MINOR POLYPHENOLS FROM *Maackia amurensis* WOOD

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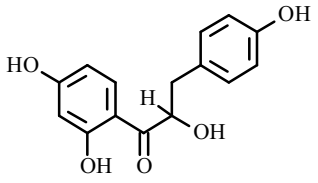
The rare α,4,2′,4′-tetrahydroxydihydrochalcone, flavanones liquiritigenin and naringenin, isoflavones calycosin and 5-methoxydaidzein, and reduced stilbene dihydroresveratrol were isolated for the first time from Maackia amurensis *wood. The structures of the pure compounds were established using 2D NMR COSY, NOE, HMBC, and HSQC experiments.*

Key words: *Maackia amurensis*, Fabaceae, α-hydroxydihydrochalcone, flavanones, isoflavones.

Maackia amurensis is the single woody representative of the Fabaceae family in the Russian Far East. The polyphenol complex obtained from its wood possesses strong antioxidant properties. The hepatoprotective preparation Maksar based on it has greater activity than the known hepatoprotectors Carsil and Legalon [1]. Therefore, the chemical composition of *M. amurensis* is interesting not only for scientific curiosity but also for complete characterization of the preparation.

Herein we report on minor components of the *M. amurensis* wood polyphenol complex. Six known compounds were isolated and characterized: α,4,2′,4′-tetrahydroxydihydrochalcone (**1**) [2], liquiritigenin (**2**) [3], naringenin (**3**) [4], calycosin (**4**) [5], 5-methoxydaidzein (**5**) [6], and dihydroresveratrol (**6**) [7]. We observed in the heartwood and characterized earlier isoflavonoids, stilbenes, dimeric stilbenes, stilbenolignan, and isoflavostilbene [8]. The rare dihydrochalcone that was hydroxylated in the α-position and flavanones were observed for the first time in *M. amurensis* wood.



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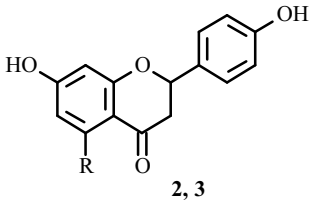
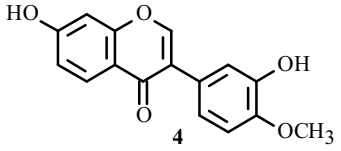


2, 3

2: R = H; **3:** R = OH



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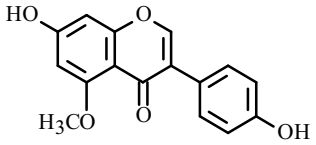

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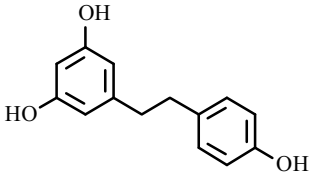

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About ten α-hydroxydihydrochalcones have been isolated and characterized until now. These are luonogenin [9], nubigenol [10], α,2′-dihydroxy-4,4′-dimethoxydihydrochalcone [11], α,2′,4,4′-tetrahydroxydihydrochalcone [2], coatlines A and B [12], α-hydroxy-2′,4,4′-trimethoxydihydrochalcone [13], and kanzonol Y [14].

Mass spectra of the isolated compounds showed that **4** and **5** had the same mass, 284 amu. The presence in the PMR spectra of these compounds of a 1H singlet at 8.05-8.34 ppm indicated that they were isoflavonoids. Compounds **4** and **5** were structural isomers. Their separation on KSK silica gel and Sephadex LH-20 was unsatisfactory. Therefore, they were chromatographed over a column (210 × 30 mm) packed with Toypearl HW-50F sorbent equilibrated with alcohol:water (1:9)

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containing 0.05% formic acid. A linear gradient with alcohol content increasing from 10 to 80% was used. Fractions were detected using light with λ = 280 nm. Structures of the isolated pure compounds were established using 2D NMR COSY, NOE, HMBC, and HSQC experiments.

α,4,2′,4′-Tetrahydroxydihydrochalcone was first isolated and characterized in 1983 [2]. It differed from **1** in the magnitude and sign of the angle of rotation, [α] +59° and –34°, respectively. Its proton spectrum was poorly resolved. Data from the ^{13}C spectrum were not reported. NMR spectra of **1** agreed best with those of prenylated hydroxydihydrochalcone kanzonol Y [14]. The PMR spectrum of **1** exhibited resonances for methylene protons at 2.87 ppm (dd, J = 6.31, 13.9) and 3.06 (1H, dd, J = 4.20, 13.9) and hydroxymethine protons at 3.81 (1H, br.s, α-OH) and 5.18 (1H, dd, J = 4.20, 6.31). Broad singlets at 8.20 and 9.68 corresponded to protons of two aromatic hydroxyls; a singlet at 12.49, to a hydroxyl involved in a H-bond. The remaining resonances corresponded to protons of two aromatic rings substituted in the 1,2,3- and 1,4-positions. The ^{13}C NMR spectrum contained resonances for 15 C atoms. The carbonyl C appeared at 204.4 ppm; methylene and methine C atoms, at 41.8 and 73.9, respectively.

EXPERIMENTAL

UV spectra in methanol were recorded on a Cary-Varian 219; IR spectra, on a Specord M 82; electron-impact (EI) mass spectra, in an AMD-604S mass spectrometer with electron ionization; PMR and ^{13}C NMR spectra, on DPX-300 and DRX-500 (Germany) spectrometers at operating frequencies 300 and 500 MHz for ^{1}H and 75 and 125 MHz for ^{13}C (δ, ppm, 0 = TMS, acetone-d_6, DMSO-d_6). Analytical HPLC was performed on an Agilent Technologies (Series 1100) chromatograph equipped with QuatPump G1311A high-pressure pumps, a G1322A degasser, a G1328B injector, and a VWD G1314A detector. Optical rotation was determined on a Perkin—Elmer 131 spectropolarimeter.

Extraction and Isolation of Flavonoids. Plant material was collected in 2005 in Primorskii Krai. Ground wood was exhaustively extracted with ethanol. The yield of alcohol extract was 5.6%. The dry alcohol extract was dissolved in the minimum amount of alcohol, diluted with water, and subsequently re-extracted with hexane, benzene, and ethylacetate. The ethylacetate extract was used for the investigations.

Chromatography of the ethylacetate extract was performed in glass columns packed with KSK (70-100 μm) and Sigma (40-63 μm) silica gel. Solvent systems were benzene:ethanol and $CHCl_3$:ethanol with a gradient of increasing ethanol content. Additional separation of pure compounds was carried out in columns packed with Sephadex LH-20 using $CHCl_3$:ethanol (5:1 and 4:1) and Toypearl HW-50F using water:ethanol with detection by a Uvicord S LKB Bromma.

α,4,2′,4′-Tetrahydroxydihydrochalcone (1), $C_{15}H_{14}O_5$, 14.0 mg, amorphous powder, [α] –34.0° (*c* 1.0, C_2H_5OH). UV spectrum (MeOH, λ_{max}, nm): 249, 280, 316. IR spectrum (KBr, ν, cm^{-1}): 3340, 1640, 1512. EIMS (*m*/*z*, I_{rel}, %): 274 (5) $[M]^+$, 256 (84), 168 (56), 137 (100), 123 (10), 107 (90).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 2.87 (1H, dd, J = 6.31, 13.9, H-β), 3.06 (1H, dd, J = 4.20, 13.9, H-β), 3.81 (1H, br.s, α-OH), 5.18 (1H, dd, J = 4.20, 6.31, H-α), 6.37 (1H, d, J = 2.1, H-3′), 6.47 (1H, dd, J = 2.5, 8.8, H-5′), 6.72 (2H, d, J = 8.8, H-3,5), 7.06 (2H, d, J = 8.8, H-2,6), 7.91 (1H, J = 8.8, H-6′), 8.18 (1H, br.s, OH), 9.68 (1H, br.s, OH), 12.37 (1H, br.s, OH).

^{13}C NMR spectrum (acetone-d_6, δ, ppm): 41.79 (C-β), 73.90 (C-α), 103.2 (C-3′), 108.8 (C-5′), 111.5 (C-1′), 115.6 (C-3,5), 128.7 (C-1), 131.1 (C-2,6), 133.2 (C-6′), 156.7 (C-4), 165.7 (C-2′), 166.4 (C-4′), 204.4 (C=O).

Liquiritigenin (2), $C_{15}H_{12}O_4$, 18.0 mg, amorphous powder, [α] –10.0° (*c* 1.0, C_2H_5OH). UV spectrum (MeOH, λ_{max}, nm): 231.6, 274.2, 309.6. IR spectrum (KBr, ν, cm^{-1}): 3360, 1640, 1600, 1510. EIMS (*m*/*z*, I_{rel}, %): 256 (89) $[M]^+$, 239 (13), 228 (16), 163 (33), 150 (28), 137 (100), 120 (70), 107 (24).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 2.67 (1H, dd, J = 2.93, 16.87, H-3a), 3.05 (1H, dd, J = 13.2, 16.87, H-3b), 5.45 (1H, dd, J = 2.9, 13.2, H-2), 6.42 (1H, d, J = 2.2, H-8), 6.57 (1H, dd, J = 2.2, 8.6, H-6), 6.89 (2H, d, J = 8.6, H-3′,5′), 7.40 (2H, d, J = 8.6, H-2′,6′), 7.73 (1H, d, J = 8.6, H-5).

^{13}C NMR spectrum (acetone-d_6, δ, ppm): 44.5 (C-3), 80.05 (C-2), 103.5 (C-8), 111.0 (C-6), 115.1 (C-10), 115.9 (C-3′,5′), 128.7 (C-2′,6′), 129.2 (C-5′), 131.1 (C-1′), 158.6 (C-4′), 164.3 (C-9), 165.0 (C-7), 190.3 (C-4).

Naringenin (3), $C_{15}H_{12}O_5$, 12.0 mg, amorphous powder, [α] 0° (*c* 1.0, C_2H_5OH). UV spectrum (MeOH, λ_{max}, nm): 215, 290. IR spectrum (KBr, ν, cm^{-1}): 3220, 1640, 1610, 1520. EIMS (*m*/*z*, I_{rel}, %): 272 (28) $[M]^+$, 256 (28), 228 (100), 211 (17), 199 (19), 181 (32), 153 (27), 137 (32), 107 (17).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 3.08 (1H, dd, J = 3.2, 16.5, H-3a), 3.30 (1H, dd, J = 12.0, 16.5, H-3b), 5.60 (1H, dd, J = 3.2, 12.0, H-2), 6.30 (1H, d, J = 1.97, H-8), 6.33 (1H, d, J = 1.97, H-6), 6.90 (2H, d, J = 8.6, H-3′,5′), 7.40 (2H, d, J = 8.6, H-2′,6′), 9.70 (2H, br.s, 2×OH), 11.50 (1H, br.s, OH).

^{13}C NMR spectrum (acetone-d_6, δ, ppm): 35.2 (C-3), 80.9 (C-2), 101.8 (C-6), 101.9 (C-8), 107.3 (C-10), 115.9 (C-3′,5′), 128.7 (C-2′,6′), 130.5 (C-1′), 143.1 (C-4′), 143.2 (C-5), 158.5 (C-7), 165.0 (C-9), 165.2 (C-4).

Calycosin (4), $C_{16}H_{12}O_5$, 9.2 mg, cream powder. UV spectrum (MeOH, λ_{max}, nm): 250, 288. IR spectrum (KBr, ν, cm^{-1}): 3389, 2800, 1698, 1630, 1516, 1461. EIMS (*m*/*z*, I_{rel}, %): 284 (100) $[M]^+$, 269 (22), 241 (23), 213 (22), 137 (22), 133 (16).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 3.83 (3H, s, OCH_3), 6.90 (1H, d, J = 1.9, H-8), 6.97 (1H, d, J = 8.3, H-5′), 7.00 (1H, dd, J = 1.9, 8.3, H-6′), 7.07 (1H, dd, J = 1.9, 8.3, H-6), 7.17 (1H, d, J = 1.9, H-2′), 8.05 (1H, d, J = 8.3, H-5), 8.15 (1H, s, H-2).

^{13}C NMR spectrum (acetone-d_6, δ, ppm): 56.0 (OCH_3), 102.9 (C-8), 112.0 (C-5′), 115.4 (C-6), 116.7 (C-2′), 118.3 (C-10), 120.8 (C-6′), 124.8 (C-3), 126.0 (C-1′), 128.2 (C-5), 146.8 (C-3′), 148.0 (C-4′), 153.2 (C-2), 158.5 (C-9), 162.9 (C-7), 175.3 (C-4).

5-Methoxydaidzein (5), $C_{16}H_{12}O_5$, 14 mg, amorphous powder. UV spectrum (MeOH, λ_{max}, nm): 260, 281. IR spectrum (KBr, ν, cm^{-1}): 3360, 2800, 1645, 1510. EIMS (*m*/*z*, I_{rel}, %): 284 (100) $[M]^+$, 241 (27), 137 (33), 131 (11).

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 3.89 (3H, s, OCH_3), 6.83 (2H, d, J = 8.8, H-3′,5′), 6.91 (1H, s, H-8), 7.38 (2H, d, J = 8.8, H-2′,6′), 7.42 (1H, s, H-6), 8.25 (1H, s, H-2).

^{13}C NMR spectrum (DMSO-d_6, δ, ppm): 55.8 (OCH_3), 102.8 (C-8), 104.7 (C-6), 114.9 (C-3′,5′), 115.9 (C-10), 122.8 (C-3), 122.9 (C-1′), 130.1 (C-2′,6′), 147.2 (C-4′), 151.9 (C-7), 152.4 (C-2), 153.5 (C-9), 157.1 (C-5), 174.3 (C-4).

Dihydroresveratrol (6), $C_{14}H_{14}O_3$, 7.3 mg, amorphous powder. UV spectrum (MeOH, λ_{max}, nm): 230, 281. IR spectrum (KBr, ν, cm^{-1}): 3360, 1600, 1510. EIMS (*m*/*z*, I_{rel}, %): 230 (30) $[M]^+$, 137 (100), 122 (7).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 2.68-2.80 (4H, m, 2×CH_2), 6.19 (1H, t, J = 2.2, H-4), 6.23 (2H, d, J = 2.2, H-2,6), 6.75 (2H, d, J = 8.5, H-3′,5′), 7.05 (2H, d, J = 8.5, H-2′,6′), 8.12 (2H, s, 2×OH), 8.15 (1H, s, OH).

^{13}C NMR spectrum (acetone-d_6, δ, ppm): 36.5 (CH_2), 38.0 (CH_2), 99.9 (C-4), 106.5 (C-2), 106.6 (C-6), 114.7 (C-3′,5′), 128.9 (C-2′,6′), 132.5 (C-1′), 144.0 (C-1), 155.0 (C-3), 155.2 (C-5), 158.1 (C-4′).

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