New Preparation of Pure Petroselinic Acid from Fennel Oil (*Foeniculum vulgare*)

A.-S. Charvet[a,*], L.C. Comeau[a] and E.M. Gaydou[b]

[a]Laboratoire de Chimie Biologique Appliquée, Université d'Aix-Marseille III, 13331 Marseille Cedex 3, France and [b]Laboratoire de Phytochimie, Faculté des Sciences et Techniques de Saint Jérôme, 13397 Marseille Cedex 13, France

Pure petroselinic acid (*cis*-6-octadecenoic acid) has been isolated from fennel oil by acid soap crystallization at 4°C in methanol, followed by two urea segregations at room temperature and crystallization at −30°C in acetone. The purity control of petroselinic acid was effected by combined gas chromatography, and ^{13}C nuclear magnetic resonance. This petroselinic acid preparation was compared to other previous crystallization or enzymatic methods, showing that this method is both short (four steps) and easy to apply.

KEY WORDS: ^{13}C NMR analysis, crystallization, fennel oil, petroselinic acid, purification.

Petroselinic acid (*cis*-6-octadecenoic acid; PA) is a characteristic fatty acid of the *Umbelliferae* family, and particularly of fennel oil (*Foeniculum vulgare dulce*) (1). This acid is interesting because of its antimicrobial activity (2,3) and, above all, because its oxidation gives lauric acid (C12:0), a very important fatty acid used in the soap, cosmetic, medical and perfume industries (4–7). As fennel oil is a by-product of anethole extraction (used for anise drinks), and because the content of PA in this oil is high, this acid might have many applications in the detergent industry.

Unfortunately, PA and oleic acid (*cis*-9-octadecenoic acid; OA) are always combined in *Umbelliferae* oils and are difficult to separate. Several methods for the isolation of PA have been published. Several authors (8–10) isolated PA from alcohol solutions of lead salts of ivy (*Hedera helix* L.) or parsley (*Petroselinum sativum* Hoff.) seed. Vanin and Chernoyarova (11) isolated PA by centrifuging the mixed fatty acids from coriander (*Coriander sativum* L.) oil and by recrystallizing the solid fraction from ethanol. In another attempt to isolate fractions rich in PA (12), the mixed fatty acids of coriander seed oil were complexed with urea and fractionally crystallized, employing methanol as solvent. Fore *et al.* (13) and Privett *et al.* (14) used a low-temperature crystallization of the mixed fatty acids of parsley seed oil, followed by urea complex formation and then, after a fractional distillation, the acids were crystallized from petroleum ether to obtain PA. In order to avoid the low temperature, Chobanov *et al.* (15) applied the acid soap crystallization technique in combination with urea complexes. Finally, a recent method based on the selectivity of enzymatic hydrolysis of fennel oil was published (16) for preparing PA.

Another problem in PA preparation is the analysis of the obtained compounds. In fact, PA and OA are almost impossible to separate by classical chromatographic methods. Usually, the position of the double bond in the monounsaturated fatty acids was determined by cleavage of the ethylenic linkage either with potassium permanganate (8), ozone and hydrogen peroxide (9,12), or periodic acid (14–16). The resulting acids were quantitatively characterized either by their melting points or by analysis of their methyl esters by means of gas chromatography (GC). Later, by using trimethylsilyloxy derivatives, it was shown (17,18) that these fatty acids can be separated by GC-mass spectroscopy (GC-MS). Following literature that proved ^{13}C nuclear magnetic resonance (NMR) spectroscopy to be an elegant method for quantitation of unsaturated fatty acids (19–21), Mallet and Gaydou (22) published a new quantitative determination of PA and OA in *Umbelliferae* by combined GC and ^{13}C NMR, and that is the method we used in this experiment.

EXPERIMENTAL PROCEDURES

Fennel oil extraction and saponification. Oil was extracted from ground fennel seeds (*Foeniculum vulgare dulce*), obtained from the Casanis Society, with n-hexane in a Soxhlet type, all-glass extractor. Seeds (150 g) gave about 30 g of oil (20% yield, based on seven extractions). Samples of the mixed fatty acids were prepared by the usual saponification method (23) with potassium hydroxide (1 M) in ethanol. The unsaponifiables were removed with n-hexane and then, after acidification with hydrochloric acid (5 M), the fatty acids were extracted into n-hexane (three times), which was washed with water, dried and evaporated; 37.8 g of fatty acids were obtained from 50 g of fennel oil (75.6% yield).

Acid soap crystallization. The fatty acids mixture (37.8 g) was dissolved in 190 mL of methanol, containing half the quantity (66.5 mL) of sodium hydroxide (1 M) required for neutralization. The mixture was allowed to stand for 6 hr at 4°C and the crystals formed were filtered and washed on the pad with cold methanol. The crystals were dissolved in a solution of water-methanol (50:50, v/v), which was then acidified with hydrochloric acid (5 M) and extracted three times with n-hexane. The extract was washed with water until neutral; the solvent was removed to give 30.5 g of impure PA.

Urea adduct separations. PA was purified by removing palmitic acid and residual OA as urea complexes. Impure PA (30.5 g) and urea (9 g) were dissolved in 30 mL of methanol and heated to reflux according to Chobanov *et al.* (15). The solution was allowed to crystallize overnight at room temperature. The resulting crystals were filtered and washed on the pad with cold methanol saturated with urea. The filtrate was diluted with water, acidified with hydrochloric acid (5 M) and extracted three times with n-hexane. The extract was washed with water until neutral, dried, and the solvent was removed. The resulting fatty acids (29.1 g) were treated once more in the same manner with 10.5 g of urea in 75 mL of methanol (15).

Crystallization in acetone. The above impure PA (25 g) was dissolved in 250 mL of acetone and the solution

*To whom correspondence should be addressed at: Laboratoire de Chimie Biologique Appliquée, Université d'Aix-Marseille III, Place Victor Hugo, 13331 Marseille Cedex 3, France.

TABLE 1

^{13}C NMR Chemical Shifts of the Olefinic Carbon Atoms of Methyl Esters (FAME) of petroselinic (PA), Oleic (OA) and Linoleic Acids (LA)

FAME	Signal number[a]	Chemical shifts[b] (ppm)
PA (C7)[c]	1	130.58
PA (C6)	6	128.99
OA (C10)	3	130.04
OA (C9)	5	129.72
LA (C13)	2	130.19
LA (C12)	4	129.98
LA (C10)	7	128.18
LA (C9)	8	127.97

[a]Signal numbers are taken from Figure 2.

[b]δ Values from TMS ($CDCl_3$).

[c]Refers to the number of the carbon atoms in the molecule.

was allowed to crystallize overnight at −30°C. The crystals were filtered at −30°C and washed with acetone at −30°C. All the solvent was removed to give PA crystals, free of linoleic acid, purity checked by GC and ^{13}C NMR was 99%. The yield was 54% based on the starting content of PA in fennel oil, m.p. 32-32.5°C (Literature values: 32-33°C, ref. 3).

Preparation of fatty acid methyl esters (FAME). FAME were prepared by refluxing acids 10 min in hydrochloric methanol (methanol with 10% of acetyl chloride). FAME were extracted three times with n-hexane, washed with a 5% potassium bicarbonate solution, washed with water until neutral, dried over sodium sulphate, and then the solvent was removed.

Gas chromatography. An Intersmat IGC16 chromatograph equipped with a flame ionization detector was used for the analyses. FAME were separated on a fused silica capillary column (30 m × 0.32 mm I.D.) coated with DBWax 30M (phase thickness 0.15 μm). Column temperature was 180°C and detector and inlet temperatures were 250°C. Helium was used as carrier gas at pressure of 0.7 bar. The injections averaged 1 μL of a 2% solution of FAME in n-hexane.

Nuclear magnetic resonance (NMR). ^{13}C NMR spectra were recorded on a Bruker AC-100 (Bruker Analytische, Karlsruhe, Germany). Samples were prepared in a 5 mm O.D. tube by mixing the FAME with $CDCl_3$ in a volume ratio 1:4. Tetramethylsilane was used as internal standard. The F.T. ^{13}C NMR spectra were obtained under the following conditions: frequency 25.2 MHz, spectral width 6000 Hz, pulse delay 5 sec, acquisition time 1.4 sec, number of data points 16 K. Relative composition of PA and OA was calculated from peak intensity ratios of ethylenic carbons, as described by Mallet and Gaydou (22), and by comparison with the quantity of both acids found by GC analysis.

RESULTS AND DISCUSSION

PA is a characteristic fatty acid of the order *Umbelliflorae* and in certain seed oils, like fennel oil, this acid has been detected at levels as high as 70-80% (1,3,22). Although PA represents by far the main fatty acid in fennel oil, its preparation at a high level of purity, *vide supra*, is quite difficult (8,16). Since Chobanov's method (15) seemed to us the most interesting (high level of purity and high yield), we evaluated which steps were most important. We used GC [palmitic acid, OA+PA and linoleic acid (LA)] and combined GC-^{13}C NMR (see Table 1 for chemical shifts) for complete quantitation. The acid soap fractionation was studied by quantitative FAME analysis of each filtrate and each crystalline fraction. After the first crystallization, the analysis of the crystals revealed that an important part of LA remained in the filtrate (10.1% *vs.* 4.4%, see Table 2). The other important steps were the two urea segregations, which removed almost all the palmitic acid (see Table 2), but also a part of PA. This experiment proved that urea segregations were not as specific as desired for palmitic acid, since portions of PA and OA were also removed. Using Chobanov's method we have obtained PA in high purity (99% by combined GC-^{13}C NMR method). If the purity was of the same order as that given by Chobanov *et al.* (15), then the yield obtained with this method (25%) was much lower than the yield claimed in the literature (64%; ref. 15).

In our method, we checked the important steps of PA preparation to give a quicker and simpler method (Fig. 1). After the oil saponification and extraction of the unsaponifiables, the fatty acids then were half neutralized and crystallized in methanol. The distribution of the individual fatty acids among the fractions corresponded to their solubilities—LA remained in the filtrate to a greater extent than OA (Table 3). During the crystallization, a recombination as acid soap dimers occurred (15). Then the crystals were extracted and submitted to two urea segregations. The analysis of the resulting product revealed no OA and only 1% of palmitic acid (see Table 3).

TABLE 2

FAME Compositions at Several Stages of Petroselinic Acid (PA) Purification According to our Practice of Chobanov's Method (15)

FAME	Starting oil (%)	Cyrstallization steps (%)			Urea complexation steps (%)	
		1	4	10	11	12
Palmitate	4.3	4.3	2.7	4.0	3.2	1.0
Oleate + PA[a]	85.6	91.1	93.6	96.0	96.8	99.0
Linoleate	10.1	4.4	3.7	0.0	0.0	0.0
Oleate[b]	7.7	6.4	1.1	0.0	0.0	0.0
PA[b]	77.9	84.9	92.5	96.0	96.8	99.0

[a]Obtained by GC.

[b]Obtained by GC and ^{13}C NMR combined method.

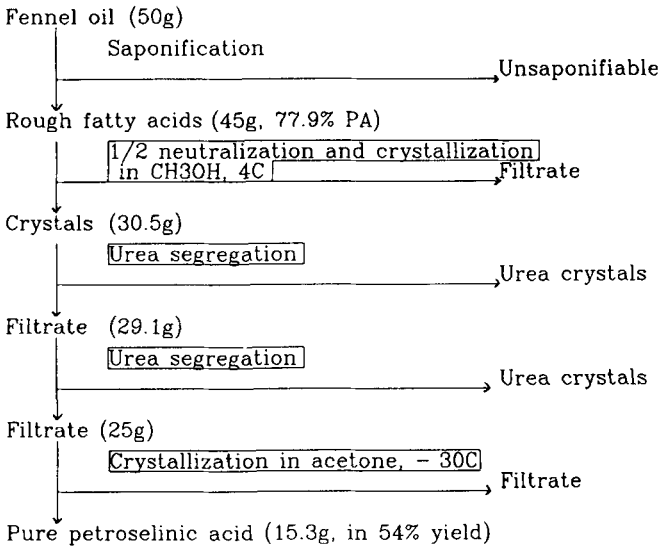

FIG. 1. Purification scheme for obtaining petroselinic acid from fennel oil. Yields are expressed based on petroselinic acid found in starting oil.

TABLE 3

FAME Composition of Fractions Obtained by the PA Preparation Scheme Shown in Figure 1

FAME	Starting oil (%)	Soap crystal[a] (%)	Urea complex steps (%) 1	2	Crystallization[b]
Palmitate	4.3	4.3	2.6	0.9	1.0
Oleate + PA[c]	85.6	91.1	90.7	92.1	99.0
Linoleate	10.1	4.4	6.7	7.0	0.0
Oleate[d]	7.7	6.4	0.9	0.0	0.0
PA[d]	77.9	84.9	89.8	92.1	99.0

[a]After unsaponifiables were removed, in methanol.
[b]In acetone.,
[c]Obtained by GC.
[d]Obtained by combined GC and ^{13}C NMR methods.

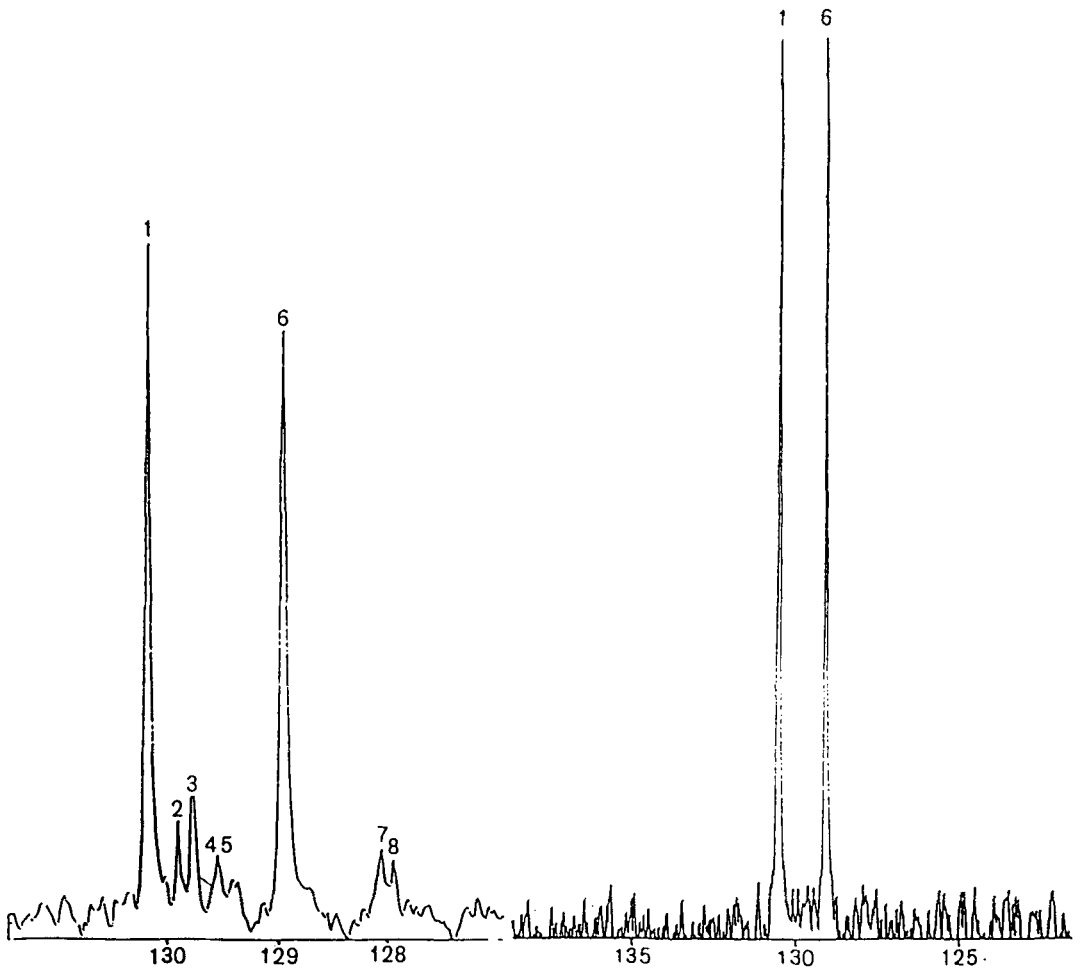

FIG. 2. ^{13}C NMR spectra of olefinic carbons of fennel oil fatty acids (left) and of purified petroselinic acid (right). See Table 1 for peak identification.

This result was interesting because, as we saw above, the most difficult step in this preparation was to eliminate OA. After a last crystallization in acetone at −30°C, residual linoleic acid (7%) was removed and very pure PA (99%) was obtained. The fatty acid compositions of products from representative steps are given in Table 3. Figure 2 showed the ethylene part of ^{13}C NMR spectra of fennel oil fatty acids and purified PA. This new method was compared to other results published previously. The order of unsaponifiable matter removal was important because the yield obtained from Chobanov's method increased from 25% to 48% if unsaponifiables were removed prior to purification. Although Chobanov's method gave pure PA, the method was tedious (12 steps) and the yield was relatively low. The Mbayhoudel and Comeau (16) method gave a higher yield with only four steps, but the purity of PA was only 96%. Our method gave purer PA in good yield (54%) with only four steps.

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