Fragrances

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The (+)-*cis*- and (+)-*trans*-Olibanic Acids: Key Odorants of Frankincense

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Dedicated to Dr. Roman Kaiser on the occasion of his 70th birthday

Abstract: *Frankincense (olibanum) is one of the oldest aromatic materials used by humans, but the key molecular constituents contributing to its characteristic odor remained unknown. Reported herein is the discovery that (1S,2S)-(+)-trans- and (1S,2R)-(+)-cis-2-octylcyclopropyl-1-carboxylic acids are highly potent and substantive odorants occurring in ppm amounts in all of the frankincense samples analyzed, even those showing radically different volatile compositions. These cyclopropyl-derived acids provide the very characteristic old churchlike endnote of the frankincense odor.*

The first perfuming devices were based on the combustion of fragrant natural raw materials, as suggested by the etymology of the word perfume itself ("per fumum" = through smoke in latin). Thus, their use might be almost as old as the domestication of fire. Among these materials, frankincense (also known as olibanum) has a history dating back to the late 4th millennium B.C.,[1] and has been often considered as one of the first aromatic materials used by humans.[2] This gum resin naturally exudes from the bark of *Boswellia* species (Burseraceae), which grows mostly in arid mountainous regions on both sides of the Gulf of Aden and the Red sea. It was burned as incense in a domestic context and during religious ceremonies in the old Civilizations of Arabia, Mesopotamia, Persia, and Egypt, and later in Greece and Roma.[1] This extremely early history of use is supported by substantial archaeochemical evidence, thanks to the stability of specific constituents of frankincense which could be detected in various containers and incense burners in archeological sites of Egypt, Yemen, France, and Belgium.[1,3]

Frankincense is mentioned 22 times in the Bible, notably as one of the presents offered to the Christ by the three Wise Men, and its use as incense has been perpetuated up to the present in Christian religious ceremonies. Indeed, its typical odor is frequently associated with the "smell of old churches"[4] as churches today are the only places in Occident where frankincense is used as a single fragrant ingredient.

Surprisingly, despite the millenial use of frankincense for its odorant properties, the exact identity of its typical odor-active constituents is poorly understood, eventhough the composition of this material has been extensively investigated. This situation is common to several other raw materials used in perfumery[5] but is more paradoxical when it concerns one of the oldest perfumes. We report hereafter on the identification of (+)-*trans*- and (+)-*cis*-2-octylcyclopropyl-1-carboxylic acids [(+)-**1** and (+)-**2**, respectively], the two new extremely potent and substantive odorants responsible for the characteristic endnote of frankincense.

We performed aroma extract dilution analysis (AEDA) experiments by gas chromatography-olfactometry (GC-O) on a carefully selected, good quality standard sample of *Boswellia carterii* essential oil (EO), and a total of 26 odor zones were detected by the four panelists involved in the study. The most interesting zone, at retention index $RI_{\text{HP-5}} = 1560$, showed the second highest mean flavor dilution (FD) factor and was strongly reminiscent of the typical balsamic, old churchlike endnote of frankincense. It could not be initially correlated with any identified constituent, and we focused our efforts on the chemical characterization of this odorant. Therefore, a 3 kg sample of the EO was distilled under reduced pressure, and the fractions were further submitted to basic liquid–liquid extraction and flash chromatography. The GC-O evaluations of each fraction indicated that this odorant was contained in the acidic part of the distillation residue, thus representing about 0.2% (*w*/*w*) of the oil. This fraction contained some of the acids previously reported as frankincense constituents[6] along with many unknown components. When comparing the GC-MS and GC-O profiles, we could deduce that the typical frankincense-like odor zone was likely caused by a pair of unknown compounds eluting close to lauric acid and showing similar mass spectra. This acidic part was further fractionated by successive flash chromatography on silica gel and $AgNO_3$ coated silica gel, and eventually by HPLC. In this last series of separations, the most efficient way to quickly localize this odorant in the chromatographic fractions proved to be their direct olfactory evaluation, on smelling strips dipped in the chromatographic tubes. Indeed, their TLC, GC-MS, and HPLC-UV analyses were not particularly helpful, as none of the corresponding detection systems was sensitive enough,

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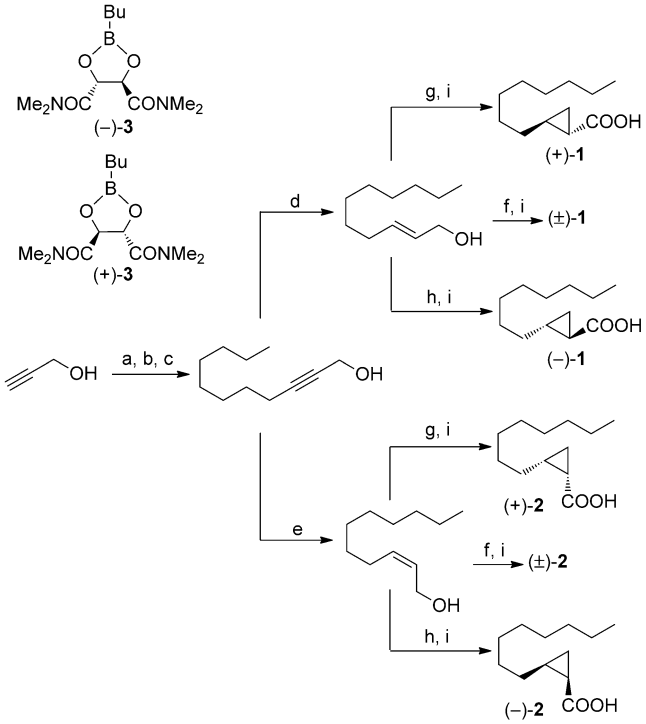
compared to the human nose. Eventually, about 1 mg of about a 94:6 mixture of the two suspected odorants was obtained at $RI_{\text{DB-WAX}} = 2546$ and 2538. ^{1}H and ^{13}C NMR, as well as COSY, HSQC and HMBC experiments suggested that the main component of this mixture was 2-octylcyclopropyl-1-carboxylic acid. This structure was consistent with the existence of two (*trans* and *cis*) isomers (**1**–**2**), and could explain the presence of two closely eluting peaks showing similar mass spectra.

To confirm the above identification, to attribute each peak to its corresponding isomer, and to determine their enantiomeric distribution in frankincense, **1** and **2** were both synthesized in racemic and enantiopure forms using the asymmetric cyclopropanation reported by Charette and coworkers (Scheme 1). When compared with the two unknown



Scheme 1. Reagents and conditions: a) 3,4-dihydropyrane, Amberlyst® 15, Petroleum ether, RT, 7 h, 89%; b) NaH, DMSO (4 equiv), THF, RT, 15 h, then 1-bromooctane, RT, 29 h, 82%; c) Amberlyst® 15, MeOH, 45 °C, 40 h, 84%; d) $LiAlH_4$, THF, reflux 2 h, 64%; e) H_2, P2-Ni, 1,2-ethylenediamine, MeOH, RT, 17 h, 87%; f) Et_2Zn, CH_2I_2, *n*-hexane, −35 °C→RT, 13 h; g) (−)-**3**, CH_2Cl_2, −15 °C, then $Zn(CH_2I)_2$-DME, CH_2Cl_2, −15 °C→RT, 15 h; h) (+)-**3**, CH_2Cl_2, −15 °C, then $Zn(CH_2I)_2$-DME, CH_2Cl_2, −15 °C→RT, 15 h; i) Jones reagent, acetone, RT, 20 h (yields over two steps: (±)-**1**, 56%; (+)-**1**, 67%; (−)-**1**, 79%; (±)-**2**, 68%; (+)-**2**, 66%; (−)-**2**, 80%). DME = dimethoxyethane, DMSO = dimethylsulfoxide, THF = tetrahydrofuran.

frankincense constituents, the synthetic samples of (±)-**1** and (±)-**2** showed identical retention times and mass spectra, and this observation was confirmed by coinjection experiments (Figure 1). The main natural isomer possessed the *trans* stereochemistry and its NMR data were identical with those of synthetic (±)-**1**.

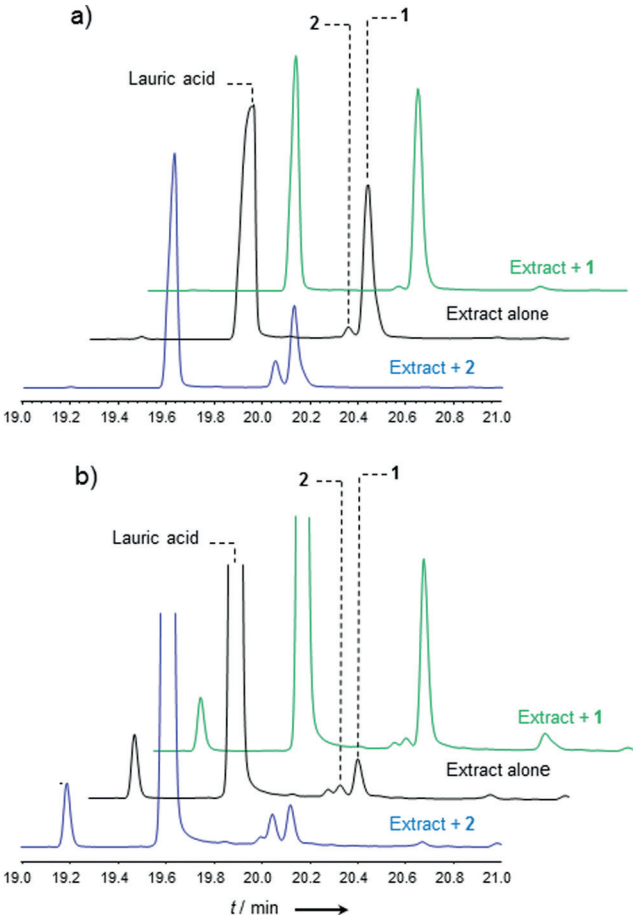


Figure 1. Coinjection experiments on acidic extracts of two samples of frankincense EO: a) monoterpene-type and b) octyl-acetate-type. GC-MS (SIM at *m/z* 97) profiles of the acidic extract alone (black) and of the extract spiked with (±)-**1** (green) or with (±)-**2** (blue).

To the best of our knowledge, **1** and **2** have never been identified as natural products. Consequently, we tried to understand to what extent they could be present in different types of commercial frankincense gum resins, by quantifying **1** and **2** in the EOs of 12 other gum resin samples, which were selected to cover a broad diversity of chemical compositions and botanical species. To also obtain a large overview of the different market qualities, the gum resins were purchased from 10 independent traders. After a liquid–liquid extraction of the acidic constituents in these EO samples, **1** and **2** were detected in ppm amounts in all of the 12 acidic extracts obtained from *B. carterii* and *B. frereana* EOs (see Table S1 in the Supporting Information) Interestingly, they were also present in the octyl-acetate-type EO (presumably *B. papyrifera*). These acids are therefore classical olibanum trace volatiles, and could not be detected in EOs obtained from other gum resins (Elemi, Myrrh, Galbanum, Opopanax; see Figure S6). Consequently, we propose to name **1** and **2** *trans*- and *cis*-olibanic acids, respectively.

As these acids are chiral, we determined their enantiomeric distribution in these frankincense samples. To avoid long and tedious fractionations of the acidic extracts, we had to develop an analytical methodology for their direct

enantioselective analysis. Our approach was based on enantioselective GC-MS analyses in single-ion monitoring (SIM) mode, but required the testing of a large set of chiral stationary phases to achieve these measurements without being disturbed by unwanted coelutions. Fortunately, the high number of different chiral phases we used[7] helped us to select the best methodology. Eventually, only (+)-**1** and (+)-**2** could be identified in the selected samples, without detection of any signal for their optical antipodes.

As part of our ongoing chirality-related studies,[8] we determined, unambiguously, their absolute configuration. The experimental electronic circular dichroism (ECD) spectra of the (+)-**1** natural sample isolated above, and those of the synthetic (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2** were compared with the time-dependent density functional theory (TD-DFT) calculated ECD spectra performed on the most stable conformers of (+)-**1** and (+)-**2** (Figure 2). **1** and **2** display an

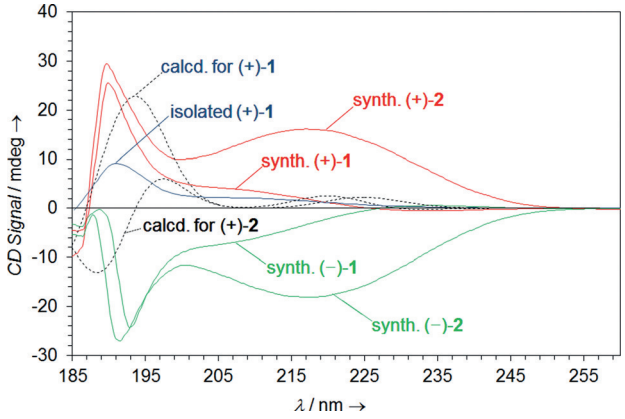


Figure 2. CD spectra of isolated (+)-**1** (blue), synthesized (+)-**1** and (+)-**2** (red), and synthesized (−)-**1** and (−)-**2** (green) in CH_3CN (1×10^{-3} M, 25 °C). TD-DFT calculated ECD spectra for (+)-**1** and (+)-**2**.

acid function chromophore close to the chiral centers of the cyclopropane ring. The acidic function implied two possible electronic transitions, one $\pi\rightarrow\pi^*$ transition with a high ε value (at ca. $\lambda = 185$ nm) and an $n\rightarrow\pi^*$ transition which has a far less important ε value (at ca. $\lambda_{max} = 210$ nm).

The experimental ECD spectrum of the isolated natural (+)-**1** showed two positive Cotton effects, at $\lambda_{max} = 192$ nm and $\lambda_{max} = 214$ nm (Figure 2). The TD-DFT calculated ECD spectrum for (+)-**1**, performed at the B3LYP/6-31 + G(d,p) level of theory in an implicit solvent modeled with the polarizable continuum model (IEFPCM, CH_3CN), also had two positive Cotton effects, one at $\lambda_{max} = 193$ nm and the second at $\lambda_{max} = 219$ nm, which reproduced the signs, positions, and differences in amplitude of the experimental Cotton effects. The slight differences between the theoretical values and the experimental observations can be explained by a small bathochromic effect of the aliphatic chain (Woodward–Fieser rules).

These results demonstrate definitely that the main natural enantiomers contained in frankincense are (1*S*, 2*S*)-(+)-**1** and (1*S*, 2*R*)-(+)-**2**.

The olfactory evaluations showed that (+)-**1** and (+)-**2** were both extremely potent odorants, and their GC-O analysis enabled us to confirm unambiguously that they were the main contributors of the characteristic old church-like, olibanum endnote odor zone in the olfactogram of the frankincense sample. The relative detection threshold of all four isomers was determined by GC-O with a panel of four judges. The data of each individual panelist showed the same tendency: their qualitative olfactory properties were similar but (+)-**2** was the most potent odorant, followed by (−)-**2** and (+)-**1**, and the weakest was (−)-**1**, which displayed a GC-O threshold at least 200 times higher than (+)-**2**. To confirm the key olfactory contribution of (+)-**1** and (+)-**2**, we performed additional blind sensorial evaluations of pure (+)-**2** with panelists familiar with frankincense EO and raw materials in general, as well as reformulation experiments using specific EO components. The results of this study are reported in Table S4 and clearly demonstrate that olibanic acids play a key role in the specific base note of frankincense. The extremely high substantivity of these components is also noteworthy: paper smelling strips impregnated with either (+)-**1** or (+)-**2** are still odorant even after several months, and this may account for their contribution to the constant olfactory atmosphere of churches. Indeed, periodical incense burnings supply a recurrent delivery of olibanic acids, which may settle on walls, furniture, and drapes and then allow a continuous diffusion of their odor.

To the best of our knowledge, natural 2-alkylcyclopropane-1-carboxylic acids are scarce: *trans*- and *cis*-2-pentylcyclopropane-1-carboxylic acids (**4** and **5**) are trace constituents of patchouli EO[9] and (1*S*, 2*R*)-**5** has been detected in *Mentha gracilis* EO (Figure 3).[10] The nor-derivative of (+)-**2**

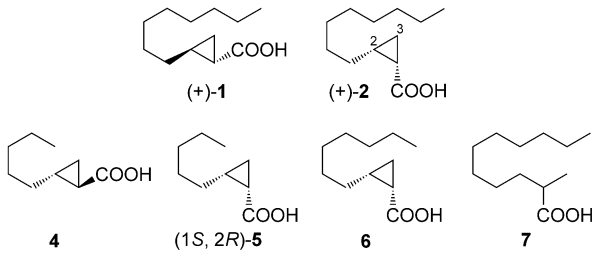


Figure 3. Olibanic acids homologues (**4**–**6**) and the analogue **7**.

(*cis*-isocascarillic acid **6**) has been discovered in a distillation residue of orange oil and described as possessing a "strong flowery, olibanum-like note".[11] In these studies, the important olfactory contributions of **4**–**6** could be demonstrated, and their use for fragrance formulation was patented.[12] We may note that the synthetic odorant 2-methylundecanoic acid (Mystikal®) **7** has been patented for the same purpose by Givaudan[13] and described as "the only perfumery material that conveys the odor of olibanum".[2] **7** can be viewed as a C2–C3 *seco*-analogue of **1** and **2** and this fact probably explains the proximity of their olfactory properties.

It is surprising that **1** and **2** remained unidentified up to now, while a detailed survey of the litterature shows that many phytochemical studies focused extensively on the

volatile fraction of frankincense.[14] When considering the main species used in incense burners (i.e. *Boswellia carterii*, *B. sacra*, *B. papyrifera*, and *B. frereana*) the major volatiles are generally either classical monoterpenes (α-pinene, α-thujene, limonene) or octan-1-ol and its acetate.[14] Interestingly, some old references noticed that both of these types share some common olfactory properties,[4b] thus suggesting that they may contain common odorants. The first mention of the odor-donating constituents of frankincense is due to Obermann,[6] who described the composition of the acidic fraction of two olibanum EO samples, of either octyl acetate or α-pinene types. He mentioned that in both cases, monoterpenic acids played an important role in the characteristic frankincense odor. De Rijke et al.[9] and later Maupetit[15] also underlined the olfactory contribution of the acidic fraction, in which α-campholytic acid (**8**) was identified and described as possessing "a rather strong odor reminiscent of the oil" (Figure 4).[9]

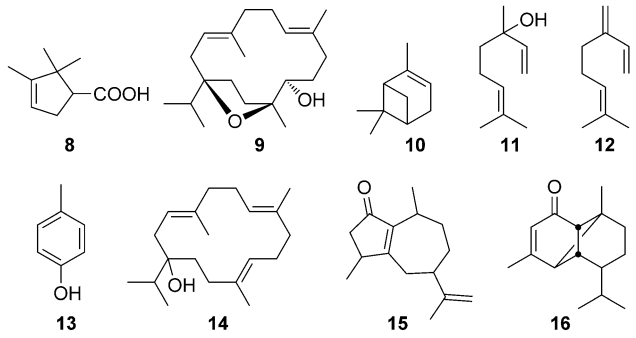


Figure 4. Frankincense constituents reported to contribute to its typical odor.

More recently, Hasegawa et al. mentioned that diterpenoids such as incensole (**9**) may be important odor components.[16] Finally, Niebler and Buettner[17] performed a sophisticated GC-O (AEDA) investigation on SAFE extracts of frankincense gum resin. Among the constituents initially identified, α-pinene, linalool, myrcene, and *p*-cresol (**10**–**13**) displayed the highest flavor dilution (FD) factors, but these constituents are classical natural volatiles and their qualitative olfactory character is not typical of frankincense odor. In this study, the only odorant with an incense-like odor quality was serratol (**14**) but its FD factor was one of the lowest and the authors admitted that its sensory relevance was probably insignificant. Besides, according to Ohloff et al., **9** and **14** are odorless.[2] Very recently, the same authors eventually elucidated the structures of the two odorants possessing the highest FD factor in these analyses as rotundone (**15**) and mustakone (**16**).[18] Both of these ketones are highly potent trace odorants, and their characterization required the use of an elegant combination of fractionation techniques. In this work, the odor of **16** was described as "spicy, woody, slightly fatty, meat-broth-like, and balsamic" while **15**, a well-known spicy and peppery odorant,[19] was reported to display "woody, coniferous, incense like" odor close to its detection threshold.

In conclusion, we have discovered the two typical key odorants of frankincense, (+)-**1** and (+)-**2**, which are present in trace amounts in all of the various chemotypes investigated. This result provides an additional example of biologically relevant cyclopropane derivatives which complement the already known fascinating series of natural cyclopropane architectures.[20] Interestingly, Buettner and co-workers had wisely pointed out that in view of its qualitative olfactory properties, the homologous acid **6** might be a constituent of frankincense, but had never been identified so far.[14] Indeed, the extremely low natural amount of **1** and **2** considerably complicates their identification, but their very low detection thresholds explain why they are nevertheless key odorant contributors. The identification of such new trace odorants has to be based on analytical studies conducted on large amounts of starting material. Obviously, it should also necessarily involve sensorial analyses, since the extraordinarily high selectivity and sensitivity of the human olfactory system is after all the heart of the matter.

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[1] J. Baeten, K. Deforce, S. Challe, D. De Vos, P. Degryse, *PLoS One* **2014**, *9*, e113142.
[2] G. Ohloff, W. Pickenhagen, P. Kraft in *Scent And Chemistry—The Molecular World of Odors*, Wiley-VCH & Verlag Helv. Chim. Acta Zürich, Weinheim, **2012**, pp. 322–324.
[3] R. P. Evershed, P. F. van Bergen, T. M. Peakman, E. C. Leigh-Firbank, M. C. Horton, D. Edwards, M. Biddle, B. Kjolbye-Biddle, P. A. Rowley-Conwy, *Nature* **1997**, *390*, 667–778.
[4] a) M. Paul, Thesis, Saarland University (Saarbrücken), **2012**. http://scidok.sulb.uni-saarland.de/volltexte/2012/4999/pdf/Dissertation_Fertig_211112.pdf; b) L. Peyron, J. Acchiardi, D. Bignotti, P. Pellerin in *8th Int. Congr. Essent. Oils* **1980**, 309–314.
[5] N. Baldovini, J.-J. Filippi in *Springer Handbook of odor* (Ed.: A. Buettner), Springer, in press.
[6] H. Obermann, *Dragoco Rep.* **1978**, 55–60.
[7] a) C. Bicchi, A. D'Amato, V. Manzin, A. Galli, M. Galli, *J. Chromatogr. A* **1996**, *742*, 161–173; b) C. Cagliero, B. Sgorbini, C. Cordero, E. Liberto, P. Rubiolo, C. Bicchi in *Importance of Chirality to Flavor Compounds*, *Vol. 1212*, American Chemical Society, Washington, **2015**, pp. 15–34; c) E. Liberto, C. Cagliero, B. Sgorbini, C. Bicchi, D. Sciarrone, B. D. Zellner, L. Mondello, P. Rubiolo, *J. Chromatogr. A* **2008**, *1195*, 117–126.
[8] C. Meinert, S. V. Hoffmann, P. Cassam-Chenai, A. C. Evans, C. Giri, L. Nahon, U. J. Meierhenrich, *Angew. Chem. Int. Ed.* **2014**, *53*, 210–214; *Angew. Chem.* **2014**, *126*, 214–218.
[9] D. De Rijke, P. C. Traas, R. Ter Heide, H. Boelens, H. J. Takken, *Phytochemistry* **1978**, *17*, 1664–1666.

[10] T. Tsuneya, M. Ishihara, H. Takatori, F. Yoshida, K. Yamagishi, T. Ikenishi, *J. Essent. Oil Res.* **1998**, *10*, 507 – 516.
[11] S. Widder, J. Looft, W. Pickenhagen, T. Voessing, S. Trautzsch, U. Schaefer, G. Krammer in *Recent Highlights in Flavor Chemistry and Biology*, 27/02 – 2/03 **2007**, 280 – 283.
[12] a) S. Widder, J. Looft, A. Van Der Kolk, T. Voessing, W. Pickenhagen, B. Kohlenberg (Symrise), DE10254265A1, **2004**; b) R. L. Chappell (IFF), US3926860A, **1975**.
[13] J.-P. Bachmann (Givaudan), WO2010063133A1, **2010**.
[14] M. Mertens, A. Buettner, E. Kirchhoff, *Flavour Fragrance J.* **2009**, *24*, 279 – 300.
[15] P. Maupetit, *Perfum. Flavor.* **1985**, *9*, 19 – 37.
[16] T. Hasegawa, A. Kikuchi, H. Saitoh, H. Yamada, *J. Essent. Oil Res.* **2012**, *24*, 593 – 598.
[17] J. Niebler, A. Buettner, *Phytochemistry* **2015**, *109*, 66 – 75.
[18] J. Niebler, K. Zhuravlova, M. Minceva, A. Buettner, *J. Nat. Prod.* **2016**, *79*, 1160 – 1164.
[19] C. Wood, T. E. Siebert, M. Parker, D. L. Capone, G. M. Elsey, A. P. Pollnitz, M. Eggers, M. Meier, T. Vossing, S. Widder, G. Krammer, M. A. Sefton, M. J. Herderich, *J. Agric. Food Chem.* **2008**, *56*, 3738 – 3744.
[20] R. Faust, *Angew. Chem. Int. Ed.* **2001**, *40*, 2251 – 2253; *Angew. Chem.* **2001**, *113*, 2312 – 2314.

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