Chemistry of silybin

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Chemistry of silybin

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Silybin, a secondary metabolite isolated from the seeds of the blessed milk thistle (*Silybum marianum*) was discovered as the first member of a new family of natural compounds called flavonolignans in 1959. Over the years it has received the research attention of many organic chemists. This research has resulted in a number of semisynthetic derivatives prepared in an effort to modulate and better target the biological activities of silybin or to improve its physical properties, such as solubility. A fundamental breakthrough in silybin chemistry was the determination of the absolute configurations of silybin A and silybin B, and the development of the methods for their separation. This review covers articles dealing with silybin chemistry and also summarizes all the derivatives prepared. Covering: 1959-2013.

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1 Introduction

1.1 Flavonoids and flavonolignans

Flavonoids are a broad class of secondary metabolites contained in most plants and are responsible for the yellow, red or blue pigments in their flowers. These important plant pigments are also the most common secondary metabolites in the human diet. Because of their beneficial biological effects and their dietary uptake has increased in recent years.

Among flavonoids, flavonolignans are a relatively small subclass of compounds, where the flavonoid part of the molecule is fused with a lignan. Flavonolignans were first discovered in the seeds of *Silybum marianum* (Fig 1) and silybin (**1**) is by far the most researched flavonolignan.[1] Its beneficial activites include hepatoprotective[2] and recently discovered anticancer activity.[3]

Large amounts of Milk thistle seeds are processed to silymarin by the pharmaceutical industry. The milled or pressed seeds (botanically fruits - *cypsela* - containing only one seed; for the sake of simplicity we are using the term "seed" in this work) are extracted with ethanol, methanol, acetone or ethyl acetate. Lipids and polar impurities are removed from the extract and the resulting dry mixture of flavonolignans is called silymarin.[1] Silybin (**1**) is isolated from silymarin by methanolic extraction. Except silybin A (**1a**) and silybin B (**1b**), silymarin contains another five major compounds – taxifolin (**1g**), isosilybin A (**1c**), isosilybin B (**1d**), silychristin A (**1e**) and silydianin (**1f**)

(Fig 2). Minor compounds include silychristin B[4] and isosilychristin.[5] Further silybin congeners such as silandrin,[6] isosilandrin,[7] silymonin,[6] silyamandin[8] were isolated from white flowering variety of *Silybum marianum*. Silymarin also contains about 30% of undefined polyphenolic compounds often referred to as a „polymeric fraction". Silybin A (**1a**), silybin B (**1b**) and isosilybin A (**1c**) were recently detected in the culture of endophytic fungus *Aspergillus iizukae* growing on *S. marianum*.[9]

Flavonolignans are found in various plants (Fig 3), for example in the common oat (*Avena sativa*, several unnamed compounds e.g., **2**),[10] in the amazon tree *Hymenea palustris* (5',5'-dimethoxyhydnocarpin D, palstatin, **3**),[11] in *Hyparrhenia hirta* (four unnamed compounds e.g., **2** and its 7-glucopyranoside **4**)[12] or in *Hydnocarpus wightiana* seeds (hydnocarpin, **5**).[13]

Fig. 1: Milk thistle depiction in the Leonhart Fuchs herbal (1542)

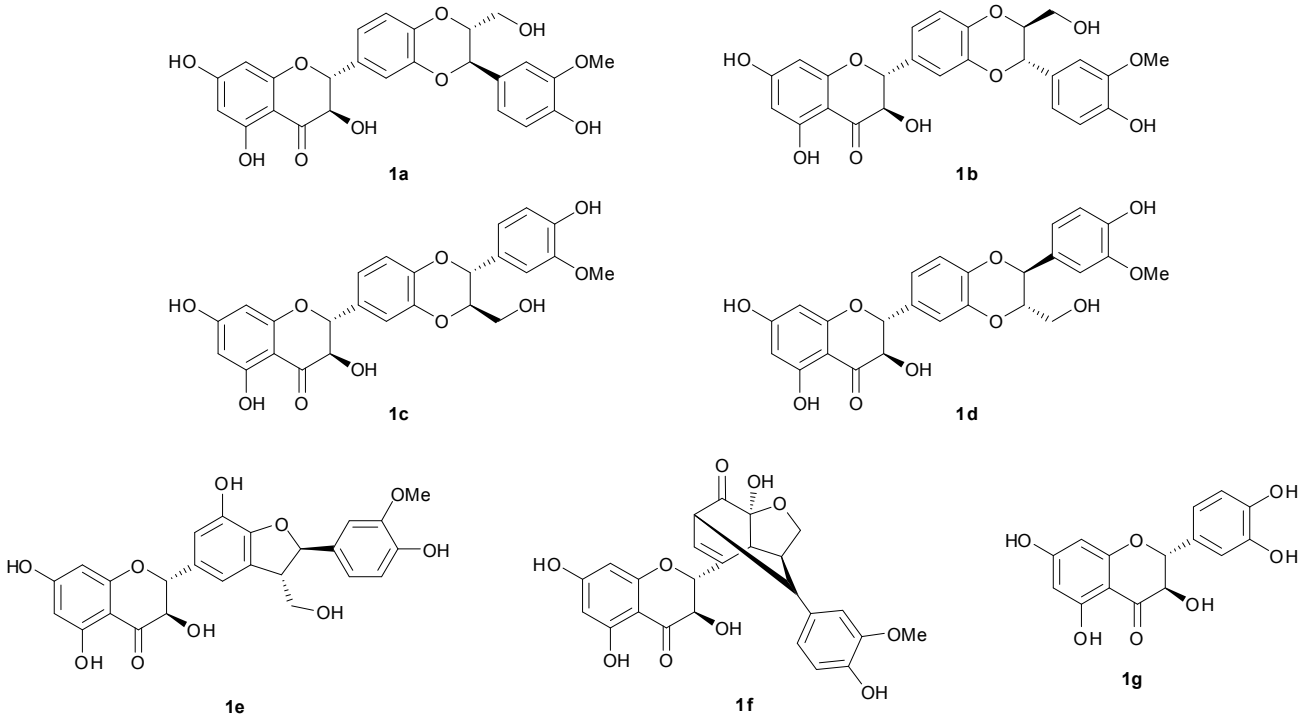

Fig. 2: Major silymarin components, silybin A (**1a**), silybin B (**1b**), isosilybin A (**1c**), isosilybin B (**1d**), silychristin A (**1e**), silydianin (**1f**) and taxifolin (**1g**).

1.2 Milk thistle – research history

The plant – the milk thistle (*Silybum marianum* (L.) Gaertn., Asteraceae, Fig 1) – was already known to the ancient Greeks. Both Theoprastus of Eresos (ca 371 – ca 287 BC) and Pedanios Dioscurides (ca 40 – 90 AD) mention it. At that time, however, it was considered a crop plant without much medicinal merit. The medicinal properties of the plant were first recorded by renaissance and humanistic naturalists and physicians. Johannes Niger, Adam Lonitzer, Leonhart Fuchs and Hieronimus Bock all included articles about the milk thistle into their herbals. Most notable, the article in Pietro Andrea Mattioli's herbal (1544) mentions the usage of crushed seeds for „side pain". In Hieronymus Bock's book (1539) in a text similar to Mattioli,

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the author adds „*Das gebrandt wasser ist güt zü der enzündten Lebern*.“ (The burned water is good for an inflamed liver), possibly referring to an alcoholic extract of the seeds used for treating liver disorders. The plant was then mentioned in a number of herbals up to modern times.

2 Silybin – properties and nomenclature

2.1 Nomenclature and numbering

Natural silybin (**1**) (CAS No. 22888-70-6; in pharmacopoeias the name *silibinin* is used) is an approximately equimolar mixture of two diastereomers: silybin A (**1a**) − (2*R*,3*R*)-2-((2*R*,3*R*)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-1,4-benzodioxin-6-yl)-2,3-dihydro-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one and silybin B (**1b**) − (2*R*,3*R*)-2-((2*S*,3*S*)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxy-methyl)-1,4-benzodioxin-6-yl)-2,3-dihydro-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one (typically **1b** is slightly prevalent in most preparations). Their basic properties are summarized in Table 1. The proprietary and systematic numbering of silybin (**1**) is shown in Fig 4. In this paper the proprietary numbering will be used. For the compound numbering of silybin derivatives the following convention will be used: Derivatives made from diastereomeric mixtures are described by a number only. Derivatives made from pure diastereomers bear “**a**” or “**b**” relating to respective parent diastereomers **1a** or **1b**.

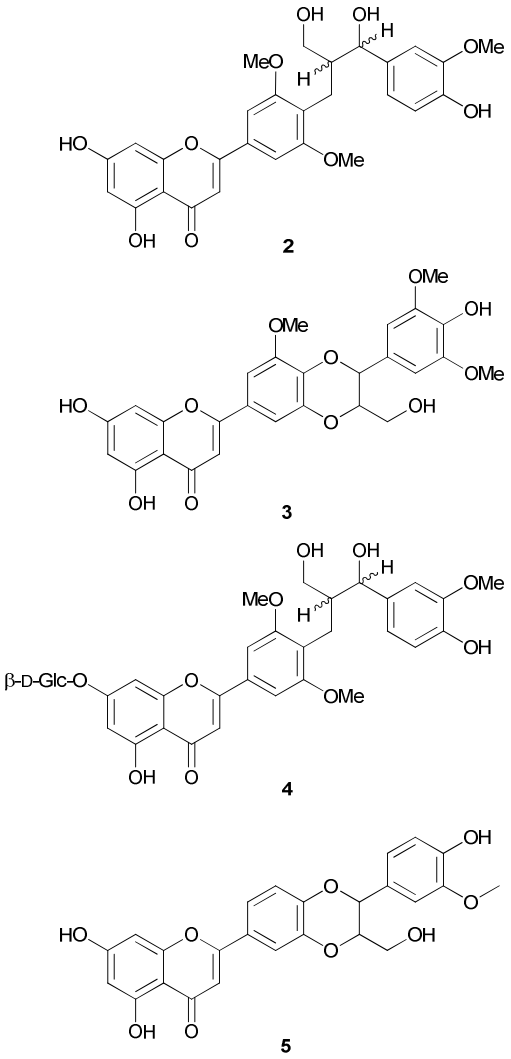

Fig. 3: Examples of flavonolignans not originating from *Silybum marianum*.

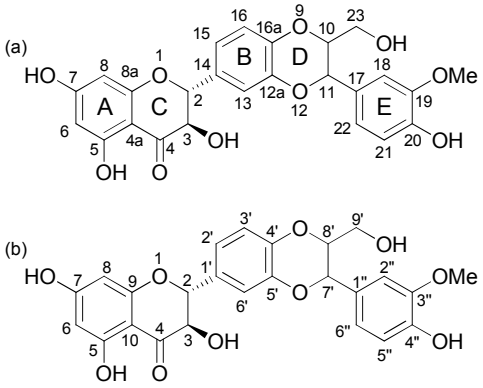

Fig. 4: Proprietary (a) and systematic (b) numbering of silybin (**1**).

2.2 Chemical properties of silybin

Silybin (**1**) can be characterized as a small, highly functionalized molecule with alternating carbo- and heterocycles. Generally, silybin (**1**) is stable under Brönsted acidic conditions, but not stable in the presence of Lewis acids

or under basic conditions. The molecule is quite resistant to reduction, but oxidizes easily to 2,3-dehydrosilybin (**114**). Strong bases or prolonged heating over 100 °C cause disruptions of its skeleton.

The five hydroxy groups of silybin (**1**) are the primary targets of derivatization. These hydroxy groups can be divided into three categories according to their nature – three phenolic, one secondary and one primary alcoholic group. Of the phenolic hydroxyls, the hydroxyl group in position C-5 is somehow exceptional because of its strong hydrogen bonding to the adjacent oxo group. The oxo group is in the conjugation with the aromatic ring and acts as a free electron pair donor to the hydrogen bond with the 5-OH group. The other two phenolic hydroxy groups are similar in behavior; the C-7 OH group being more reactive than the 20-OH group due to lower steric hindrance and the presence of the hydrogen bond. The primary alcoholic group at C-23 can be readily esterified and oxidized to a carboxylic acid. A plethora of esters but few ethers have been prepared at this position so far. The secondary alcoholic group at C-3 can be easily oxidized (even with atmospheric oxygen) to a ketone, which exists exclusively in enol form, giving rise to 2,3-dehydrosilybin (**114**).

Table 1: Basic physical constants of silybin.

	silybin (**1**)	silybin A (**1a**)	silybin B (**1b**)
m.p	162 -163 °C[14]	158 – 160 °C[15]	157 – 159 °C[15]
$[\alpha]_D$[c]	+11.38 (*c* 0.23, acetone)[16] +11.0 (*c* 0.23, acetone) [15, 17,b]	+20.0 (c 0.3, acetone)[14] +6.1 (*c* 0.3, MeOH) [15] +18.28 (*c* 0.29,acetone)[16, a] +13.7 (c 0.218, acetone)[18]	-1.07 (c 0.3, acetone)[14] +0.6 (*c* 0.3, MeOH) [15] +6.09 (*c* 0.23, acetone)[16, a] +6.3 (c 0.223, acetone)[18]
UV	217, 230, 288[15]	217, 230, 288[15]	217, 230, 288[15]

[a]The notation of the silybins was changed in 2003 by Lee and Liu[14] when determining their absolute configuration. In the older literature, the names of silybin A and B were swapped. In this table we use the current notation. The authenticity of the respective diastereomers can be simply ascribed by their optical rotation.

[b]Up to its 14th Edition, the Merck Index gives the data for the natural silybin (mixture **1a**/**1b** 1:1) but erroneously gives the formula of silybin B. This error was corrected only in the 15th Edition, in which the data of pure **1a** and **1b** and also isosilybin A and B (**1c** and **1d**) were given.

[c]Optical rotation data of the respective silybin preparations are often influenced by the minor impurities – often *cis*-silybins (see Section 5.6) – that are not easy to detect due to their coelution with the major peaks (**1a** and **1b**); e.g., the $[\alpha]_D$ of 2,3-*cis*-silybin A is -51.6 (*c* 0.091, acetone), that of 9,10-*cis*-silybin B is -40.4 (*c* 0.39, acetone) and the $[\alpha]_D$ of 9,10-*cis*-silybin A is +69,7 (*c* 0.11, acetone).[19] Due to the big differences in their $[\alpha]_D$, even minor amounts of these impurities strongly influence the final $[\alpha]_D$ of silybins.

2.3 Physical properties of silybin

Although silybin (**1**) contains several hydrophilic ionizable groups, its overall character is hydrophobic and its solubility in water is low. It is poorly soluble in polar protic solvents (EtOH, MeOH) and insoluble in nonpolar solvents (chloroform, petrolether), but is soluble in polar aprotic solvents (acetone, DMF, THF).

In neutral aqueous solutions, silybin (**1**) behaves as a weak acid with a pKa of 7.95 for the 7-OH and 6.63 for the 5-OH.[20] Van Wenum *et al.* reported a pKa of 7.7 for 7-OH and 11.0 for the 20-OH group.[21] The solubility of silybin (**1**) was studied in 1980 by Koch *et al.*[22] and later in 2007 by Bai *et al*.[20], who also employed quantum chemical calculations to investigate solvation effects. Both of these papers found that the solubility of silybin in water increases steeply with pH (Fig. 6) and moderately with temperature. Koch *et al.*[22] found its solubility to increase linearly with temperature, however, Bai *et al*.[20] observed a polynomial increase (Fig. 5).

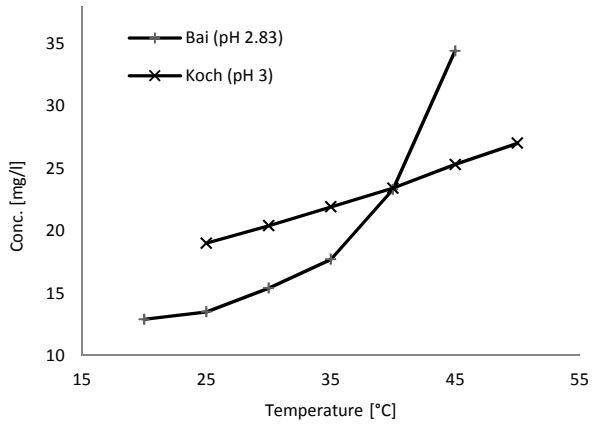

Fig. 5: Solubility of silybin (**1**) at ca pH 3 for various temperatures.

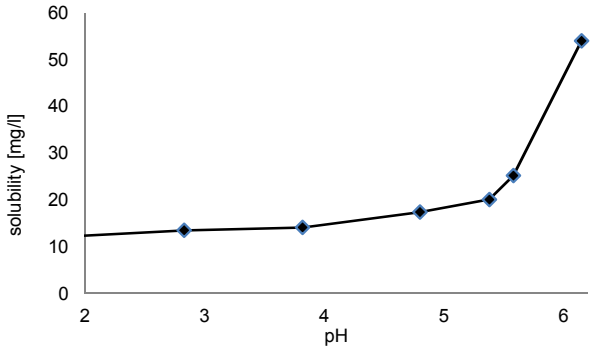

Fig. 6: Solubility of silybin (**1**) at 25 °C for various pH values.

3 Structure of Silybin

3.1 Isolation and structure determination of silybin

Silybin (**1**) was first isolated by G. Möschlin in 1959[23] within his dissertation work. Janiak and Hänsel in 1960[24] were the first to publish the isolation of flavonoids from the fruit extract of *Silybum marianum* as Silybum substances E_5 and E_6 in their journal. They determined their basic physical constants, empirical formula and based on its colorific reaction (blue with Rhodamin B etc.), they hypothesized about the 3□-methoxy-3-

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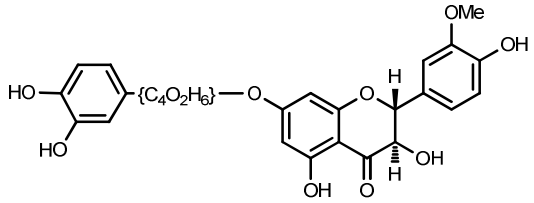
hydroxyflavanone nature of the substance. Substance E_6 was further investigated in 1965 by Wagner *et al*.[25] The authors were the first to call substance E_6 „Silybin“ and they found out that silybin (**1**) has five hydroxyl groups that can be acetylated, three hydroxyl groups that can be methylated and that it adds one equivalent of bromine. On the basis of ^{1}H-NMR spectroscopy (frequency not given, $CDCl_3$, CCl_4) of the prepared derivatives the authors suggested the first tentative partial structure (**6**), which established the 3'-methoxy-3'-hydroxyflavanon nature of the compound.



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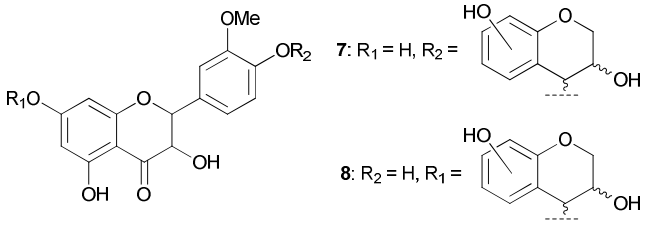
In 1967 – 1968 first Hänsel[26] (**7**) and then Wagner[27] (**7**, **8**) both published updated structures. Both structures were the same, the only difference being in the chromadiol residue position that was regarded by Wagner as being equally possible in positions 4' and 7.



The above structures were corrected in 1968 by Pelter and Hänsel,[28] based on careful examination of ^{1}H-NMR (100 MHz, DMSO-d_6) and MS spectra of silybin and 2,3-dehydrosilybin peracetates and silybin and 2,3-dehydrosilybin permethyl ethers. The authors inferred two new tentative structures (now called isosilybins (**1c**, **1d**)), first incorporating a coniferyl alcohol moiety into the molecule and established relative configurations at all stereogenic centers. The authors also suggested an oxidative coupling between taxifolin and coniferyl alcohol as a plausible biosynthetic route to silybin and coined a generic name flavonolignans for this class of compounds. It is notable that these authors, possibly by mistake, reported the wrong configurations at positions C-2 and C-3.

These structures were further corroborated by Hänsel a year later by the synthesis of the benzodioxane part of the structure.[29] At that moment it was clear that the 1,4-dioxane ring of silybin adopts a half-chair conformation with both substituents in quasi-equatorial positions. Ambiguity remained in the position of benzodioxane substituents and in the absolute configuration of the chiral centers. Later the same authors published[30] a total synthesis of racemic 2,3-dehydrosilybin pentamethylether and 2,3-dehydroisosilybin pentamethylether. Comparison of these derivatives with the compounds synthetized from natural silybin clearly established the correct structure of **1**, except for the absolute configurations. The absolute configuration in positions C-2 and C-3 remained unknown until 1975, when Pelter and Hänsel published results of degradative experiments of silybin (**1**).[31] The absolute configuration of this part of silybin molecule was inferred from ^{1}H NMR coupling constants and from CD spectra using an analogy with catechin and epicatechin.[31] The authors also proposed absolute configurations at C-10 and C-11, but failed to recognize silybin as the mixture of two diastereomers and their assignments were, therefore, only partly correct. One year later HPLC analysis was published showing for the first time silybin as two peaks - at that time an unexplained phenomenon.[32] This observation was explained by recognizing silybin (**1**) as mixture of two diastereomers (**1a** and **1b**) in the work by A. Arnone in 1979.[33] The publication of X-ray diffraction data by Lotter and Wagner[34] confirmed the existence of two diastereomers of silybin co-crystallizing in an equimolar ratio, determined absolute configuration of the taxifolin moiety to be 2*R*, 3*R*, common to both stereoisomers, but failed to assign absolute configuration at the dioxane ring that differentiate both diastereomers. Early structural determination issues of flavonolignans were discussed in detail in a review by Kurkin *et al.*[35] The debate about the absolute configuration in positions C-10 and C-11 was finally settled by Kim *et al*.[15] who assigned configuration 2*R*,3*R*,10*R*,11*R* to silybin A (**1a)** and 2*R*,3*R*,10*S*,11*S* to silybin B (**1b**). The assignment was based on comparison of CD spectra with model compound (3-methyl-2-phenyl-1,4-benzodioxane).

3.2 Total syntheses of silybin

The first synthetic studies trying to confirm the structure of silybin focused on racemic dehydrosilybin pentamethyl ethers (**9** – **13**).[30] This is an obvious target for synthesis, as dehydrosilybin lacks two chiral centers at C-2 and C-3 and it is readily available from natural silybin. Authors started from protected quercetin, building the dioxane ring in several steps (Scheme 1). Later, Merlini *et al*.[36] and Schrall *et al.*[37] published a simple biomimetic synthesis, (Scheme 2) yielding a diastereomeric mixture of silybin (**1**). In Merlini's paper, the formation of isosilybins (**1c**, **1d**) is also reported. Schrall[37] also reports the formation of silybin in the *Silybum marianum* cell culture. The nature of the radical coupling in silybin biosynthesis was thus established. For a recent insight into mechanism of the biomimetic synthesis of silybin see Althagafy *et al.*[38]

Using a methoxymethyl ether protecting group instead of methyl ether Tanaka *et al.* were able to obtain regioselectively diastereomeric mixture of silybin starting from simple precursors (**15** – **27**, Scheme 3).[39]

Synthetic efforts towards silybin were concluded in 2000 by the enantioselective formal total synthesis of both silybin A (**1a**) and silybin B (**1b**, not shown) by Gu *et al.*[40] (**14**, **23**, **28** – **33**, Scheme 4) starting from ferulic acid and using Sharpless oxidation as the key step.

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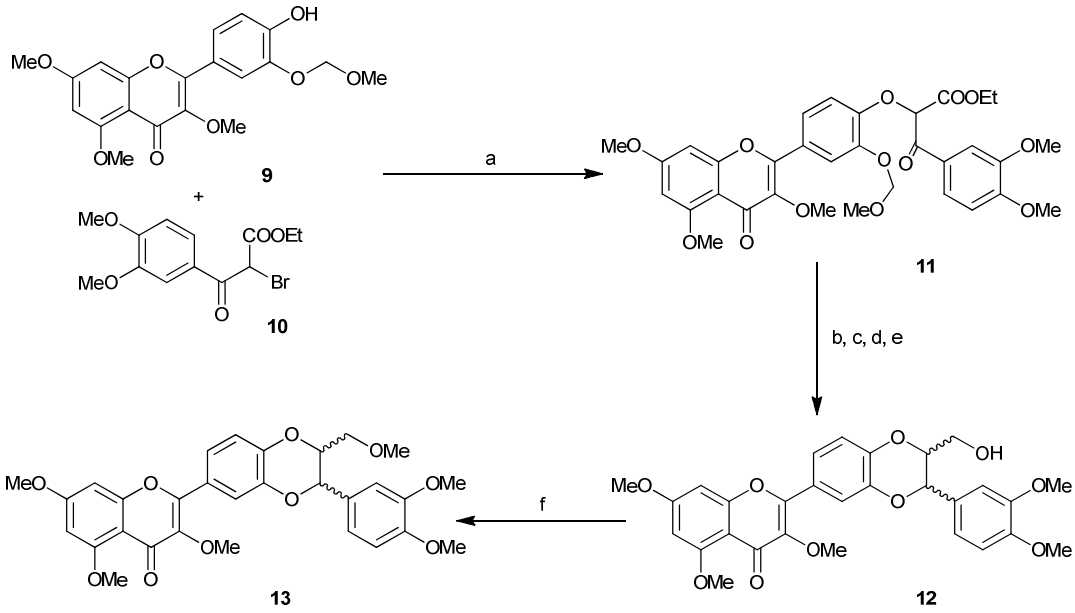
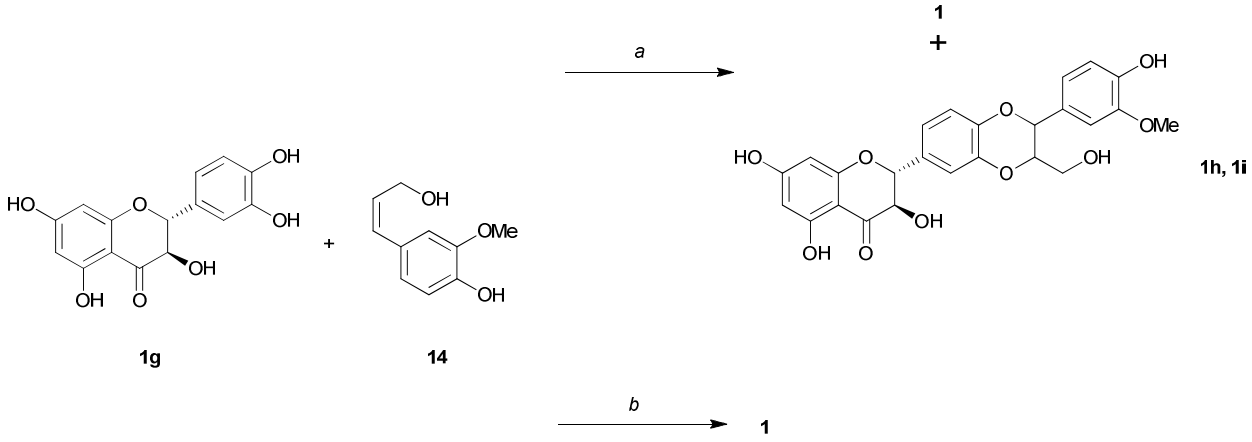
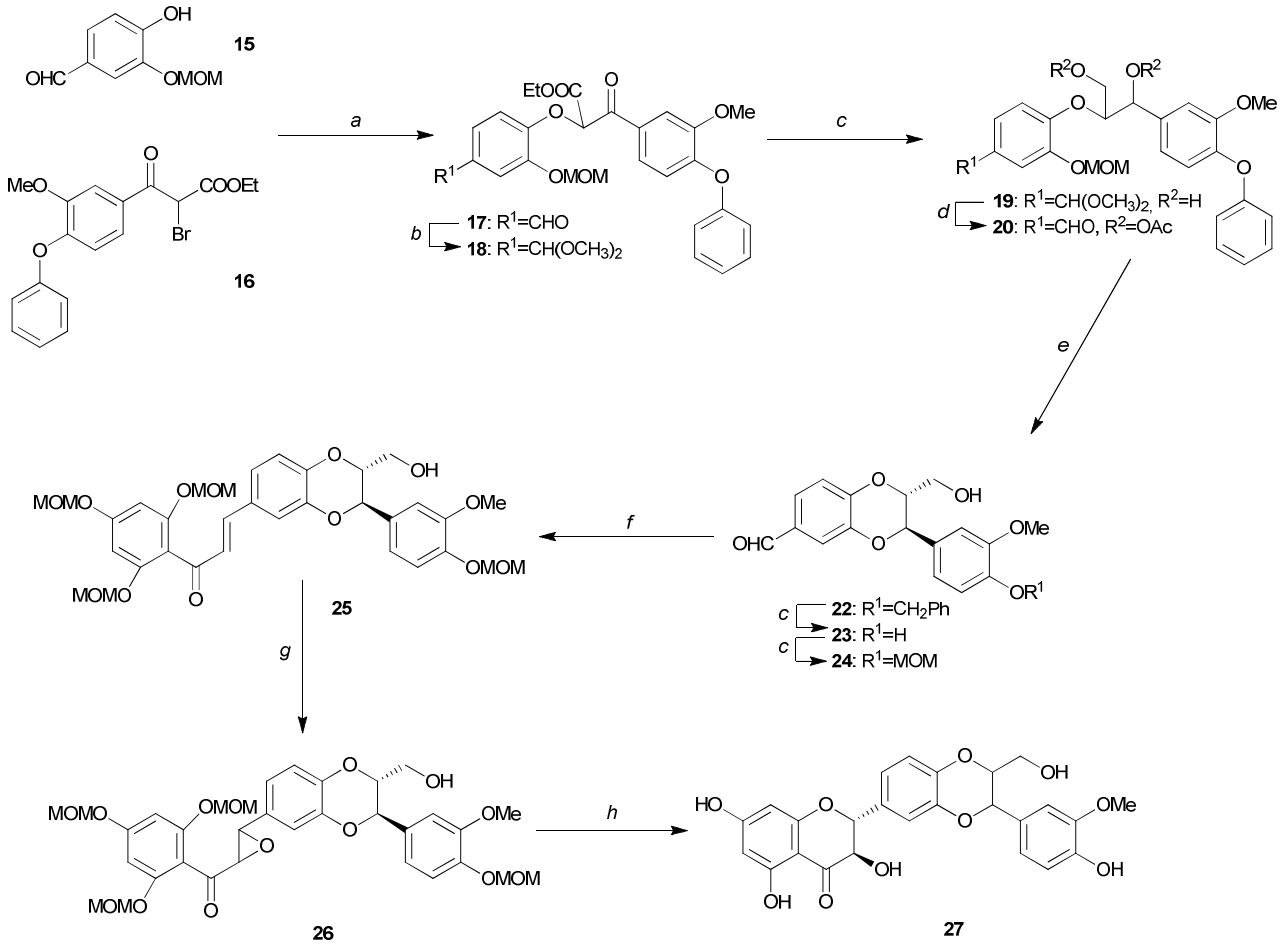


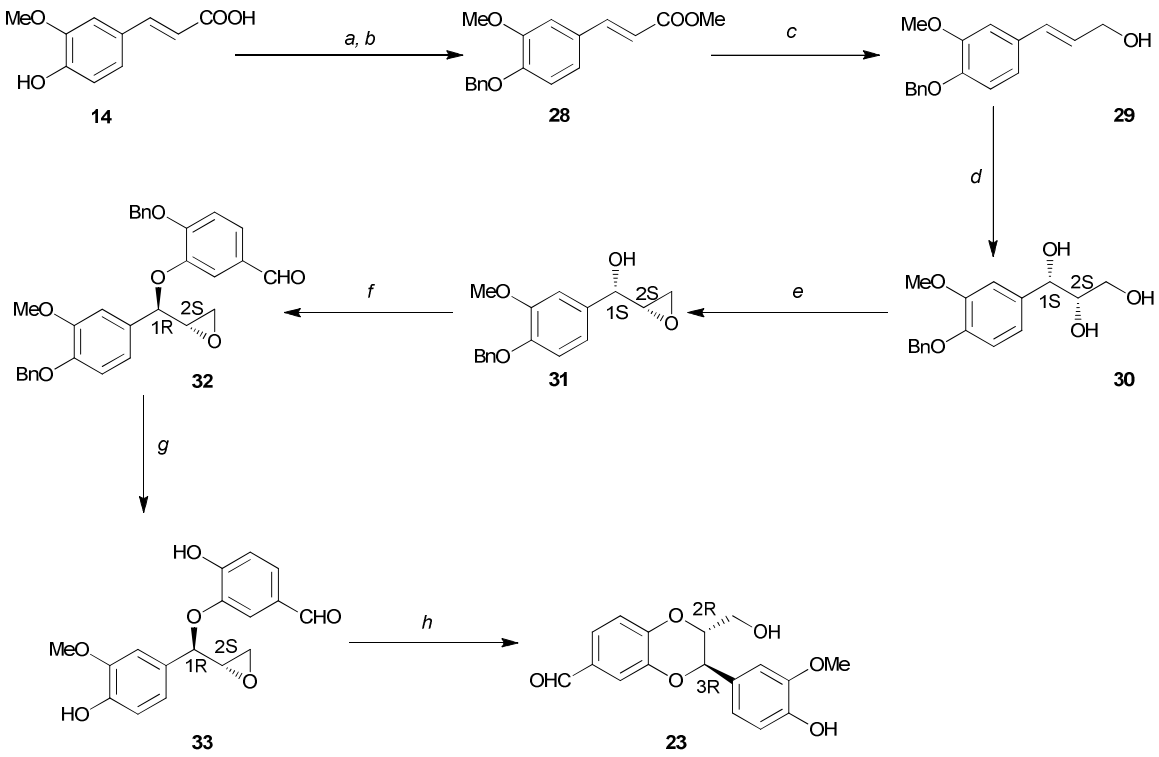
Scheme 1: Synthesis of racemic dehydrosilybin hexamethylether.[30] *Reaction conditions: a*: K_2CO_3; *b*: $NaBH_4$; *c*: CH_3COOH, H_2SO_4; *d*: $POCl_3$; *e*: $LiAlH_4$; *f*: MeI, Ag_2O.



Scheme 2: Merlini's biomimetic synthesis of silybin (top).[36] *Reaction conditions: a:* Ag_2O, benzene, acetone, 55 °C, 45 h. Biomimetic synthesis of silybin by Schrall (bottom).[37] *Reaction conditions: b: horseradish peroxidase, phosphate buffer pH 5.8.*



Scheme 3: Total synthesis of silybin by Tanaka *et al.*[39] *Reaction conditions: a: t*-BuOK, DMF; *b*: $HC(OCH_3)_3$, NH_4Cl, MeOH; *c*: $NaBH_4$, MeOH; *d*: Ac_2O, pyridin; *e*: CH_3COOH, H_2SO_4; *f*: NaOH, EtOH, 1-(2,4,6-*tris*(methoxymethoxy)phenyl)ethanone (**21**); *g*: H_2O_2, NaOH, MeOH; *h*: HCl, MeOH, THF.



Scheme 4: Formal total synthesis of silybin A (**1a**) by Gu *et al.*[40] *Reaction conditions: a:* MeOH, H_2SO_4, 90°C, 16 h; *b*: BnCl, K_2CO_3, DMF, 160°C, 3h; *c*: LAH, THF, -10°C, 1 h; *d*: AD-mix-α, $MeSO_3NH_2$, *t*-BuOH, H_2O; *e*: N-tosylimidasole, NaH, THF; *f*: PPh_3, DEAD, THF, 4-benzyloxy-3-hydroxybenzaldehyde; *g*: H_2, Pd/C, EtOAc; *h*: K_2CO_3, MeOH, r.t., 1 h.

4 Preparative resolution of silybin diastereomers

The identity and absolute configurations of both silybin diastereomers were determined by Kim *et al.*[15] and partly corrected by Lee & Liu[14] more than twenty years after silybin was first isolated. This substantial delay was caused by the fact that the chemical reactivities of both silybin distereomers are very similar (in isotropic milieu). Therefore, without diastereomeric discrimination using anisotropic systems (e.g., enzymes), it is very hard to distinguish between both diastereomers.

4.1 Chromatographic methods

Although the analytical separation of silybin diastereomers in an RP HPLC column is quite feasible (for a recent paper on the analytical separation of silybin and its congeners, see Marhol *et al.*[41]), a preparative HPLC separation can only be used to obtain minute amounts of pure silybin diastereomers and such procedures are extremely laborious. Li *et al*.[42] used preparative chromatography to separate silybin diastereomers. Using reverse-phase silica with MeOH/water/HCOOH 45/55/0.1 and a column load of just 0.6 mg per injection, the authors were able to isolate roughly 20 mg of each silybin diastereomer (97% purity) in 80 injections. A similar approach was employed by Graf *et al.*[43] In a rather complex procedure with precipitation (MeOH/water) as a key step and 154 HPLC injections, the authors prepared ca 5 g of each silybin diastereomer in purity over 98%. Preparative HPLC was also used in a number of other studies to prepare pure silybin diastereomers.[44-46]

4.2 Chemical methods

Křen *et al.*[16] succeeded in the first diastereomeric resolution using peracetylated silybin *β*-glycosides. The separation of silybin diastereomers can be facilitated by the introduction of a sterically demanding substituent adjacent to stereogenic centers C-10 and C-11 to increase the steric difference between both diastereomers. Flash chromatography on silica gel could then be employed. The closest primary OH group at C-23 was an obvious target for substitution. It turned out that even the glycosidic substituents were barely large enough to enable the separation of both diastereomers using non-chiral chromatography. In principle, this method was the first to provide a routine supply of multigram amounts of pure silybin diastereomers. This method was later optimized using the respective β-galactopyranosides, and deglycosylation was accomplished with the β-galactosidase from *Aspergillus oryzae*.[18] Quite recently, however, we have found that Lewis acids ($BF_3.OEt_2$) used as catalysts in silybin glycosylation cause isomerizations leading to various *cis*-silybins (in minor proportions; see Section 5.6)[19], which are inseparable from the products. Traces of these isomeric impurities may be detected indirectly by the shifts in $[\alpha]_D$.

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4.3 Chemo-enzymatic methods

Stereoselective enzymatic kinetic resolution is often the method of choice for separation of enantiomers or diastereomers. Diastereomeric discrimination of silybin A (**1a**) and silybin B (**1b**) was observed during combination of enzymatic acylation and alcoholysis using lipases. In this procedure, unresolved silybin (**1**) was *O*-acetylated at position C-23 by Novozym435 in acetone using vinyl acetate as an acetyl donor. Following alcoholysis using the same enzyme as catalyst in methyl *t*-butyl ether with *n*-butanol produced silybin B (**1b**, d.e. ca 45%) and 23-*O*-acetylsilybin A (**55a**, d.e. ca 95%). The products were then separated by chromatography on silica gel and silybin B (**1b**) was subjected to the same procedure again. Novozym 435 can be used for preparation of silybin diastereomers in high yields and purity (silybin A (**1a**) – 42% yield, >95% d.e.; silybin B (**1b**) – 67% yield, 98% d.e.). To avoid silybin isomerization the acetylation is performed also by the catalysis of Novozyme435 (instead of acid-catalyzed esterification). This method is robust and scalable and allows the routine production of multigram amounts of pure silybins (**1**) and it was used in another studies.[47, 48]

5 Silybin derivatives

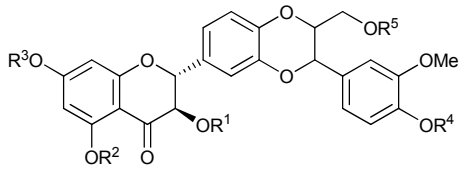
5.1 Ethers and silylethers

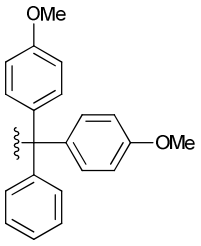
Silybin contains three phenolic hydroxyl groups (positions C-5, C-7 and C-20), one secondary hydroxyl group (C-3) and one primary hydroxyl group (C-23). The relative reactivity of the phenolic hydroxyl groups towards the etherification reaction is approximately 7-OH > 20-OH >> 5-OH. Only a few silybin ether derivatives in positions C-3 have been reported so far. Etherification of the OH groups at positions C-7 and C-20 requires mild conditions and results in mixtures of compounds unless the reaction conditions are carefully optimized (Table 2). Early etherifications (methylations) were connected with structure elucidation experiments[27] and the prepared compounds were not fully characterized. The methylation of phenolic hydroxyl groups was studied by Gažák *et al*.[49, 50] Compounds **34** – **38** were prepared by Wilkinson-type reaction using K_2CO_3 as the base and MeI as the alkyl donor. Methylation with dimethylsulfate is also possible, but it always gives rich mixtures which must be separated on HPLC (compound **39b** – **43b**).[51]

Protected compounds **44** – **51** were prepared during the syntheses of other derivatives. Benzyl ether preparation produced mixtures unless the reaction conditions were optimized. Tritylation always occured at positions C-7 and C-5. Dimethoxytrityl chloride attacked position C-23 and it was used by Zarelli *et al.*[52] to prepare a tetraacetyl derivative (**57**).

Silylation is an alternative to dimethoxytrityl protection of the silybin primary alcohol. The *t*-butyldimethylsilyl group was used by Gažák *et al.*[53] as an alternative route to silybin tetraacetate (**57**, see Scheme 5), to silybinic acid (**118**) and to some gallates (**90 – 93b**).

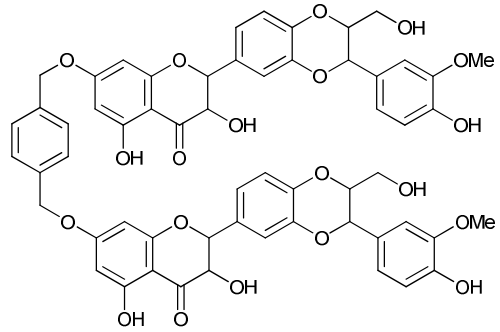
Table 2: Etheric derivatives of silybin.



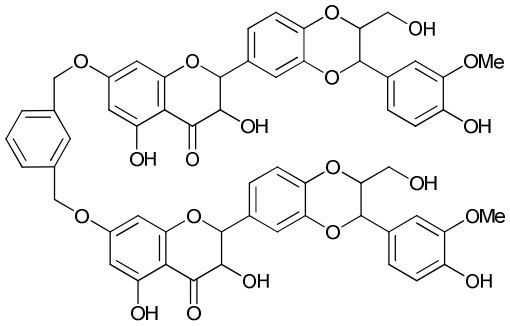
	R^1	R^2	R^3	R^4	R^5	ref.
34	Me	H	H	H	H	50
35	H	H	Me	H	H	49
36	H	H	H	Me	H	49
37	H	H	Me	Me	H	49
38	H	Me	Me	Me	H	49
39b	H	Me	Me	Me	H	51
40b	H	H	Me	H	H	51
41b	H	H	Me	Me	H	51
42b	Me	Me	Me	Me	Me	51
43b	H	H	Me	Me	Me	51
44	H	Trt	Trt	H	H	54
44a	H	Trt	Trt	H	H	55
45	H	H	H	H		52
46	H	H	H	H	TBDMS	53
47	Ac	Ac	Ac	Ac	TBDMS	53
48	H	H	Bn	H	H	49
49	H	Bn	Bn	Bn	TBDMS	54
50	H	Bn	Bn	Bn	H	54
51	MOMGal	H	H	H	TBDMS	54

Dimer-type ethers (**52a** – **54**) were recently prepared by Vavrikova *et al*.[56] using *p*- or *m*- xylene dibromide to form in a low yield a 1,4- or 1,3- phenylene*bis*(methylene) linker connecting two silybin molecules (Table 3). The same authors also prepared other dimeric structures using enzymatic methods.

Table 3: Dimer-like silybin ethers



	partner 1	partner 2	ref.
52a	**1a**	**1a**	[56]
52b	**1b**	**1b**	[56]
53	**1**	**1**	[56]



54

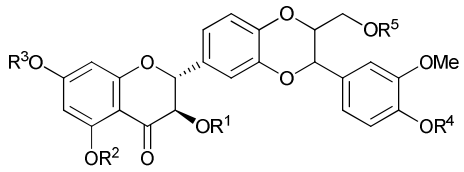
5.2 Esters

Approximately 60 different silybin esters have been prepared so far, mostly in an effort to discover new biologically active derivatives. Many esters can be easily hydrolyzed in tissues by various esterases. The biological effect, however strongly pronounced *in vitro*, could be, therefore, lost *in vivo*. This is supported by the study by Koch *et al.* where authors report the complete hydrolysis of silybin *bis*-hemisuccinate by bovine liver esterases in 30 minutes.[57] On the other hand, flavonoids including silybin can inhibit lipases or esterases increasing the stability of the esters.[48]

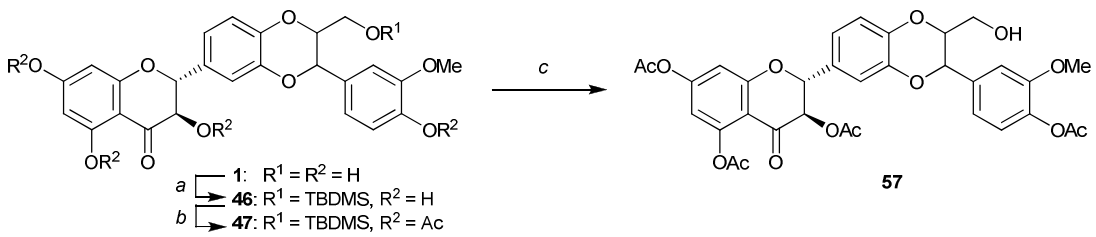
5.2.1 Acetates

From the acetates published so far (**55 – 59b**) silybin pentaacetate (**59**) and silybin 23-acetate (**55**) are readily available (Table 4). Preparation of other acetates is rather laborious but it can open access to selectively protected derivatives. Silybin pentaacetate (**59**) was originally prepared by Pelter and Hänsel,[31] but it was fully characterized by Purchartova *et al*.[58] Silybin 23-*O*-acetate (**55**) recently gained importance as the starting material for the diastereomeric resolution of silybin diastereomers and is readily accessible by Novozym435 transesterification.[48] Alcoholysis of silybin pentaacetate (**59**) by Novozym435 yields mixture of acetates, but the preparation of several silybin polyacetates (most importantly 3,5,7,20-tetra-*O*-acetyl silybin (**57**)) is feasible and probably easier than using traditional protection/deprotection strategies.[58] Gažák *et al.* prepared 3,5,7,20-tetra-*O*-acetyl silybin (**57**) by protecting C-23 OH with a TBDMS group (Scheme 5).[53] However, the use of $BF_3.OEt_2$ is somehow problematic, it can cause – according to our recent observations - isomerizations at some stereogenic centers.[19] Another suitable method for C-23 OH protection is dimethoxytritylation, which predominantly substitutes the primary alcoholic group of silybin.[52]

Table 4: Silybin acetates



	R^1	R^2	R^3	R^4	R^5	ref.
55	H	H	H	H	Ac	[48]
55a	H	H	H	H	Ac	[48]
55b	H	H	H	H	Ac	[48]
56	Ac	H	H	Ac	Ac	[58]
56a	Ac	H	H	Ac	Ac	[58]
56b	Ac	H	H	Ac	Ac	[58]
57	Ac	Ac	Ac	Ac	H	[53]
58	Ac	Ac	H	Ac	Ac	[58]
58a	Ac	Ac	H	Ac	Ac	[58]
58b	Ac	Ac	H	Ac	Ac	[58]
59	Ac	Ac	Ac	Ac	Ac	[31, 58]
59a	Ac	Ac	Ac	Ac	Ac	[58]
59b	Ac	Ac	Ac	Ac	Ac	[58]



Scheme 5: Preparation of silybin tetraacetate.[53] *Reaction conditions: a:* TBDMSCl, $AgNO_3$, pyridin*; b*: Ac_2O, pyridin; *c*: BF_3. OEt_2, $CHCl_3$.

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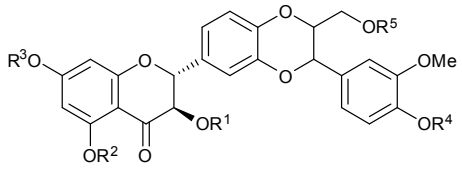
5.2.2 Hemisuccinates

Silybin hemisuccinates (**60 – 63**, Table 5) are another group of compounds, prepared with the aim of increasing silybin solubility in water. Silybin 3,23-*bis*hemisuccinate (**62**) is used clinically for the treatment of severe *Amanita phalloides* poisoning. Silybin possesses the ability to block the uptake of *Amanita* toxins into hepatocytes, but its use is hampered by poor oral availability and water solubility. Silybin 3,23-*bis*hemisuccinate (**62**), although not orally available, is water soluble and can be used intravenously.[59, 60] It is transformed back to silybin by liver esterases, thus concentrating it in the liver, where it is required.[59] The authors, however, incorrectly used the structure of silybin A (**1a**) instead of the silybin diastereomeric mixture (**1**). The preparation of silybin 3,23-*bis*hemisuccinate (**62**) is described only in a patent.[61] Silybin is treated with an excess of succinic anhydride in pyridine to form 3,23-*bis*hemisuccinate (**62**) in a good yield, which was isolated as sodium salt.

5.2.3 Aliphatic and olefinic esters

A good regioselectivity of esterification can be achieved with some long chain carboxylic acids (Table 6). Acylation with acyl chlorides under basic conditions (pyridin) yielded C-7 acylderivatives (**63 – 69**), whereas the use of a Lewis acid (BF_3) as the promoter gave C-23 (**70 – 78**) esters in overall yields of ca 40 %.[49, 62] For the ethylmalonyl ester preparation, the Mitsunobu reaction was used with a 45% yield. Chemical esterification of the silybin primary alcohol group was recently complemented by a mild chemo-enzymatic method (see Section 5.9), which offered a convenient route to some C-23 esters of long chain acids in high yields.[63]

Table 5: Silybin hemisuccinates.



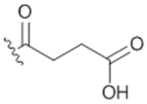
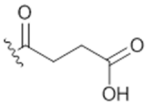
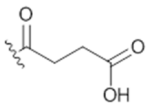
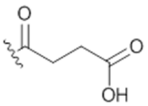
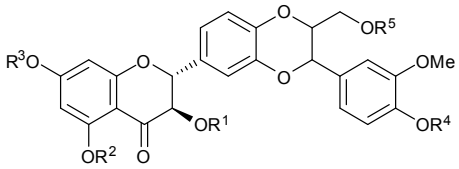
	R^1	R^2	R^3	R^4	R^5	ref.
60	H	H		H	H	60
61	H	H	H	H		60
62		H	H	H		57, 59

Table 6: Aliphatic and olefinic esters of silybin



	R^1	R^2	R^3	R^4	R^5	ref.
63	H	H	butanoyl	H	H	62
64	H	H	octanoyl	H	H	62
65	H	H	dodecanoyl	H	H	62
66	H	H	*cis*-9-hexadecenoyl	H	H	62
67	H	H	*cis, cis*-9,12-octadecadienoyl	H	H	62
68	H	H	*cis*-9-octadecenoyl	H	H	62
69	H	H	*cis*-11-eicosenoyl	H	H	62
70	H	H	H	H	butanoyl	62
71	H	H	H	H	octanoyl	62
72	H	H	H	H	dodecanoyl	62
73	H	H	H	H	*cis*-9-hexadecenoyl	62
74	H	H	H	H	*cis, cis*-9,12-octadecadienoyl	62
75	H	H	H	H	*cis*-9-octadecenoyl	62
76	H	H	H	H	*cis*-11-eicosenoyl	62
77	H	H	H	H	ethylmalonyl	64
78	H	H	H	H	pivaloyl	49

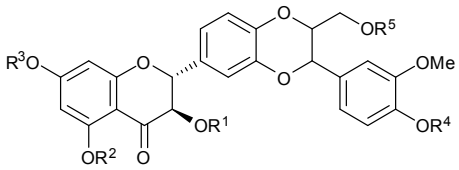
5.2.4 Aromatic esters

Wang *et al.* prepared series of 23-esters of silybin (**79 – 89**), which were designed to improve the antioxidant activity of silybin.[64] Aromatic esters with electron withdrawing, electron donating substituents and with their combination were synthesized by the Mitsunobu reaction. Another group of aromatic esters are gallates prepared by Gažák *et al.* (**90 – 98b**).[54] Silybin reacted with galloyl chloride protected with benzylic groups, which were subsequently removed by hydrogenation (Table 8).

5.2.5 Aminoacid esters

Series of five aminoacid esters of silybin (**99 – 103**) was recently prepared by Dai *et al.*[65] The authors used Mitsunobu esterification with several BOC protected aminoacids to prepare new antiviral agents (Table 7).

Table 7: Aminoacid esters of silybin



	R^1	R^2	R^3	R^4	R^5	ref.
99	H	H	H	H	Gly	[65]
100	H	H	H	H	Ala	[65]
101	H	H	H	H	Phe	[65]
102	H	H	H	H	Leu	[65]
103	H	H	H	H	Ser	[65]

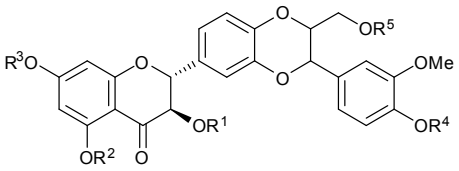
5.3 Glycosides

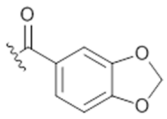
The glycosides prepared by Křen *et al.*[16] (**104 - 113**) were the first silybin glycosides, and were prepared by classical chemical synthesis (Table 9). In the case of silybin (**1**) Koenigs-Knorr glycosylation failed and instead Helferich glycosylation with $BF_3.OMe_2$ as promoter was successfully employed. Diastereomeric silybin glycosides were separated using flash chromatography during this work. Later, syntheses of silybin glycosides utilized a biotransformation approach and are discussed below.

5.4 Oxidations

The most important oxidized silybin derivative is 2,3-dehydrosilybin (**114**, Scheme 6). The hydroxy group at position C-3 of silybin readily oxidizes under basic conditions to a ketone which undergoes enolization, connecting the ring A and B aromatic systems in silybin. Gažák *et al.*[53, 66] studied the base-catalysed oxidation of silybin (**1**) and also provided some mechanistic clues. The authors concluded that dehydrosilybin (**114**) can be also formed without oxygen. In the opinion of the authors of this review,

Table 8: Aromatic esters of silybin



	R^1	R^2	R^3	R^4	R^5	ref.
79	H	H	benzoyl	H	H	[49]
80	H	H	H	H	benzoyl	[64]
81	H	H	H	H	4-methoxybenzoyl	[64]
82	H	H	H	H	3-chlorbenzoyl	[64]
83	H	H	H	H	4-aminobenzoyl	[64]
84	H	H	H	H	4-nitrobenzoyl	[64]
85	H	H	H	H	3,4-dimethoxybenzoyl	[64]
86	H	H	H	H		[64]
87	H	H	H	H	3-amino-5-nitrobezoyl	[64]
88	H	H	H	H	3,5-dinitrobenzoyl	[64]
89	H	H	H	H	3,4,5-trimethoxybenzoyl	[64]
90	galloyl	H	H	H	H	[67]
91	H	H	galloyl	H	H	[67]
92	H	H	H	galloyl	H	[67]
93	H	H	H	H	galloyl	[67]
93a	H	H	H	H	galloyl	[67]
93b	H	H	H	H	galloyl	[67]
94	H	H	H	H	3,4,5-tri-*O*-methoxymethylgalloyl	[67]
95	H	H	H	H	3,4,5-tri-*O*-benzylgalloyl	[67]
96	3,4,5-tri-*O*-benzylgalloyl	H	H	H	H	[67]
97	H	H	3,4,5-tri-*O*-benzylgalloyl	H	H	[67]
98a	H	H	3,4,5-tri-*O*-benzylgalloyl	H	H	[67]

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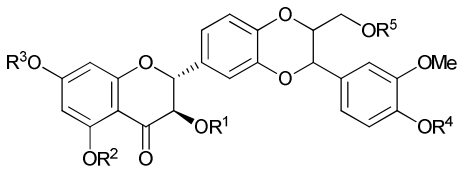
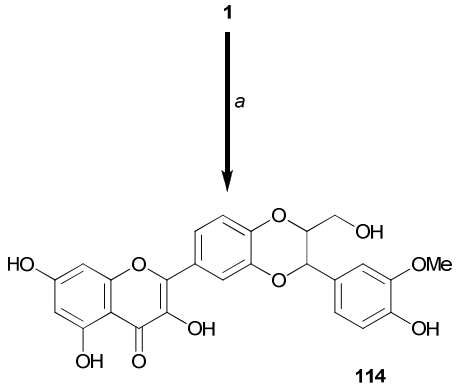
98b	H	H	3,4,5-tri-*O*-benzylgalloyl	H	H	[67]

Table 9: Silybin glycosides



	R^1	R^2	R^3	R^4	R^5	ref.
104	*O*-β-D-galactopyranosyl	H	*O*-β-D-galactopyranosyl	H	H	[16]
105	H	H	*O*-β-D-glucopyranosyl	H	H	[16]
106	H	H	H	H	*O*-β-D-glucopyranosyl	[16]
107	H	H	H	H	*O*-β-D-galactopyranosyl	[16]
108	H	H	H	H	*O*-β-D-lactosyl	[16]
109	H	H	H	H	*O*-β-D-maltosyl	[16]
110	H	H	H	H	*O*-β-D-glcAc$_4$	[16]
111	H	H	H	H	*O*-β-D-galAc$_4$	[16]
112	H	H	H	H	*O*-β-D-lacAc$_4$	[16]
113	H	H	H	H	*O*-β-D-malAc$_4$	[16]

this observation requires further corroboration and reproduction under strictly anaerobic conditions. Whether dehydrosilybin (**114**) is already present in the natural source or whether is it an artifact of processing remains unclear. Silymarin, however, always contains minor amounts of dehydrosilybin (**114**).



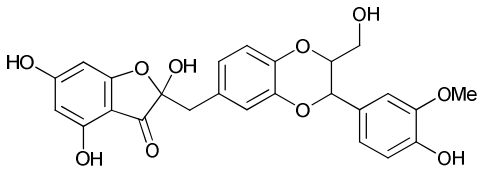
Scheme 6: Preparation of dehydrosilybin. *Reaction conditions: a*: see Table 10.

The oxidation of silybin to 2,3-dehydrosilybin (**114**) removes two of the four asymmetric centers at C-2 and C-3. Oxidation of a natural diastereomeric mixture of silybin A (**1a**) and B (**1b**), therefore results in the formation of a racemate. Several methods for silybin oxidation have been designed over the years and are summarized in Table 10. In some cases partial rearrangement of **114** into hemiacetal **115** was observed.[53, 68]

Table 10: Reaction conditions for dehydrosilybin preparation.

Yield (%)	Time (h)	Solvent	Temperature (°C)	Reagents	Ref.
90	4.5	acetic acid	reflux	I_2, CH_3COOK	[69]
78	0.5	DMF	50	CH_3COOK	[52]
51	100	pyridine	95	oxygen	[53]
13	10	water	reflux	$NaHCO_3$, 3% H_2O_2	[70]

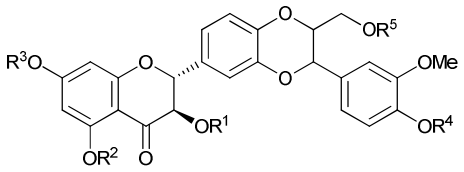
C-23 OH group of silybin can be oxidized to either aldehyde or acid (Table 11). Swern oxidation of a tritylated silybin gave the aldehyde **116** in a low yield, which was used for the preparation



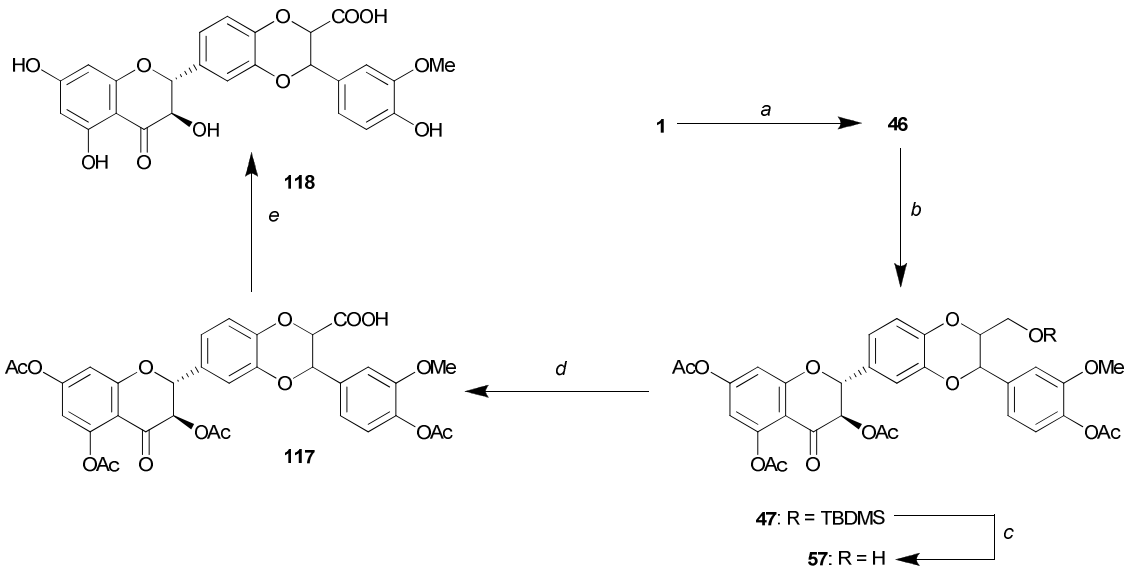
115

of radioactively labeled silybin (**1**).[55] The preparation of silybinic acid **118** requires the protection of all OH groups except the primary and was accomplished with a mixture of H_5IO_6 and CrO_3 (Scheme 7).[53]

Table 11: Oxidized silybin derivatives.



	R^1	R^2	R^3	R^4	R^5	ref.
116	H	Trt	Trt	H	CH_2O	[55]
117	Ac	Ac	Ac	Ac	COOH	[53]
118	H	H	H	H	COOH	[53]



Scheme 7: Preparation of silybinic acid.[53] *Reaction conditions: a*: TBDMSCl, $AgNO_3$, pyridine; *b*: Ac_2O, pyridine; *c*: $BF_3.Et_2O$, $CHCl_3$, r.t., 12 h; *d*: H_5IO_6, CrO_3, CH_3CN; *e*: K_2CO_3, MeOH, r.t., 24 h.

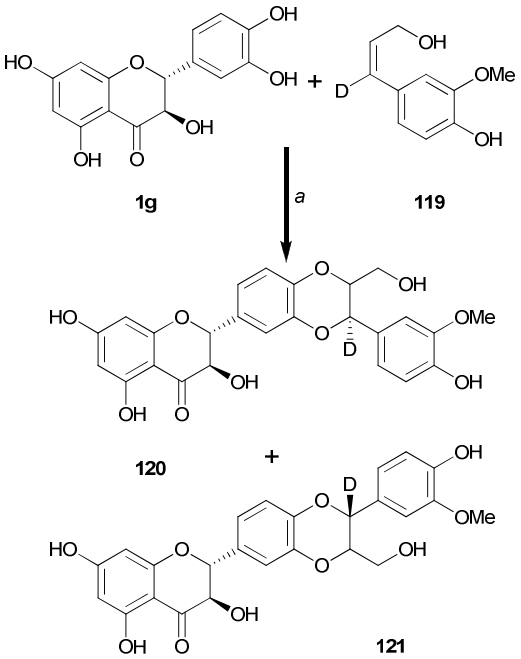
5.5 Isotopic labeling of silybin

To enable the detailed mass spectroscopic investigation of silybin, Ferenczi *et al.*[71] developed a synthesis of deuterium-labeled silybin. The synthesis is based on Merlini's biomimetic synthesis of silybin[36] (Scheme 2) and uses Ag_2O for coupling of a deuterated coniferyl alcohol (**119)** with taxifolin (**1g**) to obtain the diastereomeric mixtures of deuterated silybin (**120**) and deuterated isosilybin (**121,** Scheme 8).

Lee *et al.*[55] developed a method for the preparation of tritium labeled silybin. The primary alcoholic group of partially protected silybin was oxidized by Swern oxidation to give the aldehyde **122** in a lower yield (35%). Reduction with $NaBT_4$ and deprotection yielded tritiated silybin **123** (Scheme 9).

5.6 Isomerization of silybin

Novotná *et al.*[19] recently performed a detailed study on the isomerization of silybin. Epimerization at position C-3 of taxifolin[72] is known, but it was observed with silybin for the first time. The reaction was catalyzed by $BF_3.OEt_2$ and gave rather low yields.



Scheme 8, Preparation of deuterium-labeled silybin.[71] *Reaction conditions: a*: Ag_2O, benzene, aceton, r.t., 48 h.

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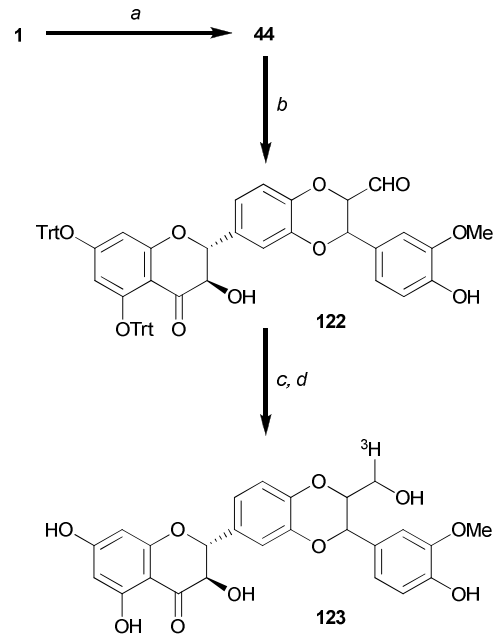
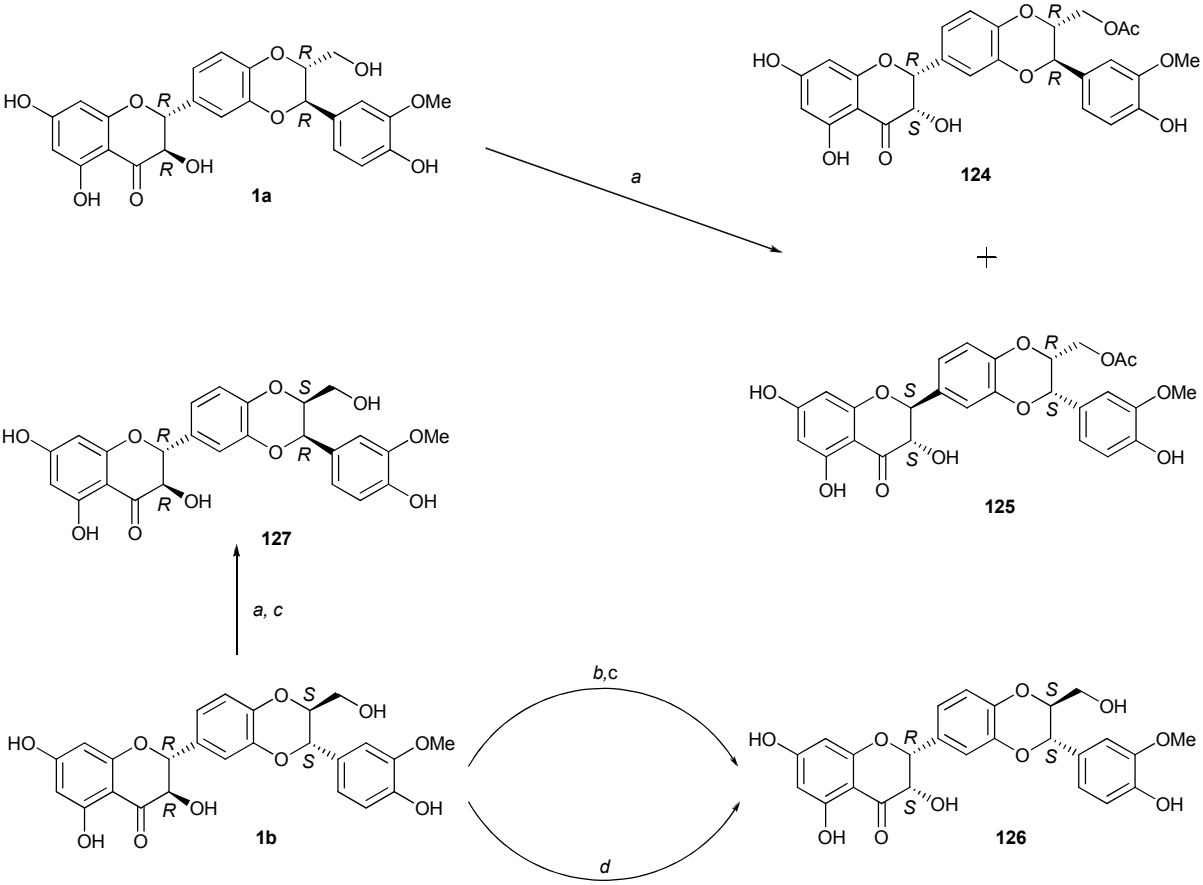


Scheme 9: Preparation of tritium labeled silybin.[55] *Reaction conditions: a*: TrtCl, Et_3N; *b*: Swern oxidation; *c*: NaB^3H_4, -60 °C *d*: TFA, r.t.

In the case of silybin B (**1b**), short reaction times (3 h) results in the epimerization at C-3, yielding 2,3-*cis* (2*R*,3*S*,10*S*,11*S*) epimer **126**. Longer reaction times (48 h) result in the formation of the 10,11-*cis* (2*R*,3*R*,10*S*,11*R*) epimer **127**. Silybin A (**1a**) gives almost exclusively the 2,3-*cis* (2*R*,3*S*,10*R*,11*R*) epimer **124**. An unusual diastereomer **125** with inverted configuration at C-2, C-3 and C-11 was isolated in very low yield. Reactions performed in ethyl acetate were in all cases accompanied with acid-catalyzed transacetylation on C-23 hydroxyl. Separation of the diastereomers was accomplished by chemoenzymatic diastereomeric kinetic resolution using *C. antarctica* lipase Novozym 435 (Scheme 10).

5.7 Other silybin derivatives

Pifferi *et al.*[73] prepared silybin-23-phosphate (**128**) and its sodium salt (**129**) in multigram amounts using $POCl_3$. The sodium salt (**129**) is soluble in water and can be administered intravenously. Increased solubility was also the motivation for the preparation of phosphates and sulphates **130 - 134**.[52] The same authors prepared also one of the few nitrogen containing derivatives of silybin (**135**, **136**). Agarwal *et al.*[74] prepared other water soluble derivative, disulfate **137** using pyridinium sulfate in DMF (Table 12).



Scheme 10: Lewis acid catalysed epimerisation of silybin.[19] *Reaction conditions:*a:$BF_3.Et_2O$, EtOAc, 48 h, 80 °C; *b*: $BF_3.Et_2O$, EtOAc, 3 h, 80 °C; *c*: Novozyme 435, *n*-BuOH, TBME; *d*: $BF_3.Et_2O$, DMF, 1 h, 50 °C;

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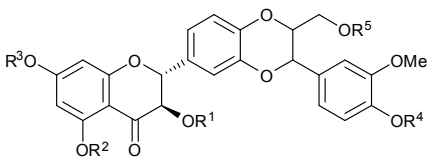
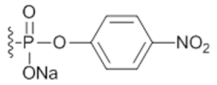
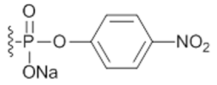
Table 12: Other silybin derivatives



	R^1	R^2	R^3	R^4	R^5	ref.
128	H	H	H	H	PO_3H_2	75
129	H	H	H	H	PO_3HNa	75
130	H	H	H	H		52
131	Ac	Ac	Ac	Ac		52
132	H	H	H	H	SO_3Na	52
133	Ac	Ac	Ac	Ac	SO_3Na	52
134	Ac	Ac	Ac	Ac	OSO_2CH_3	52
135	H	H	H	H	N_3	52
136	H	H	H	H	NH_2	52
137	H	H	SO_3H	H	SO_3H	74

5.8 Chemoenzymatic modifications of silybin

Generally, biocatalytic routes are characterized by mild reaction conditions and often with high chemoselectivity. This is especially important for silybin, which is sensitive to a number of conditions and due to its multiple reactive moieties, chemoselectivity is a must. Biotransformations enable easier product isolation, are environmentally friendly, and when using immobilized enzymes are easily scalable. On the other hand, silybin derivatives suffer from solubility issues, which are often limiting for chemoenzymatic synthesis (Table 13).

5.9 Reactions with lipases

Lipases (EC 3.1.1.3) can work well in organic solvents or at water/organic interfaces and they are frequently used for enantio-, chemo- or regioselective transesterification and ester hydrolysis. Lipases have often been used to modify flavonoids,[76] silybin being no exception. In this particular case, esterification occurs selectively at C-23 OH. Several flavonoids were, however, identified as lipase inhibitors[77, 78] and substrate inhibition must be considered when planning chemoenzymatic synthesis.

The separation of silybin A and B with stereoselective transesterification and/or stereoselective alcoholysis were tested with a wide range of lipases, proteases and acylases in methyl *t*-butyl ether and vinyl acetate. The best results for transesterification were obtained with Novozym 435 (33% conversion after 3 hours, E = 2.6 (**1b**)). In contrast, *C. rugosa* lipase gave a very good conversion rate and quite significantly, it exhibited the opposite diastereoselectivity (17% conversion after 3 hours E = 4.4 (**1a**)). Alcoholysis with *C. rugosa* lipase produced an enriched silybin A (**1a**), while Novozym 435 preferentially produced silybin B (**1b**). The same diastereoselective conversion was also observed with alcoholysis. Novozym 435 acting at a mixture of 23-*O*-acetyl silybin (**52**) hydrolyzed silybin B only (34% conversion, 3 hours, E = 6.5 (**1b**)) and the lipase from *C. rugosa* released silybin A (29% conversion, 5 hours E = 21.1 (**1a**)). These successful attempts proved to be very useful for the production of optically pure silybins in multigram quantities.[48] The selection of a suitable solvent for chemoenzymatic reaction is fundamental for better yields, shorter reaction time and higher stereo-discrimination. The best selectivity for the alcoholysis of 23-*O*-acetylsilybin (**55**) by Novozym 435 was reached in toluene, MTBE and *t*-amyl alcohol. This enzymatic reaction is fully scalable and gives a quantitative yield with no traces of side products. Good regioselectivity was achieved (34 - 40%) and workup of the reaction mixture was rather simple. The purity of the obtained diastereoisomers was over 95% d.e.[47]

Regioselectivity of lipase-catalyzed reactions can be employed in protection/deprotection chemistry of **1**. Optically pure pentaacetylated silybins (**59**) were selectively deacetylated with lipase AK yielding (40%) 3,20,23-tri-*O*-acetyl-silybin (**56**) and 3,5,20,23-tetra-*O*-acetyl-silybin (**58**).[58]

Silybin was selectively transesterified with vinyl butyrate at C-23 OH with Novozym 435 in *t*-butyl alcohol, *t*-amyl alcohol, acetonitrile and acetone with high conversion yields (78, 95, 98 and 100%, respectively). A large excess of acyl donor is required to obtain acceptable reaction rates.[79]

The regioselective acylation of silybin (**1**) was performed in various organic solvents, as well as in imidazolium-based ionic liquids containing either BF_4^- or PF_6^- anions. Silybin 23-*O*-butanoate (**70**) was formed in a one-step biocatalytic process; higher yields (up to 76%) were obtained in the BF_4^-- in contrast with the PF_6^--containing ionic liquids; quantitive yield was obtained in acetone.[80]

Novozym 435 was used for single-step silybin acylation by dicarboxylic acid in various reactant ratios and in various solvents – acetone, acetonitrile, 2-methyl-2-butanol and 2-methyl-2-propanol. Total conversion of silybin to the mono- and diester was achieved in acetone and acetonitrile. In acetone the highest conversion was observed to hexadecanedioate **140** (50%). Dodecandioate **139** and hexandioate **138** was formed in lower yields (30% and 37% respectively).[63] In extension of this approach Vavříková *et al.*[56] used stepwise transesterification of divinyl dodecandioate with Novozym 435 to prepare intermediate **141** and compounds (**149** – **151**, Table 14) formed by two silybin molecules linked with aliphatic chain.

5.10 Chemoenzymatic glycosidations

Silybin glucuronides are important metabolites of Phase II biotransformation. Silybin is quickly metabolized[81] mainly by glucuronate and sulfate conjugation. Exact determination of the type of metabolite is a complicated task, because HPLC or LC-MS methods can only determine the level of conjugation, but not the exact location of the substitution. Isolation of the metabolites from plasma (for NMR) is also a very complicated task. Therefore, preparing the authentic metabolites with defined structures is of paramount importance. Chemical conjugation is neither easy nor cheap, thus the enzymatic methods are an excellent choice.

Silybin was glucuronidated using both pure diastereomers to silybin 7-*O*-β-D-glucuronide (**142a**, **142b**) and silybin 20-*O*-β-D-glucuronide (**143a**, **143b**). The glucuronidation of silybin B (**1b**) was much preferred at position 20, while for silybin A (**1a**), the transformation rate was similar at both positions 7 and 20 (for citation[44] see Table 1, footnote a).[44, 81]

The glucuronidation of optically pure silybin A (**1a**) was performed by ovine liver glucuronyltransferase. The main silybin conjugate was 20-*O*-β-D-glucuronide (**143a**). A C-7 substituted isomer (**144a**) was also formed, but in a lower amount. Its antioxidant activity was higher than silybin (**1**). As a minor product, 5-*O*-β-D-glucuronate (**145a**) was produced in a 2.5% yield.[82]

Silybin (**1**) and its pure diastereoisomers (**1a**, **1b**) were incubated with human liver microsomes or human hepatocytes inherently containing UDP-glucuronosyltransferase (UGT) forming four silybin glucuronides: silybin 7-*O*-β-D-glucuronide A (**142a**), 20-*O*-β-D-glucuronide A (**143a**), 7-*O*-β-D-glucuronide B (**142b**) and 20-*O*-β-D-glucuronide B (**143b**). The glucuronosyltransferases preferred silybin B conjugation and thus the level of silybin B conjugates was higher than that of silybin A derivatives (which was observed also *in vivo*). Additionally, nine human hepatic forms of UGT were active towards the glucuronidation of silybin diastereoisomers. Recombinant UGT1A1, UGT1A3, UGT1A6 and UGT1A9 were responsible for the formation of silybin 7-*O*-β-D-glucuronide A (**142a**) and silybin 7-*O*-β-D-glucuronide (**142b**) when a mixture of silybins or individual diastereoisomers were added. 20-*O*-β-D-glucuronide (**143b**) was produced by UGT1A10 from optically pure silybin B (**1b**). The results outlined the importance of the study of silybin metabolic pathways using optically pure diastereoisomers and confirmed the rather different behavior of pure silybin diastereoisomers.[83]

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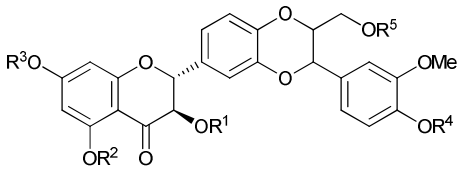
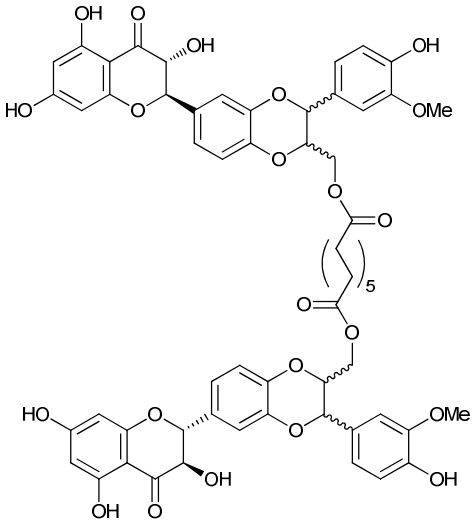
Table 13: Chemoenzymatically prepared derivatives of silybin



	R^1	R^2	R^3	R^4	R^5	Ref.
138	H	H	H	H	$OCO(CH_2)_4COOH$	63
139	H	H	H	H	$OCO(CH_2)_{10}COOH$	63
140	H	H	H	H	$OCO(CH_2)_{14}COOH$	63
141	H	H	H	H	$OCO(CH_2)_{10}COOC_2H_3$	56
142a	H	H	*O*-β-D-glucuronyl	H	H	44 83
142b	H	H	*O*-β-D-glucuronyl	H	H	44 83
143a	H	H	H	O-β-D-glucuronyl	H	44 44 83
143b	H	H	H	O-β-D-glucuronyl	H	44 44 83
144a	H	H	O-β-D-glucuronyl	H	H	44 44 83
145a	H	O-β-D-glucuronyl	H	H	H	82
146a	H	H	*O*-β-D-glucopyranosyl	H	H	84
146b	H	H	*O*-β-D-glucopyranosyl	H	H	84
147a	*O*-β-D-glucopyranosyl	H	H	H	H	84
147b	*O*-β-D-glucopyranosyl	H	H	H	H	84
148a	H	H	H	SO_3H	H	85
148b	H	H	H	SO_3H	H	85, 86

The biotransformation of silybin was studied in the culture of fungus *Trichoderma koningii*. Silybins **1a** and **1b** were transformed into two pairs of glucosylated derivatives: silybin 3-*O*-β-D-glucopyranoside A (**147a**), silybin 7-*O*-β-D-glucopyranoside A (**146a**), silybin 3-*O*-β-D-glucopyranoside B (**147b**) and silybin 7-*O*-β-glucopyranoside B (**146b**). It is noticeable that the OH group at C-3, which is not easy to substitute chemically, was glucosylated.[84]

Table 14: Deodecandioate esters of silybin



	partner 1	partner 2	ref.
149	**1a**	**1a**	56
150	**1b**	**1b**	56
151	**1a**	**1b**	56

5.11 Other chemoenzymatic reactions

The preparation and complete characterization of sulfate derivatives of silybin is of utmost importance for metabolic studies of biotransformation processes. The aryl-sulfate sulfotransferase from the bacterium *Desulfitobacterium hafniense* efficiently sulfated both silybins **1a** and **1b** into silybin sulfate **137a** (yield 58%) and silybin sulfate **148b** (yield 62%).[85] Purchartová *et al.*[86] accomplished an analogous production of silybin sulfate **148b** employing recombinant aryl sulfotransferase IV from rat liver overexpressed in *E. coli* (EC 2.8.2.1). The sulfation of silybin B (**1b**) was selective yielding exclusively silybin sulfate **148b** (48% yield). **1a** was not sulfated with this mammalian enzyme, indicating that the metabolic transformation of silybin is strictly stereoselective and each silybin diastereomer can have a different metabolic pathway.[86]

Cyclodextrin glucanotransferase (CGTase) from *Bacillus stearothermophillus* was used for the enzymatic synthesis of complex silybin oligoglycosides (**152a** – **155b**). In glycosides with a terminal D-glucopyranosyl moiety, the transfer of another α-glucopyranosyl moiety is selective for the 4-OH position. 23-*O*-β-Glucopyranosyl silybin and 23-*O*-β-galactopyranosyl silybin were further glucosylated to an oligomer with an α(1→4) and α(1→3) bond, respectively. The products were tested in a study of low-density lipoprotein oxidation by Cu, the better antioxidant effect of the glycosylated product than silybin was probably connected to its better solubility and/or bioavailability.[87]

Silybin B (**1b**; *cf.* also Table 1, footnote[a]) has been glucosylated at the C-7 position using a plant cell culture of *Papaver somniferum* in a very good yield.[88]

Lacase from *Trametens pubescens* was used for the oxidation of 7-*O*-methyl silybin (**35**) to two dimeric products in ca 2.5:1 ratio (**156**, **157**). In contrast, the oxidation of 20-*O*-methyl silybin (**36**) did not proceeded at all.[89]

6 Antioxidant properties and radical scavenging activity of individual OH groups of silybin

Several structural motifs of silybin and 2,3-dehydrosilybin molecules are distinguished by their individual antiradical activities, which were well documented in several structure-activity relationship studies.[90, 91] Two basic structural patterns can be found in the flavonoid structure – the presence of a catechol group in ring B and the association of the 3-OH group with the 2,3-double bond and C-4 carbonyl group. A conjugated system of double bonds is able to stabilize the cation-free radicals by delocalization.[92]

The antioxidant activity of individual functional groups of silybin and 2,3-dehydrosilybin was systematically studied for the first time by Gažák *et al.*, who chosed the method of selective methylation of silybin and 2,3-dehydrosilybin.[93] The authors prepared selectively protected derivatives with one or more free hydroxyl group(s) and determined their antioxidant activity. These compounds were also examined in various chemical and biological tests for their interaction with free radicals, e.g., by the DPPH scavenging test, the inhibition of microsomal lipid peroxidation (LP) and the inhibition of ROS production during the oxidation of glycerophosphate (GP). The authors concluded that the DPPH radical scavenging activities of silybin and its methyl ethers were considerably lower than 2,3-dehydrosilybin and its methyl derivatives. Similar results were observed in the DPPH test and GP test for silybin. 3-methyl silybin (**34**) and 7-methyl silybin (**35**) were slightly worse antioxidants than silybin. Based on the DPPH test, the 20-OH group was established to be the most active radical-scavenging moiety and also the most important group responsible for the lipoperoxidation inhibitory activity. According to the DPPH test, the 7-OH group may possess pro-oxidant activity, as it easily forms a rather unstable (nonstabilized) 7-O$^{\cdot}$ radical.[93]

Generally, 2,3-dehydrosilybin (**114**) is more lipophilic and less water-soluble than silybin. Due to the 2,3-double bond, which contributes to antioxidative potency, 2,3-dehydrosilybin (**114**) is a several times better antioxidant than silybin, however, it is worse than taxifolin (**1g**) or quercetin. Specifically, it is a 25-fold better radical scavenger and 10-fold better inhibitor of lipid peroxidation than silybin (**1**).[53] Substitution of the 7-OH group causes a 40% decrease in the total DPPH scavenging activity of 2,3-dehydrosilybin (**114**). The 3-OH group is responsible for the electron delocalization of the B-C ring system and is able to react with radicals. The high radical-scavenging activity of the 3-OH group in 2,3-dehydrosilybin (**114**) can be explained by the structure resonance stabilization. The 20-OH and 23-OH groups in **114** are less important for the inhibition of lipid peroxidation.[93] From the results of the DPPH scavenging test, the methylation of 5-OH and 7-OH of **114** decreased the activity of 3-OH, which means that the 5-OH and 7-OH groups enhance the activity of 3-OH *via* their electron-donating effect. Accordingly, methylation of the 3-OH group significantly decreases DPPH radical scavenging activity. In addition, the lower activity of the 5-OMe derivative of **114** is attributed to the loss of the H bond between the 5-OH and 4-C=O leading to a slight increase in the 3-OH bond dissociation energy. Based on the LP test, 3-OH is responsible for the inhibition of lipid peroxidation in **114**.[94]

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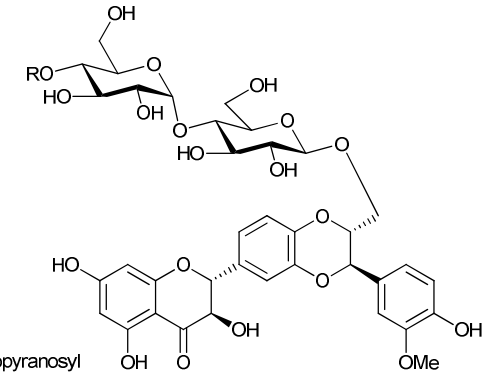
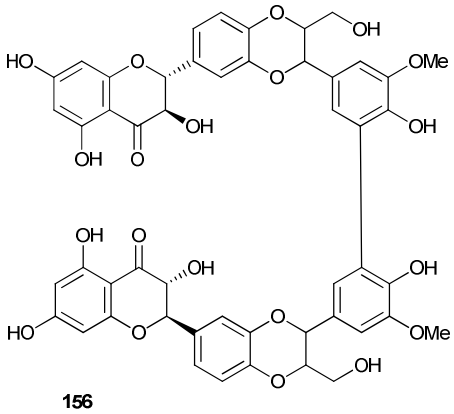
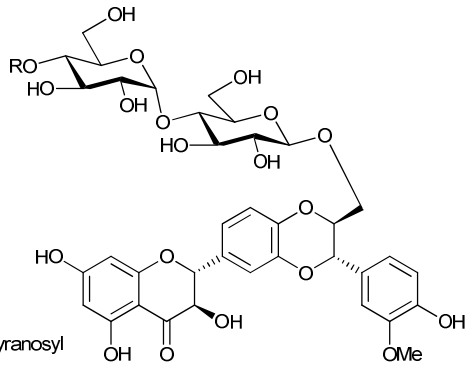


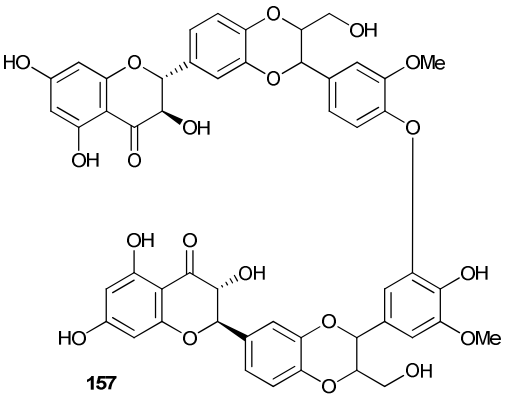
152a: R = H
153a: α-D-glucopyranosyl



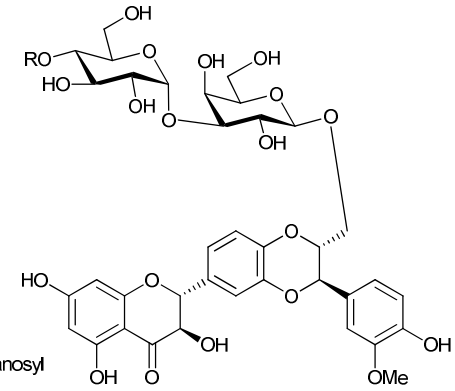
156



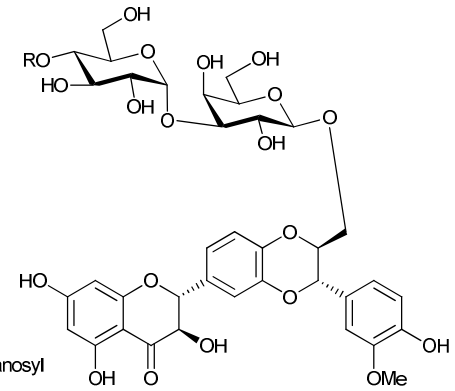
152b: R = H
153b: α-D-glucopyranosyl



157



154a: R = H
155a: α-D-glucopyranosyl



154b: R = H
155b: α-D-glucopyranosyl

7 Conclusions

Silybin (**1**) exhibits a wide range of biological activities, but none of them dominate. Synthetic efforts have been aimed towards two main goals. First, there is an effort to derivatize silybin (**1**) to discover new biological activities of the derivatives and second, the syntheses aim to make its biological activities more pronounced and selective. In the more than 50 years since the discovery of silybin (**1**) about 200 derivatives of silybin (**1**) have been prepared and one silybin derivative is now used clinically against mushroom intoxication. Silybin however, has seen its success as a widely used nutraceutic and the production of silymarin is steadily rising. Synthetically, the most promising and unexplored way in our opinion is skeletal modifications, either in the form of skeletal rearrangements or partial degradations giving access to new structural motifs. For skeletal rearrangements, ring C is the likely target, whereas the demethylation of the phenolic methoxy group (aiming at *nor*-silybin) still remains a challenge. Another promising possibility for the future lies in further refinement and scale up of the separation processes for silybin diastereomers and other pure silymarin components.

8 Acknowledgements

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