Synthesis of Unnatural Mono- and Oligosaccharides of Farnesol, Geraniol, and (*S*)-Perillyl Alcohol by Biocatalytic Glycosylations

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Farnesol and geraniol were glucosylated to the corresponding unnatural β-glucosides, which were two new biotransformation products, by cultured plant cells of *Eucalyptus perriniana*, *Strophanthus gratus*, and *Phytolacca americana*. Cultured *E. perriniana* cells glycosylated (*S*)-perillyl alcohol to two β-glycosides, i.e., β-glucoside and unnatural β-gentiobioside, whereas only perillyl β-glucoside was produced by *S. gratus* and *P. americana*. Perillyl β-glucoside was further converted into two unnatural β-maltooligosaccharides, i.e., β-maltoside and β-maltotrioside, by cyclodextrin glucanotransferase.

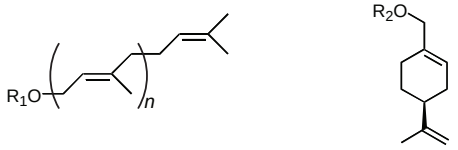
Plant cell culture is one of an ideal system for studying the synthesis and metabolism of endogenous and exogenous substances, and for production of scarce organic compounds that are hardly accessible by conventional chemical synthesis, especially towards new and unnatural glycosides. Terpene alcohols are widespread in essential oils of plants. Farnesol, geraniol, and (*S*)-perillyl alcohol are clinically useful and important terpenes, as they have chemotherapeutic and chemopreventive activities against cancer cells.[1] Irrespective of such pharmacological activity, their use as drugs is limited, as they are unstable, scarcely soluble in water, and moreover, prone to sublime. Glycosylation would be a clue so as to make such annoying properties be improved, hydrophilic and stable in the biological systems. Indeed, glycosylation of clinically useful phenolic compounds such as polyphenols and tocopherols by cultured plant cells has been reported.[2] However, little attention has been paid to the glycosylation of terpenes such as farnesol, geraniol, and (*S*)-perillyl alcohol by cultured plant cells.

In some plants, terpene alcohols such as geraniol have been reported to be generated from their disaccharide precursors, e.g., plant primeverosidases hydrolyze the disaccharide of geraniol to give the aglycone and disaccharose unit.[3] Mono-glucosides of geraniol and farnesol, however, have not so far been isolated from any plants. We report, herein, the glycosylation of farnesol, geraniol, and (*S*)-perillyl alcohol by cultured plant cells of *Eucalyptus perriniana*, *Strophanthus gratus*, and *Phytolacca americana* to unnatural monosaccharides and disaccharide. We also report the synthesis of unnatural oligosaccharides of (*S*)-perillyl alcohol by glycosylation of perillyl glucoside with cyclodextrin glucanotransferase (CGTase).

After incubation of farnesol (**1**) with cultured *E. perriniana* cells, a glycosylation product **4** was isolated from the MeOH extract of the cells.[4] The isolated yield of **4** was 69%. The structure of the product **4** was elucidated through spectroscopic studies with HRFABMS, ^{1}H and ^{13}C NMR, H–H COSY, and C–H COSY as farnesyl β-D-glucoside (Figure 1).[5] On the other hand, *S. gratus* and *P. americana* glucosylated **1** to **4** in 22% and 7%, respectively.

Geraniol (**2**) was subjected to the same biotransformation system. Incubation of **2** with *E. perriniana* cells yielded unnatural glucoside, geranyl β-D-glucoside (**5**), in 75% yield, so far having not been reported. *S. gratus* and *P. americana* converted **2** into **5** in 24% and 10%, respectively. On the other hand, (*S*)-perillyl alcohol (**3**) was converted into two β-glycosides, perillyl β-D-glucoside (**6**, 4%) and unnatural perillyl β-gentiobioside (**7**, 60%), which has not been identified before. In the case of biotransformation with *S. gratus* and *P. americana*, **6** was obtained as the sole product in 16% and 5%, respectively.

A time course experiment was studied for glycosylation of (*S*)-perillyl alcohol (**3**) with *E. perriniana* cells. Figure 2 shows that formation of perillyl β-D-glucoside (**6**) first occurred,



1: n = 2, R_1 = H; **2**: n = 1, R_1 = H; **3**: R_2 = H; **6**: R_2 = β-Glc; **7**: R_2 = β-Glc-(1-6)-β-Glc;
4: n = 2, R_1 = β-Glc; **8**: R_2 = α-Glc-(1-4)-β-Glc;
5: n = 1, R_1 = β-Glc. **9**: R_2 = [α-Glc-(1-4)-]$_2$$\beta$-Glc.

Figure 1. Structures of substrates **1**–**3** and glycoside products **4**–**9**.

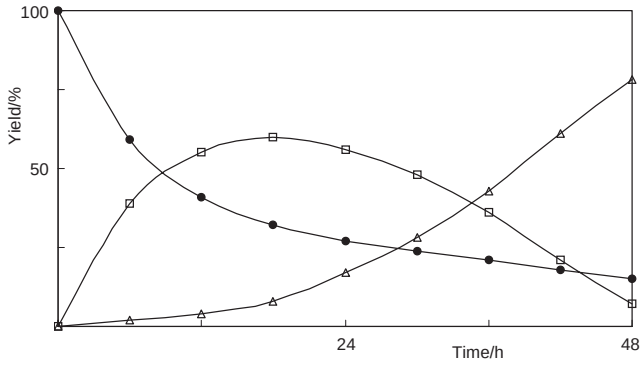


Figure 2. Glycosylation of (*S*)-perillyl alcohol (**3**) by cultured cells of *E. perriniana*. Yield was determined by peak area from HPLC. Yields of **3** (●), **6** (□), and **7** (△) are plotted.

followed by further glucosylation to give perillyl β-gentiobioside (**7**) in high yield.

Moreover, unnatural β-maltooligosaccharides of (*S*)-perillyl alcohol were synthesized by glycosylation of perillyl β-glucoside (**6**) with CGTase.[6] Two products were obtained and identified as perillyl β-maltoside (**8**, 27%) and perillyl β-maltotrioside (**9**, 7%), which were two new compounds.

In summary, this study disclosed the synthesis of unnatural β-glycosides of terpenes, including farnesol, geraniol, and (*S*)-perillyl alcohol, by biocatalytic glycosylations. Cultured *E. perriniana* cells showed considerably higher potential for the production of unnatural terpene β-glycosides than *S. gratus* and *P. americana*. The β-gentiobioside was selectively formed in the case of glycosylation of cyclic terpene (*S*)-perillyl alcohol by *E. perriniana*, and *E. perriniana* cells converted acyclic terpenes, farnesol and geraniol, into only β-D-glucosides. Sequential glycosylation of (*S*)-perillyl alcohol by plant cell culture and CGTase afforded perillyl β-maltooligosaccharides. The present tandem use of two glycosylation systems would be applicable to the production of unnatural terpene glycosides, and further studies on the physiological properties of these terpene β-glycosides are now in progress.

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4 Biotransformation of substrates with plant cell cultures was carried out as follows. Prior to this experiment, 100 g of cultured plant cells were individually transplanted to 1-L conical flasks containing 500 mL of Murashige and Skoog's medium (pH 5.7). The cells were grown for an additional 1 month on a rotary shaker (120 rpm) at 25 °C in the dark. Substrate (a total amount of 10 mmol) was administered to ten 1-L conical flasks (1 mmol/flask) containing the suspension cultured cells. After incubation of the cultures for 3 days on a rotary shaker (120 rpm), the cells and medium were separated by filtration with suction. The cells were homogenized (×3) with MeOH. The MeOH-extract was concentrated, and 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: YMC-Pack R&D ODS column (150 × 30 mm); solvent: CH_3CN-H_2O (3:7, v/v); detection: UV (280 nm); flow rate: 1.0 mL/min] to give products.

5 Spectral data for new compounds; product **4**: HRFABMS: *m/z* 407.2411 $[M + Na]^+$; ^{1}H NMR (400 MHz, CD_3OD): δ 1.61 (6H, s, H-13, 14), 1.67 (3H, s, H-12), 1.69 (3H, s, H-15), 2.05 (4H, m, H-8, 9), 2.11 (4H, m, H-4, 5), 3.16–3.36 (4H, m, H-2′, 3′, 4′, 5′), 3.67 (1H, dd, *J* = 12.0, 5.6 Hz, H-6a), 3.86 (1H, dd, *J* = 12.4, 2.4 Hz, H-6b), 4.23 (1H, d, *J* = 8.0 Hz, H-1a), 4.28 (1H, d, *J* = 8.0 Hz, H-1′), 4.34 (1H, d, *J* = 8.0 Hz, H-1b), 5.10 (2H, t, *J* = 7.0 Hz, H-6, 10), 5.37 (1H, t, *J* = 7.0 Hz, H-2); ^{13}C NMR (100 MHz, CD_3OD): δ 16.5 (C-14), 17.8 (C-15), 25.9 (C-12), 27.4 (C-5), 27.8 (C-9), 40.8 (C-4), 41.0 (C-8), 62.8 (C-6′), 66.3 (C-4′), 71.7 (C-2′), 75.0 (C-5′), 77.9 (C-3′), 102.7 (C-1′), 121.5 (C-2), 125.0 (C-6, C-10), 132.4 (C-7, C-11), 141.7 (C-3). Product **5**: HRFABMS: *m/z* 339.1790 $[M + Na]^+$; ^{1}H NMR (CD_3OD): δ 1.61 (3H, s, H-9), 1.68 (3H, s, H-8), 1.71 (3H, s, H-10), 2.10 (4H, m, H-4, 5), 3.32–3.49 (4H, m, H-2′, 3′, 4′, 5′), 3.79 (1H, dd, *J* = 12.4, 4.8 Hz, H-6a), 3.89 (1H, dd, *J* = 12.0, 1.2 Hz, H-6b), 4.33 (1H, dd, *J* = 12.0, 6.0 Hz, H-1a), 4.35 (1H, dd, *J* = 12.0, 7.0 Hz, H-1b), 4.42 (1H, d, *J* = 8.0 Hz, H-1′), 5.14 (2H, t, *J* = 6.4 Hz, H-6), 5.41 (1H, t, *J* = 6.8 Hz, H-2); ^{13}C NMR (CD_3OD): δ 16.9 (C-10), 18.3 (C-9), 26.3 (C-8), 27.3 (C-5), 40.4 (C-4), 61.8 (C-6′), 66.4 (C-4′), 70.5 (C-2′), 74.1 (C-5′), 77.1 (C-3′), 101.8 (C-1′), 120.5 (C-2), 125.2 (C-6), 132.5 (C-7), 143.2 (C-3). Product **7**: Mp: 150–152 °C; HRFABMS: *m/z* 499.2155 $[M + Na]^+$; ^{1}H NMR (CD_3OD): δ 1.49 (1H, m, H-5ax), 1.73 (3H, s, H-10), 1.83 (1H, m, H-5eq), 1.97–2.19 (5H, m, H-3, 4, 6), 3.18–3.91 (12H, m, H-2′, 2″, 3′, 3″, 4′, 4″, 5′, 5″, 6′, 6″), 4.02 (1H, d, *J* = 11.6 Hz, H-7a), 4.22 (1H, d, *J* = 11.6 Hz, H-7b), 4.23 (1H, d, *J* = 8.0 Hz, H-1″), 4.71 (2H, s, H-9), 4.87 (1H, d, *J* = 8.0 Hz, H-1′), 5.77 (1H, brs, H-2); ^{13}C NMR (CD_3OD): δ 20.9 (C-10), 27.5 (C-6), 28.7 (C-5), 31.6 (C-3), 42.4 (C-4), 62.7 (C-6″), 68.4 (C-6′), 72.6 (C-4″), 73.1 (C-4′), 74.3 (C-7), 74.7 (C-2″), 75.0 (C-2′), 77.7 (C-5″), 78.7 (C-3″, C-5′), 78.7 (C-3′), 98.9 (C-1′), 102.8 (C-1″), 109.1 (C-9), 125.8 (C-2), 135.4 (C-1), 150.8 (C-8). Product **8**: Mp: 157–160 °C; HRFABMS: *m/z* 499.2151 $[M + Na]^+$; ^{1}H NMR (CD_3OD): δ 1.48 (1H, m, H-5ax), 1.73 (3H, s, H-10), 1.81 (1H, m, H-5eq), 1.99–2.16 (5H, m, H-3, 4, 6), 3.22–3.89 (12H, m, H-2′, 2″, 3′, 3″, 4′, 4″, 5′, 5″, 6′, 6″), 4.02 (1H, d, *J* = 11.2 Hz, H-7a), 4.20 (1H, d, *J* = 11.6 Hz, H-7b), 4.27 (1H, d, *J* = 8.0 Hz, H-1′), 4.71 (2H, s, H-9), 5.15 (1H, d, *J* = 4.0 Hz, H-1″), 5.75 (1H, brs, H-2); ^{13}C NMR (CD_3OD): δ 20.9 (C-10), 27.5 (C-6), 28.8 (C-5), 31.7 (C-3), 42.5 (C-4), 62.2 (C-6″), 62.7 (C-6′), 71.5 (C-4″), 74.1 (C-2″), 74.5 (C-7), 74.7 (C-2′, C-5″), 75.0 (C-3″), 76.5 (C-5′), 77.8 (C-3′), 81.3 (C-4′), 102.8 (C-1′), 102.9 (C-1″), 109.1 (C-9), 125.9 (C-2), 135.4 (C-1), 150.9 (C-8). Product **9**: Mp: 191–195 °C; HRFABMS: *m/z* 661.2682 $[M + Na]^+$; ^{1}H NMR (CD_3OD): δ 1.48 (1H, m, H-5ax), 1.73 (3H, s, H-10), 1.82 (1H, m, H-5eq), 1.96–2.19 (5H, m, H-3, 4, 6), 3.22–3.89 (18H, m, H-2′, 2″, 2‴, 3′, 3″, 3‴, 4′, 4″, 4‴, 5′, 5″, 5‴, 6′, 6″, 6‴), 4.02 (1H, d, *J* = 11.6 Hz, H-7a), 4.20 (1H, d, *J* = 11.2 Hz, H-7b), 4.28 (1H, d, *J* = 7.6 Hz, H-1′), 4.71 (2H, s, H-9), 5.14 (1H, d, *J* = 3.6 Hz, H-1″), 5.16 (1H, d, *J* = 4.0 Hz, H-1‴), 5.75 (1H, brs, H-2); ^{13}C NMR (CD_3OD): δ 20.9 (C-10), 27.5 (C-6), 28.8 (C-5), 31.7 (C-3), 42.5 (C-4), 62.2 (C-6″, C-6‴), 62.7 (C-6′), 71.5 (C-4‴), 73.3 (C-5″), 73.8 (C-2″), 74.2 (C-2‴), 74.5 (C-7), 74.7 (C-3‴, C-5‴), 74.9 (C-2′), 75.1 (C-3″), 76.5 (C-5′), 77.8 (C-3′), 81.3 (C-4′, C-4″), 102.6 (C-1‴), 102.8 (C-1′, C-1″), 109.1 (C-9), 125.9 (C-2), 135.4 (C-1), 150.9 (C-8).

6 Glycosylation with CGTase was carried out as follows. To a solution containing 10 mL of 25 mM sodium phosphate buffer (pH 7.0), 0.5 mmol of perillyl β-D-glucoside (**6**), and 5 g of soluble starch was added 300 units of CGTase from *Bacillus macerans*. After incubation of the mixture for 24 h at 40 °C, the mixture was centrifuged at 3000 g for 10 min. The supernatant was subjected to Sephadex G-25 column chromatography. The glycoside fractions were lyophilized and purified by preparative HPLC to give products.

Published on the web (Advance View) May 5, 2008; doi:10.1246/cl.2008.556