New γ-Pyrone Glucoside, and Dimeric Ellagitannins from *Gordonia axillaris*[1)]

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A new acylated γ-pyrone glucoside was isolated from the leaf extract of *Gordonia axillaris* (Theaceae), and was characterized as 3-*O*-(6′-*O*-galloyl)-β-D-glucopyranosylmaltol (7) based on two-dimensional NMR experiments and other spectroscopic analyses. 5-*O*-(6′-*O*-Galloyl)-β-D-glucopyranosylgentisic acid (6), and two dimeric hydrolyzable tannins [camelliin B (4) and schimawalin B (5)] were also isolated.

Keywords *Gordonia axillaris*; Theaceae; galloylated maltol glucoside; ellagitannin; camelliin B

Many theaceous plants are known to be rich in polyphenols and various types of tannins, such as galloylated catechin derivatives in green tea, and complex tannins and hydrolyzable tannins isolated from *Camellia* and *Schima* species.[2)] In a screening study of Epstein–Barr virus (EBV)-specific antigens in Chinese medicinal plants, the leaf extract of *Gordonia axillaris* (ROXB.) DIETR. exhibited a significant inhibitory effect on EBV-specified DNA polymerase.[3)] This finding prompted us to investigate the chemical constituents of this plant, resulting in the isolation of the active dimeric hydrolyzable tannins, camelliin B (**4**) and schimawalin B (**5**),[4)] and a new glucoside (**7**). This paper deals with the isolation and structure elucidation of the new glucoside.

The dried leaf of *G. axillaris* was homogenized in aqueous acetone and the concentrated filtrate was extracted with ether, ethyl acetate and *n*-BuOH, successively. The ethyl acetate extract was subjected to repeated column chromatography over Diaion HP-20, MCI-gel CHP 20P and/or TSK HW-40 to give gallic acid (**1**), ellagic acid (**2**), valoneic acid dilactone (**3**),[5)] 5-*O*-(6′-*O*-galloyl)-β-D-glucopyranosylgentisic acid (**6**)[6)] and a new glucoside (**7**). Two dimeric ellagitannins were also obtained from the *n*-BuOH extract and the water-soluble portion that remained after extraction with *n*-BuOH, respectively, and were identified as camelliin B (**4**) and schimawalin B (**5**).[2b)]

The new glucoside (**7**), an off-white amorphous powder, showed a dark blue coloration with the $FeCl_3$ reagent. The fast-atom bombardment mass spectrum (FAB-MS) of **7** exhibited prominent peaks at *m/z* 441 and 463, attributable to $(M+H)^+$ and $(M+Na)^+$, respectively. The molecular formula $C_{19}H_{20}O_{12}$ of **7** was assigned by

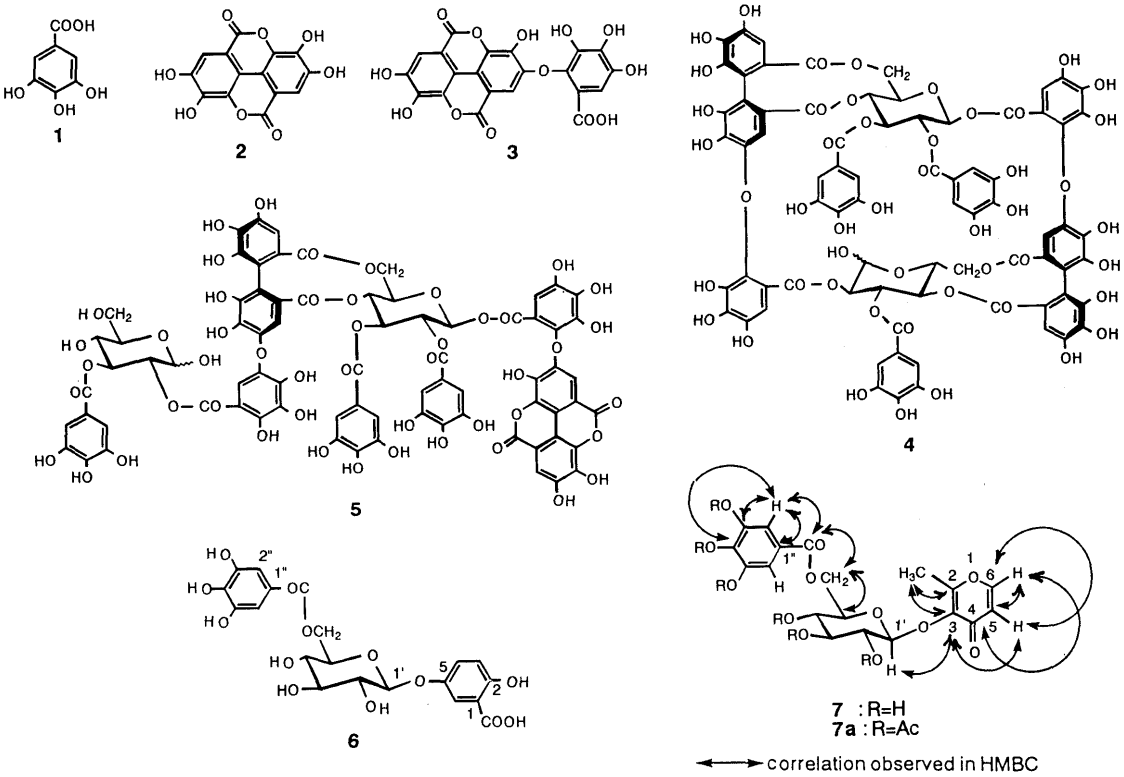

Chart 1

high-resolution mass spectroscopy (HRMS). The presence of a galloyl group in the molecule was indicated by a 2H singlet at δ 6.90 in the ^{1}H-NMR spectrum (dimethyl sulfoxide-d_6 (DMSO-d_6)), and by the characteristic carbon resonances [δ 108.69 (2C), 119.81, 138.35, 145.48 (2C) and 165.87] in the ^{13}C-NMR spectrum. The chemical shifts of six aliphatic carbon signals in the region of δ 63—104 ppm were closely similar to those of the glucose carbon signals of **6** (see Experimental), implying the presence of a β-glucoside residue bearing a galloyl group at O-6′. This location of the galloyl group on the glucose core was substantiated by the low-field shifts of C-6 methylene proton signals [δ 4.41 (dd, J=2, 12 Hz) and 4.25 (dd, J=6, 12 Hz)] in the ^{1}H-NMR spectrum. Three secondary hydroxyl proton signals in addition to three phenolic hydroxyl protons of the galloyl group, which are exchangeable with D_2O, were observed at δ 5.56 (d, J=3 Hz), 5.27 (d, J=5 Hz), 5.20 (d, J=5 Hz), 9.25 (2H, s) and 8.95 (s). These data, together with the formation of a hexa-acetate (**7a**) upon acetylation of **7** in a usual manner, indicated that the aglycone moiety ($C_6H_5O_2$) in **7** has no free hydroxyl group.

The carbon signals due to the aglycone moiety of **7** were associated with a ketonic carbon (δ 174.40), four sp^2 carbons (δ 161.75, 155.85, 141.63 and 116.20) and a methyl carbon (δ 15.14). These data, together with the appearance of AB-type doublets (J=5.5 Hz) at δ 6.38 and 8.01 and a vinyl methyl signal at δ 2.19 in the ^{1}H-NMR spectrum, indicated that the aglycone is maltol.[7] The gross structure of **7** was confirmed by the ^{1}H–^{13}C shift correlation spectrum (COSY), and by the result of HMBC measurement (J_{CH}=7.5 Hz). Based on the above data, compound **7** was characterized as 3-*O*-(6′-*O*-galloyl)-β-D-glucopyranosylmaltol [3-(6′-*O*-galloyl)-β-D-glucopyranosyloxy-2-methyl-4*H*-pyran-4-one].

This is the first report of the isolation of an acylated glucoside of maltol from one of the theaceous plants, although maltol glucoside occurs in plant species of Pinaceae,[8] Cercidiphyllaceae[9] and Araliaceae,[10] and its *p*-coumarate was found in *Evodiopanax innovans* (Araliaceae).[11]

Experimental

^{1}H-NMR (500 MHz) and ^{13}C-NMR (126 MHz) spectra were recorded on a Varian VXR 500 instrument and chemical shifts are given in δ (ppm) values relative to tetramethylsilane. FAB-MS were measured on a VG-70SE mass spectrometer. Column chromatography was performed on Diaion HP-20, MCI-gel CHP-20P (Mitsubishi Kasei Co. Ltd.) and TSK HW-40 (fine) (Merck). Solvents were evaporated under reduced pressure below 40 °C.

Isolation of Polyphenols The dried leaves (1.1 kg) of *G. axillaris*, collected in Taipei county, Taiwan, R. O. C., in September, were homogenized in 70% acetone and the combined homogenates were filtered. After evaporation of acetone, the aqueous concentrate was extracted successively with ether, EtOAc and *n*-BuOH saturated with water to yield the ether extract (30 g), EtOAc extract (43 g), *n*-BuOH extract (240 g) and the residue from the mother liquor (aqueous residue: 152 g). A part (27 g) of the EtOAc extract was chromatographed over a Diaion HP-20 column (5.6 cm i.d. × 56 cm) with H_2O containing increasing amounts of MeOH. The 40% MeOH and 80% MeOH eluates gave valoneic acid dilactone (**3**) (128.2 mg) and ellagic acid (**2**) (47.7 mg), respectively. The eluate with H_2O was further subjected to column chromatography over TSK HW-40 (3.5 cm i.d. × 27 cm) with aqueous MeOH to yield 3-*O*-(6′-*O*-galloyl)-β-D-glucopyranosylmaltol (**7**) (31 mg) from the 5% MeOH eluate, gallic acid (**1**) (14.7 mg) from the 15% MeOH eluate and 5-*O*-(6′-*O*-galloyl)-β-D-glucopyranosylgentisic acid (**6**) (12.5 mg) from the 20% MeOH eluate.

A part (40 g) of the *n*-BuOH extract was chromatographed on Dia-ion HP-20 (5 cm i.d. × 62 cm) with H_2O containing increasing amounts of MeOH in a stepwise gradient mode. The 40% MeOH eluate was further chromatographed over TSK HW-40 (coarse) (3.8 cm i.d. × 27 cm) with a solvent system of 70% EtOH–70% acetone. The eluate with 70% EtOH–70% acetone (8 : 2) was finally purified by column chromatography over MCI-gel CHP-20P (2.4 cm i.d. × 18 cm) with H_2O–MeOH to afford camelliin B (**4**) (65 mg).

A part (35 g) of the aqueous residue was similarly fractionated by column chromatography over Diaion HP-20 (5 cm i.d. × 62 cm) with H_2O containing increasing amounts of MeOH in a stepwise gradient mode. Repeated purification of the 50—70% MeOH eluate by similar column chromatography over TSK HW-40 and MCI-gel CHP 20P gave schimawalin B (**5**) (55.8 mg).

Compounds **1**—**6** were identified by direct comparisons of their spectral data with those of authentic samples.

3-*O*-(6′-*O*-Galloyl)-β-D-glucopyranosylmaltol (7) An off-white amorphous powder. FAB-MS *m/z*: 441 $(M+H)^+$, 463 $(M+Na)^+$. HRMS *m/z*: 441.1119. Calcd for $C_{19}H_{21}O_{12}$: *m/z* 441.1033. ^{1}H-NMR (DMSO-d_6) δ: 9.25 (2H, s, OH), 8.95 (1H, s, OH), 8.01 (1H, d, J=5.5 Hz, H-5), 6.90 (2H, s, galloyl), 6.38 (1H, d, J=5.5 Hz, H-6), 5.56 (1H, d, J=3 Hz, OH), 5.27 (1H, d, J=5 Hz, OH), 5.20 (1H, d, J=5 Hz, OH), 4.76 (1H, d, J=7.5 Hz, H-1′), 4.41 (1H, dd, J=2, 12 Hz, H-6′), 4.25 (dd, J=6, 12 Hz, H-6′), 3.5—3.1 (overlapped by H_2O signal, H-2′–H-5′), 2.19 (3H, s, Me). ^{13}C-NMR (DMSO-d_6) δ: 15.14 (Me), 63.23 (C-6′), 69.86 (C-4′), 73.86 (C-2′), 74.28 (C-5′), 75.98 (C-3′), 103.52 (C-1′), 108.69 (C-2″ and C-6″), 116.20 (C-5), 119.81 (C-1″), 138.35 (C-4″), 141.63 (C-3), 145.48 (C-3″ and C-5″), 155.85 (C-6), 161.75 (C-2), 165.87 (ester carbonyl), 174.40 (C-4).

Acetylation of 7 Compound **7** (2 mg) was acetylated overnight with Ac_2O and pyridine (each 0.1 ml) at room temperature. The reaction mixture was evaporated *in vacuo* and purified by preparative TLC (Kieselgel PF_{254}; $CHCl_3$–acetone 6 : 1) to give the hexa-acetate (**7a**) (1.2 mg). Colorless syrup, ^{1}H-NMR ($CDCl_3$) δ: 7.69 (2H, s, galloyl), 7.47 (1H, d, J=5.5 Hz, H-5), 6.15 (1H, d, J=5.5 Hz, H-6), 5.44 (1H, d, J=8 Hz, H-1′), 5.30 (1H, t, J=10 Hz, H-3′), 5.16 (1H, dd, J=8, 10 Hz, H-2′), 5.09 (1H, t, J=10 Hz, H-4′), 4.54 (1H, dd, J=6, 12 Hz, H-6′), 4.23 (1H, dd, J=3, 12 Hz, H-6′), 3.77 (1H, ddd, J=3, 6, 10 Hz, H- 5′), 2.20 (6H, s, OAc), 2.29, 2.10, 2.02, 2.01 (each 3H, s, OAc).

References and Notes

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