

The Anomalous Chemiluminescence of Phthalic Hydrazide¹

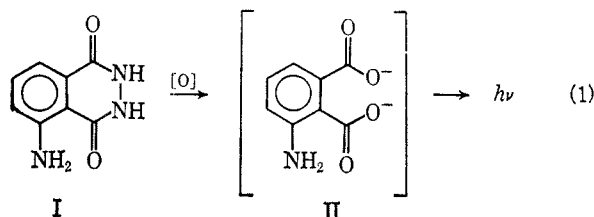
EMIL H. WHITE, DAVID F. ROSWELL, AND OLIVER C. ZAFIRIOU

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

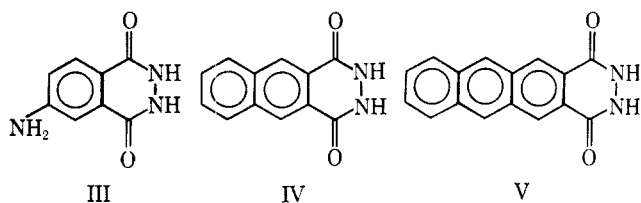
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Phthalic hydrazide (VI), the simplest member of a series of chemiluminescent compounds that includes luminol (I), is chemiluminescent in aprotic solvents. Other members of this series emit *via* fluorescence from the corresponding phthalate ion with concentration-independent quantum yields. Phthalate ion itself is nonfluorescent, however, and the quantum yield of the emission at *ca.* 525 nm from phthalic hydrazide increases with the concentration of VI. The emission spectrum of the chemiluminescence matches the fluorescence of solutions of the monosodium salt of VI in aprotic solvents; these facts and other evidence suggest that the mononegative ion of phthalic hydrazide (VI) is the light emitter in this anomalous case of hydrazide chemiluminescence.

The chemiluminescence of luminol (I) involves the chemical production of excited 3-aminophthalate ion.

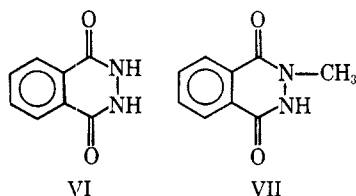


Fluorescence from II* is responsible for the light emission, and the ground-state 3-aminophthalate ion produced is the sole organic product of the reaction.² This reaction pathway—formation of an excited *o*-dicarboxylate ion, followed by fluorescence—also describes the chemiluminescence of other cyclic aromatic hydrazides which have been studied, such as isoluminol (III) and the unsubstituted hydrazides IV and V.³ This scheme



is applicable to hydrazide chemiluminescence carried out in either the "aqueous system" (hydrazide plus aqueous alkaline hydrogen peroxide and a peroxide-decomposing catalyst or oxidizing agent) or the "aprotic system" (hydrazide in an aprotic polar solvent plus oxygen and a strong base).²

The general scheme (eq 1) accounts for the chemiluminescence of all the hydrazides that have been examined with the exception of the "parent" hydrazide, 2,3-dihydrophthalazine-1,4-dione (VI). Although phthal-



(1) Taken in part from the Doctoral Dissertations of O. C. Zafiriou and D. F. Roswell, The Johns Hopkins University.

(2) (a) E. H. White and M. M. Bursley, *J. Amer. Chem. Soc.*, **86**, 941 (1964); (b) E. H. White in "Light and Life," W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1961, p 183; (c) E. H. White, O. C. Zafiriou, H. M. Kagi, and J. H. M. Hill, *J. Amer. Chem. Soc.*, **86**, 940 (1964).

(3) (a) O. C. Zafiriou, unpublished work; (b) K. D. Gundermann and M. Drawert, *Chem. Ber.*, **95**, 2018 (1962).

ate ion appears to be completely nonfluorescent, compound VI chemiluminesces in the aprotic system. Furthermore, the yellow chemiluminescence emission of VI (~ 525 nm) occurs at *longer* wavelengths than the emission from the more highly conjugated analogs IV (deep violet emission at ~ 360 nm) and V (blue emission at ~ 420 nm). The trend established by IV and V leads to the prediction that phthalic hydrazide (VI) should emit at very short wavelengths (~ 300 nm). We have studied the chemiluminescence of compound VI in order to clarify these discrepancies.

Results

Samples of 2,3-dihydrophthalazine-1,4-dione from various sources were assayed for light production; no variation in the chemiluminescence was found. The infrared and ultraviolet spectra of the various samples were identical, and a sample carefully prepared from purified materials did not change in chemiluminescence or spectroscopic properties upon sublimation, crystallization from acetic acid, or crystallization of its monosodium salt from methanol-water.⁴ All purified samples were homogeneous by paper chromatography. We therefore proceeded on the assumption that the chemiluminescence of VI is an intrinsic property, and not the result of impurities.

Preliminary observations revealed several important features of the chemiluminescent reaction. Colorless solutions of VI in dimethyl sulfoxide (DMSO) or hexamethylphosphoramide (HPT) became yellow when potassium *t*-butoxide was added, and these solutions fluoresced yellow. Both the fluorescence and the yellow color faded upon addition of small amounts of water and they returned when large amounts of the aprotic solvent were added. Solutions of the sodium salt of VI in DMSO or HPT are yellow, and they show the same behavior on dilution with water; paper chromatography of these solutions revealed only the presence of phthalic hydrazide. These oxygen-stable solutions of the monosodium salt of VI chemiluminesce yellow when excess potassium *t*-butoxide is added; during the reaction, a fine white precipitate appears (dipotassium phthalate) and the color eventually disappears.

A series of ultraviolet spectra of salts of VI and its N-methylated analog, VII, were taken in DMSO-water mixtures of varying composition. As shown in Table I, the absorption maxima of both compounds shift markedly to longer wavelengths as the proportion of water in

(4) A purification of phthalic hydrazide which removes a chemiluminescent impurity not found in our work has been reported: M. M. Rauhut, A. M. Semsel, and B. G. Roberts, *J. Org. Chem.*, **31**, 2431 (1966).

TABLE I
ABSORPTION SPECTRA OF THE
MONOSODIUM SALTS OF VI AND VII

Solvent ^a	—Absorption maximum ^b —	
	Salt of VI	Salt of VII
0.1 N KOH	310	313
DMSO-H ₂ O (9:1) ^c	342	345
DMSO-0.1 N KOH (9:1) ^c	342	345
DMSO-H ₂ O (100:1) ^c	360	365
DMSO-0.1 N KOH (100:1) ^c	360	365
DMSO ^e	364 ^d	368 ^e
DMSO-KO- <i>t</i> -Bu ^c	<i>f</i>	367

^a Volume/volume. ^b Longest wavelength absorption band.
^c Solutions yellow owing to tailing into the visible region. ^d Log ϵ 3.55. ^e Log ϵ 3.56. ^f Oxidation occurs.

the solvent decreases. The shape and intensity of the long-wavelength band remains nearly constant, and there is no isosbestic point, or appearance of additional peaks.

The fluorescence spectra of the monosodium salt of phthalic hydrazide in HPT (Figure 1) and DMSO are shown in Table II; the excitation spectra are in good agreement with the absorption spectra determined in the same solvent. The chemiluminescence emission spectrum of a reacting solution of phthalic hydrazide in HPT containing *t*-butoxide ion, measured with a spectrophotofluorimeter, is compared in Figure 1 with the fluorescence of solutions of the mono sodium salt of phthalic hydrazide. It can be seen that the fluorescence of the mononegative ion of VI matches the chemiluminescence emission of VI. Relevant data for DMSO and HPT systems and for 4-methylphthalic hydrazide, which also exhibits this same identity of initial fluorescence and chemiluminescence spectral distributions, are presented in Table II.

TABLE II
FLUORESCENCE-CHEMILUMINESCENCE
EMISSION WAVELENGTHS

Compound	Solvent	—Emission maxima ^a —	
		Fluorescence	Chemiluminescence
Phthalic hydrazide (VI) monosodium salt	H ₂ O	467 ^c	<i>c</i>
	DMSO	520	530
	HPT	525	525
N-Methylphthalic hydrazide (VII) monosodium salt	DMSO	515	
4-Methylphthalic hydrazide monosodium salt	HPT	526	522
Dipotassium phthalate ^b	H ₂ O	None ^d	
	DMSO	None	

^a ± 5 nm, uncorrected spectra. Extra base added to observe chemiluminescence. ^b This salt is very sparingly soluble in DMSO. ^c Extremely low intensity. ^d Phosphorescence has been detected in glasses at ~ 420 nm at 77°K.

The fluorescence quantum yield of dilute solutions of the salt of VI in HPT was determined to be 0.002 ± 0.001 ; the value for the N-methylated derivative VII is similar. The total amount of light emitted per mole of hydrazide VI that reacted was found to depend on the concentration of VI, as shown in Table III. The quantum yield of the chemiluminescence of 5×10^{-4} M VI was determined to be $\sim 3 \times 10^{-5}$ (photons/mole of VI reacted) by comparison with the known light yield of

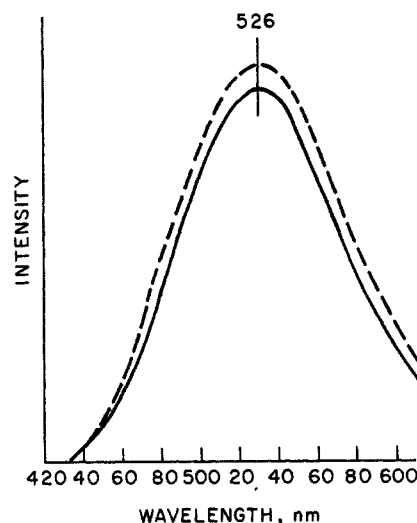


Figure 1.—The fluorescence (---) and chemiluminescence (—) spectra of the monosodium salt of phthalic hydrazide (VI).

luminol (I). Since these reactions are carried to completion, the quantum yields measured are average values reflecting the decrease of VI to zero during the reaction. In contrast to the concentration-dependent quantum yield of the chemiluminescence of VI, the fluorescence yield of the sodium salt of VI is constant (fluorescence intensity linearly related to concentration).

TABLE III

TOTAL LIGHT FROM VI vs. INITIAL CONCENTRATION		
$10^4 \times [\text{VI}]$	$10^6 \times \text{coulombs}^a$	
10.0	22.8, 22.2	
5.0	11.2, 12.1	
4.0	8.1, 7.5	
2.5	2.45, 2.15	
1.25	0.55, 0.65	

^a Charge collected on capacitor. Values are linearly related to total light emission.

Efforts to alter either the emission spectrum or the quantum yield of the chemiluminescence by adding foreign fluorescers did not result in the appearance of new emission peaks or changes in the quantum yield (Table IV). Similarly, 1-methoxy-4-hydroxyphthalazine had no effect on the reaction. Compound VII was shown to be fluorescent in the reaction medium, and stable to it for the duration of the experiments.

TABLE IV

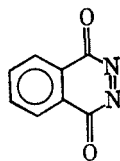
EFFECT OF ADDED FLUORESCERS ON THE
CHEMILUMINESCENCE OF VI

Added fluorescer	$10^4 \times$ [fluorescer]	$10^4 \times$ [VI]	$10^6 \times \text{coulombs}^a$
None		2.02	1.45 ± 0.03
VII sodium salt	1.95	2.02	1.47 ± 0.03
None		1.75	1.20 ± 0.05
8-Aminonaphthalene-2-sulfonic acid	1.63	1.75	1.20 ± 0.05
Anthranilic acid	2.12	1.75	1.0^b

^a Charge collected on capacitor; values (average of duplicate determinations) are linearly related to total light emitted.
^b Solution colored at end of reaction.

The precipitate formed during the reaction is dipotassium phthalate, and only phthalic acid (and occasionally some VI) could be detected in the spent reaction solutions by paper chromatography. No fluorescence from phthalic acid, or its mono- and dinegative ions, could be detected in aqueous solution or in mixtures of water and DMSO. Sodium phthalate is too insoluble to be studied in anhydrous aprotic solvents, but quaternary ammonium salts soluble in these media were also found to be nonfluorescent; high concentrations were also nonfluorescent indicating that emission from phthalate eximers was not occurring.

The two-electron oxidation product of VI, phthalazine-1,4-dione, VIII,⁵ is, *a priori*, a reasonable intermediate in the chemiluminescence of VI.⁶ This compound reacts



VIII

rapidly with butadiene, even at -80° , to yield a Diels-Alder adduct. However, addition of butadiene to a chemiluminescing solution of VI neither decreases the light intensity nor yields detectable amounts of adduct⁷ (presumably because of the high rate of reaction of VIII with peroxide ion). Compound VIII was prepared and isolated as a green solid at -80° and aliquots were treated in DMF at -40° with potassium superoxide, sodium peroxide, and *t*-BuOK in *t*-BuOH; the latter reagent yielded a weak flash of light. In contrast, compound VI chemiluminesces in this system for several minutes with an intensity comparable to that of the flash.

Discussion

The interpretation of low-yield chemiluminescence reactions in terms of molecular species is complicated by the possibility that efficient impurities cause the emission. However, in the present case the data strongly imply that the mononegative ion of phthalic hydrazide is an essential reactant and also the light emitter. The spectral, chromatographic, and chemiluminescence properties of samples do not depend on their origin or mode of purification. Furthermore, the absorption spectra of solutions of the sodium salt of phthalic hydrazide in HPT or DMSO are identical with the fluorescence excitation spectra of these solutions (Table II). Thus, the emitting species is linked to the major absorbing species in solution. The continuous shifts in the absorption spectra of these solutions with changing solvent composition (Table I) are caused by a change in solvation and not by the formation of new chemical species in DMSO solution, as shown by the continuous shift, the failure of the shape or intensity of the band to change markedly, and the absence of isosbestic points.⁸

(5) T. J. Kealy, *J. Amer. Chem. Soc.*, **84**, 966 (1962).

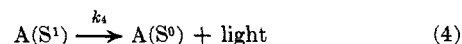
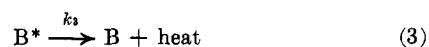
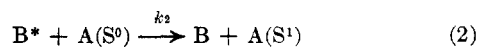
(6) (a) H. O. Albrecht, *Z. Phys. Chem.*, **A136**, 321 (1928); (b) H. Kautsky and K. H. Kaiser, *Z. Naturforsch.*, **5b**, 353 (1950); (c) E. H. White, E. G. Nash, D. R. Roberts, and O. C. Zafriou, *J. Amer. Chem. Soc.*, **90**, 5932 (1968).

(7) A similar result has also been reported by Y. Omote, T. Miyake, and N. Sugiyama [*Bull. Chem. Soc. Jap.*, **40**, 2446 (1967)].

(8) H. H. Jaffé and Milton Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley & Sons, Inc., New York, N. Y., 1966, Chapter 9.

This observed narrowing of the S^0 - S^1 gap of the anions of VI and VII is expected to occur in media (such as DMSO) which solvate anions poorly, provided that the transition involves greater charge delocalization in the excited state than in the ground state; the spectrum of VII, the *N*-methyl analog of VI, rules out the possibility that dinegative ion formation is involved. The same medium effects which vary the S^0 - S^1 gap in VI anion probably cause the great loss in fluorescence efficiency in aqueous solution (by shifting or reordering the levels responsible for nonradiative decay of its S^1 state, for example by narrowing the S^1 -T gap and thereby enhancing intersystem crossing).⁹ It is noteworthy that the quantum yield of chemiluminescence of VI in the aqueous peroxide system has been reported¹⁰ to be 10^{-10} , five orders of magnitude lower than the optimum yield in the aprotic system. Since neither phthalate ion nor phthalic hydrazide anion is detectably fluorescent under these conditions, no effective means exists for emission of light in the water system.

The main features of the chemiluminescence of phthalic hydrazide require a new mechanism for light emission. The nonfluorescence of phthalate ion clearly indicates that it is not the emitter; thus the scheme common to other hydrazide chemiluminescence reactions is not operative. Furthermore, the identity of the fluorescence of the initial solutions of phthalic hydrazide sodium salt to the chemiluminescence emission spectrum strongly implies that a key feature of the mechanism is the formation of an excited species which transfers energy to the anion of phthalic hydrazide itself. We reject alternative mechanisms involving reaction products which are formed in an excited state having the same fluorescence properties as phthalic hydrazide anion. This latter mechanism requires that the similarity of the fluorescence of phthalic hydrazide anion to the chemiluminescence is fortuitous and that the hypothetical product is rapidly destroyed after it emits; furthermore, it offers no explanation for the observed concentration dependence of the light yield (*vide infra*). An energy transfer mechanism of the type given by eq 1-4 fits the

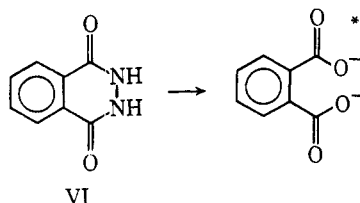


data. An electronically excited product, B^* , is formed from A in the slow step (eq 1) of the reaction. It transfers energy to the initially present fluorescer A, in a bimolecular reaction (eq 2), and it also undergoes competing unimolecular (or pseudounimolecular) deactivation (eq 3). Since the energy transfer and the decay processes are of first and zero kinetic orders, respectively, the fraction of B^* which excites the emitter, A, $[k_2A/(k_2A + k_3)]$ is concentration dependent. The linear plot of apparent quantum yield *vs.* initial concentration of A (Figure 2) fits this expression if $k_3 \gg k_2A$. This linearity implies that even at the higher concentrations studied most of the B^* is deactivated. This prediction

(9) N. J. Turro, "Molecular Photochemistry," W. A. Benjamin, Inc., New York, N. Y., 1965, pp 27-29.

(10) J. Stauff and G. Hartmann, *Ber. Bunsenges. Phys. Chem.*, **69**, 145 (1965).

is consistent with the observation that the fluorescence quantum yield of phthalic hydrazide anion is *ca.* 100 times greater than the observed chemiluminescence quantum yield at the highest concentration studied. The results indicate that reaction 1 must produce B* in a yield of at least several per cent.¹¹ Since VI is the source of chemiluminescence in this system and phthalate ion is the reaction product, the chemical reaction leading to the primary excited state is probably analogous to that of other hydrazides.^{2a, 3a}



Thus, the nonfluorescence of phthalate ion and the availability of a fluorescent acceptor of the electronic energy of excited phthalate would appear to account for the anomalous behavior of VI. However, the failure of added fluorescers to compete with the anion of VI in intercepting the primary excited state (Table IV) compromises this simple mechanism. Particularly striking is the inability of added VII to enhance the quantum yield; the structural and spectral similarities of the anions of VII and VI (Table I) imply that they are interchangeable if either an exchange or a dipole-dipole interaction energy transfer mechanism is involved.¹² Also important is the nonfluorescence of phthalate ion, which indicates a short lifetime for its excited singlet state;¹³ a short-lived singlet is not expected to transfer to an acceptor ($<5 \times 10^{-4} M$) with the required 1–2% efficiency by normal pathways. Finally, attempts to detect appreciable direct energy transfer from phthalate ion to the mononegative ion of VI in DMSO have been unsuccessful. Not much weight can be attached to this result, however, because of serious experimental difficulties, chief of which is the lack of a suitable "window" for irradiating phthalate ion in the presence of VI. This necessitates using high concentrations of phthalate ion (*ca.* $10^{-2} M$) and at this concentration self-quenching will be serious, especially for triplet states.

The experimental results could be accounted for if the energy transfer were facilitated by hydrogen bonding between excited phthalate ion and the acceptor.¹⁴ The substitute acceptor VII in its anionic form could not function as a hydrogen-bond donor, and hydrogen-bonding acceptors would be restricted to a narrow range of acidities ($pK_a \sim 13$), since stronger acids would react fully with potassium *t*-butoxide, and much weaker acids would not give sufficiently strong hydrogen bonds to permit energy transfer. Strong support for this idea comes from the observation that small amounts of potassium *t*-butoxide added to VI in DMSO lead to light emission, whereas large amounts lead to a quenching of the light emission. Presumably in the latter case, all of VI is converted into the dinegative ion and hydrogen

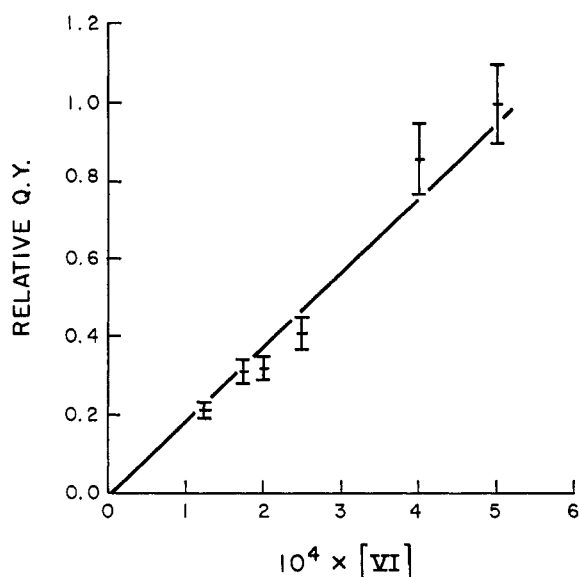
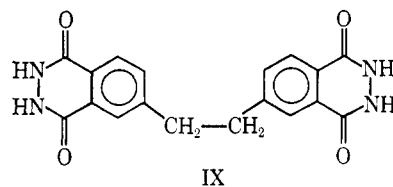


Figure 2.—Relative chemiluminescence quantum yield of phthalic hydrazide (VI) vs. initial concentration of VI.

bonding to the excited-state product (phthalate ion) is thus impossible. Further, if *t*-butyl alcohol or traces of water are added to these dark solutions containing an excess of potassium *t*-butoxide, the chemiluminescence emission returns to its former level, presumably because of the regeneration of the mononegative ion of VI. This effect of butoxide ion on the chemiluminescence of VI is far greater than the effect of butoxide ion on the fluorescence yield of the anion of VI.

Compound IX was synthesized in the hope that proximity would enhance the probability of transfer. However, IX is no more efficient than phthalic hydrazide.



Since intramolecular hydrogen bonding is prohibited in IX and the relative orientations of the donor and acceptor are restricted, this result is inconclusive.

An alternative approach to the difficulties posed by the excited phthalate mechanism is the hypothesis that the uniqueness of the chemiluminescence of VI lies in the chemistry. A mechanism which has been proposed for luminol^{6a, b} would in this case provide for the transfer of chemical energy in a step which yields excited VI anion directly, as shown in Figure 3. Since both diimide and the azaquinone required should have very short lifetimes under the conditions, this mechanism might account for the observed concentration dependence of the light yield and for the inability of VII to substitute for VI. However, it has been conclusively demonstrated that this mechanism is not operative for either hydrazide I^{2a, b} or IV,^{6c} and our experiments with the azaquinone VIII show that this compound leads to only weak chemiluminescence; a much brighter flash would be required to yield an amount of light comparable with that emitted by similar amounts of VI over a much longer time span. Furthermore, the generation of diimide in chemiluminescing solutions of VI in DMSO-*t*-butoxide

(11) The arguments presented in this paragraph do not depend on the assignment of molecular structure to the species involved; these considerations apply even in the unlikely event that an impurity is responsible for the chemiluminescence.

(12) N. J. Turro, "Molecular Photochemistry," W. A. Benjamin, Inc., New York, N. Y., 1965, Chapter 5.

(13) See ref 12, pp 48–50.

(14) This possibility was suggested to us by Dr. N. J. Turro.

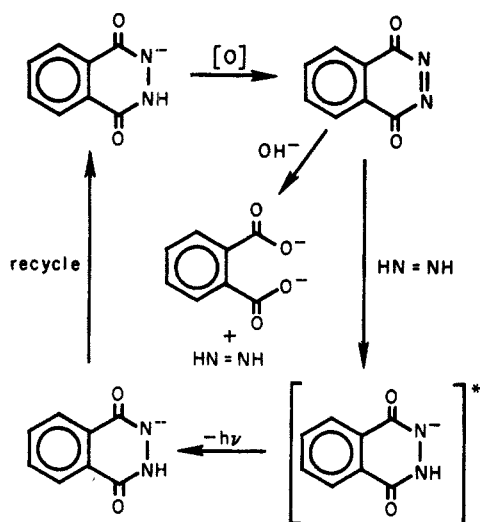
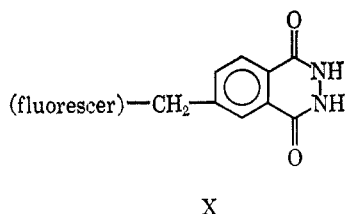


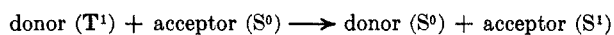
Figure 3.—Alternative mechanism for the chemiluminescence of phthalic hydrazide.

did not lead to an enhancement of the light level, but actually a decrease. Sources used were *p*-toluenesulfonic hydrazide,^{15a} chloroacetic hydrazide,^{15b} and hydrazine-copper mixtures.^{15c, 16}

This rejection of a chemical mechanism of energy transfer again indicates the involvement of an electronic energy transfer step which possesses some unusually selective feature. Recently it has been suggested^{17a} that the chemiluminescence of donor-acceptor compounds, X, utilizing phthalic hydrazide as the energy



source, involves triplet-singlet energy transfer.^{17b} This scheme predicts reasonably well the relative efficiency



of a hydrazide X in terms of the overlap between the long-wavelength absorption band of the acceptor and the phosphorescence of phthalate ion. The spin-forbidden nature¹⁸ of this process makes it particularly susceptible to competition from allowed processes, and differences in these relative rates¹⁹ may account for the unusual features of phthalic hydrazide energy transfer.

In conclusion, it appears that the chemiluminescence of phthalic hydrazide (VI) in DMSO and in HPT in-

(15) (a) S. Hünig, H. R. Müller, and W. Thier, *Tetrahedron Lett.*, 353 (1961); (b) R. Buyle, A. Van Overstraeten, and F. Eloy, *Chem. Ind. (London)*, 839 (1964); (c) E. J. Corey, W. L. Mock, and D. J. Pasto, *Tetrahedron Lett.*, 347 (1961).

(16) Lastly, attempts to detect a radical ion mechanism [D. M. Hercules, R. C. Lansbury, and D. K. Roe, *J. Amer. Chem. Soc.*, **88**, 4578 (1966); E. A. Chandross, J. W. Longworth, and R. E. Visco, *ibid.*, **87**, 3259 (1965)] by light emission during the electrolysis of a mixture of VI and potassium *t*-butoxide in DMSO were unsuccessful.

(17) (a) E. H. White, D. R. Roberts, and D. F. Roswell in Proceedings of the International Conference on Molecular Luminescence, Chicago, Ill., Aug 1968, E. C. Lim, Ed., W. A. Benjamin, Inc., New York, N. Y., 1968; (b) V. L. Ermolaev and E. B. Sveshnikova, *Opt. Spectrosc. (USSR)*, **16**, 320 (1964).

(18) R. G. Bennett, R. P. Schwenker, and R. E. Kellogg, *J. Chem. Phys.*, **41**, 3040 (1964).

(19) E. F. Ullman and N. Baumann, *J. Amer. Chem. Soc.*, **90**, 4158 (1968).

volves the formation of the mononegative ion of VI in an excited state; the mechanism whereby the chemical energy of oxidation is converted into the excitation energy of the hydrazide has not been established with certainty, but it appears reasonably certain that intramolecular energy transfer is occurring within a hydrogen-bonded complex from excited phthalate ion to the mononegative ion of VI.

Experimental Section

I. Methods.—Melting points were taken with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed either by Mr. Joseph Walters or by Galbraith Laboratories (Knoxville, Tenn.). Paper chromatography was performed in the descending manner using prewashed Whatman paper (No. 1 or No. 3MM). The eluent was 95% ethanol-water-concentrated ammonium hydroxide, 8:1:1. Spots were detected by long- and short-wavelength ultraviolet light and by spraying with either 0.1% methyl red in ethanol or 0.1% acridine in ethanol.

Infrared spectra were determined on either Model 137 or 337 Perkin-Elmer instruments. Ultraviolet and visible spectra were determined on a Cary Model 14 instrument and proton magnetic resonance spectra (nmr) were determined on a Varian Associates A-60 instrument. Chemical shifts are reported in τ units relative to internal tetramethylsilane (TMS).

Chemical screening experiments were performed in a dark room after dark adaptation of the eye, and were witnessed by a second observer. Chemiluminescence relative efficiencies were determined using either RCA IP21 or IP28 phototubes, 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 Biochemistry, The Johns Hopkins University). The amplifier signal was collected on a capacitor and recorded for total light yields, reported as coulombs per micromole at 700-V phototube bias.

Fluorescence and chemiluminescence emission spectra were determined on Aminco-Bowman spectrophotofluorimeters and recorded on a Houston Instrument Co. HR-96T X-Y recorder. Many of these spectra were rerun on a Hitachi Model MPF-2A recording spectrophotofluorimeter. Spectra are not corrected 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 reproducible to within ± 3 nm.

II. Materials.—Anthranilic acid, 8-aminonaphthalene-2-sulfonic acid, and 3-aminophthalic acid were commercial materials, homogeneous by paper chromatography. Potassium *t*-butoxide (MSA Research Corp.) was used as received. Dimethyl sulfoxide (Matheson Coleman and Bell) and hexamethylphosphoramide (Eastman) were each stirred overnight over crushed potassium hydroxide, decanted, and distilled from potassium *t*-butoxide. Distillations were performed with grease-free joints under oil pump vacuum (less than 1 torr) and at temperatures from 30 to 60°. A center cut of about 80% of the material was used.

2,3-Dihydrophthalazine-1,4-dione (VI). A.—Phthalic anhydride was prepared by heating an intimate mixture of potassium acid phthalate (Malinkrodt "Primary Standard," Lot No. 6074, 5 g) and potassium bisulfate (Merck "Reagent Grade," 20 g) to 150° in a large sublimation apparatus for 3 hr. A slow stream of dry nitrogen was swept through the apparatus. Phthalic anhydride sublimed in long needles; after 3 hr, 1 g was collected and stored in a desiccator over silica gel. A sample possessed sharp absorption bands (chloroform) at 1855, 1810, 1790, 1775, 1450, and 1260 cm^{-1} .

B.—Reagent grade acetic acid was purified by the method of Fieser (distillation from potassium permanganate).²⁰

C.—Hydrazine, 95%, was purified by distillation of commercial material at aspirator pressure. A central cut was used immediately.

D.—2,3-Dihydrophthalazine-1,4-dione was prepared from A, B, and C. Phthalic anhydride (0.50 g, 3.4 mmol) and acetic

(20) L. F. Fieser, "Experiments in Organic Chemistry," 3rd ed, D. C. Heath and Co., Boston, Mass., 1955, p 281.

acid (25 ml) were placed in a Carius tube under a nitrogen stream, and hydrazine (220 mg, 6.6 mmol) was added. The tube was degassed by two freeze-thaw cycles and sealed under vacuum. It was heated at 140° for 2 hr and then allowed to cool. The white crystals which formed were filtered off on a sintered-glass funnel, washed three times with acetic acid, and dried for 2 hr at 0.005-mm pressure: infrared (KBr) 3450, 3170, 3030, 2900 (all broad), and 1660, 1500, 1380, 1350, 1330, 1310, 1265, 1080, 795, 780, 685, and 630 cm^{-1} .

2-Methyl-2,3-dihydrophthalazine-1,4-dione (VII).—Methyl hydrazine (4.6 g, 0.10 mol) was added to phthalic anhydride (10 g, 0.0675 mol) in 150 ml of acetic acid and the mixture was refluxed for 2 hr. The cooled mixture was poured into 150 cc of water and filtered. The solid was washed with water and crystallized from water-acetic acid (1:1): mp 238.5–240° (lit.^{21a} mp 239–240°); ir (KBr) 3400 (broad), 3100 (broad), 1645, 1625, 1590, and 1575 cm^{-1} .

1-Methoxy-4-hydroxyphthalazine was prepared by the method of Rowe and Peters:^{21a} mp 189–190° (lit. mp 188°, ^{21a} 189° ^{21b}).

Dimethyl 4-Methylphthalate.—Fischer esterification (HCl-methanol) of 4-methylphthalic anhydride (20 g, 0.12 mol) yielded the corresponding dimethyl ester. Distillation at 150° (ca. 1 torr) gave 21.5 g (0.10 mol, 83%) of a clear liquid: infrared (neat) 1730, 1610, 1470 and 1290 cm^{-1} ; nmr (CCl_4) τ 2.3–2.9 (3.02 H, multiplet), 6.19 (6.08, H singlet), 7.61 (3.00 H, singlet) [lit.²² bp 147–151° (9 torr)].

6-Methyl-2,3-dihydrophthalazine-1,4-dione.—4-Methyl phthalic anhydride (1.62 g, 0.01 mol) was dissolved in glacial acetic acid (20 ml) and heated at 90° with hydrazine hydrate (3.0 g, 0.06 mol) until the solution turned turbid and then refluxed 1 hr, cooled, and filtered. The solid was dissolved in dilute aqueous potassium hydroxide, and precipitated with dilute acetic acid. Sublimation at 200–220° (1 torr) yielded 4-methylphthalic hydrazide: mp >350°; ir (KBr) 3200 (broad) and 1720 cm^{-1} .

Hydrazide Sodium Salts.—The hydrazide (10 mmol) was dispersed in 25 cc of water and 10 cc (10 mmol) of 1.00 *N* sodium hydroxide solution was added rapidly. After this solution became homogeneous, a small amount of 95% ethanol was added to precipitate the salt. The precipitate was filtered, washed with 95% ethanol, and dried *in vacuo*. These materials dissolved in water to give a solution of pH 8–9.

5-Amino-2,3-dihydrophthalazine-1,4-dione (Luminol) (I).—Powdered luminol was purified and isolated as the hydrobromide by crystallization from hot hydrobromic acid.

Phthalic Acid.—Solutions of phthalic acid in water were prepared by dissolving potassium acid phthalate (Malinkrodt "Primary Standard," Lot No. 6704) in aqueous hydrochloric acid.

Trimethylbenzylammonium Salt of Phthalic Hydrazide.—Phthalic hydrazide (ca. 1 g) was suspended in 10 ml of methanol, and "Triton B" (40% in methanol) was added dropwise with agitation until an aliquot diluted with water was strongly basic. After all of the material dissolved, the solution was evaporated to a thick glass, and the material was purified by crystallization from acetonitrile: mp 162–164.5° dec; nmr in D_2O (δ from sodium 2,2-dimethyl-2-silapentane-5-sulfonate; singlets at 3.27, 4.59, and 7.78, and a multiplet centered at 8.39 with the relative weightings 9/2/5/4).

Anal. Calcd for $\text{C}_{18}\text{H}_{21}\text{N}_3\text{O}_2$: C, 69.43; H, 6.80. Found: C, 69.13; H, 7.16.

Bis(trimethylbenzylammonium) Phthalate.—Phthalic acid (ca. 2 g) was suspended in 20 ml of methanol, and "Triton B" (40% in methanol) was added dropwise until all the material dissolved and a drop of the solution, on dilution to ca. 1 ml with water, was strongly basic (pH 11–12). The solution was concentrated to a thick oil, further concentrated under high vacuum, and crystallized three times from acetonitrile: mp 186–189° dec; nmr in D_2O (δ from sodium 2,2-dimethyl-2-silapentane-5-sulfonate; singlets at 3.22, 4.37, and 7.96, and a multiplet at ca. 7.92).

Anal. Calcd for $\text{C}_{28}\text{H}_{36}\text{N}_2\text{O}_4 \cdot \text{H}_2\text{O}$: C, 69.68; H, 7.94. Found: C, 70.18; H, 8.03.

Dipotassium, Disodium, and Dicesium Phthalate.—Stirred suspensions of phthalic acid water were titrated to pH 9 (pHydron paper) with aqueous solutions of metal hydroxide and freeze

dried. The salts were crystallized from methanol and dried *in vacuo* at 50°.

Phthalazine-1,4-dione (VIII).—The method of Kealy⁵ was used to prepare VIII after stirring 3 hr at -45° the emerald green solution was filtered through a sintered disk at -80° . The filtrate was rapidly transferred (in a drybox) to a cold flask, which was connected to a diffusion pump. The solution was evaporated by pumping for 10 hr at -80° with continuous stirring; a bright green solid remained. About 10% of the solid was redissolved in cold acetone and treated with butadiene. Evaporation of the solvent left a crystalline white adduct (12.6 mg): mp 264–268° dec (lit.⁵ mp 263–268° dec); nmr (DCCl_3) τ 1.80 (m, 2.1 H), 3.83 (broad s, 1.0), 5.30 (broad s, 3.1). The adduct weight indicates a 70% yield of VIII.

Dimethyl 4-Bromomethylphthalate.—Dimethyl 4-methylphthalate (6.5 g, 29 mmol) was dissolved in 100 cc of carbon tetrachloride and 5.5 g (30 mmol) of *N*-bromosuccinimide was added. The reaction mixture was irradiated under reflux with a sun lamp (GE 275 W) for 45 min. The succinimide was filtered, and the carbon tetrachloride was removed *in vacuo* to give a yellow oil; the nmr spectrum of this material indicated 60% bromination. Distillation gave one fraction (150–160°, 0.1 torr) of a clear liquid which on crystallization from ether-pentane gave a total of 3.65 g (12.5 mmol, 44%) of white crystals: mp 49–51°; infrared (KBr) 1720, 1600 and 1425 cm^{-1} ; nmr (CDCl_3) τ 2.1–2.5 (3.00 H, multiplet), 5.50 (1.97 H, singlet), 6.08 (6.08 H, singlet).

Anal. Calcd for $\text{C}_{11}\text{H}_{11}\text{O}_4\text{Br}$: C, 44.45; H, 3.70. Found: C, 44.37; H, 3.81.

Triphenyl-3,4-dicarbomethoxybenzylphosphonium Bromide.—Triphenylphosphine (2.25 g, 8.6 mmol) and dimethyl-4-bromomethylphthalate (2.5 g, 8.4 mmol) were heated in 50 cc of benzene at reflux for 8 hr. After standing overnight the white crystalline precipitate was filtered and washed with two portions of dry benzene. A total of 4.2 g (7.5 mmol, 89%) of product was isolated: dec >150°; infrared (KBr) 1715, 1425 and 1370 cm^{-1} .

***cis*- and *trans*-1,2-Bis(3,4-dicarbomethoxyphenyl)ethylene.**—Triphenyl-3,4-dicarbomethoxybenzylphosphonium bromide (1.58 g, 3.0 mmol) was dissolved in 25 cc of methanol (distilled from magnesium turnings) and to this solution 8.1 cc of 0.37 *N* sodium methoxide (3.0 mmol) in methanol was added. This reaction mixture was stirred under nitrogen for 15 min and then 0.63 g (3.0 mmol) of 4-formyldimethylphthalate in 10 cc of methanol was added. After stirring for 10 min, a precipitate appeared, which when filtered off gave 0.30 g (0.73 mmol, 24%) of white material shown to be the pure *trans*-tetracarbomethoxystilbene. Crystallization from a chloroform-ether mixture gave translucent needles: mp 134–135°; infrared (KBr) 1720, 1605 and 1445 cm^{-1} ; nmr (CDCl_3) τ 2.1–2.4 (2.96 H, multiplet), 2.80 (0.96 H, singlet), 6.03 (6.00 H, singlet); ultraviolet ($\text{C}_2\text{H}_5\text{OH}$) 328 μ ($\log \epsilon$ 4.48) and 235 (4.11).

Anal. Calcd for $\text{C}_{22}\text{H}_{20}\text{O}_8$: C, 64.08; H, 4.89. Found: C, 63.98; H, 4.96.

The methanol filtrate was evaporated to dryness *in vacuo* and extracted with hot ether. The ether extract, containing the *cis*-tetracarbomethoxystilbene as well as some triphenylphosphine oxide, was chromatographed on 20 g of silica gel with chloroform as the eluent. The initial fractions contained 0.8 g (20 mmol, 64%) of colorless oil which when crystallized from ether-pentane gave pure *cis*-tetracarbomethoxystilbene as white needles: mp 71–72.5°; infrared (KBr) 1720, 1595 and 1430 cm^{-1} ; nmr (CDCl_3) τ 2.1–2.8 (3.12 H, multiplet), 3.25 (0.90 H, singlet), 6.08 (6.00 H, singlet); ultraviolet ($\text{C}_2\text{H}_5\text{OH}$) 300 μ ($\log \epsilon$ 4.25) and 235 (4.49).

Anal. Calcd for $\text{C}_{22}\text{H}_{20}\text{O}_8$: C, 64.08; H, 4.89. Found: C, 64.03; H, 4.89.

1,2-Bis(3',4'-dicarbomethoxyphenyl)ethane.—*cis*-Tetracarbomethoxystilbene (85 mg, 0.19 mmol) in 20 cc of methanol was stirred with 30 mg of 10% palladium-charcoal under an atmosphere of hydrogen for 4 hr. The catalyst was then filtered off and the methanol was evaporated. Crystallization of the crude product from anhydrous ether gave 71 mg (0.17 mmol, 84%) of white crystals: mp 114–115°; infrared (KBr) 1740 cm^{-1} ; nmr (CDCl_3) τ 2.2–2.8 (3.00 H, multiplet), 6.08 (5.91 H, two almost superimposed singlets), 7.00 (1.97 H, singlet).

Anal. Calcd for $\text{C}_{22}\text{H}_{22}\text{O}_8$: C, 63.76; H, 5.35. Found: C, 63.53; H, 5.20.

Subjecting the *trans*-tetracarbomethoxystilbene to the above procedure gave material identical in all respects with that obtained from the *cis* starting material.

(21) (a) F. Rowe and J. J. Peters, *J. Chem. Soc.*, 1331 (1933); (b) J. A. Elvidge and A. P. Redman, *ibid.*, 1710 (1960).

(22) K. Alder, R. Munders, W. Krane, and P. Wirtz, *Ann. Chem.*, **624**, 59 (1959).

1,2-Bis(6',2',3'-dihydro-1',4'-diketophthalazyl)ethane.—1,2-Bis(3,4-dicarbomethoxyphenyl)ethane (130 mg, 0.31 mmol) was added to 5 cc of ethanol containing 0.5 cc of 95% hydrazine. This solution was degassed and sealed, and heated in a steam bath for 2.5 hr. The white crystalline product was filtered off, washed with ethanol, and triturated with boiling glacial acetic acid to give 115 mg (0.30 mmol, 96%) of white material: mp >300°; infrared (KBr) 1650, 1610, and 1400 cm^{-1} ; ultraviolet (in hexamethylphosphoramide) 312 $\text{m}\mu$ ($\log \epsilon$ 4.02). Paper chromatography on Whatman No. 1 paper showed one spot, R_f 0.32.

Anal. Calcd for $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_4$: C, 61.71; H, 4.03; N, 15.99. Found: C, 60.05; H, 4.20; N, 15.19.

A portion of the material was sublimed with a cool flame under high vacuum.

Anal. Found: C, 61.53; H, 4.21; N, 15.85.

Total Light Emission Studies.—A sample of the hydrazide solution, including any additives, was added to the cell of the light detector-integrator described. The cell was placed in the light-tight compartment, and *ca.* 50 mg of dry, solid potassium *t*-butoxide was added through a light-tight port with the phototube in operation, and the samples, agitated by a small magnetic stirrer bar, were monitored until no further light output occurred; additional butoxide was then added to ascertain that the reaction was complete.

Chemiluminescence Spectral Distribution Studies.—Chemiluminescence spectral distribution was determined by scanning samples in the spectrophotofluorimeter, using no exciting light. Samples were prepared as were the total light emission samples, except that oxygen and agitation was provided *via* air introduced into the cell through a long syringe needle. Two methods were used to ascertain that intensity decay during scan was not distorting curves.

(1) The sample was rescanned after the elapse of several scan times; curves were accepted if the intensity drop between successive scans was <20% at peak maximum.

(2) The Aminco-Bowman spectrometer was modified so that two phototubes monitored the emission, one before and one after emitted light was dispersed.

The "total light monitor" phototube output was time synchronized with the "dispersed light monitor" *via* an electrical marker signal. The total light decay curve was used to correct the dispersed curve for intensity-decay distortion.

Quantum Yield Determinations.—The chemiluminescence quantum yield of $5 \times 10^{-4} M$ VI in HPT was determined to be $\sim 3 \times 10^{-5}$ by comparison of total light yields relative to luminol,

I.²³ The relative total light yields were normalized for phototube sensitivity at the wavelength emission maxima.

The fluorescence quantum yield of the sodium salt of VI in HPT was determined by a comparison with *D*-luciferin in aqueous solution at pH 4.8. The fluorescence measurements were performed by Dr. T. A. Hopkins (Department of Biochemistry, The Johns Hopkins University) using a 150-W xenon arc, a Perkin-Elmer Corp. Model 98 monochromator, and a W. Fasti-type spectrophotometer. This spectrophotometer contained an EMI 9558 phototube with known spectral response in the region of interest (500–580 nm). Excitation of the samples was at 360 nm and the areas under the corrected emission curves were compared. From the known fluorescence quantum yield of 0.25 for *D*-luciferin,²⁴ the fluorescence quantum yield of the sodium salt of VI was calculated to be 0.002 ± 0.001 .

Registry No.—VI, 1445-69-8; VI (Na salt), 20116-60-3; VI (trimethylbenzylammonium salt), 20116-61-4; VII, 18393-54-9; VII (Na salt), 20116-63-6; VIII, 20116-64-7; dimethyl 4-methylphthalate, 20116-65-8; 6-methyl-2,3-dihydrophthalazine-1,4-dione, 20116-66-9; bis(trimethylbenzylammonium) phthalate, 20116-39-6; dimethyl 4-bromomethylphthalate, 20116-67-0; triphenyl-3,4-dicarbomethoxybenzylphosphonium bromide, 20116-68-1; *cis*-1,2-bis(3,4-dicarbomethoxyphenyl)ethylene, 20122-46-7; *trans*-1,2-bis(3,4-dicarbomethoxyphenyl)ethylene, 20122-47-8; 1,2-bis(3',4'-dicarbomethoxyphenyl)ethane, 20116-69-2; 1,2-bis(6'-2',3'-dihydro-1',4'-diketophthalazyl)ethane, 20116-70-5; 4-methylphthalic hydrazide (Na salt), 20116-71-6.

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