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速 報

Mass Spectral Analysis of 10-Hydroxy-octadec-12-enoic Acid Found in Rice Bran Oil

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1 Introduction

In our previous communication,[1] we presented our findings indicating that ricinoleic acid (12-hydroxy-octadec-9-enoic acid) occurs in common vegetable oils and also in common oil seeds. Since then, we continued our investigation on monohydroxy monoenoic fatty acids in those common vegetable oils, and obtained gas chromatographic data suggesting the possible existence of an unknown fatty acid in rice bran oil. We thought it was probably one in a series of isomers of ricinoleic acid, but it was not isoricinoleic acid (9-hydroxy-octadec-12-enoic acid), which is well known as a major acyl component of some seed oils.[2-6] In order to identify the unknown fatty acid, we had to obtain direct evidence indicating the positions of a double-bond and a hydroxy group in its aliphatic carbon chain.

In this rapid communication, we first explain our techniques using dimethyl disulfide (DMDS) adducts to determine the double-bond position of *O*-trimethylsilylated (O-TMS) ricinoleic acid methyl ester (authentic standard) and *O*-acetylated (O-Ac) ricinoleic acid methyl ester (authentic standard) as a model experiment, and then we describe applying these techniques to successfully identify this novel isomer. The usefulness of DMDS adducts in fatty acid analysis by gas chromatography/mass spectrometry (GC/MS) is summarized in reviews.[7-10] To our knowledge, however, there are not any papers describing the application of DMDS adducts to analyze monohydroxy monoenoic fatty acids except for our report[11] on 2-hydroxy monoenoic fatty acids of *Arabidopsis thaliana* leaf glucosylceramides.

2 Materials and Methods

2.1 Chemicals

Authentic standard of ricinoleic acid methyl ester (purity 90%) was obtained from Wako Pure Chemical Industries (Osaka, Japan), and rice bran oil was purchased at a local market. All other chemicals were analytical reagent grade, and all the solvents were distilled before use.

2.2 Methylthiolation, trimethylsilylation and acetylation of ricinoleic acid methyl ester

Authentic standard of ricinoleic acid methyl ester (2 mg) was subjected to the I_2-catalyzed reaction[12] with DMDS at 35°C for 3 h. After adding 0.5 mL of hexane with stirring, 0.5 mL of a saturated $Na_2S_2O_3$ aqueous solution was added with vigorous shaking until the color of I_2 disappeared. The mixture stood for a few minutes, and the upper phase containing the resultant DMDS adducts was withdrawn.

One half of the DMDS adducts was dissolved in 0.3 mL of dehydrated pyridine and trimethylsilylated with 0.2 mL of a reagent mixture [trimethylchlorosilane/*N*-trimethylsilylimidazole /*N,O*-bis(trimethylsilyl)trifluoroacetamide, 1:1:1, by volume] under the usual conditions.[13] After standing for 10 min at room temperature, the O-TMS derivative of DMDS adducts of ricinoleic acid methyl ester thus obtained was directly injected into a GC/MS system.

The remaining half of the DMDS adducts (described above) was dissolved in 0.5 mL of a reagent mixture of dehydrated pyridine/acetic acid anhydride (1:2, by volume) and was acetylated at room temperature for 16 h. After adding 8 mL of a saturated NaCl aqueous solution with stirring, the O-Ac derivative of DMDS adducts of ricinoleic acid methyl ester thus obtained was extracted with 3 mL of hexane three times. The combined hexane layers were dried over anhydrous Na_2SO_4, concentrated and injected into a GC/MS system.

2.3 Preparation of monohydroxy monoenoic fatty acid methyl esters from rice bran oil and their derivatizations

Rice bran oil (100 mg) was methanolyzed with 0.5 mol/L KOH methanol and successively methylated with 14% BF_3/methanol.[13] After adding a saturated NaCl solution, the resulting fatty acid methyl esters were extracted with hexane three times. The combined hexane layers were dehydrated and evaporated to dryness. The residue containing

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fatty acid methyl esters was placed on a glass column packed with 8 g of Wakogel C-300 (Wako Pure Chemical Industries, Osaka, Japan) and was developed with a solution of 10% ether/hexane (50 mL) to remove esters having straight carbon chains. The remaining esters having monohydroxy groups on their carbon chains were recovered from the column by passing acetone (50 mL). The recovered fraction containing monohydroxy monoenoic fatty acid methyl esters was evaporated to dryness and was purified by thin layer chromatography[13] with a developing system of hexane/ether/acetic acid (70:30:1, by volume).

The purified fraction containing monohydroxy monoenoic fatty acid methyl esters was methylthiolated to yield DMDS adducts; one half of the amount of the adducts was trimethylsilylated, and the remaining half was acetylated in the manner described in Section 2.2.

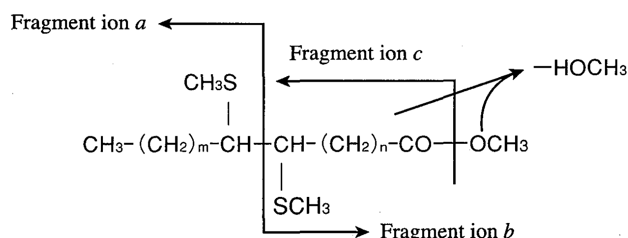
2.4 Capillary GC/MS

The O-TMS derivative of and the O-Ac derivative of the DMDS adducts were independently analyzed on a DB-5ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Agilent Technologies, Baloalt, CA) linked to a Shimadzu GCMS QP2010 mass spectrometer with a computer on-line system. The column temperature was programmed at 180°C for 2 min isothermally, then raised to 300°C at a rate of 3°C/min and held at 300°C for 3 min. The carrier gas was helium at a linear gas velocity of 35.0 cm/s. Electron impact mass spectra were measured by scanning from 70 to 520 *m/z* (0.5 s/cycle) at an ionizing energy at 70 eV.

3 Results and Discussion

3.1 Fragmentation of DMDS adducts

Electron impact ionization of DMDS adducts of monoenoic fatty acid methyl ester (having a straight carbon chain) always gives the recognizable molecular ion and the set of key fragment ions *a*, *b* and *c* derived from fragmentation (as illustrated in Scheme 1). Key fragment ions *a* and *b* come from the cleavage between the methylthio-substituted carbons; key fragment ion *c* is yielded due to the loss of methanol from key fragment ion *b*.[14] Consequently, the original double-bond position in the starting molecule can be confirmed easily by using these representative key fragment ions.



Scheme 1 Fragmentation pattern of dimethyl disulfide adducts derived from monoenoic fatty acid methyl ester (having a straight carbon chain).

When a hydroxy group, however, is at the carbon chain of a monoenoic ester, as it is in ricinoleic ester, the mass spectrum of the DMDS adducts can not be used for structural elucidation. Blocking the hydroxy group (by techniques such as O-TMS and O-Ac coverings) is first necessary before electron impact ionization analysis of DMDS adducts can be done.

3.2 Mass spectra of the O-TMS derivative of and the O-Ac derivative of DMDS adducts of ricinoleic acid methyl ester

Fig. 1A shows the mass spectrum of the O-TMS derivative of DMDS adducts of ricinoleic acid methyl ester (authentic standard). The fragment ions at *m/z* 217 and *m/z* 185 corresponded to the key fragment ions *b* and *c* (cf. Scheme 1), suggesting that the original double-bond was at the Δ-9 position in the C18 chain. There was no fragment ion at *m/z* 261 corresponding to the key fragment ion *a* (cf. Scheme 1). The base peak ion at *m/z* 187 was derived from the α-cleavage[1,15,16] at the trimethylsilyl ether group. This indicated that the position of the hydroxy group was at the C12.

Fig. 1B shows the mass spectrum of the O-Ac derivative of DMDS adducts of ricinoleic acid methyl ester (authentic standard). The relative intensities of the key fragment ions b at *m/z* 217 and *c* at *m/z* 185 were higher than those in the mass spectrum of the O-TMS derivative (Fig. 1A). In addition, the relative intensity of the molecular ion at *m/z* 448 was higher than the molecular ion at *m/z* 478 (Fig. 1A). The base peak ion at *m/z* 171 could be attributed to the loss of acetic acid from the key fragment ion *a* (at *m/z* 231). The presence of these key fragment ions *b* and *c* and of the base peak ion clearly indicated that the original double-bond was at the Δ-9 position in the C18 chain. Mass spectrum B (Fig. 1B) shows no information on the position of the hydroxy group.

On the basis of these mass spectral elucidations, we could easily determine the positions of the double-bond and the hydroxy group at the aliphatic carbon chain of monohydroxy monoenoic fatty acid.

3.3 10-Hydroxy-octadec-12-enoic acid found in rice bran oil

In our previous communication,[1] we found ricinoleic acid in seven kinds of common vegetable oils and in four kinds of common oil seeds. During our continuing investigation, we obtained gas chromatograms showing several minor peaks which eluted near the peak corresponding to ricinoleic acid methyl ester-O-TMS. Because the number of these minor peaks on those gas

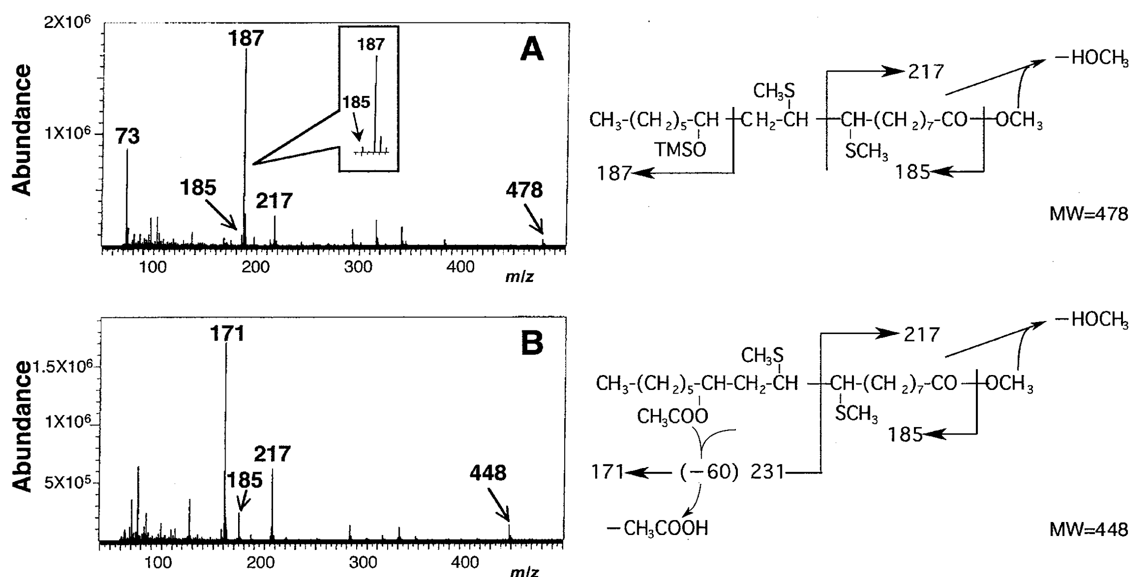


Fig. 1 Mass spectra of dimethyl disulfide adducts of ricinoleic acid methyl ester (authentic standard): A, *O*-trimethylsilylated derivative; B, *O*-acetylated derivative.
Mass spectral assignments are given in the text.

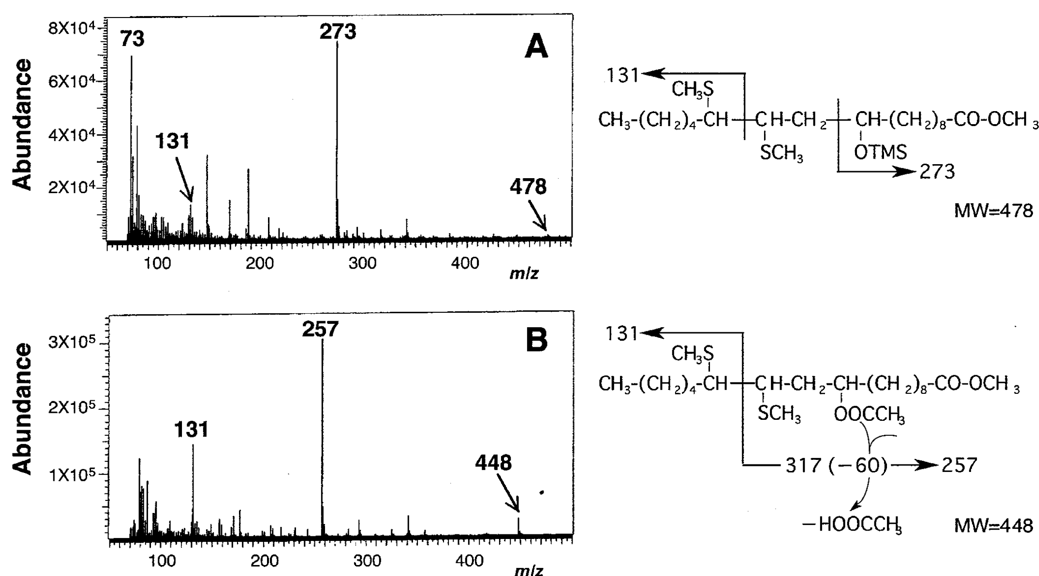


Fig. 2 Mass spectra of dimethyl disulfide adducts of the methyl ester (prepared from rice bran oil): A, *O*-trimethylsilylated derivative; B, *O*-acetylated derivative.
Mass spectral assignments are given in the text.

chromatograms was especially high in rice bran oil, we prepared a fraction rich in monohydroxy monoenoic fatty acid methyl esters from this oil. Then we analyzed the esters by GC/MS in the manner described in Section 3.2.

Fig. 2A shows the mass spectrum of the O-TMS derivative of DMDS adducts of an ester. The base peak ion at *m/z* 273 was derived from the α-cleavage[1,15,16] at the trimethylsilyl ether group. This indicated that the position of the hydroxy group was at the C10. Since the fragment ion at *m/z* 131 corresponded to the key fragment ion *a* (cf. Scheme 1), we could speculate that the original double-bond was at the Δ-12 position in the C18 chain.

In mass spectrum B (Fig. 2B) of the O-Ac derivative of DMDS adducts of the ester, the relative intensities of the key fragment ion *a* at *m/z* 131 was higher than that in the mass spectrum of the O-TMS derivative (Fig. 2A). In addition, the relative intensity of the molecular ion at *m/z* 448 was higher than the molecular ion at *m/z* 478 (Fig. 2A). Though the key fragment ions *b* at *m/z* 317 and c at *m/z* 285 were not clearly observed, the base peak ion at *m/z* 257 could be

attributed to the loss of acetic acid from the key fragment ion *b* (at *m/z* 317). This strongly suggested that the original double-bond was at the Δ-12 position in the C18 chain.

From these results, we could conclude that the starting molecule was 10-hydroxy-octadec-12-enoic acid. To our knowledge, this fatty acid, one of the isomers of isoricinoleic acid and also of ricinoleic acid, is a novel fatty acid found in natural lipids.

In addition, from common vegetable oils we obtained preliminary experimental data (not shown) indicating the possibility of the presence of a series of unknown mono- and dihydoxy mono- and dienoic fatty acids. The amounts of these hydroxy fatty acids were very low, just as the amount of 10-hydroxy-octadec-12-enoic acid in rice bran oil was very low [being about half of the amount of ricinoleic acid found in rice bran oil (ca. 200 ppm)[1]]. We should, however, consider the possible effects of these acids on human nutrition since common vegetable oils are a major part of the human diet.

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