SEPARATION AND IDENTIFICATION OF THE PRODUCTS OF THE PHOTODEGRADATION OF 7-AMINO-4-METHYLCOUMARIN AND THE MECHANISM OF ITS PHOTODEGRADATION

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Summary

The separation of the products of the photodegradation of 7-amino-4-methylcoumarin (C120), a laser dye, has been achieved by a reverse phase high performance liquid chromatography method. Two reverse phase columns (C6 and ODS) were used to optimize the separation conditions and various mixtures of methanol and water served as the mobile phase. The chromatograms representing the separation of the photolyte from the photoproducts under various experimental conditions are also shown.

The fluorescent photodegradation product in an oxygenated methanolic solution of the dye has been separated by column chromatography assisted by thin layer chromatography and high performance liquid chromatography. The structure of the compound has been ascertained by mass spectrometric and IR spectroscopic analysis and found to be 7-amino-3-hydroxy-4-methylcoumarin. A mechanism is suggested for the formation of this product by the interaction of singlet oxygen and the dye. On the basis of the behaviour of the dye in degassed solutions, a general scheme is postulated to represent the photobleaching of C120.

1. Introduction

7-Amino-4-methylcoumarin (C120), an important laser dye [1] operating in the blue region, degrades when irradiated with UV photons [2]. The rate of photodegradation is enhanced by increasing the oxygen concentration of the system. The total laser output which is dependent on the concentration of the dye dwindles as the lasing operation progresses. This may not be in direct proportion to the dye concentration as the products themselves may be lasing under the excitation conditions or they may be absorbing the laser emission themselves [3].

In general, the stability of dye lasers is a measure of the stability of laser dyes [4]. This can only be established by separating the photoproducts and identifying them.

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Although the gas chromatographic separation of coumarins has been extensively attempted [5, 6], liquid chromatographic methods [7, 8] have received scant attention. Here we present the optimum conditions for the separation of the products of photodegradation from the parent molecule by reverse phase high performance liquid chromatography (HPLC) for degassed and oxygenated solutions. The separation of the fluorescent photodegradation product of C120 in oxygenated photolysed solutions was also achieved by column chromatography and the structural identity established. In addition, a probable path of formation of products is suggested.

2. Experimental details

2.1. Materials

The C120 used in this study was synthesized in our laboratory by Pechmann condensation [9] and purified by repeated crystallization from methanol. It was found to contain a single component under the conditions of the experiments described. Methanol (GR) was used in preparing solutions of the dye, and methanol (HPLC grade) and triply distilled water were employed in the HPLC experiments.

2.2. Apparatus

A Rayonet photochemical chamber reactor with a merry-go-round facility was used for photolysis. A series of low pressure mercury arc lamps coated with a phosphor to emit a band centred at 350 nm provided the light source and the temperature in the chamber was 32 ± 1 °C.

A Laboratory Data Control HPLC instrument equipped with a 20 μl loop injector was used for the HPLC separations. ODS and C6 columns (spheriosorb support) served as the stationary phases. Both a spectromonitor (variable-wavelength type; LDC model-II) and a fluoromonitor were used as detectors. Mixtures of methanol and water were employed in an isochratic mode as the mobile phase at a flow rate of 1 ml min^{-1}.

2.3. Sample preparation

Two different concentrations (about 10^{-3} and 10^{-5} mol dm^{-3}) of C120 were prepared in methanol. Degassing was done by several freeze–pump–thaw cycles on a vacuum line.

2.4. Preparative photochemistry

About 1 g of C120 was dissolved in 1 l of methanol saturated with oxygen and photolysed with 350 nm light at an intensity of 1.4×10^{16} photons ml^{-1} s^{-1} for 10 h. The progress of this preparative photochemical reaction was followed by thin layer chromatography (TLC) on silica gel using methylene dichloride as the eluent. The R_f values for C120 and the fluorescent product were 0.84 and 0.19 respectively. The reaction mixture

after evaporation to dryness was chromatographed on a silica gel column using methylene dichloride as the eluent. Three different fractions eluted from the column were found to be cross-contaminated with undegraded C120, from which one major and one minor product could be recognized. The first fraction containing most of the major product was rechromatographed on silica. A mixture of 90% benzene and 10% acetone eluted the undegraded C120. It was then eluted with acetone (fraction P) followed by methanol (fraction Q). The other two parent fractions contained mainly undegraded C120.

3. Results and discussion

A solution of 10^{-5} mol dm^{-3} of C120 was photolysed in aerated methanol for 30 min. The optimum conditions for the separation of the reactant from the products were arrived at by a trial and error method. Figure 1 shows the chromatograms obtained when C18 ODS was used as the column and various mixtures of methanol and water were used as the mobile phase. The eluted fractions were detected using a spectromonitor at 300 nm. From a consideration of various chromatographic parameters such as the retention time and capacity factor, a solvent composition of 50% methanol and 50% water at a flow rate of 1 ml min^{-1} was chosen for regular analysis. At this composition, C120 and its photoproduct were found to have retention times of 6.69 min and 4.13 min and capacity factors of 1.28 and 0.41 respectively. Under these conditions a similar chromatogram was also

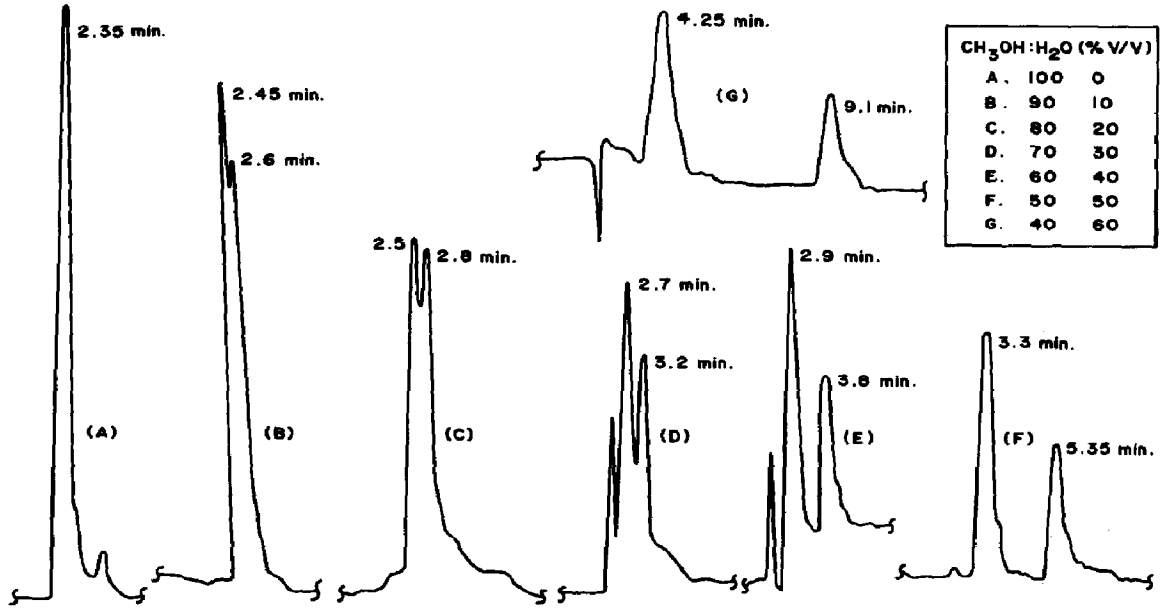


Fig. 1. Effect of solvent polarity on the HPLC separation of photolysed C120.

observed with the fluoromonitor. However, the fluorescence detector was used for better sensitivity in all the analyses. The amount of C120 left in each sample was estimated from the fluorescence response using a linear calibration curve drawn from samples of known concentrations. Solutions of the dye photolysed for various durations were analysed for the C120 content and it was found that the amount of C120 decreased progressively until it disappeared completely after 135 min of photolysis. A corresponding increase in the product was also observed during the initial period of photolysis (up to 50 min).

To observe the behaviour of C120 under high concentration and photon doses, a solution of it in methanol at a concentration of 10^{-3} mol dm^{-3} was photolysed for longer durations. Samples were prepared under vacuum-degassed and aerated conditions. They were separated on a C6 column and detected using a spectromonitor employing a solvent composition of 60% water and 40% methanol to obtain maximum resolution as shown in Fig. 2; chromatograms a, b and c respectively represent the chromatograms of an unphotolysed solution, a solution photolysed for 24 h under degassed conditions and a solution photolysed for 3 h under aerated conditions. A comparison of chromatograms b and c in Fig. 2 indicates that oxygenated polar compounds appear in the photolysed aerated solutions before the C120 peak and less polar compounds after it. Peaks c and s represent C120 and the solvent respectively.

The fluorescent photoproduct separated by column chromatography was checked for its purity by means of HPLC. Figure 3 represents the chromatograms of fraction P and fraction Q. Fraction P was found to be a pure

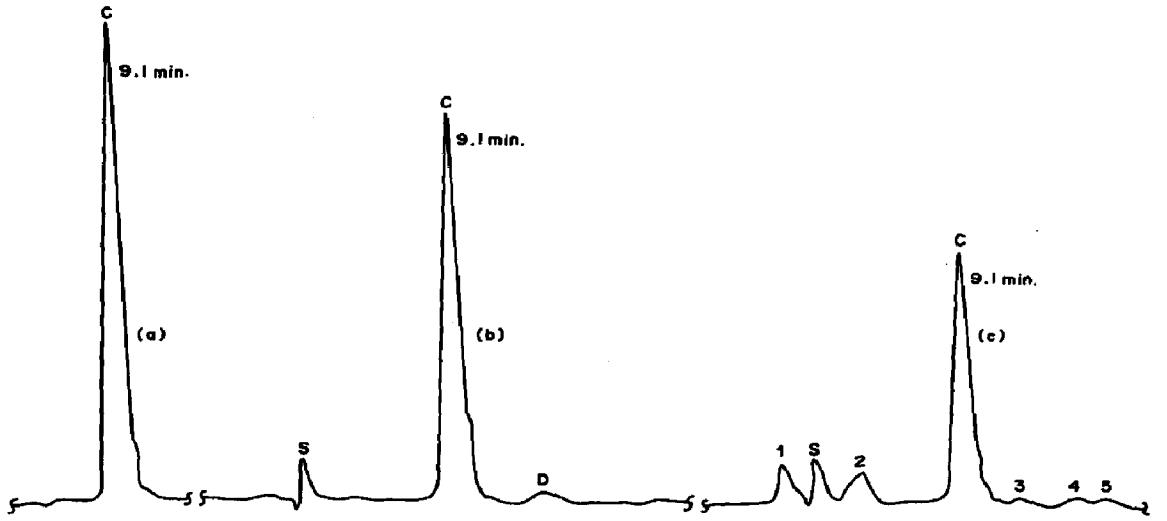


Fig. 2. Reverse phase HPLC analysis of 10^{-3} mol dm^{-3} methanolic solutions of C120 under degassed (curve b) and oxygen-saturated (curve c) conditions. Curve a represents unirradiated C120 (column, C6; eluent, methanol:water mixture (40:60 by volume); flow, 1 ml min^{-1}; pressure, 275 psi; detector, spectromonitor (300 nm)).

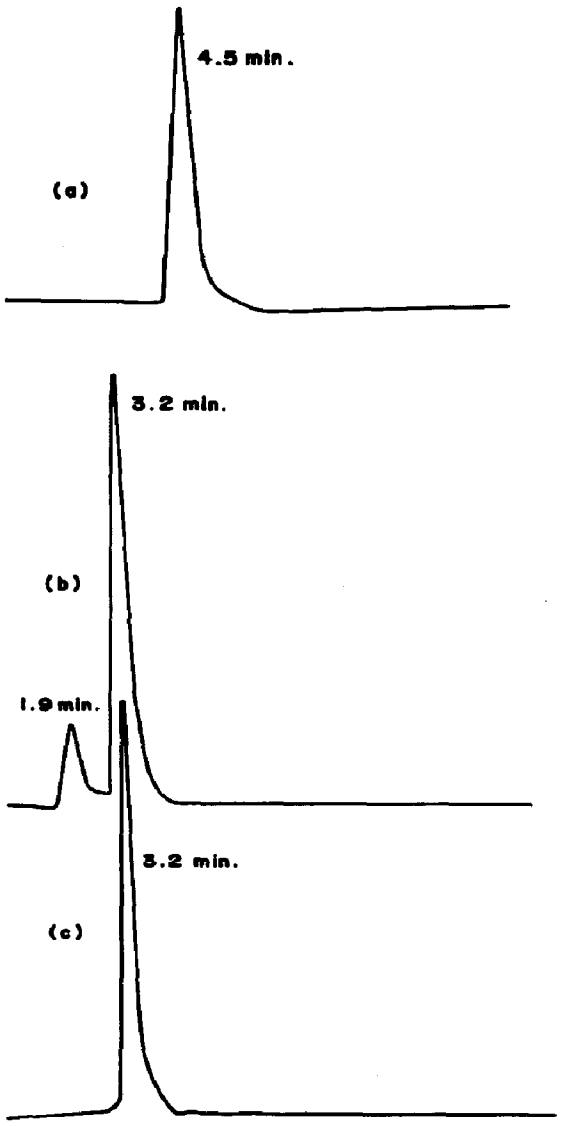


Fig. 3. Chromatograms of pure C120 (curve a), fraction Q (curve b) and fraction P (curve c).

compound and corresponded to that which immediately preceded the C120 peak in the chromatogram of the oxygenated photolysed solution (peak 2 in Fig. 2, chromatogram c). Fraction Q was found to be a mixture of the most polar product (minor component) observed in the chromatogram of the photolysed samples of the oxygenated concentrated solution (peak 1 in Fig. 2, chromatogram c) and fraction P (major component).

Table 1 represents the mass spectral data of C120 and its major photoproduct (fraction P) in which the relative abundances of the various frag-

TABLE 1

Mass spectral data for C120 and its photoproduct

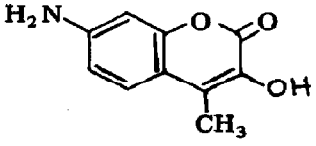
m/e	*Relative abundance* (%)	
	Photoproduct (fraction P)	*C120*
192	12.5	
191	100.0	
189	14.8	
176	8.0	12.0
175	55.7	100.0
174		6.0
163	37.5	
148		20.0
147	42.1	74.0
146	27.3	
143	43.2	
142	10.2	
135	10.2	
134	56.8	
133	11.4	
119	30.0	
118	20.0	
117	6.0	
106	23.9	
104	17.1	
102	19.3	
91	15.0	18.0
64		18.0

ment ions are listed against their respective *m/e* values. The IR spectrum of this in KBr showed strong absorption around 3500, 1600 and 1375 cm^{-1}.

This product was found to have a molecular weight of 191 and, being formed directly from C120 (molecular weight, 175) by its photolysis in the presence of oxygen, may be assumed to be formed from it by the addition of an oxygen atom. The ratio of the M^+ and $(M + 1)^+$ peak intensities for a molecular formula of $C_{10}H_9O_3N$ calculated on the basis of the natural isotopic abundance of ^{13}C and ^2H worked out to be 100:11.7. This compares well with the observed ratio of 100:12.5, reinforcing the suggested molecular formula for the compound. The IR data show the presence of a hydroxyl group. Also, the methyl group in the original molecule is found to remain intact as seen from its bending vibration at 1375 cm^{-1} [10].

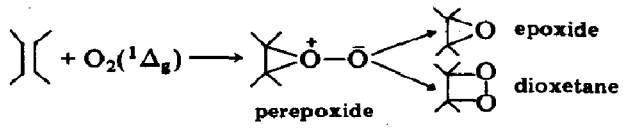
The question now arises about the exact location of the hydroxyl group in the product. The mass spectrum of C120 consists of a peak at an *m/e* value of 91. This corresponds to the fragmentation ion $(H_2N \cdot C_6H_3)^+$ formed by the scission of the α-pyrone ring of the dye molecule. If the hydroxyl group was present on the phenyl group as a substitute, then the product

would have given rise to a peak at a mass number of 107, *i.e.* (91 + 16). The absence of such a peak shows that the hydroxyl group could only be on the α-pyrone ring. Other than the methyl group which is found to remain intact in the product, only two positions are available for accommodating the hydroxyl group. These are the carbon atoms 3 and 4 connected by a double bond of which position 4 is less accessible than position 3 because of the steric hindrance caused by the methyl group at position 4. Thus the product is assigned as 7-amino-3-hydroxy-4-methylcoumarin:



The formation of oxygenated organic compounds during photolysis is usually considered to be through singlet oxygen [11] produced by the quenching of the triplet excited molecule by ground state oxygen. The most vulnerable point with respect to oxygen reactivity is the double bond at position 3. The addition of a singlet oxygen molecule to the double bond may produce the dioxetane and perepoxide derivatives. There has been some disagreement as to whether the system actually goes through a stable perepoxide in the course of forming the dioxetane [12, 13].

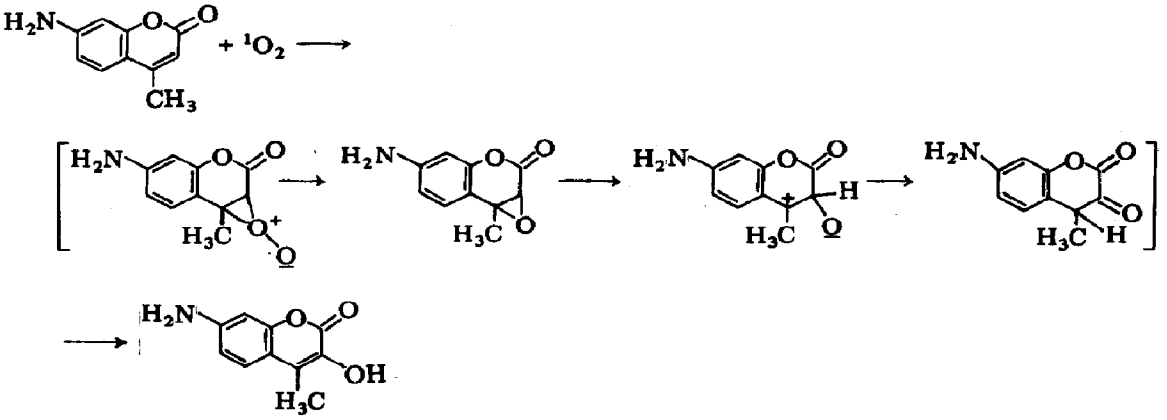
A quantitative treatment of a theoretical model of such a system carried out by Politzer and Daiker [14] arrived at the conclusion that a perepoxide is the probable initial product which dissociates into an epoxide rather than into a dioxetane derivative. This takes place at the instant that a second olefin molecule produces a singlet oxygen atom, $O(^1D)$, as the second product:



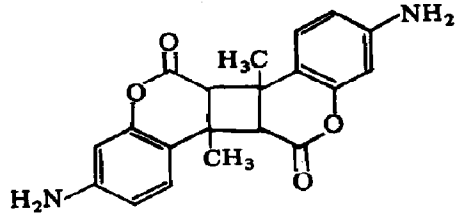
An interesting property of aromatic epoxides is their facile rearrangement to phenols [15]. This was postulated to occur through an intramolecular migration of a hydrogen atom which subsequently yields the phenol.

On the basis of the above facts, the following mechanism is suggested for the photodegradation of C120 in the presence of oxygen. The triplet state of the dye molecule is quenched by an oxygen triplet yielding singlet oxygen and the ground state dye molecule. The reactive singlet oxygen molecule forms an epoxide through a perepoxide which then yields the hydroxyl compound via a hydrogen atom shift:

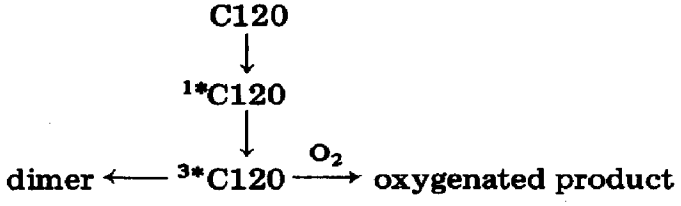
$$^3(C120) + {}^3O_2 \longrightarrow {}^1(C120) + {}^1O_2$$



One of us had earlier noted [16] that a vacuum-degassed solution of C120 did not yield the fluorescent oxygenated product observed in aerated solutions although there is a decrease in the C120 concentration. This may be presumed to be due to the disappearance of the dye via a dimerization reaction centred at the double bond at position 3 as in the case of coumarin proper [17]. This can happen by the ground state quenching [18] of the triplet state of the molecule by a head-to-tail collison leading to a less crowded trans configuration. It may be represented by the following structure:



From the foregoing account, the photodegradation of C120 can be represented by the following general scheme:



4. Conclusion

The photochemical degradation of C120 in the presence of oxygen at 350 nm leads primarily to the formation of 7-amino-3-hydroxy-4-methylcoumarin, a highly fluorescent compound. Deoxygenated solutions yield

a non-fluorescent dimer under similar conditions. Reverse phase HPLC may be conveniently employed in separating the photoproducts from the parent dye.

References

1 K. H. Drexhage, in F. P. Schafer (ed.), *Dye Lasers*, Springer, New York, 1973, p. 144.
2 J. T. Kunjappu, P. N. Moorthy and K. N. Rao, *Annual Convention of Chemists, Cuttack, India, December, 1983* (ORG-44).
3 F. P. Schafer, *Laser Chem.*, *3* (1983) 265.
4 A. N. Fletcher, *Appl. Phys.*, *B*, *31* (1983) 19.
5 T. Furuya and H. Kojima, *J. Chromatogr.*, *29* (2) (1967) 382.
6 T. Furuya, H. Kojima, H. Sato, *Chem. Pharm. Bull. (Tokyo)*, *15* (9) (1967) 1362.
7 D. G. Walters, B. G. Lake, R. C. Cottrell, *J. Chromatogr.*, *196* (3) (1980) 501.
8 C. V. C. Prasad and K. N. Rao, *J. Liq. Chromatogr.*, *6* (1983) 951.
9 V. Pechmann, *Ber. 17* (1884) 929.
10 W. Kemp, *Organic Spectroscopy*, English Language Book Society, Macmilan, London, 1982, p. 53.
11 H. Kautsky, *Biochem. Z.*, *291* (1937) 271.
12 M. J. S. Dewar and W. Thiel, *J. Am. Chem. Soc.*, *97* (1975) 3978.
13 S. Inagaki and K. Fukui, *J. Am. Chem. Soc.*, *97* (1975) 7480.
14 P. Politzer and K. C. Daiker, *Excited States in Organic Chemistry and Biochemistry*, Reidel, (1974) p. 331.
15 J. W. Daly, D. M. Jernia and B. Witkop, *Experientia*, *28* (1972) 1129.
16 J. T. Kunjappu, *Ph.D. Thesis*, Bombay University, Bombay, 1985, p. 149.
17 R. Anet, *Can. J. Chem.*, *40* (1962) 1249.
18 G. S. Hammond, C. A. Stout and A. A. Lamola, *J. Am. Chem. Soc.*, *86* (1964) 3103.