Identification of a Cannabinoid Analog as a New Type of Designer Drug in a Herbal Product

Nahoko UCIIYAMA, Ruri KIKURA-HANAJIRI, Nobuo KAWAHARA, Yuji HAISHIMA, and Yukihiro GODA*

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

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A new type of designer drug, a cannabinoid analog (1), was found in a herbal product distributed on the illegal drug market in Japan in expectation of its narcotic effect. The structure of 1 was identified by LC-MS, GC-MS, high-resolution MS, and NMR analyses. Compound 1 showed a molecular weight of 332, and accurate mass measurement exhibited its elemental composition to be C22H36O2. Together, the mass and NMR spectrometric data revealed that 1 was (1RS,3SR)-3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]cyclohexan-1-ol, which was first synthesized in 1979 by a group at Pfizer Inc. and reported as a potent cannabinoid analog possessing cannabinoid receptor binding activity and analgesic activity in the 1990s. This is the first report to identify a cannabinoid analog in an illegal drug.

Key words cannabinoid analog; designer drug; herbal product; (1RS,3SR)-3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]cyclohexan-1-ol; drug abuse

Many types of chemicals are widely distributed and abused as psychotropic substances. In Japan every year this decade, a market survey of illegal drugs is performed by the Ministry of Health, Labour and Welfare.1–9 Following the results of the survey, the compound identified and recognized as a designer drug10–12 came to be strictly controlled by the Narcotics and Psychotropic Control Law or by the Pharmaceutical Affairs Law as designated substances (Shitei-Yakubutsu).13–16 In 2008, seven new designer drugs were classified as narcotics or designated substances, and all of them are analogs of phenylethylamine or tryptamine.

Cannabis sativa L. (cannabis, hemp, marijuana, marihuana) is widely abused around the world because it contains psychoactive cannabinoids, such as Δ9-tetrahydrocannabinol (Δ9-THC), which contains no amine groups (Fig. 1). In the past few decades, a number of analogs of Δ9-THC were synthesized, and their structure–activity relationships were studied.17,18 In the 1980s, a group at Pfizer Inc. explored the development of analgesics using potent synthetic cannabinoids.19–22 After the discovery of cannabinoid receptors, type 1 (CB1, central type) and type 2 (CB2, peripheral type), as well as the discovery of an endogenous cannabinoid, their physiological roles were elucidated; a number of cannabinoid analogs were then newly synthesized, and their pharmacological activity for the treatment of various diseases was studied.23,24

Recently, cannabis abuse seems to have spread in Japan. In this study, we identified a novel designer drug (1) possessing cannabinoid activity as an adulterant in a herbal product (Fig. 1). Compound 1 was first synthesized by Pfizer Inc. in 1979,25 and reported as a cannabinoid analog in the 1990s.26–30 Although many designer drugs having phenethylamine, tryptamine, and pipеразine structures have been found,10–12 this is the first report to identify a non-nitrogenated compound, a phencylclohexane derivative having cannabinoid activity.

Experimental

Chemicals and Reagents HPLC-grade acetonitrile and all other chemicals (analytical grade) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Centrifugal filter devices (Ultrafree-MC, 0.45 μm filter unit) were from Millipore (Bedford, MA, U.S.A.). Samples A product was purchased via the Internet (June 2008). The product was described as a herbal mixture and had the appearance of dried plants. The ingredients were listed as “Baybean,” “Blue lotus,” “Dwallf skull-cap,” “Indian warrior,” “Lion’s tail,” “Maconha brava,” “Marshmallow,” “Pink lotus,” “Red clover,” “Rose,” “Siberian motherwort,” “Vanilla,” and “Honey.” Preparation of Sample Solution A product (20 mg) was crushed into powder and extracted with 2 ml of methanol under ultrasonication for 10 min. After centrifugation (5 min at 3000 rpm), the solution was filtered through a centrifugal filter device.

Instrumentation Gas chromatography-mass spectrometry (GC-MS) in the electron impact (EI) mode at 70 eV of electron energy was used. Analysis was performed on a Hewlett-Packard 6890N GC with a 5975 mass selective detector using a capillary column (HP-1 MS capillary, 30 m × 0.25 mm i.d., 0.25 μm film thickness) and helium gas as a carrier. An initial column temperature of 80°C was employed and the temperature was increased at a rate of 5°C/min to 190°C and at a second rate of 10°C/min to 310°C. Data were obtained in a full scan mode with a scan range of m/z 40–550. An ultra-performance liquid chromatography-electrospray ionization-mass spectrometer (UPLC-ESI-MS), consisting of a Waters ACQUITY UPLC system equipped with a Single Quadrupole Detector (SQD) mass detector and a photo diode array detector (PDA) Waters, Milford, MA, U.S.A.), was also used. The sample solutions were separated using a Waters ACQUITY UPLC HSS T3 column (2.1 × 100 mm i.d., 1.8 μm; Waters) at 40°C. The mobile phase A : B (acetonitrile) delivered at 0.3 ml/min; A : B 50 : 50 (0 min): 80 : 20 (40–40 min). The injection volume was 5 μl. The wavelength of the PDA detector for screening was set from UV 190 to 400 nm, and chromatographic peaks were monitored at UV 254 and 280 nm. Mass analysis by the ESI was used in both a positive and a negative mode. Nitrogen gas was used for desolvation at a flow rate of 600 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 150–700). Preparative TLC was carried out using a silica gel plate (silica gel 60, 20 × 20 cm, 0.5 mm, Merck, Dormardstadt, Germany).

Isolation of Compound 1 A product (3 g) was extracted with 100 ml of methanol by ultrasonication for 1 h. After the extraction was performed three times, the supernatant was evaporated to dryness. The extract was subjected to preparative silica gel TLC using CHCl3–acetone (4/1) as developing solvent. A portion of the silica gel in the TLC plate was taken and eluted with CHCl3–MeOH (1/1) to give a fraction 1. Repeated fractionation of fr. 1 by preparative silica gel TLC with CHCl3–MeOH (20/1) gave compound 1 (15 mg) as an off-white solid.

Measurement of Accurate Mass The accurate mass of the target compound was measured by the LTQ Orbitrap XL instrument (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) with the direct-infusion ESI positive and negative ion modes under the following conditions: solvent flow rate 5 μl/min, sheath gas flow rate 20 arb, auxiliary gas flow rate 10 arb, spray voltage 5 kV, capillary temperature 275°C, capillary voltage 4 V, and tube voltage 3 kV.

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Results and Discussion

In the sample solution of the product, an unknown main peak was detected by GC-EI-MS and by LC-ESI-MS analyses. The former found a peak at 47.9 min and showed four ion peaks at m/z 333 [M+H]+, 315 [M+H–18]+ in the positive scan mode and at m/z 331 [M–H]– in the negative scan mode. The PDA-sliced UV spectrum of the peak exhibited maxima at 220 and 275 nm and minima at 212 and 249 nm. The accurate mass of 1 revealed [M+H]+ at m/z 333.27918 in the positive scan mode and [M–H]– at 331.26442 in the negative scan mode, suggesting molecular formulae of C22H37O2 and C22H36O2, respectively. The errors between the observed mass and theoretical formula of [M+H]+ and [M–H]– are +0.71 and −0.18 m/mu, respectively. The 1H- and 13C-NMR spectra of 1 exhibited 36 protons and 22 carbons. These results suggested that 1 contained oxygen atoms but no nitrogen atoms.

The 1H-NMR spectrum of 1 exhibited 36 non-exchangeable protons, including three methyl signals at δ 1.22 (6H, s) and 0.83 (3H, t, J = 7.2 Hz), as well as ABX-type aromatic proton signals at δ 7.06 (1H, d, J = 8.2 Hz), 6.84 (1H, dd, J = 8.2, 2.0 Hz), and 6.67 (1H, d, J = 2.0 Hz), as shown in Table 1. In addition, the 1H-NMR spectrum also showed two methine proton signals at δ 2.86 (1H, tt, J = 12.4, 3.1 Hz) and 3.76 (1H, tt, J = 11.0, 4.1 Hz), and a characteristic signal assignable to hydroxy proton at δ 4.51 (1H, brd, J = 4.6 Hz) and 9.01 (1H, brs), respectively. The 13C-NMR spectrum of 1 showed 22 carbon signals, including three methyls, ten methylenes, two methines with one oxygenated carbon (δ 71.2) and one quaternary carbon, three aromatic carbons (δ 113.1, 118.5, 126.3), and three quaternary carbons (δ 128.7, 149.1, 152.3). The presence of three partial structures (1,3-substituted cyclohexyl group, 1,1-dimethyloctyl group, and 1,2,4-substituted phenyl) was suggested from its DQF-COSY, HMOC, and HMBC spectra (Table 1, Fig. 2). The connectivity of these groups was deduced from the HMBC spectrum (Table 1, Fig. 2). A methine proton at δ 2.86 (H-3) of the cyclohexyl group correlated to the phenyl carbons at δ 152.3 and 126.3 (C-2′, C-6′), and two aromatic protons, at δ 6.67 and 6.84 (H-3′, H-5′) of the phenyl group, showed correlations to the quaternary carbon at δ 37.3 (C-1′). In addition, the irradiation of the hydroxyl proton at δ 9.01 (2′-OH) resulted in ROE on the aromatic proton (H-3′), as shown in Fig. 3. The relative configuration between two methine protons at C-1 and C-3 established a cis configuration by the ROE

<table>
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<th>No.</th>
<th>13C</th>
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<tr>
<td>4</td>
<td>31.7</td>
<td>1.30</td>
<td>2, 3, 5, 1</td>
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</table>

(a) Recorded in CDCl3 at 60 and 800 MHz (1H) and 150 and 200 MHz (13C), respectively; data in ppm (J in Hz). (b) J=8 Hz, the proton signal correlated with the indicated carbons. (c) J=4 Hz. (d) Recorded in DMSO-d6.
correlations (Fig. 3). Therefore, the structure of 1 is finally elucidated as (1RS,3SR)-3-[4-(1,1-dimethylcyclohexan-1-ol.

The deduced structure has already been synthesized by Pfizer Inc. and reported as a cannabinoid analog.25,26) Pharmacological studies showed that 1 has potent cannabinoid receptor binding activity in vitro and analgesic activity in vivo mice.27—30) Compton et al. reported that compound 1 was approximately 5-fold more potent than Δ9-THC at the viewpoint of pharmacological activity.28)

This is the first case in which 1 has been detected as a designer drug and an ingredient in a herbal product. Pfizer Inc. has also reported many analogs of 1 and has described their synthesis with pharmacological data.19,22,31,32) Additionally, various cannabinoid analogs are synthesized one after another and their pharmacological activity studied for the development of new useful drugs for the treatment of a number of diseases.23,24) This situation alerts us that these described cannabinoid analogs other than 1 may be found as designer drugs or adulterants in illegal products in the near future. To avoid health problems and abuse caused by new designer drugs, we have to continuously monitor such compounds during our surveillance.

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References