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Tannins and Related Compounds. XX.[1] Two New Ellagitannins Containing a Proto-quercitol Core from *Quercus stenophylla* MAKINO. (4)

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A new class of ellagitannins (**1** and **2**) possessing a proto-quercitol moiety has been isolated from the bark of *Quercus stenophylla* MAKINO (Fagaceae), and their structures have been characterized from the chemical and spectroscopic evidence as 1,5-di-*O*-galloyl-3,4-(*S*)-hexahydroxydiphenoyl proto-quercitol (**1**) and 5-*O*-galloyl-3,4-(*S*)-hexahydroxydiphenoyl proto-quercitol (**2**).

Keywords——*Quercus stenophylla*; Fagaceae; ellagitannin; proto-quercitol; tannase; ^{1}H-NMR; spin-decoupling

In the preceding paper,[1] we demonstrated the presence of eight gallotannins based on a proto-quercitol core in the bark of *Quercus stenophylla* MAKINO (Fagaceae). In continuing the chemical examination of tannin constituents of this plant, we have now isolated two new ellagitannins (**1** and **2**) containing a proto-quercitol moiety. This paper presents a detailed account of the structure determination of these compounds.

The aqueous solution,[3] obtained previously by partition of the extract with ethyl acetate, was made acidic (pH 2) by addition of citric acid, and the solution was extracted with ethyl acetate to furnish a tannin mixture containing compounds **1** and **2**. Repeated chromatography of the ethyl acetate extract over Sephadex LH-20 and Diaion HP-20 using a variety of solvent systems yielded compounds **1** and **2**.

Compound **1** (**1**), an off-white amorphous powder, $[\alpha]_D$ +75.9° (acetone), $C_{34}H_{26}O_{21} \cdot 7/2H_2O$, contained a hexahydroxydiphenoyl and two galloyl groups as revealed by analysis of the proton nulcear magnetic resonance (^{1}H-NMR) spectrum (δ 6.42 and 6.66, each 1H, s; δ 7.13 and 7.18, each 2H, s). On hydrolysis with 1 N H_2SO_4, **1** afforded gallic acid, ellagic acid and proto-quercitol,[1] while **1** quickly liberated gallic acid and a hydrolysate (**1a**) on addition of tannase. The ^{1}H-NMR spectrum (in pyridine-d_5 solution) of **1a** showed the presence of a hexahydroxydiphenoyl group (δ 7.04 and 7.20, each 1H, s), along with proto-quercitol signals, two of which were shifted considerably downfield (δ 6.40, 2H, m). These lowfield signals were shown not to be coupled with the C(6)-methylene signals (δ 2.7, 2H, m) by the spin-decoupling techniques (Fig. 1), indicating that these signals could be attributed to the C(2)–, C(3)– or C(4)-protons. Furthermore, three upfield multiplets (δ 4.66, 4.77 and 5.04) due to hydroxy-bearing methines were assigned to the C(1)-, C(2)- and C(5)-protons, respectively, on the basis of their half-width values and also the results of spin-decoupling experiments (Fig. 1). From these observations, the location of the hexahydroxydiphenoyl group was concluded to be at the C(3)- and C(4)-positions in the proto-quercitol moiety.

The ^{1}H-NMR spectrum of **1** revealed a triplet-like signal (δ 4.38, J=2 Hz) which was shifted upfield due to the lack of an acyl group. Irradiation at the frequency of the C(6)-methylene signals (δ 2.4) caused no change of this signal, and this finding coupled with the fact

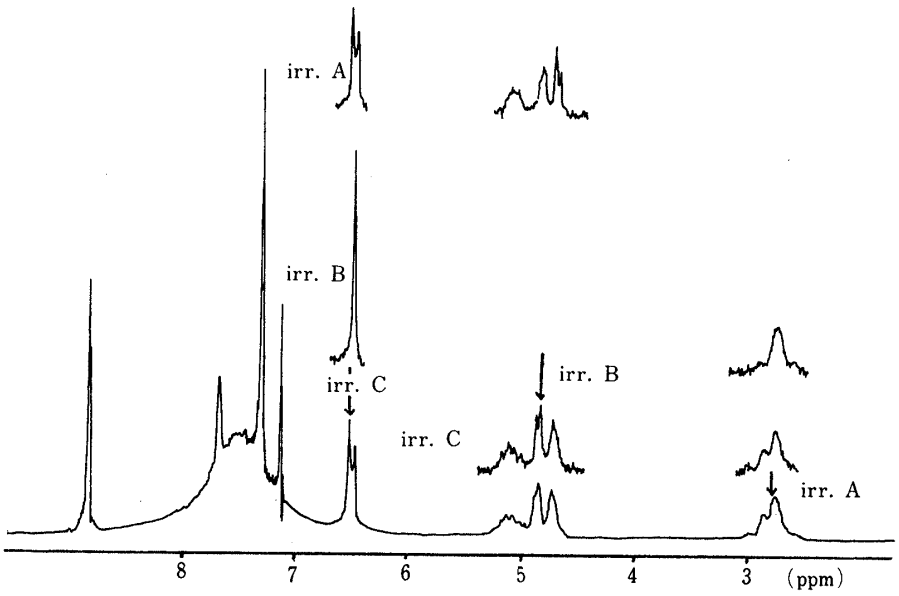
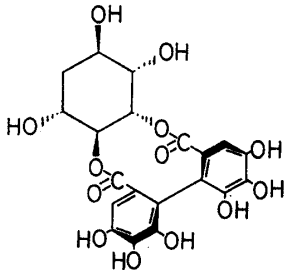
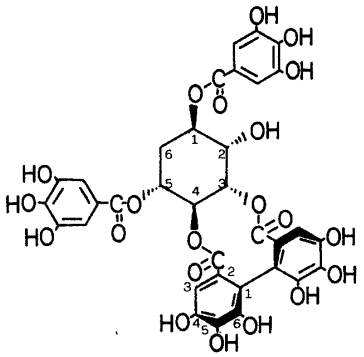


Fig. 1. ^{1}H-NMR Spectrum (Spin-decoupled) of Compound **1a** (in Pyridine-d_5)
"irr." indicates irradiation.



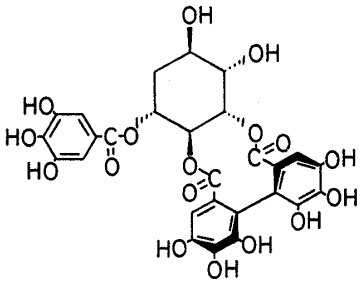
1a

Chart 1



1

Chart 2



2

Chart 3

that the hexahydroxydiphenoyl group occupied the C(3)- and C(4)-positions indicated that this upfield signal was due to the C(2)-proton. The chirality of the hexahydroxydiphenoyl group was determined to be in the *S*-series on the basis of the circular dichroism (CD) properties of **1a** ($[\theta]_{261}$ -2.89×10^4, $[\theta]_{284}$ $+9.63\times10^3$, $[\theta]_{314}$ -1.20×10^3), which were in good agreement with those obtained previously.[4-6] Based on the above-mentioned evidence, the structure of compound **1** was concluded to be 1,5-di-*O*-galloyl-3,4-(*S*)-hexahydroxydiphenoyl proto-quercitol.

Compound **2** (**2**), an off-white amorphous powder, $[\alpha]_D$ $+70.1°$ (MeOH), $C_{27}H_{22}O_{17}\cdot H_2O$, was shown by analysis of the ^{1}H-NMR features (δ 7.12, 2H, s; δ 6.64 and 6.36, each 1H, s) to be an ellagitannin possessing one galloyl group and one hexahydroxydiphenoyl group. Acid hydrolysis confirmed the presence of proto-quercitol, together with ellagic acid and gallic acid. On partial hydrolysis with tannase in the same manner as described above, **1** gave gallic acid and **1a**, indicating the location of the (*S*)-hexahydroxydiphenoyl group at the C(3)- and C(4)-positions. Since in the ^{1}H-NMR spectrum (in pyridine-d_5 solution) of **1**, the C(1)- and C(2)-methine protons appeared upfield as broad singlet-like signals (δ 4.66 and 4.77, respectively), these positions were free from acyl groups

and hence the galloyl group should be located at the C(5)-position. Accordingly, the structure of compound **2** was characterized as 3,4-hexahydroxydiphenoyl-5-*O*-galloyl proto-quercitol.

Compounds **1** and **2** are the first reported example of ellagitannins containing a proto-quercitol moiety.

Experimental

Details of the instruments and chromatographic conditions used throughout this work were the same as described in the previous paper[5] except in the following respects. For detection of a polyalcohol on thin-layer chromatography (TLC) plates, ammoniacal silver nitrate spray reagent was used. For high-performance liquid chromatography (HPLC) analysis, a Toyo Soda apparatus equipped with an SP 8700 solvent delivery system and a UV-8 model II spectrometer, and a TSK-410 column (4 mm i.d. × 300 mm) were used [mobile phase: CH_3CN–50 mM aqueous NaH_2PO_4 (3 : 17)]. For preparative-scale HPLC, a TSK-410 column (25 mm i.d. × 300 mm) connected with a Toyo Soda RT-8 differential refractometer was used.

Isolation——The aqueous layer,[3] obtained by partition of the aqueous acetone extract with ethyl acetate, was made acidic (pH 2) addition of citric acid, and the solution was extracted with ethyl acetate. The ethyl acetate-soluble portion thus obtained was subjected to Sephadex LH-20 chromatography with H_2O containing an increasing amount of MeOH (0—100%) to give four fractions; fr. I (9.3 g), II (18.8 g), III (7.9 g) and IV (4.0 g). Fr. II was chromatographed over Sephadex LH-20 with EtOH and Diaion HP-20 with a mixture of H_2O–MeOH (9 : 1) to yield a fraction containing compound **2**. A pure sample (15 mg) was obtained by preparative-scale HPLC [solvent: CH_3CN–50 mM aqueous NaH_2PO_4 (3 : 17)]. Fr. III was repeatedly chromatographed over Sephadex LH-20 with 60% aqueous MeOH and with a mixture of EtOH–H_2O (1 : 0—4 : 1) to afford compound **1** (42 mg).

Compound **1** (**1**): An off-white amorphous powder, $[\alpha]_D^{17}$ +75.9° (c=0.62, acetone), *Anal.* Calcd for $C_{34}H_{26}O_{21} \cdot 7/2H_2O$: C, 48.99; H, 3.99. Found: C, 49.07; H, 4.26. ^{1}H-NMR (acetone-d_6): 2.4 [2H, m, C(6)–H], 4.39 [1H, t, J=2 Hz, C(2)–H], 5.24—5.80 [4H, m, C(1)–, C(3)–, C(4)– and C(5)–H], 6.42, 6.66 (each 1H, s, HHDP[7]–H) 7.13, 7.18 (each 2H, s, galloyl H). Carbon-13 nuclear magnetic resonance (^{13}C-NMR) (acetone-d_6 + D_2O): 32.8 [C(6)], 68.7, 69.3, 71.8, 76.0, 76.4 [C(1), C(2), C(3), C(4) and C(5)], 107.2, 107.8 (HHDP–C(3) and C(3′)], 109.9 [galloyl C(2)], 114.4 [HHDP–C(1) and C(1′)], 120.5, 120.7 [galloyl C(1)], 126.5, 126.7 [HHDP–C(2) and C(2′)], 136.2, 136.3 [HHDP–C(5) and C(5′)], 139.6 [galloyl C(4)], 144.3, 145.2, 146.1 [HHDP–C(4), C(4′), C(6), C(6′) and galloyl C(3)], 165.9, 166.6, 169.4, 169.7 (–COO–).

Compound **2** (**2**): An off-white amorphous powder, $[\alpha]_D^{21}$ +70.1° (c=0.65, MeOH), *Anal.* Calcd for $C_{27}H_{22}O_{17} \cdot H_2O$: C, 50.95; H, 3.80. Found: C, 50.81; H, 4.26. ^{1}H-NMR (pyridine-d_5): 2.70 [2H, m, C(6)–H], 4.60 [1H, m, C(1)–H], 4.77 [1H, m, C(2)–H], 6.30—6.60 [3H, m, C(3)–, C(4)– and C(5)], 7.03, 7.09 (each 1H, s, HHDP–H), 7.79 (2H, s, galloyl H). ^{1}H-NMR (acetone-d_6 + D_2O): 2.20 [2H, m, C(6)–H], 4.10—4.30 [2H, m, C(1)– and C(2)–H], 5.36—5.66 [3H, m, C(3)–, C(4)– and C(5)–H], 6.36, 6.64 (each 1H, s, HHDP–H), 7.12 (2H, s, galloyl H).

Acidic Hydrolysis of 1 and 2——A solution of **1** or **2** in 1 N aqueous H_2SO_4 (3 mg/1 ml) was heated at 90 °C for 12 h, then cooled. The resulting precipitate was filtered off, and shown to be identical with ellagic acid by co-chromatography on a cellulose plate [solvent: *n*-BuOH–AcOH–H_2O (6 : 1 : 2) (Rf 0.36)]. The filtrate was neutralized with Amberlite IRA-400 (OH^- form), and subjected to TLC analysis: Rf 0.65 (gallic acid), 0.15 (proto-quercitol) [cellulose plate; *n*-BuOH–AcOH–H_2O (6 : 1 : 2)]; Rf 0.74 (gallic acid), 0.35 (proto-quercitol) [cellulose plate; *n*-BuOH–pyridine–H_2O (6 : 4 : 3)]; Rf 0.76 (gallic acid), 0.08 (proto-quercitol) [Silica gel plate; benzene–ethyl formate–formic acid (1 : 7 : 1)].

Enzymatic Hydrolysis of 1 with Tannase——A solution of **1** (30 mg) in H_2O (5 ml) was incubated with tannase at 37 °C for 2 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was treated with MeOH. The insoluble material was filtered off, and the filtrate, after removal of the solvent by evaporation, was subjected to Sephadex LH-20 chromatography. Elution with H_2O yielded gallic acid (5 mg) and **1a** (11 mg), an off-white amorphous powder, $[\alpha]_D^{19}$ +78.9° (MeOH), ^{1}H-NMR (pyridine-d_5): 2.7 [2H, m, C(6)–H], 4.66 [1H, m, C(1)–H], 4.77 [1H, m, C(2)–H], 5.04 [1H, m, C(5)–H], 6.40 [2H, m, C(3)– and C(4)–H], 7.04, 7.20 (each 1H, s, HHDP–H). CD (c = 8.31×10^{-5}, MeOH) $[\theta]^{25}$ (nm): -1.20×10^3 (314) (negative maximum), $+9.63 \times 10^3$ (284) (positive maximum), -2.89×10^4 (261) (negative maximum), 1.14×10^5 (233) (positive maximum).

Enzymatic Hydrolysis of 2 with Tannase——A solution of **2** (10 mg) in H_2O (2 ml) was incubated with tannase at 37 °C for 30 min. Work-up as before yielded gallic acid and a hydrolysate; the latter was shown to be identical with **1a** by comparisons of their physical data and ^{1}H-NMR spectra.

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References and Notes

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7) HHDP = hexahydroxydiphenoyl.