Steroidal Alkaloid Glycosides from Tomato (*Lycopersicon esculentum*)

Shoji Yahara,*,† Noriko Uda,§ Eri Yoshio,§ and Emiko Yae§

Medicinal Plant Garden, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan, and Faculty of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan

Received August 18, 2003

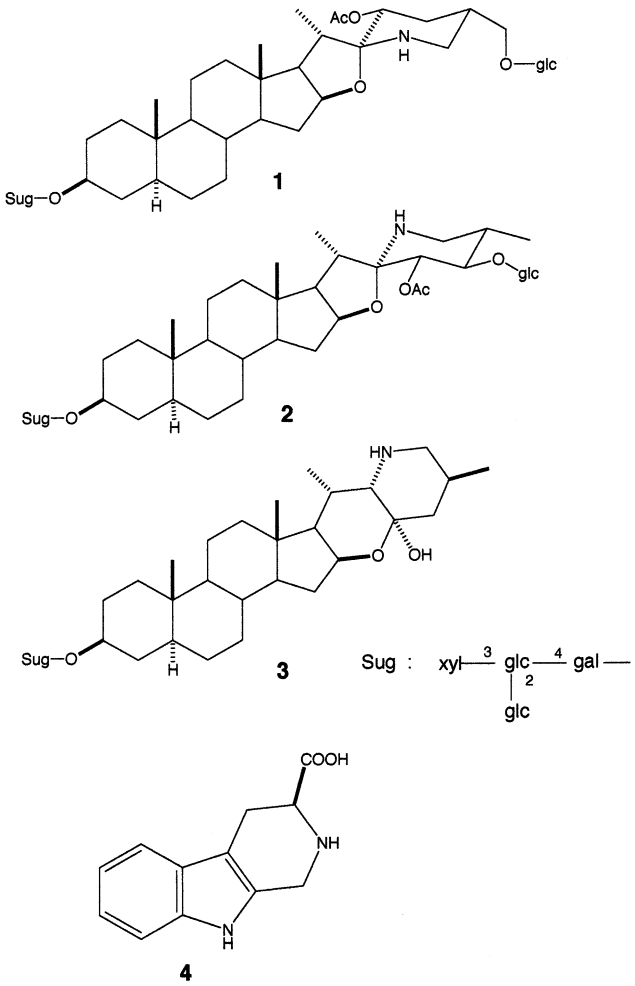
Three new steroidal alkaloid glycosides, lycoperosides F−H (**1**−**3**), were isolated from tomato fruits (*Lycopersicon sculentum*) along with lycoperosides A−D, esculeoside A, and rutin. The structures of these glycosides were characterized as the 3-*O*-*β*-lycotetraosides of 23(*R*)-23-acetoxy-27-hydroxy-27-*O*-*β*-D-glucopyranosyltomatidine (**1**), (23*S*,24*R*)-23-acetoxy-24-*O*-*β*-D-glucopyranosylsoladulcidine-24-ol (**2**), and 22-isopimpifolidine (**3**), by means of their spectroscopic data. Also obtained was the new natural product lycoperodine-1 (**4**).

Tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), and eggplant (*Solanum melongena* L.) are popular vegetables of the Solanaceae. A bitter principle, TFT,[1] isolated from tomato seeds, tomatine,[2] and lycoperosides A−D[3] and esculeoside A[4] from tomato leaves and fruits,[1] and several spirosolane[5] derivaives from the root of the tomato were reported. Continuing our investigation on solanaceous plants, we have further surveyed the steroidal glycosidic constituents of *L. esculentum* fruits.

The plant material was extracted with MeOH and treated as described in the Experimental Section to afford nine compounds, including three new steroidal alkaloid glycosides (**1**−**3**) and a new natural product, lycoperodine-1 (**4**). The known compounds rutin, lycoperosides A−D, esculeoside A, and tomatine were identified by direct comparison with standard samples.

Lycoperoside F (**1**), $C_{58}H_{95}NO_{29}$, a white powder, showed a quasi-molecular ion peak $[M + H]^+$ at *m*/*z* 1270 and a fragment ion peak $[M + H - AcOH]^+$ at *m*/*z* 1210 in the positive FABMS. The ^1H and ^{13}C NMR spectra of **1** were similar to those of lycoperoside A[3] except for the carbon signals assignable to a *β*-glucopyranoside (*δ* 104.9, 75.0, 78.6, 71.5, 78.6, 62.7) and C-24 to C-27 on the F ring. As listed in Table 1, the respective shifts of −2.7 [*δ* 32.4], +6.0 [*δ* 36.5], −3.9 [*δ* 45.3], and +53.6 [*δ* 72.3] were observed for C-23 to C-27 in the aglycon of **1**, compared with those of lycoperoside A, and cross-peaks in the $^1H-^{13}C$ COSY spectrum occurred between *δ* 72.3 (C-27) and 4.12 (m) and 4.58 (m) and between 36.5 (C-25) and 2.05 (m), indicating that the *O*-*β*-glucopyranoside moiety was attached to the C-27 hydroxy group of the aglycon. NOE experiments on **1** showed correlations between *δ* 1.13 (H_3-21) and 5.22 (H-23) and between *δ* 5.22 and 2.05 (H-25), thus suggesting H-23 and H-25 to be axial protons. The configurations at C-22 and C-25 were judged to be the same as in tomatine. The above evidence indicated **1** to have the structure 3-*O*-*β*-lycotetraosyl-23(*R*)-23-acetoxy-27-hydroxy-27-*O*-*β*-D-glucopyranosyltomatidine.

Lycoperoside G (**2**), $C_{58}H_{95}NO_{29}$, showed a quasi-molecular ion peak $[M - H]^-$ at *m*/*z* 1268 and a fragment ion peak $[M - H - \text{hexose}]^-$ at *m*/*z* 1106 in the negative FABMS; thus **2** was suggested to be an isomer of **1**. The ^1H and ^{13}C NMR spectra of **2** were similar to those of lycoperoside B,[3] except for C-22 to C-27 for the aglycon and



the *β*-glucopyranoside [δ_C 105.6 (C-1), δ_H 4.89 (d, J = 7.3 Hz, H-1)] signals. As listed in Table 1, respective shifts of +2.2 [*δ* 100.6], +2.2 [*δ* 73.3], +48.1 [*δ* 84.1], +7.5 [*δ* 39.2], −1.5 [*δ* 45.0], and −3.2 [*δ* 15.6] were observed for C-22 to C-27 in the aglycon of **2**, compared with those of lycoperoside B.[3] The HMBC spectrum of **2** showed correlations of *δ* 4.89 (glc H-1) and 84.1 (C-24), and *δ* 5.55 (d, J = 9.2 Hz, H-23) and 84.1 (C-24), thus, suggesting a *β*-glucopyranosyl moiety attached at the C-24 hydroxymethine moiety. Consequently, **2** was characterized as 3-*O*-*β*-lycotetraosyl-(23*S*,24*R*)-23-acetoxy-24-*O*-*β*-D-glucopyranosylsoladulcidine-24-ol.

* To whom correspondence should be addressed. Tel/Fax: +81-96-371-4381. E-mail: yaharas1@gpo.kumamoto-u.ac.jp.
† Medicinal Plant Garden.
§ Faculty of Pharmaceutical Sciences.

10.1021/np030382x CCC: $27.50
Published on Web 02/05/2004

Table 1. ^{13}C NMR Data for Lycoperosides F (**1**), G (**2**), and H (**3**) in Pyridine-d_5

carbon	1	2	3
1	37.1	37.2	37.2
2	29.8	29.9	29.8
3	77.3	77.4	78.2
4	34.7	34.8	34.7
5	44.6	44.7	44.6
6	28.9	28.9	28.8
7	32.3[a]	32.2	32.1
8	35.0	35.2	35.1
9	54.4	54.3	53.2
10	35.7	35.8	35.8
11	21.4	21.2	20.7
12	40.4	40.1	37.2
13	41.1	41.3	42.8
14	55.4	56.4	53.2
15	34.0	32.3	33.7
16	82.3	79.6	69.1
17	63.4	62.4	57.8
18	17.0	16.5	15.0
19	12.3	12.3	12.3
20	43.3	35.8	26.6
21	15.9	15.1	19.5
22	100.7	100.6	59.3
23	75.1	73.3	93.4
24	32.4[a]	84.1	44.8
25	36.5	39.2	27.3
26	45.3	45.0	51.2
27	72.3	15.6	18.0
OAc-23	21.4	21.5	
	169.8	171.6	
gal-1	102.3	102.4	102.3
2	73.1	73.2	72.8
3	75.3	75.6	75.1[a]
4	79.8	79.9	79.7
5	75.0	75.1	75.0
6	60.5	60.6	60.7
inner glc-1	104.5	105.1	104.6
2	81.3	81.3	80.7
3	86.7	86.7	87.0
4	70.9	70.4	69.9
5	78.3[b]	77.7	77.9[b]
6	62.9	63.0	62.5
terminal glc-1	104.9	104.9	104.4
2	76.1	76.2	75.6
3	78.4[b]	77.6	77.1[b]
4	70.4	71.0	70.4
5	77.7	78.4	77.4[b]
6	62.4	62.4	62.1
xyl-1	105.1	104.8	104.2
2	75.0	75.3[a]	74.7[a]
3	77.5	78.6[b]	77.1[b]
4	70.7	70.7	70.8
5	67.3	67.3	66.9
27-*O*-glc-1 or 24-*O*-glc-1	104.9	105.6	
2	75.0	75.7[a]	
3	78.6[b]	77.8[b]	
4	71.5	70.0	
5	78.6[b]	78.7[b]	
6	62.7	63.1	

[a,b] Signal assignments may be reversed in each column.

Lycoperoside H (**3**), $C_{50}H_{83}NO_{22}$, showed a quasi-molecular ion peak $[M - H]^-$ at *m/z* 1048 in the negative FABMS. The ^1H and ^{13}C NMR spectra of **3** were similar to those of tomatine, **1**, and **2**, except for the signals assignable to C-16 to C-27 on the D, E, and F rings, whose signals were almost similar to those of 22-isopimpifolidine, which was isolated from the roots of the wild tomato.[6] The HMBC spectrum of **3** showed correlation of δ 3.65 (H-22) and δ 19.5 (C-21), 26.6 (C20), and 93.4 (C-23), δ 2.85 (H-20) and δ 19.5 (C-21), 42.8 (C-13), 59.3 (C-22) and 93.4 (C-23), δ 0.83 (H_3-27) and δ 27.3 (C-25), 44.8 (C-24), and 51.2 (C-26), δ 1.17 (H-17) and δ 15.0 (C-18), 19.5 (C-21), 26.6 (C-20), 42.8 (C-13), 59.3 (C-22), and 69.1 (C-26), and δ 3.72 (H-26) and δ 59.3 (C-22). This was confirmed by a comparative study of the NMR data of the aglycone part of **3** with those of 22-isopimpifolidine, pimpifolidine, and 3-deamino-3β-hydroxysolanocapsine.[6] Consequently, **3** was characterized as 3-*O*-β-lycotetraosyl-22-isopimpifolidine.

Lycoperodine-1 (**4**), a pale yellow powder, $[\alpha]_D$ −30.3°, was positive to the Van Urk reagent, suggesting the presence of an indole moiety. The negative FABMS of **4** showed a quasi-molecular ion peak $[M - H]^-$ at *m/z* 215. The ^1H and ^{13}C NMR spectra of **4** disclosed 12 carbon signals constituting a tryptophan unit [δ 125.9 (s), 104.3 (s), 118.8 (d), 117.5 (d), 121.0 (d), 111.8 (d), 136.1 (s), 128.5 (s), 18.0 (t), 55.3 (d), and 165.6 (s)] by comparison with the data of tryptophan and an additional methylene unit [δ 40.3 (t)]. The positions of linkage of the tryptophan and methylene unit in **4** were determined from the above data, thus suggesting that C-2 and NH_2 of the tryptophan moiety and methylene unit participated in the cyclization to structure **4**.[7] This compound was synthesized from formaldehyde and tryptophan by Kirkup et al.[7] This compound has been isolated for first time as a natural product from the tomato and has been named lycopedine-1.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter, and values are given in 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a JEOL JIR-6500W spectrophotometer, and a microspectroscopy FTIR method was used. The one- and two-dimensional NMR spectra were recorded on JEOL GX-400 and A-500, ^1H (400 and 500 MHz) and ^{13}C (100 and 125 MHz), models using standard pulse sequences with TMS as the internal standard. Chemical shifts are reported in δ, and coupling constants (*J*) are given in Hz. FABMS and HR-FABMS were obtained using a JEOL DX303HF spectrometer. TLC was performed on precoated Kieselgel 60 F_{254} (Merck), and spots were visualized by heating with 10% H_2SO_4. Open column chromatographies were Kieselgel (270−400 mesh, Merck), Chromatorex ODS (Fuji Silisia Ltd., Nagoya, Japan), Chromatorex NH (Fuji Silisia Ltd.), and MCI gel CHP-20P (Mitsubishi Chemical Ind., Tokyo, Japan).

Plant Material. The fruits of *Lycopersicon esculentum* cv. *momotaro* (cultivated tomato, Japanese name "Momotaro") were cultivated in the Medicinal Plant Garden of the Faculty of Pharmaceutical Sciences, Kumamoto University, and were collected in July 1996. The plant material voucher specimen has been deposited at Takii & Co., Ltd., where the seeds were purchased.

Extraction and Isolation. Fresh fruits of tomato (10.0 kg) were extracted with MeOH, and the extract (350 g) was subjected to column chromatography on MCI gel CHP-20P eluting with $H_2O \rightarrow$ 50% MeOH → 80% MeOH → 100% MeOH, to afford five fractions. Fraction 3 (1.22 g, 50% MeOH eluate) was subjected to column chromatography on silica gel ($CHCl_3$−MeOH−H_2O, 7:3.5:0.6) and Chromatorex ODS (55−65% MeOH) to give lycoperoside H (**3**, 6 mg, 0.6 × 10^{-4}%) and lycoperodine-1 (**4**, 3 mg, 0.3 × 10^{-4}%). Fraction 5 (2.91 g, 100% MeOH eluate) was subjected to column chromatography over Chromatorex ODS (55−80% MeOH) and Chromatorex NH ($CHCl_3$−MeOH−H_2O, 7:3:0.5 → 7:3.5:0.5) to give tomatine (320 mg, 3.2 × 10^{-3}%), rutin (44 mg, 4.4 × 10^{-4}%), lycoperosides A (5 mg, 0.5 × 10^{-4}%), B (15 mg, 1.5 × 10^{-4}%), and C (45 mg, 4.5 × 10^{-4}%), and esculeosides A (57 mg, 5.7 × 10^{-4}%), F (**1**, 13 mg, 1.3 × 10^{-4}%), and G (**2**, 11 mg, 1.1 × 10^{-4}%).

Lycoperoside F (1): white, amorphous powder; $[\alpha]_D^{29}$ −38.5° (*c* 0.80, MeOH); IR (neat) ν_{max} 3405, 2927, 1722, 1650 cm^{-1}; ^1H NMR (pyridine-d_5) δ 0.51 (1H, br t, J = 8.8 Hz, H-9), 0.62 (3H, s, H_3-19), 0.85 (3H, s, H_3-18), 1.13 (3H, d, J = 7.3 Hz, H_3-21), 1.89 (1H, m, H-24), 2.05 (1H, m, H-25), 2.17 (3H, s, CH_3CO−), 2.22 (1H, m, H-24), 3.10 (2H, m, H_2-26), 3.90 (1H,

m, H-3), 4.12 (1H, m, H-27), 4.58 (1H, m, H-27), 4.80 (1H, d, J = 8.0 Hz, gal H-1), 4.91 (1H, d, J = 8.0 Hz, 27-glc H-1), 5.01 (1H, dd, J = 3.5, 12.3 Hz, H-16), 5.22 (1H, dd, J = 5.0, 11.0 Hz, H-23), 5.22 (1H, d, J = 7.0 Hz, inner glc H-1), 5.26 (1H, d, J = 8.0 Hz, xyl H-1), 5.59 (1H, d, J = 7.3 Hz, terminal glc H-1); positive FABMS m/z 1270 $[M + H]^+$, 1210 $[M + H - AcOH]^+$; positive HRFABMS m/z 1292.5895 (calcd for $C_{58}H_{95}NO_{29}Na$, 1292.5888, $[M + Na]^+$).

Lycoperoside G (2): white, amorphous powder; $[\alpha]_D^{20}$ −44.1° (c 0.68, MeOH); IR (neat) ν_{max} 3386, 2927, 1722, 1651 cm^{-1}; ^1H NMR (pyridine-d_5) δ 0.50 (1H, br t, J = 8.8 Hz, H-9), 0.68 (3H, s, H_3-19), 0.90 (3H, s, H_3-18), 1.17 (3H, d, J = 7.3 Hz, H_3-21), 1.30 (1H, d, J = 6.7 Hz, H_3-27), 2.07 (1H, m, H-25), 2.40 (1H, m, H-20), 2.59 (3H, s, CH_3CO−), 2.73 (H, m, H-26), 2.86 (1H, m, H-26), 3.91 (1H, m, H-3), 4.18 (1H, m, H-24), 4.43 (1H, m, gal H-2), 4.57 (1H, m, H-16), 4.89 (2H, d, J = 7.3 Hz, gal H-1, 24 glc H-1), 4.91 (1H, d, J = 8.0 Hz, 27-glc H-1), 5.20 (1H, d, J = 7.9 Hz, inner glc H-1), 5.22 (1H, d, J = 7.3 Hz, xyl H-1), 5.55 (1H, d, J = 9.2 Hz, H-23), 5.68 (1H, d, J = 7.3 Hz, terminal glc H-1); negative FABMS m/z 1268 $[M - H]^-$, 1106 $[M - H -$ hexsose$]^-$; positive FABMS m/z 1270 $[M + H]^+$; positive HRFABMS m/z 1292.5883 (calcd for $C_{58}H_{95}NO_{29}Na$, 1292.5888, $[M + Na]^+$).

Lycoperoside H (3): white, amorphous powder; $[\alpha]_D^{20}$ −29.8° (c 1.20, MeOH); IR (neat) ν_{max} 3381, 2929, 1697, 1662 cm^{-1}; ^1H NMR (pyridine-d_5) δ 0.64 (1H, br t, J = 9.0 Hz, H-9), 0.65 (3H, s, H_3-19), 0.73 (1H, m, H-14), 0.83 (3H, d, J = 6.7 Hz, H_3-27), 0.92 (1H, m, H-5), 1.05 (3H, s, H_3-18), 1.17 (1H, br d, J = 6.7 Hz, H-17), 1.26 (1H, m, H-4), 1.45 (1H, m, H-15), 1.69 (3H, d, J = 6.8 Hz, H_3-21), 1.83 (1H, m, H-4), 2.16 (1H, m, H-15), 2.34 (1H, br d, J = 10.4 Hz, H-24), 2.65 (1H, m, H-25), 2.85 (1H, m, H-20), 3.15 (1H, dd, J = 12.2, 12.2 Hz, H-26), 3.65 (1H, d, J = 6.7 Hz, H-22), 3.72 (1H, m, H-26), 3.95 (1H, m, H-3), 4.93 (1H, m, H-16), 4.92 (1H, d, J = 7.3 Hz, gal H-1), 5.15 (1H, d, J = 8.0 Hz, inner glc H-1), 5.18 (1H, d, J = 7.9 Hz, xyl H-1), 5.57 (1H, d, J = 7.0 Hz, terminal glc H-1); negative FABMS m/z 1048 $[M - H]^-$, positive FABMS m/z 1050 $[M + H]^+$; positive HRFABMS m/z 1072.5305 (calcd for $C_{50}H_{83}NO_{22}Na$, 1072.5304, $[M + Na]^+$).

Lycoperodine-1 (4): pale yellow, amorphous powder; $[\alpha]_D^{19}$ −30.3° (c 0.26, MeOH); UV (MeOH) λ_{max} (log ϵ) 274 (3.72), 291 (sh 3.50) nm; IR (neat) ν_{max} 3165, 3051, 1702, 1646 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.96[a] (1H, t, J = 8.2 Hz, H-5), 7.06[a] (1H, t, J = 8.2 Hz, H-6), 7.38 (1H, d, J = 8.2 Hz, H-7), 7.44 (1H, d, J = 8.2 Hz, H-4), 10.66 (1H, s, 1-NH), (H_2-8, H-9, 10-NH and H_2-11 were overlapped H_2O; [a]signal assignments may be reversed); ^{13}C NMR (DMSO-d_6) δ 18.0 (C-8), 40.3 (C-11), 55.3 (C-9), 104.3 (C-3), 111.8 (C-7), 117.5 (C-5), 118.5 (C-4), 121.1 (C-6), 128.5 (C-3a), 136.1 (C-6a), 165.6 (COOH); negative FABMS m/z 215 $[M - H]^-$.

Acknowledgment. We are grateful to Dr. F. Abe, Pharmaceutical Sciences, Fukuoka University, and Mr. K. Takeda and T. Iriguchi, Analytical Center of Kumamoto University, for MS and NMR measurements. We thank Prof. T. Nohara, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, for helpful discussions.

References and Notes

(1) Sato, H.; Sakamoto, S. *Agr. Biol. Chem*. **1973**, *37*, 225−231.
(2) Sinden, S. L.; Schalk, J. M.; Stoner A. K. *J. Am. Soc. Hortic. Sci*. **1978**, *103*, 596−600.
(3) Yahara, S.; Uda, N.; Nohara, T. *Phytochemistry* **1996**, *42*, 169−172.
(4) Fujiwara, Y.; Yahara, S.; Ikeda, T.; Ono, M.; Nohara, T. *Chem. Pharm. Bull.* **2003**. *51*, 234−235.
(5) Nagaoka, T.; Yoshihara, T.; Ohra, J. *Phytochemistry* **1993**, *34*, 1153−1157.
(6) Ripperger, H.; Porzal, A. *Phytochemistry* **1994**, *35*, 813−815.
(7) Kirkup, M. P.; Shankar, B. B.; McCombie, S.; Ganguly, A. K. *Tetrahedron Lett.* **1989**, *30*, 6809−6812.

NP030382X