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# THE STEROIDAL GLYCOSIDES FROM THE CAUDEX OF *YUCCA GLORIOSA**

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**Key Word Index**—*Yucca gloriosa*; Agavaceae; steroidal saponins; smilagenin glycosides; samogenin glycoside.

**Abstract**—Five steroidal glycosides were isolated from the fresh caudex of *Yucca gloriosa* together with YG-1 and Ps-1 previously obtained from flowers and the structures of these glycosides were established to be smilagenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (Ys-I), 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside (YS-II), 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (YS-III), 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranoside (YS-IV) and samogenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside (YS-V), respectively.

## INTRODUCTION

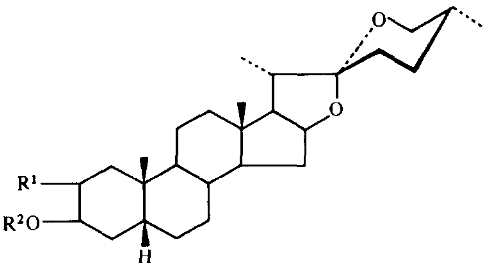
In the previous paper we reported [1] the isolation of four steroidal compounds from the fresh flowers of *Yucca gloriosa* L. The structures were elucidated to be tigogenin and gitogenin glycosides (3-*O*-β-D-xylopyranosyl-β-lycotetraosides, 3-*O*-α-L-rhamnopyranosyl-β-lycotetraoside and 26-*O*-β-D-glucopyranosyl 3-*O*-β-D-xylopyranosyl-β-lycotetraoside, respectively). The present paper describes the isolation and characterization of five new steroidal glycosides from the methanolic caudex extract of the same plant.

## RESULTS AND DISCUSSION

Five steroidal glycosides were obtained from the methanolic caudex extract of *Y. gloriosa* as described in the Experimental and gives the trivial assignments Ys-I, II, III, IV, and V. Ys-I, II, III, IV and V all gave positive Liebermann–Burchard tests, but were negative to Ehrlich's reagent. Their IR spectra showed the characteristic absorption bands for a (25*R*)-spiroketal side chain.

After acid hydrolysis of Ys-I (**1**), II (**2**), III (**3**) and IV (**4**), the aglycone was identified as smilagenin by mp, $[\alpha]_D$, IR, MS, $^1$H and $^{13}$C NMR [2]. TLC analysis of the sugars obtained from the hydrolysates of **1** and **3** showed glucose, while **2** and **4** showed glucose and galactose. The FAB mass spectra and $^{13}$C NMR spectra of **1** and **2** revealed the presence of two sugar units, while **3** and **4** revealed the presence of three sugar units. The permethylates (**1a**–**4a**) of **1**–**4**, prepared by Hakomori's method [3], showed that the anomeric proton linkages were all β in the $^1$H NMR spectrum. On methanolysis methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside were identified from **1a**, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 3,4,6-tri-*O*-methyl-D-galactopyranoside were identified from **2a**, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 4,6-di-*O*-methyl-D-glucopyranoside were identified from **3a** and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 4,6-di-*O*-methyl-D-galactopyranoside were identified from **4a**, respectively. Based on these results, Ys-I–IV can be formulated as having structures **1**–**4**, respectively.

On acid hydrolysis Ys-V (**5**) afforded the aglycone (**6**) $C_{27}H_{44}O_4$ ($M^+$ at *m/z* 432.3200), glucose and galactose. The $^{13}$C NMR spectrum of **6** exhibited the signals of 5β-steroidal sapogenin [2] (C-5; δ 36.1; C-9, 41.5; C-19, 24.1) and five signals in the carbinyl carbon region at δ 63.1 (*d*), 66.8 (*t*), 67.5 (*d*), 70.2 (*d*), and 81.1 (*d*), and three of them at δ 63.1, 66.8 and 81.1 were assigned to C-17, C-26 and C-16, respectively, in comparison with those of smilagenin. Acetylation of **6** with acetic anhydride and pyridine gave a diacetate **6a**, which showed two singlet signals at δ 0.76 and 1.04 due to C-18 and C-19 methyls, two doublet



**1** R¹ = H, R² = -β-D-glc —2— β-D-glc
**2** R¹ = H, R² = -β-D-gal —2— β-D-glc
**3** R¹ = H, R² = -β-D-glc$_3$ —2— β-D-glc
 β-D-glc
**4** R¹ = H, R² = -β-D-gal$_3$ —2— β-D-glc
 β-D-glc
**5** R¹ = β-OH, R² = -β-D-gal —2— β-D-glc

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*Part 2 in the series 'The constituents of *Yucca gloriosa*'.

Table 1. $^{13}$C NMR data of Ys-I, Ys-II, Ys-III, Ys-IV, Ys-V and **6** ($d_5$-pyridine at room temperature)

| C | Ys-I (**1**) | Ys-II (**2**) | Ys-III (**3**) | Ys-IV (**4**) | Ys-V (**5**) | (**6**) |
|---|---|---|---|---|---|---|
| 1 | 30.7 | 30.9 | 30.9 | 30.7 | 40.5 | 39.2 |
| 2 | 26.8 | 26.7 | 26.8 | 26.8 | 67.2 | 70.2 |
| 3 | 76.8 | 76.4 | 76.4 | 76.3 | 81.7 | 67.5 |
| 4 | 30.9 | 30.9 | 30.8 | 30.7 | 31.9 | 33.5 |
| 5 | 36.8 | 36.9 | 36.8 | 36.5 | 36.5 | 36.1 |
| 6 | 26.8 | 26.7 | 26.8 | 26.8 | 26.3 | 26.3 |
| 7 | 27.0 | 27.0 | 27.0 | 27.0 | 26.8 | 26.8 |
| 8 | 35.5 | 35.5 | 35.6 | 35.7 | 35.6 | 35.7 |
| 9 | 40.3 | 40.3 | 40.4 | 40.3 | 41.4 | 41.5 |
| 10 | 35.2 | 35.2 | 35.3 | 35.2 | 37.1 | 37.0 |
| 11 | 21.1 | 21.1 | 21.2 | 21.2 | 21.3 | 21.3 |
| 12 | 40.3 | 40.3 | 40.4 | 40.3 | 40.3 | 40.4 |
| 13 | 40.9 | 40.9 | 40.9 | 40.9 | 40.8 | 40.9 |
| 14 | 56.5 | 56.5 | 56.5 | 56.5 | 56.4 | 56.5 |
| 15 | 31.9[a] | 31.9[a] | 31.9[a] | 31.9[a] | 32.1[a] | 32.1[a] |
| 16 | 81.2 | 81.2 | 81.3 | 81.2 | 81.2 | 81.1 |
| 17 | 63.1 | 63.2 | 63.2 | 63.2 | 63.2 | 63.1 |
| 18 | 16.6 | 16.6 | 16.6 | 16.6 | 16.5 | 16.6 |
| 19 | 24.0 | 24.0 | 24.0 | 23.9 | 23.9 | 24.1 |
| 20 | 42.0 | 42.0 | 42.0 | 42.0 | 42.0 | 42.0 |
| 21 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 14.9 |
| 22 | 109.1 | 109.2 | 109.2 | 109.2 | 109.1 | 109.1 |
| 23 | 32.1[a] | 32.1[a] | 32.2[a] | 32.2[a] | 31.9[a] | 31.8[a] |
| 24 | 29.3 | 29.3 | 29.3 | 29.3 | 29.3 | 29.2 |
| 25 | 30.6 | 30.6 | 30.6 | 30.6 | 30.6 | 30.6 |
| 26 | 66.9 | 66.9 | 66.9 | 66.9 | 66.9 | 66.8 |
| 27 | 17.3 | 17.3 | 17.3 | 17.3 | 17.3 | 17.3 |
| glc (gal) | | | | | | |
| 1′ | 101.8 | 102.4 | 101.8 | 101.8 | 103.2 | |
| 2′ | 83.0 | 81.7 | 79.9 | 77.7 | 81.8 | |
| 3′ | 77.8 | 75.4 | 88.4 | 84.0 | 76.8 | |
| 4′ | 71.6 | 69.8 | 70.0 | 69.8 | 69.7 | |
| 5′ | 78.3[b] | 76.7 | 77.8 | 76.3 | 76.8 | |
| 6′ | 62.7[c] | 62.1[b] | 63.4 | 63.5 | 62.9[b] | |
| glc 1″ | 105.8 | 105.9 | 104.2 | 105.2 | 106.1 | |
| 2″ | 75.2 | 75.1 | 75.3[b] | 75.0[b] | 75.1 | |
| 3″ | 78.1[b] | 77.9[c] | 78.3[c] | 78.3 | 78.3[c] | |
| 4″ | 71.8 | 71.7 | 71.6 | 71.5 | 71.7 | |
| 5″ | 78.0[b] | 78.2[c] | 78.5[c] | 78.3 | 78.0[c] | |
| 6″ | 62.9[c] | 62.9[b] | 62.4[d] | 62.3[c] | 62.0[b] | |
| glc 1‴ | | | 104.8 | 104.4 | | |
| 2‴ | | | 75.5[b] | 75.2[b] | | |
| 3‴ | | | 78.1[c] | 78.3 | | |
| 4‴ | | | 72.5 | 72.7 | | |
| 5‴ | | | 78.5[c] | 78.3 | | |
| 6‴ | | | 62.5[d] | 62.5[c] | | |

[a–d] Assignments may be interchanged in each column.

signals (both $J = 6.3$ Hz) at $\delta$ 0.78 and 0.96 ascribable to C-27 and C-21 methyls and two acetoxyl signals at $\delta$ 1.98 and 2.09. Moreover, the signals at $\delta$ 3.37 (1H, *t*, $J = 10.5$ Hz), 3.46 (1H, *m*), 4.39 (1H, *m*) and 5.30 (1H, *br d*, $J = 3$ Hz) could be assigned to the 26-axial and -equatorial protons, 16α-H and 3α-H, respectively. Irradiation of the broad doublet signal at $\delta$ 5.30 collapsed a double double doublet at $\delta$ 4.86 (1H, $J = 3$, 4, 11 Hz) into a double doublet, which could be assigned to 2α-H. Therefore, the remaining two carbon signals at $\delta$ 67.5 and 70.2 could be assigned to C-3 and C-2. Thus, compounds **6** and **6a** were inferred to be (25*R*)-5β-spirostan-2β,3β-diol (samogenin) and its acetate, respectively, and this was confirmed by comparisons with the data of authentic samples [4].

The FAB mass spectra and $^{13}$C NMR spectra of **5** suggested the presence of two sugar units. Methanolysis of the permethylate (**5a**) of **5** gave monomethylated samogenin (**6b**) and two methylated sugars which were identified as methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-galactose.

In the $^1$H NMR spectrum, the acetate (**6c**) of **6b** showed an acetoxyl signal at $\delta$ 2.09, one methoxyl signal at 3.32 and one methine signal at 5.40 (1H, *br s*, $W_{1/2} = 7$ Hz) ascribable to 3α-H, that is, hydrogen attached to the carbon bearing an acetoxyl group. Furthermore, the $^{13}$C NMR spectrum of **5** indicated that the C-3 signal of the aglycone was shifted downfield, while the C-2 and C-4 signals were shifted upfield, and the sugar moiety was the same as that of **2**.

Based on the above data the structure of Ys-V has been established as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-(25*R*)-5β-spirostan-2β,3β-diol (**5**).

## EXPERIMENTAL

Almost all the instrumentation techniques were described in ref. [1]. TLC and CC using the solvent system: *a*, $CHCl_3$–MeOH–$H_2O$ 40:10:1; *b*, $CHCl_3$–MeOH–$H_2O$ 14:6:1; *c*, $CHCl_3$–MeOH–$H_2O$ 6:4:1; *d*, $CHCl_3$–MeOH–AcOEt–$H_2O$ 4:4:10:1; *e*, hexane–$Me_2CO$ 5:1.

*Extraction and isolation of saponins.* The fresh caudex (8 kg) of *Yucca gloriosa* before flowering, which is frequently cultivated as a garden plant in Japan, was obtained at the campus of this Faculty in April 1985, and extracted three times with hot MeOH. The extract was concd *in vacuo* and the residue (934 g) was defatted with *n*-hexane. The insoluble portion (878 g) was partitioned between *n*-BuOH and $H_2O$, and the BuOH layer was concd under red. pres. to afford a brown powder (132 g) which was subjected to CC on silica gel with solv. *a*→*c* to provide 10 fractions (Fr.1–Fr.10). Fr. 2 (18.8 g) was recrystallized from MeOH to afford Ys-II (**2**, 492 mg). Concentration of the mother liquors yielded the residue, which was subjected to CC on silica gel with solv. *d* to afford Ys-I (**1**, 57 mg) and Ys-II (**2**, 350 mg). Fr. 3 (3.8 g) was subjected to CC on silica gel with solv. *a* and *d*, to afford Ys-II (**2**, 75 mg), Ys-III (**3**, 74 mg) and Ys-V (**5**, 182 mg). Fr. 4 (6.1 g) was recrystallized from MeOH and $CHCl_3$ (1:1) to afford colourless needles which was identified as a mixture (930 mg) of YG-1 and Ps-1 [1] by FAB MS and $^{13}$C NMR spectra. The mother liquor was subjected to CC on Sephadex LH-20 with MeOH and silanized silica gel with 60% MeOH, to afford Ys-IV (**4**, 175 mg), Ys-VI and Ys-VII together with Ps-1 (73 mg).

*Properties of Ys-I, II, III, IV and V.* Ys-I (**1**), colourless needles, mp 247–249°, $[\alpha]_D^{26}$ −0.2° (pyridine; *c* 1.0), IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3200–3500 (OH), 980, 920, 900, 860 (intensity 900 > 920, 25*R*-spiroketal), FAB MS *m/z*: 740 $[M]^+$. Ys-II (**2**), colourless needles, mp 252–255°, $[\alpha]_D^{26}$ −51.0° ($CHCl_3$–MeOH 1:1, *c* 1.0), IR$\nu_{max}^{KBr}$ cm$^{-1}$: 3200–3500 (OH), 980, 920, 900, 860 (900 > 920, 25*R*-spiroketal), FABMS *m/z*: 763 $[M+Na]^+$. Ys-III (**3**), colourless needles, mp 258–260° (dec.), $[\alpha]_D^{22}$ +66.7° ($CHCl_3$–MeOH 1:1; *c* 1.1), IR$\nu_{max}^{KBr}$ cm$^{-1}$: 3200–3500 (OH), 980, 920, 900, 860 (900 > 920, 25*R*-spiroketal), FABMS *m/z*: 925 $[M+Na]^+$. Ys-IV (**4**), colourless needles, mp 265–267° (dec.), $[\alpha]_D^{19}$ −43.0° (MeOH; *c* 1.0), IR$\nu_{max}^{KBr}$ cm$^{-1}$: 3200–3500 (OH), 980, 920, 900, 860 (900 > 920, 25*R*-spiroketal), FABMS *m/z*: 925 $[M+Na]^+$. Ys-V (**5**), colourless needles, mp 258–261° (dec.), $[\alpha]_D^{26}$

−75.0° ($CHCl_3$–MeOH 1:1; *c* 1.0), IR$\nu_{max}^{KBr}$ $cm^{-1}$: 3200–3500 (OH), 990, 930, 905, 870 (905>930, 25*R*-spiroketal), FABMS *m*/*z*: 757 $[M+H]^+$.

*Acidic hydrolysis of compounds* **1**, **2**, **3**, **4** *and* **5**. Compounds **1** (10 mg), **2** (50 mg), **3** (10 mg), **4** (10 mg) and **5** (30 mg) were hydrolysed with 2 M HCl–MeOH by refluxing for 2 hr on a water bath, then the reaction mixture was neutralized with 3% KOH–MeOH and evapd to dryness under red. pres. From the residues of **1**, **2**, **3** and **4**, smilagenin was identified. Colourless needles, mp 182–185°, $[\alpha]_D^{26}$ −53.0° ($CHCl_3$, *c* 1.0), $^1H$ NMR ($CDCl_3$): $\delta$0.76 (3H, *s*), 0.79 (3H, *d*, *J* = 6.3 Hz), 0.96 (3H, *d*, *J* = 6.3 Hz), 0.98 (3H, *s*), 3.40 (2H, *m*, 26-$H_2$), 4.11 (1H, *m*, $W_{1/2}$ = 7 Hz, 3α-H), 4.37 (1H, *m*, 16-H). The residue of **5** was subjected to CC on silica gel with $CHCl_3$–MeOH (10:1) to afford the aglycone **6** (22 mg), colourless needless, mp 203–205°, $[\alpha]_D^{23}$ −73.9° ($CHCl_3$, *c* 1.0), HRMS *m*/*z*: 432.3200 [$M^+$, calcd for $C_{27}H_{44}O_4$: 432.3241]. The sugar components were examined by TLC (solv. *b*, **1** and **3**; $R_f$ 0.34 glucose, **2**, **4** and **5**; $R_f$ 0.31 galactose, $R_f$ 0.34 glucose).

*Acetylation of* **6**. Acetate **6a** was prepared from **6** with $Ac_2O$ and pyridine and crystallized from MeOH, mp 197–199°, $[\alpha]_D^{19}$ −15.5° ($CHCl_3$; *c* 0.9), $^1H$ NMR ($CDCl_3$): $\delta$0.76 (3H, *s*), 0.78 (3H, *d*, *J* = 6.3 Hz), 0.96 (3H, *d*, *J* = 6.3 Hz), 1.04 (3H, *s*), 1.98, 2.09 (each 3H, *s*, OAc), 3.37 (1H, *t*, *J* = 10.5 Hz, 26-Ha), 3.46 (1H, *m*, 26-He), 4.39 (1H, *m*, 16-H), 4.86 (1H, *ddd*, *J* = 3, 4, 11 Hz, 2α-H), 5.30 (1H, *br d*, *J* = 3 Hz, 3α-H).

*Methylation of compounds* **1**, **2**, **3**, **4** *and* **5**. Compounds **1** (30 mg), **2** (50 mg), **3** (10 mg), **4** (50 mg) and **5** (70 mg) were permethylated with NaH and MeI by Hakomori's method. The product was purified by CC (solv. hexane–$Me_2CO$ 3:1) to afford permethylates **1a** (5 mg), **2a** (40 mg), **3a** (6 mg), **4a** (22 mg) and **5a** (50 mg). $^1H$ NMR ($CDCl_3$): $\delta$ **1a**; 4.30 (1H, *d*, *J* = 7.8 Hz), 4.67 (1H, *d*, *J* = 7.8 Hz), **2a**; 4.27 (1H, *d*, *J* = 7.6 Hz), 4.70 (1H, *d*, *J* = 7.8 Hz), **3a**; 4.24, 4.79, 4.84 (each 1H, *d*, *J* = 7.8 Hz), **4a**; 4.27 (1H, *d*, *J* = 7.6 Hz), 4.80, 4.89 (each 1H, *d*, *J* = 7.8 Hz), **5a**; 4.23 (1H, *d*, *J* = 7.9 Hz), 4.73 (1H, *d*, *J* = 7.6 Hz).

*Methanolysis of compounds* **1a**, **2a**, **3a**, **4a** *and* **5a**. Compounds **2a** (20 mg), **4a** (15 mg), **5a** (30 mg) and a small amount of **1a**, and **3a** were separately refluxed with 2 M HCl–MeOH. The neutralized (KOH–MeOH) and the concd hydrolysates were examined by TLC and GLC and identified as methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside ($R_f$ 0.54; $C_6H_6$–$Me_2CO$ 2:1, $R_t$ 3.60, 4.50) methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside ($R_f$ 0.30, $R_t$ 6.30, 6.75) from **1a**, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 3,4,6-tri-*O*-methyl-D-galactopyranoside ($R_f$ 0.23, $R_t$ 7.2, 8.6) from **2a** and **5a**, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 4,6-di-*O*-methyl-D-glucopyranoside ($R_f$ 0.10, $R_t$ 9.7, 10.9) from **3a**, methyl 2,3,4,6-tetra-*O*-methyl-D-gluco-pyranoside and methyl 4,6-di-*O*-methyl-D-galactopyranoside ($R_f$ 0.08, $R_t$ 9.3, 12.5) from **4a**. The methanolysate of **5a** was subjected to CC on silica gel (solv. e) to afford **6b** (20 mg), colourless needles, $[\alpha]_D^{19}$ −19.5° ($CHCl_3$, *c* 0.9), which was acetylated in the usual way to afford an acetate **6c** (9.4 mg), colourless needles, mp 173–176°, $[\alpha]_D^{19}$ −67.7° ($CHCl_3$; *c* 0.94), $^1H$ NMR ($CDCl_3$): $\delta$0.76 (3H, *s*), 0.78, 0.96 (each 3H, *d*, *J* = 6.8 Hz), 1.02 (3H, *s*), 2.09 (3H, *s*, OAc), 3.24 (1H, *ddd*, *J* = 3,4,11 Hz, 2α-H), 3.32 (3H, *s*, OMe), 4.38 (1H, *m*, 16-H), 5.40 (1H, *br s*, $W_{1/2}$ = 7 Hz, 3α-H).

## REFERENCES

1. Nakano, K., Matsuda, E., Tsurumi, K., Yamasaki, T., Murakami, K., Takaishi, Y. and Tomimatsu, T. (1988) *Phytochemistry* **27**, 3235.
2. Tori, K., Seo, S., Terui, Y., Nishikawa, J. and Yasuda, F. (1981) *Tetrahedron Letters* **22**, 2405.
3. Hakomori, S. (1964) *J. Biol. Chem.* **55**, 205.
4. Marker, R. E. and Lopez, J. (1947) *J. Am. Chem. Soc.* **69**, 2375.