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Hexahydrocannabinol (HHC) on the light cannabis market: the latest "new" entry

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Title page

Full title
Hexahydrocannabinol (HHC) on the light cannabis market: the latest "new" entry

Authors

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Short running title
HHC on hemp market

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Keywords
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Abstract

Hexahydrocannabinols (HHCs), referred to (9*R*)-HHC and (9*S*)-HHC diastereoisomers, are low-studied cannabinoids naturally found in small concentrations in the pollen and the seeds of the hemp plants. Despite the lack of in-depth studies about HHCs activity, potency, toxicity, and safety, these cannabinoids are emerging on the light-cannabis (hemp) market probably because legislations still do not clearly regulate them. Here, we describe for the first time the finding of HHCs (42% of (9*R*)-HHC and 24% of (9*S*)-HHC) in two samples of hemp-derived resin. The achievement of reference standards by semi-synthetic or isolation approach allows us to develop and validate a gas chromatography mass spectrometry (GC-MS) method for the identification and quantification of HHCs in hemp-derived products. A thorough investigation could be carried out to reveal the possible addition of "new" compounds that might be a matter of safety.

1. Introduction

Hexahydrocannabinols (HHCs, refer to as (9*R*)-HHC and (9*S*)-HHC diastereoisomers, compounds **4**(*R*) and **4**(*S*), Figure 1) are relatively low-studied cannabinoids naturally found in small concentrations in the pollen and the seeds of the hemp plants that are emerging on the light-cannabis (hemp) market.[1] Firstly described by Roger Adams in 1944 [2], HHCs have recently kept the attention of toxicology researchers and analysts since laboratories all over the world started to synthesize them, applying old semi-synthetic approaches.[3] Indeed, HHCs can be obtained in two steps, by acidic treatment and successive hydrogenation, starting from cannabidiol (CBD, **1**), or by direct reduction of Δ^9-tetrahydrocannabinol (Δ^9-THC, **2**) or Δ^8-tetrahydrocannabinol (Δ^8-THC, **3**) present in hemp [4], as shown in Figure 1, in order to add them to hemp-derived products. However, in addition to these semi-synthetic procedures, stereoselective strategies for obtaining (9*R*)-HHC **4**(*R*) have been reported in the literature.[5–9]

Despite the lack of in-depth studies about HHCs activity, potency, toxicity and safety, these cannabinoids are used by hemp derived products industries probably because legislations still do not clearly regulate them (thus making them legal) and consumers report some effects, that it is still unclear whether they may be THC-like or not. In fact, studies showed that the stereoisomer (9*R*)-HHC apparently has more affinity for the endocannabinoid receptors 1 and 2 (the same ones that Δ^9-THC and other cannabinoids bind)[10] than its epimer, (9*S*)-HHC [11] and, for this reason, the composition in (9*R*)- or (9*S*)-HHC of the hemp-derived products might influence their potency and effects.

In our laboratory, we analyzed two samples of hemp-derived resin brought by a retailer for the determination and quantification of the main cannabinoids by gas chromatography mass spectrometry (GC-MS) analysis.[12] We noticed interfering compounds at Δ^9-THC retention time so we decided to investigate their nature and structures.

The aim of this work was the identification, the obtaining of reference standards by a semi-synthetic and isolation approach and the quantification of (9*R*)- or (9*S*)-HHC by a developed and validated GC-MS method.

2. Materials and Methods

2.1 Chemicals

All chemicals and reagents used were of synthetic or analytical grade and purchased from Sigma-Aldrich (Milan, Italy). The progress of reactions and the compounds isolation from natural matrix were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Sigma-Aldrich silica gel plates (60 F254) using UV light, anisaldehyde/H_2SO_4/EtOH solution. Flash chromatography

was performed with normal phase silica gel (Sigma-Aldrich 230–400 mesh silica gel). Nuclear magnetic resonance spectra were recorded at 298K on a Bruker AM-500 spectrometer equipped with a 5-mm inverse-geometry broadband probe and operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C (see Supplementary Materials for NMR attribution and spectra).

2.2 *Cannabinoids analysis of two industrial hemp resin by GC-MS*

Cannabinoids analysis was assessed by previously published methods.[12] Briefly, an aliquot of 50 mg of industrial hemp resin, brought at the Forensic Toxicology Lab of University of Milan by a retailer, were extracted with 5 mL hexane and sonicated (ca. 20 min at room temperature). 10 µL aliquot of the supernatant was collected, added to 25 µL of THC-d_3 (10 µg/mL) as internal standard and dried. The residue was derivatized by adding MSTFA (50 µL) at 70 °C for 15 min and 0.2 µL aliquot was injected into GC-MS system for Δ^9-THC, THC-A, CBD and CBD-A determination. In addition, general unknown screening was conducted by GC-MS in order to identify additional compounds.

2.3 Isolation and synthesis of diastereomeric mixture of (9R)-HHC and (9S)-HHC

2.3.1 Isolation of diastereomeric mixture of (9R)-HHC and (9S)-HHC

Starting from 100 mg of matrix, purification by flash chromatography (Hexane/AcOEt 95:5, v:v) afforded a mixture of (9*R*)-HHC and (9*S*)-HHC as a light brown oil (30 mg). ^{1}H NMR analysis of the crude mixture showed a diastereomeric ratio of 7:3 (9*R*)-HHC: (9*S*)-HHC. The diastereomeric ratio was confirmed by GC-MS analysis as described at 2.4.1 (72.2: 27.8, (9*R*)-HHC: (9*S*)-HHC; 6.79 min (major), 7.36 min (minor)).

2.3.2 Synthesis of diastereomeric mixture of (9R)-HHC and (9S)-HHC staring from (-)-Δ^9-THC

(-)-Δ^9-THC (1 mg) in methanol (2 ml) was catalytically hydrogenated with Pd 10% wt. on activate carbon catalyst (1 mg) at atm. press. The reaction was stirred at room temperature and monitored by TLC (Hexane/AcOEt/ 9:1, v:v). After 1h of stirring the catalyst was filtered off and the solvent was removed under vacuum. ^{1}H NMR analysis of the crude mixture showed a (9*S*)-HHC as the major diastereomer (> 90% diasteromeric excess) as a light brown oil (0.7 mg). The diastereomeric ratio was confirmed by GC-MS analysis as described at 2.4.1 (92.5: 7.5, (9*S*)-HHC: (9*R*)-HHC; 6.79 min (minor), 7.36 min (major)).

2.3.3 Partial purification (9R)-HHC from diastereomeric mixture

Starting from 30 mg of diastereomeric mixture (ratio of 7:3) of (9*R*)-HHC and (9*S*)-HHC, two purifications by flash chromatography (Hexane/Et_2O, 95:5, v:v) afforded a light brown oil (4 mg). ^{1}H NMR analysis of the crude mixture showed a (9*R*)-HHC as the major diastereomer (> 95% diastereomeric excess. The diastereomeric ratio was confirmed by GC-MS analysis as described at 2.4.1 (95.5: 4.5, (9*R*)-HHC: (9*S*)-HHC; conditions described at paragraph 2.4.1; 6.80 (major), 7.37 min (minor)).

2.4 HHCs confirmation analysis

Since no HHCs reference standards were available in our laboratory, their obtaining was performed as described at paragraph 2.3. Once HHC reference compounds were obtained, a GC-MS method was developed and validated for their identification and quantitation in the above-mentioned samples, according to FDA guidelines for drugs.[13]

2.4.1 Instrumental

Our GC-MS routine method for cannabinoids determination was adapted for HHC analysis.[12] HHC analysis was performed on an Agilent 6890 Plus gas chromatograph interfaced with a single quadrupole 5973 N detector (Palo Alto, CA, USA). The GC separation was carried out on an Agilent capillary column CP sil 8 CB (15 m x 0.25 mm i.d., 0.25 µm film thickness) using the following conditions: from 120 °C to 200 °C at 40 °C/min, then to 250 °C at 6 °C/min and finally to 300 °C at 60 °C/min; injector temperature: 280 °C; ion source temperature: 230 °C; carrier gas (helium) flow 1.1 mL/min; injection mode: splitless; injection volume: 0.2 µL; run time: 13 min; mass spectrometer mode: electron ionization by Single Reaction Monitoring (SIM) mode, using the target and qualifier ions shown in Table 1.

2.3.4 Preparation of standard solutions, calibrators and quality control samples

Stock standard solution of THC-d_3 (0.1 mg/mL), (9*R*)-HHC (0.7 mg/mL) and (9*S*)-HHC (0.3 mg/mL) were stored at −20 °C in the dark. Working solutions were prepared in ethanol from stock solutions and used for the preparation of calibration curves and quality control (QC) samples, at the concentration of 0.01 mg/mL for THC-d_3 and 0.1 mg/mL for (9*R*)- and (9*S*)-HHC. Calibration standards were prepared by adding suitable amounts of working solutions at the following concentrations: 0.35, 0.7, 1.75, 3.5, 7 % for (9*R*)-HHC and 0.15, 0.3, 0.75, 1.5, 3 % for (9*S*)-HHC.

2.3.5 Samples preparation for HHC analysis

The two resin samples were prepared as described at paragraph 2.2. After derivatization, the samples were diluted 1:10 (v/v) in MSTFA and 0.2 µL aliquots were injected into GC-MS system for HHCs determination.

2.3.6 Method validation

A GC-MS procedure for HHCs determination was adapted from in-house previous validated and published method [12] in accordance with international recommendations for the validation of new analytical methods endorsed by FDA guidelines.[13] Calibration standards and quality controls were obtained by spiking appropriate amounts of (9*R*)- and (9*S*)-HHC working solutions (0.1 mg/mL). Six points calibration curves (0, 0.35, 0.7, 1.75, 3.5, 7 % for (9*R*)-HHC and 0, 0.15, 0.3, 0.75, 1.5, 3 % for (9*S*)-HHC) were generated based on the peak area ratios of the analytes to the IS against nominal analyte concentration using a linear regression. The correlation was tested over the whole range of concentration. Linearity was considered satisfactory if $r^2 \geq 0.990$ and CV≤15%. Sensitivity was expressed in terms of LOD (limit of detection) and LOQ (limit of quantification). The LOQ was determined as the lowest concentration with values for precision and accuracy within ±20% and a signal-to-noise (S/N) ratio of the peak areas ≥10. The LOD was determined as the lowest concentration with a signal-to-noise (S/N) ratio of the peak areas ≥3. Precision and accuracy of the method were determined through the analysis of six independent replicates of QC materials. Precision and accuracy were determined by calculating the coefficient of variation (CV%) and the Bias (BIAS%).

3. Results and discussion

As a first step, a routine GC-MS analysis for the quantitation of Δ^9-THC, CBD, THC-A and CBD-A was applied to the two hemp resin samples as described at paragraph 2.2. The resulting

chromatograms (see Supplementary Materials Figure S1) allowed us to reveal two uncommon abundant signals, one of these at the retention time close to that of Δ^9-THC and THC-d_3. The following unknown GC-MS screening in scan mode applied to the underivatized samples enabled to identify them as HHC by a good fit of the obtained mass spectra with the NIST14 library installed on the Agilent Chemstation (as described in Figure 2).

Then, a sample of light cannabis resin was subjected to a chromatographic evaluation using thin layer chromatography (TLC). The principal UV absorbing spot was isolated by flash chromatography using an eluent mixture system (Hexane/AcOEt 95:5, v:v, R_f= 0.3). The separated compounds responsible for the principal spot were subjected to mono- and bi-dimensional NMR analyses. These compounds structurally correspond to a diastereoisomeric mixture (7:3 ratio) of (9*R*)-HHC and (9*S*)-HHC. The correct stereochemistry attribution was done by chemical comparison of the ^{1}H-NMR signals obtained with those reported in the literature [5–9,14,15] for the (9*R*)-HHC epimer, achieved by stereoselective synthesis. Remarkably, the signals attributable to the compound present in greater quantity (70%) refer to the (*9R*)-isomer, while those present as minor components are of the (*9S*) epimer (30%) (Figure 3, A). Otherwise, the ^{13}C-NMR analysis, to the best of our knowledge, has been reported here for the first time.

However, given the high signals overlapping in the alkyl carbon area and the presence of disagreeing in ^{1}H NMR reported data,[3,5,6] further experiments were performed. For this purpose, catalytic hydrogenation of (-)-(Δ^9)-THC, marketed as an analytical standard, was carried out. Under, these synthetic conditions a mixture enriched in (9*S*)-HHC is obtained, as known from the literature.[3] Curiously, ^{1}H-NMR and GC-MS analyzes reveal an elevate diastereomeric excess of (9*S*)-HHC (> 90%) (Figure 3, B). Additionally, both the diastereomeric mixture of (9*R*)-HHC and (9*S*)-HHC isolated from the hemp resin (Figure 3, A) and synthetized from (-)-(Δ^9-THC (Figure 3, B) were injected into a 1290 Infinity liquid chromatography system (Agilent Technologies, USA) coupled to a Q Trap 5500 triple quadrupole linear ion trap mass spectrometer (Sciex, Germany). Interestingly, the two diastereoisomers separated on an Acquity Fluoro-Phenyl Column (130Å, 1.7 µm, 2.1 × 100 mm) (Waters, USA) using an isocratic elution with 0.1% formic acid in H_2O (35%) and 0.1% formic acid in ACN (65%) showed an opposite elution sequence compared to GC (see Supplementary Materials Figure S2). This aspect should be taken in consideration in order to avoid a misassignment of the two compounds.

Finally, it was attempted to find the chromatographic conditions suitable for the separation of the two epimers. Based on the literature,[3,6] a solvent mixture of hexane and diethyl ether was selected. Under the best conditions, performing two consecutive chromatographic columns (hexane: Et_2O), it was thus possible to obtain a sample enriched in (9*R*)-HHC epimer (> 95%). Noteworthy, by comparison of the ^{1}H- and ^{13}C-NMR spectra of the samples, enriched in (9*S*)-HHC or (9*R*)-HHC, it was possible to define some diagnostic signals. Specifically, the hydrogens on the C10 resonate with a different chemical shift (3.02 H-10α and 0.78 H-10β in (9*R*)-HHC and 2.86 H-10α and 1.31 H-10β in (9*S*)-HHC). Moreover, the C10 chemical shift is considerably different, 39.0 (9*R*)-HHC and 36.2 (9*R*)-HHC.

As soon as the (9*R*)- and (9*S*)-HHC standards were available, a GC-MS method for their identification and quantitation was developed and validated as described at paragraph 2.4. In detail, both compounds were introduced into a routine procedure for cannabinoids research in hemp derived products. (9*R*)- and (9*S*)-HHC calibration curves (n=6 each) showed good linearity (r^2 > 0.997) over the entire investigated range when using linear correlation. LOD and LOQ values have been calculated for both analytes and are listed in table 2 as well as an overview of the assessed validation data.

The validated method was then applied to *n*=2 samples of light cannabis resin, which were prepared and analyzed as described in paragraph 2.3.3. Table 3 shows the percentages of common cannabinoids (Δ^9-THC, THC-A, CBD, CBD-A) and (9*R*)- and (9*S*)-HHC present in the samples. Total concentration of Δ^9-THC (responsible for the psychoactive effects of cannabis) indicates that both samples do not fall within the definition of psychotropic drug for the Italian law. Indeed, for the Italian legislation, the minimum concentration (determined by the scientific community) of Δ^9-THC that can lead to mind-altering effects is 0.5%.[16] However, the discovery of other cannabinoids, such as Δ^8-THC, Δ^9-tetrahydrocannabivarin (THCV) and now HHCs, in light cannabis derived products is an increasing phenomenon. In detail, the two analyzed samples showed percentage of 42.5 and 41.5 for (9*R*)-HHC and of 23.6 and 23.6 for (9*S*)-HHC. Considering the low concentrations of these two compounds in nature, our finding might suggest an addition of semi-synthetic products during the production phase for unclear and suspicious purposes. Moreover, the prevalence of (9*R*)-HHC and its high concentration should be taken into consideration since it seems to be the more active between the two stereoisomers,[10,11] leading to possible unknown psychoactive and toxic effects in unaware users.

Conclusions

The low-studied phytocannabinoids (9*R*)-HHC and (9*S*)-HHC, naturally found in low concentrations in the pollen and the seeds of the hemp plants, for the first time, were noticed in two commercialized hemp-derived products at the amount of 42% and 24%, respectively. These cannabinoids are emerging on the light-cannabis (hemp) market probably because legislations still do not clearly regulate them. In Italy, light cannabis products are sold exclusively for collection with the indication “not for human consumption”, although it is well known that people actually consume them by smoking (as joints) or drinking (as herbal infusions). Since analytical assay for hemp-derived products usually include only Δ^9-THC, THC-A, CBD and CBD-A, a thorough investigation could be carried out in order to identify the presence of other cannabinoids or adulterants. This phenomenon should be considered a matter of public health since activity, safety and toxicity of compounds are still poorly studied. Further in-vitro and in-vivo studies are necessary to elucidate HHCs roles.

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CRediT authorship contribution statement

Sara Casati: Conceptualization, Data curation, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Paola Rota**: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Roberta Bergamaschi**: Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Erika Palmisano**: Validation, Formal analysis, Investigation. **Paolo La Rocca**: Formal analysis. **Alessandro Ravelli**: Investigation, Supervision. **Ilaria Angeli**: Supervision. **Mauro Minoli**: Supervision. **Gabriella Roda**: Supervision, Funding acquisition. **Marica Orioli**: Supervision, Funding acquisition.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure legends

Figure 1. Syntheses of HHCs by CBD and/or Δ^8-THC and/or Δ^9-THC

Figure 2. Mass spectra relative to one of the two unknown signals from hemp-derived product (above) and reference from NIST library (below) recognized as HHC

Figure 3. SIM chromatograms of the diastereomeric mixture of (9*R*)-HHC and (9*S*)-HHC isolated from the hemp resin (A) and synthetized from (-)-Δ^9-THC (B)

Tables

Table 1. GC-MS parameters for (9*R*)-HHC, (9*S*)-HHC and THC-d_3: retention time (RT), target and qualifier ions

Compound	RT	Target Ion	Qualifier ion 1	Qualifier ion 2
9*R*-HHC	6.67	265.2	332.2	345.3
9*S*-HHC	7.26	265.2	332.2	345.3
THC-d_3	7.18	306.1	374.3	389.3

Table 2. Validation parameters for selected analytes

Compound	CV %	Analytical range %	LOD %	LOQ %	Precision %	Accuracy %
(9*R*)-HHC	9.5	0.35-7	0.02	0.07	<12.3	< 9.9
(*9S*)-HHC	12.2	0.15-3	0.003	0.01	< 7.8	< 12.1

Table 3. Amount (expressed in percentage) of cannabinoids in the considered hemp-derived samples (*n*=2)

Sample	Δ^9THC%	THC-A%	THC tot%	CBD%	CBD-A%	CBD tot%	(*9R*)-HHC%	(*9S*)-HHC%
1	0.2	<LOQ	0.2	2.4	3	5.03	42.5	23.6
2	0.2	<LOQ	0.2	0.5	5	4.89	41.5	23.6

Supplementary Materials

Hexahydrocannabinol (HHC) on the light cannabis market: the latest "new" entry

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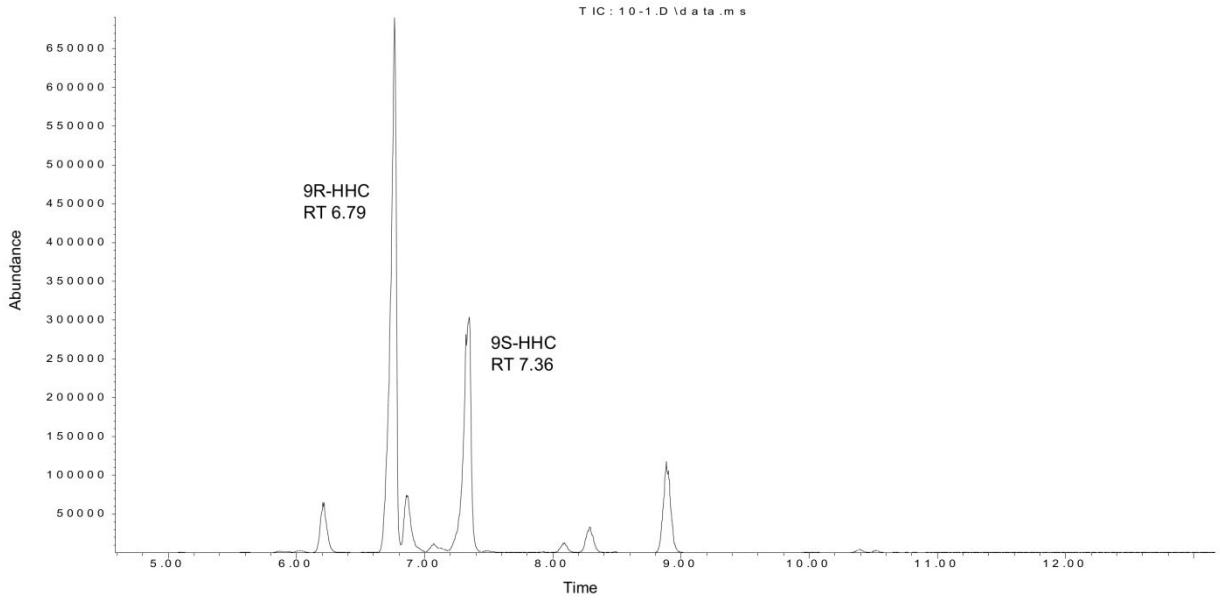
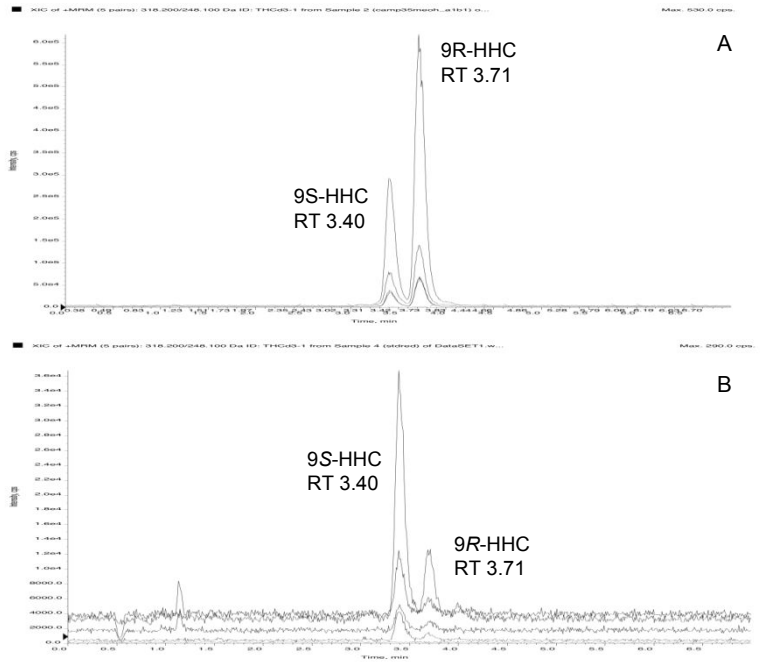


Figure S1. *SIM chromatogram of GC-MS analysis for cannabinoids relative to the considered hemp-derived product with uncommon consistent signals*

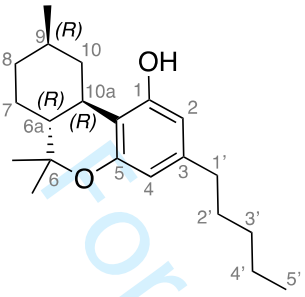


MRM parameters	Precursor ion (Da)	Product ions (Da)	Collision energy (eV)	Declustering potential (eV)
HHC-1	317.5	137.5	32	100
HHC-2	317.5	193.0	35	100
HHC-3	317.5	81.0	45	100

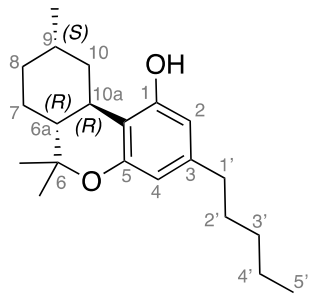
Figure S2. *Multiple Reaction Monitoring (MRM) parameters and chromatograms of the diastereomeric mixture of (9S)-HHC and (9R)-HHC isolated from the hemp resin (A) and synthetized from (-)-Δ⁹-THC (B) by liquid chromatography-tandem mass spectrometry*

Chemistry

Nuclear magnetic resonance spectra were recorded at 298K on a Bruker AM-500 spectrometer equipped with a 5-mm inverse-geometry broadband probe and operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. Chemical shifts are reported in parts per million and are referenced for ^{1}H spectra to a solvent residue proton signal (δ = 7.26 ppm for $CDCl_3$) and for ^{13}C spectra, to solvent carbon signal (central line at δ = 77.00, for $CDCl_3$). The ^{1}H and ^{13}C resonances were assigned by ^{1}H-^{1}H (COSY) and ^{1}H-^{13}C (HSQC and HMBC) correlation 2D experiments. The ^{1}H NMR data are tabulated in the following order: multiplicity (s=singlet, d=doublet, t=triplet, br=broad, m=multiplet, app=apparent), coupling constant(s) are given in Hz, number of protons, and assignment of proton(s). ^{1}H and ^{13}C NMR assignments for (9*R*)-hexahydrocannabinol and (9*S*,)-hexahydrocannabinol refer to these structures:



(9*R*)-HHC



(9*S*)-HHC

Isolation of diastereomeric mixture of (9R)-HHC and (9S)-HHC

Starting from 100 mg of matrix, purification by flash chromatography (Hexane/AcOEt 95:5, v:v) afforded a mixture of (9*R*)-HHC and (9*S*)-HHC as a light brown oil (30 mg). ^{1}H NMR analysis of the crude mixture showed a diastereomeric ratio of 7:3. The diastereomeric ratio was confirmed by GC-MS analysis (72.2: 27.8, (9*R*)-HHC: (9*S*)-HHC).

$[a]_D^{25}$ = -109.0 (c = 1, $CHCl_3$) of the diastereomeric mixture.

(9R)-HHC (Major): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.25 (br s app, overlapping, 1H, H-4), 6.07 (br s app, overlapping, 1H, H-2), 4.76 (s, 1H, OH), 3.03 (br d, 1H, J = 12.9 Hz, H-10α), 2.48-2.39 (overlapping, 3H, H-10a and 2H at C1'), 1.88-1.03 (overlapping, 11H, 2H at C7, 2H at C8, H-9, 2H at C2', 2H at C3' and 2H at C4'), 1.45 (overlapping, 1H, 6a), 1.37 (overlapping, 3H, 6α-CH_3), 1.08 (overlapping, 3H, 6β-CH_3), 0.94 (d, J = 6.6 Hz, 3H, CH_3 at C9), 0.88 (overlapping, t app, J = 6.7 Hz, 3H, H-5'), 0.80 (q app, 1H, J = 12.9 Hz, H-10β); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 154.9, 154.7 (2C, C1, C5), 142.5 (C3), 110.3 (C11), 110.0 (C4), 107.6 (C2), 77.0 (C6), 49.1 (C6a), 39.0 (C10), 35.5, 35.4 (3C, C1', C10α and C8), 32.8 (C9), 31.6 (C3'), 30.6 (C2'), 28.1 (C7), 27.7 (CH_3 at 6α), 22.6 (C4'), 22.5 (CH_3 at C9), 19.0 (CH_3 6β), 14.0 (C5'). The ^{1}H-NMR attribution was superimposable with that reported in the literature for the (9*R*)-HHC isomer obtained by stereo and regioselective synthesis.[5,6]

(9S)-HHC (Minor): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.25 (br s app, overlapping, 1H, H4), 6.07 (br s app, overlapping, 1H, H2), 4.69 (s, 1H, OH), 2.87 (br d, 1H, J = 13.0 Hz, H-10α), 2.67 (ddd, 1H, J = 2.3, J = 11.3, J = 13.0 Hz, H-10a), 2.48-2.39 (overlapping, 2H, 2H at C1'), 2.20-0.98 (overlapping, 15H, 2H at C7, 2H at C8, H-9, 2H at C2', 2H at C3', 2H at C4', H-10β and CH_3 at C9), 1.45 (overlapping, 1H; 6a), 1.37 (overlapping, 3H, 6α-CH_3), 1.08 (overlapping, 3H, 6β-CH_3), 0.88 (overlapping, t app, J = 6.7 Hz, 3H, H-5'); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 155.2, 154.6 (2C, C1, C5), 142.4 (C3), 110.5 (C11), 110.0 (C4), 107.6 (C2), 76.8 (C6), 49.9 (C6a), 36.2 (C10), 35.4 (2C, C1'and C8), 32.2 (C9), 31.6 (C3'), 30.6 (C2'), 29.3 (C10α), 27.9, 27.6 (2C, CH_3 at 6α and C7), 23.1 (C4'), 19.1, 18.8 (2C, CH_3 6β and CH_3 at C9), 14.0 (C5').

Synthesis of diastereomeric mixture of (9R)-HHC and (9S)-HHC staring from (-)-(Δ^9)-THC

(-)-(Δ^9)-THC (1 mg) in methanol (2 ml) was catalytically hydrogenated with Pd 10% wt. on activate carbon catalyst (1 mg) at atm. press. The reaction was stirred at room temperature and monitored by TLC (Hexane/AcOEt 9:1, v:v). After 1h of the catalyst was filtered off and the solvent was removed under vacuum. ^{1}H NMR analysis of the crude mixture showed a (9*S*)-HHC as the major diastereomer (> 90% diasteromeric excess) as a light brown oil (0.7 mg). The diastereomeric ratio was confirmed by GC-MS analysis (92.5: 7.5, (9*S*)-HHC: (9*R*)-HHC).

(9S)-HHC: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.24 (br s app, 1H, H4), 6.07 (br s app, 1H, H2), 4.60 (s, 1H, OH), 2.86 (br d, 1H, J = 12.9 Hz, H-10α), 2.66 (ddd, 1H, J = 2.3, J = 11.3, J = 12.9 Hz, H-10a), 2.48-2.39 (overlapping, 2H, 2H at C1'), 2.20-0.98 (overlapping, 15H, 2H at C7, 2H at C8, H-9, 2H at C2', 2H at C3', 2H at C4', H-10β and CH_3 at C9), 1.45 (overlapping, 1H; 6a), 1.36 (overlapping, 3H, 6α-CH_3), 1.08 (overlapping, 3H, 6β-CH_3), 0.88 (overlapping, t app, J = 6.7 Hz, 3H, H-5').

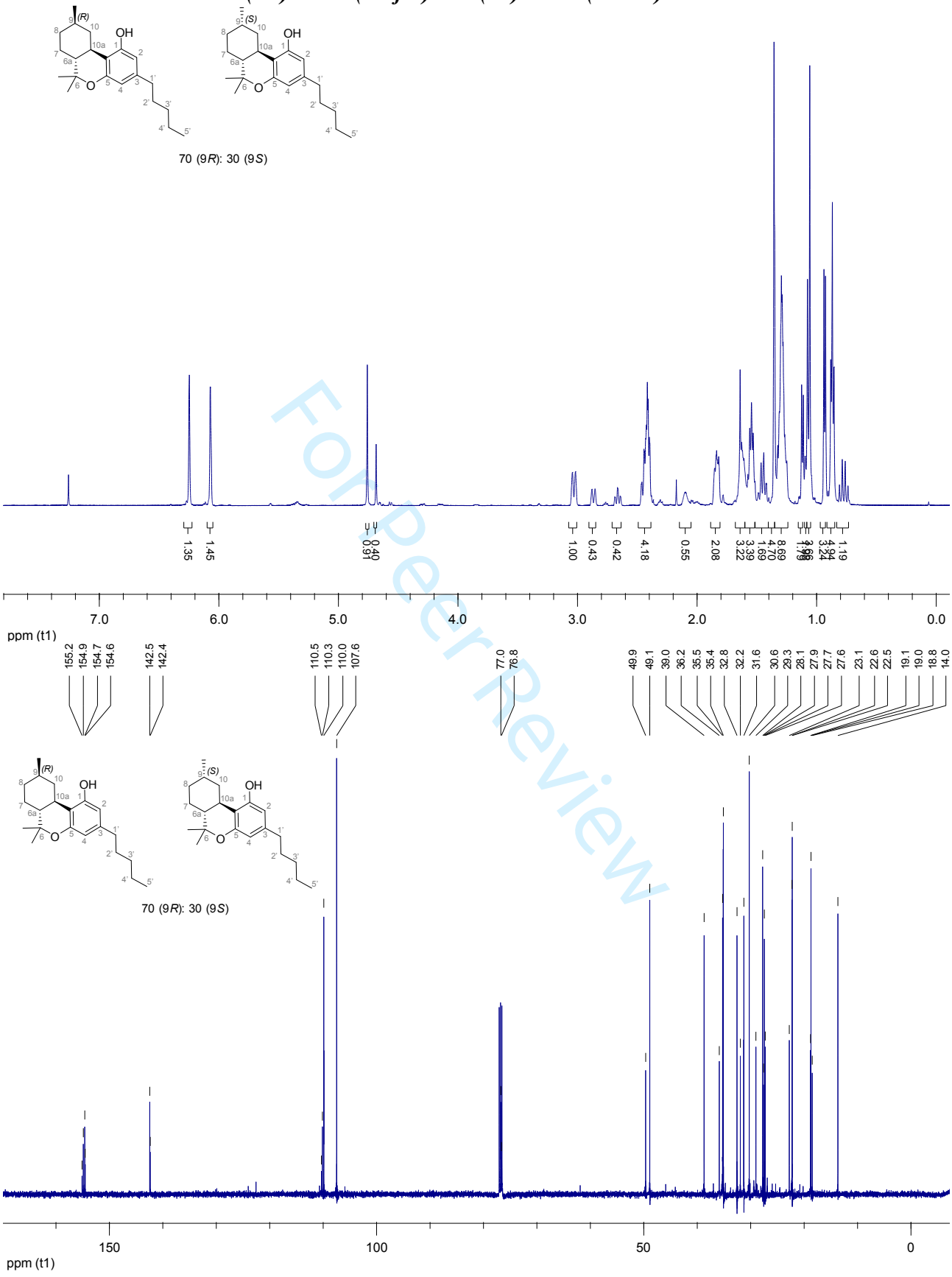
Partial purification (9R)-HHC from diastereomeric mixture

Starting from 30 mg of diastereomeric mixture (7:3 ratio) of (9*R*)-HHC and (9*S*)-HHC, two purification by flash chromatography (Hexane/Et_2O, 95:5, v:v) afforded a light brown oil (4 mg). ^{1}H NMR analysis of the crude mixture showed a (9*R*)-HHC as the major diastereomer (> 95% diastereomeric excess. The diastereomeric ratio was confirmed by GC-MS analysis (95.5: 4.5, (9*R*)-HHC: (9*S*)-HHC).

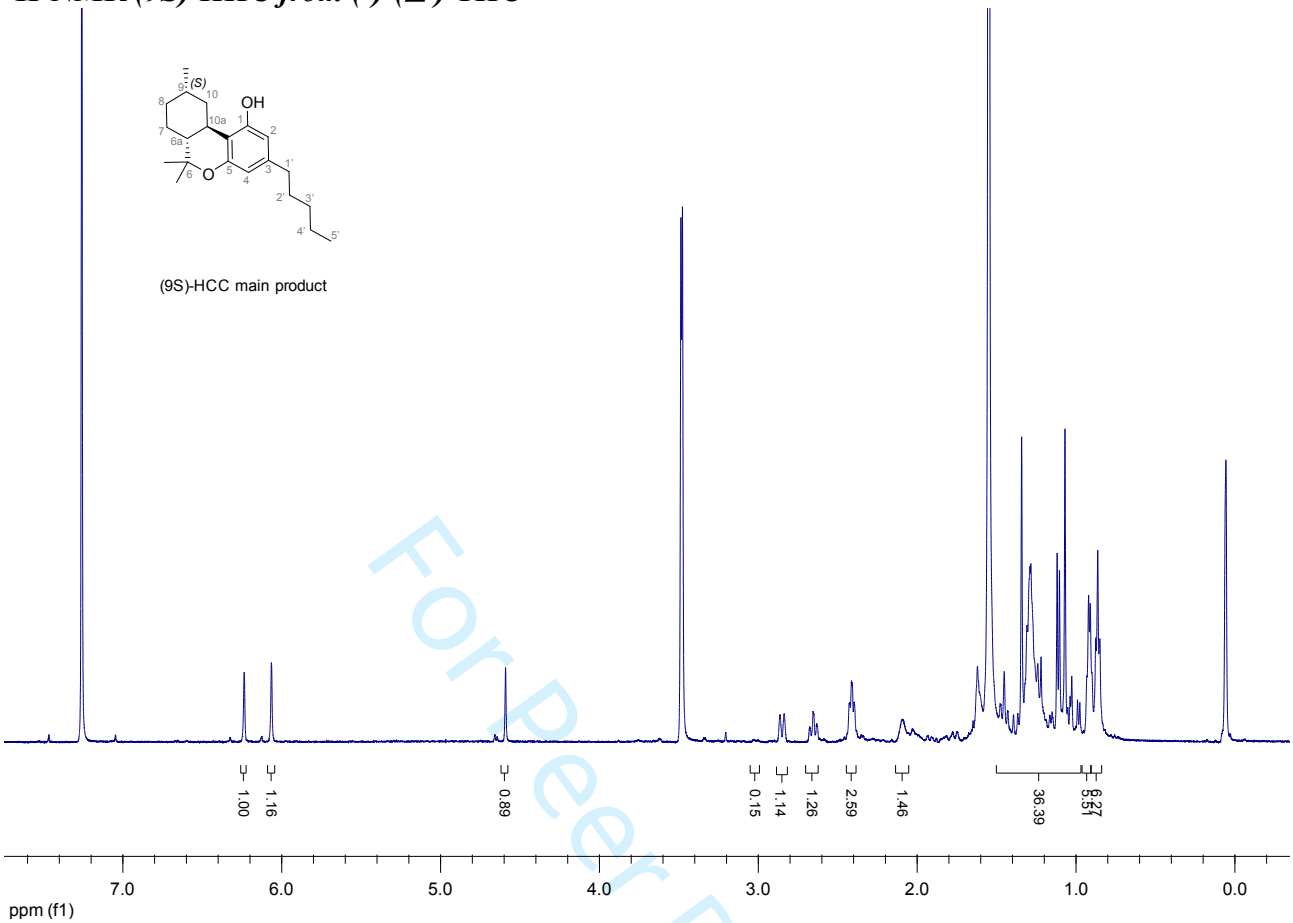
$[a]_D^{25}$ = -81.3 (c = 1, $CHCl_3$) of this diastereomeric mixture. [lit. $[a]_D$ of pure 9*R*)-HHC $[a]_D$ = -85.4 (c = 0.3, $CHCl_3$) (7); $[a]_D$ = -93.6 (c = 0.7, $CHCl_3$) (6); $[a]_D$ = -73.9 (c = 0.014, $CHCl_3$) (5); -74.1 (c = 1, $CHCl_3$).[8]

(9R)-HHC:^{1}H NMR (500 MHz, $CDCl_3$) δ 6.24 (d, J = 1.5 Hz, 1H, H-4), 6.08 (d, J = 1.5 Hz, 1H, H-2), 4.65 (s, 1H, OH), 3.02 (br d, J = 12.9 Hz, 1H, H-10α), 2.49-2.35 (overlapping, 3H, H-10a and 2H at C1'), 1.88-1.03 (overlapping, 11H, 2H at C7, 2H at C8, H-9, 2H at C2', 2H at C3' and 2H at C4'),1.44 (m, 1H; 6a), 1.36 (overlapping, 3H, 6α-CH_3), 1.06 (overlapping, 3H, 6β-CH_3), 0.94 (d, J = 6.6 Hz, 3H, CH_3 at C9), 0.88 (overlapping, t app, J = 6.9 Hz, 3H, H-5'), 0.78 (q app, 1H, J = 12.9 Hz, H-10β);^{13}C-NMR (125 MHz, $CDCl_3$) δ 155.0, 154.6 (2C, C1, C5), 142.5 (C3), 110.2 (C11), 110.0 (C4), 107.6 (C2), 76.9 (C6), 49.1 (C6a), 39.0 (C10), 35.5, 35.4 (3C, C1', C10α and C8), 32.9 (C9), 31.6 (C3'), 30.6 (C2'), 28.1 (C7), 27.8 (CH_3 at 6α), 22.6 (C4'), 22.5 (CH_3 at C9), 19.0 (CH_3 6β), 14.0 (C5').

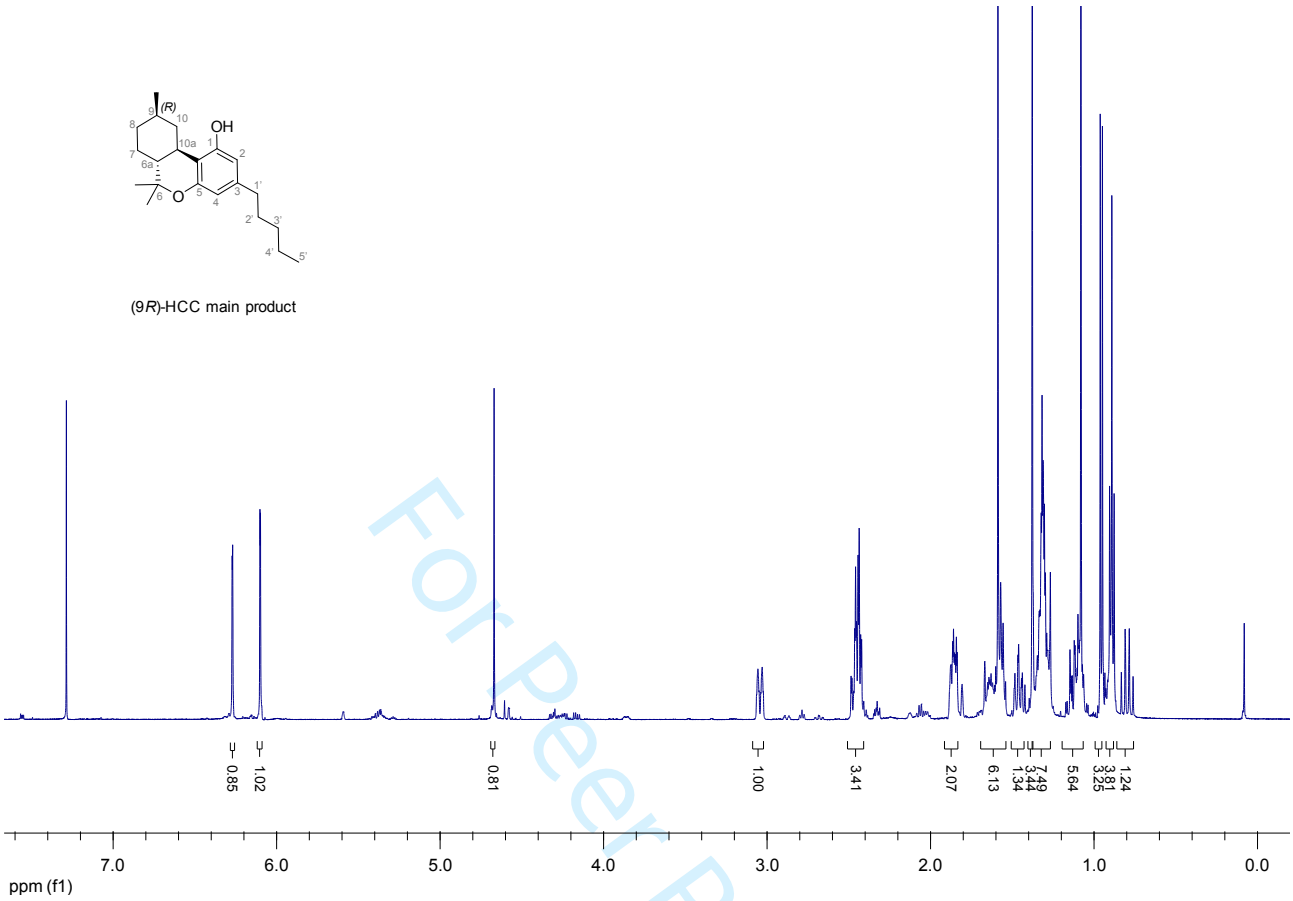
^{1}H-NMR and ^{13}C-NMR *(9R)-HHC (Major) and (9S)-HHC (Minor)*

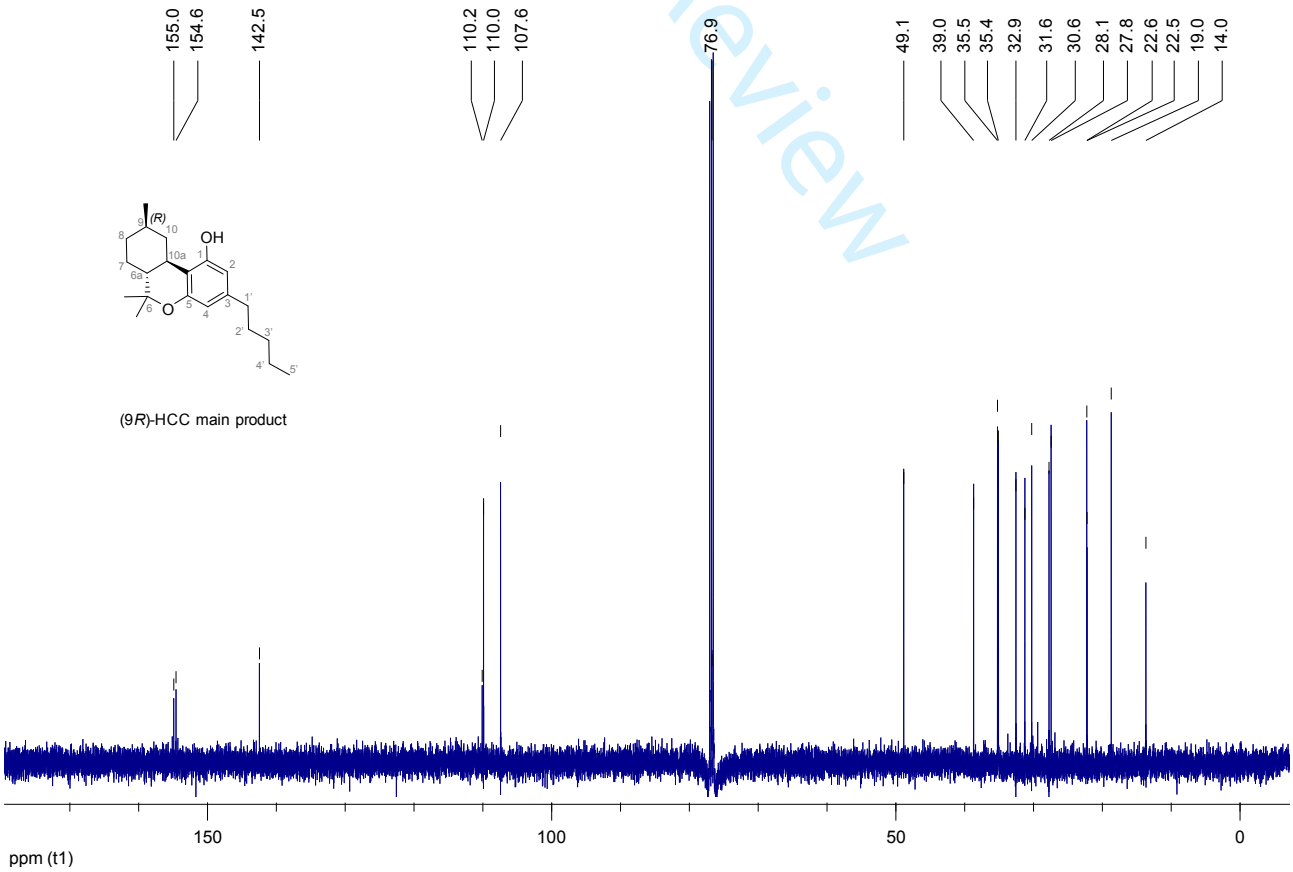


^{1}H-NMR *(9S)-HHC from (-)-(Δ^{9})-THC*



^{1}H-NMR and ^{13}C-NMR *(R)-HHC*





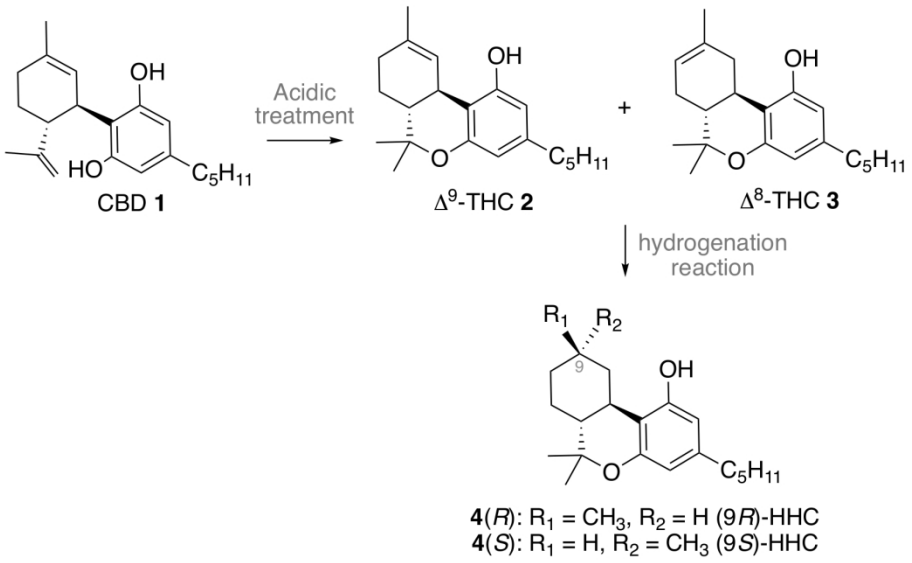


Figure 1. Syntheses of HHCs by CBD and/or Δ8-THC and/or Δ9-THC

163x99mm (300 x 300 DPI)

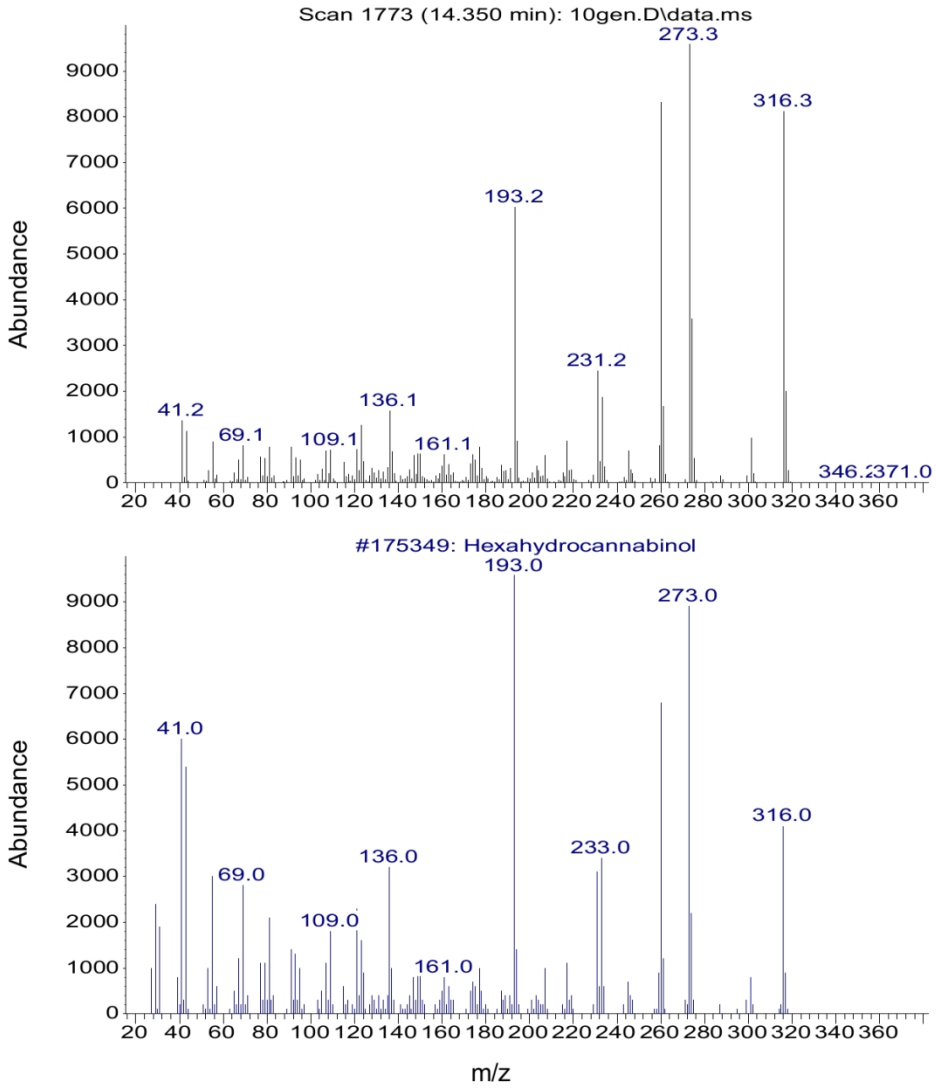


Figure 2. Mass spectra relative to one of the two unknown signals from hemp-derived product (above) and reference from NIST library (below) recognized as HHC

109x134mm (300 x 300 DPI)

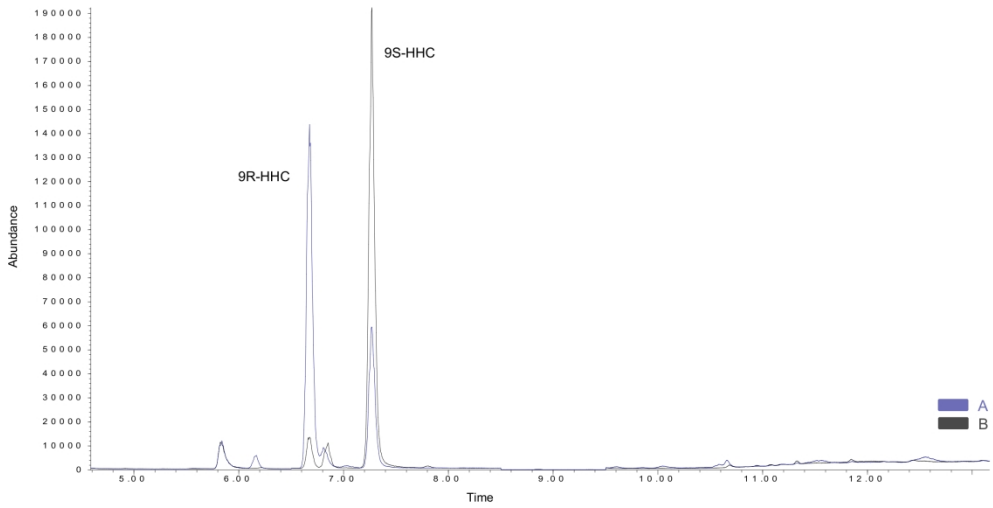


Figure 3. SIM chromatograms of the diastereomeric mixture of (9R)-HHC and (9S)-HHC isolated from the hemp resin (A) and synthetized from (-)-Δ9-THC (B)

231x125mm (300 x 300 DPI)