Conversion of Tomato Saponins to Pregnane Derivatives

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Here reports new conversions methods of tomato saponins, esculeoside A (1) and a mixture of esculeosides B-1 (2) and B-2 (3), (the latter two were obtained from tomato cans) into pregnane derivative (5) by an alkal treatment followed by acid treatment. Compound 1 or a mixture of 2 and 3 were each refluxed with 1N KOH to afford a characteristic pyridine steroidal glycoside (4), which was then treated with 2N HCl–MeOH to afford a pregnane derivative, 3β-hydroxy-5α-pregn-16-en-20-one (5). The results of the above two reactions indicated that tomato saponins are chemically closely related to pregnane hormones. We assume that the assimilated tomato saponins via the small intestine are metabolized into pregnane derivatives, demonstrating various bioactivities such as anti-cancer, anti-osteoporosis, and anti-menopausal disorder activities.

Key words tomato saponin; esculeoside A; esculeoside B; alkali treatment; pyridine steroidal derivative; pregnane

In 2003, Nohara and colleagues isolated a tomato saponin, named as esculeoside A (1),1,2) from the mature fruits of tomato, Solanum lycopersicum L., and determined its structure. Tomato saponin is a significant constituent of ripe tomatoes and is present in approximately four-fold higher levels than lycopene. Thus far, the bioactivity of tomato has been attributed solely to lycopene. Therefore, the pharmacological evaluation of 1 is important.

Recently, Fujiwara et al.3) reported that the oral administration of 1 to apolipoprotein E-deficient mice significantly reduced the serum levels of cholesterol glycerides, low-density lipoprotein cholesterol, and the severity of atherosclerotic lesions without any detectable side effects. In addition, we found an interesting chemical conversion: esculeogenin A, the sapogenol of 1, was easily converted to a pregnane derivative, 3β-hydroxy-5α-pregn-16-ene-20-one (5), by refluxing in aqueous pyridine,4,5) as shown in Chart 1, indicating that tomato saponin is closely related to the pregnane hormone from the viewpoint of chemical reaction.

Furthermore, we found a new method for the respective conversions of 1 or a mixture of esculeosides B-1 (2) and B-2 (3)6) to the pregnane derivative as follows:

Esculeoside A (1) was refluxed in 1N KOH for 1h. The reaction mixture was separated by silica gel column chromatography using a mixture of CHCl3–MeOH–H2O (8:2:0.2) to afford the major product (4), amorphous powder, [α]D

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−96.2°(pyridine), in 32% yield. The high-resolution negative-ion fast-atom-bombardment mass spectroscopy (HR-FAB-MS) of 4 exhibited the [M−H]− peak at m/z 1044.5009 [Calcd for C50H78O22N: 1044.5016]. The 1H-NMR (pyridine-d5) spectrum showed four methyl groups at δ 0.63 (3H, s), 1.16 (3H, s), 1.59 (3H, d, J = 6.9 Hz), and 2.11 (3H, s); two aromatic protons at δ 7.18 (1H, br s) and 8.18 (1H, br s); and four anomeric protons at δ 4.87 (1H, d, J = 7.5 Hz), 5.17 (1H, d, J = 7.5 Hz), 5.21 (1H, d, J = 7.4 Hz), and 5.54 (1H, d, J = 6.9 Hz). The 13C-NMR (pyridine-d5) spectrum showed five aromatic carbons at δ 123.2, 131.3, 140.8, 150.0, and 151.0; two oxygen-bearing carbons at δ 77.2, and 77.4; four methyl carbons at δ 12.1, 13.7, 17.6, and 19.4; eight methylene carbons at δ 21.0, 28.8, 29.7, 32.1, 34.6, 35.2, 37.0, and 40.3; six methine carbons at δ 35.6, 37.0, 44.5, 54.4, 54.6, and 62.8; two quaternary carbons at δ 35.6, and 42.8; and a β-lycotetraosyl moiety at δ 102.2, 71.9, 74.9, 79.8, 75.4, 60.3 (β-galactopyranosyl C-1–C-6), 104.8, 81.2, 86.5, 70.5, 78.6, 61.5 (inner β-glucopyranosyl C-1–C-6), 105.0, 74.9, 78.5, 70.8, 78.5, 62.2 (terminal β-glucopyranosyl C-1–C-6), 104.7, 75.2, 76.0, 70.3, 67.2 (β-xylopyranosyl C-1–C-5). The heteronuclear multiple-bond correlation (HMBC) showed that the methyl group at δ 2.11 correlated to the three aromatic carbons at δ 123.2, 131.3, and 140.8, indicating this methyl group as C-27; the aromatic protons at δ 7.18 and 8.18 were assigned.
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The HR-FAB-MS and HMBC results showed that the E-ring is open, and the hydroxyl groups are present at the C-16 and C-23 positions. Therefore, the structure of 4 was deduced to be a pyridine derivative as shown in Chart 2.

On the other hand, a mixture of esculeoside B-1 (2) and esculeoside B-2 (3) was refluxed in 1N KOH for 1 h. After neutralizing with 1N HCl, the reaction mixture was concentrated under reduced pressure and passed through Diaion HP-20 to afford the crude material, which was purified by using silica gel chromatography using a mixture of CHCl₃–MeOH–H₂O (8 : 2 : 0.2) to afford the major compound, amorphous powder, [α]D −98.5°(pyridine), in 34% yield, as shown in Chart 3. The negative FAB-MS showed the [M−H]⁻ peak at m/z 1044.5012 [Calcd for C₅₀H₇₈O₂₂N]; the ¹H-NMR (pyridine-d₅) and ¹³C-NMR (pyridine-d₅) spectra were identical to those of compound 4.

Next, compound 4 was refluxed in 2N HCl–MeOH for 1 h, and the resulting reaction mixture was purified by using a mixture of n-hexane–acetone (5:1) to afford compound 5 (in 27% yield from 4). The HR positive FAB-MS showed the [M+H]⁺ peak at m/z 317.2484 (Calcd for C_{21}H_{33}O_{2}: 317.2481). The ¹H-NMR spectrum (in CDCl₃) showed three methyl groups at δ 0.77 (3H, s), 0.81 (3H, s), and 2.18 (3H, s); an oxygen-bearing methane proton at δ 3.53 (1H, m); and an olefinic proton at δ 6.62 (1H, dd, J=1.8, 3.1 Hz). Moreover, the ¹³C-NMR spectrum showed a total of 21 carbons including three methyl groups at δ 12.2, 15.9, and 28.5; one oxygen-bearing methine carbon at δ 71.3; two olefinic carbons at δ 144.4 and 155.5; and a carbonyl carbon at δ 196.8. Thus, which The product was identified as 3β-hydroxy-5α-pregn-16-en-3β-ol-20-one, as shown in Chart 4. The above these reactions are summarized in Chart 5.

Our recent studies on the constituents of Solanum plants
have revealed that pregnant glycosides are accompanied with normal spirostanol and furostanol glycosides. Esculeogenin A was easily converted into a pregnane derivative, 3β-hydroxy-5α-pregn-16-ene-20-one (5) by refluxing in aqueous pyridine. Both 1, and a mixture of esculosesides B-1 (2) and B-2 (3) were converted to the same pregnane derivative by refluxing in a KOH solution, followed by the reaction with HCl/MEOH. Moreover, a pregnane glycoside has been isolated from over-ripe tomato. The above facts strongly indicate that orally administered steroidal glycosides can be metabolized into pregnane derivatives. These androsterone analogs are normally excreted; however, because no such analogs were detected in the control samples, their occurrence indicated the production of progestosterone by the subjects that had consumed tomatoes. We hypothesize that the orally administered steroidal glycosides such as spirostanol, furostanol and spirostanol glycosides are metabolized, thus introducing a hydroxyl group at C-23. These intermediates may then be metabolized into pregnane derivatives. We conclude that the tomato saponin 1 is metabolized into various steroidal hormones such as pregnane, with anti-estrogenic, anti-menopausal disorder, and anti-tumor bioactivities in the body.

Experimental

General Procedure

Optical rotations were measured with a JASCO P-1020 (l=0.5) digital polarimeter. FAB-MS were obtained with a JEOL JMS-DX300 and a JMS-DX 303 HF FAB-MS were obtained with a glycerol matrix in the positive ion mode using a JEOL JMS-DX300 and a JMS-DX 303 HF FAB-MS. HR-FAB-MS was performed with a JEOL JMS-DX300 and a JEOL JMS-DX 303 HF FAB-MS. 

Conversion of Esculeoside A (1) into Pyridine Derivative (4)

A solution of esculoseide A (1, 432 mg) in 1N KOH (22 mL) was refluxed for 1 h on the oil bath. After neutralization with 1N HCl, the reaction mixture was concentrated under reduced pressure to give the reaction mixture, to which was water added and passed through Diaion HP-20. It was first washed with water and next eluted with methanol to give the crude material, which was then separated and purified by silica gel chromatography with CHCl3-MeOH for 1 h and neutralized with 1N HCl, the reaction mixture was concentrated under reduced pressure and passed through Diaion HP-20. It was first washed with water and next eluted with methanol to give the crude material, which was purified by using silica gel chromatography with CHCl3-MeOH-H2O (8:2:0.2) to provide major compound (3) and B-2 (3). 

Conversion of a Mixture of Esculeosides B-1 (2) and B-2 (3) into Pyridine Derivative (4)

A mixture of esculoseide B-1 (2, 255 mg) and esculoseide B-2 (3) was refluxed with 1N KOH for 1 h. After neutralization with 1N HCl, the reaction mixture was concentrated under reduced pressure and passed through Diaion HP-20. It was first washed with water and next eluted with methanol to give the crude material, which was purified by using silica gel chromatography with CHCl3-MeOH-H2O (8:2:0.2) to provide major compound, an amorphous powder (74 mg), [α]D = -98.5°(pyridine), in 3% yield. The negative FAB-MS exhibited m/z 1044.5009 [Calcd for C50H78O22N: 1044.5016].

Conversion of Pyridine Derivative (4) into Pregnane Derivative (5)

Compound 4 (165 mg) was refluxed with 2N HCl-MeOH for 1 h and neutralized with 2N KOH-MeOH, concentrated under reduced pressure to give a syrup, which was added with water, and passed through Diaion HP-20. First water eluate was discarded and second methanolic eluate was evaporated to dryness, which was then separated and purified with silica gel column chromatography with n-hexane- acetone (5:1) to give compound 5 (14 mg, in 27% yield from 4). Colorless needles, mp 203–205°C, [α]D = +48.2° (c=1.0, CHCl3). HR-FAB-MS (m/z): m/z 317.2484 [M+H]+ (Calcd for C15H20O7: 317.2481). 1H-NMR spectrum (CDCl3): δ 0.81 (3H, H3-18), 2.18 (3H, s, H3-21), 3.53 (1H, m, H-16). 13C-NMR spectrum (CDCl3): δ: 0.81 (3H, H3-18), 2.18 (3H, s, H3-21), 3.53 (1H, m, H-3), 6.62 (1H, d, J=1.8, 3.1Hz, H-16). 13C-NMR spectrum (CDCl3): δ: 3.7 (C-17), 32.2 (C-2), 71.3 (C-3), 36.7 (C-4), 45.0 (C-5), 27.1 (C-6), 31.5 (C-7), 31.9 (C-8), 56.3 (C-9), 35.6 (C-10), 104.7, 75.2, 76.0, 70.3, 74.6, 79.4, 78.5, 70.8, 78.5, 62.2; xylopyranosyl C-1,C-5 at δ 104.7, 75.2, 76.0, 70.3, 67.2.

Conversion of Pyridine Derivative (4) into Pregnane Derivative (5) Compound 4 (165 mg) was refluxed with 2N HCl-MeOH for 1 h and neutralized with 2N KOH-MeOH, concentrated under reduced pressure to give a syrup, which was added with water, and passed through Diaion HP-20. First water eluate was discarded and second methanolic eluate was evaporated to dryness, which was then separated and purified with silica gel column chromatography with n-hexane–acetone (5:1) to give compound 5 (14 mg, in 27% yield from 4). Colorless needles, mp 203–205°C, [α]D = +48.2° (c=1.0, CHCl3). HR-FAB-MS (m/z): m/z 317.2484 [M+H]+ (Calcd for C15H20O7: 317.2481). 1H-NMR spectrum (CDCl3): δ 0.81 (3H, H3-18), 2.18 (3H, s, H3-21), 3.53 (1H, m, H-16). 13C-NMR spectrum (CDCl3): δ: 0.81 (3H, H3-18), 2.18 (3H, s, H3-21), 3.53 (1H, m, H-3), 6.62 (1H, d, J=1.8, 3.1Hz, H-16). 13C-NMR spectrum (CDCl3): δ: 3.7 (C-17), 32.2 (C-2), 71.3 (C-3), 36.7 (C-4), 45.0 (C-5), 27.1 (C-6), 31.5 (C-7), 31.9 (C-8), 56.3 (C-9), 35.6 (C-10), 21.0 (C-11), 38.1 (C-12), 46.3 (C-13), 54.8 (C-14), 34.7 (C-15), 144.4 (C-16), 155.5 (C-17), 15.9 (C-18), 12.2 (C-19), 196.8 (C-20), 28.5 (C-21), which was identified with 3β-hydroxy-5α-pregn-16-ene-3β-ol-20-one.

References


