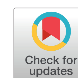
Journal of Pharmaceutical and Biomedical Analysis 152 (2018) 94–101

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

# Metabolism of flavonolignans in human hepatocytes



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ARTICLE INFO

*Article history:*
Received 20 November 2017
Received in revised form 22 January 2018
Accepted 23 January 2018

*Keywords:*
Silybin
Silymarin
Cytochrome P450
Bactosomes
Hepatocytes
LC/MS

ABSTRACT

This study examined the *in vitro* biotransformation of eight structurally related flavonolignans, namely silybin, 2,3-dehydrosilybin, silychristin, 2,3-dehydrosilychristin, silydianin, 2,3-dehydrosilydianin, isosilybin A and isosilybin B. The metabolic transformations were performed using primary cultures of human hepatocytes and recombinant human cytochromes P450 (CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4). The metabolites produced were analyzed by ultra-performance liquid chromatography coupled with tandem mass spectrometry. We found that each of the tested compounds was metabolized *in vitro* by one or more CYP enzymes, which catalyzed *O*-demethylation, hydroxylation, hydrogenation and dehydrogenation reactions. In human hepatocytes, silybin, 2,3-dehydrosilybin, silychristin, 2,3-dehydrosilychristin, and isosilybins A and B were directly conjugated by sulfation or glucuronidation. Moreover, isosilybin A was also converted to a methyl derivative, while isosilybin B was hydroxylated and methylated. Silydianin and 2,3-dehydrosilydianin were found to undergo hydrogenation and/or glucuronidation. In addition, 2,3-dehydrosilydianin was found to be metabolically the least stable flavonolignan in human hepatocytes, and its main metabolite was a cleavage product corresponding to a loss of CO. We conclude that the hepatic biotransformation of flavonolignans primarily involves the phase II conjugation reactions, however in some cases the phase I reactions may also occur. These results are highly relevant for research focused on flavonolignan metabolism and pharmacology.

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## 1. Introduction

Flavonolignans are plant secondary metabolites formed by the coupling of a flavonoid with a phenylpropanoid (lignan precursor) unit. They have been identified in a relatively small number of species, from which *Silybum marianum* (milk thistle) is the source of flavonolignans relevant for pharmacological applications [1]. Silymarin, an extract from the fruits of *S. marianum*, which is used in the complementary therapy of liver disorders caused by hepatotoxic agents and viral infections [2,3], contains as its main flavonolignans silybin, silychristin, silydianin and isosilybin (Fig. 1). All of these compounds are biosynthetically derived from the flavanonol taxifolin (2,3-dihydroquercetin) and coniferyl alcohol and, with the exception of silydianin, they are produced as pairs of 10,11-*trans*-configured diastereomers, denoted A and B [4]. The hepatoprotective potential of silymarin depends on the pleiotropic effects of its flavonolignan constituents, which exert antioxidant/prooxidant action and inhibition/activation of enzymes and receptors [2,5]. The most abundant and most extensively studied silymarin flavonolignan is silybin [6], which is also used in the form of the water-soluble prodrug silybin 3,23-*O*-bishemisuccinate as an antidote for the treatment of poisoning by the mushroom *Amanita phalloides* (death cap); this effect is based on the interaction of silybin with the respective cyclic peptide receptors [7]. However, interesting biological activities have been reported for less abundant silymarin constituents as well. For instance, silychristin and isosilybin B have demonstrated higher cytoprotective [8] and antiproliferative effects, respectively, than silybin *in vitro* [9]. In addition, a number of flavonolignan derivatives have been prepared [7,10,11]. Of these the 2,3-dehydroflavonolignans (Fig. 1), *i.e.* the oxidized derivatives and putative minor silymarin components [2,12], seem to be particularly interesting due to their increased antioxidant activity and chemoprotective action [8,13,14].

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https://doi.org/10.1016/j.jpba.2018.01.048
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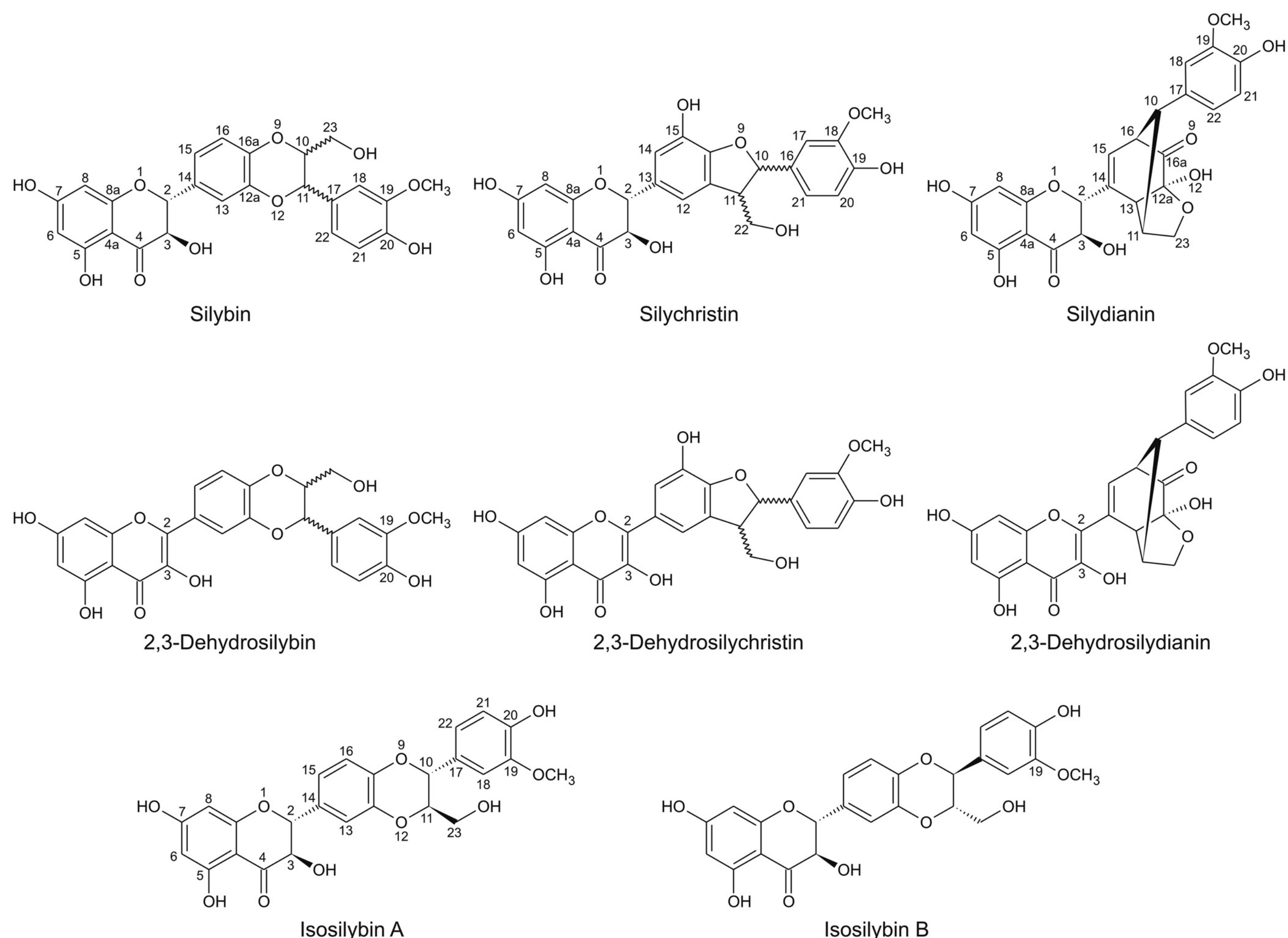


**Fig. 1.** Chemical structures of tested flavonolignans.

An important issue in the characterization of any drug, including phytopharmaceuticals, is the elucidation of the metabolic fate of a given compound [15]. In general, the metabolism of xenobiotics may be divided into four phases. Phase I involves the introduction or unmasking of a polar group in the parent molecule by oxidation, reduction, hydration and hydrolysis reactions. Phase II then represents the conversion of the functionalized molecule by conjugation reactions such as glucuronidation, sulfation, methylation, acetylation and glutathionylation. The other two phases of xenobiotic metabolism, phase 0 and phase III, refer to the cellular uptake and elimination, respectively, of either the parent compound or its metabolites [16,17]. It has been shown using human liver microsomes, recombinant biotransformation enzymes, and microbial transformations that silybin may undergo oxidative and conjugation reactions *in vitro* [18–20]. *In vivo* studies, however, demonstrate the preferential involvement of phase II conjugation enzymes in the metabolism of silybin. For instance, silybin B-20-*O*-glucuronide was found to be the main silybin metabolite in a healthy volunteer after the oral administration of silymarin [21]. A recent study with pure silybin diastereomers, given to rats by intragastric gavage, showed that silybin B was absorbed to a greater extent than silybin A. The metabolites identified in rat plasma included silybin B-7-*O*-glucuronide, silybin B-20-*O*-sulfate and 20-*O*-methylsilybin B [22]. In contrast to silybin, the metabolism of other flavonolignans has not been investigated in detail yet [23]. In this study, we examined the metabolic biotransformation of silybin, 2,3-dehydrosilybin, silychristin, 2,3-dehydrosilychristin, silydianin, 2,3-dehydrosilydianin, isosilybin A and isosilybin B using recombinant human cytochrome P450 (CYP) enzymes and primary cultures of human hepatocytes.

## 2. Materials and methods

### 2.1. Tested flavonolignans

Silybin, silychristin, silydianin, isosilybin A and isosilybin B were isolated from silymarin (Liaoning Senrong Pharmaceutical, Panjin, China; batch No. 120501) as described previously [12,24]. 2,3-Dehydrosilybin, 2,3-dehydrosilychristin and 2,3-dehydrosilydianin were prepared by the oxidation of silybin, silychristin and silydianin, respectively [8,25]. The standards of sulfated flavonolignans, *i.e.* silybin A-20-*O*-sulfate, silybin B-20-*O*-sulfate, silychristin 19-*O*-sulfate and 2,3-dehydrosilybin 20-*O*-sulfate were prepared by sulfation of the respective flavonolignans using the arylsulfate sulfotransferase from *Desulfitobacterium hafniense* [26,27]. The purity of all flavonolignans was at least 95% (HPLC). For biotransformation incubations, fresh 25 mM stock solutions of the tested compounds in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) were use.

### 2.2. Isolation of human hepatocytes

Samples of human liver were obtained from multi-organ donors according to the protocols approved by the local ethics commit-

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