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N-pentylindole: a marker for the detection of synthetic cannabinoids

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Abstract

Aim: Since the first detection of JWH-018 as the active ingredient in "Spice" herbal blends in 2007, the number of synthetic cannabinoids is constantly increasing and novel compounds steadily appear on the illicit drug market. Hence, our aim was to facilitate the detection of such compounds by identification of a generic marker or key compound that allows the tracing of synthetic cannabinoids in herbal blends and/or body fluids without even knowing their exact structure.

Methods: Representative illicit drug preparations (herbal blends and powders) from seizures in NRW and pure samples of N-pentylindole (synthesized following literature methods) were used. Examinations were mainly carried out by GC/MS spectrometry.

Results: N-pentylindole was detected in herbal blends containing the cannabimimetic compounds JWH-081, -122, -203, -210 and -250, respectively. Additionally, it was detected in the smoke of such materials. A preliminary test with drug sniffing dogs indicated their ability to detect pure N-pentylindole as well as herbal mixtures with JWH-compounds dosed with low concentrations of N-pentylindole.

Discussion and Conclusion: The detection of N-pentylindole in various herbal blends, powder samples and in the smoke of respective samples by GC/MS spectrometry is a successful approach on the identification of a generic marker for cannabimimetics. Considering the similarity of such compounds in basic structural elements, similar approaches could help to identify a wide range of cannabimimetic substances more easily.

1. Introduction

Among the ever-growing number of cannabimimetic substances, the fastest growing group are aminoalkylindoles (AAIs), in particular derivatives of N-pentylindole. Up to date at least 25 N-pentylindole-based synthetic cannabimimetics, ranging from "classical" JWH-018 to more recently identified compounds like APICA or UR-144 [1-5], have been found in drug preparations. Due to the ease of their syntheses and the possibilities of structure variation, authorities and forensic laboratories are likely to encounter previously unknown as well as "literature-known" cannabimimetic compounds. Since reference materials of such new compounds are not readily available, our idea was to identify a molecule that allows their facile detection regardless of their actual structure.

A promising candidate for the most widespread group of cannabimimetics was found in N-pentylindole (NPI **1**), which represents a key intermediate in the synthetic pathways of indole

based cannabimimetics as depicted in Figure 1 [6-7]. Consequently, NPI was synthesized, purified and investigated for its analytical behaviour.

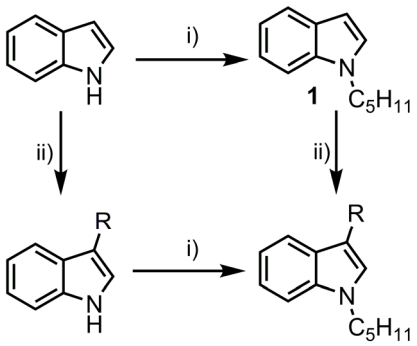

Fig. 1. Synthetic pathways to NPI-based cannabimimetics.

2. Material and Methods

2.1 Instrumentation

NMR spectra were recorded at ambient temperature on a Bruker DPX-400. Chemical shifts are reported relative to the residual undeuterated solvent peak at 7.24 ppm for ^{1}H. GC/MS spectra were recorded on a Agilent 6890N gas chromatograph fitted with a 5975 inert mass selective detector Column: HP-5-MS, 30 m; 0.25 mm, 0.25 µm; Temperature: 70°C – 350°C with 20°C/min, Gas: Helium 5.0, const flow 1.0 ml/min; Injector: SSL 250°C; Split ratio: 50; Transfer line 280°C; Source: 230°C; Range: TIC: 20 – 600 amu; Solvent delay: 4.5 min.

2.2. Synthesis and Characterization of N-Pentylindole

All solvents and reagents were purchased from commercial sources in p.a. grade and used without further purification. NPI was synthesized from indole and 1-bromopentane following literature procedures [8], purified by Kugelrohr-distillation and characterized by means of NMR and GC/MS. $C_{13}H_{17}N$; MW =187.2847; ^{1}H NMR (200 MHz, $CDCl_3$) δ = 7.72 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.35-7.20 (m, 1H), 7.16 (d, *J* = 3.2 Hz, 1H), 6.57 (d, *J* = 3.1 Hz, 1H), 4.17 (t, *J* = 7.1, 7.1 Hz, 1H), 2.06-1.75 (m, 1H), 1.54-1.30 (m, 1H), 0.98 (t, *J* = 6.7, 6.7 Hz, 1H); GC/MS (EI) *m/z* (relative intensity) 187 (81), 172 (6), 158 (8), 144 (5), 130 (100), 117 (13), 103 (15), 90 (7), 77 (14), 63 (5), 51 (3).

2.3. Herbal Blends and JWH-Compounds

All materials investigated in this work were seized by the NRW police and received for forensic analysis in the years 2011 – 2013. A total of 141 GC/MS spectra recorded during qualitative analysis of those materials was screened for NPI.

2.4. Quantitative Analysis by GC/MS

100 mg of herbal blend were extracted with 1 mL ethanol. In case of high concentrations of the JWH compounds, the resulting extract was diluted (1:50). Repeat determinations were measured in splitless mode with single ion monitoring (instrumentation see 2.1). The limits of detection and quantitation for the method were ascertained at 1.89 ng/mL and 6.30 ng/mL.

2.5. Samples for Drug Sniffing Dogs

Four samples of N-pentylindole (NPI on cloth, NPI on *Damiana* herb, NPI and JWH-210 on cloth, NPI and JWH-210 on *Damiana* herb) were prepared. They were hidden in rooms of the JVA Castrop-Rauxel and four sniffing dogs of the Justiz NRW searched the rooms separately.

3. Results

3.1. Screening of GC/MS-Chromatograms for NPI

A total of 141 GC/MS-Chromatograms of powder samples and herbal blends containing JWH-018, -081, -122, -203, -210, -250, -251 and RCS-4 were screened for residual NPI, which was identified in 46 samples (Table 1). A quantitative analysis of 10 samples showed an enormous variation in the amount of cannabimimetics (0.2 – 26 mg/mL), depending on the number of different cannabimimetic compounds in the blend. Similar observations were made for the amounts of NPI that ranged from 0 – 3.5 µg/mL. Since all data were derived from archived GC/MS spectra of qualitative analysis, it remains uncertain whether the samples in which no NPI was detected did not contain any NPI at all or if its concentration was below detection level.

Tab. 1. Results of the GC/MS screening of herbal blends.

Compound	Number of samples	Number of samples with N-pentylindole
JWH-018	18	0
JWH-081	18	2
JWH-122	26	9
JWH-203	8	5
JWH-210	62	26
JWH-250	5	4
JWH-251	2	0
RCS-4	2	0

3.2. Detection of NPI in Herbal Blend Smoke

Various samples of herbal blends containing JWH compounds were incinerated in a smoking apparatus and the smoke collected in ether/ethanol. The resulting solutions were investigated by means of GC/MS. NPI was detected in the smoke of both samples containing NPI as an impurity and in samples that contained pure JWH compounds without any NPI. The active amounts of NPI after smoking a herbal blend which contained no NPI amounted to 0.7 – 1.3 µg/mL and to 0.4 – 1.8 µg/mL in blends which were found to contain NPI impurities. Besides NPI and the respective JWH compound, a great number of decomposition products such as naphthalenol, napthol, naphthalene, naphthalenecarboxylicacidethylester, naphthoquinone, coumarine, N-pentyl-isatine and indole were identified in the smoke. Consequently, these compounds should be detectable in the body of consumers, which is in the focus of our current investigations.

3.3. Detection of NPI by Drug Sniffing Dogs

All prepared samples were individually detected by the four dogs. Further tests with a greater number of dogs from different public authorities and different JWH-compounds are in progress.

4. Conclusions

This work is a successful approach on the identification of a generic marker for various indole-based cannabimimetics. The title compound N-pentylindole, presumably a synthesis by-product or residue, was detected and quantified in herbal blends, powder samples and in the smoke of respective samples by GC/MS spectrometry. Moreover, we have shown that NPI is detectable by drug sniffing dogs even in complex matrices such as herbal blends. Given the number of known cannabimimetic compounds and their similarity in basic structural elements such as N-alkylated indoles, it is likely that similar approaches could help to enable authorities to identify forbidden or suspicious substances more easily.

5. References

[1] Bovens M, Schläpfer M. Designer Drugs/ Research Chemicals/ Legal Highs - A survey of recent seizures and an attempt to a more effective handling from a Swiss perspective. Toxichem Krimtech 2011;78:167-175.

[2] Uchiyama N, Kawamura M;, Kikura-Hanajiri R, Goda Y. Identification of two new-type synthetic cannabinoids, N-(1-adamantyl)-1-pentyl-1H-indole-3-carboxamide (APICA) and N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA), and detection of five synthetic cannabinoids, AM-1220, AM-2233, AM-1241, CB-13 (CRA-13), and AM-1248, as designer drugs in illegal products. For Tox 2012;30:114-125.

[3] Simolka K, Lindigkeit R, Schiebel H-M, Papke U, Ernst L, Beuerle T. Analysis of synthetic cannabinoids in “spice-like” herbal highs: snapshot of the German market in summer 2011. Analyt Bioanalyt Chem 2012;404:157-171.

[4] Jankovics P, Váradi A, Tölgyesi L, Lohner S, Németh-Palotás J, Balla J. Detection and identification of the new potential synthetic cannabinoids 1-pentyl-3-(2-iodobenzoyl)indole and 1-pentyl-3-(1-adamantoyl)indole in seized bulk powders in Hungary. For Sci Int 2012;214:27-32.

[5] Denooz R, Vanheugen J-C, Frederich M, de Tullio P, Charlier C. Identification and structural elucidation of four cannabimimetic compounds (RCS-4, AM-2201, JWH-203 and JWH-210) in seized products. J Anal Toxicol 2013;37:56-63.

[6] Huffman JW, Zengin G, Wu M-J, Lu J, Hynd G, Bushell K, Thompson A, Bushell A, Tartal C, Hurst DP, Reggio PH, Selley DE, Cassidy MP, Wiley JL, Martin BR. JWH-syntheses: Structure–activity relationships for 1-alkyl-3-(1-naphthoyl)indoles at the cannabinoid CB1 and CB2 receptors: steric and electronic effects of naphthoyl substituents. New highly selective CB2 receptor agonists. Bioorg Med Chem 2005;13:89-112.

[7] Huffman JW, Szklennik PV, Almond A, Bushell K, Selley DE, He H, Cassidy MP, Wiley JL, Marti BR. 1-Pentyl-3-phenylacetylindoles, a new class of cannabimimetic indoles. Bioorg Med Chem Lett 2005;15:4110-4113.

[8] Smith VJ. Synthesis and pharmacology of N-alkyl-3-(halonaphtoyl)indoles. Ph.D. Dissertation, 2008, Clemson University, South Carolina, USA.