Tetrahedron. Vol. 28, pp. 481 to 492. Pergamon Press 1972. Printed in Great Britain

ENERGY TRANSFER INVOLVING DERIVATIVES OF LUMINOL

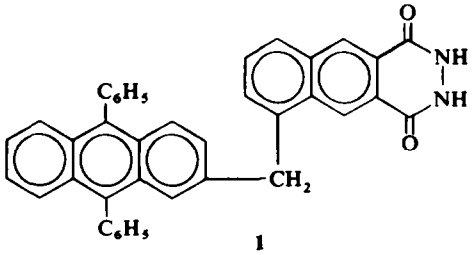
M. A. RIBI, C. C. WEI and E. H. WHITE

Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland 21218

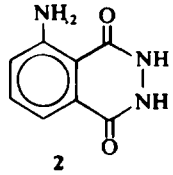
(*Received in the USA* 2 *September* 1971; *Received in the UK for publication* 18 *October* 1971)

Abstract—Two chemiluminescent energy transfer compounds were synthesized (3 and 4), in which benzacridone was a common acceptor portion and in which phthalic hydrazide and aminophthalic hydrazide, respectively, served as energy generators. A cleavage reaction during the chemiluminescence of 4 defeated attempts to compare the two energy generators quantitatively. Compound 3 was about 2% efficient in chemiluminescence in the DMSO system.

CHEMILUMINESCENCE may be defined as the emission of light by chemically produced excited states. We found it possible recently to separate the energy producing function in this process from that of light emission through the use of bifunctional molecules X–Y.[1] Hydrazides were used as energy generators (X) and highly fluorescent molecules such as 9,10-diphenylanthracene and acridone were used as light emitters (Y) (as in structure **1**).[1] Central problems in this area are: (1) the low efficiency of energy generation in most cases, and (2) the measurement of that efficiency. Since the amino

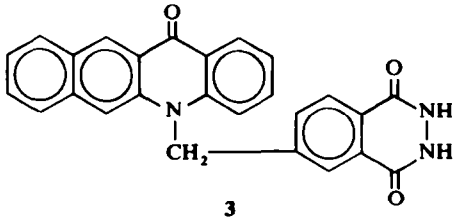


1

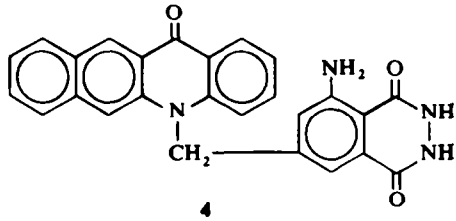


2

group is known to increase the efficiency of light emission in hydrazide chemiluminescence (compound **2** = luminol),[2] it was decided to attack both problems *via* a comparison of compounds **3** and **4**. Assuming for the moment that all the light emitted will stem from the 2,3-benzacridone portion of the molecules and that the transfer efficiencies will be the same, the ratio of light emission should give us directly



3



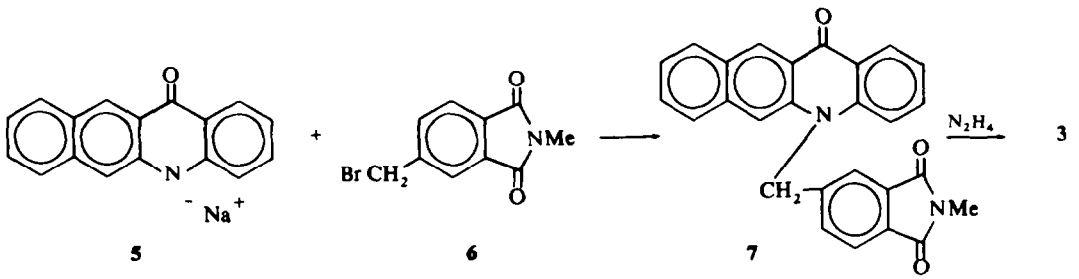
4

the ratio of excited state production. The synthesis and testing of compounds **3** and **4** is the subject of this article.

Synthesis

The synthesis of **3** was patterned after that of a lower homolog[1b] and it proceeded without incident (Chart 1). The synthesis of **4**, on the other hand, encountered several

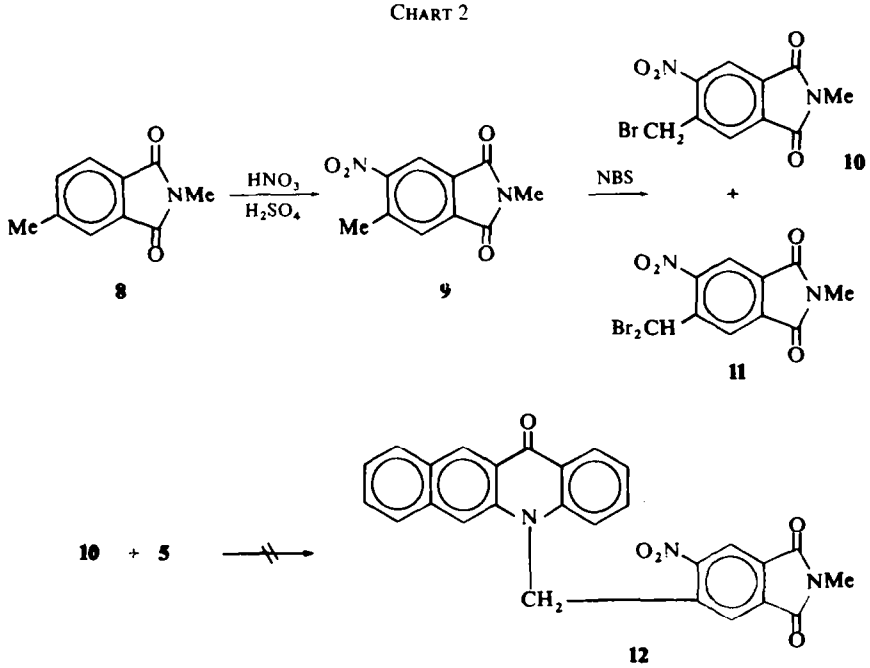
CHART 1



5 6 7

difficulties. In our first approach, the nitrogen function was introduced by nitration of N,4-dimethylphthalimide (Chart 2). An unwanted isomer, 5-nitro-N-4-dimethylphthalimide (**9**), was the chief product of the nitration, however. The structure was assigned largely on the basis of the NMR spectrum which showed that the two aromatic hydrogens were *para* to one another; the signals were quartets (J = 0·6 and 0·4 Hz) because of coupling with the Me group; no significant coupling between the

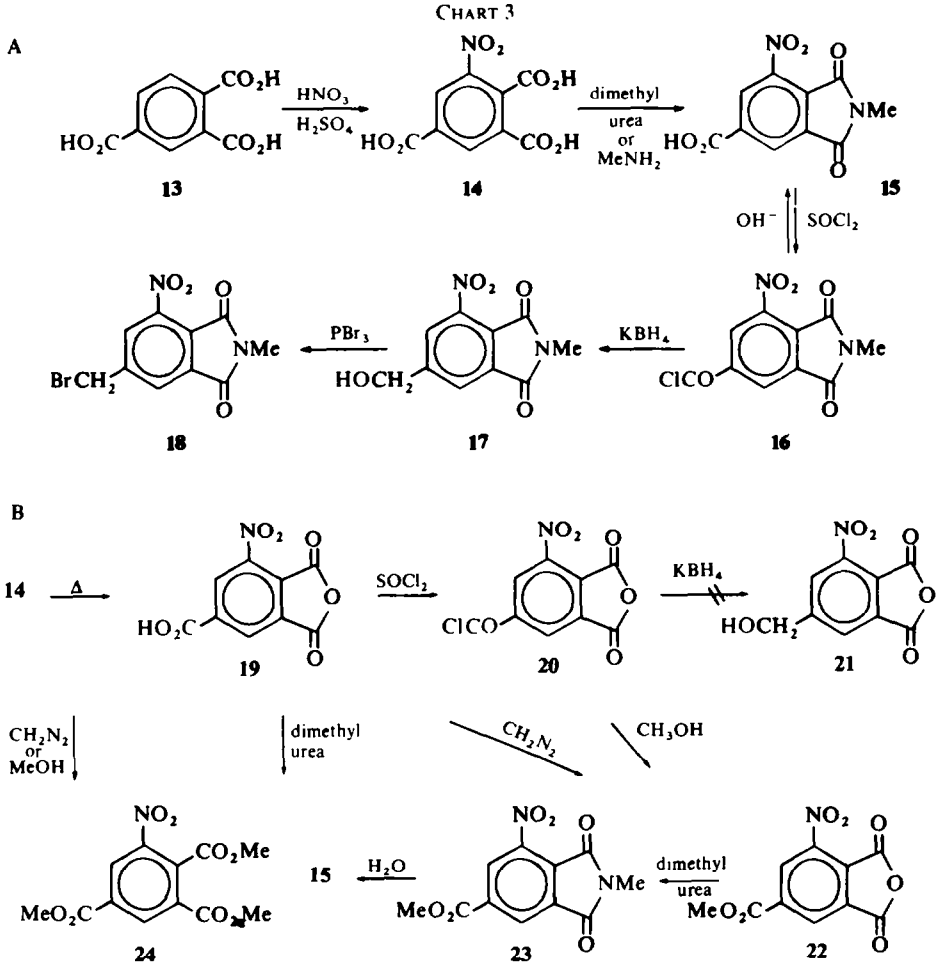
CHART 2



8 9 10 11

aromatic hydrogens was revealed by double resonance experiments. While an amino function *ortho* to the carbonyl function enhances the chemiluminescence of hydrazides (as in luminol, **2**), an amino function in the corresponding *meta* position is far less effective.[2] In any event, attempts to alkylate **5** with **10** were unsuccessful. Since the

CHART 3

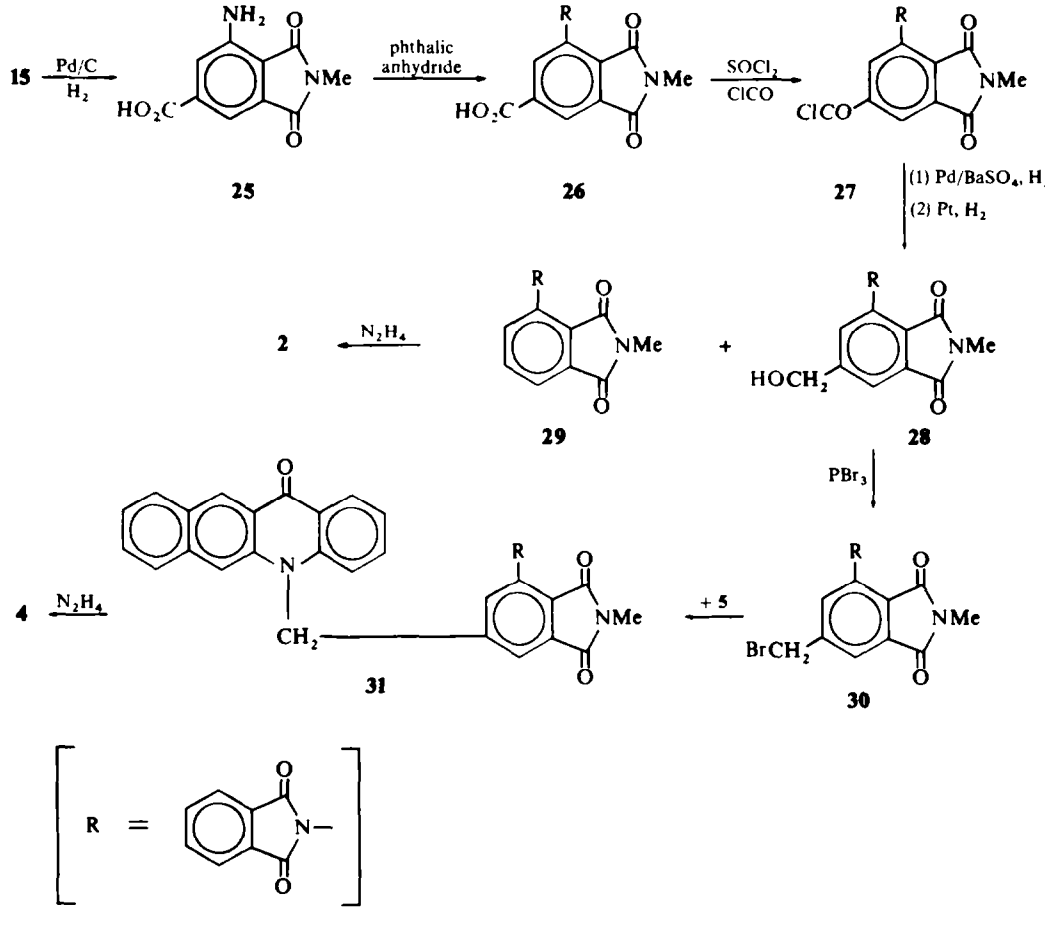


analogous reaction of **5** with **6** was successful (Chart 1), it is felt that the nitro group of **10** facilitates an electron transfer from anion **5** to **10** which competes with the desired displacement reaction.[3] The radical ion so formed probably would eject bromide ion to form a substituted benzyl radical, which in turn could go on to other products.[3]

A successful method for introducing the nitrogen function into the desired C-6 position is given in Chart 3A. Nitration of benzene-1,2,4-tricarboxylic acid (**13**) was

achieved with KNO_3 and H_2SO_4 using essentially the procedure of Prelog and Schneider[4] for the nitration of benzene-1,2,3-tricarboxylic acid. The structures of compound **14** and derivatives **15–23** follow from the analytical and spectral data, especially the NMR spectra which showed an AB quartet (J = 1·4–1·6 Hz) for the aromatic hydrogens of **15–23** characteristic of *meta* orientation.[5] 5-Nitrobenzene-1,2,4-tricarboxylic acid has been reported by Cahn[6] and by Franc,[6] but in view of the broad m.p. of this acid (probably a result of dehydration), it isn't clear whether the lower m.ps given by these authors is significant.

CHART 4

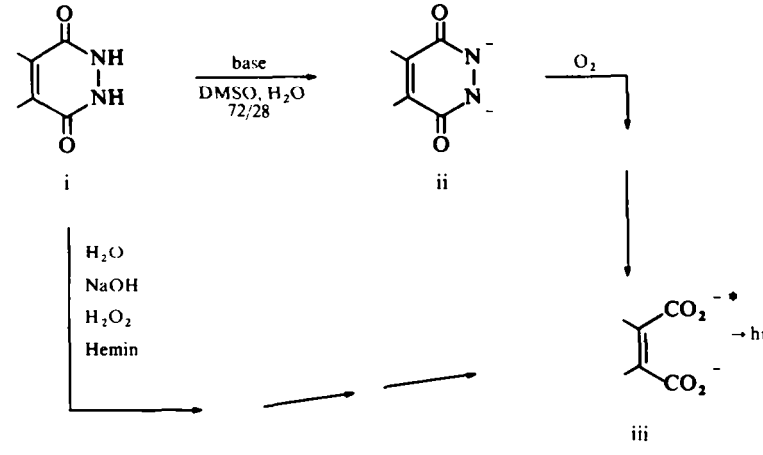


Further conversions of **14** (Chart 3A), led to the bromomethylimide **18**. This compound was also synthesized *via* compound **19**, although less expeditiously, by the reactions outlined in Chart 3B. Unfortunately, the condensation of benzacridone anion (**5**) with imide **18**, as with compound **10**, did not lead to the desired coupled product, presumably for the reason outlined above in the discussion of compound **10**.

It was then decided to reduce the nitro function in order to circumvent radical anion formation. The bromomethyl and amino functions being incompatible, the amino group was protected with the phthaloyl group (Chart 4). The compounds illustrated were readily prepared with the exception of **28**, **31**, and **4**. The reduction of compound **27** with $NaBH_4$ led to low yields of **28** (14%), presumably because ring opening of the imide grouping occurred. Prolonged reduction under conditions of the Rosenmund reaction[7] led to somewhat better yields (37%). Still better yields (50%) were obtained by hydrogenation to the aldehyde ($Pd/BaSO_4$), followed by hydrogenation to the alcohol stage (Pt). In the hydrogenations, considerable decarbonylated material (**29**) was formed, the yield increasing at high temperatures; similar decarbomonylations have been reported to accompany the Rosenmund reduction.[7] The coupling of compounds **30** and **5** proved surprisingly difficult. The best yield obtained, 9%, resulted from the reaction of equimolar amounts of the components in dimethoxyethane. Hydrazide **4** was prepared from **31** using hydrazine as the reactant and solvent; in AcOH, some cleavage to benzacridone occurred (see below).

Results

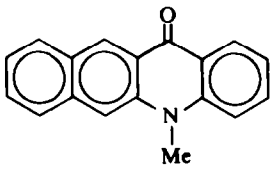
The chemiluminescence of simple hydrazides (i) involves an oxidative reaction that produces an electronically excited state of the corresponding carboxylate (eq. 1).[2] In



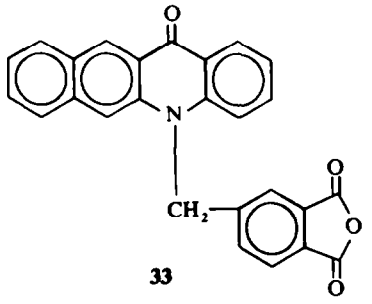
aprotic solvents such as DMSO and in the presence of a strong base, the dianion of the hydrazide (II) is an intermediate and oxygen is the only other requirement for chemiluminescence.[8] In water and other protic media, the requirements for efficient chemiluminescence are base, H_2O_2, and a transition metal such as Fe (used in a chelated form, hemin in the present study); oxidizing agents such as ferricyanide can replace the transition metal.[9]

In view of the first statement in the above paragraph, the spectrum of light emitted in the chemiluminescence of a simple hydrazide should be the same as the fluorescence spectrum of the corresponding carboxylate (iii). For a difunctional hydrazide such as **1**, however, light emission can also stem from the emitter portion of the molecule.[1]

Thus, in the present study, compounds **32** (N-methyl-2,3-benzacridone) and **33** were prepared for use in fluorescence studies. Solutions of carboxylate **34** were prepared by treatment of **33** with base.



32



33

The energy relationships in compounds **3** and **4** are proper in that the chemically produced excited states (carboxylate moieties) are sufficiently energetic to excite the emitter portions of the molecules. Excited phthalate ions are capable of transferring their excitation energy to acridone itself.[1b] Thus, ample energy should be available for the benzacridone moiety in **3** [the longest wavelength absorption band of N-methyl acridone is at 404 nm (log ε 3·98)[10] and that of compound **32** is at 472 nm (log ε 3·77)]. Similarly, since compound **2** emits at 425 nm in the water system and 490 in DMSO, ample energy is available for energy transfer in **4**, at least in the water system.[11]

TABLE 1. EMISSION MAXIMA[a]

Compound	Chemiluminescence maxima (nm)	Fluorescence
3	495,520 (sh)	
Spent reaction mixture		495,520 (sh)
34		495,520 (sh)
32		503,521 (sh)
4	420,505	
Spent reaction mixture		405 and 495 (weak); 585 (strong)
5		587

[a] Uncorrected spectra in H_2O/DMSO (28·4/71·6 mole %), 0·05 M in KOH.

Compound **3** proved to be chemiluminescent in both the DMSO and water systems (eq. 1). The chemiluminescence emission peaked at 495 nm with a shoulder at 520 nm (Table 1). Exactly the same emission was found for the fluorescence of the spent reaction mixture and for the fluorescence of **34**. Since phthalate ion is non-fluorescent,[12] all of the light in this instance comes from the benazcridone portion of the molecule.* The fluorescence of N-methylbenzacridone (**32**) is similar to, but

* Intermolecular energy transfer is not expected to occur under the reaction conditions[1a,b]

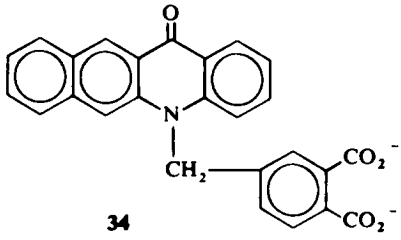
slightly different from the fluorescence of **34** (Table 1); some interaction must be occurring between the two aromatic portions in the excited state of **34**.

TABLE 2. CHEMILUMINESCENCE QUANTUM YIELDS (Einsteins/mole)[a]

Compound	$\Phi_{ch} \times 10^2$	
	H_2O system	DMSO system
3	0·16	2·6
4	0·01	0·006

[a] Uncorrected for phototube response; true values about 25% higher.

The quantum yield of the chemiluminescence of **3** was found to be $1{\cdot}6 \times 10^{-3}$ in the water system and $2{\cdot}6 \times 10^{-2}$ in the DMSO system (Table 2). In comparison, the chemiluminescence quantum yield of luminol (**2**) is $1{\cdot}25 \times 10^{-2}$ (in both systems).[13]



34

Hydrazide **4** was also chemiluminescent in both systems, but the efficiency of light production was quite low (Table 2). The emission characteristics were also anomalous (Table 1), and both results were traced, in part, to a cleavage reaction that yielded the anion of benzacridone (**5**). Benzacridone was also obtained from the chemiluminescence of **3** initiated by t-BuOK in *dry* DMSO, and from the last step in the synthesis of **4** using AcOH as the solvent. These cleavage reactions probably involve an oxidation of the benzylic methylene to a carbonyl group and the saponification of the amide linkage so formed since they did not occur in the absence of oxygen. For the reason cited above, a comparison of the yields of chemically produced excited states generated from compounds **3** and **4** was not possible. Presumably, the use of two or more carbons in the linkage, rather than one carbon as in **3** and **4**, would permit such a comparison to be made.

EXPERIMENTAL

1,2,4-Benzenetricarboxylic acid (Aldrich Chemical Co.), 3-hydroxy-2-naphthoic acid (Aldrich Chemical Co.) 4-methylphthalic acid (K and K, Inc.) were used as received. 4-Bromomethyl-N-methylphthalimide[1b] was obtained as an analytically pure sample from Dr. D. F. Roswell. t-BuOK (MSA Research Corp.) was used as received. DMSO (Matheson Coleman and Bell) was stirred overnight over crushed KOH, decanted, and distilled from t-BuOK. The distillations were performed with grease-free systems under oil pump vacuum (< 1 torr) and at temperatures from 30 to 60°. A center cut of about 80% of the material was used. The NMR chemical shifts are relative to TMS.

2,3-*Benzacridone* (**5**). The title compound was synthesized after the method of Albert *et al.*[14] The crude material was crystallized from DMF-EtOH, followed by sublimation at 250 ~ 270° (5×10^{-2} mm) to give pure 2,3-benzacridone (57·5%), m.p. 304–305° (lit[14] 303). The IR bands were in agreement with those reported.[18]

N-*Methyl*-4-[(12-*oxo*-5-*benz*[b]*acridanyl*)*methyl*]*phthalimide* (**7**). 2,3-Benzacridone (490 mg, 2 mmol) was dissolved in dry 1,2-dimethoxyethane (100 ml) and 57% NaH (93 mg, 2·2 mmol) was added. After the mixture had been stirred and heated at 80° for 30 min, N-methyl-4-bromomethylphthalimide[2, 1b] (508 mg, 2 mmol) was added at room temp, and the solution was stirred at room temp for 20 hr. The mixture was then stirred at 40–50° for 6 hr. During this time, NaH (0·47 mmol) followed by N-methyl-4-bromomethyl phthalimide (90 mg, 0·35 mmol) were added twice at intervals of 3 hr. The solvent was evaporated and water added. The brown residue was filtered and purified by dry column chromatography (250 g, alumina, neutral Woelm activity III, $CHCl_3$ as solvent) to give N-methyl-4-[(12-oxo-5-benz[b]acridanyl)methyl]-phthalimide, (323 mg, 38%), m.p. 307–308° after recrystallization from C_6H_6 containing a trace of $CHCl_3$: IR (KBr) 1770, 1710 (imide), 1645, 1620, 1600 (benzacridone); NMR ($CDCl_3$) 3·13 (s, N-Me) 5·72 (s, $-CH_2-$), 8·1–7·1 (m, aromatics), 8·60 (q, J = 2 and 8 Hz, aromatic), 9·14 ppm (s, aromatic).

4-[(12-*oxo*-5-*benz*[b]*acridanyl*)*methyl*]*phthalic hydrazide* (**3**). N-Methyl-4-[(12-oxo-5-benz[b]acridanyl)-methyl]phthalimide (50 mg, 0·120 mmol) was added to glacial AcOH (3 ml) containing hydrazine (95%, 0·5 ml). This mixture, after degassing (three freeze-thaw cycles) was sealed in glass and heated at 120° for 8 days. The mixture was poured into water and the precipitate collected, washed with water and dried under vacuum (60°, 20 mm). The crude sample was purified by crystallization from $AcOH-H_2O$, followed by sublimation at 320 ~ 35° (5×10^{-2} mm) to give the pure product (17 mg, 35%): m.p. 340–345°, (dec): UV (95% EtOH) λ_{max} (log ε) 463 (3·77), 443 (3·77), 328 sh (3·95), 316 sh (4·01), 295 sh (4·45), 272 (4·96), 240 (4·48); IR (KBr) 3115 (N—H), 3030, 1645, 1620, 1595 (Benzacridone). Further bands at 1515, 1490, 1480, 1455, 1385, 1350, 1310, 1295, 1220, 1190, 1145, 1120, 1090, 1065, 1018, 1010, 946, 920, 905, 870, 845, 793, 740, 732 cm^{-1}; R_f 0·7 (cellulose, EtOH : H_2O : NH_3 = 8 : 1 : 1). (Found: C, 74·34; H, 4·04; N, 9·93. $C_{26}H_{17}N_3O_3$ requires: C, 74·45; H, 4·09; N, 10·02%).

N,4-*Dimethylphthalimide* (**8**). The title compound was synthesized after the method of Roswell[15] by heating 4-methylphthalic anhydride with 1,3-dimethyl urea.

N,4-*Dimethyl*-5-*nitrophthalimide* (**9**). A procedure similar to that reported for 3-nitrophthalic acid[16] was used. N,4-Dimethylphthalimide (12·31 g, 0·071 mol) was slowly dissolved in HNO_3 (d = 1·42, 24 ml) and then H_2SO_4 (d = 1·84, 24 ml) added with stirring in portions of 2 ml at room temp. The resulting mixture, after being heated on the steam bath for 2 hr, was poured into ice-cold water. The resulting mixture was filtered and the residue washed with water. Crystallization of the crude product from C_6H_6-cyclohexane gave the pure N,4-dimethyl-5-nitrophthalimide (8·8 g, 0·040 mol, 57%): m.p. 165–166°; IR (KBr) 1770, 1710 (imide), 1540, 1365 cm^{-1} ($-NO_2$); NMR ($CDCl_3$) 2·71 (s, C-4 Me), 3·19 (s, N-Me), 7·82 (q, J = 0·6 Hz, aromatic), 8·26 ppm (q, J = 0·4 Hz, aromatic); the latter two signals were singlets at 60 MHz. (Found: C, 54·30; H, 3·69; N, 12·58. $C_{10}H_8N_2O_4$ requires: C, 54·55; H, 3·66; N, 12·72).

The mother liquid of the crystallization yielded a solid on evaporation (2·55 g, 0·012 mol, 16·5%) which appeared to be a mixture of N,4-dimethyl-5-nitrophthalimide and N,4-dimethyl-3-nitrophthalimide.

N-*Methyl*-4-*bromomethyl*-5-*nitrophthalimide* (**10**) *and* N-*Methyl*-4-*dibromomethyl*-5-*nitrophthalimide* (**11**). N,4-Dimethyl-5-nitrophthalimide (1·0 g, 4·55 mmol), freshly recrystallized bromosuccinimide[17] (2·43 g, 13·8 mmol) and a trace of dibenzoylperoxide (20 mg) were stirred and heated in boiling CCl_4 for 4 days. During this time, light from a sun lamp (275 W) was directed on the flask twice over a period of 40 min. The solvent was evaporated, and the residue dissolved in C_6H_6. The C_6H_6 solution was washed with water and dried over $MgSO_4$. The crude material was purified by prep TLC (silica gel PF_{254}, Merck, first development with pentane:EtOAc = 8·5:1·5, second and third developments with pentane:EtOAc = 7:3) to give: (A) N-methyl-4-dibromomethyl-5-nitrophthalimide (**11**) (90 mg of needles, 5%), m.p. 105–106°, after one recrystallization from $CHCl_3$–cyclohexane and one from ether. The analytical sample was sublimed at 120–135° (10^{-2} mm) followed by trituration with ether to induce crystallization. (Found: C, 32·22; H, 1·75; Br, 42·25. $C_{10}H_6Br_2N_2O_4$ requires: C, 31·78; H, 1·60, Br, 42·28). IR (KBr) 1785, 1720, 1545, and 1355 cm^{-1}; NMR ($CDCl_3$) 3·29 (s, N-Me), 7·40 (s, $CHBr_2$), 8·33 (s, aromatic), 8·75 (s, aromatic); and (B) N-methyl-4-bromomethyl-5-nitrophthalimide (**10**) 280 mg, 20·5%), m.p. 146–8°, after recrystallization from $CHCl_3$-cyclohexane and sublimation at 80–115° and 5×10^{-2} mm Hg: IR (KBr) 1775, 1710 (imide), 1545, 1355 cm^{-1} (C—NO_2); NMR ($CDCl_3$) 3·28 (s, N—Me), 4·90 (s, C-4 bromomethyl), 8·14 (s, aromatic) 8·42 ppm (s, aromatic). (Found: C, 40·32; H, 2·27; Br, 26·77. $C_{10}H_7BrN_2O_4$ requires: C, 40·16; H, 2·36; Br, 26·72%).

6-Nitro-1,2,4-benzenetricarboxylic acid (**14**) *and ester* 24[4,7] 1,2,4-Benzenetricarboxylic acid (60 g, 0·288 mol) was dissolved in H_2SO_4 (97·4%, 450 ml). KNO_3 (156 g, 1·33 mol) was added in portions with vigorous shaking over a period of 2 hr at 65–95°. The mixture was heated at 120° for 20 hr. Another portion of KNO_3 (60 g, 0·59 mol) was added and heated for 6 hr. After a final portion of KNO_3 (60 g, 0·59 mmol) had been added, the mixture was heated for 16 hours, and then poured on ice, and extracted with ether (2 l). After evaporation of solvent, the residue was crystallized from C_6H_6 to give 6-nitro-1,2,4-benzenetricarboxylic acid (34·4 g, 47%); m.p. 255–65°; IR (KBr) 3400–2250, 1710 (—COOH), 1550, 1350 cm^{-1} (C–NO_2). The trimethyl ester (**24**) of the acid was obtained by treating the acid in ether with CH_2N_2; m.p. 104–105° after recrystallized from ether: NMR ($CDCl_3$) 3·98 (s, OMe), 4·03 (s, two —OMe), 8·90 (d, J = 1·6 Hz aromatic), and 8·96 (d, J = 1·6 Hz, aromatic). (Found: C, 48·61; H, 3·86; N, 4·68. $C_{12}H_{11}NO_8$ requires: C, 48·49; N, 3·73; N, 4·71%).

4-Carboxy-6-nitro-N-methylphthalimide (**15**). 6-Nitro-1,2,4-benzenetricarboxylic acid (16·0 g, 62·5 mmol) and 1,3-dimethyl urea (11·0 g, 125 mmol) were melted for 30 min on an oil bath at 150–160° with stirring. The mixture was cooled to room temp and dissolved in a mixture of EtOAc and H_2O. The EtOAc extract was washed with H_2O and extracted with sat. $NaHCO_3$ aq. Upon acidification of the extract with concentrated HCl, a yellow precipitate formed and was filtered off. The crude sample was crystallized from EtOH–H_2O to give 4-carboxy-6-nitro-N-methylphthalimide (6·046 g, 24·6 mmol, 39·5%): m.p. 221–223°; IR (KBr) 3500–2500 (COOH), 1780, 1770 (imide), 1725 (C=O), 1550, 1350 cm^{-1} (C—NO_2). A side product (0·95 g, 3·78 mmol, 6%), m.p. 106–9° was also obtained. The title compound was also synthesized by heating 4-carboxyl-6-nitrophthalic anhydride (50 mg, 0·12 mmol) with 1,3-dimethyl urea (37 mg, 0·42 mmol) at 150 to 165° for 10 min. With a similar work-up procedure, the desired imide (26 mg, 57%) was obtained. Compound **15** was also obtained (in 61% yield) by the hydrolysis of ester **23** with 1 N HCl in 50% aqueous dioxane at 98% for 8 hr.

4-Chloroformyl-6-nitro-N-methylphthalimide (**16**). 4-Carboxy-6-nitro-N-methylphthalimide (0·90 g, 3·62 mmol) was dissolved in $SOCl_2$ (15 ml) and heated for 5 hr under reflux at 90–100°. Excess $SOCl_2$ was evaporated and the crude material sublimed (90–125°, 5 × 10^{-2} mmHg) to give 4-chloroformyl-6-nitro-N-methylphthalimide (0·811 g, 85%): m.p. 142–4°; IR (KBr) 1785, 1715 (imide), 1755 (—COCl), 1550, 1365 cm^{-1} (C—NO_2). (Found: C, 44·57; H, 1·90; Cl, 13·36. $C_{10}H_5ClN_2O_5$ requires: C, 44·72; H, 1·87; Cl, 13·20%).

4-Hydroxymethyl-6-nitro-N-methylphthalimide (**17**). KBH_4 (108 mg, 2 mmol) was suspended in 1,2-dimethoxyethane (10 ml, freshly distilled over NaH). 4-Chloroformyl-6-nitro-N-methylphthalimide (537 mg, 2 mmol) dissolved in dry 1,2-dimethoxyethane was added to the reduction medium at 0°. The resulting mixture was stirred at 0° for 1 hr and then at room temp for 1 hr. After reaction, the solvent was evaporated and ice, then 2 N HCl slowly added. The aqueous solution was extracted with EtOAc and after washing with sat $NaHCO_3$, was purified by prep TLC (silica gel PF_{254} Merck, development with EtOAc MeOH = 95:5) to give 4-hydroxymethyl-6-nitro-N-methylphthalimide (97 mg, 20·5%), m.p. 145–147° after sublimation at 100–130° (10^{-1} mmHg): IR (KBr) 3440 (—OH), 1765, 1700 (imide), 1535, 1365 (C—NO_2), 1445 (N—Me): NMR (d_6-acetone) 3·12 (s, N—Me), 3·12 (broad, —OH), 4·93 (s, —CH_2—OH), 8·20–8·08 ppm (m, aromatic). 4-Carboxy-6-nitro-N-methylphthalimide (137 mg, 27%) was also isolated from the mixture.

4-Bromomethyl-6-nitro-N-methylphthalimide (**18**). 4-Hydroxymethyl-6-N-methylphthalimide (77 mg, 0·326 mmol) was dissolved in dry 1,2-dimethoxyethane (5 ml). PBr_3 (40 mg, 0·148 mmol) in 1,2-dimethoxyethane (0·5 ml) was added at 0° and after stirring at 0° for 30 min another portion of PBr_3 (0·148 mmol) was added. The mixture was stirred at 0°, 25°, and 50° for 30 min, 1·5 hr and 20 min, respectively. After reaction, the solvent was evaporated and water added. The organic material was extracted with $CHCl_3$ and purified on prep TLC (silica gel PF_{254} Merck, development with EtOAc pentane = 6:4) to give 4-bromomethyl-6-nitro-N-methylphthalimide (78 mg, 80%): m.p. 154–156°; IR (KBr) 1780, 1715 (imide), 1545 (—C—NO_2), 1450 cm^{-1} (N—Me); NMR (d_6-acetone) 3·15 (s, N—Me), 4·95 (s, Br —CH_2—), 8·27 (d, aromatic), 8·35 ppm (d, aromatic). (Found: C, 40·08; H, 2·38; Br, 26·84. $C_{10}H_7BrN_2O_4$ requires: C, 40·16; H, 2·36; Br, 26·72%).

The reaction of benzacridone anion (**5**) *with* N-*methyl-4-bromomethyl* 5 (*and also* 6)-*nitrophthalimide*. The same conditions used for the synthesis of compound **7** led to a brownish color when the reagents were mixed, and the formation of a brown, insoluble tar. Chromatography of the product on alumina yielded only benzacridone (~ 45%). A second set of conditions (equimolar amounts of benzacridone and compound **10** (or **18**) and an excess of dry K_2CO_3 in refluxing glyme under N_2) led to similar results.

4-Carboxy-6-nitrophthalic anhydride (**19**). Two sublimations of 6-nitro-1,2,4-benzenetricarboxylic acid (51·4 g) resulted in an impure fraction (4·3 g, m.p. 180–230°, sublimation at 180–230°, 10^{-1} mm) and the

pure anhydride (22·15 g, 32·5%, sublimation at 230°–260°, 10^{-1} mm): m.p. 237–239°: IR (KBr) 1875, 1795 (anhydride) and 1715, 2300–3600 cm^{-1} (carboxylic acid); NMR (acetone-d_6) 8·10 (s, —COOH), 8·42 (d, J = 1·4 Hz, aromatic) and 8·54 ppm (d, J = 1·4 Hz, aromatic). (Found: C, 45·61; H, 1·33; N, 5·90. $C_9H_3NO_7$ requires: C, 45·59; H, 1·27; N, 5·91%).

4-*Chloroformyl*-6-*nitrophthalic anhydride* (**20**). $SOCl_2$ (130 ml) was added to well pulverized 4-carboxy-6-nitrophthalic anhydride (21·11 g, 0·089 mol). The resulting mixture was heated at 100° for 24 hr. $SOCl_2$ was evaporated under vacuum and the residue was treated with hot C_6H_6 and filtered. The insoluble residue (4·3 g, 20%) seemed to be the intermolecular anhydride. The filtrate was concentrated and cooled to give the desired acid chloride (12·78 g, 0·05 mmol, 56%). Purification was by sublimation at 80–140° (5×10^{-2} mm) to give the pure compound: m.p. 89–91°; IR (KBr) 1870, 1795 (anhydride), 1765 (acid chloride). (Found: C, 42·49; H, 0·81; Cl, 14·04. $C_9H_2NO_6Cl$ requires: C, 42·30; H, 0·78; Cl, 13·87).

4-*Carbomethoxy*-6-*nitrophthalic anhydride* (**22**). 4-Carboxy-6-nitrophthalic anhydride (4·24 g, 0·018 mol) dissolved in 20 ml of dioxane was treated with CH_2N_2 (50% excess). After evaporation of solvent, an oily product resulted: IR ($CHCl_3$) 1870, 1795 (anhydride), 1740 (ester), 1555 (C—NO_2). The same material was obtained from acid chloride **20** and MeOH in dioxane at 12° for 30 min.

4-*Carbomethoxy*-6-*nitro*-N-*methylphthalimide* (**23**). 4-Carbomethoxy-6-nitrophthalic anhydride (obtained in the above experiment—from 4·24 g, 0·018 mol of the acid anhydride) was mixed with 1,3-dimethyl urea (3·2 g, 0·036 mol) in a test tube. The mixture was heated in an oil bath at 165–180° for 20 min. After cooling, EtOAc was added and the organic solution washed with water, followed by extraction with sat. $NaHCO_3$. Crystallization of the crude material from C_6H_6-cyclohexane gave pure crystals, m.p. 151–154° (2·17 g, 46% based on 4-carboxy-6-nitrophthalic anhydride); IR (KBr) 1780, 1715 (imide), 1735 (ester), 1545 cm^{-1} (C—NO_2); NMR (acetone-d_6), 3·17 (s, N—Me), 4·03 (s, COOMe), 8·53 (d, J = 1·4 Hz aromatic) and 8·66 ppm (s, J = 1·4 Hz, aromatic).

3-*Amino*-5-*carboxy*-N-*methylphthalimide* (**25**). Pd/C (10%, 0·780 g) suspended in MeOH, was saturated with hydrogen by stirring in an hydrogen atmosphere for 1 hr. 4-Carboxy-6-nitro-N-methylphthalimide (7·74 g, 31·0 mmol) dissolved in MeOH (200 ml) was added with stirring at room temp. After 2390 ml (91 mmol) of hydrogen had been consumed, the mixture was filtered and the insoluble product mixed with catalyst was separated by Soxhlet extraction using MeOH as solvent. Evaporation of the filtrate and the extract gave 3-amino-5-carboxy-N-methylphthalimide (5·39 g, 24·5 mmol, 79%); m.p. 295° dec; IR (KBr) 3465, 3355 (N—H_2), 1735, 1680 (imide), 1640 cm^{-1} (COOH): UV (95% EtOH) λ_{max} 398, 260–67 (sh), 235, 213 nm.

3-*Phthalimido*-5-*carboxy*-N-*methylphthalimide* (**26**). 3-Amino-5-carboxylic-N-methylphthalimide (2·60 g, 11·8 mmol) and phthalic anhydride (6·8 g, 48 mmol) were heated in glacial AcOH (40 ml) for 56 hr at 130°. AcOH was evaporated and the crystalline residue fractionally sublimed. The first fraction (100–120°, 5×10^{-2} mmHg) consisted of phthalic anhydride and the yellow colored starting material. The second fraction (210–270°, 5×10^{-2} mmHg) was the desired colorless product containing a small amount of the starting material as impurity. Resublimation of the second fraction gave pure 3-phthalimido-5-carboxy-N-methylphthalimide (2·72 g, 66%): m.p. 275–277°; UV (95% EtOH) λ_{max} 298 nm; IR (KBr) 3290 (—COOH), 1785, 1770, 1740 (sh), 1725 (sh), 1710 (two imides and —COOH); NMR (DMSO-d_6) 3·03 (s, N—Me), 8·05 (m, aromatic), 8·35 (d, J = 1·3, aromatic) 8·46 ppm (d, J = 1·3, aromatic). (Found: C, 61·91; H, 2·82. $C_{18}H_{10}N_2O_6$ requires: C, 61·72; H, 2·88%).

3-*Phthalimido*-5-*chloroformyl*-N-*methylphthalimide* (**27**). 3-Phthalimido-5-carboxy-N-methylphthalimide (6·56 g, 18·75 mmol) was heated in $SOCl_2$ (70 ml) for 5 hr at 110°, after which $SOCl_2$ was evaporated. C_6H_6 was added twice, followed by evaporation to remove all the $SOCl_2$. The crude material was sublimed at 175–185° (5×10^{-2} mm), to give 3-phthalimido-5-chloroformyl-N-methylphthalimide (5·09 g, 73·5%): m.p. 184–187°; IR (KBr) 1790, 1735 (imide), 1780, 1715 (imide), 1755 (—COCl).

3-*Phthalimido*-5-*hydroxymethyl*-N-*methylphthalimide* (**28**). 3-Phthalimido-5-chloroformyl-N-methylphthalimide (4·5 g, 12·1 mmol) was hydrogenated using 5% Pd–$BaSO_4$ (3·6 g) in boiling toluene for 4 hr. The mixture was filtered and evaporated to dryness. This intermediate, dissolved in MeOH, was further hydrogenated on Pt black (0·5 g) under high pressure (50 lb/in^2) for 2 hr. After removing the catalyst by filtration, the mixture was purified through column chromatography (silica gel, eluting with $CHCl_3$) to give the desired alcohol, (2·06 g, 6·13 mmol, 50%) and a decarbonylated compound, **29** (1·10 g, 30%), m.p. 203–206. Recrystallization of the former from EtOAc-cyclohexane gave pure 3-phthalimido-5-hydroxymethyl-N-methylphthalimide: m.p. 213–214°; IR (KBr) 3485 (—OH), 1790, 1775, 1735, 1705 cm^{-1} (two imides); NMR (d_6-acetone) 3·08 (s, N-Me), 4·90 (s, —CH_2— OH), 7·85 (d, J = 1·5 aromatic), 8·02 p.p.m. (m, aromatic). (Found: C, 64·34; H, 3·53. $C_{18}H_{12}N_2O_5$ requires: C, 64·29; H, 3·60).

Treatment of the decarbonylated material with hydrazine in refluxing EtOH for 8 hr followed by chromatography on cellulose (NH_3:H_2O:C_2H_5OH = 1:1:8) yielded luminol (65%), identified by the R_f and the IR spectrum.

3-*Phthalimido*-5-*bromomethyl*-N-*methylphthalimide* (**30**). The title compound was synthesized by the procedure used for 4-bromomethyl-6-nitro-N-methylphthalimide. The crude product was purified on prep TLC (silica gel PF_{254} Merck, development with $CHCl_3$), followed by sublimation (200–225°, 5×10^{-2} mm). Pure 3-phthalimido-5-bromomethyl-N-methylphthalimide (494 mg, 73%), m.p. 209–210°, was obtained from 3-phthalimido-5-hydroxymethyl-N-methylphthalimide (564 mg, 1·68 mmol): IR (KBr) 1785, 1735 (imide), 1770, 1705 cm^{-1} (imide). (Found: Br, 19·99. $C_{18}H_{11}BrN_2O_4$ requires: Br, 20·02%).

N-*Methyl*-3-*phthalimido*-5-[(12-*oxo*-5-*benz*[b]*acridanyl*)*methyl*]*phthalimide* (**31**). 2,3-Benzacridone (185 mg, 0·75 mmol) was dissolved in 30 ml of dry 1,2-dimethoxyethane (freshly distilled from NaH) and 57% NaH (38 mg, 0·8 mmol) was added. The mixture was gently refluxed under dry N_2 for 1 hr N-Methyl-3-phthalimido-5-bromomethylphthalimide (300 mg, 0·75 mmol) in 20 ml of dry 1,2-dimethoxyethane was added at one time. After the mixture had stirred at room temp under N_2 for 4 hr, NaH (7 mg) followed by the bromomethylphthalimide (125 mg, 0·3 mmol) was added and the mixture stirred at room temp for 20 hr. Solvent was evaporated and H_2O, then sat. $NaHCO_3$ was added. The aqueous mixture was extracted with $CHCl_3$. The organic extract was purified by prep. TLC (silica gel PF_{254} Merck, development with $CHCl_3$) to give the desired product (51 mg, 9%), m.p. 343·5–345° (dec) after recrystallized from C_6H_6 with a trace of $CHCl_3$: IR 1790, 1725 (imide), 1780, 1720 (imide), 1650, 1620, 1600 (benzacridone). (Found: C, 74·73; H, 3·75; N, 7·20. $C_{35}H_{21}N_3O_5$ requires: C, 74·59; H, 3·76; N, 7·46%).

3-*Amino*-5-([12-*oxo*-5-*benz*[b]*acridanyl*]*methyl*)*phthalic hydrazide* (**4**). N-Methyl-3-phthalimido-5-([12-oxo-5-benz(b)acridanyl]methyl)phthalimide (40 mg, 0·071 mmol) was dissolved in 95% hydrazine (1 ml). The solution was degassed 3 times and sealed *in vacuo*, and then heated at 125° for 6 hr. The solvent was evaporated and the residue washed with $CHCl_3$, followed by warm-EtOH to give a yellow solid, m.p. 346–348° (dec): UV (95% EtOH) λ_{max} (log ε) 464 (3·79), 443 (3·83), 330 sh (3·83), 294 (4·30), 272 nm (4·84): IR 3420, 3305 (—NH_2), 3155 (hydrazide), 3050, 2920, 1645, 1620 (sh), 1600 (benzacridone characteristics), further bands at 1550 (sh), 1480, 1450, 1380, 1350, 1325, 1290, 1245, 1225, 1190, 1175, 1150, 1120, 1050, 1020, 950, 910, 850, 750 cm^{-1}: R_f, 0·63 (cellulose, NH_3:H_2O:EtOH = 1:1:8). (Found: C, 69·80; H, 4·18. $C_{26}H_{18}N_4O_3$ − 1/2 H_2O requires: C, 70·45; H, 4·32%).

N-*Methyl*-2,3-*benzacridone* (**32**). The method of Acheson and Jefford[18] was followed. The crude product was crystallized from EtOH to give yellow needlelike crystals. Sublimation at 130–170°, (5×10^{-2} mm) gave pure N-methyl-2,3-benzacridone (44·5%), m.p. 212–213 (lit[18] 215°). The UV absorption and IR spectrum were in agreement with the published data.[18]

4-[(12-*oxo*-5-*benz*[b]*acridanyl*)*methyl*]*phthalic Anhydride* (**33**). To N-methyl-4-[(12-oxo-5-benz[b]-acridanyl)methyl]phthalimide (50 mg, 0·120 mmol), MeOH (10 ml), and aqueous NaOH (1 N, 100 ml) were added. The mixture was stirred and refluxed for 4 days. The reaction was followed by TLC (cellulose-EtOH:1 M NH_4AcO aq = 7:3, R_f = 0·50 for the dicarboxylic acid). The mixture was poured into dilute HCl and then heated on a steam bath for 15 min. The precipitate which formed during cooling was filtered, washed with water and dried under vacuum. The crude product was sublimed at 250–290° (5×10^{-2} mm), to give the pure anhydride (26 mg, 53%): m.p. 302–304° (dec); IR (KBr) 1855, 1835, 1770 (anhydride), 1645, 1615, 1600 (benzacridone): UV (95% EtOH) λ_{max} (log ε), 464 (3·69), 444 (3·69), 324–330 sh (3·64), 317 (3·67), 295 (4·28), 273 (4·85). (Found: C, 76·93; H, 3·66. $C_{26}H_{15}NO_4$ requires: C, 77·03; H, 3·73%).

Identification of the products in the reaction of 5-[(12-*oxo*-5-*benz*[(b)]-*acridanyl*)*methyl*]*phthalic hydrazide* (**13**) *with oxygen and potassium hydroxide in* DMSO. A solution of $1·72 \times 10^{-4}$ M hydrazide in DMSO (5 ml) containing 0·5 N KOH (0·5 ml) was bubbled with oxygen for 2 hr. The resulting mixture was acidified with HCl, and the solvent removed. The residue was dissolved in H_2O and extracted with $CHCl_3$. The $CHCl_3$ solution (as shown by TLC and comparison with authentic samples) contained 5[(12-oxo-5-benz[b]-acridanyl)methyl]phthalic acid (>90%), a trace of starting material, and a trace of a compound at R_f 0·85 [cellulose; NH_3:H_2O:EtOH (1:1:8)] which was probably benzacridone.

Emission spectra.[1c] All emission spectra, both fluorescence and chemiluminescence, were measured on a Hitachi–Elmer MPF-2A spectrophotofluorimeter, with a stabilized Xenon arc source and a R106 photomultiplier detector. Spectra are uncorrected for phototube sensitivity, instrumental distortion, or source intensity fluctuation. Wavelengths were determined by superposition of a low-pressure mercury arc spectrum (Pen-Ray lamp) on the recorded spectrum. Reported maxima are estimated to be accurate to within ± 3 nm.

Chemiluminescence spectra were obtained by reacting solutions of the hydrazides ($< 10^{-4}$ M in 0·1 M K_2CO_3) with aqueous solutions of hemin (od = 0·2, at 414 nm) and H_2O_2 ($\sim$0·1%) and running the spectrophotofluorimeter with the source off. For emission spectra in the aprotic system, the hydrazides were dissolved in air saturated DMSO, and KOH (0·5 M) was added to a final concentration of 0·05 M.

Chemiluminescence quantum yields.[1c] Chemiluminescence relative efficiencies were determined using a RCA IP 21 photomultiplier, biased by a Fluke Model 4128 dc power supply. The output of the phototube was amplified with a unit designed and built by Mr. John Veise (Department of Biology, The Johns Hopkins University). The amplifier signal was collected on a capacitor and recorded on a Techni-rite chart recorder. Absolute quantum yields were determined by direct comparison with luminol using the method of Lee and Seliger.[19]

Acknowledgement—We thank the Public Health Service for its financial support (Research Grant No. 5 RO1 NS-07868 from the National Institute of Neurological Diseases and Stroke). We also thank Mr. Joseph Ahnell for the 100 MHz NMR spectra and Mr. William Reichert for valued help in several of the syntheses.

REFERENCES

1 [a] E. H. White and D. F. Roswell, *J. Am. Chem. Soc.* **89**, 3944 (1967);
[b] D. F. Ruswell, V. Paul and E. H. White, *Ibid.* **92**, 4855 (1970);
[c] D. R. Roberts and E. H. White, *Ibid.* **92**, 4861 (1970)
2 E. H. White and D. F. Roswell, *Accts. Chem. Research* **3**, 54 (1970)
3 G. A. Russell and E. G. Janzen, *J. Am. Chem. Soc.* **84**, 4143 (1962);
R. C. Kerber, G. W. Ury and N. Kornblum, *Ibid.* **87**, 4520 (1965)
4 V. Prelog and R. Schneider, *Helv. Chim. Acta* **32**, 1632 (1949)
5 John R. Dyer, *Applications of Absorption Spectroscopy of Organic Compounds*, p 99, Prentice-Hall, Englewood Cliffs, New Jersey (1965)
6 [a] R. S. Cahn, *J. Chem. Soc.* 990 (1930); 1342 (1932);
[b] J. Franc, *Chem. Abs.* **52**, 17254h (1958); *Chem. Listy.* **52**, 1108 (1958)
7 E. Mosettig and R. Mozingo in *Organic Reactions* **IV**, 362 (1948)
8 E. H. White, *J. Chem. Ed.* **34**, 275 (1957)
9 E. H. White in *Life and Light*, p 183, (Edited by W. D. McElroy and B. Glass). Johns Hopkins, Baltimore, Md. (1961)
10 R. D. Brown, F. N. Lahey, *Aust. J. Sci. Res.* A3 (1950)
11 E. H. White, O. Zafiriou, H. H. Kagi and John H. M. Hill, *J. Am. Chem. Soc.* **86**, 940 (1964); E. H. White and M. M. Bursey, *Ibid.* **86**, 941 (1964)
12 E. H. White, D. F. Roswell and O. C. Zafiriou, *J. Org. Chem.* **34**, 2462 (1969)
13 J. Lee and H. H. Seliger, *Photochem. Photobiol.* **4**, 1015 (1965)
14 A. Albert, D. J. Brown and H. Duewell, *J. Chem. Soc.* 1284 (1948)
15 D. Roswell, *Ph.D. Thesis*, p 70. The Johns Hopkins University (1968)
16 E. R. Littman, *J. Am. Chem. Soc.* **47**, 1980 (1925)
17 H. J. Dauben, Jr. and L. L. McCoy, *Ibid.* **81**, 4863 (1959)
18 R. M. Acheson and C. W. Jefford, *J. Chem. Soc.* 2676 (1956)
19 J. Lee, A. S. Wesley, J. F. Ferguson, III, and H. H. Seliger, *Bioluminescence in Progress*, p 35. (Edited by F. H. Johnson and Y. Haneda), Princeton University Press, Princeton, N. J. (1966).