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Occurrence of *cis*-4-Tetradecenoic Acid in the Oils of Kernels of *Lindera erythrocarpa* Seeds, as a Major Component

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Abstract Kernel oils of *Lindera erythrocarpa* seeds had high level of unusual fatty acid. Picolinyl ester of this unknown fatty acid showed molecular ion at m/z = 317 with other diagnostic ions such as m/z = 151, 191 (40 amu between two peaks), 204, and 218 on GC-MS. Characteristic peak at 720 cm^{-1} appeared in IR spectrum. In ^{1}H-NMR spectrum both methylene protons at C-3 and C-6 resonated at δ2.309 and δ2.012, and methine protons of double bond resonated in lower magnetic field centered at δ5.296 (C-4) and δ5.387 (C-5) as multiplet (J = 9.7Hz). In ^{13}C-NMR, signals at δ22.669 and δ27.048 were due to C-3 and C-6 of Δ^4-monoenoic acid. Results obtained from spectroscopic measurements confirmed unknown fatty acid as *cis*-4-tetradecenoic acid (*cis*-4-$C_{14:1}$). Main fatty acid components of oils were *cis*-4-$C_{14:1}$ (44.5-45.1%), oleic acid ($C_{18:1}$, 20.4-21.3%), and lauric acid ($C_{12:0}$, 11.6-12.4%), along with trace amounts of *cis*-4-$C_{12:1}$ and *cis*-4-$C_{16:1}$.

Keywords: *cis*-4-tetradecenoic acid, *cis*-4-dodecenoic acid, *Lindera erythrocarpa*, silver$^+$-chromatography, picolinyl ester

Introduction

The plant family Lauraceae can be divided into 7 genera with over 50 species. It is widely distributed in the subtropical and temperate zones (1). Analysis of some genera of this family showed high levels of lauric acid in their seed oils, with a considerable variation in its content among species (2). Moreover, seed oils of the genus *Lindera* are also noted for the occurrence of Δ^4-monoenoic fatty acids with medium carbon chain (2), although Δ^4- tetradecenoic acid and Δ^4-hexadecenoic acid were found in the seed oils of *Thunbergia alata* of the family Acanthaceae (3) and *Hydnocarpus anthelmintica* of the Flacourtiaceae (4) as minor components, respectively.

For the purpose of obtaining more information on the oil chemistry of the seeds of the Lauraceae, *L. erythrocarpa* was included in our consecutive lipid research project on unutilized seeds. *L. erythrocarpa* is a deciduous and medium-sized tree of 4-5 m in height, and grows in valleys and house garden throughout the southern region of Korea. Mature red seed with a diameter of 8 mm in November had been used in traditional medicines as an antifebrile and a cardiac tonic in remote mountainous areas in the Orient (1).

In the present study, oils of the kernels of *L. erythrocarpa* seeds were examined, with special regard to the possible occurrence of C_{10}-C_{14} unsaturated fatty acids. The oils of *L. erythrocarpa* seeds, not reported previously, were found to have *cis*-4-tetradecenoic acid as the major fatty acid component.

Materials and Methods

Lipid samples and reagents The seeds of *L. erythrocarpa* were collected in November 1999, and the kernels were removed from the seeds. The air-dried, smashed kernels were extracted according to the method of Bligh & Dyer (5) in a stream of nitrogen. All solvents and reagents were HPLC-grade and supplied by Merck Co., Ltd. (Darmstadt, Germany).

Gas-liquid chromatography (GC) of fatty acid methyl esters (FAMEs) The sample was transmethylated with sodium methoxide-methanol solution for 5 min at ambient temperature in the presence of an internal standard (methyl heneicosanoate) and BHT, and the methyl esters were then recovered with hexane (6). A Hewlett-Packard Model 5890 Series II capillary gas chromatograph, fitted with a split/splitless and equipped with a capillary column (25 m × 0.22 mm, i.d., 25 μm film) of fused silica coated with BPX 70 (70% cyanopropyl polysilphenylenesiloxane, SGE, Austin, TX, USA), was used for analyzing the methyl ester derivatives quantitatively. The column temperature was held at 160°C for 3 min and then programmed up to 220°C at 3°C/min, with a final hold of 10 min. Hydrogen was the carrier gas, and the fatty acid composition of total oils was calculated by measuring the area of each peak on GC chromatogram and multiplying it by a correction factor revised by Christie (7). Each value is a mean of triplicate measurements.

Fractionation of total FAMEs according to double bond, by silver-ion chromatography (6, 7) Disposable solid-phase extraction columns packed with a bonded-sulfonate acid phase (SCX disposable column, Analytical Bond Elut^{TM}, Varian Inc., PaloAlto, CA, USA) were impregnated with silver ions and equilibrated with dichloromethane (DCM) before use. An aliquot (1-3 mg) of total FAMEs dissolved in a small amount of DCM was loaded on the column. The sample was then resolved into saturated acid fraction with 100% DCM (10 mL), monoenes fraction with DCM-acetone (10 mL; 9:1, v/v), and finally dienes fraction with 100% acetone (5 mL). FAMEs present in each fraction, if necessary, were

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hydrolyzed with 5% KOH-ethanol solution, and the free fatty acids obtained were derivatized into their picolinyl esters.

Fourier-transform infrared spectroscopy The sample dissolved in a small portion of chloroform was smeared on the center of a KBr disc. The spectrum was recorded according to Chapman's method (8) using a Bruker IFS 66 FTIR spectrometer (Bruker Spectrospin Ltd., Coventary, UK).

Preparation of picolinyl esters Picolinyl derivatives of fatty acids were prepared according to the method recommended by Balazy and Nies (9). Briefly, a solution of 1,1'-carbonyl diimidazole in DCM freshly prepared (100 μL, 100 mg/mL) was added to free fatty acids (1-2 mg) dissolved in DCM (100 μL). After 1 min, a solution of 3-hydroxymethylpyridine (10 mg) and 4-pyrrolidinopyridine (2 mg) in DCM (100 μL) was added, followed by triethylamine (100 μL). The mixture was left for 10 min at 37°C and was then dried in a stream of nitrogen. The products were taken up in hexane (5 mL) and washed with water (2 mL), and the hexane layer was concentrated under nitrogen. The derivatives recovered were purified with Florisil™ (0.5 g) on a short column (Pasteur pipette); non-polar impurities were discarded by eluting with hexane/acetone solution (10 mL; 9:1, v/v). Pure picolinyl derivatives were finally obtained using hexane/acetone (10 mL; 8:2, v/v).

GC/Mass spectrometry (GC/MS) (10) Micromass model Autospec Ultima High Resolution Trisector EBI Ion Optics Mass GC/MS Spectrometer (Micromass, Wythenshawe, Manchester, UK) was used for GC/MS. A fused silica Ultra-2 (50 m × 0.25 mm, i.d., 0.17 μm film) coated with a cross-linked 5% diphenyldimethylsiloxane stationary phase (Hewlett-Packard, Palo, CA, USA) was connected directly to the ion source. The column temperature was held for 3 min, and then programmed from 80 to 160°C at 30°C/min, then to 315°C at 4°C/min, and finally held at this temperature for 20 min.

^{1}H-and ^{13}C-Nuclear magnetic resonance (NMR) (11) All NMR measurements were run on a Bruker DMX 600 spectrometer (Bruker Elektronik GmbH, Karlsruhe, Germany), operating at 600 MHz for ^{1}H-NMR and at 150 MHz for ^{13}C-NMR. The sample was dissolved in $CDCl_3$ (0.6 mol/L), which also served as an internal deuterium lock, and TMS was then added to the solution as an internal standard for ^{1}H-NMR measurement. Chemical shifts are given as δ-values in ppm downfield from TMS.

Results and Discussion

The total fatty acids in the kernels of *L. erythrocarpa* seeds were analyzed. The GC trace of the FAMEs of total lipids was quite simple, but three unknown fatty acids (peak no. 3, 6, and 8 on GC chromatogram, Fig. 1) were detected together with the common fatty acids. For a better resolution of GC-MS spectra of these unidentified components, total FAMEs were fractionated into saturated, monoene, and diene fractions, respectively, by silver-ion chromatography. The fatty acids in the saturated and the diene fractions were easily identified by comparing their retention times with those of authentic fatty acids on GC (22). However, all the unidentified fatty acids were present in the monoene fraction and these fatty acids were then converted into their picolinyl esters. An unusual fatty acid (peak no. 6 in Fig. 1) eluted between peaks corresponding to tetradecanoic acid ($C_{14:0}$) and tetradecenoic acid (*cis*-9-$C_{14:1}$) was the most abundant component. In the GC-mass spectrum of this fatty acid, this particular derivative has molecular ion at m/z=317 and could be distinguished from others by their mass spectra as picolinyl esters, because they do not have readily identifiable fragmentations at the double bond and have the abundant peaks at m/z=151 and 218 (Fig. 2-a). Other diagnostic ions such as m/z=165, 191 (40 amu between two peaks), 204, 232, 246, and 288, were clearly observed (3, 10, 12). Based on the results obtained and the chromatographic characteristics on silver-ion chromatography (3, 13), this unusual fatty acid was considered to be *cis*-4-$C_{14:1}$. The peak nos. 3 (Fig. 2-b) and 8 (data not shown) fatty acid derivatives showed molecular ions at m/z=289 and 345, respectively, as well as pronounced ion fragments at m/z=151 (150+H) and 218, with other diagnostic ions at m/z=165, 191 (40 amu between two peaks), 204, 232, 246, and 260; they were tentatively identified as *cis*-4-$C_{12:1}$ and *cis*-4-$C_{16:1}$,

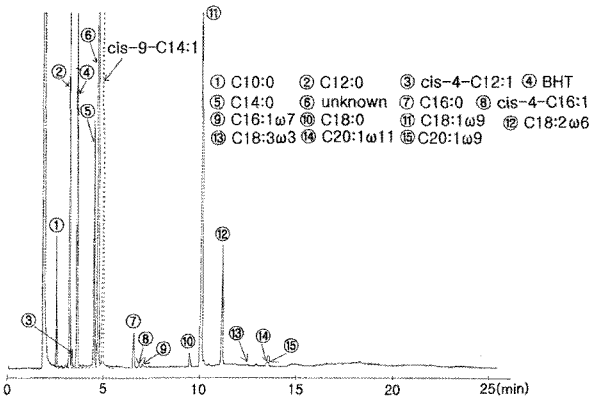

Fig. 1. GLC chromatogram of the methyl esters of fatty acid isolated from the kernel oils of *Lindera erythrocarpa*.

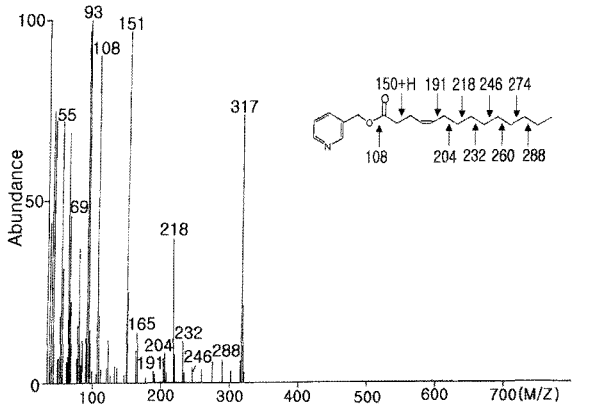

Fig. 2-a. The mass spectrum of the picolinyl ester of the second unidentified fatty acid (no. 6 peak on GLC chromatogram) isolated from the kernel oils of *Lindera erythrocarpa*.

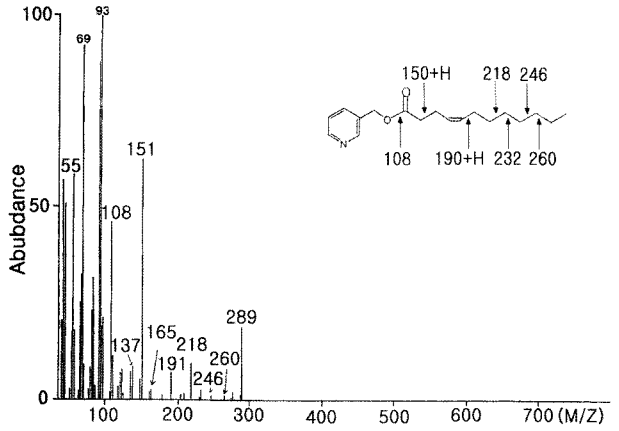

Fig. 2-b. The mass spectrum of the picolinyl ester of the first unidentified fatty acid (no. 3 peak on GLC chromatogram) isolated from the kernel oils of *Lindera erythrocarpa.*

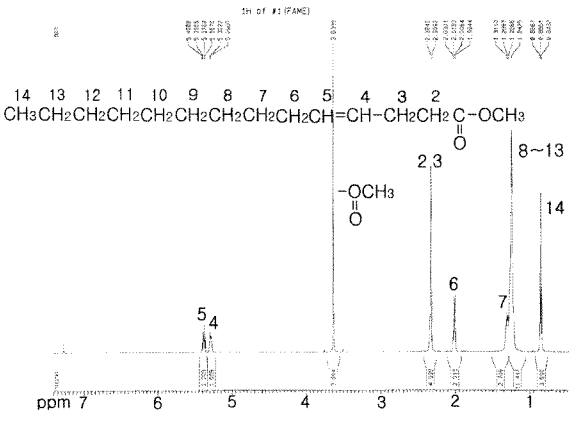

Fig. 4-a. ^{1}H-NMR spectrum of the methyl ester of the second unidentified fatty acid (no. 6 peak on GLC chromatogram) isolated from the kernel oils of *Lindera erythrocarpa.*

respectively.

For further structural elucidation and confirmation of *cis*-4-$C_{14:1}$ (peak no. 6) by IR and NMR, large quantities (2.1 mg) of this fatty acid were isolated from total FAMEs by silver-ion chromatography with a mixture of DCM acetone, followed by reversed-phase HPLC using a solution of acetonitrile/water (80:20, v/v). The procedure was repeated until the final purity of the unknown FAME exceeded 98.5%. The absorption peaks at 3030, 1670, and 720 cm^{-1} in the IR spectrum indicate the presence of double bond(s) with *cis*-configuration in the molecule (Fig. 3). Based on these results, no. 6 unknown fatty acid was identified as *cis*-4-$C_{14:1}$ (14).

Figure 4-a and 4-b show the ^{1}H-NMR and ^{13}C-NMR chemical shifts of the methyl ester of the peak no. 6 fatty acid. The ^{13}C-NMR spectrum contained six singlets, one doublet, and one overlapped signal, excluding those due to C-1 and the methyl ester methoxyl carbon. The doublet can be easily assigned to C-3 (δ22.669) and C-13 (δ22.547) from the ^{1}H-^{13}C COSY spectrum (Fig. 4-c). The signals at δ34.000 (C-2) and δ22.669 (C-3) indicated the presence of double bond between C-4 and C-5 in the chain of an FAME molecule. Resonance peaks at δ127.123, δ131.409, and δ27.048 can be easily assigned to C-4, C-5, and C-6 of a Δ^4-monoenoic fatty acid. In addition, the

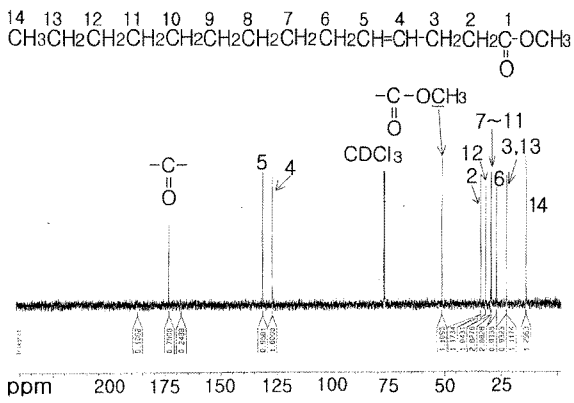

Fig. 4-b. ^{13}C-NMR spectrum of the methyl ester of the second unidentified fatty acid (no. 6 peak on GLC chromatogram) isolated from the kernel oils of *Lindera erythrocarpa.*

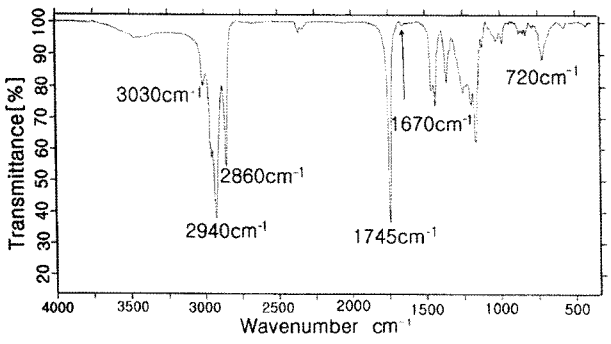

Fig. 3. IR spectrum of the methyl ester of the second unidentified fatty acid (no. 6 peak on GLC chromatogram) isolated from the kernel oils of *Lindera erythrocarpa.*

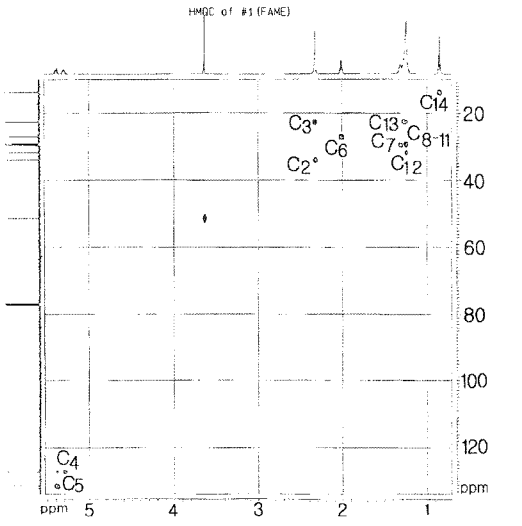

Fig. 4-c. The ^{1}H-^{13}C two-dimensional shift correlation nuclear magnetic resonance spectrum of the methyl ester of the second unidentified fatty acid (no. 6 peak on GLC chromatogram) isolated from the kernel oils of *Lindera erythrocarpa.*

Table 1. ^{1}H- and ^{13}C-NMR chemical shifts of *cis*-4-tetradecenoic acid methyl ester

Carbon	Shift	
	δ^1H	δ^{13}C
1	–	173.347
2	2.309-2.324 (4H,*m*)[a]	34.000
3		22.669
4	5.296 [1H,*m*,*J*=9.7(d)]	127.123
5	5.387 [1H,*q*,*J*=9.7(d),7.4(t)]	131.409
6	2.012 [2H,*q*,*J*=7.4(d)]	27.048
7	1.298 (2H,*m*)[a]	29.722
8	1.242-1.311 (12H,*m*)[a]	29.216[b]
9		29.483
10		29.518
11		29.438[b]
12		31.790
13		22.547
14	0.855 [3H,*t*,*J*=7.1]	13.911
OCH_3	3.638 (3H,*s*)	51.216

[a]Signals not resolved.
[b]Signals may be reversed.

Table 2. Fatty acid composition of total lipids extracted from the kernel and the pulp of seeds of *Lindera erythrocarpa* (wt %)

Fatty acid	Kernel		Pulp[b]
	A[a]	B	
$C_{10:0}$	3.2	3.4	2.0
$C_{12:0}$	12.4	11.6	
Cis-4-$C_{12:1}$	0.6	0.4	
$C_{14:0}$	9.0	8.1	0.8
Cis-4-$C_{14:1}$	45.1	44.5	
$C_{16:0}$	1.4	2.6	24.7
Cis-4-$C_{16:1(?)}$	0.1	0.3	
$C_{16:1\omega7}$	0.2	0.3	0.4
$C_{18:0}$	0.6	0.7	1.3
$C_{18:1\omega9}$	20.4	21.3	49.7
$C_{18:1\omega7}$	0.3	0.5	1.8
$C_{18:2\omega6}$	6.2	5.5	17.3
$C_{18:3\omega3}$	0.1	0.3	1.8
$C_{20:1\omega11}$	0.2	0.2	
$C_{20:1\omega9}$	0.2	0.2	
$C_{22:1\omega9}$			0.2

[a]A and B; collected from the valleys and house gardens (B) in the suburbs of Busan, respectively.
[b]From the sample A.

coupling constant of 9.7 Hz observed between split signals at both C-4 and C-5 in the ^{1}H-NMR spectrum confirmed that the double bond system has a *cis*-configuration. The signal at δ29.722 among the overlapped ones can be ascribed to C-7 from the ^{1}H-^{13}C COSY experiment, and those at δ29.483 and δ29.518 can be also attributed to C-9 and C-10 according to the references (15-17), while those at δ29.216 and δ29.438 were tentatively assigned to C-8 and C-11, respectively. The chemical assignments of each hydrogen and carbon atom are listed in Table 1.

The main fatty acid components of the oils were *cis*-4-tetradecenoic acid (44.5-45.1%), stearic acid ($C_{18:0}$, 20.4-21.3%), and lauric aicd ($C_{12:0}$, 11.6-12.4%), with small amounts of myristic acid ($C_{14:0}$, 8.1-9.0%), linoleic acid ($C_{18:2\omega6}$, 5.5-6.2%), capric acid ($C_{10:0}$, 3.2-3.4%), and palmitic acid ($C_{16:0}$, 1.4-2.6%) (Table 2). *Cis*-4-tetradecenoic acid was the most abundant fatty acid in the oils of all the seeds collected, along with trace amounts of *cis*-4-$C_{12:1}$ and *cis*-4-$C_{16:1}$; however, these unusual fatty acids were not detected in the pulp lipids.

Hopkins (2) classified Lauraceae species into three groups on the basis of fatty acid composition of the seed oils of the few species studied: (a) those whose acids are mainly saturated C_{10} and C_{12}, *e.g. Lindera benzoin, L. praecox, Cinnamomun camphora, and Umbellularia californica*; (b) those whose acids are mainly saturated medium-chain (C_{12}) and unsaturated long-chain (C_{18}), *e.g. Laurus nobilis*; (c) those whose acids are mainly medium-chain saturated and unsaturated (C_{10}-C_{14}), *e.g. L. umbellata*. However, the fatty acid composition of the seed oils of *L. erythrocarpa* was distinctly different from those of other species of the Lauraceae analyzed so far (1); because it contained both the medium-chain unsaturated fatty acid (*cis*-4-tetradecenoic acid, 44.5-45.1%) and long-chain unsaturated fatty acid (oleic acid, 20.4-21.3%) as the major fatty acids, it cannot belong to any of the classifications mentioned above.

The presence of three monoenoic acids having a double bond at position C-4 suggested that these fatty acids were produced by bio-synthetic routes strikingly different from that of the ordinary one in seeds. In general, a series of three or more monoenoic acids in plant lipids are formed by chain elongation at the carboxyl end, thereby resulting in a positional change of the double bond from the carboxyl to the methyl end as the chain length increases.

Several possible pathways are conceivable in the formation of Δ^4-monoenoic acids in the seed of *L. erythrocarpa*. The possibility cannot be ruled out that the Δ^4-acids were produced from the appropriate saturated fatty acids by desaturation of a Δ^4-desaturase in the seed, as seen in the formation of an intermediate (*cis*-4-$C_{16:1}$) in the biosynthesis pathway of petroselinic acid (*cis*-6-$C_{18:1}$) from $C_{16:0}$ (18).

Although the seeds have been utilized as traditional medicine, from the nutritional point of view, the seeds and the oils of *L. erythrocarpa* cannot be recommended, because the unusual fatty acids are still yet unexplored.

The unusual abundance of this uncommon fatty acid in this species and the paucity of literature on Lauraceae seed oils prompted this study, because this fatty acid can be also split oxidatively into capric acid ($C_{10:0}$), which is a valuable material for production of surfactants, cosmetics, and pharmaceuticals (19, 21).

Acknowledgments

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