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A SYNOPSIS OF RECENT LAB FINDINGS

DELTA-8-THC AND ITS DERIVATIVES

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- The 2018 Farm Bill defined hemp and removed hemp from the definition of marijuana in the Controlled Substance Act (CSA). As a result, cannabis plants and derivatives containing no more than 0.3% delta-9-tetrahydrocannabinol (THC) on a dry weight basis no longer fall under the purview of the CSA.

- The language in the Farm Bill has been widely interpreted to allow commercialization of minor cannabinoids that are found in hemp including those that are made by chemically modifying cannabidiol (CBD) isolated from hemp.

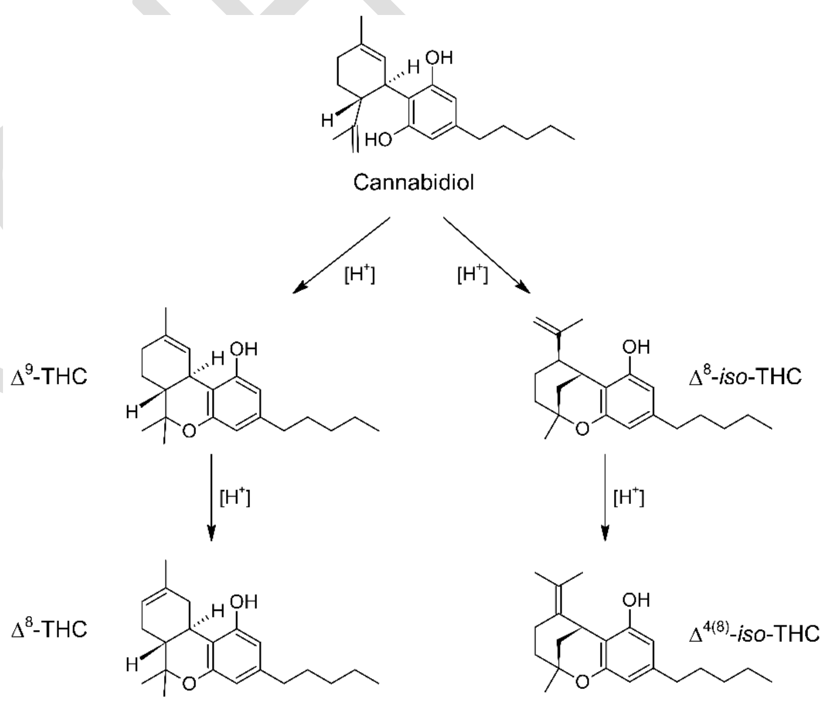
- The first evidence for this interpretation of the Farm Bill was the appearance of commercial products containing (-)-Δ^8-*trans*-THC, a positional isomer of (-)-Δ^9-*trans*-THC, the major psychoactive component of Cannabis, in late 2019.

- (-)-Δ^8-*trans*-THC is a psychoactive substance that binds to the same CB_1 receptor as (-)-Δ^9-*trans*-THC. The potency of (-)-Δ^8-*trans*-THC is less (approximately 2/3) than that of (-)-Δ^9-*trans*-THC.

- (-)-Δ^8-*trans*-THC is obtained from hemp by treating cannabidiol with catalytic amounts of a strong acid such as *p*-toluenesulphonic acid (pTSA) in a suitable solvent for a suitable period under a variety of different experimental conditions that affect the yield of Δ^8-THC as well as that of Δ^9-THC and several side-products that are not found in Cannabis.

- The synthesis of (-)-Δ^8-*trans*-THC proceeds from CBD through (-)-Δ^9-*trans*-THC to (-)-Δ^8-*trans*-THC. A parallel pathway proceeds from CBD to Δ^8-*iso*-THC to $\Delta^{4(8)}$-*iso*-THC as shown in Figure 1.

Figure 1. Acid-catalyzed intramolecular ring closure of cannabidiol to create (-)-Δ^8-trans-THC and various side-products including (-)-Δ^9-trans-THC.



- Some of the earliest Δ^8-THC samples submitted for analysis contained less than 70% of the labelled quantity of Δ^8-THC. These samples contained (-)-Δ^9-*trans*-THC plus other unidentified substances.

- The concentration of (-)-Δ^9-*trans*-THC in these samples was often greater than the 0.3% threshold established in the 2018 Farm Bill.

- Two of the previously unidentified substances are now known to be Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC (see Figure 1). Cayman Chemical released reference standards of both substances in 2022.

- Laboratory reports for Δ^8-THC based on high performance liquid chromatographic methods validated for determining cannabinoids in hemp and Cannabis are frequently incorrect because Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC are not resolved from Δ^8-THC and Δ^9-THC, respectively, under reversed-phase conditions. Consequently, estimates of the concentration of Δ^8-THC are inflated by interference from the Δ^8-*iso*-THC that co-elutes with Δ^8-THC under reversed-phase conditions. Note that the molecular formulas and UV absorption spectra of the *iso*-THCs, Δ^8-THC, and Δ^9-THC are identical, so the UV detector is not able to differentiate them. Furthermore, the concentration of Δ^9-THC is either ignored due to the slight retention time difference between it and $\Delta^{4(8)}$-*iso*-THC or it is overestimated when $\Delta^{4(8)}$-*iso*-THC is misidentified as Δ^9-THC.

- Based on these observations, we have concluded that reversed-phase HPLC methods with UV detection are not fit-for-purpose for determining Δ^8-THC that has been obtained by acid-catalyzed conversion of CBD. On the other hand, we have demonstrated that GC-MS is fit-for-purpose. The *iso*-THC side-products are completely resolved from Δ^8-THC and Δ^9-THC and the latter are completely resolved from each other chromatographically.

- Despite our concerns about the reliability of the testing methodology for Δ^8-THC, most consumer products containing it have been tested using methods that we would not use for this purpose. When chromatograms for these products obtained by these methods are included on certificates of analysis, experienced analysts can usually discern evidence for the side-products because Δ^8-*iso*-THC distorts the Δ^8-THC peak because these substances have slightly different retention times in reverse-phase systems .

- When we use fit-for-purpose methods such as GC-MS to determine Δ^8-THC, we usually find that samples contain less Δ^8-THC than indicated on the label, that Δ^9-THC is above the limit of 0.3%, and that the sample contains highly variable amounts of Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC as well as other unidentified side-products that are not naturally found in plant materials.

- Very little is known about the pharmacologic effects or toxicity of Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC particularly when inhaled except that they are not psychoactive. Could the side-products be responsible for recent reports of adverse events from Δ^8-THC products [1] be due to the presence of the side-products?

- A few contemporary samples of Δ^8-THC contain near the labelled amount of Δ^8-THC, less than 0.3% Δ^9-THC, and amounts of Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC near the lower limits of detection.

- Within several months of the commercial appearance of Δ^8-THC, products containing its acetate ester appeared with the claim that it is more potent than Δ^8-THC.

- Preliminary evidence for the presence of the acetate esters of Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC in these materials was obtained by GC-MS analysis of samples of Δ^8-THC acetate. They cannot be definitively identified or quantified because reference standards are not available. These substances are new chemical entities for which no pharmacologic effect or toxicity studies have been reported.

- More recently, Δ^8-THC obtained as described before from the acid-catalyzed intramolecular ring closure of CBD is being hydrogenated to produce hexahydrocannabinol HHC). Hydrogenation creates a new stereogenic center but does not affect the existing ones in Δ^8-THC so the resulting product is a mixture of the diastereomers (9R)-HHC and (9S)-HHC. These substances are known substances for which reference standards have existed since 2022.

- However, any Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC that is present in the Δ^8-THC used to make HHC is hydrogenated under the conditions used to prepare HHC, so they are present in commercial products. The identifications are preliminary because reference standards are not available and are based on our analysis of retention time and mass spectral data.

- Hydrogenated Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC were synthesized in the late 1960s but were not further characterized [2].

- Recent laboratory submissions have included acetates of HHC which also contain acetates of hydrogenated Δ^8-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC. These latter substances are new chemical substances for which no toxicological data exist. Identifications of these substances are preliminary based on analysis of retention time and mass spectral data because reference standards do not exist.

- More recent submissions include positional isomers of Δ^8-THC in which the double bond has been relocated as in (9R)-Δ^{10}-THC, (9S)-Δ^{10}-THC, and $\Delta^{6a,10a}$-THC. These substances are all known as minor cannabinoids but are being produced from CBD and therefore available for commercial use in large quantities. Reference standards of all of those listed are available so they can be identified and quantified but products typically contain multiple components including numerous unidentified substances.

- Other recent submissions include substances in which the alkyl side chains of Δ^8-THC, Δ^9-THC, and CBD have been shortened or elongated. Since psychoactivity increases from five carbons to seven carbons, homologues with six, seven, or eight carbon side chains are being marketed for their psychoactivity. Since the alkyl chain length cannot be altered directly, CBD homologues with different alkyl chains are being synthesized and then treated with catalytic amounts of acid to achieve ring closure to obtain the corresponding Δ^8-THC homologues. Analysis reveals that the reaction mixtures also contain the corresponding Δ^{8}-*iso*-THC and $\Delta^{4(8)}$-*iso*-THC homologues. In most cases the structures of the side products must be inferred from mass spectral data because they are new chemical entities for which no reference standards exist.

- Furthermore, these alkyl chain homologues are also being acetylated and hydrogenated. The reaction mixtures also contain the acetylated and/or hydrogenated *iso*-THC homologues. Nearly all of these substances are new chemical entities that have not been tested in any in vitro or in vivo test system to assess toxicological effects.

- One recent submission was identified as HHCP corresponding to the hydrogenated product of Δ^{8}-tetrahydrocannabiphorol or (-)-Δ^{8}-*trans*-THCP. Hydrogenation of THCP produces a mixture of (9R)-HHCP and (9S)-HHCP. References standards exist so they were identified and quantified in the product but they accounted for less then 5 % of the total mass. Most of the sample appeared to be $\Delta^{6a,10a}$-THCP with smaller amounts of (9R)-Δ^{10}-THCP, (9S)-Δ^{10}-THCP, exo-THCP, and hydrogenated Δ^{8}-*iso*-THCP and $\Delta^{4(8)}$-*iso*-THCP. Most of these substances are new chemical entities for which nothing is known about their pharmacology or toxicity particularly after inhalation which is a common route of administration of these substances.

- In conclusion, the 2018 Farm Bill includes a definition of hemp that has led to the proliferation of consumer products that contain substances previously identified as minor cannabinoids but which are now available in larger quantities. Hence, consumers are being exposed to much larger amounts of these substances than they were in the past. Furthermore, most of the materials contain side-products that are not present in hemp, and which have not been tested for pharmacologic effects or toxicity.

REFERENCES

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