

# Flavor Authenticity Studies by $^2\text{H}/^1\text{H}$ Ratio Determination Using On-line Gas Chromatography Pyrolysis Isotope Ratio Mass Spectrometry

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Based on  $^2\text{H}/^1\text{H}$  ratio measurements of commercial synthetic and “natural” references, the recently developed on-line gas chromatography pyrolysis isotope ratio mass spectrometry (HRGC-P-IRMS) technique was used to determine the  $\delta^2\text{H}_{\text{SMOW}}$  values of the flavor compounds decanal, linalool, and linalyl acetate, as well as those of *E*-2-hexenal and *E*-2-hexenol in foods and essential oils. In preceding model studies, the influence of sample preparation steps (simultaneous distillation extraction, SDE; solvent extraction, SE; liquid liquid extraction, LLE) on the  $\delta^2\text{H}$  values was found to be negligible. For decanal, the typical  $^2\text{H}$  abundance, with higher content of  $^2\text{H}$  for synthetic material ( $\delta^2\text{H}_{\text{SMOW}}$  from  $-90$  to  $-156\%$ ) and lower  $^2\text{H}$  content for natural references ( $\delta^2\text{H}_{\text{SMOW}}$  from  $-138$  to  $-262\%$ ) was observed. Although the  $\delta^2\text{H}$  data recorded for linalool did not allow one to distinguish between synthetic ( $\delta^2\text{H}_{\text{SMOW}}$  from  $-207$  to  $-301\%$ ) and natural ( $\delta^2\text{H}_{\text{SMOW}}$  from  $-234$  to  $-333\%$ ) materials, the situation was somewhat more encouraging for linalyl acetate;  $\delta^2\text{H}_{\text{SMOW}}$  values from  $-199$  to  $-239\%$  and from  $-213$  to  $-333\%$  were found for synthetic and natural samples, respectively. *E*-2-Hexenal and *E*-2-hexenol showed clear-cut origin-dependent differences in their  $^2\text{H}/^1\text{H}$  ratios; that is,  $\delta^2\text{H}_{\text{SMOW}}$  values from  $-14$  to  $-109\%$  and from  $-263$  to  $-415\%$  as well as from  $-41$  to  $-131\%$  and from  $-238$  to  $-348\%$  were recorded for products from synthetic and natural origins, respectively.

**Keywords:** Flavor authenticity; origin assignment; stable isotope ratio analysis; decanal; *E*-2-hexenal; *E*-2-hexenol; linalool; linalyl acetate; HRGC-P-IRMS,  $^2\text{H}/^1\text{H}$  ratio

## INTRODUCTION

In flavor authenticity studies, two principles, (i) enantioselectivity and (ii) isotope ratio, are used to control the origin of chiral and achiral flavor compounds, respectively (1). Whereas the fundamental studies of enantioselectivity go back to the beginning of the 1980s (2, 3) and nowadays well-established analytical techniques such as multidimensional gas chromatography (MDGC) and MDGC mass spectrometry (MDGC-MS) (4, 5) are available, mass spectrometrical measurements of isotope ratios were limited to “off-line” determinations of  $^{13}\text{C}/^{12}\text{C}$  and  $^2\text{H}/^1\text{H}$  ratios for a long time. In the past decade, the on-line coupling of gas chromatography (HRGC) with isotope ratio mass spectrometry (IRMS) via a combustion interface (HRGC-C-IRMS) has opened the access to the analysis of  $^{13}\text{C}/^{12}\text{C}$  ratios of individual constituents in complex flavorings (6). Recently, the measurement of  $^{18}\text{O}/^{16}\text{O}$  ratios was made available in both “off-line” and “on-line” modes using pyrolysis (P) IRMS (7–11).

The large variations known to exist in the  $^2\text{H}/^1\text{H}$  ratio in nature have made it a very attractive target for IRMS studies. However, technical problems have precluded for a long time successful measurement of  $^2\text{H}/^1\text{H}$  ratios of individual peaks eluting from a capillary column. Re-

cently, these problems have been overcome, and  $^2\text{H}/^1\text{H}$  determinations of GC peaks are possible using commercially available equipment (12). Most recently, pioneering information on the  $^2\text{H}/^1\text{H}$  ratios obtained by on-line HRGC-P-IRMS of the flavor compounds benzaldehyde and citral (neral/geranial) has been provided (13, 14). In this paper, we report  $\delta^2\text{H}_{\text{SMOW}}$  data of decanal, linalool, and linalyl acetate, as well as *E*-2-hexenal and *E*-2-hexenol, each determined in foods and essential oils by this new “on-line” HRGC-IRMS technique.

## MATERIALS AND METHODS

**Chemicals, Foods and Essential Oils.** Synthetic and “natural” samples of decanal, linalool, and linalyl acetate, as well as *E*-2-hexenal and *E*-2-hexenol, were from ABCR, Karlsruhe, Germany; Acros, Geel, Belgium; Aldrich, Steinheim, Germany; BASF, Ludwigshafen, Germany; BFA, Le Cannet, France; Chemos, Regenstauf, Germany; Fluka, Deisenhofen, Germany; Lancaster, Mühlheim, Germany; Phoenix, Norwood, USA; Roth, Karlsruhe, Germany; SAM, Mannheim, Germany; and Sigma, Steinheim, Germany.

Various essential oils were from Adrian, Marseille, France; Ayus, Bühl, Germany; BFA, Le Cannet, France; Caelo, Hilden, Germany; Flavex, Rehlingen, Germany (CO<sub>2</sub> extracts); Handa, Nottingham, U.K.; Hevea, Vallauris, France; Primavera, Sulzberg, Germany; Roth, Karlsruhe, Germany; Serva, Heidelberg, Germany; SAM, Mannheim, Germany; Taoasis, Lemgo, Germany; and Vieille, Vallauris, France. Samples of apple recovery aroma were obtained by SAM, Mannheim, Germany.

Oranges and grapefruits (from Spain and Israel, respectively), apples (i.e., Granny Smith from Chile and South Africa; Golden Delicious and Braeburn from Italy), and peaches and

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nectarines (from Italy and France, respectively) were purchased from a local market. Dried thyme, lavender, coriander, and basil were obtained from a local drugstore and from Klenk, Schwebheim, Germany. Fresh Rosaceae leaves (apple, sloe tree, and dogrose) were collected in the Würzburg area.

**Sample Preparation.** Synthetic and natural samples of decanal, linalool, linalyl acetate, *E*-2-hexenal, *E*-2-hexenol, and the essential oils were dissolved (1 mg/mL) in diethyl ether and the solutions directly analyzed by HRGC-MS and HRGC-P-IRMS.

Samples of aqueous apple recovery aroma and various apples—after homogenization and centrifugation—were subjected to liquid–liquid extraction (LLE) for 48 h using a pentane/dichloromethane mixture (2+1, v/v).

Dried thyme, lavender, coriander, and basil as well as the fresh Rosaceae leaves (50 g fresh/dry) were homogenized in a Waring blender and, after the addition of 800 mL of distilled water, subjected to simultaneous distillation extraction (SDE) (3 h) using a pentane/diethyl ether mixture (1+1, v/v).

Peels of citrus fruits (oranges and grapefruits) were rubbed off with a grater, and the peel oil was extracted with 200 mL of pentane/diethyl ether mixture (1+1).

All of the extracts were dried over anhydrous sodium sulfate, filtered, and carefully concentrated to an appropriate volume using a Vigreux column (40 °C).

Model experiments comprised LLE (pentane/dichloromethane, 2+1) and SDE (pentane/diethyl ether, 1+1) of *E*-2-hexenal and *E*-2-hexenol solutions (each containing 2 mg in 250 mL of 2% ethanolic solution and in 800 mL of water, respectively). In addition, solvent extraction (SE) (pentane/diethyl ether, 1+1) and SDE of decanal, linalool, and linalyl acetate solutions (each containing 2 mg in 800 mL of water) were carried out with subsequent Vigreux distillation (cf. above).

**Gas Chromatography Pyrolysis Isotope Ratio Mass Spectrometry (HRGC-P-IRMS).** A Finnigan Delta<sup>plus</sup> XL isotope ratio mass spectrometer coupled by an “open-split” via a pyrolysis interface to an HP 6890 gas chromatograph (GC) was used. The GC was equipped with a J&W DB-Wax fused silica capillary column (60 m × 0.32 mm i.d.; df = 0.25 μm). The following conditions were employed: 1 μL splitless injection (250 °C); temperature program, raised from 50 to 200 °C at 5 °C/min; helium flow, 3 mL/min; pyrolysis interface temperature, 1440 °C; hot ion source. The effluent from the GC passes the high temperature through a ceramic tube (Al<sub>2</sub>O<sub>3</sub>, l = 320 mm; 0.5 mm i.d.).

The separated compounds eluting from the GC column are converted into H<sub>2</sub> in the pyrolysis interface. Analysis is performed in the mass spectrometer by simultaneous recording of masses 2 (<sup>1</sup>H<sup>1</sup>H) and 3 (<sup>1</sup>H<sup>2</sup>H) (12). In addition, coupling of the IRMS to an elemental analyzer (EA) (HT Sauerstoff Analysator HEKAtech, Wegberg, Germany) was realized for “off-line” control determinations of reference substances. System stability check was carried out routinely by measuring an IAEA (International Atomic Energy Agency) standard (NBS 22 oil).

The isotope ratios <sup>2</sup>H/<sup>1</sup>H are expressed in per mil (‰) deviation relative to the standard mean ocean water (SMOW) international standard. The mass spectrometer was calibrated against H<sub>2</sub> gas (Messer Griesheim, Frankfurt, Germany) with a defined <sup>2</sup>H/<sup>1</sup>H content relative to the SMOW standard (δ<sup>2</sup>H<sub>SMOW</sub> = −200‰). Results are expressed in δ<sup>2</sup>H<sub>SMOW</sub> units as

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

where *R* is the isotope ratio <sup>2</sup>H/<sup>1</sup>H.

Six-fold determinations were carried out and standard deviations calculated. Additional control of peak recognition was performed by reference compounds and HRGC-MS registered under identical separation conditions as samples.

**Gas Chromatography–Mass Spectrometry (HRGC-MS).** A Fisons GC 8000 series gas chromatograph with split injection (220 °C; 1:30) was directly coupled to a Fisons

**Table 1.** δ<sup>2</sup>H<sub>SMOW</sub> Values of Commercial Synthetic Reference Chemicals<sup>a</sup> Determined (i) Off-line via Elemental Analysis (EA) and (ii) On-line via HRGC-P-IRMS

|                     | δ <sup>2</sup> H <sub>SMOW</sub> (‰) ± sd |             |
|---------------------|---|-------------|
|                     | EA-P-IRMS                                 | HRGC-P-IRMS |
| decanal             |   |             |
| A                   | −101 ± 2                                  | −137 ± 2    |
| B                   | −78 ± 1                                   | −90 ± 2     |
| C                   | −95 ± 3                                   | −126 ± 1    |
| D                   | −83 ± 2                                   | −105 ± 3    |
| E                   | −93 ± 1                                   | −115 ± 3    |
| F                   | −122 ± 2                                  | −156 ± 2    |
| linalool            |   |             |
| A                   | −186 ± 2                                  | −207 ± 1    |
| B                   | −187 ± 2                                  | −212 ± 1    |
| C                   | −187 ± 2                                  | −212 ± 2    |
| D                   | −266 ± 3                                  | −301 ± 1    |
| E                   | −269 ± 3                                  | −295 ± 3    |
| linalyl acetate     |   |             |
| A                   | −171 ± 4                                  | −206 ± 1    |
| B                   | −189 ± 2                                  | −224 ± 2    |
| C                   | −202 ± 1                                  | −239 ± 1    |
| D                   | −184 ± 1                                  | −215 ± 2    |
| E                   | −170 ± 1                                  | −199 ± 1    |
| <i>E</i> -2-hexenal |   |             |
| A                   | −2 ± 4                                    | −14 ± 8     |
| B                   | −63 ± 2                                   | −81 ± 3     |
| C                   | −8 ± 2                                    | −42 ± 4     |
| D                   | −25 ± 1                                   | −17 ± 1     |
| E                   | −91 ± 2                                   | −109 ± 1    |
| <i>E</i> -2-hexenol |   |             |
| A                   | −101 ± 3                                  | −131 ± 1    |
| B                   | −11 ± 3                                   | −41 ± 3     |
| C                   | −16 ± 2                                   | −44 ± 6     |
| D                   | −16 ± 2                                   | −47 ± 2     |
| E                   | −103 ± 3                                  | −129 ± 1    |

<sup>a</sup> Mean values of six determinations and standard deviation (sd). Purity determined by HRGC-MS varied from 90% (*E*-2-hexenal) to 97% (linalool).

Instruments MD 800 mass spectrometer. The same type of J&W DB-Wax fused silica column was used under identical conditions as mentioned above (HRGC-P-IRMS). The temperature of the ion source was 230 °C and that of the connecting parts, 200 °C. The electron energy for the EI mass spectra was 70 eV, and the cathodic current was 4.1 mA.

## RESULTS AND DISCUSSION

First, the δ<sup>2</sup>H<sub>SMOW</sub> values of defined references of decanal, linalool, and linalyl acetate, as well as *E*-2-hexenal and *E*-2-hexenol, were reproducibly determined off-line via the equipped elemental analyzer (EA). The data obtained for each of the synthetic compounds under study are summarized in Table 1. One of these reference substances (each from supplier A; cf. Table 1) was used for the subsequent HRGC-P-IRMS analysis, in which peak recognition was additionally made according to the retention data of reference substances. The aim of the series of HRGC-P-IRMS studies with authentic references was to determine the range of sample amounts, in which not only reproducibility but also linearity of data can be obtained. In our previous studies we had observed that the <sup>2</sup>H/<sup>1</sup>H ratios measured by HRGC-P-IRMS were not independent of sample size (13, 14). The data obtained for our target compounds by each of six-fold determinations carried out in the range of 0.2–>1 μg (on column) were found to be 3, 0.5, 0.5, 1.5, and 1 μg for decanal, linalool, linalyl acetate, *E*-2-hexenal, and *E*-2-hexenol, respectively. Only when at least the above-mentioned amounts (on-column) were used was the required linearity given. The on-line determined mean

**Table 2. Influence of Sample Preparation (SDE, SE, and LLE, Each with Subsequent Vigreux Distillation) on the  $\delta^2\text{H}_{\text{SMOW}}$  Values Determined by HRGC-P-IRMS Model Studies Using Reference Compounds (Each "A" Listed in Table 1)<sup>a</sup>**

| step/compound            | $\delta^2\text{H}_{\text{SMOW}}$ (‰ $\pm$ sd) |
|--------------------------|---|
| SDE/decanal              | -130 $\pm$ 5                                  |
| SE/decanal               | -133 $\pm$ 5                                  |
| SDE/linalool             | -202 $\pm$ 6                                  |
| SE/linalool              | -200 $\pm$ 4                                  |
| SDE/linalyl acetate      | -215 $\pm$ 3                                  |
| SE/linalyl acetate       | -204 $\pm$ 5                                  |
| SDE/ <i>E</i> -2-hexenal | -10 $\pm$ 4                                   |
| LLE/ <i>E</i> -2-hexenal | -7 $\pm$ 5                                    |
| SDE/ <i>E</i> -2-hexenol | -133 $\pm$ 1                                  |
| LLE/ <i>E</i> -2-hexenol | -126 $\pm$ 2                                  |

<sup>a</sup>Mean values of six determinations and standard deviation (sd).

**Table 3. Range of  $\delta^2\text{H}_{\text{SMOW}}$  Data<sup>a</sup> Determined by HRGC-P-IRMS Analysis of Decanal from Various Origins**

| origin ( <i>n</i> = no. of samples)     | range $\delta^2\text{H}_{\text{SMOW}}$ (‰) |
|---|--|
| synthetic ( <i>n</i> = 6)               | -90 to -156                                |
| "natural" ( <i>n</i> = 3)               | -138 to -262                               |
| "ex orange" ( <i>n</i> = 3)             | -175 to -236                               |
| sweet orange oil ( <i>n</i> = 5)        | -170 to -187                               |
| orange <sup>b</sup> ( <i>n</i> = 5)     | -164 to -177                               |
| grapefruit <sup>b</sup> ( <i>n</i> = 3) | -166 to -196                               |

<sup>a</sup>Mean values of six determinations. <sup>b</sup>Self-prepared samples.

$\delta^2\text{H}_{\text{SMOW}}$  values represented in Table 1 sufficiently agreed with those measured off-line by EA analysis. The observed differences from the off-line recorded values can be explained by the quite high impurities (3–10%) in the samples influencing the  $\delta^2\text{H}$  values analyzed by the EA technique.

The next step in our analytical procedure comprised model studies carried out again with one of the synthetic references (each A in Table 1), but now aimed to check the influence of sample preparation on the  $^2\text{H}/^1\text{H}$  isotope ratio. LLE, SDE, and SE were used with subsequent Vigreux distillation. The results of these model studies are summarized in Table 2. From the data it is obvious that the influence of the sample preparation steps on the  $^2\text{H}/^1\text{H}$  ratio was negligible.

In the following, the  $\delta^2\text{H}_{\text{SMOW}}$  data recorded by HRGC-P-IRMS of decanal, linalool, and linalyl acetate, as well as *E*-2-hexenal and *E*-2-hexenol, are represented separately. With regard to authenticity, it has to be considered that most samples under study were commercial products and only in a few cases were self-prepared materials used.

**Decanal.** Decanal is a component of many essential oils, such as various citrus peel oils. Its odor is reminiscent of orange peel, which changes to a fresh citrus odor when diluted. Decanal is used in blossom fragrances and in the production of citrus flavors (15). Although limited  $\delta^{13}\text{C}$  data of decanal are already available (6),  $\delta^2\text{H}$  values have not been reported to date. Ranges of  $\delta^2\text{H}_{\text{SMOW}}$  values determined by HRGC-P-IRMS analysis of decanal from various origins are summarized in Table 3. The typical  $^2\text{H}$  abundance, with higher content of  $^2\text{H}$  (range of  $^2\text{H}$  from  $\pm 0$  to -200‰) for synthetic material and lower  $^2\text{H}$  content for natural (range of  $^2\text{H}$  from -200 to -400‰), as generally found for higher alcohols, acids, and aldehydes (6), is reflected in Table 3. Owing to overlapping data between the group of synthetic samples ( $\delta^2\text{H}_{\text{SMOW}}$  from -90 to -156‰) and the natural ones ( $\delta^2\text{H}_{\text{SMOW}}$  from -138 to

**Table 4. Range of  $\delta^2\text{H}_{\text{SMOW}}$  Data<sup>a</sup> Determined by HRGC-P-IRMS Analysis of Linalool from Various Origins**

| origin ( <i>n</i> = no. of samples)        | range $\delta^2\text{H}_{\text{SMOW}}$ (‰) |
|--|--|
| synthetic ( <i>n</i> = 5)                  | -207 to -301                               |
| "natural" ( <i>n</i> = 1)                  | -297                                       |
| "ex Orange" ( <i>n</i> = 9)                | -237 to -333                               |
| basil oil <sup>b</sup> ( <i>n</i> = 4)     | -248 to -319                               |
| thyme oil <sup>c</sup> ( <i>n</i> = 3)     | -306 to -332                               |
| bergamot oil ( <i>n</i> = 6)               | -273 to -294                               |
| coriander oil <sup>b</sup> ( <i>n</i> = 4) | -245 to -267                               |
| ho-oil ( <i>n</i> = 2)                     | -328 to -331                               |
| laurel leaf oil ( <i>n</i> = 3)            | -293 to -331                               |
| lavender oil <sup>b</sup> ( <i>n</i> = 4)  | -228 to -308                               |
| lavandin oil ( <i>n</i> = 3)               | -278 to -307                               |
| neroli oil ( <i>n</i> = 4)                 | -234 to -253                               |
| orange <sup>c</sup> ( <i>n</i> = 5)        | -235 to -238                               |
| sweet orange oil ( <i>n</i> = 2)           | -244 to -257                               |
| petitgrain oil ( <i>n</i> = 7)             | -254 to -310                               |
| rosewood oil ( <i>n</i> = 6)               | -264 to -330                               |
| tea tree oil ( <i>n</i> = 2)               | -291 to -319                               |

<sup>a</sup>Mean values of six determinations. <sup>b</sup>Including one self-prepared sample. <sup>c</sup>Self-prepared.

**Table 5. Range of  $\delta^2\text{H}_{\text{SMOW}}$  Data<sup>a</sup> Determined by HRGC-P-IRMS Analysis of Linalyl Acetate from Various Origins**

| origin ( <i>n</i> = no. of samples)       | range $\delta^2\text{H}_{\text{SMOW}}$ (‰) |
|---|--|
| synthetic ( <i>n</i> = 5)                 | -199 to -239                               |
| "natural" ( <i>n</i> = 2)                 | -303 to -333                               |
| bergamot oil ( <i>n</i> = 6)              | -252 to -280                               |
| lavender oil <sup>b</sup> ( <i>n</i> = 3) | -246 to -280                               |
| lavandin oil ( <i>n</i> = 2)              | -270 to -272                               |
| neroli oil ( <i>n</i> = 2)                | -213 to -232                               |
| petitgrain oil ( <i>n</i> = 6)            | -215 to -282                               |

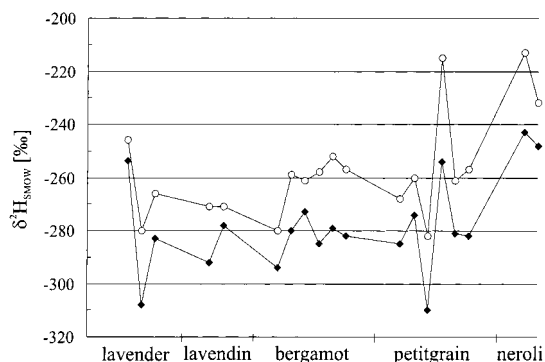
<sup>a</sup>Mean values of six determinations. <sup>b</sup>Including one self-prepared sample.

-262‰), however, origin assignment by means of  $\delta^2\text{H}$  data remains questionable.

**Linalool and Linalyl Acetate.** Both of these substances with their fresh flowery and bergamot–lavender odors, respectively, are very important among the monoterpenes for the flavor and fragrance industry. They can originate not only from lavender, bergamot, petitgrain oils, and coriander but also from "semisynthetic" and synthetic origins (15). Efforts to differentiate natural and "nature-identical" samples by means of  $\delta^{13}\text{C}$  data failed, whereas the  $\delta^2\text{H}$  values of natural linalool and linalyl acetate have been reported to be significantly lower than those of the synthetic counterparts. However, in some cases contradictory results have also been described (6).

The  $\delta^2\text{H}_{\text{SMOW}}$  data of linalool and linalyl acetate determined by HRGC-P-IRMS in the course of our studies are outlined in Tables 4 and 5, respectively. Whereas the  $\delta^2\text{H}$  data recorded for linalool do not allow one to distinguish between synthetic ( $\delta^2\text{H}_{\text{SMOW}}$  from -207 to -301‰) and natural ( $\delta^2\text{H}_{\text{SMOW}}$  from -234 to -333‰) origins, the situation was somewhat more encouraging for linalyl acetate. For the ester, little overlapping of data was found between the groups of synthetic ( $\delta^2\text{H}_{\text{SMOW}}$  from -199 to -239‰) and natural materials ( $\delta^2\text{H}_{\text{SMOW}}$  from -213 to -333‰).

In general, it is advantageous to apply knowledge of (bio)chemical reaction mechanisms and/or implied isotope effects on the interpretation or even prediction of isotopic patterns (6). For example, by the formation of esters, remarkable hydrogen isotope effects are implausible. However, as shown from the graph in Figure 1, distinct differences in  $\delta^2\text{H}$  values were evaluated be-



**Figure 1.**  $\delta^2\text{H}_{\text{SMOW}}$  values of linalool (◆) and its acetate (○) in various essential oils. A depletion of linalool by 7–30‰ can be observed.

**Table 6. Range of  $\delta^2\text{H}_{\text{SMOW}}$  Data<sup>a</sup> Determined by HRGC-P-IRMS Analysis of *E*-2-Hexenal from Various Origins**

| origin ( <i>n</i> = no. of samples)          | range $\delta^2\text{H}_{\text{SMOW}}$ (‰) |
|--|--|
| synthetic ( <i>n</i> = 5)                    | −14 to −109                                |
| "natural" ( <i>n</i> = 4)                    | −365 to −415                               |
| apple juice ( <i>n</i> = 4)                  | −312 to −360                               |
| apple juice <sup>b</sup> ( <i>n</i> = 3)     | −263 to −350                               |
| apple recovery aroma ( <i>n</i> = 12)        | −312 to −338                               |
| nectarine/peach <sup>b</sup> ( <i>n</i> = 4) | −311 to −341                               |
| Rosaceae leaves <sup>c</sup> ( <i>n</i> = 3) | −324 to −339                               |

<sup>a</sup> Mean values of six determinations. <sup>b</sup> Self-prepared. <sup>c</sup> Self-prepared from apple, sloe tree, dogrose.

**Table 7. Range of  $\delta^2\text{H}_{\text{SMOW}}$  Data<sup>a</sup> Determined by HRGC-P-IRMS Analysis of *E*-2-Hexenol from Various Origins**

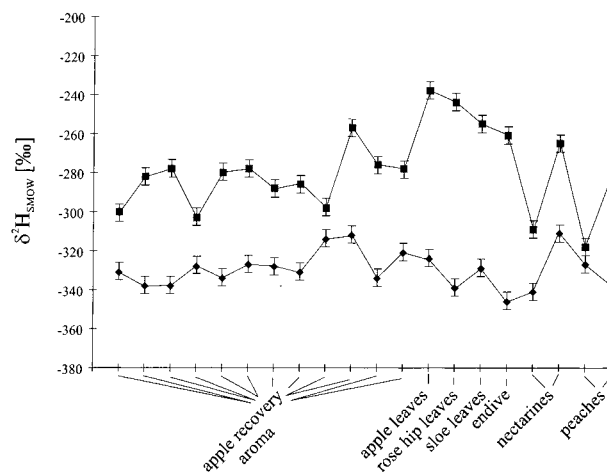
| origin ( <i>n</i> = no. of samples)          | range $\delta^2\text{H}_{\text{SMOW}}$ (‰) |
|--|--|
| synthetic ( <i>n</i> = 5)                    | −41 to −131                                |
| apple juice ( <i>n</i> = 11)                 | −248 to −300                               |
| apple juice <sup>b</sup> ( <i>n</i> = 3)     | −253 to −348                               |
| apple recovery aroma ( <i>n</i> = 17)        | −257 to −303                               |
| nectarine/peach <sup>b</sup> ( <i>n</i> = 4) | −265 to −318                               |
| Rosaceae leaves <sup>c</sup> ( <i>n</i> = 3) | −238 to −255                               |

<sup>a</sup> Mean values of six determinations. <sup>b</sup> Self-prepared. <sup>c</sup> Self-prepared from apple, sloe tree, hawthorn.

tween linalool and its acetate from various essential oils. In all cases, the  $\delta^2\text{H}$  values of linalool were depleted by ~7–30‰ in relation to that of linalyl acetate.

***E*-2-Hexenal and *E*-2-Hexenol.** The so-called "leaf aldehyde" and its reduction product have intense herbal green odors and are used in perfumes to obtain a green leaf note and in fruit flavors for green nuances (15). Their intensively studied formation in disrupted tissues of green plants via the enzyme cascade of acylhydrolyase–lipoxygenase–aldehyde lyase–isomerase–aldehyde dehydrogenase (16) has been used for their biotechnological production (17). Information about  $^2\text{H}/^1\text{H}$  ratios of these industrially important flavor compounds has not been provided to date.

In Tables 6 and 7 the  $\delta^2\text{H}_{\text{SMOW}}$  data of *E*-2-hexenal and *E*-2-hexenol from various origins recorded by HRGC-P-IRMS are summarized. The high differences found in the  $\delta^2\text{H}$  ranges for the products from synthetic and natural origins, that is, from −14 to −109‰ against −263 to −415‰ for *E*-2-hexenal as well as from −41 to −131‰ against −238 to −348‰ for *E*-2-hexenol, allowed clear-cut authenticity assessment by the  $^2\text{H}/^1\text{H}$  ratios. Graphical evaluation of  $^2\text{H}/^1\text{H}$  ratios of *E*-2-hexenal and *E*-2-hexenol from defined sources revealed a distinct depletion of  $\delta^2\text{H}$  values of the aldehyde in



**Figure 2.**  $\delta^2\text{H}_{\text{SMOW}}$  values of the aldehyde *E*-2-hexenal (◆) in relation to the alcohol *E*-2-hexenol (■).

relation to the alcohol (Figure 2). The quite obvious differences in the abundance of depletion can actually not be explained; detailed information about isotope discrimination in the course of the above-mentioned enzymic steps might be helpful to rationalize them.

In summary, despite the limited number of samples under study, the importance of the on-line HRGC-P-IRMS technique was demonstrated. There is no doubt that it will open a wide access to authenticity studies of flavor constituents in complex natural matrices. The limitation of the method will be overcome in the future by multielement HRGC-IRMS analysis.

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#### LITERATURE CITED

- Mosandl, A. Enantioselective capillary gas chromatography and stable isotope ratio mass spectrometry in the authenticity control of flavors and essential oils. *Food Rev. Int.* **1995**, *11*, 597–664.
- Tressl, R.; Engel, K. H. Formation and analysis of optically active aroma compounds. In *Analysis of Volatiles*; Schreier, P., Ed.; de Gruyter: Berlin, Germany, 1983; pp 323–342.
- Mosandl, A.; Heusinger, G. Stereoisomers of fruit flavour substances. Some aspects of synthesis and analysis. In *Analysis of Volatiles*; Schreier, P., Ed.; de Gruyter: Berlin, Germany, 1983; pp 343–356.
- Wright, D. W. Application of multidimensional gas chromatography techniques to aroma analysis. In *The Techniques for Analyzing Food Aroma*; Marsili, R., Ed.; Dekker: New York, 1997; pp 113–141.
- Mosandl, A. Enantioselective analysis. In *Flavourings*; Ziegler, E., Ziegler, H., Eds.; Wiley-VCH: Weinheim, Germany, 1998; pp 595–633.
- Schmidt, H. L.; Rossmann, A.; Werner, R. A. Stable isotope ratio analysis in quality control of flavourings. In *Flavourings*; Ziegler, E., Ziegler, H., Eds.; Wiley-VCH: Weinheim, Germany, 1998; pp 539–594.
- Koziet, J. Isotope ratio mass spectrometric method for the on-line determination of oxygen-18 in organic matter. *J. Mass Spectrom.* **1997**, *32*, 103–108.
- Begley, I. S.; Scrimgeour, C. High precision  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  measurement for water and volatile organic compounds by continuous-flow pyrolysis isotope ratio mass spectrometry. *Anal. Chem.* **1997**, *69*, 1530–1535.

- (9) Hener, U.; Brand, W. A.; Hilker, A. W.; Juchelka, D.; Mosandl, A.; Podebrad, F. Simultaneous on-line analysis of  $^{18}\text{O}/^{16}\text{O}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios of organic compounds using GC-pyrolysis IRMS. *Z. Lebensm. Unters.-Forsch.* **1998**, *206*, 230–232.
- (10) Schmidt, H. L.; Gleixner, G. Isotopic patterns in natural compounds origin and importance in authenticity analysis. In *Natural Product Analysis*; Schreier, P., Herderich, M., Humpf, H. U., Schwab, W., Eds.; Vieweg: Wiesbaden, Germany, 1998; pp 271–280.
- (11) Schmidt, H. L.; Weber, D.; Rossmann, A.; Werner, R. A. The potential of intermolecular and intramolecular isotopic correlations for authenticity control. In *Flavor Chemistry: 30 Years of Progress*; Teranishi, R., Wick, E. L., Hornstein, I., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999; pp 55–61.
- (12) Hilker, A. W.; Douthitt, C. B.; Schlüter, H. J.; Brand, W. A. Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1226–1230.
- (13) 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**, in press.
- (14) Ruff, C.; Hör, K.; Weckerle, B.; Schreier, P.; König, T.  $^2\text{H}/^1\text{H}$  Ratio analysis of flavor compounds by on-line gas chromatography-pyrolysis isotope ratio mass spectrometry (HRGC-P-IRMS): Benzaldehyde. *J. High Resolut. Chromatogr.* **2000**, *23* (5), 357–359.
- (15) Bauer, K.; Garbe, D.; Surburg H., Eds. *Common Fragrance and Flavor Materials*, 3rd ed.; Wiley-VCH: Weinheim, Germany, 1997.
- (16) Hatanaka, A. The fresh green odor emitted by plants. *Food Rev. Int.* **1996**, *12*, 303–350.
- (17) Muller, B.; Gautier, A.; Dean, C.; Kuhn, J. C. Process for the enzymatic preparation of aliphatic alcohols and aldehydes from linoleic acid, linolenic acid or a natural precursor. U.S. Patent 5464761, 1995.

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