

Flavor Authentication Studies of α -Ionone, β -Ionone, and α -Ionol from Various SourcesMARÍA DEL MAR CAJA,[#] CHRISTINA PRESTON, MICHAEL KEMPF, AND
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In addition to the already available information on the authenticity of α - (**1**) and β -ionone (**2**) from plant tissues, there is an interest in the stable isotope data of **1** and **2** available by synthesis from citral and acetone, as European Union regulations, in contrast to the United States and other countries, do not allow a product to be declared as 'natural' that has been chemically synthesized (e.g., by using a natural catalyst) from natural educts. Analyses performed by on-line capillary gas chromatography–isotope ratio mass spectrometry in the combustion and pyrolysis modes (HRGC-C/P-IRMS) as well as by elemental analyzers (EA-C/P-IRMS) measuring $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values provide for the first time isotope data of such 'natural' **1** and **2** as well as of synthetic and 'ex plant' α -ionol (**3**). The isotope data recorded for synthesized **1** and **2** reflected the influence of the origin of the used citral, whereas that of acetone was less remarkable. For instance, 'natural' **1** ex citral from lemongrass showed, as expected for a C4 plant, an enriched $\delta^{13}\text{C}_{\text{V-PDB}}$ value of -18.5‰ . In addition, the use of synthetic citral resulted in an enriched $\delta^2\text{H}_{\text{V-SMOW}}$ value of -43‰ , whereas with citral ex *Litsea cubeba* and ex lemongrass values of -242 and -232‰ , respectively, were recorded. IRMS analyses of 'natural' **2** revealed $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values that were nearly identical to that recorded for 'natural' **1**. As to both **1** and **2**, variations of synthesis conditions led to distinct changes in the $\delta^{13}\text{C}_{\text{V-PDB}}$ but not the $\delta^2\text{H}_{\text{V-SMOW}}$ values. Synthetic **3** showed $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values of -24.5 and -184‰ , respectively. These data differed from those found in raspberry fruit under study ($n = 8$), that is, ranging from -33.6 to -36.6‰ for $\delta^{13}\text{C}_{\text{V-PDB}}$ and from -200 to -225‰ for $\delta^2\text{H}_{\text{V-SMOW}}$. The values determined additionally for **1** and **2** in raspberry fruit samples ranged from -30.3 to -35.1‰ and from -176 to -221‰ for $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$, respectively, and thus corresponded to the already known literature information.

KEYWORDS: Flavor authenticity; α -ionone; β -ionone; α -ionol; IRMS; HRGC-C/P-IRMS; $^{13}\text{C}/^{12}\text{C}$ ratio; $^2\text{H}/^1\text{H}$ ratio; raspberry (*Rubus idaeus*)

INTRODUCTION

Ionones are a group of carotenoid-derived aroma compounds that are widely used in the flavor and perfume industries (1). The biogenesis of apocarotenoids by plant carotenoid cleavage oxygenases has recently been reviewed (2). As a chiral compound, α -ionone (**1**) has found early attention in authentication analysis through enantiodifferentiation; thus, the occurrence of enantiopure (*R*)-**1** in plant tissues has become a decisive parameter of authentication (3–6). Stable isotope analysis has also been applied to assess the authenticity of α - (**1**) and β -ionone (**2**) and the state of the art has recently been summarized (7).

Despite the already existing fundamental information about the isotope ratios of **1** and **2**, in particular, regarding those from

raspberry fruit, isotope data are still lacking concerning various synthetic ways to produce these ketones. The initial synthetic route to ionones is based on the condensation of citral and acetone with subsequent cyclization of the resulting isomeric pseudoionones (8, 9) (Figure 1). Later, the selective preparation of ionones was investigated, with growing interest over the past decade (11). For instance, the biotechnological production of **1** and **2** has been reported (12), and by this way **1** can be produced in high enantiomeric excess from a carotenoid-rich extract. To our best knowledge, however, this route to **1** has not been commercialized. Regardless, the laborious techniques of asymmetric synthesis of ionones (11) are out of the focus of this paper, but the traditional Tiemann route (8, 9) allows easy production of **1** and **2** from synthetic and/or natural educts. As racemic **1** declared to be natural is on the flavor market, the application of this route has to be considered. To avoid adulterations in food flavoring, it is of interest to differentiate such products analytically. In contrast to the United States and

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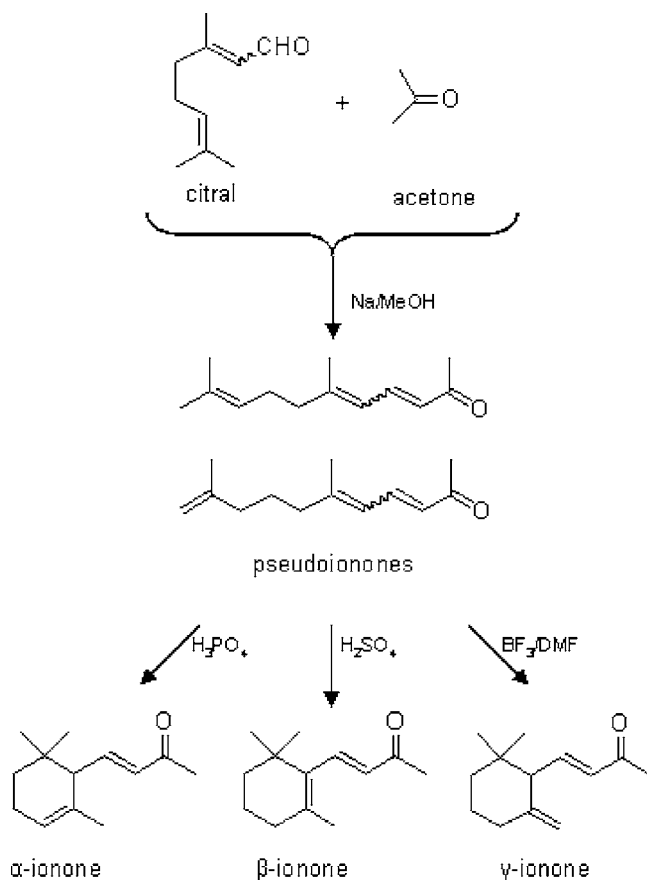


Figure 1. Production of ionones by condensation of citral and acetone with subsequent acid-catalyzed cyclization of intermediary regioisomeric pseudoionones (8, 9); stereochemical control by the catalyst, for example, predominance of α - (1), β - (2), and γ -ionone using phosphoric acid, sulfuric acid, and a Lewis acid such as BF₃/DMF, respectively (10).

other countries, European Union (EU) regulations do not allow a product that has been chemically synthesized (e.g., by using natural catalyst) from natural educts to be declared as natural. In the following, the term natural is used for EU-conforming educts and products, whereas the quoted ('natural') characterizes U.S.- but not EU-conforming materials.

In this paper we report $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ data of synthetic and 'natural' products of **1** and **2**, pointing out the influence of the use of natural and/or synthetic educts and different catalysts. Corresponding data of the enantiomers of 'natural' **1**, separated and isolated by HPLC on chiral phase, are also given. In addition, the $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values of natural and synthetic α -ionol **3** are reported for the first time. Finally, the already known database on **1** and **2** from raspberry fruit is extended by a number of examples.

MATERIALS AND METHODS

Samples and Chemicals. Raspberry fruits ($n = 8$) were purchased at local markets in Würzburg, Germany. *Litsea cubeba* oil and lemongrass oil were from Vieille (Vallauris, France), Adrian (Marseille, France), and Symrise (Holzminden, Germany). References of α -ionone (**1**), β -ionone (**2**), and α -ionol (**3**) were purchased from Fluka (Deisenhofen, Germany) and Sigma-Aldrich (Steinheim, Germany). Synthetic and natural citral references were obtained from Fluka (Deisenhofen, Germany) and Symrise (Holzminden, Germany), respectively. Synthetic and natural acetone samples were from Sigma-Aldrich (Steinheim, Germany). All others chemicals employed were from Sigma-Aldrich (Steinheim, Germany). Solvents were redistilled before use.

Sample Preparation. Raspberries (1 kg; origin Germany, Spain, and France, cultivar not determined) were blended with a mixer and homogenized with 600 mL of distilled water. The slurry was subjected to simultaneous distillation/extraction (SDE) for 2 h using 120 mL of a pentane/diethyl ether mixture (1:1, v/v). The extract was dried over anhydrous sodium sulfate, carefully concentrated to approximately 1 mL using a Vigreux column (40 °C), and subjected to LC separation on silica gel (cf. Syntheses) for subsequent HRGC-MS and HRGC-C/P-IRMS analyses.

The essential oils under study were analyzed directly, that is, without sample preparation. Pure references **1–3** were dissolved in diethyl ether (1 mg/mL) and subjected to GC-MS and HRGC-C/P-IRMS analyses and directly measured by EA-C/P-IRMS.

Syntheses. Syntheses of **1** and **2** were performed using synthetic and natural educts as well as various conditions for condensation and cyclization steps (cf. Table 1). Product isolation comprised vacuum distillation with subsequent LC purification on silica gel (glass column, 2 × 30 cm, filled with silica gel 60 Merck, 0.2–0.5 mm; pentane/diethyl ether gradient, from 0 to 100% diethyl ether in 10% steps, each 150 mL; flow rate, 3 mL/min). The fraction eluted with 10% diethyl ether contained **1** and **2**. After evaporation of the solvent in vacuo, the fraction was applied to GC-MS and GC-C/P-IRMS analyses.

Synthetic **1** and **2** were prepared according to the literature (13, 14) by base-catalyzed condensation each of synthetic citral and acetone using sodium in methanol and subsequent acid-catalyzed cyclization of the resulting *E,Z*/*E,E*-pseudoionones using sulfuric acid/acetic acid.

'Natural' **1** and **2** were prepared similarly but using phosphoric acid for the acid-catalyzed cyclization step. As educts synthetic citral and natural acetone, citral ex *L. cubeba* and synthetic acetone as well as citral ex lemongrass and synthetic acetone were used.

'Natural' **1** and **2** ('natural'^a) were prepared as were synthetic **1** and **2** using natural citral (ex *L. cubeba* and ex lemongrass) and natural acetone.

In addition, 'natural' **1** ('natural'^b) was synthesized using natural citral (ex *L. cubeba* oil and ex lemongrass) with natural acetone and phosphoric acid as acid catalysts. This 'natural'^b **1** was used for subsequent determination of isotope values of the enantiomers obtained by separation on chiral HPLC phase.

Finally, 'natural' **1** ('natural'^c) was prepared using calcium oxide as natural basic catalyst for the condensation of natural acetone with natural citral ex *L. cubeba* as well as ex lemongrass oil, respectively (15). Cyclization of the pseudoionones was catalyzed using tartaric acid/acetic acid.

The influence of sample preparation on the ²H/¹H and ¹³C/¹²C isotope ratios was checked for compounds **1–3** by model SDE and for compounds **1** and **2** by distillation and subsequent LC separation on silica gel. No significant isotope discrimination effects were observed by the applied workup procedure.

Gas Chromatography–Mass Spectrometry (GC-MS). An Agilent 6890 series gas chromatograph (GC) with split injection (220 °C, 1:20) was directly coupled to an 5973 Network mass spectrometer (Agilent Technologies Inc., Palo Alto, CA). The GC was equipped with a DB-Wax (J&W, Agilent, Waldbronn, Germany) fused silica capillary column (30 m × 0.25 mm i.d.; $d_f = 0.25 \mu\text{m}$). The temperature program was 3 min isothermal at 50 °C and then raised at 4 °C/min to 220 °C. Helium was used as the carrier gas, 1 mL/min being the flow rate. The temperatures of the ion source and the transfer line were 220 and 200 °C, respectively. The electron energy for the EI mass spectra was 70 eV. Identification was performed by comparison of linear retention indices and mass spectrometric data for sample constituents with that of authentic reference compounds.

Multidimensional Gas Chromatography–Mass Spectrometry (MDGC-MS). A double-oven MDGC system (oven I, Fisons GC 8160; oven II, Fisons GC 8130) coupled to a Fisons MD 800 mass spectrometer was used. α -Ionone (**1**) was transferred from the precolumn to the main column via the moving column stream switching (MCSS) system at a defined cut time. The prepreparation was performed using a J&W DB-Wax fused silica capillary column (30 m × 0.25 mm i.d., $d_f = 0.25 \mu\text{m}$); GC oven I was programmed from 60 °C at 10 °C/min to 240 °C; the cut time (10–12.3 min) was selected with a FID as monitor detector. As transfer lines between the GC ovens I and

Table 1. Educts and Catalysts Used To Synthesize **1** and **2**

educts	base-catalyzed condensation ^a	acid-catalyzed cyclization ^b	products
citral, synthetic; acetone, synthetic	i	a	synthetic 1 , 2
citral, synthetic; acetone, natural	i	b	'natural' 1 , 2
citral, ex <i>L. cubeba</i> ; acetone, synthetic	i	b	'natural' 1 , 2
citral, ex lemongrass; acetone, synthetic	i	b	'natural' 1 , 2
citral, ex <i>L. cubeba</i> ; acetone, natural	i	a	'natural' ^a 1 , 2
citral, ex lemongrass; acetone, natural	i	a	'natural' ^a 1 , 2
citral, ex <i>L. cubeba</i> ; acetone, natural	i	b	'natural' ^b 1
citral, ex lemongrass; acetone, natural	i	b	'natural' ^b 1
citral, ex <i>L. cubeba</i> ; acetone, natural	ii	c	'natural' ^c 1
citral, ex lemongrass; acetone, natural	ii	c	'natural' ^c 1

^a i, sodium methoxide; ii, CaO. ^b a, sulfuric acid/acetic acid; b, phosphoric acid; c, tartaric acid/acetic acid.

II and the GC oven II and the mass spectrometer deactivated fused silica capillaries (0.5 m × 0.25 mm i.d.) were used, each kept at 200 °C. The enantiomeric separation was carried out using a modified cyclodextrin fused silica capillary column, that is, 30% 2,3-diethyl-6-*tert*-butyldimethylsilyl- β -cyclodextrin in PS 86 (25 m × 0.25 mm i.d.; $d_f = 0.15 \mu\text{m}$) (Mega, Legnano, Italy). The temperature program for the GC oven II was as follows: 20 min isothermal at 100 °C and then raised at 3 °C/min to 200 °C. The MS conditions were the same as above outlined for GC-MS analysis.

Gas Chromatography–Isotope Ratio Mass Spectrometry (HRGC-IRMS). A Finnigan Delta plus XL isotope ratio mass spectrometer was coupled by an open-split via a combustion/pyrolysis (C/P) interface to an HP 6890 gas chromatograph (GC). The GC was equipped with a J&W DB-Wax fused silica capillary column (60 m × 0.32 mm i.d.; $d_f = 0.25 \mu\text{m}$). The following conditions were employed: 1 μL “splitless” injection (250 °C); temperature program, from 50 to 200 °C at 5 °C/min; helium flow, 2 mL/min.

Interfaces. For combustion to CO₂ an oxidation reactor (Al₂O₃; 0.5 mm i.d., 1.5 mm, o.d. = 320 mm) with Cu/Ni/Pt metal (each 240 mm × 0.125 mm) at 960 °C was used. Water separation was performed with a Nafion membrane. For pyrolysis to H₂ a ceramic tube (Al₂O₃, 0.5 mm i.d., 320 mm) kept at 1440 °C was employed.

In addition, off-line determinations of reference samples were performed by coupling elemental analyzers (EA) (¹³C/¹²C, Euro Vector EA 3000 Milano, Italy; temperature, 1000 °C; ²H/¹H, HT Sauerstoff-Analysator, HEKA Tech, Wegberg, Germany; temperature, 1460 °C) to the IRMS.

Daily system stability checks were carried out by measuring reference samples with known ¹³C/¹²C and ²H/¹H ratios. Stability check of the used reference gases was performed continuously by measuring International Atomic Energy Agency (IAEA, Vienna, Austria) standards with defined ¹³C/¹²C and ²H/¹H ratios (for ¹³C/¹²C IAEA-CH-7 and for ²H/¹H IAEA-CH-7, NBS 22 oil, and V-SMOW).

The isotope ratios are expressed in per mil (‰) deviation relative to the V-PDB and V-SMOW international standards. For ¹³C/¹²C determinations, the mass spectrometer was calibrated against reference CO₂ gas (Messer Griesheim, Frankfurt, Germany) with a defined $\delta^{13}\text{C}_{\text{V-PDB}}$ value of −24.9‰. Results are expressed in $\delta^{13}\text{C}_{\text{V-PDB}}$ values as

$$\delta^{13}\text{C}_{\text{V-PDB}} (\text{‰}) = \left(\frac{R_{\text{sample}} - R_{\text{V-PDB}}}{R_{\text{V-PDB}}} \right) \times 1000$$

where R is the isotope ratio ¹³C/¹²C.

The isotope ratios for ²H/¹H are expressed in per mil (‰) deviation relative to the Vienna standard mean ocean water (V-SMOW) international standard. The mass spectrometer was calibrated against reference H₂ gas (Messer Griesheim) with a defined $\delta^2\text{H}_{\text{V-SMOW}}$ value of −270 ± 10‰. Results are expressed in $\delta^2\text{H}_{\text{V-SMOW}}$ values as

$$\delta^2\text{H}_{\text{V-SMOW}} (\text{‰}) = \left(\frac{R_{\text{sample}} - R_{\text{V-SMOW}}}{R_{\text{V-SMOW}}} \right) \times 1000$$

where R is the isotope ratio ²H/¹H.

In general, 5-fold determinations were carried out and standard deviations were calculated. The latter were ± 0.1 and ± 5‰ for $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$, respectively. Additional peak recognition was performed by reference compounds and HRGC-MS registered under identical separation conditions as samples.

To determine the $\delta^2\text{H}_{\text{V-SMOW}}$ values of **1–3**, the system reliability was checked by measuring commercial references via the equipped EA. Data recorded by EA-C/P-IRMS were in good agreement with those determined by GC-C/P-IRMS analysis (data not shown). The areas of linearity for the $\delta^2\text{H}_{\text{V-SMOW}}$ determinations were from 1 to 5 μg (on column) for the compounds under study. Finally, the influence of sample preparation on the ²H/¹H and ¹³C/¹²C isotope ratios checked by model SDE, silica gel column separation, and distillation were found to be within the range of standard deviation and thus negligible (data not shown).

High-Performance Liquid Chromatography (HPLC). α -Ionone enantiomers were collected separately using a Knauer (Berlin, Germany) HPLC system equipped with a UV detector (selected wavelength of 225 nm). Chiral separation was performed according to the method of Zenoni et al. (16) on a Nucleodex- β -PM (Macherey-Nagel, Düren, Germany) column (20 cm × 4 mm; particle diameter = 5 μm). As mobile phase a mixture of methanol/water (70:30; v/v) with a flow rate of 0.5 mL/min was used.

RESULTS AND DISCUSSION

$\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values of α -ionone (**1**), β -ionone (**2**), and α -ionol (**3**) originating from various sources were measured. In all cases, LC pre-separation/purification was performed to exclude interferences from peak overlapping (7).

Using citral and acetone as natural educts in the traditional Tiemann route, ‘natural’ **1** and **2** will be obtained. Natural acetone is available by acetone–butanol fermentation of molasses or corn with *Clostridium acetobutylicum* cultures (17). Natural citral is obtained by steam distillation of essential oils, the most commonly used being lemongrass (ex *Cymbopogon flexuosus* and *citriatus*, up to 85% citral) and *L. cubeba* (up to 75% citral) (1). Isotope data ($\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$) of citral have already been reported (18, 19). Syntheses of **1** and **2** were performed using synthetic and natural educts as well as various conditions for condensation and cyclization steps (Table 1).

α -Ionone (1). In Table 2 the $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values recorded for **1** from different sources are summarized. Commercial ($n = 1$) and self-prepared synthetic ($n = 1$) references showed practically the same $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values (−29.0 and −28.0‰ as well as −26 and −27‰, respectively), which clearly differ from those determined for **1** from raspberry ($n = 8$; $\delta^{13}\text{C}_{\text{V-PDB}}$ from −30.3 to −34.8‰ and $\delta^2\text{H}_{\text{V-SMOW}}$ from −187 to −221‰). In both cases, the $\delta^{13}\text{C}_{\text{V-PDB}}$ values are in good agreement with those of the

Table 2. $\delta^2\text{H}_{\text{V-SMOW}}$ and $\delta^{13}\text{C}_{\text{V-PDB}}$ Values^a of Self-Prepared Ionones **1** and **2** Synthesized from Educts of Synthetic and Natural Origin^b

compound	$\delta^2\text{H}_{\text{V-SMOW}}$ (‰)	$\delta^{13}\text{C}_{\text{V-PDB}}$ (‰)
acetone		
synthetic	-160	-28.0
natural	-250	-5.8
citral		
synthetic	-30	-27.2
synthetic, Hör et al. (18); Nhu-Trang et al. (19)	+62 to -59	-28.1 to -30.1
natural from <i>L. cubeba</i> oil	-284	-26.6
natural, from <i>L. cubeba</i> , Hör et al. (18); Nhu-Trang et al. (19)	-206 to -297	-24.6 to -27.9
natural from lemongrass oil	-260	-10.7
natural from lemongrass oil, Hör et al. (14); Nhu-Trang et al. (19)	-207 to -288	-8.8 to -14.2
α -ionone (1)		
synthetic	-27	-28.0
synthetic, commercial	-26	-29.0
synthetic, Braunsdorf et al. (3)		-24.3 to -27.0
synthetic, Casabianca et al. (6)		-23.0 to -28.0
'natural' from synthetic citral + natural acetone	-43	-25.6
'natural' from citral ex <i>L. cubeba</i> + synthetic acetone	-242	-26.9
'natural' from citral ex lemongrass + synthetic acetone	-232	-18.5
'natural' ^a from citral ex <i>L. cubeba</i>	-203	-24.1
'natural' ^a from citral ex lemongrass	-230	-12.0
'natural' ^b from citral ex <i>L. cubeba</i>	-257	-22.0
'natural' ^b from citral ex lemongrass	-225	-16.5
'natural' ^c from citral ex <i>L. cubeba</i>	-252	-19.8
'natural' ^c from citral ex lemongrass	-213	-9.1
'biotechnological', Braunsdorf et al. (3)		-9.1
'biotechnological', Sewenig et al. (7)	-205 to -296	-10.2 to -31.7
ex raspberry fruit (<i>n</i> = 8)	-187 to -221	-30.3 to -34.8
ex raspberry fruit, Sewenig et al. (7)	-190 to -214	-29.0 to -35.1
β -ionone (2)		
synthetic	-45	-28.0
synthetic, commercial	-47	-28.0
synthetic, Braunsdorf et al. (3)		-25.6 to -28.6
'natural' from synthetic citral + natural acetone	-43	-25.6
'natural' from citral ex <i>L. cubeba</i> + synthetic acetone	-222	-28.0
'natural' from citral ex lemongrass + synthetic acetone	-202	-18.8
'natural' ^a from citral ex <i>L. cubeba</i>	-199	-22.8
'natural' ^a from citral ex lemongrass	-206	-12.0
'biotechnological', Sewenig et al. (7)		-8.6
ex raspberry fruit (<i>n</i> = 8)	-176 to -203	-31.0 to -35.1
ex raspberry fruit, Sewenig et al. (7)	-164 to -202	-28.3 to -33.4

^a Standard deviations of ± 5 and ± 0.1 for $\delta^2\text{H}_{\text{V-SMOW}}$ and $\delta^{13}\text{C}_{\text{V-PDB}}$ determinations, respectively. ^b Data are compared with those of **1** and **2** from raspberry fruit and in relation to the literature. ^{a-c}Cf. catalysts given in Table 1.

literature exhibiting values from -23.0 to -28.0‰ for synthetic standards (3, 6) and from -29.0 to -35.1‰ for raspberry (7).

Considering the isotope values of 'natural' **1** (produced from synthetic/natural educts), the influence of the origin of citral can be clearly recognized, whereas that of acetone seems to be negligible. As expected for a C4 plant, the use of citral from lemongrass led to an enriched $\delta^{13}\text{C}_{\text{V-PDB}}$ value of -18.5‰. In addition, the use of synthetic citral resulted in an enriched $\delta^2\text{H}_{\text{V-SMOW}}$ value of -43‰, whereas with citral ex *L. cubeba* and ex lemongrass values of -242 and -232‰, respectively, were recorded.

The strong influence of citral ex lemongrass on the $\delta^{13}\text{C}_{\text{V-PDB}}$ values was also obvious in the syntheses of 'natural'^{a,b,c} **1**. With data of -12.0, -16.5, and -9.1‰, respectively, the influence of the C4 origin was remarkable in each case. Similar enrichment of the $\delta^{13}\text{C}_{\text{V-PDB}}$ values was observed for 'natural'^{a,b,c} **1** synthesized from citral ex *L. cubeba*, whereas in all essays the

Table 3. Enantiomeric Excess (ee) as well as $\delta^2\text{H}_{\text{V-SMOW}}$ and $\delta^{13}\text{C}_{\text{V-PDB}}$ Values^a of α -Ionone (**1**) Enantiomers Separated and Collected by HPLC on Chiral Phase

	ee (%)	$\delta^2\text{H}_{\text{V-SMOW}}$ (‰)	$\delta^{13}\text{C}_{\text{V-PDB}}$ (‰)
α -ionone 'natural' ^b from citral ex lemongrass			
<i>S</i> - α -ionone	100	-225	-16.8
<i>R</i> - α -ionone	97	-225	-16.2
α -ionone 'natural' ^b from citral ex <i>L. cubeba</i>			
<i>S</i> - α -ionone	98	-257	-22.0
<i>R</i> - α -ionone	97	-257	-22.4

^a Standard deviations of ± 5 and ± 0.1 for $\delta^2\text{H}_{\text{V-SMOW}}$ and $\delta^{13}\text{C}_{\text{V-PDB}}$ determinations, respectively.

corresponding $\delta^2\text{H}_{\text{V-SMOW}}$ values (ranging from -203 to -257‰) remained more or less unaffected. The explanation for the process-related enrichment of $\delta^{13}\text{C}_{\text{V-PDB}}$ values requires a systematic insight into the kinetics of the syntheses, an approach going beyond the scope of the practical targets of the present work.

Taking into account upcoming techniques such as simulated moving bed (SMB) for the large-scale resolution of racemates to be used in industry, the enantiomers of **1** were separated by chiral HPLC and submitted separately to GC-IRMS (16). The enantiomeric purity of each collected fraction was checked by enantio-MDGC-MS. Recorded enantiomeric excess as well as the corresponding $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ data are summarized in Table 3. For this approach 'natural'^b **1** was synthesized. As expected, $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values were found to be nearly identical for both enantiomers.

β -Ionone (2**).** Byproducts present in self-prepared **2** from synthetic and from natural educts were studied by GC-MS in comparison with those occurring in synthetic, unspecified and declared to be natural commercial references. 1,1,6-Trimethyltetraline and α -ionone that have previously been reported as isomerization products of β -ionone in the presence of catalytic amounts of sulfuric acid (20), as well as *p*-cymene, 3,4-dehydro-7,8-dihydro- β -ionone, 5,6-epoxy- β -ionone, 4-oxo- β -ionone, and 4-hydroxy- β -ionone, appeared as byproducts in self-prepared **2**. Furthermore, α -ionone, 3,4-dehydro-7,8-dihydro- β -ionone, 5,6-epoxy- β -ionone, 4-oxo- β -ionone, and 4-hydroxy- β -ionone were found in synthetic, unspecified, and commercial references declared to be 'natural'. In the latter, in addition, 1,1,6-trimethyltetraline was also detected. This fact suggested synthetic origins for both of these commercial samples.

In Table 2 the $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values of **2** from different sources are summarized. For a synthetic reference the isotope values (-28.0 and -45‰, respectively) clearly differed from those recorded for **2** from raspberry (*n* = 8), ranging from -31.0 to -35.1‰ and from -176‰ to -203‰ for $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$, respectively. The data were in good agreement with those available from the literature that provide for **2** $\delta^{13}\text{C}_{\text{V-PDB}}$ values from -25.6 to -28.6‰ (3) for synthetic references, as well as $\delta^{13}\text{C}_{\text{V-PDB}}$ values ranging from -28.3 to -33.4‰ (3, 5, 6) and $\delta^2\text{H}_{\text{V-SMOW}}$ values from -164 to -202‰ from raspberry (7).

IRMS analyses of synthesized 'natural' **2** obtained from synthetic/natural educts revealed $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values that were nearly identical to those recorded for 'natural' **1** (Table 2). The strong influence of citral from C4 origin on the $\delta^{13}\text{C}_{\text{V-PDB}}$ values was again evident in the syntheses in which natural acetone was used. Similar to the results obtained for **1**, the used variations of process conditions did not influence the $\delta^2\text{H}_{\text{V-SMOW}}$ values of 'natural' **2**.

α -Ionol (3). The isotope data recorded for **3** from raspberry ($n = 8$; $\delta^{13}\text{C}_{\text{V-PDB}}$ from -33.6 to -36.6% ; $\delta^2\text{H}_{\text{V-SMOW}}$ from -200 to -225%) clearly differed from those of synthetic **3** ($\delta^{13}\text{C}_{\text{V-PDB}} = -24.5\%$; $\delta^2\text{H}_{\text{V-SMOW}} = -184\%$). To our best knowledge, there were no isotope data for **3** available in the literature to date.

In summary, our analyses performed by isotope ratio mass spectrometry in the combustion and pyrolysis modes measuring $\delta^{13}\text{C}_{\text{V-PDB}}$ and $\delta^2\text{H}_{\text{V-SMOW}}$ values provide for the first time isotope data of 'natural' **1** and **2** produced in the traditional Tiemann route, as well as of synthetic and 'ex plant' α -ionol (**3**). Together with the recently given recommendations (7) this work is a contribution to facilitate authentication assessment of 'natural' **1** and **2**. Further research is required to provide supplementary IRMS data for **1** and **2** from additional origins (11).

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LITERATURE CITED

- (1) Surburg, H.; Panten, J. *Common Fragrance and Flavor Materials*, 5th ed.; Wiley-VCH: Weinheim, Germany, 2006.
- (2) Auldridge, M. E.; McCarty, D. R.; Klee, H. J. Plant carotenoid oxygenases and their apocarotenoid products. *Curr. Opin. Plant Biol.* **2006**, *9*, 315–321.
- (3) Braunsdorf, R.; Hener, U.; Lehmann, D.; Mosandl, A. Analytical differentiation of natural, fermented and synthetic (nature-identical) aromas. Part 1. Origin-specific analysis of (*E*)- $\alpha(\beta)$ -ionones. *Dtsch. Lebensm. Rundsch.* **1991**, *87*, 277–280.
- (4) Werkhoff, P.; Brennecke, S.; Bretschneider, W.; Güntert, M.; Hopp, R.; Surburg, H. Chiro-specific analysis in essential oil, fragrance and flavor research. *Z. Lebensm. Unters.-Forsch.* **1993**, *196*, 307–328.
- (5) Casabianca, H.; Graff, J. B. Enantiomeric and isotopic analysis of flavour compounds of some raspberry cultivars. *J. Chromatogr.* **1994**, *684*, 360–365.
- (6) Casabianca, H.; Graff, J. B.; Jame, P.; Perrucchiotti, C.; Chastrette, M. Application of hyphenated techniques to the chromatographic authentication of flavors in food products and perfumes. *J. High Resolut. Chromatogr.* **1995**, *18*, 279–285.
- (7) Sewenig, S.; Bullinger, D.; Hener, U.; Mosandl, A. Comprehensive authentication of *E*- $\alpha(\beta)$ -ionone from raspberries, using constant flow MDGC-C/P-IRMS and enantio-MDGC-MS. *J. Agric. Food Chem.* **2005**, *53*, 838–844.
- (8) Tiemann, F.; Krüger, P. Über Veilchenaroma. *Ber. Dtsch. Chem. Ges.* **1893**, *26*, 2675–2708.
- (9) Tiemann, F. Über die Veilchenketone und die in Beziehung dazu stehenden Verbindungen der Citral(Geranal)reihe. *Ber. Dtsch. Chem. Ges.* **1898**, *31*, 808–866.
- (10) Ohloff, G.; Schade, G. Darstellung von γ -Jonon aus all-trans-Pseudojonon. *Angew. Chem.* **1962**, *74*, 944.
- (11) Brenna, E.; Fuganti, C.; Serra, S.; Kraft, P. Optically active ionones and derivatives: preparation and olfactory properties. *Eur. J. Org. Chem.* **2002**, 967–978.
- (12) Ropert, F.; Dumont, B.; Belin, J. M. Biotechnological method of ionone production in non conventional media. In *Bioflavour '95*; Etiévant, P., Schreier, P., Eds.; Editions INRA: Paris, France, 1995; pp 275–278.
- (13) Hibbert, H.; Cannon, L. T. Condensation of citral with ketones and synthesis of some new ionones. *J. Am. Chem. Soc.* **1924**, *46*, 119–130.
- (14) Krishna, H. J. V.; Joshi, B. N. The preparation of β -ionone. *J. Org. Chem.* **1957**, *22*, 224–226.
- (15) Noda, C.; Alt, G. P.; Werneck, R. M.; Henriques, C. A.; Monteiro, J. L. F. Aldol condensation of citral with acetone on basic solid catalyst. *Braz. J. Chem. Eng.* **1998**, *15*, 120–125.
- (16) Zenoni, G.; Quattrini, F.; Mazzotti, M.; Fuganti, C.; Morbidelli, M. Scale-up of analytical chromatography to the simulated moving bed separation of the enantiomers of the flavour norterpeneoids α -ionone and α -damascone. *Flavour Fragrance J.* **2002**, *17*, 195–202.
- (17) Dill, B.; Eisenbrand, G.; Fugmann, B.; Gamse, T.; Heiker, F. R.; Hulpke, H.; Kirschning, A.; Pohnert, G.; Pühler, A.; Schreier, P. *Roempp On-Line Chemical Encyclopedia*; Thieme: Stuttgart, Germany, 2006.
- (18) Hör, K.; Ruff, C.; Weckerle, B.; König, T.; Schreier, P. $^2\text{H}/^1\text{H}$ ratio analysis of flavor compounds by on-line gas chromatography–pyrolysis–isotope ratio mass spectrometry (HRGC-P-IRMS): citral. *Flavour Fragrance J.* **2001**, *16*, 344–348.
- (19) Nhu-Trang, T. T.; Casabianca, H.; Grenier-Loustalot, M. F. Authenticity of essential oils containing citronellal and citral by stable-isotope gas-chromatographic analysis. *Anal. Bioanal. Chem.* **2006**, *386*, 2141–2152.
- (20) Vasilchenko, S. A.; Fadeev, G. N.; Lebedeva, O. N.; Dryuk, L. F. Isomerization of β -ionone series. *Vopr. Khim. Khim. Tekhnol.* **1988**, *86*, 96–99.

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