Journal of Pharmaceutical and Biomedical Analysis 155 (2018) 202–209

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Isomerization of astilbin and its application for preparation of the four stereoisomers from *Rhizoma Smilacis Glabrae*

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ARTICLE INFO

Article history:
Received 5 February 2018
Received in revised form 4 April 2018
Accepted 4 April 2018
Available online 6 April 2018

Keywords:
Rhizoma Smilacis Glabrae
Astilbin
Isomerization
Purification

ABSTRACT

Astilbin is the most predominant flavonoid in *Rhizoma Smilacis Glabrae* (*RSG*) with many bioactivities. The interconversion of the astilbin and its three stereoisomers was found with incubation of *RSG* extract at different temperatures, and the equilibrium ratios were calculated. Under certain conditions, neoastilbin would replace astilbin and become the predominant flavonoid in *RSG* extract. The effects of ascorbic acid, sucrose, sodium benzoate, β-cyclodextrin (β-CD) and common metal ions on the isomerization and decomposition of astilbin were studied. Ascorbic acid showed the best protective effect on the decomposition of astilbin and its isomers, which may be attributed to its reducing and radical scavenging ability. Besides, ascorbic acid also accelerated the isomerization of astilbin. β-CD suppressed both isomerization and decomposition of astilbin through complexation between them. Most metal ions had inhibition effects on the isomerization of astilbin. Al^{3+} could almost completely inhibit the isomerization. The presence of Fe^{3+} caused the rapid decomposition of astilbin, and Cu^{2+} also showed weak effects. Based on the isomerization study, a novel and simple method for preparative separation of astilbin and neoastilbin from *RSG* sample was developed. Astilbin and neoastilbin with purity of 93% and yield of 0.86% and 0.48% were obtained, respectively, which represent 46.8% of total flavonoids in *RSG* sample. By controlling the isomerization conditions, astilbin and neoastilbin could be used as the initial reactants to produce neoisoastilbin and isoastilbin, respectively.

1. Introduction

Astilbin is a dihydroflavonol rhamnoside commonly found in many plants and plant-based foods, such as *Engelhardia roxburghiana* [1], *Rhizoma Smilax glabra* (*RSG*) [2], *Rhizoma Smilax Chinae* [3], turtle jelly [4], grape and wine [5]. It is the main effective component of *RSG*, a key herbal material used for syphilis treatment in ancient China. The Chinese pharmacopoeia requires that astilbin content in *RSG* should be greater than 0.45% [6]. Modern pharmacological studies showed that astilbin has a variety of biological activities, such as selective immunosuppression [7–11], regulation of fat metabolism [12,13], antioxidative [14], antibacterial [15], and anti-tumor [16,17]. In particular, its selective immunosuppressive activity has attracted increasing attention. Xu et al. found that astilbin could significantly inhibit delayed hypersensitivity [7,8], collagen arthritis [9] and immune liver injury [10]. Compared with cyclosporin A, a commonly used immunosuppressant at present, astilbin has more selective immunosuppressive effect. Astilbin inhibits excess cellular immunity without affecting humoral immunity. It selectively facilitates the apoptosis of activated T cells but has no effect on naive T cells. Other drugs or compounds with similar characteristics of selective immunity have not been reported. The potential clinical significance of astilbin deserves further attention and is expected to avoid long-term adverse drug reactions in the treatment of chronic immune diseases [18].

According to the molecular structure of astilbin, it has four stereoisomers depending on the configuration at C-2 and C-3 positions, which are astilbin (2R, 3R), neoastilbin (2S, 3S), neoisoastilbin (2S, 3R) and isoastilbin (2R, 3S) [19]. The four stereoisomers are usually present simultaneously in plants. However, astilbin is always the predominant one, and the contents of the other three isomers are very low, e.g. in *RSG* [1,2]. Hence, there are a lot of studies focused on the purification of astilbin from plant materials [1,20,21]. And its bioactivities have been extensively studied. In contrast, because it is difficult to obtain the pure products of the other three astilbin isomers in an easy way, very little research has been done on them. The isomerism of compounds may significantly affect their biological activity, and some drugs have completely different pharmacological effects between the two enantiomers.

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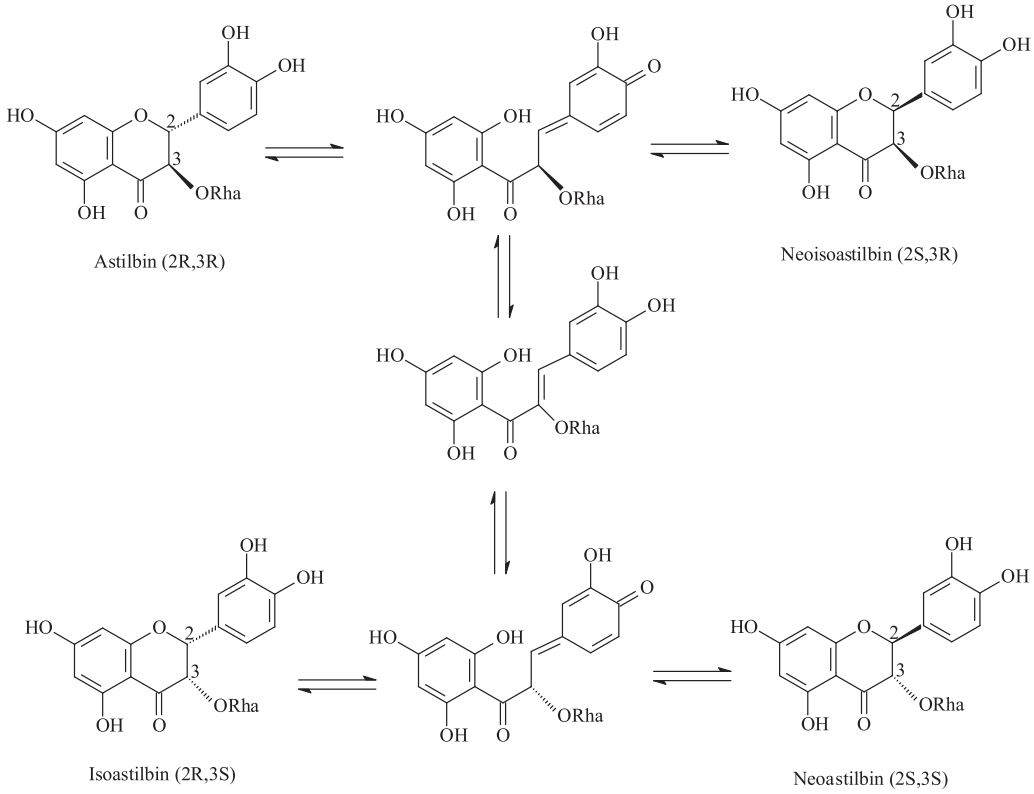
https://doi.org/10.1016/j.jpba.2018.04.005
0731-7085/



Scheme 1. A proposed route of the interconversion between astilbin and its stereoisomers.

Thus, for systematically comparing the bioactivities between astilbin and its isomers, developing a feasible method for formation and purification of other three astilbin isomers is in demand. In our previous study, the effects of temperature, pH value, and solvent on the stability of astilbin were investigated [22]. Results showed that astilbin was stable under acidic conditions. With the rise of pH value and temperature, the isomerization and decomposition of astilbin became fast. An isomerization pathway was proposed in Scheme 1. It is deduced that the four stereoisomers can convert to each other through a chalcone intermediate. However, in the study, the main isomerization product of astilbin was neoisoastilbin, and the interconversion between the four stereoisomers was unfound. More studies are needed to prove the deduction. Furthermore, the existence of astilbin in foods and pharmaceutical is not alone. There are a lot of coexisting components, such as food additives and metal ions. Besides, when investigating the bioactivity of astilbin using cell model, there are a lot of coexisting metal ions in the cultural medium. Thus, more factors affecting the isomerization and decomposition of astilbin need to be revealed.

In the present study, the interconversion between astilbin and its three stereoisomers was found with incubation of *RSG* extract at different temperatures. The effects of some food additives and metal ions on the isomerization and decomposition of astilbin were studied. Ascorbic acid could accelerate the isomerization and prevent the decomposition of the flavonoids. Based on the data, the isomerization conditions were optimized, and a strategy for preparative separation of the four stereoisomers from *RSG* was developed.

2. Material and methods

2.1. Chemicals and materials

RSG sample was purchased from Guandong shixin Pharmaceutical Co., Ltd. (Jieyang city, Guangong province, China). Astilbin (>98%) were purified from *RSG* in our laboratory, and was identified by UV, IR, MS, and NMR. HPLC grade acetonitrile was purchased Anhui Tedia High Purity Solvents Co., Ltd (Anqin city, Anhui province,China). Milli-Q water was used throughout the study. All other reagents used were analytical grade.

2.2. HPLC analysis

An Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, CA, USA) and a Symmetry C18 column (250 mm × 4.6 mm i.d., 5 μm) (Waters Corporation, Milford, MA,USA) was used for HPLC analysis. The mobile phase consisted of acetonitrile (A) and 0.1% acetic acid aqueous solution (B). The flow rate was 1 mL/min with linear gradient program of 0–15 min, 16–20% A; 15–40 min, 20–40% A. The detection wavelength was set at 291 nm and the injection volume was 10 μL.

2.3. RSG extract isomerization study

Dry *RSG* was smashed by high speed pulverizer and filtered through 40 mesh sieve. 0.5 g of *RSG* sample was immersed with 25 mL of 50% ethanol for 30 min with occasional shaking at room temperature. After centrifugation at 3000 rpm for 5 min, the supernatant was concentrated to about 10 mL by vacuum rotary evaporation at 60 °C. After adjusting the pH value to 7.0 by 1 M NaOH, the extract was incubated in a water bath with different temperatures (25, 40, 60, and 80 °C). At different time intervals, 0.25 mL of sample was taken by a pipette and used for HPLC analysis after filtering by a 0.22-μm pore size filter.

2.4. Effect of food additives on the isomerization of astilbin

Astilbin working solution was freshly prepared by diluting its stock solution (2 mg/mL in 50% methanol) 10 times with 0.2 M