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

(+)-*cis*- and (+)-*trans*-Olibanic Acids as 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. Nowadays, it is still burned for liturgical purposes in orthodox and catholic churches, but the key molecular constituents contributing to its characteristic odor remained unknown. We discovered that (1*S*, 2*S*)-(+)-trans- and (1*S*, 2*R*)-(+)-cis-2-octylcyclopropyl-1-carboxylic acids are highly potent and substantive odorants occuring in frankincense. They were identified 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 church-like base note of 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), growing mostly in arid mountainous regions on both sides of the Gulf of Aden and the Red sea. It was burned as an incense in a domestic context and during religious ceremonies in the old Civilizations of Arabia, Mesopotamia, Persia, 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 an incense was perpetuated until now in Christian religious ceremonies. Indeed, its typical odor is frequently associated with the "smell of old churches"[4] since the churches are today 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, even if the composition of this material was extensively

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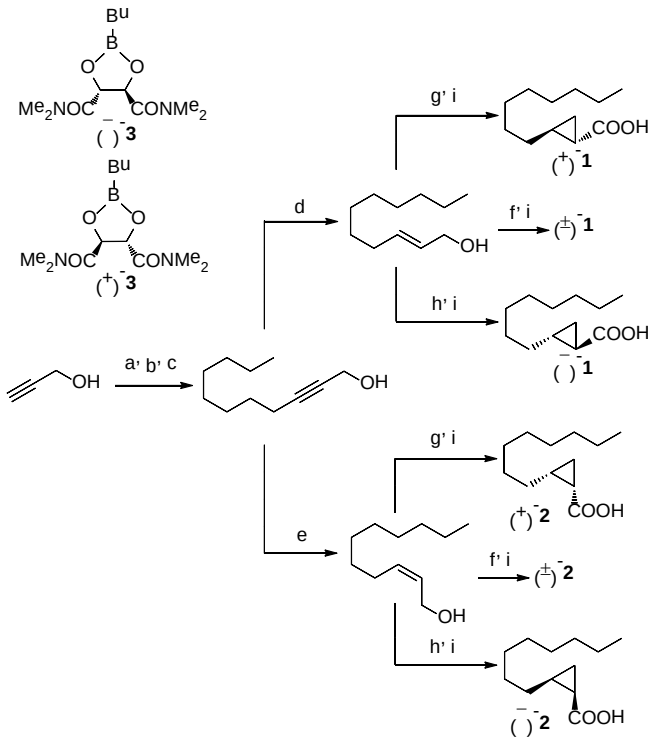
investigated. This situation is common to several other raw materials used in perfumery[5] but is more paradoxical when it concerns one of the oldest perfumes. We report hereafter on the identification of (+)-*trans*- and (+)-*cis*-2-octylcyclopropyl-1-carboxylic acids ((+)-**1** and (+)-**2**), the two new extremely potent and substantive odorants responsible for the characteristic base note 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 church-like base note 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, representing ca. 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 due to 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, compared to the human nose. Eventually, about 1 mg of a ca. 94/6 mixture of the two suspected odorants was obtained, at $RI_{\text{DB-WAX}}$ = 2546 and 2538, respectively. ^{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**), which could explain the presence of two closely eluting peaks showing similar mass spectra.

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



Scheme 1. Reagents and conditions: a) DHP, Amberlyst® 15, Petroleum ether, RT, 7 h, 89%; b) NaH, DMSO (4 eq.), 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, Ni-P2, 1,2-ethylenediamine, MeOH, RT, 17 h, 87%; f) Et_2Zn, CH_2I_2, 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%).

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 selected in order 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 all of the 12 acidic extracts, in amounts ranging from 55 to 746 ppm for **1** and from 3 to 36 ppm for **2** in *B. carterii* EOs. **2** content was even higher (73 ppm) in *B. frereana* EO. Interestingly, **1** and **2** were also present in the octyl acetate type EO (presumably *B. papyrifera*). These acids are therefore classical olibanum trace volatiles, and 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 further 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 to test 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 to work with[7] helped us to select the best methodology. Eventually, only (+)-**1** and (+)-**2** could be identified in the selected samples, without detecting 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).

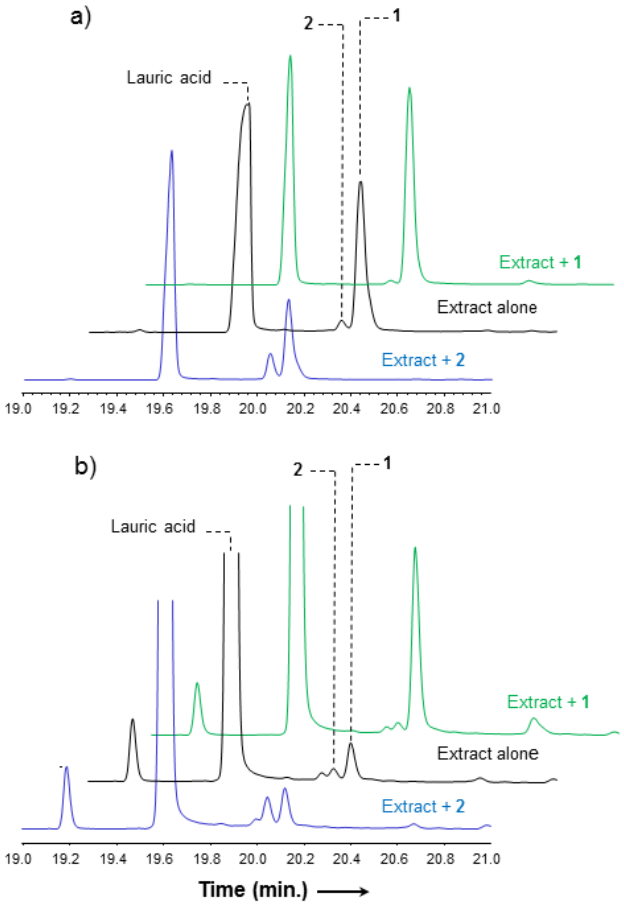


Figure 1. Coinjection experiments on acidic extracts of two samples of frankincense EO: a) monoterpene type b) octyl acetate type. GC-MS (SIM at *m/z* = 97) profiles of the acidic extract alone (black) and of the extract spiked with (±)-**1** (green) or with (±)-**2** (blue).

1 and **2** display an 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.* λ = 185 nm) and an $n \rightarrow \pi^*$ transition which has a far less important ε value (at *ca.* λ_{max} = 210 nm).

The experimental ECD spectrum of the isolated natural (+)-**1** showed two positive Cotton effects, at λ_{max}= 192 nm and λ_{max}= 214 nm. The TD-DFT calculated ECD spectrum for (+)-**1,** performed at the B3LYP/6-31+G(d,p) level of theory in an implicit solvent modelized with the polarizable continuum model (IEFPCM, CH_3CN), also had two positive Cotton effects, one at λ_{max}= 193 nm and second at λ_{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 bathochrome 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**.

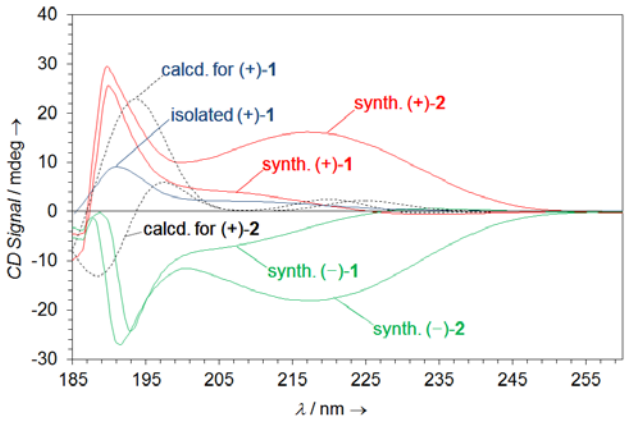


Figure 2. CD spectra of isolated (+)-**1** (blue); CD spectra of synthesized (+)-**1** and (+)-**2** (red); CD spectra of synthesized (–)-**1** and (–)-**2** (green) in CH_3CN ($1 \cdot 10^{-3}$ M, 25 °C). TD-DFT calculated ECD spectra for (+)-**1** and (+)-**2**.

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 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 4 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 (–)-**1** displayed a GC-O threshold at least 200 times higher than (+)-**2**.

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.[10] The eleven carbon homologue of (+)-**2** (*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 contribution 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 C_2-C_3 *seco*-analog of **1** and **2** and this fact probably explains the proximity of their olfactory properties.

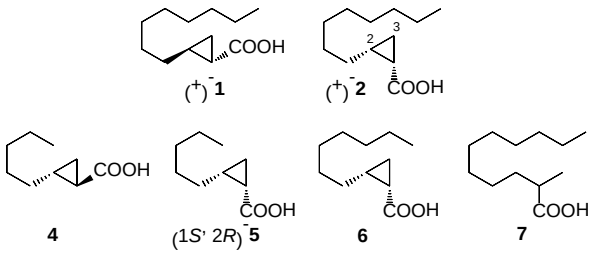


Figure 3. Olibanic acids homologues (**4**-**6**) and analogue (**7**).

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] 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[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 odour reminiscent of the oil".[9] More recently, Hasegawa 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 know spicy and peppery odorant,[19] was reported to display "woody, coniferous, incense like" odor close to its detection threshold.

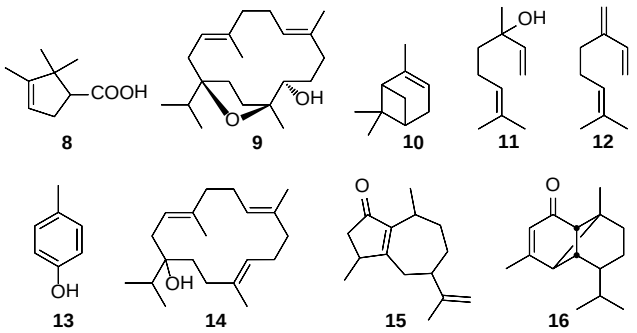


Figure 4. Frankincense constituents reported to contribute to its typical odor.

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 complements the already known fascinating series of natural cyclopropane architectures.[20] Despite the crucial role they play in the olfactory character of one of the oldest fragrant material, it is not surprising that **1** and **2** remained unknown until now, even though a large number of analytical investigations on frankincense have been published up to now. Interestingly, Buettner 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. These properties, together with their high substantivity, make them very attractive as nature-identical fragrant ingredients for perfume formulations. 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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Keywords: *Boswellia* • Frankincense • Fragrances • GC-O • Small ring systems

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