Mass Spectrometric Investigation of Structures of Intermediates in Acridine Chemiluminescence[†]

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Liquid secondary ionization mass spectra (LSIMS) of methylsulfate salts of phenyl, isopropyl, and methyl 10-methyl-acridinium-9-carboxylate were examined under chemiluminescent conditions. Under chemiluminescence conditions, oxygenated acridinium ions $[M + 16]^+$ were observed, whose structures were elucidated as 9-oxy derivatives on the basis of their fragmentation processes obtained by linked scanning at constant B/E. The oxygenated ions are probably derived from the mass spectrometric reaction of the hydroperoxy intermediates in the chemiluminescence proposed by McCapra.

1. Introduction

Recently we have shown that mass spectrometry is a potent tool for elucidation of the structures of intermediates in chemiluminescence of coelenterate luciferin analogues.¹⁾ Since McCapra discovered the bright chemiluminescence of 10-methyl-9-cyanoacridane in 1965,²⁾ numerous investigations on the mechanisms of this interesting reaction have revealed the reaction process. In addition the structures of intermediates which participate in the chemiluminescence have been deduced as shown in eq. 1.^{3), 4)}

We report here that the structures of intermediates of acridine chemiluminescence have also been elucidated by liquid secondary ionization (LSIMS) coupled with linked scanning at constant B/E.

Our results demonstrate that modern mass spectrometry is a potent tool for investigations of dynamic chemical reactions.

[†] Dedicated to the late Professor Toshio Sugiura.

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2. Experimental

Materials

Methylsulfate salts of phenyl (1), isopropyl (2), and sulfate of methyl (3) 10-methyl-acridinium-9-carboxylate were prepared according to the literature²⁾ and characterized by ¹H-NMR spectra; **1** mp 187–188°C, δ (CD₃OD) 8.89 (2H, d, $J=9.5$ Hz), 8.57 (4H, m), 8.19 (2H, t), 7.61 (5H, m), 5.03 (3H, s), 3.67 (3H, s, $\text{CH}_3\text{OSO}_3^-$); **2** mp 149–150°C, δ (CD₃OD) 8.82 (2H, d, $J=9.5$ Hz), 8.47 (4H, m), 8.10 (2H, t), 5.79 (1H, sept. $J=7$ Hz), 4.97 (3H, s), 3.68 (3H, s, $\text{CH}_3\text{OSO}_3^-$), 1.59 (6H, d, $J=7$ Hz); **3** mp 166°C, δ (CD₃OD) 8.82 (2H, d, $J=9.5$), 8.49 (4H, m), 8.09 (2H, t), 4.98 (3H, s), 4.36 (3H, s).

Scheme 1

Mass spectrometry

Positive and negative-ion LSIMS spectra as well as B/E constant linked scan spectra were obtained with a Hitachi M-80B mass spectrometer with a Hitachi M-0101 mass data system. For LSIMS measurements, a mixture of glycerol and triethanolamine (100:1) was used as a matrix and the SIMS gun (xenon ion) was operated at 8 kV. For the measurements of positive-ion LSIMS spectra under chemiluminescent conditions, the ester was first dissolved in the matrix (70 mmol/l), and 100 μl of this solution was loaded on the holder and the spectra were measured, then 31% hydrogen peroxide aqueous solution (100 μl) was added and the spectra were taken after an appropriate interval (0–30 min). After allowing the mixture to stand on the SIMS target for 24 hours, the spectra were taken finally. The light intensity of the reaction mixture was recorded with a Labo Science Model TD-8000 Lumiphotometer.

3. Results and Discussion

Strong chemiluminescence of salts of acridinium esters has been observed under basic conditions in the presence of an oxidation agent such as hydrogen peroxide (H_2O_2) or molecular oxygen (O_2).⁴⁾ For measurements of LSIMS during chemiluminescence reaction we used triethanolamine which worked as a matrix as well as a base, and 31% H_2O_2 aqueous solution was used as an oxidizing agent. Because the half life of the reaction was too short to measure mass spectra during chemiluminescence, the triethanolamine was diluted in glycerol to a 1% solution. Under these conditions the emission maxima of **1** and **2** were observed at around 450 nm, indicating the generation of the singlet excited state of 10-methylacridone as reported previously.^{2), 3)} Unfortunately, **3** scarcely chemiluminesced under similar conditions. Because the formation of dioxetanone is the rate determining step in the chemiluminescence reactions of acridinium salts, the ability of chemiluminescence of a particular compound depends on the ability of the leaving group in the ester alcohol as the alkoxide.

Fig. 1. LSIMS of 1. (a) measured before an addition of H_2O_2 ; (b) measured at 6 min after an addition of H_2O_2 ; (c) measured in glycerol alone not containing triethanolamine.

Phenyl ester 1

The LSIMS of phenyl ester 1 are shown in Fig. 1. a) measured before an addition of H_2O_2 and b) measured at 6 min after an addition of H_2O_2 , *i.e.*, during chemiluminescence. In the negative ion mode, we could not observe any characteristic ion except the methylsulfate anion under both conditions. In the positive ion mode, however, we could obtain characteristic spectra. Before addition of hydrogen peroxide, in Fig. 1a, an intense peak of acridinium carboxylate (M) was observed accompanied by the peak at m/z 210, which corresponded to the protonated 10-methylacridone 4. M means the molecular mass of acridinium carboxylate cation not containing the methylsulfate ion. In the control experiment LSIMS of a methylsulfate salt of 1 in glycerol not containing triethanolamine (Fig. 1c) does not give the m/z 210 ion. The generation of 4 in the glycerol-triethanolamine matrix suggests that the oxidation of acridinium proceeds even under alkaline conditions in the presence of oxygen. In fact, weak chemiluminescence was observed in the matrix mixture without addition of H_2O_2 .

After addition of H_2O_2 a peak at m/z 330 ($M+16$) appears in addition to the molecular ion (M) of 1 in Fig. 1b, which means oxygenation may take place during the chemiluminescence reaction. To elucidate the structure of this ion at m/z 330, linked scanning at constant B/E (Fig. 2) was carried out. From this spectrum it is clear that the ion fragments to m/z 314 [loss of 16 (oxygen)], corresponds to the molecular ion of the

Fig. 2. Liked scan of ion at m/z 330 at constant B/E.

starting material **1**], 302 [**1c**, loss of 28 (C=O)] and 210 (corresponding to the protonated **4**). From these fragments the structure of the ion at m/z 330 is deduced to be **1a**. This oxygenated ion appeared when light emission took place and disappeared when chemiluminescence ceased. Since a hydroperoxy group easily decomposes under SIMS conditions,⁵⁾ the intermediate **1b** proposed in a series of chemiluminescence reactions (eq. 1)³⁾ probably gives the oxygenated ion **1a** by mass spectrometric reaction under SIMS conditions. The observed mass spectrometric reactions are summarized in eq. 2.

Isopropyl ester 2

The LSIMS of before and after H_2O_2 treatments are shown in Figs. 3a and 3b, respectively. Here again the $[\text{M}+16]^+$ peak was observed at m/z 296. The linked

Fig. 3. LSIMS of **2**. (a) measured before an addition of H_2O_2 ; (b) measured at 3 min after an addition of H_2O_2 .

Fig. 4. Linked scan of ion at m/z 296 at constant B/E.

scanning at constant B/E indicates fragmentation to give ions at m/z 280 (loss of oxygen to generate the molecular ion of **2**), 254 (**2c**, loss of C_3H_6 , the McLafferty rearrangement product), 238 (loss of C_3H_6 from m/z 280), and 210 (protonated **4**) as shown in Fig. 4. On the basis of these fragmentation the structure of the ion at m/z 296 is deduced to be **2a** and thus the possible intermediate in the chemiluminescence reaction is **2b**, which is in accord with the case of **1**. As is shown in eq. 3, the McLafferty rearrangement product ion (**2c**) at m/z 254 corresponds to the protonated dioxetanone intermediate originally assumed by McCapra.³⁾ In the normal LSIMS in Figs. 3a and 3b, a strong peak at m/z 238 was observed. The structure of this ion corresponds to 9-carboxyacridinium ion, which is probably produced by ester exchange under LSIMS conditions as similar reactions have been reported previously.^{6), 7)}

Methyl ester 3

In contrast to the above two cases the methyl ester **3** scarcely chemiluminesces, but the oxygenation reaction takes place slowly to give **4**, which is similar to the cases mentioned above. The $[M+16]^+$ peak at m/z 268 was observed weakly in the LSIMS of **3** at 20 min after addition of H_2O_2 and the linked scanning at constant B/E indicates the peaks at m/z 252 (loss of oxygen to give the molecular ion of **3**), 238 (loss of CH_2O) and 210 (protonated **4**). Figure 5 shows the LSIMS of **3** measured at 20 min after addition of H_2O_2 and Fig. 6 the linked scanning of the $[M+16]^+$ peak (**3a**) at m/z 268. In Fig. 5 the peak at m/z 238 may arise from the same origin as described in the case of **2**, corresponding to the structure of 9-carboxyacridinium ion.

Fig. 5. LSIMS of **3** measured at 20 min after an addition of H_2O_2 .

4. Conclusion

The positive ion LSIMS of the reaction mixture of a 10-methyl-9-acridiniumcarboxylate ester with hydrogen peroxide under the chemiluminescence conditions exhibits the oxygenated ion $[M+16]^+$ together with the molecular ion. The oxygenated ion $[M+16]^+$ was presumed as the ion related to the intermediates of the chemiluminescence reactions and its structure was deduced based on the linked scan at constant B/

Fig. 6. Linked scan of ion at m/z 268 at constant B/E.

E. The generation and the structure of oxygenated ion $[M+16]^+$ can be explained by the mass spectrometric reaction under LSIMS conditions of the 9-hydroperoxy adduct of acridinium ester, which was proposed as the intermediate in the chemiluminescence reactions.³⁾ Thus, the mass spectrometric investigation has proved to be useful for elucidation of the structures of unstable intermediates in a series of reactions under chemiluminescence conditions.

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Keywords

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