A New Cannabinoid, Δ^6-Tetrahydrocannabinol 2′-*O*-*β*-D-Glucopyranoside, Biotransformed by Plant Tissue

Hiroyuki Tanaka, Ryuji Takahashi, Satoshi Morimoto, and Yukihiro Shoyama*

Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashiku, Fukuoka 812, Japan

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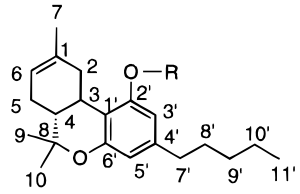
Δ^6-Tetrahydrocannabinol (Δ^6-THC, **1**) was converted mainly to **1** 2′-*O*-*β*-D-glucopyranoside (**2**) using tissue segments of *Pinellia ternata* tubers. In time−course experiments, **1** was absorbed rapidly by the tissues and glucosylated.

Many studies on the metabolism of cannabinoids have been performed on animals, their organs, and microsomal fractions.[1−5] The biotransformation of cannabidiol (CBD) and Δ^1-tetrahydrocannabinol (Δ^1-THC) have been investigated by using suspension cultures of *Cannabis sativa* and *Saccharum officinarum* to give cannabielsoin[6] and cannabicoumaronon,[7] respectively, without further biotransformation products. In a previous paper,[8] we presented the biotransformation of cannabinol into its glucoside and hydroxylated glucoside. The objectives of the prompt study were to obtain new cannabinoids that are pharmacologically active, to produce cannabinoids difficult to synthesize by other means, and to improve their aqueous solubility. In this study, Δ^6-tetrahydrocannabinol (Δ^6-THC, **1**) was administered to tissue cultures of the tubers of *Pinellia ternata* Breit. (Araceae),[9] and the biotransformation products were investigated.



1: R=H
2: R=Glc

Biotransformation product **2** was isolated from MeOH extracts of the cultured protocorm and EtOAc extracts of the growth medium after incubation of **1**. The negative FABMS of **2** showed a peak at *m*/*z* 475 [M − H]$^-$, indicating that **2** was composed of **1** and one molecule of hexose. The ^{1}H-NMR spectrum of **2** suggested the presence of the aromatic protons of an olivetol moiety and the protons of a terpenoid moiety reported for **1**,[10] as well as the presence of a sugar moiety (Table l). In the ^{13}C-NMR spectrum, the signals assignable to the sugar moiety were observed to be in good agreement with those of glucose. The large coupling constant (*J* = 8 Hz) of the anomeric proton in **2** suggested a *β*-anomer. NOESY NMR cross peaks from the anomeric proton of glucose and the H-3′ proton of **2** showed that the glucose moiety was attached to the hydroxyl group at C-2′. From these results, **2** was deduced to be a new cannabinoid, **1** 2′-*O*-*β*-D-glucopyranoside.

Table 1. ^{1}H-NMR (270 MHz) and ^{13}C-NMR (67.5 MHz) Data for Δ^6-Tetrahydrocannabinol 2′-*O*-*β*-D-Glucopyranoside[a]

	^{1}H	^{13}C
1		136.0
2	1.73−1.92 (m); 3.13 (dd, *J* = 2, 18 Hz)	38.0
3	2.62-2.72 (m)	33.0
4	1.73-1.92	46.9
5	1.73−1.92 (m); 2.12−2.18 (m)	29.0
6	5.43 (d, *J* = 5 Hz)	120.4
7	1.69 (s)	23.8
8		77.6
9	1.08 (s)	18.7
10	1.37 (s)	28.0
1′		114.3
2′		158.3
3′	7.26 (s)	107.7
4′		143.7
5′	7.26 (s)	112.5
6′		155.5
7′	2.48 (t, *J* = 8 Hz)	36.9
8′	1.51−1.62 (m)	32.1
9′	1.26−1.33 (m)	32.7
10′	1.26−1.33 (m)	23.6
11′	0.88 (t, *J* = 7 Hz)	14.4
Glc-1	4.92 (d, *J* = 8 Hz)	101.8
2	3.66−3.74 (m)	75.3
3	3.66−3.74 (m)	78.6
4	3.66−3.74 (m)	71.5
5	3.54−3.59 (m)	78.2
6	3.88 (dd, *J* = 5, 12 Hz); 3.98 (dd, *J* = 4, 12 Hz)	62.7

[a] Spectra were obtained in $CDCl_3$.

Time−course experiments were also carried out in this investigation. Figure 1A shows the growth curve of a protocorm culture. Protocorm segments were grown up the hard tissue mass as previously described,[9] and the dry weight increased gradually. In the tissue mass (Figure 1A), **2** appeared on the first day after the administration of **1** (2000 *μ*g/flask), gradually increasing to reach a maximum (ca. 203 *μ*g/flask) on day 20 when the conversion ratio was about 7%. Compound **2** was detected from the first day after the incubation of **1**, reaching a maximum (ca. 145 *μ*g/flask) on day 30 in the medium. The conversion ratio of **1** to **2** reached approximately 1:20. For the production of **2**, therefore, it was found to be desirable to harvest tissues after 20 days of incubation.

Cell suspension culture was also investigated for the biotransformation of **1**. However, the conversion ability of the cells was lower than that of the protocorm tissues (data not shown). Therefore, it became evident that the protocorm segment of *P. ternata* could be used for the biotransformation of cannabinoids such as **1** as a plant catalytic enzyme without any pretreatment such as encapsulating with polymers.

* To whom correspondence should be addressed. Phone: 092-641-1151 (6131). FAX: 092-641-8154. E-mail: shoyama@shoyaku.phar.kyushu-u.ac.jp.

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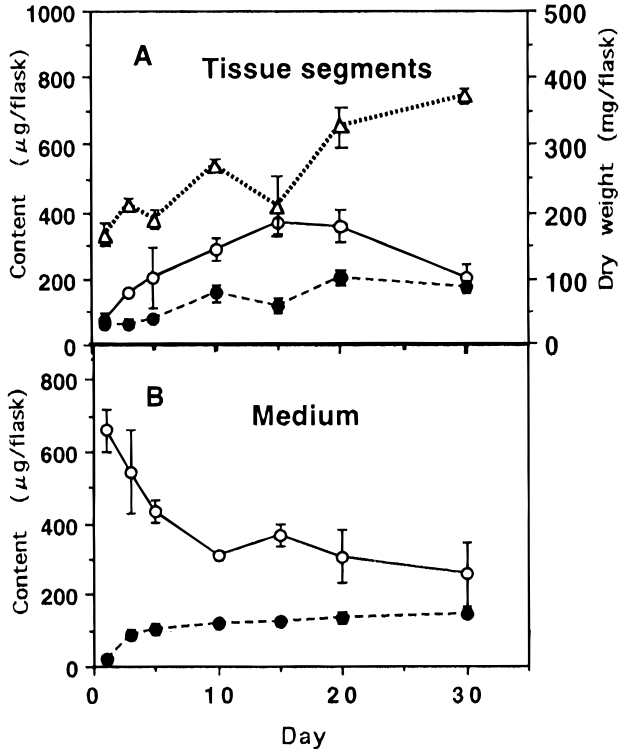

Figure 1. Time−course of biotransformation for Δ^6-tetrahydrocannabinol 2′-*O*-*β*-D-glucopyranoside (**2**) from Δ^6-tetrahydrocannabinol (**1**) in tissue segments (A) and medium (B) of *P. ternata* suspension culture. Key: −○−, **1**; - -●- - **2**; - -△- -, dry weight.

The new cannabinoid **2** was examined for its binding to CB_1 (the cannabinoid receptor found mainly in the brain) according to a published method,[11,12] and weak binding was observed at a concentration of 8 μM.

Experimental Section

General Experimental Procedures. ^{1}H-NMR (270 MHz) and ^{13}C-NMR (67.5 MHz) spectra were recorded on a JEOL GX-270 NMR spectrometer in $CDCl_3$ with TMS as internal standard. Chemical shifts are reported in units (ppm downfield from TMS). The MS was determined on a JEOL DX-300 mass spectrometer. The UV spectrum was recorded on a Hitachi 100−50 type spectrometer. The optical rotation was recorded on a JASCO digital polarimeter DIP-4. Analytical TLC was performed with E. Merck precoated TLC plates (Kieselgel 60 F_{254}, 0.2 mm). Column chromatography was carried out on E. Merck Kieselgel 60 (70−230 mesh) as the stationary phase. HPLC was carried out on a GL Science HPLC pump Model 576 and a 502T detector.

Material. Δ^6-THC (**1**) was synthesized from a dioxane solution of cannabidiol with borontrifluoride etherate, as previously described.[13]

Culture Method. A tissue segment of the tubers of *P. ternata* (ca. 1 cm in diameter) precultured in Murashige−Skoog medium[14] supplemented with 2,4-dichlorophenoxyacetic acid (0.25 mg/L) for 7 days was aseptically cut into 3-mm cubes as previously reported.[15] These segments were cultured in the same medium on a reciprocating shaker (60 rpm) at 25 °C under a 16-h photoperiod before the substrate **1** was added to the medium. An EtOH solution of **1** was sterilized by filtration using a sterile Millex-HV 0.45-μm filter unit (Millipore Products Division, Bedford, MA).

For time−course experiments, an EtOH solution of **1** (2 mg) was added to 30 mL of a tissue suspension, which was then incubated for various time periods in the dark (Figure 1). Tissue segments were collected by filtration, lyophilized, and weighed.

Extraction and Isolation. An EtOH solution (9.0 mL) of **1** (180 mg) was added to the culture (2.7 L), which was then incubated with tissue segments of *P. ternata* at 25 °C in the dark for 5 days. The medium was concentrated to a volume of approximately 500 mL and extracted with EtOAc (500 mL × 5), and the pooled EtOAc solution was evaporated to dryness. The extract (170 mg) was subjected to column chromatography (Si gel) using $CHCl_3$−MeOH−H_2O (6:1:0.05) as solvent to afford **2** (5.5 mg).

Δ^6-Tetrahydrocannabinol 2′-*O*-*β*-D-glucopyranoside (2): amorphous powder; $[\alpha]^{20}_D$ −144.8° (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 272 (3.11), 280 (3.11) nm; ^{1}H-NMR and ^{13}C-NMR data, see Table 1; negative FABMS *m*/*z* $[M - H]^-$ 475.

Quantitative Analysis. The levels of compounds **2** in the medium and tissue segments were determined by HPLC using Cosmosil 5C_{18}-MS (4.6 × 150 mm)-(Tosoh Type, Nacalai Tesque, Inc., Kyoto, Japan) with monitoring of UV absorption at 280 nm. The mobile phase used was a mixture of MeCN and H_2O (50 mM H_3PO_4) in which the proportion of MeCN was increased from 20% to 100% over a 17-min period; the flow rate was 1.0 mL/min. For time−course experiments, a tissue suspension culture derived from segments of *P. ternata* tubers was separated from the medium by filtration. The medium (2 mL) was lyophilized, and the residue was dissolved in MeOH (500 μL). The MeOH solution was filtered, and the filtrate (10 μL) was subjected to HPLC in triplicate. The freeze-dried powder (50 mg) of cultured tissue segments was extracted with MeOH (5 mL) under sonication at room temperature for 10 min five times. The MeOH solution was filtered and evaporated *in vacuo*. The extract was dissolved in MeOH (500 μL) and was filtered. The filtrate (10 μL) was subjected to HPLC. Three replicated samples incubated under the same culture conditions were analyzed, and standard deviations were calculated.

Ligand-Binding Assay. The ligand-binding assay with the biotransformation products was performed by Prof. R. Mechoulam, Department of Natural Products, Hebrew University, Jerusalem, Israel, as described elsewhere.[11,12]

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References and Notes

(1) Burstein, S. H. In *Marijuana, Chemistry, Pharmacology, Metabolism and Clinical Effects*; Mechoulam, R., Ed.; Academic Press: New York, 1973; pp 167−190.
(2) Crombie, L.; Crombie, W. M. L. In *Cannabis and Health*; Graham, J. D. P., Ed.; Academic Press: London, 1976; pp 43−76 .
(3) Mechoulam, R.; McCallum, N. K. ; Burstein, S. *Chem. Rev.* **1976**, *76*, 75−112.
(4) Agurell, S.; Dewey, W. L.; Willette, R. E. In *The Cannabinoids*: *Chemical, Pharmacologic, and Therapeutic Aspects*; Academic Press: New York, 1984; pp 165−327.

(5) Yamamoto, I. *Yakugaku Zasshi* **1986**, *106*, 537−561.
(6) Hartsel, S. C.; Loh, W. H. T.; Robertson, W. *Planta Med.* **1983**, *48*, 17−19.
(7) Braemer, R.; Paris, M. *Plant Cell Rep.* **1987**, *6*, 150−152.
(8) Tanaka, H.; Morimoto, S.; Shoyama, Y. *J. Nat. Prod.* **1993**, *56*, 2068−2072.
(9) Shoyama, Y.; Nishioka, I.; Hatano, K. In *Biotechnology in Agriculture and Forestry 19, High-Tech and Micropropagation III*; Bajaj, Y. P. S., Ed.; Springer-Verlag: Berlin; 1992, pp 464−480.
(10) Mechoulam, R.; Shvo, Y. *Tetrahedron* **1963**, *19*, 2073−2078.
(11) Devane, W. A.; Breuer, A.; Sheskin, T.; Jarbe, T. U. C.; Eisen, M. S.; Mechoulam, R. *J. Med. Chem.* **1992**, *35*, 2065−2069.
(12) Vogal, Z.; Barg, J.; Levy, R.; Saya, D.; Heldman, E.; Mechoulam, R. *J. Neurochem.* **1993**, *61*, 352−355.
(13) Shoyama, Y.; Yamauchi, T.; Nishioka, I. *Chem. Pharm. Bull.* **1970**, *18*, 1327−1332.
(14) Murashige, T.; Skoog, F. *Physiol. Planta* **1962**, *15*, 473−497.
(15) Shoyama, Y.; Hatano, K.; Nishioka, I. *Planta Med.* **1983**, *49*, 14−16.

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