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LYCOPEROSIDES A–C, THREE STEREOISOMERIC 23-ACETOXYSPIROSOLAN-3β-OL β-LYCOTETRAOSIDES FROM *LYCOPERSICON ESCULENTUM**

SHOJI YAHARA,† NORIKO UDA and TOSHIHIRO NOHARA

Faculty of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi 5-1, Kumamoto 862, Japan

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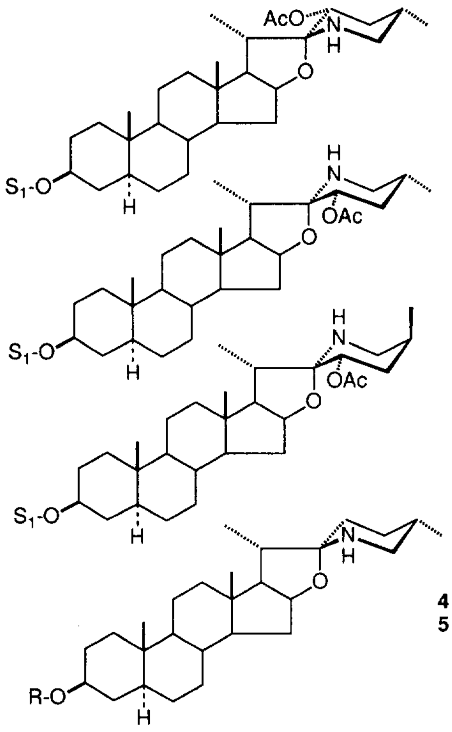
Key Word Index—*Lycopersicon esculentum*; Solanaceae; lycoperosides; steroidal glycosides; 23-acetoxyspirosolanes; (23*R*)-23-acetoxytomatidine; (23*S*)-23-acetoxysoladulcidine; (23*S*, 25*S*)-23-acetoxy-5α,22αN-spirosolan-3β-ol; tomato.

Abstract—Three new steroidal alkaloid glycosides, lycoperosides A–C, were isolated from leaves and fruits of tomato, *Lycopersicon esculentum*, and structurally elucidated as the 3-*O*-β-lycotetraosides of (23*R*)-23-acetoxytomatidine, (23*S*)-23-acetoxysoladulcidine and (23*S*,25*S*)-23-acetoxy-5α,22αN-spirosolan-3β-ol. In addition to these glycosides, leaves and fruits were found to contain the known major alkaloid tomatine (tomatidine 3-*O*-β-lycotetraoside) and leaves a small amount of γ-tomatine (tomatidine 3-*O*-[β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside], lycoperoside D).

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is an important vegetable. With regard to the constituents of this plant, tomatine from the leaves [1], bitter furostanol glycoside (TFI) from seeds [2], some steroid alkaloids, such as tomatidine, (23*R*)-23-acetoxytomatidine, (23*S*)-23-acetoxysoladulcidine and (23*S*,25*S*)-23-acetoxy-5α,22αN-spirosolan-3β-ol from the roots [3, 4], in addition to carotenes from the fruits have been reported. Our study has focused on the water-soluble constituents of the leaves of tomato and we have isolated four new spirosolane glycosides, named lycoperosides A–D (**1**–**4**), alone with tomatine (**5**). This paper deals with the structural characterization of these glycosides.



1

2

3

4 : R= S$_2$
5 : R= S$_1$

RESULTS AND DISCUSSION

Tomatine (**5**) was identified by comparison of its physical and spectral data with those of an authentic specimen; ^{13}C NMR signals were assigned by HMQC and HMBC at 500 MHz (Table 1). Lycoperosides A–D (**1**–**4**) were positive with Dragendorff's reagent, indicating that those compounds were steroidal alkaloids.

Lycoperoside A (**1**), a white powder, showed quasi ion peaks $[M + H]^+$ at m/z 1092 and $[M + Na]^+$ at m/z 1114 in the positive FAB-mass spectrum. The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of **5**,

Table 1. ^{13}C NMR data for lycoperosides A(**1**), B(**2**), C(**3**) and D(**4**), and tomatine (**5**) (in pyridine d_5)

	1	**2**	**3**	**4**	**5**
C-1	37.0	37.1	37.1	37.1	37.0
2	29.7	29.8	29.8	29.9	29.7
3	77.4*	77.5*	77.5*	77.1	77.4*
4	34.7	34.7	34.7	34.7	34.7
5	44.6	44.6	44.6	44.5	44.5
6	28.8	28.8	28.9	28.9	28.8
7	32.3	32.3	32.3	32.4	32.3
8	35.0	35.2	35.2	35.1	35.0
9	54.3	54.2	54.3	54.4	54.4
10	35.7	35.7	35.7	35.7	35.7
11	21.2	21.1	21.1	21.2	21.1
12	40.3	40.1	40.1	40.3	40.2
13	41.1	41.4	41.3	41.0	40.9
14	55.4	56.4	56.3	55.7	55.7
15	33.9	32.2	32.2	32.9	32.9
16	82.3	79.1	78.9	78.8	78.7
17	63.4	62.8	62.7	62.2	62.3
18	17.0	16.4	16.3	17.0	17.0
19	12.2	12.2	12.2	12.2	12.2
20	43.2	35.0	35.2	42.8	42.8
21	15.8	15.1	15.1	16.0	16.0
22	100.3	98.4	99.2	99.1	99.1
23	75.1	71.1	68.0	26.9	26.9
24	35.1	36.0	33.6	29.0	29.0
25	30.5	31.7	29.3	31.1	31.1
26	49.2	46.5	44.0	50.3	50.3
27	18.7	18.8	17.7	19.6	19.6
23-OAc	21.1	21.1	21.3		
	169.8	170.6	169.8		
gal C-1	102.3	102.3	102.3	102.4	102.3
2	73.0	73.1	73.1	72.1	73.0
3	75.4	75.5	75.5	75.1	75.4
4	79.7	79.8	79.8	79.9	79.7
5	75.1	75.2	75.2	75.8	75.1
6	60.5	60.5	60.5	60.9	60.5
inner					
glc C-1	105.0**	104.8**	104.8**		104.7
2	81.2	81.2	81.2		81.2
3	86.7	86.7	86.7		86.7
4	70.8	70.9	70.9		70.3
5	77.6*	77.6*	77.6*		77.6
6	62.8	62.9	62.9		62.8
terminal					
glc C-1	105.0**	105.1**	105.0**	106.6	104.9
2	76.0	76.1	76.1	75.3	76.0
3	77.3*	77.3*	77.3*	78.3*	77.2*
4	70.3	70.4	70.4	73.3	70.8
5	78.5	78.5***	78.5***	78.5*	78.5
6	62.3	62.3	62.3	63.0	62.3
xyl C-1	104.7**	104.7**	104.7**		104.7
2	74.9	74.9	74.9		74.9
3	78.5	78.6***	78.6***		78.5
4	70.6	70.6	70.6		70.6
5	67.2	67.2	67.2		67.2

*, **, *** Signal assignments may be reversed in each column.

except for carbon signals assignable to an acetoxyl group and C-20 to C-27 on the E- and F-ring. On the basis of the chemical shifts of C-16 to C-26, both configurations at C-22 and C-25 were judged to be of the same type as in tomatine. As listed in Table 1, the respective shifts by +48.2 [δ 75.1 (*d*)] and +6.1 [δ 35.1 (*t*)] were observed at C-23 and C-24 in the aglycone of **1**, compared with those of **5** and cross-peaks in the ^1H–^{13}C COSY occurred between δ 75.1 (C-23) and 5.19 (*m*), and δ 35.1 (C-24) and 1.69 (*m*)/2.01 (*m*), indicating that the acetoxyl moiety was attached to C-23 of the aglycone. The signal at δ 5.19 (*m*, H-23) overlapped with the glucosyl anomeric proton signal in pyridine-d_5 and the ^1H NMR and ^1H-^1H COSY spectra of **1** in CD_3OD exhibited a C-23 axial proton signal (*dd*, $J = 4.4$, 11.0 Hz) at δ 4.78. Therefore, the structure of **1** could be expresed as (23*R*)-23-acetoxytomatine. Its aglycone was identified by NMR as (23*R*)-23-acetoxytomatidine, which has been isolated previously from the roots of a *L. esculentum* and *L. hirsutumthis* hybrid [4].

Lycoperoside B (**2**) showed quasi-molecular ion peaks $[M + H]^+$ at *m/z* 1092 and $[M + Na]^+$ at *m/z* 1114 in the positive FAB-mass spectrum. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the C-20 to C-26 signals for the E- and F-ring, whose signals were superimposable on those of (23*S*)-23-acetoxysoladulcidine [4]. Accordingly, **2** was suggested to be the C-22, C-23 and C-25 isomer of **1**. This was confirmed by a comparative study of the ^{13}C NMR data of the aglycone part in **2** with those of (23*S*)-23-acetoxysoladulcidine [4].

Lycoperoside C (**3**) showed quasi-molecular ion peaks $[M + H]^+$ at *m/z* 1092 and $[M + Na]^+$ at *m/z* 1114 in the positive FAB-mass spectrum; thus, **3** was suggested to be an isomer of **1** and **2**. The ^1H NMR and ^1H-^1H COSY spectra of **3** were similar to those of **1** and **2**, except for the signals of δ 2.42 (1H, *br d*, $J = 11.0$ Hz), 3.30 (1H, *dd*, $J = 3.7$, 11.0 Hz) and 5.43 (1H, *dd*, $J = 5.0$, 12.0 Hz), ascribable to the H_2-26 and H-23 axial protons, respectively. Hence, the structure of **3** was considered to be a 23-acetoxy spirosolane with the (25*S*) configuration. By comparing the ^{13}C NMR spectrum of **3** with those of **1** and **2**, the C-20 signal in **3** appeared at δ 35.2; the configuration of C-22 in **3** was therefore determined to be *S*. The aglycone of **3** was identified as (23*S*,25*S*)-23-acetoxy-5α,22α*N*-spirosolan-3β-ol [4]. Consequently, **3** was characterized as shown in the formula.

Lycoperoside D (**4**) showed a quasi-molecular ion peak $[M + H]^+$ at *m/z* 740 in the positive FAB-mass spectrum. The ^{13}C NMR data indicated the presence of one terminal β-glucopyranosyl moiety and one β-galactopyranosyl moiety substituted at C-4 (δ 79.9). The aglycone moiety signals were analogous to those of tomatine (**5**). Consequently, **4** was shown to be identical to γ-tomatine [5].

EXPERIMENTAL

^1H (400 MHz) and ^{13}C NMR (100 MHz) NMR: TMS int. standard. TLC: precoated Kieselgel 60 F_{245} (Merck). CC: Kieselgel (270–400 mesh, Merck), Chromatorex ODS (Fuji Silisia Ltd) and MCI gel CHP-20P (Mitsubishi Chemical Ind.).

Extraction and separation. Dried leaves of *L. esculentum* Miller (1.5 kg) were extracted with MeOH and the extract (350 g) shaken with benzene and H_2O. The aq. phase yielded a residue of 222 g. This was subjected to CC on MCI gel CHP-20P eluting with $H_2O \rightarrow 40\% \rightarrow 60\% \rightarrow 80\%$ MeOH $\rightarrow$ MeOH $\rightarrow$ 2% NH_3/MeOH to provide nine frs. Fr. 6 (1.2 g, MeOH eluate) was subjected to a combination of various CC on silica gel ($CHCl_3$–MeOH–H_2O–NH_4OH, 7:2.2:0.2:0.1) and Chromatorex ODS (70–75% MeOH) to give **1** (41 mg, $2.7 \times 10^{-3}\%$), **2** (31 mg, $2.1 \times 10^{-3}\%$) and **3** (34 mg, $2.3 \times 10^{-3}\%$). Fr. 7 (2.6 g, 2% NH_3/MeOH eluate) was subjected to a combination of various CC over silica gel ($CHCl_3$–MeOH–H_2O–NH_4OH, 7:3:0.5:0.1) and Chromatorex ODS (80–85% MeOH) to give **4** (14 mg, $9.3 \times 10^{-4}\%$) and **5** (1.7 g, 0.11%).

Lycoperoside A (**1**). Amorphous powder. $[\alpha]_D^{27}$ −31.6° (*c* 0.80, MeOH). Positive FABMS (*m/z*): 1114.5402 (calcd for $C_{52}H_{85}NO_{23}Na$: 1114.5410, $[M + Na]^+$), 1092 $[M + H]^+$. ^1H NMR (pyridine-d_5): δ 0.51 (1H, *br t*, $J = 10.0$ Hz, H-9), 0.63 (3H, *s*, H_3-19), 0.78 (3H, *d*, $J = 5.9$ Hz, H_3-27), 0.88 (3H, *s*, H_3-18), 1.15 (3H, *d*, $J = 8.0$ Hz, H_3-21), 1.69 (1H, *m*, H_a-24), 2.01 (1H, *m*, H_b-24), 2.21 (3H, *s*, Ac), 2.72 (1H, *br d*, $J = 10.8$ Hz, H_a-26), 2.87 (1H, *t*, $J = 10.8$ Hz, H_b-26), 3.67 (1H, *t*, $J = 10.2$ Hz, xyl H-5), 3.80–4.60 (22H, *m*, sugar), 4.68 (1H, *t*, $J = 9.8$ Hz, gal H-6), 4.88 (1H, *d*, $J = 7.3$ Hz, gal H-1), 5.02 (1H, *ddd*, $J = 7.3$, 7.3, 8.4 Hz, H-16), 5.18 (1H, *d*, $J = 7.3$ Hz, inner glc H-1), 5.19 (1H, *m*, H-23) (in CD_3OD (δ 4.78, *dd*, $J = 4.4$, 11.0 Hz)), 5.22 (1H, *d*, $J = 7.7$ Hz, terminal glc H-1), 5.56 (1H, *d*, $J = 7.3$ Hz, xyl H-1).

Lycoperoside B (**2**). Amorphous powder. $[\alpha]_D^{27}$ −40.3° (*c* 0.56, MeOH). Positive FABMS (*m/z*): 1114.5403 (calcd for $C_{52}H_{85}NO_{23}Na$: 1114.5410, $[M + Na]^+$), 1092 $[M + H]^+$. ^1H NMR (pyridine-d_5): δ 0.50 (1H, *br t*, $J = 11.0$ Hz, H-9), 0.66 (3H, *s*, H_3-19), 0.79 (3H, *d*, $J = 6.0$ Hz, H_3-27), 0.92 (3H, *s*, H_3-18), 1.12 (3H, *d*, $J = 7.0$ Hz, H_3-21), 2.20 (3H, *s*, Ac), 2.68 (1H, *dd*, $J = 5.0$, 11.0 Hz, H_a-26), 2.74 (1H, *t*, $J = 11.0$ Hz, H_b-26), 3.68 (1H, *t*, $J = 10.4$ Hz, xyl H-5), 3.79–4.60 (22H, *m*, sugar), 4.55 (1H, *m*, H-16), 4.69 (1H, *t*, $J = 9.5$ Hz, gal H-6), 4.88 (1H, *d*, $J = 7.7$ Hz, gal H-1), 5.18 (1H, *d*, $J = 7.7$ Hz, inner glc H-1), 5.20 (1H, *m*, H-23) (in CD_3OD (δ 4.81, *dd*, $J = 4.8$, 11.8 Hz), 5.23 (1H, *d*, $J = 7.7$ Hz, terminal glc H-1), 5.57 (1H, *d*, $J = 7.3$ Hz, xyl H-1).

Lycoperoside C (**3**). Amorphous powder. $[\alpha]_D^{27}$ −26.3° (*c* 0.63, MeOH). Positive FABMS (*m/z*):

(3H, *s*, H_3-18), 1.13 (3H, *d*, $J = 7.0$ Hz, H_3-21), 1.22 (3H, *d*, $J = 7.0$ Hz, H_3-27), 2.17 (3H, *s*, Ac), 2.42 (1H, *br d*, $J = 11.0$ Hz, H_a-26), 3.30 (1H, *dd*, $J = 3.7$, 11.0 Hz, H_b-26), 3.68 (1H, *t*, $J = 10.2$ Hz, xyl H-5), 3.80–4.60 (22H, *m*, sugar), 4.48 (1H, *ddd*, $J = 7.2$, 7.2, 8.4 Hz, H-16), 4.69 (1H, *t*, $J = 9.9$ Hz, gal H-6), 4.88 (1H, *d*, $J = 7.7$ Hz, gal H-1), 5.19 (1H, *d*, $J = 8.0$ Hz, inner glc H-1), 5.23 (1H, *d*, $J = 7.7$ Hz, terminal glc H-1), 5.43 (1H, *dd*, $J = 5.0$, 12.0 Hz, H-23), 5.56 (1H, *d*, $J = 7.0$ Hz, xyl H-1).

Lycoperoside D (**4**). Amorphous powder. $[\alpha]_D^{27}$ $-10.8°$ (*c* 0.60, MeOH). Positive FABMS (*m/z*): 740 $[M + H]^+$. ^{1}H NMR (pyridine d_5): δ 0.56 (1H, *br t*, $J = 10.0$ Hz, H-9), 0.65 (3H, *s*, H_3-19), 0.81 (3H, *d*, $J = 6.6$ Hz, H_3-21), 0.86 (3H, *s*, H_3-18), 1.07 (3H, d, $J = 7.0$ Hz, H_3-27), 2.82 (1H, *br d*, $J = 10.7$ Hz, H-26), 2.94 (1H, *t*, $J = 10.7$ Hz, H-26), 3.93 (1H, *m*, H-3), 4.05–4.32 (8H, *m*, sugar), 4.21 (1H, *m*, H-16), 4.41 (1H, *t*, $J = 9.2$ Hz, gal H-2), 4.62 (1H, *br d*, $J = 9.2$ Hz, glc H-6), 4.69 (1H, *t*, $J = 9.4$ Hz, gal H-6), 4.73 (1H, *t*, $J = 3.7$ Hz, gal H-4), 4.90 (1H, *d*, $J = 7.7$ Hz, gal H-1), 5.30 (1H, *d*, $J = 8.0$ Hz, glc H-1).

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