Mechanism for Conversion of Spirosolane Derivative into Pregnane

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Previously, we reported an interesting reaction by which esculeogenin A [(5α,22*S*,23*S*,25*S*)-3β,23,27-trihydroxyspirosolane], a sapogenol of tomato-saponin, esculeoside A, was easily converted into a pregnane derivative, 5α-pregn-16-en-3β-ol-20-one, merely by refluxing with pyridine and water. Its chemical mechanism including air oxidation is here described.

Key words tomato; spirosolane; pregnane

Previously, we discovered a novel reaction by which a sapogenol named esculeogenin A[1,2] [(5α,22*S*,23*S*,25*S*)-3β,23,27-trihydroxyspirosolane], of tomato-glycoside esculeoside A[1,2] was converted into a pregnane derivative, 5α-pregna-16-ene-20-one.[3] This reaction is regarded caused by the presence of the hydroxyl group at C-23, which would make the E and F rings fragile. Also, this reaction appears very important since it is expected that when steroidal glycosides such as spirostanol, furostanol, and spirosolane glycosides are administered orally, they would be metabolized to the hydroxylated C-23 and these intermediates would next be metabolized into pregnane derivatives showing various pharmacological bio-activities. Here, we report its reaction mechanism.[4,5]

Esculeoside A was refluxed with 2 N HCl–MeOH for 1.5 h to give the hydrolysate (**1**) in a yield of 38%. The molecular formula of **1** was estimated as $C_{33}H_{55}NO_9$ by FAB-MS. Its various 2D-NMR (FG-COSY, HMQC, HMBC) made the following assignments: The respective signals due to H_3-19, H_3-18, H_3-21, H-17, Ha-26, H-20, Hb-26, and H-16 appeared at δ 0.75 (3H, s), 1.00 (3H, s), 1.05 (3H, d, J=7.5 Hz), 1.81 (1H, d-like, J=8.2 Hz), 2.95 (1H, d, J=11.6 Hz), 3.00 (1H, t-like, J=7.3 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 data also suggested the existence of sapogenol C-1—27, together with one β-D-glucopyranosyl moiety C-1—6. The HMBC between anomeric proton and C-27 indicated β-D-glucopyranosyl moiety links to the C-27 hydroxyl group. Therefore, the structure of **1** was determined to be esculeogenin A 27-*O*-β-D-glucopyranoside.

Next, a solution of compound **1** in pyridine was kept at rt for 3 d at rt to provide compound **2** in a 68% yield. In the ^{1}H-NMR spectrum, the signal due to H_3-21 appeared at δ 1.51 (3H, d, J=7.5 Hz) and that 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 **1**. This indicated that the C-23-hydroxy group in equatorial configulation approached H_3-21 and H-16, causing extreme lower shifts for H_3-21 and H-16.[6] That is, the F-ring was reversed at C-22. The E-ring once opened to give enamine-imine-type intermediates, to which the 16-OH recyclization took place as shown in Chart 2.

A solution of compound **2** in pyridine was kept at rt for a further 2 d to afford compound **3** in a yield of 66%. Compound **3** 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 exhibited 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.

Subsequently, compound **3** was refluxed with pyridine and water (1 : 1) to provide compound **4** in a yield 52%. The mo-

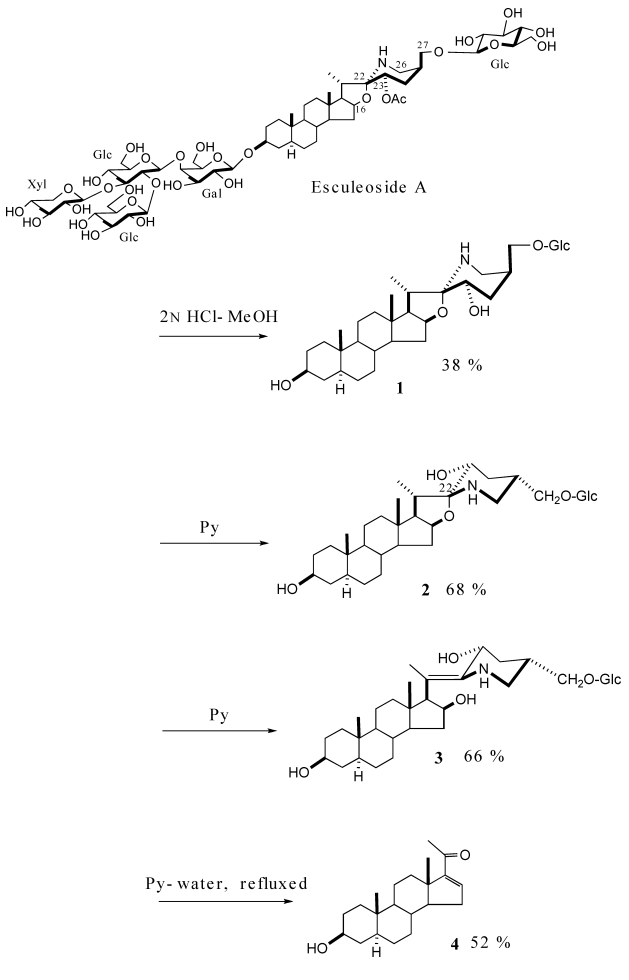

Chart 1

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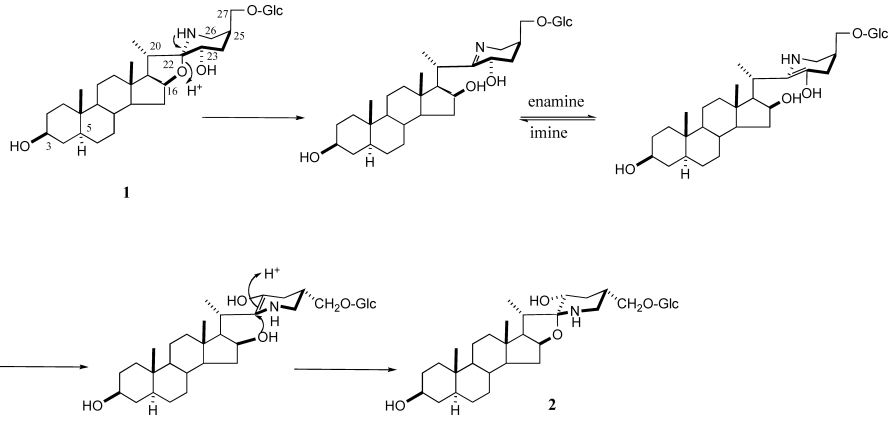

Chart 2

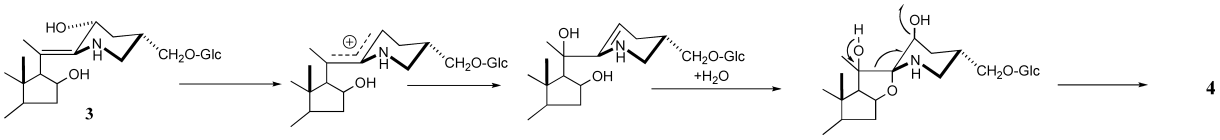

Chart 3

lecular formula was expressed as $C_{21}H_{32}O_2$ by the evidence of ^{13}C-NMR and EI-MS. Its ^{1}H-NMR spectrum displayed the signal at δ 2.26 due to H_3-21 and an olefic proton signal at δ 6.62 due to H-16. The ^{13}C-NMR spectrum had 21 carbon signals in total, among which one α,β-unsaturated carbonyl system was observed at δ 155.5, 144.7, and 196.3. Its structure was characterized as 5α-pregn-16-en-3β-ol-20-one, namely allopregnenolone.[7,8] This last reaction comprising a significant sensation was deduced to be as shown in Chart 3. Opening of E-ring was followed by formation of a double bond between C-20 and C-22. This was followed by transformation of the double bond into C-22 and C-23, and introduction of a hydroxyl group into C-20. To the double bond between C-22 and C-23, molecular H_2O was added. Next, elimination of the hydroxyl group at C-23 took place, followed by bond fission between C-20 and C-22 to provide compound **4** carrying a carbonyl group at C-20. Taking into consideration that this last reaction was not accelerated by introduction of oxygen and did not progress without H_2O, therefore, the reaction mechanism for this last stage was deduced as shown in Chart 3.

This reaction might be applied as a new synthetic pathway for production of steroidal hormone. Moreover, it might be predicted that when steroidal glycosides such as spirostanol and furostanol glycosides are administered orally, they would be metabolized to the C-23 hydroxylate and these intermediates would next be metabolized into pregnane derivatives showing various pharmacological bio-activities.

Experimental

General 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, respectively; band chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as 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.

Extraction and Separation of Esculeoside A The ripe fruits (865 g) of commercial mini tomato, *Lycopersicon esculentum* Mill., were smashed with water by mixer for 5—10 s and filtered with paper filter to give a filtrate, which was then passed through Diaion HP20 first with water then MeOH. The methanolc eluate was evaporated to give a residue. It was subsequently subjected to Sephadex LH20 with 95% MeOH to afford fractions (432 mg) including esculeoside A.

Acid Hydrolysis of Esculeoside A A solution of esculeoside A (420 mg) in 2 N HCl–MeOH (5 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 with water then MeOH, which was evaporated to give the residue. It was chromatographed on silica gel column chromatography with $CHCl_3$–MeOH–water=9 : 2 : 0.1 to 8 : 2 : 0.2 to give compound **1** (76.6 mg) as a major component. An amorphous powder, FAB-MS (*m/z*) 632 $[C_{33}H_{55}NO_9+Na]^+$, ^{1}H-NMR (in pyridine-d_5) δ: 0.75 (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), 2.95 (1H, d, J=11.6 Hz, H*a*-26), 3.00 (1H, t-like, J=7.3 Hz, H-20), 3.23 (1H, dd, J=3.2, 11.6 Hz, H*b*-26), 4.36 (1H, dd, J=4.9, 11.6 Hz, glc H*a*-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, 40.7, 41.5, 56.5, 32.4, 79.3, 63.0, 17.3, 12.5, 34.7, 15.1, 101.5, 65.7, 17.3, 12.5, 34.7, 15.1, 101.6, 65.8, 33.2, 40.3, 35.9, 71.3 (glucosyl C-1-6) δ: 105.0, 75.3, 78.5, 71.8, 78.4, 62.9.

Compound 2 A solution of compound **1** (120 mg) in pyridine (8 ml) was left to stand at rt for 3 d. Thereafter, the solvent was evaporated to provide a residue, which was separated by silica gel with $CHCl_3$–MeOH–water=8 : 2 : 0.2 to give compound **2** (81.6 mg). An amorphous powder, FAB-MS (*m/z*) 632 $[C_{33}H_{55}NO_9+Na]^+$, ^{1}H-NMR (in pyridine-d_5) δ: 0.75 (3H, s, H_3-19), 0.89 (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=7.9 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, 15.1, 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 3 A solution of compound **2** (96 mg) in pyridine (9 ml) was further kept standing at rt for 2 d. The solution was then evaporated to afford a residue, which was chromatographed on silica gel with $CHCl_3$–MeOH–water=8 : 2 : 0.2 to provide compound **3** (63.3 mg). An amorphous powder, FAB-MS (*m/z*) 632 $[C_{33}H_{55}NO_9+Na]^+$, ^{1}H-NMR (in pyridine-d_5) δ: 0.80 (3H, s, H_3-19), 0.82 (3H, s, H_3-18), 1.72 (3H, s, H_3-21), 4.81 (1H, d, J=7.5 Hz, glc H-1). ^{13}C-NMR (in pyridine-d_5) δ: 37.2, 32.4, 70.4, 39.1, 42.0, 28.9, 33.3, 34.9, 54.8, 35.6, 20.8, 39.8, 39.8, 54.6, 31.7, 73.2, 53.9, 12.3, 13.2, 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, 78.6, 62.7.

Compound 4 After a solution of compound **3** (67 mg) in pyridine (10 ml) was refluxed for 3 h, it was evaporated to give a residue, which was chromatographed on silica gel using *n*-hexane–acetone=3 : 1 to provide

compound **4** (18.1 mg), 5α-pregn-16-en-3β-ol-20-one, allopregnenolone. Colorless needles, mp 203—205 °C, EI-MS (*m*/*z*) 316 $[C_{21}H_{32}O_2]^+$, HR-EI-MS (*m*/*z*) 316.2432 (Calcd for $C_{21}H_{32}O_2$ 316.2402), ^{1}H-NMR (in pyridine-d_5) δ: 0.82 (3H, s, H_3-19), 0.94 (3H, s, H_3-18), 2.26 (3H, s, H_3-21), 3.85 (1H, m, H-3), 6.62 (1H, dd, J=1.8, 3.1 Hz, H-16). ^{13}C-NMR (in pyridine-d_5) δ: 32.5, 32.2, 70.6, 37.3, 45.5, 29.1, 32.3, 34.0, 56.6, 36.0, 21.4, 39.3, 46.6, 55.1, 35.4, 144.7, 155.5, 16.3, 12.4, 196.3, 30.5.

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