Tomato New Sapogenols, Isoesculeogenin A and Esculeogenin B

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Two novel sapogenols, isoesculeogenin A (1) and esculeogenin B (2) of steroidal alkaloid glycosides, lycoperoside F and esculeoside B, respectively, isolated from the ripe tomato have been characterized as (5α,22*R*,23*R*,25*S*)-3β,23,27-trihydroxyspirosolane and (5α,22*S*,23*R*,25*S*)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxycholestane, respectively.

Key words *Lycopersicon esculentum*; tomato; steroidal alkaloid sapogenol; spirosolane; solanocapsine

Tomato, the fruit of *Lycopersicon esculentum* MILL., is widely used as a fresh vegetable and in cooking. The species of tomato found in markets can be roughly classified into two groups, the red color-type and pink color-type; the red color-type tomato (*e.g.* Italian San Marzano) is mainly used for pasta sauce and in cooking, and the pink color-type tomato (*e.g.* Momotaro) as a fresh vegetable. The tomato has received much attention due to the presence of lycopene which has having strong anti-oxidant activity. Recently, we isolated a new major spirosolane-type glycoside, named esculeoside A,[1,2] from the fruit of a Cherry tomato [*L. esculentum* var. *cerasiforme* (DUNAL) ALEF.] and the pink color-type tomato, and a novel major solanocapsine-type glycoside, named esculeoside B,[2] from the red color-type tomato. The sapogenol, esculeogenin A,[1,2] was obtained in a crystalline state by acid hydrolysis of esculeoside A and characterized as (5α,22*S*,23*S*,25*S*)-3β,23,27-trihydroxyspirosolane. In order to confirm the structures of their new sapogenols and to investigate the bio-activities of their sapogenols, two novel sapogenols, isoesculeogenin A (**1**) and esculeogenin B (**2**) have been obtained by acid hydrolysis of lycoperoside F[3] (**3**) and by enzymic hydrolysis of esculeoside B,[2] respectively, and their structures have been established.

Acid hydrolysis of lycoperoside F (**3**), obtained from the ripe fruits of Meddy Red tomato as a major glycoside, with 2 N HCl–MeOH provided a new sapogenol, named as isoesculeogenin A (**1**), as colorless needles with mp 206—213 °C, $[\alpha]_D$ −87.2° (pyridine). Its high resolution (HR)-EI-MS indicated a molecular ion at *m*/*z* 447.3367 due to $[C_{27}H_{45}NO_4, M]^+$ being identical with that of esculeogenin A. The ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) spectrum of **1** as illustrated in Fig. 1 revealed that **1** is a spirosolane derivative with hydroxyl groups at C-23 and C-27. The above evidence suggested **1** is an isomer of esculeogenin A. By comparing the ^{1}H-NMR signals (pyridine-d_5) with those of esculeogenin A, they were assigned as follows: two quaternary methyl signals at δ 0.80 (3H, s, H_3-19), 0.95 (3H, s, H_3-18), one secondary methyl signal at δ 1.54 (3H, d, *J*=6.8 Hz, H_3-21), which was shifted toward a lower field than that of esculeogenin A by 0.47 ppm, two proton signals of a nitrogen-bearing methylene group at δ 3.17 (2H, m, H_2-26), one hydroxymethyl proton signal at δ 3.73 (2H, m, H_2-27), three proton signals of an oxygen-bearing methine group at δ 3.80 (1H, m, H-3), 4.28 (1H, dd, *J*=3.0, 10.0 Hz, H-23), and 5.29 (1H, m, H-16), which is also lower-shifted by 0.80 ppm by comparing with that of esculeogenin A. The lower shifts of H_3-21 and H-16 suggested that they are close to the C-23 hydroxyl group, therefore, the configuration at C-22 could be estimated as 22β*N*, that is 22*R*. This conclusion was also supported by the chemical shift at C-20.[4] In 22α*N*-spirosolane derivatives, the C-20 signals appeared around δ 35.0, while in 22β*N*-series (C-22*R*), it occurred around δ 43.0. Isoesculeogenin A (**1**) appeared at δ 44.1, suggesting the configuration at C-22 to be C-22β*N* (C-22*R*). The hydroxyl group at C-23 was estimated as *equatorial*, namely C-23*R*, based on the coupling constant of H-23 (1H, dd, *J*=3.0, 10.0 Hz, at δ 4.27). Moreover, the C-27-hydroxymethyl group was also judged to be *equatorial*, C-25*S*, from the evidence of the coupling pattern of the C-26 methylene protons at δ 3.17 (2H, m, H_2-26), which were apparently different from those [1H, d, *J*=11.0 Hz, H*ax*-26, at δ 3.05 and 1H, dd, *J*=3.4, 11.0 Hz, H*eq*-26, at δ 3.34] of esculeogenin A. Other carbon signals were coincident with those at C-1—14 of esculeogenin A. Consequently, the structure of **1** was (5α,22*R*,23*R*,25*S*)-3β,23,27-trihydroxyspirosolane.

Next, esculeogenin B (**2**) was obtained by enzymic hydrolysis; that is, firstly esculeoside B was enzymic-hydrolyzed with tomatinase[5] to give a prosapogen (**4**), which was subsequently subjected to an analogous enzymic hydrolysis with β-glucosidase to provide a sapogenol, named as esculeogenin B (**2**), as an amorphous powder showing $[\alpha]_D$ −96.2° (pyridine). The HR-EI-MS of **2** showed a peak at *m*/*z* 447.3298 corresponding to a molecular formula $[C_{27}H_{45}NO_4, M]^+$. The ^{1}H-NMR spectrum (in pyridine-d_5) showed two tertiary methyl signals at δ 0.81 and 1.01, one secondary methyl sig-

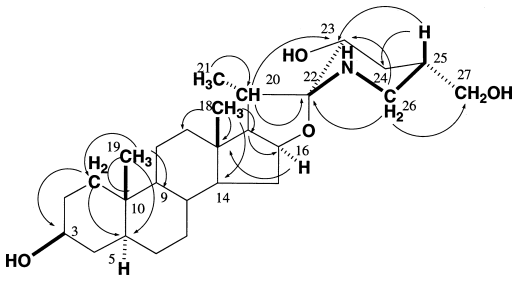

Fig. 1. Key HMBC Observed in Isoesculeogenin A (**1**)

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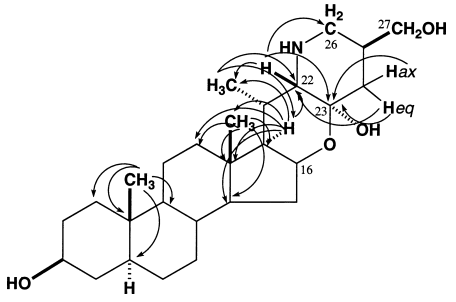

Fig. 2. Key HMBC Observed in Esculeogenin B (**2**)

nal at δ 1.67 (d, J=6.7 Hz), two nitrogen-bearing methylene protons at δ 3.02 (1H, t-like, J=12.1 Hz) and 3.30 (1H, br d, J=12.1 Hz), two hydroxymethyl protons at δ 3.73 (2H, d, J=6.7 Hz), and two oxygen-bearing methine protons at δ 3.85 (1H, m) and 4.63 (1H, m). The ^{13}C-NMR signals (in pyridine-d_5) displayed a total of twenty-seven carbon signals comprised of three methyls (δ 12.6, 15.4, 17.8), one hydroxymethyl (δ 65.4), one hemiketal carbon (δ 96.8), one nitrogen-bearing methine carbon (δ 63.0), one nitrogen-bearing methylene carbon (δ 43.8), and two oxygen-bearing methine carbons (2×δ 70.6). By the aid of proton–proton chemical shift correlated spectroscopy (^{1}H–^{1}H-COSY), ^{1}H-detected heteronuclear correlation through multiple quantum coherence (HMQC) and HMBC, all of the carbon signals of **2** were assigned as follows: C-1—27 of sapogenol: δ 37.6, 32.1, 70.6, 39.3, 45.4, 29.1, 32.6, 35.3, 54.8, 35.9, 21.4, 40.7, 42.1, 53.6, 33.8, 70.6, 62.7, 15.4, 12.6, 27.6, 17.8, 63.0, 96.8, 39.3, 25.2, 43.8, 65.4. On this assignment, especially, the HMBC between H_3-21 and C-22 as illustrated in Fig. 2, and the occurrence of the hemiketal carbon function conclusively characterized a novel sapogenol moiety, which has a rare natural product, solanocapsine-type framework.[6,7] Next, NOESY led to the assignments of the configurations at C-22 and C-23. Namely, the observation of NOESY between H-20 and H-22, and between H-26 and H-22 revealed the configurations 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_2-26 signals at δ 3.02 (1H, t-like, J=12.1 Hz, H*ax*-26), 3.30 (1H, br d, J=12.1 Hz, H*eq*-26). The configuration of the hydroxyl group at C-23 was estimated as α-*axial* based on the fact that H_3-21 signals shifted toward a lower field at δ 1.67 in a 1,3-*diaxial* correlation with the C-23-OH group. Consequently, the structure of **2** could be represented as (5α,22*S*,23*R*,25*S*)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxycholestane.

Experimental

General Procedure Melting points were determined on a Yanagimoto micromelting point apparatus without correction. Optical rotations were measured on a JASCO DIP-1000 KUY digital 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. HR-EI-MS were obtained with a JEOL JMS-MS-700 spectrometer. Column chromatography was carried out with silica gel 60 (0.063—0.200 mm, Merck), Diaion HP-20P (Mitsubishi Chemical Industries Co., Ltd.) and Chromatorex ODS (Fuji Silysia Chemical Co., Ltd.), and TLC was performed on a precoated silica gel 60 F_{254} (Merck) and RP-18 F_{254}S (Merck), and detected by spraying with 10% H_2SO_4 in 50% MeOH, followed by heating on a hot plate. The HPLC system consisted of a pump (Hitachi C-6000), detector (JASCO 830-RI), column [Kanto Chem. Co. Mightysil RP-18GP 250-4.6 (5mm)], and mobile phase (60% MeOH; flow rate, 0.8 ml/min.).

Isolation of Lycoperoside F (3) Meddy Red tomatoes (762 g) were smashed in water and then filtered to give a filtrate, which was passed through Diaion HP-20P and eluted with water and then MeOH. The MeOH eluate (1.42 g) was chromatographed on ODS with a 40% MeOH—60% MeOH–MeOH gradient to give three fractions. A part (23 mg) of fraction 3 (320.8 mg) was subjected to HPLC to provide esculeoside A (5 mg) and lycoperoside F (**3**, 9 mg). Colorless amorphous powder, $[\alpha]_D$ −44.8° (c=0.75, pyridine). Positive HR-FAB-MS (m/z) 1292.5872 (Calcd for $C_{58}H_{95}NO_{29}Na$, 1292.5888 [M+Na$^+$]. ^{1}H-NMR (pyridine-d_5) δ: 0.66 (3H, s, H_3-19), 0.88 (3H, s, H_3-18), 1.18 (3H, d, J=6.7 Hz, H_3-21), 2.23 (3H, s, acetyl), 4.74 (1H, d, J=7.9 Hz, gal H-1), 4.91 (1H, d, J=7.9 Hz, 27-*O*-glc H-1), 5.12 (1H, d, J=7.9 Hz, inner glc H-1), 5.16 (1H, d, J=7.9 Hz, xyl H-1), 5.55 (1H, d, J=7.9 Hz, term. glc H-1). ^{13}C-NMR (pyridine-d_5), sapogenol C-1—27: δ 36.9, 29.5, 77.9, 35.1, 44.7, 28.6, 32.0, 35.5, 54.2, 35.0, 21.2, 40.1, 41.1, 56.2, 34.4, 82.4, 63.2, 17.0, 12.0, 42.9, 15.2, 100.5, 74.9, 32.0, 36.9, 44.7, 72.5, OAc: 21.8, 170.2, gal C-1—6: 102.0, 73.0, 74.9, 79.1, 74.6, 61.8, inner glc C-1—6: 104.3, 80.4, 86.7, 70.4, 77.2, 62.2, terminal glc C-1—6: 103.9, 76.8, 77.9, 71.2, 77.9, 62.2, xyl C-1—5: 104.3, 75.3, 77.6, 70.1, 66.6, 27-*O*-glc C-1—6: 104.1, 74.9, 79.1, 71.1, 77.9, 63.2.

Isoesculeogenin A (1) After lycoperoside F (**3**, 124 mg) was hydrolyzed with 2 N HCl (3 ml), the reaction mixture was extracted with AcOEt. The organic layer was evaporated *in vacuo* to afford a residue which was purified by silica gel column chromatography with $CHCl_3$–MeOH–water=9 : 1 : 0.1 to give isoesculeogenin A (**1**, 9 mg). Colorless needles, mp 206—213 °C, $[\alpha]_D$ −87.2° (c=0.64, pyridine), HR-EI-MS (m/z): 447.3367 [M]$^+$ (Calcd for $C_{27}H_{45}NO_4$: 447.3349; ^{1}H-NMR (pyridine-d_5) δ: 0.80 (3H, s, H_3-19), 0.95 (3H, s, H_3-18), 1.54 (3H, d, J=6.8 Hz, H_3-21), 3.17 (2H, m, H_2-26), 3.73 (2H, m, H_2-27), 3.80 (1H, m, H-3), 4.28 (1H, dd, J=3.0, 10.0 Hz, H-23), 5.29 (1H, m, H-16). ^{13}C-NMR (pyridine-d_5), C-1—27: δ 37.5, 32.3, 70.6, 39.2, 45.2, 29.1, 32.6, 35.3, 54.8, 35.9, 21.5, 40.7, 40.1, 56.7, 34.3, 82.7, 63.7, 17.3, 12.5, 44.1, 16.5, 102.5, 72.2, 32.4, 35.3, 45.9, 65.6.

Prosapogenin (4) A mixture of esculeoside B (96 mg) and tomatinase (5 ml) in citric acid buffer (12 ml) was left to stand at r.t. for 1 d. After filtration, the filtrate was passed through a Diaion HP-20 column with water and then MeOH. The MeOH eluate was subjected to silica gel column chromatography with $CHCl_3$–MeOH–water=9 : 1 : 0.1→7 : 3 : 0.5 to give prosapogenin (18 mg). An amorphous powder, ^{13}C-NMR (pyridine-d_5), Sapogenol moiety C-1—27: δ 37.6, 32.5, 70.6, 39.3, 45.3, 29.1, 32.6, 35.3, 54.8, 35.9, 21.4, 37.7, 42.1, 53.6, 33.7, 70.6, 62.4, 15.4, 12.6, 27.6, 17.7, 63.0, 96.4, 40.7, 29.9, 43.7, 72.7; 27-*O*-Glucosyl moiety C-1—6: 104.7, 75.2, 78.5, 71.8, 78.6, 63.0.

Esculeogenin B (2) A mixture of prosapogenin (13 mg) and β-glucosidase in citric acid buffer (4 ml) was incubated for one day at 38 °C. After the reaction mixture was filtered and washed with MeOH, the filtrate was passed through a Diaion HP-20 column first with water and then MeOH. The MeOH eluate was subjected to silica gel column chromatography with $CHCl_3$–MeOH–water=9 : 1 : 0.1→8 : 2 : 0.5 to give esculeogenin B (**2**, 6 mg), an amorphous powder, $[\alpha]_D$ −96.2° (c=0.05, pyridine), HR-EI-MS (m/z): 447.3298 [M]$^+$ (Calcd for $C_{27}H_{45}NO_4$: 447.3349; ^{1}H-NMR (pyridine-d_5) δ 0.81 (3H, s, H_3-19), 1.01 (3H, s, H_3-18), 1.67 (3H, d, J=6.7 Hz, H_3-21), 3.02 (1H, t-like, J=12.1 Hz, H*ax*-26), 3.30 (1H, br d, J=12.1 Hz, H*eq*-26), 3.73 (2H, d, J=6.7 Hz, H_2-27), 3.85 (1H, m, H-3), 4.63 (1H, m, H-16). ^{13}C-NMR (pyridine-d_5), C-1—27: δ 37.6, 32.1, 70.6, 39.3, 45.4, 29.1, 32.6, 35.3, 54.8, 35.9, 21.4, 40.7, 42.1, 53.6, 33.8, 70.6, 62.7, 15.4, 12.6, 27.6, 17.8, 63.0, 96.8, 39.3, 25.2, 43.8, 65.4.

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