New Solanocapsine-Type Tomato Glycoside from Ripe Fruit of *Solanum lycopersicum*

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A new solanocapsine-type tomato glycoside, a novel and interesting natural steroidal glycoside, was isolated from a mini tomato, *Solanum lycopersicum* L. The chemical structure of the new minor glycoside, esculeoside B-5 (3), was determined to be (5*S*,22*R*,23*S*,24*R*,25*S*)-22,26-epimino-16 β ,23-epoxy-3 β ,23,24-trihydroxycholestane 3-*O*- β -lycotetraoside.

Key words tomato fruit; *Solanum lycopersicum*; solanocapsine-type glycoside; tomato glycoside

For metabolic analysis of steroidal glycosides,¹⁾ we selected the tomato as part of our systematic investigation of the constituents of *Solanum* plants.²⁾ We recognized that the tomato would be appropriate because we were convinced that it had steroidal glycosides, as its aerial parts and immature fruits are rich sources of tomatine. Hence, we isolated steroidal glycosides from the ripe fruit. In Japanese tomatoes, mini tomatoes, Momotaro tomatoes, and midi tomatoes, we were the first to identify a steroidal saponin named esculeoside A (1) as a major tomato saponin in a yield of 0.0015–0.046%.^{3–5)} From Italian tomatoes, we isolated esculeoside B-1 (2) in a yield of 0.019%.^{3,4)}

As other minor constituents of the ripe fruits of mini, midi, and Momotaro tomatoes, we have isolated many steroidal compounds so far by using various column chromatographies on Diaion HP-20, Chromatorex NH, and silica gel, as well as high-performance liquid chromatography on octadecylsilyl (ODS) silica, as shown in Fig. 1.^{6–8)}

Esculeoside B-1 (2) corresponds to an isomer of esculeoside A (1).⁹⁾ Detailed investigation of the constituents of

local Italian tomatoes, processed tomato products, and imported canned tomatoes, tomato juice, and ketchup is currently in progress.

Esculeoside B-1 (2) is the first example of a solanocapsine-type steroidal alkaloid glycoside, the fundamental skeleton of which is very rare and interesting in terms of its chemical structure and unknown pharmacological activity.

Here, we report one example of its rare solanocapsine-type glycoside, esculeoside B-5 (3). A commercial mini tomato was blended briefly with water by a mixer and filtered with a filter paper; the resulting filtrate was then passed through a highly porous polystyrene gel (Diaion HP-20) after elution with water. The water eluate was discarded, and further elution with MeOH was performed to produce an eluate that was evaporated to produce a residue. The MeOH eluate was also subjected to reversed-phase silica gel column chromatography on ODS and elution with 60% MeOH, of which the eluate was evaporated to give a residue. It consisted almost entirely of esculeoside A; it was further chromatographed on silica gel with CHCl₃:MeOH:H₂O=7:3:0.5 to

Fig. 1. Steroidal Constituents in Ripe Fruits of Tomato Obtained in Our Group

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produce five fractions. Fraction 4 was further rechromatographed on silica gel with CHCl_3 : MeOH : H_2O =6:4:1 to provide a steroidal glycoside, esculeoside B-5 (**3**).

Esculeoside B-5 (**3**) was obtained as an amorphous powder having $[\alpha]_D -52.5^\circ$ (pyridine). Positive fast atom bombardment mass spectroscopy (FAB-MS) revealed a quasimolecular ion peak at m/z 1130.5357 due to $[\text{C}_{52}\text{H}_{85}\text{NO}_{24}\text{Na}: 1130.5359]^+$. The ^1H -NMR (in pyridine- d_5) spectrum of **3** indicated two tertiary methyl groups at δ 0.62 (3H, s) and 0.68 (3H, s) and two secondary methyl groups at δ 1.16 (3H, d, $J=6.9$ Hz) and 1.58 (3H, d, $J=7.3$ Hz), which are characteristic of a typical steroidal sapogenol. We also observed one acetyl signal at δ 2.13 (3H, s); two nitrogen-bearing methylene protons at δ 2.98 (1H, dd, $J=2.7, 10.4$ Hz) and 3.04 (1H, t-like, $J=10.4$ Hz); one acetoxyl-bearing methine proton at δ 5.66 (1H, d, $J=7.8$ Hz); and four anomeric proton signals at δ 4.90 (1H, d, $J=7.2$ Hz), 5.20 (1H, d, $J=7.9$ Hz), 5.25 (1H, d, $J=8.0$ Hz), and 5.49 (1H, d, $J=7.3$ Hz). The ^{13}C -NMR (in pyridine- d_5) spectrum showed signals due to a β -lycotetraosyl moiety at δ 103.6 (gal C-1), 74.3 (gal C-2), 76.2 (gal C-3), 82.5 (gal C-4), 77.6 (gal C-5), 61.8 (gal C-6), 106.3 (inner glc C-1), 81.0 (inner glc C-2), 88.6 (inner glc C-3), 76.9 (inner glc C-4), 79.8 (inner glc C-5), 63.7 (inner glc C-6), 106.1 (term. glc C-1), 76.8 (term. glc C-2), 79.8 (term. glc C-3), 72.3 (term. glc C-4), 78.8 (term. glc C-5), 64.1 (term. glc C-6), 106.0 (xyl C-1), 76.5 (xyl C-2), 78.6 (xyl C-3), 71.8 (xyl C-4), and 68.5 (xyl C-5). When these signals were subtracted, 29 signals remained. They consisted of four methyl carbons at δ 13.4, 15.1, 18.8, and 24.9; nine methylene carbons at δ 22.0, 30.1, 30.7, 33.4, 35.2, 35.8, 38.4, 39.2, and 41.1; six methine carbons at δ 36.2, 36.2, 46.0, 56.1,

55.0, and 31.1; two quaternary carbons at δ 37.0 and 44.1; four oxygen-bearing methine carbons at δ 71.6, 78.9, 97.6, and 88.0; one nitrogen-bearing methine carbon at δ 59.7; one acetal carbon at δ 97.6; and one acetyl group at δ 18.2 and 169.2. The heteronuclear multiple bond coherence (HMBC) correlations around the steroidal D, E, and F rings are from H_3 -21 at δ 1.58 to C-17 at δ 59.7, C-20 at δ 31.1, and C-22 at δ 59.7; from H_3 -27 at δ 1.16 to C-25 at δ 36.2, C-26 at δ 41.1, and C-24 at δ 88.0; from H-24 at δ 5.66 to C-23 at δ 97.6; and from H-22 at δ 3.57 (1H, d, $J=7.8$ Hz) to C-23 and C-26. These results revealed that esculeoside B-5 (**3**) has the fundamental skeleton of a solanocapsine-type glycoside, as shown in Fig. 2. Moreover, nuclear Overhauser enhancement was observed between H-20 at δ 3.60 (m) and H-22 at δ 3.57, and between H_3 -27 at δ 1.16 and OAc at δ 2.13. Since the proton signal of H_3 -21 is shifted down slightly to δ 1.58 because of the 23-OH group, they lie in a 1,3-diaxial orientation. Therefore, the structure of esculeoside B-5 (**3**) is represented as (5*S*,22*R*,23*S*,24*R*,25*S*)-22,26-epimino-16 β ,23-epoxy-3 β ,23,24-trihydroxycholestane 3-*O*- β -lycotetraoside, as shown in Fig. 3. This compound might be biosynthesized from lycoperoside G.⁹⁾

Solanocapsine-type glycosides such as esculeoside B-5 (**3**) are novel and interesting very natural products.

Experimental

General Procedure Optical rotations were measured with a JASCO P-1020 ($l=0.5$) automatic digital polarimeter. FAB-MS were obtained with a glycerol matrix in the positive ion mode using a JEOL JMS-DX300 and a JMS-DX 303 HF spectrometer. The ^1H - and ^{13}C -NMR spectra were measured in pyridine- d_5 with JEOL α -500 spectrometer, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard. Column chromatographies were carried out on a Diaion HP-20 (Mitsubishi Chemical Ind., Japan), and silica gel 60 (230–400 mesh, Merck, Germany). TLC was performed on silica gel plates (Kieselgel 60 F₂₅₄, Merck) and RP C₁₈ silica gel plates (Merck). The spots on TLC were visualized by UV light (254/366 nm) and sprayed with 10% H_2SO_4 , followed by heating.

Extraction and Isolation of Compound 3 Commercial mini tomato (783 g) was blended with water using a mixer for a short time (10–20 s) and filtered using filter paper to obtain a filtrate. The filtrate was then passed through a highly porous polystyrene gel (Diaion HP-20) and first eluted with water. The water eluate was discarded, and elution was then carried out using MeOH to obtain an eluate. This eluate was evaporated to obtain a residue (23.4 g), which was subjected to reversed-phase silica gel column chromatography, ODS, eluting with 60% MeOH, the eluate of which was evaporated to obtain the residue (6.8 g). That residue was then chromatographed on silica gel with CHCl_3 : MeOH : H_2O =7:3:0.5 to obtain five fractions. Fraction 2 was almost composed of esculeoside A (320 mg). Furthermore, fraction 4 was rechromatographed on silica gel with CHCl_3 : MeOH : H_2O =6:4:1 to provide a steroidal glycoside, esculeoside B-5 (**3**, 12 mg).

Esculeoside B-5 (3**)** An amorph. powder, $[\alpha]_D -52.5^\circ$ ($c=0.5$, pyridine).

Positive high resolution (HR)-FAB-MS (m/z): 1130.5357 (Calcd for $\text{C}_{52}\text{H}_{85}\text{NO}_{24}\text{Na}$: 1130.5359).

^1H -NMR (pyridine- d_5) δ : 0.62 (3H, s, H_3 -19), 0.68 (3H, s, H_3 -18), 1.16 (3H, d, $J=6.9$ Hz, H_3 -27), 1.58 (1H, d, $J=7.3$ Hz, H_3 -21), 2.13 (3H, s, OAc), 2.98 (1H, dd, $J=2.7, 10.4$ Hz, Hb-26), 3.04 (1H, t-like, $J=10.4$ Hz, Ha-26), 3.57 (1H, d, $J=7.8$ Hz), 4.90 (1H, d, $J=7.2$ Hz, gal H-1), 5.20 (1H, d, $J=7.9$ Hz, inner glc H-1), 5.25 (1H, d, $J=8.0$ Hz, xyl H-1), 5.49 (1H, d, $J=7.3$ Hz, term. glc H-1), 5.66 (1H, d, $J=7.8$ Hz, H-24).

^{13}C -NMR (pyridine- d_5) δ : 39.2 (C-1), 30.1 (C-2), 78.9 (C-3), 35.2 (C-4), 46.0 (C-5), 30.7 (C-6), 35.8 (C-7), 36.2 (C-8), 56.1 (C-9), 37.0 (C-10), 22.0 (C-11), 38.4 (C-12), 44.1 (C-13), 55.0 (C-14), 33.4 (C-15), 71.6 (C-16), 59.7 (C-17), 15.1 (C-18), 13.4 (C-19), 31.1 (C-20), 18.8 (C-21), 59.7 (C-22), 97.6 (C-23), 88.0 (C-24), 36.2 (C-25), 41.1 (C-26), 24.9 (C-27), 18.2, 169.2 (acetyl group), 103.6 (gal C-1), 74.3 (gal C-2), 76.2 (gal C-3), 82.5 (gal C-4), 77.6 (gal C-5), 61.8 (gal C-6), 106.3 (inner glc C-1), 81.0 (inner glc C-2),

Fig. 2. Key HMBC and NOESY around D, E, F-Rings

Fig. 3. Structure of Compound 3

88.6 (inner glc C-3), 76.9 (inner glc C-4), 79.8 (inner glc C-5), 63.7 (inner glc C-6), 106.1 (term. glc C-1), 76.8 (term. glc C-2), 79.8 (term. glc C-3), 72.3 (term. glc C-4), 78.8 (term. glc C-5), 64.1 (term. glc C-6), 106.0 (xyl C-1), 76.5 (xyl C-2), 78.6 (xyl C-3), 71.8 (xyl C-4), 68.5 (xyl C-5).

Sugar Analysis A solution of each compound (**3**) (3.0 mg) in 2 M HCl:dioxane (1:1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H₂O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C₁₈ cartridge to give a sugar fraction. The sugar fraction was concentrated to dryness *in vacuo* to give a residue, which was dissolved in CH₃CN:H₂O (3:1, 250 μ l). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (6.0 mm i.d. $\times$ 150 mm, Showa-Denko, Tokyo, Japan); solvent, CH₃CN:H₂O (3:1); flow rate; 1.0 ml/min; column temperature, 70 °C; detection, refractive index (RI) and optical rotation (OR). The *t_R* (min) of sugars were as follow: D-xylose 4.2 (+), D-galactose 7.0 (+), D-glucose 7.2 (+), [reference: D-xylose 4.2 (positive optical rotation: +), D-galactose 7.0 (positive optical rotation: +), D-glucose 7.2 (positive optical rotation: +)].

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