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Microwave-assisted oxidation of silibinin: a simple and preparative method for the synthesis of improved radical scavengers

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ABSTRACT

A new and preparative oxidation of silibinin has been developed to give access to two different silibinin derivatives known for their enhanced antioxidant properties. Conventional heating methods were compared with results obtained from microwave (MW) heating. The base-catalysed oxidation of silibinin under MW heating is a very efficient method for the preparation of 2,3-dehydrosilybin and a related silybin rearrangement product. This latter compound shows enhanced radical scavenging properties. Optimised conditions were used to prepare 2,3-dehydrosilybins A and B from optically pure silybins A and B. An efficient, preparative purification method was also developed to enable isolation of different products in high purity.

Flavolignans are polyphenolic compounds found abundantly in edible plants (e.g., fruits and vegetables) that are extremely important for human nutrition. Silibinin (Fig. 1) is a prominent component (approximately 30%) of the silymarin complex extracted from milk thistle [*Silybum marianum* (L.) Gaertn. Carduus marianus L., Asteraceae]. Silibinin exists as two diastereomers: silybin A and silybin B. The two isomers occur naturally as a mixture in approximately 1:1 ratio and are epimeric at positions C-7″ and C-8″ in the lignan moiety.[1] Several reviews have suggested the use of the term 'Silibinin' for this mixture to prevent confusion with the pure compounds silybin A (7″*R*, 8″*R*) or silybin B (7″*S*, 8″*S*).[2,3]

Silibinin has long been recognised for its various pharmacological properties.[4] It has been shown to exhibit antioxidant, hypocholesterolemic,[5] antitumour[6–8] cardioprotective, neuroprotective and antiviral activity.[9] Many components of silymarin (Fig. 1) occur as pairs of diastereomers (silibinin, isosilybin, silychristin) or enantiomers (2,3-dehydrosilybin), some of which possess very attractive pharmacological properties. The oxidation product of silibinin, 2,3-dehydrosilybin, shows more potent antioxidant activity than its parent compound. This compound also appeared to be more effective than silibinin in biological assays comparing their antitumour and antiproliferative potencies.[10] The oxidation product has also shown positive effects against some skin diseases (*e.g.*, psoriasis and atopic eczema).[11] Extracts from seeds of *Silybum marianum* were commonly found to contain traces of 2,3-dehydrosilybin.

This compound most likely causes the characteristic yellow colour of silybin preparations. As it is not easily isolated as a natural product, 2,3-dehydrosilybin was utterly neglected in studies on the biological activity of silibinin and silymarin. Therefore, simple synthetic methods were developed to prepare 2,3-dehydrosilybin[12,13] and its analogues.[14,15]The preparation of 2,3-dehydrosilybin from silibinin was accomplished by different methods, including treatment with H_2O_2 in a solution of $NaHCO_3$ or with *N*-methylglucamine.[12] Recently, this oxidation was effected by reaction with pyridine at reflux.[16] An alternate approach employed potassium acetate in DMF at 50 °C.[17] An important byproduct obtained from alkaline treatment of silibinin is hemiacetal **3** (Scheme 1). This compound was first isolated and characterised by Křen, et al. and was found to be a more potent antioxidant than either silibinin or 2,3-deydrosilibin. Nevertheless, little is known about its biological properties, as to date it has been obtained only as an undesired side product.[16]

The recent report of base-catalysed oxidation of silibinin, silybin and isosilybin generated wide discussion.[18] Different reaction conditions were described that employed various solvents and bases. In each case the reaction was carried out in solvents (e.g., MeOH or EtOH) in which silibinin shows very limited solubility (less than 10 mg/mL). Reaction yields never exceeded 50%. Furthermore, 2,3-dehydrosilybin (**2**) was the only product isolated and could only be recovered through long and tedious crystallisation steps. Silibinin is virtually insoluble in nonpolar solvents (DCM, toluene, hexane, diethyl ether) and is relatively soluble in polar

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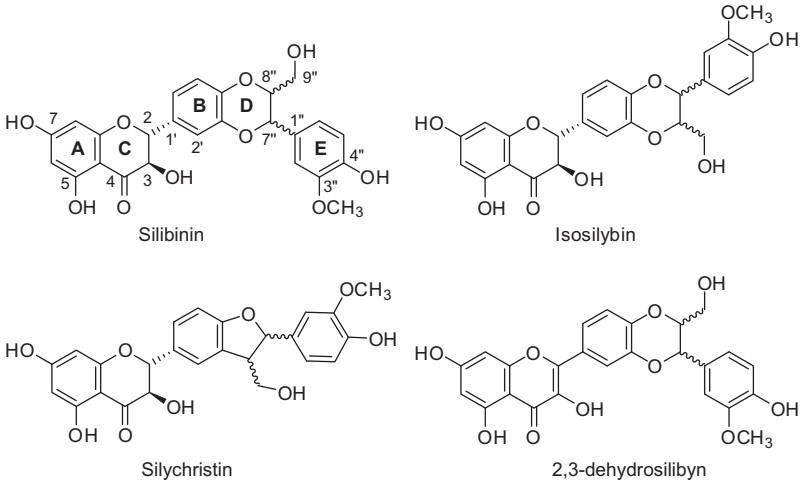


Figure 1. Some components of the extract from milk thistle.

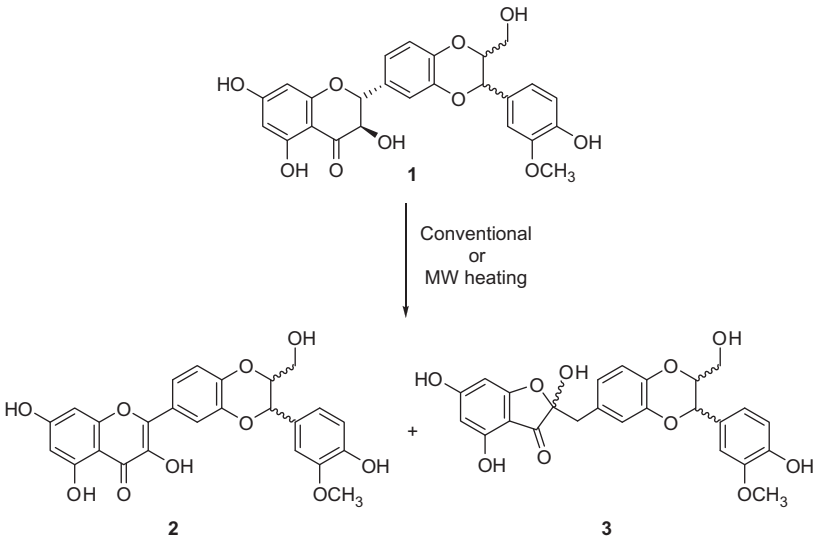
solvents. Its solubility increases in the presence of a base due to the deprotonation and the formation of the phenolate ion.

We recently took an interest in the oxidation of silibinin (**1**) to 2,3-dehydrosilybin (**2**). We also sought to develop a preparative HPLC method to separate the diastereomers of silibinin[19] and to exploit the enhanced solubility of silibinin in tetrahydrofuran (THF) or 1,4-dioxane solvents in which this metabolite is highly soluble (>120 mg/mL). It is our view that an efficient, preparative base-catalysed oxidation of silibinin has yet to be developed and that consistently effective purification methods are still required. In the last year, we focused our research on the use of microwave heating in conjunction with solvents in which silibinin is readily soluble. In addition to being energy-efficient, microwaves can also enhance reaction rates and in many cases improve product yield. The increasing interest in this area is evidenced by the large number of papers and reviews that have appeared in the literature in recent years.[20]

In this study, we report new preparative conditions for the oxidation of silibinin and silybin. We explored conventional heating methods and microwave (MW) heating to promote the reaction. We have also developed a preparative purification method to isolate 2,3-dehydrosilbin (**2**) and hemiacetal **3**, compounds with remarkable antioxidant activity that continue to spark much interest. We examined the effect of several solvents in our procedure, including 1,4-dioxane, THF, MeOH, pyridine and DMF. We also explored the utility of solvent mixtures. We investigated the effect of two bases, AcOK and Et_3N. Reactions were monitored by RP-HPLC[21] (Fig. 2) and quenched by removing the solvent under reduced pressure.

The strength of the bases chosen was a significant consideration because exposure to strong bases, such as alkali metal hydroxides, leads to the rapid decomposition of silybin. Reaction yields were calculated by weighing the products obtained after chromatographic purification over a pre-packed RP-18 column. Products were eluted with a ternary mixture of $CH_3OH/CH_3CN/H_2O$ containing increasing proportions of CH_3CN.[22] The formation of product **3** was not observed under conventional heating. The best reaction yields were obtained when the solvent was DMF (78%), consistent with previous reports. Yields from reactions in other solvents never exceeded 60% (Table 1). The purified product **2** was fully characterised by NMR (^1H, ^{13}C) and ESI-MS analysis, and these data were compared with published results to confirm the structure. The purity of **2** was greater than 97% in all cases, as determined by analytical HPLC.

The base-catalysed oxidation of silibinin was subsequently carried out under microwave heating. We varied several conditions



Scheme 1. Base-catalysed oxidation of Silibinin **1**.