Tetrahedron Letters 54 (2013) 6279–6282

Contents lists available at ScienceDirect

Tetrahedron Letters

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

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

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ARTICLE INFO

Article history:
Received 23 July 2013
Revised 4 September 2013
Accepted 11 September 2013
Available online 19 September 2013

Keywords:
Silibinin
Silybin
2,3-Dehydrosilybin
Oxidation
Microwave assisted reactions

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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0040-4039/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2013.09.035

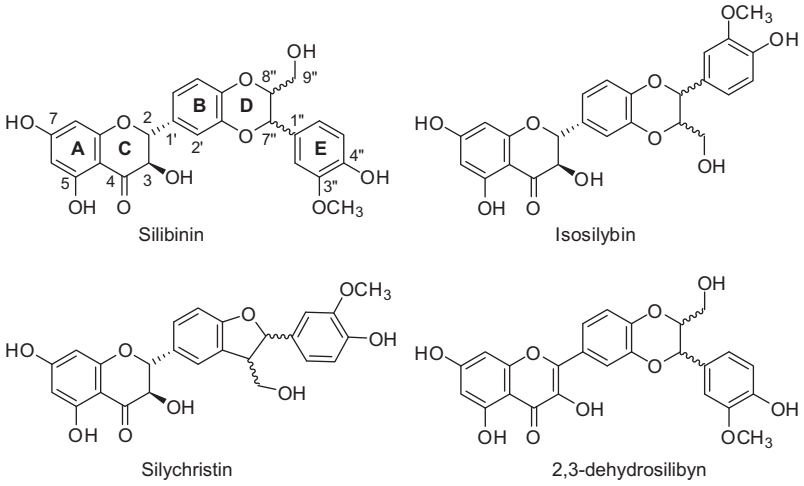


Figure 1. Some components of the extract from milk thistle.

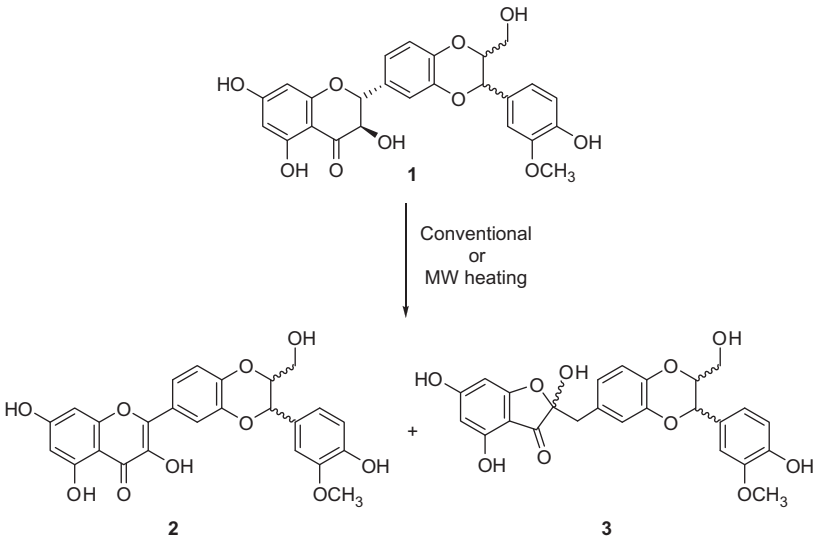
solvents. Its solubility increases in the presence of a base due to the deprotonation and the formation of the phenolate ion.

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**.

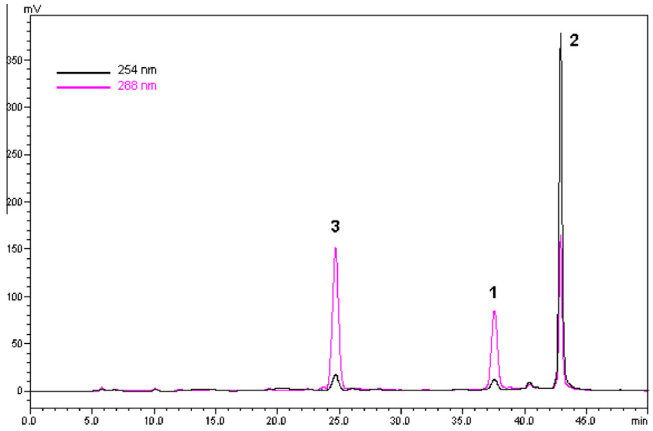


Figure 2. A typical HPLC profile of a reaction under MW heating: analytical Phenomenex LUNA RP-18 column (5 μm particle size, 10.0 mm × 250 mm i.d.), eluting with H_2O/CH_3CN (10 to 100% in 50 min), flow rate 1.0 mL/min, monitored at 254 and 288 nm.

Table 1
Base catalysed oxidation of silibinin exploiting the classical heating method (oil bath)

Base (3 equiv)	Solvent	Temperature (°C)	Time (h)	Yield[a] (%)	
				[2]	[3]
KOAc	THF	Reflux	24	25	—
KOAc	THF	Reflux	48	40	—
TEA	THF	Reflux	24	25	—
TEA	THF	Reflux	48	40	—
KOAc	Dioxane	Reflux	24	25	—
KOAc	Dioxane	Reflux	48	50	—
TEA	Dioxane	Reflux	24	25	—
TEA	Dioxane	Reflux	48	45	—
KOAc	DMF	80	0.5	78[b]	—
—	Pyridine	95	100	60 (51)[c]	— (<10)[c]

[a] See experimental details.
[b] See Ref. 18.
[c] See Ref. 17.

(solvent, temperature, time) and all reactions were carried out in a minimum volume of solvent. As expected, reaction yields (Table 2) were very low in slightly polar solvents with low dielectric constants, such as THF and 1,4-dioxane (yields ⩽25%).[23] Similar results were obtained using solvent mixtures (see Table 2) composed of CH_3OH and H_2O, which have high dielectric constants.

In some cases the harsh reaction conditions led to rapid and substantial decomposition of starting material and the formation of a brown and insoluble residue. When the reaction was conducted in 1,4-dioxane: MeOH (8:2, v/v) at 80 °C in the presence of AcOK, we observed partial degradation of silibinin and the formation of pitchy and insoluble brown material (ca. 40 wt %). Reactions in DMF or pyridine gave very interesting results. Conducting the reaction in DMF at 50 °C led to the formation of **2** and **3** in 70% and 15% yield, respectively, within 30 min. When the reaction was carried out in pyridine, **2** and **3** were formed in variable yields depending on the reaction temperature. At 110 °C, the product distribution favoured formation of **3** (62%) over **2** (32%). At a slightly lower temperature (100 °C), formation of product **2** (28%) predominated over **3** (18%). All reactions were monitored by RP-HPLC (Fig. 2) and were quenched by removing the solvent under reduced pressure. In all cases, the yield of **3** (15–62%) was improved over previously reported yields.[17] We subjected pure silybins A and B to the MW oxidation conditions. Heating in DMF at 50 °C gave 2,3-dehydrosilybins A and B in average yields of 72%. Conducting the MW oxidation in pyridine at 110 °C gave good yields of the corresponding hemiacetals. Crude products

Table 2
Base catalysed oxidation of silibinin exploiting the heating promoted by microwave (MW)

Base (3 equiv)	Solvent	Temperature (°C)	Time (h)	Yield[a] (%)	
				[2]	[3]
KOAc	THF	50	1.5	15	—
KOAc	THF/H_2O (9:1, v/v)	50	1.5	18	—
—	THF/TEA (2:1, v/v)	60	2.5	25	—
—	THF/TEA (1:1, v/v)	60	2.5	25	—
KOAc	Dioxane/CH_3OH (8:2, v/v)	110	0.5	n.r.	
KOAc	Dioxane/CH_3OH (8:2, v/v)	90	0.5	n.r.	
KOAc	Dioxane/CH_3OH (8:2, v/v)	80	0.5	20	5
TEA	Dioxane/CH_3OH (8:2, v/v)	80	0.5	20	5
KOAc	Dioxane/CH_3OH (8:2, v/v)	50	10	35	—
KOAc	DMF	50	0.16	70	15
—	Pyridine	150	1.5	n.r.	
—	Pyridine	110	3.5	32	62
—	Pyridine	100	3.5	28	18

[a] See experimental details; n.r.= was observed degradation of the reaction mixture.

were purified on a pre-packed RP-18 column, eluting with a ternary mixture of $CH_3OH/CH_3CN/H_2O$ containing increasing proportions of CH_3CN.[24] The purified products **2** and **3** were fully characterised by NMR (^1H, ^{13}C) and ESI MS analysis, and these data were compared with published results to confirm the structures.[12] The purity of products was greater than 97% in all cases, as determined by analytical HPLC.

In conclusion, we have described an efficient new preparative base-catalysed oxidation of silibinin. The oxidation can be carried out under conventional or microwave (MW) heating. The MW procedure in minimum solvent volumes gave rapid access to good yields of 2,3-dehydrosilybin (**2**) and hemiacetal **3** (Table 2). The optimised conditions were then used to oxidise optically pure silybins A and B to 2,3-dehydrosilybins A and B, respectively. These results show that the advantages of MW heating are shortened reaction times and the production of hemiacetal **3**, in particular when pyridine is used as the solvent A preparative purification method has also been developed to enable the isolation of **2** and **3** in high purity, which creates the possibility of large scale preparations of **2** and **3**. Access to significant quantities of these compounds will facilitate the evaluation of their pharmacological properties, which have not been explored to date. Having optimised oxidation conditions promoted by microwave (MW) heating, we intend to apply this method to the synthesis of other silybin analogues with improved solubility and antioxidant properties.

Acknowledgments

This study was supported by Università degli Studi di Napoli 'Federico II' (*Progetto FARO*) and AIPRAS *Onlus* (Associazione Italiana per la Promozione delle Ricerche sull'Ambiente e la Salute umana). We also thank Tecno Bios® for the HPLC facilities.

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21. *General procedure for HPLC analysis:* HPLC analysis was performed using a Waters 1525 HPLC equipped with a binary gradient pump and a Waters 2996 Photodiode Array Detector using a Phenomenex LUNA RP18 column (5-μm particle size, 10.0 mm × 250 mm i.d.). The column was eluted with H2O/CH3CN (10–100% in 50 min), flow rate 1.5 mL/min, monitored at 254 and 288 nm.
22. *General purification procedure:* The crude material (1 g) was purified by chromatography over a pre-packed column RP-18 (Biotage® KP-C-18-HS 25 g) on a Biotage® Isolera Spektra one eluting with a ternary mixture of $CH_3OH/CH_3CN/H_2O$ containing increasing proportions of CH_3CN (from 4:1:5 to 4:3:3, v/v/v). The flow rate was 25 mL/min.
23. *General procedure for oxidation under conventional heating:* a solution of silibinin 1 (1.0 g, 2.1 mmol) and base (KOAc or Et_3N, 6.22 mmol) in an appropriate solvent (15 mL for 1,4-dioxane, THF or their mixture; 8 mL for DMF; 6 mL for pyridine) was heated to reflux. The reaction mixture was quenched by removal of the solvent in vacuo. The crude product was purified by chromatography over a pre-packed RP-18 column (see Ref. 23).
24. *General procedure for oxidation under MW heating:* all irradiation experiments were carried out in a dedicated CEM-Explorer and CEM Discover monomode microwave apparatus, operating at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300 W using the standard maximum power absorbance level of 300 W. A solution of silibinin 1 (1.0 g, 2.1 mmol) and base (6.22 mmol) in an appropriate solvent (8 mL for THF, THF/H2O, 1,4-dioxane/MeOH and 4 mL for pyridine and DMF) was placed in a 10 mL glass tube. The tube was sealed with a Teflon septum, placed in the microwave cavity and irradiated. Initial microwave irradiation used the minimum wattage required to ramp the temperature from room temperature to the desired temperature. The reaction mixture was held at this temperature for the required time. After the irradiation period, the reaction vessel was cooled rapidly to ambient temperature by gas jet cooling.