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Metal coordination protocol for the synthesis of-2,3-dehydrosilybin and 19-*O*-demethyl-2,3-dehydrosilybin from silybin and their antitumor activities

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ABSTRACT

An efficient and practical method to access bioactive 2,3-dehydrosilybin and 19-*O*-demethyl-2,3-dehydrosilybin using naturally abundant flavonolignan silybin in the presence of metal salt as a chelating agent is described. The procedure presented here has several advantages including one-pot, synthetic ease, and products in high yields with no side reactions, and large-scale feasibility. The dehydrogenation and demethylation proceed smoothly via a one-pot process using the $AlCl_3$/Pyridine system and I_2 as the additive. Furthermore, 2,3-dehydrosilybin and 19-*O*-demethyl-2,3-dehydrosilybin can inhibit the expression of intracellular mature miRNA-21 with IC_{50} values of 4.46 μM and 8.25 μM, respectively, and show moderate anticancer activities against HeLa cell lines.

Introduction

Silymarin applied in the treatment of cirrhosis, hepatitis, and alcohol-induced liver disease is a crude extract of milk thistle (*Silybum marianum* L. Gaertn., Asteraceae), which contains more than 10 structurally closely related flavonolignans such as silybin, silychristin, isosilybin, silydianin, taxifolin, quercetin, and 2,3-dehydrosilybin *etc.*[1–3] Among the flavonolignans, silybin (SIL, Fig. 1) is the most abundant flavonolignan and the easiest to isolate from silymarin, but it exhibits quite poor biological activities. The structure-activity relationship analysis of silymarin flavonolignans reveals that 2,3 double bond or phenolic hydroxyl group enhance biological activities. For example, 2,3-dehydrosilybin (DHS, Fig. 1) characterized by the presence of the 2,3 double bond possesses higher biological activities than silybin, such as anticancer,[4–6] antioxidant,[7–12] and modulation of P-glycoprotein.[13] Besides, 19-*O*-demethyl-2,3-dehydrosilybin (DHDMS, Fig. 1) replaced methoxy group by the hydroxyl group at C-19 exhibits a much higher antioxidant activity compared to SIL or DHS, and better inhibitory activity against lipid peroxidation than quercetin.[14] Unfortunately, DHS and DHDMS are minor constituents in silymarin, therefore the synthesis of the 2,3-dehydro derivative and minor silymarin constituent DHS and DHDMS from the abundant silybin is of high interest due to their biological activities.

The first synthesis of 2,3-dehydrosilybin (DHS) was via the oxidation of silybin with iodine in K-acetate buffer.[15,16] However, these methods have certain drawbacks, including no more than 50% yield, side products, the use of harmful solvents, hydrolysis of the formation of acetates prior to final purification, and low solubilities of silybin and iodine in acetic acid to scale-up difficult. In another method, 2,3-dehydrosilybin has been synthesized by the aerial or anaerobic oxidation of silybin with base as catalyst, such as *N*-methylglucamine, pyridine and alkaline milieu.[17] The disadvantage of the base-catalyzed method was the occurred side-reaction due to the unstable silybin in base condition, including the free radical polymerization and rearrangement reaction, to produce the insoluble substances and decomposition products.[7,18–20] 19-*O*-Demethyl-2,3-dehydrosilybin (DHDMS) was prepared by the radical-coupling reaction of quercetin with (*E*)-3-*O*-benzyl-3,4-dihydroxy-cinnamyl alcohol,[21] only giving 9.6% yield which greatly limits it's biological applications. To the best of our knowledge, no reports existed to date for the synthesis of DHDMS from

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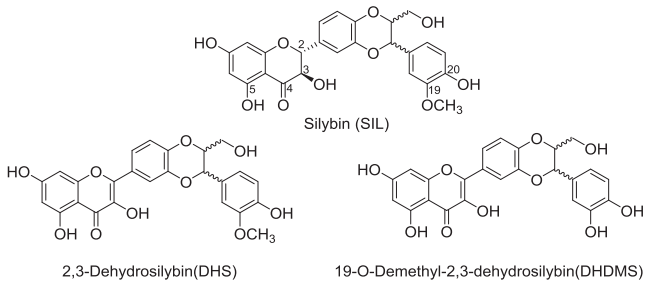


Fig. 1. Structures of SIL, DHS and DHDMS.

SIL or DHS due to the selective demethylation of 19-OMe was rather difficult.

Structurally, silybin is characterized by a flavonoid moiety associated with a phenylpropanoid unit. The flavonoid part consists of carbonyl and hydroxyl groups, they can coordinate metal ions and form complexes. In fact, a large number of experimental and theoretical studies demonstrate that metal ions such as Mg^{2+}, Zn^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+} and Al^{3+} ions can chelate with silybin at 3-OH, 5-OH, and 4-carbonyl group.[22,23,8] Besides, a survey of literature show that methoxy group of *ortho*-hydroxyphenyl alkyl ethers can be cleaved to provide catechol in a direct route with aluminum trichloride-pyridine.[24] Considering the unique ability of flavonoids to coordinate metal ions, it is envisaged that introduction of the metal ions to coordinate with the carbonyl and hydroxyl groups to stabilize the structure of silybin in base condition to avoid being oxidized or rearranged. On the other hand, due to the oxophilicity of aluminum ions, the ether cleavage reaction of 19-methoxy in phenylpropanoid unit of SIL possibly occurred during $AlCl_3$-pyridine induced the dehydrogenation of silybin. Hence, in the present work, we focus on the synthesis of DHS and DHDMS from silybin by dehydrogenation or(and) demethylation via metal coordination (Scheme 1), along with the determination of their antitumor activities.

Results and discussion

According to the O_2-induced oxidation protocols and the condition reported by Křen and co-workers,[17] the reaction of silybin in the presence of various metals in the air using various solvents at reflux temperature for 24 h was initially conducted. Unfortunately, the desired products were obtained in only low isolated yields and most of original materials were observed (Table 1, entries 1–7), when the metal salts such as $MgCl_2$, $CaCl_2$, $ZnCl_2$, $CuCl_2$, $FeCl_2$, $FeCl_3$ and $AlCl_3$ were used. The effect of the solvents, including MeOH, EtOH, DMF, and DMSO, did not show any affect in the transformation yields (Table 1, entries 8–11). Encouragingly, excellent yields were achieved when the I_2 was used as oxidizing agent with the addition of metal salts for 1 h (Table 2, entries 1–7). The reaction was found to give high conversions for all metal salts. Among the common metal salts studied for this reaction, $MgCl_2$ was found to be the most effective additive for this transformation since it resulted in the highest conversion to the desired product (Table 2, entry 1). Further optimization of the temperature revealed that without improving effect on the transformation of DHS when the temperature was decreased to 100 °C (Table 2, entry 8), but further decrease in temperature to 90 and 80 °C gave the DHS in 86% and 75% yield, respectively (Table 2, entries 9 and

Table 1
Survey of metal salts and solvents for the oxidation of SIL to DHS.[a]

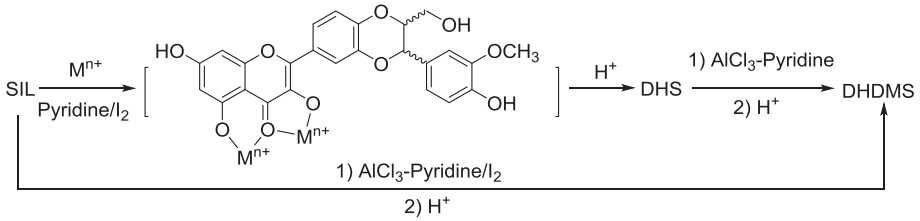
Entry	Metal	Solvent	Temp (°C)	Yield (%)[b]
1	$MgCl_2$	Pyridine	110	53
2	$CaCl_2$	Pyridine	110	37
3	$ZnCl_2$	Pyridine	110	55
4	$CuCl_2$	Pyridine	110	43
5	$FeCl_2$	Pyridine	110	38
6	$FeCl_3$	Pyridine	110	44
7	$AlCl_3$	Pyridine	110	56
8	$AlCl_3$	MeOH	Reflux	Trace
9	$AlCl_3$	EtOH	Reflux	Trace
10	$AlCl_3$	DMF	110	Trace
11	$AlCl_3$	DMSO	110	Trace

[a] Unless stated otherwise, reactions were performed on 0.2 mmol scale with silybin/metal salts as 1:2 ratio in 4.0 mL of solvent in air for 24 h.
[b] Isolated yield.

Table 2
Optimization of the reaction conditions.[a]

Entry	Metal Salt (equiv)	Temp (°C)	Time (h)	Yield (%)[b] DHS	DHDMS
1	$MgCl_2$ (2.0 eq)	110	1	91	Trace
2	$ZnCl_2$ (2.0 eq)	110	1	89	Trace
3	$CaCl_2$ (2.0 eq)	110	1	84	N.r.[c]
4	$CuCl_2$ (2.0 eq)	110	1	60	N.r.[c]
5	$FeCl_2$ (2.0 eq)	110	1	87	Trace
6	$FeCl_3$ (2.0 eq)	110	1	85	Trace
7	$AlCl_3$ (2.0 eq)	110	1	75	19
8	$MgCl_2$ (2.0 eq)	100	1	91	N.r.[c]
9	$MgCl_2$ (2.0 eq)	90	1	86	N.r.[c]
10	$MgCl_2$ (2.0 eq)	80	1	75	N.r.[c]
11	$MgCl_2$ (2.0 eq)	100	2	92	N.r.[c]
12	$MgCl_2$ (1.8 eq)	100	1	86	N.r.[c]
13	$MgCl_2$ (1.6 eq)	100	1	79	N.r.[c]
14	$MgCl_2$ (2.2 eq)	100	1	91	N.r.[c]
15	$AlCl_3$ (2.3 eq)	110	2	62	32
16	$AlCl_3$ (2.5 eq)	110	2	50	43
17	$AlCl_3$ (3.0 eq)	110	2	Trace	92
18	$AlCl_3$ (3.5 eq)	110	2	Trace	91
19	$AlCl_3$ (3.0 eq)	110	2.5	Trace	90
20[d]	$AlCl_3$ (3.0 eq)	110	2		Trace
21[e]	$AlCl_3$ (3.0 eq)	110	2		Trace
22[f]	$AlCl_3$ (3.2 eq)	110	2		85
23[h]	AlI_3 (3.2 eq)	110	2		92

[a] Unless stated otherwise, reactions were performed on 0.2 mmol scale with silybin in 1 equiv. I_2 in 4.0 mL of pyridine in air.
[b] Isolated yield.
[c] Not reaction.
[d] 0.2 mmol of DHS instead of silybin as substrate without I_2.
[e] 0.2 mmol of DHS instead of silybin as substrate in 1 equiv. I_2.
[f] 0.2 mmol of DHS instead of silybin as substrate in 2 equiv. KI.
[h] 0.2 mmol of DHS instead of silybin as substrate without I_2.



Scheme 1. Synthesis of SHS and SHSMS.