GC/IRMS Analysis of Mandarin Essential Oils. 2. $\delta^{13}C_{PDB}$ Values of Characteristic Flavor Components

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The $\delta^{13}C_{PDB}$ values of characteristic flavor components of mandarin essential oils have been measured by gas chromatography/isotope ratio mass spectrometry. For genuine mandarin oils a characteristic authenticity profile was established and used for the authenticity control of commercially available mandarin oils. In addition to cold-pressed mandarin essential oils, distilled mandarin and sweet orange oils were investigated to assess blends of cold-pressed mandarin oils with these products.

Keywords: *GC/IRMS; $\delta^{13}C_{PDB}$ values; authenticity profile; authenticity control; mandarin essential oil; Citrus reticulata Blanco*

INTRODUCTION

Mandarin essential oils are widely used in the food and perfumery industries to enhance the bouquet of flavor and fragrance compositions. The characteristic feature of this oil is mainly attributed to its content of α-sinensal, methyl *N*-methylanthranilate, and thymol.

Mandarin essential oils are obtained by cold-pressing the peel of the fruits of *Citrus reticulata* Blanco. These essential oils are articles of commerce in many parts of the world, and Italian oils especially are of economic importance.

Therefore, reliable analytical methods for the origin assessment and quality assurance of mandarin oils are of special interest. In this respect chirality evaluation and comprehensive investigations on the quantitative composition of mandarin essential oils related to the ripeness of the fruits and the production method have been reported (Dugo et al., 1992a).

Nevertheless, reliable assessments on the authenticity of essential oils remain difficult, as synthetic analogues of essential oil components are more and more commercially available.

Stable isotope ratio analysis has proved to be a further useful tool in the origin assignment and authenticity control of essential oils (Faber et al., 1995; Frank et al., 1995; Hener et al., 1995).

In this context the investigation of $\delta^{13}C$ and $\delta^{15}N$ values of methyl *N*-methyl-anthranilate in mandarin essential oils could render additional information (Faulhaber et al., 1997).

This paper describes the application of gas chromatography/isotope ratio mass spectrometry (GC/IRMS) measurements for multicomponent analysis in the authenticity assessment of mandarin essential oil. The scope and limitation of the method are discussed.

MATERIALS AND METHODS

Essence Oil Sources. *Commercially Available.* Cold-pressed mandarin oils were from Italy, Greece, Brazil, and Argentina and of unknown origin. Distilled mandarin oils and cold-pressed sweet orange oils were from Italy (Misitano & Stracuzzi, Messina, Italy; Ziegler, Aufsess, Germany; Kaders, Hamburg, Germany; Liebich, Nürnberg, Germany; Dufti, Melle, Germany).

Laboratory Prepared. Italian mandarin oils were prepared by solvent extraction. The peel from 15 fruits (*Citrus reticulata* Blanco) was rubbed off using a grater and extracted with 700 mL of *n*-pentane/diethyl ether 1:1 (v/v) overnight (room temperature). The organic layer was filtered, dried with Na_2SO_4, and concentrated using a Vigreux column. Italian mandarin oil was also distilled. The essential oil was obtained from the rubbed peel by steam distillation according to the DAB 10 method (*Deutsches Arzneibuch*, 1991). The oil was separated from the water phase by liquid−liquid extraction, three times with 50 mL of portions of *n*-pentane/diethyl ether 1:1 (v/v). The organic layer was dried with Na_2SO_4 and concentrated using a Vigreux column. Italian sweet orange oil was prepared by solvent extraction. The peel from eight fruits (*Citrus sinensis* L. Osbeck) was prepared as described above.

Preparative Layer Chromatography (PLC). Hydrocarbons are the major components of mandarin oil. Therefore, minor components such as methyl *N*-methylanthranilate, linalool, octanal, and α-sinensal must be enriched by PLC. For isolation, 300 μL of the oil sample was separated using preparative layer plates (Merck, Darmstadt, Germany, silica gel 60 F_{254}, 2 mm layer thickness) with silica gel as sorbent and *n*-pentane/diethyl ether 97:3 (v/v) as eluent. Methyl *N*-methylanthranilate is detectable at UV 254 nm ($R_f = 0.25$), whereas the other components of interest (linalool, octanal, and α-sinensal) are not UV-detectable. The selected solvent composition makes it possible to enrich these components by removing the sorbent in a range of $R_f = 0.30-0.00$. The position of this range was tested by reference experiments. The sorbent was extracted with 10 mL of diethyl ether. After concentration (Vigreux column) to about 0.5 mL, the solution was ready to use for GC and GC/IRMS. To exclude isotopic fractionation during sample cleanup (Braunsdorf et al.,1992), the PLC preparation was checked with reference substances.

Gas Chromatography. For quantification and control of the PLC fraction, a Fisons GC 8000 series equipped with a flame ionization detector connected to a Shimadzu Chromatopac C-R3A integrator was used. An OV-1701 fused silica capillary column (60 m × 0.32 mm i.d., 0.6 μm film thickness, homemade) was used for the analysis. Conditions were as follows: 1 μL split injection; split ratio, 1:18; flow rate of carrier gas (helium), 1.2 mL/min; injector temperature, 220 °C. The temperature program was as follows: the column was held at 50 °C for 5 min and then programmed at 3 °C/min to 240 °C, which was held for 20 min.

Enantio-MDGC (Enantioselective Multidimensional Gas Chromatography). A Siemens Sichromat 2.8 double-oven system with two independent temperature controls, two flame ionization detectors, and a "switching valve" as coupling piece (Live-T-piece) was used (Siemens, Karlsruhe, Germany).

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Table 1. $\delta^{13}C_{PDB}$ Values of the Investigated Citrus Peel Oils[a]

sample	α-sinensal	limonene	γ-terpinene	α-thujene	β-pinene/ sabinene	myrcene	terpinolene	methyl *N*-methyl-anthranilate	linalool	octanal
Authentic Mandarin Oils from Italy Au 1−10*										
min	−28.83	−31.01	−30.16	−28.60	−30.08	−27.49	−30.37	−31.07	−28.60	−30.90
max	−26.41	−29.40	−28.83	−27.18	−28.76	−25.83	−28.63	−29.54	−26.39	−28.30
MW	−27.95	−30.25	−29.60	−27.81	−29.32	−26.56	−29.17	−30.24	−27.79	−29.68
(σ)	(0.77)	(0.52)	(0.46)	(0.43)	(0.44)	(0.47)	(0.56)	(0.50)	(0.71)	(1.03)
Commercial Mandarin Oils from Italy										
Co 1	−27.30	−30.27	−29.86	−28.55	−29.95	−27.51	−29.52	−32.42	−27.62	−28.48
Co 2	−27.99	−30.92	−30.49	−29.29	−30.56	−27.94	−30.56	−31.42	−28.39	−29.81
Co 3	−26.90	−30.10	−29.66	−27.37	−28.87	−26.32	−29.06	−29.97	−27.00	−29.67
Commercial Mandarin Oils from Greece										
Co 4	−26.05	−29.41	−28.34	−27.06	−28.20	−25.35	−28.54	−29.11	−26.42	−28.25
Co 5	−26.81	−29.36	−29.10	−27.35	−29.03	−26.48	−29.08	−30.37	−27.10	−29.31
Commercial Mandarin Oil from Brazil										
Co 6	−30.35	−32.36	−32.00	−30.53	−31.30	−28.55	−31.29	−32.66	−29.68	−31.01
Commercial Mandarin Oil from Argentina										
Co 7	−30.22	−31.91	−30.95	−28.94	−30.62	−27.95	−31.47	−32.66	−29.40	−30.22
Commercial Mandarin Oils of Unknown Origin										
Co 8	−27.02	−30.14	−29.50	−28.16	−29.84	−27.05	−29.21	−30.22	−27.30	−
Co 9	−27.46	−29.94	−28.97	−27.41	−29.11	−25.30	−28.38	−30.42	−27.41	−28.59
Co 10	−29.92	−30.50	−31.52	−30.01	−31.16	−28.82	−29.19	−32.05	−28.81	−31.31
Distilled Mandarin Peel Oils from Italy										
Co 11	−	−30.32	−30.50	−29.37	−30.04	−26.93	−29.75	−32.48	−28.03	−30.47
Co 12	−	−31.57	−29.66	−29.18	−30.05	−27.36	−29.97	−33.42	−28.29	−30.89
Au 13*	−28.97	−30.45	−30.82	−28.58	−29.73	−26.92	−29.25	−30.30	−28.60	−31.76
Orange Peel Oils from Italy										
Au 15	−	−29.48	−	−	−	−25.78	−	−	−27.07	−27.01
Au 16	−	−28.86	−	−	−	−25.45	−	−	−26.02	−25.60
Au 17	−	−28.68	−	−	−	−26.29	−	−	−26.30	−26.54
Au 18*	−	−29.61	−	−	−	−26.25	−	−	−27.50	−28.00

[a] $\delta^{13}C_{PDB}$ values (‰) average of three measurements; standard deviation (σ); authentic samples Au 1−10, Au 13, Au 15−18 (Au 7−10* and Au 18* are laboratory prepared by solvent extraction; Au 13* is laboratory prepared by distillation); commercial samples Co 1−7 and Co 8−12; (−) not available in sufficient amounts or not contained.

A OV-215 Duran-glass capillary column (30 m × 0.23 mm i.d., 0.5 μm film thickness, homemade) was used as precolumn and a Duran-glass capillary column (25 m × 0.23 mm i.d., 0.2 μm film thickness, homemade) coated with a film of heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (45%) and OV-1701-vi (55%) as main column (Dietrich et al., 1992). Conditions were as follows: 1 μL split injection; split, 15 mL/min; injector temperature, 220 °C; detector temperature, 250 °C each.

Addtional Precolumn Conditions. The carrier gas was H_2 at 1.32 bar. The temperature program was as follows: the column was held at 50 °C for 10 min and then programmed at 2 °C/min to 80 °C followed by a 3 °C/min increase to 220 °C held for 10 min.

Additional Main Column Conditions. The carrier gas was H_2 at 1.08 bar. The temperature program was as follows: the column was held at 50 °C for 30 min and then programmed at 1 °C/min to 60 °C followed by a 2.5 °C/min increase to 150 °C.

Cut times were as follows: α-pinene, 13.32−13.45 min; β-pinene, 17.00−17.15 min; limonene, 19.60−19.61 min. The order of elution was assigned by enantiomerically pure or enriched references of defined chirality.

Gas Chromatography/Mass Spectrometry. The GC/MS analyses were performed with a Fisons GC 8000 Series equipped with a Fisons MD 800 mass spectrometer. A DB-5 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness, Durabond, J&W Scientific, Folsom, CA) was used as stationary phase. Conditions were as follows: 0.3 μL split injection: split ratio, 1:30; flow rate of carrier gas (helium), 1.4 mL/min; injector temperature, 220 °C; interface temperature, 250 °C; ion source temperature, 220 °C. The temperature program was as follows: the column was held at 40 °C for 5 min and then programmed at 2.5 °C/min to 280 °C, which was held for 20 min.

Gas Chromatography/Isotope Ratio Mass Spectrometry. Isotope ratio measurements were performed with a Finnigan MAT delta S isotope mass spectrometer, on-line coupled to a Varian 3400 GC via a combustion interface (Finnigan MAT, Bremen, Germany). The GC conditions were as follows.

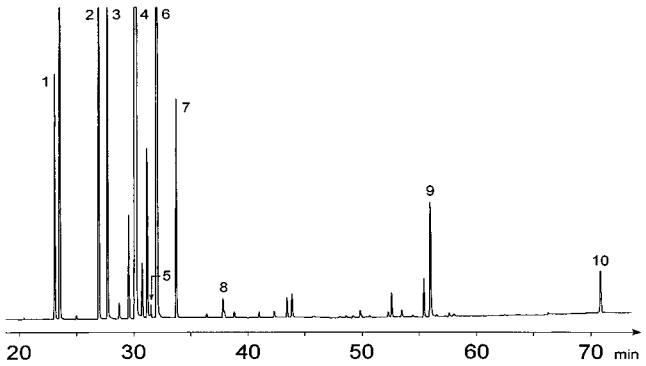

Figure 1. GC analysis of mandarin oil (OV-1701; for conditions, see Materials and Methods): (1) α-thujene; (2) β-pinene/sabinene; (3) myrcene; (4) limonene; (5) octanal; (6) γ-terpinene; (7) terpinolene; (8) linalool; (9) methyl *N*-methyl-anthranilate; (10) α-sinensal.

System I was a fused silica capillary column (OV-1701 60 m × 0.32 mm i.d., 0.6 μm film thickness, homemade); 1 μL split injection (split ratio 1:15); flow rate of carrier gas (helium), 1.9 mL; injector temperature, 220 °C.

(a) Determination of Limonene and γ-Terpinene by Direct Injection of the Essential Oil (Concentrated 0.02% and 0.2%). The column was held at 50 °C for 5 min and then programmed at 3 °C/min to 240 °C, which was held for 20 min; backflush (BF) 0−800 s or 0−800, 1555−1620 s (to fade out limonene, for the measurement of γ-terpinene).

(b) Determination of α-Thujene, β-Pinene/Sabinene, Myrcene, and Terpinolene by Direct Injection of the Essential Oil (Concentrated 1%). The column was held at 50 °C for 5 min

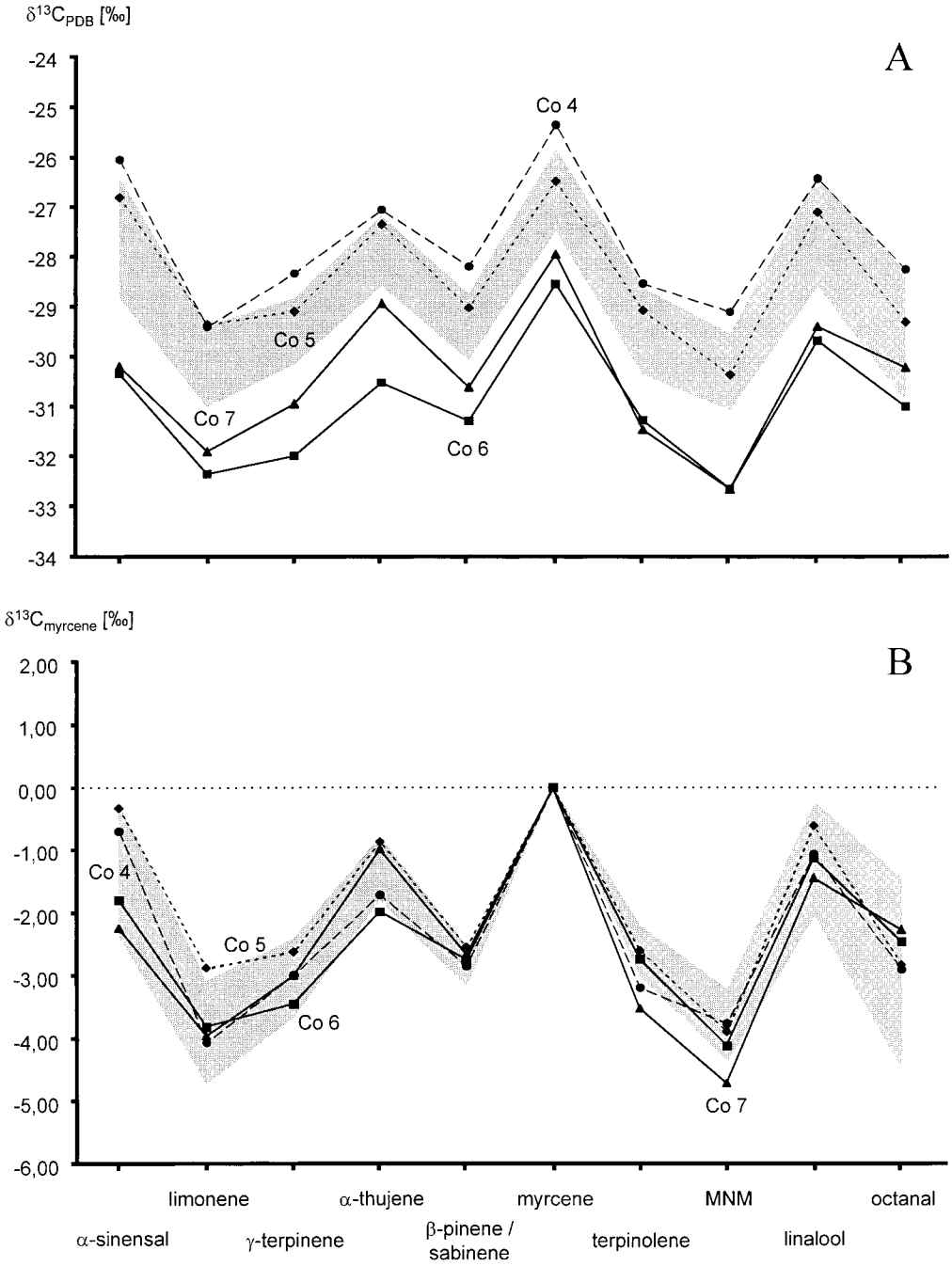

Figure 2. Authentic cold-pressed mandarin oils from Italy (shaded ranges) compared with commercial samples of different provenance (Greece Co 4, 5; Brazil Co 6; and Argentina Co 7): (A) authenticity range (shaded range, including minimum and maximum $\delta^{13}C_{PDB}$ values of samples Au 1−10); (B) authenticity profile (shaded range, calculated for myrcene as i-IST). MNM, methyl *N*-methylanthranilate.

and then programmed at 3 °C/min to 75 °C, held for 4 min, followed by a 3.5 °C/min increase to 240 °C, which was held for 20 min; BF 0−800, 1590−1730 s (to fade out limonene and γ-terpinene).

(c) Determination of Methyl N-Methylanthranilate and α-Sinensal after PLC Preparation. The column was held at 100 °C for 5 min and then programmed at 3 °C/min to 150 °C, held for 5 min, followed by a 3 °C/min increase to 200 °C, held for 5 min, followed by a 3 °C/min increase to 240 °C, which was held for 20 min; BF 0−600 s.

System II was a fused silica capillary column (BPX-35 60 m × 0.32 mm i.d., 0.25 μm film thickness, SGE, Weiterstadt, Germany); 1 μL split injection (split ratio 1:16); flow rate of carrier gas (helium), 1.9 mL; injector temperature, 220 °C.

Determination of Linalool and Octanal after PLC Preparation. The column was held at 50 °C for 5 min and then programmed at 3.5 °C/min to 91 °C, held for 3 min, followed by a 3.5 °C/min increase to 100 °C, held for 4 min, followed by a 3.5 °C/min increase to 115 °C, held for 5 min, followed by a 4 °C/min increase to 260 °C, which was held for 10 min; BF 0−800 s.

Isotope ratios are expressed as δ values (‰) versus a standard:

$$\delta^{13}C_{PDB} = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{PDB}}{(^{13}C/^{12}C)_{PDB}} \times 1000$$

The isotope ratio mass spectrometer was calibrated against CO_2 gas with a defined ^{13}C content relative to the PDB standard. Furthermore, the system was calibrated by introducing a homemade mixture of references with well-known $\delta^{13}C_{PDB}$ values (references: 5-nonanone, −28.05‰; menthol, −26.55‰; γ-octalactone, −23.17‰; γ-decalactone, −30.12‰).

RESULTS AND DISCUSSION

Cold-pressed mandarin oil possesses a characteristic odor and is a green to orange liquid, depending on the

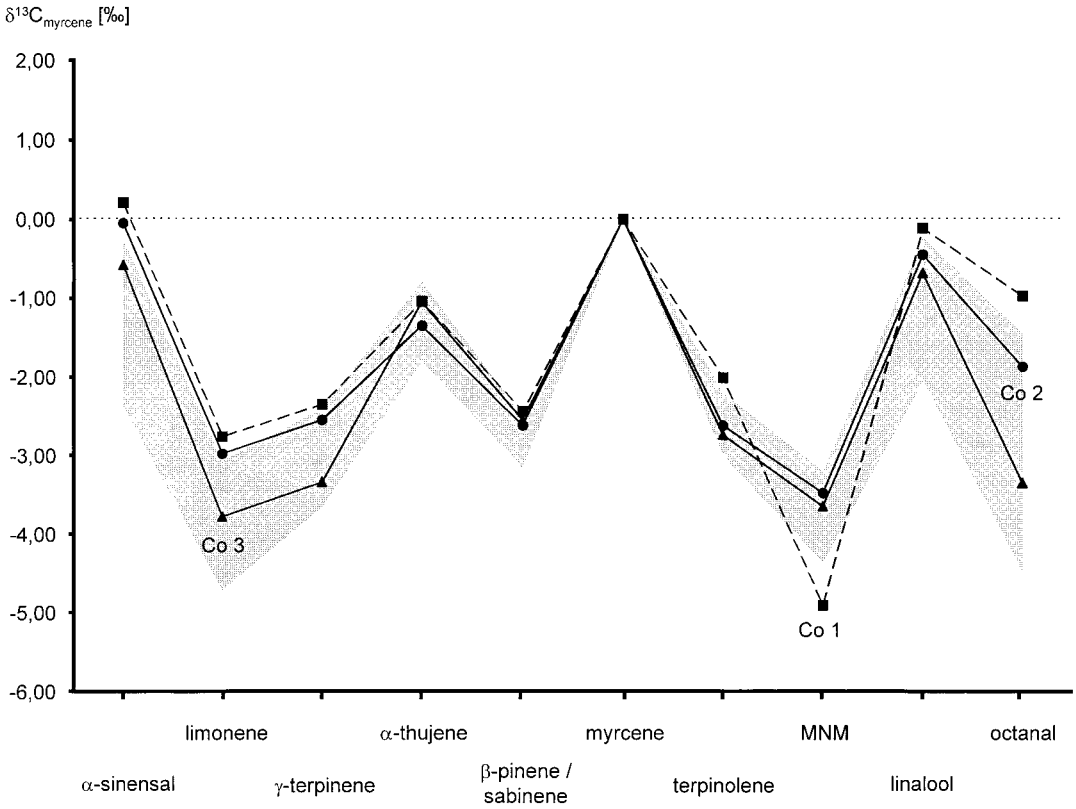

Figure 3. $\delta^{13}C_{myrcene}$ values of the commercial samples from Italy (Co 1−3) compared with the authenticity profile (shaded range, calculated for myrcene as i-IST; including minimum and maximum values of the cold-pressed Italian mandarin oils Au 1−10). MNM, methyl *N*-methylanthranilate.

degree of fruit ripeness. Besides cold-pressed essential oils, there are less valuable distilled and reconstituted oils available on the market. Whereas the distilled oils are obtained from the liquid of the screw-pressed residues of the cold extract, the reconstituted oils are obtained by mixing orange terpenes or sweet orange oil, γ-terpinene, methyl *N*-methylanthranilate, thymol, and other components to imitate a mandarin oil (Dugo et al., 1992b).

For economic reasons blended mandarin oils are available on the market.

The following adulterations are known for mandarin essential oil (Dugo et al., 1992a,b; Verzera et al., 1992): addition of sweet orange oil terpenes to cold-pressed mandarin oils; addition of reconstituted oils to cold-pressed mandarin oils; addition of distilled oils to cold-pressed mandarin oils.

Against this background not only genuine cold-pressed mandarin oils but also distilled oils and orange peel oils are investigated.

The main components of mandarin oil are monoterpene hydrocarbons (∼98%) with limonene (∼69%) and γ-terpinene (∼20%) as predominant constituents. Besides these main components, the characteristic fragrance of the essential oil is mainly determined by α-sinensal (∼0.3%) and methyl *N*-methylanthranilate (∼0.4%), which occur in small amounts (Dugo, 1994) (Figure 1).

To get reliable isotopic data from GC/IRMS measurements, the components of interest must be separated well by GC. Because of the high content of terpene hydrocarbons, there is no problem evaluating $\delta^{13}C_{PDB}$ values of these components by direct GC/IRMS measurements. However, an effective depletion of the main components must be achieved by PLC to increase the concentration of the minor components α-sinensal, methyl *N*-methylanthranilate, linalool, and octanal. The applied sample cleanup allows the separation of the components without any isotope fractionation. The cleanup method was proved by experiments with reference substances. These references were measured after PLC cleanup, compared with untreated components (S. Faulhaber, dissertation in preparation, 1997).

The components β-pinene and sabinene could not be separated well by the applied GC column (OV-1701). Nevertheless, reliable summary $\delta^{13}C$ values, with a good standard deviation, could be determined, because of the

Table 2. Enantiomeric Ratios of α-Pinene, β-Pinene, and Limonene in Mandarin Essential Oils[a]

	α-pinene		β-pinene		limonene	
sample	*S*-(−)	*R*-(+)	*R*-(+)	*S*-(−)	*S*-(−)	*R*-(+)
Au 1	55.0	45.0	98.5	1.5	2.3	97.7
Au 2	55.6	44.4	98.9	1.1	1.7	98.3
Au 3	54.9	45.1	98.3	1.7	2.2	97.8
Au 4	55.8	44.2	97.6	2.4	2.9	97.1
Au 5	55.4	44.6	97.8	2.2	1.9	98.1
Au 6	54.8	45.2	99.1	0.9	2.0	98.0
Au 7	55.0	45.0	97.9	2.1	2.4	97.6
Au 8	52.8	47.2	98.0	2.0	1.7	98.3
Au 9	52.6	47.4	98.8	1.2	1.9	98.1
Au 10	52.1	47.9	98.7	1.3	1.6	98.4
av	54.4	45.6	98.4	1.6	2.0	98.0
Co 1	63.4	36.6	57.1	42.9	2.3	97.7
Co 2	55.6	44.2	98.8	1.2	2.0	98.0
Co 3	54.5	45.5	97.0	3.0	1.9	98.1
Co 4	54.2	45.8	99.1	0.9	1.8	98.2
Co 5	53.7	46.3	99.1	0.9	1.7	98.3
Co 6	54.5	45.5	99.1	0.9	1.7	98.3
Co 7	40.2	59.8	97.5	2.5	1.1	98.9
Co 8	53.7	46.3	97.0	3.0	2.0	98.0
Co 9	52.3	47.7	90.4	9.6	5.1	94.9
Co 10	56.0	44.0	99.2	0.8	1.9	98.1

[a] Authentic samples Au 1−10; commercial samples Co 1−10.

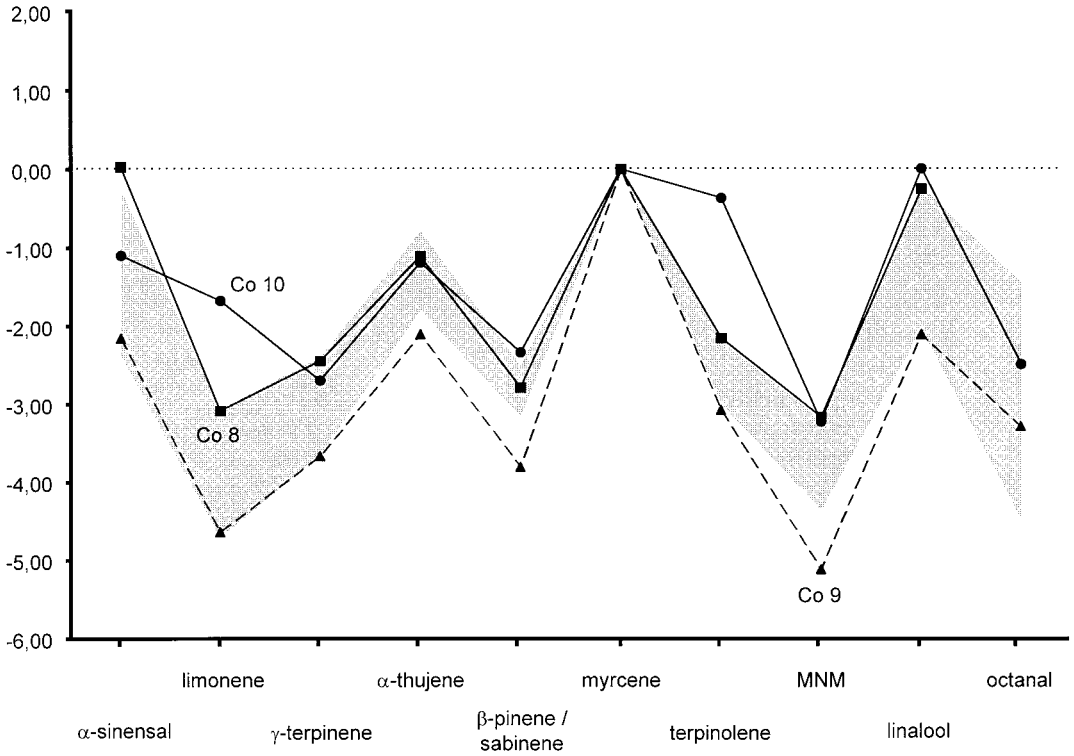

Figure 4. $\delta^{13}C_{myrcene}$ values of the commercial samples without declaration of the provenance (Co 8−10) compared with the authenticity profile (shaded range, calculated for myrcene as i-IST; including minimum and maximum values of the cold-pressed Italian mandarin oils Au 1−10). MNM, methyl *N*-methylanthranilate.

nearly constant quantitative proportions of both components in the oils.

By determination of the $\delta^{13}C_{PDB}$ values of the mentioned components, an authenticity profile of mandarin essential oil (*C.reticulata* Blanco) is established.

For that purpose 10 well-defined authentic oils from Italy are examined and an authenticity range is defined.

As shown in Table 1, the isotopic values for the components only vary in a small range, indicated by the standard deviation of 10 samples.

The $\delta^{13}C_{PDB}$ values of mandarin essential oils from Greece (Co 4, 5), Argentina (Co 7), and Brazil (Co 6) are shown in Figure 2A. Compared with the authenticity range of the Italian oils, the oils from Argentina and Brazil are extremely depleted, whereas the Greek oil Co 4 is enriched in the ^{13}C contents of the components investigated.

Isotope effects caused by growing conditions (for example, differences in location, climate, or harvest time) influence the $\delta^{13}C_{PDB}$ values of plant material. Using one of the flavor components as an internal isotopic standard (i-IST), these effects are eliminated (Braunsdorf et al., 1993).

In this investigation myrcene is used as an i-IST for the following reasons: myrcene is (i) available in sufficient amounts and free of isotope discrimination during sample cleanup, (ii) biogenetically related to the most components investigated, and (iii) a genuine characteristic component of lesser sensorial importance.

The $\delta^{13}C_{myrcene}$ values of 10 authentic samples represent the authenticity profile of mandarin essential oil. This profile is used to prove the genuineness of commercially available mandarin oils.

In Figure 2B the calculated $\delta^{13}C_{myrcene}$ values of Greek, Argentine, and Brazilian oils are presented in comparison to the authenticity profile. This kind of presentation eliminates the individual influences of isotope discrimination, caused by CO_2 fixation during photosynthesis, and only the influences of enzymatic reactions during secondary biogenetic pathways are investigated. Therefore, by introducing the i-IST method, individual influences of provenance are eliminated, shown for the mandarin oils from Greece, Argentina, and Brazil (Figure 2B). The $\delta^{13}C_{myrcene}$ values of Greek and Brazilian oils are similar to the authenticity profilee; therefore, the genuineness of the oils is confirmed. The authenticity could also be confirmed by chiral analysis. Compared with the authentic samples, the Argentine oil shows small differences. For this oil a remarkable quantitative composition is detected, especially a higher amount of δ-3-carene (0.04%), which is normally a trace compound in mandarin oil (≤0.005%). This fact indicates the addition of sweet orange oil, which contains δ-3-carene in an amount >0.1% (Dugo et al., 1992a).

Compared with the authenticity profile, the commercial Italian oils Co 1−3 are represented in Figure 3. The $\delta^{13}C_{myrcene}$ values of samples Co 2 and Co 3 show no or only small deviations from the profile. These commercial oils have to be assessed as authentic. By chiral analysis these results were confirmed. Sample Co 1 deviates from the authenticity profile. The authenticity of this sample is in doubt because of a remarkably changed enantiomeric distribution of β-pinene. For the authentic samples (*R*)-(+)-β-pinene of high enantiomeric purity was found (>97.6%), whereas a significantly decreased enantiomeric ratio of β-pinene (*R*:*S* = 57.1:42.9) was detected in the case of commercial oil Co 1 (Table 2).

In Figure 4 the $\delta^{13}C_{myrcene}$ values of commercial mandarin oils without declaration of the provenance are shown. Sample Co 8 shows a graph very similar to that of the authentic oils, confirming the authenticity of this oil. This fact could also be confirmed by chirospecific analysis. Samples Co 9 and Co 10 do not correspond with the genuine mandarin oils. Co 10 differs extremely in the $\delta^{13}C_{myrcene}$ values of limonene and terpinolene (1.4‰ and 1.8‰). The adulteration was definitely

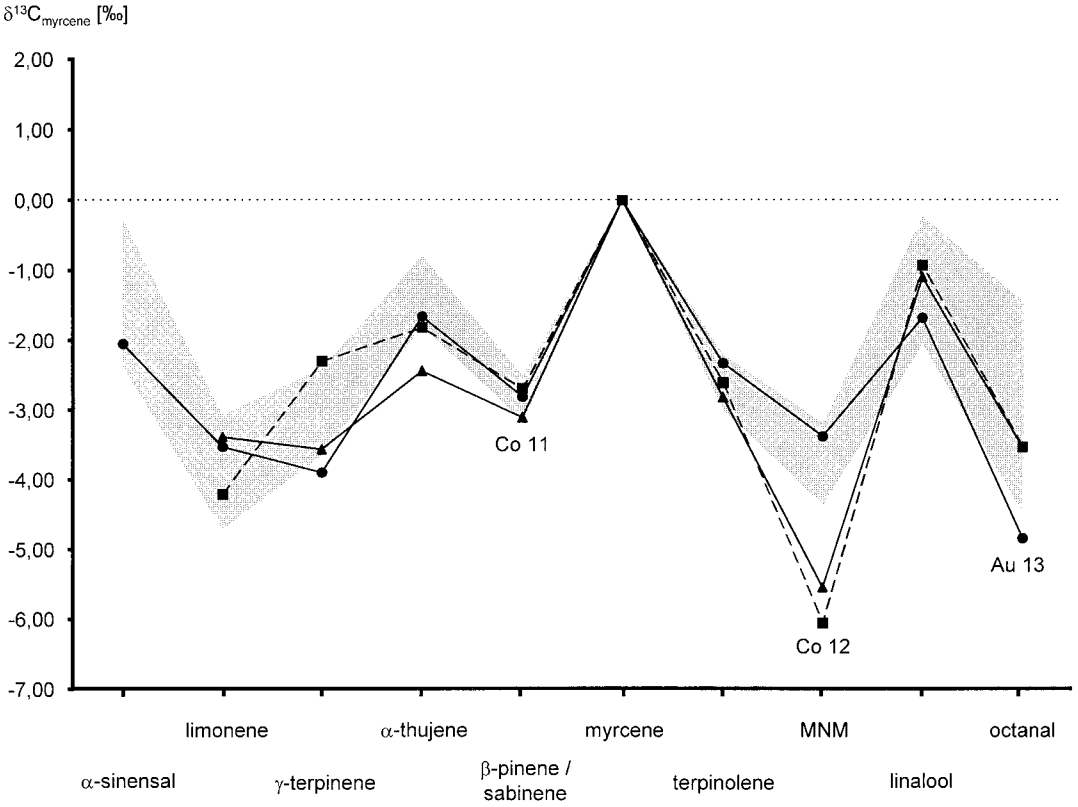

Figure 5. $\delta^{13}C_{myrcene}$ values of distilled mandarin oils (Co 11, 12; Au 13) compared with the authenticity profile of cold-pressed mandarin oils (shaded range, calculated for myrcene as i-IST; including minimum and maximum values of the Italian authentic samples Au 1−10). MNM, methyl *N*-methylanthranilate.

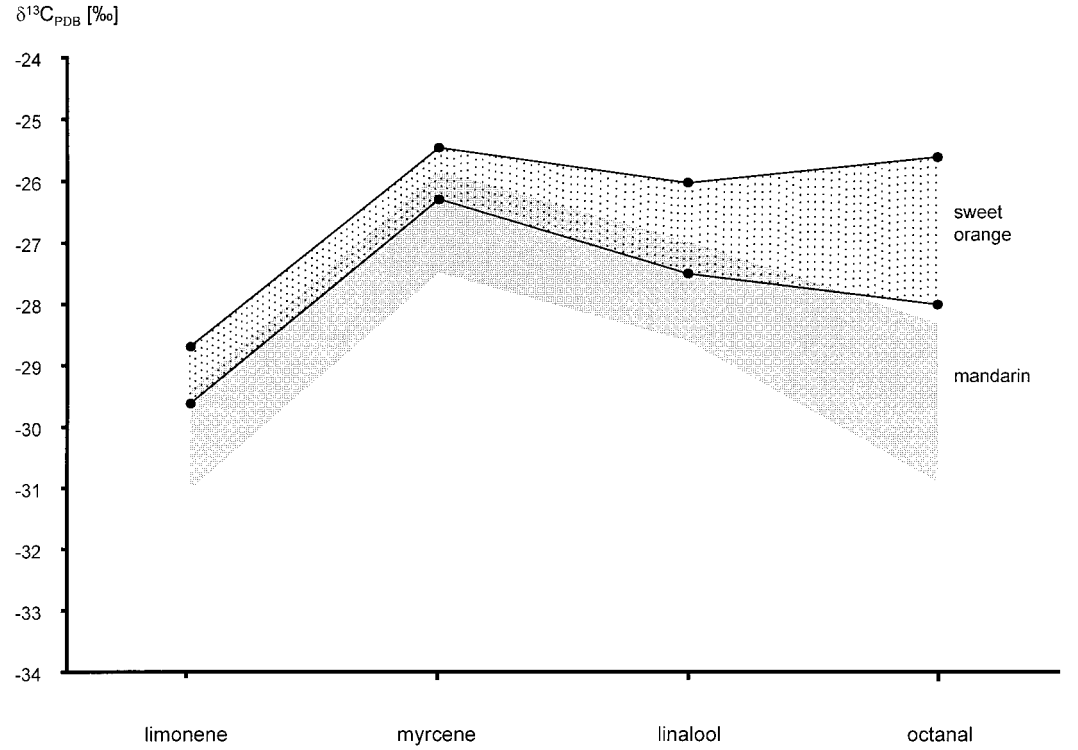

Figure 6. Authenticity ranges of Italian mandarin (shaded range, including minimum and maximum values of the cold-pressed authentic samples Au 1−10) and sweet orange oils (pointed range, including minimum and maximum values of the cold-pressed orange oils Au 15−18).

detected by IRMS, although the results of the chirospecific analysis of this sample were not suspicious.

In contrast to all other samples investigated, sample Co 9 contains nature-identical methyl anthranilate (Faulhaber et al., 1997). Moreover, a higher amount of δ-3-carene (0.05%) is detected, which indicates the addition of sweet orange oil. The adulteration of this oil is also indicated by chiroselective analysis. For the authentic samples (*R*)-(+)-limonene of high enantiomeric purity is detected (>97.1%), whereas sample Co 9 shows a decrease of (*R*)-(+)-limonene (94.9%) (Table 2). This result indicates the addition of reconstituted oil. These products, which are obtained by mixing orange terpenes or sweet orange oil, γ-terpinene, methyl

N-methylanthranilate, and other components, have an optical rotation higher than those of genuine mandarin oils owing to their content of sweet orange oil. During the quality control of essential oils the optical rotation is routinely measured. To decrease the optical rotation of reconstituted mandarin oil (*S*)-(−)-limonene is generally added (Dugo et al., 1992b).

The $\delta^{13}C_{myrcene}$ values of distilled mandarin oils are outlined in Figure 5. The commercially distilled oils are extremely depleted in the ^{13}C content of methyl *N*-methylanthranilate. The δ values of the laboratory-prepared distilled sample are in accordance with the authenticity profile of the cold-pressed oils. The difference between the commercial and the laboratory-prepared samples could be effected by the distillation parameters. By this method a differentiation between cold-pressed and distilled oils should be possible; a blend of cold-pressed mandarin oil with distilled oil is still difficult to detect.

Figure 6 shows the authenticity range of orange peel oils from Italy (*Citrus sinensis* L. Osbeck) compared with the authenticity range of mandarin essential oils from Italy. Mandarin and sweet orange oils possess a large number of identical components in variable concentrations. Therefore, the concentration of the components in sweet orange oil only permit the measurement of limonene, myrcene, linalool, and octanal in comparison to mandarin oil. The other components measured for mandarin oil are not present in sweet orange oil or are only available in trace amounts.

The analysis of sweet orange oils yields nearly the same authenticity range as the mandarin oils, only the ^{13}C contents of linalool and octanal are enriched.

Whereas the ^{13}C content of octanal in mandarin oils is depleted in comparison with linalool, in orange oils these components show nearly the same values.

As can seen in Figure 6, the differences in the δ values of both citrus peel oils are not significant enough to prove a blend of mandarin oil with sweet orange oil.

In view of the genuineness of the oils investigated, the results confirm a previous investigation dealing with the $\delta^{13}C$ and $\delta^{15}N$ measurement of methyl *N*-methylanthranilate. Mandarin oil Co 1, which was suspicious in the previous investigation, is confirmed as falsified (Figure 3). The adulteration of sample Co 9 could be confirmed (Figure 4).

Commercial oil Co 10, which could be a Brazilian oil because of its ^{13}C and ^{15}N contents of methyl *N*-methylanthranilate, also was proved as adulterated (Figure 4).

The isotopic values of mandarin essential oils were determined using GC/IRMS. By examination of 10 authentic samples, a characteristic authenticity range was established. By defining one compound as an internal isotopic standard (i-IST), a characteristic authenticity profile was calculated and used for authenticity control of commercial cold-pressed mandarin oils.

In comparison to this authenticity profile, adulterated mandarin oils could be proved. Moreover, the genuineness of the mandarin essential oils was checked by chiroselective analysis. For the most adulterated oils under investigation the falsification could also be proved by this method.

However, the chiral analysis fails in the case of imitated natural enantiomeric ratios and—of course—in the case of nonchiral aroma active components. So far, IRMS analysis is the method of choice to evaluate the genuineness of essential oil components.

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