马肾果枝条的化学成分研究

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摘要:采用多种色谱方法从马肾果(*Aglaia testicularis* C. Y. Wu)枝条中分离鉴定了 11 个化合物,分别是火焰花内酯 B (1)、火焰 花内酯苷 A (2)、(*E*)-aglawone (3)、eichlerianic acid (4)、shoreic acid (5)、3β-羟基-5α,8α-表-二氧麦角-6,22-二烯 (6)、豆甾-5-烯-3β,7α-二醇 (7)、β-谷甾醇 (8)、胡萝卜苷 (9)、东莨菪内酯 (10)、碳二十六酸 (11)。化合物 1 ~ 3,8 ~ 11 均为首次从该植物中分离得到, 而松香烷二萜内酯类衍生物 1 和 2 则是首次从米仔兰属植物中得到。化合物 1 对人肺癌细胞株 AGZY 83-a 表现出弱的抑制 作用, IC₅₀ 值为 20.5 µg mL⁻¹。这为国产米仔兰属植物的开发应用提供了理论依据。 **关键词:** 马肾果;火焰花内酯 B;火焰花内酯苷 A;细胞毒活性;化学成分

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Chemical Constituents from Twigs of Aglaia testicularis C. Y. Wu

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Abstract: Eleven compounds were isolated from the twigs of *Aglaia testicularis* C. Y. Wu (Meliaceae) by means of chromatographic technology. On the basis of spectral data, their structures were identified as phlogacantholide B (1), phlogacanthoside A (2), (*E*)-aglawone (3), eichlerianic acid (4), shoreic acid (5), 3β-hydroxy-5α,8α-epidioxyergosta-6,22-diene (6), stigmast-5-en-3β,7α-diol (7), β-sitosterol (8), daucosterol (9), scopoletin (10), and hexacosanoic acid (11). Compounds 1 - 3 and 8 - 11 were isolated from the plant for the first time, and the abietane diterpene derivatives, compounds 1 and 2, were not reported previously from the genus *Aglaia*. Compound 1 showed weak activity against AGZY 83-a (human lung cancer) cell with the IC₅₀ value of 20.5 µg mL⁻¹. The study establishes a theoretical basis for the application of the genus *Aglaia* distributed in China. **Key words:** *Aglaia testicularis*; Phlogacantholide B; Phlogacanthoside A; Cytotoxicity; Chemical constituents

The genus *Aglaia* Lour. (Meliaceae) consists of about 130 species, which are distributed mainly in the Indo-Malayan region, southern mainland China, and the Pacific Islands^[1]. The cyclopenta[b] benzofurans and two structurally related groups, the cyclopenta[bc]benzopyrans and benzo[b]oxepines, are considered characteristic secondary metabolites of the genus *Aglaia*, because they have been isolated only from this taxon^[2]. Many rocaglamide derivatives exhibit significant insecticidal and antiproliferative activities^[3–4]. Our previous papers reported the phytochemical investigation on *Aglaia perviridis*^[5–6]. Here we reported the chemical constituents from the twigs of *Aglaia testicularis* C. Y. Wu, which is regarded as an endemic species of the limestone area of southeastern Yunnan Province, China^[7]. Eleven compounds including an abietane diterpene phlogacantholides B (1), its glucoside phlogacanthoside A (2), and nine other

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compounds, (*E*)-aglawone (**3**), eichlerianic acid (**4**), shoreic acid (**5**), 3 β -hydroxy-5 α ,8 α -epidioxyergosta-6,22-diene (**6**), stigmast-5-en-3 β ,7 α -diol (**7**), β -sitosterol (**8**), daucosterol (**9**), scopoletin (**10**), and hexacosanoic acid (**11**), were also isolated and identified (Fig. 1). Among them, compounds **1** – **3** and **8** – **11** were obtained from this plant for the first time, and abietane-type diterpene lactone (compounds **1** and **2**) were firstly obtained from the genus *Aglaia*.

Compounds 1 and 2 were also screened for anticancer activity against AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells).

1 Experimental method

1.1 Plant material

The twigs of *Aglaia testicularis* were collected in Malipo County of Yunnan Province, China, in September 2003, and identified by Professor De-ding TAO, Kunming Institute of Botany, Chinese Academy of Sciences, China.

1.2 Instrument

Melting points were measured on an XRC-1 apparatus and uncorrected. Optical rotations were measured with a JASCO DIP-370 polarimeter. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. ¹H NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometer with TMS as internal standard, δ in ppm, *J* in Hz, ¹³C NMR spectra were recorded on Bruker AM-400 spectrometer. MS data recorded on a API Qstar Pulsar I spectrometer. The silica gel for TLC (GF₂₅₄) and column chromatography (CC, 200 – 300 mesh) were obtained from Qingdao Meijing Chemical Inc., China. RP-C18 silica gel (40 – 75 µm, Fuji silysia Chemical Ltd, Aichi, Japan) and Sephadex LH-



20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography.

1.3 Extraction and Isolation

Air-dried and crashed twigs of A. testicularis (11.5 kg) were extracted three times with methanol at room temperature. The solvent was evaporated in vacuo to give a crude extract, which was suspended in water and extracted four times with ethyl acetate. After the evaporation of ethyl acetate in vacuo, extracts (123.4 g) were obtained. 109.6 g of them were subjected to silica gel column chromatography (CC, 200 - 300 mesh) using petroleum etherethyl acetate (100:0 - 30:70, V/V) as eluent. By combining the fractions with TLC (GF_{254}) monitoring, 7 fractions (A - G) were obtained. Fraction B was repeatedly chromatographed over silica gel eluted with petroleum ether-acetone (100:0 - 85:15) to produce 11 (457 mg, yield 0.0040%); fraction C was repeatedly chromatographed over CC silica gel eluted with petroleum ether-ethyl acetate (95:5 -85:15) to give 8 (2.0 g, yield 0.017%); fraction C was subject to repeated on CC silica gel eluted with chloroform-acetone (97:3 - 85:15) to obtain 3 (9 mg, yield 0.000078%), 6 (17 mg, yield 0.00015%), and 7 (24 mg, yield 0.00021%); fraction E was submitted to repeated on CC silica gel eluted with chloroform-ethyl acetate (95:5 - 3:1), and then purified on a RP-18 and Sephadex LH-20 column eluted with methanolwater (50:50 - 100:0) to yield 4 (213 mg, yield 0.0019%) and 5 (135 mg, yield 0.0012%); fraction F (2.5 g) was subjected to CC silica gel eluted with chloroform-ethyl acetate (7:3 - 1:1), then purified on a RP-18 and Sephadex LH-20 column successively eluted with methanol-water (50:50 - 100:0) to give 1 (210 mg, yield 0.0018%) and 10 (28 mg, yield 0.00024%); fraction G was repeatedly chromatographed over CC silica gel eluted with chloroform-acetone (70:30 - 50:50) to afford **9** (2.7 g, yield 0.023%).

The water layer after extracted with ethyl acetate was subjected to D101 eluted with water and then with methanol. After the evaporation of methanol in *vacuo*, the residues (93.5 g) were chromatographed over CC silica gel eluted with chloroform-methanol (95:5 - 6:4) to give 6 fractions (H – M). Fraction G (18.9 g) was submitted to CC silica gel eluted with chloroform-methanol (95:5 - 60:40) and then purified on a RP-18 and Sephadex LH-20 column successively eluted with methanol-water (from 50:50 - 100:0) to yield **2** (46 mg, yield 0.0004%).

1.4 Bioassays

The anticancer active evaluation of compounds **1** and **2** towards AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells) by MTT method was examined, the experimental detail was just as what had been reported previously^[11].

2 Structural identification

Phlogacantholide B (1)^[8] C₂₀H₂₈O₄; Colorless needles (acetone); mp 196 °C – 197 °C, $[\alpha]_D^{26.3}$ –204.9° (c 1.02, MeOH); IR (KBr) v: 3421, 2963, 2927, 2854, 1736, 1680, 1635, 1458, 1381, 1153, 1127, 1057, 1015, 990 cm⁻¹; EI-MS m/z (%): 332 [M]⁺ (2), 314 (11), 301 (38), 283 (26), 271 (100), 241 (7), 231 (26), 219 (35), 201 (21), 192 (27), 180 (84), 162 (53), 154 (45), 145 (23), 135 (28), 123 (100), 109 (40), 105 (46), 91 (54), 79 (36), 67 (32), 55 (37); HR-ESIMS m/z: 355.1896 (calcd. for C₂₀H₂₈O₄Na 355.1885); ¹H NMR (C₅D₅N, 500 MHz): δ 5.17 (1H, s, H-14), 4.88 (1H, t, J = 8.1 Hz, H-12), 3.98, 3.68 (1H each, both d, J)J = 10.8 Hz, H₂-19), 2.34 (3H, s, Me-17), 1.16 (3H, s, Me-18), 1.13 (1H, d, J = 12.6 Hz, H-5), 1.00 (3H, s, Me-20); ¹³C NMR (C₅D₅N, 100 MHz): δ 36.0 (t, C-1), 19.1 (t, C-2), 36.1 (t, C-3), 39.1 (s, C-4), 52.5 (d, C-5), 18.8 (t, C-6), 29.6 (t, C-7), 130.7 (s, C-8), 137.2 (s, C-9), 38.4 (s, C-10), 33.3 (t, C-11), 78.2 (d, C-12), 162.8 (s, C-13), 70.0 (d, C-14), 120.8 (s, C-15), 175.2 (s, C-16), 9.5 (q, C-17), 27.9 (q, C-18), 64.3 (t, C-19), 19.5 (q, C-20).

Phlogacanthoside A (2)^[8] Whiter powder; $C_{26}H_{38}O_9$; $[\alpha]_D^{27.6}$ –182.4° (*c* 1.53, MeOH); IR (KBr) *v*: 3440, 2927, 2853, 1740, 1679, 1638, 1381, 1159, 1074, 1036, 1017, 615, 585 cm⁻¹; FAB⁺-MS *m/z* (%): 495 [M + 1]⁺ (65), 479 (100), 407 (16), 315 (28),

203 (20), 145 (65), 85 (92); HR-ESIMS m/z (%): 517.2421 (calcd. for C₂₆H₃₈O₉Na 517.2413); ¹H NMR (CD₃OD, 500 MHz): δ 4.94 (1H, s, H-14), 4.77 (1H, t, *J* = 8.1 Hz, H-12), 4.20 (1H, d, *J* = 7.8 Hz, H-1'), 4.08, 3.36 (1H each, both d, J = 9.5 Hz, H₂-19), 3.87 (1H, dd, J = 14.8, 2.2 Hz, H-6'a), 3.68 (1H, dd, J = 14.8, 5.4 Hz, H-6'b), 3.36 (1H, m, H-3'), 3.30 (1H, m, H-4'), 3.25 (1H, m, H-5'), 3.18 (1H, brt, J = 8.0 Hz, H-2'),1.97 (3H, s, Me-17), 1.27 (1H, d, J = 12.6 Hz, H-5), 1.15 (3H, s, Me-18), 1.05 (3H, s, Me-20); ¹³C NMR (CD₃OD, 100 MHz): δ 37.2 (t, C-1), 19.8 (t, C-2), 37.0 (t, C-3), 38.9 (s, C-4), 53.8 (d, C-5), 19.5 (t, C-6), 30.1 (t, C-7), 130.9 (s, C-8), 138.7 (s, C-9), 39.4 (s, C-10), 33.4 (t, C-11), 79.8 (d, C-12), 163.8 (s, C-13), 70.8 (d, C-14), 121.7 (s, C-15), 177.3 (s, C-16), 9.1 (q, C-17), 28.1 (q, C-18), 73.8 (t, C-19), 19.7 (q, C-20), 105.0 (d, C-1'), 75.2 (d, C-2'), 78.3 (d, C-3'), 71.7 (d, C-4'), 77.8 (d, C-5'), 62.8 (t, C-6').

(E)-Aglawone (3)^[9] $C_{21}H_{32}O_{2}$; White needles (acetone), mp 123 °C – 125 °C; ¹H NMR (CDCl₃, 500 MHz): δ 6.49 (1H, q, J = 7.5 Hz, H-20), 4.06 (1H, t, J = 2.6 Hz, H-3), 2.20 (1H, dd, J = 17.0, 7.4 Hz, H-15a), 1.96 (1H, dd, J = 17.0, 14.5 Hz, H-15b), 1.85 (3H, d, J = 7.5 Hz, Me-21), 1.01 (3H, s, Me-18), 0.83 (3H, s, Me-19); ¹³C NMR (CDCl₃, 100 MHz): δ 31.9 (t, C-1), 28.9 (t, C-2), 66.5 (d, C-3), 36.3 (t, C-4), 39.0 (d, C-5), 28.3 (t, C-6), 31.9 (t, C-7), 34.2 (d, C-8), 54.0 (d, C-9), 36.2 (s, C-10), 20.5 (t, C-11), 35.8 (t, C-12), 43.4 (s, C-13), 50.1 (d, C-14), 37.9 (t, C-15), 206.8 (s, C-16), 148.0 (s, C-17), 17.8 (q, C-18), 11.2 (q, C-19), 128.8 (d, C-20), 13.2 (q, C-21).

Eichlerianic acid (4)^[10] $C_{30}H_{50}O_4$; Colorless crystal (acetone), mp 124 °C – 126 °C; FAB⁻-MS *m/z* (%): 473 [M – 1]⁻ (100), 371 (12), 339 (5); ¹H NMR (CDCl₃, 500 MHz): δ 4.78, 4.60 (1H each, both s, H₂-28), 3.55 (1H, m, H-24), 1.67 (3H, s, Me-29), 1.23 (3H, s, Me-27), 1.09 (3H, s, Me-26), 1.07 (3H, s, Me-21), 0.96 (3H, s, Me-30), 0.83 (3H, s, Me-18), 0.79 (3H, s, Me-19); ¹³C NMR (CDCl₃, 100 MHz): δ 24.5 (t, C-1), 34.3 (t, C-2), 177.2 (s, C-3), 147.5 (t, C-4), 41.0 (d, C-5), 31.3 (t, C-6), 33.7 (t, C-7), 39.8 (s, C-8), 49.6 (d, C-9), 38.9 (s, C-10), 22.2 (t, C-11), 25.7 (t, C-12), 42.8 (d, C-13), 50.3 (s, C-14), 28.1 (t, C-15),

26.7 (t, C-16), 50.5 (d, C-17), 15.2 (q, C-18), 20.1 (q, C-19), 86.6 (s, C-20), 23.8 (q, C-21), 34.5 (t, C-22), 26.3 (t, C-23), 83.2 (q, C-24), 70.5 (s, C-25), 24.5 (q, C-26), 27.3 (q, C-27), 113.2 (s, C-28), 23.1 (q, C-29), 16.1 (q, C-30).

Shoreic acid $(5)^{[11]}$ C₃₀H₅₀O₄; Colorless crystal (acetone), mp 103° C – 104° C; ¹H NMR (CDCl₃, 500 MHz): δ4.87, 4.66 (1H each, both brs, H₂-28), 3.65 (1H, m, H-24), 1.73 (3H, s, Me-29), 1.19 (3H, s, Me-27), 1.14 (3H, s, Me-21), 1.11 (3H, s, Me-26), 1.01 (3H, s, Me-18), 0.89 (3H, s, Me-30), 0.85 (3H, s, Me-19); ¹³C NMR (CDCl₃, 100 MHz): δ 28.2 (t, C-1), 33.9 (t, C-2), 179.2 (s, C-3), 147.5 (t, C-4), 41.2 (d, C-5), 24.6 (t, C-6), 34.3 (t, C-7), 40.1 (s, C-8), 50.8 (d, C-9), 39.1 (s, C-10), 22.2 (t, C-11), 26.9 (t, C-12), 42.9 (d, C-13), 50.4 (s, C-14), 31.5 (t, C-15), 25.8 (t, C-16), 49.8 (d, C-17), 15.3 (q, C-18), 20.2 (q, C-19), 86.6 (s, C-20), 27.1 (q, C-21), 34.8 (t, C-22), 26.3 (t, C-23), 86.3 (q, C-24), 70.4 (s, C-25), 27.8 (q, C-26), 24.0 (q, C-27), 113.4 (s, C-28), 23.2 (q, C-29), 16.3 (q, C-30).

3β-Hydroxy-5α,8α-epidioxyergosta-6,22-diene **(6)**^[12] C₂₈H₄₄O₃; Colorless needles (acetone), mp $180^{\circ}\text{C} - 182^{\circ}\text{C}$; EI-MS m/z (%): 428 [M]⁺(2), 410 (4), 396 (100), 376 (5), 363 (17), 352 (3), 337 (8), 271 (4), 253 (8), 197 (2), 175 (2), 143 (3), 107 (3), 95 (4), 69 (10), 58 (15); ¹H NMR (CDCl₃, 500 MHz): δ 6.48 (1H, d, J = 8.1 Hz, H-7), 6.22 (1H, d, J = 8.3 Hz, H-6),5.19 (1H, dd, J = 15.2, 7.6 Hz, H-23), 5.12 (1H, dd, *J* = 15.2, 7.6 Hz, H-22), 3.94 (1H, m, H-3), 0.91 (3H, d, J = 6.4 Hz, Me-28), 0.88 (3H, s, Me-19), 0.84 (3H, d, J = 6.6 Hz, Me-26), 0.83 (3H, s, Me-18), 0.82 (3H, d, J = 6.4 Hz, Me-27); ¹³C NMR (CDCl₃, 100 MHz): δ 34.6 (t, C-1), 30.1 (t, C-2), 66.4 (d, C-3), 36.9 (t, C-4), 82.1 (s, C-5), 135.4 (d, C-6), 130.7 (d, C-7), 79.4 (s, C-8), 51.4 (d, C-9), 37.1 (s, C-10), 23.4 (t, C-11), 39.3 (t, C-12), 44.5 (s, C-13), 51.6 (d, C-14), 20.6 (t, C-15), 28.6 (t, C-16), 56.2 (d, C-17), 12.8 (q, C-18), 18.2 (q, C-19), 39.7 (d, C-20), 20.9 (q, C-21), 135.1 (d, C-22), 132.4 (d, C-23), 42.7 (d, C-24), 33.0 (d, C-25), 19.9 (q, C-26), 19.6 (q, C-27), 17.6 (q, C-28).

Stigmast-5-en-3 β ,7 α -diol (7)^[13] C₂₉H₅₀O₂; White needles (acetone), mp 218°C – 220°C; EI-MS

m/z (%): 430 [M]⁺ (25), 412 (100), 398 (35), 271 (8), 252 (7), 229 (6), 211 (6), 175 (8), 161 (12), 147 (11), 135 (15), 109 (10), 93 (13), 81 (19), 69 (21), 55 (35); ¹H NMR (CDCl₃, 400 MHz): δ 5.58 (1H, dd, J = 6.5, 1.8 Hz, H-6), 3.83 (1H, brs, H-7), 3.56 (1H, m, H-3), 1.03 (3H, s, Me-19), 0.90 (3H, d, J = 6.4 Hz, Me-21), 0.82 (3H, t, J = 7.8 Hz, Me-29), 0.78 (3H, d, J = 4.4 Hz, Me-27), 0.77 (3H, d, J = 6.7 Hz, Me-26), 0.66 (3H, s, Me-18); ¹³C NMR (CDCl₃, 100 MHz): δ 37.1 (t, C-1), 31.4 (t, C-2), 71.4 (d, C-3), 42.1 (t, C-4), 146.3 (s, C-5), 123.9 (d, C-6), 65.4 (d, C-7), 37.6 (d, C-8), 42.2(d, C-9), 37.3 (s, C-10), 20.8 (t, C-11), 39.2 (t, C-12), 42.3 (s, C-13), 49.5 (d, C-14), 24.3 (t, C-15), 29.3 (t, C-16), 55.8 (d, C-17), 11.7 (g, C-18), 19.1 (g, C-19), 36.1 (d, C-20), 18.3 (q, C-21), 34.0 (t, C-22), 28.3 (t, C-23), 45.9 (d, C-24), 29.3 (d, C-25), 18.8 (q, C-26), 19.8 (q, C-27), 23.1 (t, C-28), 12.0 (q, C-29).

 β -sitosterol (8), daucosterol (9), and scopoletin (10) were identified by TLC with standard samples, respectively.

Hexacosanoic acid (11)^[14] $C_{26}H_{52}O_2$; White powder, EI-MS *m/z* (%): 396 [M]⁺ (6), 382 (20), 368 (75), 354 (73), 340 (64), 325 (23), 311 (30), 297 (34), 283 (31), 269 (35), 255 (33), 241 (44), 227 (35), 213 (29), 199 (32), 185 (83), 171 (49), 157 (21), 143 (23), 129 (100), 111 (36), 97 (43), 85 (22).

3 Result and discussion

Compounds 1 - 3 and 8 - 11 were obtained from this plant for the first time, and abietane-type diterpene lactone 1 and 2 were firstly obtained from the genus *Aglaia*. Rocaglamides, the characteristic components of the genus *Aglaia*, were not isolated in our experiment.

Compounds 1 and 2 were screened for anticancer activity against AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells). Compound 1 exerted weak activity against AGZY 83-a with the IC_{50} value of 20.5 µg mL⁻¹, Compound 2 exhibited weaker activities against AGZY 83-a and SMMC-7721 with IC_{50} values of 52.6 µg mL⁻¹ and

49.2 µg mL⁻¹, respectively.

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