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Note

Biotransformation of (+)-catechin by plant cultured cells of *Eucalyptus perriniana*

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Abstract To examine the biotransformation of (+)-catechin by the plant cultured cells of *Eucalyptus perriniana*, three new biotransformation products, (+)-catechin-3′-*O*-*β*-D-glucoside, (+)-catechin-5-*O*-*β*-D-glucoside and (+)-catechin-7-*O*-*β*-D-glucoside were isolated after 3 days incubation. The cultured cells of *E. perriniana* were found to be capable of glycosylation at the 5, 7-positions on the A ring and the 3′-position on the B ring of (+)-catechin.

Key words: (+)-catechin, glycosylation, *Eucalyptus*.

Biotransformation by plant cultured cells serves as an important tools in the structural modification of compounds possessing useful therapeutic activity and is of considerable interested in green chemistry field (Orihara et al. 1991; Furuya et al. 1998a). Consequently, a great number of biotransformation studies have been carried out with plant cells in cultures (Braemer et al. 1987; Furuya et al. 1998a; Orihara et al. 1992). We have investigated the biotransformation of target substrates by plant cultured cells and clarified the plant conversion abilities such as hydroxylation, reduction, oxidation and glycosylation (Hirata et al. 1982; Naoshima et al. 1989; Hamada et al. 1997, 2002). As (+)-catechin (3,3′,4′,5,7-flavanpentol) is widely distributed in the plant kingdom and has many physiological functions such as antioxidative activity, antibacterial activity and the regulation of cholesterol levels in mammalian blood, the biotransformation of (+)-catechin by plant cells is of considerable interest in the medical field (Hara 2001). Also, the use of catechins is limited because they are poorly soluble in water and easily degraded by light irradiation in an aqueous solution resulting in rapid browning (Nonaka et al. 1983; Sato et al. 2000). As plant cultured cells of *Eucalyptus perriniana* have a high glycosylation ability we use this cell line in this experiment (Furuya et al. 1998b; Hamada et al. 2002). In this paper, we describe the biotransformation of (+)-catechin by plant cultured cells of *E. perriniana* and its biotransformation ability such as glycosylation.

Eucalyptus cultured cells were prepared as described in the literature (Furuya et al. 1987) . The feeding and incubation experiments were carried out in a manner similar to that reported in previous papers (Hamada et al. 1994, 2001). Just prior to use in this study, part of the callus tissues (30 g fresh weight) was transplanted to freshly prepared Murashige and Skoog's medium (100 ml in a 300 ml conical flask, pH 6.2) containing 1 ppm of benzyl-adenine and 3% sucrose and grown with continuous shaking for 5 days at 25°C in the dark. (+)-Catechin (20 mg/flask, no solvent, 99% or greater, purity) was added to the cultured cells and the cells were incubated at 25°C on a rotary shaker (120 rpm) in the dark.

After 3 days incubation, the cultured cells were harvested and products **2**–**4** were isolated from the MeOH extract of the cells (Figure 1).

The cells were extracted by homogenization with methanol and the extract was concentrated. The residue was applied to a Diaion HP-20 column and the column was washed with H_2O followed by elution with methanol. The methanol eluate was subjected to an ODS chromatography (100–200 mesh) and HPLC (column: 150×20 mm) to give products **2**–**4**. No products **2**–**4** were observed in the medium. The yields of the products were determined on the basis of the peak area from HPLC and are expressed as a relative percentage to the total amount of the whole reaction products extracted (Table 1). Products **2**–**4** were identified by comparison of the ^{1}H NMR, ^{13}C NMR and LC/MS spectra with those in the literature (Friedrich et al. 2002; Kashiwada et al.

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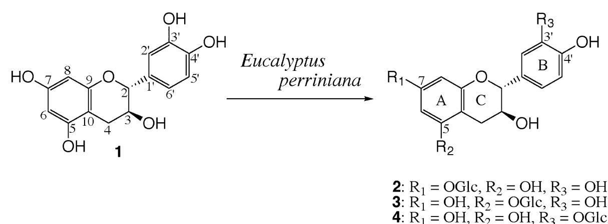

Figure 1. The glucosylation of (+)-catechin (**1**) by plant cultured cells of *E. perriniana*.

Table 1. Biotransformation of **1** by *E. perriniana*.

Substrate	Product	Yield (%)[a]
1	(+)-catechin-7-*O*-β-D-glucoside (**2**)	44
	(+)-catechin-5-*O*-β-D-glucoside (**3**)	13
	(+)-catechin-3′-*O*-β-D-glucoside (**4**)	33

[a] Weight (%) of the product relative to the substrate administered.

1986).

Spectral data of **2**; (+)-catechin-7-*O*-β-D-glucoside. Brown solid, LC-MS *m/z*: 475 $[M+Na]^+$. ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): 2.53 (1H, dd, J=16.0, 8.4 Hz, H-4ax), 2.86 (1H, dd, J=16.0, 5.4 Hz, H-4eq), 3.40–3.88 (6H, m, H-2″~H-6″), 3.99 (1H, m, H-3), 4.58 (1H, d, J=7.2 Hz, H-2), 4.80 (1H, d, J=7.2 Hz, H-1″), 6.15 (1H, d, J=2.0 Hz, H-8), 6.20 (1H, d, J=2.0 Hz, H-6), 6.70 (1H, dd, J=8.0, 2.0 Hz, H-6′), 6.75 (1H, d, J=8.0 Hz, H-5′), 6.82 (1H, d, J=2.0 Hz, H-2′). ^{13}C NMR (CD_3OD): 28.5 (C-4), 62.4 (C-6″), 68.5 (C-3), 71.3 (C-4″), 74.5 (C-2″), 77.9 (C-3″), 78.0 (C-5″), 82.9 (C-2), 96.7 (C-8), 98.0 (C-6), 102.1 (C-1″), 103.2 (C-10), 115.1 (C-2′), 115.9 (C-5′), 119.1 (C-6′), 131.9 (C-1′), 146.1 (C-3′, C-4′), 156.7 (C-9), 157.4 (C-5), 158.5 (C-7).

Spectral data of **3**; (+)-catechin-5-*O*-β-D-glucoside. Brown soild, LC-MS *m/z*: 475 $[M+Na]^+$. ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): 2.56 (1H, dd, J=16.0, 8.4 Hz, H-4ax), 3.01 (1H, dd, J=16.0, 5.4 Hz, H-4eq), 3.42–3.90 (6H, m, H-2″~H-6″), 3.97 (1H, m, H-3), 4.58 (1H, d, J=7.2 Hz, H-2), 4.83 (1H, d, J=7.2 Hz, H-1″), 6.01 (1H, d, J=2.0 Hz, H-8), 6.25 (1H, d, J=2.0 Hz, H-6), 6.66 (1H, dd, J=8.0 Hz, 2.0 Hz, H-6′), 6.75 (1H, d, J=8.0 Hz, H-5′), 6.81 (1H, d, J=2.0 Hz, H-2′). ^{13}C NMR (CD_3OD): 28.4 (C-4), 62.5 (C-6″), 68.5 (C-3), 71.2 (C-4″), 74.8 (C-2″), 78.1 (C-3″), 78.1 (C-5″), 82.8 (C-2), 96.7 (C-8), 98.0 (C-6), 102.4 (C-1″), 103.2 (C-10), 115.0 (C-2′), 115.9 (C-5′), 119.8 (C-6′), 131.9 (C-1′), 146.1 (C-3′, C-4′), 156.5 (C-9), 157.8 (C-7), 157.9 (C-5).

Spectral data of **4**; (+)-catechin-3′-*O*-β-D-glucoside. White amorphous powder, LC-MS *m/z*: 475 $[M+Na]^+$. ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): 2.51 (1H, dd, J=16.0, 8.4 Hz, H-4ax), 2.86 (1H, dd, J=16.0, 5.4 Hz, H-4eq), 3.40–3.85 (6H, m, H-2″~H6″), 4.00 (1H, m, H-3), 4.60 (1H, d, J=7.2 Hz, H-2), 4.70 (1H, d, J=7.2 Hz, H-1″), 5.83 (1H, d, J=2.0 Hz, H-6), 5.92 (1H, d, J=2.0 Hz, H-8), 6.84 (1H, d, J=8.0 Hz, H-5′), 6.96 (1H, dd, J=8.0, 2.0 Hz, H-6′), 7.19 (1H, d, J=2.0 Hz, H-2′).

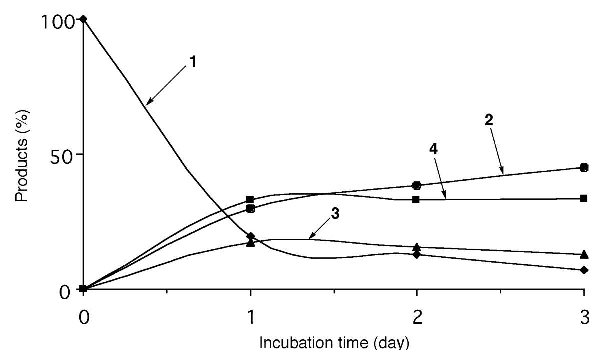

Figure 2. Time-course of biotransformation of **1** by *E. perriniana*.

^{13}C NMR (CD_3OD): 28.7 (C-4), 62.3 (C-6″), 68.6 (C-3), 71.2 (C-4″), 74.8 (C-2″), 77.5 (C-3″), 78.2 (C-5″), 82.8 (C-2), 95.4 (C-6), 96.3 (C-8), 100.8 (C-10), 103.7 (C-1″), 116.7 (C-5′), 117.1 (C-2′), 123.6 (C-6′), 132.3 (C-1′), 146.4 (C-4′), 147.9 (C-3′), 156.7 (C-9), 157.5 (C-5), 157.8 (C-7).

The biotransformation yield of (+)-catechin by the cultured cells of *E. perriniana* is shown in Table 1. The yield of products **2**, **3** and **4** was 44, 13 and 33%, respectively. We then studied the time-course experiment of biotransformation of **1** by *E. perriniana* (Figure 2). After a 1-day incubation, the biotransformation product such as **2**, **3** and **4** occurred, and after 3 days incubation, **2** and **4** become the main products with **3** as a minor product.

From the results of this experiment, it was found that the cultured cells of *E. perriniana* glycosylate at C-5 and C-7 on the A ring and at C-3′ on the B ring of (+)-catechin. This method is of considerable interest in green chemistry. Also, the biological activity as an antioxidant and the stability against light and pH of these products, **2**, **3** and **4** are now in progress.

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