

A Styrylpyrone Dimer from the Bark of *Goniothalamus leiocarpus*

Qing MU^{3*}, Yi Neng HE¹, Wei Dong TANG², Chao Ming LI¹, Li Guang LOU²,
Han Dong SUN¹, Bin XU², Guo Xun YANG³, Chang Qi HU³

¹Phytochemistry Laboratory, Kunming Institute of Botany, Chinese Academy of Sciences,
Kunming 650204

²Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Science, Chinese
Academy of Sciences, Shanghai 200032

³School of Pharmacy, Fudan University, Shanghai 200032

Abstract: A dimer of styrylpyrone derivative, leiocarpin E (**1**), was isolated from the bark of *Goniothalamus leiocarpus*. Its structures was elucidated by means of spectral and chemical methods. The cytotoxicity of leiocarpin E against HL-60 cells was tested.

Keywords: *Goniothalamus*, *G. leiocarpus*, cytotoxicity, styryllactone, leiocarpin E.

Continuous phytochemical studies on *Goniothalamus leiocarpus* have led to the isolation of several new styryllactones, which were found to possess cytotoxic activities against several human tumor cell lines^{1,2}. In this paper a styrylpyrone derivative, leiocarpin E (**1**), was reported. Its structure was elucidated as a dimer of styrylpyrone by means of spectral and chemical methods. The cytotoxicity of leiocarpin E against HL-60 cells was tested in MTT method.

The powdered stem bark of *G. leiocarpus* (5 kg) was extracted with 95% EtOH (10 L × 3) for 72 h at rt. The alcohol solution was concentrated and then dried to give 830 g of the dark brown resin. 200g of EtOH extract was separated into three fractions by silica gel column (500 g) chromatography with CHCl₃, EtOAc and MeOH, successively. The CHCl₃ fraction (88 g) was carried out silica gel chromatography with gradient mixture of CHCl₃ and MeOH, and the crude crystals of **1** (30 mg in MeOH) was obtained in the elution of CHCl₃-MeOH (90:10).

Leiocarpin E (**1**) was obtained as colorless needles. The presence of δ-lactone and hydroxyl groups was showed by the absorption brands at 1707 and 3343 cm⁻¹ in the IR spectrum⁵. ¹³C NMR data of **1** showed the presence of two styryllactone moieties^{6,7}, including signals of two similar α, β-unsaturated δ-lactones, a mono-substituted benzene, as well as an oxygenated methine. These signals were assigned by means of ¹H, ¹H-¹H COSY and HMQC spectra of **1** (Figure 1). ¹³C NMR data of **1** indicated the presence of a bi-substituted phenyl group (B' ring) besides the mono-substituted benzyl group. The position of H-14' was appointed by the correlation between H-8' (5.17, d) and the

* E-mail: muqing2008@sohu.com

H-14 (7.95, d), and the other positions in B'-ring were following determined by the ^1H , ^{13}C , HMQC, HMBC, and NOESY spectra of **1** (Table 1). Moreover, comparing with the carbon spectral data of goniodiol, the appearance of signal at δ 45.5 ppm, which was corresponded with the proton signal at δ 4.25 ppm (d, 9.6 Hz, H-8) in HMQC spectrum, together with the disappearing of an oxygenated methine (70-79 ppm) revealed that C-8 (PhCH-) in **1** attached with an aromatic carbon in the another styryllactone moiety forming a dimer of styryllactones, other than with oxygen group as in goniodiol. The connecting position at aromatic ring in the dimer was established at C-10' by the correlation between H-8 and C-10', C-9' as well as C-11' in HMBC spectra. While, the obvious coupling signals between H-8' and C-7 (77.04 ppm), H-7 (4.67, dd, 9.6, 3.6 Hz) and C-8' (5.17, d, 8.3 Hz) in the HMBC spectra identified the linkage of C-7 and C-8' across an oxygen group. The only hydroxyl group in the compound **1** was therefore arranged at position of 7' by an analysis of carbon spectral data of leiocarpin E (Figure 1).

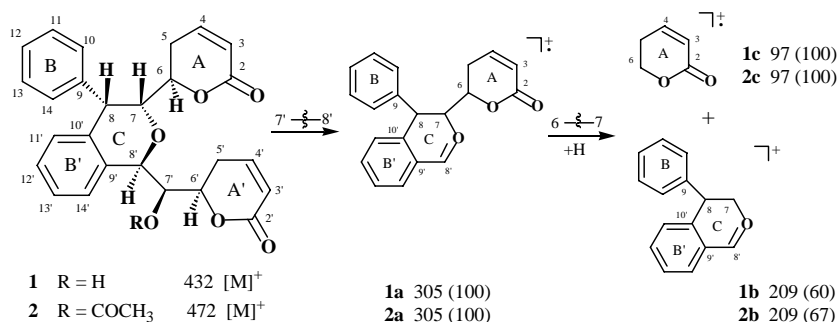
Table 1 ^1H and ^{13}C NMR data of compound **1** and **2** (δ ppm, J Hz, in $\text{C}_5\text{D}_5\text{N}$)

Position	1		2	
	H	C	H	C
2	----	163.66	----	163.79
3	5.98 dd, 9.8, 2.4	121.05	5.98 br d, 9.5	121.08
4	6.82 m	145.85	6.82 m	146.28
5	3.10 ddt, 18.5, 12.0, 3.6; 2.80 ddt, 18.5, 12.0, 3.6	23.66	2.94 br t, 19.0; 2.85 br t, 19.0	23.65
6	4.49 dt, 12.0, 3.6	77.44	4.50 br d, 11.8	77.48
7	4.67 dd, 9.6, 3.6	77.04	4.84 dd, 9.6, 2.4	77.23
8	4.25 d, 9.6	45.27	4.24 d, 9.6	45.06
9	----	142.16	----	142.32
10, 14	7.15-7.31 m	129.82	7.12-7.39 m	130.33
11, 13	7.15-7.31 m	129.46	7.12-7.39 m	129.93
12	7.15-7.31 m	130.08	7.12-7.39 m	130.86
2'	----	164.67	----	163.80
3'	6.11 dd, 9.8, 2.1	120.94	6.10 br d, 10.0	120.94
4'	6.87 m	146.64	6.85 m	147.07
5'	2.64 m; 2.61 m	23.76	2.62 m	25.66
6'	5.46 dt, 12.0, 3.2	79.04	5.38 br	76.62
7'	4.83 dd, 8.3, 3.2	73.81	6.50 dd, 8.0, 2.4	72.70
8'	5.17 d, 8.3	74.92	5.22 d, 8.0	71.27
9'	----	136.93	----	137.23
10'	----	135.71	----	133.71
11'	7.31 overlap	126.46	7.12-7.39 m	126.96
12'	7.17 overlap	127.72	7.12-7.39 m	127.84
13'	7.31 overlap	127.07	7.12-7.39 m	128.23
14'	7.95 d, 7.2	127.82	7.12-7.39 m	126.48
7'-OCOCH ₃	----	----	2.03 s	20.84
7'-OCOCH ₃	----	----	----	169.80

Acetylation of **1** with $\text{Ac}_2\text{O}/\text{Py}$ yielded a monoacetate **2**⁸. The peak at m/z 432 $[\text{M}]^+$ in the EIMS and the prominent peak at m/z 433 $[\text{MH}]^+$ in the FABMS spectrum of **1** agreed with the structural elucidation. The abundance 60 % of the peak at m/z 414 $[\text{M}-\text{HOAc}]^+$ in **2** proved the presence of the hydroxyl group in compound **1**, though the abundance at m/z 414 $[\text{M}-\text{H}_2\text{O}]$ m/z in **1** was only 0.5 %. Further more, the base peaks

at m/z 305 both in **1** and **2** firmly supported the presence of the C ring, and the upfielding of chemical shifts at C-7', 8' and 6' in compound **2** approved the existence of 7'-OH.

Figure 1 Structure of leiocarpin E **1**, **2** and their EIMS cleavage scheme



In the molecular model of **1**, the larger coupling constants (9.6 Hz) between H-8 and H-7 as well as the presence of NOE effect of H-8/H-7 in the NOESY spectrum arranged H-8 and H-7 as *synperiplanar*; so did H-8' and H-7' (*synperiplanar*). Meanwhile, the absence of NOE effects of H-8/H-8', H-8/H-7' and H-7/H-8', H-7' determined that H-8 and H-7 were not at the same plane with H-8' and H-7'. On the bases of goniodiol and the biogenetic consideration, the relative configuration of C-6 and C-6' were assigned as *R^{4,9}. Finally, the structure of leiocarpin E was established as **Figure 1**.

IC₅₀ value of leiocarpin E (**1**) was 1.2 μg/mL against HL-60 cells, while that of the positive control cheliensisin A was 0.07 μg/mL³.

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- Leiocarpin E **1** C₂₆H₂₄O₆ mp 251-252°C, UVλ_{max} nm (log ε): 204.5 (3.40); IR (KBr)v 3343 (OH), 1707, 1638, 1451, 1210, 817, 761 cm⁻¹; FABMS m/z : 433[MH]⁺; EIMS m/z (%): 433 (2) [MH]⁺, 414 (0.5) [M-H₂O]⁺, 335 (4), 305 (100), 209 (60), 115 (22), 97 (99).
- 10 mg of leiocarpin C was acetylated (AC₂O-pyridine; 24h, room temp.), and the mixture was partitioned between ice water and CHCl₃. The CHCl₃ extract on concentration showed one spot one TLC and purification by chromatography afforded compound **2**. Acetylleiocarpin E (**2**) EIMS m/z (%): 474 (4) [M]⁺, 414 (60) [M-AcOH]⁺, 377 (5), 317 (10), 305 (100). FABMS m/z : 475 [MH]⁺.
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