A Pregnane Glycoside from Overripe Tomato

Toshihiro NOHARA,*[,a] Eriko IWAKAWA,[a] Sayaka MATSUSHITA,[b] Yukio FUJIWARA,[b] Tsuyoshi IKEDA,[b] Hiroyuki MIYASHITA,[b] Masateru ONO,[c] and Hitoshi YOSHIMITSU[a]

[a] *Faculty of Pharmaceutical Sciences, Sojo University; 4–22–1 Ikeda, Kumamoto 860–0082, Japan:* [b] *Faculty of Medical and Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan: and* [c] *School of Agriculture, Tokai University; Kawayou, Minamiaso-mura, Aso-gun, Kumamoto 869–1404, Japan.*

Received February 6, 2008; accepted April 2, 2008; published online April 18, 2008

A new pregnane glycoside, 3-*O*-β-lycotetraosyl 5α-pregna-3β,26β-diol-20-one was isolated from overripe tomato, the fruit of *Lycopersicon esculentum* MILL.

Key words pregnane glycoside; tomato; *Lycopersicon esculentum*

For the first time, we isolated a pregnane glycoside together with a significant quantity of diosgenin glycosides from *Paris polyphilla*,[1] which has been extensively used in Chinese medicine. In recent years, many pregnane glycosides had been obtained from *Dioscorea*,[2] *Allium*,[3] *Tacca*,[4] *Solanum*,[5] and *Cestrum*[6] genera. The occurrence of the pregnane compounds suggests that they might be biosynthesized in the plant internally from furostanol and spirostanol glycosides by a reaction similar to Marker degradation.[7] Furthermore, this indicates that administered steroidal glycosides might be metabolized into pregnane derivatives possessing various activities.

Meanwhile, we isolated a 3-*O*-β-lycotetraosyl pregnane[8] as a minor component from the overripe tomato, *Lycopersicon esculentum* MILL., fruit. This indicated that the type of steroidal glycoside varies as tomato matures, that is, tomatine in the green immature fruit is oxidized at C-23 and C-27 in the ripe fruit to give a tomato major steroidal glycoside, esculeoside A.[9] Further, esculeoside A is converted into the pregnane glycoside in the overripe fruit. This seasonal variation also suggests variation in internal metabolism of this fruit in humans.

Here, in addition to the previously isolated 3-*O*-β-lycotetraosyl 5α-pregna-3β-ol-16-ene-20-one, we have obtained a valuable pregnane glycoside.

The overripe mini-tomato was blended by mixer with water for several seconds. The mixture was filtered by filter paper to give yellow transparent filtrate, which was subsequently passed through high-porous polystyrene gel (Diaion HP20) firstly with water then with MeOH. The methanolic eluate was next evaporated to dryness to give a residue, which was then subjected to Sephadex LH-20 by eluting with 90% MeOH to give two fractions containing steroid glycosides and aromatic compounds. The former fraction was subjected to ODS column chromatography to afford compound **1** as an amorphous powder (yield 0.0067%), showing $[\alpha]_D$ −55.2° (MeOH), together with esculeoside A[9] and 3-*O*-lycotetraosyl 5α-pregna-3β-ol-16-ene-20-one.[8] The HR-FAB-MS of **1** showed a peak at *m*/*z* 975.5960 due to $C_{44}H_{72}NO_{22}$. The ^{1}H-NMR spectrum displayed three tertiary methyl groups at δ 0.63 (3H, s), 1.23 (3H, s), and 2.10 (3H, s) and four anomeric protons at δ 4.88 (1H, d, J=7.3 Hz), 5.20 (1H, d, J=7.9 Hz), 5.25 (1H, d, J=7.9 Hz), and 5.59 (1H, d, J=7.3 Hz). Regarding the ^{13}C-NMR spectrum, signals due to the sugar moiety were assigned by comparing with those of the β-lycotetraosyl moiety in esculeoside A as follows: inner β-D-galactopyranosyl C-1—6, δ 102.6, 73.4, 74.6, 78.7, 74.6, 60.7, inner β-D-glucopyranosyl C-1—6: δ 105.0, 81.3, 86.9, 70.5, 76.2, 62.6, terminal β-D-glucopyranosyl C-1—6: δ 105.1, 75.1, 78.7, 71.8, 77.8, 62.6, terminal β-D-xylopyranosyl C-1—5, δ 104.8, 73.2, 77.6, 70.8, 67.4. When these signals were deducted from the whole signals, the remainder constituted of 21 carbons, namely pregnane skeleton, of which ^{13}C signals were assigned by the help of FG-COSY, HMQC, HMBC (from H_3-18 at δ_H 1.23 to C-13 at δ_C 44.6, to C-12 at δ_C 39.2, to C-14 at δ_C 53.8, and C-17 at δ_C 70.5; from H_3-19 at δ_H 0.63 to C-10 at δ_C 35.5, C-5 at δ_C 45.3, to C-1 at δ_C 37.1, and to C-9 at δ_C 54.4, from H_3-21 at δ_H 2.10 to C-20 at δ_C 211.0 and to C-17 at δ_C 70.5. Thus the respective carbon signals of the sapogenol moiety were assigned as follows: δ 37.1 (C-1), 29.9 (C-2), 77.6 (C-3), 34.9 (C-4), 45.3 (C-5), 29.0 (C-6), 32.2 (C-7), 35.2 (C-8), 54.4 (C-9), 35.5 (C-10), 21.1 (C-11), 39.2 (C-12), 44.6 (C-13), 53.8 (C-14), 37.4 (C-15), 71.1 (C-16), 70.5 (C-17), 14.9 (C-18), 12.3 (C-19), 211.0 (C-20), 32.0 (C-21). Moreover, the HMBC was observed between H-16 at δ 5.07, and between H-1 at δ 4.38 of the inner galactosyl moiety and C-3 at δ 77.6. Therefore **1** was deduced to be 3-*O*-β-lycotetraosyl pregnane derivative. Compound **1** would be a precursor of 3-*O*-β-lycotetraosyl pregnane.[8]

Next, **1** was enzymatically hydrolyzed with tomatinase[10] to afford compound **2** as colorless needles showing mp 193—197 °C and $[\alpha]_D$ +67.4° (pyridine). Various 2D NMR

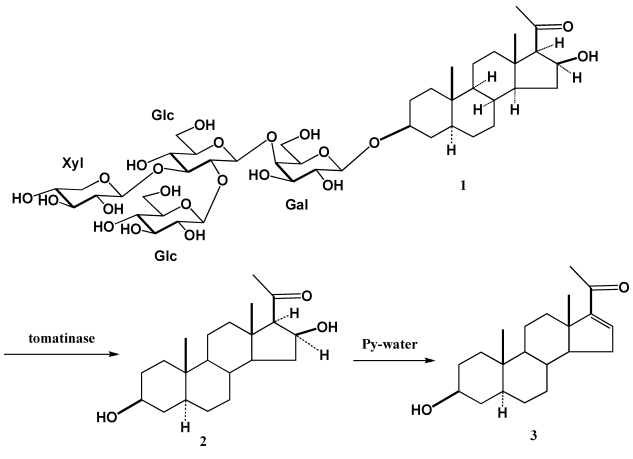

Chart 1

* To whom correspondence should be addressed. e-mail: none@ph.sojo-u.ac.jp

measurements made the following ^{1}H and ^{13}C signals assignments: δ_H 0.82 (3H, s, H_3-19), 1.43 (3H, s, H_3-18), 2.33 (3H, s, H_3-21), 3.86 (1H, m, H-3), 4.94 (1H, m, H-16); δ_C 37.5 (C-1), 32.4 (C-2), 70.6 (C-3), 39.0 (C-4), 45.3 (C-5), 29.1 (C-6), 32.5 (C-7), 34.9 (C-8), 54.9 (C-9), 35.9 (C-10), 21.0 (C-11), 39.3 (C-12), 42.4 (C-13), 54.4 (C-14), 38.2 (C-15), 71.8 (C-16), 69.3 (C-17), 14.7 (C-18), 12.5 (C-19), 208.5 (C-20), 30.8 (C-21). The NOESYs were observed between H_3-18 and H_3-21, and between H-17 and H-16. No NOESY between H_3-18 and H-17 was observed. Therefore the structure of **2** was determined to be 5α-pregna-3β,16β-diol-20-one, lycopersiconol[11] isolated from tomato stock roots.

Next, **2** was refluxed with pyridine and water (1 : 1) to provide compound **3**. Compound **3** was obtained as colorless needles showing mp 203—205 °C, $[\alpha]_D$ +48.2° (pyridine). Measurements of 2D NMR spectra led to assignment of the following ^{1}H and ^{13}C signals: δ_H 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): δ_C 32.5 (C-1), 32.2 (C-2), 70.6 (C-3), 37.3 (C-4), 45.5 (C-5), 29.1 (C-6), 32.3 (C-7), 34.0 (C-8), 56.6 (C-9), 36.0 (C-10), 21.4 (C-11), 39,3 (C-12), 46.6 (C-13), 55.1 (C-14), 35.4 (C-15), 144.7 (C-16), 155.5 (C-17), 16.3 (C-18), 12.4 (C-19), 196.3 (C-20), 30.5 (C-21). Threfore **3** was characterized as 5α-pregna-16-ene-3β-ol-20-one, allopregnenolone.[12] Consequently, the structure of **1** was elucidated as 3-*O*-β-D-xylopyranosyl(1—3)-[β-D-glucopyranosyl-(1—2)]-β-D-glucopyranosyl-(1—4)-β-D-galactopyranosyl (β-lycotetraosyl) 5α-pregna-3β,16β-diol-20-one.

Taking into consideration the result that administered tomato excreted many androstane derivatives in urine probably metabolized *via* the production of progesterone by the consumed tomatoes,[13] it might be possible to predict 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.[14]

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, respectively; 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.

Extraction and Separation of Esculeoside A The overripe fruits (840 g) of commercial mini tomato, *Lycopersicon esculentum* MILL., were smashed with water by mixer for 5—10 s then filtered with filter paper to give a filtrate, which was then passed through Diaion HP20 first with water, then with MeOH. The methanolic eluate was evaporated to give a residue. It was subsequently subjected to Sephadex LH20 with 90% MeOH to afford the first and second fractions (815, 132 mg, respectively). The first eluate was then subjected to ODS column chromatography with 65% MeOH to provide Compound **1** (56 mg, 0.00067%) together with esculeoside A (212 mg) and pregnane glycoside (60 mg, 0.0071%).

Compound 1 An amorphous powder, $[\alpha]_D$ −55.2° (c=1.0, MeOH). HR-FAB-MS (m/z) 952.5960 $[C_{44}H_{72}NO_{22}]^+$ (Calcd for 952.5962), ^{1}H-NMR (in pyridine-d_5) δ: 0.63 (3H, s, H_3-19), 1.23 (3H, s, H_3-18), 2.10 (3H, s, H_3-21), 4.88 (1H, d, J=7.3 Hz, galactosyl H-1), 5.07 (1H, m, H-16), 5.20 (1H, d, J=7.9 Hz, inner glucosyl H-1), 5.25 (1H, d, J=7.9 Hz, xylosyl H-1), 5.59 (1H, d, J=7.3 Hz, terminal glucosyl H-1). ^{13}C-NMR (in pyridine-d_5+D_2O) δ: sapogenol: 37.1 (C-1), 29.9 (C-2), 77.6 (C-3), 34.9 (C-4), 45.3 (C-5), 29.0 (C-6), 32.2 (C-7), 35.2 (C-8), 54.4 (C-9), 35.5 (C-10), 21.1 (C-11), 39.2 (C-12), 44.6 (C-13), 53.8 (C-14), 37.4 (C-15), 71.1 (C-16), 70.5 (C-17), 14.9 (C-18), 12.3 (C-19), 211.0 (C-20), 32.0 (C-21). 3-*O*-Gal: δ 102.6 (C-1), 73.4 (C-2), 74.6 (C-3), 78.7 (C-4), 74.6 (C-5), 60.7 (C-6), inner Glc 105.0 (C-1), 81.3 (C-2) , 86.9 (C-3), 70.5 (C-4), 76.2 (C-5), 62.6 (C-6), Xyl 104.8 (C-1), 73.2 (C-2), 77.6 (C-3), 70.8 (C-4), 67.4 (C-5), terminal Glc 105.1 (C-1), 75.1 (C-2), 78.7 (C-3), 71.8 (C-4), 77.8 (C-5), 62.6 (C-6).

Compound 2 A crude tomatinase solution was prepared as reported in a previous paper.[10] The tomatinase solution extracted with phosphate buffer at pH 7.0 (2.0 ml) was added to compound **1** (50 mg) dissolved in DMSO (0.2 ml) and the reaction mixture was kept at room temperature overnight. After finishing the reaction check by TLC, MeOH (2.0 ml) was added to the mixture and a precipitate was removed by centrifuge; the supernatant was passed through a MCI gel (5 ml) and eluted with H_2O (20 ml) and MeOH (20 ml). The MeOH eluate was concentrated under reduced pressure to give crude compound **2** and was further purified by silica gel column chromatography ($CHCl_3$–MeOH=30 : 1) to afford compound **2** (9 mg). Colorless needles, mp 195—197 °C, $[\alpha]_D$ +67.4° (c=1.00, pyridine). ^{1}H-NMR (in pyridine-d_5) δ: 0.82 (3H, s, H_3-19), 1.43 (3H, s, H_3-18), 2.33 (3H, s, H_3-21), 3.86 (1H, m, H-3), 4.94 (1H, m, H-16). ^{13}C-NMR (in pyridine-d_5) δ: sapogenol: 37.5 (C-1), 32.4 (C-2), 70.6 (C-3), 39.0 (C-4), 45.3 (C-5), 39.1 (C-6), 32.5 (C-7), 34.9 (C-8), 54.9 (C-9), 35.9 (C-10), 21.0 (C-11), 39.3 (C-12), 42.4 (C-13), 44.4 (C-14), 38.2 (C-15), 71.8 (C-16), 69.3 (C-17), 14.7 (C-18), 12.5 (C-19), 208.5 (C-20), 30.8 (C-21).

Compound 3 A solution of compound **2** (11 mg) dissolved in pyridine (3 ml) and water (3 ml) was refluxed for 2 h. The reaction mixture was evaporated to give a residue, which was chromatographed with *n*-hexane–acetone (4 : 1) to afford compound **3** (5 mg). Colorless needles, mp 203—205 °C, $[\alpha]_D$ +48.2° (c=1.00, pyridine). ^{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, 3.4 Hz, H-16). ^{13}C-NMR (in pyridine-d_5+D_2O) δ: sapogenol: 32.5 (C-1), 32.2 (C-2), 70.6 (C-3), 37.3 (C-4), 45.5 (C-5), 29.1 (C-6), 32.3 (C-7), 34.0 (C-8), 56.6 (C-9), 36.0 (C-10), 21.4 (C-11), 39.3 (C-12), 46.6 (C-13), 55.4 (C-14), 35.4 (C-15), 144.7 (C-16), 155.5 (C-17), 16.3 (C-18), 12.4 (C-19), 196.3 (C-20), 30.5 (C-21).

Acknowledgements This work was supported by a Grant-in-Aid for the Takeda Science Foundation and JSPS Asian Core Program.

References and Notes

1) Nohara T., Yabuta H., Suenobu H., Hida R., Miyahara K., Kawasaki T., *Chem. Pharm. Bull.*, **21**, 1240—1247 (1973).
2) Dong M., Feng X., Wang B., Wu L., Ikejima T., *Tetrahedron Lett.*, **57**, 501—106 (2001).
3) Ikeda T., Tsumagari H., Okawa M., Nohara T., *Chem. Pharm. Bull.*, **52**, 142—145 (2004).
4) Yokosuka A., Mimaki Y., Sashida Y., *J. Nat. Prod.*, **65**, 1293—1298 (2002).
5) Zhu X., Tsumagari H., Honbu T., Ikeda T., Ono M., Nohara T., *Tetrahedron Lett.*, **42**, 8043—8046 (2001).
6) Mimaki Y., Watanabe K., Sakagami H., Sashida Y., *J. Nat. Prod.*, **65**, 1863—1868 (2002).
7) Marker R. E., Tsukamoto T., Turner D. L., *J. Am. Chem. Soc.*, **62**, 2525—2532 (1940).
8) Fujiwara Y., Yoshizaki M., Matsushita M., Yahara S., Yae E., Ikeda T., Ono M., Nohara T., *Chem. Pharm. Bull.*, **53**, 580—585 (2005).
9) Fujiwara Y., Takaki A., Uehara Y., Ikeda T., Okawa M., Yamauchio K., Ono M., Yoshimitsu H., Nohara T., *Tetrahedron*, **60**, 4915—4920 (2004).
10) Eto S., Eto T., Tanaka S., Yamauchi N., Takahara H., Ikeda T., *FEBS Lett.*, **571**, 31—34 (2004).
11) Yoshihara T., Nagaoka, T. Sakamura S., *Phytochemistry*, **27**, 3982—3984 (1988).
12) Sato H., Sakamura S., *Agric. Biol. Chem.*, **37**, 225—231 (1973).
13) Noguchi E., Fujiwara Y., Matsushita S., Ikeda T., Ono M., Nohara T., *Yakugaku Zasshi*, **126**, 104—105 (2006).
14) Nohara T., Ikeda T., Fujiwara Y., Matsushita S., Noguchi E., Yoshimitsu H., Ono M., *J. Nat. Med.*, **61**, 1—13 (2007).