Historical Perspective on Early Cannabinoid Isolation

Prolific inventor and chemist offers a historical perspective on the isolation of cannabinoids.

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Fifty-six years ago, Professor Raphael Mechoulam published a landmark paper on the isolation and structural elucidation of delta-9-tetrahydrocannabinol (THC).[1] The paper described the isolation of THC from hashish using column chromatography followed by the preparation of a crystalline 3,5-dinitrophenyl urethane derivative in order to crystallize it to purity. The pure crystalline derivative was hydrolyzed to afford THC, which was distilled at a temperature of 155 to 157°C at a vacuum of 0.05 mm Hg and characterized by nuclear magnetic

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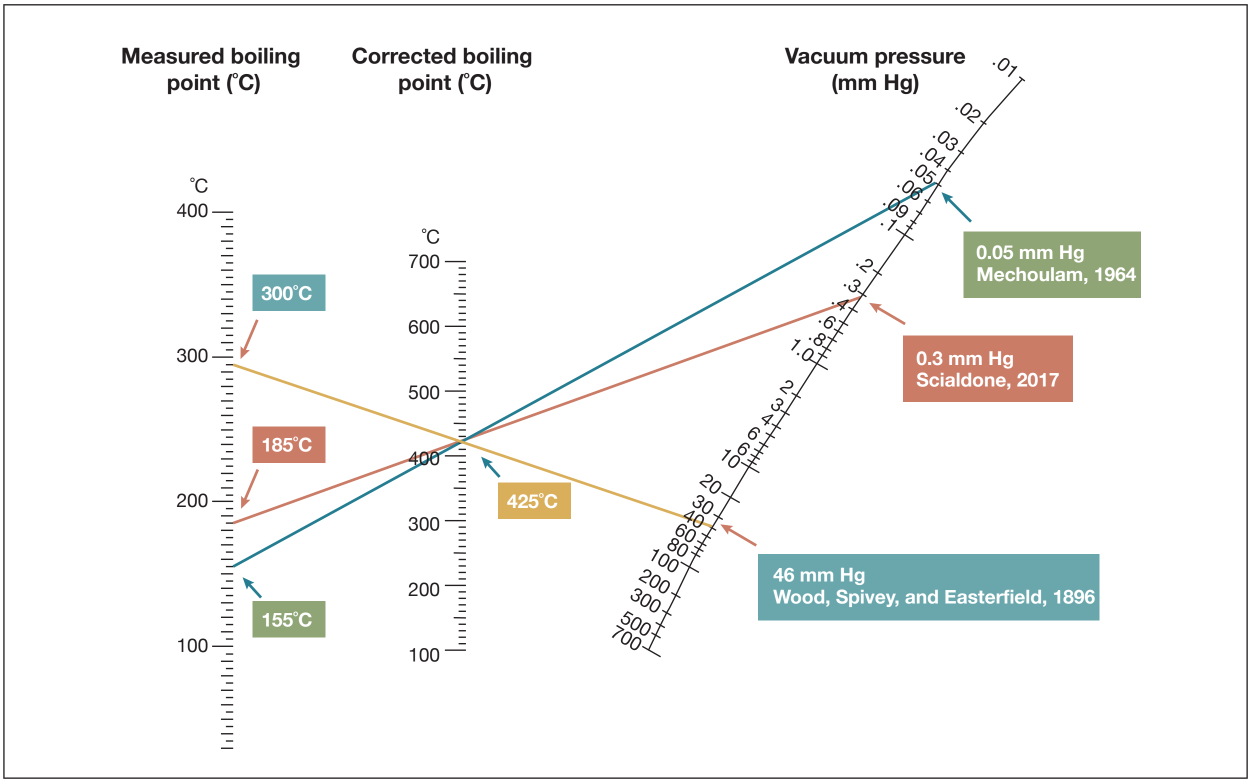

FIGURE 1. Pressure-temperature nomograph of the boiling point of THC.

THC, delta-9-tetrahydrocannabinol.

Image courtesy of Mark Scialdone, PhD.

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TABLE. Boiling Points at Atmospheric Pressure of THC-Containing Distillates[1,3–5]

Source/Year	Measured boiling point (°C)	Vacuum pressure (mm Hg)	Nomograph corrected boiling point at 760 mm Hg (°C)
Mechoulam and Gaoni/1964	157	0.05	428.6
Wood et al, fraction I/1896	265	20	410.6
Wood et al, fraction II/1896	300	46	421.0
Wood et al, fraction III/1896	315	100	404.0
Cahn fraction I/1931	268	25	405.6
Cahn fraction II/1931	285	25	425.7
Scialdone/2016	185	0.3	427.8

Adapted from references 1, 3–5 and unpublished results from Dr. Scialdone.

resonance spectroscopy. Given the mild conditions, isomerization of the cyclohexene double bond did not occur during isolation, keeping the naturally occurring delta-9 configuration intact.

Before Mechoulam's work, researchers across the globe had been engaged in exploring the active components of cannabis for more than 50 years.[2] This included the pioneering work of Sir Thomas Hill Easterfield and his associates Thomas Barlow Wood and WT Newton Spivey, who published their seminal paper in 1896.[3] The authors described fractionation of extracts of Charas, the resinous exudate of buds from the unfertilized female *Cannabis indica* plant by vacuum distillation.

The scientists isolated lower boiling monoterpene and higher boiling sesquiterpene fractions, a waxy, paraffinic fraction, and a higher boiling fraction that they termed toxic red oil, which was obtained in about 30% overall yield from the starting material. On redistillation, it boiled constantly at 265°C (20 mm Hg), 300°C (46 mm Hg), and 315°C (100 mm Hg). Wood et al. found the substance to have 79.15% carbon and 9.06% hydrogen by combustion analysis and assigned the formula of $C_{18}H_{24}O_2$. They confirmed that the physiologic action of Indian hemp is due to the presence of the red oil and compared some effects against cannabis preparations manufactured by Merck. They concluded that the compound possessed a hydroxyl group and named the compound cannabinol (CBN). Three years later, they reported that the red oil consisted of 2 compounds, one having the formula of $C_{21}H_{26}O_2$, which they proposed should keep the name cannabinol.[4] The authors state:

> *"Crude cannabinol" is, therefore, a mixture of cannabinol, $C_{21}H_{26}O_2$ with one or more compounds probably of lower molecular weight."*

The question remains, was this compound that they isolated in the 1890s CBN as they thought or was it THC, the principle psychoactive cannabinoid present in cannabis?

There's no chemical step in their isolation procedure that would cause the complete aromatization of the cyclohexene ring to form CBN from THC. It was likely a mixture that contained mostly THC isomers as evident by the observed boiling point range of the red oil fractions compared with Mecholuam's measured boiling point on the pure delta-9 THC sample. In using combustion analysis, there could have been adventitious oxidation of the THC to CBN in their combustion analysis method which complicated distinguishing between the 2 compounds by such a method. For reference, THC is 80.13% carbon and 9.62% hydrogen, whereas CBN is 81.18% carbon and 8.44% hydrogen.

The boiling point of a liquid is the temperature at which the vapor pressure of the liquid is equal to the external pressure such that the liquid undergoes a phase transition from liquid to vapor. Boiling points of pure compounds are a unique indication of compound purity but measured boiling points can change during the course of a distillation of a changing composition in the boiling flask. The pressure-temperature nomograph (Figure 1) used to estimate the boiling point of a liquid at atmospheric pressure (760 mm Hg) shows the boiling point of pure THC to be about 429°C atmospheric pressure, which corresponds within experimental error to the fraction II distilled by Wood, Spivey and Easterfield more than century ago. Regarding the distillate, they noted:

> *"When the oil was cooled below 60°, it set to a sticky, semi-solid, odourless mass, insoluble in water, but dissolving easily in alcohol, ether, benzene, glacial acetic acid, and organic solvents generally."*

FIGURE 2. THC distillate from Dr. Scialdone's laboratory.

THC, delta-9-tetrahydrocannabinol. Image courtesy of Mark Scialdone, PhD.

This characterization sounds pretty typical for THC-enriched distillate (Figure 2).

The reported boiling point of CBN is 185°C at a vacuum of 0.05 mm Hg, which corresponds to an estimated nomograph corrected boiling point of 470.6°C.

The measured boiling points suggest that a major component of the red oil isolated from Charas made from Indian hemp described by Wood, Spivey, and Easterfield was indeed THC, making them the first to report its isolation.

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