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Biotransformation of low-molecular-weight alcohols by *Coleus forskohlii* hairy root cultures

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Abstract

Coleus forskohlii hairy root cultures were shown to biotransform methanol and ethanol to the corresponding β-D-glucopyranosides and β-D-*ribo*-hex-3-ulopyranosides, and 2-propanol to its β-D-glucopyranoside. © 2003 Elsevier Science Ltd. All rights reserved.

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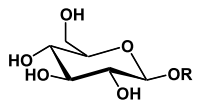
Plant cell, root or hairy root cultures are considered to be an important source of useful secondary metabolites. Furthermore, the ability of some of the cultures to specifically convert administered organic compounds into useful analogues has been the subject of increasing attention. The reactions involved in the biotransformation include oxidation, reduction, hydroxylation, esterification, methylation, isomerization, and also glycosylation, which occurs readily in plant cells but with difficulty in microorganisms. Cultures with the ability of glycosylation have been reported in some plant cell, root or hairy root cultures, such as that of *Panax ginseng*,[1,2] *Eucalyptus perriniana*,[3] *Artemisia annua*,[4] *Coffea arabica*,[5] etc.

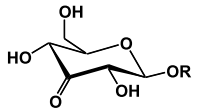
Coleus forskohlii hairy roots induced from *Agrobacterium rhizogenes* are reported to be a producer of forskolin.[6] Herein, we report the *C. forskohlii* hairy roots also have the ability to biotransform low-molecular-weight alcohols into the corresponding β-D-glucopyranosides or β-D-*ribo*-hex-3-ulopyranosides.

The *C. forskohlii* hairy root cultures induced from *A. rhizogenes* MAFF 03-01724 were subcultured on Gamborg B5 basal liquid medium[7] at 4-week intervals. Methanol was administered to hairy roots cultured for 3 weeks in the same medium (120 mL), to a final concentration of 1% (v/v). The cultures not administered any alcohol were used as negative controls. The hairy roots were further cultured for 1 week. Each culture was separated into roots and medium by filtration through a filter paper. The methanol extracts of the roots and the medium were analyzed by TLC (6:4:1 $CHCl_3$–MeOH–H_2O). Two biotransformation products (**1**, R_f 0.25 and **2**, R_f 0.35) were found in the methanol extracts of the roots administered methanol, but none were found in the medium or negative controls. Further separation of the extract by silica gel open column (solvent as 60:29:6 $CHCl_3$–MeOH–H_2O) gave products **1** (30.0 mg) and **2** (11.5 mg).

Product **1** revealed an $[M + Na]^+$ ion peak at m/z 217.1 in the positive-ion ESIMS spectrum. The ^1H NMR spectrum showed a methoxy signal and a set of β-glucopyranose signals, with the resonance for the anomeric proton at δ 4.16 (1 H, d, J 7.8 Hz). Further comparison of the $[\alpha]_D$ of **1** with that of the standard compound showed that it is methyl β-D-glucopyranoside.



1 R = CH_3
3 R = CH_2CH_3
5 R = $CH(CH_3)_2$



2 R = CH_3
4 R = CH_2CH_3

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Product **2** revealed an $[M + Na]^+$ ion peak at m/z 215.2 in the positive-ion ESIMS spectrum, which was 2 mass units smaller than that of **1**. Comparing the ^1H NMR spectrum of **2** with **1**, H-3 of **2** disappeared, and H-2 (δ 4.10) and H-4 (δ 4.22) showed a lower field shift of about 1 ppm. The ^{13}C NMR spectrum of **2** showed a ketone signal at δ 207.0. Further 2D NMR analysis suggested that **2** is a methyl D-*ribo*-hex-3-ulopyranoside (the 3-keto derivative of **1**). Reduction of **2** with $NaBH_4$ afforded two products, of which one product (**2**a) was identical with **1** by its NMR spectrum and $[\alpha]_D$ data. The other product (**2**b) was characterized as the methyl β-D-allopyranoside. All these data show **2** to be methyl β-D-*ribo*-hex-3-ulopyranoside.

Although the α-form is known to be thermodynamically more stable than the β-form of methyl D-glucopyranoside, we could not find the α-glucoside as a product. The result shows that the biotransformation was an enzyme-catalyzed reaction.

With the same experimental method, ethanol was administered to *C. forskohlii* hairy root cultures, similar to the case of methanol, and ethyl β-D-glucopyranoside (**3**, 23.4 mg) and ethyl β-D-*ribo*-hex-3-ulopyranoside (**4**, 4.6 mg) were isolated as biotransformation products. When 2-propanol was administered, 2-propyl β-D-glucopyranoside (**5**, 15.0 mg) was isolated as the biotransformation product. The lack of 2-propyl β-D-*ribo*-hex-3-ulopyranoside as a product is probably due to the higher toxicity of 2-propanol to the hairy roots.

β-D-*ribo*-Hex-3-ulopyranosides rarely exist in the plant kingdom and have only been reported as iridoid[8–10] and cardenolide[11,12] glycosides. Interestingly, it has been reported that β-D-glucopyranose-3-oxidase was present in *Agrobacterium tumeffaciens*, which belongs to the same genus with *A. rhizogenes* used to induce the hairy roots.[13] We are now working to determine whether the β-D-glucopyranose-3-oxidase activity present in the hairy roots is due to the fact that the genome of this enzyme is transferred into *C. forskohlii* hairy roots from *A. rhizogenes*.

1. Experimental

1.1. General methods

Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5-dm cell. The ESIMSs were taken on an LCQ mass analyzer. The ^1H and ^{13}C NMR spectra were measured with a JEOL ECP-500 spectrometer in MeOH-d_4 solution with Me_4Si as the internal reference, and chemical shifts are expressed in δ (ppm). For HPLC, a JASCO PU-2080 HPLC system, equipped with a Shodex RI-101 Differential Refractometer detector, was used. Column chromatography was carried out using Kieselgel 60 (E. Merck). TLC was conducted in Kieselgel 60 F_{254} plates (E. Merck).

1.2. Culture methods and feeding experiments

The root cultures were established as described in a previous paper.[6] The root cultures were subcultured on Gamborg B5 basal liquid medium[7] containing 2% sucrose at 25 °C in the dark at 60 rpm on a rotary shaker at 4-week intervals. Methanol, EtOH or 2-propanol (each 1.2 mL) was administered to hairy roots (approx 6 g, Fr. Wt.) cultured for 3 weeks in B5 liquid medium (120 mL per flask), respectively. The cultures devoid of any alcohol were used as negative controls. The hairy roots were further cultured for 1 week.

1.3. Extraction and separation procedures of biotransformation products

Each culture was separated into roots and medium by filtration through a filter paper. The roots were extracted with MeOH under ultrasonic treatment three times for 1 h each at room temperature. The MeOH extracts and the media were analyzed by TLC (6:4:1 $CHCl_3$–MeOH–H_2O). Two biotransformation products were found in the methanolic extract of roots administered MeOH and EtOH, whereas only one product was found in the methanolic extract of roots administered 2-propanol. No product was found in the media or negative controls. Further separation of each extract by silica gel open-column chromatography gave products **1** (30.0 mg, R_f 0.25 on TLC) and **2** (11.5 mg, R_f 0.35) from the MeOH extract of roots administered MeOH, products **3** (23.4 mg, R_f 0.32) and **4** (4.6 mg, R_f 0.43) from the methanolic extract of roots administered EtOH, and product **5** (15.0 mg, R_f 0.38) from the methanolic extract of roots administered 2-propanol.

1.4. Methyl β-D-glucopyranoside (1)

Powder, $[\alpha]_D^{24}$ −33.7° (*c* 1.03, MeOH). ESIMS (positive-ion) m/z: 217.1 $[(M + Na)^+]$. ^1H NMR (500 MHz, MEOH-d_4): δ 3.15 (1 H, dd, *J* 9.1, 7.8 Hz, H-2), 3.26 (1 H, m, H-5), 3.27 (1 H, dd, *J* 9.1, 9.1 Hz, H-4), 3.34 (1 H, dd, *J* 9.1, 9.1 Hz, H-3), 3.52 (3 H, s, CH_3), 3.66 (1 H, dd, *J* 11.9, 5.2 Hz, H_a-6), 3.86 (1 H, dd, *J* 11.9, 2.1 Hz, H_b-6), 4.16 (1 H, d, *J* 7.8 Hz, H-1). ^{13}C NMR (125 MHz, MeOH-d_4): δ 57.3 (CH_3), 62.8 (C-6), 71.7 (C-4), 75.0 (C-2), 77.9 (C-5), 78.1 (C-3), 105.4 (C-1).

1.5. Methyl β-D-*ribo*-hex-3-ulopyranoside (2)

Powder, $[\alpha]_D^{24}$ −27.8° (*c* 0.76, MeOH). ESIMS (positive-ion) m/z: 215.2 $[(M + Na)^+]$. ^1H NMR (500 MHz, MeOH-d_4): δ 3.31 (1 H, ddd, *J* 10.1, 4.9, 2.1 Hz, H-5),

3.58 (3 H, s, CH_3), 3.79 (1 H, dd, *J* 12.1, 4.9 Hz, H_a-6), 3.94 (1 H, dd, *J* 12.1, 2.1 Hz, H_b-6), 4.10 (1 H, dd, *J* 7.9, 1.8 Hz, H-2), 4.22 (1 H, dd, *J* 10.1, 1.8 Hz, H-4), 4.27 (1 H, d, *J* 7.9 Hz, H-1). ^{13}C NMR (125 MHz, MeOH-d_4): δ 57.5 (CH_3), 62.5 (C-6), 73.6 (C-4), 78.2 (C-5), 78.3 (C-2), 106.8 (C-1), 207.0 (C-3).

$NaBH_4$ (5.0 mg) was added to a solution of **2** (4.8 mg) in EtOH (1 mL), and the mixture was stirred at room temperature for 30 min. The reaction solution was neutralized by adding Dowex 50W-X8 (H^+) and then filtered through a filter paper to remove the Dowex. Further separation by HPLC (H_2O as solvent) gave two products (**2a**, 0.8 mg and **2b**, 0.8 mg). Compound **2a** showed the same R_f value on TLC and t_R on HPLC and was identical with **1** by its NMR spectrum and $[\alpha]_D$ data.

1.6. Methyl β-D-allopyranoside (2b)

Powder, $[\alpha]_D^{24}$ −40.0° (*c* 0.07, MeOH). ESIMS (positive-ion) *m*/*z*: 217.1 [(M + Na)$^+$]. ^{1}H NMR (500 MHz, MeOH-d_4): δ 3.27 (1 H, dd, *J* 8.0, 3.0 Hz, H-2), 3.47 (1 H, dd, *J* 9.6, 3.0 Hz, H-4), 3.51 (3 H, s, CH_3), 3.65 (1 H, dd, *J* 11.3, 5.7 Hz, H_a-6), 3.68 (1 H, ddd, *J* 9.6, 5.7, 2.1 Hz, H-5), 3.84 (1 H, dd, *J* 11.3, 2.1 Hz, H_b-6), 4.51 (1 H, d, *J* 8.0 Hz, H-1). ^{13}C NMR (125 MHz, MeOH-d_4): δ 57.2 (CH_3), 63.2 (C-6), 69.1 (C-4), 72.4 (C-3), 72.9 (C-2), 75.5 (C-5), 103.0 (C-1).

1.7. Ethyl β-D-glucopyranoside (3)

Powder, $[\alpha]_D^{24}$ −35.7° (*c* 0.78, MeOH). ESIMS (positive-ion) *m*/*z*: 231.2 [(M + Na)$^+$]. ^{1}H NMR (500 MHz, MeOH-d_4): δ 1.22 (3 H, dd, *J* 7.1, 7.1 Hz, CH_3), 3.16 (1 H, dd, *J* 9.1, 7.9 Hz, H-2), 3.26 (1 H, m, H-5), 3.27 (1 H, dd, *J* 9.1, 9.1 Hz, H-4), 3.34 (1 H, dd, *J* 9.1, 9.1 Hz, H-3), 3.61 (1 H, dd, *J* 9.5, 7.1 Hz, CH_2), 3.65 (1 H, dd, *J* 11.9, 5.5 Hz, H_a-6), 3.85 (1 H, dd, *J* 11.9, 2.0 Hz, H_b-6), 3.95 (1 H, dd, *J* 9.5, 7.1 Hz, CH_2), 4.25 (1 H, d, *J* 7.9 Hz, H-1). ^{13}C NMR (125 MHz, MeOH-d_4): δ 57.3 (CH_3), 62.8 (C-6), 66.2 (CH_2), 71.7 (C-4), 75.1 (C-2), 78.0 (C-5), 78.2 (C-3), 104.2 (C-1).

1.8. Ethyl β-D-*ribo*-hex-3-ulopyranoside (4)

Powder, $[\alpha]_D^{24}$ −55.8° (*c* 0.45, MeOH). ESIMS (positive-ion) *m*/*z*: 229.2 [(M + Na)$^+$]. ^{1}H NMR (500 MHz, MeOH-d_4): δ 1.25 (3 H, dd, *J* 7.3, 7.3 Hz, CH_3), 3.30 (overlapped by MeOH-d_4, H-5), 3.67 (1 H, dd, *J* 9.6, 7.3 Hz, CH_2), 3.78 (1 H, dd, *J* 12.4, 5.0 Hz, H_a-6), 3.92 (1 H, dd, *J* 12.4, 2.3 Hz, H_b-6), 3.99 (1 H, dd, *J* 9.6, 7.3 Hz, CH_2), 4.10 (1 H, dd, *J* 7.8, 1.8 Hz, H-2), 4.21 (1 H, dd, *J* 10.1, 1.8 Hz, H-4), 4.36 (1 H, d, *J* 7.8 Hz, H-1). ^{13}C NMR (125 MHz, MeOH-d_4): δ 15.4 (CH_3), 62.6 (C-6), 66.5 (CH_2), 73.7 (C-4), 78.4 (C-2 and C-5), 105.6 (C-1), 207.2 (C-3).

1.9. 2-Propyl β-D-glucopyranoside (5)

Powder, $[\alpha]_D^{24}$ −34.4° (*c* 1.00, MeOH). ESIMS (positive-ion) *m*/*z*: 245.2 [(M + Na)$^+$]. ^{1}H NMR (500 MHz, MeOH-d_4): δ 1.18 (3 H, d, *J* 6.2 Hz, CH_3), 1.22 (3 H, d, *J* 6.2 Hz, CH_3), 3.13 (1 H, dd, *J* 9.0, 7.8 Hz, H-2), 3.25 (1 H, m, H-5), 3.27 (1 H, dd, *J* 9.2, 9.2 Hz, H-4), 3.34 (1 H, dd, *J* 9.0, 9.2 Hz, H-3), 3.65 (1 H, dd, *J* 11.9, 5.5 Hz, H_a-6), 3.84 (1 H, dd, *J* 11.9, 2.3 Hz, H_b-6), 4.03 (1 H, m, CH), 4.25 (1 H, d, *J* 7.9 Hz, H-1). ^{13}C NMR (125 MHz, MeOH-d_4): δ 22.1 (CH_3), 23.8 (CH_3), 62.9 (C-6), 71.8 (C-4), 72.6 (CH), 75.2 (C-2), 77.9 (C-5), 78.2 (C-3), 102.6 (C-1).

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