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Gas chromatography–mass spectrometry of the picolinyl ester derivatives of deuterated acetylenic fatty acids

M. S. F. Lie Ken Jie and Y. C. Choi

Department of Chemistry, University of Hong Kong, Pokfulam Road, Hong Kong (Hong Kong)

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ABSTRACT

Deuteration (with Wilkinson's catalyst) of methyl 12:1(3*a*), 14:1(5*a*), 15:1(6*a*), 16:1(7*a*), 18:1(9*a*), 18:2(9*a*,11*a*), 17:2(10*a*,12*a*) and 18:2(5*a*,10*c*) gave the corresponding deuterium-labelled saturated fatty esters. The mass spectral analysis of their picolinyl esters gave predictable diagnostic ion fragments for most isomers. In the case of 12:1(3*a*) and 14:1(5*a*) isomers, hydrogen–deuterium scrambling occurred during electron impact to give unique fragmentation patterns. This method allows the accurate determination of the positions of the unsaturated centres in the alkyl chain of the fatty esters.

INTRODUCTION

Acetylenic bonds in fatty acids have been located by mass spectrometry following conversion into keto groups by oxymercuration and demercuration [1,2], or more simply as the pyrrolidides [3] or 4,4-dimethyloxazoline derivatives [4]. Christie *et al.* [5] have studied the mass spectra of the picolinyl ester derivatives of a series of positional isomers of dimethylene-interrupted octadecadiynoic acids. This study shows that the triple bond more remote from the carboxyl group is identifiable because of the presence of diagnostic ions, but the proximal triple bond is not. Mass spectral study of the picolinyl esters of a series of positional isomers of conjugated diacetylenic acids has also been performed [6].

We have recently reported the single and accurate determination of the positions of the double bonds (up to six) in the alkyl chain of fatty esters, by examining the mass spectra of the picolinyl esters of the corresponding deuterated fatty acids [7]. As heterogenous catalytic deuteration causes extensive hydrogen–deuterium scrambling during the reduction process [8], palladium and platinum catalysts cannot be used to "add" deuterium across unsaturated centres. Homogeneous catalysts, such as Wilkinson's catalyst [$(Ph_3P)_3RhCl(I)$], permit deuteration of unsaturated fatty acids to take place without scrambling [9,10].

This paper describes the gas chromatography–mass spectrometry (GC–MS) of picolinyl esters of deuterated 12:1(3*a*), 14:1(5*a*), 15:1(6*a*), 16:1(7*a*), 18:1(9*a*), 18:2(9*a*,11*a*), 17:2(10*a*,12*a*) and 18:2 (5*a*,10*c*) acids.

EXPERIMENTAL

Wilkinsons's catalyst [$(Ph_3P)_3RhCl(I)$] was purchased from Strem Chemical (Newburyport, MA, USA). Deuterium was obtained from Alpha Products (Ward Hill, MA, USA). The mono-acetylenic fatty acids were synthesized by known methods [11], and the conjugated diacetylenic acids [6] and enynoic acid [12] were prepared as described elsewhere. Deuteration of the unsaturated methyl esters was performed according to the procedure described by Rakoff and Emken [13], and the deuterium-labelled saturated methyl esters were trans-

Correspondence to: Dr. M. S. F. Lie Ken Jie, Department of Chemistry, University of Hong Kong, Pokfulam Road, Hong Kong, Hong Kong. Telefax: (852) 5170217.

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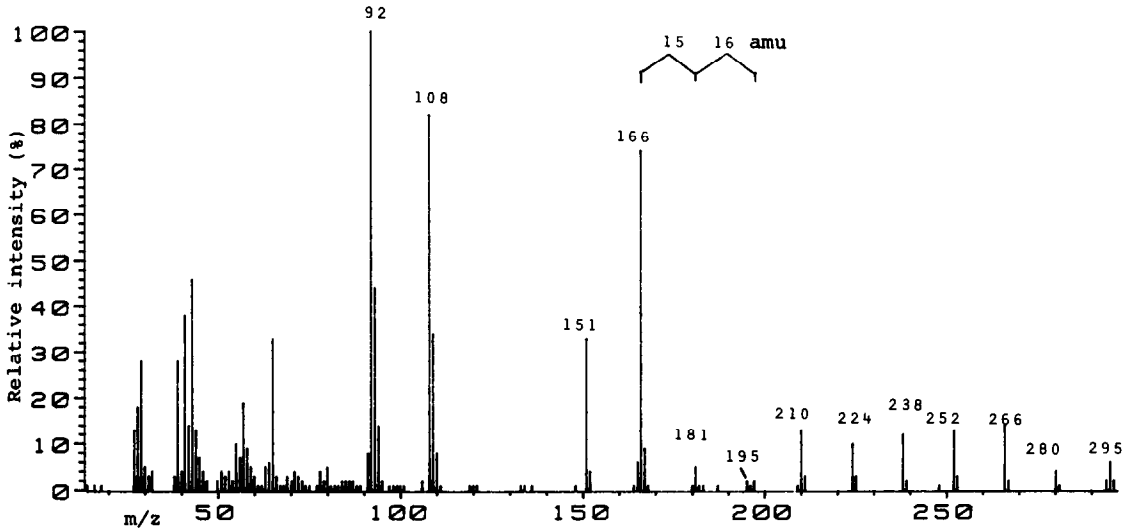

Fig. 1. Mass spectrum of deuteriated 12:1(3*a*).

formed into the picolinyl esters as described by Christie and Stefanov [14].

The picolinyl esters were submitted to GC–MS by means of on-column injection into a Hewlett Packard GC (Model HP5970). The GC column was a fused-silica capillary (12 m × 0.2 mm I.D., 0.33 μm film thickness) coated with cross-linked methyl silicone gum, Ultra 1. Helium was used as the carrier gas. The column oven temperature was 190°C. The outlet of the column was connected directly to the source of a Hewlett Packard mass selective detector, operated at an ionization energy of 70 eV (Hewlett-Packard Asia, Hong Kong, Hong Kong).

RESULTS AND DISCUSSION

The mass spectra of the picolinyl esters of deuteriated 12:1(3*a*), 14:1(5*a*), 15:1(6*a*), 16:1(7*a*) and 18:1 (9*a*) acids are shown in Figs. 1–5. In all cases the molecular ion (M^+) indicated the addition of two

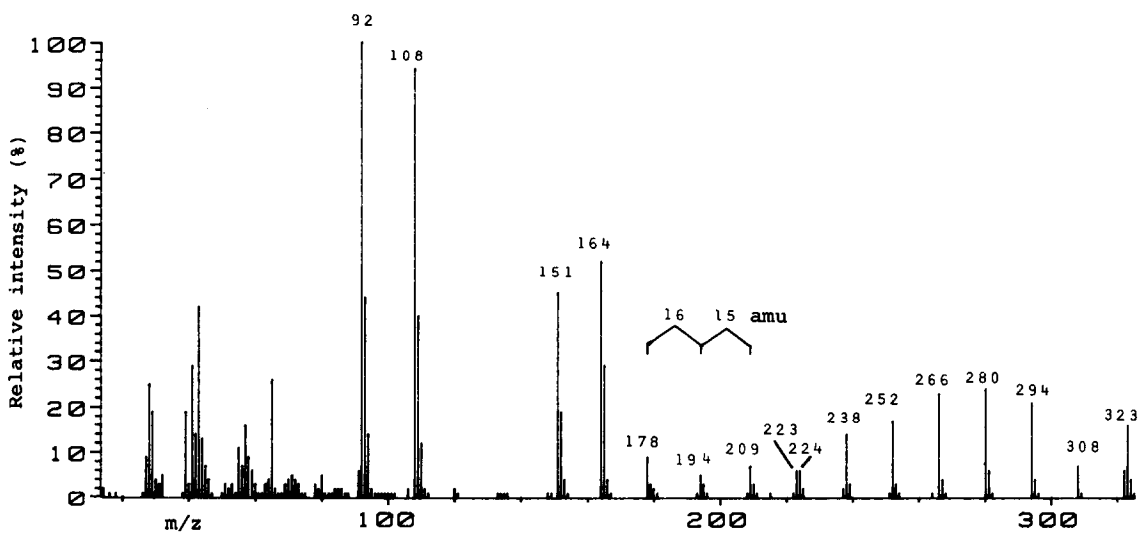

Fig. 2. Mass spectrum of deuteriated 14:1(5*a*).

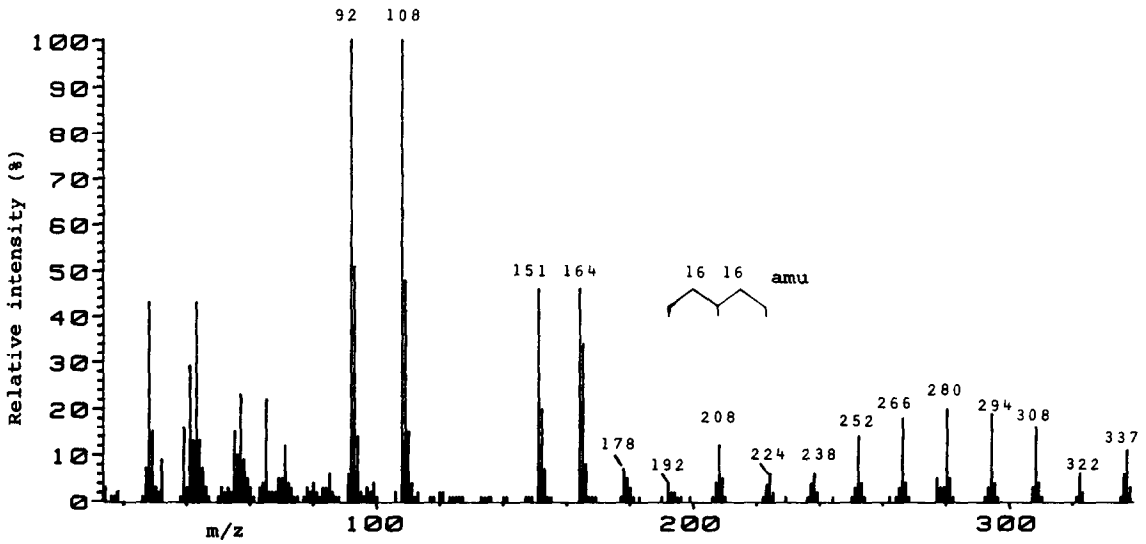

Fig. 3. Mass spectrum of deuteriated 15:1(6*a*).

molar equivalents of deuterium to the unsaturated centre of the mono-acetylenic substrate. Ion fragments of *m/z* greater than 200 were generally of medium intensity (10–25% relative intensity), except for the M − 15 ion fragment (*ca.* 5%). Ion fragments of *m/z* between 170–200 were low in relative abundance (5–10%). The picolinyl moiety was readily characterized by the appearance of very intense peaks at *m/z* 92, 108, 151 and 164. However, in the spectrum of the deuterated 12:1(3*a*) isomer, the peak at *m/z* 164 was replaced by a very intense peak at *m/z* 166 (72%), which indicated that the C-3 methylene carbon of the alkyl chain carried two deuterium atoms (Fig. 1). Cleavage of the C-4/C-5 bond gave an unexpected peak at *m/z* 181 instead of 182, implying that there was an exchange of one of

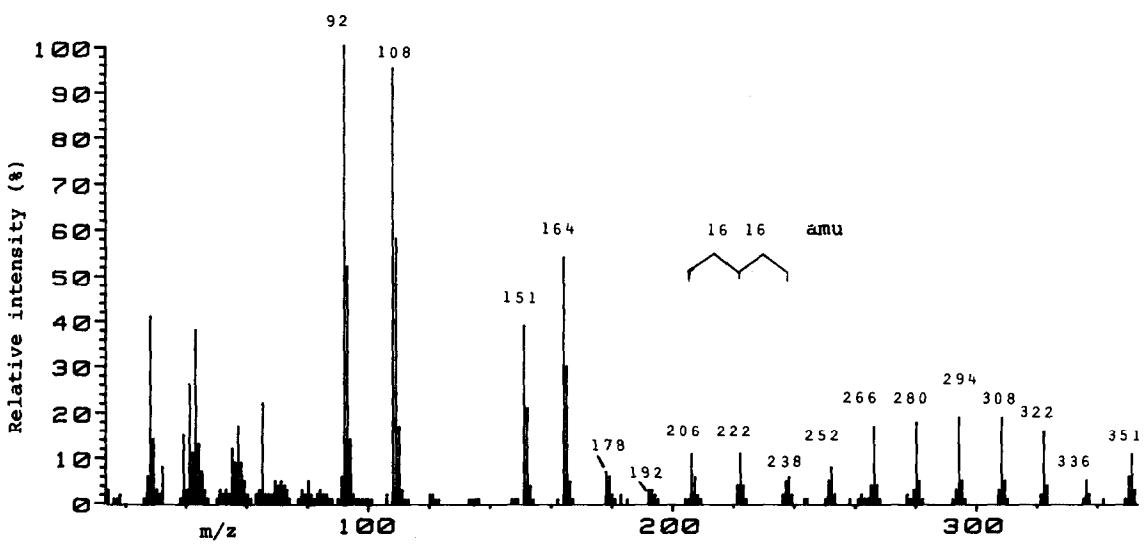

Fig. 4. Mass spectrum of deuteriated 16:1(7*a*).

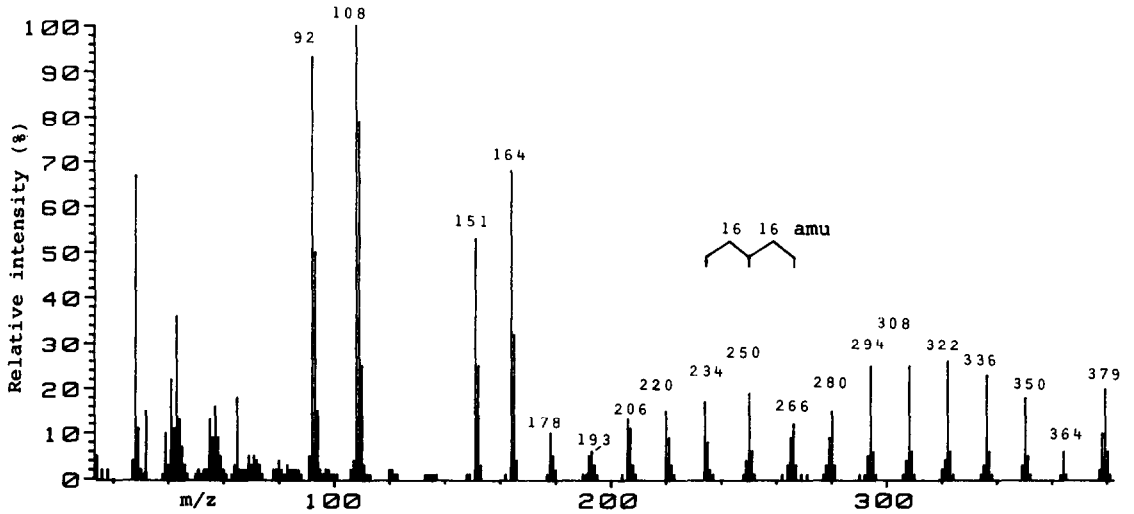

Fig. 5. Mass spectrum of deuteriated 18:1(9*a*).

the two deuterium atoms for a hydrogen at the C-4 carbon atom. Cleavage at the C-5/C-6 bond resulted in a cluster of three peaks of very low intensity at *m/z* 195 (2%), 196 (1%) and 197 (2%). This phenomenon was indicative of further hydrogen–deuterium scrambling. However, these hydrogen–deuterium exchanges appeared to be limited to the C-4 and C-5 carbon atoms of the alkyl chain of this isomer, as the cleavage of the C-6/C-7 bond gave the expected single ion fragment of *m/z* 210. The remaining peaks of the spectrum were spaced 14 amu apart until reaching the terminal methyl group, where the M − 15 peak appeared. From these results, it can be concluded that isomers containing a triple bond at the C-3/C-4 position are characterized by peaks at *m/z* 166 and 181 in their mass spectra.

In the analysis of the deuterated 14:1(5*a*) isomer (Fig. 2), cleavage of the C-5/C-6 bond gave a characteristic peak at *m/z* 194 (5%), which was 16 amu apart from the peak at *m/z* 178 (9%). This ion fragment showed that the C-5 carbon atom contained two deuterium atoms. However, scrambling involving one of the two deuterium atoms at C-6 resulted in two sets of low intensity twin peaks (1 amu apart) at *m/z* 209 (7%), 210 (3%) and 223 (6%), 224 (6%). The remaining ion fragments were 14 amu apart except for the M − 15 peak. From these two positional isomers, it was evident that hydrogen–deuterium scrambling occurred only when C–C bond cleavage took place at the α- and β-positions from the site containing the deuterium atoms.

In the spectrum of the deuteriated 15:1(6*a*) isomer (Fig. 3), there were two distinct 16-amu gaps between the peaks at *m/z* 192, 208 and 224, which allowed the position of the triple bond to be fixed at C-6/C-7 of the alkyl chain. A simple method of calculating the position of the triple bond from the values obtained in the mass spectrum is to substract 164 from the *m/z* value of the middle peak (the peak flanked by the two 16-amu gaps), divide the remainder by 14 and add 3 to the quotient. The whole number obtained corresponds to the first carbon atom of the triple bond in the alkyl chain. Thus in the case of the deuterated 15:1 isomer, the position of the triple bond is calculated as follows: [(208 − 164):14 + 3 = 6.14]. This result locates the triple bond at the C-6/C-7 position of the alkyl chain.

In the spectrum of the picolinyl ester of the deuterated 16:1(7*a*) isomer (Fig. 4), the 16-amu gaps appeared at *m/z* 206, 222 and 238. The position of the triple bond was located between the C-7 and C-8 atoms of the alkyl chain. The 16-amu gaps in the deuterated 18:1 isomer (Fig. 5) were found at *m/z* 234, 250 and 266, which confirmed the structure of 18:1(9*a*). From the spectra of the last three isomers [15:1(6*a*), 16:1(7*a*) and 18:1(9*a*)], it was evident that

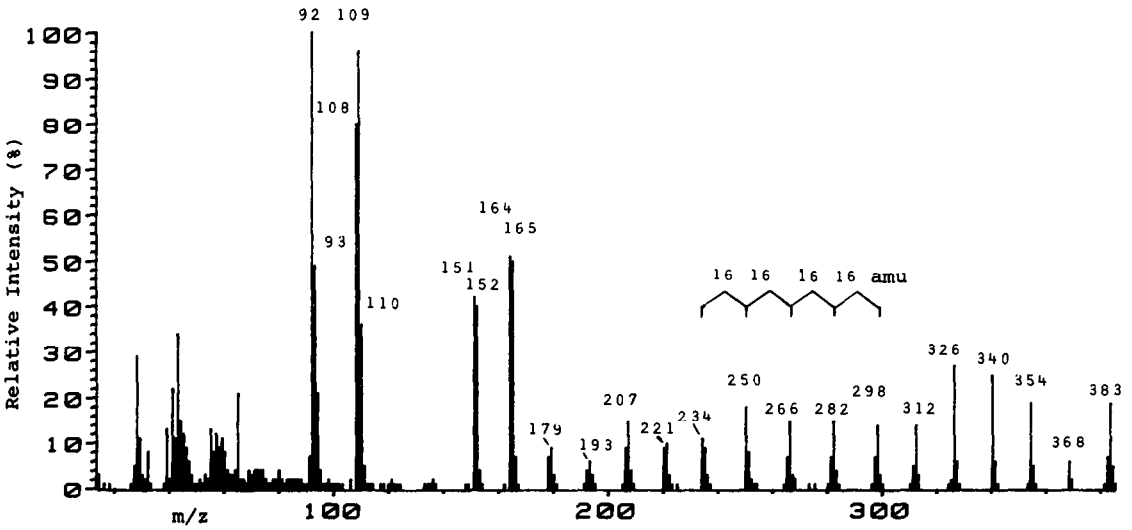

Fig. 6. Mass spectrum of deuteriated 18:2(9*a*,11*a*).

isomers with triple bonds located at the C-6 atom or at positions more distant than C-6 from the ester function produce distinct and diagnostic ion fragmentation patterns, which allow the accurate location of the triple bonds. There was no sign of significant hydrogen–deuterium scrambling during mass spectral analysis of these positional isomers.

In the mass spectrum of the picolinyl ester derivative of the 18:2(9*a*,11*a*) isomer, four distinct and consecutive 16-amu gaps at *m/z* 234, 250, 266, 282 and 298 were observed (Fig. 6). The positions of the triple bonds were therefore located between the C-9 and C-10 and the C-11 and C-12 atoms of the alkyl chain. In the case of the 17:2(10*a*,12*a*) isomer, a similar set of four consecutive 16-amu gaps at *m/z* 248, 264, 280, 296 and 312 were found (Fig. 7). From this fragmentation pattern, the locations of the triple bonds were in full agreement with the structure of 17:2(10*a*,12*a*). No hydrogen–deuterium scrambling was observed during the analysis of these isomers.

We extended this study to a mixed diunsaturated

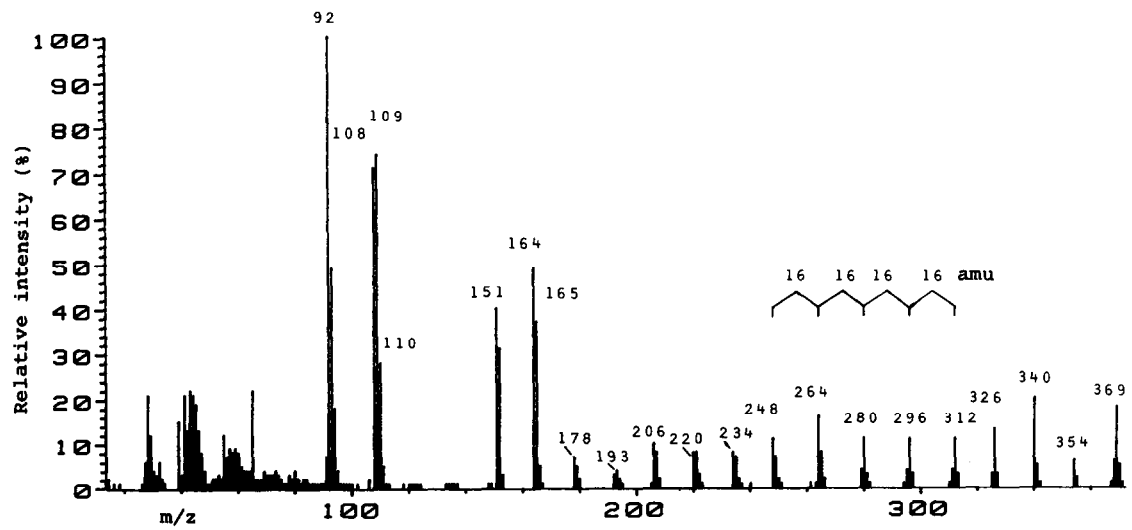

Fig. 7. Mass spectrum of deuteriated 17:2(10*a*,12*a*).

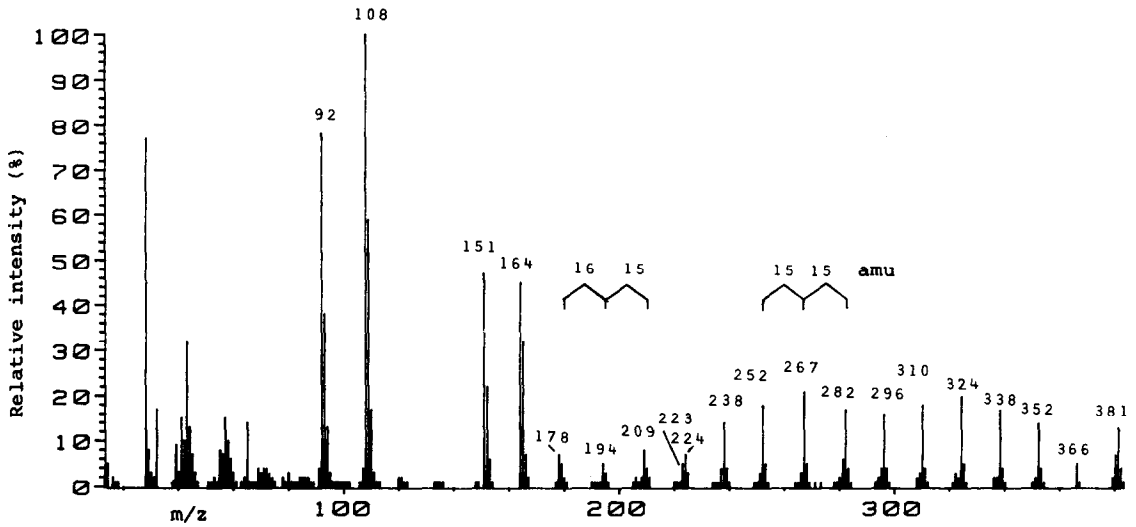

Fig. 8. Mass spectrum of deuteriated 18:2(5*a*,10*c*).

fatty acid, 18:2(5*a*,10*c*). Analysis of the corresponding picolinyl ester of the deuterated substrate gave a set of low intensity peaks at *m/z* 178 (8%), 194 (6%), 209 (9%), 210 (4%), 223 (6%) and 224 (7%) (Fig. 8). This part of the spectrum matched the characteristic pattern observed for the 14:1(5*a*) homologue (Fig. 2). From this result, the position of the triple bond was located at C-5/C-6 of the alkyl chain. In the same spectrum, there were two 15-amu gaps at *m/z* 252, 267 and 282, which confirmed the position of the double bond at C-10/C-11 of the alkyl chain.

This study demonstrates that mass spectral analysis of picolinyl esters of deuterated unsaturated fatty acids gives a predictable fragmentation pattern, which provides a simple and accurate means of locating the positions of triple bonds. Isomers with unsaturated centres in the proximal part in the alkyl chain from the ester function furnish unique mass spectral fragmentation patterns of their own, owing to hydrogen–deuterium scrambling.

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