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Hiroyuki Tanaka, Satoshi Morimoto, and Yukihiro Shoyama

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CANNABIS, 21.[1] BIOTRANSFORMATION OF CANNABINOL TO ITS GLYCOSIDES BY IN VITRO PLANT TISSUE

HIROYUKI TANAKA, SATOSHI MORIMOTO, and YUKIHIRO SHOYAMA*

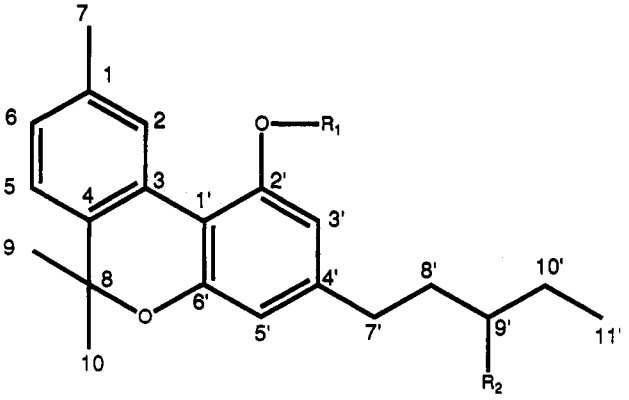
Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashiku, Fukuoka 812, Japan

ABSTRACT.—A marijuana compound, cannabinol [**1**], was converted to two metabolites using in vitro tissue of *Pinellia ternata*. The structures of the metabolites were determined to be cannabinol-*O*-β-D-glucopyranoside [**2**] and 9′-hydroxycannabinol-*O*-β-D-glucopyranoside [**3**] by ^{1}H nmr and ^{13}C nmr. From the time course experiments, **1** was absorbed rapidly in the tissues and glycosylated. Hydroxylation at the pentyl group occurred, and its metabolite was secreted in the medium.

Cannabinoids, especially tetrahydrocannabinol [**4**], are unstable compounds with respect to high temperature (1), light (2), or an acidic medium (3). However, cannabinol [**1**] is somehow stable compared with other cannabinoids. Many metabolic studies on cannabinoids have been carried out using animals, their organs, and microsomal fractions (4–8). These studies were concentrated almost entirely on **4**, causing pharmacological activity. Nearly 70 metabolites of analogues of **4** have been found. There are only a few metabolic studies on other cannabinoids.

The plant tissue culture method is used to study the metabolism of various kinds of compounds (9–12). The biotransformation of cannabidiol and **4** have been investigated by using suspension cultures of *Cannabis sativa* and *Saccharum officinarum* to give cannabielsoin (13) and cannabicoumaronon (14), respectively. However, there are no reports on the biotransformation of **1** by plant cells. Primarily for this reason, we have chosen **1** for our investigation.

The objectives of this study were to create new cannabinoids that were pharmacologically more active, to synthesize chemically difficult cannabinoids, and to improve their H_2O solubility. In this study **1** was administered to cell and tissue cultures of *Pinellia ternata* Breit. (Araceae) (15), and the biotransformation products were investigated.



1 $R_1=R_2=H$
2 $R_1=glc$, $R_2=H$
3 $R_1=glc$, $R_2=OH$

[1]For Part 20, see M. Okada, A. Urae, K. Mine, Y. Shoyama, K. Iwasaki, and M. Fujiwara, *Neurosci. Lett.*, **140**, 55 (1992).

Biotransformation products **2** and **3** were isolated from MeOH extracts of cultured protocorm and EtOAc extracts of medium after incubation of **1** with the tissue of *P. ternata.*

The fdms of compound **2** showed a peak at *m/z* 472 $[M]^+$, suggesting that **2** possessed one molecule each of **1** and glucose. The ^1H nmr of **2** showed the presence of the aromatic protons of the olivetol moiety as well as the protons from the other aromatic nucleus as shown in the **1** unit (16) in addition to those due to the sugar moiety as indicated in Table 1. In the ^{13}C nmr (Table 1), the signals assignable to the sugar moiety were observed in good agreement with those of glucose. The large coupling constant (J=8 Hz) of the anomeric protons in **2** appeared to be β. Finally **2** was unequivocally identified with the synthetic 1-*O*-β-D-glucopyranoside obtained from **1** and acetobromo-D-glucose (17). Thus, **2** is cannabinol-*O*-β-D-glucopyranoside.

The fdms of **3** showed a peak at *m/z* 488 $[M]^+$, consistent with the introduction of an additional hydroxyl group into **2**. The ^1H- and ^{13}C-nmr spectra of **3** showed signals assignable to glucose moiety in addition to those due to the monohydroxylated **1** unit. H-7′ at 2.58 (t, J=8 Hz) was found coupled to H-8′ at δ 1.59–1.66 (m) (COSY), and H-8′ coupled to shifted H-9′ at δ 3.60–3.65 (m) (COSY). From these data it was deduced that hydroxylation occurred at C-9′. In the ^{13}C nmr, the hydroxy function was

TABLE 1. ^1H- and ^{13}C-nmr Data of the Biotransformation Products **2** and **3** and 3-Pentanol (18) (270 MHz, CD_3OD). G: glucose moiety.

Position	Compound				
	2		**3**		
	^1H	^{13}C	^1H	^{13}C	
1	—	138.2[a]	—	138.2[a]	
2	8.47, s	123.3	8.47, s	123.2	
3	—	145.6	—	145.3	
4	—	137.8[a]	—	137.7[a]	
5	7.13, d, 7 Hz	109.7[b]	7.14, d, 8 Hz	109.7[b]	
6	7.04, d, 7 Hz	113.0[b]	7.05, d, 8 Hz	113.1[b]	
7	2.35, s	21.6	2.36, s	21.5	
8	—	71.4	—	71.4	
9,10	1.51, 1.54, s	27.3	1.51, 1.54, s	27.3	
1′	—	145.6	—	145.3	
2′	—	156.2	—	156.2	
3′	6.73, s	128.6	6.75, s	128.6	
4′	—	129.1	—	129.1	
5′	6.45, s	128.6	6.47, s	128.6	
6′	—	155.6	—	155.6	3-Pentanol
7′	2.56, t, 8 Hz	36.9	2.58, t, 7 Hz	36.9	C-1 10.1
8′	1.61–1.69, m	32.7	1.59–1.66, m	30.7[c]	C-2 30.0
9′	1.34–1.43, m	31.9	3.60–3.65, m	68.4	C-3 74.1
10′	1.34–1.43, m	23.6	1.42–1.45, m	30.4[c]	C-4 30.9
11′	0.92, t, 7 Hz	14.4	0.89, t, 7 Hz	14.4	C-5 10.1
G-1	5.16, d, 8 Hz	101.8	5.16, d, 8 Hz	101.8	
2	3.55–3.65, m	75.3	3.60–3.65, m	75.2	
3	3.43, t, 9 Hz	78.3	3.45, t, 8 Hz	78.3	
4	3.51, t, 9 Hz	71.4	3.51, t, 9 Hz	71.4	
5	3.71, dd, 5, 12 Hz	78.7	3.72, dd, 6, 12 Hz	78.7	
6	3.50, dd, 5, 12 Hz	62.6	3.48–3.50, m	62.6	
	3.89, dd, 2, 12 Hz		3.91, dd, 2, 12 Hz		

[a–c]Assignments may be reversed in each vertical column.

unambiguously determined as C-9′ on a pentyl group compared with those of 3-pentanol (18) as indicated in Table 1. The absolute configuration of the hydroxyl group at C-9′ is still unknown. The large coupling constant (J=8 Hz) of the anomeric proton in **3** is β. The above evidence is consistent with the formulation of **3** as 9′-hydroxycannabinol-*O*-β-D-glucopyranoside.

To investigate the accumulation of metabolites, time course experiments were carried out. The medium and cells or protocorms were separately extracted by the methods described in the Experimental section for the quantitative analysis of **1**, **2**, and **3** by hplc.

Product **2** was detected from the first day after incubation of **1**, increasing up to 9 days in the protocorm tissue (Figure 1). The conversion ratio of **1** to **2** reached approximately 35%, then decreased gradually for 30 days. Product **2** was also detected from the first day after incubation in the medium. The level of **2** in the protocorm tissue, however, was threefold compared to the medium.

The conversion of **1** into **3** was slower than the conversion of **2**. This phenomenon may be expected, as the hydroxylation of a side chain of **2** occurred only after **1** was glycosylated. The hydroxylation of **2** in the side chain occurred 2 days after incubation. Product **3** accumulated gradually and reached a maximum (transformation ratio about 25%) 23 days after incubation in the medium. The content of **3** in the protocorm tissue was rather low compared to that of the medium due to the release of **3** into the medium. Therefore, it can be assumed that **1** is absorbed in the tissue, and the first glycosylation takes place following hydroxylation at the pentyl group to give **3**. It is possible that suitable conditions prevail for the production of **2** to allow harvesting of the protocorm tissue after a 9-day incubation. When **3** is needed the medium should be harvested 23 days after incubation.

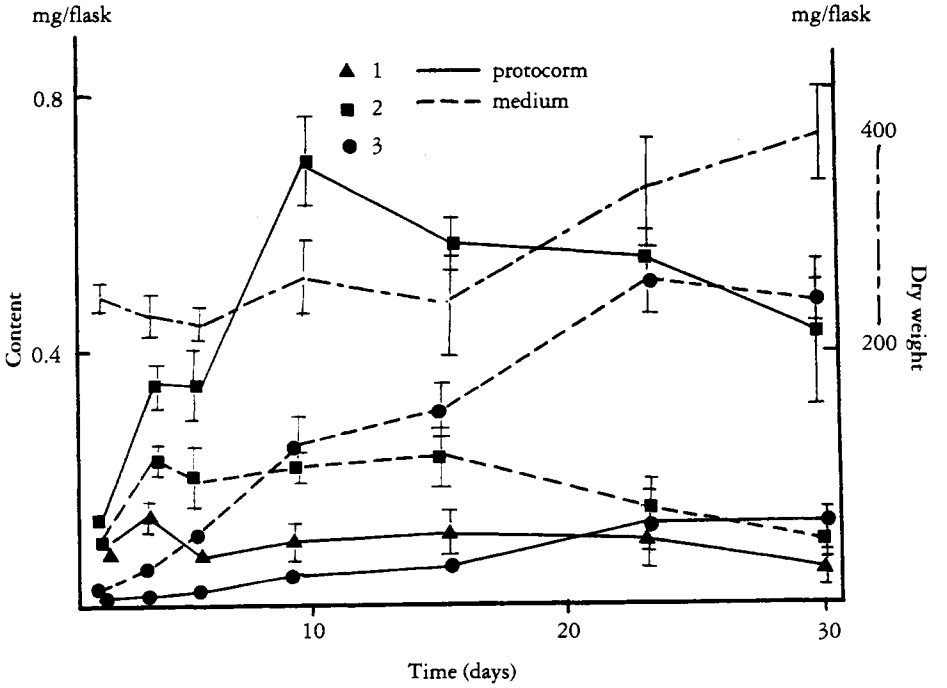


FIGURE 1. Time course of biotransformation products **2** and **3** from **1** in protocorm suspension culture of *Pinellia ternata*.

The cell suspension culture was also investigated for the biotransformation of **1**. The conversion ability of cells was, however, lower than that of the protocorm tissue as indicated in Figure 2.

This is the first report of such glycosylation occurring in cannabinoids. The results of the investigation of their pharmacological activities will be presented elsewhere.

EXPERIMENTAL

CULTURE METHOD.—The cell and protocorm suspension of *P. ternata* was cultured in Murashige-Skoog medium (19) supplemented with 2,4-D (1 mg/liter) on a shaker (60 rpm) at 25° under a 16-h photoperiod as previously established (15). Cells and protocorms were subcultured every 4 weeks into 100-ml Erlenmeyer flasks containing 30 ml of culture medium.

For the time course experiments, EtOH solution of **1** (2 mg) was added to 30 ml of suspension culture and cultured for individual incubation periods in dark as indicated in Figure 1. Cells and protocorms were collected by filtration, lyophilized, and weighed.

EXTRACTION AND ISOLATION PROCEDURE.—The compounds were detected by spraying the developed tlc plates with diazotized benzidine reagent after spraying with diluted HCl and concentrated H_2SO_4.

To isolate the conversion products, EtOH solution (8.75 ml) of **1** (175 mg) was added to the medium (2,625 ml) and incubated with the tissue of *P. ternata* at 25° in the dark for 7 days. The medium was extracted with EtOAc, and the EtOAc extract was pooled. The MeOH extract of cells and protocorms was evaporated and redissolved in H_2O, then extracted with EtOAc. The combined EtOAc extract (1.59 g) was chromatographed on Sephadex LH-20 using MeOH/H_2O as the eluent and then preparative tlc developing with $CHCl_3$-MeOH-H_2O-diethylamine (7:3:1:1 drop) to give **2** (10 mg) and **3** (6.1 mg). Compound **2**: colorless powder; $[\alpha]_D$ −31.0° (c=0.62, MeOH); fdms m/z $[M]^+$ 472, uv λ max (MeOH) nm (log ε) 280 (4.09); ^1H and ^{13}C nmr see Table 1. Compound **3**: colorless powder; $[\alpha]_D$ −9.7 (c=0.54, MeOH); fdms m/z $[M]^+$ 488; ^1H and ^{13}C nmr see Table 1.

SYNTHESIS OF **2**.—Compound **2** was synthesized following the method of Hanessian and Banoub (17). Compound **2**: colorless powder; fdms m/z $[M]^+$ 472, uv λ max (MeOH) nm (log ε) 280 (4.09); ^1H nmr (CD_3OD) 0.92 (3H, t, J=7 Hz, H-11′), 1.34–1.42 (4H, m, H-9′, -10′), 1.51, 1.54 (3H×2, each s, H-9, -10), 1.61–1.70 (2H, m, H-8′), 2.36 (3H, s, H-7), 2.56 (2H, t, J=8 Hz, H-7′), 3.43 (1H, t, J=9 Hz, glc

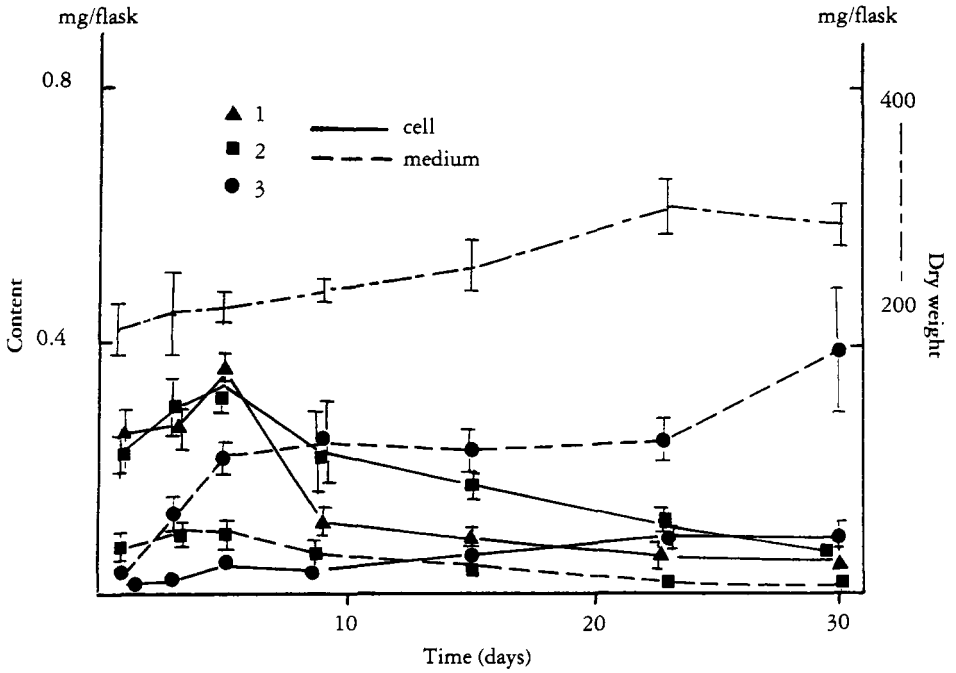


FIGURE 2. Time course of biotransformation products **2** and **3** from **1** in cell suspension culture of *Pinellia ternata*.

H-3), 3.50 (1H, dd, J=5, 12 Hz, glc H-6), 3.51 (1H, t, J=9 Hz, glc H-4), 3.55–3.65 (1H, m, glc H-2), 3.71 (1H, dd, J=5, 12 Hz, glc H-5), 3.89 (1H, dd, J=2, 12 Hz, glc H-6), 5.15 (1H, d, J=8 Hz, glc H-1), 6.45 (1H, s, H-5′), 6.73 (1H, s, H-3′), 7.06 (1H, d, J=7 Hz, H-6), 7.14 (1H, d, J=7 Hz, H-5), 8.48 (1H, s, H-2).

QUANTITATIVE ANALYSIS.—Compounds **1**, **2**, and **3** in the medium and cell or protocorm were determined by hplc using Cosmosil 5C18 (4.6×250 mm; Toso) MeCN-H_2O (5:3 plus 50 mM H_3PO_4) as eluent with monitoring of uv absorption at 280 nm. Compounds **1** (5.0 mg), **2** (4.1 mg), and **3** (3.4 mg) were dissolved in MeCN-H_2O (5:3 plus 50 mM H_3PO_4) and diluted stepwise; 10 μl was used for calibration of the system. From individual weight and peak height the calibration of the system. From individual weight and peak height the calibration curve was made.

For time course experiments the suspension culture of *P. ternata* was separated from the medium by filtration. Media (10 μl) were used for analysis. Dried powders of cells and protocorms were extracted with MeOH. The MeOH extract was dissolved in MeCN-H_2O (5:3 plus 50 mM H_3PO_4), and injected to hplc. Three samples incubated under the same condition were analyzed and the standard deviations were determined.

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