Studies on the Constituents of *Lonicera* Species. VII.[1] Three New Polyhydric Alcohol Glycosides from the Leaves of *Lonicera gracilipes* var. *glandulosa* MAXIM.

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Three new polyhydric alcohol glycosides (1—3) were isolated from the leaves of *Lonicera gracilipes* var. *glandulosa* MAXIM. (Caprifoliaceae). Their structures were determined to be erythritol-1-*O*-(6-*O*-*trans*-caffeoyl)-β-D-glucopyranoside (1), 1,2,3,4-tetrahydroxy-2-methylbutane-4-*O*-(6-*O*-*trans*-caffeoyl)-β-D-glucopyranoside (2) and arabitol-5-*O*-(6-*O*-*trans*-caffeoyl)-β-D-glucopyranoside (3) on the basis of chemical and spectral data.

Key words *Lonicera gracilipes* var. *glandulosa*; Caprifoliaceae; polyhydric alcohol glycoside; NMR; caffeate

We recently reported the isolation and the structure elucidation of a new coumarin glycoside, aesculetin 6-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside[2] and three methyl dicaffeoyl quinates[1] from the leaves of *Lonicera gracilipes* var. *glandulosa* MAXIM. (Caprifoliaceae). In the course of further studies on the constituents of this plant, three new polyhydric alcohol glycosides have been isolated. This paper deals with the isolation and the structure elucidation of these three new compounds, erythritol-1-*O*-(6-*O*-*trans*-caffeoyl)-β-D-glucopyranoside (**1**), 1,2,3,4-tetrahydroxy-2-methylbutane-4-*O*-(6-*O*-*trans*-caffeoyl)-β-D-glucopyranoside (**2**) and arabitol-5-*O*-(6-*O*-*trans*-caffeoyl)-β-D-glucopyranoside (**3**).

The isolation and purification of the new compounds are described in detail in the Experimental section.

Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{30}$ −15.2°. Its molecular formula, $C_{19}H_{26}O_{12}$, was confirmed by FAB-MS [$(M+H)^+$ at *m/z* 447, $(M+Na)^+$ at *m/z* 469] and ^{13}C-NMR spectrum. The IR spectrum suggested the presence of hydroxyl groups (3480 cm^{-1}), a carbonyl group of α,β-unsaturated ester (1695 cm^{-1}), a double bond (1630 cm^{-1}) and aromatic rings (1600, 1524 cm^{-1}). The ^{1}H-NMR spectrum of **1** exhibited the presence of a *trans* caffeoyl moiety [an ABX signal due to aromatic protons, δ 6.78 (d, *J*=8.2 Hz, H-5″), 6.96 (dd, *J*=1.7, 8.2 Hz, H-6″), 7.05 (d, *J*=1.7 Hz, H-2″) and a pair of *trans*-olefinic signals, δ 6.29 (d, *J*=15.8 Hz, H-8″), 7.57 (d, *J*=15.8 Hz, H-7″)] and sugar moieties. In the ^{13}C-NMR spectra, the signals of a *trans* caffeoyl moiety and glucosyl moiety were confirmed. The aglycone part was characterized as 1,2,3,4-tetrahydroxy butane with the aid of distortionless enhancement by polarization transfer (DEPT), ^{13}C–^{1}H shift correlation spectroscopy (^{13}C–^{1}H COSY) and ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) spectrum. In the ^{1}H-detected multiple-bond connectivity (HMBC) spectrum, the correlation was observed between H-1 of aglycone part and C-1′ of glucosyl moiety, so that the glucosyl moiety is attached to the C-1 hydroxyl group. The correlation between H-6′ of glucosyl moiety and C-9″ of caffeoyl moiety suggested the location of the caffeoyl moiety at the C-6″ hydroxyl group.

From the $J_{H2,H3}$ (14.2 Hz) coupling constant resulting from the Homonuclear *J*-resolved spectrum experiments of **1**, it was deduced that the aglycone part was the erythritol. Subjected to acidic hydrolysis, compound **1** afforded erythritol, D-glucose and caffeic acid. The erythritol and D-glucose were identified by gas chromatography (GC) as their trimethylsilyl ether derivatives, and caffeic acid was identified by thin layer chromatograpy (TLC). On the basis of the above evidence, the structure of **1** was determined to be erythritol-1-*O*-(6-*O*-*trans*-caffeoyl)-β-D-glucopyranoside.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D^{31}$ −8.0°, and the molecular formula, $C_{19}H_{26}O_{12}$, was confirmed by FAB-MS [$(M+H)^+$ at *m/z* 461, $(M+Na)^+$ at *m/z* 483] and ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum of **2** exhibited the presence of a *trans* caffeoyl moiety, glucosyl moiety and a methyl proton at δ 1.10 (s). The ^{13}C-NMR and the DEPT spectrum showed the signals of a *trans* caffeoyl moiety, glucosyl moiety, a tertiary carbon, two secondary carbons and a methyl carbon. The aglycone part was characterized as 1,2,3,4-tetrahydroxy-2-methylbutane with the aid of ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectrum. In the HMBC spectrum, the correlation was observed between H-1′ of glucosyl moiety and C-4 of the aglycone part, so that the glucosyl moiety is attached to the C-4 hydroxyl group.

The configuration of the aglycone part of **2** was inferred from the nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum where correlations of the following signals were observed: H-1 and H-3; H-3 and

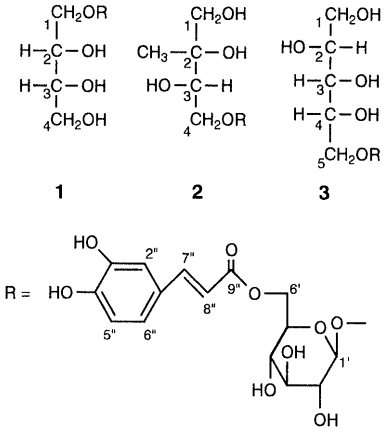

Chart 1

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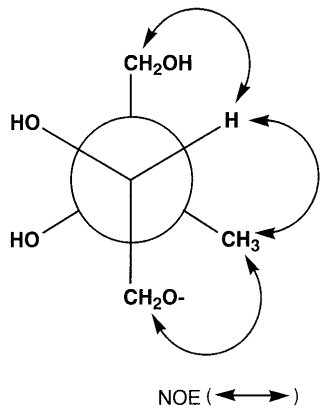

Chart 2. Conformation (2*S*,3*S* form) of Aglycone Part of **2**

2-methyl protons; 2-methyl protons and H-4. These findings demonstrate that the aglycone part is *threo* form (2*R*,3*R* or 2*S*,3*S*, Chart 2).[3] On the basis of the above evidence, the structure of **2** was elucidated as 1,2,3,4-tetrahydroxy-2-methylbutane-4-*O*-(6-*O*-*trans*-caffeoyl)-*β*-D-glucopyranoside.

Compound **3** was obtained as an amorphous powder, $[\alpha]_D^{30}$ −11.1°, and the molecular formula, $C_{20}H_{28}O_{13}$, was confirmed by FAB-MS [$(M+H)^+$ at *m/z* 477, $(M+Na)^+$ at *m/z* 499] and ^{13}C-NMR spectrum. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of a *trans* caffeoyl moiety and glucosyl moiety. In DEPT spectrum, the signals were further confirmed to be three tertiary carbons and two secondary carbons. From the ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectrum data, the aglycone part was characterized as 1,2,3,4,5-hydroxyl pentane.

In order to assign the chemical shift and coupling constant of aglycone-protons, we carried out the Homonuclear *J*-resolved spectrum experiments and the results are described in the Experimental section. Acid hydrolysis of **3** gave an aglycone, which was identical with arabitol by comparing ^{1}H-NMR data and GC with the authentic sample, and D-glucose was determined by GC. In the HMBC spectrum of **3**, the correlation was observed between H-1′ of glucosyl moiety and C-5[4] of arabitol moiety, so that the glucosyl moiety is attached to the C-5 hydroxyl group. On the basis of the above evidence, the structure of **3** was elucidated as arabitol-5-*O*-(6-*O*-*trans*-caffeoyl)-*β*-D-glucopyranoside.

The relative configuration of these three compounds was proved, but the absolute configuration could not be determined because the amount of the sample was too small. These three compounds are new constituents from a natural source.

Experimental

Optical rotations were determined with a JASCO DIP-360 digital polarimeter. Infrared (IR) spectra were recorded with a Perkin-Elmer 1725X FT-IR instrument and UV spectra with a Beckman DU-64 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JNM-GSX 400 (400 and 100 MHz, respectively) spectrometer. Chemical shifts are given on a *δ* (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; dd, double doublet; ddd, double double doublet; m, multiplet). MS were recorded on a JEOL JMS-DX 300 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230—400 mesh) and Sephadex LH-20 (Pharmacia Fine Chemicals).TLC was carried out with precoated Kieselgel 60 plates (Merck) and detection was achieved by spraying 50% H_2SO_4 followed by heating. Preparative HPLC was carried out on a Tosoh HPLC system (pump, CCPM; detector, UV-8000) using TSK gel ODS-120A (Tosoh) column. Illumination intensity was measured with a ANA 500S (SIBATA). GC: column; G-column(Chemicals Inspection and Testing, Japan), column length; 40 m, column i.d.; 1.2 mm, liquid phase; G-205, film thickness; 5 *μ*, carrier gas; N_2, flow rate; 20 ml/min (0.6 kg/cm^2), detector; flame ionization detector (FID).

Table 1. ^{13}C-NMR Chemical Shifts (100 MHz, CD_3OD)

Carbon	1	2	3
1	73.0	72.5	73.5
2	72.5	74.8	71.7[a]
3	73.5	74.5	71.9
4	64.6	68.5	71.6[a]
5			64.8
CH_3		19.7	
1′	105.0	105.0	105.0
2′	75.2	75.3	75.2
3′	77.7	77.8	77.7
4′	71.7	71.6	71.6[a]
5′	75.6	75.6	75.5
6′	64.6	64.6	64.6
1″	127.7	127.7	127.7
2″	115.2	115.2	115.2
3″	149.7	149.7	149.7
4″	146.8	146.9	146.8
5″	116.5	116.5	116.5
6″	123.1	123.1	123.1
7″	147.3	147.3	147.3
8″	114.8	114.8	114.8
9″	169.2	169.2	169.2

a) Assignments may be interchanged.

Isolation Fresh leaves of *L. gracilipes* var. *glandulosa* (0.2 kg) collected in October 1990, in Sendai, Japan, were extracted with MeOH at room temperature for two months. The MeOH extract was concentrated under reduced pressure and the residue was suspended in a small excess of water. This suspension was successively extracted with $CHCl_3$, Et_2O, AcOEt and *n*-BuOH. The *n*-BuOH-portion fraction was concentrated under reduced pressure to produce a residue (15.2 g). This residue was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1) and a Sephadex LH-20 column (MeOH–H_2O, 1 : 1), and then subjected to HPLC (MeOH–H_2O, 3 : 7) to give compounds **1** (5.3 mg), **2** (3.2mg) and **3** (2.1 mg).

Erythritol-1-*O*-(6-*O*-*trans*-caffeoyl)-*β*-D-glucopyranoside (1) An amorphous powder, $[\alpha]_D^{30}$ −15.2° (*c*=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3480, 1695, 1630, 1600, 1524. UV λ_{max}^{MeOH} nm (log *ε*): 215 (4.15), 243 (3.99), 300 sh (4.07), 328 (4.19). FAB-MS *m/z*: 447 $(M+H)^+$, 469 $(M+Na)^+$. ^{1}H-NMR (400 MHz, CD_3OD) *δ*: 3.59 (1H, ddd, *J*=14.2, 6.5, 2.7 Hz, 3-H), 3.63 (1H, dd, *J*=11.0, 6.5 Hz, 4-H_A), 3.67 (1H, dd, *J*=9.6, 6.2 Hz, 1-H_B), 3.68 (1H, dd, *J*=11.0, 2.7 Hz, 4-H_B), 3.74 (1H, ddd, *J*=14.2, 6.2, 2.5 Hz, 2-H), 4.09 (1H, dd, *J*=9.6, 2.5 Hz, 1-H_A), 4.26 (1H, dd, *J*=12.0, 6.2 Hz, 6′-H_A), 4.35 (1H, d, *J*=7.9 Hz, 1′-H), 4.51 (1H, dd, *J*=12.0, 1.7 Hz, 6′-H_B), 6.29(1H, d, *J*=15.8 Hz, 8″-H), 6.78 (1H, d, *J*=8.2 Hz, 5″-H), 6.96 (1H, dd, *J*=8.2, 1.7 Hz, 6″-H), 7.05 (1H, d, *J*=1.7 Hz, 2″-H), 7.57 (1H, d, *J*=15.8 Hz, 7″-H). ^{13}C-NMR (100 MHz, CD_3OD): Table 1.

Acid Hydrolysis of 1 Compound **1** (2.0 mg) was refluxed with 5% HCl aq. (1 ml) for 1 h in a water bath. The solution was extracted with AcOEt (3 ml × 3) to give caffeic acid. The H_2O layer was neutralized with Ag_2O, filtered and the excess Ag^+ of filtrate was removed with H_2S. The solution was concentrated *in vacuo* and dried to give monosaccharide. The composition was determined by GC analysis to be erythritol and D-glucose as their TMSi derivatives. Erythritol, t_R (column temp.: 180 °C) 23.4 min (standard; erythritol, t_R 23.4 min, threitol, t_R 22.8 min), D-glucose, t_R (column temp.: 230 °C) 21.7, 27.3 min (standard; D-glucose, t_R 21.7, 27.3 min).

1,2,3,4-Tetrahydroxy-2-methylbutane-4-*O*-(6-*O*-*trans*-caffeoyl)-*β*-D-glucopyranoside (2) An amorphous powder, $[\alpha]_D^{31}$ −8.0° (*c*=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3480, 1695, 1630, 1600, 1524. UV λ_{max}^{MeOH} nm

(log ε): 213 (4.10), 240 (3.92), 300 sh (4.00), 327 (4.10). FAB-MS *m/z*: 461 $(M+H)^+$, 483 $(M+Na)^+$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 1.10 (3H, s, CH_3), 3.42 (1H, d, J=11.3 Hz, 1-H_A), 3.52 (1H, d, J=11.3 Hz, 1-H_B), 3.57 (1H, dd, J=10.2, 8.6 Hz, 4-H_A), 3.80 (1H, dd, J=8.6, 2.6 Hz, 3-H), 4.18 (1H, dd, J=10.2, 2.6 Hz, 4-H_B), 4.31 (1H, dd, J=11.8, 5.6 Hz, 6′-H_A), 4.35 (1H, d, J=7.6 Hz, 1′-H), 4.50 (1H, dd, J=11.8, 1.9 Hz, 6′-H_B), 6.29 (1H, d, J=15.8 Hz, 8″-H), 6.77 (1H, d, J=8.2 Hz, 5″-H), 6.95 (1H, dd, J=8.2, 1.9 Hz, 6″-H), 7.04 (1H, d, J=1.9 Hz, 2″-H), 7.57 (1H, d, J=8.2, 1.9 Hz, 7″-H). ^{13}C-NMR (100 MHz, CD_3OD) δ: Table 1.

Arabitol-5-*O*-(6-*O*-*trans*-caffeoyl)-β-D-glucopyranoside (3) An amorphous powder, $[\alpha]_D^{30}$ −11.1° (*c*=0.2, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3480, 1695, 1630, 1600, 1524. UV λ_{max}^{MeOH} nm (log ε): 213 (4.16), 240 (3.97), 300 (4.04), 327 (4.14). FAB-MS *m/z*: 477 $(M+H)^+$, 499 $(M+Na)^+$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 3.55 (1H, dd, J=8.6, 2.0 Hz, 3-H), 3.61 (2H, dd, J=6.3, 6.3 Hz, 1-H_2), 3.71 (1H, dd, J=10.2, 6.2 Hz, 5-H_A), 3.82 (1H, ddd, J=8.6, 6.2, 2.3 Hz, 4-H), 3.89 (1H, td, J=6.3, 2.0 Hz, 2-H), 4.13 (1H, dd, J=10.2, 2.3 Hz, 5-H_B), 4.30 (1H, dd, J=12.0, 5.6 Hz, 6′-H_A), 4.35 (1H, d, J=7.6 Hz, 1′-H), 4.52 (1H, dd, J=12.0, 1.9 Hz, 6′-H_B), 6.29 (1H, d, J=15.8 Hz, 8″-H), 6.77 (1H, d, J=8.2 Hz, 5″-H), 6.96 (1H, dd, J=8.2, 1.9 Hz, 6″-H), 7.05 (1H, d, J=1.9 Hz, 2″-H), 7.57 (1H, d, J=15.8 Hz, 7″-H). ^{13}C-NMR (100 MHz, CD_3OD) δ: Table 1.

Acid Hydrolysis of 3 Compound **3** (1.5 mg) in 5% HCl aq. (1 ml) was refluxed for 1 h in a water bath and then treated in the same manner as compound **1**. The monosaccharides were determined by GC analysis to be arabitol and D-glucose as their TMSi derivatives. Arabitol, t_R (column temp.: 210 °C) 18.2 min (standard; arabitol, t_R 18.2 min, xylitol, t_R 17.4 min), D-glucose, t_R (column temp.: 230 °C) 21.7, 27.3 min (standard; D-glucose, t_R 21.7, 27.3 min).

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