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DITERPENOID COMPOUNDS FROM *Vitex agnus-castus*

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*A new diterpenoid alkaloid having an α,β-unsaturated γ-lactam moiety, 9α-hydroxy-13(14)-labden-16,15-amide (**1**), together with five known ones, was isolated from the fruits of* Vitex angus-castus*. The structure of **1** was elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques.The cytotoxicity of each compound was evaluated against human chronic myelogenous leukemia (K562) cell lines. Compound **1** was found to be the most potent with IC_{50} value of 0.70 μg/mL.*

Keywords: diterpenoid, *Vitex agnus-castus*, Verbenaceae, vitexlactam, cytotoxicity.

Vitex agnus-castus L. (Verbenaceae) is a medicinal plant distributed in tropical and temperate zones, the Mediterranean region, Central Asia, and Southern Europe [1]. It is also cultivated in Jiangsu and Shanghai of China. It is used in traditional medicine for the treatment of female hormonal disorders [2, 3]. In recent years, several papers have described phytochemistry investigations that show that plants in this genus produce diterpenoids [4–7], flavonoids [8–10], terpenoids, steroids [10], and iridoids [11]. The fruits of *V. agnus-castus* are popular phytomedicines in Europe [12, 13].

Vitex agnus-castus L. is widely distributed in Northwestern China. In previous work, some bioactive compounds were isolated from this plant [4–7, 10]. Motivated by a search for bioactive compounds from this plant, a reinvestigation of the chemical compounds of the fruits of *V. agnus-castus* was carried out. As a result, a new diterpenoid alkaloid (**1**), together with five known diterpenoids,was isolated from this plant. In addition, all the compounds (**1–6**) were evaluated for their cytotoxic activity against human-tumor K562 cells,with cisplatin as a positive control (IC_{50} = 1.10 μg/mL). Compound **1** showed the most potent effect against K562 cells with an IC_{50} = 0.70 μg/mL. Compounds **2**, **3**, and **4** displayed modest activities, and compounds **5** and **6** showed weak activities (Table 1).

The air-dried fruits of *V. agnus-castus* (5.75 kg) were milled sequentially and extracted three times with 70% EtOH. Evaporation of the solvent under reduced pressure provided a 70% EtOH extract (335 g). This crude extract was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18, and Chromatorex ODS to afford compounds **1–6**, including a new diterpenoid alkaloid (**1**), together with five known ditepenoids: vitexlactam (**2**) [14], vitexilactone and rotundifurans (**3**, **4**) [15], 8-epimanoyl oxide (**5**) [16], and vitetrifolin D (**6**) [17].

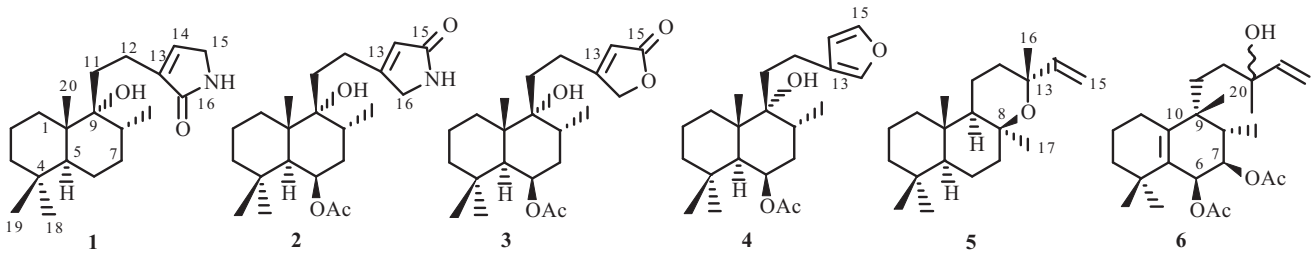

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TABLE 1. Cytotoxic Effects of Compounds **1–6** in K562 Cell Lines

Compound	IC_{50}, μg/mL	Compound	IC_{50}, μg/mL
Cisplatin (control)	1.10	**4**	2.91
1	0.70	**5**	4.56
2	1.15	**6**	6.72
3	2.73		

TABLE 2. ^{13}C NMR Data of Compounds **1–6** ($CDCl_3$, δ, ppm)

C atom	**A** [7]	**1**	**2**	**3**	**4**	**5**	**6**
1	33.7 t	32.5 t	33.7 t	33.8 t	33.9 t	36.5 t	25.9 t
2	18.8 t	18.7 t	18.6 t	18.9 t	18.7 t	20.7 t	19.4 t
3	43.8 t	41.7 t	43.6 t	43.9 t	43.7 t	42.3 t	39.4 t
4	33.9 s	33.3 s	34.0 s	34.3 s	34.8 s	32.9 s	34.6 s
5	47.5 d	46.2 d	47.6 d	48.0 d	47.5 d	46.3 d	132.5 s
6	70.6 d	21.7 t	69.9 d	70.1 d	70.3 d	21.0 t	66.2 d
7	36.3 t	36.8 t	36.1 t	36.4 t	36.1 t	37.9 t	72.7 d
8	32.1 d	31.4 d	31.9 d	32.3 d	33.6 d	74.1 s	36.4 d
9	76.4 s	76.8 s	76.7 s	76.8 s	76.8 s	61.2 d	42.9 s
10	44.0 s	43.3 s	43.8 s	44.1 s	43.7 s	38.9 s	141.5 s
11	32.3 t	32.0 t	32.3 t	31.9 t	31.8 t	18.6 t	29.3 t
12	21.7 t	22.0 t	26.5 t	25.7 t	21.5 t	45.1 t	38.6 t
13	140.6 s	140.8 s	163.6 s	171.3 s	125.5 s	73.6 s	73.0 s
14	137.1 d	136.9 d	121.2 d	115.3 d	110.8 d	146.1 d	144.5 d
15	46.6 t	46.4 t	175.3 s	171.3 s	142.9 d	111.1 t	112.1 t
16	175.3 s	175.8 s	50.5 t	73.4 t	138.5 d	27.4 q	27.8 q
17	16.4 q	16.6 q	16.0 q	16.3 q	16.1 q	32.0 q	11.1 q
18	33.6 q	33.7 q	33.6 q	33.8 q	33.6 q	33.1 q	29.3 q
19	23.7 q	22.1 q	23.6 q	23.9 q	23.7 q	21.3 q	28.1 q
20	18.9 q	16.2 q	19.0 q	19.2 q	19.0 q	24.7 q	28.0 q
AcO (C=O)	170.5 s		170.3 s	170.6 s	170.7 s		170.8 (2C, s)
AcO (CH_3)	21.9 q		21.8 q	22.1 q	21.9 q		21.4 q
							20.9 q

Compound **1** was isolated as white crystals with molecular formula $C_{20}H_{33}NO_2$, which was confirmed by high-resolution EI mass spectrometry (found: *m/z* 319.2509, calcd 319.2511). The existence of a nitrogen atom was supported by its odd-numbered molecular weight. The ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.50 (1H, t, J = 11.0, H-5), 1.75 (1H, m, H-8), 1.78 (1H, m, H-11a), 1.67 (1H, m, H-11b), 2.36 (2H, br.t, J = 8.2, H_2-12), 6.69 (1H, br.s, H-14), 3.89 (2H, br.s, H_2-15), 0.88 (3H, d, J = 6.6, CH_3-17), 0.85 (3H, s, CH_3-18), 0.80 (3H, s, CH_3-19), 0.90 (3H, s, CH_3-20), 6.61 (1H, br.s, NH), and ^{13}C NMR (Table 1) spectra of **1**, being very similar to vitexlactam (**A**) [7], suggested that **1** is a labdane diterpenoid alkaloid closely related to **A** (Table 2) having an α,β-unsaturated γ-lactam moiety at the C-9 side chain **1** and differing from **A** only by the absence of the signals for an acetyl group and the replacement of an oxygen-bearing methine at δ_C 70.6 by a methylene at δ_C 21.7, which indicates that **1** is the 6-deacetoxy derivative of **A**. This was supported as well by the fact that **A** was 58 atomic mass units less than **1** and there was no acetoxy group observed in the IR spectrum of **1**.

EXPERIMENTAL

General. UV spectral data were obtained on a UV 210A spectrometer. Optical rotations were carried out on a Horiba SEPA-300 High Sensitive Polarimeter or a Perkin–Elmer model 241 Polarimeter. 1D and 2D NMR experiments were performed either on a Bruker AM-400 or a DRX-500 spectrometer. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. FAB-MS and HR-FAB-MS were taken on a VG Auto Spec-3000 or on a Finnigan MAT 90 instrument. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. Column chromatography was

performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), silica gel H (10-40 μ, Qingdao Marine Chemical Inc.,China), Diaion HP-20 (Shandong Lukang Pharmaceutical Co., Ltd., China), Chromatorex ODS (Fuji Silysia Chemical Corporation, Ltd., Japan), and RP_{18} gel (40–63 μm, Merck, Darmstadt, Germany). The fractions were monitored by TLC, and spots were visualized by heating the silica gel plates sprayed with 5%H_2SO_4 in EtOH.

Plant Material. The fruits of *V. agnus-castus* were purchased from Caojian town in Yunnan Province in China and identified by Prof. Xiao Cheng at Kunming Institute of Botany. A voucher specimen (KI-2007-25) was deposited in the Department of Taxonomy, Kunming Institute of Botany, Kunming, China.

Extraction and Isolation. The air-dried fruits of *V. agnus-castus* (5.75 kg) were milled sequentially and extracted three times with 70% EtOH. Evaporation of the solvent under reduced pressure provided a 70% EtOH extract (335 g). A suspension of the resulting extract in water was partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH successively. The $CHCl_3$ extract (110 g) was fractionated by silica gel column chromatography (200–300 mesh) eluting with petroleum ether–acetone (100:1–100:100) to give seven fractions. Fraction 3 was separated by silica gel column chromatography (200–300 mesh) eluting with petroleum ether–acetone (8:1) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to give compound **1** (20 mg). Fraction 5 was subsequently chromatographed using silica gel (petroleum ether–acetone, 100:20–50) and Chromatorex ODS (70% MeOH, 80% MeOH, 85% MeOH) to furnish compounds **2** (15 mg), **3** (13 mg), and **4** (18 mg). Fraction 6 was also subjected to silica gel column chromatography and eluted with $CHCl_3$–acetone (100:1–100:30) to give five subfractions. Subfraction 1 ($CHCl_3$–acetone, 100:5) was purified on chromatorex ODS (85% MeOH) to afford compound **5** (15 mg). Subfraction 3 ($CHCl_3$–acetone, 100:30) was isolated on Chromatorex ODS (80% MeOH) to yield **6** (20 mg).

9α-Hydroxy-13(14)-labden-16,15-amide (1) having an **α,β-unsaturated γ-lactam moiety**. White crystals, mp 162°C, $C_{20}H_{33}NO_2$, $[\alpha]_D^{23.5}$ +18.7° (*c* 0.2, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3473, 3187, 3055, 2924, 2682, 1684, 1648, 1442, 1379, 1296, 1254, 1228, 1140, 1085, 1057, 1041, 1018, 972, 962, 943, 909, 832, 791, 777, 698. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.50 (1H, t, J = 11.0, H-5), 1.75 (1H, m, H-8), 1.78 (1H, m, H-11a), 1.67 (1H, m, H-11b), 2.36 (2H, br.t, J = 8.2, H_2-12), 6.69 (1H, br.s, H-14), 3.89 (2H, br.s, H_2-15), 0.88 (3H, d, J = 6.6, CH_3-17), 0.85 (3H, s, CH_3-18), 0.80 (3H, s, CH_3-19), 0.90 (3H, s, CH_3-20), 6.61 (1H, br.s, NH). For ^{13}C NMR data, see Table 1. EI-MS (*m/z*, I_{rel}, %): 319 $[M]^+$ (81), 304 (7), 286 (8), 206 (7), 194 (19), 180 (100), 167 (75), 152 (11), 138 (47), 123 (17), 110 (81), 96 (86), 82 (58), 69 (72), 55 (97); HR-EI-MS *m/z* found 319.2509 $[M]^+$, calcd 319.2511.

Cell Cultures. Chronic myelogenous leukemia (K562) human cell lines were obtained from the Shanghai Cell Bank, Chinese Academy of Sciences. The cells were maintained in RPMI 1640 medium with hormone-free 15% heat-inactivated FBS (fetal bovine serum). In each case, 2 mM glutamine, 100 U/mL penicillin G, and 100 μg/mL streptomycin were added.

Cytotoxic Assays. The cytotoxicity tests for the isolates were performed using the reported protocol [18]. All treatments were performed in triplicate, and the results are shown in Table 1.

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