

Identification of a new synthetic cannabinoid in a herbal mixture: 1-butyl-3-(2-methoxybenzoyl)indole

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Abstract Two unknown cannabimimetic compounds were detected in a seized herbal mixture after gas chromatography–mass spectrometry (GC–MS) screening. To elucidate the chemical structures, 0.3 g of the dried plant material was extracted with methanol and concentrated under reduced pressure. The extract was purified by silica gel column chromatography with methylene chloride and methanol. Pure compounds were isolated by preparative high-performance liquid chromatography (HPLC) and then analyzed by electrospray ionization (ESI) mass spectrometry (MS) with direct flow injection, high-resolution ESI-time-of-flight (TOF)–MS and one-dimensional and two-dimensional nuclear magnetic resonance (NMR) spectroscopy. GC–MS spectra showed that the base ion at m/z 321 for compound **1** was the same as that of 1-pentyl-3-(4-methoxybenzoyl)indole (RCS-4), and the fragment ions were almost the same as those of RCS-4. The GC–MS

spectrum of compound **2** was very similar to that of compound **1** except that the mass numbers of the fragment ions at m/z 290, 200, 186, and 173 of compound **2** were equally smaller than those of compound **1** by 14 amu. From these GC–MS results, compound **1** was assumed to be the 2- or 3-methoxy isomer of RCS-4, and compound **2** was assumed to be a 1-butylindole homologue of compound **1**. The ESI mass spectra showed a single peak at m/z 322.33 for compound **1** and a single peak at m/z 308.25 for compound **2**, which showed the masses of the protonated ions. High-resolution TOF–MS spectra showed the accurate mass numbers of protonated molecular ions at m/z 322.180512 for compound **1** and at m/z 308.164895 for compound **2**, suggesting the molecular formulas of $C_{21}H_{23}NO_2$ and $C_{20}H_{21}NO_2$, respectively. The 1H NMR spectra showed signals that suggested 23 and 21 protons for compounds **1** and **2**, respectively, while the respective ^{13}C NMR spectra showed 21 and 20 carbon signals. All protons and carbons were assigned by their couplings and correlations observed in 1H – 1H correlation spectroscopy (COSY), 1H – ^{13}C heteronuclear multiple bond correlation (HMBC), and 1H – ^{13}C heteronuclear single quantum coherence (HSQC) spectra. On the basis of the spectral data, compound **1** was identified as the 2-methoxy isomer of RCS-4; compound **2** was identified for the first time as 1-butyl-3-(2-methoxybenzoyl)indole. Phenazepam and 5-methoxy-*N,N*-diallyltryptamine (5-MeO-DALT) were also identified as coexisting drugs in the herbal mixture. The contents of compounds **1** and **2** in the mixture were calculated to be 22.4 and 3.45 mg/g, respectively.

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Introduction

Herbal blends such as “Spice” have been sold in European countries mainly through the Internet since 2004 [1–3]. These products have gained great popularity and spread worldwide. Their listed ingredients are only herbs and they are declared as incense, but consumers have described cannabis-like effects after smoking. It was revealed that synthetic compounds were added clandestinely, and various pharmacological active compounds were identified. Since JWH-018 and CP-47,497-C8 homologs were identified as active ingredients of Spice [4–6], many other compounds such as JWH-398, JWH-073, JWH-081, JWH-251, JWH-122, AM-2201, RCS-4, JWH-250, AM-2202, APICA, and APINACA have been found in herbal mixtures [7–15].

Among the various kinds of cannabimimetic compounds, Huffman et al. [16–18] synthesized many JWH compounds. Their chemical structures were mainly naphthylindoles, benzoylindoles, and phenylacetylindoles. The structure–activity relationships between JWH compounds and CB₁ and CB₂ receptors were investigated [19–23]. CB₁ is known to be expressed primarily in the central nervous system and directly related to typical cannabinoid pharmacology. Some of the JWH compounds showed affinities to the receptor that are hundreds of times higher than that of Δ^9 -tetrahydrocannabinol (THC) [24].

Many countries have controlled synthetic cannabinoids [25, 26], but new chemical analogues are constantly emerging to circumvent legal regulation. Some years ago, herbal products such as “Spice”, “Skunk”, and “Yucatan Fire” were actively marketed. Currently in South Korea, however, herbal mixtures are imported and synthetic cannabinoids in the powder form are smuggled separately; solutions of synthetic cannabinoids are then prepared and sprayed on the herbal mixture. The dried herbal mixtures are then packaged in transparent plastic bags and sold without labels.

During gas chromatography–mass spectrometry (GC–MS) screening experiments of seized herbal mixtures, we encountered two compounds that were suspected synthetic cannabinoids. One was found to be identical to a benzoylindole reported recently. The other was found to be a novel benzoylindole, which had neither been synthesized nor detected in herbal products. The details of identification by instrumental analyses of the two compounds are presented in this report.

Materials and methods

Herbal materials

A herbal mixture, packed in a transparent plastic bag and weighing 0.53 g, was seized from a suspect. For compound

isolation, 0.3 g of the sample was used and the remains served for quantitation of the compounds in the mixture.

Extraction and isolation

The 0.3-g portion of the herbal material was extracted three times with 10 ml of methanol under ultrasonication for 10 min. The extracts were combined, filtered, and evaporated to dryness under reduced pressure. The resulting residue was suspended in distilled water and then extracted with three successive portions of dichloromethane. The combined organic layer was concentrated to a small volume under reduced pressure, and was then subjected to column chromatography on silica gel (50–200 mesh) under atmospheric pressure. Gradient elution was performed using solvent mixtures of dichloromethane/methanol (50:1, v/v) to dichloromethane/methanol (20:1, v/v). The fractions containing the target compounds were concentrated under reduced pressure, and subjected to preparative high-performance liquid chromatography (HPLC) using a 90–100 % acetonitrile/water gradient program to separate compounds **1** and **2**. The HPLC instrument was a Waters HPLC system consisting of a 515 HPLC pump, a 2996 photodiode array detector (Waters, Milford, MA, USA), and a YMC ODS H-80 column (150 × 20 mm i.d., YMC Europe, Dinslaken, Germany). The flow rate was 6.0 ml/min.

Quantitation

Quantitative analysis was conducted to determine the amounts of compounds **1** and **2** in the herbal mixture. In the absence of any authorized reference materials, quantitation was done using the two compounds derived from preparative HPLC isolation. Working solutions of 100, 50, 25, and 10 µg/ml in methanol were prepared and *n*-eicosane (C₂₀; 50 µg/ml; Sigma-Aldrich, St. Louis, MO, USA) was used as internal standard.

Gas chromatography–mass spectrometry

The GC–MS system was a 5975C mass selective detector equipped with an HP6890 GC instrument and an HP7673 autosampler (Agilent, Santa Clara, CA, USA). The detector was operated in the electron ionization mode at 70 eV with a scan range of *m/z* 40–550, and the transfer line temperature was 280 °C. The GC capillary column was an HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film, Agilent) and the temperature of the injection port was 250 °C. After an initial oven temperature of 100 °C for 1 min, the temperature was increased to 300 °C at 20 °C/min and held for 15 min. Helium was used as carrier gas at a constant column flow rate of 1 ml/min and 1 µl of the sample was injected to the GC–MS system in the splitless mode.

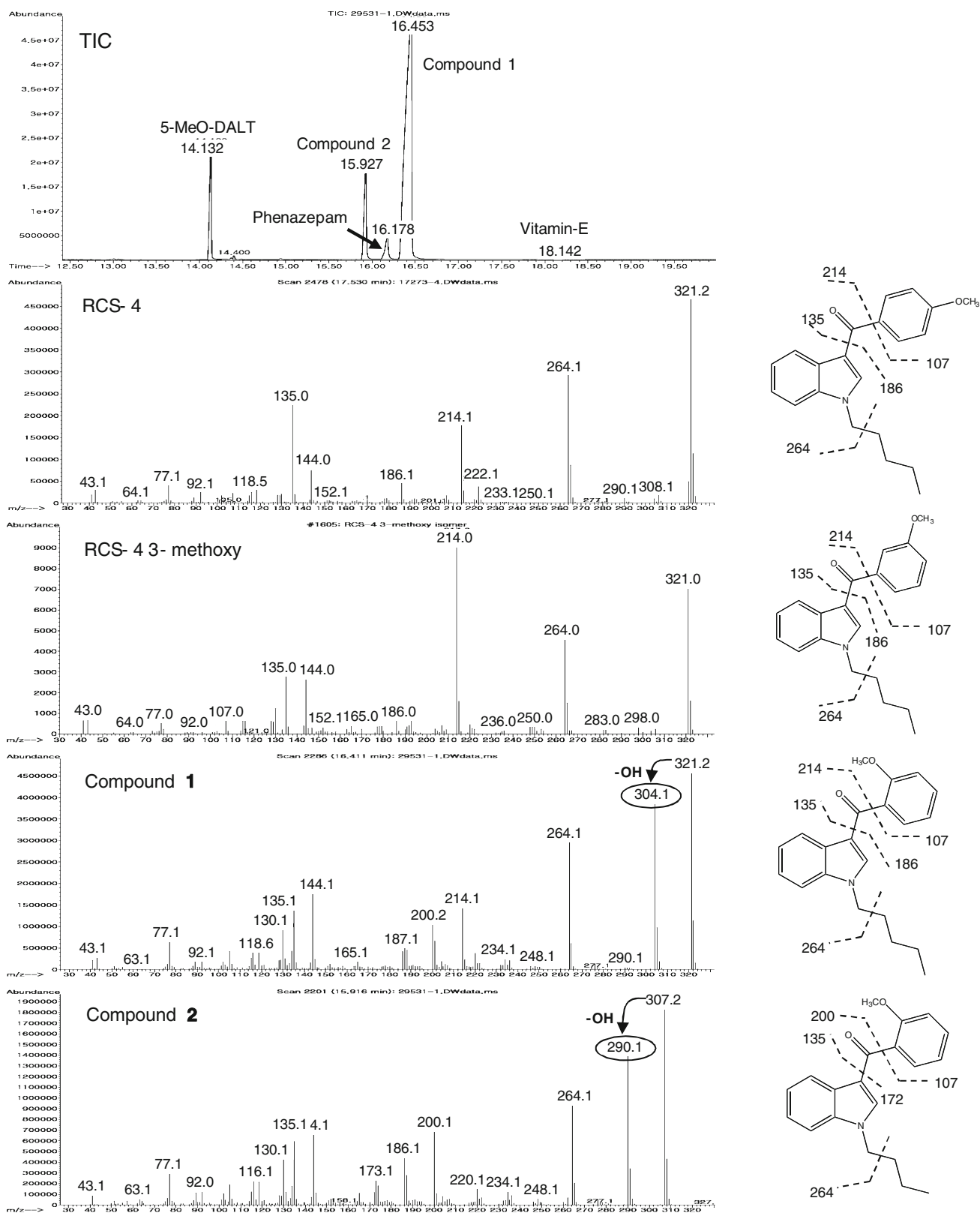


Fig. 1 Total ion chromatogram (TIC) by gas chromatography–mass spectrometry obtained from the seized mixture, and mass spectra of RCS-4, RCS-4 3-methoxy isomer, compound 1, and compound 2. The mass spectrum of RCS-4 was obtained from a previously seized

herbal mixture; that of RCS-4 3-methoxy isomer was obtained from the SWGDRUG library. Only the *ortho*-substitution of a methoxyl group on the benzene ring showed the loss of hydroxyl radical

Table 1 Appearance and nonappearance of a fragment peak due to the loss of hydroxyl radical in GC–MS mass spectra of various synthetic cannabinoids

Compound group	Fragment after loss of hydroxy radical	
	Observed	Not observed
Naphthoylindoles	JWH-015	JWH-200
	JWH-018	WIN 55, 212-2
	JWH-019	WIN 55, 212-3
	JWH-073	
	JWH-081	
	JWH-122	
	JWH-210	
	JWH-398	
	AM-2201	
Benzoylindoles	Compound 1	RCS-4
	Compound 2	RCS-4 3-methoxy isomer
Phenylacetylindoles	–	JWH-203
		JWH-250
		JWH-251
		RCS-8

Electrospray ionization–mass spectrometry

Electrospray ionization–mass spectrometry (ESI–MS) spectra were recorded on an LCQ fleet ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Parameters were: software, Xcalibur[®] system manager; direct flow injection, 5 μ l/min (Hamilton 500 μ l, methanol); MS range, m/z 140–450; spray voltage, 5 kV; capillary temperature, 275 $^{\circ}$ C; capillary voltage, 35 V; tube lens, 120 V; multipole 0 offset, –4.89 V; multipole 1 offset, –12.5 V; sheath gas flow rate, 10 (N₂); collision gas, helium.

High-resolution electrospray ionization time-of-flight–mass spectrometry

To obtain accurate mass spectra of compounds **1** and **2**, a maXis 4G electrospray ionization (ESI) time-of-flight–mass spectrometry (TOF–MS) instrument (Bruker Daltonics, Bremen, Germany) was used. Acquisition parameters were: source type, ESI; ion polarity, positive; nebulizer, 0.3 bar; capillary, 4500 V; dry heater, 180 $^{\circ}$ C; scan range, m/z 100–2900; end plate offset, –500 V; dry gas, 4.0 l/min; collision cell radiofrequency, 2000 Vpp. Data analysis was carried out using Bruker Compass DataAnalysis 4.0.

Nuclear magnetic resonance spectroscopy

¹H Nuclear magnetic resonance (NMR), ¹³C NMR, and two-dimensional (2D) NMR data, including ¹H–¹³C

heteronuclear single quantum coherence (HSQC), ¹H–¹³C heteronuclear multiple bond correlation (HMBC), and ¹H–¹H correlation spectroscopy (COSY) spectra were recorded at room temperature on a Bruker Avance DRX-500 spectrometer (Bruker Daltonics) with tetramethylsilane as internal standard. The resonance frequencies were 500.13 MHz for ¹H NMR spectra and 125.77 MHz for ¹³C NMR spectra.

Results and discussion

Isolation and quantitation of compounds **1** and **2**

After column chromatography using dichloromethane/methanol gradient elution, compounds **1** and **2** eluted together in the third fraction. To separate them into pure compounds, preparative HPLC was used and **1** and **2** were isolated as single compounds for NMR spectroscopy. By GC–MS screening in full scan mode, compound **1** was detected as the major compound with ratio of compounds **2**:**1** of 0.14. The correlation coefficient of the calibration curve was 0.9945 for compound **1** and 0.9983 for compound **2**. The contents of compounds **1** and **2** were 22.4 and 3.45 mg/g, respectively, in the herbal mixture.

GC–MS analysis

The molecular formula of RCS-4 is C₂₁H₂₃NO₂ and the calculated accurate molecular weight is 321.1729. The base ion of compound **1** was identical to that of RCS-4 with m/z 321.2 and the mass fragment ion profile was similar to that of RCS-4 (Fig. 1). However, the peak at m/z 304.1 appeared only in the mass spectrum of compound **1** and it was smaller than the molecular mass number by 17 amu. This difference is a well-known phenomenon that is observed in the mass spectra of many 3-naphthoylindoles by the loss of the hydroxyl radical after a rearrangement as shown in Table 1. The 1-pentyl-indoloyl fragment peak at m/z 214 and the methoxybenzoyl fragment peak at m/z 135 were also observed for compound **1**. From these results, compound **1** could be assumed as a positional isomer of RCS-4 and the methoxyl group might be substituted at the *ortho*- or *meta*-position, but not at the *para*-position, of the benzene ring. By careful comparison of the fragment ions with the reference mass spectrum reported by Nakajima et al. [12], compound **1** could be assumed to be an *ortho*-methoxy analogue of RCS-4, 1-pentyl-3-(2-methoxybenzoyl)indole.

The mass spectrum profile of compound **2** was very similar to that of compound **1** (Fig. 1). The fragment ions at m/z 264, 248, 234, 220, 144, 135, 130, 119, 105, 92, and 77 were identical between spectra, whereas the mass numbers of the base ion and some fragment ions at m/z 290,

Table 2 ^1H NMR and ^{13}C NMR data of compounds **1** and **2** in MeOD (ppm) and HMBC correlations

No.	Compound 1			Compound 2		
	^{13}C	^1H	HMBC	^{13}C	^1H	HMBC
2	139.35	7.60, 1H, s	3, 3a, 7a, 7', 1''	139.37	7.58, 1H, s	3, 3a, 7a, 7', 1''
3	116.34	–	–	116.33	–	–
3a	126.61	–	–	126.61	–	–
4	121.76	8.25, 1H, d, $J = 7.5$ Hz	6, 7a	121.75	8.22, 1H, d, $J = 8.0$ Hz	6
5	122.32	7.28, 1H, m, overlapped	3a, 7	122.32	7.28, 1H, m, overlapped	3a
6	123.12	7.33, 1H, m, overlapped	4, 7a	123.11	7.33, 1H, m, overlapped	–
7	110.14	7.51, 1H, m, overlapped	3a, 5	110.14	7.52, 1H, m, overlapped	–
7a	137.19	–	–	137.19	–	–
1'	130.74	–	–	130.73	–	–
2'	156.75	–	–	156.76	–	–
3'	111.43	7.16, 1H, d, $J = 8.5$ Hz	1', 5'	111.41	7.14, 1H, d, $J = 8.5$ Hz	5'
4'	130.88	7.50, 1H, m, overlapped	2', 6'	130.88	7.49, 1H, m, overlapped	–
5'	119.96	7.08, 1H, ddd, $J = 1.0, 1.0, 0.5$ Hz	1', 3'	119.97	7.05, 1H, ddd, $J = 1.0, 1.0, 0.5$ Hz	1'
6'	128.42	7.36, 1H, m, overlapped	2', 4', 7'	128.42	7.35, 1H, m, overlapped	7'
7'	191.60	–	–	191.62	–	–
1''	46.49	4.21, 2H, t, $J = 7.0$ Hz	2, 7a, 2'', 3''	46.28	4.21, 2H, t, $J = 7.5$ Hz	2, 7a, 2'', 3''
2''	29.19	1.84, 2H, quintet, $J = 7.5$ Hz	1'', 3'', 4''	31.60	1.80, 2H, quintet, $J = 7.5$ Hz	–
3''	28.50	1.28, 2H, m, overlapped	4''	19.50	1.30, 2H, m	–
4''	21.85	1.34, 2H, m, overlapped	3'', 5''	12.51	0.92, 3H, t, $J = 7.5$ Hz	2'', 3''
5''	12.86	0.89, 3H, t, $J = 7.0$ Hz	3'', 4''	–	–	–
1'''	54.69	3.80, 3H, s	2'	54.67	3.78, 3H, s	2'

Three consecutive aromatic proton signals of indoles and H-4' and H-6' proton signals of the benzene ring were overlapped in the ^1H NMR spectra; 2D NMR spectra were used to assign them conclusively

200, and 186 of compound **2** were lower than those for compound **1** by 14 amu. The fragment peak at m/z 135 suggested that compound **2** had the same methoxybenzoyl group as that of compound **1**.

Phenazepam was also identified between the two compounds in the chromatogram (Fig. 1). Phenazepam is a benzodiazepine drug that is used in the treatment of neurological disorders such as epilepsy, alcohol withdrawal syndrome, and insomnia. Phenazepam is not a controlled substance in many countries in spite of being a benzodiazepine. However, it has recently gained popularity in some countries as a recreational drug [27, 28]. In addition, 5-methoxy-*N,N*-diallyltryptamine (5-MeO-DALT) was identified in the same herbal mixture.

ESI-MS and high-resolution ESI-TOF-MS

In the ESI positive full scan mass spectra, single peaks at m/z 322.33 for compound **1** and m/z 308.25 for compound **2** appeared as the respective protonated molecular ions. Accurate mass spectra were obtained by direct injection of each of the methanolic solutions of the two compounds to the high resolution ESI-TOF-MS instrument. In addition to the protonated molecular ions, sodium adducts were also

observed for both compounds. The accurate mass spectrum of isolated compound **1** revealed $[\text{M} + \text{H}]^+$ at m/z 322.180512 (calcd. 322.180155) and $[\text{M} + \text{Na}]^+$ at m/z 344.162535 (calcd. 344.162100) in the positive scan mode, suggesting a molecular formula of $\text{C}_{21}\text{H}_{23}\text{NO}_2$. The accurate mass spectrum of isolated compound **2** revealed $[\text{M} + \text{H}]^+$ at m/z 308.164895 (calcd. 308.164505) and $[\text{M} + \text{Na}]^+$ at m/z 330.147022 (calcd. 330.146450), suggesting a molecular formula of $\text{C}_{20}\text{H}_{21}\text{NO}_2$. The errors between the observed and theoretical mass numbers of $[\text{M} + \text{H}]^+$ were smaller than 1.3 ppm.

Peak assignment for compounds **1** and **2** for one-dimensional (1D) NMR spectra

From the GC-MS results, compound **1** was assumed to be 1-pentyl-3-(2-methoxybenzoyl)indole. The chemical shifts in the ^1H NMR and ^{13}C NMR spectra of compound **1** are listed in Table 2. Twenty-three protons, including 9 aromatic and 14 aliphatic protons, were detected in the ^1H NMR spectrum for compound **1**. The chemical shifts of the indole proton appeared at δ 8.25 (1H, d, $J = 7.5$ Hz, H-4), 7.28, 7.33, 7.51 (each 1H, m, overlapped, H-5, H-6, H-7), and 7.60 (1H, s, H-2). Four aromatic proton signals of the

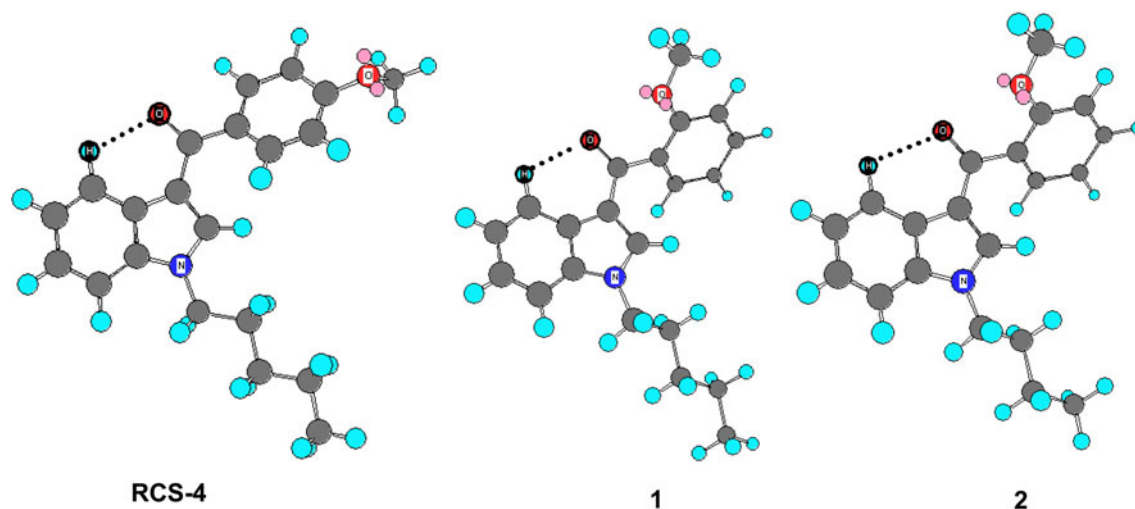


Fig. 2 Energy-minimized spatial structures of RCS-4, compound **1**, and compound **2** obtained from MM2 force field molecular modeling by CS ChemDrawPro (CambridgeSoft, Cambridge, MA, USA). Lone pairs were added to oxygen of the methoxyl group before steric

energy computation. Hydrogen bonds between the oxygen of the carbonyl group and H-4 proton of the indole moiety are represented by *dotted lines*. These bonds resulted in downfield shifts of the H-4 proton in ^1H NMR spectra

methoxybenzoyl group appeared at δ 7.16 (1H, d, $J = 8.5$ Hz, H-3'), 7.50 (1H, m, overlapped, H-4'), 7.08 (1H, ddd, $J = 1.0, 1.0, 0.5$ Hz, H-5'), and 7.36 (1H, m, overlapped, H-6'). Also present were three methylene signals at δ 1.84 (2H, quintet, $J = 7.5$ Hz, H-2''), 1.28 (2H, m, overlapped, H-3''), and 1.34 (2H, m, overlapped, H-4''), one methylene group connected to the indole nitrogen at δ 4.21 (2H, t, $J = 7.0$ Hz, H-1''), and one methyl group at δ 0.89 (3H, t, $J = 7.0$ Hz, H-5''). One methoxyl proton signal was present at δ 3.80 (3H, s, H-1''').

Twenty-one carbon signals were observed in the ^{13}C NMR spectrum for compound **1**. One carbonyl carbon signal at δ 191.60 (C-7'), five quaternary carbon signals (δ 116.34, 126.61, 137.19, 130.74, 156.75), nine aromatic carbon signals, four methylene carbon signals (δ 46.49, 29.19, 28.50, 21.85), one aliphatic methyl carbon at δ 12.86 (C-5''), and one methoxyl carbon signal of the benzoyl group at δ 54.69 were detected. The chemical shifts of the ^1H NMR and ^{13}C NMR spectra of compound **1** coincided well with the reference data [12] and they are listed in Table 2.

The ^1H NMR spectrum of compound **2** showed 21 proton signals, which was very similar to that of compound **1** (Table 2). Nine aromatic proton signals, 1 methoxyl signal at δ 3.78 (3H, s, H-1'''), and 1 methyl signal at δ 0.92 (3H, t, $J = 7.5$ Hz, H-4'') were detected. Two methylene signals were observed at δ 1.80 (2H, quintet, $J = 7.5$ Hz, H-2'') and 1.30 (2H, m, overlapped, H-3''), and 1 methylene connected to the indole nitrogen was observed at δ 4.21 (2H, t, $J = 7.5$ Hz, H-1''). The spin–spin splitting of methylene groups according to the $n + 1$ rule and the integrated values of protons enabled the peaks to be

assigned. To determine the position of the methoxyl group of the benzene ring, the spin–spin splitting of the aromatic proton signals was checked carefully. If the methoxyl group were substituted at C-4', which is the case for RCS-4, two sets of *ortho*-coupled signals (H-2' with H-3', and H-5' with H-6') for the benzene ring would have appeared. If the methoxyl group were substituted at C-3', a *meta*-coupled signal between H-2' and H-4' would have appeared. However, one *ortho*-coupled signal was observed at δ 8.22 (1H, $J = 8.0$ Hz, H-4'), which indicated that the methoxyl group was substituted at the C-2' position. In addition, the peak shape of the proton signal at δ 7.05 (1H, ddd, H-5') revealed the presence of four successive protons for two *ortho*-couplings ($J = 7.5, 7.5$ Hz) and one *meta*-coupling ($J = 1.0, 1.0, \text{ and } 0.5$ Hz).

The energy-minimized structures of the compounds are shown in Fig. 2. The downfield shift of the doublet signal at δ 8.22 (1H, $J = 8.0$ Hz, H-4) could be explained by weak hydrogen bonding between the proton and the carbonyl oxygen of the benzoyl group, due to their close spatial separation (Fig. 2). Hydrogen bonding is known to be formed when the distance between the oxygen and the hydrogen atom is < 2.55 Å. From the energy-minimized structure using MM2 force field molecular modeling by CS ChemDrawPro (CambridgeSoft, Cambridge, MA, USA), the distance between the carbonyl oxygen and H-4 proton of the indole moiety was calculated as 2.474 Å for RCS-4, 2.476 Å for compound **1**, and 2.478 Å for compound **2**. These values are less than the minimum value for hydrogen bonding.

The ^{13}C NMR spectrum of compound **2** showed 1 carbonyl carbon signal at δ 191.62 (C-7'), 14 aromatic carbon

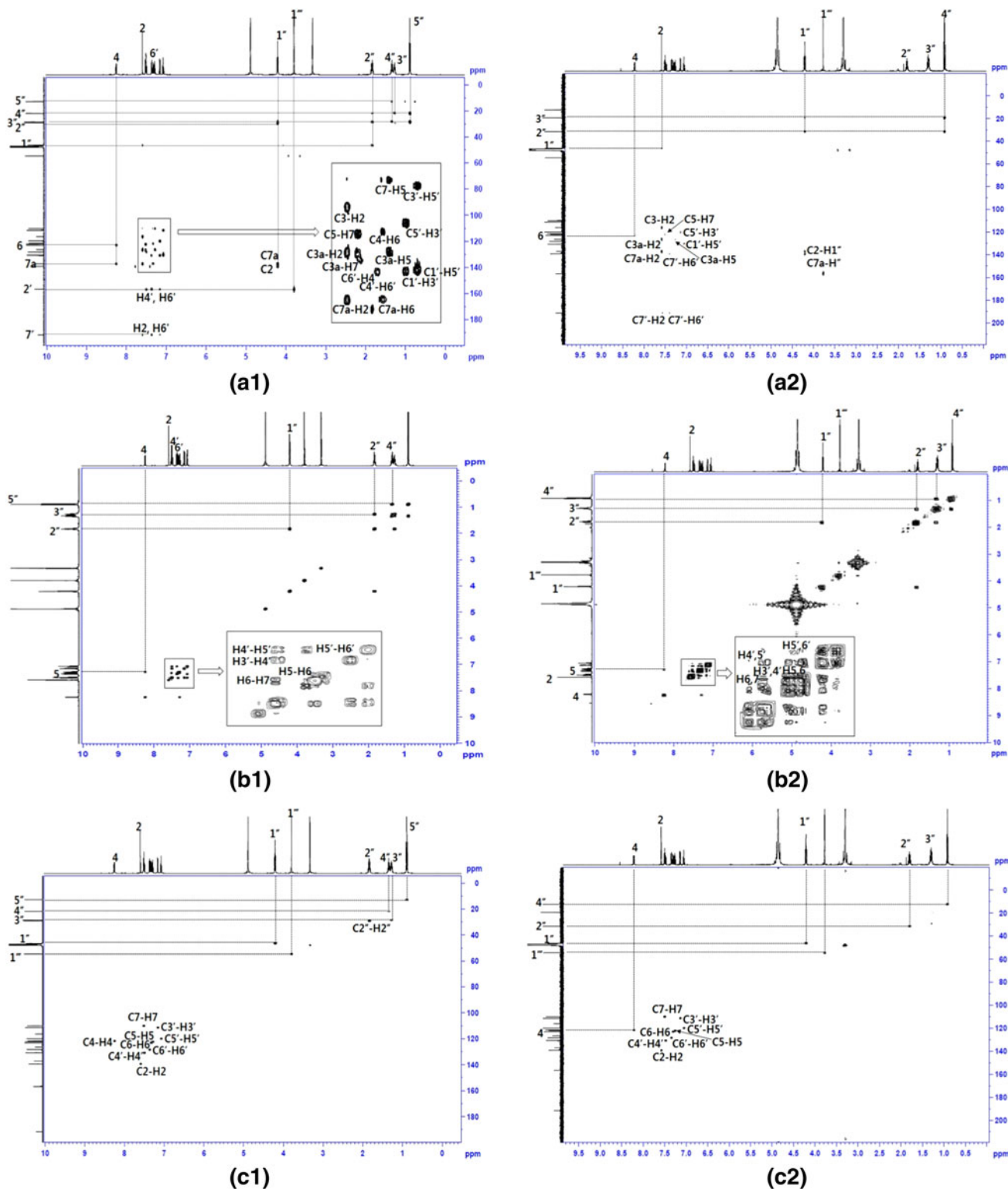


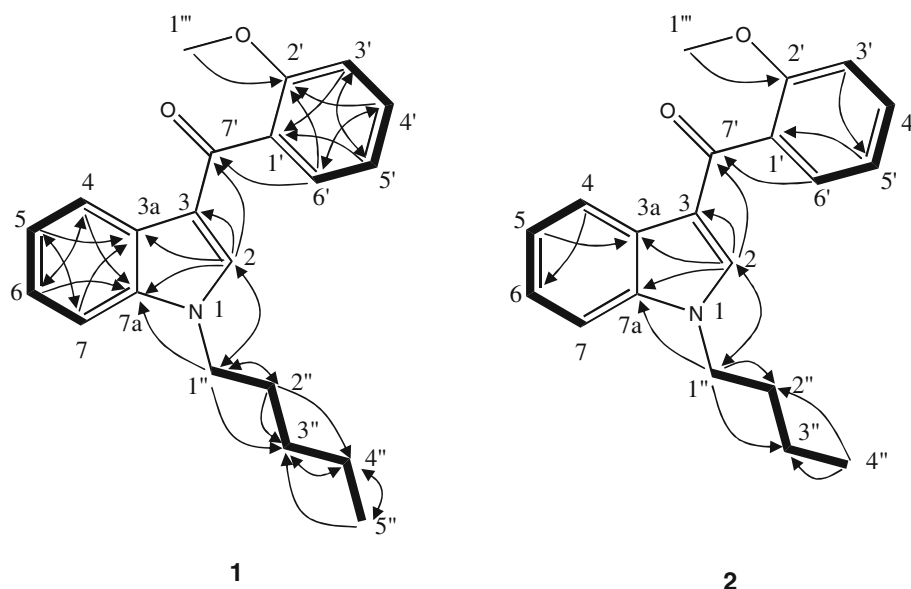
Fig. 3 Two-dimensional ^1H - ^{13}C heteronuclear multiple bond correlation (HMBC) (a1, a2), ^1H - ^1H correlation spectroscopy (COSY) (b1, b2), and ^1H - ^{13}C heteronuclear single quantum coherence

signals, 3 methylene carbon signals (δ 46.28, 31.60, 19.5), 1 aliphatic methyl carbon signal at δ 12.51 (C-4''), and 1 methoxyl carbon attached to the benzene ring at δ 54.67

(HSQC) (c1, c2) spectra for compounds 1 (left) and 2 (right) isolated from the seized herbal mixture. Some aromatic regions are magnified in large boxes for clarity

(C-1''') (Table 2). Considering the downfield shift of C-2' observed at δ 156.76, the methoxyl substitution was placed at C-2' of the benzene ring. On the other hand, the

Fig. 4 HMBC correlations (arrows) and COSY correlations (thick lines) for compounds **1** and **2**. Using the heteronuclear correlations over longer ranges of 2–4 bonds, quaternary carbons of the indole and benzene ring were assigned



downfield shift for C-4' of RCS-4 was observed at δ 162.2 in the ^{13}C NMR spectrum [11].

Two-dimensional (2D) NMR spectra

Complete assignment of protons and carbons for compounds **1** and **2** was established on the basis of ^1H - ^1H COSY, ^1H - ^{13}C HMBC, and ^1H - ^{13}C HSQC spectra, as shown in Fig. 3. The quaternary carbons of the indole and benzene ring were assigned from the correlations with tertiary carbons of the HMBC spectrum. These correlations are shown in Fig. 4 and Table 2.

In the ^1H - ^1H COSY spectrum of compound **2**, the δ 7.05 (H-5'), 7.14 (H-3'), and 7.49 (H-4') signals showed strong vicinal coupling with each other, indicating that the methoxyl group was substituted at C-2' or C-6'. The δ 7.35 (H-6') signal was correlated with the carbonyl carbon at δ 191.62 in the HMBC spectrum, which revealed that the methoxyl group was attached at C-2' rather than C-6'. In addition, the methoxyl protons were correlated with the C-2' carbon and the downfield shift of the quaternary C-2' appeared at δ 156.76. The proton signals at δ 7.05, 7.14, 7.35, and 7.48 corresponded to carbon signals at δ 119.97, 111.41, 128.42, and 130.88, respectively, in the HSQC spectrum, and they were assigned to H-5', H-3', H-6' and H-4', respectively. The quaternary carbons of the methoxybenzene ring (C-1') were assigned from the correlations of the HMBC spectrum with the protons bonded to tertiary carbons. Because compound **1** has the same methoxyl group as compound **2**, they exhibited the same correlations in the ^1H - ^1H COSY spectrum.

The H-2 signal is a characteristic signal of the indole group and it was predicted as a singlet. The peak appeared at δ 7.58 in the ^1H NMR spectrum of compound **2**. It corresponded to δ 139.37 (C-2) in the HSQC spectrum and was correlated with the carbonyl carbon in the HMBC spectrum. Although H-5, H-6, and H-7 proton peaks in the ^1H NMR spectrum appeared as a multiplet in the range of δ 7.28–7.52, the signals could be assigned from the ^1H - ^1H COSY spectrum. The signal at δ 8.22 (H-4) showed a coupling with the upfield part of the mixed range in the COSY spectrum; therefore, the peak of the upfield part was assigned to H-5, and the signal at δ 122.32 in the ^{13}C NMR spectrum was assigned to C-5 from the HSQC spectrum. The H-4 proton was correlated with a carbon at δ 123.11 in the HMBC spectrum, and this carbon corresponded to the H-6 proton in the HSQC spectrum. The C-7 carbon usually appears at a slightly more upfield region than other tertiary carbons of the indole moiety, and because the H-7 proton at δ 7.52 was correlated with the carbon signal at δ 110.14 in the HSQC spectrum, this carbon was assigned to C-7. While all possible correlations between the aromatic proton and the carbons were observed in the HMBC spectrum of compound **1**, some correlations were not observed in the HMBC spectrum of compound **2**. The quaternary carbons of the indole (C-3, C-3a, C-7a) of compound **1** were assigned from the correlations of the HMBC spectrum with the protons bonded to tertiary carbons. The C-3 carbon was correlated with the H-2 proton, the C-3a carbon with H-2, H-5, and H-7 protons, and the C-7a carbon with H-4, H-6, and H-1''. The H-2 proton and the carbonyl carbon were also correlated in the HMBC spectrum. The indole carbons

of compound **2** were assigned according to the HMBC spectrum and by comparing the chemical shifts of the ^{13}C NMR spectrum with those of compound **1**.

The difference between compounds **1** and **2** was the alkyl group attached to the nitrogen of the indole moiety. Four methylene signals were detected in compound **1** and three methylene signals for compound **2** in the ^1H NMR spectra. The H-1'' methylene protons appeared as a triplet at downfield shift, and correlated with C-2, C-7a, C-2'', and C-3'' in the HMBC spectrum. A multiplet signal of a methylene proton was observed for compound **1**, but overlapping multiplet signals of two methylene proton signals were observed for compound **2** in the aliphatic region. The end methyl protons of the alkyl group of compound **1** (H-5'') showed vicinal coupling with the downfield part of the multiplet signal in the COSY spectrum. Therefore, the proton signals at δ 1.34 and 1.28 were assigned as H-4'' and H-3'', respectively.

Conclusions

In this study, two cannabimimetic compounds were detected in a seized herbal mixture after GC–MS screening. The fragmentation patterns were very similar to that of RCS-4. We isolated the pure compounds by silica gel column chromatography and preparative HPLC. The chemical structures of the compounds were elucidated by GC–MS, ESI–MS, high-resolution ESI–TOF–MS, and NMR spectroscopy. Based on the instrumental data and in comparison with reference data, compound **1** was identified as the 2-methoxybenzoyl derivative of RCS-4, 1-pentyl-3-(2-methoxybenzoyl)indole. Compound **2** was identified as the 1-butylindole derivative of compound **1**, 1-butyl-3-(2-methoxybenzoyl)indole. Compound **1** has been found in herbal products [12], but compound **2** has neither been reported as a synthetic compound in the literature nor as an adulterant in a dubious product; this is an entirely novel compound. Compound **2** contains an *N*-1 butyl side chain structure in place of the very common *N*-1 pentyl side chain structure (Fig. 4). The synthetic cannabinoids known to have the *N*-1 butyl side chain structure are JWH-073, JWH-016, JWH-047, JWH-080, JWH-096, and JWH-095 [29]. All these naphthoylindoles showed K_i values of 5.6–140 nM for the CB₁ receptor. The relation of compound **2** to compound **1** is comparable with that of JWH-073 to JWH-018. JWH-073 and JWH-018 showed similar K_i values of 8.9 and 9.0 nM for the CB₁ receptor. Compound **1** [1-pentyl-3-(2-methoxybenzoyl)indole] is reported to show a strong cannabimimetic effect [12], and so it is expected that compound **2** [1-butyl-3-(2-methoxybenzoyl)indole] will also show a strong cannabimimetic effect in humans, although such experiments remain to be explored.

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