FLAVONOIDS FROM *Daphne holosericea*

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Plants of *Daphne* L. (Thymelaeaceae) are distributed widely in the world, and pharmacological research into this genus revealed that some chemical components of *Daphne* L. could be used as antileukemia, antithrombosis, antiatherosclerosis, antipregnancy, and antibacterial agents [1]. In the course of our systematic chemical research on *Daphne* L. [2–5], we investigated the constituents of the plant *Daphne holosericea* (Diels) Hamaya, and nine flavonoids have been isolated and elucidated from the EtOAc extract of this plant.

The stem barks of *Daphne holosericea* (46 kg) were collected in July 2006, in Yunnan Province (P. R. China), and were authenticated by Prof. Li-shan Xie of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 2006071010) was deposited in the School of Pharmacy, Second Military Medical University. The $CHCl_3$ extract was obtained after the barks were crushed into pieces and percolated three times with chloroform. The residue was percolated again with acetone, whose water suspension was partitioned with EtOAc and *n*-BuOH. The $CHCl_3$ extract (300g) and the EtOAc extract (300g) were repeatedly subjected to silica gel column chromatography, respectively, eluting with gradient $CHCl_3/CH_3OH$. The fractions obtained were purified through reverse phase ODS colunm chromatography to afford **1** (50mg), **2** (12 mg), **3** (20mg), **4** (66 mg), **5** (100 mg), **6** (78 mg), **7** (18 mg), **8** (2 g), **9** (35 mg), and **10** (15 mg).

On the basis of ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), HSQC, HMBC, and ESI-MS spectral analysis, 10 compounds were identified as stelleranol (**1**) [6], isochamaejasmin (**2**) [7], neochamaejasmin B (**3**) [8], tiliroside (**4**) [9], daphnodorin B (**5**) [10], 5-*O*-methylafzelechin (**6**) [11], (–)-afzelechin (**7**) [11], genkwanin (**8**) [12], apigenin (**9**) [12], and kaempferol (**10**) [12]. All spectral data were in good agreement with the literature data. Compounds **1**–**3** were first obtained from the genus *Daphne*, while compounds **4**–**10** were isolated from *D. holosericea* for the first time.

Compound **1**: yellow amorphous powder, mp 264–265°C, ESI/MS *m/z*: 557[M–1]$^-$. ^{1}H NMR(600 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.35 (1H, dd, J = 16.0, H-4a), 2.46 (1H, dd, J = 16.0, H-4b), 3.96 (1H, br.s, H-3), 4.56 (1H, d, J = 3.6, 3-OH), 4.85 (1H, s, H-2), 5.69 (1H, s, H-6), 5.96 (1H, s, H-2″), 6.03 (1H, d, J = 2.0, H-6″), 6.07 (1H, d, J = 2.0, H-8″), 6.49 (2H, d, J = 9.0, H-3′, H-5′), 6.52 (1H, s, 3″-OH), 6.56 (2H, d, J = 9.0, H-2′, H-6′), 6.72 (2H, d, J = 8.4, H-3‴, H-5‴), 7.08 (2H, d, J = 8.4, H-2‴, H-6‴), 9.22 (1H, s, 4′-OH), 9.56 (1H, s, 4‴-OH), 11.30 (1H, s, 5″-OH), 11.30 (1H, s, 7″-OH). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 80.0 (C-2), 63.3 (C-3), 26.9 (C-4), 108.5 (C-4a), 187.0 (C-5), 100.7 (C-6), 168.4 (C-7), 85.2 (C-8), 156.3 (C-8a), 128.3 (C-1′), 127.2 (C-2′, C-6′), 114.2 (C-3′, C-5′), 158.1 (C-4′), 89.9 (C-2″), 79.9 (C-3″), 190.8 (C-4″), 99.9 (C-4″a), 163.4 (C-5″), 96.8 (C-6″), 167.6 (C-7″), 96.3 (C-8″), 160.4 (C-8″a), 122.2 (C-1‴), 114.6 (C-3‴, C-5‴), 129.8 (C-2‴, -6‴), 58.6 (C-4‴).

Compound **2**: pale yellow amorphous powder, $[\alpha]_D^{20}$ 0° (*c* 0.5, MeOH), ESI/MS *m/z*: 541 [M–1]$^-$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 3.73 (2H, d, J = 12, H-3, H-3″), 4.91 (2H, d, J = 12, H-2, H-2″), 5.75 (2H, d, J = 2.0, H-6, H-6″), 5.88 (2H, d, J = 2.0, H-8, H-8″), 6.78 (4H, dd, J = 8.0, H-3′, H-3‴, H-5′, H-5‴), 7.01 (4H, dd, J = 8.0, H-2′, H-2‴, H-6′, H-6‴). ^{13}C NMR(150 MHz, CD_3OD, δ, ppm): 82.5 (C-2, C-2″), 96.1 (C-6, C-6″), 97.3 (C-8, C-8″), 102.8 (C-4a, C-4a′), 116.6 (C-3′, C-3‴, C-5′, C-5‴), 129.0 (C-2′, C-2‴, C-6′, C-6‴), 130.8 (C-1′, C-1‴), 159.8 (C-4′, C-4‴), 164.4 (C-8a, C-8a′), 165.5 (C-5, C-5″), 168.4 (C-7, C-7″), 196.9 (C-4, C-4″).

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Compound **3**: pale yellow amorphous powder, $[\alpha]_D^{20}$ 162° (*c* 0.7, MeOH), ESI/MS *m/z*: 541 $[M–1]^-$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 3.14 (1H, d, J = 5.0, H-3), 3.26 (1H, d, J = 12.0, H-3″), 5.13 (1H, d, J = 12.0, H-2″), 5.54 (1H, d, J = 5.0, H-2), 5.75 (1H, d, J = 2.0, H-6), 5.77 (1H, d, J = 2.0, H-6″), 5.86 (1H, d, J = 2.0, H-8″), 5.97 (1H, d, J = 2.0, H-8), 6.64 (2H, d, J = 8.0, H-3′, H-5′), 6.78 (2H, d, J = 8.0, H-3‴, H-5‴), 6.92 (2H, d, J = 8.0, H-2′, H-6′), 7.14 (2H, d, J = 8.0, H-2‴, H-6‴). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 49.4 (C-3), 50.8 (C-3″), 81.5 (C-2), 83.3 (C-2″), 96.0 (C-8), 96.3 (C-8″), 97.0 (C-6), 97.2 (C-6″), 103.8 (C-4a), 105.1 (C-4a′), 116.1 (C-3′, C-5′), 116.4 (C-3‴, C-5‴), 128.5 (C-2′, C-6′), 128.8 (C-1′), 129.0 (C-1‴), 130.2 (C-2‴, C-6‴), 158.6 (C-4′), 159.0 (C-4‴), 163.4 (C-8a, C-8a′), 165.1 (C-5), 165.5 (C-5″), 168.1 (C-7), 168.3 (C-7″), 196.3 (C-4), 198.6 (C-4″).

Compound **4**: yellow amorphous powder, mp 246–248 °C, ESI/MS *m/z*: 593 $[M–1]^-$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 3.31–3.50 (4H, t, H-2″, H-3″, H-4″, H-5″), 4.19 (1H, dd, J = 6.0, 12.0, H-6″b), 4.30 (1H, d, J = 10.0, H-6″a), 5.24 (1H, d, J = 8.0, H-1″), 6.07 (1H, d, J = 16.0, H-8‴), 6.13 (1H, s, H-6), 6.30 (1H, s, H-8), 6.79 (2H, d, J = 9.0, H-3‴, H-5‴), 6.82 (2H, d, J = 9.0, H-3′, H-5′), 7.30 (2H, d, J = 8.0, H-2‴, H-6‴), 7.40 (1H, d, J = 16.0, H-7‴), 7.99 (2H, d, J = 9.0, H-2′, H-6′). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 158.4 (C-2), 135.2 (C-3), 179.4 (C-4), 105.6 (C-4a), 163.0 (C-5), 94.8 (C-6), 165.9 (C-7), 100.0 (C-8), 161.5 (C-8a), 122.7 (C-1′), 116.0 (C-3′, C-5′), 159.3 (C-4′), 131.2 (C-4′, C-6′), 71.7 (C-1″), 75.7 (C-2″), 78.0 (C-3″), 104.0 (C-4″), 75.8 (C-5″), 64.3 (C-6″), 127.1 (C-1‴), 132.2 (C-2‴, C-6‴), 116.8 (C-3‴, C-5‴), 161.2 (C-4‴), 146.5 (C-7‴), 114.8 (C-8‴), 168.8 (C-9‴).

Compound **5**: yellow amorphous powder, mp 214–216°C, ESI/MS *m/z*: 541$[M–1]^-$. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.52 (1H, dd, J = 16.0, 9.0, H-4b), 2.76 (1H, dd, J = 16.0, 5.5, H-4a), 3.73 (1H, m, H-3), 4.57 (1H, d, J = 8.0, H-2), 5.00 (1H, d, J = 5.5, 3-OH), 5.70 (2H, s, H-7″, H-9″), 6.57 (2H, m, H-13″, H-15″), 6.59 (1H, s, H-6), 6.77 (2H, m, J = 9.0, H-3′, H-5′), 6.86 (2H, m, J = 9.0, H-12″, H-16″), 7.39 (2H, m, J = 9.0, H-2′, H-6′), 9.22 (1H, s, 10″-OH), 9.59 (1H, s, 14″-OH), 9.72 (1H, s, 8″-OH), 10.45 (1H, s, H-6″). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 80.4 (C-2), 66.3 (C-3), 28.5 (C-4), 103.2 (C-4a), 153.7 (C-5), 89.5 (C-6), 147.4 (C-7), 109.6 (C-8), 148.9 (C-8a), 129.0 (C-1′), 127.3 (C-2′, C-6′), 114.4 (C-3′, C-5′), 157.4 (C-4′), 146.6 (C-2″), 117.1 (C-3″), 194.4 (C-4″), 105.8 (C-5″), 165.8 (C-6″), 94.5 (C-7″), 165.8 (C-8″), 94.3 (C-9″), 165.8 (C-10″), 121.2 (C-11″), 126.6 (C-12″, C-16″), 115.6 (C-13″, C-15″), 156.3 (C-14″).

Compound **6**: gray needles, ESI/MS *m/z*: 287 $[M–1]^-$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 2.49 (1H, dd, J = 16.0, 5.5, H-4a), 2.85 (1H, dd, J = 16.0, 8.0, H-4b), 3.76 (3H, OCH_3), 3.98 (1H, ddd, J = 8.0, 5.5, H-3), 4.60 (1H, d, J = 7.5, H-2), 5.94 (1H, d, J = 2.0, H-8a), 6.03 (1H, d, J = 2.0, H-7), 6.79 (2H, d, J = 8.5, H-4′, H-5′), 7.21 (2H, d, J = 8.5, H-3′, H-6′). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 82.8 (C-2), 68.7 (C-3), 28.7 (C-4), 101.7 (C-4a), 160.1 (C-5), 92.7 (C-6), 158.3 (C-7), 96.3 (C-8), 156.7 (C-8a), 131.4 (C-1′), 129.6 (C-2′), 116.1 (C-3′), 158.4 (C-4′), 55.8 (OCH_3).

Compound **7**: brown amorphous powder, ESI/MS *m/z*: 273 $[M–1]^-$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 2.50 (1H, dd, J = 16.0; 5.5, H-4a), 2.87 (1H, dd, J = 16.0; 8.0, H-4b), 3.98 (1H, t, H-3), 4.58 (1H, d, J = 7.5, H-2), 5.84 (1H, d, J = 2.0, H-8a), 5.92 (1H, d, J = 2.0, H-7), 6.77 (2H, d, J = 8.5, H-4′, H-5′), 7.21 (2H, d, J = 8.5, H-3′, H-6′). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 82.8 (C-2), 68.9 (C-3), 28.9 (C-4), 100.9 (C-4a), 158.4 (C-5), 95.5 (C-6), 157.6 (C-7), 96.4 (C-8), 157.0 (C-8a), 131.5 (C-1′), 129.6 (C-2′,6′), 116.1 (C-3′, C-5′), 157.9 (C-4′).

Compound **8**: yellow amorphous powder, mp 275–277°C, ESI/MS *m/z*: 283$[M–1]^-$. ^{1}H NMR (600 MHz, C_5D_5N, δ, ppm, J/Hz): 3.79 (3H, s, $–OCH_3$), 6.60 (1H, d, J = 2.0, H-6), 6.69 (1H, d, J = 2.0, H-8), 6.88 (1H, s, H-3), 7.27 (2H, d, J = 8.0, H-3′,5′), 7.94 (2H, d, J = 8.0, H-2′,6′), 13.58 (5-OH). ^{13}C NMR (150 MHz, C_5D_5N, δ, ppm): 165.0 (C-2), 104.2 (C-3), 182.9 (C-4), 159.1 (C-5), 98.6 (C-6), 166.0 (C-7), 93.0 (C-8), 158.2 (C-9), 107.6 (C-10), 122.3 (C-1′), 129.0 (C-2′, C-6′), 117.0 (C-3′, C-5′), 162.9 (C-4′), 56.3 ($–OCH_3$).

Compound **9**: yellow amorphous powder, mp 352–354°C, ESI/MS *m/z*: 269$[M–1]^-$. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.20 (1H, d, J = 2.0, H-6), 6.48 (1H, d, J = 2.0, H-8), 6.73 (1H, s, H-3), 6.93 (2H, d, J = 8.5, H-3′, H-5′), 7.90 (2H, d, J = 8.5, H-2′,6′), 12.92 (5-OH).

Compound **10**: green amorphous powder, mp 292–293°C, ESI/MS *m/z*: 287 $[M+1]^+$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 6.09 (1H, d, J = 2.0, H-6), 6.31 (1H, d, J = 2.0, H-8), 6.81 (2H, d, J = 8.0, H-3′, 5′), 7.99 (2H, d, J = 8.0, H-2′,6′).

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