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Title page

GC-MS Metabolite profile and identification of unusual homologous cannabinoids in high potency *Cannabis sativa*

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Abstract

Phytochemical investigation of the lipids extracted from seeds of *Cannabis sativa* by GC-MS showed 43 cannabinoids, 16 of which are new. The extract is dominated by Δ^9-tetrahydrocannabinolic acid (A) and its neutral derivative *trans*-Δ^9-tetrahydrocannabinol-C_5 (THC). *Cis* and *trans*-Δ^9-tetrahydrocannabinol-C_7 isomers with an ethyl-pentyl branched chain together with minor amounts of *trans*-Δ^9-tetrahydrocannabinol with a methyl-pentyl C_6 branched side chain were identified as new natural compounds. Four cannabichromene isomers with a C_5 side chain are postulated that may be derived from the double bond migration at the terminal isoprenyl unit. A C_7 cannabichromene together with the neutral and acidic forms of the cannabinol-C_7 were also detected. The mass spectrum of these homologues as their trimethylsilyl (TMS) derivatives are presented and the fragmentation patterns are discussed.

Keywords

Cannabinoids, *Cannabis sativa*, Cannabaceae, Phytochemical analysis, GC-MS

Introduction

Hemp (*Cannabis sativa* L.) is an herbaceous annual dioecious crop plant that belongs to the family Cannabaceae. It is native to Northeast Asia where it has been grown since 5000 years [1]. Hemp has been used not only for recreational purposes due to its euphoric effects but also for medicinal uses. Its broad therapeutic potential for the treatment of many diseases is increasingly recognized [2]. The dried flowering tips and leaves are used as products known as marijuana. Trichomes, especially the capitate-stalked glandular hairs grouped together at specific parts of the female inflorescence are the main sites of cannabinoid production and are also found in the resinous secretion.

Hundreds of compounds have been isolated from hemp [3]. The number of elucidated constituents has been increasing in recent decades. Among the enormous variety of chemicals detected, noncannabinoid-type compounds are the main constituents while cannabinoids represent approximately 20% of the identified metabolites. Some cannabinoids such as Δ^9-tetrahydrocannabinol (Δ^9-THC) which is present in high amount in high potency *C. sativa* are psychoactive substances, and hemp has become the most frequently consumed illicit drug of abuse in the world [4].

Phytocannabinoids are C_{21} terpenophenolic secondary metabolites including both alkylresorcinol and monoterpene units in their molecular structure. Consensual current knowledge assumes that these constituents are not widespread in the plant kingdom but almost unique to cannabis [5]. Cannabinoids are synthesized in hemp as carboxylic acid congeners [6]. These acidic cannabinoids can be converted or degraded into their neutral decarboxylated analogs by action of heat, including that of the injector port of a chromatography apparatus, sunlight or storage, releasing their carboxyl-group in the form of CO_2 [7]. The most abundant cannabinoids are characterized by a *n*-pentyl side-chain. Further homologues have been reported in the literature including those with C_1, C_2, C_3 and C_4 side chains. Furthermore, the enlargement of the length of the side chain from C_1 to C_5 increases the psychoactivity of the cannabinoids [8]. We report in this study longer homologues up to C_6-C_7.

This work aimed to detect the presence of new cannabinoids in *C. sativa* and interpret their mass spectral fragmentation patterns. For this purpose, we conducted a detailed phytochemical analysis of lipid extract of seeds from hemp.

Results and discussion

The lipid extract of *C. sativa* seeds was analyzed by GC-EIMS after silylation. While hemp contains both noncannabinoid-type constituents and cannabinoids, we have focused in this study on the latter due to their chemical diversity and bioactivity.

The phenotypic system of hemp classification [9] defines two chemotypes based on the combined quotient of Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabinol (CBN) and cannabidiol (CBD) contents which are the most abundant cannabinoids in the majority of samples reported in the literature. Any sample with a value of this ratio greater than 1 is classified as drug-type marijuana and have high potent psychotropic effects while quotients lower than 1 indicate a fiber-type. Using this criterion of classification, we obtained the following phenotype ratio:

$$Phenotype = \frac{\Delta^9 - THC + CBN}{CBD} = 1160$$

Such value clearly indicates that our cannabis sample was of high-potency and drug-type.

Forty-three cannabinoids were identified in the crude extract. The most abundant were the *trans*- isomers of Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA (A)-C_5) and its neutral derivative Δ^9-THC-C_5 followed by cannabigerolic acid (CBGA (A)-C_5), cannabinolic acid (CBNA (A)-C_5) and cannabinol (CBN-C_5). In the extract, the amount of acidic cannabinoids was high, especially that of Δ^9-tetrahydrocannabinolic acid A probably because of the preventing action of trimethylsilyl derivatization [10].

Since cannabinoids are classified into acidic or neutral derivatives, depending on whether they carry a carboxyl moiety or not at C-2 (type A) or C-4 (type B) we have distinguished in the following sections both forms for each type and included them in the descriptive tables separately although they have been considered to belong to the same family e.g. Δ^9-THC and Δ^9-THCA (A). We report in this paper the identified cannabinoids as belonging to some of the 11 subclasses indicated in previous reviews [3, 11]. The total ion current (TIC) corresponding to the lipid extract of the hemp plant is shown in Figure 1. The new cannabinoids found in this work are listed in the tables below, which group them according to the class to which they belong. For atom

numbering of the carbon skeleton we have followed the benzopyran system [12]. The molecular structure of the new cannabinoids found is also presented in Figure 2. Nine Δ^9-tetrahydrocannabinol (Δ^9-THC)-type isomers (Table 1) were identified from the seeds of *C. sativa*. In all series, the member with C_5 side chain was the most abundant.

Six homologues (C_1, C_3, C_4, C_5, C_6, and C_7) of Δ^9-THC-type cannabinoids were identified in the seeds of our hemp sample. C_6 and C_7 Δ^9-THCs present as both *cis*- and *trans*-isomers, are described here for the first time as natural products. Such isomers result from the different configuration at 6a and 10a carbon atoms [3]. *Cis*- and *trans*-Δ^9-THCs have been synthesized and characterized [13]. When both compounds were present, the *trans*-isomer was always the most abundant and eluted after the *cis*- isomer in agreement with published results [14]. Δ^8-THC has not been detected in our study.

Δ^9-Tetrahydrocannabinol (Δ^9-THC-C_5) was the most abundant homologue of the series. This cannabinoid is considered the primary psychoactive constituent from *C. sativa* [15] and represented the second most abundant of all identified constituents after Δ^9-tetrahydrocannabinolic acid A (Δ^9-THCA (A)).

A new THC-type cannabinoid was identified from the chromatographic analysis of the hemp extract as *trans*-Δ^9-tetrahydrocannabinol-C_7 (*trans*-Δ^9-THC-C_7). The *cis*- isomer was also found eluting before. The mass spectrum of the TMS derivative is shown in Figure 3 and the EI-induced (70 eV) fragmentation is rationalized below by interpreting the mechanistic origin of the main ions with that of the *n*-pentyl homologue previously described in the literature [12]:

i. The molecular ion ($[M^{\cdot+}]$) at *m/z* 414 was very abundant reflecting the stability of this homologous series of Δ^9-THC-type of cannabinoids.
ii. The ($[M-15]^{\cdot+}$) fragment (*m/z* 399) is nearly as abundant as the parental ion. This argument together with the absence of the retro Diels-Alder (rDA) ion at *m/z* 346 which corresponds to the loss of 68 Da ($[M-68]^{\cdot+}$) allowed us to discard the possibility of an Δ^8-THC-C_7 isomer [16]. Similarly, to what happens in the fragmentation pathway of the pentyl homologue (Δ^9-THC-C_5), this fragment can be originated by means of three mechanisms: ii.1) the loss of C-11 carbon atom, being the most probable; ii.2) the elimination of one of the geminal methyl groups (C-12

or C-13); ii.3) minor amount of another equivalent structure corresponding to the loss of a methyl group from the TMS moiety at the hydroxyl group.

iii. The fragment at *m/z* 371 ($[M-43]^{+\cdot}$) has been previously interpreted through three main mechanisms: iii.1. the loss of three carbon atoms from benzopyran ring at C-6 together with both *gem*-methyl groups (C-12 and C-13) [17]; iii.2. the opening of the terpene ring with the loss of C-7 and C-8 and also the loss of one of the *gem*-methyl groups [15]; iii.3.: the partial side chain cleavage involving the three more distal carbon atoms (3', 4' and 5') [17].

iv. The most significant fragment at *m/z* 358 [18] is the ion due to the partial cleavage of the right side chain with a butylene elimination (C_4H_8) ($[M-56]^{+\cdot}$). The mechanism of this McLafferty (McL) rearrangement is depicted in Figure 4. Brown and Harvey [19] synthesized the linear hexyl-Δ^9-THC, its mass spectrum contained the fragment *m/z* 330 which was absent here. Therefore, the natural products C_6 and C_7-Δ^9-THCs reported in this work should not be linear, but consistent with a branched ethyl-pentyl side-chained Δ^9-THC. The synthetic dimethyl-heptyl-Δ^9-THC, also known as nabilone, which contains two geminal methyl groups, allows a McLafferty rearrangement together with a number of other even-mass peaks which are characteristic of the quaternary carbon atom protonation as its side chain broke down. However, these even-mass fragments were not present in this spectrum.

v. The loss of two additional carbon atoms from the ion at *m/z* 371 produced a fragment at *m/z* 343 ($[M-71]^{+\cdot}$). The direct fragmentation of the molecular ion involving the terpene ring together with a geminal methyl group [12] released an ion at *m/z* 331 ($[M-83]^{+\cdot}$) and the loss of the entire side-chain gave the ion [M-99; $M-C_7H_{15}]^{+\cdot}$ at *m/z* 315 according to Harvey [16].

vi. Finally, the trimethylsilyl (TMS) fragment ion (*m/z* 73), *m/z* 75 [$(CH_3)_2$-Si-OH] and *m/z* 147 are characteristic of silyl derivatives.

The addition of a methyl or ethyl group to the alkyl side chain of Δ^9-THC-C_5 would be biosynthetically consistent with that observed in steroids with 28 and 29 carbon atoms [20, 21, 22].

Acids found are listed in Table 2 including both *cis*- and *trans*-isomers. *cis*-Δ^9-THCA (A)-C_4 and *cis*-Δ^9-THCA (A)-C_5 have not been previously reported in the

literature [3]. Δ^9-THC-*n*-C_5 acid A was previously described as the TMS derivative [10]. This cannabinoid was found together with two other isomers with a butyl and propyl alkylic side chain. Silylated Δ^9-THC acid A shows a molecular ion peak at *m/z* 502. The loss of a methyl group at *m/z* 487 together with lower amounts of the ion at *m/z* 419 ($[M-83]^{+\cdot}$) are characteristic of THCA (A). The fragment at *m/z* 413 ($[M-89]^{+\cdot}$) may arise from the loss of the OTMS group of the carboxylic moiety, probably in a two-step mechanism consisting first on the demethylation followed by the loss of the rest of the group.

Four neutral cannabinols (CBN) were found. Homologues with *n*-propyl, *n*-butyl, *n*-pentyl and a new C_7 homologue were identified whereas CBN-C_5 was the most abundant. Their mass spectra are characterized by fragments M, M-15 and M-72 [12].

Our results given in Table 3 include a new homologue CBN-C_7. The main fragments of the mass spectrum are also given. It is characterized by the mass peak at *m/z* 410, the loss of a *gem*-methyl group (*m/z* 395) and finally a minor ion resulting from the loss of 72 Da at *m/z* 338 corresponding to the TMS group.

We have also found three cannabinolic acids (C_3, C_5 and C_7) (Table 4) whereas the most abundant was the pentyl-cannabinolic acid (CBNA-C_5). The mass spectrum of this fully aromatized cannabinoid was dominated by the fragment M-15 which is the major peak (*m/z* 483) and very low amounts of the molecular ion ($M^{+\cdot}$) [23].

As to the neutral cannabinol distribution, only C_5-cannabinolic acid (CBNA-C_5) was abundant while trace amounts of C_3 and C_7 homologues were found, with the latter being a new compound. The mass spectrum of the trimethylsilyl derivative of C_5-CBNA which to our knowledge has not been published before is presented in Fig. 1S, Supporting information.

Silylated CBNA-C_5 showed a molecular ion peak at *m/z* 498. The base peak in this spectrum corresponded to a loss of mass 15, a methyl group, leading to the formation of the ion at *m/z* 483. This loss is assigned to any TMS of the molecule [10]. The ion at *m/z* 409 can be accounted for the cleavage of the carbon-oxygen bond in the carboxyl moiety to produce a stable acylium ion. This ion can arise through the loss of TMS from the acid group which is more labile than the alcohol. The peak at *m/z* 321 may

correspond to the loss of both TMS groups. The mass spectrum of the non-silylated compound has been reported previously [24].

Three neutral homologues of cannabichromene (CBC)-type cannabinoids, propyl- ($[M]^{+\cdot}$ = 358; CBC-C_3), pentyl- ($[M]^{+\cdot}$ = 386; CBC-C_5) and C_7-canabichromene ($[M]^{+\cdot}$ = 414; CBC-C_7) were identified as their trimethylsilylated derivatives (Table 5).

CBC-C_5 appeared as 4 isomers with 3 of them as minor components eluting before the major one and having a very similar mass spectrum (Fig. 2S). The molecular ion ($[M]^{+\cdot}$ = 386) and the fragment resulting from the loss of a methyl group ($[M]^{+\cdot}$ = 371) showed low abundance. The base peak at *m/z* 303, corresponding to the chromenyl ion arose from the loss of the methyl-pentenyl left side chain. In addition, the fragment at *m/z* 246 resulted from the loss of a butylene alkyl group (C_4H_9; $[M-56]^{+\cdot}$) in the right-handed alkyl side chain from the chromenyl ion [12, 25] to produce a tropylium fragment by McL rearrangement. Ions at *m/z* 231 and 174 are equivalent to ions *m/z* 303 and 246, respectively after the loss of the TMS group.

Four isomers of CBC-C_5 were also previously described [5, 26] whereas two of them have been only synthesized i.e. *iso*-R and *iso*-S. Based on studies performed with a similar column [27, 28], it can be concluded that these 4 CBC isomers detected in our stud did not include any cannabicyclol (CBL) because this would be expected to elute between Δ^9-THC-C_3 (tetrahydrocannabivarin, THCV) and CBD. In order to explain the other three CBC isomers, we propose a mechanism consisting of a double bond migration on the terminal isoprenyl unit to the two contiguous positions giving on one side a methylene terminal unsaturation and on the other side two *Z*- and *E*- isomers.

Cannabichromene with a C_7 side chain (CBC-C_7) attached to the benzene ring was detected in low amounts and is reported for the first time (Table 5). The mass spectrum of the TMS derivative is characterized by a major ion at *m/z* 331. Besides this important fragment three other diagnostic peaks were detected at *m/z* 414 ($[M]^{+\cdot}$), *m/z* 399 ($[M-15]^{+\cdot}$) and *m/z* 274 which was a minor fragment resulting from the cleavage of the bonds that fuses both side chains to the bicycle core, consistent with an ethyl-pentyl side chain. Cannabichromenic acid and three additional isomers were also identified similarly to those previously described for cannabichromene (Table 6).

The mass spectrum of pentyl cannabichromenic acid (CBCA (A)-C_5) is shown in Fig. 3S. The fragmentation pattern was deduced by comparison with mass spectra of cannabinoid derivatives [12]. The molecular ion ([M = 502]$^{+\cdot}$ and the loss of a methyl group (*m/z* 487) were low abundant ions. The fragment at *m/z* 419 ([M-83]$^{+\cdot}$) resulted, as in the neutral isomer, from the loss of the methyl-pentenyl left side chain and was the base peak [25]. The TMSi group at *m/z* 73 was also an abundant fragment and the peak at *m/z* 147 indicated the presence of two close trimethylsilyloxy groups. The mass spectra of the other three isomers are also included (Fig. 3S) showing the diagnostic ion at *m/z* 419.

Cannabinerol (CBNR) and cannabigerol (CBG) together with cannabinerolic acid (CBNRA) and cannabigerolic acid (CBGA) are recognized as cannabigerol (CBG)-type cannabinoids [3, 29]. Their biosynthetic precursors are respectively nerol and geraniol. Such terpenoid alcohols have been widely described. The order of elution of these isomeric alcohols determined in a non-polar column such as DB-5 through their Kovats indexes [30] were: first *Z*-nerol (KI 1229) and then *E*-geraniol (KI 1252). The biosynthesis of cannabigerolic acid has been recently discussed in relation to cannabigerol and geraniol [31]. A similar relationship could be proposed to the biosynthesis of cannabinerolic acid in relation to cannabinerol and nerol. The four CBG-type cannabinoids found are showed in Fig. 4S. Although these classes of cannabinoids were scarcely present in previous studies, here cannabigerolic acid (CBGA) was the most abundant after Δ^9-THC and Δ^9-THCA (see Figure 1).

Z-Cannabinerol was synthesized from *Z*-nerol and its retention time was measured [32]. Recently, the non-silylated mass spectrum of this compound was described for the first time as part of the analysis of an Indian hashish sample [33]. It was characterized by a molecular ion at *m/z* 316 and the base peak at *m/z* 193. To the best of our knowledge the mass spectrum of CBNR as its silyl derivative is reported here for the first time (Fig. 5aS) although it was previously synthesized [34]. The spectrum shows the mass peak ([M]$^{+\cdot}$ = 460), the loss of a methyl group at *m/z* 445, an ion at *m/z* 391 ([M-C_5H_9]$^{+\cdot}$), a characteristic peak at *m/z* 337 ([M-C_9H_{15}]$^{+\cdot}$ corresponding to the fragment [M-123]$^{+\cdot}$) and finally a fragment at *m/z* 268 related to the base peak at *m/z* 193 of the non-TMS counterpart. Mass spectrometric data of this compound and others cannabinoids of this series are shown in Table 7.

Z-Cannabinerolic acid (CBNRA-C_5) was isolated for the first time by Taura *et al*. [35]. Additionally, this cannabinoid has been also reported by another analysis conducted on an HPLC column [36]. The mass spectrum of the silyl derivative should be similar to that reported for cannabigerolic acid as it is observed with nerol and geraniol TMS mass spectra.

E-Cannabigerol was isolated [37] in *C. sativa*, then synthesized from olivetol and geraniol [32] and further compared with cannabinerol by Taura *et al*. [38]. It is considered the biochemical precursor of others cannabinoids [34]. The mass spectrum of this cannabinoid has been previously reported [27, 28] and includes the mass peak ($[M]^{+\cdot}$ = 316), an intense fragment at *m/z* 231 ($[M-85]^{+\cdot}$; $[M-C_6H_{13}]^{+\cdot}$; base peak), and a fragment at *m/z* 193 ($[M-C_9H_{15}]^{+\cdot}$) [18]. The mass spectrum of the trimethylsilylated derivative [34] is quite close to that shown in Fig. 5bS and consists of the mass peak ($[M]^{+\cdot}$ = 460), less amounts of the fragment M-15, an intense ion at *m/z* 391 ($[M-C_5H_9]^{+}$), and finally a characteristic peak at *m/z* 337 ($[M-123]^{+\cdot}$ corresponding to the fragment $[M-C_9H_{15}]^{+\cdot}$) which was the base peak apart of the TMS group (*m/z* 73).

Finally, *E*-cannabigerolic acid (CBGA-C_5) also known as (*E*)-3-(3,7-dimethyl-2,6-octadienyl)-2,4-dihydroxy-6-pentylbenzoic acid was synthesized [6] and isolated from hemp leaves [35]. The mass spectrum of this non-silylated cannabinoid and that of its silyl derivative have been recently reported [39, 40]. In agreement with our results the mass spectrum of this compound includes the mass peak ($[M]^{+\cdot}$ = 576), a fragment at *m/z* 561 ($[M-CH_3]^{+\cdot}$); base peak), an ion at *m/z* 486 ([M- 90; $TMSO]^{+\cdot}$), a fragment at *m/z* 453 ([M-123; $C_9H_{15}]^{+\cdot}$) and finally another peak at *m/z* 417 corresponding to the fragment ($[M-159]^{+\cdot}$). Looking to the fragment at *m/z* 561 corresponding to the base peak, the mass chromatogram (Fig. 4bS) shows an intense peak corresponding to CBGA and a very minor peak. Based on their chromatographic characteristics, the latter could be CBNRA in agreement with abundances reported in the hemp leaves [35].

Further cannabinoids, such as cannabidiol [41], cannabidiolic acid [42], cannabitriol [43], together with three isomers of dihydrocannabinol [27, 44] and hexahydrocannabinol [45, 46], were identified through the comparison of their mass spectra with those previously published in the literature. They were detected in low amounts.

Materials and Methods

Plant material.

Seeds of *C. sativa* were collected after the grain maturation (28th 8, 2014) at Sant Fruitós de Bages (Bages County, Catalonia). The coordinates were 41º 44' 09.5" N by 1º 52' 54.4" E, and the elevation was 302 m. The seeds were stored in a stainless steel container and transported to the laboratory. The plant material was identified by Dr. Joan Simon (Faculty of Pharmacy – Universitat de Barcelona)

Analytical procedures.

GC/MS pre-analytical conditions: sample treatment, extraction and derivatization.

Fresh seeds were air-dried at room temperature, and then crushed and homogenized in a glass mortar, using a glass pestle together with 25 g of previously cleaned sea-sand. All inert materials and tools were previously cleaned and rinsed with acetone before use. The ground sample was introduced into cellulose thimbles, and then extracted in a Söxhlet apparatus for 26 h, using a 7:3 (v/v) mixture of pentane/dichloromethane. Seeds (3.73 g) were extracted in darkness to avoid any photo-oxidation reactions. The extract was silylated prior to GC-EIMS analysis to obtain the corresponding TMS-ethers (of hydroxyl groups) and TMS-esters (of carboxyl groups). After treatment with 300 μL N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Merck) the resulting mixture was heated at 70 ºC for 1 h prior to analysis.

GC-EIMS analysis.

The derivatized sample was injected (275 ºC) into a gas chromatograph (Fisons Instruments) that was operating in splitless mode and was coupled to a mass detector (GC 8000/MD 800), which was operating in electronic impact (EI) ionization mode (70 eV). The compounds were separated on a fused silica capillary column (DB-5ms;

length: 30 m; i.d.: 0.32 mm) coated with a 0.25-μm low-polarity liquid-phase film (5% methylpolysiloxane; J&W Scientific). The mass scanning in total ion count (TIC) was acquired from 50 Daltons to 650 Daltons over a period of 1 s. The oven temperature was programmed as follows: start at 40 ºC (1 min); ramp up to 230 ºC (20 ºC/min); ramp up to 300 ºC (2 ºC/min); and finally, hold at 300 ºC (20 min). Helium was used as the carrier gas (flow rate: 1.0 mL/min). The inlet temperature was 300 ºC; the transfer-line temperature, 310 ºC; the ion-source temperature, 200 ºC.

Identification of compounds.

Compounds were analyzed by GC-EIMS. The analytes were identified by comparing their characteristic mass fragmentation patterns and retention times to those reported in the literature.

Quantitation.

The relative compositions and total amounts of the homologues were estimated from the integrated area of the peaks in the TIC using MassLab software.

Supporting information

The supporting information contains the mass spectrum of the trimethylsilyl derivatives of C_5-cannabinerolic acid, C_5-cannabichromenes, cannabichromenic acids and cannabigerol-type cannabinoids together with the mass chromatogram of C_5-cannabichromenes, C_5-cannabichromenic acids and cannabigerol-type cannabinoids.

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Conflict of interest

The authors declare no conflict of interest.

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Tables

Table 1. Cannabinoid compounds belonging to the Δ^9-tetrahydrocannabinol series identified on the seeds of *C. sativa*.

Compound	Retention time (min)	M^+	Main ions *m/z*
cis-Δ^9-THC-C_1	*Not detected*	-	-
trans-Δ^9-THC-C_1	14.27	330 (9)	315 ([M-15]$^+$, 10), 287 ([M-43]$^+$, 3), 274 ([M-56]$^+$, -), 259 ([M-71]$^+$, -), 247 ([M-83]$^+$, 5), 73(71), 67(9)
cis-Δ^9-THC-C_2	*Not detected*	-	-
trans-Δ^9-THC-C_2	*Not detected*	-	-
cis-Δ^9-THC-C_3	*Not detected*	-	-
trans-Δ^9-THC-C_3	16.03	358 (48)	343 ([M-15]$^+$, 50), 315 ([M-43]$^+$, 38), 301 ([M-57]$^+$, 3) , 287 ([M-71]$^+$, 4), 275 ([M-83]$^+$, 28), 73(100), 67(11)
***cis*-Δ^9-THC-C_4**†	**16.67**	**372 (-)**	**357 ([M-15]$^+$, 8), 329 ([M-43]$^+$, 4), 315 ([M-57]$^+$, -), 301 ([M-71]$^+$, -), 289 ([M-83]$^+$, -), 73(100), 67(13)**
trans-Δ^9-THC-C_4	17.42	372 (36)	357 ([M-15]$^+$, 39), 329 ([M-43]$^+$, 15), 315 ([M-57]$^+$, 28), 301 ([M-71]$^+$), 289 ([M-83]$^+$), 73(100), 67(12)
cis-Δ^9-THC-C_5	18.70	386 (35)	371 ([M-15]$^+$, 35), 343 ([M-43]$^+$, 17), 330 ([M-56]$^+$, 10), 315 ([M-71]$^+$, 23), 303 ([M-83]$^+$, 27), 73(100), 67(8)
trans-Δ^9-THC-C_5‡	19.27	386 (97)	371 ([M-15]$^+$, 100), 343 ([M-43]$^+$, 30), 330 ([M-56]$^+$, 18), 315 ([M-71]$^+$, 67), 303 ([M-83]$^+$, 48), 73(100), 67(7)
cis-Δ^9-THC-C_6	*Not detected*	-	-
***trans*-Δ^9-THC-C_6**†	**22.89**	**400 (11)**	**385 ([M-15]$^+$, 12), 357 ([M-43]$^+$, -), 344 ([M-56]$^+$, 9), 329 ([M-71]$^+$, -), 317 ([M-83]$^+$, -), 73(100), 67(9)**
***cis*-Δ^9-THC-C_7**†	**28.42**	**414 (7)**	**399 ([M-15]$^+$, 12), 371 ([M-43]$^+$), 358 ([M-56]$^+$, 3), 343 ([M-71]$^+$, 2), 331 ([M-83]$^+$, 5), 73(100), 67(8)**
***trans*-Δ^9-THC-C_7**†	**28.80**	**414 (97)**	**399 ([M-15]$^+$, 100), 371 ([M-43]$^+$, 56), 358 ([M-56]$^+$, 43), 343 ([M-71]$^+$, 44), 331 ([M-83]$^+$, 42), 73(45), 67(13)**

†in bold is indicated the cannabinoids identified for the first time in *Cannabis sativa*.

‡is specified the most abundant member of this family of cannabinoids.

Table 2. Cannabinoid compounds belonging to the Δ^9-tetrahydrocannabinolic acid series identified on the seeds of *C. sativa*.

Compound	Retention time (min)	M^+	Main ions *m/z*
cis-Δ^9-THCA (A)-C_3	*Not detected*	-	-
trans-Δ^9-THCA (A)-C_3	21.77	474 (4)	459 ([M-15]$^+$, 100), 403 ([M-71]$^+$, 1), 391 ([M-83]$^+$, 3), 385 ([M-89]$^+$, 4), 357 ([M-117]$^+$, 3), 147(12), 73(80), 69(5)
***cis*-Δ^9-THCA (A)-C_4**	**22.32**	**488 (-)**	**473 ([M-15]$^+$, 2), 417 ([M-71]$^+$, 7), 405 ([M-83]$^+$, -), 399 ([M-89]$^+$, 1), 371 ([M-117]$^+$, 2), 147(14), 73(100), 69(9)**
trans-Δ^9-THCA (A)-C_4	23.47	488 (3)	473 ([M-15]$^+$, 83), 417 ([M-71]$^+$, -), 405 ([M-83]$^+$, 2), 399 ([M-89]$^+$, 3), 371 ([M-117]$^+$, 6), 147(20), 73(100), 69(8)
***cis*-Δ^9-THCA (A)-C_5**	**24.52**	**502 (-)**	**487 ([M-15]$^+$, 18), 431 ([M-71]$^+$, -), 419 ([M-83]$^+$, 2), 413 ([M-89]$^+$, 1), 385 ([M-117]$^+$, 4), 147(12), 73(100), 69(34)**
trans-Δ^9-THCA (A)-C_5‡	25.99	502 (5)	487 ([M-15]$^+$, 100), 431 ([M-71]$^+$, 3), 419 ([M-83]$^+$, 18), 413 ([M-89]$^+$, 4), 385 ([M-117]$^+$, 4), 1487(13), 73(100), 69(3)

†in bold is indicated the cannabinoids identified for the first time in *Cannabis sativa*.

‡is specified the most abundant member of this family of cannabinoids.

Table 3. Cannabinoid compounds belonging to the cannabinol series identified on the seeds of *C. sativa*.

Compound	Retention time (min)	M^+	Main ions *m/z*
CBN-C_3	17.27	354 (7)	339 ([M-15]$^+$, 57), 282 ([M-72]$^+$, -), 267 ([M-87]$^+$, -), 210 ([M-144]$^+$, -), 181 ([M-173]$^+$, -), 73(100), 69(44)
CBN-C_4	18.90	368 (-)	353 ([M-15]$^+$, 12), 296 ([M-72]$^+$, -), 281 ([M-87]$^+$, 2), 224 ([M-144]$^+$, -), 195 ([M-173]$^+$, 2), 73(100), 69(9)
CBN-C_5‡	20.80	382 (11)	367 ([M-15]$^+$, 100), 310 ([M-72]$^+$, 8), 295 ([M-87]$^+$, 5), 238 ([M-144]$^+$, 6), 209 ([M-173]$^+$, 2), 73(21), 69(1)
CBN-C_6	*Not detected*	-	-
CBN-C_7†	**29.00**	**410 (4)**	**395 ([M-15]$^+$, 48), 338 ([M-72]$^+$, 2), 323 ([M-87]$^+$, 9), 266 ([M-144]$^+$, -), 237 ([M-173]$^+$, 2), 73(100), 69(20)**

†in bold is indicated the cannabinoids identified for the first time in *Cannabis sativa*.

‡is specified the most abundant member of this family of cannabinoids.

Table 4. Cannabinoid compounds belonging to the cannabinolic acid series identified on the seeds of *C. sativa*.

Compound	Retention time (min)	M^+	Main ions *m/z*
CBNA-C_3	17.92	470 (-)	455 ($[M-15]^+$, 14), 397 ($[M-73]^+$, -), 381 ($[M-89]^+$, -), 367 ($[M-103]^+$, -), 307 ($[M-163]^+$, -), 293 ($[M-177]^+$, -), 147(22), 73(100), 69(15)
CBNA-C_4	*Not detected*	-	-
CBNA-C_5‡	27.74	498 (3)	483 ($[M-15]^+$, 100), 425 ($[M-73]^+$, 2), 409 ($[M-89]^+$, 3), 395 ($[M-103]^+$, 10), 335 ($[M-163]^+$, 4), 321 ($[M-177]^+$, 14), 147(14), 73(100), 69(1)
CBNA-C_6	*Not detected*	-	-
CBNA-C_7†	**36.75**	**526 (-)**	**511 ($[M-15]^+$, 5), 457 ($[M-73]^+$, -), 437 ($[M-89]^+$, -), 323 ($[M-103]^+$, -), 363 ($[M-163]^+$, -), 349 ($[M-177]^+$, -), 147(20), 73(79), 69(38)**

†in bold is indicated the cannabinoids identified for the first time in *Cannabis sativa*.

‡is specified the most abundant member of this family of cannabinoids.

Table 5. Cannabinoid compounds belonging to the cannabichromene series identified on the seeds of *C. sativa*.

Compound	Retention time (min)	M^+	Main ions *m/z*
CBC-C_3	15.63	358 (4)	371 ($[M-15]^+$, 6), 275 ($[M-83]^+$, 100), 218($[M-140]^+$, 10), 203 ($[M-155]^+$, 3), 75(5), 73(20), 69(7), 55(7)
CBC-C_4	*Not detected*	-	-
CBC-C_5†	**17.12**	**386 (9)**	**371 ($[M-15]^+$, 6), 303 ($[M-83]^+$, 71), 246 ($[M-140]^+$, 5), 231 ($[M-155]^+$, -), 75(94), 73(100), 69(18), 55(30)**
CBC-C_5	**17.69**	**386 (11)**	**371 ($[M-15]^+$, 11), 303 ($[M-83]^+$, 60), 246 ($[M-140]^+$, 6), 231 ($[M-155]^+$, -), 75(88), 73(100), 69(23), 55(30)**
CBC-C_5	**18.15**	**386 (11)**	**371 ($[M-15]^+$, 12), 303 ($[M-83]^+$, 60), 246 ($[M-140]^+$, 3), 231 ($[M-155]^+$, -), 75(80), 73(100), 69(15), 55(27)**
CBC-C_5‡	18.40	386 (4)	371 ($[M-15]^+$, 6), 303 ($[M-83]^+$, 100), 246 ($[M-140]^+$, 10), 231 ($[M-155]^+$, 3), 75(5), 73(20), 69(7), 55(7)
CBC-C_6	*Not detected*	-	-
CBC-C_7	**26.60**	**414 (4)**	**399 ($[M-15]^+$, 3), 331 ($[M-83]^+$, 30), 274 ($[M-140]^+$, -), 259 ($[M-155]^+$, 3), 75(45), 73(100), 69(18), 55(19)**

†in bold is indicated the cannabinoids identified for the first time in *Cannabis sativa*.

‡is specified the most abundant member of this family of cannabinoids.

Table 6. Cannabinoid compounds belonging to the cannabichromenic acid A series identified on the seeds of *C. sativa*.

Compound	Retention time (min)	M^+	Main ions *m/z*
CBCA (A)-C_5†	**22.69**	**502 (-)**	**487 ($[M-15]^+$, 11), 419 ($[M-83]^+$, 52), 331 ($[M-171]^+$, 4), 271 ($[M-231]^+$, -), 257 ($[M-245]^+$, 10), 147(21), 73(100), 69(18), 55(19)**
CBCA (A)-C_5	**23.77**	**502 (2)**	**487 ($[M-15]^+$, 4), 419 ($[M-83]^+$, 54), 331 ($[M-171]^+$, 2), 271 ($[M-231]^+$, 1), 257 ($[M-245]^+$, 2), 147(15), 73(100), 69(16), 55(17)**
CBCA (A)-C_5	**25.07**	**502 (2)**	**487 ($[M-15]^+$, 15), 419 ($[M-83]^+$, 40), 331 ($[M-171]^+$, -), 271 ($[M-231]^+$, 2), 257 ($[M-245]^+$, 5), 147(22), 73(100), 69(7), 55(11)**
CBCA (A)-C_5‡	26.04	502 (3)	487 ($[M-15]^+$, 20), 419 ($[M-83]^+$, 100), 331 ($[M-171]^+$, 5), 271 ($[M-231]^+$, 3), 257 ($[M-245]^+$, 15), 147(13), 73(50), 69(8), 55(7)

†in bold is indicated the cannabinoids identified for the first time in *Cannabis sativa*.

‡is specified the most abundant member of this family of cannabinoids.

Table 7. Cannabinoid compounds belonging to the cannabigerol-type cannabinoids identified on the seeds of *C. sativa*.

Compound	Retention time (min)	M^+	Main ions *m/z*
(Z)-CBNR-C_5	19.72	460 (5)	445 ($[M-15]^+$, 2), 417 ($[M-43]^+$, 2), 403 ($[M-57]^+$, 4), 391 ($[M-69]^+$, 17), 377($[M-83]^+$, 10), 351($[M-109]^+$, 3), 337($[M-123]^+$, 58), 75(9), 73(100), 69(30), 67(3)
(E)-CBG-C_5	20.24	460 (10)	445 ($[M-15]^+$, 3), 417 ($[M-43]^+$, -), 403 ($[M-57]^+$, 2), 391 ($[M-69]^+$, 12), 377($[M-83]^+$, 8), 351($[M-109]^+$, 1), 337($[M-123]^+$, 35), 75(32), 73(100), 69(40), 67(6)
(Z)-CBNRA-C_5	25.97	576 (1)	561 ($[M-15]^+$, 2), 486 ($[M-90]^+$, -) , 471 ($[M-105]^+$, 1)
(E)-CBGA-C_5‡	20.24	576 (3)	561 ($[M-15]^+$, 77), 486 ($[M-90]^+$, 6) , 471 ($[M-105]^+$, 5), 453($[M-123]^+$, 12), 417($[M-159]^+$, 20), 147 (17), 73(100), 69(28)

‡is specified the most abundant member of this family of cannabinoids.

Table legends

Table 1. Cannabinoid compounds belonging to the Δ^9-tetrahydrocannabinol series identified on the seeds of *C. sativa*.

Table 2. Cannabinoid compounds belonging to the Δ^9-tetrahydrocannabinolic acid series identified on the seeds of *C. sativa*.

Table 3. Cannabinoid compounds belonging to the cannabinol series identified on the seeds of *C. sativa*.

Table 4. Cannabinoid compounds belonging to the cannabinolic acid series identified on the seeds of *C. sativa*.

Table 5. Cannabinoid compounds belonging to the cannabichromene series identified on the seeds of *C. sativa.*

Table 6. Cannabinoid compounds belonging to the cannabichromenic acid A series identified on the seeds of *C. sativa.*

Table 7. Cannabinoid compounds belonging to the cannabigerol-type cannabinoids identified on the seeds of *C. sativa*.

Figure legends

Figure 1. Total ion current (TIC) corresponding to the total extract of the lipids of the seeds from the hemp plant (*C. sativa* L). *New cannabinoid.

Figure 2. Benzopyran system of numbering carbon atoms exemplified in the Δ^9-tetrahydrocannabinol (Δ^9-THC) and new cannabinoids reported in this work.

Figure 3. Mass spectrum of the new cannabinoid ethyl-pentyl *trans*-Δ^9-tetrahydrocannabinol (*trans*-Δ^9-THC-C_7) as its TMS derivative identified in the seeds of *C. sativa*.

Figure 4. McLafferty rearrangement for the *m/z* 358 ion of the ethyl-pentyl Δ^9-THC.