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

† 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, which has been extensively used in Chinese medicine. In recent years, many pregnane glycosides had been obtained from Dioscorea, Allium, Tacca, Solanum, and Cestrum 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. Furthermore, this indicates that administered steroid glycosides might be metabolized into pregnane derivatives possessing various activities.

Meanwhile, we isolated a 3-O-β-lycotetraosyl pregnane 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, esculetoside A. Further, esculetoside A is converted into the pregane 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,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 subjected to ODS column chromatography to afford compound 1 as colorless needles showing mp 193–197 °C and [α]D 67.4° (pyridine). Various 2D NMR spectra of compound 1 included a peak at m/z 975.5960 due to C22H26NO12. The 1H-NMR spectrum displayed three tertiary methyl groups at δ 0.63 (3H, s), 1.23 (3H, s), and 2.10 (3H, s) and four anomic 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 13C-NMR spectrum, signals due to the sugar moiety were assigned by comparing with those of the β-lycotetraosyl moieties in esculetoside 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-xylpyranosyl 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 13C signals were assigned by the help of FG-COSY, HMOC, HMBC (from H2-18 at δH 1.23 to C-13 at δC 44.6, to C-12 at δC 39.2, to C-14 at δC 53.8, and C-17 at δC 70.5; from H-19 at δH 0.63 to C-10 at δC 35.5, C-5 at δC 45.3, to C-1 at δC 37.1, and to C-9 at δC 54.4, from H2-21 at δH 2.10 to C-20 at δC 211.0 and to C-17 at δC 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 to afford compound 2 as colorless needles showing mp 193–197 °C and [α]D 67.4° (pyridine). Various 2D NMR
measurements made the following $^1$H and $^{13}$C signals assignments: $\delta_{H}$ 0.82 (3H, s, H$_3$-19), 1.43 (3H, s, H$_2$-18), 2.33 (3H, s, H$_2$-21), 3.86 (1H, m, H-3), 4.94 (1H, m, H-16); $\delta_{C}$ 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 NOEYs were observed between H$_2$-18 and H$_2$-21, and between H-17 and H-16. No NOEYs between H$_2$-18 and H$_2$-17 was observed. Therefore the structure of 2 was determined to be 5$\alpha$-pregn-3b,16b-diol-20-one, lycopersiconol$^{11}$ isolated from tomato stock roots.

Next, 2 was refluxed with pyridine and water (1:1) to produce Compound 3. Compound 3 was obtained as colorless needles showing mp 203—205 °C, [a]$_{D}^{20}$ +48.2 ° (pyridine).

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 (JEOL). $^{13}$C-NMR (in pyridine-$d_5$) $\delta$: 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), 46.6 (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-Agal: $\delta$: 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), 62.6 (C-6).

Compound 2

A crude tomatine solution was prepared as reported in a previous paper.$^{10}$ The tomatine 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 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 centrifugation; the supernatant was passed through a MCI gel (5 ml) and eluted with H$_2$O (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, [a]$_{D}^{20}$ +6.74 ° (c=1.00, pyridine). $^{13}$C-NMR (in pyridine-$d_5$) $\delta$: 82.0 (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), 3.68 (1H, m, H-1, 4.94 (1H, m, H-16). $^{13}$C-NMR (in pyridine-$d_5$) $\delta$: 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), 39.2 (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).

Therefore the structure was characterized as 5$\alpha$-pregn-16-ene-3$\beta$-ol-20-one, allopregnenolone.$^{12}$ Consequently, the structure of 1 was elucidated as 3-O-$\beta$-xylopyranosyl(1—3)-[3$\beta$-$\beta$-glucopyranosyl(1—2)-]$\beta$-$\beta$-glucopyranosyl(1—4)-$\beta$-$\alpha$-galactopyranosyl(1-$\beta$-lycotetraosyl) 5$\alpha$-pregn-3b,16b-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 steroid glycosides such as spironolactone 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.$^{14}$

Acknowledgements

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

References and Notes