Formation of the [M+H]⁺ and Abundant Fragment Ions of Methyl Stearate under Low Energy Electron Ionization Conditions†

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Mass spectra of methyl stearate (I) obtained under low energy (8-13 eV) electron ionization (EI) conditions showed intense [M+H]⁺ ions and abundant fragment ions in the positive-ion mode, and intense [M-H]⁻ ions and characteristic fragment ions in the negative-ion mode. Positive-ion mass spectra of I under 8-13 eV/EI conditions showed an interesting ion at m/z 75 which may originate from a McLafferty rearrangement reaction of the [M+H]⁺ ions, an even electron species. The detailed experimental conditions to reproduce these spectra have been explored. It was found that the most important condition is the introduction into the ionizing cell of a relatively high-pressure sample vapor from a liquid state, similar to a spray process, and that the abundant fragment ions come from the [M+H]⁺ ions. Possible mechanisms are discussed to explain the formation of [M+H]⁺, [M−H]⁻, and abundant fragment ions under the 8-13 eV/EI conditions. Furthermore, unusual ion species such as [M+K]⁺, [M+Na]⁺, [M+H]⁺, [M−H]⁻, K⁺, and Na⁻ were observed in the mass spectrum of I obtained at the lowest energy (2 eV) EI conditions, suggesting that thermal surface ionization occurred on the Re filament.

1. Introduction

The ionization and fragmentation of fatty acid methyl esters (FAMEs) under electron ionization (EI) have been studied in detail by Ryhage and Stenhagen.1 One of the remarkable characteristics of EI mass spectra of FAMEs is the appearance of an abundant ion at m/z 74, corresponding to the well-known McLafferty reaction (McR)5 with a single hydrogen rearrangement (SHR), and a related peak at m/z 87. While studying the effect of electron energy on the abundance and metastable ion decay (MID) for the fragment ions of FAMEs,9 we found that the EI mass spectra of methyl stearate (I) gave abundant protonated molecules [M+H]⁺ at m/z 299 and abundant fragment ions at electron energies near 8-13 eV, while at 15 eV the mass spectrum of I showed the expected M⁺⁺ and weak fragment ions.4 Since the ionization energy (IE) of FAMEs is in the range of 10-11 eV (e.g., 10.40 eV for methyl pentanoate),9 the 8-13 eV/EI conditions seemed to be critical for the formation of M⁺⁺ ions. We hypothesized4,6 that the formation of abundant fragment ions at m/z 267, 255, 199, 143, 101, 87, 75, 74, 55,

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and 43 under the 8–13 eV/EI conditions could be explained by the thermal activation of [M+H]⁺ ions formed by thermal surface ionization (TSI) on a hot Re filament as follows:

\[ M + M \rightarrow ([M+H]⁺ \cdots [M-H]⁻)⁺ \]
\[ \rightarrow ([M+H]⁺)⁺([M-H]⁻) \quad (1.1) \]
\[ ([M+H]⁺)⁺ \rightarrow \text{considerable fragmentation} \quad (1.2) \]

or by the EI-induced fragmentation followed by TSI mode as shown below:

\[ ([M+H]⁺)⁺ + e⁻ \rightarrow ([M+H]⁺)⁺ + e⁻ \quad (\text{EI mode}) \quad (2.1) \]
\[ ([M+H]⁺)⁺ \rightarrow \text{considerable fragmentation} \quad (2.2) \]

where \([M+H]⁺ \cdots [M-H]⁻)⁺\) represents a proton transfer complex on the Re surface. It should be also considered that there are other possibilities for the formation of [M+H]⁺ ions, such as the EI-induced ion-pair formation by a two-body process (3) or through a dimer (4) in the gas-phase as follows:\(^6\):\(^6\):

\[ M + M + e⁻ \rightarrow [M+H]⁻ + [M-H]⁻ + e⁻ \quad (3) \]
\[ M_₂ + e⁻ \rightarrow [M+H]⁺ + [M-H]⁻ + e⁻ \quad (4) \]

However, neither the mechanism(s) for the formation of [M+H]⁺ ions of 1 which can undergo considerable fragmentations under the 8–13 eV/EI conditions nor the detailed experimental requirements for the peculiar observations described above was known. To determine the experimental conditions for the reproducible appearance of [M+H]⁺ and abundant fragment ions of 1, a homemade Re filament was used and direct observation of sample states in the sample tube was performed. Charge-exchange ionization (CEI) methodology was also employed.

2. Experimental

All experiments were performed with a JEOL JMS-DX303 double-focusing mass spectrometer equipped with a JEOL JMA-DA5100 data system. The sample was introduced into the ion source heated at 160°C via a direct inlet system with a glass sample tube of 16 mm long × 1 mm inner diameter. The ionizing voltage was varied at an ionizing current of 100 µA. The pressure, as read by a gauge attached to the pumping line for the ion source, was typically at 1.0 × 10⁻⁷ Torr. The ion accelerating voltage was 3 kV. The reagent gas used in the CEI experiment was Xe. Methyl stearate (melting point: 37°C) was purchased from Tokyo Kasei (Tokyo, Japan). A schematic

Fig. 1. Schematic layout of the ion source with a normal Re filament.
layout of the ion source is shown in Fig. 1. Metastable ion (MI) and collision-induced dissociation (CID) spectra were obtained by using the B/E=constant linked-scanning experiments without and with helium collisions, respectively. The pressure of the collision gas was adjusted to reduce the parent ion beam by 50%.

3. Results and Discussion

Mass spectra under low electron energy conditions

When relatively low-pressure sample vapor was introduced into the ionizing cell by adjusting the position of the sample tube (see Fig. 1), the relative abundances of the usual fragment ions at m/z 267, 255, 199, 143, 87, and 74 decreased with decreasing electron energy as expected, and the M⁺⁺ and fragment ions disappeared at 10–11 eV. Typical EI mass spectra of methyl stearate (I) obtained at 18 and 15 eV are shown in Fig. 2. Using the direct inlet probe, however, the EI mass spectrum of I often showed an intense peak that corresponded to the protonated molecules [M+H]+ in addition to the
typical pattern in Fig. 2. This was ascribed to a self-Cl process owing to the high volatility of the analyte.

The introduction of a relatively high-pressure of sample vapor led to the preferential formation of [M+H]$^+$ and abundant characteristic fragment ions at electron energies of 8–13 eV. A typical mass spectrum of 1 obtained under 12 eV/EI conditions is shown in Fig. 3, though the intensities of fragment ions fluctuated with the electron energy and the amounts of sample. When this spectrum is compared with the mass spectra in Fig. 2, the characteristic feature of the appearance of lower mass fragments at $m/z$ 101, 83, 74, 69, 55, 43, 31, and 15 is apparent. It has been confirmed by using CID that the abundant fragment ions in Fig. 3 originate mainly from the protonated molecules [M+H]$^-$. Although the CID pattern was in good agreement with that of the [M+H]$^+$ ions formed under chemical ionization (Cl) conditions, neither spectrum showed a characteristic fragment at $m/z$ 75.

The fragment ion of $m/z$ 75 in Fig. 2 has been observed in the MI spectrum of the M$^-$ ions of 1 and also as a metastable peak $m^*$ at $m/z$ 18.88 in the normal spectrum. According to Ryhage and Stenhagen, the fragment ion at $m/z$ 75 in the EI mass spectra of FAMES originates from a double hydrogen rearrangement (DHR), though the source of the second hydrogen is not clear. On the other hand, it could be considered that the fragment ion at $m/z$ 75 in Fig. 3 originates from the McR reaction of the [M+H]$^+$ ion which is an even electron species. The proposed fragmentation pathways for the formation of the ion at $m/z$ 75 in Figs. 2 and 3 are shown in Schemes 1 and 2, respectively.

The mass spectrum of 1 obtained by setting the electron energy as low as possible showed characteristic peaks corresponding to [M+K]$^+$, [M+Na]$^-$, [M+H]$^+$, [M−H]$^+$, K$^-$, and Na$^+$ ions which form under TSI conditions (Fig. 4), while the mass spectrum in the absence of sample showed only K$^+$ and Na$^+$ peaks. These metal ions originate from the heated Re filament. The electron energy at this setting was 2 eV. Decreasing the

![Fig. 3. Mass spectrum of methyl stearate at 12 eV/EI, obtained by introducing relatively large amounts of sample vapor.](image)
electron energy leads to an increase in the filament current to maintain a constant emission current, and thus leads to an increase in the filament temperature due to Joule heating. It is possible that gas-phase sample molecules interacted with the Re surface by diffusion of the sample vapor into the ionizing cell owing to the high volatility of 1. The results obtained suggested that the TSI processes clearly occur under 2 eV/El conditions.

**Influence of the position of Re filament**

To test the hypothesis that the TSI processes occur on the Re surface, a homemade Re filament (18 mm long × 0.2 mmϕ) was used which protruded into the ionizing cell to
increase the direct contact between the analyte molecules and the Re surface (Fig. 5), in contrast to the original source design where the Re filament (9 mm long × 0.2 mmφ) was located outside the ionizing cell (Fig. 1). If the TSI processes occur as a major reaction, the use of the homemade filament would increase the rate of ion formation under the 8–13 eV/EI conditions. The use of the homemade filament, however, did not show any increase in the absolute abundance of the [M+H]+ ions and/or of the characteristic fragment ions. The mass spectrum obtained at 10 eV with the homemade filament is shown in Fig. 6. The most important condition for obtaining the mass spectrum in Fig. 6 was the introduction of a relatively high-pressure sample vapor into the ionizing cell, which could be done by adjusting the position of the sample tube. This suggests that the surface of the Re filament does not play an important role in the formation of [M+H]+ and the characteristic fragment ions, although it does seem to be essential for the formation of TSI characteristic ions such as [M+Na]+, [M+K]+, Na+, and K+ ions at the lowest electron energy.
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Direct observation of sample states
In order to determine the exact experimental conditions for the formation of \([M+H]^+\) and the characteristic fragment ions of 1 under the 8–13 eV/EI conditions, the state of the sample in the glass tube was directly observed through a glass window attached to the ion source. The states of the condensed sample in the glass tube inserted into the ionizing cell changed as follows:
1) melting of sample crystal,
2) bubble formation in glass tube,
3) vaporization from the melt surface,
4) rupture of the bubble.

The series of steps observed is shown in Scheme 3. Stable and reproducible formation of the \([M+H]^+\) and characteristic fragment ions under the 8–13 eV/EI conditions was observed during step 3. The rate of vaporization of the front part of the sample (see 3) in Scheme 3) was roughly estimated as several 10 nl/s. This seems to be a spray condition which is rather similar to thermospray. This condition 3) was maintained for several 10 s. The large amount of sample may result in a large number of collisions of analyte molecules in the ionizing cell, so the formation of clusters and/or ion/molecule

![Scheme 3](image)

Fig. 7. Negative-ion mass spectrum of methyl stearate at 12 eV/EI, obtained by introducing relatively large amounts of sample vapor.
reactions can take place. In fact, a small peak corresponding to \([2M+H]^−\) ion at \(m/z 597\) was observed in the 8–13 eV mass spectra.

Negative-ion mass spectra of 1 were obtained, under the 8–13 eV/EI and spray conditions. The spectrum at 12 eV/EI clearly showed an abundant peak corresponding to deprotonated molecules \([M−H]^−\) at \(m/z 297\) (Fig. 7). A dimer ion such as \([2M−H]^−\) was not observed. The observation of the \([M−H]^−\) ion seemed to depend strongly on the electron energy. An electron energy of 12 eV/EI was most efficient for the abundant formation of the \([M−H]^−\) ion, and the optimal conditions for its formation were roughly similar to those for the \([M+H]^+\) ion formation. Thus, conditions for the formation of \([M+H]^+\) and \([M−H]^−\) ions may be closely related. Since thresholds for ion-pair formation under EI conditions lie in the range of 10–15 eV,9 it is likely that the formation of \([M+H]^+\) and \([M−H]^−\) ions occurs through reaction (4). Thus the formation of a dimeric species, such as \(M_2\), is necessary for the production of these ions, and the spray condition produced here seems to be effective for the formation of dimeric species.

**Charge exchange ionization with Xe\(^{+\cdot}\) ions**

A positive-ion CEI mass spectrum of 1 with Xe\(^{+\cdot}\) ions, which have recombination energies of 12.1 and 13.4 eV,9 was obtained under the spray conditions described in step 3) above, and is shown in Fig. 8a. The mass spectrum showed abundant ions corresponding to dimeric ions \([2M+H]^+\) at \(m/z 597\) and \([M_2]^+\) at \(m/z 596\), as well as the monomeric \([M−H]^+, M^{+\cdot}\), and \([M+H]^+\). The electronic excitation of Xe gas due to electron impact may result in the formation of metastable species, Xe\(^\ast^\ast\) and Rydberg atoms, Xe\(^\ast\) with excess energy of 8–13 eV, as well as the ionic species Xe\(^{+\cdot}\). These electronically excited species act as the reagent species to form the ions in Fig. 8a. The dimeric and molecular-related ions in Fig. 8a may be formed by the following reactions:

\[
M_2 + Xe^{+\cdot} \rightarrow [M_2]^{+\cdot} + Xe \tag{5}
\]

\[
M + Xe^{+\cdot} \rightarrow M^{+\cdot} + Xe \tag{6.1}
\]

\[
[M−H]^+ + M → [2M]^{+\cdot} + M \tag{6.2}
\]

\[
M_2 + Xe^\ast \text{ or } Xe^{\ast\ast} \rightarrow [M+H]^+ + [M−H]^− + Xe \tag{7.1}
\]

\[
[M+H]^+ + M \rightarrow [2M+H]^+ \tag{7.2}
\]

Although the ion-pair formation (7.1) is energetically similar to that involved in electron ionization processes at 8–13 eV, the EI and CEI mass spectra of 1 are quite different from each other. Furthermore, the negative-ion CEI mass spectrum of 1 (Fig. 8b) also gave a quite different pattern from the negative ion EI mass spectrum (Fig. 7). This means that the ionization mechanism(s) of 1 under the 8–13 eV/EI conditions is different from those generated by reacting 1 with Xe at 8–13 eV excess energy.

**Formation of the abundant fragment ions**

A significant characteristic of the mass spectra obtained under low electron energy conditions was the appearance of abundant fragment ions at \(m/z 267, 255, 213, 199, 185, 157, 143, 129, 101, 87, 83, 75, 74, 69, 55, 43, 31, \) and 15, as shown in Fig. 3. The appearance of these fragment ions coincided with the appearance of \([M+H]^+\) ions. This indicates that these fragments originate from the \([M+H]^+\) ions. In order to examine the decomposition of the \([M+H]^+\) ions formed under the 8–13 eV/EI conditions, the MI and CID spectra of \([M+H]^+\) ions formed at 12 eV were obtained, and are shown in Fig. 9. The MI spectrum gave poor reproducibility and low signal/noise ratios, while the CID
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Fig. 8. Charge-exchange mass spectra of methyl stearate with Xe^2+ ions as the reagent ion in (a) positive-ion and (b) negative-ion mode.

spectrum gave clearly defined peaks. This means that the fragmentation processes of the [M+H]^+ ions proceed only with relatively high collisional activation energies. In other words, abundant fragment ions can only be formed by passing over the relatively high energy barrier for the fragmentation reaction.

From the result described above, it can be presumed that the initially formed [M+H]^+ ions in the ionizing cell contain relatively large amount of internal energy which contributes to their fragmentation. Considering the low electron energies, it would not be expected that vibrationally excited ion species would be formed under the experimental conditions used here. It does seem reasonable to consider the influence of thermal radiation from the heated Re surface, however, because filament temperature increases with decreasing electron energy. In fact, the Re filament was red hot at 10 eV or above, and changed to white hot when electron energy was decreased to 2 eV. According to Cotter et al.,8 the Re filament temperature which leads to the formation of Na^+ and K^+ ions ranges from 700 to 1000°C white hot. Consequently, the formation and degradation of the [M+H]^+ ions of 1 formed under the 8−13 eV/EI and spray conditions may be due to the influence of electron and thermal radiation from the hot surface of Re filament. The internal energy of the [M+H]^+ ions may mainly originate from thermal activation after ion formation.

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Varying the electron energy in EI is used for the estimation of the ionization energy of a given organic compound. On the other hand, the introduction of a relatively high-pressure sample vapor into the EI ionizing cell often results in ion/molecule reactions leading to the formation of ions such as \([\text{M-H}]^+\) and \([\text{M+H}]^-\), which is a characteristic of CI. Here we ascribed the unexpected ionization behavior of methyl stearate (I) to TSI on the heated Re filament at 2 eV or 8–13 eV electron energies in EI:

1) the formation of the \([\text{M+H}]^+\) and surprisingly abundant fragment ions in the positive-ion mode and the formation of \([\text{M-H}]^-\) ions in the negative-ion mode, under low electron energy conditions \(\sim\) (8–13 eV).

2) the formation of the alkali-metal and adduct ions, \(\text{Na}^+, \text{K}^+, [\text{M+Na}]^+, \) and \([\text{M+K}]^+\), under 2 eV/EI conditions.

It was confirmed that the former phenomenon occurs when a large amount of sample

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vapor enters the source, similar to a thermospray process. The formation of the [M+H]+ and abundant fragment ions could be explained by two- or many body collision processes in conjunction with the electron and thermal radiation from the hot Re surface. Direct interaction of analyte molecules with the Re surface did not significantly affect ion yields. The abundant fragment ions originating from the decomposition of the [M+H]+ ions could be explained by thermal vibrational excitation from the heated filament. The results of the filament experiments were, however, in good agreement with the TSI processes reported so far. That is, alkali-metal ions originate from the surface components of the Re material, and the metal ions act as reagent ions for adduct ion formation.

The appearance of the fragment ion at m/z 75 was ascribed to the McR reaction of the [M+H]+ ions produced under 12 eV/Ei conditions, while the fragment ion at m/z 75 in the normal EI spectrum originated from the DHR reaction of the M+ ions. The fragmentation pathways were proposed for these fragment ions.

It should be noted that these various unexpected phenomena regarding the ionization and related events were hidden in seemingly routine experimental conditions. Such hidden phenomena may possibly lead to new techniques in mass spectrometry.

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References


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