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# 菜蕨的化学成分研究

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**摘要:**采用硅胶柱层析和凝胶柱层析对菜蕨的丙酮提取物进行分离纯化,首次从中分离得到10个化合物,通过波谱学数据并和已知化合物数据比较,鉴定它们分别为<sup>-</sup>sitosterol(1), Stigmast-4-ene-6<sup>-</sup>ol-3-one(2), Stigmast-4-ene-3, 6-dione(3), Benzeneacetic acid(4), 3<sup>-</sup>Hydroxy-5<sup>,</sup>8<sup>-</sup>epidioxyergosta-6, 22-diene(5), Stigmast-4-ene-3<sup>,</sup>6<sup>-</sup>diol(6), Stigmast-5-ene-3<sup>,</sup>7<sup>-</sup>diol(7), Stigmast-4-ene-6<sup>-</sup>ol-3-one(8), Glycerol-1, 3-dihexadecanoate(9)以及Daucosterol(10)。

**关键词:**菜蕨; 化学成分; 豆甾类

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## Study on the Chemical constituents of Callipteris esculenta (Athyriaceae)

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**Abstract:** Ten compounds were firstly isolated from the acetone extraction of the fern *Callipteris esculenta* (Retz.) J. Sm. ex Moore et Houlst. The structures of the compounds were identified as <sup>-</sup>sitosterol(1), Stigmast-4-ene-6<sup>-</sup>ol-3-one(2), Stigmast-4-ene-3, 6-dione(3), Benzeneacetic acid(4), 3<sup>-</sup>Hydroxy-5<sup>,</sup>8<sup>-</sup>epidioxyergosta-6, 22-diene(5), Stigmast-4-ene-3<sup>,</sup>6<sup>-</sup>diol(6), Stigmast-5-ene-3<sup>,</sup>7<sup>-</sup>diol(7), Stigmast-4-ene-6<sup>-</sup>ol-3-one(8), Glycerol-1, 3-dihexadecanoate(9) and Daucosterol(10) on the basis of NMR spectroscopy and comparison with the data of known compounds.

**Key words:** *Callipteris esculenta*; Chemical constituents; Stigmasterols

## Introduction

*Callipteris esculenta* (Retz.) J. Sm. ex Moore et Houlst, a kind of big evergreen hygrophilous terrestrial lepto sporangiate fern, belongs to the family Athyriaceae widely distributed in the tropical, subtropical of Asia and the islands of Polynesia. They usually inhabit the valleys and ravines of shady forests. The tender fronds are of emplastic and tasty. However, the chemical constituents of this plant have not been studied up to now. In this study, we have isolated and purified ten compounds from the fronds of *Callipteris esculenta*. The structure of the compounds were identified as <sup>-</sup>sitos-

terol(1), Stigmast-4-ene-6<sup>-</sup>ol-3-one(2)<sup>[1,2]</sup>, Stigmast-4-ene-3, 6-dione(3)<sup>[1,3,4]</sup>, Benzeneacetic acid(4)<sup>[5]</sup>, 3<sup>-</sup>Hydroxy-5<sup>,</sup>8<sup>-</sup>epidioxyergosta-6, 22-diene(5)<sup>[6-9]</sup>, Stigmast-4-ene-3<sup>,</sup>6<sup>-</sup>diol(6), Stigmast-5-ene-3<sup>,</sup>7<sup>-</sup>diol(7)<sup>[1,10-12]</sup>, Stigmast-4-ene-6<sup>-</sup>ol-3-one(8)<sup>[1]</sup>, Glycerol-1, 3-dihexadecanoate(9) and Daucosterol(10) mainly by NMR spectra data and by comparison the data with those of known compounds.

## Experimental

### General

The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker AM-400 spectrometers with TMS as internal standard. The chemical shifts were reported in ppm using CDCl<sub>3</sub> as solvent. Silica gel(200-300 mesh) for column chromatography and GF<sub>254</sub> (10-40μ) for TLC were supplied by the Qingdao Marine Chemical Inc. China. Spots on

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TLC were monitored under UV lamp and by heating silica gel plates sprayed with 10% H<sub>2</sub>SO<sub>4</sub> in EtOH. Sephadex LH-20 (25–100 μm, pharma) were used for gel chromatography and MCI(MCI-gel CHP-20P) for decolorizing.

### Plant material

The fronds of *Callipteris esculenta* were collected from Da Wei Shan Mountain, He Kou in June, 2006. A voucher specimen Cheng 20060620 was deposited in the KUN.

### Extraction and Isolation

The dried and powdered fronds of *Callipteris esculenta* (8.0 kg) were extracted with acetone (10 L × 3, each one day) at room temperature. The solvent was removed under vacuum, and a residue (134 g) was obtained. After decolorized on MCI column eluted with 95% EtOH, the residue was subjected to silica gel column (8 × L 21 cm) eluted with petroleum ether: acetone (10: 0, 9: 1, 7: 3, 6: 4) to give fractions 1–4. Through recrystallization from petroleum ether, compound **1** (5.5 g) was purified from fraction 2 and compound **2** (4 g) from fraction 3. The fraction 3 was further isolated and purified through silica gel column chromatography and gel chromatography repeatedly, to give the compound **3** (3 mg), **4** (240 mg), **5** (8 mg), **6** (26 mg), **7** (118 mg), **8** (49 mg) and Compound **9** (190 mg). Compound **10** (about 1 g) was purified from fraction 4.

### Identification

Compound **1** and **10** were identified as β-sitosterol and Dauosterol by comparing with authentic samples on TLC.

**Stigmast-4-ene-6-ol-3-one (2)** Colorless plates (acetone); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) : 5.80 (1H, s, H-4), 4.34 (1H, t, J = 7.5 Hz, H-6), 0.73 (3H, s, H-18), 1.37 (3H, s, H-19), 0.92 (3H, d, J = 6.5 Hz, H-21), 0.84 (3H, d, J = 7.3 Hz, H-26), 0.82 (3H, d, J = 6.8 Hz, H-27), 0.85 (3H, t, J = 7.6 Hz, H-29); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) : 37.03 (t, C-1), 34.23 (t, C-2), 200.56 (s, C-3), 126.24 (d, C-4), 168.68 (s, C-5), 73.17 (d, C-6), 38.51 (t, C-7), 29.68 (d, C-8), 53.57 (d, C-9), 37.96 (s, C-10), 20.93 (t, C-11), 39.55 (t, C-12), 42.46 (s, C-13), 55.84 (d, C-14), 24.12 (t, C-15), 28.16 (t, C-16), 56.00 (d, C-17),

11.94 (q, C-18), 19.47 (q, C-19), 36.08 (d, C-20), 18.70 (q, C-21), 33.84 (t, C-22), 26.00 (t, C-23), 45.76 (d, C-24), 29.08 (d, C-25), 19.80 (q, C-26), 18.99 (q, C-27), 23.02 (t, C-28), 11.99 (q, C-29).

**Stigmast-4-ene-3, 6-dione (3)** Colorless solids; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) : 6.17 (1H, s, H-4), 0.72 (3H, s, H-18), 1.17 (3H, s, H-19), 0.93 (3H, d, J = 6.4 Hz, H-21), 0.83 (3H, d, J = 7.1 Hz, H-26), 0.81 (3H, d, J = 7.0 Hz, H-27), 0.84 (3H, t, J = 6.8 Hz, H-29); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) : 35.52 (t, C-1), 33.98 (t, C-2), 199.57 (s, C-3), 125.45 (d, C-4), 161.10 (s, C-5), 202.43 (s, C-6), 46.83 (t, C-7), 34.20 (d, C-8), 50.96 (d, C-9), 39.80 (s, C-10), 20.86 (t, C-11), 39.11 (t, C-12), 42.51 (s, C-13), 55.82 (d, C-14), 23.97 (t, C-15), 28.02 (t, C-16), 56.53 (d, C-17), 11.97 (q, C-18), 17.51 (q, C-19), 36.03 (d, C-20), 18.70 (q, C-21), 33.80 (t, C-22), 25.98 (t, C-23), 45.77 (d, C-24), 29.09 (d, C-25), 19.83 (q, C-26), 18.99 (q, C-27), 23.04 (t, C-28), 11.89 (q, C-29). The above spectra data were in accordance with report of Shen et al<sup>[3]</sup>; while in the reports of Greca et al<sup>[1]</sup> and Gaspar et al<sup>[4]</sup>, the <sup>13</sup>C NMR chemical shifts of C-8, C-10 and C-12 were 39.1, 34.2 and 39.8 ppm respectively, the assignments of the three carbons' signals should be interchanged.

**Benzeneacetic acid (4)** Colorless plates (petroleum ether); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) : 7.25–7.35 (5H, m), 3.65 (2H, s); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) : 178.02 (s, C-2), 133.18 (s, C-1), 129.36 (d, C-2), 129.36 (d, C-6), 128.63 (d, C-3), 128.63 (d, C-5), 127.35 (d, C-4), 41.03 (t, C-1). The structure establishment of compound 4 was mainly by comparison data with those of ester of Benzeneacetic acid<sup>[5]</sup>.

### 3-Hydroxy-5, 8-epidioxyergosta-6, 22-diene (5)

Colorless needles (MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) : 6.51 (1H, d, J = 8.5 Hz, H-6), 6.25 (1H, d, J = 8.5 Hz, H-7), 5.20 (1H, dd, J = 7.4, 15.10 Hz, H-22), 5.16 (1H, dd, J = 8.1, 15.04 Hz, H-23), 3.96 (1H, m, H-3); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) : 34.65 (t, C-1), 30.07 (t, C-2), 66.45 (d, C-3), 36.92 (t, C-4), 82.14 (s, C-5), 135.38 (d, C-6), 130.72 (d, C-7), 79.40 (s, C-8), 51.02 (d, C-9), 36.88 (s, C-10), 23.37 (t, C-11), 39.29 (t, C-12), 44.52 (s, C-13),

51.64(d, C-14), 20.60(t, C-15), 28.64(t, C-16), 56.14(d, C-17), 12.84(q, C-18), 18.16(q, C-19), 39.73(d, C-20), 20.85(q, C-21), 135.17(d, C-22), 132.26(d, C-23), 42.73(d, C-24), 33.03(d, C-25), 19.93(q, C-26), 19.62(q, C-27), 17.53(q, C-28).

**Stigmast-4-ene-3, 6-diol(6)** Colorless crystals (MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) : 5.74(1H, d, J = 3.9 Hz, H-4), 4.25(2H, m, H-3, H-6); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) : 36.70(t, C-1), 31.41(t, C-2), 71.35(d, C-3), 120.70(d, C-4), 146.10(s, C-5), 73.91(d, C-6), 42.27(t, C-7), 37.14(d, C-8), 42.69(d, C-9), 37.41(s, C-10), 20.77(t, C-11), 39.00(t, C-12), 42.05(s, C-13), 49.03(d, C-14), 24.25(t, C-15), 28.24(t, C-16), 55.62(d, C-17), 11.45(q, C-18), 18.24(q, C-19), 36.12(d, C-20), 18.79(q, C-21), 33.90(t, C-22), 25.90(t, C-23), 45.79(d, C-24), 29.09(d, C-25), 19.82(q, C-26), 18.99(q, C-27), 23.03(t, C-28), 11.98(q, C-29).

**Stigmast-5-ene-3, 7-diol(7)** Colorless needles (MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) : 5.61(1H, dd, J = 5.4, 1.33 Hz, H-6), 3.85(1H, brs, H-7), 3.55(1H, m, H-3), 0.68(3H, s, H-18), 0.99(3H, s, H-19), 0.93(3H, d, J = 6.5 Hz, H-21), 0.84(3H, d, J = 6.9 Hz, H-26), 0.82(3H, d, J = 6.8 Hz, H-27), 0.86(3H, t, J = 7.5 Hz, H-29); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) : 36.99(t, C-1), 31.35(t, C-2), 71.31(d, C-3), 41.99(t, C-4), 146.22(s, C-5), 123.84(d, C-6), 65.33(d, C-7), 37.50(d, C-8), 42.25(d, C-9), 37.39(s, C-10), 20.68(t, C-11), 39.15(t, C-12), 42.12(s, C-13), 49.40(d, C-14), 24.27(t, C-15), 28.24(t, C-16), 55.70(d, C-17), 11.60(q, C-18), 18.22(q, C-19), 36.07(d, C-20), 18.78(q, C-21), 33.90(t, C-22), 25.92(t, C-23), 45.81(d, C-24), 29.12(d, C-25), 19.76(q, C-26), 19.00(q, C-27), 23.04(t, C-28), 11.96(q, C-29). The data were resemble to those of literatures<sup>[10, 12]</sup>, while in the literatures<sup>[1, 11]</sup>, the <sup>13</sup>C NMR chemical shifts of C-5(143.88), C-15(25.90), and C-16(29.22), C-23(28.88) were different, they should be corrected.

**Stigmast-4-ene-6-ol-3-one(8)** Colorless crystals (MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) : 6.17(1H, d, J = 1.8 Hz, H-4), 4.33(1H, m, H-6), 0.72(3H, s, H-18), 1.19(3H, s, H-19), 0.82(3H, d, J = 6.1 Hz, H-

27), 0.85(3H, d, J = 6.0 Hz, H-26), 0.93(3H, d, J = 6.5 Hz, H-21), 0.86(3H, t, J = 6.8 Hz, H-29); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) : 36.23(t, C-1), 34.11(t, C-2), 199.52(s, C-3), 119.62(d, C-4), 171.54(s, C-5), 68.69(d, C-6), 41.46(t, C-7), 33.79(d, C-8), 53.71(d, C-9), 39.01(s, C-10), 20.99(t, C-11), 39.40(t, C-12), 42.11(s, C-13), 55.51(d, C-14), 24.15(t, C-15), 28.10(t, C-16), 55.94(d, C-17), 11.91(q, C-18), 18.25(q, C-19), 36.07(d, C-20), 18.66(q, C-21), 33.57(t, C-22), 25.96(t, C-23), 45.75(d, C-24), 29.07(d, C-25), 19.81(q, C-26), 18.98(q, C-27), 23.01(t, C-28), 11.95(q, C-29).

**Glycerol-1, 3-dihexadecanoate(9)** White powder; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) : 0.88(6H, t, J = 6.4 Hz, H-16), 1.26(44H, overlap, H-4-H-14), 1.36(4H, m, H-15), 1.62(4H, m, H-3), 2.35(4H, t, J = 7.5 Hz, H-2), 2.49(1H, brs, OH), 4.08-4.21(5H, m, H-1, 2, 3); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 500 MHz) : 173.91(s, C-1), 68.38(d, C-2), 65.02(t, C-1, 3), 34.09(t, C-2), 31.91(t, C-14), 29.67-29.11(t, C-4-C-13), 24.88(t, C-3), 22.68(t, C-15), 14.10(q, C-16).

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品、熏肉、烟熏、坚果等食用香精。已报道在天然依兰、肉豆蔻、丁香、水仙、晚香玉、香石竹等精油中存在。在香根油中检出该物质也属首次发现。

### 3.2.2 香兰素(组分 11) Vanilin

分子式为  $C_8H_8O_3$ , 相对质量 152.14。FEMA 编号为 3107, FDA 182.60, COE 107, 中国 GB2760-1996 批准为允许使用的食品香料。香兰素是重要的香荚兰的香料之一, 作粉底香料, 几乎用于所有香型, 如紫罗兰、兰草、葵花、玫瑰、茉莉等。香兰素在食品烟酒中应用也很广泛, 在香子兰、巧克力、太妃香型中是必不可少的香料。天然存在于秘鲁香脂、丁子香兰油、香子兰、咖啡、葡萄、白兰地、威士忌中。在香根油中检出该物质属首次发现。

## 4 问题与研究方向

香根油化学成分研究与提取工艺、香根草种植有密切关系, 不同提取工艺、种植时间可能合成的化学物质不同, 本研究将计划测定传统蒸馏法提取的香根油化学成分, 并与浸提法比较, 分析香根油成分与提取工艺之间的关系。

对已发现的新香料成分的分离、化学性质、应用、合成等研究工作需要进一步进行, 工业化生产及应用有待进一步研究。

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