QUANTITATIVE STRUCTURE-FRAGMENTATION RELATIONSHIP TECHNIQUE APPLIED FOR DISCRIMINATION OF *cis*-β-OCIMENE AND *trans*-β-OCIMENE FROM LAVENDER OIL

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Abstract: *Elucidation by mass spectrometry of isomeric structures only by using a library search presents difficulties due to the high similarity of the spectra. So, the GC-MS analysis of trans-β-ocimene and cis-β-ocimene, biologically active compounds from lavender oil, offers contradictory results when are used mass spectral libraries and retention indeces database. Under these circumstances, supplementation of analysis with an independent path is absolutely necessary to clarify the configuration of these compounds. Quantitative structure-fragmentation relationships (QSFR) techniques offer such a possibility. These techniques use thermochemical data obtained through quantum chemical calculation (QCC) for structures which should be discriminated against. In our paper we opted for an ordering algorithm (ORD) which gives good results in the case of high similarity spectra. ORD uses the inverse sorting of the relative intensities' row of the main isobaric ions with the corresponding enthalpies' row. The result thus obtained validates the structures achieved by retention indices. One of the advantages of this approach is that the use of these three analytical variants can provide high accuracy of analysis without the direct use of chemical standards.*

Keywords: quantitative structure-fragmentation relationship, quantum chemical calculation, differential mass spectrometry, lavender oil, ocimene

INTRODUCTION

The limitations of search library in mass spectrometry regarding elucidation of isomeric structures with similar mass spectra are well documented (Stein 2012). In these cases, the discrimination between isomers through the interpretation of fragmentation patterns is very difficult to achieve, because the mass spectra of these isomeric analytes contain the same signals. The use of spectral libraries can give erroneous results because of (i) the low sensitivity of the search algorithm in the case of spectra with high similarity or (ii) the absence of the analyte's spectrum from the database. We have circumvented this problem by additional use of analytical information contained in the intensity of ionic currents of, for example in the case of tetrachlorinated biphenyls (Dinca et al. 2012). In this case, an additional search for the analyte is done in a database calculated for the group of possible structures provided by library search. It includes the formation enthalpies for primary fragmentations of each molecular structure considered. Because no unified enthalpies databases exist, they were calculated by us using quantum chemical methods, usually semi-empirical, which easily provide values with good relativity, on ordinary computers (Dinca et al. 2012). The ORD algorithm compares the experimental and calculated data (e.g., the difference in the relative intensity of ionic current with the corresponding fragmentation enthalpy difference) for some main ions in all possible variants of structural assignments. The result of chemical structure identification (CSI) is presented as a list of the decrease in the probability of these variants (Bettendorf *et al.* 2007). Therefore, the aim and the novelty of our work consists of: (i) the calculation of a database containing the fragmentation enthalpies involved in chemical structure identification (CSI) of *trans* β-ocimene and *cis*-β-ocimene, two biologically active compounds from lavender oil, and (ii) the use of this database for the discrimination with ORD algorithm of the above stereoisomers.

MATERIALS AND METHODS

Lavender essential oil was obtained by steam distillation using classic Clevenger equipment. The separation and identification of different compounds had been done using a GC-MS system Shimadzu 2010 Plus gas chromatography apparatus (Shimadzu, Kyoto, Japan) and triple quadrupole mass spectrometer (TQ 8040). The column used was a capillary column DB-1 (30 m length; 0.25 mm i.d.; 0.25 μm film thickness) with helium as gas carrier at 0.93 $L \cdot min^{-1}$. The oven temperature setting was 70 ºC for 11 min, then 5 ºC/min to 190 ºC and 20 ºC/min to 240 ºC, and then left at 240 ºC for 5 min runs. The injector temperature and MS source were maintained at 250 ºC and 200 ºC, respectively. The ionization energy was 70 eV.

The strategy of $\Delta_f H$ *database calculation.* The heats of formation ($\Delta_f H$) were calculated with the semi-empirical method RM1 using the *HyperChem* 8.0.10 software. The geometries of the ocimenes molecules and radicals were optimized with the MM+ force field and re-optimized, using the RHF operators for molecules or ions and UHF for radicals (Rocha *et al.* 2006), the molecule being considered in vacuum. The fragmentation enthalpies ($\Delta_f H$(frag)) were calculated according to eqn. (1):

$$\Delta_f H(\text{frag}) = \Delta_f H(\text{ion}) + \Sigma\Delta_f H(\text{F}) - \Delta_f H(\text{M}) \qquad (1)$$

where $\Delta_f H$(ion) is the formation enthalpy of the main ion, $\Sigma\Delta_f H$(F) is the sum of the formation enthalpies of secondary fragments (radicals and molecules) and $\Delta_f H$(M) the formation enthalpy of the molecule.

We calculated the mass spectra similarities and the probabilities using the CSI-Diff-MS 3.1.1 software (for the ORD algorithm). Chemical structure identification was conducted by strictly adhering to the protocols for ORD as reported previously (Dinca *et al*. 2012).

RESULTS AND DISCUSSIONS

The partial chromatogram with the most volatile compounds of lavender oil is shown in Figure 1.

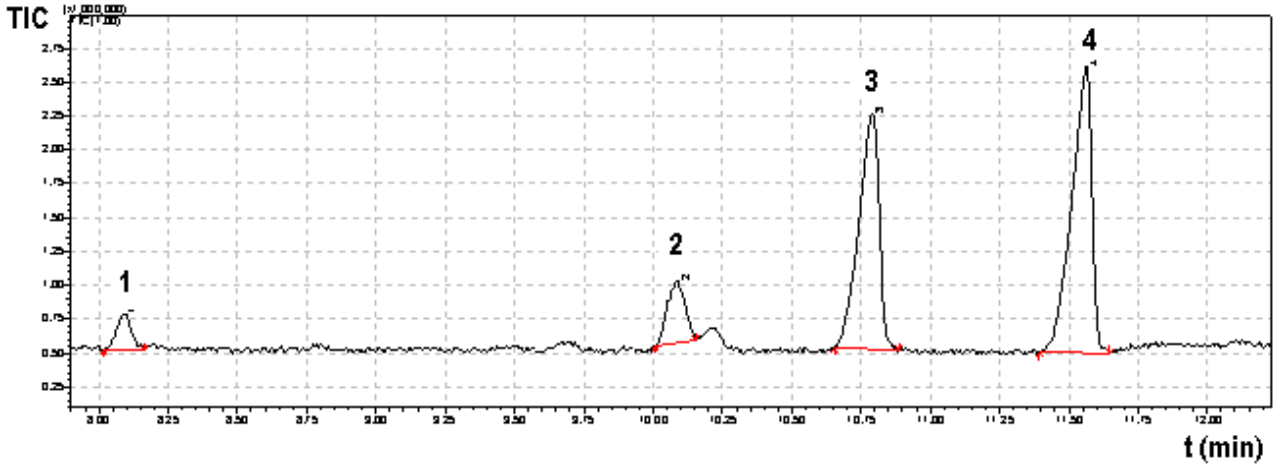


Figure 1. The first four peaks of GC-MS chromatogram of lavender oil

The search in mass spectral reference library identified the gas chromatographic peaks 3 and 4 as belonging *trans*-β-ocimene and *cis*-β-ocimene respectively (Table 1). This result is in contradiction with the structure search in retention indices (RI) database of targeted ocimenes because the retention index of *trans*-β-ocimene is greater than that of *cis*-β-ocimene (http://webbook.nist.gov). This mismatch is explicable due to the high similarity of mass spectra and very close retention indices of these compounds (Kovats RI: 1029 for *cis*-β-ocimene and 1036 for *trans*-β-ocimene). It should also be mentioned that only ocimen's formula ($C_{10}H_{16}$) corresponds to more than 240 known species of whom about 60 are isoprenoids (http://webbook.nist.gov/cgi/formula/). Under these circumstances, an additional analysis path was necessary to clarify the configurations.

Table 1. The spectral library search results

GC Peak	Retention time (min)	Compound	Molecular formula	MW
1	8.09	β-Myrcene	$C_{10}H_{16}$	136.24
2	10.07	Eucalyptol	$C_{10}H_{18}O$	154.25
3	10.78	*trans*-β-Ocimene	$C_{10}H_{16}$	136.24
4	11.54	*cis*-β-Ocimene	$C_{10}H_{16}$	136.24

The correlation between fragmentation enthalpies and differential mass spectrum provides a different analytical way that can confirm one or other of results obtained by the above mentioned methods: (i) the structure search in mass spectral reference library and (ii) in retention indices database.

Figure 2. The mirrored mass spectra of GC peak 4 (above) and GC peak 3 (down)

The mass spectra (70 eV) of *cis*-β-Ocimene and *trans*-β-Ocimene (Figure 2) are mirrored very well due to their high similarity (91%). It is obvious why the two diastereomers cannot be distinguished using only the spectral libraries. The differential mass spectrum (ΔMS) of GC *peak 4 versus* GC *peak 3* (Figure 3) clearly highlight the intensification of the allyl fragmentations: $[M-C_4H_7^{\bullet}]^+$ at m/z 81, $[M-C_5H_9^{\bullet}]^+$ at m/z 67, and the loss of methyl at m/z 121 (Scheme 1), in the diastereomer with higher energy, *trans*-β-ocimene.

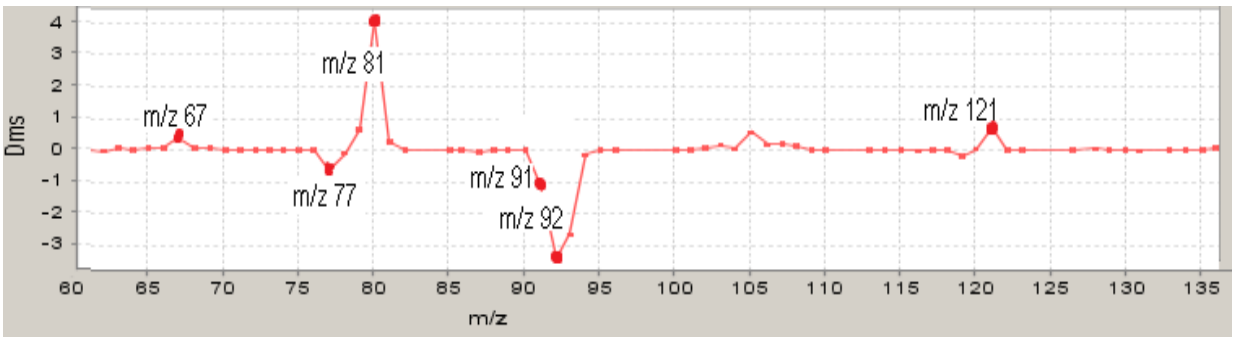
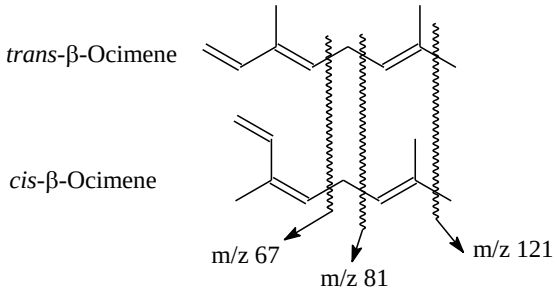


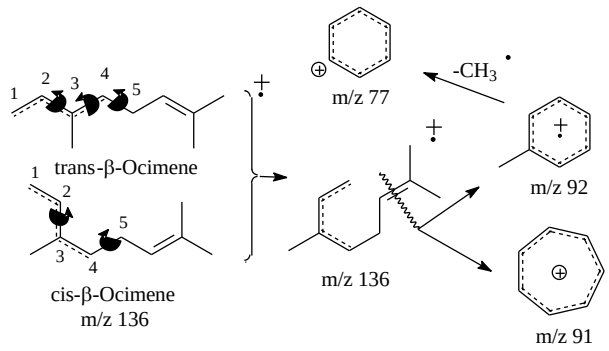
Figure 3. The differential mass spectrum (ΔMS) corresponding to the chromatogram peak 4 versus peak 3.

At the same time, one can notice a decrease in the intensity of the resulting ions by cyclization and fragmentation, m/z 92, m/z 91, and m/z 77 (Scheme 2). The decreasing of the cyclization rate of *trans*-β-ocimene is due to an additional (C3-C4) rotation which he has to do compared to *cis*-β-ocimene (Scheme 2). Its energy barrier is 11 kcal/mol (Fig. 4).



Scheme 1

Even only the common rotation C2-C3 (0.6 kcal/mol) consumes the energy excess (0.63 kcal/mol) of *trans*-β-ocimene. Values used in the ORD database (Table 2) are obtained by differential accounting (Dinca *et al.* 2012) of rotation and fragmentation energies for m/z 92, m/z 91, and m/z 77 ions of the two isomers.



Scheme 2

For the algorithm ORD applied to the isobaric ions, a good relativity of these values is sufficient even if they could have systematic errors.

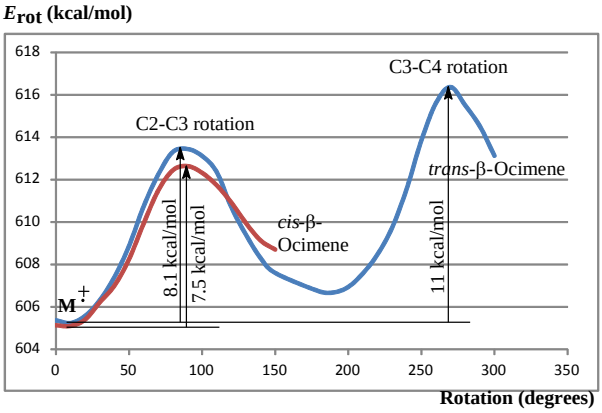


Figure 4. The rotation energy (E_{rot}) diagram of the ocimenes calculated from $\Delta_f H$ of conformers resulted by C2-C3 and C3-C4 rotations in $M^{+\cdot}$ (Scheme 2).

Table 2. The ORD probability calculation is based on the rule that the more stable ion has the higher relative intensity. The window represents the probabilities list obtained by the ordering algorithm of CSI-Diff-MS 3.1.1 software.

m/z	ΔMS (GC peaks 4 vs.3)	$\Delta_f H$(frag) difference (*cis*- vs. *trans*-)	$\Delta_f H$(frag) difference (*trans*- vs. *cis*-)
67	0.413	0.63	-0.63
77	-0.630	-2.87	2.87
81	0.250	0.45	-0.45
91	-1.079	-2.87	2.87
92	-3.409	-2.87	2.87
121	0.718	0.51	-0.51
ORD probability	Correlation results: Probability [%] | 04 peak.csv | 03 peak.csv 100.000 | OCIMENE, TRANS | OCIMENE, CIS 0.000 | OCIMENE, CIS | OCIMENE, TRANS	0 %	100 %

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The window of probabilities list resulted by software (Table 2) shows that the GC peak 3 is *cis*-β-ocimene and the GC peak 4, *trans*-β-ocimene (100% probability). This result is consistent with the retention indices of the two isomers.

CONCLUSIONS

In the computational simulations made for diastereomeric discrimination between of *trans*-β-ocimene and *cis*-β-ocimene, ORD algorithm based on QSFR strategy, invalidate the configuration achieved by the search in library spectra but it was consistent with the retention indices of the two diastereomers.

ACKNOWLEDGEMENTS

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