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ISOLATION OF CHEMICAL CONSTITUENTS FROM *Daphne odora* VAR. *Margirmt* BY HIGH-SPEED COUNTER-CURRENT CHROMATOGRAPHY

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Daphne odora var. *Margirmt*, family Thymelaeaceae, is a famous flower and medical plant in the regions along the Yangzi River. The radix of the genus has been used as a herbal remedy for treating arthritis, fever, and mange. Modern pharmacological studies indicate that *Daphne odora* var. *Margirmt* has anti-lipid peroxidation, analgesic, antibacterial, anti-inflammation, immunoloregulation, anti-hypoxia, and antitumor activity [1–6].

In this paper, the preparative high-speed counter-current chromatographic (HSCCC) separation and purification of daphnetin, daphnodorin B, *p*-hydroxybenzoic acid, and daphnodorin D_1 will be described. The purity of these constituents was 99.6, 99.7, 99.5, and 99.2%, determined by high-performance liquid chromatography (HPLC), respectively. Their chemical structures were elucidated by UV, IR, ESI-MS, EI-MS, PMR, and ^{13}C NMR analyses. Daphnodorin B, daphnodorin D_1, and *p*-hydroxybenzoic acid were isolated from this plant for the first time.

The dried whole plant of *Daphne odora* var. *Margirmt* (245 kg) was chopped into small pieces and extracted with a mixture of solvents (1500 kg) composed of 95% ethanol and ethyl acetate (1:1, v/v), under continuous dynamic countercurrent for 4 h to obtain an organic solvent extract (Fr1, 39.8 kg) and a brown sludge (Fr2, 3 kg).

Fraction Fr1 was evaporated to dryness under reduced pressure to form a syrup. A mixture of the syrup (40 g), acticarbon (25 g), and diatomaceous earth (2.8 g) was successively extracted with light petroleum (bp. 60–90°C), chloroform, and ethyl acetate. The ethyl acetate fraction (Fr3, 24 g) was obtained.

Fraction Fr2 (40 g) mixed with acticarbon (25 g) and diatomaceous earth (2.8 g) was successively extracted with light petroleum (bp. 60–90°C) and ether. The ether fraction (Fr4, 19 g) was obtained.

Daphnetin (**1**, 62 mg), daphnodorin B (**2**, 56 mg), and *p*-hydroxybenzoic acid (**3**, 67 mg) from fraction Fr3 (2.8 g) was separated and purified by HSCCC using a two-phase solvent system composed of *n*-hexane–ethyl acetate–ethanol–water 2:5:2:5 (v/v/v/v). The upper phase is the stationary phase and the lower phase is the mobile phase. The flow rate was 1.8 mL/min, the speed of the HSCCC apparatus was 850 rpm, and the separation temperature was 31°C. The effluent from the outlet of the column was continuously monitored at 254 nm. Each peak fraction was manually collected according to the chromatogram.

The two-phase solvent system composed of *n*-hexane–ethyl acetate–ethanol–water 3:5:3:5 (v/v/v/v) was used for separating and purifying daphnodorin D_1 (**4**, 6 mg) from fraction Fr4 (300 mg) by HSCCC. The upper phase is the stationary phase and the lower phase is the mobile phase. The flow rate was 2.0 mL/min, the speed of HSCCC apparatus was 800 rpm, and the separation temperature was 26°C. The effluent from the outlet of the column was continuously monitored at 254 nm. Each peak fraction was manually collected according to the chromatogram.

The collected fractions were analyzed by HPLC using CH_3CN and 0.1% H_3PO_4 as mobile phase in gradient mode (CH_3CN: 0–15 min, 0–30%; 15–20 min, 30–35%; 20 – 30 min, 35% – 35%). The peak fractions obtained from the HSCCC chromatograph were identified using UV, IR, ESI-MS, EI-MS, PMR, and ^{13}C NMR spectral data.

Daphnetin (1). White powder, mp 262–264°C, ESI-MS (*m*/*z*): 177 $[M–H]^-$, showing that the molecular ion was 178, which was in agreement with the molecular formula $C_9H_6O_4$. UV (MeOH, λ_{max}, nm): 206, 260, 326. IR (KBr, ν_{max}, cm^{-1}): 3457, 1680, 1609, 1508, 1412, 1343, 1305, 1168, 834. PMR spectrum (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.82 (1H, d, J = 9.6, H-4), 6.99 (1H, d, J = 8.4, H-5), 6.80 (1H, d, J = 8.4, H-6), 6.17 (1H, d, J = 9.6, H-3). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 163.4 (C-2), 151.1 (C-7), 146.6 (C-4), 144.9 (C-9), 133.4 (C-8), 120.1 (C-5), 113.9 (C-6), 113.7 (C-10), 112.1 (C-3).

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Compared with the reported data, the UV, IR, MS, and NMR data were in agreement with those of daphnetin in the literature [7].

Daphnodorin B (2). Yellow powder, mp 230–234°C. ESI–MS (*m/z*): 541 $[M–H]^-$, showing that the molecular ion was 542, which was in agreement with the molecular formula $C_{30}H_{23}O_{10}$. UV (MeOH, λ_{max}, nm): 308. IR (KBr, ν_{max}, cm^{-1}): 3422, 2924, 2855, 1631, 1513, 1456, 1379, 1247, 1171, 1099, 1068, 829. PMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.47 (1H, br.s, 5-OH), 11.18 (1H, br.s, 4′-OH), 10.45 (1H, br.s, 6″-OH), 9.71 (1H, br.s, 8″-OH), 9.59 (1H, br.s, 14″-OH), 9.21 (1H, br.s, 10″-OH), 7.39 (2H, d, J = 9, H-2′, 6′), 6.86 (2H, d, J = 8.4, H-12″, 16″), 6.77 (2H, d, J = 8.4, H-3′, 5′), 6.59 (1H, s, H-6), 6.57 (2H, d, J = 8.4, H-13″, 15″), 5.70 (2H, s, H-7″, 9″), 5.00 (1H, d, J = 3.6, 3-OH), 4.56 (1H, d, J = 7.8, H-2), 3.73 (1H, m, H-3), 2.75 (1H, dd, J = 14.4, 5.4, H-4), 2.52 (1H, dd, J = 14.4, 5.4, H-4). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 194.43 (C-4″), 165.82 (C-8″), 157.45 (C-4′), 156.34 (C-14″), 153.71 (C-5), 152.44 (C-8a), 147.46 (C-7), 146.66 (C-2″), 129.07 (C-1′), 127.35 (C-2′, 6′), 126.61 (C-12″, 16″), 121.27 (C-11′), 117.15 (C-3″), 115.65 (C-13″, 15″), 114.44 (C-3′, 5′), 109.67 (C-8), 105.82 (C-5″), 103.30 (C-4a), 94.56 (C-7″), 94.41 (C-9″), 89.55 (C-6), 80.4 (C-2), 66.4 (C-3), 28.5 (C-4). Compared with the reported data, the UV, IR, MS, and NMR data were in agreement with those of daphnodorin B in the literature [8].

***p*-Hydroxybenzoic Acid (3).** Yellowish crystal, mp 213–216°C. EI-MS (*m/z*): 138 $[M]^+$, 121, 93, 65. This showed that the molecular ion was 138, which was in agreement with the molecular formula $C_7H_6O_3$. UV (MeOH, λ_{max}, nm): 252. IR (KBr, ν_{max}, cm^{-1}): 3400, 1678, 1603, 1512, 1447, 1421, 1318, 1288, 1242, 1167, 852. All of these data were in agreement with those reported in the literature [9].

Daphnodorin D_1 (4). Yellow powder, mp 206–207°C. ESI-MS (*m/z*): 525 $[M–H]^-$, showing that the molecular ion was 526, which was in agreement with the molecular formula $C_{30}H_{22}O_9$. UV (MeOH, λ_{max}, nm) 216, 268. PMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 1.73 (2H, s, H-3), 1.86 (2H, s, H-3), 2.44 (2H, dd, J = 6.0, H-4), 2.53 (2H, dd, J = 12.0, H-4), 4.29 (1H, d, J = 9.2, H-2), 5.92 (1H, s, H-6), 6.10 (1H, s, H-6″), 6.25 (1H, s, H-8″), 6.55 (4H, t, J = 7.6, H-2′, 6′), 7.17 (2H, d, J = 8.0, H-10′, 14′). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 194.3 (C-4″), 169.8 (C-5), 165.4 (C-7), 163.3 (C-8a), 160.7 (C-4′), 159.1 (C-2″), 158.2 (C-5″), 157.8 (C-7″), 155.6 (C-8a″), 155.4 (C-12″), 133.5 (C-1′), 130.5 (C-10″, 14″), 129.0 (C-2′, 6′), 126.2 (C-19″), 118.4 (C-3′, 5′), 117.1 (C-11″,13″), 115.6 (C-3″), 109.6 (C-4a″), 104.0 (C-4a), 103.8 (C-8), 101.2 (C-6″), 99.5 (C-6), 94.4 (C-8″), 80.4 (C-2), 36.4 (C-3), 20.5 (C-4). Compared with the reported data, the UV, MS, and NMR data were in agreement with those of daphnodorin D_1 in the literature [10].

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