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Regioselective alcoholysis of silybin A and B acetates with lipases

Kateřina Purchartová[a,b], Petr Marhol[a], Radek Gažák[a], Daniela Monti[c], Sergio Riva[c], Marek Kuzma[a], Vladimír Křen[a,*]

[a] Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20 Prague, Czech Republic
[b] Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 8, CZ-12840 Praha 2, Czech Republic
[c] Istituto di Chimica del Riconoscimento Molecolare, C.N.R., Via Mario Bianco 9, I-20131 Milano, Italy

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ABSTRACT

Large screening identified lipase AK to be capable of the selective deacetylation of pentaacetyl silybins A and B to yield 3,5,20,23-tetra-*O*-acetyl-silybins A and B, and 3,20,23-tri-*O*-acetyl-silybins A and B, respectively. Deacetylation occurred at phenolic OH groups, only. These new compounds prepared from the optically pure silybins can serve as new stereochemically pure synthons for selective silybin modifications.

1. Introduction

The chemistry of flavonoids, analogously to that of other polyols like carbohydrates, is complicated by the need for complex protection/deprotection strategies. Further problems stem from the high sensitivity of flavonoids towards oxidative agents and alkaline conditions, and their tendency to form complexes with some metal cations. This strongly limits the use of certain protection/deprotection strategies. The use of enzymes can circumvent these limitations, both in terms of selectivity and mild conditions; and is also compatible with food and drug applications.

Lipases, which are obvious candidates for the selective derivatization of flavonoids, have been often used for selective modifications of various natural compounds [1,2].

Most of the so far described lipase-catalyzed acylations were focused on flavonoid glycoside acylations where the acyl preferentially substitutes the primary OH group of the glycone (typically glucose) [3–6]; other positions at the flavonoid moiety are seldom hit [5,7]. This was also demonstrated by molecular modeling using the CalB (*Candida antarctica* lipase B) and series of quercetin glycosides (e.g. rutin, isoquercitrin) [8] showing that the only position susceptible to enzymatic acylation on the aglycone part (quercetin) is its 3′-OH. A comprehensive review of enzymatic acylations of flavonoids has been recently published by Chebil et al. [9].

Studies dealing with selective lipase-catalyzed deacylations are scarce and are mostly limited to quercetin, catechin and morin. Lambusta et al. [7] has demonstrated partial deacetylation of quercetin and catechin pentaacetates with three lipases (from *Pseudomonas cepacia*, *Mucor miehei* and *Candida rugosa*). Another study dealing with lipase-catalyzed (*P. cepacia* lipase) deacetylation of luteolin, kaempferol, kaempferide, and quercetin peracetates, found 7- and 4′-acetates to be preferentially removed in these types of compounds [10].

However, the use of lipases for the modification of flavonoids is complicated by technical limitations, e.g. low solubility in water (but also in highly nonpolar solvents) that is circumvented by the use of suitable organic solvents and by another, often neglected fact, that many flavonoids are potent inhibitors of lipases. This effect has been demonstrated e.g. with 3-hydroxyflavone, 5-hydroxyflavone, catechin or kaempferol that even display their potential as inhibitors of lipases [11], or some flavonoids contained in citruses [12] and in medicinal plants [13].

The flavonolignan silybin (**1**) (CAS No. 22888-70-6) is the major component of the silymarin complex extracted from the seeds of *Silybum marianum* (L.) Gaertn. (*Carduus marianus* L., Asteraceae) (milk thistle) [14] occurring as an equimolar mixture of two diastereomers, silybin A (**1a**), and silybin B (**1b**) (Fig. 1). We have recently developed a preparatory-scale chemoenzymatic separation method [15–17], which made it possible to study biological activities with optically pure diastereomers of silybin and also to perform chemical transformations with optically pure and well defined compounds.

* Corresponding author. Tel.: +420 296 442 510; fax: +420 296 442 509.
E-mail address: kren@biomed.cas.cz (V. Křen).

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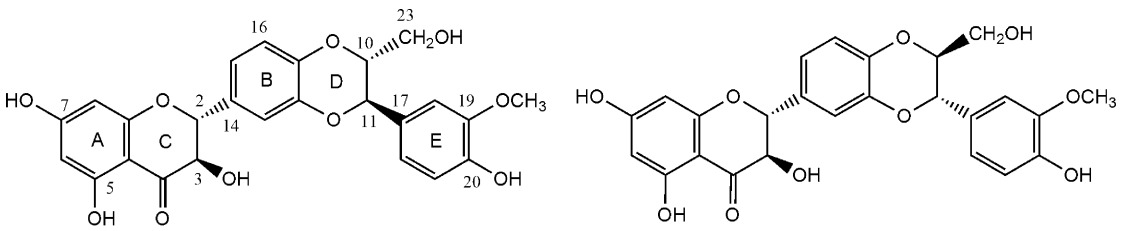

Fig. 1. Diastereomeric silybins (**1a** and **1b**).

Silybin is currently advocated for the treatment of cirrhosis, chronic hepatitis, and liver diseases associated with alcohol consumption and environmental toxin exposure [18], as it is an efficient antioxidant and chemoprotectant. Silybin is considered to be very safe and no serious adverse effects have been reported [19]. Other beneficial effects of silybin include chemopreventive as well as hypocholesterolemic, cardioprotective, and neuroprotective effects [20,21]. For example, silybin is in phase-II clinical trials in the US for the treatment of prostate adenocarcinoma [22]. Silybin activities are linked to a large number of effects at the cellular and molecular level, such as estrogenic activity, the modulation of drug transporters (P-glycoprotein) [23] and a specific action on DNA expression *via* the suppression of nuclear factor-κB [20,24]. The importance of silybin is demonstrated, for example, by the high frequency of relevant papers (ca 200 per year).

Silybin has a unique flavonoid structure, differing from most known flavonoids so far functionalized with lipases. It does not have a double bond at position 2,3 (like quercetin or rutin), which makes it very sensitive towards oxidation even under weak alkaline conditions [25]. Being a flavonolignan, it possesses a lignan moiety with a primary alcoholic 23-OH group. These structural features intrinsically create four stereocenters and this makes silybin chemistry much more complicated than in other more simple flavonoids.

The importance of regioselectively acylated derivatives of silybin (prepared mostly by chemical synthesis) has been well established. Several acyl derivatives of silybin have significantly better biological activity than silybin [26–28], but the synthesis of derivatives other than the 23-*O*-acyl is quite problematic due to poor regioselectivity and low yields [26].

In this work, we report the screening of a library of commercially available hydrolases (lipases, proteases, and acylases) for the regioselective deacylation of peracetylated silybins. An optimized protocol for obtaining optically pure 3,5,20,23-tetra-*O*-acetyl-silybins and 3,20,23-tri-*O*-acetyl-silybins using lipase AK from *Pseudomonas* sp. is described.

2. Experimental

2.1. General methods

NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer (600.23 MHz for ^{1}H, 150.93 MHz for ^{13}C at 30 °C) in DMSO-d_6 (99.9 at.% D, Sigma–Aldrich, Steinheim, DE). The residual signal of the solvent was used as an internal standard (δ_H 2.500 ppm, δ_C 39.60 ppm). NMR experiments: COSY, HSQC, and HMBC were performed using the manufacturer's software. ^{1}H NMR and ^{13}C NMR spectra were zero-filled to fourfold data points and multiplied by a window function prior to Fourier transformation. A two-parameter double-exponential Lorentz–Gauss function was applied for ^{1}H to improve resolution and line broadening (1 Hz) was applied to obtain a better ^{13}C signal-to-noise ratio. Chemical shifts are given on a δ-scale with digital resolution justifying the reported values to three (δ_H) or two (δ_C) decimal places.

2.2. HPLC

Chromatographic analyses were performed on a Shimadzu Prominence system (Kyoto, JP) consisting of a DGU-20A mobile phase degasser, two LC-20AD solvent delivery units, a SIL-20AC cooling autosampler, CTO-10AS column oven and SPD-M20A diode array detector with semi-micro cell volume (2.5 μl). Shimadzu LC solution software (Kyoto, JP) was used to collect and process chromatographic data.

A Chromolith Performance RP-18e monolithic column (100 mm × 3 mm i.d., Merck, DE) was used with solvent system A: MeCN/MeOH/H_2O/HCO_2H (2/37/61/0.1, v/v/v/v) and B: 100% MeCN. The optimal gradient was 0–4 min 0% B, 4–25 min 0–40% B, 25–26 min 40% B, 26–28 min 40–0% B; flow rate 1.2 ml/min. The column oven temperature was set to 25 °C and the samples were kept at 20 °C in the autosampler. The PDA data was acquired in the 200–350 nm range and the 285 nm signal was extracted.

2.3. Chemistry

2.3.1. 3,5,7,20,23-Penta-O-acetyl-silybin (2)

Natural silybin (mixture **1a** and **1b**, 1:1) (**1**, 500 mg, 1.04 mmol) was acetylated using the standard acetylation procedure (Ac_2O/pyridine 4 ml, 1:1, v/v, overnight) [29], which yielded 705 mg (98.2%) of the title compound **2** as a yellow amorphous solid. Acetylations of pure silybins A (**1a**) and B (**1b**), prepared as described in [17], were performed analogously yielding peracetates **2a** and **2b**, respectively. The ^{1}H and ^{13}C NMR data for **2a** and **2b**, which was compatible with their structures, is given in supplementary material.

2.3.2. Screening of enzymatic alcoholysis of 3,5,7,20,23-penta-O-acetyl-silybin (2)

The screening of lipases for the alcoholysis of silybin peracetate **2** was accomplished by dissolving **2** (5 mg, 7.2 μmol) in a mixture of *tert*-butyl methyl ether (MTBE) or toluene/*n*-butanol (1.1 ml, 10:1, v/v) containing the respective lipase (5 mg; see Table 1). The reaction mixture was incubated at 45 °C and 450 rpm in a Thermomixer (Eppendorf, DE), reaction progress was monitored by TLC (mobile phase: $CHCl_3$/toluene/acetone/HCO_2H—80:10:5:1) and HPLC. No diastereomeric selectivity, e.g. discrimination between **2a** and **2b**, was observed in any enzymatic deacetylation.

2.3.3. Preparative reactions catalyzed by lipase AK

3,5,7,20,23-Penta-*O*-acetyl-silybin (**2**, 100 mg, 0.144 mmol) was dissolved in a mixture of MTBE/*n*-butanol (22 ml, 10:1, v/v) with the addition of lipase AK from *Pseudomonas* sp. (Amano, JP) (100 mg). The reaction mixture was incubated in a 50 ml Falcon tube at 45 °C under shaking (450 rpm) for 48 h. The residue after filtration and evaporation was purified by column chromatography on silica gel (mobile phase: $CHCl_3$/acetone/toluene/HCO_2H—95:5:5:1) yielding 3,20,23-tri-*O*-acetyl-silybin (**3**, 9 mg, 15.3%) and 3,5,20,23-tetra-*O*-acetyl-silybin (**4**, 42 mg, 77.9%), both as yellowish amorphous