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SESQUITERPENOIDS FROM *CYNARA SCOLYMUS*

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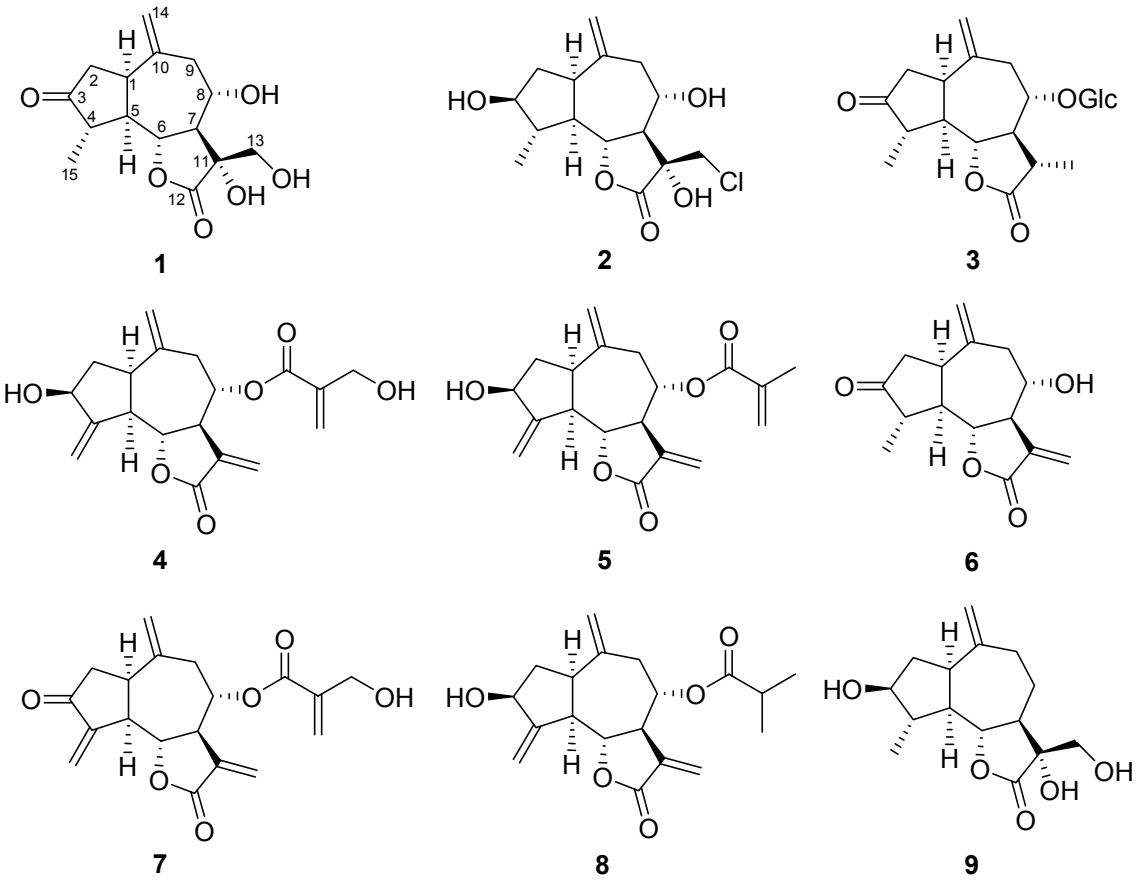
Abstract –Two new guaiane-type sesquiterpene lactones, cynarinins A (**1**) and B (**2**), together with seven known compounds, cynarascoloside C (**3**), cynaropicrin (**4**), aguerin B (**5**), grosheimin (**6**), dehydrocynaropicrin (**7**), aguerin A (**8**) and cynartriol (**9**), were isolated from aerial part of *Cynara scolymus*. The structures of new compounds were elucidated by means of 1D NMR, 2D NMR and HRMS spectral analysis. Cynaropicrin (**4**) and grosheimin (**6**) showed weak cytotoxicity against MCF-7 cancer cell line, and other compounds had no obvious activity against the same cell line.

INTRODUCTION

Cynara scolymus (family composite) is an ancient herbaceous perennial plant originating from the Southern Mediterranean parts of North Africa.[1] Today *C. scolymus* is widely cultivated in Europe and America. Its sprout is edible as a vegetable, and its leaves are used for the treatment of hepatitis and hyperlipidemia in Eropean traditional medicine.[2] In Kunming of Yunnan province, People's Republic of China, *C. scolymus* is also planted as a vegetable, and its flowers are exported to Europe as a vegetable. We studied the chemical constituents of the aerial part of this plant and gained nine compounds, including two new guaiane-type sesquiterpene lactones, cynarinin A (**1**) and cynarinin B (**2**), as well as seven

known compounds, cynarascoloside C (**3**),[2] cynaropicrin (**4**),[2,4 - 6] aguerin B (**5**),[2,5] grosheimin (**6**),[2] dehydrocynaropicrin (**7**),[3] aguerin A (**8**)[5] and cynartriol (**9**).[3] The structure elucidation of new compounds was done by means of 1D NMR, 2D NMR and HRMS spectral analysis. Cynaropicrin (**4**) and grosheimin (**6**) showed weak cytotoxicity against MCF-7 cancer cell line, and other compounds had no obvious activity against the same cell line.



Results and Disscussion

Cynarinin A (1) was obtained as a white powder. The HRESIMS spectrum showed a $[M+Na]^+$ ion peak at *m/z* 319.1157 (calcd for $C_{15}H_{20}NaO_6$, 319.1158) corresponding to the molecular formula $C_{15}H_{20}O_6$. The ^1H, ^{13}C and DEPT spectra are showed in Table 1 and indicated that the structure and relative stereochemistry of **1** were similar to those of grosheimin (**6**)[2] differing in ring C. There was a double bond between C-11 and C-13 in **6**, while it was absent in **1**. The HMBC correlation established the skeletal structure of **1**. The signal at δ 179.8 (s, C-12) showed HMBC correlation with the signal at δ 4.63 (1H, t, *J* = 9.8 Hz, H-6), which clearly indicated the presence of a lactone moiety at C-12 and C-6. The AB system signals at δ 5.03 (1H, d, *J* = 10.1 Hz, H-13a) and δ 4.56 (1H, d, *J* = 10.1 Hz, H-13b) coupling each other in H-H COSY spectrum and the oxygenated quaternary carbon signal at δ 79.8 (s, C-11) showing HMBC correlations with H-13 confirmed two hydroxyl groups attached to C-11 and C-13, respectively.

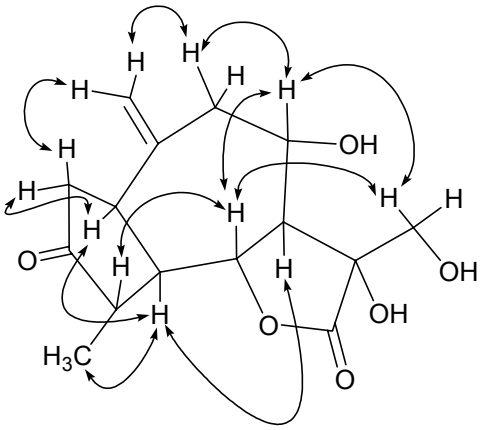


Figure 1. The key ROESY correlation of **1**

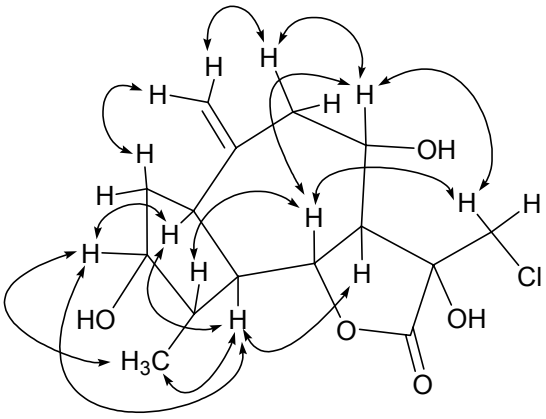


Figure 2. The key ROESY correlation of **2**

The relative stereochemistry of **1** was established by ROESY experiment (see Figure 1). The ROESY correlations of H-5/H-1, H-7, Me-15 suggested that Me-15 was in α-orientation. The correlation of H-6/H-4 indicated H-6 was β orientated, while correlation of H-7/H-5 revealed H-7 was α orientated. Furthermore, the correlation of H-6/H-8 confirmed H-8 was in β orientation. Based on the above elucidation, Cynarinin A was established as **1**.

Table 1. The NMR Spectral Data of **1** and **2** (400 MHz; C_5D_5N, δ in ppm, J in Hz)

No.	**1**		**2**	
	C	H	C	H
1	40.2 d	3.50 (1H, m)	42.7 d	2.80 (1H, m)
2	43.7 t	2.48 (2H, m)	39.2 t	2.18 (1H, m, H-2α)
				2.03 (1H, m, H-2β)
3	218.6 s	--	77.7 d	3.87 (1H, m)
4	47.5 d	2.33 (1H, m)	47.6 d	2.23 (1H, m)
5	52.2 d	2.18 (1H, m)	52.3 d	1.98 (1H, m)
6	82.4 d	4.63 (1H, t, 9.8)	81.6 d	4.52 (1H, t, 10.3)
7	57.4 d	3.21 (1H, t, 10.2)	61.7 d	3.01 (1H, t, 10.3)
8	70.5 d	4.84 (1H, m)	70.7 d	4.46 (1H, m)
9	49.6 t	3.13 (1H, m, H-9β)	48.4 t	3.14 (1H, dd, 4.0, 12.0, H-9β)
		2.56 (1H, t, 11.4, H-9α)		2.41 (1H, m, H-9α)
10	145.9 s	--	145.2 s	--
11	79.8 s	--	78.7 s	--
12	179.8 s	--	177.2 s	--
13	65.0 t	5.03 (1H, d, 10.1, H-13a)	44.7 t	4.75 (1H, d, 10.7, H-13a)
		4.56 (1H, d, 10.0, H-13b)		4.29 (1H, d, 10.7, H-13b)
14	113.7 t	4.98 (1H, s, H-14a)	114.3 t	5.08 (1H, s, H-14a)
		4.69 (1H, s, H-14b)		5.06 (1H, s, H-14b)
15	15.0 q	1.24 (3H, d, 7.1)	19.0 q	1.39 (3H, d, 6.5)

Cynarinin B (**2**) was a white powder. Its molecular formula was determined as $C_{15}H_{21}O_5Cl$ by HRESIMS spectrum at *m/z* 339.0977 $[M+Na]^+$ (calcd for $C_{15}H_{21}ClNaO_5$, 339.0975), which showed a chlorine atom in **2**. Compound (**2**) was very similar to **1**, except for ring A and C. Inspection of ^{13}C NMR spectrum of **2**

suggested the presence of a hydroxyl group at C-3 and a chlorine atom at C-13, which was consistent with the HMBC correlations of the signal at δ 3.87 (1H, m, H-3) with C-1, C-2, C-4, C-15 and upfield shift of C-13 at δ 48.4 (t) relative to those of **1**. Another evidence for a chlorine atom at C-13 was supported from the HMBC correlation between C-12 and the AB system signals at δ 4.75 (1H, d, J = 10.7 Hz, H-13a) and 4.29 (1H, d, J = 10.7 Hz, H-13b).

On the basis of the ROESY experiment (see Figure 2), the stereochemistry of **2** was ambiguously determined. The ROESY correlation between H-3 and H-1, H-5 indicated H-3 was in α orientation. Correlation of H-3 and Me-15 suggested that Me-15 was in α configuration. The correlation of H-6/H-4 indicated H-6 was in β orientation, and the correlation of H-7/H-5 revealed H-7 was in α orientation. Furthermore, the correlation of H-6/H-8 confirmed H-8 was in β orientation. Finally, the structure of cynarin B was determined as **2**.

Cytoxicities of the sesquiterpenoids isolated in this study from *C. scolymus* were assessed using MTT assay. Cynaropicrin (**4**) [IC_{50} = 65.0 μg/mL] and grosheimin (**6**) [IC_{50} = 61.5 μg/mL] showed weak cytotoxicity against MCF-7 cancer cell line, and other compounds [IC_{50} > 100 μg/mL] had no obvious activity against the same cell line.

EXPERIMENTAL

General Experimental Procedures. Both 1D and 2D NMR experiments were performed on a Bruker AM-400 or on a DRX-500 spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. MS was taken on a VG Auto Spec-3000 or on a Finnigan MAT 90 intrument. IR spectra were recorded on a Bio-Rad FTS-135 spectrophotometer with KBr pellets. UV spectral data were obtained on a UV 210A spectrophotometer. Optical rotations were carried out on a HORIBA SEPA-300 high sensitive polarimeter or a Perkin–Elmer model 241 polarimeter. Melting points were measured on a YUHUA X-4 point apparatus and uncorrected. Column chromatography was performed either on silica gel (200-300 mesh, Qingdao Marine Chemical, China), silica gel H (10-40 μm, Qingdao Marine Chemical, China), or MCI gel CHP20P (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), half-preparative HPLC was performed on a Hewlett Packard instrument (column: Zorbax SB-C18, 250×9.4 mm; UV detector). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Materials. The aerial part of *Cynara scolymus* were collected in the Xiaoshao in Kunming of Yunnan Province, P. R. China, in November 2003, and identified by Professor Deding Tao of Kunming Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (KIB 03117) is deposited.

Extraction and Isolation. The dried and powdered aerial parts of *C. scolymus L.* (11 kg) were extracted four times with 70% Me_2CO (40 L) at rt for 24 h and filtered. The filtrate was concentrated and the residue (400 g) was extracted four times with EtOAc. After removing the solvent, the crude part (70 g) was fractioned by column chromatography on Sephadex LH 20 (MeOH), yielding three fractions. Fraction Ⅰ and fraction Ⅱ were purified by repeated column chromatography over silica gel (chloroform-Me_2CO, 9:1) to afford **2** (350 mg), **4** (30 g), **6** (900 mg), and **9** (40 mg), the trace parts of fraction Ⅰ and fraction Ⅱ was isolated by half-preparation of HPLC, and lead to the separation of **1** (25mg), **3** (20mg), **5** (4 mg), **7** (4 mg), and **8** (4 mg).

Cynarinin A (**1**): white powder, mp 188-190 °C, $[\alpha]_D^{22}$ +90.5º (c 1.59, MeOH); UV (MeOH) λ_{max} (log ε) 261 (0.10), 255 (0.14), 250 (0.13), 203 (0.76) nm; IR (KBr) ν_{max} 3479, 3423, 1771, 1749, 1134, 977 cm^{-1}; ^1H NMR and ^{13}C NMR spectrum see Table 1; HRESIMS *m/z* 319.1157 $[M+Na]^+$ (calcd for $C_{15}H_{20}O_6Na$, 319.1158).

Cynarinin B (**2**): white powder, mp 151-153 °C, $[\alpha]_D^{23}$ +89.5º (c 0.65, MeOH); UV (MeOH) λ_{max} (log ε) 288 (0.06), 204 (1.03) nm; IR (KBr) ν_{max} 3415, 2926, 1764, 1640, 1347, 1277, 980 cm^{-1}; ^1H NMR and ^{13}C NMR spectrum see Table 1; HRESIMS *m/z* 339.0977 $[M+Na]^+$ (calcd for $C_{15}H_{21}O_5ClNa$, 339.0975).

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