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PINORESINOL AND 3,4',5,7-TETRAHYDROXY-3'-METHOXYFLAVANONE FROM THE FRUITS OF *SILYBUM MARIANUM* (L.) GAERTN.

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SUMMARY

*From the fruits of *Silybum marianum* (L.) Gaertn. cultivated in the North of Vietnam, (±)2,6-bis(4'-hydroxy-3'-methoxy-phenyl)-3,7-dioxabicyclo[3.3.0]octane (pinoresinol) (1) and 3,4',5,7-tetrahydroxy-3'-methoxyflavanone (2) were isolated. Their structures were elucidated by analyses of the NMR and mass spectra in comparison with the reported data. This is the first report of both 1 and 2 from *Silybum* species.*

I - INTRODUCTION

In previous papers, we have reported the isolation and structural elucidation of flavanolignan and flavonoid compositions of the fruits of *Silybum marianum* (L.) Gaertn. (Asteraceae) cultivated the North of Vietnam and used in the folk medicines [1 - 3]. This paper deals with further investigation of the bioactive compositions from *S. marianum*. By various chromatography methods, (±)2,6-bis(4'-hydroxy-3'-methoxy-phenyl)-3,7-dioxabicyclo[3.3.0]octane (Pinoresinol) (1) and 3,4',5,7-tetrahydroxy-3'-methoxyflavanone (2) were isolated from the methanolic extract of the fruits from this plant. The structures were elucidated by analyses of the NMR and ESI mass spectra in comparison with the reported data. This is the first report of both 1 and 2 from *Silybum* species. To the best of our knowledge, no ¹³C-NMR data of 2 has been reported previously.

II - EXPERIMENTAL

1. General experimental procedures

The Electrospray Ionization (ESI) mass spectrum was obtained using a AGILENT 1100 LC-MSD Trap spectrometer. The ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) 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 μm, 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** (5.2 mg) as colorless amorphous and **2** (6.5 mg) as yellow crystals.

III - RESULTS AND DISCUSSION

Compound **1** was isolated as colorless amorphous substance, mp. 120 - 121°C. The ¹H-NMR spectrum showed three peaks of the aromatic protons at δ 6.89 (1H, d, *J* = 1.5 Hz), 6.82 (1H, dd, *J* = 8.0, 1.5 Hz) and 6.88 (1H, d, *J* = 8.0 Hz) suggesting that the aromatic ring has three substituted positions. Oxymethine proton was evident at δ 4.73 ppm as a doublet and the methylene groups bearing oxygen atom at δ 4.24 and 3.88 ppm as two doublets of doublets. A methine proton was observed at δ 3.10 ppm (multiplet) and methoxy group was confirmed at 3.90 ppm (singlet).

The ¹³C-NMR spectrum of **1** exhibited signals of 10 carbons at δ 146.73, 145.28, 132.96, 118.99, 114.30, 108.65, 85.90, 71.69, 55.99 and 54.20 as listed in table 1. The partial structures were deduced from the HOMOCOSY and HMQC spectra, and were connected based

on long-range correlations in the HMBC spectrum of **1**. In the HOMOCOSY, cross peaks between the proton at δ 3.10 (H-5) and protons at δ 4.73 (H-6)/δ 4.24 (Ha-4)/δ 3.88 (Hb-6), and between proton at δ 6.88 (H-5'') and proton at δ 6.82 (H-6'') were observed. In addition, H-C long-range correlations between the proton at δ 3.10 (H-5) and the carbon at δ 85.90 (C-6)/δ 71.70 (C-4)/δ 132.9 (C-1''), between the proton at δ 4.73 (H-6) and carbons at δ 132.90 (C-1'')/δ 118.99 (C-6'')/δ 108.65 (C-2'') confirming the lignan skeleton of **1**. The ESI mass spectra of **1** showed an ion peak at *m/z* 341 [M-H₂O+H]⁺ with strongest intensity corresponding to the molecular formula of C₂₀H₂₂O₆. These findings suggested the structure of **1** as shown in Fig. 1. All NMR assignments of **1** were confirmed by detailed analyses of HSQC and HMBC spectra (table 1), which are in good agreement with those reported in the literature [4]. Thus, compound **1** was determined to be (±)2,6-bis(4'-hydroxy-3'-methoxy-phenyl)-3,7-dioxabicyclo[3.3.0]octane (Pinoresinol), the compound was isolated from *Festuca argentina* [4]. However, this is the first report of **1** from Asteraceae family.

Compound **2** was isolated as yellow crystals suggesting that **2** is a flavonoid. The ESI mass spectrum showed an ion peak at *m/z* 319 corresponding to the molecular formula of C₁₆H₁₄O₇. The ¹³C-NMR and DEPT spectra of **2** confirmed the presence of 16 carbon atoms including 8C, 7CH and 1CH₃ of the flavanon

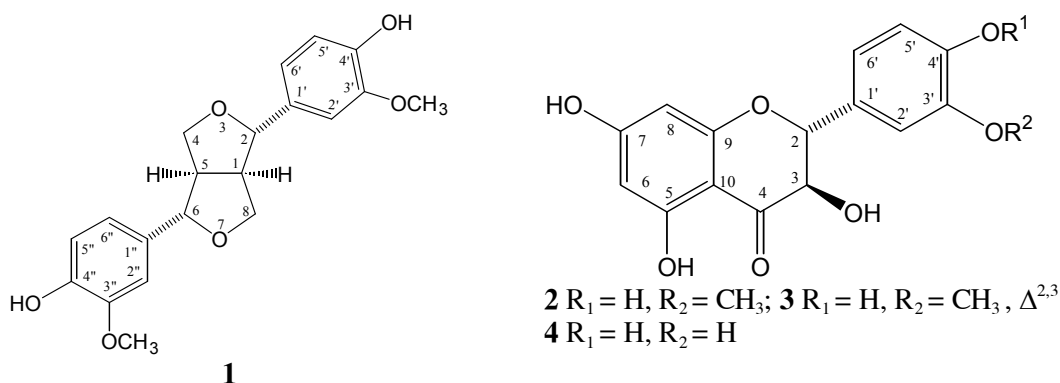


Figure 1: The structures of **1** - **4**

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

C	δ_C^*	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (J, Hz)	HMBC (H to C)
1, 5	54.2	54.20	3.10 (2H, m)	C-2, 4, 1'
2, 6	85.9	85.90	4.73 (2H, d, 5.0)	C-1, 5, 8, 1', 2', 6'
4, 8	71.7	71.70	4.24 (2H, dd, 7.0, 9.5) 3.88 (2H, dd, 4.0, 9.5)	C-1, 2 C-1, 2
1', 1''	132.9	132.90	-	
2', 2''	108.7	108.65	6.89 (2H, d, 1.5)	C-2, 1', 3', 4', 6'
3', 3''	146.7	146.73	-	
4', 4''	145.3	145.28	-	
5', 5''	114.3	114.30	6.88 (2H, d, 8.0)	C-1', 3', 4', 6'
6', 6''	118.9	118.99	6.82 (2H, dd, 1.5, 8.0)	C-2, 1', 2', 4'
3', 3''-OCH ₃	56.0	55.99	3.90 (3H, s)	C-3'

* δ_C of pinoresinol [4], ^aMeasured in CDCl₃, ^b125 MHz, ^c500 MHz.

Table 2: The NMR data of **2 - 4**

C	δ_C of 3 [5]	δ_C of 4 [6]	$\delta_C^{a,b}$ of 2	$\delta_H^{a,c}$ (J, Hz)
2	147.1	84.3	85.2	5.01 (1H, d, 6.5)
3	136.1	73.0	73.6	4.59 (1H, d, 6.5)
4	176.3	198.0	198.4	-
5	161.2	164.8	165.3	-
6	98.6	97.0	97.4	5.94 (1H, d, 1.5)
7	164.4	167.8	168.9	-
8	93.9	96.0	96.3	5.91 (1H, d, 1.5)
9	156.8	164.0	164.5	-
10	103.5	101.4	101.8	-
1'	122.6	129.6	129.8	-
2'	112.7	115.7	112.5	7.13 (1H, d, 1.5)
3'	149.4	145.6	148.9	-
4'	147.9	146.4	148.3	-
5'	116.0	115.7	115.9	6.85 (1H, d, 8.0)
6'	122.2	120.8	122.2	7.98 (1H, dd, 1.5, 8.0)
OCH ₃	56.2		56.5	3.89 (3H, s)

^aMeasured in CDCl₃, ^b125 MHz, ^c500 MHz.

skeleton. The spin-coupling of three protons of ring C suggested the hydroxyl and methoxyl at C-4' and C-3'. The chemical shifts at δ 85.2 (CH), 73.6 (CH) and 198.4 (C=O) were very similar to that of the flavanone reported in the literature [6]. The methoxyl group evidenced at δ_C 56.5 (q) and δ_H 3.89 (3H, s). The NMR assignments of ring C of **2** were deduced from those of **3** [5], and the NMR assignments of ring A were compared with that of **4** [6]. All these data led to determine the structure of **2** as 3,4',5,7-tetrahydroxy-3'-methoxyflavanone, which is first isolated from *Silybum* species.

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