Short Communication

Analytical Data for Identification of the Cannabimimetic Phenylacetylindole JWH-203

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Abstract

We identified the synthetic cannabinoid JWH-203 [1-pentyl-3-(2'-chlorophenylacetyl) indole] by coordination of various spectroscopic analytical procedures; the data that we obtained may be useful for confirming the presence of this phenylacetylindole in illegally distributed products. In previous studies, several molecules that were identified as synthetic cannabinoids were found in different products advertised as herbal mixtures; the identification of new psychotropic molecules surreptitiously included in products allows them to be added to the official list of psychotropic substances possessing cannabimimetic activity. We used liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry techniques to determine basic information about the structure of JWH-203; our data were corroborated by the results of liquid chromatography–ultraviolet spectroscopy and confirmed by nuclear magnetic resonance analysis and high-resolution accurate mass detection.

Introduction

Various psychotropic substances are being sold, transported, and distributed worldwide, both legally and illegally. Various papers refer on the structure-activity relationship for some cannabimimetic substances at the cannabinoid CB_1 and CB_2 receptors (1–4) and more recently, various synthetic cannabinoids were identified as psychoactive adulterants in herbal products (5–10). Analytical data that would be useful for identifying these compounds are not readily available in the literature, although several authors have published the analytical details of single or limited numbers of molecules with cannabimimetic structures that were isolated from herbal products (11–21). In effect, not all data necessary to identify the structures of all cannabimimetic substances by instrumental analysis are currently available. Moreover, it is only possible to include substances on a country's official list of psychotropic substances after the presence of individual synthetic cannabinoids have been identified in products distributed within that country. The publication of analytical details produced by various spectroscopic methods is an important means by which to fight the adulteration of herbal products.

In collaboration with Carabinieri Headquarters for Healthcare NAS (Anti Fraud Squad of Milan), Carabinieri Military Police (IT)—Ministry of Health, our analytical research laboratories were able to identify some synthetic cannabinoids in various herbal products. Recently, following an official mission of N.A.S. dated November 15, 2010, we identified the molecule JWH-203 in a sample withdrawn from a 2.3-kg sized powder imported from China and declared as a "chemical". The information about JWH 203 was disseminated via the "Early Warning System" of the European Monitoring Centre of Drugs and Drug Abuse (EMCDDA) on Septempber 29, 2010, after the Latvian Institute of Organic Synthesis identified 1.8 kg seized powder coming from China as JWH-203 (22). Actually only the EI-MS and ATR-IR spectra of JWH-203 result to be available in Toxichem Krimtech (23).

As the source of the analytical approach, we have adapted the methods of Auwärter et al. (13) and Uchiyama et al. (14).

A combination of spectroscopic methods was used. JWH-203 has a chlorophenylacetyl group in place of the naphthoyl ring present in most aminoalkylindole cannabinoid compounds which were identified in "herbal" products so far. The structure differs from JWH-250 and JWH-251 (20,21), which have a methoxy and a methyl substituent, respectively, in the 2' posi-

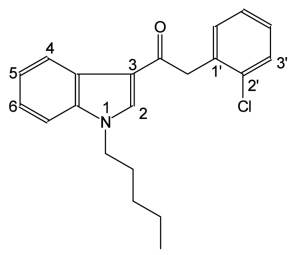

Figure 1. Structure of the cannabinomimetic compound 1-pentyl-3-(2'-chlorophenylacetyl) indole (JWH-203).

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tion. The pharmacological cannabimimetic activity of JWH-203 is described by Huffman (4). Concerning the affinities for the cannabinoid CB_1 and CB_2 receptors, Huffman et al. (3) affirms that 3-(2-substituted phenylacetyl) indoles have good to high affinity for the CB_1 receptor and that JWH-203 is one showing the highest affinity. The work presented herein describes the methods that we used to identify and then confirm the structure of JWH-203, also known as 1-pentyl-3-(2'-chlorophenylacetyl) indole, as reported in Figure 1.

Experimental

Chemicals and reagents

HPLC-grade formic acid, methanol, and water were purchased from VWR International (Milan, Italy). Pall GH Polypro filters (hydrophilic polypropylene membrane) with 0.45-µm pore size were also purchased from VWR International.

Sample preparation and extraction

The sample delivered from the Anti Fraud Squad of Milan derived from a greater quantity of a substance declared as "chemical" and imported from China. The analytical question was the qualitative identification of the presumed synthetic cannabinoid substance.

A 250-mg aliquot of the sample was placed in a 10-mL glass vial, and 10 mL of methanol was added. This was sonicated for 10 min, then left to settle for 10 min, and then filtered through a GH Polypro 0.45-µm filter (first solution). The extract was diluted 1:20 with methanol.

Instrumental analysis

The sample solution was qualitatively analyzed by liquid chromatography–mass spectrometry (LC–MS–MS) with positive electrospray ionization (ESI^+). The instrument was a Shimadzu integrated HPLC system (Shimadzu Italia), which consisted of a Shimadzu CBM 20 A system controller, two Shimadzu LC 20 AD XR pumps (including a Shimadzu degasser DGU A3), a Shimadzu SIL 20 ACXR autosampler, and a Shimadzu CTO 20 AC column oven. The analytes were detected using an Applied Biosystems 2000 MS with Analyst software (Version 1.4.1) equipped with a TurboIonSpray Source. A stainless steel column (Supelcosil) (150 mm × 3 mm) with a 120-Å pore diameter (3-µm particle size) was used. The analysis was carried out with a binary mobile phase consisting of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol). The elution program was 40% B (5 min hold) to 60% B (5–15 min), then 60% B with 5 min hold (15–20 min), then 80% B (20–35 min), followed by 98% B (35–50 min) at a flow rate of 0.3 mL/min.

The LC–MS–MS-ESI^+ conditions for multiple reaction monitoring (MRM) were established using the first solution (see Sample preparation and extraction section). This solution was injected by continuous infusion at 10 µL/min in +Q1 mode and +MS2 mode. The following conditions were adopted: declustering potential (DP; 34 V), focusing potential (FP; 343 V), entrance potential (EP; 11 V), cell entrance potential (CEP; 15 V), collision energy (CE; 40 V), and cell exit potential (CXP; 34 V). The mass spectrum of JWH-203 contains the protonated molecule $[M+H]^+$ at *m/z* 340; this ion produces abundant daughter ions at *m/z* 125 and *m/z* 214.

The same chromatographic conditions (without formic acid in A and B phases) were adopted in the LC–PDA analysis to produce the UV spectrum. LC was performed with an HPLC Shimadzu system consisting of a system controller SCL–10 A vp; two LC-10AD VP pumps; a Rheodyne injection valve (7725i, 20-µL loop); a diode-array detector (DAD) SPD-M10A VP; and Shimadzu Class-VP-5 acquisition software. Separation was achieved on a CHROMPACK C_{18} 110 A column (250 × 4.6 mm, 5 mm) from Phenomenex (Milan, Italy) using the sample solution diluted as described in the Sample preparation and extraction section. The wavelength of the PDA detector was set from 190 to 400 nm.

GC–MS analyses were carried out with a Shimadzu 2010 GC coupled to a Shimadzu 2010 MSD quadrupole MS (Shimadzu, Italia). Helium was used as the carrier gas at a flow rate of 1 mL/min. Suitable identification of the analyte was achieved using an Equity-5, a poly (5% diphenyl, 95% dimethylsiloxane) stationary phase column with 30 m × 0.25-mm internal diameter and 0.25-µm film thickness (Supelco, Milan, Italy). The oven temperature program was initially 80°C (held for 1 min.) and then increased to 300°C at a rate of 5°C/min (held for 10 min). The injector temperature was 240°C, and the splitless injection mode (1 min) was used. The ion source and the transfer line temperatures were 200°C and 320°C, respectively. The MS instrument was operated in positive electron ionization mode (EI^+) with automatic gain control, with 70 eV of electron energy and 250 mA of emission current. The MS was operated in the full scan mode from *m/z* 40 to 400. An aliquot (1 µL) of the sample solution, diluted as described in the Sample preparation and extraction section, was injected for the GC–MS analysis.

The nuclear magnetic resonance (NMR) spectrometer AV-600 MHz Bruker was operated at a proton frequency of 600.13 MHz and a carbon frequency of 151.91 MHz; it was equipped with a 5-mm TXI Z-GRAD probe. The ^1H and ^{13}C NMR spectra were recorded at 25°C. DMSO-d_6 was the optimal solvent. By performing a set of mono- and two-dimensional experiments, we were able to establish the complete assignment of the following signals: ^1H NMR, correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple bond coherence (HMBC), and heteronuclear multiple quantum coherence (HMQC).

To evaluate the accurate mass, the sample was analyzed on an Agilent 1200SL, 6210 TOF-$ESI(^+)$. For this system, a Hypersil GOLD column (100 × 2.1 mm, 3-µm particle size, Thermo Scientific, Bellefonte, PA) was used; it was operated in gradient mode. The mobile phases were as follows: solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The elution program was 50% B (5 min hold) to 100% B (5–8 min), then 100% B (8–20 min) at a flow rate of 0.4 mL/min. Dry gas (N_2) was set at 10 L/min and interface temperature at 350°C, fragmentor at 120 V, and skimmer at 60 V. The MS data were recorded with a range of *m/z* 100–1200 in the scan mode.

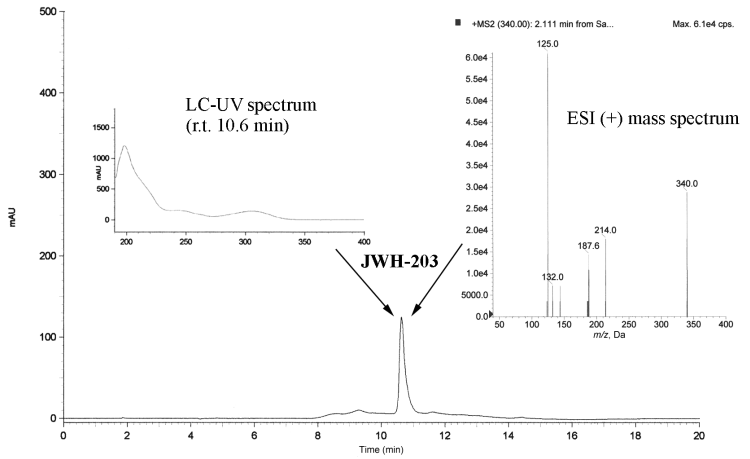

Figure 2. Data from HPLC–DAD (UV spectrum at λ = 305.8 nm) and LC–MS–MS-ESI+ for the intense peak appearing at retention time 10.6 min (JWH-203).

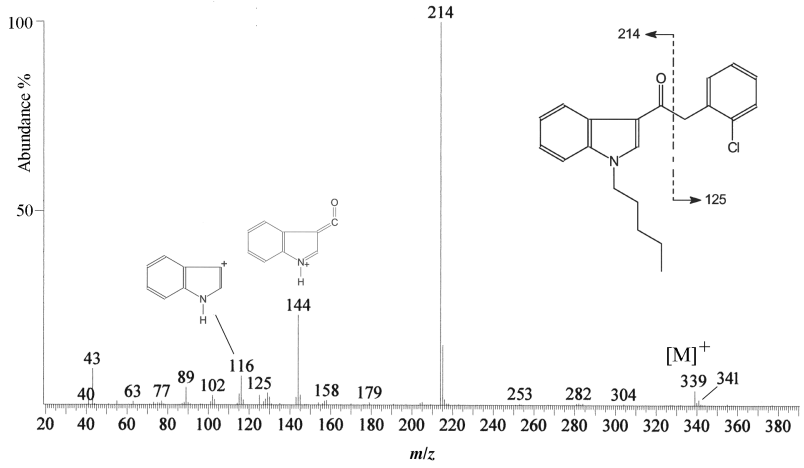

Figure 3. GC–MS mass spectrum of the intense peak appearing at retention time 44.5 min (JWH-203) and principal ion attributions.

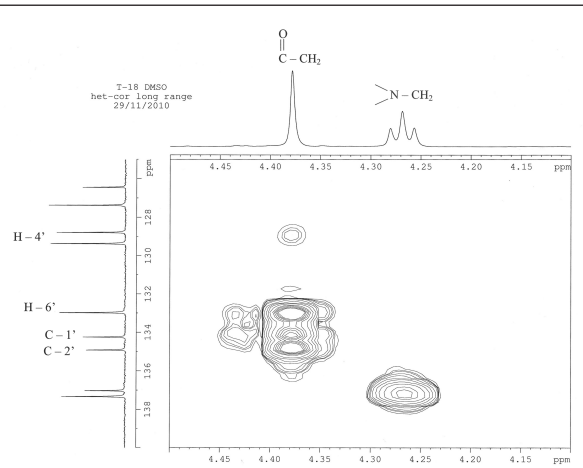

Figure 4. NMR HETCOR long-range C–H coupling, which identifies the ortho Cl substitution.

Results and Discussion

The unknown chemical, detected as a peak at 10.6 min in the HPLC–DAD trace of the test solution, was characterized by LC–UV spectroscopy (maxima at 198.3, 244.2, and 305.8 nm) (Figure 2). This UV spectrum shows some similarities to the spectra of other synthetic cannabinoids. The LC–MS–MS spectrum in Figure 2 has a major signal at *m/z* 340 $[M+H]^+$. In the accurate mass spectrum, the major ion peak showed a protonated molecular ion signal at *m/z* 340.1471 in the positive mode, and the molecular weight was compatible with the molecular formula $C_{21}H_{22}NOCl$. The LC–MS–MS spectrum shown in Figure 2 provided evidence that a *m/z* 125 ion was very abundant, and the GC–MS spectrum in Figure 3 revealed a very abundant *m/z* 214 ion. Considering that two other ions, *m/z* 144 and *m/z* 116, are present in the same GC–MS spectrum characteristic of the known structure of the *m/z* 214 ion in similar synthetic cannabinoids (e.g., JWH-018 and JWH-250) (17,20,21), it is evident that the *m/z* 125 ion completes the structure of the synthetic cannabinoid with a molecular weight of *m/z* 339. The structure of the *m/z* 125 ion, which contains Cl as substitute in a benzylic ring, was confirmed by an isotopic ion at *m/z* 341, as revealed in the GC–MS spectrum, where the ion $[M]^+$ 339 represents the molecular ion.

The ^1H NMR and 2D COSY and TOCSY experiments permitted us to assign spin systems to indole and bisubstituted benzyl structures. The absence of a symmetric system AA' BB', typical of para substitution, was evident and allowed us to exclude this isomer. The presence of clear symmetry in the region between 7.5 and 7.2 ppm (DMSO) permitted us to exclude the meta substitution. The long-range heteronuclear chemical shift correlation (HETCOR) (C–H) coupling revealed the following useful data that allowed us to identify the ortho substitution with certainty: the benzylic CH_2 showed one three-bond coupling with a quaternary C and one three-bond coupling with only an aromatic CH at 132.8 ppm (Figure 4). This C–H coupling would occur only with ortho substitution, because in the case of meta substitution, the benzylic CH_2 would have connections with two CH aromatics. Our results can be simplified in Figure 5.

The accurate mass measurement revealed $[M+H]^+$ at 340.1471; this measurement is compatible with the molecular structure of 1-pentyl-3-(2'-chlorophenylacetyl) indole (JWH-203).

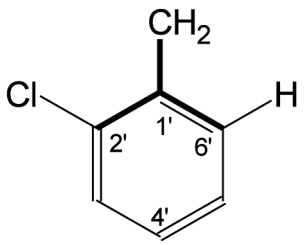

Figure 5. Chemical structure corresponding to the long-range HETCOR (C–H) coupling data: the benzylic CH_2 coupling with a quaternary C and with an aromatic CH.

Conclusions

Our research focused on applying methods to identify new cannabinoids as they appear in the market, and to provide simple analytical information that will be useful for molecular identification. In the present paper, we identified a substance that has not yet been found by us in herbal mixtures, but was imported to Italy as a raw material around November 2010. Our analytical data confirmed the identity of JWH-203 [1-pentyl-3-(2'-chlorophenylacetyl) indole], and our method permitted us to rapidly identify this synthetic cannabinoid and include it in a database containing JWH compounds that have been identified in the illegal market.

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