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醉魂藤的化学成分研究

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摘 要:运用多种色谱技术从云南产醉魂藤 *Heterostemma alatum* wight中分离得到 10个化合物。通过理化鉴别和波谱数据确定了他们的化合物结构分别为 β谷甾醇 (**1**)、正二十四烷酸 (**2**)、芹菜素 (**3**)、胡萝卜苷 (**4**)、芹菜素 -7-*O*-β-D葡萄糖苷 (**5**)、醉魂藤碱 A (**6**)、醉魂藤碱 B (**7**)、醉魂藤碱 C (**8**)、醉魂藤碱 D (**9**)和醉魂藤碱 F (**10**)。这些化合物均为首次从该植物中分离得到。

关键词:醉魂藤;β谷甾醇;胡萝卜苷;芹菜素;芹菜素 -7-*O*-β-D葡萄糖苷;醉魂藤碱

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Study on the Chemical Constituents of Heterostemma alatum Wight

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Abstract: Ten compounds were obtained from *Heterostemma alatum* wight. By spectral analysis, they were determined as β-sitosterol (**1**), (n)-tetracosanoic acid (**2**), Apigenin (**3**), daucosterol (**4**), apigenin-7-*O*-β-D-glucopyranoside (**5**), heteromines A (**6**), B (**7**), C (**8**), D (**9**) and F (**10**). They were isolated from the plant for the first time.

Key words: *Heterostemma alatum*; steroids; flavonoids; Alkalolids

Introduction

Heterostemma alatum wight is a Chinese folk medicine, used as an expelling dampness and detoxifying agent in southwest of china[1]. Previously studies reported the isolation of some purine derivatives from this genus, which showed anti-tumor activity *in vitro* and *in vivo*[2-4]. In the course of phytochemical investigation on the plant, we obtained 10 known compounds, including two steroids, β-sitosterol (**1**), daucosterol (4); two flavonoids, apigenin (3), apigenin-7-*O*-β-D-glucopyranoside (**5**); four purine derivatives, heteromines A (**6**), B (**7**), C (**8**), and D (**9**); a pyrimidine derivative Heteromine F (**10**); and (n)-tetracosanoic acid (**2**). The above compounds were obtained from the title plant for the first time. Here, we describe the isolation and structure elucidation of these compounds.

Experimental

General

All melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Optical rotations were measured using a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet Magna 750 FTIR (KBr) spectrometer. EIMS data were obtained with a MAT-95 mass spectrometer. NMR spectra were recorded on a Bruker Avance DRX-300 or Varian Mercury VX 400 NMR spectrometers, the chemical shift values are reported in ppm (δ) and coupling constants (J) are given in Hz. Silica gel (200-300, 400 mesh) and precoated plates of silica gel (HSGF-254) (Qing dao Haiyang Chemical Group Co., Qingdao) were used for column chromatography (CC) and TLC, respectively.

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Plant material

The aerial parts of *Heterostemma alatum* wight were collected in Xishuangbanna County, Yunnan Province, China, in July 2006. The plants were identified by Prof. Jing-Yun Cui, Xishuangbanna Tropical Botanical Garden, Academica Sinica, China. A voucher specimen (No. 2006-64) was deposited in our laboratory.

Extraction and isolation

The aerial parts of *Heterostemma alatum* wight (7.0 kg) was soaked with 95% ethanol (60 L ×3, each 7 d) at room temperature. The solvents were evaporated under reduced pressure to give 524 g residue. The concentrated extract was suspended in H_2O (3 L) and partitioned successively with petroleum ether (PE, 60-90 ℃), $CHCl_3$, EtOAc. The EtOAc-soluble fraction (30 g) was subjected repeatedly to CC on silica gel eluted with CH_2Cl_2-MeOH, and further purified successively through ODS, and Sephadex LH-20 columns to yield successively compounds **1** (4 mg), **2** (4 mg), **3** (17 mg), **4** (26 mg), **5** (23 mg), **6** (102 mg), **7** (47 mg), and **8** (16 mg). The H_2O-soluble fraction was divided into H_2O, 30%, 60% and 95% EtOH subfractions through a macropore resin D1400 (Yangzhou Pharmaceutical Factory, Yangzhou, China) column (ϕ10 ×85 cm). The 30% (55 g) and 60% EtOH (4.4 g) subfractions were further purified successively through silica gel, and Sephadex LH-20 columns to afford compounds **5** (53 mg), **6** (5.3 g), **7** (0.6 g), **8** (0.2 g), **9** (21 mg) and **10** (22 mg), respectively.

Identification

β-sitosterol (1) $C_{29}H_{50}O$. Colorless needles (EtOAc), mp. 135-137 ℃. EI-MS *m/z*: 414 $[M]^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.68 (3H, s, H-18), 0.81 (3H, d, *J* = 6.3 Hz, H-27), 0.83 (3H, d, *J* = 6.3 Hz, H-26), 0.85 (3H, t, *J* = 7.2 Hz, H-29), 0.93 (3H, d, *J* = 6.3 Hz, H-21), 1.02 (3H, s, H-19), 5.35 (1H, m, H-6), 3.51 (1H, m, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.8 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.7 (C-12), 42.3 (C-13), 56.7 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 12.0 (C-18), 19.4 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.0 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.0 (C-28), 11.8 (C-29). The structure was identified as β-sitosterol by comparison of its physical and spectral data with those reported in the literature[5].

(n)-tetracosanoic acid (2) $C_{24}H_{48}O_2$. White powder, mp. 80-85 ℃. EI-MS *m/z*: 368 $[M]^+$, 354, 340, 326, 312, 298, 284, 269, 255, 241, 99, 85, 71, 57, at an interval of CH_2. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.34 (2H, t, *J* = 7.5 Hz, CH_2COOH), 1.63, 1.30 (CH_2), 0.88 (3H, t, *J* = 6.7 Hz); 13 C NMR ($CDCl_3$, 100 MHz) δ: 179.3, 33.9, 31.9, 29.7, 29.4, 29.4, 29.2, 29.0, 24.7, 22.7, 14.1. The spectral data are matched with a characteristic (n)-tetracosanoic acid, so it was identified as (n)-tetracosanoic acid[6].

Apigenin (3) $C_{15}H_{10}O_5$. Yellow powder. ESI-MS *m/z*: 269 $[M-H]^-$, 271 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.89 (2H, d, *J* = 8.6 z, 2H, 2′, 6′-H), 6.90 (2H, d, *J* = 8.6Hz, 3′, 5′-H), 6.73 (1H, s, 3-H), 6.46 (1H, br s, 8-H), 6.17 (1H, br s, 6-H). Spectral data were in agreement with the reported values[7].

Daucosterol (4) $C_{35}H_{60}O_6$. White grain. The FAB-MS and IR data were identical with those of daucosterol[8], and TLC behavior was identical with those of authentic daucosterol.

Apigenin-7-O-β-D-glucopyranoside (5) $C_{21}H_{20}O_{10}$. Yellow amorphous powder. ESI-MS *m/z*: 431 $[M-H]^-$, 433 $[M+H]^+$. ^{1}H NMR (DMSO-d_6 300 Hz) δ: 8.07 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 7.13 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 6.69 (1H, s, H-3), 6.86 (1H, d, *J* = 2.2Hz), 6.46 (1H, d, *J* = 2.2Hz), 5.08 (1H, d, *J* = 7.4 Hz, H-1″); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 182.0 (C-4), 163.8 (C-2), 163.0 (C-7), 162.4 (C-5), 161.4 (C-4′), 156.9 (C-9), 128.4 (C-2′, 6′), 120.7 (C-1′), 114.6 (C-3′, 5′), 105.4 (C-10), 103.8 (C-3), 99.5 (C-6), 94.9 (C-8), glc (C-1 – C-6): 105.4, 99.9, 73.1, 77.2, 69.5, 76.4, 60.6. The data of ^{1}H NMR and ^{13}C NMR were consistent with those of the reference[9].

Heteromine A (6) $C_{10}H_{16}N_5OCl$, was presumed to be a quaternary ammonium chloride because it formed a precipitate with $AgNO_3$. White powder. ESI-MS *m/z*: 222 $[M]^+$. ^{1}H NMR (CD_3OD, 300 MHz) δ: 4.18

(3H, s, O-CH_3), 4.09 (3H, s, 7-CH_3), 3.87 (3H, s, 9-CH_3), 3.26 (6H, s, N-(CH_3)2); ^{13}C NMR (CD_3OD, 100M Hz) δ: 162.4 (C-2), 160.1 (C-6), 154.5 (C-4), 141.2 (C-8), 106.3 (C-5), 38.2 (N-(CH_3)2), 31.9 (9-CH_3), 37.2 (7-CH_3), 55.7 (O-CH_3). The data of ^{1}H NMR and ^{13}C NMR were in agreement with those of the reference[3].

Heteromine B(7) $C_9H_{14}N_5OCl$, was a quaternary ammonium chloride due to giving AgCl precipitation as reaction with $AgNO_3$. White powder. ESI-MS *m/z* 208$^{[M]+}$. ^{1}H NMR (CD_3OD, 300 MHz) δ: 4.13 (3H, s, O-CH_3), 4.08 (3H, s, 7-CH_3), 3.87 (3H, s, 9-CH_3), 2.97 (6H, s, N-CH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ: 163.6 (C-2), 160.1 (C-6), 154.3 (C-4), 140.1 (C-8), 106.9 (C-5), 29.1 (N-CH_3), 32.0 (9-CH_3), 37.2 (7-CH_3), 55.0 (O-CH_3). The data of ^{1}H NMR and ^{13}C NMR were consistent with those of the reference[3].

Heteromine C(8) $C_8H_{12}N_5OCl$, was a quaternary ammonium chloride due to giving AgCl precipitation as reaction with $AgNO_3$. White powder. ESI-MS *m/z*: 194 $[M]^+$. ^{1}H NMR (CD_3OD, 300 MHz) δ: 3.94 (3H, s, O-CH_3), 3.92 (3H, s, 7-CH_3), 3.02 (3H, s, 9-CH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ: 162.6 (C-2), 159.9 (C-6), 151.9 (C-4), 140.1 (C-8), 106.5 (C-5), 31.6 (9-CH_3), 35.3 (7-CH_3), 55.2 (O-CH_3). The data of ^{1}H NMR and ^{13}C NMR were consistent with those of the reference[3].

Heteromine D(9) $C_9H_{14}N_5OCl$, also was a quaternary ammonium chloride due to giving AgCl precipitation as reaction with AgNO3. White powder. ESI-MS *m/z* 208 $[M]^+$. ^{1}H NMR (CD_3OD, 300 MHz) δ: 4.11 (3H, s, 7-CH_3), 3.81 (3H, s, 9-CH_3), 3.21 (6H, s, N-(CH_3)2), 8.98 (H, s, 8-H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 156.5 (C-2), 156.3 (C-6), 152.1 (C-4), 140.5 (C-8), 108.5 (C-5), 38.9 (N-(CH_3)2), 32.1 (9-CH_3), 36.6 (7-CH_3). The data of ^{1}H NMR and ^{13}C NMR were consistent with those of the reference[4].

Heteromine F(10) $C_{10}H_{16}N_5O$. White powder. ESI-MS *m/z*: 240 $[M+H]^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.89 (1H, br s, CHO), 4.64 (1H, br s, N-H), 3.86 (3H, s, O-CH_3), 3.16 (6H, s, N-(CH_3)2), 2.98 (3H, s, 7-CH_3), 2.96 (3H, d, *J* = 4.9 Hz, 9-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.0 (C-8), 165.3 (C-6), 161.0 (C-4), 160.1 (C-2), 94.0 (C-5), 36.8 (N-(CH_3)2), 27.7 (9-CH_3), 31.7 (7-CH_3), 53.0 (O-CH_3). The data of ^{1}H NMR and ^{13}C NMR were consistent with those of the reference[4].

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