Improvement in the Gas Chromatographic Resolution of Petroselinate from Oleate

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ABSTRACT: In the extraction of oils from seeds of the genus *Coriandrum,* GC separations of petroselinate from oleate often gave poor resolution of these two isomers. Oleic and petroselinic acids were esterified with a series of alcohols (methanol, ethanol, 1-propanol, 2-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol, and 2-ethyl-1-hexanol). GC resolution of the Δ6 from the Δ9 and Δ11 octadecenoates was examined for all ester derivatives on a polar phase column. The Δ6 and Δ9 isomers were unresolved as methyl esters; however, the 2-ethyl-1-hexyl esters gave baseline separation of all three isomers under temperature programming conditions. When isothermal conditions were optimized for each ester, separation of these isomers was possible with good resolution values (>89%) for all the alcohols except methanol, which had a partial resolution of 51%. The rates of esterification of all the alcohols were determined for reactions with both oleic acid and triolein using potassium hydroxide as the esterification catalyst. Methanol gave the largest rate constant in both acid and oil esterification reactions with a rate constant 10-fold better than all of the other alcohols. Based on rates of reaction, resolution of petroselinate from oleate, and removal of residual alcohol, the ethyl ester derivative appears to be the best choice for seed oils containing petroselinic acid.

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Petroselinic acid is present in a number of seed oils but is most commonly found in the Umbelliferae family. One interest in this acid is that it could serve as a useful raw material for producing adipic and lauric acids *via* oxidative cleavage of the Δ6 double bond in petroselinic acid. Adipic acid is commonly used as a monomer for nylon synthesis, and lauric acid is used in detergent applications. Analysis of petroselinic acid in natural oils by GC is typically confounded by the presence of oleic acid. When methyl esters of petroselinic and oleic acids are synthesized, the GC separation of these two isomers is incomplete (1,2). Separation of petroselinate from oleate was reported to have only a 0.04 equivalent chain length (ECL) unit resolution, which made quantification of the individual isomers difficult. In an effort to overcome these quantification problems, several tedious chromatographic separation or derivatization techniques have been devised. Kleiman (3) used a combination of silver ion chromatography to isolate the monoene fraction of Umbelliflorae seed oils followed by ozonolysis and GC quantification of the cleavage products. Santinelli (4) epoxidized the olefins and then converted the epoxides to chlorohydrins *via* a hydrochloric acid-mediated ring opening. The resulting chlorohydrins were converted to their trimethylsilyl ethers, which were partially resolved by GC. Thies (5) treated the FA with dimethyldisulfide to make the bis-methylthio derivatives, which gave only partial separation by GC.

Good separation of the Δ6 and Δ9 isomers has been reported using phenylethyl (6), isopropyl (7), and butyl (8–10) esters. The phenylethyl ester gives near baseline resolution on a 15-m polar phase (DB-23) column when the esterification reaction conditions are mild using acid or base catalysis. The authors reported that purification of the ester by a silica-gel TLC plate separation was necessary to remove residual phenylethanol. The isopropyl esters also provided near baseline separation, but derivatization was slow due to the secondary nature of the alcohol. The best resolution reported appeared to be when butyl esters were used but the authors report (8,9) that resolution was lost after several injections due to fouling of the precolumn when butanol was used as the derivatization solvent. This problem was overcome using *n*-hexane as the solvent in the esterification step.

Based on these literature reports, one might conclude that the driving force for good resolution of the Δ6 and Δ9 octadecenoates is dependent on the ability to differentiate small polarity differences between these two isomers. Accordingly, masking of the polar carboxyl group with larger alkyl ester groups limits the interaction of carboxyl oxygens with the polar phase of the capillary column. A detailed study of the effect of alcohol chain length and branching on ester resolution for petroselinate from oleate, however, has not been reported. Christie (10) refers to a good resolution of the Δ6 and Δ9 octadecenoates and cites unpublished data for a series of short-chain alcohols. In addition, the esterification rate of these FA with this alcohol series will be important since rapid derivatization is often a requirement for analyzing large numbers of germplasm samples associated with breeding programs.

EXPERIMENTAL PROCEDURES

Materials. Coriander oil was a gift from International Flora Technologies Ltd. (Gilbert, AZ). Hexanes, acetone, methanol,

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1-propanol, 2-propanol, 1-butanol, 2-methyl-1-propanol, and 3-methyl-1-butanol were from Sigma-Aldrich (St. Louis, MO). 2-Ethyl-1-hexanol was from Acros (Fairlawn, NJ). Ethanol was obtained from Aaper (Shelbyville, KY). FAME standards were from Alltech Associates (Deerfield, IL).

GC. GC was performed with a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA), equipped with a FID and an autosampler/injector. Analyses were conducted on an SP-2380 column (0.25 mm × 30 m × 0.2 μm film thickness; Supelco, Bellefonte, PA). GC analyses were conducted as follows: column flow 1.0 mL/min with helium head pressure of 16.9 psi (116 kPa) using electronic pressure control set to maintain constant flow; split ratio 200:1. Temperature ramp analysis: programmed ramp 150 to 265°C at 10°C/min with injector and detector temperatures set at 250°C. Under isothermal analysis conditions, all GC parameters were the same for each alcohol series, except the temperature was held at the values listed in Table 3 (presented shortly) for each ester to obtain a 10 min run. Saturated C_8–C_{30} FAME provided standards for calculating ECL values, which were used to make FAME assignments.

Resolution calculation. The resolution for the separation of Δ6 from the Δ9 octadecenoates and the separation of the Δ9 from the Δ11 octadecenoates was calculated using the method of Kaiser (11). The following equation was used to calculate the resolution; $\theta = f/g$ where θ is the resolution as a value between 0 and 1.0, which can be represented as a percentage by multiplying θ by 100. Figure 1 provides a graphical depiction of the calculation. The term *f* represents the distance between the valley at point v and point m on a line that connects the maxima of peaks 6 and 9. The term *g* represents the distance from the baseline to point m on the same line connecting peaks 6 and 9. This method is particularly suited to adjacent peaks whose heights differ greatly. The method calls for a graphical determination of the point m. We calculated the equation for this line to eliminate the graphical errors that would result from determining an accurate intersect point (m) on a line with this steep of a slope.

Relative ramp rate calculation. The relative ramp rate for each alcohol studied under the temperature program analysis conditions was calculated from the change in oven temperature to elute the C18:0 peak compared with the C18:2 peak divided by the change in ECL as expressed in Equation 1:

$$\text{relative ramp rate} = \Delta T_{C18:2 - C18:0}/\Delta ECL \quad [1]$$

The temperature ramp program described previously had an initial temperature of 150°C with a temperature ramp rate of 10°C/min. Therefore, the oven temperature for the retention time of each isomer was determined by Equation 2:

$$150°C + (10°C \times \text{retention time}) = T_{peak} \quad [2]$$

Stearate (18:0) and linoleate (18:2) were present in all the samples and were used as an internal standard to calculate ECL values across the octadecenoate isomer region. Therefore, the change in ECL was the same value (ΔECL = 1.952) for all the calculations and was determined by the ECL for 18:0 = 18.0 and the ECL for 18:2 = 19.952, which were calculated as previously described.

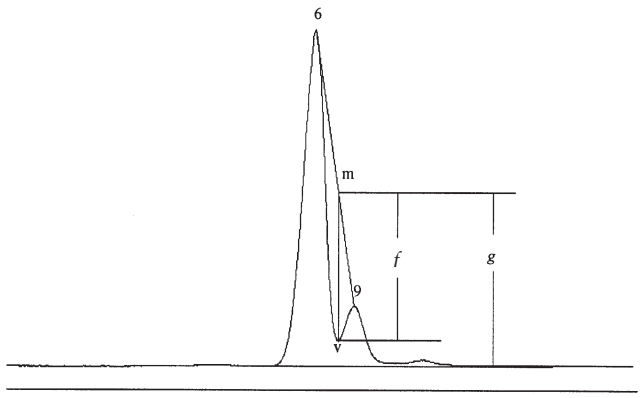

FIG. 1. Graphical depiction of calculation for resolution of unresolved peaks, $\theta = f/g$.

HPLC for esterification rate study. HPLC analyses were performed on a Thermo Separations P2000 binary gradient pump (San Jose, CA) with a Spectra System AS3000 autosampler/injector from Thermo Separation Products (Fremont, CA) coupled to an ELSD from Alltech Associates (Deerfield, IL). A Dynamax (250 mm × 4.6 mm, 60 Å, 8 μm) silica column purchased from Rainin Instrument Co., a division of Varian (Walnut Creek, CA), was used to separate the mixtures. Components were eluted from the column using the following gradient elution: 0–1 min, isocratic hexane/ethyl acetate 95:5; gradient to 90:10 at 2 min then hold until 5 min followed by reverse gradient to 95:5 at 5.5 min then isocratic to 7.0 min. A flow rate of 1 mL/min was used. The ELSD drift tube was set at 50°C with the nebulizer set at 20 psi (138 kPa) N_2 to give a flow rate of 2.0 standard liters per minute (SLPM). Standard curves for both triolein and oleic acid were developed by serial dilution of a 0.50 mg/mL standard to cover the range 0.50–0.03 mg/mL for a total of seven concentrations. Response curves for triolein and oleic acid were then developed under these analysis conditions.

General esterification reactions for GC resolution study. Esters were made by dissolving 2–10 mg of coriander oil in 0.5 mL of a solution of the appropriate alcohol that is 0.5 M in potassium hydroxide in a 2-mL crimp-top vial. The vial was sealed with a crimp cap and placed in a heating block maintained at 60°C for 1 h. The vial was removed from the heating block and, after cooling to room temperature, the cap was removed and 0.5 mL of 1.0 M sulfuric acid in the same alcohol was added to the vial. The vial was sealed with a new cap and placed back in the heating block at 60°C. After 15 min the vial was removed and allowed to cool to room temperature; then the cap was removed. The vial content was transferred to a 2-dram vial using 2 mL of hexane; 1.0 mL of saturated brine was added to the vial, and the solution was then mixed thoroughly. After the water phase separated, 1.0 mL of the upper hexane layer was drawn off with a Pasteur pipette and placed in a 2.0-mL target vial and analyzed by the GC method previously described.

TABLE 1
Rate of Esterification of Oleic Acid

Alcohol	Rate constant (k)	Relative rate
Methanol	1.78×10^{-1}	22.1
Ethanol	2.37×10^{-2}	2.9
1-Propanol	1.64×10^{-2}	2.0
2-Propanol	8.06×10^{-3}	1.0
2-Methyl-1-propanol	9.41×10^{-3}	1.2
1-Butanol	1.59×10^{-2}	2.0
3-Methyl-1-butanol	1.47×10^{-2}	1.8
2-Ethyl-1-hexanol	9.37×10^{-3}	1.1

TABLE 2
Rate of Esterification of Triolein

Alcohol	Rate constant (k)	Relative rate
Methanol	1.23×10^{-2}	70.3
Ethanol	3.29×10^{-3}	18.8
1-Propanol	3.02×10^{-3}	17.2
2-Propanol	1.75×10^{-4}	1.0
2-Methyl-1-propanol	3.27×10^{-3}	18.7
1-Butanol	4.34×10^{-3}	24.8
3-Methyl-1-butanol	3.97×10^{-3}	22.7
2-Ethyl-1-hexanol	3.82×10^{-3}	21.8

Esterification rate studies. Pseudo first-order reaction conditions were made by dissolving oleic acid (0.60 g, 2.13 mmol) or triolein (0.50 g, 0.56 mmol) in a solution of 1.0 M sulfuric acid in alcohol (100 mL) and placed in a constant-temperature reactor connected to a constant-temperature circulating bath maintained at 30°C. All reactions were mixed *via* a magnetic stir bar. Consumption of oleic acid or triolein was followed by removing 1-mL aliquots at designated time intervals, diluting with 1 mL of hexane, and then injecting on the high-performance liquid chromatograph and analyzing as previously described. All reactions were run in duplicate, and initial rates were determined by plotting the log of the oleic or triolein concentration vs. time. A linear regression of the first 4–8 data points of each plot provided a slope, which represents the initial rate of esterification. The average of the two esterification rates for each alcohol studied (Tables 1, 2) was used to calculate the rate constant k ($k = -2.303 \times$ slope) for all reactions.

The esterification rate study was conducted at 30°C (rather than 60°C) so that the rate of reaction was slow enough for a sufficient number of data points to be collected before the reaction reached its half-life. In practice, the esterification reaction would typically be run at the highest temperature possible so that the reaction could reach completion in minimal time with no deleterious side product formation. We found 60°C to be a suitable temperature to meet this criterion.

RESULTS AND DISCUSSION

Esterification rate studies. A number of rate studies for esterification of oleic acid with several of the alcohols used in this study have been reported (12–16). Unfortunately, reaction conditions between these studies varied widely in terms of catalyst concentration, type of catalyst, reaction temperature, and alcohol concentration, making direct comparisons difficult. Because of these discrepancies, a series of straight and branched-chain alkyl alcohols was used to examine the esterification rate of oleic acid and triolein under pseudo first-order kinetics. These data are presented in Tables 1 and 2.

In the reactions of oleic acid with this series of alcohols (Table 1), the rate constant ranged across two orders of magnitude; methanol provided the fastest rate and 2-propanol the slowest. The rate for methanol was significantly faster (nearly 10-fold) than for any of the other alcohols studied. As expected, as the alkyl chain length increased within the straight-chain primary alcohols, the rate of reaction decreased owing to decreased nucleophilicity of the alcohol (methanol >>> ethanol > 1-propanol > 1-butanol). Furthermore, the addition of branching at the β-carbon of the primary alcohol increased the steric bulk of the alcohol, further diminishing the rate of esterification, as can be seen by comparison of 1-propanol with 2-methyl-1-propanol. The only secondary alcohol studied, 2-propanol, gave the slowest rate, as expected.

For esterifications with triolein and this series of alcohols (Table 2), the rate constant ranged across two orders of magnitude, where methanol again gave the fastest rate and 2-propanol the slowest. The rate of methyl ester formation compared with the rest of this series was not as dramatic as those reactions with oleic acid, with only an approximately threefold faster rate. The leveling of the rate constant across the entire series was most likely a result of increased solubility of the triolein in the longer-chain alcohols (1-butanol > ethanol). In addition, branching at the β-carbon of the alcohol appears to have little impact on the rate of these primary alcohols (1-propanol vs. 2-methyl-1-propanol). However, esterification with the secondary alcohol, 2-propanol, is quite slow. Lastly, all the triolein esterifications are nearly an order of magnitude slower than their corresponding reaction with oleic acid.

GC resolution of octadecenoate positional isomers. The alcohols listed in Tables 1 and 2 were used to esterify coriander oil and examine the GC resolution of Δ6 (petroselinic), Δ9 (oleic), and Δ11 (vaccenic) esters. The resulting coriander esters were analyzed by GC using both temperature programming and isothermal analysis conditions on a polar (90% biscyanopropyl/10% cyanopropylphenyl siloxane) 30 m capillary column. A temperature-programmed ramp that we typically use for screening seed oils with FA profiles having ECL values that vary from C6 to C30 was used for the initial evaluation. Using this temperature program, separation of the petroselinate from the oleate and vaccenate esters was conducted, and these aligned chromatograms are shown in Figure 2. Good separation of the isomers is observed as the chain length or the branching of the ester increased. Under these analysis conditions, the 2-ethyl-1-hexyl ester provided the highest resolution value for the separation of the Δ6 from the Δ9 isomer (Table 3, Resol. = 0.957 Δ6-Δ9 column) whereas the methyl ester provided no resolution of these two isomers. These data suggested that sterically masking the polarity of the carboxyl group, so the main interaction would be between the small polarity differences of the olefins and the

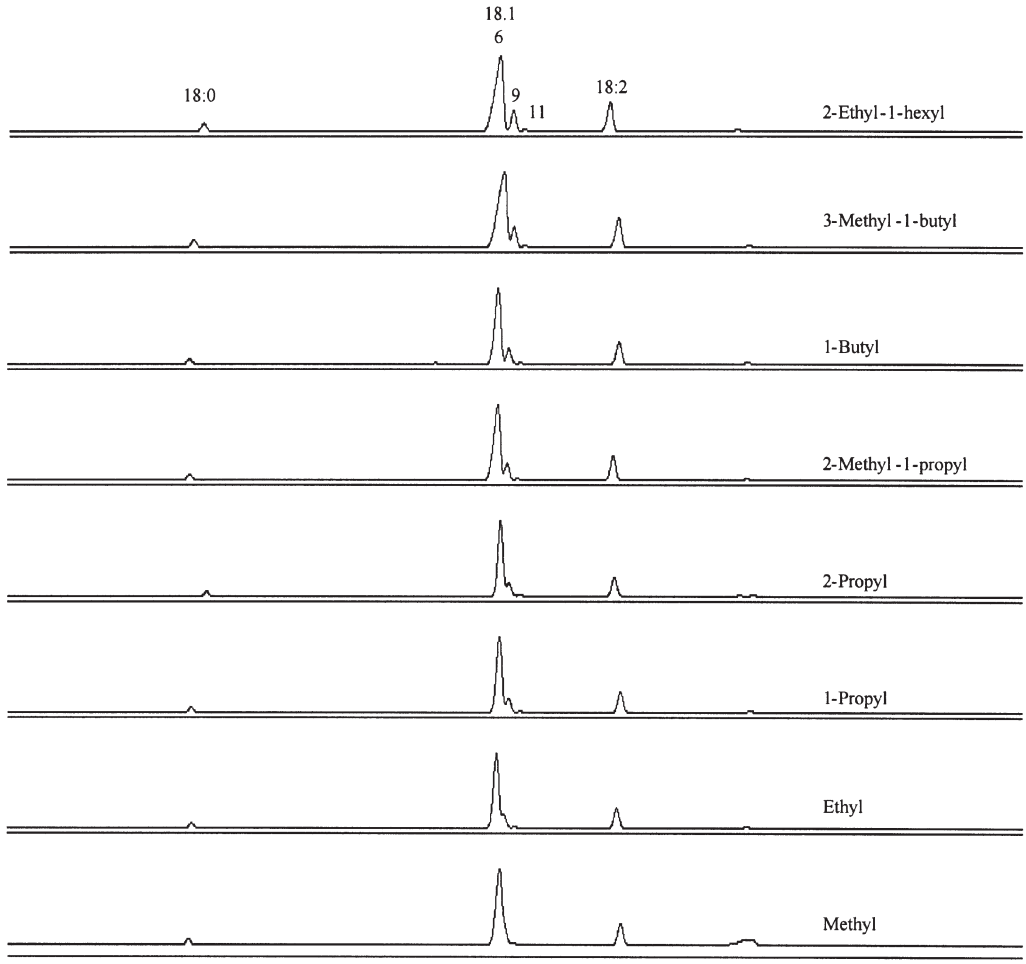

FIG. 2. Stack of GC traces of coriander esters from an SP-2380 column (0.25 mm × 30 m × 0.2 μm film thickness), temperature ramp 150 to 265°C at 10°C/min. Chromatograms aligned based on the Δ6 octadecenoate.

polar liquid phase of the column, caused the observed separations. However, the longer-chain esters have longer retention times under this temperature ramp program, providing increased time for partitioning with the column liquid phase, thereby enhancing resolution. As a result, Table 3 shows that the shorter-chain esters have higher relative ramp rates per ECL (°C/ECL column of Table 3) over the 18:1 retention time range being studied, thus making comparisons between the resolution data of these 18:1 isomers under temperature ramp conditions tenuous. To ascertain the effects of alcohol chain length and branching on the resolution of the 18:1 isomers, the ramp rate per ECL should be equal in the region of interest. Although isothermal conditions for the analysis of a broad range of FA chain lengths typically give poor results, use of an isothermal run over a narrow retention time span containing the desired FA, particularly with the same chain length, can provide good resolution. Thies (8) has reported the use of isothermal conditions for the separation of the butyl ester of oleic acid isomers at 220°C with a total run time of 13 min. Thies saw good resolution of the butyl esters under these conditions, but baseline separation was not observed. We optimized the isothermal runs for each set of esters derived from the individual alcohols, and these chromatographic separations are shown in Figure 3. To optimize the isothermal runs, conditions were found such that all the esters would have retention times between 5 and 9 min, and good resolution of the isomers was observed. Under these conditions, all of the esters except methyl gave a Δ6, Δ9, and Δ11 separation that would be suitable for quantitative purposes. The temperature, retention time, and resolution data for these esters are shown in the bottom half of Table 3. In the series ethyl through 2-ethyl-1-hexyl, the resolution indices are all >89% resolved for the Δ6-Δ9 octadecenoate peaks, and even the methyl ester is now 51% resolved. The 2-ethyl-1-hexyl ester is completely resolved under the isothermal conditions with baseline separation. Of particular note, the branched esters all tended to have leading peaks. Leading peaks can be caused by a number of parameters including column overload, poor injection techniques, poor solubility of the analyte in the stationary phase, and sample condensation in the system caused by too low of a column temperature. Because the straight-chain esters gave good peak symmetry even though they were prepared to have similar concentrations and injected by the same auto-injector as the

TABLE 3
GC Resolution of Esters

Ester	Temperature (°C)	Relative ramp rate[a] (°C/ECL)	Ret. time Δ6 (min)	Ret. time Δ9 (min)	Ret. time Δ11 (min)	Resol. Δ6-Δ9	Resol. Δ9-Δ11
Methyl	Ramp	2.9	4.710	4.710	4.770	0.000	0.987
Ethyl	Ramp	2.8	4.793	4.845	4.932	0.745	0.945
1-Propyl	Ramp	2.8	5.114	5.140	5.174	0.616	0.970
2-Propyl	Ramp	2.5	4.679	4.736	4.782	0.855	0.997
2-Methyl-1-propyl	Ramp	2.6	5.220	5.250	5.276	0.845	0.969
1-Butyl	Ramp	2.7	5.449	5.479	5.513	0.910	0.968
3-Methyl-1-butyl	Ramp	2.6	5.609	5.638	5.670	0.819	0.983
2-Ethyl-1-hexyl	Ramp	2.3	6.426	6.464	6.497	0.957	0.989
Methyl	165	0	8.728	8.791	8.963	0.512	0.985
Ethyl	170	0	7.756	7.848	8.009	0.894	0.922
1-Propyl	175	0	7.875	7.991	8.163	0.955	0.998
2-Propyl	165	0	8.838	8.956	9.163	0.935	0.983
2-Methyl-1-propyl	190	0	5.121	5.175	5.255	0.922	0.949
1-Butyl	185	0	6.784	6.892	7.043	0.977	0.997
3-Methyl-1-butyl	195	0	5.291	5.360	5.447	0.967	0.988
2-Ethyl-1-hexyl	200	0	6.825	6.956	7.105	0.992	0.992

[a]Relative ramp rate (°C/ECL) was calculated from the change in oven temperature to elute the C18:0 peak compared with the C18:2 peak divided by the change in ECL, which is 1.952 for all the entries.

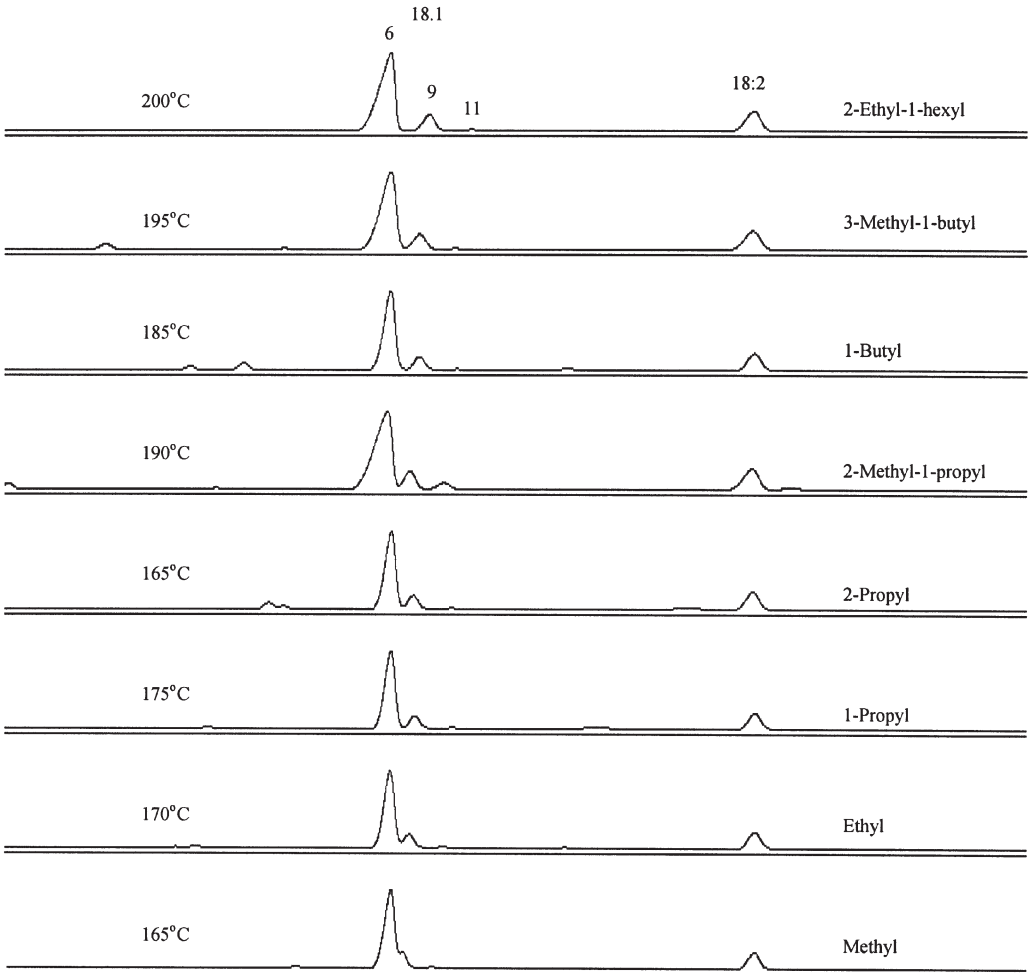

FIG. 3. Stack of GC traces of coriander esters from an SP-2380 column (0.25 mm × 30 m × 0.2 μm film thickness). Isothermal temperature programs with temperature are shown on trace. Chromatograms aligned based on the Δ6 octadecenoate.

branched esters, column overload and poor injection technique can be readily eliminated as possible causes of the leading peaks. However, it is possible that the branched esters exhibit lower solubility in the stationary phase and therefore produce leading peaks. To investigate the possibility that analyte was condensing in the system due to low column temperature, we increased the isothermal temperature above the corresponding temperature of a straight-chain ester having the same number of carbon atoms (2-methyl-1-propyl, 190°C vs. 1-butyl, 185°C). This increase in temperature resulted in improved peak symmetry. Unfortunately, the leading peak could not be sharpened for the 2-ethyl-1-hexyl ester even with increased temperature. To explore the possibility of improving peak resolution, lower isothermal temperatures, which result in longer retention times, were examined (reported in Table 3). However, decreasing the temperature did not improve peak resolution and leading peaks persisted.

Based on the rates of esterification and the resolution of isomers, the 2-ethyl-1-hexyl ester would appear to be promising for the quantitative analysis of oils containing a mixture of petroselinic, oleic, and vaccenic acids. However, 2-ethyl-1-hexanol was not appreciably soluble in water, and removal of excess alcohol in the esterification step by a water wash did not occur in those alcohols larger than 1-propanol. As a result, residual alcohol causes longer separation times during the extraction step of the esterification reaction due to emulsion formation. In addition, injection of these residual alcohols onto the gas chromatograph is known (8,9) to be detrimental to isomer resolution owing to fouling of the injection liner. With these practical considerations in mind, ethanol may be the alcohol of choice to provide a reasonable rate of esterification and good isomer resolution along with rapid partitioning and easy removal of residual alcohol from the reaction mixture.

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