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Full Length Research Paper

Biotransformation of 4-phenylcoumarin by transgenic hairy roots of *Polygonum multiflorum*

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In order to find a new method for preparation of coumarin glycosides, the glycosylation of 4-phenylcoumarin was achieved for the first time by biotransformation using transgenic hairy roots of *Polygonum multiflorum*. Two substrates, 7-hydroxy-4-phenylcoumarin (1) and 5,7-dihydroxy-4-phenylcoumarin (2) were synthesized through Pechmann condensation. Two biotransformed products, 4-phenylcoumarin-7-*O*-*β*-D-glucopyranoside (3) and 7-hydroxy-4-phenylcoumarin-5-*O*-*β*-D-glucopyranoside (4), biosynthesized by transgenic hairy roots of *P. multiflorum* were isolated and identified using ^{1}H-NMR, ^{13}C-NMR, HMBC and ESI-MS spectral technologies. Biosynthesis product 4 was a novel compound. Co-culture time curve on conversion was also established. Time course experiment revealed that the optimal co-culture time of compounds 1 and 2 was 4 and 2 day, respectively. In conclusion, biotransformation was shown to be an effective method to prepare 4-phenylcoumarin glycoside.

Key words: Biotransformation, 4-phenylcoumarin, *Polygonum multiflorum*, transgenic hairy roots.

INTRODUCTION

Biotransformation, also called bioconversion or biocatalysis, is an enzymatic reaction catalyzed by plant cells, microorganisms and purified enzymes. The biochemical reactions occurring in plant cells have certain advantages over organic synthesis in terms of high stereo- and regio-selectivity, fewer side reactions, mild reaction conditions, simpler operation procedures, easier separation of products, lower cost and environmentally friendly properties (Rozzell, 1999; Liu and Yu, 2010). The reactions involved in the biotransformation of organic compounds by plant cells include oxidation, reduction, hydroxylation, esterification, methylation, isomerization, acetylation, and glycosylation (Liu and Yu, 2010).

Coumarins, a class of naturally occurring compounds, are present either in their free state or as glycosides. Coumarin derivatives exhibit a broad range of pharmacological properties and biological activities, including anticoagulant (Omaima et al., 2010), antibiotic (Musiciki et al., 2000), anticancer (Koneni et al., 2010), anti-HIV (Kostova et al., 2006), anti-inflammatory and antioxidant activities (Georgia et al., 2009). However, the pharmaceutical applications of most coumarins are limited by their low water solubility. Their glycosides, on the other hand, are more soluble in water and therefore are likely to be safer as ways of drug-administration and could play essential roles in pharmaceutical utilization of coumarins. As a result, the preparation of coumarin glycoside has been greatly of research interest.

4-Phenylcoumarins are known as antivirus agents (Queiroz, 1997). Glycosylation of 4-phenylcoumarins was investigated in different ways of chemosynthesis for many years (Garazd et al., 2005; Semeniuchenko et al., 2009). However, applications of chemosynthesis methods were limited by their low yield and regioselectivity. Glycosylation using plant-cultured cells is useful for preparing water-soluble and stable glycosides from water-insoluble and unstable compounds (Shimoda et al., 2010). Work on the 4-phenylcoumarin biotransformation using plant cell has not been reported up to now. In this study, biotransformation of 4-phenylcoumarin by transgenic hairy roots of *Polygonum multiflorum* was preformed to obtain 4-phenylcoumarin glycosides. The relationship of co-culture time to conversion rate was detected by high performance liquid chromatography (HPLC). Time course experiment also helped to

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determine the optimal co-culture time.

MATERIALS AND METHODS

Substrate synthesis

Substrates 1 and 2 were chemosynthesized in our laboratory. The ethyl benzoylacetate and resorcinol upon Pechmann reaction yielded compound 1. The ethyl benzoylacetate and phloroglucinol upon Pechmann reaction obtained compound 2 (Daniel et al., 2008). Structures of the two compounds were confirmed by ^{1}H-NMR and characterized by thin layer chromatography (TLC).

Cultivation of transgenic hairy roots of *P. multiflorum*

The transgenic hairy roots of *P. multiflorum* were induced in our laboratory (Wang et al., 2002). Cultivated hairy roots of *P. multiflorum* were sub-cultured at 14 days intervals on solid Murashige and Skoog (MS) medium (100 ml in a 250 ml conical flask) containing 3% sucrose and 1% agar (adjusted the pH to 5.75) at 25°C in the dark. A suspension culture was started by transferring the cultured hairy roots to 200 ml of liquid medium in 500 ml conical flask, and then incubated on rotary shaker (110 rpm) at 25°C in the dark.

Methods of biotransformation

The hairy roots were transplanted to freshly prepared liquid MS medium (200 ml in a 500 ml conical flask) and grown with continuously shaking for 10 days on a rotary shaker (110 rpm). After cultivation for 10 days, 0.5 ml of 1 and 2 solution (60 mg/ml, DMSO as solvent) was added to the system, respectively, and co-cultivated for 7 days. At the same time, the control group was added with 0.5 ml of DMSO.

Detection of biotransformation products

After 7 days of co-culture period, the medium was filtered under vacuum. The filtrate was concentrated by evaporation *in vacuo*. Then the medium was extracted 3 times by EtOAc, and extracted another 3 times by *n*-BuOH. The hairy roots were dried at 55°C, soaked in MeOH for 24 h and extracted by ultrasonic method for 30 min. After vacuum filtration, the filtrate was condensed to dryness, followed by addition of water for re-suspension. The suspension was extracted by EtOAc and *n*-BuOH as the previous procedure. For the control group, the parallel procedures were employed. The substrates and samples of control as well as experimental groups were detected by TLC simultaneously. The chloroform-methanol-formic acid system was selected as developing agent; meanwhile the 10% sulfuric acid alcohol solution was used as color reagent. Each of the MeOH, EtOAc and *n*-BuOH fractions was detected by the HPLC. The chromatography conditions of HPLC were as follows: column temperature: 35°C; mobile phase: methanol-water (65:35, v/v); flow rate: 0.75 ml/min; detection wavelength: 332 nm; sample loading: 10 µl.

Separation and purification of biotransformed products 3 and 4

The EtOAc-extracts were developed on silica gel column (200-300 mesh) using petroleum ether-ether acetate (1:1), ether acetate and ether acetate-methanol (9:1) as eluents to give biotransformed products 3 as white needle crystals and 4 as white massive crystals in eluent of ether acetate-methanol (9:1).

Structural elucidation of the biotransformed products

Structures of the biotransformed products were elucidated on the basis of their ESI-MS, ^{1}H NMR, ^{13}C NMR and HMBC spectra. The chemical shifts were expressed in δ (ppm), referring to TMS. The spectral data of the new compound (4) was as follows: ESI-MS m/z: 417.5 $[M+H]^+$, 255.0 $[M-162-H]^-$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 5.82 (1H, *s*, H-3), 6.44 (1H, *d*, *J* = 12.4 Hz, H-6), 10.70 (1H, *s*, 7-OH), 6.47 (1H, *d*, *J* = 17.7, H-8), 7.33-7.38 (5H, *m*, H-2', 3', 4', 5',6'), 4.72 (1H, *d*, *J* = 7.6 Hz, 1''-H), 4.94 (1H, *d*, *J* = 5.0 Hz, sugar-OH), 4.98 (1H, *d*, *J* = 4.2 Hz, sugar-OH), 4.54 (1H, *t*, *J* = 5.0 Hz, sugar-OH), 2.93-3.87 (sugar protons).
^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 160.02 (C-2), 111.77 (C-3), 155.95 (C-4), 156.20 (C-5), 99.06 (C-6), 162.10 (C-7), 97.07 (C-8), 156.59 (C-9), 102.54 (C-10), 139.67 (C-1'), 127.61 (C-2', 6'), 127.86 (C-4'), 128.10 (C-3', 5'), 99.81 (C-1''), 72.93 (C-2''), 76.74 (C-3''), 69.53 (C-4''), 77.18 (C-5''), 60.82 (C-6'').

Time course of biotransformation

The transgenic hairy roots (90 g) of *P. multiflorum* were partitioned to 18 flasks containing 200 ml of liquid MS medium. Substrate (10 mg) was added to each of the flasks, under the same culture conditions as described above. Three flasks for one time were processed at 1, 2, 3, 4, 5 and 6 days, respectively. The medium was collected after filtration. The cultures, dried at 55°C, were weighed and powderized. The powders (10 mg) were extracted in 5 ml of MeOH. All of the extracts were detected by HPLC.

RESULTS AND DISCUSSION

As shown in Figures 1 and 2, there is a new chromatographic peak in the MeOH extract of experimental cultures. According to the results of TLC and HPLC, the biotransformation products of 1 and 2 was detected. After incubation for 7 days, the biotransformation products were isolated andidentified as 4-phenylcoumarin-7-*O*-*β*-D-glucopyranoside (3) and 7-hydroxy-4-phenylcoumarin-5-*O*-*β*-D-glucopyranoside (4), and no additional transformation products were observed under HPLC analysis.

The molecular formula of 4 was established as $C_{21}H_{20}O_9$ based on its ESI-MS spectrum, which included a molecular ion, peak at m/z: 417.5 (calcd. 417.5 for $C_{21}H_{20}O_9H$). The results of NMR spectrum demonstrated that the sugar was D-glucopyranose. Its ^{1}H-NMR spectrum showed anomeric proton signals at δ 4.72 (1H, *d*, *J* = 7.6 Hz); the sugar component was indicated to be *β*-D-glucopyranose, according to the chemical shift of the sugar hydrogen resonances (Shimoda et al., 2010). In the HMBC spectrum, signals at δ 6.44 (1H, *s*, H-6) and 6.47 (1H, *s*, H-8) correlated to carbon resonance at δ 162.10 (C-7). In the ^{1}H-NMR signal, compared with 2, a phenolic hydroxyl signal of 4 disappeared. The anomeric proton signal at δ 4.72 (1''-H) correlated to the carbon resonance at 156.20 (C-5). These findings confirmed that

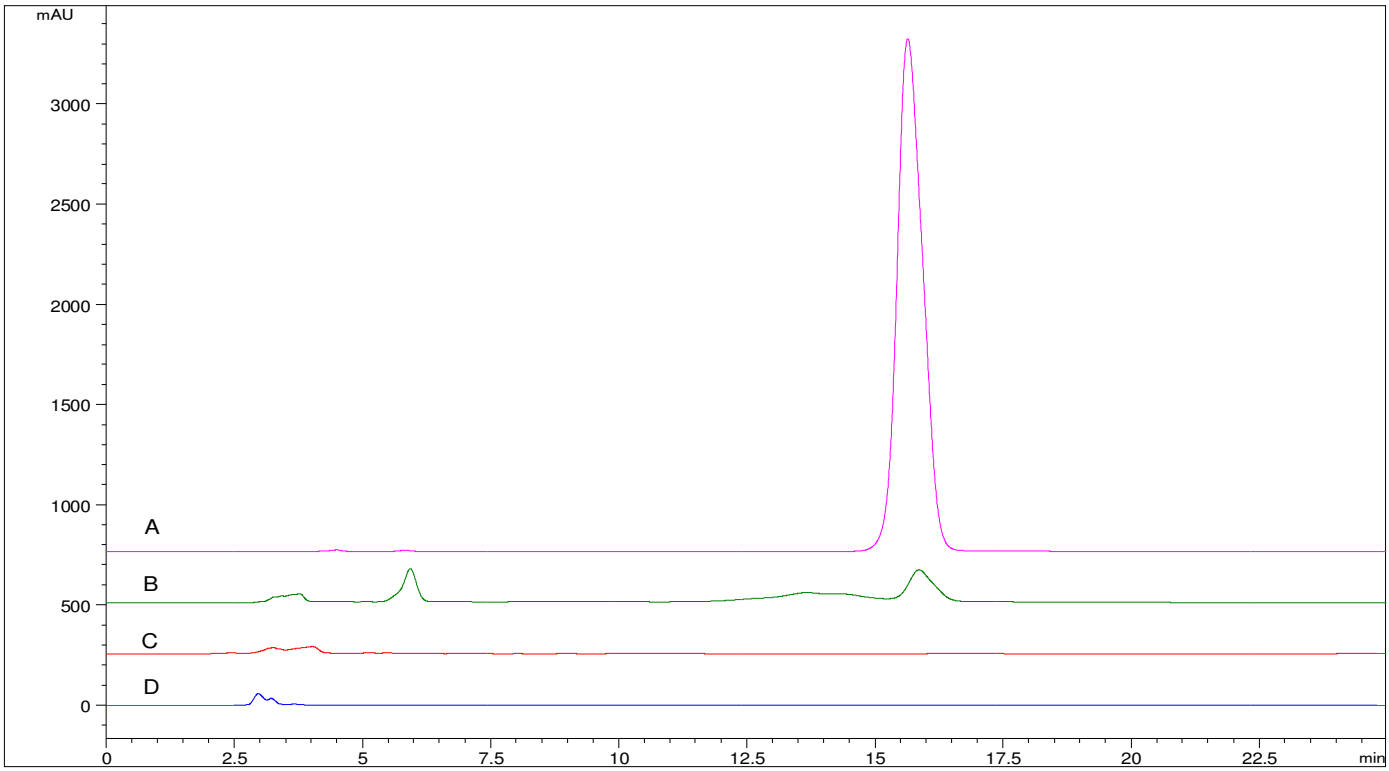

Figure 1. HPLC of the biotransformation of **1** by transgenic hairy roots of *P. multiflorum* (A) 7-hydroxy-4-phenylcoumarin, (B) MeOH extract of experimental cultures, (C) MeOH extract of control cultures, (D) control medium; a: the new chromatographic peak.

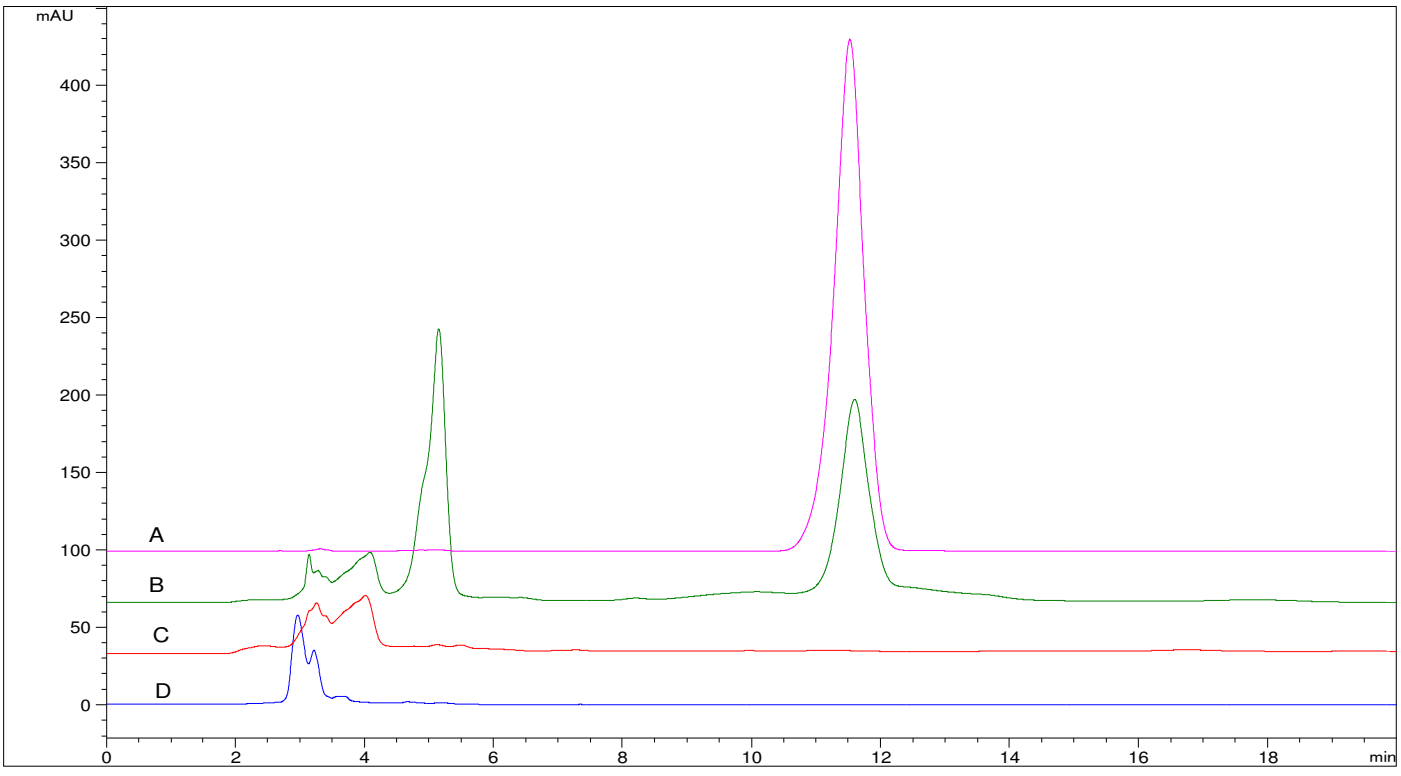

Figure 2. HPLC of the biotransformation of **2** by hairy roots of *P. multiflorum* (A) 5,7-dihydroxy-4-phenylcoumarin, (B) MeOH extract of experimental cultures, (C) MeOH extract of control cultures, (D) control medium; b: the new chromatographic peak.

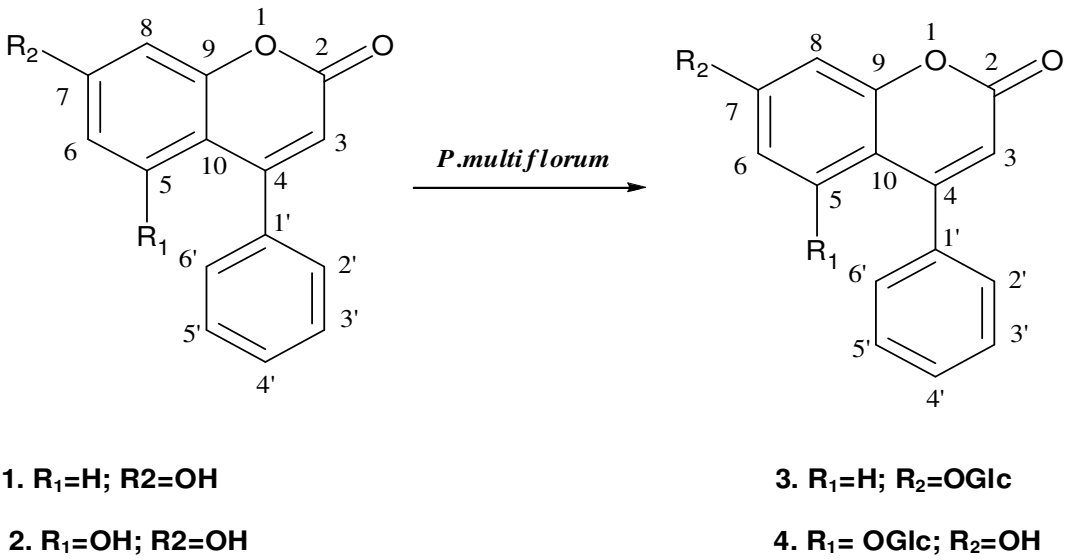

Figure 3. Biotransformation pathway of substrates **1** and **2** by transgenic hairy roots of *P. multiflorum*.

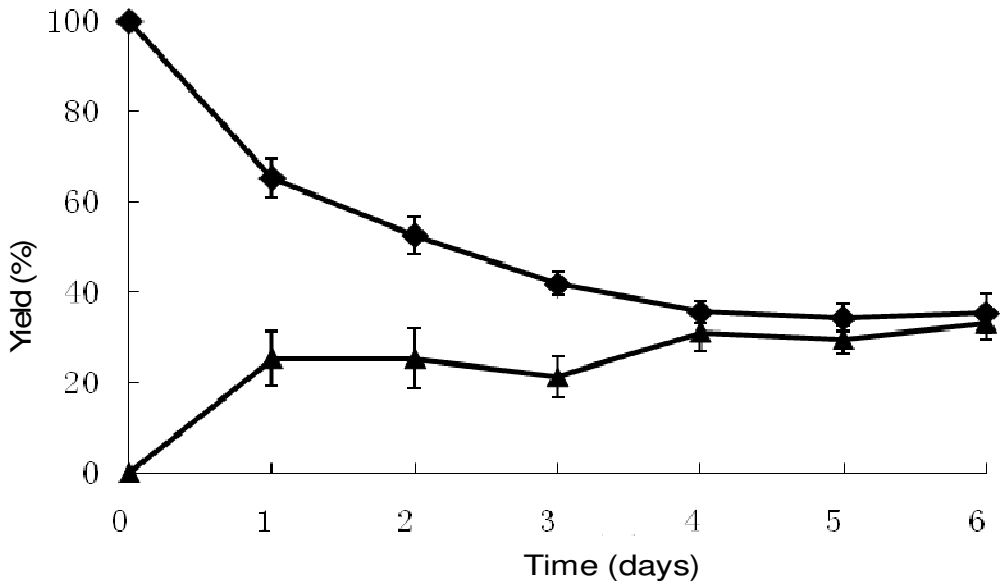

Figure 4. Time-course of biotransformation of substrate **1** by transgenic hairy roots of *P. multiflorum* yields of **1** (■), **3** (▲) are plotted. Yield is expressed as relative percentage of products to the total amount of substrates. The points are the means of three determinations. Bars represent mean standard deviation.

the glucopyranosyl residue was attached to the phenolic hydroxyl group at C-5 position of 2. Thus, biosynthesis product 4 was identified as 7-hydroxy-4- phenylcoumarin-5-*O*-*β*-D-glucopyranoside. This is a new compound. The biotransformation pathways of 1 and 2 were shown in Figure 3.

In order to determine the ability of transgenic hairy roots of *P. multiflorum* to biotransform 1 and 2, the time courses of the conversion of 1 and 2 were followed. As Figures 4 and 5 indicated, the date for optimal yields of biotransformation products 3 and 4 were stable at 4 and 2 days, respectively.

In conclusion, we developed a new method for preparation of 4-phenylcoumarin glucoside by transgenic hairy roots of *P. multiflorum*. This method was capable of regioselectively glycosylating the phenolic hydroxyl group (Yu et al., 2008), and the reaction was found to be unaffected by the steric hindrance of the phenyl group at

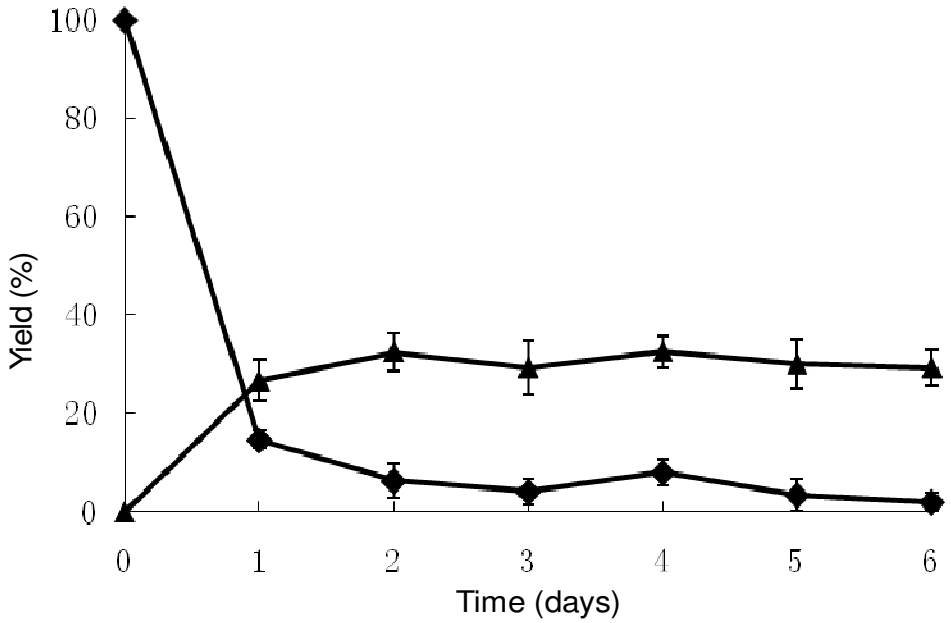

Figure 5. Time-course of biotransformation of substrate **2** by transgenic hairy roots of *P. multiflorum* yields of **2** (■), **4** (▲) are plotted. Yield is expressed as relative percentage of products to the total amount of substrates. The points are the means of three determinations. Bars represent mean standard deviation.

C-4 position. The time course experiment indicated that the optimal time for biotransformation of 1 and 2 was 4 and 2 days, respectively. The yields of biotransformation products 3 and 4 were 31.16 and 32.42%, respectively. Studies on the physiological activities of 4-phenylcoumarin glycosides are now in progress.

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REFERENCES

Daniel OB, Diego MR, Gustavo PR, Graciela TB, Horacia JT, Juan CA (2008). Efficient microwave solvent-free synthesis of flavones, chromones, coumarins and dihydrocoumarins. Lett. Org. Chem., 5: 607-615.

Garazd YL, Garazd MM, Khilya VP (2005). Modified coumarins. 19. synthesis of neoflavone D-glycopyranosides. Chem. Nat. Comp., 41(6): 663-668.

Georgia M, Antreas A, Olga IM, Anastasia D, Maria K, Christos K, Dimitra JHL (2009). Synthesis and evaluation of the antioxidant and anti-inflammatory activity of novel coumarin-3-aminoamides and their alpha-lipoic acid adducts. Eur. J. Med. Chem., 44: 3020-3026.

Kostova I, Raleva S, Genova P, Argirova R (2006). Structure-activity relationships of synthetic coumarins as HIV-1 inhibitors. Bioinorg. Chem. Appl., pp. 1-9.

Koneni VS, Abdhesh K, Manoj K, Jayanta S, Sinha S (2010). Synthesis and *in vitro* evaluation of novel coumarin–chalcone hybrids as potential anticancer agents. Bioorg. Med. Chem. Lett., 20: 7205-7211.

Liu JH, Yu BY (2010). Biotransformation of bioactive natural products for pharmaceutical lead compounds. Curr. Org. Chem., 14: 1400-1406.

Musiciki B, Periers AM, Laurin P, Ferroud D, Benedetti Y (2000). Improved antibacterial activities of coumarin antibiotics bearing 5', 5'-dialkylnoviose: biological activity of RU79115. Bioorg. Med. Chem. Lett., 10: 1695-1699.

Omaima MA, Kamelia MA, Rasha ZB, Timothyl JM, Somaia AN, Shalini S (2010). Synthesis, anticoagulant and PIVKA-II induced by new 4-hydroxycoumarin derivatives. Bioorg. Med. Chem., 18: 3371-3378.

Queiroz MD (1997). Pharmaceutical compositions comprising a 4-arylcoumarin for the treatment of viral infections. WO9825608.

Rozzell JD (1999). Commercial scale biocatalysis: myths and realities. Bioorg. Med. Chem., 7: 2253-2261.

Semeniuchenko V, Garazd Y, Garazd M, Shokol T, Groth U, Khilya (2009). Highly efficient glucosylation of flavonoids. Monatsh. Chem., 140: 1503-1512.

Shimoda K, Naoji K, Koji T, Sato D, Nakajima N, Hamada H, Hamada H (2010). Biotransformation of naringin and naringenin by cultured *Eucalyptus perriniana* cells. Phytochem., 71: 201-205.

Wang L, Yu RM, Zhang H, Cheng KD (2002). Hairy-roots culture of *Polygonum multiflorum* Thunb and the production of its active constituents-anthraquinones. Chin. J. Biotechnol., 18: 69-72.

Yu RM, Zhou LB, Yan CY, Duan GY, Zhao Y (2008). Two new coumarin glucosides biosynthesized by transgenic hairy roots of *Polygonum multiflorum*. Chin. Chem. Lett., 19: 76-78.