Plant Biotechnology 23, 291–296 (2006)

Original Paper

Regioselective hydroxylation, reduction, and glycosylation of diphenyl compounds by cultured plant cells of *Eucalyptus perriniana*

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Received December 8, 2005; Accepted February 6, 2006 (Edited by K. Yazaki)

Abstract Bisphenol A and benzophenone, diphenyl compounds, were regioselectively hydroxylated, reduced, and glycosylated by the cultured plant cells of *Eucalyptus perriniana*. Three known biotransformation products and two new products, 2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)-2-(4-β-D-glucopyranosyloxyphenyl)propane and 2-(3-β-D-glucopyranosyloxy-4-β-D-glucopyranosyloxyphenyl)-2-(4-hydroxyphenyl)propane, were isolated after seven days' incubation of bisphenol A. This shows that the cultured cells of *E. perriniana* regioselectively hydroxylate at C-6 and C-12 of bisphenol A and that the glycosides can be formed at the hydroxyl group at C-6, 7, 12, and 13. On the other hand, two known products and two new products, 4-*O*-[6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]benzophenone and diphenylmethyl 6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside, were obtained from the biotransformation of benzophenone, showing that the cultured cells of *E. perriniana* regioselectively hydroxylate at C-4 and reduce the carbonyl group of benzophenone and that glycosylation can occur at the hydroxyl group at C-4 and C-7. Thus, both diphenyl compounds were removed from the culture medium and accumulated in cells as glycosides. The biotransformation with *E. perriniana* would provide a very useful process for the phytoremediation of endocrine disrupting chemicals such as diphenyl compounds. This procedure using enzymatic reactions is simple and environmentally friendly.

Key words: Benzophenone, biotransformation, bisphenol A, diphenyl compounds, *Eucalyptus perriniana*.

Some aromatic chemicals such as bisphenol A (BPA) and benzophenone (BZP) are widely used as the starting material for the production of polyacrylates, ether resins, phenol resins, photostabilizers, insecticides, fragrance ingredients, agricultural chemicals, pharmaceuticals, and coatings and are released as pollutants and toxic compounds into the environment (Sandell et al. 1987). Recently, BPA and BZP, diphenyl compounds, have attracted considerable attention as they exhibited estrogenic activity in bioassays (Howdeshell et al. 1999) and have been listed among "chemicals suspected of having endocrine disrupting effects" by the World Wildlife Fund, the National Institute of Environmental Health Sciences in the USA and the Japanese Environment Agency. From the viewpoint of pollution control, many studies on the metabolites of aromatic compounds have been reported, e.g., the benzene rings of aromatic compounds are degraded through the gentisic acid intermediate by some soil bacteria (Harpel et al. 1990; Atkinson et al. 1995; Yokota et al. 1999; Hirano et al. 2000). However, little attention has been paid to the biological degradation of endocrine disrupting chemicals such as diphenyl compounds. On the other hand, the metabolic pathway of aromatic compounds in plant cells is quite different from that in microorganisms; plant cells glycosylate phenols and benzoic acids including gentisic acid and accumulate them as glycosides in the cells (Mizukami et al. 1986; Mizutani et al. 1987; Tabata et al. 1988; Ushiyama et al. 1989; Shimoda et al. 2002).

Recently, the biotransformation of exogenous substrates by cultured plant cells has been reported (Suga et al. 1990; Ishihara et al. 2003). The cultured plant cells have the abilities of hydroxylation, glycosylation, oxido-reduction, hydrogenation, and hydrolysis for various organic compounds. Particularly, hydroxylation, reduction, and glycosylation seem to be efficient procedures for the phytoremediation of environmental pollution.

Abbreviations: BPA, bisphenol A; BZP, benzophenone.

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With respect to the phytoremediation of diphenyl compounds, a callus strain of *Eucalyptus perriniana* with a high potential for regioselective hydroxylation and glycosylation of BPA was screened out of various plant callus strains in our laboratory. In our recent communication, only three disaccharide products have been isolated after the incubation of BPA with the cultured cells of *E. perriniana* (Hamada et al. 2002), however, there are two other disaccharide products which are new compounds. In addition, *E. perriniana* cells were able to regioselectively hydroxylate and reduce BZP and were capable of glycosylation of the newly generated hydroxyl groups. This paper describes in detail the regioselective hydroxylation, reduction, and glycosylation of diphenyl compounds by the cultured cells of *E. perriniana*.

Materials and Methods

General

Chemicals such as BPA and BZP used as substrates were purchased from Aldrich Chemical Co. The ^{1}H 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 FABMS and HRFABMS spectra were measured using a JEOL The MStation JMS-700 spectrometer.

Plant materials and culture methods

Eucalyptus cultured suspension cells were prepared as described previously (Furuya et al. 1987). Just prior to use for this work, part of the callus tissues (fresh weight 40 g) 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 benzyladenine and 3% sucrose and grown with continuous shaking for 1 week at 25°C in the dark.

Biotransformation of BPA by the cultured cells of E. perriniana

A total of 120 mg of BPA was administered to the 10 flasks (12 mg/flask, none solvent) containing the suspension cultured cells of *E. perriniana* and the cultures were incubated at 25°C for seven days on a rotary shaker (120 rpm) in the dark. After the incubation, the cells and medium were separated by filtration with suction. The filtered medium was extracted with EtOAc. The medium was further extracted with *n*-BuOH to give no glycoside products. The cells were extracted (×3) by homogenization with MeOH. The MeOH fraction was concentrated and partitioned between H_2O and EtOAc. The EtOAc fractions were combined and concentrated to give no BPA. The H_2O fraction was applied to a Diaion HP-20 column and 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^{-1}] to give products **1** (43%; t_R=15.1 min), **2** (21%; t_R=13.4 min), **3** (6%; t_R=19.2 min), **4** (13%; t_R=22.8 min), and **5** (7%; t_R=17.9 min). 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. No further conversion products were observed in spite of the careful HPLC analyses. Spectral data for the products; product **3**, 2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)-2-(4-β-D-glucopyranosyloxyphenyl)propane: FABMS: m/z 591 $[M+Na]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 1.61 (6H, s, H-1, 3), 4.60 (1H, d, J=7.2 Hz, H-1″), 4.88 (1H, d, J=8.0 Hz, H-1′), 6.72 (1H, d, J=8.5 Hz, H-8), 6.81 (1H, dd, J=8.5, 2.0 Hz, H-9), 6.90 (1H, d, J=2.0 Hz, H-5), 6.99 (2H, d, J=8.5 Hz, H-12, 14), 7.16 (2H, d, J=8.5 Hz, H-11, 15); ^{13}C NMR (100 MHz, CD_3OD) see Table 1. Product **4**, 2-(3-β-D-glucopyranosyloxy-4-β-D-glucopyranosyloxyphenyl)-2-(4-hydroxy phenyl)propane: FABMS: *m/z* 591 $[M+Na]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 1.61 (6H, s, H-1, 3), 4.71 (1H, d, J=7.2 Hz, H-1″), 4.82 (1H, d, J=7.5 Hz, H-1′), 6.68 (2H, d, J=9.0 Hz, H-12, 14), 6.90 (1H, dd, J=8.5, 2.0 Hz, H-9), 7.05 (2H, d, J=9.0 Hz, H-11, 15), 7.08 (1H, d, J=2.0 Hz, H-5), 7.12 (1H, d, J=8.5 Hz, H-8); ^{13}C NMR (100 MHz, CD_3OD) see Table 1.

Table 1. ^{13}C chemical shifts of the products **1**–**5** in the biotransformation of BPA by the cultured cells of *E. perriniana*.

Position	1	2	3	4	5
C-1	31.5	31.4	31.5	31.4	31.4
C-2	42.9	42.9	43.0	43.0	43.0
C-3	31.5	31.4	31.5	31.4	31.4
C-4	146.1	148.2	144.3	148.7	148.1
C-5	128.7	116.1	117.9	119.5	116.4
C-6	117.2	147.8	146.2	148.8	147.8
C-7	157.0	144.6	146.0	146.8	144.6
C-8	117.2	118.5	116.4	120.3	118.4
C-9	128.7	119.2	122.7	122.9	119.2
C-10	146.1	146.0	146.1	142.6	144.2
C-11	128.7	128.7	128.8	128.8	117.9
C-12	117.2	117.2	117.1	115.7	146.2
C-13	157.0	157.0	156.9	156.2	146.0
C-14	117.2	117.2	117.1	115.7	116.2
C-15	128.7	128.7	128.8	128.8	122.6
C-1′	102.4	104.6	102.4	104.1	104.5
C-2′	75.0	74.9	74.8	75.0	74.8
C-3′	78.0	77.7	77.7	77.8	77.6
C-4′	71.4	71.3	71.1	71.1	71.1
C-5′	78.1	78.1	78.1	78.1	78.1
C-6′	62.5	62.4	62.2	62.2	62.2
C-1″	102.4	102.4	104.6	104.2	104.5
C-2″	75.0	75.0	75.0	75.1	74.9
C-3″	78.0	78.0	78.0	77.8	77.7
C-4″	71.4	71.4	71.4	71.4	71.4
C-5″	78.1	78.3	78.1	78.2	78.3
C-6″	62.5	62.5	62.5	62.5	62.5

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Biotransformation of BZP by the cultured cells of E. perriniana

Feeding and incubation experiments were carried out in the same manner as described in Section 4.3. The MeOH extracts from the cells were purified by Diaion HP-20 column chromatography and then HPLC with a YMC-Pack R & D ODS column to give **6** (23%; t_R=16.9 min), **7** (16%; t_R=2.4 min), **8** (29%; t_R=20.2 min), and **9** (24%; t_R=10.3 min). No further conversion products were observed in spite of the careful HPLC analyses. Spectral data for the products; product **6**, 4-*O*-β-D-glucopyranosylbenzophenone: *m/z* 361 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 3.72 (1H, dd, *J*=12.4, 5.6 Hz, H-6b′), 3.92 (1H, dd, *J*=12.4, 2.0 Hz, H-6a′), 5.06 (1H, d, *J*=7.2 Hz, H-1′), 7.20 (2H, d, *J*=8.8 Hz, H-3,5), 7.49 (2H, t, *J*=8.0 Hz, H-10, 12), 7.60 (1H, t, *J*=7.6 Hz, H-11), 7.71 (2H, d, *J*=8.4 Hz, H-9, 13), 7.77 (2H, d, *J*=8.8 Hz, H-2, 6); ^{13}C NMR (100 MHz, CD_3OD) see Table 2. Product **7**, 4-*O*-[6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]benzophenone: FABMS: m/z 507 $[M+H]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 1.18 (3H, d, *J*=6.0 Hz, H-6″), 4.70 (1H, d, *J*=1.2 Hz, H-1″), 5.04 (1H, d, *J*=7.2 Hz, H-1′), 7.19 (2H, d, *J*=8.8 Hz, H-3, 5), 7.52 (2H, t, *J*=7.6 Hz, H-10, 12), 7.62 (1H, t, *J*=7.6 Hz, H-11), 7.74 (2H, d, *J*=7.2 Hz, H-9, 13), 7.80 (2H, d, *J*=8.8 Hz, H-2, 6); ^{13}C NMR (100 MHz, CD_3OD) see Table 2. Product 8, diphenylmethyl β-D-glucopyranoside: FABMS: *m/z* 369 $[M+Na]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 3.67 (1H, dd, *J*=12.0, 6.4 Hz, H-6b′), 3.88 (1H, dd, *J*=12.0, 2.4 Hz, H-6a′), 4.27 (1H, d, *J*=7.6 Hz, H-1′), 6.05 (1H, s, H-7), 7.18 (1H, t, *J*=7.6 Hz, H-4), 7.23 (3H, t, *J*=7.6 Hz, H-3, 5, 11), 7.33 (2H, t, *J*=8.0 Hz, H-10, 12), 7.40 (2H, d, *J*=7.6 Hz, H-2, 6), 7.47 (2H, d, *J*=7.2 Hz, H-9, 13); ^{13}C NMR (100 MHz, CD_3OD) see Table 2. Product **9**, diphenylmethyl 6-O-(β-D-glucopyranosyl)-β-D-glucopyranoside: FABMS: *m/z* 531 $[M+Na]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 3.66 (1H, dd, *J*=11.6, 5.6 Hz, H-6b″), 3.81 (1H, dd, *J*=11.2, 5.2 Hz, H-6b′), 3.86 (1H, dd, *J*=11.6, 2.0 Hz, H-6a″), 4.15 (1H, d, *J*=11.2 Hz, H-6a′), 4.30 (1H, d, *J*=7.6 Hz, H-1″), 4.46 (1H, d, *J*=7.6 Hz, H-1′), 6.05 (1H, s, H-7), 7.18 (1H, t, *J*=8.0 Hz, H-4), 7.26 (3H, t, *J*=7.6 H, H-3, 5, 11), 7.33 (2H, t, *J*=8.0 Hz, H-10, 12), 7.40 (2H, d, *J*=7.6 Hz, H-2, 6), 7.51 (2H, d, *J*=7.2 Hz, H-9, 13); ^{13}C NMR (100 MHz, CD_3OD) see Table 2.

Table 2. ^{13}C chemical shifts of the products **6**–**9** in the biotransformation of BZP by the cultured cells of *E. perriniana*.

Position	6	7	8	9
C-1	132.3	132.7	142.1	142.1
C-2	133.1	133.4	128.0	128.0
C-3	117.0	117.2	128.8	128.8
C-4	162.5	162.7	128.0	128.0
C-5	117.0	117.2	128.8	128.8
C-6	133.1	133.4	128.0	128.0
C-7	197.2	197.7	81.0	81.1
C-8	139.0	139.3	143.8	143.7
C-9	130.5	130.9	128.9	128.9
C-10	129.2	129.5	129.3	129.2
C-11	133.1	133.4	128.6	128.5
C-12	129.2	129.5	129.3	129.2
C-13	130.5	130.9	128.9	128.9
C-1′	101.4	101.5	100.9	101.1
C-2′	74.6	74.8	75.2	75.1
C-3′	78.1	78.0	77.9	77.8
C-4′	71.1	71.5	71.7	71.5
C-5′	77.7	77.1	77.9	77.8
C-6′	62.3	67.8	62.8	69.6
C-1″		102.2		104.8
C-2″		72.2		75.1
C-3″		72.4		77.8
C-4″		74.0		71.5
C-5″		69.9		77.8
C-6″		18.0		62.6

Results and discussion

Biotransformation of BPA by the cultured cells of E. perriniana

After seven days' incubation of BPA with the cultured cells of *E. perriniana*, products 1–5 were isolated from the extracts of the cells with MeOH and no products were observed in the medium. Neither BPA nor additional conversion products were observed in spite of the careful HPLC analyses. The structures of the products were determined on the basis of their FABMS, ^{1}H and ^{13}C NMR (Table 1), H-H COSY, C-H COSY, and NOE spectra as 2,2-bis(4-β-D-glucopyranosyloxyphenyl)-propane (**1**, 43%), 2-(4-β-D-glucopyranosyloxy-3-hydroxyphenyl)-2-(4-β-D-glucopyranosyloxyphenyl)-propane (**2**, 21%), 2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)-2-(4-β-D-glucopyranosyloxyphenyl)-propane (**3**, 6%), 2-(3-β-D-glucopyranosyloxy-4-β-D-glucopyranosyloxyphenyl)-2-(4-hydroxyphenyl)-propane (**4**, 13%), and 2-(4-β-D-glucopyranosyloxy-3-hydroxyphenyl)-2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)propane (**5**, 7%). Disaccharide products **3** and **4** were two new compounds. No formation of **2** was observed in the incubation of **1** with *E. perriniana* cells under the same transformation conditions. It was also confirmed that no formation of **5** was observed during the incubation of **3** with the cells. These results suggest that not **1**, but 2-(4-hydroxyphenyl)-2-(3,4-dihydroxyphenyl)-propane was converted into **2** and that not **3**, but 2,2-bis(3,4-dihydroxyphenyl)propane was transformed into **5**. The biotransformation scheme of BPA by the cultured cells of *E. perriniana* is shown in Figure 1.

The FABMS spectrum of the product **3** showed a pseudomolecular ion $[M+Na]^+$ peak at *m/z* 591. The ^{1}H NMR spectrum of **3** exhibited characteristic signals due to protons on the 1,4-disubstituted and 1,3,4-trisubstituted benzene rings at δ 6.99 (2H, d, *J*=8.5 Hz), 7.16 (2H, d, *J*=8.5 Hz) and 6.72 (1H, d, *J*=8.5 Hz), 6.81 (1H, dd, *J*=8.5, 2.0 Hz), 6.90 (1H, d, *J*=2.0 Hz),

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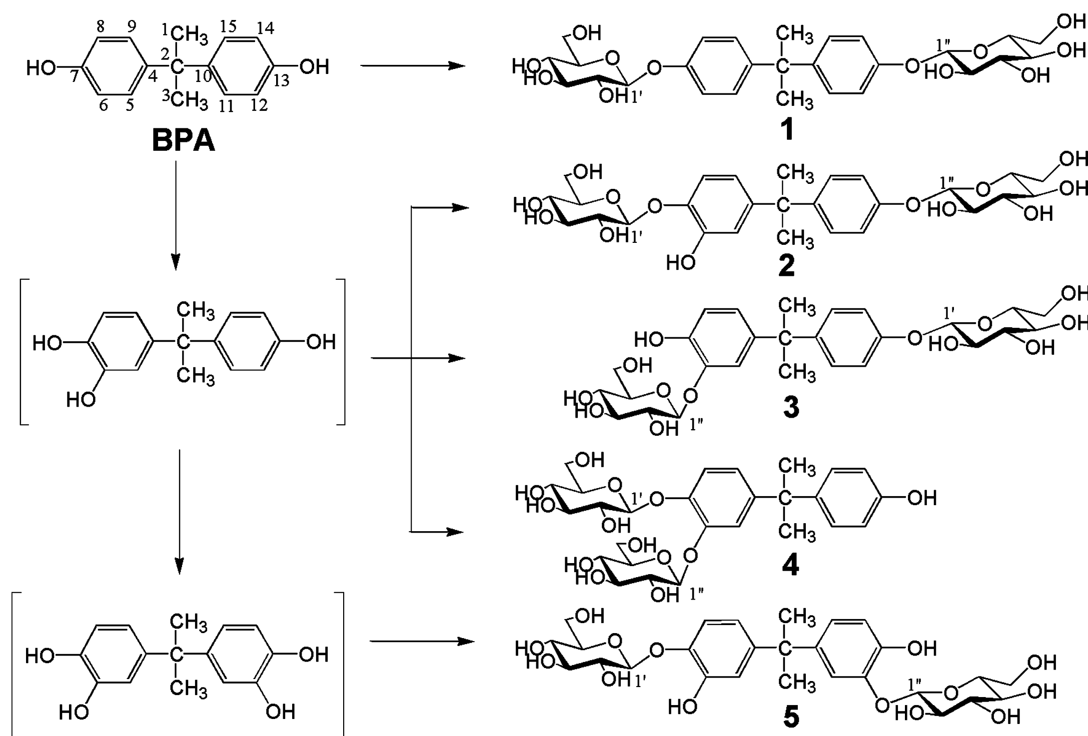

Figure 1. Biotransformaiton of BPA by the cultured cells of *E. perriniana*. **1**, 2,2-bis(4-β-D-glucopyranosyloxyphenyl)propane; **2**, 2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)-2-(4-β-D-glucopyranosyloxyphenyl)propane; **3**, 2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)-2-(4-β-D-glucopyranosyloxyphenyl)propane; **4**, 2-(3-β-D-glucopyranosyloxy-4-β-D-glucopyranosyloxyphenyl)-2-(4-hydroxyphenyl)propane; **5**, 2-(4-β-D-glucopyranosyloxy-3-hydroxyphenyl)-2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)propane.

respectively. The spectrum also showed two anomeric proton signals at δ 4.60 (1H, d, J=7.2 Hz) and 4.88 (1H, d, J=8.0 Hz). The ^{13}C NMR spectrum of **3** showed 27 carbon signals with two anomeric carbon signals at β 102.4 and 104.6. From the coupling constants of the proton signals and pattern of the carbon signals of the sugar moiety, the component sugar in **3** was indicated to be β-D-glucopyranose. The bonding position of the glucose moieties was determined by NOE difference spectrum. Upon irradiation of the anomeric protons at δ 4.60 (H-1″) and 4.88 (H-1′), NOEs were observed for the signals δ 6.99 (H-12 and H-14) and 6.90 (H-5), respectively. Thus, the structure of **3** was determined as 2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)-2-(4-β-D-glucopyranosyloxyphenyl)propane. The FABMS spectrum of the product **4** showed a pseudomolecular ion $[M+Na]^+$ peak at m/z 591. In the ^{1}H NMR spectrum of **4**, signals of the protons on the 1,4-disubstituted and 1,3,4-trisubstituted benzene rings were observed at δ 6.68 (2H, d, J=9.0 Hz), 7.05 (2H, d, J=9.0 Hz) and 6.90 (1H, dd, J=8.5, 2.0 Hz), 7.08 (1H, d, J=2.0 Hz), 7.12 (1H, d, J=8.5 Hz), respectively. The spectrum also showed two anomeric proton signals at δ 4.71 (1H, d, J=7.2 Hz) and 4.82 (1H, d, J=7.5 Hz). The ^{13}C NMR spectrum of **4** showed 27 carbon signals with two anomeric carbon signals at δ 104.1 and 104.2. The component sugar in **4** was determined to be β-D-glucopyranose from the coupling pattern of the sugar proton signals and the chemical shifts of the sugar carbon signals. The bonding position of the glucose moieties was determined by the NOE difference spectrum. Upon irradiation of the anomeric protons at δ 4.71 (H-1″) and 4.82 (H-1′), NOEs were observed for the signals δ 7.08 (H-5) and 7.12 (H-8), respectively. Therefore, the structure of **4** was established as 2-(3-β-D-glucopyranosyloxy-4-β-D-glucopyranosyloxyphenyl)-2-(4-hydroxyphenyl)propane.

Thus, these results demonstrate that the cultured cells of *E. perriniana* regioselectively hydroxylate at C-6 and C-12 of BPA and that the glycosides can be formed at the hydroxyl group at C-6, 7, 12, and 13.

Biotransformation of BZP by the cultured cells of E. perriniana

After the seven days' incubation period, products **6**–**9** were obtained from the extracts of the cells with MeOH. Neither BZP nor additional conversion products were observed in spite of the careful HPLC analyses. The structures of the products were determined on the basis of their FABMS, ^{1}H and ^{13}C NMR (Table 2), H-H COSY, C-H COSY, NOE, and HMBC spectra as 4-*O*-β-D-glucopyranosylbenzophenone (**6**, 23%), 4-*O*-[6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]benzophenone (**7**, 16%), diphenylmethyl β-D-glucopyranoside (**8**, 29%), and diphenylmethyl 6-*O*-(β-D-glucopyranosyl)-α-D-glucopyranoside (**9**, 24%). Disaccharide products **7** and **9**

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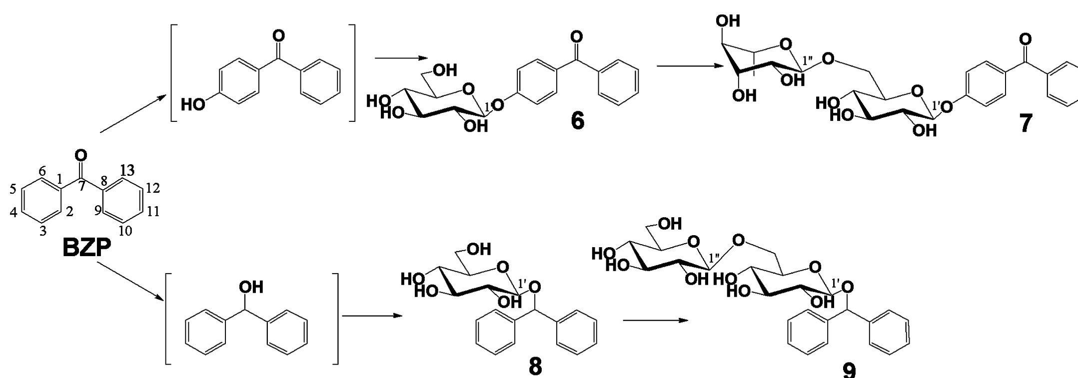

Figure 2. Biotransformaiton of BZP by the cultured cells of *E. perriniana*. **6**, 4-*O*-β-D-glucopyranosylbenzophenone; **7**, 4-*O*-[6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]benzophenone; **8**, diphenylmethyl β-D-glucopyranoside; **9**, diphenylmethyl 6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside.

were two new compounds. The biotransformation scheme of BZP by *E. perriniana* cells is shown in Figure 2.

The FABMS spectrum of **7** showed a pseudomolecular ion $[M+H]^+$ peak at *m*/*z* 507. The ^{1}H NMR spectrum of **7** showed two anomeric proton signals at δ 4.70 (1H, d, $J=1.2$ Hz) and 5.04 (1H, d, $J=7.2$ Hz), indicating the presence of α- and β-anomers in the sugar moiety. The ^{13}C NMR data of the sugar moiety of **7** was in good agreement with that of β-rutinose (Ishimaru et al. 2003). The spectrum also showed two anomeric carbon signals at δ 101.5 and 102.2. Upon irradiation of the anomeric proton at δ 5.04 (H-1′), NOE was observed for the signal δ 7.19 (H-3 and H-5). An HMBC correlation was observed between the anomeric proton signal at δ 4.70 (H-1″) and the carbon signal at δ 67.8 (C-6′). These results established that the inner β-D-glucopyranosyl residue was attached to the 4-hydroxyl group of 4-hydroxybenzophenone and that the second α-L-rhamnopyranosyl residue and the inner β-D-glucopyranosyl residue were 1,6-linked. Thus, the structure of 7 was determined as 4-*O*-[6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]benzophenone. The FABMS spectrum of **9** showed a pseudomolecular ion $[M+Na]^+$ peak at *m*/*z* 531, suggesting the composition of **9** to be one molecule of diphenylmethanol and two hexoses. The ^{1}H NMR spectrum of **9** showed two anomeric proton signals at δ 4.30 (1H, d, $J=7.6$ Hz) and 4.46 (1H, d, $J=7.6$ Hz). The ^{13}C NMR spectrum of **9** exhibited 25 carbon signals including two anomeric carbon signals at δ 101.1 and 104.8. From the coupling pattern of the proton signals and the chemical shifts of the carbon signals due to the sugar moiety, the component sugar in **9** was indicated to be β-D-glucopyranose. The ^{13}C NMR chemical shift of C-6′ comparatively shifted downfield to δ 69.6. In addition, the HMBC correlations were observed between the anomeric proton signal at δ 4.46 (H-1′) and the carbon signal at δ 81.1 (C-7) and between the anomeric proton signal at δ 4.30 (H-1″) and the carbon signal at δ 69.6 (C-6′) to establish that the inner glucopyranosyl residue was attached to the hydroxyl group of diphenylmethanol and that the pair of β-D-glucopyranosyl residues was 1,6-linked. Thus, the structure of **9** was determined to be diphenylmethyl 6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside.

These results demonstrate that the cultured cells of *E. perriniana* regioselectively hydroxylate at C-4 and reduce the carbonyl group of BZP and that glycosylation can occur at the hydroxyl group at C-4 and C-7.

The results of this experiment revealed that the cultured cells of *E. perriniana* are able to regioselectively hydroxylate and reduce diphenyl compounds and that the regioselective glycosylation that follows produces glycosides which have little endocrine disruption activity (Nishikawa et al. 1999; Hamada et al. 2002). The plant enzymes responsible for these biotransformations should be available for addition to the group of biocatalysts used for the chemical modification of endocrine disrupting chemicals. Also, this method is of considerable interest and important in green chemistry. Studies on the characterization of these enzymes from *E. perriniana* are now in progress. Further phytoremediation studies such as biotransformations of BPA and BZP using immobilized Eucalyptus cells are now in progress.

Acknowledgements

The authors are grateful to the Research Instruments Center of the Okayama University of Science for the NMR and FAB-MS spectra.

References

Atkinson A, Roy D (1995) *In vitro* conversion of environmental estrogenic chemical bisphenol A to DNA binding metabolite(s). *Biochem Biophys Res Commum* 210: 424–433

Furuya T, Orihara Y, Hayashi C (1987) Triterpenoids from eucalyptus perriniana cultured cells. *Phytochem* 26: 715–719

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Hamada H, Tomi R, Asada Y, Furuya T (2002) Phytoremediation of bisphenol A by cultured suspension cells of *Eucalyptus perriniana*-regioselective hydroxylation and glycosylation. *Tetrahedron Lett* 43: 4087–4089

Harpel MR, Lipscomb JD (1990) Gentisate 1,2-dioxygenase from pseudomonas. Purification, characterization, and comparison of the enzymes from Pseudomonas testosteroni and Pseudomonas acidovorans. *J Biol Chem* 265: 6301–6311

Hirano T, Honda Y, Watanabe T, Kuwahara M (2000) Degradation of bisphenol A by the lignin-degrading enzyme, manganese peroxidase, produced by the white-rot basidiomycete, *Pleurotus ostreatus*. *Biosci Biotechnol Biochem* 64: 1958–1962

Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, Saal FS (1999) Exposure to bisphenol A advances puberty. *Nature* 401: 763–764

Ishihara K, Hamada H, Hirata T, Nakajima N (2003) Biotransformation using plant cultured cells. *J Mol Catal B: Enz* 23: 145–170

Ishimaru K, Osabe M, Yan L, Fujioka T, Mihashi K, Tanaka N (2003) Polyacetylene glycosides from *Pratia nummularia* cultures. *Phytochem* 62: 643–646

Mizukami H, Terao T, Amano A, Ohashi H (1986) Glucosylation of salicyl alcohol by *Gardenia jasminoides* cell cultures. *Plant Cell Physiol* 27: 645

Mizutani H, Hirano A, Ohashi H (1987) Effect of substituent groups on the glucosyl conjugation of xenobiotic phenols by cultured cells of *Gardenia jasminoides*. *Plant Sci* 48: 11–15

Nishikawa J, Saito K, Goto J, Dakeyama F, Matsuo M, Nishihara T (1999) New screening methods for chemicals with hormonal activities using interaction of nuclear hormone receptor with coactivator. *Toxicol Appl Pharmacol* 154: 76–83

Sandell J, Schuller-Goetzbung VV, Tashiro M (1987) in *Bisphenol A, CEH Product Review, Chemical Economic Handbook*. SRI International

Shimoda K, Yamane S, Hirakawa H, Ohta S, Hirata T (2002) Biotransformation of phenolic compounds by the cultured cells of *Catharanthus roseus*. *J Mol Catal B: Enz* 16: 275–281

Suga T, Hirata T (1990) Biotransformation of exogenous substrates by plant cell cultures. *Phytochem* 29: 2393–2406

Tabata M, Unetani Y, Oya M, Tanaka S (1988) Glucosylation of phenolic compounds by plant cell cultures. *Phytochem* 27: 809–813

Ushiyama M, Furuya T (1989) Glucosylation of phenolic compounds by root culture of *Panax ginseng*. *Phytochem* 28: 3009–3013

Yokota H, Iwano H, Endo M, Kobayashi T, Inoue H, Ikushiro S (1999) Glucuronidation of the environmental oestrogen bisphenol A by an isoform of UDP-glucuronosyltransferase, UGT2B1, in the rat liver. *Biochem J* 340: 405–409

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