J. of Korean Oil Chemists' Soc.,
Vol. 22, No. 4. December, 2005. 323~331

Identification of Petroselinic Acid (*Cis*-6-octadecenoic Acid) in the Seed Oils of Some of the Family Umbelliferae (*Panax schinseng*, *Aralia continentalis* and *Acanthopanax sessiliflorus*) by GC-MS, IR, ^{1}H-and ^{13}C-NMR Spectroscopic Techniques

Seong-Jin Kim†

Department of Food Science and Nutrition, Dong-A University, Busan 604-714, Korea

(Received July 7, 2005 ; Accepted September 12, 2005)

Abstract : Fatty acid compositions of the seed oils of *P. schinseng*, *A. continentalis* and *A. sessiliflorus*, were analyzed by gas chromatography (GC) equipped with a capillary column. A large unusual peak was observed just before the peak corresponding to oleic acid (*cis*-9-$C_{18:1}$). This unknown fatty acid was isolated by silver ion chromatography and then derivatized into the picolinyl ester. The mass spectrum of the picolinyl ester showed molecular ion at m/z=373 with other diagnostic ions such as m/z=178, 218, 232, 246, 274, 288, 302 and 344. Characteristic absorption peaks at 720 cm^{-1}, 1640 cm^{-1} and 3010 cm^{-1} in IR spectrum indicated the presence of *cis*-configurational double bond in the molecule. The ^{1}H-NMR spectrum of this acid gave two quintets centered at δ1.638 (2H, C-3) and δ1.377 (2H, C-4), and two multiplets centered at δ2.022~2.047 (2H, C-5) and δ2.000~2.022 (2H, C-8), and multiplet signals of olefinic protons centered at δ5.3015~5.3426 (C-6, J=9.5 Hz) and δ 5.3465~5.3877 (C-7, J=9.5 Hz). The ^{13}C-NMR spectrum showed 18 carbon resonance signals including an overlapped signal at δ29.7002 for C-12 and δ 29.6520 for C-13 (or they can be reversed), and other highly resolved signals at δ33.950, δ24.558, δ 26.773 and δ27.205 due to C-2, C-3, C-5 and C-8 of a Δ^{6}-octadecenoic acid, respectively. From analysis results this unknown fatty acid could be identified as *cis*-6-octadecenoic acid. The seed oils of *P. schinseng* and *A. sessiliflorus* contained petroselinic acid (59.7 %, 56.0%), oleic acid (18.3%, 6.1%) and linoleic acid (16.2 %, 30.4%) with small amount of palmitic acid (3.0%, 3.1%) while the seed oil of *A. continentalis* comprised mainly oleic acid (30.2%), petroselinic acid (29.0%), linoleic acid (24.1%) and palmitic acid (13.1%).

Keywords : *Panax schinseng, Aralia continentalis, Acanthopanax sessiliflorus, petroselinic acid, silver ion chromatography.*

† 주저자 (e-mail : sjkim@kicm.re.kr)

1. Introduction

The families Araliaceae, Umbelliferae and Garryaceae of the order Umbelliflorae are characterized by high levels of petroselinic acid (*cis*-6-$C_{18:1}$, $C_{18:1\omega12}$) in their seed oils, with a considerable variation of its content among the species[1]. Petroselinic acid is less soluble in organic solvent than one of its positional isomers, oleic acid, so it is easily separated from the other isomer by crystallization[2]. Petroselinic acid has higher melting point (33℃) than that of oleic acid (12℃) which may be advantageous for certain industrial and nutritional uses of this fatty acid. In addition, this fatty acid is less sensitive to pancreatic lipase than oleic acid in the triacylglycerol (TG)[3]. As such, TGs rich in petroselinic acid may offer an "low-fat" substitute for conventional vegetable oils. Petroselinic acid can be also split oxidatively to lauric ($C_{12:0}$) and adipic acid (C_6-dicarboxylic) with are valuable materials for production of surfactants, cosmetics and pharmaceuticals[4~6]. Our government imports annually considerable amounts of lauric rich oils, about half of which is used as surfactants and the other half as edible products. For the purpose of obtaining more information on the oil chemistry of the seeds of the Umbelliflorae, the species, *Panax schinseng*, *Aralia continentalis* and *Acanthopanax sessiliflorus* of the family Araliaceae were included in our consecutive lipid research project on unutilized seeds. *P. schinseng* usually using the root has been used as a cure-all medicine in the Far East, and they thus have been cultivated agronomically for centuries in Korea and the northern part of China. The other species, *A. continentalis* and *A. sessiliflorus*, have been also used as traditional folk medicines such as an anticancer, an antifebrile and a cardiac and a tonic in this area[7].

In the present works, an unusual fatty acid was isolated purely from the seed oils of *P. schinseng*, *A. continentalis* and *A. sessiliflorus* by silver ion solid-phase extraction chromatography and silver ion column chromatogrphy, and was identified as *cis*-6-octadecenoic acid ($C_{18:1\omega12}$) by GC-MS, IR and ^{1}H- and ^{13}C-NMR spectroscopy. The oils of *P. schinseng*, *A. continentalis* and *A. sessiliflorus* seeds, which had not been reported previously, were found to have *cis*-6-octadecenoic acid ($C_{18:1\omega12}$) as its major fatty acid.

2. Experimental

2.1. Lipids samples and reagents

The seeds of *P. schinseng*, *A. continentalis* and *A. sessiliflorus* grown in herb-cultivating fields, were collected at farmer's markets in Sunchun, Chun-Nam Province, Korea, in November 1999. The air-dried, smashed seeds were extracted according to the method of Bligh & Dyer[8] in a stream of nitrogen. All solvents and reagents were HPLC-grade, and supplied by Merck Ltd. Fatty acid and TG standards were purchased from Sigma-Aldrich and Nu-Chek Prep, Inc (Elysian, MN, USA)

2.2. Gas-liquid chromatography (GC) of FAMEs[9]

The sample was transmethylated with sodium methoxide-methanol solution for 5 min at ambient temperature in the presence of an internal standard of methyl henecosanoate ($C_{21:0}$, 25 μL, 5.8 mg/25 mL hexane) along with BHT (1 mL, 50 mg/25 mL hexane), and a mixture of the methyl esters was then recovered with hexane. Methyl esters of fatty acids were analyzed with a Hewlett-Packard Model 5890 Series II gas chromatograph, fitted with split/splitless injection system, and equipped with a capillary column (25 m × 0.22 mm, i.d., 0.25 μm film) of fused silica coated with BPX 70 (70% cyanopropylpoly-silphenylene siloxane,

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts of Methyl ester of *Cis*-6-octadecenoic Acid Isolated from the Seed Oils of *P. schinseng*

Carbon	Shift	
	δ^{1}_{H}	δ^{13}_{C}
1	-	174.0740
2	2.3073 (2H, *t*)	33.9496
3	1.6382 (2H, *qnt*)	24.5576
4	1.3774 (2H, *qnt*)	29.1864
5	2.0344 (2H, *qt*)	26.7726
6	5.3196 (1H, *m*)	129.0120
7	5.3760 (1H, *m*)	130.4290
8	2.0111 (2H, qt)	27.2052
9	1.3256 (2H, *m*)[a]	29.7374
10		29.3192
11		29.5309
12		29.7002[b]
13	1.2591~1.2958	29.6520[b]
14	(14H, *m*)	29.6179
15		29.2914
16		31.8939
17	1.3736 (2H, *m*)[a]	22.6518
18	0.8773 (3H, *t*)	14.0473
OCH_3	3.6594 (3H, *s*)	51.3480

[a]Signals are not fully resolved
[b]Signals may be reversed

SEG, Austin, TX, USA). The column temperature was held at 160℃ for 3 min and then programmed up to 220℃ at 3℃/min with a final hold for 10 min. Hydrogen was the carrier gas and the area of each peak on GC chromatogram was quantified by electronic integration. The analysis results corrected according to Christie method[9], are given in Table 2. Each figure is the mean of triplicate measurements.

2.3. Fractionation of total fatty acid methyl esters (FAME) according to double bond by silver-ion chromatography[10,11]

Disposable solid-phase extraction columns packed with a solid-phase extraction column (SCX disposable column, Varian Bond Elut^{TM}SCX, Varian Inc., Palo Alto, CA, USA), were impregnated with silver ions (Ag^{+}-SEC) and equilibrated with dichloromethane (DCM) before use. An aliquot (1~3 mg) of total FAME dissolved in a small amount of DCM was loaded on the column. The samples were then resolved into saturated acid fraction with 100% DCM (10 mL), monoenoic acid fraction with DCM-acetone (10 mL, 9 : 1, v/v) and finally dienoic acid fraction with 100%

Table 2. Fatty Acid Composition of Total Lipids from the Seeds of *P. schinseng*, *A. continentalis* and *A. sessiflorus* (wt %)

Fatty acid	*P. schinseng*	*A. continentalis*	*A. sessiliflorus*
$C_{16:0}$	3.0	13.1	3.1
$C_{16:1\omega7}$	0.4	0.7	0.1
$C_{18:0}$	0.4	1.3	0.7
$C_{18:1\omega12}$	59.7	29.0	56.0
$C_{18:1\omega9}$	18.3	30.2	6.1
$C_{18:1\omega7}$	1.4	1.0	1.1
$C_{18:2\omega6}$	16.2	24.1	30.4
$C_{18:3\omega3}$	tr*	tr	2.3
$C_{20:1\omega9}$	0.6	0.5	0.2

tr*; trace

acetone (5 mL). Fraction containing this unusual fatty acid was concentrated and then hydrolyzed with 5% KOH-ethanol solution. Free fatty acids released were recovered with diethyl ether, and the solvent layer was evaporated under a stream of nitrogen, and then the free fatty acids were derivatized into their picolinyl esters. For measuring IR and ^{1}H-& ^{13}C-nuclear magnetic resonance (NMR) spectra of the unknown component, silver ion column chromatography was used instead of Ag^{+}-SEC[12] ; a glass-column (1.5 × 50 cm) was packed with Silica Gel 60 (70~230 mesh, Merck, Darmstadt, Germany) impregnated with silver nitrate (16% to absorbent weight) and was equilibrated with hexane overnight. A portion (400 mg) of total FAME dissolved in a small amount of hexane was loaded onto the column, which was then eluted step-wisely with 15% hexane/ benzene (300 mL, saturated acid fraction), followed with 30% hexane/benzene (500 mL, monoenoic acid fraction) and 100% benzene (500 mL, dienoic and trienoic acid fraction). Excess solvent in the fraction containing unknown fatty acid was removed in a stream of nitrogen. Semi-solid residues obtained was rechromatographed repeatedly four~five times on silver ion-loaded columns freshly prepared.

2.4. Preparation of picolinyl esters

Picolinyl derivatives of fatty acids were prepared essentially according to the method recommended by Balazy and Nies[13], *i. e.*, a solution of 1, 1'-carbonyl diimidazole in DCM freshly prepared (100 μL, 100 mg/mL) was added to the 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 with triethylamine (100 μL). The mixture was left for 10 min at 37℃ and was then taken to dryness 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 on a short column (Pasteur pipette) of Florisil^{TM} (0.5 g); non-polar impurities were washed out with a solvent of hexane/acetone (10 mL; 9 : 1, v/v), and then the picolinyl derivatives required could be purely isolated by elution with hexane/acetone (10 mL, 8 : 2, v/v).

2.5. GC/Mass spectrometry (GC/MS)[14]

The instrument used was a Micromass model Autospec Ultima High Resolution Trisector EBI Ion Optics Mass GC/MS Spectrometer (Micromass, Wythenshawe, Manchester, UK). 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 Alto, CA, USA) was connected directly to the ion source. The column temperature was held for 3 min and then programmed from 80℃ to 160 ℃ at 30℃/min, and then 315℃ at 4℃/min. The column was held at this temperature for 20 min.

2.6. Fourier transform infrared spectroscopy (IR)

The spectrum was measured on a KBr disc and recorded using a Bruker IFS 66 FTIR spectrometer (Bruker Spectrospin Ltd., Conventary, UK) according to Chapman[15].

2.7. ^{1}H-and ^{13}C-nuclear magnetic resonance (NMR)

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

3. Results and Discussion

Fatty acid components in the seed oils of *P. schinseng*, *A. continentalis* and *A. sessiliflorus* belonging to the family Umbelliferae, were analyzed. As shown in Fig. 1 (GC chromatogram of *P. schinseng* was typically shown here) the fatty acid composition of total lipids from all the seeds tested was very simple, and most of the peaks appeared on GC chromatogram could be easily identified by comparing their retention times with those of authentic fatty acids. But a peak eluted just a few second earlier than that of oleic acid could not be identified. A FAME mixture (below 5 mg) of each of the seed oils was classified into saturated, monoenoic and dienoic (plus trienoic) acid fraction by elution of solvent

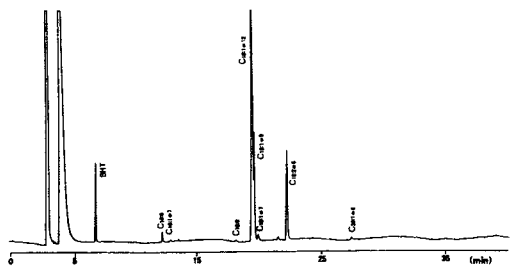

Fig. 1. GLC chromatogram of fatty acid methyl esters of total lipids from the seeds of *P. schinseng*.

system of 100% DCM, DCM-acetone (9 : 1, v/v) and 100% acetone on Ag^{+}-SEC, respectively, and the unknown fatty acid existed in the monoenic acid fraction (DCM-acetone, 9 : 1, v/v) with oleic acid. Free fatty acids released by hydrolysis of the monoenoic acid fraction was recovered with diethyl ether and then derivatized into their picolinyl esters prior to injection onto GC-MS. In the GC-mass spectrum of this fatty acid shown in Fig. 2, the derivative of this fatty acid gives molecular ion at m/z=373 which corresponds to octadecamonoenoic acid

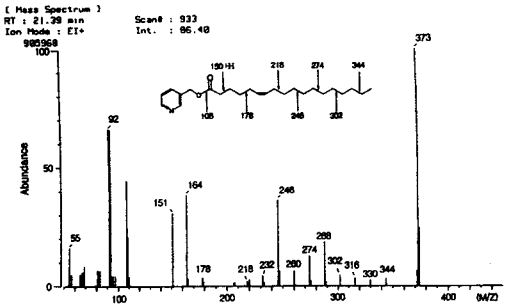

Fig. 2. The mass spectrum of the picolinyl ester of *cis*-6-octadecenoic acid isolated from the seed oils of *P. schinseng*.

($C_{18:1}$), and a gap of 40 amu between m/z=178 and m/z=218 indicates the presence of a double bond between C-6 and C-7, and other diagnostic ions such as m/z=151, 164, 232, 246, 274, 288, 302 and 344 are clearly observed[16]. And the absorption peaks at 720 cm^{-1}, 1458 cm^{-1}, 1640 cm^{-1}, 1744 cm^{-1}, 2854 cm^{-1}, 2925 cm^{-1} and 3010 cm^{-1} in the IR spectrum indicate the presence of an unsaturated fatty acid with *cis*-configurational double bond [15,17] (Fig. 3). From these results this unexpected fatty acid can be tentatively identified as petroselinic acid (*cis*-6-$C_{18:1}$).

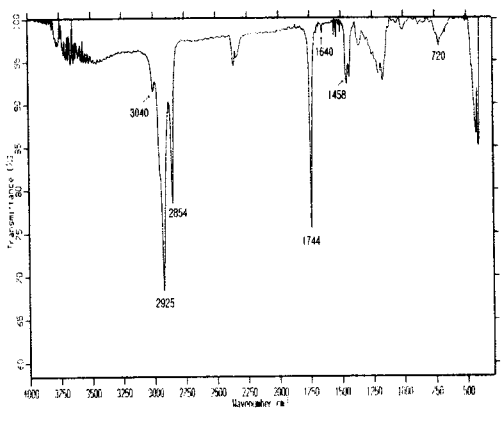

Fig. 3. The IR spectrum of the picolinyl ester of *cis*-6-octadecenoic acid isolated from the seed oils of *P. schinseng*.

A mixture of FAMEs (400~600 mg) of each of the seed oils was classified into saturated acid fraction, monoene and diene (plus triene) acid fraction on silicic acid column impregnated with silver nitrate, by elution of 15%, 30% hexane/benzene, and 100% benzene, respectively. The monoene acid fraction (300~400 mg) also contained the unusual fatty acid, and this fraction was re-chromatographed repeatedly four~five times to remove contaminated oleic acid, on freshly prepared silver ion loaded silicic acid columns with developing the solvent system

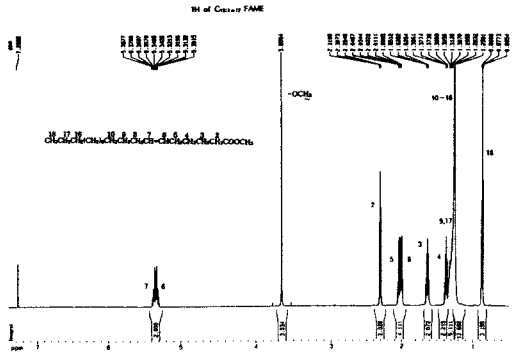

Fig. 4. ^{1}H-NMR spectrum of the methyl ester of *cis*-6-octadecenoic acid isolated from the seed oils of *P. schinseng.*

above-mentioned. Thus the unknown fatty acid could be obtained purely (purity, up to 97%).

Both of the ^{1}H- and ^{13}C-NMR spectra gave more useful information to elucidate the structure of this acid and particularly ^{1}H-^{13}C two-dimensional shift correlation NMR techniques also facilitated the assignment of all resonance signals. In the ^{1}H-NMR spectrum (Fig. 4), the singlet at δ 3.6594 was the resonance signal of methyl protons of the methoxy and two triplets centered at δ 0.8773 and δ 2.3073 each could be easily ascribed to the protons of the end methyl (C-18) and the methylene radical at carbon 2 (C-2) in the molecule. And both the methylene protons of C-5 and C-8 were deshielded by induction current produced from π-electrons of vicinal methine radical in the double bond between C-6 and C-7, and then their resonance signals appeared as multiplets at lower magnetic field of δ 2.0222~2.0467 and δ 1.9995~2.0222 respectively[17,18]. ^{13}C-NMR spectrum (Fig. 5-a & 5-b) showed fifteen singlets, one doublet and one overlapped signal, neglecting those due to C-1 and the methoxyl carbon. The resonance peaks at δ 14.0473, δ 22.6518, δ 24.5576, δ 31.8939 and δ 33.9496 could be attributable to C-18, C-17, C-3, C-16 and C-2 in the fatty acyl chain

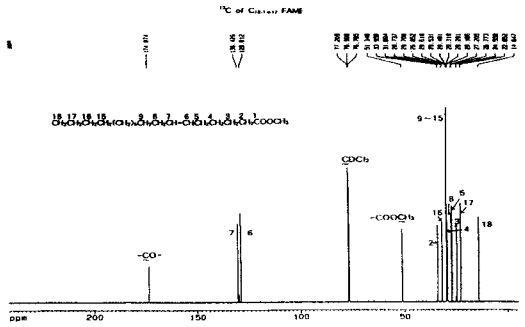

Fig. 5-a. ^{13}C-NMR spectrum of the methyl ester of *cis*-6-octadecenoic acid isolated from the seed oils of *P. schinseng.*

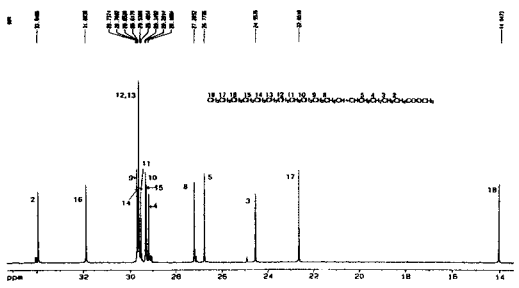

Fig. 5-b. ^{13}C-NMR spectrum of methylene radicals in the methyl ester of *cis*-6-octadecenoic acid isolated from the seed oils of *P. schinseng.*

respectively[19,20]. The multiplet centered δ 5.3015~5.3877 (2H) in proton spectrum, together with the signals in the range of δ 129.0120 and δ 130.4290 in the carbon spectrum, suggested the presence of one olefinic structure unit from the ^{1}H-^{13}C-NMR two-dimensional shift correlation spectrum (HMQC spectrum, Fig. 6). In addition, coupling constant (*J*) of 9.5 Hz observed between split signals at each of C-6 and C-7 in the ^{1}H-NMR spectrum indicated that the olefinic protons were in *cis*-configuration. In this way two multiplets centered at δ 1.3256 (2H) and δ 1.3736 (2H) were found to be the signals of C-9 and C-17 respectively, from the ^{1}H-^{13}C-NMR two- dimensional shift correlation spectrum. The chemical assignments of each hydrogen and carbon atom in the molecular chain are listed in Table 1. From the analytical results obtained the unknown fatty acid could be identified as *cis*-6-octadecenoic acid (petroselinic acid, an isomer of oleic acid). To the best of our knowledge, it is first time that petroselinic acid was isolated from seed oils purely, and identified by ^{1}H-^{13}C two dimensional shift correlation NMR techniques.

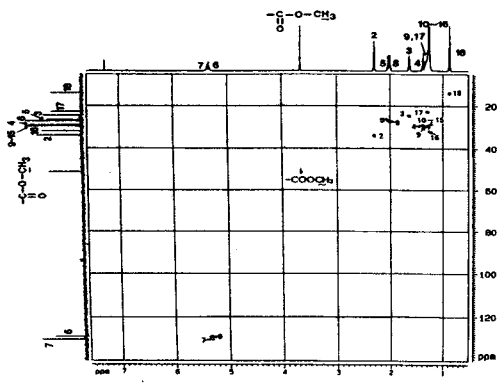

Fig. 6. ^{1}H-^{13}C two-dimensional shift correlation NMR spectrum of the methyl ester of *cis*-6-octadecenoic acid isolated from the seed oils of *P. schinseng*.

The fatty acid composition of the seed oils of *P. schinseng* and *A. sessiliflorus* consists of *cis*-6-octadecenoic acid (59.7%, 56.0%), oleic acid (18.3%, 6.1%) and linoleic acid (16.2%, 30.4%), with a small amount of palmitic acid (3.0%, 3.1%) as shown in Table 2. On the other hand, the seed oils of *A. continentalis* have oleic acid (30.2%), petroselinic acid (29.0%) and linoleic acid (24.1%) in a nearly equal amount as major constituents, followed with palmitic acid (13.1%). Kleiman *et al* [1] investigated the fatty acid composition of total oils from the seeds of the families Umbelliferae, Garryaceae, Araliaceae, Cornaceae, Davidiaceae, Nyssaceae and Alangiaceae of the order Umbelliflorae, for the purpose of selecting plant species containing higher level of petroselinic acid. They concluded that petroselinic acid was widely distributed in the seed oils of all the families tested, and occurred in amounts up to 85% in the Umbelliferae, 81% in the Garryaceae and 83% in the Araliaceae. These levels of petroselinic acid were much higher than those of our samples. Avato [21] also reported that the seed oils of the genus *Thapsia* of the family Umbelliferae contained high contents of petroselinic acid (69.7~82.3%) in the fatty acid composition.

4. Conclusions

The seed oils of *P. schinseng*, *A. continentalis* and *A. sessiliflorus*, have petroselinic acid (*cis*-6-$C_{18:1}$) as main component. This fatty acid known as potential resources of lauric and adipic acid, was purely isolated from total FAMEs of the oils by silver ion chromatography. The silver ion-SEC is found to be excellent to classify small amounts of fatty acid methyl esters (below 50 mg) into simple fractions according to the number of double bond while the silver column chromatography is suitable for

isolating and purifying large amounts of specific fatty acids (up to 1~3 g). ^{1}H-and ^{13}C two-dimensional shift correlation NMR makes it easy to confirm the chemical structure of unknown fatty acid.

Acknowledgments

The authors thank Prof. Dr. J. C. Park at Sunchun University for helping collect the samples and Dr. E. H. Kim at the Korea Basic Science Institute in Daejon, Korea, for carrying out NMR measurements.

References

1. R. Kleiman and G. F. Spencer, Search for New Industrial Oils : XVI. Umbelliforae-Seed Oils Rich in Petroselinic Acid, *J. Am. Oil Chem. Soc.*, **59**(1), 29 (1982).
2. F. D. Gunstone, *"Fatty Acid and Lipid Chemistry"*, p. 79, Blackie Academic & Professional, London (1996).
3. K. Mbayhoudel and L.-C. Comeau, Obtention Se'lective de L'acide Pe'tro-se'linique a' Partir de L'huile de Fenouil par Hydrolyse Enzymatique, *Rev. Fr. Corps Gras*, **36**, 427 (1989).
4. E. B. Cahoon, J. Shanklin, and J. B. Ohlrogge, Expression of a Coriander Desaturase Results in Petroselinic Acid Production in Transgenic Tobacco, *Proc. Natl. Acad. Sci. USA*, **89**, 1184 (1992).
5. D. J. Murphy, Identification and Characterization of Genes and Enzyme for the Genetic Engineering of Oilseed Crops for Production of Oils for the Oleochemical Industry : A Review, *Ind. Crops Prod.*, 1, 251 (1993).
6. J. B. Ohlrogge, Design for New Plant Products : Engineering of Fatty Acid Metabolism, *Plant Physiol.*, **104**, 821 (1994).
7. J. T. Song, H. B. Chung, B. W. Kim, and H. S. Jin, *"Colorful Korean Flora Encyclopedia"*, vol. 1, pp. 724~730, Che-Il Pub. Co., Ltd, Seoul (1989) (in Korean).
8. E. G. Bligh and W. J. Dyer, A Rapid Method of Total Lipid Extraction and Purification, *Canad. J. Physiol.*, **37**, 911 (1959).
9. W. W. Christie, *"GC and Lipids"*, p. 128, Oily Press, Ayr (1989).
10. W. W. Christie, Silver Ion Chromatography Using Solid-Phase Extraction Columns Packed with a Bonded-Sulfonic Acid Phase, *J. Lipid Res.*, **30**, 1471 (1989).
11. W. W. Christie, *"GC and Lipids"*, pp. 69-70, Oily Press, Ayr, Scotland (1989).
12. Y. G. Joh, K. H. Lee, and Y. J. Cho, Nonrandom Combination of Fatty Acid and Alcohol Moieties in Wax Esters from *Liza carinata* Roe, *Korean J. Food Sci. Technol.*, **21**(5), 623 (1989).
13. M. Balazy and A. S. Nies, Characterization of Epoxides of Polyunsaturated Fatty Acids by Mass Spectrometry via 3-Pyridinylmethyl Esters, *Biomed. Environ. Mass Spectrom.*, **18**, 328 (1989).
14. W. W. Christie, E. Y. Brechany, and K. Stefanov, Silver Ion High-Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry in the Analysis of Complex Fatty Acid Mixtures : Application to Marine Invertebrates, *Chem. Phys. Lipids*, **46**, 127 (1988).
15. D. Chapman, Infrared Spectroscopy of Lipids, *J. Am. Oil Chem. Soc.*, **42**, 353 (1965).
16. W. W. Christie, E. Y. Brechany, and T. Holman, Mass Spectra of the Picolnyl Esters of Isomeric Mono- and Dienoic Fatty Acids, *Lipids*, **22**, 224 (1987).
17. D. H. Williams and J. Fleming, *"Spectroscopic Methods in Organic Chemistry"*, McGraw-Hill, New York

(1966).

18. M. Silverstein, G. C. Bassler, and T. C. Morrill, *"Spectrometric Identification of Organic Compounds"*, 4th ed., pp. 181-238, John Wiley & Sons, New York (1981).
19. J. Bus, I. Sies, and M. S. F. Lie Ken Jie, ^{13}C-NMR of Methyl, Methylene and Carbonyl Carbon Atoms of Methyl Alkenoates and Alkynoates, *Chem. Phys. Lipids*, **17**, 501 (1976).
20. J. G. Batchelor, R. J. Cushley, and J. H. Prestegard, Carbon-13 Fourier Transform Nuclear Magnetic Resonance Ⅷ. Role of Steric and Electric Field Effects in Fatty Acids Spectra, *J. Org. Chem.*, **39**, 1698 (1974).
21. P. Avato, F. P. Fanizzi, and I. Rosito, The Genus *Thapsia* as a Source of Petroselinic Acid, *Lipids*, **36**, 845 (2001).