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Short Communication

Re-examination of the fatty acid composition of *Biota orientalis* seed oil by gas chromatography–mass spectrometry of the picolinyl ester derivatives

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ABSTRACT

Re-examination of the fatty acid composition of *Biota orientalis* (*Platycladus orientalis* or *Thuja orientalis* or *Arbor-vitae*) by gas chromatography–mass spectrometry of the picolinyl ester derivatives showed the presence of 20:3(5*c*,11*c*,14*c*) (4.3%) and 20:3(11*c*,14*c*,17*c*) (0.4%), which was not in agreement with the results published previously. The positions of the unsaturated centres in the alkyl chain of the fatty acids were readily determined by examining the mass spectral fragmentation pattern of the picolinyl derivatives.

INTRODUCTION

Gas chromatography–mass spectrometry (GC–MS) of picolinyl ester derivatives of unsaturated fatty acids has proved to be a reliable means of determining the chain length and the position of the unsaturated centres in long-chain fatty acids. Similarly to the methyl ester derivatives of unsaturated fatty acid molecules, the picolinyl esters are readily separated by GC on polar and non-polar stationary phases. Also, prior to GC analysis, reversed-phase high-performance liquid chromatography can be utilized to fractionate the picolinyl esters to enhance the detectability of minor components present in natural samples [1,2]. However, unlike methyl ester derivatives, there is no double bond migration during mass spectral analysis of the picolinyl esters

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of unsaturated fatty acids. This observation permits the direct analysis of a mixture of picolinyl esters of saturated and unsaturated fatty acids without the need to derivatize the unsaturated centres prior to mass spectral analysis in order to determine the positions of the ethylenic bonds present [3–7]. This method is also applicable to highly unsaturated fatty acids as demonstrated by Christie [8] in the structural elucidation of 18:3(5*c*,9*c*,12*c*).

The fatty acid composition of the seed oil of *Biota orientalis* (also known as *Platycladus orietalis* or *Thuja orientalis* or more commonly as *Arbor-vitae*) [9] was reported earlier by our group to contain a relatively high proportion of 20:4 (5*c*,11*c*,14*c*,17*c*) (10.5%) [10]. This seed oil is used in Chinese medicine to alleviate problems related to the male reproductive system [11]. Our purpose in re-examining the fatty acid composition stems from our intensive investigations into the biological effects of this seed oil on rats maintained on an essential fatty acid-free diet [12].

EXPERIMENTAL

Materials

B. orientalis seeds were purchased from herb shops in Guangzhou, China. Isolation of the oil and conversion of the neutral lipid–glycolipid fraction to the free fatty acid and methyl ester derivatives and separation of methyl esters by silver ion thin-layer chromatography as described previously [10]. 1,1′-Carbonyldiimidazole, 3-hydroxymethylpyridine and 4-pyrrolidinopyridine were purchased from Aldrich (Milwaukee, WI, U.S.A.). All solvents were distilled before use.

Preparation of picolinyl ester derivatives

1,1′-Carbonyldiimidazole in dichloromethane (75 μl, 100 mg/ml) was added to the free fatty acid mixture (5 mg) in dichloromethane (75 μl). After standing for 1 min at room temperature, a solution of 3-hydroxymethylpyridine (7.5 mg) and 4-pyrrolidinopyridine (1.5 mg) in dichloromethane (75 μl) was added, followed by triethylamine (75 μl). The mixture was left for 10 min at 37°C, then evaporated to dryness in a stream of nitrogen. The products were dissolved in *n*-hexane (5 ml), washed with water (2 ml) and dried over sodium sulphate. The *n*-hexane solution was loaded on a column of Florisil (0.5 g) in *n*-hexane contained in a pasteur pipette and eluted with 8 ml of *n*-hexane–diethyl ether (95:5, v/v) to remove non-polar material. The column was then eluted with 10 ml of *n*-hexane–diethyl ether (1:4, v/v), which gave the required picolinyl ester derivatives after evaporation of the solvent of the eluent under reduced pressure.

GC–MS

GC–MS was carried out on a Hewlett-Packard Model HP5970 gas chromatograph fitted with a 12 m × 0.2 mm I.D. capillary glass column (0.33 μm film thickness, cross-linked methylsilicone gum, Ultra 1), with helium as the carrier gas at a flow-rate of *ca.* 2 ml/min and temperature programming from 170 to 240°C at 5°C/min. The outlet of the column was connected to a Hewlett-Packard mass-selective detector.

GC of the methyl ester derivatives was carried out on the same chromatograph, fitted with a 30 m × 0.25 mm I.D. glass capillary column (0.20 μm film thickness, SP2330) isothermally at 220°C using helium as the carrier gas at a flow-rate of *ca.*

2 ml/min and a flame ionization detector. A mixture of methyl esters of 14:0, 16:0, 18:0, 20:0 and 22:0 fatty acids was used as an external standard.

RESULTS AND DISCUSSION

The fatty acid composition of the *B. orientalis* seed oil was compared to that obtained in a previous study [10], which utilized a microbore non-polar column and a 2-m SP2300 packed column (Table I). The present investigation showed the clear separation of the picolinyl ester derivatives of the 20:3 component into two distinct positional isomers (Fig. 1), which were identified by GC–MS as 20:3(5*c*,11*c*,14*c*) (4.3%) and 20:3(11*c*,14*c*,17*c*) (0.4%). The relative abundance of these two isomers is consistent with that occurring in the seed oils of many gymnospermae [13]. The mass spectra of these isomers are presented in Figs. 2 and 3, respectively. In the mass spectum of 20:3(5*c*,11*c*,14*c*), the unsaturated centre at the C-5/C-6 position was confirmed by the gap between m/z = 178 and 204, and positions C-11/C-12 and C-14/C-15 were determined from gaps at m/z = 260 and 286 and m/z = 300 and 326, respectively (Fig. 2). The mass spectrum of 20:3(11*c*,14*c*,17*c*) displayed gaps at m/z = 262 and 288, m/z = 302 and 328 and m/z = 342 and 368, which confirmed the positions of the double bonds at C-11/C-12, C-14/C-15 and C-17/C-18, respectively (Fig. 3). Three minor components [20:1(11*c*) (0.4%), 20:2(5*c*,11*c*) (0.8%) and 20:2 (11*c*,14*c*) (0.8%)] were also identified by GC–MS of the picolinyl ester derivatives, which eluded our earlier investigation. The structure of 20:4 (11.3%) was reconfirmed by mass spectral analysis of the picolinyl derivative (Fig. 4). In the mass spectral analysis of 20:4(5*c*,11*c*,14*c*,17*c*), the unsaturated centre at the C-5/C-6 position was confirmed by the gap between m/z = 178 and 204, while the positions C-11/C-12, C-14/C-15 and C-17/C-18 were determined from the gaps at m/z = 260 and 286, m/z = 300 and 326 and m/z = 340 and 366, respectively.

From the above results, it is evident that GC–MS of picolinyl ester derivatives of

TABLE I

FATTY ACID COMPOSITION OF *BIOTA ORIENTALIS* SEED OIL

Fatty acid	Content (mol%)	
	This work	Ref. 10
16:0	4.7	5.1
18:0	3.5	3.4
18:1(9*c*)	11.6	15.3
18:2(9*c*,12*c*)	26.0	25.6
18:3(9*c*,12*c*,15*c*)	36.2	34.7
20:0	Trace	Trace
20:1(11*c*)	0.4	–
20:2(5*c*,11*c*)	0.8	Trace
20:2(11*c*,14*c*)	0.8	Trace
20:3(5*c*,11*c*,14*c*)	4.3	–
20:3(11*c*,14*c*,17*c*)	0.4	4.9
20:4(5*c*,11*c*,14*c*,17*c*)	11.3	10.5

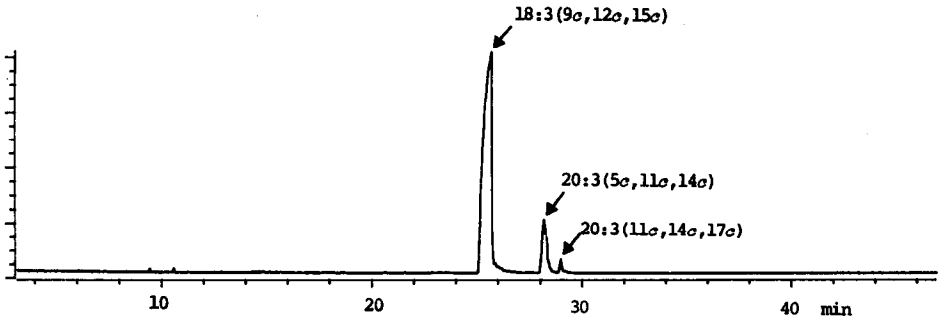

Fig. 1. Chromatogram of picolinyl esters of 18:3 and 20:3 components of *Biota orientalis* seed oil. For conditions, see *GC–MS*.

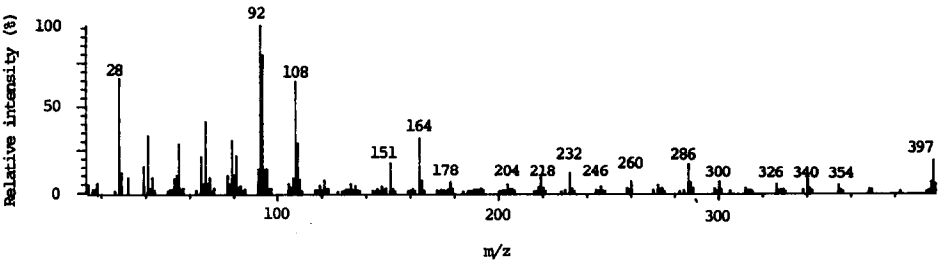

Fig. 2. Mass spectral analysis of picolinyl ester of 20:3(5*c*,11*c*,14*c*).

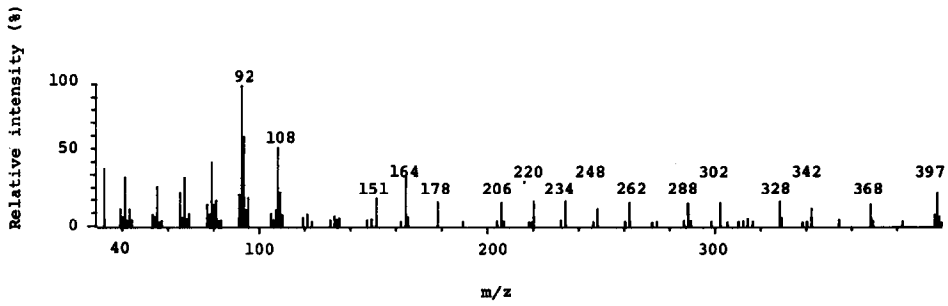

Fig. 3. Mass spectral analysis of picolinyl ester of 20:3(11*c*,14*c*,17*c*).

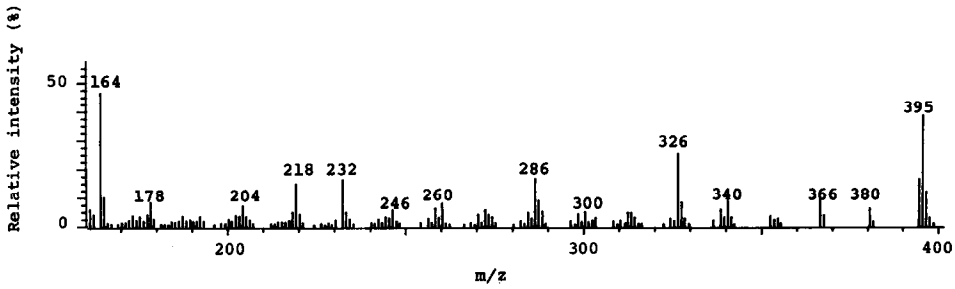

Fig. 4. Mass spectral analysis of picolinyl ester of 20:4(5*c*,11*c*,14*c*,17*c*).

fatty acids constitutes a facile and reliable method for determining the chain length and the positions of the unsaturated centres in the alkyl chain of fatty acid molecules.

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