Thermal Degradation of Synthetic Cannabinoids Containing a Cyclopropyl Group

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Abstract

❖ The newest wave of synthetic cannabinoids, e.g. UR-144 and XLR-11, contain cyclopropyl rings and therefore circumvent the new S.3187 law. The analysis of cyclopropyl containing molecules can be challenging because the chromatograms of the standards (and casework) contain multiple related peaks. Standards of UR-144 and XLR-11 were heated and then both the Unheated and the Heated samples were analyzed using GC-MS, LC-MS, solid-phase GC-IR, FT-IR, Raman, and pyrolysis GC-MS. It was concluded that the first peak was the original molecule and the second was a thermodynamic product where the cyclopropyl ring was thermally opened. This research provided methods to identify cyclopropyl-containing synthetic cannabinoids, as well as answer what was happening to create two peaks in the chromatogram.

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Introduction

❖ The first wave of synthetic cannabinoids was detected in herbal smoking packages in late 2008 and included JWH-018, JWH-073, and CP-47,497. In 2009, JWH-018, JWH-073, and CP-47,497 were explicitly controlled in several European countries.[1]

❖ A second wave of synthetic cannabinoids hit the market in 2010 and included JWH-081, AM-2201, JWH-210, and JWH-122. Several European countries enacted generic bans that controlled synthetic cannabinoids based on general chemical structures.[1]

❖ In March 2011, the DEA temporarily placed JWH-018, JWH-073, JWH-200, CP-47,497, and cannabicyclohexanol and their salts, isomers, and salts of isomers into Schedule I of the Controlled Substances Act for twelve months. The ban was later extended an additional six months.[2]

❖ On July 9, 2012 Senate bill S.3187 was signed into law. This bill classified cannabimimetic agents' as Schedule I controlled substances and defined them as "any substance that was a cannabinoid receptor type 1 (CB1 receptor) agonist as demonstrated by binding studies and functional assays within any of the following structural classes" (**Fig. 1**). The bill also listed 16 synthetic cannabinoids by name, making them Schedule 1 controlled substances including the five temporarily scheduled in 2011.[3]

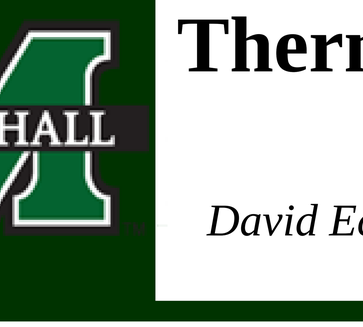

Figure 1. The seven general structures that are in the five classes of generically controlled structures that are now Schedule 1 drugs.

❖ Recently, a third wave of synthetic cannabinoids have been detected in herbal smoking mixtures. Some of these new synthetic cannabinoids, such as UR-144 (**Fig. 2A**) and XLR-11 (**Fig. 2B**), contain cyclopropyl rings. The structure-activity relationships (SAR) of many synthetic cannabinoids and how they interact with the cannabinoid receptors have been identified.[4] The SAR of UR-144 identified that it binds better to the CB_2 receptor[5] and there is currently no SAR for XLR-11. A paper was recently published about UR-144 pyrolysis products.[6]

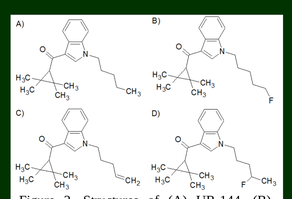

Figure 2. Structures of (A) UR-144, (B) XLR-11, (C) XLR-11 4-Pentenyl Analog (4-PA), and (D) XLR-11 4-Fluoropentyl Isomer (4-FPI).

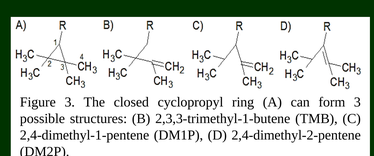

Figure 3. The closed cyclopropyl ring (A) can form 3 possible structures: (B) 2,3,3-trimethyl-1-butene (TMB), (C) 2,4-dimethyl-1-pentene (DM1P), (D) 2,4-dimethyl-2-pentene (DM2P).

❖ This study was conducted because many samples that involve compounds containing cyclopropyl rings have multiple peaks in the chromatogram and analysts are questioning what is causing the extra peaks. It was previously reported that two cyclopropyl ketones do thermally rearrange to homoallylic ketones.[7]

❖ It is hypothesized that the heat produced when smoking the synthetic cannabinoids as well as the injection port temperature causes the cyclopropyl ring (**Fig. 3A**) to open, creating one of three thermodynamic products (**Fig. 3 B-C**).

Materials and Methods

❖ Purchased following standards from Cayman Chemical:

- UR-144 (1-pentyl-1H-indol-3-yl) (2,2,3,3-tetramethylcyclopropyl)methanone
- XLR-11 (1-(5-fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
- XLR-11 4-Pentenyl Analog (1-(pent-4-en-1-yl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl) methanone) (**Fig. 2C**)
- XLR-11 4-Fluoropentyl Isomer (1-(4-fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl) methanone) (**Fig. 2D**)

Materials and Methods

❖ Heated standards:

- Heated hot plate to 300ºC
- ~1mg standard into well of a spot well plate (SWP)
- Small watchglass placed on top
- Placed SWP onto hot plate (~10 min)
- Rinsed both SWP and watchglass with MeOH

❖ Analyzed both Unheated and Heated cannabinoid standards using:

- Gas Chromatography – Mass Spectrometry (GC-MS)

Oven: 220ºC (2min), 5ºC/min, 280ºC (2min)
Inlet: Initial Temp: 250ºCTotal Flow: 205 ml/min
Split Ratio: 100:1
Split Flow: 200 ml/min
Gas: He
Flow: 2 ml/min
Columns: Agilent Technologies: HP-5MS (5% Phenyl Methyl Siloxane) 325ºC: 30m x 250µm x 0.25µm
Injector: Volume: 1µl

- Liquid Chromatography – Mass Spectroscopy (LC-MS)

Control: Column Flow: 500.00µl/min
Stoptime: 10.00min
Solvents: Solvent A: 35.0% (15mM ammonium acetate pH~4)
Solvent B: 65.0% (ACN)
Pressure Limits: Minimum Pressure: 0 bar
Maximum Pressure: 400 bar
Injection: 0.50 µl

- Solid-phase GC-Infrared Detection (GC-IR)

GC:
Oven: 220ºC (2min), 5ºC/min, 280ºC (2min)
Injector: Volume: 1µl
Inlet: Initial Temp: 250ºC
Total Flow: 15 ml/min
Split Ratio: 5:1
Split Flow: 10 ml/min
Gas: He
Flow: 2 ml/min
Column: Restek: Rxi-35Sil MS (35% Phenyl Methyl Siloxane) 360ºC: 30m x 250µm x 0.25µm

IR:
Transfer Line: 250ºC
Oven: 250ºC
Restrictor: 250ºC
Dewar Cap: 30ºC
Disk: -40ºC
Disk Speed: 3mm/minute
Chamber: 1.00x10^{-4}torr.
FTIR Detector: 4000-650cm^{-1} MCT; 4cm^{-1} resolution

Results and Discussion

❖ The following spectra are for UR-144. Similar spectra were observed for XLR-11, 4-PA, and 4-FPI standards.

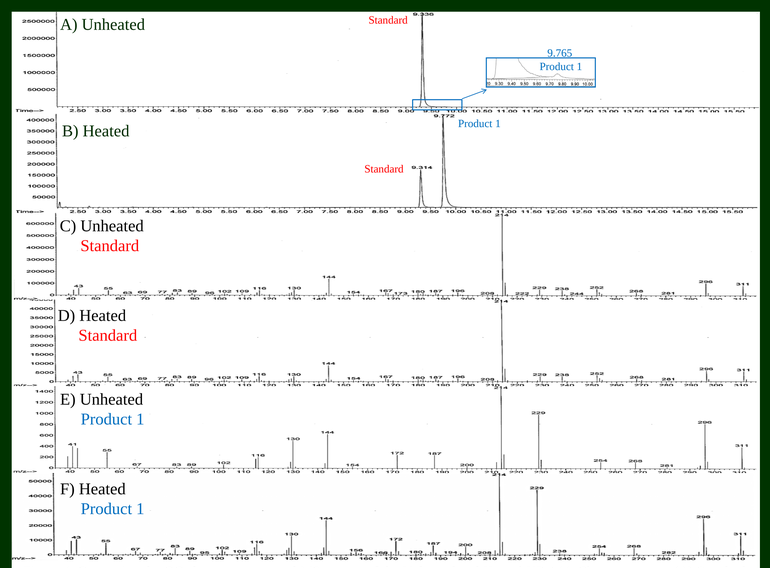

Figure 4. Comparing the GC chromatograms and MS spectra of the Unheated and Heated UR-144. Chromatograms: (A) Standard Rt = 9.34min and Product 1 was very small (close-up in box) Rt = 9.77min. (B) Standard Rt = 9.31min and Product 1 Rt = 9.77min. Mass Spectra: (C) Unheated Standard, (D) Heated Standard, (E) Unheated Product 1, and (F) Heated Product 1.

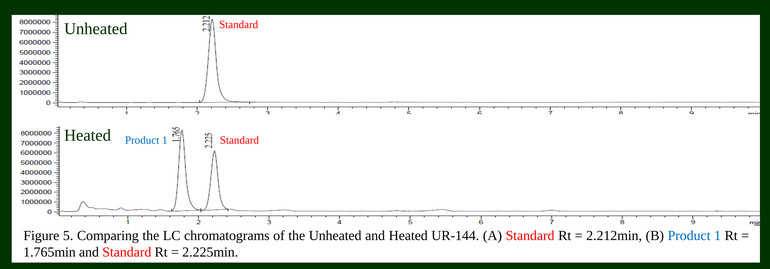

Figure 5. Comparing the LC chromatograms of the Unheated and Heated UR-144. (A) Standard Rt = 2.212min, (B) Product 1 Rt = 1.765min and Standard Rt = 2.225min.

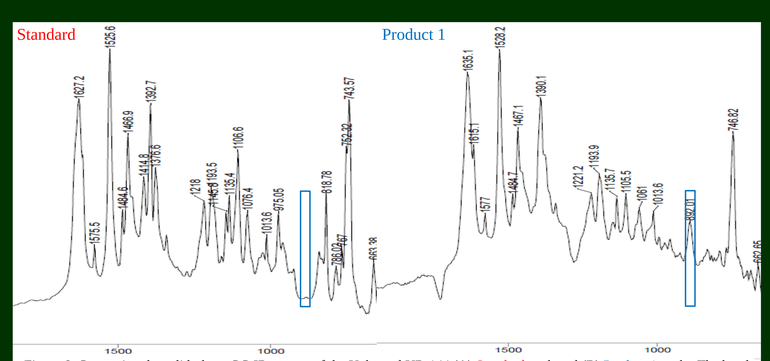

Figure 6. Comparing the solid-phase GC-IR spectra of the Unheated UR-144 (A) Standard peak and (B) Product 1 peak. The band at 892cm^{-1} was only in the Product 1 spectrum. Bands between 885-895cm^{-1} are interpreted as C=CH_2 (terminal double bond), therefore UR-144 with DM2P cannot be Product 1 because it does not have a terminal double bond.

Results and Discussion

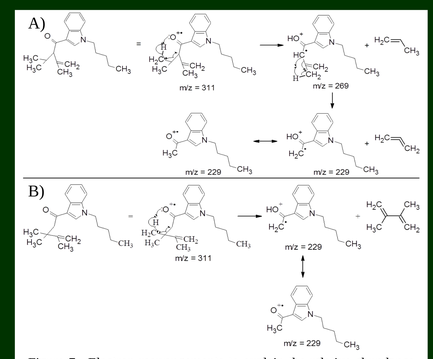

Figure 7. Electron rearrangements to explain the relative abundance of the m/z 229 seen in the Standard and Product 1 MS (**Fig. 4**). (A) UR-144 with DMIP would need to break 2 bonds, therefore not very efficient. (B) UR-144 with TMB would need to break 1 bond, therefore very efficient.

❖ **Figure 5** showed that Product 1 was created using heat and was not a impurity in the standard.

❖ It was hypothesized, based on the 892cm^{-1} band (**Fig. 6**) and the efficiency of breaking one bond (**Fig. 7**), that Product 1 included TMB as the substituent.

❖ At the request of USACIL, UR-144 with TMB (3,3,4-trimethyl-1-(1-pentyl-1H-indol-3-yl)pent-4-en-1-one) was synthesized by Cayman Chemical. To synthesize the new molecule, Cayman heated UR-144 and then followed with purification by prep-HPLC.[8]

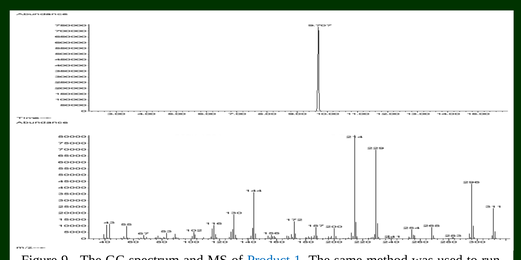

Figure 9. The GC spectrum and MS of Product 1. The same method was used to run Product 1 and UR-144. Rt = 9.707min.

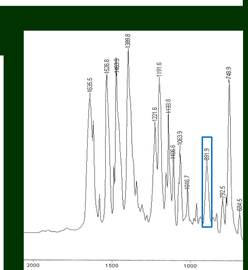

Figure 10. The solid-phase GC-IR spectrum Product 1. The same method was used to run Product 1 and UR-144. The spectrum contained a band at 891cm^{-1}.

Conclusion

❖ **Figure 9** and **Figure 10** confirmed that 3,3,4-trimethyl-1-(1-pentyl-1H-indol-3-yl)pent-4-en-1-one (**Fig. 11**) was Product 1 of UR-144 and it does contain TMB as the substituent. Based on the similarities between spectra, XLR-11, 4-PA, and 4-FPI probably have TMB as substituents. **Figure 12** was provided by John Krstenansky to help explain the production of TMB. **Figure 11** shows the structure of UR-144 with TMB and the probable structures of Product 1 for XLR-11, 4-PA, and 4-FPI."

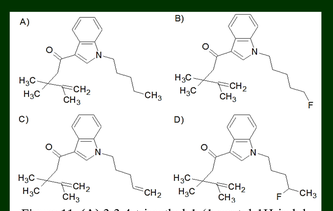

Figure 11. (A) 3,3,4-trimethyl-1-(1-pentyl-1H-indol-3-yl)pent-4-en-1-one. (B-D) The probable structures for Product 1 (B) XLR-11, (C) 4-PA, and (D) 4-FPI.

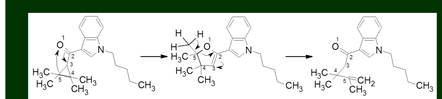

Figure 12. The dihydrofuran intermediate may help to explain the likelihood of producing TMB in preference to the other two openings of the cyclopropane.

References

1) European Monitoring Centre for Drugs and Drug Addiction Thematic paper. Understanding the 'spice' phenomenon. Luxembourg: Office for Official Publications of the European Communities 2009: 1-34.
2) Drug Enforcement Administration, Department of Justice. Schedules of Controlled Substances: Placement of Five Synthetic Cannabinoids Into Schedule I. *Federal Register* Mar 2012; **77**(41): 12508-14.
3) United States Congress Senate. S.3187: Food and Drug Administration Safety and Innovation Act. *112th Congress 2nd Session* May 2012: 1-388.
4) Huffman, JW. Cannabimimetic Indoles, Pyrroles, and Indenes: Structure-Activity Relationships and Receptor Interactions. Located within: Reggio PH (ed.). The Cannabinoid Receptors. ©Humana Press 2009; 49-93.
5) Frost JM, Dart MJ, Tietbje KR, Garrison TR, Grayson GK, Daza AV, El-Kouhen OF, Yao BB, Hsieh GC, Pai M, Zhu CZ, Chandran P, Meyer MD. Indol-3-ylcyclalkyl ketones: Effects of N1 substituted indole side chain variations on CB_2 cannabinoid receptor activity. *J. Med. Chem*. 2010; **53(1)**: 295-315.
6) Kavanagh P, Grigoryev A, Savchuk S, Mikhura I, Formanovsky A. UR-144 in products sold *via* the Internet: Identification of related compounds and characterization of pyrolysis products. *Drug Testing and Analysis* Jan 2013: 1-10. Published online. Web Accessed: April 15, 2013. http://onlinelibrary.wiley.com/doi/10.1002/dta.1456/abstract. DOI 10.1002/dta.1456.
7) Roberts RM, Landolt RG. Thermal Rearrangement of Cyclopropyl Ketones to Homoallylic Ketones, Relationship to the 'Abnormal Claisen Rearrangement'. *Journal of the American Chemical Society* May 1965; **87(10)**: 2281-82. Print.
8) Cayman Chemical. UR-144 Degradant *Product Information*. Dec. 2012. Web Accessed: April 9, 2013. https://www.caymanchem.com/app/template/Product.vm/catalog/11928.

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