Characterization of Hexahydrocannabinol Diastereomers by NMR, HPLC, GC-MS, and TLC

Alicyn I. Stothard, Nathan K. Layle, Michael J. Martin, Jianmei Liu, Jonathon R. Bassman, Jeffrey B. Williams, and Kirk W. Hering

Cayman Chemical Company, Forensic Chemistry Division, Ann Arbor, MI

KEY FINDING

The identity of two hexahydrocannabinol (HHC) diastereomers is described based on analytical characterization by NMR.

INTRODUCTION

Hydrogenation of tetrahydrocannabinols (Δ^8- or Δ^9-THC) leads to formation of a mixture of hexahydrocannabinols (HHCs) comprised of the 9(*S*)- and 9(*R*)-HHC diastereomers. These compounds retain some psychoactivity but avoid classification as THCs as well as any THC-related regulations. Separation of the HHC diastereomers is possible by TLC, HPLC, and GC-MS, but identity cannot be inferred without verified reference standards. Full NMR characterization of the two HHC diastereomers is necessary for confirmation and has not previously been described.

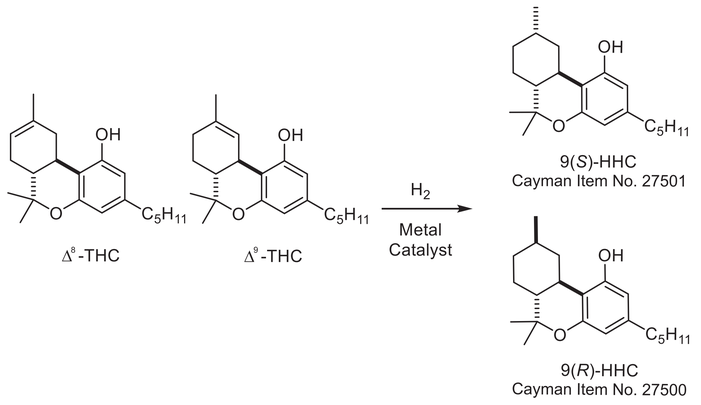


FIGURE 1 – Hydrogenation of either Δ^8- or Δ^9-THC provides two diastereomers 9(*S*)- and 9(*R*)-HHC

STEREOCHEMICAL RELATIONSHIP OF THC & HHC

Δ^9-THC is the primary psychoactive substance in *Cannabis*. Acid-catalyzed reactions with Δ^9-THC or hemp-derived cannabidiol (CBD) result in formation of another psychoactive product, Δ^8-THC.[1] Current regulations at both the Federal and State levels have resulted in marketplace demand for psychoactive substances that are not classified as "THC."

- The numbering system between THC and HHC is retained
- The *trans* stereochemistry of the 6a(*R*),10a(*R*) is retained
- Position of the C_{11}-methyl is the key stereochemical difference
- Psychoactivity reported in the literature is higher for 9(*R*)-HHC than the 9(*S*)-HHC diastereomer[2]
- 9(*S*)-HHC and 9(*R*)-HHC are diastereomers separable by chromatography

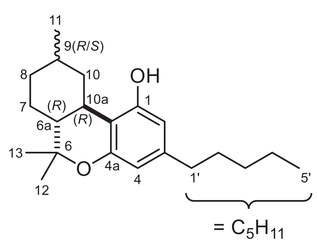
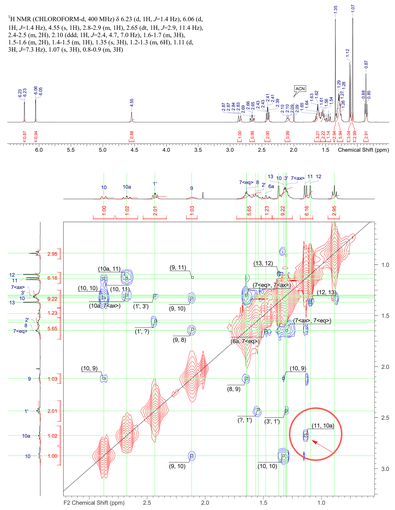


FIGURE 2 – Benzopyran numbering of the THCs and HHCs

FIGURE 3 – TLC of 9(*S*)- and 9(*R*)-HHC on silica gel developed with 10% MTBE in heptane and visualized by ceric ammonium molybdate (CAM)

NMR STRUCTURAL DETERMINATION OF HHC DIASTEREOMERS

9(*S*)-HHC NMR



- ^{1}H-NMR shows C_{11}-methyl as a doublet at 1.15 ppm
- COSY and HSQC established chemical shifts of H_9, H_{10}, H_{6a}, and H_{10a}
- NOESY shows a correlation between the axial C_{11}-methyl group and the axial H_{10a}
- Given the known stereochemistry of H_{10a}, this corresponds to 9(*S*)-HHC

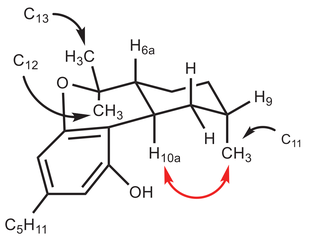
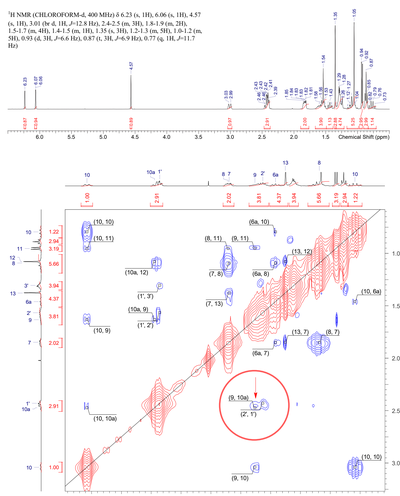


FIGURE 4 – ^{1}H-NMR and NOESY of the 9(*S*)-HHC diastereomer

9(*R*)-HHC NMR



- ^{1}H-NMR shows C_{11}-methyl as a doublet at 0.95 ppm, and a pseudo quartet coupling at 0.8 ppm (J=12Hz) for the axial H_{10} proton
- COSY and HSQC established chemical shifts of H_9, H_{10}, H_{6a}, and H_{10a}
- NOESY shows the key correlation between the axial H_9 and axial H_{10a}, and an absence between the C_{11}-methyl and H_{10a}
- Given the known stereochemistry of H_{10a}, this means the C_{11}-methyl group is equatorial, this corresponds to 9(*R*)-HHC

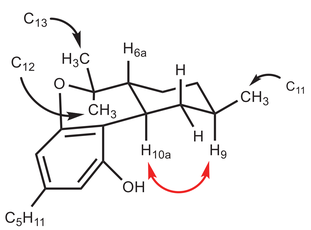
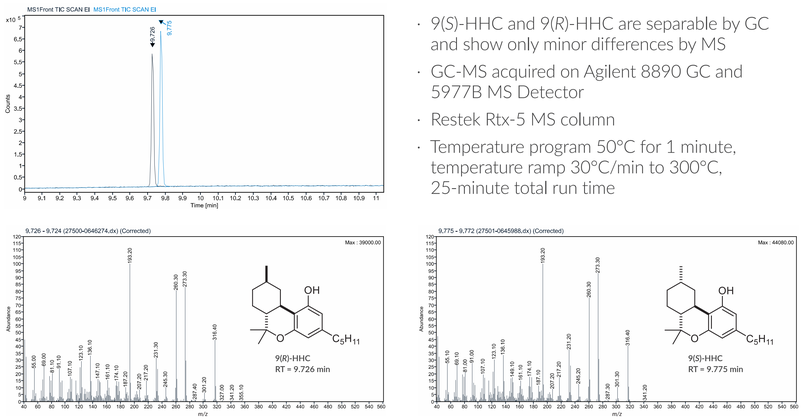


FIGURE 5 – ^{1}H-NMR and NOESY of the 9(*R*)-HHC diastereomer

NMR spectra ^{1}H and ^{13}C were acquired in chloroform-d on a JEOL ECZ-400S spectrometer, 2D experiments included COSY, HSQC, and NOESY.

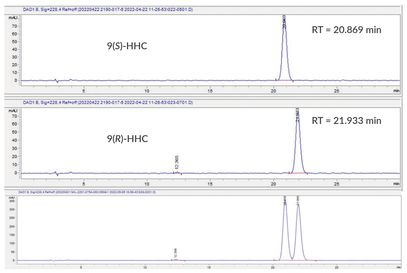
GC-MS ANALYSIS OF HHC DIASTEREOMERS



- 9(*S*)-HHC and 9(*R*)-HHC are separable by GC and show only minor differences by MS
- GC-MS acquired on Agilent 8890 GC and 5977B MS Detector
- Restek Rtx-5 MS column
- Temperature program 50°C for 1 minute, temperature ramp 30°C/min to 300°C, 25-minute total run time

FIGURE 6 – GC-MS separation and EI spectra of HHC diastereomers

HPLC SEPARATION OF HHC DIASTEREOMERS



- 9(*S*)-HHC and 9(*R*)-HHC are separable by HPLC-C18 using an isocratic method
- Gemini-C18 250 x 4.6 mm, 5 µm
- Mobile phase 20:80:0.1 Water/Methanol/ Acetic Acid
- 1 ml/min, column oven 40°C
- UV 228 nm

FIGURE 7 – HPLC separation of HHC diastereomers

CONCLUSIONS & FUTURE WORK

Hexahydrocannabinol diastereomers, 9(*S*)-HHC and 9(*R*)-HHC, have been fully characterized. The information provided herein can aid in the correct identification and differentiation of the products resulting from hydrogenation of Δ^8- or Δ^9-THC. The identification and confirmation of presumptive HHC metabolites is ongoing.[3]

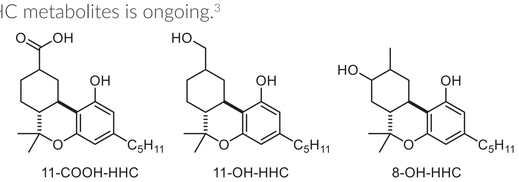


FIGURE 8 – Presumptive metabolites of HHC

References

1. Marzullo, P., Foschi, F., Coppini, D.A., *et al.* Cannabidiol as the substrate in acid-catalyzed intramolecular cyclization. *J. Nat. Prod.* **83(10)**, 2894-2901 (2020).
2. Mechoulam, R., Lander, N., Varkony, T.H., *et al.* Stereochemical requirements for cannabinoid activity. *J. Med. Chem.* **23(10)**, 1068-1072 (1980).
3. Harvey, D.J. and Brown, N.K. *In vitro* metabolism of the equatorial C_{11}-methyl isomer of hexahydrocannabinol in several mammalian species. *Drug Metab. Dispos.* **19(3)**, 714-716 (1991).