PHOTOCHEMISTRY OF COUMARIN LASER DYES: THE ROLE OF SINGLET OXYGEN IN THE PHOTO-OXIDATION OF COUMARIN 311

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Summary

Coumarin 311 was shown to sensitize the formation of singlet oxygen, a reactive intermediate proposed to be responsible for degradation of coumarin dyes in dye lasers. The reactivity of Coumarin 311 and various oxidized states of Coumarin 311 with both the $^1\Delta$ and $^1\Sigma$ states of molecular oxygen was determined. Both singlet states of oxygen react predominantly at the dimethylamino substituent. Although singlet oxygen is formed in coumarin dye lasers and oxidizes the dyes, the products resulting from singlet-oxygen oxidation do not interfere with stimulated emission.

1. Introduction

The utility of dye lasers is often limited by photochemical degradation of the dyes to molecules which absorb light at the lasing wavelength of the dye, interfering with stimulated emission. The mechanism of these photo-degradation reactions is not well understood and has been the subject of investigation by several research groups.

Coumarin laser dyes are popular dyes for the blue–green spectral region. Winters *et al.* studied the aerobic photochemistry of Coumarin 1 (7-diethylamino-4-methylcoumarin) and identified several products from the photoreaction [1]. One of these products, the carboxylic acid resulting from oxidation of the 4-methyl substituent, was found to interfere with stimulated emission in a Coumarin 1 dye laser. No mechanism was proposed to account for the formation of these photoproducts.

In 1970, Marling *et al.* reported that oxygen can quench the triplet excited state of Coumarin 1 and other dyes [2]. The quenching of triplet excited states by molecular oxygen often results in the generation of singlet oxygen. *trans*-Stilbene is also known to quench the triplet state of Coumarin 1 [3]. Consequently, the triplet energy of Coumarin 1 cannot be significantly below that of *trans*-stilbene (50 kcal mol^{-1} [4]), and triplet Coumarin 1 should be capable of sensitizing the formation of both the $^1\Delta$ and $^1\Sigma$ states

of oxygen [5]. Both states can serve as oxidizing agents and could play a role in the photo-oxidation of coumarin dyes. We have also been concerned about the possible role of these states of oxygen in dye degradation because earlier we observed that addition of 1,4-diazabicyclo[2.2.2]octane (DABCO), an efficient singlet oxygen quencher [6], to dye laser solutions can increase the useful lifetime of several of the dyes [7]. Therefore, we have investigated the aerobic photochemistry of Coumarin 311 (7-dimethylamino-4-methyl-coumarin (1)) and the reaction of both the $^1\Delta$ and $^1\Sigma$ states of singlet oxygen with 1 and its photoproducts.

2. Results

Irradiation of 1 in oxygen-saturated ethanol using a medium pressure mercury lamp at 366 nm resulted in the formation of 7-methylamino-4-methylcoumarin (2), 7-dimethylamino-4-hydroxymethylcoumarin (3), 7-dimethylaminocoumarin-4-carboxyaldehyde (4) and 7-dimethylamino-coumarin-4-carboxylic acid (5) as summarized in eqn. (1). These products

$$\text{(scheme: 1 Coumarin 311} \xrightarrow[\text{EtOH}]{\substack{h\nu \\ O_2}} \text{2 + 3 + 4 + 5 + 6)} \tag{1}$$
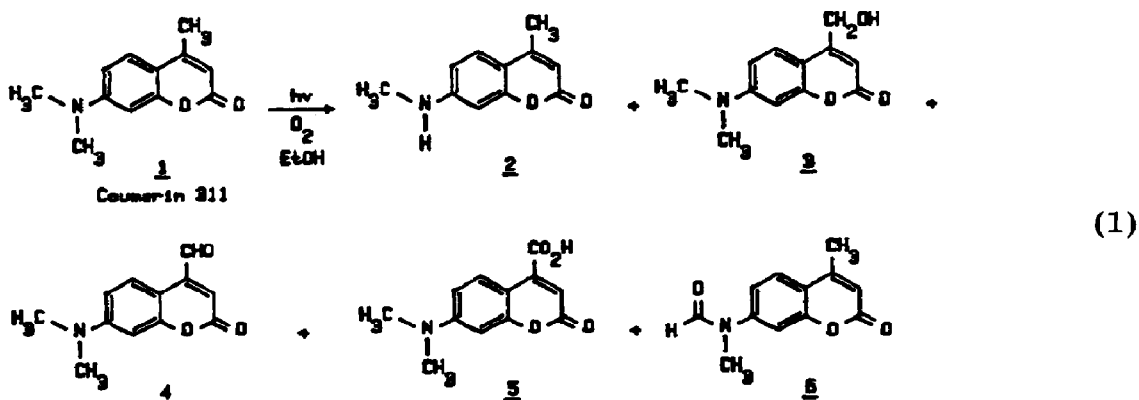
are all analogous to the photoproducts reported by Winters et al. from the aerobic irradiation of Coumarin 1 [1]. We have confirmed Winters' finding that carboxylic acids such as 5 absorb light at 460 nm, the approximate lasing wavelengths of both Coumarin 1 and 1, and their presence would interfere with stimulated emission. The aldehyde 4 has a chromophore similar to 5 and might be expected to absorb light at the lasing wavelengths as well; however, 4 spontaneously forms an acetal or hemiacetal in ethanol solvent as shown in eqn. (2). The proton nuclear magnetic resonance (^{1}H NMR) spectrum of 4 in hexadeuteriodimethyl sulfoxide (DMSO-d_6) solvent

$$\text{(scheme: 4} \xrightarrow{\text{EtOH}} \text{HCOEt derivative, R = H or Et)} \tag{2}$$
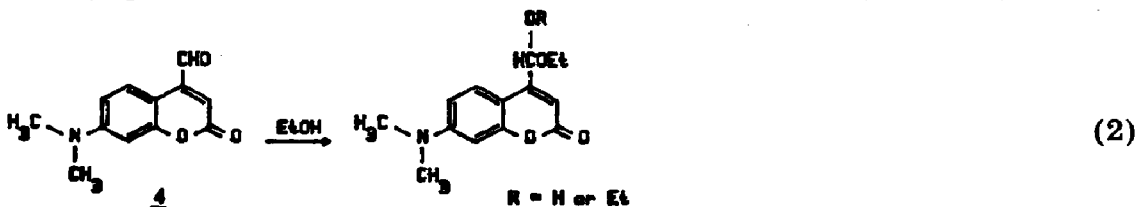
shows an aldehyde proton at δ 10.20 ppm, whereas, in tetradeuteriomethanol or ethanol solvent no aldehyde proton appears. Consequently, **4** as its acetal or hemiacetal in ethanol solvent does not absorb light significantly at 460 nm.

An additional product observed from the irradiation of **1** in oxygen-saturated ethanol was 7-formylmethylamino-4-methylcoumarin (**6**). Amide **6** is similar to products reported by Jones *et al.* [8] from the irradiation of Coumarin 153 (1,2,4,5,3*H*,6*H*,10*H*-tetrahydro-8-trifluoromethyl(1)benzopyrano(9,9*a*,1-*gh*)quinolizin-10-one) in oxygen-saturated acetonitrile solvent. The Coumarin 153 amide photoproducts were proposed to result from oxygen quenching of the singlet excited state of the dye [8]. Amide **6** absorbs at much shorter wavelengths than **1** and, consequently, does not interfere with stimulated emission.

In order to determine whether **1** could sensitize the formation of singlet oxygen, an oxygen-saturated solution of 6.7×10^{-4} M **1** and 0.20 M 2,3-dimethyl-2-butene (**7**) in methanol was irradiated with a Pyrex-filtered medium pressure mercury lamp. This resulted in the formation of 2,3-dimethyl-3-hydroperoxy-1-butene (**8**) in 28% isolated yield (eqn. (3)). The hydroperoxide **8** is the known product of the reaction of $O_2(^1\Delta)$ with **7** [9]; hence, **1** at least can sensitize the formation of the $^1\Delta$ state of oxygen.

$$\tag{3}$$

The reactions of singlet oxygen with **1** and its photoproducts were studied by irradiation of oxygen-saturated ethanol solutions of the coumarin dye and the singlet oxygen photosensitizers Rose Bengal for production of predominantly $^1\Delta$ or fluorescein for $^1\Sigma$ and $^1\Delta$ states of oxygen. The solutions were irradiated with filtered light which only the sensitizer absorbed, thus preventing interference from photoreactions of the coumarin dye itself.

A solution of 5.0×10^{-2} M **1** and 8×10^{-4} M Rose Bengal in oxygen-saturated ethanol was irradiated for 3 h with a 400 W medium pressure mercury lamp in a Pyrex immersion well surrounded with a cupric chloride–calcium chloride filter solution. The filter solution transmitted light of wavelengths 500 - 575 nm, measured at 50% transmittance [10]. Two products were isolated from the reaction mixture by silica-gel flash chromatography [11] and characterized by ^{1}H NMR and mass spectrometry as the demethylated product **2** and the formamide **6**. The identification of **2** was confirmed by its synthesis via the reaction of 7-amino-4-methylcoumarin with formaldehyde and sodium cyanoborohydride in 95 vol.% ethanol adjusted to pH 5 - 6 with dilute HCl. The formamide **6** was then synthesized from **2** by reaction with formic acid and acetic anhydride. A control experiment was performed in which 5.0×10^{-2} M **1** without Rose Bengal was similarly irradiated for 5 h. No products were observed by high performance

liquid chromatography (HPLC) or thin layer chromatography (TLC). Analogous reactions of $O_2(^1\Delta)$ are demethylation at the methylamino group of codeine [12] and oxidation of the methylamino group of pseudopelletierine to a formamide group [13]. Singlet oxygen may also have been involved in the oxidation of Coumarin 153 to two isomeric amides [8].

The coumarin photo-oxidation products 2 and 6 possibly resulted from reaction of 1 with $O_2(^1\Delta)$ and 3, 4 and 5 from reaction with the higher energy $O_2(^1\Sigma)$. Kearns et al. [14] have reported two products from singlet-oxygen oxidation of a steroid. With Rose Bengal as the sensitizer, one product predominated and it was attributed to oxidation by $O_2(^1\Delta)$ oxygen. With fluorescein as the sensitizer the other product predominated and it was attributed to oxidation by $O_2(^1\Sigma)$ [14]. The following experiment was performed to test whether $O_2(^1\Sigma)$ from fluorescein sensitization would oxidize 1 to 3, 4 or 5.

A solution of 1.0×10^{-2} M 1 and 1.0×10^{-2} M fluorescein in oxygen-saturated ethanol was irradiated for 12 h with a 500 W slide projector lamp filtered with an Oriel 51490 glass filter which transmits light of wavelengths longer than 450 nm. Again, the only products observed were 2 and 6. Even though no new products were observed which could have been attributed to $O_2(^1\Sigma)$, the possibility still exits that 3, 4 or 5 might result from $O_2(^1\Sigma)$ oxidation. The lifetime of $O_2(^1\Sigma)$ in solution is unknown [5]; however, it is much shorter than the lifetime of $O_2(^1\Delta)$ [14]. Therefore, the reaction of $O_2(^1\Sigma)$ produced from fluorescein sensitization with 1 may not have occurred because the $O_2(^1\Sigma)$ may have internally converted to $O_2(^1\Delta)$ before it encountered 1 by diffusion. When 1 is the sensitizer, $O_2(^1\Sigma)$ is created adjacent to a 1 molecule and diffusion is not required.

Even though $O_2(^1\Delta)$ or $O_2(^1\Sigma)$ oxygen did not appear to react with 1 to yield 3, 4 or 5, experiments were also performed to determine whether singlet oxygen would react with 3 or 4 to produce 5, the product which interferes with stimulated emission in the dye laser. For these experiments 3, 4 and 5 were synthesized by the sequence of steps summarized in Scheme
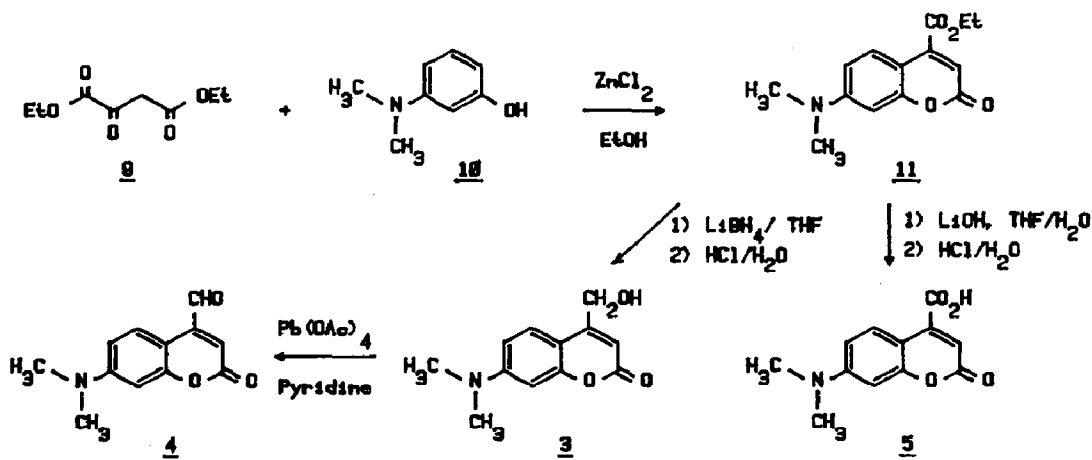
Scheme 1.

1. Diethyl oxalacetate (9) was prepared by acidification of an aqueous solution of the sodium salt of oxalacetic acid diethyl ester, extraction with dichloromethane and vacuum distillation. The reaction of 9 with 3-dimethyl-aminophenol (10) using anhydrous zinc chloride as a catalyst yielded ethyl-7-dimethylaminocoumarin-4-carboxylate (11) in 63% yield. Hydrolysis of 11 with lithium hydroxide in 50vol.%tetrahydrofuran–50vol.%water followed by acidification afforded 5 in 86% yield. Reduction of 11 with lithium borohydride in tetrahydrofuran gave 3 in 37% yield after silica-gel chromatography. Oxidation of 3 with lead tetraacetate in pyridine [15] afforded 4 in 8% yield after silica-gel chromatography.

Irradiation of 4.6×10^{-3} M 3 and 1.5×10^{-3} M Rose Bengal in oxygen-saturated ethanol with the filtered mercury lamp for 4 h resulted in no observable product formation as determined by HPLC. Similarly, irradiation of 1.0×10^{-2} M 3 and 1.0×10^{-2} M fluorescein with the filtered projector lamp for 20 h yielded no observable products. However, irradiation of 1.6×10^{-3} M 3 and 1.5×10^{-3} M Rose Bengal in 50vol.%ethanol–50vol.%water resulted in efficient formation of a new product isolated by preparative HPLC and characterized by ^{1}H NMR and mass spectrometry as the de-methylated product 7-methylamino-4-hydroxymethylcoumarin (12), as shown in eqn. (4). No other products were observed by HPLC.

$$\text{(4)}$$
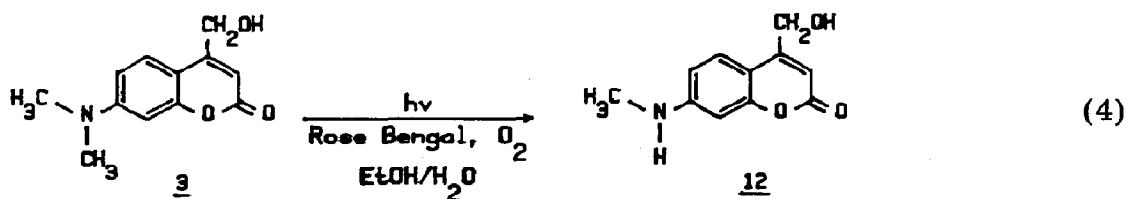
Irradiation of a solution of 1.0×10^{-2} M 4 and 1.0×10^{-2} M fluorescein in oxygen-saturated ethanol with the filtered projector lamp for 20 h resulted in the formation of a small amount of 5 as determined by HPLC. Although this result does suggest that singlet oxygen can oxidize 4 to 5, the reaction is so inefficient that it cannot be an important pathway for the formation of 5 in dye lasers. Furthermore, the concentration of 4 never rises to an appreciable level during the photoreaction of 1.

3. Conclusions

The results of these experiments suggest that, although 1 can sensitize the formation of singlet oxygen and singlet oxygen does react with 1 and some of its photoproducts, singlet-oxygen oxidation does not appear to be a significant pathway to products such as 5 which have deleterious effects in coumarin dye lasers.

Earlier we reported that some of the photochemistry of 1 in oxygen-free ethanol solvent occurred via a bimolecular reaction, possibly of the

triplet state, which resulted in the formation of free radicals [16]. The reaction of molecular oxygen with these free radicals could account for the formation of **3**, **4** and **5**. However, preliminary experiments indicate that the quantum yields of formation of **3**, **4** and **5** in oxygen-saturated ethanol show only a slight dependence on coumarin concentration over the range (1×10^{-2}) - (2×10^{-4}) M, which is inconsistent with a bimolecular reaction pathway.

Another possible mechanism for the formation of **3**, **4** and **5** is electron transfer from singlet excited coumarin to oxygen followed by proton transfer from the 4-methyl substituent and radical combination as shown in Scheme 2. Oxygen has been shown to quench the excited singlet state of
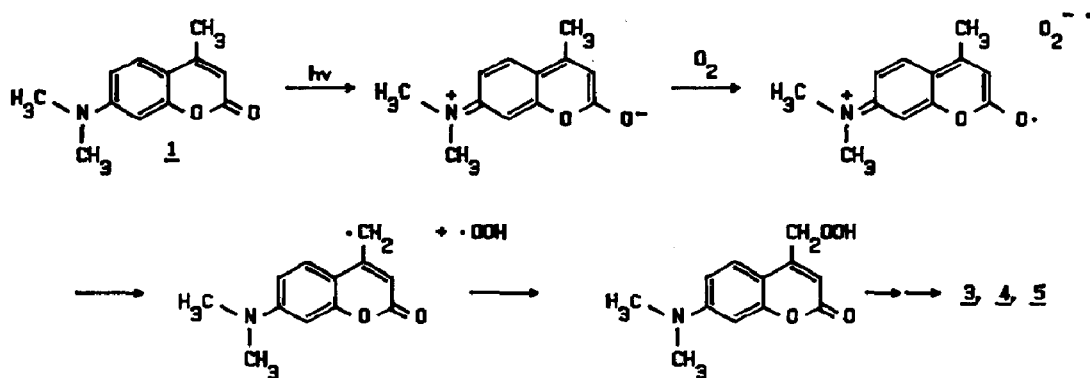
Scheme 2.

coumarin dyes from the effect of oxygen on dye fluorescence [17]. Oxygen quenching of the singlet excited state of the dye resulting in oxidation at the 4-methyl substituent rather than the dialkylamino group is consistent with the proposal that the excited singlet state of 7-aminocoumarin dyes is an intramolecular charge transfer state in which the amino substituent is electron poor and the lactone group electron rich [18]. Further work is underway to determine whether this mechanism explains oxidation at the 4-methyl substituent of 7-amino-4-methylcoumarin laser dyes and whether it is a reasonable explanation for degradation in dye laser output under aerobic conditions.

4. Experimental details

4.1. Instruments and methods used for analysis

Melting and boiling points are uncorrected and reported in degrees Celsius. All melting points were measured with a Thomas—Hoover melting point apparatus. IR spectra were recorded using a Perkin—Elmer Model 337 IR spectrometer. UV and visible absorption spectra were recorded with a Hewlett—Packard 8450 A spectrophotometer using quartz cuvettes 1 cm

square. ^{1}H NMR spectra were obtained using a Varian Associates EM 390, a Chemagnetics A200 or a Bruker 250 MHz spectrometer. NMR chemical shifts are reported in parts per million on the δ scale using tetramethylsilane (TMS) as a standard. Electron impact mass spectra were obtained with a Varian Mat CH-5 or a VG Instruments 7070 EQ-HF mass spectrometer. Gas–liquid chromatography (GLC) was done using a Varian Aerograph Series 1700 GLC instrument with a thermal conductivity detector. Micro-analyses were performed by Atlantic Microlabs, Inc., Atlanta, Georgia.

HPLC was performed with either a Tracor system or a Hewlett–Packard 1090A system. The Tracor system included a Tracor 950 HPLC pump with a Tracor 970 variable wavelength UV detector. A 6.35 mm × 240 mm Alltech RSIL C-18 HL 10 μm column was used with the Tracor system. This system was used for preparative HPLC and for monitoring some of the synthetic reactions.

Most of the reactions of singlet oxygen with 1, 3 and 4 were monitored with a Hewlett–Packard 1090A HPLC instrument equipped with a diode array detector, a data processing unit and a 2.1 mm × 100 mm HP Hypersil ODS 5 μm column. The eluting solvent was 60vol.%methanol–40vol.%water or an acetonitrile–water gradient at 0.3 ml min^{-1}. Products were identified by comparison of retention times and UV–visible spectra with those of authentic samples.

4.2. Irradiation equipment

Irradiations were generally carried out in Pyrex 18 mm × 150 mm test tubes equipped with a serum stopper with inlet and outlet syringe needles for oxygen. The 400 W medium pressure mercury lamp was a 400 W street-lamp (Westinghouse H33GL-400/DX) with the outside envelope removed. The light source for the fluorescein irradiations was a slide projector with a 500 W DAY lamp. The filter used to isolate the 366 nm band of the mercury lamp consisted of a Corning 7-51 glass filter and a 0.04 wt.% solution of 2,3-dihydro-5,7-dimethyl-1H-1,4-diazepinium iodide in distilled water with a path length of 1 cm [10, 19]. The 500 - 575 nm region of the mercury lamp was isolated by a filter solution prepared by dissolving 20 g cupric chloride and 27 g anhydrous calcium chloride in 100 ml distilled water, acidifying slightly with HCl and filtering [20]. An Oriel 51490 glass filter and a 20 cm path length water filter were used to filter out IR radiation and wavelengths of less than about 450 nm from the slide projector lamp for the experiments using fluorescein as the sensitizer.

4.3. Flash chromatography

The procedure for flash chromatography was essentially the same as that described by Still et al. [11]. The silica gel used was Merck silica gel 60 (40 - 63 μm). A slightly different technique, referred to as "dry column chromatography", was used in some of the syntheses [21].

4.4. Solvents and chemicals

All organic solvents were J. T. Baker or Fisher reagent grade and were used without further purification. Ethanol was U.S.P. grade absolute ethanol. Other chemicals were obtained from Aldrich, Sigma or Fisher and were used as received.

4.5. Irradiation of Coumarin 311 (1) in oxygen-saturated ethanol solution

In the first experiment 50 mg 1 in 10 ml ethanol was irradiated at 366 nm with a 400 W medium pressure mercury lamp for 15 h. Oxygen was bubbled through the solution throughout the irradiation. After the irradiation several products could be observed by TLC on silica gel eluting with 5vol.%(ethyl acetate)—95vol.%dichloromethane. One product was barely visible as a brown spot (R_f = 0.35). Another spot with R_f = 0.16 fluoresced blue under UV light. 1 had an R_f of 0.26. The first product was purified three times by flash chromatography, eluting with 5vol.%(ethyl acetate)—95vol.%dichloromethane. A total of 3.8 mg product, which appeared as a brown spot by TLC, was obtained. This product was identified as the aldehyde 4 based on its NMR and mass spectra and subsequent synthesis. The other fluorescent product was isolated by flash chromatography with 30vol.%(ethyl acetate)—70vol.%dichloromethane eluent. This yielded 1.5 mg material which was identified as the alcohol 3 based on its NMR and mass spectra and comparison with synthetic material.

Another irradiation was done similarly except that 81.2 mg 1 in 10 ml of ethanol was irradiated for 22 h. The crude reaction mixture was purified by flash chromatography on a 10 mm silica-gel column, eluting with 10vol.% (ethyl acetate)—90vol.%dichloromethane. After 20 fractions (each 5 ml) were collected, the column was eluted with 60vol.%ethanol—40vol.%(ethyl acetate) and an additional ten 8 ml fractions were collected. Aldehyde 4 appeared to elute in fractions 2 - 3. 1 eluted in fractions 2 - 4. The demethylated product 2 eluted in fractions 3 - 6. The amide 6 eluted in fractions 6 - 10. Fraction 22 contained the alcohol 3. The carboxylic acid 5 eluted in fractions 26 - 30. The identification of 2, 5 and 6 was based on NMR and mass spectra and subsequent syntheses.

4.6. Photosensitized generation of singlet oxygen by Coumarin 311

A Pyrex immersion well equipped with an inlet and outlet for oxygen was charged with 1.90 g 7, 15 mg 1 and 110 ml methanol. The solution was irradiated with a Pyrex-filtered medium pressure mercury lamp for 3.25 h. Oxygen was rapidly bubbled through the solution during the irradiation. The reaction was monitored by GLC using a 6.35 mm × 1.83 m column of 7.5% SE-30 on 60/80 mesh acid-washed DMCS Chromasorb W at a column temperature of 80 °C. The alkene (7) was observed to disappear and a new product with a longer retention time appeared. The reaction was complete after 3.25 h. The solvent was rotary evaporated to yield a yellow oil. Molecular distillation at 65 - 70 °C and 12 Torr yielded 0.74 g (28%) of a colorless liquid which was identified as 8 based on comparison of its NMR and mass

spectra with those of an authentic sample prepared by the reaction of 7 with singlet oxygen generated by Rose Bengal photosensitization [9].

4.7. Reaction of Coumarin 311 with singlet oxygen

A 5 ml solution of 5.0×10^{-2} M 1 and 8×10^{-4} M Rose Bengal in methanol was irradiated for 3 h with a 400 W mercury lamp filtered to transmit light in the 500 - 575 nm region. Oxygen was bubbled through the solution during the irradiation. After the irradiation the reaction mixture was purified by flash chromatography on a 10 mm column, eluting with 10vol.%(ethyl acetate)−90vol.%dichloromethane. A total of 20 fractions (each 5 ml) were collected. The demethylated product 2 eluted in fractions 5 - 12. The formamide 6 eluted in fractions 14 - 17. The products were characterized by NMR and mass spectroscopy and by comparison with authentic material synthesized later. No other products were observed.

4.8. Reaction of 7-dimethylamino-4-hydroxymethylcoumarin (3) with singlet oxygen

Three 5 ml samples were prepared containing 3.5 mg of the alcohol 3 and 15.0 mg Rose Bengal in 50vol.%ethanol−50vol.%water. The samples were irradiated with a 400 W medium pressure mercury lamp, filtered to transmit the 500 - 575 nm region of the lamp. Oxygen was bubbled through the solutions during the irradiation. After 4 h of irradiation, HPLC with the Tracor system, eluting with 50vol.%methanol−50vol.%water at 2 ml min^{-1}, and monitoring at 380 nm, showed that the alcohol (retention time, 4.95 min) was about 75% destroyed and a new product (retention time, 2.7 min) had formed. The three samples were combined and the solvent was removed by rotary evaporation. The residue was slurried in 25 ml ethyl acetate and filtered through a 15 mm column of silica gel to remove the Rose Bengal. The ethyl acetate was removed by evaporation and the crude product was purified by preparative HPLC using the Tracor system and eluting with 50vol.%methanol−50vol.%water. Evaporation of the solvent yielded 0.6 mg of a product which was identified as 12 based on the following spectral properties: ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.77 (d, J = 5 Hz, 3H), 4.66 (d, J = 5 Hz, 2H), 5.52 (t, J = 5 Hz, 1H), 6.05 (s, 1H), 6.37 (d, J = 3 Hz, 1H), 6.54 (dd, J = 9 Hz, J = 3 Hz, 1H), 6.69 (q, J = 5 Hz, 1H), 7.37 (d, J = 9 Hz, 1H); mass spectrum m/z (relative intensity) 205 (base), 206 (14%), 177 (51%), 176 (21%), 160 (16%), 148 (56%).

Similar irradiations in absolute ethanol resulted in little or no reaction after 4.5 h. Likewise, direct irradiation of 3 at 366 nm in absolute ethanol or 50vol.%ethanol−50vol.%water for 4 h resulted in no reaction observable by HPLC.

4.9. Preparation of 7-methylamino-4-methylcoumarin (2)

A 500 ml round bottom flask equipped with a magnetic stirrer was charged with 500 mg (2.86 mmol) of 7-amino-4-methylcoumarin, 1.00 ml (1.08 g, 13.3 mmol) 37 vol.% formaldehyde in water, 899 mg (14.3 mmol)

sodium cyanoborohydride and 250 ml of 95 vol.% ethanol. The pH of the solution was adjusted to pH 5 - 6 using 10 vol.% HCl as measured by moist pH paper. At this pH the solution turned a deep yellow color. More 10 vol.% HCl was added periodically to maintain the pH at 5 - 6. The reaction was allowed to stir for 15 h, and then the reaction mixture was analyzed by HPLC using a 6.35 mm × 24 cm Alltech RSIL C-18 HL 10 μm column, eluting with 75vol.%methanol–25vol.%water at 2 ml min^{-1}. The UV detector was set at 365 nm. After 15 h the reaction mixture appeared to be about 63% of the desired product (retention time, 5.25 min), 33% unreacted starting material (retention time, 4.2 min) and 4% of the dimethylated product 1 (retention time, 7.3 min). The solvent was rotary evaporated from the reaction mixture and the residue was taken up in 100 ml dichloromethane. This organic solution was washed with 50 ml saturated sodium bicarbonate solution, then 50 ml water and then dried over anhydrous magnesium sulfate. The solution was filtered, the solvent was rotary evaporated and the yellow residue was purified by flash chromatography on a 40 mm diameter column packed with 15 cm silica gel. The column was eluted with 10vol.%-(ethyl acetate)–90vol.%dichloromethane, and 30 fractions (each 10 ml) were collected. The desired product eluted in fractions 13 - 25. These fractions were combined and rotary evaporated to yield a yellow solid which was recrystallized from ethanol–water to yield 163 mg (0.86 mmol, 30% yield) 2 (melting point, 188 - 189 °C), which had the following spectral properties: ^{1}H NMR (200 MHz, CDCl$_3$) δ 2.34 (s, 3H), 2.92 (s, 3H), 4.31 (s, broad, 1H), 5.96 (s, 1H), 6.43 (d, J = 2 Hz, 1H), 6.48 (dd, J = 2 Hz, J = 9 Hz, 1H), 7.34 (d, J = 9 Hz, 1H); mass spectrum m/z (relative intensity) 189 (base), 190 (14%), 160 (81%), 159 (25%), 146 (11%), 132 (15%), 76 (11%); IR (CHCl$_3$) 2.90, 5.83, 6.14, 6.20, 6.41, 7.14, 7.28 μm; UV (ethanol) 363 nm (log ϵ = 4.34), 236 nm (log ϵ = 4.19). The analysis was as follows. Calculated for C$_{11}$H$_{11}$NO$_2$: C, 69.83%; H, 5.86%; N, 7.40%. Found: C, 69.96%; H, 5.90%; N, 7.39%.

4.10. Preparation of 7-formylmethylamino-4-methylcoumarin (6)

To a 10 ml round bottom flask equipped with a reflux condenser and magnetic stirrer was added 74.4 mg (0.394 mmol) 2 and 0.44 ml (0.53 g, 10 mmol) 88 vol.% formic acid in water. This mixture was heated to 75 - 80 °C with an oil bath and then 0.15 ml (0.16 g, 1.6 mmol) acetic anhydride which had been distilled from magnesium was added. The mixture was stirred for 30 min at 75 - 80 °C and then cooled. Addition of a few drops of water to the solution caused the product to precipitate as white crystals which were collected by vacuum filtration and washed with water. The crude product was purified by flash chromatography on a 20 mm diameter column packed with 15 cm silica gel. The column was eluted with 50vol.%-(ethyl acetate)–50vol.%dichloromethane and 10 fractions (each 10 ml) were collected. The desired product eluted in fractions 3 - 5. These fractions were combined and rotary evaporated to yield 60.0 mg (0.28 mmol, 70% yield) 6. The product was recrystallized twice from 95 vol.% ethanol to give fine white

crystals (melting point, 165 - 166 °C) with the following spectral properties: ^{1}H NMR (90 MHz, CDCl$_3$) δ 2.40 (d, J = 1 Hz, 3H), 3.32 (s, 3H), 6.22 (q, J = 1 Hz, 1H), 7.11 (m, 2H), 7.60 (d, J = 9 Hz, 1H), 8.62 (s, 1H); mass spectrum m/z (relative intensity) 217 (base), 218 (14%), 189 (14%), 176 (22%), 160 (28%), 148 (25%); IR (CHCl$_3$) 5.79, 6.18, 7.20, 7.48, 7.73, 8.45, 8.90, 9.28 μm; UV (ethanol) 321 nm (log ϵ = 4.15). The analysis was as follows. Calculated for C$_{12}$H$_{11}$NO$_3$: C, 66.35%; H, 5.10%; N, 6.45%. Found: C, 66.17%; H, 5.14%; N, 6.39%.

4.11. Preparation of diethyl oxalacetate (9)

A 200 ml Erlenmeyer flask was charged with 20.0 g (95.2 mmol) of the sodium salt of oxalacetic acid diethyl ester and 190 ml distilled water. The pH of this solution was adjusted to 4 - 5 with about 9 ml concentrated HCl. The resulting cloudy solution was extracted with four 40 ml portions of dichloromethane. The combined organic layers were dried over anhydrous magnesium sulfate, filtered and rotary evaporated to yield a yellow oil which was distilled at 64 °C and 0.15 Torr to yield 6.69 g (35.6 mmol, 37% yield) of colorless diethyl oxalacetate which appeared to be about 96% pure by GLC. The product had the following spectral properties: ^{1}H NMR (90 MHz, CCl$_4$) δ 1.33 (t, J = 7 Hz, 3H), 1.36 (t, J = 7 Hz, 3H), 4.25 (q, J = 7 Hz, 2H), 4.27 (q, J = 7 Hz, 2H), 5.90 (s, 1H), 11.57 (broad s, 1H); mass spectrum m/z (relative intensity) 188 (4%), 133 (59%), 115 (base), 87 (31%), 86 (59%), 43 (66), 42 (35%), 29 (84%).

4.12. Preparation of ethyl-7-dimethylaminocoumarin-4-carboxylate (11)

A 10 ml round bottom flask equipped with a magnetic stirrer, reflux condenser and drying tube was charged with 0.92 g (6.7 mmol) 10 which had been recrystallized from Skellysolve B and then sublimed at 65 °C and 0.025 Torr before use (melting point, 86 - 87 °C), 1.47 g (7.8 mmol) 9, 0.46 g (3.4 mmol) anhydrous zinc chloride and 3 ml dry ethanol. The mixture was heated to reflux for 12 h and then cooled. The solvent was removed from the dark brown reaction mixture by rotary evaporation and the residue was taken up in 25 ml distilled water. This solution was extracted with three 25 ml portions of dichloromethane. The combined organic layers were dried over anhydrous magnesium sulfate, filtered and rotary evaporated to yield a dark brown solid which was recrystallized from about 30 ml 95 vol.% ethanol to yield 1.09 g (4.2 mmol, 63% yield) of dark green crystalline 11 with the following spectral properties: ^{1}H NMR (90 MHz, CDCl$_3$) δ 1.39 (t, J = 7 Hz, 3H), 3.03 (s, 6H), 4.39 (q, J = 7 Hz, 2H), 6.55 (multiplet, 3H), 8.00 (d, J = 9 Hz, 1H); mass spectrum m/z (relative intensity) 261 (base), 262 (31%), 263 (5%), 233 (63%), 232 (23%), 205 (63%), 204 (37%), 189 (35%), 132 (27%); IR (CHCl$_3$) 3.33, 5.82, 6.17, 6.29, 6.54, 6.72, 7.08, 7.29, 7.95, 8.72 μm; UV (ethanol) 406 nm (log ϵ = 4.15). Recrystallization of the product from 95 vol.% ethanol yielded dark green crystals (melting point, 124 - 125 °C). The analysis was as follows. Calculated for C$_{14}$H$_{15}$NO$_4$: C, 64.36%; H, 5.79%; N, 5.36%. Found: C, 64.25%; H, 5.80%; N, 5.31%.

4.13. Preparation of 7-dimethylaminocoumarin-4-carboxylic acid (5)

A 100 ml round bottom flask equipped with a reflux condenser and magnetic stirrer was charged with 500 mg (1.92 mmol) 11, 80 mg (1.90 mmol) lithium hydroxide monohydrate, 25 ml tetrahydrofuran and 25 ml distilled water. The solution was heated to reflux for 2 h and then cooled. The pH of the solution was adjusted to pH 2 - 3 with concentrated HCl as determined by pH paper; about 2 ml acid was needed. Most of the tetrahydrofuran was removed by rotary evaporation. Cooling of the resulting mixture caused orange crystals to precipitate which were collected by vacuum filtration, washed with water and dried under vacuum to yield 382 mg (1.64 mmol, 86% yield) of orange crystals which were identified as 5 based on the following spectral data: ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.02 (s, 6H), 5.85 (s, 1H), 6.48 (d, J = 2 Hz, 1H), 6.66 (dd, J = 2 Hz, J = 9 Hz, 1H), 7.58 (d, J = 9 Hz, 1H) (the carboxylic acid proton appeared as part of the water peak at δ 3.30); mass spectrum m/z (relative intensity) 233 (base), 234 (13%), 232 (20%), 205 (36%), 204 (40%), 189 (7%), 161 (24%), 160 (14%), 132 (14%); IR (KBr pellet) 3.50 (broad), 5.90, 6.21, 6.34, 6.57, 7.20, 7.84, 8.78, 11.56, 12.38, 15.30 μm; UV (ethanol) 393 nm (log ϵ = 4.18; log ϵ at 460 nm, 3.50). The product was recrystallized from ethanol to yield fine dark orange-brown crystals (melting point, 233 - 234 °C). The analysis was as follows. Calculated for $C_{12}H_{11}NO_4$: C, 61.80%; H, 4.75%; N, 6.01%. Found: C, 61.68%; H, 4.80%; N, 5.97%.

4.14. Preparation of 7-dimethylamino-4-hydroxymethylcoumarin (3)

A 500 ml round bottom flask equipped with a drying tube and magnetic stirrer was charged with 3.00 g (11.5 mmol) of the ester 11 and 150 ml of tetrahydrofuran which was freshly distilled from sodium and benzophenone. Lithium borohydride (330 mg, 15.1 mmol) was added and the reaction was stirred for 10 min during which time the reaction mixture turned yellow-orange in color. The reaction was stopped by adding 10 vol.% HCl dropwise until the evolution of hydrogen ceased and the solution was pH 5 as indicated by moist pH paper. Distilled water (100 ml) was added and most of the tetrahydrofuran was removed by rotary evaporation, causing orange crystals to precipitate. The aqueous mixture was extracted with two 75 ml portions of ethyl acetate and the organic layers were combined, dried over anhydrous magnesium sulfate and filtered. The solvent was removed to yield 2.44 g of crude crystals which were purified by dry column flash chromatography, eluting with mixtures of ethyl acetate and dichloromethane. The yield was 933 mg (4.26 mmol, 37%) of pale yellow crystals with the following spectral properties: ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.98 (s, 6H), 4.68 (dd, J = 5 Hz, J = 1 Hz, 2H), 5.47 (t, J = 5 Hz, 1H), 6.10 (t, J = 1 Hz, 1H), 6.52 (d, J = 2 Hz, 1H), 6.67 (dd, J = 9 Hz, J = 2 Hz, 1H), 7.43 (d, J = 9 Hz, 1H); mass spectrum m/z (relative intensity) 219 (base), 220 (38%), 218 (28%), 191 (72%), 190 (53%), 174 (14%), 162 (79%), 134 (15%); IR (KBr pellet) 2.96, 3.50, 5.96, 6.22, 6.53, 7.10, 7.27, 7.48, 7.97, 8.12 μm; UV (ethanol) 369 nm (log ϵ = 4.27). The product (melting point,

194 - 196 °C) was recrystallized from ethanol. The analysis was as follows. Calculated for $C_{12}H_{13}NO_3$: C, 65.74%; H, 5.98%; N, 6.39%. Found: C, 65.61%; H, 6.00%; N, 6.33%.

4.15. Preparation of 7-dimethylaminocoumarin-4-carboxaldehyde (4)

A 250 ml round bottom flask equipped with a drying tube and magnetic stirrer was charged with 3 (600 mg, 2.74 mmol), lead tetraacetate (1.212 g, 2.73 mmol) and 100 ml pyridine. The solution was stirred for 40 h and then the pyridine was removed by rotary evaporation. The residue was taken up in 75 ml ethyl acetate and extracted with 75 ml portions of water, then saturated sodium bicarbonate and then again with water. The organic layer was dried over anhydrous magnesium sulfate and then filtered. The solvent was removed by rotary evaporation and the crude product was purified by dry column flash chromatography on a 45 mm (diameter) X 50 mm silica-gel column, eluting with 30 ml portions of ethyl acetate–dichloromethane mixtures. The fractions containing the non-fluorescent product were combined and the solvent was evaporated to yield 76.5 mg of crystals. The product was recrystallized from ethyl acetate to yield 48.7 mg (0.224 mmol, 8.2% yield) of dark red crystals (melting point, 214.5 - 216 °C) with the following spectral properties: ^1H NMR (90 MHz, DMSO-d_6) δ 3.07 (s, 6H), 6.68 (d, J = 3 Hz, 1H), 6.72 (s, 1H), 6.87 (dd, J = 9 Hz, J = 3 Hz, 1H), 8.33 (d, J = 9 Hz, 1H), 10.22 (s, 1H); ^1H NMR (90 MHz, CD$_3$OD) δ 3.03 (s, 6H), 5.65, (s, 1H), 6.23 (s, 1H), 6.50 (d, J = 2 Hz, 1H), 6.72 (dd, J = 9 Hz, J = 2 Hz, 1H), 7.68 (d, J = 9 Hz, 1H) (this latter NMR spectrum is the spectrum of the acetal or hemiacetal of the aldehyde); mass spectrum m/z (relative intensity) 217 (base), 218 (15%), 216 (17%), 190 (33%), 188 (41%), 161 (28%), 160 (14%), 132 (14%); IR (KBr pellet) 3.45 (weak), 5.78, 5.89, 6.16, 6.32, 6.58, 7.03, 7.16, 7.31, 7.83, 8.03, 8.61, 9.52, 11.66, 11.96, 12.55 μm; UV (ethanol) 373 nm (log ϵ = 4.28; log ϵ at 460 nm, 2.70); the UV spectrum in ethanol is the spectrum of the acetal or hemiacetal (It was not possible to determine from the available data whether the aldehyde 4 forms an acetal or hemiacetal in ethanol solution. The reaction with ethanol is reversible, however, since the aldehyde can be recovered by removing the ethanol under vacuum. This suggests that 4 reacts with ethanol to form a hemiacetal.); UV (ethyl acetate) 430 nm (log ϵ = 4.01; log ϵ at 460 nm, 3.89). The analysis was as follows. Calculated for $C_{12}H_{11}NO_3$: C, 66.35%; H, 5.10%; N, 6.45%. Found: C, 66.31%; H, 5.10%; N, 6.44%.

Acknowledgment

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