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Biotransformation of raspberry ketone and zingerone by cultured cells of *Phytolacca americana*

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Abstract

The biotransformation of raspberry ketone and zingerone were individually investigated using cultured cells of *Phytolacca americana*. In addition to (2*S*)-4-(4-hydroxyphenyl)-2-butanol (2%), (2*S*)-4-(3,4-dihydroxyphenyl)-2-butanol (5%), 4-[4-(β-D-glucopyranosyloxy)phenyl]-2-butanone (19%), 4-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (23%), and (2*S*)-4-(4-hydroxyphenyl)but-2-yl-β-D-glucopyranoside (20%), two biotransformation products, i.e., 2-hydroxy-4-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (12%) and 2-hydroxy-5-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (11%), were isolated from suspension cells after incubation with raspberry ketone for three days. On the other hand, two compounds, i.e., (2*S*)-4-(4-hydroxy-3-methoxyphenyl)but-2-yl-β-D-glucopyranoside (17%) and (2*S*)-2-(β-D-glucopyranosyloxy)-4-[4-(β-D-glucopyranosyloxy)-3-methoxyphenyl]butane (16%), together with (2*S*)-4-(4-hydroxy-3-methoxyphenyl)-2-butanol (15%), 4-[4-(β-D-glucopyranosyloxy)-3-methoxyphenyl]-2-butanone (21%), and 4-[(3*S*)-3-hydroxybutyl]-2-methoxyphenyl-β-D-glucopyranoside (24%) were obtained upon addition of zingerone. Cultured cells of *P. americana* can reduce, and regioselectively hydroxylate and glucosylate, these food ingredients to their β-glycosides.

Keywords: *Phytolacca americana*; Phytolaccaceae; Cultured plant cells; Biotransformation; Raspberry ketone; Zingerone; Glycosides; β-Glucoside; Di-β-Glucoside

1. Introduction

Over the past few decades, biotransformation has been extensively studied because it is considered to be an important method for converting inexpensive and plentiful organic compounds into costly and scarce ones. Recently, plant cell cultures have been studied as useful agents for biotransformation reactions because of their biochemical potential to produce specific secondary metabolites such as flavors, pigments, and agrochemicals (Suga and Hirata, 1990; Ishihara et al., 2003). The reactions involved in the biotransformation of organic compounds by cultured plant cells include oxidation, reduction, hydroxylation, esterification, methylation, isomerization, hydrolysis, and glycosylation (Suga and Hirata, 1990; Ishihara et al., 2003). Particularly, glycosylation by cultured plant cells has been the subject of increasing attention, since an one-step enzymatic glycosylation by cultured plant cells is more convenient than chemical glycosylation, which requires tedious steps such as protection and deprotection of the hydroxyl groups of the sugar moieties. Glycosylation occurs readily in plant cells, i.e., many kinds of secondary metabolites such as saponins and anthocyanins are produced in the

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form of glycosides in higher plants and most of them are accumulated in the vacuole of cells. Many of these secondary metabolites have specific physiological activities and have been widely used in folk medicines (Mastelic et al., 2004). Therefore, the glycosylation of organic compounds by cultured plant cells is of pharmaceutical importance and has been carried out for many exogenous compounds (Furuya et al., 1987, 1988, 1989; Moyer and Gustine, 1987; Tabata et al., 1988; Upmeier et al., 1988; Ushiyama et al., 1989a; Lewinson et al., 1996).

4-(4-Hydroxyphenyl)butan-2-one (raspberry ketone (**1**)) and 4-(4-hydroxy-3-methoxyphenyl)butan-2-one (zingerone (**2**)) are major aromatic compounds of red raspberry *Rubus idaeus* and zinger *Zingiber officinale*, respectively, and have been used worldwide as food additives and spices (Govindarajan, 1982; Borejsza-Wysocki and Hrazdina, 1994). Recently, it has been reported that these compounds, the structures of which are similar to those of capsaicinoids, showed stronger anti-obesity activity than capsaicin (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-6-nonamide) and synephrine (1-(4-hydroxyphenyl)-2-methylaminoethanol) (Govindarajan, 1982; Morimoto et al., 2005). Furthermore, raspberry ketone (**1**) has antibacterial, anticancer, and depigmenting activities, and zingerone (**2**) produces antiemetic, anti-inflammatory, anticancer, anxiolytic, antithrombotic, and cardiovascular effects (Fukuda et al., 1998; Reddy et al., 2001). Despite such bio- and physiological activities, their use as lipid degradation ingredients and medicines has been limited, due to their insolubility in water and decomposition under light. Glycosylation allows water-insoluble and unstable organic compounds to be converted into the corresponding water-soluble and stable compounds to improve their bio- and pharmacological properties. From a physiological point of view, the glycosides of raspberry ketone (**1**) and zingerone (**2**) may be of pharmacological interest. However, there have been no reports on their enzymatic glycosylation by cultured plant cells. We report here the biotransformation of raspberry ketone (**1**) and zingerone (**2**) into β-glucosides and di-β-glucoside, with greater water-solubility, by cultured plant cells of *Phytolacca americana*.

2. Results and discussion

2.1. Biotransformation of raspberry ketone (*1*)

After cultured cells of *P. americana* were incubated with raspberry ketone (**1**) for three days, the glycosylated products **5**–**9** were isolated from the cells by extraction with MeOH. On the other hand, none were detected in the medium. No additional glycosylation products were detected in the MeOH extracts of the cells despite careful HPLC analyses. On the basis of their HRFABMS, ^{1}H and ^{13}C NMR (Table 1), H–H COSY, C–H COSY, and NOE-spectroscopic analyses, the products were determined to be (2*S*)-4-(4-hydroxyphenyl)-2-butanol (**3**, 2%, 98% ee), (2*S*)-4-(3,4-dihydroxyphenyl)-2-butanol (**4**, 5%, 98% ee), 4-[4-(β-D-glucopyranosyloxy)phenyl]-2-butanone (**5**, 19%), 4-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (**6**, 23%), (2*S*)-4-(4-hydroxyphenyl)but-2-yl-β-D-glucopyranoside (**7**, 20%), 2-hydroxy-4-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (**8**, 12%), and 2-hydroxy-5-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (**9**, 11%), of which **8** and **9** are new. The HRFABMS spectrum of **8** showed a pseudomolecular ion $[M+Na]^+$ peak at *m*/*z* 367.1370, consistent with a molecular formula of $C_{16}H_{24}O_8$ (calcd. 367.1369 for $C_{16}H_{24}O_8Na$). The ^{1}H NMR spectrum of **8** had a signal at δ 4.69 (1H, *d*, $J = 7.6$ Hz) corresponding to its attachment to the anomeric carbon (C-1′). The ^{13}C NMR spectrum of **8** exhibited 16 carbon signals including the anomeric carbon signal at δ 104.8. From the coupling pattern of the proton signals and the chemical shifts of the carbon resonances due to the sugar moiety, the sugar component in **8** was concluded to be β-D-glucopyranose. Hydrolysis of **8** using almond β-glucosidase gave the aglycone, (2*S*)-4-(3,4-dihydroxyphenyl)-2-butanol, the optical purity of which was determined to be 97% ee by chiral GLC analysis. An HMBC correlation was also observed between the proton at δ 4.69 (H-1′) and the carbon signal at δ 148.4 (C-8), which confirms that the glucopyranosyl residue was attached to the phenolic hydroxyl group at the 8-position of (2*S*)-4-(3,4-dihydroxyphenyl)-2-butanol. Thus, the structure of **8** was determined to be 2-hydroxy-4-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside. The HRFABMS spectrum of the product **9** ($[M+Na]^+$ peak at *m*/*z* 367.1369) suggested a molecular formula of $C_{16}H_{24}O_8$ (calcd. 367.1369 for $C_{16}H_{24}O_8Na$). From the coupling pattern of the proton signals and the chemical shifts of the carbon resonances due to the sugar moiety, the sugar component in **9** was concluded to be β-D-glucopyranose. Hydrolysis of **9** using almond β-glucosidase gave the aglycone, (2*S*)-4-(3,4-dihydroxyphenyl)-2-butanol, the

Table 1
^{13}C NMR spectroscopic assignments for compounds **3**–**9**

Product	3	4	5	6	7	8	9
Aglycone							
1	23.5	23.6	30.0	23.5	22.3	23.6	23.5
2	67.8	67.8	210.7	67.8	75.3	67.9	67.7
3	32.2	32.2	45.9	32.3	31.9	32.6	32.4
4	42.4	42.5	30.0	42.3	40.6	42.2	42.1
5	134.3	135.0	136.1	137.4	134.5	139.6	135.2
6	130.1	118.7	130.0	130.1	130.4	119.2	118.8
7	115.9	145.0	117.6	117.7	116.0	145.0	146.1
8	156.1	146.7	157.3	157.1	156.3	148.4	146.4
9	115.9	116.8	117.6	117.1	116.0	117.1	116.7
10	130.1	124.3	130.0	130.1	130.4	120.8	124.3
Glc							
1′			102.3	102.5	102.2	104.8	104.3
2′			74.8	74.9	75.1	74.9	74.8
3′			78.0	78.0	78.1	78.3	78.2
4′			71.3	71.4	71.6	71.3	71.3
5′			78.0	78.0	77.8	77.7	77.5
6′			62.4	62.5	62.8	62.5	62.4