Phenylethyl Esters of Fatty Acids for the Analytical Resolution of Petroselinate and Oleate

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ABSTRACT: 2-Phenylethyl esters of fatty acids were prepared readily by esterification of free fatty acids or transesterification of other lipids. Compared with methyl esters, phenylethyl esters greatly improve the resolution of oleate and petroselinate by both gas and high-performance liquid chromatography, and the ultraviolet absorption of the phenylethyl esters facilitates detection of the derivatives by high-performance liquid chromatography (HPLC) ultraviolet detectors. The fatty acid compositions of corn and soybean oil obtained by analysis of phenylethyl esters agreed with those obtained with methyl esters. The phenylethyl esters were resolved and eluted on C-18 HPLC columns with much smaller solvent volumes than those reported for other aromatic esters. *JAOCS 72*, 749–751 (1995).

KEY WORDS: Fatty acid analysis, gas chromatography, high-performance liquid chromatography, petroselinic acid, phenylethyl esters.

Fatty acid compositions typically are analyzed as methyl esters by gas chromatography (GC), but methyl petroselinate and oleate are poorly resolved on most stationary phases (1). In metabolic studies of the fatty acids, high-performance liquid chromatography (HPLC) separations also may be necessary, and methyl esters have little absorption in the ultraviolet (UV) detectors that are commonly available. Phenyl, naphthacyl, and benzyl esters of the fatty acids have been suggested for such HPLC separations (2), but the preparation of these derivatives has required preliminary saponification of the samples, which obviously is inconvenient for routine use. We have developed simple procedures to prepare phenylethyl esters of fatty acids by esterification and transesterification, and we demonstrate the advantages of this derivative in the chromatographic determination of fatty acid composition.

MATERIALS AND METHODS

Reagents. Phenylethyl alcohol was purchased from Aldrich Chemical Company (Milwaukee, WI) and used without further purification. HPLC-grade hexane and methanol were from Fisher Scientific (Pittsburgh, PA); all other reagents came from Sigma Chemical Company (St. Louis, MO). Phenylethyl alcohol containing 1% sulfuric acid was prepared by mixing the alcohol and acid in an ice bath. Sodium phenylethoxide (0.5 M) was prepared by reacting freshly cut sodium metal with phenylethyl alcohol.

Acid-catalyzed phenylethylation. Approximately 10 mg of lipid was dissolved in 0.5 mL hexane and combined with 1 mL of 1% sulfuric acid in phenylethyl alcohol, and the mixture was heated in a capped vial for 1 h in a boiling water bath or overnight in a 50°C oven. Aqueous sodium chloride (1%) was added, and the phenylethyl esters were recovered by extracting twice with 1 mL of hexane. The hexane extract could be used directly for GC analysis, but it contained some phenylethyl alcohol. The alcohol could be removed by thin-layer chromatography on a silica-gel G plate developed with hexane/ether (80:20). For HPLC analysis, the hexane was evaporated with a stream of nitrogen, and 1 mL methanol was added to dissolve the derivatives.

Base-catalyzed phenylethylation. Approximately 10 mg of lipid was dissolved in 0.5 mL hexane, and 0.5 mL of 0.5 M sodium phenylethoxide in phenylethyl alcohol was added. After 30 min at 50°C or 1 h at room temperature, 1% aqueous sodium chloride was added, and the derivatives were extracted and purified as before.

GC. The phenylethyl and methyl esters of fatty acids were analyzed isothermally at 220°C on an Hewlett-Packard (Wilmington, DE) 5890 instrument equipped with a flame-ionization detector and 15 m x 0.244 mm J&W (Deerfield, IL) DB-23 capillary column. Helium was the carrier gas at 1 mL/min.

HPLC. Shimazu (Kyoto, Japan) LC-9A and Beckman Gold (Fullerton, CA) instruments were used with Supelco (Bellefonte, PA) C-18 4 x 250 mm columns and UV detectors at a wavelength of 210 nm. Acetonitrile or methanol combined with water were the mobile phases.

UV spectrophotometry. Samples in methanol were scanned between 200–300 nm with a Hitachi (Tokyo, Japan) U-2000 spectrophotometer.

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RESULTS AND DISCUSSION

Many combinations of acetonitrile or methanol with water can resolve the phenylethyl esters of fatty acids by HPLC on Supelco C-18 columns, but 5% water in methanol gave adequate resolution in the shortest time. The phenylethyl derivatives of common fatty acids were separated and eluted in about 30 min by 5% water in methanol at 1 mL/min. These elution times are significantly shorter than those reported for other aromatic derivatives of fatty acids (Table 1), thus economizing on time and solvents. Figure 1 illustrates the separation that can be achieved with phenylethyl esters in this system.

TABLE 1
The High-Performance Liquid Chromatography Elution Times Reported for Various Aromatic Fatty Acid Derivatives

Fatty acids	Phenacyl[a]	Bromophenacyl[b]	Naphthacyl[c]	Phenyl ethyl[d]	Phenyl ethyl[e]
18:3	28	13	38	5	7
18:2	34	18	54	6	14
18:1	50	26	85	8	17
18:0	57	44	—	11	23

[a]90 x 0.64 cm μ-Bondapak C-18 (Waters Associates, Milford, MA), acetonitrile/water mobil phase at 2 mL/min (Ref. 2).
[b]25 x 0.5 cm Applied Science Lab (State College, PA) micropat C-18, 1.5 mL/min of 90:10 methanol/water (Ref. 3).
[c]90 x 0.18 Corosil C-18 (Waters Associates), 12 mL/h of 85:15 methanol/water (Ref. 4).
[d]25 x 0.4 cm Supelco (Bellefonte, PA), C-18, 2 mL/min of acetonitrile.
[e]25 x 0.4 cm Supelco C-18, 1 mL/min of 95:5 methanol/water.

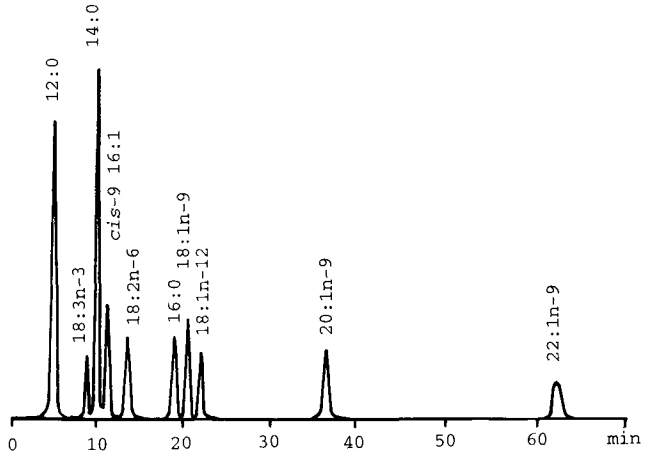

FIG. 1. Separation of phenylethyl esters by high-performance liquid chromatography on a C-18 column with methanol/water 95:5 (vol/vol) at 2 mL/min.

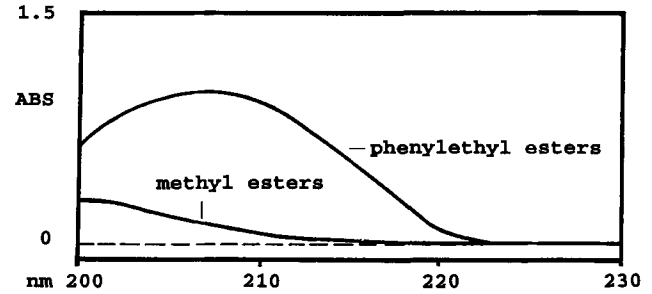

FIG. 2. Spectrum of a 5 μM solution of soybean oil esters in methanol illustrating phenylethyl esters and methyl esters; ABS, absorbance.

TABLE 2
The Relative Response Factors to Give Weight Percentage of Phenylethyl Esters of Some Common Fatty Acids in an Ultraviolet (UV) High-Performance Liquid Chromatographic and Hydrogen Flame Gas Chromatographic Detector

Fatty ester	UV detector[a]	Hydrogen flame detector[b]
16:0	0.968	1.012
18:0	1.000	1.000
18:1	0.976	0.995
18:2	0.888	0.990
18:3	0.620	0.984

[a]Empirical values.
[b]Calculated values.

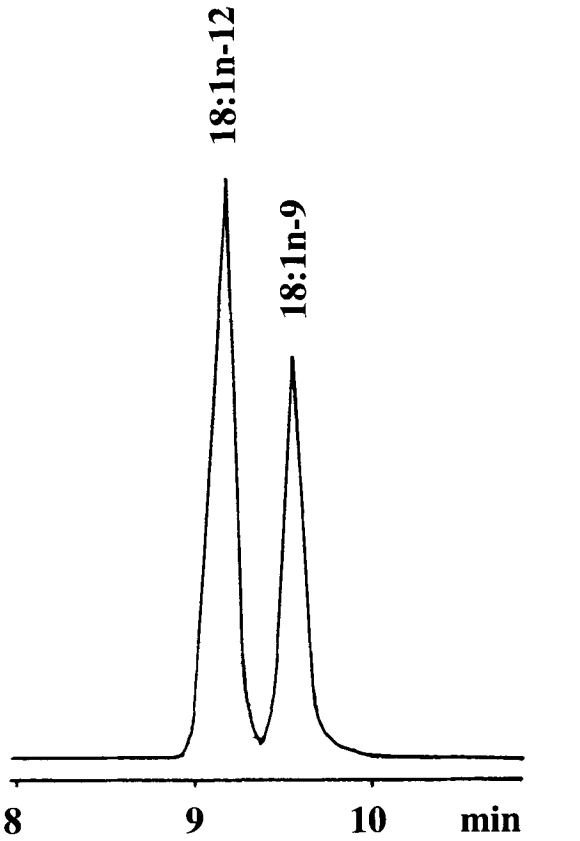

FIG. 3. Separation of phenylethyl petroselinate and oleate on a DB-23 15-M capillary column (J&W, Deerfield, IL) operated isothermally at 220°C with helium as carrier gas at 1 mL/min.

The phenylethyl esters were detected with the greatest sensitivity at 210 nm where they have an absorption maximum (Fig. 2). Table 2 shows the relative response factors of several fatty acid phenylethyl esters on a UV detector. The UV factors were determined experimentally with phenylethyl stearate arbitrarily set to 1.0. The factors deviate from 1.0 because of the absorbance of the carboxyl group and isolated double bonds in the acyl groups at 210 nm (Fig. 2) and the conversion of mole percentages to weight percentages.

The separation of phenylethyl oleate and petroselinate achieved on a 15 m x 0.224 mm J&W DB-23 column is shown in Figure 3. The hydrogen flame correction factors

TABLE 3
The Fatty Acid Compositions of Corn and Soybean Oils as Weight Percent Methyl Esters Determined as Methyl and Phenylethyl Esters by Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC)

Oils	Derivatives/methods	16:0	18:0	18:1	18:2	18:3
Soy	Methyl/GC	11.4	3.8	25.8	52.1	6.9
	Phenylethyl/HPLC	11.1	4.3	25.3	52.5	6.8
	Phenylethyl/GC	11.8	4.4	26.9	51.2	5.8
Corn	Methyl/GC	12.2	1.9	25.4	59.4	1.0
	Phenylethyl/HPLC	11.8	2.4	25.5	59.0	1.0
	Phenylethyl/GC	12.0	2.1	26.5	59.0	0.8

(Table 2) were calculated according to Ackman and Sipos (5). The fatty acid compositions in Table 3 compare the results obtained from methyl and phenylethyl esters by GC and HPLC. For repeated injections, percentages for the various esters by GC are replicated with standard deviations ranging from 0.07 to 0.12. For HPLC, the corresponding standard deviations are 0.03 to 0.21.

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