1H-NMR Study of Unheated and Heated Marijuana and Hashish Extracts from Cannabis Seizures in Egypt

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Abstract

Objective: Is to detect the presence of delta-9-tertrahydrocannabinol (THC) and other cannabinoids in samples of cannabis seized in Egypt with the use of nuclear magnetic resonance (1H-NMR). **Methods:** Extracts of cannabis seizures were prepared from marijuana (the dried flowering tops and leaves) or from hashish (the compressed plant resin). Cannabis extraction was carried out on unheated or heated samples using either chloroform or methanol. 1H-NMR spectrum for cannabis extract was determined in $CDCl_3$ with a JEOL ECA-500. **Results:** 1H-NMR indicate that the extracts of the cannabis samples contains THC as the major constituent. The 1H-NMR of heated cannabis extracts showed only THC while the 1H-NMR spectra of unheated extracts showed the presence of THC as a major compound beside minor fraction of cannabinol (CBN), both are decraboxylated. **Conclusion:** The 1H-NMR spectra indicate the presence of THC as the major compound.

1. Introduction

Cannabis is still the most widely abused substance in the world and according to a recent World drug report, there are about 182.5 million consumer world-wide [1]. Cannabis is the term used to describe the material obtained from the female plant of *Cannabis sativa* (*Cannabidaceae*). and these are mainly marijuana from the plant leaves and flowers, and hashish which is the compressed resin and flowers. Cannabis is usually smoked in cigarettes or but can be eaten or prepared as cannabis tea [2, 3]. Users of recreational are aware of the psychotropic effects of cannabis such as mild euphoria or "high", intense sensory phenomena [2,4]. Cannabis, however, is not an innocent plant and impairment of memory, cognition [5], thinking [6], academic functioning [7], social relationships [8] and the propensity for developing psychosis [9] are among the spectrum of its health hazards. The chemistry of cannabis is complex with over 600 chemical compounds. The most important constituents are a group of C21 terpenophenolic compounds known as cannabinoids and these are produced only in the *Cannabis sativa* plant [10]. About 120 of these cannabinoids have been identified till now [11, 12] and among them is Δ^9- Tetrahydrocannabinol (THC) is the principal and most abundant one [10,13] and the compound responsible for the psychotropic effects of the *Cannabis sativa* plant [14]. Other important cannabinoids are cannabinol (CBN), cannabidiol (CBD) (CBN) and Δ9- tetrahydrocannabivarin (THCV). These are found in much lower concentrations [10] and exert pharmacological effects distinct from effects from those of THC [15].

Cannabinoids are produced in the form of carboxylic acids in the *Cannabis sativa* plant. Therefore in fresh plant samples, it is expected that there are acidic but not neutral cannabinoids [10]. When the plant material is exposed to heat, light or after prolonged storage, there is decarboxylation i.e., loss of CO_2 from the carboxylic group with the resultant formation of neutral cannabinoids [3]. Thus, in the fresh plant THC is present in its acidic form THC-acid (THCA) to be converted by heat eg., smoking to its psychotropic active form THC. Consequently, analytical methods aim mainly to detect the presence of THC in seized samples of cannabis [13]. Several analytical techniques eg., gas chromatography/mass spectrometry [13] and liquid chromatography coupled with mass spectrometry [16] and high-performance liquid chromatography [17] have been used for the direct analysis of the plant metabolites. Recently, there has been renewed interest in

the use of nuclear magnetic resonance (^{1}H-NMR) for detecting THC and other cannabinoids in the cannabis plant. These studies indicated the usefulness of ^{1}H-NMR being an accurate, highly reproducible and a time saving analytical tool [18-20].

2. Material and Methods

2.1. Cannabis seizures and chemicals

Cannabis seizures were officially obtained from the Ministry of Justice, Egypt. The seizures were marijuana (flowering tops and leaves) and hashish (compressed resin).

2.2. Extraction of cannabis

A weighed amount (10 g) of each sample of marijuana or hashish was divided into portions and well-grounded in a mortar. Each sample was then divided into two parts; one was heated in a glass beaker in a boiling water bath at 100 ℃ for two hours to decarboxylate all its cannabinolic acids content [21] and the other part was directly extracted without being heated. All samples were extracted either with chloroform or methanol overnight and filtered. The filtrates were then evaporated under a gentle stream of nitrogen to yield 2 g of dry extract and were protected from light and heat by placing it in an aluminum-covered container and stored at 4°C [22]. We in addition examined the presence of THC in an old sample of marijuana dating more than 10 years ago.

2.3. [1]H-Nuclear magnetic resonance (^{1}H-NMR)

^{1}H-NMR spectrum for cannabis extracts was determined in $CDCl_3$ with a JEOL ECA-500 (Japan), run for ^{1}H-NMR at 500 MHz. The chemical shifts were expressed as δ-values against TMS as internal standard.

3. Results

The major cannabinoids that could be encountered in the *Cannabis sativa* plant are Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinol-carboxylic acid (THC-COOH), cannabinol (CBN), and cannabidiol (CBD) (Fig. 1).
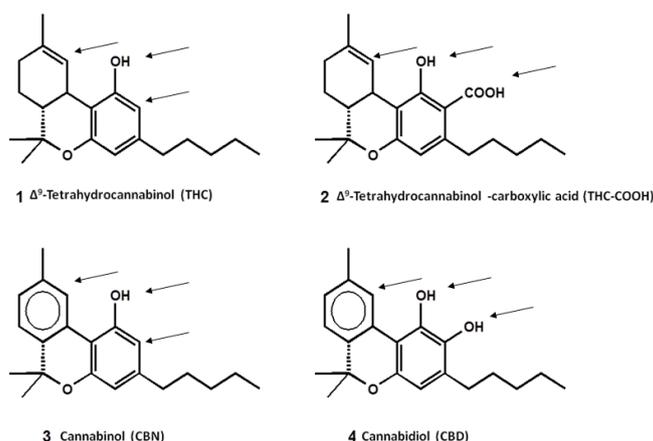

Fig. (1): Structures of major cannabinoids. The specific target protons are illustrated by arrows.

^{1}H-NMR detected THC in the unheated or heated preparations whether hashish (resin) or marijuana. In this study, the decarboxylation was carried out by heat in water bath at 100 °C for 2h (Fig. 2).
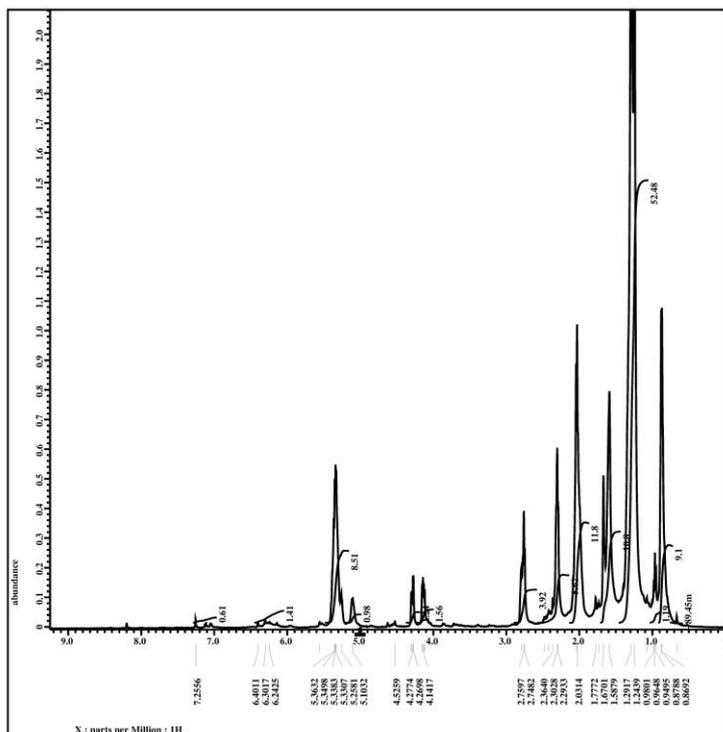
Fig. 2. decarboxylation of THCA by heat.

The ^{1}H-NMR spectra of extracts obtained from the cannabis material after extraction in chloroform are shown in Fig. 3. Both methanolic and cholorform extraction was efficient and the latter showed more clear signals. ^{1}H-NMR of heated cannabis extracts showed no signal corresponding to the carboxylic acid (COOH) around 12 ppm. The ^{1}H-NMR data of heated extracts thus indicates that heating of the extract converted all the acidic cannabinoids to neutral cannabinoids.There are traces of the THC-COOH in ^{1}HNMR displayed as integration, not as a peak with very low concentration in the unheated extracts.

On the other hand, ^{1}H-NMR of old marijuana extract indicated absence of THC (data not shown).

Fig. 3A. ^{1}H-NMR charts of extracted unheated marijuana dissolved in deuderated chloroform.

Fig. 3B. ^{1}H-NMR charts of extracted heated marijuana dissolved in deuderated chloroform.

4. Discussion

In this study, we used ^{1}H-NMR for the detection of THC, the major psychotropic constituent in illicit cannabis. The examination was carried out on both heated and unheated extracts from either leaves or hashish (resin). Either chloroform or methanolic extraction was used. The presence of THC, THC-carboxylic acid, CNB and CBD in the *Cannabis sativa* plant can be identified by ^{1}H-NMR [18-20]. In this study, the ^{1}H-NMR data indicates the following: (i) THC is present; (ii) THC-COOH is not present since the ^{1}H-NMR chart does not have the signal of the acid group around 12 ppm, which is in agreement with the fact shown in the equation in figure (2) i.e., decarboxylation of acidic THC occurred. The ^{1}H-NMR data also indicate the absence of CBD in the samples. This is because the ^{1}H-NMR proved the existence of one hydroxylic group at 8.20 ppm and CBD contains two hydroxyl groups. Our results also indicate the absence of CNB in these heated samples as the[1]H-NMR chart does not have an aromatic protons corresponding to the full aromatic ring of the compound. To the contrary, in heated samples, we identified only three peaks corresponding to the double bond of THC which proves the existence of THC in neutral form only (see figure 1 to review the change in structure between each of the cannabinoids). The three saturated protons of cyclohexane displayed around 4.5 ppm are in accordance with the structure of THC and THC-COOH and excludes the probability of CBN and CBD. Collectively, the above data indicate that THC-non-carboxylic is the major cannabinoid structure present in the samples. ^{1}H-NMR detects the major compound in an extract [23]. Since the ^{1}H-NMR detects grade 10^{-3} g [24, 25], this means that other cannabinoids could be present in the extracts in amounts smaller than that. Unheated extracts showed the presence of a minor fraction of CBN as the ^{1}H-NMR displayed aromatic protons in the region 6-7.5 ppm as minor signals.

Cannabinoids are enzymatically biosynthesised in the plant as their corresponding carboxylic acid forms [10]. Neutral cannabinoids are formed via decarboxylation (loss of CO_2) of the acidic cannabinoids during exposure to light, heat (e.g. smoking), or as a result of prolonged storage [16, 26] (see Fig. 2). In this study, the charts obtained from ^{1}H-NMR for the unheated and heated hashish or leaves extracts revealed the same structure as a major compound, that is THC as illustrated in figure 1. It was expected that the heated cannabis samples

contain no signal for carboxylic acid and this meets our findings. It was also expected that unheated extracts contain the carboxylic proton around 12 ppm. Surprisingly, the ^{1}H-NMR does not show that. Our explanation is the occurrence of decarboxylation in the process of hashish production and/or prolonged storage since these were obviously not fresh samples.

The ^{1}H-NMR thus show that; (i) unheated extracts contain both THC (as the major compound) and CBN (as minor product) which is in agreement with the literature since CBN is a THC storage degradation product [27] and (ii) heated extracts show only THC. From the above data, we conclude that heating of the cannabis material not only converts the carboxylic form of cannabinoids to the neutral one, but also destroys CNB.

On the other hand, performing ^{1}H-NMR on the old marijuana extract indicated the presence of most of the same moiety except that the phenolic OH group was lost (by time) and was replaced with an H atom. This finding was inferred from: (1) The absence of the phenolic OH group signal; (2) The single signal of the H atom in position (6), which was displayed in the charts of the recent resin or leaves, was replaced with a doublet peak, indicating that it is no more an isolated H atom and ensures the presence of a neighboring H atom that replaced the OH group. Moreover, the phenolic H atom displayed at in the charts of the recent resin or leaves was absent and another doublet arose, indicating the replacement of the phenolic group with a sp2 proton attached to aromatic ring and having the same J value of the other doublet mentioned above.

In summary, the present study indicate that THC can be detected in the *Cannabis sativa* plant material using ^{1}H-NMR. Our results indicate that THC was the major cannabinoid structure in the samples examined.

Conflicts of interests

The authors declare that there are no conflicts of interest

Acknowledgements

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