SUPPLEMENTARY MATERIAL

Two new *ent*-atisanes from the Root of *Euphorbia fischeriana* Steud.

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Abstract

Two new *ent*-atisanes *ent*-1 β ,3 β ,16 β ,17-tetrahydroxyatisane (**1**), *ent*-1 β ,3 α ,16 β ,17-tetrahydroxyatisane (**2**) together with 11 known diterpenes were isolated from the anti-tumor activity fraction of *Euphorbia fischeriana* Steud. The compounds were identified by detailed spectroscopic analysis, including extensive 2D-NMR experiments. X-ray analysis was applied to determine the structure of compound **2**. All the 13 compounds were screened for cytotoxicity *in vitro* against human tumor MCF-7, HepG-2 and SGC-7901 cell lines. Compounds **1** and **2** showed the inhibitory effects against MCF-7 with IC₅₀ levels of 23.21 and 15.42 μ M, simultaneously compounds **4**, **6**, **8** and **11** also had definite inhibitory effect against different cell lines.

Keywords: *ent*-atisane; *Euphorbia fischeriana* Steud.; X-ray; stereoismers; anti-tumor activity

Experimental

The optical rotations were measured in MeOH, using a PE 343 polarimeter made by Perkin-Elmer Company of USA under conditions of 589 nm, 23 °C, Na⁺ light source, and the concentrations of two compounds were 0.24 mg/mL (compound **1**) and 0.28 mg/mL (compound **2**). The NMR samples of compounds were prepared by pyridine-*d*₅ with TMS as an internal standard, coupling constant *J* in Hz. The HR-ESI-MS was determined on IonSpec Ultima 7.0 T FTICR. Preparative HPLC (Waters, Delta 600-2487) was performed on Hypersil-ODS II (10 μ m, 20 $\times$ 300 mm, Yilite; Da Lian). Column chromatography was performed with silica gel (200-300 mesh; Qingdao Haiyang Chemical Group Co. Ltd.). Analytical TLC spots were detected on Silica gel 60 F254 (Merck) by spraying with 10% ethanolic H₂SO₄ reagent followed by heating. The UV and NMR spectra were recorded on Shimadzu UV-1601 and Bruker DPX 400 (400 MHz for ¹H-NMR and 100 MHz for ¹³C-NMR).

52 **Table S1.** NMR data in pyridine-*d*₅ for compounds **1** and **2**

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	3.67-3.71(t,7.2)	79.6	4.48(dd,11.4,4.4)	76.1
2	2.31(s)	39.1	2.19(t,3.8)	37.5
	2.29(s)		2.36(d,2.5)	
3	3.55(t,8.52)	75.9	3.75(t,2.6)	76.7
4		39.6		38.0
5	0.75-0.78(m)	53.7	1.74(d,10.3)	48.6
6	1.43-1.49(m)	18.5	1.45-1.48(m)	18.8
			1.56(d,3.1)	
7	1.41(s)	40.8	1.25(td,13.6,4.3)	40.9
	1.22-1.25(m)		1.37(s)	
8		33.6		33.8
9	1.86-1.88(m)	52.8	2.10(dd,10.7,6.9)	52.8
10		43.5		43.5
11	2.26-2.28(m)	27.1	2.32(td,6.8,2.3)	27.1
	2.94(td,11.2,3.0)		2.97(tt,12.7,3.1)	
12	2.23-2.24(m)	33.1	2.22-2.23(m)	33.2
13 α	1.80-1.84(m)	24.3	1.60(td,6.3,2.6)	24.3
13 β	1.57(d,3.0)		1.84(td,12.8,2.9)	
14 α	1.91-1.97(m)	28.4	0.84(td12.6,6.2)	28.4
14 β	0.86(td,12.4,6.2)	28.4	1.96(t,12.8)	28.4
15 α	1.58-1.61(m)	53.6	1.51-1.54(m)	53.8
15 β	1.38(s)	53.6	1.39-1.40(m)	53.8
16		73.7		73.7
17	3.82(d,10.9)	69.5	3.80(d,10.9)	69.6
	3.93(d,10.9)		3.90(d,10.9)	
18	1.10(s)	16.4	0.95(s)	22.8
19	1.20(s)	28.7	1.17(s)	29.1
20	1.32(s)	11.3	1.34(s)	11.1

53 NMR was measured at 400 MHz (¹H) and 100 MHz (¹³C) (C₅D₅N₅)

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55 **Table S2.** Cytotoxicity of the isolated compounds

Compound	IC ₅₀ (μM)		
	HepG-2	SGC-7901	MCF-7
Compound 1	67.10±9.9	> 100	23.21±2.14
Compound 2	76.35±18.9	95.0±14.2	15.42±13.2
Compound 4	23.12±2.75	22.20±1.66	21.28±1.47
Compound 8	21.92±3.22	> 100	> 100
Compound 13	21.49±3.32	92.90±8.3	23.79±2.73
5-Fu	8.3±2.65	5.4±2.32	8.4±3.02

56 Other selected compounds not listed in the table were inactive (IC₅₀ > 100 μM) for all cell lines.

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Figure S1. ^1H NMR spectrum of compound **1**

Figure S2. ^{13}C NMR spectrum of compound **1**

67 **Figure S3:** DEPT NMR spectrum of compound **1**.

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71 **Figure S4:** ^1H - ^1H COSY spectrum of Compound **1**

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Figure S5: HSQC spectrum of Compound **1**

Figure S6: HMBC spectrum of compound **1**

Figure S7: NOESY spectrum of Compound 1

Figure S8: MS spectrum of compound 1

Figure S9: ^1H NMR spectrum of compound 2

Figure S10: ^{13}C -NMR spectrum of compound 2

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100

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102
103

104

105

Figure S13: HSQC spectrum of compound 2

Figure S14: HMBC spectrum of compound 2

Figure S15: NOE spectrum of compound 2

Figure S16: MS spectrum of compound 2

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128
129

NOESY for compound **1** NOESY for compound **2**