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ORIGINAL PAPER

Steffi Bilke · Armin Mosandl

Authenticity assessment of lavender oils using GC-P-IRMS: $^2H/^1H$ isotope ratios of linalool and linalyl acetate

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Abstract Gas chromatography-pyrolysis-isotope mass spectrometry (GC-P-IRMS) is applied to the authenticity assessment of lavender oils. For this reason, self-prepared lavender oils, samples of linalool and linalyl acetate labeled as synthetic and natural, and commercially available lavender oils have been investigated. $^2H/^1H$ isotope ratios of linalool and linalyl acetate have been determined, and characteristic ranges of authenticity were deduced. Samples of natural linalool and linalyl acetate are clearly differentiated from those of synthetic origin by their $\delta^2H_{V\text{-}SMOW}$ values. Besides enantioselective multidimensional gas chromatography-mass spectrometry (enantio-MDGC-MS), online GC-P-IRMS has proven to be an additional and powerful tool in the detection of lavender oils adulteration.

Furthermore, the influence of different processing parameters (steam distillation and diethyl ether extraction) on the $\delta^2H_{V\text{-}SMOW}$ values of linalool and linalyl acetate in lavender oils is discussed.

Keywords GC-P-IRMS · $^2H/^1H$ isotope ratio analysis · Lavender oil · Linalool · Linalyl acetate · Authenticity assessment

Introduction

There is a great demand for lavender oils in perfumery, cosmetic, and soap industries. Lavender oil is obtained by steam distillation from the fresh flowering tops of *Lavandula angustifolia* Miller (*Lavandula angustifolia* Chaix) [1]. According to the European Pharmacopoeia the authenticity assessment of lavender oil is based on the quantification of characteristic compounds, such as linalool, linalyl acetate, limonene, 3-octanone, 1,8-cineol, camphor, terpinen-4-ol, lavandulyl acetate, lavandulol and α-terpineol by means of capillary GC [1].

Blends of lavender oils with lavandin oil or spike oil, or the addition of synthetic linalool and/or linalyl acetate are well-known adulterations. Due to the higher amounts of 1,8-cineol and camphor in lavandin and spike oils, this kind of adulteration of lavender oil can be easily detected by quantitative GC-analysis.

On the other hand, (*R*)-linalyl acetate and (*R*)-linalool of high enantiomeric purity are known to be characteristic, genuine lavender oil compounds. Thus, enantioselective analysis of linalool and linalyl acetate has proven to be a powerful tool to detect adulterations of lavender oils with (partially) racemic, synthetic linalool and linalyl acetate [2, 3, 4]. Consequently, the enantioselective analysis of linalool and linalyl acetate is promising for the authenticity assessment of lavender oil and will hopefully soon become established in the European Pharmacopoeia.

Besides enantioselectivity, isotope discrimination during biosynthesis is another phenomenon that may serve as an endogenous parameter, provided that suitable methods and comprehensive data from authentic sources are available. Several authors reported the determination of $\delta^{13}C_{V\text{-}PDB}$ values of synthetic and natural linalool and linalyl acetate by using IRMS [5, 6, 7]. Culp et al. also published $\delta^2H_{V\text{-}SMOW}$ values of linalool determined via IRMS [6]. They found that the values of natural linalool were significantly lower than those of the synthetic counterparts (–297±26 and –196±16, respectively).

The on-line determination of $^2H/^1H$ isotope ratios using a gas chromatograph coupled with an isotope ratio mass spectrometer was demonstrated by Tobias et al. and Begley et al. [8, 9]. In 1999, Hilkert et al. presented a commercially available technique for the on-line determination of $^2H/^1H$ isotope ratios [10]. Thereafter, this method has been applied to authenticity assessments of flavor compounds [11, 12, 13]. Schreier et al. determined the $\delta^2H_{V\text{-}SMOW}$ values of synthetic and natural linalool and linalyl acetate [11]. They concluded that the $\delta^2H_{V\text{-}SMOW}$ values of linalool and linalyl acetate do not

S. Bilke · A. Mosandl (✉)
Institut für Lebensmittelchemie,
Biozentrum J.W. Goethe-Universität,
Marie-Curie-Str. 9, 60439 Frankfurt/Main, Germany
e-mail: Mosandl@em.uni-frankfurt.de
Tel.: +49-69-79829202/203, Fax: +49-69-79829207

allow the differentiation between synthetic and natural origins. In contrast to these results, the results of Culp et al. have now been confirmed by GC-P-IRMS. In this paper the $\delta^2H_{V\text{-}SMOW}$ values of linalool and linalyl acetate from self-prepared lavender oils (*Lavandula angustifolia* Miller), synthetic linalool and linalyl acetate, and commercially available lavender oils are presented. These results demonstrate the determination of $\delta^2H_{V\text{-}SMOW}$ values of linalool and linalyl acetate by GC-P-IRMS as a new sophisticated method for the authenticity assessment of lavender oils.

Materials and methods

Chemicals and essential oils

Samples of linalool and linalyl acetate labeled as synthetic and natural were obtained from Fluka (Buchs, Switzerland), Sigma-Aldrich (Seelze, Germany), Merck Eurolab (Darmstadt, Germany), Roth (Karlsruhe, Germany), Acros Organics (Geel, Belgium), and Kaders GmbH (Hamburg, Germany). Commercial lavender oils were from Kaders GmbH (Hamburg, Germany), Sigma-Aldrich (Seelze, Germany), Caesar & Loretz GmbH (Hilden, Germany), Mainland GmbH (Frankfurt, Germany), Rose Egert (Sachsenheim, Germany), Bergland Pharma (Heimertingen, Germany), Allos Walter Lang (Mariendrebber, Germany), and Spinnrad (Gelsenkirchen, Germany). Lavender samples (*Lavandula angustifolia* Miller) were obtained from several botanical gardens.

Sample preparation

Steam distillation

Lavender oils were obtained using a glass steam extractor (40 cm×5 cm i.d.), equipped with a filter bottom and a steam-heated jacket (extraction period: 30 min, according to GMP-conditions).

Diethyl ether extraction

The lavender samples were extracted with diethyl ether for a period of 2 h. The extracts were dried over sodium sulfate, filtered, and the solvent was evaporated in a stream of nitrogen.

Enantioselective multidimensional gas chromatography-mass spectrometry (enantio-MDGC-MS)

The enantioselective analysis of linalool and linalyl acetate was carried out with an enantio-MDGC-system. This system consists of a Siemens SiChromat 2.8 double-oven gas chromatograph (with two independent temperature controls) coupled to the transfer line of a Finnigan MAT ITD 800, using an open split interface. Pre- and main column were connected by a live-switching coupling device (live-T-device).

Pre-column: 30 m×0.25 mm i.d. fused silica column coated with SE 52, film thickness 0.25 µm, prepared according to Grob [14]; carrier gas: He 2.4 bar; injector temperature: 240 °C; split flow: 30 ml min^{-1}; detector: flame ionization detector (temperature: 250 °C); temperature program: starting from 50 °C, isothermal for 20 min, increasing by 2 °C min^{-1} to 200 °C, isothermal for 30 min; cut time:

linalool	28.6–29.0 min
linalyl acetate	43.3–43.4 min

Main column: 30 m×0.25 mm i.d. BGB-174 [heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in BGB 1701], film thickness 0.25 µm, BGB-Analytik, Schloßböckelheim; carrier gas: He 1.5 bar; detector: ITD 800, transfer line temperature 250 °C, open split interface temperature 250 °C, helium sweeping flow 1 ml min^{-1}, ion trap manifold temperature 195 °C, EI 70 eV; temperature program: starting from 50 °C, isothermal for 20 min, increasing by 0.5 °C min^{-1} to 75 °C, after a further isothermal step for 10 min, increasing by 0.5 °C min–1 to 200 °C

Retention times:

(*R*)-linalyl acetate	76.5 min
(*S*)-linalyl acetate	77.1 min
(*R*)-linalool	78.8 min
(*S*)-linalool	82.3 min

High temperature conversion elemental analyzer-isotope ratio mass spectrometry (TC/EA-IRMS)

The determination of the $^2H/^1H$ isotope ratios of the secondary standards was carried out with an elemental analyzer (TC/EA), coupled with a Delta^{plus}XL isotope ratio mass spectrometer via a ConFlo III-Interface (ThermoFinnigan MAT, Bremen, Germany). The following conditions were employed: reactor temperature 1450 °C, GC column temperature 110 °C, flow (Helium) 120 ml min^{-1}.

Gas chromatography-pyrolysis-isotope ratio mass spectrometry (GC-P-IRMS)

The on-line determination of the $^2H/^1H$ isotope ratios was performed with an HP 6890 gas chromatograph (GC), coupled with a Delta^{plus}XL isotope ratio mass spectrometer via a pyrolysis reactor [ceramic tube (Al_2O_3), 0.5 mm i.d., length 320 mm, reactor temperature 1440 °C] and an open split (ThermoFinnigan MAT, Bremen, Germany). The GC was equipped with a VB 5 column (ValcoBond, Gig Harbor, USA) (30 m×0.25 mm i.d., d_f=0.5 µm). The following conditions were employed: splitless injection (injector temperature 240 °C); temperature program: starting from 40 °C, isothermal for 30 min, increasing by 2 °C min^{-1} to 120 °C, then increasing by 5 °C min^{-1} to 240 °C; carrier gas flow: He 0.8 mL min^{-1}.

The pyrolysis reactor was regularly conditioned by passing methane through the reactor in the backflush mode (at operating temperature for a period of five minutes) [15]. Fivefold determinations were carried out, the standard deviations of the GC-P-IRMS measurements are 1–3 ‰. The isotope ratios are expressed in per mill (‰) versus V-SMOW (Vienna Standard Mean Ocean Water).

Calibration strategies

The reference gas (Messer-Griesheim, Frankfurt, Germany) was calibrated against the IAEA-standards CH 7 ($\delta^2H_{V\text{-}SMOW}$=–100.3± 2.0 ‰) and NBS 22 ($\delta^2H_{V\text{-}SMOW}$=–118.5±2.8 ‰). The $\delta^2H_{V\text{-}SMOW}$ values of five tertiary standards (5-nonanone, linalool, menthol, linalyl acetate, and 4-decanolide) were determined via TC/EA-IRMS (number of measurements 10–15, standard deviation <3 ‰). The reproducibility and the accuracy of the measurements made by GC-P-IRMS were routinely checked by means of these tertiary standards.

Results and discussion

To determine the natural range of the $\delta^2H_{V\text{-}SMOW}$ values of linalool and linalyl acetate in lavender oils, the essential oils of fresh lavender were prepared by means of steam distillation and diethyl ether extraction. Diethyl

Table 1 Ranges of $\delta^2H_{V\text{-}SMOW}$ values of linalool

Linalool	$\delta^2H_{V\text{-}SMOW}$ range (‰)	Number of samples
Synthetic	–185 to –209	6
Natural	–265 to –307	2
Self-prepared lavender oils	–241 to –274	11
Commercial lavender oils	–190 to –294	24

Table 2 Ranges of $\delta^2H_{V\text{-}SMOW}$ values of linalyl acetate

Linalyl acetate	$\delta^2H_{V\text{-}SMOW}$ range (‰)	Number of samples
Synthetic	–173 to –197	5
Natural	–276	1
Self-prepared lavender oils	–238 to –270	11
Commercial lavender oils	–187 to –274	24

ether extraction was used for sample work-up as this avoids any possibility of hydrogen exchange. According to the European Pharmacopoeia, lavender oils are produced by steam distillation. The determined $\delta^2H_{V\text{-}SMOW}$ values of linalool and linalyl acetate of the essential oils produced by the above mentioned procedures correspond well within the range of standard deviations. The influence of the different kinds of sample preparation on the determined $\delta^2H_{V\text{-}SMOW}$ values is negligible.

The $\delta^2H_{V\text{-}SMOW}$ values of linalool from different origins are summarized in Table 1. The $\delta^2H_{V\text{-}SMOW}$ values of the linalool samples labeled as synthetic were in the range of –185 to –209 ‰, whereas the values of linalool from self-prepared lavender oils were between –241 and –274 ‰.

As shown in Fig. 1, these values allow us to distinguish the synthetic linalool from those of lavender oil samples. The values of commercial samples of linalool labeled as natural were well below the values of the synthetic ones. $\delta^2H_{V\text{-}SMOW}$ values of commercially available lavender oils were found to be between –190 and –294 ‰.

The values for linalyl acetate are outlined in Table 2. It is also possible to differentiate between the synthetic and natural linalyl acetate on the basis of the $\delta^2H_{V\text{-}SMOW}$ values as shown in Fig. 2. The values for linalyl acetate of the commercial lavender oils were in a wide range (–187 to –274 ‰) analogous to those of linalool.

The $\delta^2H_{V\text{-}SMOW}$ values of linalyl acetate were plotted against the values of linalool as shown in Fig. 3. This plot shows that the $\delta^2H_{V\text{-}SMOW}$ values of linalool and linalyl acetate of five commercial lavender oils (S1–S5) are clearly differentiated from those of the authentic samples. Obviously, the commercial samples do not correspond with the authenticity range determined for the genuine samples. Consequently, these samples are identified as adulterated with synthetic linalool and linalyl acetate, respectively. Furthermore, the enantiomeric ratios of linalool and linalyl acetate of the lavender oils were determined by enantio-MDGC-MS. (*R*)-Linalyl acetate

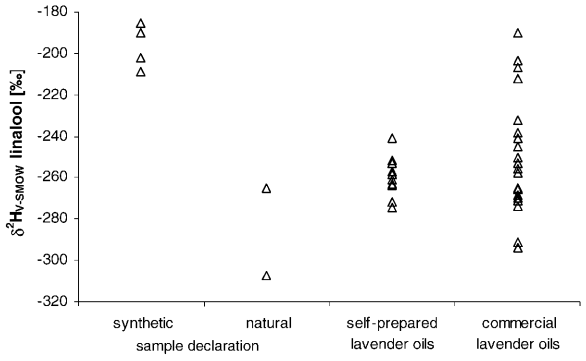

Fig. 1 $\delta^2H_{V\text{-}SMOW}$ values of linalool from different origins

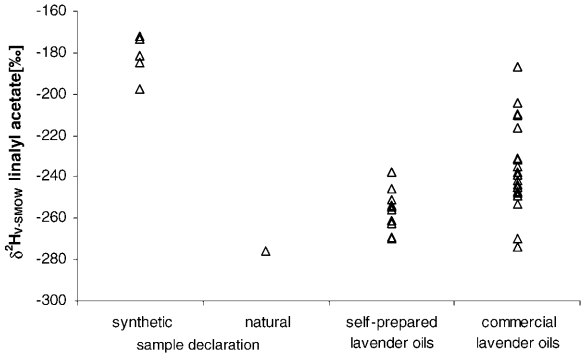

Fig. 2 $\delta^2H_{V\text{-}SMOW}$ values of linalyl acetate from different origins

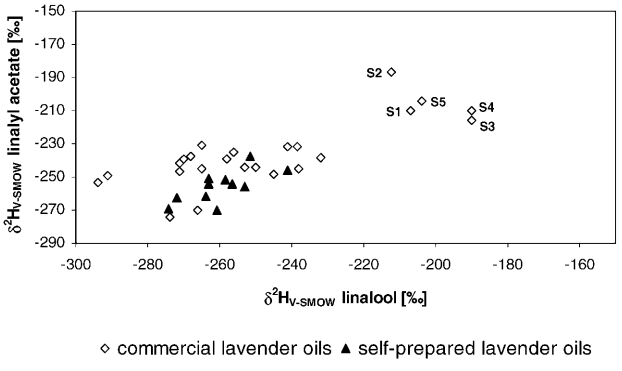

Fig. 3 Correlation of the $\delta^2H_{V\text{-}SMOW}$ values of linalyl acetate/linalool from lavender oils, self-prepared (▲) and commercial (◇) samples

and (*R*)-linalool of high enantiomeric purity were found for the self-prepared lavender oils. The same results were obtained for most of the commercial oils. Only the enantiomeric ratios of eight analyzed oils were different. The results of these oils are outlined in Table 3.

According to these results, the samples S1–S5 were obviously adulterated by blending with synthetic linalool and linalyl acetate. According to Kreis et al., a content of <95% (*R*)-linalyl acetate and <85% (*R*)-linalool in lavender oils has to be interpreted as falsification [3]. Thus, the enantiomeric ratios of linalyl acetate of the samples S6–S8 definitely indicate a blend with synthetic linalyl

Table 3 Enantiomeric ratios of linalyl acetate and linalool from commercial lavender oils

Sample	Linalyl acetate		Linalool	
	(*R*)	(*S*)	(*R*)	(*S*)
S1	52.9	47.1	70.7	29.3
S2	55.7	44.3	55.5	44.6
S3	51.8	48.2	62.0	38.0
S4	52.0	48.0	69.7	30.3
S5	53.3	46.7	60.8	39.2
S6	80.7	19.3	>99	<1
S7	91.4	8.6	87.5	12.5
S8	86.9	13.1	91.4	8.6

acetate, whereas the enantiomeric ratios of linalool in the samples S7 and S8 are in the lower range of natural linalool. Alternatively, the lavender oils (S1–S5), which are obviously adulterated with synthetic linalool and linalyl acetate according to the determined enantiomeric ratios, can clearly be identified on the basis of the $\delta^2H_{V\text{-}SMOW}$ values of linalool and linalyl acetate (Fig. 3). On the other hand, the samples S6–S8, which are blended with linalyl acetate, cannot be differentiated from the authentic samples due to similar $\delta^2H_{V\text{-}SMOW}$ values of linalyl acetate.

Conclusions

In summary, the presented data show that the determination of $\delta^2H_{V\text{-}SMOW}$ values of linalool and linalyl acetate in lavender oils by using GC-IRMS is a valuable new method for the determination of the authenticity of lavender oils. This method represents an alternative to the enantioselective analysis; in particular it can be applied to the detection of possible adulteration of essential oils with synthetic compounds of high enantiomeric purity. Furthermore, multi-element GC-IRMS may provide more explicit information in authenticity studies of flavor compounds.

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