Characterization of Hexahydrocannabinol (HHC) Diastereomers, and Hexahydrocannabidiol (H_4CBD) Diastereomers Using NMR, HPLC, and GC-MS

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Short Report

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Abstract

The characterization of any compound is important in the field of chemistry, as the field of cannabinoid chemistry grows so does the need for the characterization of new cannabinoids or rare cannabinoids that gain popularity within the consumer and research fields. Hexahydrocannabinol (HHC) a hydrogenated analogue of Δ9-THC, also found in trace amounts naturally within the *cannabis sativa* plant, has been gaining attention and popularity within the cannabis industry. Hexahydrocannabidiol (H_4CBD) is a synthetic hydrogenated analogue to Cannabidiol (CBD) used in conjunction with or substitute for CBD. Identifying the Diastereomers of the cannabinoids with instrumentation plays a huge role within the chemistry field adding valuable information of the structure and the parameters for others to identify such cannabinoids. Elucidation and characterization of HHC and H_4CBD was performed using current analytical techniques such as NMR, HPLC, and GC-MS, effectively characterizing both the Diastereomers of HHC and H_4CBD.

Introduction

Hexahydrocannabinol (HHC) is a naturally occurring cannabinoid found in trace amounts within the cannabis sativa plant[1]. It is a structurally similar to the Δ9-THC compound, except HHC is lacking the double bond within the cyclohexyl ring, making it a hydrogenated analogue to Δ9-THC. Since the cannabinoid is found in trace amounts the cannabinoid is synthesized within facilities to mass produce the cannabinoid for research and consumer purposes. Hexahydrocannabidiol (H_4CBD) is a Hydrogenated analogue of CBD[2]. The cannabinoid has several safety studies providing evidence on the safety of the cannabinoid[3,4]. As the field of cannabinoid chemistry grows, the need for characterization of cannabinoids and constituents of the extracts are pertinent to the fields of chemistry. Providing information on the parameters used as well as the structure allows for others to identify such cannabinoids within extract mixtures, or for purification measures, including identification of product during purification methods.

Results

Presented below are the various spectra listed as Fig. 1(a-d). In Fig. 1a below, the MS spectra of H_4CBD is shown with the diastereomers separated clearly.

The Fig. 1b as shown below depicts the diastereomers of HHC with the Left peak being the S-isomer and the right peak being the R-isomer.

Listed below in Fig. 1c and Fig. 1d, is the Mass Spectra of the 9R-HHC isomer and 9S-HHC isomer respectively.

In Fig. 2a below is the compounds while Fig. 2b and 2c below are the Mass spectrometry data for the H4CBD isomers R and S respectively.

Below in Fig. 2c and 2d are the pertinent NMR spectra all other in-depth spectra are in the supporting information. The conformation analysis was configured through the various forms of NMR techniques that allowed for specific confromation analysis to be pieced together.

HPLC of Fig. 3a as shown below for elucidation of HHC.

Below in Fig. 3b and Fig. 3c is the conformation analysis of H4CBD of the R/S isomers.

HPLC of the R/S-H4CBD isomers are observed within the Fig. 3d below.

Discussion

The elucidation of the isomers R and S of HHC and H4CBD were presented through the analysis of GC-MS, NMR, and Mass spectrometry, to identify the fragments and to identify the parameters of the spectra of the given compounds. The R and S isomers can be easily identified with the help of NOESY and COSY spectra techniques which might not be easily accessible, with the utility of proton NMR the shifts of the given isomers change slightly and can be deduced from the given changes of which isomer the given compounds are. Through the mass spectrometry, the identification of the isomers are not clearly shown since similar fragmentation patterns occur. HPLC using RP-C_{18} column with an isocratic mobile phase, the separation of the isomer is possible showing distinct R/S peaks of HHC and H4CBD.

Conclusion

The isolation and elucidation of H_4CBD and HHC R/S isomers can be deduced definitively through proton and carbon NMR and various 2D NMR techniques, when the 2D NMR techniques are lacking, the use of ^{13}C and ^{1}H NMR techniques can help provide insight into the deduction of the isomers. The other instrumental techniques used help provide an in-depth understanding of how the isomers look under various conditions.

Methods And Materials

HHC and H_4CBD were dissolved in acetonitrile-d3 and ^{1}H, ^{13}C, COSY, HSQC, HMBC and NOESY data were acquired on a 500MHz Bruker AVANCE II system at 25*C. ^{1}H, ^{13}C, COSY, HSQC, HMBC data sets were analyzed to yield complete ^{1}H and ^{13}C peak assignment. Once the peaks were assigned, NOESY data were analyzed to yield stereochemistry information about the orientation of the hexanyl-methyl group. Instruments used were the Shimadzu TQ8050 NX Mass Spectrometer, Shimadzu Nexis GC-2030 Gas Chromatograph, Shimadzu AOC-20i Plus Autosampler, the method is custom developed GC-MS/MS method with KCA Labs SOP number TP-402.1. Agilent 1100 series HPLC with Diode Array Detector was used to determine and separate isomers using C_{18}-RP column.

Declarations

Supplementary Information is available for this paper.

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Author Contributions

GAR, and WC wrote the manuscript. GAR, ACC, TTT carried out the experiments.

Competing interests

All authors declare that they have no conflicts of interest.

References

1. A) Hanuš LO, Meyer SM, Muñoz E, et al. Phytocannabinoids: A Unified Critical Inventory. Nat Prod Rep 2016;33(12):1357–1392; doi: 10.1039/c6np00074f. B) Bases-Jamandreu J, Xavier C. de Las Heras F, GC-MS Metabolite Profile and Identification of Unusual Homologous Cannabinoids in High Potency Cannabis sativa, Planta Med, 2020;86(5):338-347; doi: 10.1055/a-1110-1045
2. A) Jacob A, Todd AR, Cannabis Indica Part II. Isolation of Cannabidiol from Egyptian Hashish. Observations on the Structure of Cannabinol. J Chem Soc 1940; 649-653; doi: 10.1039/JR9400000649 B) Ben-Shabat S, Hanuš LO, Katzavian G, Gallily R, New Cannabidiol Derivatives: Synthesis, Binding to Cannabinoid Receptor, and Evaluation of their Antiinflammatory Activity, J Med Chem 2006; 49; 1113-1117; doi: 10.1021/jm050709m C) Lago-Fernandez A, Redondo V, Hernandez-Folgado L, Figuerola-Asencio L, Jagerovic N, New Methods for the Synthesis of Cannabidiol Derivatives. Methods in Enzymology (Reggio PH eds) Elsevier 2017 pp 237- 257
3. Collins A, Tesfatsion T, Ramirez G, Ray K, Cruces W, *Cannabis Science and Technology®* Vol. **5**(7), 23-27 (2022).
4. Tesfatsion T, Ramirez G, Collins A, et al. Evaluation of in Vitro Cytotoxic, Cardiac Safety and Genotoxic Effects of Hydrogenated Cannabidiol (H_4CBD) on Cells. Preprint: doi.org/10.21203/rs.3.rs-2256889/v1

Figures

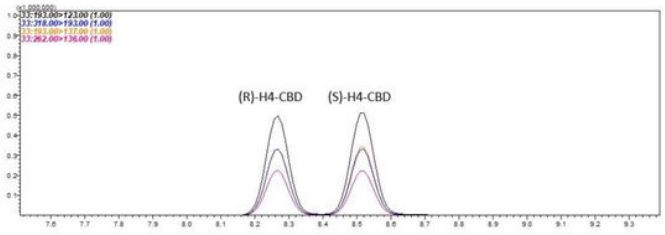
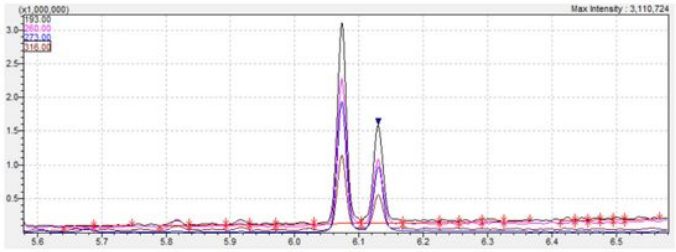


Figure 1a.



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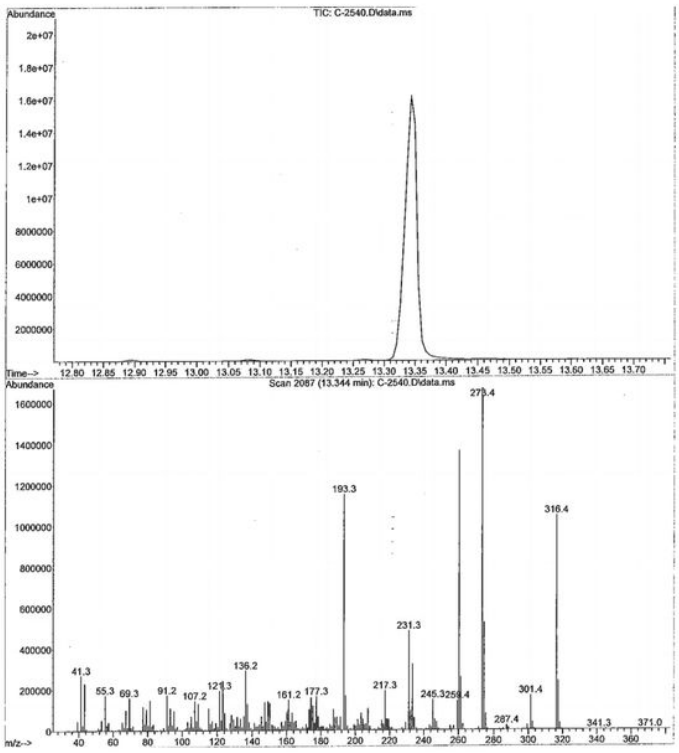

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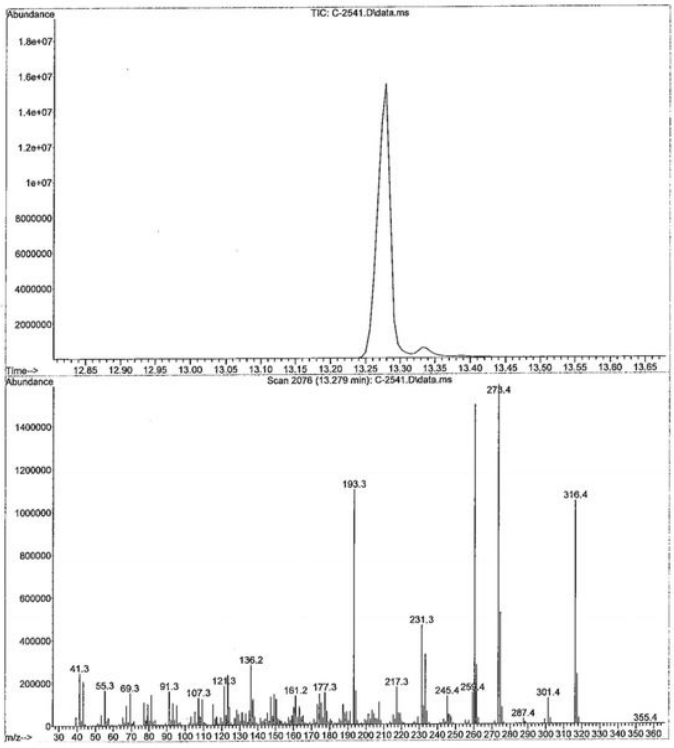


Figure 1d.

Figure 1

1a. The GC-MS of the diastereomers of H4CBD

1b. The GC-MS of the Diastereomers of HHC (S-isomer on the Right and the R-isomer on the left)

1c. The mass spectra of 9R-HHC isomer

1d. The mass spectra of 9S-HHC isomer

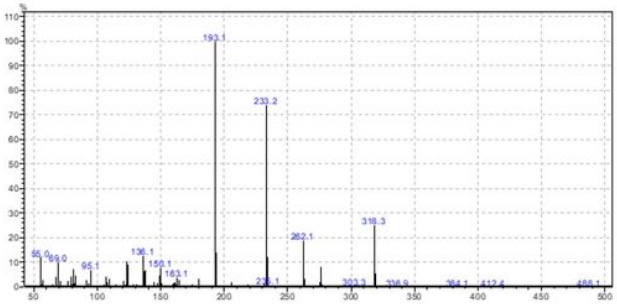
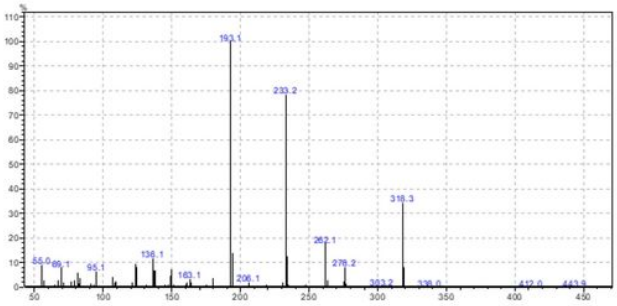


Figure 2a.



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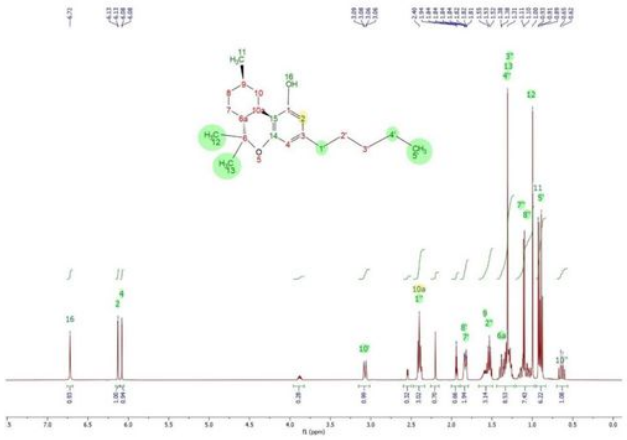

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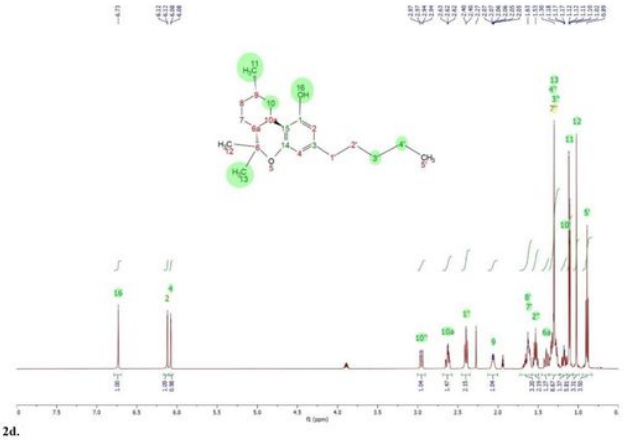


Figure 2d.

Figure 2

2a. The mass spectra of R-H4CBD isomer

2b. The mass spectra of S-H4CBD isomer

2c. NMR of HHC R-isomer

2d. NMR HHC S-isomer

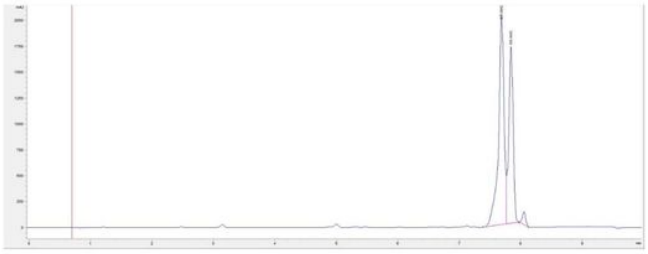
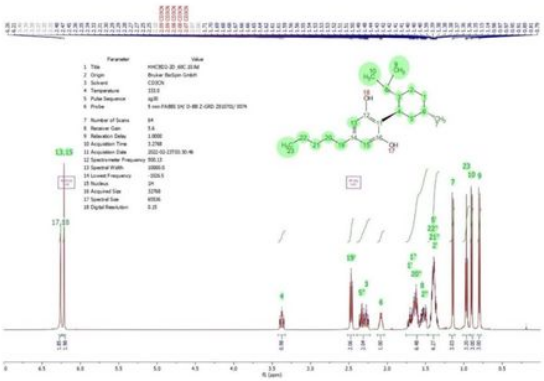


Figure 3a.



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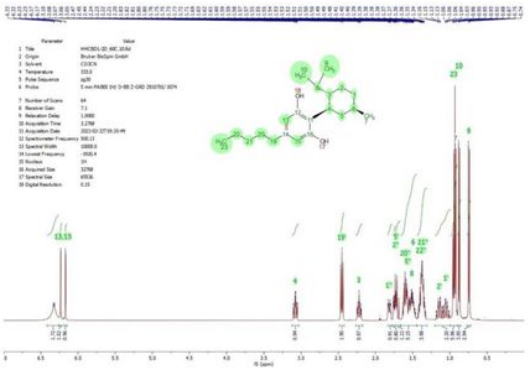

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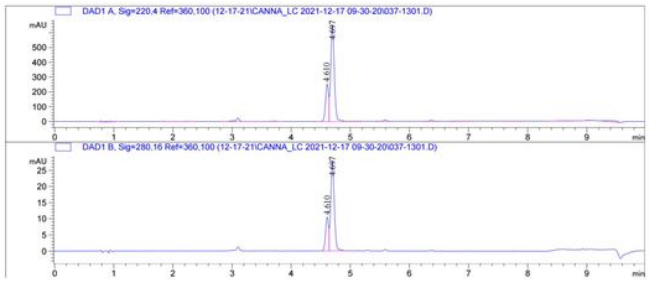

Figure 3d.

Figure 3

3a. HPLC of the R/S-isomer of HHC

3b. The conformation analysis of S-H4CBD isomer

3c. The conformation analysis of R-H4CBD isomer

3d. The R-isomer the left peak and S-isomer on the right peak of H4CBD

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