Chemiluminescence of Linear Hydrazides

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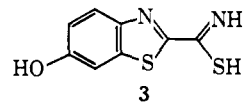
Received June 16, 1971

Abstract: Derivatives of two efficient chemiluminescent linear hydrazides, 9-acridinecarboxylic acid hydrazide (**4d**) and 2-(6′-hydroxy-2′-benzothiazolyl)thiazole-4-carboxylic acid hydrazide (**1b**), were synthesized. The chemiluminescence emission spectra of these compounds in dimethyl sulfoxide (with potassium *tert*-butoxide and oxygen) suggest that the corresponding acid anion is the light emitter. Different alkyl substituents on the hydrazide nitrogens were introduced, and a study of the chemiluminescent reaction products under different conditions was carried out. Base-catalyzed autoxidation followed by electron transfer is discussed as a likely mechanism.

Chemiluminescence in liquid solutions has been interpreted in terms of two general mechanisms leading to electronically excited states (followed by light emission). There are several examples in which a four-membered peroxide (dioxetane) is believed to be generated during a chemiluminescent reaction.[1a] Such four-membered peroxides have been isolated recently and shown to decompose thermally to give electronically excited products.[1c] This type of decomposition has been interpreted in terms of the Woodward–Hoffman rules of orbital symmetry[2] or an antiaromatic transition state for the decomposition.[1b] The second type of chemiluminescent reaction involves simple electron transfer,[3] which can lead directly to excited states.

Chemiluminescent cyclic hydrazides have been studied extensively[4] in both protic[5] and aprotic[6] systems. The best known example of the chemiluminescent hydrazides is luminol (5-amino-2,3-dihydro-1,4-phthalazinedione).[7] Its chemiluminescence has been studied by several groups,[4,7,8] and 3-amino phthalate was suggested as the light emitter in both protic and aprotic media.[4] Any substitution on the heterocyclic ring of the cyclic hydrazides renders the compounds nonchemiluminescent.[9] It was because of this limitation that we looked for efficient chemiluminescent linear hydrazides and studied their reactivity with different substitution patterns on the hydrazide nitrogens. Chemiluminescent linear hydrazides have been reported previously,[10] but the low quantum yield for emission in most of these cases was the main difficulty in carrying out mechanistic studies. We now report the synthesis of several efficient monoacylhydrazines and their chemiluminescence in aprotic media; a strong base and oxygen are the only requirements for light emission.

Synthesis. The reaction of 2-cyano-6-hydroxybenzothiazole[11] with hydrogen sulfide in pyridine and triethylamine[12] afforded 6-hydroxybenzothiazole-2-thiocarboxamide (**3**). The thioamide **3** reacted smoothly



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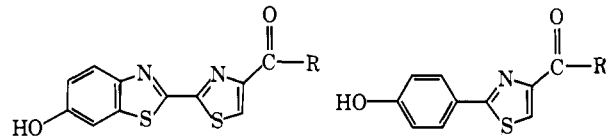


1 **2**

a, R = –OEt
b, R = –$NHNH_2$
c, R = –$NHNHCH_3$
d, R = –OH

with ethyl bromopyruvate in methanol to give the mixed ethyl and methyl esters of dehydroluciferin (**1a**). Treatment of the esters with 95% hydrazine gave the parent hydrazide **1b**, and treatment with monomethylhydrazine in refluxing ethanol gave the 1-acyl-2-methylhydrazine **1c**.

It has been reported that the reaction of an ester, bearing a large acyl group with monomethylhydrazine, yields the 1-acyl-2-methylhydrazine with only small amounts of the 1-acyl-1-methylhydrazine.[13] Confirmation of structure **1c** was obtained from spectral data; the nmr spectrum showed the methyl proton absorption at τ 7.38 (3 H, singlet),[14] whereas the methyl protons of a 1-acyl-1-methylhydrazine absorb around τ 6.5 (see Experimental Section). Saponification of the ester **1a** led to 2-(6′-hydroxy-2′-benzothiazolyl)thiazole-4-carboxylic acid (dehydroluciferin, **1d**), which

(1) (a) E. H. White and M. J. C. Harding, *Photochem. Photobiol.*, 4, 1129 (1965); F. McCapra, D. G. Richardson, and Y. C. Chang, *ibid.*, 4, 1111 (1965); M. M. Rauhut, *Accounts Chem. Res.*, 2, 80 (1969); (b) F. McCapra, *Chem. Commun.*, 155 (1968); (c) K. R. Kopecky and C. Mumford, *Can. J. Chem.*, 47, 709 (1969); E. H. White, J. Wiecko, and C. C. Wei, *J. Amer. Chem. Soc.*, **92**, 2167 (1970).

(2) R. Hoffman and R. B. Woodward, *Accounts Chem. Res.*, 1, 17 (1968); D. R. Kearns, *J. Amer. Chem. Soc.*, **91**, 6554 (1969).

(3) (a) D. M. Hercules, *Accounts Chem. Res.*, 2, 301 (1969); (b) E. A. Chandross and F. I. Sonntag, *J. Amer. Chem. Soc.*, 88, 1089 (1966).

(4) E. H. White and D. F. Roswell, *Accounts Chem. Res.*, 3, 54 (1970).

(5) E. H. White and M. M. Bursey, *J. Org. Chem.*, 31, 1912 (1966); K. D. Gundermann, "Chemilumineszenz Organischer Verbindungen," Springer-Verlag, New York, N. Y., 1968.

(6) E. H. White, *J. Chem. Educ.*, 34, 275 (1957).

(7) H. O. Albrecht, *Z. Phys. Chem.*, 136, 321 (1928).

(8) M. M. Rauhut, A. M. Semsel, and B. G. Roberts, *J. Org. Chem.*, 31, 2431 (1966).

(9) H. D. K. Drew and R. F. Garwood, *J. Chem. Soc.*, 836 (1939).

(10) (a) A. A. M. Witte, *Recl. Trav. Chim. Pays-Bas*, 64, 471 (1935); (b) J. S. Wasserman and G. P. Mikluchin, *Zh. Obshch. Khim.*, **9**, 606 (1939); (c) H. Ojima, *Naturwissenschaften*, 48, 600 (1961); (d) E. H. White, M. M. Bursey, D. F. Roswell, and J. H. M. Hill, *J. Org. Chem.*, **32**, 1198 (1967); (e) J. Kroh and J. Luszczewski, *Rocz. Chem.*, 30, 647 (1956).

(11) (a) E. H. White, H. Wörther, G. F. Field, and W. D. McElroy, *J. Org. Chem.*, 30, 2344 (1965); (b) S. Keto, K. Ogura, and Y. Nishiyana, *Bull. Chem. Soc. Jap.*, 36, 331 (1963).

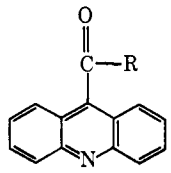
(12) A. E. S. Fairfull, J. L. Lowe, and D. A. Peak, *J. Chem. Soc.*, 742 (1952).

(13) R. L. Hinman and O. Fulton, *J. Amer. Chem. Soc.*, 80, 1895 (1958).

(14) W. J. Theuer and J. A. Moore, *J. Org. Chem.*, **29**, 3734 (1964).

was used in the fluorescence studies. Derivatives of another fluorescent acid, 2-(4′-hydroxyphenyl)thiazole-4-carboxylic acid (**2d**), were prepared by analogous reactions starting with 4-hydroxybenzonitrile.

The 9-acridinoyl system **4** proved to be the most convenient for our purposes because of the availability



4a, R = -OH
b, R = -Cl
c, R = -OMe
d, R = $-NHNH_2$
e, R = $-NHNHCH_3$
f, R = $-N(CH_3)N{=}CHCH_3$
g, R = $-N(CH_3)NH_2$
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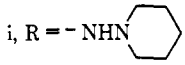
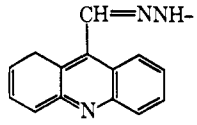
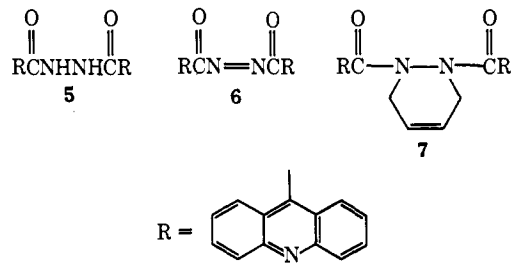
i, R = 
j, R = 

of the acid chloride **4b** and the efficient light emission from certain derivatives. 9-Acridinecarboxylic acid[15] (**4a**), 9-chlorocarbonylacridine[16] (**4b**), and 9-carbomethoxyacridine[16] (**4c**) were prepared according to literature procedures. Treatment of the methyl ester **4c** with 95% hydrazine in refluxing ethanol gave the parent hydrazide **4d** whereas the same reaction with monomethylhydrazine was unsuccessful, leading instead to oxidation products. The acid chloride **4b** reacted with monomethylhydrazine to give a mixture of the two monoacyl–monomethyl hydrazines **4e** and **4g**. The two isomers were treated with acetaldehyde in 2-propanol, followed by column chromatography, to give the 1-acyl-1-methyl-2-ethylidene derivative **4f**, which in turn was treated with acid to give the pure 1-acyl-1-methylhydrazine **4g**. The second isomer **4e** could not be isolated in a pure state from the column. Hydrazine derivatives **4h** and **4i** were prepared by the reaction of the acid chloride **4b** with *sym*-dimethylhydrazine and with *N*-aminopiperidine, respectively, in methylene chloride–pyridine solutions. The 9-acridinoylhydrazone of 9-formylacridine (**4j**) was isolated from the reaction of hydrazide **4d** with 9-formylacridine[17] in 2-propanol. Treatment of the acid chloride **4b** with 0.5 equiv of hydrazine in methylene chloride–pyridine afforded 1,2-bis(9-acridinoyl)hydrazine (**5**). Oxidation of **5** with *N*-bromosuccinimide in methylene chloride–pyridine gave azobis-9-acridinoyl (**6**). The spectroscopic properties of **6** [infrared absorption at 1745 and 1705 cm^{-1} and visible absorption at 480 nm (log ϵ 3.14)], are in agreement with those of bisdiacylazo compounds.[18] Bubbling butadiene through a



5 **6** **7**

R =

solution of **6** in benzene followed by column chromatography gave 1,2-di(9-acridinoyl)-1,2-diazacyclohex-4-ene (**7**), which serves as another proof of the structure of **6**.

Chemiluminescence and Fluorescence Results. The chemiluminescence experiments reported here were performed in dimethyl sulfoxide solutions with *tert*-butoxide as base. Oxygen was necessary for light emission from all the reactive hydrazides; water or alcohol quenched the chemiluminescent emission in all cases. The unsubstituted hydrazides had the highest quantum yield for emission in each series. Quantum yields of 3×10^{-4} and 5×10^{-4} were found for **4d** and **1b**, respectively; these values are relatively high for acyclic hydrazides.[19] Both values were very sensitive to the reaction conditions and they probably do not represent maximum values. Quantum yields for chemiluminescent emission in the 9-acridinoyl series decreased in the order **4d** > **6** > **4g** > **4h** > **4j**.

Product analysis of the chemiluminescent oxidation of 9-acridinecarboxylic acid hydrazide (**4d**) showed as reaction products the corresponding acid **4a** (80% yield), acridone (5%), and acridine (1.5%). All the other chemiluminescent linear hydrazides gave the corresponding acids as the major product (shown by thin-layer chromatography compared with authentic samples of the acids). Carbon monoxide was identified as a minor product in the chemiluminescent reaction of derivatives **4d**, **4g**, **4h**, and **6**.

The chemiluminescence emission spectra of hydrazides **1b** and **2b** match the fluorescence emission spectra of the spent reaction mixtures, and also the fluorescence emission spectra of the corresponding acid anions **1d** and **2d**, respectively (Table I). In the case of the active derivatives of 9-acridinecarboxylic acid hydrazide, the chemiluminescence emission spectra matched the fluorescence emission spectra of the spent reaction mixtures, and the fluorescence emission spectrum of a mixture of 9-acridinecarboxylic acid (95%) and acridone (5%). These data suggest that light emission is a result of the formation of the corresponding acid anions in an electronically excited state. In the case of 9-acridinoyl derivatives, emission from electronically excited acridone accompanies the emission from the excited acid anion.[20] Several alkyl derivatives of chemiluminescent parent hydrazides did not produce any observable light emission in our system. These include the 1-acyl-2-methylhydrazines **1c** and **2c**, the 1-acyl-1-methyl-2-ethylidenehydrazine **4f**, and the 1-acyl-2,2-dialkylhydrazine **4i**. 1,2-Bis-9-acridinoylhydrazine (**5**) was also "dark."

Acyl Anions as Reaction Intermediates. The base-catalyzed autoxidation of linear[21–23] and cyclic[24,25]

(15) K. Lemstedt and E. Wirth, *Chem. Ber.*, **61**, 2044 (1928).
(16) M. M. Rauhut, D. Sheehan, R. A. Clarke, B. G. Roberts, and A. M. Semsel, *J. Org. Chem.*, 30, 3587 (1965).
(17) A. Albert, "The Acridines," E. Arnold, London, 1951, p 148.
(18) E. Fahr and H. Lind, *Angew. Chem., Int. Ed. Engl.*, **5**, 372 (1966).
(19) Reference 4.
(20) Light emission from electronically excited acridone is discussed in the following paper: E. Rapaport, M. W. Cass, and E. H. White, *J. Amer. Chem. Soc.*, **94**, 3160 (1972).

Table I. Chemiluminescence and Fluorescence Results[a]

Compd	Wavelength max, nm[b] Chemiluminescence emission	Wavelength max, nm[b] Fluorescence of spent reaction mixture	Wavelength max, nm[b] Fluorescence
1b	551 (54)	550 (52)	
1c	None		
1d			551 (53)
2b	447 (44)	444 (42)	
2c	None		
2d			443 (42)
4a			468, 498, 527 (sh)[c]
4d	468, 498, 523 (sh)[d]	468, 498, 525 (sh)[d]	
4f	None		
4g	467, 498, 524 (sh)[d]	467, 498, 525 (sh)[d]	
4h	467, 498, 525 (sh)[d]	467, 498, 525 (sh)[d]	
4i	None		
4j	467, 499, 527 (sh)[d]	466, 498, 526 (sh)[d]	
5	None		
6	466, 497, 529 (sh)[d]	466, 498, 529 (sh)[d]	
Acridone			471, 498, 524 (sh)[e]
4a (95%) + acridone (5%)			468, 498, 525 (sh)[d]

[a] Slit width and scanning speeds constant in all runs; concentration 10^{-4} *M* in dry dimethyl sulfoxide with excess potassium *tert*-butoxide. [b] Full width at half-maximum intensity is reported in parentheses; the accuracy was estimated to be $\lambda_{max} \pm 2$ nm and fwhm ± 5 nm. [c] The peak-height ratio of 468:498 nm is about 2. [d] The peak-height ratio of 467:498 nm is 1–1.5. [e] The peak-height ratio of 471:498 nm is 0.9–1.0.

hydrazides is a well-known reaction and acyldiimides have been proposed as intermediates. It is likely, therefore, that the first step in the chemiluminescent autoxidation of linear hydrazides yields the corresponding acyldiimides (eq 1).

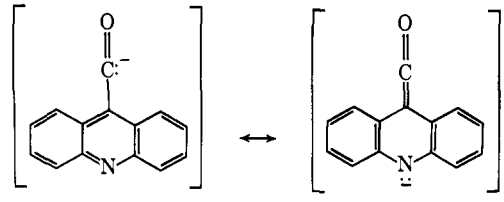
$$\mathrm{RCNHNH_2} \xrightarrow[\text{DMSO}]{\textit{tert}\text{-BuO}^-} \mathrm{RCNNH_2} \xrightarrow{O_2} \mathrm{RCN{=}NH} \qquad (1)$$

The related alkyldiimides are intermediates in the basic cleavage of 2-alkylarylsulfonylhydrazines,[26] the reductive deamination of aliphatic amines,[27] and the reaction of difluoramine with some amines.[28] In the presence of a strong base they appear to generate carbanions, while in the absence of a base or in the presence of a weak base the alkyldiimides decompose by other pathways.[29]

The activity of an alkoxide toward N–H bonds is greatly enhanced in dimethyl sulfoxide for much the same reason that it is enhanced toward C–H bonds.[30] A pK_a of 26.9 was reported[31] for methanol in dimethyl sulfoxide and *tert*-butoxide is considered to be a stronger base than methoxide.[32] It is therefore reasonable to assume that the second step of the reaction of monoacylhydrazines under our conditions involves the loss of a proton, and the third step is the loss of nitrogen. An acyl anion as the reactive intermediate

$$\mathrm{RCN{=}NH} \xrightarrow[\text{DMSO}]{\textit{tert}\text{-BuO}^-} \mathrm{RCN{=}N^-:} \longrightarrow \mathrm{RC:^-} \qquad (2)$$

(eq 2) would be consistent with our experimental results. In the case of 9-acridinoyl derivatives this intermediate will be resonance stabilized (eq 3)[33] and therefore its formation will be favored compared to the other two systems (**1** and **2**) which were studied. This fact may explain the relatively high quantum yields for chemiluminescent emission of 9-acridinecarboxylic acid hydrazide derivatives despite the fact that the fluorescence emission of 9-acridinecarboxylic acid (**4a**) is considerably weaker than that of the acids **1d** and **2d** under the reaction conditions.

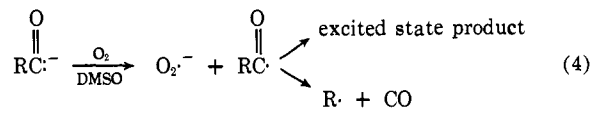


(3)

Russell, *et al.*,[34] felt that reactive carbanions are oxidized with molecular oxygen in dimethyl sulfoxide to the corresponding free radical and superoxide ion. A similar route can account for the presence of carbon monoxide as a minor product (eq 4).

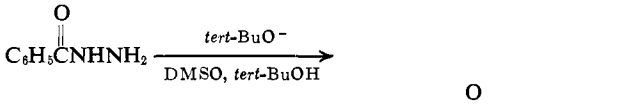
The autoxidation products of benzhydrazide in dimethyl sulfoxide–*tert*-butyl alcohol solutions with *tert*-butoxide as base are also consistent with a reactive carbanion as an intermediate. The yield of benzaldehyde

(21) E. Mossetig, *Org. React.*, 8, 233 (1954).
(22) C. Niemann and J. T. Hays, *J. Amer. Chem. Soc.*, 74, 5796 (1952).
(23) For a recent review on base-catalyzed autoxidation see G., Sosnovsky and E. H. Zaret in "Organic Peroxides," Vol. 1, D. Swern, Ed., Wiley-Interscience, New York, N. Y., 1970, p 517.
(24) E. H. White, E. G. Nash, D. R. Roberts, and O. C. Zafiriou, *J. Amer. Chem. Soc.*, 90, 5932 (1968).
(25) (a) Y. Omotoe, T. Miyake, and N. Sugiyama, *Bull. Chem. Soc. Jap.*, 40, 2446 (1967); (b) K. D. Gunderman, H. Fiege, and G. Klockenbring, *Justus Liebigs Ann. Chem.*, 738, 140 (1970).
(26) D. J. Cram and J. S. Bradshaw, *J. Amer. Chem. Soc.*, 85, 1108 (1963).
(27) A. Nickon and A. S. Hill, *ibid.*, 86, 1152 (1964).
(28) C. L. Bumgardner and J. P. Freeman, *ibid.*, 86, 2233 (1964).
(29) D. J. Cram in "Fundamentals of Carbanion Chemistry," Academic Press, New York, N. Y., 1965, p 213.
(30) (a) D. J. Cram and M. R. V. Sahynn, *J. Amer. Chem. Soc.*, 84, 1734 (1962); (b) D. J. Cram, B. Rickborn, C. A. Kingsburg, and P. Haberfield, *ibid.*, 83, 3678 (1961).
(31) E. C. Steiner, presented at the 153rd National Meeting of the American Chemical Society, Miami, Fla., April 1967.
(32) C. D. Ritchie and R. E. Uschold, *J. Amer. Chem. Soc.*, 89, 2960 (1967).
(33) For an example of basic autoxidation of a resonance-stabilized carbanion, see: G. A. Russell, A. G. Bemis, E. J. Geels, E. G. Janzen, and A. J. Moye, *Advan. Chem. Ser.*, No. 75, 195 (1968).
(34) (a) G. A. Russell and E. G. Janzen, *J. Amer. Chem. Soc.*, 84, 4153 (1962); (b) G. A. Russell and A. G. Bemis, *ibid.*, 88, 5491 (1966).



(4)

(as its condensation product with benzhydrazide) increases with an increase in the amount of *tert*-butyl



alcohol in the solvent mixture (Table II). Also basic

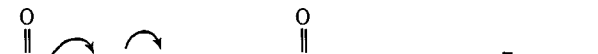
Table II. Autoxidation Products of Benzhydrazide in Basic Solutions[a]

Run no.	Ratio DMSO:*tert*-butyl alcohol	% *N*-benzoylhydrazone of benzaldehyde	% benzoic acid
1	10:0	None	107
2	9:1	None	104
3	7:3	22	69
4	2:8	51	35

[a] Potassium *tert*-butoxide was used in excess. All solutions were of the same concentration of benzhydrazide and base (Experimental Section).

oxidation of aromatic monoacylhydrazides[21,22] in aqueous solution is known to yield the corresponding aldehydes. These conditions are much milder than those employed in the present study. In fact 9-formylacridine is oxidized under our reaction conditions to the corresponding acid **4a** with light emission from the excited state of the acid.[20] This aldehyde, and its derivative **4j**, were eliminated from being necessary intermediates in the light reaction because they have lower quantum yields for emission than the corresponding hydrazide **4d**.

Consistent with an acyl anion as the reactive intermediate is the high reactivity of azobis-9-acridinoyl (**6**), which chemiluminesces brightly in dimethyl sulfoxide with *tert*-butoxide as base in the presence of oxygen. By analogy to the known reaction of 1-acyl-2-methylazo derivatives which react with alkoxide to give esters plus methyl carbanion (eq 5),[35] the bisdiacylazo compound **6** should yield the acyl anion which we propose to be the reactive intermediate in the light reaction of the hydrazides (eq 6).



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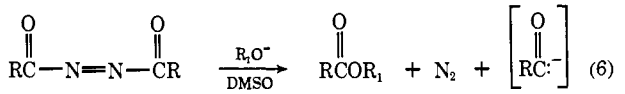
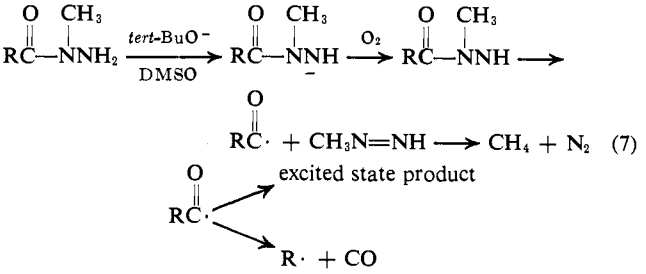


(6)

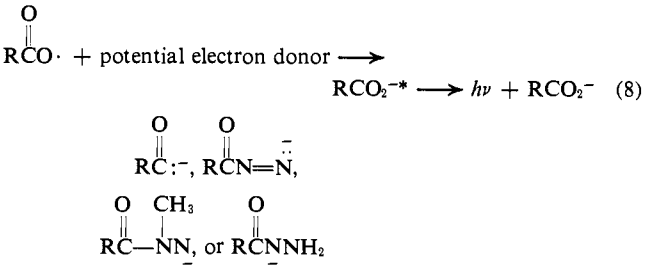
The 1-acyl-1-methylhydrazine **4g** and 1-acyl-1-methyl-2-methylhydrazine **4h** are reactive in our chemiluminescent system and their emission spectra match the fluorescence emission spectrum of the corresponding acid **4a**. These two hydrazides are stable in dimethyl sulfoxide solutions with excess *tert*-butoxide in the absence of oxygen. In the presence of oxygen under the same conditions, hydrazide **4g** produces methane as a major product and carbon monoxide as a minor product.



(7)

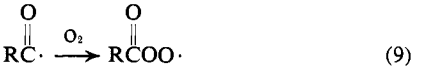
Equations 4 and 7 account for the formation of these products.

Excitation *via* Electron Transfer. Hercules, *et al.*, have reported that an electronically excited state is produced when hydrazine reduces the radical cation of 1,6-diaminopyrene;[36] presumably, an electron is transferred to an antibonding orbital of the radical cation. A similar process involving carboxylate radicals as acceptors and various anionic intermediates as donors (eq 8) seems reasonable for the chemiluminescence of the hydrazides since carboxylate ions are the light emitters in the reaction. The carboxylate radicals required (eq 8)



(8)

could be produced by (1) oxidation of the acyl anion[34] (eq 4), (2) addition of oxygen to the resulting radical[37a] (eq 9), and (3) dimerization of the peroxy radicals, followed by loss of oxygen[37b] (eq 10).



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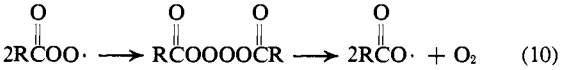

(10)

The compounds which do not chemiluminesce in our system provide further support for the proposed mechanism. The 1-acyl-2-methylhydrazines **1c** and **2c** did not produce any light emission although a dark reaction did take place leading to the corresponding acid (shown by thin-layer chromatography). Autoxidation of **1c** and **2c** in basic solutions should give 1-acyl-2-methylazo derivatives (eq 11) which react with bases at the carbonyl group[35] (eq 5) and thus do not lead to acyl anions. The trisubstituted hydrazide **4f**, the 1-acyl-2,2-disubstituted derivative **4i**, and 1,2-bis-9-acridinoyl-

(35) (a) R. W. Hoffman, *Chem. Ber.*, **97**, 2763, 2772 (1965); (b) *Angew. Chem., Int. Ed. Engl.*, **2**, 222 (1965).

(36) (a) D. M. Hercules, R. C. Lansbury, and D. K. Roe, *J. Amer. Chem. Soc.*, 88, 4578 (1966); (b) D. M. Hercules and F. E. Lytle, *ibid.*, 88, 4745 (1966).

(37) (a) W. A. Pryor in "Free Radicals," McGraw-Hill, New York, N.Y., 1966; (b) J. A. Howard and K. U. Ingold, *J. Amer. Chem. Soc.*, **90**, 1056 (1968).

$$RCNHNHCH_3 \xrightarrow[DMSO]{tert\text{-}BuO^-} RCNNHCH_3^- \xrightarrow{O_2} RCN{=}NCH_3 \quad (11)$$

hydrazine (**5**) are stable under the reaction conditions (shown by thin-layer chromatography).

From the fluorescence emission spectrum of 9-acridine carboxylate (Table I), 2.65 eV (61 kcal/mol) is required to produce the excited singlet state. The electrode potential has been determined[38a] to be 2.41 eV for the couple $CH_3CO_2^-|CH_3CO_2\cdot + e^-$ and 1.66 eV for the couple $C_6H_5CO_2^-|C_6H_5CO_2\cdot + e^-$. Using the latter value as a model for the acridine system, an additional 1 eV is required from the electron transfer reaction to produce excited acridine carboxylate. The energies available from the anions we have invoked as reasonable donors (eq 8) are unknown, but they are surely higher than the energy available from the oxidation of highly stabilized anions such as triphenylmethide ion.[38b]

Experimental Section

Melting points were taken with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories (Knoxville, Tenn.). Infrared spectra were determined on a Perkin-Elmer Model 337 instrument. Ultraviolet and visible spectra were determined on a Cary Model 14 instrument. Proton magnetic resonance spectra (pmr) were determined on Varian Associates A-60 and HR-100 instruments; chemical shifts are reported in τ units relative to tetramethylsilane (TMS). Solvents, except where stated, were reagent grade and used as received.

4-Hydroxybenzenethiocarboxamide. Hydrogen sulfide was bubbled for 4 hr through a solution of 4-hydroxybenzonitrile (5.0 g, 0.041 mol) in pyridine (70 ml) and triethylamine (3 ml). The solution was allowed to stand overnight and the solvent evaporated *in vacuo*. The dry residue was triturated with benzene, filtered, and recrystallized from ethanol to give the thioamide (5.4 g, 0.035 mol) in 85% yield, mp 182–184°. Two more recrystallizations from ethanol raised the melting point to 185–186°: ir (KBr) 3180 and 1635 cm^{-1}; uv (95% ethanol) 308 (log ϵ 3.87) and 278 nm (3.89).

Anal. Calcd for C_7H_7NOS: C, 54.90; H, 4.57; N, 9.51. Found: C, 55.04; H, 4.71; N, 9.30.

Methyl 2-(4′-Hydroxyphenyl)thiazole-4-carboxylate (2a). Ethyl bromopyruvate (3.9 g, 20 mmol) was added to a suspension of 4-hydroxybenzenethiocarboxamide (1.5 g, 9.9 mmol) in methanol (40 ml), and the reaction mixture was stirred at room temperature for 12 hr. It was then heated on a steam bath to yield upon cooling a cream product, recrystallized from methanol to give white plates (1.7 g, 7.3 mmol, 74%): mp 195–196°; ir (KBr) 3350 and 1705 cm^{-1}; uv (95% ethanol) 298 (log ϵ 3.72) and 208 nm (3.94). Elementary analysis showed almost complete ester interchange.

Anal. Calcd for $C_{11}H_9NO_3S$: C, 56.17; H, 3.83; N, 5.95; S, 13.61. Found: C, 56.32; H, 4.04; N, 5.83; S, 13.88.

2-(4′-Hydroxyphenyl)thiazole-4-carboxylic Acid Hydrazide (2b). Hydrazine (95%, 0.5 ml) was added to a stirred solution of **2a** (0.2 g, 0.83 mmol) in methanol (50 ml) at 40°. The resulting solution was refluxed for 6 hr and then concentrated to 15 ml *in vacuo*. Hydrazine (95%, 3 ml) was added and the solution refluxed for 10 min. Cooling gave a crude product (0.131 g, 0.56 mmol, 68%) which was recrystallized twice from methanol: mp 247–249°; ir (KBr) 3200–2500 (broad) and 1650 cm^{-1}; uv (95% ethanol) 304 (log ϵ 4.15) and 205 nm (4.34).

Anal. Calcd for $C_{10}H_9N_3O_2S$: C, 51.06; H, 3.83; N, 17.87. Found: C, 50.89; H, 3.58; N, 17.74.

2-(4′-Hydroxyphenyl)thiazole-4-carboxylic Acid (2d). A solution of **2a** (0.2 g, 0.8 mmol) in 10% aqueous sodium hydroxide (30 ml) was refluxed for 3 hr. The solution was cooled, filtered, and acidified to give a white product, mp 215–217° (0.135 g, 0.65 mmol, 81%). An analytical sample was prepared by dissolving the acid in sodium hydroxide and slowly acidifying with 1 *N* hydrochloric acid: ir (KBr) 2500–3300 (broad) and 1670 cm^{-1}; uv (95% ethanol) 300 nm (log ϵ 4.38).

Anal. Calcd for $C_{10}H_7NO_3S$: C, 54.30; H, 3.16; N, 6.33; S, 14.48. Found: C, 54.38; H, 3.49; N, 6.09; S, 14.24.

1-[2-(4′-Hydroxyphenyl)thiazole-4-carboxy]-2-methylhydrazine (2c). Monomethylhydrazine (4.0 ml) was added to a solution of **2a** (2.0 g, 8.3 mmol) in ethanol (40 ml), and the resulting solution was refluxed for 30 hr. The solution was concentrated *in vacuo* to about 10 ml and white needles precipitated after cooling. The product was recrystallized from ethanol (1.44 g, 5.8 mmol, 70%): mp 194–195°; ir (KBr) 3100–3300 (broad) and 1660 cm^{-1}; uv (95% ethanol) 304 nm (log ϵ 4.36); nmr (DMSO-d_6) τ 1.78 (1 H, s), 2.04 (2 H, d, J = 9 Hz), 3.06 (2 H, d, J = 9 Hz), and 7.39 (3 H, broad singlet).

Anal. Calcd for $C_{11}H_{11}N_3O_2S$: C, 53.01; H, 4.42; N, 16.87. Found: C, 53.20; H, 4.35; N, 16.59.

6-Hydroxybenzothiazole-2-thiocarboxamide (3). 2-Cyano-6-hydroxybenzothiazole[11] (50 mg, 0.28 mmol) was dissolved in pyridine (3 ml) and 2 drops of triethylamine, and hydrogen sulfide was bubbled through the solution for 2 hr. The solvent was removed *in vacuo* and the residue triturated with benzene and collected. The crude product was recrystallized from aqueous methanol to give the pure thioamide (52 mg, 0.25 mmol, 90%): mp 243–244°; ir (KBr) 3430, 3350, and 1620 cm^{-1}; uv (95% ethanol) 360 (log ϵ 4.30), 319 (sh) (4.03), and 271 nm (3.93).

Anal. Calcd for $C_8H_6N_2OS_2$: C, 45.71; H, 2.86; N, 13.33; S, 30.48. Found: C, 45.81; H, 2.92; N, 13.10; S, 30.51.

2-(6′-Hydroxy-2′-benzothiazolyl)thiazole-4-carboxylic Ester (Ethyl and Methyl Dehydroluciferin) (1a). Ethyl bromopyruvate (0.42 g, 1.3 mmol) was added to a suspension of **3** (0.1 g, 0.48 mmol) in methyl alcohol (10 ml). The reaction mixture was stirred at room temperature for 15 hr and then heated on a steam bath for 2 hr. A yellow colored product (0.11 g, 0.36 mmol, 75%) precipitated upon cooling. The crude product was recrystallized from methanol: mp 256° (sinters at 235°); ir (KBr) 3300 and 1730 cm^{-1}; uv (95% ethanol) 353 (log ϵ 4.33) and 271 nm (3.77). Elementary analysis showed partial ester interchange.[10d]

Anal. Calcd for $C_{12}H_8N_2O_3S_2$ (methyl ester): C, 49.33; H, 2.76; N, 9.59. Found: C, 50.21; H, 2.97; N, 9.31.

2-(6′-Hydroxy-2′-benzothiazolyl)thiazole-4-carboxylic Acid Hydrazide (1b). Hydrazine (95%, 0.25 ml) was added to a solution of **1a** (0.1 g, 0.32 mmol) in ethanol (60 ml), and the resulting solution was refluxed under nitrogen for 6 hr. It was concentrated to about 20 ml total volume and the hydrazide was collected upon cooling (0.056 g, 0.20 mmol, 63%). An analytical sample was recrystallized twice from methyl alcohol: mp 292–294; ir (KBr) 2800–3300 (broad) and 1650 cm^{-1}; uv (95% ethanol) 356 (log ϵ 4.36) and 271 nm (3.74).

Anal. Calcd for $C_{11}H_8N_4O_2S_2$: C, 45.10; H, 2.74; N, 19.17. Found: C, 44.81; H, 2.71; N, 18.86.

1-[2-(6′-Hydroxy-2′-benzothiazolyl)thiazole-4-carboxy]-2-methylhydrazine (1c). A solution of **1a** (0.230 g, 0.74 mmol) and monomethylhydrazine (2 ml) in ethanol (40 ml) was refluxed under nitrogen for 48 hr. The solution was cooled and part of the solvent (20 ml) evaporated. The yellowish precipitate was collected and recrystallized from ethanol to give a pure product (0.206 g, 0.6 mmol, 82%): mp 246–247°; ir (KBr) 2800–3300 (broad) and 1650 cm^{-1}; uv (95% ethanol) 356 (log ϵ 4.34) and 271 nm (3.79); nmr (DMSO-d_6) τ 0.02 (1 H, s), 1.52 (1 H, s), 2.04 (1 H, d, J = 9 Hz), 2.49 (1 H, d, J = 2 Hz), 2.90 (1 H, pair of doublets, J = 2, 9 Hz), 4.90 (1 H, broad singlet), and 7.38 (3 H, s).

Anal. Calcd for $C_{12}H_{16}N_4O_2S_2$: C, 47.36; H, 3.29; N, 17.76. Found: C, 47.37; H, 3.37; N, 17.88.

2-(6′-Hydroxy-2′-benzothiazolyl)thiazole-4-carboxylic Acid (Dehydroluciferin) (1d). This compound was obtained as an analytically pure sample prepared by White, McCapra, and Field.[39]

(38) (a) L. Eberson, *Acta Chem. Scand.*, 17, 2004 (1963). (b) The reduction of triphenylmethyl radical under reversible conditions has been recorded at −0.83 V relative to a silver perchlorate electrode, which in turn has a potential of +0.32 V *vs.* a saturated calomel electrode (R. Breslow and K. Balasubramanian, *J. Amer. Chem. Soc.*, **91**, 5182 (1969)). The calculated value for the reverse process of oxidation of triphenylmethide ion (relative to the standard hydrogen electrode) is +0.18 V. The corresponding value for the oxidation of the anion of triphenylcyclopropene is +0.91 V.

(39) E. H. White, F. McCapra, and G. F. Field, *J. Amer. Chem. Soc.*, 85, 337 (1963).

Acridine-9-carboxylic Acid (4a). The compound was prepared according to Lehmstedt and Wirth.[15] Pure acid was obtained by dissolving the crude material in 1 *N* sodium hydroxide and acidifying slowly with 6 *N* sulfuric acid: uv (0.1 *N* aqueous sodium hydroxide) 384 (sh) (log ϵ 3.49), 368 (sh) (3.66), 356 (3.97), and 340 nm (3.72).

9-Chlorocarbonylacridine Hydrochloride (4b). 9-Acridinecarboxylic acid (1.73 g, 0.01 mmol) was dissolved in thionyl chloride (25 ml) and the solution was refluxed for 6 hr. The solution was concentrated *in vacuo* and heptane was added slowly to precipitate the product, mp 218–219° (lit.[16] 218°).

9-Carbomethoxyacridine (4c). 9-Chlorocarbonylacridine (5 g, 0.02 mol) was added slowly to 100 ml of absolute methanol at room temperature. The resulting solution was neutralized with saturated aqueous sodium bicarbonate and extracted with methylene chloride. The methylene chloride solution was dried and the solvent evaporated to dryness. The crude product was recrystallized from heptane to give 2.9 g (0.013 mol, 65%) of yellowish needles, mp 126–127° (lit.[16] 127–128°).

9-Acridinecarboxylic Acid Hydrazide (4d). A solution of 9-carbomethoxyacridine (**4c**) (1.4 g, 5.8 mmol) and 95% hydrazine (3 ml) in ethanol (100 ml) was refluxed under nitrogen for 6 hr. The solution was concentrated *in vacuo* to 50-ml total volume and refluxed for another 20 min with an additional 3 ml of 95% hydrazine. Upon cooling the crude hydrazide precipitated. It was recrystallized from methanol to give 0.97 g (4.1 mmol, 70%) of pure material: mp 244–245°; ir (KBr) 3370, 3310, and 1650 cm^{-1}; uv (95% ethanol) 384 (log ϵ 3.65), 359 (4.05), and 345 nm (3.86).

Anal. Calcd for $C_{14}H_{11}N_3O$: C, 70.88; H, 4.64; N, 17.72. Found: C, 71.09; H, 4.54; N, 17.45.

Acridine-9-*N*-(1-piperidino)carboxamide (4i). A solution of *N*-aminopiperidine (0.650 g, 6.5 mmol) in methylene chloride (30 ml) was added at room temperature to a stirred solution of 9-chlorocarbonylacridine (**4b**) (0.6 g, 2.1 mmol) in methylene chloride (30 ml). The resulting solution was stirred for an additional 2 hr and the solvent evaporated to dryness. The residue was extracted wth ether and the insoluble *N*-aminopiperidine hydrochloride removed by filtration. The ethereal solution was dried and the solvent evaporated to yield 0.488 g (1.6 mmol, 76%) of the crude hydrazide. Pure product was obtained by recrystallization from ethanol: mp 241–242°; ir (KBr) 2990, 2920, and 1660 cm^{-1}; uv (95% ethanol) 383 nm (log ϵ 3.61), 359 (4.03), and 345 (3.84).

Anal. Calcd for $C_{19}H_{19}N_3O$: C, 74.73; H, 6.27; N, 13.76. Found: C, 74.52; H, 6.53; N, 13.64.

9-Acridinecarboxhydrazone of 9-Formylacridine (4j). A solution of 9-acridinecarboxylic acid hydrazide (**4d**) (0.100 g, 0.42 mmol) and 9-formylacridine[15] (0.087 mg, 0.42 mmol) in ethanol (15 ml) was refluxed for 15 min. The solution was filtered while hot, part of the solvent was removed *in vacuo*, and upon cooling yellow needles precipitated. The crude material was recrystallized from ethanol (0.149 g, 3.5 mmol, 83%): mp 231–232°; ir (KBr) 3050 and 1670 cm^{-1}; uv (95% ethanol) 385 (sh) (log ϵ 4.05), 359 (4.25), and 343 nm (4.06).

Anal. Calcd for $C_{26}H_{18}N_4O$: C, 78.86; H, 4.25; N, 13.14. Found: C, 78.57; H, 4.20; N, 13.36.

1-(9-Acridinoyl)-1-methylhydrazine and 1-(9-Acridinoyl)-2-methylhydrazine. 9-Chlorocarbonylacridine (**4b**) (0.6 g, 2.1 mmol) dissolved in methylene chloride (30 ml) was added at room temperature to a solution of monomethylhydrazine (0.3 g, 6.5 mmol) in methylene chloride (30 ml). The reaction solution was stirred for 1 hr when the initially formed oil solidified. The solvent was removed *in vacuo*, and the residue triturated with water and filtered. Sublimation of the crude material (140° at 40 μ) gave a pure product (0.355 g, 1.5 mmol, 71%): mp 152–154°; ir (KBr) 3500, 3250, 1660, and 1630 cm^{-1}; uv (95% ethanol) 384 (log ϵ 3.64), 359 (4.02), and 344 nm (3.85); nmr ($CDCl_3$) τ 1.58–2.63 (8 H, m), 4.86 (0.9 H, s), 6.11 (0.5 H, s), 6.48 (1 H, s), and 7.13 (2 H, s). The nmr spectrum showed the product to be a mixture of both isomers.

Anal. Calcd for $C_{15}H_{13}N_3O$: C, 71.71; H, 5.18; N, 16.73. Found: C, 71.74; H, 5.16; N, 16.68.

1-(9-Acridinoyl)-1-methyl-2-ethylidenehydrazine (4f). A mixture of 1-(9-acridinoyl)-1-methylhydrazine and 1-(9-acridinoyl)-2-methylhydrazine (4.0 g, 1.59 mmol) was dissolved in 2-propanol (100-ml) and acetaldehyde (15 ml) was added. The solution was refluxed for 1 hr and filtered, and the solvent evaporated. The residue was absorbed on silica gel (Davison Grade 62, 10 g) by dissolving it in chloroform, adding the silica gel, and evaporating the solvent. The residue was transferred to the top of a dry packed silica gel column (Davison Grade 62, 400 g) which was eluted with chloroform. The expected product was the first compound to come off the column (3.05 g, 1.1 mmol, 68%). An analytical sample was recrystallized from ethanol: mp 176–177°; ir (KBr) 2995, 2920, 1660, and 1630 cm^{-1}; uv (95% ethanol) 384 (log ϵ 3.78), 359 (4.15), and 343 nm (4.00); nmr ($CDCl_3$) τ 1.57–2.60 (8 H, m), 2.81 (1 H, q, J = 6 Hz), 6.38 (3 H, s), and 8.55 (3 H, d, J = 6 Hz).

Anal. Calcd for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15. Found: C, 73.73; H, 5.60; N, 14.93.

1-(9-Acridinoyl)-1-methylhydrazine (4g). A solution of **4f** (5.2 g, 19 mmol) in ethanol (50 ml) and hydrochloric acid (15 ml) was refluxed for 2 hr. The solution was left overnight, filtered, and concentrated *in vacuo* to a total volume of 10 ml. It was diluted with water (50 ml) and extracted with chloroform (three times). The aqueous layer was neutralized with sodium carbonate and extracted twice with chloroform (100 ml). The chloroform extracts were dried and evaporated to dryness. The crude product was purified by column chromatography on basic alumina (Alcoa F-20, 1.5 g of compound on 220 g of alumina) using chloroform as an eluent. Fractions of 100 ml were collected; the fractions that did not contain any impurity (by tlc) were combined. Yellowish needles appeared upon removal of the solvent (3.1 g, 12 mmol, 63%) and were proven to be analytically pure: mp 163–164°; ir (KBr) 3300 and 1650 cm^{-1}; uv (95% ethanol) 384 (log ϵ 3.63), 359 (3.98), and 344 nm (3.81); nmr (DMSO-d_6) τ 2.02–2.83 (8 H, m), 5.33 (2 H, s), and 6.63 (3 H, s).

Anal. Calcd for $C_{15}H_{13}N_3O$: C, 71.71; H, 5.18; N, 16.73. Found: C, 71.49; H, 5.18; N, 16.61.

1-(9-Acridinoyl)-1,2-dimethylhydrazine (4h). A solution of 9-chlorocarbonylacridine (**4b**) (3.0 g, 11 mmol) in methylene chloride (10 ml) was added dropwise to a stirred solution of *sym*-dimethylhydrazine dihydrochloride (3.3 g, 25 mmol) in pyridine (100 ml) and triethylamine (10 ml) at 0°. The resulting solution was stirred at 0° for 1 hr and left overnight at room temperature. The solvent was removed *in vacuo* and the residue dissolved in chloroform (150 ml) and washed twice with aqueous 5% sodium bicarbonate and twice with water. The chloroform solution was treated with Norit A, dried, and evaporated to dryness. The crude product was recrystallized from benzene–cyclohexane to yield 1.72 g (6.5 mmol, 59%) of yellow needles. An analytical sample was prepared by sublimation at 120° and 30 μ and another recrystallization: mp 170–171°; ir (KBr) 3180 and 1640 cm^{-1}; uv (95% ethanol) 384 (log ϵ 3.55), 359 (3.96), and 343 nm (3.77); nmr ($CDCl_3$) τ 1.57–2.60 (8 H, m), 6.49 (3 H, s), 7.08 (1 H, broad singlet), and 7.15 (3 H, s).

Anal. Calcd for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.20; H, 6.01; N, 15.73.

1,2-Bis-9-acridinoylhydrazine (5). A solution of 9-chlorocarbonylacridine (**4b**) (3.5 g, 12.5 mmol) in methylene chloride (100 ml) was added dropwise to a well-stirred solution of 95% hydrazine (0.208 g, 6.3 mmol) and triethylamine (1.26 g, 12.5 mmol) in pyridine (75 ml) at 0°. The resulting solution was stirred at room temperature for 1 hr and left overnight. The solvent was removed *in vacuo*, the residue was treated with 5% aqueous sodium bicarbonate (100 ml), and the solid material was collected. The crude material was recrystallized from methanol and from methanol–chloroform to give 1.77 g (4.1 mmol, 66%) of the hydrazide: mp >280°; ir (KBr) 3090 and 1670 cm^{-1}; uv (95% ethanol) 385 (log ϵ 3.70), 359 (4.06), and 344 nm (3.87); nmr (DMSO-d_6) τ 1.59–2.79 (8 H, m) and 6.83 (2 H, s). The nmr spectrum and the elemental analysis show the compound to crystallize with half a molecule of methanol per one molecule of the diacylhydrazine.

Anal. Calcd for $C_{28}H_{18}N_4O_2$: C, 76.01; H, 4.10; N, 12.66. Found: C, 75.13; H, 4.22; N, 12.23. Calcd for $C_{28}H_{18}N_4O_2 + 0.5CH_3OH$: C, 74.67; H, 4.36; N, 12.22.

Azodi-9-acridinoyl (6). *N*-Bromosuccinimide (20 mg, 0.12 mmol) was added slowly at 0° to a suspension of **5** (50 mg, 0.12 mmol) in methylene chloride (15 ml) with 3 drops of pyridine.[40] The resulting mixture was stirred at room temperature for 2 hr. The organic solution was washed with water, 10% aqueous sodium carbonate, and again with water. The solution was dried and the solvent was removed *in vacuo* at room temperature. The crude reddish product (38 mg, 0.076 mmol, 63%) was purified on a silica gel dry column with chloroform as eluent to give orange needles: mp 267–268°; ir (KBr) 1745 and 1705 (weak) cm^{-1}; uv (95% ethanol) 480 (log ϵ 3.14), 384 (sh) (3.98), 360 (4.43), and 343 (sh) nm (4.25).

(40) L. A. Carpino, P. H. Terry, and P. J. Crowley, *J. Org. Chem.*, **26**, 4336 (1961); H. Bock and E. Baltin, *Chem. Ber.*, **98**, 2054 (1965).

Anal. Calcd for $C_{28}H_{16}N_4O_2$: C, 76.35; H, 3.66. Found: C, 74.12; H, 3.68, probably due to a partial decomposition.

1,2-Di-(9-acridinoyl)-1,2-diazacyclohex-4-ene (7). Butadiene was bubbled through a solution of azodi-9-acridinoyl (**6**) (200 mg, 0.48 mmol) in benzene (350 ml). Bubbling was continued until the initial orange color of the solution turned yellow (about 2 hr). The solvent was dried and evaporated to dryness. The crude product was chromatographed on a basic alumina column (Alcoa F-20, 30 g) with chloroform as eluent. The chromatography was followed by tlc of each fraction (50 ml); the fractions showing one spot were combined and collected. Evaporation of the solvent gave 123 mg (0.25 mmol, 52%) of yellow product: mp >280°; ir (KBr) 2960, 2925, 2850, and 1700 cm^{-1}; uv (95% ethanol) 385 (log ϵ 3.81), 359 (4.20), and 363 nm (4.01).

Anal. Calcd for $C_{32}H_{22}N_4O_2$: C, 77.72; H, 4.48; N, 11.33. Found: C, 77.58; H, 4.70; N, 11.29.

Autoxidation of Benzhydrazide in Dimethyl Sulfoxide–*tert*-Butyl Alcohol with *tert*-Butoxide as Base. Benzhydrazide (1.00 g, 7.35 mmol) was dissolved in each of four solutions (100 ml) of dimethyl sulfoxide[41] and *tert*-butyl alcohol. The ratios of dimethyl sulfoxide to *tert*-butyl alcohol were 10:0, 9:1, 7:3, and 2:8. Potassium *tert*-butoxide[42] (3.0 g, 25.9 mmol) was added to each solution and the resulting solutions were stirred for 3 days in the presence of oxygen. At that time 10% aqueous sodium bicarbonate (50 ml) was added and the solvent removed *in vacuo*. Water (50 ml) was added to each run and the basic solution was extracted with chloroform. The chloroform extract contained benzoylhydrazone of benzaldehyde (identified by melting point, mixture melting point (202°), and infrared spectrum). The aqueous solution was acidified with 5 *N* hydrochloric acid and extracted with chloroform. The acidic material was identified as benzoic acid by its melting point, mixture melting point 123°, and infrared spectrum. The quantitative results are summarized in Table II.

Quantitative Determination of the Chemiluminescent Reaction Products. 9-Acridinecarboxylic acid hydrazide (**4d**) (70 mg, 0.3 mmol) was dissolved in dimethyl sulfoxide (50 ml) and potassium *tert*-butoxide (1.00 g, 6.8 mmol) was added in one portion to the vigorously stirred solution. Aqueous sodium bicarbonate (5%, 50 ml) was added immediately when light emission stopped, and the solution was extracted with two 50-ml portions of chloroform. The chloroform solution was dried and evaporated to dryness, and the residue was dissolved in 50 ml of ethanol. The ethanolic solution was shown by tlc (silica gel–chloroform) to contain only acridine and acridone. Acridone was determined quantitatively by its ultraviolet absorption at 398 nm (log ϵ 3.96), where acridine does not absorb. Acridine was determined by its ultraviolet absorption at 339 nm (log ϵ 3.83) where acridone does not absorb. An authentic mixture of acridone (2.13 mg, 0.015 mmol, 5%) and acridine (0.825 mg, 0.004 mmol, 1.5%) gave a superimposable ultraviolet spectrum in 95% ethanol. The aqueous solution (5% sodium bicarbonate) was shown to contain 9-acridinecarboxylic acid. Acidification followed by tlc (cellulose:ethanol–water–ammonia, 8:1:1) showed one spot at R_f 0.88 identical with an authentic sample. The ultraviolet spectrum of the basic aqueous solution [384 (sh), 368 (sh), 356, and 340 nm] was identical with the spectrum of 9-acridinecarboxylic acid. Quantitative determination was done by the 356-nm (log ϵ 3.91) absorption, and the solution was shown to contain 52.92 mg (0.24 mmol, 80%) of 9-acridinecarboxylic acid.

Gas–Liquid Chromatography[43] of Gaseous Reaction Products. Each of the following three active chemiluminescent materials, 9-acridinecarboxylic acid hydrazide (**4b**), 1-(9-acridinoyl)-1-methylhydrazine (**4g**), and azodi-9-acridinoyl (**6**) (0.1 mmol) was dissolved in dimethyl sulfoxide (20 ml). Each solution was placed in a 25-ml two-necked, round-bottomed flask where the side arm was closed with a rubber septum. Solid potassium *tert*-butoxide (100 mg, 0.85 mmol) was added and the flask was closed tightly. The reaction flask was well shaken until light emission stopped and a gaseous sample was drawn (5 cm^3 using a thermal conductivity detector and 1 cm^3 using a flame ionization detector). Carbon monoxide and methane (Matheson Chemicals Inc.) were used as standards. Carbon monoxide was identified as a product of all three chemiluminescent reactions, using a molecular sieve 5-Å column at room temperature with helium as carrier gas at 110 ml/min. Retention time for carbon monoxide under these conditions was 7′ 50″. A control experiment was done by adding potassium *tert*-butoxide to dimethyl sulfoxide under the same conditions; no carbon monoxide was observed in three different runs. The chemiluminescent reaction of 1-(9-acridinoyl)-1-methylhydrazine (**4g**) carried out under the same conditions showed methane as well as carbon monoxide as a product. Retention time for methane (molecular sieve 5Å, thermal conductivity detector, room temperature, and helium at 110 ml/sec) was 9′ 55″. Methane was also identified using a silica gel plus 5% squalene column and flame ionization detector. Control experiments did not produce any methane. The ratio of methane to carbon monoxide produced by the chemiluminescent reaction of **4g** was about 17:1.

The Stability of 1-(9-Acridinoyl)-1-methylhydrazine (4g) in Dimethyl Sulfoxide with Potassium *tert*-Butoxide under Degassed Conditions. A solution of **4g** (250 mg, 1.0 mmol) in dimethyl sulfoxide (50 ml) in a three-necked flask was connected by a side arm to a round-bottomed flask in which potassium *tert*-butoxide (900 mg, 8 mmol) was dissolved in dimethyl sulfoxide (20 ml). The whole system was degassed (six freeze–pump–thaw cycles), and the two solutions were mixed. No light emission was observed, while in the presence of oxygen there is light emission, and the solutions react completely in 5 min. The combined solution was stirred *in vacuo* for 18 hr and was decomposed by degassed water (five cycles) which was distilled directly into the reaction flask. The resulting solution was evaporated to dryness and the dry residue was extracted with chloroform. The organic solution was treated with Norit A and dried, and the solvent was removed. The dry residue (220 mg, 88% of starting material) was shown to be unreacted starting material; uv (95% ethanol) 384 (sh) (log ϵ 3.69), 359 (4.04), and 342 nm (3.75). The infrared spectrum of the residue (KBr), 3300 and 1650 cm^{-1}, was the same as for the starting material.

Determination of Reaction Products and Stability of the Starting Hydrazides by Thin-Layer Chromatography. Each reaction was carried out on 5 mg of starting hydrazide dissolved in dimethyl sulfoxide (30 ml). Potassium *tert*-butoxide (50 mg) was added and the reaction solution was stirred for 10 min in the presence of oxygen. Aqueous bicarbonate (30 ml, 5%) was added and the solvent evaporated to dryness. The dry residue was triturated with methanol from which a sample was withdrawn for tlc. The acids were determined on cellulose (ethanol–water–ammonia, 8:1:1) by comparison with authentic samples. Acridone, acridine, and unreacted hydrazides were compared with authentic samples on alumina (chloroform or 10% methanol in chloroform).

Emission Spectra. Fluorescence and chemiluminescence emission spectra were determined on a Hitachi-Perkin-Elmer MPF-2A recording spectrophotofluorimeter. Spectra were not corrected for phototube sensitivity, instrumental distortion or source intensity fluctuation. Chemiluminescence spectra were obtained by reacting solutions of the hydrazides (10^{-4} *M*) with excess potassium *tert*-butoxide in dimethyl sulfoxide and running the spectrophotofluorimeter with the source off.

Total Light Emission Studies. Chemiluminescence relative efficiencies for **1b** and **4d** were determined using a photomultiplier (RCA IP21) powered by a Fluke 4128 dc power supply. The signal from the photomultiplier after amplification was collected on a capacitor and recorded for total light yields. The determinations were performed as described for luminol[44] except that the quantum yields of the hydrazides were determined in media strictly free of water and alcohols. Higher yields were obtained when solutions of the hydrazides in dry DMSO were added to an excess of powdered potassium *tert*-butoxide.

Acknowledgments. We thank the Public Health Service for its financial support (Research Grant No. 5 RO 1 NSO 7868 from the National Institute of Neurological Diseases and Stroke). We are also grateful for a Sonneborn Graduate fellowship in chemistry for E. R. and we thank Mr. Robert B. Brundrett for the quantum yield measurements.

(41) In all experiments, dimethyl sulfoxide (Matheson Coleman and Bell) was stirred overnight over crushed potassium hydroxide, decanted, and distilled from potassium *tert*-butoxide.

(42) MSA Research Corp. was used as received.

(43) Carried out on a Varian Aerograph series 1800 instrument.

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