Metabolism of Tomato Steroidal Glycosides in Humans

Eishin NOGUCHI,[a] Yukio FUJIWARA,[a] Sayaka MATSUSHITA,[a] Tsuyoshi IKEDA,[a] Masateru ONO,[b] and Toshihiro NOHARA*,[a]

[a] *Faculty of Medical and Pharmaceutical Science, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan: and* [b] *School of Agriculture, Kyushu Tokai University; Choyo 5435, Aso, Kumamoto 869–1404, Japan.*
Received March 13, 2006; accepted May 15, 2006

Pregnane glycosides have been isolated in small amounts, along with the major components furostanol and spirostanol glycosides, from Dioscoreaceae, Taccaceae, and Solanaceae, suggesting that pregnane glycosides might be biosynthesized from furostanol and spirostanol glycosides. Recently, commercial natural foods composed of diosgenin have been used for the treatment of diseases such as osteoporosis and premenstrual syndrome in women. It is anticipated that diosgenin would be metabolized into a type of steroidal hormone, for instance progesterone, however, this metabolism has not been confirmed. Therefore, we have examined the metabolites in the urine of subjects who ingested tomatoes, which contain a considerable amount of the steroidal glycoside esculeoside A. The occurrence of steroidal hormones in the metabolites has been recognized. It has been proven that when a steroidal glycoside is administered, it is partly metabolized into a type of steroidal hormone exhibiting various physiological activities.

Key words steroidal glycoside metabolism; pregnane; tomato steroidal glycoside

Pregnane glycoside was first isolated from *Paris polyphylla* (Liliaceae) by Nohara *et al.*[1] Recently, pregnane (*g* in Chart 1) and 16-*O*-acylpregnane glycosides (*c*) have been occasionally isolated from various plants of various genera such as Dioscoreacee,[2—5] Taccaceae,[6] and Solanaceae.[7] We have also isolated nigrosides A (*d*),[8] B (*e*) and tuberoside C (*f*)[9] along with the usual major components, furostanol (*a*) and spirostanol (*b*) glycosides. The occurrence of a 20,22-dicarbonyl cholestane glycoside skeleton in 16-*O*-acylpregnane glycoside (*c*) strongly suggests a biogenetic pathway of oxidation between C-20 and C-22 *via* C-20(22)-pseudomerization in furostanol glycoside. Therefore, we have hypothesized that pregnane glycoside (*g*) might be biosynthesized from furostanol (*a*) and/or spirostanol (*b*) glycosides as shown in Chart 1. Recently, natural foods containing diosgenin, a constituent of Mexican yam (*Dioscorea mexicana*), are marketed in Japan and promoted as being effective in treating diseases such as osteoporosis and premenstrual syndrome in women. This indicates that diosgenin may be metabolized into a type of steroidal hormone. Therefore, we have speculated that when steroidal glycoside is administered, it might be partially metabolized into a steroidal hormone. However, this has not yet been proven.

For the metabolic experiment, we isolated the tomato glycoside, esculeoside A (*h* in Chart 2), (5α,22*R*,23*R*,25*S*)-3β,27-dihydroxy-23-acetoxy-spirosolane 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (β-lycotetraoside), at a yield of 0.025% from the fruit of Cherry tomato, *Lycopersicon esculentum* var. *cerasiforme* (DUNAL) ALEF.[10,11] Moreover, a pregnane glycoside, 3-*O*-β-lycoteraosyl 5α-pregn-16-ene-20-one, was isolated from over-ripe tomatoes that showed decreasing amounts of esculeoside A. This fact suggests that esculeoside A could be converted to pregnane derivatives as the tomatoes mature, suggesting that pregnane derivatives are biosynthesized from spirosolane glycoside. Furthermore, we have observed that esculeogenin A (*i*) corresponding to the sapogenol moiety of esculeoside A (*h*) can be easily converted to a pregnane derivative, 3β,16β-dihydroxy-5α-pregna-20-one, by merely refluxing with water and pyridine.[12] The presence of the hydroxy group at C-23 may cause the E and F-rings of the steroidal nucleus to be fragile. So that we have designed metabolic experiment of tomato glycoside.

Eight males consumed Cherry tomatoes at the rate of 2 kg per adult person over a period of 2 d: their urine samples were collected for 48 h and passed through a polystyrene gel (Diaion HP-20). The first eluate with water was discarded,

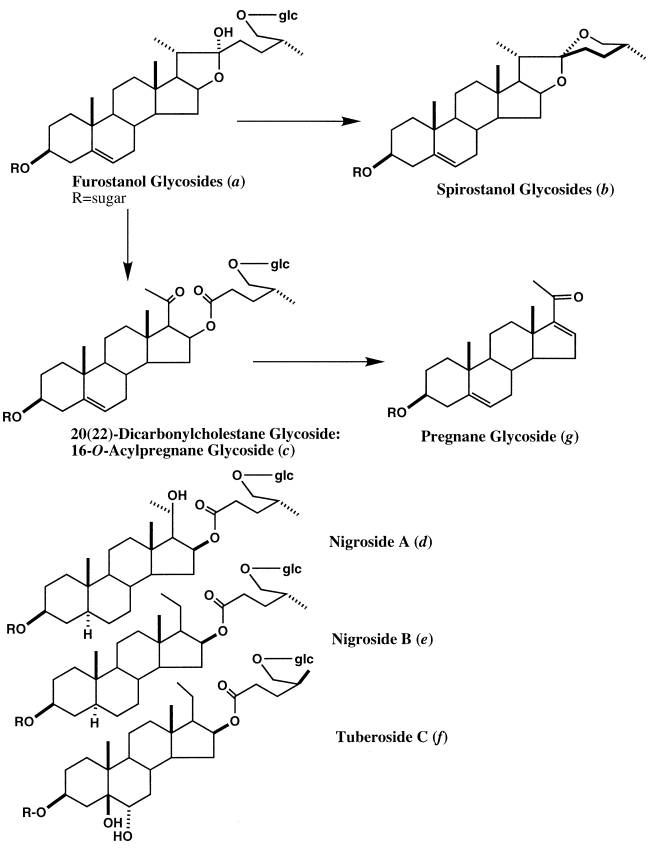

Chart 1. Hypothetical Biogenesis of Steroidal Glycosides in Plants

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

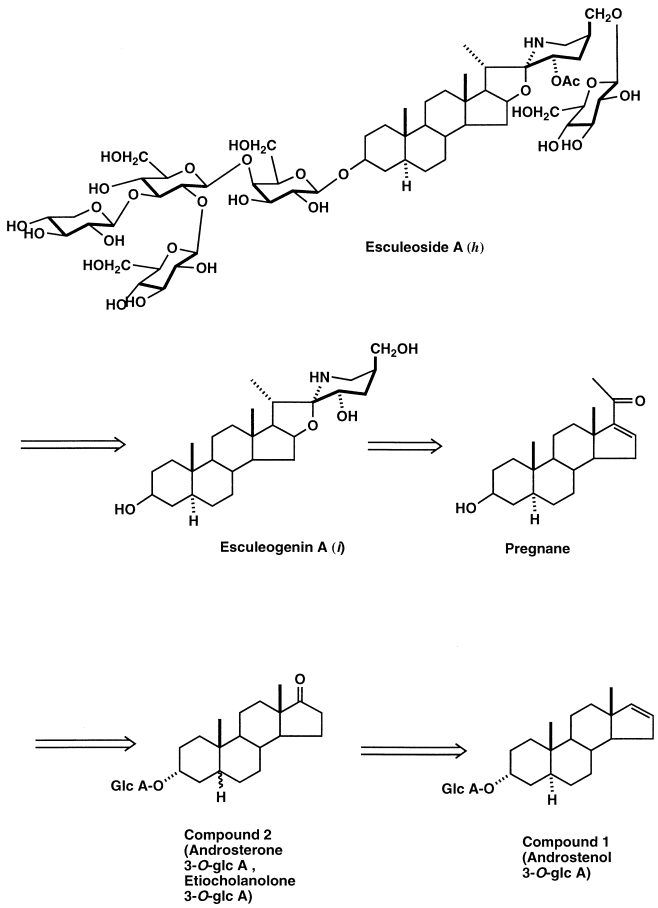

Chart 2. Hypothetical Metabolism of Steroidal Glycosides in Humans

and the second eluate with MeOH was collected. The methanolic residue (7.42 g) was subjected to Sephadex LH-20, silica gel and ODS column chromatographies to afford two compounds, namely compound **1** (7.9 mg) and compound **2** (11.9 mg).

Compound **1** was obtained as an amorphous powder and showed a quasi molecular peak at *m/z* 473 due to $[M(C_{25}H_{38}O_7)+Na]^+$ in the positive FAB-MS. In the ^{1}H-NMR spectrum, the presence of two tertiary methyl groups at δ 0.73 (3H, s) and 0.76 (3H, s), and two olefinic protons at δ 5.74 (1H, br s) and 5.89 (1H, br s) along with an anomeric proton at δ 4.93 (1H, d, J=7.9 Hz) were detected. The ^{13}C-NMR spectrum showed signals due to two methyl carbons at δ 11.6 and 17.6, eight methylene carbons at δ 21.6, 25.6, 28.7, 2×32.3, 35.0, 36.3, 45.8; four methine carbons at δ 34.2, 45.8, 54.9, 56.3; two quaternary carbons at δ 39.8 and 45.8; one oxygen-bearing methine carbon at δ 75.0; and two sp^2 methine carbons at δ 129.3 and 144.3 along with a β-D-glucuronopyranosyl moiety (C-1-6 δ: 102.5, 74.1, 77.6, 73.4, 78.2, 170.2). Based on the experiment using 2D-NMR [^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiquantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC)] and the reported data for androstenol,[13] the respective ^{1}H and ^{13}C signals were assigned: the methyl proton signals at δ 0.73 were assigned to H_3-18, which correlated to the sp^2 carbon at δ 144.3. Therefore, the double bond was located between C-16(17). Consequently, the structure of compound **1** was determined as 3-*O*-β-D-glucuronopyranosyl androsterol.

Compound **2**, an amorphous powder, exhibited a quasi molecular ion peak at *m/z* 489 due to $[M(C_{25}H_{38}O_8)+Na]^+$ in the positive FAB-MS. The ^{1}H-NMR spectrum exhibited four singlet methyl groups at δ 0.73, 0.74, 0.75 and 0.85. HMBC showed signals at δ 0.73 and 0.85 that were assigned to H_3-19, and the rest of the signals were assigned to H_3-18: therefore, compound **2** was detected as a mixture of two steroids. The ^{13}C-NMR spectrum showed signals due to the aglycone moiety with slightly complicated signals due to the carbonyl functions at δ 219.8 and oxygen-bearing carbons at δ 73.2 and 78.1 along with a β-D-glucuronopyranosyl moiety (δ 102.4, 73.6, 77.1, 73.2, 78.1, 170.1). The H_3-18 correlated to the carbonyl function at δ 219.8. Based on the 2D-NMR spectra and the reported data,[14] the respective carbon signals at C-1-19 of the sapogenol moiety in compound **2** were assigned to be a mixture of 3α-hydroxy-5α-androstan-17-one (androsterone) and 3α-hydroxy-5β-androstan-17-one (etiocholanolone) as follows: C-1-19 of 5α-compound, δ 32.7, 27.1, 73.2, 34.4, 39.6, 28.5, 31.0, 35.1, 54.2, 36.1, 20.3, 32.2, 47.8, 51.7, 21.9, 36.0, 219.8, 13.8, 11.5. C-1-19 of 5β-compound, δ 35.9, 28.5, 78.1, 32.7, 42.2, 27.1, 25.5, 35.1, 40.9, 36.1, 20.3, 32.2, 47.8, 51.7, 21.9, 36.0, 219.8, 13.8, 23.4. Consequently, the structure of compound **2** was represented as a mixture of 3α-hydroxy-5α-androstan-17-one (androsterone) and 3α-hydroxy-5β-androstan-17-one (etiocholanolone) β-D-glucuronopyranoside.

In a normal person, these androsterone analogues are usually excreted. However, since these excreted analogues were not detected in the control sample, the occurrence of androsterone analogues indicates that they would be excreted *via* the production of progesterone due to the consumption of tomatoes, that is, the tomato steroidal glycoside might stimulate hormone secretion or it would be metabolized to pregnane. Their metabolites might be produced by liver enzymes (cytochrome P450) or bacteria in the intestine.

Now, we would like to propose a novel theory that an orally administered steroidal glycoside such as spirostanol and furostanol glycosides would be metabolized to pharmacologically active pregnane derivatives *via* the introduction of a hydroxyl group at C-23 (Chart 2). The final metabolites are excreted as androsterone analogues in urine.

Summarizing the effectiveness of steroidal glycosides; when steroidal glycoside is administered, a portion of it remains on the surface of the small intestine and affects the receptor or mediator of the nervous system that controls the rise in blood sugar level.[15] On the other hand, the assimilated portion of the steroidal glycoside is metabolized to a type of pregnane hormone that exhibits various physiological activities such as the prevention of osteoporosis and premenstrual syndrome in women, namely, a part of steroidal glycosides might be a pro-drug of steroidal hormone. Moreover, steroidal glycosides are absorbed *via* the skin and act against herpes virus and skin tumor,[16,17] as shown in Chart 3.

Experimental

Optical rotations were measured on a JASCO DIP-1000 KUY digital polarimeter (l=0.5). NMR spectra were measured in pyridine-d_5 using a JEOL α-500 spectrometer and chemical shifts were referenced to TMS. Positive FAB-MS was obtained with a JEOL JMS-MS-700 spectrometer. Column chromatography was carried out using a silica gel 60 (0.063—0.200 mm; Merck), Diaion HP-20P (Mitsubishi Chemical Industries Co., Ltd.) and Chromatorex ODS (Fuji Silysia Chemical Co., Ltd.), and TLC was per-

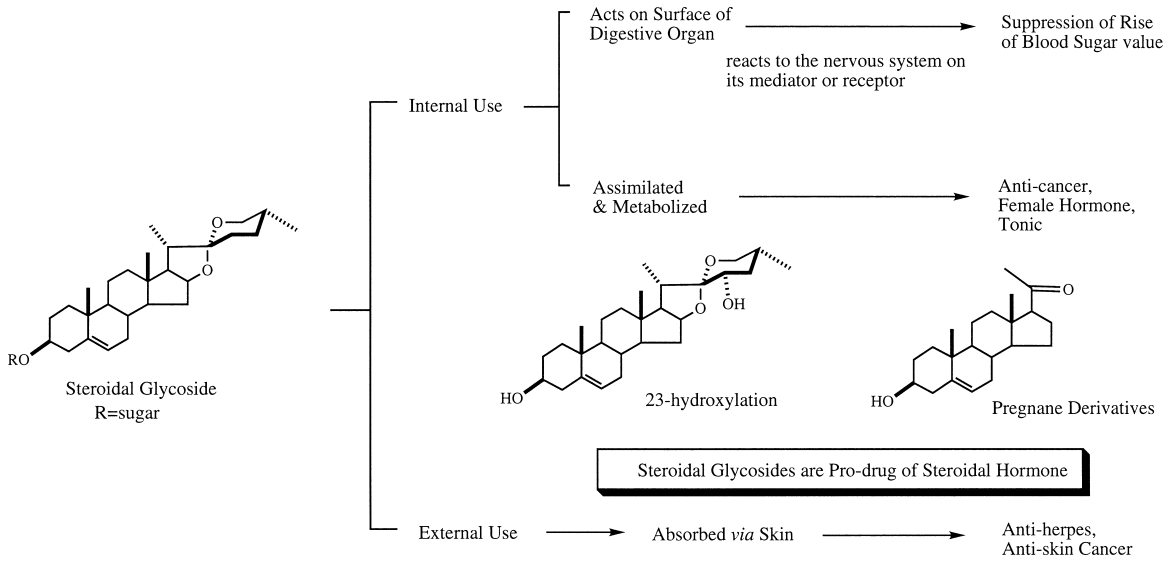

Chart 3. Effectiveness of Steroidal Glycosides

formed on a precoated silica gel 60 F_{254} (Merck) and RP-18 $F_{254}S$ (Merck), and the compound was detected by spraying with 10% H_2SO_4 in 50% MeOH, followed by heating on a hot plate.

Metabolic Experiment Using Tomatoes Tomatoes were ingested by eight adult males at the rate of 2 kg per person over two days. Their urine samples were collected, passed through a Diaion HP-20 column, and the column was washed with distilled water. Subsequently, elution was carried out with MeOH, and the methanolic eluate was evaporated to obtain a residue (7.42 g). A portion (2.29 g) of the residue was introduced into a Sephadex LH-20 column; elution was carried out with water, increasing concentration of MeOH (20%—40%—60%—80%) and MeOH (100%), successively, to obtain eight fractions. Fraction (Fr.) 2 (2006.2 mg) was chromatographed on ODS and then eluted with water—20%→40%→60%→80% MeOH→MeOH, successively, to afford eight fractions. Fr. 6 (297.3 mg) was then chromatographed on a silica gel with $CHCl_3$: MeOH : water= 9 : 1 : 0.1→8 : 2 : 0.2→7 : 3 : 0.5→6 : 4 : 1 to obtain eleven fractions. Fr. 5 (44.0 mg) was subsequently subjected to ODS column chromatography with water→20%→40%→60%→80% MeOH, MeOH to afford compound **2** (11.9 mg). On the other hand, Fr. 7 (115.1 mg) from the Sephadex LH-20 column was chromatographed on a silica gel with $CHCl_3$: MeOH : water= (9 : 1 : 0.1→8 : 2 : 0.2→7 : 3 : 0.5→6 : 4 : 1) to obtain eight fractions. Fr. 4 (28.8 mg) was then subjected to ODS chromatography with 20%→40%—60%—80% acetone→acetone to obtain compound **1** (7.9 mg).

Compound **1**: An amorphous powder. Positive FAB-MS (*m*/*z*): 473 $[M(C_{25}H_{38}O_7)+Na]^+$. ^{1}H-NMR ($D_2O$) δ 0.73 (3H, s, H_3-18), 0.76 (3H, s, H_3-19), 4.93 (1H, d, J=7.9 Hz, glc A H-1), 5.74 (1H, br s, H-16), 5.89 (1H, br s, H-17). ^{13}C-NMR (D_2O) sapogenol C-1-19: δ 32.6, 25.6, 75.0, 35.0, 45.8, 28.7, 32.3, 34.2, 54.9, 39.8, 21.6, 36.3, 45.8, 56.3, 32.3, 129.3, 144.3, 17.6, 11.6. glc A C-1-6: δ 102.5, 74.1, 77.6, 73.4, 78.2, 170.2.

Compound **2**: An amorphous powder. Positive FAB-MS (*m*/*z*): 489 $[M(C_{25}H_{38}O_8)+Na]^+$. ^{1}H-NMR ($D_2O$) δ: 0.73, 0.85 (each s, H_3-19 of 5α and 5β-compound, respectively), 0.74, 0.75 (each s, H_3-18 of 5α and 5β-compound, respectively). ^{13}C-NMR (D_2O) C-1-19 of 5α-compound, δ 32.7, 27.1, 73.2, 34.4, 39.6, 28.5, 31.0, 35.1, 54.2, 36.1, 20.3, 32.2, 47.8, 51.7, 21.9, 36.0, 219.8, 13.8, 11.5; C-1-19 of 5β-compound, δ 35.9, 28.5, 78.1, 32.7, 42.2, 27.1, 25.5, 35.1, 40.9, 36.1, 20.3, 32.2, 47.8, 51.7, 21.9, 36.0, 219.8, 13.8, 23.4. glc A C-1-6: δ 102.4, 73.6, 77.1, 73.2, 78.1, 170.1.

References

1) Nohara T., Yabuta H., Suenobu M., Hida R., Miyahara K., Kawasaki T., *Chem. Pharm. Bull.*, **21**, 1240—1247 (1973).
2) Dong M., Feng X.-Z., Wang B.-X., Wu L.-J., Ikejima T., *Tetrahedron*, **57**, 501—506 (2001).
3) Tran Q.-L., Tezuka Y., Banskota A. H., Tran Q. K., Saiki I., Kadota S., *J. Nat. Prod.*, **64**, 1127—1132 (2001).
4) Yin J., Kouda K., Tezuka Y., Tran Q. L., Miyahara T., Chen Y., Kadota S., *J. Nat. Prod.*, **66**, 646—650 (2003).
5) Liu H., Xioong Z., Li F., Qu G., Kobayashi H., Yao X., *Chem. Pharm. Bull.*, **51**, 1089—1091 (2003).
6) Yokosuka A., Mimaki Y., Sashida Y., *J. Nat. Prod.*, **65**, 1293—1298 (2002).
7) Mimaki Y., Watanabe K., Sakagami H., Sashida Y., *J. Nat. Prod.*, **65**, 1863—1868 (2002).
8) Zhu X.-H., Tsumagari H., Honbu T., Ikeda T., Ono M., Nohara T., *Tetrahedron Lett.*, **42**, 8043—8046 (2001).
9) Ikeda T., Tsumagari H., Okawa M., Nohara T., *Chem. Pharm. Bull.*, **52**, 142—145 (2004).
10) Fujiwara Y., Yahara S., Ikeda T., Ono M., Nohara T., *Chem. Pharm. Bull.*, **51**, 234—236 (2003).
11) Fujiwara Y., Takaki A., Uehara Y., Ikeda T., Ono M., Yoshimitsu H., Nohara T., *Tetrahedron*, **60**, 4915—4920 (2004).
12) Matsushita S., Yoshizaki M., Fujiwara Y., Ikeda T., Ono M., Okawara T., Nohara T., *Tetrahedron Lett.*, **46**, 3549—3551 (2005).
13) Eggert H., Djerassi C., *J. Org. Chem.*, **46**, 5399—5401 (1981).
14) Blunt J. W., Stothers J. B., *Org. Mag. Res.*, **9**, 439—464 (1977).
15) Yoshikawa M., *Kagaku to Seibutsu*, **40**, 172—178 (2002).
16) Cham B. E., Daunter B., *Drugs Today*, **26**, 55—58 (1990).
17) Ikeda T., Ando J., Miyazono A., Zhu X.-H., Tsumagari H., Nohara T., Yokomizo K., Uyeda M., *Biol. Pharm. Bull.*, **23**, 363—364 (2000).