

## SILYCHRISTIN AND ISOSILYCHRISTIN FROM THE FRUITS OF *SILYBUM MARIANUM* (L.) GAERTN.

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### SUMMARY

By various chromatography methods, two flavanolignan silychristin (**1**) and isosilychristin (**2**) were isolated from the fruits of *Silybum marianum* (L.) Gaertn. cultivated in the North of Vietnam. Their structures were elucidated by analyses of the NMR (1D-, 2D-NMR) and ESI spectra. This is the first report of both **1** and **2** from *S. marianum* cultivated in Vietnam.

### I - INTRODUCTION

In previous papers, we have reported the isolation and structural elucidation of flavanolignan, flavonoid, and lignan compositions of the fruits of *Silybum marianum* (L.) Gaertn. cultivated in the North of Vietnam and used in folk medicine [1 - 4]. Here, we report the isolation and structural determination of two flavanolignan silychristin (**1**) and isosilychristin (**2**) isolated from the fruits of *Silybum marianum* (L.) Gaertn. cultivated in the North of Vietnam. The structures were elucidated by analyses of the NMR and ESI spectra, and by the reported data. This is the first report of both **1** and **2** from *S. marianum* cultivated in Vietnam.

### II - EXPERIMENTAL

#### 1. General experimental procedures

The Electrospray Ionization (ESI) mass spectrum was obtained using a AGILENT 1100 LC-MSD Trap spectrometer. The NMR spectra were recorded on a Bruker AM500 FT-NMR

spectrometer using TMS as the internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck) or YMC RP-18 resins (30 - 50  $\mu$ m, FujiSilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F254 (Merck 1.05715) or RP18 F254s (Merck) plates.

#### 2. Plant material

The fruits of *Silybum marianum* (L.) Gaertn. were collected in Sapa, Lao Cai Province in June, 2004 and were identified by Dr Nguyen Tap, Department of Botany and Medicinal Plant Resources, National Institute of Medicinal Materials.

#### 3. Extraction and isolation

Air-dried and powdered fruits of *Silybum marianum* (L.) Gaertn (200 g) were defatted with petroleum and then extracted with methanol. After removal of the solvent under vacuum, the residue (5.8 g) was obtained, which was then repeatedly chromatographed on silica gel columns to get **1** (30 mg) as colorless amorphous powder and **2** (50 mg) as yellow crystals.

### III - RESULTS AND DISCUSSION

Compound **1** was obtained as colourless amorphous powder from methanolic extract of the fruits of *S. marianum* (L.), mp. 174 - 176°C. The <sup>1</sup>H-NMR spectrum of **1** exhibited two doublets at δ<sub>H</sub> 5.85 and 5.90 ppm (*J* = 1.5 Hz), two doublets at δ<sub>H</sub> 6.82 and 6.87 ppm, three other aromatic protons at δ<sub>H</sub> 6.79 (d, *J* = 8.0 Hz), 6.81 (dd, *J* = 8.0, 1.0 Hz) and 6.99 (d, *J* = 1.0 Hz). Two doublet of doublets of two protons

at *trans* configuration were at δ 5.00 (dd, *J* = 11.5, 2.5 Hz) and 4.49 (dd, *J* = 11.5, 5.5 Hz) [5], a doublet at δ 5.46 (*J* = 7.0 Hz), a multiplet at δ 3.49. The methylene group bearing oxygen atom was at δ 3.64 (dd, *J* = 10.0, 10.5 Hz) and 3.71 (dd, *J* = 10.5, 5.5 Hz), and methoxyl group was at 3.75 as a singlet signal. All the <sup>1</sup>H-NMR data suggested the flavonolignan type of **1**, which was similar to the isolated compounds from the same sources [1, 2].

Table 1: The NMR data of **1**

C	δ <sub>C</sub> <sup>a</sup>	δ <sub>C</sub> <sup>b,d</sup>	δ <sub>H</sub> <sup>c,d</sup> ( <i>J</i> in Hz)
2	85.2	83.20	5.00 (dd, 11.5, 2.5)
3	73.8	71.66	4.49 (dd, 11.5, 5.5)
4	198.2	197.53	-
5	165.2	163.25	-
6	97.4	96.01	5.90 (d, 1.5)
7	168.7	166.97	-
8	96.3	94.96	5.85 (d, 1.5)
9	164.4	162.49	-
1'	130.0	128.99	-
2'	116.6	115.26	6.87 (d, 1.5)
3'	147.5	147.02	-
4'	142.1	140.66	-
5'	131.5	129.90	-
6'	116.9	115.58	6.82 (d, 1.5)
α	89.1	86.96	5.46 (d, 7.0)
β	55.5	53.29	3.49 (m)
γ	64.8	62.94	3.64 (dd, 10, 10.5); 3.71 (dd, 5.5, 10.5)
1''	134.8	132.38	-
2''	110.6	110.43	6.99 (d, 1.0)
3''	149.0	147.5	-
4''	147.5	146.33	-
5''	116.2	115.26	6.79 (d, 8.0)
6''	119.7	118.64	6.81 (dd, 1.0, 8.0)
OCH <sub>3</sub>	56.4	55.64	3.75 (3H, s)
5-OH			11.89 (br s)

<sup>a</sup> δ<sub>C</sub> of silychristin [5], <sup>b</sup>125 MHz, <sup>c</sup>500 MHz, <sup>d</sup>Measured in DMSO-d<sub>6</sub>.

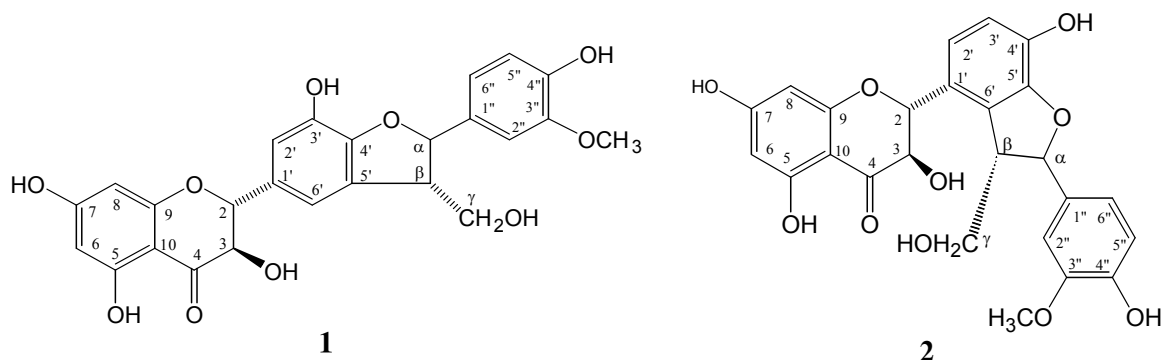


Fig.1. The structures of **1** & **2**

The  $^{13}\text{C}$ -NMR spectrum of **1** showed the signals of 25 carbons including 12 quaternary, 11 methine, 1 oximethylene and 1 methoxyl carbons, suggesting a flavonolignan compound. The carbonyl group was at  $\delta$  197.53, the methylene bearing oxygen atom was at  $\delta$  62.94 and methoxyl group was at  $\delta$  55.64. All NMR assignments of **1** were made from analysis of HSQC, HMBC spectra and by comparison with those of isolated compound (dehydrosilychristin) [3] as well as with those of silychristin reported in the literature [5]. In the HMBC spectrum, H-2 ( $\delta$  5.00) correlated with C-3 ( $\delta$  71.66)/C-4 ( $\delta$  197.53)/C-1' ( $\delta$  128.99)/C-2' (115.26), and H-3 ( $\delta$  4.49) had only correlations with C-2 ( $\delta$  83.2) and C-1' (128.99) confirming the position of two oximethine carbons at C-2 and C-3. In addition, the H-C long-range correlations were observed between H- $\alpha$  ( $\delta$  5.46) and carbons C- $\beta$  ( $\delta$  53.29)/C-1'' ( $\delta$  132.38)/C-6'' ( $\delta$  118.64)/C-2'' ( $\delta$  110.43)/C- $\gamma$  (62.94), as well as the H-C long-range correlations were observed between H- $\beta$  ( $\delta$  3.49) and carbons C1'' ( $\delta$  132.38)/C- $\gamma$  (62.94) also confirming the positions of C- $\alpha$ , C- $\beta$  and C- $\gamma$  of two oximethine and oximethylene groups. The selected H-C correlations in the HMBC spectrum of **1** were shown in Fig. 2. Furthermore, the negative ESI mass spectrum of **1** exhibited an ion peak at  $m/z$  481  $[\text{M}-\text{H}]^+$  with strongest intensity, corresponding to the molecular formula of  $\text{C}_{25}\text{H}_{22}\text{O}_{10}$ . From the above data, compound **1** was determined to be

silychristin, which was first isolated from *Silybum marianum* (L.) Gaertn. cultivated in the North of Vietnam. This compound also was reported to be inhibitor of horseradish peroxidases and lipoxygenase and antihepatotoxic agent [5].

Compound **2** was obtained as yellow crystals from methanolic extract of the fruits of *S. marianum* (L.), mp. 155 - 157°C and had similar NMR spectra as compound **1**, suggesting the same flavonolignan type. 25 carbons corresponding to 25 carbon signals were observed in the  $^{13}\text{C}$ -NMR spectrum of **2** (including 12 quaternary, 11 methine, 1 oximethylene and 1 methoxy carbons as **1**). Detailed comparison from NMR spectrum of **2** with those of **1**, especially at C-2, C-3, C- $\alpha$  and C- $\beta$  showed that **2** must be a derivative of **1**, as isosilychristin, which was also isolated from *Silybum marianum* (L.) Gaertn [6]. The ESI mass spectrum of **2** was taken confirming the molecular formula of  $\text{C}_{25}\text{H}_{22}\text{O}_{10}$  of **2** from the appearance of an ion peak at  $m/z$  483  $[\text{M}+\text{H}]^+$  in the positive and an ion peak at  $m/z$  481  $[\text{M}-\text{H}]^+$  in the negative spectra. All NMR assignments of **2** were made carefully from HSQC and HMBC and from the comparison with those of **1** as shown in table 2. The H-C long-range correlations in the HMBC were shown in Fig.2 further confirming the structure of **2** as isosilychristin, which also was first isolated from *Silybum marianum* (L.) Gaertn. cultivated the North of Vietnam

Table 2: The NMR data of 2

C	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (J in Hz)
2	79.87	5.17 (d, 12.0)
3	71.48	4.66 (dd, 12.0, 6.0)
4	197.99	-
5	163.33	-
6	96.10	5.92 (d, 1.5)
7	166.83	-
8	95.03	5.87 (d, 1.5)
9	162.55	-
10	100.49	-
1'	128.85	-
2'	119.42	6.95 (d, 8.0)
3'	116.08	6.74 (d, 8.0)
4'	145.92	-

C	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (J in Hz)
5'	141.56	-
6'	124.44	-
$\alpha$	86.36	5.57 (d, 2.0)
$\beta$	51.97	3.68 (m)
$\gamma$	63.49	3.46 (dd, 10, 10.5) 3.72 (dd, 5.5, 10.5)
1''	132.83	-
2''	110.28	6.86 (d, 1.5)
3''	147.44	-
4''	146.27	-
5''	115.15	6.71 (d, 8.5)
6''	118.48	6.76 (dd, 8.5, 1.5)
OCH <sub>3</sub>	55.59	3.71 (3H, s)
5-OH		11.90 (br s)

<sup>a</sup>Measured in DMSO-d<sub>6</sub>, <sup>b</sup>125 MHz, <sup>c</sup>500 MHz.

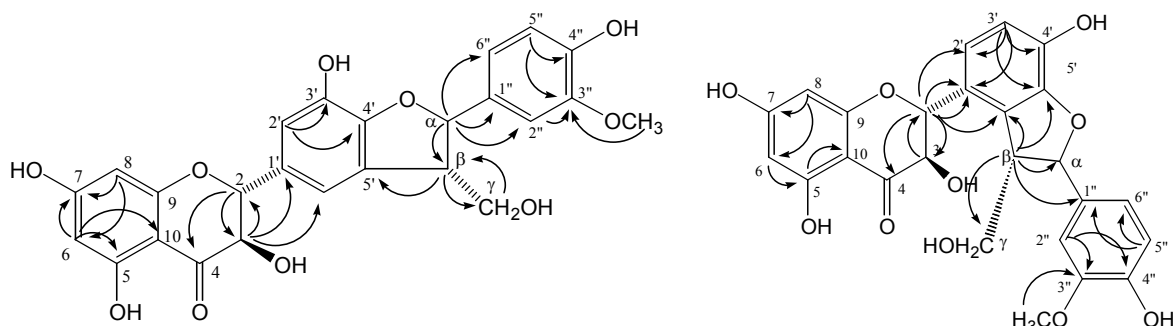


Figure 2: H-C long-range correlations in the HMBC of 1 and 2

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