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# Base-catalyzed oxidation of silybin and isosilybin into 2,3-dehydro derivatives

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ABSTRACT

The base-catalyzed oxidation of the flavonolignans, silybin, and isosilybin into the corresponding 2,3-dehydro derivatives has been studied and optimized. Various bases, solvents, and reaction conditions were tested to achieve the best yields. The influence of water on the course of the reaction was also observed. It was found that this oxidation reaction was probably based on the disproportionation of the (iso)silybin (or some intermediate) molecule, as the reaction also proceeds in the absence of oxygen. We report here for the first time the preparation of optically pure 2,3-dehydroisosilybins A and B.

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The flavonolignan silybin (**1**, Fig. 1; synonym 'silibinin') is a very commonly reported component of the milk thistle (*Silybum marianum*). However, the crude extract from the seeds of this plant—so-called silymarin—contains other constituents (isosilybin, silychristin, silydianin, 2,3-dehydrosilybin, taxifolin, and polymeric polyphenolic compounds) whose biological activities have been neglected. The situation is complicated by the fact that most silymarin components occur as pairs of diastereoisomers (silybin, isosilybin, silychristin) or enantiomers (2,3-dehydrosilybin).

At least two of these compounds—2,3-dehydrosilybin (**2**) and isosilybin (**3**)—appear to be more potent than silybin in various complex biological tests focusing on, for example, their antitumor and antiproliferative activities.

2,3-Dehydrosilybin is a minor component in silymarin[1] with significantly better anticancer[2–4] and antioxidant[5,6] activity than silybin. Moreover, some C-prenylated[7] and certain O-alkylated[8] and 8-prenylated[9] derivatives of 2,3-dehydrosilybin are effective modulators of P-glycoprotein (Pgp). The 2,3-dehydro derivative **2** is an effective inhibitor of *Plasmodium* strains.[10]

2,3-Dehydrosilybin is a potent protectant against $H_2O_2$-induced oxidative stress in human keratinocytes and mouse fibroblasts,[11] it suppresses UVA-caused oxidative stress in the skin[12] and also decreases reactive oxygen species (ROS) formation in rat heart mitochondria.[13] Compounds **1** and **2** are inhibitors of glucose uptake in adipocytes and Chinese hamster ovarian (CHO) cells.[14]

Natural isosilybin (**3**)—a silybin regioisomer—is, similar to silybin, a mixture of the two diastereoisomers A (**3a**) and B (**3b**). Recent studies have demonstrated that **3** is probably the most potent anticancer agent present in silymarin. Compound **3** possesses in vivo antiproliferative, anti-angiogenic, pro-apoptotic, and cell-cycle modulatory properties.[15] Moreover, isosilybin treatment inhibits the growth of advanced human prostate cancer cells in vivo without any toxic effects. Isosilybin B has more profound effects upon most cell regulatory pathways than isosilybin A in various prostate cancer cell lines.[16,17]

Although **4** has not yet been detected in silymarin, it should be present in this natural mixture at least as a result of isosilybin oxidation—as in compound **2**. The oxidation of **1** into **2** improves significantly its antioxidant and anticancer activities, therefore, we expect that the analogous oxidation of **3** (being a more potent anticancer agent than silybin) into **4** (which has not been described as yet) should provide a more active anticancer compound than both 2,3-dehydrosilybin and isosilybin.

The major aim of this work was to develop a novel, robust, and scaleable method for the oxidation of silybin and isosilybin to the corresponding 2,3-dehydro derivatives; the method is based on base-catalyzed disproportionation. The key part of this work was the preparation and structural description of the optically pure 2,3-dehydrosilybins A and B **2**, and 2,3-dehydroisosilybins A and B **4**.

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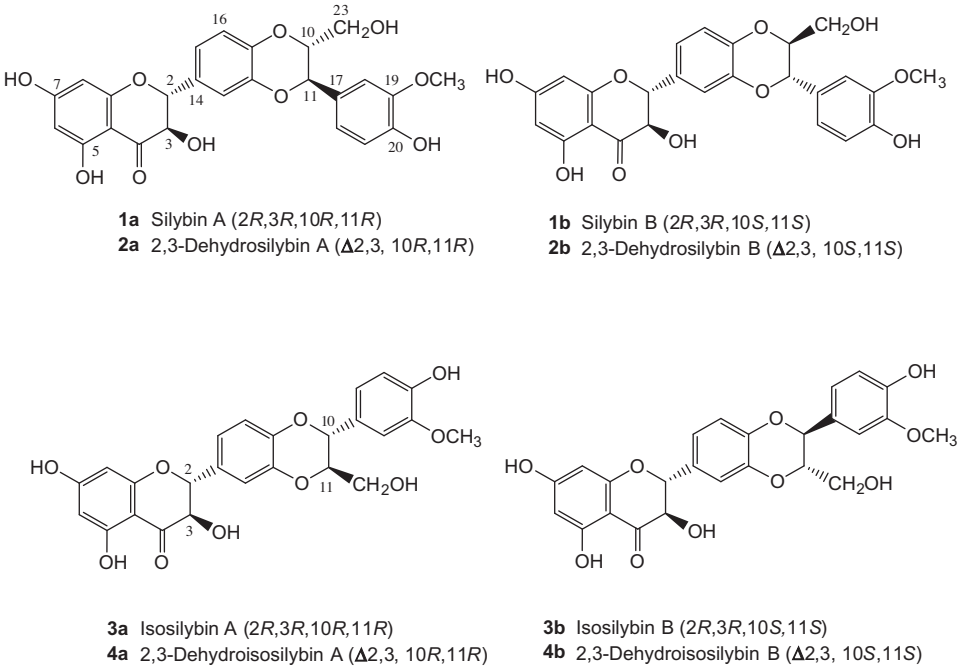


**1a** Silybin A (2*R*,3*R*,10*R*,11*R*)
**2a** 2,3-Dehydrosilybin A (Δ2,3, 10*R*,11*R*)

**1b** Silybin B (2*R*,3*R*,10*S*,11*S*)
**2b** 2,3-Dehydrosilybin B (Δ2,3, 10*S*,11*S*)

**3a** Isosilybin A (2*R*,3*R*,10*R*,11*R*)
**4a** 2,3-Dehydroisosilybin A (Δ2,3, 10*R*,11*R*)

**3b** Isosilybin B (2*R*,3*R*,10*S*,11*S*)
**4b** 2,3-Dehydroisosilybin B (Δ2,3, 10*S*,11*S*)

**Figure 1.** Silybin, isosilybin, and respective dehydro derivatives. Structures **1**, **2**, **3**, and **4** occur as equimolar mixtures (natural) of both respective stereoisomers **a** and **b**.

The first method for the synthesis of 2,3-dehydrosilybin (**2**) involved the oxidation of silybin with iodine in K-acetate buffer.[18,19] This method gave a high yield of 2,3-dehydrosilybin (**2**, 70–80%), which contained side products that were difficult to separate, and an unreacted starting material. The reaction only gave a ca. 50% yield of pure **2** after repetitive crystallizations. Another drawback of this method was the formation of acetates that had to be hydrolyzed (HCl) prior to final purification. Moreover, both silybin and iodine have rather low solubilities in acetic acid, which made scale-up difficult. Another approach leading to **2** is based on (supposed) atmospheric oxidation of silybin in the presence of a base, for example, treatment of silybin with *N*-methylglucamine where **2** was formed as one of the side products,[20] or dehydrogenation of silybin to **2** in the presence of air in boiling pyridine.[5] Recently, the oxidation of silybin to 2,3-dehydrosilybin was achieved in the presence of air using potassium acetate in DMF at 50 °C.[21] However, the yield strongly depended on the work-up (as observed by ourselves) (Table 1).

The major disadvantage of the above base-catalyzed procedures was the use of harmful solvents (pyridine or DMF), which are incompatible with the large-scale preparation of 2,3-dehydrosilybin intended for pharmaceutical applications.

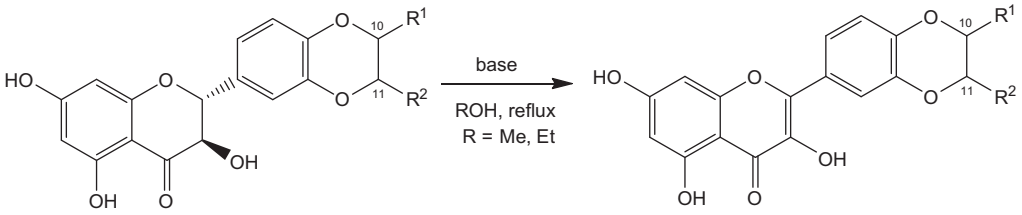
All previous methods for 2,3-dehydrosilybin preparation, with the exception of iodine oxidation, are similar in principle as they proceed via oxidation of silybin by oxygen, in an alkaline milieu. We assumed that this reaction was applicable to other 2,3-saturated flavonolignans and its course depended only on the solvent, base, and reaction conditions. The choice of suitable solvents was rather limited by the relatively poor solubility of silybin in most organic solvents as well as in water. Silybin is virtually insoluble in solvents such as $CH_2Cl_2$, toluene, hexane, and diethyl ether. It is, however, relatively soluble in polar solvents. Moreover, its solubility increases in the presence of bases due to phenolate formation. Accordingly, acetone and simple alcohols, such as MeOH and EtOH, were tested as solvents for this reaction. Since most inorganic bases, for example, carbonates and hydroxides, are rather insoluble in organic solvents, solvent mixtures containing water were also tested. Only negligible differences in the yields of **2** were observed with the different solvents, keeping the conditions identical. The presence of water in the solvent (up to 10%) did not affect the course of the reaction.

The reactions using various inorganic and organic bases were monitored by HPLC (Scheme 1, Table 1). The choice of the base was limited by its strength, because strong bases such as alkali metal hydroxides cause rapid decomposition of silybin. The reaction rate was relatively slow at room temperature, therefore all the reactions were tested under reflux. Four equivalents of the base versus **1** or **3** were found to be optimal for the product yield. Finally, the reaction was tested both in the presence and absence of atmospheric oxygen. Surprisingly, the reaction proceeded similarly in both cases, which indicated that molecular oxygen was not required for this oxidation reaction. This observation can be corroborated by the behavior of silybin during alkylation reactions, as it always forms a small amount of 2,3-dehydrosilybin analogues, despite working under strict anaerobic conditions. Moreover, a similar observation was reported recently in a study concerning the

**Table 1**
Effect of the base on silybin oxidation

| Base (equiv) | Solvent | Time (h) | Yield of **2** (%) |
|---|---|---|---|
| $K_2CO_3$ (4) | EtOH/$H_2O$ (9:1) | 16 | 45 |
| $K_2CO_3$ (4) | EtOH | 16 | 40–45 |
| $K_2CO_3$ (4) | MeOH | 16 | 40–45 |
| $K_2CO_3$ (4) | MeOH | 1.5 | 48 |
| $Na_2CO_3{\cdot}10H_2O$ (4) | MeOH | 16 | 37 |
| $NaHCO_3$ (4) | MeOH | 16 | 51 |
| $Cs_2CO_3$ (4) | MeOH | 16 | 20 |
| $Et_3N$ (4) | MeOH | 24 (72) | 20 (40) |
| KOAc (4) | MeOH | 16 (48) | 37 (49) |
| $Ba(OH)_2$ (4) | MeOH | 16 | n.r.[b] |
| DMAP (4) | MeOH | 16 (94) | 20 (50) |
| $NH_4OH$ (4) | MeOH | 16 (42) | 32 (50) |
| $Li_2CO_3$ (4) | MeOH | 16 (48) | 28 (40) |
| DBU (4) | MeOH | 16 (48) | 10 (27) |
| KOAc (3)[a] | DMF[a] | 0.5[a] | 21[a] (78)[c] |

[a] Experiment was performed according to the reported method[21] with a slight modification in the work-up that consisted of precipitation from an aqueous solution of HCl.
[b] No reaction.
[c] Yield reported in the literature.[21]




**1** $R^1 = CH_2OH$, $R^2$ = 4-hydroxy-3-methoxyphenyl
**3** $R^1$ = 4-hydroxy-3-methoxyphenyl, $R^2 = CH_2OH$
**1a** 10*R*,11*R*, **1b** 10*S*,11*S*
**3a** 10*R*,11*R*, **3b** 10*S*,11*S*

**2** $R^1 = CH_2OH$, $R^2$ = 4-hydroxy-3-methoxyphenyl
**4** $R^1$ = 4-hydroxy-3-methoxyphenyl, $R^2 = CH_2OH$
**2a** 10*R*,11*R*, **2b** 10*S*,11*S*
**4a** 10*R*,11*R*, **4b** 10*S*,11*S*

**Scheme 1.** Base-catalyzed oxidation of silybin and isosilybin into 2,3-dehydro derivatives.

thermal rearrangement of taxifolin (flavanonol) into alphitonin, a process during which quercetin (2,3-dehydroflavanonol) was formed as a side product despite the work being performed under a strictly inert atmosphere with a degassed solvent.[22] Based on these results, we conclude that an alternative oxidation mechanism takes place in this situation. Since no oxidizing agent is present and the oxidation reaction is always accompanied by decomposition products, we infer that the formation of dehydrosilybins from the respective silybin under alkaline conditions is a disproportionation reaction. This hypothesis is also supported by the fact that the maximum yield of 2,3-dehydrosilybin was very close to 50%. During the reaction, the formation of polar compounds (TLC) was observed, which however decomposed quickly into polymers. These might be the reduced products of disproportionation, but without knowledge of their structure(s), we cannot really speculate on the reaction mechanism.

The strength of the base influenced positively the kinetics of 2,3-dehydrosilybin formation. However, the best results were more likely to be obtained with relatively weak bases such as $NaHCO_3$, KOAc, $NH_4OH$, or DMAP after a longer reaction time (16–94 h). Probably, stronger bases ($Cs_2CO_3$ or DBU) cause simultaneous decomposition of product **2**, which results in the lower yield.

The optimized conditions[23] were used for the first preparation of both optically pure 2,3-dehydrosilybins A and B (**2a** and **2b**) starting from stereochemically pure **1a** and **1b**. Subsequently, the synthesis of 2,3-dehydroisosilybins A and B starting from **3a** and **3b** was accomplished (the experimental procedure and analytical data for these new compounds are described in the Supplementary data associated with this work).

In conclusion, we have described the base-catalyzed preparation of optically pure 2,3-dehydrosilybins A and B and 2,3-dehydroisosilybins A and B. This oxidation reaction was probably based on the disproportionation of the silybin or isosilybin (or some intermediate) molecule, as the reaction was found to proceed in the absence of oxygen.

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## Supplementary data

Supplementary data (supplementary data ($^1H$ and $^{13}C$ NMR, MS, HPLC, [α]D, and CD)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.11.049. These data include MOL files and InChiKeys of the most important compounds described in this article.

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23. Silybin (2.5 g, 5.183 mmol) and $NaHCO_3$ (1.74 g, 20.798 mmol) were dissolved in MeOH (100 mL) and the mixture was heated under reflux for 16 h. The mixture was then left to cool to room temperature and poured into ice-cold water containing HCl (400 mL, 5% v/v). The precipitate formed was filtered off, washed with $H_2O$, dissolved in a mixture of EtOAc/acetone (1:1), and evaporated to give 2.17 g of dry residue. The solid was crystallized from MeOH (1000 mg, 40% yield). The mother liquor was filtered through a silica gel pad ($CHCl_3$/acetone/HCOOH 90:10:1–70:30:1) to obtain, after concentration, another portion of the product, which after recrystallization from MeOH yielded pure **2** (270 mg, 11%). Thus, the total yield of **2** was 51%. Full spectral characterization of compounds **2a**, **2b**, **4a,** and **4b** ($^1H$ and $^{13}C$ NMR, MS, HPLC, $[\alpha]_D$, and CD) are given in the Supplementary data.