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Multidimensional analysis of Cannabis volatile constituents: identification of 5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane as a volatile marker of Hashish, the resin of *Cannabis sativa* L.

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Abstract: The volatile constituents of drug samples derived from *Cannabis sativa* L. were investigated by means of headspace solid phase microextraction (HS-SPME) and gas chromatography techniques (GC-MS, GC × GC-MS). Samples of cannabis herb and hashish showed clear differences in their volatile chemical profiles, mostly resulting from photo-oxidation processes occurring during the transformation of fresh cannabis herb into hashish. Most unexpectedly, we could demonstrate hashish samples as containing remarkable amounts of a rare and unusual monoterpene—5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane—among the volatile compounds detected in their headspaces. We gave evidence for the formation of this compound from the light induced rearrangement of β-myrcene during the manufacture of hashish. In view of its high abundance among volatile constituents of cannabis resin and its scarce occurrence in other natural volatile extracts, we propose to rename this specific monoterpene hashishene.

Keywords: *Cannabis sativa* L., hashish, volatile marker, myrcene rearrangement, photo-oxidation processes.

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1. Introduction

Among all drugs of abuse, cannabis remains the most consumed worldwide under all of its various forms (marijuana, hashish, cannabis oil). Cannabis is particularly popular in western countries owing to its low price compared to other common illegal drugs, and it is commonly considered as a drug of lower impact since discussions about bringing it to legality are still ongoing in some European countries. The resin of *Cannabis sativa* L. known as hashish is a product of interest for drug dealers because of its malleability that allows easy concealment and transportation, and its high content of psychoactive principle (THC) compared to the raw herb. It is produced in various countries around the world but the main producers are undoubtedly Morocco and Afghanistan [1-4]. Hashish is produced via a relatively long cottage industry process that consists in successively drying, sieving, and finally pressing the resin-rich buds of cannabis plants that have been harvested at full blooming stage. It results in a medium hard greenish-brownish paste that softens upon heating. The demand for hashish is still high in Europe, making its illegal market a major concern for European authorities, requiring more and more means for efficient traffic control.

Research of illegal drugs by customs squads at borders or during police controls usually requires canine assistance for detection of target substances. The use of trained dogs is currently the most efficient mean for rapid drug detection but has a certain drawback since a dog provides a working time limited to a few hours a day. Moreover, the high cost of a canine team is also the reason why people have long sought to replace, or rather say, complement detection dogs by the use of electronic devices designed for the specific detection of illicit substances such as drugs and explosives. While different ways to carry out detection are possible, those addressing target-associated volatile organic compounds are more likely to mimic canine recognition processes [5-7], and doing so, to reach low detection limits, provided that the chosen analytical technology is sensitive enough, robust, and reliable. The identification of specific and stable-in-time volatile target markers is therefore of crucial importance in this field of technological applications.

In this frame, we undertook the chemical investigation of different forms of cannabis (fresh and dried herb, and hashish) in order to search for potential candidates intended to trigger remote detection of cannabis derived products. The volatile constituents of *Cannabis sativa* L. have been explored since the early developments of gas chromatography [8]. Nearly all studies published to date have reported the existence of a complex mixture of mono- and

sesquiterpenic derivatives. Surprisingly, while the headspace chemical composition of cannabis herb is now well documented, hashish has received much less attention. We therefore want to report on a multi-directed parallel analysis of various forms of cannabis by means of headspace sampling methods and hyphenated GC techniques (GC-MS, GC × GC-MS). Our investigations led us to observe noticeable differences between cannabis herb and hashish samples, and eventually, to identify a new volatile marker in hashish.

2. Experimental

2.1. Chemicals and products

Samples of resins and herb of *Cannabis sativa* L. were obtained from the *French Police Forensic Institute (INPS, Ecully - France).* All chemicals and solvents were purchased from Sigma-Aldrich (Illkrich, France) and used without further purification otherwise stated.

2.2. Extraction of hashish and isolation of the terpenic fraction

15 g of hashish (probably originating from Morocco[1]) were extracted in a 200 mL Soxhlet apparatus with 250 mL of pentane for 5 hours. The resulting extract (~10 g) mainly contained cannabinoids and was subsequently chromatographed on silica gel with eluents of increasing polarity. The first fraction (1.1 g), eluted with pentane, contained mostly monoterpenes and sesquiterpenes, accompanied with a limited series of diterpenes. In this fraction, compound **11** accounted for ~4.5% of the total chromatogram area, as determined by GC-MS analysis.

2.3. SPME analysis

100% Polydimethysiloxane (PDMS; 100 µm) and Divinylbenzene/Carboxen/Polydimethyl-siloxane (DVB/CAR/PDMS; 50/30 µm) SPME fibers were purchased from Supelco Europe. Optimized parameters for SPME analyses (incubation/extraction time/temperature, nature of the fiber coating) were selected following an experimental design inspired from a methodology described by Vanot and Sergent [9].

[1] internal communication from INPS

2.4. Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were run on two GC-MS systems. For SPME analyses, gas chromatographs were operated in splitless mode with a constant flow (1 mL/min) of helium. For other routine analyses, GC systems were operated in split mode (1/50). At UNS, an Agilent 6890N/5973N GC-MS system was equipped with a J&W HP-1 capillary column (50 m × 0.2 mm; 0.33 µm film thick.) and operated as follows: initial oven temperature, 50 °C, then increased to 250 °C with a constant rate of 2 °C/min. At INPS, an Agilent 7890A/5975C system was mounted with a Varian VF-1MS capillary column (50 m × 0.25 mm; 0.25 µm film thick.) and operated as follows: initial oven temperature, 60 °C, then increased to 250 °C with a constant rate of 3 °C/min. For both systems, the transfer-line temperature was set at 280 °C. Electron impact mass spectra were acquired over a 35-350 m/z range. Volatile constituents were identified upon cross-correlation of their retention indices (RI) calculated from a series of *n*-alkanes, and their mass spectra matched against commercial librairies (Wiley275, NIST08, MassFinder 2.1) or in-house MS databases built from literature information [10,11] and isolated or synthesized substances whenever possible.

2.5. Comprehensive two-dimensional gas chromatography-mass spectrometry (GC × GC-MS)

GC × GC-MS analyses were performed using an Agilent 6890N/5973N GC-MS system equipped with a GC × GC ZOEX kit (cryogenic dual jet/loop modulator and a secondary oven installed in the main GC oven). The two-dimensional GC column set was composed of a Varian VF-5MS (30 m × 0.25 mm; 0.2 µm film thick.; referred to as column 1) and a J&W DB-Wax (1.25 m × 0.10 mm; 0.1 µm film thick.; referred to as column 2) coupled through a deactivated fused silica capillary tube (1.25 m × 0.10 mm) installed as a loop passing twice the cryogenic modulator. Column flow (He) 0.8 mL/min; Splitless injection mode; temperature program for oven #1: 50 °C (5 min isotherm.) to 250 °C at 4 °C/min (20 min isotherm.); temperature program for oven #2: 60 °C (5 min isotherm.) to 252 °C at 8 °C/min (46 min final isotherm.). Modulation time, P_M = 5.5 s. The mass spectrometer was operated at 70 eV in fast scan mode (10 000 amu/s) over a 50–250 m/z range, giving a 24.67 Hz acquisition rate. Volatile constituents were identified upon cross-correlation of their retention

indices calculated from a series of *n*-alkanes, and their mass spectra matched against commercial libraries (Wiley275, NIST08) or in-house MS databases built from literature information, and isolated or synthesized substances whenever possible.

2.6. Enantioselective gas chromatography

Separation of the two enantiomers of 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane **11** was carried out using a Macherey-Nagel Hydrodex β-6TBDM capillary column mounted on a Agilent 6890N/5973N GC-MS system. Split ratio 1/100; Oven temperature program: 80 °C to 220 °C at 2 °C/min. Constant flow of helium: 1.0 mL/min. MS parameters were used as described above for SPME experiments.

*2.7. UV-mediated synthesis of 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane **11***

Compound **11** was synthesized according to a protocol inspired from Liu and Hammond [12]. Typically, a mixture of β-myrcene (1.63 g; 12 mmol) and 2-acetylnaphtalene (23 mg) was dissolved in 70 mL of dry diethylether and placed in reactor system equipped with a medium pressure UV lamp (Heraeus, Germany). The mixture was irradiated under argon atmosphere until reaching total consumption of β-myrcene as monitored by GC analysis of aliquots withdrawn from the reaction mixture. The solvent was carefully removed under vacuum, and the crude product was obtained as nearly colorless oil. (crude yield > 90%; ~67% GC purity). The reaction was repeated several times and all crude products combined in order to increase the amount of material for distillation. A mixture of crude reaction products (12.5 g) was submitted to distillation under reduced pressure. Compound **11** (6.5 g) was isolated with 87% purity (bp 50 °C under 0.3 mbar) and subsequently separated by column chromatography with $AgNO_3$-impregnated silica gel for structural elucidation by NMR (see Figures S2-7 in supporting information).

2.8. Nuclear magnetic resonance

High resolution NMR spectra were recorded in $CDCl_3$ at 298 K on a Bruker Avance DRX 500 spectrometer operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. In order to increase sensitivity, ^{13}C-NMR spectra such as broadband-^{13}C and JMOD were run with a direct probe head (5 mm PADUL ^{13}C-1H Z-GRD). 1D- and 2D-NMR spectra such as 1H, COSY, NOESY, HSQC, HMBC were run with an inverse probe head (5 mm PHTXI ^{1}H-$^{13}C/^{15}N$ ZGRD). Spectrum calibration was performed by using $CDCl_3$ signal as internal

reference (7.26 ppm for ^{1}H, 77.16 ppm for ^{13}C). Chemical shifts (δ) are expressed in parts per million (ppm) and coupling constants (*J*) in hertz. All NMR experiments were carried out using pulse sequences supplied by the spectrometer manufacturer (Bruker Topspin™) and processed via Mestrelab MestreNOVA software (v6.0.2-5475). All spectra are given as supplementary data.

3. Results and discussion

3.1. Headspace sampling of volatile constituents

In relation with its psychotropic activity, *Cannabis sativa* L. has been mainly studied for the determination of its content in cannabinoids. A large variety of molecules has been reported within this family [13,14], and the most representative are cannabidiol (CBD), cannabinol (CBN), and Δ^{9}-tetrahydrocannabinol (Δ^{9}-THC) which is known as the main psychoactive principle of the drug [15]. In comparison with cannabinoid derivatives, the volatile constituents of cannabis have received much less attention. Nevertheless, the chemical composition of the volatile fraction of cannabis derived products is well documented and has been mainly established through the analysis of their essential oils [16-25] or by means of headspace sampling techniques [25-29]. Hence, a large variety of volatile compounds representing all chemical classes (hydrocarbons, alcohols, ketones, esters) has been detected in cannabis samples [20]. Some authors also attempted to establish a chemical profile for each form of cannabis, and eventually determined a more or less steady odour that could be used as a chemical basis for detection of cannabis in the frame of criminal investigations [21]. Most of the time, those studies reported a high content of monoterpenes and sesquiterpenes. Among the monoterpene family, α- and β-pinene, β-myrcene, and limonene were the most cited instances, while β-caryophyllene and α-humulene were reported as predominant in sesquiterpenes. A quick overview of the main terpenoids reported as volatile constituents of *Cannabis sativa* L. is given in Table S1 (see supporting information).

Indeed, the chromatographic profile of a cannabis sample strongly depends on 1) its nature and 2) the method applied for extraction of its volatile constituents. Essential oils extracted from fresh cannabis herb were previously reported to show a chemical composition qualitatively similar to that obtained from air-dried herb, but that drying resulted in a loss of the most volatile compounds, particularly affecting the content in monoterpenes [21].

176 Moreover, the direct comparison of the chemical composition of a cannabis essential oil with
177 that of a headspace sample is not often straightforward since thermolabile or water-sensitive
178 constituents might be dramatically affected by hydrodistillation [25,26].

179 Consequently, methods involving headspace sampling appear as the most opportune option to
180 investigate cannabis samples in order to obtain representative profiles of their volatile
181 constituents, but also allow avoiding interferences potentially brought by the predominant
182 cannabinoids in the resulting chromatograms. Headspace sampling is usually carried out by
183 using gas-tight syringes, sorbent materials placed in glass/metal tubes for purge-and-trap, or
184 SPME fibers. Direct headspace sampling and purge-and-trap methods actually give a
185 representative profile of the volatile constituents emitted by a sample but usually require
186 sampling large volume of gas, and involve the use of specific analytical equipment
187 (headspace autosampler, large volume injectors, thermodesorption units, PTV). In contrast,
188 HS-SPME allows an easier analytical setup since it can be run on any GC system with
189 minimal modifications. However, the chromatographic profiles obtained after SPME actually
190 depend on the nature of the fiber selected for extraction, and other parameters such as
191 temperature and time allowed for sample incubation/extraction, so that an optimization step is
192 often necessary to define the best conditions prior to GC analysis.

193 In our case, this optimization step was carried out by following a simple design of experiment
194 (DoE) according to a protocol inspired by Vanot and Sergent [9]. We applied this procedure
195 to the analysis of hashish samples in order to define the best operating conditions for
196 extraction of their volatile constituents. We decided to evaluate the influence of five
197 parameters at two different levels (Table S2-4; see supporting information). It resulted in the
198 Hadamard matrix presented in Table S3. The results of the experimental design enlightened
199 the crucial influence of the temperature applied for incubation and extraction (X3) (Table S4).
200 Hence, an increase of temperature generally resulted in higher signal abundance (Fig. 1a), and
201 not surprisingly, in conjunction with a longer time of extraction (X5), sesquiterpenes were
202 predominantly concentrated on the fiber (Fig. 1b). In order to be compliant with remote
203 sensing scenarii, and because the most volatile constituents are more likely to be detected in
204 real field measurements, we selected the parameters allowing for a better preconcentration of
205 monoterpenes. Consequently, the highest selectivities for monoterpene extraction were
206 obtained when analyses were performed at room temperature while the extraction time was
207 limited to 20 min (exp. 7 and 8). The nature of the fiber had here a limited effect as well as the
208 incubation time. Optimal conditions deduced from in Figure 1b were used to investigate

various cannabis samples. Thus, samples of resin and cannabis herb (1.0 g) were allowed to equilibrate in a 20 mL cap-sealed vial for 60 min at room temperature, then a 100 μm PDMS SPME fiber was used to perform the extraction of the volatile constituents at the same temperature for 20 min. Representative profiles for each form of cannabis are depicted in Figure 2.

3.2.Identification of an unusual monoterpene in hashish

In a first series of analyses (samples 20-24; Table 5), we observed that all hashish samples presented high amounts of an unusual and rare monoterpene **11** eluting well before α-pinene **13**. This compound was identified as 1-vinyl-5,5-dimethyl[2.1.I]bicyclohexane by its mass spectrum (Figure 3) available in only one commercial MS databank (MassFinder 4.0, Germany). It was rather surprising to find **11** in such high abundance among the volatile constituents of hashish samples since the natural occurrence of this compound was reported only once in the past as a minor constituent (<0.1%) of the essential oil of *Mentha cardiaca* L. [30].

In order to go in further details, a second series of analyses was performed with a higher number of samples at the forensic department of the French Institute of scientific police (INPS, Ecully, France). In total, 11 samples of hashish and 10 samples of cannabis herb (fresh and dried) were investigated by means of HS-SPME-GC-MS (Figure 4). In almost all hashish samples, compound **11** was detected among the most abundant volatile compounds present in the headspace, ranging from 1.1 to 14.9% (median value: 10.2 %), while it was detected either at trace levels or in low amounts in fresh and dried herb (Table 1).

Not surprisingly, the literature related to the GC-MS analysis of cannabis (essential oil and headspace constituents) does not contain any mention of **11** among all the previously identified volatile constituents. In contrast, other common monoterpenes (α-pinene **13**, β-pinene **24**, limonene **37**) and sesquiterpenes (β-caryophyllene **159** and α-humulene **168**) were frequently reported as abundant. Among them, β-myrcene **25**, a monoterpene widespread in essential oils, has often been cited as a major volatile constituent and has thus been proposed as specific marker of cannabis and marijuana [21,22,25]. As a matter of fact, among the samples of cannabis herb investigated in here, seven out of ten contained huge amounts of β-myrcene (19.5-28.7 %).

Interestingly, compound **11** was previously reported as a UV light-mediated rearrangement product of **25** when irradiated in presence of sensitizers such as 2-acetylnaphtalene, benzophenone, or clay-supported materials [12,31-33] (Figure 5). Yet the literature related to **11** is scarce since only 6 bibliographic references dealing mainly with its synthetic preparation can be found registered by the Chemical Abstract Service (as observed by using SciFinder) [12,31-34].

Quite obviously, the intriguing high abundance of **11** in hashish samples led us to assume that its presence was directly linked with the high abundance of **25** in samples of fresh cannabis herb. As already mentioned, hashish is manufactured according to a lengthy process involving repetitive steps of sieving and drying of the resin-rich female heads of cannabis plants. The process is carried out until the resin naturally aggregates, producing hashish after a final compression [2,4]. Any exposure to sunlight during those drying steps could therefore be reasonably considered responsible for the photolytic formation of **11** from **25**.

Several experiments were additionally performed to ensure that the presence of **11** was not due to any potential artifact related to the chosen analytical procedure. Therefore, 15 g of hashish were extracted in a Soxhlet apparatus by using dichloromethane as a solvent, then the concentrated extract was separated by column chromatography in order to obtain the hydrocarbon fraction. As observed by subsequent GC-MS analysis, **11** was detected among other apolar constituents such as mono-, sesqui-, and diterpenes (dimyrcene isomers essentially in the latter case). In addition, we could determine that **11** was present as a racemic mixture in this fraction by means of enantioselective GC-MS (Figure 6). It thus strongly supported the photolytic origin of **11**, where an enzymatic formation would have more likely induced some enantiomeric excess. Any attempts of isolation were unfortunately unsuccessful. Compound **11** was thus synthesized according to a protocol inspired by Liu *et al.*[12] in order to bring final evidence for its presence as a constituent of hashish (its complete characterization by 1D- and 2D-NMR is given as supplementary material to this article). Owing to the rare occurrence of **11** in natural extracts and its unexpected high abundance in hashish, we therefore propose to rename it hashishene.

3.3.Investigation of photo-oxidation volatile products

As described above, all hashish and herb samples released substantial amounts of mono- and sesquiterpene hydrocarbons in their headspace. In addition, all samples appeared as containing small amounts of oxygenated terpenoids in both series. Several of them were already detected and identified by GC-MS but the whole family could not be sufficiently investigated on the overall because of a generally high extent of coelution in this part of the chromatogram, associated with their low abundances in comparison with their hydrocarbon counterparts. We therefore aimed at using comprehensive two-dimensional gas chromatography-mass spectrometry (GC × GC-MS) in order to investigate in more detail the chemistry of hashish samples, especially regarding their composition in oxygenated derivatives. GC × GC brings several advantages compared to conventional GC, among which an increased sensitivity, a higher resolution and, eventually, the structured distribution of analytes on the 2D-plots are probably the most outstanding specific features of the technique [35-42]. Four different resins and one sample of cannabis herb were analyzed in our laboratory by means HS-SPME-GC × GC-MS. Parameters for HS-SPME were identical to those used for conventional GC-MS analysis. A comparison of the resulting two-dimensional chromatographic profiles is presented in figures 7 and 8. Both hashish and bud samples provided two distinct regions in their 2D-chromatogram, roughly corresponding to the distribution areas of monoterpenic and sesquiterpenic derivatives. A total of 186 constituents were identified in our samples (Table 2). The resulting chromatographic profiles were identical to those obtained by conventional GC-MS except that the number of identified constituents was greatly improved owing to the better resolution of the chromatograms. At first sight, hashish samples presented a greater diversity in oxygenated terpenes than cannabis herb (Figure 7). This was particularly observed in the monoterpene family where no less than 50 constituents were actually identified. Besides the common ketones and alcohols that appeared in both types of samples (linalool, α-fenchol, terpinen-4-ol, α-terpineol), hashish samples contained other minor oxygenated monoterpenes presenting higher abundances compared to the herb. Most of these alcohols, aldehydes or ketones could be structurally related to the hydrocarbons initially present in the herb. For instance, the two stereoisomers of 2-methyl-6-methylene-octa-3,7-dien-2-ol **55** and **64**, ipsdienol **79**, and 2-methyl-6-methylene-octa-1,7-dien-3-ol **86** are all derivatives of β-myrcene **25**. The same phenomenon was observed for limonene **37**, producing *trans*- and *cis*-mentha-2,8-dien-1-ol, **69** and **93**, *cis*- and *trans*-mentha-1(7),8-dien-2-ol, **109** and **122**, and *trans*- and *cis*-carveol **119** and **121**. From a general point of view, most of these oxygenated derivatives corresponded to secondary photo-oxidation products of the initial terpenes. In the presence of light and singlet oxygen, terpenes

are known to undergo photo-oxidation leading to the formation of allylic hydroperoxides (Figure 9) [43,44]. Since hydroperoxides are generally not stable-in-time, they will generate the corresponding alcohols by reductive loss of an oxygen atom. At some point, those alcohols may be further oxidized into aldehydes or ketones. By considering the possibility of an overall process of photo-oxidation occurring during the manufacture of hashish from cannabis, we could retrace the molecular relationships existing between volatile constituents of hashish and those present in cannabis herb. In view of the compounds identified by GC × GC-MS, a clear scheme of molecular transformations can now be pictured out (Figure 10).

Interestingly, GC × GC-MS gave us the opportunity to explore the sesquiterpene region in more details. While most of the commonly reported sesquiterpenes (Table S1) were detected, it was possible to observe that a complete series of caryophyllane/humulane derivatives was present among hashish volatile consituents. Hence, in addition to the frequently cited isocaryophyllene **156**, β-caryophyllene **159**, α-humulene **168**, and caryophyllene oxide **200**, we identified three more isomers of caryophyllene, **161**, **166**, and **169**, but also various (photo)-oxidation products. These compounds, somewhat rare in nature, were identified on the basis of their mass spectra, in all respects comparable with those of the literature (Figure S1; see supporting information) [11,43].

In addition to terpenic derivatives that accounted for more than 80% of the detected constituents, it was possible to notice the presence of other organic compounds commonly found in natural extracts such as linear alkanes, and fatty acids, esters or alcohols. Only a few phthalates could actually be identified but their presence was more likely attributed to the packaging used to wrap the resin (especially in case of hashish ovules; see hashish 24). Other traces of unknown organic pollutions were detected but could not be considered as indicative of any potential adulteration. On the overall, no significant contamination from any potential cutting agent was detected. It thus gives a clue about the actual composition of cannabis resin, while it is often believed that bad quality resins are often cut with undesired and/or harmful substances such as wax, grease, or even rubber. Although these experiments have been performed on a limited number of samples, the results obtained here are consistent with that of HS-SPME-GC-MS. Moreover, GC × GC brought us the opportunity to explore in more detail their volatile profiles, hence providing a deeper insight towards minor constituents.

Conclusion

The volatile constituents of *Cannabis sativa* L. have been widely studied in the past, and most studies have reported the predominance of mono- and sesquiterpenes. Our analytical investigations of cannabis herb and resin (hashish) led us to observe clear differences in the chemical composition of their headspaces. It appeared that most of the major terpenes present in fresh cannabis herb undergo various transformations during hashish manufacture, including isomerization, dehydration, cyclisation, and more specifically, photo-oxidation. By means of GC × GC-MS analysis, hashish samples were determined to contain a huge variety of oxygenated terpenes which could be related to the initial terpenes identified in fresh herb.

Most importantly, hashish samples were determined to contain significant amounts of a rare monoterpene, 1-vinyl-5,5-dimethyl[2.1.I]bicyclohexane, that we wish to rename hashishene. We demonstrated hashishene as being formed during the manufacture of hashish upon photolytic rearrangement of β-myrcene, a monoterpene that is particularly abundant in fresh cannabis herb. Owing to its rare natural occurrence in other essential oils and to its high abundance in hashish, this compound might thus prove useful as a hashish marker for remote-sensing applications.

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Note

The use of 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane as volatile marker of hashish has been submitted to patent protection (European patent application filed on 04/28/14 under the serial number EP 14305628.1). A supporting information file containing various spectroscopic data is available free of charge at the editor website.

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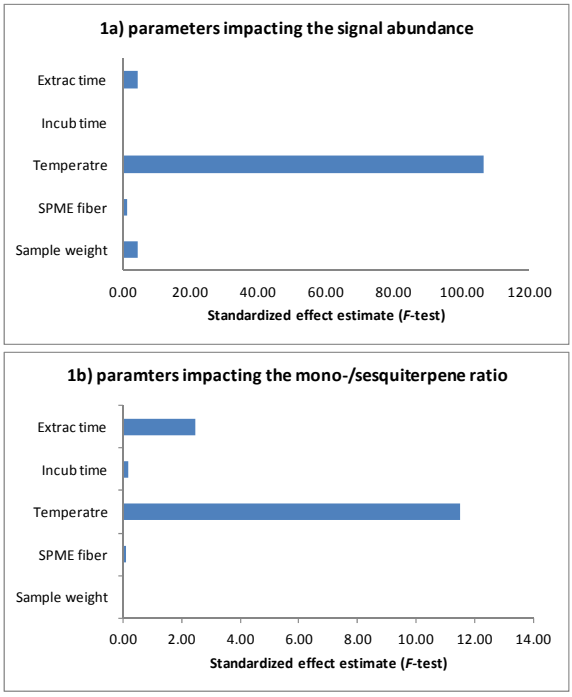

Fig. 1. Pareto charts depicting the influence of parameters studied during SPME optimisation in regard to the total signal abundance (1a) and the mono-/sesquiterpene ratio (1b). Caculation of *p*-value for temperature in each case gave 0.009 (1a) and 0.077 (1b), other parameters did not reveal significant influence with *p*-values > 0.1. (single column figure)

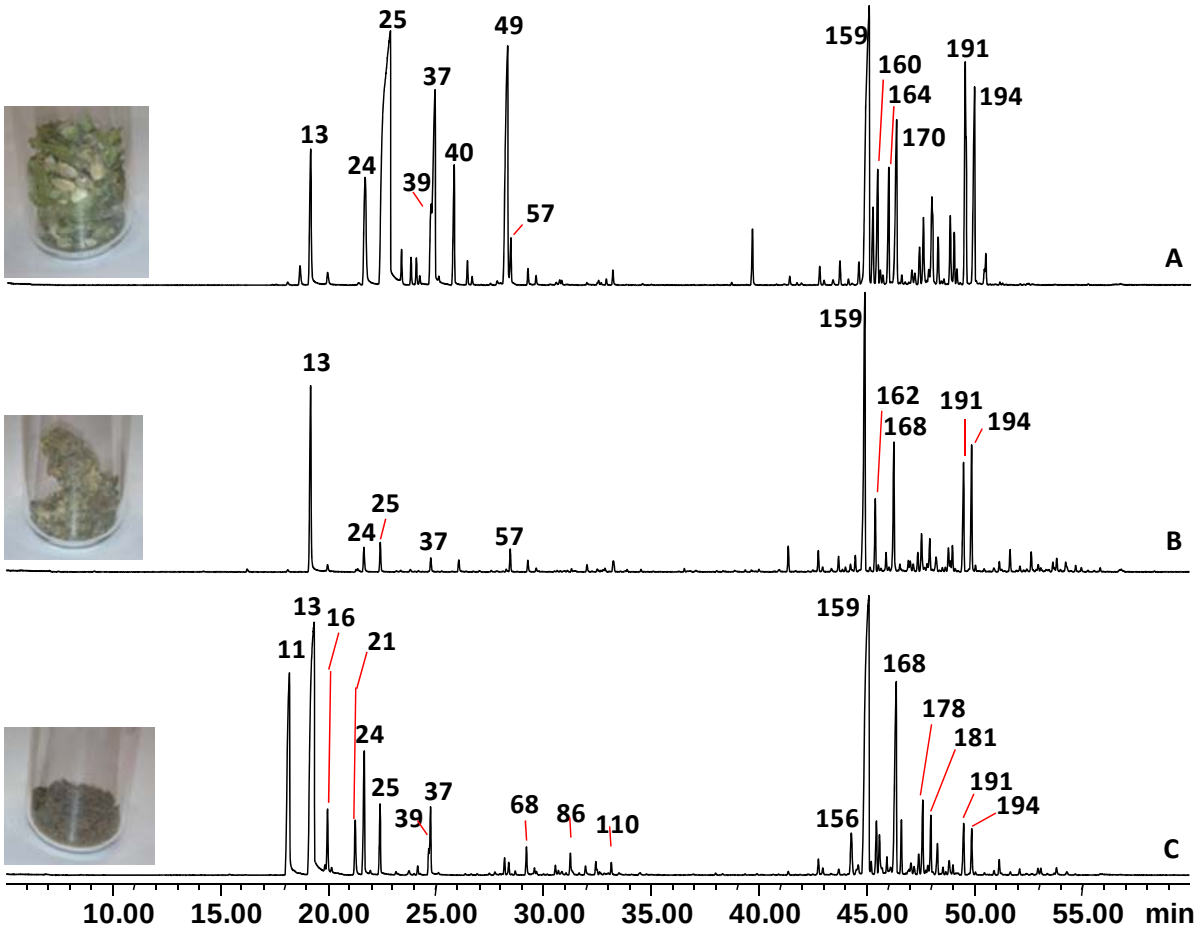

Fig. 2. Comparative HS-SPME-GCMS analyses of cannabis samples. A: fresh cannabis herb; B: dry herb; C: hashish. SPME was performed by using 100 µm PDMS fibers (Supelco). Compound numbers are given according to Tables 1 and 2. (double column figure)

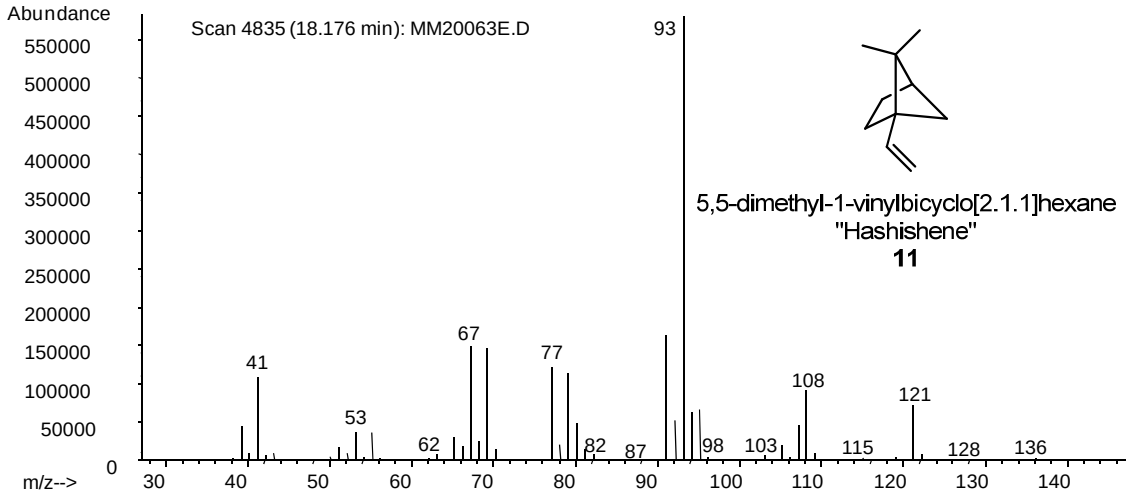

Fig. 3. Structure and EI-mass spectrum of 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane **11**.

(double column figure)

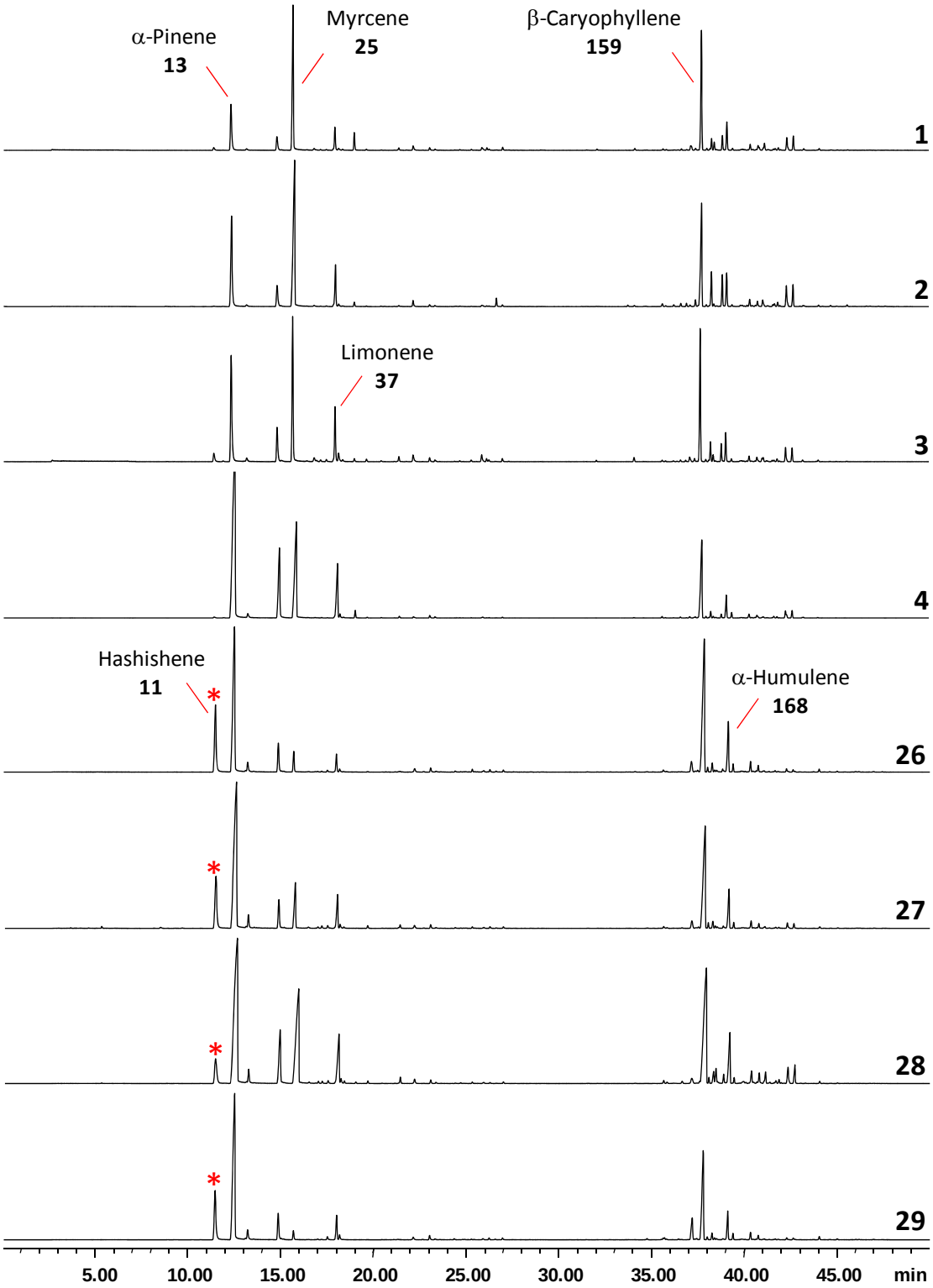

Fig. 4. Comparative HS-SPME-GCMS analyses of multiple samples of cannabis herb (1-4) and hashish (26-29) performed at the *Institut National de Police Scientifique* (Ecully, France). While several terpenes are common to all forms of cannabis, hashish samples are characterized by the presence of hashishene **11** eluting before α-pinene **13**. It is moreover interesting to notice how a great amount of myrcene **25** coincides with a smaller amount of hashishene **11** by simply comparing chromatographic profiles of hashish samples 26, 27 and 28. The complete description of their chemical composition is given in Table 5. (double column figure)

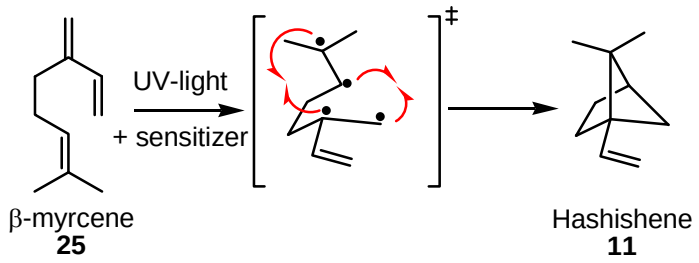

Fig. 5. UV-light mediated synthesis of hashishene **11** from β-myrcene **25**. (single column figure)

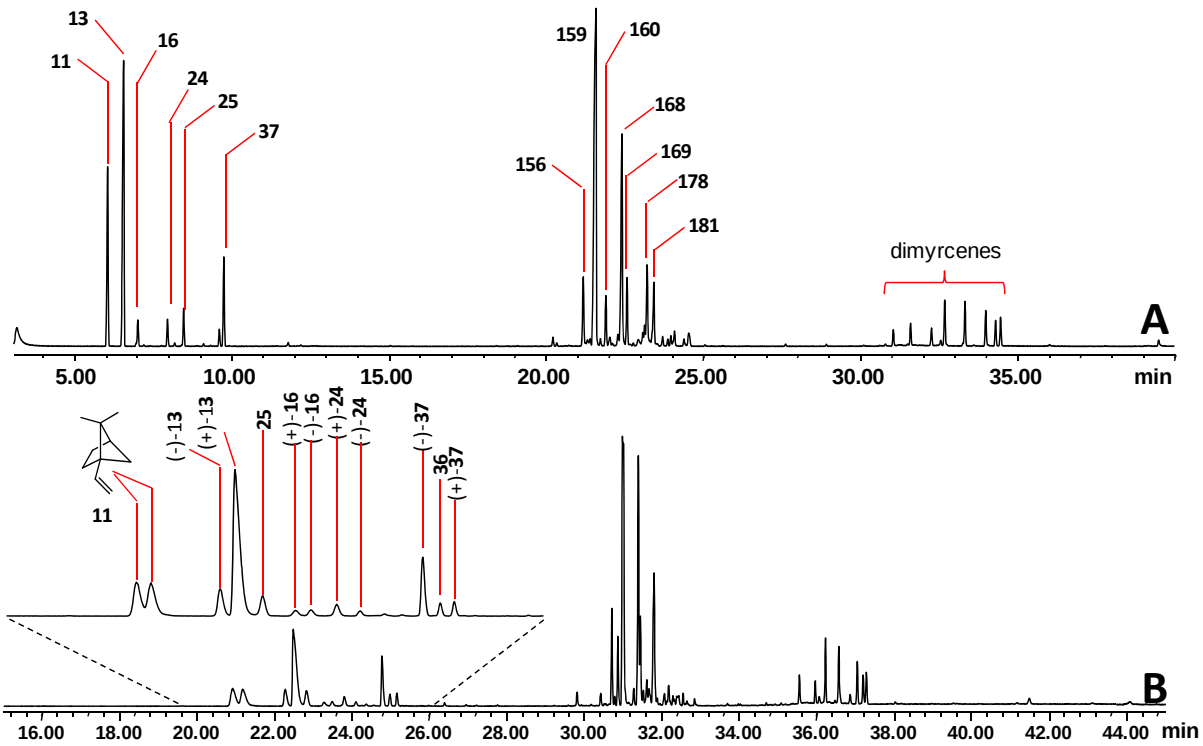

Fig. 6. GC-MS analysis of a hydrocarbon fraction obtained from hashish by Soxhlet extraction and subsequent flash column chromatography (A). Hydrocarbons were distributed in three main families including mono-, sesqui- and diterpenes (traces of *n*-alkanes could also be detected). The enantioselective GC-MS analysis of the same fraction (B) revealed that hashishene **11** was present as a racemic mixture (analysis performed on heptakis(2,3-di-*O*-methyl-6-*O*-tert-butyldimethylsilyl)-β-cyclodextrin; elution order is deduced from reference substances or literature data (see *J. Essent. Oil Res.*, **1996**, *8*, 487-497). (double column figure)

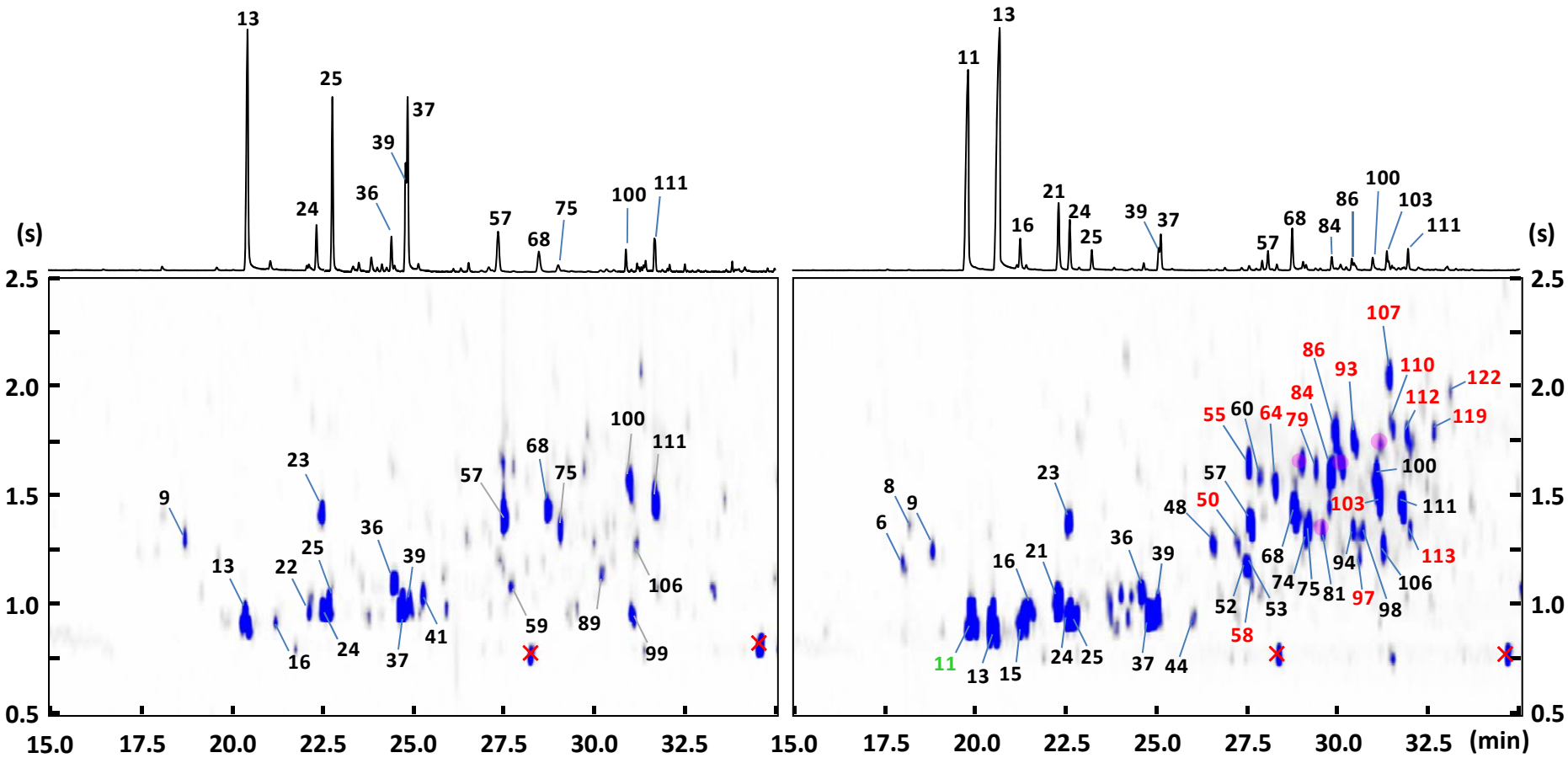

Fig. 7. Expansions of two-dimensional GC plots resulting from the GC × GC-MS analysis of cannabis herb (left) and hashish (right). These plots clearly enlightened the differences observed in the monoterpenic region (RI HP-5 ~800-1300) between the two sample types. Compounds are numbered according to tables 5 and 6. Those appearing with red numbers are assumed to be photo-oxidation products. (double column figure)

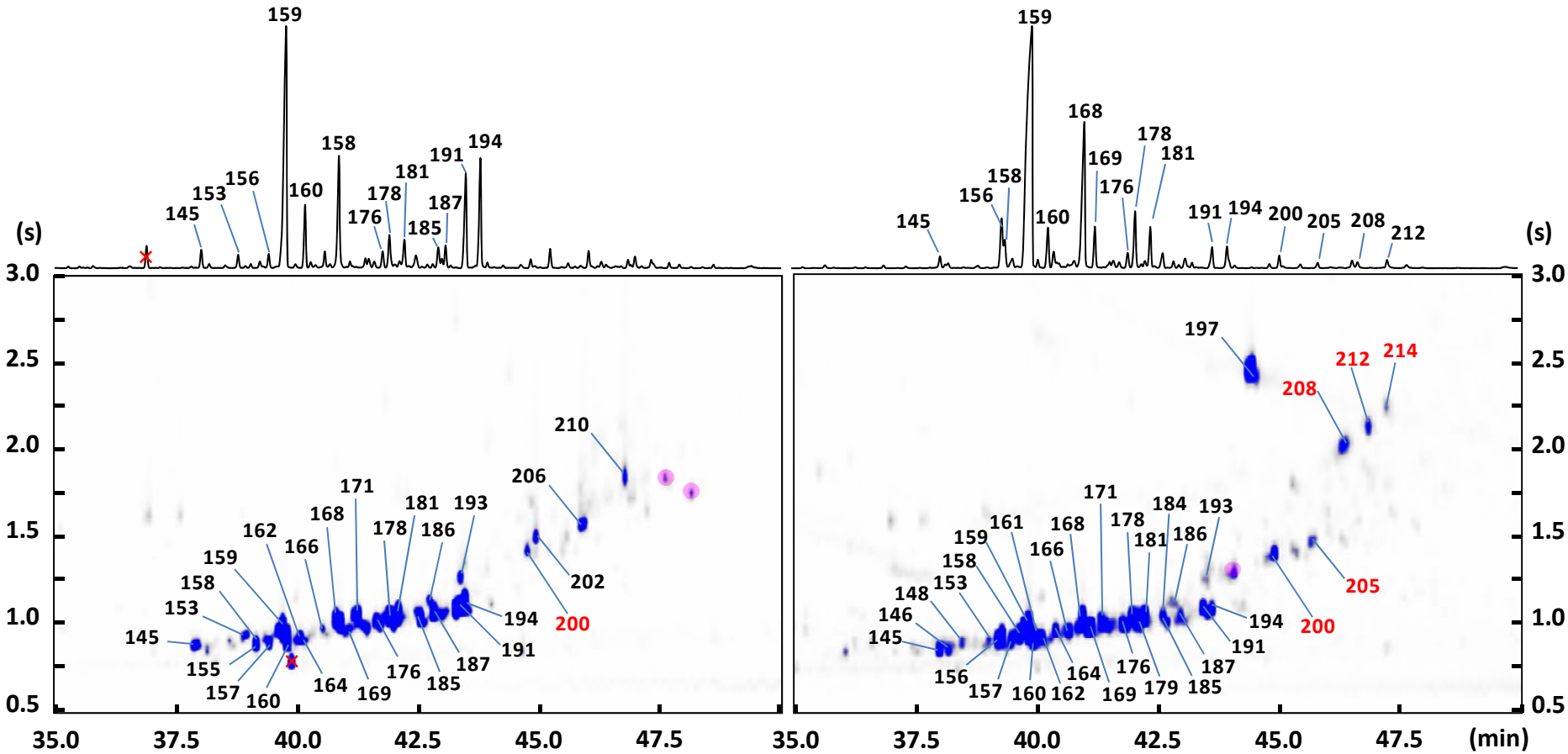

Fig. 8. Expansions of GC × GC plots of cannabis herb (left) and hashish (right) in the sesquiterpenic region (RI HP-5 ~1300-1700). Compounds are numbered according to tables 5 and 6.Those appearing with red numbers are assumed to be photo-oxidation products. (double column figure)

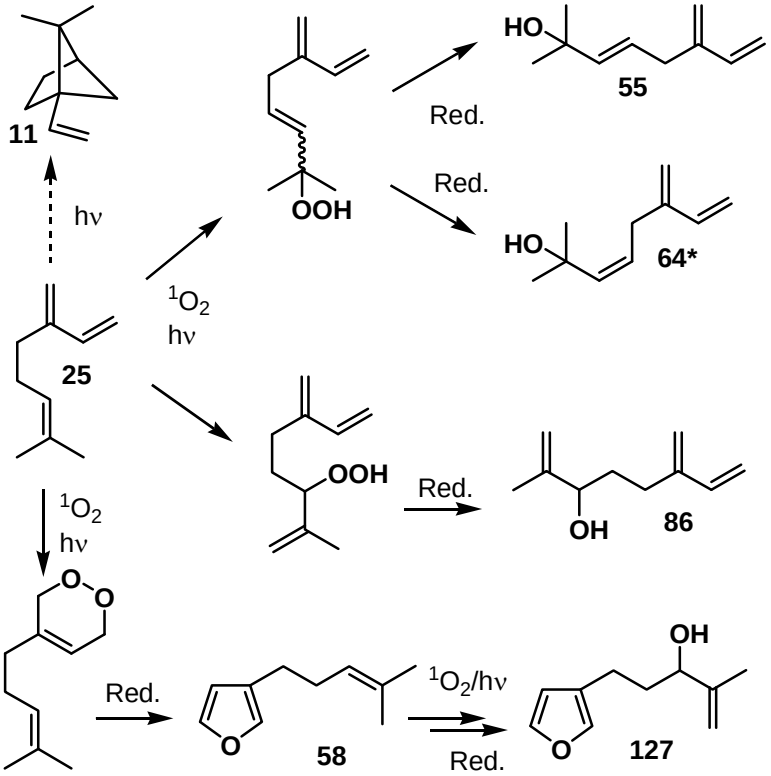

Fig. 9. Photo-oxidation reactions of β-myrcene **25** leading to the formation of compounds **55**, **58**, **64**, **86**, and **127**, all identified in hashish. (double column figure)

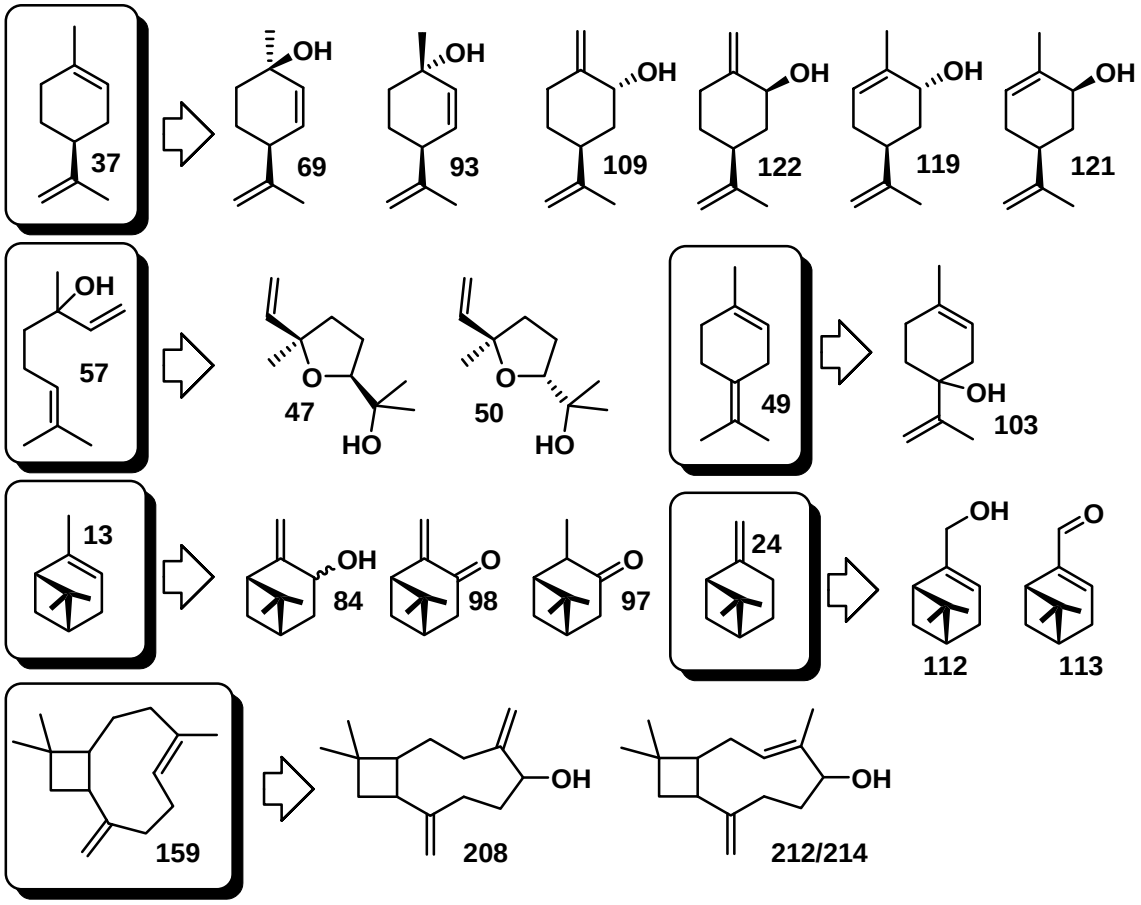

Fig. 10. Main photo-oxidation products observed in hashish samples. Their corresponding precursors are depicted within frames. (double column figure)

Table 1. Main volatile constituents of cannabis herb and hashish samples determined by HS-SPME-GC-MS.

						Cannabis herb									Hashish										
#[a]	t_r (min)	RI[b] calc.	RI[c] Liter.	Compound name	Chemical class	1 %	2a[i] %	2b[i] %	2c[i] %	2d[i] %	3 %	4 %	5 %	20[j] %	21[j] %	22[j] %	23[j] %	24[j] %	25 %	26 %	27 %	28 %	29 %	30 %	31[k] %
11	11.47	919	931	« Hashishene »	Monoterpenes	0.64	tr.	tr.	0.43	0.27	1.55	0.18	tr.	0.14	14.87	12.00	10.17	11.68	2.50	11.50	9.30	3.30	10.70	5.60	1.10
12	11.93	927	932	α-Thujene	Monoterpenes	tr.	tr.	tr.	0.66	1.11	tr.	0.06	tr.	tr.	-	0.08	-	-	tr.	-	-	tr.	tr.	-	0.16
13	12.50	936	936	α-Pinene	Monoterpenes	10.25	14.93	17.49	4.02	9.62	17.94	33.84	33.34	12.15	24.45	17.26	21.31	20.22	15.10	28.80	37.40	29.50	38.50	10.60	10.60
15	13.10	946	941	α-Fenchene	Monoterpenes	tr.	-	-	-	-	tr.	-	-	tr.	0.47	0.19	0.31	0.40	0.08	0.20	0.30	0.20	0.30	0.10	0.06
16	13.24	948	950	Camphene	Monoterpenes	tr.	0.28	tr.	0.17	0.20	0.65	0.62	0.92	0.40	2.65	1.18	1.69	2.07	0.70	1.50	1.60	1.10	1.80	0.50	0.51
21	14.86	974	-	*43 55 67 79 95 (109) 111 119 123 137 152**	Monoterpenoids	-	-	tr.	0.30	0.69	-	-	-	-	2.26	2.64	1.58	2.11	*	*	*	-	*	*	-
24	14.90	975	978	β-Pinene	Monoterpenes	2.99	3.51	4.43	1.70	3.22	5.61	11.46	11.43	1.39	2.32	1.82	3.56	4.18	3.00	3.80	3.50	5.20	4.40	2.50	4.00
23	15.04	977	978	6-Methylhept-5-en-2-one	Miscellaneous	tr.	tr.	0.38	tr.	tr.	0.21	-	-	0.12	-	-	-	-	tr.	tr.	tr.	-	0.24	0.16	0.22
25	15.73	988	987	β-Myrcene	Monoterpenes	26.93	27.69	28.75	5.33	1.90	19.54	19.56	20.69	1.43	2.58	0.68	1.88	6.96	2.40	2.30	6.10	15.90	1.30	2.60	1.90
36	17.51	1018	1015	*p*-Cymene	Monoterpenes	0.13	tr.	0.17	0.33	0.22	0.30	tr.	0.04	0.07	0.65	0.23	0.21	0.44	0.10	0.20	0.30	0.20	0.40	0.20	0.60
37	18.02	1027	1025	Limonene	Monoterpenes	3.91	5.13	5.43	1.56	1.46	7.15	8.26	6.92	0.83	4.67	1.23	1.72	4.41	1.60	2.00	4.00	5.00	3.50	1.70	7.70
39	18.17	1029	1024	Eucalyptol	Monoterpenoids	0.44	0.38	0.53	7.51	11.71	1.42	0.52	0.29	*	0.68	0.65	0.54	0.49	0.47	0.40	0.40	0.40	0.70	0.60	1.40
40	19.01	1043	1029	(*E*)-β-Ocimene	Monoterpenes	2.71	0.52	0.51	tr.	tr.	0.37	0.78	1.09	0.04	tr.	-	tr.	0.09	0.01	tr.	tr.	0.11	tr.	tr.	tr.
44	19.70	1055	1051	γ-Terpinene	Monoterpenes	0.16	0.06	0.08	0.29	0.25	0.32	0.08	tr.	0.04	0.02	tr.	tr.	0.18	0.06	0.05	0.25	0.15	0.03	0.05	0.18
48	20.47	1068	1053	*cis*-Sabinene hydrate	Monoterpenoids	tr.	tr.	0.08	0.51	0.67	0.13	0.06	tr.	0.03	0.02	tr.	0.03	0.02	0.04	0.02	tr.	0.02	0.03	tr.	0.11
49	21.44	1084	1082	α -Terpinolene	Monoterpenes	0.37	0.14	0.18	0.29	0.17	0.64	0.20	0.16	tr.	0.15	0.28	tr.	0.31	0.18	0.10	0.30	0.40	0.04	0.10	0.70
57	22.20	1097	1086	Linalool	Monoterpenoids	0.90	0.77	1.05	0.53	0.23	0.97	0.14	0.47	1.10	0.35	0.45	0.31	0.22	0.50	0.50	0.40	0.30	0.40	0.80	0.40
62	22.29	1099	1082	*trans*-Sabinene hydrate	Monoterpenoids	-	-	0.08	0.46	0.36	0.15	tr.	-	-	0.02	-	-	-	-	-	tr.	-	-	-	-
68	23.07	1113	1099	α-Fenchol, exo	Monoterpenoids	0.45	0.24	0.50	0.59	0.27	0.54	0.28	0.23	0.60	1.02	1.07	0.70	0.55	0.31	0.50	0.40	0.20	0.60	0.70	0.70
74	23.35	1118	1119[d]	*trans*-Pinene hydrate	Monoterpenoids	0.22	0.16	0.29	0.29	0.09	0.28	0.16	0.14	0.21	0.13	0.29	0.23	0.12	0.14	0.14	0.07	0.07	0.11	0.23	0.42
86	25.32	1155	1143[e]	2-Methyl-6-methylene-octa-1,7-dien-3-ol	Monoterpenoids	0.15	tr.	0.09	0.17	-	0.21	tr.	tr.	0.14	0.14	0.24	0.64	-	0.12	0.28	0.14	0.09	0.09	0.40	0.06
100	25.94	1167	1165[d]	Borneol	Monoterpenoids	tr.	0.12	tr.	-	-	tr.	0.11	0.12	0.39	0.30	0.38	0.27	0.18	0.14	0.17	0.11	0.08	0.20	0.25	0.21
101	26.15	1171	1172	Menthol	Monoterpenoids	0.39	0.09	0.24	0.73	0.38	0.37	tr.	tr.	-	-	-	-	-	0.02	0.03	-		tr.	-	-
103	26.27	1173	1189[f]	1,8-Menthadien-4-ol	Monoterpenoids	0.17	0.08	0.16	0.43	0.21	0.26	0.05	tr.	-	0.28	0.42	0.30	0.07	0.10	0.30	0.16	0.06	0.24	0.40	0.30
107	26.60	1180	1169	p-Cymen-8-ol	Monoterpenoids	tr.	tr.	tr.	0.08	tr.	tr.	tr.	tr.	0.07	0.07	0.18	0.09	0.09	0.03	0.11	0.05	tr.	0.10	0.17	0.14
99	26.67	1181	1176	Hexyl butyrate	Miscellaneous	tr.	0.88	0.60	-	-	-	tr.	tr.	0.40	-	-	-	-	0.02	-	tr.	-	tr.	0.44	-
111	27.00	1187	1176	α-Terpineol	Monoterpenoids	0.47	0.20	0.30	0.48	0.19	0.38	0.08	0.12	0.45	0.38	0.51	0.31	0.24	0.18	0.21	0.14	0.10	0.24	0.32	0.33
145	35.62	1373	1376	α-Ylangene	Sesquiterpenes	0.25	0.38	0.28	0.35	0.20	0.20	0.11	0.09	1.01	0.38	0.51	0.41	0.34	0.42	0.20	0.20	0.20	0.20	0.30	0.70
146	35.81	1377	1379	α-Copaene	Sesquiterpenes	-	0.10	0.09	0.20	0.17	0.11	tr.	tr.	0.24	0.08	0.20	0.21	0.19	0.21	0.09	0.07	0.10	0.12	0.34	0.74
153	36.63	1396	1394	Sativene	Sesquiterpenes	0.22	0.36	0.30	0.35	0.50	0.20	tr.	0.20	0.75	0.03	0.14	0.16	0.04	0.50	tr.	tr.	0.16	0.12	tr.	0.36
154	36.94	1403	1399	Sesquithujene	Sesquiterpenes	tr.	0.34	0.27	0.22	0.50	tr.	tr.	0.09	0.21	-	-	-	-	tr.	tr.	tr.	tr.	-	tr.	0.17
156	37.14	1408	1409	Isocaryophyllene	Sesquiterpenes	0.61	tr.	0.35	1.17	0.46	0.57	0.16	tr.	0.41	2.26	2.28	1.45	1.73	1.10	1.70	1.10	0.60	4.00	2.20	1.60
158	37.15	1408	1411	Longifolene	Sesquiterpenes	0.53	-	tr.	tr.	tr.	-	-	-	0.09	-	1.05	-	-	-	-	-	-	-	-	-

						Cannabis herb									Hashish										
#[a]	t_r (min)	RI[b] calc.	RI[c] Liter.	Compound name	Chemical class	1 %	2a[i] %	2b[i] %	2c[i] %	2d[i] %	3 %	4 %	5 %	20[j] %	21[j] %	22[j] %	23[j] %	24[j] %	25 %	26 %	27 %	28 %	29 %	30 %	31[k] %
155	37.57	1419	1411	*cis*-α-Bergamotene	Sesquiterpenes	0.35	0.74	0.53	0.87	1.42	0.43	0.15	0.27	0.77	0.20	0.56	0.36	0.23	0.43	0.24	0.15	0.14	0.22	0.41	0.45
159	37.90	1427	1421	β-Caryophyllene	Sesquiterpenes	20.22	15.53	14.88	31.99	26.86	16.22	12.27	7.54	20.28	15.60	24.02	22.27	17.39	30.90	27.30	20.20	19.60	17.60	43.30	32.10
161	38.02	1430	-	« exo-Caryophyllene »[g]	Sesquiterpenes	tr.	tr.	tr.	0.53	0.55	0.28	0.17	tr.	0.27	0.25	0.32	0.31	0.26	0.40	0.40	0.40	0.30	0.30	0.70	0.70
160	38.32	1437	1434	*trans*-α-Bergamotene	Sesquiterpenes	1.72	3.88	2.76	4.14	7.02	2.28	0.66	1.46	3.38	1.27	1.57	1.38	1.40	2.50	1.00	0.70	0.90	0.80	1.90	2.80
162	38.46	1441	1440	α-Guaiene	Sesquiterpenes	1.24	0.33	0.38	1.10	1.06	0.81	0.18	0.72	tr.	0.05	0.70	1.19	tr	3.00	0.20	0.20	1.10	0.20	0.20	0.90
164	38.84	1450	1446	(*Z*)-β-Farnesene	Sesquiterpenes	2.17	3.49	2.48	2.78	3.26	2.07	0.36	1.52	1.00	0.11	0.15	0.42	0.44	1.30	0.30	0.30	0.60	0.20	0.50	0.90
168	39.19	1459	1455	α-Humulene	Sesquiterpenes	4.41	3.96	3.39	7.54	5.36	3.48	2.50	2.18	7.09	5.50	7.82	7.37	6.36	8.10	6.40	4.60	4.60	3.90	9.90	7.80
169	39.42	1464	1462	9-epi-(*E*)-Caryophyllene	Sesquiterpenes	tr.	tr.	0.23	1.12	1.26	0.36	0.54	tr.	0.37	1.36	1.49	1.17	1.32	0.60	0.70	0.50	0.30	0.70	1.40	0.60
176	39.93	1477	1475	Selina-4,11-diene	Sesquiterpenes	-	-	-	-	-	-	-	-	1.01	0.18	0.37	0.31	0.47	-	0.10	0.10	0.20	0.10	0.20	0.90
177	39.94	1477	1474	γ-Muurolene	Sesquiterpenes	tr.	-	-	-	-	-	-	-	0.20	0.12	0.25	0.36	-	-	-	-	-	-	-	0.90
178	40.32	1487	1486	β-Selinene	Sesquiterpenes	1.01	0.87	0.77	1.48	0.87	0.74	0.44	0.48	2.16	1.45	2.23	1.81	1.60	2.60	1.10	0.70	0.90	1.00	1.70	1.80
181	40.73	1497	1494	α-Selinene	Sesquiterpenes	0.75	0.69	0.58	1.17	0.87	0.72	0.28	0.42	1.80	0.95	1.52	1.37	1.11	2.00	0.70	0.50	0.70	0.60	1.00	1.10
179	41.07	1506	1503	β-Bisabolene	Sesquiterpenes	tr.	0.73	0.77	0.37	0.59	0.40	-	tr.	1.06	0.14	0.65	0.81	0.11	0.30	0.10	0.10	0.10	0.10	0.20	0.90
183	41.12	1507	1503	α-Bulnesene	Sesquiterpenes	1.13	-	tr.	0.69	0.67	0.51	0.17	0.79	-	-	0.69	-	-	2.40	0.13	0.17	0.70	0.10	0.10	0.40
185	41.66	1521	1523	Spirovetiva-1(10),7(11)-diene[h]	Sesquiterpenes	tr.	0.37	0.26	0.43	0.36	tr.	0.20	0.21	-	0.60	tr.	tr.	-	0.60	0.20	0.10	0.20	0.10	0.27	0.50
187	41.83	1526	1527	Eremophila-1(10),7(11)-diene	Sesquiterpenes	tr.	0.45	0.34	0.53	0.61	0.34	0.14	0.30	1.26	0.04	0.21	0.22	0.08	0.70	0.10	0.10	0.20	0.10	0.09	0.40
191	42.28	1538	1534	Selina-4(15),7(11)-diene	Sesquiterpenes	1.96	2.38	1.64	2.92	3.59	1.69	0.77	1.81	6.02	0.10	0.88	0.38	0.46	3.90	0.30	0.40	1.20	0.20	0.40	1.60
193	42.38	1540	1541	α-Calacorene	Sesquiterpenes	tr.	tr.	tr.	tr.	tr.	tr.	0.25	tr.	**	0.03	**	0.11	**	tr.	0.10	0.10	0.10	0.04	0.10	0.10
194	42.72	1549	1542	Selina-3,7(11)-diene	Sesquiterpenes	2.26	2.38	1.58	2.54	3.16	1.66	0.74	2.18	6.40	0.06	1.03	1.10	0.21	4.00	0.20	0.40	1.20	0.10	0.20	1.40
200	44.04	1584	1578	Caryophyllene oxide	Sesquiterpenoids	0.13	0.16	0.19	0.59	0.35	0.21	0.06	0.04	0.50	0.51	0.45	0.34	0.35	0.29	0.30	0.10	0.10	0.30	0.40	0.70

[a] Compounds are numbered according to those cited in both tables 5 and 6. [b] Retention indices calculated against a series of C6-C24 n-alkanes; [c] Retention indices from the Terpenoids Library list (available at massfinder.com); [d] Retention indices from "Identification of Essential Oil Components By Gas Chromatography/Mass Spectrometry", R.P Adams, 4th Ed. [e] Retention indices obtained from NIST webbook; [f] Retention indices obtained from *J Chrom A*, **2003**, *998*, 201-211. [g] Exo-caryophyllene : 10,10-dimethyl-2,6-bis(methylene)bicyclo[7.2.0]undecane (CAS# 136296-38-3); [h] often coeluted with minor sesquiterpenes such as δ-cadinene ; [i] Samples of herb 2a-d indexed by INPS as coming from the same seizure; [j] samples 20-24 correspond to analyses performed at University of Nice (all other samples were analyzed at INPS); [k]Hashish sample #31 indexed by INPS as probably originating from Afghanistan; * unknown compound often coeluted with β-pinene; ** α-calacorene: often coeltued with Selina-4(15),7(11)-diene.

Table 2. Analysis of cannabis herb and hashish samples by HS-SPME-GC × GC-MS.

#	RI calc. HP5[b]	Compound Name	Group Name	Constellation Name	$^{1}t_r$ (min)	$^{2}t_r$ (s)	Hash. 21 %	Hash. 22 %	Hash. 23 %	Hash. 24 %	Herb 20 %
1		2,4-Hexadiene	Alkenes	Hydrocarbons	8.80	0.97	-	-	0.05	-	-
2		Toluene	Hydrocarbons	Aromatics	12.83	1.54	-	-	-	-	-
3		Hexanal	Aldehydes		14.11	1.50	-	-	-	-	0.14
4		*67 81 91 (109) 124*	unknown		15.76	0.85	-	-	-	-	-
5		Hexan-1-ol	Alcohols		17.05	2.72	-	-	-	-	0.53
6		1-Acetylcyclohexene	Ketones	Norterpenoids	17.87	1.18	-	0.06	tr.	0.02	tr.
7		Cyclofenchene	Hydrocarbons	Monoterpenes	17.87	0.89	0.04	-	tr.	-	-
8		Heptan-2-one	Ketones		18.05	1.34	0.05	0.03	tr.	0.10	0.04
9	900	*57 (72) 84 100 114 127*	unknown		18.70	1.22	0.03	0.08	-	0.02	0.16
10	904	Bornylene	Hydrocarbons	Monoterpenes	18.88	0.85	0.05	tr.	tr.	0.02	-
11	924	Hashishene	Hydrocarbons	Monoterpenes	19.70	0.89	11.95	10.30	10.07	10.06	tr.
13	939	α-Pinene	Hydrocarbons	Monoterpenes	20.35	0.89	19.40	14.04	19.68	18.03	3.45
14	954	2(5H)-Furanone, 5,5-dimethyl-	Esters		20.99	3.94	-	-	tr.	0.04	-
15	959	α-Fenchene	Hydrocarbons	Monoterpenes	21.17	0.89	0.30	1.44	0.19	1.67	-
16	961	Camphene	Hydrocarbons	Monoterpenes	21.26	0.93	3.04	0.33	2.59	0.92	0.13
17	965	Thuja-2,4(10)-diene	Hydrocarbons	Monoterpenes	21.45	0.93	0.19	0.06	0.22	0.11	-
18	965	Verbenene	Hydrocarbons	Monoterpenes	21.45	0.97	0.09	-	0.04	0.08	-
19	970	*53 (67) 82 93 107 121 136*	unknown		21.63	0.89	0.05	-	-	0.05	-
20	972	Benzaldehyde	Aldehydes	Aromatics	21.72	2.64	-	-	tr.	-	0.07
21	980	*67 79 95 (109) 111 137 152*	unknown		22.09	0.97	3.04	4.73	2.47	3.89	0.13
22	985	Sabinene	Hydrocarbons	Monoterpenes	22.27	0.89	-	-	0.06	0.09	0.11
23	987	6-Methylhept-5-en-2-one	Ketones	Norterpenoids	22.36	1.34	0.33	0.28	0.13	0.46	0.51
24	989	β-Pinene	Hydrocarbons	Monoterpenes	22.45	0.93	2.55	1.87	4.70	3.83	0.46
25	991	β-Myrcene	Hydrocarbons	Monoterpenes	22.55	0.89	3.87	0.37	1.86	8.70	2.12
26	1000	Decane	Alkanes	Hydrocarbons	22.91	0.73	-	tr.	tr.	-	-
27	1006	*(56) 69 84 100 110*	unknown		23.19	1.18	-	-	-	-	tr.
28	1013	*(56) 61 69 73 84*	unknown		23.46	1.06	-	-	-	-	0.05
29	1015	Nonanal	Aldehydes		23.55	1.46	0.25	-	-	-	-

#	RI calc. HP5[b]	Compound Name	Group Name	Constellation Name	$^{1}t_r$ (min)	$^{2}t_r$ (s)	Hash. 21 %	Hash. 22 %	Hash. 23 %	Hash. 24 %	Herb 20 %
30	1015	α-Phellandrene	Hydrocarbons	Monoterpenes	23.55	0.97	0.08	0.14	0.07	0.73	0.11
31	1017	Δ3-Carene	Hydrocarbons	Monoterpenes	23.65	0.89	0.44	-	0.10	-	-
32	1021	*53 65 (79) 91 105 119 134*	unknown		23.83	1.02	-	0.08	0.12	0.46	tr.
33	1026	α-Terpinene	Hydrocarbons	Monoterpenes	24.01	0.93	0.13	0.06	0.09	0.40	0.03
34	1028	*55 67 82 (93) 96 109 119 137 152*	unknown		24.10	1.02	0.03	-	tr.	-	-
35	1032	2-Ethylhexan-1-ol	Alcohols		24.29	1.58	-	-	-	0.06	0.05
36	1034	*p*-Cymene	Hydrocarbons	Monoterpenes	24.38	1.02	1.75	0.47	0.49	1.45	0.72
37	1038	Limonene	Hydrocarbons	Monoterpenes	24.56	0.93	5.43	1.68	3.00	9.10	4.14
38	1045	Benzylalcohol	Alcohols	Aromatics	24.84	5.48	-	-	-	0.75	0.04
39	1045	Eucalyptol	Oxides	Monoterpenoids	24.84	0.93	0.83	1.58	1.11	0.91	0.48
41	1051	Isobutyl angelate	Esters		25.11	1.02	-	-	-	-	0.41
42	1055	Phenylacetaldehyde	Aldehydes	Aromatics	25.30	2.31	-	-	-	-	tr.
43	1062	γ-Hexalactone	Esters		25.57	2.76	-	-	-	-	0.08
44	1066	γ-Terpinene	Hydrocarbons	Monoterpenes	25.75	0.89	-	0.06	0.08	0.48	0.10
45	1072	2-Acetylpyrrole	Amines		26.03	0.73	-	-	-	-	tr.
46	1072	*(56) 69 84 130*	unknown		26.03	1.62	-	-	-	-	0.04
47	1077	*cis*-Linalool oxide (furanoid)	Oxides	Monoterpenoids	26.21	1.22	-	-	tr.	0.01	0.07
48	1079	*cis*-Sabinene hydrate	Alcohols	Monoterpenoids	26.30	1.26	0.10	0.13	0.08	0.09	0.09
49	1094	α-Terpinolene	Hydrocarbons	Monoterpenes	26.95	0.93	0.04	tr.	0.06	0.80	0.03
50	1096	*trans*-Linalool oxide (furanoid)	Oxides	Monoterpenoids	27.04	1.26	0.06	0.07	0.03	0.05	0.03
51	1100	Undecane	Alkanes	Hydrocarbons	27.22	1.18	-	-	tr.	-	tr.
52	1100	*p*-Cymenene	Hydrocarbons	Sesquiterpenes	27.22	1.14	0.11	0.13	0.05	0.64	0.08
53	1100	Fenchone	Ketones		27.22	1.14	0.17	0.13	0.08	0.02	0.05
54	1102	*55 67 79 (93) 104 113 120 134*	unknown		27.31	1.87	-	-	-	-	0.03
55	1102	(*E*)-2-Methyl-6-methylene-octa-3,7-dien-2-ol A	Alcohols	Monoterpenoids	27.31	1.62	0.18	0.31	0.47	0.65	0.22
56	1105	*51 77 (105) 136*	unknown		27.40	1.79	-	-	-	-	0.03
57	1105	Linalool	Alcohols	Monoterpenoids	27.40	1.34	0.68	0.76	0.40	0.52	2.01
58	1105	Perillene	Oxides	Monoterpenoids	27.40	1.06	0.09	tr.	0.07	0.20	-
59	1107	Nonanal	Aldehydes		27.50	1.06	-	-	93	-	0.17
60	1109	6-Methylhepta-3,5-dien-2-one	Ketones	Norterpenoids	27.59	1.54	0.05	0.08	0.04	0.06	0.10
61	1111	*(79) 107 122 137 152*	unknown		27.68	1.06	0.04	tr.	tr.	0.03	-

#	RI calc. HP5[b]	Compound Name	Group Name	Constellation Name	$^{1}t_r$ (min)	$^{2}t_r$ (s)	Hash. 21 %	Hash. 22 %	Hash. 23 %	Hash. 24 %	Herb 20 %
62	1111	*trans*-Sabinene hydrate	Esters	Monoterpenoids	27.68	1.38	0.03	-	0.03	0.02	0.04
63	1118	α-Thujone	Ketones	Monoterpenoids	27.95	1.14	-	-	-	-	0.04
64	1120	2-Methyl-6-methylene-octa-3,7-dien-2-ol B	Alcohols	Monoterpenoids	28.05	1.50	0.19	0.24	0.13	0.25	0.03
65	1123	*(56) 69 83 97 111 119 139 180 208*	unknown		28.14	1.79	-	-	-	0.05	-
66	1123	*54 (74) 87 101 127*	unknown		28.14	1.02	-	-	-	-	tr.
67	1130	β-Thujone	Ketones	Monoterpenoids	28.41	1.18	-	-	-	-	0.03
68	1134	exo-Fenchol	Alcohols	Monoterpenoids	28.60	1.38	1.55	1.88	1.08	0.92	0.64
69	1134	*trans*-Mentha-2,8-dien-1-ol	Alcohols	Monoterpenoids	28.60	1.50	0.03	0.06	0.06	0.05	-
70	1136	*51 64 81 (91) 109 134*	unknown		28.69	2.03	-	-	-	-	0.03
71	1136	*(55) 67 69 81 83 93 109 119 123 134 137 152*	unknown		28.69	1.58	0.12	-	0.08	0.14	-
72	1139	Campholenal	Oxides	Monoterpenoids	28.78	1.22	-	tr.	tr.	-	tr.
73	1139	*(55) 67 77 82 91 106 119 134*	unknown		28.78	1.62	-	0.18	-	-	0.03
74	1141	*trans*-Pinene hydrate	Alcohols	Monoterpenoids	28.87	1.30	0.09	0.19	0.11	0.10	0.04
75	1143	4-Acetyl-1-methylcyclohexene	Ketones		28.96	1.34	0.22	0.19	0.16	0.15	0.32
76	1145	*56 71 84 (89) 101*	unknown		29.05	0.93	-	-	-	-	0.08
77	1145	*(95) 123 138*	unknown		29.05	1.34	-	-	tr.	-	-
78	1148	*(55) 67 82 97 111 141 152*	unknown		29.15	1.26	-	-	-	-	tr.
79	1148	Ipsdienol	Alcohols	Monoterpenoids	29.15	1.58	0.05	0.12	0.12	0.12	0.08
80	1152	Isoamyl angelate	Esters		29.33	0.97	-	-	-	-	0.08
81	1152	*(69) 79 93 107 122 135 150*	unknown		29.33	1.30	0.05	0.09	0.04	0.09	-
82	1155	*55 67 (83) 93 111 118 136 161*	unknown		29.42	1.42	-	-	-	-	tr.
83	1157	*(55) 67 81 93 111 153*	unknown		29.51	1.30	-	-	-	-	tr.
84	1157	*trans*-Pinocarveol	Alcohols	Monoterpenoids	29.51	1.54	0.37	0.62	0.35	0.44	0.11
85	1157	Nopinone	Ketones	Norterpenoids	29.51	1.42	0.03	0.07	tr.	0.04	-
86	1161	2-Methyl-6-methylene-octa-1,7-dien-3-ol	Alcohols	Monoterpenoids	29.70	1.75	0.24	0.40	0.64	0.91	0.09
87	1166	Camphor	Ketones	Monoterpenoids	29.88	1.22	-	tr.	tr.	0.14	0.09
88	1166	*67 (79) 91 93 96 105 109 119 123 134 137 152*	unknown		29.88	1.58	0.16	0.21	0.11	0.17	-
89	1168	Menthone	Ketones	Monoterpenoids	29.97	1.10	-	-	-	-	0.16
90	1168	Decanal	Aldehydes		29.97	1.14	0.30	-	-	-	-
91	1173	*trans*-β-Terpineol	Alcohols	Monoterpenoids	30.15	1.30	-	-	-	-	0.06
92	1173	*57 67 79 83 95 (109) 121 133 137*	unknown		30.15	1.75	-	-	-	-	0.03

#	RI calc. HP5[b]	Compound Name	Group Name	Constellation Name	$^{1}t_r$ (min)	$^{2}t_r$ (s)	Hash. 21 %	Hash. 22 %	Hash. 23 %	Hash. 24 %	Herb 20 %
93	1173	*cis*-Mentha-2,8-dien-1-ol	Alcohols	Monoterpenoids	30.15	1.75	0.15	0.23	-	0.17	-
94	1173	Camphene hydrate	Alcohols	Monoterpenoids	30.15	1.34	0.08	0.13	0.07	0.02	-
95	1177	Mentha-1,5-dien-8-ol	Alcohols	Monoterpenoids	30.34	1.50	-	-	0.05	0.08	-
96	1177	Isomenthone	Ketones	Monoterpenoids	30.34	1.18	-	-	-	-	0.05
97	1177	Pinocamphone	Ketones	Monoterpenoids	30.34	1.18	0.03	0.06	tr.	0.04	-
98	1180	Pin-2(10)-en-3-one	Ketones	Monoterpenoids	30.43	1.30	0.04	0.08	0.07	0.07	0.04
99	1189	Hexyl butyrate	Esters		30.80	0.93	-	-	-	-	0.32
100	1189	Borneol	Alcohols	Monoterpenoids	30.80	1.54	0.75	0.56	0.37	0.22	0.42
102	1189	*(69) 84 95 97 109 123 137 152*	unknown		30.80	1.06	0.04	tr.	-	-	-
103	1191	*p*-Mentha-1,8-dien-4-ol	Alcohols	Monoterpenoids	30.89	1.46	0.09	0.73	0.35	0.59	0.12
104	1191	*(55) 67 79 81 93 109 119 137*	unknown		30.89	1.71	0.06	0.08	0.04	0.07	-
105	1191	(*Z*)-Hex-3-enyl acetate	Esters		30.89	2.84	tr.	-	-	-	-
106	1193	Terpinen-4-ol	Alcohols	Monoterpenoids	30.98	1.26	0.15	0.22	0.13	0.14	0.16
107	1198	*p*-Cymen-8-ol	Alcohols	Monoterpenoids	31.16	2.03	0.14	0.21	0.10	0.16	0.11
108	1200	Dodecane	Alkanes	Hydrocarbons	31.25	0.73	-	0.05	0.04	0.10	0.06
109	1200	*trans*-Mentha-1(7),8-dien-2-ol	Ketones	Monoterpenoids	31.25	1.79	0.07	0.11	0.06	0.10	0.03
110	1209	Decanal	Aldehydes		31.53	1.06	-	-	-	-	0.06
111	1209	α-Terpineol	Alcohols	Monoterpenoids	31.53	1.42	0.55	0.67	0.33	0.30	1.10
112	1212	Myrtenol	Alcohols	Monoterpenoids	31.62	1.75	0.11	0.20	0.11	0.15	0.07
113	1215	Myrtenal	Aldehydes	Monoterpenoids	31.71	1.30	-	0.09	0.04	0.06	-
114	1215	*79 (91) 107 121 134 150*	unknown		31.71	1.38	-	-	-	-	tr.
115	1226	*(55) 67 79 81 93 109 119 137*	unknown		32.08	1.87	-	tr.	tr.	0.02	-
116	1229	Verbenone	Ketones	Monoterpenoids	32.17	1.50	-	tr.	tr.	-	-
117	1232	Fenchyl acetate	Esters	Monoterpenoids	32.26	0.97	-	-	tr.	0.01	tr.
118	1232	Methoxybenzofuran (unknown isomer)	Oxides	Aromatics	32.26	2.56	-	-	tr.	-	0.03
119	1235	*trans*-Carveol	Alcohols	Monoterpenoids	32.35	1.75	0.05	0.07	0.04	0.05	0.04
120	1241	*57 85 (103)*	unknown		32.54	0.93	-	-	-	-	tr.
121	1250	*cis*-Carveol	Alcohols	Monoterpenoids	32.81	1.79	-	-	tr.	-	-
122	1250	*cis*-Mentha-1(7),8-dien-2-ol	Alcohols	Monoterpenoids	32.81	1.95	0.04	0.04	tr.	0.04	0.14
123	1262	*53 67 81 95 109 137 (152)*	unknown		33.18	1.34	-	-	-	-	tr.
124	1262	*51 (69) 93 109 135*	unknown		33.18	1.71	-	-	-	-	0.03

#	RI calc. HP5[b]	Compound Name	Group Name	Constellation Name	$^{1}t_r$ (min)	$^{2}t_r$ (s)	Hash. 21 %	Hash. 22 %	Hash. 23 %	Hash. 24 %	Herb 20 %
125	1268	Carvone	Ketones	Monoterpenoids	33.36	1.46	-	tr.	tr.	0.02	0.08
126	1285	*57 71 (85) 98 125 168*	unknown		33.91	0.77	-	-	-	-	tr.
127	1297	2-Methyl-5-(fur-3-yl)-pent-1-en-3-ol	Alcohols		34.28	2.36	-	-	-	-	0.03
128	1300	Tridecane	Alkanes	Hydrocarbons	34.37	0.73	-	0.21	tr.	0.01	0.07
129	1307	Undecanal	Aldehydes		34.65	1.10	0.12	-	-	-	-
130	1310	Bornyl acetate	Esters	Monoterpenoids	34.74	1.06	-	0.06	0.04	0.04	0.06
131	1312	Anethole	Oxides	Aromatics	34.83	1.54	-	-	-	-	0.07
132	1319	Sabinyl acetate	Esters	Monoterpenoids	35.10	1.14	-	-	tr.	-	-
133	1326	Perillyl alcohol	Alcohols	Monoterpenoids	35.38	2.11	-	-	tr.	-	0.03
134	1340	*55 67 81 (94) 107 121 136 149 177 192*	unknown		35.93	0.89	0.05	0.07	-	0.03	-
135	1343	Myrtenyl acetate	Esters	Monoterpenoids	36.02	1.18	-	-	tr.	-	-
136	1348	*55 66 79 93 108 (121) 136 160*	unknown		36.20	1.18	-	-	-	-	tr.
137	1352	*56 (71) 83 89 98 111 143*	unknown		36.39	1.66	0.07	-	-	-	-
138	1355	*91 105 (119) 133 147 175 190*	unknown		36.48	0.97	0.05	0.05	tr.	0.04	-
139	1364	Piperitenone	Ketones	Monoterpenoids	36.85	1.79	0.06	0.05	tr.	0.01	0.18
140	1367	*(57) 71 85 97 126 154 183 207*	unknown		36.94	0.89	-	-	-	-	0.03
141	1367	*(56) 71 89*	unknown		36.94	1.46	0.12	tr.	-	-	-
142	1379	α-Cubebene	Hydrocarbons	Sesquiterpenes	37.40	0.89	0.04	0.08	tr.	-	-
143	1381	*56 (71) 89 143 173*	unknown		37.49	1.50	0.07	-	-	tr.	-
144	1381	Piperitenone oxide	Oxides	Monoterpenoids	37.49	1.79	0.05	tr.	tr.	0.01	0.11
145	1390	α-Ylangene	Hydrocarbons	Sesquiterpenes	37.85	0.89	0.39	0.47	0.38	0.26	0.73
146	1393	α-Copaene	Hydrocarbons	Sesquiterpenes	37.95	0.93	0.16	0.09	0.22	0.12	0.25
147	1400	Tetradecane	Alkanes		38.22	0.77	0.03	0.04	tr.	0.01	0.09
148	1403	Longicyclene	Hydrocarbons	Sesquiterpenes	38.31	0.93	-	0.16	-	-	-
149	1403	Biphenyl	Hydrocarbons	Aromatics	38.31	1.79	-	-	0.04	-	-
150	1406	Dodecanal	Aldehydes		38.40	1.14	0.10	-	-	-	-
151	1406	β-Bourbonene	Hydrocarbons	Sesquiterpenes	38.40	0.93	0.04	-	0.04	0.02	0.20
152	1412	*55 67 77 (93) 105 119 133 147 161 189 204*	unknown		38.59	0.93	-	0.04	-	0.04	-
153	1421	Sativene	Hydrocarbons	Sesquiterpenes	38.86	0.97	-	0.16	0.18	-	0.42
155	1426	*cis*-α-Bergamotene	Hydrocarbons	Sesquiterpenes	39.05	0.85	-	-	tr.	0.34	0.89
156	1429	Isocaryophyllene	Hydrocarbons	Sesquiterpenes	39.14	1.02	2.27	2.53	1.61	1.01	-

#	RI calc. HP5[b]	Compound Name	Group Name	Constellation Name	$^{1}t_r$ (min)	$^{2}t_r$ (s)	Hash. 21 %	Hash. 22 %	Hash. 23 %	Hash. 24 %	Herb 20 %
157	1435	α-Santalene	Hydrocarbons	Sesquiterpenes	39.32	0.93	0.33	0.08	0.12	0.23	0.72
158	1441	Longifolene	Hydrocarbons	Sesquiterpenes	39.50	1.02	tr.	1.45	0.11	-	-
159	1447	β-Caryophyllene	Hydrocarbons	Sesquiterpenes	39.69	1.06	12.43	19.50	19.46	9.68	16.82
160	1447	trans-α-Bergamotene	Hydrocarbons	Sesquiterpenes	39.69	0.89	tr.	tr.	0.33	0.46	4.40
161	1453	Caryophyllene, exo-[c]	Hydrocarbons	Sesquiterpenes	39.87	1.02	0.06	0.13	0.10	0.67	-
162	1456	α-Guaiene	Hydrocarbons	Sesquiterpenes	39.96	0.97	-	0.81	0.84	0.06	0.23
163	1456	Geranylacetone	Ketones	Norterpenoids	39.96	1.26	-	0.03	-	-	-
164	1462	*(Z)*-β-Farnesene	Hydrocarbons	Sesquiterpenes	40.15	0.89	tr.	tr.	0.16	0.18	1.00
165	1465	Guaia-6,9-diene	Hydrocarbons	Sesquiterpenes	40.24	1.06	0.14	0.43	0.10	0.07	-
166	1474	Caryophyll-3-ene[d]	Hydrocarbons	Sesquiterpenes	40.51	1.02	0.36	0.60	0.15	0.21	0.31
167	1476	1-Dodecanol	Alcohols		40.60	1.50	0.08	-	-	-	-
168	1482	α-Humulene	Hydrocarbons	Sesquiterpenes	40.79	1.10	4.32	6.80	6.49	3.48	6.92
169	1488	9-epi-(*E*)-Caryophyllene	Hydrocarbons	Sesquiterpenes	40.97	1.06	1.31	1.85	1.13	1.88	0.46
170	1494	Valencene	Hydrocarbons	Sesquiterpenes	41.15	0.97	0.13	tr.	0.17	0.10	-
171	1497	α-Curcumene	Hydrocarbons	Sesquiterpenes	41.25	1.10	0.13	0.80	0.09	0.14	0.61
172	1500	Vetiselinene	Hydrocarbons	Sesquiterpenes	41.34	1.06	-	-	0.31	0.12	0.69
172	1500	Pentadecane	Alkanes		41.34	0.81	0.03	tr.	tr.	-	0.04
173	1503	α-Muurolene	Hydrocarbons	Sesquiterpenes	41.43	1.02	0.14	tr.	-	-	0.43
174	1509	*55 67 79 93 105 119 133 147 161 177 189 204*	unknown		41.61	1.06	-	-	-	0.09	-
175	1512	*55 67 79 81 105 119 133 147 161 175 189 204*	unknown		41.70	1.18	0.18	-	-	-	-
176	1519	Selina-4,11-diene	Hydrocarbons	Sesquiterpenes	41.89	1.10	0.36	1.29	0.55	0.20	0.80
178	1519	β-Selinene	Hydrocarbons	Sesquiterpenes	41.89	1.14	1.12	1.40	1.61	1.61	4.69
179	1519	β-Bisabolene	Hydrocarbons	Sesquiterpenes	41.89	1.06	tr.	-	-	0.09	0.22
180	1522	*53 (69) 79 93 109 119 135 161 204*	unknown		41.98	0.93	-	-	-	0.46	tr.
181	1522	α-Selinene	Hydrocarbons	Sesquiterpenes	41.98	1.14	0.51	0.87	0.51	0.19	1.96
182	1525	*53 (69) 79 93 105 119 133 147 161 189 204*	unknown		42.07	0.97	-	tr.	-	0.05	-
183	1525	α-Bulnesene	Hydrocarbons	Sesquiterpenes	42.07	1.14	0.06	1.22	1.43	0.27	0.03
184	1538	δ-Cadinene	Hydrocarbons	Sesquiterpenes	42.44	1.14	0.20	0.46	0.37	-	-
185	1541	Spirovetiva-1(10),7(11)-diene	Hydrocarbons	Sesquiterpenes	42.53	1.06	-	0.04	-	-	2.00
186	1544	*trans*-Calamenene	Hydrocarbons	Sesquiterpenes	42.62	1.26	0.08	0.06	0.04	-	0.30
187	1550	Eremophila-1(10),7(11)-diene	Hydrocarbons	Sesquiterpenes	42.80	1.14	0.12	0.36	0.21	0.02	2.23

#	RI calc. HP5[b]	Compound Name	Group Name	Constellation Name	$^{1}t_r$ (min)	$^{2}t_r$ (s)	Hash. 21 %	Hash. 22 %	Hash. 23 %	Hash. 24 %	Herb 20 %
188	1553	*55 65 77 91 105 119 131 145 (161) 187 202*	unknown		42.90	1.22	-	-	0.04	-	-
189	1553	α-Bisabolene	Hydrocarbons	Sesquiterpenes	42.90	1.10	0.04	0.14	0.23	-	0.46
190	1556	*55 67 81 91 105 119 133 147 161 175 189 (204)*	unknown		42.99	1.14	-	-	0.03	-	-
191	1566	Selina-4(15),7(11)-diene	Hydrocarbons	Sesquiterpenes	43.26	1.18	0.07	0.79	0.64	0.04	4.90
192	1569	Nerolidol	Alcohols	Sesquiterpenoids	43.35	1.46	0.07	0.07	0.04	0.03	0.14
193	1569	α-Calacorene	Hydrocarbons	Sesquiterpenes	43.35	1.38	0.03	0.12	0.08	0.03	0.45
194	1572	Selina-3,7(11)-diene	Hydrocarbons	Sesquiterpenes	43.45	1.18	0.08	0.38	0.76	0.07	13.57
195	1588	*55 65 77 91 105 119 133 149 161 175 (189) 204*	unknown		43.90	1.18	-	-	0.03	0.04	-
196	1588	*55 69 79 81 96 107 123 135 149 (164)*	unknown		43.90	1.42	0.14	0.30	0.08	0.10	-
197	1600	Diethyl Phthalate	Esters	Plasticizers	44.27	2.84	1.18	2.05	0.33	1.84	0.04
198	1600	Hexadecane	Alkanes	Hydrocarbons	44.27	0.89	0.05	tr.	tr.	-	0.03
199	1617	1-Benzyl-3,5-dimethylbenzene	Hydrocarbons	Aromatics	44.73	1.91	-	-	-	-	0.17
200	1617	Caryophyllene oxide	Oxides	Sesquiterpenoids	44.73	1.58	0.67	0.56	0.31	0.29	0.37
201	1617	Tetradecanal	Aldehydes		44.73	1.30	0.11	-	-	-	-
202	1620	Guaiol	Alcohols	Sesquiterpenoids	44.82	1.66	-	-	-	-	0.52
203	1630	*(161) 187 205 220*	unknown		45.10	2.03	0.04	0.07	-	0.03	-
204	1633	*41 55 67 80 (93) 107 121 138 205 220*	unknown		45.19	1.58	0.09	0.11	0.04	0.03	-
205	1643	Humulene oxide	Oxides	Sesquiterpenoids	45.46	1.62	0.22	0.18	0.10	0.08	0.21
206	1653	10-epi-γ-Eudesmol	Alcohols	Sesquiterpenoids	45.74	1.75	-	-	-	-	0.82
207	1660	Methyl dihydrojasmonate, *trans-*	Esters		45.92	2.36	0.03	-	-	-	-
208	1670	Caryophylla-4(12),8(13)-dien-5-ol	Alcohols	Sesquiterpenoids	46.20	2.31	0.23	0.43	0.18	0.10	tr.
209	1683	Hexadecan-1-ol	Alcohols		46.56	1.75	0.09	-	-	-	-
210	1687	α-Eudesmol	Alcohols	Sesquiterpenoids	46.65	2.07	-	-	-	-	0.63
211	1687	*unknown nitrogenated (base peak 100)*	unknown		46.65	1.34	-	-	-	-	0.03
212	1687	Caryophylla-3,8(13)-dien-5-ol A	Alcohols	Sesquiterpenoids	46.65	2.44	0.17	0.25	0.09	0.05	-
213	1690	Bulnesol	Alcohols	Sesquiterpenoids	46.75	1.95	-	-	-	-	0.16
214	1700	Caryophylla-3,8(13)-dien-5-ol B	Alcohols	Sesquiterpenoids	47.02	2.56	0.09	0.12	0.05	0.03	-
215	1700	Heptadecane	Alkanes	Hydrocarbons	47.02	0.93	0.09	tr.	tr.	0.04	0.04
216	1703	α-Bisabolol	Alcohols	Sesquiterpenoids	47.11	1.83	-	-	tr.	-	0.10
217	1716	*91 129 (207) 222*	unknown		47.48	2.07	-	-	-	-	0.24
218	1719	Pentadecanal	Aldehydes		47.57	1.42	0.12	-	-	-	-

#	RI calc. HP5[b]	Compound Name	Group Name	Constellation Name	$^{1}t_r$ (min)	$^{2}t_r$ (s)	Hash. 21 %	Hash. 22 %	Hash. 23 %	Hash. 24 %	Herb 20 %
219	1753	Palmitic acid	Acids		48.58	4.71	0.05	-	-	-	-
220	1800	Octadecane	Alkanes	Hydrocarbons	49.95	1.02	0.08	-	tr.	0.01	0.03
221	1809	Hexadecanal	Aldehydes		50.14	1.54	0.12	-	-	-	-
222	1835	Farnesol	Alcohols	Sesquiterpenoids	50.69	1.95	0.05	-	-	-	-
223	1870	*unknown nitrogenated (base peak 100)*	unknown		51.42	3.00	-	0.11	-	-	0.06
224	1870	Diisobutyl phthalate	Esters	Plasticizers	51.42	3.17	0.56	-	-	0.10	-
225	1874	Nonadecene	Alkenes	Hydrocarbons	51.51	1.14	0.19	-	-	-	-
226	1887	Hexadecan-1-ol	Alcohols		51.79	2.15	0.08	-	-	-	-
227	1900	Nonadecane	Alkanes	Hydrocarbons	52.06	1.10	0.49	0.08	0.05	0.12	tr.
228	1908	*unknown nitrogenated*	unknown		52.25	1.22	-	-	0.99	0.03	0.05
229	1928	Heptadecanal	Aldehydes		52.70	1.71	0.08	-	-	-	-
230	1928	Methyl palmitate	Esters	Phthalates	52.70	1.62	0.08	0.06	-	-	-
231	1956	(*E*,*E*)-Farnesylacetone	Ketones	Norterpenoids	53.35	3.65	0.46	-	-	-	-
232	1972	Dibutyl phthalate	Esters	Plasticizers	53.71	3.86	0.09	0.11	0.03	0.04	-
233	2000	Eicosane	Alkanes	Hydrocarbons	54.35	1.14	0.12	-	tr.	0.01	-
234	2029	Octadecanal	Aldehydes		55.00	1.95	0.05	-	-	-	-
235	2104	Eneicosane	Alkanes	Hydrocarbons	56.65	1.30	0.14	0.03	tr.	0.03	0.04
236	2104	Octadecan-1-ol	Alcohols		56.65	3.98	0.13	-	-	-	-
237	2108	*unknown nitrogenated (base peak 100)*	unknown		56.74	2.07	-	-	-	-	0.08
238	2200	Docosane	Alkanes	Hydrocarbons	59.03	1.46	0.08	-	tr.	tr.	-
239	2300	Tricosane	Alkanes	Hydrocarbons	61.87	1.66	0.04	-	0.06	tr.	tr.
240		Dioctyl adipate	Esters		65.08	4.06	0.36	0.92	0.14	0.63	-
241		Squalene	Hydrocarbons	Triterpenes	67.83	0.97	-	3.19	-	-	-

[a] Compounds are numbered according to those cited in both tables 5 and 6. [b] Retention indices calculated against a series of C6-C24 *n*-alkanes and compared with those contained in the MS databases cited above. [c] Exo-caryophyllene: 10,10-dimethyl-2,6-bis(methylene)bicyclo[7.2.0]undecane (CAS# 136296-38-3; MS available on SciFinder); [d] Caryophyll-3-ene: 4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene (CAS# 889360-49-0; MS available on SciFinder).

Supporting Information for

Multidimensional analysis of Cannabis volatile constituents: identification of 5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane as a volatile marker of Hashish, the resin of *Cannabis sativa* L.

Marie Marchini, Céline Charvoz, Laurence Dujourdy, Nicolas Baldovini, and Jean-Jacques Filippi[*]

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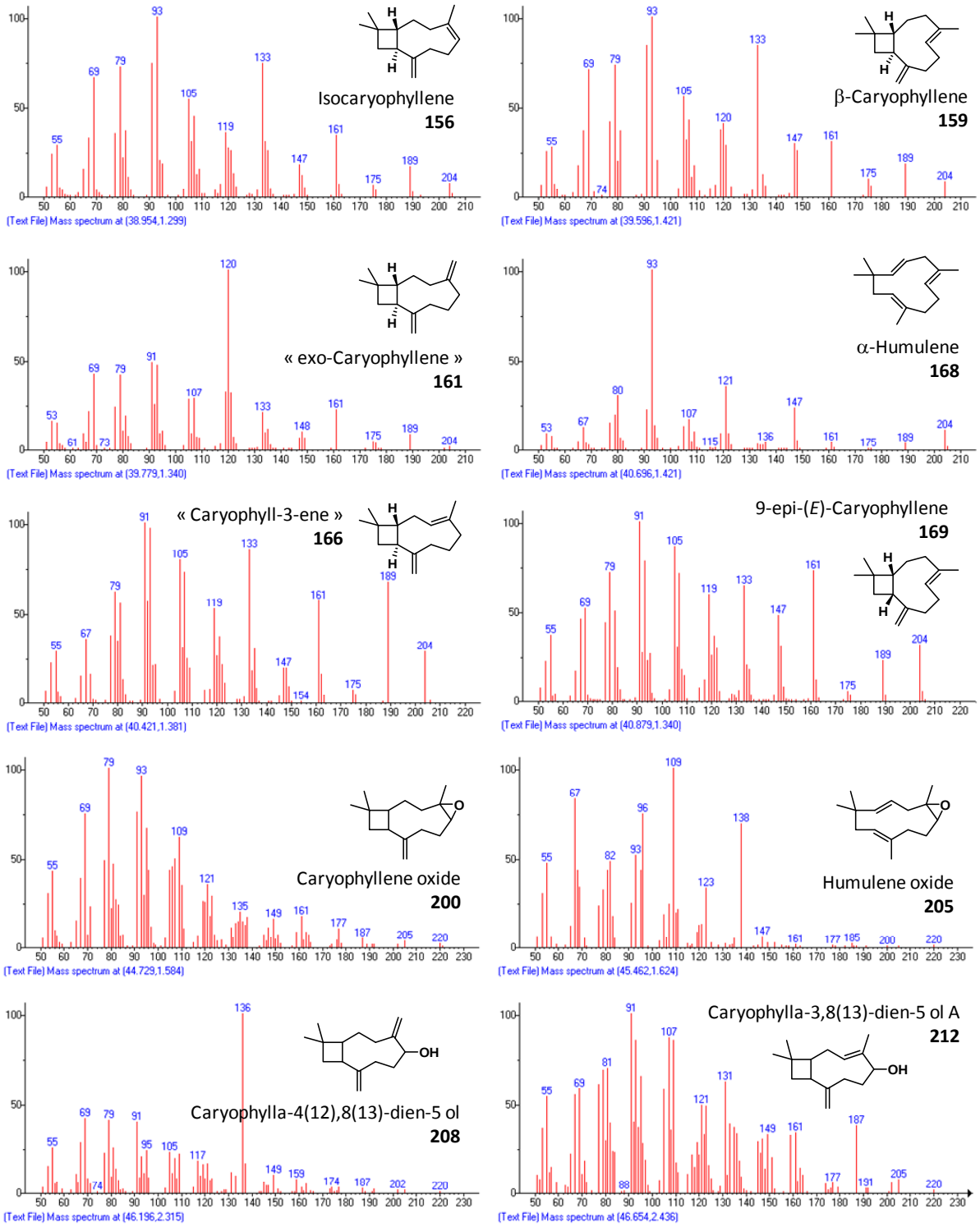

Fig. S1. Caryophyllane sesquiterpenes and sesquiterpenoids identified as volatile constituents of hashish samples (compounds **212** and **214** show similar mass spectra).

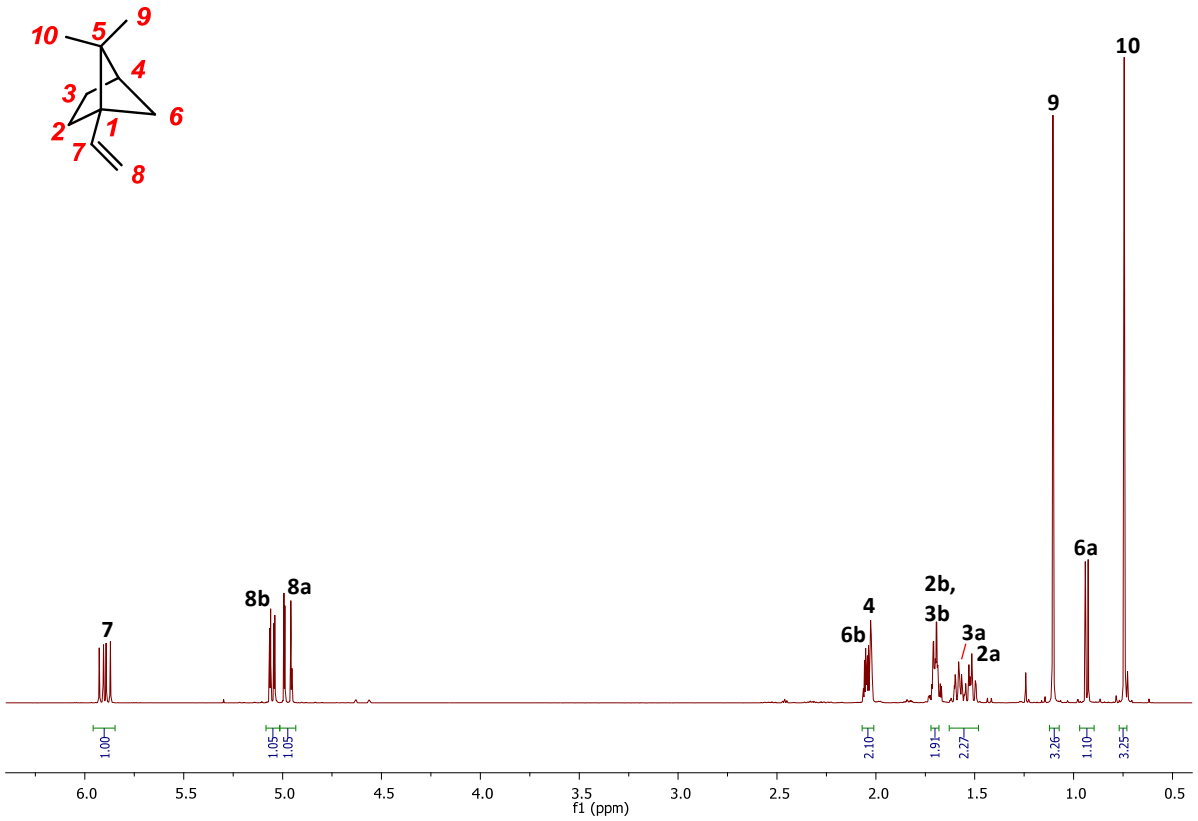

Figure S2. ^{1}H-NMR spectrum for 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane.

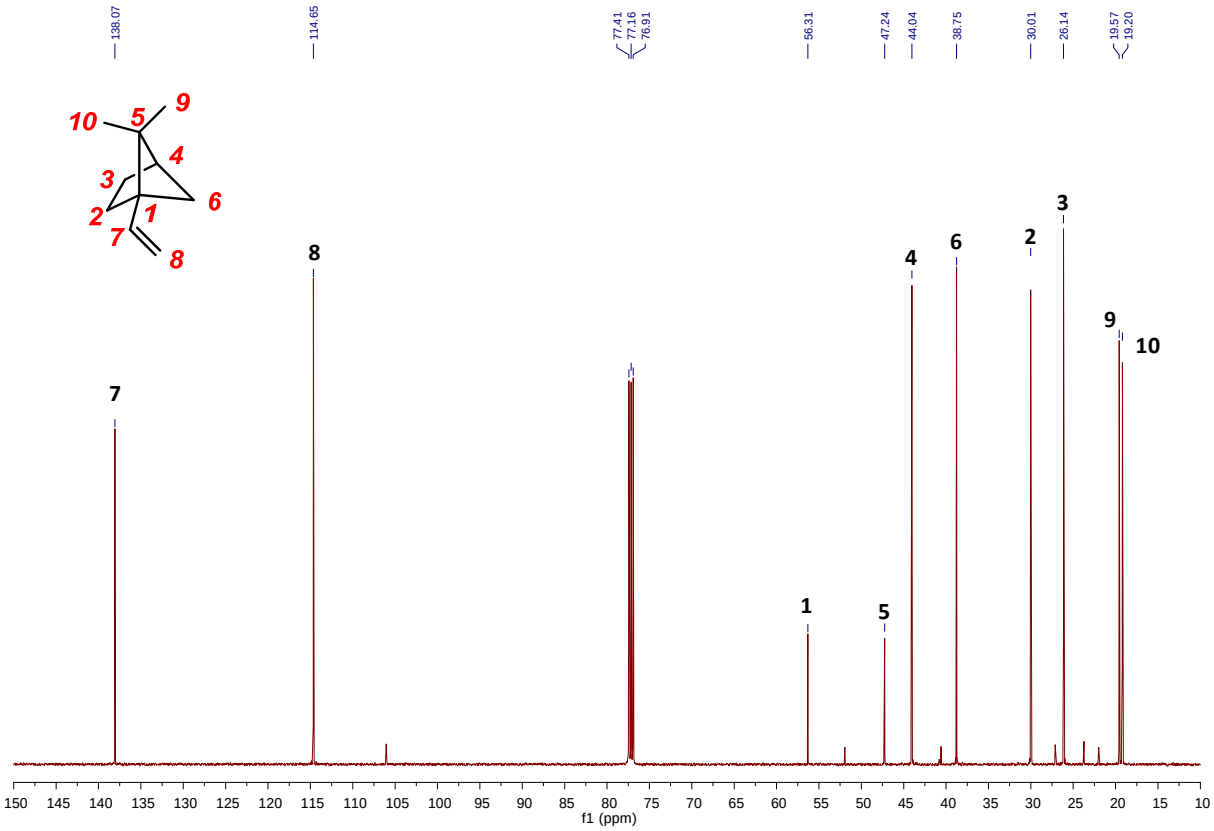

Figure S3. ^{13}C-NMR spectrum for 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane.

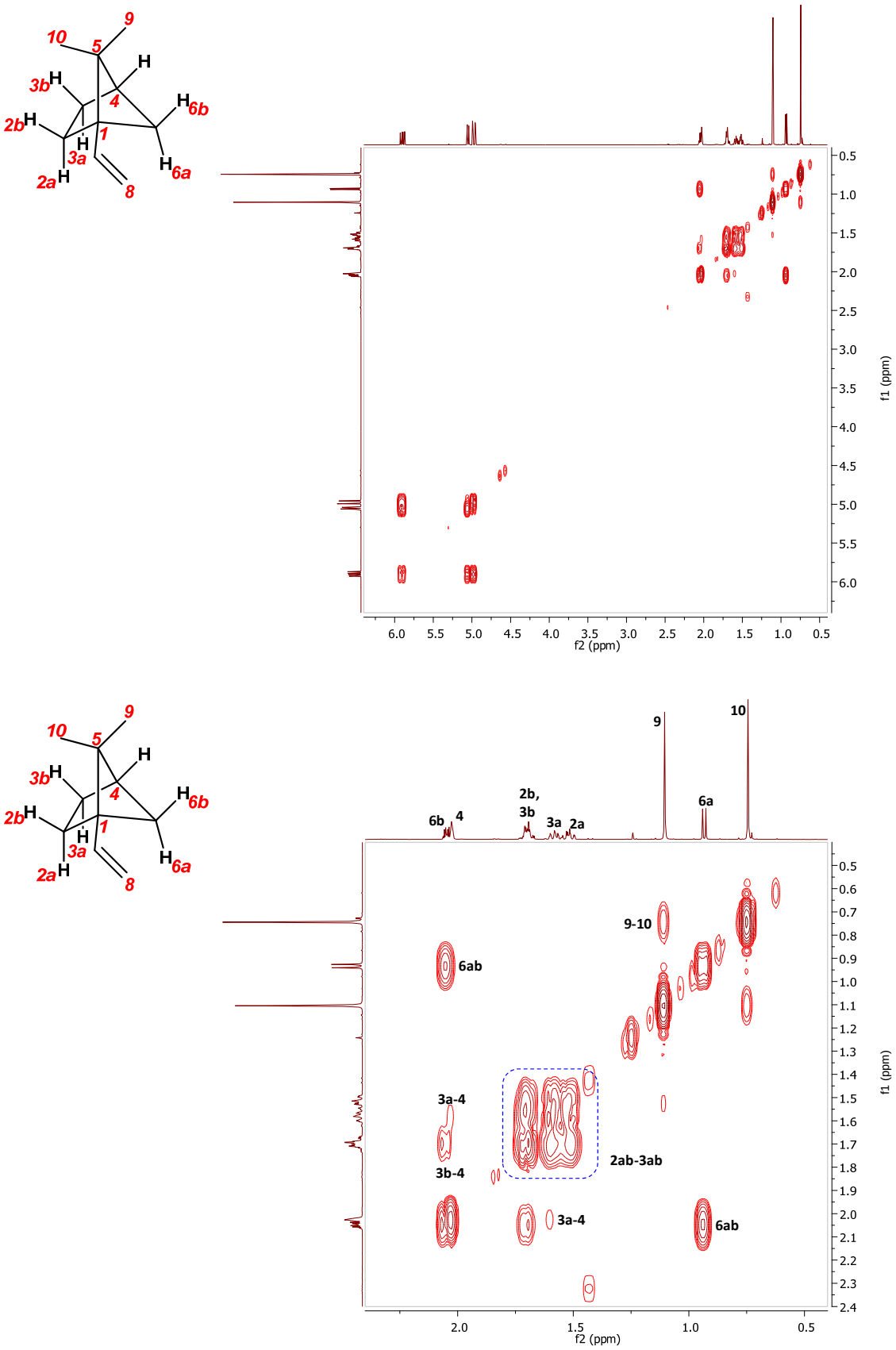

Figure S4. COSY spectrum for 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane. Full spectrum (top), expansion in the aliphatic region (bottom).

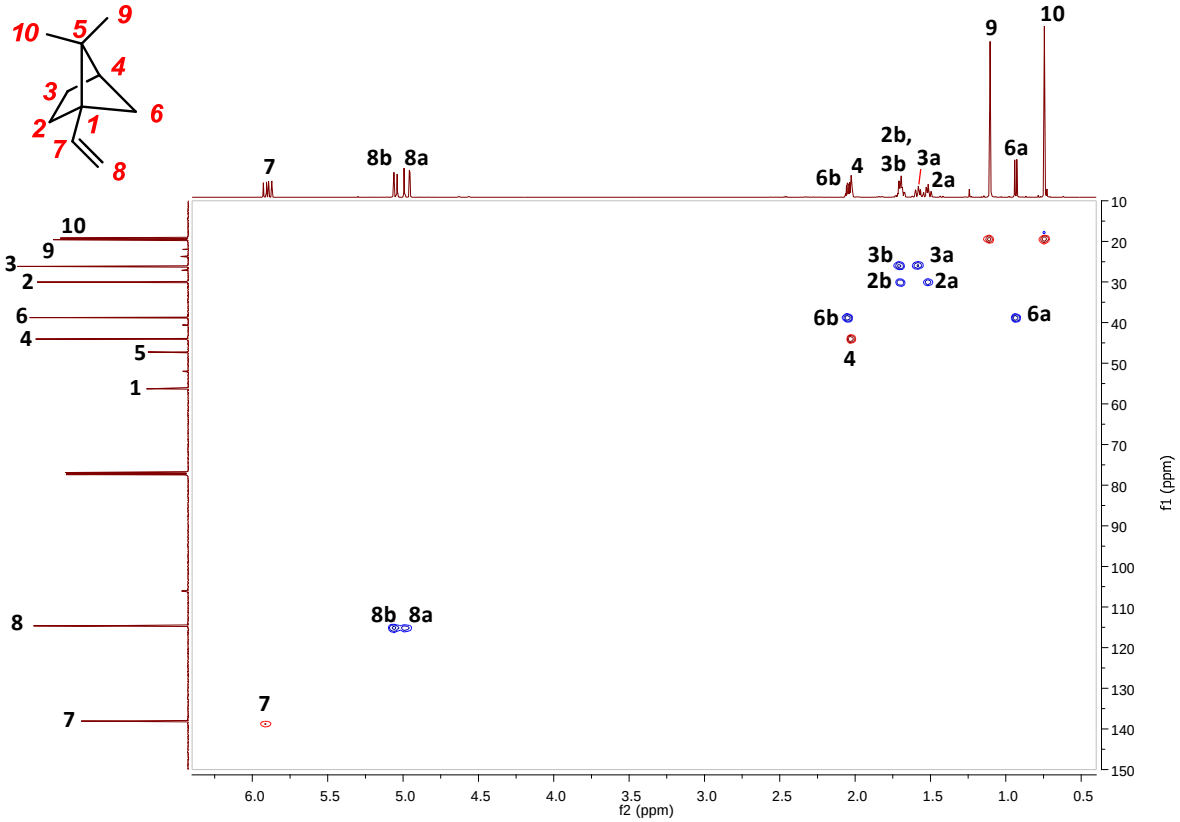

Figure S5. HSQC spectrum for 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane.

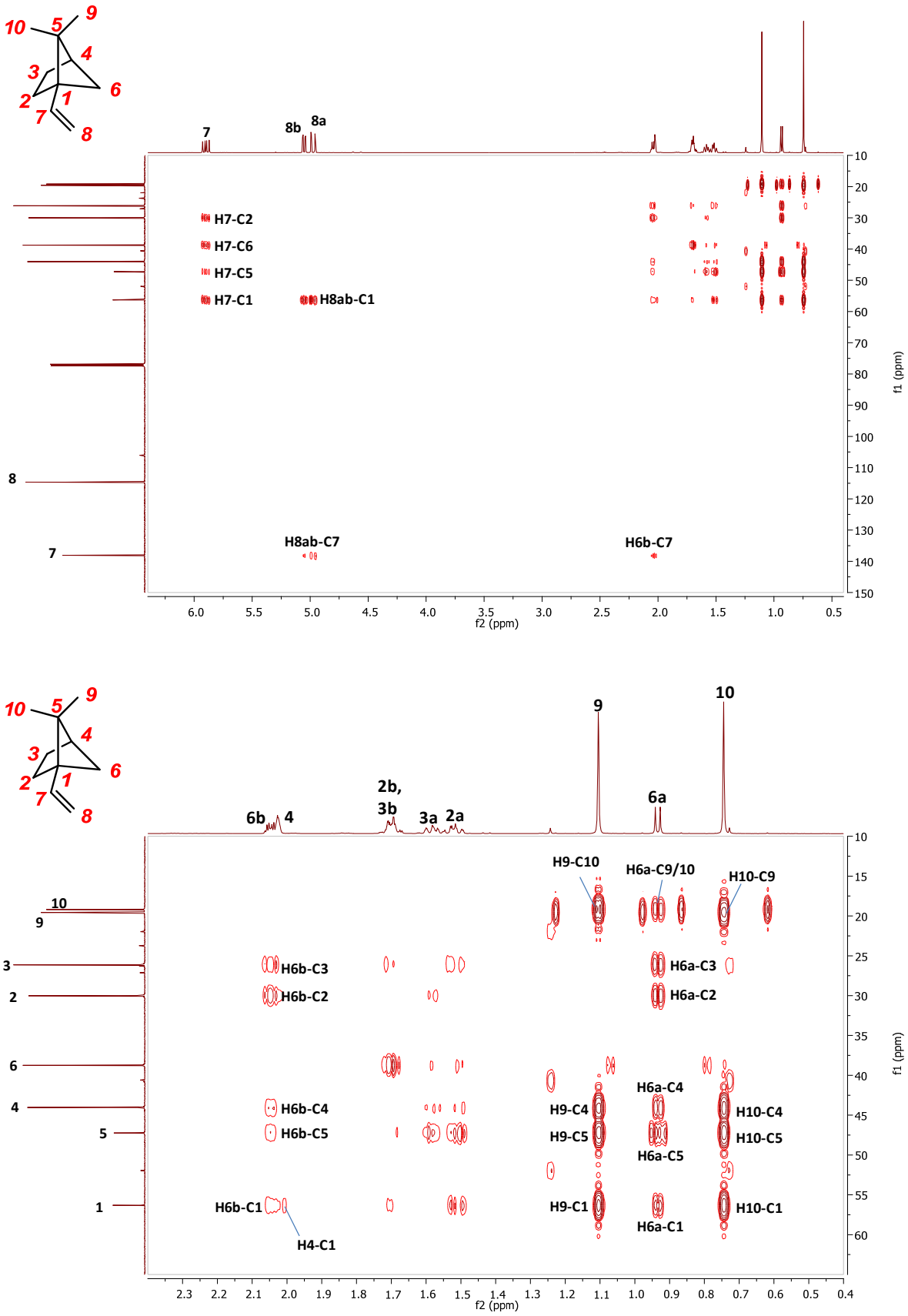

Figure S6. HMBC spectrum for 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane. Full spectrum (top), expansion in the aliphatic region (bottom).

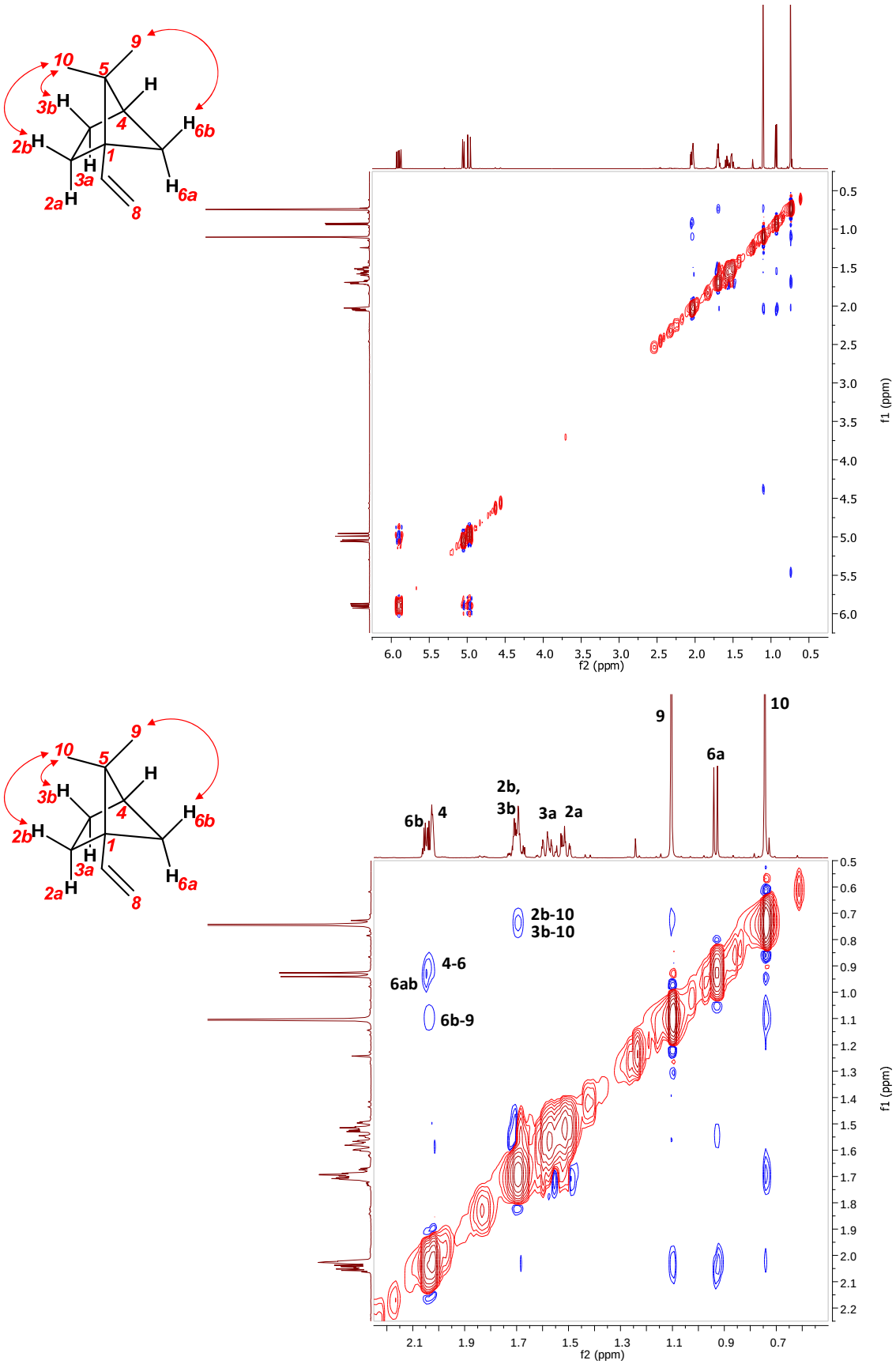

Figure S7. NOESY spectrum for 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane. Full spectrum (top), expansion in the aliphatic region (bottom).

Table S1. Most cited terpenoids previously identified as volatile constituents of *Cannabis sativa* L. samples.

Compound name	RI liter.[a]	Citations[b,c, d]
Monoterpenes		
Tricyclene	927	24, 25
α-Thujene	932	**19***, 20, 21, 22, 24, 25
α-Pinene	936	**16**, 18, **19***, 20, **21**, **22**, **23**, **24**, **25**, **26**, **27**, 28, **29**
Camphene	950	16, 18, 19, 20, 21, 22, 24, 25, 26, **27**, 28, 29
Sabinene	973	17, 21, 24, 25
β-Pinene	978	16, 19, 20, **21**, **22**, **23**, **24**, **25**, **26**, **27**, 28, **29**
β-Myrcene	987	**16**, 18, 19, 20, **21**, **22**, **23**, **24**, **25**, **26**, **27**
α-Phellandrene	1002	16, 20, 22, 24, 25
Δ^3-Carene	1010	16, 20, **22**, 23, 24, 25, 26, 27, 28
α-Terpinene	1013	16, 19, 20, 21, 22, 24, 25, 26, 27
p-Cymene	1015	16, 19, 20, 24, 25, 26, 28
β-Phellandrene	1023	**16**, 19, 20, 25, **26**, 27
Limonene	1025	16, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29
(Z)-β-Ocimene	1029	16, 20, 21, 22, 23, 24, 25, 26, 27, 29
(E)-β-Ocimene	1041	18, 19, 20, **22**, **24**, **25**, **26**, 27
γ-Terpinene	1051	**16**, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28
α-Terpinolene	1082	16, 20, 21, **22**, 23, **24**, **25**, 26, 27
Monoterpenoids		
Eucalyptol (1,8-cineole)	1024	17, 21, 22, 24, 25, 28, 29
trans-Sabinene hydrate	1053	16, 20
Fenchone	1069	17, 21
cis-Linalool oxide (furanoid)	1072	16, 20
Linalool	1086	16, 20, **21**, 23, 24, 25, 26
Fenchol	1099	17, 21, 23, 24, 26, 28
Ipsdienol	1123	18, 24
Borneol	1150	17, 21, 24, 25, 26
Terpinen-4-ol	1164	16, 20, 21, 22, 24, 25
α-Terpineol	1176	16, 20, 21, 22, 23, 24, 25, 26
Sesquiterpenes		
α-Cubebene	1355	17, 24
α-Ylangene	1376	18
α-Copaene	1379	17, 21
β-Elemene	1389	17 (γ), 21
Isocaryophyllene (syn. *(Z)*-caryophyllene)	1409	**19**, 20, 22, **29**
Longifolene	1417	**17**, 20
cis-α-Bergamotene	1418	**16**, 21, 22
(E)-β-Caryophyllene	1427	**16**, **17**, 18, **19***, 20, **21**, **22**, **23**, **24**, **25**, **26**, **27**, 28, **29**
trans-α-Bergamotene	1434	**16**, **19***, 20, 21, **22**, **23**, 24, 25, 26, 27
α-Guaiene	1440	18, 23, 24, 25
(*E*)-β-Farnesene[e]	1446	**16**, **19**, 20, 21, **23**, 24, 25, 26, 27
α-Humulene[f]	1455	**16**, **17**, 18, **19**, 20, **21**, **22**, **23**, **24**, **25**, **26**, **27**, 28, **29**
allo-Aromadendrene	1462	17, 21, 22, 24, 25
Curcumene, α- (RI for β: 1503)	1473	**16**, 20, 26
γ-Muurolene	1474	24, 25
β-Selinene	1486	**19**, 20, 21, 22, 24, 25, **29**
α-Selinene	1494	**16**, 18, **19**, 20, 21, 22, 24, 25, 26
Valencene	1494	22, **29**
β-Bisabolene	1503	18, 22, 24, 25, 26
γ-Cadinene	1507	17, 21, 24, 25
δ-Cadinene	1520	17, 24, 25
Selina-4(15),7(17)-diene	1534	**19**, 20
Selina-3,7(17)-diene	1542	**19**, 20, 24, 25, **29**
Germacrene-B	1552	22, 24, 25
Sesquiterpenoids		

Compound name	RI liter.[a]	Citations[b,c, d]
Nerolidol (RI for *Z*: 1522 ; *E*: 1553)		20, 24 (*E*-), 25 (*E*-)
Caryophyllene oxide	1578	**16**, 20, 21, **22**, **23**, 24, **25, 26**
Humulene oxide	1593	20, 24
α-Guaiol	1593	20, **21**, 23, **29**
γ-Eudesmol	1618	20, 21, 23, 24, 25
β-Eudesmol	1641	21, 23, 25
α-Eudesmol	1653	21, 25
β-Bisabolol	1659	21, 25
α-Bisabolol	1673	20, 24, 25
epi-α-Bisabolol		21, 22

[a] Retention indices from the literature mainly taken from the Terpenoids Library (Mass Finder 4.0) available online at http://massfinder.com/wiki/Terpenoids_Library_List
[b] compounds listed here have been cited at least twice in the literature.
[c] citations appearing in bold indicate the compound has been reported as present above 1%.
[d] references 16-24 are dealing with essential oils (EO) of cannabis; references 25 and 26 are dealing with both EO and headspace analysis; references 27-29 are dealing with headspace constituents only.
[e] probably wrongly reported as the (*Z*)-β-farnesene in references 21 and 23, no stereochemistry indicated in 16.
[f] probably wrongly reported as β-humulene in references 16, 19, 20 and 25.
*an asterisk indicate that compounds were reported to coelute in the corresponding reference.

Table S2. Factors and their levels evaluated during the optimization of HS-SPME of hashish samples.

	Parameters	Level -	Level +
X1	Sample weight (g)	0.5	1.0
X2	SPME fiber	100µm PDMS	CAR/DVB/PDMS (Tri.)
X3	Temperature (°C)	22 (R.T.)	65
X4	Incubation time (min)	30	60
X5	Extraction time (min)	20	40

Table S3. Hadamard matrix for the experimental design.

Experim. #	X1	X2	X3	X4	X5
1	+	+	+	-	+
2	-	+	+	+	-
3	-	-	+	+	+
4	+	-	-	+	+
5	-	+	-	-	+
6	+	-	+	-	-
7	+	+	-	+	-
8	-	-	-	-	-

Table S4. Experimental datasheet summarizing the full experimental design.

Experim. #	Sample weight (g)	SPME fiber	Temp. (°C)	Incub. Time (min)	Extract. time (min)	Total area monoterpenes	Total area sesquiterpenes	Area ratio mono./sesqui.
1	1.0	Tri.	65	30	40	2970050887	4030196821	0.74
2	0.5	Tri.	65	60	20	2340631996	3388024051	0.69
3	0.5	PDMS	65	60	40	3096816610	4123095709	0.75
4	1.0	PDMS	R.T.	60	40	2776641137	1580795373	1.76
5	0.5	Tri.	R.T.	30	40	1941254226	1618686461	1.20
6	1.0	PDMS	65	30	20	3255986890	3567518678	0.91
7	1.0	Tri.	R.T.	60	20	2921018612	1062763525	2.75
8	0.5	PDMS	R.T.	30	20	2292967457	946031499	2.42