Effects of Additives to Methyl Hydroperoxyoleates on the Formation of Protonated Molecules and/or Molecular Adduct Ions in Liquid Ionization Mass Spectrometry

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Effects of additives, introduced to a sample through vapor phase, on the formation of protonated molecules and/or molecular adduct ions were investigated in liquid ionization mass spectrometry for the analysis of methyl hydroperoxyoleates. Water and formic acid having small proton affinities were useful for increasing ion abundance of protonated molecules $[M+H]^+$. 2-Aminoethanol and pyridine having slightly greater proton affinities were useful for forming molecular adduct ions $[M+H+L]^+$ (L: additive molecule). The use of additives, acting as matrix for producing $[M+H]^+$ and/or as *reagent* for forming $[M+H+L]^+$ in liquid ionization mass spectrometry, provided useful mass spectral characteristics of the molecular weight and structure of unknown compounds.

1. Introduction

Lipid hydroperoxides are easily produced by the reaction between unsaturated fatty acids and active oxygen species such as singlet oxygen, superoxide anion radical, and hydroxyl radical.^{1), 2)} Many scientists have much interests in biological consequences of lipid hydroperoxides arising from *in vivo* reactions,³⁾ and have studied about their formation⁴⁾⁻⁶⁾ and decomposition⁷⁾⁻⁹⁾ mechanisms. The structural characterization of lipid hydroperoxides has also been studied by gas chromatography/mass spectrometry (GC/MS)^{10), 11)} and chemical ionization (CI) mass spectrometry,¹²⁾⁻¹⁴⁾ in which preliminary derivatization such as trimethylsilylation of sample is generally required.

Liquid ionization (LI) mass spectrometry^{15), 16)} has been applied to the structural elucidation of methyl hydroperoxyoleates (OMH) without any derivatization. OMH is thermally labile, and has four positional isomers as shown in Fig. 1. Their protonated molecules, $[M+H]^+$ (m/z 329), which have not given by other soft ionization methods such as fast atom bombardment (FAB) and CI, and characteristic fragment peaks (m/z 173, 187, 199, 201, and 213) indicative of the positional isomers have been observed in the LI mass spectra.¹⁷⁾

Matrix plays the following important roles in soft ionization techniques such as FAB and LI: decreasing intermolecular forces between solute molecules by dispersing the molecules in the matrix; promoting ionization and desorption of analyte molecules; increasing abundance of molecular ion species including molecular adduct ions; and

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OMH

formic acid

1,2-ethanediol

DMF

1,2-ethanediamine

pyridine

2-aminoethanol

Fig. 1. Structure of OMH and several additives.

reducing fragmentation and thermal decomposition.

In the LI method, introducing some compounds (additives) to a sample through vapor phase is often useful to observe molecular ion species.¹⁸⁾⁻²⁰⁾ Some of them play a role of matrix, assisting the formation of protonated molecules $[\text{M} + \text{H}]^+$. Proton affinity of matrix should be smaller than those of analyte molecules. Some of additives (L) produce molecular adduct ions $[\text{M} + \text{H} + \text{L}]^+$. Molecular adduct ions are often observed as intense peaks in CI mass spectra when using NH_3 ,²¹⁾⁻²⁴⁾ *iso*- C_4H_{10} with additives,^{22), 25)} and $\text{NO}^{26)}$ as reagent gas. The adduct ion formation techniques have been applied to the structural elucidation.²⁶⁾⁻²⁹⁾

This paper describes the effects of additives on the LI mass spectra of OMH.

2. Experimental

2.1 Materials

OMH (a mixture of isomeric methyl 8-, 9-, 10-, and 11-hydroperoxyoctadecenoates, Fig. 1) was synthesized from methyl oleate and refined by the same procedure described in a previous paper.¹⁷⁾ Approximately 1% methanol solution of OMH was prepared for the measurements. Other chemicals of analytical reagent grade were purchased from Tokyo Kasei (Tokyo, Japan) or Wako (Osaka, Japan).

Benzene, chlorobenzene, acetone, acetonitrile, methanol, ethanol, isopropanol, 1,2-ethanediol, propane-1,3-diol, 2-aminoethanol, 1-amino-2-propanol, 3-amino-1-propanol, 2-amino-1-butanol, 2-mercaptoethanol, 1,2-ethanediamine, aniline, pyridine, dimethylsulfoxide (DMSO), dimethylformamide (DMF), formic acid, acetic acid, 1M-ammonia water, and deionized water were examined as additives to OMH. Among them, pyridine and 1,2-ethanediamine were diluted to 10% (v/v) aqueous solution before use, and others were used as purchased. These additives were supplied to the sample by vaporiz-

ing near the needle tip (sample holder) in the ion source. Additives producing mainly protonated molecules have been referred to as *vapor-matrix*,^{18), 19)} and those producing mainly molecular adduct ions have been referred to as *reagent*.²⁰⁾ The difference in the action is predictable from the chemical properties to some extent in advance of experiment.

Glycerol, thioglycerol, *m*-nitrobenzylalcohol (NBA) were also examined in expectation of similar effects. These compounds were directly mixed with the sample, and were termed *liquid-matrix*. In this study, a 1- μ l aliquot of aqueous solution (0.1%, v/v) of each additive was mixed with the sample on the sample holder prior to measurement. A 0.1% (w/v) urea aqueous solution was also examined as *liquid-matrix*.

2.2 Apparatus

All mass spectra were obtained by using a modified JEOL (Tokyo, Japan) JMS-QH 100 quadrupole mass spectrometer equipped with an LI ion source,³⁰⁾ coupled with a JMA-3100 data processing system.³⁰⁾⁻³²⁾

In the LI method, a pointed needle type of sample holder^{31), 32)} is fundamentally used, because ions produced at the needle tip can get through the pinhole to be observed. Since the methanol solution of OMH was used, the sample diffused around the whole needle due to its low surface-tension. The ion formation did not continue for enough time to measure mass spectra under several different experimental conditions. In order to keep a sample longer period of time, another type of sample holder was tested.

A new sample holder was made of a molybdenum wire (1.5 mm in diameter) cut at a 25° bevel and processed by a mirror finish to have an elliptic flat surface. This new sample holder was used in place of the ordinary one, and its flat surface was set to upside. The other conditions³²⁾ of the needle were unchanged. The ions were produced around the sharp edge as the same manner as the ordinary pointed needle tip was used.¹⁵⁾ Because of flat sample holder, the sample in methanol with low surface tension could be loaded always within the surface area. The sample loading therefore was easy and reproducible. The sample could easily be mixed with *liquid-matrix* on the sample holder.

A device used in this study for introducing additives through vapor phase was similar to that reported previously.^{18), 19)} A 5-cm long PTFE tube (2 mm i.d., 3 mm o.d.) padded with an absorbent cotton (3 mm in length) wetted with an additive was used in place of the glass tube,^{18), 19)} and the end of PTFE tube (vapor exit) was placed near the needle edge.

2.3 Mass spectrometry

A 0.5- μ l aliquot of the OMH solution was deposited on the sample holder, which was adjusted to the optimum position³³⁾ to the pinhole beforehand. While vaporizing additives in the ion source, LI mass spectrum between m/z 10 and 800 was scanned in 1 s and the scan was repeated every 1.5 s for 150 s with increasing the sample temperature from 25 to 120°C by controlling the heater current.

3. Results and Discussion

In our previous work,¹⁷⁾ OMH dissolved in methanol has been measured without any additives. The protonated molecules have been observed in about half of the base peak

Fig. 2. An LI mass spectrum of OMH with H₂O introduced through vapor phase.

Fig. 3. An LI mass spectrum of OMH with ethanol.

intensity, and the mass spectra have been dominated by the fragment peaks such as $[M + H - H_2O]^+$. Since solvent (methanol) molecules vaporize easily by heating the sample holder and do not remain between the sample molecules, the action of solvent is not enough as matrix.

It was found that water introduced through vapor phase to the sample deposited onto the needle was effective to obtain $[M + H]^+$ ion of OMH, although OMH is insoluble in water. An LI mass spectrum of OMH, measured by using water vapor from the PTFE tube, exhibited the intense $[M + H]^+$ peak as shown in Fig. 2. The ion abundances of the base peak were increased more than 50% compared with those obtained without water vapor. Water vapor seemed to play a role of matrix to OMH. Formic acid introduced through vapor phase showed a similar effect to water vapor on the mass spectra of OMH. Water and formic acid having small proton affinities successfully acted as proton donor to OMH and increased the abundance of $[M + H]^+$ ions.

On the other hand, no effect was observed when using other compounds of relative-

Fig. 4. An LI mass spectrum of OMH with DMF.

ly small proton affinities,¹⁹⁾ such as acetic acid, methanol, acetonitrile, benzene, chlorobenzene, and acetone. Benzene and chlorobenzene giving stable $M^{+\cdot}$ ions of themselves in LI could not act as proton donor to OMH. Although proton affinities of acetic acid and acetone are greater than those of formic acid and water, the difference in the action is inexplicable by only difference in proton affinity. These additives could not assist OMH to form stable $[M+H]^+$ ions.

Ethanol seemed to be a good matrix for observing $[M+H]^+$ ions and also acted as *reagent* giving a small peak of molecular adduct ion at m/z 375 corresponding to $[M+H+L]^+$ as shown in Fig. 3. "L" stands for ethanol molecule in this case. The use of ethanol resulted in the increase of about 50% in the total ion abundance.

Figure 4 shows an LI mass spectrum of OMH by the use of DMF, where the intense peak of the adduct ion $[M+H+L]^+$ is observed at m/z 402 in addition to intense $[M+H]^+$ and $[M+H-H_2O]^+$ peaks at m/z 329 and 311, respectively. The peak at m/z 147 is corresponding to $[2L+H]^+$ ion of DMF. This spectral pattern was similar to that obtained by the use of DMSO. This kind of dipolar aprotic solvent gave both molecular and adduct ions, leading to somewhat complicated mass spectra. Therefore, the use of them seems to be not so suitable for the analysis of OMH in a mixture.

Several volatile compounds having two functional groups such as $-OH$, $-SH$, and $-NH_2$ were examined in expectation of formation of more stable molecular adduct ions. Among them, both 1,2-ethanediol and 2-mercaptoethanol did not show any significant effects on the formation of adduct ions of OMH. The mass spectra were similar to those obtained without any additives, as reported previously.¹⁷⁾

In contrast, alkaneaminoalcohols were useful for observing the adduct ions, $[M+H+L]^+$, which were more abundant than $[M+H]^+$ ions. The effect of 2-aminoethanol on the mass spectrum is shown in Fig. 5. The base peak at m/z 390 is corresponding to the adduct ion and the peaks at m/z 372 and 356 are its fragment ions, due to the elimination of H_2O and H_2O_2 , respectively. The peak intensity ratios of fragment ions to the adduct ion (m/z 372 and 356 from m/z 390) were different from those to $[M+H]^+$ (m/z 311 and 295 from m/z 329). The mass spectrum indicates that the molecular

Fig. 5. An LI mass spectrum of OMH with 2-aminoethanol.

Fig. 6. An LI mass spectrum of OMH with 1,2-ethanediamine (10% aqueous solution).

adduct ions are more stable than the protonated molecules. The other amino alcohols examined so far showed similar effect on the mass spectra of OMH, while the mass spectrum obtained by the use of 1,2-ethanediamine was very complicated as shown in Fig. 6, exhibiting the intense fragment peaks from $[M+H]^+$ in addition to those from $[M+H+L]^+$. If abundant molecular adduct ions are observed by using some additive, such additive is a good *reagent* for the analyte molecule and the structure of $[M+H+L]^+$ ion must be stable. In such case, proton affinities of additives, like aminoalcohols, are often greater than that of sample.²⁴⁾ It is considered that the stability of molecular adduct ions is dependent mainly on the strength of hydrogen bonding between solute and additive molecules. Thus bifunctional compounds having different functional groups, such as amino and hydroxyl groups, were adequate to the formation of stable adduct ions of OMH.

Figure 7 shows an LI mass spectrum obtained by the use of vapor of 1M ammonia water. The mass spectrum exhibited the intense peak at m/z 346 corresponding to $[M$

Fig. 7. An LI mass spectrum of OMH with ammonia water (1M).

Fig. 8. An LI mass spectrum of OMH with pyridine (10% aqueous solution).

$+H+L)^{-}$ ions ($L: NH_3$) and its fragment peaks at m/z 328, $[M+H+L-H_2O)^{-}$, and 312, $[M+H+L-H_2O_2)^{-}$, in addition to the peaks related to the molecular ion species at m/z 329, 311, and 295. The mass spectral pattern obtained by LI was different from that by ammonia CI. In general, NH_3 CI often gives $[M+H+NH_3]^+$ ions,²¹⁾⁻²⁴⁾ but no peak corresponding to $[M+H+NH_3]^+$ has been observed in NH_3 CI spectra of OMH.¹⁷⁾ In LI, ammonia seemed to be one of good *reagents* for the measurement of OMH, although the mass numbers for the adduct ion species were close to those for the molecular ion species.

The mass spectral pattern obtained by the use of aniline was similar to that by ammonia water, showing intense $[M+H+L]^+$ peak at m/z 422 and weak $[M+H]^+$ peak at m/z 329 with $[M+H-H_2O]^+$ at m/z 311.

Figure 8 shows an LI mass spectrum of OMH by the use of pyridine. The intense $[M+H+L]^+$ type ion peak and its fragment peak due to H_2O loss were observed at m/z 408 and 390, respectively. Less intense peaks related to the molecule, *e.g.*, mass of 311, were

observed. The peak at m/z 159 is corresponding to $[2L+H]^+$ ion of pyridine. It is considered that pyridine, having proton affinity greater than proton affinity of OMH, is one of good *reagents* producing stable molecular adduct ions of OMH.

The mass spectra of OMH, measured by mixing the sample with glycerol, thioglycerol, or NBA, exhibited neither intense $[M+H]^+$ nor $[M+H+L]^+$ peak. The urea solution also showed no significant effect, although a weak $[M+H+L]^+$ peak was observed at m/z 389. The mass spectral patterns obtained by the use of these additives were similar to each other, and were almost the same as those obtained without additives. Compounds adequate to *liquid-matrix* are not found so far.

4. Conclusion

Additives acting as matrix introduced through a vapor phase, such as water and formic acid, are useful for observing protonated molecule of OMH, and also for increasing the ion abundance. Proton affinity of OMH seems to be greater than that of DMF, because protonated molecules of both compounds are observed in the mass spectrum. Additives acting as *reagent*, such as ethanolamine and pyridine, are useful for producing molecular adduct ions of OMH. These two independent mass spectra give useful information to determine the molecular weights of compounds, even if the sample is unknown. Moreover, the use of *vapor-matrix* adequate to analytes promotes the desorption of molecular ions of even thermolabile compounds such as OMH. Additives play important roles in LI mass spectrometry.

References

- 1) "Free Radicals, Lipid Peroxidation and Cancer," ed. by D. McBrien and T. Slater, Academic Press, London (1982).
- 2) C. Nagata, M. Kodama, Y. Ioki, and T. Kimura, "Free Radicals and Cancer," Marcel Dekker, New York (1982), p. 1.
- 3) R. A. Froyd, "Free Radicals in Biology," Vol. IV, Academic Press, New York (1980), p. 187.
- 4) F. D. Gunstone, *JAOCS*, **61**, 441 (1984).
- 5) E. N. Frankel, *JAOCS*, **61**, 1908 (1984).
- 6) N. A. Porter and D. G. Wujek, *J. Am. Chem. Soc.*, **106**, 2626 (1984).
- 7) H. W.-S. Chan, F. A. A. Prescott, and P. A. T. Swoboda, *JAOCS*, **53**, 572 (1976).
- 8) Y. Mochida and T. Yamada, *Yukagaku*, **30**, 182 (1981).
- 9) Y. Mochida and T. Yamada, *Yukagaku*, **32**, 163 (1983).
- 10) H. W. Gardner and R. D. Plattner, *Lipids*, **19**, 294 (1984).
- 11) E. N. Frankel, W. E. Neff, and E. Selke, *Lipids*, **19**, 790 (1984).
- 12) R. D. Plattner, H. W. Gardner, and R. Kleiman, *JAOCS*, **60**, 1298 (1983).
- 13) R. D. Plattner and H. W. Gardner, *Lipids*, **20**, 126 (1985).
- 14) E. N. Frankel, W. E. Neff, and R. D. Plattner, *Lipids*, **21**, 333 (1986).
- 15) M. Tsuchiya and H. Kuwabara, *Anal. Chem.*, **56**, 14 (1984).
- 16) M. Tsuchiya, H. Kuwabara, and K. Musha, *Anal. Chem.*, **58**, 695 (1986).
- 17) Y. Mochida, Y. Yokoyama, T. Kawai, S. Nakamura, and M. Tsuchiya, *J. Mass Spectrom. Soc. Jpn.*, **42**, 207 (1994).
- 18) M. Tsuchiya, "Advances in Mass Spectrometry," Vol. 13, ed. by I. Cornides, Gy. Horváth, and K. Vékey, Wiley (1995), pp. 333-346.
- 19) M. Tsuchiya, *J. Mass Spectrom. Soc. Jpn.*, **43**, 373 (1995).
- 20) Y. Niki and M. Tsuchiya, *Org. Mass Spectrom.*, **22**, 157 (1987).
- 21) J. B. Westmore and M. M. Alauddin, *Mass Spectrom. Rev.*, **5**, 381 (1986).
- 22) D. I. Carroll, J. G. Nowlin, R. N. Stillwell, and E. C. Horning, *Anal. Chem.*, **53**, 2007 (1981).

- 23) T. Keough and A. J. DeStefano, *Org. Mass Spectrom.*, **16**, 527 (1981).
- 24) P. Rudewicz and B. Munson, *Anal. Chem.*, **58**, 2903 (1986).
- 25) D. V. Bowen and F. H. Field, *Org. Mass Spectrom.*, **9**, 195 (1974).
- 26) H. Budzikiewicz, B. Schneider, E. Busker, W. Boland, and W. Francke, *Org. Mass Spectrom.*, **22**, 458 (1987).
- 27) R. Chai and A. G. Harrison, *Anal. Chem.*, **53**, 34 (1981).
- 28) J-C. Tabet, Y. Tondeur, Y. Hirano, A. Wegmann, P. Tecon, and C. Djerassi, *Org. Mass Spectrom.*, **19**, 473 (1984).
- 29) B. Schneider, M. Breuer, H. Hartmann, and H. Budzikiewicz, *Org. Mass Spectrom.*, **24**, 216 (1989).
- 30) M. Tsuchiya, E. Aoki, and H. Kuwabara, *Int. J. Mass Spectrom. and Ion Processes*, **90**, 55 (1989).
- 31) Y. Yokoyama, M. Hashimoto, M. Tsuchiya, and R. Yabe, *Int. J. Mass Spectrom. and Ion Processes*, **111**, 263 (1991).
- 32) Y. Yokoyama, M. Aragaki, H. Sato, and M. Tsuchiya, *Anal. Chim. Acta*, **246**, 405 (1991).
- 33) Y. Yokoyama, H. Kakinuma, and M. Tsuchiya, *Shitsuryou Bunseki*, **37**, 79 (1989).

Keywords

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