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金刚纂的化学成分及其抗 HIV 和抗肿瘤活性研究

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摘　要:从金刚纂全草中分离得到一个新的天然来源的阿替生烷型二萜,3-methyl-agallochaol C(**1**),以及 13 个已知化合物。其中,化合物 **4** 和 **9** 具有一定的抗 HIV-1 活性,化合物 **10** 具有一定的抗肿瘤活性。

关键词:金刚纂;3-methyl-agallochaol C;抗 HIV;抗肿瘤

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Chemical Constituents from *Euphorbia neriifolia* and Their Related Anti-HIV and Anti-cancer Activities

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Abstract: Systematic investigation on the whole plant of *Euphorbia neriifolia* led to the isolation of a new natural atisane diterpenoid, 3-methyl-agallochaol C (**1**), along with thirteen known compounds (**2-14**). Among them, compounds **4** and **9** exhibited moderate anti-HIV-1 activity, and compound **10** possessed moderate anti-cancer activity.

Key words: *Euphorbia neriifolia*; 3-methyl-agallochaol C; anti-HIV; anti-cancer

Introduction

Human immunodeficiency virus (HIV) is the etiologic agent of the acquired immunodeficiency syndrome (AIDS), a disease that already claimed the lives of more than 25 million people. The global incidence of HIV infection in 2010 was estimated to be approximately 33.2 million people [1]. Current antiretroviral drugs are vitally important to improve the quality and prolong the life of HIV/AIDS patients. Nevertheless, these drugs have many disadvantages including resistance, toxicity, limited availability, high cost and lack of any curative effect [2]. Thus, the need and demand has prompted an intense research effort to discover new, selective and safe drugs for the treatment of HIV/AIDS. Natural sources, particularly plants, are an excellent source of anti-HIV agents. Southern China, especially Yunnan Province, possess an abundant plant biodiversity and a long history of medicinal use of plants, so many plants may contain novel anti-HIV constituents. *Euphorbia neriifolia* Linn. (Euphorbiaceae), traditional Dai medicine in China, is a landscape plant widely cultivated in the south and southwest of Yunnan Province and used for hedges [3]. This plant produces milky latex which possesses several applications in folk medicines, such as irritant, emetic, purgative and diuretic [4]. The plant extracts were demonstrated to exhibit antihepatotoxic and cytotoxic activities [5]. In order to discover anti-HIV agents of natural origin, different parts (EtOAc, n-BuOH and H_2O parts) of 95% EtOH extracts of *E. neriifolia* were evaluated for their anti-HIV-1 activities, using AZT as positive control (EC_{50} = 0.008 μg/mL). Results showed that EtOAc part exhibited potential anti-HIV-1 activity with an EC_{50} value of 1.26 μg/mL. Bioassay-guided isolation of the EtOAc part led to the purification of fourteen compounds (**1-14**), including a new natural atisane diterpenoid, 3-methyl-agallochaol C (**1**). Among them, compounds **4** and **9** possessed moderate anti-HIV-1 activity. In addition, in view of the cytotoxic activity of this plant reported previously, the cytotoxicities of isolated compounds were also tested.

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Experimental

General

Optical rotation was run on a Jasco DIP-370 digital polarimeter (JASCO Corporation, Tokyo, Japan). NMR spectra were recorded over Bruker AM-400, DRX-500 and AVANCE III-600 instruments with tetramethylsilane (TMS) as an internal standard (Bruker BioSpin Group, Germany). ESI-MS was obtained with an API-Qstar-TOF instrument. Column chromatography (CC) was performed with silica gel (200-300 mesh, Qingdao Marine Chemical and Industrial Factory, China), MCI (MCI-gel CHP-20P, 75-150 μm, Mitsubishi Chemical Corporation) and Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden). Fractions were monitored by TLC plates (Si gel GF_{254}, Qingdao Marine Chemical and Industrial Factory, China), and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4-EtOH.

Plant material

The whole plant of *E. neriifolia* was collected from Xishuangbanna, Yunnan Province, PR China, in September 2008, and was identified by Mr. Jing-yun Cui. A voucher specimen (No. 20080901) was deposited at the Laboratory of Phytochemistry, Faculty of Life Science and Technology, Kunming University of Science of Technology.

Extraction and isolation

The air-dried and powdered woods of *E. neriifolia* (4.0 kg) were extracted with 95% EtOH (3 × 6 L, 24 h each) at room temperature, and then concentrated under vacuum to yield an extract (125 g), which was suspended in H_2O (2 L) and then extracted with EtOAc (4 × 2 L). The EtOAc extract (66.0 g) was separated by MCI, eluting with MeOH/H_2O (gradient 30%, 60%, 90% and 100%), to afford fractions A-E. Fr. C (1.6 g) was subjected to silica gel CC (200-300 mesh), using petroleum ether/acetone (5∶1) as eluent to give six subfractions, C-1 ~ C-6. Compound **1** (10 mg) was obtained from C-5 (45 mg) by silica gel CC eluted with petroleum ether/EtOAc (3∶1).

3-Methyl-agallochaol C (1) colorless oil, $C_{21}H_{34}O_4$, $[\alpha]_D^{19}$-15.49 (*c* 0.57, $CHCl_3$). ESI-MS (neg.) *m/z* 373 (61, $[M+Na]^+$).

Bioactivities

HIV-1_{NL4-3} Replication Inhibition Assay

A previously described HIV-1 infectivity assay was used [6,7].

Cytotoxicity analysis

Cytotoxicity was determined by the sulforhodamine B (SRB) colorimetric assay [8].

Results and Discussion

Compound **1** was isolated as colorless oil. Its molecular formula, $C_{21}H_{34}O_4$, was determined by ESI-MS (*m/z* 373, $[M+Na]^+$), in combination with ^1H and ^{13}C NMR data (Table 1), indicating five degrees of unsaturation. In the ^1H-NMR spectrum, a methoxyl group at δ_H3.63 (3H, s), two tertiary methyls at δ_H 1.76 and

Table 1 ^1H (600 MHz) and ^{13}C (150 MHz) NMR data of compound 1 in C_5D_5N

No.	δ_H (mult., *J*, Hz)	δ_C (mult.)
1	1.68 (2H, m)	34.7 (t)
2	2.37 (1H, overlap)	29.4 (t)
	2.46 (1H, m)	
3	–	175.0 (s)
4	–	148.5 (s)
5	2.02 (1H, dd, *J* = 2.6, 12.8)	51.4 (d)
6	1.27 (2H, m)	25.6 (t)
7	1.16 (1H, m)	39.1 (t)
	1.28 (1H, m)	
8	–	33.7 (s)
9	1.63 (1H, m)	44.0 (d)
10	–	40.4 (s)
11	1.22 (1H, m)	24.1 (t)
	2.39 (1H, overlap)	
12	2.25 (1H, m)	33.4 (d)
13	1.84 (1H, overlap)	24.1 (t)
	1.44 (1H, m)	
14	1.84 (1H, overlap) 0.85 (1H, m)	28.1 (t)
15	1.37 (1H, d, *J* = 13.6)	54.1 (t)
	1.52 (1H, dd, *J* = 13.6, 2.8)	
16	–	74.2 (s)
17	3.85 (1H, d, *J* = 11.0)	70.0 (t)
	3.93 (1H, d, *J* = 11.0)	

18	1.76 (3H, s)	24.3 (q)
19	4.80 (1H, br s)	114.1 (t)
	4.93 (1H, br s)	
20	0.93 (3H, s)	18.5 (q)
-OCH_3	3.63 (3H, s)	51.9 (q)

0.93 (each 3H, s), two protons of terminal double bond at δ_H 4.80 and 4.93 (each 1H, br s), as well as a pair of hydroxylmethyl protons at δ_H 3.85 and 3.93 (each 1H, d, J = 11.0) were observed. Apart from the methoxyl group (δ_C51.9, q), twenty carbons were observed in the ^{13}C NMR spectrum, including two methyls, nine methenes (including an oxygenated one and a terminal double bond), three methines, four quaternary carbons (containing an oxygenated one and an olefinic one) and one carbonyl group. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data with those of agallochaol C [9] showed they were very similar, except for the presence of the methoxyl group (δ_H 3.63, s; δ_C 51.9, q) in **1**. This methoxyl was attached to C-3 (δ_C 175.0, s) because it showed HMBC correlation with C-3. Thus, the structure of **1** (Fig. 1) was determined and named as 3-methyl-agallochaol C, which was a new natural product synthesized by Guo *et al.* in order to further confirm the structure of agallochaol C [9].

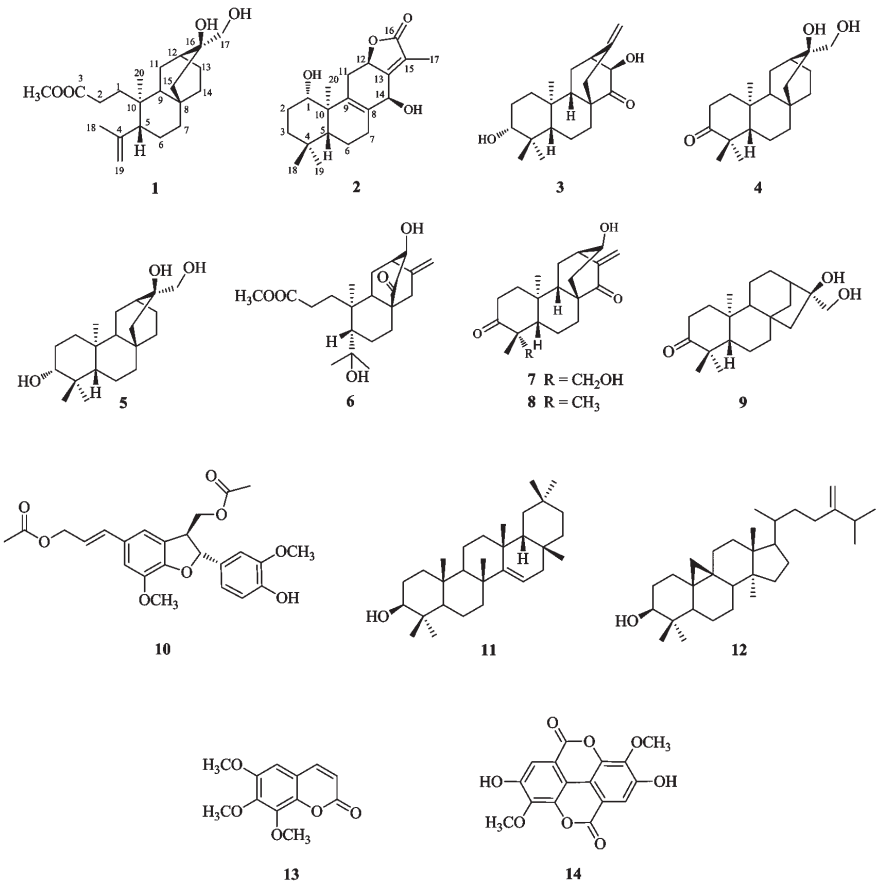

Fig. 1 Chemical structures of compounds 1-14

The known compounds were determined to be eurifoloid D (**2**) [5], *ent*-3β, (13*S*)-dihydroxyatis-16-en-14-one (**3**) [10], *ent*-16α, 17-Dihydroxyatisan-3-one (**4**) [10], *ent*-atisane-3β, 16α, 17-triol (**5**) [11], 4, 13β-dihydroxy-14-oxo-3, 4-secoatis-16-en-3-oic acid methyl ester (**6**) [12], 13β, 19-dihydroxy-3, 15-dioxoatis-16-ene (**7**) [12], 13β-hydroxy-3, 15-dioxoatis-16-ene (**8**) [12], *ent*-16α, 17-dihydroxykauran-3-one (**9**) [13], 3-acetoxymethyl-5-[(*E*)-3-acetoxy-propen-1-yl)]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2, 3-dihydrobenzofuran (**10**) [14], taraxerol (**11**) [15], 9β, 19-cyclolanostan-3β-ol (**12**) [15], 6, 7, 8-trimethoxyl-coumarin

(**13**)[15], and 3,3′-di-*O*-methylellagic acid (**14**)[15] by comparison of their spectral data with literature values.

The anti-HIV-1 activity of compounds **3-5**,**8**,**9** and **11-13** were evaluated using AZT as positive control (EC_{50} = 0.0086 ± 0.0015 μg/mL), compounds **4** and **9** exhibited moderate anti-HIV-1 activity with EC_{50} values of 6.55 ± 2.24 and 12.3 ± 3.75 μg/mL, respectively. In addition, the cytotoxicities of compounds **3-5** and **8-13** against a panel of human cancer cell lines were tested with Paclitaxel as positive control, and compound **10** possessed moderate anti-cancer activity (Table 2).

Table 2 Cytotoxicity SRB assay of compounds 3-5,8-13 and paclitaxel

Compounds	Cell Line (IC_{50}, μM)			
	A549	MDA-MB-231	KB	KB-VIN
3	> 10	> 10	> 10	> 10
4	> 10	> 10	> 10	> 10
5	> 10	> 10	> 10	> 10
8	> 10	> 10	> 10	> 10
9	> 10	> 10	> 10	> 10
10	7.248 ± 0.136	4.796 ± 0.044	4.659 ± 0.011	5.519 ± 0.107
11	> 10	> 10	> 10	> 10
12	> 10	> 10	> 10	> 10
13	> 10	> 10	> 10	> 10
Paclitaxel	0.005230 ± 0.000852	0.004354 ± 0.000978	0.002899 ± 0.000202	1.298787 ± 0.060698

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