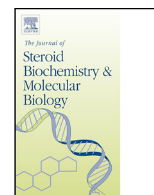
Journal of Steroid Biochemistry & Molecular Biology 156 (2016) 53–63

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb



Glyceollins and dehydroglyceollins isolated from soybean act as SERMs and ER subtype-selective phytoestrogens

Milou G.M. van de Schans[a], Jean-Paul Vincken[a,*], Pieter de Waard[b], Astrid R.M. Hamers[c], Toine F.H. Bovee[c], Harry Gruppen[a]

[a] *Laboratory of Food Chemistry, Wageningen University, Wageningen, The Netherlands*
[b] *Wageningen NMR Centre, Wageningen University, Wageningen, The Netherlands*
[c] *Business Unit of Toxicology and Bioassays, RIKILT-Institute of Food Safety, Wageningen, The Netherlands*

ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form 23 November 2015
Accepted 27 November 2015
Available online 30 November 2015

Keywords:
hERα-CALUX
hER yeast bioassay
Molar extinction coefficient
6a-Hydroxy-pterocarpan
6a,11a-Pterocarpene
Prenylation

ABSTRACT

Seven prenylated 6a-hydroxy-pterocapans and five prenylated 6a,11a-pterocarpenes with different kinds of prenylation were purified from an ethanolic extract of fungus-treated soybean sprouts. The activity of these compounds toward both human estrogen receptors (hERα and hERβ) was determined in a yeast bioassay and the activity toward hERα was additionally tested in an U2-OS based hERα CALUX bioassay. In the yeast bioassay, compounds with chain prenylation showed in general an agonistic mode of action toward hERα, whereas furan and pyran prenylation led to an antagonistic mode of action. Five of these antagonistic compounds had an agonistic mode of action in the U2-OS based hERα CALUX bioassay, implying that these compounds can act as SERMs. The yeast bioassay also identified 8 ER subtype-selective compounds, with either an antagonistic mode of action or no response toward hERα and an agonistic mode of action toward hERβ. The ER subtype-selective compounds were characterized by 6a-hydroxy-pterocarpan or 6a,11a-pterocarpene backbone structure. It is suggested that either the extra D-ring or the increase in length to 12–13.5 Å of these compounds is responsible for an agonistic mode of action toward hERβ and, thereby, inducing ER subtype-selective behavior.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Isoflavonoids (3-phenyl benzopyrans) are phenolic compounds, which can be found in plants of the Leguminosae family [1,2]. Within the Leguminosae family, soybean (*Glycine max*) is a rich source of isoflavonoids. The content and structural diversity of isoflavonoids can be influenced by stimulation of the plant's defense system [3,4]. The main compounds accumulating after stimulation of soybean sprouts by fungus and/or light are prenylated 6a-hydroxy-pterocarpans, the so-called glyceollins. These 6a-hydroxy-pterocarpans can be completely converted into their respective, more stable, 6a,11a-pterocarpenes after a combined acid and heat treatment (100 °C, 0.56 M HOAc, 30 min) [5]. Both the 6a-hydroxy-pterocarpans and 6a,11a-pterocarpenes have structural similarities with the female sex hormone 17β-estradiol (E_2). Therefore, many of these compounds are able to interact with the human estrogen receptors (hERs), which in turn can result in adverse or beneficial health effects [6,7].

Previous studies revealed that a mixture of prenylated 6a-hydroxy-pterocarpans had an agonistic mode of action toward hERα and hERβ in yeast estrogen bioassays [8] and showed also an agonistic mode of action in a MCF-7 based proliferation assay (E-screen) [9]. However, in other studies it was shown that glyceollin I showed an antagonistic mode of action in MCF-7 cells and inhibits estrogen-induced tumor progression [10,11] . Glyceollins II and III also showed only antagonistic hER activity, although less pronounced [10]. Taken together, these data imply that the activity of glyceollins is bioassay or tissue dependent, which indicates that glyceollins might act as selective estrogen receptor modulators (SERMs).

SERMs, like tamoxifen and raloxifene, are used for the treatment of ER-positive breast cancer. This is due to their anti-proliferative effects on breast cancer cells. This anti-proliferative effect is caused by an antagonistic mode of action toward hERα in these cancer cells [12] . However, while tamoxifen has often an antagonistic mode of action in breast cancer cells, it acts as a full agonist in the uterus, increasing the risk of developing endometrial cancer [13]. Tamoxifen and raloxifene also exhibit an agonistic mode of action in bone cells, which is regarded as beneficial as it increases bone strength in postmenopausal women [14,15]. Ever

* Corresponding author at: Laboratory of Food Chemistry, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands.
E-mail address: jean-paul.vincken@wur.nl (J.-P. Vincken).

http://dx.doi.org/10.1016/j.jsbmb.2015.11.020
0960-0760/© 2015 Elsevier Ltd. All rights reserved.

since these observations, SERMs are of great pharmaceutical interest, as they can modulate the response in one tissue differently from that in another. SERM behavior might be caused by differences in cofactors, the abundance of receptor types (hERα vs hERβ) and intrinsic E_2 levels between the different tissues. ER subtype-selective compounds are also of interest, because they might also be used in the treatment of ER-positive breast cancer [16]. Theoretically, breast cancer patients should respond most positively to treatment with a compound that is an hERα-antagonist and an hERβ-agonist. Subtype-selectivity is mostly associated with differences in affinity toward the hERs, *e.g.*, compounds which exhibit an agonistic mode of action toward hERβ and (almost) no response toward hERα [17]. However, subtype-selective compounds can also show a different mode of action toward the different hERs. For example, (*R*,*R*)-5,11-diethyl-5,6,11,12-tetrahydro-2,8-chrysenediol (*R*,*R*-THC) shows an agonistic mode of action toward hERα and an antagonistic mode of action toward hERβ [17].

Previously, it was demonstrated that the conversion of mixtures of glyceollins into mixtures of dehydroglyceollins changed the mode of action toward the estrogen receptors in yeast based estrogen bioassays [5]. Whereas glyceollins showed an agonistic mode of action toward hERα, the more planar dehydroglyceollins caused an antagonistic mode of action toward hERα. Moreover, chain prenylated naringenin showed a strong full agonistic activity, whereas genistein with the prenyl chain at the same position showed reduced estrogenic activity in MCF-7 cells [18]. This indicates that the backbone structure is an important parameter in determining estrogenicity.

Besides the backbone structure, it has been speculated that the kind of prenyl group might also influence the estrogenicity of prenylated isoflavonoids and flavonoids [8,19]. Chain prenylation of the isoflavone daidzein into kwakhurin resulted in a stronger agonistic activity compared to daidzein [20], although it should be mentioned that kwakhurin also had both an extra hydroxyl group and methyl group compared to daidzein. Pyran prenylation of daidzein led to an antagonistic mode of action for 7,8-(2,2-diMe-pyrano)daidzein [21].

From the above it is clear that different structural features can induce different estrogenic responses *in vitro* . However, structure-activity relationships for these compounds need to be established further. In this research, first glyceollins and their corresponding dehydroglyceollins were purified from an ethanolic extract from elicited soybean seedlings. Next, their agonistic and antagonistic activity toward the hERα and hERβ were investigated using yeast estrogen bioassays and an hERα CALUX bioassay. It is hypothesized that planar backbones (as in 6a,11a-pterocarpenes) induce antagonism more efficiently than non-planar ones (as in 6a-hydroxy-pterocarpans) and that pyran prenylation induces antagonism more efficiently than chain prenylation. Moreover, it is suggested that glyceollins and dehydroglyceollins might act as SERMs and/or exhibit ER subtype-selectivity.

2. Materials and methods

2.1. Materials

Soybeans, *Glycine max* (L.) Merrill, were provided by Frutarom (Londerzeel, Belgium). An extract of germinated and *Rhizopus* challenged soybean seedlings were prepared as described previously [4]. L-Leucine, L-histidine, sodium hydroxide (NaOH) pellets, chloroform-d_1 (99.8% atom %) and 17β-estradiol (E_2) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethanol (100%, analytical grade), D-(+)-glucose, ammonium sulfate, methanol-d_4 (99.8% atom%), acetone-d_6 (99.8% atom%) and dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany). Yeast nitrogen base without amino acids and without ammonium sulfate, and agar were obtained from Becton-Dickinson (Franklin Lakes, NJ, USA). Acetic acid (HOAc) (ULC/MS grade), acetonitrile (ACN) (ULC/MS and HPLC-R grade), silica gel (60 Å, 70–230 mesh), water (ULC/MS grade) and ethyl acetate (LC–MS grade) were purchased from Biosolve BV (Valkenswaard, The Netherlands). Water for purposes other than UHPLC was prepared using a Milli-Q water purification system (Millipore, Molsheim, France). The reporter yeast strains were provided by RIKILT (Wageningen, The Netherlands) and the hERα-CALUX U2-OS cells were provided by BioDetection Systems (Amsterdam, The Netherlands).

2.2. RP-UHPLC-ESI-MS analysis

RP-UHPLC-ESI-MS analysis was performed as described previously [5].

2.3. Purification using ethyl acetate partitioning

600 mg of the soybean extract was solubilized in 70% (v/v) EtOH (10 mg/mL). Solubilization was enhanced with 5 min sonication at 25 °C. The solubilized soybean extract was added to 120 mL water, 180 mL ethyl acetate and 150 mg NaCl. This liquid–liquid partitioning was performed three times. The pooled ethyl acetate fractions were evaporated under vacuum, solubilized in *tert*-butanol and lyophilized.

2.4. Purification using RP Flash chromatography

A Grace Reveleris™ Flash system was used as first purification step. The soybean extract after ethyl acetate partitioning was injected onto a 12 g Reveleris C18 RP cartridge (particle size 38.6 μm) using a solid loader (5 g cartridges) (Grace Davison Discovery Science, Columbia, MD, USA). Therefore, the soybean extract after ethyl acetate partitioning (~250 mg) was mixed with 2 g silica. The mixture was transferred into an empty 5 mL cartridge and closed with a plunger. The cartridge was placed upstream of the C18 RP column. Water (Milli-Q) containing 1% (v/v) HOAc (HPLC grade) + 1% (v/v) ACN (HPLC-R grade), eluent A, and ACN (HPLC-R grade) acidified with 1% (v/v) HOAc (HPLC-R grade), eluent B, were used as eluents. The flow rate was 30 mL/min and the separation was performed at room temperature. The following elution profile was used: 0–5 min, linear gradient from 0 to 40% B; 5–9 min, linear gradient from 40 to 50% B; 9–14 min, isocratic on 50% B; 14–19 min, linear gradient from 50 to 60% B; 19–24 min, linear gradient from 60 to 70% B; 24–27 min, linear gradient from 70 to 80% B; 27–28 min, linear gradient from 80 to 84% B; 28–33 min, linear gradient from 84 to 96% B; 33–35 min, linear gradient from 96 to 100% B. Absorption was measured at 280 nm. During the entire run, fractions of 10 mL were collected. All fractions were analyzed with UHPLC-MS. Fractions containing similar prenylated isoflavonoids were pooled. Next, the ACN from the pools was evaporated under vacuum at RT and the remaining water phase was immediately frozen and lyophilized in order to obtain 6a-hydroxy-pterocarpans. The pools were re-solubilized in *tert*-butanol, frozen and lyophilized in order to get a fine powder. Afterwards, the pools were re-solubilized in 70% (v/v) ethanol for analysis with UHPLC-MS and in 100% EtOH for further purification using preparative RP-HPLC.

2.5. Formation of prenylated 6a,11a-pterocarpenes

To generate prenylated 6a,11a-pterocarpenes, Flash fractions containing prenylated 6a-hydroxy-pterocarpans were evaporated to dryness. During evaporation the HOAc level increased, and

Download English Version:

https://daneshyari.com/en/article/1991278

Download Persian Version:

https://daneshyari.com/article/1991278

Daneshyari.com