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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

enantiomeric composition of which was determined to be 97% ee by chiral GLC analysis. The ^{1}H NMR spectrum of **9** had a proton signal at δ 4.73 (1H, *d*, $J = 7.2$ Hz, H-1′) and analyses by the ^{13}C NMR data indicated its anomeric carbon resonance was at δ 104.3. An HMBC correlation was also observed between the proton signal at δ 4.73 (H-1′) and the carbon resonance at δ 146.1 (C-7). This confirms that the β-D-glucopyranosyl residue was attached to the phenolic hydroxyl group at C-7 of (2*S*)-4-(3,4-dihydroxyphenyl)-2-butanol. Thus, compound **9** was identified as 2-hydroxy-5-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside. To investigate the biotransformation pathway, the time course of the conversion of **1** was examined. As shown in Fig. 1, **1** was converted into (2*S*)-4-(4-hydroxyphenyl)-2-butanol (**3**) and β-glucoside **5** after incubation for 6 h, whereas products **4**, **6** and **7** predominantly accumulated after incubation for 12 h with concomitant decrease in amounts of **3**. This indicated that the reduction product **3** was formed first, with hydroxylated and glucosylated products **4**, **6** and **7** subsequently formed, as shown in Scheme 1.

2.2. Biotransformation of zingerone (2)

Zingerone (**2**) was subjected to the same biotransformation system. Glycoside products **11**–**14** were obtained from the MeOH extracts of the cells, whereas product **10** was isolated from EtOAc extract of the medium. The products were identified as (2*S*)-4-(4-hydroxy-3-methoxyphenyl)-2-butanol (**10**, 15%, 98% ee), 4-[4-(β-D-glucopyranosyloxy)-3-methoxyphenyl]-2-butanone (**11**, 21%), 4-[(3*S*)-3-hydroxybutyl]-2-methoxyphenyl-β-D-glucopyranoside (**12**, 24%), (2*S*)-4-(4-hydroxy-3-methoxyphenyl)but-2-yl-β-D-glucopyranoside (**13**, 17%), and (2*S*)-2-(β-D-glucopyranosyloxy)-4-[4-(β-D-glucopyranosyloxy)-3-methoxyphenyl]butane (**14**, 16%), of which **13** and **14** are new. The HRFABMS spectrum of product **13** ([M+Na]$^+$ peak at *m*/*z* 381.1528) suggested a molecular formula of $C_{17}H_{26}O_8$ (calcd. 381.1525 for $C_{17}H_{26}O_8Na$). The sugar component in **13** was concluded to be β-D-glucopyranose based on the coupling pattern of the proton signals and the chemical shifts of the carbon resonances due to the sugar moiety. The optical purity of the aglycone, (2*S*)-4-(4-hydroxy-3-methoxyphenyl)-2-butanol which was obtained by hydrolysis of **13** with almond β-glucosidase, was 97% ee. The ^{1}H NMR spectrum of **13** had a signal at δ 4.34 (1H, *d*, $J = 8.0$ Hz) corresponding to its attachment to the anomeric carbon (C-1′) and the ^{13}C NMR spectroscopic data of **13** showed anomeric carbon signal at δ 102.1 (Table 2). An HMBC correlation was observed between the proton signal at δ 4.34 (H-1′) and the carbon resonance at δ 75.2 (C-2). This result confirms that the β-D-glucopyranosyl residue was attached to the secondary hydroxyl group at the 2-position of (2*S*)-4-(4-hydroxy-3-methoxyphenyl)-2-butanol. Thus, compound **13** was identified as (2*S*)-4-(4-hydroxy-3-methoxyphenyl)but-2-yl-β-D-glucopyranoside. The HRFABMS spectrum of **14** showed a pseudomolecular ion [M+Na]$^+$ peak at *m*/*z* 543.2055, suggesting a molecular formula of $C_{23}H_{36}O_{13}$ (calcd. 543.2054 for $C_{23}H_{36}O_{13}Na$). The ^{1}H NMR spectrum of **14** showed proton signals for H-1′ and H-1″ at δ 4.35 (1H, *d*, $J = 8.0$ Hz) and 4.83 (1H, *d*, $J = 8.0$ Hz), respectively. The ^{13}C NMR spectrum of **14** exhibited 23 carbon signals including carbon signals for C-1′ and C-1″ at δ 102.1 and 103.0, respectively. The sugar component in **14** was concluded to be β-D-glucopyranose based on the coupling pattern of the sugar proton signals and the chemical shifts of the sugar carbon resonances. The optical purity of the aglycone, (2*S*)-4-(4-hydroxy-3-methoxyphenyl)-2-butanol, was determined to be 98% ee by the same method as was used for **8**, **9**, and **13**. HMBC correlations were observed between the proton signal at δ

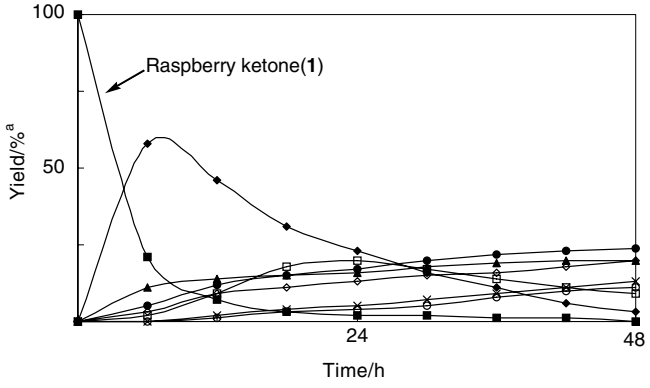
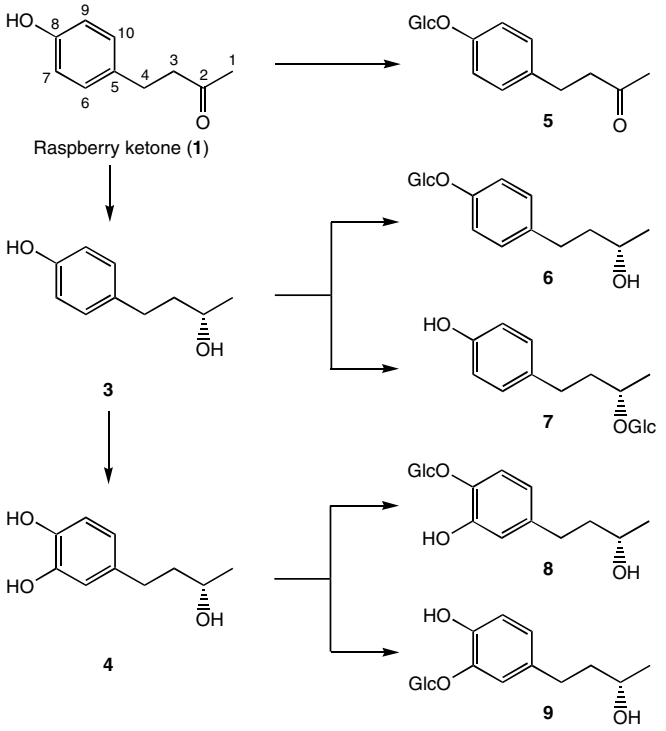


Fig. 1. Time course of the biotransformation of raspberry ketone (**1**) by cultured cells of *P. americana*. [a]Yields are expressed as a percentage of the total amounts of reaction products. Yields of **1** (■), **3** (◇), **4** (□), **5** (▲), **6** (●), **7** (◇), **8** (×), and **9** (○) are plotted.



Scheme 1. Glycosylation of raspberry ketone (**1**) by cultured cells of *P. americana*.

Table 2
^{13}C NMR spectroscopic assignments for compounds **10**–**14**

Product	10	11	12	13	14
Aglycone					
1	22.2	30.0	23.6	19.9	20.0
2	67.2	210.8	67.7	75.2	75.2
3	31.2	45.9	32.7	32.3	32.4
4	40.4	30.4	42.1	40.6	40.6
5	134.8	137.4	138.6	135.3	138.6
6	112.5	113.8	113.8	113.0	113.8
7	147.3	150.5	150.5	148.6	150.5
8	143.0	146.1	145.9	145.2	145.9
9	115.2	118.1	118.1	115.9	118.1
10	120.8	121.6	121.7	121.7	121.7
OMe	55.8	56.6	56.6	56.3	56.6
Glc					
1′		102.9	103.0	102.1	102.1
2′		74.8	74.8	75.1	75.0
3′		78.1	78.1	78.1	78.1
4′		71.2	71.2	71.6	71.7
5′		77.7	77.7	77.8	77.8
6′		62.4	62.4	62.7	62.7
1″					103.0
2″					74.8
3″					77.8
4″					71.2
5″					77.7
6″					62.4

4.35 (H-1′) and the carbon resonance at δ 75.2 (C-2), and between the proton signal at δ 4.83 (H-1″) and the carbon resonance at δ 145.9 (C-8). These findings confirm that the glucopyranosyl residues were attached to the secondary hydroxyl group at the C-2 position and the phenolic hydroxyl group at the C-8 position of (2*S*)-4-(4-hydroxy-3-methoxyphenyl)-2-butanol, respectively. Thus, compound **14** was identified as (2*S*)-2-(β-D-glucopyranosyloxy)-4-[4-(β-D-glucopyranosyloxy)-3-methoxyphenyl]butane. The time course of the conversion of **2** was examined. As shown in Fig. 2, **2** was reduced to (2*S*)-4-(4-hydroxy-3-methoxyphenyl)-2-butanol (**10**) and glucosylated to β-glucoside **11** after incubation for 6 h, while **12**, **13**, and **14** were produced after incubation for 12 h. This result indicated that (2*S*)-4-(4-hydroxy-3-methoxyphenyl)-2-butanol (**10**) was produced first and further glucosylation gave β-glucosides **12** and **13** and di-β-glucoside **14**, as shown in Scheme 2.

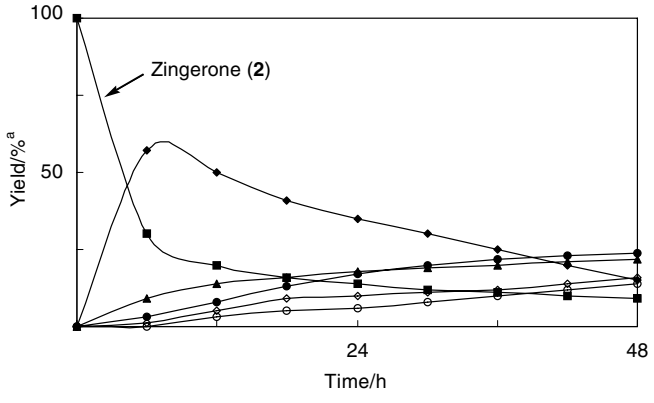
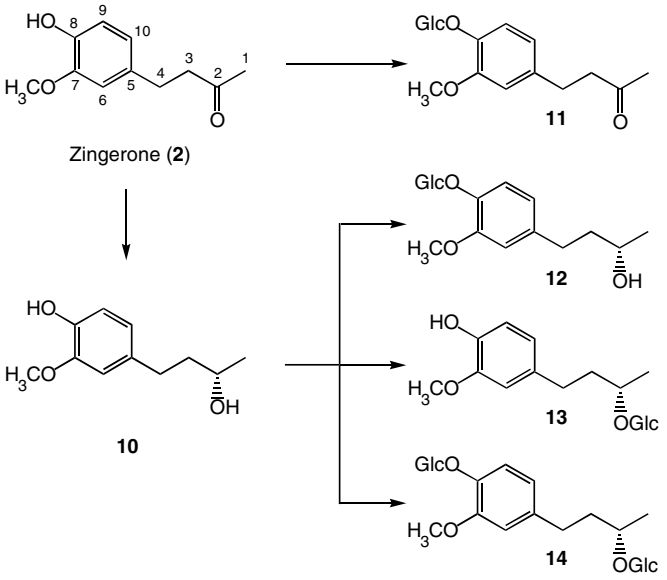


Fig. 2. Time course of the biotransformation of zingerone (**2**) by cultured cells of *P. americana*. [a]Yields are expressed as a percentage of the total amounts of reaction products. Yields of **2** (■), **10** (◇), **11** (▲), **12** (●), **13** (◇), and **14** (○) are plotted.



Scheme 2. Glycosylation of zingerone (**2**) by cultured cells of *P. americana*.

3. Conclusion

The results of this study established that cultured cells of *P. americana* can convert raspberry ketone and zingerone into the corresponding β-glucosides and di-β-glucoside, which accumulate in the cells. Cultured cells of *P. americana* regioselectively hydroxylated C-7 of raspberry ketone, and glycosides were formed at the hydroxyl groups at C-2, -7, and -8. The hydroxyl groups at C-2 and C-8 of zingerone were glucosylated by *P. americana* to give β-glucosides and di-β-glucoside. Disaccharide formation occurred only in the case of the biotransformation of zingerone, probably due to the substrate specificity of phenol-glucosyltransferases and/or secondary alcohol glucosyltrasnferases. Phenolic glycosylation by plant cell cultures is a very well known phenomenon (Tabata et al., 1976, 1988; Ushiyama and Furuya, 1989b), whereas little attention has been paid to the biological glycosylation of secondary alcohols. This is the first report on the introduction of individual glucose residues onto both phenolic and secondary hydroxyl groups by cultured plant cells. Furthermore, it is well known that glycosylation by plant cells contribute to the detoxification of toxic phenolic compounds which could arise either from normal plant metabolism or from the environment (Tabata et al., 1976; Kamel et al., 1992). The present results suggest that exogenous phenolic compounds such as raspberry ketone and zingerone, in high concentrations, act as chemical stresses on cultured *P. americana* cells. Although reduction products of raspberry ketone (**1**) and zingerone (**2**) have been isolated from

Taxus species (Das et al., 1993; Chattopadhyay et al., 2004), the biotransformation pathway of these compounds in cultured plant cells has not yet been elucidated. This is the first description of the reduction, hydroxylation, and glycosylation of weight-loss ingredients such as raspberry ketone (**1**) and zingerone (**2**) by cultured plant cells. The plant enzymes responsible for these biotransformations should be useful for the preparation of more water-soluble derivatives of raspberry ketone and zingerone. This procedure is simple and environmentally friendly. This method is useful for the practical preparation of these glycosides as food additives. Studies on the physiological activities of the glycosides and the characterization of these enzymes from *P. americana* are now in progress.

4. Experimental

4.1. Substrates

The substrates raspberry ketone (**1**) and zingerone (**2**) were purchased from Aldrich Chemical Co.

4.2. Cell line and culture conditions

Cultured suspension cells of *P. americana* were prepared as described previously (Hamada et al., 2001). Just prior to use for this work, part of the callus tissues (fr. wt 40 g, dry wt. ca. 20 g) was transplanted to freshly prepared Murashige and Skoog's medium (100 ml in a 300 ml conical flask, pH 5.7) containing 3% sucrose and grown with continuous shaking for 2 weeks on a rotary shaker (120 rpm) at 25 °C under illumination (4000 lx).

4.3. Biotransformation and purification of products

Substrate (0.08 mmol) dissolved in EtOH (300 μl) was individually administered to each of 10 flasks (0.8 mmol/l) containing suspension cultured cells of *P. americana*. The cultures were then incubated at 25 °C for three days on a rotary shaker (120 rpm) under illumination (4000 lx). After incubation, the cells and medium were separated by filtration with suction. The filtered medium (ca. 80 ml) was extracted with EtOAc (100 ml × 3). The medium was further extracted with *n*-BuOH (100 ml × 3). EtOAc and *n*-BuOH fractions were analyzed by HPLC, with the cells extracted with MeOH for 12 h (100 ml × 3) and sonicated for 5 min. The yields of the glycosylation products were calculated on the basis of the peak area from HPLC using calibration curves prepared by HPLC analyses of the authentic glycosides. The MeOH fraction was conc. and partitioned between H_2O (30 ml) and EtOAc (40 ml × 3). The EtOAc fractions were combined and analyzed by HPLC. The H_2O fraction was applied to a Diaion HP-20 column, with the column was washed with H_2O followed by elution with MeOH. The MeOH eluate was subjected to HPLC [column: YMC-Pack R& D ODS column (150 × 30 mm); solvent: MeOH–H_2O (9:11, v/v); detection: UV (280 nm); flow rate: 1.0 ml/min] to give glycosylated products.

4.4. Analysis

GLC analyses were carried out with a FID and a capillary column (0.25 mm × 25 m) coated with 0.25 μm CP cyclodextrin β 236M-19 (Chrompack) using N_2 as carrier gas (column temp: 100 °C, split ratio: 50, make up: 50 ml min^{-1}). The ^1H and ^{13}C NMR, H–H COSY, C–H COSY, NOE, and HMBC spectra were recorded using a Varian XL-400 spectrometer in CD_3OD solution and the chemical shifts were expressed in δ (ppm) referring to TMS. The HRFABMS spectra were measured using a JEOL MStation JMS-700 spectrometer. The structures of the products were determined on the basis of analysis of their HRFABMS, ^1H and ^{13}C NMR, H–H COSY, C–H COSY, NOE, and HMBC spectra. The enantiomeric excess of **3**, **4**, and **10** was determined by chiral GLC analyses on CP cyclodextrin β 236M-19. In order to examine the enantiomeric composition of aglycone moiety of glycosides **5**–**9**, and **11**–**14**, the glycosides were hydrolyzed by almond β-glucosidase purchased from Wako Pure Chemical Int. Ltd. Each glycoside was incubated at 37 °C for 24 h with 100 U of almond β-glucosidase and 1.0 ml phosphate buffer (0.1 M, pH 6.0). The glycosides were quantitatively hydrolyzed to give the corresponding aglycone alcohols as judged by HPLC analyses. The mixture was extracted with EtOAc and the ee of the aglycone alcohols obtained was determined by chiral GLC analyses on CP cyclodextrin β 236M-19.

Spectral data of new compounds are as follows:

2-Hydroxy-4-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (**8**), white powder (96 μmol): m.p. 127–129 °C; UV: λ_{max}^{MeOH} 282 nm; IR: 3370 cm^{-1}; HRFABMS: *m*/*z* 367.1370 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 1.17 (3H, *d*, *J* = 6.4 Hz, H-1), 1.66 (2H, *m*, H-3), 2.58 (2H, *m*, H-4), 3.30–3.52 (4H, *m*, H-2′, 3′, 4′, 5′), 3.70 (1H, *dd*, *J* = 12.0, 5.1 Hz, H-6a′), 3.72 (1H, *m*, H-2), 3.89 (1H, *d*, *J* = 10.8 Hz, H-6b′), 4.69 (1H, *d*, *J* = 7.6 Hz, H-1′), 6.62 (1H, *dd*, *J* = 8.4, 2.0 Hz, H-10), 6.69 (1H, *s*, H-6), 7.08 (1H, *d*, *J* = 7.6 Hz, H-9); for ^{13}C NMR (100 MHz, CD_3OD), see Table 1; chiral GLC: 97% ee.

2-Hydroxy-5-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (**9**), white powder (88 μmol): m.p. 130–131 °C; UV: λ_{max}^{MeOH} 278 nm; IR: 3388 cm^{-1}; HRFABMS: *m*/*z* 367.1369 $[M+Na]^+$; ^1H NMR (400 MHz, CD_3OD, δ in ppm): δ 1.17 (3H, *d*, *J* = 6.4 Hz, H-1), 1.68 (2H, m, H-3), 2.58 (2H, *m*, H-4), 3.30–3.48 (4H, *m*, H-2′, 3′, 4′, 5′), 3.71 (1H, *dd*, *J* = 11.6, 5.2 Hz, H-6a′), 3.72 (1H, *m*, H-2), 3.90 (1H, *d*, *J* = 12.0 Hz, H-6b′), 4.73 (1H, *d*, *J* = 7.2 Hz, H-1′), 6.74 (2H, *s*, H-9, 10), 7.05 (1H, *s*, H-6); for ^{13}C NMR (100 MHz, CD_3OD), see Table 1; chiral GLC: 97% ee.

(2*S*)-4-(4-Hydroxy-3-methoxyphenyl)but-2-yl-β-D-glucopyranoside (**13**), white powder (136 μmol): m.p. 125–127 °C; UV: λ_{max}^{MeOH} 280 nm; IR: 3353 cm^{-1}; HRFABMS: *m*/*z* 381.1528 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 1.21 (3H,

d, $J = 6.0$ Hz, H-1), 1.73 (1H, *m*, H-3a), 1.88 (1H, *m*, H-3b), 2.62 (2H, *m*, H-4), 3.17–3.41 (4H, *m*, H-2′, 3′, 4′, 5′), 3.71 (1H, *dd*, $J = 12.0$, 5.5 Hz, H-6a′), 3.83 (3H, *s*, OCH_3), 3.88 (1H, *d*, $J = 12.0$, 2.0 Hz, H-6b′), 3.93 (1H, *m*, H-2), 4.34 (1H, *d*, $J = 8.0$ Hz, H-1′), 6.65 (1H, *d*, $J = 8.4$ Hz, H-10), 6.70 (1H, *d*, $J = 8.4$ Hz, H-9), 6.77 (1H, *d*, $J = 1.6$ Hz, H-6); for ^{13}C NMR (100 MHz, CD_3OD), see Table 1; chiral GLC: 97% ee.

(2*S*)-2-(β-D-glucopyranosyloxy)-4-[4-(β-D-glucopyranosyloxy)-3-methoxyphenyl] butane (**14**), white powder (128 μmol): m.p. 138–140 °C; UV: λ_{max}^{MeOH} 277 nm; IR: 3367 cm^{-1}; HRFABMS: *m*/*z* 543.2055 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 1.20 (3H, *d*, $J = 6.0$ Hz, H-1), 1.73 (1H, *m*, H-3a), 1.87 (1H, *m*, H-3b), 2.63 (2H, *m*, H-4), 3.17–3.49 (8H, *m*, H-2′, 2″, 3′, 3″, 4′, 4″, 5′, 5″), 3.68 (1H, *dd*, $J = 12.4$, 5.6 Hz, H-6a″), 3.72 (1H, *dd*, $J = 12.0$, 5.5 Hz, H-6a′), 3.84 (3H, *s*, OCH_3), 3.88 (2H, *m*, H-6b′, 6b″), 3.94 (1H, *m*, H-2), 4.35 (1H, *d*, $J = 8.0$ Hz, H-1′), 4.83 (1H, *d*, $J = 8.0$ Hz, H-1″), 6.73 (1H, *dd*, $J = 8.4$, 2.0 Hz, H-10), 6.85 (1H, *d*, $J = 2.0$ Hz, H-6), 7.06 (1H, *d*, $J = 8.4$ Hz, H-9); for ^{13}C NMR (100 MHz, CD_3OD), see Table 1; chiral GLC: 98% ee.

4.5. Time course experiments

Fifty grams (fr. wt, dry wt ca. 25 g) of the suspension cells of *P. americana* was partition in to each of eight flasks containing 100 ml of MS medium. Substrate (0.1 mmol) dissolved in EtOH (300 μl) was administered to each flask (1 mmol/l) and the mixtures were incubated on a rotary shaker at 25 °C. At a 6 h interval, one of the flasks was taken out from the rotary shaker, and the cells and medium were separated by filtration. The extraction and analysis procedures were as described in Section 4.3. The yield of the products was determined on the basis of the peak area from HPLC, and expressed as a relative percentage to the total amount of the whole reaction products extracted.

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