Structural Analysis of 9-Acridinylmethyl Derivatives of Fatty Acids by Mass Spectrometry

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Characterization of electron impact mass spectra of 9-acridinylmethyl esters of fatty acids was reported. The esters gave molecular ions with relatively strong intensity and simple fragmentation patterns. Fragmentation ions appeared with 14 mass unit separations in the spectra of saturated fatty acid esters. In the spectra of unsaturated fatty acid esters, the 26 mass unit separations were observed at the position of double bonds in the molecule. Ions (M-30)+ and (M-31)+ suggested the existence of a hydroxymethyl group and the 29 and 30 mass unit separations suggested the positions of hydroxy groups located in the molecule. These fragmentations were useful for the structural analysis of fatty acids.

Keywords 9-Acridinylmethyl ester, fatty acid, mass spectrum, structural analysis

Fatty acids and their esters play very important roles in vivo. Their separation and quantitative analysis are performed by gas chromatography and their mass spectral data give information about their structures. Methyl or trimethylsilyl esters are usually employed in gas chromatography/mass spectrometry (GC/MS), but they give unsatisfactory information about the position of their double bonds. To determine the position of double bonds by GC/MS, some derivatization methods were proposed. Among them, picolinyl derivatives are useful for the structural determination of polyenoic acids, hydroxy acids, diacids and related compounds.

The rapid development of high performance liquid chromatography (HPLC) made it possible to analyze small amounts of unstable or unvolatile compounds with high sensitivity, and many kinds of labeling reagents were developed for HPLC analysis. Previously, we reported 9-bromomethylacridine (9-Br•Ma) as one of fluorescent labeling reagents for fatty acids. 9-Acridinylmethyl (9-AM) esters, the fluorescent products of the labeling reaction, were good markers for the separation and were expected to be useful for the structural analysis of fatty acids by mass spectrometry because of their similarity to picolinyl derivatives which stabilized the double bond migration. In this article, characterization of electron impact (EI) mass spectra of 9-AM derivatives of fatty acids is reported.

Experimental

9-Br•Ma was synthesized by the method previously reported. Free fatty acids were purchased from Sigma Chemical Co. (St. Louis, USA). Other reagents were purchased from Wako Pure Chem. Co. (Osaka, Japan).

The preparations of 9-AM derivatives of fatty acids were as follows. Five milliliters of 9-Br•Ma DMF solution (4 - 40 mM) and 5 ml of tetraethylammonium carbonate DMF solution (2 - 20 mM) were added to the excess amounts of a fatty acid (5 -100 mg) and then the mixture was reacted at room temperature for more than 30 min. Then 20 ml of water was added to it and the 9-AM derivative was extracted with 20 ml of hexane. After being dried with magnesium sulfate, followed by evaporation of the solvent, the 9-AM derivative was purified by silica gel chromatography (Silicagel 60, 70 - 230 mesh, Merck; eluant, benzene and ethylacetate) or preparative TLC (Silicagel 60 GF254; developing solvent, benzene and ethylacetate). Mass spectra of 9-AM derivatives were measured with a JEOL JMS HX-105 mass spectrometer (Japan Electron Optics Laboratory Co. Ltd., Tokyo, Japan). Operating conditions were: ionization, EI; injection, direct; chamber temperature, 150 - 250°C; electron energy, 70 eV; ion species, positive.

Results and Discussion

The mass spectrum of 9-AM palmitate (heptadecanoate) is shown in Fig. 1. The molecular ion (M+) was observed at m/z 447, and (M-15-14n)+ (n=1, 2, ..., 12) were observed as fragmentation ions. These fragmentations were attributed to the radical-induced
cleavage, as shown in Fig. 1-a, through each carbon-carbon bond, similar to the spectra of picolinyl esters.\textsuperscript{3} The resultant ions were very stable and few further rearrangements were observed. The ion at \textit{m/z} 251 was a result of McLafferty rearrangement with cleavage of the C-2/C-3 bond (Fig. 1-b). The ion at \textit{m/z} 264 was derived by the cleavage of C-3/C-4 bond (Fig. 1-c). This ion appeared with relatively strong intensity because of its stability. The ions at \textit{m/z} 193 and 209 were attributed to the chemical structures shown in Fig. 1-d. Other saturated fatty acid esters such as laurate, myristate and stearate gave mass spectra similar to this spectrum.

The mass spectra of 9-AM olate (cis-9-octadecenoate), linolate (cis-9- and cis-12-octadecadienoate) and linolenates (cis-9-, cis-12- and cis-15-octadecatrienoate) are shown in Fig. 2. Their molecular ions were observed clearly at \textit{m/z} values of 473, 471 and 469, respectively. The ions at \textit{m/z} 193, 209, 251 and 264 were observed similarly to the spectra of the saturated fatty acid esters. The 14 mass unit separated ions were derived by the cleavage of carbon-carbon single bonds. By the cleavage at a double bond, a 13 mass unit separation was observed. However, the 26 mass unit separation was not observed. In Fig. 2(A), the 26 mass unit separation was observed between \textit{m/z} 334 and 360 which were formed by the cleavages of C-8/C-9 and C-10/C-11 bonds, respectively. This result showed that a double bond of oleic acid was located between the C-9 and C-10 carbon atoms. The 26 mass unit separated ions were also observed between \textit{m/z} 334 and 360, and \textit{m/z} 374 and 400 in the spectrum of 9-AM linolate (Fig. 2(C)), respectively. These 26 mass unit separations gave useful information for the determination of the positions of double bonds in their molecules. The mass spectrum of cis-4-, cis-7-, cis-10-, cis-13-, cis-16- and cis-19-docosahexaenoic acid esters is shown in Fig. 3. The 26 mass unit separations were observed, depending on each position of the double bonds in the molecule, but they were not so obvious as those of monoenoic acid derivatives.

Figure 4 shows the mass spectra of 16-hydroxyhexadecanoic acid and dl-12-hydroxystearic acid derivatives. These spectra were obtained without further derivatizations of esters such as trimethylsilylation. The ion (M-17)\textsuperscript{+} was a result of an elimination of a hydroxy group and (M-18)\textsuperscript{+} was formed by dehydration in a bombardment chamber. In Fig. 4(A), the ions at \textit{m/z} 432 and 433 were derived by the cleavage of C-15/C-16 bond. This means that the hydroxy group connected to the C-16 carbon atom. In Fig. 4(B), the spectrum was characterized by the ions at \textit{m/z} 376, 377 and 406. The ions at \textit{m/z} 376 and 377 were produced...
by the cleavage of C-11/C-12 bond, and the ion at $m/z$ 406 was derived by the cleavage of C-12/13 bond. These 29 and 30 mass unit separations suggested that the hydroxy group was located at C-12 carbon atom. In this spectrum, the contamination of 11- and 12-octadecenoic acid derivatives was observed. They were probably produced by dehydration during the sample preparation or sample injection. The ions (M-30)$^+$ and (M-31)$^+$ suggest the existence of a hydroxymethyl group, and the 29 and 30 mass unit separations indicate the position of a hydroxy group in the molecule.

Figure 5 shows the mass spectrum of 9-AM ricinolate ((R-(Z))-12-hydroxy-9-octadecenoic acid ester). This ester possesses both a hydroxy group and a double bond. The ion at $m/z$ 471 indicated the existence of a hydroxy group because this ion was attributed to dehydration of ricinolate. The 29 and 30 mass unit separations were observed between the ions at $m/z$ 375 and 404 and $m/z$ 374 and 404, respectively. These characteristic separations showed that the C-12 carbon atom possessed a hydroxy group. The 26 mass unit separation was also observed between $m/z$ 334 and 360. These ions were formed by the cleavage of C-8/ C-9 and C-10/ C-11 bonds, respectively. Therefore, the position of the double bond in the molecule of ricinolate was assigned to be between the C-9 and C-10 carbon atoms.

Figure 6 shows a mass spectrum of 4-methyloctanoic acid derivative, which has a branched structure at the C-4 carbon atom. The molecular ion was observed at $m/z$ 349. The ions at $m/z$ 264 and 292, which were observed with relatively strong intensities, were derived by the cleavage of C-3/C-4 and C-4/C-5 bonds, respectively. The results show that the cleavage readily occurs at the branching bond.

Mass spectra of 9-AM derivatives of the fatty acids gave information about not only their molecular
weights but also the positions of double bonds, hydroxy groups and branched chains in the molecules. The labeling reaction proceeded in moderate conditions and the resultant esters could be separated by HPLC. Therefore, this method is expected to be applied in both the off-line and on-line LC-MS systems.

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