The Fatty Acid Composition of the Seeds of *Ginkgo biloba*

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ABSTRACT: The fatty acid composition of seeds of *Ginkgo biloba* has been examined by a combination of capillary gas chromatography, silver ion high-performance liquid chromatography and gas chromatography/mass spectrometry. Some of the fatty acids identified are unusual in plants and were rather different from those reported earlier. These include an *anteiso*-methyl branched fatty acid, 14-methylhexadecanoic acid, 5,9-octadecadienoic acid, and 5,9,12-octadecatrienoic acid. Fourier-transform infrared spectroscopy confirmed that all of the double bonds were of the *cis*-configuration.
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KEY WORDS: Fatty acids, *Ginkgo biloba*, mass spectrometry, silver ion chromatography.

Ginkgo biloba, the maidenhair tree, is an ancient gymnosperm relict, known from fossil evidence of near-identical leaves to have existed almost unchanged for 200 million years. It was discovered growing in the wild in China and was first cultivated in China and now elsewhere. Ginkgo seeds have long been consumed as a food and as an herbal medication in the Far East.

There are only a few studies of the fatty acid composition of oils from Ginkgo seeds, and all of them date back 20–30 yr (1–3). Only Schlenk and Gellerman (1) gave details of the position of double bonds in fatty acids, and they reported unusual structures, mainly the presence of fatty acids with a double bond in position 5, separated by more than one methylene group from other double bonds, as well as an eicosatetraenoic fatty acid. However, the techniques used to identify the compounds, such as ozonization–hydrogenation–gas–liquid chromatography (GLC), could give rise to misinterpretations of structure.

In this study, the total fatty acids of the seeds of *Ginkgo biloba* have been examined by methods involving fractionation of the mixture according to degree of unsaturation by high-performance liquid chromatography (HPLC) in the silver ion mode, followed by gas chromatography/mass spectrometry (GC/MS) of the picolinyl ester derivatives. The fatty acid composition was found to be distinctive and rather different from that reported earlier.

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EXPERIMENTAL PROCEDURES

Sample. Ripened stony fruits were plucked early in November 1994, from a *Ginkgo biloba* tree, which has grown for approximately 60–70 yr on the outskirts of Masan, Korea. The seeds were taken from the fruits and washed with water twice before air-drying in a shady place. The kernels collected from the crushed seeds were chopped and ground in a mortar. The total lipids were extracted under an atmosphere of nitrogen according to the method of Bligh and Dyer (4). The crude lipid level was 2.2% on a wet weight basis.

Analytical GC. Methyl esters of fatty acids were analyzed with a Hewlett-Packard Model 5890 Series II (Hewlett Packard Ltd., Stockport, United Kingdom) gas chromatograph, fitted with split/splitless injection, and equipped with a capillary column (25 m × 0.25 mm × 0.2 μm film thickness) of fused silica coated with CP-Wax 52CB (Chrompack UK Ltd., London, United Kingdom). The carrier gas was hydrogen at a flow rate of 1 mL/min. The initial temperature in the column was 170°C for 3 min, temperature-programmed to 210°C at 4°C/min, and held at this point for a further 25 min. Components were quantified by electronic integration.

Silver ion HPLC. A Spectra-Physics Model 8700 solvent delivery system was used (Spectra-Physics Ltd., St. Albans, United Kingdom), together with a Cunow model DDL 21 evaporative light-scattering detector (Severn Analytical Ltd., Shefford, United Kingdom). A stream-splitter (10:1) was inserted between the column and the detector to enable collection of fractions. A column (4.6 × 250 mm) of Nucleosil 5SA (HPLC Technology Ltd, Macclesfield, United Kingdom) was converted to the silver ion form as described elsewhere (5). The column was eluted with a gradient of dichloromethane/1,2-dichloroethane (1:1, vol/vol) (solvent A) and dichlorometh-ane/1,2-dichloro-ethane/acetonitrile/methanol (45:45:5:5, by vol) (solvent B). There was a linear gradient from 100% A to 80% A/20% B over 40 min. Methyl esters of fatty acids were converted into *p*-methoxyphenacyl esters by the method of Wood and Lee (6). For micropreparative purposes, 1 mg of phenacyl esters was applied to the column in 10 μL of 1,2-dichloroethane.

GC/MS. The fractions were hydrolyzed to the free fatty acids before conversion to the picolinyl ester derivatives as described elsewhere (7). The derivatives were analyzed on a

Carlo Erba (Rome, Italy) Mega Series gas chromatograph connected to a Kratos 8/90 double-focusing magnetic sector instrument (Kratos Analytical, Manchester, United Kingdom). The picolinyl esters were separated on a fused-silica capillary column, DB5-MS (30 m × 0.25 mm × 0.25 μm film thickness), coated with a cross-linked 5% phenylmethylsilicone stationary phase (J&W Scientific, Folsom, CA). Samples were injected by cold on-column injection at 80°C (held for 3 min), temperature-programmed to 160°C at 30°C/min, then to 325°C at 4°C/min, then held at this point for a further 15 min. In addition, total fatty acids as picolinyl esters were analyzed on a polar capillary column, BPX70 (50 m × 0.22 mm × 0.25 μm film thickness), coated with a biscyanopropylsiloxane-silphenylene polymer (SGE, Milton Keynes, United Kingdom). Samples were injected as before onto the column, which was operated at the same temperature range.

Fourier-transform infrared spectroscopy. A Bruker IFS 66 FTIR spectrometer (Bruker Spectrospin Ltd., Coventry, United Kingdom) was used to record spectra of the total fatty acid methyl esters from Ginkgo seeds.

RESULTS AND DISCUSSION

Figure 1 shows the GC analysis of the total fatty acid methyl esters of Ginkgo seeds. More than 20 peaks were evident, many of which did not correspond to the common range of standards. With the aim of identifying the fatty acids, the sample was simplified by silver ion HPLC, which separates components according to the degree of unsaturation. Five fractions were collected *via* a stream-splitter, and the fatty acids were converted to the picolinyl ester derivatives for examination by GC/MS.The fatty acids identified and their relative proportions are given in Table 1, together with the fraction number in which they appear.

The saturated fatty acids (fraction 1) were mainly $C_{16:0}$, 14-methyl-16:0, and 18:0, with small amounts of other straight-chain fatty acids, such as 12:0, 14:0, 20:0, and 22:0. The saturated branched fatty acid has not been described previously in Ginkgo seeds, and the mass spectrum of its picolinyl ester derivative is shown in Figure 2. There was a molecular ion at *m/z* = 361 and an interval of 28 amu between *m/z*

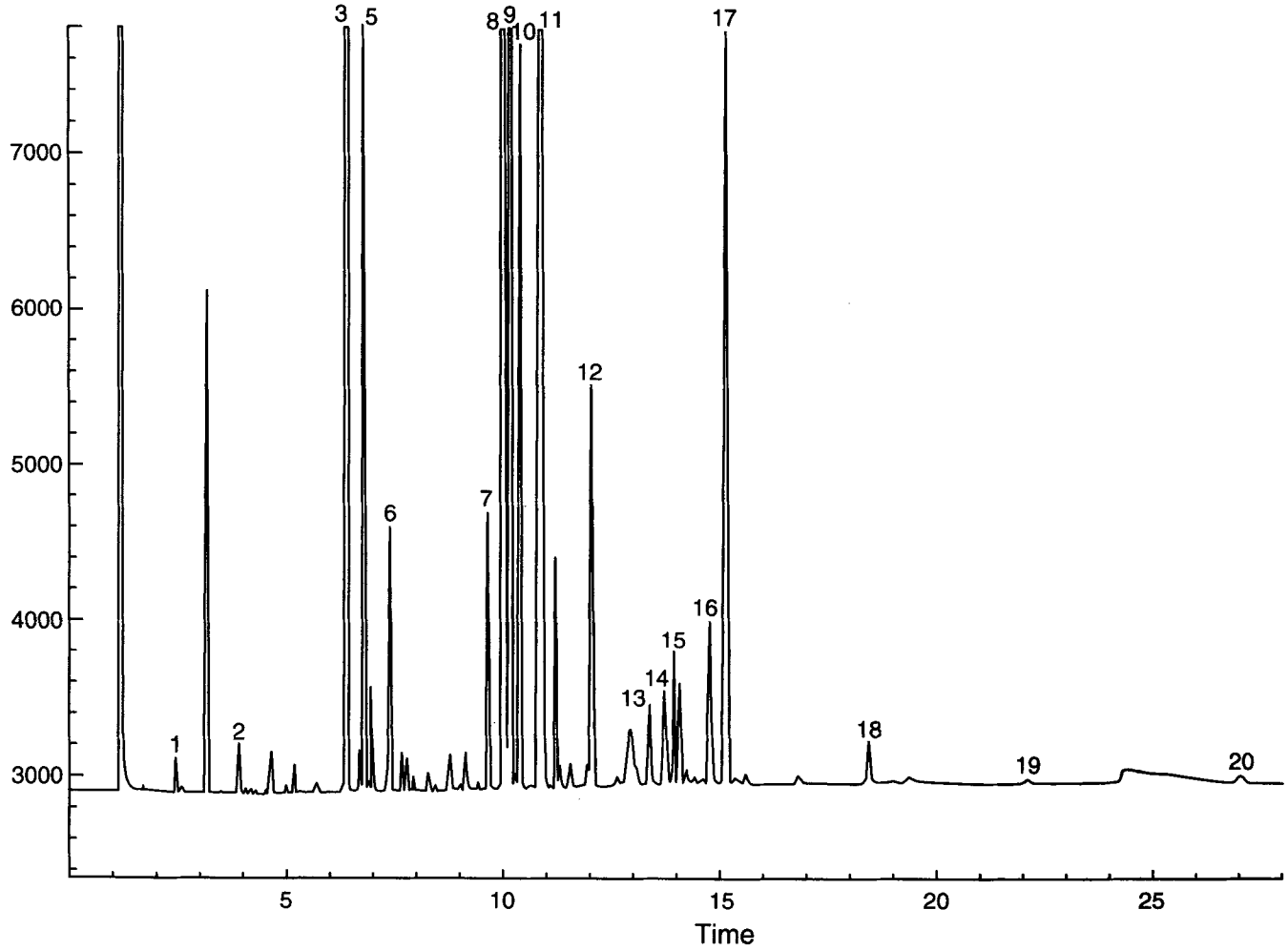

FIG. 1. Capillary gas chromatography chromatogram shows separation of fatty acids in *Ginkgo biloba* as methyl esters. For peak identification, see Table I (peak 4 is the minor component at the leading edge of peak 5).

TABLE 1
Fatty Acid Composition (wt% of the total) from Ginkgo Nuts, Fraction Number in the Silver Ion High-Performance Liquid Chromatography (HPLC) and Peak Number in the Gas–Liquid Chromatography (GLC) Chromatogram

Peak number	Fatty acid	Fraction number[a]	%
1	12:0	1	trace
2	14:0	1	trace
3	16:0	1	6.62
4	7-16:1	3	0.13
5	9-16:1	3	3.30
6	14-methyl-16:0	1	0.86
7	18:0	1	0.99
8	9-18:1	3	13.76
9	11-18:1	3	21.53
10	5,9-18:2	5	2.77
11	9,12-18:2	4	38.99
12	5,9,12-18:3	—	1.61
12	9-19:1	3	trace
13	20:0	1	0.37
14	11-20:1	3	0.44
15	13-20:1	3	0.66
16	11,14-20:2	4	0.90
17	5,11,14-20:3	5	5.70
18	22:0	1	0.40
19	23:0	1	trace
20	24:0	1	trace
21	26:0	1	trace

[a]Fraction 2 contained no detectable fatty acid derivatives.

= 304 and 332 for the *anteiso*-methyl branch. Saturated methyl-branched fatty acids are not common in the plant kingdom, although some have been found in the seed oils of species of the family Flacourtiaceae (8). Traces of some long-chain saturated fatty acids also were found (23:0, 24:0, and 26:0). Some of these fatty acids previously have been reported to be present in the sterol ester fraction of the Ginkgo seed (2).

The retention characteristics of fraction 2 in silver ion chromatography were identical to those of *trans*-monoenoic isomers (9). However, the GC/MS analyses appeared to indicate that the compounds were not fatty acids.

Cis-monoenes were found in the third fraction; the main components were Δ9-16:1, Δ9-18:1, and Δ11-18:1, and they comprised 38.6% of the total fatty acids. There were also low proportions of 16:1 with the double bond in position 7, Δ10-19:1, and two isomers of 20:1 with the double bonds in positions 11 and 13. Iyoda and Noguchi (3) and Urakami *et al.* (2) reported that 20:1 comprised between 1 and 1.5% of the total fatty acids in Ginkgo seeds, although they did not specify double bond position.

Fraction 4 contained two dienoic acids with methylene-interrupted double bonds (Δ9,12-18:2 and Δ11,14-20:2). Δ9,12-18:2 was the major compound and comprised 39% of the total fatty acids.

Fraction 5 from the silver ion column is mainly a trienoic fraction, but a dienoic fatty acid with a 5,9-diene system was also present. It is known from studies with model compounds in silver ion chromatography that a 5,9-diene system is re-

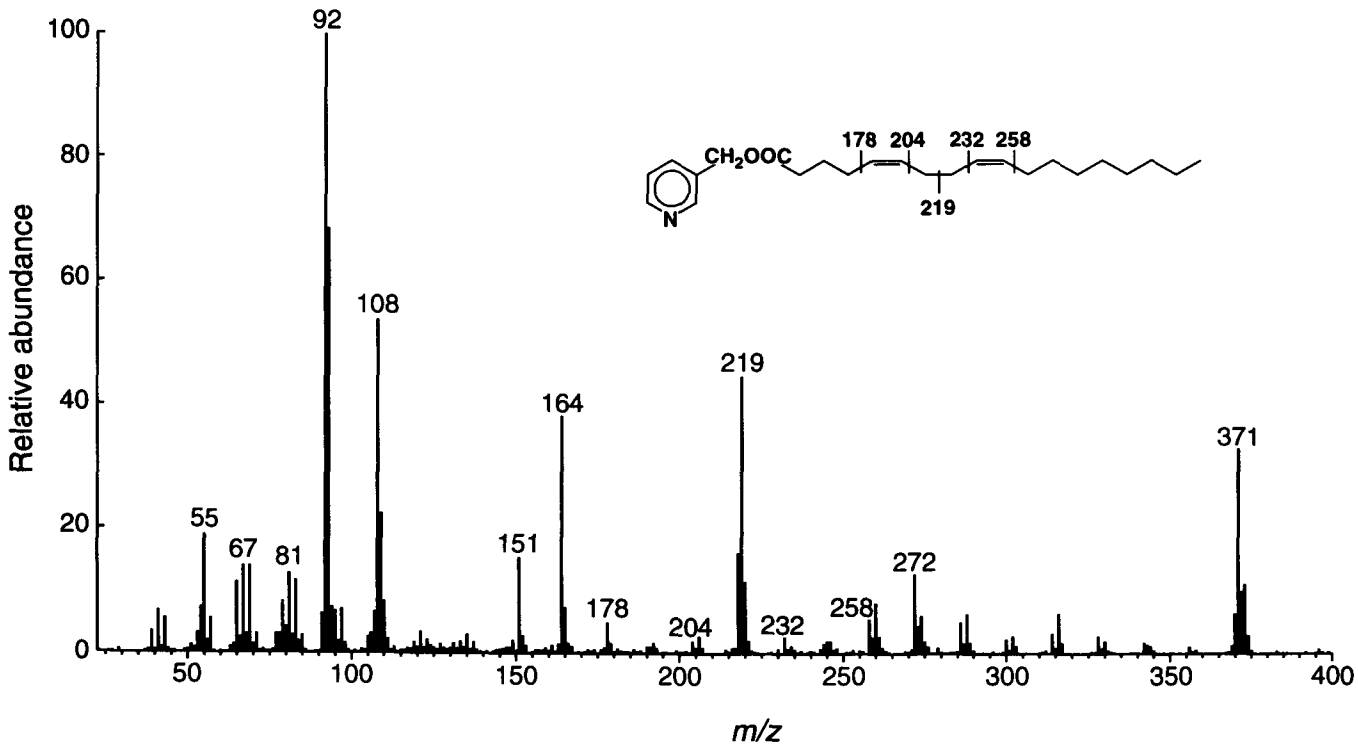

FIG. 2. Mass spectrum of the picolinyl ester derivative of 14-methyl-16:0.

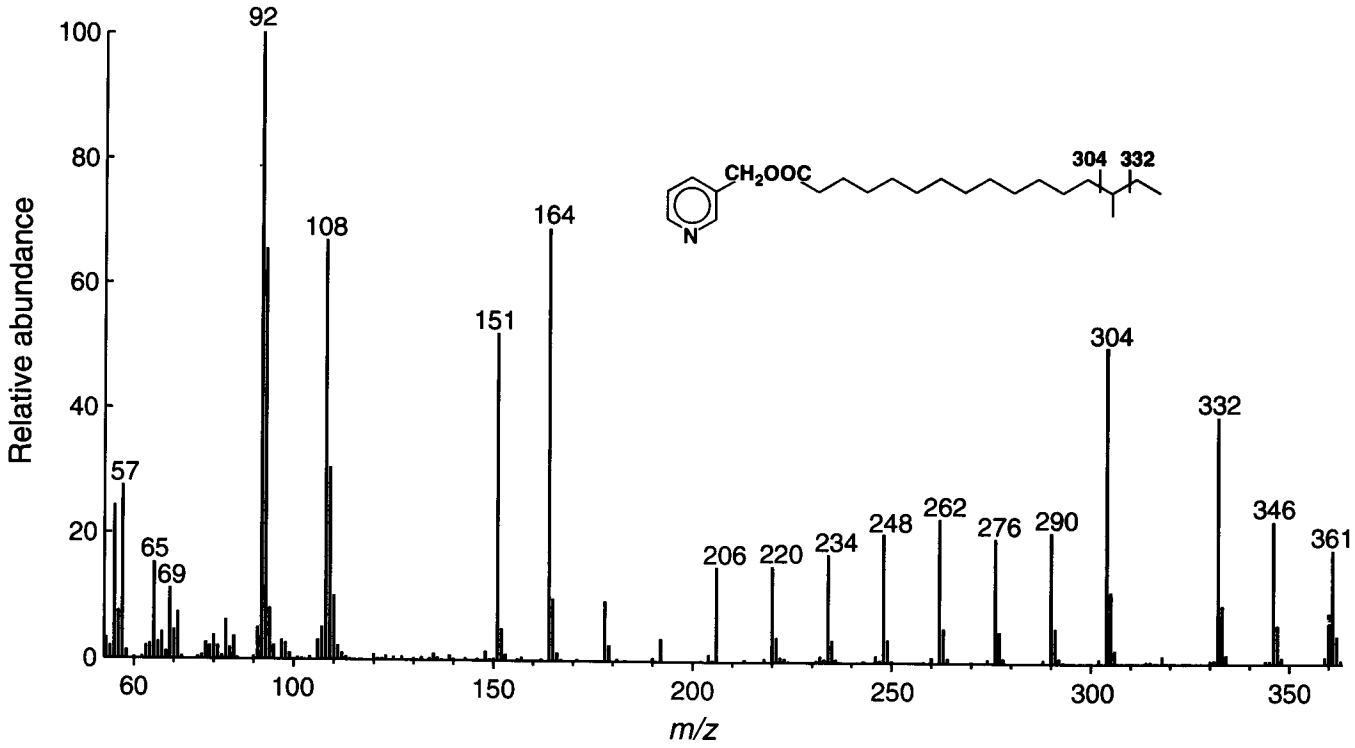

FIG. 3. Mass spectrum of the picolinyl ester derivative of Δ5,9-18:2.

tained more strongly than most other dienes (10). The main compound in fraction 5 was the triene Δ5,11,14-20:3, comprising 5.7% of the total fatty acids.

Figure 3 shows the picolinyl ester for Δ5,9-18:2. It has a molecular ion at *m/z* 371. The most important diagnostic feature was an ion formed by cleavage between the two methylene groups that separate the double bonds, which, unusually for a picolinyl ester, has an odd mass number (*m/z* = 219). In addition, gaps of 26 amu between *m/z* = 178 and 204 and between 232 and 258 confirmed the presence of double bonds in positions 5 and 9, respectively.

The mass spectrum (not shown) of the picolinyl ester of Δ5,11,14-20:3 had a molecular ion at *m/z* 397, and gaps of 26 amu between *m/z* 178 and 204, 260 and 286, and 300 and 326 were indicative of double bonds in positions, 5, 11, and 14, respectively. This compound was the same 20:3 fatty acid described by Schlenk and Gellerman (1).

A similar feature was found in the spectrum of Δ5,9,12-18:3, but a further gap of 26 amu between 272 and 298 confirmed the presence of the double bond in position 12. These results do not agree with those of Schlenk and Gellerman (1), who reported several members of the "delta 5" group but with 5,11-diene configuration. Δ5,9,12-18:3 was not found in fraction 5 with the other triene, which presumably eluted later and was not detected. However, this triene was identified when the picolinyl derivatives of the total sample were analyzed on the BPX-70 column. Again, the Δ5,9-double bond structure forms a more stable complex with the silver ions than other configurations. Finally, the determination of the content of *trans* fatty acids was carried out by utilizing the characteristic absorbance of *trans* double bonds at 967 cm^{-1} in the IR spectrum. The results showed no detectable *trans* isomers in the samples.

Δ5,9-18:2 and Δ5,9,12-18:3 fatty acids are unusual in plants, although Δ5,9-18:2 has been described in some pine species (11) and slime molds (12). The occurrence of Δ5,9,12-18:3 fatty acid has been reported in *Xeranthemum annuum* (13), and there are reports that this fatty acid is also a major constituent of the seeds of other Gymnospermae (14,15). Seed oils of the Ranunculaceae family (Angiospermae) have been found to contain some Δ5,9,12-18:3 fatty acids, but in most instances the double bond in position 5 had the *trans* configuration (16). Ginkgo seeds, therefore, contain a number of unusual fatty acids, including a C16 branched-chain component, C18 fatty acids with double bonds in the Δ5,9-position and Δ5,11,14-20:3. No eicosatetraenoic fatty acid was detected [as described earlier by Schlenk and Gellerman (1)].

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