Identification of the synthetic cannabinoid (1-(cyclohexylmethyl)-1*H*-indol-3-yl)(4-methoxynaphthalen-1-yl)methanone on plant material

Rohan Edmunds*, Oliver Locos, David Brown and Dominic Reynolds
Illicit Drugs Section, Forensic Science Laboratory, ChemCentre
PO Box 1250, Bently WA 6893
[redmunds@chemcentre.wa.gov.au]

ABSTRACT: In this article we report the isolation and characterisation of a new synthetic cannabinoid. The mass spectrum highlighted structural similarities to JWH-081, however the spectrum showed no match in available libraries. The use of nuclear magnetic resonance spectroscopy along with high resolution LC/MS allowed for structural elucidation and identification of the unknown as (1-(cyclohexylmethyl)-1*H*-indol-3-yl)(4-methoxynapthalen-1-yl)methanone.

KEYWORDS: synthetic cannabinoids, naphthoylindole, spice, kronic, forensic chemistry

Since early 2011, synthetic cannabinoids have gained prominence in Australia as a form of legal highs. Governments in Australia have reacted and changed legislation banning certain synthetic cannabinoids [1-3]. These changes have resulted in a wide range of new, and currently unscheduled cannabinoids being seen across the world. The ability for these drugs to affect the same cannabinoid receptors as THC, CB_1 and CB_2, has been well documented over the past decade with studies carried by many people including those by John William Huffman and co-workers [4-13]. Identification of synthetic cannabinoids is a challenging task facing forensic laboratories, with new cannabinoids continuously being detected [14-25]. Gas chromatography alone is not sufficient to identify novel synthetic cannabinoids with nuclear magnetic resonance spectroscopy and high resolution mass spectrometry required for the structural elucidation process [21-25].

Synthetic cannabinoids were first detected in plant material submitted for analysis to this laboratory in January 2011. Since this time there has been a rapid increase in the number of samples submitted with products bearing branded trademarks like "Kronic" and "Spice," as well as unlabelled samples. In the initial stages most samples contained only a few synthetic cannabinoids, predominately JWH-018, JWH-073 and CP47, 497 [2]. Western Australian legislation was initially changed banning specific cannabinoids such as JWH-018, but with the dramatic variety in synthetic cannabinoids being detected listing each one would not be practical. Legislation was further changed, this time into eight different classifications based on structure. The classes were benzoylindoles, cyclohexylphenols, dibenzopyrans, naphthoylindoles, naphthylmethylindoles, naphthoylpyrroles, naphthylmethylindenes, and phenylacetylindoles [1-3]. Any synthetic cannabinoid having a structure belonging to one of these categories is considered a scheduled substance in Western Australia [1-3].

Here we report the isolation and identification of the previously unknown synthetic cannabinoid (1-(cyclohexylmethyl)-1*H*-indol-3-yl)(4-methoxynaphthalen-1-yl)methanone (compound **1**) found in a herbal mixture branded "Northern Lights Skunk" labelled as "Passionfruit Skunk" variety. The presence of this synthetic cannabinoid had been seen previously within the laboratory, but only in the presence of other synthetic cannabinoids and only at trace levels. This sample was the first exhibit submitted to our laboratory where this compound was the only synthetic cannabinoid present. This facilitated the isolation and characterisation.

Experimental

Chemical and reagents

All chemicals and solvents were analytical or high performance liquid chromatography (HPLC) grade. Solvents were obtained from Thermo Fisher Scientific. Chemicals were obtained from Rowe Scientific.

*Extract and Isolation of Compound **1***

The plant material was enclosed in a packet labelled "Northern Lights Skunk" and was red in colour. The plant material (6 g) was suspended in dichloromethane (10 mL) and the mixture was vortexed for 3 minutes. The suspension was then filtered through filter paper by vacuum filtration. The filtrate was then purified using column chromatography (60-120 mesh Silica Gel, dichloromethane eluant) to afford a pale yellow solid.

Gas Chromatography/Mass Spectrometry (GC/MS)

GC/MS was run using an Agilent model 6890N GC equipped with an Agilent model 5975 mass-selective detector (MSD). The GC was fitted with a 12 m x 0.2 mm I.D. silica column coated with 0.33 µm 100% dimethylpolysiloxane (HP-1 Ultra). The injection port was maintained at 240°C and injections were performed in splitless mode. The oven temperature program was as follows: Initial temperature 60°C (0.5 min), ramped to 310°C at 25°C/min (final hold 3.5 min). Helium was used as the carrier gas at 2.5mL/min. The MSD was tuned to operate at 69.9 eV for the electron impact energy and 20.4 V for the repeller plate.

High Resolution Mass Spectrometry (HRMS)

High resolution mass spectrometry was run using an Agilent model 6540 UHD accurate mass QTOF and an Agilent 1290 Infinity LC system. The column used was a Phenomenex Kinetex 2.6 µm C18, 100 x 3.00 mm. The mobile phase was a 10 mmol ammonium formate buffer adjusted to pH 9 using 28% ammonia solution and the organic solvent was acetonitrile. The mobile phase was initially 10% organic, then after seven minutes

50%, and after 12 minutes 100% organic. The ionization source was an Agilent Jet Stream Electron Spray source with an ionization gas of nitrogen. The column heater was set to 50°C and the flow rate was 0.5 mL/min.

Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectrum was acquired using a Thermo Scientific Nicolet 6700 FTIR with a Smart iTR diamond ATR accessory. Data was collected between 4000 cm^{-1} and 550 cm^{-1} with a resolution of 4 cm^{-1} for 16 scans.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Sample Preparation: The sample was dissolved in deuterated chloroform ($CDCl_3$). The $CDCl_3$ was passed through a plug of sodium sulphate before being subjected to NMR.

NMR spectra were recorded using a Bruker AVN400 (400.13 MHz for ^1H, 100.62 MHz for ^{13}C) spectrometer at ambient conditions. ^1H and ^{13}C chemical shifts were referenced to residual solvent resonances. Assignment of NMR spectra were made with the aid of DEPT, ^1H-^1H COSY, HSQC (one bond ^1H-^{13}C correlations) and HMBC (two- and three- bond ^1H-^{13}C correlations) experiments.

^1H NMR ($CDCl_3$, 400.13 MHz): δ 0.93 (m, 2H, H3'/H7' 2 x C*H*H), 1.15 (m, 2H, H4'/H6' 2 x C*H*H), 1.55 – 1.73 (m, 6H, H3'/H7' 2 x CH*H*, H4'/H6' 2 x CH*H* and H5'), 1.83 (m, 1H, H2'), 3.91 (d, J = 7.2 Hz, 2H, H1'), 4.09 (s, 3H, OCH_3), 6.84 (d, J = 8 Hz, 1H, H3"), 7.33 – 7.38 (m, 3H, H4, H5 and H6), 7.37 (s, 1H, H2), 7.49 – 7.52 (m, 2H, H6" and H7"), 7.67 (d, J = 8 Hz, 1H, H2"), 8.31 (m, 1H, H8"), 8.35 (m, 1H, H5") and 8.46 (m, 1H, H7).

^{13}C NMR ($CDCl_3$, 100.61 MHz): δ 25.7 (CH_2, C4'/6'), 26.2 (CH_2, C5'), 31.0 (CH_2, C3'/7'), 38.4 (CH, C2'), 53.7 (CH_2, C1'), 102.3 (CH, C3"), 110.3, 122.7, 123.5 (3 x CH, C4, C5 and C6), 117.6, 127.3, 137.4 (3 x C, C3, C8 and C9), 122.2 (CH, C5"), 123.0 (CH, C7), 125.78, 127.4 (2 x CH, C6" and C7"), 125.84 (C, C1"), 126.0 (CH, C8"), 128.0 (CH, C2"), 131.6 (C, C10"), 132.3 (C9"), 138.2 (CH, C2), 157.2 (C, C4") and 191.9 (C=O).

Ultraviolet/Visible Spectroscopy (UV-Vis)

UV spectra were acquired on a Perkin Elmer Lambda 25 UV-Vis spectrometer, using a 1 cm path length. The sample was diluted in methanol and scanned over a range of 210-400 nm at a rate of 450 nm/min.

Results and Discussion

Isolation of compound **1** was relatively straight forward with an eluant of dichloromethane proving adequate for separating compound **1** from the plant derived impurities. Figure 1 shows the total ion chromatogram for the GC/MS before and after column chromatography. Purification resulted in a single peak

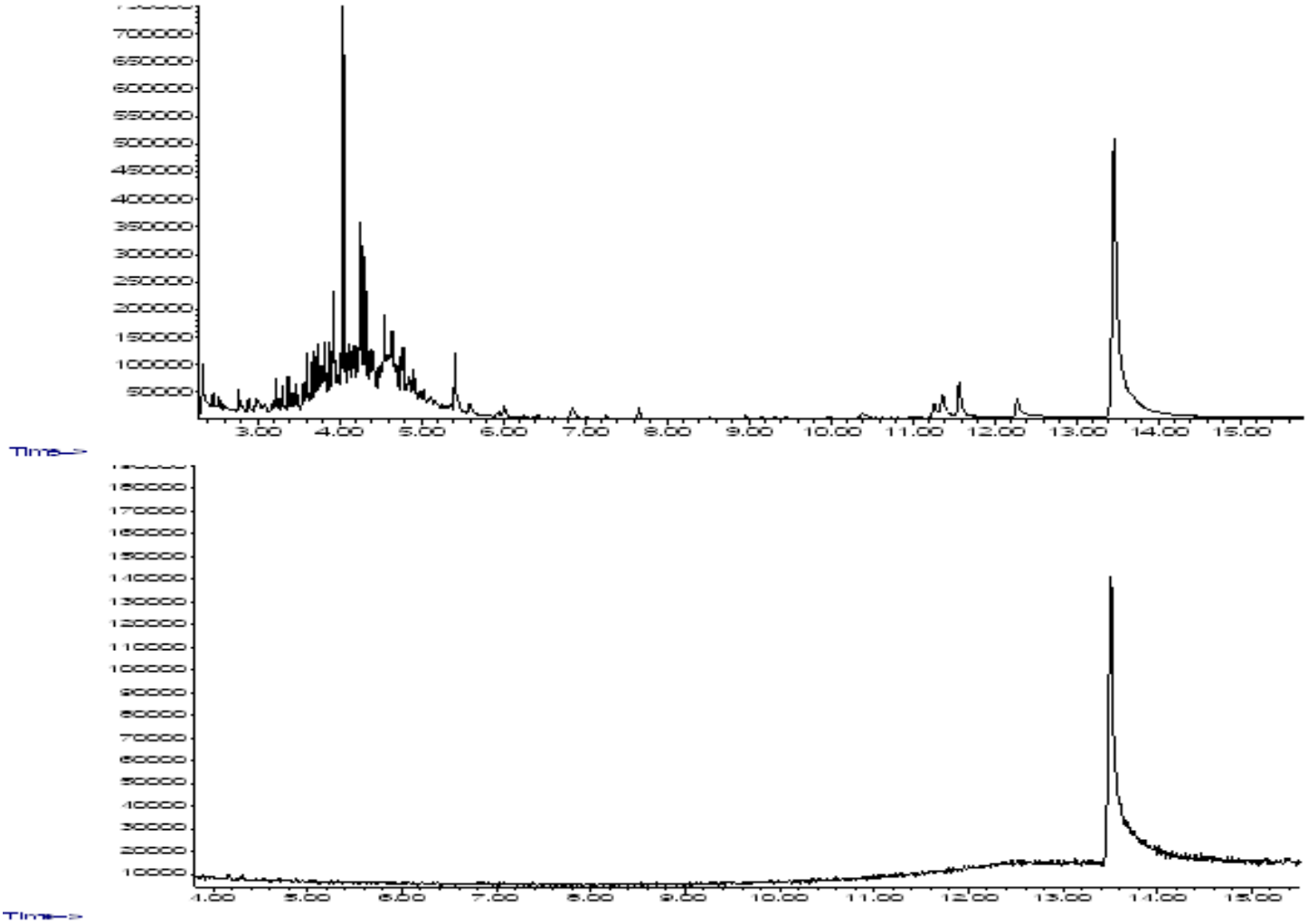

Figure 1 - TIC chromatograms (a) before and (b) after purification.

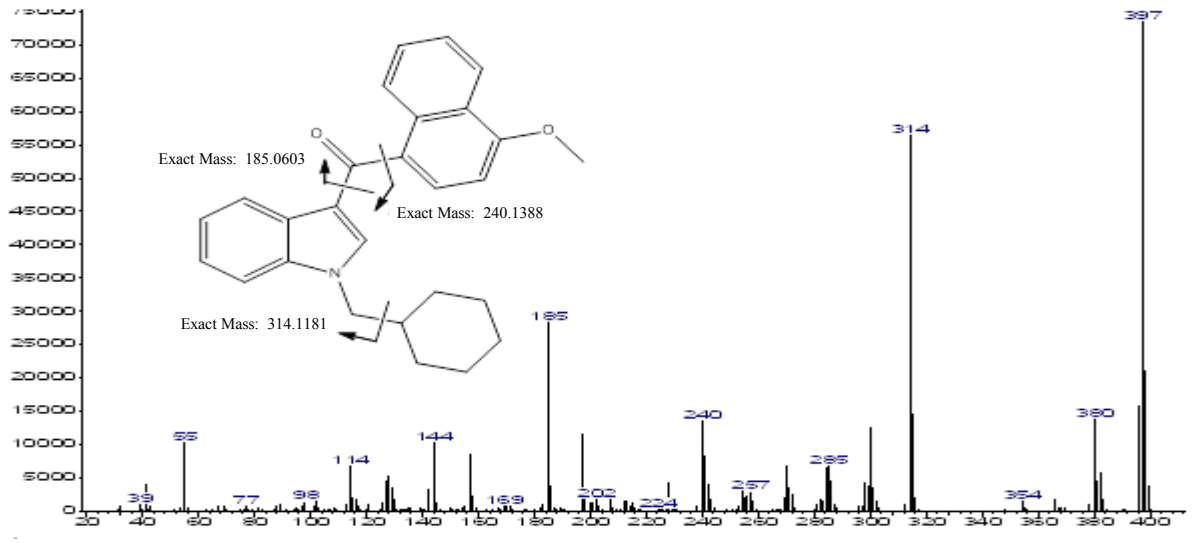

Figure 2 - Mass spectrum for compound **1**.

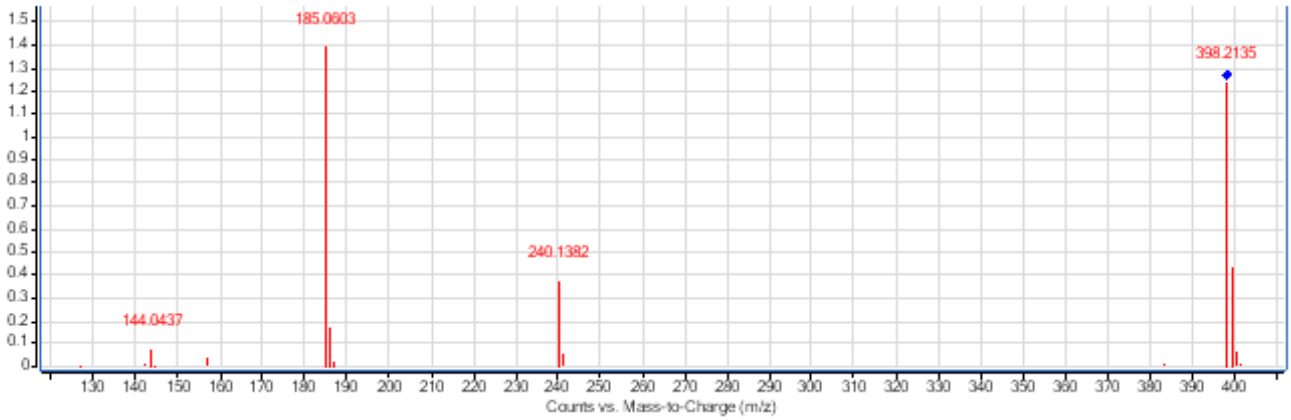

Figure 3 - HRMS MS^2 data for compound **1**.

for compound **1** at 13.5 minutes. This peak contained no matches in any library databases available at the time. The mass spectrum of the compound had a base peak of m/z (relative intensity %) 397 (100) and three other key ions 314 (75), 185 (38) and 240 (18). Based on the mass spectrum the unknown was suspected to contain a naphthoylindole structure due to similarities in the mass spectrum with JWH-081. Cleavage of the cyclohexane ring explains the m/z of 314, whilst the m/z 185 is from cleavage of the bond between the indole and ketone carbons. The third key ion m/z 240 is explained by the cleavage of methoxynaphthylene ring as shown in figure 2.

HRMS was performed to determine a molecular ion and a molecular formula. The HRMS mass spectrum produced a molecular ion (MH^+) of m/z = 398.2135, which suggested a calculated molecular formula of $C_{27}H_{27}NO_2$ (mass accuracy 4.7 ppm) with a calculated accurate mass for the molecular ion of 398.2115 Da. Using the accurate mass and retention time a targeted MS/MS analysis was carried out. The two major fragments present were m/z 185.0613 and 240.1397 with 157.0654 and 144.0449 as minor ions as shown in figure 3. The calculated molecular formula for the ions observed in the MS/MS spectrum was $C_{12}H_9O$ for m/z 185.0613 (mass difference 5.4 ppm) and $C_{16}H_{18}NO$ for m/z 240.1397 (mass difference 3.74 ppm). This data is consisted with the structure suggested for compound **1**.

NMR Experiments

The final structural elucidation of **1** was completed using ^1H and ^{13}C NMR experiments. Key signals in the ^1H NMR spectrum (Figure 4) include a downfield doublet at 3.91 ppm, due to the N-bound methylene protons. The doublet indicated that the adjacent group was a CH and thus a site of additional branching. The symmetry of the cyclohexyl group is consistent with the numbers of signals in the alkyl regions of the ^1H and ^{13}C NMR spectra. Standout features in the aromatic region of the ^1H NMR spectrum include two doublets at 6.84 and 7.67 ppm, which show no additional ^1H-^1H coupling. These doublets are due to the ortho H2" and H3" protons on the naphthyl ring.

UV-Vis spectroscopy

The UV-Vis spectrum was recorded for compound **1**, with two major peaks at λ_{max} = 212.7 and 320.7 nm, which is very similar to the spectrum of JWH-081 to which compound **1** is structurely related.

FTIR Spectroscopy

The key features in the FTIR spectrum are the CH stretches at 2921 and 2847 cm^{-1}. Due to the high level of conjugation within compound **1** the key carbonyl stretch has likely been shifted to a lower wave number and cannot be clearly identified due to the large number of absorbances below 1607 wavenumbers.

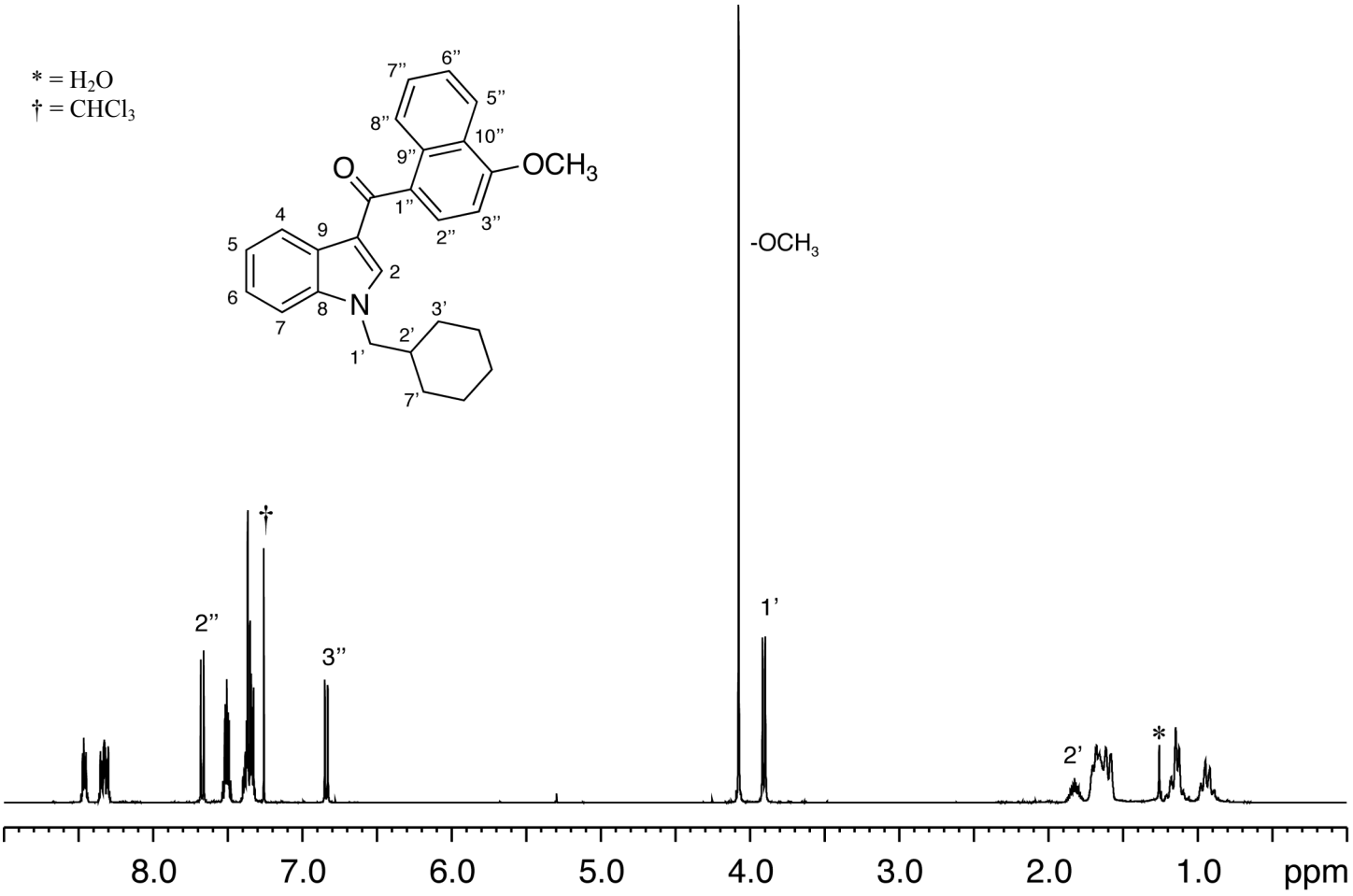

Figure 4 - ^{1}H NMR spectrum for compound **1**.

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