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Biotransformation of umbelliferone by *Panax ginseng* root cultures

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Abstract—*Panax ginseng* root cultures biotransformed umbelliferone (**1**) to its 7-*O*-β-D-glucopyranoside (**2**), 7-*O*-β-D-glucopyranosyl (1→6) β-D-glucopyranoside (**3**), 7-*O*-β-D-xylopyranosyl (1→6) β-D-glucopyranoside (**4**) and 7-*O*-α-L-rhamnopyranosyl (1→2) β-D-glucopyranoside (**5**). The roots showed high glycosylation ability to the 7-hydroxycoumarin molecule. The glycosylation was catalyzed by glycosyltransferase rather than glycosidase, as was demonstrated by administration of inhibitors.

A great number of biologically active natural products exist as glycosides. Chemical synthesis of glycosides is sometimes limited by the unstable structure of the glycosyl acceptor or donor. An alternative route to obtaining glycoconjugates would be by using enzymatic synthesis, such as glycosyltransferase (or glycosidase) as biocatalysts.[1] But the high cost of purified enzyme or glycosyl-donor, e.g. UDP-sugars, led us to turn our interest to biological in vivo synthesis of the glycosides by plant whole cells or organ cultures. Coumarin is a kind of very important secondary metabolite of the plant kingdom and has been reported to have various pharmaceutical and biological activities.[2] The low water solubility of most coumarins and the lack of their natural glycosides led us to investigate an approach to bio-synthesize the coumarin glycosides by plant tissue cultures. Herein, we report the biotranformation of umbelliferone (**1**), a simple 7-hydroxycoumarin widespread in the plant kingdom, by *Panax ginseng* root cultures.

The *P. ginseng* root cultures were established as described in a previous paper.[3] The root cultures were subcultured on Murashige and Skoog's basal medium[4] containing 5 mg/l IBA and 0.1 mg/l kinetin (B5K medium) at 25°C in the dark at 140 rpm on a rotary shaker at 4 weeks intervals. 50 mg of umbelliferone (Nihon kaisei. Co.) dissolved in ethanol (2 ml) were administered to hairy roots (ca. 12 g, Fr. Wt.) cultured for 3 weeks in B5K liquid medium (120 ml per flask) and then cultured for 1 week. The cultures were separated into roots and medium by filtration through a filter paper. The roots were extracted with 200 ml methanol under supersonic, 1 h for three times at room temperature. The methanol extract and the medium were then analyzed by HPLC. Four biotransformation products were found in the methanol extracts of roots, but none from the medium. Further separation of the extract gave a main product **2** (40.0 mg, biotransformation yield, 40.1%) and three minor products **3** (10.2 mg, 6.8%), **4** (4.1 mg, 2.9%) and **5** (1.9 mg, 1.3%). **2** was confirmed as umbelliferone 7-*O*-β-D-glucopyranoside by its $[\alpha]_D$, FAB-MS and NMR spectra.[5] The structures of new compounds **3**–**5** were further determined by spectral and chemical analysis.[6]

Product **3** was obtained as a colorless powder, $[\alpha]_D$ −63.3 (*c* 0.67, MeOH, 24°C). Negative high-resolution FAB-MS analysis of **3** provided a molecular formula of $C_{21}H_{25}O_{13}$ $[(M-H)^-]$ (found 485.1305, calcd 485.1296), suggesting that **3** is larger than **2** by a hexose unit. Acid hydrolysis of **3** by 1 M hydrochloric acid in dioxane/water (1:1) gave only D-glucose as component sugars, which was identified by HPLC analysis following its conversion to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxy-alditol acetate derivatives.[7] The β-anomeric configurations for glucose were determined from their large $^3J_{H1,H2}$ coupling constants (7–8 Hz). The position of the sugar moiety attached to the aglycon and the interglycosidic linkage were confirmed by

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observing the correlations between H-1 (δ 5.03) of the inner glucose and C-7 (δ 160.7) of aglycone, H-1 (δ 4.36) of the terminal glucose and C-6 (δ 69.0) of the inner glucose in the HMBC spectrum. Based upon all of the above evidence, the structure of **3** was elucidated as umbelliferone 7-*O*-β-D-glucopyranosyl (1→6) β-D-glucopyranoside.

Product **4** was obtained as a colorless powder, $[\alpha]_D$ −66.0 (*c* 0.24, MeOH, 24°C). Negative high-resolution FAB-MS analysis of **4** provided a molecular formula of $C_{20}H_{23}O_{12}$ $[(M-H)^-]$ (found: 455.1180; calcd 455.1189), which is larger by a pentose unit than **2**. Acid hydrolysis of **3** gave D-glucopyranose and D-xylopyranose in a ratio of 1:1, which were identified by HPLC analysis following their conversion to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxy-alditol acetate derivatives.[7] The β-anomeric configurations for the glucose and xylose were determined from their large $^3J_{H1,H2}$ coupling constants (7–8 Hz). In the HMBC spectrum, correlations of H-1 (δ 4.31) of the xylose with C-6 (δ 69.0) of the glucose gave the linkage of the sugar units to be β-D-xylopyranosyl(1→6) β-D-glucopyranose. Thus, the structure of **4** was established as umbelliferone 7-*O*-β-D-xylopyranosyl(1→6) β-D-glucopyranoside.

Product **5** was obtained as a colorless powder, $[\alpha]_D$ −76.2 (*c* 0.13, MeOH, 24°C). Negative high resolution-FAB-MS of **5** gave the molecular formula as $C_{21}H_{25}O_{12}$ $[(M-H)^-]$ (found: 469.1360; calcd 469.1346). On the basis of the ^1H–^1H COSY spectrum, all proton signals were assigned as shown in Table 1, indicating that component sugars in **5** are one β-glucopyranoses and one α- or β-rhamnopyranose. Two sugar units were further identified as D-glucose and L-rhamnose by HPLC analysis of the acid hydrolysate.[7] The ^{13}C NMR data of rhamnose were compared with those of methyl-α-L and methyl-β-L rhamnopyranosides. They agreed well with those of methyl-α-L- rhamnopyranoside. The interglycosidic linkage was determined unambiguously by the HMBC spectrum. Namely, the correlation between H-1 (δ 5.28) of rhamnose and C-2 (δ 77.8) of glucose indicated that rhamnose was linked to C-2 of glucose. Based upon all the above evidence, the structure of **5** was elucidated as umbelliferone 7-*O*-α-L-rhamnopyranosyl(1→2) β-D-glucopyranoside.

The biotransformation pathway of umbelliferone (**1**) by *P. ginseng* root cultures is considered as shown in Fig. 1. *P. ginseng* root cultures do not contain coumarins as metabolites. To investigate the enzyme responsible for this glycosylation, glucosyltransferase inhibitors [hexadecylpyridinium chloride (final conc. 4.2 mM), hexadecyltrimethyl ammonium bromide (4.1 mM) and sodium dodecyl sulfate (17.3 mM)], and β-glucosidase inhibitors [imidazole (4.6 mM), benzimidazole (6.5 mM) and 2-aminopyridine (16.0 mM)], were administered to *P. ginseng* root cultures that were pre-cultured for 2 weeks, respectively. After 3 days, umbelliferone (each 30 mg) was administered to the root cultures, and the root cultures were further cultured for 1 week, then harvested and extracted with 20 ml methanol, respec-

Table 1. ^1H (500 MHz) and ^{13}C NMR (125 MHz) data for the biotransformation products **3**–**5** in methanol-d_4 at 30°C

Position	**3**		**4**		**5**	
	δ ^1H (mult, *J* in Hz)	δ ^{13}C	δ ^1H (mult, *J* in Hz)	δ ^{13}C	δ ^1H (mult, *J* in Hz)	δ ^{13}C
2		161.9		161.9		161.8
3	6.28 (d, 9.6)	112.9	6.28 (d, 9.6)	112.9	6.28 (d, 9.6)	113.0
4	7.89 (d, 9.6)	144.3	7.89 (d, 9.6)	144.3	7.89 (d, 9.6)	144.2
5	7.57 (d, 8.5)	129.2	7.57 (d, 8.8)	129.2	7.56 (d, 8.8)	129.1
6	7.12 (dd, 8.5, 1.8)	114.0	7.11 (dd, 8.8, 2.3)	114.0	7.03 (dd, 8.8, 2.3)	113.7
7		160.7		160.8		160.5
8	7.16 (d, 1.8)	103.8	7.15 (d, 2.3)	103.8	7.04 (d, 2.3)	103.4
9		155.4		155.4		155.3
10		114.0		114.0		114.0
	Glc		Glc		Glc	
1′	5.03 (d, 7.8)	100.4	4.99 (d, 7.3)	100.6	5.16 (d, 7.8)	98.8
2′	3.49[a]	73.5	3.48[a]	73.4	3.69 (dd, 9.2, 7.8)	77.8
3′	3.49[a]	76.5	3.48[a]	76.5	3.61 (dd, 9.6, 9.2)	77.7
4′	3.40 (dd, 8.7, 8.7)	70.2	3.37 (dd, 8.7, 8.7)	70.2	3.39 (dd, 9.6, 9.6)	70.1
5′	3.82 (m)	76.1	3.75 (ddd, 8.7, 6.5, 1.8)	76.1	3.51 (ddd, 9.6, 5.9, 1.8)	76.9
6′	4.16 (dd, 12.0, 2.5)	69.0	4.10 (dd, 11.4, 1.8)	69.0	3.89 (dd, 11.9, 1.8)	61.1
	3.83 (dd, 12.0, 5.5)		3.79 (dd, 11.4, 6.5)		3.68 (dd, 11.9, 5.9)	
	Glc		Xyl		Rham	
1″	4.36 (d, 7.3)	103.8	4.31 (d, 7.3)	104.4	5.28 (d, 1.8)	101.2
2″	3.26 (dd, 8.7, 7.3)	73.9	3.26[a]	73.7	3.93 (dd, 1.8, 1.8)	70.9
3″	3.33 (dd, 8.7, 8.7)	76.8	3.26[a]	76.5	3.56 (dd, 9.6, 1.8)	70.9
4″	3.35 (dd, 8.7, 8.7)	70.4	3.56 (ddd, 10.2, 8.7, 5.5)	69.8	3.40 (ddd, 9.6, 9.6)	72.6
5″	3.24 (ddd, 8.7, 6.2, 2.3)	76.8	3.14 (dd, 11.5, 10.2)	65.6	3.93 (dq, 9.6, 6.4)	68.6
			3.87 (dd, 11.5, 5.5)			
6″	3.86 (dd, 11.9, 2.3)	61.5			1.29 (dd, 6.4)	16.8
	3.66 (dd, 11.9, 6.2)					

[a] Overlapped signals.

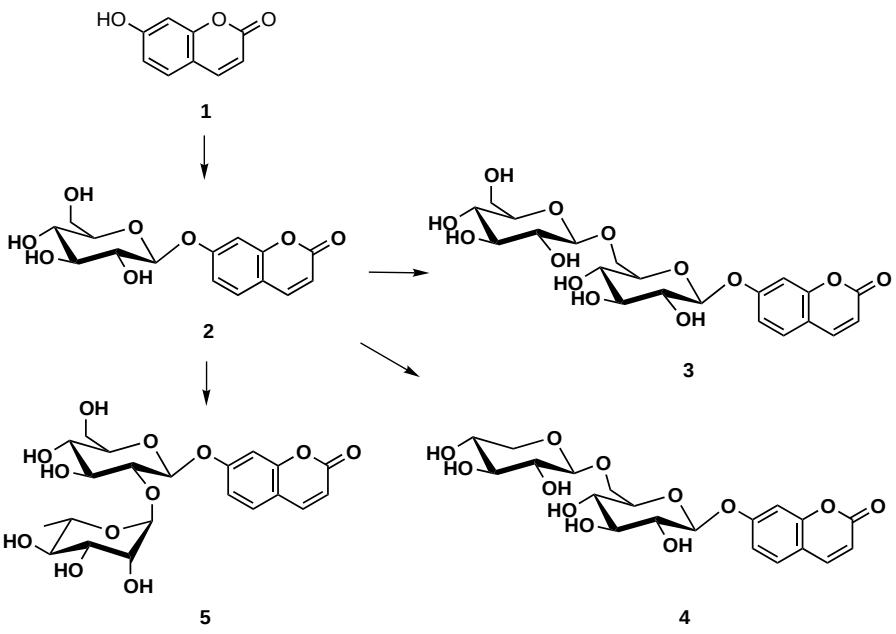

Figure 1. Biotransformation of umbelliferone (**1**) by *P. ginseng* root cultures.

tively. Further HPLC analysis[8] of main biotransformed product **2** showed that glucosyltransferase inhibitors (hexadecylpyridinium chloride, hexadecyltrimethyl ammonium bromide and sodium dodecyl sulfate) inhibited the glycosylation completely, on the contrary to the fact that the glucosidase inhibitors showed almost no effect on the glycosylation to umbelliferone. Thus, the glycosylation was most likely catalyzed by the glycosyltranferase. The glycosyltransferase is now under further investigation.

Acknowledgements

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References

1. Koeller, K. M.; Wong, C.-H. *Chem. Rev.* **2000**, *100*, 4465.
2. Dewick, P. M. *Medicinal Natural Products*: *A Biosynthetic Approach*; Wiley Press: Chichester, 1997 chapter 4.
3. Furuya, R.; Yoshikawa, T.; Orihara, Y.; Oda, H. *J. Nat. Prod.* **1984**, *47*, 70.
4. Murashige, T.; Skoog, F. *Physiol. Plant* **1962**, *15*, 473.
5. Cussans, N. J.; Huckerby, T. N. *Tetrahedron* **1975**, *31*, 2719.
6. Data for new compounds. Umbelliferone 7-*O*-β-D-glucopyranosyl(1→6) β-D-glucopyranoside (**3**): Powder, $[\alpha]_D$ −63.3 (*c* 0.67, MeOH, 24°C). IR *v* cm^{-1}: 3430, 1702, 1617. FAB-MS (negative) *m*/*z*: 485 [(*M*−H)$^-$]. HR-FAB-MS (negative) *m*/*z*: Found: 485.1305; Calcd for $C_{22}H_{25}O_{13}$ [(*M*−H)$^-$]: 485.1296. ^{1}H NMR (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4): see Table 1. Umbelliferone 7-*O*-β-D-xylopyranosyl(1→6) β-D-glucopyranoside (**4**): Powder, $[\alpha]_D$ −66.0 (*c* 0.24, MeOH, 24°C). IR *v* cm^{-1}: 3446, 1702, 1619. FAB-MS (negative) *m*/*z*: 455 [(*M*−H)$^-$]. HR-FAB-MS (negative) *m*/*z*: Found: 455.1180; Calcd for $C_{20}H_{23}O_{12}$ [(*M*−H)$^-$]: 455.1189. ^{1}H NMR (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4): see Table 1. Umbelliferone 7-*O*-β-D-rhamnopyranosyl(1→2) β-D-glucopyranoside (**5**): Powder, $[\alpha]_D$ −76.2 (*c* 0.13, MeOH, 24°C). IR *v* cm^{-1}: 3432, 1706, 1619. FAB-MS (negative) *m*/*z*: 469 [(*M*−H)$^-$]. HR-FAB-MS (negative) *m*/*z*: Found: 469.1360; Calcd for $C_{21}H_{25}O_{12}$ [(*M*−H)$^-$]: 469.1346. ^{1}H NMR (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4): see Table 1.
7. (a) Oshina, R.; Kumanotani, J. *Chem. Lett.* **1981**, 943; (b) Oshina, R.; Yamauchi, Y.; Kumanotani, J. *Carbohydr. Res.* **1982**, *107*, 169; (c) HPLC conditions for 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxy-alditol acetate derivatives of the monosaccharides: Column, Senshu Pak Pegasil ODS, 4.6×150 mm; Solvent: 33% CH_3CN; flow rate, 0.8 ml/min; detection, UV 230 nm. The derivatives of D-glucose, D-xylose and L-rhamnose were detected as follows: t_R (min) 38.30 (derivative of D-glucose), 26.50 (derivative of D-xylose) and 40.24 (derivative of L-rhamnose).
8. HPLC condition for analysis of main product **2**. Detector: SPD-M10 Avp Diode Array (Shimadzu); 280 nm. Column: Shenshu Pak, Pegasil ODS II (4.6×250 mm). Solvent: MeOH/H_2O (0.06% TFA), Linear gradient; 0% ~100% MeOH in 45 min, then 100% MeOH at flow rate 0.7 ml/min. Column temperature: 40°C. t_R: 26.0 min.