Conversion of Esculeoside A into Esculeogenin B

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Conversion of the spirosolane-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-trihydroxycholestane, and esculeogenin B-1, (5α,22R,23S,25S)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxycholestane, which are rare 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: Momo-Taro tomato and Mini tomato), the fruits of *Lycopersicon esculentum*, a spirosolane-type glycoside, esculeoside A (**1**),[1,2] 3-*O*-β-lycotetraosyl (5α,22*S*,23*S*,25*S*)-23-acetoxy-3β,27-dihydroxyspirosolane 27-*O*-β-D-glucopyranoside, was obtained, and its bioactivity anti-arteriosclerotic has been revealed.[3] On the other hand, from Italian San Marzano tomato (red-type), a solanocapsine-type glycoside,[4,5] esculeoside B,[2] 3-*O*-β-lycotetrosyl (5α,22*S*,23*R*,25*S*)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxycholestane 27-*O*-β-D-glucopyranoside, was isolated. Its framework is rare naturally occurring compound; therefore investigation of the bio-activity of its derivatives is desired. However, raw fresh Italian tomato is difficult to acquire. Although Italian can tomatoes are imported, it has been revealed that esculeoside B is not present in large amounts probably owing to decomposition during heat treatment of the can procedure. Therefore we planned a conversion of the spirosolane derivative esculeoside A (**1**) into the solanocapsine derivative esculeogenin B[6] by acid hydrolysis, because esculeogenin A,[1,2] the sapogenol of esculeoside A, is an isomer of esculeogenin B.

Since we desire esculeogenin B for pharmacological tests, we attempted conversion by acid hydrolysis of esculeoside A. First, we treated esculeoside A with 2 N HCl for 1.5 h by refluxing to give compound **2** in a yield of 35% along with compound **3** in a yield of 9%. The molecular formula of major compound **2** was estimated as $C_{33}H_{55}NO_9$ by HR-FAB-MS. Its various 2D-NMR spectra (^{1}H–^{1}H COSY, HMQC, HMBC) made the following assignments: The respective signals due to H_3-19, H_3-18, H_3-21, Ha-26, Hb-26, and H-16 appeared at δ 0.74 (3H, s), 1.00 (3H, s), 1.05 (3H, d, J=7.5 Hz), 2.94 (1H, d, J=11.6 Hz), 3.23 (1H, dd, J=3.2, 11.6 Hz), and 4.44 (1H, m). The signal due to one anomeric proton was also observed at δ 4.88 (1H, d, J=7.9 Hz). The ^{13}C-NMR spectrum showed signals due to the sapogenol C-1—27, which were almost coincident with those of esculeogenin A except around C-27, together with the presence of oneβ-D-glucopyranosyl moiety C-1—6. The HMBC between the anomeric proton of the glucosyl moiety and the C-27 indicated that the β-D-glucopyranosyl moiety links to the C-27 hydroxyl group. Therefore the structure of **2** was determined to be esculeogenin A 27-*O*-β-D-glucopyranoside. The molecular formula of **3** was measured as $C_{35}H_{57}NO_{10}$ by HR-FAB-MS. The ^{1}H-NMR signals were assigned as follows: δ 0.56 (1H, m, H-9), 0.76, 0.79 (each s, H_3-18), 0.81 (3H, s, H_3-19), 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 ^{13}C-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 ^{1}H- and ^{13}C-NMR signals appeared as split pattern at H_3-18, H_2-26, OAc, and glucosyl H-1, and C-16—C-23, C-25—27, the compound was conceivably a 1 : 1 mixture of C-22*S* and C-22*R*. The HMBC between anomeric proton and C-27 indicated that the β-D-glucopyranosyl moiety links to the C-27 hydroxyl group. Coupling constants due to H-23 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 isoesculeogenin A[4] 27-*O*-β-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 2 N HCl–MeOH for 1.5 h provided compound **4** and compound **5** in yields 21% and 32%, respectively. In the ^{1}H-NMR spectrum of **4**, the signal due to H_3-21 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_3-21 and H-16, causing extreme lower shifts for H_3-21 and H-16.[7] That is, the F-ring was reversed at C-22 configuration. The E-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 olefic methyl signal at δ 1.72 in the ^{1}H-NMR spectrum; on the other hand, the ^{13}C-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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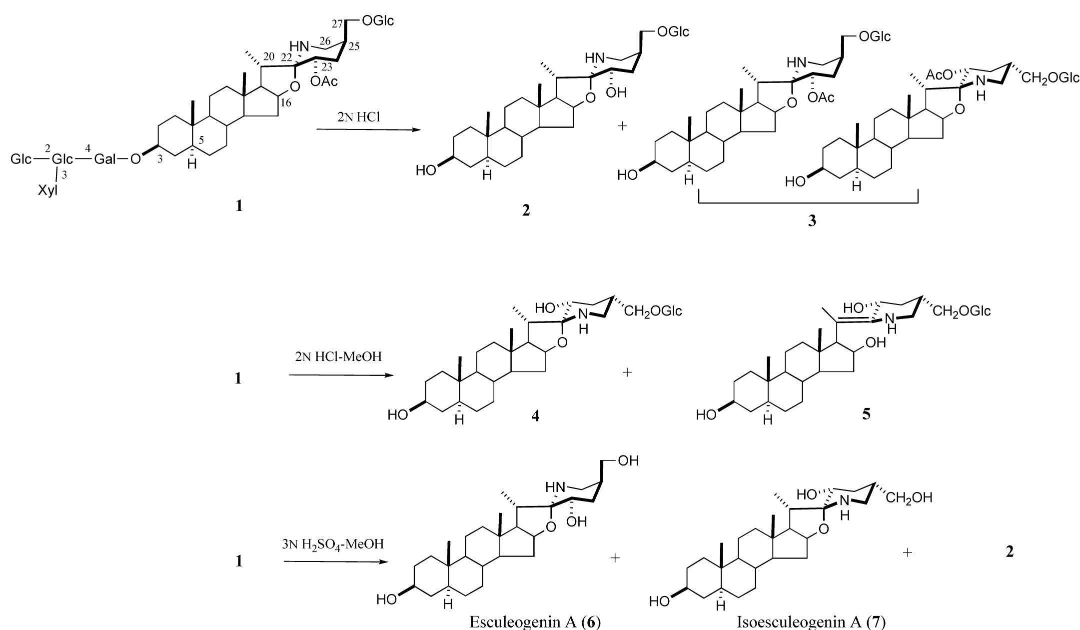


Chart 1

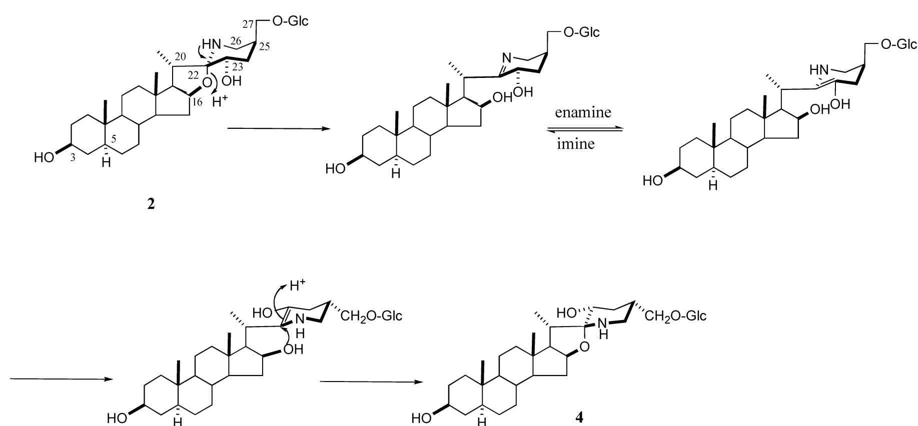


Chart 2

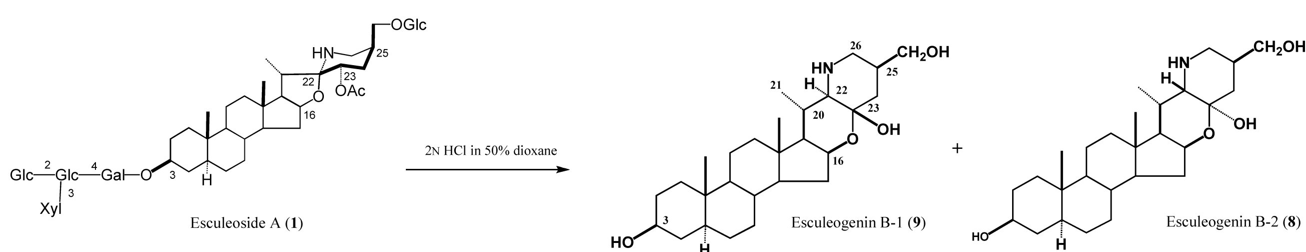


Chart 3

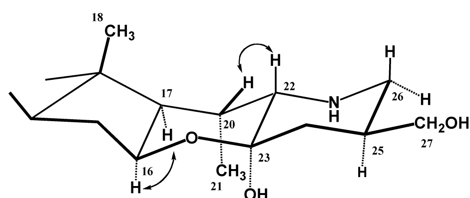
tions. Namely, esculeoside A (**1**) was hydrolyzed with 3 N H_2SO_4 and MeOH to provide esculeogenin A (**6**) in a yield of 25% and isoesculeogenin A (**7**)[4] in a yield of 13% along with compound **2** as shown in Chart 1. Here, we first could obtain the sapogenols; however, esculeogenin A was not 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 ^{1}H-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**).

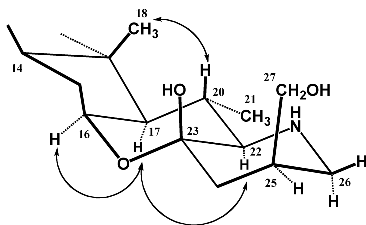
Esculeogenin B-2 (**8**) showed $C_{27}H_{45}NO_4$ by the HR-EI-MS and $[\alpha]_D$ $-96.2°$ (pyridine). In the ^{1}H-NMR spectrum

(in pyridine-d_5) displayed signals at δ 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, Ha-26), 3.40 (1H, br d, J=10.1 Hz, Hb-26), 3.75 (2H, d, J=10.2 Hz, H_2-27), 3.85 (1H, m, H-3), 4.92 (1H, m, H-16). 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 +0.32 ppm and H_3-21 by +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 (δ 1.23), and between H-20 (δ 2.99) and H-22 (δ 3.57) indicated that the configuration at C-22 was S.



Esculeoside B-2 (**8**)



Esculeoside B-1 (**9**)

Fig. 1. Key NOESYs of Esculeosides B-1 (**9**) and B-2 (**8**)

Consequently, esculeogenin B-2 (**8**) was characterized as (5α,22S,23R,25S)-22,26-epimino-16β,23-epoxy-3β,23,27-trihydroxycholestane, which was identical with the compound, esculeogenin B, previously obtained by enzymatic hydrolysis with tomatinase and β-glucosidase, in turn.[8]

Esculeogenin B-1 (**9**) showed $C_{27}H_{45}NO_4$ by the HR-EI-MS and $[\alpha]_D$ −68.2° (pyridine). In the ^{1}H-NMR spectrum (in pyridine-d_5) displayed signals at δ 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, Ha-26), 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 (5α,22R,23S,25S)-22,26-epimino-16β,23-epoxy-3β,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 δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Silica gel 60 (Merk, 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 2 N HCl A solution of esculeoside A (**1**, 920 mg) in 2 N 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) δ: 0.74

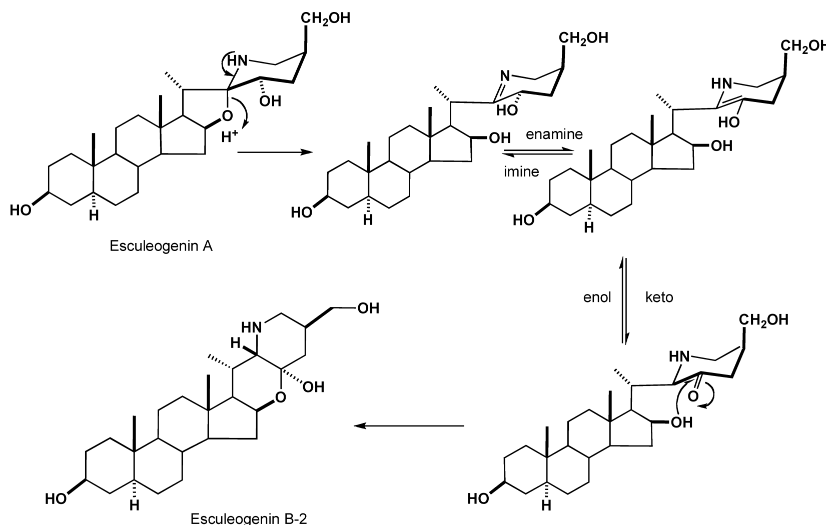


Chart 4

(3H, s, H_3-19), 1.00 (3H, s, H_3-18), 1.05 (3H, d, J=7.5 Hz, H_3-21), 1.81 (1H, d-like, J=8.2 Hz, H-17), 2.94 (1H, d, J=11.6 Hz, Ha-26), 2.99 (1H, t-like, J=7.3 Hz, H-20), 3.23 (1H, dd, J=3.2, 11.6 Hz, Hb-26), 4.36 (1H, dd, J=4.9, 11.6 Hz, glc Ha-6), 4.44 (1H, m, H-16), 4.88 (1H, d, J=7.9 Hz, glc H-1). ^{13}C-NMR (in pyridine-d_5) δ: 37.5, 32.3, 70.6, 39.3, 45.2, 29.1, 32.6, 35.2, 54.6, 35.9, 21.4, 40.8, 41.5, 56.5, 32.4, 79.3, 63.0, 17.3, 12.5, 34.7, 15.1, 101.6, 65.7, 17.3, 12.5, 34.7, 15.1, 101.6, 65.7, 33.2, 40.3, 35.9, 71.3, (glc C-1—6) δ: 105.0, 75.3, 78.4, 71.8, 78.4, 62.9.

Compound **3**: An amorphous powder, HR-FAB-MS (m/z): 674.3865 (Calcd for $C_{35}H_{57}NO_{10}+Na^+$ 674.3880). ^{1}H-NMR (in pyridine-d_5) δ: 0.56 (1H, m, H-9), 0.76, 0.79 (each s, H_3-18), 0.81 (3H, s, H_3-19), 2.14, 2.19 (each s, OAc), 2.96 (d, J=11.5 Hz, Ha-26), 3.30 (dd, J=3.5, 11.5 Hz), 3.06 (m, H_2-26), 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). ^{13}C-NMR (in pyridine-d_5): sapogenol C-1—27 at δ 37.5 (C-1), 32.4 (C-2), 70.6 (C-3), 39.2 (C-4), 45.3 (C-5), 29.1 (C-6), 32.4 (C-7), 35.2 (C-8), 54.5 (C-9), 35.9 (C-10), 21.4 (C-11), 40.3 (C-12), 41.2 (C-13), 56.6 (C-14), 32.4 (C-15), 79.8, 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), 97.6, 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 2 N HCl–MeOH A solution of esculeoside A (**1**, 542 mg) in 2 N HCl–MeOH (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 $CHCl_3$–MeOH–water=9 : 2 : 0.1 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-FAB-MS (m/z): 632.3781 (Calcd for $C_{33}H_{55}NO_9+Na^+$ 632.3774). ^{1}H-NMR (in pyridine-d_5) δ: 0.76 (3H, s, H_3-19), 0.90 (3H, s, H_3-18), 1.51 (3H, d, J=7.5 Hz, H_3-21), 3.10 (1H, t-like, J=11.5 Hz, Ha-26), 3.16 (1H, dd, J=3.1, 11.5 Hz, Hb-26), 4.78 (1H, d, J=7.6 Hz, glc H-1), 5.30 (1H, m, H-16). ^{13}C-NMR (in pyridine-d_5) δ: 37.3, 32.3, 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) δ: 104.5, 74.9, 78.4, 71.7, 78.4, 62.6.

Compound **5**: An amorphous powder, HR-FAB-MS (m/z): 632.3796 (Calcd for $C_{33}H_{55}NO_9+Na^+$ 632.3774). ^{1}H-NMR (in pyridine-d_5) δ: 0.79 (3H, s, H_3-19), 0.81 (3H, s, H_3-18), 1.72 (3H, s, H_3-21), 4.88 (1H, d, J=7.9 Hz, glc H-1). ^{13}C-NMR (in pyridine-d_5) δ: 37.2, 32.3, 70.4, 39.1, 42.0, 28.9, 33.3, 34.9, 54.8, 35.6, 20.8, 39.8, 39.8, 54.5, 31.7, 73.2, 53.9, 12.3, 13.1, 95.7, 19.4, 165.2, 66.5, 24.9, 45.1, 47.1, 70.2 (glucosyl C-1—6) δ: 104.9, 75.0, 78.5, 71.8, 78.5, 62.7.

Acid Hydrolysis of Esculeoside A (1) with 3 N H_2SO_4–MeOH A solution of esculeoside A (**1**, 850 mg) in 3 N H_2SO_4 (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 $CHCl_3$–MeOH–water=9 : 2 : 0.1 to 8 : 2 : 0.2 to give compound **6** (75 mg, 25%) and compound **7** (53 mg, 13%), which were identified with esculeogenin A and isoesculeogenin A, respectively, as a major component and compound **2** (45 mg, 11%).

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 $CHCl_3$–MeOH–water=9 : 2 : 0.1 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 esculeogenin B, named esculeogenin B-1 (**9**, 67 mg, 16% yield from **1**) and esculeogenin B-2 (**8**, 89 mg, 21% yield from **1**).

Esculeogenin B-1 (**9**): An amorphous powder, HR-EI-MS (m/z): 447.3256 (Calcd for $C_{27}H_{45}NO_4$: 447.3349) and $[\alpha]_D$ −68.2° (c=0.1, pyridine). ^{1}H-NMR spectrum (in pyridine-d_5) δ: 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), 2.99 (1H, m, H-20), 3.01 (1H, d, J=11.1 Hz, Ha-26), 3.30 (1H, d, J=11.1 Hz, Hb-26), 4.60. (1H, m, H-16). ^{13}C-NMR (in pyridine-d_5) 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), 55.3 (C-9), 36.0 (C-10), 21.0 (C-11), 37.5 (C-12), 43.0 (C-13), 53.6 (C-14), 33.9 (C-15), 69.1 (C-16), 58.3 (C-17), 15.0 (C-18), 12.5 (C-19), 27.3 (C-20), 18.0 (C-21), 62.2 (C-22), 94.1 (C-23), 39.3 (C-24), 42.0 (C-25), 48.8 (C-26), 65.0 (C-27).

Esculeogenin B-2 (**8**): An amorphous powder, HR-EI-MS (m/z): 447.3298 (Calcd for $C_{27}H_{45}NO_4$: 447.3349) and $[\alpha]_D$ −96.2° (c=0.1, pyridine). ^{1}H-NMR spectrum (in pyridine-d_5) δ: 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, Ha-26), 3.40 (1H, br d, J=10.1 Hz, Hb-26), 3.75 (2H, d, J=10.2 Hz, H_2-27), 3.85 (1H, m, H-3), 4.92 (1H, m, H-16). ^{13}C-NMR data were assigned as follows: δ 37.5 (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), 55.3 (C-9), 36.0 (C-10), 21.0 (C-11), 37.5 (C-12), 43.0 (C-13), 55.6 (C-14), 33.7 (C-15), 69.1 (C-16), 58.3 (C-17), 15.2 (C-18), 12.5 (C-19), 27.3 (C-20), 19.8 (C-21), 60.0 (C-22), 96.5 (C-23), 39.3 (C-24), 41.1 (C-25), 48.8 (C-26), 64.2 (C-27).

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