A New Steroidal Glycoside and a New Phenyl Glycoside from a Ripe Cherry Tomato

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A new steroidal glycoside and a new phenyl glycoside have been isolated from a ripe cherry tomato [*Lycopersicon esculentum* var. *cerasiforme* (DUNAL) ALEF., Solanaceae] along with two known steroidal alkaloid glycosides, esculeoisides A and B, and five aromatic compounds, zizibeoside I, benzyl alcohol β-gentiobioside, rutin, methyl caffeate, and phenylalanine. Their chemical structures were determined on the basis of spectroscopic data as well as chemical evidence.

Key words *Lycopersicon esculentum*; cherry tomato; Solanaceae; steroidal glycoside; phenyl glycoside

In previous papers,[1—4] we reported the isolation and structural elucidation of a steroidal alkaloid glycoside, esculeoside A, from a ripe pink color-type tomato (*Lycopersicon esculentum*, Momotaro, Solanaceae), a solanocapsine-type glycoside, esculeoside B, from a ripe red color-type tomato (*Lycopersicon esculentum*, Italian San Marzano), esculeosides A, B, C, and D and lycoperoside G from a ripe cherry tomato [*Lycopersicon esculentum* var. *cerasiforme* (DUNAL) ALEF.], and a pregnane glycoside, tomato-pregnane, from an overripe cherry tomato. As part of our ongoing study on the chemical constituents of tomatoes, the present study deals with the isolation and structural characterization of a new steroidal glycoside and a new phenyl glycoside along with two known steroidal alkaloid glycosides and five known aromatic compounds from ripe cherry tomatoes.

A ripe cherry tomato was crushed and extracted with MeOH. The MeOH extract was successively subjected to Diaion HP-20, Sephadex LH-20, Chromatorex ODS, and silica gel column chromatography, as well as HPLC on C18, C8, and polyamine to yield three steroidal glycosides (**1**, **3**, **4**) and six aromatic compounds (**2**, **5**—**9**).

Compounds **3**—**9** were identified as esculeoside A (**3**),[1,2] esculeoside B (**4**),[2] zizibeoside I (**5**),[5] benzyl alcohol β-gentiobioside (**6**),[6] rutin (**7**), methyl caffeate (**8**), and phenylalanine (**9**), respectively, based on the comparison of their physical and spectral data with authentic or previously reported samples (Fig. 1).

Compound **1** was obtained as an amorphous powder, and its positive FAB-MS exhibited an $[M+Na]^+$ ion peak at *m*/*z* 1367. The molecular formula of **1** was determined to be $C_{61}H_{100}O_{32}$ by using high-resolution (HR)-positive FAB-MS. In the ^{1}H-NMR spectrum of **1**, signals due to two tertiary methyl groups (δ 0.70, 0.66), two secondary methyl groups [δ 1.10 (br d, J=5.0 Hz), 1.06 (d, J=6.0 Hz)], and six anomeric protons [δ 5.57 (d, J=8.0 Hz), 5.19 (d, J=8.0 Hz), 5.16 (d, J=8.0 Hz), 4.94 (d, J=6.0 Hz), 4.93 (d, J=7.5 Hz), 4.77 (d, J=8.0 Hz)] were observed. The ^{13}C-NMR spectrum of **1** exhibited 61 carbon signals including two carbonyl carbons (δ 218.5, 214.1) and six anomeric carbons (δ 105.0, 104.6, 104.6, 104.5, 104.3, 102.4). From these data, **1** was concluded to be a steroidal hexaglycoside. Acidic hydrolysis of **1** yielded D-glucose, D-galactose, D-xylose, and L-arabinose, which were identified by using optical rotation chiral detection in the HPLC analysis, along with several unidentified artificial aglycones. A detailed analysis of the ^{1}H- and ^{13}C-NMR signals of **1** using the ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and total correlation spectroscopy (TOCSY) indicated that the planar structure of **1** was a 3,26-bisdesmoside of 3,26-dihydroxy cholestan-16,22-dione, as shown in Fig. 2. Further, the ^{13}C-NMR data of C-1—C-11 and C-19 of the aglycone (Ag) moiety and sugar chain attached to C-3 of Ag were superimposable on those of **3**[2]; on the other hand, those of C-12—C-18 and C-20—C-27 of the Ag moiety were quite similar to those of anguivioside XV (**10**).[7] The remaining sugar chain, which was attached to C-26, was determined to be an α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl group based on the following evidence. The ^{13}C-NMR spectrum of **1** exhibited signals due to a terminal α-L-arabinopyranosyl group (δ 105.0, 72.0, 74.0, 68.8, 66.3)[8] and a β-D-glucopyranosyl group (δ 104.5, 74.8, 78.0, 71.0, 76.7, 69.4), which indicated the glycosylation shifts[8,9] at C-5 and C-6 with magnitudes −2.0 ppm and +6.5 ppm, respectively, as compared to those of **10**. Further, the values of the coupling constants of the anomeric proton signals confirmed the mode of glycosidic linkages of the glucopyranosyl group and the arbinopyranosyl group to be β and α, respectively. From these data, the structure of **1** was determined to be 3-*O*-β-lycotetraosyl 3β,26-dihydroxy cholestan-16,22-dione 26-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **2** was obtained as an amorphous powder. The positive FAB-MS of **2** exhibited an $[M+Na]^+$ ion peak at *m*/*z* 457, and the molecular formula of **2** was determined to be $C_{18}H_{26}O_{12}$ by using HR-positive FAB-MS. The ^{1}H-NMR spectrum of **2** exhibited signals due to four aromatic protons for an AA′BB′ pattern [δ 7.46 (d, J=7.0 Hz), 7.14 (d, J=7.0 Hz)] and two anomeric protons [δ 5.40 (d, J=8.0 Hz), 5.36 (d, J=7.5 Hz)]. The ^{13}C-NMR spectrum of **2** was similar to that of **5**, except for the presence of signals due to a 4-hydroxyphenyl group and the absence of signals due to a benzyl group. On acidic hydrolysis, **2** yielded hydroquinone and

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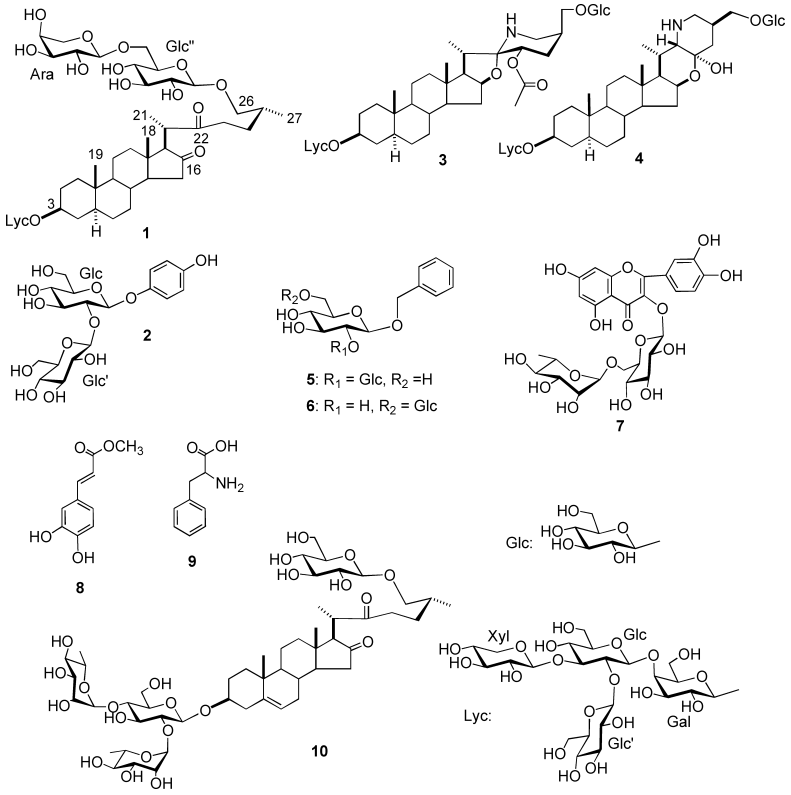

Fig. 1. Structures of **1**—**10**

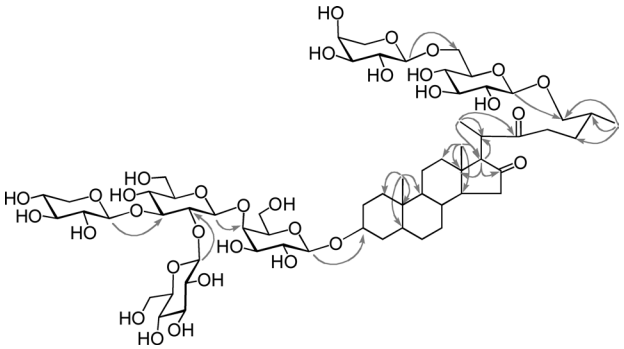

Fig. 2. ^{1}H–^{13}C Long-Range Correlations Observed for **1** in the HMBC Spectrum (in Pyridine-d_5, 500 MHz)

D-glucose, and the β-glycosidic linkage of the glucose units was confirmed to be based on the coupling constants of the anomeric protons. Consequently, the structure of **2** was concluded to be 4-hydroxyphenyl β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Table 1. ^{13}C-NMR Data for **1** (in Pyridine-d_5, 125 MHz)

Ag-1	36.8	Ag-19	12.2	Gal-1	102.4	Xyl-1	104.6
Ag-2	29.7	Ag-20	43.8	Gal-2	72.8	Xyl-2	74.7[a)]
Ag-3	77.2	Ag-21	15.6	Gal-3	75.2[a)]	Xyl-3	78.1
Ag-4	34.7	Ag-22	214.1	Gal-4	79.6	Xyl-4	70.5
Ag-5	44.6	Ag-23	37.4	Gal-5	75.2[a)]	Xyl-5	67.0
Ag-6	28.7	Ag-24	27.6	Gal-6	60.7	Glc″-1	104.5
Ag-7	32.1	Ag-25	33.4	Glc-1	104.6	Glc″-2	74.8
Ag-8	34.4	Ag-26	75.2	Glc-2	80.8	Glc″-3	78.0
Ag-9	54.0	Ag-27	17.2	Glc-3	87.0	Glc″-4	71.0
Ag-10	35.7			Glc-4	70.1	Glc″-5	76.7
Ag-11	20.9			Glc-5	77.5	Glc″-6	69.4
Ag-12	38.9			Glc-6	62.6	Ara-1	105.0
Ag-13	42.1			Glc′-1	104.3	Ara-2	72.0
Ag-14	51.0			Glc′-2	75.7	Ara-3	74.0
Ag-15	39.7			Glc′-3	78.2	Ara-4	68.8
Ag-16	218.5			Glc′-4	71.5	Ara-5	66.3
Ag-17	66.7			Glc′-5	77.8		
Ag-18	13.1			Glc′-6	62.2		

δ in ppm from TMS. *a*) Assignments may be interchangeable. Ag, aglycone; Gal, galactopyranosyl; Glc, glucopyranosyl; Xyl, xylopyranosyl; Ara, arabinopyranosyl.

Experimental

All the instruments and materials used were the same as those cited in a previous report,[10] unless otherwise specified.

Plant Material Cherry tomatoes (Senka) were grown in a hothouse and harvested in Kumamoto prefecture, Japan, in February 2003.

Extraction and Isolation The crushed fresh ripe cherry tomatoes (1936.7 g) were extracted with MeOH (1000 ml×2) for 95 d at room temperature, and the solvent was removed under reduced pressure to yield a syrup (137.8 g). The MeOH extract was chromatographed over Diaion HP-20 column (H_2O, MeOH, and acetone) to yield fractions (frs.) 1—3. Fr. 2 (6341 mg) was subjected to Sephadex LH-20 column chromatography (MeOH) to yield frs. 2.1—2.4. The chromatography of fr. 2.1 (3972 mg) on Chromatorex ODS column (40% MeOH, 50% MeOH, 60% MeOH, 70% MeOH, 90% MeOH, MeOH) yielded frs. 2.1.1—2.1.9. Fr. 2.1.1 (1644 mg) was subjected to silica gel column [Merck. Art 9385, $CHCl_3$–MeOH–H_2O (10 : 2 : 0.1, 8 : 2 : 0.2, 8 : 2 : 0.1, 7 : 3 : 0.5, 6 : 4 : 1)] to yield frs. 2.1.1.1—2.1.1.12. Frs. 2.1.1.5 (63 mg), 2.1.1.6 (145 mg), and 2.1.1.7 (132 mg) were each subjected to HPLC (Cosmosil 5C18 AR-II, Nacalai Tesque, Inc., 250 mm×20 mm i.d.; 30% MeOH) to yield **5** (3 mg) from fr. 2.1.1.5, **9** (16 mg), **6** (8 mg) from fr. 2.1.1.6, and **2** (7 mg) from fr. 2.1.1.7. Fr. 2.1.1.10 (160 mg) was successively subjected to Diaion HP-20 (70% MeOH, 80% MeOH, 90% MeOH, MeOH) and HPLC [YMC-Pack Polyamine II, YMC Co., Ltd., 250 mm×20 mm i.d.; $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to yield **4** (6 mg). HPLC of fr. 2.1.5 (132 mg), which was performed under the same conditions as those used for fr. 2.1.1.10, yielded **1** (20 mg). Fr. 2.2 (1420 mg) was subjected to silica gel column [Merck. Art 9385, $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 5 : 5 : 1, 0 : 1 : 0)] to yield frs.

2.2.1—2.2.8 and **7** (44 mg). Fr. 2.2.1 (90 mg) was subjected to HPLC (Cosmosil 5C8 MS, Nacalai Tesque, Inc., 250 mm×20 mm i.d.; 50% MeOH) to yield **8** (25 mg). The chromatography of fr. 3 (1373 mg) on silica gel column [Merck. Art 9385, $CHCl_3$–MeOH–H_2O (50 : 1 : 0, 20 : 1 : 0, 14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 8 : 2 : 0.1, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] yielded frs. 3.1—3.20 and **3** (81 mg).

1: Amorphous powder. $[\alpha]_D^{21}$ −65.8° (c=2.3, MeOH). Positive FAB-MS m/z: 1367 $[M+Na]^+$. HR-positive FAB-MS m/z: 1367.5839 $[M+Na]^+$ (Calcd for $C_{61}H_{100}O_{32}Na$: 1367.6096). ^{1}H-NMR data (in pyridine-d_5, 500 MHz) δ: 5.57 (1H, d, J=8.0 Hz, H-1 of Glc′), 5.19 (1H, d, J=8.0 Hz, H-1 of Xyl), 5.16 (1H, d, J=8.0 Hz, H-1 of Glc), 4.94 (1H, d, J=6.0 Hz, H-1 of Ara), 4.93 (1H, d, J=7.5 Hz, H-1 of Gal), 4.77 (1H, d, J=8.0 Hz, H-1 of Glc″), 1.10 (3H, br d, J=5.0 Hz, H_3-21), 1.06 (3H, d, J=6.0 Hz, H_3-27), 0.70 (3H, s, H_3-18), 0.66 (3H, s, H_3-19). ^{13}C-NMR data: see Table 1.

2: Amorphous powder. $[\alpha]_D^{28}$ −7.9° (c=0.4, pyridine). Positive FAB-MS m/z: 457 $[M+Na]^+$. HR-positive FAB-MS m/z: 457.1329 (Calcd for $C_{18}H_{26}O_{12}Na$: 457.1322). ^{1}H-NMR data (in pyridine-d_5, 500 MHz) δ: 7.46 (2H, d, J=7.0 Hz), 7.14 (2H, d, J=7.0 Hz), 5.40 [1H, d, J=8.0 Hz, H-1 of Glc], 5.36 (1H, d, J=7.5 Hz, H-1 of Glc′). ^{13}C-NMR (in pyridine-d_5, 125 MHz) δ: 153.8 (C-1 of Ag), 151.6 (C-4 of Ag), 119.2 (C-2 of Ag, C-6 of Ag), 116.5 (C-3 of Ag, C-5 of Ag), 105.6 (C-1 of Glc), 102.1 (C-1 of Glc′), 83.3 (C-2 of Glc), 78.2, 78.0, 77.7, 77.6 (Glc-5, Glc-5′, Glc-3′, Glc-3), 76.1 (C-2 of Glc), 71.2, 70.8 (C-4 of Glc, C-4 of Glc′), 62.3, 62.0 (C-6 of Glc, C-6 of Glc′).

Acidic Hydrolysis of 1 and 2 Compounds **1** (14 mg) and **2** (2 mg) were each heated in 2 M HCl (2 ml) at a temperature of 95° for 3 h. The reaction mixture was diluted with H_2O (10 ml) and then extracted with AcOEt (10 ml×2). The aqueous layer was neutralized with Amberlite MB-3 (Organo Co.) and then evaporated under reduced pressure to give a monosaccharide fraction. This monosaccharide fraction was extracted with MeOH, and the MeOH extract was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (6.0 mm i.d.×150 mm, Showa Denko); solvent, CH_3CN–H_2O (3 : 1); flow rate, 1.0 ml/min; column temperature, 70 °C; detector, JASCO OR-2090 plus; pump, JASCO PU-2080; and column oven, JASCO CO-2060. The retention time (t_R) and optical activity of each of the monsaccharides were detected as follows. D-xylose [t_R (min) 5.5; optical activity, positive], L-arabinose [t_R (min) 6.1; optical activity, positive], D-glucose [t_R (min) 7.3; optical activity, positive] and D-galactose [t_R (min) 8.0; optical activity, positive] for **1**; D-glucose [t_R (min) 7.3; optical activity, positive] for **2**. HPLC analysis [detector, Shimadzu SP-6A (UV at 293 nm); column, Cosmosil 5SL-II, Nacalai Tesque, Inc., 250 mm×4.6 mm i.d.; solvent, hexane–acetone (3 : 1); flow rate, 0.8 ml/min] of the AcOEt extract of the reaction mixture of **2** revealed the presence of hydroquinone (t_R 12.4 min). However, the AcOEt extract of the reaction mixture of **1** exhibited several spots by TLC, and the Ag of **1** was not obtained.

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