Synthesis and chemiluminescent evaluation of a series of phenyl *N*-alkylacridinium 9-carboxylates

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Abstract

The nitrogen atom of phenyl acridine-9-carboxylate was alkylated with several alkylating agents using thermal, photochemical and sonochemical techniques. Thermal reactions were only suitable for methylation and ethylation, but irradiation with UV light or ultrasound allowed the synthesis of *n*-propyl, iso-propyl and benzyl derivatives. Use of ultrasound produced cleaner product mixtures than UV irradiation. The quantum yields and kinetics of the chemiluminescent reactions of the products with alkaline hydrogen peroxide were found to be independent of the nature of the *N*-alkyl group.

1. Introduction

The production of light from a chemical reaction is called chemiluminescence ("cold light"). This phenomenon is observed when the excited product of a chemical reaction emits photons upon relaxation to its ground state [1]. Several classes of molecules are capable of undergoing chemiluminescent reactions; the examples include acridinium esters of type **1** [2].

Acridinium esters are capable of exhibiting chemiluminescence under aqueous conditions in the absence of a catalyst, emission occurring in the presence of dilute alkaline hydrogen peroxide. The reaction has been proposed to occur via a concerted multiple bond cleavage mechanism involving a dioxetanone intermediate **3**. When $R \equiv CH_3$, the excited emitter arising from the decomposition of this intermediate is *N*-methylacridone (**4a**) which emits photons at 426 nm (Scheme 1) [3].

The quantum yield of the reaction ϕ_{CL} is a product of the yields ϕ_c, ϕ_e, and ϕ_f which respectively represent the chemical yield, the excited state yield and the fluorescence quantum yield of the emitter. Structural changes in the molecule may change one or more of these parameters, hence altering the quantum yield of the reaction. For most *N*-methylacridinium esters, $\phi_{CL} = 0.05$.

In recent years, acridinium molecules have gained importance as replacements for radioisotopes in immunoassays [4] and DNA hybridization assays [5]. In these situations the *N*-methylated acridinium salts have been used and little is known about the effectiveness of other *N*-alkylacridinium salts. *N*-methylacridinium salts are produced

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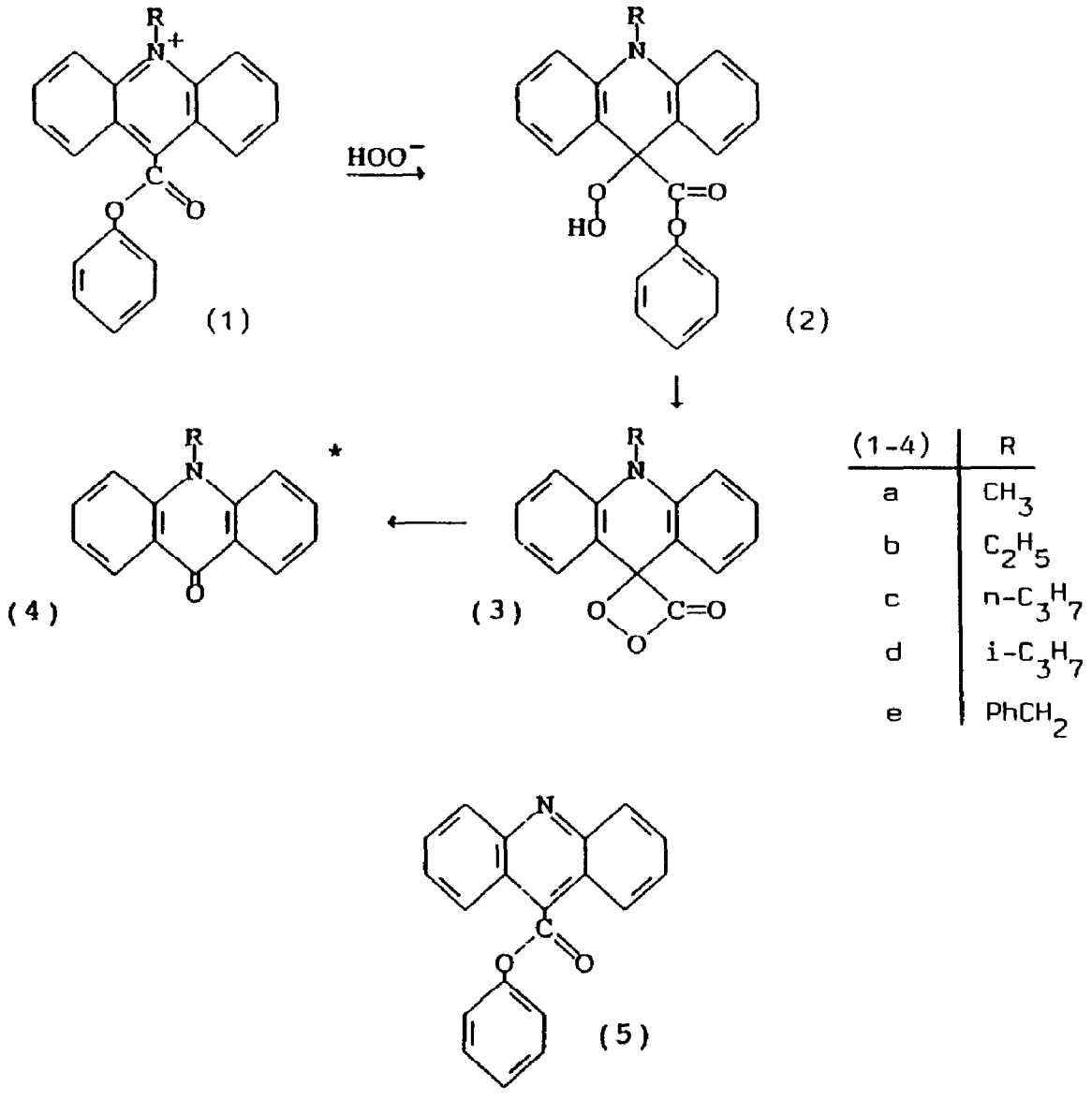

Scheme 1.

from the neutral acridine species by methylation with methyl fluorosulphonate at room temperature [6] or with methyl iodide [7] or dimethyl sulphate at elevated temperatures [8]. Simple acridines have also been alkylated with methyl *p*-toluenesulphonate [9] and allyl bromide [10]. However, alkylation with higher alkyl groups is more difficult and other methods for the production of quaternary acridines have therefore been reported [11, 12].

2. Experimental details

2.1. Synthesis of phenyl acridine-9-carboxylate (5)

Acridine-9-carbonyl chloride was prepared by established methods [13]. Thus, this compound (5.32 g, 22 mmol) was dissolved in anhydrous pyridine (25 ml). Dried phenol (2.2g, 23 mmol) was added and the mixture was stirred at room temperature for 18 h, then poured into ice cold water (250 ml). The product thus obtained was filtered and dried in air (6.63 g, 22 mmol, 96%), melting point 180–181 °C (from diethyl ether) (ref. 14 189–190 °C): ν_{max} (KBr) 3050 (C−H) and 1755 cm^{-1} (C=O); ^1H nuclear magnetic resonance $\delta(CDCl_3)$ 8.5–7.3 (13H, m, arom); *m/z* 299 (M^+, 22%), 206 (100%); $C_{20}H_{13}NO_2$ (M^+) requires 299.0934, found 299.1090.

2.2. Synthesis of N-alkylacridinium-9-carboxyphenyl esters (1a–1e)

Various methods were used for the synthesis of these compounds. Representative procedures are given below.

2.2.1. Ambient temperature

Here, methyl trifluoromethanesulphonate was used as the methylating agent in a manner described previously [6].

2.2.2. Thermal reactions

A solution of phenyl acridine-9-carboxylate (0.5 mmol) in methyl or ethyl iodide (5 ml) was placed in a thick-walled glass tube (Carius tube). The contents were frozen in liquid nitrogen and sealed. After warming to room temperature the tube was heated to 100–110 °C for the appropriate period of time. The tube was allowed to cool, refrozen and the seal broken to facilitate removal of the contents. The residue obtained by evaporation under reduced pressure was purified by flash column chromatography (silica gel, chloroform–methanol) followed by evaporation of the solvent under reduced pressure to yield pure products **1a** and **1b**.

2.2.3. UV irradiation

In a round-bottom flask equipped with a reflux condenser was placed a solution of phenyl acridine-9-carboxylate (0.1 g, 0.33 mmol) in excess alkylating agent (5 ml). The solution was irradiated with light from a medium pressure mercury vapour arc lamp (250 W) positioned 2 cm from the reaction vessel. Incubation times were 8–25 h. Following irradiation, the contents were evaporated under reduced pressure and the residue purified by flash column chromatography as described above.

2.2.4. Ultrasound irradiation

A solution of phenyl acridine-9-carboxylate (0.15 g, 0.5 mmol) in excess alkylating agent (5 ml) was placed in a stoppered flask and placed in a 40 kHz ultrasound bath for 18–80 h. The residue obtained by evaporation under reduced pressure was purified by chromatography as described above. The analytical data for the compounds **1a–1e** are given in Table 1.

2.3. Preparation of samples for chemiluminescence measurements

Solutions of the products **1a–1e** were made in acetonitrile (0.5 mg ml^{-1}). These solutions were then diluted further as necessary by making serial ten-fold dilutions in a sodium phosphate buffer (0.1 M, pH 6.3). Aliquots of 10 μl were taken for luminometry.

TABLE 1

Analytical data for *N*-alkylacridinium esters (IR data for KBr discs, ^1H nuclear magnetic resonance data expressed as δ for d_6-DMSO solutions; mass spectrometry data for fast atom bombardment from thioglycerol)

Product	Melting point (°C)	Analysis
1a	201–202 (decomposition)	ν_{max} 3000 (C−H) and 1770 cm^{-1} (C=O); δ, 9.16–7.52 (13H, m, arom) and 5.04 (3H, s, CH_3); *m/z* 314 (m^+, cation, 100%), $C_{21}H_{16}NO_2$ requires 314.1176, found 314.1190.
1b	220–222 (decomposition)	ν_{max} 3000 (C−H) and 1770 cm^{-1} (C=O); δ, 9.18–7.52 (13H, m, arom), 5.64 (2H, q, $J=8$ Hz, CH_2) and 1.76 (3H, t, $J=6$ Hz, CH_3); *m/z* 328 (m^+, cation, 100%), $C_{22}H_{18}NO_2$ requires 328.1332, found 328.1328.
1c	216–219 (decomposition)	ν_{max} 3000 (C−H) and 1760 cm^{-1} (C=O); δ, 9.15–7.5 (13H, m, arom), 5.60 (2H, t, $J=8$ Hz, CH_2), 5.40 (2H, m, CH_2) and 5.05 (3H, t, $J=6$ Hz, CH_3); *m/z* 342 (m^+, cation, 50%), $C_{23}H_{20}NO_2$ requires 342.1494, found 342.1494.
1d	231–235 (decomposition)	ν_{max} 3000 (C−H) and 1760 cm^{-1} (C=O); δ, not determined, insufficient sample; *m/z* 342 (m^+, cation, 30%), $C_{23}H_{20}NO_2$ requires 342.1494, found 342.1490.
1e	189–190 (decomposition)	ν_{max} 3090–3000 (C−H) and 1745 cm^{-1} (C=O); δ, 8.46–7.42 (18H, m, arom) and 5.90 (2H, s, CH_2); *m/z* 390 (m^+, cation, 50%), $C_{27}H_{20}NO_2$ requires 390.1488, found 390.1481.

Melting points are uncorrected.

3. Results

The aim of this study was to investigate alternative methods of alkylating the relatively unreactive acridine heteroatom and to produce a range of *N*-alkylated derivatives to obtain information on the effect of alkylation on the quantum yield and kinetics of the chemiluminescent reactions of acridinium salts. The study involved a comparison of thermal, photochemical and ultrasound methods.

A range of *N*-alkylacridinium esters (**1a–1e**) was produced using these methods. Initial studies involved reactions in sealed tubes heated to temperatures in excess of 100 °C (care). Usable products were only obtained in the case of *N*-methylation and *N*-ethylation. This technique is not advisable because of the risk of explosion at these elevated temperatures. Methyl trifluoromethanesulphonate was found to be a useful methylating agent though like methyl fluorosulphonate it is highly toxic [15].

The use of ultrasound irradiation of the reaction mixtures at ambient temperature yielded the *N*-alkylated products with a range of alkylating agents. Though ultrasound-

TABLE 2

Summary of reaction times and yields

Alkylating agent	Thermal		UV		Ultrasound	
	Time[a] (h)	Yield (%)	Time[b] (h)	Yield (%)	Time[b] (h)	Yield (%)
CH_3I	24	85	8	50	18	60
C_2H_5I	120	88	18	45	35	50
n-C_3H_7I	–	–	20	10	80	15
i-C_3H_7I	–	–	25	3	80	5
$C_6H_5CH_2Br$	–	–	14	40	72	50

[a]At 100–110 °C. [b]At ambient temperature.

TABLE 3

Chemiluminescence emission intensities

N-alkylacridinium salt	RLU ($\times 10^6$)	$t_{1/2}$ (s)
1a	6.45	0.50
1b	5.22	0.55
1c	5.32	0.49
1d	5.26	0.50
1e	5.12	0.50

RLU, relative light unit, with background subtraction of 1000 RLU.

driven reactions proceeded more slowly than photolytic reactions, cleaner product mixtures were obtained. In all cases *N*-methylation took place more rapidly and gave a better yield than *N*-ethylation or *N*-benzylation, while reactions with higher alkylating agents proceeded slowly and gave poor yields. Nevertheless, the reactions were successful in producing samples of the desired products. The results are summarized in Table 2, where yields refer to crude reaction products prior to purification.

The chemiluminescence emission intensities for peroxide decomposition of the *N*-alkylated products were measured using a Magic Lite Analyser (Ciba Corning Diagnostics, Medfield, MA 02052, U.S.A.). The instrument added sequentially 300 μl of a hydrogen peroxide solution then 300 μl of sodium hydroxide solution (reagents supplied by Ciba Corning) and integrated the photon counts (relative light units, RLU) over 2 s. The results are shown in Table 3. It is apparent that neither the quantum yield nor the kinetics of emission are affected by the change in the nature of the alkyl groups. However, the ester **1e** can be modified so that it can react with proteins and other biological molecules via its *N*-benzyl group. This will give rise to a new class of acridinium ester labels where during hydrogen peroxide decomposition, *N*-alkylacridone would still be attached to the analyte. Other uses of this type of labelling capitalize on the highly fluorescent nature of *N*-alkylacridones.

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