Supporting Information for the Note

Direct Identification of α-Bisabolol Enantiomers in an Essential Oil Using a Combined Ion Mobility - Mass Spectrometry/Quantum Chemistry Approach

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SUMMARY

ADDITIONAL EXPERIMENTAL DETAILS

NMR experiments

^{13}C-NMR experiments were recorded using one-pulse excitation pulse sequence (90° excitation pulse) with ^{1}H decoupling during signal acquisition (performed with WALTZ-16); the relaxation delay was set at 2s. For each analyzed sample, depending on the compound concentration, 3k up to 5k free induction decays (FID) 64k complex data points were collected using a spectral width of 30000 Hz (240 ppm). Chemical shifts (δ in ppm) were reported relative to residual signal of CDCl$_3$ (δ$_C$ 77.16 ppm).

MS experiments

Samples were introduced at 10μL/min flow rate (capillary voltage +2.8 kV, sampling cone voltage: varied between +20 V and +60 V) under a curtain gas (N$_2$) flow of 100 L/h heated at 35 °C. Accurate mass experiments were performed using reference ions from CH$_3$COONa internal standard. Depending on the experiment, samples were dissolved and further diluted in methanol doped with formic acid (1% v/v) or 0.1mM silver trifluoroacetate (AgTFA) prior to analysis. IM-MS spectra acquired on the samples prepared in presence of silver adducts were all recorded in the 50–1200 m/z range, with trap bias DC voltage of 35 V, helium cell gas flow of 180 mL/min, and the TWIMS cell operated at 3.45 mbar of N$_2$ with the following wave parameters: 750 m/s wave velocity and 27 V wave heights. These parameters have been slightly varied in the case of H$^+$ adduct measurements.

Computational details

The geometry optimization of the two α-bisabolol enantiomers, α- and τ-cadinol, and farnesol in complex with Ag$^+$ was performed by DFT calculations using the 6-31+G** + LANL2DZ mixed basis set[1] and the effective core potential for Ag$^+$ developed by Hay and Wadt.[2]

Analysis of further peaks in the ^{13}C NMR spectra of the EO samples from *X. italicum* fruits

Somewhat unexpectedly, when the commercial sample of (-)-α-bisabolol was analyzed by NMR, ^{13}C signals of epi-α-bisabolol (aproximatively 3%) were also recorded, as shown in **Figure S1**.
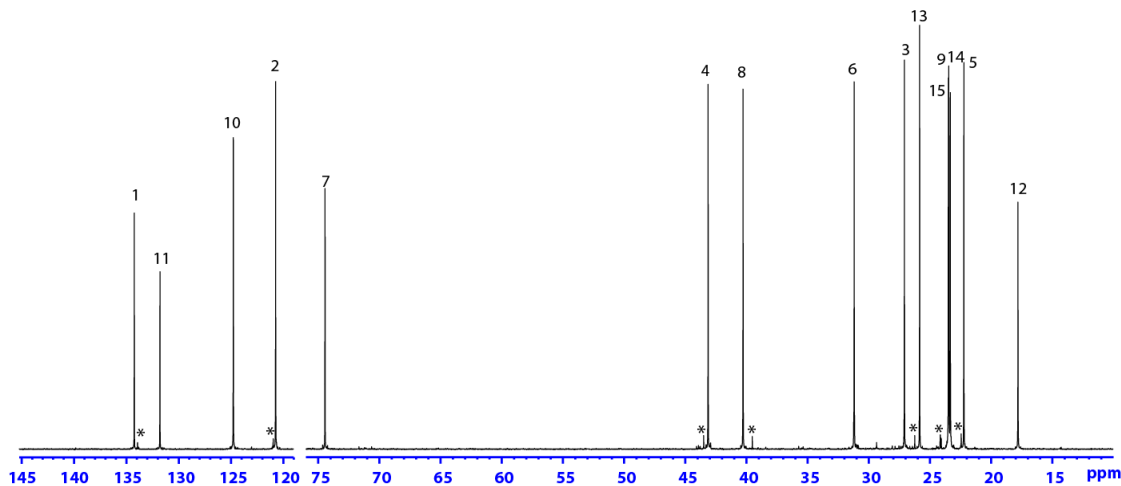

Figure S1. ^{13}C NMR data of commercial sample of (-)-α-bisabolol (signals numbered from 1 to 15) showing the presence (in traces) of epi-α-bisabolol (peaks labeled with *).

Besides the expected presence of α-bisabolol (approx. 40% w/w), the NMR data of the EO samples exhibited many other components, as evident from the numerous, alternative peaks (**Figure 1b** in main text). These signals can be generally attributed to secondary metabolites which, according to previously reported composition of essential oil extracted from *X. italicum* fruits,[3] account for 2.5 to 5% by weight of the oil components, as shown in **Table S1**.

Table S1. Main components (>2.5%) detected in essential oil of *Xanthium italicum*[3]

EO Component	%	Formula
borneol	2.9	$C_{10}H_{18}O$
bornyl acetate	2.6	$C_{12}H_{20}O_2$
germacrene B	3.8	$C_{15}H_{24}$
τ-cadinol	2.5	$C_{15}H_{26}O$
α-cadinol	5.0	$C_{15}H_{26}O$
α-bisabolol	43.0	$C_{15}H_{26}O$
(*E,E*)-farnesol	4.3	$C_{15}H_{26}O$

^{13}C NMR peaks recognition of these further oil components was performed using the extraction-NMR method proposed by Gerhard *et al.*[4], which allows the assignment of miscellaneous molecules based on the ^{13}C data recorded on the complex mixtures. The ^{13}C chemical shifts and the structure of these minority EO component molecules are reported in **Table S2** and **Figure S2**, respectively.

Table S2. [13]C chemical shifts of the main oxygenated compounds detected in the EO samples from *Xanthium italicum* fruit

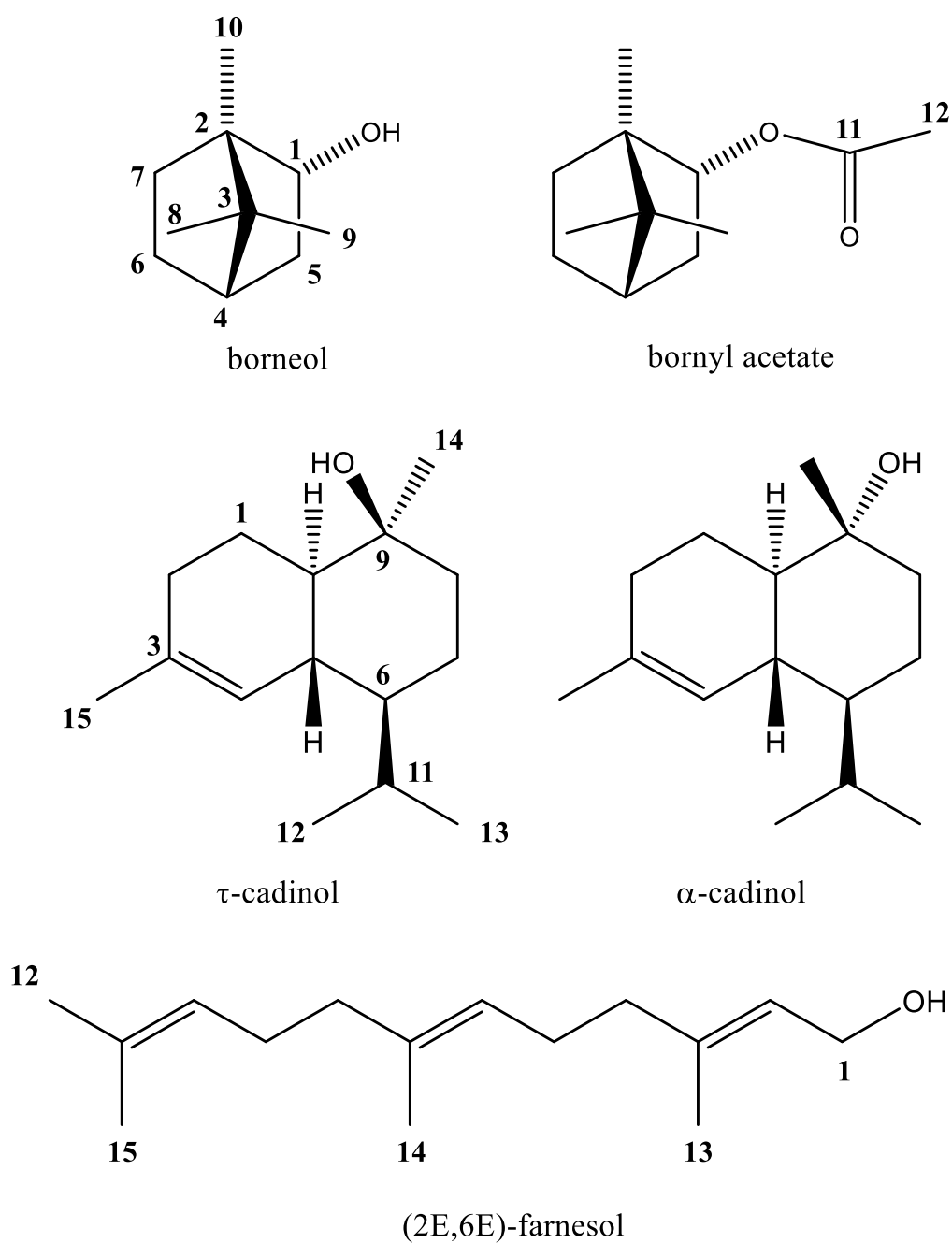| Atom No. | δ (^{13}C), ppm in CDCl$_3$ | | | | | |
	α-bisabolol	borneol[5]	bornyl acetate[6]	α-cadinol[7]	τ-cadinol[8]	(E,E)–farnesol[9]
1	134.15	77.35	80.02	21.98	20.97	59.3
2	120.62	49.46	48.76	30.98	31.3	123.8
3	26.97	48.00	47.83	134.98	133.52	139.5
4	42.98	45.16	44.92	122.38	124.89	39.7
5	22.12	39.10	36.72	39.89	34.53	26.4
6	31.09	28.31	28.07	46.72	43.99	123.5
7	74.53	25.87	27.05	22.72	19.41	135.3
8	40.14	20.22	19.75	42.14	34.64	39.6
9	23.35	18.72	18.87	72.68	72.55	26.8
10	124.64	13.37	13.52	49.95	46.05	124.4
11	131.70	-	171.7	26.01	26.58	131.2
12	17.72	-	21.21	21.57	21.65	25.6
13	25.76	-	-	15.67	15.46	15.9
14	23.22	-	-	20.71	29.33	16.2
15	23.41	-	-	23.89	23.67	17.6

Figure S2. Structures of the secondary metabolites found in the EO samples from *X. italicum* fruits identified by ^{13}C NMR spectroscopy.

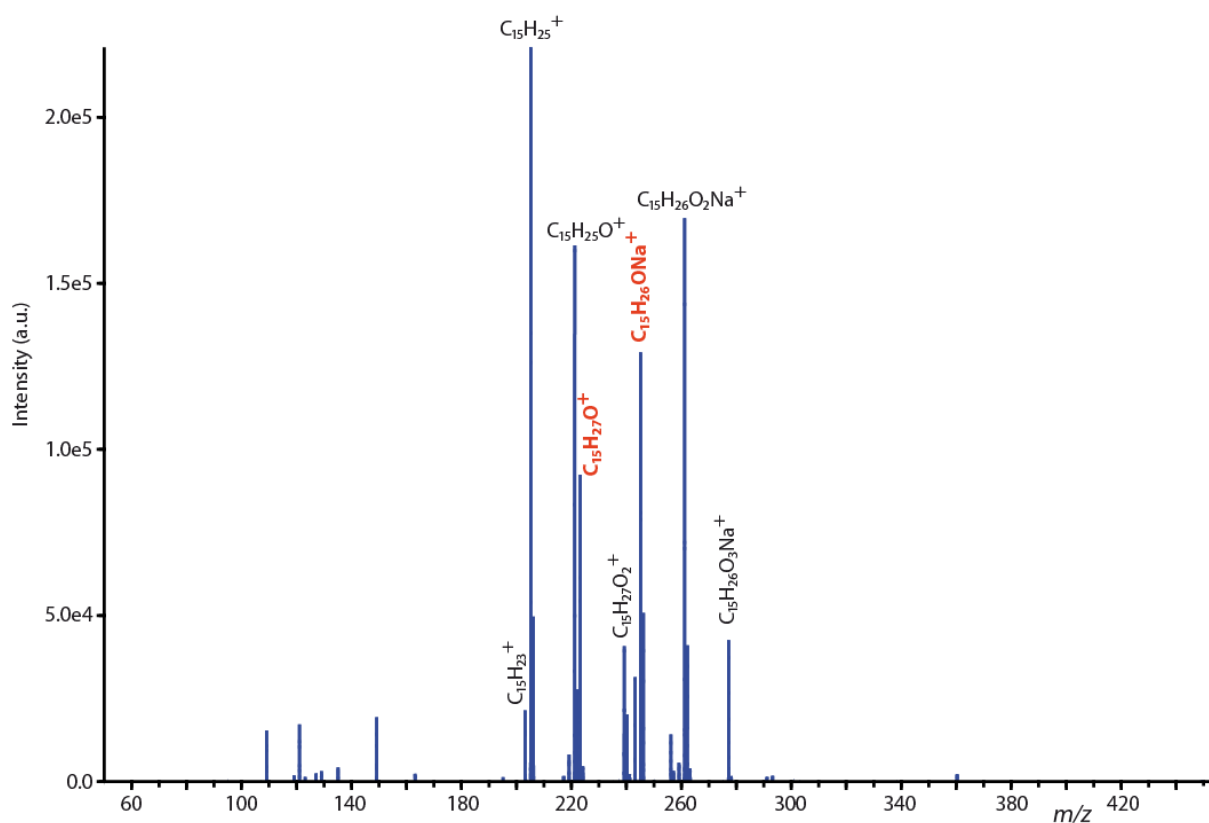**ESI-MS and IM-MS experiments for α-bisabolol enantiomer identification**

Figure S3. (+)-ESI-MS spectrum recorded on *Xanthium italicum* crude essential oil sample prepared in acidified methanol (DP=30V).
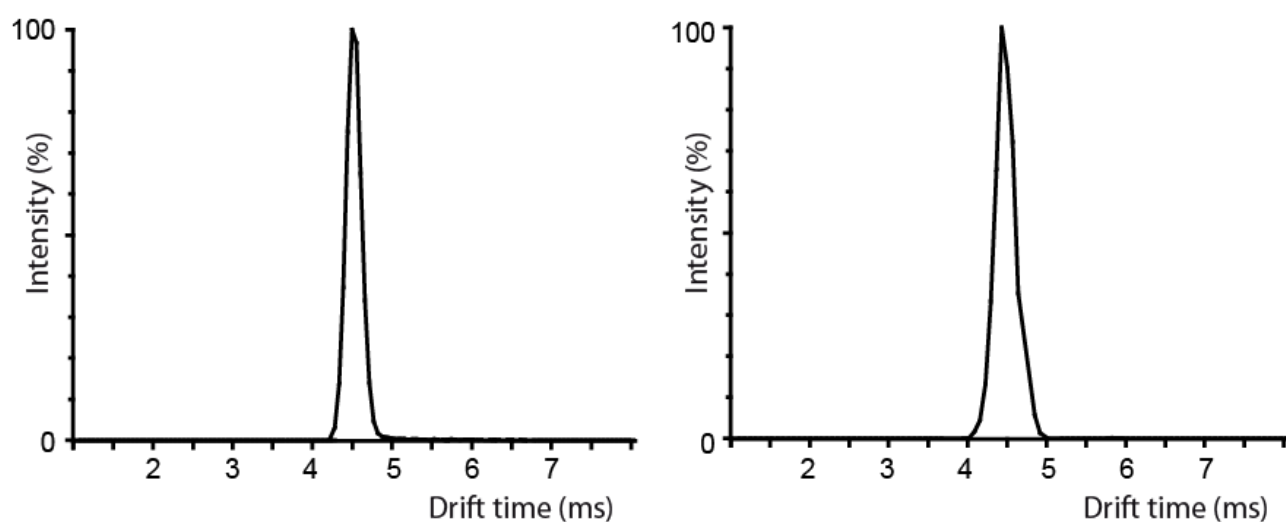

Figure S4. Arrival time distribution of $C_{15}H_{27}O^+$ ions (*m/z* 223) in (+)-ESI-IM-MS experiments recorded on(-)-α-bisabolol (left panel) and *X. italicum* EO sample (right panel), both dissolved in acidified methanol.

Molecular simulations revealed the structures of the two □-bisabolol enantiomer silver (I) adducts

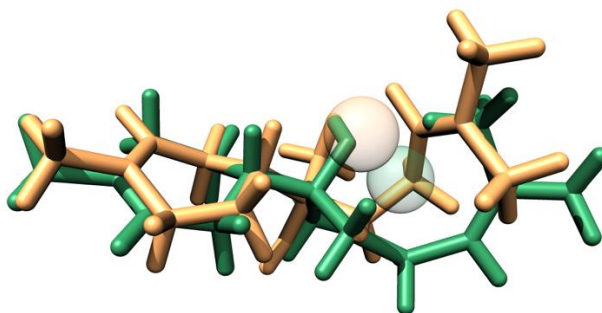**Figure S5.** Superposition of the optimized structure for the Ag⁺-adducts of (-)-α-bisabolol (sea green) and (+)-α-bisabolol (sandy brown). The two Ag⁺ ions are shown as color-matching transparent CPK spheres.
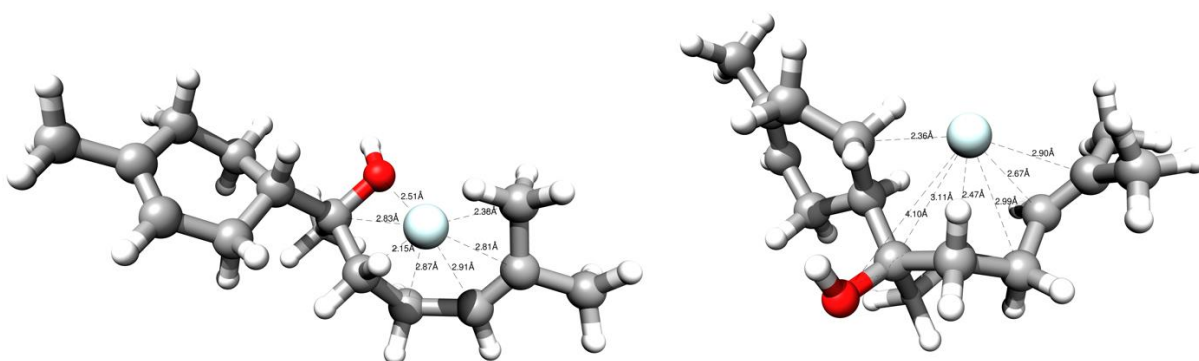

Figure S6. Optimized interatomic distances between the silver ion and selected atoms of the (-)-α-bisabolol (left) and (+)-α-bisabolol (right) as obtained from DFT calculations.

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