Forensic Science International 198 (2010) 31–38

Contents lists available at ScienceDirect

Forensic Science International

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

Chemical analysis of synthetic cannabinoids as designer drugs in herbal products

Nahoko Uchiyama, Ruri Kikura-Hanajiri, Jun Ogata, Yukihiro Goda *

National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

ARTICLE INFO

Article history:
Received 19 August 2009
Received in revised form 5 January 2010
Accepted 7 January 2010

Keywords:
Cannabicyclohexanol
JWH-018
Oleamide
CP-47,497
JWH-073
Synthetic cannabinoid

ABSTRACT

Several synthetic cannabinoids were found in 44 of 46 different kinds of herbal products that are currently distributed on the illegal drug market in Japan due to their expected narcotic effects. Gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) analyses indicated that most of the products contained two major synthetic cannabinoids: (1*RS*,3*SR*)-3-[2-hydroxy-4-(2-methylnonan-2-yl)phenyl]cyclohexan-1-ol, renamed cannabicyclohexanol with the agreement of Pfizer Inc., and/or 1-naphthalenyl(1-pentyl-1*H*-indol-3-yl)methanone, named JWH-018. Oleamide (*cis*-9,10-octadecenoamide), which is an endogenous cannabinoid, was also detected in 7 products. Additionally, two synthetic cannabinoids were identified as minor components in some products. One was (1*RS*,3*SR*)-3-[2-hydroxy-4-(2-methyloctan-2-yl)phenyl]cyclohexan-1-ol, which is named CP-47,497 and is a homolog of cannabicyclohexanol. The other was 1-naphthalenyl(1-butyl-1*H*-indol-3-yl)methanone, which is named JWH-073 and is a homolog of JWH-018. These compounds were reported as synthetic cannabinoids possessing pharmacological cannabimimetic activity. The concentrations of cannabicyclohexanol, JWH-018 and oleamide in the products ranged from 1.1 to 16.9 mg/g, 2.0 to 35.9 mg/g and 7.6 to 210.9 mg/g, respectively, and showed considerable variation. In this study, details of the analysis and identification of these synthetic cannabinoids in herbal products being sold on the Japanese drug market are described.

1. Introduction

Cannabis sativa L. (cannabis, marijuana) is widely abused throughout the world because it contains psychoactive cannabinoids such as Δ^9-tetrahydrocannabinol. In Japan, the abuse of cannabis has recently increased, along with the abuse of a number of herbal products that are also distributed on the drug market for their cannabis-like effects when smoked. Although the active components of these herbal products were not identified, we recently found two synthetic cannabinoids as adulterants in herbal products that had been sold commercially as incense. One was (1*RS*,3*SR*)-3-[2-hydroxy-4-(2-methylnonan-2-yl)phenyl]cyclohexan-1-ol (cannabicyclohexanol, **1**), which was detected together with its *trans*-diastereomer (**2**) [1]; the other was 1-naphthalenyl(1-pentyl-1*H*-indol-3-yl)methanone (JWH-018, **3**) [2] (Fig. 1). Compound **1**, which is a non-classical cannabinoid, was first synthesized by Pfizer Inc. in 1979 [3] and reported as a potent cannabinoid analog in the 1990s [4–8]. In consideration of its general properties, this compound was renamed cannabicyclohexanol with the agreement of Pfizer Inc. Compound **3**, which is an aminoalkyl naphthoyl indole derivative, was first synthesized by Huffman et al. in 1998 and reported as a potent cannabinoid receptor agonist possessing *in vivo* pharmacological cannabinoid analog activity [9–12]. Auwärter et al. also reported the identification of these compounds from some herbal products around the same time [13]. In January 2009, Germany's Health Minister announced that compounds **1** and **3** and their homologs had been identified as active components in a mislabeled mixture of herbs. Control of these compounds was begun in Germany immediately thereafter (on 22 January 2009) [14]; Austria, France and other countries initiated legal actions to ban or otherwise control these synthetic cannabinoids over the preceding months [15].

In this study, we report the analysis and identification of several synthetic cannabinoids as adulterants in herbal products using gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS). In addition, the results of a survey of herbal products being sold on the Japanese market are described.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade acetonitrile, betamethasone valerate (internal standard, IS) and α-tocopherol were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Authentic cannabicyclohexanol (**1**) and JWH-018 (**3**) were isolated from herbal

* Corresponding author. Tel.: +81 3 3700 9154; fax: +81 3 3707 6950.
E-mail address: goda@nihs.go.jp (Y. Goda).

0379-0738/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2010.01.004

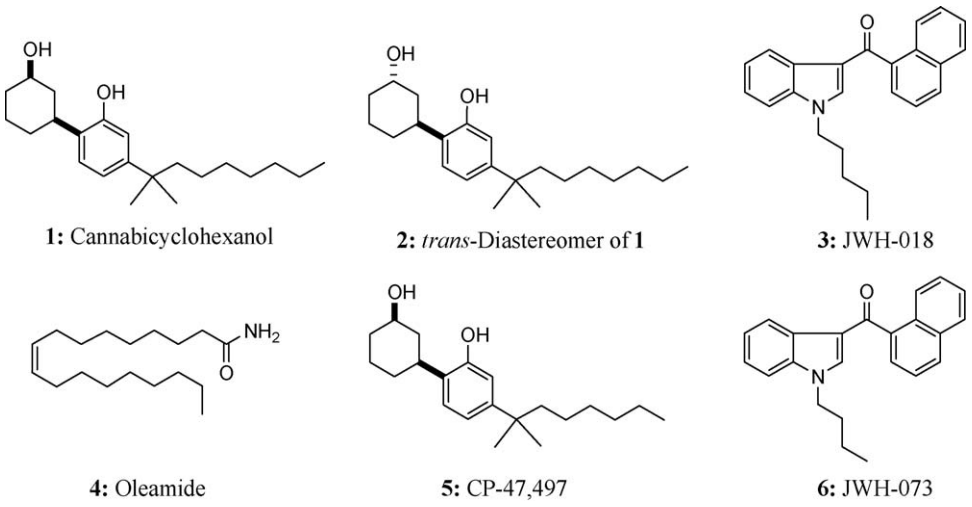

Fig. 1. Structures of detected cannabimimetic compounds.

products and identified in our previous studies [1,2]. Oleamide (**4**) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). CP-47,497 (**5**) and JWH-073 (**6**) were purchased from Cayman Chemical (Ann Arbor, MI). All other common chemicals and solvents were of analytical reagent grade or HPLC-grade.

2.2. Samples

Forty-six herbal products being sold in Japan for their expected cannabis-like effects were purchased via the Internet from June 2008 to June 2009. All products had different names and were contained in different packages. Thirty-nine products were in the form of dried leaves and 7 products were in the form of cigarettes. In most cases, the labels on the packages indicated that the products contained between 1 and 3 g of a mixture of plants.

2.3. Instrumentation

The sample solutions were qualitatively and quantitatively analyzed by using an ultra-performance liquid chromatography-electrospray ionization-mass spectrometer (UPLC-ESI-MS), consisting of an ACQUITY UPLC system equipped with a Single Quadrupole Detector (SQD) mass detector and a photo diode array (PDA) (Waters, Milford, MA). The sample solutions were separated using an ACQUITY UPLC HSS T3 column (2.1 mm i.d. × 100 mm, 1.8 μm; Waters) protected by a Van Guard column (2.1 mm i.d. × 5 mm, 1.8 μm; Waters) at 40 °C. The following gradient system was used with a mobile phase A (0.1% formic acid in water) and a mobile phase B (0.1% formic acid in acetonitrile) delivered at 0.3 ml/min: A:B 50:50 (0–3 min) followed by −20:80 (5–10 min). The injection volume was 1 μl. The wavelength of the PDA detector for screening was set from UV–vis 190 to 500 nm. ESI mass analysis was carried out in positive mode. Nitrogen gas was used for desolvation at a flow rate of 650 l/h at 350 °C. The capillary voltage was 3000 V, and the cone voltage was 30 V. MS data were recorded in the full scan mode (*m/z* 50–500). For qualitative analysis of cannabicyclohexanol (**1**) and JWH-018 (**3**), the protonated molecular peaks ([M+H$^+$]) of these compounds and IS were monitored in the scan mode. The monitoring ions were as follows: cannabicyclohexanol (**1**) (*m/z* 333), JWH-018 (**3**) (*m/z* 342) and betamethasone valerate (IS, *m/z* 477).

MS analysis was also performed by GC-MS in electron impact (EI) mode at 70 eV electron energy. The GC-MS analysis was performed on a Hewlett-Packard 6890N GC with a 5975 mass selective detector using a capillary column (HP1-MS capillary; 30 m × 0.25 mm i.d., 0.25 μm film thickness) at 0.7 ml/min and helium gas as a carrier. The injector temperature was 200 °C and splitless injection was employed with a split value on-time of 1.0 min. The initial column temperature was 80 °C (held for 1 min), and was increased at a rate of 5 °C/min to 190 °C (held for 15 min) followed by 10 °C/min to 310 °C (held for 5 min). The mass selective detector was kept at 280 °C. Data were obtained in full scan mode with a scan range of *m/z* 40–550. The analysis was performed under the established methods and conditions as used in the analysis of designated drugs (Shitei-Yakubutsu) controlled by the Pharmaceutical Affairs Law of Japan described in our previous report [16]. In the case of oleamide (**4**), the relative content was calculated from the area of the target molecular ion peak to that of the standard sample (1 mg/ml methanol) following GC-MS.

2.4. Standard solutions

For qualitative analysis, standard solutions were prepared for each compound (cannabicyclohexanol (**1**), JWH-018 (**3**), oleamide (**4**), CP-47,497 (**5**), JWH-073 (**6**) and α-tocopherol) at a concentration of 1.0 or 0.1 mg/ml in methanol.

2.5. Calibration curves

The concentrations of cannabicyclohexanol (**1**) and JWH-018 (**3**) in the samples were calculated using the peak area ratios of **1** versus IS at 275 nm, and those of **3** at 314 nm versus IS at 240 nm, respectively. Cannabicyclohexanol (**1**) and JWH-018 (**3**) were diluted with methanol to prepare calibration solutions containing 10, 25, 50, 100, 250 and 500 μg/ml. The solutions also included IS (betamethasone valerate) at 100 μg/ml.

2.6. Precision and accuracy of the method

The precision and accuracy of the method were evaluated by analyzing triplicates of the standard solutions containing 10, 50, 500 μg/ml of each compounds. Accuracy, expressed as bias, was calculated as difference between the amounts of each compound added and recovered.

2.7. Preparation of sample solution

For quantitative analysis, the product (10 mg) was crushed into powder and extracted with 1 ml of methanol including IS (100 μg/ml) under ultrasonication for 10 min. After centrifugation (5 min at 3000 rpm), the solution was passed through a centrifugal filter (Ultrafree-MC, 0.45 μm filter unit; Millipore, Bedford, MA). For qualitative analysis, the product (50 mg) was crushed into powder and extracted with 2 ml of methanol under ultrasonication for 10 min. After evaporation to dryness, the extract was dissolved with 200 μl of methanol and the solution was filtered through the centrifugal filter. If necessary, the solution was diluted with methanol to a suitable concentration.

3. Results

3.1. Analyses of herbal products obtained from the Japanese market

Forty-six herbal products currently being sold in Japan for their expected cannabis-like effects were purchased via the Internet. These products appeared in primarily two forms i.e., as bits of dried leaves or as cigarettes (Table 1).

GC-MS and LC-MS analyses indicated that most of the products contained the two major compounds (**1** and **3**) (Figs. 1 and 2a–p) and these compounds were reported in our previous studies [1,2]. Oleamide (*cis*-9,10-octadecenoamide, **4**), which shows cannabinoid-like behavioral effects [17,18], was also detected at 42.96 min in some products by GC-MS analysis, and this was also in agreement with a previous study (Figs. 1 and 2a and e) [13]. Compound **4** was not clearly detected by LC-MS analysis. Compound **2**, which is detected at 47.30 min, was considered as *trans*-diastereomer of **1** based on comparison with the previously determined mass spectrum of reported data of the compound (**2**) (Figs. 1 and 2a, c, f, h, l, and o) [13]. Furthermore, it was revealed that 37 products contained α-tocopherol by the direct comparison of the GC-MS data to those of the authentic sample (Table 1).