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The Effect of Storage Conditions on Cannabinoid Stability

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Cannabinoids are chemical compounds found in the cannabis plant. There are more than 100 cannabinoids that have been isolated from cannabis.[1] Cannabidiol (CBD), tetrahydrocannabinol (THC) and cannabinol (CBN) are the most frequently studied species. Monitoring the cannabinoid composition of the extracts and purity of the isolated compounds is important to ensure the potency of prepared formulations as well as proper sample handling in the laboratory. Here we want to review the common routes of cannabinoid decomposition and highlight our observations for stock solution storage and preparation.

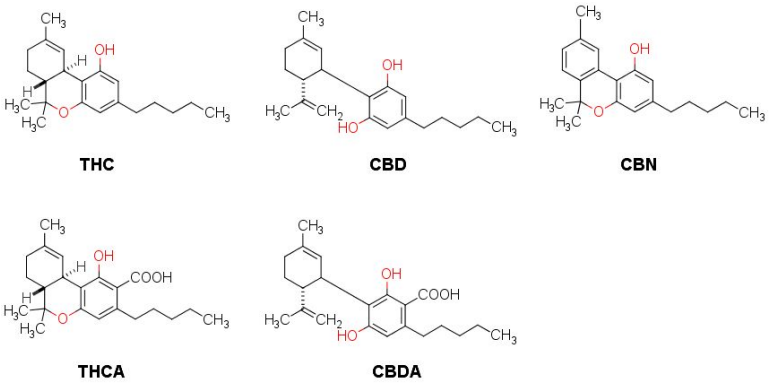

Figure 1: Cannabinoid Structures

The stability of different cannabinoids depends on the storage form and storage conditions.[2] In the living plant, the precursors of THC and CBD are found in their acidic forms, THCA and CBDA. Neutral (THC, CBD) and acidic (THCA, CBDA) forms have different stability towards temperature and light exposure. Neutral cannabinoids are stable in the darkness at room temperature up to two weeks. However, exposure to light can lead to a significant decrease in the THC and CBD content. Acidic forms of cannabinoids decarboxylate and turn into neutral forms in both daylight and darkness. According to several studies this is a temperature dependent process.[3,4]

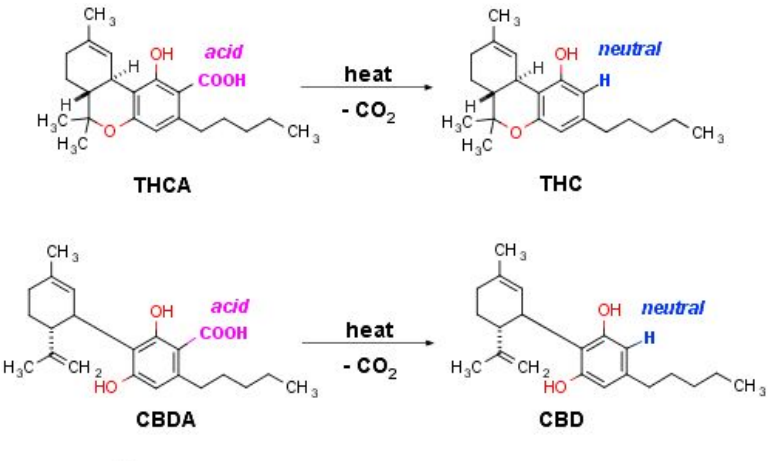

Figure 2: Decarboxylation of Cannabinoids

Different organic solvents can be used for the extraction and subsequent storage of cannabinoids. Solvents have been shown to have a significant effect on the compounds' stability. Alcohols and alcohol mixtures (methanol, ethanol, methanol:chloroform) yield more stable solutions of both neutral and acidic forms of THC and CBD.[3,5] Using chloroform is not advisable for the long-term storage of cannabinoids.[6]

In the presence of atmospheric oxygen, THC is oxidized to CBN.[7] The proposed mechanism of this conversion is shown in the scheme below.

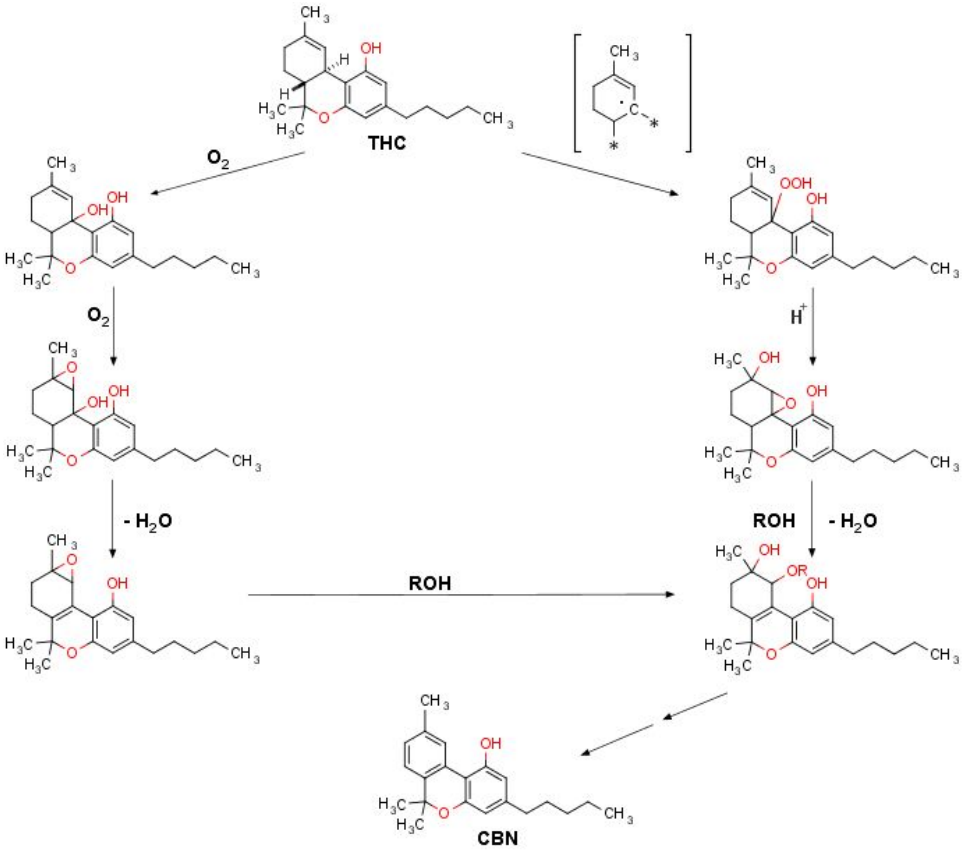

Figure 3: Oxidative Decomposition of THC

Cannabidiol also undergoes chemical transformations when the pH is changed. Under acidic conditions it may transform into Δ9–THC by acid-catalyzed cyclization[8] and, under basic conditions in the presence of oxygen, it is oxidized to monomeric and dimeric hydroxyquinones.[9] A characteristic sign of such transformation is a colour change of the solution where a purple colour is produced due to the accumulation of the anions of hydroxyquinone 1 and its dimer 2.

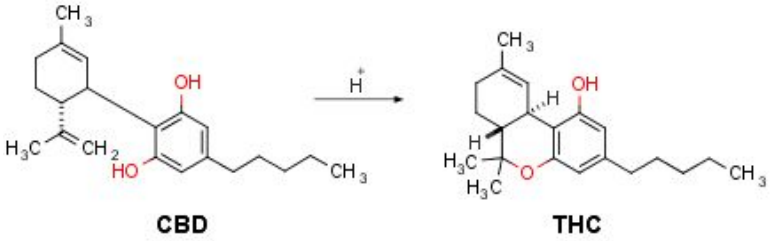

Figure 4: CBD Decomposition in Acidic Conditions

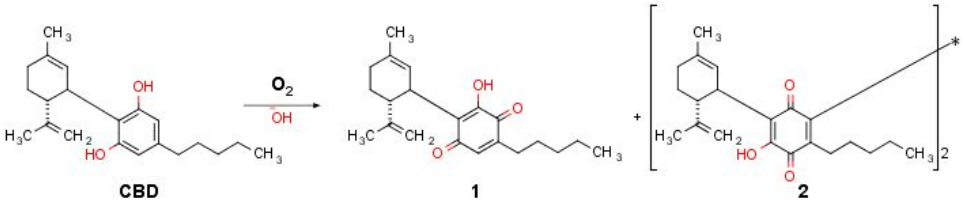

Figure 5: CBD Decomposition in Basic Conditions

The common practice in our laboratory is to prepare concentrated stock solutions of cannabinoids in organic solvents for use in nanoparticle formulations. Such stock solutions have cannabinoid concentration of 30 – 50 mg/mL and are typically stored for 2 – 4 weeks. Based on the existing data on cannabinoid solution stability and nanoparticle formulation requirements, we have chosen ethanol to prepare stocks. Ethanol provides stable cannabinoid solutions and is compatible with our microfluidic nanoparticle formulation equipment (NanoAssemblr Benchtop).

In order to confirm stability of the stock, we have prepared a solution of THC distillate in ethanol (50 mg/mL) and analyzed cannabinoid content of the sample after preparation and after 2 months storage at various conditions. Samples were stored at room temperature in amber and clear vials, at 4 °C and at -20 °C. Cannabinoid content has been determined by HPLC.

Materials and Methods

Extract from biomass of a high Δ^9-THC chemotype of *C. sativa* has been used for the experiments. Anhydrous ethanol, HPLC-grade acetonitrile and water were purchased from Sigma Aldrich.

Sample Preparation

THC distillate was dissolved in anhydrous ethanol (50 mg/mL). Three 1 mL aliquots were placed in 10 mL clear falcon tubes and stored at room temperature, 4 °C and at -20 °C, respectively. One 1 mL aliquot was placed in a 10 mL amber falcon tube and stored at room temperature. Before analyses, 10 µL of sample were placed into an HPLC vial and diluted with 490 µL HPLC-grade acetonitrile. After this, samples were analyzed for major cannabinoid content (Δ^9-THC, CBD and CBN).

HPLC Protocol

HPLC analyses were carried out on an Agilent 1260 Infiniti II HPLC chromatograph. The HPLC system consisted of a quaternary pump system (Agilent, 1260 VL), autosampler (Agilent, 1260 TCC) and UV/VIS with diode array detector (Agilent, 1260 DAD VL). Chromatography was achieved on a 4.6 mm × 100 mm, 4 µm EC-C18 Poroshell 120 column at oven temperature 55 °C. The HPLC operated with constant flow at 0.3 mL/min mobile phase (gradient acetonitrile – water). The absorbance of all three compounds of interest (Δ^9-THC, CBD and CBN) was monitored at 214 nm. The identification of peaks was performed by comparing the retention times and UV absorption spectra of the samples with those of the standard solutions.

Results

The initial content of major cannabinoids in the THC resin sample

Cannabinoid	Concentration, ppm
Δ^9-THC	62.14
CBD	3.42
CBN	2.63

The content of major cannabinoids in the THC resin sample after 2 months

Cannabinoid	Concentration, ppm			
	Light, rt	*Darkness, rt*	*Darkness, 4 °C*	*Darkness, -20 °C*
Δ^9-THC	55.57	57.72	59.10	61.27
CBD	0.96	1.06	1.94	1.95
CBN	3.92	3.85	2.98	2.93

The experimental results indicate a small but clear difference between cannabinoid content of the samples depending on storage conditions. Table 1 shows the initial content of the major cannabinoids in the freshly prepared solution of the THC distillate in ethanol. Cannabinoid content in the samples stored at the different conditions for 2 months are summarized in Table 2. The Δ^9-THC content decreases during storage and is higher in the samples stored in the darkness at lower temperatures (4 °C and -20 °C) than in the sample stored at room temperature exposed to light. The same trend can be also observed for CBD content. The CBN content increases during storage period and is higher in the sample stored at room temperature exposed to light than in the samples stored in darkness and at lower temperatures. However, differences between samples stored at 4 °C and -20 °C in the darkness are minimal.

The results of this experiment indicate that ethanolic stocks of cannabinoids can be safely stored at 4 °C in the laboratory fridge for the required period of time (2 – 4 weeks).

References

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