*Phytochemistry*, Vol. 30, No. 2, pp. 633–636, 1991
Printed in Great Britain.

0031–9422/91 $3.00+0.00


# 12-KETO STEROIDAL GLYCOSIDES FROM THE CAUDEX OF *YUCCA GLORIOSA**

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(*Received* 12 *June* 1990)

**Key Word Index**—*Yucca gloriosa*; Agavaceae; caudex; 12-keto steroidal saponins; mexogenin glycoside; gloriogenin glycosides; manogenin glycosides.

**Abstract**—Eight new steroidal glycosides, tentatively named YS-VI, -VII, -VIII, -IX, -X, -XI, -XII and -XIII were isolated from the caudex of *Yucca gloriosa* along with P-1, YG-2 and YG-3 previously obtained from flowers. The structures of five of these compounds were elucidated as mexogenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranoside (YS-VI), gloriogenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside (YS-VII) and 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-galactopyranoside (YS-VIII), manogenin 3-*O*-*β*-lycotetraoside (YS-IX) and 3-*O*-*α*-L-rhamnopyranosyl-*β*-lycotetraoside (YS-X), respectively, on the basis of chemical and spectral evidence.

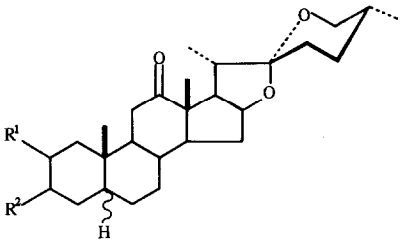
## INTRODUCTION

In the preceding papers of this series [1, 2], we reported the isolation and structure determination of nine steroidal saponins, tentatively named YG-1, -2, -3 and -4 (tigogenin and gitogenin glycosides from flowers), YS-I, -II, -III, -IV and -V (smilagenin and samogenin glycosides from caudex). Further investigation of caudex led to the isolation of eight additional saponins, named YS-VI, -VII, -VIII, -IX, -X, -XI, -XII and -XIII. This paper reports the structure elucidation of YS-VI (**1**), -VII (**2**), -VIII (**3**), -IX (**4**) and -X (**5**).

## RESULTS AND DISCUSSION

The remaining material from fractions 4 and 5 obtained in the previous work [2] were rechromatographed as described in the Experimental to afford YS-VI (**1**), -VII (**2**), -VIII (**3**), -IX (**4**), -X (**5**), -XI, -XII and -XIII. Compounds **1**–**5** produced a yellow colour with $H_2SO_4$ on TLC. Their IR spectra showed the characteristic absorption bands for a (25*R*)-spiroketal side chain, strong absorptions at 1700–1720 $cm^{-1}$ (carbonyl on six-membered ring) and 3200–3500 $cm^{-1}$ (hydroxyls).

Hydrolysis (2 M HCl–MeOH) of **2** and **3** gave the same sapogenin (**6**), $C_{27}H_{42}O_4$ ([M]$^+$ at *m/z* 430.3061), mp 186–188°, $[\alpha]_D^{22}+44.4°$ ($CHCl_3$). Its IR spectrum showed a broad band at 3500 $cm^{-1}$ for OH, a strong band at 1706 $cm^{-1}$ for a six-membered ring carbonyl and the characteristic bands for the spirostan ring system. The ORD curve of **6** showed a strong positive Cotton effect associated with the carbonyl group, which was similar to that of hecogenin [3]. The $^1$H NMR spectrum of **6** showed signals ascribable to the C-18 and C-19 methyl groups at $\delta$1.04 and 1.05 and the C-27 and C-21 methyls at 0.79 (*d*, *J* = 6.1 Hz) and 1.07 (*d*, *J* = 7.1 Hz). Using the Tori *et al.* [4] additivity rules for predicting the chemical shifts of methyl proton resonances in steroidal sapogenins, the observed values for compound **6** were found to be in best agreement with those calculated for 5*β*(25*R*)-spirostan-3*β*-ol-12-one. Moreover, the $^{13}$C NMR spectrum of **6** exhibited the signals of a 5*β*-steroidal sapogenin [5] (C-5: $\delta$36.6; C-9: 41.8; C-19: 23.4), a carbonyl signal at $\delta$212.9 and three signals in the carbinyl carbon region at 65.7 (CH), 67.0 ($CH_2$) and 79.8 (CH) which could be assigned to C-3, C-26 and C-16 by comparison with the corresponding signals of smilagenin and hecogenin [6].



**1** $R^1$ = *β*-OH, $R^2$ = -*β*-D-Gal—(2)—*β*-D-Glc, 5*β*-H
**2** $R^1$ = H, $R^2$ = -*β*-D-Glc—(2)—*β*-D-Glc (3)—*β*-D-Glc 5*β*-H
**3** $R^1$ = H, $R^2$ = -*β*-D-Gal—(2)—*β*-D-Glc (3)—*β*-D-Glc 5*β*-H
**4** $R^1$ = *α*-OH, $R^2$ = -*β*-D-Gal—(4)—*β*-D-Glc—(2)—*β*-D-Glc (3)—*β*-D-Xyl 5*α*-H
**5** $R^1$ = *α*-OH, $R^2$ = -*β*-D-Gal—(4)—*β*-D-Glc—(2)—*β*-D-Glc (3)—*β*-D-Xyl—(4)—*α*-L-Rha 5*α*-H
**6** $R^1$ = $R_2$ = H, 5*β*-H
**7** $R^1$ = *β*-OH, $R^2$ = H, 5*β*-H
**8** $R^1$ = *α*-OH, $R^2$ = H, 5*α*-H

*Part 3 in the series 'The Constituents of *Yucca gloriosa*'. For Part 2 see ref. [2].

Thus, compound **6** was represented as 5β(25*R*)-spirostan-3β-ol-12-one and identified with gloriogenin by comparison with the published data [7, 8]. TLC analysis of the sugars obtained from the hydrolysate of **2** showed D-glucose, while **3** showed D-glucose and D-galactose. The FAB mass and $^{13}$C NMR spectra of **2** and **3** revealed the presence of three sugar units in each compound.

Methanolysis of the permethylate (**2a**) of **2**, prepared by Hakomori's method [9] yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 4,6-di-*O*-methyl-D-glucose. From the methanolysate of the permethylate (**3a**) of **3** was obtained 2,3,4,6-tetra-*O*-methyl-D-glucose and 4,6-di-*O*-methyl-D-galactose. The identities of the methylated sugars were established by comparison with the authentic samples [2]. Furthermore the comparison of the $^{13}$C NMR signals of **2** and **3** (Table 1) with those of YS-III and -IV [2] showed them to have the same glycosidic linkages.

Thus, YS-VII and -VIII were characterized as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-5β(25*R*)-spirostan-3β-ol-12-one (**2**) and 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranosyl-5β(25*R*)-spirostan-3β-ol-12-one (**3**), respectively.

Acid hydrolysis of **1** gave the aglycone (**7**), $C_{27}H_{42}O_5$ ([M]$^+$ at *m/z* 446.3021), mp 225–227° (dec.), $[\alpha]_D^{26}$ −16.5° (MeOH) which was also considered to be a 12-keto steroid sapogenin from the IR, ORD and $^{13}$C NMR spectra. Acetylation of **7** gave the diacetate (**7a**), which showed the presence of a 2β- and 3β-acetoxy group (δ1.98, 2.09, 3H each *s*; 4.75 1H, *ddd*, *J* = 2.4, 4.6, 10.8 Hz; 5.32 1H, *br d*, *J* = 2.4 Hz) in the $^1$H NMR spectrum. Irradiation of the broad doublet signal at δ5.32 collapsed a double double doublet at 4.75 into a double doublet (*J* = 4.6, 10.8 Hz). The $^{13}$C NMR spectrum of compound **7** exhibited the signals of a 5β-steroidal sapogenin [5] with a carbonyl signal at δ212.8 and four carbinyl carbon signals at 67.0 ($CH_2$), 67.3 (CH), 70.2 (CH) and 79.8 (CH), which could be assigned to C-26, C-3, C-2 and C-16 by comparison with the corresponding signals of samogenin [2] and **6** (gloriogenin). Thus, compound **7** was inferred to be 5β(25*R*)-spirostan-2β,3β-diol-12-one (mexogenin) and this was confirmed by comparison with the data of an authentic sample [10].

The aqueous hydrolysate of **1** showed the presence of D-glucose and D-galactose (TLC). The FAB mass and $^{13}$C NMR spectra of **1** suggested the presence of two sugar units. Methanolysis of the permethylate (**1a**) of **1** gave monomethylated mexogenin (**7b**) 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 (**7c**) of **7b** showed an acetoxyl signal at δ2.11, one methoxyl signal at 3.36 and one methine signal at 5.42 (*br d*, *J* = 2.4 Hz) ascribable to 3α-H, that is, hydrogen attached to the carbon bearing an acetoxyl group. Furthermore, the $^{13}$C NMR spectrum of **1** 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 YS-V [2]. Based on the above data the structure of YS-VI has been established as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-5β(25*R*)-spirostan-2β,3β-diol-12-one (**1**).

Acidic hydrolysis of **4** and **5** gave the same sapogenin (**8**), $C_{27}H_{42}O_5$ ([M]$^+$ at *m/z* 446.3078), mp 243–244°, $[\alpha]_D^{22}$ −10.7° (MeOH) which was also considered to be a 12-keto steroid sapogenin from the IR, ORD and $^{13}$C NMR spectra. The $^{13}$C NMR spectrum of **8** exhibited the signals of a 5α-steroidal sapogenin [5, 11] (C-5: δ44.4; C-9: 55.3; C-19: 13.1), a carbonyl signal at δ212.5 and four carbinyl carbon signals at 66.9 ($CH_2$), 72.8 (CH), 76.4 (CH) and 79.7 (CH), which could be assigned to C-26, C-2, C-3 and C-16 by comparison with the signals of gitogenin and **6**. Acetylation of **8** gave the diacetate (**8a**), the $^1$H NMR spectrum of which showed the presence of a 2α- and 3β-acetoxy group (6H, *s*, δ2.02; 1H, *m*, 4.77; 1H, *m*, 5.03). Thus, compound **8** was inferred to be 5α(25*R*)-spirostan-2α,3β-diol 12-one (manogenin) and this was confirmed by comparison with the data of an authentic sample [10].

The hydrolysate from **4** was examined by TLC to prove the occurrence of D-galactose, D-glucose and D-xylose, while the hydrolysate from **5** revealed L-rhamnose in addition. The FAB mass and $^{13}$C NMR spectra of **4** suggested the presence of four sugar units. The permethylate (**4a**) of **4** showed terminal permethylated -hexosyl, -pentosyl and -pentosyl dihexosyl cations at *m/z* 219, 175 and 583, respectively, in the mass spectrum. In the $^1$H NMR spectrum of **4a**, four doublet signals with *J* = 7–8 Hz at 4.35, 4.72, 4.91 and 5.04 ascribable to the anomeric protons were detected, indicating that all glycosidic linkages are β. Methanolysis of **4a** afforded an aglycone derivative (**8b**) and four methylated sugars which were identified as methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3,6-tri-*O*-methyl-D-galactose and 4,6-di-*O*-methyl-D-glucose by TLC analysis. The above evidence suggested that the sugar moiety might be β-lycotetraose. Therefore, the $^{13}$C NMR spectrum of compound **4** was compared with that of Ps-1 [1]. The signals due to the sugar were coincident with those of β-lycotetraoside. In the $^1$H NMR spectrum, the acetate (**8c**) of **8b** showed an acetoxyl signal at δ2.06, one methoxyl signal at 3.36 and one methine signal at 4.67 (1H, *m*) ascribable to 3α-H attached to the carbon bearing an acetoxyl group. Consequently, the structure of YS-IX was assigned as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-5α-(25*R*)-spirostan 2α,3β-diol-12-one (**4**).

The FAB mass spectrum of compound **5** showed a peak due to [M + Na]$^+$ at *m/z* 1233 indicating that **5** is a manogenin pentaglycoside constituted from three molecules of hexose and one molecule each of rhamnose and xylose. On enzymic hydrolysis with crude hesperidinase, **5** liberated a glycoside and rhamnose. The former was identical with **4** as judged by TLC. The $^{13}$C NMR spectrum of **5** was compared with that of YG-3 [1]. The signals due to the sugar moieties of both substances were identical and the signals of the aglycone were superimposable on those of **4**. Thus, YS-X was characterized as 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-5α-(25*R*)-spirostan 2α,3β-diol-12-one (**5**).

## EXPERIMENTAL

Mp: uncorr. $^1$H and $^{13}$C NMR spectra were recorded on a 400 MHz spectrometer. Chemical shifts are given in δ (ppm) values referred to int. TMS, and assigned by using INEPT.

*Isolation of saponins.* Refer to the preceding paper [2], the remaining material from Frs 4 and 5 were separately rechromatographed over silanized silica gel 60 (Merck) and Sephadex LH-

Table 1. $^{13}C$ NMR data of compounds YS-VI, -VII, -VIII, -IX, -X, **6–8** (in pyridine-$d_5$)

| C | 7 | YS-VI | 6 | YS-VII | YS-VIII | 8 | YS-IX | YS-X |
|---|---|---|---|---|---|---|---|---|
| 1 | 39.1 | 40.2 | 30.3 | 30.4 | 30.5 | 45.9 | 44.9 | 44.9 |
| 2 | 70.2 | 66.8 | 28.3 | 26.7 | 26.4 | 72.8 | 70.7 | 70.3 |
| 3 | 67.3 | 81.6 | 65.7 | 76.4 | 77.3 | 76.4 | 83.9 | 83.9 |
| 4 | 33.5 | 31.8 | 34.2 | 30.6 | 30.5 | 37.0 | 33.9 | 33.9 |
| 5 | 35.9 | 36.1 | 36.6 | 36.2 | 35.9 | 45.0 | 44.4 | 44.4 |
| 6 | 26.2 | 26.1 | 26.5 | 26.4 | 26.4 | 28.0 | 27.8 | 27.8 |
| 7 | 26.5 | 26.5 | 27.0 | 26.7 | 26.7 | 31.7 | 31.5 | 31.6 |
| 8 | 34.8 | 34.7 | 34.8 | 34.7 | 34.7 | 33.8 | 33.7 | 33.7 |
| 9 | 42.7 | 42.7 | 41.8 | 41.9 | 42.0 | 55.6 | 55.3 | 55.3 |
| 10 | 37.5 | 37.5 | 36.0 | 35.7 | 35.7 | 37.9 | 37.3 | 37.3 |
| 11 | 38.0 | 37.9 | 37.8 | 37.7 | 37.7 | 38.2 | 38.0 | 38.0 |
| 12 | 212.8 | 212.7 | 212.9 | 213.2 | 213.0 | 212.5 | 212.4 | 212.4 |
| 13 | 55.7 | 55.6 | 55.7 | 55.6 | 55.5 | 55.4 | 55.3 | 55.3 |
| 14 | 56.0 | 55.8 | 56.2 | 56.0 | 56.0 | 55.9 | 55.7 | 55.7 |
| 15 | 31.8 | 31.8 | 31.8 | 31.8 | 31.8 | 31.8 | 31.8 | 31.8 |
| 16 | 79.8 | 79.4 | 79.8 | 79.8 | 79.7 | 79.7 | 79.4 | 79.3 |
| 17 | 54.3 | 54.3 | 54.3 | 54.3 | 54.3 | 54.3 | 54.3 | 54.3 |
| 18 | 16.1 | 16.0 | 16.1 | 16.0 | 16.0 | 16.1 | 16.1 | 16.1 |
| 19 | 23.4 | 23.1 | 23.4 | 23.1 | 23.1 | 13.1 | 12.8 | 12.8 |
| 20 | 43.0 | 42.9 | 42.7 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 |
| 21 | 14.0 | 13.9 | 13.9 | 13.9 | 13.8 | 13.1 | 13.8 | 13.8 |
| 22 | 109.3 | 109.3 | 109.3 | 109.3 | 109.2 | 109.3 | 109.3 | 109.3 |
| 23 | 31.5 | 31.5 | 31.5 | 31.4 | 31.4 | 31.4 | 31.4 | 31.4 |
| 24 | 29.2 | 29.2 | 29.2 | 29.2 | 29.2 | 29.2 | 29.2 | 29.2 |
| 25 | 30.6 | 30.5 | 30.3 | 30.5 | 30.5 | 30.5 | 30.5 | 30.5 |
| 26 | 67.0 | 67.0 | 67.0 | 66.9 | 66.9 | 66.9 | 66.9 | 66.9 |
| 27 | 17.3 | 17.3 | 17.3 | 17.3 | 17.3 | 17.3 | 17.3 | 17.3 |
| Gal-1′ | | 103.1 | | 101.6 | 101.4 | | 103.2 | 103.2 |
| (Glc) 2′ | | 81.6 | | 79.7 | 77.6 | | 73.4 | 73.8 |
| 3′ | | 76.9 | | 88.1 | 84.2 | | 75.5 | 75.2 |
| 4′ | | 69.8 | | 70.0 | 69.6 | | 79.4 | 79.7 |
| 5′ | | 77.0 | | 78.2 | 76.1 | | 75.9 | 75.6 |
| 6′ | | 62.9 | | 63.2 | 63.4 | | 60.6 | 60.6 |
| Glc-1″ | | 106.1 | | 104.2 | 105.0 | | 104.6 | 103.2 |
| 2″ | | 75.2 | | 75.2 | 75.1 | | 81.1 | 81.1 |
| 3″ | | 78.1 | | 78.6 | 78.3 | | 87.1 | 86.9 |
| 4″ | | 71.8 | | 71.5 | 71.4 | | 70.3 | 70.2 |
| 5″ | | 78.5 | | 78.5 | 78.1 | | 78.1 | 78.3 |
| 6″ | | 62.0 | | 62.3 | 62.3 | | 62.7 | 62.7 |
| Glc-1‴ | | | | 104.7 | 104.2 | | 104.9 | 104.6 |
| 2‴ | | | | 75.2 | 74.6 | | 75.0 | 75.4 |
| 3‴ | | | | 78.3 | 78.1 | | 78.3 | 78.0 |
| 4‴ | | | | 72.3 | 72.6 | | 71.3 | 71.3 |
| 5‴ | | | | 78.5 | 78.1 | | 78.6 | 77.4 |
| 6‴ | | | | 62.3 | 62.1 | | 62.9 | 62.9 |
| Xyl-1⁗ | | | | | | | 104.9 | 104.6 |
| 2⁗ | | | | | | | 75.7 | 75.2 |
| 3⁗ | | | | | | | 77.5 | 75.9 |
| 4⁗ | | | | | | | 70.2 | 76.0 |
| 5⁗ | | | | | | | 67.2 | 64.0 |
| Rha-1⁗′ | | | | | | | | 99.7 |
| 2⁗′ | | | | | | | | 72.4 |
| 3⁗′ | | | | | | | | 72.4 |
| 4⁗′ | | | | | | | | 74.8 |
| 5⁗′ | | | | | | | | 69.8 |
| 6⁗′ | | | | | | | | 18.5 |

20 with a mixt. of $H_2O$ and MeOH to give YS-VI (52 mg), YS-VII (63 mg), YS-VIII (185 mg), YS-IX (122 mg) and YS-X (57 mg), -XI (58 mg), -XII (34 mg) and -XIII (27 mg), together with P-1, YG-2 and YG-3. YS-VI (**1**), needles, mp 245–247°, $[\alpha]_D^{26} - 13.8°$ (MeOH; *c* 0.58), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3200–3500 (OH), 1708, 980, 915, 900, 860 (915 < 900). FABMS *m/z* 793 $[M+Na]^+$, 809 $[M+K]^+$. YS-VII (**2**), needles, mp 260–261° (dec.), $[\alpha]_D^{24} - 13.6°$ ($CHCl_3$–MeOH 1:1; *c* 1.0), IR $\nu_{max}^{KBr}$ $cm^{-1}$:

3200–3500 (OH), 1705, 982, 920, 900, 863 (900 > 920). FABMS *m/z* 939 $[M+Na]^+$, 955 $[M+K]^+$. YS-VIII (**3**), needles, mp 279–281° (dec.), $[\alpha]_D^{26}$ −5.33° (MeOH; *c* 0.75), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3200–3500, 1718, 1650, 990, 920, 900, 870 (900 > 920). FABMS *m/z* 939 $[M+Na]^+$. YS-IX (**4**), needles, mp 258–259° (dec.), $[\alpha]_D^{26}$ −23.7° (MeOH; *c* 0.85), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3200–3500, 1700, 980, 920, 900, 860 (900 > 920). FABMS *m/z* 1087 $[M+Na]^+$. YS-X (**5**), amorphous powder, $[\alpha]_D^{28}$ −38.0° ($CHCl_3$–MeOH 1:1; *c* 1.6), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3200–3500, 1700, 980, 920, 900, 862 ((900 > 920). FABMS *m/z* 1233 $[M+Na]^+$, 1249 $[M+K]^+$.

*Acidic hydrolysis of compounds* **1**–**5**. Compounds **1** (10 mg), **2** (5 mg), **3** (15 mg), **4** (10 mg) and **5** (6 mg) were hydrolysed with 2 M HCl–MeOH for 2 hr. The usual work-up afforded the aglycone **6** (8 mg), needles, mp 186–188°, $[\alpha]_D^{22}$ +44.4° ($CHCl_3$; *c* 0.27), HRMS *m/z* 430.3061 $([M]^+$, calcd for $C_{27}H_{42}O_4$: 430.3081), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500, 2851, 1706, 983, 923, 900, 866 (900 > 923), ORD (MeOH; *c* 0.1) $\lambda_{ext}$ 306 nm ($[\phi]$ +2715), 266 (−3211), $^1H$ NMR ($CDCl_3$): 0.79 (3H, *d*, *J* = 6.1 Hz), 1.04, 1.05 (3H each, *s*), 1.07 (3H, *d*, *J* = 7.1 Hz), 3.29–3.48 (2H, *m*, 26-$H_2$), 4.10 (1H, *br s*, $W_{1/2}$ = 7 Hz, 3-H), 4.36 (1H, *m*, 16-H), **7** (4 mg) needles, mp 225–227° (dec.), $[\alpha]_D^{26}$ −16.5° (MeOH; *c* 0.2), HRMS *m/z* 446.3021 $([M]^+$, calcd for $C_{27}H_{42}O_5$ 446.3010), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500, 2870, 1702, 984, 922, 901, 864 (901 > 922), ORD (MeOH; *c* 0.2) $\lambda_{ext}$ 306 nm ($[\phi]$ +1422), 269 (−2407), $^1H$ NMR ($CDCl_3$): 0.79 (3H, *d*, *J* = 6.4 Hz), 1.04 (3H, *s*), 1.06 (3H, *d*, *J* = 6.9 Hz), 1.07 (3H, *s*), 3.35–3.49 (2H, *m*, 26-$H_2$), 3.67 (1H, *m*, 2-H), 3.99 (1H, *br s*, 3-H), 4.33 (1H, *m*, 16-H) and **8** (5 mg), needles, mp 243–244°, $[\alpha]_D^{22}$ −10.7° (MeOH; *c* 0.28), HRMS *m/z* 446.3078 $([M]^+$, calcd for $C_{27}H_{42}O_5$ 446.3032), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500, 2861, 1707, 983, 923, 902, 864 (902 > 923), ORD (MeOH; *c* 0.04) $\lambda_{ext}$ 310 nm ($[\phi]$ +1890), 261 (−3720). From the hydrolysates of **2** and **5**, the aglycones **6** and **8**, respectively, were identified by TLC. The sugar components were examined by TLC ($CHCl_3$–MeOH–$H_2O$, 14:6:1, **2**; $R_f$ 0.34 glucose, **1** and **3**; $R_f$ 0.31 galactose and 0.34 glucose, **4**; $R_f$ 0.31 galactose, 0.34 glucose and 0.52 xylose, **5**; $R_f$ 0.31 galactose, 0.34 glucose, 0.52 xylose and 0.57 rhamnose).

*Acetylation of compounds* **7** *and* **8**. Acetates **7a** and **8a** were prepared from **7** and **8** with $Ac_2O$ and pyridine and purified on CC (hexane–EtOAc, 3:1). $^1H$ NMR ($CDCl_3$) **7a**: δ0.79 (3H, *d*, *J* = 6.3 Hz), 1.03 (3H, *s*), 1.07 (3H, *d*, *J* = 6.9 Hz), 1.12 (3H, *s*), 1.98, 2.09 (3H each, *s*, OAc × 2), 3.35 (1H, 26-Ha), 3.49 (1H, 26-He), 4.35 (1H, *m*, 16-H), 4.75 (1H, *ddd*, *J* = 2.4, 4.6, 10.8 Hz, 2-H), 5.32 (1H, *br d*, *J* = 2.4 Hz, 3-H). **8a**: δ0.78 (3H, *d*, *J* = 6.3 Hz), 1.00, 1.04 (3H each, *s*), 1.06 (3H, *d*, *J* = 7.1 Hz), 2.02 (6H, *s*, OAc × 2), 3.4 (2H, *m*, 26-$H_2$), 4.33 (1H, *m*, 16-H), 4.77 (1H, *m*, 3-H), 5.03 (1H, *m*, 2-H).

*Methylation of compounds* **1**–**4**. Compounds **1** (20 mg), **2** (10 mg) **3** (20 mg) and **4** (30 mg) were permethylated with NaH and MeI by Hakomori's method. The products were purified by CC (hexane–$Me_2CO$, 5:1) to afford permethylates **1a** (4 mg), amorphous powder, $^1H$ NMR ($CDCl_3$): 4.22, 4.74 (each 1H, *d*, *J* = 7.8 Hz), **2a** (5.2 mg), amorphous powder, $[\alpha]_D^{28}$ −3.2° ($CHCl_3$; *c* 0.62), $^1H$ NMR ($CDCl_3$): 4.28 (1H, *d*, *J* = 7.6 Hz), 4.93, 4.96 (1H each, *d*, *J* = 7.8 Hz), **3a** (17.3 mg), amorphous powder, $[\alpha]_D^{23}$ +18.5° ($CHCl_3$; *c* 0.8), $^1H$ NMR ($CDCl_3$): 4.25, 4.81 (1H each, *d*, *J* = 7.6 Hz), 4.81 (1H, *d*, *J* = 7.8 Hz), MS *m/z* 413, 219, 187, **4a** (13.8 mg), amorphous powder, $[\alpha]_D^{23}$ +20.3° ($CHCl_3$: *c* 0.69, $^1H$ NMR ($CDCl_3$): δ4.35 (1H, *d*, *J* = 7.8 Hz), 4.72 (1H, *d*, *J* = 7.3 Hz), 4.91 (1H, *d*, *J* = 7.4 Hz), 5.04 (1H, *d*, *J* = 7.6 Hz), MS *m/z* 583, 443, 391, 219, 187, 175.

*Methanolysis of compounds* **1a** *and* **4a**. Compounds **1a** (5 mg), **2a** (5 mg), **3a** (8 mg) and **4a** (13.8 mg) were separately refluxed with 2 M HCl–MeOH. The neutralized (KOH–MeOH) and the concd hydrolysates were examined by TLC and identified with the aid of authentic samples as methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 3,4,6-tri-*O*-methyl-D-galactopyranoside from **1a**, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 4,6-di-*O*-methyl-D-glucopyranoside from **2a**, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 4,6-di-*O*-methyl-D-galactopyranoside from **3a** and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside, methyl 2,3,6-tri-*O*-methyl-D-galactopyranoside and methyl 4,6-di-*O*-methyl-D-glucopyranoside from **4a**.

The methanolysates of **1a** and **4a** were subjected to CC over silica gel (hexane–$Me_2CO$, 10:1) to afford mexogenin monomethyl ether (**7b**, 2 mg) and manogenin monomethyl ether (**8b**, 7 mg). **7b** and **8b** were acetylated with $Ac_2O$–pyridine (each 1 ml) in the usual manner to give the corresponding monoacetate (**7c**, 1.5 mg and **8c**, 4.5 mg). $^1H$ NMR ($CDCl_3$) 7c; δ0.80, 1.02 (3H each, *d*, *J* = 6.3 Hz), 1.12, 1.16 (3H each, *s*), 2.11 (3H, *s*, OAc), 3.36 (3H, *s*, OMe), 3.25–3.47 (2H, *m*, 26-$H_2$), 3.51 (1H, *m*, 2-H), 4.35 (1H, *m*, 16-H), 5.42 (1H, *br d*, *J* = 2.4 Hz, 3-H), **8c**; 0.78 (3H, *d*, *J* = 6.3 Hz), 0.94, 1.05 (3H each, *s*), 1.06 (3H, *d*, *J* = 7.5 Hz), 2.06 (3H, *s*, OAc), 3.36 (3H, *s*, OMe), 3.30–3.50 (3H, *m*, 26-$H_2$ and 2-H), 4.34 (1H, *m*, 16-H), 4.67 (1H, *m*, 3-H).

*Enzymic hydrolysis of* **5**. Compound **5** (5 mg) was dissolved in MeOH (0.2 ml), HOAc–NaOAc buffer (pH 4.5, 0.5 ml), crude hesperidinase (3 mg) added, and the mixt. incubated at 37° overnight. The reaction mixt. was evapd to dryness *in vacuo*, the residue examined by TLC and identified as **4** and rhamnose.

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