Conversion of Esculeoside A into Esculeogenin B

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Conversion of the spirostanol-type glycoside, esculeoside A, a major component contained in the ripe tomato Lycopersicon esculentum fruits, into a solanocapsine-type sapogenol, esculeogenin B-2, (5α,22S,23R,25S)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxycholestan-3-one, and esculeogenin B-1, (5α,22R,23S,25S)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxycholestan-3-one, which are rarely naturally occurring compounds was attained by acid hydrolysis with 2 N HCl in dioxane and water (1:1).

Key words tomato; solanocapsine-type; esculeogenin B

For the first time, from the ripe fruits of Japanese tomato (pink-type: Momotaro tomato and Mini tomato), the fruits of Lycopersicon esculentum var. cerasiforme, a solanocapsine-type glycoside, esculeoside A (1), 3-O-β-lactotetraosyl (5α,22S,23R,25S)-23-acetoxy-3β,27-dihydroxy-spirosolane 27-O-β-D-glucopyranoside, was isolated, and its bioactivity anti-arteriosclerosis was obtained, and therefore the structure of esculeoside A except around C-27, together with the presence of one C-22 by the HMBC. Its chemical structure was represented as shown in Chart 1.

The molecular formula of 3 was measured as C_{33}H_{55}NO_{9} by HR-FAB-MS. The 1H-NMR signals were assigned as follows: δ 0.56 (1H, m, H-9), 0.78, 0.79 (each s, H-3, H-19), 0.81 (3H, s, H-17), 2.14, 2.19 (each s, OAc), 2.96 (d, J=11.5 Hz, H-26), 3.30 (1H, dd, J=3.5, 11.5 Hz, H-26), 3.06 (m, H-26), 4.77, 4.87 (each d, J=7.6 Hz, glc H-1), 5.00 (1H, m, H-16), and 5.18 (1H, dd, J=3.2, 9.4 Hz, H-23). The 13C-NMR data also suggested the existence of the sapogenol C-1—27 together with one β-D-glucopyranosyl moiety as shown in Experimental. Since the above 1H- and 13C-NMR signals appeared as split pattern at H-18, H-26, OAc, and glucosyl H-1, and C-22 showed that both acetoxyl groups oriented to equatorial accompanied by steric inversion. Therefore the structure of 3 was determined to be a mixture of 23-O-acetyl esculeogenin A 27-O-D-glucopyranoside and 23-O-acetyl isosceleogenin A 27-O-D-glucopyranoside. This acid hydrolysis was regarded as not completed owing to insolubility of the products without organic solvent; therefore next we tried acid hydrolysis by addition of MeOH.

Refluxing of esculeoside A (1) with 2N HCl–MeOH for 1.5 h provided compound 4 and compound 5 in yields 21% and 32%, respectively. In the 1H-NMR spectrum of 4, the signal due to H-21 appeared at δ 1.51 (3H, d, J=7.5 Hz) and the signal due to H-16 at δ 5.30, both of which were lower shifted by +0.46 and +0.86 ppm by comparing with those of 2. This indicated that the C-23-hydroxy group in equatorial configuration approaches to the H-21 and H-16, causing extreme lower shifts for H-21 and H-16.7 That is, the F-ring was reversed at C-22 configuration. The F-ring once opened to give enamine-imine type intermediates, to which the 16-OH took place recyclization as shown in Chart 2.

Compound 5 showed a singlet olefinic methyl signal at δ 1.72 in the 1H-NMR spectrum; on the other hand, the 13C-NMR spectrum displayed the occurrence of one double bond at δ 95.7 and 165.2, which latter were assigned to C-20 and C-22 by the HMBC. Its chemical structure was represented as shown in Chart 1.

Even the above reaction did not give the sugar-free compound; thus we next tried reacting in stronger acid condi-

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tions. Namely, esculeoside A (1) was hydrolyzed with 3 N H₂SO₄ and MeOH to provide esculeogenin A (6) in a yield of 25% and isoesculeogenin A (7) in a yield of 13% along with compound 2 as shown in Chart 1. Here, we first could obtain the sapogenols; however, esculeogenin A was not isomerized into esculeogenin B.

Next, to elevate refluxing temperature, we used 2 N HCl in a solution of dioxane and water (1:1). After refluxing for 1.5 h, the reaction mixture was neutralized and evaporated under reduced pressure to give a residue, to which water was added and it was then subjected to polystyrene gel. First it was eluted with water and the products were recovered with MeOH. Major product was measured with the ¹H-NMR spectrum suggesting it to be a mixture of esculeogenin B analogues. Therefore we separated using ODS with 65% MeOH to give two kinds of esculeogenin B, named esculeogenin B-1 (9, 16% yield from 1) and esculeogenin B-2 (8, 21% yield from 1).

Esculeogenin B-2 (8) showed C₂₇H₄₅NO₄ by the HR-EI-MS and [α]D -96.2° (pyridine). In the ¹H-NMR spectrum
(in pyridine-$d_5$) displayed signals at $\delta$ 0.77 (3H, s, H$_3$-19), 1.01 (3H, s, H$_3$-18), 1.67 (3H, d, $J$=6.7 Hz, H$_3$-21), 3.10 (1H, t-like, $J$=10.1 Hz, H$_3$-18), 3.40 (1H, brd, $J$=10.1 Hz, Hb-26), 3.85 (1H, m, H-3). The $^{13}$C-NMR data were assigned by $^1$H-$^1$H COSY, HMQC and HMBC as shown in Experimental.

Next, the stereo configuration at C-22, C-23, and C-25 was discussed. First, as regards the configurations at C-23, remarkable lower shifts were observed at H-16 by $\delta$ 0.96 ppm and H$_3$-21 by $\delta$ 0.44 ppm by comparing with those of esculeogenin B-1 (9). This suggested the hydroxyl group at C-23 to be both 1,3-diaxial conformations against H-16 and H$_3$-21; therefore the configuration of the hydroxyl group at C-23 was deduced to be a (C-23: R). Moreover, NOESY (Fig. 1) between H-16 and H-17 ($\delta$ 1.23), and between H-20 ($\delta$ 3.57) and H-22 ($\delta$ 3.75) indicated that the configuration at C-22 was S.

Consequently, esculeogenin B-2 (8) was characterized as (5$\alpha$,22S,23R,25S)-22,26-epimino-16$\beta$,23-epoxy-3$\beta$,23,27-trihydroxycholestane, which was identical with the compound, esculeogenin B, previously obtained by enzymatic hydrolysis with tomatinase and $\beta$-glucosidase, in turn. Esculeogenin B-1 (9) showed C$_{27}$H$_{45}$NO$_4$ by the HR-EI-MS and $[\alpha]_D^{20}$ = -68.2° (pyridine). In the $^1$H-NMR spectrum (in pyridine-$d_5$) displayed signals at $\delta$ 0.77 (3H, s, H$_3$-19), 0.96 (3H, s, H$_3$-18), 1.23 (3H, d, $J$=6.7 Hz, H$_3$-21), 3.01 (1H, d, $J$=11.1 Hz, H$_3$-21), 3.30 (1H, d, $J$=11.1 Hz, Hb-26), 4.60 (1H, m, H-16). The $^{13}$C-NMR data were assigned by the $^1$H-$^1$H COSY, HMQC and HMBC as shown in Experimental. NOESYS (Fig. 1) were observed between H$_3$-18 and H-20, between H-16 and H-17, and between H-17 and H-22, suggesting the configurations at C-22 and C-23 to be R and S, respectively. The remaining configuration at C-25 was determined to be S by the coupling constants of H$_2$-26.

Therefore the structure of esculeogenin B-1 (9) was characterized as (5$\alpha$,22R,23S,25S)-22,26-epimino-16$\beta$,23-epoxy-3$\beta$,23,27-trihydroxycholestane.

Thus conversion of spirosolane skeleton-type, esculeoside A, into solanocapsine-type skeleton, esculeogenin B, has successfully been attained. Its mechanism of conversion is deduced to be as shown in Chart 4.

**Experimental**

**General** Optical rotations were performed with a JASCO DIP-1000 KYU digital polarimeter (JASCO, Tokyo). MS were recorded on a JEOL JMS-700. $^1$H- and $^{13}$C-NMR spectra were recorded with a JEOL alpha 500 spectrometer at 500 and 125 MHz; band chemical shifts were given on a $\delta$ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Silica gel 60 (Merck, Art. 9385), Sephadex LH20 (Pharmacia Fine Chemicals), Chromatorex ODS (Fuji Silysia Chemical, Ltd.), and Diaion HP20 (Mitsubishi Chemical Industries Co., Ltd.) were used for column chromatography.

**Acid Hydrolysis of Esculeoside A (1) with 2N HCl** A solution of esculeoside A (1), 920 mg in 2N HCl (25 ml) was refluxed for 1.5 h. After neutralization with 3% KOH, the mixture was concentrated, added with water (100 ml), and passed through Diaion HP20 eluted first with water; then MeOH. The MeOH eluate was evaporated to give a residue, which was chromatographed on silica gel column with CHCl$_3$-MeOH-water=9:2:0.1 to 8:2:0.2 to give compound 2 (154 mg, 35%) as a major component and compound 3 (42 mg, 9%).

Compound 2: An amorphous powder, HR-FAB-MS (m/z): 632.3793 (Calcd for C$_{33}$H$_{55}$NO$_9$Na $^+$ 632.3774), $^1$H-NMR (in pyridine-$d_5$) $\delta$ 0.74
Acid Hydrolysis of Esculeoside A (1) with 2 N HCl in Dioxane and Water (1:1) Esculeoside A (1, 1200 mg) in 2 N HCl (55 ml) in a solution of dioxane and water (1:1) was refluxed for 1.5 h. After neutralization with 3% KOH, the mixture was concentrated, added with water (160 ml), and passed through Diaion HP20 eluted first with water, then MeOH. The MeOH eluate was evaporated to give a residue, which was chromatographed on silica gel column with CHC13–MeOH–water=9:2:1 to 8:2:0.2 to give a mixture of compound 8 and compound 9 (220 mg, 52%.

Next, separation using ODS with 65% MeOH led to the isolation of two kinds of escuegenic B, named escuengenic B-1 (9, 67 mg, 16% yield from 1) and escuegenic B-2 (8, 89 mg, 21% yield from 1).

Esuclegenin B-1 (9): An amorphous powder, HR-El-MS (m/z): 447.3256 (Caled for C33H55NO9. 447.3349) and [m/z] 96.2° (c=0.1, pyridine). 1H-NMR spectrum (in pyridine-d5) δ: 0.77 (3H, s, H3-19), 0.96 (3H, s, H3-18), 1.23 (3H, d, J=6.7 Hz, H21), 2.99 (1H, m, H-16), 3.01 (H, m, J=11.1 Hz, H-20), 3.30 (1H, d, J=11.1 Hz, H-26), 4.60 (1H, m, H-16). 1C-NMR (in pyridine-d5) δ: 38.5 (C-1), 32.5 (C-2), 70.6 (C-3), 37.6 (C-4), 45.4 (C-5), 29.2 (C-6), 32.5 (C-7), 35.4 (C-8), 53.5 (C-9), 36.0 (C-10), 21.4 (C-11), 40.3 (C-12), 41.2 (C-13), 56.6 (C-14), 32.4 (C-15), 78.9, 82.4 (C-16), 62.8, 62.9 (C-17), 16.9, 17.2 (C-18), 12.4, 12.5 (C-19), 35.2, 45.3 (C-20), 15.9, 16.6 (C-21), 25.1, 100.8 (C-22), 73.1, 75.0 (C-23), 36.5 (C-24), 35.3, 37.5 (C-25), 41.1, 45.2 (C-26), 63.5, 65.1 (C-27), β-D-glucopyranosyl moiety C-1 at δ 105.0, 75.3, 78.4, 71.8, 78.4, 62.9.

Acid Hydrolysis of Esculeoside A (1) with 3 N H2SO4–MeOH A solution of esculeoside A (1, 542 mg) in 3 N H2SO4 (22 ml) was refluxed for 1.5 h. After neutralization with 3% KOH, the mixture was concentrated, added with water (100 ml) and passed through Diaion HP20 eluted first with water, then MeOH. The MeOH eluate was evaporated to give a residue, which was chromatographed on silica gel column with CHCl3–MeOH–water=9:2:1 to 8:2:0.2 to give compound 4 (54.6 mg, 21%) as a major component and compound 5 (83.2 mg, 32%).

Compound 4: An amorphous powder, HR-El-MS (m/z): 632.3781 (Caled for C33H55NO9. 632.3774). 1H-NMR (in pyridine-d5) δ: 0.76 (3H, s, H3-19), 0.90 (3H, s, H3-18), 1.51 (3H, d, J=7.5 Hz, H21), 3.10 (1H, t- like, J=11.5 Hz, H-26), 4.78 (IH, d, J=7.6 Hz, glc H-1), 5.30 (IH, m, H-16). 13C-NMR (in pyridine-d5) δ: 37.3, 32.7, 70.4, 39.1, 45.5, 28.9, 32.4, 35.1, 54.5, 35.7, 21.4, 40.8, 40.5, 55.7, 34.1, 82.6, 63.5, 16.6, 12.4, 45.0, 16.3, 102.2, 72.7, 27.7, 43.8, 41.0, 71.5, (glucosyl C-1—6 at δ 104.5, 74.9, 78.4, 71.7, 78.4, 62.6.

Acid Hydrolysis of Esculeoside A (1) with 3 N H2SO4–MeOH A solution of esculeoside A (1, 850 mg) in 3 N H2SO4 (45 ml) was refluxed for 1.5 h. After neutralization with 3% KOH, the mixture was concentrated, added with water (140 ml), and passed through Diaion HP20 eluted first with water, then MeOH. The MeOH eluate was evaporated to give a residue, which was chromatographed on silica gel column with CHCl3–MeOH–water=9:2:1 to 8:2:0.2 to give compound 6 (75 mg, 25%) and compound 7 (53 mg, 13%), which were identified with escuegenic A and isoescuegenic A, respectively, as a major component and compound 2 (45 mg, 11%).

References