Cytotoxic Major Saponin from Tomato Fruits

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A major novel steroidal alkaloid glycoside, possessing cytotoxic activity has been isolated from the fruits of *Lycopersicon esculentum*.

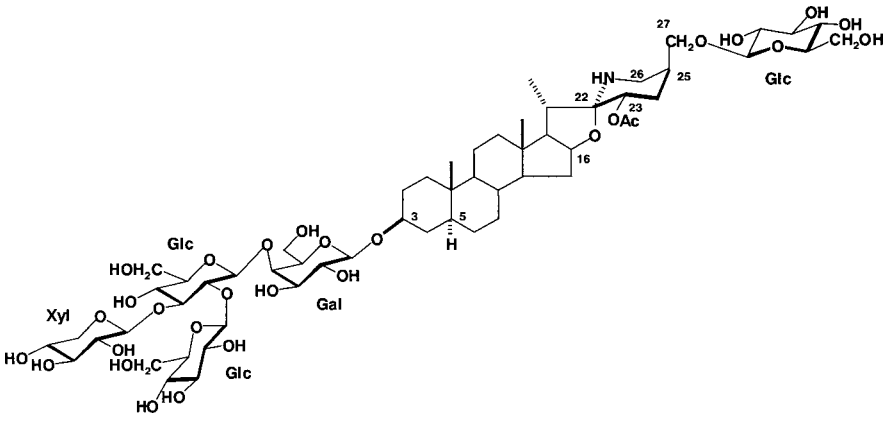
Key words *Lycopersicon esculentum*; steroidal alkaloid; cytotoxic activity

With regard to recent studies on the constituents of tomato (*Lycopersicon esculentum* MILLER; Japanese "Momotaro" species), a bitter principle, named TFI[1)] isolated from tomato seeds, tomatine[2)] and some spirosolane glycosides[3)] obtained from the stems and leaves, and lactone,[4)] pregnane,[5)] and several spirosolane[6)] derivatives from the roots of a tomato stock were reported. Now we have isolated a novel major spirosolane glycoside, named esculeoside A (**1**), having cytotoxic activity by using various column chromatographies of polystyrene high-porous gel, and normal and reversed silica gel in a yield[7)] of 91 mg from the methanolic extract (275.5 g) of the commercial ripe tomato fruits (7.28 kg). This paper deals with the first isolation of the steroidal alkaloid glycoside from the tomato fruits and its characterization.

Esculeoside A (**1**), obtained as a white powder, $[\alpha]_D$ −52.5° (MeOH), has a molecular formula of $C_{58}H_{95}NO_{29}$ based on the high resolution (HR)-FAB-MS.[8)] Two angular methyl signals at δ 0.66 (s, H_3-18), 0.89 (s, H_3-19), one secondary methyl signal at δ 1.07 (d, J=6.7 Hz), and one acetyl methyl signal at δ 2.11 (s) together with five anomeric proton signals (each 1H, d, J=7.3 Hz, δ 4.88; J=7.9 Hz, δ 4.89; J=7.9 Hz, δ 5.19; J=7.9 Hz, δ 5.23; J=7.3 Hz, δ 5.57) were observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR signals revealed the presence of a β-lycotetraosyl and a β-D-glucopyranosyl moieties[9)] as sugar component. The remaining signals were composed of twenty-seven carbons, which comprised of two oxygen-bearing methine carbons (δ 77.8, 78.7), a hydroxymethyl carbon (δ 70.8), an acetoxyl carbon (δ 21.2, 190.8), a spirosolane center carbon (δ 99.1) and a nitrogen-bearing methylene carbon (δ 39.9). The spirosolane center carbon at C-22 appeared at δ 99.1 exhibited a little lower field shift than the usual shift around at δ 98.0, therefore, it was deduced that the acetoxyl group attached to C-23. The methine proton signal at C-23 appeared at δ 5.38 (1H, dd, J=5.5, 11.0 Hz), which indicated α-configuration of the acetoxyl group. Since the signal due to H_3-21 was assigned to δ 1.07 in the correlation of the heteronuclear multiple band connectivity (HMBC) between H_3-21 and C-20/C-17/C-22 in the ^{1}H-NMR spectrum, a methyl signal at C-27 was oxygenated to the hydroxymethyl group at δ 70.8 in **1**. Mutual coupling signals at δ 2.87 (br d, J=11.0 Hz) and 3.24 (br d, J=11.0 Hz) were assigned to H_2-26 by the ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and HMBC. Their coupling constants were indicative to be 25*S* configuration. The hydroxymethyl group at C-27 was lower-shifted at δ 70.8 compared with that of solaparnaine,[10)] possessing the 27-hydroxymethyl group, therefore, one glucopyranosyl moiety should attach to the C-27-OH. Moreover, the configuration of the C-22 was determined by comparison with the chemical shifts of the ^{13}C signals at C-20 of Alkaloids 2, 3, 4[6)] and lycoperosides A, B, C, D.[3)] Normally, the signal due to C-20 in case of 22α*N*-spirosolan, even though an acetoxyl group bounded to C-23, appeared around at δ 35.0, on the other hand those of 22β*N*-isomers displayed around at δ 43.0. Escleoside A (**1**) exhibited at δ 35.3, therefore, **1** was characterized as (23*S*,25*S*)-23-acetoxy-5α,22α*N*-3β,27-dihydroxyspirosolan 3-*O*-β-lycotetraosyl 27-*O*-β-D-glucopyranoside. This steroidal oligoglycoside is included as a major saponin.

Growth inhibition of esculeoside A (**1**) and tomatine against MCF-7 cells have been evaluated. Cytotoxicity of these compounds was mesured using the WST-8 proliferation[11)] reagent. The IC_{50} value of **1** was 24.5 μM (tomatine: 15 μM).

The isolation of the steroidal alkaloid having anti-cancer activity from tomato fruits has firstly been attained, and the intake of tomato is supposed to be effective for reducing the risk of cancer in cooperation with the occurrence of lycopene.



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7) The yield would be raised by the improvement of the extraction and separation.
8) Positive HR-FAB-MS (*m*/*z*): 1292.5869 $[M+Na]^+$ ($C_{58}H_{95}NO_{29}Na$, Calcd for 1292.5883).
9) 3-*O*-β-Lycotetraosyl moiety: gal C-1-6, 102.5, 73.2, 75.3, 79.9, 75.3, 60.7; inner glc C-1-6, 105.1, 81.3, 86.9, 70.8, 77.6, 62.8; terminal xyl C-1-5, 104.8, 75.1, 77.5, 70.8, 67.3; terminal glc C-1-6, 104.9, 76.2, 78.3, 70.5, 77.6, 62.5; 27-*O*-β-D-glucopyranosyl moiety: C-1-6, 104.9, 75.3, 78.7, 70.8, 78.7, 62.3.
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