Minor Cannabinoids of *Cannabis sativa* L.

Fabian Thomas\(^a\), Oliver Kayser\(^b\)

TU Dortmund University, Technical Biochemistry, Germany

ABSTRACT

Cannabinoids from *Cannabis sativa* L. play an important role as natural products in clinics. The major cannabinoids compromise tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) and its decarboxylated analogs. In this review, we focus on often neglected minor cannabinoids and discuss biosynthetic and chemical degradation routes to other neglected cannabinoids in *Cannabis sativa* starting from THCA, CBDA and cannabichromenic acid (CBCA). Based on the literature, patents and scientific reports, essential routes for the chemical modification of cannabinoids are discussed to explain chemical diversity chemical conversion and degradation by UV light, as well as temperature and pH leading to the formation of structurally unusual cannabinoids in planta called as minor cannabinoids. Based on known bioorganic reaction schemes and organic chemistry, principles for minor cannabinoid formation like [2+2] cycloaddition, Markonov condensation, radical introduction, or aromatization are discussed. Finally, the non-aqueous environment in *Cannabis sativa* trichomes is analyzed to clarify their role of a miniaturized bioreactors the light-induced conversion in a non-aqueous enviroment. The overall objective is to bridge from metabolic profiling via cannabinomics to structural and chemical diversity that allows the definition of patterns with consequences also to pharmacology and plant breeding.

Keywords: Cannabis sativa, Cannabinoids, Tetrahydrocannabinol, Cannabidiol, Cannabichromene, Cannabielsoin, Cannabitriol, Cannabinomics.

Biosynthesis of cannabinoids

Cannabinoids seem to be a unique class of natural products limited to *Cannabis sativa* L. only. In recent time, prenylated olivetolic acid derivates and other structurally related prenylated phenolics have also been identified in various genera and species like *Helichrysum umbraculigerum* Less. [1] or the liverwort *Radula marginata* TAYLOR [2, 3]. So far, the biosynthesis of cannabinoids towards to the biogenetically hybrid derived from the mevalonate and polyketide pathway as we understand today has only been detected in *Cannabis sativa* L., *Cannabis indica*, and *Cannabis ruderalis*, but recently as well in the New Zealand liverwort *Radula marginata* TAYLOR and *R. peritittoni* [3]. Without going into the details of molecular biology, genetics and spatial resolution of biosynthesis (Figure 1) [4], all committed biosynthetic enzymes involved in the formation of THCA-C5, CBDA-C5 and CBCA-C5 (Figure 1), cannabidiolic acid (Figure 1) and cannabichromenic acid (Figure 1) have a common precursor in cannabinolic acid (CBGA-C5). and all conversion products have identical masses and differ only structurally.

Major structural differences refer to the alkyl chain length of the classic THCA-C5 and the short one in cannabivarin known as THCA-C3 (Figure 2A). Interestingly, no other biosynthetically relevant gene or enzyme has been found yet that may extend on the biosynthetic pathway from above-mentioned cannabinoids on. In contrast to more than 150 found so-called minor cannabinoids [5] this observation is provoking the question if these cannabinoids, detected at very low
concentrations in planta, are simply degradation products of chemical conversions induced by light, temperature or abiotic ecological factors.

**Chemistry of cannabinoids**

Structural diversity of cannabinoids is ruled by complex chemistry [6]. In principle, it can be distinguished between the polar carboxylated cannabinoids and the neutral lipophilic decarboxylated cannabinoids. Going deeper into structural aspects, all cannabinoids show a resorcinol core and mostly a side chain of three or five carbons. Flanking alkyl regions in the bicyclic region (menthyl-core) and the aliphatic chain are sensitive to oxidation [7]. Even the single double bond in position $\Delta^9$ shows poor oxidative stability during storage of dried plant material. A closer look to the isoprenyl residue (C10) that has been formerly been the C-C attached GPP moiety (Figure 1), can be found in four topological arrangements [5]:

1. The simple connection via a Friedel-Crafts C-C alkylation as an electrophilic aromatic substitution towards CBG or in a second step reaction induced by UV light starting from CBCA-C5 to cannabicyclol type cannabinoids like CBL-C5 (Figure 2B). This closure of an additional carbon bond is by some authors considered to be an additional fourth topological arrangement [5].

2. Closure of the attached GPP-unit with the resorcinol hydroxyl group to chromenes as CBCA-C5.

3. Internal C-C reaction to a cyclohexane ring like in CBD followed by a nucleophilic reaction with one of the resorcinol hydroxyl groups resulting in hydrocannabinols like THCA-C5.
or canabielsoin-type cannabinoids (reaction with C₂-OH) like cannabielsoin E (CBE) (reaction with C₁-OH).

4. Aromatization of the cyclohexane ring from CBDA-C₅ or THCA-C₅ after oxidation in the positions C₁, C₂, C₃, and C₅ or C₉, C₁₀, C₁₀a, C₇, C₈, respectively. Aromatization gives CBN and CBDN-type cannabinoids.

Decarboxylation of cannabinoids is a spontaneous non-enzymatical process that is highly temperature dependent. Smoking will accelerate decarboxylation at high temperatures, but during storage decarboxylation with an estimated rate of 5–10% a year is known in dried plant material at room temperature (20–25°C) as well [8].

Oxidation of the alkyl side chain is also reported in the literature. Here, oxidative processes are linked to microbial degradation [9]. The chain length can also be reduced by β-oxidation and the loss of a C₂-unit. It is not anticipated that this is the major route to THCA-C₁ (oricoids), because the presence of specific polyketide synthases (PKS) is responsible by biosynthesis for C₁ but also C₃ cannabinoids (viridinoids) [10].

### Classification of cannabinoids

The chemical taxonomy and classification of cannabinoids are not ruled by the enzymatic or biosynthetic route. Here, we follow the standard classification as applied in organic chemistry based on the structure an arrangement of cyclic systems. In Figure 3 a broad overview of the most important classes is depicted. Based on topological arrangements as discussed above, cannabidiolic acid (CBDA-C₅) seems to be an important player. Mostly, CBDA-C₅ is accepted as a cannabinoid on its own and catalyzed by the enzyme CBGA-C₅ synthase. But from the mode of catalysis and a theoretical carbocation in the CBD-like intermediate, we may have to consider CBDA-C₅ cations as intermediates towards to THCA-C₅ (Figure 4). This has the implication, that CBDA-C₅

![Figure 3](image-url)
is rather unstable and can be activated by appropriate conditions. This may explain, why CBD-C5 can be converted to THC-C5 at harsh conditions like pH 1.

Although we know that the homology between CBDA Synthase and THCA Synthase is 88% [11], we need to address the question how the carbocation is relocated in the intermediate activated CBDA-C5 intermediate and how it is stabilized in the active center of the enzymes. This question cannot be answered today, but it is of high interest under the proven assumption that each one of the three enzymes (CBDA Synthase, THCA Synthase, and CBC Synthase) can convert in principle CBGA-C5 to all three products THCA-C5, CBDA-C5 and CBCA-C5. If so, we may have an answer to why the fiber hemp the plant still produces THC-C5 at concentrations below 0.3% and we never get a Cannabis plant that is free of THC-C5.

If we consider this basic question of carboxylation relocation and structural diversity, we may have a first approach towards a better understanding for classification based on a hierarchy of chemical reactions. First, C-C coupling is based on an enzymatic reaction by a prenyltransferase, second, carbocation formation and another C-C coupling to a bicyclic system, third, additional cyclization based on nucleophilic reaction with hydroxyl groups or electrophilic addition with a carbocation to three-membered aliphatic, aromatic or heteroaromatic ring systems.

**Formation of minor cannabinoids**

**Δ8/Δ10- THC**

In *Cannabis sativa* L. the position of the double bond is due to the localization in the GPP and its addition to the aromatic ring at the position C9. Commonly this is indicative for the nomenclature of Δ8 and represents 99% of THCA-C5 and THC-C5 in the plant. Other positions of the double bond in the positions C8 and C10 are considered as artifacts introduced by acids or oxidatively promoted a shift of the endocyclic double bond. Main reason, as observed in aged plant material, is oxidation under UV light influence or in the lab at drastic acidic (pH1) conditions, as observed for CBD-C5 cultivations also yielding Δ8/Δ10- THC (Figure 5). For CBD-C5 it must be mentioned, that UV light exposure is irrelevant for the conversion to THC as documented by unpublished experiments of the author.

**Cannabinol (CBN-C5)**

Presence of aromatized THC known as cannabinol (CBN-C5) (12,13) is mostly detectable in aged *Cannabis sativa* L. If cannabis material or the pure isolated THC is exposed to UV light or sun light for an extended amount of time, the menthyl-ring system will be oxidized as suggested in Figure 6. Oxidation followed by dehydration resulting additional double bonds is the main road to full aromatization. The order of stepwise
induced double bonds is not clear, but it can be assumed that the first oxygen attack is at position C10α as energetically most favored followed by a C6 attack. In parallel, other various intermediates like C6-C9 overbridged peroxide derivatives as instable intermediates are discussed as well. A C9-C10 epoxide was detected, but seems unlikely to contribute to the aromatization process [14]. In general, oxidation in the menthyl ring is often observed in plants and represents the most prominent way of oxidation in this kingdom. For instance, double oxidation like in 10-Hydroxy-

![Figure 5. Chemical conversion of CBD-C5 to Δ8/Δ10-THC at pH 1](image)

![Figure 6. Proposed oxidation of THC under UV light towards CBN-C5](image)
9-oxo-Δ⁵-tetrahydrocannabinol (Figure 2B), double oxidation to Δ⁸-trans-Tetrahydrocannabinol glycol (Cannabiripsol) [15], triple oxidation to 6a,7,10a-Trihydroxy-Δ⁵-Tetrahydrocannabinol (Figure 2C) or simple α/β-oxidation are typical reactions in plants. It is of interest, that microsomal oxidation by human liver cytochromes and by certain microorganisms take place in the exocyclic methyl group at C11 or in the alkyl chain [9]. It must be mentioned that CBDN-C5 as aromatized product from CBD-C5 follows the same chemical routes as described above. Here, we will not go into detail again, but the steps of oxidation and the same photochemistry can be applied as well.

In recent publications (-)-trans-Cannabitriol (CBT-C5) is mentioned as a minor cannabinoid on its own [5, 16]. Here, we do not consider CBT-C5 as a minor cannabinoid of elevated importance for the plant. We regard CBT-C5 to be a highly oxidized intermediate. Doubtful is the identification of ethoxy structures which are more likely to be artifacts by ethanol extraction [16].

Cannabichromene (CBC-C5) and Cannabicyclol (CBL-C5)

To our knowledge, the acid form of cannabichromene (Figure 2C) [17] is the last out of the three major compounds that are biosynthesized by an enzymatic reaction (CBCA synthase) [18]. Although it is not considered a minor cannabinoid in sensu stricto, CBCA accumulates in concentrations of around 0.2–1% [17] in various Cannabis sativa L. strains, which is significantly lower than their respective THCA-C5 and CBDA-C5 analogs. CBCA-C5 and CBC-C5 is racemic and can also easily be made by synthesis [19].

Cannabicyclol (CBL-C5) as shown in Figure 2E is a conversion product of CBC-C5 under the influence of light (Figure 7) [19]. The chemical mechanism can be explained as followed: After UV irradiation, a Lewis acid-catalyzed intramolecular ene reaction between two partners occurs. For CBC, one ene in the chromene ring of CBC-C5 reacts with remaining one in the former GPP rest to give a carbocyclic four-membered ring in CBL-C5 [21]. It must be mentioned that in cannabis CBL-C3 known as CBLV-C3 and the carboxylated CBCA-C3 are also detected.

Cannabielsoin (CBE-C5)

This cannabinoid type is characterized by a furan ring as a result of a formal intramolecular opening of the 9-epoxide THC (Figure 6). It is not clear if the CBE-type compounds are natural products or artifacts in the isolation process. By mechanism, a radical initiation is also likely and shown for the conversion of CBD in plant cell cultures [22]. The absolute stereochemistry is 5aS,6S,9R,9aR [19]. In planta, carboxylated metabolites like cannabielsoic acid A, B, C1-C3 are known. Both occur with a C5 or a C3 aliphatic chain. Cannabielsoin is also detected after administration of CBD-C5 to humans and guinea pigs [23, 24]. The mode of liver metabolization is unclear, but radical formation of a reactive intermediate by a cytochrome and coupling with the C1 hydroxyl group is likely.

Discussion

Minor cannabinoids by the definition of low concentrated cannabinoids in Cannabis sativa L. are an exciting group of natural products. We learned that it is likely that most of the minor cannabinoids are unlikely to be biosynthesized by an enzymatic biotransformation that rather photochemical conversion or other reactions are important as we know from organic chemistry (Table 1). Chemical diversity in cannabinoid species is a consequence of the catalytic promiscuity of THCAS, CBDAS and CBCAS at the beginning of biosynthesis followed by a series of non-rational oxidation and C-C coupling steps in a non-stringent order. If we consider photochemical reactions, these will primarily occur in the trichomes as
main biosynthetic organ. Trichomes are appendages or aerial epidermal surface hairs on plants with two main features. First, trichomes consist of secretory cells and second, secreted exudate is stored in an extracellular cavity also called essential oil container. The localization of the biosynthetic proteins for CBGA-C5 and THCA-C5 is still unclear. Most authors assume membrane bound (CBGAS) and cytosolic (THCAS) localization of both enzymes. THCA and CBDA or decarboxylated analogs are secreted or diffuse into the essential oil container. But in recent reports by Mahlberg et al. 1992 [25] reported THCAS to be detectable outside of the cell wall of the glandular cell, stucked in the membrane and catalyzing at non-aqueous conditions. This was confirmed by Rodziewicz and Kayser, 2019 [26] who clearly documented by proteome analysis the presence of THCAS in the essential oil. Trichome may play in the physical way an important role as well. Specific physical properties may also contribute to the unique reaction conditions inside of trichomes. A near perfect spherical shape, observable in the lower micrometer range, strongly promotes light refraction and focusing through lensing effects in a bulb filled with essential oil. These exact conditions of extreme sphericity in shape and structure are found in the globular essential oil reservoirs of trichomes. An increase of temperature up to 50°C in direct sunlight is likely and accelerates radical formation for the discussed C-C binding. Other reactions as well as mentioned in Table 1 are temperature dependent and may promote conversion to minor cannabinoids. These physical conditions for chemical conversion in trichomes have not even raised so far and yet may play an important role for the understanding of non-aqueous organic chemistry in these biobased reactors.

We have not discussed minor cannabinoids with a C3 or C1 alkyl chain here. In total, the number of minor cannabinoids is estimated to compromise at least 150 compounds. Many of these compounds seems to be artifacts of downstream processes and some like quinones are only stable after acetylation or O-methylation. In addition, dimerization is known although not considered as a biogenic process and isolated structures are known in very low concentrations only. Dimerization is anticipated as a radial-initiated organic reaction of the resorcinyl-core. It is interesting that plants mostly modify the pattern by oxidation in the aromatic and menthyl-cores of cannabinoids, while mammalians oxidize C11 methyl group and microorganisms mostly impact the alkyl chain. We do not know about or just have overlooked this diversity and it is worth it to look closer with sophisticated analytical mass spectrosopy.

The biological role and function of minor cannabinoids for the plant is not known. On a closer look, the postulate that all natural products must have a specific benefit for the simple sake of energy optimized plant physiology, may not be true in this case. The previously discussed extracellular non-regulated synthesis being out of the control of the plant is special. Nevertheless, the usability in human medicine seems most obvious and in the future minor cannabinoids will definitively be in the interest of pharmaceutical companies.

### Table 1. Chemical organic reactions of cannabinoids

| Type of reaction                      | Type of reaction and position in the cannabinoid structure | Example for cannabinoid conversion               |
|---------------------------------------|----------------------------------------------------------|--------------------------------------------------|
| Makonovikov-type protonation          | Endocyclic double bond and tertiary C9                   | cannabicitran                                    |
| Radical reaction                      | Epoxidation                                              | cannabinolson                                    |
| [2+2] Cycloaddition                   | Ene coupling                                             | cannabicyclol                                    |
| Oxidation epoxidation                 | Oxidation of non-aromatic double bond                    | cannabitriol, cannabidiol glycol and more         |
| Dimerization                          | Radical induced aromatic coupling                        |                                                  |
| Steric rearrangement                  | Wagner-Meerwein relocation                               | cannabicyclol                                    |

Conclusion

After the legislation of Cannabis sativa L. for medical use, research and development is exploding (Figure 8) and still huge amounts of investments take place to push pharmacological studies. After only 5 years of legalized research in the USA, Israel, Canada and Germany, we merely can see the tip of the iceberg and more research in the interest of both the patient and consumer is welcome and needed. If we can see today a great interest in THC-C5 and CBD-C5 by industry and academia,
it is more than likely that companies and scientists will focus in the near future on the unexplored world of minor cannabinoids in the near future. A limiting factor for extensive research is the low availability and high price for minor cannabinoids. Extraction and purification of these compounds from cannabis extracts is challenging due to extreme low yield and low stability during downstreaming. Alternative strategies like organic synthesis or biotechnological production will be attractive and competitive to plant-based production in the future. The chemical diversity of cannabinoids is high and still unexplored, but we know that the biosynthetic impact by enzymatic catalysis on diversity was overestimated and photochemical conversion in a non-aqueous trichome “bioreactor” is an accepted hypothesis to explain pattern and occurrence. Beyond the horizon of CBD-C5 and THC-C5 dominated research and business today, we can expect new disruptive findings to establish minor cannabinoids in pharmacology and clinics, why the story of the one-billion-dollar plant is not told yet.

Acknowledgements

Conflict of interest statement
The authors have no conflict of interest with any commercial enterprise.

Funding sources
There are no sources of funding to declare.

References
1. Pollastro F, De Petrocellis L, Schiano-Moriello A, Chianese G, Heyman H, Appendino G, et al. Amor-frutin-type phytocannabinoids from Helichrysum umbaculigerum. Fitoterapia. 2017.
2. Nagashima F, Asakawa Y. Terpenoids and bibenzyls from three argentine liverworts. Molecules. 2011.
3. Asakawa Y, Hashimoto T, Takikawa K, Tori M, Ogawa S. Prenyl bibenzyls from the liverworts Radula perrottetii and Radula complanata. Phytochemistry. 1991;30(1):235–51.
4. Degenhardt F, Stehle F, Kayser O. The Biosynthesis of Cannabinoids. In: Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis, and Treatment. 2017. p. 13–23.
5. Hanuš LO, Meyer SM, Muñoz E, Taglialetela-Scafati O, Appendino G. Phytocannabinoids: a unified critical inventory. Nat Prod Rep [Internet]. 2016;33(12):1357–92.
6. ElSohly MA, Radwan MMM, Gul W, Chandra S, Galal A. Phytochemistry of Cannabis sativa L. In: Progress in the chemistry of organic natural products [Internet]. 2017. p. 1–36.
7. Muntendam R, Flemming T, Steup C, Kayser O. Chemistry and biological activity of Tetrahydrocannabinol and its derivatives. In: Topics in Heterocyclic Chemistry Bioactive Heterocycles IV. 2007. p. 1–42.
8. Citti C, Pacchetti B, Vandelli MA, Forni F, Cannazza G. Analysis of cannabinoids in commercial hemp seed oil and decarboxylation kinetics studies of cannabidiolic acid (CBDA). J Pharm Biomed Anal. 2018;149:532–40.
9. Muhammad T, Akhtar, Shaari K, Verpoorte R. Biotransformation of Cannabinoids. 10. Flores-Sanchez IJ. Polyketide synthases in Cannabis sativa L. Phytochemistry Reviews. 2008.
11. Onofri C, De Meijer EPM, Mandolino G. Sequence heterogeneity of cannabinergic- and tetrahydrocannabinolic acid-synthase in Cannabis sativa L. and its relationship with chemical phenotype. Phytochemistry. 2015.
12. Tuner S, Williams C, Iversen L, Whalley B. Molecular Pharmacology of Phytocannabinoids. Prog Chem Org Nat Prod. 2017;103:81–101.
13. Wood TB, Spiye WTN, Easterfi TH. Cannabinol. Part1. J Chem Soc Trans. 1898;75(20):20–36.
14. Uliss DB, Razdan RK, Dalzell HC, Handrick GR. Synthesis of racemic and optically active Δ1- and Δ6-3,4-cis-tetrahydrocannabinols. Tetrahedron. 1977.
15. Radwan MM, ElSohly MA, El-Alfy AT, Ahmed SA, Slade D, Husni AS, et al. Isolation and Pharmacological Evaluation of Minor Cannabinoids from High-Potency Cannabis sativa. J Nat Prod. 2015.
16. Carbone M, Castelluccio F, Daniele A, Sutton A, Ligresti A, Di Marzo V, et al. Chemical characterisation of oxidative degradation products of Δ9-THC. Tetrahedron. 2010.
17. Smith RN. High-pressure liquid chromatography of cannabis. Identification of separated constituents. J Chromatogr A. 1975;115(1):101–6.
18. Sirikantaramas S, Taura F, Morimoto S, Shoyama Y. Recent advances in Cannabis sativa research: biosynthetic studies and its potential in biotechnology. Curr Pharm Biotechnol [Internet]. 2007;8(4):237–43.
19. Turner CE, Elsohly MA, Boeren EG. Constituents of cannabis sativa L. XVII. a review of the natural constituents. J Nat Prod. 1980.
20. Kane V V. Structure of cannabicyclol, a detailed NMR study of a synthetic analog. Tetrahedron Lett. 1971.
21. de Zeeuw RA, Vree TB, Breimer DD, van Ginneken CAM. Cannabivarichromene, a new cannabinoid with a propyl side chain in cannabis. Experientia. 1973.
22. Braemer R, Paris M. Biotransformation of cannabinoids by a cell suspension culture of Cannabis sativa L. Plant Cell Rep. 1987;6(2):150–2.
23. Ikuo Yamamoto, Hiroshi Gohda, Shizuo Narimatsu, Kazuhito Watanabe, Hidetoshi Yoshimura. Cannabielsoin as a new metabolite of cannabidiol in mammals. Pharmacol Biochem Behav. 1991.
24. Yamamoto I, Gohda H, Narimatsu S, Yoshimura H. Identification of cannabielsoin, a new metabolite of cannabidiol formed by guinea-pig hepatic microsomal enzymes, and its pharmacological activity in mice. J Pharmacobiodyn. 2011.
25. Mahlberg PG, Kim E. Secretory Vesicle Formation in Glandular Trichomes of Cannabis sativa (Cannabaceae). Am J Bot. 1992;79(2):166–73.
26. Rodziewicz P, Loroch S, Marczak Ł, Sickmann A, Kayser O. Cannabinoid synthases and osmoprotective metabolites accumulate in the exudates of Cannabis sativa L. glandular trichomes. Plant Sci. 2019.

Acceptance for editing: 2019-07-09
Acceptance for publication: 2019-09-30