A New Tomato Pregnane Glycoside from the Overripe Fruits

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A new pregnane glycoside has been isolated from the overripe fruits of Cherry tomato (Mini tomato), *Lycopersicon esculentum* var. *cerasiforme* (DUNAL) ALEF. The structure was determined to be 3-*O*-β-lycotetraosyl 3β-hydroxy-5α-pregn-16-en-20-one on the basis of spectroscopic analysis. The seasonal variation of the tomato saponin is discussed.

Key words *Lycopersicon esculentum* var. *cerasiforme*; tomato; tomato-pregnane; pregnane β-lycotetraoside; seasonal variation

Recently, we isolated a new major spirosolane-type glycoside, named esculeoside A,[1,2] from the fruits of Cherry tomato [*Lycopersicon esculentum* var. *cerasiforme* (DUNAL) ALEF.] and the pink color-type tomato (*Lycopersicon esculentum* MILL., Momotaro), and a novel major solanocapsine-type glycoside, named esculeoside B,[2] from the red color-type tomato (*Lycopersicon esculentum* MILL., Italian San Marzano).

Previously, Schreiber and Aurich found 3β-hydroxy-5α-pregn-16-en-20-one (allopregnenolone), in the epigeous parts of the primitive tomato species, *Lycopersicon pimpinellifolium* MILL., and suggested that it might be a degradation product of tomatidine.[3] Heftmann and Schwimmer reported that tomatine-4-^{14}C incubated in ripe tomato was rapidly converted to 3β-hydroxy-5α-pregn-16-en-20-one.[4] Taking into consideration the above evidence, finding the pregnane glycoside would be significant suggesting a seasonal variation of tomato saponin; tomatine in the immature fruits, *via* esculeoside A in the ripe fruits, into the pregnane glycoside in the overripe fruits. Therefore, we have searched for the pregnane glycoside in the overripe tomato. This paper deals with the first isolation of tomato pregnane glycoside from the overripe fruit and its structural characterization.

Tomato-pregnane (**1**), an amorphous powder, $[\alpha]_D$ −4.0° (pyridine), was obtained by column chromatography of high-porous polystyrene gel (Diaion HP-20), and reversed silica gel in a yield of 30 mg together with esculeoside A (1.15 g) from the aqueous extract of the overripe Cherry tomato (10 kg). Compound **1** has a molecular formula of $C_{44}H_{70}O_{21}$ based on the HR-positive FAB-MS: 935.4481 $[(M+H)^+$, $C_{44}H_{70}O_{21}$+H: Calcd 935.4488]. Three tertiary methyl signals at δ 0.65 (3H, s, H_3-19), 0.91 (3H, s, H_3-18) and 2.25 (3H, s, H_3-21), one olefinic proton signal at δ 6.62 (1H, s, H-16) together with four anomeric proton signals (1H, d, J=7.3 Hz, δ 4.88; 1H, d, J=7.9 Hz, δ 5.18; 1H, d, J=7.9 Hz, δ 5.23; 1H, d, J=7.3 Hz, δ 5.57) were observed in the ^{1}H-NMR spectrum (in pridine-d_5). On the other hand, the ^{13}C-NMR signals due to a total of 21 carbon signals (in pyridine-d_5) originating from the sapogenol were composed of three methyl groups at δ 12.2, 16.2, and 27.1, one oxygen-bearing methine carbon at δ 77.8, one carbonyl group at δ 196.3, two quaternary carbons at δ 35.9 and 46.5, four methine carbons at δ 33.8, 44.9, 54.9 and 56.4, eight methylene carbons at δ 21.3, 28.9, 29.9, 32.1, 32.2, 34.8, 35.3 and 37.0, and two olefinic carbons at δ 144.6 and 155.5. The HMBC as illustrated in Fig. 1 disclosed the connectivities of the above functional carbons to form a pregnane skeleton. Furthermore, the ^{13}C-NMR signals attributable to the sugar moiety suggested the presence of a β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl (1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-(β-lycotetraosyl) residue (gal C-1-6 δ: 102.4, 73.1, 75.3, 79.8, 75.1, 60.7, inner glc C-1-6 δ: 104.8, 81.3, 86.9, 70.8, 78.6, 62.5, terminal glc C-1-6 δ: 104.9, 76.2, 78.6, 71.1, 77.5, 62.9, terminal xyl C-1-5 δ: 105.3, 75.1, 77.6, 70.4, 67.3).

Therefore, the structure of **1** has been determined as 3-*O*-β-lycotetraosyl 3β-hydroxy-5α-pregn-16-en-20-one, which was derived from tomatine by Miyahara.[5] This is the first example of a report concerning the occurrence of glycosyl pregnane from tomato fruit.

A seasonal variation of tomato steroidal glycosides, that esculeosides A and B might be produced from tomatine in the immature tomato as tomato grows, they would decrease and partly derived into the pregnane glycoside in the overripe season, would be proposed.

The first pregnane glycoside was isolated from *Paris polypylla* by one of our authors,[6] and recently several pregnane glycosides,[7,8] except digitanol pregnane glycosides, were obtained. This evidence indicates that the pregnane glycoside coexists in small amounts together with steroidal glycoside, and simultaneously might mean that pregnane glycoside could be biosynthesized from steroidal glycoside, because the intermediates of the pregnane, 16-acylated pregnane derivatives,[7,9] corresponding to the intermediate in the Marker's degradation procedure, were also found. Steroidal

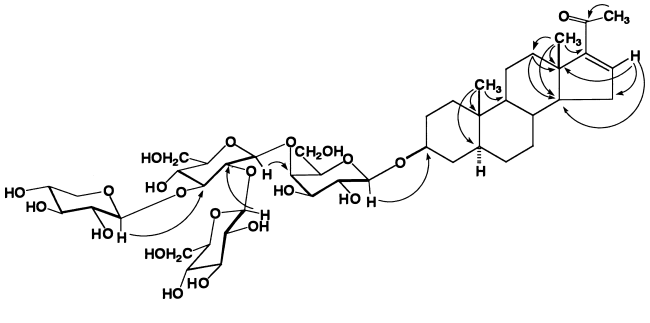

Fig. 1. Key HMBC of Tomato-Pregnane (**1**)

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glycoside may be metabolized into pregnane derivative, the raw material of steroidal hormones that have various physiological activities in the human body.

Experimental

Msp: uncorr. Optical rotations were measured with a JASCO DIP-1000 KUY polarimeter (l=0.5). NMR spectra were measured in pyridine-d_5 on a JEOL α-500 spectrometer and chemical shifts were referenced to TMS. Column chromatography was carried out with silica gel 60 (Art. 7734 and Art. 9385, Merck), Diaion HP-20P (Mitsubishi Chemical Industries Co., Ltd.) and Chromatorex ODS (Fuji Silysia Chemical Co., Ltd.). TLC was performed on a precoated silica gel 60 F_{254} (Merck) and RP-18 $F_{254}S$ (Merck).

Extraction and Isolation of Tomato-Pregnane The fresh overripe fruits (usually summer-tomatoes are harvested in May at Kumamoto Prefecture, but overripe tomatoes are harvested one month later, 10 kg) of Cherry tomato (*Lycopersicon esculentum* var. *ceraciforme*) purchased in Kumamto city were smashed, water added, and then filtered. The filtrate was passed through Diaion HP-20 and eluted with water and MeOH, successively. The MeOH eluate was then subjected to an ODS column eluted with a 40% aq. MeOH to MeOH gradient. The 80% eluate provided pregnane (**1**, 30 mg) together with esculeoside A (1.15 g).

3-*O*-β-Lycotetraosyl 5α-Pregn-16-en-3β-ol-20-one (**1**): An amorphous powder, $[\alpha]_D$ −4.0° (c=0.1, pyridine), HR-positive FAB-MS (m/z): 935.4481 $[M+H]^+$, $C_{44}H_{70}O_{21}$+H: Calcd 935.4488, ^{1}H-NMR (pyridine-d_5) δ: 0.65 (3H, s, H_3-19), 0.91 (3H, s, H_3-18), 2.25 (3H, s, H_3-21), 6.62 (1H, s, H-16), 4.88 (1H, d, J=7.3 Hz), 5.18 (1H, d, J=7.9 Hz), 5.23 (1H, d, J=7.9 Hz), 5.57 (1H, d, J=7.3 Hz), ^{13}C-NMR (pyridine-d_5), C-1-21: δ 37.0, 29.9, 77.8, 34.8, 44.9, 28.9, 32.2, 33.8, 54.9, 35.9, 21.3, 35.3, 46.5, 56.4, 32.1, 144.6, 155.5, 16.2, 12.2, 196.3, 27.1.

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