Mass Spectral Studies of Deuterium-Labelled Picolinyl Fatty Esters in the Determination of Double-Bond Positions

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Deuteration (with Wilkinson's catalyst) of methyl 12:1(3*c*), 14:1(5*c*), 18:1(9*c*), 18:2(9*c*,12*c*), 18:3(9*c*,12*c*,15*c*), 20:4(5*c*,11*c*,14*c*, 17*c*), 20:5(5*c*,8*c*,11*c*,14*c*,17*c*) and 22:6(4*c*,7*c*,10*c*,13*c*,16*c*,19*c*) fatty acids gave the corresponding deuterium-labelled saturated fatty esters. The mass spectral analysis of their picolinyl esters gave clear diagnostic ion fragments, which permitted the facile and accurate determination of the positions of the double bonds (up to six) in the alkyl chain of the fatty ester.

KEY WORDS: Deuterium-labelled fatty esters, double bond position, mass spectra, picolinyl esters, Wilkinson's catalyst.

Schmitz and Klein (1) have described many methods for determining the positions of double bonds in fatty acids and other unsaturated organic compounds by means of mass spectrometry. Two commonly applied methods involve esterification of unsaturated fatty acids to the pyrrolidide (2) or picolinyl esters (3). Harvey has recently reviewed the mass spectrometry of picolinyl and other nitrogen-containing derivatives of lipids (4). Picolinyl esters have been used extensively for the determination of the position of double bonds in natural and in synthetic long-chain fatty acid molecules (5–9).

Anderson *et al.* (10–12) have studied the mass spectrometric fragmentation pattern of pyrrolidide esters of deuterated unsaturated fatty acids and have described the location of the positions of the double bonds in monoenes, dienes and trienes using this approach. However, Kawaguchi *et al.* (13) have noted that pyrrolidide esters of deuterated fatty acids containing four or five double bonds give less satisfactory results. Klein and Schmitz (14) have examined the isotopic effects during the deuteration of polyunsaturated fatty acids and within the mass spectrometer.

One of the main drawbacks of the mass spectra of pyrrolidide esters of fatty acids is that the diagnostic ions are often weak in intensity (1–3% relative intensity). Picolinyl derivatives of fatty acids exhibit diagnostic ions of higher intensities (5–40% relative intensity) than pyrrolidide esters do. As a result, there has been an inclination to use picolinyl esters of unsaturated fatty acids for mass spectrometric analysis. In a comparative study of pyrrolidide and picolinyl esters for the structural identification of fatty acids by mass spectrometry, Christie *et al.* (15) envisaged that picolinyl esters of deuterated fatty acids may be preferred to pyrrolidide esters to permit a facile identification of double-bond positions in the fatty acid molecule. However, no report on picolinyl esters of deuterated fatty acids has been published yet.

Because heterogeneous catalytic deuteration causes extensive hydrogen-deuterium scrambling during the reduction process (16), palladium and platinum catalysts cannot be used to "add" deuterium across the unsaturated centers. Homogeneous catalysts, such as Wilkinson's catalyst [$(Ph_3P)_3RhCl(I)$], permit deuteration of unsaturated fatty acids to take place without scrambling (16–20).

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This paper describes the superior results obtained in the analysis of the picolinyl esters of deuterated polyunsaturated (1–6 double bonds) fatty acids.

MATERIALS AND METHODS

Wilkinson's catalyst [$(Ph_3P)_3RhCl(I)$] was purchased from Strem Chemical Co. (Newburyport, MA). Deuterium was obtained from Alpha Products (Ward Hill, MA). Monounsaturated fatty acids [12:1(3*c*) and 14:1(5*c*)] were synthesized by known methods (21); methyl oleate [18:1(9*c*)], linoleate [18:2(9*c*,12*c*)] and linolenate [18:3(9*c*,12*c*,15*c*)] were obtained from Sigma Chemical Co. (St. Louis, Mo). Methyl all *cis*-5,11,14-eicosatrienoate [20:3(5*c*,11*c*,14*c*)] and all *cis*-5,11,14,17-eicosatetraenoate [20:4(5*c*,11*c*,14*c*,17*c*)] were isolated from *Biota orientalis* seed oil (7), and methyl all *cis*-5,8,11,14,17-eicosapentaenoate [20:5(5*c*,8*c*,11*c*,14*c*,17*c*)] and methyl all *cis*-4,7,10,13,16,19-docosahexaenoate [22:6(4*c*,7*c*,10*c*,13*c*,16*c*,19*c*)] were isolated from cod liver oil (22).

General method for the deuteration of unsaturated methyl fatty esters (23). A 50-mL pear-shaped flask containing sodium-dried benzene (1 mL) was evacuated and filled with dried nitrogen. The solvent was deoxygenated by stirring vigorously for 2 min. The process was repeated thrice with nitrogen and twice with deuterium. Wilkinson's catalyst (3 mg) and unsaturated methyl ester (25 mg) were added, and the flask was flushed twice with deuterium. The reaction mixture was stirred at room temperature for 8 h. The color of the reaction mixture turned from red to yellow during the first hour, and after six hours of stirring, the color of the reaction reverted to a reddish-brown hue. The solvent was evaporated under reduced pressure. Diethyl ether (20 mL) was added to the residue, and the ethereal solution was percolated through a silica (5 g) gel column. The solvent was evaporated and saturated deuterium-labelled methyl ester was obtained (25 mg).

Picolinyl esters. The deuterated methyl fatty esters were hydrolyzed and transformed to the corresponding picolinyl esters, as described previously (6).

Gas-liquid chromatography-mass spectrometry (GLC-MS). Mass spectra of picolinyl esters were obtained by means of on-column injection into a Hewlett Packard GC (Model HP5890). The GLC column used was a fused silica capillary column (12 m × 0.2 mm, 0.33 μm film thickness) coated with cross-linked methyl silicone gum (Ultra 1, Hewlett Packard, Palo Alto, CA). Helium was the carrier gas. The column oven temperature was 190°C. The outlet of the column was connected directly into the source of a Hewlett-Packard 5970 Mass Selective Detector, operated at an ionization energy of 70 eV (Hewlett-Packard Asia Ltd., Hong Kong).

RESULTS AND DISCUSSION

The mass spectra of the picolinyl esters of deuterated 12:1(3*c*), 14:1(5*c*) and 18:1(9*c*) are shown in Figures 1–3, respectively. The *m/z* value of the molecular ion (M+) in

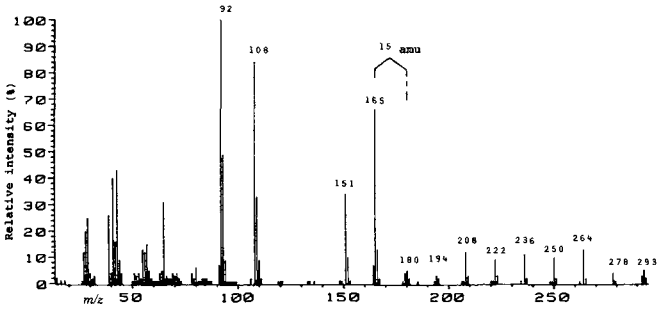

FIG. 1. Mass spectrum of deuterated 12:1(3*c*).

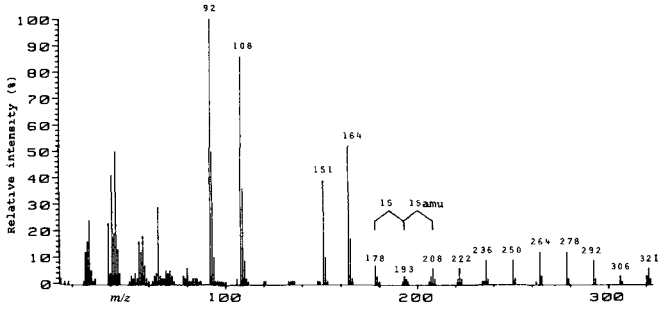

FIG. 2. Mass spectrum of deuterated 14:1(5*c*).

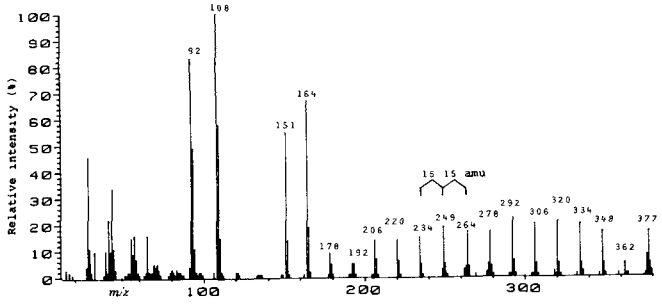

FIG. 3. Mass spectrum of deuterated 18:1(9*c*).

each case indicated the addition of one molar equivalent of deuterium to the unsaturated center of the monoene substrate. Ion fragments of *m/z* value greater than 200 were normally of medium intensities (10–20% relative intensity), except for the M—15 ion fragment (*ca.* 5% relative intensity). However, ion fragments of *m/z* between 170 and 200 were generally low in relative abundance (5–10% relative intensity). The picolinyl moiety was readily characterized by the intense peaks at *m/z* = 92, 108, 151 and 164. For the deuterated 12:1(3*c*) isomer, the peak at *m/z* 164 was replaced by an intense peak at *m/z* 165 (66% relative intensity), which implied that one of the two deuterium atoms occupied the C-3 carbon atom of the alkyl chain (Fig. 1). The adjacent 15 amu gap was not distinct, as two higher mass peaks (*m/z* 179 and 180) appeared with almost equal intensities (4 and 5% relative intensity, respectively). The remaining peaks of the spectrum, however, were spaced at 14 amu apart until reaching the terminal methyl group where the M—15 ion was found. From these results it could be concluded that the position of the double bond in the original 12:1 fatty acid was located between the C-3 and C-4 carbon atoms of the alkyl chain, and characterized by the peak at *m/z* 165.

There were two distinct gaps of 15 amu at *m/z* 178, 193 and 208 in the spectrum of the deuterated 14:1 isomer (Fig. 2). The remaining peaks were all 14 amu apart except for the M—15 ion. From the peaks with the 15 amu gaps, the position of the double bond in the original unsaturated fatty acid, 14:1, could be determined to occupy the C-5/C-6 position of the alkyl chain. A simple means of determining the position of the double bond from the values obtained in the mass spectrum was to subtract 164 from the *m/z* value of the middle peak (*i.e.*, peak flanked by the two 15 amu gaps), divide the remainder by 14 and add 3 to the quotient. The whole number obtained constituted the position of the first carbon atom of the double bond in the alkyl chain. Thus, in the case of the deuterated 14:1 isomer, the double bond was located between the C-5/C-6 carbon atoms [(193—164) ÷ 14 + 3 = 5.07] of the alkyl chain. The 15 amu gaps in 18:1 (Fig. 3) were at *m/z* 234, 249 and 264. The position of the double bond was therefore located between the C-9/C-10 carbon atoms of the alkyl chain.

The spectrum of the picolinyl ester of deuterated linoleate is presented in Figure 4. There were four distinct 15 amu gaps at *m/z* (234, 249, 264) and (278, 293, 308). The positions of the double bonds in picolinyl linoleate could be readily determined from the values at 249 and 293, which placed the double bonds between the C-9/C-10 and C-12/C-13 carbon atoms of the alkyl chain.

The mass spectra of the picolinyl ester of the deuterated 18:3(9*c*,12*c*,15*c*), 20:4(5*c*,11*c*,14*c*,17*c*), 20:5(5*c*,8*c*,11*c*,14*c*,17*c*) and 22:6(4*c*,7*c*,10*c*,13*c*,16*c*,19*c*) are presented in Figures 5–8, respectively. The 15 amu gaps in the spectrum of 18:3 (Fig. 5) were at *m/z* (234, 249, 264), (278, 293, 308) and (322, 337, 352). From these results, it can be seen that the positions of the double bonds were located between the C-9/C-10, C-12/C-13 and C-15/C-16 carbon atoms of the alkyl chain. For the 20:4 ester (Fig. 6), the 15 amu gaps were found to be at *m/z* (178, 193, 208), (264, 279, 294), (308, 323, 338) and (352, 367, 382). This fragmentation pattern confirmed the structure of 20:4(5*c*,11*c*,14*c*,17*c*). Extending the analysis to the deuterated 20:5 isomer (Fig. 7), the 15 amu gaps were at *m/z* (178, 193, 208), (222, 237, 252), (266, 281, 296), (310, 325, 340) and (354, 369, 384). The positions of the five double bonds in this isomer agreed with the structure of the original unsaturated fatty acid [20:5(5*c*,8*c*,11*c*,14*c*,17*c*)]. The ultimate test for this method was to determine the positions of the double bonds by analyzing a deuterated 22:6 isomer. The spectrum (Fig. 8) of the C_{22} hexaenoate isomer distinctly showed the 15 amu gaps at *m/z* (164, 179, 194) (208, 223, 238), (252, 267, 282), (296, 311, 326), (340, 355, 370) and (384, 399, 414). From this fragmentation pattern, the locations of the double bonds were in full agreement with the reported structure of 22:6(4*c*,7*c*,11*c*,13*c*,16*c*,19*c*) (22).

This study demonstrates that the mass-spectral fragmentation pattern of picolinyl esters of deuterated unsaturated fatty acids provides a facile and accurate means of determining the positions of the double bonds (up to six) in the alkyl chain of the fatty acid molecule. The peaks in the spectra of the picolinyl esters of deuterium-labelled saturated fatty acids are intense (10–20% relative intensity) and diagnostic to permit the accurate location

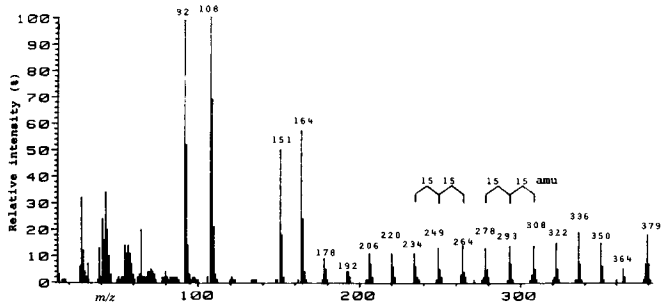

FIG. 4. Mass spectrum of deuterated 18:2(9*c*,12*c*).

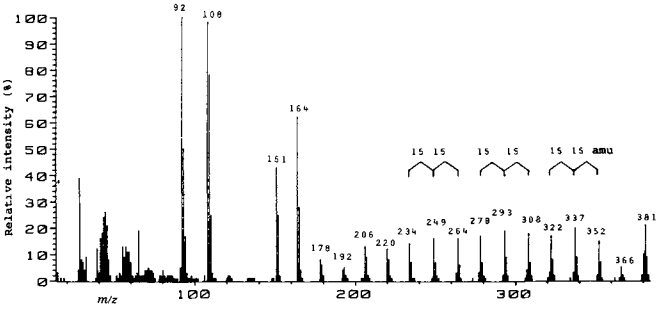

FIG. 5. Mass spectrum of deuterated 18:3(9*c*,12*c*,15*c*).

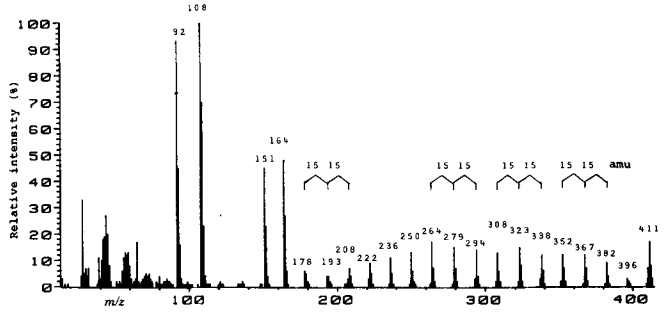

FIG. 6. Mass spectrum of deuterated 20:4(5*c*,11*c*,14*c*,17*c*).

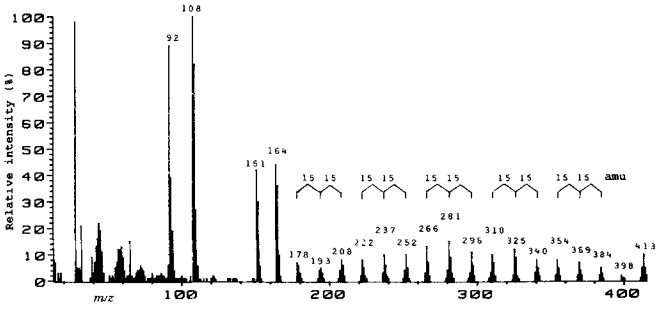

FIG. 7. Mass spectrum of deuterated 20:5(5*c*,8*c*,11*c*,14*c*,17*c*).

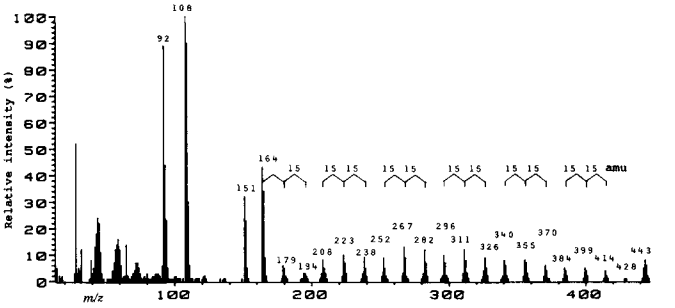

FIG. 8. Mass spectrum of deuterated 20:6(4*c*,7*c*,10*c*,13*c*,16*c*,19*c*).

of the positions of the double bonds in the alkyl chain of the original unsaturated fatty acid prior to deuteration.

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