Forensic Science International 191 (2009) 58–63

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Spice: A never ending story?

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ARTICLE INFO

Article history:
Received 21 April 2009
Received in revised form 5 June 2009
Accepted 8 June 2009
Available online 8 July 2009

Keywords:
Spice
CP 47,497-C8
JWH-018
Cannabimimetic
Alkylaminoindole
GC–MS

ABSTRACT

On January 22nd 2009, the German Health Authorities prohibited several non-traditional cannabinoids, that proved to be the active components in popular "Bio-Designer-Drugs" like "Spice" and analogous products. The recent detection of CP 47,497-C8 in Europe and Japan documents that these products have already spread world wide. We synthesized several potentially interesting alkylaminoindoles (alkylchain C_3 to C_7) and isolated CP 47,497-C8 from "Spice Gold". The compounds were purified and characterized by NMR and mass spectrometry methods. With the aid of these authentic references we were able to detect and quantify added psychoactive compounds in different herbal blends. All samples that were acquired before the prohibition in December 2008 contained either CP 47,497-C8 (5.4–11.0 mg/g) or JWH-018 (2.3 mg/g). Some samples acquired in March 2009, 4 weeks after the prohibition took place, still contained CP 47,497-C8 (3.0–3.3 mg/g) but JWH-018 was not detected anymore. Instead it was replaced by its non-regulated C_4-homolog JWH-073 (5.8–22.9 mg/g). Furthermore some of the new products did not contain any non-traditional cannabinoids. To our knowledge this is the first report of the appearance JWH-073 as a new designer drug. The data and method presented here will facilitate and accelerate the detection of these compounds in complex matrices.

1. Introduction

Tracking the World Wide Web, the appearance of "Spice" can be traced back to the year 2006. The popularity of "Spice" and analogous herbal blends peaked in the second half of 2008 after several reports in German television and local newspapers covered the issue. Shortly after these reports, these products had sold out and were only available online. To our knowledge, these herbals were sold in many western European countries. Although declared as incense and not for human consumption, these blends are consumed as herbal drugs via smoking, much like cannabis (as for instances documented, on www.youtube.com). In several internet blogs consumers described cannabis-like effects after smoking, although preliminary chemical and botanical analysis showed no indication of cannabis in the mixtures. The list of ingredients indicated a mixture of plant components like "Lion's Tail", "Indian Warrior" etc., with very vaguely described intoxicating effects, but no clinical evidence. Based on these accounts these products were not banned by the authorities. Instead their popularity as "legal drugs" rapidly increased based their reputation of being potent herbal intoxicants and "legal" alternatives to the strictly regulated cannabis. Towards the end of 2008 at least eight effectively similar products were available on the German market demonstrating both the popularity and financial lucrativity of these products.

However, there was strong suspicion that added synthetic compounds or plant extracts are the real source of the described narcotic pharmacological effects.

In December 2008 the German company THC Pharma (Frankfurt, Germany) reported JWH-018 as an active ingredient in "Spice". Shortly afterwards two research groups at the University of Freiburg (Germany) [1] and at the National Institute of Health Sciences, Japan [2] concurrently identified and characterized the CP 47,497-C8 homolog (and its isomer as a synthetic byproduct) in these incenses (Fig. 1). Both substances are among the dozens of synthetic cannabinoids already described and *in vitro* tested for cannabinoid-action in scientific journals. As a consequence, on January 22nd 2009, the German Health Authorities prohibited the detected synthetic cannabinoids JWH-018 and CP 47,497-C8 [3]. While the German regulation included several homologues of CP 47,497-C8 (alkyl side chain C_6 to C_9), only one representative of the alkylaminoindoles (JWH-018) was banned. In the meantime some European countries have undertaken similar steps. However, *in vitro* data suggest that JWH-018 analogues

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doi:10.1016/j.forsciint.2009.06.008

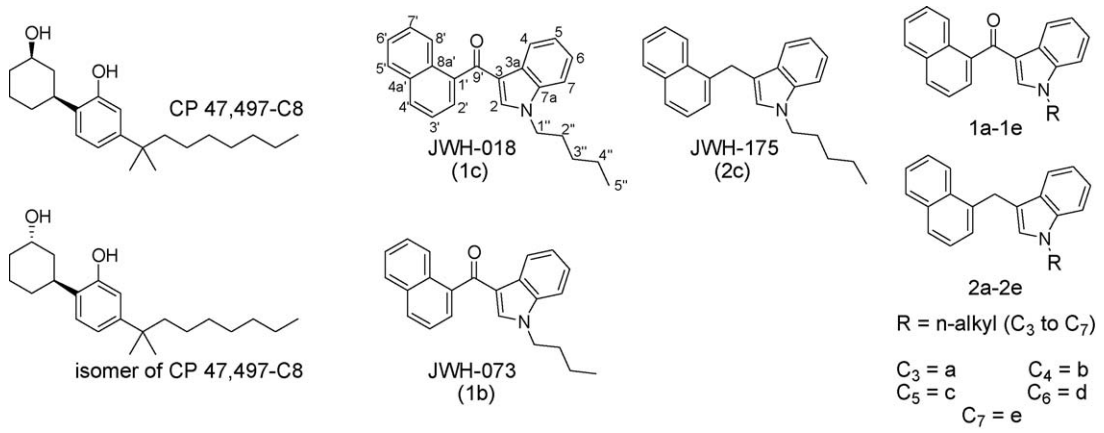

Fig. 1. Structures of compounds related to "Spice" and structures of synthesized alkylaminoindols **1a**–**2e**.

(especially the side chain length C_4 to C_6; **1b**–**1d**) possess equal or higher affinity to the CB1 and CB2 receptor than Δ^9-THC [4]. The same is true for compounds that lack the carbonyl functionality [5]. Here, only *in vitro* data for **2c** is available and shows receptor affinity in the same range as reported for Δ^9-THC.

During the last 15 years several dozens of alkylaminoindols were synthesized to study structure–activity relationships and receptor affinities for the CB1 and CB2 receptors [5–8]. While CB1 is primarily expressed in the central nervous system and exhibits the typical cannabinoid pharmacology, CB2 is also found in peripheral immune cells and seems to be involved in pain perception. Hence, it seems especially desirable to discover compounds with strong binding affinity towards CB2 but low affinity for CB1 [9].

Because of the ease of synthesis analogues of JWH-018 could easily be used as legal "Spice replacement products". In order to provide a resource for facilitating the analysis of these potentially narcotic substances we synthesized several JWH-018 analogues. The structures were verified by NMR and characterized by mass spectrometry to provide the chemical information needed for rapid target screening. The gathered information were then used to analyze the second generation of "Spice-like-products" that were available on the German market as of March 2009.

2. Materials and methods

2.1. General experimental procedures

GC–MS parameters: An Agilent 6890 gas chromatograph equipped with a 30 m analytical column (ZB5MS, Phenomenex, 30 m × 0.32 mm ID, ft = 0.25 μm) and helium as carrier gas (1.0 ml/min; constant flow mode). Temperature program 70 °C (3 min)–10 °C/min–330 °C (5 min). The GC was coupled directly to a JMS-T100GC (GCAccuTOF, JEOL, Japan) time of flight mass spectrometer in electron ionization (EI) mode at 70 eV and JEOL MassCenter™ workstation software was used. The source and transfer line temperature were set at 200 °C and 310 °C, respectively. The detector voltage was set at 2100 V. The acquisition range was from *m/z* 41–600 with a spectrum recording interval of 0.4 s. The system was tuned with PFK to achieve a resolution of 5000 (FWHM) at *m/z* 292.9824.

NMR: 600 MHz ^{1}H- and 151 MHz ^{13}C NMR spectra were obtained of $CDCl_3$ solutions of **1a**–**2e** on a Bruker Avance II 600 spectrometer with a 5 mm TCI

Table 1
^{13}C NMR chemical shifts (ppm) of **1a**–**2e**[a,b].

	1a	1b	1c	1d	1e	2a	2b	2c	2d	2e
C-2	138.04	137.95	137.98	137.97	137.97	126.67	126.61	126.61	126.62	126.62
C-3	117.48	117.44	117.42	117.45	117.42	113.49	113.50	113.49	113.49	113.49
C-3a	126.97	126.93	126.92	126.94	126.92	127.91	127.89	127.89	127.89	127.90
C-4	122.91	122.85	122.84	122.86	122.84	119.17	119.17	119.16	119.16	119.16
C-5	123.58[b]	123.54[b]	123.55[b]	123.55[b]	123.54[b]	118.66	118.65	118.65	118.64	118.65
C-6	122.85[b]	122.79[b]	122.80[b]	122.81[b]	122.79[b]	121.37	121.36	121.37	121.36	121.36
C-7	109.98	109.97	109.97	109.97	109.97	109.39	109.37	109.37	109.38	109.38
C-7a	137.02	136.97	136.97	136.97	136.96	136.37	136.34	136.31	136.31	136.31
C-1′	139.06	139.01	139.00	139.05	139.03	136.97	136.97	136.97	136.98	136.98
C-2′	125.80	125.77	125.79	125.78	125.76	126.55	126.55	126.55	126.55	126.56
C-3′	124.53	124.51	124.50	124.51	124.50	125.63	125.63	125.62	125.62	125.62
C-4′	129.94	129.91	129.92	129.91	129.90	126.75	126.75	126.75	126.75	126.75
C-4′a	133.70	133.67	133.66	133.68	133.67	133.81	133.80	133.80	133.80	133.81
C-5′	128.14	128.11	128.11	128.12	128.11	128.54	128.53	128.53	128.53	128.53
C-6′	126.27	126.23	126.23	126.24	126.23	125.44	125.43	125.43	125.43	125.43
C-7′	126.74	126.69	126.69	126.71	126.69	125.70	125.69	125.70	125.71	125.70
C-8′	125.97	125.93	125.92	125.94	125.92	124.42	124.43	124.43	124.42	124.43
C-8′a	130.76	130.72	130.72	130.74	130.72	132.18	132.17	132.17	132.17	132.18
C-9′	192.04	191.97	192.01	192.00	191.98	28.90	28.91	28.92	28.91	28.92
C-1″	48.76	46.87	47.10	47.12	47.09	47.85	45.92	46.18	46.19	46.18
C-2″	23.10	31.75	29.41	29.67	29.69	23.52	32.31	29.93	31.35	31.67
C-3″	11.32	19.97	28.82	26.39	26.66	11.48	20.13	29.06	30.17	30.22
C-4″	–	13.52	22.11	31.16	28.66	–	13.68	22.28	26.57	28.85
C-5″	–	–	13.83	22.40	31.54	–	–	13.94	22.50	26.87
C-6″	–	–	–	13.90	22.45	–	–	–	13.98	22.53
C-7″	–	–	–	–	13.97	–	–	–	–	14.05

[a] Solvent and internal chemical shift reference: $CDCl_3$ (δ_C = 77.01 ppm).
[b] Values with identical superscripts are exchangeable within the same column.