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Trace level determination of enantiomeric monoterpenes in terrestrial plant emission and in the atmosphere using a β-cyclodextrin capillary column coupled with thermal desorption and mass spectrometry

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Abstract

For the first time, enrichment on solid sorbents followed by thermal desorption has been used for the determination of the enantiomeric signature of monoterpenes in the gaseous emission of terrestrial plants. A β-cyclodextrin capillary column has been used for the separation of critical pairs. The temperature program and column loading were optimized for making the accurate quantification of individual enantiomers possible by mass spectrometry. The resolution achieved was sufficient for separating enantiomeric monoterpenes from other biogenic and anthropogenic volatile organic compounds present in air and vegetation emission samples. The method has been applied to the determination of the enantiomeric ratios of monoterpenes in the gaseous emissions of some evergreen plants and in the open atmosphere.

Keywords: Enantiomer separation; Gaseous plant emissions; Monoterpenes; β-Cyclodextrins; Volatile organic compounds

1. Introduction

With the advent of highly enantioselective cyclodextrin derivatives as stationary phases for capillary gas chromatography (GC) [1,2], chiral analysis has been widely applied to the quality assurance of natural flavors and essential oils [2–4]. Only recently, however, has the enantiomeric composition of monoterpenes produced by terrestrial plants been exploited to assess the potential impact of gaseous pollutants on plant metabolism [5] and to investigate the complex host–insect relationships existing in forest ecosystems [6–8]. These latter studies showed that some insect taxa can use the enantiomeric signature of monoterpenes produced by coniferous trees for their own pheromone production. The predominance of (−)-enantiomers of the monoterpenes in the emission of some *Pinus* and *Abies* species of Europe was explained [7] with the attitude that these plants act as an aggregation source of

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insects for breeding purposes. The need to hinder mass attacks of these insects on the plant during the reproduction season was invoked to explain the high variations in the enantiomeric composition of monoterpenes in the leaf and xylem extracts of the same tree. It is believed that the enantiomeric signature of monoterpenes could also be used by plants to prevent the attack of herbivores on leaves, trunks and twigs. In this case, the plant could exploit either the different toxicity of the (+)- and (−)-enantiomers toward ants, bugs and beetles [9] or might use specific enantiomers as semiochemicals to reveal the presence of the herbivore to its natural enemies who can then come to assist the plant [10].

Although insect–plant interactions mostly occur in the gas phase, all these studies have been conducted on liquid extracts from different plant tissues under the assumption that volatile organic compound (VOC) production was reflecting the emission. To get a realistic view of the whole gaseous emission, a large number of extracts were analyzed and the final figures on the emission obtained through a statistical treatment of the data [7,8]. This procedure is quite valid when chemicals are emitted as a response to herbivores as they are rapidly transferred from the liquid to the gas phase through the wounds. It is less accurate in the case of intact plants because not all production is transferred into the atmosphere at the same rate. It should be recalled that isoprenoids can be produced in different cell compartments and their transfer to the atmosphere depends upon the vapor pressure of VOCs, the total resistance of the path followed by the organic components to reach the atmosphere and the environmental conditions to which the plant is exposed [11]. Because of this, low volatile isoprenoids (such as some mono- and sesquiterpenes) accumulated in resin ducts, which are separated from the stomatal chamber by strong cellular structures surrounding the secretory cells [12], could be emitted at very low rates although they are abundant in leaf extracts. Studies performed with ^{13}C-labeled CO_2 have shown that the transfer of monoterpenes into the atmosphere is quite rapid (10–20 min) in plants lacking of specialized organs for their storage [13] whereas it is very slow (some days) in plants where VOCs are accumulated in resin ducts [14]. Even in plants characterized by a fast turnover of assimilated carbon, it was found that the leaf content of monoterpenes does not correspond to the gaseous emission and monoterpenes respond quite differently to light induction [15]. Whenever plant insect interactions are driven by the VOC emissions from intact tissues, the direct analysis of the gaseous emissions seems, therefore, the most reliable approach to follow.

Although several selective and sensitive methods exist for the determination of monoterpenes in the gaseous plant emission [16], only recently has an attempt been made to extend these methods to the identification and quantification of enantiomeric components [17]. Traps filled with active carbons were used to enrich enantiomeric monoterpenes. They were separated on a β-cyclodextrin capillary column after liquid extraction. Although this method has been successfully applied to the determination of the enantiomeric signature of two plant species (*Cedrus atlantica* L. and *Pinus halepensis* L.) growing in Algeria, it requires large air volumes (ca. 100 l) to meet the sensitivity of the detection system. The sampling times are, however, too long (8 h) to follow the response of the plant to environmental and ecological factors and for an accurate determination of the basal emission (i.e., the emission occurring at 30°C and 1000 μmol m^{-2} s^{-1}) under field conditions. The effect of light and/or temperature can be, for instance, so rapid that sampling times exceeding 30 min are today considered too long for physiological investigations. Another severe limitation of methods using liquid extraction for VOC determination is represented by the impossibility to detect the whole range of isoprenoids present in the emission because of the selective evaporation of VOCs from the solution and the interference in the chromatographic analysis caused by the elution of the solvent peak and its impurities. The simultaneous detection of monoterpenes and isoprene is crucial for physiological and environmental studies because the latter component is, on a global scale, the most abundant biogenic compound emitted by terrestrial vegetation [18].

In this work, a method capable of providing the full isoprenoid composition of vegetation emission together with the enantiomeric signature of monoterpenes has been developed and tested. The use of cartridges filled with solid sorbents able to quantitatively retain VOCs from C_5 to C_{15} and to release

them by thermal desorption allowed the detection of the organic emission in volumes of air ranging from 0.2 to 1 l. The chromatographic conditions of a capillary column coated with β-cyclodextrin were optimized to better separate enantiomeric monoterpenes and to make the quantification of other biogenic VOCs (such as isoprene and some sequiterpenes) possible in the same GC run. Because of the low volumes required for the analysis, sampling times ranging between 1 to 10 min were sufficient for an accurate quantification of the emission. Unambiguous identification of desorbed compounds was achieved by connecting the column to a mass spectrometer. The method has been used to derive the enantiomeric ratios of monoterpenes [ERs, defined as the ratio of the (+)-enantiomers with respect to the (−)-enantiomers] released by different plant species. The sensitivity afforded was sufficient for the accurate determination of the enantiomeric composition of monoterpenes in air samples where these components were present at sub-ppbv levels.

2. Experimental

Biogenic VOCs emitted from *Quercus ilex* L., *Pinus radiata* D. Don., *Pinus pinea* L., *Cupressus glabra* Sudw., *Cupressus sempervirens* L., *Eucalyptus globulus*, *Cedrus deodora* Loud. and *Citrus aurantium* L. and from dead needles of *Pinus radiata* D. Don. deposited on soil were collected using static branch cuvettes. They were made by Tedlar bags, 3 l in volume, equipped with inlet and outlet lines for air circulation. Samples were collected 5 min after branch enclosure to prevent heat stress to the plant leaves. All experiments were carried out under light saturation conditions (PAR> 1000 μmol m^{-2} s^{-1}) with leaf temperatures ranging from 27 to 30°C. All plants investigated with this type of enclosure were located inside the research area of Montelibretti (Rome, Italy) near our laboratory. The emission from *Pinus pinaster* L. was, instead, collected using dynamic branch cuvettes having a volume of 20 l. They were flushed with a flow-rate of 20 l min^{-1} of purified air. They were quite similar to those commonly used during the BEMA Project [19] to investigate the biogenic emission from Mediterranean vegetation. A full description of this type of cuvette can be found in Ref. [20]. Dynamic enclosures were installed at the top of the canopy of a pine forest located in San Rossore, near Pisa (Italy).

Air samples were collected 50 m away from any tree, in a large space where local emission was well mixed with air masses transported inside the Tiber valley during the central hours of the day.

Air and emission samples were enriched on glass tubes (15 cm×0.3 cm I.D.) filled with a bed of Carbopack C (0.034 g) and Carbograph 1 (0.17 g), set in series. The graphitic materials from 20 to 40 mesh particle size were supplied by Supelco (Bellefonte, PA, USA) and LARA (Rome, Italy), respectively. The features of these new materials and their efficiency were discussed elsewhere [21]. Before sample collection, cartridges were cleaned at 300°C under a flow-rate of helium (100 ml min^{-1}). After 10 min purging, the traps were closed with tight connectors and stored in large (10 l) sealed glass containers till ambient air sampling was performed. Air was drawn through the adsorption traps by using battery operated samplers (Alpha 1 type) supplied by Ametek (Mansfield & Green Division, Largo, FA, USA). Flow rates ranging from 200 to 330 ml min^{-1} were used for sample collection. After sample collection, traps were wrapped in aluminum foil and stored at room temperature until they were subjected to chemical determinations. After removing oxygen and the excess of water from the adsorbents by a back-flushing step, traps were thermally desorbed at 250°C and VOCs cryofocused in an empty liner kept at −120°C. Injection into the capillary column was achieved by fast heating the liner from −150 to +150°C in 10 s. Duplicate analyses of some standard and emission samples were also performed by using tubes filled with 0.125 g of Tenax TA particles ranging between 20 and 35 mesh. The polymeric adsorbent was provided by Alltech Associates (Deefield, IL, USA). Due to the lower thermal stability of Tenax TA with respect to graphitic carbon adsorbents, a temperature of 250°C was used for the cleaning process. The complete recovery of retained VOCs was, instead, obtained by heating the traps at 235°C.

The separation of desorbed VOCs was performed on a Cyclodex-B capillary column, 30 m×0.256 mm I.D., 0.25 μm film thickness supplied by J & W

Scientific (CA, USA). The internal coating was composed of a permethylated β-cyclodextrin dissolved into a cyanopropyl–dimethyl polysiloxane liquid characterized by a polarity equivalent to that of OV 1701 [1], but a better long-term stability. It was operated at a flow-rate of helium of 0.94 ml min^{-1}, corresponding to a linear velocity of 30 cm s^{-1}. Mass spectrometric determinations were performed using a HP 5890 gas chromatograph coupled with a HP 5970B mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA). Thermal desorption of the sampled tubes, cryofocusing of released vapors and their injection into the capillary column were performed with a TCT/PT1 CP4001 unit supplied by Chrompack (Middleburg, The Netherlands). For peak identification the mass spectrometer was operated in scan conditions by collecting all ions from 20 to 250 m/z. A scan frequency of 3 scans s^{-1} was used for generating the mass chromatogram. Selective detection of enantiomeric monoterpenes was achieved by plotting the current profiles generated by the most specific fragments generated by electron impacts. Positive identification was obtained by combining the mass spectral information with the elution sequence obtained through the analysis of pure compounds.

Since both the initial temperature and the temperature program are quite critical for the separation of enantiomeric pairs on cyclodextrin coated columns [22], several tests were performed to achieve the best resolution. They were conducted by using liquid standard solutions containing the enantiomeric pairs of α-pinene, camphene, limonene and linalool at fixed ratios. High-purity chiral components were obtained from Fluka (Buchs, Switzerland). Methanol was used as a solvent to allow the complete mixing of enantiomeric monoterpenes characterized by the presence of hydroxyl groups in the molecule. To simulate the adsorption and desorption processes to which vegetation samples were subjected, liquid standard mixtures were injected into the capillary column using the thermal desorption system. Small aliquots (usually 1–2 μl) of the standard mixture were injected into the adsorption traps under a flow of helium of 100 ml min^{-1}. The sample was ready for the analysis after the bulk of methanol was removed by the trap and biogenic VOCs distributed through the adsorbing beds. This was obtained by passing 4 l of helium through the cartridge.

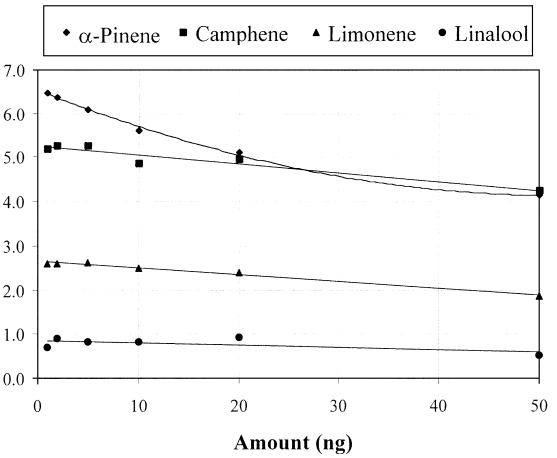

Fig. 1. Changes of resolution (measured at 50% valley) of selected enantiomeric monoterpenes as a function of the amount injected (ng) into the Cyclodex-B column. For the experimental conditions used see the text.

Tests carried out by changing the initial temperature of the column from 30 to 60°C and temperature gradient from 1 to 3°C min^{-1} showed that the best compromise between analysis time, the resolution of the enantiomeric pairs present in the standard mixture and their separation from all other isoprenoids was achieved by keeping the column at 40°C for 5 min and by increasing the temperature of the oven up to 200°C at a rate of 1.5°C min^{-1}. The effect of column loading on the chromatographic resolution of enantiomeric monoterpenes was also investigated. Fig. 1 shows the changes in resolution (R_s) of selected enantiomeric monoterpenes recorded when increasing amounts of individual components were injected into the β-cyclodextrin column. Results indicate that column loading was a quite critical parameter with this type of columns and specific attention should have been paid to sample volumes where individual components were below 20 ng.

3. Results and discussion

The first experiments were aimed at assessing that the trapping materials and the thermal desorption process were not a source of artifacts in the analysis of enantiomeric monoterpenes. These tests were performed by comparing the theoretical values of the enantiomeric ratios of monoterpenes present in a

standard mixture with those obtained by submitting the mixture to GC–MS analysis after adsorption of the enantiomeric mixture on solid sorbents and its transfer into the chiral column by thermal desorption. To check for possible decomposition effects arising from the chemical nature of the solid sorbents, experiments were performed with traps filled with graphitic carbons and Tenax TA. The latter adsorbent was selected as reference material in our experiments because it is the most widely used for the quantification of monoterpenes in plant emissions and it is sufficiently inert to prevent the thermal degradation of these components in air samples free from ozone [23]. The results obtained during these experiments are reported in Table 1. They show that the combination of carbon adsorbents and Tenax TA provided consistent results that only slightly deviated from theoretical values. Direct injection of pure compounds into the columns showed that these small discrepancies were not originated by the thermal treatment of the sample but by changes in composition of the liquids stored in the bottles. Pure enantiomeric components showed a lower purity than that declared by the company, probably due to contamination or partial degradation. This was possible because liquids were stored for long time in the laboratory (ca. 2 years) and often used for the preparation of standard mixtures of different monoterpenes.

The second important step was to check that the monoterpene composition measured with β-cyclodextrin columns was the same as that obtained with non-chiral columns commonly used for the analysis of monoterpene compounds in plant emissions. This comparison was performed on a real emission sample using *Quercus ilex* L. as a test case. This evergreen tree, which is one of the few oak species in the world that emits monoterpenes instead of isoprene [24,25], was selected because it is a strong emitter, the composition of monoterpene is much more constant through the year than that of coniferous trees and tree-by-tree variations are quite limited [25]. It represented a good test for evaluating the separation properties of our β-cyclodextrin column also because the emission contains a fair mixing of chiral and non chiral components. Although the major constituent is α-pinene, components lacking of an asymmetric carbon (such as myrcene, tricyclene and sometimes *cis*- and *trans*-β-ocimenes) are also present in detectable amounts. Fig. 2a reports the reconstructed mass chromatographic profiles of monoterpenes obtained by analyzing the gaseous emission of *Quercus ilex* L. on a β-cyclodextrin column. The sample was collected with the static branch enclosure described in the previous section and VOCs were enriched on traps filled with graphitic carbon adsorbents. Also in this case, duplicate samples were collected on Tenax TA adsorbent. Their analysis fully confirmed the data obtained with traps filled with carbon material. For the sake of comparison, we have reported in Fig. 2b the profile of the standard mixture used for the calibration of enantiomeric monoterpenes. Both profiles were generated by plotting the current produced by the ion with m/z 93, a fragment quite specific of monoterpene compounds that, in many instances, is the base peak of the mass spectrum. The percent composition of monoterpenes obtained with capillary columns internally coated with β-cyclodextrin and silicon phases are reported in Table 2. The comparison was made with data from the literature obtained by using the same type of carbon adsorbents for collecting VOCs but with different dynamic enclosures [15,26,27]. Data reported in Table 2 show that no substantial differences in composition were observed on the dominant components but some clear discrepancies occurred in the compounds present at trace levels. It should be

Table 1
Comparison between the theoretical values of ER in the monoterpene standard mixture and those experimentally measured by using adsorption traps filled with graphitic carbons and Tenax TA

Compound	Theoretical	Graphitic carbon adsorbent	Tenax TA
(−)/(+)-α-Pinene	1.20±0.05	1.27±0.03	1.15±0.03
(+)/(−)-Camphene	3.35±0.05	3.42±0.03	3.35±0.03
(−)/(+)-Limonene	1.25±0.05	1.27±0.03	1.32±0.03
(−)/(+)-Linalool	1.00±0.05	1.02±0.03	1.05±0.03

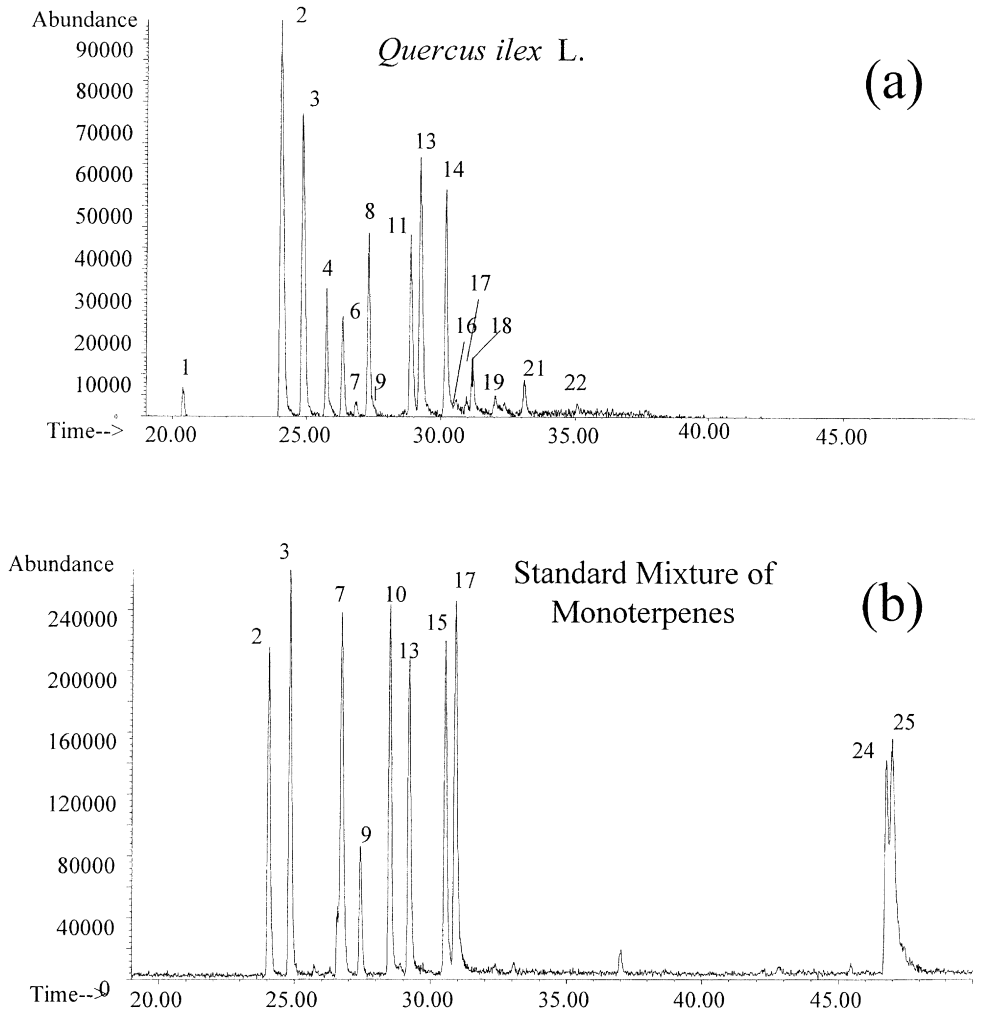

Fig. 2. Reconstructed mass chromatographic profiles (*m*/*z* 93) of monoterpenes present in: (a) the gaseous emission of the evergreen oak *Quercus ilex* L. and (b) the standard mixture used for calibration. Numbers in the figure refer to the components listed in Table 3.

noticed, however, that these differences were not related to the separation system used. Small variations in *Quercus ilex* L. emission can actually take place because of the different phenological stage of leaves and the presence of fruit and flowers in the plant. Higher contents of *cis*- and *trans*-β-ocimene are indeed common in leaves younger than 30 days whereas emission of linalool can be detected during the flowering season [28]. By considering that data were obtained with different enclosures and that seasonal and tree-by-tree variations, although small, affect the emission, differences between chiral and silicon columns fall inside the statistical uncertainty of field studies.

The capability of the β-cyclodextrin column to provide an accurate quantification of all isoprenoids emitted by terrestrial vegetation together with the enantiomeric signature of monoterpenes is well summarized in the examples shown in Fig. 3a and b, where the mass chromatographic profiles obtained by submitting to GC–MS analysis the gaseous emission of *Cupressus glabra* Sudw. and *Eucalyptus globulus*,

Table 2
Comparison between the percent monoterpene emission from *Quercus ilex* L. measured with chiral and non-chiral columns

Compound	Cyclodex-B	CP-SIL 5 CB[a]	DB-1[b]	DB-1[c]
α-Thujene	1.0	n.r.	1.9	1.7
(−)/(+)-α-Pinene	36.9	32.9	32.3	39.0
Myrcene	6.1	14.4	2.4	11.3
Tricyclene	0.6	n.r.	0.2	0.4
(+)/(−)-Sabinene	11.6	7.1	8.8	13.8
(+)/(−)-Camphene	2.1	2.3	3.1	2.7
(+)/(−)-β-Pinene	23.3	25.1	22.2	24.3
cis-β-Ocimene	10.2	3.8	–	–
(−)/(+)-Limonene	1.5	1.8	4.1	4.7
p-Cymene	0.4	n.r.	4.2	4.2
trans-β-Ocimene	3.2	1.1	–	–
(+)-β-Phellandrene	0.9	n.r.	1.0	0.5
γ-Terpinene	1.4	n.r.	4.5	3.2
α-Terpinolene	0.6	n.r.	1.8	1.2

n.r., Not reported as individual components.
[a] Data from Ref. [15], single leaf cuvette, laboratory conditions.
[b] Data from Ref. [27], branch cuvette, field conditions.
[c] Data from Ref. [28], branch cuvette flushed with ambient air, field conditions.

respectively, are reported. They were obtained by recording the total ion current (TIC) of all fragments with mass to charge ratios (m/z) ranging from 20 to 250. Data in Fig. 3a show that chiral chromatography combined with MS detection allowed to identify and quantify all enantiomeric monoterpenes in the presence of high amounts of sesquiterpene compounds. The resolution of the β-cyclodextrin column was good enough to prevent substantial overlapping of monoterpenes with higher molecular mass isoprenoids and to allow the collection of clear spectra of both mono- and sesquiterpenes. In spite of this, it was impossible to derive the enantiomeric signature of sesquiterpenes because of the large number of pure components needed for positive identification.

The possibility to detect the enantiomeric signature of monoterpenes in samples where isoprene was the dominant component is shown, instead, in Fig. 3b. It is worth nothing that the trace level determination of chiral monoterpenes in the emission of *Eucalyptus globulus* was possible only with the use of multistage traps filled with graphitic sorbents. Since up to 5 l of air can be enriched on these traps without substantial losses of isoprenoids from the carbon adsorbents [21] they were also suitable for measuring the enantiomeric signature of monoterpenes in atmospheric samples where individual constituents are present at levels of pptv.

The high resolving power exhibited by the β-cyclodextrin column was confirmed by further experiments carried out on plants characterized by a large complexity in monoterpene emissions. The examples reported in Fig. 4a and b, serve well to illustrate this point. They show the mass chromatographic profiles of monoterpene emission from *Pinus radiata* D. Don. and *Pinus pinea* L. obtained by plotting the ion current from the fragment with m/z 93. By combining the data of previous figures with those collected by screening additional plant species, it was found that capillary chromatography on columns coated with β-cyclodextrin allowed an accurate determination of the enantiomeric pairs of α-pinene, sabinene, camphene, Δ-3-carene, β-pinene, limonene, β-phellandrene and 4-terpineol in all vegetation samples and in the atmosphere. Reliable information could have also been obtained in the case of linalool and α- and γ-terpineol if the enantiomeric pairs of these components were present in comparable amounts in plant emission or in the atmosphere.

Table 3 summarizes the information obtained by submitting different types of gaseous samples to GC–MS analysis. Data refer to the gaseous emission from terrestrial plants and pine vegetation deposited on soil and to atmospheric samples collected during daytime hours in the suburban area of Montelibretti.

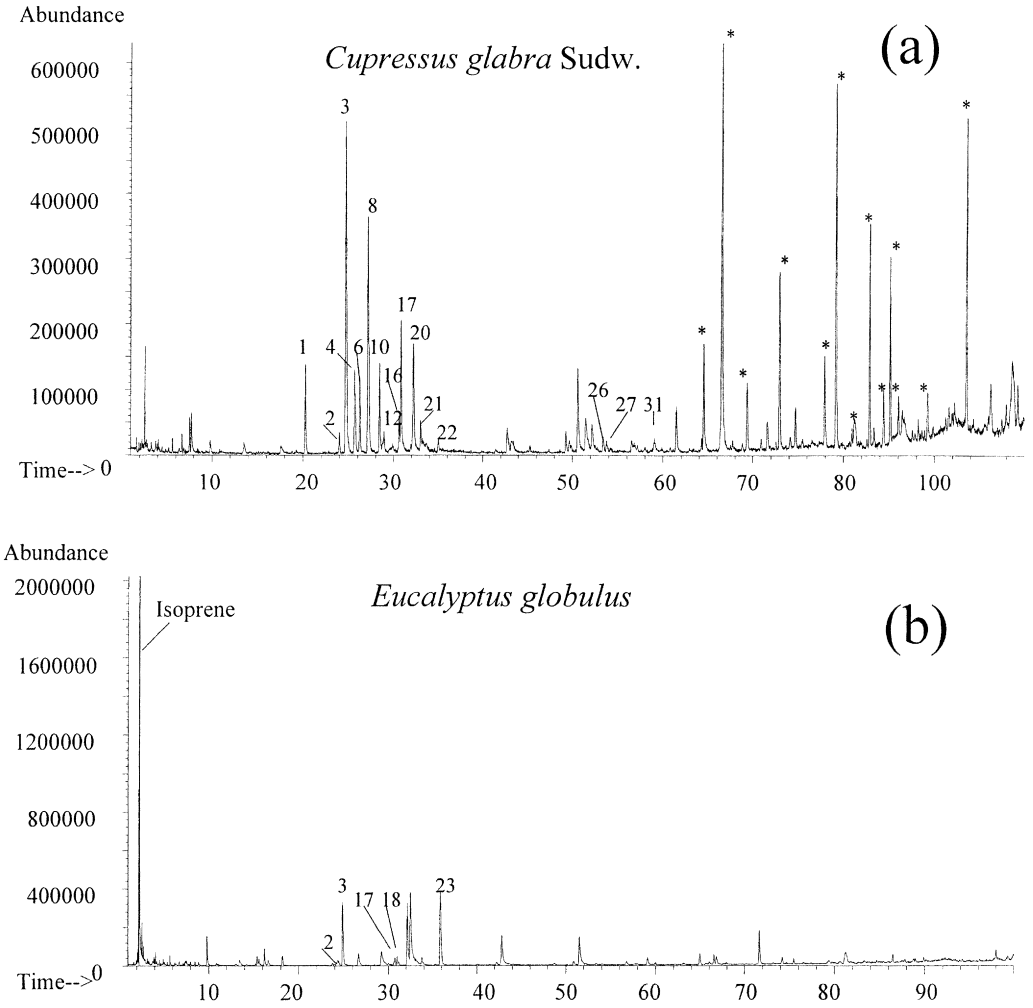

Fig. 3. Total ion current profiles of VOCs present in the gaseous emission of: (a) a strong mono- and sesquiterpene emitter (*Cupressus glabra* Sudw.) and (b) a strong isoprene emitter releasing monoterpenes at trace levels (*Eucalyptus globulus*). Numbers in the figure refer to the components listed in Table 3; the asterisk identifies sesquiterpene compounds.

In the Table are reported the percent composition of individual monoterpenes present in each sample, the percent fraction of the (+)- and (−)-enantiomeric forms (when they exist) and the ER values. Numbers from 1 to 31 were assigned to enantiomeric monoterpenes on the basis of the retention time measured on the β-cyclodextrin column. No common trends were detected by looking at the enantiomeric ratios of individual monoterpenes emitted by terrestrial plants and litter-covered soil. The case of α-pinene illustrates well this point, although similar considerations apply to limonene, sabinene and β-pinene, also abundant in vegetation emissions. From Table 3 we can see that the ER values of α-pinene ranged from 0.08 in *Cedrus deodora* Loud. to 36.88 in *Cupressus glabra* Sudw. This behavior was somehow expected because each plant could use specific defense–attraction strategies as a function of the type of insect taxa the plant interacts with. Since plants belonging to the same family might use similar strategies to attract flying insects or defend themselves from herbivores we looked in more detail at the enantiomeric signatures of monoterpenes emitted by pine and cypress plants. It was found that all pine species investigated

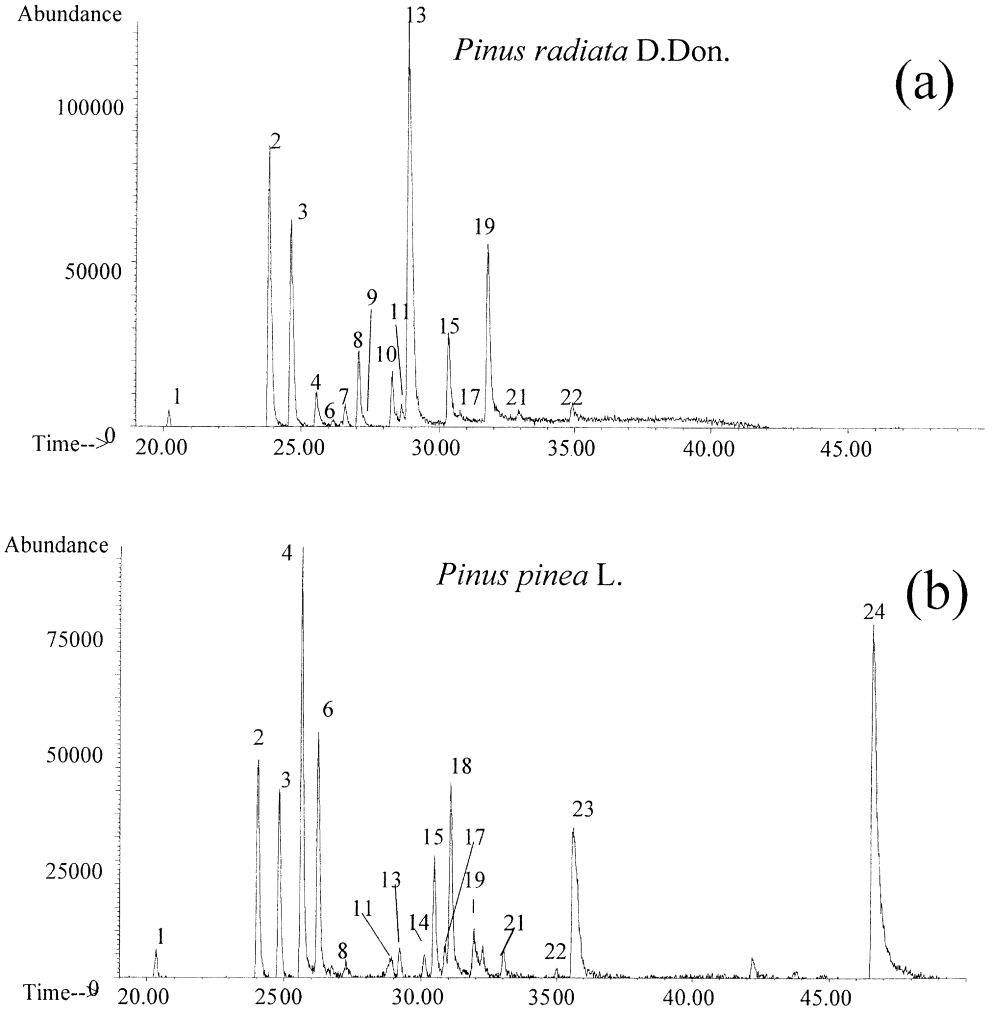

Fig. 4. Reconstructed mass chromatographic profiles (m/z 93) of monoterpenes present in the gaseous emission of (a) *Pinus radiata* D. Don. and (b) *Pinus pinea* L. Numbers in the figure refer to the components listed in Table 3.

showed a clear predominance of the (−)-enantiomers of α-pinene and limonene over the corresponding (+)-enantiomers, although one tree species was from north America (*Pinus radiata* D. Don.) and the other two (*Pinus pinaster* L. and *Pinus pinea* L.) were typical of southern Europe. Another common features of pine trees was the preferential emission of (+)-β-phellandrene with respect to the (−)-enantiomer. A species to species variability was, instead, observed in the enantiomeric ratios of β-pinene and sabinene. Distinctive features of southern European pines were the close consistency in the preferential emission of (−)-α-4-terpineol and (−)-linalool, that were lacking in the emission of the north American pine, and in the enantiomeric signature of sabinene. In terms of total monoterpene emission, the (−)-enantiomers accounted for ca. 70 to 90% of chiral components. Similar considerations can be made for cypress trees that showed, however, a preferential emission of the (+)-enantiomers of monoterpenes over the corresponding (−)-enantiomers. In the north American species (*Cupressus glabra* Sudw.) they accounted for 73% of total chiral monoterpenes whereas in the indigenous species a value of 90%

Table 3
Enantiomeric composition of monoterpenes in the emission of some terrestrial plants and in the open atmosphere

Peak No.	t_R (min)	Compound	*Quercus ilex* L.			*Pinus radiata* D. Don.			*Pinus pinaster* L.			*Pinus pinea* L.			Leaves of *Pinus radiata* D. Don. deposited on soil		
			%[a]	%[b]	ER[c]	%[a]	%[b]	ER[c]	%[a]	%[b]	ER[c]	%[a]	%[b]	ER[c]	%[a]	%[b]	ER[c]
1	20.379	α-Thujene	1.0	–	–	0.5	–	–	0.04	–	–	0.35	–	–	n.d.	–	–
2	24.022	(−)-α-Pinene	21.5	58.3	0.72	16.3	57.0	0.76	8.9	73.6	0.36	3.7	56.1	0.77	14.7	28.5	2.50
3	24.831	(+)-α-Pinene	15.4	41.7		12.3	43.0		3.2	26.4		2.9	43.9		36.8	71.5	
4	25.728	Myrcene	6.1	–	–	2.9	–	–	12.7	–	–	9.7	–	–	0.64	–	–
5	25.854	Tricyclene	0.6	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–
6	26.344	(+)-Sabinene	4.3	37.4	0.60	0.21	5.2	0.06	1.4	92.1	11.67	4.5	95.1	19.57	n.d.	–	–
8	27.299	(−)-Sabinene	7.2	62.6		3.8	94.8		0.12	7.9		0.23	4.9		n.d.	–	
7	26.839	(+)-Camphene	0.9	42.9	0.75	1.6	69.6	2.27	0.27	100	–	n.d.	–	–	1.1	64.0	1.77
9	27.420	(−)-Camphene	1.2	57.1		0.7	30.4		n.d.	–		n.d.	–		0.62	36.0	
10	28.530	(+)-Δ-3-Carene	n.d.	–	–	2.5	100	–	n.d.	–	–	n.d.	–	–	n.d.	–	–
12	28.940	(−)-Δ-3-Carene	n.d.	–		n.d.	–	–	n.d.	–		n.d.	–		n.d.	–	
11	28.860	(+)-β-Pinene	9.1	39.1	0.64	1.2	3.1	0.03	0.36	3.5	0.04	0.53	53.0	1.13	0.88	2.0	0.02
13	29.216	(−)-β-Pinene	14.2	60.9		37.4	96.9		10.0	96.5		0.47	47.0		43.4	98.0	
14	30.173	*cis*-β-Ocimene	10.2	–	–	n.d.	–	–	n.d.	–	–	0.37	–	–	n.d.	–	–
15	30.577	(−)-Limonene	0.6	40.0	1.50	7.9	89.8	0.12	9.6	88.9	0.13	3.0	80.4	0.24	0.75	72.1	0.39
17	30.957	(+)-Limonene	0.9	59.0		0.9	10.2		1.2	11.1		0.73	19.6		0.29	27.9	
16	30.697	*p*-Cymene	0.4	–	–	n.d.	–	–	0.19	–	–	0.35	–	–	0.07	–	–
18	31.172	*trans*-β-Ocimene	3.2	–	–	n.d.	–	–	n.d.	–	–	4.5	–	–	n.d.	–	–
19	32.023	(+)-β-Phellandrene	0.9	100	–	10.3	100	–	0.91	72.2	2.60	0.63	100	–	0.68	100	–
20	32.360	(−)-β-Phellandrene	n.d.	–		n.d.			0.35	27.8		n.d.	–		n.d.	–	
21	33.113	γ-Terpinene	1.4	–	–	0.5	–	–	0.11	–	–	0.41	–	–	n.d.	–	–
22	35.081	α-Terpinolene	0.6	–	–	0.9	–	–	0.16	–	–	n.d.	–	–	n.d.	–	–
23	35.830	1,8-Cineole	n.d.	–	–	n.d.	–	–	9.9	–	–	33.3	–	–	n.d.	–	–
24	46.810	(−)-Linalool*	n.d.	–	–	n.d.	–	–	37.0	100	–	24.3	100	–	n.d.	–	–
25	47.221	(+)-Linalool*	n.d.	–		n.d.	–		n.d.	–		n.d.	–		n.d.	–	
26	53.915	(−)-4-Terpineol	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–
27	54.242	(+)-4-Terpineol	n.d.	–		n.d.	–		n.d.	–		n.d.	–		n.d.	–	
28	57.613	(+)-α-Terpineol	n.d.	–	–	n.d.	–	–	0.42	12.1	0.14	1.2	12.0	0.14	n.d.	–	–
30	57.912	(−)-α-Terpineol	n.d.	–		n.d.	–		3.04	87.9		8.8	88.0		n.d.	–	
29	57.660	(+)-γ-Terpineol	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–
31	58.041	(−)-γ-Terpineol	n.d.	–		n.d.	–		n.d.	–		n.d.	–		n.d.	–	

Table 3. Continued

Peak No.	t_R (min)	Compound	*Cupressus glabra* Sudw.			*Cupressus sempervirens* L.			*Eucalyptus globulus*			*Cedrus deodora* Loud.			*Citrus aurantium* L.			Ambient air		
			%[a]	%[b]	ER[c]	%[a]	%[b]	ER[c]	%[a]	%[b]	ER[c]	%[a]	%[b]	ER[c]	%[a]	%[b]	ER[c]	%[a]	%[b]	ER[c]
1	20.379	α-Thujene	4.6	–	–	1.9	–	–	0.03	–	–	0.01	–	–	0.21	–	–	n.d.	–	–
2	24.022	(−)-α-Pinene	1.3	2.6	37.31	6.5	11.6	7.60	1.5	4.3	22.47	28.7	92.6	0.08	1.5	79.8	0.25	24.2	40.1	1.49
3	24.831	(+)-α-Pinene	48.5	97.4		49.4	88.4		33.7	95.5		2.3	7.4		0.38	20.2		36.1	59.9	
4	25.728	Myrcene	4.7	–	–	4.5	–	–	0.38	–	–	33.5	–	–	36.7	–	–	n.d.	–	–
5	25.854	Tricyclene	2.2	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–	1.6	–	–
6	26.344	(+)-Sabinene	3.6	20.9	0.26	9.2	96.8	30.67	n.d.	–	–	0.51	100	–	0.60	27.3	0.38	n.d.	–	–
8	27.299	(-)-Sabinene	13.6	79.1		0.30	3.2		n.d.	–	–	n.d.	–	–	1.6	72.7	–	n.d.	–	
7	26.839	(−)-Camphene	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–		n.d.	–		6.2	49.6	0.98
9	27.420	(−)-Camphene	n.d.	–		0.22	100		n.d.	–		n.d.	–	–	n.d.	–	–	6.3	50.4	
10	28.530	(+)-δ-3-Carene	3.8	66.7	2.00	22.4	92.9	13.18	n.d.	–	–	1.0	3.1	0.03	n.d.	–	–	3.5	100	–
12	28.940	(−)-δ-3-Carene	1.9	33.3		1.7	7.1		n.d.	–		31.1	96.9		n.d.	–		n.d.	–	
11	28.860	(+)-β-Pinene	n.d.	–	–	n.d.	–	–	0.22	32.4	0.48	n.d.	–	–	11.5	100	–	n.d.	–	–
13	29.216	(−)-β-Pinene	n.d.	–		n.d.	–		0.46	67.6		n.d.	–		n.d.	–		3.1	100	
14	30.173	*cis*-β-Ocimene	n.d.	–	–	n.d.	–	–	0.46	–	–	n.d.	–	–	9.6	–	–	n.d.	–	–
15	30.577	(−)-Limonene	0.09	1.4	71.11	0.20	16.7	5.00	1.1	26.2	2.82	0.74	67.3	0.49	3.4	23.8	3.21	5.4	45.8	1.19
17	30.957	(+)-Limonene	6.4	98.6		1.0	83.3		3.1	73.8		0.36	32.7		10.9	76.2		6.4	54.2	
16	30.697	*p*-Cymene	1.5	–	–	0.62	–	–	3.5	–	–	0.10	–	–	0.18	–	–	7.2	–	–
18	31.172	*trans*-β-Ocimene	n.d.	–	–	n.d.	–	–	1.9	–	–	n.d.	–	–	19.0	–	–	n.d.	–	–
19	32.023	(+)-β-Phellandrene	0.36	6.0	0.06	0.66	77.6	3.47	n.d.	–	–	1.7	100	–	n.d.	–	–	n.d.	–	–
20	32.360	(−)-β-Phellandrene	5.6	94.0		0.19	22.4		n.d.	–		n.d.	–		n.d.	–		n.d.	–	
21	33.113	γ-Terpinene	1.4	–	–	0.55	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–
22	35.081	α-Terpinolene	0.33	–	–	0.52	–	–	n.d.	–	–	n.d.	–	–	1.9	–	–	n.d.	–	–
23	35.830	1,8-Cineole	n.d.	–	–	n.d.	–	–	53.1	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–
24	46.810	(−)-Linalool*	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–	2.4	100	–	n.d.	–	–
25	47.221	(+)-Linalool*	n.d.	–		n.d.	–		n.d.	–		n.d.	–		n.d.	–		n.d.	–	
26	53.915	(−)-4-Terpineol	0.03	37.5	1.67	0.02	100	–	0.10	55.6	0.80	n.d.	–	–	n.d.	–	–	n.d.	–	–
27	54.242	(+)-4-Terpineol	0.05	62.5		n.d.	–		0.08	44.4		n.d.	–		n.d.	–		n.d.	–	
28	57.613	(+)-α-Terpineol	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	–
30	57.912	(−)-α-Terpineol	n.d.	–		n.d.	–		n.d.	–	–	n.d.	–	–	n.d.	–	–	n.d.	–	
29	57.660	(+)-γ-Terpineol	n.d.	–	–	n.d.	–	–	0.07	21.9	0.28	n.d.	–	–	n.d.	–	–	n.d.	–	–
31	58.041	(−)-γ-Terpineol	0.02	100		n.d.	–		0.25	78.1		n.d.	–	–	n.d.	–	–	n.d.	–	

[a] Relative content of individual monterpenes in the monoterpene fraction.

[b] Relative content of enantiomeric pairs.

[c] Ratio of the content of the (+)-enantiomer with respect to the (−)-enantiomer (ER).

*Data affected by a large uncertainty due to the limited resolution between the enantiomeric pairs.

was reached. In both species, (+)-α-pinene, (+)-Δ-3-carene, (+)-limonene were the components contributing most to the enantiomeric signature of monoterpene emission. The close similarities between plants belonging to the same family, if confirmed, can add an additional impulse to recent studies in which the chemical signature of isoprenoid emission has been used for assessing the evolutionary aspects of various plant taxa [29,30]. However, the example reported in Fig. 2a opens the question if the enantiomeric signature of monoterpenes is sufficient for taxonomical and ecological studies in those case where substantial emission of enantiomeric sesquiterpenes takes place. It is worth noting, here, that among the four plant species showing a preferential emission of the (+)-enantiomers of monoterpenes, three of them (namely *Cupressus glabra* Sudw., *Cupressus sempervirens* L. and *Citrus aurantium* L.) were strong sesquiterpene emitters whereas the last one (*Eucalyptus globulus*) was basically an isoprene emitter. It is thus possible that the dominance of (+)-enantiomers of monoterpenes in cypress and orange plants is only a part of the complex attraction–defense strategies available to these plants and a major role is played by enantiomeric sesquiterpenes. The possibility that these plants exhibit a chiral signature of the total isoprenoid emission not different from that of the pines, the cedar and the holm oak cannot be ruled out until a detailed analysis of enantiomeric sesquiterpenes is performed. The profile of Fig. 2a shows that these components are eluted by the column and a positive identification of major chiral components could be attempted providing that enough standards compounds are available.

Data reported in Table 3 also show that the proposed method is suitable for determining the enantiomeric signature of monoterpenes in atmospheric samples where biogenic VOCs account for a small fraction (<10%) of the total organic content and they are present at levels of few pptv [20 pptv for (+)-enantiomer of α-pinene in the sample reported in Table 3]. Previous investigations carried out in Montelibretti have shown that transport of anthropogenically polluted air masses from the urban area of Rome is the main source of VOCs in our site [31] and that local sources are not strong enough to explain the content of monoterpenes in air. It cannot be excluded, therefore, that exogenous sources contributed to a large extent to the enantiomeric ratios of α- and β-pinene in these samples. Although the GC profile was dominated by alkanes, alkenes, arenes and oxygen and halogen-containing VOCs, the resolution was good enough to allow the identification and quantification of enantiomeric monoterpenes by GC–MS. The method can thus be used in complex ecosystems to assess the contribution of different vegetation species to the total input of monoterpenes in the atmospheric boundary layer or to follow the degradation pathways of biogenic VOCs in the atmosphere whenever the chirality of emitted compounds (both gases or aerosols) is maintained in the secondary products. At the present time, it is hard to envision if the monitoring of chiral monoterpenes in air can contribute to improve the present knowledge of atmospheric processes because it is not known to what extent the chirality of precursors can be preserved in the various secondary products formed by the reaction of VOCs with ozone, OH radicals and NO_3 radicals.

4. Conclusions

The combined use of adsorption traps, thermal desorption and GC–MS on β-cyclodextrin capillary columns has made possible the positive identification and quantitative determination of enantiomeric monoterpenes in terrestrial plant emission and, for the first time, in the atmosphere. Enantiomeric ratios of α-pinene, sabinene, camphene, Δ-3-carene, β-pinene, limonene, β-phellandrene, linalool, 4-terpineol, α-terpineol and γ-terpineol were determined in emission samples of different evergreen species common in Europe and in the North America. They cover a wide range of isoprenoid composition. A marked dominance of the (−)-enantiomers was observed in five of the nine species investigated. None of them was emitting sesquiterpenes. Preferential emission of the (+)-enantiomers was found in four plant species. Three of them were also strong sesquiterpene emitters whereas the last one was a strong isoprene emitter. Plant species belonging to the same families showed similar enantiomeric signatures of monoterpenes. The proposed method is

suitable for the monitoring of chiral monoterpenes in the atmosphere. This approach might be useful for the apportionment of biogenic sources in complex ecosystems and for better assessing the chemical transformation of biogenic VOCs in the atmospheric environment.

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