

# 白木香果皮中的五个葫芦素类化合物及其细胞毒活性

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**摘要:**从白木香(*Aquilaria sinensis*)果皮中分离得到5个葫芦素类化合物,通过波谱数据分析分别鉴定为双氢葫芦素 E(1)、endecaphyllacins B(2)、2-*O*- $\beta$ -D-glucopyranosylcucurbitacin I(3)、葫芦素 E(4)、葫芦素 I(5)。其中化合物 1~3 为首次从沉香属植物中分离得到;化合物 1~5 具有细胞毒活性。

**关键词:**白木香;果皮;化学成分;葫芦素;细胞毒活性

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## Five Cucurbitacins from *Aquilaria sinensis* Peels and Their Cytotoxic Activities

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**Abstract:** Five cucurbitacins were isolated from the peels of *Aquilaria sinensis*. On the basis of spectral data, their structures were identified as dihydrocucurbitacin E (1), endecaphyllacins B (2), 2-*O*- $\beta$ -D-glucopyranosylcucurbitacin I (3), cucurbitacin E (4), cucurbitacin I (5). Among them, compounds 1-3 were isolated from plants of the genus *Aquilaria* for the first time. Compounds 1-5 showed cytotoxic activities against tested human cell lines.

**Key words:** *Aquilaria sinensis*; peel; chemical constituent; cucurbitacin; cytotoxic activity

*Aquilaria sinensis* (Lour.) Gilg, a tropical and subtropical evergreen trees, belonging to the family Thymelaeaceae, is mainly distributed in Guangdong, Guangxi, Hainan, Yunnan, Hong Kong and Macao<sup>[1]</sup>. *A. sinensis* can secrete a fragrant defensive black resin when suffering an injury or infected by fungi. The black resin, called Chinese eaglewood, is a rare traditional Chinese medicine<sup>[2]</sup>. To investigate the chemical constituents of *A. sinensis*, luteolin, epifriedelanol, friedelin, genkwanin have been isolated from the leaves of *A. sinensis*<sup>[3]</sup>, the flavonoid and benzenoid derivatives from the stem of *A. sinensis*<sup>[4,5]</sup>, and the cucurbitacins from the fruits of *A. sinensis*<sup>[6]</sup>. We have shown, in our previous research,

that the extract from the peels of *A. sinensis* displayed cytotoxic activity against MCF-7 cell line<sup>[7]</sup>. To further clarify the cytotoxic active ingredients in *A. sinensis* peels, five cucurbitacins were isolated from the peels of *A. sinensis* and identified as dihydrocucurbitacin E (1), endecaphyllacins B (2), 2-*O*- $\beta$ -D-glucopyranosylcucurbitacin I (3), cucurbitacin E (4), cucurbitacin I (5). Compounds 1-3 were isolated from plants of the genus *Aquilaria* for the first time. The chemical structure of compounds 1-5 are shown in Figure 1.

## Materials and Methods

### Apparatus and reagents

The NMR spectra were recorded on a Bruker AVANCE 500 spectrometer and referenced to the signals of TMS as an internal standard. EI-MS: Thermo DSQ mass spectrometer and/or Thermo MAT95XP mass spectrometer; in *m/z*. Column chromatography (CC): commercial silica gel (SiO<sub>2</sub>; 200-300 mesh; Qingdao Marine

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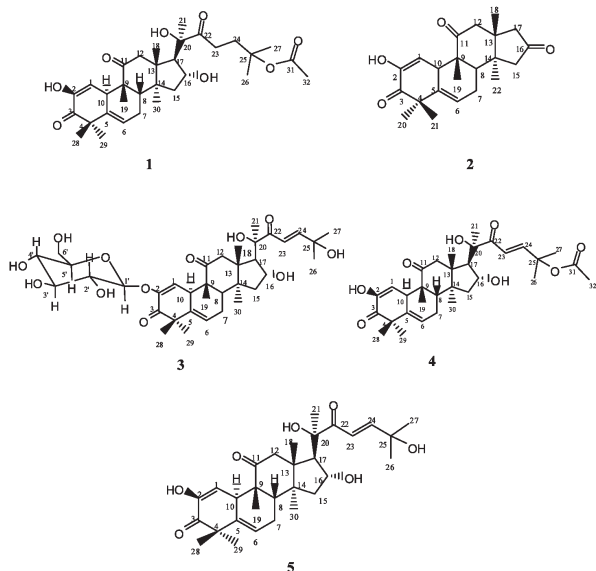


Fig. 1 Structures of compounds 1-5

Chemical Group Co, Qingdao, P. R. China), ChromatorexODS (40-75  $\mu\text{m}$ ; Fuji Silysia), and Sephadex LH-20 (Amersham Biosciences). TLC: Merck precoated silica gel plates GF<sub>254</sub>. All reagents were of analytical grade and purchased from Guangzhou Chemical Factory.

### Plant material

The fruits of *Aquilaria sinensis* were collected in Xinyi, Guangdong Province of China in 2010 and the seeds and peels were separated by hand. The material was authenticated by the Department of Medicine Plants and Chinese Medicine Appraisal, Guangdong Pharmaceutical University. The voucher specimen were deposited in the Guangdong Institute of Microbiology.

### Extraction and isolation

The air-dried peels of *A. sinensis* (10.0 kg) were powdered and then extracted three times with 95% EtOH. The solvent was removed under vacuum to give a dark brown gum, which was suspended in water and successively partitioned with petroleum ether and EtOAc.

The EtOAc fraction (167.9 g) was separated by column chromatography with a gradient system of increasing polarity (petroleum ether/ EtOAc/MeOH) to afford 11 fractions (Fr. A – Fr. K). Fr. C was submitted to column chromatography on silica gel C<sub>18</sub> with MeOH-H<sub>2</sub>O (40:60) to afford 8 subfractions (Fr. C-1 – Fr. C-8). Fr. C-2, Fr. C-3 and Fr. C-5 were purified over Sephadex LH-20 using CHCl<sub>3</sub>-MeOH (1:1) to yield

compound **1** (42.3 mg), compound **4** (20.5 mg) and compound **5** (11.2 mg). Fr. E afforded compound **2** (29.8 mg) by Sephadex LH-20 using CHCl<sub>3</sub>-MeOH (1:1) and recrystallization technique. Fr. G was subjected to Sephadex LH-20 using CHCl<sub>3</sub>-MeOH (1:1) to yield compound **3** (13.5 mg).

### Identification

**Compound 1** Yellow amorphous powder (CHCl<sub>3</sub>). ESI-MS  $m/z$ : 557 [M-H]<sup>-</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 5.93 (1H, d,  $J$  = 2.6 Hz, H-1), 5.76 (1H, m, H-6), 4.30 (1H, m, H-16), 3.23 (1H, d,  $J$  = 14.5 Hz, H-12a), 2.82 (1H, m, H-23), 2.71 (1H, d,  $J$  = 14.5 Hz, H-12b), 2.51 (1H, m, H-7b), 2.37 (1H, m, H-7a), 1.95 (3H, s, 32-CH<sub>3</sub>), 1.44 (3H, s, 27-CH<sub>3</sub>), 1.42 (3H, s, 26-CH<sub>3</sub>), 1.41 (3H, s, 21-CH<sub>3</sub>), 1.39 (3H, s, 30-CH<sub>3</sub>), 1.34 (3H, s, 28-CH<sub>3</sub>), 1.23 (3H, s, 29-CH<sub>3</sub>), 1.01 (3H, s, 19-CH<sub>3</sub>), 0.96 (3H, s, 18-CH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$ : 214.9 (C-22), 213.9 (C-11), 199.6 (C-3), 171.4 (C-31), 145.5 (C-2), 137.6 (C-5), 121.7 (C-6), 115.8 (C-1), 82.2 (C-25), 79.8 (C-20), 71.8 (C-16), 58.7 (C-17), 51.5 (C-14), 49.8 (C-12), 49.7 (C-9), 49.3 (C-13), 48.5 (C-4), 46.5 (C-15), 42.4 (C-8), 35.6 (C-10), 35.5 (C-24), 31.6 (C-23), 28.8 (C-28), 27.1 (C-27), 26.7 (C-26), 25.4 (C-21), 24.5 (C-7), 23.3 (C-32), 21.1 (C-29), 20.9 (C-18), 20.7 (C-19), 19.1 (C-30). It was identified as dihydrocucurbitacin E by comparing with spectral data of the literature<sup>[8]</sup>.

**Compound 2** White amorphous powder (MeOH). ESI-MS  $m/z$ : 357 [M + H]<sup>+</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 5.93 (1H, dd,  $J$  = 2.8, 0.6 Hz, H-1), 5.78 (1H, m, H-6), 3.18 (1H, d,  $J$  = 14.5 Hz, H-12a), 2.56 (1H, d,  $J$  = 17.6 Hz, H-17a), 2.45 (1H, d,  $J$  = 14.5 Hz, H-12b), 2.26 (1H, d,  $J$  = 17.2 Hz, H-15a), 2.09 (1H, d,  $J$  = 17.2 Hz, H-15b), 2.00 (1H, d,  $J$  = 17.6 Hz, H-17b), 1.37 (3H, s, 20-CH<sub>3</sub>), 1.28 (3H, s, 21-CH<sub>3</sub>), 1.26 (3H, s, 22-CH<sub>3</sub>), 1.10 (3H, s, 19-CH<sub>3</sub>), 1.03 (3H, s, 18-CH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$ : 216.9 (C-16), 212.0 (C-11), 199.4 (C-3), 145.6 (C-2), 137.8 (C-5), 121.1 (C-6), 115.4 (C-1), 51.2 (C-9), 51.1 (C-15), 50.1 (C-17), 48.5 (C-4), 46.8

(C-12), 46.0 (C-14), 45.2 (C-13), 43.0 (C-8), 35.8 (C-10), 28.7 (C-21), 25.1 (C-18), 24.9 (C-7), 21.1 (C-20), 20.9 (C-19), 19.4 (C-22). It was identified as endecaphyllacins B by comparing with spectral data of the literature<sup>[9]</sup>.

**Compound 3** White amorphous powder (MeOH). ESI-MS  $m/z$ : 677  $[M + H]^+$ ;  $^1H$  NMR (500 MHz,  $CD_3OD$ )  $\delta$ : 6.98 (1H, d,  $J = 15.8$  Hz, H-24), 6.85 (1H, d,  $J = 15.8$  Hz, H-23), 6.11 (1H, d,  $J = 2.6$  Hz, H-1), 4.65 (1H, d,  $J = 7.4$  Hz, H-1'), 4.05 (1H, dd,  $J = 12.3, 3.3$  Hz, H-6'a), 3.87 (1H, dd,  $J = 12.3, 3.3$  Hz, H-6'b), 3.69 (1H, br s, H-10), 3.42 (1H, d,  $J = 14.8$  Hz, H-12a), 2.61 (1H, d,  $J = 14.8$  Hz, H-12b), 2.57 (1H, d,  $J = 7.5$  Hz, H-17), 1.58 (3H, s, 30- $CH_3$ ), 1.55 (3H, s, 21- $CH_3$ ), 1.42 (3H, s, 26- $CH_3$ ), 1.42 (3H, s, 27- $CH_3$ ), 1.31 (3H, s, 28- $CH_3$ ), 1.28 (3H, s, 29- $CH_3$ ), 1.02 (3H, s, 19- $CH_3$ ), 0.90 (3H, s, 18- $CH_3$ );  $^{13}C$  NMR (125 MHz,  $CD_3OD$ )  $\delta$ : 216.1 (C-11), 204.8 (C-22), 199.3 (C-3), 151.1 (C-24), 146.7 (C-2), 137.0 (C-5), 123.0 (C-1), 122.2 (C-6), 121.8 (C-23), 100.6 (C-1'), 80.6 (C-20), 79.8 (C-5'), 77.6 (C-3'), 77.1 (C-2'), 73.8 (C-25), 71.4 (C-16), 70.1 (C-4'), 61.4 (C-6'), 59.7 (C-17), 51.1 (C-4), 49.9 (C-9), 49.6 (C-13), 49.5 (C-14), 49.1 (C-12), 46.2 (C-15), 42.7 (C-8), 35.9 (C-10), 27.8 (C-27), 26.4 (C-26), 25.9 (C-28), 24.9 (C-21), 24.2 (C-7), 21.4 (C-19), 20.3 (C-18), 20.1 (C-29), 18.2 (C-30). It was identified as 2-O- $\beta$ -D-glucopyranosylcucurbitacin I by comparing with spectral data of the literature<sup>[10]</sup>.

**Compound 4** White amorphous powder ( $CHCl_3$ ). ESI-MS  $m/z$ : 555  $[M - H]^-$ ;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$ : 7.06 (1H, d,  $J = 15.6$  Hz, H-24), 6.46 (1H, d,  $J = 15.6$  Hz, H-23), 5.96 (1H, d,  $J = 2.6$  Hz, H-1), 5.77 (1H, m, H-6), 4.37 (1H, m, H-16), 3.50 (1H, br s, H-10), 3.22 (1H, d,  $J = 14.4$  Hz, H-12a), 2.74 (1H, d,  $J = 14.4$  Hz, H-12b), 2.48 (1H, d,  $J = 7.1$  Hz, H-17), 2.01 (3H, s, 32- $CH_3$ ), 1.57 (3H, s, 27- $CH_3$ ), 1.54 (3H, s, 26- $CH_3$ ), 1.44 (3H, s, 21- $CH_3$ ), 1.39 (3H, s, 30- $CH_3$ ), 1.36 (3H, s, 28- $CH_3$ ), 1.25 (3H, s, 29- $CH_3$ ), 1.03 (3H, s, 19- $CH_3$ ), 1.00 (3H, s, 18- $CH_3$ );  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )

$\delta$ : 212.9 (C-22), 202.5 (C-11), 198.7 (C-3), 170.3 (C-31), 152.0 (C-24), 144.5 (C-2), 136.7 (C-5), 120.8 (C-6), 120.3 (C-23), 114.8 (C-1), 79.3 (C-25), 78.2 (C-20), 71.3 (C-16), 58.2 (C-17), 50.7 (C-13), 48.9 (C-12), 48.5 (C-9), 48.1 (C-14), 47.5 (C-4), 45.5 (C-15), 41.6 (C-8), 34.7 (C-10), 29.7 (C-29), 27.9 (C-27), 26.5 (C-26), 25.9 (C-21), 23.9 (C-7), 21.9 (C-28), 20.2 (C-30), 20.1 (C-32), 19.8 (C-18), 18.4 (C-19). It was identified as cucurbitacin E by comparing with spectral data of the literature<sup>[8]</sup>.

**Compound 5** White amorphous powder (MeOH). ESI-MS  $m/z$ : 515  $[M + H]^+$ ;  $^1H$  NMR (500 MHz,  $CD_3OD$ )  $\delta$ : 6.98 (1H, d,  $J = 15.4$  Hz, H-24), 6.85 (1H, d,  $J = 15.4$  Hz, H-23), 5.81 (1H, m, H-6), 5.76 (1H, d,  $J = 2.8$  Hz, H-1), 4.51 (1H, m, H-16), 3.40 (1H, d,  $J = 14.7$  Hz, H-12a), 2.66 (1H, d,  $J = 14.7$  Hz, H-12b), 2.61 (1H, d,  $J = 7.5$  Hz, H-17), 1.87 (1H, m, H-15a), 1.73 (1H, m, H-15b), 1.42 (3H, s, 30- $CH_3$ ), 1.41 (3H, s, 21- $CH_3$ ), 1.34 (3H, s, 29- $CH_3$ ), 1.33 (3H, s, 26- $CH_3$ ), 1.31 (3H, s, 27- $CH_3$ ), 1.26 (3H, s, 28- $CH_3$ ), 0.99 (3H, s, 18- $CH_3$ ), 0.94 (3H, s, 19- $CH_3$ );  $^{13}C$  NMR (125 MHz,  $CD_3OD$ )  $\delta$ : 215.7 (C-11), 204.5 (C-22), 199.5 (C-3), 154.8 (C-24), 146.3 (C-2), 137.9 (C-5), 121.2 (C-6), 120.8 (C-23), 116.3 (C-1), 79.4 (C-20), 71.2 (C-16), 71.0 (C-25), 59.1 (C-17), 51.3 (C-14), 49.7 (C-12), 49.5 (C-9), 49.0 (C-13), 48.8 (C-4), 46.3 (C-15), 42.7 (C-8), 35.5 (C-10), 28.7 (C-26), 28.7 (C-27), 27.9 (C-28), 24.9 (C-21), 24.1 (C-7), 20.3 (C-29), 20.2 (C-19), 19.8 (C-18), 18.4 (C-30). It was identified as cucurbitacin I by comparing with spectral data of the literature<sup>[8]</sup>.

### Cytotoxicity assay

Compounds **1-5** were investigated for their cytotoxic activities against SF-268, MCF-7, NCI-H460 and HePG-2 cell lines by using SRB method<sup>[11]</sup>, with cisplatin as a positive control. The result showed that all compounds exhibited cytotoxic activities with inhibitory rates ranging from 51.9% to 94.6% at a concentration of 100  $\mu$ mol/L (Table 1) and their  $IC_{50}$  values were also determined (Table 2).

**Table 1** Inhibitory rates of compounds 1-5 against four tumor cell lines( % )

Compounds	Concentration ( $\mu\text{mol/L}$ )	Inhibitory rates			
		SF-268	MCF-7	NCI-H460	HePG-2
1	100	94.6	85.6	91.1	90.8
2	100	75.5	78.6	85.2	85.3
3	100	79.5	71.9	88.4	51.9
4	100	80.4	60.6	90.9	60.1
5	100	88.9	73.2	88.5	80.5
Cisplatin	100	99.2	94.0	94.9	92.6

**Table 2** IC<sub>50</sub> values of compound 1-5 against four tumor cell lines (  $\mu\text{mol/L}$  )

Compounds	IC <sub>50</sub>			
	SF-268	MCF-7	NCI-H460	HePG-2
1	3.6	2.7	4.5	16.9
2	5.6	12.6	2.9	19.5
3	3.7	14.6	4.1	18.2
4	12.7	17.9	3.6	19.2
5	3.8	2.3	1.5	16.9
Cisplatin	5.2	4.1	1.9	2.7

## Results and Discussion

Cucurbitacins were originally isolated as the bitter components of the Cucurbitaceae, and were later found to be present in plants of the families Brassicaceae, Scrophulariaceae, Begoniaceae, Elaeocarpaceae, Datisceae, Desfontainiaceae, Polemoniaceae, Primulaceae, Rubiaceae, Sterculiaceae, Rosaceae and Thymelaeaceae<sup>[12]</sup>. They are a series of compounds called tetracyclic triterpenes with strong and unique biological activities such as antitumor, anti-inflammatory, liver-protective activities and so on<sup>[13]</sup>. Recently, five cucurbitacins; hexanorcucurbitacin I, cucurbitacin I, cucurbitacin D, isocucurbitacin D, and neocucurbitacin B, were reported from fruits of *A. sinensis*, among them the four cucurbitacins except cucurbitacin D showed different degrees of cytotoxic activities against K562, SGC-7901 and SMMC-7721<sup>[6]</sup>. The cucurbitacin E (4) were isolated from the hulls of *Aquilaria agallocha* and used in treatment of cancer<sup>[14]</sup>. In this study, five cucurbitacins were also isolated from the peels of *A. sinensis* and i-

dentified as dihydrocucurbitacin E (1), endecaphyllacins B (2), 2-*O*- $\beta$ -D-glucopyranosylcucurbitacin I (3), cucurbitacin E (4), cucurbitacin I (5) (Fig. 1). Compounds 1-3 were isolated for the first time from plants of the genus *Aquilaria*, as well as from the family Thymelaeaceae. Cytotoxic assay revealed all compounds showed inhibitory activities against SF-268, MCF-7, NCI-H460 and HePG-2 cell lines, which appeared to be responsible for the cytotoxic activity of the extract from *A. sinensis* peels. Endecaphyllacins B was first isolated from the tubers of *Hemsleya endecaphylla* and its HIV-1 inhibitory activity was examined<sup>[9]</sup>. In this study, the cytotoxic activity of endecaphyllacins B was investigated for the first time and showed a strong inhibitory activity against above four cell lines with IC<sub>50</sub> values of 5.6, 12.6, 2.9 and 19.5  $\mu\text{mol/L}$ , respectively.

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