Energy Transfer in Chemiluminescence. III.[1,2] Intramolecular Triplet–Singlet Transfer in Derivatives of 2,3-Dihydrophthalazine-1,4-dione

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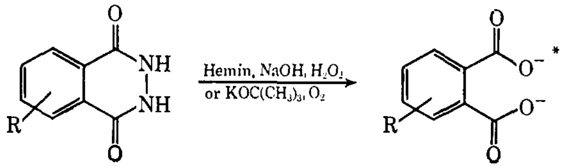
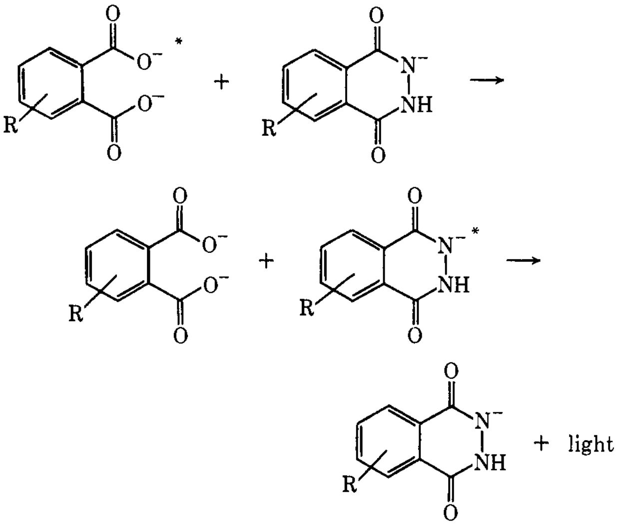
Abstract: The seemingly anomalous chemiluminescence properties of a series of four 6-substituted 2,3-dihydrophthalazine-1,4-diones, in which the substituents were acridone (**3a**), 2,3-benzcarbazole (**4a**), 3,4-benzcarbazole (**5a**), and carbazole (**6a**) attached *via* a methylene group, have been investigated. The relative chemiluminescence quantum yields of **3a**–**6a** and the fluorescence quantum yields of the corresponding phthalic acids have been measured. These, together with the emission properties of phthalic acid, indicate that the intramolecular energy transfer involved is not singlet–singlet in nature, but more probably triplet–singlet of a mixed dipole–dipole and exchange character.

In chemiluminescent reactions, electronic excited states of molecules are produced by chemical energy.[3–5] Once formed, the excited molecules behave in a manner identical with those produced by irradiation. Thus both radiative and nonradiative processes occur (*e.g.*, fluorescence, intersystem crossing, internal conversion, and energy transfer). The use of chemical excitation, however, has both advantages and disadvantages: uniform excitation throughout the solution and specific location of the excitation energy in one species can be advantageous, especially in the latter case if donor and acceptor have overlapping absorption bands; the presence of oxygen (a reactant in many cases), on the other hand, can be disadvantageous. The need for oxygen means that triplet states are *usually* effectively quenched and consequently play no significant role in chemiluminescence emission.[5b]

The chemiluminescent reactions of cyclic aromatic hydrazides have been well investigated,[3–7] both in aqueous and aprotic media, with that of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione), by far the most widely studied. The reaction is an oxidation of the hydrazide by either oxygen or hydrogen peroxide (in the latter case, along with a cooxidant or with certain metal ions). The main, if not the sole product is the appropriately substituted phthalate ion; this ion, in every case so far investigated, except for the parent unsubstituted system and the 3- and 4-methyl-substituted derivatives, has been shown also to be the emitter.[4,8,9] The exceptional compounds are only chemiluminescent in aprotic solvents such as dimethyl sulfoxide or hexamethylphosphoric triamide. The emitter here is the anion of the hydrazide, which is fluorescent in these solvents.[9–11] The corresponding phthalate dianions are completely nonfluorescent in either aqueous or aprotic solution.[10] It appears, therefore, that intermolecular energy transfer must be occurring in these systems.[11]





Intramolecular energy transfer has been shown to occur[1,10] in certain bifunctional compounds of the type B–A in which the energy producing part B (an aromatic hydrazide) is different, and separated, from the emitting part A, which is a highly fluorescent group.

$$B\text{--}A \longrightarrow D^*\text{—}A \longrightarrow D\text{—}A^* \longrightarrow D\text{—}A + h\nu$$

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[1] For part II, see D. F. Roswell, V. Paul, and E. H. White, *J. Amer. Chem. Soc.*, **92**, 4855 (1970).

[2] Presented in part at the International Conference on Molecular Luminescence, Chicago, Ill., Aug 1968.

[3] E. H. White in "Light and Life," W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1961, p 183.

[4] K. D. Gundermann, "Chemilumineszenz Organischer Verbindungen," Springer-Verlag, Berlin, 1968.

[5] (a) F. McCapra, *Quart. Rev., Chem. Soc.*, 20 485 (1966). (b) A notable exception is the work of Vassil'ev on autooxidation chemiluminescence, involving low concentrations of oxygen and ultimate weak emission from various carbonyl compounds formed as products (R. F. Vassil'ev, "Progress in Reaction Kinetics," Vol. 4, G. Porter, Ed., Pergamon Press, Oxford, 1967, p 305, and references cited therein).

[6] E. H. White, O. C. Zafiriou, H. M. Kägi, and J. H. M. Hill, *J. Amer. Chem. Soc.*, **86**, 940 (1964).

[7] E. H. White and M. M. Bursey, *ibid.*, **86**, 941 (1964).

[8] M. M. Bursey, Ph.D. Thesis, The Johns Hopkins University, Baltimore, Md., 1963.

[9] O. C. Zafiriou, Ph.D. Thesis, The Johns Hopkins University, Baltimore, Md., 1966.

[10] D. F. Roswell, Ph.D. Thesis, The Johns Hopkins University, Baltimore, Md., 1968.

[11] E. H. White, D. F. Roswell, and O. C. Zafiriou, *J. Org. Chem.*, **34**, 2462 (1969).

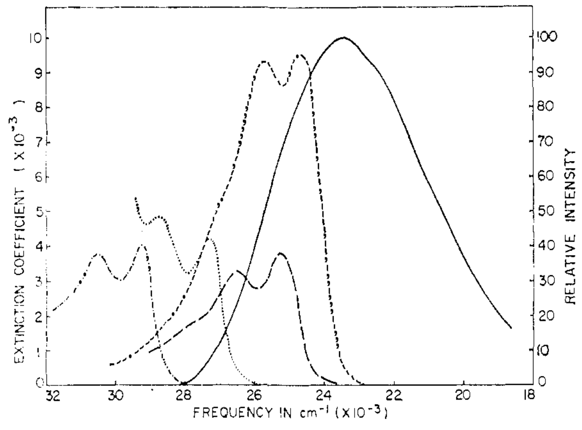
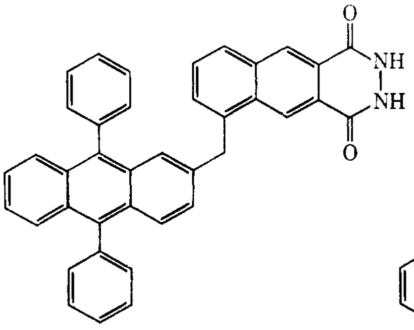


Figure 1. The phosphorescence emission spectrum of dipotassium phthalate (———) and the low-frequency ultraviolet absorption bands of the acceptors [**3b** (----), **4b** (——), **5b** (· · · · ·), and **6b** (- · · · -)].

D* represents the primary excited product (a phthalic acid moiety). This molecular coupling has the effect of increasing the quantum yield of chemiluminescence relative to that of B itself. Thus 6-[(9,10-diphenyl-2-anthryl)methyl]-2,3-dihydrobenzo[*g*]phthalazine-1,4-dione (**1**) is 4.3 times as efficient in light production as 2,3-dihydrobenzo[*g*]phthalazine-1-4-dione (**2**) itself.[1] In this case the fluorescence emission of the 2,3-naphthalenedicarboxylate dianion overlaps well the first absorption band (^1L_a) of diphenylanthracene, leading to efficient singlet–singlet transfer, presumably of the dipole–dipole type proposed by Förster.[12]



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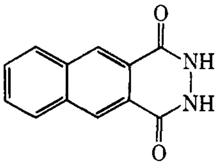

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Results

In this paper we wish to report the seemingly anomalous chemiluminescence of a series of four compounds **3a–6a**. These compounds are of the type in which an energy generator is connected to an efficient fluorescer. The common energy producing part B is phthalic hydrazide and the four different acceptors are acridone in **3a**, 2,3-benzocarbazole in **4a**, 3,4-benzocarbazole in **5a**, and carbazole in **6a**. The emission maxima of chemiluminescence, the fluorescence of the total reaction mixture, and the fluorescence of the postulated products (the substituted phthalic acids **3b–6b**) are given in Table I, together with the fluorescence emission maxima of the N-methyl derivatives of the acceptors (**3d–6d**). The chemiluminescence of **6a** was too dim to enable a meaningful emission spectrum to be recorded. The relative chemiluminescence quantum yields of the four hydrazides and the fluorescence quantum yields of the four acids are given in Table II, together with the overlap integrals between the phosphorescence of dipotassium phthalate and the absorption bands of the acids **3b–6b**, shown in Figure 1. The chemiluminescent reactions were studied in aqueous solution, since potassium *t*-butoxide in aprotic solvents caused cleavage of the carbon–nitrogen bond between the methylene group and the acceptor.

Table I. Emission Maxima[a]

	3a	**4a**	**5a**	**6a**
Chemiluminescence maxima, nm[b]	430,448	413,430	395[c]	*d*
	3b	**4b**	**5b**	**6b**
Fluorescence[e] maxima, nm	425,445 427,446	410,430 411,430	377,394 379,393	350,367 351,364
	3a product	4a product	5a product	6a product
Fluorescence[f] maxima, nm	427,447	410,433	377,395	351,367
	3d	**4d**	**5d**	**6d**
Fluorescence maxima, nm	423,442	410,433	376,395	353,366

[a] The values quoted are as directly measured and are not corrected. They are for internal comparison only. [b] The relative weakness of the chemiluminescence emission, using dilute solutions to give undistorted spectra, necessitated relatively wide slits. [c] Emission slit here gave 16 nm band pass, hence the weaker 377 maximum was lost. [d] Too dim to record spectra. [e] Upper at $\sim 3 \times 10^{-6}$ *M*, lower at $\sim 10^{-4}$ *M*. [f] Fluorescence of total reaction mixture.

Table II. Quantum Yields and Overlap Integrals

Φ_{Ch}[a]		φ_F[b]		$J_{DA}(\times 10^{15})$[c]	
3a	100.0	**3b**	0.89	**3b**	7.41
4a	50.0	**4b**	0.45	**4b**	1.80
5a	5.8	**5b**	0.21	**5b**	0.13
6a	0.3	**6b**	0.02	**6b**	0.00

[a] Relative chemiluminescence quantum yield. [b] Fluorescence quantum yield relative to quinine bisulfate at 0.55: W. H. Melhuish, *J. Phys. Chem.*, **65**, 229 (1961). [c] The overlap integral between the phosphorescence of dipotassium phthalate and the absorption bands of the acceptors.[12]

Discussion

It can be seen that there is a marked decrease in chemiluminescence quantum yield on going down the series **3a–6a**, only partly explicable by the decreasing fluorescence quantum yields of the products. This constitutes the first of two major anomalies if the chemiluminescence is interpreted on the basis of singlet–singlet resonance transfer. It is anomalous because, though the phthalate anion is completely nonfluorescent, extrapolation from the next higher benzolog, the 2,3-naphthalenedicarboxylate dianion, which is fluorescent, would place the maximum of the $S_1 \rightarrow S_0$ energy distribution near 305 nm. Consequently there should be good overlap with the absorption bands of all four acceptors. Indeed, because of the relative extinction coefficients, the 3,4-benzocarbazole compound, **5a**, should have the greatest chemiluminescence quantum yield since it absorbs

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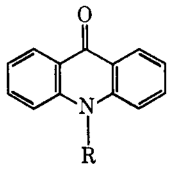
(12) Th. Förster, *Discuss. Faraday Soc.*, **27**, 7 (1959).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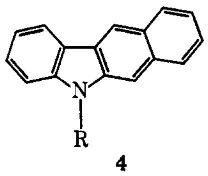
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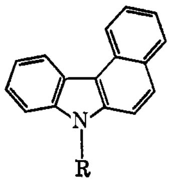
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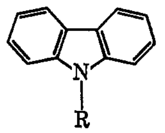
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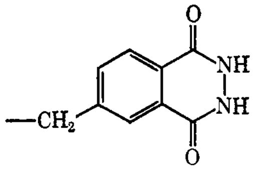
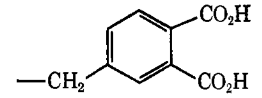
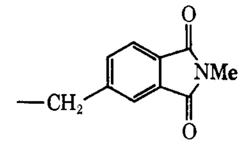
a, R =



b, R =



c, R =



d, R = $—CH_3$
e, R = —H

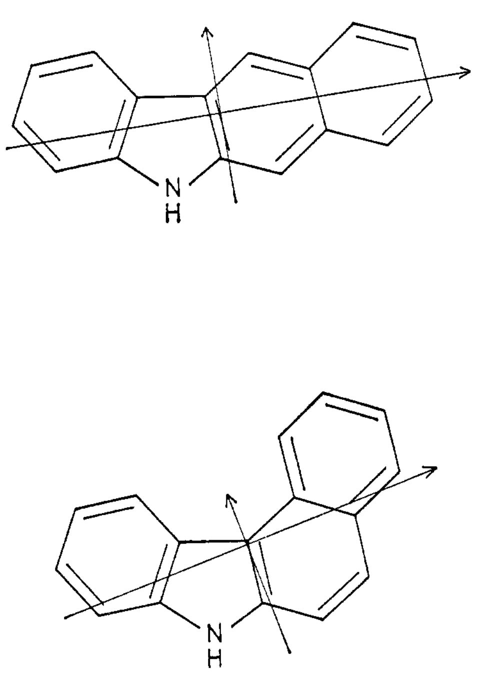


Figure 2. The postulated directions of transition dipoles for 2,3-benzcarbazole and 3,4-benzcarbazole.

most strongly in that region. It should be noted that provided the $S_1 \rightarrow S_0$ maximum is at a shorter wavelength than about 360 nm, the exact position is not critical to the argument. The foregoing does assume that the gross differences in chemiluminescence quantum yields are not due to the concomitant molecular changes which alter the relative angles of the transition dipoles, the population of different conformations, and the distance between the centers of the transition dipoles. Inspection of molecular models shows that the variations in the acceptor portions of the molecules are sufficiently remote from the donor portion that the preferred conformers should differ only slightly. However, little is known of the direction of the transition dipoles of the relevant absorption bands. For the dipolar interaction to be near zero, the orientation factor κ given by[12]

$$\kappa = \cos \theta_{DA} = 3 \cos \theta_D \cos \theta_A \qquad (1)$$

must be near zero. In eq 1, θ_{DA} is the angle between the transition moment vectors while θ_D and θ_A are the angles between these respective vectors and the direction DA. It can be seen therefore that there are an infinite number of orientations which give zero interaction. However, using the observation of Zander[13] that the electronic absorption and emission properties of the carbazoles are very similar to those of the analogous phenanthrenes, and from the known symmetry properties of such aromatic compounds, reasonable transition dipole directions may be postulated. Thus for acridone and carbazole they would be parallel to one of the molecular axes; for 2,3-benzcarbazole and 3,4-benzcarbazole they would be approximately as shown in Figure 2. Such directions all give nonzero interactions. Furthermore, the efficiency of energy transfer is not a very sensitive function of κ, since κ^2 can only vary between 0 and 4. Also, the compounds are not sufficiently rigid for κ to be zero for all conformations. It therefore seems unlikely that this mechanism alone could explain the observed variations in relative chemiluminescence quantum yields.

The second major anomaly concerns the absence of fluorescence in dipotassium phthalate. This means that radiationless processes must be depopulating the first excited singlet state at a rate of at least 10^{10}–10^{11} sec^{-1}.[14] To compete successfully for excitation energy, energy transfer must occur at a rate not too different from that of the deactivating processes. However, in the systems so far studied at least,[15] intramolecular singlet–singlet energy transfer takes place at a rate of 10^7–10^8 sec^{-1}. Clearly, then, singlet–singlet transfer would be very inefficient, and consequently unable to account for more than a minor proportion of the chemiluminescence of even **6a**, the least efficient of the hydrazides. While dipotassium phthalate is nonfluorescent, however, it is highly phosphorescent. This raises the possibility that the chemiluminescence emission is a result of a triplet–singlet transfer process. Förster has shown[12] that the rate of singlet–singlet energy transfer, *via* the weak coulombic interaction of the transition dipoles, is given[16] by the relation

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(13) M. Zander, "Phosphorescence," Academic Press, New York, N. Y., 1968, p 98.

(14) F. Wilkinson, "Advances in Photochemistry," Vol. 3, W. A. Noyes, Jr., G. S. Hammond, and J. N. Pitts, Jr., Ed., Interscience Publishers, New York, N. Y., 1964, p 241.

(15) A. A. Lamola, P. A. Leermakers, G. W. Byers, and G. S. Hammond, *J. Amer. Chem. Soc.*, **87**, 2322 (1965).

(16) In ref 12 the expression for k_{ET} contains a π^6. This apparently is an error, the correct factor being π^5. See footnote 15 in ref 17.

(17) S. A. Latt, H. T. Cheung, and E. R. Blout, *J. Amer. Chem. Soc.*, **87**, 995 (1965).

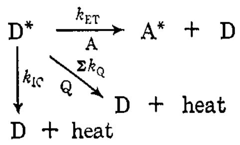

Figure 3. Pathways for loss of excitation energy.

$$k_{ET} = \frac{9000 \ln 10 \kappa^2}{128 \pi^5 n^4 N \tau R^6} \int_0^\infty f_D(\bar{\nu}) \epsilon_A(\bar{\nu}) \frac{d\bar{\nu}}{\bar{\nu}^4} \qquad (2)$$

Here $\bar{\nu}$ is the wave number, $\epsilon(\bar{\nu})$ the molar decadic extinction coefficient, $f(\bar{\nu})$ the spectral distribution of fluorescence (measured in quanta and normalized to unity on a wave-number scale), N Avagadro's number, and τ is the radiative lifetime of the donor. R is the distance between the molecules, n is the refractive index of the solvent, and κ is the orientation factor, given by eq 1. Förster also noted[12] that eq 2 is equally applicable to triplet–singlet energy transfer, *via* a dipole–dipole mechanism, provided the spectral distribution of phosphorescence and the phosphorescence radiative lifetime are substituted for the corresponding fluorescence values. There have been a number of examples of triplet–singlet energy transfer reported.[18-23] With only one exception, these have been investigated in solid media, either at room or liquid nitrogen temperature, and all of them were intermolecular. The only system examined in fluid solution contained chemically excited cyclohexanone as the donor and substituted anthracene derivatives as the acceptors.[23]

From eq 2 it can be seen that the rate of energy transfer should be proportional to the overlap integral J_{DA} defined by

$$J_{DA} = \int_0^\infty f_D(\bar{\nu}) \epsilon_A(\bar{\nu}) \frac{d\bar{\nu}}{\bar{\nu}^4} \qquad (3)$$

Figure 3 shows the different pathways by which the excited triplet donor can lose its excitation energy. The excited donor, D*, can either transfer its energy at a rate $k_{ET}[D^*]$ to the acceptor A, or be quenched. The quenching can either be unimolecular, at a rate $k_{IC}[D^*]$, where k_{IC} is the rate constant for intersystem crossing, or bimolecular, at a rate $\Sigma k_Q[Q][D^*]$, where k_Q is the rate constant for the quencher Q. The rate of loss of excited donor is given by

$$-\frac{d[D^*]}{dt} = \{k_{IC} + \Sigma k_Q[Q] + k_{ET}\}[D^*] \qquad (4)$$

while the rate of production of excited acceptor is given by

$$\frac{d[A]}{dt} = k_{ET}[D^*] \qquad (5)$$

The relative efficiency of energy transfer is given by

$$\varphi_{ET} = -\frac{d[A]}{dt} \Big/ \frac{d[D^*]}{dt} \qquad (6)$$

$$= \frac{k_{ET}}{(k_{IC} + \Sigma k_Q[Q] + k_{ET})} \qquad (7)$$

Hence

$$1/\varphi_{ET} = 1 + \frac{(k_{IC} + \Sigma k_Q[Q])}{k_{ET}} \qquad (8)$$

Now the overall efficiency of chemiluminescence Φ_{Ch} is a function of the efficiency of excitation φ_{Ex}, the efficiency of energy transfer φ_{ET}, and the efficiency of fluorescence of the acceptor, φ_F. Hence

$$\Phi_{Ch} = k\varphi_{Ex}\varphi_{ET}\varphi_F \qquad (9)$$

Since the donor in the systems under discussion is common to all, the assumption is made that φ_{Ex} is constant for all four hydrazides under the same conditions. Thus

$$\Phi_{Ch} = k'\varphi_{ET}\varphi_F \qquad (10)$$

and from (8)

$$1/\Phi_{Ch} = 1/k'\varphi_F\left\{1 + \frac{k_{IC} + \Sigma k_Q[Q]}{k_{ET}}\right\} \qquad (11)$$

However

$$k_{ET} = k''J_{DA} \qquad (12)$$

therefore

$$1/\Phi_{Ch} = 1/k'\varphi_F\left\{1 + \frac{k_{IC} + \Sigma k_Q[Q]}{k''J_{DA}}\right\} \qquad (13)$$

It is clear from (12) that φ_F/Φ_{Ch} should vary linearly with $1/J_{DA}$ if this mechanism is correct. The values of these functions for the four hydrazides **3a**–**6a** and their corresponding acids are shown in Table III. It is immediately obvious that the relationship is anything but linear and, consequently, that the dipole–dipole mechanism is not adequate to explain the results. It should be noted at this point that it is implicitly assumed that the phosphorescence emission spectrum of dipotassium phthalate measured at 77°K in a methanol glass is a good approximation to the emission from the triplet state of the phthalate moiety in a phosphate buffer at room temperature if it could be observed.

Table III. Energy Transfer Functions

φ_F/Φ_{Ch} ($\times 10^2$)		$1/J_{DA}$ ($\times 10^{-15}$)	
3b/3a	0.89	**3b**	0.135
4b/4a	0.89	**4b**	0.555
5b/5a	2.7	**5b**	7.69
6b/6a	6.0	**6b**	Very large

Vassil'ev, while investigating the chemiluminescence arising from autoxidation of hydrocarbons, discovered that the addition of fluorescent compounds, such as substituted anthracenes, markedly increased the intensity of emission.[24] However, there was an inverse correlation with efficiency of chemiluminescence and fluorescence quantum yields. Thus 9,10-dibromoanthracene was 26 times as efficient for chemiluminescence

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

(19) V. Ermolaev and E. Sveshnikova, *Dokl. Akad. Nauk SSSR*, **149**, 1295 (1963); English translation, *Sov. Phys. Dokl.*, **8**, 373 (1963).

(20) V. Korsunskii and A. Faidysh, *Dokl. Akad. Nauk SSSR*, **150**, 771 (1963); English translation, *Sov. Phys. Dokl.*, **8**, 564 (1963).

(21) M. Höfert, "International Conference on Photochemistry, Munich, 1967, preprints, p 607.

(22) D. Phillips, V. Anissimov, O. Karpukhin, and V. Shiliapintokh, *Nature*, **215**, 1163 (1967).

(23) R. F. Vassil'ev, *ibid.*, **200**, 773 (1963).

(24) R. F. Vassil'ev, *ibid.*, **196**, 668 (1962).

as 9,10-diphenylanthracene, while having a fluorescence quantum yield one-eighth as large. Since the absorption spectra for the series of anthracene derivatives used are all very similar, the overlap integrals should also be very close in value. Thus a dipole–dipole mechanism is unlikely and Vassil'ev considered[23] the transfer to be predominantly of an exchange type.

The rate of energy transfer *via* an exchange mechanism should be proportional to the square of the matrix element of the operator of the exchange interaction.[25] Thus the rate of transfer is given by

$$k_{\mathrm{ET}}\alpha|\langle H_1\rangle|^2 = \left|\int \psi_{\mathrm{D}}{}'^*(1)\psi_{\mathrm{A}}{}^*(2)H_1\psi_{\mathrm{A}}{}^1(1)\psi_{\mathrm{D}}(2)\mathrm{d}\tau\right|^2 \quad (14)$$

where

$$H_1 = e^2/r_{12} \quad (15)$$

$\langle H_1\rangle$ is nonzero only if the multiplicity of the two excited states are equal, and the multiplicity of the two ground states are also equal, though the two pairs need not have the same spin; that is

$$\chi_{\mathrm{D}}' = \chi_{\mathrm{A}}' \quad (16)$$

$$\chi_{\mathrm{D}} = \chi_{\mathrm{A}} \quad (17)$$

but the χ' need not equal the χ. This provides the basis for the exchange mechanism of the well-known triplet–triplet energy transfer. However it also means that *via* an exchange mechanism, triplet–singlet energy transfer is forbidden, except for the part of $\langle H_1\rangle$ corresponding to the pure triplet–triplet transfer. Since, in general, spin–orbit coupling mixes the singlet and triplet states, the first excited "singlet" state can be written

$$\psi_{\mathrm{A}}' = {}^1\psi_{\mathrm{A}}' + \lambda{}^3\psi_{\mathrm{A}}' \quad (18)$$

Combining eq 14 and 18 gives

$$k_{\mathrm{ET}}\alpha\lambda^2|\langle H_1\rangle|_{3,3}{}^2 \quad (19)$$

where $|\langle H_1\rangle|_{3,3}{}^2$ is the part of $|\langle H_1\rangle|$ corresponding to the "pure" triplet–triplet transfer. Thus the greater λ is, that is, the more spin–orbit coupling present, the greater is the rate of transfer.

In the series of compounds **3b–6b**, since there is only one methylene group separating the donor and acceptor portions of the molecule, they are well within the generally accepted limits for exchange processes (~15–20 Å).[26] Thus it is quite likely that transfer *via* such a mechanism contributes to a considerable extent to the emission seen as chemiluminescence in these compounds, especially since it could be as fast as 10^{10} sec^{-1}.[23] Furthermore, if it is assumed that for large aromatic molecules the sum of the fluorescence quantum yield and the triplet formation efficiency approaches unity,[27,28] then the compounds with the lower fluorescence quantum yields should have higher triplet formation efficiencies and therefore, presumably, greater spin–orbit coupling parameters. This would then help to explain the large deviation from linearity in the relationship between $\varphi_{\mathrm{F}}/\Phi_{\mathrm{Ch}}$ and $1/J_{\mathrm{DA}}$ for the 3,4-benzcarbazole, and more especially, the carbazole compounds. Furthermore, a mechanism for transfer similar to that of thermally activated fluorescence[29] may be operating. Thus, thermal excitation to higher vibrational sublevels of the triplet manifold may need to occur prior to transfer in these compounds.

It should be noted here, however, that another effect may be present in the fluorescence of **6b**. Thus, while the fluorescence quantum yield of acridone is similar to that of **3b**, the fluorescence quantum yield of carbazole is considerably greater[30] than that of **6b**. This may be solely due to the difference in solvents, cyclohexane as against water, or may be an example of intramolecular charge transfer quenching of fluorescence, similar to that observed previously intermolecularly.[31] However while such an interaction may be present in the excited state, the absorption spectra of **3b–6b** and **3d–6d** are sufficiently similar to neglect any strong interaction in the ground state.

In summary, the chemiluminescence of the four hydrazides **3a–6a** cannot be explained satisfactorily within the framework of the current knowledge of singlet–singlet energy transfer. However, through a combination of dipole–dipole and exchange interactions, in which the latter most probably predominates, it is possible to rationalize the observed results in terms of triplet–singlet transfer. Furthermore, because of the disposition of the energy levels necessary for this type of energy transfer, chemiluminescence provides a unique way of observing the phenomenon, since the initial excitation energy is localized on a single, known species.

Experimental Section[32]

Materials. Acridone (3e), carbazole (6e), N-methylacridone (3d), N-methylcarbazole (6d), N-methyl-4-bromomethylphthalimide (7), N-methyl-4-[(9-oxo-10-acridanyl)methyl]phthalimide (3c), N-methyl-4-[(9-carbazolyl)methyl]phthalimide (6c), 2,3-dihydro-6-[(9-oxo-10-acridanyl)methyl]phthalazine-1,4-dione (3a), **2,3-dihydro-6-[(9-carbazolyl)methyl]phthalazine-1,4-dione (6a), and dipotassium phthalate** were obtained as analytically pure samples from Dr. D. F. Roswell.[1,10]

2,3-Benzcarbazole (4e). This was obtained from three sources, of which two were commercial (Aldrich and Columbia) and the third was a preparation of the method of Bucherer and Sonnenburg[33] from sodium 2-naphthol-1-sulfonate,[34] phenylhydrazine (Fisher certified), and sodium bisulfate solution. The crude material was sublimed at 150° (50 μ) and recrystallized from a large volume of toluene to give material pure by tlc (silica gel–chloroform), mp 335° (lit.[35] mp 337°); ultraviolet (95% C_2H_5OH) 392 nm (log ε 3.53), 372 (3.52) [lit.[35] 345 nm (log ε 3.6), 375 (3.6)]. The Aldrich sample was pure enough by tlc (silica gel–chloroform) to be used directly; the Columbia sample was recrystallized from toluene to give material pure by tlc (silica gel–chloroform).

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[25] D. L. Dexter, *J. Chem. Phys.*, **21**, 836 (1953).

[26] J. R. DeMember and N. Filipescu, *J. Amer. Chem. Soc.*, **90**, 6425 (1968).

[27] T. Medinger and F. Wilkinson, *Trans. Faraday Soc.*, **61**, 620 (1965).

[28] C. A. Parker and T. A. Joyce, *ibid.*, **62**, 2785 (1966).

[29] C. A. Parker, "Photoluminescence of Solutions," Elsevier Publishing Co., New York, N. Y., 1968, p 44.

[30] I. B. Berlman, "Handbook of Fluorescence Spectra of Aromatic Molecules," Academic Press, New York, N. Y., 1965, p 120.

[31] K. Kaneta and M. Koizumi, *Bull. Chem. Soc. Jap.*, **40**, 2254 (1967).

[32] Melting points were taken with a Thomas-Hoover capillary melting point apparatus or with a hot stage microscope and are uncorrected. Infrared spectra were determined on Perkin-Elmer Model 137 or 337 instruments and were calibrated using polystyrene film. Ultraviolet spectra were determined on a Cary 14 instrument. Elemental analyses were performed by either Mr. Joseph Walters in this department or Galbraith Laboratories, Knoxville, Tenn. Solvents, except where stated, were reagent grade and used as received.

[33] H. T. Bucherer and E. F. Sonnenburg, *J. Prakt. Chem.*, **81**, 29 (1910).

[34] Elsevier's Encyclopedia of Organic Chemistry, Series III, Vol. 12B, p 5288.

[35] G. M. Badger and B. J. Christie, *J. Chem. Soc.*, 3438 (1956).

3,4-Benzcarbazole (5e). This synthesis was based on that of Fuchs and Niszel.[36] 2-Naphthol (14.4 g, 0.10 mol) and phenylhydrazine (10.8 g, 0.10 mol) were refluxed in sodium sulfite solution (250 ml, 36%) for 17 hr. The reaction mixture was then treated with concentrated hydrochloric acid (200 ml) under reflux for 2.5 hr. The oily layer which separated was isolated by decanting the upper, aqueous, layer and was triturated with ether to give a red-brown solid. The solid was extracted with sodium hydroxide solution and filtered. The remaining yellow solid was dried and sublimed (100° (15 μ) and the sublimate recrystallized from aqueous methanol to give white needles (2.5 g, 11.5 mmol, 11.5%): mp 136–137° (lit.[36] mp 135°); ultraviolet (95% C_2H_5OH) 363 nm (log ε 3.68), 345 (3.75) [lit.[35] 365 nm (log ε 3.7), 345 (3.7)].

N-Methyl-2,3-benzcarbazole (4d). To **4e** (110 mg, 0.5 mmol) in dry hexamethylphosphoric triamide (HPT) (15 ml) was added excess sodium hydride (50% dispersion in oil) in a stoppered flask. Periodically the pressure was released. After 30 min the hydrogen evolution was essentially complete and methyl iodide (Fisher) (0.5 ml, 8.1 mmol) was added. The orange color of the solution was immediately discharged. The mixture was allowed to stand overnight, after which chloroform (100 ml) was added and the organic layer washed continuously with water for 2 hr. The organic layer was separated, dried (anhydrous $MgSO_4$), and evaporated to an oil which solidified on trituration with water. The solid was filtered and recrystallized from toluene–isooctane to give pale yellow crystals (70 mg, 0.3 mmol, 60%): mp 139–140°; infrared 3040 and 2900 (weak, broad), 1630, 1600, 1475 cm^{-1}; ultraviolet 397 nm (log ε 3.61), 377 (3.54); tlc (silica gel–chloroform) showed no starting material and only one spot.

Anal. Calcd for $C_{17}H_{13}N$: C, 88.28; H, 5.67; N, 6.06. Found: C, 88.57; H, 5.70; N, 6.18.

N-Methyl-3,4-benzcarbazole (5d). This compound was prepared from **5e** (220 mg, 1.0 mmol) by the method used for **4d** to give white needles from toluene–isooctane (Norit) (90 mg, 0.39 mmol, 39%): mp 117–118°; infrared 3030 and 2930 (weak, broad), 1625, 1590 cm^{-1}; ultraviolet 369 nm (log ε 3.72); tlc (silica gel–chloroform) showed no starting material and only one spot.

Anal. Calcd for $C_{17}H_{13}N$: C, 88.28; H, 5.67; N, 6.06. Found: C, 88.49; H, 5.70; N, 6.17.

N-Methyl-4-[9-(2,3-benzcarbazolyl)methyl]phthalimide (4c). 2,3-Benzcarbazole (110 mg, 0.50 mmol) was dissolved in dry HPT (15 ml) and excess sodium hydride was added to the stoppered reaction flask. The flask was flushed with nitrogen and left for 3 hr, with initial periodic release of the pressure. The orange, green fluorescent solution was filtered through glass wool to remove excess sodium hydride. Compound **7** (125 mg, 0.50 mmol) was added to the reaction mixture to give a deep purple solution. After standing at room temperature overnight, the reaction mixture was heated on the steam bath for 3 hr. Evaporation of the solvent on the vacuum line, followed by chromatography of the residue on a preparative plate (silica gel) with chloroform as eluent, gave, on extraction with acetone, a yellow-brown solid (60 mg, 0.15 mmol, 30%). The solid was recrystallized from benzene–isooctane to give yellow-brown microcrystals: mp 166–169° dec; infrared (KBr) 1770, 1720 cm^{-1}; ultraviolet (95% C_2H_5OH) 392 nm (log ε 3.64), 373 (3.56).

Anal. Calcd for $C_{26}H_{18}N_2O_2$: C, 79.98; H, 4.65. Found: C, 79.86; H, 4.74.

N-Methyl-4-[9-(3,4-benzcarbazolyl)methyl]phthalimide (5c). This compound was prepared essentially as for **4c**, except on a 1 m*M* scale. The pure, chromatographed, product was a yellow, fluffy solid (60 mg, 0.15 mmol, 15%). Recrystallization from benzene–isooctane gave material with mp 140–143° dec; infrared (KBr) 1775, 1715 cm^{-1}; ultraviolet (95% C_2H_5OH) 365 nm (log ε 3.75), 347 (3.77).

Anal. Calcd for $C_{26}H_{18}N_2O_2 \cdot C_6H_6$: C, 82.03; H, 5.16. Found: C, 81.84; H, 5.32.

2,3-Dihydro-6-[9-(2,3-benzcarbazolyl)methyl]phthalazine-1,4-dione (4a). Compound **4c** (30 mg, 0.075 mmol) was added to 95% ethanol (3 ml) containing 95% hydrazine (*ca.* 0.1 ml, 3 mmol). This reaction mixture, after degassing (three freeze–thaw cycles), was sealed off in a glass tube. The tube was heated at 100° for 8 hr, then cooled and opened, and the contents were poured into water. The resulting precipitate was collected and crystallized from glacial acetic acid to give **4a** as a yellow solid (22 mg, 0.056 mmol, 75%), decomposing above 300°; infrared (KBr) 3000 (broad), 1660, 1620 cm^{-1}; ultraviolet (95% C_2H_5OH) 392 nm (log ε 3.66), 373 (3.57).

Anal. Calcd for $C_{25}H_{17}N_3O_2 \cdot C_2H_4O_2$: C, 71.83; H, 4.69; N, 9.31. Found: C, 71.75; H, 4.74; N, 9.37.

2,3-Dihydro-6-[9-(3,4-benzcarbazolyl)methyl]phthalazine-1,4-dione (5a). This compound was prepared by the method used for **4a**. From **5c** (50 mg, 0.13 mmol) the product was obtained as a white solid (30 mg, 0.08 mmol, 60%). Recrystallization from glacial acetic acid gave pure material, decomposing above 300°: infrared (KBr) 3050 (broad), 1720, 1660, 1610 cm^{-1}; ultraviolet (95% C_2H_5OH) 364 nm (log ε 3.75), 347 (3.77).

Anal. Calcd for $C_{25}H_{17}N_3O_2 \cdot C_2H_4O_2$: C, 71.83; H, 4.69. Found: C, 71.84; H, 4.91.

4-[(9-Oxo-10-acridanyl)methyl]phthalic Acid (3b). Compound **3c** (60 mg, 0.16 mmol) was stirred for 3 days with a solution of sodium hydroxide (10 ml, 0.1 *N*). A small amount of acetone was added periodically to aid the solubility of **3c** for ease of reaction. The solution obtained was extracted with two 10-ml portions of chloroform and filtered. Glacial acetic acid was then added to the solution until the initial precipitate just dissolved on boiling. On cooling the acid crystallized and was filtered off and dried to yield 43 mg (0.11 mmol, 72%). Several recrystallizations were necessary to obtain analytically pure material: mp 179–180° (rapid heating); infrared 1710, 1605 cm^{-1}; ultraviolet (1 *M* phosphate buffer) 405 nm (log ε 3.98), 388 (3.97); tlc (cellulose: ethanol–ammonia–water 8:1:1) showed one spot.

Anal. Calcd for $C_{22}H_{15}NO_5$: C, 70.77; H, 4.05. Found: C, 70.91; H, 4.13.

4-[9-(2,3-Benzcarbazolyl)methyl]phthalic acid (4b). This compound was prepared as for **3b** from **4c** (60 mg, 0.15 mmol) to give a fine yellow powder by very slow crystallization (20 mg, 0.05 mmol, 33%): mp 214–215°; infrared 1715, 1605 cm^{-1}; ultraviolet (1 *M* phosphate buffer) 397 nm (log ε 3.58), 377 (3.51); tlc (cellulose: ethanol–ammonia–water 8:1:1) showed one spot.

Anal. Calcd for $C_{25}H_{17}NO_4$: C, 75.94; H, 4.33. Found: C, 75.50; H, 4.48.

4-[9-(3,4-Benzcarbazolyl)methyl]phthalic acid (5b). This acid was prepared as for **3b** from **5c** (60 mg, 0.15 mmol) to give a pale yellow powder (40 mg, 0.10 mmol, 66%). Several recrystallizations were necessary to obtain analytically pure material: mp 169–172° (rapid heating); infrared 1705, 1605 cm^{-1}; ultraviolet (1 *M* phosphate buffer) 365 nm (log ε 3.62), 348 (3.68); tlc (cellulose: ethanol–ammonia–water 8:1:1) showed one spot.

Anal. Calcd for $C_{25}H_{17}NO_4 \cdot 0.5H_2O$: C, 74.28; H, 4.48. Found: C, 74.66; H, 4.51.

4-[(9-Carbazolyl)methyl]phthalic Acid (6b). This acid was prepared as for **3b** from 6c (60 mg, 0.17 mmol) to give an off-white powder (35 mg, 0.10 mmol, 60%), decomposing above 280°: infrared 1705, 1600 cm^{-1}; ultraviolet (1 *M* phosphate buffer) 342 nm (log ε 3.60), 327 (3.56); tlc (cellulose: ethanol–ammonia–water 8:1:1) showed one spot.

Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.04; H, 4.38. Found: C, 73.35; H, 4.54.

Quinine Bisulfate. This was obtained from Pfaltz and Bauer and was recrystallized three times from water, and dried in a vacuum oven (12 mm (80°)). The compound was kept in a black bottle in a desiccator until used. It had ϵ_{346} 5.14 × 10^3 (lit.[37,38] 1.09 × 10^4, 1.04 × 10^4 for quinine sulfate). The compound was pure by tlc (silica gel: ethanol–ammonia–water 8:1:1). Suitable scanning of both excitation and emission monochromators showed quinine to be the only fluorescent compound present.

Emission Spectra. All emission spectra, both photoluminescence and chemiluminescence, were measured on a Hitachi-Perkin-Elmer MPF-2A spectrophotofluorimeter, with a stabilized Xenon arc source and a R106 photomultiplier detector. The wavelength scale of the emission monochromator was checked with a mercury lamp and found to be accurate to within 1 nm. The instrument was calibrated so that the measured spectra could be corrected for nonlinearity of both the source and the detector. The source and excitation monochromator combination were calibrated using a solution of Rhodamine B (4 g/l. in glycerol) as a quantum counter. The excitation spectrum of the Rhodamine B gave directly the calibration curve for the excitation portion of the instrument. The detector and emission monochromator combination were calibrated using a quartz–iodine spectral irradiance standard (Electro Optics Associates, type L101). The light was directed onto the entrance slit of the monochromator by means of a diffusing surface of freshly prepared magnesium oxide. From the experimentally

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[36] W. Fuchs and F. Niszel, *Chem. Ber.*, 60, 213 (1927).

[37] J. W. Eastman, *Photochem. Photobiol.*, 6, 55 (1967).

[38] R. Rusakowicz and A. C. Testa, *J. Phys. Chem.*, 72, 793 (1968).

obtained spectrum and the known emission curve of the source, the correction curve between 300 and 600 nm was determined.

The fluorescence emission spectra of **3b–6b** were measured using both $\sim 10^{-4}$ *M* and $\sim 3 \times 10^{-6}$ *M* solutions in 1 *M* phosphate buffer (96.6 g of KOH and 348.4 g of K_2HPO_4 in 2 l. of H_2O, measured pH 11.8). The spectra measured at $\sim 3 \times 10^{-6}$ *M* were used to calculate the fluorescence quantum yields.

The phosphorescence emission spectrum of dipotassium phthalate in a methanol glass was measured using the phosphorescence accessory provided with the MPF-2A instrument. This was corrected by a computer program (see below).

The overlap integrals were calculated using a graphical integration procedure. The function $F(\bar{\nu})$ given by

$$F(\bar{\nu}) = p(\bar{\nu})\epsilon(\bar{\nu})/\bar{\nu}^4$$

where $p(\bar{\nu})$ was the emission intensity of the phosphorescence of dipotassium phthalate (normalized such that $\int p(\bar{\nu})d\bar{\nu}$ was unity) and $\epsilon(\bar{\nu})$ was the extinction coefficient of the acceptor (**3b–6b**), was calculated at intervals of 250 cm^{-1}. The function $F(\bar{\nu})$ was then plotted against $\bar{\nu}$ and the area under each curve determined by weighing and comparing the weight to that of a standard rectangle. Since the overlap integrals J_{DA} are given by

$$J_{DA} = \int F(\bar{\nu})d\bar{\nu}$$

this gave the required integrals directly.

Chemiluminescence emission spectra were obtained by reacting solutions of the hydrazides ($\sim 10^{-4}$ *M* in 1 *M* phosphate buffer) with aqueous solutions of hemin (OD_{414} ~ 0.5) and hydrogen peroxide ($\sim 0.5\%$) and running the spectrofluorimeter with the source off. Wide slits (up to 16 nm band pass) and fast scanning rates were necessary because of the low light levels and the relatively rapid decay of the light emission. In this manner reasonably undistorted spectra were obtained. However, it is for this reason that the quoted chemiluminescence emission maxima may be in error by ±3 nm.

Fluorescence Quantum Yields. These quantum yields were measured relative to quinine bisulfate (3.4×10^{-6} *M* in 1 *N* H_2SO_4). The optical densities of all the solutions used for these determinations were 0.02 or less. The five emission spectra (**3b–6b** and the standard) were tabulated for intervals of 2 or 3 nm and the resulting data used, in conjunction with a computer program containing the instrumental correction factors, to correct the spectra and calculate the quantum yields. From the data (presented as relative intensity on a wavelength scale), the optical densities of the solutions, the quantum yield of the standard, the refractive indices of the solvents, the instrumental sensitivity factors, and the relative lamp output at the different excitation wavelengths, the program calculated the quantum yields of the unknowns and presented the corrected spectra on a wave-number scale.

Chemiluminescence Quantum Yields. The relative chemiluminescence quantum yields of the four hydrazides, **3a–6a**, were determined using a photomultiplier (RCA 1P21) powered by a Fluke 4128 DC power supply. A glass vial (25 mm × 75 mm) in a totally light-tight enclosure formed the reaction cell, and the detector was placed about 12 cm above the surface of the reaction mixture. The signal from the photomultiplier, after amplification, was fed to a capacitor. The capacitor was connected to a Varian G-10 chart recorder, the deflection of which at any instance was proportional to the integrated light output from the start of the reaction to that time. The measured light yields were corrected for the nonlinear response of the photomultiplier using the published S-4 response curve, given in the RCA handbook, and the already corrected fluorescence emission spectra.

Solutions of the hydrazides (about 5×10^{-5} *M*) were made up accurately by dissolving the weighed material in five drops of dimethyl sulfoxide before adding 1 *M* phosphate buffer to the mark. The hydrogen peroxide solution was 0.1% and the hemin had an optical density of 0.2 at 414 nm.[39] Aliquots (1 ml) of the hydrazides were used and 0.2-ml aliquots of both hemin and hydrogen peroxide were injected in rapid sequence to initiate the reaction. Additional hemin and peroxide was added to complete the reaction. For **4a** the solution had to be heated to about 40° before reaction occurred at a reasonable rate. This was presumably because the extreme insolubility of this hydrazide meant that it was not in true solution at room temperature and required a somewhat higher temperature to allow the rate of dissolution to be comparable to the rate of reaction. The product solutions were checked, using the spectrofluorimeter, for the appearance of the fluorescence of the acceptor portion of the molecules (the initial solutions were completely nonfluorescent). The final values obtained were the mean of at least three determinations and are probably accurate to ±15%.

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(39) J. Lee, A. S. Wesley, J. F. Ferguson, and H. H. Seliger in "Bioluminescence in Progress," F. H. Johnson and Y. Hameda, Ed., Princeton University Press, Princeton, N. J., 1966, p 35.