Saponins, Esculeosides B-1 and B-2, in Tomato Juice and Sapogenol, Esculeogenin B₁

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It has been shown that commercial tomato juice packaged in 900 g plastic bottles contains rare, naturally occurring steroidal solanocapsine-type tomato glycosides in which the saponins consist of esculeosides B-1 (2) and B-2 (3) in 0.041% as major components lacking esculeoside A. We suggest that these saponins are derived from esculeoside A (1) when the juice in plastic bottles is prepared by treatment with boiling water, similar to the process used in preparing canned tomatoes. Herein, the obtained tomato saponins (2) and (3) provided sapogenols esculeogenin B₁ (4) and B₂ (5), respectively, by acid hydrolysis. The former was identical to esculeogenin B previously reported, and the latter was a new sapogenol characterized to be (5α,22S,23S,25S)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxycholestan-24-oic acid.

Key words Solanum lycopersicum; tomato juice; tomato saponin; esculeoside B-1; esculeoside B-2; esculeogenin B₁

Every Solanum lycopersicum L. tomato reviewed in this study including Momotaro, mini, medium, yellow and black tomatoes from Japan, and European and American varieties contains tomato saponin, esculeoside A (1),1-4 as a major ingredient occurring at levels approximately four times higher than those of lycopene.3) The structure of this saponin was determined to be spirostol bidesmoside, as shown in Chart 1. Thus far, the bioactivity of tomatoes has been attributed solely to lycopene. Therefore, pharmacological examination of esculeoside A in the near future should be important.

Fujiwara et al.Δ3) revealed that oral administration of esculeoside A (1) to apolipoprotein E-deficient mice significantly reduces levels of serum cholesterol glycerides and low-density lipoprotein-cholesterol in addition to the areas of atherosclerotic lesions with no detectable side effects. Zhou and colleaguesΔ5) reported that oral administration of esculeoside A (1) improved the effects of atopic dermatitis like disease on mice caused by 2,4-dinitrochlorobenzene.

In previous work, we performed qualitative analyses of fresh tomato, tomato boiled in water, tomato heated in a microwave oven, and freeze-dried tomato prior to their development for health foods.7) In all cases, esculeoside A (1) was a major component. Next, we investigated the tomato saponins in Italian canned tomatoes. Such tomatoes contain tomato saponins, rare, naturally occurring steroidal solanocapsine-type glycosides, a 0.095% mixture of esculeosides B-1 (2) and B-2 (3) lacking esculeoside A (1).8) We refluxed esculeoside A (1) with water for 6.5 h, providing a mixture of esculeosides B-1 (2) and B-2 (3) quantitatively; therefore, we hypothesized that these substances might be derived from esculeoside A (1) when the cans are processed with boiling water.Δ8)

In the present study, we analyzed the amount of tomato saponin in tomato juice commercially processed in 900 g plastic bottles to compare the respective ingredients in each bottle. Five kinds juice produced by different companies were individually treated and passed through highly porous polystyrene gel (Diaion HP-20), and the resulting methanol residue was then subjected to dextran gel (Sephadex LH-20) column chromatography, and then eluted with 90% methanol. The first eluate includes tomato saponins and the following fraction without saponin was collected. The 90% methanol eluates from the dextran column apparently lacked esculeoside A (1) but contained esculeosides B-1 (2) and B-2 (3) on TLC with chloroform(CHCl₃)–methanol (MeOH)–water (6:4:1). We recognized that the esculeoside A in fresh tomato converts to esculeosides B-1 (2) and B-2 (3) when these tomatoes are packed in bottles via sterilization with boiling water similar to that in canned preparation.Δ8) Next, a mixture of esculeosides B-1 (2) and B-2 (3) obtained from juice was separated with octadeylsilane (ODS) to give two saponins. Esculeoside B-2 (3) was hydrolyzed by refluxing with 2 N HCl for 1 h to obtain a sapogenol, esculeogenin B₂ (5) as colorless needles, melting point (mp) 223–226°C (decomp), which was identical to the esculeogenin B previously reported.Δ9) Esculeoside B-1 was similarly hydrolyzed with 2 N HCl to give a new sapogenol, named esculeogenin B₁ (4). Esculeogenin B₁ (4) was obtained as a colorless needles showing mp 232–235°C (decomp) and [α]D -89.6° (pyridine). Positive high-resolution fast atom bombardment mass spectrometry (HR-FAB-MS) exhibited a quasi-molecular ion at m/z 448.3424 corresponding to the molecular formula [C₂₇H₂₆NO₄+H]. The 1H-NMR spectrum (in pyridine-d₅) showed two tertiary methyl signals at δ 0.78 (3H, s) and 0.99 (3H, s), one secondary methyl signal at δ 1.26 (3H, d, J=6.3 Hz), two nitrogen-bearing methylene protons at δ 2.67 (1H, t-like, J=11.9 Hz) and 3.34 (1H, d, J=11.9 Hz), one nitrogen-bearing methine proton at δ 3.05 (1H, d, J=11.5 Hz), two hydroxymethyl protons at δ 3.73 (2H, d, J=5.7 Hz), and two oxygen-bearing methine protons at δ 3.84 (1H, m) and 4.66 (1H, m). The 13C-NMR signals (in pyridine-d₅) displayed a total of twenty-seven carbon signals comprised of three

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methyls (δ 12.4, 15.2, 17.6), one hydroxymethyl (δ 65.1), one hemiketal carbon (δ 96.6), one nitrogen-bearing methine carbon (δ 62.7), one nitrogen-bearing methylene carbon (δ 43.8), and two oxygen-bearing methine carbons (δ 70.4, 70.7). By the aid of proton–proton chemical shift correlated spectroscopy (1H–1H-COSY), 1H-detected heteronuclear correlation through multiplet quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC; Fig. 1), all of the carbon signals of 4 were assigned as follows: C-1–C-27 of sapogenol: δ 37.3, 32.3, 70.7, 39.1, 45.0, 28.9, 32.3, 35.1, 54.5, 35.7, 21.2, 40.5, 41.9, 53.4, 33.5, 70.4, 62.4, 15.2, 12.4, 27.4, 17.6, 62.7, 96.6, 38.7, 38.0, 43.8, 65.1. On this assignment, the HMBC between H-3-21 (δ 1.26) and C-22 (δ 62.7), and the occurrence of the hemiketal carbon function (δ 96.6) in particular conclusively characterized a novel sapogenol moiety, which has a rare, natural solanocapsine-type framework. 10) Next, nuclear Overhauser effect spectroscopy (NOESY; Fig. 2) led to the assignments of the configurations at C-22 and C-23. Namely, the observation of NOESY between H-20 (δ 2.12, m) and H-22 (δ 3.05), and between H-3-21 (δ 1.26) and H-22 revealed the configuration of both H-20 and H-22 to be cis-correlation. The configuration of the hydroxymethyl group at C-25 was also deduced to be equatorial on the basis of the coupling constants of H-26 signals at δ 2.67 (1H, t-like, J=11.9 Hz, Hax) and 3.34 (1H, d, J=11.9 Hz, Heq). The configuration of the hydroxyl group at C-23 was estimated as β-axial because the H-3-21 signals stayed at δ 1.26 in the usual chemical shift, whereas it appeared at δ 1.59 in a 1,3-diaxial correlation11) with the C-23-OH group in esculeogenin B1 (5). Therefore, the structure of 4 could be represented as (5α,22S,23S,25S)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxycholestane, and the configuration at C-22 in esculeoside B-1 (2)8 should be revised to S from R. Thus, we have shown that tomato juices packaged in plastic bottles contain tomato saponins, esculeosides B-1 (2) and B-2 (3), which provide esculeogenins B1 (4) and B2 (5), respectively, by 2N HCl hydrolysis. We concluded that tomato saponins in juice packaged in plastic bottles were derived from esculeoside A (1) when the juice is processed with boiling water.
Acid Hydrolysis of Respective Esculeosides B-1 (2) and B-2 (3) After a solution of esculeoside B-2 (3, 350 mg) in 2N HCl (5 mL) was refluxed for 1 h, its reaction mixture was neutralized with 2N KOH and added with water, which was passed through Diaion HP-20 and washed with water, then eluted with methanol to give a sapogenol. Crude sapogenol was purified on silica gel column chromatography with CHCl₃–MeOH–water=9:1:0.1 to afford the sapogenol (25 mg), which was identical with esculeogenin B obtaining by enzymatic hydrolysis of 3. Similarly, esculeoside B-1 (2, 280 mg) was treated with 2N HCl and purified on silica gel chromatography to give a sapogenol (4, 18 mg), named esculeogenin B₁.

**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 ¹³C-NMR spectra were measured in pyridine-d₅ 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.), silica gel 60 (230–400 mesh, Merck), and ODS (preparative C₁₈, 55–105 µm, Waters). 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% H₂SO₄, followed by heating.

**Extraction of Tomato Juice in Plastic Bottles** Five types of juice products prepared by different companies were individually blended with water and were centrifuged to give a supernatant, which was then passed through Diaion HP-20 and rinsed with water. Methanol was then passed through the gel to produce an eluate, which was evaporated to produce a residue. This methanolic residue was subjected to Sephadex LH-20 column chromatography and was then eluted with 90% methanol. The first eluate was checked by TLC (CHCl₃–MeOH–water=6:4:1) including tomato saponins, and the following fraction excluding tomato saponin was collected. The respective yields are shown as follows. MeOH eluate: residue (0.129–0.216%; average, 0.166%), esculeosides B-1 (2) and B-2 (3): 0.027–0.072%; average, 0.41%, others (including aromatic compounds): 0.074–0.114%, average, 0.101%.

This first running 90% methanolic eluate from the Sephadex LH-20 column gave a residue (1834.7 mg, 0.024%), a part of which (1550 mg) was then chromatographed on ODS column with 45% methanol to provide two tomato sapogenins, identical with esculeosides B-1 (2, 320 mg) and B-2 (3, 410 mg).³

**References**