apparently groups that extend further above and below the ring plane will be required.

Since overall quantum yields (Φ_{ch}) are products of several efficiencies (eq 2), substituent effects will not be readily interpretable on this basis. Consequently, we have measured Φ_{fl} for several of the hydrazides and have factored out Φ_{es} ($\approx \Phi_r\Phi_{es}$) from Φ_{ch} (eq 2). The fluorescence quantum yields are also affected by substituents; 6-methyl-3-aminophthalate (from Va) has about the same fluorescence efficiency as 3-aminophthalate (from I), whereas 6-isopropyl 3-aminophthalate (from Vc) shows a decrease. This decrease in efficiency may stem from the additional vibrational modes available to the latter compound as well as from steric inhibition of resonance of the C-1 carboxyl group by the isopropyl group (see discussion that follows).

The efficiencies of production of excited states (Φ_{es}) obtained from eq 2 are listed in Table I; the 6-methyl and isopropyl compounds (Va and Vc) have about the same efficiency (9-10%),[13] which is approximately double the efficiency found for the parent luminol (I). These values should be compared with the entries in the last column of Table I

which lists the efficiencies expected for these compounds based on a linear relationship between Φ_{es} and the wavelength of light emission that has been found to hold for cyclic hydrazides free of steric complications.[10,14] On this basis luminol (I) is about twice as efficient as predicted, and the C-6 substituted compounds Va and Vc are about four-five times as efficient. Since the measured and expected values of Φ_{es} are the same for isoluminol (VIa) (Table I), the twofold effect observed for luminol probably stems from a neighboring group interaction of the amino group. Intramolecular hydrogen bonding to the carbonyl group (XI)

XI
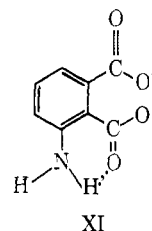
would be expected to increase the planarity of the system and increase the resonance interactions of the C-2 carboxylate with the ring. The hydrogen bridge illustrated in XI has been implicated in the chemiluminescence of luminol in aprotic solvents.[15] We assume that similar interactions in compounds Va and Vc can account for a factor of 2 in Φ_{ch}. Thus, of the factor of 4-5 separating $\Phi_{es(found)}$ from $\Phi_{es(expected)}$ for Va and Vc, a factor of about 2-2.5 remains. We are inclined to believe that the remaining factor originates in the steric interaction of the C-6 substituent with the carbonyl group at C-1, since the effect shows up for C-6 substituents but not for those at C-5. A twisting of the C-1 carbonyl group out of the plane of the aromatic ring could, in principle, lead to a larger fraction of molecules crossing over to the excited state surface if the geometry of the transition state leading from the critical intermediate to the phthalate product were forced to resemble the excited state more than the ground state species. Such a departure from coplanarity could also explain the shift of the emission wavelength to the blue range (423 nm for luminol and $\sim$405 nm for Va-d; Table I). At the extreme limit of noncoplanarity, the carboxylate group would be perpendicular to the aromatic ring and not in conjugation with it. 2-Aminobenzoate, which lacks the carboxylate group at C-3 is a model for this extreme limit; it fluoresces at 395 nm.[15]

As expected, substituents in the 5-position show no abnormal steric effects; isoluminol (VIa) has a Φ_{es} near that predicted from its emission wavelength (Table I), and 5-methylisoluminol (VIb) and 5-*tert*-butylluminol (Ve) resemble the corresponding unalkylated compounds (VIa and I, respectively), in both the emission wavelength and Φ_{ch}. In

contrast, the Φ_{ch} of 3-methylisoluminol (VIc) is only about half that of isoluminol (VIa). This decrease probably results from a decrease in Φ_{fl}, since the wavelength of emission is about the same as that of isoluminol (Table I). Steric inhibition of resonance of the amino group by the methyl substituent,[16] buttressed in this case by the carbonyl group,[17] could lead to an effect of this nature.

The discussion above deals with the chemiluminescence of the cyclic hydrazides in aqueous media. Preliminary evidence indicates that similar substituent effects[18] occur in the aprotic system in which DMSO, tert-butoxide, and oxygen are utilized. The Φ_{ch} of luminol (I) and 6-methylluminol (Va) in DMSO containing 1 M tert-butyl alcohol, potassium tert-butoxide, and oxygen are 0.013 and 0.043, respectively. The Φ_{fl} of the emitters in this solvent are 0.10 and 0.19, respectively. Thus, the Φ_{es} of I is 0.13 and that of Va is 0.23. Both I and Va emit at the same wavelength in this solvent (475 nm). The increase in Φ_{es} from I to Va (a factor of about 2) is the same as that found in the aqueous system (Table I). If the substituent effects on Φ_{es} in the protic and aprotic systems are found to be similar for other compounds, it would suggest that the final excitation step in the two systems is the same despite differences in the initial chemistry.

Several factors are thus of importance in the design of efficient chemiluminescent cyclic hydrazides: the presence of electron releasing substituents, avoidance of steric inhibition of resonance of these substituents, a degree of steric interaction of other substituents with the carbonyl group at C-1, and emission at long wavelengths. In addition to these factors, hydrogen bonding by an amino group ortho to one of the carbonyl groups appears advantageous. More generally, the compounds should not be degraded by the reagents used and they should be reasonably soluble in the reaction media.

Experimental Section[19]

Dimethyl 3-n-Propylphthalate. A mixture of 1,3-heptadiene (5.0 g, 52 mmol), dimethyl acetylenedicarboxylate (7.4 g, 49 mmol), and benzene (20 ml) was refluxed for 20 hr. The solvent was then removed under vacuum. Then 10% Pd/C (0.1 g) was added to the resulting oil, and the mixture was heated at 250–260° for 3 hr. Fractional distillation (at 0.1 Torr, 90–92°) gave 9.5 g (39 mmol, 80%) of a colorless liquid: ir (neat) 1730 cm^{-1}; nmr (CCl₄) δ 7.9–7.2 (3 H, m), 3.8 (6 H, 2s), 2.5 (2 H, t), 1.5 (2 H, m), 0.9 (3 H, t). An impurity gives a multiplet at 3.6 in the nmr which integrates for 10% of the δ 3.8 peak.

3-Nitro-6-n-propyl-N-methylphthalimide. Fuming nitric acid (2 ml, specific gravity 1.60) was added dropwise over 3 hr to a solution of dimethyl 3-n-propylphthalate (1.5 g, 6.1 mmol, unpurified product of previous synthesis) dissolved in sulfuric acid (6 ml). The mixture was heated at 80–85° for 1 hr. Water (25 ml) was added and the mixture was extracted with chloroform. The organic phase was separated and dried, and the solvent was removed under vacuum. The resulting material was heated at 160–165° with 1,3-dimethylurea (0.75 g, 8.5 mmol) for 1 hr. Then more of the urea (0.35 g) was added and heating was continued for 30 min more. The resulting material was dissolved in a mixture of water and chloroform, the organic phase was separated and dried, and the solvent was removed under vacuum to give an oil. The oil was separated by preparative tlc (silica gel developed three times with 1:4 chloroform:benzene). The large yellow nonfluorescent mobile band was cut out and extracted with chloroform; the solvent was removed under vacuum to give a yellow oil (1.1 g, 4.4 mmol, 70%): ir (neat) 1780, 1720, and 1540 cm^{-1}; nmr (CDCl₃) δ 8.1 (1 H, d), 7.8 (1 H, d), 3.2 (5 H, s + t), 1.7 (2 H, m), 1.0 (3 H, t).

Anal. Calcd for C₁₂H₁₂N₂O₄: C, 58.06; H, 4.87. Found: C, 58.61; H, 5.29.

3-Amino-6-n-propyl-N-methylphthalimide. A mixture of 3-nitro-6-n-propyl-N-methylphthalimide (400 mg, 1.6 mmol), 10% Pd/C (40 mg), and ethanol (40 ml) was stirred under 1 atm of hydrogen for 4 hr. The solution was filtered and the solvent was re-

moved under vacuum. The resulting solid was separated by preparative tlc (silica gel developed three times with chloroform). The large yellow second most mobile band was cut out and extracted with methanol. The solvent was removed under vacuum, and the resulting solid was recrystallized from carbon tetrachloride to give yellow crystals (250 mg, 1.1 mmol, 70%): mp 118–119°; ir (KBr) 3450, 3330, 1750, and 1680 cm^{-1}; nmr (CDCl₃) 7.2 (1 H, d), 6.8 (1 H, d), 5.2 (2 H, broad s), 3.1 (3 H, s), 2.9 (2 H, t), 1.5 (2 H, m), 0.9 (3 H, t).

Anal. Calcd for C₁₂H₁₄N₂O₂: C, 66.04; H, 6.47. Found: C, 65.45; H, 6.46.

5-Amino-8-n-propyl-2,3-dihydrophthalazine-1,4-dione (Vb). 3-Amino-6-n-propyl-N-methylphthalimide (100 mg, 0.46 mmol) was refluxed under nitrogen with 95% hydrazine (1 ml) for 4 hr. The reaction mixture was then diluted with water and acidified with glacial acetic acid. The precipitate was collected, reprecipitated from 10% ammonium hydroxide with acetic acid, and sublimed (0.01 mm, 200°) to give an off-white solid (80 mg, 0.37 mmol, 80%): ir (KBr) 1640 and 1600 cm^{-1}; uv (0.1 M K₂CO₃) 310 nm (log ε 3.88) and 360 (3.90).

Anal. Calcd for C₁₁H₁₃N₃O₂: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.30; H, 6.08; N, 19.36.

3-Isopropylcyclohexene-4,5-dicarboxylic Acid Anhydride. Freshly sublimed maleic anhydride (8 g, 82 mmol) and 5-methyl-1,3-hexadiene (8 g, 84 mmol) were stirred together in benzene (32 ml) at 50° for 1 hr. The solution was then refluxed for 23 hr. To the hot solution was added hot cyclohexane (64 ml) and the mixture was cooled on ice. The resulting crystals were collected and recrystallized from cyclohexane (150 ml) to yield 12.3 g of product. The two filtrates were combined and the solvents were removed under vacuum. The resulting solid was crystallized from cyclohexane (75 ml) to yield 2.0 g of product. The two crops of white crystals were combined (14.3 g, 74 mmol, 90%): mp 88–89° (lit.[20] 90°); ir (KBr) 1840, 1770, 1370, and 1380; nmr (CDCl₃) δ 6.0 (2 H, m), 3.5 (2 H, m), 1.3–2.8 (4 H, m), 1.1 (6.0 H, t). The triplet in the nmr is probably two overlapping doublets, one from each of the geminal methyl groups, which are nonequivalent because the isopropyl side chain cannot freely rotate.

3-Isopropylphthalic Anhydride. A solution of bromine (16 g, 0.1 mol) in acetic acid (14 ml) was added dropwise over 3 hr to a stirred solution of 3-isopropylcyclohexene-4,5-dicarboxylic acid anhydride (8 g, 0.042 mol) in acetic acid (13 ml) maintained at 95°. The solution was then refluxed for 19 hr. Most of the acetic acid was removed under vacuum, and the resulting material was heated at 180–190° for 24 hr. The material was then distilled (0.1 mm) keeping the fraction coming off between 150–230° pot temperature. A low boiling fraction comes off before this. The solid crystallized very slowly from benzene (1 ml) to give greasy white crystals (3.2 g, 0.017 mol, 40%): mp ∼50°; ir (KBr) 1850 and 1780; nmr (CCl₄) δ 7.8 (3 H, s), 4.0 (1.2 H, septet, J = 7 Hz), 1.3 (6 H, d, J = 7 Hz). This compound was impure by nmr. The impurity shows up as a singlet at δ 3.8 under the septet and is responsible for the larger than expected integral of this absorption.

3-Isopropyl-6-nitro-N-methylphthalimide. To the impure 3-isopropylphthalic anhydride (1.5 g, 8.0 mmol) dissolved in concentrated sulfuric acid heated at 80° was added dropwise over 1 hr fuming nitric acid (0.6 ml, specific gravity 1.60). Then concentrated nitric acid (3 ml, specific gravity 1.42) was added which caused an oil to separate out. Heating was continued for 1 hr. Water was added to the reaction mixture and the product was extracted into chloroform. The chloroform solution was dried and the solvent was removed under vacuum. The resulting material was heated at 160–165° with 1,3-dimethylurea (0.75 g, 8.5 mmol) for 1 hr. Then more of the urea (0.30 g) was added and heating continued for 15 min. The product was dissolved in a mixture of water and chloroform. The chloroform layer was separated and dried, and the solvent was removed under vacuum. The material was sublimed (0.1 mm, 130–140°) and then crystallized from cyclohexane-ethyl acetate to give yellow crystals of the desired product (1.3 g, 5.2 mmol, 64%): mp 103–105°; ir (KBr) 1780, 1700, 1610, and 1530 cm^{-1}; nmr (CDCl₃) δ 8.0 (2 H, AB quartet, J = 8 Hz), 4.3 (1 H, septet, J = 6.5 Hz), 3.2 (3 H, s), 1.4 (6 H, d, J = 6.5 Hz).

Anal. Calcd for C₁₂H₁₂N₂O₄: C, 58.06; H, 4.87. Found: C, 58.53; H, 4.49.

3-Amino-6-isopropyl-N-methylphthalimide. A mixture of 3-isopropyl-6-nitro-N-methylphthalimide (500 mg, 1.9 mmol), 10%

Pd/C (50 mg), and ethanol (35 ml) was stirred under hydrogen (1 atm) for 4 hr. The solution was filtered through Celite, and the solvent was removed under vacuum. The solid was chromatographed on silica gel column with 20% chloroform in benzene as eluent. The fraction containing the first yellow band was collected and the solvent was removed under vacuum to give a bright yellow solid (380 mg, 1.7 mmol, 90%): mp 172–173°; ir (KBr) 3440, 3320, 1740, and 1680 cm^{-1}; nmr (CDCl$_3$) δ 7.3 (1 H, d, J = 9 Hz), 6.9 (1 H, d, J = 9 Hz), 5.2 (2 H, broad s), 4.0 (1 H, septet, J = 7 Hz), 3.1 (3 H, s), 1.2 (6 H, d, J = 7 Hz).

Anal. Calcd for C$_{12}$H$_{14}$N$_2$O$_2$: C, 66.04; H, 6.47. Found: C, 66.27; H, 6.33.

5-Amino-8-isopropyl-2,3-dihydrophthalazine-1,4-dione (Vc). 3-Amino-6-isopropyl-N-methylphthalimide (100 mg, 0.46 mmol) was refluxed under nitrogen with 95% hydrazine for 4 hr. Water (10 ml) was added and the solution was acidified with acetic acid. The solid was collected and was reprecipitated from 10% ammonium hydroxide by acidification with acetic acid. The solid was collected and sublimed (0.01 mm, 250–260°) to give a light yellow solid (80 mg, 0.37 mmol, 80%): ir (KBr) 3440, 3310, and 1650 cm^{-1}; uv (0.1 M K$_2$CO$_3$) 310 nm (log ϵ 3.88) and 360 (3.90).

Anal. Calcd for C$_{11}$H$_{13}$N$_3$O$_2$: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.30; H, 6.00; N, 19.12.

3-(Bromomethyl)-N-methyl-6-nitrophthalimide (VIII). To a solution of N, 3-dimethyl-6-nitrophthalimide[10] (VII) (1.5 g, 6.8 mmol) in carbon tetrachloride refluxing over a 100-W bulb, was added 1,3-dibromo-5,5-dimethylhydantoin (0.99 g, 3.5 mmol) in portions over 1 hr. The mixture was allowed to reflux for 1 hr more. The mixture was extracted with water, the organic phase was dried, and the solvent was removed under vacuum. The resulting oil was separated by preparative tlc (silica gel developed three times with benzene). The middle band of three large bands (dark under uv light) was cut out and extracted with chloroform and the solvent was removed under vacuum to give a pale yellow solid (0.8 g, 2.7 mmol, 40%): mp 91–93°; ir (neat melt) 1780, 1720, and 1540 cm^{-1}; nmr (CDCl$_3$) δ 8.1 (2 H, s), 5.1 (2 H, s), 3.2 (3 H, s).

Anal. Calcd for C$_{10}$H$_7$N$_2$O$_4$: C, 40.16; H, 2.36. Found: C, 40.64; H, 2.48.

The presumed dibrominated product (IX) gave nmr (CDCl$_3$) δ 8.5 (1 H, d), 8.3 (1 H, d), 8.0 (1 H, s), 3.3 (3 H, s).

3-(Methoxymethyl)-6-nitro-N-methylphthalimide. 3-(Bromomethyl)-6-nitro-N-methylphthalimide (650 mg, 2.2 mmol) was refluxed in methanol (10 ml) for 6 days. The resulting hot solution was filtered and cooled to give pale yellow crystals (420 mg, 1.7 mmol, 80%): mp 104–106°; ir (KBr) 1780, 1720, and 1550 cm^{-1}; nmr (CDCl$_3$) δ 8.1 (2 H, s), 5.1 (2 H, s), 3.6 (3 H, s), 3.2 (3 H, s).

Anal. Calcd for C$_{11}$H$_{10}$N$_2$O$_5$: C, 52.80; H, 4.03. Found: C, 53.03; H, 3.98.

3-Amino-6-(methoxymethyl)-N-methylphthalimide. A mixture of 3-(methoxymethyl)-6-nitro-N-methylphthalimide (250 mg, 1.0 mol), 5% Rh/C (25 mg), and glyme (25 ml) was stirred under hydrogen (1 atm) for 40 hr. The mixture was filtered through Celite, and the solvent was removed under vacuum. The resulting solid was purified by preparative tlc (silica gel developed three times with chloroform). The large yellow, mobile band was cut out and extracted with ether. Removal of the solvent under vacuum gave a bright yellow solid (200 mg, 0.9 mmol, 90%): mp 126–127°; ir (KBr) 3450, 3330, 1750, and 1680 cm^{-1}; nmr (CDCl$_3$) δ 7.5 (1 H, d), 6.9 (1 H, d), 5.4 (2 H, broad s), 4.8 (2 H, s), 3.45 (3 H, s), 3.1 (3 H, s).

Anal. Calcd for C$_{11}$H$_{12}$N$_2$O$_3$: C, 59.99; H, 5.49. Found: C, 59.75; H, 5.24.

5-Amino-8-(methoxymethyl)-2,3-dihydrophthalazine-1,4-dione (Vd). 3-Amino-6-(methoxymethyl)-N-methylphthalimide (80 mg, 0.36 mmol) was refluxed under nitrogen with 95% hydrazine (3 ml) for 4 hr. Water (10 ml) was added, and the mixture was cooled on ice and acidified with glacial acetic acid. The precipitate was collected, reprecipitated from cold 10% ammonium hydroxide with acetic acid, and dried under vacuum (0.1 mm, 80°, 5 hr) to give a white solid (60 mg, 0.27 mmol, 75%): ir (KBr) 1680, 1650, and 1600 cm^{-1}.

Anal. Calcd for C$_{10}$H$_{11}$N$_3$O$_3$: C, 54.30; H, 5.01; N, 18.99. Found: C, 54.39; H, 5.01; N, 19.09.

3-Nitro-5-*tert*-butyl-*o*-xylene. To 4-*tert*-butyl-*o*-xylene[21] (7 g, 0.04 mol), cooled to −12° and stirred, was added a mixture of concentrated sulfuric acid (10 g) and 70% nitric acid (5 g, 0.052 mol

of HNO$_3$) dropwise over 30 min. The mixture was stirred for another 30 min before cold water (20 ml) and ether (30 ml) were added. The ether layer was separated, washed with 10% NaOH until the wash was basic, and washed with water. After drying, the mixture was distilled under aspirator vacuum and the 180–185° fraction was saved. During the distillation, decomposition took place and the product was impure (by nmr). The product was redistilled, as before, giving a yellow liquid (5 g, 0.024 mol, 60%): nmr (CCl$_4$) δ 7.6 (1 H, d, J = 2 Hz), 7.4 (1 H, d, J = 2 Hz), 2.4 (3 H, s), 2.3 (3 H, s), 1.3 (9 H, s).

5-*tert*-Butyl-3-nitrophthalic Anhydride. 3-Nitro-5-*tert*-butyl-*o*-xylene (700 mg, 3.4 mmol) was heated with 6 ml of nitric acid (specific gravity 1.15) at 180–185° for 5 hr in a sealed thick walled glass tube. After cooling, the solid formed was filtered off and sublimed (0.01 mm, 250°). Crystallization from ethyl acetate–pentane gave white needles (200 mg, 0.8 mmol, 23%): mp 157–158° (lit.[11] 147–149°); ir (KBr) 1860, 1780, and 1540 cm^{-1}; nmr (CDCl$_3$) δ 7.3 (1 H, d, J = 2 Hz), 7.5 (1 H, d, J = 2 Hz), 1.5 (9 H, s).

3-Amino-5-*tert*-butyl-N-methylphthalimide. 5-*tert*-Butyl-3-nitrophthalic anhydride (0.2 g, 0.8 mmol) was heated at 160–165° with 1,3-dimethylurea (0.1 g, 1.1 mmol) for 1 hr. Then an addition portion of the urea (0.05 g) was added and the heating continued for 15 min more. The resulting material was dissolved in a mixture of ether and water. The ether layer was separated and dried, and the ether was removed under vacuum. The resulting solid was dissolved in ethanol (20 ml) and stirred under hydrogen (1 atm) with 10% Pd/C (0.02 g) for 5 hr. The solvent was removed under vacuum. The resulting yellow solid was chromatographed on alumina with 30% chloroform in benzene. A small yellow band was eluted first followed by a large bright yellow band of the desired material. The solvent was removed under vacuum from this fraction to give an oil which crystallized to a yellow solid (0.12 g, 0.51 mmol, 63%): mp 147–149°; ir (KBr) 3460, 3340, 1750, and 1700 cm^{-1}; nmr (CDCl$_3$) δ 7.2 (1 H, d, J = 1 Hz), 6.8 (1 H, d, J = 1 Hz), 5.2 (2 H, broad s), 3.1 (3 H, s), 1.2 (9 H, s).

Anal. Calcd for C$_{13}$H$_{16}$N$_2$O$_2$: C, 67.22; H, 6.94. Found: C, 67.27; H, 6.93.

5-Amino-7-*tert*-butyl-2,3-dihydrophthalazine-1,4-dione (Ve). 3-Amino-5-*tert*-butyl-N-methylphthalimide (75 mg, 0.32 mmol) was refluxed with 95% hydrazine (1.5 ml) for 4 hr under nitrogen. Water (10 ml) was added to the reaction mixture, and the resulting solution was acidified with acetic acid. The precipitate was collected by vacuum filtration, dissolved in 10% ammonium hydroxide, and reprecipitated by acidification with acetic acid. The solid was collected and sublimed (0.01 mm, 260–270°) to give a white solid (41 mg, 0.17 mmol, 55%): ir (KBr) 3470, 3340, 1650, and 1590 cm^{-1}; uv (0.1 M K$_2$CO$_3$) 305 nm (log ϵ 3.79) and 350 (3.91).

Anal. Calcd for C$_{12}$H$_{15}$N$_3$O$_2$: C, 61.79; H, 6.48; N, 18.01. Found: C, 62.08; H, 6.34; N, 18.25.

4-Amino-N, 5-dimethylphthalimide. 4-Nitro-N, 5-dimethylphthalimide[22] (1 g, 0.45 mmol), ethanol (100 ml), and 10% Pd/C (0.1 g) were stirred together under hydrogen (1 atm) for 4 hr. During this time, 320 ml (1.4 mmol, 105%) of hydrogen was consumed. The reaction mixture was heated to dissolve the product and was filtered through Celite to remove the catalyst. The solvent was removed under vacuum, and the resulting solid was crystallized from ethanol to give light yellow needles (0.67 g, 0.35 mmol, 77%): mp 210–211°; ir (KBr) 3400, 3340, 1760, 1720 cm^{-1}; nmr (DMSO-d_6) δ 7.4 (1 H, s), 7.0 (1 H, s), 6.0 (2 H, broad s), 3.0 (3 H, s), 2.2 (3 H, s).

Anal. Calcd for C$_{10}$H$_{10}$N$_2$O$_2$: C, 63.15; H, 5.30. Found: C, 63.12; H, 5.20.

6-Amino-7-methyl-2,3-dihydrophthalazine-1,4-dione (VIb). 4-Amino-N, 5-dimethylphthalimide (200 mg, 1.05 mmol) was refluxed with 95% hydrazine (2 ml) for 4.5 hr under nitrogen. Water (10 ml) was added to the reaction mixture, and the resulting solution was acidified with acetic acid. The precipitate was collected by vacuum filtration, dissolved in 10% ammonium hydroxide, and reprecipitated with acetic acid. The solid was collected and sublimed (0.01 mm, 260–270°) to give a white solid (140 mg, 0.73 mmol, 70%): ir (KBr) 3460, 3360, and 1630 cm^{-1}; uv (0.1 M K$_2$CO$_3$) 270 nm (log ϵ 4.29) and 310 (3.73).

Anal. Calcd for C$_9$H$_9$N$_3$O$_2$: C, 56.54; H, 4.74; N, 21.98. Found: C, 55.85; H, 4.70; N, 21.82.

4-Amino-N,3-dimethylphthalimide. The carbon tetrachloride mother liquors from the preparation of N,3-dimethyl-6-nitrophthalimide[10] were combined and evaporated to dryness. A portion of the solid obtained (1 g) was stirred overnight under hydrogen (1 atm) with 10% Pd/C (0.1 g) in ethanol (100 ml). The mixture was boiled and filtered hot through Celite. The Celite was washed with hot ethanol, and all the hot filtrates were combined and allowed to cool. The resulting crystals were recrystallized from ethanol and dried under vacuum to give a yellow powder (0.20 g): mp >260°; ir (KBr) 3460, 3370, 1750, and 1700 cm^{-1}; nmr (DMSO-d_6) δ 7.2 (1 H, d, J = 8 Hz), 6.8 (1 H, d, J = 8 Hz), 6.1 (2 H, s), 2.9 (3 H, s), 2.4 (3.0 H, s); uv (95% ethanol) 380 nm (log ε 3.70) and 305 (3.49).

Anal. Calcd for $C_{10}H_{10}N_2O_3$: C, 63.15; H, 5.30. Found: C, 63.21; H, 5.40.

6-Amino-5-methyl-2,3-dihydrophthalazine-1,4-dione (VIc). 4-Amino-N,3-dimethylphthalimide (75 mg, 0.39 mmol) was refluxed in 95% hydrazine (2 ml) for 5 hr under nitrogen. Water (15 ml) was added to the reaction mixture, and then the product was precipitated by acidification with glacial acetic acid. The solid was crystallized from 15% hydrobromic acid. The resulting hydrobromide was stirred with a solution of sodium bicarbonate (25 mg) in water for 30 min. The solid was collected and sublimed (0.01 mm, 250–260°) to yield a white solid (24 mg, 0.13 mmol, 32%): ir (KBr) 3480, 3390, 3360, 1630, and 1600 cm^{-1}; uv (0.1 M K_2CO_3) 265 nm (log ε 4.08) and 340 (3.74).

Anal. Calcd for $C_9H_9N_3O_2$: C, 56.54; H, 4.74. Found: C, 56.44; H. 4.72.

3-Amino-6-isopropylphthalic Anhydride. 3-Isopropyl-6-nitro-N-methylphthalamide (100 mg, 0.4 mmol) was refluxed for 3 weeks in an ethanol (2 ml)–hydrochloric acid (2 ml) mixture. The resulting solution was extracted with ether, dried, and evaporated to dryness. The residue was sublimed (130°, 0.01 mm) and crystallized from carbon tetrachloride to give white crystals (70 mg, 0.3 mmol, 75%): mp 125–126°; (KBr) 1860, 1780, and 1540 cm^{-1}. This 3-isopropyl-6-nitrophthalic anhydride was stirred with Drierite (1 g) and 10% Pd/C (20 mg) in tetrahydrofuran (50 ml) under hydrogen (1 atm) for 3 hr. The solution was filtered and evaporated to a bright yellow crystalline solid (60 mg, 0.3 mmol, 100%): mp 132–135°; ir (KBr) 3500, 3380, 1830, and 1760 cm^{-1}; nmr (CDCl$_3$) δ 7.4 (1 H, d, J = 8 Hz), 6.9 (1 H, d, J = 8 Hz), 5.2 (2 H, broad s), 3.8 (1 H, heptet, J = 7 Hz), 1.2 (6 H, d, J = 7 Hz); uv (0.1 M K_2CO_3) λ_{max} 304 nm (log ε 3.26).[23] Dissolving this anhydride in aqueous base gives the phthalate, X, which is the emitter in Vc chemiluminescence.

Chemiluminescence Emission Spectra. Emission spectra were measured on a Hitachi-Perkin-Elmer MPF-2A spectrophotofluorimeter with a stabilized xenon arc source and R 106 photomultiplier detector and were not corrected for nonlinearity of the source or detector. Chemiluminescence spectra were determined by treating solutions of hydrazide ($\sim 10^4$ 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. By comparison with fluorescence spectra of the phthalates (where available) or spent reaction mixtures, this shift is found to be usually 2–3 nm.

Fluorescence Quantum Yields. The fluorescence yields were measured relative to quinine bisulfate in 0.1 N sulfuric acid.[24] 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.[25]

Chemiluminescence Quantum Yields. The chemiluminescence yields were determined relative to the luminol standard of Seliger[26] using a RCA 1P21 photomultiplier powered by a Fluke 4128 DC power supply. No correction was made for photomultiplier sensitivity as this is at most a few per cent in this wavelength range.[27] 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 ($\sim 10^{-5}$ 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 $\sim 3 \times 10^{-6}$ M hemin solution. Additional peroxide and hemin were injected to ensure that the first injection caused complete reaction (usually the case).

References and Notes

(1) These compounds are correctly named as 2,3-dihydrophthalazine-1,4-diones. For simplicity In this paper, except in the experimental section, they will be named as derivatives of luminol (I) and isoluminol (VIa) with the numbering of phthalic acid.

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(12) E. J. Bowen, *Trans. Faraday Soc.*, **56**, 97 (1954).

(13) Since the 6-alkyl luminols (Va–d) all emit at about the same wavelength (~ 405 nm), we assume that they all have about the same value of Φ_{es} as do Va and Vc, the variations In Φ_{ch} being due to variations in Φ_{fl} of the emitters.

(14) The relationship between Φ_{es} and λ has been extended to longer wavelengths by the addition of the pair 7-(dimethylamino)naphthalene-1,2-dicarboxylic acid hydrazide (Φ_{ch} = 0.014, Φ_{es} = 0.054) and 7-(dimethylamino) naphthalene-1,2-dicarboxylate (Φ_{fl} = 0.26, λ_{max} 515 nm, ν_{min} = 23,300 cm^{-1}). We thank Dr. K. G. Gundermann[6c] for generous samples of the hydrazide and of dimethyl 7-aminonaphthalene-1,2-dicarboxylate from which the desired carboxylate was prepared. Methylation with methyl iodide and separation by preparative tlc gave dimethyl 7-dimethylaminonaphthalene-1,2-dicarboxylate in 12% yield: mp 130–131°; ir (KBr) 1740 and 1725 cm^{-1}. *Anal.* Calcd for $C_{16}H_{17}NO_4$: C. 66.89; H, 5.96. Found: C, 66.64; H. 6.18. Hydrolysis of this ester with 1 M hydrochloric acid gave the desired dicarboxylic acid.

(15) P. D. Wildes and E. H. White, *J. Amer. Chem. Soc.*, **95**, 2610 (1973).

(16) An example of this effect in the luminol series has been provided by Gundermann and Drawert,[6b] who found that the substitution of the amino hydrogens of I by methyl groups led to a 100-fold decrease in light emission.

(17) H. Gilman, Ed., "Organic Chemistry," Vol. I, 2nd ed, Wiley, New York, N.Y., 1938, 1943, p. 368.

(18) The effect of the 3- (or 6-) methyl group on Φ_{ch} was first noted by us in the case of 3-methylphthalic hydrazide in the aprotic system. The Φ_{ch} of 3-methylphthalic hydrazide is about ten times that of phthalic hydrazide or 4-methylphthalic hydrazide. In the presence of dibromoanthracene its Φ_{ch} is five times that of phthalic hydrazide. In these reactions energy transfer is necessary for light emission.[9]

(19) Melting points are 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 internal tetramethylsilane. Ultraviolet spectra were taken on a Cary 14 instrument. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Solvents and reagents, unless otherwise stated, were reagent grade and used as received.

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(23) 3-Aminophthalic acid gives 304 nm (3.35): J. Lee and H. H. Seliger, *Photochem. Photobiol.*, **11**, 247 (1970). 3-Amino-6-methylphthalic acid gives 306 nm (3.36).[10]

(24) See ref. d, Table I.

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(26) See ref b, Table I.

(27) Tube specifications from Photovolt Corp., New York, N.Y.