cribed manner afforded a dibromide mixture (300 mg) that was directly subjected to elimination with LiF and Li_2CO_3 in HMPA as before. There was isolated 150 mg (82%) of a mixture of **3** and **4** identical with that encountered earlier (^1H NMR analysis).

Hydrolysis–Oxidation of (−)-*endo*-Bornyltriazolinedione Adduct 16b. A solution of **16b** ($[\alpha]^{24}_D$ + 39.6°, 100 mg, 0.226 mmol) and sodium hydroxide (350 mg, 8.75 mmol) in isopropyl alcohol was heated at reflux under nitrogen for 24 h. The reaction mixture was cooled in an ice bath, acidified to pH 1 with 3 N hydrochloric acid, and finally made basic to pH 9 with 3 N ammonium hydroxide. Pentane (10 mL) and activated manganese dioxide (300 mg, 3.45 mmol) were added, and the resulting mixture was stirred at room temperature for 40 min before being filtered. The solid was washed once with cold pentane (10 mL). The aqueous phase of the filtrate was extracted with pentane (10 mL), and the combined organic layers were washed with cold water (3 × 10 mL) and brine (2 × 10 mL) prior to drying and solvent removal at room temperature on a rotary evaporator. The brown oily residue was chromatographed on Florisil (6 g) at −78 °C (pentane elution) to give 30 mg of a colorless oil. This was dissolved in $CDCl_3$, and the ^1H NMR spectrum showed ca. 30% of **4** to be present along with **3**. The $CDCl_3$ was carefully removed and to the remaining 29 mg was added 1.07 g of diglyme and the specific rotation was determined: $[\alpha]^{30}_D$ −76.7°, $[\alpha]^{30}_{578}$ −81.9°, $[\alpha]^{30}_{546}$ −100.0°, $[\alpha]^{30}_{436}$ −279.1°.

Determination of Racemization Rates for (−)-3. The procedure described in the accompanying paper[1] was followed. A summary of the resulting data is given in Table I.

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Chemiluminescence Mechanism of Cyclic Hydrazides Such as Luminol in Aqueous Solutions

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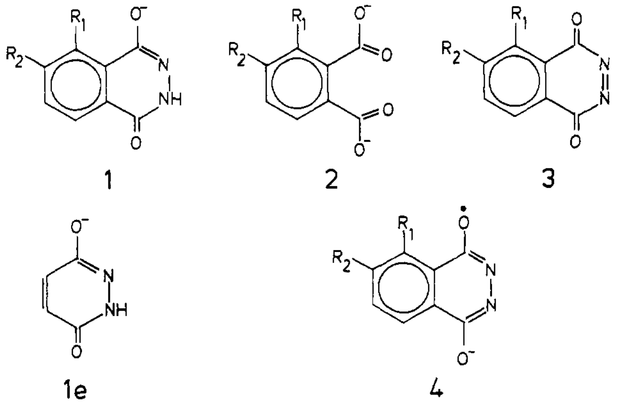
Abstract: Independently of the mode of oxidation, the first critical intermediate on the chemiluminescent pathway of luminol is a hydroperoxide. Below its pK_a it decomposes into oxygen and the parent hydrazide. At higher pH, the monoanion of the hydroperoxide expels nitrogen and yields a monoprotonated dicarboxylate. The latter is chemiexcited to some extent.

Being among the longest known synthetic chemiluminescing compounds, cyclic hydrazides **1** have been the subject of countless investigations starting with the famous paper by Albrecht[1] in 1928 (Chart I). Though the literature is replete with many suggestions, not much has emerged in the way of hard facts concerning the reaction mechanism. Elegant work in the laboratory of White et al.[2,3] has nevertheless revealed the main cornerstones of hydrazide chemistry. In aprotic solvents such as Me_2SO the overall stoichiometry was shown to involve the consumption of 1 mol each of the hydrazide dianion and O_2 to yield 1 mol of the dicarboxylate **2** and N_2. In each case the corresponding dicarboxylates have been proved to be the emitting species. Furthermore, the reaction of the two-electron oxidized hydrazide (the diazaquinone **3**) with H_2O_2 was shown to yield chemiluminescence as well.[4,5] In protic solvents, particularly water, chemiluminescence presupposes not only the presence of the hydrazide and a dioxygen species but also that of an oxidant.[6] Due to the sluggishness of the initial reaction step, kinetic investigations beyond it were not possible in the above studies.

In aqueous solutions the rate of the primary oxidation can be dramatically enhanced by means of radiolysis.[7] In a recent publication the quantum yield of radiolytically initiated luminol chemiluminescence was shown to be around 1%.[8] Characteristic features of radiolysis will now be briefly described insofar as they are relevant to hydrazide oxidation. Subject to ionizing radiation water decomposes to yield the radicals e_{aq}^-, H·, and OH·. By addition of N_2O e_{aq}^- is converted into OH· radicals. In oxygenated solutions e_{aq}^- and H· reduce O_2 to superoxide (O_2^-·). In the presence of the anions N_3^-, CO_3^{2-}, SCN^-, Br^-, and ClO_2^- the OH· radical produces the one-electron oxidants N_3·, CO_3^-·, $(SCN)_2^-$·, Br_2^-·, and ClO_2·, respectively. When the solution contains several solutes they compete for the radicals present and the competition is governed by the rate constants and the concentration of the solutes.

Chart I[a]



[a] a, R_1 = H, R_2 = H; b, R_1 = NH_2, R_2 = H; c, R_1 = H, R_2 = NH_2; d, R_1 = H, R_2 = $N(CH_3)_2$.

The above-mentioned one-electron oxidants produce one single radical species **4** upon reaction with a particular hydrazide.[9,10] This species, identified as the diazasemiquinone radical, quantitatively dismutes upon self-recombination (reaction 1) into the

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parent hydrazide and the corresponding diazaquinone **3**.[9,10]

$$\mathbf{4} + \mathbf{4} \xrightarrow[H_2O]{2k_1} \mathbf{3} + \mathbf{1} + OH^- \tag{1}$$

Oxygen (O_2) has been found to be relatively inert toward the diazasemiquinone radical **4**. Neither the second-order decay kinetics of the latter nor the γ-radiolytic yields of the products found in N_2O saturated solutions were modified by O_2 (10–50%) added to the N_2O gas.[9,10]

By means of pulse radiolysis the two stoichiometrically equivalent reactions 2 and 3 and the decomposition of the hydroperoxide have become amenable to study.[11,12] Whenever the radiolytic oxidation triggers off a chemiluminescent reaction sequence, the kinetics of this process can be studied by observing the time dependent light emission. It is a general observation that the dynamics of the chemiluminescence consists of a buildup and a decay portion, and it can be shown that they reflect the two slowest reaction steps.

$$\mathbf{4} + O_2^{-}\cdot \xrightarrow{k_2} \mathbf{5} \tag{2}$$

$$\mathbf{3} + HO_2^{-} \xrightarrow[H_2O]{k_3} \mathbf{5} \tag{3}$$

Under appropriate conditions, reaction 2 or 3 was always found to be followed by a pH-dependent first-order reaction, which we have attributed to the decomposition of the hydroperoxide. Our previous studies revealed that the deprotonated hydroperoxide **5b** yields light and 3-aminophthalate (**2b**) upon decomposition.[11,12] However, the undissociated form appeared to show different chemical behavior depending on whether it was formed in reaction 2 or 3. The latter observation prompted us to suggest that the hydroperoxides produced in reactions 2 and 3, respectively, were different.[12] Underlying this suggestion was the assumption that the chemiluminescent reaction was unaffected by the oxidative system used.

It will be recalled that reaction 3 was studied in a system where $ClO_2\cdot$ generated radiolytically from ClO_2^- was employed as the primary oxidant of luminol.[12] However, in this system, the simultaneous formation of OCl^-, a well-known cooxidant in luminol chemiluminescence, could not be prevented.[13] The presence of hypochlorite, whose precise mode of interaction with luminol and dioxygen species still awaits clarification,[14,15] may well have distorted the chemiluminescent dynamics ensuing reaction 3.

In ref 11 reaction 2 was preceded by the initial attack of the $OH\cdot$ radical on luminol. However, a later study has demonstrated that only a part of the luminol molecules (**1b**) transfers an electron to the $OH\cdot$ radicals.[9] In fact, about 50% of the latter adds instead to the aromatic ring of luminol. The propensity of $OH\cdot$ to add to cyclic hydrazides has been examined in detail in the case of maleic hydrazide (**1e**).[16]

With the above complications in mind the question whether the hydroperoxides formed in reactions 2 and 3 are identical or not still perseveres. Consequently one of the objectives of this work is the clarification of this point.

At the heart of any chemiluminescent process lies the formation of the emitter from its immediate precursor as this step determines the efficiency of chemiexcitation. Obviously, a plausible assessment of the chemiexcitation process requires the identification of this intermediate. With this aim in mind we will study the temperature dependence of the decomposition of hydroperoxide **5**.

Finally, the precise nature of the emitting molecule will be further pursued. To motivate this last objective we will provide a brief description of the prevailing picture of the emitter. Ever since the work of White et al.[3] the good spectral match in aqueous solutions of the hydrazide chemiluminscence and of the fluorescence of the dianion of the dicarboxylic acid has been known. The assumption was therefore natural that the latter is the emitter at all accessible pH values.

However, the issue of the emitter is somewhat obscured by reports in the literature to the effect that the chemiexcited and photophysically excited dicarboxylates are not identical with respect to quenching characteristics.[17,18] In addition, the pH dependence of luminol (**1b**) chemiluminescence and 3-aminophthalate (**2b**) fluorescence at pH >12 are known to differ. At $pH_{1/2}$ = 12.5 the quantum yield of fluorescence of 3-aminophthalate is halfed.

Alteration or quenching of the fluorescence of excited aromatic amines has been shown to be due to the deprotonation of the amino group by OH^-.[19,20] The chemiluminescence quantum yield also decreases with increasing pH, but the $pH_{1/2}$ is found to be ~13. The different pH profiles have been rationalized by assuming the existence of two excited states differing with respect to hydration.[17] The connection between photo- and chemiexcitation was given by the following scheme where A^{2-} is the dianion **2b**:

$$\begin{tikzcd}
(A^{2-}\cdots H_2O) + h\nu \arrow[r] & (A^{2-}\cdots H_2O)^* \arrow[r, "OH^-"] \arrow[d] & \text{dark} \\
\text{chemiexcitation} \arrow[r] & (A^{2-})^*\ H_2O \arrow[r, "OH^-"] \arrow[d] & \text{dark} \\
 & h\nu &
\end{tikzcd}$$

The transformation of $(A^{2-}\cdots H_2O)^*$ to $(A^{2-})^*\ H_2O$ would merely involve intermolecular rearrangements of hydrogen bonds. Therefore the maximum lifetime of $(A^{2-}\cdots H_2O)^*$ should not exceed 10^{-10} s.[21] In order to quench such a short-lived state with OH^- ions a rate constant $>10^{11}\ M^{-1}\ s^{-1}$ would be required. Obviously, such a high rate constant is unreasonable, and thus the above scheme has to be modified.

Experimental Section

The microtron accelerator[22] and the computerized detection system[23] have been described elsewhere. The dose/pulse was measured with a secondary emission chamber calibrated against an aerated 10^{-2} M KSCN solution taking $G[(SCN)_2^-\cdot] = 2.14 \times 10^4$ at 500 nm.[24]

γ-radiolysis was carried out in a AECL220 $^{60}Co\gamma$ source with a dose rate of 0.9 Gy/s as determined with the Fricke dosimeter.

HPLC analyses were performed on a C-18 reversed phase column, the eluent being an aqueous phosphate solution buffered at pH 6.7 with various (3–20%) admixtures of methanol. In all determinations of product yields care was taken to keep the overall conversion below 20%. Test experiments have shown that conversion up to 30% was linear with the dose delivered.

The pK_a values of the substituted phthalic acids **2** were determined by UV–vis spectrophotometry. Fluorimetric measurements were not corrected for the spectral response of the PM tube (R-106). This was not deemed necessary as the recorded emissions lie in a spectral region (400–500 nm) where the response is almost linear.

Chemicals. Phthalhydrazide (**1a**), luminol (**1b**), 3-aminophthalic acid hydrochloride (**2b**) (all EGA Chemie), 4-aminophthalhydrazide (**1c**) (Sigma), 4-aminophthalic acid (**2c**) (ICN), and phthalic acid (**2a**) (Merck) were used as received. 4-(Dimethylamino)phthalhydrazide (**1d**)

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and 4-(dimethylamino)phthalic acid (**2d**) were kindly provided by P. Sand of the Department of Organic Chemistry. HPLC analyses of the above compounds revealed no appreciable amount of impurities.

Acid-Base Equilibria for Species 1, 2, and 4. Cyclic hydrazides **1** are dibasic acids. Their pK_1 values range between 6 and 7.[6] Maleic hydrazide (**1e**) is somewhat more acidic with a $pK_1 = 5.7$.[16] The pK_2 values lie around 14.[6] Therefore, these compounds are monoanions in the pH range (8–13) where chemiluminescence is usually observed. The one-electron-oxidized hydrazide radicals **4** are monobasic acids with pK_a values ranging between 6.8 and 7.7 (see Table I). Again the maleic hydrazide radical **4e** is more acidic. Formally, the radicals will be depicted as semiquinones with the unshared electron on an oxygen atom. However, quantum chemical calculations indicate that this electron is smeared over several atoms, where the density is highest on the oxygen and the hydrazine nitrogen while the carbonyl carbon has a lower radical character. For the acid–base equilibria of the fluorescent phthalic acids **2b,c,d** see Table VI. The structural formulas depicted in the substance definition refer to the protonation state that predominates in the chemiluminescent pH region (pH 8–14). Protonation equilibria in water are always maintained for all the species **1**–**5** excepting for the excited **2** discussed below.

Results and Discussion

In the present work we have chosen to use $N_3\cdot$ and $CO_3^-\cdot$ for the generation of the diazasemiquinone radicals. These oxidants introduce no complications in the presence of dioxygen species.[25,26]

In N_2O-saturated 10^{-3} M luminol (**1b**) solutions containing 1 M CO_3^{2-} or 0.01 M N_3^- we assume a G value of 5.5 ± 0.3 for the **4b** radical. The extinction coefficient for the corresponding diazaquinone **3b** is known.[27] From this ϵ and the measured absorbance of **3b** appearing upon the self-recombination of **4b** (reaction 1), $G(3b) = 2.3$ was found for luminol in the present system. We attribute the fact that it is somewhat lower than 2.7–2.9 expected from the primary **4b** yield to recombination of some H· atom adduct with the latter.

In the γ-radiolysis experiments the steady-state concentration of radicals is about 3×10^{-8} M. Pulse irradiation generated initial concentrations of radicals in the range 5×10^{-7} to 5×10^{-5} M. Within the above concentration regime of the radicals the following important observations were made on potentially chemiluminescent hydrazides **1b,d**: (1) No chemiluminescence occurs nor is any hydrazide **1** consumed ($G(\text{–hydrazide}) \leq 0.01$) when only $O_2^-\cdot$ is generated in the presence of the parent hydrazide (no diazasemiquinone radical **4** present). (2) In solutions purged with N_2O/O_2 mixtures the chemiluminescence yield is strictly proportional to the amount of $O_2^-\cdot$ formed by e_{aq}^- and H· reacting with O_2. (3) Chemiluminescence occurs only either when the diazasemiquinone **4** recombines with $O_2^-\cdot$ (reaction 2) or when **3** reacts with HOO^- (reaction 3).

Observation 1 clearly demonstrates that luminol cannot be oxidized by $O_2^-\cdot$ to any appreciable extent. Observation 2 is a neat confirmation of the aforementioned unreactivity of O_2 toward diazasemiquinone (**4**) radicals. In analogy with semiquinone/quinone redox systems[28] the following equilibrium can be written:

$$4 + O_2 \rightleftarrows 3 + O_2^-\cdot$$

However, this equilibrium is only a formal one as the diazaquinone **3** is constantly depleted due to its rapid hydrolysis[27,29] ($k(OH^- + 3) \sim 10^7\ M^{-1}\ s^{-1}$). As a consequence of this all **4** that reacts with O_2 should yield $O_2^-\cdot$.

Superoxide might then undergo reaction 2 to yield dicarboxylate and hence chemiluminescence. The above reaction sequence is strongly dependent on radical concentration as the oxygen reaction competes with the self-recombination of **4** (reaction 1). In γ-or pulsed e^--beam irradiated solutions of **1b** purged with mixtures of N_2O/O_2 the yields of the dicarboxylate **2b** were found to be identical (within 0.1 G unit). These two types of measurements deal with quite different radical concentrations (see above). Knowledge of the rate constant of reaction 1 ($2k_1 = 5 \times 10^8\ M^{-1}\ s^{-1}$), the radical concentration (3×10^{-8} M in γ-radiolysis), and the accuracy of analysis ($\Delta G = \pm 0.05$) enables us to estimate the upper limit for the rate constant $k(4b + O_2)$ to be $10^3\ M^1\ s^{-1}$. At low radical concentration the slow reaction between **4b** and O_2 is likely to be responsible for chemiluminescence. This appears to be the case for the weak chemiluminescence observed upon anodic oxidation[30] of luminol (**1b**) or upon its addition to aerated aqueous solutions containing transition-metal ions.[6]

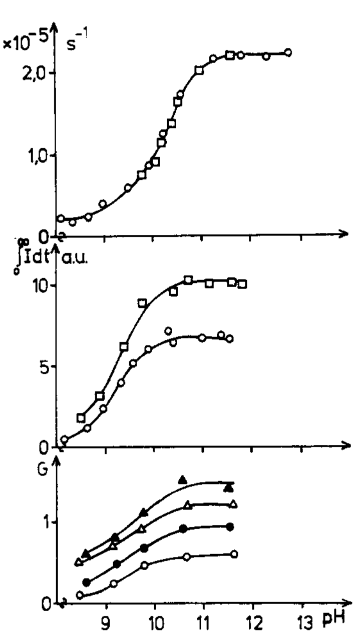

Figure 1. (Top) pH dependence of the rate constant for hydroperoxide decomposition as extracted from the build up of e^--beam initiated chemiluminescence: ○, 10^{-3} M luminol (**1b**), O_2 saturated (reaction 2); □, 10^{-2} M luminol, H_2O_2 varied from 0.03 M at pH >11 to 0.1 M at pH 9.8, N_2O saturated (reaction 3). (Middle) pH dependence of the total chemiluminescence yield: ○, 10^{-3} M luminol, O_2 saturated (reaction 2); □, 10^{-3} M luminol, 10^{-2} M H_2O_2 N_2O saturated (reaction 3). (Bottom) variation of radiolytic G values with pH as found in e^--beam irradiated solutions: ○, G(3-aminophthalate) (**2b**), 10^{-3} M luminol, O_2-saturated solutions (reaction 2); ●, G(–luminol), same as above; △, G(3-aminophthalate), 10^{-3} M luminol, 10^{-2} M H_2O_2, N_2O-saturated solutions (reaction 3); ▲, G(–luminol), same as above. All experiments were carried out at 20 °C in solutions containing 1 M CO_3^{2-} throughout.

Before leaving radical chemistry we will briefly digress on the part played by the OH· radical. Oxidation of luminol by the OH· radical produces light in the presence of O_2.[7] In a previous paper it was shown[9] that in its reaction with luminol OH· produces both **4b** and an OH adduct and that the latter reacts rapidly with O_2, $k(\text{adduct} + O_2) = 10^9\ M^{-1}\ s^{-1}$. In the present work it is found that in N_2O/O_2 purged solutions the light yield in OH· initiated chemiluminescence is twice that expected from the initial yield of superoxide. The above observation clearly shows that the OH· adduct is instrumental in reducing O_2 to $O_2^-\cdot$ and transferring the latter to **4b** with more chemiluminescence as the result. Similarly, it was found previously[16] that OH· radicals in part add to the C–C double bond in maleic hydrazide (**1e**). In the presence of O_2 the OH· adduct yields a peroxy radical that is able to transfer $O_2^-\cdot$ to **4e** and thus yield maleic acid.

The Hydroperoxide Intermediate. The decomposition of the hydroperoxide intermediate **5** formed in reactions 2 and 3 was studied directly by time-dependent chemiluminescence measurements and/or product analysis. The results with luminol are depicted in Figure 1.

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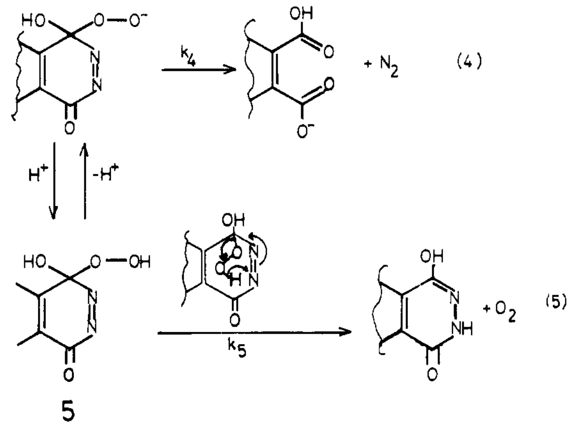
Table I[a]

parent hydrazide	pK_a	$(pH_{1/2})_{light}$	pK_a[d]	$10^{-5}k_4$, s^{-1}	$10^{-4}k_5$, s^{-1}	φ_3, %
1e	6.3[b]	7.5				100
1a	6.9[c]	8.2 ± 0.3				90
1b	7.7[c]	9.2 ± 0.2	10.4 ± 0.1	2.2	2.0	40
1c	7.5[c]	8.9 ± 0.2	10.1 ± 0.2	1.6	1.0	55
1d	7.7[c]	9.2 ± 0.1	10.5 ± 0.1	2.2	1.8	50

[a] All experiments were carried out at 20 °C. [b] pK_a of the diazasemiquinone **4** from ref 10. [c] pK_a of **4** from ref 9. [d] pK_a of hydroperoxide **5**.

Scheme I



First of all, we will point out that the pH dependence of each parameter is unique irrespective of whether it is observed subsequent to reaction 2 or 3. It should be stressed that since the pK_a of the luminol diazasemiquinone **4b** is 7.7 (see Table I) the protonation state of the latter has obviously no influence on the pH behavior of the yields.

Most important, within the accessible pH range the measured rate constant of decomposition of the hydroperoxide is independent of the mode of its generation. Since this rate constant is measured from the chemiluminescent kinetics it stands to reason that in all cases only one unique hydroperoxide exists on the light producing pathway. The $pH_{1/2}$ at which the experimental light yield reaches half of its maximum value is readily explained by the pH dependent reactivity of this hydroperoxide. When dissociated, it yields light through reaction 4 whereas the acidic form undergoes a dark reaction (eq 5) (see Scheme I).

The relation between the observed $pH_{1/2}$ and the pK_a of the hydroperoxide (obtained from the measured pH dependent rate constant) is expressed by the equation

$$pH_{1/2} = pK_a(\mathbf{5b}) - \log (k_4/k_5)$$

The $pH_{1/2}$ calculated according to this expression neatly coincides with the experimental $pH_{1/2}$, thus showing the consistency of the reaction scheme.

The decrease in the light yield is parallelled by similar decreases in both the consumption of the parent hydrazide and the yield of the dicarboxylic acid (Figure 1). The lowering of hydrazide consumption with decreasing pH shows that the dark reaction (i.e., the reaction of the neutral hydroperoxide **5**) is the regeneration of luminol (reaction 5). Even at pH >10.5 the yields are lower than expected from $G(\mathbf{3}) = 2.3$.

The partial regeneration of the hydrazide at pH >11 has been noted earlier.[12] At that time this was thought to be due to a competetive decomposition of the hydroperoxide. As the Arrhenius plots obtained for the rate of decomposition of the hydroperoxide are perfect straight lines (see below), this explanation can be ruled out. Rather it appears that neither reaction 2 nor 3 yields hydroperoxide **5** quantitatively.

The Efficiency of Hydroperoxide Formation. The yields of the hydroperoxide **5** in reactions 2 and 3 cannot be compared directly. Allowance must be made for that fraction of the diazasemiquinone radicals **4** which undergoes self-recombination (reaction 1) in competition with reaction 2. In the absence of H_2O_2 diazaquinone **3**, the product of reaction 1, hydrolyzes and does not contribute to chemiluminescence.

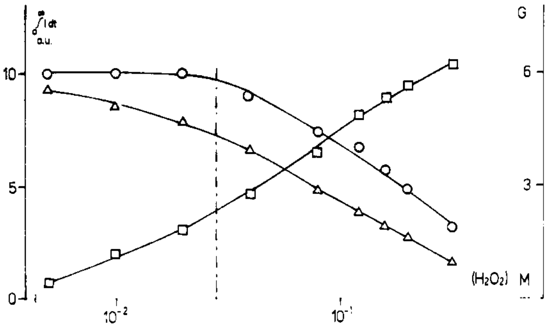


Figure 2. The total pulse radiolytical yields of chemiluminescence and of radicals formed initially as functions of H_2O_2 concentration (conditions: pH 10.6, 10^{-3} M luminol (**1b**), 0.5 M CO_3^{2-}, N_2O saturation): ○, total experimental light yield; △, measured initial yield of diazasemiquinone radicals **4b**; □, initial yield of $O_2^-\cdot$; full line, through circles; light yield calculated by use of the initial radical yields and the measured ratio $2k_1/k_2 = 2.2$ and assuming equal chemiluminescence efficiencies in reactions 2 and 3; broken line, H_2O_2 concentration at which reactions 2 and 3 have equal weights.

The most accurate way to investigate the efficiencies of hydroperoxide formation in reactions 2 and 3 is to measure pulse radiolytically the light yield at varying H_2O_2 concentrations. With[29] $k(HOO^- + \mathbf{3}) \sim 10^8$ M^{-1} s^{-1} a H_2O_2 concentration in excess of 5×10^{-3} M is sufficient for reaction 3 to completely suppress the hydrolysis of the diazaquinone. By increasing the H_2O_2 concentration, a growing fraction of the primary oxidants ($CO_3^-\cdot$, $OH\cdot$) is forced to yield $O_2^-\cdot$ by reacting with H_2O_2. The light yield as a function of the H_2O_2 concentration at constant pH is presented in Figure 2.

The relative light yields can be calculated numerically if the following parameters are known: (1) the initial yields of the diazasemiquinone **4** and superoxide radicals; (2) the ratio of the rate constants of reactions 1 and 2; (3) the ratio of the chemiluminescence efficiencies in reactions 2 and 3, i.e., the efficiencies of formation of the hydroperoxide **5**, φ_2/φ_3.

By absorbance measurements at 410 nm the initial yield of **4b** was determined. $O_2^-\cdot$ was calculated from the total radical yield allowing for the increase due to increasing scavenger concentration.[31] The rate constant $2k_1$ was measured in N_2O saturated solutions while k_2 was extracted from the pseudo-first-order decay when $O_2^-\cdot$ was generated in 10-fold excess over **4b**. As seen from Figure 2 an excellent fit between calculated and experimental light yield vs. H_2O_2 was obtained with the assumption that $\varphi_2 = \varphi_3$. The conclusion is thus inescapable that reactions 2 and 3 lead to the same yield of the critical hydroperoxide intermediate. This finding might indicate that reaction 3 is an electron-transfer process, as a result of which species **4** and superoxide are formed in a cage. The subsequent step, i.e., the radical recombination, would then be identical with reaction 2. The activation parameters (see discussion below) for the decomposition of the hydroperoxide **5** strongly suggest that it is carbon centered.

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(31) I. G. Draganic and Z. D. Draganic, "The Radiation Chemistry of Water", Academic Press, New York, 1971, p 128.

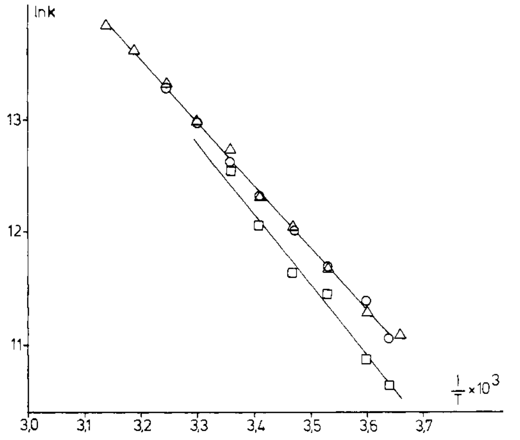
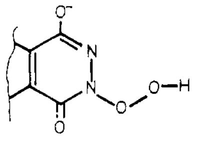


Figure 3. Arrhenius plots for the decomposition of the hydroperoxide **5** generated from (□) **1c** (4-aminophthalhydrazide) at pH 12.4, (○) **1b** (luminol) at pH 12.4, (△) **1d** (4-(dimethylamino)phthalhydrazide) at pH 14.0. All solutions were saturated with O_2 and contained 10^{-3} M hydrazide and 0.1 M N_3^-.

Table II. Activation Parameters for the Decomposition of the Hydroperoxide 5

starting hydrazide	E_a, kcal/mol	$10^{-13}A$, s^{-1}	$\Delta S^{\ddagger}$, eu
1c	12.5 ± 0.5	30	+5.8
1d	11.1 ± 0.1	4	+1.8
1b	11.1 ± 0.2	4	+1.8

In Table I we have summarized the measured parameters for a number of cyclic hydrazides. The rate constants for the decomposition of the hydroperoxide can only be monitored when the reaction leads to a reasonably efficient chemiluminescence. For nonchemiluminescent hydrazides, $(pH_{1/2})_{light}$ means the pH at which the yield of the dicarboxylate is halfed. The efficiencies φ_3 are calculated from the amount of dicarboxylate formed in H_2O_2 containing solutions divided by the initial amount of the diazaquinone **3**. The efficiency of reaction 2 or 3 is clearly dependent on the substituent. Below follows the simplest explanation consistent with the experimental findings. Reactions **2** and 3 are branced, with a branching ratio φ_3. In addition to species **5**, which reacts according to Scheme I, a nitrogen-centered hydroperoxide, **6**, is also formed. Species **6** partly regenerates the parent hydrazide and partly forms an unidentified byproduct.



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Activation Parameters for the Decomposition of the Light Yielding Hydroperoxide. Figure 3 presents Arrhenius plots for three strongly chemiluminescent hydroperoxides. The rate constants were determined from the buildup of the chemiluminescence. The initial concentrations of **4** and of $O_2^{-}\cdot$ were kept $<3 \times 10^{-6}$ M. In this way the effective rate of radical recombination was at least an order of magnitude lower than that of the hydroperoxide decomposition. In all experiments the pH was kept sufficiently high for the hydroperoxide to be dissociated at all temperatures.

All three plots yield perfect straight lines with no tendency to curve. This straight-line behavior is in itself an important mechanistic clue as it reveals that the decomposition of the hydroperoxide **5** is not a branched reaction. In Table II are given the activation parameters extracted from Figure 3. The low activation energies indicate that the process is concerted and not a sequential breaking and making of bonds. The evidence at hand strongly favors the chemiluminescent hydroperoxide to be carbon centered as a corresponding nitrogen-centered species could hardly undergo a smooth rearrangement to yield dicarboxylate **2**.

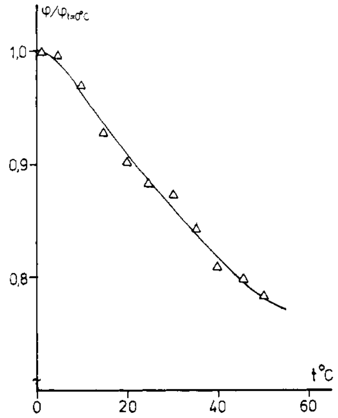
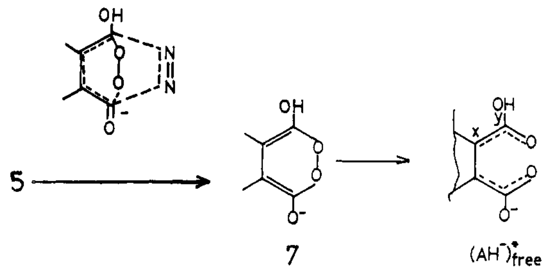


Figure 4. Temperature dependence of the quantum yields of chemiluminescence of **1d** (4-(dimethylamino)phthalhydrazide) and of fluorescence (4-(dimethylamino)phthalate, **2d**): △, chemiluminescence; full line, fluorescence. The fluorescence was measured in a pH 12 solution containing 5×10^{-5} M phthalate **2d**. The excitation wavelength was 255 nm. The chemiluminescence yield was measured upon pulsed e⁻-beam irradiation of an O_2-saturated solution containing 0.1 M N_3^-, 10^{-3} M hydrazide **1d** holding pH 14.

Scheme II



The activation entropies are positive and rather small. The overall picture is that the transition state is quite similar to but somewhat looser than the starting hydroperoxide. The fact that the reaction occurs only when the hydroperoxide is dissociated discloses that it must involve an intramolecular nucleophilic attack on the carbonyl carbon. However, this cannot be the whole truth since in this case the transition state would be a highly strained species with a substantial negative entropy of activation. Therefore, the loss of the degrees of freedom of the hydroperoxy moiety must be outweighed by a corresponding relief of the strain in some other part of the molecule, i.e., stretching of the carbon–nitrogen bonds. This transition state appears therefore to be a bicyclic endoperoxide with the nitrogens half way out of the molecule (see Scheme II).

The concerted decomposition of **5** could yield either (1) the dicarboxylate **2** directly or (2) a short-lived endoperoxide **7**. Since the direct formation of the dicarboxylate from **5** is an allowed process in the ground state,[32] alternative 1 is hard to reconcile with efficient chemiexcitation. On the other hand, the decomposition of **7** to yield the dicarboxylate has been shown by Michl[33] to be a potential source of chemiexcitation. In ref 34 the act of chemiexcitation has been considered in detail. Therefore, from the point of view of the total chemiluminescence mechanism the second alternative has a much stronger likelihood.

Finally, it should be mentioned that ever since the suggestion of Rauhut et al.[35] a bicyclic intermediate, formally similar to the

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(32) R. B. Woodward and R. Hoffman, "The Conservation of Orbital Symmetry", Academic Press, New York, 1970.

(33) J. Michl, *Photochem. Photobiol.*, **25**, 141 (1977).

(34) S. Ljunggren, G. Merényi, and J. Lind, *J. Am. Chem. Soc.*, following paper in this issue.

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

(36) J. Lee and H. H. Seliger, *Photochem. Photobiol.*, **11**, 247 (1970).

Table III. Yields Found in γ-Irradiated Solutions of **1d** at Different Temperatures[a]

t, °C	G(-hydrazide)	G(dicarboxylate)
0	1.8	1.14
25	1.7	1.04
90	1.3	0.97

[a] The solutions were pH 11.3, contained 2×10^{-3} M N_3^-, 10^{-4} M **1d**, and were saturated with O_2 gas.

Table IV. Apparent pK_a Values for the pH Titration of the Quantum Yields of Dicarboxylate Fluorescence and Hydrazide Chemiluminescence, Respectively

excited phthalate	$(pK_a)_{fl}$	$(pK_a)_{cl}$[a]
2b	12.6	13.1
2c	12.25	12.3
2d		13.5

[a] Determined by pulse radiolysis of O_2 saturated solutions containing 10^{-3} M hydrazide and 0.1 M N_3^-.

Table V. pH Dependence of Radiolytic Yields[a]

hydrazide	pH	G(-hydrazide)	G(dicarboxylate)
1d	12	1.7	0.68
	14	1.8	0.67
1b	12	1.2	0.63
	14	1.1	0.65

[a] O_2-saturated solutions containing 0.1 M N_3^- and 10^{-3} M hydrazide were irradiated by e^- beams.

above suggested transition state, has been assumed to exist as a real species en route toward the emitter. The present work, however, strongly suggests that such a species has no stability at all.

Temperature Dependence of Quantum Yields. In Figure 4 the chemiluminescence quantum yield of **1d** divided by the yield of **2d** is presented as a function of the temperature. Compound **1d** was chosen because it enables the reaction to be studied at pH 14. High pH is essential in order to ensure that the emitting molecule is the dianion of the dicarboxylic acid **2d** at all experimental temperatures (see below). The yield of the dicarboxylate at any temperature is interpolated from the yields in Table III. As can be seen from the figure an excellent agreement is obtained between the fluorescence and chemiluminescence quantum yields, which shows that the efficiency of chemiexcitation is independent of the temperature. The fact that the yield of the dicarboxylate is almost invariant with temperature clearly shows that the total mechanism does not contain branching steps with substantial difference in activation energies. Actually, since all the branching steps, i.e., reaction 2, and the competition between reactions 1 and 2 are radical recombinations, they are expected to have low and similar activation energies. For reactions 1 and 2 we have determined the activation energies to be 3.9 and 4.1 kcal/mol, respectively.

The Emitter: A New Model. In the present work we have undertaken comparative studies on the pH-dependent luminescence of the hydrazides **1b–d** and of their corresponding dicarboxylates **2b–d**. In the case of **b** and **c** the luminescence decreases to zero with increasing pH as discussed above. The corresponding $pH_{1/2}$ values are given in Table IV. Lacking amino protons, **2d** in alkaline solutions fluoresces with a pH-invariant quantum yield. Interestingly, however, the chemiluminescence quantum yield of **1d** increases between pH 13 and 14 as is shown in Figure 5. That these pH effects are not due to variations of the dicarboxylate yields clearly transpires from Table V. Furthermore, the rate constant for the decomposition of the hydroperoxide intermediate **5** is constant with all three hydrazides from pH 11 and up (5 M NaOH in the case of **5d**).

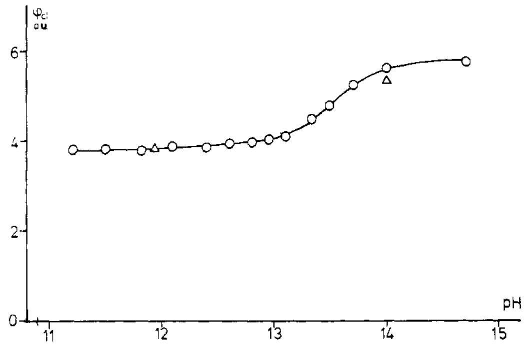


Figure 5. pH dependence of the chemiluminescence quantum yield of **1d** (4-(dimethylamino)phthalhydrazide): ○, pulse radiolytically determined light yield in O_2-saturated solutions containing 10^{-3} M hydrazide and 0.1 M N_3^-; △, light yield obtained in the reaction between the hydrazide **1d** and H_2O_2 with hemine as the catalyst (ref 39). The values are relative and normalized to the one obtained at pH 12.

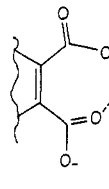
Table VI. Acid-Base Equilibria and Fluorescence Characteristics of Some Substituted Phthalic Acids

phthalic acid	protonation state	pK_a	λ_{max},[a] nm	$\Delta\nu$,[b] cm^{-1}	φ_{fl},[c] %	φ_{fl} lit. values
2b	AH_2	3.0	455	4180	10	4[d]
	AH^-	5.7	451	4270	23	
	A^{2-}		424	4430	30	
2c	AH_2	3.3	455	4120	9	
	AH^-	5.9	450	4180	9	
	A^{2-}		420	4450	41	11,[d] 13[e]
2d	AH_2	3.1	470	3760	0.3	
	AH^-	5.8	465	3810	0.6	
	A^{2-}		438	4010	64	25,[e] 60[f]

[a] Wavelength at maximum emission. [b] Band width of emission peak at half maximum height. [c] Fluorescence quantum yields based on the value of 30% reported for the dianion of 3-aminophthalic acid.[36] [d] From ref 36. [e] From ref 37. [f] From ref 38.

In the case of **1d** it is found that the chemiluminescence spectrum is slightly blue shifted (≃3 nm) and broadened (≃100 cm^{-1}) upon raising the pH from 11 to 14. It should be pointed out that the precursors (the hydroperoxide **5** and the endoperoxide **7** are expected to contain a hydroxylic proton. This follows from the lower acidity of the hydroxy group as compared to the hydroperoxy group and the pH invariance of the decomposition rate of the hydroperoxide (not even in 5 M NaOH solutions is any effect on the rate observed). In summary, all clues point to the formation of a monoprotonated excited state of the dicarboxylate **2**.

One immediately wonders: why is the chemiluminescence spectrum similar to the fluorescence spectrum of the dianion and not to that of the monoanion? The answer to this question will be approached in two steps. First one can notice that photoexcitation of the monoanion occurs from an intramolecularly hydrogen bonded ground state, **8**, to a similarly hydrogen bonded



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(37) R. B. Brundrett, D. F. Roswell, and E. H. White, *J. Am. Chem. Soc.*, **94**, 7536 (1972).

(38) V. N. Emokhonov, A. L. *Roschchin* and B. A. Rusin, 1978, VINITI, 1975–78.

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

excited state, **8***. On the other hand, chemiexcitation as proposed in the reaction scheme will produce an excited monoanion without intramolecular hydrogen bonding, and the fluorescence spectrum of this species may be almost identical with that of the dianion. Our surmise that the presence of a "free" proton has little effect on the fluorescence spectra is corroborated by a comparison of the values of AH_2 and AH^- compiled in Table VI.

Secondly, molecular orbital considerations[34] require that the excited singlet state have a substantial double bond character in the C_x–C_y bonds (see Scheme II). Evidently rotation around this bond must precede intramolecular hydrogen-bond formation. The rotational barrier around the C_x–C_y bond may be sufficiently high to freeze the excited state during its lifetime in the rotational conformation it had at the time of its formation.

The fluorescence quantum yield of the chemiexcited $(AH^-)^*_{free}$ containing a "free" proton cannot be measured directly since it cannot be generated by photoexcitation. A reasonable guess would be that the fluorescence quantum yield ratios are rather similar in the conjugate acid base couples $(AH^-)^*/(AH_2)^*$ and $(A^{2-})^*/(AH^-)^*_{free}$.

In Figure 5 the observed $pH_{1/2}$ of the chemiluminescence yield of **1d** reflects the kinetics of deprotonation, which can be represented by the scheme

$$h\nu \leftarrow (AH^-)^*_{free} \xrightarrow{OH^-} (A^{2-})^* \rightarrow h\nu$$

The observed procentual increase in chemiluminescence quantum yield (Figure 5) is of the same order as that for the increase of the φ_{fl} of **2d** when going from AH_2 to AH^- (Table VI). With the knowledge of the fluorescence lifetime of the dianion of 3-aminophthalic acid (**2b**) (6 ns),[40] the ratio of chemiluminescence yields of **1d** at pH 12 and 14, and by comparing the appropriate absorption band areas we estimate the fluorescence lifetime of the chemiexcited $(AH^-)^*_{free}$ of **2d** to be 4 ns. From this lifetime we calculate the rate constant for OH^- attack to be 8×10^8 M^{-1} s^{-1}, which is a reasonable value. By means of simple photophysical arguments based on experiments the rate constant of deprotonation against H_2O of the excited $(AH^-)^*_{free}$ can be shown to be less than 10^5 s^{-1}. Thus protonation equilibrium cannot be attained during the lifetime of the excited state.

When there are removable protons on the amino group the following reaction sequence operates:

$$h\nu \leftarrow (NH_2\text{–}AH^-)^*_{free} \xrightarrow{OH^-} ({}^-NH\text{–}AH^-)^*_{free} \rightarrow \text{dark}$$

Generally, the following relationship holds,

$$\tau = \tau_0\phi_{fl}$$

where τ, τ_0, and ϕ_{fl} denote respectively the fluorescence lifetime, radiative lifetime, and fluorescence quantum yield of the excited species in question. Since the absorption band areas of AH_2 and AH^- are almost identical with both **2b** and **2c** it follows that the τ^0 values for $(AH_2)^*$ and $(AH^-)^*$ are equal. Therefore, proportionality is expected between τ and ϕ_{fl}; i.e., $\tau(AH^-)^*/\tau(AH_2)^* \simeq \phi_{fl}(AH^-)^*/\phi_{fl}(AH_2)^*$. With the φ_{fl} values in Table VI at hand we predict the lifetime of $(A^{2-})^*$ to be equal to that of $(AH^-)^*_{free}$ of 4-aminophthalic acid (**2c**). The proximity of the $pH_{1/2}$ values observed for the OH^- quenching of the chemiluminescence and the fluorescence is in keeping with this prediction (see Table IV).

Similarly, in the case of 3-aminophthalic acid (**2b**) the lifetime of $(A^{2-})^*$ should be longer by a factor of 2.3 than that of $(AH^-)^*_{free}$. If so, OH^- should be more effective by about the same factor in quenching the fluorescence as compared to the chemiluminescence. This tallies with the observed $\Delta pH_{1/2} = 0.5$. In view of the good agreement between prediction and experiment it seems probable that chemiexcitation to a monoprotonated dicarboxylate singlet is a general feature of hydrazide chemiluminescence in aqueous solution.

The Efficiency of Chemiexcitation. It is customary[6] to partition the total chemiluminescence quantum yield, φ_{cl}, into the three factors, φ_{ch}, φ_{fl}, and φ_{ex}. φ_{ch} is the fraction of reagent molecules that traverses the chemiluminescent pathway, φ_{fl} is the quantum yield of fluorescence of the emitter, and φ_{ex} is the efficiency of chemiexcitation. From what has been said before it is evident that in the case of luminol φ_{fl} cannot be measured at pH values where $(AH^-)^*_{free}$ is the emitter. The best that can be done is to come up with a reasonable estimate.

Now, it is known that in dimethyl sulfoxide molecular oxygen reacts with the dianion of luminol to yield the excited dianion of 3-aminophthalic acid. In this case φ_{fl} can be measured, and φ_{ex} has been determined to be 9%.[17] In water where previously $(A^{2-})^*$ with the well-known $\varphi_{fl} = 30\%$ was assumed to be the chemiexcited species, the calculated φ_{ex} amounted merely to 4%.[8,17] The reason for this discrepancy was not understood. The present model predicts the φ_{fl} of $(AH^-)^*_{free}$ to be 0.13, which yields a $\varphi_{ex} = 9\%$ in excellent agreement with the Me_2SO value. Now, since in this model the act of chemiexcitation is conceived of as a purely intramolecular process, one would really expect its efficiency to be independent of the solvent.

Conclusions

(1) The carbon-centered hydroperoxide **5** is a key intermediate in the chemiluminescence of cyclic hydrazides **1**. It is formed with equal efficiency in reactions 2 and 3.

(2) In the decomposition of the neutral **5**, the parent hydrazide **1** is regenerated in a dark reaction.

(3) The chemiluminescent decomposition of the monoanion of **5** is a concerted process in which the endoperoxide **7** is likely to form in one single step.

(4) At the time of its formation the chemiexcited dicarboxylate singlet retains the protonation state of **5**; i.e., it is a monoanion.

(5) At moderate pH values the emission of light occurs from this monoanion. Unless the dicarboxylate singlet contains more easily removable protons (such as amino protons), a sufficiently high concentration of OH^- deprotonates the carboxylic group of the excited monoanion. In this case light emission occurs from the dianion singlet.

Acknowledgment. We are greatful to Dr. S. Ljunggren for many stimulating discussions. The Swedish Natural Research Council is acknowledged for its financial support.

Registry No. Phthalhydrazide, 1445-69-8; luminol, 521-31-3; 4-aminophthalhydrazide, 87781-78-0; 4-(dimethylamino)phthalhydrazide, 18697-31-9; 3-aminophthalic acid hydrochloride, 6946-22-1; 4-aminophthalic acid, 5434-21-9; 4-(dimethylamino)phthalic acid, 39519-77-2.

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(40) E. Würzberg and Y. Haas, *J. Phys. Chem.*, **83**, 2687 (1979).