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Spice: A never ending story?

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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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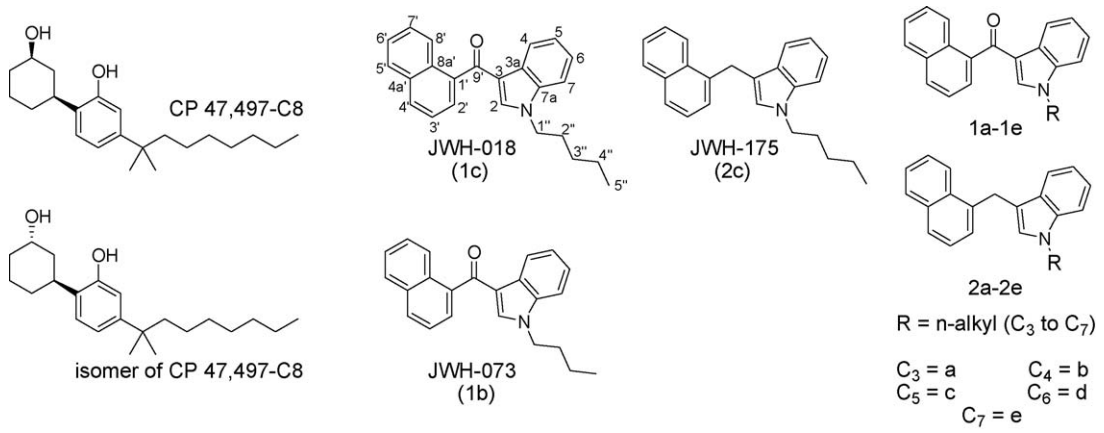

Fig. 1. Structures of compounds related to "Spice" and structures of synthesized alkylaminoindols **1a**–**2e**.

(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.

CryoProbe at a sample temperature of 20 °C. Internal chemical shift references were tetramethylsilane (δ_H = 0.00 ppm) and the solvent (δ_C = 77.01 ppm), respectively. Sample concentrations were of the order of 4 mg/0.6 ml.

2.2. Chemical synthesis

Compounds **1a**–**1e** and **2a**–**2e** (Fig. 1) were synthesized with standard chemical procedures. Since we do not want to promote the illegal use of these compounds the reaction details are not given. Instead we focus on the physical/chemical description to provide the basis for the fast and facile detection of the compounds in complex matrices.

All synthesized compounds were analyzed with elevated resolution by GC–EI-MS affording accurate masses to establish elemental compositions of molecular and fragment ions ($\Delta m < 3$ mmu). The retention index (RI) was calculated using a set of hydrocarbons (even numbered from C_{10} to C_{34}) as reference compounds by linear interpolation [10].

In addition the structures and purity of compounds were confirmed by NMR (Tables 1 and 2).

1a EIMS 70 eV, *m/z* (rel. int.): 313 $[M]^+$ (100), 296 (45), 284 (61), 270 (24), 256 (10), 254 (13), 241 (12), 226 (2), 213 (4), 186 (70), 167 (16), 155 (12), 144 (35), 127 (32), 116 (14), 101 (4), 89 (5), 77 (4).
RI(ZB5MS) 3083
Accurate mass (theor./meas.): 313.14666/313.14826.

1b EIMS 70 eV, *m/z* (rel. int.): 327 $[M]^+$ (100), 310 (44), 284 (67), 270 (23), 256 (10), 254 (12), 241 (11), 226 (2), 213 (4), 200 (61), 167 (14), 155 (15), 144 (30), 127 (30), 116 (11), 101 (3), 89 (4), 77 (3), 57 (2), 41 (3).
RI(ZB5MS) 3166
Accurate mass (theor./meas.): 327.16231/327.16049.

1c EIMS 70 eV, *m/z* (rel. int.): 341 $[M]^+$ (100), 324 (47), 284 (74), 270 (24), 256 (10), 254 (13), 241 (10), 226 (2), 214 (56), 186 (3), 167 (14), 155 (21), 144 (27), 127 (31), 116 (10), 101 (3), 89 (3), 77 (3), 43 (5).
RI(ZB5MS) 3261
Accurate mass (theor./meas.): 341.17796/341.17713.

1d EIMS 70 eV, *m/z* (rel. int.): 355 $[M]^+$ (100), 338 (47), 326 (2), 284 (78), 270 (24), 256 (9), 254 (14), 241 (10), 228 (51), 215 (3), 200 (4), 167 (13), 155 (25), 144 (25), 127 (32), 116 (8), 101 (2), 89 (2), 77 (2), 43 (6).
RI(ZB5MS) 3371
Accurate mass (theor./meas.): 355.19361/355.19228.

1e EIMS 70 eV, *m/z* (rel. int.): 369 $[M]^+$ (100), 352 (46), 284 (77), 270 (22), 256 (7), 254 (11), 242 (49), 214 (4), 191 (2), 167 (9), 155 (30), 144 (23), 127 (35), 116 (6), 101 (2), 73 (5), 57 (3), 43 (3).
RI(ZB5MS) > 3400
Accurate mass (theor./meas.): 369.20926/369.20957.

2a EIMS 70 eV, *m/z* (rel. int.): 299 $[M]^+$ (100), 270 (57), 256 (16), 254 (25), 241 (6), 226 (6), 202 (2), 172 (40), 158 (3), 153 (3), 141 (16), 135 (6), 130 (16), 115 (9), 103 (2), 77 (3).
RI(ZB5MS) 2757
Accurate mass (theor./meas.): 299.16740/299.16870.

2b EIMS 70 eV, *m/z* (rel. int.): 313 $[M]^+$ (100), 270 (58), 256 (16), 254 (23), 241 (6), 226 (5), 202 (2), 153 (3), 141 (19), 130 (20), 115 (9), 103 (2), 77 (3).
RI(ZB5MS) 2841
Accurate mass (theor./meas.): 313.18305/313.18309.

2c EIMS 70 eV, *m/z* (rel. int.): 327 $[M]^+$ (100), 312 (1), 270 (58), 256 (13), 254 (20), 241 (5), 226 (4), 200 (27), 186 (18), 164 (3), 153 (3), 141 (19), 135 (7), 130 (17), 115 (7), 103 (2), 43 (3).
RI(ZB5MS) 2936
Accurate mass (theor./meas.): 327.19870/327.19793.

2d EIMS 70 eV, *m/z* (rel. int.): 341 $[M]^+$ (100), 312 (1), 270 (55), 256 (14), 254 (20), 241 (4), 226 (4), 214 (26), 153 (2), 141 (23), 135 (7), 130 (20), 115 (8), 77 (1), 43 (3).
RI(ZB5MS) 3030
Accurate mass (theor./meas.): 341.21435/341.21558.

2e EIMS 70 eV, *m/z* (rel. int.): 355 $[M]^+$ (100), 312 (1), 270 (52), 256 (13), 254 (19), 241 (4), 228 (24), 214 (21), 153 (2), 141 (24), 130 (18), 115 (8), 77 (1).
RI(ZB5MS) 3129
Accurate mass (theor./meas.): 355.23000/355.23010.

2.3. Extraction of herbal mixtures

Several commercially available herbal mixtures were purchased on the local market or via internet shops. The plant mixtures were ground in a coffee mill and the extracts were obtained by a 2-step Soxhlet-extraction starting with petroleum ether (40–60 °C) and followed by methanol (12 turns each per solvent). Both extracts were analyzed separately.

2.4. Isolation of CP 47,497-C8

6 g of Spice Gold were extracted with petroleum ether as described above. The extract was concentrated and CP 47,497-C8 was isolated by column chromatography on silica gel using ethyl acetate:dichloromethane (1:9) as a solvent system. Fractions containing pure CP 47,497-C8 were combined and concentrated. R_f = 0.35 (TLC, SiO_2, ethyl acetate:dichloromethane (2:8)). Purity and authenticity were confirmed by GC–MS and NMR and were in accordance with published data [1,2].

2.5. Quantification

CP 47,497-C8 was quantified by a standard addition method using purified CP 47,497-C8. Mixtures containing JWH-018 were quantified by addition of the C_4 analogue (JWH-073) as the internal standard. In turn JWH-073 instead was quantified using JWH-018 (C_5 analogue). Since these two compounds are structurally closely related and show similar GC–MS behavior, a response factor of 1 was assumed.

3. Results

The ^{13}C and ^1H NMR data and the signal assignments for **1a**–**2e** are given in Tables 1 and 2, respectively. The NMR spectra of JWH-

Table 2
^1H NMR chemical shifts (ppm) of **1a**–**2e**[a].

	1a	1b	1c[b]	1d	1e	2a	2b	2c[c]	2d	2e
H-2	7.36	7.34	7.34	7.34	7.33	6.60	6.60	6.60	6.59	6.59
H-4	8.50	8.50	8.49	8.50	8.50	7.62	7.62	7.62	7.62	7.62
H-5	7.34–7.41	7.33–7.39	7.33–7.39	7.33–7.39	7.33–7.39	7.10	7.10	7.10	7.10	7.10
H-6	7.34–7.41	7.33–7.39	7.33–7.39	7.33–7.39	7.33–7.39	7.22	7.22	7.22	7.22	7.22
H-7	7.34–7.41	7.33–7.39	7.33–7.39	7.33–7.39	7.33–7.39	7.32	7.32	7.32	7.32	7.32
H-2′	7.66	7.64	7.64	7.64	7.64	7.36	7.36	7.37	7.36	7.36
H-3′	7.53	7.51	7.51	7.51	7.51	7.39	7.39	7.39	7.39	7.39
H-4′	7.97	7.96	7.95	7.95	7.95	7.75	7.75	7.75	7.75	7.75
H-5′	7.91	7.89	7.89	7.90	7.89	7.87	7.87	7.87	7.87	7.87
H-6′	7.51	7.50	7.50	7.50	7.50	7.47	7.47	7.47	7.47	7.46
H-7′	7.47	7.45	7.45	7.46	7.45	7.44	7.44	7.44	7.44	7.43
H-8′	8.19	8.19	8.19	8.19	8.19	8.10	8.10	8.10	8.10	8.10
H-9′	–	–	–	–	–	4.55	4.54	4.55	4.55	4.54
H-1″	4.04	4.04	4.03	4.04	4.03	3.95	3.99	3.98	3.98	3.97
H-2″	1.84	1.76	1.78	1.78	1.77	1.76	1.72	1.73	1.72	1.72
H-3″	0.89	1.27	1.22	1.22–1.26	1.16–1.27	0.85	1.26	1.20	1.20–1.25	1.15–1.26
H-4″	–	0.88	1.27	1.22–1.26	1.16–1.27	–	0.87	1.26	1.20–1.25	1.15–1.26
H-5″	–	–	0.84	1.22–1.26	1.16–1.27	–	–	0.83	1.20–1.25	1.15–1.26
H-6″	–	–	–	0.83	1.16–1.27	–	–	–	0.83	1.15–1.26
H-7″	–	–	–	–	0.84	–	–	–	–	0.84

[a] Solvent: $CDCl_3$, internal chemical shift reference: tetramethylsilane (δ_H = 0.00 ppm).
[b] Multiplicities and coupling constants for **1c**: H-2: br. s; H-4: m; H-5,6,7: m; H-2′: dd, 7.0, 1.3 Hz; H-3′: dd, 8.2, 7.0 Hz; H-4′: dt, 8.2, 1.0 Hz; H-5′: dm, 8.1 Hz; H-6′: ddd, 8.1, 6.8, 1.3 Hz; H-7′: ddd, 8.4, 6.8, 1.5 Hz; H-8′: dm, 8.4 Hz; H-1″: t, 7.3 Hz; H-2″: qi, 7.4 Hz; H-3″-4″: m; H-5″: t, 7.2 Hz.
[c] Multiplicities and coupling constants for **2c**: H-2: t, 1.1 Hz; H-4: ddd, 7.9, 1.1, 0.9 Hz; H-5: ddd, 8.0, 7.0, 1.0 Hz; H-6: ddd, 8.2, 7.0, 1.2 Hz; H-7: dt, 8.3, 0.9 Hz; H-2′: ddt, 7.0, 1.6, 0.8 Hz; H-3′: dd, 7.9, 7.1 Hz; H-4′: dm, 8 Hz; H-5′: dm, 8 Hz; H-6′, -7′: m; H-8′: dm, 8 Hz; H-9′: s; H-1″: t, 7.2 Hz; H-2″: qi, 7.4 Hz; H-3″, -4″: m; H-5″: t, 7.2 Hz.

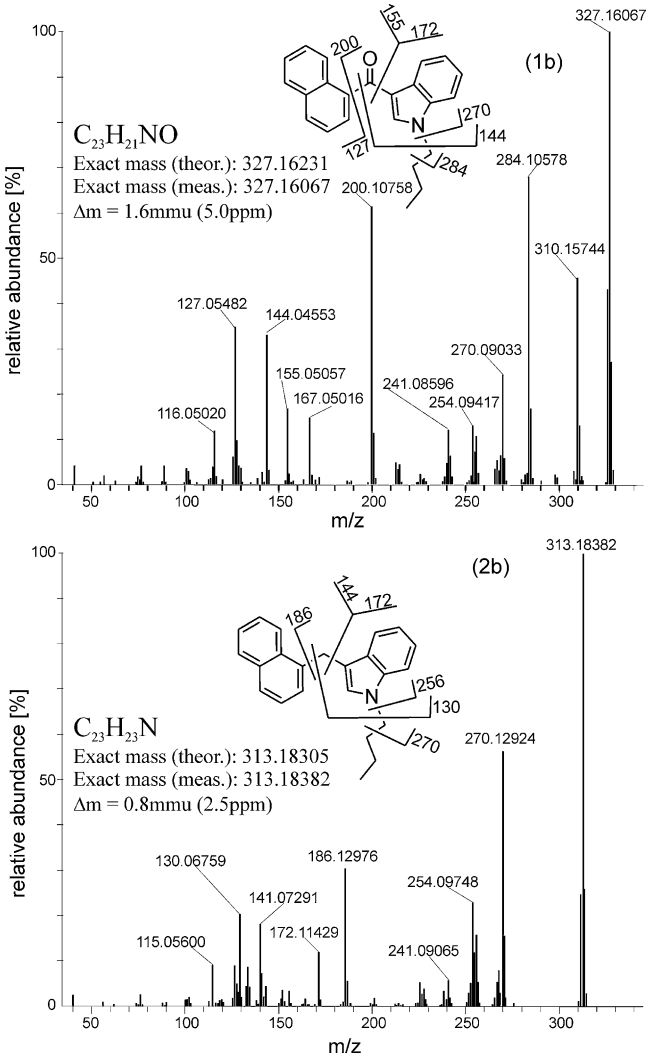

Fig. 2. Proposed EI-fragmentation of compounds **1b** and **2b**, based on accurate masses and elemental compositions of the fragment ions.

018 (**1c**) and of its analogue **2c** were fully assigned by means of two-dimensional H,H-COSY and -NOESY and H,C-HSQC and -HMBC experiments, which provided definite proof of the structures. Due to the high similarity of the spectra of these compounds with the remaining ones of the respective series, the NMR spectra of the latter could be assigned by analogy.

Table 3
Quantification of cannabimimetics in herbal insences.

	CP 47,497-C8 [mg/g]	JWH-018 [mg/g]	JWH-073 [mg/g]
Products acquired December 2008			
Spice Gold 1	11.0	n.d	n.d
Spice Gold 2	5.4	n.d	n.d
Chill out 1	5.7	n.d	n.d
Smoke	n.d.	2.3	n.d
Products acquired March 2009			
Forest Humus	n.d	n.d	7.6
Scope Vanilla	n.d	n.d	22.9
Scope Wildberry	n.d	n.d	5.8
Chill X	3.3	n.d	n.d
Chill out	3.0	n.d	n.d
Space	n.d.	n.d	n.d
Silent Black	n.d.	n.d.	n.d

n.d. not detected.

The GC–MS data are exemplified for the two homolog structures **1b/2b**. The assignment of fragment ions is based on the prediction of the elemental composition obtained by accurate mass measurements ($\Delta m < 3$ mmu) and was unambiguous in all cases (Fig. 2, data only shown for **1b** and **2b**).

With these authentic references we were able to quantify the added psychoactive compounds. To our knowledge this is the first time that these additives were quantified in these herbal blends. The result is summarized in Table 3. Typically, only one sample per product was available. In all cases both extracts per sample (petroleum ether and methanol) contained substantial amounts of the respective analyte. The concentration of each individual extract was measured by preparing duplicate samples of each extract. The values per individual analysis differed by less than 10% and were subsequently averaged. The contents of both extracts per sample were summed and represent the total amount found in each sample. The amount of added cannabimimetics varied over one order of magnitude and ranged from 2.3 mg/g up to 22.9 mg/g. The range is in accordance to the estimated 1% of CP 47,497-C8 reported for "Spice Diamond" [1].

One batch of samples was acquired before the German prohibition (December 2008) and each sample contained CP 47,497-C8 (together with its isomeric byproduct as reported in [1]) or JWH-018. In contrast the second batch of samples (acquired in March 2009) showed a more complex pattern. While CP 47,497-C8

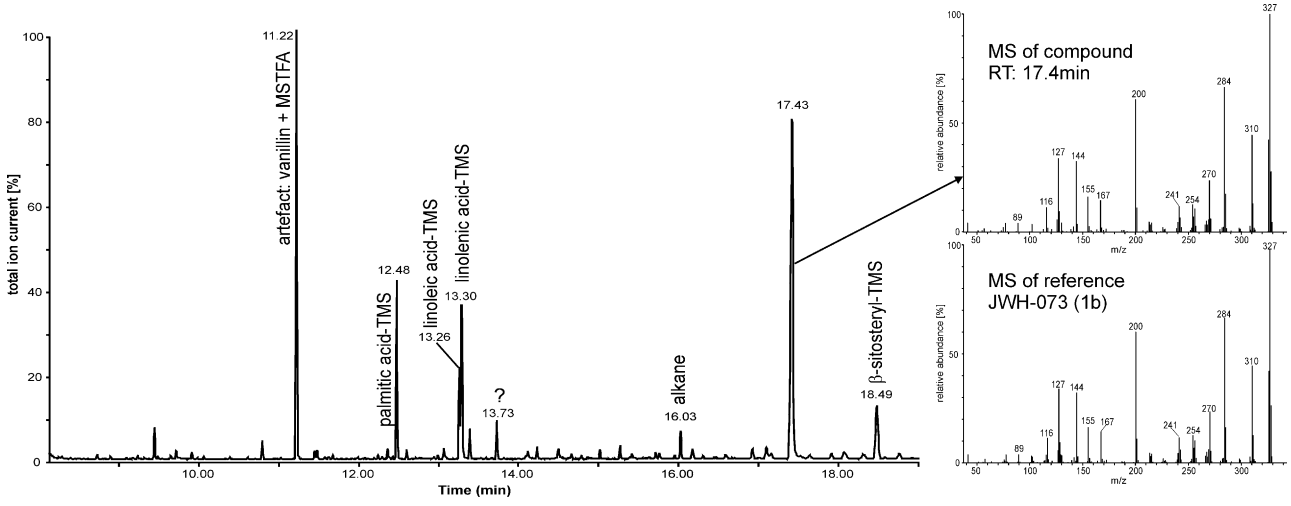

Fig. 3. GC–EI-MS analysis and detection of JWH-073 (**1b**) in the petrol ether extract "Scope Vanilla" after derivatization with MSTFA.

can be still found on the market, we report here the first appearance of JWH-073 (C_4-homologue) as replacement for JWH-018 on the German market (Fig. 3). Furthermore, products, without any apparent additives appeared on the market.

4. Discussion

The recent success of "Spice" and related products reflects the large demand for "legal-drugs" in our society, but the observed phenomena show some additional peculiarities. Up to the legal ban, "Spice" was probably the most popular product defining a new class of so-called "herbal highs" with high popularity in user circles because of its obvious psychoactive properties. In retrospect, the trade name "Spice" together with the imprinted symbol of a wide open eye seems a blatant allusion to Frank Herberts science fiction novel "Dune" where the fictional drug "Melange" usually is referred to as "[the] Spice" and where a "*characteristic effect of a diet high in melange wherein the whites and pupils of the eyes turn a deep blue* (*indicative of deep melange addiction*)" [11].

It is evident that the producers of these products have gone about in a very methodical manner to mine the scientific literature for promising psychoactive compounds. Most likely the published CB1 binding affinities were exploited as primary criterion. Since all compounds detected in this study are synthetic non-traditional cannabinoids it seems apparent that their "marketing strategy" aims at producing "legal" alternatives for cannabis. The design of the packaging is very conspicuous and professional. Therefore it seems rational that these products are designed for young people that are eager to try cannabis but are afraid of the judicial consequences and/or the reputation of the narcotics scene. These herbal mixtures are sold for 10 €/g, which is rather expensive compared to local street prices of cannabis (5–7 €/g). Hence, buying a "non-illegal" product online or in local headshops despite of the high price seems to be an attractive alternative but might likely represent the first step towards drug abuse. The innocuous label "incense" is only used to conceal the real purpose of these products against legal actions and in the case of the German legislation, to avoid troubles with existing tobacco regulations from the beginning. But of course, the users utilize these products exactly like cannabis by inhaling the smoke.

Recently, there are an increasing number of suspects in police stop-and-search controls. These cases showed all symptoms of cannabis abuse (red eyes, impaired motoric and linguistic abilities and/or conspicuous cardiovascular symptoms) but negative test results for THC-consumption or any other tested drug (personal communications).

Our analysis (Table 3) demonstrated that just 4 weeks after the prohibition took effect a multitude of second generation products were flooding the market. The speed of introduction of new products and the use of JWH-073 as a substitute for JWH-018 not only showed that the producers are well aware of the legal frameworks, but that they likely anticipated the prohibition and already had an array of replacement products on hand (JWH-073-positive products are still available on the German market; last checked: June 5th, 2009).

The example "Chill out" (sample 1 vs. sample 2, Table 1) and "Chill X" demonstrated, that even already prohibited compounds (CP 47,497-C8) are still available on the German market (last checked: April 6th, 2009; Updated: Chill out and Chill X were withdrawn from the market; last checked: June 5th, 2009) while Scope products and Forest Humus are still available). On the web pages of internet stores "Chill out/Chill X" was even advertised as "*Does not contain JWH-018 or CP 47,497*". This statement might be due the fact that "Chill out/Chill X" were not yet tested [2] and therefore still seemed to be safe to sell for the vendors.

In addition it appeared that the success of "Spice" has reared some mimics that offer the same image, but might be of "real" herbal origin and without the desired psychoactive effects (Table 3). Because of their lack of psychoactive effects, products like "Space Diamond" and "Silent Black" share a very bad reputation in consumers' internet blogs.

In contrast the Spice story puts the focus on a very serious problem. The producers use chemicals with likely psychoactive properties but without any knowledge of clinical data or hazardous consequences for the consumer. All compounds introduced into the market, lack any published *in vivo* testing even in animal models. Only limited data on the pharmacological and cannabimimetic properties of CP 47,497 in animal models and the metabolism of JWH-015 (**1a**) in rat liver microsomes are available [12,13,9]. It is evident that the producers are purposely risk the health of consumers to skim high profits. In fact there are first clinical reports that clearly demonstrate signs of addiction syndrome and withdrawal symptoms that were unequivocally linked to the chronic abuse of "Spice Gold" and were similar to syndromes observed in cannabis abuse [14]. Within this context the analogy to "Melange" appears cynical and tasteless but alarmingly real. "Spice" is "*...mildly addictive when taken in small quantities*, *severely addictive when imbibed in quantities above two grams daily per seventy kilos of body weight*" and withdrawal is described as a fatal process [11].

The synchronous detection of CP 47,497-C8 in Europe and Japan indicates that these products are already spread world wide. Since the legal status differs from country to country there is a high probability that the delay between appearance of a substance on the market and its analysis and prohibition by health authorities will be methodically exploited by producers in the future. Furthermore there are dozens of compounds which fit into the same scheme as it is just observed for JWH-analogues; it seems that the producers are moving on to the next product, always one step ahead of the law. Hence the spice story will keep us occupied for quite some time unless the legal framework adjusts to take deal with the quick evolution of new products which fill the gap created by a set of banned substances.

To facilitate the screening and to complement existing databases the MS-data (NIST-format) and NMR-data (JCAMP-DX-format) of the compounds described here can be obtained from the authors. In addition, full EI-MS spectra are available as supplementary material.

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References

[1] V. Auwärter, S. Dresen, W. Weinmann, M. Müller, M. Pütz, N. Ferreirós, Spice and other herbal blends: harmless incense or cannabinoid designer drugs? J. Mass Spectrom. 45 (2009), doi:10.1002/jms.1558 (online February 2).
[2] N. Uchiyama, R. Kikura-Hanajiri, N. Kawahara, Y. Haishima, Yukihiro Goda, Identification of a cannabinoid analog as a new type of designer drug in a herbal product, Chem. Pharm. Bull. 57 (2009) 439–441.
[3] Bundesgestzblatt, 22. BtMÄndV, 21, January 2009.
[4] M.M. Aung, G. Griffin, J.W. Huffman, M.-J. Wu, C. Keel, B. Yang, V.M. Showalter, M.E. Abood, B.R. Martin, Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB1 and CB2 receptor binding, Drug Alcohol. Depend. 60 (2000) 133–140.
[5] J.W. Huffman, D. Dai, Design, synthesis and pharmacology of cannabimimetic indoles, Bioorg. Med. Chem. Lett. 4 (1994) 563–566.
[6] M.M. Aung, G. Griffin, J.W. Huffman, M.-J. Wu, C. Keel, B. Yang, V.M. Showalter, M.E. Abood, B.R. Martin, Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB1 and CB2 receptor binding, Drug. Alcohol Depend. 60 (2000) 133–140.

[7] J.W. Huffman, R. Mabon, M.-J. Wu, J. Lu, R. Hart, D.P. Hurst, P.H. Reggio, J.L. Wileyc, B.R. Martin, 3-Indolyl-1-naphthylmethanes: new cannabimimetic indoles provide evidence for aromatic stacking interactions with the CB1 cannabinoid receptor, Bioorg. Med. Chem. 11 (2003) 539–549.
[8] J.W. Huffman, P.V. Szklennik, A. Almond, K. Bushell, D.E. Selley, H. He, M.I.P. Cassidy, J.L. Wiley, B.R. Martin, 1-Pentyl-3-phenylacetylindoles, a new class of cannabimimetic indoles, Bioorg. Med. Chem. Lett. 15 (2005) 4110–4113.
[9] Q. Zhang, P. Ma, R.B. Cole, G. Wang, Identification of in vitro metabolites of JWH-015, an aminoalkylindole agonist for the peripheral cannabinoid receptor (CB2) by HPLC-MS/MS, Anal. Bioanal. Chem. 386 (2006) 1345–1355.
[10] L.S. Ettre, Retention index expression, Chromatographia 58 (2003) 491–494.
[11] F. Herbert, Dune, Chilton Books, New York, 1965, Terminology of the Imperium.
[12] D.R. Compton, M.R. Johnson, L.S. Melvin, B.R. Martin, Pharmacological profile of a series of bicyclic cannabinoid analogs: classification as cannabimimetic agents, J. Pharm. Exp. Ther. 260 (1992) 201–209.
[13] A. Weissmann, G.M. Milne, L.S. Melvin Jr., Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol, Pharm. Exp. Ther. 223 (1982) 516–523.
[14] U.S. Zimmermann, P.R. Winkelmann, M. Pilhatsch, J.A. Nees, R. Spanagel, K. Schulz, Fallbericht über körperliche Entzugszeichen und Abhängigkeitssyndrom nach chronischem Konsum von "Spice Gold", Deutsches Ärzteblatt, in press.