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ANTHRAQUINONES AND BIFLAVONOIDS FROM *Selaginella delicatula*

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Selaginella delicatula (Desv.) Alston, a perennial herb belonging to the *Selaginella* genus of the Selaginellaceae family, is mainly distributed in the south regions of the Yangtze River. Many cytotoxic biflavonoids [1–3], most of which were rubustaflavone derivatives, were isolated from *S. delicatula* and showed anti-cancer prospect. Current phytochemical research into *S. delicatula* led to the isolation of compounds **1**–**9**. All the identified compounds were obtained from *S. delicatula* for the first time. The anthraquinone glycosides were first reported in *Selaginella* genus.

S. delicatula was purchased from Hebei Province in China and identified by Prof. Zhen-ji Li, School of Life Science, Xiamen University. The corresponding voucher specimens (No. 20080909) were deposited at the Department of Medicinal Chemistry, Central South University. The air-dried herbs of *S. delicatula* (16 kg) were extracted three times with 75% (v/v) ethanol (320 L) by heat (two hours for each time). The combined solutions were evaporated in vacuum to yield 2 kg crude extract, which was subjected to polyamide column chromatography with EtOH–H_2O (v/v) gradient elution (0%, 30%, 70%, 95%) to afford four fractions (A–D). Fraction C (30–70%) (269 g) was submitted to silica gel column chromatography using $CHCl_3$–CH_3OH–H_2O (1:0:0–0:1:1) gradient elution to provide 40 fractions, which were further purified by a series of purification techniques, such as silica gel column chromatography (200–300 mesh), polyamide column chromatography (100–200 mesh), Sephadex LH-20, and recrystallization, yielding compounds **1**–**9**.

Identification of compounds **1**–**9** were performed by comparing NMR data with those reported in the literature [4–7] or further supported by RP18-HPLC-DAD comparison (retention time and UV spectra) with authentic compounds.

7,4′,7″,4‴-Tetra-*O*-methylamentoflavone (1): $C_{34}H_{26}O_{10}$, yellow powder, mp 305–306°C. ESI-MS *m/z* 595 $[M + H]^+$. IR spectrum (KBr, ν, cm^{-1}): 3434 (OH), 2931, 2840, 1374 (C-H), 1655 (C=O), 1604, 1590, 833 (Ar), 1028 (C-O). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 13.04 (1H, br.s, OH-5″), 12.72 (1H, br.s, OH-5), 7.90 (1H, dd, J = 8.5 and 2.5, H-6′), 7.80 (1H, d, J = 2.5, H-2′), 7.39 (2H, d, J = 8.5, H-2‴, 6‴), 7.09 (1H, d, J = 8.5, H-5′), 6.75 (2H, d, J = 8.5, H-3‴, 5‴), 6.55 (1H, s, H-3), 6.52 (1H, s, H-3″), 6.45 (1H, s, H-6″), 6.36 (1H, d, J = 2.0, H-8), 6.28 (1H, d, J = 2.0, H-6), 3.78 (3H, s, OMe), 3.76 (3H, s, OMe), 3.73 (3H, s, OMe), 3.71 (3H, s, OMe). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ): 182.9 (C-4″), 182.4 (C-4), 165.5 (C-7), 164.0 (C-2″), 163.8 (C-2), 162.7 (C-4‴), 162.5 (C-5), 162.4 (C-7″), 162.2 (C-4′), 160.7 (C-5″), 157.8 (C-9), 154.1 (C-9″), 131.1 (C-6′), 127.9 (C-2′), 127.7 (C-2‴), 127.7 (C-6‴), 123.4 (C-1‴), 123.4 (C-1′), 122.0 (C-3′), 114.5 (C-3‴), 114.5 (C-5‴), 111.2 (C-5′), 105.7 (C-10), 105.1 (C-10″), 104.6 (C-8″), 104.5 (C-3), 103.6 (C-3″), 98.2 (C-6), 95.4 (C-6″), 92.6 (C-8), 56.3 (OMe), 55.9 (OMe), 55.8 (OMe), 53.4 (OMe) [4].

Heveaflavone (2): $C_{33}H_{24}O_{10}$, yellow powder, mp 310°C. IR spectrum (KBr, ν, cm^{-1}): 3429 (OH), 2940, 2834, 1373 (C-H), 1655 (C=O), 1603, 833 (Ar), 1028 (C-O). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.22 (1H, br.s, OH-5″), 12.95 (1H, br.s, OH-5), 10.37 (1H, br.s, OH-4′), 8.04 (1H, d, J = 8.5, H-6′), 8.03 (1H, br.s, H-2′), 7.66 (2H, d, J = 8.5, H-2‴, 6‴), 7.16 (1H, d, J = 8.5, H-5′), 6.93 (1H, s, H-3), 6.92 (2H, d, J = 8.5, H-3‴, 5‴), 6.89 (1H, s, H-3″), 6.73 (1H, br.s, H-8), 6.67 (1H, s, H-6″), 6.34 (1H, br.s, H-6), 3.83 (3H, s, OMe), 3.80 (3H, s, OMe), 3.76 (3H, s, OMe). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ): 182.3 (C-4″), 181.9 (C-4), 165.0 (C-7), 163.9 (C-2), 163.5 (C-2″), 162.7 (C-7″), 162.3 (C-4‴), 161.4 (C-5), 161.2 (C-5″), 159.5 (C-4′), 157.2 (C-9), 153.6 (C-9″), 131.3 (C-2′), 128.0 (C-2‴, C-6‴, C-6′), 122.8 (C-1′), 121.0 (C-3′), 119.6 (C-1‴), 116.2 (C-5′), 114.5 (C-3‴, C-5‴), 104.9 (C-8″), 104.7 (C-10), 104.1 (C-10″), 103.2 (C-3, C-3″), 97.9 (C-6), 95.5 (C-6″), 92.6 (C-8), 56.4 (OMe), 55.9 (OMe), 55.5 (OMe) [5].

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7,7″-Di-*O*-methylamentoflavone (3): $C_{32}H_{22}O_{10}$, yellow powder. IR spectrum (KBr, ν, cm^{-1}): 3424 (OH), 2935, 2845, 1372 (C-H), 1655 (C=O), 1602, 835 (Ar), 1019 (C-O). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.25 (1H, br.s, OH-5″), 12.96 (1H, br.s, OH-5), 8.05 (1H, d, J = 8.5, H-6′), 8.04 (1H, br.s, H-2′), 7.56 (2H, d, J = 8.5, H-2‴, 6‴), 7.16 (1H, d, J = 8.5, H-5′), 6.91 (1H, s, H-3), 6.85 (1H, s, H-3″), 6.77 (1H, d, J = 2.0, H-8), 6.72 (2H, d, J = 8.5, H-3‴, 5‴), 6.67 (1H, s, H-6″), 6.36 (1H, d, J = 2.0, H-6), 3.84 (3H, s, OMe), 3.82 (3H, s, OMe). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ): 182.3 (C-4″), 181.9 (C-4), 165.0 (C-7), 164.0 (C-2), 163.9 (C-2″), 162.7 (C-7″), 161.4 (C-4‴), 161.2 (C-5), 161.1 (C-5″), 159.5 (C-4′), 157.3 (C-9), 153.6 (C-9″), 131.3 (C-2′), 128.2 (C-2‴, C-6‴), 128.0 (C-6′), 121.2 (C-1′), 120.9 (C-3′), 119.6 (C-1‴), 116.1 (C-5′), 115.8 (C-3‴, 5‴), 104.9 (C-8″), 104.7 (C-10), 104.1 (C-10″), 103.1 (C-3), 102.5 (C-3″), 98.0 (C-6), 95.4 (C-6″), 92.7 (C-8), 56.4 (OMe), 55.9 (OMe). The structure of **3** was elucidated on the basis of a comparison with the NMR data of compounds **1**, **2**, and **4**, which showed that the chemical shifts of C-6, C-8, and C-6″ were obviously affected by the methyl-etherification of OH-7 and OH-7″ [4]. The ^{13}C NMR data of **3** was reported for the first time.

Amentoflavone (4): $C_{30}H_{18}O_{10}$, yellow powder. PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.15 (1H, br.s, OH-5″), 13.03 (1H, br.s, OH-5), 8.14 (1H, d, J = 2.5, H-2′), 7.95 (1H, dd, J = 8.5 and 2.5, H-6′), 7.63 (2H, d, J = 8.5, H-2‴, 6‴), 7.03 (1H, d, J = 8.5, H-5′), 6.81 (1H, s, H-3), 6.75 (1H, s, H-3″), 6.64 (2H, d, J = 8.5, H-3‴, 5‴), 6.39 (1H, d, J = 2.0, H-8), 6.23 (1H, s, H-6‴), 6.18 (1H, d, J = 2.0, H-6) [4].

Physcion-8-*O*-β-D-glucopyranoside (5): $C_{22}H_{22}O_{10}$, yellow needle crystral (CH_3OH/H_2O), mp 238.5–239.5°C. IR spectrum (KBr, ν, cm^{-1}): 3432 (OH), 2959, 2923, 2860, 1377 (C-H), 1630 (C=O), 1595 (Ar), 1083 (C-O). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.09 (1H, br.s, OH-1), 7.47 (1H, d, J = 2.0, H-4), 7.34 (1H, d, J = 2.0, H-2), 7.17 (2H, br.s, H-5, 7), 5.17 (1H, d, J = 7.0, H-1′), 4.70–5.16 (4H, sugar-OH), 3.96 (3H, s, OMe-6), 3.18–3.75 (sugar protons), 2.41 (3H, s, Me-3). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ): 186.4 (C-9), 181.8 (C-10), 164.6 (C-6), 161.6 (C-8), 160.6 (C-1), 147.0 (C-3), 136.2 (C-10a), 132.0 (C-4a), 124.1 (C-2), 119.3 (C-4), 114.4 (C-8a), 114.4 (C-9a), 107.3 (C-7), 106.4 (C-5), 21.3 (Me-3), 100.6 (C-1′), 77.4 (C-5′), 76.5 (C-3′), 73.2 (C-2′), 69.7 (C-4′), 60.7 (C-6′), 56.0 (OMe-6) [6].

Chrysophanol-8-*O*-β-D-glucopyranoside (6): $C_{21}H_{20}O_9$, yellow needle crystral (CH_3OH/H_2O). IR spectrum (KBr, ν, cm^{-1}): 3399 (OH), 2923, 2853, 1390 (C-H), 1637, 1630 (C=O), 1088 (C-O). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.84 (1H, s, OH-1), 7.86 (1H, d, J = 7.5, H-5), 7.87 (1H, br.s, H-7), 7.71 (1H, dd, J = 7.5, 2.5, H-6), 7.49 (1H, br.s, H-4), 7.19 (1H, br.s, H-2), 5.17 (1H, d, J = 7.6, H-1′), 4.61–5.14 (4H, sugar-OH), 3.20–3.80 (sugar protons), 2.42 (3H, s, Me-3). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ): 187.8 (C-9), 182.3 (C-10), 161.9 (C-1), 158.5 (C-8), 147.9 (C-3), 136.2 (C-6), 135.0 (C-10a), 132.4 (C-4a), 124.3 (C-2), 122.8 (C-7), 120.8 (C-8a), 120.8 (C-5), 119.6 (C-4), 115.0 (C-9a), 100.8 (C-1′), 77.5 (C-5′), 76.8 (C-3′), 73.6 (C-2′), 69.8 (C-4′), 60.9 (C-6′), 21.7 (Me-3) [6].

Emodin-8-*O*-β-D-glucopyranoside (7): $C_{21}H_{20}O_{10}$, yellow needle crystral (CH_3OH/H_2O). IR spectrum (KBr, ν, cm^{-1}): 3299, 1367 (C-H), 1677, 1626 (C=O), 1595 (Ar), 1071 (C-O). PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.18 (1H, br.s, OH-1), 7.43 (1H, br.s, H-5), 7.27 (1H, br.s, H-4), 7.14 (1H, br.s, H-2), 6.99 (1H, br.s, H-7), 4.66–5.15 (4H, sugar-OH), 5.05 (1H, d, J = 7.5, H-1′), 3.23–3.73 (sugar protons), 2.39 (3H, s, Me-3). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ): 186.8 (C-9), 182.5 (C-10), 164.7 (C-6), 162.0 (C-8), 161.4 (C-1), 147.3 (C-3), 136.8 (C-10a), 132.4 (C-4a), 124.5 (C-2), 119.6 (C-4), 114.8 (C-9a), 113.6 (C-8a), 108.7 (C-7), 108.6 (C-5), 21.8 (Me-3), 101.1 (C-1′), 77.6 (C-5′), 76.7 (C-3′), 73.6 (C-2′), 69.8 (C-4′), 60.9 (C-6′) [6].

Chrysophanol (8): $C_{15}H_{10}O_4$, orange crystral. PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 11.89 (2H, br.s, OH-1, 8), 7.78 (1H, dd, J = 8.0 and 8.0, H-6), 7.68 (1H, d, J = 8.0, H-7), 7.51 (1H, br.s, H-4), 7.36 (1H, d, J = 8.0, H-5), 7.19 (1H, br.s, H-2), 2.42 (3H, s, Me-3). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ): 192.5 (C-9), 182.0 (C-10), 162.7 (C-8), 162.4 (C-1), 149.3 (C-3), 136.9 (C-6), 133.6 (C-10a), 133.3 (C-4a), 124.5 (C-2), 124.4 (C-7), 121.4 (C-5), 119.9 (C-4), 115.8 (C-8a), 113.7 (C-9a), 22.3 (Me-3) [6].

Aloe-emodin (9): $C_{15}H_{10}O_5$, orange powder. ^{1}H PMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 11.90 (2H, br.s, OH-1, 8), 7.65 (1H, br.s, H-4), 7.29 (1H, br.s, H-2), 7.77 (1H, br.s, H-6), 7.68 (1H, br.s, H-7), 7.35 (1H, br.s, H-5), 5.64 (1H, br.s, 3-OH), 4.62 (2H, s, 3-CH_2) [7].

Identifications of compounds **2**, **4**, **8**, and **9** were further supported by RP18-HPLC-DAD comparison (retention time and UV spectra) with authentic compounds.

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