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Original Paper

Metabolism of administered (2*RS*)-naringenin in flavonoid-producing cultured cells of *Sophora flavescens*

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Abstract Cultured cells of *Sophora flavescens* produce (2*S*)-naringenin-derived prenylated flavanone sophoraflavanone G and liquiritigenin-derived trifolirhizin 6′-*O*-malonate. The regulation of flavonoid biosynthesis was examined by analyzing the metabolites produced in the cultured cells fed (2*RS*)-naringenin. The amount of sophoraflavanone G in cells fed 0.1 or 0.3 mM (2*RS*)-naringenin was two-fold that in control cells, although the conversion ratio was only 5 to 10% of the administered (2*S*)-naringenin. On the other hand, (2*R*)-naringenin, which does not occur naturally, was efficiently converted into its 4′,7-di-*O*-*β*-D-glucoside. (2*S*)-Naringenin prenylation activity was higher at the logarithmic growth stage. The cells fed (2*RS*)-naringenin at a lower concentration (below 0.1 mM), accumulated sophoraflavanone G as the main prenylated flavanone. In contrast, cells fed 0.3 mM (2*RS*)-naringenin accumulated 8-prenylnaringenin and leachianone G, intermediates of sophoraflavanone G in large amounts. Accumulation of trifolirhizin 6′-*O*-malonate was suppressed by the addition of naringenin.

Key words: Naringenin; (2*R*)-naringenin 4′,7-di-O-*β*-D-glucoside; *Sophora flavescens*; sophoraflavanone G.

Sophora flavescens, a leguminous plant, produces diverse 8-lavandulylated flavanones, such as sophoraflavanone G (SFG), kurarinone and kushenol I (Hatayama and Komatsu 1971; Wu et al. 1985; Kuroyanagi et al. 1999; Kang et al. 2000), which are exclusively accumulated in the cork layer of intact roots (Yamamoto et al. 1992). Recent pharmaceutical studies showed that the lavandulyl side chain was essential for the antitumor activity and phospholipase-Cγ1-inhibition activity of flavonoids isolated from this plant, and that SFG had the most potent activities (Lee et al. 1997; Ko et al. 2000). Nevertheless, neither biosynthetic enzymes nor the regulatory mechanisms of these lavandulylated flavanones has been clarified.

To elucidate the regulatory mechanism for the biosynthesis of lavandulylated flavanones, we have established cell cultures of this plant that are capable of producing SFG as well as a pterocarpan glucoside, trifolirhizin 6′-*O*-malonate (TM), as major secondary metabolites (Figure 1) (Yamamoto et al. 1991a, 1991b). Using these cell cultures, we have demonstrated that the lavandulyl group of SFG was not directly transferred to the flavanone skeleton, but was biosynthesized by a discontinuous two-step dimethylallylation. That is, naringenin was first dimethylallylated to afford 8-prenylnaringenin (8PN) by plastid-located naringenin 8-dimethylallyltransferase, a stereospecific enzyme for (−)-(2*S*)-naringenin (Yamamoto et al. 2000), then hydroxylated to leachianone G (LG) by endoplasmic reticulum-associated cytochrome P450 mono-oxygenase, 8-prenylnaringenin 2′-hydroxylase (Yamamoto et al. 2001a). Finally, another dimethylallyl moiety was transferred to the 2″ position of LG by leachianone G 2″-dimethylallyltransferase exist in the plastid to form SFG (Zhao et al. 2003a).

For the efficient production of SFG, we also showed that some elicitors such as methyl jasmonate and yeast extract stimulated the production of SFG in cultured cells. Moreover, we found that the addition of commercially available cork tissue increased the production of SFG by about three- to five-fold compared to that of cells cultured alone, without affecting the cell growth (Yamamoto et al. 1996; Zhao et al. 2003b). Interestingly, these additives had different effects; methyl jasmonate stimulated, yeast extract inhibited, and cork tissue scarcely affected TM production. These different responses suggested that in *S. flavescens* cell cultures, biosyntheses of SFG and TM are regulated in a different manner.

In the present study, we added (2*RS*)-naringenin, the

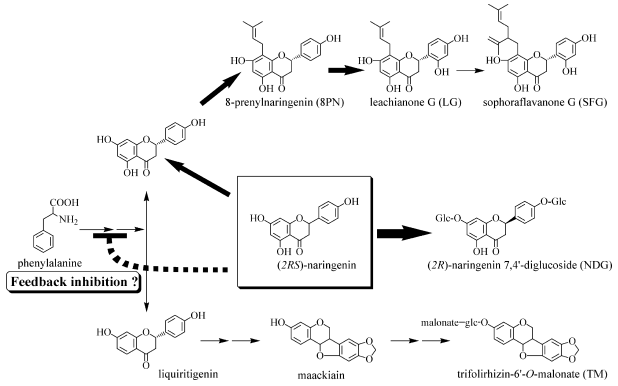

Figure 1. Metabolism of administered (2*RS*)-naringenin in *Sophora flavescens* cultured cells.

precursor of SFG but not TM, to the culture medium, and examined its effect on the production of these secondary metabolites.

Materials and methods

General

(2*RS*)-Naringenin was purchased from Nacalai Tesque (Kyoto, Japan). HPLC-photodiode array analysis was carried out using a Waters Alliance PDA system (Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 with spectrometers (Varian Unity plus 500 and Varian Gemini 300) operating at 500 MHz and 300 MHz for ^{1}H, and 125 MHz for ^{13}C, respectively. Mass spectra were recorded on a JEOL JMS DX-303 spectrometer. UV and CD spectra were measured with a UV-1600 visible spectrophotometer and a J-725N spectrometer, respectively.

Plant materials and culture methods

The origin and subculturing of callus cultures (Yamamoto et al. 1991a) and the establishment of cell suspension cultures (Yamamoto et al. 1996) of *S. flavescens* were described previously. Cell suspension cultures (0.5 g) were subcultured in 100 ml-flasks containing 20 ml of Murashige and Skoog (1962) medium with 1 μM 2,4-D and 1 μM kinetin at 14 days interval on a rotary shaker at a speed of 100 rpm in the dark at 25°C. For the experiment, filter-sterilized 300 mM (2*RS*)-naringenin solution in 20 μl of DMSO was aseptically added to the flask (final concentration 0.3 mM), and cultured for another one day. Twenty μl of DMSO was added to the control cells.

Isolation and identification of the metabolites

The naringenin-fed cells collected from 60 flasks (120 g) were extracted three times with MeOH by ultrasonication in ice-water bath for 30 min. The MeOH extract after concentration was resuspended in H_2O, partitioned with $CHCl_3$ and *n*-BuOH, successively. The residual H_2O layer was passed through Diaion HP-20 column (Mitsubishi Chem, Tokyo, Japan), washed with H_2O, and eluted with MeOH. The *n*-BuOH fraction (0.70 g) and HP-20 eluate (0.24 g) were combined, and chromatographed on Toyopearl HW-40C (Tosoh, Tokyo, Japan) using MeOH as a solvent. Naringenin glycoside containing fraction was further purified by prep. HPLC to afford 20 mg of compound 1. Preparative HPLC was performed at 40°C, with $CH_3OH/H_2O/HCOOH$ (25/75/0.1) as a solvent at a flow rate of 5 ml/min, whilst monitoring the absorbance at 294 nm.

Ten mg of compound 1 dissolved in 50 mM sodium citrate buffer (pH 5.0, 100 ml) was partially hydrolyzed by incubating with 1 mg Cellulase Onozuka RS (Yakult, Pharmaceutical, Tokyo, Japan) for 75 min at 30°C. After terminating the reaction by the addition of 1 M HCl (10 ml), the solution was applied to Diaion HP-20, eluted with MeOH, concentrated, and further purified by prep. HPLC using $CH_3OH/H_2O/HCOOH$ (30/70/0.1) as a solvent to obtain compounds 2 (2.7 mg) and 2′ (2.3 mg).

Compound 1: (2*R*)-naringenin 4′,7-di-*O*-β-D-glucopyranoside

UV λ_{max}^{MeOH} nm (log ε): 284 (4.21), 330 (3.48); CD (MeOH) nm ($\Delta\varepsilon$): 284 (+7.08), 333 (−2.05), [α]D: −35.7° (c=0.025, MeOH); Positive FABMS (*m*/*z*): 619 ([M+Na]$^+$), 435 ([M+H−Glc]$^+$), 273 ([M+H−Glc−Glc]$^+$); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.44 (2H, *d*, *J*=8.4 Hz, H-2′, 6′), 7.06 (2H, *d*, *J*=8.4 Hz, H-3′, 5′), 6.15 (1H, *broad s*, H-8), 6.12 (1H, *broad s*, H-6), 5.56 (1H, *broad d*, *J*=12.5 Hz, H-2), 4.97 (1H, *d*, *J*=7.5 Hz, 7-glc H-1), 4.89 (1H, *d*, *J*=7.5 Hz, 4′-glc H-1), 3.76−3.21 (*m*, 7-glc H-2-6, 4′-glc H-2-6, and H-3*ax*), 2.85 (1H, *dd*, *J*=17.5, 3.0 Hz, H-3*eq*); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 196.8 (C-4), 165.3 (C-7), 165.2 (C-5), 162.6 (C-9), 157.6 (C-4′), 131.7 (C-1′), 128.1 (C-2′, 6′), 116.2 (C-3′, 5′), 103.3 (C-10), 100.3 (4′-glc C-1), 99.6 (7-glc C-1), 96.6 (C-6), 95.4 (C-8), 78.3 (C-2), 77.0 (4′-glc C-3, 7-glc C-3), 76.6 (7-glc C-5), 76.3 (4′-glc C-5), 73.2 (7-glc C-2), 73.0 (4′-glc C-2), 69.7 (4′-glc C-4), 69.5 (7-glc C-4), 60.7 (4′-glc C-6), 60.5 (7-glc C-6), 42.1 (C-3).

Compound 2: naringenin 4′-O-β-D-glucopyranoside

Positive FABMS (*m*/*z*): 457 ([M+Na]$^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.43 (2H, *d*, *J*=8.0 Hz, H-2′, 6′), 7.15 (2H, *d*, *J*=8.0 Hz, H-3′, 5′), 5.91 (1H, *s*, H-8), 5.89 (1H, *s*, H-6), 5.42 (1H, *broad d*, *J*=11.5 Hz, H-2), 4.90 (1H, *d*, *J*=7.5, Hz 4′-glc H-1), 3.90 (1H, *broad d*, *J*=11.3 Hz, 4′-glc H-6a), 3.70 (1H, *m*, 4′-glc H-6b), 2.80 (1H, *broad d*, *J*=17.0 Hz, H-3*eq*).

Compound 2′: naringenin 7-*O*-β-D-glucopyranoside

Positive FABMS (*m*/*z*): 457 ([M+Na]$^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.33 (2H, *d*, *J*=8.0 Hz, H-2′, 6′), 6.82 (2H, *d*, *J*=8.0 Hz, H-3′, 5′), 6.21 (1H, *s*, H-8), 6.19 (1H, *s*, H-6), 5.39 (1H, *br d*, *J*=12.4 Hz, H-2), 4.97 (1H, *d*, *J*=7.5, Hz 7-glc H-1), 3.88 (1H, *br d*,

J=12.4 Hz, 7-glc H-6a), 3.67 (1H, *m*, 7-glc H-6b), 2.75 (1H, *br d*, J=17.0 Hz, H-3*eq*).

Quantitative analysis of flavonoids

One g of harvested cells was extracted with MeOH (3 ml) for 30 min by ultrasonication at room temperature. As an internal standard, 0.15 mg 1-naphthalene acetic acid dissolved in MeOH (0.3 ml) was added to each extract. After centrifugation, each supernatant was subjected to reversed phase HPLC; column: Shodex silica 18M4E (5 μm, 4.6 i.d.×250 mm, Showa Denko, Tokyo, Japan), solvent: 1% AcOH containing an MeOH-H_2O gradient of 25–100% MeOH in 75 min, flow rate: 0.9 ml/min, oven temp: 40°C. The quantities of all flavonoids were calculated from the peak area at 294 nm recorded using Chromatopac C-R4A (Shimadzu, Kyoto, Japan).

Results and discussion

Identification of the compounds accumulated by the administration of (2RS)-naringenin

HPLC photodiode array analysis revealed accumulation of many hydrophilic compounds as well as two lipophilic compounds, besides the increase of the peak area of SFG, in 6-day old *S. flavescens* cells fed 0.3 mM (2*RS*)-naringenin and incubated for another 1 day (Figure 2). Among the compounds detected, UV spectra of hydrophilic compounds 1 and 2 resembled that of naringenin. Compound 1 was isolated from 120 g of cells treated with 360 μmol (2*RS*)-naringenin by Toyopearl HW-40 column chromatography and prep. HPLC as described in Materials and methods. Complete hydrolysis of compound 1 by β-glucosidase afforded naringenin as the aglycone, indicating that compound 1 was the β-glucoside of naringenin. Positive FABMS, ^{1}H and ^{13}C NMR spectra as well as UV spectrum of compound 1 indicated that this compound was di-*O*-β-D-glucopyranoside of naringenin. The two glucosylation sites were confirmed as 7-OH and 4′-OH by the ^{1}H-Detected Heteronuclear Multiple Bond Connectivity experiment, in which correlations of one anomeric proton (δ_H4.97) with C-7 (δ165.3) and another (δ_H4.89) with C-4′ (δ157.6) were observed. The absolute configuration at C-2 was, interestingly, assigned as 2*R* by the observation of a positive Cotton effect at 284 nm. Thus, the structure of compound 1 was characterized as (2*R*)-naringenin 4′,7-di-*O*-β-D-glucopyranoside (NDG). It was noteworthy that compound 1 was easily racemized when kept in DMSO, and in repeated ^{1}H NMR analyses, the doublet signal assigned to the anomeric proton of the glucose bound to 7-OH (δ_H4.98 and 4.97, J=7.5 Hz) was changed to the broad triplet (δ_H4.98, 4.96 and 4.93, J=7.5 Hz) presumably owing to the racemization at C-2. The trace signal at δ_H4.93 was also observed in the first

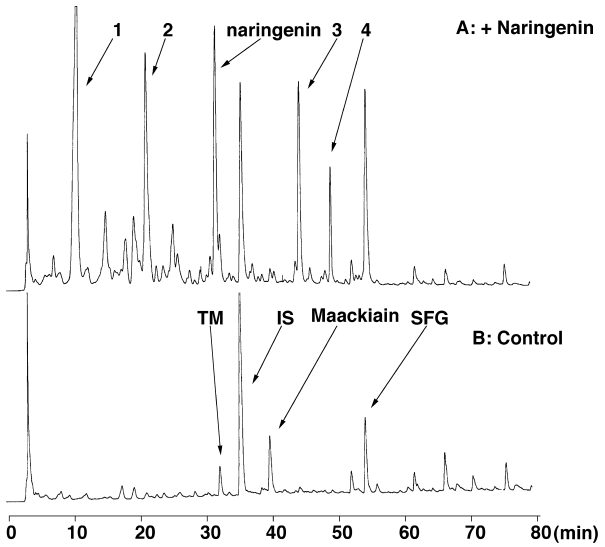

Figure 2. HPLC chromatograms of MeOH extract of *S. flavescens* cells administrated 0.3 mM (2*RS*)-naringenin (A) and control cells (B) monitored at 294 nm. (2*RS*)-Naringenin was fed to 6-day cultured cells, and then incubated for one day. Abbreviations: IS; internal standard, SFG; sophoraflavanone G, TM; trifolirhizin 6′-*O*-malonate.

^{1}H NMR analysis, suggesting that compound 1 isolated in the present study contained a small amount of (2*S*)-derivative, although its optical purity was not determined.

Partial hydrolysis of NDG by β-glucosidase treatment suggested that compound 2 was the monoglucoside of naringenin, and it was finally confirmed as naringenin 4′-*O*-β-D-glucopyranoside by the comparison of ^{1}H NMR and FABMS spectral data of the partial hydrolysate with the authentic data (Esaki et al. 1994). In the β-glucosidase treatment, the formation of naringenin 7-*O*-β-D-glucopyranoside (Saito et al. 1994) was also detected.

Lipophilic compounds 3 and 4 were identified as 8PN and LG respectively, by direct comparisons of their retention times and UV spectra with those of authentic samples by HPLC-photodiode array analyses. These prenylated flavanones, both of which are intermediates of SFG, were hardly detectable in a normal culture condition. Some of the other hydrophilic compounds (observed at retention times between 15 to 30 min) showed genistein-like UV spectra, although they were not identified in the present study.

Diglucosylation of (2R)-naringenin

Six day-cultured *S. flavescens* cells efficiently glucosylated (2*R*)-naringenin; when 0.3 mM of (2*RS*)-naringenin was fed, almost 60% of (2*R*)-naringenin was converted to NDG (Figure 3). At a higher concentration of (2*RS*)-naringenin (1 mM), formation of NDG could not be observed owing to cell death. This glucosylation ability was almost unchanged during the entire culture period (data not shown).

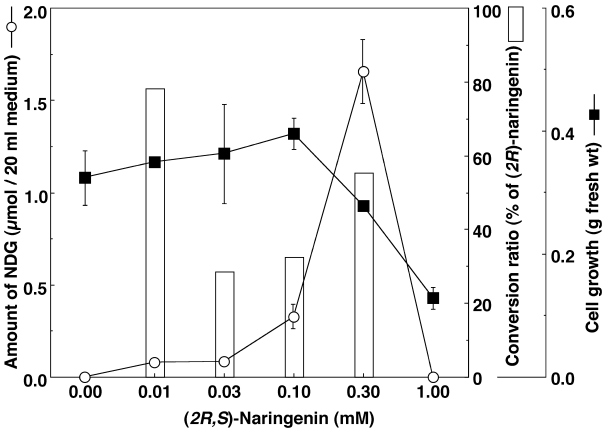

Figure 3. Formation of naringenin diglucoside by the administration of (2*RS*)-naringenin to 6-day cultured *S. flavescens* cells. Values are the means of triplicate determinations; error bars are SEs.

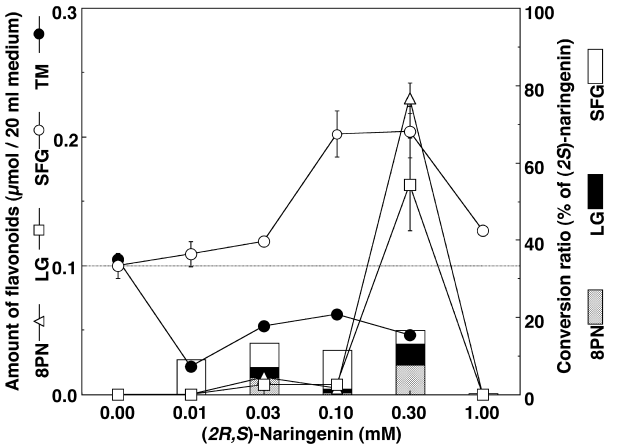

Figure 4. Amounts of flavonoids in (2*RS*)-naringenin-fed cells of *S. flavescens*. (2*RS*)-Naringenin was fed to 6-day cultured cells and incubated for one day. Values are the means of triplicate determinations; error bars are SEs. The dashed line indicates the amount of SFG in control cells. Conversion ratio was calculated as follows; {(amount of the compound in naringenin administered cells)−(amount of the compound in control cells)}/(amount of administered (2*S*)-naringenin)×100 (%). The amount of TM in 1 mM naringenin-fed cells could not be determined, because the peak of TM was overlapped with naringenin in the present assay condition.

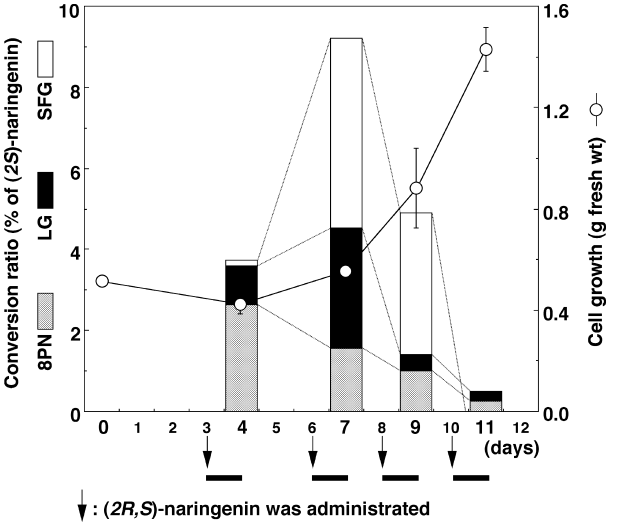

Figure 5. Relationship between cell growth and the conversion of (2*S*)-naringenin into prenylated flavanones in *S. flavescens* cultured cells. 0.3 mM (2*RS*)-naringenin was fed to the cells at days 3, 6, 8 and 10, and incubated for one day. Values are the means of triplicate determinations; error bars are SEs.

Prenylation of (2S)-naringenin

Compared to glucosylation of (2*R*)-naringenin, conversion of (2*S*)-naringenin into prenylated flavanone SFG and its prenylated precursors LG and 8PN was rather low in 6 day-cultured *S. flavescens* cells (Figure 4). The amount of SFG in cells fed 0.1–0.3 mM (2*RS*)-naringenin was about two-fold that in the control cells, but the conversion ratio of (2*S*)-naringenin into SFG was only 5 to 10%. In cells fed (2*RS*)-naringenin at a lower concentration (below 0.1 mM), SFG was the most accumulated of the three prenylated flavanones. On the other hand, in cells fed 0.3 mM (2*RS*)-naringenin notable accumulation of two intermediates 8PN and LG was observed, although the content of SFG was almost the same as that in cells fed 0.1 mM (2*RS*)-naringenin-fed cells. These results suggest that the second prenylation step catalyzed by leachianone G 2″-dimethylallyltransferase (Zhao et al. 2003a) is one of the regulatory steps in SFG biosynthesis. The amount of TM, derived from 5-deoxyflavanone liquiritigenin but not from 5-hydroxylated naringenin, decreased by the addition of (2*RS*)-naringenin. Probably the excessive (2*S*)-naringenin suppressed the supply of the common precursor of SFG and TM such as phenylalanine by the feedback inhibition mechanism.

Figure 5 shows the relationship between the cell growth and the (2*S*)-naringenin prenylation ability in *S. flavescens* cultured cells. Obviously, prenylation ability was the highest at the logarithmic growth stage (day 6). At day 8, conversion of (2*S*)-naringenin into SFG was still observed, whereas the amount of intermediates was only one third of that at day 6. At the late straight growth stage (day 10), the cells scarcely prenylated (2*S*)-naringenin.

Many attempts have been made to increase the amount of useful secondary metabolites in plant cells by supplying their precursors or intermediates (Ibrahim 1987). Construction of a new “shunt” or “bypass” to supply more precursors by genetic engineering techniques have also been examined. In most cases, however, the increase of the final products remained marginal level (Sommer et al. 1999; Boehm et al. 2000). Similarly, only 10–20% of (2*S*)-naringenin fed to the *S. flavescens* cells in culture was converted into SFG presumably owing to the feedback inhibition induced by (2*S*)-naringenin and the existence of the alternative pathway to convert (2*S*)-naringenin into unidentified compounds (Figure 1). Nevertheless, the fact that the

SFG content in (2*RS*)-naringenin-fed cells was twice that in control cells, suggests that *S. flavescens* cells still have the latent ability to biosynthesize more SFG. The present findings also suggested that the leachianone 2″-dimethylallyltransferase-catalyzed second prenylation is one of the regulatory steps in SFG biosynthesis. Moreover, the timing of the administration of precursor / intermediates was also important for the efficient conversion. Further examinations concerning regulatory mechanisms of the supply and the metabolism of the intermediates as well as the transport and accumulation of SFG are necessary.

It is unlikely that (2*R*)-naringenin-specific glucosyltransferase(s) exists in *S. flavescens* cells, because (2*R*)-naringenin does not occur in the cells, and naringenin glucosides have been detected in neither cultured cells nor intact plants of *S. flavescens*. In preliminary experiments using crude cell-free extracts from *S. flavescens* cultured cells, the glucosyltransferase activity against (2*RS*)-naringenin was much lower than that against maackiain, suggesting that naringenin glucosylation takes place very slowly in *S. flavescens* cells. Presumably in the cells, (2*S*)-naringenin was rapidly transformed to prenylated flavanones and other unidentified metabolites by the "normal" pathway, and the remaining (2*R*)-derivative was glucosylated as the xenobiotic.

Acknowledgements

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References

Boehm R, Sommer S, Li S-M, Heide L (2000) Genetic engineering on shikonin biosynthesis: expression of the bacterial *ubiA* gene in *Lithospermum erythrorhizon*. *Plant Cell Physiol* 41: 911–919

Esaki S, Nishiyama K, Sugiyama N, Nakajima R, Takao Y, Kamiya S (1994) Preparation and taste of certain glycosides of flavanones and of dihydrochalcones. *Biosci Biotechnol Biochem* 58: 1479–1485

Hatayama K, Komatsu M (1971) Studies on the constituents of *Sophora* species. V. Constituents of the root of *Sophora angustifolia* Sieb. Et Zucc. (2). *Chem Pharm Bull* 19: 2126–2131

Ibrahim RK (1987) Regulation of synthesis of phenolics. In: Constabel F, Vasil IK (eds.) Cell culture and Somatic Cell Genetics of Plants, Vol. 4, Cell culture in phytochemistry. Academic Press, London, pp. 77–95.

Kang T-H, Jeong S-J, Ko W-G, Kim N-Y, Lee B-H, Inagaki M, Miyamoto T, Higuchi R, Kim Y-C (2000) Cytotoxic lavandulyl flavanones from *Sophora flavescens*. *J Nat Prod* 63: 680–681

Ko W-G, Kang T-H, Kim N-Y, Lee S-J, Kim Y-C, Ko G-I, Ryu S-Y, Lee B-H (2000) Lavandulylflavonoids: a new class of *in vitro* apoptogenic agents from *Sophora flavescens*. *Toxicol In Vitro* 14: 429–433

Kuroyanagi M, Arakawa T, Hirayama Y, Hayashi T (1999) Antibacterial and antiandrogen flavonoids from *Sophora flavescens*. *J Nat Prod* 62: 1595–1599

Lee H-S, Ko H-R, Ryu S-Y, Oh W-K, Kim B-Y, Ahn S-C, Mheen T-I, Ahn J-S (1997) Inhibition of phospholipase Cγ1 by the prenylated flavonoids from *Sophora flavescens*. *Planta Medica* 63: 266–268

Murashige T, Skoog F (1962) Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15: 473–497

Saito N, Cheng J, Ichimura M, Yokoi M, Abe Y, Honda T (1994) Flavonoids in the acyanic flowers of *Pharbitis nil*. *Phytochem* 35: 687–691

Sommer S, Köhle A, Yazaki K, Shimomura K, Bechtold A, Heide L (1999) Genetic engineering of shikonin biosynthesis. hairy root cultures of *Lithospermum erythrorhizon* transformed with the bacterial *ubiC* gene. *Plant Mol Biol* 39: 683–693

Wu L-J, Miyase T, Ueno A, Kuroyanagi M, Noro T, Fukushima S (1985) Studies on the constituents of *Sophora flavescens*. Ait. III. *Yakugaku Zasshi* 105: 736–741

Yamamoto H, Kawai S, Mayumi J, Tanaka T, Iinuma M, Mizuno M (1991a) Prenylated flavanone production in callus cultures of *Sophora flavescens* var. *angustifolia*. *Z Naturforsch* 46c: 172–176

Yamamoto H, Ichimura M, Tanaka T, Iinuma M, Mizuno M (1991b) A trifolirhizin malonate from *Sophora flavescens* var. *angustifolia* and its stability. *Phytochem* 30: 1732–1733

Yamamoto H, Ichimura M, Ishikawa N, Tanaka T, Iinuma M, Mizuno M (1992) Localization of prenylated flavonoids in *Sophora flavescens* var. *angustifolia* plants. *Z Naturforsch* 47c: 535–539

Yamamoto H, Yamaguchi M, Inoue K (1996) Absorption and increase in the production of prenylated flavanones in *Sophora flavescens* cell suspension cultures by cork pieces. *Phytochem* 43: 603–608

Yamamoto H, Senda M, Inoue K (2000) Flavanone 8-dimethylallyltransferase in *Sophora flavescens* cell suspension cultures. *Phytochem* 54: 649–655

Yamamoto H, Yatou A, Inoue K (2001a) 8-Dimethylallylnaringenin 2′-hydroxylase, the crucial cytochrome P450 mono-oxygenase for lavandulylated flavanone formation in *Sophora flavescens* cultured cells. *Phytochem* 58: 671–676

Yamamoto H, Yato A, Yazaki K, Hayashi H, Taguchi G, Inoue K (2001b) Increases of secondary metabolite production in various plant cell cultures by co-cultivation with cork. *Biosci Biotechnol Biochem* 65: 853–860

Zhao P, Inoue K, Kouno I, Yamamoto H (2003a) Characterization of leachianone G 2″-dimethylallyltransferase, a novel prenyl side-chain elongation enzyme for the formation of the lavandulyl group of sophoraflavanone G in *Sophora flavescens* Ait. cell suspension cultures. *Plant Physiol* 133: 1306–1313

Zhao P, Inoue K, Yamamoto H (2003b) Efficient production and capture of 8-prenylnaringenin and leachianone G—biosynthetic intermediates of sophoraflavanone G—by the addition of cork tissue to cell suspension cultures of *Sophora flavescens*. *Phytochem* 62: 1093–1099