Determination of Petroselinic Acid in *Umbelliflorae* Seed Oils by Combined GC and ^{13}C NMR Spectroscopy Analysis

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Synthetic triolein and tripetroselinin mixtures were examined by ^{13}C NMR spectroscopy, showing marked chemical shift differences of the olefinic carbon atoms. Peak height ratios were compared to weight values for quantitative determination of oleic and petroselinic acids in seed oils, since these two fatty acids are quantitated together by GC analysis. Values observed for NMR peak height ratios were fairly close and agreed well with weight ratios. From overall compositions of oleic and petroselinic acids obtained by GC and relative compositions given by ^{13}C NMR, petroselinic acid has been determined in ten *Umbelliflorae* seed oils.

KEY WORDS: ^{13}C NMR, fatty acid, gas chromatography, oleic acid, petroselinic acid, seed oil, *Umbelliflorae*.

Petroselinic acid (*cis*-6-octadecenoic acid) (PA) is a characteristic fatty acid of the order *Umbelliflorae*, and its occurrence had been widely investigated, particularly throughout the *Umbelliflorae* family (1-9). In certain seed oils, this acid has been detected at levels as high as 80-83%. Although PA represents, by far, the main fatty acid in most of the species, its identification and quantification are quite difficult because of the coexistence of positional isomers like oleic acid (*cis*-9-octadecenoic acid) (OA) and *cis*-vaccenic acid (*cis*-11-octadecenoic acid).

Generally, PA is determined by reductive or oxidative ozonolysis by gas chromatography (GC) of the resulting products (1-6,10-13). However, it has been shown that only 75-82% of adipic acid, one of the oxidative products, is recovered (10). Recent progress in GC phases and procedures brought effective improvement in the separation and identification of certain fatty acids. For instance, it has been possible to detect *cis*-vaccenic acid in the presence of other isomeric monoenoic acids (14), but until now, OA and PA could not be differentiated by GC of their fatty acid methyl esters (FAME). Partial separation of the latter two acids was finally obtained using new derivatization techniques like epoxidation of the double bonds followed by gas chromatography-mass spectrometry (GC-MS) analysis (15,16). Mallet *et al.* (17) introduced the use of trimethylsilyloxy (TMS) derivatives for the identification of PA in seed oils, and more recently, quantitative MS analysis has been investigated (18).

All the reviewed methods include combined analytical procedures which are time-consuming. ^{13}C NMR spectrometry has been presented as a rapid, nondestructive and quantitative method for analysis of seed oils (19,20). Fatty acids are characterized by the chemical shift of their different olefinic carbon atoms. We, therefore, used the ^{13}C NMR technique for the identification and determination of the relative composition of OA and PA in mixtures of synthetic triglycerides such as tripetroselinin and tripolein. In a second step, we have applied this method, in combination with GC, to the determination of PA in some *Umbelliflorae* seed oils.

EXPERIMENTAL PROCEDURES

Synthetic triacylglycerols. Triolein and tripetroselinin were purchased from Sigma Chemical Co., St. Louis, MO.

Oils extractions. Seed samples were obtained from the Comptoir Agricole (Juan les Pins, France). Seeds were ground to a powder with an electric mill and extracted during 6 hr with hexane using a Soxhlet apparatus. Oil contents expressed on a dry basis are given in Table 4.

Preparation of methyl esters. FAME were prepared from oil by ambient temperature transmethylation with sodium methoxide (21).

Gas chromatography (GC). A Girdel 300 gas chromatograph equipped with a flame ionization detector (FID) was used for the analyses. FAME were separated on a fused silica capillary column (30 m × 0.32 mm I.D.) coated with DB Wax 30 M (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 a pressure of 0.7 bar. The injections averaged 1 μL of a 2% solution of FAME in hexane.

Nuclear magnetic resonance (NMR). ^{13}C NMR spectra were recorded on a Brüker AC-100 (Ecole Supérieure de Chimie de Marseille). Samples were prepared in a 5 mm o.d. tube by mixing the various triolein and tripetroselinin mixtures or oils with $CDCl_3$ in a volume ratio 1:4. Tetramethylsilane (TMS) was used as internal standard. The FT ^{13}C NMR were measured under the following conditions: frequency, 25.2 MHz; spectral width, 6,000 Hz; pulse delay, 5 sec; acquisition time, 1.4 sec; number of data points, 16 K. Relative compositions of OA and PA in synthetic triglyceride mixtures were calculated from peak height ratios and for seed oils. When possible, average peak heights of carbon atoms of the same acid were used as described by Tulloch and Bergter (22) and Gaydou *et al.* (20).

RESULTS AND DISCUSSION

^{13}C NMR chemical shifts of olefinic carbons of OA and PA. It has been shown that the olefinic carbons of various fatty acids or their methyl esters have different chemical shifts and can be distinguished in the carbon-13 NMR spectrum (23,24). Recently, Ng and

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Koh (25) observed that ^{13}C NMR analysis of synthetic triacylglycerols gave close and comparable results with those obtained for free fatty acid and FAME, showing that the glycerol skeleton has little influence on the chemical shifts of the olefinic carbons, at least for double bonds remote to the acyl group. The ^{13}C NMR spectrum of the olefinic carbons of a synthetic mixture of triolein and tripetroselinin is given in Figure 1. The different peaks can be unambiguously assigned to the corresponding unsaturated carbon atoms of the two fatty acids. Table 1 lists the chemical shifts of the olefinic carbon atoms for each isomer in triacylglycerols and FAME.

Quantitative ^{13}C NMR analysis of synthetic triolein and tripetroselinin mixtures. Mixtures of triolein and tripetroselinin were constituted and submitted to ^{13}C NMR analysis. Peak ratios (OA/PA) were determined using either peak areas or peak heights. When peak ratios were calculated from peak heights, adequate response was achieved after 250-300 scans. Such results contribute to the rapidity of the ^{13}C NMR method, since each sample analysis was completed in less than 25 min. For each mixture of triolein and tripetroselinin and from the different peak ratios, relative proportions of the two fatty acids were calculated. PA percentages were determined by the following equation,

$$\% \text{ PA} = \frac{100}{1 + \frac{\text{OA}_i}{\text{PA}_j}}$$

where i and j refer to signal numbers given in Figure 1.

^{13}C NMR results, expressed in percent of PA, were compared to weight ratios, as shown in Table 2. It appears that for every peak ratio considered, ^{13}C NMR values are in good agreement with weight values within the NMR sensitivity, since these two acids are detected at a level of 2% (Table 2) as previously observed (19). A linear regression analysis of data in Table 2 shows that the best correlation between wt and NMR PA percentages is obtained using the height ratio of the carbons nearest the carbonyl end of the chain, i.e., C9 and C6 of OA and PA, respectively (OA_3/PA_4, Table 2). The possibility of using various peak ratios seems interesting for it provides alternatives to peak overlapping occurring between some olefinic carbon atoms of unsaturated fatty acids encountered in natural products. Peak ratios established from peak areas showed very poor correlation, and as a result, were not taken into consideration.

Seed oil analyses. Seed oil samples of one *Araliaceae* species (*Hedera helix*) and five *Umbelliferae* species (*Apium graveolens, Daucus carota, Foeniculum vulgare, Cuminum cyminum* and *Anthriscus cerefolium,* including some varieties) were investigated using ^{13}C NMR. Figure 2 shows a partial ^{13}C NMR spectrum of one seed oil (*Daucus carota* "Amsterdam") and chemical shift assignments are given in Table 3. Since no peak overlapping is observed, the relative composition of OA and PA was determined from averaged peak heights (Table 3):

$$\frac{\text{OA}}{\text{PA}} = \frac{\Sigma \text{OA}}{\Sigma \text{PA}} = \frac{4.14 + 3.91}{16.30 + 15.37} = 0.254$$

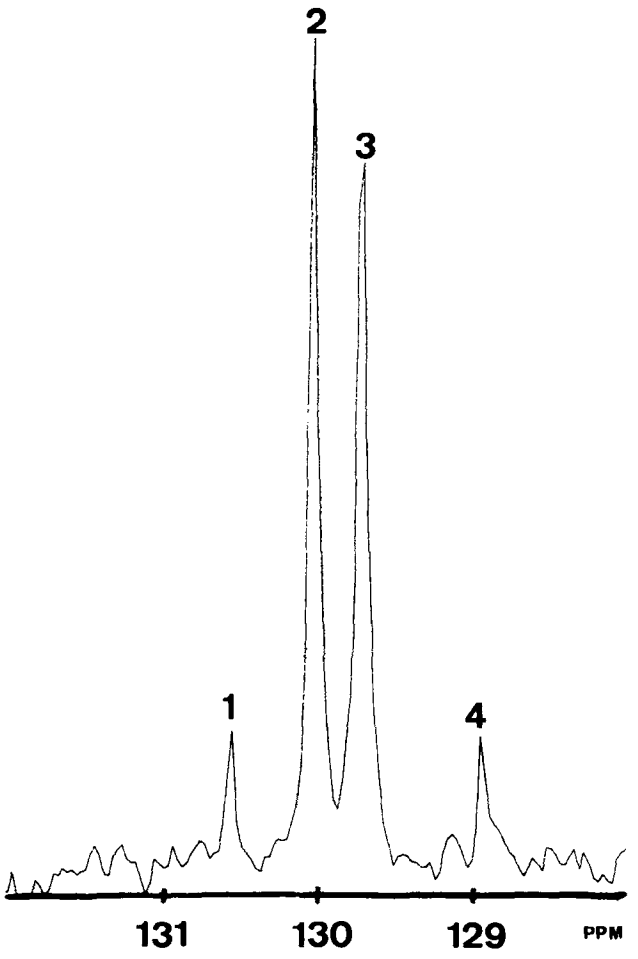

FIG. 1. ^{13}C NMR spectrum of the olefinic carbons of a synthetic triolein and tripetroselinin mixture (weight ratio 4.5: 1, respectively). Peak 1 and 4 belong to C-7 and C-6 of PA, respectively, while peaks 2 and 3 belong to C-10 and C-9 of OA, respectively.

TABLE 1

^{13}C NMR Chemical Shifts of the Olefinic Carbon Atoms of Petroselinic Acid (PA) and Oleic Acid (OA) in Triacylglycerol Mixture and FAME

Signal number[b]	Chemical shift[a]		
	This work	Gunstone *et al.* (23)	Bus *et al.* (24)
	Triacylglycerols	FAME	
1 (PA, C-7)	130.53	130.55	130.48
2 (OA, C-10)	130.00	130.10	130.02
3 (OA, C-9)	129.69	129.80	129.78
4 (PA, C-6)	128.94	129.15	129.18

[a]The δ values from TMS ($CDCl_3$).
[b]Signal numbers are taken from Figure 1.

TABLE 2

Petroselinic Acid (PA) Determination Using ^{13}C NMR Peak Height Ratios in Various Triolein and Tripetroselinin Synthetic Mixtures

% PA (by weight)	% PA (by ^{13}C NMR)[a]			
	OA_2/PA_1[b]	OA_3/PA_1	OA_2/PA_4	OA_3/PA_4
93.52	92.80 ± 0.75	92.45 ± 0.44	93.05 ± 0.74	93.73 ± 0.42
78.89	78.01 ± 0.57	76.33 ± 0.54	76.10 ± 0.34	78.39 ± 0.44
39.98	42.00 ± 0.76	40.65 ± 0.41	41.98 ± 0.55	40.66 ± 0.41
18.18	17.86 ± 0.85	17.88 ± 0.57	17.05 ± 0.46	18.11 ± 0.33
7.71	6.98 ± 0.55	8.72 ± 0.62	7.35 ± 0.45	8.52 ± 0.36

[a]Mean value and standard deviation obtained from 8 experiments.
[b]Indices refer to signal numbers given in Figure 1.

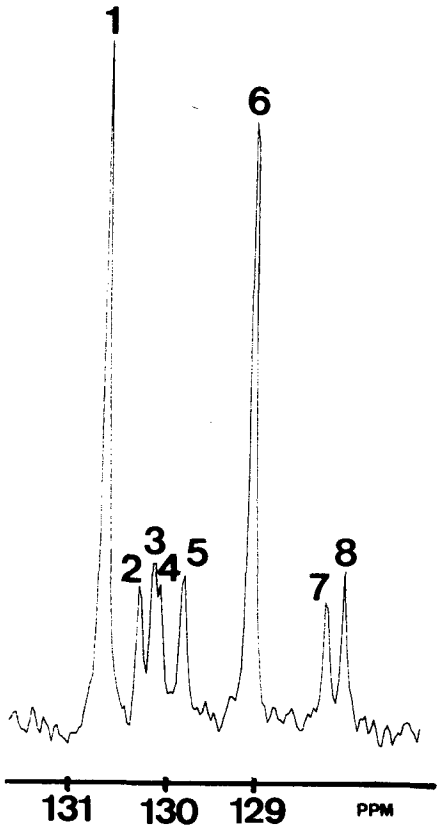

FIG. 2. ^{13}C NMR spectrum of olefinic carbons of *Daucus carota* "Amsterdam." For signal assignment see Table 3.

TABLE 3

^{13}C NMR Chemical Shifts of Olefinic Carbon Atoms of *Daucus carota* ("Amsterdam") Seed Oil

Signal number[a]	Chemical shift[b]	Assignment	Peak height
1	130.58	PA	16.30
2	130.19	LA[c]	3.60
3	130.04	OA	4.14
4	129.98	LA	3.60
5	129.72	OA	3.91
6	128.99	PA	15.37
7	128.18	LA	3.35
8	127.98	LA	3.92

[a]Signal numbers are taken from Figure 2.
[b]The δ values from TMS ($CDCl_3$).
[c]LA: linoleic acid.

Chemical shifts were assigned on the basis of fatty acid compositions obtained by GC analysis of the corresponding FAME. In some other seed oil spectra, peak overlapping occurred principally between the C-10 peak of OA and the C-9 peak of linoleic acid (*cis, cis*-9, 12-octadecadienoic acid). Relative compositions were however calculated from other available peak ratios. Since overall compositions of OA and PA were known by GC analyses, quantitation of the two acids could finally be established. For example, in the case of *D. carota* "Amsterdam", GC analysis yielded 76.9% of unresolved acids, and ^{13}C NMR gave an OA/PA ratio of 0.254. Hence, from these values, we found 61.3% and 15.6% of PA and OA, respectively. Results, together with comparative literature data, are reported in Table 4. For the same species, the OA/PA ratio is almost unchanged for cultivars within a species, as observed in *D. carota* (0.22–0.26) and *A. graveolens* (0.09–0.13). Our results are in agreement with those given by Kleiman and Spencer (5), as shown in Table 4.

TABLE 4

Combined GC and ^{13}C NMR Quantitation of Petroselinic acid (PA) in some *Umbelliflorae* Seed Oils and Comparison of OA/PA Ratios with Literature

Species		Seed oil (%)	GC OA + PA (%)	^{13}C NMR + GC PA (%)	^{13}C NMR OA/PA	Ozonolysis (5) OA/PA
A. graveolens[a]	1	16.7	67.3	61.6	0.09	0.12
	2	23.6	62.9	55.6	0.13	
D. carota[b]	1	17.3	77.4	63.5	0.22	0.14-0.18
	2	9.4	74.4	59.2	0.26	
	3	14.0	76.9	61.3	0.25	
F. vulgare[c]	1	13.9	62.5	60.6	0.03	0.04 -0.12
	2	13.0	73.5	73.5	n.d.[d]	
C. cyminum		18.4	61.1	49.0	0.25	0.3
A. cerefolium		7.2	65.6	65.6	n.d.[d]	0.14
H. helix		20.0	73.3	53.5	0.37	0.10

[a]*Apium graveolens* 1, "Grand vert"; 2, "Géant de Prague."
[b]*Daucus carota* 1, "Touchon"; 2, "Chatenay"; 3, "Amsterdam."
[c]*Foeniculum vulgare* 1, "Géant mammouth perfection"; 2, "Doux de Florence."
[d]OA was not detected.

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