Hello!

Thank you for your purchase of Baked HHC products!

HHC stands for Hexahydrocannabinol. It is a hemp derived product that contains less than .3% thc of any kind.

Below you will find a lab test outlining the THC contents. Due to how extremely new HHC is, a complete HHC COA is not yet available. We are working diligently with our testing laboratory to create a new HHC specific COA.

In order to get a HHC COA created there must first be a baseline, essentially pure HHC molecules that will be compared to HHC product testing to determine how pure the product actually is. Thankfully for us, and you, our HHC is what is being used to create this baseline/standard.

We hope to have this done by early August, at which point this COA will be updated with a complete cannabinoid analysis outlining the HHC content.

We thank you for your patience, and hope you love our products!

For questions or inquiries please contact Admin@distromike.com

Gobi Hemp

Analytical Report - Certificate of Analysis

Manifest:	2107200002
Sample Id:	1A-GHEMP-2107200002-0001
Sample Name:	D10 ML
Sample Type:	Concentrate
Client Id:	CID-50161
Client:	Baked HHC
Address:	Po Box 15598, , Las Vegas, NV 89114

Test Performed:	Hemp Lab
Report No:	P-2107200002-V1
Receive Date:	2021-07-20
Test Date:	2021-07-09
Report Date:	2021-07-20
Sample Condition:	Good
Method Reference:	GH-OP-06

Scope

The content of sixteen cannabinoids was determined by an in-house developed method for solvent extraction followed by High Performance Liquid Chromatography with Diode Array Detection.

Cannabinoids	Percent	mg/gram
CBDV	ND	ND
CBDA	ND	ND
CBGA	ND	ND
CBG	ND	ND
CBD	ND	ND
THCV	ND	ND
CBN	0.13	1.26
Δ9-THC	ND	ND
CBC	ND	ND
THCA	ND	ND
CBDVA	ND	ND
THCVA	ND	ND
CBNA	ND	ND
Δ8-THC	0.12	1.19
CBL	ND	ND
CBCA	ND	ND

ND - not detected; T - trace; ULOQ - limit of quantitation

	Percent	mg/gram
Total Δ9-THC	0.00	0.00
Total CBD	0.00	0.00
Total CBG	0.00	0.00
Total Cannabinoids	0.24	2.45

Total Δ9-THC = Δ9-THC + (THCA x 0.877)
Total CBD = CBD + (CBDA x 0.877)
Total CBG = CBG + (CBGA x 0.877)

Laboratory Comments:
Presence of two large peaks at 6.043 min and 6.249 min that are unidentified by our standards. Ref# 2107200002

2021-07-20

Jerry Hogan - Director of Operations | Date

Gobi Hemp
● 3940 Youngfield St. ●
● Wheat Ridge CO 80033 ●
● **ISO/IEC 17025:2017 Accredited** ●
● (303) 955-4934 ●

PJLA
Testing
Accreditation #103051

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Summary

Colorado Chromatography Labs, a leading cGMP certified manufacturer of rare cannabinoids, has recently accessed Hexahydrocannabinol (HHC) at commercial scale. Our HHC concentrate contains three different stereoisomers of HHC and less than 0.2% other cannabinoids. There is currently no Certified Reference Standard commercially available for HHC. We are working with various third-party cannabis testing labs including KCA Labs and Green Scientific Labs to purify and isolate the various HHCs and have them turned into Certified Reference Materials for the industry. Once we have created the Certified Reference Standard, we will have exclusive testing rights on HHC for a period of 6 months. We expect to have the standard certified within the next 30 to 45 days.

HHC is typically found in both the seeds and pollen of cannabis, meaning that it is a naturally occurring cannabinoid. We have attached our in-house HPLC chromatogram which shows no other cannabinoids detected other than HHC and trace levels of Delta-8 THC. There are three versions of HHC present in our product each of which corresponds to an individual peak on the chromatogram. A COA from Gobi shows that our HHC is truly Farm Bill Compliant, meaning that this product is below 0.3% Total THC. The technician at Gobi also noticed unidentified peaks which they do not have a standard for.

We have a third-party NMR which confirms that the structure of what we made is HHC. We have also sent a sample to KCA labs to verify the identity of these unknown peaks and their Mass Spec data confirms that the sample is HHC. Colorado Chromatography is also working with toxicology and product safety labs to verify the safety of HHC using the latest *in vitro* techniques.

Thieme

GC-MS Metabolite Profile and Identification of Unusual Homologous Cannabinoids in High Potency *Cannabis sativa*

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Supporting information available online at http://www.thieme-connect.de/products

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 to be derived from the double bond migration at the terminal isoprenyl unit. C_7 cannabichromene together with the neutral and acidic forms of cannabinol-C_7 were also detected. The mass spectrum of these homologues as trimethylsilyl (TMS) derivatives are presented, and the fragmentation patterns are discussed.

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 for 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 amounts 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, re-

leasing 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. We report in this study longer homologues up to C_6–C_7. Interestingly, the enlargement of the length of the side chain from C_1 to C_5 increases the psychoactivity of the cannabinoids [8].

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 a 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 2 chemotypes based on the combined quotient of 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 has potent psychotropic effects while quotients lower than 1 indicate a fiber-type. Using this criterion of classification, we obtained the following phenotype ratio:

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

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 considered in previous reviews [3, 11]. The total ion current (TIC) corresponding to the lipid extract of the hemp plant is shown in ▶ **Fig. 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 ▶ **Fig. 2**. Nine 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 ▶ **Fig. 3**, and the EI-induced (70 eV) fragmentation is rationalized below by interpreting the mechanistic origin of the main ions based on the fragmentation of the *n*-pentyl homologue previously described in the literature [12]:

i. The molecular ion ([M^+]) at *m/z* 414 was very abundant, reflecting the stability of this homologous series of Δ^9-THC-type of cannabinoids

ii. The $[M - 15]^+$ fragment (*m/z* 399) is nearly as abundant as the parental ion. This argument, together with the absence of the retro Diels-Alder ion at *m/z* 346 which corresponds to the loss of 68 Da ($[M - 68]^{+\bullet}$), 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 3 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 ($[M - 43]^{+\bullet}$) has been previously interpreted through 3 main mechanisms: iii.1. the loss of 3 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 3 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) ($[M - 56]^{+\bullet}$). The mechanism of this McLafferty (McL) rearrangement is depicted in ▶ **Fig. 4**. Brown and Harvey [19] synthesized the linear hexyl-Δ^9-THC. Its mass spectrum contained a fragment at *m/z* 330 that 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 2 geminal methyl groups, allows an McL rearrangement to-
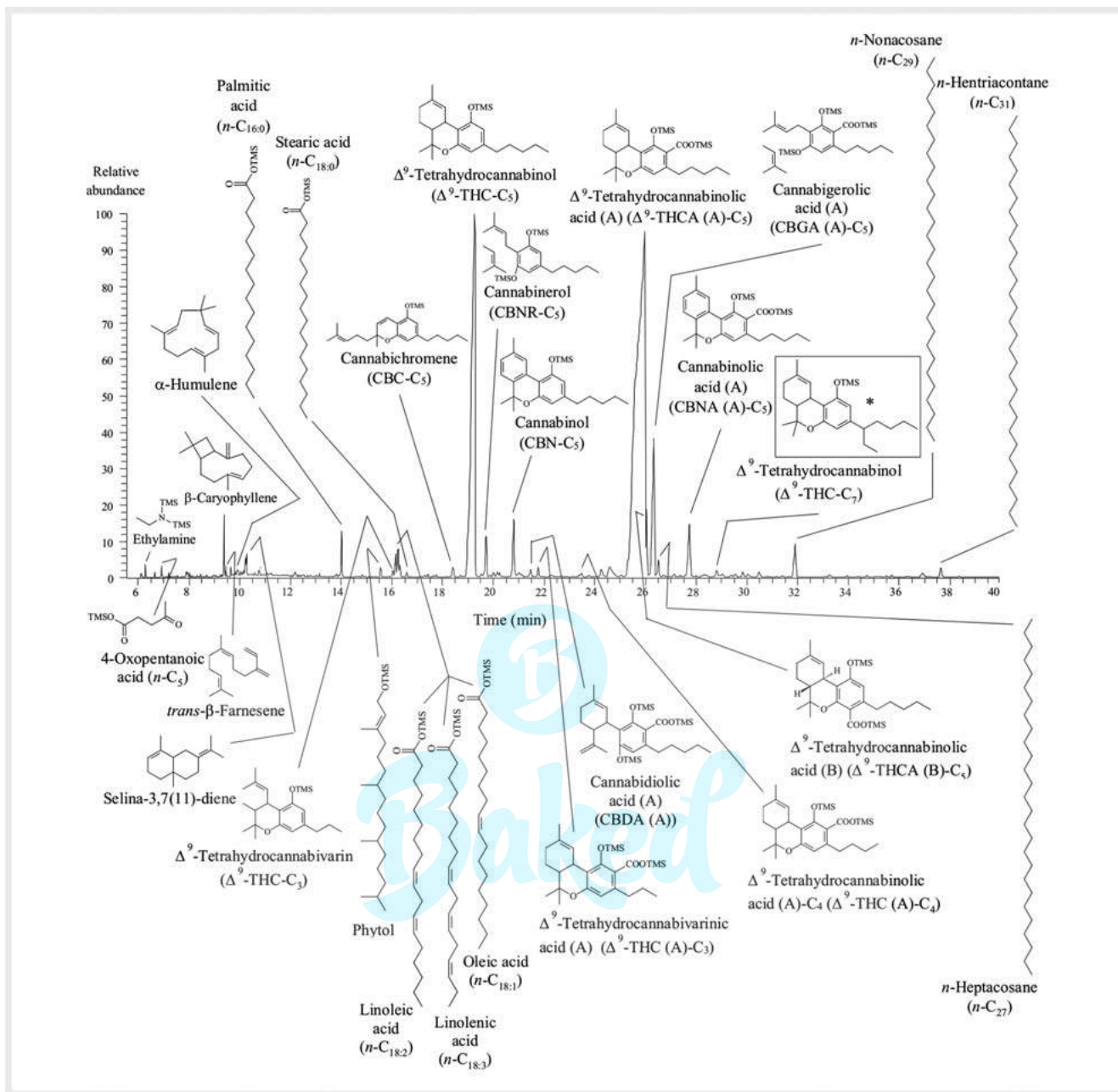
▶ **Fig. 1** Total ion current (TIC) corresponding to the total extract of the lipids of the seeds from the hemp plant (*C. sativa* L). *New cannabinoid.

gether with a number of other even-mass peaks which result from 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 2 additional carbon atoms from the ion at *m/z* 371 produced a fragment at *m/z* 343 ([M − 71]⁺). The direct fragmentation of the molecular ion at *m/z* 331 ([M − 83]⁺) and the loss of the entire side-chain gave the ion [M − 99; M − C_7H_{15}]⁺· 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 Δ⁹-THC-C_5 would be biosynthetically consistent with that observed in steroids with 28 and 29 carbon atoms [20–22].

Acids found are listed in ▶ **Table 2** including both *cis*- and *trans*-isomers. *cis*-Δ⁹-THCA (A)-C_4 and *cis*-Δ⁹-THCA (A)-C_5 have not been previously reported in the literature [3]. Δ⁹-THC-*n*-C_5 acid A was previously described as TMS derivative [10]. This cannabinoid was found together with 2 other isomers with a butyl and propyl alkylic side chain. Silylated Δ⁹-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]⁺), are characteristic of THCA (A). The fragment at *m/z* 413 ([M − 89]⁺) may
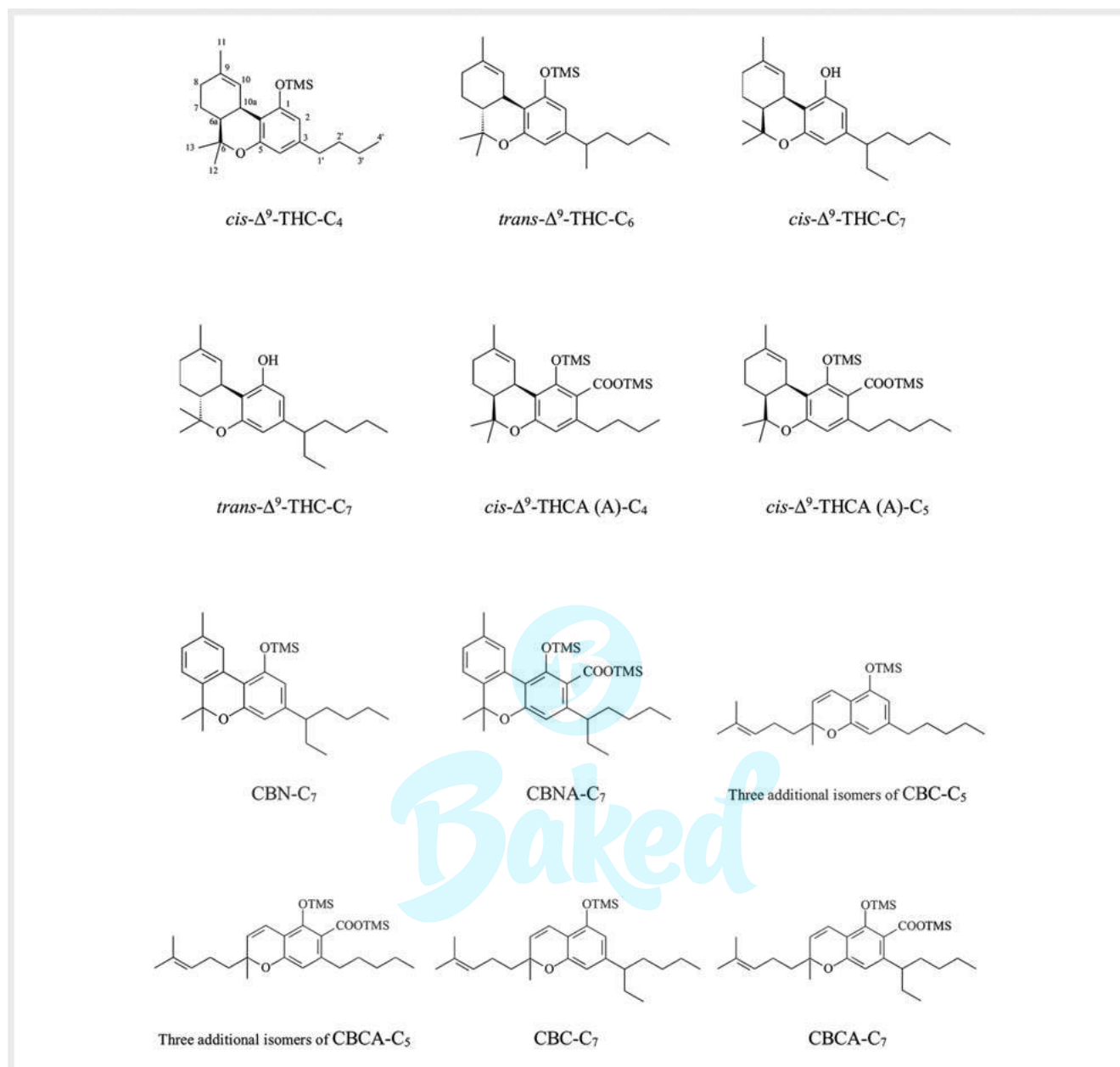
▶ **Fig. 2** Benzopyran system of numbering carbon atoms exemplified in the cis-Δ⁹-tetrahydrocannabinol-C₄ (Δ⁹-THC-C₄) and new cannabinoids reported in this work.

arise from the loss of the trimethylsilyloxy (OTMS) group of the carboxylic moiety, probably in a 2-step mechanism consisting first on the demethylation followed by the loss of the rest of the group.

Four neutral CBNs 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 the molecular peak M and the fragments 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.

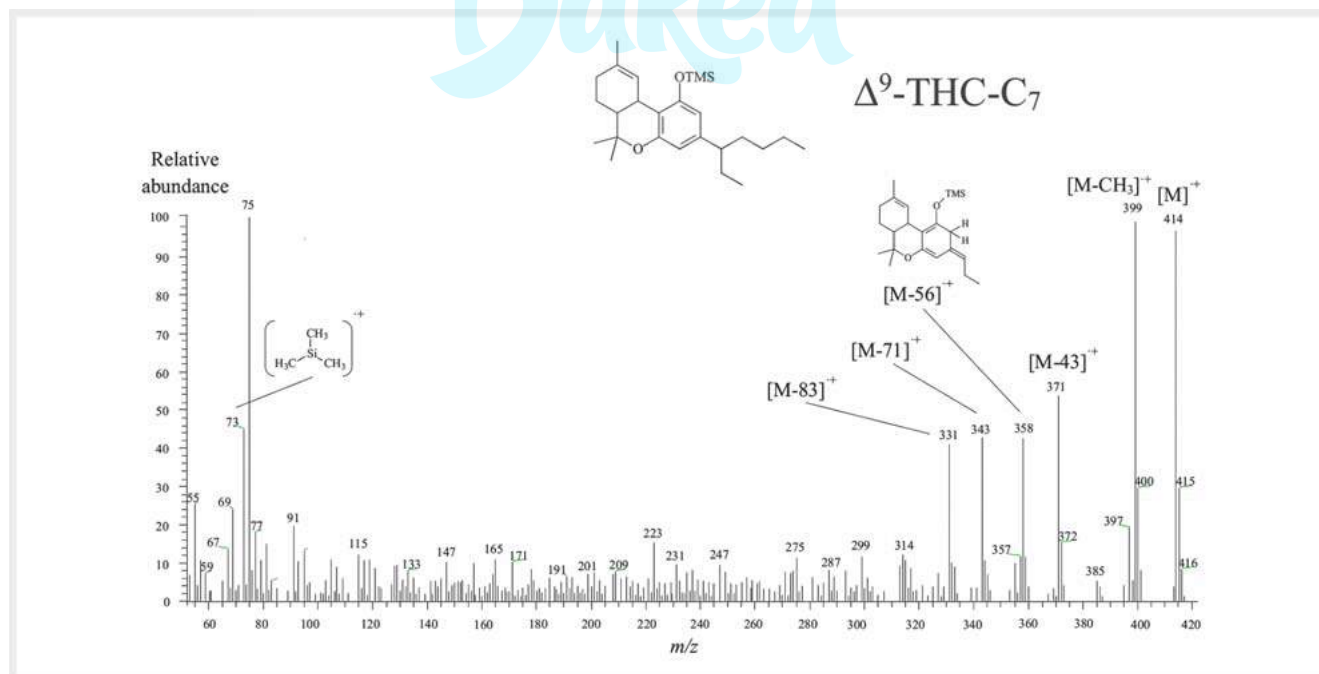Three cannabinolic acids (C_3, C_5, and C_7) (▶ **Table 4**) were also found, whereas the most abundant was pentyl-cannabinolic acid (CBNA-C_5). The mass spectrum of this fully aromatized cannabinoid was dominated by the fragment M-15, which is the major peak (m/z 483) and very low amounts of the molecular ion [M⁺·] [23].
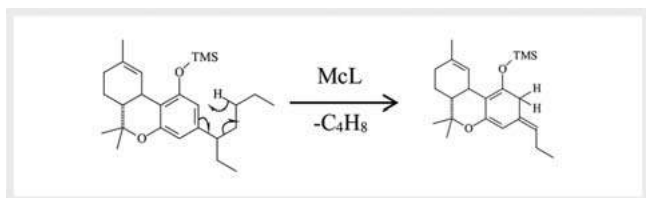
As to the neutral cannabinol distribution, only C_5-cannabinolic acid (CBNA-C_5) was abundant, while trace amounts of C_3 and C_7 homologues were found, with the latter being a new compound.

► **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

► **Fig. 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*.

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

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 would 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**, Supporting information). 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; $[M - 56]^+$) 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 at 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 2 of them have only been 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 study did not include any cannabicyclol because this would be expected

▶ **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

to elute between Δ⁹-THC-C$_3$ (tetrahydrocannabivarin, THCV) and CBD. In order to explain the other 3 CBC isomers, we propose a mechanism consisting of a double bond migration on the terminal isoprenyl unit to the 2 contiguous positions, giving on 1 side a methylene terminal unsaturation and on the other side 2 *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, 3 other diagnostic peaks were detected at *m/z* 414 ([M]+·), *m/z* 399 ([M − 15]+·), 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 3 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)** Supporting information). The fragmentation pattern was deduced by comparison with mass spectra

of cannabinoid derivatives [12]. The molecular ion ([M = 502]+·) and the loss of a methyl group (*m/z* 487) were low abundant ions. The fragment at *m/z* 419 ([M − 83]+·) 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 2 close OTMS groups. The mass spectra of the other 3 isomers are also included (**Fig. 3S**, Supporting information), showing a 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] was 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 for the biosynthesis of cannabinerolic acid in relation to cannabi-

► **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)-C5[†]	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)-C5	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)-C5	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)-C5[‡]	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-C5	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-C5	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-C5	25.97	576 (1)	561 ([M − 15]+, 2), 486 ([M − 90]+, -), 471 ([M − 105]+, 1)
(E)-CBGA-C5[‡]	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

nerol and nerol. The 4 CBG-type cannabinoids found are shown in **Fig. 4S** (Supporting information). 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 ► **Fig. 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. 5 aS**, Supporting information) although it was previously synthesized [34]. The spectrum shows the mass peak ([M]+· = 460), the loss of a methyl group at *m/z* 445, an ion at *m/z* 391 ([M − C$_5$H$_9$]+·), a characteristic peak at *m/z* 337 ([M − C$_9$H$_{15}$]+· corresponding to the fragment [M − 123]+·), 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-C5) was isolated for the first time by Taura et al. [35]. Additionally, this cannabinoid has been identified by HPLC analysis after enzymatic biotransformation by olivetolic acid [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]. 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]+· = 316), an intense fragment at *m/z* 231 ([M − 85]+·; [M − C$_6$H$_{13}$]+·; base peak), and a fragment at *m/z* 193 ([M − C$_9$H$_{15}$]+·) [18]. The mass spectrum of the trimethylsilylated derivative [34] is quite close to that shown in **Fig. 5 bS** (Supporting information) and consists of the mass peak ([M]+· = 460), less amounts of the fragment M − 15, an intense ion at *m/z* 391 ([M − C$_5$H$_9$]+·), and finally a characteristic peak at *m/z* 337 ([M − 123]+· corresponding to the fragment [M − C$_9$H$_{15}$]+·) which was the base peak apart of the TMS group (*m/z* 73).

Finally, *E*-cannabigerolic acid (CBGA-C5) 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 [38, 39]. In agreement with our results, the mass spectrum of this compound includes the mass peak ([M]+· = 576), a fragment at *m/z* 561 ([M − CH$_3$]+·; base peak), an ion at *m/z* 486 ([M − 90; TMSO]+·), a fragment at *m/z* 453 ([M − 123; C$_9$H$_{15}$]+·), and finally another peak at *m/z* 417 corresponding to the fragment ([M − 159]+·). Looking to the fragment at *m/z* 561 corresponding to the base peak, the mass chromatogram (**Fig. 4 bS**, Supporting information) 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 CBD [40], cannabidiolic acid [41], cannabitriol [42], together with 3 isomers of dihydrocannabinol [27,43] and hexahydrocannabinol [44,45], 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 (August 28, 2014) at Sant Fruitós de Bages (Bages County, Catalonia). The coordinates were 41°44′ 09.5′′ N by 1°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 °C for 1 h prior to analysis.

GC-EIMS analysis

The derivatized sample was injected (275 °C) 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-5 ms; length: 30 m; i.d.: 0.32 mm) coated with a 0.25 μL low-polarity liquid-phase film (5% methylpolysiloxane; J&W Scientific). The mass scanning in total ion current (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 °C (1 min); ramp up to 230 °C (20 °C/min); ramp up to 300 °C (2 °C/min); and finally, hold at 300 °C (20 min). Helium was used as the carrier gas (flow rate: 1.0 mL/min). The inlet temperature was 300 °C; the transfer-line temperature, 310 °C; the ion-source temperature, 200 °C.

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 that they have no conflict of interest.

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Lumír Ondřej Hanuš, Giovanni Appendino *et al.*
Phytocannabinoids: a unified critical inventory

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Phytocannabinoids: a unified critical inventory

Lumír Ondřej Hanuš,*[a] Stefan Martin Meyer,[b] Eduardo Muñoz,[c] Orazio Taglialatela-Scafati[d] and Giovanni Appendino*[e]

Covering up to January 2016

Cannabis sativa L. is a prolific, but not exclusive, producer of a diverse group of isoprenylated resorcinyl polyketides collectively known as phytocannabinoids. The modular nature of the pathways that merge into the phytocannabinoid chemotype translates in differences in the nature of the resorcinyl side-chain and the degree of oligomerization of the isoprenyl residue, making the definition of phytocannabinoid elusive from a structural standpoint. A biogenetic definition is therefore proposed, splitting the phytocannabinoid chemotype into an alkyl- and a β-aralklyl version, and discussing the relationships between phytocannabinoids from different sources (higher plants, liverworts, fungi). The startling diversity of cannabis phytocannabinoids might be, at least in part, the result of non-enzymatic transformations induced by heat, light, and atmospheric oxygen on a limited set of major constituents (CBG, CBD, Δ^9-THC and CBC and their corresponding acidic versions), whose degradation is detailed to emphasize this possibility. The diversity of metabotropic (cannabinoid receptors), ionotropic (thermos-TRPs), and transcription factors (PPARs) targeted by phytocannabinoids is discussed. The integrated inventory of these compounds and their biological macromolecular end-points highlights the opportunities that phytocannabinoids offer to access desirable drug-like space beyond the one associated to the narcotic target CB$_1$.

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1. Introduction

Over the past decades, the name "cannabinoid" has become increasingly vague. Originally coined in a phytochemical context to refer to a structurally homogenous class of meroterpenoids typical of cannabis (*Cannabis sativa* L.), the name "cannabinoid" has then been associated to the biological profile of the psychotropic constituent of marijuana (Δ^9-THC), substantially losing its structural meaning and being growingly associated, in accordance with the rules of pharmacological research,[1] to compounds showing affinity to the two GPCR known as cannabinoid receptors (CB_1 and CB_2), independently from any structural or biogenetic relationship with the cannabis meroterpenoids. To compound semantics even more, CB_1 and CB_2 are actually Δ^9-THC receptors, since, within the almost 200 known cannabinoids, only Δ^9-THC, its isomer Δ^8-THC, and, to a lower extent, their aromatized derivative CBN (Fig. 1), bind with significant affinity the ligand recognizing site of these receptors.[1] The endogenously produced biological analogues of THC are referred to as endocannabinoids,[1] and it seems

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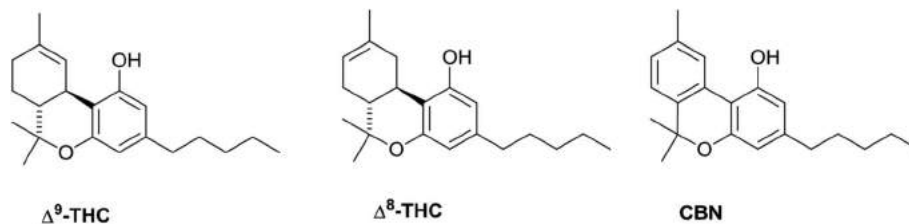This journal is © The Royal Society of Chemistry 2016

Fig. 1   High-affinity phytocannabinoid ligands of cannabinoid receptors.

therefore logical to refer to cannabis meroterpenoids and their analogues of plant origin as phytocannabinoids, emphasizing their botanical origin.

The phytocannabinoid structural motif is biogenetically hybrid, and results from the convergence of the mevalonate and the polyketide pathways. Since both of them are intrinsically modular, variation in terms of polyketide starter and prenyl oligomerization are possible, and indeed Nature has deftly capitalized on this modularity to create chemical diversity that complements the one resulting from the oxidative cyclase phase of isoprenyl diversification. As a result, the name phytocannabinoid is also vague from a structural standpoint. The biogenetic hallmark of phytocannabinoids is a resorcinyl core decorated with *para*-oriented terpenyl and pentyl groups, but compounds with a different degree of isoprenylation (prenyl, sesquiterpenyl) or with a shortened alkyl group (methyl, propyl, or more rarely ethyl and butyl) are also present in *C. sativa*. Phytocannabinoids derived from aliphatic ketide starters are typical of *C. sativa* and are otherwise of limited distribution in Nature, while their analogues derived from an aromatic ketide starter and with a phenetyl-type substituent have a much broader distribution, encompassing not only plants but also liverworts and fungi. Many of these compounds are referred to in the literature as prenylated bibenzyls, a name that hides their relationship with their more famous analogues from cannabis.

To cope with the biogenetic abundance associated with the production of cannabinoids, we propose the classification summarized in Table 1 to address variation of the substituents of the resorcinyl core and of their topological relationships. According to this proposal, "classic" phytocannabinoids are those whose resorcinyl side-chain is derived from a linear aliphatic polyketide starter, while their analogues derived from aromatic starters could be referred to as aralkyl phytocannabinoids. Regarding the relationship between the substituents of the resorcinyl moiety, in most compounds isoprenyl and the resorcinyl side-chain are *para*-related, while analogues where

these groups are in an *ortho*-relationship are assigned to the "abnormal" series. Finally, compounds characterized by an elongated or a shortened terpenyl residue should be referred to as sesquicannabinoids when the isoprenyl residue is of the sesquiterpenyl type, and deprenylcannabinoids when the isoprenyl residue is a simple dimethylallyl. Most cannabinoids have so far been isolated as artifacts from their carboxylated forms (pre-cannabinoids or acidic cannabinoids) from plant sources, and are therefore phytocannabinoids, but the generality of the biogenetic origin does not make it unconceivable that compounds of this type could also occur in fungi or bacteria, and some examples of fungal cannabinoids are indeed known. While phytocannabinoids from the abnormal- and the sesquiterpenyl-series occur in cannabis, phytocannabinoids derived from an aromatic ketide starter have never been reported from this plant source.

This review article aims at providing a comprehensive inventory of phytocannabinoids of different botanical origin. Most phytocannabinoids chemotypes were characterized in the 60ties and 70ties,[2–5] but, after a three-decade gap, new structural types have been discovered, as exemplified by sesquicannabinoids[6] and by the isoprenyl esters of pre-cannabinoids.[7] Furthermore, technological advancement, the growth of the natural product community, and the availability of new cannabis breeds are expected to further expand the current inventory of these compounds. Most phytochemical studies on cannabis precede the identification of cannabinoid and TRPs receptors that occurred in the 90ties, and bioactivity was mostly evaluated with the cannabinoid tetrad test in mice, a combination of four different behavioural tests (hypothermia, hypomotility, catalepsy, analgesia) that, although *per se* unspecific, when all four positive were indicative of a Δ^9-THC-type activity.[8] Activities unrelated to the activation of CB_1 and the replication of the biological profile of Δ^9-THC were therefore missed.

Various articles have regularly updated the inventory of phytocannabinoids from *C. sativa*,[2–5] but no attempt has so far

Table 1   Major classes of phytocannabinoids *sensu lato*

Compound class	Ketide starter	Side-chain/isoprenyl topological relationship	Isoprenyl residue
Alkyl phytocannabinoids	Aliphatic	*para*	Terpenyl (C10)-type
Aralkyl phytocannabinoids	Aromatic	*para*	Terpenyl (C10)-type
Abnormal series	Aliphatic or aromatic	*ortho*	Isoprenyl
Sesqui (deprenyl)-series	Aliphatic or aromatic	*ortho* or *para*	Sesquiterpenyl (C15) Deprenyl (C5)

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been done to include in this survey also phytocannabinoids from additional natural sources. Apart from this, we have also tried to outline the basic chemical and biological profile of the various structural types of phytocannabinoids, and to discuss their biogenetic relationships, chemical interconversions, and biomimetic synthesis from terpene derivatives and resorcinols.

The most important phytocannabinoids are commonly referred to using a three-letter acronym system originating from the first investigators in the field, and later updated by ElSohly to include all the major structural types (Fig. 2).[5] Regrettably, there is no single numbering throughout the various classes of phytocannabinoids, and at least five different systems are documented in the literature. As a rule, the reference system is given simple numbers, while positions in the other elements are referred to with primed or doubly primed numbers. There is no agreement, however, on the identification of the reference system. It used to be the terpene moiety in all cases, but it is now growingly considered the aromatic ring in CBG derivatives

(but not in CBD). When oxygen bridges are present between the terpenyl and the resorcinyl system, the reference system becomes the corresponding fused heterocycle in accordance with the IUPAC rules, even though this hides relationships between biogenetically corresponding carbons (Fig. 2). Thus, all p-menthane-type phytocannabinoids were originally numbered in the same way, using the isoprenoid moiety as a basic system, but, also because of ambiguities in the identification of the starting carbon of the menthane moiety (benzylic carbon $vs.$ the methyl-bearing olefin carbon), the terpenoid numbering has now been replaced by the heterocyclic numbering. As a result of this change, Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD), although structurally related (Scheme 1), are numbered in a different way (Fig. 2). The terpenoid system is still often used for cannabichromene (CBC) and for cannabicyclol (CBL), both numbered according to CBG, while cannabielsoin (CBE) is numbered according to THC. To avoid confusion, especially when tabulating NMR data, it would be
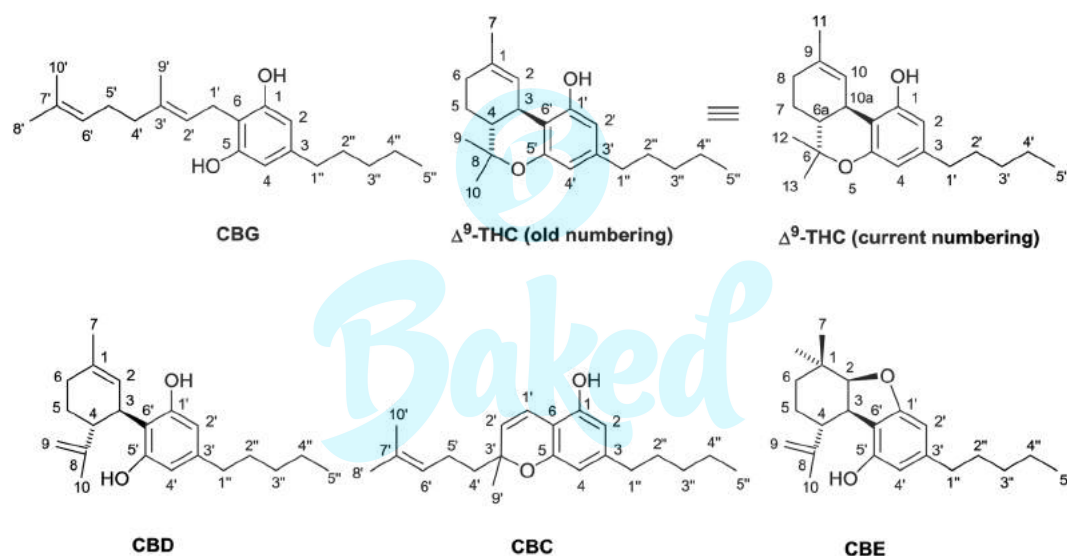
Fig. 2   Phytocannabinoid numbering systems.
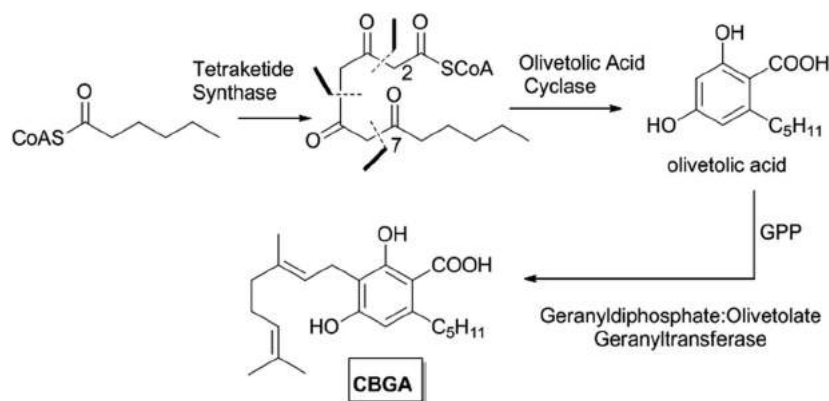
Scheme 1   Formation of cannabigerolic acid (CBGA) in *C. sativa*.

practical to have a reference numbering system capable to accommodate all phytocannabinoids having the same type of isoprenyl residue, independently from the closure of oxygenated heterocyclic with the resorcinyl moiety.

2.   Biogenesis of phytocannabinoids

Neutral phytocannabinoids were long assumed to be genuine natural products, but, while investigating fresh samples of fiber hemp, Schulz and Haffner[9] discovered that their major constituent was not CB, but, rather, its carboxylated version (cannabidiolic acid, CBDA or pre-CBD, Scheme 2), a compound first described by Krejčí and Šantavý in 1955.[10] It is currently assumed that all neutral phytocannabinoids originate from the mostly non-enzymatic decarboxylation of their corresponding carboxylated forms. Consequently, olivetolic acid and not olivetol, was their actual aromatic precursor, and the early biogenetic schemes were elaborated on the basis of the biosynthesis of polyketides, identifying some basic relationships between the small pool of the compounds known at that time. The first step in cannabinoid biosynthesis was correctly considered the condensation of a hexanoylCoA and three activated acetate units to generate the diketo tautomer of olivetolic acid. Farmilo's biogenetic proposal[11] was the first to consider phytocannabinoids in their native carboxylated form, anticipating the existence of THCA before its actual isolation.

Guided by this proposal, the enzymology of phytocannabinoids biosynthesis was substantially clarified. A polyketide origin for the resorcinyl moiety of phytocannabinoids is consistent with the finding that a close relationship exists in *Cannabis* tissues (female flowering tops, leaves, stems and roots) between the levels of hexanoylCoA and the concentrations of the carboxylated form of CBD (pre-CBD, CBDA). A gene encoding a novel type III polyketide synthase (PKS) was cloned from *C. sativa* and named olivetol synthase,[12] but the enzyme actually failed to produce olivetol or olivetolic acid in the
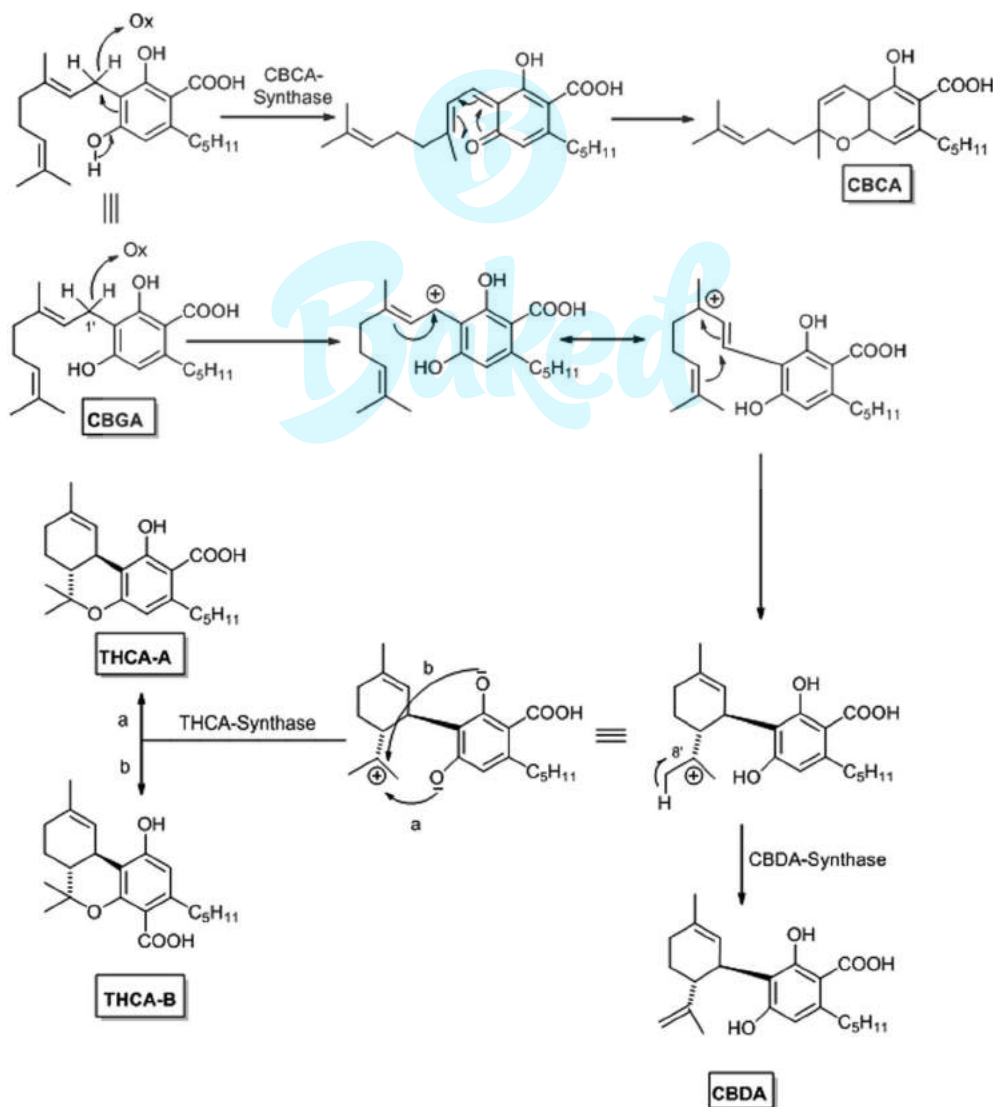
Scheme 2   Biosynthetic origin of the major phytocannabinoids.

absence of a polyketide cyclase enzyme, named olivetolic acid cyclase (OAC) that was cloned from the glandular trichomes of cannabis.[13] This enzyme catalyzes a C-2/C-7 intramolecular aldol condensation, retaining the carboxylic group and forming olivetolic acid. Interestingly, OAC is a dimeric α + β barrel (DABB) protein structurally similar to polyketide cyclases from *Streptomyces* species, indicating evolutionary parallels between polyketide biosynthesis in plants and bacteria.[14]

Regarding the isoprenoid residue, Mechoulam recognized CBG as the precursors of all other types of phytocannabinoids already in 1964,[15] reasoning that this compound has the lowest oxidation level for the isoprenyl moiety. Accordingly, CBG can be formed by the *C*-isoprenylation of olivetolic acid with geranyl diphosphate, and then be converted to CBD, THC and, eventually, CBN. Two years later, the biogenesis of cannabinoids from geranyldiphosphate and olivetolic acid was indeed reported.[16] This biogenetic blueprint was next extended[17] to include the possibility to generate both acidic and neutral cannabinoids, with, however, growing awareness that neutral phytocannabinoids might actually be artifacts formed during harvest and storage of *Cannabis*.[18]

Progress was done in the discovery of the enzymes responsible for the isoprenylation of olivetolic acid, and a specific enzyme, named geranyldiphosphate:olivetolate geranyltransferase, was characterized in young leaves of *C. sativa*.[19] This enzyme catalyzes the first step in cannabinoid formation in hemp, namely the prenylation of olivetolic acid, and accepts geranyldiphosphate (in turn derived from the plastidial 2-methyl-D-erythritol-4-phosphate pathway) as a substrate. In the presence of olivetolic acid (olivetol is not accepted as a substrate), a *ca.* 2 : 1 mixture of cannabigerolic- and cannabinerolic acids is formed. The replacement of geranyldiphosphate with neryldiphosphate changed the ratio to 1 : 1, with rate being only 20% of the one observed with geranyldiphosphate.[19]

The isoprenylation step is next followed by an oxidative cyclase activity that, through the agency of specific enzymes, generates CBCA, CBDA and Δ^9-THCA from CBGA. From a mechanistic standpoint (Scheme 2), the reaction formally involves hydride abstraction from the benzallylic terpenyl carbon. The formation of the resulting cation scrambles the configuration of the adjacent double bond, making it possible the generation of the cyclohexene ring of CBDA and Δ^9-THCA by electrophilic cyclization. Alternatively, the isomerized benzallyl cation can evolve into a quinone methide and generate CBCA by an electrocyclic reaction. The electrophilic cyclization is enzyme-promoted and generates chiral products, while the electrocyclic reaction is probably spontaneous, since CBCA is generated as a racemate.

The electrophilic cyclization step is highly specific in terms of termination. In one version of the process, the C-8 cation (menthane numbering) behaves as a Broensted acid, and is quenched by loss of a proton from C-9 to generate the exocyclic double bond of CBDA (Scheme 2). In the alternative version of the termination, the C-8 menthyl cation behaves as an electrophilic sink for one of the two *ortho*-hydroxyls, generating Δ^9-THCA-A from the hydroxyl *para*- to the carboxylate, and Δ^9-THCA-B from the other phenolic hydroxyl. The oxidative- and the electrophilic cyclase activities are

closely associated, and the menthyl cation is not released or leaking from the enzymatic cleft where it is generated, making the two termination process biogenetically orthogonal. This is consistent with the paradoxical observation that, while CBD is easily converted into Δ^8- and Δ^9-THC by acidic treatment under laboratory conditions, CBDA is not converted into THCA in cannabis tissues. CBDA-synthase and THCA-synthase have been cloned from the storage cavity of the glandular trichomes of cannabis,[20,21] and they exclusively produced their corresponding phytocannabinoids. THCA synthase has also been crystallized, and the FAD and substrate-binding sites identified.[22] Apparently, the enzyme selectively produce one of the two isomeric THC acids present in nature, THCA-A.[22] THCA- and CBDA-synthases are similar in terms of mass (both are 74 kDa monomeric proteins), pI, v_{max} and K_m for CBGA, and are 84% identical in their amino-acid sequence.[21,23] Both THCA- and CBDA-synthases show a domain with high homology with the enzyme involved in the oxidative cyclization step of the biosynthesis of berberine, a benzophenantridine alkaloid, in the Californian poppy (*Eschscholtzia californica*). Both processes require molecular oxygen for their activity and form hydrogen peroxide during the oxidative cyclization of the substrate.[24] Also cannabichromenic acid (CBCA) synthase, the enzyme catalyzing the oxidocyclization of CBGA to CBCA has been identified in young leaves of cannabis and next purified and characterized.[25] A summary of the biogenic relationship between the main phytocannabinoids in *Cannabis sativa* L. is reported in Scheme 2.

Genuine oxidative capacity has been detected in cannabis tissues, as shown by the observation that suspension cultures of the plant can convert primary and secondary allylic alcohols into the corresponding carbonyls.[26] It is unclear, however, whether phytocannabinoids are substrates for this activity.

Labelling experiments with ^{14}C-CBG, and ^{14}C-olivetolic acid were used to study the production of phytocannabinoids in cannabis roots. These experiments confirmed that C-3 phytocannabinoids derive from an independent biosynthesis and not from the enzymatic shortening of the C-5 side chain by either plant or contaminating fungal tissue.[27] Thus, all the CBGA alkyl-homologs could be used as substrate for the different cannabinoid synthases *in vitro*, although the efficiency of conversion was different within the various homologues.[27] It was also shown that decarboxylation of cannabinoid acids is a continuous process, generating neutral cannabinoids already in the early stages of the plant growth, and next continuing during all the vegetation stage.[27]

There is currently great interest in the expression of the key enzymes involved in the production of phytocannabinoids in fermentable organisms, and in 2015 it was announced that the methylotropic yeast *Pichia pastoris* has been engineered to produce Δ^9-THCA from CBGA.[28] Functional expression of Δ^9-tetrahydrocannabinolic acid synthase (THCAS) was also obtained in baker's yeast (*Saccharomyces cerevisiae*), although an overall lower fermentation yield was obtained.[28]

The genetic of inheritance of the enzymes responsible for the formation of the major cannabinoids is complex, and has been extensively investigated as regards CBDA and THCA synthases. These two enzymes are assumed to be coded for by two

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co-dominant alleles, respectively B_D and B_T, while a defective form of the allele could be responsible for the accumulation of CBG *via* the production of an inactive or minimally active oxydocyclizing enzyme. The situation is, however, complicated by the presence of a host of THCA- and CBDA-synthase-related pseudogenes that make the inheritance of phytocannabinoids substantially deviating from a simple Mendelian model.[29]

Nature is a biogenetically tinkerer, and prefers to re-use, recycle and re-assemble rather than creating *ex novo* something new. This so called "law of stinginess" is exemplified by the observation that certain isoprenylated ketides replicate, within the framework of compounds derived from an aromatic starter, the features of phytocannabinoids from cannabis (aldol-type derivation of the prenylated aromatic moiety, resorcinyl-type hydroxylation pattern, *C*-monoprenylation), fully qualifying as "phytocannabinoids", as will be discussed in Section 4.2 to highlight the difference between phytocannabinoids and phytocannabinoid-like compounds.

3. Naturally occurring phytocannabinoids

3.1 Structural diversity

The diversity of natural phytocannabinoids is the result of differences in their three moieties, namely the isoprenyl residue, the resorcinyl core, and the side-chain. These differences are generally orthogonal, that is, biogenetically unrelated. Although impressive, the inventory of alkyl-cannabinoids might have been inflated by the poor oxidative stability of some of the major phytocannabinoids, Δ^9-THC in particular. Furthermore,

many investigations were carried out on aged samples of seized marijuana or hashish, and some compounds were only observed as GC peak and tentatively identified by their mass spectrum, without never actually have been isolated.

3.1.1 The isoprenyl residue. Apart from its oligomerization degree (prenyl-, terpenyl-, sesquiterpenyl), the isoprenyl moiety of phytocannabinoids can occur in nine basic topological arrangements (Scheme 3), classified according to:

(a) The carbon–carbon connectivity of their isoprenyl moiety, that can be linear (cannabigerol-type compounds), monocyclic (*para*-menthane-type and thymyl-type) or bicyclic (cannabicyclol-type phytocannabinoids)

(b) The closure of oxygen bridges between the isoprenyl and the resorcinyl moieties, that generates cannabichromene (CBC)-type compounds from linear precursors and hydrocannabinol-, cannabielsoin (CBE)- and cannabifuran (CBF)-type compounds from monocyclic precursors.

(c) The aromatization of the *p*-menthyl moiety to a thymyl moiety, that generates cannabinol-type and cannabinodiol-type derivatives from, respectively, THC- and CBD-type precursors.

(d) The closure of additional carbon-bonds, as exemplified by cannabicyclol derivatives.

3.1.2 The resorcinyl moiety. The resorcinyl core of native phytocannabinoids is carboxylated, and these compounds are referred to as acidic phytocannabinoids or pre-cannabinoids. In compounds with a single bond between the isoprenyl residue and the aromatic moiety, the two unsubstituted aryl carbons are equivalent. However, when one of the two phenolic oxygens is bound to the isoprenyl residue, the two positions are not identical, and isomeric carboxylated forms have been isolated
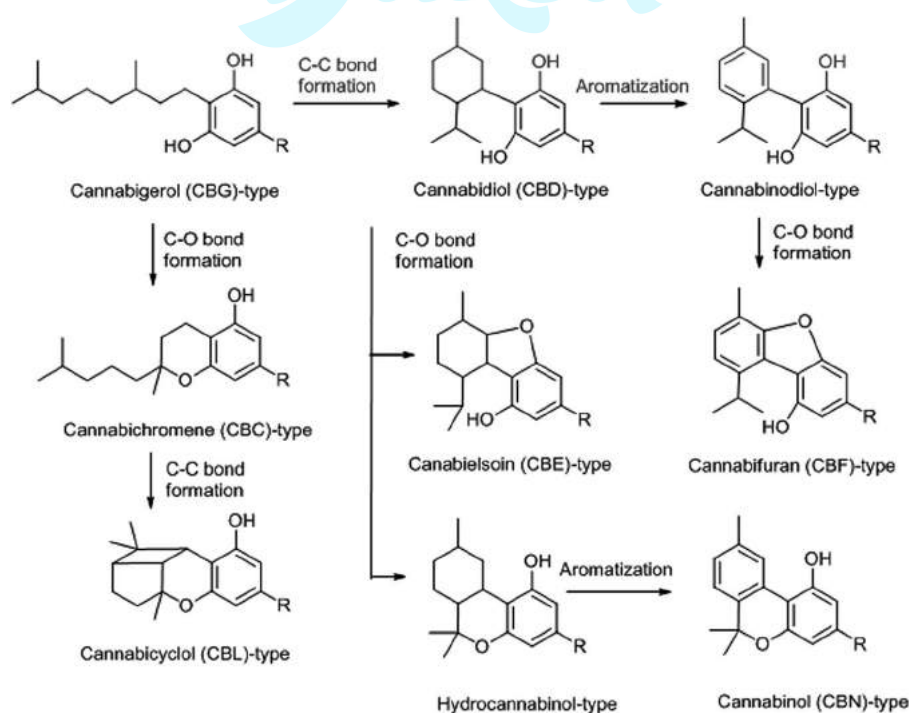
Scheme 3 Topological classification of the major skeletal types of phytocannabinoids.

(Fig. 2, type 1 and type 2 pre-cannabinoids). The spectroscopic properties of the two isomeric forms are rather different, since in type 1 pre-cannabinoids the carboxyl group is hydrogen-bonded to the adjacent *ortho*-hydroxyl, while this bond is not possible in their type-2 isomers.[30] This reflects in their carbonyl IR frequencies (*ca.* 1615 cm^{-1} for the hydrogen bonded carboxyl, and *ca.* 1715 cm^{-1} for the non-hydrogen bonded isomeric form) and UV maxima, with the hydrogen-bonded isomers absorbing at a lower frequency (λ_{max} *ca.* 250–257 nm) compared to the other type of pre-cannabinoids (λ_{max} *ca.* 260–270 nm).[30] Decarboxylation can occur spontaneously in the plant material, and is accelerated by heating at high temperature (>100 °C). The reaction is much faster with intramolecular hydrogen-bonded pre-cannabinoids, despite their higher thermodynamic stability compared to their isomers.[30] The higher thermal stability of type-2 pre-cannabinoids makes it likely that they are absorbed as such from cannabis preparation even from heated products. Nevertheless, virtually nothing is known on the bioactivity of type-2 pre-cannabinoids.

Acidic cannabinoids have been detected in historical samples of *Cannabis* tincture over 100 year old,[31] and these compounds are not decarboxylated under physiological conditions.[32] Up *ca.* 70% decarboxylation has been reported in controlled smoking experiments,[32] but the half-life of acidic phytocannabinoids in plant material at room- or lower temperatures is in the range of hundreds of days.[32] Therefore these compounds are the major form of phytocannabinoids present in edible marijuana. Despite their low volatility, pre-cannabinoids are absorbed from smoked cannabis, and the detection of pre-THC derivatives has even been proposed as a diagnostic test to distinguish the recreational use of marijuana, that contains pre-THC, from positivity due to the assumption of mainstream medications originating from semi-synthetic THC (Marinol®).[32] There is currently great interest for pre-cannabinoids, fostered by the discovery that pre-THC retains activity at both CB$_1$ and CB$_2$, but is not narcotic due to its very poor brain penetration.[33] Pre-cannabinoids can also occur as thermally-stable complex esters with terpenic and sesquiterpenic alcohols, and the pharmacology of these compounds is still unexplored, probably because of the difficulty to purify them from the highly lipophilic fractions of cannabis extracts. Methyl esters of pre-cannabinoids were often prepared to facilitate their purification, but hydrolysis by basic treatment to regenerate the native acids has been reported to be unsuccessful.[34] Pre-cannabinoids show strong anti-bacterial activity, similar to the one of their corresponding neutral derivatives.[35]

Further structural diversity in the resorcinyl moiety can involve *O*-alkylation, generally with a methyl group, or oxidation to the quinol and hydroquinol level. Cannabinoids from the quinol series are intensively purple-colored in non-acidic conditions, and their easy formation from CBD and CBG is at the basis of the Beam test, a forensic identification method for marijuana.[36] Cannabinoid quinols are unstable toward dimerization and further degradation,[36] and have so far been isolated only in traces from the abnormal series,[37] as their stable acetates from the normal series,[36] or in deoxygenated form.[38] They might also be involved in the mammalian metabolism of phytocannabinoids, but their instability and the lack of reference compounds have combined to leave this issue unsettled.[39] Cannabinoid quinols show interesting bioactivity, and those derived from CBD (HU-313)[40] and CBG (VCE-003)[41] (Fig. 3) are non-adipogenic PPARγ agonists and have been considered for clinical development respectively, as anticancer agent and as neuroprotectory agents.[40–42] These compounds could be stabilized as rapidly re-oxidized aza-Michael adducts without loss of anti-fibrotic activity as in VCE-004-8 (Fig. 3).[43]

The carbon-substitution pattern of the resorcinyl core is generally 1,4, with the isoprenyl and the side-chain *para*-related. Few alkyl phytocannabinoids belong to the so called "abnormal series", where the two carbon substituents are in an *ortho*-relationship (Fig. 2), but these compounds are more common in aralkyl phytocannabinoids. Compounds from the abnormal series derive by a process of prenylation at the carbon in *ortho* or *para* relationship to the resorcinyl hydroxyls, while cannabinoids from the normal series derive from the alkylation of the carbon adjacent to the two resorcinyl hydroxyls (Fig. 4).

3.1.3 The resorcinyl side-chain.

The ketide substituent of the resorcinyl core can be alkylic or aralkylic. The alkyl residue of the resorcinyl moiety has generally an odd number of carbons, five (olivetoids) or, less frequently three (viridinoids) and one (orcinoids), with the names making reference to their corresponding non-prenylated resorcinyl derivatives (olivetol, divarinol, and orcinol, Fig. 5). Orcinoids are the major phytocannabinoids from *Rhododendron* species, but are otherwise rare in cannabis. Alkyl side chains with an even number of carbons (two or four) are very rare, although compounds of this type have been reported as trace constituents of cannabis. Since hashish is often attack by molds, it was suggested that phytocannabinoids with an even number of carbons might be artifacts derived by fungal ω-oxidation and decarboxylation of their corresponding homologues.[44] However, enzymatic studies provided evidence for the presence of specific ketide synthases
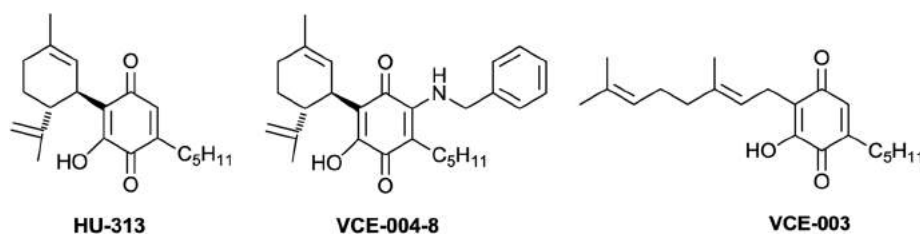

Fig. 3 Bioactive cannabinoid quinols under preclinical/clinical development.
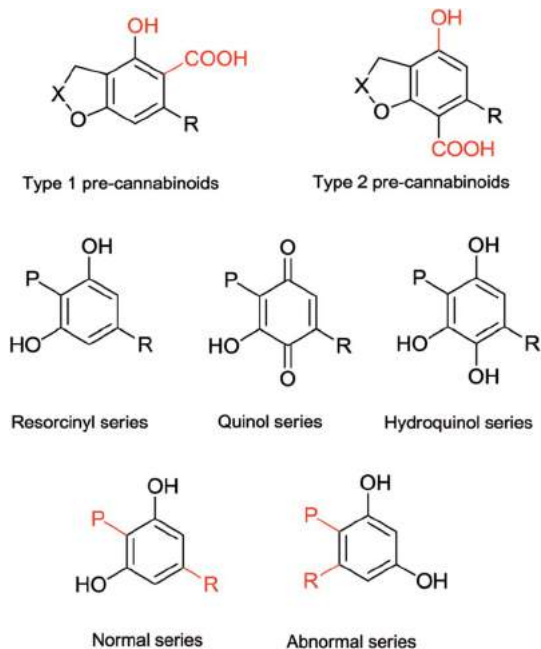
Fig. 4 Diversity of the resorcinyl moiety of cannabinoids (P = prenyl; R = alkyl).

responsible for the generation of these "shortened" alkyl phytocannabinoids.[27] The alkyl residue is a critical element for the phytocannabinoid pharmacophore, and its manipulation can lead to an increased potency compared to the natural compounds.[45]

Aralkyl phytocannabinoids do not occur in *Cannabis*, but have an otherwise broad distribution in plants, both higher (*Helichrysum*, *Amorpha*, *Glycyrrhiza* and other genera) and lower (liverworts from the *Radula* species), with even a single report from a parasitic fungus. The aralkyl residue can be of the phenethyl-, stiryl-, or benzofuranyl type (Fig. 6), and the corresponding compounds have been named bibenzyl-, stilbenyl- and benzofuranyl phytocannabinoids.
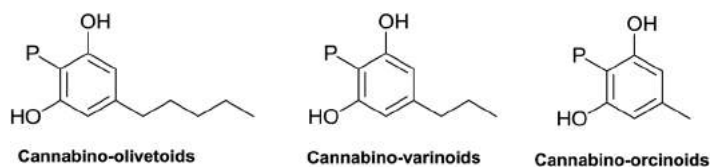
4. Phytocannabinoids inventory

Depending on the nature of the resorcinyl side-chain, compounds will be sorted out in alkyl- and β-aralkyl phytocannabinoids. Within the two classes, compounds are classified according to the nature of the isoprenyl residue (linear, carbomonocyclic) and the presence of oxygen bridges with the resorcinyl core, making reference to a set of archetypal major chemotypes.

4.1 Alkyl phytocannabinoids

4.1.1 Cannabigerol (CBG)-type compounds.
The structural hallmark of these compounds is the presence of a linear isoprenyl residue, as exemplified by cannabigerol (CBG, **1c**), structurally elucidated in 1964, and also the first natural cannabinoid to be synthesized.[15] The isoprenyl residue of CBG is non-oxygenated, and is therefore at the lower oxidation- and earliest biogenetic state within phytocannabinoids. Although CBG was not identified as a major constituents of *C. sativa* during the first studies on this plant, varieties enriched in this compound have recently been generated by hybridization.[29] Remarkably, a South-African species of everlasting (*Helichrysum umbraculigerum* Less.), is also a major producer of CBG (**1c**) and CBGA (**1d**) (overall *ca.* 0.2% of the aerial parts) as well as of abnormal CBGA (**10a**).[46] Cannabigeroids are one of the most structurally diversified class of phytocannabinoids, with structural changes being associated to the isoprenyl residue (oxidation, double bond isomerization, prenylogation), the resorcinyl core (hydroxylation or oxygenative dehydrogenation), and its substituents (esterification of the C-2 carboxylate with isoprenyl alcohols, acetylation or methylation of one of the two phenolic hydroxyls). The parent compound shows only marginal affinity for CB$_1$, and, based on the SAR of Δ^9-THC that emphasize the relevance of the pyrane B ring for significant binding,[45] all the natural modifications are also expected to be only marginally active on CB$_1$ and CB$_2$. On the other hand, prenylogation increases affinity for CB$_2$,[6] and a systematic evaluation of the

Fig. 5 Major classes of alkyl phytocannabinoids.

Fig. 6 Major classes of aralkyl phytocannabinoids.

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activity on other phytocannabinoids ionotropic- or transcription factor targets should be worth evaluation. Thus, CBG is a powerful antagonist of the menthol receptor TRPM8, a target of relevance for prostate cancer,[47] potently activates α-2 adrenergic receptors, and inhibits with moderate potency 5HT$_{1A}$ serotonin receptors.[48] The activation of α-2 receptors inhibits the liberation of catecholamine, and has been associated to sedation, muscle relaxation and analgesia.[49]

Apart from the parent compounds (CBG and CBGV) and their carboxylic forms, all the other derivatives are minor or trace constituents of cannabis, with the exception of the monomethyl ether of CBG (1e), that occurs in significant concentrations in some Asian strain of *Cannabis*.[50] Dihydroxylation of CBG affords chemoselectively the ω-epoxide, identical to the racemic compound (carmagerol, 4), isolated from the Carmagnola variety of fiber hemp.[51] Also the proximal epoxides were isolated as a racemic mixture, from both the geranyl (CBG) and the neryl (cannabinerolic) series of neutral and acidic cannabinoids.[52] Analogues with an oxidized resorcinyl residue have also been characterized, both in the quinol and the hydroxyhydroquinone form. Quinol cannabinoids are very unstable,[36] and the isolation of 9a is undoubtedly due to the acetylation of one of the hydroxyl.[53]

It is not clear if the various oxidized versions of cannabigerol are natural products or rather isolation artifacts. The geranylation of olilvetol gives a mixture of CBG and its positional isomer, the so called "abnormal" cannabigerol (10a).[15] While abnormal cannabigerol has never been reported from cannabis and only occurs in *H. umbraculigerum*, both its actylated hydroquinol (10b) and quinol (11) forms have been detected in a high potency Δ^9-THC-strain.[37] The only sesquiterpenyl cannabinoid isolated so far belongs to the cannabigerol series, but it is likely that sesqui-cannabinoids also occur in other structural types biogenetically derived from linear isoprenyl cannabinoids.[6] The deprenyl derivative of *O*-methylcannabigerolic acid (amorfrutin 2, 7), a constituent of leguminous plants (see 4.2.1), is one of the few *n*-pentyl-type phytocannabinoids not isolated from cannabis.[54]

CBG is unstable to acids and bases. Mineral acids cyclize the terpenyl moiety,[15] while in strong bases (heating with BuLi in HMPA), the proximal (Δ$^{2'}$) double bond is isomerized to the

phenyl-conjugated *E*-Δ$^{1'}$-isomer, a reaction mediated by deprotonation at C-1'.[55] Removal of the benzylic proton might involve proton transfer mediated by a phenate ion, since bis-*O*-methyl CBG was stable in these conditions (Scheme 4).[55] Compound 12,[37] although structurally a chromene, is most likely derived from the intramolecular cyclization of ω-epoxycannabigerol, a compound so far unknown from natural sources, and, as a cyclo-CBG, is therefore included in this group of phytocannabinoids.

4.1.2 Cannabichromene (CBC)-type compounds. In this type of phytocannabinoids, the isoprenyl residue is oxidatively fused to the resorcinyl ring. The parent compound (CBC, 13f) was independently isolated in 1966 by Mechoulam[62] and Claussen,[63] who assigned the same trivial name to the compound, thus avoiding semantic confusion in the literature. In many varieties of cannabis, the presence of CBC is associated to the one of Δ^9-THC, suggesting an inheritance relationship between the oxidase involved in the generation of CBC and THC from CBG.[29] Conversely, no relationship seems to exist with oxidase involved in the generation of CBD.[29] The concentrations of CBC-type phytocannabinoids has been found higher in the vegetative compared to the reproductive stage of cannabis.[57] CBC is the only major phytocannabinoid that shows a bluish fluorescence under UV light. When thoroughly purified, natural CBC is racemic, and does not show any activity related to activation of CB$_1$.[64] CBC is, however, a potent non-covalent activator of TRPA1.[47]

CBC is the simplest natural phytocannabinoid to obtain by synthesis, being available, apart from CBG by oxidative dehydrogenation, also from the one step condensation of citral and olivetol (see Section 4.1.5 for a discussion on the mechanism of the reaction).[65] CBC is stable, and has been detected in century-old historical samples of cannabis.[31] As with CBG, diversity in the derivatives of CBC is associated to oxidation of the prenyl group and the aromatic ring, with the hydroquinol hydroxylation pattern being stabilized by acetylation. The configurational aspects of hydroxylated cannabichromenes 14 and 16 have not been elucidated. Since natural CBC is racemic, these compounds are most probably a mixture of diastereomers. Remarkably, the orcinol-type cannabichromenes 13b and 13c are of fungal and not plant origin, and have been obtained from *Cylindrocarpon olidum* Wollenw., a parasite of the root knot
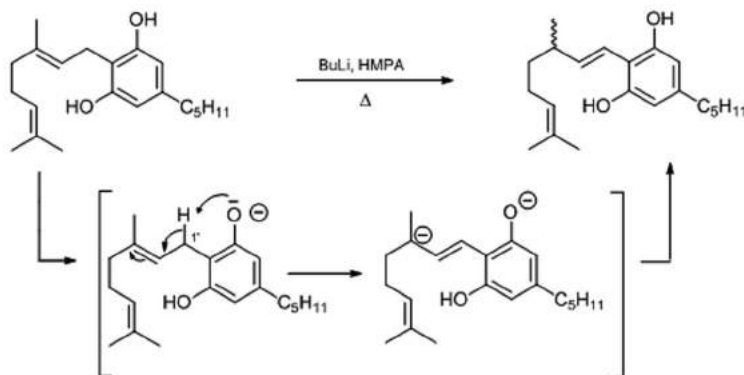


Scheme 4 Isomerization of CBG in basic conditions.

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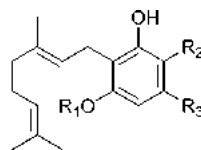
		R_1	R_2	R_3	Ref.
1a	Cannabigerovarin (CBGV)	H	H	C_3H_7	[56]
1b	Cannabigerovarinic acid (CBGVA)	H	COOH	C_3H_7	[57]
1c	Cannabigerol (CBG)	H	H	C_5H_{11}	[15]
1d	Cannabigerolic acid (CBGA)	H	COOH	C_5H_{11}	[34]
1e	O-Methylcannabigerol	Me	H	C_5H_{11}	[50]
1f	Cannabigerolic acid methylether	Me	COOH	C_5H_{11}	[58]
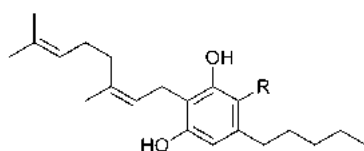
		R	Ref.
2a	Cannabinerolic acid	COOH	[59]
2b	Cannabinerol	H	[60]
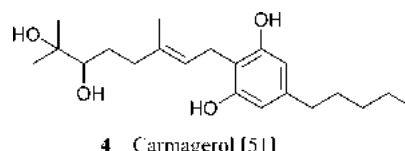
4 Carmagerol [51]
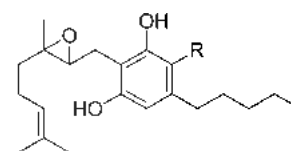
		R	Configuration	Ref.
5a	rac-6'-Epoxycannabigerol	H	(2'S*, 3'R*)	[52]
5b	rac-6'-Epoxycannabigerolic acid	COOH	(2'S*, 3'R*)	[52]
5c	rac-6'-Epoxycannabinerol	H	(2'R*, 3'R*)	[52]
5d	rac-6'-Epoxycannabinerolic acid	COOH	(2'R*, 3'R*)	[52]

nematode *Meloidogyne incognita*, a major pest of some cultivated plants,[66] while the sesquicannabinoids confluentin (13k) and the anti-HIV agent daurichromenic acid (13l) have been isolated from a rhododendron species (*Rhododendron dauricum* L.) with confluentin having also been reported as a constituent of the mushroom from the genus *Albatrellus*.[67] In accordance with the racemic nature of CBC, confluentin (13j) was reported as a racemate, while daurichromenic acid (13k) as well as several functionalized analogues were isolated in an optically active form.[67] This suggests that racemization *via* an electrocyclic mechanism might be slowed by the presence of a carboxylic group *para* to the chromenic oxygen.

4.1.3 Cannabidiol (CBD)-type compounds. CBD (16e) was the first genuine phytocannabinoid to be isolated in 1940,[70] but its correct structure elucidation had to wait the advent of NMR spectroscopy, and was only reported more than two decades later, revising the location of the endocyclic double bond (originally reported at C-3, C5-, and C-8 by different authors), and establishing its relative configuration.[71,72] The clarification of the absolute configuration was done by correlation with natural (−)-menthol,[72] although a wrong absolute configuration for this monoterpene was originally assumed.[71] Since CBD is the major phytocannabinoid in fiber hemp, its carboxylated form was also the first pre-cannabinoid to be isolated,[10] and its relationship with CBD was correctly established by the Czech chemist Šantavý. Along with Cahn, Adams and Todd, Šantavý is one of the founding fathers of the chemistry of cannabinoids, but his contributions appeared, mostly in Czech, in scientific
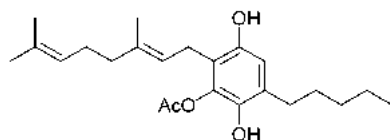
3a	γ-Eudesmyl cannabigerolate	R = A	[7]
3b	γ-Cadinyl cannabigerolate	R = B	[7]

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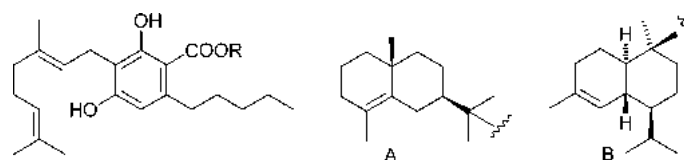
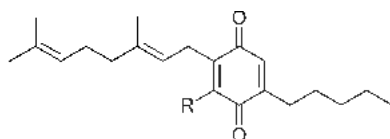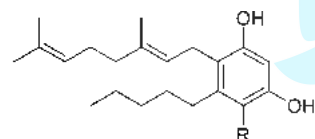*Nat. Prod. Rep.*, 2016, **33**, 1357–1392 | **1367**
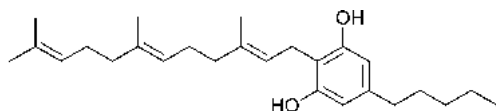
6 Sesquicannabigerol [6]
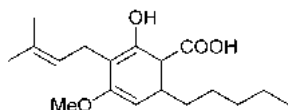
7 Deprenyl *O*-methyl cannabigerolic acid (= Amorfrutin 2) [54]
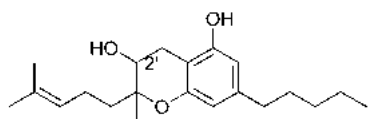

8 5-Acetyl-4-hydroxycannabigerol [37]

	R	Ref.
9a Acetylcannabigeroquinol	OAc	[53]
9b Cannabigeroquinone	H	[61]

	R	Ref.
10a Abnormal cannabigerol	H	[46]
10b Acetyl abnormal hydrocannabigeroquinol	OAc	[37]
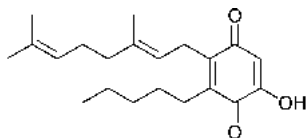
11 Abnormal cannabigeroquinol [37]
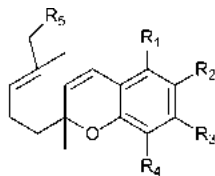

12 2'-Hydroxy-1',2'-dihydrocannabichromene (Cyclo-CBG) [37]

journals of limited distribution outside the Iron Courtain that divided Europe during the cold war, and are still largely overlooked in the phytocannabinoid community. Various modifications[65] of the original synthesis of CBD according to Petrzilka (condensation of *p*-menthadienol and olivetol under mild acidic conditions)[73] have been published. Depending on the strength of the acid, the reaction can stop at the CBD level, or further proceed to a mixture of Δ^8- and Δ^9-THC.[65] During the reaction, abnormal CBD is also formed by a retro Friedel–Craft process, and Razdan carried out a detailed investigation on this remarkable reaction and its subtleties (see also Section 4.1.5).[65]

The isolation of a prenylogue orcinoid analogue of CBD (**17**) was reported from the Alpine rhododendron (*Rododendron ferrugineum* L.).[74] This compound showed only negligible affinity for CB$_1$ and CB$_2$, not unlike CBD.[74] Despite the structural similarity between CBD and Δ^9-THC, the two compounds show a distinct biological profile, and, even though CBD can be electrophilically cyclized to Δ^9-THC by treatment with acids,[65] the two compounds are the result of independent oxidative cyclizations of their common precursor CBGA, and are not interconverted in cannabis tissues.[29] Δ^9-THC and CBD have also quite different oxidative stability. While THC is roughly planar and removal of the benzallylic proton (H-10a) leads to a conjugated radical, the two rings of CBD lie in different planes,[75] and the benzyl radical generated from CBD cannot therefore benefit from conjugation with the aromatic ring. The slow (relatively to the NMR time scale) rotation around the terpenyl–resorcinyl bond is an interesting case of aryl-C(sp^3) hindered rotation en route to atropisomerism, and is responsible for the temperature-dependence of the NMR spectra of CBD.[75] The impossibility to attain planarity and conjugation due to *E*-strain is also responsible the different behaviour of CBD and Δ^9-THC in bases. While the latter generates the conjugated Δ^{10} isomer, CBD is isomerized to its further de-conjugated Δ^6-isomer, a compound of unknown bioactivity (Scheme 5).[55]

The acid-catalyzed cyclization of CBD to a mixture of narcotic THC isomers might be of relevance for the biological profile of CBD, rationalizing, for instance, the high incidence of somnolence observed in pediatric studies.[76] In simulated gastric fluid (pH = 1), the conversion of CBD, solubilized with sodium dodecyl sulfate, to a mixture of Δ^9 and Δ^8-THC was 98% complete after 2 hours, although the insolubility of CBD might slow down the reaction under physiological conditions.[76] This could also rationalize the observation that CBD is unable to generate significant amounts of Δ^9-THC on smoking marijuana,[77] whose water suspensions are mildly basic (pH *ca.* 8). On the other hand, CBD can do so in the more acidic (pH *ca.* 5.7) tobacco cigarettes when they are spiked with CBD or CBD-containing cannabis oil, a popular practice within cannabis consumers.[3] The pyrolysis of CBD under conditions mimicking smoking gave a complex mixture of products. Apart from small amounts of Δ^8- and Δ^9-THC, the major products identified were cannabielsoin (**39c**) and its C-1 epimer.[78]

Some of the naturally occurring analogues of CBD show interesting structural features, like the presence of an alkyl residue with an even number of carbons (nor-CBD, **16d**) or *O*-alkylation with propyl- and pentyl residues. The isolation of an

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		R_1	R_2	R_3	R_4	R_5	Ref.
13a	Cannabiorcichromene	OH	H	CH₃	H	H	[60, 67]
13b	Cannabiorcichromenic acid	OH	COOH	CH₃	H	H	[66]
13c	Chlorcannabiorcichromenic acid	OH	COOH	CH₃	Cl	H	[66]
13d	Cannabivarichromene (CBCV)	OH	H	C_3H_7	H	H	[56, 67]
13e	Cannabichromevarinic acid	OH	COOH	C_3H_7	H	H	[57]
13f	Cannabichromene (CBC)	OH	H	C_5H_{11}	H	H	[66, 68]
13g	Cannabichromenic acid	OH	COOH	C_5H_{11}	H	H	[69]
13h	4-Acetoxycannabichromene	OH	H	C_5H_{11}	OAc	H	[37]
13i	Anthopogochromenic acid	H	OH	COOH	Me	H	[67]
13j	Confluentin	OH	H	CH₃	H	Prenyl	[67]
13k	Daurichromenic acid	OH	COOH	CH₃	H	Prenyl	[67]
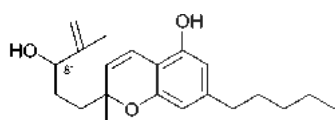
14 8'-Hydroxyisocannabichromene [37]
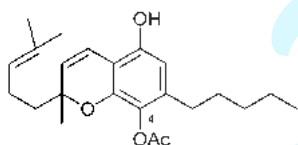
15 4-Acetoxycannabichromene [37]

ester of cannabidiolic acid with a dihydroxylated $\Delta^{6a,10a}$-tetra-hydrocannabinol derivative (**16j**) has also been reported. This compound was the first complex ester of pre-cannabinoids to be isolated.[79]
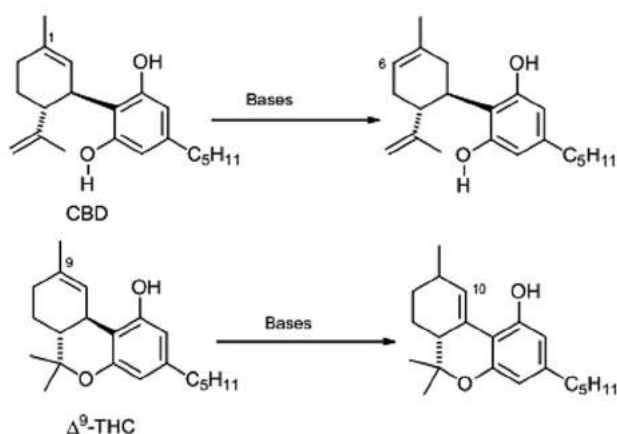
CBD is an allosteric inhibitor of CB₁,[80] and further modulates the activity of Δ^9-THC by interfering with its hepatic allylic hydroxylation, a reaction that generates a metabolite (11-hydroxy Δ^9-THC) with a higher brain penetration and similar potency on CB₁.[39] Despite the enormous current interest for the clinical uses of CBD, the first studies for the bioactivity of CBD were actually triggered by its modulating activity on cytochromes and the potential for drug interaction, with the synergizing activity of CBD on the hypnotic effects of barbiturates being already reported in 1942 by Adams himself.[39] CBD seems to have a host of biological targets, including various thermos-TRP channels and the serotonin receptor 5-HT₁ₐ,[64] and its overall biological profile cannot probably be summarized by the modulation of any single end point of the growing list of CBD biological targets. Currently, the major area of clinical research on CBD is the management of pediatric epilepsy, a use reminiscent of the first report on the medicinal use of *Cannabis* in colonial India by W. B. O'Shaughnessy in 1838.[81]

4.1.4 Thymyl-type phytocannabinoids (cannabinodiol- and cannabifuran type compounds). This type of compounds is characterized by aromatization of the menthyl moiety of CBD to give a thymyl group. Cannabinodiol (**18b**) has a checkered history, and its original isolation report most probably actually referred to its oxidatively cyclize analogue cannabi-furan (**19a**).[88] Cannabifuran (**19a**) and dehydrocannabifuran (**19b**) were isolated from aged samples of hashish,[89] while cannabioxepane (**20**) was obtained from fiber hemp using a mild isolation protocol.[90] Since CBD is air-stable, its aromatization could be the result of enzymatic activity, and these thymyl-type compounds might therefore be genuine phytochemicals. Also the orcinoid form of cannabinodiol is known,[91] and, just like with many other phytocannabinoids, the syntheses of cannabinodiol predates the actual isolation,


Scheme 5 Base-catalyzed isomerization of CBD and Δ^9-THC.

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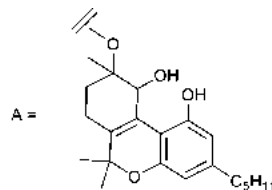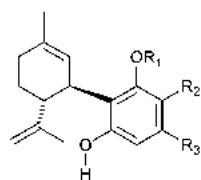
		R_1	R_2	R_3	Ref.
16a	Cannabidiorcol	H	H	CH_3	[82]
16b	Cannabidivarin	H	H	C_3H_7	[83]
16c	Cannabidivarinic acid	H	COOH	C_3H_7	[57]
16d	nor-Cannabidiol	H	H	C_4H_9	[84,85]
16e	Cannabidiol (CBD)	H	H	C_5H_{11}	[70]
16f	O-Methylcannabidiol	CH_3	H	C_5H_{11}	[86]
16g	O-Propylcannabidiol	C_3H_7	H	C_5H_{11}	[87]
16h	O-Pentylcannabidiol	C_5H_{11}	H	C_5H_{11}	[87]
16i	Cannabidiolic acid	H	COOH	C_5H_{11}	[10]
16j	CBDA-THC ester	H	COOA	C_5H_{11}	[79]
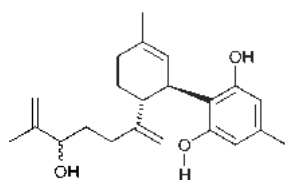
17 Ferruginene C [74]
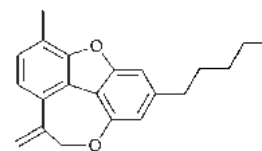
20 Cannabioxepane [90]

being the major photodegradation product of CBN.[92] Nothing is known on the biological profile of this type of phytocannabinoids.
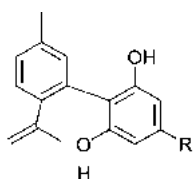
		R	Ref
18a	Cannabinodivarin	C_3H_7	[91]
18b	Cannabinodiol	C_5H_{11}	[91,92]
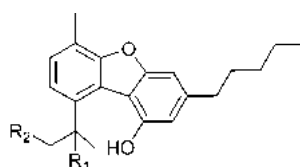
		R_1, R_2	Ref.
19a	Cannabifuran	H,H	[89]
19b	Dehydrocannabifuran	Δ	[89]

4.1.5 Tetrahydrocannabinol-type compounds. *Cannabis* contains a bouquet of bis-reduced forms of cannabinol, differing for the location of the remaining double bond, the configuration of the stereogenic centers, or both isomeric options. The major constituent, and the flagship constituent of cannabis, is *trans*-Δ^9-THC (**23g**. Δ^9-THC for short), but regio- and stereo-isomers also occur as minor constituents.[93–96,118,121] It is not clear if these compounds are enzymatically produced or if, conversely, they are artifacts originating from the degradation of Δ^9-THC or of CBD.

4.1.5.1. Δ^8-tetrahydrocannabinol (Δ^8-THC)-type compounds. Compounds of this class might be isolation artifacts resulting from Δ^9-THC by acid- or oxidatively promoted shift of the endocyclic double bond, or from CBD by electrophilic cyclization. The Δ^8 location is thermodynamically more stable than the Δ^9 location, and this drives the isomerization.[65] The major spectroscopic difference between the two isomeric series is the chemical shift of the olefinic proton, that, because of the proximity to the aromatic ring, is more deshielded in the Δ^9-isomer (δ *ca.* 6.40 in $CDCl_3$) compared to the Δ^8-isomer (δ *ca.* 5.50 in $CDCl_3$).[93] The electrophilic cyclization of CBD can afford the Δ^8- or the Δ^9-isomer depending on the conditions, with mild acidic conditions favoring the Δ^9-isomer and more forced conditions in terms of acidity and temperature the Δ^8-isomer.[93] Δ^8-THC and Δ^9-THC show a similar profile of activity on cannabinoid receptors, with Δ^8-THC being only slightly less active than Δ^9-THC.[45] It should, however, be interesting to evaluate the profile of the two isomers also in terms of other

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targets, like thermo-TRPs and transcription factors of the PPAR family, since this could provide interesting clues to clarify the role of the non-metabotropic targets in the pharmacological profile of Δ^9-THC.

Compounds from the Δ^8 series can be converted into their Δ^9 isomers by addition of hydrochloric acid and base-mediated dehydrohalogenation (Scheme 6).[94] The counter-thermodynamic course of the reaction has been rationalized by assuming that deprotonation occurs intramolecularly *via* a phenate ion, thus favoring deprotonation from C-10 rather than from the other carbons adjacent to C-9.[94] This reaction is of great relevance, since Δ^8-THC is much easier to synthesize than Δ^9-THC (one step from verbenyl olivetol).[95] The isolation of a compound oxygenated at C-11 is interesting, since this is a major route in the human metabolism of Δ^9-THC. In general, compounds from the Δ^8-series are much more stable than their Δ^9-series, and Δ^8-THC has even been detected in a burial tomb dating from the fourth century B.C.[96] Because of the improved stability compared to Δ^9-THC and its easier synthesis, Δ^8-THC proved a better lead structure for phytocannabinoid-inspired probes to explore the biological space around cannabinoid receptors.[45]

location of the double bond turned out to be the only one never considered in all the previous investigations on the elusive narcotic principle of cannabis.[102] As with CBD, Šantavý came independently to the same conclusions, also establishing the absolute configuration of the active narcotic principle by correlation of Δ^9- and Δ^8-THC with CBD.[72] Δ^9-THC belongs to the largest class of phytocannainoids, but the investigation on the phytochemistry of cannabis was long biased on the recreational chemotypes, and future studies on fiber hemp might reveal a different scenario. Diversity within this class of phytocannabinoids is mostly related to oxidation of the *p*-menthene moiety, possibly related to spontaneous degradation of the natural product (see infra), and to the esterification of pre-THC with various isoprenyl alcohols.

Δ^9-THC acts as a partial agonist at both CB$_1$ and CB$_2$,[45] but, unexpectedly, its shorter analogue from the bis-nor type (THCV, 23c) is instead an antagonist at CB$_1$, an important discovery in the light of the observation that rimonabant and most synthetic inhibitors of CB$_1$ are actually reverse-agonist and not antagonists.[103] The phenolic hydroxyl is critical for the activity, but, surprisingly, branching in the alkyl residue makes it redundant for the interaction with CB$_1$.[104] The native form of Δ^9-THC is
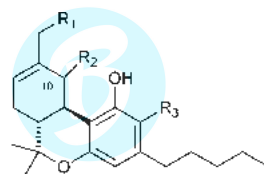
		R$_1$	R$_2$	R$_3$	Ref.
21a	*trans*-Δ^8-Tetrahydrocannabinol (Δ^8-THC)	H	H	H	[97]
21b	*trans*-Δ^8-Tetrahydrocannabinolic acid	H	H	COOH	[98]
21c	10α-Hydroxy *trans*-Δ^8-tetrahydrocannabinol	H	α-OH	H	[99]
21d	10β-Hydroxy *trans*-Δ^8-tetrahydrocannabinol	H	β-OH	H	[99]
21e	11-Acetoxy-Δ^8-tetrahydrocannabinoic acid	OAc	H	COOH	[99]
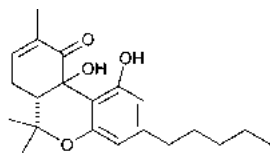
22 10-Hydroxy-9-oxo-Δ^8-tetrahydrocannabinol [100]

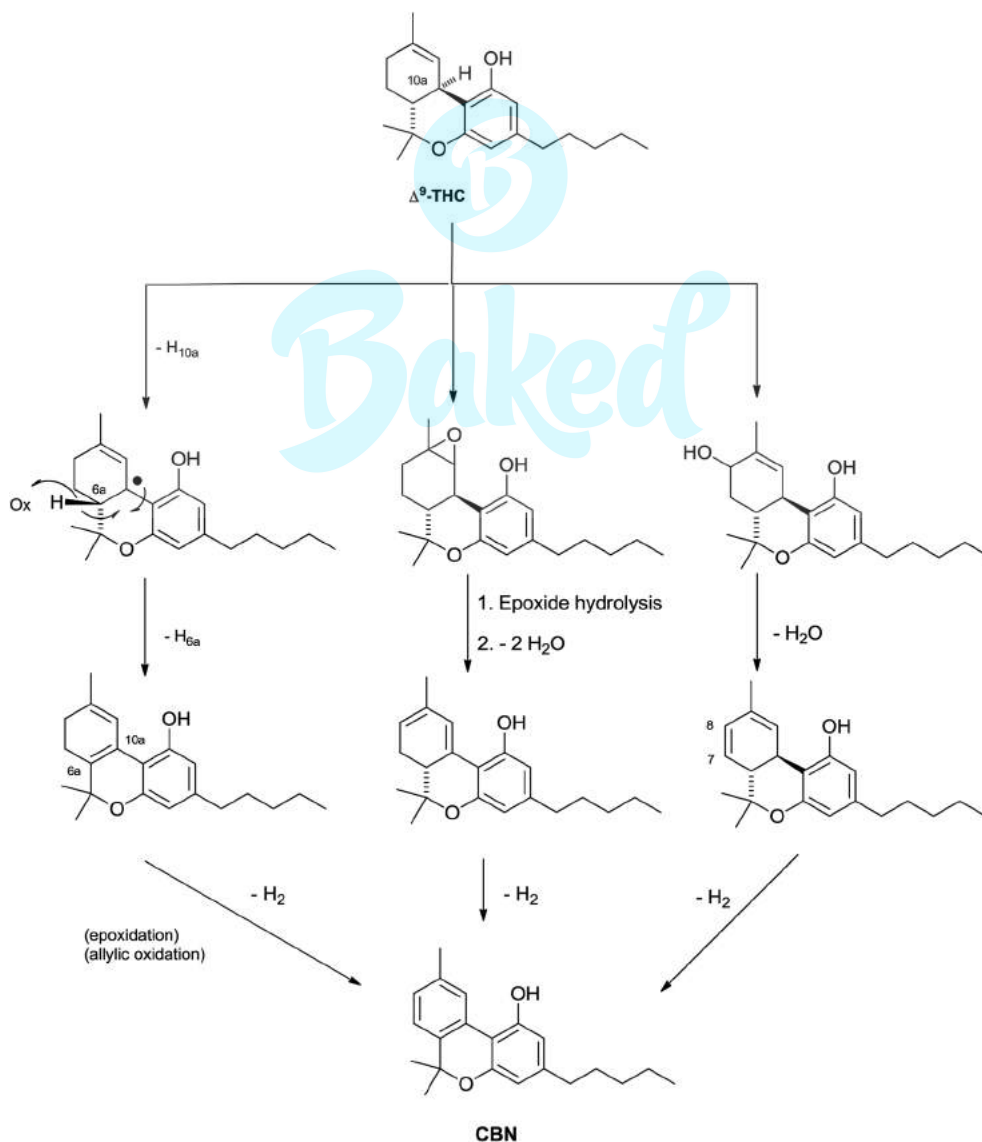*4.1.5.2. Δ^9-trans-tetrahydrocannabinol (Δ^9-THC)-type compounds.* The early investigations on the phytochemistry of cannabis came to the conclusion that the narcotic constituent of the plant was a reduced form of cannabinol, at that time the only cannabinoid whose structure was known. The nature of this "active" tetrahydrocannabinol, possibly confusingly purified as acetyl derivative already in 1942,[101] remained elusive and confusing until the seminal paper by Gaoni and Mechoulam who in 1964 disclosed its isolation and structure elucidation from a Lebanese sample of hashish.[102] Curiously, the Δ^9-

represented by a mixture of two pre-cannabinoids, THCA-A and THCA-B, very different in terms of physical state (THCA-B was investigated by crystallographic studies, while THCA-A is amorphous), stability toward decarboxylation (THCA-A is decarboxylated at 90 °C, while THCA-B is stable at this temperature), and concentration in plant tissues.[105] The acidic form of Δ^9-THC-A is stabilized toward decarboxylation by esterification with isoprenyl alcohols, and these conjugates occur, as a complex mixture, in narcotic cannabis.[37] The structure of these compounds was only tentatively assessed, and the configuration of the isoprenyl residue should be confirmed by an independent synthesis. Δ^9-THC is unstable as a pure compound, an amorphous gum that easily turns brown, but is more stable in crude form and can be stored in refrigerated methanol solution. The degradation is mainly oxidative, and is triggered by abstraction of the allylic and benzylic hydrogen at C-10a (Scheme 7). The resulting radical undergoes further hydrogen abstraction at C-6a, with formation of a conjugated

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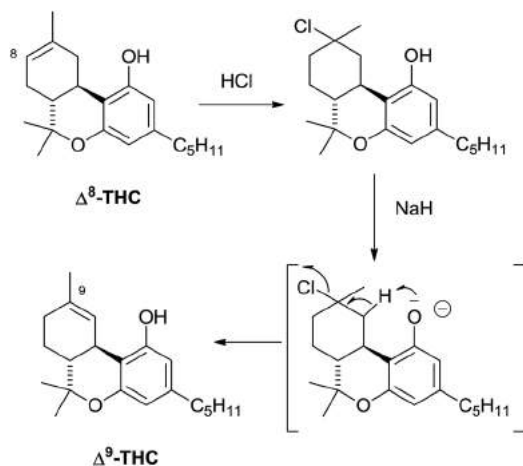
Scheme 6 Conversion of Δ^8-THC into Δ^9-THC.

double bond between C-6a and C-10a, en route to aromatization to CBN. Alternative dienes can be generated *via* either epoxidation of the endocyclic double bond, hydrolysis of the epoxide, and twofold dehydration, or *via* allylic oxidation at the C-8 methylene and dehydration. Aromatization of these dienes eventually generates CBN (Scheme 7).[106] At room temperature, the rate of degradation of Δ^9-THC in cannabis has been estimated in *ca.* 5% per month, and 10% for the pure product, but other degradations pathways have been postulated be operative in plant tissues, since the rate of appearance of CBN was significantly lower than the one of disappearance of Δ^9-THC.[106] On the other hand, this discrepancy could be related to the quick formation of intermediates that then converge to CBD at a slower rate. The mechanistic scenario for the aromatization is in accordance with the isolation of some of the intermediate compounds as well as with the detection of radicals by electron spin resonance during the degradation process.[107] There are no

Scheme 7 Possible mechanism for the oxidative degradation of Δ^9-THC to CBN.

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recent studies on the degradation of Δ^9-THC, and the development in analytical technology witnessed by the past decades should greatly help the clarification of this important process. Interestingly, a tri-hydrocannabinol (28) has been isolated from the pollen of cannabis.[108] This compound could originate by disproportion of a dihydrocannabinol intermediate.

The acidic isomerization of Δ^9-THC generates the thermodynamically more stable Δ^8-isomer, that does not undergo oxidative degradation either in plant material or as a pure product, in accordance with the minor stabilization by resonance of a C-10a radical, that would now only be benzylic and not benzallylic.[109] Δ^9-THC is characterized by an extremely low acute toxicity ($LD_{50} > 100$ mg kg^{-1} iv in rats), while CBD and other cannabinoids have a measurable toxicity (LD_{50} ca. 50 mg kg^{-1} iv in rats for CBD).[104]

while the generation of the methylene-linked dimer cannabisol (30) might be the result of a process similar to the one that forms dicoumarol from 4-hydroxycoumarin.

4.1.5.3. Δ^9-cis-Tetrahydrocannabinol-type compounds. The existence of a *cis*-isomer of Δ^9-THC in cannabis has long been known, but the structure of this compound is still unclear, and the confusing history of this compound exemplify the subtleties of cannabinoid chemistry. Δ^9-*cis*-THC is only a trace constituent of narcotic cannabis, but has been reported to occur in fiber hemp in concentrations similar to those of its *trans*-isomer, an important observation waiting, however, confirmation in modern studies.[118] Since the presence of significant amounts of Δ^9-*cis*-THC is associated to the one of large amounts of CBD, it is not unconceivable that Δ^9-*cis*-THC could actually be an artifact, derived by migration of the exocyclic $\Delta^{8(9)}$ double bond of
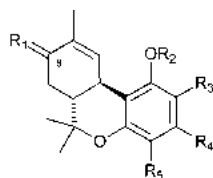
|	R₁	R₂	R₃	R₄	R₅	Ref.
23a Δ^9-*trans*-Tetrahydrocannabiorcol	H,H	H	H	CH₃	H	[82]
23b Δ^9-*trans*-Tetrahydrocannabiorcolic acid	H,H	H	COOH	CH₃	H	[84]
23c Δ^9-*trans*-Tetrahydrocannabivarin (THCV)	H,H	H	H	C₃H₇	H	[110]
23d Δ^9-*trans*-Tetrahydrocannabivarinic acid	H,H	H	COOH	C₃H₇	H	[57,111]
23e Δ^9-*trans*-nor-Tetrahydrocannabinol	H,H	H	H	C₄H₉	H	[85]
23f Δ^9-*trans*-nor-Tetrahydrocannabinolic acid	H,H	H	COOH	C₄H₉	H	[84]
23g Δ^9-*trans*-Tetrahydrocannabinol (Δ^9-THC)	H,H	H	H	C₅H₁₁	H	[102]
23h Δ^9-*trans*-Tetrahydrocannabinolic acid A	H,H	H	COOH	C₅H₁₁	H	[18,112]
23i Δ^9-*trans*-Tetrahydrocannabinolic acid B	H,H	H	H	C₅H₁₁	COOH	[113]
23j 8α-Hydroxy-Δ^9-*trans*-tetrahydrocannabinol	α-OH,H	H	H	C₅H₁₁	H	[99]
23k 8β-Hydroxy-Δ^9-*trans*-tetrahydrocannabinol	β-OH,H	H	H	C₅H₁₁	H	[99]
23l 8-Oxo-Δ^9-*trans*-tetrahydrocannabinol	=O	H	H	C₅H₁₁	H	[100]
23m O-Propyl-Δ^9-*trans*-tetrahydrocannabinol	H,H	C₃H₇	H	C₅H₁₁	H	[87]
23n O-Pentyl-Δ^9-*trans*-tetrahydrocannabinol	H,H	C₅H₁₁	H	C₅H₁₁	H	[87]
23o 2-Formyl-Δ^9-*trans*-tetrahydrocannabinol	H,H	H	CHO	C₅H₁₁	H	[100]

Hydroxylated derivatives of Δ^9-THC have been isolated as a diastereomeric mixture, as expected from a non-enzymatic oxidative process. In some cases, as in 27, the configuration at the hydroxylated carbons was not assessed, and it is unclear if the isolated compound was a mixture of isomers or, alternatively, configurationally pure.[114] The hydroxylated derivatives of Δ^9-THC have been poorly investigated in terms of bioactivity. Interestingly, microsomal hydroxylation of Δ^9-THC takes place at the allylic methyl (C-11) rather than at the endocyclic allylic methylene (C-8).[45] 11-Hydroxy Δ^9-THC, unknown as a natural product, substantially retains the affinity of the natural product toward CB_1 and CB_2, but penetrates more easily the brain.[45] Also the epoxide of Δ^9-THC (25) has been isolated from cannabis,[99]

CBD to a $\Delta^{4(8)}$ position, followed by closure of the pyran ring (Scheme 8). If so, epimerization should be at C-6a (THC numbering), but this reaction has not been clearly observed under laboratory conditions. In accordance with this, treatment with Lewis acids converts racemic Δ^9-*cis*-THC into racemic Δ^8-*trans*-THC, presumably by opening of the oxygen bridge to give a $\Delta^{4,8}$-CBD intermediate, that then re-closes to generate the *trans*-isomer (Scheme 8).[119] However, under these conditions, interconversion from the normal- to the abnormal series has also been observed, showing that also the cleavage of the resorcinyl-menthyl bond *via* a retro-Friedel Craft reaction is, in principle, possible.[119] By using optically active substrates, it was eventually demonstrated that the isomerization takes place *via*
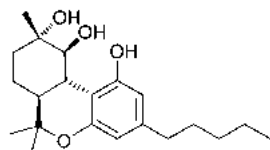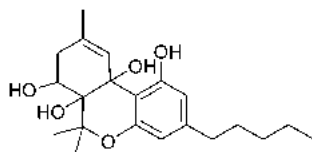
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A   B   C   D   E

F   G   H
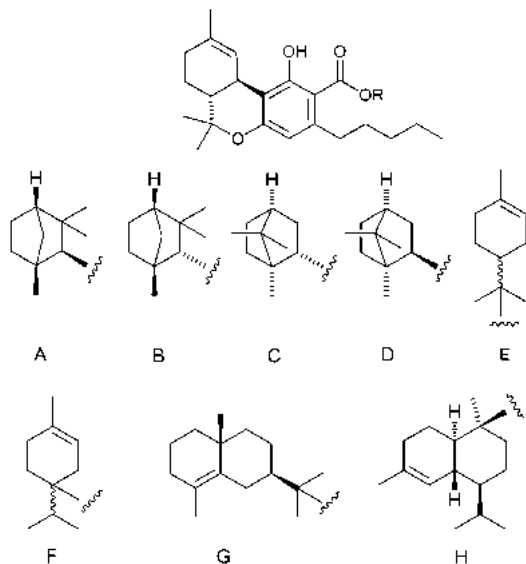
24a β-Fenchyl Δ⁹-trans-Tetrahydrocannabinolate   R = A   [7]

24b α-Fenchyl Δ⁹-trans-Tetrahydrocannabinolate   R = B   [7]

24c Bornyl Δ⁹-trans-Tetrahydrocannabinolate   R = G   [7]

24d epi-Bornyl Δ⁹-trans-Tetrahydrocannabinolate   R = C   [7]

24e α-Terpinyl Δ⁹-trans-Tetrahydrocannabinolate   R = E   [7]

24f 4-Terpinyl Δ⁹-trans-Tetrahydrocannabinolate   R = F   [7]

24g γ-Eudesmyl Δ⁹-trans-Tetrahydrocannabinolate   R = D   [7]

24h α-Cadinyl Δ⁹-trans-Tetrahydrocannabinolate   R = H   [7]
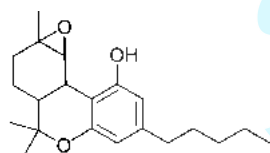
25 Tetrahydrocannabinol epoxide [99]

26 Δ⁹-trans-Tetrahydrocannabinol glycol (cannabiripsol) [115]

27 6a,7,10a-Trihydroxy-Δ⁹-tetrahydrocannabinol [116]

cleavage of the pyrane ring, but it is unclear how this relates to the configuration of natural Δ⁹-cis-THC, if this is, indeed scalemic.[119]

Racemic Δ⁹-cis-THC can be easily prepared from the condensation of citral and olivetol in acidic medium.[93]
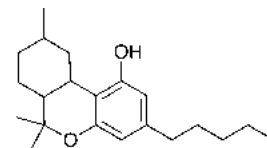
28 Hexahydrocannabinol [114]
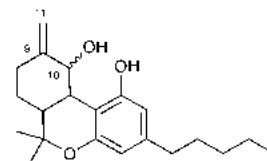
29 Hydroxy Δ⁹,¹¹-hexahydrocannabinol [61]
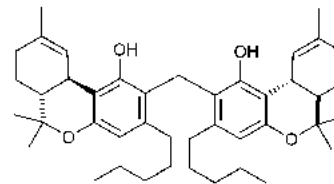
30 Methylen-bis Δ⁹-trans-Tetrahydrocannabinol (Cannabisol) [117]

According to the catalysis, the reaction can afford cannabichromene or Δ⁹-cis-THC. Presumably, the reaction has a concerted course in basic medium, going through a quinone methide intermediate. Conversely, in the presence of protic or Lewis acids, cyclization of the initial 1,2-adduct to a menthyl cation could occur, followed by cyclization to Δ⁹-cis-THC (Scheme 9). The relative configuration of the final product depends on the nature of the catalyst. While Broensted acids afford essentially the cis-isomer, Lewis acids selective for the trans-isomer have been developed.[120]

Δ⁹-Tetrahydrocannabinols from the trans and cis series can be distinguished by the chemical shift of the geminal methyls ($\Delta\delta$ 0.25–0.35 in the trans-series, and 0.08–0.15 in the cis-series) from the signal of the benzylic proton, a broad singlet at around δ 3.50 (CDCl$_3$) for the cis-isomer, and a broad doublet at around δ 3.20 (CDCl$_3$) for the trans-isomer.[55,93] The profile of bioactivity of Δ⁹-cis-THC has only been investigated for CB$_1$-related activity, with the epimerization causing a general decrease of activity. The recent development of a stereoselective total synthesis of all isomeric forms of Δ⁹-tetrahydrocannabinol should make it possible a systematic investigation of the biological translation of the epimerization, as well as a long-awaited evaluation of the configuration of the natural product, if indeed optically active.[121]

Cannabicitran (32) might derive from cis-THC epoxide by Makovnikov-type protonation of the endocyclic double bond followed by trapping of the tertiary C-9 cation by the free-hydroxyl at C-1. Cannabicitran is an interesting case of "anticipated" natural product, since it was obtained by Crombie[122] from the pyridine-promoted condensation of citral and olivetol, before its actual isolation.[123] In a rare example of fair play within natural product chemists, Crombie acknowledged the
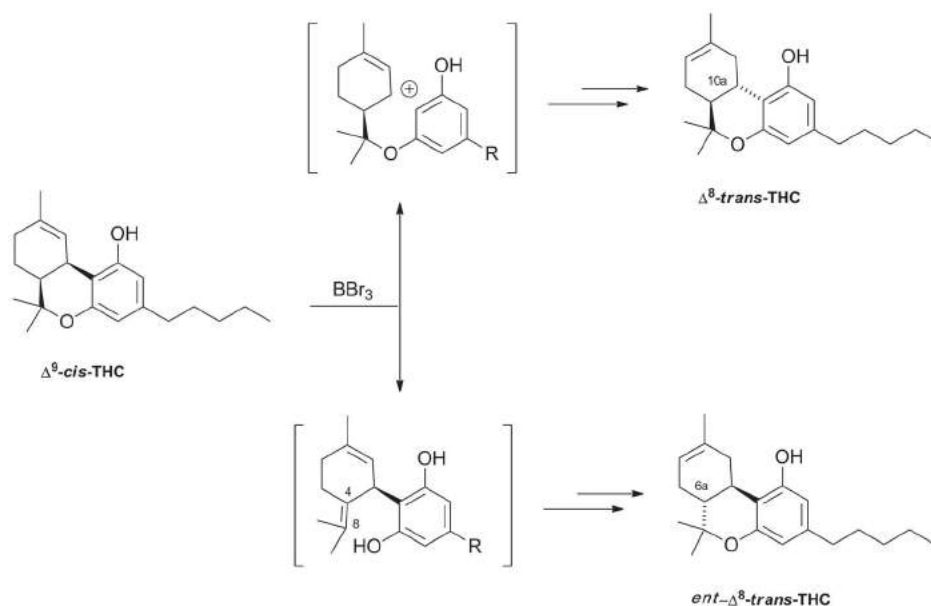
Scheme 8 Possible mechanisms for the isomerization of *cis* to *trans* tetrahydrocannabinols.

renaming of the compound she had originally named cytrilidene cannabis.

4.1.5.4. $\Delta^{6a,10a}$ Tetrahydrocannabinol and cannabitriol-type compounds. Compounds of this type are characterized by conjugation between the double bond on the terpenyl moiety and the resorcinyl residue, and are presumably intermediates in the oxidative aromatizatization of Δ^9-THC, a process triggered by the generation of a C-10a radical (Scheme 7). Although $\Delta^{6a,10a}$-THC is unknown as natural product, an oxygenated analogue (the epoxide **34**) has been isolated from cannabis,[108] and the parent compound was synthesized as a racemate by Adams and Todd during the structure elucidation of cannabinol by the preparation of a series of possible putative structures for the natural product.[124,125] Racemic $\Delta^{6a,10a}$-THC was found active in the dog ataxia assay, and the observation was confirmed by modern studies, that localized cannabinoid activity exclusively in the *S*-enantiomer of the racemate.[126] The activity was lower, but qualitatively similar to the one of Δ^9-THC, and it is therefore surprising that little information exists on compounds of this type, that are stable in ethanol solution and have been detected in historical samples of cannabis tinctures.[84]

4.1.5.5. Isotetrahydrocannabinol-type compounds. Compounds from this class originate from CBD-type phytocannabinoids by protonation of the endocyclic double bond and quenching of the positive charge at C-1 by one of the two symmetrically disposed around C-3 (CBD numbering) phenolic hydroxyl of the resorcinyl moiety. While in THC-type phytocannabinoids the pyrane ring is linearly fused with the aromatic and the terpenyl moieities, in these compounds the junction is bridged. Both the stereochemical details and the biological profile of these compounds are still largely unknown.

4.1.6. Cannabicyclol (CBL)-type compounds. Interest in CBL (**38b**), a compound originally named THC-III, was fostered by the wrong assumption of a close structural relationship with

THC.[134] After a series of structural revisions, the relative configuration was eventually established by X-ray analysis of the dibromoderivative.[135] CBL can be obtained by irradiation of CBC *via* an intramolecular stereoselective [2 + 2] cycloaddition.[135] This observation, the racemic nature of these phytocannabinoids, and the strict relationship between their concentration in plant material and the one of the corresponding cannabichromenes, strongly suggest that they are artefacts formed during storage of the plant material in the presence of light. Both the normal-(**38b**) and the abnormal (anthopogocyclolic acid, **38f**) version of the acids from the orcinoid series were isolated from a Cinese rhododendron species (*Rhododendron anthopogonoides*). Another rhododendron (*R. dauricum*) afforded the sesqui-cannabinoid rhododaurichromanic acid A (**38g**).[136] Apart from the lack of narcotic properties of CBL,[135] very little is known on the biological profile of these compounds, even though rhododaurichromanic acid A shown potent anti-HIV properties.[136]

4.1.7. Cannabielsoin (CBE)-type compounds. Compounds of this type are named after Elsa Boyanova, who isolated the first members of this class of compounds in the laboratories of Raphael Mechoulam, and who prematurely passed away.[140] These compounds are the result of the formal intramolecular opening of cannabidiol-type epoxides, as evident from the *trans*-relationship of the oxygen functions on the menthyl moiety. The process has been mimicked by epoxidation of the diacetate of CBD. Thus, hydrolysis of the acetate triggered the opening of the oxirane ring by one of the two phenolic *ortho*-hydroxyls, affording a compound identical to the one obtained by decarboxylation of cannabielsoic acid.[141] Cannabielsoic acid A could also be obtained from pre-CBD by oxidation with manganese(ɪᴠ) dioxide, or, alternatively, by irradiation in an oxygen atmosphere.[30,140] Cannabielsoin-type phytocannabinoids might well be isolation artifacts, but it is remarkable that in all their semi-

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Nat. Prod. Rep., 2016, **33**, 1357–1392 | **1375**
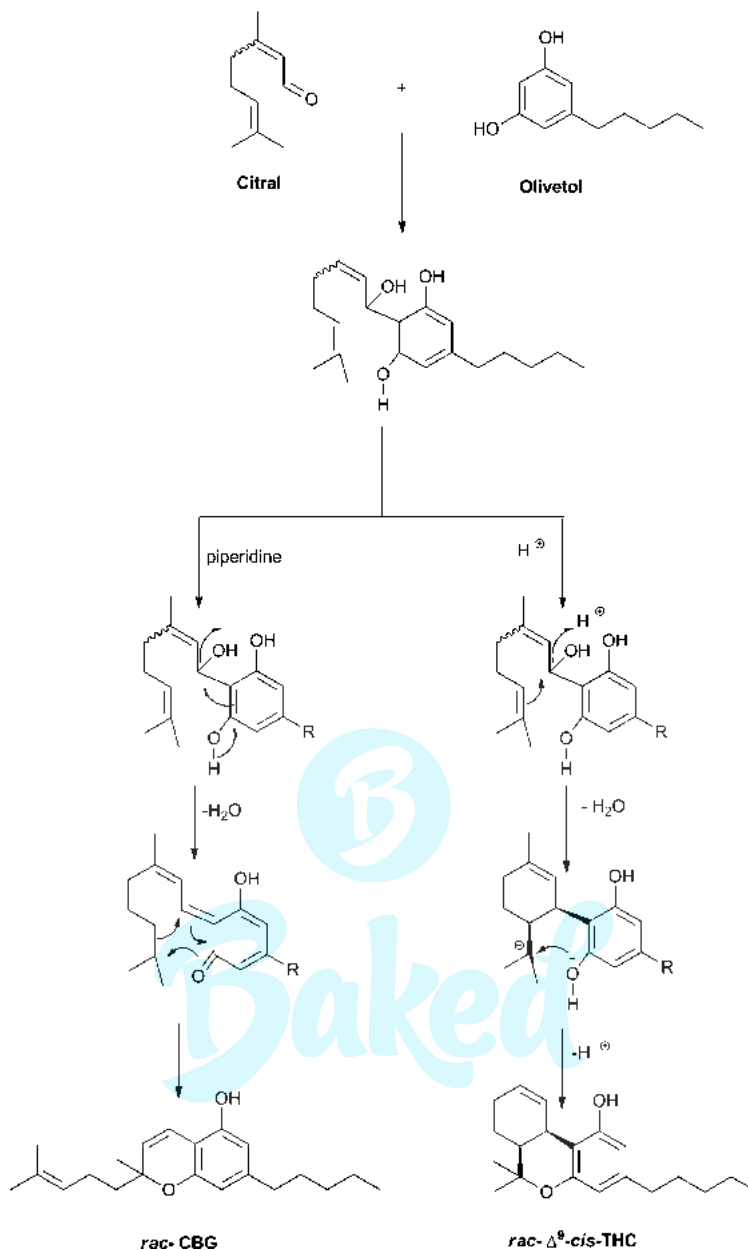
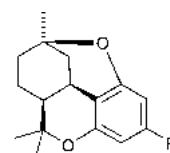**Scheme 9** Different course of the condensation of citral and olivetol depending on the conditions (R = *n*-pentyl).
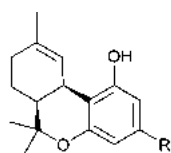
		R	Ref.
31a	Δ⁹-*cis*-Tetrahydrocannabivarin	C₃H₇	[60]
31b	Δ⁹-*cis*-Tetrahydrocannabinol	C₅H₁₁	[118]

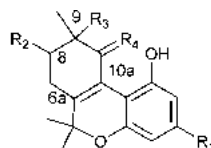|	R	Ref.
32a Cannabicitran (citrilidene-cannabis)	C₅H₁₁	[122, 123]
32b Cannabiorcicitran	CH₃	[67]

syntheses from CBD-type compounds, mixtures of compounds unknown as natural products were also obtained.[30,141] Of interest is the occurrence of cannabielsoic acid in two isomeric forms, having the carboxylate located *ortho* or *meta* to the oxygen bridge, a situation reminiscent of the one of pre-THC.[140] Cannabielsoin is a major pyrolytic product of CBD, and is

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	R_1	R_2	R_3	R_4	Ref.
33a Bis-*nor* cannabitriol	C_3H_7	H	α-OH	β-OH,α-H	[84]
33b Bis-*nor*-Cannabitriol isomer	C_3H_7	H	OH	OH, H	[84]
33c 10-*O*-Ethyl bis-*nor* cannabitriol	C_3H_7	H	α-OH	β-OH,α-H	[84]
33d Isocannabitriol	C_5H_{11}	OH	OH	H,H	[127]
33e Cannabitriol	C_5H_{11}	H	α-OH	β-OH,α-H	[128,129]
33f Cannabitriol isomer	C_5H_{11}	H	OH	OH	[127, 130]
33g 10-*O*-Ethyl cannabitriol isomer	C_5H_{11}	H	OH	OEt	[130]
33h 10-Oxo-$\Delta^{6a(10a)}$-tetrahydrocannabinol	C_5H_{11}	H	H	=O	[89]
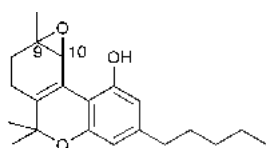
34 9,10-Anhydrocannabitriol [108]
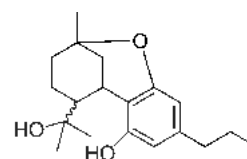
37 Cannabiglendol [133]
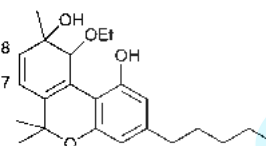
35 7,8-Dehydro-10-*O*-ethylcannabitriol [108]

therefore expected to be present in cannabis smoke.[78] It is also a metabolite of CBD in rodents,[142] and in tissue cultures by cannabis and the sugar cane.[143] Nevertheless, and despite interesting clues on the bioactivity of CBD pyrolysates,[78] very little is known on its bioactivity.

Two prenylogues analogues of CBE from the orcinoid series (ferrugienes A and B, **39f** and **39g**) have been isolated from the Alpine rhododendron (*Rhododendron ferrugineum* L.).[74]
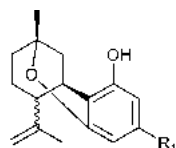
	R	Ref.
36a Δ^7-*cis*-Isotetrahydrocannabivarin	C_3H_7	[131]
36b Δ^7-*trans*-Isotetrahydrocannabivarin	C_3H_7	[132]
36c Δ^7-*trans*-Isotetrahydrocannabinol	C_5H_{11}	[132]

4.1.8.   Cannabinol (CBN)-type compounds. Cannabinol was the first phytocannabinoid isolated from cannabis. In 1896, by exploiting the crystalline nature of its acetate, Easterfield in Cambridge (UK) managed to obtain cannabinol from the high-boiling fraction of an ethereal extract from an Indian sample of cannabis.[145] Its structure was reported in 1940 by Adams,[70] and cannabinol remained for two decades the only compound of this class to be structurally elucidated. Cannabinol and its derivatives and analogues are considered isolation artifacts, derived from the oxidative aromatization of the corresponding THC-type derivatives, and the isolation of partially aromatized mentadienic derivatives like **41** (7,8-dihydrocannabinol) supports this view. CBN is highly stable toward oxidative degradation, and has been used as a marker for the identification of narcotic cannabis in archeological findings.[146] The aromatization of THC to CBN can be affected by sulfur dehydrogenation at 250 °C.[147] These harsh conditions cause the decarboxylation of pre-cannabinoids, and a milder, but poorly yielding, protocol that uses selenium dioxide and trimethylsilyl polyphosphate has been developed to prepare pre-CBN from pre-THC.[148] The significant overlapping between the diversity of CBN and THC derivatives is in accordance with the view that oxidative aromatization of THC derivatives occurs spontaneously in plant material and in cannabis extracts. Nevertheless, the presence of nor-derivatives of C_2- and C_4-phytocannabinoids is interesting, and, at least for the C_2-cannabinoid nor-cannabivarin (**40b**), unreported in compounds from the THC series, where also hydroxylation at C-7 is unknown. CBN is the only
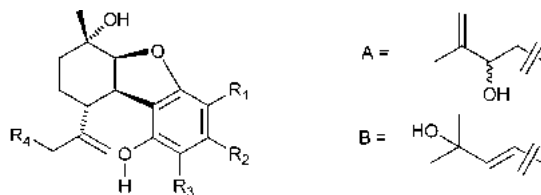
		R_1	R_2	R_3	R_4	Ref.
39a	Bisnor-cannabielsoin	H	C_3H_7	H	H	[144]
39b	Bis-*nor*-Cannabielsoic acid B	COOH	C_3H_7	H	H	[144]
39c	Cannabielsoin	H	C_5H_{11}	H	H	[142]
39d	Cannabielsoic acid A	H	C_5H_{11}	COOH	H	[140,30]
39e	Cannabielsoic acid B	COOH	C_5H_{11}	H	H	[140,30]
39f	Ferruginene A	H	CH_3	H	A	[74]
39g	Ferruginene B	H	CH_3	H	B	[74]

phytocannabinoid existing in all the alkyl versions from methyl to pentyl.

Cannabinol has only weak affinity for CB_1 and CB_2, *ca.* 10% of the one of THC.[149] nor-Cannabivarin (**40b**), the only phytocannabinoid with an ethyl side chain, and nor-CBN (**4d**) were isolated from an historical bottle of cannabis tincture dating from the first half of the 19th century and prepared from an Indian sample of cannabis resin.[84] The presence of phytocannabinoids with an even number of carbons could be typical of cannabis samples of that origin but, surprisingly, there are no modern studies on the diversity of cannabis in India.

4.1.9. 8,9-Secomenthyl cannabidiols. The oxidative cleavage of the endocyclic double bond of Δ^9-THC affords, after trapping of the C-10 aldehyde by the phenolic hydroxyl and dehydration, cannabicoumaronone (Scheme 10).[151] The configurational aspects of these compounds have not been fully clarified. When configuration of a stereocenter was assessed, it was found identical to that of Δ^9-THC (see **3b**, with a *R*-configuration at C-6).[37]

Further oxidative degradation of the furane moiety of cannabicoumaronone leads to cannabichromanones, a class of seco-10 norcannabinoids (Scheme 10). Cannabichromanone

itself was isolated from a degraded sample of hashish having as major constituent CBN,[89] and these compounds might well have a non-enzymatic origin.

Cannabimovone (**46**) is formally the result of the oxidative fragmentation of the endocyclic bond of CBD followed by intramolecular aldolization (Scheme 11)[153] Interestingly, attempt to mimic this biogenetic relationship with CBD failed to deliver the natural products, affording instead the oxy-Michael adduct of its crotonized version (anhydrocannabimovone, **47**).[153] While cannabimovone showed little affinity for CB_1 or CB_2, anhydrocannabimovone activated both CB_1 and CB_2 with a Ki of *ca.* 100 nM.[153] The configuration of the oxygen bridge of anhydrocannabimovone was revised during the total synthesis of cannabimovone.[154]
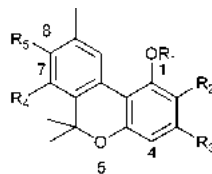
4.2 β-Aralkyl type phytocannabinoids (phytocannabinoid-like compounds, bibenzyl cannabinoids, stiryl cannabinoids)

Because of the derivation from an aromatic starter, in these compounds a β-aralkyl residue replaces the alkyl group of cannabis phytocannabinoids, while the connectivity (but not always the configuration) of the isoprenyl moiety closely mimics the one of the cannabis products, overall resulting in similarity
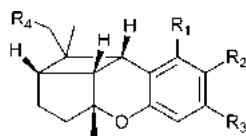

		R_1	R_2	R_3	R_4	Ref.
38a	Cannabiorcicyclol	OH	H	CH_3	H	[67]
38b	Cannabiorcicyclolic acid	OH	COOH	CH_3	H	[67]
38c	Cannabicyclovarin	OH	H	C_3H_7	H	[131,137]
38d	Cannabicyclol (CBL)	OH	H	C_5H_{11}	H	[134]
38e	Cannabicyclolic acid	OH	COOH	C_5H_{11}	H	[139]
38f	Anthopogocyclolic acid	CH_3	COOH	OH	H	[67]
38g	Rhododaurichromanic acid A	OH	COOH	CH_3	Prenyl	[136]

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		R_1	R_2	R_3	R_4	R_5	Ref.
40a	Cannabiorcol	H	H	CH_3	H	H	[82]
40b	nor-Cannabivarin	H	H	C_2H_5	H	H	[84]
40c	Cannabivarin	H	H	C_3H_7	H	H	[149]
40d	nor-Cannabinol	H	H	C_4H_9	H	H	[84,85]
40e	Cannabinol	H	H	C_5H_{11}	H	H	[70, 145]
40f	Cannabinolic acid	H	COOH	C_5H_{11}	H	H	[34]
40g	O-Methylcannabinol	CH_3	H	C_5H_{11}	H	H	[150]
40h	O-Propylcannabinol	C_3H_7	H	C_5H_{11}	H	H	[87]
40i	O-Penthylcannabinol	C_5H_{11}	H	C_5H_{11}	H	H	[87]
40j	7-Hydroxycannabinol	H	H	C_5H_{11}	OH	H	[84]
40k	8-Hydroxycannabinol	H	H	C_5H_{11}	H	OH	[37]
40l	8-Hydroxycannabinolic acid	H	COOH	C_5H_{11}	H	OH	[37]
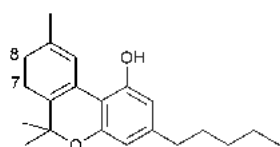
41 7,8-Dihydrocannabinol [108]
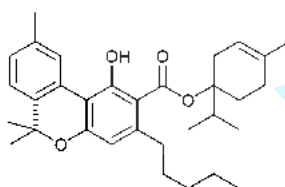
42 4-Terpenyl cannabinolate [7]
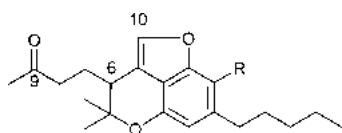
		R	Ref.
43a	Cannabicoumaronone	H	[151]
43b	Cannabicoumarononic acid	COOH	[37]

with the major phytocannabinoid chemotypes (CBG, CBC, THC). On the other hand, *O*-methylation of the resorcinyl moiety is rare within alkyl phtytocannabinoids, but is instead common in compounds from the β-aralkyl series, as are oxidative modifications of the isoprenyl residue, especially in compounds from the abnormal series.

4.2.1 Cannabigerol (CBG) analogues. Amorfrutins are the best known and investigated β-aralkyl phytocannabinoids of the cannabigerol type.[155,156] Five amorfrutins are known, distinguished by an overlapping and confusing code system of numbers and letters [A (=1), B, 2, 3, C (=4)].[157] With the exception of amorfrutin 2 (7), a pentyl-type cannabinoid, the other amorfrutins are of the phenethyl type and are structurally related to pre-cannabigerol *O*-methyl ether. All amorfrutins share a salicylate core bearing a *para*-methoxy- or hydroxy group, a *meta*-isoprenyl and an *ortho* aralkyl or alkyl substituent. The first member of the class, later named amorfrutin A (=amorfrutin 1, **48d**), was isolated in 1978 by Asakawa from a French collection of the liverwort *Radula complanata* (L.) Dum.,[158] and the following year was also reported by Bohlmann from *Helichrysum umbraculigerum* Less., a South-African species where it co-occurs with CBG.[46] Two years later, amorfrutin A was independently isolated from the seeds of the bastard indigo-bush (*Amorpha fruticosa* L.), a plant native to US, by Mitscher,[159] and from an Australian *Glycyrrhiza* species [*G. acanthocarpa* (Lindl) J. M. Black] by Ghisalberti.[160] Further amorfrutins (**48f**, **48j**, **48l**, **49b**) were obtained from the roots of the Mediterranean species *Glycyrrhiza foetida* Desf.[157] and from the leaves of the American licorice [*G. lepidota* (Nutt) Pursh],[161] while the genus *Radula* has provided a host of analogues.[162] Interestingly, the roots of better known licorices like *G. glabra* L. and *G. uralensis* L. do not contain amorfrutins.[163]

Amorfrutins were originally characterized as anti-bacterial agents,[159] but interest was re-kindled by the discovery that amorfrutin B (**48j**) is a powerful ligand of PPARγ (Ki = 19 nM), showing remarkable insulin-sensitivity activity *in vivo*.[157] The

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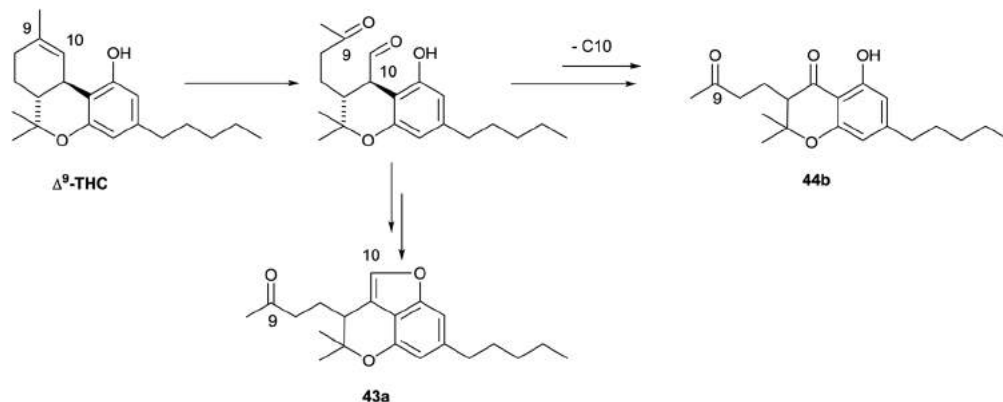
Scheme 10 Oxidative degradation of the endocyclic double bond of Δ⁹-THC.

		R₁	R₂	R₃	Ref.
44a	Bisnor-Cannabichromanone	C₃H₇	H	H,H	[152]
44b	Cannabichromanone	C₅H₁₁	H	H,H	[89]
44c	(6aR)-Cannabichromanone B	C₅H₁₁	OH	H,H	[152]
44d	(6aR)-Cannabichromanone C	C₅H₁₁	H	=O	[152]

46 Cannabimovone [153]

47 Anhydrocannabimovone [153]

45 Cannabichromanone D [152]
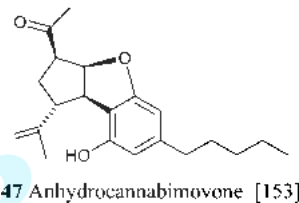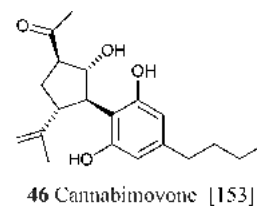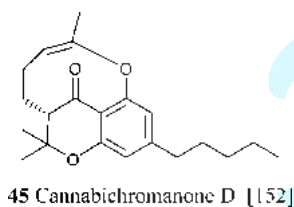
interaction of amorfrutins with PPARγ is basically different from the one of glitazones, since a crystallographic analysis has shown that amorfrutins bind PPARγ at the entry side and not at into the pocket of the ligand binding groove of this transcription factor.[164] This finding underlies the observation that the amorfrutin-PPARγ complex associates to a distinct profile of proteins compared to the glitazone-PPARγ complexes, resulting in the selective activation of only of a subset of the genes under

PPARγ control. The possibility therefore exists that the modulation of PPARγ by amorfrutins might not be associated to the side-effects typical of glitazones (fluid retention, weight gain, cardiovascular complication, bladder cancer), and animal studies have supported this suggestion.[157] Amorfrutin B (**48j**) is the most powerful compound of the series in terms of PPARγ activation. Its superior activity compared to its demethyl derivative (amorfrutin 4, **48l**) and it deprenyl derivative (amorfrutin A = amorfrutin 1, **48d**) highlights the relevance of O-methylation and the oligomerization degree of the isoprenyl residue for superior potency. A second high-affinity target for amorfrutins was identified in the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH).[165] Amorfrutins can

CBD
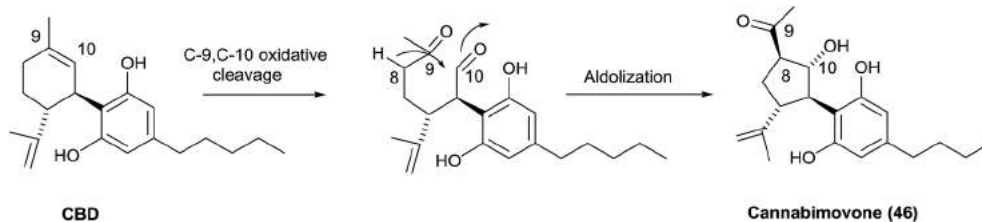
Cannabimovone (46)

Scheme 11 Oxidative degradation of the endocyclic double bond of CBD to cannabimovone (**46**).

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inhibit both its activity and its translocation to the nucleus, a process involved in neuronal death, and hold therefore promise for the management, and possibly also the prevention, of neurodegenerative diseases. Several additional targets have been identified for amorfrutins, including the inhibition of NF-κB activity, the inhibition of iNOS, the corticotropin releasing factor-binding protein, the cysteine protease ATGB4, and the photoreceptor-specific nuclear receptor NR2E3.[155,156] The multifaced profile of end-points makes it possible that amorfrutins could target, apart from diabetes, also a host of other conditions characterized by chronic inflammation, not unlike curcumin. For unclear reasons, amorfrutins and pre-cannabinoids from the phenethyl series are more resistant to decarboxylation compared to the alkyl phytocannabinoids.

Just like amorfrutins, also their analogues were isolated from taxonomically unrelated sources. Thus, the stiryl version of decarboxyamorfrutin C (amporphastilbol, **48g**) was isolated from three leguminous *Amorpha* species (*A. nana* Nutt., *A. fruticosa* L., and *A. canescens* Pursh.),[166] as well as from *H. umbraculigerum*, an asteraceous plant.[46] *H. umbraculigerum* also afforded its phenethyl analogue (**48e**), a compound first isolated from the liverwort *Radula variabilis*.[158] In this context, the phytochemistry of *H. umbraculigerum* is very interesting, since this plant is not only the major natural source of cannabigerol in terms of isolation yield, but also produces its abnormal-, phenethyl- and stiryl-analogues, undoubtedly qualifying as the biogenetically most versatile source of phytocannabinoids known. Interestingly, also amorphastilbol was reported to bind PPARγ (as well as

PPARα),[167] but a direct comparison with amorfrutins has not yet been reported.

Amorfrutin-type compounds were also isolated from peanut (*Arachis hypogea* L.) seeds infected with an *Aspergillus flavus* fungal strain.[168] Compounds **53a–c** are characterized by a shift of the prenyl double bond in conjugation to the aromatic core, a rare feature in isoprenylated phenolics. These compounds (araphyns, arachidins) act as phytoalexins, helping the plant to resist fungal attack.
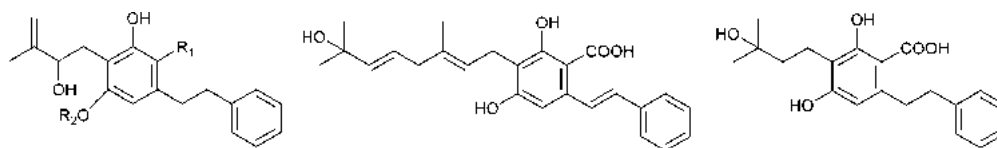
A unique feature of some phytocannabinoids from *H. umbraculigerum* is the esterification of the resorcinyl hydroxyl *para* to the carboxylate group, generally a site of methylation, with branched short-chain carboxylic acids.[46] Within the phytocannabinoids from *H. umbraculigerum*, acylation is a selective feature of compounds from the phenethyl series with a prenyl residue, and was not observed in their stiryl and terpenyl analogues. *O*-Prenylation, along with meta-hydroxylation, has also been reported in a bibenzyl cannabinoid (**55**) from *Glycyrrhiza lepidota*.[161]

From liverwort of the *Radula* genus, stilbenic phytocannabinoids with an heterocyclized isoprenyl residue have been isolated. Apart from compounds resulting from the acidic cyclization of *o*-hydroxylated prenyl phenols, like compounds **56a,b** and **57a,b**, also compounds derived from the cyclization of ω-oxygenated precursors have been described.[156] Thus, compounds **57a–c** are formally derived from the intramolecular opening of a terminal epoxide in a S_N2 fashion (attack to the least substituted carbon) by the hydroxyl *para* to the carboxylate group. This *7-endo tet* regiochemistry is unusual in
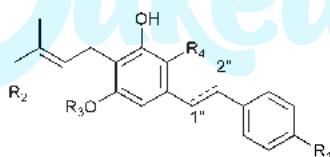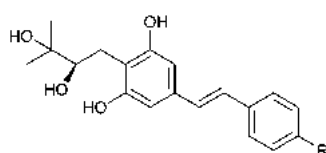

		R₁	R₂	R₃	R₄	Δ¹'',²''	Ref.
48a	Demethyldecarboxyamorfrutin A	H	H	H	H	–	[46,169-172, 174]
48b	Demethylamorfrutin A	H	H	H	COOH	–	[169,171,174]
48c	Decarboxyamorfrutin A	H	H	Me	H	–	[160,171,175]
48d	Amorfrutin A (= amorfrutin 1)	H	H	Me	COOH	–	[157,159,160, 171,172]
48e	Heli-Cannabigerol (H-CBG)	H	Prenyl	H	H	–	[46,158,172]
48f	Amorfrutin C (= amorfrutin 4, pre-H-GBC)	H	Prenyl	H	COOH	-	[46,157]
48g	Amorphastylbol	H	Prenyl	H	H	+	[46,173]
48h	Pre-amorphastylbol	H	Prenyl	H	COOH	+	[46]
48i	Hydroxy Helicannabigenol	OH	Prenyl	H	H	-	[158]
48j	Amorfrutin B	H	Prenyl	Me	COOH	-	[159]
48k	Decarboxyamorfrutin B	H	Prenyl	Me	H	-	[158]
48l	Amorfrutin 4 (=demethylamorfrutin B)	H	Prenyl	H	COOH	-	[157]
48m	Chiricanin A	H	H	H	H	+	[176]
48n	*trans*-Arachidin-2	OH	H	H	H	+	[168]

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		R₁	R₂	Ref.
49a	Glepidotin C	H	H	[161]
49b	Amorfrutin 3	COOH	Me	[156]

50 [46]   51 [174]

		R	Ref.
52a	Araphyn-3	OH	[168]
52b	Araphyn-4	H	[168]
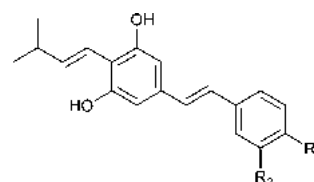
		R₁	R₂	Ref.
53a	Araphyn-1	H	H	[168]
53b	Arachidin-3	OH	H	[168]
53c	trans-Arachidin-1	OH	OH	[168]
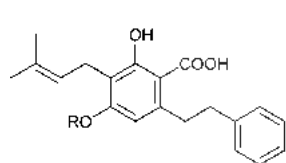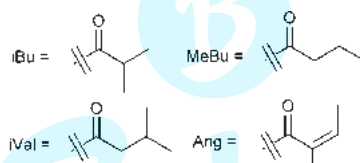
	R	Ref.		R	Ref.
54a	iBu	[46]	54b	iVal	[46]
54c	MeBu	[46]	54d	Ang	[46]
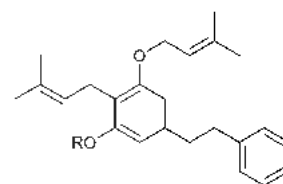
55 [161]

isoprenylated phenolics, but its permitted by the Baldwin rules. A similar regiochemistry of intramolecular cyclization is involved in the generation **58a,c** from their ω-hydroxylated precursors, with compound **58c** being originally assigned the regio-isomeric structure **59**. These compounds are, in turn, the precursor of the unusual cyclopropa-pyranes **60** and **61a,b**, whose generation might involve the protonation of the oxepine double bond and then closure of a cyclopropane ring by loss of one of the benzylic protons (Scheme 12).

The "taxonomy" of a series of chromanes from leguminous plants and liverworts is ambiguous. Biogenetically, they could

be considered either as cyclized CBG-type compounds, derived from the cyclization of a prenyl (**56a,b**) or an ω-epoxyprenyl precursor (**62a,b**). Alternatively, as hydrogenated or hydrated CBC analogues. The CBG-type derivation seems more likely, and therefore they are included in this section.

Abnormal phenethyl phytocannabinoids are widespread in liverworts from the genus *Radula*, where, like in *R. variabilis*[177] and *R. kojana*,[178] they can represent the major chemotype of bibenzyls, or even the only type of phytocannabinoids detected, as in *R. voluta*.[179,180] Remarkably, *R. perrottetii* contains abnormal phytocannabinoids from the CBG and CBC series,
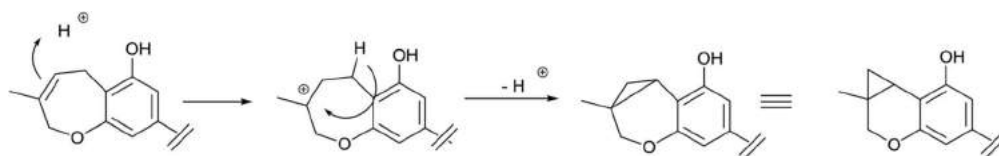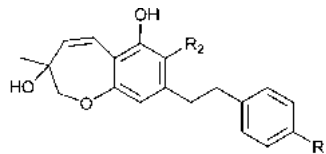
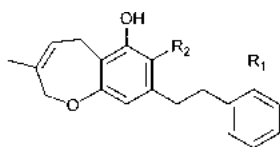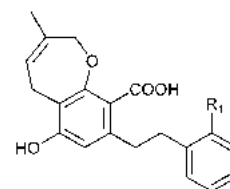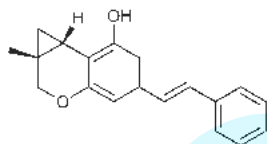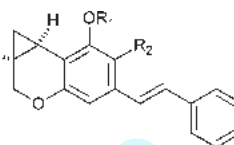**Scheme 12** Possible biogenetic origin of the cyclopropapyranes **60,61a,b**.
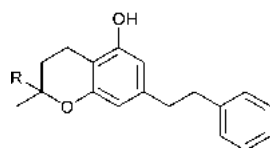
	R	Ref.
56a	H	[171]
56b	Prenyl	[171]

	R₁	R₂	Ref.
57a	H	COOH	[171]
57b	OH	H	[171]

	R₁	R₂	Ref.
58a Radulanin A	H	H	[158, 171]
58b Radulanin L	OH	H	[171, 172]
58c Radulanin H	H	COOH	[158, 171]

59 [179]

60 Radulanin I [175]

	R₁	R₂	Ref.
61a Radulanin J	Me	H	[175]
61b Radulanin K	H	COOH	[175]
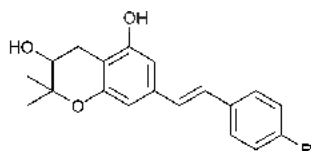
	R₂	Ref.
62a Chiricanine B	H	[176]
62b Radulanin K	OH	[176]

4.2.2 Cannabichromene (CBC) analogues. Many β-aralkyl compounds of this group belong to the abnormal series, but the modifications of the isoprenyl core are, otherwise, identical to those documented within alkyl-cannabinoids. As usual, stilbenoid structures prevail within compounds of plant origin, and bibenzyl ones from those of liverwort origin. The geranylated derivatives **72a–d** were isolated from the leaves of phyllanthaceous African tree *Hymenocardia acida* Tul.[184]

4.2.3 Mentyl cannabinoids (CBD, THC) analogues. Relatively few compounds of this type from the β-aralkyl series have been reported, and, remarkably, the configuration of at the carbon(s) involved in the junction with the resorcinyl core is different, in terms of absolute or relative configuration, from the one of their analogues from cannabis.[185]

The macheridiol chemotype is similar to the one of CBD, with the β-aralkyl moiety declined in the stiryl (**73a,b**) and benzofuranyl (**74**) form. These compounds, as well as the THC analogues from the macheriol chomotype (see infra),[186] were isolated from the stem bark of the Amazonian legumionous liana *Macherium multiflorum* Spruce.[185] The pseudo-enantiomeric configuration at C-3 and C-4 compared to CBD was

and regular phytocannabinoids from the menthyl-type (THC series).[181] The structural diversity of phenethyl abnormal phytocannabinoids closely parallels the one of their related regular phytocannabinoids (*O*-methylation, prenylation), but also "internal" hydroxylation of the prenyl residue has been reported, as in **64** and **65**. The furan **67** might derive from the degradation of the isopropyl-substituted dihydrobenzofuran derivative **68**, as usual in the biogenesis of furanocoumarins from plants.

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	R_1	R_2	R_3	R_4	R_5	Δ	Ref.
63a	H	H	H	H	H	-	[172,175,177-180,182,183]
63b	H	Me	H	H	H	-	[176,178]
63c	H	H	Me	H	H	-	[178]
63d	H	H	H	COOMe	H	-	[175]
63e	Prenyl	H	H	H	H	-	[172,175,177-180]
63f	Prenyl	Me	H	H	H	-	[178]
63g	Prenyl	H	H	COOMe	H	-	[188]
63h	H	H	H	H	OH	-	[183]
63i	H	H	H	H	H	+	[183]
63k	H	Me	H	H	H	+	[176]
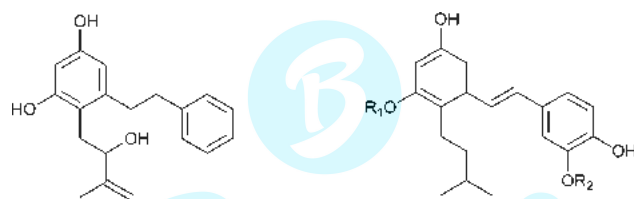
64 [177,178]

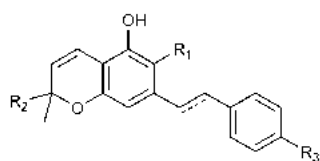|	R_1	R_2	Ref.
65a	H	H	[183]
65b	H	Me	[183]
65c	Me	H	[183]
65d	Me	Me	[183]
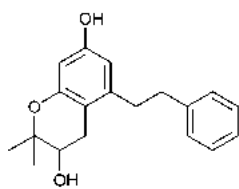
66 [177]
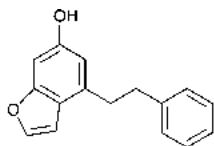
67 [174,178]
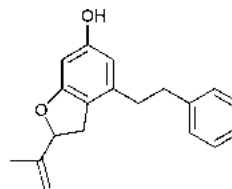
68 [175,181]

suggested by CD studies. Despite their similarity, the biological profile of machaeridiol is remarkably different, with machaeridiol B (73b) being an order of magnitude more potent of machaeridiol C (74) as an antimalarial agent.[185]
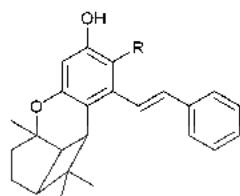
The occurrence in Nature of the phenethyl analogue of THC was predicted in 1986 by Crombie,[187] an overlooked founder of cannabinoids (and not only this class of compounds) chemistry, based on the occurrence of the phenethyl analogue of CBG, the precursor of THC in cannabis, in lieverworts[158,172] and in higher plants.[46] Two years later, Crombie synthesized the phenethyl version of THC with the aim of investigating its presence in cannabis, but no information on its bioactivity was disclosed.[188] While the phenethyl version of THC is still unknown as a natural product, its *cis* isomer [perrottettinen(e)] was isolated by Asakawa from the Japanese liverwort *Radula perrottetii*[181] and from the New Zeeland liverworth *Radula marginata*,[177] and by Becker from the Costa Rican liverwort *Radula laxiramea*,[174] with the absolute configuration being confirmed by an
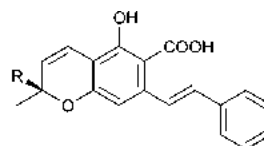
1384 | *Nat. Prod. Rep.*, 2016, **33**, 1357–1392

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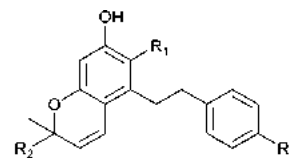
	R_1	R_2	R_3	Δ	Ref.
69a	H	H	H	-	[172,174,177,178,181]
69b	COOH	H	H	-	[178]
69c	H	Prenyl	H	-	[174,180,182]
69d	H	H	H	+	[168]
69e	H	H	OH	−	[168]

	R_1	R_2	R_3	Ref.
70a	H	H	H	[178]
70b	H	Prenyl	H	[178]
70c	COOH	Prenyl	H	[178]
70d	H	H	OH	[179]

	R	Ref.
71a	H	[182]
71b	COOH	[184]

	R	Ref.
72a		[184]
72b		[184]
72c		[184]
72d		[184]
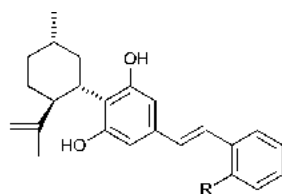
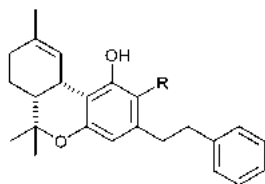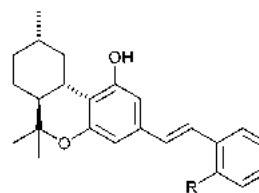|		R	Ref.
73a	Machaeridiol A	H	[185]
73b	Machaeridiol B	OH	[185]
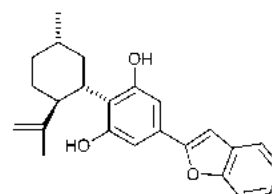
74 Machaeridiol C [185]

enantioselective synthesis.[189] Since *cis*-THC, a very minor cannabinoid in marijuana but almost equimolar with THC in fiber hemp, is not psychotropic,[85] also perottetinene should not be so. On the other hand, detailed information on the biological profile of the various isomers of THC has never been published, and the biological profile of perrottettinene is unknown, or, at least, it has not been reported in the mainstream literature,

despite undocumented claims on its psychotropic properties that circulate on the web.[190] It is remarkable that the enormous efforts of exploration of the biological space around the THC chemotype and the critical role of the C-3 substituent on bioactivity, the "hint" suggested by Nature with the existence of phenethyl versions of the pentyl cannabinoids of *Cannabis* has been so far overlooked. Since cannabinoids have additional
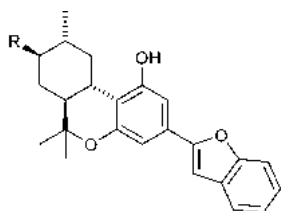
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	R	R	Ref.
75a	Perrottetinen(e)	H	[177,181,187]
75b	Perrottetinenic Acid	COOH	[177]

		R	Ref.
76a	Machaeriol A	H	[186]
76b	Machaeriol C	OH	[186]

|		R	Ref.
77a	Machaeriol B	H	[31]
77b	Machaeriol D	OH	[31]

targets to the psychotropic CB_1 receptor, the exploration around the perrottetinene chemotype seems well worth pursuing.

Machaeriols A and B from the Amazonian liana *Machaerium multiflorum* Spruce are analogues of *trans*-dihydroTHC,[186] but show an enantiomeric configuration at the ring junction, as shown by CD studies and enantioselective total syntheses.[191–193] It is not known if machaeriols bind CB_1 and are psychotropic.
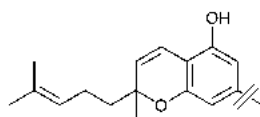
4.2.4   Spurious phytocannabinoids. The enzymatic system involved in the terpenylation of the resorcinyl core of phytocannabinoids and phytocannabinoid-like compounds is not specific, and can be operative also in other classes of phenolics, generating compounds overall similar to phytocannabinoids. However, the *meta*-relationship between the substituents of the
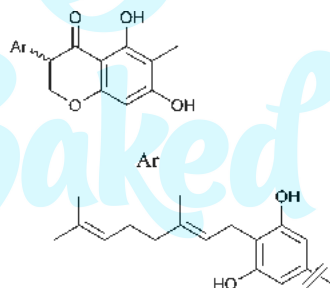
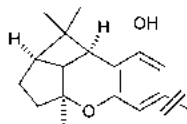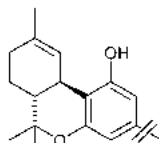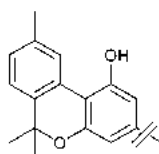|		Ar	Ref.
78a	Desmodianone C		[194]
78b	Desmodianone A		[194]
78c	Desmodianone D		[195]
78d	Desmodianone E		[195]
78e	6-Metiltetrapterol A		[195]

core aromatic ring clearly points to a different biogenetic origin, or, at least, to a different sequence of prenylation *vs.* closure of the polyphenolic aromatic core. Thus, desmodianones (**78a–e**),[194,195] a series of compounds isolated from the South American leguminous species *Desmodium canum* (Gmell) Shintz and Tellung, are basically isoprenylated flavanones with a *meta*-dihydroxylated B-ring, a rare functionalization since, being of shikimate origin, ring B of flavonoids normally bears *ortho*-oxygen groups. Desmodianones could, in principle, be viewed as terpenylated cannabinoids since the structure of this moiety mimics the one of phytocannabinoids (CBG, CBC, CBL, THC, CBN, **78a–e**, respectively), and one of them, the cannabinol analogue **78e**, has also been isolated as a 6-demethylderivative (tetrapterol A) from another leguminous plant (*Sophora tetraptera* J. S. Muell.).[196]

their aromatic core is derived from a Claisen- and not an aldol condensation of a linear ketide, and these compounds should be better considered isoprenylated flavonoids rather than "phloroglucinyl" phytocannabinoid. The two biogenetic processes are exemplified in Scheme 13 by the structure of amorphastilbol (**48g**)[46,173] and canniflavone 2 (= cannflavin A, **81**).[198] For comparison, the analogous process leading to cannabigerol is also reported. Compounds derived from both the aldol (resorcinyl) and the Claisen (phloroglucynyl) series can co-occur taxonomically unrelated plant *C. sativa* and *H. umbraculigerum*, as well as in *Radula* liverworts.[162] Polyprenylated stilbenoids should also not "*a priori*" be considered "phytocannabinoids", because this structural element is not documented within the archetypal compounds of this type from cannabis, nor should prenylated polyphenolic ketides with
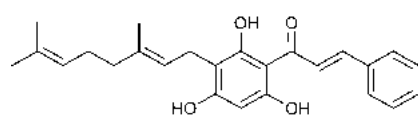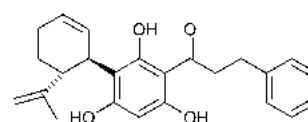

79 [46]   Linderatin **80** [197]

Similar considerations apply for the large class of isoprenylated acylphloroglucynols like **79** from *H. umbraculigerum*[46] and **80** (linderatin) from a lauraceous *Lindera* species,[197] both isoprenylated flavonoids (chalcone and dihydrochalcone, respectively) rather than phytocannabinoids. There is little reason to consider these compounds phytocannabinoids, since

a hydroxylation profile different from the resorcinol one, or at least that cannot be reconduced to the further oxidation of a resorcinol core to a quinol. Compounds of this type co-occur with phytocannabinoids, *e.g.* demethylamorfrutin A (**48a**),[167,169–172,174] the deoxystilbenoid **82**,[172] and the hydroxylated version of abnormal demethylamorfrutin A (**83**)[178] in *Radula*
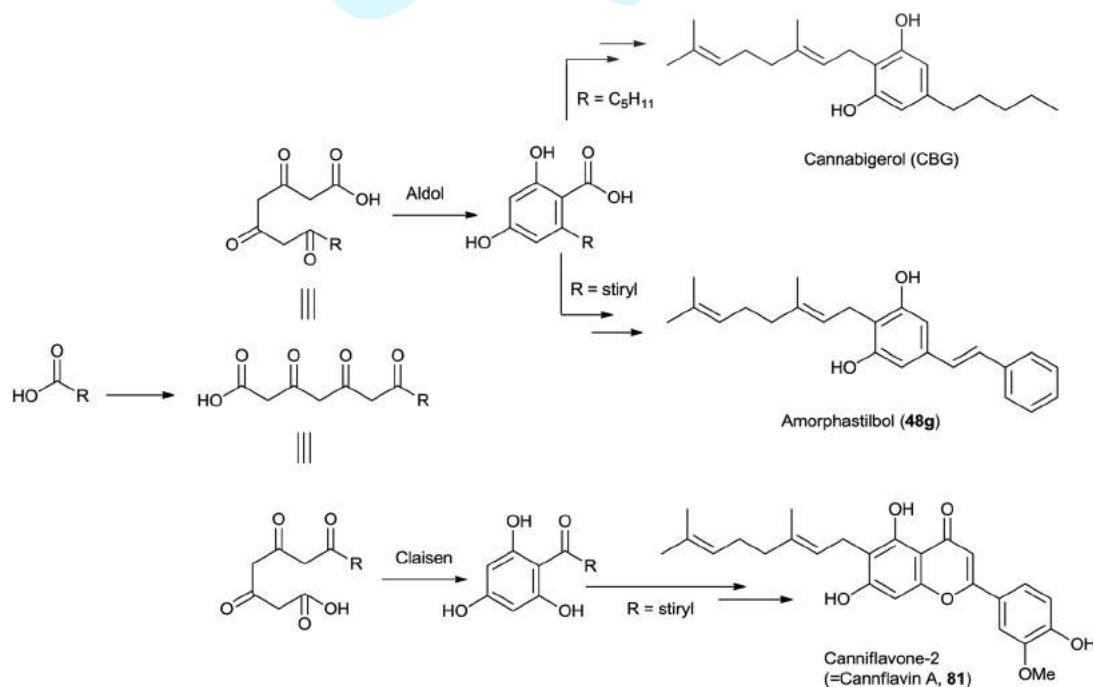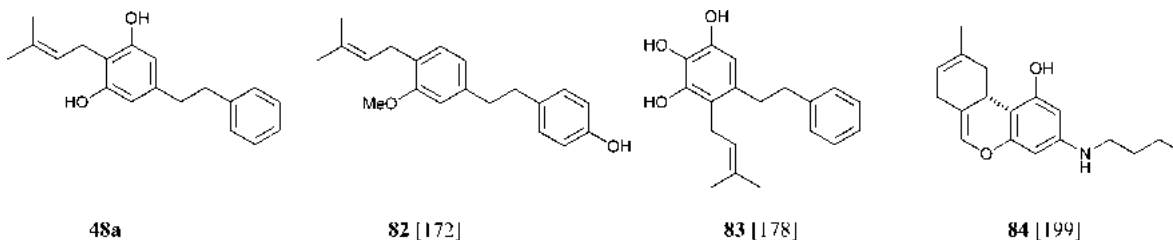

Scheme 13 Biogenetic relationship between resorcinyl (phytocannabinoids) and phloroglucynyl (flavonoids) meroterpenoids.

liverworts, but the biogenetic relationship between the two groups is unclear.

Finally, a compound named "dronabinol alkaloid" (**84**) was reported from *Cassia alata* L., a leguminous medicinal plant.[199] The structure of this compound was only tentatively established and needs confirmation. Even if the proposed structure should be confirmed, there seems to be little reason to consider it as a cannabinoid, since plant aromatic amines are generally of anthranilate origin.

observation in the light of the enormous interest for the pharmacological activity of phytocannabinoids and the messianic await for the development of cannabinoid-based medicines that permeates the media.[200]

It is tempting to predict that, given the biosynthetic plasticity of *C. sativa*, further types of alkyl phytocannabinoids will be described in the near future from both the natural and the man-induced diversity of cannabis strains. In the wake of the growing interest from amorfrutins, further additions to the

48a   **82** [172]   **83** [178]   **84** [199]

5. Conclusions

Phytocannabinoids have a limited distribution in Nature, but occur in phylogenetically unrelated sources (higher plants, liverworts, fungi). These compounds are traditionally associated to cannabis, that, with almost 150 alkyl (C-5, C-3, C-1) phytocannabinoids reported, remains their main source of diversity. However, only a few members of the class are accumulated in substantial amounts, namely the ones having the terpenyl residue in the form of a geranyl (CBG-type), a menthyl (CBD-type and THC-type), or a prenylchromanyl (CBC-type) residue. Many of the minor cannabinoids could be auto-oxidation artifacts eventually evolving into aromatized phytocannabinoid of the CBN type, but others might be genuine natural products worth investigating from a bioactivity standpoint.

Apart from the variation of the terpenyl connectivity, structural diversity in phytocannabinoids is also related to the elongation of the isoprenyl moiety from a terpenyl- to a sesquiterpenyl moiety, while shortened analogues (hemiprenyl phytocannabinoids) have only been reported in phytocannabinoids from the aralkyl series. Oxidation of the resorcinyl moiety to a quinol is also documented, but compounds of this type have only been isolated in their acetylated and more stable form. The mammalian metabolism of phytocannabinoids involves allylic oxidation rather than nuclear oxidation to quinoid metabolites, but, due to this instability, these metabolites might have been overlooked. *O*-Methylation was reported in phytocannabinoids obtained from far-East samples of cannabis but it is otherwise rare in alkyl phytocannabinoids, while it is common in compounds from the phenethyl series. Aralkyl cannabinoids have a broader distribution in Nature compared to alkyl cannabinoids, but their accumulation is point-like in terms of producing organisms, with phenethyl substitution prevailing in liverworts and styryl substitution in plant constituents. Most phytocannabinoids still await an evaluation of their biological profile and pharmacological potential, a somewhat paradoxical

phytocannabinoids inventory should also come from compounds of the aralkyl structural type. By focusing on the remarkable structural diversity of phytocannabinoids and highlighting their largely overlooked wide distribution in plants, we hope to stimulate the exploration of the biological space associated to their natural variation, going beyond the THC structural motif, and paving the way to a full opening of the Pandora's box of their biomedical potential.

6. Acknowledgements

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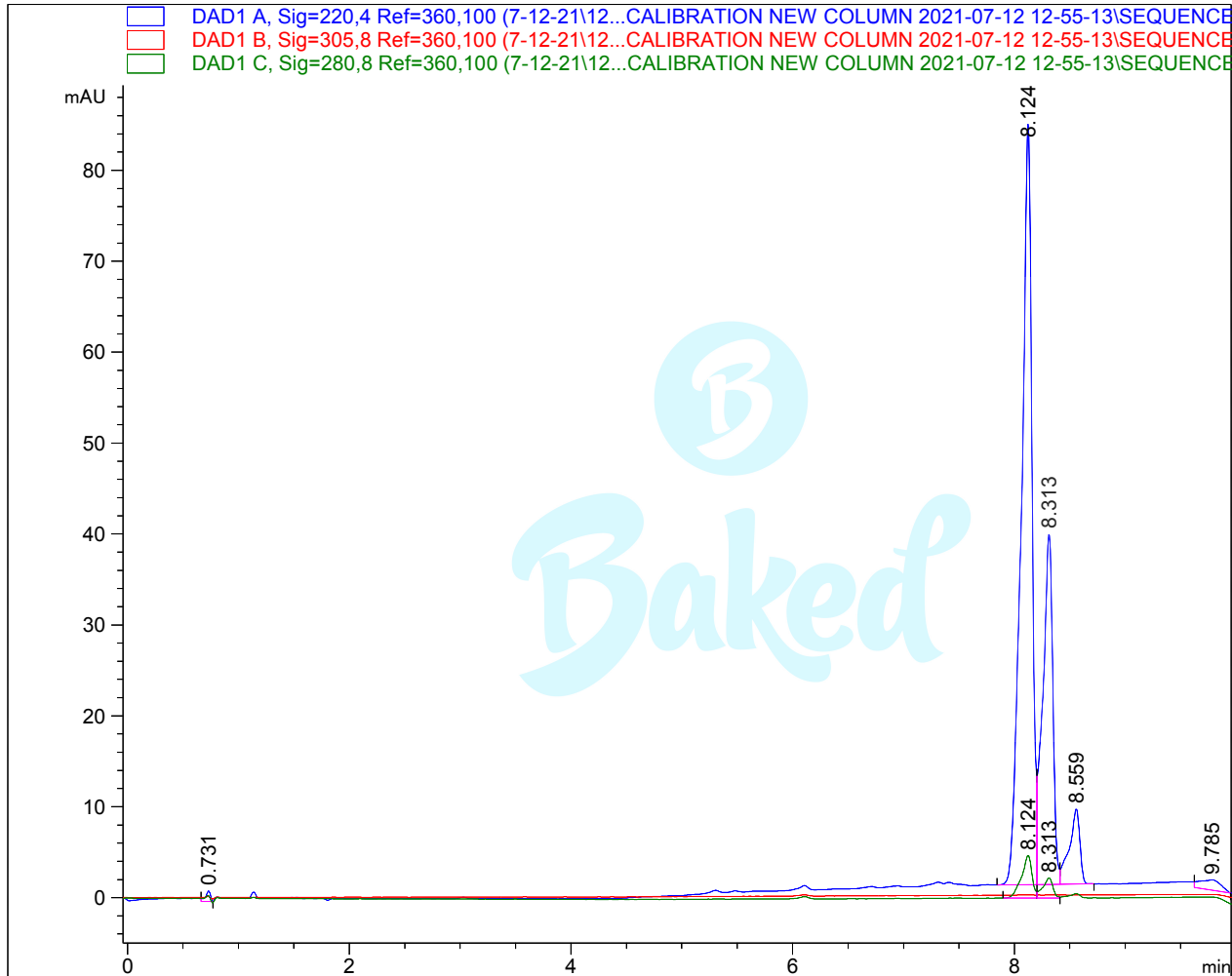
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Acq. Operator    :                           Seq. Line :   3
Acq. Instrument  : Instrument 1               Location : Vial 10
Injection Date   : 7/12/2021 1:23:54 PM              Inj :   1
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                   1100 DAD HIGH THROUGHPUT (NO CBT).M
Last changed     : 7/12/2021 1:03:17 PM
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Method Info      : First Runs Shutdown
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==========================================================================
                    Area Percent Report
==========================================================================

Sorted By          :        Signal
Calib. Data Modified :       7/6/2021 7:24:35 PM
Multiplier:                  :       1.0000
Dilution:                    :       1.0000
Use Multiplier & Dilution Factor with ISTDs
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Signal 1: DAD1 A, Sig=220,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	2.402		0.0000	0.00000	0.0000	CBDV
2	4.467		0.0000	0.00000	0.0000	CBD
3	4.718		0.0000	0.00000	0.0000	CBG
4	5.470		0.0000	0.00000	0.0000	CBDA
5	7.134		0.0000	0.00000	0.0000	D9 THC
6	7.863		0.0000	0.00000	0.0000	CBN
7	10.329		0.0000	0.00000	0.0000	D8 THC

Totals :   0.00000   0.0000

Uncalibrated Peaks:

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	0.731	VB	0.0485	3.60868	0.4125	?
2	8.124	BV	0.0936	556.16852	63.5785	?
3	8.313	VV	0.0920	244.60983	27.9626	?
4	8.559	VB	0.0962	55.31372	6.3232	?
5	9.785	VBA	0.1801	15.07346	1.7231	?

Uncalib. totals :   874.77422 100.0000

Signal 2: DAD1 B, Sig=305,8 Ref=360,100

Uncalibrated Peaks:

Signal 3: DAD1 C, Sig=280,8 Ref=360,100

Uncalibrated Peaks:

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	8.124	BV	0.0933	31.06334	68.8874	?
2	8.313	VV	0.0919	14.02956	31.1126	?

Uncalib. totals :   45.09291 100.0000

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

===
                        *** End of Report ***

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Certificate of Analysis

1 of 1

HHC

Sample ID: 2105KCA0856.2132

Cultivar: N/A
Matrix: Concentrates & Extracts
Type: Distillate
Sample Size:

Received: 05/17/2021
Completed: 05/27/2021
Batch#:

Client

Colorado Chromatography
Lic.#
10505 S Progress Way, Unit 105
Parker, CO 80134

Summary

Test	Date Tested	Result
Cannabinoids	05/27/2021	Complete

Cannabinoids by HPLC-PDA

Complete

ND	ND	1.5359%	NT	Not Tested
			Not Tested	
Total THC	Total CBD	Total Cannabinoids	Moisture Content	Foreign Matter

Analyte	LOD	LOQ	Result	Result
	%	%	%	mg/g
CBC	0.0095	0.0284	ND	ND
CBCA	0.0181	0.0543	ND	ND
CBCV	0.0060	0.0180	ND	ND
CBD	0.0081	0.0242	ND	ND
CBDA	0.0043	0.0130	ND	ND
CBDV	0.0061	0.0182	ND	ND
CBDVA	0.0021	0.0063	ND	ND
CBG	0.0057	0.0172	ND	ND
CBGA	0.0049	0.0147	ND	ND
CBL	0.0112	0.0335	ND	ND
CBLA	0.0124	0.0371	ND	ND
CBN	0.0056	0.0169	ND	ND
CBNA	0.0060	0.0181	ND	ND
Δ8-THC	0.0104	0.0312	1.5359	15.359
Δ9-THC	0.0076	0.0227	ND	ND
THCA	0.0084	0.0251	ND	ND
THCV	0.0069	0.0206	ND	ND
THCVA	0.0062	0.0186	ND	ND
Total THC			**ND**	**ND**
Total CBD			**ND**	**ND**
Total			**1.5359**	**15.359**

2105KCA0856.2132
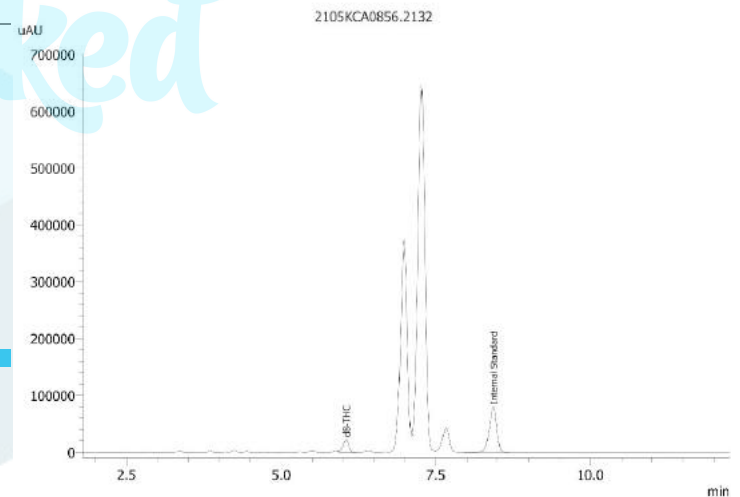
Total THC = THCA * 0.877 + Δ9-THC
Total CBD = CBDA * 0.877 + CBD
LOD = Limit of Detection
LOQ = Limit of Quantitation
ND = None Detected
For plant material, the reported result is based on a sample weight with the applicable moisture content for that sample.

Wes Rogers
Principal Scientist
05/27/2021

ISO/IEC 17025:2017 Accredited
Accreditation #108651

1H NMR and 13C NMR for HHC
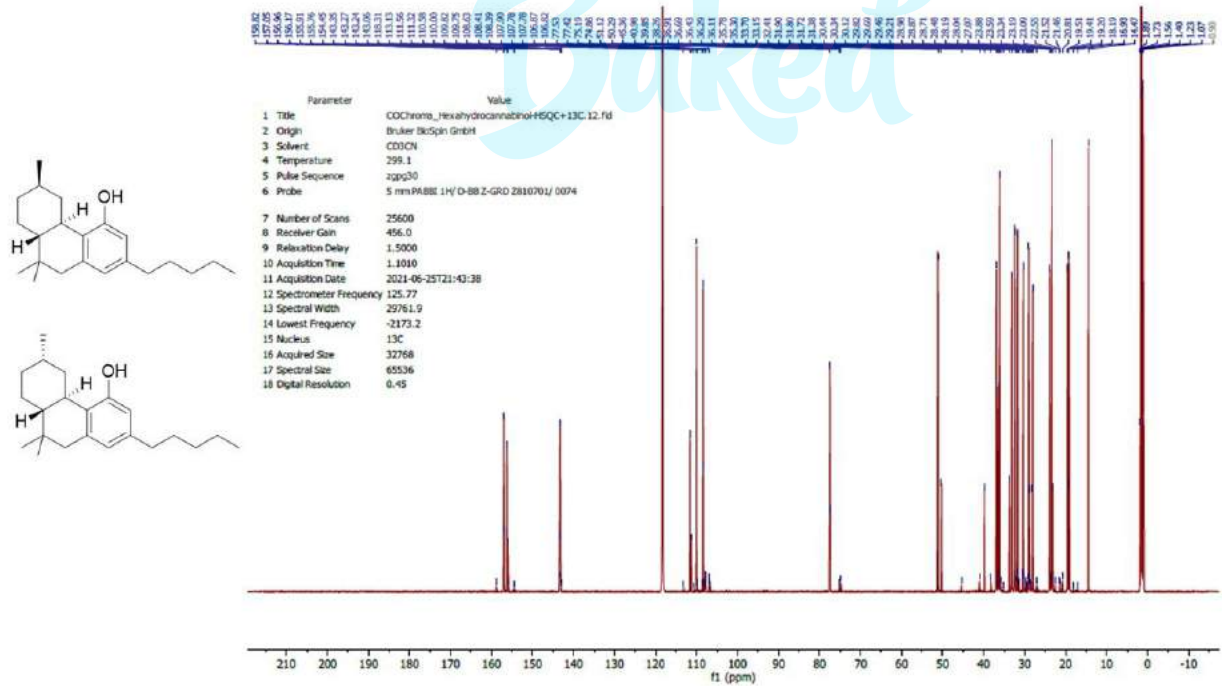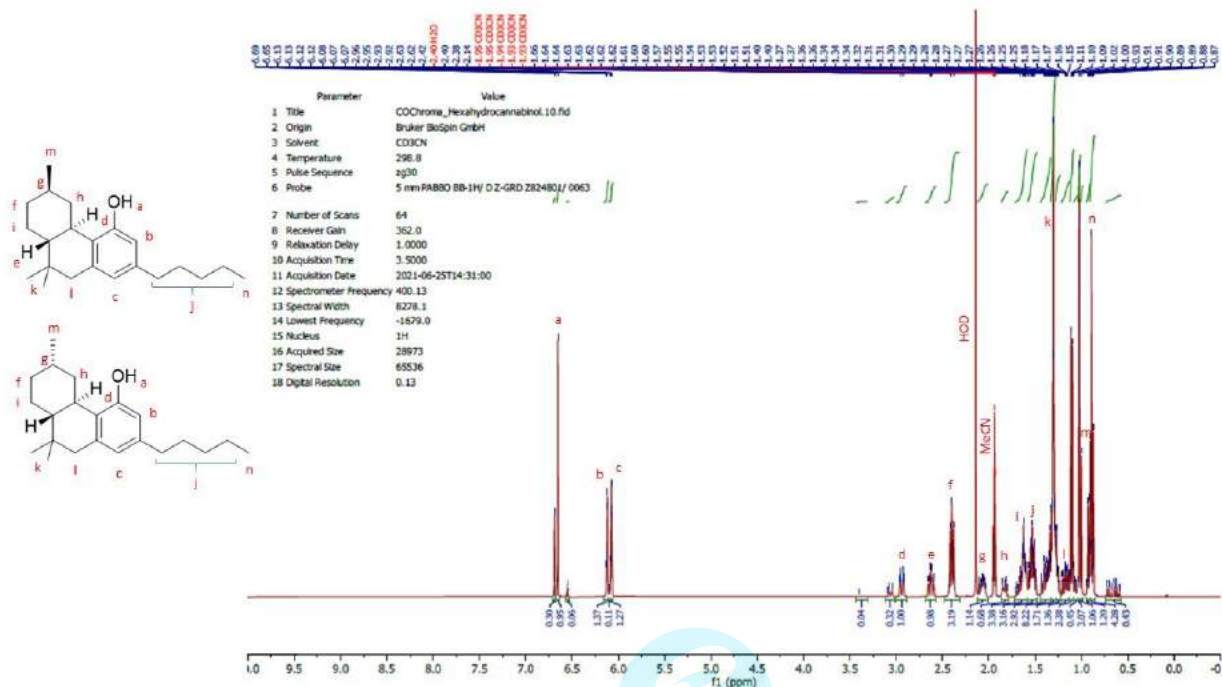
Colorado Chromatography Report 28May2021

HPLC-PDA Data Analysis

Samples were initially analyzed by HPLC-PDA to identify and quantify cannabinoids in the mixture of 18 standard cannabinoids plus additional cannabinoids (*i.e.*, CBND, CBT, CBE, Δ^{10}-THC, $\Delta^{6a,10a}$-THC etc.). Cannabinoids were identified by comparison of their relative retention times (RRT) and the UV-spectral data. Samples were analyzed twice – once at a concentrated level to detect and quantify minor cannabinoids and once after dilution to detect and quantify major cannabinoids).

Sample ...2132
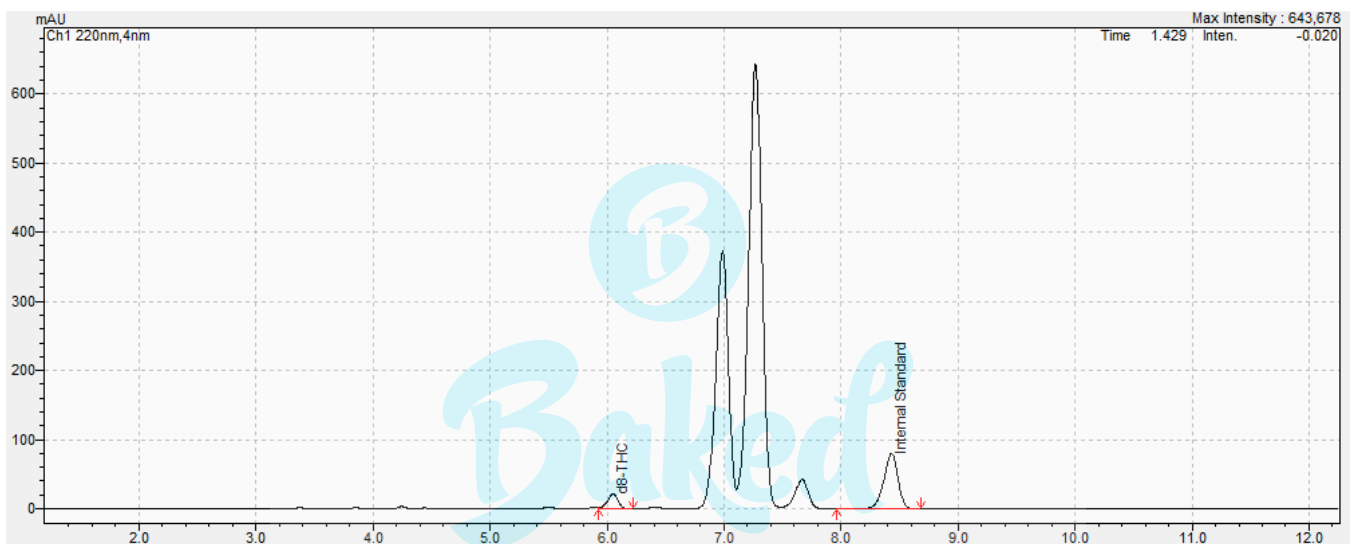
Sample ...2132 (Zoomed In)
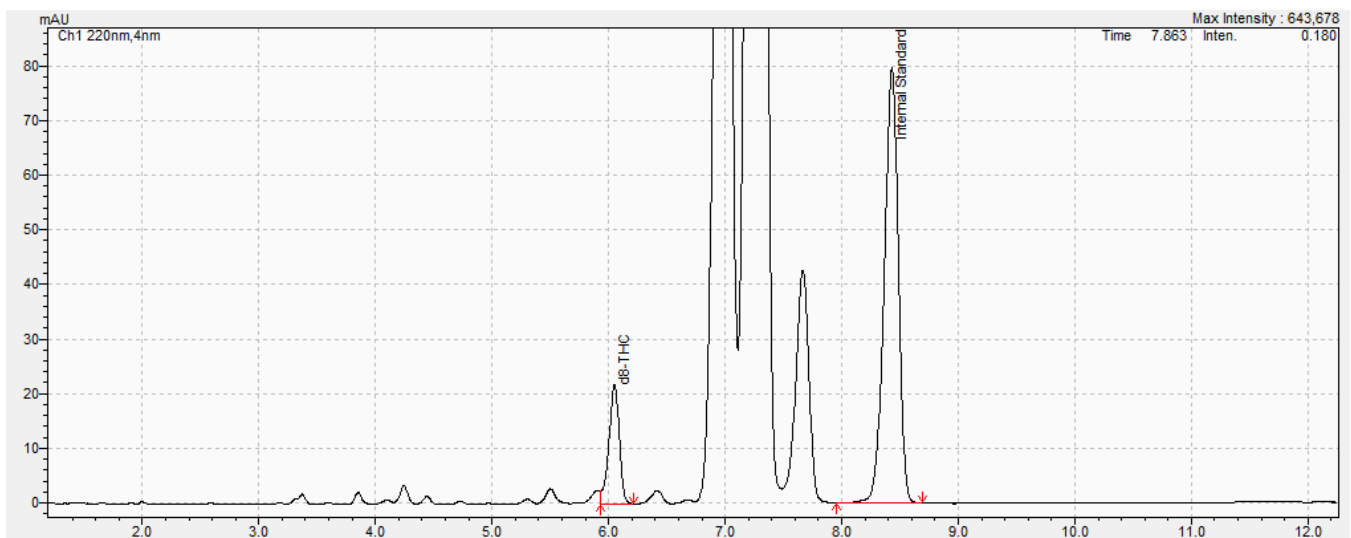
Δ⁸-THC was the only cannabinoid identified from the reference standards analyzed by this method. The spectra of unknown peaks are displayed below. At least three unidentified substances eluted after Δ⁸-THC indicating that they are more lipophilic than Δ⁸-THC. If the molar absorptivities of these substances are similar to that of Δ⁸-THC, then at least two of them are much more abundant than that of Δ⁸-THC and the third at a retention time of about 7.7 min is about twice the abundance of Δ⁸-THC.

Origins of PDA Spectral Data
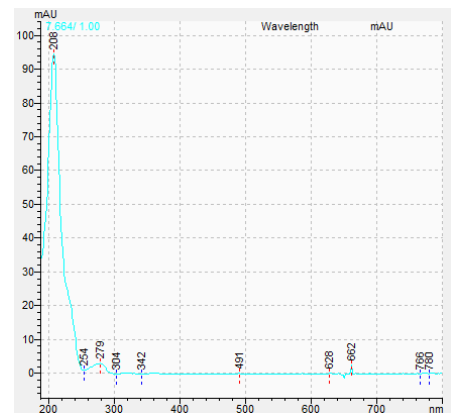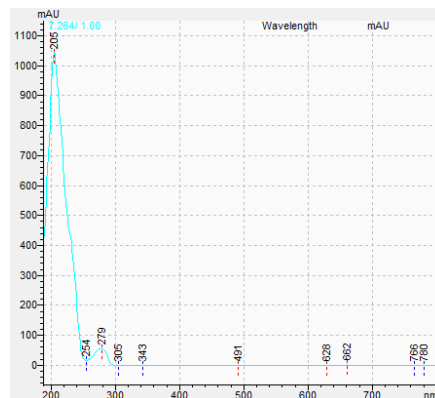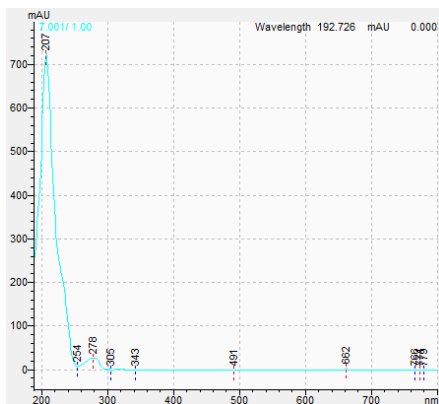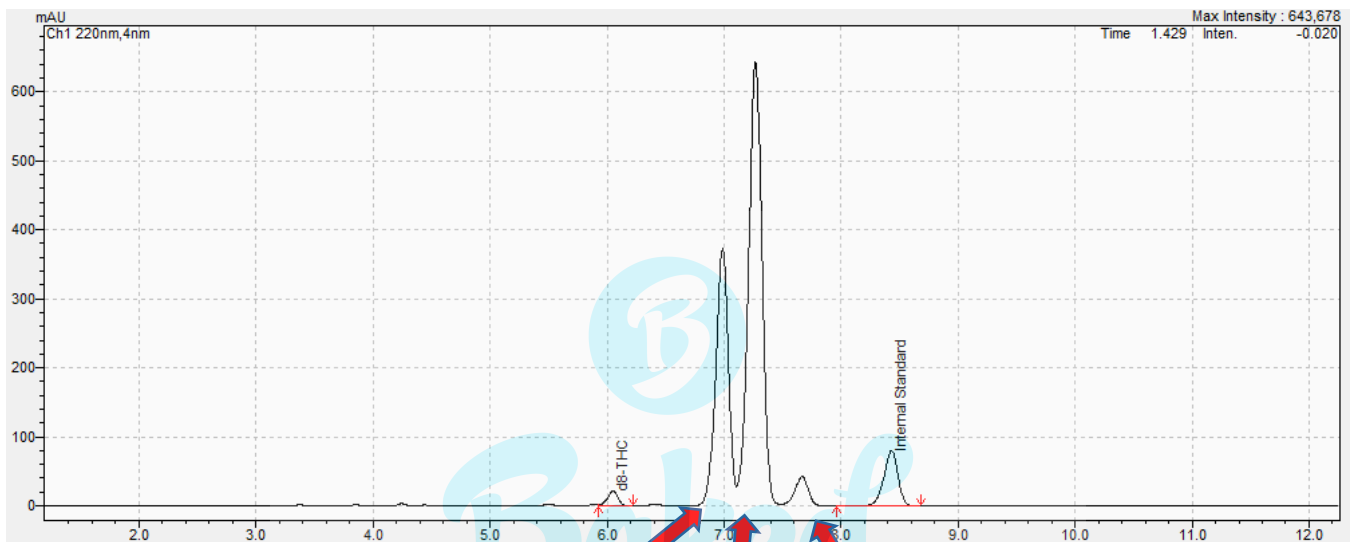
The PDA spectral data for these peaks appear similar to those of other cannabinoids such as Δ^8-THC, Δ^9-THC, and CBD without extended conjugation.

Since the major components of the sample were not identified by HPLC-PDA, we subjected the sample to LC-MS/MS analysis as shown on the following pages.

LC-MS/MS Data Analysis

Full Scan Data

Sample ...2132
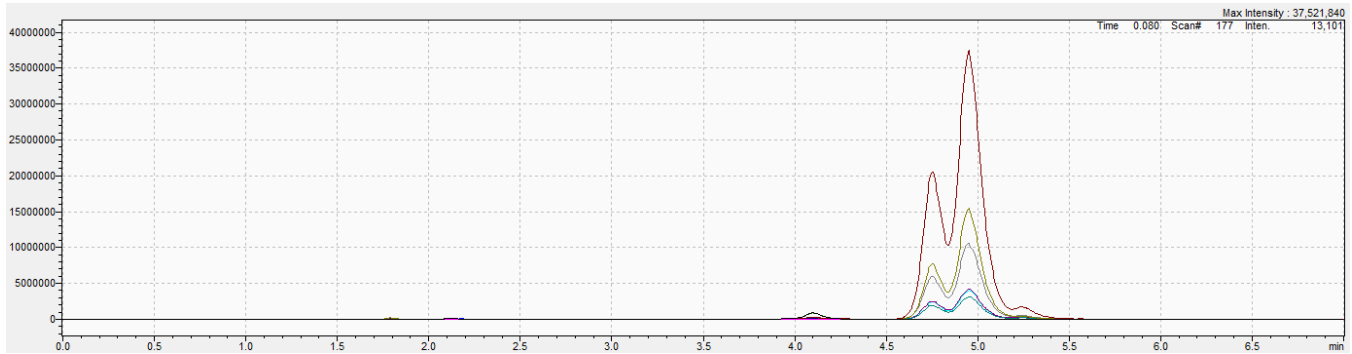
Sample ...2132 (Zoomed In)
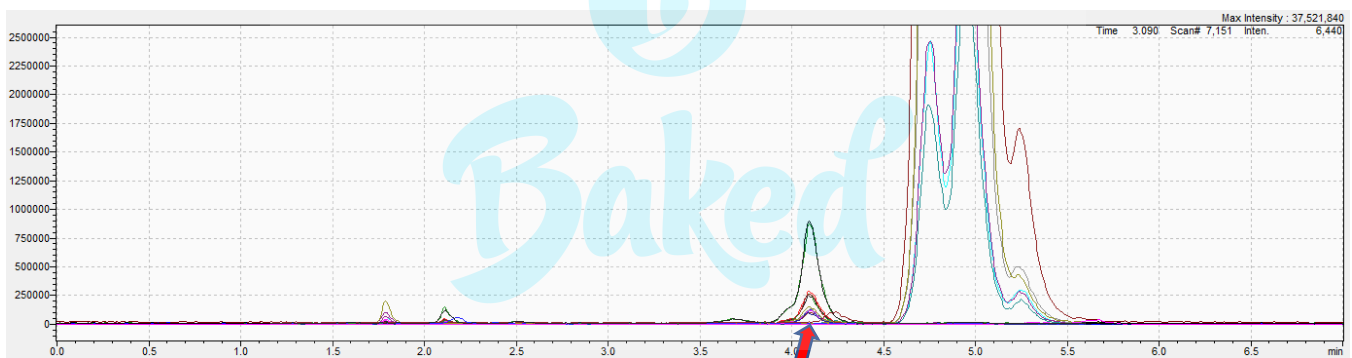
Δ8 THC

Data were then acquired in MRM (multiple reaction monitoring mode). The MRM transitions are listed to the right of the chromatogram. The "Act%" is the relative abundance (RA) of each MRM transition compared to the primary transition (calculated by area counts). To identify a compound, the relative abundances (RA) of the MRM transitions of the peak are compared to those of a reference standard.

Calibration Mix containing cannabinoid standards.

Type	m/z	Area	Act%	Ident.Range%	Display
T	315.2000>193.050	25024286	100.00	100.00	☑
Ref.1	315.2000>123.000	22560456	90.15	----	☑
Ref.2	315.2000>259.100	9042478	36.13	----	☑
Ref.3	315.2000>135.050	14394409	57.52	----	☑
Ref.4	315.2000>93.1000	11497309	45.95	----	☑

Δ9 THC   Δ8 THC

Sample …2132

Type	m/z	Area	Act%	Ident.Range%	Display
T	315.2000>193.050	38046165	100.00	100.00	☑
Ref.1	315.2000>123.000	34335464	90.25	----	☑
Ref.2	315.2000>259.100	13430819	35.30	----	☑
Ref.3	315.2000>135.050	20468446	53.80	----	☑
Ref.4	315.2000>93.1000	16567005	43.54	----	☑

The presence of Δ^8-THC was confirmed by the ions and ion area ratios of the peak at 4.10 minutes in sample #2132. The peak just to the left of the peak for Δ^8-THC has ions characteristic of Δ^9-THC but we do not believe that identification criteria were met for this identification.

Ion chromatogram for *m/z* 317.2 in positive ionization mode
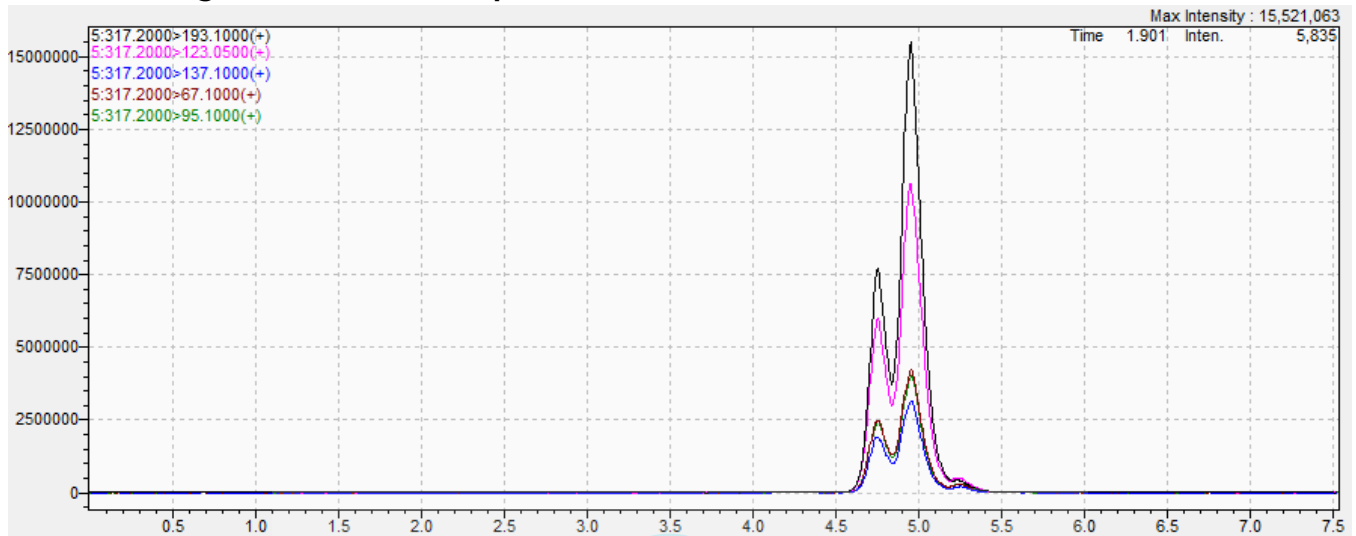
These peaks have masses and fragmentation patterns similar to those of CBG but the retention times are incorrect for CBG. The apparent molecular mass of the three substances eluting between 4.5 and 5.3 minutes is 316. This mass is identical to that of various isomers of hexahydrocannabinol.
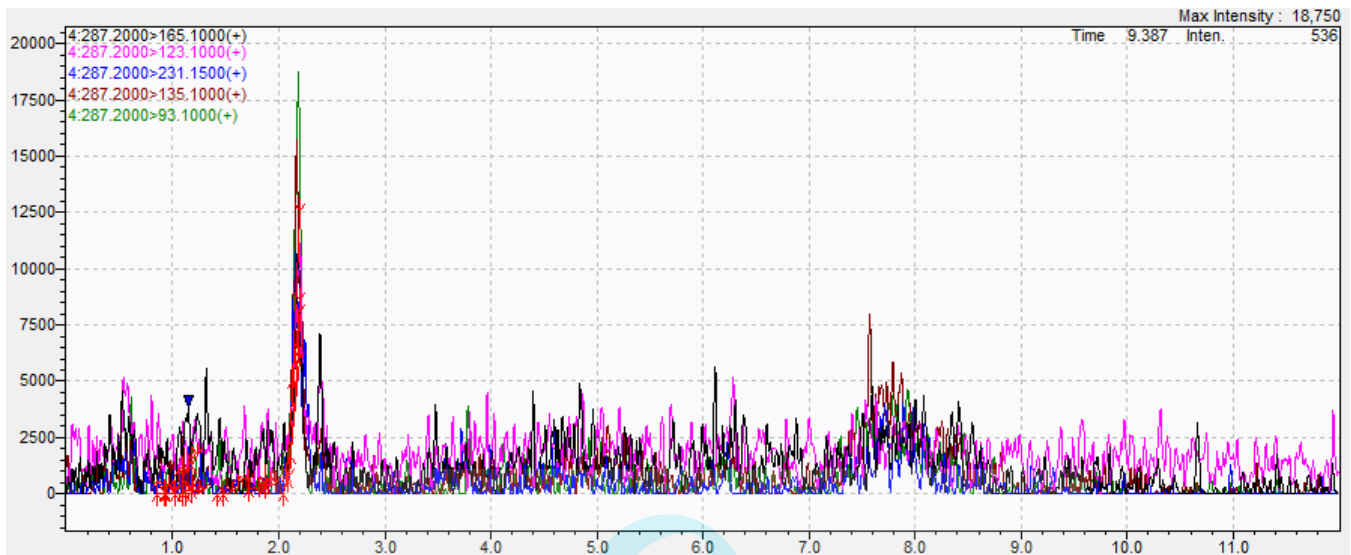
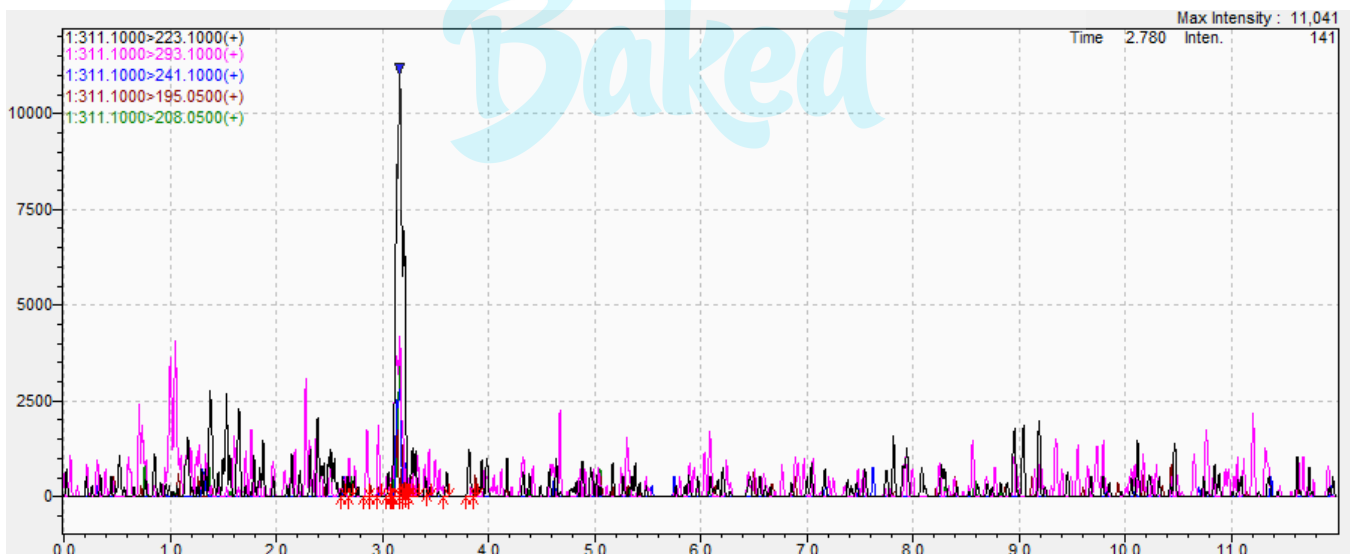**Ion chromatogram for *m/z* 315.2 in positive ionization mode**
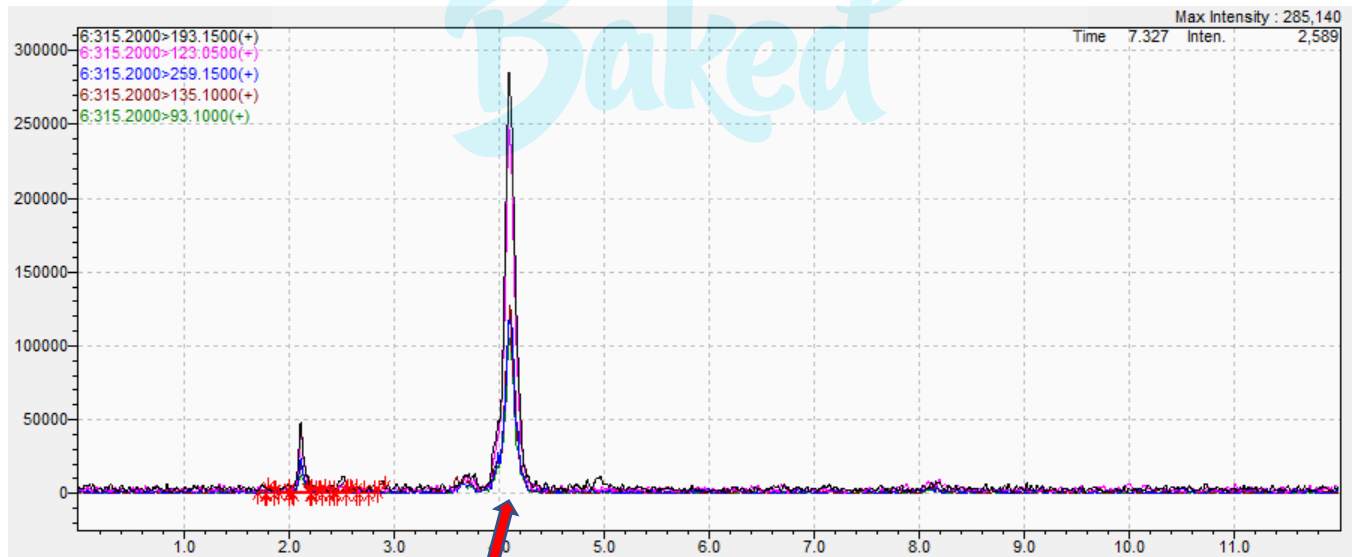
Δ^8-THC

The identification of Δ^8-THC was confirmed.

Ion chromatogram for *m/z* 287.2 in positive ionization mode

Nothing was identified in this ion chromatogram. The ions are of low intensity and probably do not represent substantial products.

Ion chromatogram for *m/z* m/z 311.1 in positive ionization mode

Nothing was identified in this ion chromatogram. The ions are of low intensity and probably do not represent substantial products.

Ion chromatogram for *m/z* 331.0 in positive ionization mode
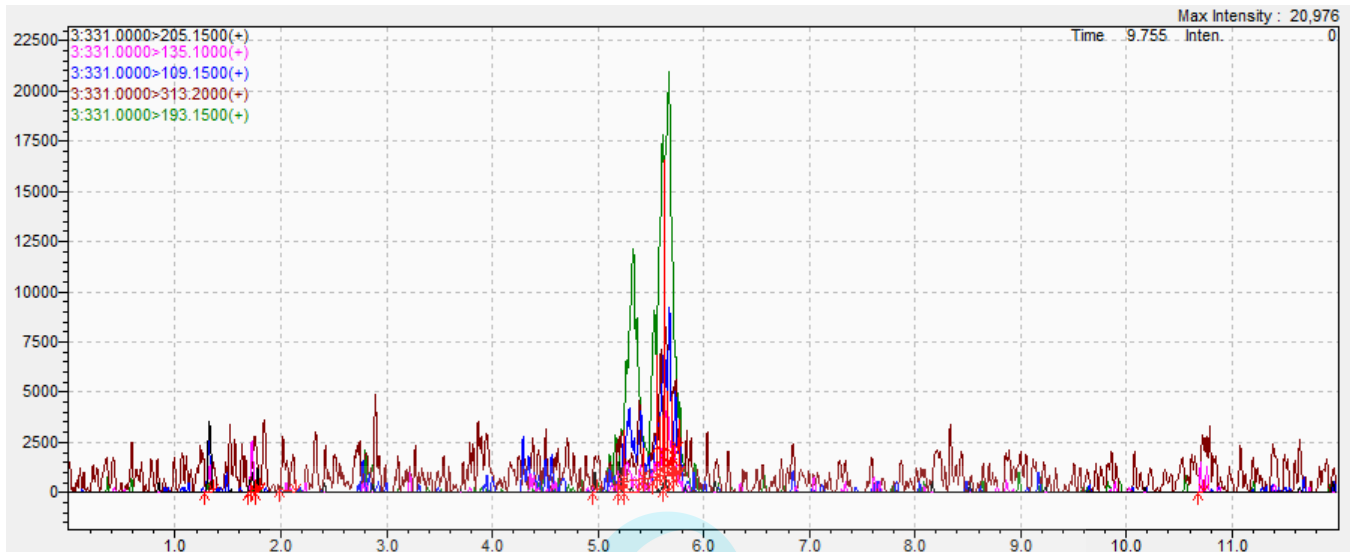
Nothing was identified in this ion chromatogram. The ions are of low intensity and probably do not represent substantial products.

The selected ion monitoring data for the ion at m/z 317 were collected and are displayed in the following ion chromatograms. The vertical line in the ion chromatogram indicates the time at which the data displayed in the second window were obtained.
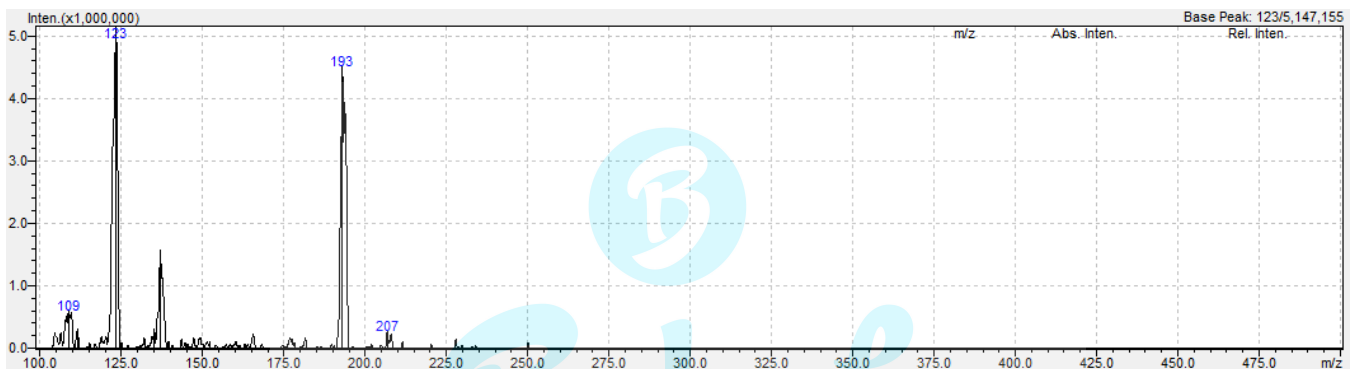
The Selected Ion Monitoring (SIM) results for *m/z* 317 in positive ionization mode are shown below. The data at 5.0 minutes were selected for analysis.
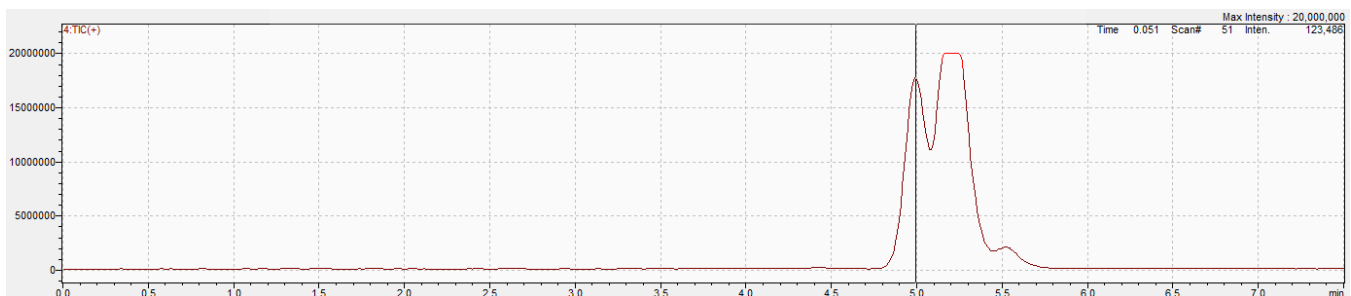
The prominent peak at 5.0 min is characterized by *m/z* 317 and the full scan spectrum is characterized by ions at *m/z* 289, 270, and 201.
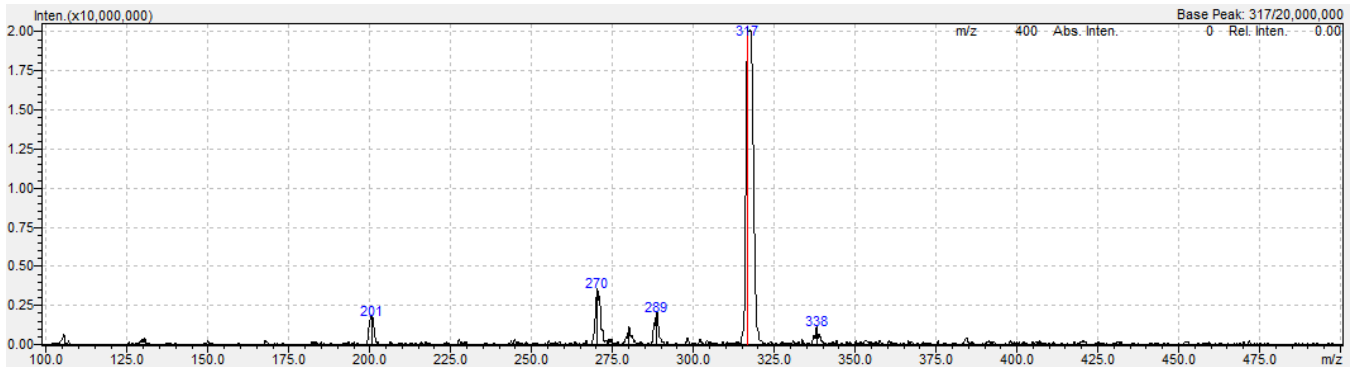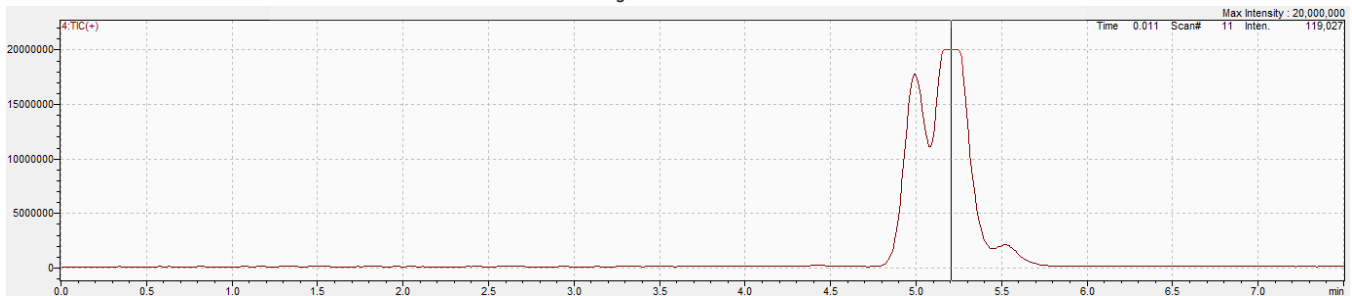
The product ion scan for m/z 317 at 5.0 min is characterized by ions at *m/z* 193 and 123.
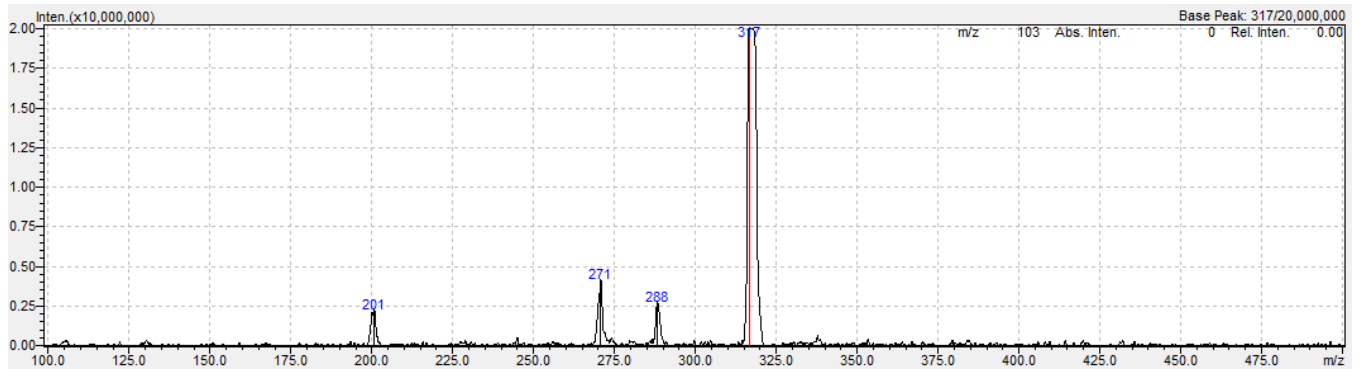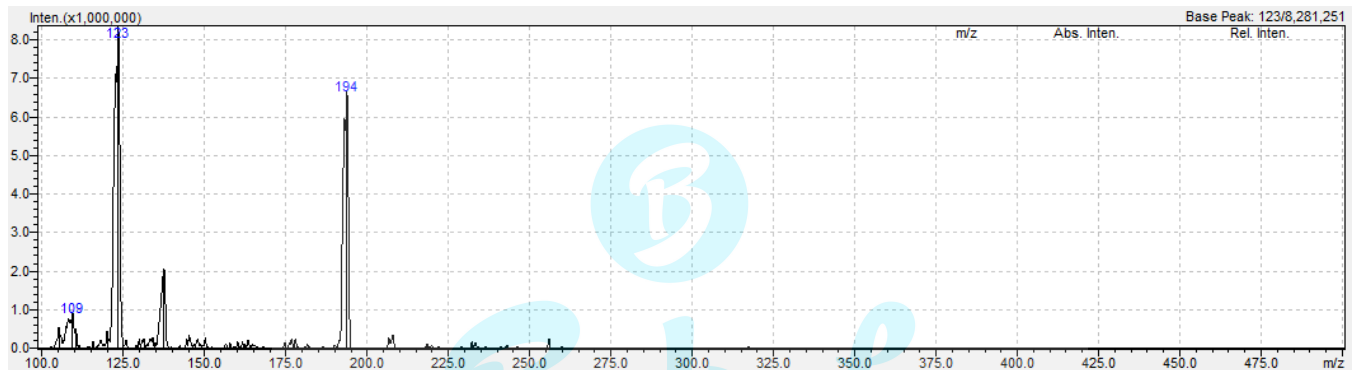

SIM of *m/z* 317

The Selected Ion Monitoring (SIM) results for *m/z* 317 in positive ionization mode are shown below. The data at 5.2 minutes were selected for analysis.

The prominent peak at 5.2 min is characterized by *m/z* 317 and the full scan spectrum is characterized by ions at *m/z* 2898 271, and 201.
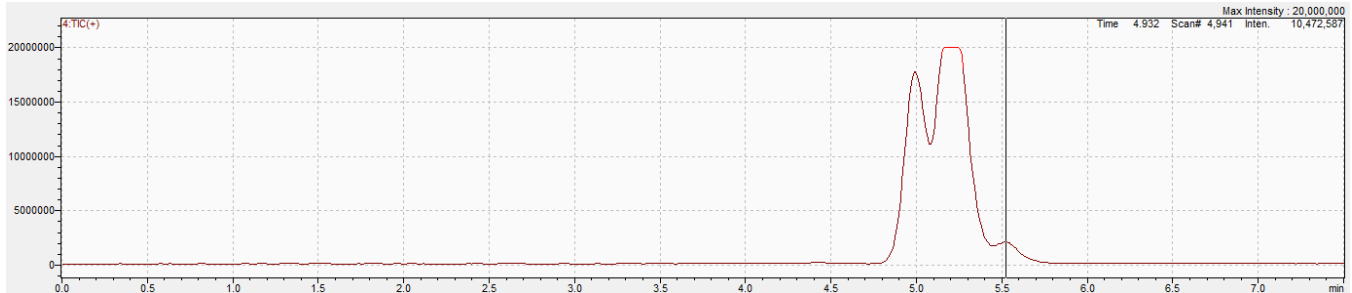
The product ion scan for *m/z* 317 at 5.2 min is characterized by fragment ions at *m/z* 194 and 123.
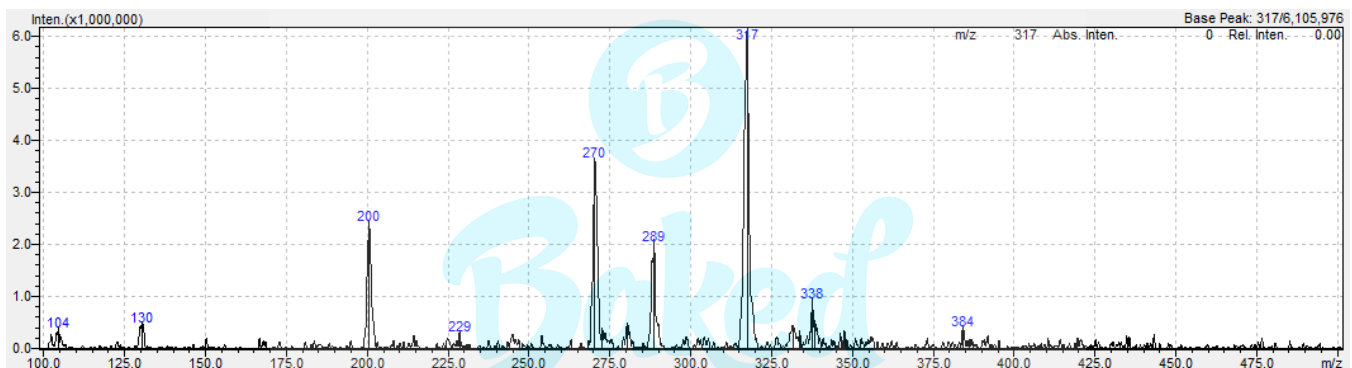
SIM of *m/z* 317

The Selected Ion Monitoring (SIM) results for *m/z* 317 in positive ionization mode are shown below. The data at 5.5 minutes were selected for analysis.

The prominent peak at 5.5 min is characterized by *m/z* 317 and the full scan spectrum is characterized by ions at *m/z* 289, 270, and 200.
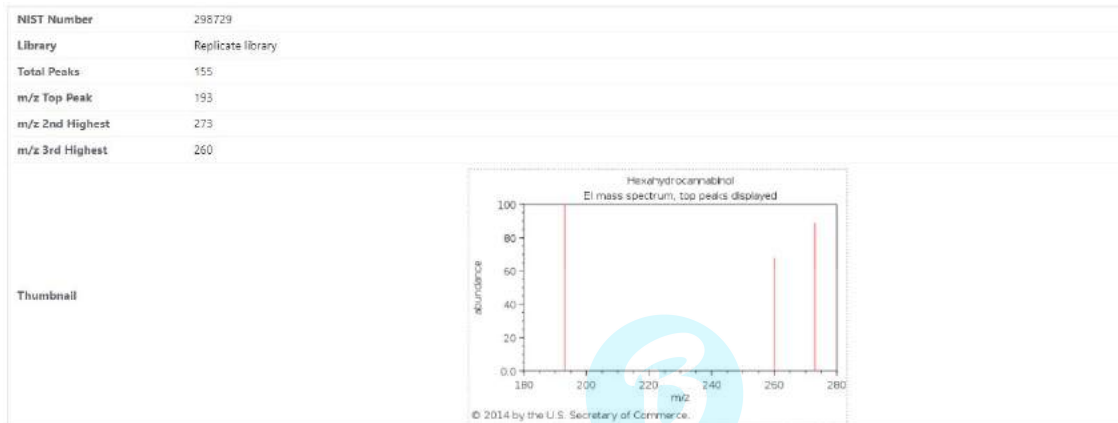
The product ion scan for *m/z* 317 at 5.5 min is characterized by fragment ions at *m/z* 194 and 124.

GC-MS/MS Analysis of Sample #2132

The sample was subjected to GC-MS/MS analysis to obtain evidence for the presence of hexahydrocannabinol diastereomers and confirm the identity of the Δ^8-THC.

The GC/MS spectrum of hexahydrocannabinol was obtained from the NIST library and is shown below. Prominent ions are reported at *m/z* 193 (base peak), *m/z* 273, and *m/z* 260.

NIST Number	298729
Library	Replicate library
Total Peaks	155
m/z Top Peak	193
m/z 2nd Highest	273
m/z 3rd Highest	260
Thumbnail	

Hexahydrocannabinol
EI mass spectrum, top peaks displayed

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Those ions characteristic of hexahydrocannabinol were monitored during the GC/MS analysis of Sample 2123. The selected ion chromatogram is shown below and the relative abundances of the ions characteristic of hexahydrocannabinol from the 1st prominent peak at about 6.08 min are shown in the table below.
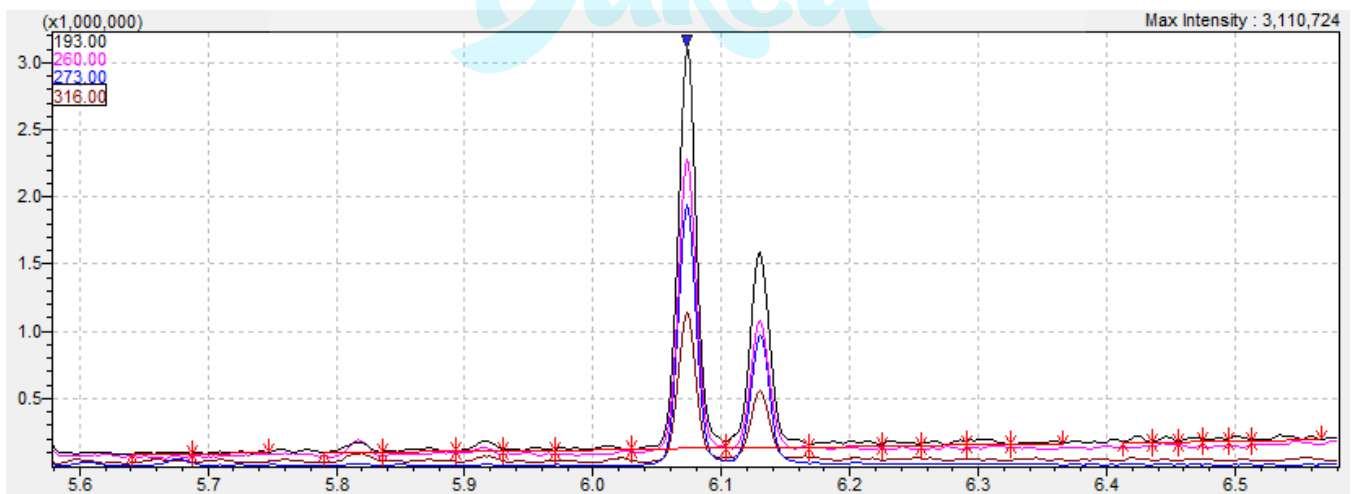
Type	m/z	Intensity	Set%	Act.%	Ref.Band
Target	193.00	3107990	100.00	100.00	---
Ref.Ion1	260.00	2249844	0.00	72.39	30
Ref.Ion2	273.00	2044301	0.00	65.78	30
Ref.Ion3	316.00	846728	0.00	27.24	30
Ref.Ion4					
Ref.Ion5					

The selected ion chromatogram is shown below and the relative abundances of the ions characteristic of hexahydrocannabinol from the 2nd prominent peak at about 6.13 min are shown in the table below.
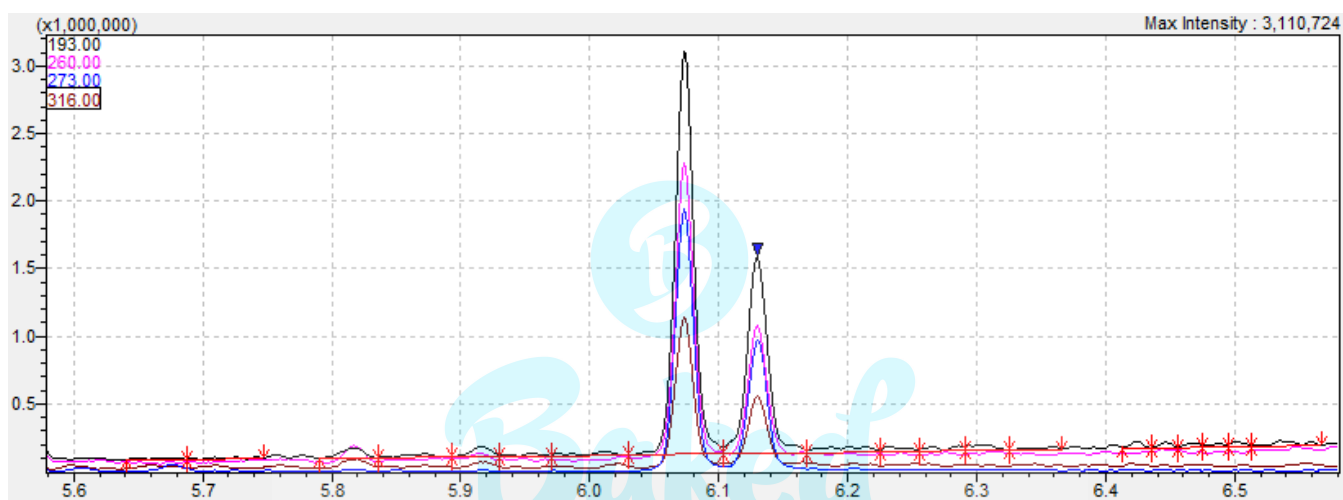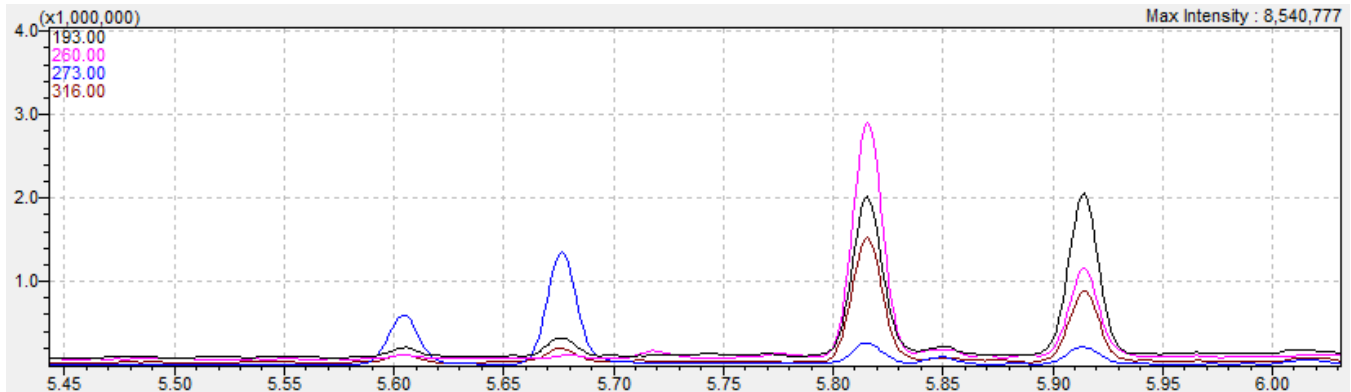

Type	m/z	Intensity	Set%	Act.%	Ref.Band
Target	193.00	1472511	100.00	100.00	---
Ref.Ion1	260.00	994232	0.00	67.52	30
Ref.Ion2	273.00	955394	0.00	64.88	30
Ref.Ion3	316.00	518360	0.00	35.20	30
Ref.Ion4					
Ref.Ion5					

The selected ion chromatograms of the ions characteristic of hexahydrocannabinol from the less abundant peaks before 6.0 min are shown below.
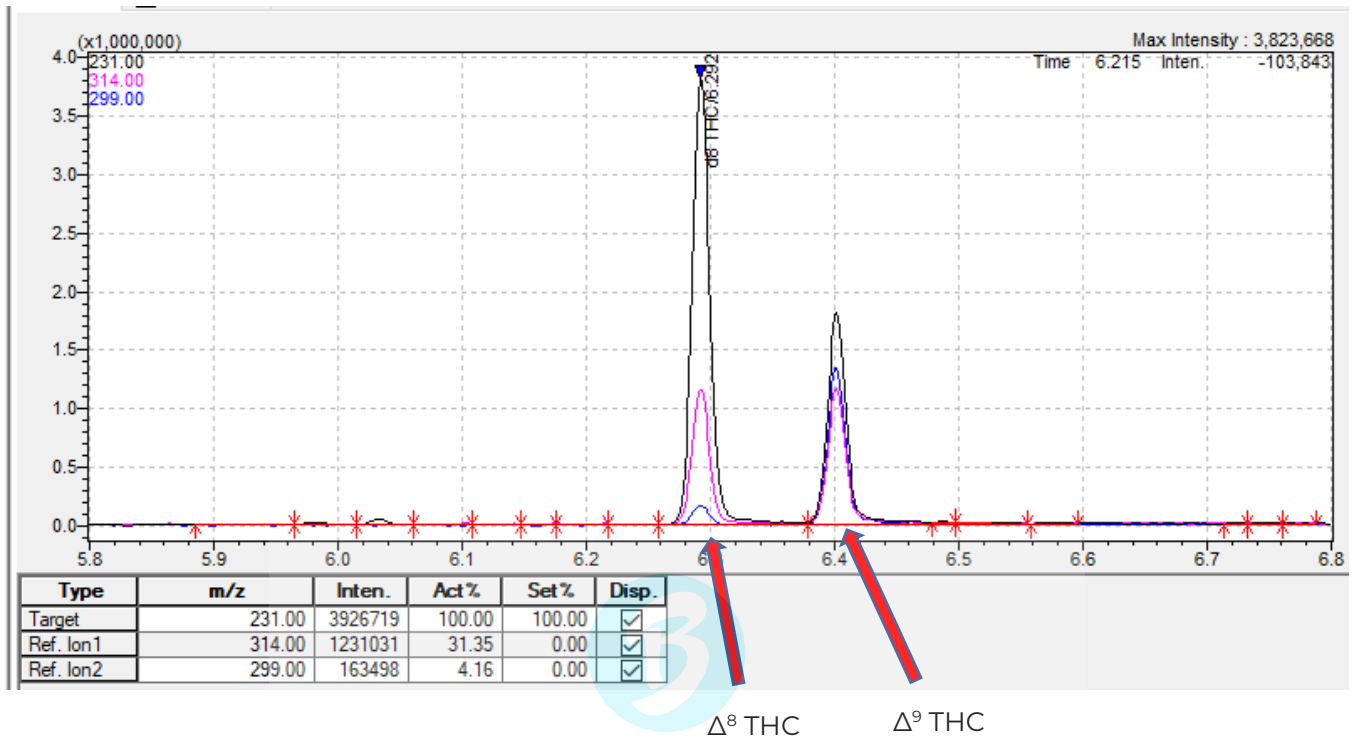

These results indicate with reasonable assurance that at least two and possibly more isomers of hexahydrocannabinol are present in the sample submitted for analysis. Proof of identity would require a reference standard.
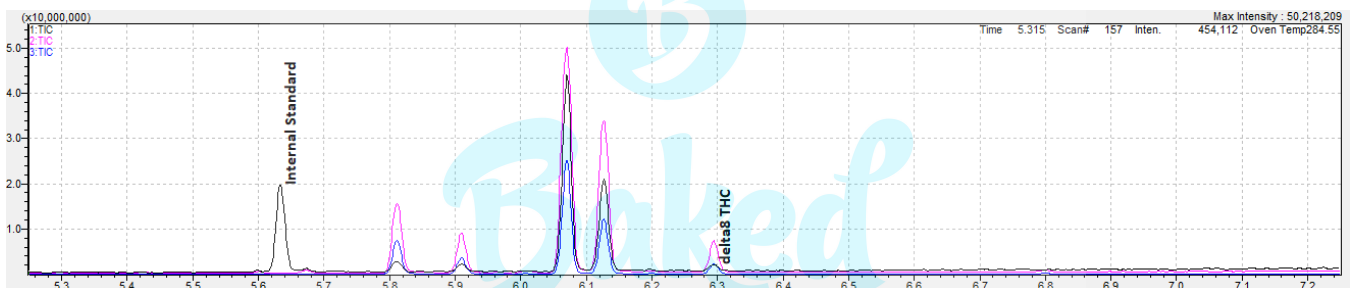
The peak at 6.3 minutes was identified as Δ^8-THC. See the following page for confirmation of the identity of Δ^8-THC.

The following is a SIM analysis of a Standards Mix showing ions and retention times for Δ^8-THC and Δ^9-THC. The table below the ion chromatogram reports the relative abundances of the most abundant ion for Δ^8-THC at m/z 231, the molecular ion at m/z 314, and a fragment ion at m/z 299.

Type	m/z	Inten.	Act%	Set%	Disp.
Target	231.00	3926719	100.00	100.00	☑
Ref. Ion1	314.00	1231031	31.35	0.00	☑
Ref. Ion2	299.00	163498	4.16	0.00	☑

Δ⁸ THC   Δ⁹ THC

The following is a SIM analysis of Sample 2132 showing ions and retention times for Δ^8-THC and Δ^9-THC. The table below the ion chromatogram reports the relative abundances of the most abundant ion for Δ^8-THC at m/z 231, the molecular ion at m/z 314, and a major fragment ion at m/z 299.

SIM analysis of Sample 2132.

Type	m/z	Inten.	Act%	Set%	Disp.
Target	231.00	2875917	100.00	100.00	☑
Ref. Ion1	314.00	854930	29.73	0.00	☑
Ref. Ion2	299.00	109787	3.82	0.00	☑
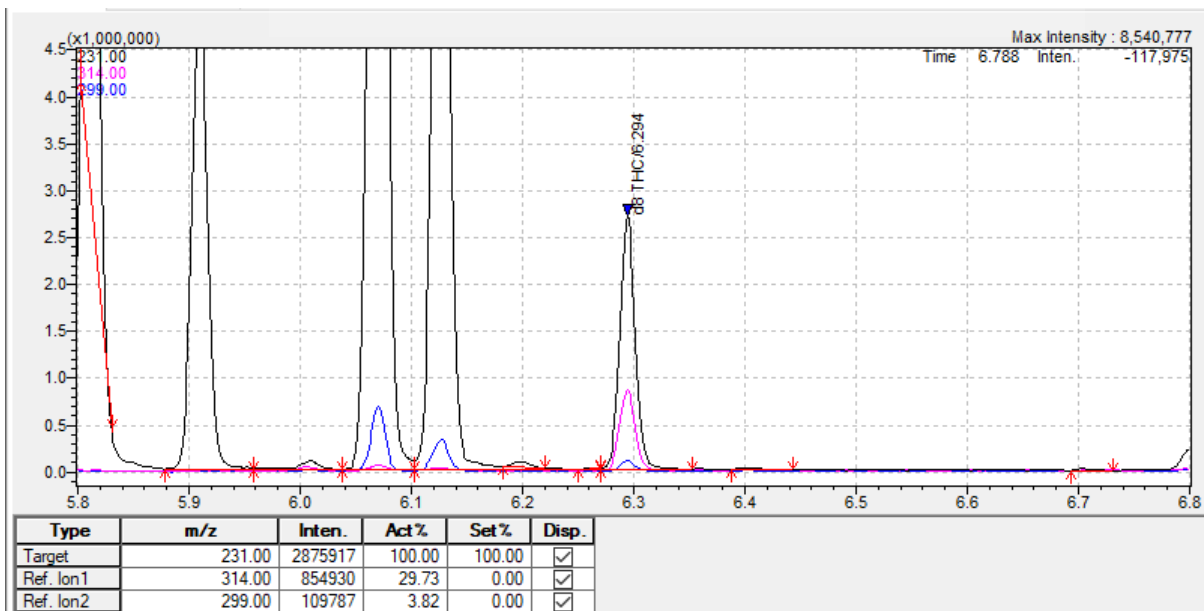
The presence of Δ^8-THC in sample #2132 was confirmed by comparison of the retention time and relative abundances of the qualifier ions with those of a certified reference standard.

Conclusions

- Sample #2132 contains a small amount of D^8-THC based on HPLC-PDA, LC-MS, and GC-MS data.

- Sample #2132 contains components characterized by greater lipophilicity than D^8-THC based on longer HPLC and LC retention times. The components eluting after D^8-THC are characterized by a pseudomolecular ion at m/z 317 based on positive ionization electrospray LC-MS analysis indicating an apparent molecular weight of 316, consistent with that of the hexahydrocannabinol isomers. Analysis of sample #2132 by GC/MS analysis indicated the presence of at least two substances with an apparent molecular mass of 316 and characterized by fragment ions at m/z 193, m/z 260, and m/z 273. These fragment ions are consistent with reported fragment ions of hexahydrocannabinol.

- Confirmation of the presence of hexahydrocannabinol in sample #2132 requires availability of a reference standard.

- Sample #2132 contains no other detectable cannabinoids from our collection of more than forty reference standards.