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by Kiyoshi Furukawa, Hiromichi Nii, Mitsuo Iwakiri, and Takashi Kubota

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The fatty acid composition of the seed oil of Lindera obtusiloba Blume

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by

Kiyoshi FURUKAWA*, Hiromichi NII*, Mitsuo IWAKIRI*
and Takashi KUBOTA**

1. Introduction

In a previous report (1), the authors threw light upon the geometrical isomerism of 4-alkene acids (number of carbon atoms: 10, 12 and 14) contained in the seed oil of Litsea glauca Sieb. of the family Lauraceae (tsuzu oil). Furthermore, these acids were synthesized (2) and their properties were clarified.

In the present report, the fatty acid composition of the seed oil from Lindera obtusiloba Blume of the family Lauraceae (tohaku oil) was studied.

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* Ibaraki Research Laboratory of Nagaoka Perfumery Co., Ltd. (218 Hatakeda, Ibaraki-shi)
** Faculty of General Education, Kinki University (3-4-1 Kowakae, Higashiosaka-shi)

The fatty acid composition of Lindera obtusiloba Blume has been studied for a long time and the presence of the following acids has been established : decanoic acid (3), dodecanoic acid (3) and tetradecanoic acid (4) as saturated fatty acids and 4-decenoic acid (tohaku oil) (5) (6), 4-dodecenoic acid (Linder oil) (3) (5) (6), 4-tetradecenoic acid (tsuzu oil) (5) (6), oleic acid (3) (5) and linoleic acid (5) as unsaturated fatty acids. However, there is no report on the geometrical isomerism of 4-alkene acids containing 10, 12 and 14 carbons. Here are the only articles available : Iwakiri (7) has synthesized the trans-form of these acids containing the above number of carbons and Komori et al. (6) have compared them with natural 4-alkene acids isolated from the seed oil of Lindera obtusiloba Blume. Also very few studies are reported concerning the components contained in little quantity.

The purpose of the present study is to isolate the tohaku oil from the seed of Korean Lindera obtusiloba Blume and to examine the fatty acid composition of this oil in all its aspects. Chemical methods as well as instrumental analysis were employed to this end.

As a result of our study, it was confirmed by comparing with standard samples (2), that all 4-alkene acids having 10, 12 and 14 carbons contained in the seed oil of Lindera obtusiloba Blume were cis-form. Furthermore, besides the known fatty acids, the presence of the following acids was confirmed : octanoic acid, undecanoic acid, tridecanoic acid, pentadecanoic acid, hexadecanoic acid and

octadecanoic acid as saturated acids and hexadecenoic acid and eicosenoic acid as unsaturated acids.

2. Experiments and results

2. 1. Measurement methods

Neither boiling point nor melting point were corrected.

The gas chromatography was carried out by a Shimazu gas chromatograph, model GC-5APTF with a column of 3 mm x 2 m, filled with DEGS (10%), PEG-20M (10%). The column temperature was 120°C - 220°C. Helium was used as carrier gas.

The infrared spectrum was measured using a Shimazu spectrometer, model IR-400, by the liquid film method with a sodium chloride cell.

A JEOL spectrometer, model JNM-MH-100 (100 MH_z) was used for the nuclear magnetic resonance analysis. The analysis was carried out in $CDCl_3$ at room temperature using TMS as the internal standard.

The mass spectrum was measured with a Shimazu spectrometer, p191 model LKB-9000 GC-MS, at an ionization voltage of 70eV.

2. 2. Standard samples

Standard samples of methyl octanoate, decanoic acid methyl ester, undecanoic acid methyl ester, dodecanoic acid methyl ester, tridecanoic acid methyl ester, tetradecanoic acid methyl ester, hexadecanoic acid methyl ester, octadecanoic acid methyl ester, methyl oleate and methyl linoleate were prepared by converting commercial acids into methyl ester by the usual method using diazomethane.

Standard samples of cis-4-decenoic acid methyl ester, cis-4-dodecenoic acid methyl ester, cis-4-tetradecenoic acid methyl ester and p-bromophenacyl ester of these acids were synthesized during the previous experiment (2).

The pentadecanoic acid methyl ester used as the standard sample was synthesized by the following method (9). 49.0 g (0.1 mol) of the [3-(methoxycarbonyl) propyl] triphenylphosphonium iodid obtained by the reaction between 4-methyl iodobutyrate and triphenylphosphine was brought into reaction with 5.4 g (0.1 mol) of dried sodium methoxide in dimethylformamide.

The [3-(methoxycarbonyl) propylidene] triphenylphosphoran* obtained by the above reaction was subjected to condensation with undecanal in order to obtain 10.5 g of cis-4-pentadecenoic acid methyl

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* Translator's note : the word triphenylphosphoran is transliterated.

ester (yield 59.0% against undecanal, bp 146-147°C/3 mmHg, n_D^{25} 1.4456, d_4^{25} 0.8743). This methyl ester was hydrolyzed to cis-4-pentadecenoic acid (bp 169°C/2 mmHg, n_D^{25} 1.4538, d_4^{25} 0.8943, p-bromophenacyl ester mp 62.0-63.0°C) which was subjected to catalytic reduction by the usual method (7) using platinum oxide. The pentadecanoic acid [mp 53.0-54.0°C (value given in the document (10) : mp 52.3°C)] thus obtained was converted into methyl ester with diazomethane.

2. 3. Sample of the seed oil of Lindera obtusiloba Blume and its fatty acid composition

419 g of air-dried seeds of Korean Lindera obtusiloba Blume 6 - 7 mm in diameter were separated into 96 g of seed coat and 323 g of nucleolus. The nucleolus was ground to rough powder which was subjected to extraction with hexane in a Soxhlet's extractor. 200 g (yield 61.9% against nucleolus) of seed oil of Lindera obtusiloba Blume was obtained after the removal of the hexane. This is a lemon yellow liquid at room temperature and presents the following constants : n_D^{25} 1.4620, d_4^{25} 0.9308 ; acid value : 1.0 ; saponification value : 272.0 ; iodine value (Wijs' method) : 68.4 ; unsaponifiable matter : 1.6%.

100.0 g of the seed oil of Lindera obtusiloba Blume, with 800 ml of 5% ethanolic potassium added, was subjected to heating reflux. After the removal of the ethanol, water was added to the residue. Subsequently, 1.6 g of unsaponifiable matter was extracted by means of ether. The saponifiable matter was acidified with hydrochloric acid.

After the extraction of acid by ether and washing with water, the saponifiable matter was dried with anhydrous sodium sulfate in order to eliminate ether. 90.0 g of fatty acid of the seed oil of Lindera obtusiloba Blume thus obtained presents the following constants : n_D^{25} 1.4482, d_4^{25} 0.9002 ; neutralization value : 273.5 ; iodine value (Wijs' method) : 68.8.

2. 4. Gas chromatographic analysis of methyl esters of fatty acid from the seed oil of Lindera obtusiloba Blume

The fatty acid of the seed oil from Lindera obtusiloba Blume was methylated by the usual method using diazomethane. This methyl ester was analyzed by means of GLC and 16 peaks of compounds (1) - (16) were observed. Furthermore, the methyl ester was analyzed by GLC after having been subjected to catalytic reduction with platinum oxide in methanol. This time, peaks of the compounds (3), (6), (9), (12), (14), (15) and (16) disappeared. This confirmed the presence of seven unsaturated fatty acids in the seed oil of Lindera obtusiloba Blume.

The composition and content of the seed oil of Lindera obtusiloba Blume were also obtained by the experiments described in 2.5. The results are shown in Table-1.

Table-1. The fatty acid composition of the seed oil from *Lindera obtusiloba* Blume.

Peak No.*	Fatty acid	Content(%)**
(1)	Octanoic acid	Tr.
(2)	Decanoic acid	4.8
(3)	*cis*-4-Decenoic acid	4.1
(4)	Undecanoic acid	Tr.
(5)	Dodecanoic acid	33.5
(6)	*cis*-4-Dodecenoic acid	37.8
(7)	Tridecanoic acid	Tr.
(8)	Tetradecanoic acid	3.6
(9)	*cis*-4-Tetradecenoic acid	5.0
(10)	Pentadecanoic acid	Tr.
(11)	Hexadecanoic acid	0.6
(12)	Hexadecenoic acid	0.2
(13)	Octadecanoic acid	0.7
(14)	Oleic acid	6.7
(15)	Linoleic acid	2.8
(16)	Eicosenoic acid	0.2

* As methyl ester.

** Content was calculated by means of SHIMAZU digital integrator ITG-2A combined GLC. GLC : column, 10% DEGS on celite 545 AW, 3mm×2m, carrier gas He, column temp. 120 (3min, hold)~180℃ (8℃/min)

2. 5. Inspection of the fatty acid composition of the seed oil from Lindera obtusiloba Blume

2. 5. 1. Fractional distillation of the fatty acid methyl ester

68.5 g of the methyl ester prepared from 70.0 g of the fatty acid contained in the seed oil of Lindera obtusiloba Blume was subjected to vacuum distillation in the nitrogenous current by means of Widmer's fractionating tower. The results are reported in Table-2. p192

Table-2. Fractional distillation of the fatty acid methyl ester from the seed oil of *Lindera obtusiloba* Blume.

Distillate No.	bp (℃/3mmHg)	Yield (g)
1	85 ~ 95	5.0
2	95 ~ 110	19.5
3	110 ~ 115	24.0
4	115 ~ 135	8.5
5	135 ~ 145	2.0
6	145 ~ 165	4.0
7	165 ~ 172	1.5
8	Residue	2.0

2. 5. 2. Identification of the unsaturated fatty acid composition

(3) : The distillate No. 1 obtained by the fractional distillation of the fatty acid methyl ester contained in the seed oil of Lindera obtusiloba Blume was used as sample. According to the GLC analysis, the sample contains 22.5% of the compound (3). The chromatography was carried out by the same method described in the previous report (1) using a silicic acid - silver nitrate column. The column was prepared as follows : to 320 g of the silicic acid for chromatographic use of the Marinchrodt Co. (100 mesh), was added a solution prepared by dissolving 80 g of silver nitrate in 600 ml of hot water. The mixture was well blended and left for a night. Then it was activated at 130°C for 8 hours. To 100 g of the packing thus obtained, was added 30 g of celite 535. After having mixed in hexane, the suspension was introduced in a glass tube 25 mm in interior diameter and 500 mm long. A hexane solution of 1.5 g of the sample was added in the column. The development was

carried out using successively :

- 100 ml of a hexane solution containing 5% of ether,
- 200 ml of a solution containing 7.5% of ether,
- 200 ml of a solution containing 10% of ether,
- 100 ml of a solution containing 20% of ether,
- 200 ml of a solution containing 30% of ether.

Each 5 g of the eluate was collected and analyzed by GLC for the purpose of gathering the eluate which contains only compound (3) as a component. After eliminating the solvent in the presence of nitrogen, 220 mg of compound (3) was obtained.

By IR spectrometry, a characteristic band due to the olefinic C-H stretching vibration was observed at 3010 cm^{-1}. Also, another characteristic band due to the C-H out-of-plane and deformation vibration of cis-olefin and another due to the C=O stretching vibration of ester were observed at 720 cm^{-1} and 1745 cm^{-1} respectively. No absorption of the substitution 2 trans-double band was observed around 960 - 980 cm^{-1}. These facts prove that the double bond is a cis-form.

By NMR spectrometry, the following signals were observed : δ 0.88 (3H, t, CH_3CH_2), 1.30 [6H, m, $(CH_2)_3$], 2.04 (2H, m, $CH_2CH=$), 2.34 (4H, d, $=CHCH_2CH_2CO$), 3.66 (3H, s, OCH_3) and 5.38 (2H, m, CH=CH).

By MS spectrometry, the following peaks were detected : m/e 184 (M^+, 6%), 152 (M^+-CH_3OH, 33%), 110 (M^+-74, 61%), 74 [$CH_2=C(\overset{+}{O}H)OCH_3$, 100% , 55 ($C_4H_7^+$, 59%), 41 ($C_3H_5^+$, 60%), etc.

Based on these results, the compound was presumed to be cis-4-decenoic acid methyl ester. The IR, NMR and MS spectrums and the retention time (Rt) of GLC presented by this compound were in accord with those of the standard sample. Furthermore, compound (3) was converted into free acid by saponification with alcoholic potash in order to synthesize p-bromophenacyl ester (mp 47.0 - 48.0°C ; recrystallized from alcohol ; white flaky crystal) by the usual method (8). This p-bromophenacyl ester was subjected to mixed examination with the standard sample. As a result, a decrease in the melting point was not observed.

(6) : Distillate No. 3 in Table-2 containing 47.5% of compound (6) was used as a sample. 580 mg of compound (6) was obtained from 1.5 g of the sample by chromatography with a silicic acid - silver nitrate column as in the case of (3).

By IR spectrometry, a characteristic band due to the olefinic C-H stretching vibration was observed at 3010 cm^{-1}. Likewise, another band due to the C-H out-of-plane and deformation vibration of cis-olefin and another due to the C=O stretching vibration of ester were observed at 720 cm^{-1} and 1740 cm^{-1} respectively. No absorption of the substitution 2 trans-double bond was observed around 960 - 980 cm^{-1}.

By NMR spectrometry, the following signals were observed : δ 0.89 (3H, t, CH_3CH_2), 1.29 [10H, s, $(CH_2)_5$], 2.05 (2H, m, $CH_2CH=$), 2.36 (4H, d, $=CHCH_2CH_2CO$), 3.68 (3H, s, OCH_3) and 5.38 (2H, m, CH=CH).

By MS spectrometry, the following peaks were detected : m/e 212 (M^+, 6%), 180 (M^+-CH_3OH, 26%), 138 (M^+-74.52%), 74 [$CH_2=C(\overset{+}{O}H)OCH_3$, 100%], 55 ($C_4H_7^+$, 62%), 41 ($C_3H_5^+$, 61%), etc.

Based on these results, the compound was presumed to be cis-4-dodecenoic acid methyl ester. The IR, NMR and MS spectrums and Rt of GLC of the compound were in accord with those of the standard sample. Furthermore, compound (6) was converted into free acid by saponification with alcoholic potash in order to synthesize p-bromophenacyl ester (mp 55.0 - 56.0°C ; recrystallized from alcohol ; white flaky crystal) by the usual method (8). According to the mixed examination with the standard sample, a decrease in the melting point was not observed.

(9) : Distillate No. 5 in Table-2 containing 33.5% of compound (9) was used as sample. 320 mg of compound (9) was obtained from 1.5 g of the sample by means of silicic acid - silver nitrate column chromatography in the same manner as (3).

By IR spectrometry, characteristic bands due to respectively the olefinic C-H stretching vibration, the C-H out-of-plane and deformation vibration of cis-olefin and the C=O stretching vibration

of ester were observed at 3010 cm^{-1}, 720 cm^{-1} and 1740 cm^{-1}. No characteristic band of the substitution 2 trans-double bond was observed around 960 - 980 cm^{-1}.

By NMR spectrometry, the following signals were observed : δ 0.89 (3H, t, CH_3CH_2), 1.28 [14H, s, $(CH_2)_7$], 2.05 (2H, m, $CH_2CH=$), 2.36 (4H, d, $=CHCH_2CH_2CO$), 3.68 (3H, s, OCH_3) and 5.38 (2H, m, CH=CH).

By MS spectrometry, the following peaks were detected : m/e 240 (M^+, 4%), 208 (M^+-CH_3OH, 23%), 166 (M^+-74, 37%), 74 [$CH_2=C(O^+H)OCH_3$, 100%], 55 ($C_4H_7^+$, 73%), 41 ($C_3H_5^+$, 76%), etc.

Based on the results, the compound was presumed to be cis-4-tetradecenoic acid methyl ester. Compared with the standard sample, IR, NMR and MS spectrums and Rt of GLC were in complete agreement. Furthermore, the compound (9) was converted into free acid by saponification with alcoholic potash in order to synthesize p-bromophenacyl ester (mp 62.5 - 63.5°C ; recrystallized from alcohol ; white flaky crystal) by the usual method (8). According to the mixed examination with the standard sample, no decrease in the melting point was observed.

(12) : By MS spectrometry, the following peaks were detected : m/e 268 (M^+, 5%), 236 (M^+-CH_3OH, 27%), 194 (M^+-74, 21%), 74 [$CH_2=C(\overset{+}{O}H)OCH_3$, 79%], 69 ($C_4H_9^+$, 63%), 55 ($C_4H_7^+$, 100%), 41 ($C_3H_5^+$, 79%), etc.

Based on the results, the compound was presumed to be hexadecenoic acid methyl ester.

(14) : By MS spectrometry, the following peaks were detected : m/e 296 (M^+, 9%), 264 (M^+-CH_3OH, 39%), 222 (M^+-74, 18%), 180 (M^+-116, 14%), 74 [$CH_2=C(\overset{+}{O}H)OCH_3$, 38%], 69 ($C_5H_9^+$, 69%), 55 ($C_4H_7^+$, 100%), 41 ($C_3H_5^+$, 66%), etc.

These results were in accord with the values given in reference (11). The compound was therefore presumed to be methyl oleate. In addition, Rt of GLC was in accord with that of the standard sample. The compound was consequently identified as methyl oleate. p193

(15) : MS spectrometry detected the following peaks : m/e 296 (M^+, 40%), 263 (M^+-CH_3O, 19%), 220 (M^+-74, 6%), 81 ($C_6H_9^+$, 88%), 74[$CH_2=C(\overset{+}{O}H)OCH_3$, 13%], 67 ($C_5H_7^+$, 100%), 55 ($C_4H_7^+$, 60%), 41 ($C_3H_5^+$, 50%), etc.

Since the results were in agreement with the values given in reference (12), the compound was presumed to be methyl linoleate. Furthermore, Rt of GLC being in accord with that of the standard sample, the compound was identified as methyl linoleate.

(16) : MS spectrometry detected the following peaks : m/e 324 (M^+, 9%), 292 (M^+-CH_3OH, 57%), 250 (M^+-74, 14%), 208 (M^+-116, 11%), 74 [$CH_2=C(\overset{+}{O}H)OCH_3$, 57%], 69 ($C_5H_9^+$, 68%), 55 ($C_4H_7$, 100%), 41 ($C_3H_5^+$, 57%), etc.

According to the results, the compound was presumed to be eicosenoic acid methyl ester.

2. 5. 3. Identification of the saturated fatty acid composition

The results of the MS spectrometry are given in Table-3. The identity of each saturated fatty acid methyl ester was presumed based on these results. Since Rt of GLC of these compounds were in agreement with that of the standard samples, they are identified as follows :

(1) methyl octanoate

(2) decanoic acid methyl ester

(4) undecanoic acid methyl ester

(5) dodecanoic acid methyl ester

(7) tridecanoic acid methyl ester

(8) tetradecanoic acid methyl ester

(10) pentadecanoic acid methyl ester

(11) hexadecanoic acid methyl ester

(13) octadecanoic acid methyl ester

Table-3 Results of MS analysis of saturated fatty acid methyl esters contained in the seed oil of *Lindera obtusiloba* Blume.

Peak No. in Table-1	Mass spectral data *m/e* (%)		
	M^+	M^+-CH_3O	$CH_2=\overset{+}{(OH)}OCH_3$
(1)	158(3)	127(17)	74(100)
(2)	186(10)	155(24)	74(100)
(4)	200(5)	169(11)	74(100)
(5)	214(10)	183(13)	74(100)
(7)	228(9)	197(7)	74(100)
(8)	242(13)	211(8)	74(100)
(10)	256(10)	225(12)	74(100)
(11)	270(18)	239(7)	74(100)
(13)	298(21)	267(6)	74(100)

3. Discussion

Komori et al. (6) isolated tohaku acid (4-decenoic acid), Linder acid (4-dodecenoic acid) and tsuzu acid (4-tetradecenoic acid) from the seed oil of Lindera obtusiloba Blume. However, the melting points of p-bromophenacyl esters which are the crystalline derivatives of the above acids are slightly lower than our results. These acids seem to contain impurities.

Hopkins et al. (13) identified the fatty acid composition contained in the seed oil of Lindera umbellata Thunb. of the family Lauraceae. They reported that the principal component was cis-4-dodecenoic acid (47%). The presence of cis-4-decenoic acid (4%) and cis-4-tetradecenoic acid (5%) was also reported. This composition closely resembles the fatty acid composition found in the seed

oil of Lindera obtusiloba Blume. Accordingly, the results of our experiments seem interesting.

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