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DETERMINATION OF ISOTOPIC PURITY AND POSITION IN DEUTERATED METHYL BEHENATE

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SUMMARY

In the course of preparing peanut oil type triglycerides for metabolism studies, methyl esters of deuterated behenic acid, a saturated 22-carbon fatty acid, with zero, two, and six deuterium atoms were synthesized. The specific positions of deuterium atoms and the completion of deuteration in the products were characterized by gas chromatography/mass spectrometry methods.

Key words: behenic acid, gas chromatography/mass spectrometry, deuterium, isotopic purity

INTRODUCTION

In the course of preparing peanut oil type triglycerides for metabolism studies, deuterated behenic acid, a saturated twenty-two carbon fatty acid, with zero, two, and six deuterium atoms were synthesized using a combination of catalytic deuteration (1) and deuterium exchange (2,3). This report describes mass spectrometric characterization of the specific positions of deuterium atoms and the completion of deuteration in the products.

EXPERIMENTAL

A Finnigan 4000 GC/MS system was used for compound identification, and the determination of deuterium position and the yield of deuteration product. The ion source of the mass spectrometer was maintained at 200 °C.

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The analyzer was scanned from m/z 33 to 450 (1 sec/cycle) for compound identification and deuterium position determination, and scanned from m/z 351 to 365 for the quantification of deuteration product yields. A 40 m SE-54 fused silica capillary column (0.22 mm ID) was used with helium (linear velocity 0.36 m/sec, inlet pressure 8 psi) as the carrier gas and temperature programmed from 100 °C to 250 °C in 10 °C/min. The injector and the interface were maintained at 275 °C.

RESULT AND DISCUSSION

Mass spectra of methyl behenate, methyl behenate-12,14-d_2 and methyl behenate-12,12,13,14,15,15-d_6 are shown in Fig. 1(A), 1(B) and 1(C), respectively. The masses of the $[(CH_2)_nCOOCH_3]^+$ ions (with

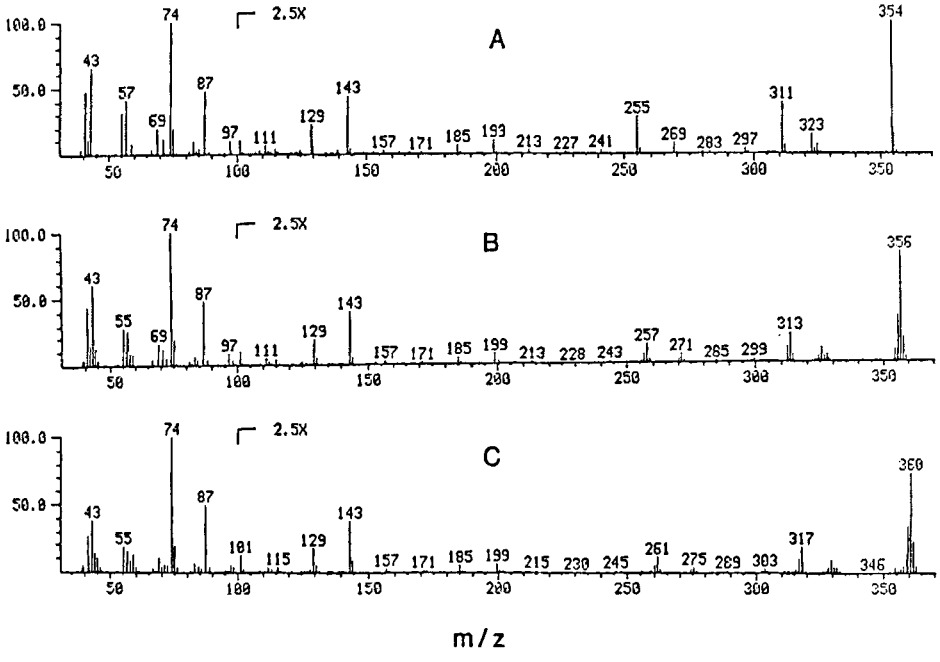

Figure 1. Mass spectra of methyl behenate, methyl behenate-13,14-d_2, and methyl behenate-12,12,13,14,15,15-d_6.

$n \geq 14$) and the molecular ions are indicative of the number of deuterium atoms in these compounds, while the masses of the characteristic

$[(CH_2)_nCOOCH_3]^+$ ion series (with n between 10 and 14) are used for the assignments of deuterium positions as shown in Table 1. For example,

Table 1. Characteristic ions derived from $[(CH_2)_nCOOCH_3]^+$ and assignments of deuterium positions in methyl behenate

Compound & Spectrum	Molecular ion	n	Characteristic ion (m/z)	Additional no. of ^2H	Deuterium position
		10	199	0	-
		11	213	0	-
$-d_0$ 1(A)	354	12	227	0	-
		13	241	0	-
		14	255	0	-
		10	199	0	-
		11	213	0	-
$-d_2$ 1(B)	356	12	228	1	C-13
		13	243	1	C-14
		14	257	0	-
		10	199	0	-
		11	215	2	C-12
$-d_6$ 1(C)	360	12	230	1	C-13
		13	245	1	C-14
		14	261	2	C-15

the masses for $[(CH_2)_{10}COOCH_3]^+$ ions in 1(A) and 1(C) are both 199, while the masses for $[(CH_2)_{11}COOCH_3]^+$ ions are 213 and 215, respectively, indicating that the two deuterium atoms in the d_6- behenate are on the C-12 position. Similarly, one additional deuterium atom is assigned to the C-13 position for the d_6- behenate since the mass for the $[(CH_2)_{12}COOCH_3]^+$ ion in spectrum 1(C) is further increased by one unit. The same process is used to complete the information shown in Table 1. These results comfortably confirm the number and positions of deuterium atoms in these compounds. Since these series of ions may also be generated (4) through the elimination of part of the $(CH_2)_n$ chain and an additional hydrogen atom, minor amounts of other ions are also observed.

d_0-, d_2- and d_6-Methyl behenates are actually separated under the gas chromatographic conditions used in this study. d_6- Elutes about 8 seconds before d_2- and 10 seconds before the d_0-counterparts. Total ion (m/z 351-

365) and single ion (m/z 354 and 360) displays in Fig. 2 show the resolution of the d_0- and d_6- derivatives of the behenic acid synthesis product.

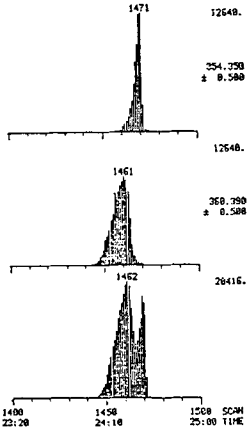

Figure 2. Reconstructed total ion (m/z 351 - 365) and single ion (m/z 354 and 360) chromatograms obtained from the methyl behenate-12,12,13,14,15,15-d_6 synthesis product.

To further determine the respective yields of the $-d_2$ and $-d_6$ products. Ion intensities of m/z 354 and 356; and 354, 356, and 360 for these two synthetic products were integrated at the retention times of the $-d_0$, $-d_2$, and $-d_6$ compounds. The resulting integration areas indicate that the methyl behenate-d_2 yield is about 95%, while the methyl behenate-d_6 yield is about 63%.

Results presented here indicate the feasibility in making deuterium atom position assignments in methyl esters of long-chain fatty acid using a series of ions generated under electron impact conditions. Selected ion monitoring of representative ions also provide quantitative information for the determination of deuteration yields.

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