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# Substituent Effect on the Chemiluminescence Quantum Efficiency of Some Acridan Derivatives

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Eight 9-benzylidene-*N*-methylacridan derivatives were synthesized and their chemiluminescence quantum yields on reaction with $NaOCl—H_2O_2$ or $O_3$ in *N*,*N*-dimethylformamide were plotted versus *Hammett*'s "$\sigma$" values. A good linear fit was obtained, the higher quantum yields being associated with negative "$\sigma$" values. Two acridans did not fit in the plot and a novel treatment of chemiluminescence quenching is outlined to account for the discrepancy.

*(Keywords: Acridans; Chemiluminescence; Hammett; Quenching)*

*Substituenteneffekt auf die Quantenausbeute der Chemilumineszenz einiger Acridin-Derivate*

Acht 9-Benzyliden-*N*-methylacridin-Derivate wurden hergestellt und ihre Chemilumineszenz-Quantenausbeute bei der Reaktion mit $NaOCl—H_2O_2$ oder $O_3$ in *N*,*N*-Dimethylformamid gemessen und gegen die *Hammet*schen „$\sigma$"-Werte aufgetragen. Es wurde eine gute lineare Abhängigkeit festgestellt, wobei die höheren Quantenausbeuten bei negativen „$\sigma$"-Werten zu finden waren. Zwei der Derivate wichen von der Linearität ab; zur Erklärung dieser Abweichungen wird ein neuer Gesichtspunkt bezüglich des Quenchprozesses der Chemilumineszenz aufgezeigt.

## Introduction

A large class of chemiluminescent reactions is associated with singlet excited carbonyl products[1, 3] and the same is true for the most striking examples of bioluminescence[2]. *N*-Methylacridone (*NMA*) with its high fluorescence efficiency in most solvents is an attractive such product and following its establishment as the primary emitter in the classical lucigenin light reaction[5], numerous chemiluminescent reactions have been designed leading to excited *NMA*. Studies on the

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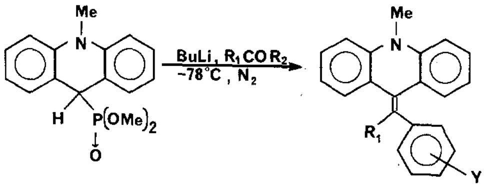
chemiluminescence of acridan derivatives have shown that the critical step leading to excited *NMA* is the highly exoenergetic decomposition of an intermediate 1,2-dioxetan and such studies have supported the idea that the light reaction of the various luciferins responsible for the bioluminescence of the firefly, marine organisms, photobacteria, etc. proceed via formation and decomposition of such four membered rings.

The large number of acridan derivatives synthesized has been reacted in a variety of ways[6] yielding high yields of singlet *NMA* or the corresponding 1,2-dioxetans have been prepared[3,4] and in many instances isolated, their induced decomposition under controlled conditions, again yielding high yields of singlet *NMA*. In contrast to simple dioxetans whose decomposition seems to follow a homolysis mechanism leading to triplet formation, the heavily substituted ones[3a] seem to decompose by concerted cleavage or radical ions[3b] and this is accompanied by strong chemiluminescence due to predominantly singlet formation. It has been argued that highly electron donating substituents increase the rate of decomposition of the dioxetan ring and although not always, cases are reported in which this rate increase is accompanied by very efficient chemiluminescence[3c]. In order to increase the general knowledge on the effect of substitution on the chemiluminescence of reactions proceedings via the dioxetan mechanism a study of the chemiluminescence of eight substituted *N*-methylacridans is herein reported together with a correlation of their quantum efficiencies with their "$\sigma$"*Hammett* values.

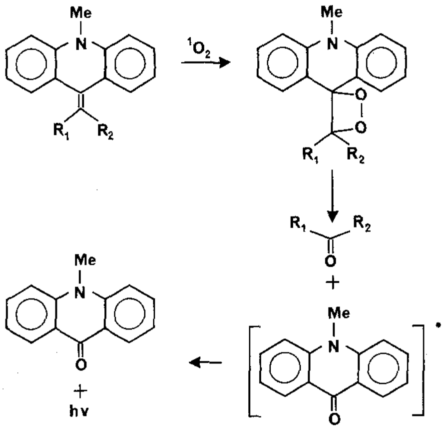
## Results and Discussion

The acridan derivatives shown in Scheme 1 (synthesized via the route shown) were employed in two types of chemiluminescent reactions. In one series of experiments the acridan derivatives as *N*,*N*-dimethylformamide (*DMF*) solutions in the presence of hydrogen peroxide were reacted with sodium hypochlorite (added dropwise), a reagent known to yield singlet oxygen[7]. This reaction yielded electronically excited *NMA**, apparently following the mechanism outlined in Scheme 2 (a similar mechanism[6a,8] has been proposed for the 10-10′-dimethyl-9,9′-biacrylidene chemiluminescence). De-excitation of *NMA** gives rise to emission of light and a typical light intensity-time diagram is shown in Fig. 1. The identity of *NMA** as primary emitter was established by comparing the reaction's chemiluminescence spectrum with the fluorescence spectrum of *NMA* and the fluorescence spectrum of the spent reaction mixture. As de-excitation of a species irrespectable of the mode of excitation ought to, *Scheme 1*



| | |
|---|---|
| I: $R_1=H$ | $Y=p\text{-}OCH_3$ |
| II: $R_1=H$ | $Y=p\text{-}Cl$ |
| III: $R_1=p\text{-}C_6H_4Cl$ | $Y=p\text{-}Cl$ |
| IV: $R_1=H$ | $Y=H$ |
| V: $R_1=H$ | $Y=m\text{-}NO_2$ |
| VI: $R_1=H$ | $Y=p\text{-}NO_2$ |
| VII: $R_1=H$ | $Y=p\text{-}N(CH_3)_2$ |
| VIII: $R_1=H$ | $Y=p\text{-}CH_3$ |

*Scheme 2*



and does result in the same emission spectrum the similarity of the *NMA* fluorescence and the reaction's chemiluminescence spectra clearly indicates that *NMA* is the primary emitter. Here, however, some energy transfer from *NMA* to unreacted starting material gives rise to emission from the starting material as well, resulting in the emission a 450 nm. It should be noted that energy transfer to, and emission from the starting material is a rather common phenomenon in chemiluminescence and especially in the chemiluminescence of systems such as the ones studied here[9]. A typical set of such spectra is shown in Fig. 2.

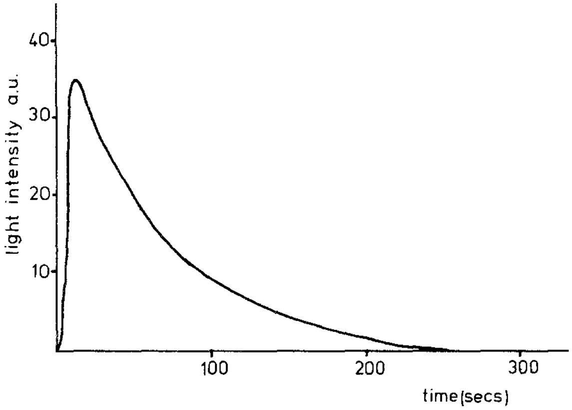


Fig. 1. A typical light intensity-time diagram for compound I

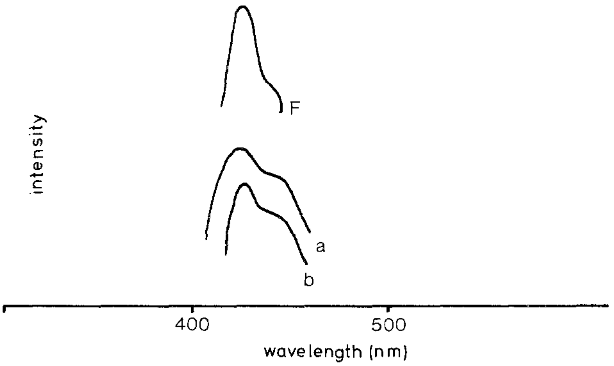


Fig. 2. A typical set of emission spectra for compound I. *a* Chemiluminescence spectrum; *b* fluorescence spectrum of the spent reaction mixture; *F* fluorescence spectrum of *NMA* under identical conditions

In another series of experiments the acridan derivatives of the present work (as *DMF* solutions) were reacted with ozone again yielding excited *NMA** (with much lower quantum yields only) as established by sets of spectra similar to the ones shown in Fig. 2, by a mechanism not too different[10] from the one shown above, leading to *NMA** via the primary ozonide.

The chemiluminescence quantum yields of the acridan derivatives obtained by integration of the light intensity-time diagrams and comparison with the integral of the luminol standard[11] were then plotted versus *Hammett*'s "$\sigma$" values as shown in Fig. 3 for the reactions

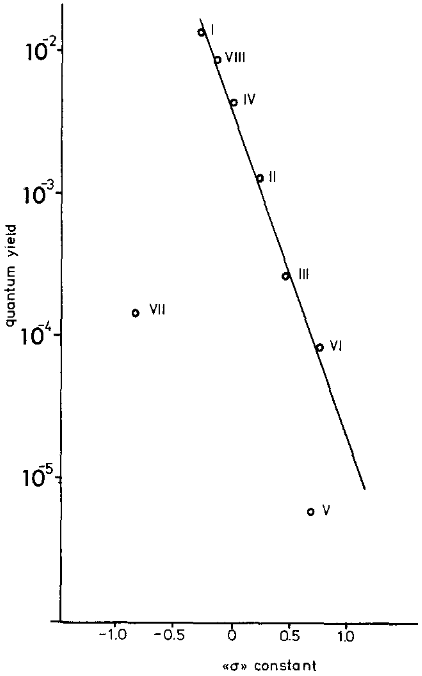


Fig. 3. Semi-logarithmic plot of the chemiluminescence quantum yields from compounds I–VII versus *Hammett*'s "σ" constants for the corresponding substituents taken from *Hine J. S.*, Physical Organic Chemistry. McGraw-Hill. 1962

with singlet oxygen and a similar plot was obtained for reaction with ozone. It is important to note the linear relation of the electron density at the double bond as reflected in *Hammett*'s "σ" values and the observed quantum yields of the chemiluminescence in both types of reaction. Taking into account the electrophilic character of both reagents employed, formation of the initial 1,2-dioxetan and the initial primary ozonide are favoured at negative "σ" values. More important, however, are the low quantum yields of the two acridans not fitting in the plot. Taking into account the CIEEL mechanism[12] of chemiluminescence especially as employed in the acridan case[4] one would expect the presence of a second nitrogen in the two deviating acridans to favour, rather than disfavour high chemiluminescence quantum yields. Furthermore, here the same compounds deviate from the plot [in the ozonization reactions it is mainly the $p$-$N(CH_3)_2$-derivative that deviates], in both types of light reaction, the second type not involving singlet oxygen in its reaction mechanism.

To explain this deviation, we believe that a case of luminescence quenching should be considered here, and the anomalous behaviour of the two acridan derivatives would be rationalized if the two aldehydes produced on decomposition of the intermediate dioxetans from compounds V and VII, were quenchers of the *NMA* fluorescence. Indeed progressive addition of those aldehydes to *DMF* solutions of *NMA* verified their quenching effect on *NMA*, yet its extent, especially at a ratio *NMA*: aldehyde equal to 1 (the ratio expected on decomposition of the intermediate dioxetan) was not so pronounced as to account for the dramatic deviation observed at the plot of Fig. 3. To further rationalize our results we now advance the following hypothesis: a) de-excitation by photon emission leaves the de-excited moiety in the light reaction with little thermal energy, b) the moving apart of the two moieties formed should be diffusion controlled, c) the moving apart of the two moieties (one is the quencher) is therefore not very fast and at a time comparable to the fluorescence lifetime at the emitter (a few nanoseconds) the quencher is still in relative proximity, d) as intermolecular energy transfer can occur at distances[13] as large as 50-100 Å, the "effective" quencher to *NMA* ratio is not 1 : 1 (the two moieties are produced in this ratio) but very much larger, compared to external equimolar addition of quencher to the excited species.

The anomalous behaviour of acridans could be explained in this way, but as, so far as we know, chemiluminescence quenching has never been treated in this way, we are presently studying this effect quantitatively with encouraging results (for example, $K_{\text{quenching}}$ of the aldehydes from compounds I, V, and VII on the *NMA* fluorescence is $1.15 \times 10^9$, $2.64 \times 10^{10}$, and $1.64 \times 10^{11}$ $\mathrm{l\,mol^{-1}\,s^{-1}}$, compared to $K_{\text{diffusion}}$ $7.7 \times 10^9$ $\mathrm{l\,mol^{-1}\,s^{-1}}$) and we hope that we will soon be able to report verification of the above postulation.

## Experimental

### *10-Dimethoxyphosphinyl-9-methyl-9,10-dihydro-9-azaanthracene*

Trimethyl phosphite (7.5 g, 61 mmol) and sodium iodide (9 g, 60 mmol) were added to a stirred solution of 9-methyl-9-azonianthracene methyl sulfate (18.5 g, 60 mmol) [prepared from acridine (22.5 g) and dimethyl sulfate (19.0 g) in benzene (55 ml)] in acetonitrile (300 ml) under nitrogen. Stirring was continued for 2 h, the solvent was then removed under reduced pressure and water (250 ml) was added to the residue; this was extracted with methylene chloride (3 × 150 ml), the extracts were dried over $MgSO_4$ and the solvent was removed under reduced pressure to leave on oily residue which was crystallized from ethyl acetate-ether to give the azaanthracene (12 g, 70%), m.p. 98-100 °C (Ref.[14]: 98-99 °C).

*9-(p-Methoxy)benzylidene-9,10-dihydroacridine-10-methyl* (I)

*Bu*Li in hexane (5 ml, 8 mmol) was added to a stirred solution of 10-dimethoxyphosphinyl-9-methyl-9,10-dihydro-9-azaanthracene (2.12 g, 7 mmol) in tetrahydrofuran (*THF*) (70 ml) at —78 °C under nitrogen and the solution immediately turned yellow due to the carbanion formed. *p*-Anisaldehyde (0.95 g, 7 mmol) in *THF* was added after 10 min, stirring was continued for another 10 min and the cooling bath was removed to let the reaction mixture reach room temperature. The solvent was then removed under reduced pressure and water (250 ml) was added to the residue followed by extraction with methylene chloride (3 × 150 ml); the combined extracts were dried over $MgSO_4$ and the solvent was removed under reduced pressure to leave a yellow solid which on recrystallization from methanol-benzene gave yellow flakes of I (1.2 g, 51%), m.p. 153.5-155.5 °C (Ref.[15]: 155-156 °C).

*9-(p-Chloro)benzylidene-9,10-dihydro-10-methylacridine* (II)

Prepared as I from the azaanthracene and *p*-chlorobenzaldehyde. Orange-coloured crystals from methanol-benzene (64%). Found: C 78.59, H 4.81, N 4.36%; $C_{21}H_{16}NCl$ requires: C 79.36, H 5.07, N 4.40%. M.p. 130-132 °C.

*9-[bis(p-chlorophenyl)-methylene]-9,10-dihydro-10-methylacridine* (III)

Prepared as I from the azaanthracene and 4,4'-dichlorobenzophenone with the modification that in this case the removal of the cooling mixture was followed by heating under reflux for an additional 45 min. Recrystallization from ethanol gave yellow-green fine crystals of III (16%), m.p. 257 °C (Ref.[14]: 257-258 °C).

*9-Benzylidene-9,10-dihydro-10-methylacridine* (IV)

Kindly offered by Prof. *F. McCapra* of the University of Sussex.

*9-(m-Nitro)benzylidene-9,10-dihydro-10-methylacridine* (V)

Prepared as I from the azaanthracene and *m*-nitro benzaldehyde. Recrystallization from methanol-benzene gave orange red fine crystals of V (54%), m.p. 183-186 °C (Ref.[15]: 182-185 °C).

*9-(p-Nitro)benzylidene-9,10-dihydro-10-methylacridine* (VI)

Prepared as I from the azaanthracene and *p*-nitro-benzaldehyde. Recrystallization from methanol-benzene gave VI as dark brown fine neddles (58%). Found: C 75.56, H 4.49, N 8.19%; $C_{21}H_{16}N_2O_2$ requires: C 76.81, H 4.91, N 8.53%. M.p. 169-171 °C.

*9-(p-Dimethylamino)benzylidene-9,10-methylacridine* (VII)

Prepared as III from the azaanthracene and *p*-dimethylaminobenzaldehyde. Recrystallization from methanol gave VII as orange coloured fine needles (46%). Found: C 83.89, H 6.15, N 7.93%. $C_{23}H_{22}N_2$ requires: C 84.63, H 6.79, N 8.58%. M.p. 111-113 °C.

*9-(p-methyl)benzylidene-9,10-dihydro-10-methylacridine* (VIII)

Prepared as I from the azaanthracene and *p*-tolualdehyde. Recrystallization from methanol gave VIII as yellow needles (53%), m.p. 128-131 °C (Ref.[14]: 127.5-128.5 °C).

*Chemiluminescence Intensity—Time Diagrams*

The light-reactions were carried out in a glass vessel securedly positioned in front of a Philips 56 AVP photomultiplier tube fed with 1700 V and connected with a Hewlett-Packard model 7044 A recorder and a "Σ" Time Electronics digital integrator. The reaction vessel-photomultiplier set-up were housed in a suitably constructed dark chamber with inlets for liquid and gaseous reactants. The chemiluminescence intensity-time diagrams were recorded on dropwise addition of aqueous sodium hypochlorite with the aid of a Gilson "minipuls 2" peristaltic pump (5 ml/min) to *N*,*N*-dimethylformamide (Aldrich, spectrophotometric grade) solutions of the compounds (10 ml, $10^{-5}$ *M*) containing hydrogen peroxide (0.3%). Where ozone was employed, it was produced with the aid of a Fischer 500 ozone generator and was bubbled through the appropriate reaction mixtures.

*Chemiluminescence Quantum Yields*

Chemiluminescence quantum yields were calculated by comparing the light intensity-time integrals of the acridan derivatives with the intensity-time integral of the standard luminol[11] light reaction, under the same optical geometry and are based on reactant employed. Corrections due to the photomultiplier's spectral response were unnecessary as both reactions emit in the same spectral region.

*Spectra*

Excitation and fluorescence spectra were recorded on an Aminco-Bowman spectrophotofluorometer calibrated with a quartz "pen-ray" lamp and are uncorrected. Absorption spectra were obtained with the aid of a Cary 14 spectrophotometer. Chemiluminescence spectra were recorded on the Aminco-Bowman instrument employing fast scanning rates and wide slits, with the excitation source off.

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