Biotransformation of the Phytoestrogen 8-Prenylnaringenin

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In order to select microorganisms capable of transforming the potent phytoestrogen 8-prenylnaringenin, preliminary screening tests on 30 fungal cultures were performed. No reports concerning successful biotransformation of 8-prenylnaringenin by this class of organisms have been known so far. *Fusarium equiseti* converted 8-prenylnaringenin into 2”-(2”’-hydroxyisopropyl)-dihydrofuran-[2”,3”:7,8]-5,4’-dihydroxyflavanon in high yield.

Key words: 8-Prenylnaringenin, Biotransformation, *Fusarium equiseti*

Introduction

8-Prenylnaringenin has received much attention due to its health-promoting properties. This compound is considered to be the most potent phytoestrogen isolated to date (Milligan *et al*., 1999). It is known to inhibit angiogenesis (Pepper *et al*., 2004) and metastasis (Rong *et al*., 2001), and to prevent bone lose in rats (Miyamoto *et al*., 1998). It also exhibits antiandrogenic activity (Zierau *et al*., 2003).

8-Prenylnaringenin occurs naturally in minor amounts in some *Wyethia* species (McCormick *et al*., 1986) and hops (Stevens *et al*., 1997). Beer is the main dietary source of the hop prenylflavonoid desmethylxanthohumol, which is efficiently converted into 8-prenylnaringenin giving a final concentration of this compound in beer of up to 240 μg/L (Tekel *et al*., 1999).

Because of the interesting biological properties and increasing commercial applications of 8-prenylnaringenin, the study of its metabolism and of new derivatives of this phytoestrogen is important. Additionally, compounds which are obtained *via* biotransformation are considered to be “natural compounds”, as well. The main goal of the presented work was to select fungi able to transform 8-prenylnaringenin. Only two literature reports concerning biotransformation of this compound have been found at all. In a *Ascochyta rabiei* culture 8-prenylnaringenin was practically not metabolized (Tahara *et al*., 1997). Possemiers *et al*. (2005) incubated 8-prenylnaringenin with the fecal samples and with the intestinal bacterium *Eubacterium limosum*.

Material and Methods

General

Microorganisms, cultivation of fungi, isolation of xanthohumol from spent hops, chemical cyclization of xanthohumol into isoxanthohumol, reaction work-up, and product analysis were performed as described by Bartmańska *et al*. (2009).

*8-Prenylnaringenin (**1**)*

8-Prenylnaringenin (**1**) was obtained by demethylation of isoxanthohumol. A solution of I_2 in anhydrous Et_2O (859 mg/30 mL), protected from light, was stirred at room temperature until the reaction mixture turned colourless (3 h). The mixture was transferred *via* a syringe into a flask equipped with a condenser and containing isoxanthohumol in anhydrous THF (400 mg/80 mL). The reaction mixture was stirred and refluxed under N_2 for 12 h, then evaporated to 10 mL and a saturated aqueous solution of NH_4Cl was added. Next the mixture was extracted with CH_2Cl_2, dried over Na_2SO_4, the solvent evaporated, and the residue subjected to column chromatography, using CH_3Cl/MeOH (98:2 v/v) as eluent. Purification of the product gave 8-prenylnaringenin (**1**) as a light yellow solid. This method of synthesis of **1** was reported by Anioł *et al*. (2008).

^{1}H NMR (acetone-d_6): δ = 12.14 (1H, s, 5-OH), 9.55 (1H, s, 7-OH), 8.60 (1H, s, 4’-OH), 7.41 (2H, d, J = 8.4 Hz, H-2’,6’), 6.90 (2H, d, J = 8.6 Hz, H-3’,5’), 6.04 (1H, s, H-6), 5.44 (1H, dd, J = 12.7, 3.0 Hz, H-2), 5.19 (1H, t, J = 7.3 Hz, H-2”), 3.22

Table I. Screening for the microorganisms that can transform 8-prenylnaringenin (**1**).

Microorganism	*	Microorganism	*
Absidia coerulea AM93	+++	*Mortierella isabellina* AM212	++
Absidia cylindrospora AM336	++	*Mortierella vinaceace* AM149	++
Absidia glauca AM177	++	*Penicillium chermesinum* AM113	++
Aspergillus glaucus AM211	++	*Penicillium notatum* KCh904	++
Aspergillus niger UPF702	++	*Penicillium purpurogenum* AM49	+++
Aspergillus ochraceus AM370	++	*Penicillium vermiculatum* AM50	–
Aspergillus ochraceus AM456	++	*Penicillium camembertii* AM51	++
Beauveria bassiana AM278	+++	*Piptoporus betulinus* ARK5213	–
Botrytis cinerea AM235	++	*Piptoporus betulinus* ARK15980	+
Chaetomium sp. AM432	+++	*Piptoporus betulinus* ARK20129	–
Chaetomium indicum AM32	++	*Rhizopus nigricans* UPF701	++
Cunnighamella japonica AM472	+++	*Spicaria divaricata* AM423	–
Fusarium equiseti AM15	+++	*Spicaria fusispora* AM136	–
Inonotus radiatus ARK15970	+	*Trametes versicolor* AM536	++
Laetiporus sulphurens AM525	++	*Yarrowia lipolytica* AR71	+

* Capability of transformation of 8-prenylnaringenin (**1**): (+) capable; (–) incapable; (+++) less than 10% of the substrate remaining (by HPLC, detection at 290 nm) and a few transformation products; (++) 10–80% of substrate remaining; (+) more than 80% of the substrate remaining; (–) no product(s) observed.

(1H, d, J = 7.2 Hz, H-1''), 3.12 (1H, dd, J = 17.0, 12.7 Hz, H-3ax), 2.75 (1H, dd, J = 17.0, 3.1 Hz, H-3eq), 1.61 (3H, s, H-4''), 1.60 (3H, s, H-5''). – ^{13}C NMR (acetone-d_6): δ = 198.4 (C-4), 166.3 (C-7), 164.0 (C-5), 162.0 (C-9), 159.6 (C-4'), 132.1 (C-3''), 132.1 (C-1'), 129.8 (C-2',6'), 124.8 (C-2''), 117.1 (C-3',5'), 109.3 (C-8), 104.1 (C-10), 97.4 (C-6), 80.7 (C-2), 44.5 (C-3), 26.9 (C-5''), 23.3 (C-1''), 18.9 (C-4''). – UV (MeOH): λ_{max} = 290, 335 nm.

*Microbial transformation of 8-prenylnaringenin (**1**)*

In the screening tests, a solution of **1** (5 mg) in methanol (1 mL) was added to the fungal cultures. After a 7-day incubation on a rotary shaker (25 °C, in the dark), the pH value was measured, and the metabolites and any remaining substrate were extracted. All experiments were performed in duplicate. A control sample consisted of substrate **1** and sterile growth medium.

In the preparative-scale transformation, a solution of **1** (70 mg) dissolved in methanol (8 mL) was distributed equally into four flasks with *Fusarium equiseti* AM15 cultures (2-day culture, pH 6.6). The incubation was carried out under the same conditions as the screening tests and continued for 5 d (until substrate **1** had been metabolized). Progress of conversion was monitored by HPLC, a neutral pH value of the culture was observed at the end of the test.

*2''-(2'''-Hydroxyisopropyl)-dihydrofuran-[2'',3'':7,8]-5,4'-dihydroxyflavanon (**2**)*

Yellow crystals. – ^{1}H NMR (acetone-d_6): δ = 12.45/12.44 (1H, s, 5-OH), 8.59 (1H, br. s, 4'-OH), 7.41 (2H, d, J = 8.3 Hz, H-2',6'), 6.90 (2H, d, J = 7.9 Hz, H-3',5'), 5.88 (1H, s, H-6), 5.51/5.49 (1H, dd, J = 12.7, 2.4 Hz, H-2), 4.75 (1H, dd, J = 9.4, 8.0 Hz, H-2''), 4.74 (1H, dd, J = 9.6, 7.6 Hz, H-2''), 3.76/3.75 (1H, s, 2'''-OH), 3.20/3.18 (1H, dd, J = 12.7, 6.3 Hz, H-3ax), 3.05 (1H, dd, J = 15.2, 7.6/15.2, 8.0 Hz, H-3''), 2.99 (1H, dd, J = 15.5, 9.6/15.4, 9.4 Hz, H-3''), 2.77/2.74 (1H, dd, J = 17.1/2.9 Hz, H-3eq), 1.26/1.25 (3H, s, H-1'''), 1.20/1.19 (3H, s, H-3'''). – ^{13}C NMR (acetone-d_6): δ = 197.9 (C-4), 170.9 (C-7), 166.9 (C-5), 159.7 (C-9), 159.3, 159.2 (C-4'), 131.8 (C-1'), 130.0 (C-2',6'), 117.2 (C-3',5'), 106.7 (C-8), 104.5, 104.4 (C-10), 93.7 (C-2''), 92.5 (C-6), 80.9, 80.8 (C-2), 72.4 (C-2'''), 44.5, 44.4 (C-3), 28.1, 28.0 (C-3''), 26.8 (C-1'''), 26.7, 26.6 (C-3'''). – HR-ESI-MS: m/z = 355.1161 [M–H$^-$]. – UV (MeOH): λ_{max} = 294 nm. – CD (MeOH): $\theta_{290.3}$ = 10.1.

Results and Discussion

In order to select fungi capable of transforming 8-prenylnaringenin (**1**), we performed screening tests with 29 strains of filamentous fungi and one strain of yeast. An overview of the results of our experiments is presented in Table I.

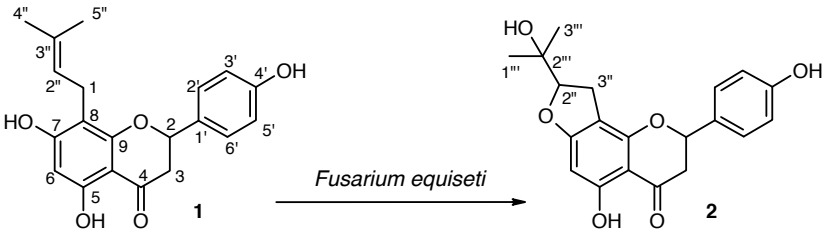

Fig. 1. Transformation of 8-prenylnaringenin (**1**) in the culture of *Fusarium equiseti* AM15.

Most of the tested strains metabolized 8-prenylnaringenin (**1**). We observed that the ability to effectively transform **1** does not depend on the taxonomical affiliation of the microorganisms. For example, *Penicillium purpurogenum* AM49 and *Penicillium camembertii* AM51 transformed the substrate with good efficiency during 7 days, while another tested *Penicillium* strain, *Penicillium vermiculatum* AM50, did not.

Fusarium equiseti AM15 was selected for preparative-scale transformation as it gave a single product in high yield compared to the other fungi. Transformation of 8-prenylnaringenin (**1**) by *F. equiseti* was carried out for 5 days (pH of the culture 6.7–7.1) to give after purification 36 mg of product **2** as light yellow crystals (51.5% yield). The UV spectrum of **2** showed a maximum absorption band at 294 nm, similar to that of substrate **1** (290 nm, respectively), which suggested that it was a flavanone derivative. The main difference in the ^{1}H NMR spectrum of **2** compared to 8-prenylnaringenin (**1**) was the disappearance of the signal of the 7-hydroxy group proton and the up-field shift of the H-2'' signal (from δ 5.19 for **1** to δ 4.75 for **2**). The presence of a –CH(O)CH_2– spin system in the dihydrofurane ring in product **2** was confirmed by a COSY experiment, which showed couplings between signals at δ 4.75 (H-2'') and δ 3.05 (H-3''). Most of the signals are doubled due to the presence of diastereomers.

In order to determine the stereochemistry of the product a CD analysis was performed. The high amplitude positive Cotton effect in the region of 280–300 nm allowed the assignment of prevalent (*R*) configuration to the C-2 stereocenter.

Compound **2** showed the [M – H$^-$] peak at *m/z* 355.1161 in HR-ESI-MS, which is in agreement with the formula $C_{20}O_6H_{20}$. On the basis of the above spectral analysis, metabolite **2** was identified as 2''-(2'''-hydroxyisopropyl)-dihydrofuran-[2'',3'':7,8]-5,4'-dihydroxyflavanon (Fig. 1).

The 2*S* isomer, named phellodensin D, occurs naturally in *Phellodendron chinese* var. *glabriusculum* (Wu *et al*., 2003). This compound was detected also as one of the minor metabolites of 8-prenylnaringenin (**1**) in human liver microsomes (*in vitro* study) (Nikolic *et al*., 2004).

The first step of the formation of product **2** was an epoxidation of the prenyl group, then an intramolecular attack of the neighbouring hydroxy group occurred, leading to a five-member-ring closure. In the cyclization process a new chiral centre at C-2'' was formed. This metabolic pathway was found to be the major route of the prenyl group metabolism in xanthohumol by human liver microsomes (Yilmazer *et al*., 2001). Earlier we reported that the tested strain transformed isoxanthohumol in a similar way (Bartmańska *et al*., 2009).

Conclusions

In the present study we observed that most of the tested fungi are capable of the transformation of 8-prenylnaringenin (**1**). 2''-(2'''-Hydroxyisopropyl)-dihydrofuran-[2'',3'':7,8]-5,4'-dihydroxyflavanon (**2**) was the transformation product of **1** by *Fusarium equiseti* AM15; it was obtained in good yield (51.5%). To the best of our knowledge, such an efficient transformation of this phytoestrogen by fungi has never been reported before.

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