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Phytoremediation of bisphenol A by cultured suspension cells of *Eucalyptus perriniana*-regioselective hydroxylation and glycosylation

Hiroki Hamada,[a,*] Ryotoku Tomi,[a] Yoshihisa Asada[b] and Tsutomu Furuya[a]

[a]*Department of Applied Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan*
[b]*School of Phamaceutical Sciences, Kitasato University, Shirokane, Minato-ku, Tokyo 108-8641, Japan*

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Abstract—On biotransformation of bisphenol A by cultured suspension cells of *Eucalyptus perriniana*, three new biotransformation products, 2,2-bis(4-β-D-glucopyranosyloxyphenyl)propane, 2-(4-β-D-glucopyranosyloxy-3-hydroxyphenyl)-2-(4-β-D-glucopyranosyloxyphenyl)propane and 2-(4-β-D-glucopyranosyloxy-3-hydroxyphenyl)-2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)propane were isolated after three days incubation. Cultured suspension cells of *E. perriniana* are capable of regioselective hydroxylation and glycosylation of bisphenol A. © 2002 Elsevier Science Ltd. All rights reserved.

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, BPA) is widely used as a starting material for the production of plastics, phenol resins, polyacrylates, polyesters and coatings.[1] Worldwide production capacity was estimated about 1,100 million pounds[1] leading to many studies concerning toxicity and metabolites of BPA.[2–7] More recently this compound is suspected of being an endocrine disturbing chemical and Howdeshell et al. reported that BPA is especially active toward females.[8] However, there are no reports concerning plant cultured suspension cells that degrade BPA and/or the chemistry of BPA biotransformation. We now report that BPA is biotransformed through regioselective hydroxylation and glycosylation.

Eucalyptus cultured suspension cells were prepared as described in[9] and feeding and incubation experiments were carried out in a manner similar to that reported in[10–12]. 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. BPA (12 mg/flask, none solvent) was added to the suspension cultures and the cultures were incubated at 25°C for 3 days on a rotary shaker (120 rpm) in the dark.

After incubation the cultures were harvested and products **1**–**3** were isolated from the extracts of the cells. The cells were extracted (×3) by homogenization with MeOH and the extract was concentrated. The residue was partitioned between H_2O and EtOAc. The H_2O layer 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: 150×20 mm) and products **1**–**3** were obtained. No products **1**–**3** were observed in the medium. The biotransformation scheme of BPA by the cultured suspension cells of *Eucalyptus perriniana* is shown in Fig. 1. Products **1**–**3** were new compounds and were identified using ^{1}H and ^{13}C NMR, MS, LC/MS and IR. The yield[13] of products, **1**, **2** and **3** was 41.7, 18.7 and 6.3%, respectively. The FAB MS spectrum of product **1** showed a pseudomolecular ion peak at m/z 575 [M+Na]$^+$ which is larger than that of BPA by two hexsose units. Its molecular formula, $C_{27}H_{36}O_{12}$, was established based on its HR-FAB spectrum. Two aromatic proton signals were observed at δ 6.89 (4H, d, $J=8.5$ Hz) and 7.13 (4H, d, $J=8.5$ Hz) in the ^{1}H NMR spectrum[14] of **1**. The spectrum also showed an anomeric proton signals at δ 4.87 (2H, d, $J=8.0$ Hz), suggesting that product **1** has a symmetric structure as dose BPA. The ^{13}C NMR spectrum[14] of **1** showed 12 carbon

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* Corresponding author. Tel.: +81-86-256-9473; fax: +81-86-256-8468; e-mail: hamada@das.ous.ac.jp

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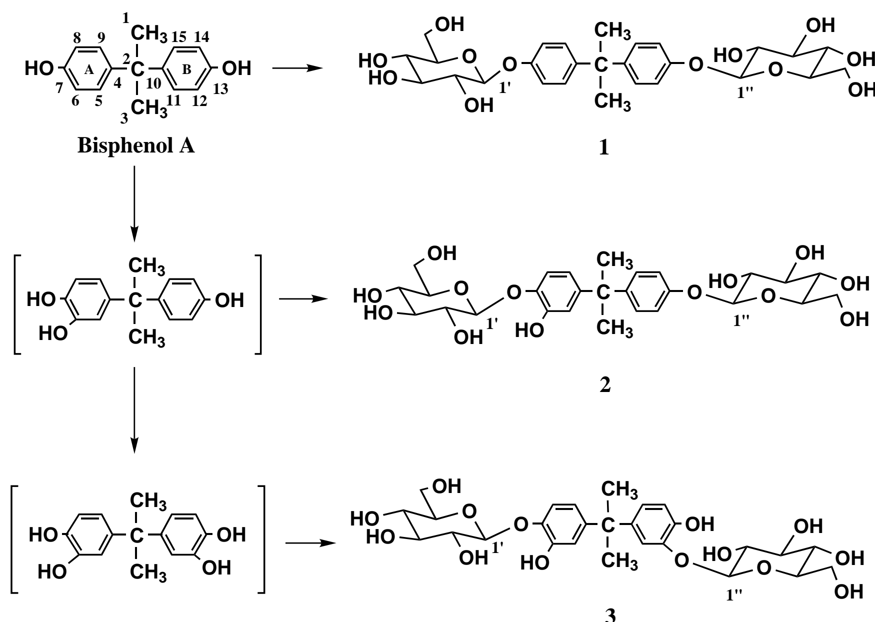

Figure 1. Biotransformation of bisphenol A by the cultured suspension cells of *E. perriniana*.

signals including an anomeric carbon at 102.4 ppm, indicating that **1** is a symmetric *O*-glycoside. From the pattern of the carbon and proton signals due to the sugar moiety, the component sugar in **1** was indicated to be β-D-glucopyranoside. Therefore, the structure of **1** was characterized as 2,2-bis(4-β-D-glucopyranosyloxyphenyl)propane. The endocrine disturbing activity of **1** was determined by the reported method[15] and activity decreased 80% in comparison with that of BPA. The HR-FAB mass spectrum of product **2** gave a pseudomolecular ion $[M+Na]^+$ at *m*/*z* 591.2042 consistent with a molecular formula $C_{27}H_{36}O_{13}$ which is larger that that of **1** by 16 mass units due to additional hydroxyl group. In the ^{1}H NMR spectrum[14] of **2**, characteristic signals due of protons on 1,3,4-tri-substituted and 1,4-di-substituted benzene rings were observed at δ 6.66 (1H, *dd*, $J=8.0$, 2.0 Hz), 6.68 (1H, *d*, $J=2.0$ Hz), 7.06 (1H, *d*, $J=8.0$ Hz) and δ 6.98 (2H, *d*, $J=8.5$ Hz), 7.14 (2H, *d*, $J=8.5$ Hz), respectively. The spectrum also showed two anomeric proton signals at δ 4.71 (1H, *d*, $J=7.8$ Hz) and δ 4.86 (1H, *d*, $J=8.0$ Hz). The ^{13}C NMR spectrum[14] of **2** showed 27 carbon signals, with two anomeric carbon signals at δ 102.4 and 104.6. The ^{13}C NMR spectra of **2** was similar to that of **1** except for the carbon signals on aromatic ring A, suggested that **2** also had two molecules of β-D-glucoside but with a second hydroxyl group attached to the A ring. The bonding position of the glucose moieties was determined by NOE difference spectrum. On irradiation of the anomeric protons at δ 4.71 and 4.86, NOEs were observed for the signals δ 7.06 (H-8) and δ 6.98 (H-12 and H-14), respectively. Thus, the structure of **2** was determined as 2-(4-β-D-glucopyranosyloxy-3-hydroxyphenyl)-2-(4-β-D-glucopyranosyloxyphenyl)propane. The HR-FAB mass spectrum of product **3** suggested the molecular formula $C_{27}H_{36}O_{14}$, indicating a second addid hydroxyl group. In the ^{1}H NMR spectrum[14] of **3**, two sets of aromatic proton signals on 1,3,4-tri-substituted benzene rings were observed at δ 6.69 (1H, *dd*, $J=8.5$, 2.0 Hz), 6.72 (1H, *d*, $J=2.0$ Hz), 7.07 (1H, *d*, $J=8.5$ Hz) and δ 6.73 (1H, *d*, $J=8.5$ Hz), 6.82 (1H, *dd*, $J=8.5$, 2.0 Hz). Two anomeric proton signals were observed at δ 4.61 (1H, *d*, $J=7.2$ Hz) and δ 4.73 (1H, *d*, $J=7.8$ Hz). The ^{13}C NMR spectrum,[14] exhibited 27 carbon signals and two anomeric carbons were observed at δ 104.47 and 104.54. A comparison of ^{13}C and ^{1}H NMR spectra of **3** with that of **2** showed that **3** has two β-D-glucoside groups and an additional hydroxyl group on B ring. On irradiation of the anomeric proton at δ 4.61 and 4.73, NOEs were observed for the signal δ 6.99 (H-11) and δ 7.07 (H-8), respectively. Consequently, the structure of **3** was established as 2-(4-β-D-glucopyranosyloxy-3-hydroxyphenyl)-2-(3-β-D-glucopyranosyloxy-4-hydroxyphenyl)propane.

From the result of this experiment it was found that the cultured suspension cells of *E. perriniana* regioselectively hydroxylate at C-6 of A ring and C-12 of B ring of BPA. Glycosides can be formed at the hydroxyl group at C-7, 12 and 13. This plant enzyme should be available addition to the group of biocatalysts used for chemical modification of endocrine disturbing chemical. Also, this method is of considerable interest in green chemistry. Studies of biotransformation of another endocrine disturbing chemical by cultured suspension cells of *E. perriniana* are now in progress.

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13. The yield 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. BPA was converted to the product in about 70% yield.
14. Spectral data for selected products; product **1**: ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): δ 1.62 (s, 6H, H-1, 3), 4.87 (d, 2H, $J=8.0$ Hz, H-1′, 1″), 6.98 (d, 4H, $J=8.5$ Hz, H-6, 8, 12, 14), 7.13 (d, 4H, $J=8.5$ Hz, H-5, 9, 11, 15); ^{13}C NMR (CD_3OD): δ 31.5 (C-1, C-3), 42.9 (C-2), 62.5 (C-6′, C-6″), 71.4 (C-4′, C-4″), 75.0 (C-2′, C-2″), 78.0 (C-3′, C-3″), 78.1 (C-5′, C-5″), 102.4 (C-1′, C-1″), 117.2 (C-6, C-8, C-12, C-14), 128.7 (C-5, C-9, C-11, C-15), 146.1 (C-4, C-10), 157.0 (C-7, C-13). Product **2**: ^{1}H NMR (CD_3OD): δ 1.60 (s, 6H, H-1, 3), 4.71 (d, 1H, $J=7.8$ Hz, H-1′), 4.86 (d, 1H, $J=8.0$ Hz, H-1″), 6.66 (dd, 1H, $J=8.0$, 2.0 Hz, H-9), 6.68 (d, 1H, $J=2.0$ Hz, H-5), 6.98 (d, 2H, $J=8.5$ Hz, H-12, 14), 7.06 (d, 1H, $J=8.0$ Hz, H-8), 7.14 (d, 2H, $J=8.5$ Hz, H-11, 15); ^{13}C NMR (CD_3OD): δ 31.4 (C-1, C-3), 42.9 (C-2), 62.4, 62.5 (C-6′, C-6″), 71.3, 71.4 (C-4′, C-4″), 74.9, 75.0 (C-2′, C-2″), 77.7, 78.0 (C-3′, C-3″), 78.1, 78.3 (C-5′, C-5″), 102.4 (C-1″), 104.6 (C-1′), 116.1 (C-5), 117.2 (C-12, C-14), 118.5 (C-8), 119.2 (C-9), 128.7 (C-11, C-15), 144.6 (C-7), 146.0 (C-10), 147.8 (C-6), 148.2 (C-4), 157.0 (C-13). Product **3**: ^{1}H NMR (CD_3OD): δ 1.59 (s, 6H, H-1,3), 4.61 (d, 1H, $J=7.2$ Hz, H-1″), 4.73 (d, 1H, $J=7.8$ Hz, H-1′), 6.69 (dd, 1H, $J=8.5$ Hz, 2.0, H-9), 6.72 (d, 1H, $J=2.0$ Hz, H-5), 6.73 (d, 1H, $J=8.5$ Hz, H-14), 6.82 (dd, 1H, $J=8.5$ Hz, 2.0, H-15), 6.99 (d, 1H, $J=2.0$ Hz, H-11), 7.07 (d, 1H, $J=8.5$ Hz, H-8); ^{13}C NMR (CD_3OD): δ 31.4 (C-1, C-3), 43.0 (C-2), 62.2, 62.5 (C-6′, C-6″), 71.1, 71.4 (C-4′, C-4″), 74.8, 74.9 (C-2′, C-2″), 77.6, 77.7 (C-3′, C-3″), 78.1, 78.3 (C-5′ C-5″), 104.47 (C-1′), 104.54 (C-1″), 116.2 (C-14), 116.4 (C-5), 117.9 (C-11), 118.4 (C-8), 119.2 (C-9), 122.6 (C-15), 144.2 (C-10), 144.6 (C-7), 146.0 (C-13), 146.2 (C-12), 147.8 (C-6), 148.1 (C-4).
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