Terpenoids and flavonoids from *Laggera pterodonta*

YANG Guang-zhong[1], LI Yun-fang[1], YU Xin[2], MEI Zhi-nan[1*]

(1. *Institue of National Medicine, South-Centml University for Nationalities, Wuhan 430074, China;*
2. *College of Phamacy, Wuhan University, Wuhan 430072, China*)

Abstract: To study the chemical constituents of aerial parts of *Laggem pterodonta* (DC.) Benth., the air-dried aerial parts of this plant were powered and extracted with boiling water and purified by silica gel column chromatography and recrystallized. Eleven compounds were obtained from *L. pterodonta*. They were identified as to be 6-*O*-β-*D*-glucopyranosyl-carvotanacetone (**1**), pterodontic acid (**2**), 1β-hydroxy pterondontic acid (**3**), pterodontoside A (**4**), pterodondiol (**5**), pterodontriol B (**6**), 5-hydroxy-3, 4′, 6, 7-tetramethoxyflavone (**7**), artemitin (**8**), chrysosplenetin B (**9**), quercetin (**10**) and β-sitosterol (**11**). Compound **1** is a new monoterpene glucoside. Compounds **10** and **11** were isolated from this plant for the first time. Compounds **2** and **5** showed moderate activity against bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Mycobacteium phlei* and *Bacillus circulans* by paper disc diffusion method, while they both displayed no activity against *Escherichia coli*.

Key words: *Laggem pterodonta*; monoterpene glucoside; terpenoids; flavonoids; antibacterial activity

CLC number: R284.1; R284.2 **Document code:** A **Article ID:** 0513 - 4870(2007)05 - 0511 - 05

臭灵丹萜类和黄酮化合物

杨光忠[1], 李芸芳[1], 喻 昕[2], 梅之南[1*]

(1. 中南民族大学 民族药物研究所, 湖北 武汉 430074; 2. 武汉大学药学院, 湖北 武汉 430072)

摘要: 臭灵丹(*Laggem pterodonta*)为菊科四棱峰属植物,是云南民间抗菌消炎的良药。本文对臭灵丹地上部分的化学成分进行了研究。其地上部分用水煎煮提取,硅胶柱色谱和重结晶等方法进行分离纯化。从该植物中分离得到 11 个化合物,其结构分别鉴定为: 6-*O*-β-*D*-glucopyranosyl-carvotanacetone (**1**), 臭灵丹酸 (**2**), 1β-hydroxy pterondontic acid (**3**), pterodontoside A (**4**), 臭灵丹二醇 (**5**), 臭灵丹三醇乙 (**6**), 5-hydroxy-3, 4′, 6, 7-tetramethoxyflavone (**7**), 洋艾素 (**8**), 金腰素乙 (**9**), 槲皮素 (**10**)和 β-谷甾醇 (**11**)。化合物 **1**为新的单萜苷,化合物 **10**和 **11**为首次从该植物中发现。应用滤纸扩散法对该植物中的两个化合物 **2**和 **5**的抑菌活性进行检测,结果表明这两个化合物对金黄葡球菌、铜绿假单孢菌、枯草芽胞杆菌、草分支杆菌和环状芽胞杆菌均呈现明显的抑菌活性,但对大肠埃希氏菌均未呈现抑菌活性。

关键词: 臭灵丹; 单萜苷; 萜类化合物; 黄酮类化合物; 抗菌活性

Introduction

Laggem pterodonta (DC.) Benth. is widely distributed in Yunnan, China. The aerial part of this plant is used as a folk medicine for its anti-inflammatory and anti-bacterial activities[1]. Previous investigations of this plant led to the isolation of 55 eudesmane sesquiterpenes and nine flavonoid compounds[2]. Some eudesmane sesquiterpenes isolated from this plant showed cytotoxicity towards tumour cells[3] and antibacterial activity[4]. These interesting activities have prompted us to reinvestigate the constituents of *L. pterodonta*. As a result, a new monoterpene

Received date: 2006-11-14.

* Corresponding author Tel: 86 - 27 - 62951645,
Fax: 86 - 27 - 67843220,
E-mail: meizhinan@163.com

glucoside, 6-*O*-β-*D*-glucopyranosyl-carvotanacetone (**1**), along with ten known compounds, pterodontic acid[5] (**2**), 1β-hydroxy pterondontic acid[5] (**3**), pterodontoside A[6] (**4**), pterodondiol[7] (**5**), pterodontriol B[7] (**6**), 5-hydroxy-3, 4′, 6, 7-tetramethoxyflavone[8] (**7**), artemitin[8] (**8**), chrysosplenetin B[8] (**9**), quercetin[9] (**10**), β-sitosterol (**11**), were isolated from this plant (Figure 1). The structure of **1** was determined by spectroscopic analysis. Compounds **2** and **5** showed moderate activity against bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Mycobacteium phlei* and *Bacillus circulans* by paper disc diffusion method, while they both displayed no activity against *Escherichia coli*.

Results and Discussion

Compound **1** was isolated as colorless oil, $[\alpha]_D^{20.0}$ -0.5° (*c* 0.035, MeOH). According to its ESIMS (*m/z* 353 [M + Na]$^+$), ^{1}H NMR and ^{13}C NMR spectral data, its molecular formula was deduced to be $C_{16}H_{26}O_7$. This result was subsequently confirmed by observation of the fragments *m/z* 353.155 2 ($C_{16}H_{26}O_7Na$; calc. 353.157 6) in the HRESI-MS. The ^{1}H NMR spectrum of **1** showed three methyl group signals [δ 0.88 (d, 3H, *J* = 6.7 Hz), 0.90 (d, 3H, *J* = 6.7 Hz), 1.75 (s, 3H)], one double bond proton at δ 6.74 (brs, 1H), and one oxygenated proton at δ 4.47 (d, 1H, *J* = 7.5 Hz). The ^{13}C NMR and DEPT spectra of **1** gave 16 carbon signals including three methyls (δ 15.5, 15.7, 15.7), one methylene (δ 24.7), one oxygenated methylene (δ 62.5), two methines (δ 25.8, 46.8), one methine connected with two oxygens (δ 104.5), five oxygenated methines (δ 82.3, 76.9, 76.0, 74.4, 70.3), and one conjugated carbonyl and double bond signal [δ 201.3 (s), 134.1 (s), 146.3 (d)]. Comparison of ^{13}C NMR data (Table 1) of **1** with that of known 6β-hydroxycarvotanacetone[10], both compounds showed very similar NMR data except for extra signals of glucose unit in compound **1**. In ^{13}C NMR spectra, the signal of anomeric carbon atom was at δ 104.5, and other carbon signals in the glucose unit were at δ 62.5, 76.9, 76.0, 74.4, 70.3, separately[11]. Therefore, compound **1** had one glucose unit compared with 6β-hydroxycarvotanacetone. The chemical shift of C (6) of **1** was down-shifted for δ 7.90 compared with 6β-hydroxycarvotanacetone, indicating the glucose unit was connected with hydroxy group at C-6. In the ^{1}H NMR spectrum, the signal of H-1′ was observed to be doublet, and the coupling constant value (*J* = 7.5 Hz) of the signal of H-1′ revealed the linkage of glucose unit with the hydroxy group of C-6 was β-linkage. Thus, the above evidence led to establish the structure of compound **1** as 6-*O*-β-*D*-glucopyranosyl-carvotanacetone. Many eudesmane sesquiterpenoids were isolated from this plant[12-14], and to the best of our knowledge, the isolation of monoterpenoid from this

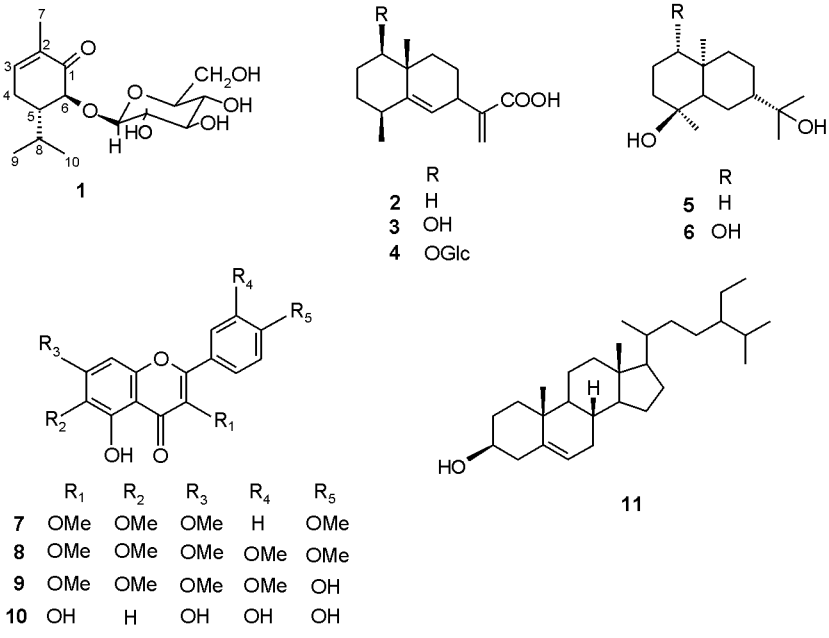

Figure 1 The chemical structures of compound **1** - **11**

Table 1 ^{1}H NMR (300 MHz) and ^{13}C NMR (150 MHz) data of compound **1** ($CDCl_3$)

No.	δ_H	δ_C	DEPT	No.	δ_H	δ_C	DEPT
1		201.3	C	9	0.88 (d, 3H, J = 6.7)	15.7	CH_3
2		134.1	C	10	0.90 (d, 3H, J = 6.7)	15.7	CH_3
3	6.74 (br s, 1H)	146.3	CH	1′	4.47 (d, 1H, J = 7.5)	104.5	CH
4	2.26 (m, 2H)	24.7	CH_2	2′	3.48 (m, 1H)	74.4	CH
5	2.14 (m, 1H)	46.8	CH	3′	3.29 (m, 1H)	76.0	CH
6	4.13 (d, 1H, J = 12)	82.3	CH	4′	3.64 (m, 1H)	70.3	CH
7	1.75 (s, 3H)	15.5	CH_3	5′	3.70 (m, 1H)	76.9	CH
8	2.22 (m, 1H)	25.8	CH	6′	3.81 (br s, 2H)	62.5	CH_2

* Assignment of ^{1}H NMR spectral data were made on the basis of HSQC spectra

plant was the first to be reported.

Experimental

Optical rotation was determined on a Perkin-Elmer 341 polarimeter. UV spectra were measured on a Shimadizu UV-210A spectrometer. IR (KBr disc) was recorded on Spectrum One FT-IR spectrometer. NMR spectra were measured on a Bruker AM-300 and Bruker AM-600 spectrometer with TMS as internal standard. ESI-MS was recorded with a Finnigan LCQ^{DECA} mass spectrometer. Silica gel (200 - 300 mesh) was used for column chromatography, and pre-coated silica gel GF254 plate (Qingdao Haiyang Chemical Plant) was used for TLC. C_{18} reversed-phase silica gel (50 μm, YMC) was also used for column chromatography.

Laggera pterodonta (DC.) Benth. aerial parts were collected in November 2002 at Xinping City, Yunnan Province of P. R. China. The plant was identified by Prof. WAN Ding-Rong. Voucher specimen is deposited at the herbarium of National Medicine Institute, South-Central University for Nationalities.

Bacteria were obtained from stock cultures maintained at China Center for Type Culture Collection, Wuhan, China.

The air-dried aerial parts of the *Laggera pterodonta* (2 kg) was powered and extracted twice with water at 100 ℃, 2 h each time. The water extracts were combined and concentrated to half of the original volume. Then it was partitioned with EtOAc and *n*-BuOH, separately. The EtOAc and *n*-BuOH extracts were concentrated *in vacuo* to afford 20 g and 22 g of residue. The EtOAc portion was subjected to column chromatography (CC) on silica gel, using petroleum ether: acetone gradients (from 9∶1 to 0∶1) as eluents. Combining the fractions with TLC (GF_{254}) monitoring, eight fractions were obtained. Then, fraction 2 (1.2 g) was subjected to CC on silica gel, eluted with $CHCl_3$-MeOH (from 99∶1 to 95∶5) gradients to give compound **2** (10 mg). Compound **5** (2.0 g) was crystallized from the fraction 4 (3.5 g), while compound **7** (7 mg) was isolated from this fraction by silica gel CC eluted with toluene-EtOAc (9∶1). Fraction 5 (825 mg) was subjected to CC on silica gel, eluted with $CHCl_3$-MeOH (from 99∶1 to 7∶3), to give compound **8** (54 mg) and **9** (20 mg). Compound **3** (10 mg) was obtained from fraction 5 by prepared TLC using $CHCl_3$-MeOH-$NH_3 \cdot H_2O$ (8∶2∶0.02) solvent system. Fraction 7 (2.3 g) and fraction 8 (1.0 g) were chromatographed over RP-18 silica gel eluting with MeOH-H_2O (from 1∶9 to 10∶0) to afford compound **6** (13 mg) and **4** (20 mg), respectively. The *n*-BuOH part was absorbed on D101 resin (500 g), eluting with EtOH-H_2O (from 0∶10 to 10∶0) to obtained 12 fractions. Fraction B (2.1 g) and fraction C (1.5 g) were chromatographed repeatedly on silica gel and RP-18 silica gel to give compound **10** (9 mg) and **1** (35 mg), respectively. Compound **11** (220 mg) was crystallized as colorless needles from the EtOH extract of *L. pterodonta*.

Biological assay

Antibacterial activities of compounds **2** and **5** on ten bacteria strains were assayed by using the disc diffusion method[15]. The results of antibacterial activity were observed after incubation at 37 ℃ for 24 h. Compounds **2** and **5** showed activities against six of the tested bacteria. The results indicated that the two compounds exhibited broad spectrum on Gram (+) bacteria. Most of Gram (-) were tolerated to the compounds except *Pseudomonas aeruginosa* (field strain) (Table 2). Compound **2** showed strong activity against *Bacillus circulans*. Some compounds isolated

Table 2 *In vitro* antibacterial activities compounds **2** and **5**[a]

Bacteria	Gram	Compound **2** 500 μg/disc	Compound **5** 500 μg/disc	Chloramphenicol 30 μg/disc
Staphylococcus aureus[b]	+	11	9	29
Staphylococcus aureus ATCC 25923[c]	+	11	7	24
Bacillus subtilis[b]	+	12	9	23
Bacillus circulans ATCC 4513[c]	+	32	9	33
Mycobacteium phlei[b]	+	12	9	20
Escherichia coli[b]	-	6	6	19
Escherichia coli ATCC 25922[c]	-	6	6	27
Escherichia coli ATCC 37197[c]	-	6	6	24
Pseudomonas aeruginosa[b]	-	11	10	23
Pseudomonas aeruginosa ATCC 27853[c]	-	6	6	26

[a] Values are means of three replications. Zone of inhitition (mm), including the diameter of the fliter paper disc (6 mm); [b] Field strain; [c] Standards strain

from this plant have been reported to exhibit cytotoxicity on tumor cell lines[3], and only ilicic acid was reported to have antibacterial activity[4]. We investigated the two main constituents of *L. pterodonta*, compound **2** and **5** whose showed antibacterial activities were reported for the first time.

Identification

6-*O*-β-*D*-glucopyranosyl-carvotanacetone (1) Colorless gum, $C_{16}H_{26}O_{27}$, $[\alpha]_D^{20.0}$ - 0.5 (*c* 0.035, MeOH). UV (MeOH) λ_{max}: 236 nm, 207 nm. HRESI-MS *m/z* 353.155 2 ($C_{16}H_{26}O_7Na$; calc. 353.157 6). ^{1}H NMR and ^{13}C NMR see Table 1.

Pterodontic acid (2) Colorless crystal (acetone), $C_{15}H_{22}O_2$, ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.31 (1H, br s, H-12), 5.68 (1H, br s, H-12), 5.18 (1H, br s, H-6), 1.25 (3H, s, H_3-14), 1.16 (3H, d, *J* = 7.5 Hz, H_3-15). The ^{13}C NMR data were identical to those recorded in reference[5].

1β-hydroxy pterondontic acid (3) Colorless gum, $C_{15}H_{22}O_3$, ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.32 (1H, br s, H-12), 5.68 (1H, br s, H-12), 5.32 (1H, br s, H-6), 3.34 (1H, dd, *J* = 3.9, 11.7 Hz, H-1α), 2.43 (1H, m, H-4), 1.25 (3H, s, H_3-14), 1.16 (3H, d, *J* = 9 Hz, H_3-15). The ^{13}C NMR data were identical to those recorded in reference[5].

Pterodontoside A (4) Yellow gum, $C_{21}H_{32}O_8$, ^{1}H NMR (300 MHz, CD_3OD) δ 5.91 (1H, br s, H-12), 5.30 (1H, br s, H-12), 5.23 (1H, br s, H-6), 4.23 (1H, d, *J* = 7.8 Hz, H-Glu1′), 1.10 (3H, s, H_3-14), 1.07 (3H, d, *J* = 7.6 Hz, H_3-15). The ^{13}C NMR data were identical to those recorded in reference[6].

Pterodondiol (5) Colorless crystal (acetone), $C_{15}H_{28}O_2$, ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (3H, s, H_3-13), 1.26 (3H, s, H_3-12), 1.08 (3H, s, H_3-15), 0.89 (3H, s, H_3-14). The ^{13}C NMR data were identical to those recorded in reference[7].

Pterodontriol B (6) Colorless gum, $C_{15}H_{28}O_3$, ^{1}H NMR (300 MHz, CD_3OD) δ 1.24 (6H, s, H_3-12, H_3-13), 1.08 (3H, s, H_3-15), 0.91 (3H, s, H_3-14). The ^{13}C NMR data were identical to those recorded in reference[7].

5-hydroxy-3, 4′, 6, 7-tetramethoxyflavone (7) Yellow powder, $C_{19}H_{18}O_7$, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (2H, d, *J* = 9.0 Hz, H-2′, 6′), 7.03 (2H, d, *J* = 9.0 Hz, H-3′, 5′), 6.51 (1H, s, H-8), 3.96 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.86 (3H, s, OCH_3). The spectra data were identical to those recorded in reference[7].

Artemitin (8) Yellow powder, $C_{20}H_{20}O_8$, UV (CH_3CN) λ_{max}: 346.25, 272.13, 254.80. IR (KBr) ν_{max}: 3 100.20, 1 657.60, 1 590.21, 1 509.89, 1 470.95 cm^{-1}. EIMS *m/z* 388 $[M]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (1H, d, *J* = 8.7 Hz, H-6′), 7.69 (1H, s, H-2′), 7.00 (1H, d, *J* = 8.7 Hz, H-5′), 6.52 (1H, s, H-8), 3.98 (9H, s, 3 × OCH_3), 3.93 (3H, s, OCH_3), 3.87 (3H, s, OCH_3). The ^{13}C NMR data were identical to those recorded in reference[8].

Chrysosplenetin B (9) Yellow powder, $C_{19}H_{18}O_8$, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (1H, s, H-2′), 7.68 (1H, d, *J* = 8.4 Hz, H-6′), 7.05 (1H, d, *J* = 8.4 Hz, H-5′), 6.51 (1H, s, H-8), 3.99 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.86 (3H, s, OCH_3). The ^{13}C NMR data were identical to those recorded in reference[8].

Quercetin (10) Yellow powder, $C_{15}H_{10}O_7$, ^{1}H NMR (300 MHz, CD_3OD) δ 7.63 (1H, s, H-2′), 7.53 (1H, d, J = 8.6 Hz, H-6′), 6.78 (1H, d, J = 8.6 Hz, H-5′), 6.28 (1H, s, H-8), 6.08 (1H, s, H-6). The ^{13}C NMR data were identical to those recorded in reference[9].

β-sitosterol (11) White needle crystal, mp 138 - 140 ℃. By comparing its NMR data and TLC behavior of compound **11** with those of β-sitosterol, this compound was determined to be β-sitosterol.

References

[1] Jiangsu New Medical College. A Dictionary of a Traditiona Chinese Drugs (中药大辞典) [M]. 1st ed. Shanghai: Shanghai Sciences and Technology Publishing House, 1977: 1889 - 1890.

[2] Mei ZN, Li YF, Yu X, et al. Advances in study on chemical constituents and pharmacological activities in plants of *Laggem* Sch. Bip. Ex Benth. & Hook [J]. J South Cent Univ Nat (中南民族大学学报), 2005, 24: 32 - 35.

[3] Xiao YC, Zheng QX, Zhang QJ, et al. Eudesmane derivatives from *Laggem pterodonta* [J]. Fitoterapia, 2003, 74: 459 - 463.

[4] Wei JX, Zhao AH, Hu JL, et al. Constituents of *Laggem pterodonta* [J]. Acad J Kunming Med Coll (昆明医学院学报), 1995, 16: 83 - 84.

[5] Li SL, Ding JK. Four new sesquiterpenoids from *Laggem pterodonta* [J]. Acta Bot Yunnan (云南植物研究), 1996, 18: 349 - 352.

[6] Zhao Y, Yue JM, Ding JK, et al. Eudesmane and eudesmanoic glucosides from *Laggem pterodonta* [J]. Chin Chem Lett, 1996, 7: 1093 - 1094.

[7] Li SL, Ding JK. Three new sespuiterpenols from *Laggem pterodonta* [J]. Acta Bot Yunnan (云南植物研究), 1993, 15: 303 - 305.

[8] Li SL, Ding JK. The flavonols from *Laggem pterodonta* [J]. Acta Bot Yunnan (云南植物研究), 1994, 16: 434 - 436.

[9] Yu DQ, Yang JS. Handbook of Chemical Analysis (分析化学手册) [M]. 7th Vol. 2nd ed. Beijing: Chemical Industry Press, 1999: 820.

[10] Onayade OA, Scheffer JJC, Schripsema J. 6-Hydroxycarvotanacetone and other constituents of the essential oil of *Laggem alata* (D. Don) Sch. Bip. ex Oliv [J]. Flavour Fragrance J, 1990, 5: 165 - 172.

[11] Wu LJ, Wu JZ. Natural Medicinal Chemistry (天然药物化学) [M]. Beijing: People's Health Press, 2004: 87 - 88.

[12] Zhao Y, Yue JM, Lin ZW, et al. Five new eudesmane deriva-tives from *Laggem pterodonta* [J]. Acta Bot Yunnan (云南植物研究), 1997, 19: 207 - 210.

[13] Zhao Y, Yue JM, Lin ZW, et al. Eudesmane sesquiterpenes from *Laggem pterodonta* [J]. Phytochemistry, 1997, 44: 459 - 464.

[14] Li SL, Ding JK, Jiang B, et al. Sesquiterpenes from *Laggem pterodonta* [J]. Phytochemistry, 1998, 49: 2035 - 2036.

[15] Xu SY, Bian RL, Chen X. The Experiment Method of Pharmacology (药理学实验方法学) [M]. 3rd ed. Beijing: People's Health Press, 2001: 1651 - 1654.