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Isolation and Characterization of Phenolic Compounds from Coptidis Rhizoma[1)]

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Six phenolic compounds were isolated from Coptidis Rhizoma and were identified as 3-(3′,4′-dihydroxyphenyl)-(2*R*)-lactic acid, 3-(3′,4′-dihydroxyphenyl)-(2*R*)-lactic acid 4′-*O*-β-D-glucopyranoside, 3′,4′-dihydroxyphenethyl alcohol 1-*O*-β-D-glucopyranoside, gentisic acid 5-*O*-β-D-glucopyranoside, 4-*O*-feruloyl-D-quinic acid and 5-*O*-feruloyl-D-quinic acid.

Keywords——Coptidis Rhizoma; Ranunculaceae; phenylpropanoid; phenyllactic acid; phenyllactic acid glucoside; phenethyl alcohol glucoside; gentisic acid glucoside; feruloylquinic acid

Previously, we reported that intraperitoneal administration of the aqueous extract of Coptidis Rhizoma caused a remarkable decrease of urea-nitrogen concentration (BUN) in rat serum,[2)] and later, the BUN-decreasing activity, after fractionation of the extract by Amberlite IR-120B column chromatography, was found in the non-adsorbed (neutral and acidic) fraction. In continuing the search for the active compounds in this fraction, we isolated six phenolic compounds. This paper deals with the isolation and structure elucidation of these compounds.

The aqueous extract of this drug was partitioned with AcOEt, and the H_2O layer was passed through an Amberlite IR-120B column to yield the neutral and acidic fraction. This fraction was repeatedly chromatographed over MCI GEL CHP-20P and Sephadex LH-20 columns to give compounds **1**—**6** (Chart 1).

Compound **1** (**1**), colorless hygroscopic prisms, mp 84—87 °C, $[\alpha]_D$ +10.8° (MeOH), showed a blue spot with ferric chloride reagent on the thin-layer chromatogram (TLC). The infrared (IR) spectrum of **1** exhibited absorption bands due to hydroxyl groups (3390 cm^{-1}) and a carboxylic acid function (1728 and 3000—2400 cm^{-1}). In the field-desorption mass spectrum (FD-MS), it showed a peak at *m/z* 198 due to the molecular ion. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** in methanol-d_4 showed signals ascribable to six aromatic carbons at δ 116.2 (d, $C_{2'}$), 117.7 (d, $C_{5'}$), 121.9 (d, $C_{6'}$), 130.2 (s, $C_{1'}$), 144.9 (s, $C_{3'}$) and 145.9 (s, $C_{4'}$), one methylene at δ 41.0 (t, C_3), one carbinol at δ 73.0 (d, C_2) and one carboxylic acid at δ 177.2 (s, C_1). Therefore, **1** was assumed to be an *ortho*-dihydroxyphenyl lactic acid.

The ^1H-NMR spectrum of **1** exhibited aromatic ABX-type signals at δ 6.60 (dd, J=2, 8 Hz), 6.72 (d, J=8 Hz) and 6.79 (d, J=2 Hz) assignable to H-6′, -5′ and -2′, respectively, and aliphatic ABX-type signals at δ 2.73 (dd, J=7, 14 Hz), 2.98 (dd, J=5, 14 Hz) and 4.32 (dd, J=7, 5 Hz) assignable to H-3, -3 and -2, respectively, of 3-(3′, 4′-dihydroxyphenyl)-lactic acid. The structural assignment of 3-(3′,4′-dihydroxyphenyl)-lactic acid was supported by the fact

that methylation of **1** with diazomethane afforded a trimethylate (**1a**), which showed the molecular ion peak at *m/z* 240 in the electron-impact mass spectrum (EI-MS). The absolute configuration of the C-2 carbon in **1** was elucidated by comparison of the circular dichroism (CD) spectrum of **1** with those of D-(+)-3,4-dihydroxyphenylalanine (DOPA) (2*R*) and L-(−)-DOPA (2*S*). The CD spectrum of **1** showed a positive Cotton effect at 200 nm, and was similar to that of D-(+)-DOPA (a positive Cotton effect at 197 nm), indicating that **1** possesses an *R*-configuration. Consequently, the structure of **1** was established as 3-(3′,4′-dihydroxyphenyl)-(2*R*)-lactic acid, which had previously been obtained by hydrolysis of rosmarinic acid.[3]

Compound **2** (**2**), a white hygroscopic powder, $[\alpha]_D$ −41.0° (MeOH), showed absorptions due to a carboxylic acid function (1735 and 3000—2400 cm^{-1}) and hydroxyl groups (3400 cm^{-1}) in the IR spectrum. In the FD-MS, it showed peaks at *m/z* 360 (molecular ion), 198 (base peak) and 163 (hexosyl ion), suggesting that **2** is a glycoside. Hydrolysis of **2** with 2 N HCl yielded compound **1** and glucose. The location of the glucosyl moiety in **2** was determined to be at the C-4′ position by comparison of the ^{13}C-NMR spectra of **2** and **1**; carbon resonances of C-1′, -3′ and -5′ in **2** were observed downfield by 4.2, 2.9 and 0.9 ppm, respectively, as compared with those of **1**.[4] The structural assignment of 3-(3′,4′-dihydroxyphenyl)-(2*R*)-lactic acid 4′-*O*-β-D-glucopyranoside was supported by measurement of the intramolecular nuclear Overhauser effect (NOE) of the dimethylate (**2a**). Namely, irradiation of the methoxyl signal (δ 3.75) caused a 22% increase in the integrated intensity of the C-2′ proton signal (δ 6.83, d, J=2 Hz). Therefore, the structure of **2** was established as 3-(3′,4′-dihydroxyphenyl)-(2*R*)-lactic acid 4′-*O*-β-D-glucopyranoside.

Compound **3** (**3**), a white amorphous powder, $[\alpha]_D$ −30.4° (MeOH), showed a blue spot with ferric chloride reagent on TLC. The ^{1}H- and ^{13}C-NMR spectra revealed the presence of an *ortho*-dihydroxyphenethyl alcohol moiety [δ 36.0 (t), 71.5 (t), 116.1 (d), 116.9 (d), 120.9 (d), 131.0 (s), 144.1 (s) and 145.5 (s)] and a carbohydrate moiety [δ 4.37 (d, J=7 Hz, anomeric H)], thus suggesting that **3** is a glycoside. Hydrolysis of **3** with 2 N HCl yielded 3′,4′-dihydroxyphenethyl alcohol (**7**) and glucose. The location of the glucosyl moiety in **3** was determined to be at the C-1 position by analysis of the ^{13}C-NMR spectrum of **3**, that is, the carbon resonance of C-1 appeared downfield [δ 71.5 (t)] as compared with that of **7**.[5] Consequently, the structure of **3** was determined as 3′,4′-dihydroxyphenethyl alcohol 1-*O*-β-D-glucopyranoside.

Compound **4** (**4**), a white amorphous powder, $[\alpha]_D$ −53.2° (MeOH), showed the molecular ion peak at *m/z* 316 in the FD-MS. The ^{1}H-NMR spectrum of **4** exhibited aromatic ABX-type signals at δ 6.86 (1H, d, J=9 Hz), 7.34 (1H, dd, J=3, 9 Hz) and 7.58 (1H, d, J=3 Hz), and an anomeric proton doublet signal at δ 4.90 (J=7 Hz). The ^{13}C-NMR spectrum showed signals due to a 2,4- or 2,5-dihydroxybenzoic acid moiety at δ 113.3 (s), 118.6 (2C, d), 126.7 (d), 150.7 (s), 157.5 (s) and 172.3 (s), and a β-glucopyranosyl moiety at δ 62.0, 70.6, 74.2, 77.1, 77.2 and 102.8. Hydrolysis of **4** with 2 N HCl yielded gentisic acid as an aglycone and glucose as a component sugar. The location of the glucosyl moiety in **4** was determined to be at the C-5 position by NOE experiments with the dimethylate (**4a**); irradiation of a methoxyl signal (δ 3.77) caused a 20% increase in the integrated intensity of the C-3 proton signal (δ 7.06, d, J=9 Hz). Therefore, the structure of **4** was established as gentisic acid 5-*O*-β-D-glucopyranoside.

Compounds **5** and **6** were identified as 4-*O*-feruloyl-D-quinic acid[6] and 5-*O*-feruloyl-D-quinic acid,[6] respectively, by examinations of the ^{1}H-NMR spectra and by hydrolytic studies.

Biological tests of these compounds are in progress.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus, and are uncorrected. Optical

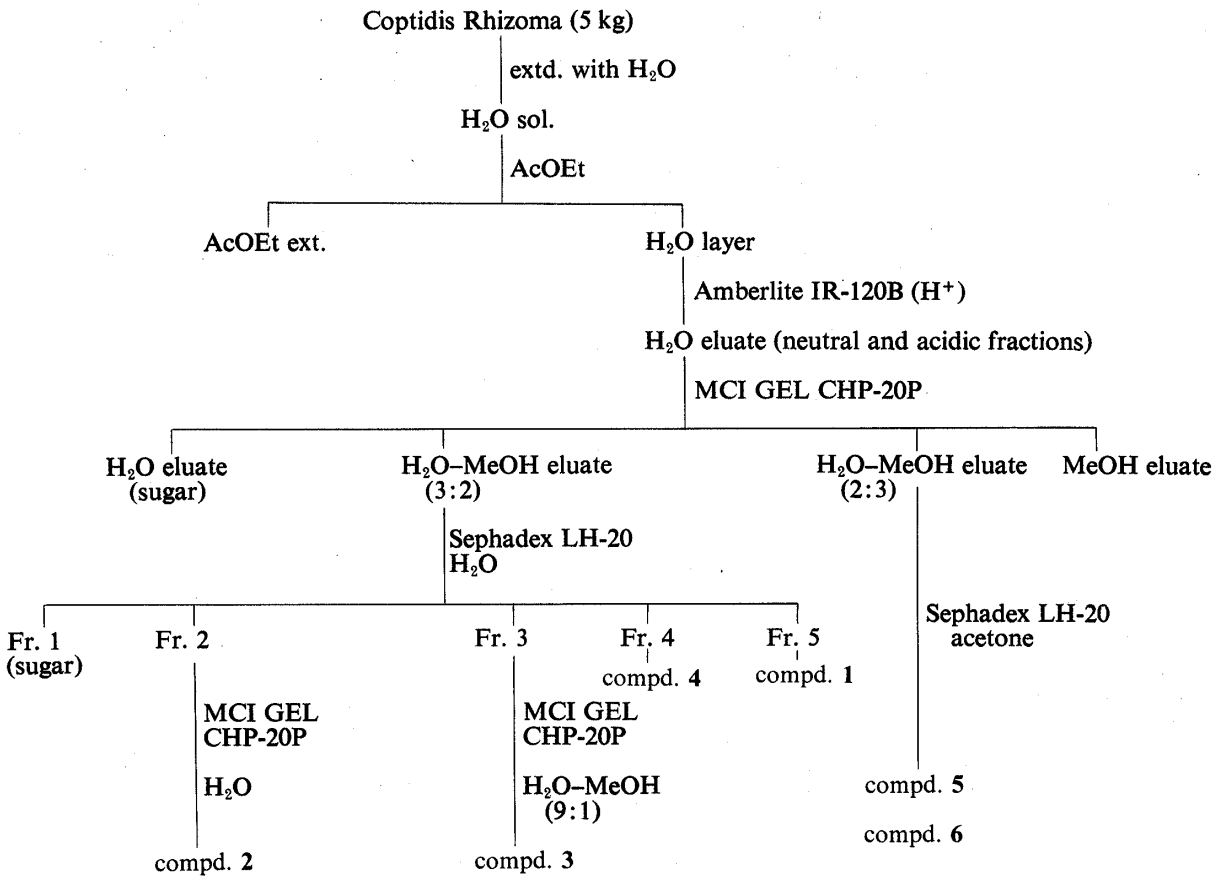

Chart 1

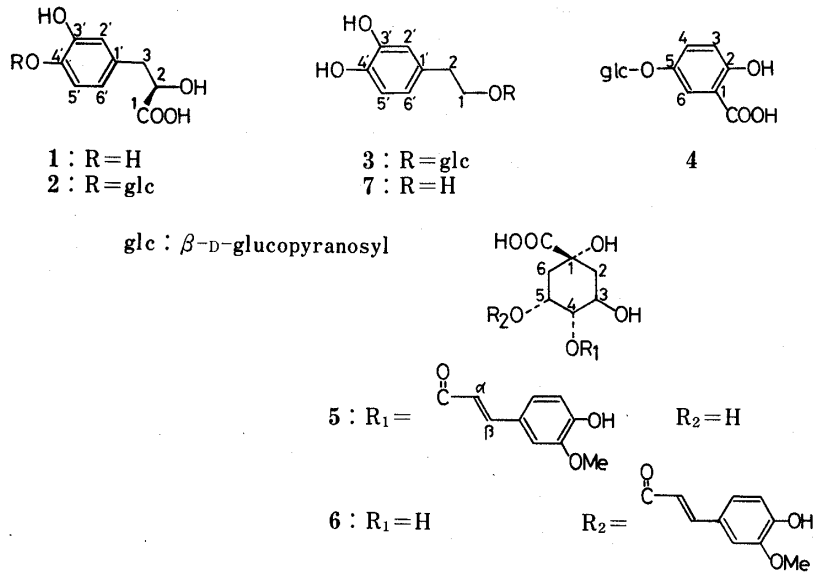

Chart 2

rotations were taken with a JÁSCO DIP-4 digital polarimeter (cell length: 0.5 dm). CD spectra were obtained with a JASCO ORD/CD J-20 unit (cell length: 0.2 mm). IR spectra were obtained with a JASCO IR-G spectrometer. EI- and FD-MS were taken on JEOL D-300 and JEOL DX-300 mass spectrometers, respectively. UV spectra were recorded on a Hitachi UV-340 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with JEOL PS-100 and JEOL FX-100 spectrometers at 100 and 25.05 MHz, respectively, with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Column chromatography was performed with Kieselgel 60 (70—230 mesh,

Merck), Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemicals) and MCI GEL CHP-20P (75—150 μ, Mitsubishi Chemical Industries, Ltd.). TLC was conducted on precoated Kieselgel 60 F_{254} plates (Merck, 0.2 mm thick) (for phenolics) and precoated Avicel SF cellulose plates (Funakoshi) (for sugars) using $FeCl_3$ reagent, 10% H_2SO_4 followed by heating (for phenolics) and aniline–hydrogen–phthalate reagent (for sugars) as detectors.

Isolation——Commercial Coptidis Rhizoma (5 kg) was extracted at room temperature with H_2O. The H_2O extract was partitioned with AcOEt. The H_2O layer was passed through an Amberlite IR-120B column (H^+ form, 5 l). The eluate (neutral and acidic fractions) was evaporated *in vacuo*, and the residue was chromatographed over an MCI GEL CHP-20P column with H_2O–MeOH (1:0, 3:2, 2:3). The H_2O–MeOH (3:2) eluate was repeatedly chromatographed over Sephadex LH-20 (H_2O) and MCI GEL CHP-20P (H_2O–MeOH = 1:0—9:1) to afford compounds **1** (1190 mg), **2** (477 mg), **3** (16 mg) and **4** (529 mg). The H_2O–MeOH (2:3) eluate was repeatedly chromatographed over Sephadex LH-20 (acetone) to give compounds **5** (718 mg) and **6** (450 mg).

Compound 1 (1)——Colorless hygroscopic prisms (AcOEt–benzene), mp 84—87 °C, $[\alpha]_D^{28}$ +10.8° (c = 3.70, MeOH). FD-MS m/z: 198 [M^+], 134, 91. IR ν_{max}^{KBr} cm^{-1}: 1728, 3000—2400 (COOH), 3390 (OH), 1606. UV λ_{max}^{MeOH} nm (ε): 283 (3270), 222 (sh., 6450), 208 (10700). CD ($c = 1.50 \times 10^{-4}$, MeOH) $[\theta]^{20}$ (nm): −7200 (218), 0 (210), +3620 (200). ^{1}H-NMR (acetone-d_6): 2.73 (1H, dd, J = 7, 14 Hz, C_3-H), 2.98 (1H, dd, J = 5, 14 Hz, C_3-H), 4.32 (1H, dd, J = 5, 7 Hz, C_2-H), 6.60 (1H, dd, J = 2, 8 Hz, $C_{6'}$-H), 6.72 (1H, d, J = 8 Hz, $C_{5'}$-H), 6.79 (1H, d, J = 2 Hz, $C_{2'}$-H).

Methylation of 1——A solution of **1** (10 mg) in methanol was methylated with ethereal CH_2N_2 at room temperature overnight. The solution was concentrated to dryness, and purified by chromatography over silica gel. Elution with benzene–AcOEt (3:1) gave the trimethylate (**1a**) (10 mg), $[\alpha]_D^{32}$ −4.5° (c = 1.00, MeOH). ^{1}H-NMR ($CDCl_3$): 2.83 (1H, dd, J = 7, 14 Hz, C_3-H), 3.08 (1H, dd, J = 5, 14 Hz, C_3-H), 3.77 (3H, s, COOMe), 3.85 (6H, s, Ar-OMe), 4.42 (1H, dd, J = 5, 7 Hz, C_2-H), 6.76 (3H, br s, Ar-H). CD ($c = 2.34 \times 10^{-4}$, MeOH) $[\theta]^{20}$ (nm): −11300 (218), 0 (208), +20000 (200).

Compound 2 (2)——A white hygroscopic amorphous powder, $[\alpha]_D^{25}$ −41.0° (c = 1.00, MeOH). *Anal.* Calcd for $C_{15}H_{20}O_{10}$: C, 50.00; H, 5.56, Found: C. 49.56; Hm 5.98. FD-MS m/z: 360 [M^+], 198, 163. IR ν_{max}^{KBr} cm^{-1}: 1735, 3000—2400 (COOH), 3400 (OH), 1600. UV λ_{max}^{MeOH} nm (ε): 277 (2330), 217 (sh., 6100), 199 (6350). CD ($c = 3.89 \times 10^{-4}$, MeOH) $[\theta]^{20}$ (nm): −14100 (215), 0 (207), +25700 (197). ^{1}H-NMR (methanol-d_4): 2.79 (1H, dd, J = 8, 14 Hz, C_3-H), 3.00 (1H, dd, J = 4, 14 Hz, C_3-H), 3.30—4.00 (m, glc. C_{2-6}-H), 4.30 (1H, dd, J = 4, 8 Hz, C_2-H), 4.77 (1H, d, J = 7 Hz, glc. C_1-H), 6.69 (1H, dd, J = 2, 8 Hz, $C_{6'}$-H), 6.80 (1H, d, J = 2 Hz, $C_{2'}$-H), 7.08 (1H, d, J = 8 Hz, $C_{5'}$-H). ^{13}C-NMR (methanol-d_4): 40.8 (t, C_3), 72.7 (d, C_2), 118.3 (d, $C_{2'}$), 118.6 (d, $C_{5'}$), 122.1 (d, $C_{6'}$), 134.4 (s, $C_{1'}$), 145.4 (s, $C_{4'}$), 147.8 (s, $C_{3'}$), 177.2 (s, C_1), 62.3 (t, glc. C_6), 71.1 (d, glc. C_4), 74.7 (d, glc. C_2), 77.4 (d, glc. C_5), 77.9 (d, glc. C_3), 104.4 (d, glc. C_1).

Methylation of 2——**2** (10 mg) was methylated with Me_2SO_4 (0.5 ml) and K_2CO_3 (1 g) in dry acetone (10 ml) under reflux for 3 h. After filtration of the inorganic precipitates, the solution was concentrated, and the residue was purified by chromatography over silica gel. Elution with $CHCl_3$–MeOH (10:1) gave the dimethylate (**2a**, 4 mg). A white amorphous powder. ^{1}H-NMR (DMSO-d_6): 2.84 (1H, dd, J = 8, 14 Hz, C_3-H), 3.22 (1H, dd, J = 4, 14 Hz, C_3-H), 3.63 (3H, s, COOMe), 3.75 (3H, s, Ar-OMe), 4.23 (1H, dd, J = 4, 8 Hz, C_2-H), 4.89 (1H, d, J = 7 Hz, glc. C_1-H), 6.70 (1H, dd, J = 2, 8 Hz, $C_{6'}$-H), 6.83 (1H, d, J = 2 Hz, $C_{2'}$-H), 6.99 (1H, d, J = 8 Hz, $C_{5'}$-H).

Acid Hydrolysis of 2——**2** (3 mg) was heated with 2 N HCl (0.5 ml) for 1 h on a hot water bath, and the reaction mixture was evaporated to dryness. The residue was checked by TLC to detect **1** (*Rf* 0.46: benzene–HCOOEt–HCOOH = 1:7:1, *Rf* 0.52: upper layer of *n*-BuOH–AcOH–H_2O = 6:3:5) and glucose (*Rf* 0.42: *n*-BuOH–pyridine–H_2O = 6:4:3).

Compound 3 (3)——A white amorphous powder, $[\alpha]_D^{25}$ −30.4° (c = 0.31, MeOH). *Anal.* Calcd for $C_{14}H_{20}O_8 \cdot H_2O$: C, 51.69; H, 6.15. Found: C, 51.19; H, 6.17. FD-MS m/z: 316 [M^+]. UV λ_{max}^{MeOH} nm (ε): 281 (2290), 220 (sh., 5140), 209 (5970). ^{1}H-NMR (acetone-d_6 + D_2O): 2.77 (2H, t, J = 8 Hz, C_2-H), 3.20—4.10 (m, glc. C_{2-6}-H), 4.37 (1H, d, J = 7 Hz, glc. C_1-H), 6.55 (1H, dd, J = 2, 8 Hz, $C_{6'}$-H), 6.72 (1H, d, J = 8 Hz, $C_{5'}$-H), 6.78 (1H, d, J = 2 Hz, $C_{2'}$-H). ^{13}C-NMR (acetone-d_6 + D_2O): 36.0 (t, C_2), 71.5 (t, C_1), 116.1 (d, $C_{5'}$), 116.9 (d, $C_{2'}$), 120.9 (d, $C_{6'}$), 131.0 (s, $C_{1'}$), 144.1 (s, $C_{4'}$), 145.5 (s, $C_{3'}$), 62.2 (t, glc. C_6), 71.0 (d, glc. C_4), 74.4 (d, glc. C_2), 77.2 (d, glc. C_5), 77.3 (d, glc. C_3), 103.6 (d, glc. C_1).

Acid Hydrolysis of 3——**3** (3 mg) was heated with 2 N HCl (0.5 ml) for 1 h on a hot water bath, and the reaction mixture was evaporated to dryness. The residue was checked by TLC to detect **7** (*Rf* 0.37: benzene–HCOOEt–HCOOH = 5:7:1) and glucose (*Rf* 0.42: *n*-BuOH–pyridine–H_2O = 6:4:3).

Compound 4 (4)——A white amorphous powder, $[\alpha]_D^{25}$ −53.2° (c = 1.00, MeOH). *Anal.* Calcd for $C_{13}H_{16}O_9 \cdot 1/4H_2O$: C, 48.68; H, 5.18. Found: C, 48.66; H, 5.49. FD-MS m/z: 316 [M^+]. UV λ_{max}^{MeOH} nm (ε): 320 (3480), 234 (6400), 218 (7900). ^{1}H-NMR (acetone-d_6): 3.40—4.00 (m, glc. C_{2-6}-H), 4.90 (1H, d, J = 7 Hz, glc. C_1-H), 6.86 (1H, d, J = 9 Hz, C_3-H), 7.34 (1H, dd, J = 3, 9 Hz, C_4-H), 7.58 (1H, d, J = 3 Hz, C_6-H). ^{13}C-NMR (acetone-d_6): 113.3 (s, C_1), 118.6 (d, $C_{3,6}$), 126.7 (d, C_4), 150.7 (s, C_5), 157.5 (s, C_2), 172.3 (s, COOH), 62.0 (t, glc. C_6), 70.6 (d, glc. C_4), 74.2 (d, glc. C_2), 77.1 (d, glc. C_5), 77.2 (d, glc. C_3), 102.8 (d, glc. C_1).

Acid Hydrolysis of 4——**4** (5 mg) was heated with 2 N HCl (0.5 ml) for 1 h on a hot water bath, and the reaction mixture was evaporated to dryness. The residue was checked by TLC to detect gentisic acid (*Rf* 0.56: benzene–HCOOEt–HCOOH = 5:7:1) and glucose (*Rf* 0.42: *n*-BuOH–pyridine–H_2O = 6:4:3).

Methylation of 4——**4** (10 mg) was methylated with Me_2SO_4 (0.2 ml) and K_2CO_3 (0.5 mg) in dry acetone (10 ml) under reflux for 3 h. The mixture was worked up in the same way as for **2** to give the dimethylate (**4a**, 3 mg). A white amorphous powder. ^{1}H-NMR (DMSO-d_6): 3.39 (3H, s, COOMe), 3.77 (3H, s, Ar-OMe), 4.70 (1H, d, J=7 Hz, glc. C_1-H), 7.06 (1H, d, J=9 Hz, C_3-H), 7.26 (1H, dd, J=3, 9 Hz, C_4-H), 7.31 (1H, br s, C_6-H).

Compound 5 (5)——Colorless needles (acetone–MeOH), mp 187—188 °C, $[\alpha]_D^{28}$ −69.0° (c=1.00, MeOH). ^{1}H-NMR (acetone-d_6): 2.00—2.43 (4H, m, $C_{2,6}$-H), 3.82 (3H, s, OMe), 4.28 (1H, m, C_3-H), 4.37 (1H, m, C_5-H), 4.85 (1H, dd, J=3, 9 Hz, C_4-H), 6.41 (1H, d, J=16 Hz, C_α-H), 6.84 (1H, d, J=8 Hz, $C_{5'}$-H), 7.13 (1H, dd, J=2, 8 Hz, $C_{6'}$-H), 7.34 (1H, d, J=2 Hz, $C_{2'}$-H), 7.64 (1H, d, J=16 Hz, C_β-H).

Acid Hydrolysis of 5——**5** (15 mg) was heated with 1 N HCl (1 ml) for 3 h on a hot water bath, and the reaction mixture was concentrated *in vacuo*. The residue was applied to a Sephadex LH-20 column (H_2O, MeOH) to afford D-quinic acid [6 mg, $[\alpha]_D^{32}$ −38.8° (c=0.30, MeOH)] and ferulic acid.

Compound 6 (6)——Colorless needles (acetone–MeOH), mp 178—180 °C, $[\alpha]_D^{25}$ +1.4° (c=0.99, MeOH). ^{1}H-NMR (DMSO-d_6): 1.80—2.20 (4H, m, $C_{2,6}$-H), 3.58 (1H, dd, J=3, 7 Hz, C_4-H), 3.81 (3H, s, OMe), 3.85 (1H, m, C_3-H), 5.22 (1H, m, C_5-H), 6.40 (1H, d, J=16 Hz, C_α-H), 6.78 (1H, d, J=8 Hz, $C_{5'}$-H), 7.08 (1H, dd, J=2, 8 Hz, $C_{6'}$-H), 7.25 (1H, d, J=2 Hz, $C_{2'}$-H), 7.53 (1H, d, J=16 Hz, C_β-H).

Acid Hydrolysis of 6——**6** (10 mg) was heated with 1 N HCl (1 ml) for 3 h on a hot water bath. The reaction mixture was worked up in the same way as described for **5** and D-quinic acid and ferulic acid were detected.

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